

EU forum

In search of a new paradigm: mechanisms of sensitization and elicitation of food allergy*

In this review of research priorities, presented for the European Commission, food allergy is established as a disease with a considerable impact on modern society. Research paradigms have changed from establishing basic symptomatology and diagnostic methods over allergen characterization to a risk-identification approach looking at which patients are at risk of developing reactions to a certain dose of allergenic food. In order to solve some of the apparent paradoxes of regional, temporal, and species-related differences in sensitization and food allergic reactions, it is suggested to study the basic underlying mechanisms in the cross-field between immunology and gastroenterology. Research priorities should include the molecular basis of cross-reactivity, the digestion and maintenance of antigenicity, the genetics of food allergy, and the immune response to food antigens and effects on the gastrointestinal immune system by food matrices and the microbial gut-flora. Necessary methods will be spanning from molecular biology to large-scale clinical and epidemiological studies.

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Key words: allergens; food; food allergy; prevention;
research priorities.

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Accepted for publication 10 January 2005

Food allergy plays an important role in the history of allergy, as it was his colleague's hypersensitivity to fish that led Carl Prausnitz to the famous demonstration of a transferable, specific serum factor (1), almost 50 years later to be identified as immunoglobulin (Ig)E (2, 3).

Since then research paradigms have been changing in food allergy, Table 1. In the 1970s the double-blinded, placebo-controlled food challenges (DBPCFC) were introduced (4), but this practice – now universally recognized as the gold standard for diagnosis (5) – only spread slowly, and it was not until the 1990s that a substantial number of publications on challenged patients began to appear. With the advent of sufficient numbers of well-characterized patients it became possible to characterize and describe the individual allergens by means of IgE binding-techniques. An early pioneering study had identified and sequenced the major allergen from cod fish (6), but the new molecular biology techniques in combination with the availability of relevant patient sera spurred an explosion in the number of reported recombinant allergens. One example (among many possible)

were studies supported by the European Commission 4th framework programme, where DBPCFC of more than 80 patients with suspected hazel-nut allergy were reported from three centres across a north–south axis in Europe (7). The collected serum samples allowed characterization of the allergens in hazel-nut extract (8), and further studies from the group included the cloning of a new hazel-nut allergen (9) and attempts to produce low-allergenic roasted hazel-nuts (10). The increased awareness (and incidence?) of serious food allergic reactions arising from hidden or undeclared allergens (11) united consumers, legislators and the food industry in requests for better risk assessment of foods, and more quantitative aspects were introduced. This included primarily the establishment of threshold values (LOAEL: lowest-observed-adverse-effect level. NOAEL: no-observed-adverse-effect level), which also called for international collaborations (12, 13). However, in order to make overall risk evaluations and risk management it is also necessary to know the true prevalence of food allergy in the general population. Throughout the 1980s and 1990s some studies had appeared indicating the prevalence of food allergy to individual foods such as milk (14) in particular in the paediatric populations, but because of sparse and few studies and the suspicion that the incidence is increasing (15) much more studies are needed to establish the true prevalence. The few population

*This paper is a revised version of a speech given at the European Commission RESEARCH DIRECTORATE GENERAL Directorate E -Biotechnology, agriculture and food: Workshop 'Food Allergy Research in Europe-Future Directions', Bruxelles, Belgium 7th July 2004.

Table 1. Research paradigms in food allergy

Period	Basic question	Typical study design
1970–	Does the disease exist, and how can it be diagnosed?	DBPCFC of suspected patients with comparison to additional diagnostic tools such as skin prick tests, specific IgE, histamine release etc.
1995–	What allergens cause it?	Characterization of individual food allergens based on specific IgE (RAST, western blot techniques), cloning of recombinant food allergens. Classification of food allergens into groups with similar primary amino acid sequence. The molecular basis of cross-reactivity.
2000–	How small amounts are too much for how many?	Threshold studies of food allergic patient populations, epidemiological studies of unselected populations.
2005?	Why do only some persons become sensitized, and why do not all sensitized get symptoms when challenged with a food to which they have IgE?	Digestion and the maintenance of antigenicity The immune response to food antigens Nonantigen specific effects of foods on the immunity of the digestive system Genetics of food allergy

DBPCFC, double-blinded, placebo-controlled food challenge; IgE, immunoglobulin E; RAST, radioallergosorbent test.

based studies of adults (15, 16), clearly demonstrated the need to confirm interviews with DBPCFC, as large discrepancies between self-reported and confirmed symptoms were found.

