

IgE binding to proteins from sesame and assessment of allergenicity: implications for biotechnology?

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Abstract Successful prediction of the potential allergenicity of a protein may be a key factor in the development of novel, genetically modified foods. The use of the decision tree approach for the prediction of allergenicity is discussed. The methods currently used for identifying allergenic proteins (including use of IgE from patient sera for recognition of proteins) are reviewed. Finally, a specific review of the literature concerning identification of allergens from sesame leads to the conclusion that in the absence of validated animal models, identification of allergenicity (and, consequently, prediction of allergenicity) may be problematic.

Keywords Food allergy · Identification of food allergens · IgE-binding proteins · Prediction of allergenicity · Safety of GM foods · Sesame allergens · Sesame allergy

Introduction

The use of recombinant techniques to generate novel, genetically modified (GM) foods may offer considerable benefits on a global scale. Such

benefits are not yet accepted by large proportions of the population, particularly in much of Europe, with issues of safety, of choice, of need, and of the possibility of increasing trends towards monoculture for particular crops being raised by opponents of the technology. One of the main scientific concerns raised about the safety of GM foods has been about the allergenicity of proteins and of the novel foods in which they are contained. Critical to safety assessment of GM foods is the ability to predict allergenicity of proteins. Here we examine the basis of allergenicity prediction, illustrating various aspects with reference to sesame, a food being increasingly associated with allergy. In the long term, it is possible that the ability to predict allergenicity successfully may determine the degree of acceptance of modern biotechnology.

Genetically modified (GM) organisms contain an altered genome that, apart from special cases involving anti-sense technology and a reduction in expression levels of particular protein(s), leads to the enhanced production of unique protein(s) conferring the agronomic trait(s) of interest. Three possible scenarios should be taken into consideration with regards to the allergenicity of GM foods. First, known allergens may be transferred into a non-allergenic target food plant, thus, causing allergic reactions in sensitised individuals. Such GM foods would currently, almost certainly, fail safety evaluations and never reach the marketplace. (It is interesting to note,

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however, that clear and accurate labelling would place sensitised consumers at no greater risk than presented by currently available foods.) Second, proteins of unknown allergenicity may be encoded by the target crop. Thus, neo-allergens may be created as a result of biotechnology and the population could be exposed to de novo sensitisation. Third, if the host plant of the newly introduced gene(s) is allergenic, it is also possible that the levels and/or nature of the endogenous allergenic proteins may be unintentionally altered as a result of the genetic modification, thus increasing the potential to cause problems to consumers.

In order to prevent adverse allergic reactions that may occur as a result of genetic modification, it is important to be able to predict the allergenic potential of proteins (and foods). In this respect, considerable efforts have been made to establish robust protocols to evaluate the allergenic potential of GM foods prior to their introduction into the market. The current decision-tree approach applied for the assessment of the allergenic potential of transgenic foods (FAO/WHO 2001) is based on the evaluation of structural and sequence similarities with known food allergens, allergenicity testing/cross-reactivity with serum from allergic patients, resistance to pepsin digestion and immunogenicity testing using animal models (Fig. 1).

The allergenicity of a given protein, however, is not an intrinsic and fully predictable characteristic. In this regard, it is worth noting that the ability of a protein to cause an allergic reaction depends not only on the specific features of the protein, but also on the temporal susceptibility and genetic variability of the atopic individuals. In addition, the decision tree approach is at least partly dependent on our ability to identify those proteins within a food able (i) to cause sensitisation and (ii) to cause allergic reactions in sensitised consumers. Moreover, the approach takes no account of the role of food processing in altering protein immuno-reactivity. The present review will discuss the depth of our knowledge in relation to the prediction of allergenicity of proteins, with particular reference to proteins from sesame, and the implications for biotechnology.

