

Relevance of Lipophilic Allergens in Food Allergy Diagnosis

Uta Jappe^{1,2} · Christian Schwager¹

© Springer Science+Business Media, LLC 2017

Abstract

Purpose of Review The purpose of this review is to provide available data on a new class of allergens, the oleosins, and their diagnostic value. There is evidence that allergen extracts used for in vivo as well as in vitro diagnostic tests do not contain oleosins because these proteins are lipophilic and nearly insoluble in saline or aqueous solutions. So far, only oleosins of peanut, sesame and hazelnut have been registered as allergens. Reports on IgE-binding tests performed with oleosins of different species with sera from allergic patients show that IgE specific for oleosins are associated with severe allergic reactions which is why they should be part of the diagnostic tests in the future.

Recent Findings Recent findings showed that oleosins purified from in shell-roasted peanuts revealed a higher IgE-binding capacity when compared to raw ones. Naturally purified as well as recombinantly produced peanut oleosins can be used in basophil activation test.

Summary The synopsis of all reports on different thermal processing of several oleosin sources and the respective data obtained with patients sera investigated via immunoblot and basophil activation test points to the recommendation that—if

naturally purified oleosins are used, they should mostly be obtained from roasted food allergen sources. For immunoblot and basophil activation test, both, naturally purified oleosins as well as recombinant modified oleosins are valuable diagnostic tools.

Keywords Food allergy · Lipophilic allergens · Marker allergens · Oleosin · Oil body · Oil seeds · Precision medicine · Peanut allergy · Single allergens

Introduction

Allergologists in molecular allergology research are often confronted with the question: Do we really need more single allergenic components to be identified? Does this bring us closer to the elucidation of allergic pathomechanisms and, subsequently, the definite cure of the disease?

The answer of the molecular allergologist is “YES”, of course, although there is still a lot of work to do. This work, however, should not only consist in further identification and characterization of additional single allergens. The more single components we know the better we shall be equipped to investigate target-oriented sensitization routes and allergenic pathomechanisms. With regard to the cure of the disease, it seems more appropriate at present to improve diagnostic measures in order to identify those patients who will (or will not) profit from already approved treatments than primarily aiming at the development of completely new therapies.

The fact that single allergens are available is not only of considerable advantage for component-resolved diagnosis (CRD) but for mechanistic research on the relationship between structure and allergenicity as well.

This article is part of the Topical Collection on *Immunologic/Diagnostic Tests in Allergy*

✉ Uta Jappe
ujappe@fz-borstel.de

¹ Division of Clinical and Molecular Allergology, Priority Research Area Asthma & Allergy, Airway Research Center North (ARC�), Member of the German Center for Lung Research (DZL), Research Center Borstel, Parkallee 35, 23845 Borstel, Germany

² Interdisciplinary Allergy Outpatient Clinic, Department of Pneumology, University of Lübeck, Lübeck, Germany

Therefore, it is of interest to look at these new single components from both sides: mainly the diagnostic but also the scientific one.

Why Is It of so Much Interest to Identify New Allergens?

There is evidence that whole allergen extracts used for in vivo as well as in vitro diagnostic tests do not contain all clinically relevant allergenic components [1, 2, 3, 4] which is why these tests are sometimes negative even if the history of the patient is positive. Although not all single allergenic components from the respective allergen sources have been identified yet, it has become obvious that during the extraction process some allergens might be missed and are, therefore, not part of the commercially available diagnostic test solutions. Availability of single allergens for diagnostic measurements, however, can improve risk assessment and management of food allergy.

Whereas important single allergens such as Bet v 1-homologues, storage proteins and lipid transfer proteins are already integral part of routine allergy diagnostic tests with some of them having the potential of becoming marker allergens for the severity of an allergic reaction (like Ara h 2) [5], defensins have only recently been identified as allergens in peanuts with some evidence of being IgE-positive only in patients with more severe reactions [6]. However, there might be still some allergens hidden in the lipophilic matrix of peanut.

What Exactly Are “Lipophilic” Allergens?

Peanut is a lipid-rich fruit and highly allergenic. It is, therefore, most probable that lipids take part in the allergenicity of peanuts, which is why we started to investigate single peanut allergens for structural features that would allow them to associate with lipids. This approach was successful in more than one aspect: First, it could be shown that the Bet v 1-homologue of peanut and relevant marker allergen for pollen-associated food (class II) allergy, Ara h 8, possesses a hydrophobic pocket where lipids are attached to the protein [7]. This is noteworthy as there is some evidence for a protective effect of lipids delaying the enzymatic digestion and supporting the uptake of allergens by intestinal mucosa cells [7, 8]. Structural association with lipids is a common feature of members of several different protein families that contain allergens: apart from the already named Bet v 1-homologues, there are the lipid transfer proteins, 2S albumins, lipocalins, secretoglobulins, oleosins and allergens of the mite group 2, 5, 7, 13 and 14 proteins [8, 9], all of them containing hydrophobic binding sites for lipid ligands, capable of inducing TH2-immunomodulation.

However, although associated with lipids, the allergens could/should not be classified as strictly lipophilic but just hydrophobic, instead, as they all, except the oleosins, are soluble in aqueous solutions. For instance, when taking the GRAVY (grand average of hydropathy) index [10], a measure of protein hydrophobicity or hydrophilicity, into account the following scoring results can be obtained for peanut: seed storage proteins (< -1.0), profilin (-0.1), Bet v 1 homologue (-0.4), nsLTP (0.3); oleosins (0.2–0.5), defensins (-0.7 to -0.5). The grade of positivity is representing hydrophobicity of the whole molecule, whereas the grade of negativity accounts for the degree of hydrophilia. According to this calculation, lipid transfer proteins are as hydrophobic as some oleosins. However, lipid transfer proteins are not consequently more hydrophobic, since the calculation takes the whole molecule into account. It, therefore, does not reflect the properties of individual domains or the folding of the protein. In fact, nsLTPs are well-soluble proteins, and some of them are able to bind and carry molecules, such as lipids and phospholipids, in their internal hydrophobic cavity [11]. In contrast to nsLTP, oleosins are oil body membrane proteins and are designed to directly interact with lipids via a specialized domain (explained in the section below) which has been found to be the longest hydrophobic stretch ever encountered in nature (GRAVY score 1.8) [12]. Therefore, their solubility in aqueous buffers is rather low which is why oleosins are quite difficult to study.

