ELSEVIER

Journal of Chromatography B, 756 (2001) 33–40

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Review

What establishes a protein as an allergen?

Reinhard Bredehorst*, Kerstin David[1]

Institute of Biochemistry and Food Chemistry, University of Hamburg, Martin-Luther-King Platz 6, D-20146 Hamburg, Germany

Received 11 May 2000; accepted 9 November 2000

Abstract

There is little known about the factors that determine the allergenicity of food proteins. Apparently, the ability of a food protein to induce an allergic response requires its presence in substantial amounts in the food supply, its durability during food processing, and its resistance to digestion in the gastrointestinal tract. In addition to the mode and degree of exposure, structural characteristics appear to play an important role for the capacity of a protein to modulate the immune response towards allergic reactions. Until now, however, there has been no indication for common structural characteristics of linear T cell or linear IgE (B cell) epitopes and the knowledge of structural characteristics of conformational IgE binding sites is very limited. Experimental data point only to certain surface areas of allergenic proteins which are important for IgE binding. Therefore, it is not possible to suggest any structural motif or conformational sequence pattern common to all allergenic proteins. Furthermore, glycosylation appears not to be a common critical determinant of allergenicity since food allergens comprise both glycoproteins and nonglycosylated proteins. Based on the few published three-dimensional structures of allergenic proteins including food proteins, one unifying feature of allergens appears to be their spherical shape. The three-dimensional structures of many more allergens have to be determined, however, to allow for a better understanding of the molecular basis of allergenicity. Most recently, new ideas have been introduced as to why certain biochemical or biologic functions such as enzymatic activities may predispose a protein to become an allergen. Proteolytically active allergens have been demonstrated to irritate the human mucosal surface, to enhance their own transmucosal uptake, and to augment IgE production. Therefore, the functional activity of some allergens may play a role among other factors in the process of sensitization and allergic responses.

Keywords: Food allergy; Proteins

Contents

*Corresponding author.
E-mail address: rbredehorst@chemie.uni-hamburg.de (R. Bredehorst).
[1]Currently at the Cancer Research Center of Hawaii, University of Hawaii at Manoa, 1236 Lauhala Street, Honolulu, HI 96813, USA.

0378-4347/01/$ – see front matter
PII: S0378-4347(01)00069-X

1. Introduction

Allergies arise in response to certain proteins, termed allergens, capable of triggering immediate (type I) hypersensitivity reactions. The development of allergy is a multistep process, and the mechanisms leading to sensitization, production of IgE antibodies, and allergic diseases are complex and not fully understood. However, the key step to a specific allergic reaction is the binding of at least two IgE antibody molecules to a multivalent allergenic protein. If the allergen–IgE complex is bound to high affinity IgE receptors (FcϵR I) present on mast cells and basophils, and at least two IgE receptors are crosslinked by this event, mediator release is induced triggering marked allergic inflammatory responses [1]. The synthesis of IgE antibodies by plasma cells is regulated by multifactorial mechanisms which are different from the mechanisms controlling IgG antibodies. For example, adjuvants containing *Bordetella pertussis* proteins are known to have the ability of enhancing IgE response, whereas complete Freund's adjuvant is known to enhance IgG response and to suppress IgE response in animals [2]. Infestation with intestinal parasites causes both in man and in animals extremely high serum levels of total IgE and specific IgE antibodies. Most human individuals with past or present helminthic infestation react with IgE production and positive skin test wealing to extracts of the parasite, independent of whether they are otherwise allergic or non-allergic [3]. On the other hand, bacterial and viral infections are not followed by an increase of total serum IgE levels as is found following exposure to known allergens. An interpretation of these puzzling observations is further complicated by the fact that only a limited number of the numerous proteins in a given allergenic material act as allergens.