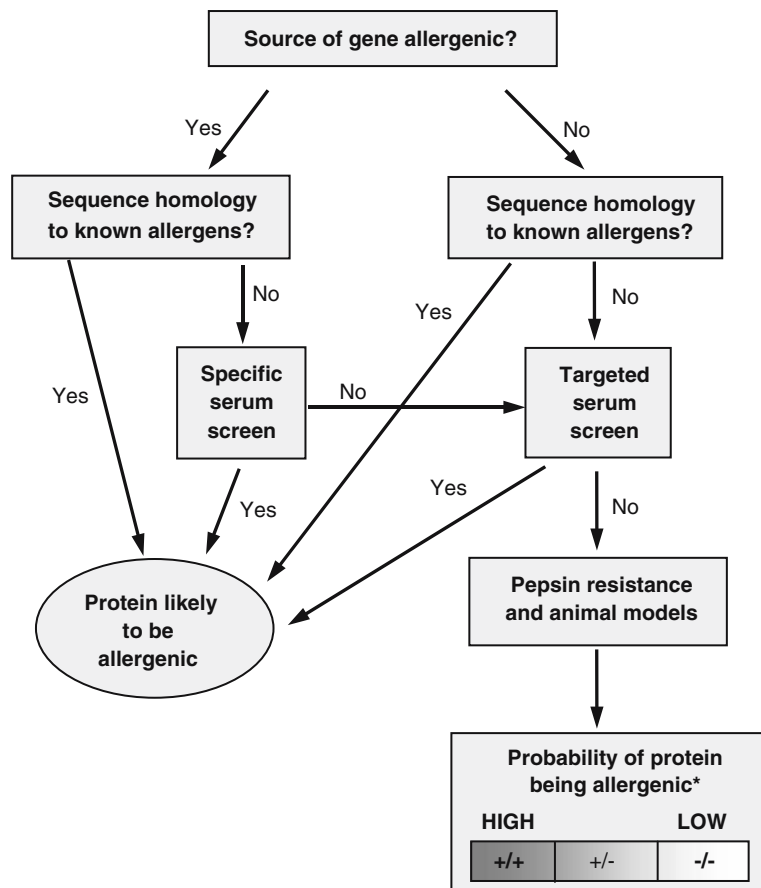
Sequence homology and structural similarity to known allergens

The evaluation of the sequence homology of newly introduced proteins to known allergens constitutes the first step in the investigation of the allergenic potential of genetically modified food proteins according to the FAO/WHO decision tree. Comparisons between amino acid sequences are generally carried out by aligning the allergen sequences of public domain protein databases (e.g. Swiss-Prot, TrEMBL, PIR-NREF, Entrez) using common algorithms, such as FASTA (Pearson and Lipman 1988) and BLAST (Altschul et al. 1990). There is currently some debate on whether the identity between the transgenic protein and known allergens should be of six or eight contiguous amino acids in order to be indicative of potential allergenicity. An epitope (the 'area' of a protein which is intimately involved in the binding interaction with antibody) will include around 10–15 amino acids. According to the FAO/WHO decision tree, an immunologically significant sequence similarity between a novel protein and a known allergen would require either a match of six contiguous identical amino acids or more than 35% identity in the amino acid sequence of the expressed protein using a window of 80 amino acids. Nevertheless, the identity of six contiguous amino acids will lead to high number of false positives (Hileman et al. 2002). When there is a considerable homology between the sequences of the novel protein and known allergens, but the identity is below 35%, the structural similarity with known allergens should be taken into consideration. Some families of structurally related proteins, such as lipocalins, 2S albumins, lipid transfer proteins or parvalbumins, are known to contain many allergens and, therefore, if a novel protein belongs to any of these families it may be more likely to be an allergenic protein.

The examination of sequence homologies offers a useful tool for predicting cross-reactivity due to shared linear epitopes between the novel protein and known allergens. Yet, this approach cannot be applied for the prediction of cross-reactivity involving conformational epitopes consisting of a discontinuous amino acid sequence along a folded protein.

Fig. 1 FAO/WHO decision-tree for the assessment of the allergenic potential of foods derived from biotechnology (FAO/WHO 2001).

