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Topic 6: Solid Phase Immunoassay, Immunoreactivity and Other Criteria

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A logical approach to investigate possible allergenicity of a food is by starting out with in vitro investigations based on binding of specific IgE to a solid phase. A negative outcome in these assays prompts further in vitro invrestigation e.g. using immunoblotting techniques folowed by in vivo testing of suitable patients by Skin Prick Testing. Negative outcome here prompts Double-Blind, Placebo-Controlled Food Challenges (DBPCFC) constituting the ultimate diagnostic tools for establishing or ruling out a food allergy.

The different methods for in vitro and in vivo testing in this approach is summarised from page 3-10, followed by Task Force's suggestion for Risk Assessment of Genetically modified foods (page 10-13), taken from the European Academy of Allergy and Clinical Immunology Task Force on "Genetically modified foods and allergenicity, which will be published later this year.

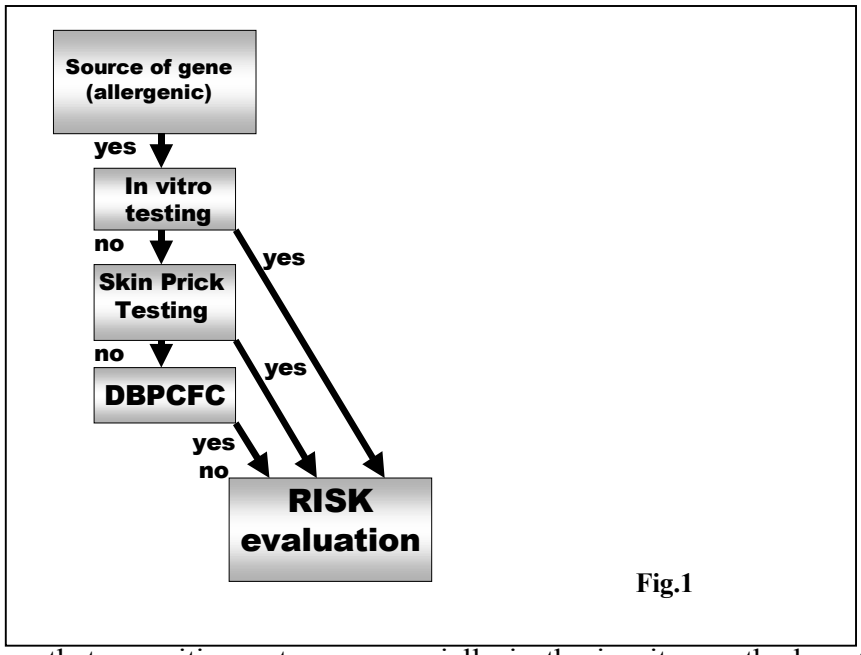

Fig.1

It is important to emphasize, that a positive outcome especially in the in vitro methods not necessarily reflects clinical significance, and this fact is also true in some cases of skin Prick Testing, whereas the Gold Standard, DBPCFC is considered having the highest level of significance. On the other hand, false negative outcomes in the in vitro or in vivo systems may be even more serious to the patients/consumers (1-3). Standardization of the procedures is therefore of central importance.

References:

1 Bruijnzeel Koomen, C.A., Ortolani, C., Aas, K., Bindslev-Jensen, C., Björksten, B., Moneret-Vautrin, D., & Wüthrich, B. (1995). EAACI Position paper: Adverse reactions to food. Allergy, 50, 623-635.

2 Bindslev-Jensen, C., & Poulsen, L.K. (1997). In vitro diagnostic methods in the evaluation of food hypersensitivity. In D. D. Metcalfe, H. A. Sampson, & R. A. Simon (Eds.), Food allergy: Adverse reactions to food and food additives. (pp. 137-150). Cambridge, Massachusetts: Blackwell Scientific Publications.

3 European Academy of Allergology and Clinical Immunology. (1989). Skin tests used in type I allergy testing. Position paper. Allergy 44: (Suppl. 10). Copenhagen: Munksgård.

4 C.Ortolani, C.Bruijnzeel-Koomen, U.Bengtson, C.Bindslev-Jensen, B.Björksten, A.Høst, M.Ispano, R.Jarish, C.Madsen, K.Nekam, R.Paganelli, LK.Poulsen & B.Wütrich: Controversial aspects of adverse reactions to food. Allergy 1999, 54: 27-46.