The question naturally arises as to why certain foreign proteins act as allergens while other foreign proteins present in the same allergenic material do not. The extensive experimental work that has been devoted in efforts to answer this urging question, points to several characteristics of allergenic proteins. First, allergens are foreign proteins or glycoproteins with a molecular mass usually ranging between 5000 and 70 000 [4]. Furthermore, a particular foreign protein must be present in substantial amounts, and preferably over prolonged periods, in the patients' environment or food in order to become an allergen. For example, Norwegians are big fish-eaters, and codfish is prepared in many places in the fish industry. Consequently, fish allergy is frequent in Norway, but does not occur among the beef-eating inhabitants of Texas. Another factor that determines the allergenic potential of such proteins appears to be the ease with which foreign proteins reach the mucosa. For example, the lack of allergies against pine pollen in the Scandinavian countries is explained by the structure of the pollen grain. The pine pollen proteins are encased in a tough cellulose layer which is resistant to the enzymes of the human respiratory passages. As a result, no antigenic material comes in contact with the mucosa. These examples, however, do not provide an explanation for the observation that allergy to fish is more common than allergy to meat, even in families eating more meat than fish. Therefore, in addition to the mode and degree of exposure, structural characteristics appear to play an important role for the capacity of foreign proteins to modulate the immune response towards allergic reactions.

In this paper we want to review recent findings in the field of structural analysis of allergens and discuss the significance of this knowledge for the understanding of the molecular basis of allergenicity. As the scope of this book is the review of food allergies, the focus will be primarily on food allergens.

2. Analysis of IgE (or B cell) epitopes

Given that allergen-specific IgE plays such a

critical role in the etiology of allergic disease, determination of allergen-specific IgE-binding epitopes (or B cell epitopes) known to be either linear (continuous, sequential) or conformational (discontinuous) appears to be of crucial importance for a better understanding of the allergenic nature of proteins.

2.1. Analysis of linear IgE epitopes

By generating synthetic, overlapping peptides representing the entire primary sequence of a given allergenic protein, multiple distinct linear IgE recognition sites have been identified for a variety of food allergens including those from cow's milk [5–7], codfish [8], soybean [9], shrimp [10], peanuts [11–13] and other sources. All analyzed allergens contain multiple linear IgE binding sites which probably reflects the polyclonal nature of the immune response and may be a necessary step in establishing a protein as an allergen. It should be kept in mind, however, that the use of overlapping peptides can lead to confusion since linear epitopes may in fact correspond to fragmented parts of large conformational (discontinuous) ones.

There are several lines of evidence that linear epitopes play an important role as IgE binding sites. For example, serum IgE recognition of the two major peanut allergens Ara h 1 (63 500) and Ara h 2 (17 000) appears to be due primarily to amino acids comprising linear epitopes in the absence of carbohydrates [11,12]. Mutational analysis of these immunodominant epitopes revealed that IgE binding could be abolished by single amino acid mutations within each epitope [11,12]. The structural analysis of a third peanut allergen, the minor Ara h 3 allergen, revealed the presence of four epitopes, between 10 and 15 amino acids in length, with no obvious shared sequence motif [13]. Mutational analysis of these epitopes demonstrated also that single amino acid exchanges within these peptides could lead to a reduction or loss of IgE binding [13]. The IgE binding sites of ovalbumin (Gal d 1), the most dominant of five major allergens of egg white, were also shown to be mainly determined by the primary structure and to be dependent on a certain peptide length [4]. Treatment with trypsin did not abolish the allergenic reactivities of ovalbumin, whereas its allergenic character was destroyed by limited pepsin hydrolysis. These data are in accordance with the observations that thermal denaturation of ovalbumin had no substantial effect on its allergenic properties and that the four fractions of ovalbumin obtained by cyanogen bromide cleavage retained their IgE reactivity [4].

To date there has been no indication for a common structural character of linear IgE epitopes. However, analysis of the major allergen of cod fish, designated Allergen M (Gad c 1 or parvalbumin p*I* 4.75), revealed an interesting feature of one IgE binding site [4]. Among the several epitopes on Allergen M recognized by IgE antibodies, the immunological reactivity of the region 41–64 of Allergen M was shown to be determined by the three tetrapeptides DELK, DEDK, and DELK, interspaced by six amino acid residues in a segment of twenty-four residues. The reactivity of these peptides was independent of the two spacer arms. The authors concluded from their studies that the four amino acid peptides interspaced by six unrelated amino acid residues represent a minimal requirement of an allergenic epitope. However, these structural characteristics appear to be a unique feature of Allergen M.