*When positive results are obtained in both the pepsin resistance and animal model protocols, the expressed protein has a high probability of becoming an allergen. When negative results are obtained in both protocols, the expressed protein is unlikely to become an allergen. When different results are obtained in the pepsin resistance and animal model protocols, the probability of allergenicity is intermediate



Stability to digestion

It is generally believed that food allergens must be relatively resistant to degradation during the digestive process in order to optimise exposure to the immune system and elicit an allergic reaction. Hence, the ability of food allergens to reach the intestinal mucosa intact (with at least some elements of structure) seems to be a likely requirement for allergenicity (Fuchs and Astwood 1996). Accordingly, it has been suggested that allergenic food proteins are inherently more stable to enzymic digestion than non-allergenic proteins (Astwood et al. 1996). On this basis, the digestive stability (pepsin resistance) is being currently used as a criterion for assessing the potential allergenicity of novel proteins derived from biotechnology (FAO/WHO 2001). As part of the FAO/WHO decision tree approach, the relative pepsin stability of the newly expressed protein is compared to that of known allergenic and

non-allergenic food proteins. However, given that many allergenic proteins from fruits and vegetables do not resist enzymatic digestion and, conversely, some non-allergenic food proteins are stable to digestion, the pepsin resistance cannot be taken as an absolute criterion for allergenicity. This does not mean that the stability to digestive enzymes is not important for the assessment of the allergenicity, but it is a reminder that caution should be exercised when assessing the digestive stability of a protein and that the information obtained from pepsin resistance experiments should be further compared with other allergenicity criteria before reaching a conclusion about the allergenic potential of a novel protein.

Stability to heat and processing

It is often forgotten that food allergy means an exposure to food as it is normally eaten, and not

to purified, native proteins. During food processing, allergenicity can be altered by various procedures, such as storage time, preparation techniques, heating, prolonged washing, interactions with other food components and texturising. As a result, the allergenic potential may be unaffected, decreased or even increased (Besler et al. 2001). Many food allergens are stable to heat and other processing treatments commonly used in food manufacturing (Burks et al. 1992; Wigotzki et al. 2000). However, exceptions exist and some fruit and vegetable allergens are very labile to heat. For example, the allergens from apples, potatoes and carrots were found to be heat sensitive (Dreborg and Foucard 1983). Heat treatment disrupts the tertiary structure of the protein, causes unfolding, cleavage and re-arrangement of disulphide bonds and aggregation of allergens (Davis and Williams 1998). As a result of heating, new epitopes may be exposed at the surface of the proteins, while former conformational epitopes are probably lost. Alterations in stability caused by processing may, of course, also alter the resistance to digestion and the nature of the interaction with the immune system.

To take a very simple example to understand the complexity involved, consumption of sesame proteins will often involve ingestion of intact seeds. Within the seeds, the proteins may have already been exposed to heating and have undergone chemical reaction with localised components or with other food components present (Davis and Williams 1998). Even when (or if) the seed coat becomes broken during chewing and digestion, the proteins may remain within localised structures such as protein bodies (Teuber 2002). Clearly, survival of proteins to be able to interact with the immune system is determined by more than just 'stability', as tested in simple, in vitro systems.

Animal models

It is difficult to carry out detailed experimentation on sensitised individuals to determine aspects of allergenicity, for obvious reasons. Consequently, validated animal models may offer the most direct approach for the determination of the

intrinsic sensitising potential of transgenic food proteins in the future. In particular, such models could have an important application in instances when the transferred genes originate from a source with unknown allergenicity. In this regard, the aim is to develop animal models that are capable of determining the allergenic potency of a novel protein relative to that of well known strong and weak food allergens as well as non-allergenic proteins. Despite the increasing research efforts in recent years to develop suitable approaches for the identification of the allergenic potential of food proteins based on responses induced in animals (Knippels et al. 1998; Dearman et al. 2000), no validated and widely accepted animal model is available.

The current methods for the determination of the allergenic potential of transgenic proteins are generally carried out with purified *E. coli*-produced proteins (Fuchs et al. 1993; Reed et al. 1996). However, it is important to note that the normal allergenicity of the proteins may be enhanced as a result of the interaction with other components present in the food matrix, such as lipids and sugars, and of wider aspects of structure and localisation. Therefore, other factors apart from the allergenic proteins themselves should be considered when assessing the allergenicity of novel food proteins. Moreover, since most foods are processed before consumption it is clear that individuals become sensitised to allergenic proteins, which are generated after processing treatments. Consequently, the real allergenic potential of transgenic proteins may be under-estimated when purified recombinant proteins are used for allergenicity testing by means of serum screen tests, pepsin resistance and animal tests.

