

GM food: The risk-assessment of immune hypersensitivity reactions covers more than allergenicity

Alexander G. Haslberger

Institute for Microbiology and Genetics, University of Vienna, Austria, c.o. Department of Food Safety, WHO, Geneva, Switzerland. email: alexander.haslberger@univie.ac.at

Received 25 September 2002, accepted 20 December 2002.

Abstract

Allergenicity of genetically modified food (GM food) has become a public concern and international expert panels e.g. WHO/FAO have depicted decision trees for a rigorous assessment and testing for GM foods, especially where no history of safe use is available. The way to use patient sera for the assessment of allergenicity is still under discussion in cases of proteins where stability and protein sequences may not be conclusive or for potential new allergies. The risk assessment of immune hypersensitivity reactions induced potentially by GM food needs also to consider effects on other type of immune responses e.g. activation of specific immune cell populations. The role of antigen presenting cells of the gut is now understood to direct immune responses resulting in humoral, cellular or IgE predominant characteristics. For GM microorganisms potential effects on the immune system need to be assessed.

Key words: GM food, allergenicity, hypersensitivity, immune cells.

Introduction

No specific international regulatory systems for GM foods safety or GMO environmental safety are currently in place. In the field of environmental safety the Cartagena Protocol on Biosafety may enter into force soon and Codex Alimentarius principles on human health risk analysis are expected to be adopted in 2003. The premise of these Codex principles dictates a premarket assessment, performed on a case-by-case basis and including an evaluation of both direct effects (from the inserted gene) and unintended effects (that may arise as a consequence of insertion of the new gene). The safety assessment of GM foods investigates:

- (a) direct health effects (toxicity)
- (b) tendency to provoke allergic reactions (allergenicity)
- (c) specific components thought to have nutritional or toxic properties
- (d) the stability of the inserted gene
- (e) nutritional effects associated with the specific genetic modification
- (f) any unintended effects which could result from the gene insertion¹.

When new foods are developed by natural methods, some of the existing characteristics of foods can be altered unintentionally, either in a positive or a negative way. New plants developed through traditional breeding techniques may not be evaluated rigorously using risk assessment techniques². In contrast to traditionally developed foods which are not generally tested for allergenicity before market introduction, protocols for testing detrimental immune responses, especially the allergenic potential of GM foods have been established by international expert panels².

The Role of the Gut Immune System and Hypersensitivity Responses

Food allergies and other food sensitivities are individualistic adverse reactions to foods because they affect only a few people in the population. Within the different types of reactions involved in adverse reactions to foods non-immunological intolerances (such as reactions to increased contents of histamins or intolerances against lactose) and reactions involving components of the immune system need to be differentiated³. In hypersensitivity reactions involving elements of the immune-system it became evident that these reactions are mainly caused by a lack of the induction of a tolerance against components of the foods in specific individuals. While research has delivered a very good understanding for the structural specificities of the protein food components which are often the cause for allergenic reactions, basic mechanisms underlying the reactions are at the focus of present research: Genetic and environmental factors are believed to influence antigen presenting cells, especially dendritic cells and T cell subsets which, using different sets of immune mediators, differentially regulate both, the synthesis of Immunoglobulin E which is the basis for humoral, immediate (or real, Type I) allergic hypersensitivity reactions and cellular reactions involving sensitized or self reactive T cells (delayed type, hypersensitivity reactions). Antigen presenting cells belong to the gut associated lymphoid tissue (GALT). Immature dendritic cells reside in the epithelia also of the gut and have the potential to sense foreign antigens. Following recognition and uptake of Ag, mature dendritic cells provide signals which polarize Th0 cells into Th1 or Th2 cells, the basis for humoral or cellular immune-responses as well as decisions for the production of enhanced IgE⁴. Systemic immune responses to soluble oral antigens are most likely induced by gut-conditioned dendritic cells that function both to initiate the gut-oriented response and to impart the characteristic features that discriminate it from responses induced parenterally⁵. Also the differential stimulation of cytokines effecting immune responses and

activation of the immune system was shown in intestinal epithelial immune cells using non-pathogenic *E. coli* and *Lactobacilli*⁶. Specific microbes in the gut microflora and sporadic infections are so thought to be important in allergy prevention. The gastrointestinal microflora promotes potentially anti-allergenic processes such as TH1-type immunity, suppression of TH2-induced allergic inflammation, induction of oral tolerance and IgA production. The gut microflora might therefore be a major postnatal counter-regulator of the universal TH2-skewed immune system in fetuses and neonates⁷. Because of its role to serve as a barrier to pathogenic bacteria and to enable an immune surveillance of the antigenic environment the local mucosal immunity of the gut is of a central importance for health. Antigens, primarily associated with intestinal microbes and dietary antigens, can stimulate production of IgA in the intestine resulting in local protective immunity. Because of its role for a stimulation and regulation of immune responses the gut has become a favourite system for developments and techniques to interfere with modified or functional foods or vaccines including DNA vaccines^{8,9,10}.

