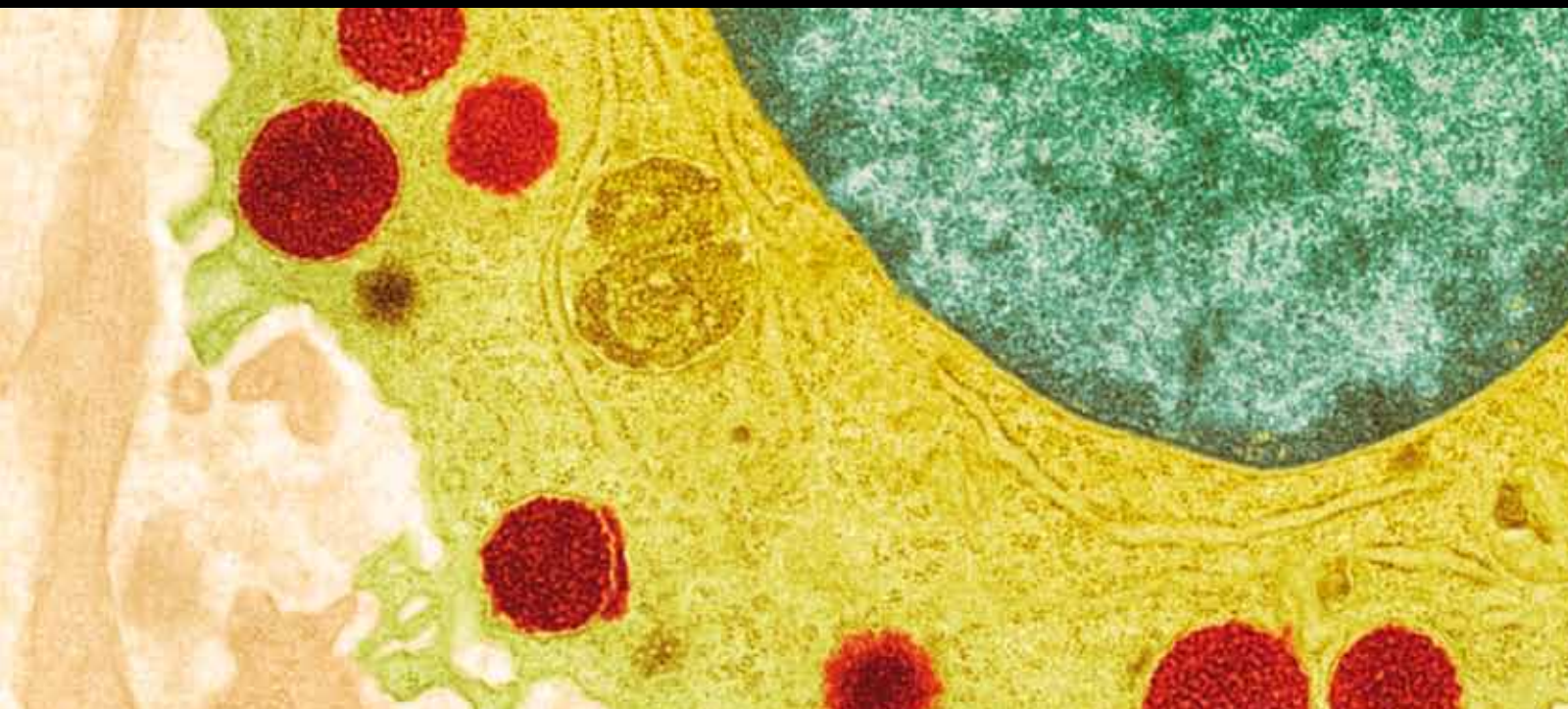


Food Hypersensitivity: Diagnosing and Managing Food Allergies and Intolerances

GUEST EDITORS: CARINA VENTER, BERBER Vlieg-BOERSTRA, AND KIRSI LAITINEN





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Journal of Allergy

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Guest Editors: Carina Venter, Berber Vlieg-Boerstra,
and Kirsi Laitinen



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Editorial

Food Hypersensitivity: Diagnosing and Managing Food Allergies and Intolerances

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Received 17 May 2012; Accepted 17 May 2012

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The last two years have seen the publication of 3 major guidelines on the diagnosis and management of food hypersensitivity (FHS) [1–3]. These guidelines have highlighted important issues in dealing with those with FHS, primarily food allergies. Dealing with adults and children with food allergies requires the healthcare professional to have an understanding of the immune mechanisms involved in the development of allergy versus tolerance, diagnostic methods, managing the patient, and the development of new treatment modalities. In this issue, we have continued to discuss topics such as the role of the epithelial barrier in the development of FHS, the importance of patient education and support groups, the effect of food production on the allergenicity of foods, and the induction of oral tolerance. This special issue is concluded with a tutorial from expert allergy dietitians on the diagnosis and management of FHS in children.

Food hypersensitivity includes a wide spectrum of symptoms and mechanisms. Many researchers and clinicians have discussed the possible role of the gut (barrier) and the mucosal immune system in the development of food allergies. Factors that have been addressed in particular include weaning and the weaning diet [4], age of solid food introduction and breast feeding [5], gut bacteria [6], and gastrointestinal infections [7]. What is clear is that a better understanding of the immunological mechanisms involved in sensitisation and tolerance will enhance our efforts of allergy prevention and discovering novel treatments. L. C.-H. Yu discusses in the review paper “*Intestinal epithelial barrier dysfunction in food hypersensitivity*” the epithelial barrier dysfunction in sensitized intestines with particular reference to the enhanced transcytotic rates of allergens and the mechanisms involved in development of sensitisation. They

discuss that recent studies demonstrate that food allergens manage to be transported across the epithelium and avoid lysosomal degradation by binding to cell surface IgE and the low-affinity receptor CD23/FcεRII—leading to investigation of anti-IgE and anti-CD23 antibodies in the prevention and management of allergic diseases.

A clear diagnosis plays a very important role in the management of food hypersensitivities. The paper “*Late type of bronchial response to milk ingestion challenge: a comparison of open and double-blind challenge*” by Z. Pelikan addresses two important issues often discussed by allergists and other health care professionals [8]. (1) Can cow’s milk cause a bronchial response in patients with diagnosed asthma and (2) can open food challenges be used for the diagnosis of bronchial responses in asthmatics? In this study the authors showed that cow’s milk allergy can lead to late-phase bronchial complaints (2 hours after ingestion) in patients with diagnosed asthma either as the sole allergen causing the problem or in addition to other aeroallergens. In addition, they showed that the open food ingestion challenge (OFICH) combined with monitoring of the objective lung function parameters can be considered as a definite confirmation of the suspected role of food allergy and involvement of a certain food (e.g., cow’s milk) in bronchial complaints of patients suffering from bronchial asthma due to various inhalants, making the use of DBPCFC superfluous.

It is well known that food hypersensitivity leads to reduced quality of life of both the parent and the child suffering from this [9–11]. The World Allergy Organisation Guidelines [2], the US Food Allergy Guidelines [1], and the UK NICE Guidelines on Food Allergy [3] stated the importance of patient support groups for sufferers of FHS.

These support groups are, however, often set up and run by allied health professionals. It is therefore very enlightening to read in the paper “A pediatric food allergy support group can improve parent and physician communication: results of a parent survey” by A. Sharma et al. that a food allergy support group decreased anxiety about food allergies for 77.7% of families. Almost 65% of parents indicated that an allergist attending the support group increased their communication with their doctor. The most important reason for joining the support group was to increase their knowledge on food allergies, followed by meeting others with food allergies, reduce their anxiety, recipe ideas, and improve their quality of life.

Patient education on food labelling is another topic of importance highlighted by the food allergy guidelines, with many questions surrounding the issue of allergen content of processed food [12]. P. A. Alvarez and J. I. Boye discussed in their paper “Food production and processing considerations of allergenic food ingredients: a review” the importance of epidemiological data, indicating the most allergenic foods in a population. Despite tests available to detect allergens in food and a constant improvement in this science, is allergen risk management right from the primary ingredients is the most important factor in reducing the risk for allergic consumers. This also includes the practice of minimising ingredient and simplifying sourcing of ingredients.

This special issue is concluded with a tutorial by allergy dietitians and guest editors, Drs. Laitinen, Venter, and Vlieg-Boerstra. The tutorial covers a general overview of FHS, addressing in particular nomenclature of food hypersensitivity, prevalence, recently published guidelines, the principles of taking an allergy focussed diet history, and the principles of the nutritional management of FHS.

Carina Venter

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Review Article

Nutritional Aspects in Diagnosis and Management of Food Hypersensitivity—The Dietitians Role

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Received 19 March 2012; Accepted 20 April 2012

Academic Editor: K. Blaser

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Many common foods including cow's milk, hen's egg, soya, peanut, tree nuts, fish, shellfish, and wheat may cause food allergies. The prevalence of these immune-mediated adverse reactions to foods ranges from 0.5% to 9% in different populations. In simple terms, the cornerstone of managing food allergy is to avoid consumption of foods causing symptoms and to replace them with nutritionally equivalent foods. If poorly managed, food allergy impairs quality of life more than necessary, affects normal growth in children, and causes an additional economic burden to society. Delay in diagnosis may be a further incremental factor. Thus, an increased awareness of the appropriate procedures for both diagnosis and management is of importance. This paper sets out to present principles for taking an allergy-focused diet history as part of the diagnostic work-up of food allergy. A short overview of guidelines and principles for dietary management of food allergy is discussed focusing on the nutritional management of food allergies and the particular role of the dietitian in this process.

1. Introduction

According to the World Allergy Organisation [1] about 1.9%–4.9% of children suffer from cow's milk allergy, with the prevalence in adults much lower; less than 0.5% [2]. It is known that perceived food allergy could be 10 times higher [3, 4] than that confirmed by appropriate tests. Although a large number of foods are suspected to cause food allergies, most studies have focused on 6 common foods which include cow's milk, hen's egg, soya, peanut/tree nuts, fish/shellfish, and wheat, clearly showing different patterns based on the population studied and the diagnostic methods used [5]. Peanut allergy is one of the most common causes of food-induced anaphylaxis, with a reported or challenge proven prevalence rate between 0.06% and 5.9% depending on the country and age group studied [2]. Tree nut allergies also show a wide range of reported or challenge-confirmed prevalence ranging between 0.2%–8.6% [2]. High rates of

reported allergies to tree nuts may, however, be due to cross-sensitisation with aeroallergens, rather than a primary food allergy, with an increased number of patients with so-called oral allergy syndrome/fruit pollen syndrome seen [6].

There is a paucity of information on the role of nutrition versus just food avoidance in the management and natural course of food allergy. Very little is also known about the effect of a nutrition consultation in this process. Furthermore, the role of the dietitian and the diagnostic and therapeutic value of the elimination diet has not been established and extensively investigated. A systematic review on the role of the dietitian and the value of the elimination diet in the diagnostic and therapeutic phase found only two papers. These only partially address the issue and focus mainly on the nutritional status of children with food allergies [7, 8]. The lack of information on how to take an allergy-focused diet history, linking symptoms of allergic disease with foods implicated in the adverse reactions, and

the impact of general nutritional intake, has been recognised by the European Academy of Allergy. A special task force initiated by the Interest Group on Allied Health is addressing this.

The paucity of information on the role of nutrition in allergy is reflected in and may even be explained by the fact that globally seen, only a few dietitians and nutritionists have both a specialty in food allergy and are academically trained in food allergy. The level of training in dietetics in general differs between countries and the UK is the only country which offers MSc degrees in allergy (<http://www.southampton.ac.uk/> and <http://www.imperial.ac.uk/>). As a result, the level of knowledge about food allergy not only differs strongly between individual dietitians but also strongly between countries. A survey conducted in the USA [9] and UK [10] shows a great need for food allergy knowledge amongst dietitians. Dietitians in the USA felt more knowledgeable about the definitions of food allergies (57% scored "high level of knowledge" in the USA versus 31% in the UK, $P < 0.001$) and intolerances (59% scored "high level of knowledge" in the USA versus 30% in the UK, $P < 0.001$). However, UK-based dietitians indicated more confidence in designing food challenge protocols (12% UK versus 8% USA scored "high", $P = 0.12$) and 18% in the UK and 19% in the USA indicated to have no proficiency in developing challenge protocols. Very interestingly, dietitians from both countries indicated that their most immediate need was standardised patient handouts/diet sheets (89% USA and 70% UK).

Having realised this problem some time ago, the International Network for Diet and Nutrition in Allergy (INDANA) (<http://www.indana-allergy-network.org/>) was established. The aim of INDANA is to encourage, support, and disseminate best evidence-based clinical practice and to influence the development of the dietitian's and nutritionist's role in the field of food hypersensitivity (FHS) at international level. In this paper, the aims and activities of INDANA will be further discussed.

The role of the dietitian differs between countries depending on the extent of the physician's role in the dietary management. In some countries the dietitians are trained to be involved in the diagnosis, while in other countries the dietitians are involved in the dietary management only.

During 2010 and 2011, three official guidelines have been published on the diagnosis and management of food allergies by international organizations or by comprehensive working groups. These are the World Allergy Organisation guidelines on the diagnosis and management of cow's milk allergy (DRACMA guidelines) [1], the USA guidelines on the diagnosis and management of food allergies in adults and children [11] and the UK NICE guidelines on the diagnosis of food allergies in children [12].

All 3 guidelines recognize the important role of nutrition education. However, the UK NICE guidelines were the only guidelines that recognized the role of the dietitian in diagnosis and management of food allergies [12]. The DRACMA guidelines mention the role of the dietitian (referred to as a nutritionist) in the management of cow's milk allergy [1].

2. Aim

This paper sets out to present nutritional aspects in diagnosis and management of food allergies, comprising

- (i) the aims and activities of the International Network for Diet and Nutrition in Allergy (INDANA);
- (ii) principles for taking an allergy-focused diet history in the diagnosis of food allergy;
- (iii) principles for the nutritional and dietary management of food allergy, including the impact of food allergy on an individual's diet and nutritional status, unnecessary avoidance diets and dietary restrictions, and psychosociological aspects of food allergy.

3. International Network for Diet and Nutrition in Allergy: INDANA

Acknowledging the need for education on food hypersensitivities for both dietitians and other professionals, the International Network for Diet and Nutrition in Allergy (INDANA) was established in 2009 by a group of academic dietitians and food scientists specializing in food allergies and intolerances. This nonprofit international network [13] aims to

- (i) develop the role of the dietitian/nutritionist in the field of food allergy and to enhance the focus on diet and nutrition when dealing with food allergy;
- (ii) provide a platform for nutritional and dietary management advice for all those involved in dietary care of food allergy;
- (iii) educate health care professionals (HCPs) involved in food allergy on nutrition and dietary management of food allergy;
- (iv) encourage international collaboration and research;
- (v) encourage a membership that is representative of all countries and continents where food allergy is highly prevalent.

INDANA aims to bridge the gap in science between food hypersensitivity, immunology, nutrition, and food science to improve the prevention, nutritional diagnosis, and management of those living with food allergies and intolerances.

The steering group consists of members with a first degree in Nutrition & Dietetics or Nutrition, plus postgraduate training in allergy or a research degree at Masters or Doctorate level. The steering committee includes representation from the USA, Europe, Australia, and South Africa. The three authors of this paper are members of the steering committee of INDANA. Membership is open to any HCP with a relevant first degree who is working in the field of food hypersensitivity (i.e. dietitian, nutritionist, nurse, clinician, researcher, and industry).

The importance of diet and nutrition in allergic disease and the role of the dietitian/nutritionist are now officially

acknowledged by the European Academy of Allergy and Clinical Immunology (EAACI), by initiating an Interest Group on Diet and Nutrition in the Allied Health (IG on AH). This creates a platform within the EAACI where people, sharing an interest and expertise in diet and nutrition in allergy, can meet, teach, exchange expertise and ideas, and support the EAACI with regard to this topic. Also recently INDANA was officially incorporated in the American Academy of Allergy, Asthma and Immunology (AAAAI).

The primary goals of INDANA and the IG on AH are to educate HCPs on food hypersensitivity, taking into account international variations in the role of the dietitian in the diagnosis and management of these conditions. The IG on AH is involved in setting up an EAACI-initiated practical training course on food allergy and a Food Allergy and Anaphylaxis Guideline. In addition, members of INDANA and the IG on AH are also presenting at international conferences of the EAACI, AAAAI and the International Congress of Dietetics (ICDs). The first educational and research tool that is being developed is a standardized allergy-focused diet history. This tool is being developed under the funding of the EAACI by the Interest Group (IG) on Allied Health (AH). This tool could be adjusted for use in the USA pending support of national allergy organisations. This may be particularly useful for physicians who do not have access to dietitians.

The next steps will be to develop educational materials for health care professionals around the globe and to initiate research projects.

4. Principles for Taking an Allergy-Focused Diet History

The aim of the allergy-focused diet history is to investigate if there is an association between food or diet and the symptoms of the patient.

When patients have immediate food allergic reactions and reactions to single foods, it is often obvious to which food the patient has reacted. However, very often patients have chronic symptoms, reactions to compound foods, or sensitisation to foods of which the clinical relevance is not clear. This may be due to unknown allergens, may be caused by cross-sensitisation between pollens and foods [14], or due to a deficient or unbalanced diet. The outcome of the allergy-focused diet history may direct the physician to further diagnostic testing supporting the final diagnosis by the physician and may direct the dietary measures to be taken. A detailed allergy-focused diet history should consist of the following.

4.1. Assessment of Signs and Symptoms and Possible Mechanisms. According to the UK NICE guidelines, the possibility of food allergy should be considered in children and young people who are presenting with one or more allergic signs and symptoms, in particular when there are persistent symptoms that involve different organ systems [12]. These symptoms may be IgE-mediated or non-IgE-mediated or may not involve the immune system.

4.2. Allergy-Focused Clinical History and Linking Allergenic Foods to Symptoms. If food allergy is suspected (by a HCP parent, carer, child, or young person), a HCP with the appropriate competencies should take an allergy-focused clinical history tailored to the presenting symptoms and age of the child, young person, or adult.

This should include [12] information on the following:

- (i) any personal history of atopic disease (asthma, eczema, or allergic rhinitis);
- (ii) any individual and family history of atopic disease (such as asthma, eczema, or allergic rhinitis) or food allergy in parents or siblings;
- (iii) details of any foods that are avoided and the reasons why;
- (iv) an assessment of presenting symptoms and other symptoms that may be associated with food allergy, including questions about:
 - (a) the age of the child or young person when symptoms first started;
 - (b) speed of onset of symptoms following food contact;
 - (c) duration of symptoms;
 - (d) severity of reaction;
 - (e) frequency of occurrence;
 - (f) setting of reaction (for example, at school or home);
 - (g) reproducibility of symptoms on repeated exposure;
 - (h) what food and how much exposure to it causes a reaction;
 - (i) cultural and religious factors that affect the foods they eat;
 - (j) who has raised the concern and suspects the food allergy;
 - (k) what the suspected allergen is;
 - (l) the child or young person's feeding history, including, the age at which they were weaned and whether they were breastfed or formula-fed, if the child is currently being breastfed, considering the mother's diet;
 - (m) details of any previous treatment, including medication, for the presenting symptoms and the response to this;
 - (n) any response to the elimination and reintroduction of foods (NICE).

Additional Information on Consumption of the Major Allergenic Foods, Food Chemicals, and Any Possible Cross-Reactions

- (i) Do you regularly eat the following foods (peanuts, tree nuts, sesame seeds, celery, milk, egg, wheat, fish, shell fish, molluscs, soya, lupin, mustard, or sulphite containing foods) and do you experience any problems when eating them?

- (ii) Do you have hay fever or are you allergic to pollens?

It is important that the person taking the allergy-focused diet history has knowledge of all aspects of food allergy. OAS or oral itch is a type of food allergy classified by a cluster of allergic reactions in the mouth in response to eating certain (usually fresh) fruits, nuts, and vegetables [15]. However, within the spectrum of OAS lies the pollen-fruit syndrome (PFS), characterised by much milder symptoms and needs much less stringent dietary restrictions. It is now widely reported that OAS is becoming a widespread problem in Europe, particularly in younger age groups [16]. In the UK, the problem in the adult population is growing [17], but the number of children suffering from this problem is unclear. Information about OAS is particularly important for successful management of fruit, vegetable, and nut allergies in order to prevent unnecessary restrictions in these people's diets. PFS is not known to cause anaphylaxis but true IgE-mediated allergies to fruit, vegetables, and nuts, which are on the other end of the OAS spectrum can be potentially fatal, and it is important to distinguish between the two.

- (iii) Do you eat mainly home-cooked or commercially prepared foods? This will enable and prompt further questioning about possible chemicals in food.

Factors to Take into Account during the Decision Making

- (i) What is the clinical relevance of positive test results if tests have been done?
- (ii) Is there any cross-sensitisation between foods and aeroallergens?
- (iii) Are there any extrinsic factors involved: medication, stress, exercise, alcohol, hormonal, infection, vitamin and mineral supplements and herbal medication.