The advent of new research paradigms or priorities have not made the previous obsolete. There are still a large number of food items for which DBPCFC-recipes have not been developed or reported and the value of diagnostic tools is unknown. The characterization of food allergens also seem to be a long story, where we still only know the first chapters, and for thresholds and epidemiology clearly just a few studies on selected foods have emerged so far. An extensive review of our current knowledge of the most common allergenic foods, that are now regulated under European legislation can be found in Ref. (17). Moreover, a full list of recent EU Commission-supported research in food allergy is given in Ref. (18). The paradigms and methods within these fields are well established however, and it is fair to expect much new knowledge arising at a steady speed during the coming years. When planning new research programmes it is essential however to identify important questions that may not be solved by the research paradigms of today. An example of such problem identification is given below in the shape of four paradoxes of food allergy.

Four paradoxes in food allergy

Regional differences. There seems to be some crude correlation between the consumption of a food item, and the occurrence of food allergy to that item in certain regions of the world. Among the abundant examples are fish allergy from Scandinavia and coastal North America (19, 20), allergy to crustaceans at the Mexican Gulf coast in Louisiana (21), peanuts in the USA in general (22), and *prunoideae* fruits in the Mediterranean region (23). With the globalization of the food market, it is striking however, that these differences exist. As mentioned above there is still lacking good epidemiological studies of food

allergy in different regions of the world. Thus the mentioned regional differences may be due to publication or awareness bias, but recent data from inter-European studies under the 5th framework programme indicate that there are indeed large differences in prevalence to different allergens across a north–south axis in Europe (Unpublished data from the FAREDAT- and SAFE-projects¹). It is possible that some of the observed variations may be explained by variations in exposure to cross-reactive inhalation allergens such as birch pollen in Northern/Central Europe and parietaria or olive pollen in the Mediterranean area, but it is not likely to provide a full explanation since some of the differences are found in the infants that have not yet developed allergic rhinitis to pollen. Differences in dietary exposure probably play an important role, and reliable methods to assess food consumption are urgently needed.

Temporal changes of food allergy prevalence. There is an impression that peanut allergy from being primarily a North American problem is spreading to Europe (and perhaps parts of Asia) with the UK as a bridgehead. Both in North America (24, 25) and in the UK (15) the prevalence is reported to increase. A comparison between two Danish birth cohort studies of children born 13 years apart (1985 vs 1998) gives the impression that egg has taken over from cow's milk as being the most frequent food allergen in infants (14) vs (26). Whether this can be ascribed to an abandoned practice of giving a cow's milk based formula during the first night after birth (27) is possible but has not been verified in prospective studies. Other explanations such as changes in patterns of food consumption have been suggested but are hard to verify.

¹FAREDAT: please see Acknowledgments. SAFE: Personal communication, Dr Karin Hoffman-Sommergruber, coordinator of SAFE: Plant food allergies: field to table strategies for reducing their incidence in Europe (<http://www.akh-wien.ac.at/safe/>).

Taxonomically related, but allergenically different foods. There are extensive cross-reactions between species. This cross-reactivity is however far from fully understood: some studies suggest that a serological cross-reactivity is far from always accompanied by a clinical cross-reactivity, i.e. peanut and soy, which are serological cross-reacting legumes (28, 29). Studies of other food items have found a close parallelism between serological and clinical cross-reactivity, i.e. different species of fish seems to be highly cross-reactive on both the serological and clinical level (30, 31). It is not known what differentiates between clinically relevant and nonrelevant serological cross-reactivity, but among the possible explanations are IgE-allergen affinity differences, variations in processing of foods, or different matrix effects of other components, such as e.g. lipids, in the foods.

Differential natural history of varying food allergies. Whereas allergy to cow's milk in infants is often outgrown in the majority of the children (14, 32), allergies to fish (T.K. Hansen, personal communication) and peanut (33–35) seems to be much more constant – in particular in children with relative high levels of specific IgE – and may last many years into adulthood. It is also a feature of allergy to cow's milk that it arises very early, milk being the first food item introduced to most children, and it may be that food allergies introduced in infancy is more easily outgrown compared with food allergies that arise at a later age.

