

Evaluation of biotechnology-derived novel proteins for the risk of food-allergic potential: advances in the development of animal models and future challenges

Varun Ahuja · Maria Quatchadze · Vaishali Ahuja ·
Daniela Stelter · Achim Albrecht · Ralf Stahlmann

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Abstract Increasing concern from the public about the safety of genetically modified food has made critical to have suitable methods for recognizing associated potential hazards. Hierarchical approaches to allergenicity determination were proposed, and these include evaluation of the structural and sequence homology and serological identity of novel proteins with existing allergens, measuring the resistance to proteolytic digestion and assessment of sensitizing potential using animal models. Allergic individuals have a predisposed (i.e. atopic) genetic background, and a close resemblance to this setup is therefore desirable in animal models, which is possible by using a strain of an animal species that is prone for allergic disorders. So far, none of the animal model has been validated for the purpose of hazard identification in the context of safety assessment. However, the available knowledge suggests that the judicious use of an appropriate animal model could provide important information about the allergic potential of novel proteins. This paper provides an up-to-date review of the progress made in the field of development of *in vivo* models in this direction and the further goals that have to be achieved.

Keywords Food allergy · Genetically modified organism · GMO · Animal models

Introduction

A number of agricultural crops have been developed with biotechnology methods in the last few years. The genetic modifications in these crops are done in order to modify nutrient content, improve insect resistance and salt tolerance and increase productivity. However, genetic modification implies accompanying risk assessment including toxicity and allergy. Besides genetic engineering, conventional breeding approaches, such as chemical and radiation mutation, can also alter levels of existing proteins (Batista et al. 2008). The incidence of food allergy in Europe and North American countries ranges from 3 to 4% in adults and 6 to 8% in children (Helm and Burks 2000; NIH 2006), and its prevalence seems to be increasing in conjunction with the increasing prevalence of the eczema, asthma and environmental allergies (Sampson and McCaskill 1985; Holt et al. 2000). In fact, anaphylaxis induced due to food is the most common cause of anaphylaxis evaluated in emergency departments in the United States and the United Kingdom (Bock et al. 2002; Yocum et al. 1999). Consumption of any protein food could result in an allergic reaction; however, eight foods, specifically peanuts, tree nuts, eggs, cow's milk, wheat, soybeans, fish and shellfish, are commonly responsible for >90% of food allergies. The remaining 10% of food allergies occur due to over 150 diverse food proteins (Hefle et al. 1996). Food allergy manifests as inflammation of the skin (hives), gut, lung and, in the most extreme cases, results in anaphylactic shock and death (Burks and Sampson 1997). Food allergy accounts for about 35–50% of emergency room visits for anaphylaxis

Varun Ahuja (✉) · D. Stelter · A. Albrecht
In Vivo Pharmacology and Toxicology,
BSL Bioservice Scientific Laboratories GmbH,
Behringstr. 6/8, Planegg/Munich 82152, Germany
e-mail: drvarunahuja@gmail.com

A. Albrecht
e-mail: aalbrecht@bioservice.com

M. Quatchadze · Vaishali Ahuja · R. Stahlmann
Institute of Clinical Pharmacology and Toxicology,
Charité-Universitätsmedizin Berlin,
Campus Benjamin Franklin, Berlin, Germany

and causes about 30,000 cases of anaphylaxis and 100–200 deaths per year in the United States (NIH 2006).

The first logical endeavor for developing a planned approach leading to the assessment of the allergenic potential of novel food proteins was organized by the International Food Biotechnology Council (IFBC) and the International Life Sciences (ILSI) Allergy and Immunology Institute (Metcalf et al. 1996). The scheme proposed in this meeting took the form of a decision tree, in which the starting point was to determine the origin of the novel gene and whether it is derived from an allergenic source. The approaches that could be used included comparison of the serologic characteristics of the novel protein with known human allergens, amino acid sequence homology with allergenic proteins, and resistance to proteolytic digestion in a simulated gastric fluid. In 2001, the Food and Agriculture Organization (FAO) and World Health Organization (WHO) formulated a special consultation panel to consider food allergenicity testing (FAO/WHO 2001). The recent approach for investigating potential allergenicity of genetically modified food is based on a weight-of-evidence approach as proposed by the Annex to the Codex Alimentarius “Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants” (Codex Alimentarius Commission 2003). European Food Safety Authority (EFSA 2006) has also expressed interest for the development of an *in vivo* model as part of the safety assessment process. Particularly important is the need for developing an animal model that could predict the inherent sensitizing potential of an allergen to which the human beings are not yet exposed. In particular, only animal models can predict the risk that involves expression of novel proteins that may become allergens *de novo*.

