Review:

IgE-Binding Properties of Recombinant Food Allergens

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SUMMARY

Recombinant DNA technology is a common procedure to produce allergenic proteins in vitro. To date, more than 40 food allergens from fruits, vegetables, nuts, milk and seafood have been expressed as recombinant proteins. Since recombinant proteins can be produced in large quantities and in controlled quality, they promise to be suitable tools for precise diagnostics and research applications. "Hypoallergenic" variants of these molecules with reduced or abolished IgE-binding activity may be candidates for immunotherapy of food allergies. Comparative investigations of recombinant food allergens and their natural counterparts may offer new insights into the structural basis of allergenicity. This review provides a collection of data on recombinant food allergens and the spectrum of IgE-binding assays with which they have been tested. In addition, possible structural differences between natural and recombinant food allergens are considered and future diagnostic and therapeutic applications of recombinant food allergens are discussed.

(Internet Symposium on Food Allergens 2001, 3(1):1-36)

KEYWORDS

recombinant allergen, food allergy, IgE-binding activity, post-translational modifications, extracts, expression system, diagnosis, immunotherapy

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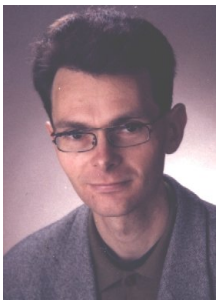

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Contents

Abbreviations:

CCD	cross-reactive carbohydrate determinants
DBPCFC	double-blind placebo-controlled food challenge
DTT	dithiothreitol
FEIA	fluorescence enzyme immunoassay
GST	glutathione-S-transferase
HPS	hydrophobic protein of soybean
LTP	lipid transfer protein
NMR	nuclear magnetic resonance
SPT	skin prick test
RAST	radioallergosorbent test

1 INTRODUCTION

Extracts from allergenic foods are the source of natural food allergens as they are applied in *in vivo* and *in vitro* tests for diagnostic and investigational purposes. Alternatively, it has become possible to produce allergens of interest *in vitro* by cultured, protein-expressing cells such as transformed *E.coli*. Expressing cells synthesise and express the protein according to an inserted DNA molecule (cDNA) which is a complementary copy of the messenger RNA (mRNA) of the protein of interest.
The main difficulty in producing recombinant food allergens is the identification and selection of the allergen-specific cDNA from the pool of diverse cDNA molecules generated from the cellular mRNA. If the amino acid or nucleotide sequence is (partly) known, the target cDNA can be amplified by PCR strategy with specific primers (Scheurer et al. 1997, Hoffmann-Sommergruber et al. 1999b, Son et al. 1999, Scheurer et al. 2000b). If there is no sequence information on the food allergen, a cDNA library has to be constructed which can be screened, for example, with sera of food-allergic subjects (Burks et al. 1995b, Scheiner et al. 1997, Kleber-Janke et al. 1999).
The expression of a recombinant allergen can be very laborious. Once established, it is possible to produce virtually unlimited amounts of the food allergen of interest. In comparison to natural allergens in extracts, recombinant food allergens are available in defined quantity and controlled quality. They may serve as pure reference materials for the standardisation of allergen extracts. During the last ten years, many recombinant allergens have been produced because they offer new prospects of an improvement of diagnosis and therapy of allergies as well as molecular allergology such as immunological and structural studies on allergenic proteins. This review summarises the data of more than 40 recombinant food allergens derived from fruits, vegetables, milk, nuts and seafood. Special attention is directed towards the structural differences between natural and recombinant food allergens and their effects for the IgE-binding and IgE-cross-linking capacity of these molecules.

2 POSSIBLE STRUCTURAL DIFFERENCES BETWEEN NATURAL AND RECOMBINANT FOOD ALLERGENS

E.coli was the most widely used cell type for the expression of approximately 40 recombinant food allergens, whereas five recombinant food allergens were produced in eukaryotic yeast cells of the species *Pichia pastoris* (see tables 1 and 2). As *E.coli* are prokaryotic they, in contrast to eukaryotic cells, do not possess the intracellular machinery for post-translational modifications such as glycosylations or disulfide bridges (Bhatia & Mukhopadhyay 1998). As a consequence, recombinant food allergens can structurally vary from the native analogues as these are produced from eukaryotic plant or animal cells. IgE antibodies bind to linear stretches of amino acids on an allergenic protein or to discontinuous epitopes formed by the secondary or tertiary structure of the protein (Kuby 1994). Therefore, changes of the structure may alter allergenic determinants and accordingly may modify the allergenic potential of a protein. The following paragraphs point out possible structural differences between recombinant food allergens and their natural counterparts.

2.1 Post-translational modifications

The lack of post-translationally added **carbohydrates** is a characteristic of the bacterial expression system. Lacking glycosylations can have an impact on the IgE-binding capacity due to a possibly altered tertiary structure or the absence of carbohydrate epitopes (Smith et al. 1996, Petersen et al. 1998, van Ree et al. 2000). Yeast cells glycosylate polypeptides, but in a different manner than other cell types. Though yeast cells attach monosaccharides to the polypeptide chain at the correct glycosylation site, they tend to hyperglycosylate and link the monomers in varying patterns (Tschopp et al. 1987, Sudbery 1996, Petersen et al. 1997, Cereghino & Cregg 2000). Thus, the carbohydrate side chains of yeast-expressed proteins can differ in size and composition. *Pichia pastoris* yeast cells are known to perform N-linked glycosylations

resulting in long carbohydrate structures of the high-mannose type without terminal alpha-1,2 mannose (Grinna & Tschopp 1989, Sudbery 1996, Verma et al. 1998). Food allergens from peanut (Ara h 1), avocado (Pers a 1), rapeseed (BnI), wheat (lipid transfer protein [LTP]) and carrot (LTP) were produced in *Pichia pastoris* cells (Sowka et al. 1998, Maid 1998, Asero et al. 2000b, Villalba et al. 2000), but none of these five recombinant allergens were applied to study glycosylations: Recombinant Pers a 1 (rPers a 1) lacked a potential N-glycosylation site and the proportion of O-linked sugars in natural Pers a 1 (nPers a 1) was very low (Sowka et al. 1998). Recombinant lipid transfer proteins of wheat and carrot were not tested on glycans (Asero et al. 2000b). The glycoprotein Ara h 1 from peanut could not be obtained in a glycosylated recombinant form because the extracellular targeting of the expressed protein failed. Finally, rAra h 1 was isolated from the cytoplasm as a non-glycosylated protein (Maid 1998).

Disulfide bridges are a second post-translational modification with a high impact on the conformation and therefore the IgE-binding activity of a protein. *E.coli* are not able to introduce disulfide bonds into polypeptide chains intracellularly but in periplasm where sulfhydryl-connecting enzymes are present. Thus, to express proteins with disulfide bonds in *E.coli*, the protein has to contain special signal sequences targeting the polypeptide to the periplasm. Recombinant milk allergen Bos d 5 was expressed according to this technique and contained at least one of two possible bonds (Chatel et al. 1996).

Further post-translational modifications, not performed in prokaryotic cells, are **hydroxylation** of prolines or N-terminal **acetylations** which can influence the IgE-binding activity of proteins (Petersen et al. 1998).

2.2 Tertiary structure

The correct folding of a recombinant protein is also of great importance, especially when the IgE antibodies bind to discontinuous epitopes. The folding machinery of eukaryotic cells is more advanced than that of *E.coli*. To benefit from the eukaryotic processing pathways and to obtain correctly folded recombinant food allergens, *Pichia pastoris* was chosen as the expression cell in the case of Pers a 1 from avocado and rapeseed allergen (Sowka et al. 1998, Villalba et al. 2000). During the expression of rPers a 1 in *Pichia pastoris*, the leader peptide was cleaved and rPers a 1 showed enzymatic activity which was comparable to that of nPers a 1. These results were taken as proof of the correct processing and folding of the protein in the yeast system (Sowka et al. 1998). Likewise, the proper folding of yeast-expressed rapeseed allergen r"BnI" was demonstrated by equivalent spectroscopic features in comparison to the natural analogue (Villalba et al. 2000). In addition, several *E.coli*-expressed food allergens were accurately folded. For example, the recombinant mustard allergen rSin a 1 showed the same resistance to trypsin digestion as nSin a 1. As trypsin resistance is regarded as a conformation-dependent property, it was concluded that rSin a 1 was properly folded (González De La Peña et al. 1996).

As frequently observed during expression in bacteria, several recombinant food allergens aggregated as insoluble "inclusion bodies" (Batt et al. 1990, González De La Peña et al. 1993, González De La Peña et al. 1996, Chatel et al. 1996, Karamloo et al. 2001). Inclusion bodies had to be solubilised under harsh denaturing conditions, for example with urea and acidic pH (Karamloo et al. 2001), urea and DTT (Chatel et al. 1996) or by guanidine-HCl treatment plus DTT (Batt et al. 1990). Subsequently, the solubilised proteins underwent successful refolding procedures (Chatel et al. 1996, Karamloo et al. 2001) according to special renaturation protocols (Cho et al. 1994, Boehm & Rösch 1997). In contrast, inclusion bodies containing aggregated rSin a 1 could not be solubilised by detergents, organic solvents, urea or guanidinium chloride (González De La Peña et al. 1996).

2.3 Codon usage

Problems in the production of peanut allergen Ara h 1 in bacteria can serve as an example to demonstrate a specific problem with bacterial expression systems. During the biosynthesis of rAra h 1, many fragments occurred and the yield of rAra h 1 was rather low. It was concluded that rare codons used by *E.coli* were responsible for these phenomena. Two of six possible arginine-coding triplets are scarce in *E.coli*, but frequent in Ara h 1-coding mRNA and the corresponding tRNA molecules are rare in *E.coli*. This fact possibly led to the termination of translation (Burks et al. 1995b, Maid 1998). Truncation or low expression rates were also observed during the production of rAra h 2, rAra h 6 and rAra h 7 which are rich in rare arginine codons (Kleber-Janke et al. 1999, Kleber-Janke & Becker 2000). However, high-level expression of the peanut allergens Ara h 1, Ara h 2 and Ara h 6 could be gained by the application of modified *E.coli* cells. Those expressing cells were transformed with extra tRNA genes specific for rare arginine codons (Kleber-Janke & Becker 2000).

2.4 Technical reasons leading to altered structures

As illustrated above, structural differences between recombinant food allergens and the natural counterparts can be due to the different protein biosynthesis pathways of bacterial and eukaryotic cells. In addition, technical aspects of the expression procedure can cause structural differences.
The majority of recombinant food allergens were cloned with fusion peptides for which specific ligands were available. This enabled the purification of the recombinant protein from the bulk of cellular proteins on an affinity column specific for the fusion peptide. A very common fusion peptide in the production of recombinant food allergens were tags consisting of six or ten histidine (his) residues at the N- or C terminus of the protein (Scheurer et al. 1997, Burks et al. 1999, Rabjohn et al. 1999, King et al. 1999, Beardslee et al. 1999, Hoffmann-Sommergruber et al. 1999b, Son et al. 1999, van Do et al. 1999, Karamloo et al. 2001). In most cases, these his-tagged recombinant proteins were purified by metal-chelate affinity chromatography on an Ni-NTA-matrix column (NTA: nitrilotriacetic acid) (Scheurer et al. 1997, Rabjohn et al. 1999, Hoffmann-Sommergruber et al. 1999b, Son et al. 1999, Karamloo et al. 2001). Several other food allergens were fused to the enzyme glutathione-S-transferase (GST) from *Schistosoma japonicum* (Leung et al. 1994, González De La Peña et al. 1996, Leung et al. 1996, Teuber et al. 1998, Leung et al. 1998a, Leung et al. 1998b, Teuber et al. 1999). These recombinant GST-fused food allergens were purified from cell lysate by using a glutathione column. Other fusion peptides used with recombinant food allergens were maltose-binding protein (MBP) (Rihs et al. 1999), beta-galactosidase (beta-gal) (Bugajska-Schretter et al. 1998, Bugajska-Schretter et al. 1999) and intein-CBD protein (CBD: chitin-binding domain) (Lüttkopf et al. 1999, Vieths et al. 2000). In some cases, the fusion peptides were cleaved by proteolytic or chemical treatment after purification to obtain the recombinant non-fusion protein (Lüttkopf et al. 2000b). In the case of enzymatic cleavage of his-tags, additional amino acids remained at the terminus of the recombinant non-fusion protein (Scheurer et al. 1997, Son et al. 1999, Karamloo et al. 2001). Moreover, several recombinant food allergens were directly subjected to IgE-binding assays without cleavage of the fusion peptide (see table 2). Fusion peptides such as his-tags with a molecular mass of about 3-4 kDa (Scheurer et al. 1997) were rather small compared to GST which had a size of about 26 kDa. In the recombinant constructs rSin a 1-GST or rJug r 1-GST, the fusion part GST even had a higher mass than the respective allergens of 18.8 kDa or 15-16 kDa (González De La Peña et al. 1996, Teuber et al. 1998).
Fusion tags are not necessarily required for cloning and purification of food allergens. For example, Mal d 1 the major allergen from apple was expressed as his-tagged protein (Son et al. 1999) as well as non-fusion protein which was purified by an anion-exchange column (Vanek-Krebitz et al. 1995). Plant profilins required no linkage to fusion peptides as they bind physiologically to proline-rich sequence stretches and therefore could be purified by poly-L-proline affinity chromatography (Karamloo 1999a, Scheurer et al. 2000).

Heterodimeric allergens such as seed storage proteins consist of two polypeptide chains linked by disulfide

bridges. They emerge from a long precursor polypeptide which is enzymatically cleaved into a large and a short chain which are then linked by disulfide bonds. Allergenic seed storage proteins were cloned in *E.coli* as the unprocessed precursor form. The cDNA coding for the precursor molecule was selected from a cDNA library and used as template for production in *E.coli*. According to that method, the 2S seed storage proteins Ara h 6, Ara h 7 and Jug r 1 from walnut as well as the 11S seed storage protein Ara h 3 were cloned (Smith et al. 1997, Kleber-Janke et al. 1999). Recombinant 2S albumins from mustard and rapeseed were produced by a similar strategy. The cDNA used for the expression was not obtained from a library but constructed by fusing DNA fragments amplified from genomic DNA, each coding for one subunit of the precursor. The resulting recombinant allergens differed from the original precursor because they carried three additional internal amino acid residues (González De La Peña et al. 1996, Villalba et al. 2000).