4.3. A General Diet History to Assess the Quality of the Diet.

Chronic symptoms may also be caused by other nonallergenic factors or may be caused by an unbalanced or deficient diet with similar presentation to food intolerance. Ask general questions focusing on nutrients of importance, particularly if a dietitian is in post. This can be done by dietitians using 2–7 days food diaries, a 24 hours recall diet history, or a more general diet history [18, 19]. The allergy specialist dietitian could be the ideal HCP to deal with this part of the diet history, having knowledge on food allergy, nutrition, and foods.

When to Refer to a Dietitian

- (i) The more foods avoided the more important it is to refer to the dietitian [7];
- (ii) general growth monitoring and nutritional assessment, particularly children with growth issues either faltering growth or malnutrition [8];

- (iii) for help and advice on infant formula/milk substitutes and weaning [1];
- (iv) for advice on the general nutritional aspects of the diet and for psychological support [20];
- (v) for information on life-style issues when living with food allergies such as, eating out, travelling, school trips/camps [21].

5. Principles for Management of Food Allergy and Intolerance

The key treatment of food allergy is avoidance of the allergenic foods. Although this sounds very simple, dietary management encompasses more than advice on avoidance of the allergenic foods. Scientific data are scarce, but there is general agreement that the aims and principles of the dietary management of food allergy are multiple and include the following:

- (1) obtaining relief of symptoms by avoidance of the allergenic foods;
- (2) preventing inadvertent exposure to the allergenic foods;
- (3) preventing the patient from unnecessary avoidance of foods;
- (4) supporting normal growth and development for age and gender in children;
- (5) providing an adequate, healthy, nutritionally dense, and balanced diet with appropriate alternatives for the excluded food allergens to minimize the impact on quality of life [22].

The dietary management of food allergies can be complex and difficult to follow in most cases and input from a dietitian is very important. For optimal dietary management it is very important to know which foods should be avoided in order to give appropriate avoidance advice [20]. Without clear identification of the allergenic foods dietary management of food allergy becomes difficult. Additionally, a clear diagnosis enhances appropriate coping strategies and determines the level/degree of avoidance required.

In general, stringent dietary advice may be needed for food-allergic patients, including the avoidance of the following [23]:

- (i) foods containing allergenic ingredients, even in small amounts;
- (ii) foods having a high risk for cross-contamination, such as chocolate in the case of peanut or cow's milk allergy/foods with precautionary labeling, although an individual risk assessment is recommended by some clinicians;
- (iii) unrefined oil derived from allergenic foods;
- (iv) meals or foods of uncertain composition, (for example, away from home).

Regulatory legislation is very helpful when allergenic foods are to be avoided as these allergenic foods must be declared on the label of prepacked foods. For the EU these are milk (including lactose), egg, soy, wheat or gluten, peanut, tree nuts, sesame, fish, crustaceans, molluscs, celery, lupin, mustard, and sulphites [24]. However, dietary management becomes much more complex when other foods leading to severe reactions have to be avoided.

The dietitian should educate the patient about this legislation, about reading labels, about high-risk foods, and high-risk settings such as eating out, to be stringent if medical care is not nearby and about early signs of severe reactions. Advice for school meals, day care, birthday parties, holidays, and other social circumstances should also be addressed. All of these points should be included and discussed in an adequate dietary management plan [23].

Another aspect of food hypersensitivity is that many patients choose to avoid too many foods and therefore over-restrict their diets [3, 25]. Patients may avoid foods related to the allergenic food, for example, peanut allergic patients may avoid all other nuts and sesame, while this is not always indicated. Anxiety of unfamiliar foods and fear of inadvertent allergic reactions may also lead to unnecessary avoidance.

Anecdotal evidence indicates that food allergic patients live with a permanent alertness as to what they are eating in numerous situations and settings. Successful avoidance of foods requires an understanding of label reading, meal preparation, and effective communication to friends and restaurant personnel providing food [20, 26]. The knowledge of the potential dangers of an accidental exposure may lead to a heightened level of anxiety [27] and negatively impacts the quality of life [28], as well as affecting lifestyle issues and welfare [29]. Previous research studies have shown that food allergic consumers experience difficulties when eating out and while shopping [30, 31].

The number of studies looking at health-related quality of life (HRQoL) in those suffering from food allergies has increased over the years. These studies showed that HRQoL in patients with food allergy and their families was significantly reduced [29, 32–35]. They found that several areas of HRQoL are affected, such as, family and social activities, emotional issues, and family economy, basically all aspects of family life. Food-hypersensitive children are to a large extent also limited in performing social activities without adult supervision.

Patients should therefore be encouraged to expand their diet using foods which have been proven to be safe in a graded fashion. Specific ready-to-use introduction schedules for use at home for first exposure to foods have been developed and published and could also be used for reintroduction of foods [36].

Several patient groups deserve specific attention in dietary management, such as, lactating women and young children who need weaning/introduction of solids advice and advice on the most appropriate choice of hypoallergenic formula, picky/faddy eaters, and vegetarians.

It is recommended that lactating mothers with food allergic children should remove the offending food from their own diet for a period of time.

These foods can be reintroduced once the infant/child improves in order to establish whether they (the mother) need to continue with the food avoidance [1, 12, 37]. Mothers avoiding cow's milk from their diet should be supplemented with calcium and vitamin D according to the national guidelines for each country [38]. In most countries, vitamin D supplementation is suggested for all breastfeeding women, irrespective of avoiding cow's milk or not.

Choosing the right formula for the patient based on the clinical presentation is a matter of huge debate with clear differences between countries. This choice should ultimately be based on clinical presentation, nutritional composition of the formula, residual allergenicity of the formula, and other components added to the formula. The DRACMA guidelines [1] performed a comprehensive review of the literature and their different indications for formulas suggest the use of an aminoacid-based formula for anaphylaxis, Heiner syndrome, and eosinophilic Eosophagitis, with the use of extensively hydrolysed formula for all other clinical presentations. Three additional papers, however, suggest the use of aminoacid-based formulas for growth faltering, severe atopic dermatitis, multiple food allergies, and infants not responding to maternal avoidance of cow's milk [37, 39, 40]. One should, however, always take national differences into account as some countries may use soya formula as a first-line approach for some clinical conditions in infants over six months [41].

Weaning is a particular problem in infants suffering from food allergies, and the most difficult question is when and how to introduce other highly allergenic foods into the diets of infants with milk/egg allergies with parents understandably being cautious about introducing these foods [42]. For both nutritional and developmental reasons [43], over restriction and delayed introduction of these foods are not recommended. Another clinical dilemma is whether to "screen" for other food allergies in children with one food allergy. There are difficulties with interpreting the results of these tests in young infants which may lead to over restriction of foods to which an infant is sensitised but not allergic. The National Institute of Allergy and Infectious Diseases (NIAID (USA)) guidelines [11] states that there is insufficient evidence to suggest whether, or which, foods should be tested prior to introduction in children at risk of food allergies (either from a high-risk family or with other existing food allergies). Testing prior to introduction could potentially prevent allergic reactions, but there is currently no practical consensus on which (if any) foods should be tested.

Until further clear evidence becomes available, each clinician may have their own preference in dealing with the problem, but it makes sense to start with low-allergenic foods first, try the cooked version of a food first, increase the amount given, and expand the diet as soon as possible. Parents should be clear on how to deal with any unexpected reactions and realise that children with coexisting asthma/wheeze are more likely to have (perhaps) severe reactions [44].

Food allergic individuals should also be followed up at regular intervals. New allergens can emerge or patients can outgrow one or more of their food allergies. It should be obvious that in multiple and complex food allergies the management of food allergy is tailored to the individual

patient and requires sophisticated skills and knowledge about food allergy and composition of foods from the dietitian. However, food allergy specialist dietitians are limited. Further education activities should be undertaken to educate more dietitians in diet history taking and the management of food allergy. It is one of the aims of INDANA to enhance the food allergy skills of dietitians around the world.

6. Impact of Allergy on Growth of the Children

Dietary antigens induce a local hypersensitivity reaction impairing the intestine's barrier function, leading to continuation of inflammation. The consequences of the inflammatory responses may be severe and manifested as impaired growth, increased symptoms, and poor quality of life. The cornerstone of the management of documented food allergies is an elimination diet and when appropriately designed and accomplished it dampens the inflammation and ensures optimal growth and well-being of the child. Nutritional inadequacies in food allergic individuals may result particularly from the elimination of multiple foods or nutritionally key foods, such as, milk or cereals [7, 8]. Early onset of symptoms, manifested during the first few months of life, compared to late onset, 6 to 10 months of age, appears to result in more seriously affected disease and the delay in growth may be more pronounced [45]. A Brazilian study [46] demonstrated the impact of food allergy on a child's nutritional status as the prevalence of poor growth was seen in as many as a quarter of children diagnosed with cow's milk allergy at the age of 24 months or less. A weight-for-age Z-score below 2 was demonstrated in 15.1% and a height-for-age Z-score below 2 in 23.9% of the children. Importantly, delay in diagnosis and thus delay in initiation of an appropriate dietary management may slow down growth. Conversely early diagnosis is associated with an appropriate growth for age, along with shorter duration of symptoms, fewer food allergies, and improved prognosis.

There are a number of factors that could lead to poor growth in children with food allergy as summarised in Table 1. Nevertheless, severe cases of growth failure are rare, affecting only a minor proportion of children following an appropriate care plan and may relate to the severity of the disease, like a large skin surface area affected in atopic eczema [47]. Lack of appropriate care or poor compliance may result in severe nutritional inadequacies, for example, where small children have been fed with rice beverages with an inappropriate nutritional composition for the needs of a small child [48]. There is little evidence on the impact of food allergy on a child's body composition or bone health, but these may be potentially affected.

The mechanisms for impaired growth may rise from a sustained inflammation and subsequent reduced bioavailability or loss of nutrients in the gastrointestinal tract [49], while metabolic requirements may be increased. Patients may consciously or unconsciously regulate symptoms of the disease by (unnecessary) elimination of foods [50]. A limited diet may also be of psychosocial origin, particularly if food allergy is potentially life threatening as in some

cases of peanut allergy [51]. Patients may also develop food aversions and anxiety, resulting in inadequate dietary intake or replacement of allergenic foods by foods that are not nutritionally equivalent. Some parents seek help for their child's symptoms from alternative practitioners, and unfortunately they may prescribe very limited diets with subsequent impact on dietary variety and nutritional status [42]. In this case, appropriate allergy testing and reintroduction of foods to the child's diet under dietetic supervision resulted in regain in weight and general improvement of health.

Differences in food and nutrient intakes between children with and without food allergy have been reported. Dietary intake studies in children with food allergy compared to figures for healthy children or reference nutrient intakes have demonstrated lower intakes of energy and protein as well as that of zinc, iron, calcium, vitamin D, and vitamin E [52]. Deviations in growth may reflect a lower dietary intake of energy and nutrients or relate to allergy itself through inflammation. Indeed, a recent study showed that despite a deviant growth between healthy children and those with food allergy, no difference in dietary intake was detected [7]. This study again points to the need for nutritional evaluation of children with food allergies, preferably by allergy specialist dietitians.

Poor growth does not seem to be a feature of allergy per se but rather a matter of inadequate dietary intake with reference to the requirements. Faltering growth may culminate in patients not being regularly monitored by HCPs specializing in allergy, including a dietitian, as the reasons for growth failure are various. Prospective follow-up studies have demonstrated that with careful monitoring of growth and management of allergy in early childhood, the growth of children remains within population reference values and only a minor proportion evince poor growth [53]. In addition, an early diagnosis of food allergy and subsequent initiation of dietary management enable catch up growth and normal adult height [54]. Nevertheless, nutritional risks persist as a study reported that at the age of 7 to 15 years lower height and weight Z-scores were detected in children who had avoided two or more foods, particularly milk, at the age of three years due to allergic symptoms [55]. This is indeed demonstrated by a range of studies, including previously presented cases [51] as well as the case study of a boy with multiple food allergies presented in the next chapter.

Case 3 presents a boy with severe eczema associated with multiple food allergies.

7. Conclusions

An early diagnosis and appropriate care of food allergy are necessary to allow a good quality of life and nutritional status of the patient. An allergy-focused diet history assisted by the allergy-specialist dietitian may direct the physician to further diagnostic testing; supporting the final diagnosis dietetic expertise is important to conduct a dietary assessment to ensure appropriate intake of energy and essential nutrients and to provide patient-oriented counselling. This includes education on reading labels, safe eating at restaurants, risks

TABLE 1: Risks for impaired growth in children with food allergy.

(i) Delayed diagnosis
(ii) Onset of disease at an early age
(iii) Multiple food allergies
(iv) Active disease
(v) Persistent (subclinical) inflammation of the gut resulting in increased requirements and/or losses and poor utilization of nutrients
(vi) Inadequate food intake due to poor appetite, regulation of gastrointestinal symptoms by modifying diet
(vii) Elimination of multiple foods from diet
(viii) Elimination of staple, nutritionally central foods from diet (milk, cereals)
(ix) Poor compliance in dietary management (unwillingness to broaden diet variety)
(x) Extreme self-restriction of foods

of cross-contamination, and potential sources of hidden allergens but also informing about support groups and online resources. These patient-oriented tools facilitate the appropriate management and followup of patients with food allergies. Nonprofit organisations, such as the recently founded INDANA, focus on increasing awareness, education, and improvement of nutritional and dietary care in food allergy.

8. Case Studies

Case 1 (a teenage girl with anaphylaxis to peanut). Lisa was referred to the allergy specialist dietitian by the paediatrician because of episodes of abdominal pain and cramps with increasing severity and several food allergic reactions over the years. The family attributed these reactions to peanut, because she was diagnosed with peanut allergy since she was young. However, they had been avoiding peanut strictly.

The paediatrician recently updated the information on sensitization to foods and inhalant allergens. Sensitization to inhalant allergens was negative, sensitization to foods was positive for the following foods: hazelnut 0.61 kU/L, peanut 76.2 kU/L, pistachio 7.14 kU/L, soy 2.72 kU/L.

Sensitisation to coconut, almond, cashew nut, milk, egg, fish, and wheat was negative.

Clinical and Allergy-Focused Diet History. There is a positive family history of atopy and asthma in her father's family. The clinical history revealed that she has suffered from abdominal pain since she was very young. She reported increasing episodes of having cramps and nausea. She has asthma which was under good control. She was carrying an epipen for her peanut allergy, however, was not confident about using it.

Firstly, the diet history focused on the clinical relevance of the foods sensitized to and on excluding dietary errors on peanut ingestion.

Lisa has been trying to avoid peanuts and other nuts since she was young. The family read labels and try to avoid foods containing peanut and nuts. Foods with advisory labeling for peanuts and nuts were avoided.

Over the years, several allergic reactions occurred after eating a food or meal, of which the family thought that peanut must have been the causative ingredient.

The reactions did not occur immediately. Remarkably, most reactions, except the reaction to M&Ms, were preceded by a form of exercise after eating (gym, playing outside, karate lessons) or stress (school party).

- (i) When she was much younger she reacted to M&Ms with vomiting.
- (ii) When she was 11 years old, she had an anaphylactic reaction following a home made Indonesian meal without nuts or peanut, for which she used her epipen. The meal included tofu and soy sauce.
- (iii) Last year, at a school party, she reacted with swollen lips, itchy ears, and dyspnoea after eating a chicken nugget.
- (iv) A few months ago she reacted with swollen lips, tachycardia, and presyncope after eating meat coated with bread crumbs.
- (v) Over the years there were several reactions of tachycardia, abdominal pain with cramps, and lip swelling having had commercially prepared meat products.
- (vi) On one occasion she had a hazelnut and a cookie with almonds without symptoms.

Secondly, the diet history focused on the nutritional composition of the food to rule out any over or under consumption of foods, because cramps and nausea may be related to fibre consumption. Lisa's diet, however, seems to be sound with no nutritional imbalances.

The dietitian also suspected the sensitisation to soy to be of clinical relevance and suspected exercise-induced anaphylaxis to soy. The paediatrician agreed with this, and the dietitian advised Lisa and her family to avoid not only peanuts and nuts, but also soy protein. Soy protein was incorporated in the Indonesian meal and could have been incorporated in the chicken nuggets, coated meat with bread crumbs, and commercially prepared meals.

Results. No anaphylactic reactions occurred from that point forward, and the complaints of abdominal pain, cramps, and nausea disappeared. The dietitian advised to continue avoiding peanuts, nuts, and soy protein and to take particular care when exercising. The avoidance of all other nuts needed further consideration. The paediatrician/allergy nurse updated the information and the use of the adrenaline autoinjector.

Teaching Points

- (i) An allergy specialist dietitian can sustain the diagnosis in taking an allergy-focused diet history.
- (ii) The diet history is the cornerstone of the diagnosis of food allergy and may direct the nature of the foods to be avoided.
- (iii) The dietary history may reveal if chronic symptoms may be caused by a deficient or unbalanced diet and if external factors may play a role, such as, exercise.

Reason for Referral to an Allergy Specialist Dietitian. In many countries, access to the dietitian is limited, and allergy specialist dietitians are scarce.

Referral is specifically important:

- (i) when an allergy-focused diet history is required to examine if certain foods provoke symptoms or, in case of chronic symptoms, these complaints may be caused by a deficiency or imbalance in the diet;
- (ii) when nutritional adequacy of the diet is questionable and needs to be checked, for example, in case of avoidance diets in young infants and toddlers (specifically cow's milk free and wheat free diets), multiple food allergy, picky eaters, faltering growth, and when symptoms do not improve despite adequate medication;
- (iii) when counselling and nutritional management are required, for example, in patients having questions about the practical implication of the avoidance diet (replacements of foods, social activities, school meals, school camps, holidays), allergic reactions despite following an avoidance diet, anxiety to food, and overrestriction of foods.

Case 2 (11 months old boy with multiple food allergies).

Referral

- (i) Oscar was referred to the allergy specialist dietitian by the paediatrician because of acute urticaria and angioedema after ingestion of egg and growth faltering. Skin prick test results indicated;
- (ii) egg allergy (8 mm SPT);
- (iii) peanut sensitisation (SPT 4 mm);
- (iv) no other positive SPT/IgE for milk, wheat, fish, soy.

Clinical History. There is a positive family history for atopy and asthma in both families. The clinical history reveals that he has suffered from eczema from about 2-3 months. He also has "loose" stools but does not wheeze.

Allergy-Focused Diet History: Enquire about Breastfeeding, Formula Feeding and Weaning onto Solids, and If Any Reactions Occurred. Oscar was breastfed until 6 months and received a top-up drink of cow's milk formula from 1 month of age. Solids were introduced at 5 months starting with baby rice, vegetables, and fruit. At six months gluten was introduced then chicken, lamb, fish, and lentils. No reactions were noticed to these.

Enquire about the Introduction of the Major Allergenic Foods

Milk given from 1 month and mother did not avoid milk during lactation with no noticeable reactions.

Wheat was introduced at 6 months with no noticeable reactions.

Fish was given at about 7 months with no noticeable reactions.

Egg was given at 10 months when he had the reaction.

He has not had peanut, tree nuts, sesame seeds, shell fish, molluscs, mustard, celery (although she thinks it might have been in a stew she made), lupin (although difficult in the UK to know which foods contain these), soya (not sure but as she cooks everything at home it seems to be unlikely).

General Diet History. Mother offers 3 meals per day which are nutritionally balanced, but he has always been a "difficult feeder". He has breakfast most days (baby rice with apple) but often refuses lunch (usually have 12 banana) and needs distraction most meal times but may eat about 90 g of dinner with pasta/rice/potato, meat, and mixed vegetables.

He has 350 mL/day of formula, which he drinks in 5-6 bottles of 60 mL/bottle.

Dietary Assessment. There are some concerns about his intake of protein and energy (60% of required calculated intake), iron (75% of UK RNI), calcium (51% of UK RNI), and vitamin D (60% of UK RNI).

Dietary Management

Initial Dietary Intervention. The initial dietary consultation included advice on an egg and nut-free diet. It was felt that the diagnosis of egg allergy was clear. The peanut result clearly only indicated sensitisation at this stage rather than clinical allergy as he has not consumed peanut until now, but it was felt that he was too young for a peanut challenge.

The formula was changed to an energy dense formula of 100 kcal/100 mL and 2.5 g protein/100 mL. A normal infant formula contains approx 67-79 kcal/mL and 1.4-1.7 g of protein. Mother was also advised to further increase his protein, kcal, and calcium intake with cheese and to add double cream to sauces. Meal times should be kept to 30 min and a long consultation about dealing with faddy eating, including, practical tips follows. No force feeding was allowed and mother was asked to allow time for messy play. Inclusion of red meat (iron) and fatty fish (vitamin D) when possible was recommended, but these deficiencies should be corrected by taking sufficient formula, to initially aim to increase formula to 500 mL per day, which would not provide all the nutrients he needs but a step in the right direction.

