

Food allergy: what do we learn from animal models?

Léon M.J. Knippels^a, Femke van Wijk^{a,b} and André H. Penninks^a

Purpose of review

This review summarizes selected articles on animal models of food allergy published in 2003. The research areas that are covered include mechanistic studies, the search for new therapies, as well as screening models for hazard identification of potential allergens.

Recent findings

Novel treatment options of both prevention and therapeutic strategies have been reported with promising results. The induction of de-sensitization to food proteins was achieved by exposure to a mixture of recombinant food allergens and T helper 1 (Th1)-skewing bacterial components. Furthermore, research in animal models has provided new insights into the role of protein structure, digestion, and gut permeability in sensitization and tolerance induction to food proteins. The Th2 hypothesis of food allergy was tested in mouse strains, linking genetic susceptibility to sensitization with differential Th1–Th2 responses. In this context, the role of the liver in development of food antigen-specific Th2 cells, and the importance of costimulatory molecules in Th2 skewing were demonstrated. Finally, rodent models to predict potential allergenicity of novel foods have been further developed using different routes of sensitization.

Summary

Currently, several animal models of food allergy are used, including mouse, rat, swine, and dog. Continuing research in these models may elucidate the immunological mechanisms that underlie the sensitization and challenge phase of food allergy and may result in improved therapeutic options. Furthermore, the development of animal models to predict relative allergenicity of novel foods remains an important topic.

Keywords

food allergy, animal models, allergenicity, therapy

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^aExperimental Immunology, TNO Nutrition and Food Research, Zeist and ^bInstitute for Risk Assessment Sciences, Immunotoxicology, Utrecht University, Utrecht, The Netherlands

Correspondence to Dr Léon M.J. Knippels, TNO Nutrition and Food Research, Toxicology and Applied Pharmacology Department, PO Box 360, 3700 AJ Zeist, The Netherlands
Tel: +31 30 6944378; fax: +31 30 6960264; e-mail: knippels@voeding.tno.nl

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Abbreviations

HKE heat-killed *Escherichia coli*
HKL heat-killed *Listeria*
OVA ovalbumin
Th T helper

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Introduction

Food allergy has emerged as a major health problem due to the severity of the reactions and its dramatic increase over the past decades. Currently, IgE-mediated food allergy affects approximately 2% of the general US population, with cow's milk, eggs, peanuts, tree nuts, soy, wheat, crustaceans and fish being the most common food allergens [1,2]. So far, no definitive therapies are available and the only remedy is avoidance of the offending food in the diet.

The normal immune response to dietary proteins is associated with the induction of oral tolerance. When this active immune suppression is abrogated, adverse reactions to food proteins may arise. Type I food allergy is characterized by the presence of antigen-specific serum IgE antibodies that can reside on mast cells, and bind circulating ingested antigen. Upon re-exposure, cross-linking of the IgE antibodies provokes degranulation of mast cells and release of mediators, leading to a variety of cutaneous, gastrointestinal, and systemic symptoms. The production of IgE antibodies is induced by the T helper 2 (Th2)-related cytokines IL-4 and IL-13 and can be cross-regulated by Th1 cytokines such as IFN- γ and IL-12. Therefore, a shift in the Th1/Th2 balance in favour of Th2 is considered a key factor for developing food allergy.

Animal models may provide a useful tool to identify new therapeutic strategies, and to expand the knowledge of mechanisms underlying the development of food allergy. Furthermore, with the development of genetically modified foods there has been a growing interest in approaches to assess the potential allergenicity of novel gene products. In the past decade, several animal models have been developed for research on food allergy. Studies initiated in small animal models including guinea pig [3], rat [4], and mouse [5] have shown that rodents can be orally sensitized to food proteins with or without the use of adjuvant. Furthermore, the allergic responses upon re-exposure to the offending food reflected the human situation [5,6] and experiments on therapeutic strategies in mice have provided promising results [7,8]. Recently, larger animals – dogs [9] and swine [10] – that more closely mimic human physiology, anatomy, and allergic disease have been used for food allergy investigation.

This review summarizes articles on recent findings in the field of food allergy obtained by animal model research. The topics that will be presented include the investiga-

tion of mechanisms underlying the development of food allergy, the search for new prophylactic and therapeutic strategies, and the prediction of allergenic potential of novel proteins.