With regard to lipid transfer proteins, a lot has been published already on the clinical significance as markers for severe reactions in the Mediterranean countries, revealing the geographical dependency of LTP-associated reactions, so that the focus of this review will be on truly lipophilic allergens, the oleosins.

Oil Bodies and Oleosins

Oil bodies, also known as oleosomes, lipid bodies or spherosomes are discrete spherical organelles found in oil-rich plant seeds but also in algae, mosses, ferns and tapetum of *Brassicaceae* [13–15]. In this review, we will mainly focus on seed oil bodies and their oleosins as they are of importance for allergologists. Oil bodies are storage organelles for lipids (triglycerides) which are used as energy resource for seed germination [16]. They have been found in diverse plant seeds like soybean, maize, rapeseed, sunflower, peanut and sesame [17]. Seed oil bodies consist of a core of lipids, surrounded by a single layer of phospholipids and embedded proteins, named oleosin (15–24 kDa), caleosin (~ 30 kDa) and steroleosin (~ 40 kDa) (Fig. 1) [20–24]. Oleosins are by far the most abundant proteins from oil bodies representing 80–90% of total protein [25]. They are thought to stabilize oleosomes, to prevent their coalescence and to possess an enzymatic

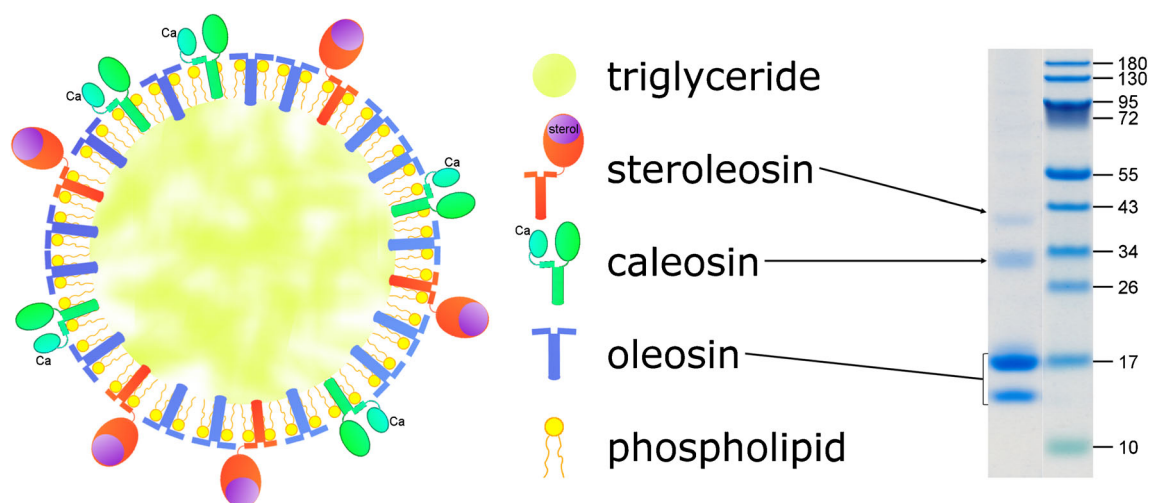


Fig. 1 Schematic model of an oil body with its components (*left*) and determination of the molecular mass of oil body proteins from peanut by SDS-polyacrylamid gel electrophoresis (*right*). Schwager et al. [18••] and Shimada et al. [19], modified

activity [26–28]. The unique characteristic of the primary sequence of all oleosins is its division into three defined structural domains: a central hydrophobic domain flanked on either side by relatively hydrophilic domains, respectively [29]. The hydrophobic domain is embedded into the oil body membrane, in contrast to the hydrophilic domains which reside on the surface of the oil body and interact with the head groups of phospholipids [16, 29]. Once removed from the oil body, strong detergents are required to keep oleosins in solution [30, 31]. Caleosin and steroleosin are minor oil body proteins that received their names because of the calcium and sterol-binding properties [32, 33]. As their allergological relevance is unknown, they will not be discussed in this review.

Clinical Significance of Oleosins

In 1998, Olszewski and co-workers described an allergenic protein present in peanut oil [34]. After its purification and identification as peanut oleosin, they could show IgE-binding in sera of 3 peanut-allergic patients in 2002 [35, 36]. Moreover, based on immunoblot experiments, this group provided first hints for an increased allergenicity of oleosins from roasted peanuts [36]. Within the next 6 years, oleosins from sesame (Ses i 4 and Ses i 5) [37•], hazelnut (Cor a 12 and Cor a 13) and peanut (Ara h 10 and Ara h 11) have been registered as allergens by the WHO/IUIS Allergen Nomenclature Sub-Committee (www.allergen.org). However, an accompanying publication for the registration of the hazelnut and peanut oleosins was missing for years. The proof for the allergenicity of Cor a 12 and Cor a 13 was provided in 2014 [2•]. One year later, the first demonstration of the IgE-binding properties of Ara h 10 and Ara h 11 was published [18••]. At the same time, the authors also provided hints for the allergenicity of a new oleosin of peanut [18••]. After substantial

evidence for IgE-binding, this oleosin was registered as Ara h 15 [38••]. In addition, the same authors also proved the allergenicity of the oleosin initially encountered in peanut oil [34] in a larger cohort [38••]. For that reason, they were able to fulfil the criteria of the WHO/IUIS and to register the oleosin which was discovered more than a decade ago as Ara h 14 [38••].