GM Food and Hypersensitivity

Molecular biology and biochemistry have significantly increased the knowledge of the nature of allergens. However, only limited information about specific properties of food allergens is presently available. The majority of known plant food allergens belong to seed storage proteins, protease and amylase-inhibitors, profilins or pathogenesis-related (PR) proteins. Less variety is found among allergenic proteins derived from animal sources. For allergic reactions it became clear that plant food allergens belong almost exclusively to one of two structurally related protein superfamilies, which share remarkable stability to processes such as heating (being stable to temperatures between 75-95°C, compared with 45-50°C for most proteins), and the extremes of pH and the proteolytic processing environment found in the digestive tract. These proteins mainly come from foods or food groups often referred to as "The Big Eight" which account for more than 90-percent of all Type I allergic reactions worldwide. These Big Eight are; milk, eggs, fish, crustacean shellfish, peanuts, soybeans, tree nuts and wheat¹¹. In response to pathogens, plants synthesize and accumulate a variety of proteins which are part of a plants defence system. As plant protection against bacteria, fungi, viruses and insects is a major challenge to agriculture world-wide, over-expression of such proteins in transgenic plants has been applied to increase the defense potential. Some of these proteins which are considered for use in the production of GMOs to increase the resistance to microbial and insectal attack include proteins with allergenic potential e.g. chitinases providing protection against fungal attack or insecticidal proteins including protease inhibitors¹².

Risk Assessment of Allergenicity

An assessment of the potential allergenicity of GM foods typically follows the generally well known decision-tree process which depends from the source of the genes transferred as outlined by international expert panels^{3, 13, 14, 15}. The most difficult assessment occurs when genes are obtained from sources with no history of allergenicity, such as viruses, bacteria or non food plants. The likelihood that the proteins derived from such sources of DNA will be allergens is not very high,

since most proteins in nature are not allergens. The key features of the allergenicity assessment for such foods than again involves a comparison of the amino acid sequence of the introduced protein with the amino acid sequences of known allergens and the digestive stability of the introduced protein. While the combination of these two criteria provides reasonable assurance that the introduced protein has limited allergenic potential, the ideal approaches to the application of these two criteria have been debated, and the desirability of adding other criteria for the allergenicity assessment of such products and additional testing has been advocated¹⁴. The development of additional criteria and additional tests to use in the assessment of the allergenicity of GM foods would be advantageous in cases where the gene is obtained from sources with no history of allergenicity. The level of expression of the introduced protein and the functional category of the introduced protein could be used as additional criteria. In addition, the development of suitable animal models for the prediction of the allergenic potential of the introduced proteins is anticipated in the future. While several animal models appear to be promising, none has been sufficiently validated for its routine use in the assessment of the allergenicity of GM foods. It must also be realised that the absence of sequence similarity with allergenic protein-epitopes and a missing stability against digestion does not necessarily prove for a missing allergenicity as examples are known which contradict to the general rules: Highly homologous sequences with allergens in case of allergen-isoforms have been shown without any allergenicity. Furthermore, proteins with a low stability have been shown to exhibit a significant potency to induce allergenicity or to sensitize for allergic reactions¹⁷⁻¹⁹. The use of patients sera for the testing of allergenicity is therefore recommended²⁰. There is also some discussion if the generally agreed system is sensitive enough to detect upcoming new allergies in time. It is likely that the first manifestations of a new allergy will occur in pre-existing adult allergic individuals and could occur as a consequence of cross-reactivity. A screening programme may be desirable to predict such cross-reactivities by employing patients sera, however, the number of sera that would need to be screened may need to be much larger than that hitherto recommended in international documents²¹.

