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Review

Recombinant food allergens

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Abstract

Allergenic (glyco)proteins are the elicitors of food allergies and can cause acute severe hypersensitivity reactions. Recombinant food allergens are available in standardised quantity and constant quality. Therefore, they offer new perspectives to overcome current difficulties in the diagnosis, treatment and investigation of food allergies. This review summarises the expression strategies and characteristics of more than 40 recombinant food allergens that have been produced until today. Their IgE-binding properties are compared to those of their natural counterparts, in addition their application as diagnostic tools, the generation of hypoallergenic recombinant isoforms and mutants for therapeutic purposes, the determination of epitopes and cross-reactive structures are described.

Keywords: Reviews; Food allergy; Recombinant food allergen

Contents

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1. Introduction

Recombinant DNA technology provides the potential for in vitro production of virtually unlimited amounts of a certain protein of constant quality. The DNA sequence coding for the desired protein is inserted into cultured *E. coli* bacteria, yeast or insect cells, which express the corresponding recombinant protein. Ten years ago, the first food allergen was cloned [1,2] and during the last 3 years the number of available recombinant food allergens has strongly increased. Recombinant food allergens offer new perspectives to solve problems of clinical and molecular allergology in diagnosis, research, and therapy of food allergies.

1.1. Diagnosis

Sensitisation to food is commonly diagnosed by serological tests or skin tests. Serological assays detect allergen-specific IgE antibodies by immunoblots or allergosorbent tests (EAST, RAST, ELISA, FEIA). In skin tests, the allergen source is brought into contact with the skin in different ways (injecting, rubbing, dropping in scratched wounds) after which the appearance of wheals caused by mediator release of IgE-sensitised cells is recorded [3,4]. Usually, allergists apply commercially available soluble extract preparations of the allergen source. Most food extracts are not standardised according to total protein content, content of single allergens or allergenic activity [5,6]. Therefore, skin test results can differ between different batches of extracts and between extracts from different manufacturers [7,8]. Similarly, semi-quantitative values of different in vitro assays for specific IgE cannot be compared. The allergen content of food extracts can vary according to the strain of the food used or to the degree of ripeness of a fruit [9–14]. It is possible that an allergen is not detected, because of its low content in certain extracts. Extracts often lose their activity during extraction procedures and storage [13,15–17]. Inherent enzymatic activity of extracts can degrade allergens and destroy their IgE-binding activity, a phenomenon which is particularly important for food extracts [7,9,13,18–20]. In addition, interactions of allergens with phenolic compounds can change allergenicity [18,21,22]. For example, Vieths et al. showed that of four commercially available apple extracts used for skin tests, none could detect allergic patients [9]. Recombinant food allergens, which are of constant quality with batch-to-batch consistency, pure and stable could be used to standardise extracts or to replace extracts in order to provide more sensitive and specific tests of sensitisation to food. Standardised tests with recombinant food allergens would supply comparable qualitative as well as quantitative results and would allow to determine the allergen pattern of a patient in a short time. However, this would require that all allergens of a certain food were known and cloned. Yet, recombinant food allergens may lack post-translational modifications and may be incorrectly folded. Consequently, the immunological properties have to be well characterised to make sure that the features are similar to the natural counterpart.

1.2. Therapy

To date, there is no immunological, causally effective therapy of food allergies and the only way of dealing with this problem is to avoid the allergy-eliciting food. Avoidance of food is not always possible, as is in the case with peanuts. Peanut oil and peanut protein are widely used in pharmaceutical preparations and food industry, and a high percentage of peanut-allergic persons accidentally consume hidden peanut proteins [23]. Peanut allergy is a very severe food allergy, because symptoms can be elicited by very low doses of peanut and can cause fatal anaphylactic reactions [24–26]. A first attempt of specific immunotherapy (SIT) with aqueous peanut extract appeared to be effective, but the subjects had systemic reactions during therapy and consequently required medication [27,28]. It is assumed that allergens with reduced IgE-binding activity and retained T cell reactivity could be effective, as well as safe for immunotherapy [29]. Recombinant mutants or fragments of allergens with low IgE-binding reactivity and recombinant hypoallergenic isoforms could be a source for pure, standardised therapeutics with reduced side-effects [30–35].

1.3. Research

Recombinant allergens can be produced in virtually unlimited amounts and therefore sufficient material is available to investigate key issues in allergy research: what makes an allergen allergenic? Are defined amino acid sequences, or two- and three-dimensional structures responsible for allergenicity? Or are post-translational modifications such as carbohydrate side chains of glycoprotein allergens involved in allergenicity? Recombinant food allergens and their fragments are tools to characterise B and T cell epitopes and cross-reactive structures on allergens. Studies with mutants may provide new information on allergenic properties. Recombinant food allergens could serve as tools to examine the influence of food processing on the allergenicity of a food protein under precisely controlled conditions. This approach could lead to foods with reduced allergenicity. Finally, the cloning of allergenic proteins opens the door to determine the tertiary structure of allergens [36–38]. In addition the analysis of allergen–antibody complexes may provide new insights into the generic structure of allergenic epitopes.

2. Production of recombinant food allergens

To produce recombinant food allergens, a DNA copy (cDNA) of the allergen-encoding mRNA is ligated into an expression vector which is transformed into the lost cell, mostly *E. coli* bacteria. The host cell expresses the recombinant food allergen which is purified from cell lysate.

In more detail, the mRNA molecules are extracted from the cells and first converted into single-stranded cDNA copies (ss-cDNA) using reverse transcriptase (RT) and an oligo-dT primer which binds to the poly-A tails in mRNA. Second, the single-stranded cDNA molecules are amplified by polymerase chain reaction (PCR) to double-stranded cDNA (ds-cDNA) or cloned into phages and used for the infection of bacteria [39].

Table 1 provides an overview of the procedures for cloning of the recombinant food allergens produced until today (February 2000). ‘‘Food allergens’’ which are so far not accepted as allergens by the WHO/IUIS allergen nomenclature subcommittee [40] are given in square brackets. When a food allergen in the cited publication has been differently denominated than in the official IUIS list of allergens [ftp://biobase.dk/pub/who-iuis/allergen.list] it is also shown in square brackets next to the official abbreviation (for example Pers a 1 and [Prs a 1]). Table 1 also indicates the cloning strategy used for selecting the cDNA as well as the applied plasmid expression vector and the expression system. It lists whether the food allergen was expressed with a fusion peptide or not, describes the yield of expression and gives some remarks about the expression.

2.1. Selection of allergen-coding cDNA by two principles

A crucial point of the cloning strategy is to select the allergen-coding cDNA out of the pool of cDNA molecules of the cell. According to whether the DNA sequence of the food allergen is (partially) known or not, there are two cloning strategies to identify the cDNA of interest: PCR-based methods or screening of cDNA libraries.

2.1.1. Polymerase chain reaction (PCR)-based identification

For amplification of the allergen-coding cDNA by PCR, sequence information is required: either a part of the amino acid sequence of the allergen or of the corresponding cDNA sequence have to be known. In many cases, the amino acid sequence of the N-terminus is analysed by Edman degradation. On the basis of protein sequence data, cDNA sequences of related proteins may be obtained from nucleotide sequence databases. This information can be used to create a degenerate oligonucleotide primer specific for a conserved region near the 5′-end of the coding region of the target cDNA. After the RT reaction primed with oligo-dT and mRNA as template, this 5′-primer and oligo-dT are used to amplify a fragment of the allergen-coding cDNA. The nucleotide sequence of this cDNA molecule is analysed. However, the 5′-untranslated region and parts of the 5′-coding region remain unknown. This fragment is reversely transcribed from the mRNA with an oligo-

Table 1
Cloning of recombinant food allergens[a]

Recombinant food allergen [ ][b]	Source	Function	Cloning strategy	Expression system	Plasmid expression vector	Fusion peptide	Yield (mg/l)	Remarks	M_r (kDa)	Ref.
Api g 1	Celery	Pathogenesis-related protein	PCR	*E. coli*	pMW 175	–	300	–	17	[43,121]
[Api g 2]	Celery	Pathogenesis-related protein	cDNA library	*E. coli*	?	?	?	–	?	[60]
[Api g 3]	Celery	Chlorophyll a/b-binding protein	cDNA library	*E. coli*	?	?	?	–	?	[60]
Api g 4	Celery	Profilin	PCR	*E. coli*	Modified pET-30a	–	1–3	Recombinant protein soluble	14.3, calculated	[50,53]
Ara h 1	Peanut	Vicilin-like seed storage protein	cDNA library	*E. coli*	pBluescript	37 amino acids of β-gal		Fragments resulting from inefficient translation	~68 with fusion peptide	[62]
Ara h 1	Peanut	Vicilin-like seed storage protein	cDNA library	*E. coli*	?	?	?	Fragments resulting from inefficient translation	47.8	[63]
Ara h 1	Peanut	Vicilin-like seed storage protein	cDNA library; phage display technology	*E. coli*	pDS56	his6	?	–	60.3, calculated	[64]
Ara h 1 2 variants	Peanut	Vicilin-like seed storage protein	PCR	Yeast: *Pichia pastoris*	pIC3	–	?	Intracellular expression; less fragments compared to *E. coli*-expressed allergen	62	[76]
Ara h 2	Peanut	Conglutin seed storage protein	?	*E. coli*	pET-24	his6	?	?	?	[104,105] MA
Ara h 2	Peanut	Conglutin seed storage protein	cDNA library; phage display technology	*E. coli*	pDS56	his6	?	–	17, calculated	[64]
Ara h 3	Peanut	Legumin-like 11S seed storage protein	cDNA library; phage display technology	*E. coli*	pET-24	One additional amino acid at N-terminus, his6 at C-terminus	?	–	~57	[65,66]
Ara h 4	Peanut	Glycinin	cDNA library; phage display technology	*E. coli*	pDS56	his6	?	–	36, calculated	[64]
Ara h 5	Peanut	Profilin	cDNA library; phage display technology	*E. coli*	pDS56	his6	?	–	14, calculated	[64]
Ara h 6	Peanut	Conglutin-like seed storage protein	cDNA library; phage display technology	*E. coli*	pDS56	his6	?	–	14.5, calculated	[64]
Ara h 7	Peanut	Conglutin-like seed storage protein	cDNA library; phage display technology	*E. coli*	pDS56	his6	?	–	15.8, calculated	[64]
Bos d 5 [β-lactoglobulin]	Cow's milk	Retinol-binding protein	cDNA library [67]	*E. coli*	pTTQ18	–	>50	Met-lactoglobulin (without the first 10 amino acids of the mature protein); inclusion bodies	18–20	[2]
Bos d 5 [β-lactoglobulin]	Cow's milk	Retinol-binding protein	cDNA from [2]	*E. coli*	pTTQ18	–	5–10	Met-lactoglobulin (without the first 10 amino acids of the mature protein); inclusion bodies	18–20	[2,90]
Bos d 5 [β-lactoglobulin]	Cow's milk	Retinol-binding protein	PCR	*E. coli*	pET26	his, C-terminal	?	Vector with signal for periplasmic localisation; detected in periplasm, cytoplasm and aggregates (inclusion bodies)	?	[77]

Table 1 (Continued)

Recombinant food allergen [ ][b]	Source	Function	Cloning strategy	Expression system	Plasmid expression vector	Fusion peptide	Yield (mg/l)	Remarks	M_r (kDa)	Ref.
Bos d 5 [β-lactoglobulin]	Cow's milk	Retinol-binding protein	cDNA from [2]	Yeast: *Pichia pastoris*	pPIC9	–	1,5	Secreted in culture medium	?	[78]
Bos d 5 [β-lactoglobulin]	Cow's milk	Retinol-binding protein	cDNA library	Yeast: *S. cerevisiae*	pYG100	–	1,1	Pre-lactoglobulin with 16 amino acid signal sequence; protein expressed in growth medium	18	[1]
Bos d 5 [β-lactoglobulin]	Cow's milk	Retinol-binding protein	PCR	COS 7 cells	pcDNA3	–	?	Recombinant protein partially soluble/insoluble	?	[77]
[carp parvalbumin]	Carp	Parvalbumin	cDNA library	*E. coli*	?	β-gal	?	–	?	[68,217] MA
[Cha f 1]	Crab	Tropomyosin	cDNA library	*E. coli*	pGEX	GST	?	Positive expression only with pGEX 1, not with pGEX 2 and pGEX 3	60 fusion protein; 34 allergen, calculated	[69]
Cor a 1.0401	Hazelnut	Pathogenesis-related protein	PCR	*E. coli*	pTYB-1	Intein-CBD	?	Recombinant protein soluble	17.45, calculated	[51,108]
[Cha m ?]	Scallop	Tropomyosin	PCR	*E. coli*	pGEX or proEXHT	?	?	?	38?	[205] MA
[Dau c 1]	Carrot	Pathogenesis-related protein	PCR	*E. coli*	pDS 56	his6	130	–	16	[44]
Gly m 1	Soybean	Hydrophobic protein of soybean (HPS)	?	*E. coli*	?	his6	?	?	?	[106] MA
Gly m 3	Soybean	Profilin	PCR	*E. coli*	pMAL-c2	MBP, C-terminal	8	–	14.1 allergen, calculated; 56.8 fusion protein, calculated	[48]
[Gly m ? 2 glycinin acid chains]	Soybean	Glycinin	?	*E. coli*	?	his6	?	?	?	[106] MA
[Hal d ?]	Abalone	Tropomyosin	PCR	*E. coli*	pGEX or proEXHT	?	?	–	38?	[205] MA
[Hom a 1]	Lobster	Tropomyosin	cDNA library [218]	*E. coli*	pGEX	GST	?	–	60 fusion protein; 34 allergen, calculated	[75]
Jug r 1	Walnut	2S albumin seed storage protein	cDNA library	*E. coli*	pGEX 2	GST	?	–	42 fusion protein; 15–16 allergen, calculated	[70]
Jug r 2	Walnut	Vicilin-like seed storage precursor protein	cDNA library [70]	*E. coli*	pGEX-4T-3	GST	?		92 fusion protein; 66 allergen, estimated	[61]
[Jug r 19 kDa]	Walnut	2S albumin seed storage protein ?	cDNA library	*E. coli*	?	?	?	?	19	[59] MA
Mal d 1	Apple	Pathogenesis-related protein	PCR	*E. coli*	pMW 175	–	?	–	17.7, calculated	[45]
Mal d 1	Apple	Pathogenesis-related protein	PCR	*E. coli*	pET-15b	his6	2.5–7.5	–	18	[10,182]
Met e 1	Shrimp	Tropomyosin	cDNA library	*E. coli*	pGEX-1	GST	?	–	60 fusion protein; 34 allergen, calculated	[56,71]
[Pan s 1]	Lobster	Tropomyosin	cDNA library	*E. coli*	pGEX	GST	?	Positive expression only with pGEX 1,3	60 fusion protein; 34 allergen, calculated	[75]
Pen a 1	Shrimp	Tropomyosin	cDNA library	*E. coli*	pcDNA II	–	?	not with pGEX 2 and pGEX –	>36 kDa	[58]
[Pern v ?]	Mussel	Tropomyosin	PCR	*E. coli*	pGEX or proEXHT	?	?	?	38?	[205] MA
Pers a 1 [Prs a 1]	Avocado	Endochitinase	PCR	Yeast: *Pichia pastoris*	pPIC9	–	50	Extracellularly expressed	32	[79]

