

Update on threshold doses of food allergens: implications for patients and the food industry

Denise Anne Moneret-Vautrin and Gisèle Kanny

Purpose of review

The purpose of this review is to bring the reader up to date on the importance of assessing a food's lowest observed adverse-effect level (LOAEL) with two aims. Firstly, to help industry choose tests with a level of sensitivity capable of detecting food allergens hidden in industrial products. Secondly, to specify protective measures for highly allergic individuals in order to prevent recurrent severe anaphylaxis. The review also seeks to highlight the present issues and unsolved questions.

Recent findings

Thanks to standardized oral-provocation tests (double-blind placebo-controlled food challenges), LOAELs have been identified for many IgE-dependent food allergies. Most studies concern the pediatric population. Data is available for milk, egg, peanut, wheat flour, and sesame. The LOAELs are commonly in the range of 1–2 mg of natural foods, representing a few hundred micrograms of protein. These minimal reactive doses characterize about 1% of people allergic to milk, egg, or peanut. The level at which no observed adverse effect is seen might be a few tens of micrograms of protein for peanut. At the present time, allergy to oil seems to be restricted to unrefined cold-pressed oils.

Summary

Concerning IgE-dependent food allergies, the threshold dose inducing symptoms is now known to vary a great deal according to the individual. A reactive dose of less than 65 mg characterizes 16 and 18% of patients allergic to egg or peanut. Less than 30 mg of milk proteins characterizes 5% of those allergic to milk. For milk, egg, and peanut, 1% of patients have a very low threshold, about 1 mg. Such data emphasize the necessity of using detection tests with a sensitivity better than 10 parts per million. The modifications of allergenicity undergone by protein ingredients that are now commonly introduced into industrially made products are not yet sufficiently known. A better knowledge of the reactive doses of these proteins is needed.

Keywords

food allergen, food labelling, reactive threshold, LOAEL, NOAEL

Abbreviations

LOAEL lowest observed adverse-effect level
NOAEL no observed adverse-effect level

© 2004 Lippincott Williams & Wilkins
1528-4050

Introduction

Food allergies are common allergic diseases that can sometimes be severe or even lethal. In the USA they might concern 8% of the pediatric population, and 1.5% of all patients [1]. Peanut allergy only affects 1% of Americans [2,3]. Food allergies result in 125–150 fatalities per year [3]. The prevalence is 3.2% in the French population [4]. The safety of patients is a constant concern because it can only be ensured by the avoidance of food allergens, as there is no therapy available. Therefore, informative labelling of industrial products is strongly requested to allow patients to carefully check the food products in order to avoid masked allergens. The food industry authorities need to obtain precise information about the characteristics of raw materials and implement strict manufacturing procedures (hazard critical control point analysis) [5]. It appears necessary to use detection tests for dangerous allergens in order to validate these procedures [6–8]. The precise estimation of reactive doses is a useful element to calculate the concentration of the allergenic food allergen in an industrial product which might elicit a reaction. Industry takes into account commonly reactive quantities. Great attention is also given to minimal reactive doses [9]. On this basis, the limit of sensitivity demanded of a detection test to meet safety needs can be established without taking into account traces which would have no clinical implications. Indeed, a zero tolerance would lead to problems that food industries would practically be unable to solve.

This review considers the precise present knowledge on risk doses and thresholds for clinical reactivity and emphasizes the relevance of studies defining minimal reactive and nonreactive thresholds [5].

Methods of assessment of reactive doses of foods

The experience of all allergologists, as well as the literature, shows that clinical reactions to masked allergens in low amounts can be serious, which therefore emphasizes the need for a better knowledge of reactive

Curr Opin Allergy Clin Immunol 4:215–219. © 2004 Lippincott Williams & Wilkins.