Problems of identification of allergenic proteins

Theoretically, the best method to verify whether a particular food protein is responsible for triggering an allergic reaction is to perform a double-blinded, placebo-controlled oral challenge on the individual. However, such an approach is problematic and very few individuals are even diagnosed using this procedure. Other in vivo human studies, such as skin prick tests (SPT) can also be

used in certain instances to prove an association between specific food proteins and allergic symptoms. One of the most significant drawbacks of the latter technique is, however, that a particular food allergen may cause a reaction in the gut after ingestion but not in the skin, resulting in a negative skin test. Nevertheless, considering the risks of conducting oral challenges or skin tests on allergic patients, the use of allergic individuals for non-diagnostic purposes is not, generally, ethically acceptable. Other important disadvantages of performing human studies to identify food allergens are the existing inter- and intra-individual variations with regards to food allergic reactions. It has been frequently observed, for example, that different individuals react to different proteins within the same allergenic food. Moreover, the amount of allergen that elicits a reaction varies from person to person, as may the type of symptoms that different individuals suffer after the ingestion of the same allergens. In addition, the same person may react differently to allergenic proteins on different days. A good example of such intra-individual variations was reported by Hourihane et al. (1997) showing that some individuals with proven peanut allergy did not react to any dose up to 50 mg peanut protein in double-blinded placebo-controlled food challenges (DBPCFC). It is also known that exercise and stress can be important co-factors in food allergic reactions (Mullins 2003; Yang et al. 2006).

Besides the inherent problems associated with the human studies, the lack of a robust and reliable animal model is an additional obstacle for the study of food allergens. Thus, currently the only alternative approach for identifying allergenic proteins are *in vitro* tests in which the health of the individuals is not put at risk. The allergenicity assessment of foods and food proteins is commonly carried out with immunoglobulin E (IgE) from the sera of allergic patients using a range of tests. These *in vitro* assays include solid-phase immunoassays, such as the RadioAllergoSorbent Test (RAST), Enzyme-Linked Immunosorbent Assay (ELISA) and EnzymeAllergoSorbent Test (EAST), used either as direct binding tests or as inhibition assays (Poulsen 2001). Extracts of the food or protein of

interest are bound to a solid matrix (e.g. polystyrene, nitrocellulose, aluminium hydroxide gel or magnetic microparticles) and exposed to the sera from allergic individuals. The allergen-specific IgE binds to the protein attached to the matrix and is detected by using a secondary labelled antibody specific for human IgE. Such *in vitro* methods allow the quantification of the IgE-binding to an allergenic source material without differentiating the individual allergenic components contained in that particular food.

When the identification of the allergenic components of a food material is required, SDS-PAGE followed by immunoblotting is generally applied. The technique is based on the electrophoretic separation of the protein components of a food and their subsequent transfer onto nitrocellulose or polyvinylidene difluoride (PVDF) membranes. The IgE-binding activity of each individual allergenic protein can then be assessed by incubation of the blots with the sera from allergic patients.

The main drawback of the *in vitro* tests described is that the demonstration of IgE recognition of food proteins does not necessarily imply that degranulation of mast cells and basophils would have resulted *in vivo* and, therefore, a positive *in vitro* test may not be correlated with adverse reactions in a particular individual. In this respect, *in vitro* mast cell or basophil degranulation tests may be more appropriate, since such methods can be used to determine whether a protein can elicit a mediator response through the cross-linking of IgE. For these assays, mast cells or basophils are isolated and sensitised with antigen-specific IgE antibodies (either using serum from sensitised humans or animals). Subsequently, the sensitised cells are incubated with the food extract and degranulation is determined by the released mediators, such as histamine. Antibodies and effector cells from various species can be used for these assays (Hoffmann et al. 1997; Opara et al. 1999). However, the results of mast cell or basophil degranulation tests should be interpreted with caution since many other factors apart from the IgE cross-linking with cell surface receptors may affect the development of an allergic reaction *in vivo*. *In vitro* techniques do not take into consideration the ‘real life’ situation

in which many other factors, such as food preparation, effect of the gastrointestinal flora, development of oral tolerance among other features, may ultimately determine the presence or absence of a clinical reaction. Whether all food proteins capable of being recognised by serum IgE from an individual are also capable of eliciting an allergic response is a question yet to be answered.

