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Clinical importance of non-specific lipid transfer proteins as food allergens

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Abstract

Non-specific lipid transfer proteins (nsLTPs) have recently been identified as plant food allergens. They are good examples of true food allergens, in the sense that they are capable of sensitizing, i.e. inducing specific IgE, as well as of eliciting severe symptoms. This is in contrast with most plant food allergens, which are recognized because of primary sensitization to related inhalant allergens (cross-reactivity), i.e. pollen allergens. The basis of the difference between the latter category and strong food allergens such as nsLTPs appears to lie in the sensitivity of the allergens to proteolytic attack and food processing. Stability allows the allergen to reach the gastrointestinal immune system in an immunogenic and allergenic conformation, allowing sensitization and induction of systemic symptoms. Stability also explains the presence of such allergens in processed foods. Together, these characteristics make nsLTPs clinically highly relevant plant food allergens and ideal tools with which to study the mechanisms involved in food allergy.

Introduction: history of the discovery of an allergen

Retrospectively, one can say that in 1992 Lleonart et al. [1] were the first to identify a low-molecular-mass (~10 kDa) allergen in peach, which was preferentially located in the peel of the fruit. This later turned out to be the non-specific lipid transfer protein (nsLTP) that was recently designated Pru p 3. In 1994, Pastorello et al. [2] described a group of patients that showed so-called oral allergy syndrome (OAS) upon consumption of peaches. In OAS, predominantly mild symptoms are restricted to the oral cavity [3]. Pastorello et al. [2]

reported a low-molecular-mass (~13 kDa) allergen which was recognized by all patients in their study, including those that were exclusively food-allergic, i.e. not allergic to pollen. Up to that date, allergy to fruits of the family of the *Rosaceae*, such as apple, pear, cherry and peach, was usually described as a phenomenon closely associated with birch pollinosis, i.e. mediated by cross-reactive IgE [4,5]. Obviously, the perception of allergy to fruits was dominated for decades by research from countries with significant exposure to birch pollen. In 1997, Fernandez-Rivas et al. [6] described a group of fruit-allergic patients from Central Spain without pollinosis. The majority of these patients had more severe symptoms than OAS. Although at that time it was not yet clear that the allergen recognized by these patients was the nsLTP, the report clearly distinguished non-pollen-related from pollen-related allergy to fruits, on the basis of the higher incidence of severe and life-threatening episodes. That same year, Pastorello and co-workers presented the identification of their low-molecular-mass allergen from peach as a nsLTP (European Academy of Allergology and Clinical Immunology Meeting, Rhodes, Greece). This milestone in plant food allergen research was finally published in 1999 [7], together with a paper from Salcedo and colleagues [8] that confirmed the identity of the major non-pollen-related peach allergen as a nsLTP. Subsequently, Asero et al. [9,10] were the first to report allergies to a broad range of non-fruit vegetable foods caused by nsLTPs. These authors also proposed the structural stability of nsLTPs as a major factor determining their allergenicity. Most recently, some of the major allergenic nsLTPs have been cloned and produced as recombinant molecules [11,12]. Now, 10 years after the first detection of peach LTP by Lleonart et al. [1], a picture has emerged of a molecule that has the typical characteristics needed to induce sensitization independent from pollen and to cause severe food allergy.

Key words: food allergy, fruit, nsLTP, sensitization, stability.

Abbreviations used: (ns)LTP, (non-specific) lipid transfer protein; OAS, oral allergy syndrome.