3 IgE-BINDING ACTIVITY OF RECOMBINANT FOOD ALLERGENS

As shown in section 2, recombinant food allergens are, in general, not an identical copy of the natural analogue. Therefore, changes in the IgE-binding property of the recombinant protein may occur. Through the loss of linear or conformational IgE-binding epitopes, the allergenic reactivity may be altered or even be eliminated. Thus, it is important that the IgE binding properties of a recombinant food allergen are well characterized before it is used in research, diagnostic or therapeutic applications. The present data collection summarises the recombinant food allergens published up to July 2000 (tables 1 and 2). The criterion for enlisting a recombinant food allergen is that it was tested for IgE-binding activity. The mere *in vitro* production of a molecule known as a food allergen (such as ovalbumin or bovine lactoglobulin) was not sufficient to be included in the list. The cloning of the postulated celery allergen Api g 3 (rApi g 3) was reported, but as rApi g 3 was not assayed with patients' sera it was not included in the list (Scheiner et al. 1997).

The level of characterization of an allergen depends on the diversity of test systems applied. In immunoblot tests under denaturing conditions, loss of conformational epitopes may occur. In inhibition tests, the inhibitor protein is in fluid phase and thus in correct conformation. Mediator release assays show the biological activity indicating that the recombinant food allergen is able to cross-link cell-bound IgE and elicit degranulation of basophils.
All recombinant food allergens listed in table 2 were at least tested in IgE immunoblotting and showed a considerable IgE-binding capacity. Recombinant Api g 1 from celery, for example, had a similar IgE reactivity to that of the natural analogue. When tested by IgE immunoblotting, both rApi g 1 and nApi g 1 in celery extract were recognized by sera from 11 patients with symptoms after ingestion of celery (Breiteneder et al. 1995). The recombinant apple allergen rMal d 1, like nMal d 1, bound IgE of 13 sera of patients in immunoblot analysis and EAST (enzyme allergosorbent test) (Son et al. 1999), and similarly, both rDau c 1 and nDau c 1 from carrot were IgE-reactive to sera of six carrot-allergic subjects (Hoffmann-Sommergruber et al. 1999b). Several recombinant food allergens underwent a panel of different tests including mediator release assay providing evidence for IgE-binding and cross-linking activity (Scheurer et al. 1997, Scheurer et al. 1999, Karamloo 1999a, Son et al. 1999, Fötisch et al. 1999, Scheurer et al. 2000b, Vieths et al. 2000, Karamloo et al. 2001). Recombinant cherry allergen rPru av 1 for example, caused histamine release in basophils of three cherry-allergic patients as did cherry extract (Scheurer et al. 1997). Three isoforms of rMal d 1 from apple were compared in mediator release assay with nMal d 1. One rMal d 1 isoform provoked release similar to nMal d 1. This isoform displayed the highest EAST classes with sera from 13 apple-allergic patients. The two other rMal d 1 isoforms with lower EAST classes elicited moderate releases (Son et al. 1999). The first and until now the only recombinant food allergen applied in skin prick tests was rApi g 1 which elicited positive skin reactions in Api g 1-sensitive subjects (Hoffmann-Sommergruber et al. 1999a).

An important question is whether structural variations of a recombinant food allergen influence the IgE-binding properties. Information on the impact of carbohydrates is rare. The known glycoprotein allergens

Ara h 1, wheat glutenins and wheat gliadins were expressed in *E.coli* and thus did not contain carbohydrate structures. However, all recombinant forms bound IgE antibodies of patients' sera in immunoblot tests or RAST (Burks et al. 1995a, Burks et al. 1995b, Maruyama et al. 1998, Kleber-Janke et al. 1999). Eight out of 11 patients' sera with IgE to nAra h 1 recognized rAra h 1 in immunoblotting indicating that a considerable number of epitopes are unaffected by carbohydrate components (Burks et al. 1995a).

A correct folding of recombinant food allergens was concluded by some authors from results of circular dichroism (CD) spectroscopy, immunological assays, and tests for enzymatic activity (González De La Peña et al. 1996, Chatel et al. 1996, Villalba et al. 2000). The evidence of IgE-binding activity supported this conclusion. Profilins for example, are regarded as allergens with a highly conformation-dependent IgE reactivity (Valenta et al. 1991, Vrtala et al. 1996, Rihs et al. 1999). Nevertheless, recombinant profilins (rApi g 4, rAra h 5, rGly m 3, rPru av 4, rPyr c 4) bound IgE in EAST tests and two of them even elicited mediator release (Kleber-Janke et al. 1999, Scheurer et al. 2000a, Karamloo 1999a, Rihs et al. 1999, Scheurer et al. 2000b). A correct conformation was assumed for *E.coli*-expressed rBos d 5 which contained at least one of two possible disulfide bonds. Studies with monoclonal and polyclonal antibodies revealed evidence that natural and recombinant Bos d 5 had a comparable conformation. Correspondingly, the IgE reactivity of nBos d 5 and that of rBos d 5 in five patients' sera were similar (Chatel et al. 1996).

In several cases, recombinant food allergens were extracted from inclusion bodies and successfully refolded by subsequent renaturation procedures. Recombinant pear allergen rPyr c 1, for example, was characterized in various tests including mediator release assay with a large number of sera and was found to have a high biological activity (Scheurer et al. 1999, Vieths et al. 2000). Recombinant Pyr c 1 detected 89% and pear extract 85% of the sera of 61 pear-allergic patients indicating that rPyr c 1 was more active than the extract (Vieths et al. 2000).

Differences in the amino acid sequence can alter the IgE-binding pattern of allergens. Mutation studies revealed that the substitution of one single amino acid drastically reduced the ability to bind IgE (Scheurer et al. 1999, Son et al. 1999). The recombinant mustard allergen rSin a 1 contained three additional amino acids. Inhibition studies with monoclonal antibodies showed slight conformational changes between natural and recombinant Sin a 1 due to the additional residues, but both forms inhibited the binding of IgE to a similar degree (González De La Peña et al. 1996). In some cases, recombinant food allergens with N- or C terminally added fusion peptides were used in IgE assays. There was no indication that the fusion tags had an influence on the IgE-binding capacity of the recombinant allergen. Even the fusion peptide GST with a relatively high molecular mass of about 26 kDa did not affect IgE-binding activity. Tropomyosins from several seafoods with a molecular mass of 34 kDa were expressed as fusion proteins with GST and bound IgE from patients with crustacean allergy (Leung et al. 1998a, Leung et al. 1994, Leung et al. 1996, Leung et al. 1998b). In rJug r 1-GST, the difference of molecular mass was considerable: the Jug r 1 had a molecular mass of 15-16 kDa while the fusion part was estimated at 26 kDa. The fusion protein bound IgE in immunoblotting whereas the binding of IgE to GST was excluded (Teuber et al. 1998). His-tags which had a molecular mass of about 3-4 kDa and were thus smaller than the respective allergen had no apparent effect on the IgE binding activity. Both the recombinant his-tagged Pru av 1 and the non-fusion rPru av 1 reacted with serum IgE antibodies. In inhibition studies, the same results were obtained with fusion and non-fusion forms (Scheurer et al. 1997, Scheurer et al. 1999).

4 APPLICATIONS OF RECOMBINANT FOOD ALLERGENS

4.1 Diagnostics

Recombinant food allergens offer new perspectives to solve current problems in diagnostics of food allergies. *In vivo* and *in vitro* tests are performed with extract preparations of the allergenic food. To date, these commercially available test extracts are standardised neither with respect to content of allergens nor with respect to biological activity (Bruijnzeel-Koomen et al. 1995, AAAAI 1997). There are differences between extracts from various manufacturers and even between batches from one manufacturer (Sampson 1988, Ortolani et al. 1989). A raw food, used as source for the production of extracts, can vary in its allergen content. In the case of fruits, factors such as degree of ripening or strain can influence the amount of allergens (Chapman et al. 1987, Vieths et al. 1993, Vieths et al. 1995, Fernández-Rivas & Cuevas 1999). Apples of the variety "Golden Delicious", for example, were found to contain eleven times more major allergen Mal d 1 than those of the variety "Gloster" (Son et al. 1999). In addition, it is possible that food extracts lose activity during extraction procedure or during storage (Bernhisel-Broadbent et al. 1992b, Norgaard et al. 1992, Norgaard et al. 1995, Vieths et al. 1998). The IgE-binding capacity of allergens in an extract can be altered by enzymatic degradation or by interaction with phenolic components (Björkstén et al. 1980, Ortolani et al. 1989, Deluze et al. 1991, Vieths et al. 1994, Vieths et al. 1995, Rudeschko et al. 1995, Varjonen et al. 1996). It was shown that of four commercially available apple skin test extracts none could verify the existing sensitisation to apple in 72 patients (Vieths et al. 1995). In conclusion, these data show that results of a test performed with an extract may be false-negative.

Pure recombinant food allergens could be used as reference material to standardise extracts for *in vitro* use and skin tests. Diagnostic results obtained with standardised preparations would be reproducible and more precise. Recombinant allergens may not only improve the quality of extracts, but also could replace extracts in *in vitro* and *in vivo* tests. Tests could be carried out with a panel of recombinant food allergens representing the allergenic components of a food. The prerequisite for this concept is that all allergens of a food are known and available in recombinant form that is active and shares the same IgE-binding capacity as the natural analogue. In screening tests, problematic food extracts could be supplemented by recombinant food allergens or mixtures thereof, to make sure that all sensitised patients are identified by the screening procedures.

A new aspect of testing with single recombinant allergens is that it will be possible to compile a patient's individual sensitisation pattern, whereas with extracts, in general, only sensitisation to the whole food is determined. A knowledge of the sensitisation pattern, will make prognostic statements as well as preventive recommendations possible. This aspect bases on the phenomenon that birch pollen-allergic patients reacting to birch pollen allergen Bet v 1, will probably develop a food allergy against a certain spectrum of fruits and vegetables, such as cherry, pear, apple and celery. This is due to allergens of approximately 17-18 kDa in the respective food which belong to the Bet v 1-family, a group of allergens with sequence homology to the Bet v 1 allergen. The symptoms are caused by cross-reactivities between the members of the Bet v 1-family, as shown by studies with recombinant proteins (Hoffmann-Sommergruber et al. 1999a, Scheurer et al. 1999, Hoffmann-Sommergruber et al. 1999b, Karamloo et al. 2001, Kazemi-Shirazi et al. 2000, Lüttkopf et al. 2000a). In these cases, it is favorable to reduce consumption of food containing Bet v 1-related allergens. If the sensitisation pattern reveals reactivity to profilin, avoidance will not be possible because profilin occurs in all eukaryotic cells (Vieths et al. 1996a, Vieths et al. 1996b). Allergenic profilins have been identified in many fruits, vegetables and pollens. Studies have demonstrated cross-reactivities between allergenic profilins from different organisms. Thus, ubiquitous profilin is regarded as a pan-allergen in plants (see table 1) (Valenta et al. 1992).

Initial experience with recombinant food allergens as diagnostic tools is promising. For example, a sample of 43 sera of patients with clinical hazelnut allergy, verified by double-blind placebo-controlled food challenges (DBPCFC), was tested by EAST with recombinant hazelnut allergen rCor a 1.0401 and hazelnut extract. The one recombinant allergen rCor a 1.0401 proved 41 of 43 sera positive while the

extract was only recognized by IgE of 32 of 43 sera (Vieths et al. 2000, Lüttkopf et al. 2000b). Pear extract bound 85% of 61 sera of pear-allergic patients while one recombinant pear allergen (rPyr c 1) bound IgE of 89% of the sera (Vieths et al. 2000). In these two examples one single recombinant food allergen was more sensitive in the EAST test than the whole extract and led to a lower number of false-negative results. This fact may be caused by higher amounts of the allergen as recombinant proteins in the tests, whereas the relative amounts of single allergens in the whole extracts were not sufficient to be detected by certain sera. The replacement of food extracts in screening tests by a panel of recombinant food allergens seems to be possible. In a recent study, six recombinant peanut allergens were able to identify all 40 peanut-sensitive individuals. Various individual sensitisation patterns were observed, but ultimately the six recombinant allergens were sufficient to detect all 40 sera (Kleber-Janke et al. 1999). Another example of recombinant allergens resembling the activity of extracts is the pear allergen rPyr c 1 in combination with the minor allergens rPyr c 4 and rPyr c 5 (Vieths et al. 2000).

It is a well-known fact that results of serological assays, skin tests or clinical tests do not always correlate (Bernhisel-Broadbent et al. 1989, Bernhisel-Broadbent et al. 1992a, Norgaard et al. 1992, Eigenmann & Sampson 1998, Majamaa et al. 1999). A group of 24 patients with a clinical history of celery allergy showed different results in three different test systems. All subjects had a positive SPT to crude celery and to celery extract, 20/24 reacted to extract in IgE immunoblotting and only 14/24 had a RAST class 1 and higher. In contrast to these results, in another group of 12 celery-allergic patients 11/12 had a positive SPT to fresh celery but only 3/12 reacted to celery extract in SPT, indicating that the extract applied in the latter group probably was not active (Hoffmann-Sommergruber et al. 1999a). With the help of recombinant food allergens as test material or as reference compound for standardisation it may be possible to highlight whether contradicting results of diagnostic tests depend on test compounds or test systems. *In vitro* tests, for example, are often false-positive with respect to clinical food allergy (Ebner et al. 1991). They determine sensitisation to IgE epitopes in foods, but do not prove clinical allergy. Thus, the predictive value of routine tests such as RAST or CAP is limited. The golden standard for verifying a food allergy is the DBPCFC, but it has to be performed by well-educated allergists and is very time-consuming and costly (Bock et al. 1988). Therefore, the DBPCFC will not become a routine technique. Clinically false-positive *in vitro* results may be due to cross-reactive structures in foods such as, for example, IgE-binding carbohydrate structures. Some authors postulate that these cross-reactive carbohydrate determinants (CCD), which are broadly present in foods (Vieths 1997, Aalberse & van Ree 1997), are clinically irrelevant and therefore cause false-positive *in vitro* tests (van der Veen et al. 1997, Aalberse 1998). Although this statement is not generally accepted (Fötisch et al. 1999), it is reasonable to investigate these reactivities to improve the predictive value of *in vitro* tests (van Ree et al. 2000). This could be another possible approach for applying recombinant food allergens.

4.2 Therapy

Food allergies may elicit severe acute hypersensitivity reactions and are a main cause of emergency treatment due to life-threatening anaphylaxis (Yunginger et al. 1988, Sampson et al. 1992, Yocum & Khan 1994, Hourihane et al. 1997a). Pumphrey et al. showed that more than 50% of anaphylactic reactions were due to food allergies (Pumphrey & Stanworth 1996). Patients with severe acute reactions to food are instructed to avoid the allergenic food, which can be very difficult to manage. When patients consume food to which they are allergic, it mostly happens accidentally and inadvertently (Sampson et al. 1992, Borelli et al. 1999, Sicherer et al. 1999). For that reason, patients with an anaphylactic history to food ingestion are equipped with emergency medications. In the case of peanut allergy it is virtually impossible to abstain from peanut because peanut proteins are widely used in food products and in the pharmaceutical industry (Moneret-Vautrin et al. 1994, Ewan 1996, Lever 1996). Since only micrograms of peanut protein are sufficient to elicit symptoms, contaminations of foods by hidden peanut proteins are a high risk for peanut-allergic subjects (Hourihane et al. 1997b).