Background to sesame allergy

During the last few years the number of sesame allergic individuals seems to have increased significantly in a number of countries, though without clear, quantitative data it is difficult to prove. Sesame seeds are used for the preparation of a range of traditional dishes and as decoration and flavouring agents by the bakery industry for the preparation of specialty breads, cakes, and other delicacies. Other common foods containing sesame seeds include vegetarian burgers, spice mixtures, salad dressings and a wide range of diet and 'health' foods. Ethnic foods containing sesame seeds and sesame oil are also becoming more common in Western countries and can lead to specific problems because the presence of sesame in such preparations may not be obvious to consumers unfamiliar with such products. The increasing consumption of sesame-containing foods, and the fact that sesame may be present as a hidden allergen, is likely to make sesame allergy become even more common in future. The prevalence of sesame allergy differs significantly from country to country and seems to be affected by genetic factors, cultural and dietary habits, and exposure to sesame products early in life (Sporik and Hill 1996; Emmett et al. 1999; Rance et al. 1999; Dalal et al. 2002).

Severe reactions to sesame, such as anaphylactic shock, have been reported in the literature (Asero et al. 1999; Pajno et al. 2000; Dalal et al. 2003). Sensitisation to sesame may be first observed at any age. In some cases, the symptoms do not begin to develop until the individuals are in their thirties or forties, though sesame hypersensitivity is also observed amongst the paediatric population (Sporik and Hill 1996; Rance et al.

1997; Levi and Danon 2001; Dalal et al. 2002). In this respect, it appears that the early introduction of sesame into the diet of Australian, Israeli and French infants has resulted in a striking increase in allergic reactions to sesame.

Not only the seeds, but also sesame oil has been implicated in allergic reactions (Chiu and Haydik 1991; Kanny et al. 1996; Morisset et al. 2003). Although the vast majority of oils consumed are highly processed and contain virtually no protein, crude oils, such as sesame oil, may contain trace amounts of protein and, therefore, have the potential to cause allergic reactions (Hefle and Taylor 1999). Despite refined sesame oil being the main grade used in pharmaceutical products and cosmetics, hypersensitivity reactions to such products have been reported, constituting a risk for atopic patients (Pecquet et al. 1998).

The threshold for elicitation of symptoms depends on the sensitivity of the individual patients towards the sesame allergens. Extremely sensitive subjects were reported to react to doses as low as 30 mg crushed sesame seeds and 1 ml sesame oil (Morisset et al. 2003).

Problems of identifying patients allergic to sesame

Identifying individuals with allergy to sesame is not an easy task. The DBPCFC (double-blind, placebo-controlled food challenge) is the gold standard for the diagnosis of sesame allergy. Besides, in many instances, it is probably the only way of establishing an association between a clinical reaction and exposure to sesame allergens. However, due to the severity of the reactions triggered by sesame, oral challenges are not generally performed in patients with a history of anaphylaxis. Often, SPTs and *in vitro* tests are the only procedures used to identify sesame allergic individuals. A negative SPT does not have a high predictive value for the diagnosis of sesame allergy. Kolopp-Sarda and et al. (1997) reported a very low efficiency of commercial sesame extracts in stimulating cutaneous responses as compared to freshly prepared extracts. Moreover, the SDS-PAGE analysis of the commercial sesame extract showed a single faint blurred band as opposed to

the 19 bands detected in the freshly prepared extract. Therefore, in many cases, negative results for SPT performed with commercial preparations are probably due to the lack of available immunogenic epitopes capable of triggering cutaneous immune responses. Another possible explanation of negative SPT for patients reacting to food challenges could be that the epitopes recognised by IgE antibodies are exposed or formed only after the food has been digested. Hence, these epitopes are unrecognisable in the undigested extracts.

