



Utility of animal models for predicting human allergenicity

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ABSTRACT

The biochemical characterization of protein structures has led to a better understanding of allergens, their structure/function relationship, and can be very powerful in identifying protein sequences with significant structural similarity to known allergens. However, for scientists, regulators and food manufacturers there exists a need for acquiring additional data on potential allergenicity of proteins, particularly, biotechnology derived molecules in food products for which minimal or no prior human exposure information is available. Since human exposure testing, while direct, is unacceptable, understanding allergy in animals has been used to investigate the allergic response on a molecular level as well as test the potential in vivo allergenicity of food proteins. Rodents seem to be the most likely candidate for assessing allergenicity. For development of an animal test system for allergenicity characterization and testing, a number of criteria are required for qualification for a model of human allergy including acceptable immunization protocols, allergic response measurements, and for standardization and validation of materials and procedures. If an animal test system can minimally provide a basis for measuring the relative physiological response to known allergens, this should be enough to establish a model that produces a relative measure of potential allergenicity. Our article will consider development of an adequate animal model for allergenicity determination that can be validated as a tool in safety assessments.

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1. Introduction

Over the past five years a number of review articles, as well as workshops and symposia, have addressed animal models of human allergenicity with respect to food allergy (Helm and AW, 2002; Helm, 2002; Helm et al., 2003; Kimber et al., 2003a; Knippels et al., 2004; McClain and Bannon, 2006; Tryphonas et al., 2003). The primary questions are (1) can an in vivo allergy response to known allergens be accurately be measured in animals and (2) is such a response in animals predictable for a similar response in humans? Of particular interest is the animal's capacity to respond to and predict the potential allergenicity of molecules for which little or no information on prior human exposure is available, i.e., newly discovered proteins or those newly created proteins derived from biotechnology. The purpose of this paper is the discussion of the use of animal models (particularly rodents—a likely candidate test system) and their application in answering these questions (Akiyama et al., 2001; Dearman and Kimber, 2001; Gaudry et al., 2004a,

2003, 2004b; Kimber et al., 2003b; Knippels and Penninks, 2002, 2003; Lehrer et al., 2003; Li et al., 2000). This report will consider issues regarding development of an adequate animal model for the assessment of allergenicity of novel proteins with an emphasis on exposure to food proteins. In particular, criteria for selection and characterization of an animal model with a particular focus on the potential for validating its use as a tool in allergy safety assessments will be addressed. Existing animal models and their limitations and future expectations are discussed.

During the past 15 years substantial biochemical and physical information has been cataloged for many of the protein allergens that cause food allergy (Bannon, 2004; Breiteneder and Mills, 2005; Vieths et al., 2002). Recent studies using three-dimensional structures of known allergens, and structural database of allergenic proteins (Schein et al., 2007) have aided our understanding of the structural basis of allergens (Oezguen et al., 2008). This, in turn, has been extended as a way to help characterize the potential allergenicity of novel proteins to which there has been no prior human exposure. Generally, food allergens have been shown to be soluble molecules adept at crossing mucosal membrane barriers; usually these molecules are stable to heat and enzymatic digestion, are immunogenic (possess B- and T-cell epitopes) and are multivalent. IgE antibody testing of sera taken from food-allergic subjects has been used for a number of years to assess immunochemical similarities that are shared among allergens. IgE-binding studies have

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also been used to describe potential allergenicity of biotechnology derived proteins (Batista et al., 2005; Hoff et al., 2007) although these studies are not required as part of the routine allergy safety assessment of novel proteins (Codex Alimentarius, 2003).