Mechanistic studies

In allergic patients, research on food allergy is limited due to the inability of accessing and investigating cell function and associated processes that take place in the gut-associated lymphoid tissue or other involved lymphoid organs. Furthermore, for ethical reasons, the process of prospective sensitization cannot be studied in humans. Therefore, animal models are used for investigating the complex mechanisms that are associated with the development of food allergy.

Most ingested proteins are digested in the gut lumen. For the induction of sensitization, however, a significant proportion of the intact protein has to be absorbed by the intestine. Therefore, both intestinal permeability and digestibility of dietary antigens are important factors that may influence the elicitation of an immune response against food proteins. One of the biochemical characteristics shared by many food allergens is that they are relatively stable proteins and resistant to denaturation [11]. Accordingly, Untersmayr *et al.* [12•] exposed mice to antacids, drugs that are used for the treatment of dyspeptic disorders and that hinder peptic digestion. BALB/c mice were orally gavaged with caviar proteins and recombinant parvalbumin from carp, *rCyp c 1*, and were treated with different antacids. Feeding with caviar alone led to antigen-specific T-cell tolerance, while the groups fed with caviar in combination with antacid treatment showed significant levels of caviar-specific IgE antibodies, T-cell reactivity, and elevated counts of gastrointestinal eosinophils and mast cells. Feeding with *rCyp c 1* in combination with antacid medication also induced allergen-specific IgE levels. The authors concluded that impairment of gastric digestion by antacid promotes IgE synthesis towards novel dietary proteins, leading to food allergy.

With this in mind, it was recently shown in our lab that protein structure plays an important role in the sensitizing capacity of an antigen (L.M.J. Knippels *et al.*, unpublished data). Reduction and alkylation of the disulphide bridges of the major Brazil nut allergen 2S-albumin (*Ber e 1*) resulted in an increased sensitivity to digestion *in vitro* (S.J. Koppelman *et al.*, unpublished data). Subsequently, it was demonstrated in Brown Norway rats orally exposed to the native or reduced/alkylated form of 2S-albumin that only the native 2S-albumin was able to induce specific IgE responses.

Besides digestion, the integrity of the intestine determines the rate of protein absorption. Although increased

protein absorption has been reported in food-allergic patients, the question remains as to whether increased permeability is a primary cause of the development of food allergy or rather a secondary phenomenon. Matysiak-Budnik *et al.* [13•] hypothesized that gastric *Helicobacter* infection would inhibit the development of oral tolerance to food antigens by increasing absorption of antigens across the digestive epithelium. In an oral mouse model they demonstrated that *H. felis* infection can inhibit the development of oral tolerance to ovalbumin (OVA) and that this inhibition is prevented by a gastro-protective agent that reinforces digestive epithelial barrier integrity. Thus, in susceptible individuals, chronic bacterial infection may increase the immunogenicity of orally absorbed dietary antigens.

Clinical manifestation of allergic reactions to food may also be localized in the gut, partly induced by cell-mediated reactions. Ogawa *et al.* [14•] established a chronic Brown Norway rat food allergy model with morphological changes in the intestinal mucosa, and T-lymphocyte migration. When rats were sensitized intraperitoneally to OVA and subsequently dosed by gastric intubation for 6 weeks with OVA, T lymphocytes significantly accumulated in villus microvessels as well as Peyer's patches via a mucosal addressin cell adhesion molecule-1-dependent process. These authors concluded that increased lymphocyte trafficking to the intestine might play a key role in the development of intestinal mucosal injury in patients with chronic food allergies.

Although T lymphocytes play a central role in the development of food allergy, the underlying mechanisms remain to be elucidated. The Th2 hypothesis in allergy has been established, signifying a Th2 bias in predisposed individuals. In mice, genetic susceptibility to food allergy has recently also been linked to differential Th2–Th1 responses [15•]. BALB/c and C3H/HeJ mice were sensitized to cow's milk or peanut by oral gavage with cholera toxin as a mucosal adjuvant and were challenged with cow's milk or peanut. Induction of food-induced hypersensitivity was found to be strain dependent, with C3H/HeJ mice being susceptible and BALB/c mice being resistant. Culture of splenocytes in the presence of specific antigen induced significantly increased levels of IL-4 and IL-10 in the C3H/HeJ mice, whereas splenocytes from BALB/c mice exhibited elevated IFN- γ secretion.