Solid-phase immunoassays are frequently used as part of the diagnosis of sesame allergy. The measurement of food-specific serum IgE is commonly carried out using commercially available diagnostic kits, such as the Pharmacia CAP System. The assay is conducted on a hydrophilic carrier polymer encased in a capsule (ImmunoCAP). Standardised food allergens are immobilised on the solid phase of the ImmunoCAP and subsequently incubated with the sera from individuals with suspected food allergy. The presence of serum food-specific IgE is then detected with labelled anti-human IgE antibodies. Nevertheless, despite the manufacturer's claims, the use of commercial kits for the detection of serum IgE may lead to incorrect results. Recently, negative ImmunoCAP results for sesame-specific IgE were reported in 13 out of 32 individuals with sesame allergy (Leduc et al. 2006). However, IgE-binding to white and black sesame extracts was demonstrated by ELISA for nine of the patients with negative ImmunoCAP results. Moreover, isoelectric focusing of white sesame extracts followed by immunoblotting showed numerous bands recognised by the serum IgE of all patients with negative ImmunoCAP.

In the view of the false negative results that may derive from the use of commercial kits, it is important to draw attention to the allergen preparations used for such diagnostic tests as the most likely source of discrepancy. First, it is essential to consider the characteristics of the protein allergen present in the extract. In this regard, several aspects, such as the degree of protein solubility and the possible masking of IgE-binding epitopes as a result of the binding to the solid support should be taken into account.

Second, commercial allergen extracts generally consist of native, unprocessed proteins. Thus, it is important to point out the absence of other components of the food matrix that may have an effect on the IgE-binding. Finally, it should be borne in mind that the allergens in the commercial extract may not be present in the same form as they would be presented to the gastrointestinal tract when the individual consumes the food. Allergic individuals are likely to become sensitised to food proteins that have previously been processed in different ways. Such processing treatments may lead to important structural changes in the allergenic proteins. Moreover, the processed food proteins may suffer further structural alterations after exposure to gastric acids and enzymes during digestion. Multi-subunit proteins may be more relevant as stable subunits; stable polypeptides as proteolysis products of larger proteins might be more relevant than the intact protein. Hence, in some instances, the unprocessed native proteins used in the diagnostic kits may not be recognised by the IgE from allergic patients.

Patient IgE-binding for sesame

Sesame proteins are interesting to study in regard to literature investigations of allergenicity because relatively few studies have been published and it is possible to make some interesting comparisons and observations. Similar studies with, for example, peanut protein are too numerous. Studies describing the IgE-binding proteins of sesame have been compiled in Table 1. The identification of putative sesame allergens has been performed by means of *in vitro* techniques. Generally, the proteins are separated by SDS-PAGE followed by immunoblotting and tested with patient sera containing sesame-specific IgE antibodies. In most cases the IgE from the serum of patients allergic to sesame appears to recognise a wide range of polypeptides with different molecular weights, whereas in others only a few polypeptides are reported as IgE-reactive. Are the diverse observations consistent with inter-individual variation or are there other effects involved? The recognition of multiple polypeptides from the sesame

Table 1 Compilation of the investigations reporting IgE-binding proteins of sesame