European Academy of Allergy and Clinical Immunology Risk Evaluation Scheme for assessment of allergy risk of genetically modified foods. Prepared by Task Force GMO (Bindslev-Jensen C, Ebner C, Madsen C, Mäkinen-Kiljunen S, Peltre G, Poulsen LK, van Ree R, Viets S) Allergy 2001, *in press*

Chapter 5, Detection methods for allergenic transgene products

There are several reasons why it is important that the content of potential allergens can be precisely and sensitively determined in GMO foods. Since it is the ultimate goal to secure safe food consumption for the individual patient, the allergic population, and the society in total, both consumers, producers, health care personal, and legislators should be able to have information on the concentration of ingredients in a given food. It is important to emphasize, that this information not only pertains to the actually products of modified genes, but to all potential allergens, since the amount of gene products that are native to the organism, may very well be changed by the introduction of a new or changed gene in the organism.

Determination of potential allergens by biological methods

The biological activity of food allergens or mixtures thereof may be determined by various in vivo and in vitro methods that may quantitatively or semi-quantitatively express the combined effects of individual allergenic molecules in a mixture. Even in the rare case of testing an individual food allergen molecule, a response will emerge that is only declared relatively to other allergenic substances or mixtures. Thus, an important feature of testing the biological activity of mixtures is the lack a response which can be directly linked to individual molecular entities, and this put special emphasis on the definition of both the test systems and the mixtures that are tested.

The biological activity of a substance in relation to food allergy may be understood in various ways. In the context of this review only food allergic diseases believed to be mediated by immunoglobulin E (IgE) will be considered even though adverse reactions to foods include other disease entities and even the term food allergy may comprise diseases elicited by several other mechanisms (1).

The term allergenic may be understood both as the capacity to sensitize, i.e. induce an IgE immune response, and as the capacity to elicit an allergic reaction in an individual already sensitized. In this chapter only the latter meaning will be discussed corresponding to the left branches of the decision trees. The induction of an allergic reaction in an already sensitized individual, has been much more successfully investigated and as described below numerous models exist for *determination of the biological potency* (Table 1), which lists the various test procedures in the opposite direction of what is seen in the decision trees .

TBL 1	Target	Species	Test system	Examples
In vivo	Entire organism	Human	Challenge of allergic patients	DBPCFC, open challenges
		Experimental animals	Peroral challenge of animals	Anaphylactic response
	Skin	Human	Skin testing of allergic patients	Skin prick tests, intrademal tests
		Experimental animals	Actively or passively sensitized animals	PCA
In vitro	Basophils	Human	Actively or passively sensitized basophils + allergen	Basophil histamine release, cord blood basophil histamine release
	Basophil or mast cells	Humanized, i.e. trans-fected with a human IgE receptor	Passively sensitized cells	Mediator release
	Mast cells	Rodents	Peritoneal mast cells	Histamine or other mediator release

Modified from *In vivo and in vitro techniques to determine the biological activity of food allergens (Review)*; Poulsen LK Submitted.

The Table illustrates the hierarchy that exist among these test systems as challenge of human patients are considered as closest to the relevant biological response, i.e. elicitation of an actual allergic response, albeit under controlled and safe circumstances. The next level in the hierarchy is to use the skin as a restricted and localized area for challenge. This system obviously involves the skin mast cells, which must be sensitized by IgE in order to respond to the offending allergen. Leaving the in vivo systems, the next step is to use the sensitized basophil granulocyte as a model for the sensitized mast cell present in the relevant organ of the patient. Moving further away from the actual patient, basophil from a non-allergic donor such as cord blood may even be used as an reagent which are then sensitized by IgE derived from an actual patient.

The above-mentioned human model systems all have their animal experimental counterparts which will not be further discussed since many of the parameters of the human systems discussed below, will also apply to animal models. The basic problem with experimental animals is to actually make them allergic. Although several immunization schemes - often parenteral - are available, which will readily produce an IgE response, it is still not known whether the mere presence of IgE specific to a food allergen gives a good prediction of allergenicity.