Table 1. Continued

Recombinant food allergen [ ][b]	Source	Function	Cloning strategy	Expression system	Plasmid expression vector	Fusion peptide	Yield (mg/l)	Remarks	M_r (kDa)	Ref.
Pru av 1 [Pru a 1]	Cherry	Pathogenesis-related protein	PCR	*E. coli*	pET-16b	his10, N-terminal	100	–	18–19	[46,115]
Pru av 4 [Pru a 4]	Cherry	Profilin	PCR	*E. coli*	pET-30a	–	1+3	–	14	[53] MA
Pyr c 1	Pear	Pathogenesis-related protein	PCR	*E. coli*	pET-16b	his10, N-terminal	20	Three additional amino acids at N-terminus left after cleavage of his-tag	18–19 in gel 17.4, calculated	[49,51]
Pyr c 4	Pear	Profilin	PCR	*E. coli*	Modified pET-30	–	1–3	Purified under denaturing conditions with urea	13.9, calculated	[51–53,163]
Pyr c 5 [Pyr c 2]	Pear	Isoflavone reductase -like protein (IRL)	PCR	*E. coli*	pET-16b	his10, N-terminal	10–30	Purified under native conditions; three additional amino acids at N-terminus after enzymatic cleavage of his-tag	33.7, calculated	[51,52,163]
Sal s 1	Salmon	Parvalbumin	cDNA library in [72]	*E. coli*	pET-19b	his10	?	–	14 fusion protein	[107]
Sin a 1	Mustard	2S albumin seed storage protein	PCR	*E. coli*	pCE17	C-LYTA	8–10	Inclusion bodies; purification of protein from bacterial homogenate was not possible	32 fusion protein	[111]
Sin a 1	Mustard	2S albumin seed storage protein	PCR	*E. coli*	pGEX-2T	GST	?	98% of recombinant protein insoluble; inclusion bodies, impossible to solubilise	20	[55]
[Wheat glutenins]	Wheat	Glutenins	PCR	*E. coli*	pET-21d, pET-24d	–	?	Expression of glutenin LMM only possible with vector pET-24d	~94 glutenin HMMx ~80 glutenin HMMy ~33 glutenin LMM	[112]
[Wheat gliadins]	Wheat	Gliadins	PCR	*E. coli*	pET-21d	–	?	–	~30 α- and γ-gliadins ~43 ω-gliadin	[112]

[a] *S. cerevisiae*, *Saccharomyces cerevisiae*; his, histidine; β-gal, β-galactosidase GST, glutathione-*S*-transferase; C-LYTA, C-terminal domain of *N*-acetylmuramoyl-L-alanine amidase of *Streptococcus pneumonia*; M_r (kDa), molecular mass in kiloDalton; HMMx glutenin, high-molecular mass glutenin x-type; HMMy glutenin, high-molecular mass glutenin y-type; LMM glutenin, low-molecular mass glutenin; MA, references are only meeting abstracts.

[ ][b] Food allergen is either not noted in the official WHO/IUIS list of allergens or is denominated differently by the authors in the cited reference.

dT primer or with a gene-specific 3′-primer. The gene-specific 3′-primer can be constructed according to the cDNA sequence data gained before. Then the 5′-RACE method (RACE, rapid amplification of cDNA ends) is performed to determine the unknown 5′-end [41,42]. The 3′-end of the ss-cDNA is extended with a homopolymeric dC tail. A degenerate homopolymeric dG primer anneals to the attached dC tail at ss-cDNA and allows the amplification of ss-cDNA to ds-cDNA by PCR. Thus, two overlapping cDNA molecules, together spanning the whole DNA region coding for the allergen, have been selected. From their sequences the full-length cDNA sequence of the food allergen can be deduced. Now, two gene-specific primers, representing the 5′- and 3′-ends of the coding region, can be synthesised. They are used in PCR amplification of the pool of cellular ss-cDNA. This third cDNA finally represents the complete coding region of the food allergen of interest. It will serve as template for the expression of the desired food allergen. According to this principle, the cDNAs of homologous food allergens belonging to the Bet v 1 family (Dau c 1, Mal d 1, Pru av 1, Api g 1, Cor a 1, Pyr c 1) and of the profilins (Api g 4, Gly m 3, Ara h 5, Pyr c 4, Pru av 4) were selected [10,43–53,64].

Differing from the above-described strategy, the cDNA molecules of mustard allergen Sin a 1 was gained. As the DNA sequence of Sin a 1 was known [54], primers for the 5′-end and the 3′-end of the coding region were constructed. The Sin a 1-specific DNA was directly amplified from genomic DNA by PCR without using mRNA molecules as templates. This strategy was possible because the Sin a 1 gene contains no introns within the polypeptide-coding sector [55] (for further description of Sin a 1 see Section 3.1).

The allergen-coding cDNA molecules selected by PCR are not identical but display a certain degree of heterogeneity. For example, they comprise different isoforms of the allergen. Therefore, they have to undergo a cloning step to identify the single allergen-coding cDNA clone of interest. For cloning, the various cDNA molecules are ligated into cloning vectors, which are then transformed into *E. coli*. Positive *E. coli* clones on the agar plates are screened for containing the correct cDNA insert by PCR screening with vector-specific and/or gene-specific primers and sequencing. The cDNA insert is excised from the cloning vector and ligated into an expression vector, which is subsequently transformed into target cells for protein synthesis. Consequently, the recombinant allergen derives from one cDNA molecule (see Fig. 1).

2.1.2. Copy DNA (cDNA) expression library

If the amino acid or DNA sequence of the required food allergen was unknown, a cDNA expression library was generated [1,56–72]. DNA copies of virtually all mRNA molecules of a cell are synthesised by RT and PCR reactions. The entire cDNA preparation is ligated into a plasmid vector or a bacteriophage λ vector and inserted into *E. coli* host cells which express the gene product. The expression libraries are immobilised on a solid phase, most frequently on nitrocellulose membranes, and screened for the desired food allergen. The cDNA of the identified clone is sequenced and the expression product is characterised.

In a variant of a phage expression library, the expression product is displayed on the surface of filamentous phages as a heterodimeric fusion protein together with a phage coat protein. Thus, phages carrying the gene product of interest can readily be adsorbed and isolated by binding to a specific ligand on a column, e.g., IgE antibody [73,74]. For example, six different peanut allergens were cloned by the pJuFo technology where the allergenic polypeptides were displayed on the pili of bacteriophage λ [64]. Advantages of this so-called phage display technology are the specific enrichment of the allergen-displaying phages out of large libraries by immunoaffinity and the linkage of the gene product with its cDNA. A disadvantage can be the enrichment of high affinity isoforms of an allergen. In contrast to this technique, screening of an expression library for a particular allergen-expressing *E. coli* clone by Western blotting is like looking for a needle in a haystack [62].

The cDNA expression libraries have to be screened for the presence of the correct allergen-coding cDNA. The screening was carried out with allergen-specific antibodies (immunoscreening), for example by IgE from sera of allergic patients [56,60,62,64,69,70,75], by allergen-specific polyclonal rabbit antiserum [2,67] or with allergen-spe-

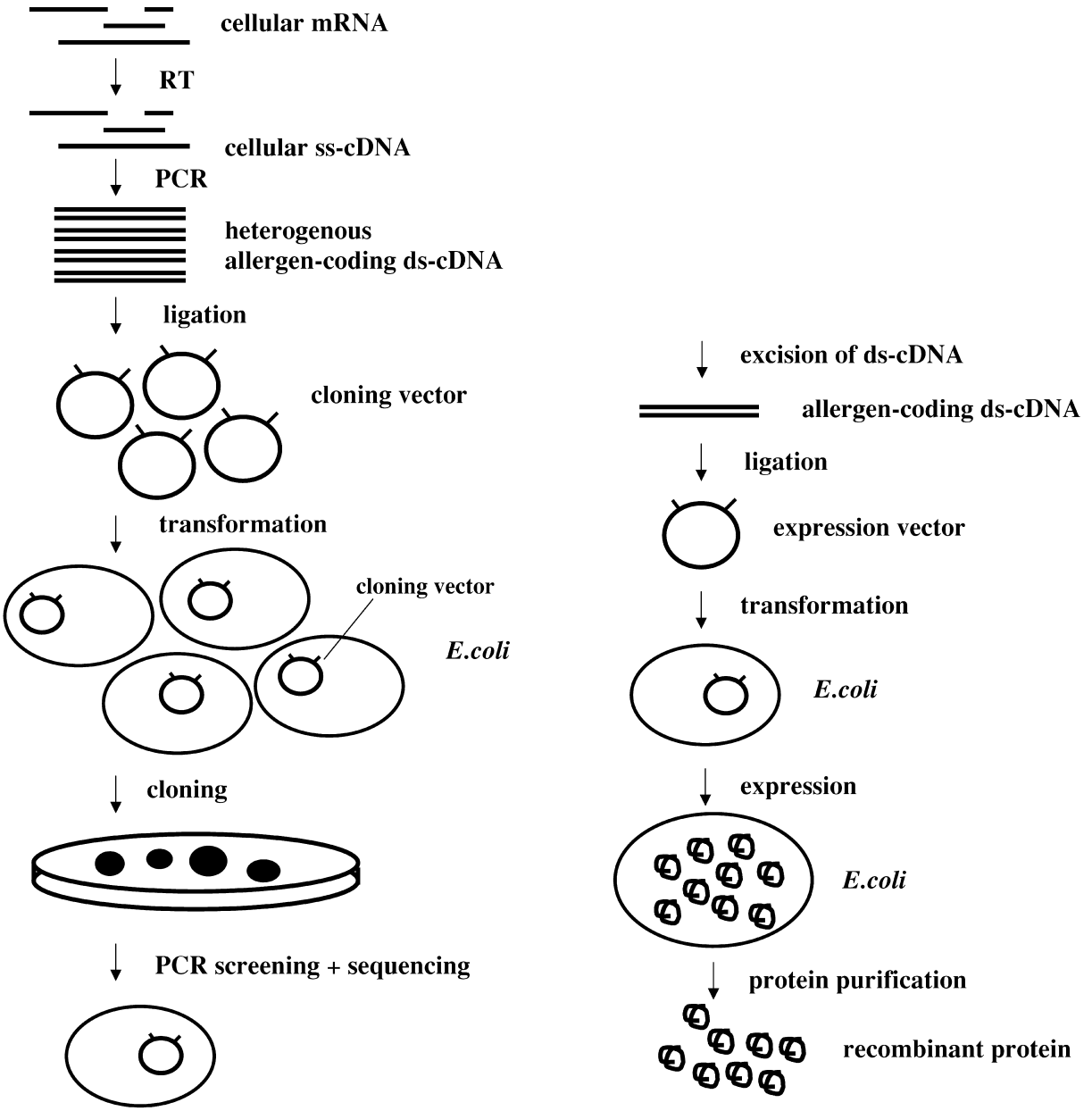

Fig. 1. Production of recombinant proteins in *E. coli*. Copy DNA (cDNA) is selected by PCR strategy.

cific monoclonal antibodies [58]. The efficiency of immunoscreening strongly depends on the quality (specificity, affinity) of the serum or the monoclonal antibody used, and the size of the cDNA expression library.

When the cDNA-encoded proteins are not expressed as is in the case with cDNA non-expression libraries, the screening is performed on the DNA level provided that DNA sequence information is available. A bovine cDNA-library [1] was screened for the β-lactoglobulin-coding cDNA by colony hybridisation in Southern blot. Two ^{32}P-labeled

oligonucleotide probes anneal specifically to complementary segments at the 5′-end and in the centre of the cDNA and identify the cDNA of interest [1].

2.2. Expression systems used for food allergens

The vast majority of recombinant food allergens were successfully expressed in prokaryotic *E. coli* cells with the use of standard plasmid expression vectors (see Table 1). Only three food allergens, namely Ara h 1, Bos d 5 and Pers a 1, were produced in the eukaryotic expression systems yeast cells and mammalian COS7 cells [76–79]. *E. coli* bacteria do not perform eukaryotic post-translational modifications such as glycosylations. Therefore, bacterially expressed glycoprotein allergens can display IgE-binding reactivity different to their natural analogue, because of an altered tertiary structure or because of lacking carbohydrate epitopes [80,81]. Yeast cells glycosylate proteins during expression at the correct glycosylation sites, but they hyperglycosylate proteins and link monosaccharides in a pattern which differs from the natural protein, since the carbohydrate parts are not modified during expression [82–87]. *E. coli* cells actually do not introduce disulfide bridges except in periplasm [88]. As disulfide bridges can determine the tertiary structure of an allergen and therefore the conformational epitopes [89], lack of them in *E. coli*-expressed allergens can alter the allergenicity of the recombinant allergen. Recombinant bovine β-lactoglobulin which naturally contains two disulfide bridges was expressed in *E. coli* by a periplasm-targeting vector. A comparison of recombinant and natural β-lactoglobulin indicated that *E. coli* had at least introduced one disulfide bridge [90]. Yeast cells provide advanced protein folding pathways and thus process and fold recombinant proteins correctly. The identical N-termini in recombinant, yeast-expressed β-lactoglobulin and in its natural analogue indicate that cleavage of the signal sequence occurred during the expression of β-lactoglobulin in yeast [1]. During the expression of avocado allergen Pers a 1, an endochitinase, in the yeast *Pichia pastoris*, the leader peptide was cleaved and recombinant Pers a 1 (rPers a 1) had enzymatic activity, which was assumed to be a proof for correct processing and folding of the protein in the yeast system [79]. One advantage of the yeast system is that the expressed protein can be secreted into the culture medium and thus can easily be purified [1,78]. Yeast cells, especially *Pichia pastoris*, are regarded as high-level expression systems compared to *E. coli* [86,91]. In contrast to this notion, the expression level of recombinant β-lactoglobulin in *E. coli* [2] was much more higher than in yeast cells [1,78]. However, the database on recombinant food allergens (see Table 1) is still too small for a final conclusion in this issue.