Department of Internal Medicine, Clinical Immunology and Allergology, University Hospital, 29 avenue de Lattre de Tassigny, 54035 Nancy, France

Correspondence to Denise Anne Moneret-Vautrin, Department of Internal Medicine, Clinical Immunology and Allergology, University Hospital, 29 avenue de Lattre de Tassigny, 54035 Nancy, France
Tel: +33 3 83 85 28 63; fax: +33 3 83 85 28 64; e-mail: s.barrat@chu-nancy.fr

Current Opinion in Allergy and Clinical Immunology 2004, 4:215–219

DOI: 10.1097/01.all.0000129454.42340.23

thresholds. There are three ways of determining the thresholds for clinical reactions. The first approach relies on recorded data concerning reactions to masked allergens which the consumer could hardly expect to find in a food product [10]. Hidden allergens were detected, and their concentration calculated, in a series of cases where the food product could be traced: the concentration varied between 0.003 and 2.3% [6]. Severe anaphylactic shock resulted from the ingestion of less than 200 mg of milk proteins, soy proteins, or ovalbumin [6], less than 50 mg of casein in salmon [11], 60 mg of hazelnut in chocolate [12], 180 μ g of lactoserum proteins in a sorbet [13], and 100 μ g of milk proteins in a baby-food cereal flour guaranteed to be milk-free [14,15]. A very low dose can clearly cause a severe reaction [16].

The cases characterized by an extremely small dose triggering a reaction are likely to be most often published, which can induce a bias exaggerating their relative frequency. The summation of anecdotal cases gives no information as to the percentage of patients likely to react to such quantities.

A second approach has been proposed based on a statistical method applied in a wide range of disciplines, which gives consideration to the methodology as well as to the quantitative results of published challenge studies. A theoretical threshold is suggested, corresponding to one case out of 1 million allergic patients: this threshold is postulated to be 5 μ g for milk, 2 μ g for egg, 1.3 μ g for soy, and 0.7 μ g for peanut [16]. Although the authors acknowledge the numerous shortcomings of this evaluation, this original approach gives plausible estimations but deals with a minute theoretical risk, which hardly corresponds to reality.

The third means of evaluation is the reference method, performed through standardized oral-provocation tests, or double-blind placebo-controlled food challenges [3,17–20]. Applied to a group of patients allergic to a given food, they help evaluate the clinical reactivity by using a range of increasing doses. The variety of methods employed in challenge studies is considerable. For example, some studies use natural foods, others use dehydrated foods. Results may be expressed according to the weight of fresh or dried food, or in terms of protein equivalence. The variety of vehicles used to mask the food may introduce a risk of interaction between food allergens and other components. The criteria for positivity differ when considering objective symptoms only, or accepting subjective symptoms when they differ from the placebo. A precise equivalence between the results of different studies cannot therefore be established [21••]. As concerns peanut, 25% of allergic children react to 100 mg [18]. The mean reactive dose could be 850 mg [22]. All children cumulatively tested at

our institution react to less than 7.11 g (Figure 1). Concerning egg and milk, a threshold of 100 mg is observed in 2.5% of allergic children [19]. Commonly reactive quantities are likely to be underestimates since oral challenges are not carried out in subjects with a history of severe anaphylaxis.

Minimal thresholds: LOAEL (lowest observed adverse-effect level) and NOAEL (no observed adverse-effect level)

