

Allergie respiratoire et santé publique Évaluation des risques

Biotechnology and allergic risk

J.M. Wal*

Laboratoire INRA-CEA d'immunoallergie alimentaire, DRM-SPI, CEA de Saclay, 91191 Gif-sur-Yvette, France

Summary

The development of biotechnology and particularly of genetic engineering raises the question of the safety of the genetically modified organisms (GMOs) and the evaluation of any risks that they could pose to Public Health. The allergic risk is considered as a special issue which raises many concerns among consumers and regulatory committees. Indeed biotechnology is a possible way of creating new allergens or of unmasking hidden and therefore non bioavailable immunoreactive structures and of increasing the expression/potential of existing ones. If the inserted DNA is well characterized, one can expect that only the trait protein (and possibly the marker gene product) will be expressed and will have to be assessed for allergenicity. However, the insertion of the transgene within the plant genome is not controlled and indirect pleiotropic effect may over express one or several endogenous allergenic protein(s) or express it (them) in a modified form. Therefore there is a pressing need to assess the allergenicity of the product(s) of the introduced gene(s) but also of the whole transgenic plant or of the foods derived therefrom. If the introduced gene encodes a known allergen, the recombinant protein may be tested for allergenicity by the classical in vitro tests using sera from allergic patients. If the source of the transgene has no history of allergenicity, alternative approaches are required based on the structure and stability of the foreign protein. However they must be critically appraised and applied on a case-by-case basis. No validated methodologies exist for evaluating or predicting the allergic risk generated by indirect and unintended effects of the genetic modification. Chemical and genetic (mRNA) fingerprinting, proteomics to detect differences in total protein expression of the transgenic plant and its conventional counterpart offer a possible approach. In addition to pre-market testing, post market surveillance could bring useful informations on the occurrence of unintended effects and particularly on new allergies after exposure to GMOs in the every day life conditions.

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allergy / biotechnology / risk assessment / genetically modified organism

Résumé – Biotechnologie et risque allergique.

Le développement des biotechnologies et notamment du génie génétique nécessite la prise en compte des risques que l'exposition aux organismes génétiquement modifiés (OGM) peut faire courir à la santé publique et en particulier du risque allergique. En effet les procédés biotechnologiques peuvent faire apparaître de nouveaux allergènes ou rendre biodisponibles des structures immunoréactives jusque là cachées et non accessibles aux anticorps. Ils peuvent également augmenter le niveau d'expression et/ou d'exposition à des allergènes existant, voire modifier leur potentiel allergisant. Si la modification génétique est bien caractérisée, on peut penser que seuls la protéine d'intérêt et le produit d'un éventuel gène marqueur seront exprimés et devront être évalués pour leur allergénicité. Cependant, l'insertion du transgène dans le génome d'une plante n'est pas contrôlée et peut donner lieu par effet pléiotropique, c'est-à-dire par interaction avec le fonctionnement d'autres gènes, à la

* Correspondence and reprints.

surexpression d'allergènes endogènes de la plante, sous leur forme naturelle ou sous une forme modifiée. C'est donc l'allergénicité des protéines étrangères exprimées mais également de l'OGM dans son entier, ou des produits qui en sont dérivés, qu'il faut évaluer. Si le transgène code un allergène ou est issu d'un organisme connu pour son allergénicité, la protéine recombinante produite pourra être évaluée pour son allergénicité à l'aide des tests in vitro classiques utilisant des sérums de patients allergiques. Si le transgène provient d'une source dont l'allergénicité n'a pas été établie et que l'on ne dispose donc pas de patients allergiques, des méthodes alternatives fondées essentiellement sur la structure et la stabilité des protéines exprimées sont proposées. Elles doivent cependant être mises en œuvre au cas par cas et de manière critique. Il n'y a pas de méthode reconnue pour évaluer les effets indirects, non recherchés et imprévisibles, et en particulier un éventuel risque allergique, liés à la modification génétique. Des comparaisons de composition entre l'OGM et l'organisme conventionnel correspondant fondées sur des empreintes chimiques ou génétiques ainsi que l'analyse du protéome ont été proposées. En complément de l'évaluation a priori, une surveillance après la mise sur le marché peut apporter des informations sur la survenue d'effets indésirables imprévus, et notamment de manifestations allergiques nouvelles ou augmentées après exposition à l'OGM, ou aux produits qui en sont dérivés, dans les conditions de la vie courante. © 2001 Éditions scientifiques et médicales Elsevier SAS

allergie biotechnologie / organismes génétiquement modifiés

There have been rapid developments in plant biotechnology but also in microorganism and animal genetic engineering within the last ten years. Genetically modified organisms (GMOs) have been used as bio-reactors to prepare biological products such as drugs, vaccines, enzymes, flavours or foods. The genetic modification allows to produce a heterologous protein in the modified organism or to optimize the conditions of preparation or of activity of the product. Biotechnology has also produced animals with increased zootechnic performances and plants with new agronomic and technologic properties or with altered composition and nutritional value. However until now the rate of introduction of new GMOs is low. GM foods/feeds mainly concern four major crops, e.g. maize, soybean, canola and cotton in which the introduced gene confers herbicide tolerance or/and insect resistance. In addition, products of marker genes of antibiotic resistance may be expressed.