2.2. Analysis of conformational B cell epitopes

For several allergens it has been demonstrated that effective IgE binding depends on their three-dimensional structural integrity. For example, dissolution of a disulfide bond of Der f 2, a major allergen of mites, by mutation of two amino acid residues resulted in a complete loss of IgE binding activity [14]. Furthermore, there are many examples of allergenicity being reduced by heat-induced unfolding of native allergens leading to the destruction of conformational epitopes [15]. For example, heat treatment reduced the allergenic activity of egg white to the extent that many (but not all) patients who were allergic to freeze-dried egg white could tolerate the thermally processed material [16]. However, heat-denatured allergens can also present new allergenic sites, uncovered by the heat-induced unfolding process or created by partial refolding processes after heat denaturation and/or new chemical reactions with other molecules present in the allergenic material [15]. For example, heat-denatured β-lactoglobulin was shown to form a stable complex with heat-denatured α-lactalbumin [17] and to present at

least one new conformational epitope which is not found in native β-lactoglobulin [18].

Despite the well documented importance of conformational epitopes for efficient IgE binding, the knowledge of structural characteristics of conformational IgE binding sites is very limited and it is not possible to suggest any structural motif or conformational sequence pattern common to all allergenic proteins. Data obtained from peptide studies and site-directed mutagenesis experiments point only to certain surface areas of allergenic proteins which are important for IgE binding. For example, the presence of important conformational IgE epitopes has been demonstrated on N- and C-terminal fragments of birch pollen profilin Bet v 2 [19], on the C-terminal part of bovine dander allergen Bos d 2 [20], and on two small areas located on opposites faces of the house dust mite allergen Der f 2 [21]. One of the latter areas is composed of polar or charged residues and the other of more hydrophobic residues [21].

2.3. Role of carbohydrate in allergenicity

The majority of food allergens are glycoproteins [22] and the carbohydrate structures of plant food allergens in particular are known to represent important IgE epitopes [23–27]. For example, IgE from wheat- or barley-allergic patients recognized an endo-Lys C peptide containing an N-linked oligosaccharide with a β1,2-linked xylose attached to the β-linked mannose of the core structure. This recognition was lost upon deglycosylation [27]. On the other hand, data of recent structural studies suggest that glycosylation is not a common critical determinant of allergenicity. The lipocalin protein family, for example, includes several animal allergens some of which are not glycosylated [28].

3. IgE epitopes of major and minor allergens

A given allergenic material usually contains only one or a very few allergenic proteins which act as denominators of the allergic reaction in the majority (more than 50%) of patients allergic to the matter (termed ‘major allergens’), while the same allergenic material may contain other allergenic proteins which elicit allergic reactions only in a minor fraction (less than 50%) of patients allergic to the matter (termed ‘minor allergens’). Furthermore, the definition of major and minor allergens depends on the population of allergic individuals. For example, Api g 1 represents a major allergen in celery-allergic patients from Switzerland, whereas celery-allergic patients from Southern France are rarely sensitized to it [29].

Although the molecular basis of major and minor allergens remains to be elucidated, several theories are discussed. According to one theory, varying levels of sequence similarity among allergens from different species may be responsible for the existence of major and minor allergens. The percentage of patients with allergen-specific IgE may depend on unique sequences not conserved between protein families of different species. For example, the two peanut (*Arachis hypogaea*) proteins Ara h 1 and Ara h 2 represent major allergens since they are recognized by >90% of peanut-allergic patients [11,12], whereas the peanut protein Ara h 3 is recognized only by a fraction of the patient population and, therefore, has been defined as minor allergen [13]. All three allergens are seed storage proteins and share similar functional properties. However, the level of sequence similarity retained between these proteins from different legumes varies significantly. Ara h 3 exhibits a higher sequence identity (62–72%) with legume storage proteins from soybean and pea than Ara h 1 with vicilins (40%) or Ara h 2 with conglutinins (39%) [11,12]. This could account for the lower percentage of peanut-allergic patients with IgE to Ara h 3 and the smaller number of linear IgE-binding regions found within this allergen. It should be mentioned, however, that allergens belonging to the lipid transfer protein (LTP) family contradict this theory. Although these allergens exhibit a high level of sequence identity (60–94%) with other proteins from the LTP family, they act as major allergens [30,31].