Important research themes

Based on solving some of the above-mentioned paradoxes, a number of food allergy research themes are suggested (Figs 1 and 2).

The molecular basis of cross-reactivity. An allergen is an antigen, to which IgE-antibodies will bind. In order to be functional, there should be at least two – not necessarily different – epitopes in order to cross-link IgE, when this is bound to its high affinity receptor, FcεRI, on the surface of effector cells, mast cells or basophil granulocytes. This definition does not *per se* imply that a particular protein can elicit allergic reactions in food allergic patients, however. It is well-known that food proteins may bind IgE from persons that are clinically tolerant to the food from which the proteins are derived, and this poses great challenges in developing better and more valid diagnostic reagents for food allergic patients. In order to better define such *clinically relevant* allergens, the strategy has been to identify patients with a clinically proven allergy to a food item, i.e. to challenge patients in a DBPCFC, and use their sera for allergen characterization. Even if a careful selection has been performed of patient sera for such studies, the definition of these proteins as clinically relevant allergens still relies on the assumption that all

proteins to which IgE has been developed in food allergic patients are in fact relevant to the disease. A formal proof of this assumption would call for clinical challenges of food allergic patients with purified or recombinant allergens – an enormous endeavour requiring large amounts of clinical research. So far there has been only limited interest in performing such studies, as for practical reasons, it is sufficient for the patient to know that he or she is allergic to a particular food, but when genes and proteins can be transferred freely from one biological material to another, there may be an increased interest in knowing whether an IgE-binding protein is in fact a clinically relevant allergen. Such knowledge may also be of importance if strategies to develop low-allergenic foods based on destruction or reduced expression of certain genes should be successful.

It may not be possible to formally prove that all newly cloned allergens are in fact clinical allergens, but model systems should be tested which would allow the evaluation of what factors on the molecular level that distinguishes between clinically relevant and nonrelevant molecules. Affinity studies on IgE and IgG binding to inhalation allergens are beginning to appear (36–38), structural data are already available for some food allergens (39) and detailed mappings of epitopes have been carried out on food allergens by site-directed mutagenesis and monoclonal antibodies (40, 41) or phage-display peptide libraries (42, 43). Thus the tools are available to discern at the molecular level whether there is any explanation of the nonclinically relevant cross-reactivity, or whether the explanation should be sought for on a different biological level as discussed below.

Digestion and the maintenance of antigenicity

The fate of food proteins and thus potential food allergens after they have been ingested still leaves some unanswered questions (Fig. 1). In a series of pioneering studies initiated in the 1920s, it was convincingly demonstrated both in adults and children that when passively skin sensitizing nonatopic persons with a serum from a food allergic patient *ad modum* Prausnitz-Küstner (1), a wheal and flare developed at the sensitization site, when the subject was subsequently exposed to the food by the oral route (44, 45).

Later studies demonstrated the absorption of allergens by directly measuring the allergens in the blood (46), and even found that minute amounts can be passed on to breastfed children when allergens are released through the milk of lactating women (13).

It has generally been assumed that if proteins are broken down in the digestion, they are less likely to become allergenic (47), and it has been suggested to use digestibility as a safety parameter in risk evaluation (48, 49). Later studies have however also identified digestion labile proteins among well-known food allergens (50). Nevertheless it seems that if proteins survive