An ideal animal model should imitate human disease with respect to route of exposure, mechanisms underlying the disease and associated symptoms and therefore possess the following: (1) having similar allergenic response as humans; (2) having a similar relative reactivity to proteins on an “allergenicity” scale compared to humans, thus discriminating allergens from non-allergens; (3) elicit response using typical routes of exposure (e.g., oral) for administration; (4) no adjuvant requirement; and (5) being reproducible, specific and sensitive. There is no animal model currently being evaluated that achieves even a majority of these criteria. However, a complete recapitulation of the human disease should not be the goal of animal model development. Rather, the objective should be to develop a model that provides useful information and, when used in combination with other available data, will permit useful decisions to be made about the hazards associated with particular food protein. Various models are needed for studying the mechanism of allergic responses, to

evaluate therapeutic approaches or to assess the allergenicity of novel proteins.

Food allergy is the consequence of altered oral tolerance i.e. a physiological mechanism of immune suppression following exposure to environmental allergens. The mucosal-associated lymphoid tissue (MALT), and in particular the intestinal immune system, develops immune tolerance to dietary antigens and components of the bacterial flora to which epithelial tissues are exposed constantly (Dubois et al. 2003). Immune tolerance induced by the oral route prevents the outcome of both systemic and local allergic reactions to proteins and is induced and maintained by regulatory T cells (Treg) (Desvignes et al. 1996, 1998, 2000). Most animal models are aiming at evading the mechanisms of normal oral tolerance. Since IgE is strongly associated with allergic reactions, its presence is commonly used as an indicator of food-allergic disease. IgE is also most susceptible to suppression by oral tolerance (Christensen et al. 2003; Saklayen et al. 1984). Various models have been proposed over the years in an effort to establish a model for assessing the allergenicity of novel food proteins.

Rat experimental model

The Brown Norway (BN) is a high immunoglobulin (especially IgE) responder rat strain. Therefore, it resembles to a certain degree to atopic humans in their genetic tendency to respond rapidly to antigens with the production of IgE.

Knippels and coworkers studied the strain-specific influence on oral sensitization using different strains of rats (Knippels et al. 1999a). They found that following oral exposure of Wistar, Piebald Virol Glaxo (PVG), Hooded Lister (HL) and BN rats to ovalbumin (OVA) by gavage for 6 weeks, only the BN rats developed OVA-specific IgE antibodies, while the highest OVA-specific IgG antibodies were detected in BN rats, followed by Wistar, HL and PVG rats. This experiment confirmed that the BN rat seems to be the most appropriate strain for oral sensitization studies. Further validation of the BN rat model was provided by studying the effects of oral challenge on OVA-sensitized rats. Following oral challenge with OVA of orally and parenterally sensitized animals, they observed local effects in all the rats, while systemic effects were observed at lesser frequency, thereby reflecting the situation in food-allergic patients after oral challenge (Knippels et al. 1999b). Local effects induced by oral challenge were studied by measuring gut permeability, which was found to be increased. Systemic effects were investigated by monitoring respiratory functions and blood pressure changes, which were found to be only slightly affected in some animals.

They further studied the effect of mode of application (gavage vs. via drinking water) and the frequency of application (daily, twice a week, once a week, once every 2 weeks) of OVA on the production of antigen-specific antibody response in BN rats (Knippels et al. 1998a). Daily intragastric administration for 6 weeks showed OVA-specific IgG and IgE production. Using less-frequent administration regimes of once or twice a week or once every week by gavage resulted in negligible or very low frequency of OVA-specific antibody response. Upon exposure in the drinking water, OVA-specific IgG but not IgE antibody was found. They concluded that the BN rat is an appropriate model for inducing antigen-specific IgG and IgE responses upon enteral exposure of antigen in the absence of adjuvant. In successive investigations, the specificity of the induced antibody response in the BN rat and food-allergic patients was studied (Knippels et al. 2000). The rats were administered with hen's egg white protein extract (HEW) and cow's milk preparation (CM) by daily gavage for 9 weeks. Both IgG and IgE antibodies present in sera of rats sensitized orally and in sera of patients allergic to HEW or CM showed a similar profile of protein recognition. These results provide further support to the BN rat model for research on the allergenicity of food proteins (Knippels et al. 2000).