In another study by Watanabe *et al.* [16•] it was shown that the liver is one of the sites at which food antigen-specific Th2 cells develop. Feeding of OVA to DO11.10 mice generated CD4⁺ T cells producing IL-4 in the liver. When these CD4⁺ T cells were transferred to a naïve host, followed by systemic challenge with OVA,

OVA-specific IgE responses were elevated, whereas OVA-specific IgG antibody and delayed type hypersensitivity responses were decreased. Mice that received CD4⁺ T cells obtained from the spleen or Peyer's patches showed no changes in antibody or delayed type hypersensitivity responses against OVA. Furthermore, the regulatory function of these hepatic CD4⁺ T cells was demonstrated to be dependent on IL-4 production and Fas ligand expression by using neutralizing antibody to Fas ligand and DO11.10 mice deficient in IL-4. Together, these results suggest that the liver might function as a generator as well as a reservoir for those Th2 cells that escape from suppression mechanisms and that predispose the host to development of food allergy.

Th2 skewing in allergic and atopic patients has been associated with CTLA-4 polymorphism [17,18]. This costimulatory molecule, which is predominantly expressed on activated T cells provides signals that down-modulate T-cell function. Currently we are investigating the role of CTLA-4 in food allergy by utilizing an established model of peanut allergy [5]. Preliminary results showed that blockade of the CTLA-4 pathway dramatically enhances specific IgE responses against peanut and the peanut allergens *Ara h 1*, *Ara h 3*, and *Ara h 6*, the production of Th2-associated cytokines IL-4, IL-5, and IL-10 in both spleen and mesenteric lymph nodes, and induces an enormous elevation of serum mouse mast cell protease-1 levels upon oral challenge with peanut (F. van Wijk *et al.*, unpublished data). These results suggest that CTLA-4 plays a role in regulation of allergic responses to food proteins.

Finally, an interesting article has been published about the effect of food allergy on brain function. Basso *et al.* [19•] demonstrated a direct relationship between food allergy and behaviour patterns. It was shown that after an oral challenge with OVA, allergic mice exhibited higher levels of anxiety, increased Fos expression in emotionality-related brain areas and aversion to OVA solution. Furthermore, treatment with anti-IgE antibody or induction of oral tolerance abrogated both food aversion and the expression of c-fos in the central nervous system. Although the authors impute an important role for mast cells on the neural correlates of food allergy, the mechanisms are still to be elucidated.

Therapeutic and prophylactic studies

At present, no satisfactory treatment for food allergy is available. The establishment of animal models for food allergy research has improved the assessment of therapeutic strategies. Specific immunotherapy with modified antigens/epitopes, regulatory cytokines, immunomodulatory microorganisms, anti-IgE antibodies, DNA vaccination, and alternative medicine has been described (reviewed by Li *et al.* [20]).

An ideal treatment should induce long-lasting de-sensitization in food-allergic individuals. To regenerate tolerance to allergens, antigen-specific immunotherapy has been used. Due to an unacceptable high rate of systemic side effects, however, this strategy needs improvement. Therefore, Li *et al.* [21••,22••] used recombinant peanut allergens *Ara h 1*, *Ara h 2*, and *Ara h 3* (*mAra h 1–3*) that were modified to reduce IgE binding, thereby reducing the risk of severe systemic reactions. To potentiate the de-sensitization a mixture of *mAra h 1–3* with heat-killed *Listeria* (HKL) or heat-killed *Escherichia coli* (HKE)-producing *mAra h 1–3* was used. In a murine model of peanut anaphylaxis, subcutaneous injections of the modified peanut allergens and HKL 10 weeks after sensitization significantly reduced peanut-specific IgE production, plasma histamine levels, and anaphylactic symptoms in HKL-*mAra h 1–3*-treated animals compared with sham-treated controls [22••]. More recently, they also demonstrated a persistent protective effect of HKE-producing recombinant peanut allergens (HKE-*mAra h 1–3*) [21••]. Different doses of HKE-*mAra h 1–3* were administered rectally 10 weeks after the initial sensitization with peanut plus cholera toxin. Upon an oral challenge with peanut several weeks after therapy, a reduction in symptom scores, specific IgE serum levels, and histamine release was found in HKE-*mAra h 1–3*-treated animals compared with sham-treated controls. The effects were most profound in the high-dose HKE-*mAra h 1–3*-treated group, and in both the medium and high-dose exposed group the mice remained protected for up to 10 weeks. In both studies HKE/L- *mAra h 1–3*-treated mice exhibited a decrease in Th2-associated cytokine (IL-4, IL-5 and IL-13) production and an increase in Th1-associated IFN- γ cytokine and IgG2a antibody production compared with sham-treated controls. Therefore, the long-lasting beneficial effect of immunotherapy in this model may be caused by a shift to Th1-driven responses. Altogether these results seem very promising for further development of antigen-specific immunotherapy and provide novel insights into the use of adjuvant and route of administration in de-sensitization protocols.