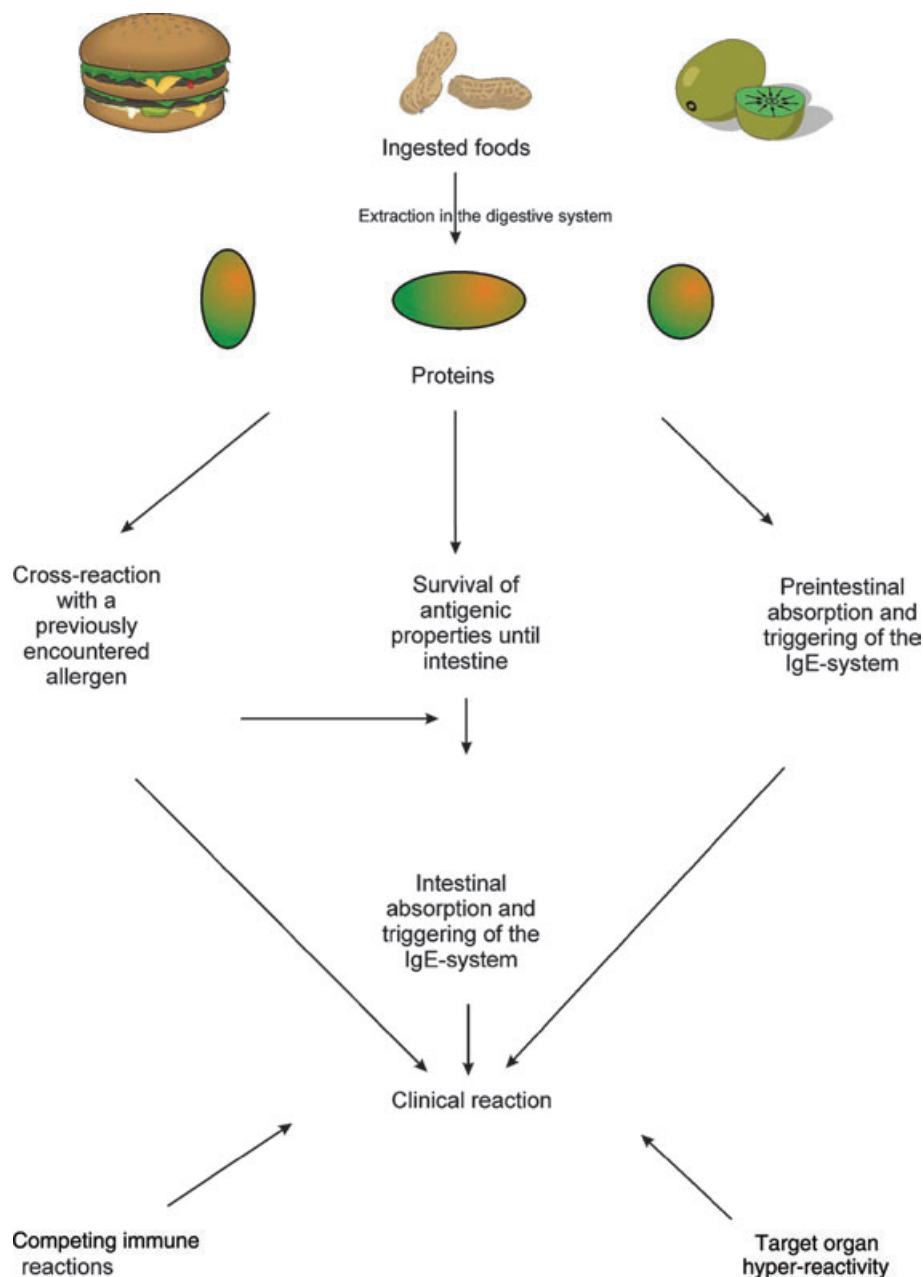


Figure 1. Sequence leading to sensitization and elicitation of a food allergic reaction to a food. A large number of host-, food- and context-specific factors (see Fig. 2) will be decisive for the degradation and absorption of the protein, development of an IgE immune response, and finally for a clinical reaction.

the digestion in the stomach – as would happen in patients treated with antacids – they may more frequently be sensitizing (51–53). Studies from our own group suggest that even the buccal mucosa, may be a site of absorption (C. Dirks, M. Pedersen, M. Platzer, C. Bindselev-Jensen, P. Skov, L. Poulsen, unpublished data), which would not only explain the sensitization by some digestion labile food allergens, but also the fast food allergic reactions experiences by some patients.

Future research should aim at giving a better description of how food allergens are digested, absorbed and further processed by the immune system, and in particular how this is influenced by factors such as age, health, eating habits, digestive system, nonallergenic components of the food, medication, and the disease itself, i.e. whether a (subclinical) allergic reaction will change the absorption of undigested proteins.