Using the BN rat model, it has been revealed that unscheduled dietary preexposure of test animals, or their parental generation, to the antigen under investigation may significantly affect the result of oral sensitization studies (Knippels et al. 1998b). BN rats were bred on a soy protein diet for many generations and placed on a soy protein-free diet during and 6 months before breeding (F0); this was continued for four generations (F1, F2, F3 and F4). F4 animals were administered with soy protein. They found the presence of soy protein-specific antibodies in F0 and F1 rats, while the other generations were tested negative. The exposure of F4 animals to soy protein resulted in enhanced soy protein-specific IgG titers. In rats exposed to soy protein diet continuously, no significant increase in soy protein-specific IgG titers was observed following exposure to soy protein. Therefore, while performing sensitization studies with proteins, care should be taken in breeding and raising the test animals as well as their parental generations on a diet free of the antigen under investigation to get reliable results.

The BN rat seems to be a useful animal model to study the oral allergenic potential of novel food proteins (Knippels and Penninks 2003). Nevertheless, additional testing with whole food and with purified non-, weak-, and strong allergenic proteins is desirable for an exhaustive evaluation of this model. However, an important point of concern is that there appears to be significant inconsistency in the production of IgE antibody responses in BN rats. In some experiments, 80% IgE responders to OVA were observed,

whereas in other experiments, specific IgE antibody was not detected in any recipient (Knippels et al. 1998a, Dearman et al. 2001). Another group of investigators report 13–38% IgE responders for male BN rats and 38–75% IgE responders for female BN rats (Pilegaard and Madsen 2004). It seems that the capability of BN rats to mount IgE responses is inherently variable and is affected by number of factors including environmental conditions, age and the presence or absence of subclinical infection (Kemeny 1994). Therefore, while using the BN rat model to study responses to a novel protein, it may be practical to incorporate a concurrent positive control group along with assessment of levels of endogenous IgG antibody to the protein of interest.

Mouse experimental models

The species most commonly preferred with respect to animal model development is the mouse. This is principally due to the availability of various immunological and molecular reagents, including transgenic animals in which typical genes of interest are overexpressed or deleted. Similar mechanisms are also shared between man and mouse for many aspects of immune regulation (Griffiths et al. 2005; Hausding et al. 2008).

BALB/c mice

The advantage of using a BALB/c mouse model to predict food-allergic potential is that it favors the development of Th2 type immune response and production of IgE antibody. As food allergy in humans is initiated through oral exposure, the oral route might be thought to be the most suitable route of exposure while developing a model for food allergy. However, some research groups have argued that oral administration is not the preferred route of exposure as it may lack the sensitivity necessary for the effective identification of sensitization potential. This might be possible because of the development of tolerance (Strobel and Mowat 2006). Therefore, many investigators have focussed mainly on systemic (intraperitoneal) route of exposure of BALB/c mice to the food protein.

Mice were administered intraperitoneally with 0.2% peanut agglutinin (a minor allergen of the peanut) (Dearman et al. 2001, 2002), 2% ovalbumin (OVA) or a crude potato protein extract (PPE; 10%) containing acid phosphatase activity and which is assumed to lack significant sensitizing activity. All the tested proteins resulted in specific IgG antibody responses, whereas strong IgE responses were observed in mice exposed to peanut agglutinin or OVA. However, PPE did not produce any significant effect on the IgE response.

The responses observed in mice following intraperitoneal administration have been compared with those observed following gavage administration. Strong IgE response was observed following systemic exposure of mice to OVA, while the oral exposure was found to be less sensitive (Dearman et al. 2001). Difficulty in generating robust IgE responses to OVA following oral dosing suggests that for the purpose of evaluation of inherent sensitizing activity of novel proteins, systemic exposure may be more appropriate. The intranasal route has also proven to be an efficient route for sensitization in BALB/c mice (Hilton et al. 1997).