Apart from avoidance of the offending foods, food allergies are treated with symptomatic medication.

Current developments of causative therapeutic approaches are focused on DNA vaccination strategies (Roy et al. 1999, Adel-Patient et al. 2000) or oral desensitisation protocols (Patriarca et al. 1984, Patriarca et al. 1998, Bauer et al. 1999).
Up to now, there is no established causally effective immunotherapy for food allergies. In patients with pollen-related food allergy who underwent specific immunotherapy (SIT) by subcutaneous injection of pollen extracts, the food allergy improved in approximately 37% to 84% of the cases (Möller 1989, Henzgen et al. 1991, Henzgen et al. 1994, Kelso et al. 1995, Herrmann et al. 1995, Asero 1998a, Henzgen et al. 1999, Asero 2000). The positive effect of pollen SIT on food allergy reflects cross-reactivity of food and pollen allergens at T cell level. It was shown that birch pollen allergen Bet v 1 stimulated T cells specific for homologous apple allergen Mal d 1 and vice versa. Cross-reactive T cell epitopes could be characterized (Fritsch et al. 1998). Since pollen-related food allergy did not improve in all patients under pollen SIT, development of food allergen-specific SIT to treat unsuccessful cases may be required.

A few years ago, initial attempts were undertaken to develop a SIT for peanut allergy. A rush immunotherapy over a period of one month performed by injecting increasing doses of aqueous peanut extracts to peanut-allergic subjects was effective; at the end of one month patients tolerated higher doses of peanut but all subjects experienced allergic side effects during the therapy (Oppenheimer et al. 1992, Nelson et al. 1997). When the therapy was prolonged up to one year by application of maintenance doses, the positive effects of the rush phase persisted or improved in three of six patients. However, all patients required symptomatic treatment during the immunotherapy (Nelson et al. 1997).
These studies indicate that classical SIT by injection could be a strategy to treat food allergies. However, side effects should be avoided. Recombinant DNA technology could provide the techniques to develop effective and safe therapeutic reagents. First, recombinant isoforms and mutants of allergens with low IgE-binding capacity and retained T cell activity may be candidates. These modified allergens may be safe, because they do not elicit side effects due to cross-linking of cell-bound IgE antibodies. However, to be effective, they have to be T cell-reactive to induce T cell non-responsiveness to allergens. Second, small peptides and fragments containing T cell epitopes may be other candidates for SIT. In two studies, SIT of bee venom- and cat-allergic subjects with T cell epitope-containing peptides were successful (Müller et al. 1998, Norman et al. 1996). Peptides for therapy could be produced as defined synthetic peptides or recombinant allergen fragments.

Hypoallergenic isoforms, mutants, and fragments of food allergens were produced. For example, five recombinant fragments of the cherry allergen Pru av 1 of 59 to 116 amino acids in length were described. One of these fragments bound no IgE antibodies whereas four were IgE antibody-reactive (Scheurer et al. 1999). Three hypoallergenic recombinant fragments of soybean profilin Gly m 3, comprising 50 to 81 amino acids and spanning the whole allergen molecule scarcely inhibited binding of IgE to the entire recombinant allergen (Rihs et al. 1999). Hypoallergenic isoforms as well as mutants of the apple major allergen Mal d 1 were cloned. One of three recombinant Mal d 1 isoforms had a weaker IgE antibody-binding activity by EAST than the others (Son et al. 1999). A mutant of Mal d 1, differing by a single point mutation from the wild-type form, showed a drastically reduced IgE-binding capacity. This mutant bound no IgE antibodies of 13 patients' sera while the wild-type rMal d 1 was recognized by all sera (Son et al. 1999). Three of seven mutants of the cherry allergen Pru av 1 with one or two point mutations showed a strongly reduced or even abolished IgE-binding activity (Scheurer et al. 1997). Hypoallergenic recombinant mutants of peanut allergens Ara h 1, Ara h 2, Ara h 3 (Burks et al. 1999, King et al. 1999, Shin et al. 1999, Burks et al. 2000), Mal d 1 and Api g 1 (Ferreira et al. 2000) and potato allergen Sol t 1 (Alibhai et al. 2000) containing amino acid substitutions in IgE-binding epitopes were reported.
It has to be considered that "hypoallergenicity" of an isoform or mutant is determined by the epitope recognition of the individual patient in serological or skin tests. A mutant of rAra h 2 was tested with 16 sera of peanut-sensitive patients. A strong decrease in IgE binding was shown in 12 of 16 sera, while three sera bound the modified allergen similar to the wild-type allergen, and in one serum the binding was even increased (Burks et al. 1999). Thus, the safety of a modified allergen for a patient has to be tested before any kind of application, and it might be necessary to develop a panel of different immunotherapeutic allergens. The search for this patient-tailored panel of hypoallergenic isoallergens or mutants may be

extensive. However, there is no risk of new sensitization to other proteins of the allergenic source, which theoretically exists when whole extracts are applied.
To be applied as therapeutic reagents, the above mentioned isoforms, mutants and fragments have to show that they have (1) low IgE-binding capacity and (2) T cell reactivity and that they are (3) therapeutically effective. So far, the incapacity to bind IgE antibodies was only shown in *in vitro* tests. Skin tests still have to be performed. Additionally, the T cell reactivity of the hypoallergenic derivatives has to be determined. So far, recombinant food allergens were not used as therapeutic reagents. Only in the case of peanut allergen Ara h 2, was the T cell-stimulatory ability of a hypoallergenic rAra h 2 mutant determined and was comparable to that of wild-type Ara h 2 (Burks et al. 1999). More information exists on recombinant non-food allergens. Several hypoallergenic isoforms, mutants and fragments displayed weak IgE-binding activity not only in *in vitro* tests but also in skin tests and furthermore induced T cell proliferation (Kauppinen et al. 1999, Takai et al. 1999, Ferreira et al. 1996, Zeiler et al. 1997). In animal model recombinant inhalant allergens proved to be effective therapeutics in SIT (Yasue et al. 1998, Yasue et al. 1999, Vrtala et al. 1995).
These data on recombinant hypoallergenic derivatives of food allergens indicate new approaches to SIT of food allergies, but more studies, especially on T cell-stimulatory capacity of the derivatives, have to be performed.

4.3 Research

Recombinant DNA technology provides tools to investigate the possible links between structure and allergenicity. With recombinant fragments of allergens, for example, one is able to characterize the location of epitopes and whether they are linear or conformational. Allergenic shrimp tropomyosin was mapped for epitopes by recombinant fragments of 13 to 22 amino acids in length and synthetic peptides. The epitopes were located at the C terminus and in the centre of the allergen sequence (Reese et al. 1997, Reese et al. 1999). Five recombinant fragments of the cherry allergen Pru av 1, with a size of 59 to 120 amino acids and spanning the whole molecule, displayed weak IgE-binding activity, with one fragment which bound no IgE antibodies (Scheurer et al. 1999). The results indicated that Pru av 1 epitopes are mainly conformational. Similarly, the IgE-binding capacity of soybean profilin Gly m 3 depends on the structural integrity of the entire molecule, as deduced from tests with recombinant fragments (50-81 amino acids) which were not able to inhibit binding of IgE antibodies to rGly m 3 (Rihs et al. 1999).

Recombinant mutants of food allergens are further tools to reveal strucutural characteristics of epitopes and relevance for IgE-binding capacity. Mutants of three related allergens of birch pollen (Bet v 1), cherry (Pru av 1) and apple (Mal d 1) with regards to the amino acid 112 (or equivalent 111 in Mal d 1) were constructed. The replacement of original serine by proline drastically reduced the IgE-binding ability of all three allergens compared to that of recombinant wild-type form (Scheurer et al. 1999, Son et al. 1999). The results showed that the amino acid at position 112 is a key residue in proteins of the Bet v 1-family with implications for IgE reactivity of the entire molecule. Similar consequences from equivalent mutations have also been reported from homologous allergens Mal d 1, Api g 1 and Bet v 1. Six substitutions in IgE-binding epitopes similarly reduced IgE-binding capacity of the allergens (Ferreira et al. 2000).

Furthermore, involvement of certain structures in allergenicity can be studied on the basis of spectroscopic and immunological data obtained by recombinant proteins. In the cherry allergen Pru av 1 two mutations affecting the P loop region (amino acids 46-52) reduced the IgE binding activity in six of seven sera of patients indicating that the P-loop region possibly displays an IgE-binding epitope or, at least, is involved in forming a conformational IgE binding structure (Scheurer et al. 1999). Recombinant proteins are well-suited test compounds in spectroscopic techniques such as circular dichroism (CD) spectroscopy or heteronuclear NMR spectroscopy because they are available as pure substances in high amounts of controlled quality. Heteronuclear NMR spectroscopy can be performed with allergens which contain special detectable isotopes (^{13}C, ^{15}N, ^{1}H) introduced during the expression of recombinant proteins. Spectroscopic studies were undertaken to investigate whether similar allergenicity of related proteins were

based on related structural elements. A highly resolved tertiary structure of cherry allergen Pru av 1 was calculated on the basis of NMR data of double-labelled recombinant protein. Apart from the conclusion that cross-reactivity is based on structural homology, studies may be useful to identify conserved surface patches that may represent the epitope regions (Schweimer et al. 1900, Neudecker et al. 2000b).

Knowledge on structural characteristics of IgE-binding epitopes may serve to develop hypoallergenic processed foods. Peanut major allergen Ara h 1 or LTP from peach are both heat-stable food allergens containing linear epitopes (Koppelman et al. 1999, Brenna et al. 2000). Therefore, other technological processes than heat treatments have to be applied to reduce the allergenicity of foods. In contrast, celery allergen Api g 1, which has conformational epitopes, is heat-labile and heating as well as non-thermal procedures were able to decrease the IgE-binding activity (Jankiewicz et al. 1996, Vieths et al. 1998). Another aspect of recombinant DNA technology is that nucleotide sequence data of a food allergen gained during cloning procedure can be applied for genetic immunisation with cDNA of an allergen. This is a new approach for an allergen-specific immunotherapy with the potency for treatment as well as for protecting from allergy (Roy et al. 1999, Adel-Patient et al. 2000).

5 CONCLUSIONS

Up to now, more than 40 recombinant food allergens have been expressed *in vitro*, most of them in *E.coli*, several in the yeast system (see table 1). The recombinant allergens may differ structurally from the natural counterpart, for various reasons. A most striking feature of *E.coli*-expressed recombinant proteins is the lack of post-translational modifications such as carbohydrate side chains, disulfide bonds (if expressed intracellularly), hydroxylated prolines or acetylations (Burks et al. 1995b, Kleber-Janke et al. 1999). Recombinant allergens cloned in yeast cells can be glycosylated but the glycans may differ in size and pattern from those of other eukaryotic cell types (Petersen et al. 1997). Some recombinant food allergens have been expressed with fusion peptides which have been applied as specific ligands for purifying expressed protein from the pool of cellular proteins (see table 2). After cleavage of the fusion peptide additional amino acid residues may remain at the recombinant food allergen (Scheurer et al. 1997, Son et al. 1999). Heterodimeric allergens have been cloned as monomeric precursor molecules (González De La Peña et al. 1996, Smith et al. 1997, Kleber-Janke et al. 1999, Villalba et al. 2000). Recombinant proteins may display conformations differing from wild-type proteins, for example, if they have to be solubilised from aggregates (inclusion bodies) (Batt et al. 1990, Chatel et al. 1996, Karamloo et al. 2001). Amino acid sequences as well as secondary and tertiary structures of a protein have an impact on linear and discontinuous IgE-binding epitopes. Therefore, structural differences may alter the IgE binding capacity of a recombinant allergen compared to the natural analogue. We have reviewed data of the recombinant food allergens cloned to date and concluded that they showed a considerable IgE-binding activity *in vitro*. Several of them even displayed biological activity in mediator release assays or skin tests (see table 2). In comparative IgE binding studies, natural and recombinant food allergens shared similar properties in many cases (Breiteneder et al. 1995, Chatel et al. 1996, Son et al. 1999, Karamloo et al. 2001). There are also examples of recombinant food allergens with an altered IgE reactivity compared to natural counterpart (Burks et al. 1995a, Burks et al. 1995b, Son et al. 1999).

The application of recombinant food allergens could possibly improve diagnostics and therapy of food allergies. They may serve as pure reference material for standardisation of food extracts. Biologically and quantitatively standardised food extracts could provide more precise results in diagnostic tests. Recombinant food allergens could be applied as diagnostic tools in addition to or instead of labile extracts. As a prerequesite, all allergens of a food have to be known and available in recombinant form of comparable IgE reactivity. In initial studies, tests with one, three or six recombinant allergens were more sensitive than the respective food extract (Kleber-Janke et al. 1999, Vieths et al. 2000). A panel of six recombinant minor and major peanut allergens were sufficient to verify 40 peanut-sensitive subjects (Kleber-Janke et al. 1999). With single recombinant food allergens, the patient's individual sensitisation pattern could be compiled with possible effects for therapy and prognostic statements.

So far, a causally effective specific immunotherapy against food allergies does not exist. The only means to handle food allergy are avoidance of the offending food, application of symptomatic medication or oral hyposensitisation strategies resulting in increased tolerance to the respective food (Patriarca et al. 1984, Bauer et al. 1999). *In vitro*-expressed "hypoallergenic" mutants, isoforms or fragments of food allergens with weak or lacking IgE binding potency are discussed as possible candidates for an immunotherapy. They could be effective if they have T cell reactivity and safe because of inability to bind and cross-link IgE antibodies. Hypoallergenic mutants, isoforms and fragments have been described, but T cell activity still has to be demonstrated (Scheurer et al. 1997, Burks et al. 1999, King et al. 1999, Son et al. 1999, Burks et al. 2000, Shin et al. 1999, Ferreira et al. 2000, Alibhai et al. 2000). Another approach for causative therapy by DNA vaccination depends on nucleotide sequence information obtained during cloning procedure (Roy et al. 1999, Adel-Patient et al. 2000).

Since high amounts of recombinant allergens can be produced and can be labelled with specific isotypes, they are suitable tools in spectroscopic techniques to resolve allergen structures and to investigate the link between conformational features and IgE binding. It has been demonstrated that cross-reactivity in homologous allergens depends on related structures (Schweimer et al. 1900, Neudecker et al. 2000b). Knowledge of the relevance of structure for allergenicity may have implications for the development of food processing techniques to create hypoallergenic foods (Vieths et al. 1998, Brenna et al. 2000).
In summary, recombinant food allergens are suitable tools to gain more insight into the allergenicity of food proteins. They offer prospects to solve current problems in the fields of diagnostics and therapy of food allergies and in molecular allergology.