Difficulties which can arise during the production of recombinant food allergens, can be illustrated by the production of the recombinant peanut allergen Ara h 1. A cDNA clone coding for the preproform of Ara h 1 could not be expressed in full length in *E. coli*. After eliminating the first 93 basepairs at the 5′-end, corresponding to the first 31 amino acids at the N-terminus, full-length rAra h 1 as well as recombinant fragments of Ara h 1 were expressed at low levels [62,92]. Burks et al. [62] and Maid [76] hypothesised that translation is terminated in the N-terminal region of the protein due to the presence of the arginine-coding triplets AGA and AGG which are rare in *E. coli* [93] and the lack of arginine-specific transfer RNA ($tRNA_{Arg}$) [94]. As arginine is the second most frequent amino acid in Ara h 1, this possibly resulted in a frame shift [95], termination of translation [96], incorrect translation of arginine into lysine [97] or low expression rates [98,99]. Inefficient translation could also be due to the formation of hair pin structures in mRNA [100]. As in the yeast *Pichia pastoris* arginine is mainly encoded by the triplet AGA [101,102] and because higher expression rates were expected from the incorporation of inserted cDNA into the yeast's genome [103], rAra h 1 was expressed in the yeast *Pichia pastoris* [76]. First, the cDNA coding for the mature Ara h 1 was inserted into a vector which attached a special leader sequence for extracellular transport. The expression of this protein construct failed without recognisable reasons. Subsequently, two cDNAs that code for the preproform of Ara h 1 and for the mature form of Ara h 1, respectively, were expressed. At its N-terminus, the mature Ara h 1 protein is 78 amino acids shorter than the preproform. Both recombinant Ara h 1 proteins were detected intracellularly, even the preproform, although it included the natural signal sequence of Ara h 1 which targets the protein

for extracellular transport. Mature rAra h 1 was expressed in higher amounts than the prepro-rAra h 1. Fragments were observed during the expression, but to a lesser extent than in *E. coli*. It was hypothesised by the author that prepro-Ara h 1 was not exported because of interactions between the (possibly incorrectly processed) signal sequence and the membrane, leading to an anchoring of the recombinant protein in the membrane [76]. From these expression experiments with two shortened rAra h 1 molecules and the prepro-rAra h 1, one can conclude that the N-terminus of the prepro-Ara h 1 was important for the translation problems in *E. coli* as well as in yeast [62,76].

2.3. Protein purification

For easier purification of the recombinant protein from the cell lysate, most proteins are expressed with a fusion peptide. The recombinant fusion protein can be purified by affinity chromatography with binding of the fusion peptide to a specific ligand coupled to a column. After the purification step, the fusion part has to be removed to release the authentic recombinant food allergen. Disadvantages of this strategy are that the cleavage efficiency rarely is 100% and that some additional amino acids of the fusion peptide may remain at one of the termini of the non-fusion proteins. Some fusion peptides are known to cause aggregates of the recombinant protein, or to yield poorly soluble fusion proteins.

The most frequently used fusion peptide in the production of recombinant food allergens were tags including six or 10 histidine (his) residues at the N- or C-terminus of the protein [10,44,46,49,65,77,104–107]. The recombinant his-tagged fusion protein was purified by metal chelate affinity chromatography on a Ni–NTA matrix column [10,44,46,49,65]. Cleavage of the his-tag was performed either on the column-bound fusion protein [10], or after elution with the histidine-analogue imidazole [46,65,107], or under acidic conditions [49]. To obtain the non-fusion protein, the his-tag was removed enzymatically with factor Xa protease [46], enterokinase [107], or thrombin [10] at specific cleavage sites between the allergen and the fusion peptide. Cleavage was also performed chemically by cyanogen bromide (CNBr) which cleaved after the first methionine residue provided that there were no more methionine residues in the insert [49]. In case of recombinant peanut allergen rAra h 3, the his-tag was not cleaved and the fusion protein was directly applied in immunological assays [65].

The second commonly used fusion peptide in the production of recombinant food allergens was glutathione-*S*-transferase (GST) from *Schistosoma japonicum*, an enzyme with a molecular mass of about 26 kDa [55,56,61,69,70,75]. Recombinant GST-food allergen fusion proteins were isolated by binding of the GST fusion part to a glutathione column [55,56,61,69,70,75].

Another fusion peptide used was Intein-CBD, applied as a self-cleavable affinity tag at the C-terminus of the hazelnut allergen rCor a 1.0401 [51,108]. The chimeric protein intein-CBD consists of intein, which is a protein-splicing element from yeast, and a bacterial chitin-binding domain (CBD). By passing the cell lysate through a chitin column, the recombinant fusion protein bound with its CBD part. The addition of DTT at 4°C induced an intein-mediated self-cleavage of the fusion protein on the column and rCor a 1.0401 was released while the intein-CBD fusion peptide remained bound to the column [51]. The cleavage efficiency in the intein–CBD system depends on the amino acid of the allergen next to the intein part. It is favourable that no overhanging amino acids remain attached to the allergen and that purification can be achieved under native conditions. However, the intein–CBD system cannot be applied when the recombinant proteins are insoluble under native conditions.

Furthermore, maltose-binding protein (MBP) was fused to the C-terminus of the recombinant soybean allergen rGly m 3 [48,109], thioredoxin to soybean allergen fragments [110], and β-galactosidase (β-gal) to recombinant carp parvalbumin or a part of β-gal to rAra h 1 from peanut. The MBP- and β-gal-containing recombinant food allergens were not cleaved before characterization for IgE-binding [48,62,68].

In case of rSin a 1 fused to the choline-binding domain of a bacterial murein hydrolase (C-LYTA), the purification by performing ion chromatography in DEAE-cellulose was impossible and rSin a 1-C-

LYTA could not be isolated from *E. coli* lysate [111].

Several recombinant food allergens were expressed as non-fusion proteins, especially those expressed in eukaryotic systems and, for example, the profilins in prokaryotic *E. coli* [1,2,43,50,52,53, 58,64,76–79,90,112]. Therefore, non-fusion proteins are without additional amino acids resulting from the fusion peptide. However, the purification procedure can be very extensive, especially when there is no specific ligand available. For purification of recombinant profilin, its property of binding to proline-rich sequence stretches was used by applying a poly-L-proline column [50,52]. In the case of one recombinant β-lactoglobulin, affinity chromatography was done with an allergen-specific monoclonal antibody [90]. Another recombinant β-lactoglobulin was purified by ion exchange [78].

At best during the purification step, the recombinant food allergens were present in soluble form in the supernatant of the cell lysate and could be purified under native conditions [1,43,50,77–79]. However, in some cases, the problem of inclusion bodies occurred, when the recombinant proteins aggregated and were poorly soluble [2,49,55, 77,90,111]. Inclusion bodies had to be solubilised under denaturing conditions, for example with urea and acidic pH [49], urea and DTT [90] or by guanidine–HCl treatment plus DTT [2]. Following the purification from inclusion bodies, the pear allergen rPyr c 1 was refolded according to a special protocol [49,113]. The expression vector for recombinant β-lactoglobulin from cow's milk contained a signal sequence for periplasmic localisation of the recombinant protein. Periplasmic expression is thought to mainly yield correctly folded proteins. Finally, recombinant β-lactoglobulin was detected in periplasm as well as in cytoplasm and in aggregates [77].

The expression of mustard allergen rSin a 1 fused to GST led to a proportion of 98% of insoluble rSin a 1-GST in inclusion bodies and only to 2% of soluble rSin a 1-GST. Several methods to solubilise the great fraction of insoluble rSin a 1-GST failed. It was hypothesised that this might be an inherent property of proteins which are rich in disulfide bonds and prolins [55].

3. Research applications of recombinant food allergens

A prerequisite for the use of recombinant food allergens is a good knowledge of their physicochemical, biological and immunological properties compared to those of the native forms. A cloned food allergen that is immunologically identical to the native one could replace the extracted allergen in diagnostic tests or research.

If not otherwise mentioned, the recombinant food allergens described in the following text have been expressed in *E. coli*.

3.1. Immunological properties of recombinant food allergens

Many studies have revealed that recombinant food allergens show a considerable degree of biological activity (see Table 2). Studies directly comparing allergenic potency of recombinant and corresponding natural food allergens are rare [10,43–45,48,49,51,58,62,63,76,90,114,118].

Table 2 lists all recombinant food allergens and the IgE-binding assays they have been characterised with. According to the panel of different assays applied, we have classified the level of characterisation for each recombinant food allergen. If no IgE-binding activity was shown and the recombinant food allergen was only immunologically characterised by polyconal or monoclonal antibodies, the level of characterisation has been regarded as ''insufficiently characterised'', marked by ''−''. Detection of IgE-binding reactivity of the recombinant allergen in Western blot (IgE immunoblot) has been taken as ''sufficiently characterised'' or ''+''. Immunoblots are usually performed under conditions which denature proteins and hence it is assumed that they mainly detect linear epitopes. However, to a certain degree a renaturation of protein and therefore reconstitution of conformational epitopes may occur on the blotting membrane [119]. In tests such as inhibition assays and allergosorbent tests, the binding of recombinant allergen and IgE occur in fluid phase or under more native conditions. At this level, characterisation has been designated as ''well characterised ++''. When a recombinant food allergen was not

Table 2
Recombinant food allergens and their biological activity[a]

Recombinant food allergen [ ][b]	Applied as fusion protein	Expression cell	Level of characterisation	Assay	Number of sera assayed	Ref.
Api g 1	–	*E. coli*	+++	IgE immunoblot, compared to extract	10	[43]
				IgE immunoblot, compared to extract	24; 12; 10	[121]
				IgE immunoblot inhibition	Pool (n=12)	[43]
				IgE immunoblot inhibition	1	[120]
				EAST, compared to extract	30	[114]
				EAST	9; 1	[120]
				RAST	24; 12	[121]
				EAST inhibition	1; 9; 3; 1	[50,120]
				SPT	24; 12	[121]
				Mediator release	1	[120]
[Api g 2]	–	*E. coli*	(+)	IgE binding reported, but data not shown	?	[60]
[Api g 3]	–	*E. coli*	−	No immunological characterisation		[60]
Api g 4	–	*E. coli*	+++	IgE immunoblot	17;	[50]
				IgE immunoblot inhibition	9; 2; pool (n=9)	[50]
				EAST reported, but data not shown	10	[53]
				EAST inhibition	1; pool (n=2)	[50]
				Mediator release	1	[50,53]
Ara h 1 (68 kDA)	37 amino acids of β–gal	*E. coli*	+	IgE immunoblot, compared to nAra h 1	18; pool (n=18?)	[62]
Ara h 1 (48 kDa)	?	*E. coli*	+	IgE immunoblot, compared to nAra h 1	11; 3	[63]
Ara h 1	his6	*E. coli*	+	IgE immunoblot	40	[64]
Ara h 1	–	yeast	+	IgE immunoblot, compared to extract	5	[76]
Ara h 2	his6	*E. coli*	+	IgE immunoblot	40	[64]
Ara h 2	–	*E. coli*	+	IgE immunoblot	16	[104,105]
				T cell proliferation		
Ara h 3	his6	*E. coli*	+	IgE immunoblot	Pool (n=?); 18	[65,66]
Ara h 4	his6	*E. coli*	+	IgE immunoblot	Pool (n=34); 40	[64]
Ara h 5	his6	*E. coli*	+	IgE immunoblot	Pool (n=34); 40	[64]
Ara h 6	his6	*E. coli*	+	IgE immunoblot	Pool (n=34); 40	[64]
Ara h 7	his6	*E. coli*	+	IgE immunoblot	Pool (n=34); 40	[64]
Bos d 5 [Bovine β-lactoglobulin]	–	yeast	−	No IgE binding shown, only immunoblot with two monoclonal antibodies		[1]
Bos d 5 [Bovine β-lactoglobulin]	–	*E. coli*	−	No IgE binding shown, only with polyclonal rabbit anti-serum		[2]
Bos d 5 [Bovine β-lactoglobulin]	–	*E. coli*	++	ELISA, compared to nBos d 5	5	[90]
[Carp parvalbumin]	β-gal	*E. coli*	(+)	IgE immunoblot	Pool (n=10)	[68,217] MA
[Cha f 1]	GST	*E. coli*	++	IgE immunoblot	Pool (n=10?); 10	[69]
				IgE immunoblot inhibition	Pool (n=10?)	
[Cha n ?]	?	*E. coli*	(++)	IgE immunoblot and inhibition studies reported, but data not shown	?	[205] MA
Cor a 1.0401	–	*E. coli*	(+++)	IgE immunoblot	34	[108] MA
				EAST, compared to extract	43	[51]
				Mediator release compared to extract reported, but data not shown	6	[51]
[Dau c 1]	his6 ?	*E. coli*	++	IgE immunoblot, compared to extract	6	[44]
				IgE immunoblot inhibition	Pool (n=6)	