Adel-Patient et al. (2000, 2001) analyzed the intensity and specificity of IgE, IgG1 and IgG2a antibody responses in BALB/c mice immunized intraperitoneally against β -lactoglobulin (BLG). They observed that the IgE epitopes on BLG recognized by the mouse were similar to those recognized by the serum IgE antibodies of humans allergic to milk. Prescott et al. (2006) reported that BALB/c mice also exhibited clinical symptoms of food allergy such as diarrhea, anaphylaxis, and eosinophil and mast cell accumulation apart from antibody responses (IgE and IgG1). Gizzarelli et al. (2006) sensitized BALB/c mice by intragastric gavage of protein extracts obtained from a wild-type and a genetically modified (GM) soybean in the presence of CT. They observed generation of Th2 immune responses which were similar for both varieties of soybeans, but could not see any specific response to the protein in the GM soybean variety.

The conditions needed to sensitize animals to food allergens through oral route in order to study the allergenicity were studied in different mouse strains (e.g. BALB/c, B10A and ASK) and BN rats by Akiyama et al. (2001). In the mouse strains examined, the effect of age, oral feeding technique and dose of protein was examined. In terms of OVA-specific IgE and IgG1 antibody, the B10A mice exhibited the highest response of the three mouse strains tested. Based on the results observed, it was also suggested that oral sensitization of mice requires low doses and intermittent protein intake. Bodinier et al. (2009) used BALB/cJ, B10.A and C3H/HeJ strains of mice to study the sensitization and elicitation by wheat gliadins (a protein fraction containing major wheat allergens) following intraperitoneal route of administration. They concluded that BALB/c mice were the most appropriate model out of the different strains tested. They also reported that the specificity of the mouse IgE against the different wheat gliadins (i.e., alpha-, beta-, gamma-, omega 1-, omega 2- and omega 5-gliadin) was similar to that observed in children allergic to wheat flour.

Strid et al. (2004) sensitized BALB/c mice using a single intragastric administration of the protein antigen first followed by an injection of the protein along with complete Freund's adjuvant (CFA) and, on day 28, a boost injection

of the protein administered in the foot pad. Oral challenge of sensitized mice was done with high doses of protein, in the hundred milligrams range. Delayed-type hypersensitivity was assessed by measuring the food pad swelling following the last boost administration. The test was performed with peanut and egg allergens, and the results showed that the sensitizing potential depends on the nature of the protein. In further experiments, Strid et al. (2005) showed that induction of oral tolerance to peanut protein can be prevented by epicutaneous exposure to peanut protein, which even might modify existing tolerance to peanut. The epidermal exposure to protein allergens selectively induces Th2 type response and could probably promote sensitization to proteins upon oral exposure.

Birmingham et al. (2007) worked with an adjuvant-free transdermal sensitization protocol using hazelnut protein in BALB/c mice with subsequent oral challenge and measure of the antibody response and clinical score for anaphylaxis. They concluded that transdermal exposure to hazelnut protein is sufficient to activate the key immune pathways necessary for sensitizing mice for clinical immediate hypersensitivity reactions.

An oral exposure model was described that used a very low sensitizing dose (0.1 mg) administered on eight consecutive days using staphylococcal enterotoxin B (SEB) toxin as adjuvant (Ganeshan et al. 2009). A challenge dose without adjuvant was administered 24 h after the last sensitization, and various physiological and immunological end points characteristic for anaphylaxis were assessed in addition to antigen-specific IgE. Promising results were obtained for peanut extract and ovalbumin. Further research on this model is needed with testing of non-allergenic food extracts.

