Regulatory Toxicology and Pharmacology 54 (2009) S2–S6

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Identifying food proteins with allergenic potential: Evolution of approaches to safety assessment and research to provide additional tools

Gregory S. Ladics [a,*], MaryJane K. Selgrade [b]

[a] DuPont Co., DuPont Crop Genetics, Wilmington, DE 19880 USA
[b] National Health and Environmental Effects Research Laboratory, Office of Research and Development, US EPA, Research Triangle Park, NC, USA

ARTICLE INFO

Article history:
Received 28 August 2008
Available online 8 November 2008

Keywords:
Protein allergenicity
Food safety assessment approaches
Transgenic proteins
Plant biotechnology
Research approaches

ABSTRACT

A safety assessment process exists for genetically engineered crops that includes the evaluation of the expressed protein for allergenic potential. The objectives of this evaluation are twofold: (1) to protect allergic consumers from exposure to known allergenic or cross-reactive proteins, and (2) protect the general population from risks associated with the introduction of genes encoding proteins that are likely to become food allergens. The first systematic approach to address these concerns was formulated by Metcalfe et al. [Metcalfe, D.D., Astwood, J.D., Townsend, R., Sampson, H.A., Taylor, S.L., and Fuchs, R.L. 1996. Assessment of the allergenic potential of foods from genetically engineered crop plants. Crit. Rev. Food Sci. Nutr. 36(5), 165–186.] and subsequently Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) [FAO/WHO, 2001. Evaluation of allergenicity of genetically modified foods. Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology. January 22–25, 2001. Rome, Italy]. More recently, Codex [Codex Alimentarius Commission, 2003. Alinorm 03/34: Joint FAO/WHO Food Standard Programme, Codex Alimentarius Commission, Twenty-Fifth Session, Rome, Italy, 30 June–5 July, 2003. 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. pp. 47–60], noting that no single factor is recognized as an identifier for protein allergenicity, suggested a weight of evidence approach be conducted that takes into account a variety of factors and approaches for an overall assessment of allergenic potential. These various recommendations are based on what is known about allergens, including the history of exposure and safety of the gene(s) source; amino acid sequence identity to human allergens; stability to pepsin digestion *in vitro*; protein abundance in the crop and processing effects; and when appropriate, specific IgE binding studies or skin-prick testing. Similarities and differences between these various suggested recommendations, as well as data gaps, are discussed. The US Environmental Protection Agency (EPA)'s Office of Research and Development (ORD) has initiated a targeted research effort to address data gaps and improve the various recommended methods/endpoints for assessing the allergenic risks associated with plant incorporated pesticides (PIPs) through both intramural and extramural (grant supported) research. The areas of primary focus for EPA include: (1) development and evaluation of animal models; (2) targeted or specific serological assays; and (3) structure–activity relationships. Details on the current as well as proposed EPA funded research are discussed. More recently US EPA has partnered with the National Institute of Allergy and Infectious Disease (NIAID), National Institutes of Health to support research in areas of mutual interest with respect to food allergy.

1. Introduction

The incorporation of new proteins into food crops using genetic engineering to promote resistance to pests and other stressors, improve nutrition, or otherwise modify the phenotype is an important, relatively new technology that has many advantages over more conventional approaches. Genetically engineered foods, however, have raised a number of concerns (see e.g., US GAO, 2002), including the possibility that introduction of a novel protein into the food supply could result in the unintentional introduction of a new or cross-reactive food allergen and could pose a risk to susceptible individuals. Importantly, conventional breeding tactics, such as chemical and radiation mutation, can also alter existing proteins. Over the last 10 years, approaches to identifying potential food allergens for purposes of safety assessment of genetically engineered crops have been developed and modified, and research

* Corresponding author. Fax: +1 302 366 5207.
E-mail address: gregory.s.ladics@usa.dupont.com (G.S. Ladics).

0273-2300/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.yrtph.2008.10.010

is underway to better understand what characteristics make a protein an allergen and provide additional predictive tools.

A food allergy is a reaction of the immune system to an otherwise harmless protein in food. Typically, such food allergic reactions are mediated by IgE and occur in atopic individuals who are genetically predisposed to allergy and who have been previously sensitized to the allergen (Sicherer, 2000). Upon initial exposure of an offending food (i.e., sensitization phase), antigen specific immunoglobulin E (IgE) is produced and binds to the surface of mast cells and basophils. Subsequent exposure of sensitized subjects to the offending food antigen produces an allergic response (i.e., the elicitation phase) by cross-linking antigen specific IgE on the surface of mast cells and basophils causing the release of various chemical mediators. These mediators in turn produce the clinical signs typically associated with food allergy (e.g., rhinitis, itching, hives, or gastrointestinal symptoms and occasionally bronchoconstriction and anaphylaxis). It should be noted that despite the large extent of food antigen exposure, only a small percentage of individuals (even among atopics) experience adverse immunologic reactions to food. The normal immune response to dietary proteins is associated with the induction of oral tolerance, a state of active inhibition of immune responses to an antigen by means of prior exposure to that antigen via the oral route. The mechanisms responsible for the development of oral tolerance are still the subject of research but involve the presentation of antigen via dendritic cells to T lymphocytes and the development of various types of regulatory T cells (Chehade and Mayer, 2005).