Up to now, the oleosins registered as allergens are derived from three plant sources: peanut, sesame and hazelnut. However, there is a growing body of evidence that oleosins from other plant species might be elicitors of allergic reactions, as well. So far, oleosins have been suspected to induce allergic reactions after contact to flax [39], ingestion of soybean [40], sunflower [41], and walnut [42, 43]. Moreover, an IgE-binding epitope of Ara h 15 was identified which is cross-reactive with buckwheat [44]. An overview of oleosins registered as allergens and oleosins that have been reported to have allergenic potential is given in Table 1.

Oleosins from different food allergen sources have already been discussed as being associated with severe allergic reactions [2•, 37•, 38••]. Interestingly, all oleosin containing foods, for which oleosins have been described as allergens, are requiring labelling on food products [51]. Manufacturing of foods affects the allergenic properties of proteins. In the course of processing, allergens are exposed to different conditions that can lead to their aggregation, degradation or modification. With regard to peanuts, it has been shown that roasting is able to increase the allergenicity of various single allergens [52, 53]. This is also true for peanut oleosins as we could show, by immunoblot and basophil activation test (BAT), that thermal treatment results in an elevated IgE-binding potential [38••]. This issue is probably a result of the Maillard reaction which has been assumed to create IgE-reactive ‘neo-epitopes’ [53–55]. In Western countries, peanuts are usually consumed as roasted products and, therefore,

Table 1 Overview of allergenic and potentially allergenic oleosins

Plant	Component	Molecular weight [kDa]	Allergenic potential	References
Hazelnut (<i>Corylis avellana</i>)	Cor a 12	17 ^a	Yes	Zuidmeer-Jongejan et al. [2•] Akkerdaas et al. [45]
	Cor a 13	14–16 ^a		
Peanut (<i>Arachis hypogaea</i>)	Ara h 10	16 ^a	Yes	Schwager et al. [18••] Pons et al. [36] Schwager et al. [38••]
	Ara h 11	14 ^a		
	Ara h 14	17.5 ^a		
	Ara h 15	17 ^a		
Sesame (<i>Sesamum indicum</i>)	Ses i 4	17 ^a	Yes	Teodorowicz et al. [3•] Leduc et al. [37•] Chen et al. [46]
	Ses i 5	15 ^a		
	15.5 kDa-oleosin	15.5 ^a		
Flax (<i>Linum usitatissimum</i>)	Not specified		Probably	Hyun et al. [47]
Soybean (<i>Glycine maxima</i>)	17 kDa-oleosin	17	Probably	Pons et al. [36] Herman EM [48]
	18 kDa-oleosin	18		
	24 kDa-oleosin	24		
Sunflower (<i>Helianthus annuus</i>)	19 kDa-oleosin	19	Probably	Macias et al. [41] Cummins et al. [49]
	20.5 kDa-oleosin	20.5		
Walnut (<i>Juglans regia</i>)	12 kDa-oleosin	12	Probably	Lacomba et al. [42] Santos et al. [43] Cao et al. [50]
	15 kDa-oleosin	15		

^a Molecular weight information as documented in the WHO/IUIS database (www.allergen.org)

individuals might be more prone to be sensitized to modified allergens, which in turn explains their higher reactivity. However, detailed data on modifications responsible for the increased IgE binding are still lacking. Interestingly, there are observations on different grades of allergenicity to the oils of peanut, soybean and sesame [37•]. The latter is cold-pressed and nonetheless associated with anaphylactic reactions [37•], whereas the discussion regarding the sensitizing potential of peanut oil remains controversial [56].

Prevalence of Oleosin Sensitization

Data about the sensitization prevalence to seed oleosins are generally rare. A study conducted by Leduc et al. demonstrated a consistent sensitization to the sesame oleosins, Ses i 4 and Ses i 5, in 29 of 32 sesame-allergic patients with systemic reactions [37•]. As their study was not intended to evaluate the sensitization prevalence to oleosins, patients displaying mild symptoms or sesame-sensitized individuals without allergic symptoms were not included. Therefore, substantial prevalence data about the oleosin sensitization in sesame-allergic patients are still missing.

There are two studies available which partially reflect the sensitization to hazelnut oleosins. The first study utilizes the oil body fraction of hazelnut for RAST to evaluate the sensitization to oleosins in a study population having different grades of severity [2•]. Positive RAST results (sIgE > 0.35 kU/L) were found in 36/91 of the tested individuals. Immunoblot analysis with 21 of these 36 individuals confirmed a sensitization to the oleosin Cor a 12. As IgE reactions

to further proteins were observable in the immunoblot, the authors described the presence of additional allergens, at least of Cor a 9 (11S globulin), in the oil body fractions. The conclusion drawn by the authors that an oleosin sensitization is associated with severe allergic symptoms in hazelnut allergy might be true but is slightly weakened by the fact that Cor a 9, which has also been associated with more severe symptoms, is present in their oil body preparations [2•].

In 2015, Datema et al. published the largest epidemiological study on oleosins so far with 423 hazelnut-allergic patients [57••]. In this study, the authors revealed a sensitization to the hazelnut oleosin, Cor a 12, between 10 and 25% across Europe. A lower prevalence (< 8%) was reported for the cities of Strasbourg and Łódź. Moreover, the authors showed that children and adolescents were more often sensitized to the oleosin than adults. This aspect was ascribed to an increasing prevalence of birch pollen-associated hazelnut allergy with age. Interestingly, a Cor a 12 sensitization correlated with sensitization to other oil-rich foods such as soybean, sesame, peanut, sunflower, but also with latex and diverse pollen. This raised the question whether a sensitization to pollen oleosins might be causative for IgE-mediated reactions to foods containing seed oleosins and prompted us to look for differences between oleosins from pollen and food allergen sources in the bioinformatics literature.