C3H/HeJ mice

The significance of mouse strain was strongly emphasized in an oral sensitization model using C3H/HeJ and BALB/c mice (Morafo et al. 2003). The reason for using C3H/HeJ strain of mice is its high production of IgE antibody and confirmable symptoms following challenge (Li et al. 1999, 2000). They sensitized mice on day 0 and dosed five times at weekly intervals with the protein, by intragastric gavage in presence of CT as adjuvant. The mice were challenged intragastrically on day 42 with protein and specific IgE antibodies measured in blood samples. Following challenge, clinical symptoms occurred including increased vascular permeability, hyperpermeability of the gut mucosa, lung inflammation and systemic anaphylaxis symptoms such as anaphylactic shock, which were evaluated with a standardized scoring system ranging from no symptoms to death. Other allergic responses determined included detection of vascular leakage, determination of plasma histamine levels and cutaneous mast cell degranulation. This model

resembles the clinical and immunological characteristics of peanut and cow's milk allergy in humans involving several organs. Thus, the model helps to study mechanisms associated with the immune pathology of IgE-mediated allergic reaction, to measure and to grade the severity of the reaction according to the serological and clinical manifestations induced, and to study the impact of any change in the structure of the protein in relation to its allergenicity. This is important as the generation of a heterologous foreign protein in a genetically modified organism might result in structural modifications. Oral exposure in the absence of CT did not induce food-specific antibody response (Bowman and Selgrade 2008a, b). It was also found that a two-time exposure at weekly interval with 1–2 mg doses of food extract is optimal instead of a four-time exposure regimen at weekly interval with 5 mg dose for observing distinct response between allergenic and non-allergenic foods.

Homogenized cow's milk and crude peanut extract with CT were used for the sensitization of BALB/c and C3H/HeJ mice. BALB/c mice produced specific IgE against peanut only; however, no clinical allergic reactions were observed. On the other hand, C3H/HeJ mice showed allergic responses to cow's milk and peanut. In splenocyte cultures of BALB/c mice, IFN- γ production (Th1 cytokine) was very high, while IL-4 (Th2 cytokine) and IL-10 levels (T regulatory cytokine) were found to be increased in C3H/HeJ mice. These results show that food allergy depends partly on cytokine responses driven by genetic variables (Morafo et al. 2003). Various investigators while working with chicken egg proteins have showed that the recognition of these proteins as allergens was dependent on the strain of mice used (Vaz et al. 1970, 1971). Some strains (C3H/HeJ) failed to mount a significant IgE antibody response to ovalbumin (OVA; Gal d 2) in spite of the presence of adjuvant. In total, it is possible that using a particular mouse strain to examine the allergenic potential of a particular protein may result in a false-negative response if the protein is not recognized by the immune system. It has been shown that the induction of specific IgE antibody is accompanied by the induction of specific IgG antibody (Yamanishi et al. 2003). Therefore, it is necessary that along with measurements of IgE antibody production, the IgG antibody responses are also determined (Dearman and Kimber 2007). A negative IgE antibody response in the presence of a vigorous IgG response is evidence of a lack of allergenic potential. In this case, it can be concluded that the protein is recognized as 'foreign' by the immune system and that exposure was sufficient to elicit immune (IgG) response.

Transgenic mouse models

Many mouse strains such as different C3H variants have been used to study the influence of the genetic background

in assessing the sensitizing potential of proteins. Alteration in IgE responses to OVA was observed in C3H/HeJ-, TLR4-deficient and thus LPS-hyporesponsive strains of mice (Vaz et al. 1971). Working with three different C3H mouse substrains with the same genetic background (H-2 k), Kaiserlian et al. (2005) demonstrated that repeated oral immunizations with BLG (β -lactoglobulin) along with CT induced variable clinical scores of immediate-type hypersensitivity upon oral challenge with BLG alone, thus reproducing the variable allergic disease severity in human. Wild-type normal C3H/HeN mice did not develop clinical symptoms; C3H/HeOuJ mice (a substrain of HeN mice) developed sensitization after several immunizations but showed only mild clinical symptoms, while C3H/HeJ mice were sensitized rapidly and developed systemic symptoms with anaphylaxis. The depletion of natural Treg increased the severity of symptoms in C3H/HeOuJ mice, which became as susceptible as C3H/HeJ mice.

Various workers have shown that C3H/HeJ strain is more susceptible to the induction of food allergy than other non-deficient mouse strains, thus making C3H/HeJ a valuable strain for food allergy research (Frossard et al. 2004a; Frossard et al. 2004b; Sicherer and Sampson 2006). However, studies on mouse strains of different genetic backgrounds including the TLR4⁺- and TLR4⁻-C3H, C57Bl/6 or BALB/c mice sensitized with either BLG or peanut extract showed that T-cell responses, susceptibility to develop an allergic reaction and intensity of the IgE response were under H2-linked genetic control and depend on both the TLR4 status of the mice and the nature of the antigen (Bashir et al. 2004; Berin et al. 2006). Goubier et al. (2008) showed that subsets of dendritic cells (plasmacytoid dendritic cells) in mice are tolerogenic and that depletion of these cells with specific monoclonal antibodies impairs oral tolerance and renders mice susceptible to sensitization by the oral route.