Table 1: List of Recombinant Food Allergens

Table 1 enlists, in alphabetical order of the latin names, all recombinant food allergens published until July 2000 which have been tested in IgE-binding assays. Asterisks * indicate food allergens which are noted in the official list of allergens of the WHO / IUIS allergen nomenclature subcommittee (ftp://biobase.dk/pub/who-iuis/allergen.list). The theoretical or experimental molecular mass refers to the recombinant form of the respective food allergen.

Common names: [Abalone] [Apple] [Avocado] [Carp] [Carrot] [Celery] [Cherry] [Crab] [Hazelnut] [Lobster: *Homarus americanus*, *Panulirus stimpsoni*] [Milk] [Mussel] [Mustard] [Peanut] [Pear] [Rapeseed] [Salmon] [Scallop] [Shrimp: *Metapenaeus ensis*, *Penaeus aztecus*] [Soybean] [Walnut] [Wheat]

Allergen Nomenclature		References
Celery *(Apium graveolens)*		
Api g 1*	***Function*** pathogenesis-related protein ***Molecular Mass*** 17 kDa, experimentally determined (1) ***Expressed in*** *E.coli*	(1) Breiteneder et al. 1995 (2) Jankiewicz et al. 1998 (3) Scheurer et al. 2000b (4) Scheurer et al. 1999 (5) Hoffmann-Sommergruber et al. 1999b (6) Scheurer et al. 1997 (7) Fötisch et al. 1999 (8) Hoffmann-Sommergruber et al. 1999a (9) Kazemi-Shirazi et al. 2000 (10) Lüttkopf et al. 2000a
Api g 1.0201	***Function*** pathogenesis-related protein ***Molecular Mass*** 17 kDa, calculated ***Expressed in*** *E.coli*	Hoffmann-Sommergruber et al. 2000
Api g 2	***Function*** pathogenesis-related protein ***Molecular Mass*** 17 kDa, calculated ***Expressed in*** *E.coli*	Scheiner et al. 1997
Api g 4*	***Function*** profilin ***Molecular Mass*** 14.3 kDa, calculated ***Expressed in*** *E.coli*	Scheurer et al. 2000a Scheurer et al. 2000b Lüttkopf et al. 2000a
Peanut *(Arachis hypogaea)*		
Ara h 1*	***Function*** vicilin-like seed storage protein ***Molecular Mass*** 60.3 kDa, calculated (2) 68 kDa as fusion protein, experimentally determined in (1) 47.8 kDa, experimentally determined in (3) 65 kDa, experimentally determined in (4) 62 kDa, experimentally determined in (4) ***Expressed in*** *E.coli* (1, 2, 3) yeast: *Pichia pastoris* (4)	(1) Burks et al. 1995b (2) Kleber-Janke et al. 1999 (3) Burks et al. 1995a (4) Maid 1998

Ara h 2*	***Function*** conglutin seed storage protein ***Molecular Mass*** 17 kDa, calculated (3) ***Expressed in*** *E.coli*	(1) Burks et al. 1999 (2) King et al. 1999 (3) Kleber-Janke et al. 1999
Ara h 3*	***Function*** glycinin ***Molecular Mass*** 57 kDa, experimentally determined ***Expressed in*** *E.coli*	Rabjohn et al. 1999
Ara h 4*	***Function*** glycinin ***Molecular Mass*** 36 kDa, calculated ***Expressed in*** *E.coli*	Kleber-Janke et al. 1999
Ara h 5*	***Function*** profilin ***Molecular Mass*** 14 kDa, calculated ***Expressed in*** *E.coli*	Kleber-Janke et al. 1999
Ara h 6*	***Function*** conglutin-like seed storage protein ***Molecular Mass*** 14.5 kDa, calculated ***Expressed in*** *E.coli*	Kleber-Janke et al. 1999
Ara h 7*	***Function*** conglutin-like seed storage protein ***Molecular Mass*** 15.8 kDa, calculated ***Expressed in*** *E.coli*	Kleber-Janke et al. 1999
Milk *(Bos domesticus)*		
Bos d 5* beta-lactoglobulin	***Function*** retinol-binding protein ***Molecular Mass*** 18-20 kDa, experimentally determined (1) ***Expressed in*** *E.coli*	(1) Chatel et al. 1996 (2) Batt et al. 1990
Rapeseed *(Brassica napus)*		
Bra n ? note: in reference denominated BnI	***Function*** 2S seed storage protein ***Expressed in*** yeast: *Pichia pastoris*	Villalba et al. 2000

Crab *(Charybdis feriatus)*		
Cha f 1	***Function*** tropomyosin ***Molecular Mass*** 60 kDa as fusion protein with glutathione S transferase, experimentally determined; without fusion part 34 kDa, estimated ***Expressed in*** *E.coli*	Leung et al. 1998a
Scallop *(Chamys nobilis)*		
Cham n ?	***Function*** tropomyosin ***Expressed in*** *E.coli*	Leung et al. 2000
Hazelnut *(Corylus avellana)*		
Cor a 1.0401*	***Function*** pathogenesis-related protein ***Molecular Mass*** 17.45 kDa, calculated ***Expressed in*** *E.coli*	Lüttkopf et al. 1999 Lüttkopf et al. 2000b Vieths et al. 2000
Carp *(Cyprinus carpio)*		
Parvalbumin	***Function*** parvalbumin ***Expressed in*** *E.coli*	Bugajska-Schretter et al. 1998 Bugajska-Schretter et al. 1999
Carrot *(Daucis carota)*		
Dau c 1	***Function*** pathogenesis-related protein ***Molecular Mass*** 16 kDa, experimentally determined ***Expressed in*** *E.coli*	Hoffmann-Sommergruber et al. 1999b Kazemi-Shirazi et al. 2000
Dau c LTP	***Function*** lipid transfer protein (LTP) ***Expressed in*** yeast: *Pichia pastoris*	Asero et al. 2000b
Soybean *(Glycine maxima)*		
Gly m 1*	***Function*** HPS (hydrophobic protein of soybean) ***Expressed in*** *E.coli*	Beardslee et al. 1999
Gly m 3*	***Function*** profilin ***Molecular Mass*** 14.1 kDa, experimentally determined ***Expressed in*** *E.coli*	Rihs et al. 1999
Gly m ? 2 glycinin chains	***Function*** glycinin ***Expressed in*** *E.coli*	Beardslee et al. 1999

Abalone *(Haliotis diversicolor)*		
Hal d ?	***Function*** tropomyosin ***Expressed in*** *E.coli*	Leung et al. 2000
Lobster *(Homarus americanus)*		
Hom a 1	***Function*** tropomyosin ***Molecular Mass*** 60 kDa as fusion protein with glutathione S transferase, experimentally determined; without fusion part 34 kDa, estimated (2) ***Expressed in*** *E.coli*	(1) Leung et al. 1998a (2) Leung et al. 1998b
Walnut *(Juglans regia)*		
Jug r 1*	***Function*** 2S albumin seed storage protein ***Molecular Mass*** 42-43 kDa as fusion protein with glutathione S transferase, experimentally determined; without fusion part 15-16 kDa, estimated ***Expressed in*** *E.coli*	Teuber et al. 1998
Jug r 2*	***Function*** vicilin-like seed storage precursor protein ***Molecular Mass*** 92 kDa as fusion protein experimentally determined, without fusion part 66 kDa, estimated ***Expressed in*** *E.coli*	Teuber et al. 1999
Jug r ?	***Function*** 2S albumin seed storage protein ***Molecular Mass*** 19 kDa including fusion peptide ***Expressed in*** *E.coli*	Teuber et al. 1996
Apple *(Malus domesticus)*		
Mal d 1*	***Function*** pathogenesis-related protein ***Molecular Mass*** 17.7 kDa, calculated (1) 17 kDa, experimentally determined in (2) ***Expressed in*** *E.coli*	(1) Vanek-Krebitz et al. 1995 (2) Son et al. 1999 (3) Scheurer et al. 1999 (4) Fritsch et al. 1998 (5) Kazemi-Shirazi et al. 2000 (6) Neudecker et al. 2000a
Shrimp *(Metapenaeus ensis)*		
Met e 1*	***Function*** tropomyosin ***Molecular Mass*** 60 kDa as fusion protein with glutathione S transferase, experimentally determined; without fusion part 34 kDa, estimated ***Expressed in*** *E.coli*	Leung et al. 1994 Leung et al. 1996 Leung et al. 1998a Leung et al. 1998b

Lobster *(Panulirus stimpsoni)*		
Pan s 1	***Function*** tropomyosin ***Molecular Mass*** 60 kDa as fusion protein with glutathione S transferase, experimentally determined; without fusion part 34 kDa, estimated (1) ***Expressed in*** *E.coli*	(1) Leung et al. 1998b (2) Leung et al. 1998a
Shrimp *(Penaeus aztecus)*		
Pen a 1*	***Function*** tropomyosin ***Molecular Mass*** > 36 kDa, experimentally determined ***Expressed in*** *E.coli*	Reese et al. 1997
Mussel *(Perna viridis)*		
Pern v ?	***Function*** tropomyosin ***Expressed in*** *E.coli*	Leung et al. 2000
Avocado *(Persea americana)*		
Pers a 1* note: in reference denominated Prs a 1	***Function*** endochitinase ***Molecular Mass*** 32 kDa, experimentally determined ***Expressed in*** yeast: *Pichia pastoris*	Sowka et al. 1998
Cherry *(Prunus avium)*		
Pru av 1* note: in references denominated Pru a 1	***Function*** pathogenesis-related protein ***Molecular Mass*** 17.7 kDa, calculated ***Expressed in*** *E.coli*	Scheurer et al. 1997 Scheurer et al. 1999 Fötisch et al. 1999 Schweimer et al. 1900 Neudecker et al. 2000a Neudecker et al. 2000b
Pru av 4* note: in reference denominated Pru a 4	***Function*** profilin ***Molecular Mass*** 14 kDa, experimentally determined ***Expressed in*** *E.coli*	Scheurer et al. 2000a
Pear *(Pyrus communis)*		
Pyr c 1*	***Function*** pathogenesis-related protein ***Molecular Mass*** 17.4 kDa, calculated ***Expressed in*** *E.coli*	Karamloo 1999a Karamloo et al. 1999b Scheurer et al. 1999 Karamloo et al. 2000 Vieths et al. 2000 Karamloo et al. 2001
Pyr c 4*	***Function*** profilin ***Molecular Mass*** 13.9 kDa, calculated ***Expressed in*** *E.coli*	Karamloo 1999a Karamloo et al. 2000 Scheurer et al. 2000a Vieths et al. 2000

Pyr c 5* note: in reference (1) denominated Pyr c 2	***Function*** isoflavone reductase-like protein (IRL) ***Molecular Mass*** 33.7 kDa, calculated ***Expressed in*** *E.coli*	(1) Karamloo et al. 1999b (2) Vieths et al. 2000 (3) Karamloo 1999a (4) Karamloo et al. 2000
Atlantic Salmon *(Salmo salar)*		
Sal s 1*	***Function*** parvalbumin ***Molecular Mass*** 14 kDa, experimentally determined ***Expressed in*** *E.coli*	van Do et al. 1999
Mustard *(Sinapis alba)*		
Sin a 1*	***Function*** 2S albumin seed storage protein ***Molecular Mass*** 20 kDa, experimentally determined ***Expressed in*** *E.coli*	González De La Peña et al. 1993 González De La Peña et al. 1996
Wheat *(Triticum aestivum)*		
Wheat glutenins	***Function*** glutenins ***Molecular Mass*** 33 / 80 / 94 kDa, experimentally determined ***Expressed in*** *E.coli*	Maruyama et al. 1998
Wheat gliadins	***Function*** gliadins ***Molecular Mass*** 30 / 43 kDa, experimentally determined ***Expressed in*** *E.coli*	Maruyama et al. 1998
Wheat LTP	***Function*** lipid transfer protein (LTP) ***Molecular Mass*** 9 kDa, expected ***Expressed in*** yeast: *Pichia pastoris*	Klein et al. 1998 Asero et al. 2000b

Table 2: IgE-Binding Activity of Recombinant Food Allergens

This table shows the spectrum of IgE-binding assays with which the respective recombinant food allergens have been tested and the number and quality of patients' sera used in the assays. When the recombinant food allergen has been used in the IgE-binding test as a fusion protein, the fusion peptide is noted. Asterisks * indicates food allergens which are enlisted in the official list of allergens of the WHO / IUIS allergen nomenclature subcommittee (ftp://biobase.dk/pub/who-iuis/allergen.list). The allergens are enlisted in alphabetical order of the latin names.