The complex structure of Ara h 3, an 11S storage protein, may provide another possible explanation for the fact that this protein is recognized only by a portion of the patient population. 11S Storage proteins are initially synthesized as 60 000 preproglobulins consisting of covalently linked acidic and basic polypeptides which are cleaved by an asparagine-dependent endopeptidase and then linked

by a disulfide bridge [32,33]. Thereafter, 11S storage proteins are assembled into their mature form as hexameric oligomers consisting of six similar subunits [34]. The ability of 11S storage proteins to oligomerize into higher-order structures may create mainly conformational IgE-binding sites which are likely to be altered during food processing. According to this theory, the allergenic potency of Ara h 3 would be higher in peanuts prior to the processing procedure which remains to be investigated.

4. Analysis of T cell epitopes

Allergens are presented to CD4+ T helper cells by antigen presenting cells (APC). CD4+ T helper cells are activated following the recognition of peptide fragments of the allergen occupying the peptide-binding site of major histocompatibility complex (MHC) class II molecules [35,36]. The immunological milieu in patients producing specific IgE antibodies is characterized by a predominance of allergen-specific CD4+ TH2 cells and the cytokines produced by TH2 cells are central in the regulation of allergic inflammatory responses [37–39]. Although limited in number, several food allergen-specific TH2 cells have been cloned from patients allergic to peanuts [40,41], milk [42], egg [43] and apples [44].

Molecular cloning techniques and the knowledge of amino acid sequences of allergens made it possible to characterize T helper cell epitopes of different allergens [45]. Using allergen-specific T cell clones obtained by density centrifugation of allergen-stimulated peripheral blood mononuclear cells from allergic patients, T cell epitopes were identified with overlapping peptides (e.g. three amino acids overlapping dodecapeptides) representing the entire primary sequence of the allergenic protein. The peptides were assayed for their ability to stimulate proliferation of the T cell clones and the production of a TH2-like cytokine pattern. The data show that each allergen molecule displays a large array of T cell epitopes distributed along the entire polypeptide chain [22,46–49]. However, none of the identified T cell epitopes displays a sequence common to all allergic proteins.

5. Cross-reactive IgE and T cell epitopes

Elucidation of the primary structures of allergens and characterization of their linear IgE binding epitopes has shown that cross-reactivity between related allergenic proteins is structurally based. For example, cross-reactivity has been demonstrated for birch pollen with apples, nuts, pears, carrots, potatoes and kiwi fruit [50–54] as well as for mugwort with celery, fennel, parsley and spices [55–57]. Recent studies have demonstrated that cross-reactive epitopes present in pollen and plant food are responsible for this phenomenon. For example, the major birch pollen allergen Bet v 1 shows a high degree of sequence homology with several food allergens including Mal d 1, the major allergen from apple [58] and Api g 1, the major allergen of celery [59]. Bet v 2, a profilin from birch [60], exhibits also a high degree of sequence homology with profilins from other plants including wheat [61], maize [62] and beans [63]. Profilins represent actin-binding and phosphoinositide (PIP)-binding proteins in eukaryotes with a high cross-reactive allergenic potential [64,65]. A variety of structurally related plant allergens with significant sequence homology have been identified recently which display a similar average molecular mass and are capable of inducing a battery of cross-reactive IgE antibodies [66].

The cross-reactivity between structurally related allergens occurs also at the cellular level as demonstrated by recent studies [44,67]. For example, Bet v 1-specific TH2 cell clones displaying reactivity with the epitope Bet v 1 139–156, recognized the major apple allergen Mal d 1 at the corresponding position [44]. Vice versa, Mal d 1-specific TH2 cell clones displaying reactivity with the epitope Mal d 1 141–155, reacted also with the corresponding Bet v 1 peptide [44].

6. Analysis of three-dimensional allergen structures

In a different approach to probing the molecular basis of allergenicity, the three-dimensional structures of some allergens have been determined during the past few years by using X-ray diffraction or NMR. Examples include plant allergens such as the

birch pollen allergens Bet v 1 [68] and Bet v 2 [19], the mouse ear cress allergen Ara t [69], and the timothy grass pollen allergen Phl p 2 [70], as well as animal allergens such as the house dust mite allergen Der f 2 [71], the mouse urine allergen Mus m 1 [72], the bovine milk allergen Bos d 5 (β-lactoglobulin) [73], and the bovine dander allergen Bos d 2 [28].