The human challenge model: Double-blinded, placebo-controlled food challenges

The ultimate determination of the biological activity of a food allergen or a mixture of these is the effect on a sensitized food allergic patient. The first report on double-blinded placebo controlled food challenges (DBPCFC) is probably a study of May, where asthmatic children were challenged with freeze-dried foods in capsules (2). By 1991 Bock and Atkins reviewed about 500 challenges performed at their pediatric centre, and a pattern emerged with relatively few placebo reactions and a high degree of safety (3). Thus DBPCFC has been said to be the gold standard of food hypersensitivity diagnosis (4), and is recommended by the European Academy for

Allergy and Clinical Immunology as *the only conclusive evidence of a food allergy, provided it is performed properly* (1).

For both practical and ethical reasons it is obvious, that patients cannot be routinely challenged to potential allergenic preparations. Thus the main reason for conducting controlled food challenges in patients is to verify or rule out a suspicion of food allergy, establishing a clinical diagnosis for the benefit of the patient. It is possible however to perform clinical trials on allergic patients in order to obtain knowledge about the allergenicity of GMO foods, provided that all other toxicologal and safety issues have been satisfactory solved.

Skin tests in humans

For the standardization of inhalation allergens the skin test has been the most important tool, and it is the recommended method for biological standardization of allergen extracts (5, 6). For this reason skin tests have also been used extensively for diagnosis of food allergy (1), and it seems well justified to use it for biological activity measurements of food allergens including GMO-foods. The rationale behind skin testing is that by introducing a small volume of allergen in the skin - either intradermally or via a small puncture of the *stratum corneum* as in the skin prick test - mast cells sensitized with specific IgE are activated via allergen cross-linking of this IgE. The activation of mast cells results in release of mediators - primarily histamine - which induces a wheal and flare reaction of the skin. Within a certain concentration range there is a dynamic response, i.e. a wheal and flare with a larger area develop after application of a higher concentration of allergen. The biological response is measured by planimetry as the area of the wheal or the flare (7, 8) and the result may be quantified by end-point-titration, i.e. the highest concentration in a titration which produces a negative response, or by comparison with a standard, typically histamine in a concentration of 10 mg/ml. The patient must - besides being well-defined as a patient - fulfill certain conditions such as an intact skin, lack of dermographism, and abstinence from drugs such as antihistamines which will dramatically inhibit the skin reaction (9). For ethical and safety reasons the test substance must be assured to be without infectious or toxicological potential besides its allergenic properties. A detailed outline of the technique is given in the guidelines, and will not be discussed here, but some points of special relevance for food allergens and GMO foods should be mentioned, however. The guidelines recommend the use of 20 patients with symptoms of moderate severity, but with the number of available patients that have undergone a DBPCFC-procedure this may present a problem in many centres, especially if - for ethical reasons - only adults or adolescents are selected. Moreover, infants and small children have a good prognosis for outgrowing their food allergy (10) early in their life, which makes them less suited for participation in safety studies. Due to the paucity of DBPCFC+ patients and the individual responses which may be quite varying, it can be difficult to perform a sufficient number of skin tests to ensure safety of the GMO.

Effector cells *in vitro*: Experimental systems for mast cell or basophil activation

As an alternative to the skin test the basophil granulocytes which are believed to be sensitized analogously to skin mast cells have been used extensively for many immunological studies of the allergic response (11,12). Being an *in vitro* method this technique has obvious advantages compared to the skin tests, since less strict requirements are posed on the test substance regarding non-toxicity and non-infectivity, albeit it should not be cytotoxic in the applied concentrations. For studies of inhalation and food allergy histamine release tests are correlating well with other measures of IgE sensitization, such as skin prick tests or determination of specific IgE in plasma

(13-16), and based on these findings it has been suggested to use the technique for biological standardization of allergen extracts including food allergens.

The direct histamine release method uses blood from a sensitized patient, and this limits the practical possibilities for running the method to patient-near centres, since the whole blood must be used within 24 h after drawing of the blood. This obstacle can be overcome by combining basophils from a non-sensitized person with serum containing specific and relevant IgE-antibodies. In its original form, basophils from adult donors were stripped of their original IgE by a brief treatment with low pH, followed by a new incubation with the sensitizing serum (12; 17). More recently cord blood basophils have been used as recipient cells, which has the advantage of eliminating the low pH IgE dissociation step, which may interfere with the biological functions of the cells. It is conceivable that basophil cell lines, such as the KU812 (18) or animal cell lines transfected with the the human FcεRI, i.e. the IgE high affinity receptor (19), may also be used as recipient cells for this purpose.