Guinea pig

Guinea pig model is widely used for the oral sensitization studies of cow's milk and infant formula (Fritsche 2003; Kitagawa et al. 1995). However, it is not the preferred model for the assessment of allergenicity of novel proteins. In guinea pigs, sensitization can be performed orally without adjuvant, but immunological reactions to proteins are different than in humans; for instance, reaginic antibody responses are of the IgG1a subtype and not IgE as in humans (Fritsche 2003). Disadvantages of using guinea pig in food allergy research are significant differences in immunophysiology when compared with other species and the lack of tools to study its immune system.

Dogs

Approximately 8% of the canine population suffers from food allergy, with no predisposing factors *viz.* age, sex or breed (Baker 1990; Jeffers et al. 1991). The clinical signs varying from gastrointestinal reactions (vomiting, diarrhea) to dermatologic reactions (pruritis, papules, erythema) are the symptoms of food allergy in both humans and dogs without predisposing factors such as age, sex or breed of canines (Halliwell 1992).

There are several advantages using this large animal model: the gut anatomy, physiology and nutritional requirements are similar to humans, it is possible to perform repeated endoscopic analysis of the GI tract, high IgE responder animals can be identified, large size of primary and secondary immune organs and blood volume supports certain analyses, the same animal can be immunized with multiple allergens (Helm et al. 2003, Teuber et al. 2002), etc. These advantages make them more useful for the mechanistic studies compared to the routine testing strategies for safety assessment. However, availability of limited strains, presence of greater interanimal variation in comparison to rodents, lack of commercially available immunological reagents, animals being expensive to maintain, etc. are the associated drawbacks. Moreover, sensitization is an extensive process, often needing up to 18 months of repeated immunization in the presence of adjuvant to achieve a stable response. Dogs are also not the preferred animal model due to animal welfare reasons.

The dogs that were first used in testing for food allergy had been derived from a colony of inbred spaniel/basenji dogs, which had genetic predisposition to allergy and history of sensitivity to pollen and foods (Buchanan and Frick 2002; Ermel et al. 1997; Jeffers et al. 1991). Dog also resembles humans in developing food allergies naturally and exhibiting same clinical symptoms (Buchanan and Frick 2002).

Dogs have been sensitized to peanut, walnut or Brazil nut extracts to see whether sensitization is accompanied by clinical reactions and whether cross-reactivity exists among the tested substances (Teuber et al. 2002). The protocol involved subcutaneously sensitizing the animals at birth with the extracts in alum and then with modified live virus vaccinations later, followed by oral challenges. The dogs showed positive intradermal skin test reactions to the nut extracts at 6 months of age. IgE immunoblotting of sera to the nuts displayed strong recognition of proteins in the aqueous preparations. At 2 years of age, most of the sensitized dogs responded on oral challenge with the corresponding primary immunogen. Little or no cross-reactivity occurred among the different preparations. On the basis of the tests performed, the hierarchy of nut and cereal allergen reactivity was also similar to that seen

clinically with humans (peanuts > tree nuts > soy > wheat > barley).

The observations from various investigators support the use of high IgE-producing dogs as a prospective model to assess the allergenicity of proteins as human food allergens. The profile of allergenic foods, response to individual allergens, clinical reactions of dogs and ranking of the potency of major food allergens are comparable to humans.

