



Recent advances using rodent models for predicting human allergenicity

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Abstract

The potential allergenicity of newly introduced proteins in genetically engineered foods has become an important safety evaluation issue. However, to evaluate the potential allergenicity and the potency of new proteins in our food, there are still no widely accepted and reliable test systems. The best-known allergy assessment proposal for foods derived from genetically engineered plants was the careful stepwise process presented in the so-called ILSI/IFBC decision tree. A revision of this decision tree strategy was proposed by a FAO/WHO expert consultation. As prediction of the sensitizing potential of the novel introduced protein based on animal testing was considered to be very important, animal models were introduced as one of the new test items, despite the fact that none of the currently studied models has been widely accepted and validated yet. In this paper, recent results are summarized of promising models developed in rat and mouse.

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Introduction

For the safety evaluation of genetically engineered crops, the potential allergenicity of the newly introduced protein(s) has become an important issue. There is, however, no universal and reliable test system for the evaluation of the allergenic potency of food products and a case-by-case approach is suggested. The first systematic attempt to develop a structured approach to assessment of the allergenic potential of novel food proteins was jointly developed by the International Biotechnology Council and the ILSI Allergy and Immunology Institute and published in 1996 (Metcalf et al., 1996). In the so-called IFBC/ILSI decision tree, a careful step-wise process should be followed. Attention should be given to several factors, like amino acid sequence homology and various physico-chemical properties, such as heat and digestive stability of the protein. In 2001, a Food and Agriculture Organization (FAO) and World Health Organization (WHO) expert

consultation proposed a revision of the IFBC/ILSI decision tree strategy including among others additional testing (FAO/WHO, 2001). Next to the introduction of targeted serum testing, also animal testing was introduced that should be focused on the prediction of the sensitizing potential of novel protein. An important advantage of animal models may become the possibility to obtain information on the sensitizing potential of new protein(s) in relation to its potency, relative to well known allergens. However, despite increasing research efforts in recent years, validated and widely accepted animal models are not available yet.

An animal model should satisfy several important criteria (Penninks and Knippels, 2001): (1) sensitization and challenge should preferably be orally because natural barriers such as the gastro-intestinal acid denaturation and enzymatic digestion and mucosal/epithelial layers, which are all known to prevent, reduce or in any other way influence the allergenicity of proteins should be taken into account, (2) preferably no use of adjuvants, although adjuvants in real-life situations may play a role in the sensitization process, it is preferred to assess the inherent allergenicity of the protein, (3) the test animal should

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produce a significant amount of IgE- and/or other Th2-specific antibody(sub)classes, (4) the test animal should tolerate most food proteins, (5) upon a challenge with the allergen clinical reactions with respect to organ sensitivities should be reflected similar to those seen in humans, (6) antibody responses should be directed to similar proteins (and allergenic epitopes) in the allergenic food proteins as found in patient sera and finally (7) the model should be relatively easy to conduct and reproducible in time and in different laboratories. All these criteria are difficult to attain in one model. Several different approaches have been used and the recent advances using these models are reviewed in this paper.

Oral exposure of Brown Norway rats

We have developed an oral sensitization protocol in Brown Norway (BN) rats as this is a high-immunoglobulin (particularly IgE) responder rat strain. Thus, to a certain degree, this BN rat strain resembles atopic humans in their genetic predisposition to react more readily with IgE production to antigens. We believe that for the evaluation of the intrinsic allergenic potential of new proteins, oral application is preferred, and that the presence of an adjuvant is to be avoided.

We showed that upon daily intra-gastric administration of 1 mg ovalbumin (OVA) for 42 days, without the use of adjuvants, the animals developed OVA-specific IgG as well as OVA-specific IgE responses as measured by both enzyme-linked immunosorbent assay and homologous passive cutaneous anaphylaxis (PCA) assay (Knippels et al., 1998). In addition, clinical reactions, like an increased gut permeability, changes in blood pressure and respiratory function, could be observed in the animals after an oral challenge (Knippels et al., 1999). Using the described model, the sensitizing potential of hen egg white (HEW), cows' milk (CM) proteins and in a separate study a peanut protein extract (PE) was examined (Table 1). Daily intra-gastric administration of different concentrations of either HEW, CM or PE during 42 days, without the use of

adjuvants, resulted in antigen-specific IgG as well as IgE responses. The kinetics of IgG antibody responses induced in Brown Norway rats by oral exposure to various proteins in different concentrations was investigated. The optimal dosing concentrations found for the different proteins, based on the height of the induced response and number of responding animals, are presented in Table 1. Although antigen-specific IgG responses were found upon daily gavage dosing of the animals with different concentrations of HEW, CM or PE, only a limited number of IgE responders was observed as measured by PCA. Oral exposure to 1 mg PE by daily gavage dosing during 42 days resulted in optimal specific IgG and IgE responses as compared to the other dosing groups tested. For the induction of maximal responses to both HEW and CM, higher doses of protein were needed, 5 mg and 10 mg protein by daily gavage dosing during 42 days, respectively. In both cases, not all animals developed a specific IgG response, whereas 1 animal in each group showed a positive PCA reaction. These data indicate that it is necessary to test different dose levels of the food protein in order to obtain a good rank order. Immunoblotting experiments using sera from rats exposed to CM- and HEW-proteins demonstrated the presence of specific IgE antibodies against both HEW-proteins and CM-proteins (Knippels et al., 2000). Moreover, both IgG and IgE antibodies present in sera of rats sensitized orally to HEW or CM and in sera of HEW- or CM-allergic patients recognized a comparable profile of allergens in these food products. These results indicate that the specific protein recognition of induced antibodies in the BN rat is comparable with that observed in sera from allergic patients (Knippels et al., 2000). In the animals exposed to PE, the IgG2a responses (Th2-mediated) were determined against three purified major peanut allergens Ara h 1, Ara h 2 and Ara h 3. After oral sensitization, IgG2a antibodies were directed against all three major peanut allergens, however, following intraperitoneal sensitization of the BN rats, IgG2a antibodies were mainly directed towards Ara h 2 and to a lesser extend against Ara h 1.

