



Oleosins: A Short Allergy Review

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Abstract

In patients having a history of anaphylaxis after consumption of peanuts, sunflower seeds, or soy and skin or blood tests negative for the allergen extracts, oleosins could be the culprit. Oleosins are common and largely underestimated allergy inducers of plant origin, causing severe allergy symptoms, including the anaphylactic shock. They are resistant to high temperatures and digestive enzymes. The consumption of heat-treated oleosins has been associated with a higher risk of a severe anaphylactic reaction. Recent studies have shown that oleosins could be a biomarker of the allergy severity to peanuts. Oleosins have a hydrophobic structure and thus, are poorly

soluble in aqueous solutions. The aqueous extraction, separation, and purification procedures do not guarantee their solubility. Oleosins dissolve only in the presence of detergents, which limits their use in both in vivo and in vitro allergy tests. Recently, a multiparameter allergy test that detects the allergen-specific immunoglobulin E (sIgE) against oleosins has become available. This capability may help to unravel the presence of oleosin source during the routine diagnostic of allergy, which is conducive to assessing the risk for severe anaphylaxis and may also help to clarify the ambiguous allergy cases.

Keywords

Allergy · Anaphylaxis · Biomarker · Diagnostic tests · Oleosin · sIgE

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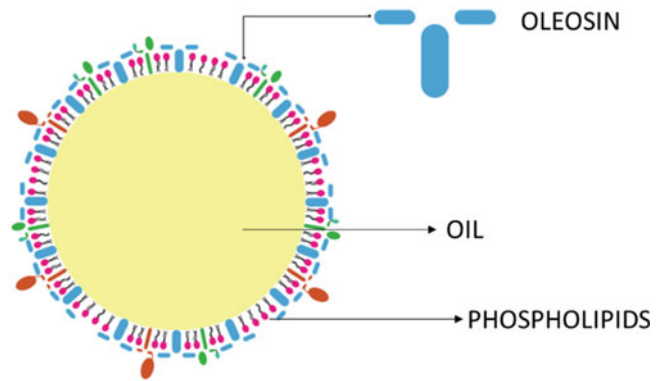
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1 Introduction

The consumption of peanuts, one of the most common allergen, may cause a severe anaphylactic reaction (Kleine-Tebbe and Jakob 2017). Allergy symptoms can be provoked by a minute quantity of peanuts (Blom et al. 2013). The early diagnosis of peanut allergy may prevent a severe anaphylactic reaction. Astonishingly, in some patients with symptoms and history of allergy to peanuts or other seeds like sesame and hazelnut, the skin prick test (SPT) and the identification of

Fig. 1 A scheme of an oleosome, oil droplet. Oleosins are the key proteins present in the membrane of an oil body



specific immunoglobulins E (sIgE) against allergen extracts may not firmly establish the sensitization (Ehlers et al. 2019; Schwager et al. 2017). Double-blind placebo-controlled food challenges are necessary in such cases. This type of examination is expensive and potentially dangerous for the patient, as it may result in anaphylactic shock. Recent advances in the molecular diagnostics, based on the *in vitro* detection of a serum sIgE level against allergen molecules, offer a way to resolve such difficult cases. That also includes allergy to traces of peanuts and other seeds in ingested food where the oleosin family of molecules might be responsible for the unexplained allergy symptoms.

Oleosins are plant-derived proteins with the molecular weight ranging from 15 kDa to 24 kDa. These protein molecules are constituents of oil bodies known as oleosomes that store triglycerides as an energy source during the germination process of seeds like peanuts, hazelnuts, walnuts, almonds, soybeans, sesame and rapeseeds (Zuidmeer-Jongejan et al. 2014). The central part of the oleosome consists of a hydrophobic domain referred to as a core, built of 72 amino acids, the longest known hydrophobic domain. The N-terminal domains, present on the outer ring, are either hydrophilic or amphipathic, whereas the C-terminal domain is always amphipathic. Oleosins, due to a unique structure, stabilize the oleosomes, preventing them from coalescence (Fig. 1). Oleosins are common energy storage proteins in plants. The first identified oleosin has been a peanut protein

(Pons et al. 2002). Since then a few more peanut oleosin allergens have been discovered, such as Ara h 10, Ara h 11, Ara h 14, and Ara h 15. The hazelnut oleosin allergens are Cor a 12, Cor a 13, and Cor a 14. The sesame seeds contain the molecules Ses i 4 and Ses i 5, belonging to the oleosin family (Jappe et al. 2019). The general characteristics of oleosins are presented in Table 1.