One unifying feature of these allergens is the similarity of their shape and dimensions. The shortest dimension for these molecules is on average 3.4 nm and the longest dimension 4.4 nm which gives them a more spherical than elliptical shape [28]. Comparison of their secondary structures revealed that they are composed mainly of β-structures. For example, the major birch pollen allergen Bet v 1 contains a seven-stranded anti-parallel β-sheet wrapping around a long α-helix, and two shorter α-helices [68]. The second birch pollen allergen Bet v 2 also has a seven-stranded anti-parallel β-sheet and, thereby, partly resembles Bet v 1 although the strand order and helix positions are slightly different [25]. The mouse ear cress allergen Ara t has a similar structure as Bet v 2 [69], and the bovine dander allergen Bos d 2 contains eight anti-parallel β-strands forming a central β-barrel, another short β-strand, and three helices [19]. The timothy grass pollen allergen Phl p 2 is composed of an eight-stranded anti-parallel β-barrel [70] and the major house dust mite allergen Der f 2 is a single domain β-protein with an immunoglobulin fold [71].

The structural similarity of the above mentioned allergens is remarkable. However, the list of denominated allergens contains more than 170 proteins from different species [74] and these proteins are structurally and functionally a heterogeneous group [75]. Furthermore, the molecular mass of allergens ranges usually between 5000 and 70 000, whereas the above-mentioned allergens listed have a molecular mass between 13 000 and 20 000. It is obvious that the three-dimensional structures of many more allergens have to be determined to allow for a better understanding of the molecular basis of allergenicity.

7. Analysis of enzymatic properties of allergens

Taken together, only a few characteristics appear to be important in establishing a food protein as an allergen. These characteristics include their increased abundance in the food supply, their durability during food processing, and their resistance to digestion in the gastrointestinal tract [76,77]. Apparently, any food protein may be allergenic if it can be adsorbed intact, or as substantial fragments, through the gut mucosa. Assuming that the three-dimensional structural stability of a protein is an essential structural property for the induction of an allergic response, the molecular stability may also secure other functional features such as enzymatic activity which could be important for the process of allergic sensitization. For example, recent studies have demonstrated that the major mite allergen Der p 1 is a cysteine protease [78] that enhances its own permeability in the bronchial epithelium by proteolytic activity [79]. Der p 1 has also been shown to cleave the low affinity receptor for IgE (CD23) on B cells and monocytes leading to an increase of soluble CD23 and, thereby, to an increased IgE production [80,81]. Furthermore, Der p 1 is capable of decreasing the proliferation of TH1 cells by proteolytic cleavage of the IL-2 receptor (CD 25) [82] which creates a bias of the immune response towards TH2 cells. While these observations do not explain how and why patients develop an allergic response to Der p 1, they demonstrate the ability of this allergen to facilitate its penetration of the bronchial epithelium and to shift the immune response towards IgE production. Other examples of enzymatically active allergens include mainly those with protease activity but also some with nuclease activity, phospholipase A2 activity, hyaluronidase activity, and lysozyme activity (for a review, see [83]). It remains to be determined, however, to what extent enzymatic activities other than proteolytic activities play a role in the process of sensitization and allergic responses.

References

[1] B.J. Sutton, H.J. Gould, Nature 366 (1993) 421.
[2] K. Ishizaka, J. Immunol. 135 (1985) i.
[3] K. Aas, Allergy 33 (1978) 3.
[4] S. Elsayed, in: M.H.V. Van Regenmortel (Ed.), Structure of Antigens, CRC Press, Boca Raton, 1993, vol. II, p. 293.
[5] G. Ball, M.J. Shelton, B.J. Walsh, D.J. Hill, C.S. Hosking, M.E. Howden, Clin. Exp. Allergy 24 (1994) 758.
[6] H. Kahlert, A. Petersen, W.M. Becker, M. Schlaak, Mol. Immunol. 29 (1992) 1191.