After Dietary Intervention. After the dietary intervention, his volume of feed was reduced and he was only managing 30 mLs per bottle that is about 180 mL per day. His "difficult feeding behaviour" turned into extreme food refusal, and he was crying during and after feeds showing clear signs of discomfort. His eczema also seemed to flare after every meal. He stopped gaining weight.

Results: Followup and Current Intervention. He started on a cows' milk and soya-free diet and changed formula to amino-acid-based formula. The decision about soya avoidance was based on the concomitant soya allergy seen in children with gut symptoms and a non-IgE-mediated (in this case) milk allergy. His eczema cleared completely, feeding standard improved, and he started to gain weight again. The patient is currently on egg- and nut-free diet with plans to review the nut-free diet and possible challenge after 12 months. Both the suspected milk and soya allergies also need to be confirmed by challenge, but there are currently no standardized procedures for performing food challenges in children with non-IgE-mediated food allergies.

Teaching Points

- (i) An allergy specialist dietitian can support the diagnostic process and may identify other nutrition-related issues.
- (ii) The diet history forms an important part of the diagnosis of food allergy and may direct the nature of the foods to be avoided particularly in the case of infants foods involved in delayed symptoms.
- (iii) The diet history may reveal any deficiencies which may relate to growth and development problems in infants.

Reasons for Referral to Dietitian. The dietitian can assist in and plays a crucial role in:

- (i) taking an allergy-focused diet history;
- (ii) identifying any additional feeding problems either behavioural or nutritional;
- (iii) assessing nutritional intake and nutritional status;
- (iv) dietary management of any issues surrounding growth;
- (v) practical advice on food avoidance and suitable replacements which can include:
 - (a) foods to avoid;
 - (b) label reading;
 - (c) suitable substitute foods for example, egg replacers/suitable infant formulas;
 - (d) recipe adaptation and suitable cook books;
 - (e) internet resources;
 - (f) support groups;
- (vi) assistance with design of food challenges;
- (vii) with young children having feeding difficulties when being weaned;
- (viii) in pregnant and lactating women following an avoidance diet for more than a few weeks, when losing weight involuntarily or when they consider stopping breastfeeding due to lack of dietary counselling and/or decrease in breast milk production.

Case 3 (a boy with severe eczema associated with multiple food allergies).

Referral

Jack presented at the allergy centre at age 3 months with severe eczema covering a large area of his body and face. A detailed history revealed that he had been born at full term, with a normal delivery. Jack was exclusively breastfed and his weight was between the 25th and 50th centile. His family history included asthma and hay fever in both parents. Following consultation with a paediatrician and a dietitian, Jack's mother was given information on skin care for eczema, in line with NICE guidelines [18]. Although she did not want him to have skin prick tests (SPT) at this stage, she was advised on avoidance of foods that commonly cause allergic reactions in breastfed infants, and she agreed to begin a diet excluding cow's milk and egg. In addition, advice on low-allergen weaning was provided, as Jack's mother wanted to start introducing solid foods in the coming months.

When Jack returned to the clinic at age 6 months, his mother was successfully avoiding dairy and egg. She had read advice that rice milk should be avoided for babies and toddlers, and she was drinking soya milk instead, consuming more than 500 mL each day. Jack's eczema was improving but not completely cleared, and his weight had dropped below the 25th centile. His mother also felt that he was irritable and not keen on feeding. Jack was otherwise well, with a normal physical examination and full blood count.

Jack had begun weaning at age 17 weeks, with baby rice, fruit, and vegetables. He had accidentally been given fromage frais (contains milk) by a relative and had experienced a severe immediate reaction, involving swelling of his lips and tongue, red facial flush, hives on his chest, and wheezing. Indeed, SPTs at age 6 months confirmed that Jack was sensitized to a number of foods, including milk, egg, and soya.

Given Jack's ongoing symptoms and his positive SPT to soya, the paediatrician advised his mother to begin excluding soya from her diet, while continuing to avoid egg and dairy. Jack's mother was feeling worn out by the constant effort of checking food labels and managing Jack's eczema and irritability. Consequently, she wanted to introduce a formula feed at bedtime, and an amino-acid-based formula was prescribed. Written information was provided explaining that it could be expected to taste and smell different from other formulas, and also that the change in diet may alter stool colour and consistency. If required, to aid introduction, we advised that it could be mixed with breast milk, pointing out that mixed feeds must be used immediately to avoid possible digestion by enzymes in the breast milk. Additionally, all foods, excluding dairy, egg, soya, and peanut, could be introduced into Jack's diet, one at a time.

Jack's mother initially introduced amino-acid-based formula in a 30:70 mix with breast milk. The proportion of formula was gradually increased and within a few days, Jack was taking full formula feeds. At age 9 months, Jack's weight had increased to just below the 50th centile and he had a varied diet of solid foods, with breast milk and night-time feeds of amino-acid-based formula. Jack's mother was also adhering well to the exclusion diet and was taking calcium

and vitamin D supplements. At age 12 months, his weight was on the 75th centile. Two days prior to the appointment, he had a reaction after eating hummus, despite the fact that his mother was eating hummus regularly and he was still receiving breast milk. The symptoms included swollen lips and a red facial flush, though there was no wheezing this time. SPTs at age 12 months confirmed that he was sensitized to sesame, among other foods.

We advised that Jack and his mother should exclude sesame from their diets, while continuing to avoid dairy, egg, and soya.

Jack's progress will be monitored at 6 to 12 monthly intervals, as appropriate. At each follow-up visit, SPTs and records of accidental ingestion will be used to determine whether oral challenges should take place. Deciding when to challenge can be difficult and should take into account both the clinical factors discussed here and the family's readiness, as the process can cause anxiety and emotional distress. A negative oral challenge for a given food will then enable recommendation of its introduction to Jack's diet. Clinical reactivity to milk and sesame has been confirmed by the reactions during oral exposure. The diagnosis of egg and soya allergy at this stage is based on sensitisation and improvement of symptoms after avoidance but will need to be confirmed by oral food challenges under medical supervision.

Acknowledgments

Dr. Rosan Meyer is acknowledged for the coauthoring of the case in Case 8. Text was revised and checked by Gill Glasbey, The David Hide Asthma and Allergy Research Centre, Isle of Wight.

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Review Article

Food Production and Processing Considerations of Allergenic Food Ingredients: A Review

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Received 4 May 2011; Revised 29 August 2011; Accepted 2 September 2011

Academic Editor: Kirsi Laitinen

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Although most consumers show no adverse symptoms to food allergens, health consequences for sensitized individuals can be very serious. As a result, the Codex General Standard for the Labelling of Prepackaged Foods has specified a series of allergenic ingredients/substances requiring mandatory declaration when present in processed prepackaged food products. Countries adhering to international standards are required to observe this minimum of eight substances, but additional priority allergens are included in the list in some countries. Enforcement agencies have traditionally focused their effort on surveillance of prepackaged goods, but there is a growing need to apply a bottom-up approach to allergen risk management in food manufacturing starting from primary food processing operations in order to minimize the possibility of allergen contamination in finished products. The present paper aims to review food production considerations that impact allergen risk management, and it is directed mainly to food manufacturers and policy makers. Furthermore, a series of food ingredients and the allergenic fractions identified from them, as well as the current methodology used for detection of these allergenic foods, is provided.

1. Introduction

Exposure to undeclared ingredients in processed foods constitutes an important source of concern for allergic individuals. Although the vast majority of consumers will not show any adverse reactions of medical concern, contact with tainted food products could translate to anaphylaxis and potentially death for sensitized individuals. The challenge to find safe ready-to-eat foods is even greater for people displaying multiple food allergies, a phenomenon of particular importance in children. As a result, proper package labelling is enforced on the food manufacturer, and active surveillance for priority allergens on finished goods has constituted one of the primary activities of governmental agencies worldwide.

Adaptations to the modern fast-paced lifestyle have led to increased commercialization of processed prepackaged food products to keep up with people's demand for convenience and variety. Some of the many changes in the way popular foods are produced include greater use of machines to reduce

processing times, improve shelf life, and develop superior textural properties, but all of these advancements have also introduced many additional ingredients to the modern industrial recipes for prepackaged foods. New ingredients or processing aids are used to help in machinability of products at intermediate steps of manufacture (e.g., glycerine in cookies). Other new ingredients improve texture of the final product (e.g., soybean flour in sausages [1]) whereas others improve shelf life (e.g., sulphites in dried fruits [2]). Many new ingredients in these complex industrial formulations are known food allergens.

An additional level of complexity is introduced when the purity and authenticity of raw materials is in question. The cascade effect of using a heavily contaminated food ingredient in a complex recipe could be a source of confusion for all parties (manufacturers, consumers, and enforcement agencies) while still posing a threat to the health of consumers. A good example of this was a Margherita pizza recipe made with tomato sauce, mozzarella cheese, basil, and

oregano, on a wheat flour base pie (wheat flour, water, bakers' yeast, and salt); although this was a simple pizza recipe judging by the number of ingredients, it was the source of an anaphylactic reaction to buckwheat hidden within the crust dough for a young woman [3]. In some cases the allergen containing ingredient is a small fraction of a formula and the dilution effect of the recipe is enough to protect the consumer, but the threshold dose to trigger clinical symptoms varies greatly and depends on the sensitization level of the individual. For some, multiple oral exposures with a minimum cumulative dosage in the order of grams is required to cause a reaction whereas others require a dosage in only micrograms levels to elicit symptoms [4]. A summary of minimum levels to elicit adverse effects to some allergenic foods can be found in Table 1. The limit of detection and method of commercial test for these allergens is also included in this table.

Regulation regarding which food allergens to consider varies globally, although the current FAO/WHO Codex General Standard for the Labelling of Prepackaged Foods contains a defined list of eight foods or substances and their derivatives [5]. Similarly, Canada currently recognizes nine priority food allergens: peanut, tree nuts, sesame seed, milk, egg, seafood (fish, crustaceans, and shellfish), soy, wheat, and sulphites [6]. The United States of America recognizes soybeans in addition to the allergens in Codex [7, 8]. Australia and New Zealand includes bee products (bee pollen, propolis, and royal jelly) besides the Codex standard [9, 10]. The European Union regulations includes soybeans, celery, mustard, sesame seeds, and lupin in addition to the Codex standard [11]. Japan enforces the labelling of five allergens: wheat, buckwheat, egg, milk, and peanut; but recommends the labelling of another twenty foods: abalone, squid, salmon roe, shrimp, orange, crab, kiwi fruit, beef, walnut, salmon, mackerel, soybean, chicken, pork, matsutake mushroom, peach, yam, apple, gelatin, and banana [12]. The severity of patients' reactions to specific allergens and worldwide or regional incidence of the allergy constitute the general guideline to include allergenic foods on priority lists.

The present paper aims to review certain food production considerations with implications on allergen risk management. Also, a series of food ingredients and the current allergenic fractions identified from them, as well as the methodology for detection of these allergens in foods are reviewed. The collected information is intended to raise awareness for food manufacturing operations as well as to help in policy making.

2. Technical and Technological Considerations for Allergen Risk Management

The precautionary statement now widely used in prepackaged foods: "may contain traces of..." arises from a potential risk of allergen contamination which could occur either during manufacturing or due to the presence of allergens in raw materials. Described below are some of the inherent risks of allergen contamination in the food manufacturing process.

TABLE 1: Lowest amount of allergenic food to elicit an observed objective adverse effect (LOAEL) and limit of detection of contaminants (allergens) in foods.

Contaminant	LOAEL (mg of protein)	Method of detection	LOD (ppm)
Peanuts	0.25–10	ELISA	0.1
Soybeans	88–522	ELISA	0.016
Tree nuts	0.02–7.5	ELISA	0.06
Sesame seeds	30	ELISA	0.2
Gluten	20–100	ELISA	0.6
Mustard seeds	1–936	ELISA	1
Milk	0.36–3.6	ELISA	0.00004
Egg	0.13–1.0	ELISA	0.05
Seafood	1–100	ELISA	0.0009
Sulphites		Monier-Williams distillation	10

Data from multiple sources [165, 171–175]; LOAEL—lowest observed adverse effect level; LOD—limit of detection; empty cell means no data was found.

2.1. Issues at Primary Food Processing. Primary food processing involves the harvesting and initial conversion of plant and animal organisms into food and includes agricultural activities such as harvesting, slaughter, cleaning, sorting, and grading.

Proper allergen risk mitigation starts at this stage. The current enforcement system only tests terminated packaged foods and responds in a reactive manner with food recalls as the instrument to protect consumers. For some highly sensitized individuals a food recall is a measure that responds too late and which is incapable of preventing severe allergic reactions.

As an example, some fish allergic individuals have very specific sensitization for certain species of fish but could be tolerant to other fish species which could provide an opportunity to enrich the diet. The misidentification and therefore mislabelling of harvested fish species constitutes a potential risk of unintended exposure for such allergic consumers. Misidentification risks are of less concern when fish is grown and harvested in an aquaculture operation. In Ireland, some 25% cod and haddock products and as much as 82% smoked fish were found mislabelled using molecular biology techniques [13]. Similarly, some 75% of the fish sold in the United States of America as red snapper (the US Food and Drug Administration's legally designated common name for *Lutjanus campechanus*) belong to another species [14].

Agricultural activity presents its own challenges; non-allergenic crops contaminated with allergenic crops are an important risk to allergic consumers and can be compared to the historical contamination of wheat with the weed-plant purple cockle (*Agrostemma githago*) whose seeds are poisonous to the population at large; this contamination of the seeds carried over to the next planting season resulting in perpetuation or even amplification of the problem [15]. Certain contaminations of grains are particularly hard to detect due to the similarity of the kernels like soybean contamination of corn, or wheat in oats. Much of the

cross-contact risk for plant foods is minimized by Good Agricultural Practice (GAP) but additional measures could be taken to protect allergic consumers.

Similar to the more widely known Good Manufacturing Practice (GMP), GAP is a collection of methods including record keeping, which is designed and implemented to achieve a particular purpose mainly quality preservation, but can also be extended to food security, food safety, sustainability, and ecology [16].

Figure 1 shows a general schematic of the agricultural processes used in seed-food production (e.g., cereals, oilseeds, and pulses). Cross-contact with other plant species can occur at any point during this process. After primary processing which includes general cleaning and sorting, seeds can be kept for the following season and replanted; or heat treated to stop enzymatic activity which can alter taste followed by transportation for further processing. Wagons, trucks, and bins (silos) previously used to transport and store other crops can easily hold remnants of the previous crop and contaminate newly harvested crop. Machinery used to harvest seeds (usually a combine harvester) and cleaning and sorting mills can also hold significant amounts of previously processed crops thus contaminating newly harvested crop. Farmers concerned about cross-contact can take additional measures. These include thoroughly cleaning harvest combines, trucks, and bins; using dedicated cleaning/sorting mills; procuring bare land around the planted plot; carefully documenting and planning crop rotation; obtaining fertilizer in bags rather than bulk format which are distributed in trucks or wagons which could have been used to hold crops beforehand. Most of these measures go beyond GAP but may be required if seeds are to be labelled as allergen-free.

Allergen contamination in finished prepackaged food products has been extensively studied and is the focus of most legislation. However, the contamination status of bulk food ingredients before and after primary food processing is often unknown.

Common agricultural practices include the use of green manure and cover crops to provide nutrient and minimize invasive weeds or earth erosion and crop rotation; leguminous plants are usually rotated (planted in alternating seasons) with other crops due to their soil nitrogen fixation abilities which lessen the need for fertilizers. Farmers generally follow a three-year rotation pattern of peanuts with cotton, corn or small grains planted on the same land in intervening years. The complete removal of the peanut plant at harvest diminishes the risk of cross-contact with other cultivated crops; also harvesting techniques and equipment are radically different between peanuts and grains, but even in the event of peanut contamination of other grains, the difference in size of the produce is large enough for the sorting/cleaning operation to be effective at removing contaminants. Besides peanuts, the tillage of cover and rotation crops eradicates most of the previously planted species, but does not eliminate the risk of cross-contact. A few of the plants turned into the earth will be able to grow back and contaminate the next crop at harvest, unless tillage is performed quite a few times enough to exhaust the plants' stored energy and/or badly injure the plant to cause its death.

Mustard seed is a relative of canola that has the advantage of being tolerant to drought, heat, and frost. It is an annual, cool-season crop that can be grown in a short growing season, commonly in rotation with cereal grains. The potential for mustard to contaminate grains like wheat, buckwheat, flax, and canola exists and, therefore, needs to be assessed.

Currently, there are several standards used when neat groats, kernels, or beans are sold. For example, the Codex standard for gluten-free foods specifies a maximum of 20 ppm of gluten; however, other Codex standards exist for "unprocessed" grains and pulses which establish a variable tolerance of 1 to 3% for contamination with extraneous matter and/or other grains. In the case of oats, as an example, this can be as high as 3% maximum edible grains other than oats. This tolerance represents an extremely high amount in terms of potential allergenic contamination since it allows up to 30 000 ppm (3%) of wheat, barley, and/or rye in oat kernels. Some currently accepted levels of contamination in various crops in different countries are provided in Table 2 [17–20].

The different levels of foreign material allowed in different crops (Table 2) are greatly influenced by the market. Higher levels of contamination are expected for lower crop grades; however, inherent technological challenges in the cleaning process also exist which helps to explain the differences across crops. Segregation of machinery and effectiveness of the cleaning milling operation is reflected in the lower limits for lentils. In cases such as sorghum, the lower economic importance for Canada is reflected in the lack of regulation.

2.2. Issues at Secondary Food Processing. Industrialized food production is a complex globalized endeavour with ingredient sourcing from many different parts of the world, tight schedules, and pressing requirements for very high productivity and profitability. As with any production operation, these systems are not always perfect. Some common production practices increase the risk of cross-contact (e.g., push-through uninterrupted production of different flavour ice creams; sharing of production equipment for manufacturing of foods with very different list of ingredients; or the indiscriminate use of rework in many food sectors including the bakery industry) [21].

Figure 2 represents a general schematic of the food manufacturing process showing rework as the incorporation of preworked packaged food into new production batches as raw materials. The risk, however, is that rework can be recuperated from all the intermediate steps of the process before packaging (i.e., after measuring, mixing, dividing, cooking, cooling, packaging, etc.). In certain industries, rework can go as far as the recycling of processed, packaged end-products which did not comply with quality controls for nonsafety-related specifications, such as appearance.

In multisector bakery products manufacturing, rework from pastry lines is occasionally incorporated into lower end bakery products regardless of the inclusion of allergenic ingredients in the dough which could result in the presence of hidden allergens and violation of local and sometimes international allergen labelling legislation.

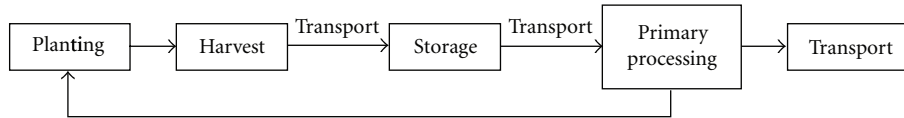


FIGURE 1: Schematic of the stages involved in food production and primary processing. Cross-contact with other crops, including allergenic organisms, can occur at any point in the process and can be magnified if harvested contaminated seeds are the primary material in the next planting season.

TABLE 2: Current maximum accepted levels of foreign material allowed in various crops in Canada, USA, and Europe.

Crops\grade	Foreign material allowed (%)										
	Canada (CE, CW)					US					EU
	1	2	3	4	5	1	2	3	4	5	
Oats	1, 0.75 w	2, 1.5 w	6, 3 w	14, 8 w							
	1, 0.75 b	2, 1.5 b	6, 3 b	14, 8 b	NA	2.0	3.0	4.0	5.0	NA	2
	NS, 1 c	NS, 2 c	NS, 3 c	NS, 8 c							
Corn	2, 2	3, 3	5, 5	7, 7	12, 12	2.0	3.0	4.0	5.0	7.0	5
Buckwheat	1	2.5	5	NA	NA						
Sorghum						1.0	2.0	3.0	4.0	NA	5
Soybean	1	2	3	5	8	1.0	2.0	3.0	5.0	NA	
Lentils	0.2	0.5	1	NA	NA	0.2	0.5	0.5	NA	NA	

CE—Canada East; CW—Canada West; w—wheat; b—barley; c—other cereals; NA—not applicable (grade does not exist for the given crop); NS—not specified; empty cell means no data was found.

Unrefined oils usually contain a higher amount of residual proteins from the starting raw material compared to purified oils; however, recently some refined oils were found to contain enough residual proteins to elicit IgE-mediated reactions in patients [22].