The development of new technologies for the production and processing of foods raises the question of the safety of these foods and the evaluation of any risks, including the allergic risk, that their consumption could pose to Public Health [17]. Indeed once a protein is ingested there is a potential risk of allergy but one can wonder why the allergenic risk of transgenic foods is thus considered as a special issue which raises so many concerns among consumers

and regulatory committees. Insertion of a new gene into a plant could result in expression of new allergen(s) or increased expression of existing allergen(s). If the inserted DNA is well characterized, one can expect that only the trait protein (and possibly the marker gene product) will be expressed and will have to be assessed for allergenicity. The question is then: is there any intrinsic characteristics in recombinant protein that make them different of conventional ones and could account for a special immunoreactivity or confer a particular allergenicity?

Moreover, the location where the transgene is inserted within the plant genome is not controlled, and the indirect pleiotropic effect by which a single genetic modification may interfere with the expression of other genes and affect numerous uncontrolled and nonrelated phenotypic characteristics is a specific issue that needs to be addressed. It might for example over express one or several endogenous allergenic protein(s) or express it (them) in a modified form. An other issue is then whether the whole genetically modified plant itself (or a food derived therefrom) can be considered a novel hazard and particularly could present a novel allergic risk which would be inherent to the process.

Therefore there is a pressing need to consider biotechnology as a possible way of creating new allergens or of unmasking hidden and therefore non

bioavailable immunoreactive structures and of increasing the expression/potential of existing ones.

Procedures/guidelines for testing allergenicity of food proteins been proposed for assessment of genetically modified plant products [13]. They have been mostly endorsed by scientific/regulatory committees including FAO-WHO [6, 7] and are applied in the applications submitted by manufacturers for marketing authorizations. A critical appraisal of these strategies first raises the preliminary question whether a recombinant protein can be considered as any conventional protein for assessment of its allergenicity or if it is intrinsically different and then, whether there are any structural, functional or chemical characteristics which may allow to definitely assess or predict the allergenic risk of any (food) protein.

ASSESSMENT OF ALLERGENICITY OF THE PRODUCTS OF THE INTRODUCED GENE(S)

The introduced gene encodes a known allergen

If in a GMO, the introduced gene encodes a known allergen, it is very likely that the transgenic organism will express the protein with its allergenic potential. This was the case when Brazil nut 2S storage albumin was engineered into soybean by Pioneer Hi-Bred in order to increase levels of sulfur-containing amino acids so as to improve the biologic value of soybean meal proteins for animal feeding. Unfortunately, this protein is a major allergen of Brazil nut which was recognized in the same way by allergic patients whether expressed in transgenic soybean or when naturally present in Brazil nut [14]. This protein molecule which is the (or one of the) component(s) responsible for allergenicity of Brazil nut was expressed in the GM soybean with all its chemical characteristics and biological properties and thus transferred to GM soybean the nutritional value but also the allergenicity of Brazil nut. Recombinant bovine β -lactoglobulin (BLG) expressed in *E. coli* also has the same immunologic and allergenic characteristics as the corresponding BLG secreted in milk [4]. Therefore a recombinant protein may be tested for allergenicity the same way as the equivalent natural protein. The classical standard in vitro immunochemical testing using sera from allergic

patients are a useful and relevant tool to assess the remaining allergenic potential of a GM food derived from an allergenic source. However, they cannot be used on a large scale since serum banks built up from patients with known allergy are limited. In vivo tests on allergic patients, including skin prick tests and oral provocation tests have also been proposed [13] but they are subject to ethical concerns.

The source of the transgene has no history of allergenicity

Such assessments cannot be performed if the source of the transgene is an organism without history of allergenicity or a limited history that precludes the availability of sera. No or very few cases of allergy have been reported for transgenic foods as yet. This is probably due to the fact that most of the expressed proteins are bacterial, having no or low glycosylation levels, which could make them poorly allergenic.