[7] K. Honma, Y. Kohno, K. Saito, N. Shimojo, T. Horiuchi, H. Hayashi, N. Suzuki, T. Hosoya, H. Tsunoo, H. Niimi, Clin. Exp. Immunol. 103 (1996) 446.
[8] K. Aas, S. Elsayed, Dev. Biol. Stand. 29 (1975) 90.
[9] A.M. Herian, S.L. Taylor, R.K. Bush, Int. Arch. Allergy Appl. Immunol. 92 (1990) 193.
[10] K.N. Shanti, B.M. Martin, S. Nagpal, D.D. Metcalfe, P.V. Rao, J. Immunol. 151 (1993) 5354.
[11] A.W. Burks, D. Shin, G. Cockrell, J.S. Stanley, R.M. Helm, G.A. Bannon, Eur. J. Biochem. 245 (1997) 334.
[12] 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.
[13] 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.
[14] T. Takai, T. Yokota, M. Yasue, C. Nishiyama, T. Yuuki, A. Mori, H. Okudaira, Y. Okumura, Nat. Biotechnol. 15 (1997) 754.
[15] P.C. Davis, S.C. Williams, Allergy 53 (Suppl. 46) (1998) 102.
[16] A. Urisu, H. Ando, Y. Morita, E. Wada, T. Yasaki, K. Yamada, S. Torii, M. Goto, T. Wakamatsu, J. Allergy Clin. Immunol. 100 (1997) 171.
[17] A. Baer, M. Oroz, B. Blanc, J. Dairy Res. 43 (1976) 419.
[18] S.C. Williams, R.A. Badley, P.J. Davis, W.C. Puijk, R.H. Meloen, J. Immunol. Methods 213 (1998) 1.
[19] A.A. Fedorov, T. Ball, N.M. Mahoney, R. Valenta, S.C. Almo, Structure (Lond.) 5 (1997) 33.
[20] T. Zeiler, A. Taivainen, M. Rytkönen, J. Rautiainen, H. Karjalainen, R. Mäntjärvi, L. Tuomisto, T. Virtanen, J. Allergy Clin. Immunol. 100 (1997) 721.
[21] C. Nishiyama, M. Fukada, Y. Usui, N. Iwamoto, T. Yuuki, Y. Okumura, H. Okudaira, Mol. Immunol. 32 (1995) 1021.
[22] W. Burks, H.A. Sampson, G.A. Bannon, Allergy 53 (1998) 725.
[23] K. Fötisch, F. Altmann, D. Haustein, S. Vieths, Int. Arch. Allergy Immunol. 120 (1999) 30.
[24] A. Jankiewicz, H. Aulepp, F. Altmann, K. Fötisch, S. Vieths, Allergo J. 7 (1998) 87.
[25] I.B. Wilson, J.E. Harthill, N.P. Mullin, D.A. Ashford, F. Altmann, Glycobiology 8 (1998) 651.
[26] E. Bantanero, J.F. Crespo, R.I. Monsalve, M. Martin-Esteban, M. Villalba, R. Rodriguez, J. Allergy Clin. Immunol. 103 (1999) 147.
[27] G. Garcia-Casado, R. Sanchez, M.J. Chrispeels, A. Armentia, G. Salcedo, L. Gomez, Glycobiology 6 (1996) 471.
[28] J. Rouvinen, J. Rautiainen, T. Virtanen, T. Zeiler, J. Kauppinen, A. Taivainen, R. Mäntyjärvi, J. Biol. Chem. 274 (1999) 2337.
[29] K. Hoffmann-Sommergruber, P. Demoly, R. Crameri, H. Breiteneder, C. Ebner, L. DaCamara, M. Machado, K. Blaser, C. Ismail, O. Scheiner, J. Bousquet, G. Menz, J. Allergy Clin. Immunol. 104 (1999) 478.
[30] E.A. Pastorello, L. Farioli, V. Pravettoni, C. Ortolani, M. Ispano, M. Monza, C. Baroglio, E. Scibola, R. Ansaloni, C. Incorvaia, A. Conti, J. Allergy Clin. Immunol. 103 (1999) 520.