Based on existing information, less than 20 protein families have been shown to contain most common allergens (i.e., lipid transfer proteins, plant defense proteins, and contractile proteins) while the vast majority of protein families are not associated with allergens. The advent of ever increasing biochemical characterization of protein structures has led to a better understanding of protein allergens and their structure/function relationship. Homology/similarity comparisons which rely on allergen sequence databanks and bioinformatic software have also improved as both the software algorithms and the supporting databases have undergone periodic review and improvement (Hileman et al., 2002; Ladics et al., 2007; Food Allergy Research and Resource Program Database, 2008; Pearson, 2000). Bioinformatic analyses can be very powerful in identifying protein sequences with significant structural similarity to known allergens. Analysis of potential IgE-binding epitopes have also been performed using bioinformatic searches of 6–8 contiguous amino acids that match between novel proteins and known allergens (Codex Alimentarius Commission, 2003). These small specific regions could potentially represent IgE-binding epitopes and are used in the characterization of potential protein allergenicity (2003); however, the epitopes from most known allergens have not been identified and therefore, the analysis remains a theoretical exercise (Silvanovich et al., 2006). In the future, more sophisticated search algorithms, using a database of conformational structures and motifs, may increase the accuracy of associating uncharacterized protein sequences with the three-dimensional structures of known allergens (Thomas et al., 2005). Although known allergens share many features, there is no single feature that is common to all protein allergens and there is no single test for reliably predicting allergenicity therefore, analyses such as bioinformatics remain just one of several characterizations that are used in a weight of evidence approach to describe allergenicity. Generally accepted methodology that has the capacity to describe the function and potency of known allergens as well as provide a tool for the assessment of in vivo allergenicity of novel proteins remains a challenge. Animal models may hold promise in this area if the technical, biological, and logistical challenges can be met.

2. Animal models

2.1. Animal models of allergenicity

For scientists, regulators and food manufacturers there exists a need for acquiring additional data on the potential allergenicity of proteins. In particular, biotechnology derived molecules in food products to which there is minimal or no prior human exposure and which share little or no properties with known allergens are

difficult to characterize without a reliable measure of allergenic potential specific for known allergens. Human exposure testing, while direct, has unacceptable ethical constraints. Alternatively, understanding allergy in animals to assess allergic responses to specific proteins has become a method of study for bridging from in silico and other indirect in vitro techniques (bioinformatics and biochemical characterizations) to the human response. Indeed, animal models have been used to investigate the allergic response on a molecular level as well as test the potential in vivo allergenicity of food proteins (Dearman et al., 2001; McClain and Bannon, 2006). Generally, there are three main questions that animal models can address: First, what are the mechanistic bases of IgE-mediated allergic responses; second, how can prophylactic intervention and therapeutic approaches to food allergy affect disease outcome; third, what are the most useful parameters to assess allergenicity and the likelihood that a novel protein is allergenic in humans? (Table 1).

Animal models have been used to address each of these questions separately, but no one model is ideal to address them all. The models that have most frequently been used are rats and mice, guinea pigs, swine and dogs. Each species has its own advantages and disadvantages with some species more naturally responsive to food allergens while others require extensive experimental manipulation to develop an allergic response. Generally, the canine and pig models have been useful for mechanistic approaches to understanding food-mediated allergic responses as they pertain to the physiologic response in humans (Buchanan and Frick, 2002; Helm et al., 2003; Helm et al., 2002; Teuber et al., 2002). Prophylactic intervention and therapeutic approaches have favored the use of rodent or guinea pig models, while several models have been used for qualitative approaches to assess allergenicity of novel proteins (Helm, 2002). The rodent model, especially murine, has been the most frequently used and is best understood in regards to the molecular and cellular cascade of events in allergic sensitization and elicitation.

2.2. Animal models: strengths and weaknesses

An animal model that most closely mimics the allergic response in man would be useful for the assessment of novel protein allergenicity and development into a validated test system. However, model selection implies considering immune responses not necessarily identical to those of humans and thus, there can be limits in extrapolating animal responses to the human response. Other considerations are the cost and logistics of using a particular model (handling, husbandry, and size). Also, analytical reagents may not be as readily available for some species as they are for other animal species, especially with regard to measuring the IgE and IgG antibody responses. Weaknesses of animal test systems, especially rodents, include oral tolerance to protein immunogens and a need in most cases for boosting the immune response with adjuvants, conditions which can limit

Table 1
Considerations for choice of animal model used for the study of food allergy.