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nsLTPs: a stable barrel of four α -helices

nsLTPs belong to a group of structurally related proteins that are often referred to as α -class proteins. These proteins all have eight cysteine residues at conserved positions, forming four disulphide bridges that keep four α -helices together, resulting in a compact barrel-like structure. This structure gives nsLTPs their extreme resistance to proteolysis, heat denaturation and other forms of processing. Their stability is in extreme contrast with the fruit allergens that are closely related to the major birch pollen allergen Bet v 1. Related proteins from fruits such as apple (Mal d 1), pear (Pyr c 1), cherry (Pru av 1) and peach (Pru p 1) are digested by pepsin within seconds [9] and rapidly lose their allergenicity upon disruption of the tissue [13]. The latter process is an oxidative process mediated by polyphenol oxidases and their substrates, polyphenolic compounds. The production of good *in vivo* diagnostic products for fruit allergy (i.e. for skin testing) has always been hampered by immediate oxidation upon extraction. Techniques used to inhibit this process (depletion of phenolic compounds by polyvinylpyrrolidone and inhibition of polyphenol oxidase by addition of chelating agents such as diethyldithiocarbamate) are not compatible with *in vivo* applications. Interestingly, nsLTPs are insensitive to the oxidative attack that destroys the allergenicity of birch pollen-related fruit allergens. Asero and co-workers [14,15] have creatively used the poor quality of commercial skin-prick test reagents in their clinical practice to identify fruit-allergic patients that are sensitized to nsLTPs. *In vivo* diagnosis of birch pollen-related fruit allergy can only be performed reliably by the so-called prick-to-prick method with fresh fruits [16,17], or by double-blind placebo-controlled oral challenges [17,18]. For some fruits, homologues of Bet v 1 have been cloned and expressed as stable reagents that can replace the labile crude extracts for both *in vivo* and *in vitro* diagnostic tests [11,19–25].

Stability and severe symptoms go hand in hand

Fruit allergy linked to birch pollinosis is almost exclusively mild and restricted to the oral cavity (OAS). The labile allergens involved in this type of food allergy simply cannot reach the effector cells of the allergic response (mast cells) at the serosal side of the gastrointestinal epithelial lining

in an IgE-binding conformation. The acidic proteolytic environment of the stomach destroys these allergens instantly [9]. In contrast, nsLTPs have been shown to survive this digestive attack as fully IgE-reactive structures that can trigger mast cells in the vicinity of the epithelial monolayer [9]. In addition, resorption of protein is more efficient at this site than at the oral mucosa. It is now generally accepted that these factors explain why allergens such as nsLTPs can cause more severe symptoms and reach multiple target organs (OAS, generalized urticaria, asthma, vomiting, diarrhoea, hypotension and even anaphylactic shock). Bet v 1-related allergens depend on the poor resorption at the oral mucosa for entering the bloodstream to reach other target organs.

Still, several questions related to clinical presentation remain to be answered. Why do some LTP-sensitive patients only present with OAS, whereas others have systemic reactions? Does this mean that some patients do in fact recognize epitopes that are lost upon passage of the allergen through the stomach? Or do individual differences in clinical presentation depend on differences in sensitivity of the target organ? There is some evidence that differences in target organ sensitivity indeed play a role. A group of patients with house dust mite-induced rhinitis and/or asthma was reported to have food allergy caused by snails [26]. These patients, almost without exception, had rhinitis and/or asthmatic episodes after eating snails. Patients with isolated food allergies rarely have food-induced asthma. By analogy, target organ sensitivity probably also determines whether nsLTPs cause, e.g., generalized urticaria, asthma or anaphylactic shock. Most likely the genetic background as well as the health of the individual patient determine which organ will be the 'weak spot'.

Stability and the route of sensitization

IgE antibodies against food homologues of Bet v 1 are rarely, if ever, found in patients not sensitized to the pollen allergen. This implies that Bet v 1 is causing sensitization, and that the cross-reactive allergens from fruits cannot sensitize directly. The observation that IgE binding to fruit homologues can be completely inhibited by Bet v 1, but not vice versa, confirms the role of Bet v 1 as primary sensitizer. A few reports claim sensitization to vegetable food homologues in the absence of IgE against Bet v 1 or with IgE only partially cross-reactive to Bet v 1 [27]. In general, though, the immune system cannot be reached in an immuno-

genic conformation at an immunopotent site by these labile food allergens. In contrast, fruit-allergic patients sensitized to nsLTPs frequently do not have any pollen sensitization [2,6]. This suggests that these allergens can sensitize by encounter with the gastrointestinal immune system. In other words, the stability of nsLTPs not only makes them into allergens with the capacity to cause severe systemic symptoms, it also turns them into molecules that have the ability to induce specific IgE antibodies.