No. of patients	Main IgE-reactive proteins	References
32 (CH, SPT, DBPCFC or LC, CAP)	Group 1— <i>11S globulin</i> : • 30–40 kDa (~70%) (acidic subunits) • 20–25 kDa (~60%) (basic subunits) under reducing conditions Group 2— <i>2S albumin</i> : • 4 kDa (small subunit) • 9 kDa (large subunit) under reducing conditions/• ~14 kDa under non-reducing conditions Group 3— <i>7S globulin</i> : • 55–60 kDa (~90%) Group 4— <i>Oleosins</i> : • 15–17 kDa (~90%)	Leduc et al. (2006)
24 (CH, SPT), No DBPCFC	14 kDa (92%, 22/24) (2S albumin precursor) 20–25 kDa and 30–35 kDa (17%, 4/24) 20–25 kDa (8%, 2/24)	Wolff et al. (2003)
20 (CH, CAP), No DBPCFC	45 kDa (75%, 15/20) (7S globulin, <i>Ses i 3</i>) 34 kDa (60%, 12/20) (homologous to SMPS) 78 kDa (50%, 10/20) (homologous to EAPS) 7 kDa (30%, 6/20) (2S albumin, <i>Ses i 2</i>) 12–13 kDa (100%, 6/6) 22–23.5 kDa (100%, 6/6) 32–36 kDa and 47 kDa (83%, 5/6) 57.5 kDa (50%, 3/6)	Beyer et al. (2002)
6 (SPT), 1 patient + DBPCFC; 1 patient + LC	Patient 1: 20, 29, 33, 40, 42, 50 and 76 kDa Patient 2: 20, 35 and 52 kDa	Fremont et al. (2002)
2 (CH, RAST)	9 kDa (100%, 10/10) (2S albumin, <i>Ses i 1</i>) 30 kDa (80%, 8/10) 14 kDa (70%, 7/10) 38.7 kDa and 43 kDa (60%, 6/10) 18 kDa and 53 kDa (50%, 5/10)	Soylemez et al. (2002)
10 (CH, SPT, RAST/CAP) No DBPCFC	10 kDa, 15–20 kDa and 30–67 kDa 12 and 25 kDa (lower intensity bands) 32–35 kDa (55%, 6/11) 66 kDa (18%, 2/11) 21 kDa and 25 kDa (9%, 1/11)	Pastorello et al. (2001)
1 (CH, SPT, RAST), No DBPCFC	25 kDa (60%; 6/10) 30 kDa (20%, 2/10) 14 kDa (10%, 1/10)	Asero et al. (1999)
11 (CH)	14 kDa	Mirzai et al. (1999)
10 (CH, SPT, RAST/CAP, DBPCFC or LC)	Protein fraction Inhibition [†] < 8 kDa 29% (54%) 8–20 kDa 60% (59%) 20–40 kDa 57% (54%) 40–62 kDa 28% (73%) 62–84 kDa 25% (69%) 84–105 kDa 12% (60%) 105–126 kDa 0% (63%)	Kolopp-Sarda et al. (1997)
1 (CH, SPT)		Alday et al. (1995)
2 (CH, RAST)		Malish et al. (1981)*
	[†] Dilution 1:1,000 (dilution 1:100)	

DBPCFC, Double-blind placebo-controlled food challenge; CH, clinical history; LC, labial challenge; RAST, radioallergen sorbent test for measurement of anti-sesame IgE levels in blood; CAP, CAP System for measurement of anti-sesame IgE levels in blood; SPT, skin prick test; SMPS, seed maturation protein of soybeans; EAPS, embryonic abundant protein of soybeans. *The IgE-binding fractions were determined by density gradient ultracentrifugation/RAST inhibition

extracts could have arisen as a result of analytical artefacts. However, the multiple IgE-binding polypeptides may also indicate the existence of multiple allergens in sesame seeds. According to the investigations, it appears that the IgE-reactivity pattern of sesame proteins varies from patient to patient.

Interpreting the IgE-binding results for sesame proteins is not an easy task due to a number of factors. First, there seems to be a lack of basic knowledge about the proteins involved. For example, the protein extraction procedure requires careful consideration to obtain an extract with sufficiently high protein concentration and yet, at the same time, prevent denaturation of the proteins during the extraction process. The solubility of the different sesame proteins needs to be taken into account to ensure that the extract contains as many potential allergens as possible before immunoblotting analysis with sesame-specific IgE. Extraction with phosphate buffer (20 mM, pH 7.0) containing 1 M NaCl ensures the presence of the main storage proteins of sesame in the extract, namely; 11S and 7S globulins and 2S albumin, rendering a high protein extraction yield (Prakash 1986). In contrast, when the extraction was carried out with water, only the 2S albumin could be observed (Prakash 1986). This may explain why the SDS-PAGE pattern of the aqueous sesame extracts reported by Alday et al. (1996) showed only a single protein (2S albumin), and, as a result, the group only found one IgE-binding protein.

