Evaluation of Allergenicity of Genetically Modified Soybean Protein Extract

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Background

Food allergies are adverse reactions to an otherwise harmless food or food component that involve an abnormal response of the body’s immune system to a specific protein(s) in foods. Food allergies are caused by a wide variety of foods. Theoretically, any food that contains protein would be capable of eliciting an allergic reaction, although foods vary widely in their likelihood of provoking allergic sensitization. Approximately 2–5% of the adult population is affected, with the prevalence among children being even greater.

The incidence of food allergy around the world is increasing in line with other forms of allergic disease. Under healthy conditions, the immune system reacts towards innocuous dietary antigens by inducing antigen-specific systemic non-responsiveness, termed oral tolerance; whereas under pathological conditions, such as food allergy, it is believed that a dysregulation in the mechanisms of oral tolerance induction prevails. Most food-allergic reactions are mediated by specific IgE antibodies, and the development of an IgE-mediated response to an allergen is the result of a series of molecular and cellular interactions involving antigen presenting cells, T cells and B cells. The production of IgE is promoted by Th2 cells and their cytokines (Fig. 1).

Most confirmed food allergies are associated with a relatively limited range of products (peanuts, crustaceans, fish, milk, eggs, tree nuts, wheat, and soybeans), although there are many other foods and food products that have also been implicated. Soybean is one of the major sources of protein in human and animal nutrition and it has also been well characterized as an important allergenic source. At least 16 IgE-binding soy proteins with molecular masses from 7.5 to 97 kDa may be involved in clinical allergy.

Advances in biotechnology have resulted in an increasing number of genetically engineered foods, and among these, soybean is one of the most widespread. The predominant genetically engineered soybean grown in the world is Roundup Ready, which is resistant to glyphosate, the active ingredient in Roundup agricultural herbicides. This resistance was obtained by introduction of the glyphosate-tolerant cp4 epsps coding sequence, derived from the common soil bacterium Agrobacterium sp. strain CP4, into the soybean genome using particle acceleration transformation. Globally, genetically modified soybean made up 60% of all transgenic crops grown in 2005.

The application of modern biotechnology to food

Figure 1: IgE-mediated anaphylaxis (modified from Epstein M.M. Pharmacology & Therapeutics 109 (2006) 107-136.)

Figure 2: FAO/WHO 2001 Decision Tree
production presents new opportunities and challenges for human health. The potential benefits to the public health sector include altering the nutrient content of foods, decreasing their allergenic potential, and improving the efficiency of food production systems. On the other hand, the potential effects on human health of the consumption of food produced through genetic modification must be carefully examined.

With the development of genetically engineered crop plants there has been a growing interest in the approaches available to assess the potential allergenicity of novel gene products. This allergenicity might derive from changes in endogenous protein levels, expression of known allergens in different foods, and the expression of novel proteins that may be allergenic. Although strategies exist for such assessments, improvements should be considered, especially in cases in which the gene of interest is derived from a source with no history of allergenicity. A report in 2001 of a joint FAO/WHO expert consultation on allergenicity of foods derived from biotechnology suggests an integrated and stepwise, case-by-case approach (Fig. 2), which incorporates consideration of the serological identity of the protein of interest with known human allergens, examination of the amino acid homology with, and/or structural similarity to, allergenic proteins, and measurement of the stability of the protein in a simulated gastric fluid.4

The same document gave an indication that, for additional assessment of the potential allergenicity of expressed proteins, informative data can be generated using animal models. These data could be used in concert with the approaches summarized above.

**Development of a murine model of soybean specific IgE sensitization**

Recently, we developed a murine model of IgE-mediated food allergy based on oral administration of soybean protein extract. The purpose of our study was to develop and characterize the immune response induced in the animal model by intragastrical immunization with wild-type soybean extract (wt-SE), or with a genetically modified soybean extract (gm-SE). Then, we used this model to compare the immunological response obtained both with wt-SE and with gm-SE to assess the potential allergenicity of gm-SE with respect to its natural counterpart.5

The Balb/c mouse has been widely utilized to evaluate the sensitizing potential of novel proteins, because it favors the development of Th2-type immune responses and the production of IgE antibody. Although oral administration is the preferred route of exposure, it has been demonstrated that such a regimen may lack the sensitivity required for effective identification of the inherent sensitizing potential. This is probably attributable, at least in part, to the fact that oral exposure to the antigen generally results in the development of tolerance. Therefore, the major general obstacle towards establishing food allergy models is the strong innate tendency of animals to develop immunological tolerance to ingested antigens. This is particularly relevant in the case of soybean allergen, as the usual mouse diet includes soybean proteins. Consequently, for our experiments we used Balb/c mice fed on soy-free feed and born in our animal facilities from females fed on soy-free feed. Recent studies have developed murine models of IgE-mediated food allergy based on oral coadministration of antigen with an optimal dose of cholera toxin (CT) to generate a robust allergen-specific antibody response.6