Table 2. Continued

Recombinant food allergen [ ][b]	Applied as fusion protein	Expression cell	Level of characterisation	Assay	Number of sera assayed	Ref.
Gly m 1	his6	*E. coli*	(+)	IgE binding reported, but data not shown, type of assay not mentioned	?	[106] MA
Gly m 3	–	*E. coli*	++	IgE immunoblot	8; pool (n=7)	[48]
				IgE immunoblot inhibition	3; pool (n=?)	
				EAST inhibition	Pool (n=?)	
Gly m 3	MBP	*E. coli*	+	IgE immunoblot	8; pool (n=7)	[48]
[Gly m ? (2 glycinin acidic chains)]	his6	*E. coli*	(+)	IgE binding reported, but data not shown, type of assay not mentioned	?	[106] MA
[Hal d ?]	?	*E. coli*	(++)	IgE immunoblot and inhibition studies reported, but data not shown	?	[205] MA
[Hom a 1]	GST	*E. coli*	++	IgE immunoblot	10	[69]
				IgE immunoblot	Pool (n=10)	[75]
				IgE immunoblot inhibition	Pool (n=10)	[75]
Jug r 1	GST	*E. coli*	++	IgE immunoblot	16	[70]
				FEIA inhibition	16	
Jug r 2	GST	*E. coli*	+	IgE immunoblot	15	[61]
				IgE immunoblot inhibition	4	
[Jug r (19 kDa)]	fp (fusion part not mentioned)	*E. coli*	(+)	IgE binding reported, but data not shown, type of assay not mentioned	12	[59] MA
Mal d 1	–	*E. coli*	++	IgE immunoblot, compared to extract	9	[45]
				IgE immunoblot inhibition	Pool (n=5)	[45]
				T cell proliferation assay, cytokine assay	19 T cell clones	[117]
Mal d 1 (three isoforms)	–	*E. coli*	+++	IgE immunoblot, compared to nMal d 1	8	[10,115,182]
				EAST, compared to nMal d 1	13	[10,118,182]
				Mediator release		[10,115,118,182]
Met e 1	GST	*E. coli*	+	IgE immunoblot	10	[69]
				IgE immunoblot inhibition reported, but data not shown	Pool (n=9)	[71]
				IgE immunoblot	Pool (n=10?)	[56]
[Pan s 1]	GST	*E. coli*	++	IgE immunoblot	10	[69]
				IgE immunoblot	Pool (n=10)	[75]
				IgE immunoblot inhibition	Pool (n=10)	[75]
Pen a 1	–	*E. coli*	+	IgE immunoblot, compared to nPen a 1	Pool (n=?)	[58]
[Pern v ?]	?	*E. coli*	(+)	IgE immunoblot and inhibition studies reported, but data not shown	?	[205] MA
Pers a 1 [Prs a 1]	–	*E. coli*	−	no immunological characterisation		[60]
Pers a 1 [Prs a 1]	–	yeast	++	IgE immunoblot	Pool (n=20?)	[79]
				IgE immunoblot inhibition	Pool (n=20?)	
Pru av 1 [Pru a 1]	–	*E. coli*	+++	IgE immunoblot	7	[115]
				IgE immunoblot inhibition	Pool (n=4)	[46,115]
				EAST	19; 7	[46,115]
				mediator release	3	[46]
				mediator release		[115]
Pru av 1 [Pru a 1]	his10	*E. coli*	+	IgE immunoblot	9; pool (n=4)	[46,115]
				IgE immunoblot inhibition	Pool (n=4)	[46,115]
Pru av 4 [Pru a 4]	–	*E. coli*	(+++)	IgE immunoblot reported, but data not shown	?	[53] MA
				EAST	13	
				Mediator release reported, but data not shown		
Pyr c 1	–	*E. coli*	+++	IgE immunoblot	23	[51,163]
				IgE immunoblot inhibition	1	[51]
				EAST, compared to extract	61	[51]
				Mediator release	5	[115,219]

Table 2. Continued

Recombinant food allergen [ ][b]	Applied as fusion protein	Expression cell	Level of characterisation	Assay	Number of sera assayed	Ref.
Pyr c 4	–	*E. coli*	+++	IgE immunoblot	23	[51,163]
				IgE immunoblot inhibition	1	[163]
				EAST reported, but data not shown	16	[53]
				EAST, compared to extract	61	[51]
				EAST inhibition	6	[163]
				Mediator release	5; ?	[51,53,163]
Pyr c 5	–	*E. coli*	+++	IgE immunoblot	23	[51,163]
				IgE immunoblot inhibition	1	[163]
				EAST, compared to extract	61; 36	[51,52]
				Mediator release	5	[51,163]
Sal s 1	his10	*E. coli*	++	IgE immunoblot	6	[107]
				ELISA	4	
Sin a 1	–	*E. coli*	++	IgE immunoblot	Pool (n=5)	[55]
				ELISA inhibition	Pool (n=5)	
Sin a 1	C-LYTA	*E. coli*	−	No IgE detection, only polyclonal rabbit anti-Sin a 1-serum		[111]
Sin a 1	GST	*E. coli*	+	IgE immunoblot	Pool (n=5)	[55]
[Wheat glutenins and gliadins]	–	*E. coli*	+	IgE immuno dotblot	10	[112]

[a] kDa, kiloDalton; fp, fusion peptide; his, histidine; β-gal, β-galactosidase; GST, glutathione-*S*-transferase; C-LYTA, C-terminal domain of *N*-acetylmuramoyl-L-alanine amidase of *Streptococcus pneumonia*; MA, references are only meeting abstracts.

[ ][b] Food allergen is not noted in the official WHO/IUIS list of allergens or is denominated differently by the authors in the cited reference.

only shown to bind specific IgE, but also to elicit mediator release and therefore being able to cause allergic/anaphylactic reactions it has been classified as ''very well characterised +++''. When there was poor information about methods and results or when the references were only meeting abstracts, the level of characterisation has been put in parenthesis and ''MA'' (meeting abstract) has been added to the reference number in the table. When the name of the recombinant food allergen given by the author differed from that in the official list of allergens of the WHO/IUIS allergen nomenclature subcommittee [40] or when the ''food allergen'' was so far not accepted as allergen by the subcommittee, it has been given in square brackets. When a recombinant food allergen was subjected to immunological assays as a fusion protein, the fusion part is mentioned in column 2.

To date (February 2000), more than 40 different food allergens from 23 foods have been cloned, of which no fewer than seven are peanut allergens. The immunological characterisation of nine recombinant food allergens (Api g 2, Api g 3, Sal s 1, Pru av 4, Ara h 1 of Burks et al. [63], one walnut allergen, carp parvalbumin, three mollusc tropomyosin and two allergens in soybean) has only been reported but not shown, as indicated by parentheses in Table 2. The remaining 39 recombinant food allergens have a level of characterisation as follows: four are ''insufficiently characterised'', 16 ''sufficiently +'', 12 ''well ++'' and seven are ''very well characterised +++''.

An example of a ''very well characterised'' recombinant food allergen is the cherry allergen rPru av 1. It bound IgE in sera of patients with oral symptoms after eating fresh cherries in immunoblot, immunoblot inhibition and the EAST test, indicating that rPru av 1 and nPru av 1 share identical epitopes [46,115]. Recombinant Pru av 1 was positive in the EAST test of 17/18 sera positive for cherry extract and elicited histamine release from basophils. In IgE immunoblotting with cherry extract and rPru av 1, all five sera of allergic individuals had IgE against rPru av 1 and 4/5 sera had IgE against the Pru av 1 band in the cherry extract. IgE of one patient bound rPru av 1 but not nPru av 1 in extract, possibly because there was not enough nPru av 1 in the blotted extract to be detected [46].

Three cloned isoforms of the apple allergen Mal d 1, differing in several amino acids, bound IgE in immunoblots similar to nMal d 1. In the EAST test with 13 nMal d 1-positive sera, two rMal d 1 isoforms were recognised by IgE from all 13 sera and one rMal d 1 isoform by IgE from 11/13 sera. However, in mediator release assays all three rMal d 1 were different, with one being as reactive as the natural allergen and two having a lower biological activity [10,115].

Another recombinant food allergen which showed allergenic activity, is rApi g 1 from celery. It bound to all 10 sera of celery-allergic patients in immunoblotting and to 24/30 in the EAST test. The 10 sera were proven positive with celery extract before [43,114]. Furthermore rApi g 1 elicited mediator release in vitro and in vivo [120,121]. Three other celery allergens, Api g 2, Api g 3 and Api g 4, were cloned. Significant IgE-binding activity was shown for rApi g 4 [50]; for rApi g 2 and rApi g 3, IgE-binding properties remain to be investigated [60].

An example for a ''sufficiently characterised'' recombinant food allergen is β-lactoglobulin (named Bos d 5) an allergen in cow's milk. Studies with the rBos d 5 cloned in *E. coli* revealed that rBos d 5 possessed at least one of two disulfide bonds present in the native form. Recombinant Bos d 5 had a similar conformation as nBos d 5 and performed similar IgE-binding activity in the ELISA test with five sera of milk-allergic individuals [90]. Bovine and the related ovine β-lactoglobulin were also expressed in different eukaryotic yeast cells (*Pichia pastoris*, *Saccharomyces cerevisiae*, *Kluveromyces lactis*) but their allergenic activity was not proven. The results of these studies, however, provided new information about the structure of β-lactoglobulin and the differences of prokaryotic and eukaryotic expression systems [1,2,77,78,122–124].

First attempts to produce the mustard allergen rSin a 1 fused to C-LYTA failed because it was impossible to harvest the recombinant fusion protein from *E. coli* lysate in free, soluble form [111]. By contrast, the expression of rSin a 1 as fusion protein with GST and its purification as soluble, non-fusion protein succeeded, although the yield of expression was very low [55]. The molecular structure of rSin a 1 differs from that of the natural molecule. While nSin a 1 consists of two polypeptide chains linked by two disulfide bonds [125], the recombinant Sin a 1 was constructed of the two polypeptide chains in line and linked together by a peptide fragment, representing the precursor molecule of the mature protein. Two monoclonal antibodies recognising conformational epitopes on nSin a 1 bound similarly to rSin a 1. Both nSin a 1 and rSin a 1 showed the same trypsin resistance, which is known to be a property highly dependent on conformation [55]. Therefore, it has been suggested that rSin a 1 and nSin a 1 share similar conformations. A monoclonal antibody specific for a linear epitope on nSin a 1 did not bind to rSin a 1, possibly due to three additional amino acids in the large chain region of rSin a 1, disrupting the linear epitope. However, changes in amino acid sequence by an additional peptide fragment and two additional amino acids at the N-terminus, causing a slight conformational difference in rSin a 1, seemed not to influence antibody-binding properties. Polyclonal rabbit IgG as well as human IgE of pooled sera bound identically to nSin a 1 and rSin a 1. However, the study lacked test sera from individual patients and a detailed specification of the serum pool [55].

The cloning of seven peanut allergens, rAra h 1–7, which is the highest number cloned from a single allergen source, has been reported. However, no other tests apart from IgE immunoblots were performed to show their allergenic activity [62–66,76,104,105,116,126].

Most of the available recombinant food allergens have been expressed as fusion proteins. Hence, it is of interest to know whether the attached fusion peptide influenced the allergenic properties of the recombinant allergens. In case of rPru av 1, the tag including 10 histidine residues seemed not to change the IgE-binding capacity of rPru av 1 [115]. The rPru av 1-his-tag fusion protein showed a molecular mass which was about 3–4 kDa greater than that of Pru av 1 (18–19 kDa) [46,115]. In comparison to the his-tag, GST has a size of about 26 kDa. Thus, in the case of rSin a 1 (18,8 kDa) and rJug r 1 (15–16 kDa), the fusion peptide alone has a higher mass than the allergen. However, the IgE-binding features of the recombinant allergens appeared to be unaffected [55,70].

Summarising these data, the vast majority of

recombinant food allergens was only characterised by the IgE immunoblotting technique and showed a considerable IgE reactivity. Further studies should investigate the IgE-binding activity of the recombinant food allergens by a panel of different in vitro tests and in comparison with their natural counterparts to gain more complete knowledge about their allergenic characteristics. Results from some single very well-characterised recombinant food allergens, which not only bound serum IgE but also cross-linked cell-bound IgE, were positive. They indicate that recombinant food allergens can constitute an immunologically similar copy of a food allergen.

3.2. Recombinant allergens as tools to characterise allergenic properties

3.2.1. Epitope mapping with recombinant fragments

Epitope mapping is a strategy to determine the location and sequence of epitopes of a protein. It can be applied to characterise B cell epitopes, i.e., the parts of the molecule that bind IgE, as well as T cell epitopes. For example, allergen fragments were screened for IgE binding in immunoblots or allergosorbent tests or, to map T cell epitopes, were used to stimulate T cells. Fragments were produced by (1) degradation of protein by enzymes such as trypsin [127], or (2) by synthesis of overlapping peptides of six to 20 amino acids, which map linear epitopes [57,65,66], or (3) as recombinant fragments. Recombinant fragments usually comprised longer parts of the allergens than synthetic peptides [48,58,106, 110,115,128]. For example, five recombinant fragments of Pru av 1 (60–120 amino acids long, spanning the whole allergen, overlapping and including a his-tag) had weak or no IgE-binding activity, indicating that binding to Pru av 1 depended on the structure of the whole molecule [115]. Epitope mapping of shrimp tropomyosin (Pen a 1) with four recombinant fragments of 13 and 20 amino acids and synthetic peptides revealed the location of IgE-binding epitopes at the C-terminus and the centre of the allergen [58,128]. One IgE-binding peptide was also characterised in tropomyosin of another variety of shrimp (*Penaeus indicus*) [127]. The epitopes are located in phylogenetically diverse and conserved parts of the tropomyosin molecule, showing that the characterisation of linear epitopes is important in tropomyosins [129]. For the soybean allergen profilin, studies with three recombinant fragments, not recognised by IgE in contrast to the whole recombinant allergen, revealed the existence of IgE-binding epitopes depending on the conformation of the allergen [48].