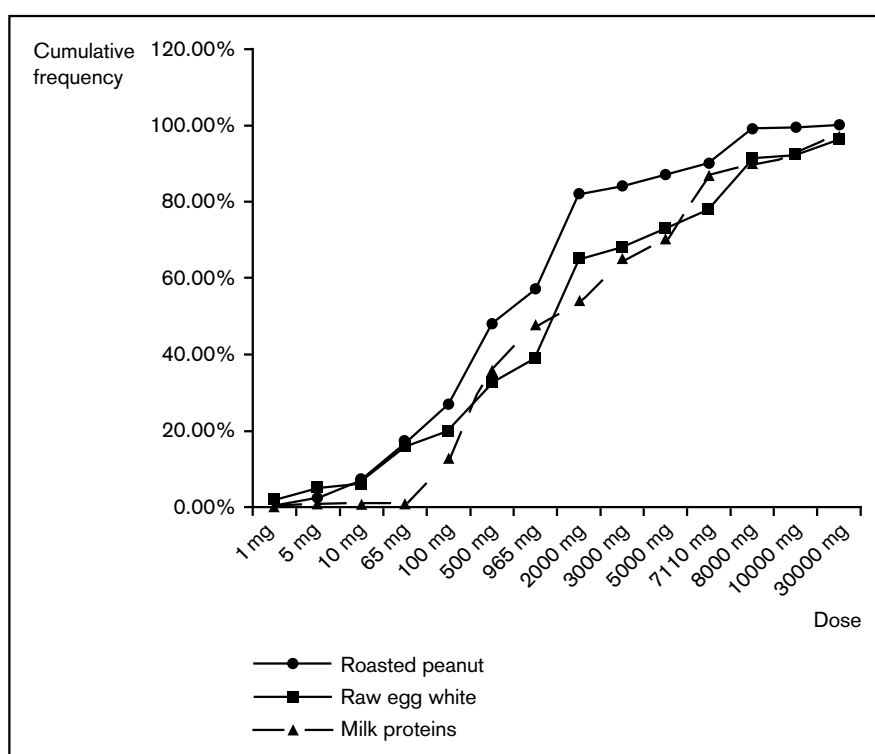
The notion of threshold doses is common in the field of toxicology and in the determination of the risk of carcinogenesis. Determining a toxic risk is based on animal experimentation. The threshold where no adverse effect is observed can be defined as the NOAEL. In the case of additives, the acceptable daily intake is fixed according to a dose 100 times lower than the NOAEL. In the case of genotoxic mutagens, no threshold can be considered as innocuous. According to animal experimentation as well as epidemiological studies, the acceptable risk is the reference, often defined as the risk of one case of cancer for 1 million patients exposed throughout their lives [8]. In the case of food allergy, neither animal models nor epidemiological studies help to determine the range of reactive doses in humans. This is why one now contemplates searching for the minimal reactive dose in large groups of food-allergic patients.

The work carried out by different teams has been summarized by Taylor *et al.* [21••]: 0.35% of patients allergic to egg may react to 130 μ g of egg-white proteins, 0.33% of children allergic to cow's milk could react to 600 μ g of milk proteins (0.03 ml), 0.32% of subjects allergic to peanut could react to 250 μ g of peanut proteins (1 mg of peanut). One study aimed specifically to establish the LOAELs of different foods; they observed a LOAEL of 2 mg for raw egg white (200 μ g of proteins) in 0.8% of patients, a LOAEL of 3.2 mg of milk proteins (0.1 ml) in 1.6% of children, and a LOAEL of 5 mg of peanut (1.25 mg of proteins) in 0.97% of patients [23••]. Double-blind placebo-controlled food challenge to hazelnut in 29 allergic patients established the LOAEL for subjective reactions in the range of 1–100 mg of hazelnut proteins, corresponding to 6–600 mg of whole hazelnuts. In the same subject the dose eliciting objective symptoms could be considerably higher, from 30–1000 mg of proteins [24].

The allergenicity of oils raises the problem of extremely low quantities of protein allergens; about a few dozen micrograms. Reactions to peanut oil, soy oil, or sunflower oil are extremely rare on account of the present refining methods [25,26]. The absence of reactions to refined peanut oil in 60 adult subjects allergic to peanut contrasted with reactions to the crude oil in 10% of

Figure 1. Results of peanut, egg, and milk reactivity: cumulative frequency of positive single-blind placebo-controlled food challenges to peanut (137), egg (163), and milk (108)

Data were collected at the University Hospital, Nancy, France. Unpublished data presented at the European Allergy, Asthma and Clinical Immunology Annual Meeting, Paris, June 2003. Doses are given in total weight for peanut and egg white and in protein equivalent for milk.



cases [27]. Reactivity to refined peanut oil could be observed only in young children [18]. However, the situation may be different for sesame oil, which is cold-pressed and is imported from Asian countries or Mexico. A real risk of severe anaphylaxis has been confirmed by double-blind placebo-controlled oral challenges [23^{••},28]. Several IgE-binding bands have been detected in sunflower and soy oils [25,26,29]. A recombinant peanut oleosin obtained from baculovirus was shown to be an allergen contained in peanut oil [30].