Swine

It is known that swine and humans have similarity with respect to anatomy, physiology and immunology of skin and gastrointestinal tract. Piglets have been used as model for sensitization/tolerance to cow's milk and soy proteins, with responses that are comparable to those recorded in young children (Bailey et al. 1993; Hankins et al. 1992). Intraperitoneal injection in the presence of cholera toxin (CT) adjuvant has also been used to determine responses to peanut proteins and the HEW allergen ovomucoid (Helm et al. 2002; Rupa et al. 2008). Helm et al. (2002) sensitized newborn piglets intraperitoneally with peanut extract and cholera toxin followed by challenge with peanut meal. The piglets showed physical symptoms mimicking those seen in double-blinded, placebo-controlled oral food challenges to peanuts in children and adults. Skin testing showed an IgE-mediated response. Histologically, the damage to the villi of the small intestine was found similar to that observed in endoscopically obtained tissue specimens from food-allergic individuals. The results confirmed that neonatal pig model mimics the physical and immunological characteristics of allergy in human beings.

The development and characterization of the swine as a model for the allergen-sensitizing capacity of novel proteins will be a valuable tool for investigations into the immunopathologic mechanisms of gastrointestinal allergy.

A summary of the selected animal models found useful for allergenicity evaluation of novel proteins has been provided in Table 1.

Conclusion

In conclusion, a number of oral animal models have been developed. The results seem to vary dependent on the study design. For oral models, the routes of exposure (oral, gavage), together with the presence/absence of adjuvant, dose administered and frequency of administration, play an important role. The oral route appears to be relevant for testing food proteins; however, the interpretation of findings is complicated by the oral tolerance process, driven by the regulatory T cells and other tolerance mechanisms.

Table 1 Summary of some of the animal models found useful for allergenicity evaluation of novel proteins

Animal model	Sensitization (route)	Adjuvant	Challenge (route)	Measurements	References
Rat (Brown Norway)	Intragastric	No	No	IgE, IgG	Knippels et al. (1998a)
Mice (BALB/c)	Intraperitoneal	No	No	IgE, IgG	Dearman et al. (2001, 2002)
Mice (BALB/c)	Transdermal	No	Oral	IgE, symptom score, temperature	Birmingham et al. (2007)
Mice (BALB/c)	Oral	SEB	Oral	Serum immunoglobulin levels, blood eosinophil count, cytokine determination, histology, plasma histamine levels	Ganeshan et al. (2009)
Mice (C3H/HeJ)	Intragastric	CT	Intragastric	IgE, symptom score, histology, plasma histamine levels, vascular leakage, intestinal permeability, splenocyte proliferation	Li et al. (1999, 2000)
Mice (C3H/HeJ)	Oral	CT	Intraperitoneal	IgE	Bowman and Selgrade (2009)
Dog (spaniel/basenji)	Subcutaneous	Alum, live vaccine	Oral	IgE, intradermal skin test, scoring of vomiting and number and quality of stools	Buchanan and Frick (2002), Teuber et al. (2002)
Swine (Landrace)	Intraperitoneal	No	Oral	Clinical symptoms, skin testing, passive cutaneous anaphylaxis, IgE, IgG, histology	Helm et al. (2002)

SEB Staphylococcal enterotoxin B, CT Cholera toxin

Several models used adjuvant for overcoming this difficulty; however, the hyperstimulation of the immune system may not reflect perfectly the type I allergic mechanisms as observed in humans. This aspect should be further evaluated with a large panel of proteins and/or food extracts as well as other strains with different genetic backgrounds.

The intraperitoneal injection models may permit the most direct assessment of the allergic potential for a novel protein, and it has been demonstrated that i.p. injections may overcome the tolerance that may occur if the protein is administered orally. Experience to date suggests that the measurement of antibody (IgE) responses in mice shows some promise for the discrimination between allergens and those materials that apparently lack allergenicity. Nevertheless, these studies have been conducted with a limited number of proteins, and further experience with a wider range of proteins of differing allergenic potential is required before their use in the risk assessment evaluation.

It is imperative to note that currently none of the approaches have been validated in the context of safety assessment. However, the available data suggest that the sensible use of an accurate animal model, along with other needed approaches, could be considerably useful in safety assessment. Useful information could be obtained by using a combination of various models in order to replicate the genetic background as well as different environmental factors and conditions of exposure to risk groups of the popula-

tion on a case-by-case basis. In future, the need is to improve the sensitivity and specificity of useful animal models developed. Also, intralaboratory validation of selected models should be carried out using a panel of allergenic (strong, moderate, weak) and non-allergenic proteins, along with whole food as well as protein extracts. The use of transgenic animals to assess sensitization to newly expressed proteins should be investigated further in greater detail.

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