Another issue of concern is the contamination of food ingredients at source which could generate finished prepackaged foods containing ingredients not normally used as a typical ingredient of the food, such as wheat contaminated rolled oatmeal (gluten contamination). Gluten contamination of Canadian commercial oats was detected in 8 of 12 tested oats samples [23], and more recently 88% of samples on a larger Canadian survey was found contaminated by gluten [24]. Similarly, a study in the USA found 9 out of 12 samples of oats to be gluten contaminated [25]; another in Europe found 13% of oats products heavily contaminated with gluten with over 200 ppm [26].

For these reasons, HACCP programs in food manufacturing plants should include the analysis of critical control points for allergen contamination in order to effectively mitigate risks for consumers. Regular testing for allergens may be necessary as part of an allergen management plan at the manufacturing level in order to establish and monitor control limits with appropriate corrective actions to resolve deviations from normal acceptable levels. Thus, allergen testing tools that are simple to use and reliable are of paramount importance for the food industry.

Although testing methods for food allergens with excellent sensitivity and selectivity have been developed and commercialized, they are still subject to inaccuracies due to matrix and processing effects and stability issues [27, 28]. Conformational epitopes can be modified by processing or

residence time within the food matrix [29], although linear epitopes will generally withstand denaturant conditions in the food. The need for processed allergen reference materials that can help in research and allergen surveillance in processed food products has been recognized and various research studies have shown that differences in food matrices can affect allergen recovery and protein structure which may alter immunodetection [30].

As many foods are likely to contain multiple allergens, there also continues to be a need for methods to detect the presence of multiple allergenic proteins. Simultaneous detection of food allergens in foodstuffs is possible by quantitative real-time polymerase chain reaction (qPCR), but this could become cumbersome and prone to unspecific DNA amplification when too many primers are involved; there are also matrix effects that can have an important impact on the technique therefore limiting a real multiplexing application of the method. Legitimate criticism has also been raised against the validity of DNA as a molecular marker to test the presence in the food of allergenic proteins or peptides, particularly after processing.

Recently, a combination technique has been developed for the recognition of multiple fish species parvalbumin. The detection is based on the hybridization of DNA probes on beads to amplified DNA of food samples [31].

Other recent developments in multiplexed detection of allergens have been made using beads-based immunoassays but the extended environmental testing is still limited to non-food allergens: dust mite, cat, dog, rat, mouse, cockroach, and ragweed [32, 33]. Advances in mass spectrometry have permitted the recent multiplexing of food allergens detection, but the technique remains price-prohibiting and testing

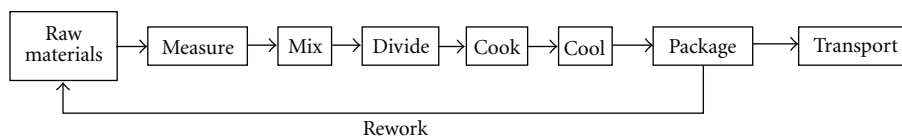


FIGURE 2: Example of rework in a food manufacturing process. Rework is the incorporation of preworked packaged food into new production batches as raw materials, but rework can be also derived from all intermediate steps before packaging. Rework is an important source of allergen cross-contact in the food manufacturing process.

times could be long when a proteolysis step (sometimes overnight) is needed [34–36].

3. Food Ingredients, Allergenic Fractions, and Recognized Allergens

3.1. Peanuts. The seeds of the leguminous crop *Arachis hypogaea* are processed to obtain a limited number of food ingredients: peanut oil, peanut butter, and peanut flour. Peanut ingredients (butter and flour) were once used to increase protein content, flavour, and taste of foods until the risks of peanut allergy were acknowledged; today, the use of these ingredients still remains popular in the food industry. Besides peanut ingredients, whole roasted peanuts also find wide use in confectionary products alongside tree nuts.

Studies carried out in Montreal, Canada found 1.5% of young school children (up to grade 3) were sensitized to peanuts [37]. Eleven relevant allergens of peanut has been identified *Ara h1* to *Ara h9* [38–43] plus two peanut oleosins designated *Ara h10* and *Ara h11* [44]. Traditionally *Ara h1* a protein of 63 kDa and *Ara h2* a 17–19 kDa doublet, have been designated the major peanut allergens based on the frequent and intense binding of IgE to these proteins on immunoblots with sera from peanut-allergic patients. Recently, *Ara h2* has been reported as the most potent allergen from peanuts [45]; additionally, *Ara h2* shows cross-reactivity to almond and Brazil nut [46]. Another cross-reactivity between *Ara h8* and *Bet v1* was observed; this is of special importance to Europeans given the abundance of birch trees in the region [47, 48].

Detection of peanuts allergens has been investigated using qPCR and ELISA with limits of detection (LODs) as low as 0.5 ng per mL [49, 50]. Also, combination techniques like liquid chromatography coupled with immunomagnetic beads has been investigated [51].

3.2. Soybeans. Botanically, soy (*Glycine max*) is a legume but similar to peanuts it is considered an oilseed from the a food technology point of view since it is not cultivated to be consumed in the pod like snow peas or for the dry grain like red kidney beans. Besides the numerous traditional dishes prepared with soy in Asia, for example, tofu, tempeh, soy sauce, soymilk, and miso; soybean ingredients have been developed for a great variety of mainstream food uses and include soybean oil, soy flour (min. 50% protein), flakes, grits, soy protein concentrate (65–85% protein), soy protein isolate (>85% protein), soybean lecithin, and soybean fibre.

A comprehensive list of primary and secondary ingredients from soy has been reported elsewhere [52].

Six different allergens in soybean have been designated: *Gly m1* and *Gly m2* are aeroallergens responsible for asthma reactivity [53]; *Gly m3* is a 14 kDa profilin that shows cross-reactivity with birch pollen profilin *Bet v2* [54]; *Gly m4* a disease resistance response protein of 17 kDa is present in soy products in variable quantities and it is also related to birch pollen *Bet v1* [55, 56]; the two major soybean storage proteins are also allergenic, β -conglycinin a vicilin, 7S globulin denominated *Gly m5* of 140–170 kDa; glycinin an 11S globulin of 320–360 kDa denominated *Gly m6* [57].

Detection methods for soybeans in food include ELISA-based methods [58], PCR, and qPCR-based methods [59], and combination methods like aggregation immunoassay involving the use of gold nanoparticles coupled with light scattering detection [60]. Detection and quantification methods for soybean allergens also depend on the protein extraction procedure from the food matrix. Specific extraction methods have been developed and standardized [61].

3.3. Tree Nuts. Tree nuts are the fruits or seeds of various tree species from the orders Rosales, Sapindales, Fagales, Ericales, Proteales, and Pinales, contained within a hard shell. These species do not form a taxonomic group but rather a functional or agronomic one. Tree nuts are consumed as mixed nuts usually roasted, or used in specialty bakery, pastry, and confections.

Allergen cross-reactivity is frequent and extensive within this group; pollinosis has also been observed persistently. In addition to serious and acute reactions including systemic reactions to tree nuts, a commonly observed reaction is oral allergy syndrome (OAS). OAS is characterized by itching or burning of the mouth, lips, tongue, and/or throat, with concomitant local inflammation [62].

Simultaneous reactions to tree nuts were observed in 12 of 62 patients studied, with the most common allergic reaction to Brazil nut plus other nuts. Also, allergy to peanuts plus other tree nuts was observed in 12 other patients [63].

Allergens from cashew (*Anacardium occidentale*) include the major allergen *Ana o1*, a 7S vicilin-like protein; a homotrimer of 45 kDa subunits. Cashew and peanut vicilins do not share linear epitopes [64]. *Ana o2* of 55 kDa, encode for a member of the legumin family (an 11S globulin) of seed storage proteins [65]. *Ana o3* of 14 kDa is a 2S albumin [66].

Pistachio (*Pistacia vera*) allergens *Pis v1* (7 kDa) and *Pis v2* (32 kDa), belong to the 2S albumin and 11S globulin

family, respectively [67]; *Pis v3* of 55 kDa is a 7S vicilin-like protein [68]; *Pis v4* a 23 kDa manganese superoxide dismutase-like protein [69]; a minor pistachio allergen *Pis v5* is an 11S globulin precursor peptide [70].

Walnut (mostly *Juglans regia* but also *J. nigra*): *Jug r1* a 2S albumin [71]; *Jug r2* a 7S vicilin-like globulin [72]; *Jug r3* a 9 kDa lipid transfer protein (LTP) [73]; *Jug r4* an 11S legumin-like globulin [74].

Hazelnut (*Corylus avellana*): *Cor a 1.04* is the major food allergen from hazelnut and it is closely related to birch pollen allergen *Bet v1*, but much less related with only 63% sequence homology to hazel pollen allergen *Cor a 1* [75]; less prevalent *Cor a2* is a profilin homologous to *Bet v 2* [76]; *Cor a8* and *Cor a9* are, respectively, an LTP and 11S globulin-like seed storage protein identified as a legumin, these two minor allergens are involved in life-threatening reactions to hazelnut [77]; *Cor a11* a vicilin-like 7S is a minor hazelnut allergen [78]; two oleosin isoforms of 17 and 14–16 kDa, now designated *Cor a12* and *Cor a13*, were identified as new allergens in hazelnut [79]; *Cor a14* is a 2S albumin of 15–16 kDa from hazelnut [80].

Almond (*Prunus dulcis*): almond major protein or amandin designated *Pru du6* is the major seed storage protein of almond with 360 kDa an 11S globulin legumin-like protein [81]; *Pru du4* a profilin, cross-reactive to ryegrass pollen profilins [82]; *Pru du3* a nonspecific LTP of 9 kDa [83]; *Pru du 5a* 10 kDa 60s acidic ribosomal protein [84].

Brazil nut (*Bertholletia excelsa*): *Ber e1* is a 9 kDa 2S seed storage albumin [85], and *Ber e2* is a 29 kDa 11S globulin legumin-like protein [86].

Macadamia nut (*Macadamia integrifolia*, *M. tetraphylla*, and their hybrids): although not as commonly consumed as other tree nuts, macadamia can occasionally cause serious allergic reactions like angioedema and dyspnoea [87, 88]. A previous case of anaphylaxis showed strong serum IgE binding to a protein of 17.4 kDa from both raw and roasted extracts [89]. There are no designated allergens for macadamia nut to date.

There are only two recognized allergens of pecan (*Carya illinoensis*). *Car i1* is a 16 kDa 2S albumin seed storage protein [90], and *Car i4* is a legumin 11S seed storage protein, hexameric with 55.4 kDa per monomer [91].

There are no designated allergens for pine nut (*Pinus* spp.) to date; although a 17 kDa allergenic protein has been detected [92]. Allergic reactions to pine nuts have been reported and include skin reactions, angioedema, hypotension, and anaphylaxis among others [93–96].

There are many protocols for detection of tree nuts in food. Some examples of analytical techniques include ELISA-based methods for detection of walnut [97], pecan [98], almond [99], and Brazil nut [100]; qPCR for detection of macadamia nut [101], hazelnut [102], pecan [103], and cashew [104]; time-resolved fluoroimmunoassay for hazelnut [105]. Simultaneous detection of multiple tree nuts is possible with qPCR-based methodology [106].

3.4. Sesame Seeds. *Sesamum indicum* seeds are mainly used whole dried or toasted for culinary purposes, and sesame oil is used in salad dressing in Oriental, Chinese, and South

American cuisines. The production and use of sesame oil is restricted to Mid and Far East and used primarily as a flavouring agent. Sesame seeds are a common sight as garnish of hamburgers' buns (breads), certain confectionary products, crackers, chips, vegetable patties (burgers), and oriental specialities.

Research on sesame seed allergens is recent and has allowed the identification of multiple important allergenic fractions: *Ses i1* a 9 kDa, 2S albumin [107]; *Ses i2* another 2S albumin of 7 kDa; *Ses i3* a 45 kDa, 7S vicilin-type globulin [108]; *Ses i4* and *Ses i5* are oleosins with 17 and 15 kDa, respectively [109]; two minor allergens *Ses i6* and *Ses i7* were identified as 11S globulins with 52 and 57 kDa respectively [110].

Detection of sesame allergens can be accomplished by qPCR assays [111, 112] or ELISA [113] with LOQ as low as 49 µg per g of sesame flour in food.

3.5. Wheat. Wheat (*Triticum* spp.) belongs to the Triticeae tribe within the Gramineae family of grasses. Of immense economic importance, wheat is the third grain grown globally after corn and rice. In the five years period from 2004 to 2008, the average world production of corn was 752 million tonnes, rice 645 million tonnes, and wheat 633 million tonnes; but adding up the production of wheat, barley, rye, and triticale (hybrid of wheat and rye) the figure goes up to 806 million tonnes which makes this group the largest cereal produced worldwide [114]. Many foods are made with wheat and its derived ingredients: flour, starch, hydrolyzed wheat protein, and so forth; therefore an avoidance diet for sensitized patients is a difficult proposition.

Food allergens identified in wheat include *Tri a12* a profilin of 14 kDa [115]; *Tri a14* a nonspecific LTP1 of 9 kDa [116]; *Tri a18* agglutinin isolectin 1 [117]; *Tri a19* omega-5 gliadin, a seed storage protein of 65 kDa [118, 119]; *Tri a25* thioredoxin [120]; *Tri a26* a glutenin of 88 kDa [121].

Aside from the IgE-mediated allergic response that wheat and related grasses can create in sensitized individuals, the importance of including wheat and other sources of gluten (or related proteins) as a priority allergen in the *Codex Alimentarius* derives from the greater and growing prevalence of celiac disease among the world population. Gluten sources have to be declared on packaging in many countries when a food contains gluten protein or modified gluten protein.

For celiac individuals it is the gluten protein or more importantly the prolamins contained in oats, barley, rye, triticale, or wheat, including kamut or spelt which causes the cell-mediated immunologic reaction with the consequent abdominal and nonabdominal symptoms. Recent studies have suggested that the prolamins from oats (avenin) is not toxic to celiacs [122–125], but the problem appears to reside in the contamination of oats by wheat, barley, or rye. Gluten contamination of commercial oats' products has been detected in various studies and therefore deserves further investigation and surveillance [24, 25].

3.6. Mustard Seeds. Canada was the top world exporter of mustard seeds in the five-year period of 2004 to 2008 [114].

There are three industrial cultivars of mustard: black mustard (*Brassica nigra*), oriental mustard (*B. juncea*), and yellow, also referred to as white mustard (*Sinapis alba* or *B. hirta*). European regulations include mustard as an allergen to be declared on food labels. Mustard has also been recently added to the Canadian list of priority allergens after extensive public consultation and review of the literature. Mustard seeds are principally used in the preparation of mustard condiments for which all three cultivars have specific uses, although *S. alba* seeds are the most frequently employed to produce the common yellow mustard condiment. Out of the three species, yellow seeds are the mildest also showing the lowest oil content. Oriental mustard seed is often used to produce spicy cooking oils utilized in traditional Asian cuisine. Mustard seeds are also used whole in spice blends or ground into flour which has multiple uses in processed foods like mayonnaise, salad dressings, soups, and processed meats for its taste, but also for emulsification and water holding capacity properties.

Mustard allergy accounts for 1.1% of food allergies in French children [126, 127]. The most predominant allergenic protein of yellow mustard, *Sin a1*, is a 2S seed storage albumin, a compact molecule with molecular mass of 14.18 kDa; this thermostable protein is resistant to *in vitro* digestion by trypsin and degradation by other proteolytic enzymes [128]. The principal allergen of *B. juncea* seed is *Bra j1* with a structure very close to *Sin a1* [129]. Another storage protein the 11S globulin *Sin a2* of 51 kDa has recently been identified as an important allergen [130, 131]. A couple of allergens derived from nonstorage seed proteins have also been identified (*Sin a3* a nonspecific LTP of 12.3 kDa and *Sin a4* a profilin of 13–14 kDa) which show IgE cross-reactivity with peach and melon fruits, respectively [132].

Quantitative detection of mustard allergens in food can be accomplished by sandwich-type ELISA with LODs as low as 1 μg of ground whole mustard seeds per mL [133, 134] or qPCR [111].

3.7. Milk. Milk is defined as the mammary glands' secretion of many animal species mostly cattle, sheep, goats, and buffalo. Milk is widely used as food ingredient after standardization, homogenization, and pasteurization. Many other food ingredients are derived from milk including cream, butter, cheese, and protein derivatives such as caseinates, whey protein, protein hydrolysates, and lactose. Due to the diverse list of ingredients derived from milk and the use of milk itself in a multitude of foods, it is a difficult allergen to avoid.

Cow's (*Bos taurus domesticus*) milk allergy is well documented and extensively studied. α_{S1} - and β -casein fractions from the milk coagulum and β -lactoglobulin from the lactoserum fraction are important allergens; in fact, all milk protein fractions display some degree of antigenicity with a multitude of conformational as well as linear epitopes [135, 136]. Formally designated allergens from milk are denominated *Bos d4* to *Bos d8* which refer respectively to α -lactalbumin, β -lactoglobulin, serum albumin, immunoglobulin, and caseins. Polysensitization and cross-reactivity

occurs between different milk protein fractions and among milk from different species making the selection for a cow's milk substitute among milk from other ungulates a very difficult task [136–138].

One popular approach for production of hypoallergenic baby formula is the use of partially hydrolyzed whey proteins. In these formulations the allergens of the casein fraction from milk are not present, and the allergens from the whey proteins are modified by hydrolysis, diminishing conformational epitopes, although linear epitopes could still remain. The degree of hydrolyzation should be controlled as extensive hydrolyzation creates bitter peptides. The use of partly-digested milk protein-based baby formulas do not eliminate all allergens, therefore it is usually advised as a preventative measure when there is a family history of atopy. Other formulations (soybeans or rice based) should be sought when milk allergy is confirmed for the infant.

As there are no cures for food allergies at the present time, complete avoidance of the allergenic food is the commonly prescribed therapy. In practical terms a zero-tolerance limit presents many challenges and allergen occurrence thresholds for enforcement agencies are often established based on detection and quantification limits of analytical techniques. There are several methods developed to detect and quantify the different allergens in milk [139]. Many ELISA-based methods have been developed and some are commercially available. Recent combination methods have been investigated based on different techniques such as liquid chromatography with mass spectrometry detection [140], specialized extraction coupled with ELISA detection [141], and surface plasmon resonance-based immunosensors [142], among others.

3.8. Eggs. Chicken (*Gallus gallus domesticus*) eggs are a very common food ingredient. They are used whole or as separated egg white and egg yolk. Eggs are a very important food ingredient from the technological stand point, since emulsifiers are found in egg yolk and foaming agents in egg white; although some of these functionalities can be simulated by other ingredients such as plant-derived emulsifiers and plant or micro-organism extracted gums, there is a price penalty.

Egg allergy is common among children, with prevalence calculated at 1.6% at 2.5 years of age [143]. The condition can be reversed, with as many as 11–50% of infants developing tolerance to eggs by age 4–4.5, and 82% by age 16 [144, 145]. The level of IgE to egg has been reported as a good predictor of clinical symptoms, and a level of ≥ 50 kU/L egg IgE as an indication of persistent egg allergy that will unlikely resolve before age 18 [144]. New oral immunotherapy has been successfully tested with potential for tolerance development [146]. A peculiar phenomenon of documented cross-reactivity is called the bird-egg syndrome [147], where sensitization for egg yolk livetins occurs via bird's aeroallergens [148].

Four allergens from hen's egg white have been documented *Gal d1* to *Gal d4* which are, respectively, ovomucoid, ovalbumin, ovotransferrin, and lysozyme. Additionally, two

allergens from egg yolk have been characterized, *Gal d5* or α -livetins [149]; YGP42 protein, a fragment of the vitellogenin-1 precursor denominated *Gal d6* [150]. All these proteins except for lysozyme exhibit different degrees of polymorphism and glycosylation [151].

Testing methodology for the presence of eggs in foods include ELISA-based tests [152] and qPCR [153].

3.9. Seafood. This group of allergenic foods is composed of crustaceans, shellfish, and fish, therefore many animal species comprising several allergenic proteins are included. Given the dominant flavour of this food group, seafood is usually not found as a contaminant of other food groups; in contrast, Asian cuisine makes intense use of seafood stock and fermented fish sauces as base flavour for many dishes.

Of all the allergenic fractions of seafood, β -parvalbumin stands out as a major allergen; this protein has been characterized and immunologically assessed in many fish species: Atlantic herring (*Clupea harengus*), Pacific pilchard (*Sardinops sagax*), Baltic cod (*Gadus callarias*), yellowfin tuna (*Thunnus albacares*), swordfish (*Xiphias gladius*), Atlantic salmon (*Salmo salar*), and ocean perch (*Sebastes marinus*) [154]. Allergic individuals usually avoid all species of fish while some people may tolerate a few, which is an indication of specific epitopes per fish species allergen.