Beyond the determination of LOAELs, would it be possible to have access to NOAELs? The NOAEL could not be determined in the studies just mentioned, since at least one patient reacted to the very first test dose. It is necessary to set a special progression of doses from a few dozen micrograms to several milligrams, so as to determine constantly nonreactive doses. Protocols are necessarily complex. They concern adults solely, or teenagers. Criteria of clinical reactions have to take into account subjective symptoms, since they have been shown to precede objective symptoms in the current procedure using a gradual increase of doses. Subjective symptoms must be validated either by the fact that they do not occur with placebo, or because higher doses trigger objective symptoms. Experience shows that a series of intermediate doses between the first dose that

causes subjective symptoms and the dose which leads to objective signs can be tolerated satisfactorily enough, which makes it difficult to set the clinically relevant threshold. Out of 14 adult patients allergic to peanut, observed by Hourihane *et al.* [31], two presented subjective signs at 100 μ g [31]. Out of 26 patients observed by Wensing *et al.* [32^{••}], one had objective symptoms at the same dose. The NOAEL would be 30 μ g in this group of 26 adults. We underline the fact that most studies commonly search for the reactive doses in allergic patients, in order to establish the diagnosis of food allergy, excluding from oral challenges the most reactive individuals with severe anaphylaxis. On the contrary, the studies focusing on the determination of LOAELs and NOAELs deal with patients who have been selected when a serious clinical sensitivity has been suspected. These two types of study thus present opposite biases.

Questions open to debate

Numerous questions are still unanswered. In most cases, studies document IgE-dependent allergies in the pediatric population. A small number of food products have been the main focus of investigation: cow's milk, egg, peanut, and fish. There are few results concerning wheat flour, sesame, tree nuts, or crustaceans [21^{••},23^{••},24]. When specific IgEs are detectable, the

fact that there is no relationship between their level and the seriousness of clinical reactions shows that parameters other than the intensity of sensitization are to be considered. Several factors are likely to modify the threshold of the reactive dose, such as exercise, the simultaneous intake of aspirin, regular treatment with β -blockers or angiotensin-converting enzyme inhibitors, the association of another food allergen, the fat content of the food, and the season of the year in the case of a crossreactivity between pollen and food allergens [33]. This is why determining a LOAEL through oral-provocation tests carried out at rest cannot ensure total safety. Detection tests, while taking into account such data, might have to measure a lower level of food allergens than that calculated on the basis of the LOAEL.

The LOAEL is equally unknown in chronic food-allergy-induced diseases such as food-protein-related eosinophilic gastroenteropathies. Test procedures other than a single oral challenge would be necessary. The evaluation of adverse effects should cover a period of 2–3 weeks with a daily ingestion of a fixed dose.

For one and the same food product, the LOAEL and NOAEL probably depend on the age group. An infant could be at a higher risk of reaction induced by traces of food allergens than an older child. This hypothesis is supported by the fact that breast-fed newborn babies are able to become allergic to cow's milk, reacting to tiny amounts of milk proteins passing through the breast milk [34]. The level of cow's-milk β -lactoglobulin in the breast milk has been evaluated in the range of 10 ng/ml to 10 μ g/ml, indicating that a small amount of a few milligrams is able to elicit reactions [35]. We have also found age-group effects in our own studies of wheat allergy. When comparing children and adults with wheat-flour allergy, the reactive doses show a considerable difference, with 83% of children reacting to <2 g of wheat protein compared with only 18% of adults reacting to the same dose (Moneret-Vautrin DA, Kanny G, P  rier P, *et al.*, unpublished data).