The immune response to food antigens and effects on the gastrointestinal immune system by food matrices and the microbial gut-flora

In spite of increasing knowledge of the IgE antibody response to food allergens – now studied to the level of individual epitopes – fewer studies have directly addressed the question as to why susceptible individuals develop IgE. As discussed above, most cases of food allergy arises in infants shortly after weaning, where the immature immune system seems to be skewed against a Th2-type response (54). While some infants outgrow their food allergy, others maintain the Th2-type IgE-response into adolescence. Besides genetic factors – discussed below – it is tempting to speculate that the microbial flora and/or components in the food influence the gut-associated immune system. A phenomenon which is also known from inhalation allergy, is the fact that IgE-reactivity seems to spread from one allergen to another. Thus rather than becoming allergic to proteins from different foods, most patients develops IgE reactivity to more than one protein, i.e. Ara h1, Ara h2, or Ara h3, in the same food. This could either be caused by adjuvant components in the peanut, which favours an IgE-response, or it could be that the inflammation itself may favour development of new IgE-reactivities. An IgG immune response to a component in a food arguably classifies the component as an antigen, but it is far from fully elucidated whether or how IgE-binding antigens, i.e. allergens, can be distinguished as a subgroup of all antigens. Accordingly measurements of human IgG or other non-IgE isotype responses to food antigens would be highly interesting.

Th1 and Th2 type cytokines seem to down regulate each other, and it is possible that the correct balance between these two CD4⁺ T-cell subsets is essential to avoid food allergy. It is very likely, however, that also regulatory T-cells (Treg) are necessary to secure a normal development. These cells are still only partly characterized but according to the present understanding, they can be divided into naturally occurring Treg, for which expression of the transcription factor Foxp3 seems to be the best marker (55), and inducible Treg, which uses IL-10 to suppress Helper cells. It is conceivable that the latter are induced by a normal gut flora (56), and perhaps further stimulated by probiotics (57). Inducible Treg have been suggested to act on other CD4⁺ T-cells via the cytokines IL-10 and TGF- β , but it is of interest that these cytokines may themselves have stimulatory effects on B-cells, resulting in isotype switch to IgG4 and IgE. Interestingly, both food allergic children with high levels of IgE to the offending allergen and nonallergic children express IgG4 and IgA with the same specificity (M. Osterballe, C. Enerold, C. Bindselev-Jensen, L. Poulsen, et al., unpublished data), but it is not known whether this non-IgE immune response to food allergens is beneficial to the patient.

As regards the natural Treg it is of interest that when genetic defects occur in the Foxp3 gene [the protein product of which has been designated scurf in the mouse (58)] the resulting phenotype in man [X-linked autoimmunity–allergic dysregulation syndrome (XLAAD) (59)] is a mixture between Th1 and Th2 pathology with not only insulin dependent diabetes 1, but also a severe IgE-mediated food allergy with severe diarrhoea and eosinophilia (59).

Although much information may be gained from animal studies, where a full control of antigen exposure from birth can be maintained, there are species differences (60) and it is necessary to embark on prospective, clinical studies carefully describing the developing immune response, as well as life style factors, exposure to foods and development of bacterial flora.

Genetics of food allergy

A twin study comparing mono- and di-zygotic twins has suggested a very high level of heritability (82%) of peanut allergy (61), which emphasize the need to look much deeper into genetic factors behind food allergy. The large number of subjects needed to carry out genetic investigations combined with the relatively small number of well-characterized food-allergic patients, have so far hindered studies of a specific genetic predisposition to food allergy, whereas a large number of candidate genes have been identified for allergy and atopy in general. With an increased knowledge of candidate genes, it may be possible in the future to address particular questions such as to whether there are specific genetic markers for gastrointestinal allergy as there seems to be for asthma, whether polymorphisms in the genes of the innate immune system are linked to the early IgE-response to foods or to a natural history where the disease becomes self-limiting within the first 3 years of life.

Possible benefits arising from mechanistic food allergy research

In the evaluation of research projects – and in particular those financed by the European Commission (18) – an increasing emphasis is being put on the possible benefit of the proposed research to the individual citizen, to the industry and to society as a whole. Without going into detail with the socioeconomic costs of food allergy, it is clear that an increased knowledge of the themes mentioned above may form the basis of large improvements for both patients, relatives and industry. The immediate improvements may be seen from the perspective of the foods and of the patients, but also from the perspective as to whether preventive measures can be made that will avoid previous nonallergic persons to react or avoid new foods from causing allergic reactions.