Determination of potential allergens by biochemical methods

A number of systems for biochemical detection of allergens are listed in Table 2. A pure system related to the allergic patient can be obtained by immunochemical assays detecting IgE-allergen binding directly or indirectly by inhibition designs. More indirect methods involve the use of animal antibodies for immunochemical detection, or molecular biology methods for detection via the DNA or mRNA levels.

Table 2. Hierarchy of biochemical test systems for testing of allergenic potency of pure substances or mixtures.

Vivo/ vitro	Target	Species	Test system	Examples
In vitro	IgE	Human	(Inhibition of) IgE-allergen binding in immunochemical assays	RAST or RAST-inhibition
	IgG/IgE	Experimental animal including monoclonal antibodies, chimeric antibodies etc.	Immunochemical assays	ELISAs, Dipsticks etc.
	Phage display antibodies	Measurement of protein con-tent in allergenic sources	Immunochemical assays	ELISA-type assays
In vitro (indirect)	PCR	Direct measurement of DNA coding for the gene in question	Polymerase chain reaction (PCR)	PCR
	RT-PCR	Direct measurement of mRNA encoding the protein in question	Reverse transcriptase-PCR	RT-PCR

Modified from *In vivo and in vitro techniques to determine the biological activity of food allergens (Review)*; Poulsen LK Submitted.

In vitro studies of IgE-allergen binding: RAST and RAST-inhibition

Since the binding between the allergen and the IgE is central in eliciting of the biological function in the test systems described above, it is obvious to use a test system that measures this binding, and the RadioAllergoSorbentTest and modifications of this play an important role in allergen standardization. The initial design of the RAST was based on the use of dextran-derived materials (20; 21) but later solid phases have comprised the widely used paper discs (22), aluminium hydroxide gel (23), polystyrene tubes (24), cellulose polymers (25; 26), and magnetic microparticles (27). Reviews of the available technologies and a discussion of method evaluation have been given in (28; 29).

Other immunochemical assays

In the Table 2 is also mentioned the production of animal antibodies to individual allergens and the use of such antibodies in ELISAs etc, forms the border between the biological assays and molecular identification of individual allergens A large number of animal antibodies has been raised against known or suspected food allergens, and may be used for testing. Several commercial assays for well-known food allergens have been described (30-33) [More references to be added, please give me all your inputs!] (summarized in Table 3) and more are to follow. A word of caution should be issued: If gene products are only slightly modified, it is important to carefully check how an antibody raised to the native protein (allergen) will react to the modified protein. In some cases it may be necessary to raise new antibodies to the modified protein.

Molecular biology assays

The final line in the Table 2 mentions the possibility of using determination of mRNA or DNA as a surrogate marker of the presence of allergens. Since the DNA may be transcribed with

varying efficiency in the plant and the correlates between mRNA levels and protein levels may also vary, these measures may only be semi-qualitatively related to the potential allergen level. On the other hand, the molecular biology are very sensitive and may be the only way to determine extremely low levels of (genes coding for) allergens in GMO foods. Moreover, since the sequence of the targetted genes is often known, these techniques may be able to differentiate between native and genetically modified versions of the same gene products, since small dissimilarities may evade detection by immunochemically based techniques.

References to this chapter

1. Bruijnzeel Koomen, C.A., Ortolani, C., Aas, K., Bindslev-Jensen, C., Bj□ksten, B., Moneret-Vautrin, D., & W□hrich, B. (1995). EAACI Position paper: Adverse reactions to food. Allergy, 50, 623-635.

2. May, C.D. (1976). Objective clinical and laboratory studies of immediate hypersensitivity reactions to foods in asthmatic children. J Allergy Clin Immunol, 58, 500-515.

3. Bock, A.S., & Atkins, F.M. (1990). Patterns of food hypersensitivity during sixteen years of double-blind, placebo-controlled food challenges. J Pediatr, 117, 561-567.

4. Bindslev-Jensen, C. (1998). Food allergy. BMJ., 316, 1299-1302.

5. European Academy of Allergology and Clinical Immunology. (1989). Skin tests used in type I allergy testing. Position paper. Allergy 44: (Suppl. 10). Copenhagen: Munksg□d.