The incidence of food allergy ranges from 1% to 2% in adults and 6% to 8% in children (US GAO, 2002; Ladics et al., 2003). The most serious manifestation, severe anaphylaxis, occurs in approximately 3 individuals per 100,000/year (Burks and Sampson, 1997). Relatively few foods are responsible for the vast majority of significant food-induced allergic reactions. In general, the most common causal foods in children are cow's milk, egg, peanut, wheat, soy, tree nuts, fish, and shellfish. In adults the most common allergies are to shellfish, peanut, tree nuts, fish, and more recently sesame seeds. Reactions to fruits and vegetables are also common but usually not severe (Sicherer and Sampson, 2006). The prevalence of food allergy to specific foods can also vary geographically (e.g., increased buckwheat allergy in Asia; celery allergy in Europe) and is believed to be increasing worldwide (Metcalfe et al., 1996; Sampson, 1997). In the United States, this translates into approximately 6–7 million individuals with a clinically documented food allergy. Importantly, food, whether developed by conventional means or through biotechnology, is a potential source of allergens.

Before marketing genetically modified crops, such products are required to undergo an evaluation of the potential allergenic activity of the protein(s) that are produced from the introduced genes. The objectives of this evaluation are twofold: (1) protect allergic consumers from exposure to known allergenic or cross-reactive proteins that may trigger an adverse reaction in those already allergic to such proteins, and (2) protect atopic individuals from risks of allergic sensitization associated with the introduction of genes encoding proteins that are likely to become food allergens. Over the last decade, there have been three key documents published that have provided recommendations for evaluating the potential allergenicity of transgenic proteins. The first systematic approach to address the potential allergenic concerns with genetically modified crops was published by the International Food Biotechnology Council (IFBC), in collaboration with the Allergy and Immunology Institute (AII) of the International Life Sciences Institute (ILSI) in 1996 (Fig. 1; Metcalfe et al., 1996). The IFBC/ILSI report suggested the use of a decision tree approach and introduced the use of bioinformatics (i.e., $\geqslant$8 contiguous identical amino acids to identify 'theoretical' IgE epitopes) and pepsin resistance (Herman et al., 2006) for the assessment of potential allergenicity. Central to the IFBC/ILSI assessment was a consideration of the source of the transgene (i.e., allergenic or non-allergenic source). If the protein was derived from an allergenic source, an IgE binding study using sera from well-characterized patients allergic to the source was suggested, followed if necessary by additional clinical studies (e.g., skin-prick testing, food challenge studies). If from a non-allergenic source, an 8 or greater contiguous identical amino acid search and a pepsin resistance study were suggested. If a significant bioinformatics match occurred, an IgE binding study and additional clinical studies were recommended.

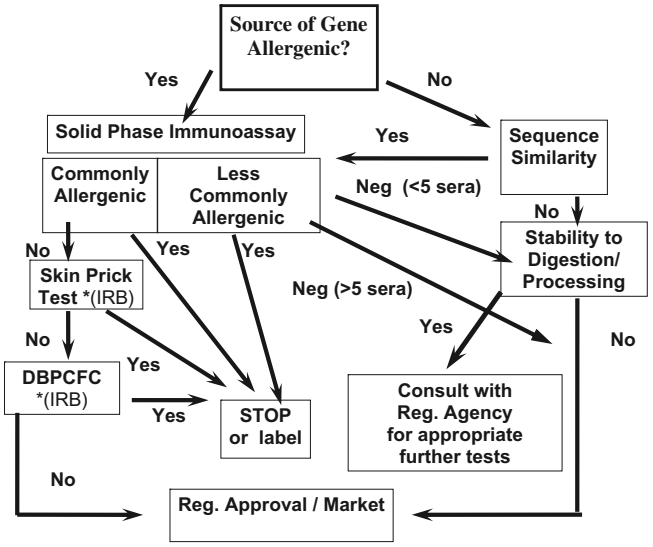

Fig. 1. 1996 International Food Biotechnology Council/the Allergy and Immunology Institute of the International Life Sciences Institute (IFBC/ILSI) Decision Tree (Metcalfe et al., 1996). Double blind placebo control food challenge (DBPCFC) studies; *institutional review board (IRB) approval required.

In 2001, the Joint Food and Agriculture Organization/World Health Organization of the United Nations (FAO/WHO) Consultation on Foods Derived from Biotechnology developed a new decision tree approach and included a number of additional recommendations (Fig. 2; FAO/WHO, 2001). Similar to the IFBC/ILSI approach, the FAO/WHO approach initially considered the source of the transgene (allergenic or non-allergenic) and subsequent bioinformatics and pepsin resistance analysis. Specific IgE binding studies using well-characterized sera from individuals allergic to

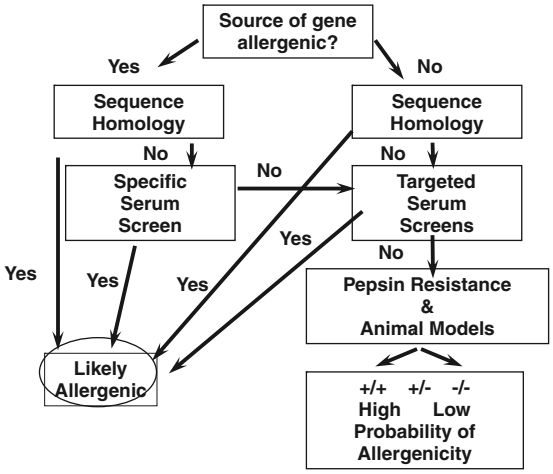

Fig. 2. The Joint Food and Agriculture Organization/World Health Organization of the United Nations (FAO/WHO) 2001 Decision Tree.