Risk Assessment of Cell Mediated Reactions and Microbial Impact on the Immune System

Although the well characterised interactions which lead to allergic immediate hypersensitivities may comprise the fare most important food derived hypersensitivity problems, the role of other type of responses, and their relevance for food hypersensitivity in general and for the safety assessment for foods from GM organisms specifically remain more unclear. Adverse food reactions are discussed and also need to be taken in mind in the assessment of GM-foods²². Such reactions could comprise delayed type hypersensitivity reactions which have been characterised to develop slowly, reaching a peak at approximately 48 hours and then slowly subsiding over 72-96 hours. They are known to involve cell mediated responses without important IgE involvement. Also reactions to cow milk, soy proteins, eggs etc known in infants and children, Celiac disease and Crohns disease show missing tolerance and mislead activation of T cells²³. Potential immune-stimulatory or immune-modulatory effects of GM microorganisms (GMMs) used as or in foods are a specific area of a risk assessment

which evaluates immune responses to GM organisms. GMMs may establish themselves within the GI tract and exert influences on the immune system via interactions with the gut immune system. Even non-viable microorganisms are known to retain functional properties (i.e. cell adhesion, binding of chemicals, immunomodulating activities), which can have direct or indirect effects on both microflora- and host associated functions²⁴. Gut-associated lymphoid tissue (GALT) has important interactions with the immune system and it is well established that microbial stimuli are the main antigenic forces in the development and maintenance of GALT and acquired immunity²⁴. Stimulation of antigen presenting, dendritic cells influencing immune type responses was shown for bacterial cell walls before²⁵ Potential safety relevant consequences from rare, but possible uptake of recombinant DNA from GM food by cells of the immune system remain to be investigated²⁶⁻²⁸.

Conclusions

In general, it seems that the present discussion on GM food safety, especially in the field of hypersensitivity reactions does not so much point towards a significantly increased safety problem of GM foods compared with conventional foods, but it reflects increased regulatory demands as well as perceived needs for a higher safety level. Conventional foods often have not been subject of hypersensitivity assessment. Public awareness as well as genuine scientific considerations in the field of GM foods has resulted in general guidelines being elaborated for allergenicity assessment of such foods. These internationally agreed guidelines are specifically important for foods which are traded globally. Standards established for the assessment of GM foods may then turn out to be a paragon for the testing of conventional foods. The detailed analysis of immune mechanisms involved in the stimulation of different type of immune responses has revealed complex ways and some of these ways are still poorly understood, such as pathways resulting in cell mediated hypersensitivity reactions to food. An improved investigation of activation pathways including antigen presenting- and T cells will not only contribute to a better understanding of these reactions but may also result in improved testing methods for allergenicity, where the possibilities for testing, especially of whole foods, in animal models are still limited.