The hydrophobic nature of oleosins makes them hardly soluble in aqueous solutions (Ehlers et al. 2017; Schwager et al. 2017). Therefore, the use of oleosins in both *in vivo* and *in vitro* allergen testing is limited, unless a detergent is added to the system (Scala et al. 2018). An important feature of oleosins is the resistance to heat and digestion. There is a biological plausibility that stability of the oleosins' structure is conducive to their high potential of evoking allergy symptoms, including anaphylactic shock (Kleine-Tebbe and Jakob 2017).

2 Clinical Implications

Schwager et al. (2017) have investigated the capacity of sIgE to bind with Ara h 10/11 and Ara h 14/15 in the serum of patients who experienced a severe anaphylactic reaction after eating peanuts. The authors found that sIgE was tightly attached to the molecules from roasted but not raw peanuts. The control group was formed by patients who were sensitized to peanuts but tolerated them and patients who were not

Table 1 Summary of oleosins' features in nuts and seed, the proteins of 15–24 kDa accumulating on the surfaces of oil bodies

Peanut: Ara h 10, Ara h 11, Ara h14, and Ara h 15
Hazelnut: Cor a 12, Cor a 13, and Cor a 14
Sesame: Ses i 4 and Ses i 5
Highly resistant to heat and digestion
Hydrophobic
Lipid soluble, which make them elude common extraction, separation, and purification procedures
Patients sensitized to oleosins from one kind of seeds are likely to be cross-sensitized to oleosins from other sources, which is explicable by the presence of the identical amino acid sequence in C-terminal
Induction of sIgE in the blood is responsible for allergy symptoms after consumption of oleosin-containing food
Food processing at high temperature, e.g., roasting, is associated with a higher risk of allergic symptoms
Introduction of the detection of sIgE against oleosins into the routine diagnostic tests would facilitate the risk assessment for anaphylaxis and help explain unequivocal symptomatic cases linked to consumption of seed-containing food
Oleosins are a candidate biomarker of allergic symptom severity to peanuts; the most widely consumed nuts

sensitized to peanuts. In both groups, serum sIgE failed to bind either to molecules derived from raw or roasted peanuts. Then, the basophil activation test was performed with peanut molecules that belong to the oleosin family. In the test, higher reactivity was noticed when oleosins from roasted peanuts were used, which was particularly evident in patients with a history of severe allergic symptoms. The explanation of these findings might be that the process of roasting deprives oleosins of a protective effect of lipids, which delays the peanut digestion (Kleine-Tebbe and Jakob 2017). Consequently, the consumption of oleosins that underwent thermal processing may increase the risk of severe anaphylaxis. Studies performed with sesame seeds and hazelnuts have also confirmed that consumption of thermally processed seeds facilitates the development of allergy symptoms (Zuidmeer-Jongejan et al. 2014; Leduc et al. 2006). Therefore, there appears a consistent impression that the most relevant explanation of severe anaphylactic reaction in peanut allergy is the presence of sIgE against oleosins in the serum (Schwager et al. 2017; Ballmer-Weber et al. 2015).

The risk assessment of severe anaphylaxis in peanut allergy seems essential since this kind of allergy is widespread. Every fourth patient sensitized to peanuts has sIgE against oleosins in the serum. Moreover, sensitization to oleosins is more common among children than among

adults (Datema et al. 2015). Therefore, the introduction of oleosins into the routine diagnostic tests would significantly improve the diagnostics and would help tailor the therapeutic recommendations to the sensitization profile of a patient. The only one test allowing the detection of sIgE against oleosins is a third-generation multiparameter ALEX2[®] that reveals if the patient is sensitized to the peanut oleosin Ara h 15.

