Animal Models to Detect Allergenicity to Foods and Genetically Modified Products: Workshop Summary

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Respiratory allergy and allergy to foods continue to be important health issues. There is evidence to indicate that the incidence of food allergy around the world is on the rise. Current estimates indicate that approximately 5% of young children and 1–2% of adults suffer from true food allergy (Kagan 2003). Although a large number of *in vivo* and *in vitro* tests exist for the clinical diagnosis of allergy in humans, we lack validated animal models of allergenicity. This deficiency creates serious problems for regulatory agencies and industries that must define the potential allergenicity of foods before marketing. The emergence of several biotechnologically derived foods and industrial proteins, as well as their potential to sensitize genetically predisposed populations to develop allergy, has prompted health officials and regulatory agencies around the world to seek approaches and methodologies to screen novel proteins for allergenicity.

One such approach was proposed initially by the International Life Science Institute (ILSI) Allergy and Immunology Institute in collaboration with the International Food Biotechnology Council (IFBC) (Metcalfe et al. 1996) and was subsequently modified by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) (FAO/WHO 2001). The FAO/WHO 2001 decision tree approach for the evaluation of allergenicity of genetically modified (GM) foods recommended a hierarchical approach to safety assessment (reviewed by Metcalfe et al. 1996; FAO/WHO 2001; Taylor and Hefle 2001). One of the recommendations in this approach was that animal models, when sufficiently developed and validated, could contribute valuable information to the process of identifying potential sensitization to food protein. On the basis of this recommendation, Health Canada undertook to organize a 2-day workshop held in Ottawa, 13–14 November 2002. Several prominent research scientists who are developing animal models of allergenicity convened to review the current status of such models, identify areas that need further development, and discuss the role these models may play in predicting the allergenic potential of foods, including GM food products. Data were presented on several murine and nonmurine animal models currently under development. In addition, emerging approaches—including the identification of antigenic epitopes on human allergens using the human leukocyte antigen (HLA) transgenic mouse and a human dendritic cell–based method to identify $CD4^+$ T-cell epitopes in potential protein allergens—were presented. This monograph consists of selected papers that are relevant to the development of animal models.

Animal models presently under development include the Brown Norway (BN) rat, the BALB/c mouse, and a transgenic mouse strain engineered to produce class II HLA molecules. The potential to sensitize BALB/c mice systemically via intraperitoneal injections of proteins has been explored in a series of experiments detailed by Kimber et al. (2003). This approach favors the initiation of vigorous humoral immune responses of the immunoglobulin (Ig)G and IgE class and could be a useful model to screen novel proteins for their allergenic potential. The BN rat is a high Ig (particularly IgE) responder rat strain. In some ways the BN rat resembles atopic humans in their genetic predisposition to develop allergies. Preliminary experiments whereby ovalbumin was used as the sensitizing antigen demonstrated that the BN rat is a promising species for the development of an oral sensitization animal model. Furthermore, immunoblotting experiments demonstrated that sera from BN rats that were sensitized orally with hen's egg white and cow's milk and sera of allergic patients to hen's egg white or cow's milk recognized a comparable profile of allergens in these allergic food products. This indicates that the specific protein recognition of induced antibodies in the BN rat is comparable to that observed in sera from allergic patients (Knippels et al. 2003).

Besides their usefulness in systemic sensitization research, the BN rat and the BALB/c mouse models exhibit many of the characteristics of allergic asthma observed in humans: immediate bronchial hyperresponsiveness and increased levels of IgE antibodies in serum and bronchoalveolar lavage fluid upon exposure to respiratory allergens such as dust mite allergens and molds. One present limitation of these models is that although many asthma symptoms are exhibited, the animals do not go on to develop chronic respiratory disease, as do humans. Eventually the rodents become tolerant to the allergen. Several possibilities are being explored to overcome this limitation, such as circumventing the potential for tolerance by exposing the animals when very young.