Therefore, complementary and/or alternative approaches are required. The most commonly used are based on sequences homologies of the foreign protein with known allergens and on the stability of the foreign protein to heat and acidic treatments and to hydrolysis using digestive proteolytic enzymes. However they must be critically appraised and applied on a case-by-case basis [18].

The first critical point is the strict chemical identity of the foreign protein which is expressed in the genetically modified plant and of the test protein which is used for allergy assessment experiments. Indeed, the foreign protein is generally expressed at a very low level in transgenic plants, and the amount of material which is necessary to perform the experiments precludes extraction of the test substance from the plant which would anyway denature the protein. Large quantities of the protein are thus produced in genetically modified microorganisms in which the same transgene, encoding the protein, is introduced. However, slight structural changes may occur, depending on the producing organism. These can affect the primary sequence of the protein due to point mutations on a few amino-acid residues or can be due to post-translational modifications; i.e., variations in the glycosylation or phosphorylation levels. Such modifications may have an effect on the immunoreactivity of the protein [2].

Assessment of the allergenicity of a novel protein based on its structure and function

The amino-acid sequence of the recombinant protein is known or deduced from the genetic construct used. Although no relationship between the primary structure of a protein and allergenicity has been established, it has been proposed that a sequence homology of at least eight contiguous identical or chemically similar amino-acid residues between the test protein and a known allergen would be a presumption of allergenicity [13]. Data bases giving the amino-acid sequence of some allergens and computer programs for comparisons of sequences are available on the Internet.

This method may provide an indication of the allergenic potential of the recombinant protein but the absence of such homology cannot be considered as a presumption of non-allergenicity as it is often concluded. Indeed, it is limited by the small number of allergens that are listed as such in data bases and by the paucity of information currently available on specific epitopes of most allergens. Moreover, these criteria are very restrictive and are limited to linear epitopes. It is worth noting that in the spatial configuration of the protein, short homologous sequences comprising fewer than eight identical amino-acid residues can be brought close together by the folding of the molecule and thus participate in the formation of conformational epitopes which could mediate allergenic reactivity.

Some protein families are known to include allergens, such as enzymes, storage proteins, pathogenesis-related proteins (stress proteins), calcium-binding proteins, lipid transfer proteins, lipocalins, profilins, etc. Some of them are widespread, highly conserved proteins with a structure that has remained quite stable during evolution. Therefore the presence of homologous sequences may be the sign of such a conservation as well as an indicator of allergenicity.

Sequences homologies thus appear to be not sufficient for a consistent assessment of (non) allergenicity and should be completed by structure analysis when such informations are available. Indeed three-dimensional structure analysis reveals data on the structure-activity relationship of some protein families known to include allergens. Lipocalins and calycins which bind hydrophobic ligands, are char-

acterized by a highly conserved common tertiary structure, called " β barrel", comprising arrangements of eight or ten antiparallel β sheets [8]. Lipid transfer proteins are basic proteins of low molecular weight (7–9 kDa), stabilised by four intramolecular disulphide bonds; the 3D structure reveals the presence of a large hydrophobic tunnel which forms the lipid binding site [5].

As far as epitopes are concerned, analysis of crystallographic structures, when available, computer analysis, and molecular structure modeling can be used to predict which are the most likely immunoreactive regions within a protein molecule. Theoretic prediction of protein antigenic determinants has also been based on amino-acid sequences, assuming that antigenic sites are surface features of the proteins and should be related to regions of high hydrophilicity along the polypeptide chain [10]. However, in food allergens, such as milk allergens, IgE often appear to be specific of destructured and denatured parts of the protein and IgE binding epitopes have been located in hydrophobic parts of the molecule [12].

Assessment of the allergic potential of a novel protein based on its physicochemical characteristics

This approach is based on the hypothesis that allergenicity is mainly due to the whole intact protein. Thus, for sufficient amounts of the protein to reach the gut, be absorbed through the intestinal mucosa, and then be presented to the immunopotent cells, a prerequisite for food protein allergenicity would be stability in acidic gastric conditions and resistance to digestion by the proteolytic enzymes present in the gut. An *in vitro* test has been proposed to simulate gastric and intestinal digestion [1]; however, the kinetics of decrease of pH and of release of enzymes are difficult to reproduce, as they occur in physiologic conditions. Moreover, performed on the isolated protein, it does not take into account the incidence of the food matrix in which the allergen is present. The stability of proteins under conditions encountered during industrial processing, particularly heat denaturation, is also considered to be an important property of food allergens.