Common names: [Abalone] [Apple] [Avocado] [Carp] [Carrot] [Celery] [Cherry] [Crab] [Hazelnut] [Lobster: *Homarus americanus*, *Panulirus stimpsoni*] [Milk] [Mussel] [Mustard] [Peanut] [Pear] [Rapeseed] [Salmon] [Scallop] [Shrimp: *Metapenaeus ensis*, *Penaeus aztecus*] [Soybean] [Walnut] [Wheat]

Recombinant Allergen	Tested by	References
Celery *(Apium graveolens)*		
Api g 1* ***Expressed in*** *E.coli* ***Fusion peptide*** --	immunoblot: ❐ sera of 10 celery-allergic patients with positive SPT and symptoms after ingestion of celery (1) ❐ sera of 24 celery-allergic patients of Central Europe with clinical history and positive SPT to extract (5) ❐ sera of 12 celery-allergic patients of Mediterranean area with positive case history and positive SPT to crude celery (5) ❐ sera of 22 celery-allergic patients proved by DBPCFC and SPT (6) immunoblot inhibition: ❐ serum of 1 patient with multiple inhalant and food allergies (4) ❐ pooled serum of 12 celery-allergic patients with positive SPT and symptoms after ingestion of celery (1) ❐ sera of 2 celery-allergic patients proved by DBPCFC and SPT (6) RAST: ❐ sera of 24 celery-allergic patients of Central Europe with clinical history and positive SPT to extract (5) ❐ sera of 12 celery-allergic patients of Mediterranean area with positive case history and positive SPT to crude celery (5) EAST: ❐ sera of 30 patients with adverse reactions after ingestion of celery tuber (2) ❐ sera of 9 patients with celery-related symptoms after ingestion of celery and IgE against Api g 1 and / or CCD and serum of 1 patient with multiple inhalant and food allergies (4) ❐ sera of 22 celery-allergic patients proved by DBPCFC and SPT (6) EAST inhibition: ❐ serum of 1 celery-allergic patient with positive DBPCFC (3) ❐ sera of 9 patients with celery-related symptoms after ingestion of celery and IgE against Api g 1 and / or CCD and serum of 1 patient with multiple inhalant and food allergies (4) ❐ sera of 3 patients with celery-related symptoms after ingestion of celery and IgE against Api g 1 and / or CCD (4) ❐ sera of 3 celery-allergic patients proved with DBPCFC and SPT (6) mediator release: ❐ serum of 1 patient with celery-related symptoms after ingestion of celery and IgE against celery tuber (4)	(1) Breiteneder et al. 1995 (2) Jankiewicz et al. 1998 (3) Scheurer et al. 2000b (4) Fötisch et al. 1999 (5) Hoffmann-Sommergruber et al. 1999a (6) Lüttkopf et al. 2000a

	SPT: ❒ sera of 24 celery-allergic patients of Central Europe with clinical history and positive SPT to extract (5) ❒ sera of 12 celery-allergic patients of Mediterranean area with positive case history and positive SPT to crude celery but not to extract (5)	
Api g 1.0201 ***Expressed in*** *E.coli* ***Fusion peptide*** his6-tag	immunoblot: ❒ sera of 10 celery-allergic patients immunoblot inhibition: ❒ pooled serum of 4 celery-allergic patients	Hoffmann-Sommergruber et al. 2000
Api g 2 ***Expressed in*** *E.coli* ***Fusion peptide*** --	❒ (reported, data not shown) test with sera from celery-allergic and birch pollen-allergic patients; test and number of sera not mentioned	Scheiner et al. 1997
Api g 4* ***Expressed in*** *E.coli* ***Fusion peptide*** --	immunoblot: ❒ sera of 17 patients: 13/17 sera of patients with positive DBPCFC to celery, 4/17 sera of patients with clinical symptoms to celery and positive allergosorbent test to birch profilin (1) ❒ sera of 22 celery-allergic patients proved by DBPCFC and SPT (3) immunoblot inhibition: ❒ sera of 9 patients: 3/9 sera of patients with positive DBPCFC to celery, 6/9 sera of patients with clinical symptoms to celery and positive allergosorbent test to birch profilin (1) ❒ sera of 2 celery-allergic patients with positive allergosorbent test to birch pollen profilin (1) ❒ pooled serum of 9 celery-allergic patients (1) ❒ sera of 2 celery-allergic patients proved by DBPCFC and SPT (3) EAST: ❒ sera of 22 celery-allergic patients proved by DBPCFC and SPT (3) ❒ (reported, data not shown) sera of 10 patients with a history to celery (2) ❒ (reported, data not shown) sera of 49 allergic subjects with positive EAST to recombinant birch profilin (2) EAST inhibition: ❒ 1 individual serum and pooled serum of 2 celery-allergic patients with IgE against birch profilin (1) ❒ sera of 22 celery-allergic patients proved by ❒ DBPCFC and SPT (3) mediator release: ❒ serum of 1 celery-allergic patient with positive allergosorbent test to birch pollen profilin (1) ❒ (reported, data not shown) (2)	(1) Scheurer et al. 2000b (2) Scheurer et al. 2000a (3) Lüttkopf et al. 2000a

Peanut *(Arachis hypogaea)*		
Ara h 1* ***Expressed in*** *E.coli* (1, 2, 3) yeast: *Pichia pastoris* (4) ***Fusion peptide*** 37 amino acids of beta-galactosidase (1); his6-tag (3); -- (4)	immunoblot: ❐ sera of 18 patients with peanut hypersensitivity confirmed by positive SPT to peanut and positive DBPCFC or clinical history of peanut anaphylaxis (1) ❐ pooled serum of patients with peanut hypersensitivity confirmed by positive SPT to peanut and positive DBPCFC or clinical history of peanut anaphylaxis (1) ❐ sera of 11 and of 3 peanut-hypersensitive patients (2) ❐ sera of 40 patients with clinical symptoms after peanut ingestion, positive SPT, RAST class to peanut > 3 (3) ❐ sera of 5 peanut-allergic patients (4)	(1) Burks et al. 1995b (2) Burks et al. 1995a (3) Kleber-Janke et al. 1999 (4) Maid 1998
Ara h 2* ***Expressed in*** *E.coli* ***Fusion peptide*** his6-tag (1, 2, 3)	immunoblot: ❐ sera of 40 patients with clinical symptoms after peanut ingestion, positive SPT, RAST class to peanut > 3 (1) ❐ (reported, data not shown) 16 peanut-sensitive patients (2, 3)	(1) Kleber-Janke et al. 1999 (2) King et al. 1999 (3) Burks et al. 1999
Ara h 3* ***Expressed in*** *E.coli* ***Fusion peptide*** his6-tag	immunoblot: ❐ sera of 18 peanut-allergic patients with positive SPT and either positive DBPCFC or history of anaphylaxis ❐ pooled serum of peanut-allergic patients with positive SPT and either positive DBPCFC or history of anaphylaxis	Rabjohn et al. 1999
Ara h 4* ***Expressed in*** *E.coli* ***Fusion peptide*** his6-tag	immunoblot: ❐ sera of 40 patients with clinical symptoms after peanut ingestion, positive SPT, RAST class to peanut > 3 ❐ pooled serum of 34 peanut-sensitised patients	Kleber-Janke et al. 1999
Ara h 5* ***Expressed in*** *E.coli* ***Fusion peptide*** his6-tag	immunoblot: ❐ sera of 40 patients with clinical symptoms after peanut ingestion, positive SPT, RAST class to peanut > 3 ❐ pooled serum of 34 peanut-sensitised patients	Kleber-Janke et al. 1999
Ara h 6* ***Expressed in*** *E.coli* ***Fusion peptide*** his6-tag	immunoblot: ❐ sera of 40 patients with clinical symptoms after peanut ingestion, positive SPT, RAST class to peanut > 3 ❐ pooled serum of 34 peanut-sensitised patients	Kleber-Janke et al. 1999
Ara h 7* ***Expressed in*** *E.coli* ***Fusion peptide*** his6-tag	immunoblot: ❐ sera of 40 patients with clinical symptoms after peanut ingestion, positive SPT, RAST class to peanut > 3 ❐ pooled serum of 34 peanut-sensitised patients	Kleber-Janke et al. 1999

Milk *(Bos domesticus)*		
Bos d 5* allergen here: met-beta-lactoglobulin (lacking the first ten amino acids of the mature protein) ***Expressed in*** *E.coli* ***Fusion peptide*** --	ELISA: ❒ sera of 5 patients allergic to beta-lactoglobulin	Chatel et al. 1996
Rapeseed *(Brassica napus)*		
Bra n ? note: in reference denominated BnI ***Expressed in*** yeast: *Pichia pastoris*	immunoblot: ❒ (reported, not shown) sera of mustard-allergic patients ELISA: ❒ (reported, data not shown) sera of mustard-allergic patients	Villalba et al. 2000
Crab *(Charybdis feriatus)*		
Cha f 1 ***Expressed in*** *E.coli* ***Fusion peptide*** glutathione S transferase	immunoblot: ❒ 10 individual sera and pooled serum of patients with type I hypersensitivity upon ingestion of crab and IgE to shrimp, lobster and crab immunoblot inhibition: ❒ pooled serum of patients with type I hypersensitivity upon ingestion of crab and IgE to shrimp, lobster and crab	Leung et al. 1998a
Scallop *(Chamys nobilis)*		
Cham n ? ***Expressed in*** *E.coli*	immunoblot and inhibition studies: ❒ (reported, data not shown) sera of shellfish-allergic subjects	Leung et al. 2000
Hazelnut *(Corylus avellana)*		
Cor a 1.0401* ***Expressed in*** *E.coli* ***Fusion peptide*** --	immunoblot: ❒ (reported, data not shown) sera of 34 hazelnut-allergic patients with positive DBPCFC (2) EAST: ❒ sera of 13 food-allergic patients with positive DBPCFC to hazelnut (1) ❒ (data reported, not shown) sera of 43 food-allergic patients with positive DBPCFC to hazelnut (3) inhibition assay: ❒ (reported, data not shown) (1) mediator release: ❒ (reported, data not shown) sera of 6 patients with positive DBPCFC to hazelnut (1)	(1) Vieths et al. 2000 (2) Lüttkopf et al. 1999 (3) Lüttkopf et al. 2000b

Carp *(Cyprinus carpio)*		
Parvalbumin ***Expressed in*** *E.coli* ***Fusion peptide*** beta-galactosidase	immunoblot: ❒ sera of 10 fish-allergic patients (1) IgE-binding assay: ❒ (reported, data not shown) sera of fish-allergic patients; test and number of sera not mentioned (2)	(1) Bugajska-Schretter et al. 1999 (2) Bugajska-Schretter et al. 1998
Carrot *(Daucis carota)*		
Dau c 1 ***Expressed in*** *E.coli* ***Fusion peptide*** his6-tag	immunoblot: ❒ sera of 6 carrot-allergic patients with positive SPT to fresh carrot and positive case history immunoblot inhibition: ❒ pooled serum of 6 carrot-allergic patients with positive SPT to fresh carrot and positive case history	Hoffmann-Sommergruber et al. 1999b
Dau c LTP ***Expressed in*** yeast: *Pichia pastoris* ***Fusion peptide*** --	RAST inhibition: ❒ serum of 1 pollen- and apple-allergic patient with sensitisation to LTP	Asero et al. 2000b
Soybean *(Glycine maxima)*		
Gly m 1* ***Expressed in*** *E.coli* ***Fusion peptide*** his6-tag	IgE-binding test: ❒ (reported, data not shown) test and sera not mentioned	Beardslee et al. 1999
Gly m 3* ***Expressed in*** *E.coli* ***Fusion peptide*** --	immunoblot: ❒ 8 individual sera and a pooled serum of 7 subjects with adverse reactions to food and IgE against soybean proteins immunoblot inhibition: ❒ 3 individual sera and pooled serum of patients with adverse reactions to food and IgE against soybean proteins EAST inhibition: ❒ pooled serum of subjects with adverse reactions to food and IgE against soybean proteins	Rihs et al. 1999
Gly m 3* ***Expressed in*** *E.coli* ***Fusion peptide*** maltose-binding protein	immunoblot: ❒ 8 individual sera and a pooled serum of 7 subjects with adverse reactions to food and IgE against soybean proteins EAST: ❒ sera of 13 subjects with adverse reactions to food and IgE against soybean proteins	Rihs et al. 1999
Gly m ? 2 glycinin chains ***Expressed in*** *E.coli* ***Fusion peptide*** his6-tag	IgE-binding test: ❒ (reported, data not shown) test and sera not mentioned	Beardslee et al. 1999

Abalone *(Haliotis diversicolor)*		
Hal d ? ***Expressed in*** *E.coli*	immunoblot and inhibition studies: ❐ (reported, data not shown) sera of shellfish-allergic subjects	Leung et al. 2000
Lobster *(Homarus americanus)*		
Hom a 1 ***Expressed in*** *E.coli* ***Fusion peptide*** glutathione S transferase	immunoblot: ❐ sera of 10 patients with type I hypersensitivity upon ingestion of crab and IgE against lobster, shrimp and crab (1) ❐ pooled serum of 10 patients with crustacean allergy (2) immunoblot inhibition: ❐ pooled serum of 10 patients with crustacean allergy (2)	(1) Leung et al. 1998a (2) Leung et al. 1998b
Walnut *(Juglans regia)*		
Jug r 1* ***Expressed in*** *E.coli* ***Fusion peptide*** glutathione S transferase	immunoblot: ❐ sera of 16 patients with allergic reactions to walnuts and FEIA class 2 and higher to walnut extract FEIA inhibition: ❐ sera of 16 patients with allergic reactions to walnuts and FEIA class 2 and higher to walnut extract	Teuber et al. 1998
Jug r 2* ***Expressed in*** *E.coli* ***Fusion peptide*** glutathione S transferase	immunoblot: ❐ sera of 15 patients with systemic reactions to walnuts immunoblot inhibition: ❐ sera of 4 patients with systemic reactions to walnuts and positive IgE immunoblot to rJug r 2	Teuber et al. 1999
Jug r ? ***Expressed in*** *E.coli* ***Fusion peptide*** not specified	IgE-binding assay: ❐ (reported, data not shown) sera of 12 walnut-allergic patients, test not mentioned	Teuber et al. 1996
Apple *(Malus domesticus)*		
Mal d 1* ***Expressed in*** *E.coli* ***Fusion peptide*** --	immunoblot: ❐ sera of 9 patients with allergy to apple and birch pollen according to positive case history and RAST class 2.5 and higher (1) ❐ sera of 8 birch pollen-allergic patients with symptoms after ingestion of fresh apples, positive SPT to apple extract and positive EAST to natural Mal d 1 class 2 and higher (2) immunoblot inhibition: ❐ pooled serum of 5 patients with allergy to apple and birch pollen according to positive case history and RAST class 2.5 and higher (1) EAST: ❐ sera of 13 birch pollen-allergic patients with symptoms after ingestion of fresh apples, positive SPT to apple extract and positive EAST to natural Mal d 1 class 2 and higher (2) mediator release: (2, 3)	(1) Vanek-Krebitz et al. 1995 (2) Son et al. 1999 (3) Scheurer et al. 1999

Shrimp *(Metapenaeus ensis)*		
Met e 1* ***Expressed in*** *E.coli* ***Fusion peptide*** glutathione S transferase	immunoblot: ❐ pooled serum of shrimp-allergic patients (1) ❐ sera of 10 patients with type I hypersensitivity upon ingestion of crab and IgE to shrimp, lobster and crab (2) immunoblot inhibition: ❐ (reported, data not shown) pooled serum of 9 patients with anaphylactic reactions after ingestion of shrimp (3)	(1) Leung et al. 1994 (2) Leung et al. 1998a (3) Leung et al. 1996
Lobster *(Panulirus stimpsoni)*		
Pan s 1 ***Expressed in*** *E.coli* ***Fusion peptide*** glutathione S transferase	immunoblot: ❐ pooled serum of 10 patients with crustacean allergy (1) ❐ sera of 10 patients with type I hypersensitivity upon ingestion of crab and IgE to shrimp, lobster and crab (2) immunoblot inhibition: ❐ pooled serum of 10 patients with crustacean allergy (1)	(1) Leung et al. 1998b (2) Leung et al. 1998a
Shrimp *(Penaeus aztecus)*		
Pen a 1* ***Expressed in*** *E.coli* ***Fusion peptide*** --	immunoblot: ❐ pooled serum of shrimp-allergic patients	Reese et al. 1997
Mussel *(Perna viridis)*		
Pern v ? ***Expressed in*** *E.coli*	immunoblot and inhibition studies: ❐ (reported, data not shown) sera of shellfish-allergic subjects	Leung et al. 2000
Avocado *(Persea americana)*		
Pers a 1* note: in reference denominated Prs a 1 ***Expressed in*** yeast: *Pichia pastoris* ***Fusion peptide*** --	immunoblot: ❐ pooled serum of patients with positive case history, positive SPT, RAST class 4 and higher and type I allergy to latex and some patients with additional symptoms after ingestion of avocado immunoblot inhibition: ❐ pooled serum of patients with positive case history, positive SPT, RAST class 4 and higher and type I allergy to latex and some patients with additional symptoms after ingestion of avocado	Sowka et al. 1998