An analysis of oleosin genes revealed that pollen oleosins of *Brassicaceae* species such as *Arabidopsis thaliana* have evolved from seed oleosins [13]. However, their N-terminal domain is truncated whereas the C-terminal domain is usually longer than that of seed oleosins. Therefore, the overall shared sequence identity between pollen and seed oleosins is rather

low, except for the conserved hydrophobic domain which is considered to be non-antigenic [24, 38•]. As a consequence, allergic cross-reactions seem to be unlikely. Our data obtained from the mapping of IgE-epitopes of Ara h 15 and other seed oleosins support this thesis (Jappe and Schwager, *personal communication*). A BLAST search of the identified IgE-binding sequences revealed a sequence similarity to other seed oleosins rather than to pollen oleosins. Nevertheless, this method is limited to the detection of sequential epitopes, and thus we cannot completely rule out the possibility of conformational epitopes that might be responsible for allergic cross-reactions between oleosins of different plant organelles.

The observation of Schwager et al.—in contrast to the study of Datema and co-workers—does not point to a pollen-associated oleosin-sensitization: They recruited 74 peanut-allergic patients with different clinical phenotypes in Northern Germany. Among them, 52 individuals displayed an oleosin sensitization. All of these patients reacted to at least two different oleosins purified from roasted peanuts in immunoblot experiments ([38•], expanded study population). All of the oleosin-sensitized peanut-allergic patients had rather severe allergic reactions after peanut consumption, pointing to oleosins as potential marker allergens for the severity of an allergic reaction (Fig. 2). 22/74 patients who had pollen-

associated peanut allergy did not reveal oleosin sensitization. One of them, although Ara h 8- and not storage protein-sensitized, had experienced a severe reaction after peanut consumption, an observation that rarely occurs when the concentration of the heat labile Bet v 1-homologue is high enough [58]. In our cohort of oleosin-sensitized peanut-allergic patients, a genuine peanut allergy developed mostly in childhood and was usually not accompanied by a pollen allergy at this time.

This is an important finding because according to Datema et al., oleosins show a sensitization pattern that is similar to profilins and CCDs [57•]. However, oleosins do not seem to be glycoproteins [59]; therefore, a cross-reactivity mediated by cross-reactive carbohydrate determinants (CCD) can be excluded.

Diagnostic Value of Lipophilic Allergens: Potential of Closing Diagnostic Gaps

Diagnostic tests are based on aqueous extracts. However, oleosins are most hydrophobic and, therefore, nearly insoluble proteins, which hampers immunoreactivity measurement. For that reason, some patients are not diagnosed adequately by skin prick tests and IgE-detection methods. Zuidmeer-Jongejan et al. showed that oleosins are virtually absent in 9 commercially available hazelnut extracts which are used for skin prick test solutions which is due to the procedure of aqueous extraction that includes defatting steps [2•]. Hence, it is highly probable that patients with a positive history of allergy to oil seeds and negative diagnostic tests may be oleosin-IgE positive. Forty-one percent of those patients who had IgE against the oil body fraction were negative in skin prick test performed with commercially available solutions. Ninety percent of those who were negative in the in vitro test with oil body fraction were positive in skin prick test. It can be hypothesized that these individuals suffered from a pollen-associated rather than a primary hazelnut allergy.

Up to now, the number of well-characterized patients investigated for specific IgE to oleosins is still small. However, from initial studies and the most recent investigation on different phenotypes of peanut allergy, there is strong evidence for their potential as marker allergens for severe allergic reactions [2•, 37•, 38•].

Already there are allergenic components used as treatment decision markers (hymenoptera venom components), treatment efficacy indicators (Bet v 1, Phl p 1 + 5), and severity markers (Ara h 2) (summarized in [60]). Whether oleosins can be used as such will depend on the allergen preparation, the method, and the number of well-characterized patients investigated in the near future.

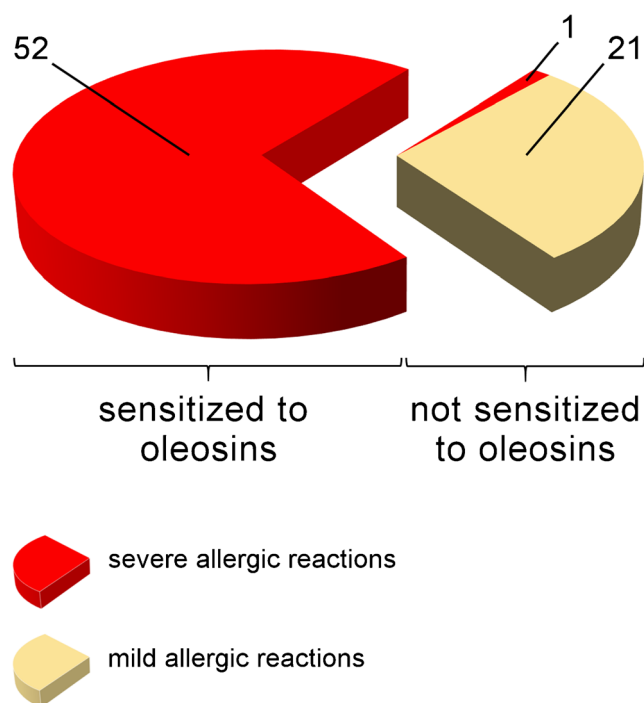


Fig. 2 Association of severe allergic reactions after peanut ingestion with IgE to peanut oleosins in different subgroups of peanut allergic patients. $N = 74$ patients were investigated, 53 experienced severe reactions, 52 had a genuine peanut allergy and had IgE to oleosins, 1 other individual with severe reactions was a patient with a pollen-associated peanut allergy. This patient was anti-oleosin-IgE negative. All those with mild symptoms were patients with a pollen-associated peanut allergy

Is the Diagnostic Capacity of Oleosin-Based Tests Probably Depending on the Method?

The identification of oleosin-sensitized individuals was realized so far by immunoblot, ImmunoCAP, RAST or the investigation of basophil reactivity. However, one has to keep in mind that in most studies, the experiments were carried out with oil body fractions which were virtually contaminated with other allergens [2•, 43, 57••].