Since the classical work of Shanti et al. [155] describing the allergenic characteristics of shrimp's (*Penaeus indicus*) major muscle protein tropomyosin, now officially denominated *Pen i1*, many food tropomyosins from other Decapoda species have been characterized and recognized in crab (*Charybdis feriatus*), shrimp (*Metapenaeus ensis* and *Penaeus aztecus*), white shrimp (*Litopenaeus vannamei*), North Sea shrimp (*Crangon crangon*), black tiger shrimp (*Penaeus monodon*), american lobster (*Homarus americanus*), spiny lobster (*Panulirus stimpsoni*), and also recognized in squid (*Todarodes pacificus*) and *Anisakis simplex* which is a nematode parasitic of marine mammals, crustacean, and fish. Tropomyosin can cause anaphylaxis in sensitized consumers who consume raw or processed seafood and fish [154].

3.10. Sulphites. Sulphites or sulfites are widely used food preservatives, employed to extend shelf life of foods and maintain food colour due to its antioxidant properties that prevent enzymatic and nonenzymatic browning. Its antimicrobial properties are also well known and historically employed in the food industry.

Sensitivity to sulphites is not an allergy *per se* but rather an adverse acute reaction to this inorganic substance, although IgE-mediated responses have been identified [156]. There are several compounds used in the food industry from which the water-soluble sulphite anion SO_3^{2-} is derived: sulphur dioxide, sodium sulphite, and potassium and sodium salts of bisulphite and metabisulphite. Incorporation of sulphites in recipes or its natural occurrence in excess of 10 ppm has to be declared in Australian, Canadian, New Zealand, and European food labels. USA standard labelling requires its declaration when present in excess of 10 ppm, but it is not part of the USA priority allergens list.

The amount of this preservative in foods markedly varies from around 10 ppm in frozen dough, corn syrup, and jellies, to up to 60 ppm in fresh shrimp, pickles, and fresh mushrooms, to up to 100 ppm in dried potatoes, wine, vinegar, and maraschino cherries, and up to 1000 ppm and beyond in dried fruit; lemon, lime, grape, and sauerkraut juice; some retail made-in-place fresh sauces [2, 157]. There is a compelling body of knowledge which indicates exacerbation of symptoms (bronchospasms) in sulphite-sensitive asthmatic individuals after ingestion of sulphites [157–162], although this has recently been challenged for sulphite-containing wines [163].

Japanese legislation requires sulphites to be declared as additives (bleaching agents) and allows from 30 ppm in squeezed fruit juice, 1500 ppm for raisins, 2000 ppm in dried fruits, up to 5000 ppm in kanpyo (dried gourd strips) [164].

Sulphites content determination in food is traditionally accomplished by the Monier-Williams distillation method [165]. Fast detection methods have also been developed like an enzyme electrode assay [166], flow injection analysis with voltametric detection system [167], ion chromatography with electrochemical detection [168], HPLC-fluorescence spectrometry method [169], ion-exchange chromatography with conductivity detection [170], and many others.

4. Concluding Remarks

Priority allergens lists are in constant review and prone to modifications to adapt them to regional epidemiological changes in allergic subpopulations. However, it is difficult to determine the accurate populations' prevalence of food allergies, and comparisons are most of the time invalid partially because of differences in methodologies and general testing criteria. Accurate food allergy incidence figures are elusive and cross-reactivity frequency estimations are even more obscure. Nonetheless, the obligation to protect the allergic public has been recognized by governments and international entities.

There is a need to apply a bottom-up approach to allergen risk management in the food manufacturing process starting from primary food processing practices in order to ensure greater food safety for allergic consumers. Assessment of the allergen contamination status of food ingredients at the primary processing level is of vital importance as it will help in the development of improved integrated solutions for allergen risk mitigation and in the establishment of a proactive food surveillance system.

For the food manufacturing industry the "clean-label" trend which calls for minimization of the number of ingredients in recipes has had a positive impact on production costs by consolidating and simplifying the sourcing of ingredients. This in turn may help in minimizing cross-contact of ingredients; however, the allergenic load in these raw materials after primary processing needs to be assured.

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Clinical Study

Late Type of Bronchial Response to Milk Ingestion Challenge: A Comparison of Open and Double-Blind Challenge

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Received 3 May 2011; Accepted 4 August 2011

Academic Editor: Carina Venter

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Background. In some asthmatics the food allergy, for example, to milk, can participate in their bronchial complaints. The role of food allergy should be confirmed definitively by food ingestion challenge performed by an open challenge with natural foods (OFICH) or by a double-blind placebo-controlled food challenge (DBPCFC). **Objectives.** To investigate the diagnostic value of these techniques for confirmation of a suspected milk allergy in bronchial asthma patients. **Methods.** In 54 asthmatics with a positive history and/or positive skin tests for milk the 54 OFICH, and DBPCFC, were performed in combination with spirometry. **Results.** The 54 patients developed 39 positive late asthmatic responses (LAR) and 15 negative asthmatic responses to OFICH and 40 positive LARs and 14 negative responses to DBPCFC. The overall correlation between the OFICH and DBPCFC was statistically significant ($P < 0.01$). **Conclusions.** This study has confirmed the existence of LAR to milk ingestion performed by OFICH and DBPCFC in combination with spirometry. The results obtained by both the techniques did not differ significantly. The OFICH with natural food combined with monitoring of objective parameter(s), such as spirometry, seems to be a suitable method for detection of the food allergy in asthmatics. The DBPCFC can be performed as an additional check, if necessary.

1. Introduction

Food allergy is a clinical manifestation of an immunologic process in which foods or their components acting as antigen(s) stimulate the production of specific antibodies or sensitize the particular T lymphocyte subsets and then interact with them [1–11]. This interaction then induces a number of intracellular and extracellular processes, defined as a hypersensitivity mechanism(s), resulting in the manifestation of the clinical symptoms [1–23].

Principally, various types of hypersensitivity can be involved in food allergy; however, the immediate type (IgE-mediated) hypersensitivity has mostly been investigated and documented [1–9, 11–19, 24–28]. Nevertheless, in recent years evidence has been found for possible involvement of other hypersensitivity types, such as late type (Type III) and delayed type (Type IV) in the food allergy [1–3, 5–8, 14, 15, 23, 24, 29–39]. The exact immunopathologic mechanisms underlying various clinical manifestations of food allergy are, however, not yet fully clarified [1–3, 7–9, 15].

Food allergy can occur in two basic forms: a primary form, where the foods act as the primary and sole cause

of the activation of the immunologic mechanism(s), and a secondary form, where food participates in an already existing hypersensitivity mechanism(s) activated by different antigens, for example, inhalant antigens. The secondary form occurs more frequently [1, 6–9, 11, 18, 40, 41]. Although the provocation tests with foods are not always performed routinely, they may be considered to be the definite confirmation of involvement of particular foods in the patient's complaints [1–13, 18, 20, 22, 25, 27, 28, 40, 42–51].

Provocation tests with foods can be performed using three basic techniques, the open food ingestion challenge (OFICH), double-blind placebo-controlled food challenge (DBPCFC), and the single-blind food ingestion challenge (SBFIC), all of them having a number of advantages and disadvantages [1–13, 18–22, 25–28, 40, 42–58].

The DBPCFC is generally considered to be “a golden standard” [1–13, 16, 25–28, 48, 49, 51, 58]. However, under some circumstances and for some reasons the OFICH may be more preferable to DBPCFC [1, 2, 6–11, 20–22, 25, 40, 43–46, 52, 53]. The purpose of this study was (a) to verify the possible involvement of milk allergy in some patients with bronchial asthma, (b) to compare the results attained by both

TABLE 1: Characteristics of the patients and control subjects.

	Total <i>n</i> = 54	Patients		Control subjects <i>n</i> = 12
		LAR <i>n</i> = 39	NAR <i>n</i> = 15	
Age (years)	31 ± 6	30 ± 7	32 ± 5	27 ± 6
Gender (M/F)	25/29	11/15	14/14	5/7
Disease history (years)	4.7 ± 1.3	3.5 ± 1.6	5.1 ± 1.0	4.2 ± 1.5
Asthmatic attacks per month	4 ± 1	5 ± 2	2 ± 1	3 ± 2
FEV ₁ (% predicted)	94.8 ± 4.2	93.1 ± 5.5	97.0 ± 3.3	95.6 ± 4.3
FVC (% predicted)	99.2 ± 1.1	96.4 ± 3.0	100.1 ± 1.4	98.0 ± 4.3
Blood leukocyte count (×10 ⁹ /L)*	7.1 ± 0.8	7.5.0 ± 1.2	8.0 ± 0.4	7.9 ± 1.5
Blood eosinophil count (×10 ⁶ /L)**	355 ± 60	387 ± 56	329 ± 70	410 ± 53
Blood neutrophil count (×10 ⁹ /L)***	5.2 ± 0.5	6.0 ± 0.3	4.5 ± 0.9	6.5 ± 0.4
Bronchial histamine threshold (BHT)□				
≤2.0 mg/mL		4	2	1
4.0 mg/mL		9	2	4
8.0 mg/mL		11	3	3
16.0 mg/mL		4	5	2
32.0 mg/mL		5	2	2
>32.0 mg/mL		6	1	0

Values: mean ± SD; *: normal value = 4.0 – 10.0 × 10⁹/L; **: normal value ≤ 300 × 10⁶/L; ***: normal value: 2.0–7.2 × 10⁹/L; □: normal value ≥ 32.0 mg/mL.

the techniques, OFICH and DBPCFC, and to assess their suitability and diagnostic values for the confirmation of food allergy involvement in patients with bronchial asthma, by monitoring the objective parameters, such as lung function using spirometry.

2. Material and Methods

2.1. Patients. Fifty-four patients suffering from perennial bronchial asthma suspected of participation of milk allergy, examined at our Department of Allergology & Immunology, Institute of Medical Sciences “De Klokkenberg,” Breda, The Netherlands, and developing 39 positive late or 15 negative asthmatic responses to OFICH, volunteered to participate in this study.

These patients, 18–39 years of age, included 46 subjects suffering from already existing bronchial asthma due to various inhalant allergens in whom the milk has been suspected to participate possibly in their bronchial complaints and/or demonstrating positive skin tests with milk and 8 subjects in whom the milk has been suspected to be a sole cause of their bronchial complaints. They showed positive skin (prick and/or intradermal) tests with milk to various degrees, and in some of them also positive specific IgE antibodies for some foods have been recorded (Tables 1 and 2). They did not suffer from any airway infections and did not use oral corticosteroids or immunotherapy.

The patients were examined by routine diagnostic procedure, acting also as an exclusion-inclusion check, consisting of (1) general part: disease history, physical examination, basic laboratory tests, X-ray of the chest and sinuses, lung function, blood gases determination, bacteriological examination of the sputum; (2) allergologic part: skin tests with inhalant and food allergens, bronchial histamine thresholds

[59], blood leukocyte differential count, determination of the serum immunoglobulins; (3) 95 bronchial provocation tests (BPT) with inhalant allergens [60–63]; (4) 54 OFICH with milk suspected from history and/or positive skin tests. The 54 food challenges with milk were then repeated by means of DBPCFC. A 5-day interval was always inserted between the consecutive tests to prevent the carryover effects and to allow the patient's recovery.

All challenges were performed in a period without manifest symptoms and during a short hospitalization of the patients. The milk and all dairy products were avoided by the patients for 3–4 weeks before the challenges.

Inhaled glucocorticosteroids (*n* = 35), long-acting β₂-sympathomimetics (*n* = 19), and oral cromolyn (*n* = 2) were withdrawn 4 weeks, inhaled cromolyn (*n* = 5), nedocromil sodium (*n* = 11), and leukotriene modifiers (*n* = 1) 2 weeks, and other treatments 48 hours before each of the challenges. The local ethical committee approved this study, and informed consent was obtained from all study participants.

2.2. Allergens. Dialyzed and lyophilized extracts of inhalant allergen as well as foods (Allergopharma, Reinbek, Germany) diluted in PBS were used for skin tests in concentrations 50–500 BU/mL, as indicated in the subsection “Skin tests.” The recommended concentrations by the manufacturer were 100–500 BU/mL for skin prick as well as for intracutaneous tests.

2.3. Skin Tests. The skin prick tests (SPTs) in concentrations of 500 BU/mL were performed [27, 28, 59, 64, 65], and evaluated after 20 minutes and 24 hours. If the SPTs were negative, then intracutaneous (intradermal) tests in concentrations of

TABLE 2: Survey of other diagnostic parameters.

	Total n = 54	Patients LAR n = 39	NAR n = 15	Control subjects [▲] n = 12
Bronchial complaints [△]				
(i) Dyspnea		++	0	0
(ii) Wheezing		++	0	0
(iii) Cough		±	0	0
(iv) Expectoration		0	0	0
Positive skin response (SPT) [◇]				
(i) Immediate	31	24	7	1°
Negative skin response (SPT) [◇]	23	15	8	11
Positive skin response (i.c.) ^{◇◇}	37	28	9	0
(i) Immediate	-15	-12	-3	
(ii) Late	-21	-15	-6	
(iii) Delayed	-1	-1	-0	
Negative skin response (i.c.)	17	11	6	
Increased total IgE (serum) ^{□□}	1	1	0	0
Positive specific IgE (serum) ^{□□□}	9	7	2	1
Increased total IgG (serum) [*]	2	2	0	0
Increased sub-classes (serum) ^{**}				
(i) IgG ₁	1	1	0	0
(ii) IgG ₂	0	0	0	0
(iii) IgG ₃	0	0	0	0
(iv) IgG ₄	2	1	1	0
Increased total IgM (serum) ^{***}	0	0	0	0
Increased total IgA (serum) [■]	3	3	0	0
Concomitant (allergic) disease				
(i) Allergic rhinitis	8	4	4	1
(ii) Atopic eczema	11	9	2	0
(iii) Urticaria	1	0	1	0
(iv) Angio-neurotic edema	0	0	0	1
(v) Gastrointestinal complaints	5	3	2	0

L: late asthmatic response; N: negative asthmatic response; [△]: Bronchial complaints accompanying the asthmatic response (author's modified score system): 0: absent, ±: very slight/incidental, +: slight, ++: moderate/intermittent, +++: pronounced/regularly, ++++: very pronounced/distinct/frequent; [◇]: skin prick test (SPT) with milk extract; ^{◇◇}: intracutaneous (intradermal) skin test with milk extract; ^{□□}: total IgE in the serum (PRIST)-normal value ≤ 500 IU/mL; ^{□□□}: positive allergen-specific IgE in the serum for milk (ImmunoCAP) ≥ 0.70 U/mL (=more than class 1); ^{*}: total IgG in the serum (Single radial immunodiffusion and ELISA)-normal value ≤ 15.0 g/L; ^{**}: normal values: IgG₁ < 5.0 g/L, IgG₂ < 2.6 g/L, IgG₃ < 0.4 g/L, IgG₄ < 0.5 g/L; ^{***}: IgM ≤ 3.8 g/L (<1.5); [■]: IgA ≤ 4.0 g/L (<3.2); [▲]: control subjects: 6 open (OFICH) + 6 double-blind (DBPCFC) food ingestion challenges with milk; [○]: positive skin response to milk extract.

100 BU/mL and 500 BU/ml were carried out [1, 6–10, 18–22, 28, 57, 60–65] and evaluated 20 minutes, 6, 12, 24, 36, 48, 72, and 96 hours after the injection. If the SPT were positive (immediate/early skin response), then the intracutaneous tests were performed in concentrations of 50 BU/mL and 200 BU/mL and evaluated 20 minutes, 6, 12, 24, 36, 48, 72 and 96 hours after the injection. Histamine diphosphate was used as a positive control, whereas PBS was used as a negative control. A skin wheal (>7.0 mm in diameter) appearing 20 minutes after the injection was considered to be positive immediate skin response, the skin infiltration observed between 6 and 12 hours was considered to be a late skin response, and the skin induration recorded later than 48 hours was designated a delayed skin response [6–10, 60–65].

2.4. Spirometry. The asthmatic responses were monitored by using spirometry (Spirograph D-75; Lode NV, Groningen,

The Netherlands), recording the FVC and FEV₁, and evaluated by the following criteria: (1) the decrease in FEV₁ of less than 10% with respect to the prechallenge values as negative, from 10% to 20% as doubtful, and of 20% or more as positive asthmatic response; (2) the decrease in FEV₁ values recorded at least at 3 consecutive time intervals was considered to be a positive response; (3) the response appearing within 2 hours after the challenge was considered to be an immediate asthmatic response (IAR), that occurring between 4 and 24 hours to be a late/asthma response (LAR), and response appearing later than 24 hours after the challenge to be a delayed asthmatic response (DYAR) [9, 18, 22, 60–63].

2.5. Food Used for the Ingestion Challenge. The quantities of milk used both for the OFICH and DBPCFC were similar to those consumed usually by the patients in order to obtain the highest degree of reproducibility. The amount

of 100 mL of natural milk (3.5 g of protein and 3.5 g of fat per 100 mL) was used for the OFICH. The amount of 20 g of powdered whole milk (containing 3.0 g of protein and 2.9 g of fat) dissolved in 80 mL water was used for DBPCFC. The 5% glucose solution was used as control (placebo) for OFICH. For the DBPCFC, 20 g of tablet inactive ingredients, so-called “excipients” (including lactose, dibasic calcium, sucrose, maize corn, starch, and microcrystalline cellulose) dissolved in 80 mL water, was used as control (placebo). Both the solutions used for DBPCFC, the powdered milk as well as the inactive tablet mass (excipients) were enriched with 4 g of glucose to mask their taste. The control challenges were performed according to the same schedule as those with the experimental foods. The DBPCFC arrangement was in principle triple-blinded, and that both for the technician preparing the test material, and for the nurse performing the challenge, and lastly for the patient himself.

2.6. Schedule of the Food Challenge. The OFICH and DBPCFC challenges as well as the spirometry monitoring of were performed according to the European and international standard procedures [2, 4, 25–27, 40, 42, 48, 49, 57, 58] modified by us [6–10, 18–22], by the following schedule: (1) recording of the initial (baseline) values at 0, 5 and 10 minutes; (2) ingestion of the food within 10 minutes, followed by a 1-hour waiting interval to allow the food to be ingested. During this interval the parameters were measured four times to exclude an unexpected or too early reaction; (3) recording of the postchallenge values at 0, 5, 10, 20, 30, 45, 60, 90, and 120 minutes, and every hour up to the 12th hour and every second hour during the 22nd and 38th hour, the 46th and 58th hour intervals [6–10, 18–22].

2.7. Control Group. Twelve patients suffering from perennial bronchial asthma, developing 12 late asthmatic responses (LAR) to BPT with *Dermatophagoides pteronyssinus*, however demonstrating negative history, skin test, and RAST for the foods, volunteered to participate as controls. In 6 patients the OFICH and in 6 patients the DBPCFC were performed with the most frequently consumed food, usually milk, cheese or peanuts, according to the same schedule as applied in the patients studied.

2.8. Statistical Analysis. Asthmatic responses were analyzed by generalized multivariate analysis of the variance (MANOVA) model [66]. The polynomials were fitted to the mean curves over time (8 time points within 120 minutes and 14 time points up to 24 hours after the challenge), and the appropriate hypotheses were tested by the modified MANOVA computerized system.

In every patient the postchallenge FEV₁ values measured at each time interval were compared with the prechallenge values and evaluated by Wilcoxon matched-pair signed-rank test. The mean postchallenge FEV₁ values were compared with corresponding post-challenge control values at each of the time points and analyzed by the Mann-Whitney *U* test. The correlation between the OFICH and DBPCFC was evaluated by Wilcoxon matched-pair signed

rank test. A *P* value < 0.05 was considered to be statistically significant.

3. Results

In 54 patients suffering from bronchial asthma, 54 open ingestion challenges with milk (OFICH) have resulted in 39 positive asthmatic responses of late type (LAR; *P* < 0.01) and 15 negative asthmatic responses (NAR; *P* > 0.1) (Table 1, Figures 1 and 2). The LARs began 4–6 hours, reached their maximum 6–10 hours, and resolved within 24 hours after the milk ingestion challenge (OFICH). The LARs were associated with various general and bronchial complaints, predominantly dyspnea (100%), wheezing (79%), cough (28%), oral itching (23%) and gastrointestinal complaints (31%) (Table 2), whereas no general or bronchial complaints were recorded during the NARs. The LARs as well as the NARs correlated with disease history, skin tests and other diagnostic parameters to various degrees (Tables 1, 2, and 3). The 39 patients developing positive LAR for milk in OFICH demonstrated positive skin tests, and positive (suspect) history in 48%, positive skin tests but unknown history in 4%, and positive (suspect) history but negative skin tests in 20%. The 15 patients developing negative asthmatic response (NAR) for milk in OFICH displayed positive skin test and suspect history in 11%, positive skin test but unknown history in 6%, and suspect history but negative skin tests in 11% (Tables 3 and 4). Survey of detailed agreement between the positive and negative asthmatic responses to OFICH with milk and the other diagnostic parameters (disease history, skin tests) in both the groups of patients, those with bronchial asthma to inhalant allergens and suspicion of milk allergy as well as those with bronchial asthma suspected of milk allergy only, is presented in Table 5. All 54 control ingestion challenges with glucose solution were negative (*P* > 0.1) and without any accompanying bronchial or general complaints.