Cherry *(Prunus avium)*		
Pru av 1* note: in references denominated Pru a 1 *Expressed in* *E.coli* *Fusion peptide* --	immunoblot: ❐ sera of 7 birch pollen-allergic patients with symptoms after ingestion of fresh cherries, positive SPT to cherry extract and birch pollen extract and EAST class 2 and higher (2) immunoblot inhibition: ❐ pooled serum of 4 birch pollen-allergic patients with symptoms after ingestion of fresh cherries and positive SPT to cherry extract (1) ❐ pooled serum of 4 birch pollen-allergic patients with symptoms after ingestion of fresh cherries, positive SPT to cherry extract and birch pollen extract and EAST class 2 and higher (2) EAST: ❐ sera of 19 birch pollen-allergic patients with clinical symptoms after ingestion of cherry and positive SPT to cherry extract (1) ❐ sera of 7 birch pollen-allergic patients with symptoms after ingestion of fresh cherries, positive SPT to cherry extract and birch pollen extract and EAST class 2 and higher (2) mediator release: ❐ sera of 3 birch pollen-allergic patients with clinical symptoms after ingestion of fresh cherries (1) ❐ mediator release (2)	(1) Scheurer et al. 1997 (2) Scheurer et al. 1999
Pru av 1* note: in references denominated Pru a 1 *Expressed in* *E.coli* *Fusion peptide* his 10-tag	immunoblot: ❐ sera of 9 birch pollen-allergic patients with clinical symptoms after ingestion of fresh cherries and positive SPT to cherry extract (1) ❐ pooled serum of 4 birch pollen-allergic patients with symptoms after ingestion of fresh cherries, positive SPT to cherry extract and birch pollen extract and EAST class 2 and higher (2) immunoblot inhibition: ❐ pooled serum of 4 birch pollen-allergic patients with symptoms after ingestion of fresh cherries, positive SPT to cherry extract and birch pollen extract and EAST class 2 and higher (1, 2)	(1) Scheurer et al. 1997 (2) Scheurer et al. 1999
Pru av 4* note: in reference denominated Pru a 4 *Expressed in* *E.coli* *Fusion peptide* --	immunoblot: ❐ (reported, data not shown) number and quality of sera not mentioned EAST: ❐ (reported, data not shown) sera of 13 patients with symptoms after ingestion of cherry ❐ (reported, data not shown) sera of 49 subjects with positive EAST to recombinant birch profilin	Scheurer et al. 2000a

Pear *(Pyrus communis)*		
Pyr c 1* ***Expressed in*** *E.coli* ***Fusion peptide*** --	immunoblot: ❒ sera of 23 patients with pollen-related allergy to pear and EAST to pear > 0.7 U/ml (1, 2) immunoblot inhibition: ❒ serum of 1 patient with pollen-related allergy to pear (1) ❒ sera of 2 subjects with EAST to pear class 2 or 3, respectively (2) EAST: ❒ sera of 36 or 61 patients with pollen-related allergy to pear (1, 2, 4) EAST inhibition: ❒ sera of 8 subjects with or without symptoms after ingestion of pear but positive EAST to pear class 2 and higher (2) mediator release: ❒ sera of 5 patients with oral allergy syndrome after ingestion of pear and EAST to pear class 1 and higher (2) ❒ mediator release (3, 5)	(1) Vieths et al. 2000 (2) Karamloo 1999a (3) Scheurer et al. 1999 (4) Karamloo et al. 1999b (5) Karamloo et al. 2000
Pyr c 4* ***Expressed in*** *E.coli* ***Fusion peptide*** --	immunoblot: ❒ sera of 23 patients with pollen-related allergy to pear and EAST to pear > 0.7 U/ml (1, 2) immunoblot inhibition: ❒ sera of 1 subject with oral allergy syndrome after ingestion of pear and EAST to pear class 2 (2) EAST: ❒ sera of 61 patients with pollen-related allergy to pear (1, 2) ❒ (reported, data not shown) sera of 16 patients with oral allergy to pear (3) ❒ (reported, data not shown) sera of 49 allergic subjects with positive EAST to recombinant birch profilin (3) EAST inhibition: ❒ sera of 6 subjects with or without symptoms after ingestion of pear but positive EAST to pear class 2 and higher (2) mediator release: ❒ sera of 5 patients with oral allergy syndrome after ingestion of pear and EAST to pear class 1 and higher (2) ❒ (reported, data not shown) (3, 4)	(1) Vieths et al. 2000 (2) Karamloo 1999a (3) Scheurer et al. 2000a (4) Karamloo et al. 2000

Pyr c 5* note: in reference (1) denominated Pyr c 2 ***Expressed in*** *E.coli* ***Fusion peptide*** ---	immunoblot: ❐ sera of 23 patients with pollen-related allergy to pear and EAST to pear > 0.7 U/ml (1, 3) immunoblot inhibition: ❐ sera of 1 subject with EAST to pear class 2 (3) EAST: ❐ sera of 36 or 61 patients with pollen-related allergy to pear (1, 2, 3) EAST inhibition: ❐ sera of 8 subjects with or without symptoms after ingestion of pear but positive EAST to pear class 2 and higher (3) mediator release: ❐ sera of 5 patients with oral allergy syndrome after ingestion of pear and EAST to pear class 3 (3) ❐ (data reported, not shown) (4)	(1) Vieths et al. 2000 (2) Karamloo et al. 1999b (3) Karamloo 1999a (4) Karamloo et al. 2000
Atlantic Salmon *(Salmo salar)*		
Sal s 1* ***Expressed in*** *E.coli* ***Fusion peptide*** his10-tag	immunoblot: ❐ sera of 6 patients with clinical histories of allergy to fish and cod fish-specific IgE of RAST class 3 and higher ELISA: ❐ sera of 4 patients with clinical histories of allergy to fish and cod fish-specific IgE of RAST class 3 and higher	van Do et al. 1999
Mustard *(Sinapis alba)*		
Sin a 1* ***Expressed in*** *E.coli* ***Fusion peptide*** ---	immunoblot: ❐ pooled serum of 5 mustard-sensitive individuals ELISA inhibition: ❐ pooled serum of 5 mustard-sensitive individuals	González De La Peña et al. 1996
Sin a 1* ***Expressed in*** *E.coli* ***Fusion peptide*** glutathione S transferase	immunoblot: ❐ pooled serum of 5 mustard-sensitive individuals	González De La Peña et al. 1996
Wheat *(Triticum aestivum)*		
Wheat gliadins ***Expressed in*** *E.coli* ***Fusion peptide*** ---	immuno dotblot: ❐ sera of 10 patients with significant RAST against wheat	Maruyama et al. 1998
Wheat glutenins ***Expressed in*** *E.coli* ***Fusion peptide*** ---	immuno dotblot: ❐ sera of 10 patients with significant RAST against wheat	Maruyama et al. 1998

Wheat LTP ***Expressed in*** yeast: *Pichia pastoris* ***Fusion peptide*** --	RAST: ❐ (reported, data not shown) sera of 34 patients with food allergies, mainly against Rosaceae	Asero et al. 2000b

REFERENCES

- AAAAI (1997) **The use of standardized allergen extracts. American Academy of Allergy, Asthma and Immunology (AAAAI)** *J Allergy Clin Immunol 99:583-586*
- Aalberse RC (1998) **Clinical relevance of carbohydrate allergen epitopes** *Allergy 53:54-57*
- Aalberse RC, van Ree R (1997) **Crossreactive carbohydrate determinants** *Clin Rev Allergy Immunol 15:375-387*
- Adel-Patient K, Creminon C, Wal JM, Chatel JM (2000) **Prévention d'une réponse IgE spécifique de la beta-lactoglobuline bovine chez la souris par immunisation génique (Prevention of a beta-lactoglobulin specific IgE response in the mouse via gene immunisation)** *Rev Fr Allergol Immunol Clin 40:171-184 (article in French)*
- Alibhai M, Astwood J, Joyce E, Pershing J, Sampson H, Purcell J (2000) **Re-engineering patatin (Sol t 1) protein to eliminate IgE binding** *J Allergy Clinical Immunol 105:S79 (abstract)*
- Asero R (1998) **Effects of birch pollen-specific immunotherapy on apple allergy in birch pollen-hypersensitive patients** *Clin Exp Allergy 28:1368-1373*
- Asero R (2000a) **Fennel, cucumber, and melon allergy successfully treated with pollen-specific injection immunotherapy** *Ann Allergy Asthma Immunol 84:460-462*
- Asero R, Mistrello G, Roncarolo D, de Vries SC, Gautier MF, Ciurana CLF, Verbeek E, Mohammadi T, Knul-Brettlova V, Akkerdaas JH, Bulder I, Aalberse RC, van Ree R (2000b) **Lipid transfer protein: a pan-allergen in plant-derived foods that is highly resistant to pepsin digestion** *Int Arch Allergy Immunol 122:20-32*
- Batt CA, Rabson LD, Wong DW, Kinsella JE (1990) **Expression of recombinant bovine beta-lactoglobulin in *Escherichia coli*** *Agric Biol Chem 54:949-955*
- Bauer A, Ekanayake Mudiyanselage S, Wigger-Alberti W, Elsner P (1999) **Oral rush desensitization to milk** *Allergy 54:894-895*
- Beardslee T, Zeece M, Markwell J, Sarath G (1999) **The use of recombinant soy proteins in detecting IgE-binding from soy sensitive sera** *J Allergy Clin Immunol 103:S104 (abstract)*
- Bernhisel-Broadbent J, Taylor S, Sampson HA (1989) **Cross-allergenicity in the legume botanical family in children with food hypersensitivity. II. Laboratory correlates** *J Allergy Clin Immunol 84:701-109*
- Bernhisel-Broadbent J, Scanlon SM, Sampson HA (1992a) **Fish hypersensitivity. I. *In vitro* and oral challenge results in fish-allergic patients** *J Allergy Clin Immunol 89:730-737*
- Bernhisel-Broadbent J, Strause D, Sampson HA (1992b) **Fish hypersensitivity. II: Clinical relevance of altered fish allergenicity caused by various preparation methods** *J Allergy Clin Immunol 90:622-629*
- Bhatia PK, Mukhopadhyay A (1998) **Protein glycosylation: Implications for *in vivo* functions and therapeutic applications** *Adv Biochem Eng Biotechnol 64:155-201*
- Björkstén B, Halmepuro L, Hannuksela M, Lahti A (1980) **Extraction and properties of apple allergens** *Allergy 35:671-677*
- Bock SA, Sampson HA, Atkins FM, Zeiger RS, Lehrer S, Sachs M, Bush RK & Metcalfe DD (1988) **Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure: a manual** *J Allergy Clin Immunol 82:986-997*
- Boehm M, Rösch P (1997) **Expression in *Escherichia coli*, purification, and spectroscopic characterization of two mutant Bet v 1 proteins** *Biol Chem 378:687-695*
- Borelli S, Anliker MD, Wüthrich B (1999) **Anaphylaxie auf Erdnuss: das Problem versteckter Allergene. (Peanut anaphylaxis: the problem of hidden allergens)** *Dtsch Med Wochenschr 124:1197-1200 (article in German)*
- Breiteneder H, Hoffmann-Sommergruber K, O'Riordain G, Susani M, Ahorn H, Ebner C, Kraft D, Scheiner O (1995) **Molecular characterization of Api g 1, the major allergen of celery *(Apium graveolens)*, and its immunological and structural relationships to a group of 17-kDa tree pollen allergens** *Eur J Biochem 233:484-489*
- Brenna O, Pompei C, Ortolani C, Pravettoni V, Farioli L, Pastorello EA (2000) **Technological processes to decrease the allergenicity of peach juice and nectar** *J Agric Food Chem 48:493-497*
- Bruijnzeel-Koomen CA, Ortolani C, Aas K, Bindslev-Jensen C, Björkstén B, Moneret-Vautrin D, Wüthrich B (1995) **Adverse reactions to food. European Academy of Allergology and Clinical Immunology Subcommittee** *Allergy*