The HLA class II transgenic mouse was shown to be an excellent model for studying the genetic and molecular basis of allergic hyperresponsiveness because, like humans, this mouse strain develops pulmonary eosinophilia, lung tissue damage, and airway hyperreactivity upon exposure to allergens. This was demonstrated by exposing the animals to extracts of short and giant ragweed. T-cell epitopes were identified, and transgenic mice with the HLA-DQ locus exhibited strong T-cell responses to short ragweed extracts. Only the HLA-DQ mice exhibited these responses, indicating that a response specificity exists for different HLA molecules. This response was also mediated by $CD4^+$ T cells. This model holds promise for identifying epitopes critical in eliciting allergenic responses as well as for developing potential immunotherapies (Chapoval and David 2003).

A functional *in vitro* assay that predicts T-cell responses to peptide epitopes in humans was also presented (Stickler et al. 2003). It was specifically developed to predict functional T-cell epitopes in individuals who had not been previously exposed to the protein in question. For the allergenic assessment of GM proteins, this is important because previous human exposure to most novel recombinant proteins has never occurred. Determining the T-cell epitopes of potentially allergenic substances may also aid in developing variants that may be hypoallergenic, thus reducing the overall allergenic potential of GM proteins and other novel substances.

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This workshop summary is an introduction to the mini-monograph on "Animal Models to Detect Allergenicity to Foods and Genetically Modified Products." The articles are based on presentations at the conference "Health Canada Workshop on Animal Models to Detect Allergenicity to Foods and Genetically Modified Products," held 13–14 November 2001, Ottawa, Ontario, Canada.

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Although some practical considerations make rodents the model of choice for many labs, several nonrodent models have proven useful in evaluating food allergenicity. The swine model and the atopic dog model were described at the workshop (Helm et al. 2003). The advantage of both models lies in their propensity to develop clinical symptoms of food allergy, primarily gastrointestinal and dermatologic reactions, after the sensitized animal is challenged with food antigens. In contrast, clinical responses to food allergens in rodent models are not as reminiscent of human responses.

Swine are used frequently in research as a surrogate for humans (Helm et al. 2003). Developing piglets have anatomic and nutritional similarities to developing humans, including a tendency to become either sensitive to or tolerant of soy and cow's milk proteins. In the swine model of food allergenicity, newborn piglets are sensitized to peanut protein by intraperitoneal injection of peanut extract plus the adjuvant cholera, followed by two subsequent booster injections 18 and 25 days later. Oral challenge of sensitized piglets produces gastrointestinal and dermatologic symptoms that can be measured with direct skin testing to elicit wheal and flare reactions or by assessing changes in gut morphology, including edema and hemorrhage. A limitation of this model, which will undoubtedly be overcome in the near future, is the lack of antibodies specific for swine IgE. As a result, the presence of food antigen-specific IgE in sensitized animals has not yet been confirmed.

Food allergy is relatively common in dogs, affecting about 8% of the canine population (Helm et al. 2003). The atopic dog model is based on an inbred colony of high IgE-producing dogs. This model has the advantage that gastrointestinal and dermatologic responses to food allergen challenge in sensitized animals have been correlated with increased circulating antigen-specific IgE. To elicit sensitization, dogs are immunized with live virus vaccine, followed by several subcutaneous injections with food antigens over a course of weeks. At this point the dogs produce antigen-specific IgE, respond with wheal and flare reactions in skin tests, and show signs of gastrointestinal edema and inflammation. The applicability of the atopic dog model has been proven in a study of the potential allergenicity of a genetically modified corn line. Both transgenic and nontransgenic corn leaf extracts were found to be essentially nonallergenic compared with common food allergens in pups sensitized from birth with either of the two leaf protein extracts and with common food allergens such as peanut, soy, and cow's milk. These data indicate that nonrodent models should not be overlooked as a source of valuable data on the potential allergenicity of genetically modified foods.

In summary, several rodent and nonrodent animal models of respiratory and systemic sensitization with allergens are currently been evaluated for their potential to predict allergenicity to food, plant, and animal substances. Although presently no single animal model meets the requirements for an ideal animal model, each of these models has merits that, when further validated, can contribute significantly to the overall assessment of allergenicity to several substances including the GM-derived protein products. The development of *in vitro* assays to predict functional T-cell epitopes in individuals who had not been exposed previously to the culprit proteins can be a very powerful tool in the study of allergenicity to substances. Perhaps the most useful application of this novel technique would be the development of hypoallergenic substance variants. This would help reduce the overall allergenic potential of GM proteins and other novel substances.

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