The 54 patients challenged with milk by means of DBPCFC developed 40 positive LAR (*P* < 0.01) and 14 NAR (*P* > 0.05) (Table 6, Figures 1 and 2). The 38 of the 40 DBPCFC positive LARs correlated with the OFICH positive LARs (=97%; *P* < 0.01), whereas 13 of the 14 DBPCFC negative responses (NARs) correlated with the OFICH negative responses (NARs) (=87%; *P* < 0.05).

The 3 noncorrelating cases showed 2 OFICH negative responses but DBPCFC positive LARs and 1 OFICH positive LAR but DBPCFC negative response. The overall correlation between the OFICH and DBPCFC responses was statistically significant (*P* < 0.01). All 54 DBPCFC control challenges with “tablet excipients” were negative (*P* > 0.1). No bronchial complaints were registered during the DBPCFC controls; however 1 patient developed diarrhea to a slight degree during this test (=2%).

3.1. Control Group. The 12 patients of the control group, in whom 6 OFICHs and 6 DBPCFCs with milk were performed, did not develop any asthmatic response. No general or bronchial complaints have been registered during these 12 NAR (*P* > 0.2).

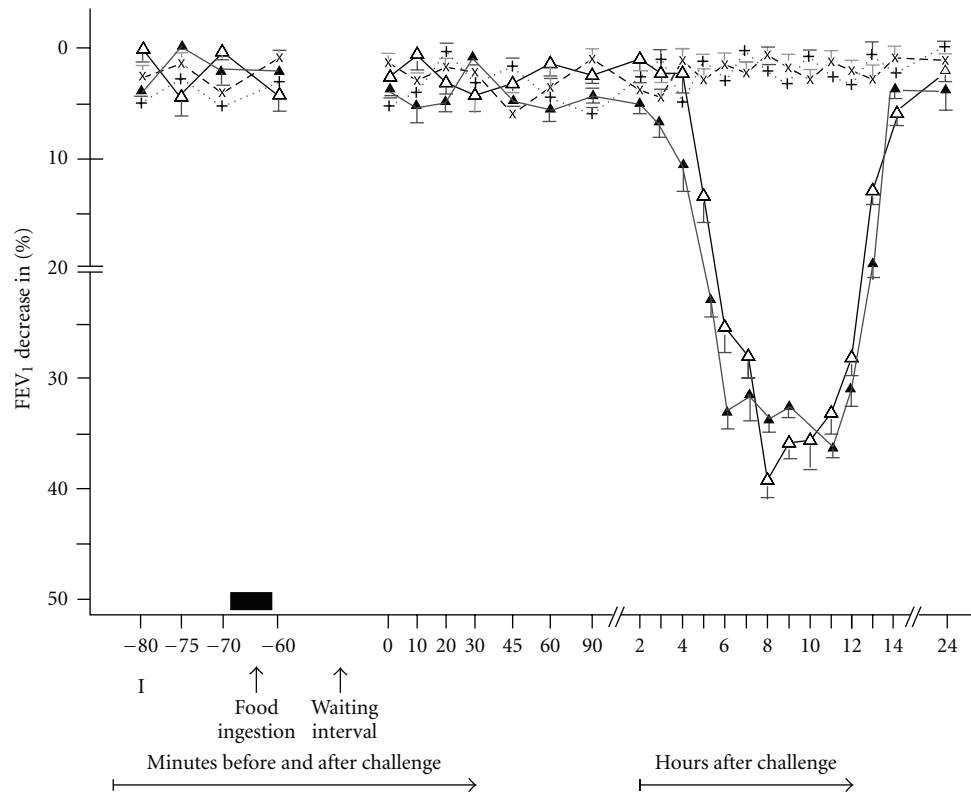


FIGURE 1: Late asthmatic responses due to the food ingestion challenge (LAR) in 39 patients with bronchial asthma. The mean percentage changes in the FEV₁ calculated from all patients with positive LAR. LAR ($n = 39$) recorded after OFICH: experimental food (Δ) and control food (\times) and after DBPCFC: experimental food (\blacktriangle) and placebo ($+$) I: initial (baseline) values; food ingestion: OFICH or DBPCFC; waiting interval: 1 hour; bars: means \pm SEM.

4. Discussion

The role of foods and food allergy in bronchial asthma in producing bronchial complaints, especially bronchospasm, through the hypersensitivity mechanisms has already been investigated from various points of views [1–9, 11–13, 15, 16, 18, 20, 22, 26, 42, 45, 53, 55, 56, 67]. The involvement of food allergy in bronchial asthma, classically attributed to the IgE-mediated hypersensitivity upon involvement of IgE antibodies, mast cells, basophils, eosinophils, and Th₂ lymphocytes, has mostly been investigated [1–3, 5, 9, 11–13, 15, 16, 24, 26, 29, 39, 44, 67]. Later, some evidence was also gathered for possible involvement of the non-IgE-mediated mechanism(s) upon participation of various cytokines, neutrophils, and Th₁ lymphocytes in the food allergy events [5–9, 14, 15, 23, 24, 29–39, 43, 53, 54, 68, 69]. The link between the BALT and GALT and the disturbed homing of T and B lymphocytes (plasma cells) may also play an important role in these processes [1, 14, 15, 24, 29, 33, 39, 68, 69].

Patients with bronchial asthma upon participation of food allergy, having been challenged with foods, may develop various types of asthmatic (bronchus-obstructive) response. The immediate/early (IAR), late (LAR), and delayed (DYAR) asthmatic responses to food ingestion challenge, described in our previous papers [9, 18, 20, 22] and some of these types reported also by other investigators [1–3, 11–13, 16,

20, 43–46, 53, 55, 56, 70], are in principle analogical to the three types of asthmatic response to the bronchial challenge with inhalant allergens [60–63]. The IAR, LAR and DYAR due to the food ingestion challenge differ substantially not only with respect to the possibly underlying immunologic mechanisms, but also in their clinical features, time course and association with other diagnostic parameters [1, 2, 7–9, 12, 13, 15, 18, 20, 22–24, 30–33, 38, 39, 53–56, 70].

The immediate/early asthmatic response (IAR/EAR) to foods, due to the immediate (IgE-mediated) hypersensitivity mechanism, has been investigated most frequently [1–5, 9, 11–13, 16, 24, 26, 39, 40]. The late asthmatic response to foods (LAR) has also been reported in the literature [53, 55, 56, 70]. However, the immunologic mechanism(s) underlying the LAR, especially the possible involvement of IgE-mediated or non-IgE-mediated hypersensitivity, is not yet sufficiently clarified [2, 9, 15, 23, 24, 29, 39]. In our previous studies we also have observed and described the DYAR response to food ingestion challenge, analogical to the DYAR to bronchial challenge with inhalant allergens [63, 71], in which the involvement of cell-mediated hypersensitivity mechanism (Type IV allergy) could be presumed [9, 15, 18, 20, 22, 63]. This presumption may be supported by other investigators' findings of possible role of various cell types, such as Th₁-cells, neutrophils, macrophages, dendritic cells, and a number of cytokines, chemokines, and other factors,

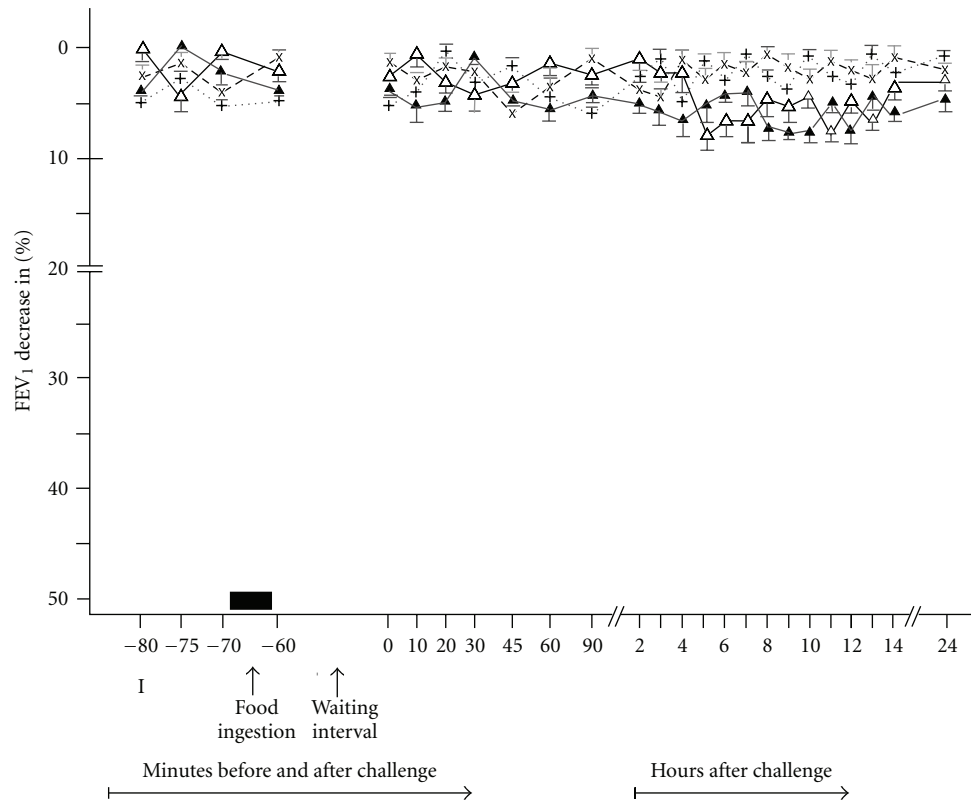


FIGURE 2: Negative asthmatic responses due to the food ingestion challenge (NAR) in 15 patients with bronchial asthma. The mean percentage changes in the FEV₁ calculated from all patients with NAR. NAR ($n = 39$) recorded after OFICH: experimental food (Δ) and control food (\times) and after DBPCFC: experimental food (\blacktriangle) and placebo ($+$) I: initial (baseline) values; food ingestion: OFICH or DBPCFC; waiting interval: 1 hour; bars: means \pm SEM.

in the clinical manifestations of food allergy, especially in its role in bronchial asthma [2, 14, 15, 24, 29–31, 33–38, 53, 54, 56]. However, there is still a dearth of information concerning the clinical features of the various types of bronchial response resulting from the ingestion (challenge) as well as their association and correlation with other *in vivo* and *in vitro* diagnostic parameters in large groups of well-defined patients [1, 2, 6–9, 11–13, 40, 41, 43, 45, 47, 49, 67].

Diagnostic confirmation of the involvement of food allergy in the clinical manifestations, especially in the bronchial asthma, is not always an easy assignment. The diagnostic parameters being customarily used in the practice, such as disease history, skin tests, determination of the total and specific IgE antibodies in the serum (PRIST, RAST, ImmunoCAP), demonstrate various degrees of correlation with the clinical manifestations due possibly to food allergy, such as bronchial asthma. None of these parameters demonstrated sufficient and statistically significant diagnostic value to predict and/or to conform arbitrarily the role of foods and food allergy in bronchial asthma in a given patient [1–13, 18, 27, 28, 41–43, 45, 58, 67, 72].

The role of foods in the bronchial asthma can definitely be confirmed only by the ingestion challenge with the suspected food(s), during which the particular asthmatic response types can be recorded quantitatively in its dynamic course [1, 2, 7–9, 12, 18, 22, 25, 27, 28, 42–44, 46, 48–

50, 53]. The food challenge is therefore a more reliable test for the detection of a bronchial reaction to foods and its clinical consequences than data obtained from single skin tests and/or RAST/ImmunoCAP tests [1, 2, 9, 12, 18, 22, 26, 39, 42, 44–48, 50, 57, 58, 72].

The importance and significance of the food challenge for the diagnostic confirmation of the food allergy has repeatedly been demonstrated in the literature [1–13, 16–22, 25–28, 30, 39, 40, 42–46, 48–50, 52–55, 57, 58, 70, 72]. Unfortunately, papers dealing with the role of food allergy and food ingestion challenge in patients with bronchial asthma are not numerous [2, 9, 11–13, 16, 18–22, 26, 27, 42, 50, 52, 57, 58].

Nevertheless, the food ingestion challenge has also some limitations and contraindications and requires therefore some special conditions, and precautions [1, 2, 6, 9, 11, 13, 22, 24, 28, 40, 42–45, 47–50, 52, 57, 58]. The absolute contraindication is the suspected anaphylactic shock to the particular food(s), pregnancy, and any life-threatening disorder or situation, whereas the relative contraindication may be considered any state or disorder leading to any undesirable complication(s) or which can distinctly influence the food ingestion challenge results, such as treatment with certain drugs [1, 2, 6, 9, 22, 27, 28, 40, 42, 45, 48, 49, 52, 57, 58]. Food challenges, where the vital organ functions should be recorded, for example, lung function,

TABLE 3: Agreement between OFICH and other diagnostic parameters.

	History + Skin +(n = 32)	History – Skin +(n = 5)	History + Skin –(n = 17)	Total (n = 54)
OFICH (n = 54)				
(i) 39 positive responses	26 (48%)	2 (4%)	11 (20%)	39 (72%)
(ii) 15 negative responses	6 (11%)	3 (6%)	6 (11%)	15 (28%)
Total	32 (59%)	5 (10%)	17 (31%)	54 (100%)

TABLE 4: Survey of detailed agreement between asthmatic response types to milk ingestion challenge (OFICH) recorded in patients of both the groups and other diagnostic parameters (disease history and skin tests) for milk.

Patients	Asthmatic responses to OFICH with milk		
	Total n = 54	LAR n = 39	NAR n = 15
Group I. (n = 46)			
(i) History + Skin +	26	21	5
(ii) History + Skin –	15	9	6
(iii) History – Skin +	5	2	3
	46	32	14
Group II. (n = 8)			
(i) History + Skin +	6	5	1
(ii) History + Skin –	2	2	0
(iii) History – Skin +	0	0	0
	8	7	1

Group I: patients with already existing bronchial asthma to inhalant allergens and additional suspicion of milk allergy.

Group II: patients in whom the milk allergy has been suspected to be a sole cause of their bronchial complaints.

+: suspect or positive; –: unknown or negative; OFICH: open food ingestion challenge with milk.

severe diarrhea, or where a late onset of the response is expected, should be performed during hospitalization under standard conditions, and for a sufficiently long period of time, 56 hours at least [1, 6, 9, 22, 25, 27, 40, 48, 52, 53]. The foods, their parts and the related foods used for the ingestion challenge should be excluded from the diet for a sufficiently long period of time before the challenge, at least 7–14 days [1, 2, 6, 9, 18–22, 25, 27, 28, 40, 42, 45, 46, 48, 49, 52, 58].

The food ingestion challenge can be performed by an open challenge (OFICH) with natural foods, by double-blind placebo-controlled challenge (DBPCFC), or rarely by single-blind challenge (SBFIC), each of them having its advantages and disadvantages [1–13, 16, 18–22, 24–28, 39, 40, 42–52, 57, 58, 67, 70].

The OFICH with natural foods and suitable placebo is relatively simple easy to perform, the results are directly available, and no special processing of the foods used for the challenge is required. In addition, it is eligible in cases where the objective parameters can be recorded. This technique is not suitable for challenges where the results can be influenced by the patient or by the investigator or where the response can only be measured by means of subjective parameters,

such as headache, (skin) itching, tiredness, and behavior changes [1–3, 6–11, 16, 22, 27, 42, 45, 48, 49, 52, 73].

The DBPCFC, considered by many investigators to be a “golden standard,” has a number of advantages as well as disadvantages [1–9, 25, 27, 42, 43, 48, 49, 51, 52, 57]. Its important advantage is exclusion of the influencing of results both by the patient and investigator, its usability in cases where the objective parameters cannot be recorded and the response can only be evaluated by means of subjective parameters, such as pain, headache, itching, gastrointestinal complaints, general malaise, behavior changes, and finally when the effects of drugs should be investigated [1–9, 25, 27, 28, 40, 42, 43, 46, 48, 49, 51, 52, 58].

Nevertheless, this technique has also some disadvantages, such as (a) processing of the food in a manner excluding its identification, which means the food must be colorless, tasteless, odorless. Such a preparation of foods can lead to essential changes of their structure and physical and/or chemical properties and sometimes it is not even possible; (b) providing a suitable placebo that matches the offending food in quantity and other properties is sometimes a technical problem; (c) the content of the capsules to be swallowed is maximally 500 mg; If the foods tested were administered in an amount equal to that used in a daily practice then the capsule number would increase enormously, otherwise the food will be taken in an amount less than the natural consumption; (d) the food administered in capsules excludes the oral cavity, tongue and oesophagus, organs which are often the site of the first reaction to foods; (e) by administering of food in capsules, the digestive process already beginning in the mouth is shifted to the gastric and duodenal mucosa and therefore prolonged; (f) the hidden placebo can sometimes induce a false-positive response [1–9, 25, 27, 42, 43, 48, 49, 51, 52, 57, 58].

The results of this study confirmed the existence of late type of asthmatic response (LAR) due to the food ingestion, which has already been described in our previous studies [7–9, 18, 20, 22] and reported by other authors [53, 55, 56, 70]. Although a possible role of an “IgE-mediated” hypersensitivity in the LAR caused by food allergy has already been suggested, the precise mechanism underlying this asthmatic response type is not yet sufficiently clarified [1–3, 5, 9, 13, 17, 18, 24, 27, 29, 34–39, 49, 53–55, 70]. These results have emphasized the importance of the food ingestion challenge for the diagnosis of food allergy in patients with bronchial asthma. The definite confirmation of this role should be provided by a food ingestion challenge combined with monitoring of lung function, for example,

TABLE 5: Survey of the asthmatic responses to inhalant allergens in patients developing positive and negative asthmatic response to OFICH with milk.

	Patients developing asthmatic response to OFICH with milk		
	Total <i>n</i> = 54	LAR <i>n</i> = 39	NAR <i>n</i> = 15
Group I (<i>n</i> = 46)			
Total bronchial challenge with inhalant allergens	72	53	19
(i) Positive asthmatic response	52	35	17
(ii) Negative asthmatic response	20	18	2
Group II (<i>n</i> = 8)			
Total bronchial challenges with inhalant allergens	23	20	3
(i) Positive asthmatic response	3	1	2
(ii) Negative asthmatic response	20	19	1
Total BPT with inhalant allergens	95	73	22

Group I: patients with already existing bronchial asthma to inhalant allergens and additional suspicion of milk allergy.

Group II: patients in whom the milk allergy has been suspected to be a sole cause of their bronchial complaints.

+: positive; -: negative; OFICH: open food ingestion challenge with milk.

TABLE 6: Correlation between OFICH and DBPCFC.

	DBPCFC (<i>n</i> = 54)		Total
	Positive (<i>n</i> = 40)	Negative (<i>n</i> = 14)	
OFICH (<i>n</i> = 54)			
(i) 39 positive OFICH	38**	1	39
(ii) 15 negative OFICH	2	13*	15
Total	40	14	54

OFICH: open food ingestion challenge; DBPCFC: double-blind placebo controlled food challenge; Statistical significance: * < 0.05; ** < 0.01.

spirometry, demonstrating the particular types of asthmatic response in their dynamic course.

Regarding the results of this study, together with our previous papers [6–10, 18–22] and other investigators' findings [2, 25, 28, 42–47, 49, 51, 53, 54, 57, 58] the diagnostic value of the food ingestion challenge seems to be superior to other diagnostic parameters. The significant correlation of the OFICH and DBPCFC results, both for the positive and for the negative asthmatic responses would suggest that in bronchial asthma, where the asthmatic responses can be measured by means of objective lung function, the DBPCFC is not superior to the OFICH [6–10, 20–22, 44–46, 52].

It can therefore be concluded that the OFICH combined with monitoring of objective diagnostic parameters, such as lung functions, can be considered to be definite confirmation of the suspected role of food allergy and involvement of certain food(s), such as cow's milk, in bronchial complaints of patients suffering from bronchial asthma. These patients may include both those suffering from bronchial asthma due to the inhalant allergens, in whom the food allergy is suspected as an additional cause of their bronchial complaints, and those in whom the food allergy, for example, for cow's milk, is suspected as an only cause of the bronchial asthma symptoms. The OFICH is a suitable and reliable technique in all cases of food allergy where the response can

be measured by using the objective parameters and recorded for a sufficiently long period of time, such as 24–48 hours. In such cases this technique would be preferable, because it is easier, cheaper, quicker, and less burdening for the patient who will not need to swallow a large number of capsules.