50:623-635

- Bugajska-Schretter A, Rumpold H, Valenta R, Spitzauer S (1998) **Molecular characterization of carp parvalbumin, a major cross-reactive fish allergen** *Allergy 53:103-104 (abstract)*
- Bugajska-Schretter A, Pastore A, Vangelista L, Rumpold H, Valenta R, Spitzauer S (1999) **Molecular and immunological characterization of carp parvalbumin, a major fish allergen** *Int Arch Allergy Immunol 118:306-308*
- Burks AW, Cockrell G, Stanley JS, Helm RM, Bannon GA (1995a) **Isolation, identification, and characterization of clones encoding antigens responsible for peanut hypersensitivity** *Int Arch Allergy Immunol 107:248-250*
- Burks AW, Cockrell G, Stanley JS, Helm RM, Bannon GA (1995b) **Recombinant peanut allergen Ara h I expression and IgE binding in patients with peanut hypersensitivity** *J Clin Invest 96:1715-1721*
- Burks AW, King N, Bannon GA (1999) **Modification of a major peanut allergen leads to loss of IgE binding** *Int Arch Allergy Immunol 118:313-314 (abstract)*
- Burks AW, King N, Rabjohn P, Buzen F, West CM, Helm RM, Stanley JS, Sampson H (2000) **Development of hypoallergenic proteins for use in treating patients with peanut hypersensitivity** *J Allergy Clin Immunol 105:S310 (abstract)*
- Cereghino JL, Cregg JM (2000) **Heterologous protein expression in the methylotrophic yeast *Pichia pastoris*** *FEMS Microbiol Rev 24:45-66*
- Chapman MD, Heymann PW, Wilkins SR, Brown MJ, Platts-Mills TAE (1987) **Monoclonal immunoassays for major dust mite *(Dermatophagoides)* allergens, Der p I and Der f I, and quantitative analysis of the allergen content of mite and house dust extracts** *J Allergy Clin Immunol 80:184-194*
- Chatel JM, Bernard H, Clement G, Frobert Y, Batt CA, Gavalchin J, Peltres G, Wal JM (1996) **Expression, purification and immunochemical characterization of recombinant bovine beta-lactoglobulin, a major cow milk allergen** *Mol Immunol 33:1113-1118*
- Cho Y, Gu W, Watkins S, Lee SP, Kim TR, Brady JW, Batt CA (1994) **Thermostable variants of bovine beta-lactoglobulin** *Protein Eng 7:263-270*
- Deluze C, Dunoyer-Geindre S, Girard JP (1991) **Comparative analysis of lyophilized fresh fruits and vegetables with commercial extracts for skin testing of patients with food allergy** *J Invest Allergol Clin Immunol 1:53-57*
- Ebner C, Birkner T, Valenta R, Rumpold H, Breitenbach M, Scheiner O, Kraft D (1991) **Common epitopes of birch pollen and apples--studies by western and northern blot** *J Allergy Clin Immunol 88:588-594*
- Eigenmann PA, Sampson HA (1998) **Interpreting skin prick tests in the evaluation of food allergy in children** *Pediatr Allergy Immunol 9:186-191*
- Ewan PW (1996) **Clinical study of peanut and nut allergy in 62 consecutive patients: new features and association** *Brit Med J 312:1074-1078*
- Fernández-Rivas M, Cuevas M (1999) **Peels of *Rosaceae* fruits have a higher allergenicity than pulps** *Clin Exp Allergy 29:1239-1247*
- Ferreira F, Hirtenlehner K, Jilek A, Godnik-Cvar J, Breiteneder H, Grimm R, Hoffmann-Sommergruber K, Scheiner O, Kraft D, Breitenbach M, Rheinberger HJ, Ebner C (1996) **Dissection of immunoglobulin E and T lymphocyte reactivity of isoforms of the major birch pollen allergen Bet v 1: potential use of hypoallergenic isoforms for immunotherapy** *J Exp Med 183:599-609*
- Ferreira F, Hebenstreit D, Kramer B, Himly M, Breiteneder H, Scheiner O, Briza P, Ebner C & Breitenbach M (2000) **Amino acid positions involved in the formation of IgE-binding epitopes of Api g 1 and Mal d 1 allergens** *J Allergy Clin Immunol 105:S137 (abstract)*
- Fötisch K, Altmann F, Haustein D, Vieths S (1999) **Involvement of carbohydrate epitopes in the IgE response of celery-allergic patients** *Int Arch Allergy Immunol 120:30-42*
- Fritsch R, Bohle B, Vollmann U, Wiedermann U, Jahn-Schmid B, Krebitz M, Breiteneder H, Kraft D, Ebner C (1998) **Bet v 1, the major birch pollen allergen, and Mal d 1, the major apple allergen, cross-react at the level of allergen-specific T helper cells** *J Allergy Clin Immunol 102:679-686*
- González De La Peña MA, Villalba M, García-López JL, Rodríguez R (1993) **Cloning and expression of the major allergen from yellow mustard seeds, Sin a I** *Biochem Biophys Res Commun 190:648-653*
- González De La Peña MA, Monsalve RI, Batanero E, Villalba M, Rodríguez R (1996) **Expression in *Escherichia coli* of Sin a 1, the major allergen from mustard** *Eur J Biochem 237:827-832*
- Grinna LS, Tschopp JF (1989) **Size distribution and general structural features of N linked oligosaccharides from the methylotrophic yeast, *Pichia pastoris*** *Yeast 5:107-115*
- Henzgen M, Schlenvoigt G, Diener C, Jäger L (1991) **Nahrungsmittelallergie bei Frühblüherpollinosis und deren Beeinflussung mittels Hyposensibilisierung (Food hypersensitivity in patients with tree pollen allergy and the influence of hyposensitization)** *Allergologie 14:90-94 (article in German)*
- Henzgen M, Frank E, Herrmann D (1994) **Der Einfluss der Hyposensibilisierung bei Baumpollenallergie auf assoziierte Nahrungsmittelunverträglichkeiten - Teil 1 (Effect of tree pollen hyposensitization on associated food**

allergy) *Allergologie 17:50-54 (article in German)*
- Henzgen M, Rudeschko O, Schlenvoigt G, Herrmann D, Frank E (1999) **Immunparameter der Apfelallergie unter Hyposensibilisierung mit Birkenpollen (Immunological parameters of apple allergy during hyposensitisation with birch pollen)** *Allergologie 22:655-664 (article in German)*
- Herrmann D, Henzgen M, Frank E, Rudeschko O, Jäger L (1995) **Effect of hyposensitization for tree pollinosis on associated apple allergy** *J Invest Allergol Clin Immunol 5:259-267*
- Hoffmann-Sommergruber K, Demoly P, Crameri R, Breiteneder H, Ebner C, Laimer da Camara Machado M, Blaser K, Ismail C, Scheiner O, Bousquet J, Menz G (1999a) **IgE reactivity to Api g 1, a major celery allergen, in a Central European population is based on primary sensitization by Bet v 1** *J Allergy Clin Immunol 104:478-484*
- Hoffmann-Sommergruber K, O'Riordain G, Ahorn H, Ebner C, Laimer da Camara Machado M, Pühringer H, Scheiner O, Breiteneder H (1999b) **Molecular characterization of Dau c 1, the Bet v 1 homologous protein from carrot and its cross-reactivity with Bet v 1 and Api g 1** *Clin Exp Allergy 29:840-847*
- Hoffmann-Sommergruber K, Ferris R, Pec M, Radauer C, O'Riordain G, Laimer da Camara Machado M, Scheiner O, Breiteneder H (2000) **Characterization of Api g 1.0201, a new member of the Api g 1 family of celery allergens** *Int Arch Allergy Immunol 122:115-123*
- Hourihane JO'B, Kilburn SA, Dean P, Warner JO (1997a) **Clinical characteristics of peanut allergy** *Clin Exp Allergy 27:634-639*
- Hourihane JO'B, Kilburn SA, Nordlee JA, Hefle SL, Taylor SL, Warner JO (1997b) **An evaluation of the sensitivity of subjects with peanut allergy to very low doses of peanut protein: a randomized, double-blind, placebo-controlled food challenge study** *J Allergy Clin Immunol 100:596-600*
- Jankiewicz A, Aulepp H, Baltes W, Bögl KW, Dehne LI, Zuberbier T, Vieths S (1996) **Allergic sensitization to native and heated celery root in pollen-sensitive patients investigated by skin test and IgE binding** *Int Arch Allergy Immunol 111:268-278*
- Jankiewicz A, Aulepp H, Altmann F, Fötisch K, Vieths S (1998) **Serological investigation of 30 celery-allergic patients with particular consideration of the thermal stability of IgE-binding celery allergens** *Allergo J 7:87-95*
- Karamloo F (1999a) **Klonierung und Charakterisierung von Allergenen der Birne *(Pyrus communis)* und Untersuchung ihrer Kreuzreaktivität mit Allergenen aus Pollen der Birke (*Betula verrucosa)*** *Johann Wolfgang Goethe-Universität, Frankfurt am Main (dissertation, in German)*
- Karamloo F, Scheurer S, Franke S, Haustein D, Vieths S (1999b) **Klonierung, Expression und Charakterisierung von pollenassoziierten Allergenen der Birne *(Pyrus communis)*** *Allergo J 8:14 (abstract, in German)*
- Karamloo F, Wangorsch A, Scheurer S, May S, Fötisch K, Haustein D, Vieths S (2000) **Molecular cloning and characterisation of birch pollen-related allergens from pear (*Pyrus communis)*** *Allergy 55:221-222 (abstract)*
- Karamloo F, Scheurer S, Wangorsch A, May S, Haustein D, Vieths S (2001) **Pyr c 1, the major allergen from pear *(pyrus communis)*, is a new member of the Bet v 1 allergen family** *J Chromatogr B (in press)*
- Kauppinen J, Zeiler T, Rautiainen J, Rytkönen-Nissinen M, Taivainen A, Mäntyjärvi R, Virtanen T (1999) **Mutant derivatives of the main respiratory allergen of cow are less allergenic than the intact molecule** *Clin Exp Allergy 29:989-996*
- Kazemi-Shirazi L, Pauli G, Purohit A, Spitzauer S, Fröschl R, Hoffmann-Sommergruber K, Breiteneder H, Scheiner O, Kraft D, Valenta R (2000) **Quantitative IgE inhibition experiments with purified recombinant allergens indicate pollen-derived allergens as the sensitizing agents responsible for many forms of plant food allergy** *J Allergy Clin Immunol 105:116-125*
- Kelso JM, Jones RT, Tellez R, Yunginger JW (1995) **Oral allergy syndrome successfully treated with pollen immunotherapy** *Ann Allergy Asthma Immunol 74:391-396*
- King N, Maleki SJ, Sampson H, Burks AW, Bannon GA (1999) **Modulation of the allergenicity of a major peanut allergen, Ara h 2 by mutagenesis of its immunodominant IgE binding epitopes** *J Allergy Clin Immunol 103:S67 (abstract)*
- Kleber-Janke T, Crameri R, Appenzeller U, Schlaak M, Becker WM (1999) **Selective cloning of peanut allergens, including profilin and 2S albumins, by phage display technology** *Int Arch Allergy Immunol 119:265-274*
- Kleber-Janke T, Becker WM (2000) **Use of modified BL21(DE3) *Escherichia coli* cells for high-level expression of recombinant peanut allergens affected by poor codon usage** *Protein Expr Purif 19:419-424*
- Klein C, de Lamotte-Guery F, Gautier F, Moulin G, Boze H, Joudrier P, Gautier MF (1998) **High-level secretion of a wheat lipid transfer protein in *Pichia pastoris*** *Protein Expr Purif 13:73-82*
- Koppelman SJ, Bruijnzeel-Koomen CA, Hessing M, de Jongh HH (1999) **Heat-induced conformational changes of Ara h 1, a major peanut allergen, do not affect its allergenic properties** *J Biol Chem 274:4770-4777*
- Kuby J (1994) **Immunology** *WH Freeman and Company, New York*
- Leung PSC, Ka HC, Wing KC, Ansari A, Bandea CI, Hoi SK, Nagy SM, Gershwin ME (1994) **Cloning, expression, and primary structure of *Metapenaeus ensis* tropomyosin, the major heat-stable shrimp allergen** *J Allergy Clin Immunol 94:882-890*

- Leung PSC, Chow WK, Duffey S, Kwan HS, Gershwin ME, Chu KH (1996) **IgE reactivity against a cross-reactive allergen in crustacea and mollusca: evidence for tropomyosin as the common allergen** *J Allergy Clin Immunol 98:954-961*
- Leung PSC, Chen YC, Gershwin ME, Wong SH, Kwan HS, Chu KH (1998a) **Identification and molecular characterization of *Charybdis feriatus* tropomyosin, the major crab allergen** *J Allergy Clin Immunol 102:847-852*
- Leung PSC, Chen YC, Mykles DL, Chow WK, Li CP, Chu KH (1998b) **Molecular identification of the lobster muscle protein tropomyosin as a seafood allergen** *Mol Mar Biol Biotechnol 7:12-20*
- Leung PSC, Wong SH, Chu KH (2000) **Identification of tropomyosin as mollusk allergens in abalone, scallop and mussel** *J Allergy Clin Immunol 105:S140-S141 (abstract)*
- Lever LR (1996) **Peanut and nut allergy. Creams and ointments containing peanut oil may lead to sensitisation (letter)** *Brit Med J 313:299*
- Lüttkopf D, Scheurer S, Haustein D, Vieths S (1999) **Klonierung, Expression und Charakterisierung des Hauptallergens aus Haselnuss** *Allergo J 8:27 (abstract, in German)*
- Lüttkopf D, Ballmer-Weber BK, Wüthrich B, Vieths S (2000a) **Celery allergens in patients with positive double-blind placebo-controlled food challenge** *J Allergy Clin Immunol 106:390-399*
- Lüttkopf D, Scheurer S, Ballmer-Weber BK, Wüthrich B, Poulsen LK, Skamstrup Hansen K, Haustein D, Vieths S (2000b) **Cloning, expression and characterization of the major allergen from hazelnut Cor a 1.0401** *Allergy 55:66 (abstract)*
- Maid B (1998) **Ara h 1: Klonierung in *Pichia pastoris* und *in vitro* Untersuchungen unter gastrointestinalen Bedingungen** Philipps-Universität Marburg / Lahn, Germany *(dissertation, in German)*
- Majamaa H, Moisio P, Holm K, Kautiainen H, Turjanmaa K (1999) **Cow's milk allergy: diagnostic accuracy of skin prick and patch tests and specific IgE** *Allergy 54:346-351*
- Maruyama N, Ichise K, Katsube T, Kishimoto T, Kawase S, Matsumura Y, Takeuchi Y, Sawada T, Utsumi S (1998) **Identification of major wheat allergens by means of the *Escherichia coli* expression system** *Eur J Biochem 255:739-745*
- Moneret-Vautrin DA, Hatahet R, Kanny G (1994) **Risks of milk formulas containing peanut oil contaminated with peanut allergens in infants with atopic dermatitis** *Pediatr Allergy Immunol 5:184-188*
- Möller C (1989) **Effect of pollen immunotherapy on food hypersensitivity in children with birch pollinosis** *Ann Allergy 62:343-345*
- Müller U, Akdis CA, Fricker M, Akdis M, Blesken T, Bettens F, Blaser K (1998) **Successful immunotherapy with T-cell epitope peptides of bee venom phospholipase A2 induces specific T-cell anergy in patients allergic to bee venom** *J Allergy Clin Immunol 101:747-754*
- Nelson HS, Lahr J, Rule R, Bock A, Leung D (1997) **Treatment of anaphylactic sensitivity to peanuts by immunotherapy with injections of aqueous peanut extract** *J Allergy Clin Immunol 99:744-751*
- Neudecker P, Nerkamp J, Boehm M, Schweimer K, Sticht H, Rösch P (2000a) **Food hypersensitivity of many birch pollen allergic patients can be explained by highly similar molecular structures of the corresponding allergens** *Allergy 55:135 (abstract)*
- Neudecker P, Schweimer K, Nerkamp J, Boehm M, Scheurer S, Vieths S, Rösch P (2000b) **Sequence-specific ^{1}H, ^{13}C and ^{15}N resonance assignments of the major cherry allergen Pru a 1** *J Biomol NMR 18:71-72*
- Norgaard A, Skov PS, Bindslev-Jensen C (1992) **Egg and milk allergy in adults: comparison between fresh foods and commercial allergen extracts in skin prick test and histamine release from basophils** *Clin Exp Allergy 22:940-947*
- Norgaard A, Bindslev-Jensen C, Skov PS, Poulsen LK (1995) **Specific serum IgE in the diagnosis of egg and milk allergy in adults** *Allergy 50:636-647*
- Norman PS, Ohman Jr JL, Long AA, Creticos PS, Gefter MA, Shaked Z, Wood RA, Eggleston PA, Hafner KB, Rao P, Lichtenstein LM, Jones NH, Nicodemus CF (1996) **Treatment of cat allergy with T-cell reactive peptides** *Am J Respir Crit Care Med 154:1623-1628*
- Oppenheimer JJ, Nelson HS, Bock SA, Christensen F, Leung DY (1992) **Treatment of peanut allergy with rush immunotherapy** *J Allergy Clin Immunol 90:256-262*
- Ortolani C, Ispano M, Pastorello EA, Ansaloni R, Magri GC (1989) **Comparison of results of skin prick tests (with fresh foods and commercial food extracts) and RAST in 100 patients with oral allergy syndrome** *J Allergy Clin Immunol 83:683-690*
- Patriarca C, Romano A, Venuti A, Schiavino D, Di Rienzo V, Nucera E, Pellegrino S (1984) **Oral specific hyposensitization in the management of patients allergic to food** *Allergol Immunopathol 12:275-281*
- Patriarca G, Schiavino D, Nucera E, Schinco G, Milani A, Gasbarrini GB (1998) **Food allergy in children: results of a standardized protocol for oral desensitization** *Hepatogastroenterology 45:52-58*
- Petersen A, Grobe K, Lindner B, Schlaak M, Becker WM (1997) **Comparison of natural and recombinant isoforms of grass pollen allergens** *Electrophoresis 18:819-825*