3.2.2. Hypoallergenic isoforms and mutants

Hypoallergenic isoforms or mutants of food allergens with reduced IgE reactivity but retained T cell reactivity are important tools to determine the sensitising characteristics of food allergens or to investigate the potential for a safe specific immunotherapy [30,130,131]. Differences in IgE reactivity were shown with three recombinant isoforms of the apple allergen Mal d 1. In the EAST test two rMal d 1 isoforms bound IgE to a similar degree as nMal d 1, whereas the third rMal d 1 isoform showed weaker activity [10]. Furthermore, mutants of Mal d 1 were generated with a single amino acid substitution of serine at position 111 by proline or cysteine (rMal d 1 [S111C,P]). The cysteine mutants showed a slight and the proline mutants a strong decrease of IgE-binding activity in the EAST test [10]. Also, corresponding recombinant mutants were constructed of the two homologous cherry and birch pollen allergens, rPru av 1 and rBet v 1, respectively. Position 111 in Mal d 1 is equivalent to position 112 in rBet v 1 and rPru av 1. Recombinant Pru av 1 cysteine and proline mutants both showed weaker IgE-binding activity than wild-type rPru av 1. The mutation of serine S112 to proline in rBet v 1 drastically reduced the binding of IgE, whereas the mutation of S112 to cysteine reduced it only slightly [115]. This indicates that the amino acid position 111/112 in these three allergens has a key function in IgE-binding activity and in maintaining the structure of a cross-reactive epitope. Two further mutants of rPru av 1 with an altered P-loop region, which comprises the residues 46–54, showed a partially reduced IgE-binding activity [115]. As the reduction of IgE binding in mutated rPru av 1 was not complete and the P-loop region in Bet v 1 was supposed to be an antibody-binding domain [132], it was assumed that the P-loop is part of an IgE-binding epitope of Bet v 1-related food allergens [115]. Additional studies with recombinant mutants could focus on the question whether the P-loop region, which is a highly conserved part of the

allergens of the Bet v 1 family such as Mal d 1, Pyr c 1, Dau c 1 and Bet v 1, forms a cross-reactive IgE epitope.

The construction of hypoallergenic recombinant mutants of peanut, apple and celery allergens was reported [104,105,126,131]. The substitution of six amino acids at the same positions of the two homologous allergens rApi g 1 and rMal d 1 reduced the IgE binding activity [131]. In case of rAra h 1, seven single amino acid substitutions within epitopes of non-conserved regions were generated and led to the reduction of the IgE-binding capacity [126]. Four single amino acid substitutions in immunodominant IgE epitopes of rAra h 2 altered the IgE-binding activity in comparison to the wild-type rAra h 2. In the majority of the tested sera IgE binding was reduced, in some sera it was unchanged and in one serum the binding of IgE to the mutant increased. However, it has been reported that the hypoallergenic rAra h 2 mutant had the same potency in T cell proliferation as the wild-type rAra h 2 [104,105]. This hypoallergenic peanut allergen mutant with retained T cell reactivity has properties which are supposed to be suitable for safe immunotherapy.

3.2.3. Cross-reactive structures

Cross-reactivity of allergen-specific antibodies is a well-known phenomenon in food allergy. It is caused by similar amino acid sequences and/or similar structures of related proteins that share common epitopes. Cross-reactive epitopes can occur on proteins from phylogenetically related or distant species, and on food and non-food allergens such as pollen, insects and latex. Recombinant food allergens are suitable to characterise the molecular basis of cross-reactivity.

Among the ''cross-reactive'' food allergies, the so-called pollen-related food allergies are the most important. Patients with pollinosis have a high risk of developing a related food allergy. In case of a birch pollen allergy, up to 70% of the patients are also allergic to fruits, vegetables or nuts. To date, three cross-reactive structures in pollens and food are known: (1) Bet v 1 homologues, (2) profilins and (3) cross-reactive carbohydrate determinants (CCD). Many proteins are homologues of the birch pollen allergen Bet v 1 [133]. They have a molecular mass between 15 and 18 kDa, are plant pathogenesis-related proteins, and are induced by stress. However, their function is as yet unclear. Recombinant food allergens of the **Bet v 1 family** are, e.g., rPru av 1 found in cherries with an amino acid sequence identity with Bet v 1 of 59% [46], various rMal d 1 isoforms from apple (56–60% amino acid sequence identity) [10,45], rApi g 1 from celery (41.6%) [43], rPyr c 1 from pear (62%) [49,52], rDau c 1 from carrot (38%) [44] and rCor a 1.0401 from hazelnut (67%) [51]. Cross-reactivities have been shown between rApi g 1 or rPru av 1 and rBet v 1, between rMal d 1 and rPru av 1, but not between rPru av 1 and rApi g 1 or between rMal d and rApi g 1. It has been assumed, that the different cross-reactive epitopes on rBet v 1 display two subsets, one being cross-reactive with fruits, the other with celery [115].

Cross-reactivity between the allergens of the Bet v 1 family is supposed to be due to their very similar three-dimensional structure. The circular dichroism (CD) spectra of rBet v 1 and rPru av 1 are highly superimposed, indicating that the secondary structure elements of the two allergens are nearly identical [115]. The three-dimensional structure of Bet v 1 was determined by nuclear magnetic resonance (NMR) spectroscopy and X-ray diffraction [36,38]. Additionally the conformation of Pru av 1 was partially analysed by NMR. In comparison, the tertiary structures of the two homologous allergens are highly similar and in corresponding areas they display the same shape charge distribution [37,115].

A second cross-reactive structure in pollen-related food allergies is profilin. Profilins are proteins of 12–15 kDa, involved in intracellular communication between the cell membrane and the cytoskeleton, participating in the regulation of actin polymerisation and signal transduction of the phosphatidylinositol pathway. As profilin occurs ubiquitously in all eukaryotic cells and is identified as an allergen in many pollens and foods, it is a pan-allergen and has to be considered as a potential allergen in almost all kinds of plants. Although of people with pollinosis fewer are affected by sensitisation to profilin than to Bet v 1 [134], profilin can cause food allergy to virtually all plants (reviewed in Refs. [135–138]). Food profilins have been cloned from celery (rApi g 4) [50], pear (rPyr c 4) [51], cherry (rPru av 4) [53] and soybean (rGly m 3) [48]. Celery profilin Api g 4 has an amino acid sequence identity of 80% to birch pollen profilin Bet v 2 [50]. Profilin is known to be a cross-reactive allergen in pollen-associated food

allergies and in the birch–mugwort–celery syndrome [139–141]. Scheurer et al. studied the cross-reactivity between purified rApi g 4, nApi g 4 and rBet v 2 by IgE inhibition experiments [50]. The results indicated that rApi g 4 and nApi g 4 share identical epitopes. IgE immunoblots showed complete inhibition of IgE binding to rApi g 4 after preincubation with celery extract and complete inhibition of nApi g 4 by rApi g 4. Also in the EAST, the binding of IgE to rApi g 4 was completely inhibited by celery extract. Inhibition experiments with the profilins rBet v 2 and rApi g 4 revealed that rBet v 2 and rApi g 4 had similar as well as different IgE epitopes. Differences in the IgE-binding epitopes of plant profilins of taxonomic families have been shown [142]. Cross-reactivity has been reported of the recombinant profilin of soybean, rGly m 3, and birch profilin, rBet v 2, by IgE-binding studies with sera of soybean-sensitive patients [48]. It is assumed that IgE reactivity of profilins strongly depends on the highly conserved conformational structure, rather than on amino acid sequence identity [143–145]. This is supported by the observation that only complete rGly m 3 and not it's recombinant fragments, which lack the tertiary structure of the natural protein, bound IgE [48]. By developing additional recombinant profilin allergens one could investigate the role of tertiary structure of profilins and their highly conserved regions for allergenic activity. Furthermore, the influence of the immune response to human ''self'' profilin on profilin-based food allergies may be studied in more detail [145].

Carbohydrate structures on glycoproteins are involved in cross-reactivity between foods and pollens. These IgE-binding epitopes are called **"cross-reactive carbohydrate determinants (CCD)"**. They are widely distributed in the plant kingdom and are present in invertebrates such as insects and molluscs. Not only can CCD mediate cross-reactions between pollens and plant foods, but also between bee venom and wheat flour, between bee venom and the plant protease bromelain and between plant foods and several spices [146–152]. So far, two IgE-binding epitopes on N-linked glycans in plants have been characterised [149,153,154]. The clinical relevance of CCD is not clear [155–158]. Bacterially produced recombinant food allergens are not suitable to investigate CCD as they do not contain carbohydrate side chains. Information on the influence of CCD on allergenicity of the allergen can only be obtained indirectly by comparing a glycoprotein allergen expressed in *E. coli* with the natural allergen or with the recombinant allergen expressed by yeast or insect cells [62,64,76].

In birch pollen, exotic fruits and other plant foods, cross-reactive minor allergens of about 35 kDa have been detected and characterised as **isoflavone reductase-like proteins (IRL)** [159–162]. It has been hypothesised that IRL constitute a fourth cross-reactive allergenic structure in pollen-related food allergies. The recombinant IRL protein from pear, rPyr c 5, was recognised by IgE of 8% of 61 patients' sera with pollen-related pear allergy [51,163].

Apart from the cross-reactivities between food and pollen, there are also cross-reactivities between shellfish and other animals, between food and latex, and among legumes. These are mediated by two more groups of allergenic proteins, the tropomyosins (5) and the chitinases (6). **Tropomyosins** are allergens in shellfish (crustaceans and molluscs) and are responsible for cross-reactivity between different species of crustaceans such as crab, shrimp and lobster [69,71]. Tropomyosins also cause cross-reactivity between crustaceans, molluscs and arthropods (house dust mite, cockroaches, chironomids, fruit fly and grass hoppers) [71,164–171]. In an IgE immunoblot, five recombinant crustacean tropomyosins of two shrimp species and two lobster species (rMet e 1 and rPen e 1 or rHum a 1 and rPan s 1, respectively) and one crab species (rCha f 1) bound IgE of sera containing IgE against crustacean extract, thereby demonstrating immunological similarity between naturally occurring and recombinant allergens [69,75,172]. Immunoblot inhibition tests revealed that recombinant lobster tropomyosin from the spiny lobster (rPan s 1) and the American lobster (rHom a 1) as well as recombinant shrimp tropomyosin rMet e 1 were cross-reactive [75]. Recombinant shrimp allergen rMet e 1 and lobster allergen rPan s 1 completely inhibited the binding of IgE to crab tropomyosin rCha f 1 in the immunoblot test [69]. Tropomyosins are a highly conserved protein family in muscle and non-muscle cells of vertebrates and invertebrates [173]. Different crustacean tropomyosins show a greater than 90% amino acid sequence identity. They have an identity of 66–69%

with fruit fly tropomyosin and of 46–60% with chicken tropomyosin [69,75]. Although vertebrates such as chicken, cow or pig contain tropomyosins, the allergenicity arising from the intake of such meats does not seem to be caused by tropomyosins [71,174]. Consequently, tropomyosin is regarded as a pan-allergen exclusively in invertebrates.

Class I **chitinases** are the sixth group of known cross-reactive proteins in food allergy. Up to now, they have been characterised in latex, avocado, banana and chestnut and they are assumed to be pan-allergens in the latex–fruit syndrome and other foods of plant origin [175–180]. Chitinases are proteins of the plant's defense mechanism against fungal pathogens and are overexpressed in transgenic crops [181]. Since one endochitinase has been identified as an allergen, the possibility of creating transgenic foods with an increased content of allergens should be taken into consideration. With the help of recombinant endochitinase from food sources, a quantitative and qualitative risk assessment would be possible. The recombinant major allergen rPers a 1 of avocado, an endochitinase, showed the same enzymatic activity as the natural Pers a 1 and inhibited fungal growth and its allergenic activity was comparable to that of nPers a 1 [79].

Extended research on the basis of recombinant cross-reactive allergens of food and non-food sources will provide further knowledge about the correlation between structure and allergenicity.

3.2.4. Diagnostic application of recombinant food allergens

Food extracts for in vitro and in vivo tests are not standardised for allergen content or biological activity. Their allergenic activity can decrease or even be lost during extraction procedures or storage and can differ between various manufacturers and even between different batches [13,15,18,21,121]. The allergen content of fruits can depend on the variety and the degree of ripeness [9–13]. For example, the content of the major apple allergen Mal d 1 in the strain ''Golden Delicious'' was 11-fold higher than in ''Gloster'' [10]. Therefore, the use of food extracts prepared from the ''wrong'' strain for in vitro and in vivo diagnosis of food allergies can lead to false negative results. Finally, a valid diagnosis strongly depends on the allergist's experience with the applied extracts. Diagnosis with recombinant food allergens which are of constant quality and known quantity could provide more sensitive, more specific and reproducible results in in vitro or in vivo tests. In the EAST test, two recombinant isoforms of the apple major allergen Mal d 1 were as sensitive as apple extract and they detected IgE in sera of all 13 apple-allergic patients. In the EAST classes, the rMal d 1 isoforms and apple extract corresponded in 9/13 or 10/13 sera, respectively, and the differences in the other three or four sera were at most one class [10,182]. Specific IgE against a single recombinant allergen can be detected in the majority of patients allergic to a certain food. The recombinant major allergen of celery, rApi g 1, bound IgE of 83% of 30 EAST-positive celery-allergic patients and the peanut major allergen, rAra h 2, bound IgE of 85% of 40 peanut-allergic patients [64,114]. In cases where food extracts are of poor quality and not suited for the detection of sensitisation, the use of recombinant food allergens as sensitive test material has to be taken into consideration. Of 13 patients with known pear allergy, one patient was not detected by the EAST test with pear extract, but all were detected with rPyr c 1 [49]. The recombinant hazelnut major allergen rCor a 1.0401 proved 41/43 hazelnut-allergic patients positive, whereas only 32/43 had a positive EAST test with extract from hazelnut [51]. These examples show that single recombinant food allergens can, in certain cases, replace extracts in in vitro tests or are even more sensitive than extracts. In 61 patients with pear allergy, 85% had a positive in vitro test with pear extract and 89% were positive with recombinant pear allergen rPyr c 1. This indicates that a first screening of patients' sera with rPyr c 1 instead of extract would be more sensitive and would at least determine a high percentage of the allergic patients. The number of false negative results may be reduced by including rPyr c 4 and rPyr c 5 as additional test allergens, which proved to be positive in 11 or 8% of the patients, respectively. Sera from non-allergic patients were not false-positive [51]. Possibly, a combination of single recombinant food allergens could be used as a test panel. A first hint has been given by studies with 40 sera of peanut-allergic patients, where all sera were positive in IgE immunoblots with a panel of six recombinant

peanut allergens [64]. To date, in vivo tests (skin tests) have been performed with some recombinant non-food allergens [183–188] and one recombinant food allergen, rApi g 1 [121,188]. A group of 24 celery-allergic patients from Switzerland with sensitization to nApi g 1 had a positive SPT to celery extract as well as to rApi g 1 and none of five controls were false-positive. The results indicated that rApi g 1 was a suitable tool for specific and sensitive skin tests [121].