The LOAEL might be very low for certain food allergens: in the case of allergy to spices (garlic, coriander), traces may trigger clinical reactions [36]. LOAELs have not been studied. Industry widely uses proteic ingredients in food products and in the production of flavors [5]. There are few data on reactive doses of food proteins used as ingredients, such as casein and wheat isolates [11,37,38]. A case of allergy to a wheat isolate has been studied, showing that neoallergenicity was characteristic of this ingredient [37]. Cases of anaphylaxis to a soy isolate have also been described in subjects who tolerate the usual soy products. A higher concentration of a minor allergen, SAM-22, in the isolate

was sufficient to induce allergy in patients allergic to birch pollen [39]. One must therefore emphasize that it will not be possible to apply the knowledge of LOAELs and NOAELs of natural foods to technologically modified proteins used as ingredients.

Conclusion

In spite of the current uncertainties, the LOAEL for the main food allergens can be determined within the range of a few milligrams of proteins. The food industry can use this information to ensure increasing safety of food products with the help of detection tests. The objective of a sensitivity limit of 5 parts per million seems to have already been achieved in some cases. Postmarketing control procedures that result in the recall of food products presenting a potential risk to the consumer are aided in reaching food-allergic consumers by such advocacy groups as the Food Allergy and Anaphylaxis Network [40]. A complementary strategy has been developed in France with the Allergo-Vigilance Network, where 300 allergologists report cases of severe anaphylaxis as well as their etiology. This information may draw the authorities' attention to risky products [41]. The compulsory labelling of the 10 most frequent allergens to present a severe risk, due to appear in 2004 in European countries, will be a significant advance in the safety of allergic patients [42]. However, the tricky problem of masked allergens has not yet been solved. More extensive research on LOAELs and NOAELs must be carried out to enable authorities to determine tolerable concentrations of masked allergens in industrial food products.

A further question arises: Could the changes in the allergenicity of proteins due to food technologies contribute to lowering the thresholds of reactive doses of natural proteins? This question should be kept in mind, however theoretical it is at the present time. In the case of allergy to a new ingredient, it is highly advisable to proceed to a precise evaluation of the LOAEL of the substance.

References and recommended reading

- Papers of particular interest, published within the annual period of review, have been highlighted as:
- of special interest
 - of outstanding interest
- 1 Sampson H. Adverse reactions to foods. In: Middleton E Jr, Reed CE, Ellis EF, *et al.*, editors. *Allergy: principles and practice*. 4th ed. Orlando: Mosby; 1993. pp. 1661–1686.
 - 2 Sicherer SH, Munoz-Furlong A, Sampson HA. Prevalence of peanut and tree nut allergy in the United States determined by means of a random digit dial telephone survey: a 5-year follow-up study. *J Allergy Clin Immunol* 2003; 112:1203–1207.
 - 3 Sampson H. Food anaphylaxis. *Brit Med Bull* 2000; 56:925–935.
 - 4 Kanny G, Moneret-Vautrin DA, Flabbee J, *et al.* Population study of food allergy in France. *J Allergy Clin Immunol* 2001; 108:133–140.