Immunoblot

SDS-PAGE and subsequent immunoblot analysis was the method of choice for allergological investigation of oleosins due to their limited solubility in detergent-free buffers. With respect to peanut, clearly visible bands indicating an IgE-binding were only obtained when using oleosins from roasted seeds, as described above [38••]. In contrast, a sensitization to hazelnut and sesame oleosins was identified by using the raw material [2•, 37•]. Another problem that had to be faced is the close similarity of molecular masses of the oleosins. Total separation of the single molecules is difficult to achieve, and if applied as mixture the proteins might be differentiable by SDS-PAGE but appear most often as smear in immunoblots [2•, 18••, 38••]. Moreover, oleosins tend to form IgE-reactive dimers and oligomers which can be observed on the membrane, too [30, 35, 38••].

Pons et al. were the first that used purified oleosins for immunoblot experiments. However, they had hardly visible protein bands [36]. Applying the oleosin-enriched membrane fraction of their baculovirus cells, they found 3/14 sera from patients with peanut allergy reactive with the 18 kDa-oleosin (later registered as Ara h 14) but also to further protein bands. In the first publication on peanut oleosins in which the natural purification of all natural oleosins from roasted peanuts was reported, sera from 3/4 patients with severe symptoms after peanut ingestion had IgE positivity in immunoblot [18••].

(Automated) ELISA Techniques

ELISA techniques allow for the use of native allergens and the detection of IgE against conformational epitopes in contrast to immunoblot where oleosins are denatured. However, no standardized natural or recombinant oleosin is available for these routine diagnostic tests, so far. Therefore, oil body preparations have been used in initial studies [2•, 43, 57••]. For the measurements, they used the soluble part of these preparations which might be problematic as oil bodies were contaminated with Cor a 9 and further unknown proteins. As a consequence, proteins that are less soluble, such as the oleosins, might be discriminated during these procedures. This might have affected the outcome of these tests as the patients' reactivity cannot be considered as solely oleosin-specific. Since the

solubility of natural oleosins in detergent-free buffers will remain a problem, attempts to create water-soluble IgE-reactive oleosins have been undertaken [38••]. The results of these efforts will be discussed in the next section.

Cell-Based In Vitro Diagnostics

Cell-based diagnostic tests like the basophil activation test became more prominent within the last years as these tests are able to mimic allergic reactions in a physiological system [61]. So far, they have not become routine measures because of restrictions such as high costs [62] and elaborate experimental logistics [61]. However, reactivity of oleosins from different sources in cellular assays, i.e. BAT revealed their ability to be mediators of allergic reactions [2•, 38••, 41]. Moreover, it confirmed differences in the recognition of oleosins of raw and roasted peanut by basophil-bound IgE, pointing to the latter as more potent to elicit allergic reactions. A ROC-curve analysis showed that the use of oleosins obtained from roasted peanuts in BAT can identify all peanut-allergic patients suffering from severe allergic reactions [38••]. However, the application of an oleosin mixture from raw peanuts showed only minor differences when it came to sensitivity and specificity of the BAT [38••]. Despite these results, the main problem of oleosins, their poor solubility, still remained, as only a low amount of oleosins was recovered after the necessary dialysis against BAT buffer. Again, recombinant molecules might overcome this issue.

Recombinant Production of a Water-Soluble Peanut Oleosin (rAra h 15)

As already stated, natural oleosins show a highly restricted solubility in detergent-free solutions which is due to the hydrophobic core domain of oleosins. This part of the molecule is responsible for the steric position of the oleosins in the oil bodies. It is thought to be non-antigenic [24], which is why approaches of its biotechnological omission or the construction of an artificial oleosin without the hydrophobic core domain deemed a sensible one for being successful in improving the solubility in aqueous solutions like aqueous-based routine diagnostics [38••]. The final product, a recombinant Ara h 15 was soluble in milligramme quantities in BAT buffer whereas the solubility of the natural oleosins was soluble in microgramme quantities [38••]. Interestingly, the single recombinant Ara h 15 was nearly as reactive in BAT as the natural oleosin mixture from raw peanuts. By providing the other peanut oleosins Ara h 10, Ara h 11 and Ara h 14 as additional recombinant analytes, the sensitivity and specificity of the BAT with oleosins might be further improved. Presently, the reactivity of recombinant molecules in immunoblot does not seem to be satisfactory ([36] and own observation). Nevertheless, they are promising molecules for component resolved diagnostics in the future.

What Are the Problems with Oleosins as Diagnostic Tool?

Natural oleosins are nearly insoluble in aqueous/saline solutions. Some of the oleosins in their respective food allergen source have a similar molecular weight (Ara h 10 and 11; Ara h 14 and 15), which makes it difficult to separate them to produce single components (see above) for methodologically different test procedures (immunoblot, ELISA). Furthermore, they tend to form dimers, which poses a problem in immunoblot. Since the insolubility remains a huge problem, immunoblots are still performed under reducing conditions with detergent treatment.

The fact that IgE-reactive epitopes are basically present on the hydrophilic domains and not on the central hydrophobic core domain was used for the production of a modified recombinant oleosin which proved soluble and moderately reactive in BAT, whereas the usability in immunoblot is unsatisfactory. Application in ELISA is under way. Once the problem of insolubility has been overcome, conformational epitopes on oleosins can be investigated as well and be used for diagnostic tests in the future.

Which Variant of Allergen Should Be Provided for Which Diagnostic Tests?

When it comes to the application of oleosins for diagnostic tests, one has to make a decision between natural and recombinant molecules. With regard to sensitivity and specificity, purified natural molecules are the best choice for patient screening by immunoblot analysis, and in case of peanut they should be obtained from roasted seeds, whereas in the case of other food allergen sources, it seems to be reasonable to use oleosins from raw material, which should take cultural habits of food consumption into account. In cellular tests, water-soluble recombinant oleosins as single molecules or mixtures should be applied due to a higher sensitivity of the test. Presently, there are no comparative data on the performance of purified natural and the respective recombinant oleosins available.

Conclusion

In general, DBPCFC is the gold standard in food allergy diagnosis, and in many cases the only proof of an allergy to a certain food allergen source. It is nonetheless risky, time consuming and costly. Therefore, it is valuable to focus research efforts on molecular allergy diagnostics and the evaluation of new allergenic molecules for clinical relevance and their marker (biomarker!) potential.

