

## Summary

It is generally assumed that the prevalence of food allergy is increasing. However, corroborative epidemiological evidence is still lacking. Furthermore, it should be mentioned that the public's-awareness of food allergy has increased in recent years allowing early recognition of food allergy. As part of the sensitization to plant foods arises due to crossreactivity with inhalant allergens, an increase in the prevalence of food allergy may be expected, however, parallel to an increase in inhalant allergies. The introduction of many novel foods in modern society results in exposure to a high variety of ingredients. This subsequently can induce sensitizations to a lot of different foods. In this thesis, different aspects of plant food allergy are investigated. We focused on sensitization patterns, diagnostic procedures and clinical reactivity, i.e. the nature and severity of allergic reactions and the individuals' sensitivity to plant foods which is expressed by the minimum provoking dose.

In *Chapter 2*, different sensitization patterns were studied. It is a well known fact that crossreactivity occurs between pollen and plant food and among plant foods mutually. It is not known, however, whether sensitization to one of the identified crossreactive structures from pollen, like Bet v 1 (major birch pollen allergen) and profilin (Bet v 2; minor birch pollen allergen) can predict the extent of the resulting crossreactivity and its clinical relevance. Investigation of 52 pollen-allergic patients with one or more sensitization(s) to plant food demonstrated a sensitization to Bet v 1 in 85 % and to profilin in 71 % of patients. Recognition of Bet v 1 mostly resulted in crossreactive IgE and clinical reactivity to apple, peach and hazelnut. Profilin-sensitization, on the other hand, resulted in IgE antibodies to many different plant foods but was accompanied by food-related symptoms in only a minority of cases. These differences in clinical expression could not be explained by the IgE-titers to Bet v 1 and profilin. This difference can possibly be explained by variability in affinity or valency in the interaction between IgE and Bet v 1 or IgE and profilin.

Food-allergic patients frequently encounter accidental exposure to the offending food due to the presence of hidden allergens in processed foods. *Chapter 3* illustrates this by describing two patients who suffered an allergic reaction due to the unexpected presence of casein and hazelnut respectively in consumer products. Furthermore, these case reports illustrate different reasons for the presence of hidden food allergens. Processed foods, comprising various 'untraditional' ingredients are increasingly consumed. Current legislation concerning labeling of foods is inadequate, allowing low doses of hidden proteins (allergens). Cross-contamination of ingredients during the production of different processed foods is a reason for the unintentional presence of allergens. To estimate the risk

for food-allergic patients of developing an allergic reaction due to hidden allergens, information about minimum provoking doses of food allergens is needed.

To obtain this information, double-blind placebo-controlled food challenges (DBPCFC) were conducted, starting with hazelnut. *Chapter 4* describes the results of challenging 31 patients. In 29 out of 31 patients the challenge was positive and a threshold dose, i.e. minimum provoking dose, could be determined. Four patients reacted to the lowest given dose of 1 mg hazelnut protein (about 1/200 hazelnut). After a dose of 100 mg hazelnut protein (~ 1/2 hazelnut) all patients had developed an allergic reaction. Extrapolation of the dose-response-curve gives information about the percentage of hazelnut-allergic patients that will react to a certain dose, for example 50 % (95 % CI, 30-70 %) of a hazelnut-allergic population will react to a dose of 6 mg hazelnut protein (1/30 hazelnut). The reactions observed during the challenges consisted of 'oral allergy syndrome' (OAS) initially in all patients, together with gastrointestinal symptoms in 5 patients and difficulty in swallowing in 1 patient. Possibly, there is a relation between the severity of symptoms and the involved hazelnut-allergen. Investigation of allergen-recognition patterns of these patients, as is shown in the *addendum to Chapter 4*, revealed, however, no clear distinction between patients subdivided according to severity of symptoms.

To diagnose hazelnut allergy with adequate accuracy, reliable diagnostic tests are necessary. In routine daily practice, skin testing is mostly used. A high negative predictive value is important to avoid false-negative responses. To achieve this, extracts used in skin testing should contain all major allergens in an IgE-binding conformation and in sufficient amounts. *Chapter 5* demonstrates the large differences concerning protein content and protein composition in nine commercially available skin test extracts for hazelnut. This was also reflected in the variable skin test responses as obtained using six of these extracts in 30 patients. To improve the diagnostic tools for plant food allergy, identification and knowledge of the properties (heat and proteolysis resistance) of the involved allergens is needed. Subsequently standardization of extracts is desirable. As this is hard to achieve using crude extracts, purified (recombinant) proteins may replace crude extracts in future.

In addition to hazelnut, minimum provoking doses were also determined for peanut. *Chapter 6* shows the results of challenging 26 peanut-allergic patients. Based on literature, lower doses of peanut were used than in the hazelnut challenges. Peanut threshold doses appeared to vary widely; from 0.1 mg up to 1000 mg peanut protein. None of the patients reacted to the lowest dose, used in this protocol, of 0.03 mg peanut protein. As mentioned above, extrapolation of the dose-response curve is informative as to the number of patients that will react to a certain peanut dose. For example, a dose of 3 mg peanut protein (about 1/50 peanut) will cause an allergic reaction in 50 % (95 % CI, 30-70 %) of 'our' peanut allergic patients. Furthermore, a correlation was found between the

severity of symptoms, as observed during DBPCFCs, and the threshold dose. Patients with more severe symptoms reacted to lower doses of peanut. Data from this chapter and from chapter 4 contribute to the determination of a distribution of threshold doses in the allergic population. This is especially important for risk assessment analyses. Hereto these data should be combined with information about the consumption patterns of allergic patients and contamination levels in consumer products.

*Chapter 7* highlights three extraordinary peanut allergic patients. In this chapter three patients are described with severe pea allergy who also developed peanut allergy later in life. Crossreactivity between pea and peanut was demonstrated using IgE-inhibition assays. The use of purified pea vicilin and Ara h 1 further confirmed that vicilin-homologues form the molecular basis of this crossreactivity, in which pea vicilin acts as the primary sensitizer. A remarkable fact is that two of these three patients spent their youth in an agricultural environment where different legumes were cultivated. As clinically relevant crossreactivity among legumes is rare in peanut allergic patients, it was speculated that the route of sensitization and the kind of sensitizing legume determine the clinical relevance.