Since it may be difficult to re-establish the Th1/Th2 balance in a sensitized patient, there is growing interest in modifying the immune system still naïve to a relevant allergen. In this regard it has been demonstrated that the prophylactic use of orally administered FIP-*fve* (an immunomodulatory protein isolated from the edible mushroom *Flammulina velutipes*) had a Th1 skewing effect on the development of allergen-specific immune responses [23•]. BALB/c mice were sensitized to OVA by intraperitoneal injection and before and during the period of sensitization mice were orally exposed to FIP-*fve*. Mice receiving the FIP-*fve* treatment showed lower OVA-specific IgE levels, were protected from systemic

anaphylaxis-like symptoms upon subsequent oral challenge with OVA, and displayed a predominant Th1 cytokine profile in the spleen compared with nontreated animals. Although safety issues remain to be extensively investigated, this approach of prophylactic treatment may be promising, especially for children of atopic parents that have a higher risk of developing an allergic disorder.

Allergenic potential assessment

Potential allergenicity of newly introduced protein(s) has become an important issue in safety evaluation of genetically engineered crops. At present, no single definitive test is available to predict allergenicity of newly expressed proteins. Therefore, a decision tree strategy, mainly based on in-vitro experiments, is recommended [24,25]. Animal tests need to be considered, however, to obtain valuable information regarding the in-vivo sensitizing capacity of novel foods. In the past decade, several animal models for the assessment of the allergenic potential of genetically engineered foods have been developed with varying success (reviewed in [26,27,28,29]).

For the development of animal models, high-immunoglobulin (particularly IgE) responder strains are used, which, to a certain degree, resemble atopic humans in their genetic predisposition to react with an overproduction of IgE to antigens. Our group has focussed on the development of a Brown Norway rat food allergy model [4]. The strength of this model is the oral application of proteins without the use of an adjuvant, which mimics the relevant route of exposure in humans. A number of allergens tested induced specific IgE levels and related clinical symptoms upon an oral challenge with the offending protein [30], while other well known allergens failed to induce an appreciable number of IgE-responding animals, which may result in a false-negative prediction of allergenicity when applied as a screening model. In order to increase the sensitivity of this model, Madsen and Pilegaard [31] used newborn Brown Norway rats and in-the-mouth exposure to OVA, but this strategy did not result in priming of the immune response.

Dearman and Kimber [32] used another approach to assess the allergic potential of proteins by examining the intrinsic ability of various food proteins to stimulate IgG (immunogenicity) and IgE (allergenicity) antibody responses following systemic (intraperitoneal) administration to the BALB/c strain mouse. In a recent study, dose-response analyses were conducted with several allergens (peanut agglutinin, OVA and bovine serum albumin) and proteins considered to lack allergenicity (purified potato protein and crude potato protein extract) [33]. All proteins were immunogenic, inducing IgG antibody responses at all doses tested. However, only

the allergens provoked substantial levels of specific IgE over the dose ranges examined. Hence, measurement of antibody responses in BALB/c mice seems to be promising to accurately identify allergens and to distinguish them from proteins that lack allergenicity. As peanut agglutinin is not considered a major allergen [34], more relevant peanut allergens should additionally be tested. Furthermore, the discussion remains whether systemic exposure to proteins is representative for the relevant route of exposure. Ignoring gastrointestinal digestion and mucosal immune responses may result in false-positive but also false-negative identification of allergens. Further research with a broader panel of (non)allergenic proteins may elucidate the possibilities of both oral and parenteral models for allergenicity screening of novel proteins.

Conclusion

The use of animal models in food allergy research is increasing. In the past year, new strategies of prophylactic and therapeutic interventions have been described. Additionally, knowledge on the mechanisms that play a role in the development of sensitization to food proteins has been extended. These new insights may contribute to the prevention of food allergy in infants at risk and may help to diminish clinical symptoms in food allergic patients.

Finally, the development of animal models to predict allergenicity of novel proteins has made progress, but a lot of research remains to be done.

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