- 5 Taylor S, Hefle S. Ingredient and labelling issues associated with allergenic foods. *Allergy* 2001; 56:64–69.
 - 6 Malmheden Yman I, Eriksson A, Everitt G, *et al.* Analysis of food proteins for verification of contamination or mislabelling. *Food Agric Immunol* 1994; 6:167–172.
 - 7 Brett GM, Bull VJ, Morgan VR, *et al.* Identification of hidden allergens within foods. *Allergy* 1998; 53:109–110.
 - 8 Madsen C. Where are we in risk assessment of food allergens? The regulatory view. *Allergy* 2001; 56:91–93.
 - 9 Hourihane JOB. The threshold concept in food safety and its applicability to food allergy. *Allergy* 2001; 56:86–90.
 - 10 Hefle SL. Hidden food allergens. *Curr Opin Allergy Clin Immunol* 2001; 1:269–271.
 - 11 Koppelman S, Wensing M, de Jong GAH, *et al.* Anaphylaxis caused by the unexpected presence of casein in salmon. *Lancet* 1999; 354:2136.
 - 12 Wensing M, Koppelman S, Penninks AH, *et al.* Hidden hazelnut is a threat to allergic patients. *Allergy* 2001; 56:191–192.
 - 13 Laoprasert N, Wallen ND, Jones RT, *et al.* Anaphylaxis in a milk-allergic child following ingestion of lemon sorbet containing trace quantities of milk. *J Food Prot* 1998; 61:1522–1524.
 - 14 Frémont S, Kanny G, Bieber S, *et al.* Identification of a masked allergen, alpha-lactalbumin, in a baby-food cereal flour guaranteed free of cow's milk proteins. *Allergy* 1996; 51:749–754.
 - 15 Kanny G, Fremont S, Talhouarne G, *et al.* Anaphylaxis to mustard as a masked allergen in "chicken dips". *Ann Allergy Asthma Immunol* 1995; 75:340–342.
 - 16 Bindslev-Jensen C, Briggs D, Osterballe M. Can we determine a threshold level for allergenic foods by statistical analysis of published data in the literature? *Allergy* 2002; 57:741–746.
 - 17 Bock S, Atkins FM. Pattern of food hypersensitivity during sixteen years of double blind, placebo-controlled food challenges. *J Pediatr* 1990; 117:561–567.
 - 18 Moneret-Vautrin DA, Rance F, Kanny G, *et al.* Food allergy to peanuts in France – Evaluation of 142 observations. *Clin Exp Allergy* 1998; 28:1113–1119.
 - 19 Sicherer SH, Morrow EH, Sampson HA, *et al.* Dose-response in double-blind, placebo-controlled oral food challenges in children with atopic dermatitis. *J Allergy Clin Immunol* 2000; 105:582–586.
 - 20 Bindslev-Jensen C. Standardization of double-blind, placebo-controlled food challenges. *Allergy* 2001; 56:75–77.
 - 21 Taylor SL, Hefle SL, Bindslev-Jensen C, *et al.* Factors affecting the
 - determination of threshold doses for allergenic foods: how much is too much? *J Allergy Clin Immunol* 2002; 109:24–30.
- A comprehensive review of problems encountered by industry and the rational link with the determination of threshold doses, as well as a thorough report of the experience of several clinical groups with a daily practice of standardized food challenges for diagnostic purposes.
- 22 Rance F, Dutau G. Peanut hypersensitivity in children. *Pediatr Pulmonol Suppl* 1999; 18:165–167.
 - 23 Morisset M, Moneret-Vautrin DA, Kanny G, *et al.* Thresholds of clinical
 - reactivity to milk, egg, peanut and sesame in immunoglobulin E-dependent allergies: evaluation by double-blind or single-blind placebo-controlled oral challenges. *Clin Exp Allergy* 2003; 33:1046–1051.
- This study reporting standardized food challenges to peanut (103), raw egg white (125), milk (59), and sesame (12) was one of the first studies dealing with the determination of LOAELs in a large series of immediate food allergies. In order to guarantee 95% safety for patients, and on the basis of consumption of 100 g of food, the detection tests should ensure a sensitivity of 10 parts per million for egg, 24 parts per million for peanut and 30 parts per million for milk proteins. Oil allergies being considered, the limit of sensitivity should fall to 5 parts per million.