Approach-objective	Species	Reagents available	Size	Relative cost	Naturally occurring food allergy	Adjuvants/systemic immunizations required
Mechanistic	Canine	Some	Lg	+++	+++	
	Swine	Some	Lg	+++	+	
Therapeutic	Rodent	Many	Sm	+		+++
	Canine	Some	Lg	+++	+++	+
	Guinea pigs	Some	Sm	++	+	+++
	Swine	Some	Lg	+++	+	
Assess Allergenicity of proteins	Rodents	Many	Sm	+		+++
	Guinea pigs	Some	Sm	++		+++

an experimental design attempting to approximate food exposure in humans.

It is well established that an IgE-mediated mechanism of allergy is present in many animal species and that they have substantial responsiveness to allergenic proteins. Sensitization, (including route, dose, number and timing of exposure and elicitation) unlike in man, can be controlled and precisely standardized in animals. Eliciting a response can be performed at a time when the reactions are most acute and most accurately measured which is critical for standardizing a quantitative measure of the response. Finally, strain selection can help minimize extraneous variability in the allergic model since many inbred strains with known genealogy are available. However, there are concerns that a particular inbred strain may not reflect the multifaceted nature of allergy in humans. In the context of developing reliable animal models, the question remains; what parameter of the immune response in which animal species best approximates a significant allergic response in humans?

2.3. Rodent models

Rodent models seem to be the most likely candidate for assessing novel protein allergenicity. Rodents can be easily tested in most laboratories with skilled personnel readily available. Rodent genetics have been well studied, there is a well documented and adequate supply of species, and numerous reagents are available to facilitate the analysis of the allergic response. Finally, the physical size of the rodent compared to larger laboratory animals provides easier manipulation, potential for increased sample size, and reduced cost of housing and care. Small rodents also require much less protein for immunizations compared to larger animals which can be an issue with heterologous novel biotechnology proteins. There is a large database of existing knowledge regarding the Major Histocompatibility complex as well as a base of knowledge regarding the effect of genetics on the immune response. In summary, the rodent is most likely to be included in the development of a broadly accepted, standardized allergy model because its flexibility would lead to the shortest development time, lowest cost, and best opportunity to include quantitative parameters under routine testing conditions.

3. An animal model for allergenicity characterization and testing

3.1. Criteria for qualifying a model of human allergy

It would be advantageous for industry safety assessments as well as regulatory agencies if a single standardized and validated model could be established. The challenge has been to develop a test system from these models that can be used for assessing novel proteins under standard conditions and methodologies. There are numerous rodent-based models that have used a variety of model allergens and various species (see the following reviews: Kimber et al., 2003a,b; Knippels et al., 2004; Helm and Burks, 2002; Helm et al., 2003; Kimber et al., 2003a; Knippels et al., 2004). Models have differed in species/strain used, immunization protocol, allergen doses and adjuvants. Additionally, standardized methods among researchers and laboratories have been difficult to bring to consensus.

A primary question to be considered for characterization and prediction of allergenicity is whether the rodent model should approximate every aspect of human allergy? Even though rodents may differ in their tolerance to orally delivered protein immunogens as compared to humans, the basic levels of the allergy disease response, and clinical manifestations may not vary significantly;

the question can then be asked as to whether or not this comparison is necessary for developing a model that may be used to characterize novel proteins, for example with the goal of determining allergenic potential. Is the lack of a full demonstration of “clinical similarity” between the animal and human responses important? Since animal models may be used to screen for the induction of an IgE response, or other parameter of allergic elicitation, the clinical relevance may actually not be as important relative to the larger goal of assessing relative allergenicity of known allergens versus non-allergens.