A basic problem in the diagnosis of food allergies is the fact that positive serum tests and skin tests do not necessarily reflect a clinically relevant food allergy. They demonstrate the existence of food allergen-specific IgE or sensitised effector cells, but this does not inevitably correlate with symptoms when eating the specific food [7,16,17,189–192]. Conducting in vitro and in vivo tests with qualitatively and quantitatively standardised test material may provide more knowledge, whether this phenomenon depends on the test or the test material.

4. Conclusion

Food allergy can cause severe acute hypersensitivity reactions and fatal anaphylaxis [25,193–197]. The diagnosis is based on serological assays and skin tests with extracts prepared from the allergenic food [3]. As the test extracts are not standardised with regard to the content of allergens, and because the allergenic activity can be lost during the extraction procedure and storage, false positive as well as false negative results may occur [5,6,8,13,15–17,198]. On the other hand, it is very important for allergic patients to have precise information about their sensitisation pattern, because avoidance is the only management for food allergy [23,197].

Recombinant food allergens are of constant quality, pure and stable and therefore offer tools to standardise extracts, to replace extracts in diagnosis and to investigate the structural characteristics of allergens. Since recombinant allergens, cloned in vitro in expression systems (see Table 1), often are not identical with their natural counterpart it has to be carefully proven whether they are immunologically similar. We have reviewed the data of more than 40 recombinant food allergens cloned until now (February 2000) with respect to diagnosis, treatment and investigation of food allergy by recombinant food allergens.

The recombinant food allergens, mostly expressed in *E. coli,* showed considerable IgE-binding activity in vitro and several of them even displayed biological activity in mediator release assays (see Table 2). In comparative IgE-binding tests, natural and recombinant food allergens shared similar properties in many cases [10,43,49,90]. There are also examples for recombinant food allergens with an altered IgE reactivity compared to their natural homologues [10,62,63]. Thus, ongoing studies should follow to examine the allergenic properties of recombinant food allergens in different test systems. If the allergenic activity of recombinant food allergens in vitro would be concordant with that of their natural counterparts their application in skin tests could be taken into consideration. So far, one recombinant food allergen has been applied in skin tests [121]. The results confirm first experiences with recombinant non-food allergens which could replace extracts in skin tests [183–186,199]. In diagnostic in vitro assays, with a large number of participants, recombinant major food allergens were recognised by IgE from the majority of patients [51,64,114]. Tests with only one or three recombinant food allergens were more sensitive than with the corresponding food extract [51]. The possibility to establish precise, sensitive, specific and comparable diagnostic tests with recombinant food allergens or with extracts, standardised by recombinant food allergens, seems to be very promising. Yet, there is still a lack of knowledge about the various isoforms of allergens and their importance for the allergenic activity within allergenic source material [10,200–204].

Recombinant food allergens were applied as tools to investigate IgE cross-reactivity in related allergens such as profilins, tropomyosins, chitinases, isoflavone reductase-like proteins (IRL) and proteins of the Bet v 1 family [44,46,50,53,69,71,75,115, 131,205]. They indicated cross-reactivity within these protein families which are the molecular reason for concomitant allergy against different foods and non-foods. These proteins now allow to investigate the structural basis of IgE cross-reactivity between pollen and food and between cross-reactive foods

[37,115]. Moreover, recombinant food allergens facilitate generic approaches for studying the tertiary structure of allergens by spectroscopic methods [36,113,132].

Studies with recombinant fragments helped to recognise whether the IgE reactivity of a food allergen depended on the conformation or not and determined the location of linear epitopes on the allergen [48,58,106,110,115,128]. Recombinant isoforms and recombinant mutants of food allergens generated by site-directed mutagenesis showed the same or an altered IgE-binding capacity [10,104,105,115,131,206]. The location and the kind of mutations allowed conclusions about the molecular basis of the food protein's allergenicity. Identical mutations in homologous food and non-food allergens caused similar changes [10,30,115,131,201]. Recombinant isoforms, mutants or peptides with reduced IgE-binding activity and retained T cell-stimulatory capacity are candidates for a safe and specific immunotherapy against food allergy [33–35,117,130,131,206–208]. So far, immunotherapy is only efficacious with non-food allergens but not with food allergens. The nucleotide sequence of food allergens, obtained by the cloning procedure, can be applied for genetic immunisation with the cDNA of an allergen. This is a new approach for allergen-specific immunotherapy with the potential for treatment as well as for protecting from allergy [209–216].

In summary, recombinant allergens have strongly improved our knowledge about the properties of food allergens. In the future, they may help to solve current problems in diagnosis, treatment and investigation of the basic mechanisms of food allergies.

5. Nomenclature

E. coli	*Escherichia coli*
C-LYTA	C-terminal domain of *N*-acetylmuramoyl-L-alanine amidase of *Streptococcus pneumonia*
C-terminus	carboxyl terminus
DEAE	diethylaminoethyl
DTT	1,4-dithiothreitol
EAST	enzyme allergosorbent test
ELISA	enzyme-linked immunosorbent assay
FEIA	fluorescence enzyme immunoassay
kDa	kiloDalton
N-terminus	amino terminus
NTA	nitrilo triacetic acid
RAST	radioallergosorbent test
SPT	skin prick test
WHO/IUIS	World Health Organization/International Union of Immunological Societies

References

[1] M. Totsuka, Y. Katakura, M. Shimizu, I. Kumagai, K. Miura, S. Kaminogawa, Agric. Biol. Chem. 54 (1990) 3111.
[2] C.A. Batt, L.D. Rabson, D.W. Wong, J.E. Kinsella, Agric. Biol. Chem. 54 (1990) 949.
[3] A.J. Frew, in: A.B. Kay (Ed.), Allergy and Allergic Diseases, Blackwell Science, Oxford, 1997, p. 1007.
[4] K.C. Bergmann, H. Müsken, J. Ring, Allergo J., Sonderdruck aus 10/92-12/92-1/93-2/93-4/93 (1993) 1.
[5] AAAAI, J. Allergy Clin. Immunol. 99 (1997) 583.
[6] C.A. Bruijnzeel-Koomen, C. Ortolani, K. Aas, C. Bindslev-Jensen, B. Björkstén, D. Moneret-Vautrin, B. Wüthrich, Allergy 50 (1995) 623.
[7] C. Ortolani, M. Ispano, E.A. Pastorello, R. Ansaloni, G.C. Magri, J. Allergy Clin. Immunol. 83 (1989) 683.
[8] H.A. Sampson, J. Allergy Clin. Immunol. 82 (1988) 718.
[9] S. Vieths, H. Aulepp, B. Schöning, R. Tschirnich, Allergologie 18 (1995) 89.
[10] D.Y. Son, S. Scheurer, D. Haustein, S. Vieths, Eur. J. Nutr. 38 (1999) 201.
[11] S. Vieths, B. Schöning, A. Jankiewicz, Food Agric. Immunol. 5 (1993) 93.
[12] M.D. Chapman, P.W. Heymann, S.R. Wilkins, M.J. Brown, T.A.E. Platts-Mills, J. Allergy Clin. Immunol. 80 (1987) 184.
[13] S. Vieths, A. Hoffmann, T. Holzhauser, U. Müller, J. Reindl, D. Haustein, Allergy 53 (1998) 65.
[14] M. Fernández-Rivas, M. Cuevas, Clin. Exp. Allergy 29 (1999) 1239.
[15] J. Bernhisel-Broadbent, D. Strause, H.A. Sampson, J. Allergy Clin. Immunol. 90 (1992) 622.
[16] A. Norgaard, C. Bindslev-Jensen, P.S. Skov, L.K. Poulsen, Allergy 50 (1995) 636.
[17] A. Norgaard, P.S. Skov, C. Bindslev-Jensen, Clin. Exp. Allergy 22 (1992) 940.
[18] B. Björkstén, L. Halmepuro, M. Hannuksela, A. Lahti, Allergy 35 (1980) 671.
[19] C. Deluze, S. Dunoyer-Geindre, J.P. Girard, J. Invest. Allergol. Clin. Immunol. 1 (1991) 53.
[20] S. Vieths, A. Jankiewicz, B. Schöning, H. Aulepp, Allergy 49 (1994) 262.
[21] E. Varjonen, B. Björkstén, J. Savolainen, Clin. Exp. Allergy 26 (1996) 436.

[22] O. Rudeschko, B. Fahlbusch, M. Henzgen, G. Schlenvoigt, D. Herrmann, S. Vieths, L. Jäger, Allergy 50 (1995) 575.
[23] J.O'B. Hourihane, Clin. Exp. Allergy 27 (1997) 1240.
[24] J.W. Yunginger, K.G. Sweeney, W.Q. Sturner, L.A. Giannandrea, J.D. Teigland, M. Bray, P.A. Benson, J.A. York, L. Biedrzycki, D.L. Squillace, R.M. Helm, J. Am. Med. Assoc. 260 (1988) 1450.
[25] W. Burks, G.A. Bannon, S. Sicherer, H.A. Sampson, Int. Arch. Allergy Immunol. 119 (1999) 165.
[26] J.O'B. Hourihane, S.A. Kilburn, P. Dean, J.O. Warner, Clin. Exp. Allergy 27 (1997) 634.
[27] J.J. Oppenheimer, H.S. Nelson, S.A. Bock, F. Christensen, D.Y. Leung, J. Allergy Clin. Immunol. 90 (1992) 256.
[28] H.S. Nelson, J. Lahr, R. Rule, A. Bock, D. Leung, J. Allergy Clin. Immunol. 99 (1997) 744.
[29] R.J.J. van Neerven, C. Ebner, H. Yssel, M.L. Kapsenberg, J.R. Lamb, Immunol. Today 17 (1996) 526.
[30] F. Ferreira, C. Ebner, B. Kramer, G. Casari, P. Briza, A.J. Kungl, R. Grimm, B. Jahn-Schmid, H. Breiteneder, D. Kraft, M. Breitenbach, H.J. Rheinberger, O. Scheiner, FASEB J. 12 (1998) 231.
[31] G. Schramm, H. Kahlert, R. Suck, B. Weber, H.T. Stüwe, W.D. Müller, A. Bufe, W.M. Becker, M.W. Schlaak, L. Jäger, O. Cromwell, H. Fiebig, J. Immunol. 162 (1999) 2406.
[32] T. Okada, I. Swoboda, P.L. Bhalla, K. Toriyama, M.B. Singh, FEBS Lett. 434 (1998) 255.
[33] M. Yasue, T. Yokota, H. Okudaira, Y. Okumura, J. Allergy Clin. Immunol. 103 (1999) S141.
[34] J. Kauppinen, T. Zeiler, J. Rautiainen, M. Rytkönen-Nissinen, A. Taivainen, R. Mäntyjärvi, T. Virtanen, Clin. Exp. Allergy 29 (1999) 989.
[35] U. Wiedermann, S. Vrtala, B. Jahn-Schmid, K. Baier, R. Valenta, C. Ebner, D. Kraft, Int. Arch. Allergy Immunol. 119 (1999) 156.
[36] M. Gajhede, P. Osmark, F.M. Poulsen, H. Ipsen, J.N. Larsen, R.J.J. van Neerven, C. Schou, H. Løwenstein, M.D. Spangfort, Nat. Struct. Biol. 3 (1996) 1040.
[37] K. Schweimer, H. Sticht, J. Nerkamp, M. Boehm, M. Breitenbach, S. Vieths, P. Rösch, Appl. Magn. Reson. 17 (1900) 449.
[38] M.D. Spangfort, O. Mirza, J. Holm, J.N. Larsen, H. Ipsen, H. Løwenstein, Allergy 54 (1999) 23.
[39] J. Sambrook, E.F. Fritsch, T. Maniatis, in: Molecular Cloning — A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.
[40] T.P. King, D. Hoffman, H. Løwenstein, D.G. Marsh, T.A.E. Platts-Mills, W. Thomas, Int. Arch. Allergy Immunol. 105 (1994) 224.
[41] M.A. Frohman, Methods Enzymol. 218 (1993) 340.
[42] M.A. Frohman, in: M.A. Innis (Ed.), PCR Protocols: a Guide to Methods and Application, Academic Press, San Diego, CA, 1990, p. 28.
[43] H. Breiteneder, K. Hoffmann-Sommergruber, G. O'Riordain, M. Susani, H. Ahorn, C. Ebner, D. Kraft, O. Scheiner, Eur. J. Biochem. 233 (1995) 484.
[44] K. Hoffmann-Sommergruber, G. O'Riordain, H. Ahorn, C. Ebner, M. Laimer da Camara Machado, H. Pühringer, O. Scheiner, H. Breiteneder, Clin. Exp. Allergy 29 (1999) 840.
[45] M. Vanek-Krebitz, K. Hoffmann-Sommergruber, M. Laimer da Camara Machado, M. Susani, C. Ebner, D. Kraft, O. Scheiner, H. Breiteneder, Biochem. Biophys. Res. Commun. 214 (1995) 538.
[46] S. Scheurer, K. Metzner, D. Haustein, S. Vieths, Mol. Immunol. 34 (1997) 619.
[47] H.P. Rihs, P. Rozynek, K. May-Taube, B. Welticke, X. Baur, Int. Arch. Allergy Immunol. 105 (1994) 190.
[48] H.P. Rihs, Z. Chen, F. Ruëff, A. Petersen, P. Rozynek, H. Heimann, X. Baur, J. Allergy Clin. Immunol. 104 (1999) 1293.
[49] F. Karamloo, S. Scheurer, A. Wangorsch, S. May, D. Haustein, S. Vieths, J. Chromatogr. B (2001) this volume.
[50] S. Scheurer, A. Wangorsch, D. Haustein, S. Vieths, Clin. Exp. Allergy (2000) in press.
[51] S. Vieths, F. Karamloo, D. Lüttkopf, S. Scheurer, K. Fötisch, S. May, F. Altmann, P.S. Skov, D. Haustein, Arb. Paul Ehrlich Inst. Bundesamt Sera Impfstoffe Frankf.a.M. 92 (2000) in press.
[52] F. Karamloo, S. Scheurer, S. Franke, D. Haustein, S. Vieths, Allergo J. 8 (1999) 14.
[53] S. Scheurer, A. Wangorsch, D. Haustein, S. Vieths, J. Allergy Clin. Immunol. 105 (2000) S131.
[54] L. Menendez-Arias, I. Moneo, J. Dominguez, R. Rodríguez, Eur. J. Biochem. 177 (1988) 159.
[55] M.A. González De La Peña, R.I. Monsalve, E. Batanero, M. Villalba, R. Rodríguez, Eur. J. Biochem. 237 (1996) 827.
[56] P.S.C. Leung, H.C. Ka, K.C. Wing, A. Ansari, C.I. Bandea, S.K. Hoi, S.M. Nagy, M.E. Gershwin, J. Allergy Clin. Immunol. 94 (1994) 882.
[57] J.S. Stanley, N. King, A.W. Burks, S.K. Huang, H. Sampson, G. Cockrell, R.M. Helm, C.M. West, G.A. Bannon, Arch. Biochem. Biophys. 342 (1997) 244.
[58] G. Reese, B.J. Jeoung, C.B. Daul, S.B. Lehrer, Int. Arch. Allergy Immunol. 113 (1997) 240.
[59] S.S. Teuber, A.M. Dandekar, W.R. Peterson, S.L. Uratsu, J. Allergy Clin. Immunol. 97 (1996) 241.
[60] O. Scheiner, W. Aberer, C. Ebner, F. Ferreira, K. Hoffmann-Sommergruber, L.S. Hsieh, D. Kraft, S. Sowka, M. Vanek-Krebitz, H. Breiteneder, Int. Arch. Allergy Immunol. 113 (1997) 105.
[61] S.S. Teuber, K.C. Jarvis, A.M. Dandekar, W.R. Petersonn, A.A. Ansari, J. Allergy Clin. Immunol. 106 (1999) 1311.
[62] A.W. Burks, G. Cockrell, J.S. Stanley, R.M. Helm, G.A. Bannon, J. Clin. Invest. 96 (1995) 1715.
[63] A.W. Burks, G. Cockrell, J.S. Stanley, R.M. Helm, G.A. Bannon, Int. Arch. Allergy Immunol. 107 (1995) 248.
[64] T. Kleber-Janke, R. Crameri, U. Appenzeller, M. Schlaak, W.M. Becker, Int. Arch. Allergy Immunol. 119 (1999) 265.
[65] P. Rabjohn, E.M. Helm, J.S. Stanley, C.M. West, H.A. Sampson, A.W. Burks, G.A. Bannon, J. Clin. Invest. 103 (1999) 535.
[66] P. Rabjohn, A.W. Burks, H.A. Sampson, G.A. Bannon, J. Allergy Clin. Immunol. 103 (1999) S101.
[67] A.C. Jamieson, M.A. Vandeyar, Y.C. Kang, J.E. Kinsella, C.A. Batt, Gene 61 (1987) 85.
[68] A. Bugajska-Schretter, A. Pastore, L. Vangelista, H. Rumpold, R. Valenta, S. Spitzauer, Int. Arch. Allergy Immunol. 118 (1999) 306.