In our study, we used a similar approach to develop our murine model, combining it with the use of Balb/c mice of the second (F2) offspring generation bred on a soy protein-free diet. We generated the first murine model of IgE-mediated soybean sensitization, in which a soybean-specific IgE response was induced by oral immunization, and reported the characterization of the antigen-specific cellular and humoral responses. To this aim, two allergenic extracts from wt-soybean and from gm-soybean seeds were prepared and also characterized. We immunized Balb/c mice with both wt-SE and gm-SE and obtained high levels of specific IgE and IgG1 vs. low levels of IgG2a. This pattern is indicative of a Th2-type response induced by the oral immunization with CT as an adjuvant.

**Comparison of immunological responses of wt-SE and gm-SE**

In our model we obtained a comparable level of IgE and IgG1 antibodies between those produced by gm-SE-sensitized mice and those obtained from wt-SE-sensitized mice. Moreover, using a specific IgE ELISA inhibition test, we observed that IgE antibodies produced after immunization with wt-SE were able to inhibit completely the binding of IgE obtained by oral administration with gm-SE. Similar results were obtained in the opposite case. These data strongly suggest that gm-SE shared the same allergenic components with wt-SE. In particular, the complete IgE inhibition (up to 100%) obtained in the assay performed on gm-SE as an antigen, wt-SE as an inhibitor, and sera from gm-SE sensitized mice sustains the hypothesis that the exogenous CP4-EPSPS protein is not able to induce an IgE response under our conditions.

T lymphocytes and cytokines have been demonstrated to play a pivotal role in the induction of IgE response. Therefore, to further assess the suitability of our animal model for testing the allergenicity of genetically engineered foods, we evaluated T cell responses to the SEs. We found that spleen cells from mice treated with gm-SE exhibited the same proliferative responses to wt-SE in vitro stimulation compared with homologous antigen stimulation and vice versa. Antigen stimulation of spleen cells from mice sensitized either with wt-SE or with gm-SE induced significantly increased and comparable production of IL-4, IL-5 and IFN-γ.

Taken together, our data on the humoral and cellular response demonstrate that in sensitized mice, we observed a predominantly Th2-type immune response, with increased food-specific IgE and IgG1 antibodies and concomitant production of IL-4 and IL-5. Similar antibodies and cytokine profiles have been found in human beings with food allergy. We could also show that gm-SE induced an immunological response comparable with that induced by wt-SE. Moreover, results obtained in our model by specific IgE ELISA inhibition and by antigen-specific T cell proliferation demonstrated that wt-SE and gm-SE shared B and T epitopes. Finally, the absence of humoral and cellular response against control proteins (irrelevant proteins) in the same assays confirms that our model is specific for the components of SE. In conclusion, considering that there is no single predictive bioassay available to assess the
allergenic potential of proteins in humans, the murine IgE sensitization model described provides valuable information regarding the allergenicity of modified soybean derived from biotechnology, and it could be a suitable system for the in vivo testing of genetically modified foods.

Conclusion

Agricultural biotechnology offers the promise to produce crops with improved agronomic characteristics and enhanced consumer benefits. Foods produced through agricultural biotechnology are already reaching the consumer marketplace, and there is a growing concern that introducing foreign genes into food plants may have an unexpected and negative impact on human health. As a result, the safety evaluation of transgenic food must be subject to a careful and complete safety assessment before commercialization. Of particular interest with genetically engineered organisms is the risk of allergic reactions. It is possible that the manipulations of genetic engineering may increase the potential risk of food allergy. Nevertheless, biotechnology may be able to modify foods to remove or change the proteins that cause allergy, offering the potential of making nutritious foods available to people who presently cannot eat them.

The safety evaluation of transgenic food is relatively easy when the allergenicity of the gene source is known. Nordlee et al. showed that an allergen (2S-albumin) from a food known to be allergenic (Brazil nut) can be transferred into another food (soybeans) by genetic engineering. On the contrary, the safety assessment approach could be a very complex problem when the expressed protein comes from a source that is not known to be allergenic, as in the case of Roundup Ready soybean, or when gene products are down-regulated for hypoallergenic purposes. Although strategies exist for such assessments, improvements should be considered to develop testing strategies to examine the allergenicity of genetically engineered food. In this context, animal models would be of considerable benefit if they facilitated a more direct evaluation of the ability of proteins to induce allergic responses in vivo.

Note: Animals were housed and treated according to the local guidelines for animal care (D.L. 116/92, which has implemented in Italy the requirements of the European Directive 86/609/EEC on laboratory animal welfare).

References


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