3.2. Immunization protocols

First and foremost in developing a standard allergic response is demonstration that the animal can be sensitized through immunization to known allergens as well as generate a reasonably broad dose-dependent response. Conversely, it is essential that non-allergens trigger minimal or negative responses. The goal should be to produce a measurable response to a panel of known allergens and a negative response to a panel of non-allergens. The class (contact or food allergen), matrix, and dose of allergen are important considerations in developing the immunization protocol. The relative measured “potency” of an allergen, as it is known in humans, should then be reflected by its “order of potency” in the animal’s response. Because the context of exposure for any allergen is important, the question of whether to use crude extracts, purified proteins, or protein within a particular food matrix should be considered. If purified, the criteria for preparation and purity standards for the immunogen should be well described. It is not necessary to have a final processed food for testing, but the base matrix in which it is expressed should be made available to act as a control to exclude non-specific responses to the matrix. The response to a purified allergen may be useful, particularly with proteins that are shown to have no similarity to known allergens through other characterization studies. Whatever test material and accompanying matrix are used, it must be well standardized and characterized as discussed later.

Understanding the route of exposure for proteins in food allergy is important. Should the route of sensitization be the one that causes the strongest response or should it be the one that most likely simulates exposure in humans (the oral response)? Oral feeding or gavages are most desirable if simulating human exposure to foods is critical. What is the timing for priming, sensitizing, and immunization intervals? Humans are generally exposed to allergens several times before an allergic reaction develops, usually over a period of several years, therefore, characterization of human exposure and replication in an animal is unlikely. The use of adjuvants is a strategy for alleviation of the oral tolerance observed in rodents but is not a well described feature of human exposure to food allergens. Thus the oral exposure route requires further development and validation (Bowman and Selgrade, 2008). In the majority of rodent models some form of adjuvant is used—success in the mouse model has also been obtained with the use of cholera toxin. Although this clearly enhances the immune response the risk is that the response is enhanced to a degree that produces false positive responses to non-allergens should be well described in any model utilizing adjuvants. As long as the model appears to recognize allergens and not recognize non-allergens in a pattern similar to humans then use of adjuvants may be appropriate.

3.3. Measuring the allergic response

The allergic parameter to be measured—whether it be IgE antibody production, basophil mediator release, IgG, or T-cell responses—should be considered in the context of the goal of characterizing allergenicity for a broad range of test proteins. An

important decision with any biological parameter is the ability to differentiate between a low and high response level and to differentiate a known allergen from a known non-allergen, since this will ultimately be used to assess the allergenicity of an unknown, test protein. Measuring IgE antibody, for example, should indicate recognition of known allergenic food proteins and react to the same amino acid sequences (epitopes) to which human IgE is directed. Certainly, human allergic responses can be quite variable and individuals can react to the many different protein allergens in a food such as those in soybean (Ballmer-Weber et al., 2007). Individual human subjects may even vary in reactivity to various epitopes (Ayuso et al., 2002a; Ayuso et al., 2002b). However, demonstration of the similarity between the animal model and humans with regard to epitope specificity and the patterns of reactivity has significant value. Connecting the animal response to the potential clinical outcome in humans for known allergens may help support the ability to predict that outcome for uncharacterized, novel proteins.

4. Requirements for a standardized and validated animal model

Any animal model which is intended for use as a standard safety assessment tool must be accurate and reproducible (See Table 2 for a summary of considerations). The animal must be able to identify an allergen from a non-allergen and produce the same response reliably over the course of time and among different laboratories. The animal test system should also include one or more non-allergens as a “negative control” for each experiment. The use of non-allergens is an issue that has not been addressed adequately in most models but is critical to describing the performance of the model; the model should not produce a false positive response to a non-allergen. Because variability exists at many different levels in human allergy, the animal model is unlikely to ever mimic humans. However, the model must have a similar scaled response that reliably shows true negative and positive responses to non-allergens and allergens, respectively. The precision of the model describes the ability of the animal to respond in a quantitative fashion and distinguish non-allergens from known allergens and if possible, less potent allergens from those more likely to cause life-threatening disease in humans. In other words, well characterized allergens should respond in the model similar to that observed for humans for a selected response parameter; e.g., peanut or peanut allergens should produce the most significant response relative to other foods such as soy, peas or corn.