[69] P.S.C. Leung, Y.C. Chen, M.E. Gershwin, S.H. Wong, H.S. Kwan, K.H. Chu, J. Allergy Clin. Immunol. 102 (1998) 847.
[70] S.S. Teuber, A.M. Dandekar, W.R. Peterson, C.L. Sellers, J. Allergy Clin. Immunol. 101 (1998) 807.
[71] P.S.C. Leung, W.K. Chow, S. Duffey, H.S. Kwan, M.E. Gershwin, K.H. Chu, J. Allergy Clin. Immunol. 98 (1996) 954.
[72] C.D.V. Lindström, T. van Do, I. Hordvik, C. Endresen, S. Elsayed, Scand. J. Immunol. 44 (1996) 335.
[73] R. Crameri, M. Suter, Gene 160 (1995) 139.
[74] R. Crameri, K. Blaser, Int. Arch. Allergy Immunol. 110 (1996) 41.
[75] P.S.C. Leung, Y.C. Chen, D.L. Mykles, W.K. Chow, C.P. Li, K.H. Chu, Mol. Mar. Biol. Biotechnol. 7 (1998) 12.
[76] B. Maid, Dissertation, Philipps-Universität Marburg/Lahn, Germany, 1998.
[77] J.M. Chatel, K. Adel-Patient, C. Creminon, J.M. Wal, Protein Expr. Purif. 16 (1999) 70.
[78] T.R. Kim, Y. Goto, N. Hirota, K. Kuwata, H. Denton, S.Y. Wu, L. Sawyer, C.A. Batt, Protein Eng. 10 (1997) 1339.
[79] S. Sowka, L.S. Hsieh, M. Krebitz, A. Akasawa, B.M. Martin, D. Starrett, C.K. Peterbauer, O. Scheiner, H. Breiteneder, J. Biol. Chem. 273 (1998) 28091.
[80] A. Petersen, G. Schramm, M. Schlaak, W.M. Becker, Clin. Exp. Allergy 28 (1998) 315.
[81] P.M. Smith, C. Suphioglu, I.J. Griffith, K. Theriault, R.B. Knox, M.B. Singh, J. Allergy Clin. Immunol. 98 (1996) 331.
[82] A. Petersen, K. Grobe, B. Lindner, M. Schlaak, W.M. Becker, Electrophoresis 18 (1997) 819.
[83] L.S. Grinna, J.F. Tschopp, Yeast 5 (1989) 107.
[84] J.F. Tschopp, G. Sverlow, R. Kosson, W. Craig, L. Grinna, Biotechnology (NY) 5 (1987) 1305.
[85] M.A. Romanos, C.A. Scorer, J.J. Clare, Yeast 8 (1992) 423.
[86] P.E. Sudbery, Curr. Opin. Biotechnol. 7 (1996) 517.
[87] R. Verma, E. Boleti, A.J. George, J. Immunol. Methods 216 (1998) 165.
[88] D. Missiakas, S. Raina, J. Bacteriol. 179 (1997) 2465.
[89] A.M. Smith, M.D. Chapman, Mol. Immunol. 33 (1996) 399.
[90] J.M. Chatel, H. Bernard, G. Clement, Y. Frobert, C.A. Batt, J. Gavalchin, G. Peltres, J.M. Wal, Mol. Immunol. 33 (1996) 1113.
[91] L.D. Vailes, M.T. Kinter, L.K. Arruda, M.D. Chapman, J. Allergy Clin. Immunol. 101 (1998) 274.
[92] L. Buschmann, W.M. Becker, Forschungszentrum Karlsruhe Forschungsbericht FZKA-PUG22, 1996, p. 207.
[93] S.P. Zhang, G. Zubay, E. Goldman, Gene 105 (1991) 61.
[94] P.M. Schenk, S. Baumann, R. Mattes, H.H. Steinbiss, Biotechniques 19 (1995) 196.
[95] R.A. Spanjaard, J. van Duin, Proc. Natl. Acad. Sci. USA 85 (1988) 7967.
[96] W. Gao, S. Tyagi, F.R. Kramer, E. Goldman, Mol. Microbiol. 25 (1997) 707.
[97] R. Seetharam, R.A. Heeren, E.Y. Wong, S.R. Braford, B.K. Klein, S. Aykent, C.E. Kotts, K.J. Mathis, B.F. Bishop, M.J. Jennings, Biochem. Biophys. Res. Commun. 155 (1988) 518.
[98] A.H. Rosenberg, E. Goldman, J.J. Dunn, F.W. Studier, G. Zubay, J. Bacteriol. 175 (1993) 716.
[99] G.T. Chen, M. Inouye, Genes Dev. 8 (1994) 2641.
[100] C.R. Wood, M.A. Boss, T.P. Patel, J.S. Emtage, Nucleic Acids Res. 12 (1984) 3937.
[101] K. Sreekrishna, in: Strategies for Optimizing Protein Expression and Secretion in the Methylotrophic Yeast *Pichia pastoris*, American Society for Microbiology (ASM), Washington, DC, 1993.
[102] J.L. Bennetzen, B.D. Hall, J. Biol. Chem. 257 (1982) 3026.
[103] J.M. Cregg, T.S. Vedvick, W.C. Raschke, Biotechnology (NY) 11 (1993) 905.
[104] A.W. Burks, N. King, G.A. Bannon, Int. Arch. Allergy Immunol. 118 (1999) 313.
[105] N. King, S.J. Maleki, H. Sampson, A.W. Burks, G.A. Bannon, J. Allergy Clin. Immunol. 103 (1999) S67.
[106] T. Beardslee, M. Zeece, J. Markwell, G. Sarath, J. Allergy Clin. Immunol. 103 (1999) S104.
[107] T. van Do, I. Hordvik, C. Endresen, S. Elsayed, Scand. J. Immunol. 50 (1999) 619.
[108] D. Lüttkopf, S. Scheurer, D. Haustein, S. Vieths, Allergo J. 8 (1999) 27.
[109] H.P. Rihs, Z. Chen, F. Ruëff, A. Petersen, P. Rozynek, H. Sippel, X. Baur, Allergo J. 8 (1999) 14.
[110] T. Beardslee, M. Zeece, J. Markwell, G. Sarath, J. Allergy Clin. Immunol. 105 (2000) S136.
[111] M.A. González De La Peña, M. Villalba, J.L. García-López, R. Rodríguez, Biochem. Biophys. Res. Commun. 190 (1993) 648.
[112] N. Maruyama, K. Ichise, T. Katsube, T. Kishimoto, S. Kawase, Y. Matsumura, Y. Takeuchi, T. Sawada, S. Utsumi, Eur. J. Biochem. 255 (1998) 739.
[113] M. Boehm, P. Rösch, Biol. Chem. 378 (1997) 687.
[114] A. Jankiewicz, H. Aulepp, F. Altmann, K. Fötisch, S. Vieths, Allergo J. 7 (1998) 87.
[115] S. Scheurer, D.Y. Son, M. Boehm, F. Karamloo, S. Franke, A. Hoffmann, D. Haustein, S. Vieths, Mol. Immunol. 36 (1999) 155.
[116] T. Kleber-Janke, R. Crameri, M. Schlaak, W.M. Becker, Allergo J. 8 (1999) 14.
[117] R. Fritsch, B. Bohle, U. Vollmann, U. Wiedermann, B. Jahn-Schmid, M. Krebitz, H. Breiteneder, D. Kraft, C. Ebner, J. Allergy Clin. Immunol. 102 (1998) 679.
[118] D.Y. Son, S. Scheurer, D. Haustein, S. Vieths, Allergo J. 8 (1999) 14.
[119] B.A. Baldo, E.R. Tovey (Eds.), Protein Blotting: Methodology, Research, and Diagnostic Applications, Karger, Basel, 1989.
[120] K. Fötisch, F. Altmann, D. Haustein, S. Vieths, Int. Arch. Allergy Immunol. 120 (1999) 30.
[121] K. Hoffmann-Sommergruber, P. Demoly, R. Crameri, H. Breiteneder, C. Ebner, M. Laimer da Camara Machado, K. Blaser, C. Ismail, O. Scheiner, J. Bousquet, G. Menz, J. Allergy Clin. Immunol. 104 (1999) 478.
[122] Y. Cho, W. Gu, S. Watkins, S.P. Lee, T.R. Kim, J.W. Brady, C.A. Batt, Protein Eng. 7 (1994) 263.
[123] T.L. Rocha, G. Paterson, K. Crimmins, A. Boyd, L. Sawyer, L.A. Fothergill-Gilmore, Biochem. J. 313 (1996) 33.
[124] Y. Katakura, A. Ametani, M. Totsuka, S. Nagafuchi, S. Kaminogawa, Biochim. Biophys. Acta 1432 (1999) 302.