Processing of foods and allergenicity

The simplest form of processing with a clear effect on the allergenicity of fruits is peeling. LTPs tend to accumulate in peel and outer (wax) layers of fruits and other plant organs [28]. Peeling of apples, for example, significantly reduces the allergenicity of the fruit for some patients with IgE antibodies against nsLTPs [29]. For most patients, however, the peeled fruit still contains sufficient quantities of LTPs to induce symptoms. Production of fruit purees and juices frequently includes heating. In general, patients allergic to LTPs do not tolerate such products, suggesting that the LTP is heat stable [9,30]. The heat stability of LTPs has been demonstrated on several occasions [31]. Recently, a group of patients was described that had strong IgE-mediated reactions to LTPs in maize-based products such as the Italian dish polenta, which is extensively heated [32]. Nevertheless, some processes that include heating steps, such as the brewing of beer, might affect the allergenicity of nsLTPs. The foam of beer contains relatively high quantities of LTPs, but only a limited number of LTP-reactors do not tolerate beer [33–38]. Products in which flour of cereals is used, such as bread and pasta, are not among the major inducers of LTP-based allergic reactions. Whether the apparent low allergenicity of wheat, rye and barley LTPs is caused by processing steps needed to make the final food product, or whether cereal LTPs have a lower allergenicity as such, is still a matter of debate. Altogether, nsLTPs are allergens that exhibit a high degree of stability during food processing. This characteristic, combined with their resistance to proteases, stresses their clinical importance.

nsLTPs: model allergens of true food allergy

The identification and characterization of plant food nsLTPs as important food allergens, as well

as their availability as purified natural and recombinant molecules, now offers unique possibilities to study the process of sensitization and symptom elicitation in true food allergy, as opposed to the milder forms of food allergy linked to pollen sensitization.

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Stability of recombinant 2 S albumin allergens *in vitro*

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Abstract

Two well known 2 S albumins, Ber e 1 from brazil nut and sunflower 2 S albumin 8 (SFA-8), have been expressed in a eukaryotic system and purified. Analysis of recombinant versions of Ber e 1 and SFA-8 revealed them to be significantly more resistant to digestion by pepsin than BSA, and to be stable for up to 30 min in simulated gastric fluid. Unfolding monitored by CD indicated that both proteins were also very resistant to denaturation induced by heat and low pH. These results suggest that, although the ability of 2 S albumins to reach the circulatory system may be a prerequisite for the allergenicity of this group

of proteins, stability is just one of a number of characteristics that provoke a selective immune response.

Introduction

In light of the growing number of genetically engineered crops and the observation that the horizontal transfer of allergens can occur unintentionally during transgenic studies [1], a decision tree has been recommended to ensure the safety of foods derived from biotechnology [2,3]. The stability of proteins, as measured by resistance to proteolysis by simulated gastric fluid (SGF), is an important factor in the assessment of the allergenicity of proteins [4]. Many allergens have been found to be consistently more resistant to digestion by pepsin than other proteins, and the ability of an intact protein to reach the circulatory system maybe a prerequisite for allergenicity [3]. 2 S seed albumins have been associated with both allergy, in the oil milling and baking industries [5], and the genetic enhancement of nutrient-poor crops [6].

Key words: allergenicity, Ber e 1, SFA-8, simulated gastric fluid, unfolding.

Abbreviations used: Ber e 1 and rBer e 1, native and recombinant brazil nut 2 S albumin respectively; SFA-8 and rSFA-8, native and recombinant sunflower 2 S albumin 8 respectively; SGF, simulated gastric fluid.

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