Because most allergens to date have not been reproducibly tested in one animal system there are still important issues to

address regarding the potential of an animal model to predict allergenicity. For example, the precision in the selected allergic response, as described above, should exist for any biological response parameter (e.g., IgE) to develop an allergenicity scale from low to high for known allergens. Sensitivity is also an important aspect of any model. Will the animal model respond with sufficient population level sensitivity; i.e., can we detect allergens that only affect a very small, but sensitive population? Alternatively, should the model simply show a separation of IgE antibody production to allergenic foods such as peanuts and shrimp as compared to less or non-allergenic foods such as peas or beef? The model should be assessed with regard to its ability to distinguish allergenicity at a categorical or qualitative level. This question reflects a developmental issue regarding application of models because it remains unclear whether or not sensitivity and selectivity are sufficient for potentially applying quantitative measures of allergenic potential. It is important to show how the allergic response will be quantified and what thresholds will be developed for each known allergen; ultimately, the model will have to be validated for its utility in characterization for all protein allergens. Can a threshold IgE response be used to distinguish between allergens and non-allergens? All of these issues require further consideration and refinement.

Finally, the issue of protein standards and test system reproducibility must be considered in the validation process. Standards should be well described in terms of sourcing foods or proteins with their particular criteria for purity. Any model will be only as robust in its performance as the standards are in their consistency. The model itself should have described parameters that cover (1) appropriate levels of accuracy (positive is always positive and negative always negative) (2) expected levels of precision and (3) reproducibility (acceptable coefficients of variation for inter- and intra-assay performance). The quantity and types of controls need to be considered: Are all controls required for the test system or only during validation?—perhaps not, but certainly positive and negative controls are essential every time the test is performed. The criteria that are the basis for a validated test system remain to be clarified, but should ultimately focus on the ability of the model to show consistent results from one laboratory to another (Dearman et al., 2003).

5. Conclusions: closing the gap between expectations and reality

Sensitization/exposure history of humans to food allergens is poorly understood: Human sensitization can be a complicated

Table 2
Animal model validation parameters.

Validation parameter	Criteria	Notes
Sensitivity	Limit of detection of model parameter(s) for known positive and negative allergens	May need to test the animal population sensitivity since each animal may not be responsive
Accuracy	Distinguish known allergens from non-allergens	
Precision	Determine whether or not known allergens can be distinguished from one another	Categorical response: weak allergens produce a lower response than strong allergens based on human prevalence or clinical severity
Reproducibility	Establish variability acceptance criteria to determine needed animal replicates per immunization group	
Assess threshold response	Determine the dose at which a physiologically significant response is measured	May need to assess non-allergens for a threshold response
Standards acceptance criteria	Purity and matrix composition description required for known positive and negative allergens	
Inter-laboratory variability	Model performs similarly in other laboratories within acceptance criteria	
Model robustness	Describe the animal species and immunization protocols that produce expected results for known positive and negative allergens	Robustness could also describe the model's ability to accept various materials (e.g., pure proteins versus whole foods) as test substances

and convoluted process. Sensitization can vary by route, frequency-rate, and number of exposures. The age of the exposed individual, genetics, as well as other yet to be identified events may affect sensitization and provocation of an allergic response. It is highly unlikely that any one animal model can mimic this entire process. Thus, an animal model should be regarded as a biological test system rather than a human surrogate since it is practically impossible to duplicate the human allergic disease process in an animal. If an animal model can minimally provide a basis for measuring the relative physiological response to known allergens, this should be enough to establish a model that produces a relative measure of potential allergenicity. The animal model of allergy may become a useful tool to place a protein in relative proportion to its potential for allergenicity by comparing it to other known allergenic and non-allergenic proteins.