[125] L. Menéndez-Arias, R.I. Monsalve, J.G. Gavilanes, R. Rodríguez, Int. J. Biochem. 19 (1987) 899.
[126] D.S. Shin, C.M. Compadre, H.A. Sampson, A.W. Burks, G.A. Bannon, J. Allergy Clin. Immunol. 103 (1999) S99.
[127] K.N. Shanti, B.M. Martin, S. Nagpal, D.D. Metcalfe, P.V. Rao, J. Immunol. 151 (1993) 5354.
[128] G. Reese, R. Ayuso, T. Carle, S.B. Lehrer, Int. Arch. Allergy Immunol. 118 (1999) 300.
[129] R. Ayuso, G. Reese, S.B. Lehrer, J. Allergy Clin. Immunol. 103 (1999) S66.
[130] H. Fiebig, G. Schramm, H. Kahlert, R. Suck, B. Weber, H.T. Stüwe, W.D. Müller, A. Bufe, W.M. Becker, O. Cromwell, Int. Arch. Allergy Immunol. 119 (1999) 158.
[131] F. Ferreira, D. Hebenstreit, B. Kramer, M. Himly, H. Breiteneder, O. Scheiner, P. Briza, C. Ebner, M. Breitenbach, J. Allergy Clin. Immunol. 105 (2000) S137.
[132] M.D. Spangfort, M. Gajhede, P. Osmark, F.M. Poulsen, H. Ipsen, J.N. Larsen, R.J.J. van Neerven, C. Schou, H. Løwenstein, Int. Arch. Allergy Immunol. 113 (1997) 243.
[133] B. Schöning, S. Vieths, A. Petersen, W. Baltes, J. Sci. Food Agric. 67 (1995) 431.
[134] E. Jarolim, H. Rumpold, A.T. Endler, H. Ebner, M. Breitenbach, O. Scheiner, D. Kraft, Allergy 44 (1989) 385.
[135] R. Valenta, M. Duchêne, C. Ebner, P. Valent, C. Sillaber, P. Deviller, F. Ferreira, M. Tejkl, H. Edelmann, D. Kraft, J. Exp. Med. 175 (1992) 377.
[136] R. Valenta, M. Duchêne, S. Vrtala, P. Valent, C. Sillaber, F. Ferreira, M. Tejkl, R. Hirschwehr, C. Ebner, D. Kraft, O. Scheiner, Int. Arch. Allergy Immunol. 99 (1992) 271.
[137] S. Vieths, A. Jankiewicz, B. Schöning, D. Haustein, Allergo J. 5 (1996) 89.
[138] S. Vieths, A. Jankiewicz, B. Schöning, D. Haustein, Allergo J. 5 (1996) 157.
[139] R. van Ree, V. Voitenko, W.A. van Leeuwen, R.C. Aalberse, Int. Arch. Allergy Immunol. 98 (1992) 97.
[140] L. Bauer, C. Ebner, R. Hirschwehr, B. Wüthrich, C. Pichler, R. Fritsch, O. Scheiner, D. Kraft, Clin. Exp. Allergy 26 (1996) 1161.
[141] B. Wüthrich, J. Stäger, S.G.O. Johansson, Allergy 45 (1990) 566.
[142] A. Vallverdú, J.A. Asturias, M.C. Arilla, N. Gómez-Bayón, A. Martínez, J. Martínez, R. Palacios, J. Allergy Clin. Immunol. 101 (1998) 363.
[143] K.S. Thorn, H.E. Christensen, R. Shigeta, D. Huddler, L. Shalaby, U. Lindberg, N.H. Chua, C.E. Schutt, Structure 5 (1997) 19.
[144] S. Vrtala, P. Wiedemann, I. Mittermann, H.G. Eichler, W.R. Sperr, P. Valent, D. Kraft, R. Valenta, Biochem. Biophys. Res. Commun. 226 (1996) 42.
[145] R. Valenta, M. Duchêne, K. Pettenburger, C. Sillaber, P. Valent, P. Bettelheim, M. Breitenbach, H. Rumpold, D. Kraft, O. Scheiner, Science 253 (1991) 557.
[146] R.C. Aalberse, R. van Ree, in: B. Wüthrich, C. Ortolani (Eds.), Monographs in Allergy: Highlights in Food Allergy, Karger, Basel, 1996, p. 78.
[147] S. Vieths, M. Mayer, M. Baumgart, Food Agric. Immunol. 6 (1994) 453.
[148] R.C. Aalberse, R. van Ree, Clin. Rev. Allergy Immunol. 15 (1997) 375.
[149] V. Tretter, F. Altmann, V. Kubelka, L. März, W.M. Becker, Int. Arch. Allergy Immunol. 102 (1993) 259.
[150] R.C. Aalberse, V. Koshte, J.G. Clemens, J. Allergy Clin. Immunol. 68 (1981) 356.
[151] P.W. Broichmann, H. Kastner, K.J. Kalveram, Allergologie 15 (1992) 295.
[152] S. Vieths, Environ. Toxicol. Pharmacol. 4 (1997) 61.
[153] R. van Ree, J.H. Akkerdaas, M. Villalba, R. Rodríguez, S. Koppelman, R.C. Aalberse, L. Faye, P. Lerouge, Allergy 53 (1998) 30.
[154] I.B. Wilson, F. Altmann, Glycoconj. J. 15 (1998) 1055.
[155] R. van Ree, R.C. Aalberse, J. Allergy Clin. Immunol. 103 (1999) 1000.
[156] M.J. van der Veen, R. van Ree, R.C. Aalberse, J. Akkerdaas, S.J. Koppelman, H.M. Jansen, J.S. van der Zee, J. Allergy Clin. Immunol. 100 (1997) 327.
[157] A. Mari, P. Iacovacci, C. Afferni, B. Barletta, R. Tinghino, G. Di Felice, C. Pini, J. Allergy Clin. Immunol. 103 (1999) 1005.
[158] R.C. Aalberse, Allergy 53 (1998) 54.
[159] S. Vieths, E. Frank, S. Scheurer, H.E. Meyer, G. Hrazdina, D. Haustein, Scand. J. Immunol. 47 (1998) 263.
[160] A. Wellhausen, B. Schöning, A. Petersen, S. Vieths, Z. Ernährungswiss. 35 (1996) 348.
[161] F. Karamloo, N. Schmitz, S. Scheurer, K. Fötisch, A. Hoffmann, D. Haustein, S. Vieths, J. Allergy Clin. Immunol. 104 (1999) 991.
[162] F. Karamloo, N. Schmitz, S. Scheurer, K. Fötisch, A. Hoffmann, D. Haustein, S. Vieths, Allergo J. 8 (1999) 14.
[163] F. Karamloo, Dissertation, Johann Wolfgang Goethe-Universität Frankfurt am Main, Germany, 1999.
[164] T. Carrillo, F. Rodríguez de Castro, C. Blanco, R. Castillo, J. Quiralte, M. Cuevas, Ann. Allergy 73 (1994) 504.
[165] A.M. Witteman, J.H. Akkerdaas, J. van Leeuwen, J.S. van der Zee, R.C. Aalberse, Int. Arch. Allergy Immunol. 105 (1994) 56.
[166] C.E. O'Neil, R.P. Stankus, S.B. Lehrer, Ann. Allergy 55 (1985) 374.
[167] J.F. Crespo, C. Pascual, R.M. Helm, S. Sanchez-Pastor, I. Ojeda, L. Romualdo, M. Martín-Esteban, J.A. Ojeda, Allergy 50 (1995) 918.
[168] A. Martínez, J. Martínez, R. Palacios, R. Panzani, Allergol. Immunopathol. 25 (1997) 118.
[169] C.Y. Pascual, J.F. Crespo, S. San Martín, N. Ornia, N. Ortega, T. Caballero, M. Muñoz-Pereira, M. Martín-Esteban, Allergy 52 (1997) 514.
[170] N.E. Eriksson, B. Ryden, P. Jonsson, Allergy 44 (1989) 305.
[171] C.B. Daul, M. Slattery, G. Reese, S.B. Lehrer, Int. Arch. Allergy Immunol. 105 (1994) 49.
[172] M. Breitenbach, B. Simon, G. Probst, H. Oberkofler, F. Ferreira, P. Briza, G. Achatz, A. Unger, C. Ebner, D. Kraft, R. Hirschwehr, Int. Arch. Allergy Immunol. 113 (1997) 114.
[173] G. Reese, R. Ayuso, S.B. Lehrer, Int. Arch. Allergy Immunol. 119 (1999) 247.
[174] R. Ayuso, G. Reese, K. Tanaka, M.D. Ibanez, C. Pascual, A.W. Burks, G.L. Sussman, A.C. Dalton, C. Lahoud, M. Lopez, S.B. Lehrer, Ann. Allergy Asthma Immunol. 80 (1998) 102.

[175] C. Blanco, A. Díaz-Perales, C. Collada, R. Sánchez-Monge, C. Aragoncillo, R. Castillo, N. Ortega, M. Alvarez, T. Carrillo, G. Salcedo, J. Allergy Clin. Immunol. 103 (1999) 507.
[176] R. Sánchez-Monge, C. Blanco, A. Díaz-Perales, C. Collada, T. Carrillo, C. Aragoncillo, G. Salcedo, Clin. Exp. Allergy 29 (1999) 673.
[177] H. Alenius, S. Mäkinen-Kiljunen, M. Ahlroth, K. Turjanmaa, T. Reunala, T. Palosuo, Clin. Exp. Allergy 26 (1996) 341.
[178] A. Posch, C.H. Wheeler, Z. Chen, A. Flagge, M.J. Dunn, F. Papenfuss, M. Raulf-Heimsoth, X. Baur, Clin. Exp. Allergy 29 (1999) 667.
[179] H. Alenius, N. Kalkkinen, T. Reunala, K. Turjanmaa, T. Palosuo, J. Immunol. 156 (1996) 1618.
[180] Z. Chen, A. Posch, R. Cremer, M. Raulf-Heimsoth, X. Baur, J. Allergy Clin. Immunol. 102 (1998) 476.
[181] W. Lin, C.S. Anuratha, K. Datta, I. Potrykus, S. Muthukrishnan, S.K. Datta, Biotechnology (NY) 13 (1995) 686.
[182] D.Y. Son, Dissertation, Technische Universität Berlin, Germany, 1999.
[183] S. Hemmann, G. Menz, C. Ismail, K. Blaser, R. Crameri, J. Allergy Clin. Immunol. 104 (1999) 601.
[184] O. Arquint, A. Helbling, R. Crameri, F. Ferreira, M. Breitenbach, W.J. Pichler, J. Allergy Clin. Immunol. 104 (1999) 1239.
[185] G. Pauli, J.P. Oster, P. Deviller, S. Heiss, J.C. Bessot, M. Susani, F. Ferreira, D. Kraft, R. Valenta, J. Allergy Clin. Immunol. 97 (1996) 1100.
[186] L. Elfman, M. Svensson, J. Lidholm, G. Pauli, R. Valenta, Int. Arch. Allergy Immunol. 113 (1997) 249.
[187] S. Heiss, V. Mahler, R. Steiner, S. Spitzauer, C. Schweiger, D. Kraft, R. Valenta, J. Invest. Dermatol. 113 (1999) 830.
[188] P.P.O. Jorge, K.R.C. Tobias, V.P.L. Ferriani, A.M. Smith, M.D. Chapman, L.K. Arruda, J. Allergy Clin. Immunol. 105 (2000) S169.
[189] J. Bernhisel-Broadbent, S.M. Scanlon, H.A. Sampson, J. Allergy Clin. Immunol. 89 (1992) 730.
[190] J. Bernhisel-Broadbent, S. Taylor, H.A. Sampson, J. Allergy Clin. Immunol. 84 (1989) 701.
[191] H. Majamaa, P. Moisio, K. Holm, H. Kautiainen, K. Turjanmaa, Allergy 54 (1999) 346.
[192] P.A. Eigenmann, H.A. Sampson, Pediatr. Allergy Immunol. 9 (1998) 186.
[193] H.A. Sampson, Allergy 53 (1998) 125.
[194] M.W. Yocum, D.A. Khan, Mayo Clin. Proc. 69 (1994) 16.
[195] M. Crivellaro, P. Bonadonna, A. Dama, G.E. Senna, P. Mezzelani, G. Mistrello, G. Passalacqua, J. Invest. Allergol. Clin. Immunol. 9 (1999) 58.
[196] T. Foucard, I. Malmheden Yman, Allergy 54 (1999) 261.
[197] T. Mori, M. Yokoyama, N. Komiyama, M. Okano, K. Kino, Biochem. Biophys. Res. Commun. 263 (1999) 166.
[198] C. Ortolani, M. Ispano, R. Ansaloni, F. Rotondo, C. Incorvaia, E.A. Pastorello, Allergy 53 (1998) 58.
[199] G. Pauli, A. Purohit, J.P. Oster, F. De Blay, S. Vrtala, V. Niederberger, D. Kraft, R. Valenta, Int. Arch. Allergy Immunol. 118 (1999) 216.
[200] M.D. Spangfort, H. Ipsen, S.H. Sparholt, S. Aasmul-Olsen, P. Osmark, F.M. Poulsen, M. Larsen, E. Mortz, P. Roepstorff, J.N. Larsen, Adv. Exp. Med. Biol. 409 (1997) 251.
[201] F. Ferreira, K. Hirtenlehner, A. Jilek, J. Godnik-Cvar, H. Breiteneder, R. Grimm, K. Hoffmann-Sommergruber, O. Scheiner, D. Kraft, M. Breitenbach, H.J. Rheinberger, C. Ebner, J. Exp. Med. 183 (1996) 599.
[202] H. Breiteneder, F. Ferreira, K. Hoffmann-Sommergruber, C. Ebner, M. Breitenbach, H. Rumpold, D. Kraft, O. Scheiner, Eur. J. Biochem. 212 (1993) 355.
[203] G.A.J. Hakkaart, M.D. Chapman, R.C. Aalberse, R. van Ree, Clin. Exp. Allergy 28 (1998) 169.
[204] G. Schramm, A. Bufe, A. Petersen, M. Schlaak, W.M. Becker, Eur. J. Biochem. 252 (1998) 200.
[205] P.S.C. Leung, S.H. Wong, K.H. Chu, J. Allergy Clin. Immunol. 105 (2000) S140.
[206] S.J. Maleki, S.Y. Chung, E.T. Champagne, J. Allergy Clin. Immunol. 105 (2000) S138.
[207] S.B. Lehrer, L.G. Wild, K.L. Bost, R.U. Sorensen, Immunol. Allergy Clin. North Am. 19 (1999) 563.
[208] S.R. Durham, S.J. Till, J. Allergy Clin. Immunol. 102 (1998) 157.
[209] K. Roy, H.Q. Mao, S.K. Huang, K.W. Leong, Nat. Med. 5 (1999) 387.
[210] J.E. Slater, A. Colberg-Poley, Arb. Paul Ehrlich Inst. Bundesamt Sera Impfstoffe Frankf.a.M. 91 (1998) 230.
[211] X. Li, C.K. Huang, B.H. Schofield, A.W. Burks, G.A. Bannon, K.H. Kim, S.K. Huang, H.A. Sampson, J. Immunol. 162 (1999) 3045.
[212] C.H. Hsu, K.Y. Chua, M.H. Tao, Y.L. Lai, H.D. Wu, S.K. Huang, K.H. Hsieh, Nat. Med. 2 (1996) 540.
[213] D.J. Lee, H. Tighe, M. Corr, M. Roman, D.A. Carson, H.L. Spiegelberg, E. Raz, Int. Arch. Allergy Immunol. 113 (1997) 227.
[214] E. Raz, H. Tighe, Y. Sato, M. Corr, J.A. Dudler, M. Roman, S.L. Swain, H.L. Spiegelberg, D.A. Carson, Proc. Natl. Acad. Sci. USA 93 (1996) 5141.
[215] A. Hartl, J. Kiesslich, R. Weiss, A. Bernhaupt, S. Mostböck, S. Scheiblhofer, C. Edner, F. Ferreira, J. Thalhamer, J. Allergy Clin. Immunol. 103 (1999) 107.
[216] M. Roman, H.L. Spiegelberg, D. Broide, E. Raz, Springer Semin. Immunopathol. 19 (1997) 223.
[217] A. Bugajska-Schretter, H. Rumpold, R. Valenta, S. Spitzauer, Allergy 53 (1998) 103.
[218] D.L. Mykles, J.L. Cotton, H. Taniguchi, K. Sano, Y. Maeda, J. Muscle Res. Cell Motil. 19 (1998) 105.
[219] P. Bradding, Clin. Exp. Allergy 29 (1999) 1445.