The available literature summarized here unanimously points to the fact that oleosins have the potential to be markers for the grade of severity in food allergies, and thus they should be included in routine measurements as soon as possible to allow for an improved risk management of the respective patients. Thereby, it is of the essence to provide tests with the most suitable allergen preparation: nearly pure natural oleosins most reactive with IgE (roasted/raw) in immunoblot and BAT, whereas recombinant molecules might be more suitable in automated ELISA techniques. In parallel, the investigation of (large) cohorts of well-characterized patients should be performed in order to consolidate the value of oleosins for precision medicine.

Acknowledgements Patients whose data were calculated for Fig. 2 provided informed consent for the study on peanut allergens, approved by the local ethics committee, University of Luebeck, Germany, approval numbers 10-126 and 16-268.

Compliance with Ethical Standards

Conflict of Interest The authors report grants and personal fees from German Research Foundation.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

Funding The oleosin project was funded by the German Research Foundation (DFG) (grant no. JA 1007/2-1).

References

Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major importance

1. Jappe U, Nikolic J, Opitz A, Homann A, Zabel P, Gavrovic-Jankulovic M. Apparent IgE negative anaphylactic reaction to banana combined with kiwi allergy-complementary diagnostic value of purified single banana allergens. *Journal of the European Academy of Dermatology and Venereology: JEADV*. 2016;30(7):1220–2.
2. Zuidmeer-Jongejan L, Fernandez-Rivas M, Winter MG, Akkerdaas JH, Summers C, Lebens A, et al. Oil body-associated hazelnut allergens including oleosins are underrepresented in diagnostic extracts but associated with severe symptoms. *Clinical and Translational Allergy*. 2014;4(1):4. **The first study investigating the content of (hazelnut) oleosins in routine allergy diagnostic extracts revealing their absence and, thereby, the poor sensitivity of routine allergy tests for anti-oleosin-IgE detection. Additionally, they demonstrated the association between oleosin sensitization and the severity of symptoms**
3. Teodorowicz M, Terlouw RJ, Jansen A, Savelkoul HF, Ruinemans-Koerts J. Immunological characterization of Dutch sesame seed-allergic patients. *Int Arch Allergy Immunol*. 2016;169(1):13–22. **This investigation showed that oleosin was identified as the**