- 24 Wensing M, Penninks AH, Hefle SL, *et al.* The range of minimum provoking doses in hazelnut-allergic patients as determined by double-blind, placebo-controlled food challenges. *Clin Exp Allergy* 2002; 32:1757–1762.
 - 25 Zitouni N, Errahali Y, Metche M, *et al.* Influence of refining steps on trace allergenic protein content in sunflower oil. *J Allergy Clin Immunol* 2000; 106:962–967.
 - 26 Moneret-Vautrin DA, Morisset M, Flabbee J, *et al.* Unusual soy oil allergy. *Allergy* 2002; 57:266–267.
 - 27 Hourihane JO, Bedwani SJ, Dean TP, Warner JO. Randomised, double blind, crossover challenge study of allergenicity of peanut oils in subjects allergic to peanuts. *BMJ* 1997; 314:1084–1088.
 - 28 Kanny G, de Hauteclouque C, Moneret-Vautrin DA. Sesame seed and sesame seed oil contain masked allergens of growing importance. *Allergy* 1996; 51:952–957.
 - 29 Errahali Y, Morisset M, Moneret-Vautrin DA. Allergens in soy oils. *Allergy* 2002; 57:648–649.
 - 30 Pons L, Chery C, Romano A, *et al.* The 18 kDa peanut oleosin is a candidate allergen for IgE-mediated reactions to peanuts. *Allergy* 2002; 57 (Suppl 72):88–93.
 - 31 Hourihane JOB, Kilburn SA, Nordlee JA, *et al.* An evaluation of the sensitivity of subjects with peanut allergy to very low doses of peanut protein: a randomized, double-blind, placebo-controlled food challenge study. *J Allergy Clin Immunol* 1997; 100:596–600.
 - 32 Wensing M, Penninks AH, Hefle SL, *et al.* The distribution of individual
 - threshold doses eliciting allergic reactions in a population with peanut allergy. *J Allergy Clin Immunol* 2002; 110:915–920.
- This was the first study that managed to evaluate a NOAEL for peanut, of 30 µg (26 patients were tested).
- 33 Moneret-Vautrin DA, Kanny G. Food anaphylaxis. A new French multicentric survey [in French]. *Bull Acad Natl Med* 1995; 179:161–184.
 - 34 Sorea S, Dabadie A, Bridoux-Henno L, *et al.* Hemorrhagic colitis in exclusively breast-fed infants. *Arch Pediatr* 2003; 10:772–775.
 - 35 Sorva R, Mäkinen-Kiljunen S, Juntunen-Backman K. β -Lactoglobulin secretion in human milk varies widely after cow's milk ingestion in mothers of infants with cow's milk allergy. *J Allergy Clin Immunol* 1994; 93:787–792.
 - 36 Moneret-Vautrin DA, Morisset M, Lemerdy P, *et al.* Food allergy and IgE sensitization caused by spices: CIBAA data (based on 589 cases of food allergy). *Allerg Immunol* 2002; 34:135–140.
 - 37 Leduc V, Moneret-Vautrin DA, Guerin L, *et al.* Anaphylaxis to wheat isolates: Immunochemical study of a case proved by means of double-blind, placebo-controlled food challenge. *J Allergy Clin Immunol* 2003; 111:897–899.
 - 38 Hefle SL, Taylor SL. How much food is too much? Threshold doses for allergenic foods. *Curr Allergy Asthma Rep* 2002; 2:63–66.
 - 39 Kleine-Tebbe J, Vogel L, Crowell DN, *et al.* Severe oral allergy syndrome and anaphylactic reactions caused by a Bet v 1-related PR-10 protein in soybean, SAM22. *J Allergy Clin Immunol* 2002; 110:797–804.
 - 40 Sicherer SH, Noone SA, Munoz-Furlong A. The impact of childhood food allergy on quality of life. *Ann Allergy Asthma Immunol* 2001; 87:461–464.
 - 41 Moneret-Vautrin DA, Kanny G, Parisot L. First survey from the "Allergy Vigilance Network": life-threatening food allergies in France. *Allerg Immunol* 2002; 34:194–198.
 - 42 A proposal to modify the rule 2000/13/CE of the European Parliament as concerns the labelling of food allergenic components to be found in the food products. *Journal Officiel des Communautés Européennes* 2002; C 331E/188: 188/193.