[31] E.A. Pastorello, F.P. D'Ambrosio, V. Pravettoni, L. Farioli, G. Giuffrida, M. Monza, R. Ansaloni, D. Fortunato, E. Scibola, F. Rivolta, C. Incorvaia, A. Bengtsson, A. Conti, C. Ortolani, J. Allergy Clin. Immunol. 105 (2000) 371.
[32] P.E. Staswick, M.A. Hermodson, N.C. Nielsen, J. Biol. Chem. 256 (1981) 8752.
[33] K.A. Barton, J.F. Thompson, J.T. Madison, R. Rosenthal, N.P. Jarvis, R.N. Beachy, J. Biol. Chem. 257 (1982) 6089.
[34] P.E. Staswick, M.A. Hermodson, N.C. Nielsen, J. Biol. Chem. 259 (1984) 13424.
[35] S. Buus, A. Sette, H.M. Grey, Immunol. Rev. 98 (1987) 115.
[36] R.E. O'Hehir, R. D Garman, J. L Greenstein, J. R Lamb, Ann. Rev. Immunol. 9 (1991) 67.
[37] T.R. Mosmann, R.L. Coffman, Ann. Rev. Immunol. 7 (1989) 145.
[38] E.A. Wierenga, M. Snoek, C. De Groot, I. Chretien, J.D. Bos, H.M. Jansen, M.L. Kapsenberg, J. Immunol. 144 (1990) 4651.
[39] S. Romangnani, Immunol. Today 18 (1997) 263.
[40] J.A. Higgins, J.R. Lamb, R.A. Lake, R.E. O'Hehir, Immunology 84 (1995) 91.
[41] E.C. De Jong, S. Spanhaak, B.P. Martens, M.L. Kapsenberg, A.H. Penninks, E.A. Wierenga, J. Allergy Clin. Immunol. 98 (1996) 73.
[42] Z. Szepfalusi, I. Nentwich, M. Gerstmayr, E. Jost, C. Ebner, T. Frischer, R. Urbanek, J. Allergy Clin. Immunol. 101 (1998) 514.
[43] P.A. Eigenmann, S.K. Huang, H.A. Sampson, Paediat. Allergy Immunol. 135 (1996) 935.
[44] 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.
[45] R.J. van Neerven, C. Ebner, H. Yssel, M.L. Kapsenberg, J.R. Lamb, Immunol. Today 17 (1996) 526.
[46] H. Yssel, K.E. Johnson, P.V. Schneider, J. Wideman, A. Terr, K. Kastelein, J.E. De Vries, J. Immunol. 148 (1992) 738.
[47] R.M. O'Brien, W.R. Thomas, B.D. Tait, J. Allergy Clin. Immunol. 93 (1994) 628.
[48] A.J. Higgins, J.C. Thorpe, J.D. Hayball, R.E. O'Hehir, J.R. Lamb, J. Allergy Clin. Immunol. 93 (1994) 891.
[49] R.J. van Neerven, M.M. van de Pol, F.J. van Milligen, H.M. Jansen, R.C. Aalberse, M.L. Kapsenberg, J. Immunol. 152 (1994) 4203.
[50] N.E. Eriksson, H. Formgren, E. Svenonius, Allergy 37 (1982) 437.
[51] S. Dreborg, T. Foucard, Allergy 38 (1983) 162.
[52] P. Calkhoven, M. Aalberse, V.L. Koshte, O. Pos, H.D. Oei, R.C. Aalberse, Allergy 42 (1987) 382.
[53] C. Ebner, T. Birkner, R. Valenta, H. Rumpold, M. Breitenbach, O. Scheiner, D. Kraft, J. Allergy Clin. Immunol. 88 (1991) 588.
[54] R. Hirschwehr, R. Valenta, C. Ebner, F. Ferreira, W.R. Sperr, P. Valent, M. Rohac, H. Rumpold, O. Scheiner, D. Kraft, J. Allergy Clin. Immunol. 90 (1992) 927.
[55] P. Vallier, C. Dechamp, O. Vial, P. Deviller, Clin. Allergy 18 (1988) 491.
[56] B. Wütrich, J. Stäger, S.G. Johansson, Allergy 45 (1990) 566.