Object of intervention	Intervention perspective	
	Primary prevention	Treatment
The food	Prediction of new allergens and allergens that will cross-react with known allergens	Labelling of foods containing allergens
The person	Identification of persons at risk and instauration of preventive measures	Identification of allergic patients, and description of their sensitivity. Treatment of food allergic patients

Strategies for predicting allergens in novel foods and in gene-modified organisms

The introduction of novel proteins into foods carries a risk of eliciting allergic reactions in individuals sensitive to the introduced protein or of sensitising susceptible individuals (e.g. atopics). So far no single predictive test can identify the allergenic potential of an unknown protein, but various schemes have been formulated. The first of these was the IFBC/ILSI decision tree for the assessment of the allergenicity of foods produced by genetic modification (62). This scheme was subsequently adopted by World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) and then extensively modified and updated in 2001 (48). Each of these decision trees identifies features of allergens which must be considered and tests such as IgE-binding, homology to known allergens, and digestibility, which should be performed to give assurance that a novel protein is not likely to elicit reactions. These schemes thus use a weight of evidence approach to assess the likelihood that a novel protein will prove allergenic, but the methods still need considerable more refinement (49).

Creation of a food market clearly divided into foods with or without allergens

*One man's meat is another man's poison*²: clearly, each allergic patient has individual needs of avoidance, but for society as whole a situation must strived for, where every food item on the market is clearly labelled with its content of food allergens. A large step in this direction has already been taken by the Commission's new directive on labelling [discussed in detail in a recent volume of Allergy (63, 64)], but the appendix of allergenic foods on which these regulations are based, must continuously be revised according to the increasing scientific knowledge on food allergens. Moreover, two large areas, i.e. allergens as contaminants of

industrial food (11), and catered foods are still not sufficiently covered by the legislation.

Identification of persons at risk of developing food allergy and possible prevention

Based on the simple question, as to whether there is a family history of allergy, it is possible to identify children, which may be at high risk of developing food allergy, and where diet restrictions may be of value (65). In the future we should aim at a much more precise description of the genetic background as well as an improved knowledge of which foods that – at different periods of a child's development – may increase the risk of developing food allergies. Other factors for which we presently only have vague ideas about their influence on an increased or reduced risk of food allergy are (in particular oro-faecal) infections, commensal bacterial flora, living environment including inhalation of tobacco smoke, toxicants, and inhalation allergens (Fig. 2).

Improved diagnosis and therapy

The knowledge of a specific food allergy is a prerequisite for a patient being able to avoid the offending food, and as discussed above and in recent guidelines from the European Academy (5) the *in vitro* or *in vivo* diagnostic tools are still not available for omission of the DBPCFC. Recent studies suggest that not only a causative treatment in the form of anti-IgE antibodies (66) but also a specific treatment in the form of immunotherapy (67) may be a possibility of the future, but many more studies are still needed to verify this. In this respect, a better knowledge of the gut-associated immune system and the balances between on one hand Th1 vs Th2 and on the other between Treg and inflammatory cells would be an important stepping stone.

Conclusion

Food allergy is a disease with a considerable impact on modern society, and from the point of view of not only the individual patients but also of society as a whole, investments in research will have a great impact. To solve some of the pertinent questions it is necessary to address the basic underlying mechanisms in the cross-field between immunology and gastroenterology that have hitherto attracted little attention. This will include methods spanning from molecular biology to large-scale clinical and epidemiological studies.

Acknowledgments

Parts of the still unpublished research on which this paper has been based have been performed under the EPI-PAT (*Epidemiology and Pathophysiology of Food Allergy*, funded by the Danish Ministry of

²Originally derived from *quod cibus est aliis, aliis est venenum* in *De Rerum Natura*, the main work by the Roman poet and philosopher Titus Lucretius Carus (c. 99–55 BC).