It is worth recalling that the sensitizing food is first degraded by digestive enzymes, it may be altered during absorption by the gut mucosa, and the antigenic/allergenic protein components are processed in antigen presenting cells before reaching the immune system. The allergic reaction may thus be induced and/or triggered not only by the native form of a food protein but also by fragments. Indeed denatured molecules and/or short peptide fragments may be potent allergens as well as the native protein [12, 15]. Digestion of a food protein may even increase allergenicity by unmasking new epitopes which were hidden in the native spatial conformation of the molecule. They were therefore inaccessible to IgE and they became bioavailable after degradation of the protein during digestive process [9, 16].

ASSESSMENT OF ALLERGENICITY OF THE WHOLE GM PLANT/FOOD

Another consideration which relates to the host organism rather than to the expressed foreign protein(s), is rarely dealt with in dossiers for the marketing authorization of GMOs. The question is whether the allergenicity of the whole GM plant or of a food product derived therefrom may be increased or modified as compared with the conventional one.

The transgene, once randomly introduced in the plant genome, may provoke pleiotropic effects on the host and deregulate the expression of other genes that are not related with the intended modification. It may particularly interfere with those genes encoding endogenous allergens, thereby increasing the amount or modifying the composition of endogenous allergens present in transgenic organisms as compared with those naturally present in classical commercial strains [3].

This aspect appears to be a real issue in terms of allergenicity. It is specific to every new product and to each new genetic modification event. Therefore it should be addressed in all applications.

No recognized and validated models or methodologies exist for evaluating or predicting the allergic risk generated by indirect and unintended effects of the genetic modification. Case-by-case analysis is needed and *in vivo* and *in vitro* tests have been proposed. Chemical and genetic (mRNA) fingerprinting, proteomics to detect differences in total protein

expression of the transgenic plant and its conventional counterpart offer a possible approach [11]. At present, these methods are difficult to put into practice, the relevance of the differences they reveal is uncertain, and their interpretation in terms of biologic significance is entirely haphazard.

In addition to pre-market testing, post market surveillance could bring useful informations on the occurrence of such unintended effects and particularly on new allergies after exposure to GMOs in the every day life conditions. However such an epidemiologic survey is difficult to organize and the hope to get reliable and unambiguous conclusions in terms of statistically significant impact of the exposure to a GMO product on disease processes or outcomes is low because of the multiplicity of factors affecting the burden of disease in the general population. It is therefore generally much criticised.

However allergic disease does not affect the general population but only an at-risk group of atopic individuals. These people are particularly aware of the different factors that may interfere with their health, they are used to read labels and keep themselves well informed about new ingredients, new processes, modifications in composition of newly commercialized foods, regulations,... just because it may be life-threatening for them. Moreover they are organized in Associations that can be reliable and efficient relays to pass and to get back the informations and will certainly be very keen to carefully scrutinize any possible impact of GM foods on occurrence of allergic reactions as they already do with conventional foods and to participate in such a kind of post-market surveillance even if no fully achieved epidemiological studies can be set up.

Our limited experience with GM foods, owing to a lack of long-term use, prevents us from having any clear-cut and well-defined ideas about what novel or increased risks of allergy can be linked to their consumption. In particular, no method is available to identify, characterize, and assess any unforeseen and unintended adverse effects caused by the insertion of a transgene which could result in emergence of new allergies. This safety assessment is even more difficult when complex constructs are concerned, or for GMOs of second generation where several traits are simultaneously expressed in the same organism,

which can result in interactions between different transgenes and between various proteins.

The predicted introduction into the market place of transgenic plants of altered nutritional characteristics will also complicate the evaluation, as foreign proteins will then be expressed in substantial amounts. The ill-fated experience of Pioneer Hi-Bred GM soybean shows that care is required.

CONCLUSIONS

No evidence has yet suggested that transgenic foods, and particularly recombinant proteins expressed by genetically modified organisms, would be more (or less) allergenic than the corresponding conventional foods. However care should be taken in interpreting and applying the results of the different available predictive procedures. These procedures should be subject to case-by-case critical appraisal. The predictive approach to determining the allergenic potential of novel foods should be allied to mandatory implementation of monitoring of the potential postmarketing impact of these new foodstuffs on public health. Such a postmarket surveillance can only be achieved through the development and organization of procedures ensuring the traceability of novel food products as part of a quality assurance policy applicable to all agribusiness sectors. Epidemiologic survey of any increasing prevalence of food allergy or emergence of new allergies in relation with the intake and exposure of 'novel' allergens derived from GMOs should mobilize all agribusiness sectors, physicians, scientific and regulatory committees in charge of public health as well as at risk groups of consumers and associations of allergic patients.

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