Besides studying allergen extracts, the relative allergenicity of selected allergenic and non-allergenic proteins, based on human experience, has been investigated in the BN rat oral exposure model. Ara h 1 purified from peanut, Pen a 1 (tropomyosin) purified from shrimp or beef tropomyosin, and Sol t 1 (patatin) purified from potatoes were used. In two separate studies, animals were exposed to OVA and in a very recent study to purified Ber e 1 (2S albumin) from Brazil nut. The animals were daily gavage dosed with 1 mg of protein for 42 days and the protein-specific IgG and IgE antibodies were assessed by ELISA and PCA, respectively (Table 2). As previously observed with the tested extracts, most animals developed a specific IgG response against the tested protein but not all animals developed a specific IgE response against the protein. However, based on the obtained results from separate studies, the oral sensitizing potential decreased in the following order OVA > Ber e 1 >

Table 1

IgG and IgE antibody production following oral exposure of Brown Norway rats to hen egg white (HEW), cows' milk (CM) or peanut protein extract (PE)

	² log IgG titer (Day 42)	Number of responders		Induction of response (Day)
		IgG	IgE	
PE (1 mg)	10 ± 1.8	10/10	4/10	7
HEW (5 mg)	7.2 ± 2	5/6	1/6	21
CM (10 mg)	2.5 ± 0.7	4/5	1/5	35

Rats received 1, 5 or 10 mg of PE, HEW or CM in 1 ml water daily by gavage during 42 days, respectively. Blood was sampled weekly and individual sera were tested for the presence of specific IgG antibody by ELISA and IgE antibody by PCA. Only day 42 sera were tested for the presence of IgE antibody.

Table 2

IgG and IgE antibody production following oral exposure of Brown Norway rats to purified proteins

	² log IgG titer	Number of responders	
		IgG	IgE
OVA	12.3	8/10	6/10
Ber e1	6.9	7/10	5/10
Ara h1	11.3	6/6	1/6
Pen a1	12.2	6/6	1/6
Sol t1	15	6/6	1/6
Beef trop.	3.75	4/6	0/6

Rats received 1 mg of protein in 1 ml water daily by gavage dosing during 42 days, respectively. Blood was sampled and individual sera were tested for the presence of specific IgG antibody by ELISA and IgE antibody by PCA in day 42 sera.

Ara h 1 > Pen a 1 > Sol t 1, with no sensitization to beef tropomyosin.

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 (Knippels and Penninks, 2003). Although for certain allergens, results were promising, 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. The results obtained up to date indicate that the BN rat might be a useful animal model to study the potential oral allergenicity of “novel” food proteins. Further testing with either whole food or with additional purified non-, weak- and strong-allergenic proteins are needed to evaluate further this Brown Norway rat model.

Systemic exposure of BALB/c mice

Dearman and colleagues (Dearman et al., 2000; Dearman and Kimber, 2001) 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 (i.p.) administration to the Balb/c strain mouse (mouse strain known to favor the development of Th2 type immune responses and the production of IgE antibody). Groups of mice receive one of the various concentrations of the test protein by i.p. injection. Seven days later, this treatment is repeated. At various periods following the initiation of exposure, mice are exsanguinated by cardiac puncture, and individual and pooled serum samples are prepared for subsequent analyses. Their strategy is to distinguish between immunogenic proteins (that are able to induce specific IgG antibody responses) and potentially allergenic proteins (that are able under these conditions to provoke both IgG

and IgE antibody production). Protein-specific IgG antibodies are measured by ELISA and protein-specific IgE antibodies by homologous PCA. This approach is designed to serve as tool for hazard identification and characterization (Dearman and Kimber, 2001; Kimber et al., 2003).

In a recent study, dose–response analyses were conducted with several allergens (peanut agglutinin, OVA and BSA) and materials considered to be lacking allergenicity, a purified potato protein and a crude potato protein extract (Dearman et al., 2003). All proteins were immunogenic in mice, 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 identify allergens accurately and to distinguish them from proteins that lack allergenicity. Although further investigations with a broader panel of (non)-allergenic proteins are needed, these data suggest that this approach could play an important role in the identification and characterization of novel proteins. However, the discussion remains whether systemic exposure to proteins is representative for the relevant route of exposure. Ignoring gastro-intestinal digestion and mucosal immune responses may result in false-positive but also false-negative identification of allergens.

Conclusion

It is evident that considerable progress has been made in developing rodent models for the assessment of the allergenic potential of novel proteins but a lot of research remains to be done. Since there are no validated animal models available yet, further efforts are needed to evaluate and validate the sensitivity, specificity, and reproducibility of these models. However, future research with the different models available may provide useful information on the potential allergenicity of novel proteins.

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