The DBPCFC should be reserved for such cases, in which objective parameters cannot be measured; the response to food can only be expressed by subjective complaints, for example, itching, headache, tiredness, distinct discrepancy among the other diagnostic parameters that occur, or in cases in which the OFICH results are dubious or not reliable. Vice versa, in the cases in which the DBPCFC results seem to be unreliable, the OFICH can be performed as an extra check.

Conflict of Interests

The author has no conflict of interests to be disclosed.

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Research Article

A Pediatric Food Allergy Support Group Can Improve Parent and Physician Communication: Results of a Parent Survey

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Received 6 June 2011; Accepted 14 July 2011

Academic Editor: Carina Venter

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Rationale. We sought to evaluate the impact of having an allergist at a food allergy support group (FASG) on the relationship between parents and their child's allergist. *Methods.* Ninety-eight online surveys were sent to parents who attend a FASG affiliated with our institution. Responses were analyzed looking for reasons for attending the support group and comfort with having an allergist present at the meetings. The main objective of this study was to evaluate the impact of having an allergist at the food allergy support group on the relationship between parents and their child's allergist. *Results.* The FASG decreased anxiety about food allergies for 77.7% of those who responded. Most (71.4%) felt the FASG improved their child's quality of life. Greater than 90% felt comfortable having an allergist at the support group meeting, and 64.3% felt that talking to an allergist at the FASG made it easier to speak with their child's allergist. *Conclusions.* FASG meetings appear to be a good way for families of children with food allergies to learn more about food allergies, improve quality of life, and increase comfort in communicating with a child's allergist.

1. Introduction

Food allergies affect up to 6% of preschool and school aged children. While the only available therapy for these children is strict avoidance of the offending foods, accidental reactions are common and occur in up to 50% of food-allergic children despite their best efforts to avoid the offending foods [1]. Food allergies have a significant impact on quality of life. Families must be vigilant about food allergen avoidance in a variety of settings including home, restaurants, schools, camps, and social gatherings. Food allergic reactions are the leading cause of emergency department visits for anaphylaxis in the United States. The burden of avoidance and fear of an accidental exposure can increase anxiety and result in reduced quality of life [2]. Food allergy has been shown to lower general health perception, limit family activities, and have a significant emotional as well as economic impact on the parent of the food allergic child [3]. Poor communication between physicians and parents may be a factor that contributes to parental anxiety, as they do not feel that their concerns are adequately addressed. Thus, with the increasing prevalence of food allergy and the absence of a cure, communication between parents of food-allergic

children and their physicians is crucial. There have been few studies done that have looked at the parent perceptions of a food allergy support group; however, none have looked at the parent's comfort with having an allergist present at the meetings and how this impacts their relationship with their child's own allergist.

In 2006, Dr. Jenifer LeBovidge's group at the Children's Hospital in Boston, Massachusetts did an initial study on food allergy support groups and developed the Food Allergy Parent Questionnaire [4]. This tool was designed to evaluate both parental adjustment to a food allergy diagnosis and parental coping with children's food allergy. They concluded that the measure may be useful in screening for parental anxiety, perceived impact of food allergies, level of family support, and coping skills. In 2008, LeBovidge et al. designed another study (where both parents and children attended workshops) to evaluate a group intervention for children with food allergy and their parents [5]. The purpose of this study was to increase parent-perceived competence in coping with food allergy and to decrease the parent-perceived burden associated with food allergy. Parent and child evaluations of the workshop were favorable, and the

results showed that parent-perceived competence in coping with food allergy increased significantly from preworkshop to postworkshop and followup, parent-perceived burden associated with food allergy decreased from preworkshop to followup. Another study, done by Gupta et al. studied focus groups which were held to obtain information for the development of validated survey instruments to assess food allergy knowledge, attitudes, and beliefs of parents, doctors, and the general public [6]. In this relatively small study, it was concluded that the quality of life for children with food allergy and their families is significantly affected due to gaps in physician knowledge and public knowledge about food allergies. Results showed that parents of food allergy had solid fundamental knowledge but had concerns about primary care physicians' knowledge of food allergy, diagnostic approaches, and treatment practices. Physicians had good basic knowledge of food allergy but differed in their approach to diagnosis and advice about feeding. The general public had wide variation in knowledge about food allergy with many misconceptions of key concepts related to prevalence, definition, and triggers of food allergy. Parents expressed concern about effect of food allergy on quality of life and the associated anxiety about keeping their children safe. Parents also reported frustration in receiving a timely diagnosis and felt that physicians of different specialties provided conflicting guidance in the diagnosis and treatment of food allergy. A novel finding was the mothers' assertions that their child's food allergy caused them to stop working outside the home. It was difficult for many mothers to entrust others with the care of their child.

The support group at our institution was designed to provide practical and emotional support that might better enable parents to handle the stress of their children's food allergies. The purpose of our study was to evaluate the impact of a food allergy support group (FASG) at our institution on quality of life and the relationship between parents and their child's allergist. We sought to understand how the interaction between parents and the allergist at the support group meetings affects the relationship between parents and their child's own allergist. This information would allow us to improve the support group experience in the future.

2. Methods

The support group at our institution is listed on the Food Allergy and Anaphylaxis Network (FAAN) website, and therefore an open support group. Patients and families do not need to be seen at our institution in order to attend group meetings or social activities. The meetings are held one evening a month at our institution. The participants that attend the FASG include about 50% parents of children at our institution, and about 50% parents from outside the institution. After IRB approval, survey consent forms were sent by email to all 98 support group members (all over 18 yrs of age). The email contained a link to the 30-question online survey. Completion of the survey implied voluntary consent to participate in the research study. The consent form described the purpose of the research (i.e., to improve future allergy support group meetings) as well as a statement of

confidentiality. The participants had subsequently met with their child's allergist when they answered the questionnaire. Our 30-question survey was designed with a few ideas in mind: to uncover reasons for attending the support group, gauge parent satisfaction with the FASG, and comfort level with having an allergist present at the meetings. The main objective of this study was to evaluate the impact of having an allergist at the food allergy support group on the relationship between parents and their child's allergist. The first set of questions is information about the person completing the survey, including their relationship to the child. The next set of questions were about the child with food allergies including type of food allergies, type of allergic reactions the child has experienced, length of time since diagnosis, and length of time seeing an allergy specialist. The next few questions were to get a sense of the reasons why the participants had joined the support group and how many meetings they had attended. We also asked about participants' level of anxiety and level of comfort before and after attending the food allergy support group, and before and after meeting with an allergist. We also asked about comfort having an allergist at the support group meetings, emotional support, and comfort with food allergies before and after the meetings. We asked questions regarding the support group's impact on coping with managing allergies at school). Responses were analyzed looking for reasons for attending the support group, parent satisfaction, and comfort with having an allergist present at the meetings.

3. Results

Demographics. A total of 29 surveys (29.6%) were completed. All respondents were mothers of children with food allergies, and 28 classified themselves as Caucasian not of Hispanic origin. 23 respondents had a college education or higher. Children's ages ranged from 1 to 11 years old, and were equally split between males and females. 26 had peanut allergy, 12 had a tree nut allergy, 15 had an egg allergy, and 15 had milk allergy. Almost 90% of children had experienced rash/hives, and 60% of the children had experienced a severe allergic reaction (vomiting, abdominal pain, difficulty breathing, face or lip swelling). 28 of the 29 respondents stated that it had been >12 months since their child was diagnosed with a food allergy. Ten of the respondents starting seeing an allergist less than a month after diagnosis; 15 of respondents did so within 1–6 months after diagnosis. All 29 respondents felt comfortable speaking to their child's allergist.

Reasons for Joining. Fourteen respondents were referred to the support group by their own allergist. The number one reason for joining the FASG was to "feel more knowledgeable about food allergies". Other reasons are shown in Figure 1 (see below).

Anxiety. The FASG decreased anxiety about food allergies for 77.7% of those who responded. Results showed that after parents attended support group meetings, almost 78% felt "very comfortable" caring for their child's food allergies,

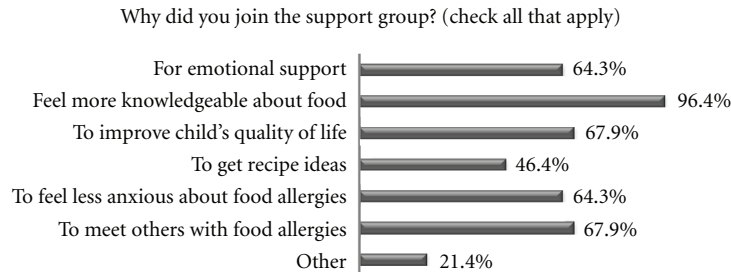


FIGURE 1

compared to only 3.7% that felt very comfortable prior to attending the FASG meetings.

Quality of Life. Most respondents (71.4%) felt the FASG improved their child's quality of life. 64.3% of respondents felt the FASG helped them manage allergies at family activities.

Relationship between Parents and Allergist. Twenty-eight out of twenty-nine respondents felt comfortable having an allergist at the support group meeting, and 64.3% felt that talking to an allergist at the FASG made it easier to speak with their child's allergist. Many of the respondents (77.8%) said they would continue to attend the support group meetings.

4. Discussion

As discussed earlier, there have been few studies done that have looked at the parent perceptions of a food allergy support group. Similar to other studies done by LeBovidge and Gupta, our study shows that a FASG has a positive effect on its members and provides many benefits including providing advice on food allergy risks and safety procedures, providing help in managing and coping with food allergies, and helping to decrease anxiety and increase comfort in dealing with food allergies.

However, none of these prior studies have looked at the parent's comfort with having an allergist present at the meetings and how this affects the relationship they have with their child's own allergist. This is the first study which, to our knowledge, has looked at the role of the physician in a FASG. From our results, it appears that having an allergist present at the meeting is a positive feature. Having the allergist available improved the parents' ability to communicate with their own allergist. Unfortunately, the availability of an allergist to be able to attend food allergy support group meetings is unclear.

These findings provided preliminary support for the effectiveness and feasibility of a group intervention for the parents of children with food allergy. FASG meetings appear to be a good way for families of children with food allergies to learn more about food allergies and become comfortable with their child's diagnosis. Parents who feel more competent in managing their child's medical condition can also help their child develop better coping skills. Participating in FASG meetings can improve quality of life and if there is an allergist

present, they can also increase comfort in communicating with a child's own allergist.

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Review Article

Intestinal Epithelial Barrier Dysfunction in Food Hypersensitivity

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Received 26 May 2011; Revised 6 July 2011; Accepted 8 July 2011

Academic Editor: Kirsi Laitinen

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Intestinal epithelial barrier plays a critical role in the maintenance of gut homeostasis by limiting the penetration of luminal bacteria and dietary allergens, yet allowing antigen sampling for the generation of tolerance. Undigested proteins normally do not gain access to the lamina propria due to physical exclusion by tight junctions at the cell-cell contact sites and intracellular degradation by lysosomal enzymes in enterocytes. An intriguing question then arises: how do macromolecular food antigens cross the epithelial barrier? This review discusses the epithelial barrier dysfunction in sensitized intestine with special emphasis on the molecular mechanism of the enhanced transcytotic rates of allergens. The sensitization phase of allergy is characterized by antigen-induced cross-linking of IgE bound to high affinity FcεRI on mast cell surface, leading to anaphylactic responses. Recent studies have demonstrated that prior to mast cell activation, food allergens are transported in large quantity across the epithelium and are protected from lysosomal degradation by binding to cell surface IgE and low-affinity receptor CD23/FcεRII. Improved immunotherapies are currently under study including anti-IgE and anti-CD23 antibodies for the management of atopic disorders.

1. Introduction

Food allergy is reported in 6–10% of the pediatric population and is more frequent in children than adults [1, 2]. The most common allergens include cow's milk, eggs, peanuts, and seafood. Although some types of food allergy remit spontaneously during the first few years of life, it is often associated with later development of extraintestinal allergies that manifest in the respiratory tract and skin [1, 2].

The sensitization phase of allergy is characterized by increased IgE synthesis and Th2-type cytokine (IL-4, IL-5, and IL-13) responses. Elevated production of IL-4 by mononuclear cells has been demonstrated in the blood and intestinal mucosa of atopic individuals [3, 4]. IL-4 induces germ line ϵ transcription for isotype class switching in B cells and promotes B cell proliferation to increase the synthesis of antigen-specific IgE [5]. In addition to its presence in serum, elevated levels of allergen-specific IgE are detected in the intestinal fluid and stool samples in food-allergic patients [6–8]. The presence of IgE in the gut lumen was also observed in parasitic infection animal models infected with parasites

[9]. The binding of IgE to the high affinity FcεRI on the surface of mast cells is the hallmark of allergy. Cross-linking of IgE by specific antigen induces mast cell degranulation and release of mediators, thereby, causing anaphylactic responses [10]. Anaphylactic reactions in food allergy are associated with enhanced epithelial ion transport with passive outflux of water which is responsible for clinical diarrheal symptoms [11, 12]. The release of mast cell mediators, for example, histamine, prostaglandin, and serotonin, is involved in the stimulation of epithelial ion secretion [13, 14].

2. Immunopathogenesis of Intestinal Sensitization: Role of Bacterial Products and Intestinal Epithelial Cells

A number of factors are involved in the onset of food sensitization, including genetic traits, allergen exposure, and environmental stimuli. Other factors that affect the outcome of allergic diseases include the age at which food antigen is introduced, formula versus breast-feeding,

dietary composition, and gastrointestinal infection status. Recent evidence has implicated a critical role of intestinal microflora in the developmental stage of food allergy. In healthy individuals, the colonic lumen hosts over 100 trillion commensal bacteria, the microfloral composition of which is established during the neonatal period by exposure to vaginal microbes through the birth canal or to bacteria of the digestive tract of the mother via food ingestion [15, 16]. Commonly identified enteric commensal bacteria include those in the phyla Firmicutes (species such as *Lactobacillus*, *Clostridium*, *Enterococcus*), Bacteroidetes (species such as *Bacteroides*), Proteobacteria (species such as *Escherichia coli*), and Actinobacteria (species such as *Bifidobacteria*) [17, 18]. These bacteria are traditionally viewed as cohabitant organisms in the gut that only require elimination in cases where abnormal translocation to systemic blood or extraintestinal organs occurs. It was only recently that our coevolved microorganisms have started to be viewed in a more positive light, with more and more evidence of their beneficial effects to the host [16, 19]. It is now generally believed that the enteric microbiota is involved in the regulation of multiple physiological functions in the gastrointestinal tract. These include competition with and the reduction of pathogen colonization, the degradation of nondigestible dietary substances, the production of short chain fatty acids, folic acids and vitamins, and the stimulation of normal epithelial turnover, as well as the shaping of the mucosal immunity [16, 19–21].

Evidence gathered in germ-free and gnotobiotic mouse models led to the identification of another beneficial role of gut bacterial flora, the induction of oral tolerance. The term “oral tolerance” has been defined as a systemic immune unresponsiveness to a specific antigen that had been previously administered via the oral route. The breakdown of oral tolerance has been suggested to be involved in the pathogenesis of food allergy. In contrast to conventionally raised animals, germ-free mice do not generate immune tolerance against fed antigens [22, 23]. The Th1-mediated responses such as the production of IgG2a and IFN γ were abolished while the Th2-mediated synthesis of IgE, IgG1, and IL-4 remained high in germ-free mice orally administered ovalbumin as a tolerogen before a systemic challenge with the same protein [22]. Interestingly, oral tolerance may be restored in germ-free mice by inoculation with a single strain of commensal bacteria such as *Escherichia coli* or *Bifidobacterium infantis* [23]. Moreover, mice given oral antibiotics that cause commensal depletion during infancy displayed increased plasma levels of IgG1 and IgE, and decreased IgG2a, in parallel with enhanced IL-4 secretion in stimulated spleen cells [24]. The polarized Th2 immune responses in antibiotic-treated mice were reversed by supplementation with *Enterococcus faecalis*, and to a lesser extent with *Lactobacillus acidophilus* [25]. These findings underscore the role of intestinal commensal bacteria in the induction of oral tolerance and the prevention of allergy.

Signaling receptors activated by microbe-associated molecular pattern (MAMP) may regulate the host susceptibility to food allergy. Polymorphism of CD14, the binding receptor for lipopolysaccharide, has been associated with the

development of nonatopic asthma and food allergy [26–28]. However, other studies found no evidence of gene polymorphism of CD14, toll-like receptor (TLR)-2 and -4 in food allergic diseases [29, 30]. Another study demonstrated increased production of tumor necrosis factor- α and interleukin-1 in cord blood mononuclear cells upon TLR2, TLR4, and TLR5 activation in newborns who later develop allergic diseases, suggesting a link between heightened perinatal TLR response and allergy development [31]. Using animal models lacking functional TLR4 in C3H mice background, it was demonstrated that TLR4-dependent signals provided by intestinal commensal bacteria inhibit the development of allergic sensitization, including Th2-skewing responses and anaphylaxis to peanut allergens [32, 33]. It is worth noting that both intestinal epithelial cells and lamina propria macrophages express CD14 and TLR4 at variable levels that change in intestinal inflammation [34, 35]. The role of MAMP signaling by epithelial cells and/or innate immune cells in the mechanism of allergic sensitization is still poorly understood.

The putative concept underlying development of oral tolerance is that feeding antigen at a high dosage results in clonal deletion or anergy of specific T cell clones in a process that involves Fas/FasL-dependent apoptosis, whereas low antigen dosage favors the pathway of active suppression following the induction of regulatory T (Treg) cells [36]. The different means of tolerance induction are not mutually exclusive but may overlap. Different subsets of dendritic cells have been described in the mouse intestine based on their expression of surface molecules such as CD11b, CD11c, CD103, CX₃CR1, and CD70; these subsets have their own functional specialization that are crucial for determining the induction of immunity or tolerance to gut antigens [37]. For example, certain subtypes of dendritic cells are involved in the differentiation of Th1, Th2, and Th17 cells, or are required for isotype switching of IgA in B cells [37–40]. On the other hand, tolerogenic CD103(+) dendritic cells isolated from the lamina propria or mesenteric lymph nodes drive the development of Treg cells that are crucial for the induction of oral tolerance [41, 42].

Recent advances have indicated that intestinal epithelial cells play critical roles in promoting the differentiation of dendritic subsets with tolerogenic phenotypes, suggesting that the local microenvironment is important for driving oral tolerance. Recent studies have indicated that epithelial-derived transforming growth factor (TGF)- β and retinoic acid were required for the upregulation of CD103 on dendritic cells, and the epithelial-conditioned dendritic cells are in turn capable of inducing the differentiation of adaptive Foxp3+ Treg cells with gut-homing properties [43, 44]. Others have reported that the expression of integrin $\alpha\beta$ 6 in epithelial-derived exosomes, when coupled with food antigen, results in the development of TGF β -producing tolerogenic dendritic cells that promote active production of TGF β in Treg cells [45]. Moreover, a transient break of epithelial barrier caused by ethanol or a *Vibrio cholerae* zonula occludens toxin hexapeptide induced the development of Treg cells through mechanisms that requires the presence of an intact microflora and dendritic cells [46].

These findings suggest that intestinal epithelial cells are involved in the development of tolerogenic dendritic cells and Treg cells that are central for the induction of oral tolerance.

As feeding of antigen alone leads to oral tolerance, the administration of food proteins with bacterial adjuvants such as pertussis toxin and cholera toxin is used to sensitize animals instead [47–50]. Coadministration of bacterial-derived toxins with antigens was shown to upregulate the expression of MHCII and costimulatory molecules on monocyte- and bone marrow-derived dendritic cells, and to induce a Th2-skewing response with elevated production of IL-4 and increased synthesis of antigen-specific IgE and IgG2a [51, 52]. Accumulating data indicate that bacterial adjuvants may affect the intestinal dendritic cells population. A recent report documented that cholera toxin induce the selective expansion of CD11c(+) dendritic cell subsets and increased maturation of all subsets of dendritic cells associated with upregulated OX40 ligand expression in the mesenteric lymph nodes and consequent promotion of Th2-driven responses. The authors suggest that the OX40L molecule may play a critical role in sensitization to food allergens [53]. Another study indicated that exposure to cholera toxins induces allergic sensitization to peanut extracts by causing a shift of the subsets of dendritic cells in the lamina propria and Peyer's patches with more inflammatory CD11b(+) and fewer tolerogenic CD103(+) cells [54]. Moreover, increased expression of T-cell immunoglobulin and mucin domain molecule (TIM)-4 was found in mouse bone marrow-derived dendritic cells *in vitro* after concurrent exposure to cholera toxins and peanut allergens compared to those treated with allergens alone [55]. Adoptive transfer of these TIM4-expressing dendritic cells sensitizes naïve mice to orally challenged peanut allergens, as evidenced by heightened Th2 cytokine responses and elevated levels of specific antibodies of IgE in the serum and intestinal tissues [55]. The interaction between TIM1 expressed on CD4(+) T cells and TIM4 expressed on dendritic cells has been suggested to play an important role in the polarization of Th2 responses and the inhibition of tolerance development [56, 57]. Similar effects of TIM4 overexpression in intestinal mucosal dendritic cells and intestinal sensitization to ovalbumin were reported after exposure to staphylococcal enterotoxin B as the adjuvant [58]. These findings all point to the regulatory role of intestinal dendritic cells in the determination of the nature of antigen-specific T cell differentiation, and the induction of Th2 skewing caused by bacterial toxins for allergic sensitization to food proteins.