- Petersen A, Schramm G, Schlaak M, Becker WM (1998) **Post-translational modifications influence IgE reactivity to the major allergen Phl p 1 of timothy grass pollen** *Clin Exp Allergy 28:315-321*
- Pumphrey RSH, Stanworth SJ (1996) **The clinical spectrum of anaphylaxis in north-west England** *Clin Exp Allergy 26:1364-1370*
- Rabjohn P, Helm EM, Stanley JS, West CM, Sampson HA, Burks AW, Bannon GA (1999) **Molecular cloning and epitope analysis of the peanut allergen Ara h 3** *J Clin Invest 103:535-542*
- Reese G, Jeoung BJ, Daul CB, Lehrer SB (1997) **Characterization of recombinant shrimp allergen Pen a 1 (tropomyosin)** *Int Arch Allergy Immunol 113:240-242*
- Reese G, Ayuso R, Carle T, Lehrer SB (1999) **IgE-binding epitopes of shrimp tropomyosin, the major allergen Pen a 1** *Int Arch Allergy Immunol 118:300-301*
- Rihs HP, Chen Z, Ruëff F, Petersen A, Rozynek P, Heimann H, Baur X (1999) **IgE binding of the recombinant allergen soybean profilin (rGly m 3) is mediated by conformational epitopes** *J Allergy Clin Immunol 104:1293-1301*
- Roy K, Mao HQ, Huang SK, Leong KW (1999) **Oral gene delivery with chitosan--DNA nanoparticles generates immunologic protection in a murine model of peanut allergy** *Nature Med 5:387-391*
- Rudeschko O, Fahlbusch B, Henzgen M, Schlenvoigt G, Herrmann D, Vieths S, Jäger L (1995) **Investigation of the stability of apple allergen extracts** *Allergy 50:575-580*
- Sampson HA (1988) **Comparative study of commercial food antigen extracts for the diagnosis of food hypersensitivity** *J Allergy Clin Immunol 82:718-726*
- Sampson HA, Mendelson L, Rosen JP (1992) **Fatal and near-fatal anaphylactic reactions to food in children and adolescents** *New Engl J Med 327:380-384*
- Scheiner O, Aberer W, Ebner C, Ferreira F, Hoffmann-Sommergruber K, Hsieh LS, Kraft D, Sowka S, Vanek-Krebitz M, Breiteneder H (1997) **Cross-reacting allergens in tree pollen and pollen-related food allergy: implications for diagnosis of specific IgE** *Int Arch Allergy Immunol 113:105-108*
- Scheurer S, Metzner K, Haustein D, Vieths S (1997) **Molecular cloning, expression and characterization of Pru a 1, the major cherry allergen** *Mol Immunol 34:619-629*
- Scheurer S, Son DY, Boehm M, Karamloo F, Franke S, Hoffmann A, Haustein D, Vieths S (1999) **Cross-reactivity and epitope analysis of Pru a 1, the major cherry allergen** *Mol Immunol 36:155-167*
- Scheurer S, Wangorsch A, Haustein D, Vieths S (2000a) **Cloning and immunological characterization of profilins Api g 4 from celery, Pru a 4 from cherry and Pyr c 4 from pear** *J Allergy Clin Immunol 105:S131 (abstract)*
- Scheurer S, Wangorsch A, Haustein D, Vieths S (2000b) **Cloning of the minor allergen Api g 4 profilin from celery *(Apium graveolens)* and its allergenic cross-reactivity with birch pollen profilin Bet v 2** *Clin Exp Allergy 30:962-971*
- Schweimer K, Sticht H, Nerkamp J, Boehm M, Breitenbach M, Vieths S, Rösch P (2000) **NMR spectroscopy reveals common structural features of the birch pollen allergen Bet v 1 and the cherry allergen Pru a 1** *Appl Magn Reson 17:449-464*
- Shin DS, Compadre CM, Sampson HA, Burks AW, Bannon GA (1999) **Modulation of the reactivity of the major peanut allergen Ara h 1 through epitope characterization, structural analysis, and mutation** *J Allergy Clin Immunol 103:S99 (abstract)*
- Sicherer SH, Furlong TJ, DeSimone J, Sampson HA (1999) **Self-reported allergic reactions to peanut on commercial airliners** *J Allergy Clin Immunol 104:186-189*
- Smith PM, Suphioglu C, Griffith IJ, Theriault K, Knox RB, Singh MB (1996) **Cloning and expression in yeast *Pichia pastoris* of a biologically active form of Cyn d 1, the major allergen of Bermuda grass pollen** *J Allergy Clin Immunol 98:331-343*
- Smith PM, Xu H, Swoboda I, Singh MB (1997) **Identification of a Ca2+ binding protein as a new Bermuda grass pollen allergen Cyn d 7: IgE cross-reactivity with oilseed rape pollen allergen Bra r 1** *Int Arch Allergy Immunol 114:265-271*
- Son DY, Scheurer S, Hoffmann A, Haustein D, Vieths S (1999) **Pollen-related food allergy: cloning and immunological analysis of isoforms and mutants of Mal d 1, the major apple allergen, and Bet v 1, the major birch pollen allergen** *Eur J Nutr 38:201-215*
- Sowka S, Hsieh LS, Krebitz M, Akasawa A, Martin BM, Starrett D, Peterbauer CK, Scheiner O, Breiteneder H (1998) **Identification and cloning of Prs a 1, a 32-kDa endochitinase and major allergen of avocado, and its expression in the yeast *Pichia pastoris*** *J Biol Chem 273:28091-28097*
- Sudbery PE (1996) **The expression of recombinant proteins in yeasts** *Curr Opin Biotechnol 7:517-524*
- Takai T, Mori A, Yuuki T, Okudaira H, Okumura Y (1999) **Non-anaphylactic combination of partially deleted fragments of the major house dust mite allergen Der f 2 for allergen-specific immunotherapy** *Mol Immunol 36:1055-1065*
- Teuber SS, Dandekar AM, Peterson WR, Uratsu SL (1996) **Cloning and sequencing of a walnut *(Juglans regia)* food allergen and its sequence similarity with other seed storage proteins** *J Allergy Clin Immunol 97:241 (abstract)*

- Teuber SS, Dandekar AM, Peterson WR, Sellers CL (1998) **Cloning and sequencing of a gene encoding a 2S albumin seed storage protein precursor from English walnut *(Juglans regia)*, a major food allergen** *J Allergy Clin Immunol 101:807-814*
- Teuber SS, Jarvis KC, Dandekar AM, Peterson WR, Ansari AA (1999) **Identification and cloning of a complementary DNA encoding a vicilin-like proportion, Jug r 2, from English walnut kernel *(Juglans regia)*, a major food allergen** *J Allergy Clin Immunol 106:1311-1320*
- Tschopp JF, Sverlow G, Kosson R, Craig W, Grinna L (1987) **High-level secretion of glycosylated invertase in the methylotropic yeast, *Pichia pastoris*** *Biotechnology (NY) 5:1305-1308*
- Valenta R, Duchêne M, Pettenburger K, Sillaber C, Valent P, Bettelheim P, Breitenbach M, Rumpold H, Kraft D, Scheiner O (1991) **Identification of profilin as a novel pollen allergen. IgE autoreactivity in sensitized individuals** *Science 253:557-560*
- Valenta R, Duchêne M, Ebner C, Valent P, Sillaber C, Deviller P, Ferreira F, Tejkl M, Edelmann H, Kraft D (1992) **Profilins constitute a novel family of functional plant pan-allergens** *J Exp Med 175:377-385*
- van der Veen MJ, van Ree R, Aalberse RC, Akkerdaas J, Koppelman SJ, Jansen HM, van der Zee JS (1997) **Poor biologic activity of cross-reactive IgE directed to carbohydrate determinants of glycoproteins** *J Allergy Clin Immunol 100:327-334*
- van Do T, Hordvik I, Endresen C, Elsayed S (1999) **Expression and analysis of recombinant salmon parvalbumin, the major allergen in Atlantic salmon *(Salmo salar)*** *Scand J Immunol 50:619-625*
- van Ree R, Akkerdaas JH, van Leeuwen WA, Fernández-Rivas M, Asero R, Knul-Brettlova V, Knulst A, Aalberse RC (2000) **New perspectives for the diagnosis of food allergy** *Allergy Clin Immunol Int 12:7-12*
- van Ree R, Cabanes-Macheteau M, Akkerdaas J, Milazzo JP, Loutelier-Bourhis C, Rayon C, Villalba M, Koppelman S, Aalberse R, Rodriguez R, Faye L, Lerouge P (2000) **Beta(1,2)-xylose and alpha(1,3)-fucose residues have a strong contribution in IgE binding to plant glycoallergens** *J Biol Chem 275:11451-11458*
- Vanek-Krebitz M, Hoffmann-Sommergruber K, Laimer da Camara Machado M, Susani M, Ebner C, Kraft D, Scheiner O, Breiteneder H (1995) **Cloning and sequencing of Mal d 1, the major allergen from apple *(Malus domestica)*, and its immunological relationship to Bet v 1, the major birch pollen allergen** *Biochem Biophys Res Commun 214:538-551*
- Varjonen E, Björkstén B, Savolainen J (1996) **Stability of cereal allergens** *Clin Exp Allergy 26:436-443*
- Verma R, Boleti E, George AJ (1998) **Antibody engineering: comparison of bacterial, yeast, insect and mammalian expression systems** *J Immunol Meth 216:165-181*
- Vieths S, Schöning B, Jankiewicz A (1993) **Occurrence of IgE binding allergens during ripening of apple fruits** *Food Agric Immunol 5:93-105*
- Vieths S, Jankiewicz A, Schöning B, Aulepp H (1994) **Apple allergy: the IgE-binding potency of apple strains is related to the occurrence of the 18-kDa allergen** *Allergy 49:262-271*
- Vieths S, Aulepp H, Schöning B, Tschirnich R (1995) **Untersuchungen zur Apfelallergie bei Birkenpollenallergikern. Hauttestergebnisse und Immunoblot-Analysen mit Tieftemperaturextrakten unterschiedlicher Apfelsorten (Investigations of apple hypersensitivity in birch pollen allergic patients: Skin prick test results and immunoblot studies using low temperature extracts of four apple strains)** *Allergologie 18:89-97 (article in German)*
- Vieths S, Jankiewicz A, Schöning B, Haustein D (1996a) **Profiline - ubiquitäre Proteine mit spezifischer physiologischer Funktion und potente Pflanzenallergene Teil 1: Regulation der Aktinpolymerisation** *Allergo J 5:89-92 (article in German)*
- Vieths S, Jankiewicz A, Schöning B, Haustein D (1996b) **Profiline - ubiquitäre Proteine mit spezifischer physiologischer Funktion und potente Pflanzenallergene Teil 2: Signaltransduktion, Molekülstruktur und Allergenität** *Allergo J 5:157-163 (article in German)*
- Vieths S (1997) **Allergenic cross-reactivity, food allergy and pollen** *Environ Toxicol Pharmacol 4:61-70*
- Vieths S, Hoffmann A, Holzhauser T, Müller U, Reindl J, Haustein D (1998) **Factors influencing the quality of food extracts for *in vitro* and *in vivo* diagnosis** *Allergy 53:65-71*
- Vieths S, Karamloo F, Lüttkopf D, Scheurer S, Fötisch K, May S, Altmann F, Skov PS, Haustein D (2000) **Recombinant allergens expressed in *E.coli*: benefits and drawbacks in the diagnosis of food allergies** *Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankf A M 93:159-168*
- Villalba M, Monsalve RI, Batanero E, Ledesma A (2000) **Cloning and expression of Bnl, a 2S albumin from *Brassica napus* seeds with allergenic properties** *Allergy 55:128 (abstract)*
- Vrtala S, Grote M, Ferreira F, Susani M, Stocker B, Kraft D, Valenta R (1995) **Humoral immune responses to recombinant tree pollen allergens (Bet v I and Bet v II) in mice: construction of a live oral allergy vaccine** *Int Arch Allergy Immunol 107:290-294*
- Vrtala S, Wiedemann P, Mittermann I, Eichler HG, Sperr WR, Valent P, Kraft D, Valenta R (1996) **High-level expression in *Escherichia coli* and purification of recombinant plant profilins: comparison of IgE-binding capacity and allergenic activity** *Biochem Biophys Res Commun 226:42-50*

- Yasue M, Yokota T, Fukada M, Takai T, Suko M, Okudaira H, Okumura Y (1998) **Hyposensitization to allergic reaction in rDer f 2-sensitized mice by the intranasal administration of a mutant of rDer f 2, C8/119S** *Clin Exp Immunol 113:1-9*
- Yasue M, Yokota T, Okudaira H, Okumura Y (1999) **Specific immunotherapy in rDer f 2-sensitized monkeys by administration of a mutant of rDer f 2, C8/119S** *J Allergy Clin Immunol 103:S141 (abstract)*
- Yocum MW, Khan DA (1994) **Assessment of patients who have experienced anaphylaxis: a 3-year survey** *Mayo Clin Proc 69:16-23*
- Yunginger JW, Sweeney KG, Sturner WQ, Giannandrea LA, Teigland JD, Bray M, Benson PA, York JA, Biedrzycki L, Squillace DL, Helm RM (1988) **Fatal food-induced anaphylaxis** *JAMA 260:1450-1452*
- Zeiler T, Taivainen A, Rytkönen M, Rautiainen J, Karjalainen H, Mäntyjärvi R, Tuomisto L, Virtanen T (1997) **Recombinant allergen fragments as candidate preparations for allergen immunotherapy** *J Allergy Clin Immunol 100:721-727*

[Summary] [Article] [Table 1] [Table 2] [Abbreveations]