Second, the analytical limitations of the techniques used for these studies should be taken into consideration. A major limitation to the SDS-PAGE/immunoblotting procedure is that the denatured proteins bound to the membrane may no longer contain the same conformational epitopes present in the sesame proteins as they encounter the immune system. Few analytical studies take any account of how the proteins are presented to the IgE antibodies. Thus, it is possible that some of the non-IgE-reactive polypeptides may have arisen as a result of such analytical artefacts. Another important limitation of the SDS-PAGE technique according to Laemmli (1970) is the insufficient resolution for proteins

below 10 kDa (Schocker and Becker 2001). To achieve better resolution and subsequent identification of low molecular weight proteins, methods such as Tricine-SDS-PAGE according to Schagger and von Jagow (1987), modifications of the Tricine-SDS-PAGE technique developed by Westermeier (1997) or NuPAGE Bis-Tris electrophoresis (from Novex) should be used instead.

Third, it is important to consider the characteristics of the patients recruited for the studies. Are the patients with a particular allergy who report to clinicians representative of the whole group of allergy sufferers? Are the patients who do see clinicians and who then volunteer for further studies representative? Experience suggests that the answer to the two questions maybe 'certainly not' or at the very least 'probably not'. Yet the pressing need of researchers for serum samples and for volunteers may be reflected in a lack of representativeness in the experimental material that even pooled samples cannot hide. Interestingly, only two out of the 11 investigations conducted DBPCFC to corroborate allergy to sesame (Kolopp-Sarda et al. 1997; Leduc et al. 2006). SPTs were carried out in seven studies. A proper and comparative diagnosis of the individuals should be conducted prior to the evaluation of the sesame proteins if the IgE-binding results are to have any meaning in terms of actual allergenicity. Yet, it should be pointed out that IgE-binding to a specific protein does not necessarily mean that such protein is capable of triggering allergic reactions in vivo. (Incidentally, the binding affinity of the IgE antibodies is generally not taken into account, which may be an important consideration with respect to mast cell degranulation).

Finally, the interpretation of the IgE-binding results for sesame is made even harder due to the fact that most of the studies describe polypeptides of a given molecular weight but do not try to identify the particular sesame protein. In this regard, it is worth noting that probably the most relevant allergens of sesame, i.e. the storage proteins, are highly heterogeneous, oligomeric proteins, consisting of groups of closely related proteins, which often make the identification of such proteins very difficult.

Implications of the review of IgE-binding proteins from sesame to biotechnology for predicting allergenic potential

Two interpretations of the literature are that (a) it is difficult, using IgE from patient sera, to reproducibly identify allergenic proteins from sesame, and (b) inter-patient variability is such that different proteins are responsible for allergenicity in different patients. Whilst both could be true, it is also intuitively likely that the allergenicity of a food resides in a limited number of proteins for the sensitised population as a whole.

Since it appears to be difficult to identify sesame-allergic patients using commercial protein extracts and reagents (Kolopp-Sarda et al. 1997), and since it is likely that the pool of patient sera available for study is unlikely to be representative, it has to be concluded that (a) may well be correct, and that if it is correct for sesame it may well be correct for other foods.

The use of patient IgE to identify allergenic proteins is clearly a limiting bottleneck on the study of food allergy, given the absence of validated animal models, the lack of availability of large amounts of representative patient sera, and the limitations in methodology available to study IgE–food protein interactions in an open, non-biased manner.

Consequently, it has to be assumed that our ability to identify those food proteins or peptides able to initiate allergic reactions *in vivo* (and, presumably also able to cause sensitisation) is such that significant further research is required. In particular, the need to move away from techniques requiring protein immobilisation, the need for improved understanding of the nature of proteins as consumed (and, in certain cases, digested), and the need to better understand the range of IgE response, are key areas for study. Until then, it may be that prediction of allergenicity is a less certain activity than we would like it to be, a view that has implications for safety assessment of novel foods such as those provided by genetic modification.

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