References

- 1 Codex, 2001. Consideration of proposed draft general principles for the risk analysis of foods derived from modern methods of biotechnology. http://www.who.int/fsf/GMfood/bt01_05e.pdf
- 2 WHO, 2002. 20 Questions on GM Foods. <http://www.who.int/fsf/GMfood/q&a.pdf>
- 3 IFT. 2001. Food Allergies and other Food sensitivities, expert panel on food safety and nutrition: Institute of food technologists. <http://www.ift.org/publications/sss/allergens.pdf>
- 4 Lambrecht B.N. 2001. Allergen uptake and presentation by dendritic cells : *Curr Opin Allergy Clin Immunol* **1**:51-59
- 5 Alpan O., Rudomen G., Matzinger P. 2001. The role of dendritic cells, B cells, and M cells in gut-oriented immune responses. *J. Immunol* **166**:4843-4852
- 6 Haller D., Bode C., Hammes W.P., Pfeifer A.M., Schiffrin E.J., Blum S. 2000. Non-pathogenic bacteria elicit a difcytokine response by intestinal epithelial cell/leucocyte co-cultures. *Gut* **47**:79-87
- 7 Kalliomaki M., Salminen S., Arvilommi H., Kero P., Koskinen P., Isolauri E. 2001. Probiotics in primary prevention of atopic disease: a randomised placebo- controlled trial. *Lancet* **357**:1076-1079
- 8 Johnson I.T., 2001. New food components and gastrointestinal health. *Proc Nutr Soc.* **6**:481-488. Review.
- 9 Bouvet JP., Decroix N., Pamoninlapham P. 2002. Stimulation of local antibody production: parental or mucosal vaccination? *Trends Immunol.* **23**:209-213. Review.
- 10 Howard K.A., Alpar H.O. 2002. The development of polyplex-based DNA vaccines. *J. Drug Target.* **10**:143-151
- 11 Institute of food research, 2002. Food allergy. <http://www.ifr.bbsrc.ac.uk/Diet/Immunology.html>
- 12 Bindslev-Jensen C., Ebner C., Madsen C., Mäkinen-Kiljunen S., Peltre G., Poulsen L.K., van Ree R., Viets S. 2000. Genetically modified foods and allergenicity., preliminary Position Paper. European Academy for Allergology and Clinical Immunology, <http://www.ig-food.org/seiten/position2k.htm>
- 13 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 food derived from genetically engineered crop plants. *Crit. Rev. Food Sci. Nutr.* **36**: 165-186
- 14 WHO/FAO 2000. Second Joint WHO/FAO Expert Consultation on Foods Derived from Biotechnology, Safety aspects of genetically modified foods of plant origin <http://www.who.int/fsf/GMfood/FAO-2000.pdf>
- 15 WHO/FAO 2001. Expert Consultation on Allergenicity of Foods Derived from Biotechnology, http://www.who.int/fsf/Documents/Biotech_Consult_Jan2001/report20
- 16 IFT 2000. Expert Report on Biotechnology and Foods: Human Food safety Evaluation of rDNA Biotechnology- derived Foods, *Food technology* **54**: 15-23 http://www.ift.org/publications/docs/hop/ft_shop/09-00/09_00_pdfs/09-00-bio-safety.pdf
- 17 Ferreira F., Hirtenlehner K., Jilek A., Godnik-Cvar J., Breiteneder H., Grimm R., Hoffmann-Sommergruber K., Scheiner O., Kraft D., Breitenbach M., Rheinerger H. J., Ebner C. 1996. Dissection of immunoglobulin E and T lymphocyte reactivity of isoforms of the major birch pollen allergen Bet v 1: potential use of hypoallergenic isoforms for immunotherapy. *J. Exp. Med.* **183**: 599-609.
- 18 Heiss S., Fischer S., Muller W D., Weber B., Hirschwehr R., Spitzauer S., Kraft D., Valenta R. 1996. Identification of a 60d cross-reactive allergen in pollen and plant-derived food. *J. Allergy Clin. Immunol.* **98**:938-947.
- 19 Vrtala S., Fischer S., Grote M., Vangelista L., Pastore A., Sperr W R., Valent P., Reichelt R., Kraft D., Valenta R. 1999. Molecular, immunological, and structural characterization of Phl p 6, a major allergen and P-particle-associated protein from Timothy grass (*Phleum pratense*) pollen. *J. Immunol.* **163**:5489- 5496.
- 20 Valenta R. 2002. p. comm.
- 21 Warner J. 2002. p. comm.
- 22 Baldwin J.L. 1997. Pharmacologic food reactions. In: Metcalfe DD, Sampson HA, Simon RA. *Food Allergy: Adverse reactions to foods and food additives* 2nd ed. Blackwell science, p. 419-429.
- 23 Janeway C.A. 2001. *Immunobiology*, New York : Garland; Edinburgh : Churchill Livingstone, Edition 5th ed., Biology Publications. <http://www.bmb.leeds.ac.uk/illingworth/icu3/lecture/09/>
- 24 WHO/FAO expert consultation, 2001. Safety assessment of foods derived from genetically modified microorganisms. Report of a Joint WHO/FAO expert consultation. http://www.who.int/fsf/Documents/GMMConsult_Final_.pdf
- 25 Haslberger A.G., Kohl G., Felnerova D., Mayr U.B., Fuerst- Ladani S., Lubitz W. 2000. Activation, stimulation and uptake of bacterial ghosts in antigen presenting cells. *J. Biotechnol.* **83**: 57-66
- 26 Schubert, R., Renz, D., Schmidt, B. and Dorfler, W. 1997. Foreign (M13) DNA ingested by mice reaches peripheral leukocytes, spleen and liver via the intestinal wall mucosa and can be

covalently linked to mouse DNA. Proceedings of the National Academy of Sciences, USA **94**: 961–966.

²⁷ Hohlweg, U. and Doerfler, W. 2001. On the fate of plant or other foreign genes upon the uptake of food or after intramuscular injection in mice. *Molecular Genetics and Genomics* **265**: 225–233.

²⁸ Einspanier, R., Klotz, A., Kraft, J., Aulrich, K., Poser, R., Schwägele, F., Jahreis, G., Flachowsky, G. 2001. The fate of forage plant DNA in farm animals: a collaborative case-study investigating cattle and chicken fed recombinant plant material. *European Food Research and Technology* **212**: 129–134.