- major allergen for patients with anaphylaxis to sesame seed, whereas one patient with only mild symptoms to sesame consumption did not have anti-oleosin-IgE. The number of patients is small. However, for peanut allergic patients, Schwager et al. could later demonstrate a similar sensitization pattern in a much larger cohort
4. Aalberse JA, Meijer Y, Derksen N, van der Palen-Merkus T, Knol E, Aalberse RC. Moving from peanut extract to peanut components: towards validation of component-resolved IgE tests. *Allergy*. 2013;68(6):748–56. **Oleosins are mentioned as potential component candidates that behave differently in RAST and immunoblots**
 5. Beyer K, Grabenhenrich L, Haertl M, Beder A, Kalb B, Ziegert M, et al. Predictive values of component-specific IgE for the outcome of peanut and hazelnut food challenges in children. *Allergy*. 2015;70(1):90–8. **This investigation showed the value of Ara h 2-specific IgE (>42.2 kU/L) to estimate the probability for a positive challenge outcome in the diagnostic work-up of peanut allergy making some food challenges superfluous, confirming previous reports on Ara h 2 as marker allergen for the severity of the allergic reaction**
 6. Petersen A, Kull S, Rennert S, Becker WM, Krause S, Ernst M, et al. Peanut defensins: novel allergens isolated from lipophilic peanut extract. *J Allergy Clin Immunol*. 2015;136(5):1295–301. **Original description of a new class of peanut allergens isolated from lipophilic extract, the defensins (Ara h 12 and Ara h 13), particularly reactive with IgE of patients with severe peanut allergy and which demonstrated their reactivity in basophil activation test**
 7. Petersen A, Rennert S, Kull S, Becker W-M, Notbohm H, Goldmann T, et al. Roasting and lipid binding provide allergenic and proteolytic stability to the peanut allergen Ara h 8. *Biol Chem*. 2014;395(2):239–50. **A study that investigated structural allergen-lipid interaction and demonstrated that IgE reactivity and thermal and proteolytic stability are reinforced in natural Ara h 8 after roasting, most likely due to Maillard reactions, lipid oxidations, and lipophilic associations**
 8. Bublin M, Eiwegger T, Breiteneder H. Do lipids influence the allergic sensitization process? *J Allergy Clin Immunol*. 2014;134(3):521–9.
 9. Kull S, Vrtala S, Jappe U. Bekannte Einzelallergene der Hausstaubmilben: Struktur, Funktion und Relevanz Allergologie. 2015;38:55–63.
 10. Kyte J, Doolittle RF. A simple method for displaying the hydrophobic character of a protein. *J Mol Biol*. 1982;157(1):105–32.
 11. Liu F, Zhang X, Lu C, Zeng X, Li Y, Fu D, et al. Non-specific lipid transfer proteins in plants: presenting new advances and an integrated functional analysis. *J Exp Bot*. 2015;66(19):5663–81.
 12. Beisson F, Ferte N, Vouloury R, Arondel V. Large scale purification of an almond oleosin using an organic solvent procedure. *Plant Physiol Biochem*. 2001;39(7):623–30.
 13. Huang M-D, Huang AH. Bioinformatics reveal five lineages of oleosins and the mechanism of lineage evolution related to structure/function from green algae to seed plants. *Plant Physiol*. 2015;169(1):453–70.
 14. Jayaram S, Bal A. Oleosomes (lipid bodies) in nitrogen-fixing peanut nodules. *Plant Cell Environ*. 1991;14(2):195–203.
 15. Huang C-Y, Chung C-I, Lin Y-C, Hsing Y-IC, Huang AH. Oil bodies and oleosins in *Physcomitrella* possess characteristics representative of early trends in evolution. *Plant Physiol*. 2009;150(3):1192–203.
 16. Huang AH. Oil bodies and oleosins in seeds. *Annu Rev Plant Biol*. 1992;43(1):177–200.
 17. Tzen J, Cao Y, Laurent P, Ratnayake C, Huang A. Lipids, proteins, and structure of seed oil bodies from diverse species. *Plant Physiol*. 1993;101(1):267–76.
 18. Schwager C, Kull S, Krause S, Schocker F, Petersen A, Becker WM, et al. Development of a novel strategy to isolate lipophilic allergens (oleosins) from peanuts. *PLoS One*. 2015;10(4):e0123419. **A novel way to isolate all known and immunologically distinct peanut oleosins simultaneously was reported. The first study in which the allergenicity of all peanut oleosins was proven. As a consequence, Ara h 14 and Ara h 15 were documented by the WHO/IUIS Allergen Nomenclature Subcommittee**
 19. Shimada TL, Hara-Nishimura I. Oil-body-membrane proteins and their physiological functions in plants. *Biol Pharm Bull*. 2010;33(3):360–3.
 20. Lin LJ, Tzen JT. Two distinct steroleosins are present in seed oil bodies. *Plant Physiol Biochem*. 2004;42(7–8):601–8.
 21. Tzen J, Wang M, Chen J, Lin L, Chen M. Seed oil body proteins: oleosin, caleosin, and steroleosin. *Current Topics in Biochemical Research*. 2003;5:133–9.
 22. Frandsen GI, Mundy J, Tzen JT. Oil bodies and their associated proteins, oleosin and caleosin. *Physiol Plant*. 2001;112(3):301–7.
 23. Murphy DJ, Keen JN, Osullivan JN, Au DMY, Edwards EW, Jackson PJ, et al. A class of amphipathic proteins associated with lipid storage bodies in plants. Possible similarities with animal serum apolipoproteins. *Biochim Biophys Acta*. 1991;1088(1):86–94.
 24. Tzen JT, Lai Y-K, Chan K-L, Huang AH. Oleosin isoforms of high and low molecular weights are present in the oil bodies of diverse seed species. *Plant Physiol*. 1990;94(3):1282–9.
 25. Tzen JT, Peng CC, Cheng DJ, Chen EC, Chiu JM. A new method for seed oil body purification and examination of oil body integrity following germination. *The Journal of Biochemistry*. 1997;121(4):762–8.
 26. Millichip M, Tatham AS, Jackson F, Griffiths G, Shewry PR, Stobart AK. Purification and characterization of oil-bodies (oleosomes) and oil-body boundary proteins (oleosins) from the developing cotyledons of sunflower (*Helianthus annuus* L.). *Biochem J*. 1996;314(Pt 1):333–7.
 27. Leprince O, Van Aelst A, Pritchard H, Murphy D. Oleosins prevent oil-body coalescence during seed imbibition as suggested by a low-temperature scanning electron microscope study of desiccation-tolerant and -sensitive oilseeds. *Planta*. 1997;204(1):109–19.
 28. Parthibane V, Rajakumari S, Venkateshwari V, Iyappan R, Rajasekharan R. Oleosin is bifunctional enzyme that has both monoacylglycerol acyltransferase and phospholipase activities. *J Biol Chem*. 2012;287(3):1946–54.
 29. Li M, Smith LJ, Clark DC, Wilson R, Murphy DJ. Secondary structures of a new class of lipid body proteins from oilseeds. *J Biol Chem*. 1992;267(12):8245–53.
 30. Pons L, Chery C, Mrabet N, Schohn H, Lapique F, Gueant JL. Purification and cloning of two high molecular mass isoforms of peanut seed oleosin encoded by cDNAs of equal sizes. *Plant Physiol Biochem*. 2005;43(7):659–68.
 31. Capuano F, Beaudoin F, Napier JA, Shewry PR. Properties and exploitation of oleosins. *Biotechnol Adv*. 2007;25(2):203–6.
 32. Chen JC, Tsai CC, Tzen JT. Cloning and secondary structure analysis of caleosin, a unique calcium-binding protein in oil bodies of plant seeds. *Plant Cell Physiol*. 1999;40(10):1079–86.
 33. Lin L-J, Tai SS, Peng C-C, Tzen JT. Steroleosin, a sterol-binding dehydrogenase in seed oil bodies. *Plant Physiol*. 2002;128(4):1200–11.
 34. Olszewski A, Pons L, Moutete F, Aimone-Gastin I, Kanny G, Moneret-Vautrin DA, et al. Isolation and characterization of proteic allergens in refined peanut oil. *Clin Exp Allergy*. 1998;28(7):850–9.
 35. Pons L, Olszewski A, Gueant JL. Characterization of the oligomeric behavior of a 16.5 kDa peanut oleosin by chromatography and