The basic function of an animal model remains the most challenging aspect of developing the test system; for measuring allergenicity of novel proteins the animal model must be able to discriminate non-allergens from known allergens. Testing non-allergens is as important as testing allergens. Current allergy safety assessments utilize the guidelines outlined in the *Codex Alimentarius Commission report (2003)* for food proteins derived from biotechnology. Novel proteins, or any other protein developed into a biotechnology food product are assessed for safety using a weight of evidence procedure that bases conclusions upon many analytical approaches. No one approach can definitively describe a novel protein as an allergen or non-allergen. Since it is likely that most proteins derived from biotechnology and developed into novel food proteins and subsequently tested in an animal model will be non-allergenic, the model must have a level of performance that ensures a low rate of false positive responses to non-allergens. Even if validated for use in a regulatory environment, it is expected that an animal model of allergenicity would function as the other regulatory characterization studies of allergenicity and provide one of many assessment strategies (weight of evidence approach) rather than a definitive test.

Scientific research in allergenicity safety assessments is moving toward development of an acceptable in vivo model that can support the determination of relative allergenic potency and ultimately, predict the allergenic risk of proteins. Remaining development goals should center on standardizing the test system itself so that the same laboratory can repeat its findings and communicate a consistent result in parallel with those from other laboratories. In addition, the technical validation of models must be performed with an agreement on a relevant measure of allergenic response as well as acceptance criteria for sensitivity, accuracy, and reproducibility of the test system and protein standards. As outlined previously, these are possible achievements but they require resources, time, and most importantly a consensus among the scientific community utilizing the expertise among academic, regulatory, and industry centers of research.