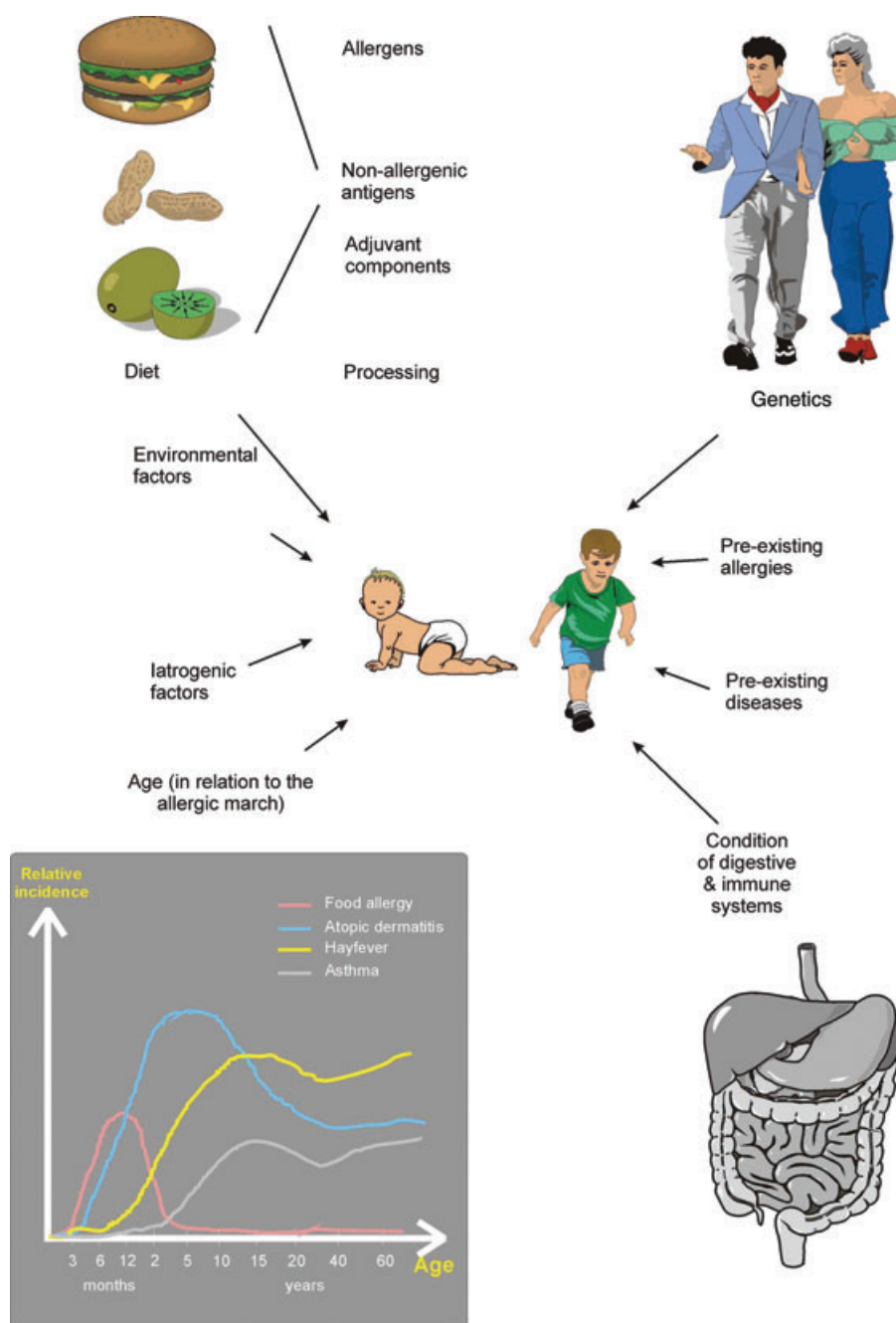


Figure 2. Factors determining the risk of becoming sensitized and later reacting to a food allergen. The contribution of the various factors may be both positive and negative. There is likely to be an extensive, but so far not resolved interplay between the different factors. In the present paper the following factors and interplays have been selected and given the highest priority for future research: (i) The cross-reactivity between proteins within foods and to inhalant allergens. (ii) The (partial and imperfect) breakdown and absorption of antigens in the digestive system. (iii) The interplay between the food antigens, adjuvant factors in the food and the commensal bacterial flora. (iv) The genetics of food allergy.

Food and Agriculture), and the FAREDAT projects (*Food Allergy Risk Evaluation based on improved Diagnosis, Allergens and Test methods*, funded by the European Commission, QLK 4-CT-2001-

00301) <http://www.lkpallgy.suite.dk/FAREDAT>. The author expresses a sincere thanks to all project partners for allowing the implicit use of data.

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