Regarding peanut allergy, another important aspect concerning the molecules Ara h 8 (a representative of PR-10 group) and Ara h 9 (belonging to nsLTP) should be mentioned. In a raw peanut, these molecules are affiliated with lipids that protect the proteins from enzyme-mediated digestion in the intestinal tract (Jappe et al. 2019). A connection between Ara h 8 and lipids explains why mono-sensitization against this molecule is responsible for peanut tolerance or mild allergic symptoms. There is evidence that roasting and long-term storage of peanuts causes oxidation of the protective lipids attached to Ara h 8, which leads to structural changes in the molecule or the formation of other compounds. Hence, resistance to heat and digestion resistance of the molecule is reduced and sIgE against Ara h 8 becomes more reactive (Bublin et al. 2014). Enhanced sIgE binding to antigens after roasting has also been noticed for other peanut molecules such as Ara h 9, Ara h 12, and Ara h 13. The last two of these molecules belong to the family of defensins (Jappe et al. 2019).

3 Cross-Reactions with Oleosins of Different Origin

As revealed in a study involving 423 patients, who lived in the European cities and were sensitized to hazelnuts, sensitization to oleosins is a common occurrence, ranging from 10% to 25% of the population (Datema et al. 2015). Referring to the cross-reactivity, the authors observe that sensitization to Cor a 12, a hazelnut oleosin, correlates with sensitization to fat-rich sesame and sunflower seeds. There also is cross-sensitivity to pollen from various plants and latex. In light of this report, the question arises of whether sensitization to pollen oleosins can result in IgE-mediated reactions caused by oleosins from seeds. Genetic analysis of oleosins might give a deeper insight into this issue. It has been shown that pollen oleosins evolved from seeds oleosins, but their N-terminal domain is shorter while the C-terminal domain is more built out. It means that there is little similarity between pollen and seed oleosins, except for the hydrophobic core. Since the core is thought to be non-antigenic, cross-reactivity between pollen and seed oleosins is rather unlikely to happen (Jappe and Schwager 2017).

Schwager et al. (2017) have formed a different hypothesis to explain cross-reactions in patients sensitized to oleosins, based on the analysis of a structure of rAra h 15 molecules in peanuts. The authors show that an amino acid sequence in the C-terminal domain of this molecule displays the highest IgE-binding affinity. It appears that the same sequence is present in oleosins from other seeds such as hazelnut, rapeseed, soybeans, sesame, and almond. Therefore, cross-reactions with oleosins from various seeds are more likely to happen in the serum of patients who are sensitized to oleosins from a seed source. Paraphrasing, patients who are sensitized to oleosins from a specific seed are more likely to have allergy symptoms after eating other seeds due to cross-reactions.

Jappe and Schwager (2017) have failed to show the association between sensitization to seed oleosins and pollen, investigating 74 peanut-

allergic patients from northern Germany. However, the authors suggested that oleosins could be regarded as a marker of symptoms severity in peanut allergy. They also found that 52 out of the 53 patients, who experienced an anaphylactic reaction after the consumption of peanuts, had sIgE against oleosins in the blood. The remaining 21 sensitized patients had neither appreciable symptoms related to peanut consumption nor sIgE against oleosins. Sensitization could result from the cross-reaction of immunoglobulins against other peanut and pollen allergen molecules. Pollen is rather doubtful as the oleosin-sensitized patients experienced first symptoms of genuine peanut allergy in childhood, which was unaccompanied by pollen allergy at the time. The lack of sIgE against peanut oleosins in the sensitized patients also puts into question the cross-reactivity with oleosins originating from other seeds. However, other possibilities remain as oleosins show sensitization pattern akin to profilins and cross-reactive carbohydrate determinants (Datema et al. 2015).

4 Benefits from Introducing Oleosins into Routine Allergy Diagnostic Tests

The development of new methods of extraction of allergen molecules extends the understanding of the mechanisms of allergic reactions. There appears a consensus that oleosins underlie the allergy to peanuts and other seeds. Therefore, these molecules should be included in routine diagnostic tests, with benefits to patients, as peanuts are considered wholesome and their consumption is ubiquitous. Peanuts are also the most common sensitizing allergens, often provoking dire health effects in the form of anaphylactic shock. The detection of sIgE against oleosins could help assess the risk of anaphylaxis. Moreover, the molecular diagnostics of sensitization against oleosins would enable the avoidance of troublesome and potentially dangerous food challenges in patients with a history of anaphylactic reaction after the contact with seeds and in

those with unequivocal results of skin prick test or sIgE against allergen extracts in vitro.

Conflicts of Interest The authors declare no conflicts of interest concerning this article.

Ethical Approval This review article does not contain any studies with human participants or animals performed by any of the authors.

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