3. Intestinal Epithelial Barrier Functions

The luminal surface of the gastrointestinal tract from the stomach to the rectum is covered by a single layer of epithelial cells. The vast epithelial surface of the gut allows for efficient nutrient uptake of energy sources in the individual. However, the epithelial layer must also form a competent line of defense since it is constantly bombarded with noxious luminal contents, such as antigenic substances and pathogens. The epithelial layers are maintained in a dynamic equilibrium governed by the balance between crypt stem

cell proliferation and villus/surface cell shedding. The newly proliferated stem cells in the crypt regions differentiate into absorptive and secretive types of epithelial cells with high expression of brush border enzymes and transporters, and concurrently migrate upward to the apex of the villi where cells then undergo apoptosis and detachment [59]. During the differentiation process, epithelial tight junctions (TJs) are formed at the cell-cell contact sites to seal off gaps between cells. This physical barrier constituted by the closely linked epithelial cells is the rate-limiting factor that determines gut permeability.

The tight junctional complexes forming the most apical portion of the lateral plasma membrane between two cells only allow molecules smaller than about 500 Daltons to cross and exclude the influx of antigenic proteins and bacteria through paracellular routes. The transmembraneous junctional proteins, for example, claudins, occludin, or junction-associated molecule (JAM) are linked to intracellular zonula occludens (ZOs) which are bridges to cytoskeletal actin and myosin filaments [60, 61] (Figure 1). The organization of TJ proteins and perijunctional actinomyosins is regulated by a complex network of signaling pathways. Contraction of the actinomyosin filaments, which opens up paracellular junctions, is mediated by the phosphorylation of myosin light chain via the activation of myosin light chain kinase or Rho-associated kinase [62–64]. In addition to the physical opening of TJs, Rho-associated kinase also mediates the endocytosis of TJ proteins into vacuolar apical compartments [64]. Different isoforms of protein kinase C (PKC) are involved in TJ opening and assembly [65]. The atypical PKCzeta is the sole isoform found at intercellular contact sites [66, 67]. Previous studies have shown that membrane translocation and phosphorylation of PKCzeta leads to decreased transepithelial epithelial resistance and relocation of ZO-1 and occludin in human intestinal T84 and Caco-2 cell cultures following infection with enteropathogenic *E. coli* [68, 69]. Other reports demonstrated that the activation of PKCzeta causes the redistribution of occludin away from the intercellular junctions by direct phosphorylation of this tight junctional protein [70]. Recent *in vivo* data have further supported a critical role of PKCzeta activation in the disruption of TJs and gut permeability increase in bowel obstruction models [71].

Structural damage to TJ proteins may also depend on excessive epithelial cell death in examples of bacterial and parasitic infection, and in metabolic and inflammatory stress. Numerous pathogens including *Helicobacter pylori* [72, 73], enterohemorrhagic *E. coli* Shiga-like toxin [74], *E. coli* lipopolysaccharide [75–77], *Salmonella enterica* [78], *Citrobacter rodentium* [79], and *Giardia spp.* [80, 81] were reported to cause epithelial cell apoptosis. It has been demonstrated that caspases (cellular proteins involved in the apoptotic cascade) may directly cleave TJ proteins [82]. Metabolic stresses, such as mesenteric ischemia/reperfusion and hemorrhagic shock, evoke epithelial cell apoptosis and necrosis that are associated with mucosal barrier dysfunction and abnormal bacterial translocation [83–88].

Transcellular transport of particles and proteins is limited by endosomal degradation within enterocytes. Although

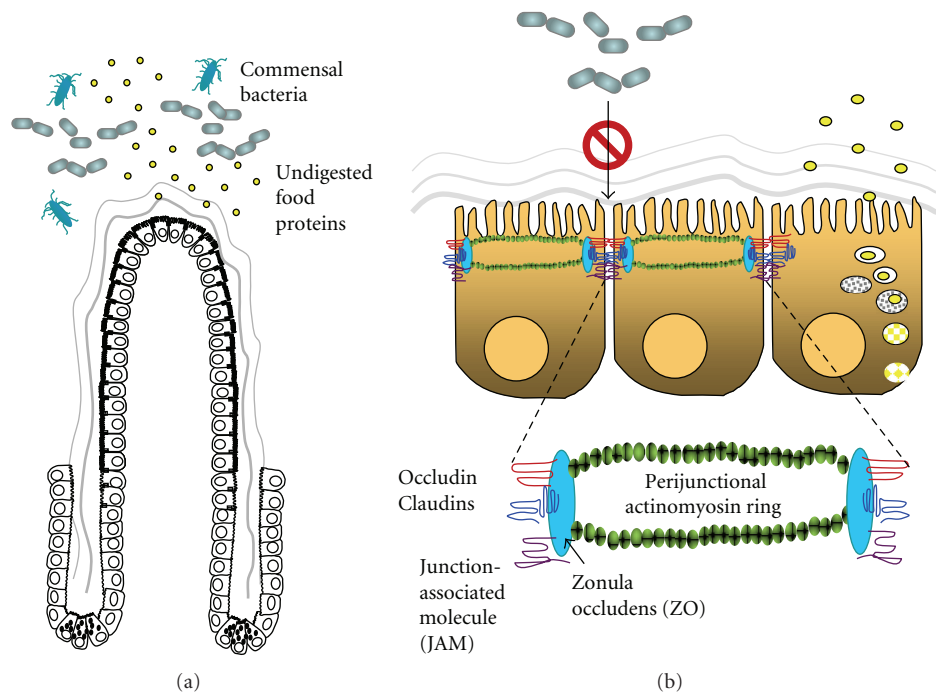


FIGURE 1: Intestinal barrier functions. (a) Differentiated intestinal villous epithelial cells and the covering mucus layer form a physical barrier to separate luminal contents from the lamina propria. The epithelial barrier prevents the entry of noxious substances, such as undigested food proteins and commensal bacteria, into the body proper. (b) Tight junctional complexes located at the most apical portion of the lateral plasma membrane between two cells excludes the influx of antigenic proteins and bacteria through paracellular routes. The transmembrane junctional proteins, for example, claudins, occludin, or junction-associated molecule (JAM), are linked to intracellular zonula occludens (ZO) which are bridges to perijunctional actinomyosin rings. Most dietary proteins are digested to small peptides and amino acids before being absorbed into enterocytes via specific transporters. A very small percentage of intact proteins may be endocytosed into epithelial cells but are degraded by lysozymes and lose their antigenic properties. The lysosomal degradation pathway thus prevents the entry of intact proteins through transcellular routes.

a small amount of intact protein may be endocytosed into epithelial cells in physiological conditions, most of it is sorted into lysosomal compartments for degradation, and, therefore, transcytosis of whole proteins with antigen properties is normally prevented [89]. An early study showed that less than 3% of proteins remain in their intact bioactive form after luminal-to-basolateral passage across the intestinal epithelial layer [90].

4. Epithelial Barrier Defects in Intestinal Allergy

Dietary proteins are mostly digested by gastric and pancreatic proteases, as well as by integral brush border enzymes, and converted to small peptides and amino acids, which are then absorbed by enterocytes via electrogenic or sodium-dependent transporters. Undigested proteins usually do not gain access to the gut lamina propria due to exclusion by the physical tight junctional barrier and intracellular degradation by the lysosomal enzymes. Nevertheless, an apparent defect in epithelial barrier was noted in food allergy. Early clinical studies in children with cow's milk allergy demonstrated intestinal permeability rise after, but not before, allergen challenge [91–93]. A recent study using small intestinal biopsy specimens exposed to food allergen *in vitro*

has shown decreased expression of tight junctional protein, that is, occluding, claudin-1 and ZO-1, in tissues obtained from patients with food allergy compared to those from normal subjects after antigen challenge [94]. These studies suggest that allergen challenge in sensitized individuals leads to enhanced intestinal permeability.

Experimental models indicate that the breach of epithelial barrier may be a consequence of Th2 switching or may possibly reflect exaggerated responses and vicious cycles caused by mast cell activation [10]. Direct effects of type 2 cytokines, for example, IL-4 and IL-13, on the modulation of intestinal epithelial cell permeability have been demonstrated in human epithelial cell cultures [95–97]. Both IL-4 and atopic serum decreased the transepithelial resistance, and selectively increased the apical-to-basal movement of a macromolecular protein, that is, horseradish peroxidase, through both transcellular and paracellular pathways across the human colonic epithelial T84 monolayer [95, 96]. Others reported that IL-4 increased the expression of pore-forming tight junctional protein claudin-2, which correlated with the enhanced epithelial permeability [98]. Recent studies have demonstrated that IL-13 decreased the transepithelial resistance of human colonic epithelial HT29/B6 cells through the induction of cell apoptosis and increased expression of claudin-2 [99, 100]. Moreover, the involvement of phos-

phatidylinositol 3-kinase has been identified in the signaling pathways of IL-4 and IL-13-increased intestinal epithelial permeability using cell culture models [96, 97]. There is also evidence that mediators released from mast cells, for example, tryptase and tumor necrosis factor (TNF)-alpha, contribute to the increased epithelial paracellular permeability [101–104].

Although the link between enhanced permeability in gut epithelial barrier and food allergy is widely accepted, it is not clear which one happens first during the sensitization phase. A previous study in rats has shown that chronic psychological stress, which increases uptake of luminal proteins, may predispose animals to sensitization of orally delivered antigens [105]. The underlying factors that contribute to gut barrier defects caused by psychological stress include corticotrophin-releasing factor and nerve growth factor, as well as mast cell activation [105–108]. However, psychological stress also modulates mast-nerve cell interaction and increases mast cell-dependent bacterial adherence and uptake in enterocytes as well as follicle-associated epithelium on Peyer's patches [109–111]. Therefore, we cannot rule out the possibility that nerve and bacteria are involved in the stress-induced intestinal sensitization by altering immune predisposition. Another report demonstrated that mast cell-dependent epithelial permeability rise predisposes mice with IL-9 overexpression to oral antigen sensitization. The intestinal sensitization may be prevented by mast cell stabilizer cromolyn that blocks mast cell activity and intestinal permeability [112]. However, there is no direct evidence that intestinal barrier dysfunction is the main initiating factor for intestinal allergic sensitization. The use of antigens with protease activity for disruption of epithelial barrier in experimental models may tease out the order between permeability change and allergic sensitization. A murine model of allergic respiratory inflammation has been recently developed by repeated intratracheal administration of proteolytically active Pen c13, a major allergen secreted by fungal *Penicillium citrinum*, without the use of adjuvant [113]. The protease activity of the allergen and the resultant tight junctional disruption of respiratory epithelial cells were found associated with the development of airway allergic sensitization [113]. To date, a direct role of intestinal permeability rises, and luminal antigen leakage in the sensitization stage of food allergy remains to be established.

5. Mechanism of Enhanced Transepithelial Antigen Transport in Allergic Intestines

It is generally accepted that intestinal anaphylactic reactions are caused by biological mediators released from mast cells in the lamina propria after antigen cross-linking of IgE on the cell surface, suggesting abnormal transepithelial transport of luminal antigens in food allergy. An intriguing question then arises: how do macromolecular food antigens cross the intestinal epithelial barrier? Abnormal antigen passage through specialized lymphoid organs, that is, Peyer's patches, in the intestinal tract has been suggested as one mechanism responsible for the lack of tolerance [114–116]. However, in comparison to the limited exposing area of the Peyer's

patches, villous epithelium with its much larger relative surface area may play a more important role in the loss of barrier integrity in intestinal allergy.

It was first noticed in rodent models that the addition of antigen to the luminal or serosal sides of the allergic intestine both induces strong ion secretory responses, though with different time frames. Antigen challenge to the serosal side of the intestine induces an immediate increase (~30 sec) in ion secretion, whereas luminal addition of antigen results in a lag phase (~3 min) before the occurrence of the mast cell-mediated epithelial secretory response [13]. The lag phase of the anaphylactic response after luminal challenge appears to reflect the time for antigen transport across the intestinal epithelial cells to activate the underlying mast cells in the lamina propria [13].

Abundant studies exist that show enhanced transcytotic rates of intact proteins across the intestinal epithelium in experimental allergy [117, 118]. Using rat models of food sensitization, the phenomenon of increased antigen uptake within the endosomal compartment was observed in jejunal enterocytes before the occurrence of mast cell activation [48, 117, 119], suggesting that heightened apical-to-basolateral transcellular transport of allergen is mast cell independent. This notion was confirmed in studies using sensitized Ws/Ws rats (mast cell deficiency due to the mutation of the gene *c-kit*) and mast cell stabilizing agents [119]. The uptake of antigen appeared to be specific and the transport pathway was exclusively transcellular within the first 2 min after challenge. This period of specific transcellular antigen transport before mast cell activation was termed phase I [48, 119]. The period following mast cell activation, as evidenced by an epithelial ion secretory response, was denoted phase II. During phase II, antigens were visualized not only inside endosomes but also within the tight junctions and paracellular regions between enterocytes in allergic animals [47, 119]. The electrical conductance (measurement of ionic permeability through the paracellular pathway) in intestinal tissues of allergic rats was comparable to that of nonsensitized control animals during phase I, suggesting that gut paracellular permeability was not modified in response to sensitization *per se*. Moreover, a gradual time-dependent increase in tissue conductance corresponds to the phenomenon of enhanced paracellular antigen transport in allergic rats during phase II. The abnormal paracellular epithelial permeability in phase II was absent in allergic mast cell-deficient Ws/Ws rats, suggesting a crucial role of mast cell activation in the induction of tight junction opening and increase of paracellular influx that was not antigen specific.

The phenomenon of enhanced transepithelial antigen transport prior to mast cell activation is specific for the allergen to which the rodents are sensitized, suggesting an immunoglobulin recognition mechanism at the epithelial level [117, 118]. Accumulating evidence suggests that a low-affinity IgE receptor (CD23/FcεRII) may contribute to enhanced antigen recognition and rapid transepithelial transport in allergic animals [11, 47, 48, 90, 120]. CD23 was previously known for its role in regulating IgE synthesis in B cells and promoting B cell proliferation [121–123]. CD23 expression was found in small intestinal epithelial cells in

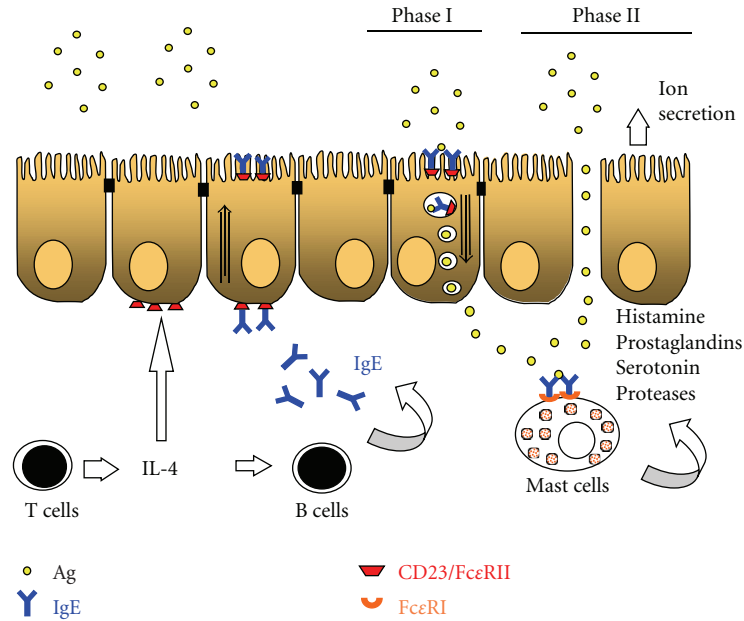


FIGURE 2: IgE/CD23-mediated transepithelial antigen transport in allergic intestines. In food allergy, Th2-skewing and IL-4 synthesis induce isotype switching in B cells to produce a large amount of IgE that is secreted into serum and gut lumen, or bound to high affinity receptor FcεRI on mast cell surface. IL-4 also acts on intestinal epithelial cells to upregulate the expression of low-affinity IgE receptor, CD23/FcεRII. Following exposure to dietary allergens, enhanced luminal-to-serosal transepithelial antigen transport is mediated by IgE/CD23 prior to mast cell activation during phase I. Transcytosed allergens reach the subepithelial lamina propria and cause IgE cross-linking on mast cells, resulting in cell degranulation and anaphylactic responses. The release of mast cell mediators, such as histamine, prostaglandin, serotonin and proteases, are known to induce epithelial ion secretion and to increase paracellular epithelial permeability in phase II.

normal and food-allergic humans and rodents, as well as in bronchial epithelial cells in asthmatic patients [48, 118, 124].

Studies in sensitized rat models have demonstrated the translocation of CD23 from the cell surface to the membrane of allergen-containing endosomes in intestinal epithelial cells, confirming the internalization of CD23 protein upon luminal antigen challenge [118]. Further studies using genetically mutant mouse models provided evidence for the role of IgE/CD23 in mediating enhanced transepithelial antigen transport in allergy [47, 48]. The phenomenon of augmented antigen uptake in allergic enterocytes was completely absent in sensitized CD23^{-/-} mice and IL-4^{-/-} mice. Moreover, the increased transepithelial antigen uptake in allergic wild-type mice was inhibited luminally with neutralizing anti-CD23 antibodies [47, 48]. Passive sensitization of naive mice by injecting immune serum from allergic mice restored the allergic response, but not if IgE was first depleted from serum, confirming the crucial role of IgE in antigen uptake. In addition, IL-4 increased the expression of CD23 transcript and protein levels in murine intestinal epithelial cell cultures, as well as allergic mouse enterocytes [48]. These findings demonstrate that enhanced transepithelial allergen transport is mediated by IgE/CD23 and regulated by IL-4 in food allergy (Figure 2). Recent evidence further supports the notion that food allergens binding to IgE/CD23 are protected from lysosomal degradation in intestinal epithelium, and therefore, intact antigenic forms of the proteins are preserved during transcytosis [120]. It is now clear that IgE/CD23 plays a major role in the mechanism of enhanced transepithelial

antigen uptake that is responsible for later mast cell activation and anaphylactic responses in experimental models.

Recent studies have indicated that various isoforms of CD23 mediate bidirectional transport of IgE across the epithelium in allergic murine and human intestine. DNA sequencing revealed the presence of classical and alternative CD23*b* transcripts lacking exon 5 (*bΔ5*) or 6 (*bΔ6*) in mouse enterocytes, all of which were translated into functional IgE receptors with distinct endocytic properties [47, 125]. Mouse intestinal epithelial CD23*bΔ5* mediated apical to basolateral transport of free IgE, whereas classical CD23*b* displayed higher efficiency in the transcytosis of IgE/allergen complexes [47, 125]. Studies using primary human intestinal epithelial cells and transformed cell lines have also shown that CD23 transports IgE in both the mucosal-to-serosal and serosal-to-mucosal directions [126]. Both CD23 isoforms *a* and *b* transfected into human intestinal epithelial cells transcytosed IgE bidirectional; however, CD23*a* transported IgE/allergen complex faster than CD23*b* in the apical-to-basolateral direction [6]. There remains controversy over which isoform of CD23 is expressed in human enterocytes, and further evidence is needed to confirm the role of IgE/CD23-mediated transepithelial transport in human food allergy [6, 126].

6. Novel Diagnostic and Therapeutic Developments Targeting CD23

To date, dietary exclusion is still the most effective measure for the prevention of allergic sensitization and anaphylactic

responses in high-risk children and adults. The efficacy of delivering allergen via subcutaneous or sublingual routes for symptom alleviation still needs to be confirmed by large-scale blinded, placebo-controlled trials [127]. A therapeutic approach involving the modulation of dendritic cell functions to disrupt their Th2-skewing ability which has been proposed for food allergy and allergic rhinitis [53–55, 128, 129]. Other immunotherapies, such as the use of anti-IgE antibody, have shown some benefits for peanut allergic patients [130]. Moreover, targeting CD23 with monoclonal antibody has been shown to decrease total serum IgE level in ~75% of allergic asthma patients in a phase I clinical trial and was proposed as candidate therapy for treating allergic diseases for some patient subgroups [131]. Additional information is needed to develop safe and effective treatments for food allergy. A better understanding of the molecular mechanism underlying intestinal sensitization and epithelial barrier defects may hasten the development of prophylactic or therapeutic interventions for the management of atopic disorders.

Acknowledgment

The author thanks the funding support by the National Science Council, Taiwan Roc (NSC 100-2325-B-002-035 and NSC99-2320-B-002-024-MY3).

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