- electrophoresis of the iodinated form. *J Chromatogr B*. 1998;706(1):131–40.
36. Pons L, Chery C, Romano A, Namour F, Artesani MC, Gueant JL. The 18 kDa peanut oleosin is a candidate allergen for IgE-mediated reactions to peanuts. *Allergy*. 2002;57(Suppl 72):88–93.
 37. Leduc V, Moneret-Vautrin DA, Tzen JT, Morisset M, Guerin L, Kanny G. Identification of oleosins as major allergens in sesame seed allergic patients. *Allergy*. 2006;61(3):349–56. **This study provided the first proof for the allergenicity of sesame oleosins, Ses i 4 and Ses i 5 (first oleosins registered as allergens). Hypothesized a correlation between oleosin sensitization and severe anaphylaxis. Suspected the absence of oleosins from commercial SPT extracts**
 38. Schwager C, Kull S, Behrends J, Röckendorf N, Schocker F, Frey A, et al. Peanut oleosins associated with severe peanut allergy—importance of lipophilic allergens for comprehensive allergy diagnostics. *J Allergy Clin Immunol*. 2017 Mar 22. pii: S0091-6749(17)30417–7. doi:10.1016/j.jaci.2017.02.020. **The first study on 52 peanut allergic patients with different phenotypes that revealed sensitization to peanut oleosins only in patients with severe peanut allergy, the effect of thermal processing on the IgE-binding capacity of naturally purified oleosins and their reactivity in basophil activation test. Production of a water-soluble IgE-reactive recombinant oleosin as a promising candidate for future component-resolved diagnosis. Identification of an IgE-binding epitope of Ara h 15.**
 39. Leonart R, Andrés B, Pineda F, Labrador M, Corominas M. Anaphylaxis caused by flaxseed. *Clinical and Translational Allergy*. 2015;5(S3):P58.
 40. Armentia Medina A, Pineda F, Martín B, San Miguel A, Gil M, Javier F, et al. Anaphylaxis caused by hidden soybean allergens in pillows. *J Allergy Clin Immunol*. 2013;131(1):228–30.
 41. Macías ML, Gómez F, Díaz A, Mayorga C, Aranda A, González M, et al. Basophil response to storage proteins and oleosins from sunflower seed. *J Allergy Clin Immunol*. 133(2):AB111.
 42. Lacomba JM, Witek JD, de la Losa FP, Cerdá VJ. Oral allergy syndrome due to nut oleosins. *J Investig Allergol Clin Immunol*. 2015;25(4):301–2.
 43. Santos A, Zuidmeer L, Summers C, Marsh J, Knulst A, van Ree R. Oleosin, the missing link in the diagnosis of allergy to walnut. *Allergy*. 2009;64:27.
 44. Kobayashi S, Katsuyama S, Wagatsuma T, Okada S, Tanabe S. Identification of a new IgE-binding epitope of peanut oleosin that cross-reacts with buckwheat. *Biosci Biotechnol Biochem*. 2012;76(6):1182–8.
 45. Akkerdaas JH, Schocker F, Vieths S, Versteeg S, Zuidmeer L, Hefle SL, et al. Cloning of oleosin, a putative new hazelnut allergen, using a hazelnut cDNA library. *Mol Nutr Food Res*. 2006;50(1):18–23.
 46. Chen JC, Lin R-H, Huang H-C, Tzen JT. Cloning, expression and isoform classification of a minor oleosin in sesame oil bodies. *The Journal of Biochemistry*. 1997;122(4):819–24.
 47. Hyun TK, Kumar D, Cho Y-Y, Hyun H-N, Kim J-S. Computational identification and phylogenetic analysis of the oil-body structural proteins, oleosin and caleosin, in castor bean and flax. *Gene*. 2013;515(2):454–60.
 48. Herman EM. Immunogold-localization and synthesis of an oil-body membrane protein in developing soybean seeds. *Planta*. 1987;172(3):336–45.
 49. Cummins I, Murphy DJ. cDNA sequence of a sunflower oleosin and transcript tissue specificity. *Plant Mol Biol*. 1992;19(5):873–6.
 50. Cao H, Zhang L, Tan X, Long H, Shockey JM. Identification, classification and differential expression of oleosin genes in tung tree (*Vernicia fordii*). *PLoS One*. 2014;9(2):e88409.
 51. Commission Directive 2006/142/EC of 22 December 2006 amending Annex IIIa of Directive 2000/13/EC of the European Parliament and of the Council listing the ingredients which must under all circumstances appear on the labelling of foodstuffs. *Official Journal of the European Union*. 2006;368(110):110–1.
 52. Mondoulet L, Paty E, Drumare MF, Ah-Leung S, Scheinmann P, Willemot RM, et al. Influence of thermal processing on the allergenicity of peanut proteins. *J Agric Food Chem*. 2005;53(11):4547–53.
 53. Maleki SJ, Chung S-Y, Champagne ET, Raufman J-P. The effects of roasting on the allergenic properties of peanut proteins. *J Allergy Clin Immunol*. 2000;106(4):763–8.
 54. Chung SY, Champagne ET. Association of end-product adducts with increased IgE binding of roasted peanuts. *J Agric Food Chem*. 2001;49(8):3911–6.
 55. Johnson KL, Williams JG, Maleki SJ, Hurlburt BK, London RE, Mueller GA. Enhanced approaches for identifying amadori products: application to peanut allergens. *J Agric Food Chem*. 2016;64(6):1406–13.
 56. Ring J, Mohrenschlager M. Allergy to peanut oil-clinically relevant? *J Eur Acad Dermatol Venereol*. 2007;21(4):452–5.
 57. Datema MR, Zuidmeer-Jongejan L, Asero R, Barreales L, Belohlavkova S, de Blay F, et al. Hazelnut allergy across Europe dissected molecularly: a EuroPrevall outpatient clinic survey. *J Allergy Clin Immunol*. 2015;136(2):382–91. **The first study in which hazelnut oleosin-sensitization (Cor a 12) was investigated in a European multicentre and multidisciplinary study (EuroPrevall project). One methodological problem, however, is that probably Cor a 12 was contaminated with storage proteins, which might have affected the sensitization pattern in childhood and adulthood**
 58. Glaumann S, Nopp A, Johansson SGO, Borres MP, Lilja G, Nilsson C. Anaphylaxis to peanuts in a 16-year-old girl with birch pollen allergy and with monosensitization to Ara h 8. *Journal of Allergy and Clinical Immunology: In Practice*. 2013;1(6):698–9.
 59. Cao Y, Zhao L, Ying Y, Kong X, Hua Y, Chen Y. The characterization of soybean oil body integral oleosin isoforms and the effects of alkaline pH on them. *Food Chem*. 2015;177:288–94.
 60. Jappe U. Molecular-allergological aspects of allergen-specific immunotherapy. *Hautarzt*. 2017;68(4):271–81.
 61. Hoffmann HJ, Santos AF, Mayorga C, Nopp A, Eberlein B, Ferrer M, et al. The clinical utility of basophil activation testing in diagnosis and monitoring of allergic disease. *Allergy*. 2015;70(11):1393–405.
 62. Chirumbolo S. Basophil activation test in allergy: time for an update? *Int Arch Allergy Immunol*. 2012;158(2):99–114.