References

- Akiyama, H., Teshima, R., Sakushima, J.-I., Okunuki, H., Goda, Y., Sawada, J.-i., Toyoda, M., 2001. Examination of oral sensitization with ovalbumin in Brown Norway rats and three strains of mice. *Immunology Letters* 78, 1–5.
- Ayuso, R., Lehrer, S., Reese, G., 2002a. Identification of continuous, allergenic regions of the major shrimp allergen Pen a 1 (tropomyosin). *International Archives of Allergy Immunology* 127, 27–37.
- Ayuso, R., Reese, G., Leong-Kee, S., Plante, M., Lehrer, S., 2002b. Molecular basis of arthropod cross-reactivity: IgE-binding cross-reactive epitopes of shrimp, house dust mite and cockroach tropomyosins. *International Archives of Allergy and Immunology* 129, 38–48.
- Ballmer-Weber, B.K., Holzhauser, T., Scibilia, J., Mittag, D., Zisa, G., Ortolani, C., Oesterballe, M., Poulsen, L.K., Vieths, S., Bindslev-Jensen, C., 2007. Clinical characteristics of soybean allergy in Europe: a double-blind, placebo-controlled food challenge study. *Journal of Allergy and Clinical Immunology* 119, 1489–1496.
- Bannon, G.A., 2004. What makes a food protein an allergen? *Current Allergy and Asthma Reports* 4, 43–46.
- Batista, R., Nunes, B., Carmo, M., Cardoso, C., Jose, H.S., de Almeida, A.B., Manique, A., Bento, L., Ricardo, C.P., Oliveira, M.M., 2005. Lack of detectable allergenicity of transgenic maize and soya samples. *Journal of Allergy and Clinical Immunology* 116, 403–410.
- Bowman, C.C., Selgrade, M.K., 2008. Differences in allergenic potential of food extracts following oral exposure in mice reflect differences in digestibility: potential approaches to safety assessment. *Toxicological Sciences* 102 (1), 100–109.
- Breiteneder, H., Mills, E.N.C., 2005. Plant food allergens—structural and functional aspects of allergenicity. *Biotechnology Advances* 23, 395–399.
- Buchanan, B.B., Frick, O.L., 2002. The dog as a model for food allergy. *Annals of the New York Academy of Science* 964, 173–183.
- Codex Alimentarius Commission, 2003. Joint FAO/WHO Food Standard Programme: Appendix III, Guideline for the conduct of food safety assessment of foods derived from recombinant DNA plants and Appendix IV, Annex on the assessment of possible allergenicity, 25th Session, Rome, Italy 30 June–5 July, pp. 47–60.
- Dearman, R., Caddick, H., Stone, S., Basketter, D., Kimber, I., 2001. Characterization of antibody responses induced in rodents by exposure to food proteins: influence of route of exposure. *Toxicology* 167, 217–231.
- Dearman, R.J., Kimber, I., 2001. Determination of protein allergenicity: studies in mice. *Toxicology Letters* 120, 181–186.
- Dearman, R.J., Skinner, R.A., Herouet, C., Labay, K., Debruyne, E., Kimber, I., 2003. Induction of IgE antibody responses by protein allergens: inter-laboratory comparisons. *Food and Chemical Toxicology* 41, 1509–1516.
- Food Allergy Research and Resource Program (FARRP) Allergen Database. 2008. University of Nebraska, Lincoln, NE, USA. University of Nebraska (Lincoln, Nebraska, USA).
- Gaudry, P., Fernandes, J., Reish, R., Rous, K., Sathe, S., Teuber, S., Reese, G., Lehrer, S.B., 2004a. Murine IgE and IgG antibody responses to allergenic and non-allergenic foods: Potential model for allergenicity assessment of foods in man? *Journal of Allergy and Clinical Immunology* 113 (2 Suppl) (Abstract 83).
- Gaudry, P., Lehrer, S.B., Roux, K., Teuber, S., 2003. IgE and IgG antibody responses to walnut and cashew allergens in orally immunized mice. *Annals of Allergy Asthma and Immunology* 92, 49.
- Gaudry, P., Roux, K., Sathe, S., Teuber, S., Lehrer, S.B., 2004b. Detecting the allergenicity of foods: IgE and IgG antibody responses to allergenic and non-allergenic foods in orally immunized mice. *Journal of Investigative Medicine* S306, Abstract #275.
- Helm, R., Aw, B., 2002. Animal models of food allergy. *Current Opinion in Allergy and Clinical Immunology* 2, 541–546.
- Helm, R.M., 2002. Food allergy animal models; an overview. *Annals of the New York Academy of Sciences* 964, 139–150.
- Helm, R.M., Ermel, R.W., Frick, O.L., 2003. Nonmurine animal models of food allergy. *Environmental Health Perspectives* 111, 239–244.
- Helm, R.M., Furuta, G.T., Stanley, J.S., Ye, J., Cockrell, G., Connaughton, C., Simpson, P., Bannon, G.A., Burks, A.W., 2002. A neonatal swine model for peanut allergy. *Journal of Allergy and Clinical Immunology* 109, 136–142.
- Hileman, R.E., Silvanovich, A., Goodman, R.E., Rice, E.A., Hollerschlag, G., Astwood, J.D., Hefle, S.L., 2002. Bioinformatic methods for allergenicity assessment using a comprehensive allergen database. *International Archives of Allergy and Immunology* 128, 280–291.
- Hoff, M., Son, D., Gubesch, M., Ahn, K., Si, L., Vieths, S., Goodman, R., Ballmer-Weber, B., Bannon, G., 2007. Serum testing of genetically modified soybeans with special emphasis on potential allergenicity of the heterologous protein CP4 EPSPS. *Molecular Nutrition and Food Research* 51, 946–955.
- Kimber, I., Dearman, R.J., Penninks, A.H., Knippels, L.M., Buchanan, R.B., Hammerberg, B., Jackson, H.A., Helm, R.M., 2003a. Assessment of protein allergenicity on the basis of immune reactivity: animal models. *Environmental Health Perspectives* 111, 1125–1130.
- Kimber, I., Stone, S., Dearman, R.J., 2003b. Assessment of the inherent allergenic potential of proteins in mice. *Environmental Health Perspectives* 111, 227–231.
- Knippels, L.M., Penninks, A.H., 2002. Assessment of protein allergenicity studies in Brown Norway rats. *Annals of the New York Academy of Science* 964, 151–161.
- Knippels, L.M., Penninks, A.H., 2003. Assessment of the allergic potential of food protein extracts and proteins on oral application using the brown Norway rat model. *Environmental Health Perspectives* 111, 233–238.
- Knippels, L.M.J., vanWijk, F., Penninks, A.H., 2004. Food allergy: what do we learn from animal models? *Current Opinion in Allergy and Clinical Immunology* 4, 205–209.
- Ladics, G., Bannon, G.A., Silvanovich, A., Cressman, R.F., 2007. Comparison of conventional FASTA identity searches with the 80 amino acid sliding window FASTA search for the elucidation of potential identities to known allergens. *Molecular Nutrition and Food Research* 51, 985–998.
- Lehrer, S., Fernandes, J., Reish, R., Gaudry, P., Dai, G., Reese, G., 2003. Murine IgE and IgG antibody responses to peanuts and shrimp allergens, major food allergens in man. *Journal of Allergy and Clinical Immunology* 111, 716.
- Li, X.-M., Serebrisky, D., Lee, S.-Y., Huang, C.-K., Bardina, L., Schofield, B.H., Stanley, J.S., Burks, A.W., Bannon, G.A., Sampson, H.A., 2000. A murine model of peanut anaphylaxis: T- and B-cell responses to a major peanut allergen mimic human responses. *Journal of Allergy and Clinical Immunology* 106, 150–158.