[57] L. Bauer, C. Ebner, R. Hirschwehr, B. Wütrich, C. Pichler, R. Fritsch, O. Scheiner, D. Kraft, Clin. Exp. Allergy 26 (1996) 1161.
[58] M. Vanek-Krebitz, K. Hoffmann-Sommergruber, M. Laimer da Camara Machado, M. Susani, C. Ebner, O. Scheiner, D. Kraft, H. Breiteneder, Biochem. Biophys. Res. Commun. 214 (1995) 538.
[59] H. Breiteneder, K. Hoffmann-Sommergruber, G. O'Riordain, M. Susani, H. Ahorn, C. Ebner, D. Kraft, O. Scheiner, Eur. J. Biochem. 233 (1995) 484.
[60] R. Valenta, M. Duchène, K. Pettenburger, C. Sillaber, P. Valent, P. Bettelheim, M. Breitenbach, H. Rumpold, D. Kraft, O. Scheiner, Science 253 (1991) 557.
[61] H.P. Rihs, P. Rozynek, K. May-Taube, B. Welticke, X. Baur, Int. Arch. Allergy Immunol. 105 (1994) 190.
[62] C.J. Staiger, K.C. Goodbody, P.J. Hussey, R. Valenta, B.K. Drobak, C. Lloyd, Plant J. 4 (1993) 631.
[63] L. Vidali, H. Perez, V. Valdez Lopez, R. Noguez, F. Zamudio, F. Sanchez, Plant Physiol. 108 (1995) 115.
[64] R. Valenta, M. Duchène, C. Ebner, P. Valent, C. Sillaber, P. Deviller, F. Feirrera, M. Tejkl, H. Edelmann, D. Kraft, O. Scheiner, J. Exp. Med. 175 (1992) 377.
[65] P. Vallier, S. Balland, R. Harf, R. Valenta, P. Deviller, Clin. Exp. Allergy 25 (1995) 332.
[66] R. Valenta, P. Steinberger, M. Duchène, D. Kraft, Immunol. Cell Biol. 74 (1996) 187.
[67] S. Schenk, H. Breiteneder, M. Susani, N. Najafian, S. Laffer, M. Duchène, R. Valenta, G. Fischer, O. Scheiner, D. Kraft, C. Ebner, J. Allergy Clin. Immunol. 96 (1995) 986.
[68] M. Gajhede, P. Osmark, F.M. Poulsen, H. Ipsen, J.N. Larsen, R.J. van Neerven, C. Schou, H. Lowenstein, M.D. Spangfort, Nat. Struct. Biol. 3 (1996) 1040.
[69] K.S. Thorn, H.E. Christensen, R. Shigeta, D. Huddler, L. Shalaby, U. Lindberg, N.H. Chua, C.E. Schutt, Structure (Lond.) 5 (1997) 19.
[70] A.A. Fedorov, T. Ball, R. Valenta, S.C. Almo, Int. Arch. Allergy Immunol. 113 (1997) 109.
[71] S. Ichikawa, H. Hatanaka, T. Yuuki, N. Iwamoto, S. Kojima, C. Nishiyama, K. Ogura, Y. Okumura, F. Inagaki, J. Biol. Chem. 273 (1998) 356.
[72] Z. Böcskei, C.R. Groom, D.R. Flower, C.E. Wright, S.E. Phillips, A. Cavaggioni, J.B. Findlay, A.C. North, Nature 360 (1992) 186.
[73] S. Brownlow, J.H.M. Cabral, R. Cooper, D.R. Flower, S.J. Yewdall, I. Polikarpov, A.C. North, L. Sawyer, Structure (Lond.) 5 (1997) 481.
[74] V. Liebers, I. Sander, V. Van Kampen, M. Raulf-Heimsoth, P. Rozynek, X. Baur, Clin. Exp. Allergy 26 (1996) 494.
[75] G.A. Stewart, P.J. Thompson, Clin. Exp. Allergy 26 (1996) 1020.
[76] J.D. Astwood, J.N. Leach, R.L. Fuchs, Nat. Biotechnol. 14 (1996) 1269.
[77] S. Vieths, J. Aulepp, W.M. Becker, L. Buschmann, in: G. Eisenband, H. Aulepp, A.D. Dayan, P.S. Elias, W. Grunow, J. Ring, J. Schlatter (Eds.), Food Allergies and Intolerances, VCH Verlagsgesellschaft, Weinheim, Germany, 1996, p. 130.
[78] E.R. Tovey, M.C. Johnson, A.L. Roche, G.S. Cobon, B.A. Baldo, J. Exp. Med. 170 (1989) 1457.
[79] C.A. Herbert, C.M. Kinh, P.C. Ring, S.T. Holgate, G.A. Stewart, P.J. Thompson, C. Robinson, Am. J. Respir. Cell. Mol. Biol. 12 (1995) 369.
[80] C.R.A. Hewitt, A.P. Brown, B.J. Hart, D.I. Pritchard, J. Exp. Med. 182 (1995) 1537.
[81] O. Schulz, P. Laing, H.F. Sewell, F. Shakib, Eur. J. Immunol. 25 (1995) 3191.
[82] O. Schulz, H.F. Sewell, F. Shakib, J. Exp. Med. 187 (1998) 271.
[83] A. Bufe, Int. Arch. Allergy Immunol. 117 (1998) 215.