- McClain, S., Bannon, G., 2006. Animal models of food allergy: opportunities and barriers. *Current Allergy and Asthma Reports* 6, 141–144.
- Oezguen, N., Zhou, B., Negi, S.S., Ivanciuc, O., Schein, C.H., Labesse, G., Braun, W., 2008. Comprehensive 3D-modeling of allergenic proteins and amino acid composition of potential conformational IgE epitopes. *Molecular Immunology* 45, 3740–3747.
- Pearson, W.R., 2000. Flexible sequence similarity searching with the FASTA3 program package. *Methods in Molecular Biology* 132, 185–219.
- Schein, C.H., Ivanciuc, O., Braun, W., 2007. Bioinformatics approaches to classifying allergens and predicting cross-reactivity. *Immunology and Allergy Clinics of North America* 27 (1), 1–27.
- Silvanovich, A., Nemeth, M.A., Song, P., Herman, R., Tagliani, L., Bannon, G.A., 2006. The value of short amino acid sequence matches for prediction of protein allergenicity. *Toxicological Sciences* 90, 252–258.
- Teuber, S.S., Del Val, G., Morigasaki, S., Jung, H.R., Eisele, P.H., Frick, O.L., Buchanan, B.B., 2002. The atopic dog as a model of peanut and tree nut food allergy. *The Journal of Allergy and Clinical Immunology* 110, 921–927.
- Thomas, K., Bannon, G., Hefle, S., Herouet, C., Holsapple, M., Ladics, G., MacIntosh, S., Priville, L., 2005. In silico methods for evaluating human allergenicity to novel proteins: international bioinformatics workshop meeting report, 23–24 February. *Toxicological Sciences* 88, 307–310.
- Tryphonas, H., Arvanitakis, G., Vavasour, E., Bondy, G., 2003. Animal models to detect allergenicity to foods and genetically modified products: workshop summary. *Environmental Health Perspectives* 111, 221–222.
- Vieths, S., Scheurer, S., Ballmer-Weber, B., 2002. Current understanding of cross-reactivity of food allergens and pollen. *Annals of the New York Academy of Sciences* 964, 47–68.