6. Nordic Council on Medicines. (1989). Registration of allergen preparations. Nordic Guidelines. (2 ed.). Uppsala: NLN Publication No 23.

7. Poulsen, L.K., Liisberg, C., Bindslev-Jensen, C., & Malling, H.-J. (1993). Precise area determination of skin-prick tests: validation of a scanning device and software for a personal computer. Clin Exp.Allergy, 23, 61-68.

8. Poulsen, L.K., Bindslev-Jensen, C., & Rihoux, J.P. (1994). Quantitative determination of skin reactivity by two semiautomatic devices for skin prick test area measurements. Agents and Actions, 41, C134-C135

9. Petersen, L.J., Bindslev-Jensen, C., Poulsen, L.K., & Malling, H.J. (1994). Time of onset of action of acrivastine in the skin of pollen- allergic subjects. A double-blind, randomized, placebo-controlled comparative study. Allergy, 49, 27-30.

10. Host, A., & Halken, S. (1990). A prospective study of cow milk allergy in Danish infants during the first 3 years of life. Clinical course in relation to clinical and immunological type of hypersensitivity reaction. Allergy, 45, 587-596.

11. Lichtenstein, L.M., & Osler, A.G. (1966). Studies on the mechanisms of hypersensitivity phenomena. XII. An in vitro study of the reaction between ragweed pollenantigen, allergic human serum and ragweed sensitive human leucocytes. J Immunol, 96, 169-179.

12. Stahl Skov, P., Permin, H., & Malling, H.-J. (1977). Quantitative and qualitative estimations of IgE bound to basophil leukocytes from hay fever patients. Scand.J Immunol., 6, 1021-1028.

13. □stergaard, P.A., Ebbesen, F., Nolte, H., & Skov, P.S. (1990). Basophil histamine release in the diagnosis of house dust mite and dander allergy of asthmatic children. Comparison between prick test, RAST, basophil histamine release and bronchial provocation. Allergy, 45, 231-235.

14. Nolte, H., Storm, K., & Schi□z, O. (1990). Diagnostic value of a glass fibre-based histamine analysis for allergy testing in children. Allergy, 45, 1-11.

15. Hansen, T.K., Bindslev-Jensen, C., Stahl Skov, P., & Poulsen, L.K. (1996). Codfish allergy in adults. Specific tests for IgE and histamine release vs double-blind, placebo-controlled challenges. Clin Exp.Allergy, 26, 1276-1285.

16. N□gaard, A., Skov, P.S., & Bindslev-Jensen, C. (1992). Egg and milk allergy in adults: comparison between fresh foods and commercial allergen extracts in skin prick test and histamine release from basophils. Clin Exp.Allergy, 22, 940-947.

17. Pruzansky, J.J., Grammer, L.C., Patterson, R., & Roberts, M. (1983). Dissociation of IgE from receptors on human basophils. I. Enhanced passive sensitization for histamine release. J Immunol, 131, 1949-1953.

18. Hara, T., Yamada, K., & Tachibana, H. (1998). Basophilic differentiation of the human leukemia cell line KU812 upon treatment with interleukin-4. Biochem.Biophys.Res.Commun., 247, 542-548.

19. Lowe, J., Jardieu, P., VanGorp, K., & Fei, D.T. (1995). Allergen-induced histamine release in rat mast cells transfected with the alpha subunits of Fc epsilon RI. J Immunol Methods, 184, 113-122.

20. Ishizaka, K., Ishizaka, T., & Hornbrook, M.M. (1967). Allergen-binding activity of □E, □G and □A antibodies in sera from atopic patients. In vitro measuremnts of reaginic antibody. J Immunol, 98, 490-501.

21. Wide, L., Bennich, H., & Johansson, S.G.O. (1967). Diagnosis of allergy by an in vitro test for allergen antibodies. Lancet, ii, 1105-1107.

22. Ceska, M., Eriksson, R., & Arga, J.M. (1972). Radio-immunosorbent assay of allergens. J.Allergy, 49, 1-9.

23. Poulsen, L.K., & Weeke, B. (1985). Aluminium hydroxide adsorbed allergens used in modified RAST. Allergy, 40, 405-416.

24. Poulsen, L.K., Pedersen, M.F., Malling, H.-J., Søndergaard, I., & Weeke, B. (1989). Maxisorp RAST. A sensitive method for detection of absolute quantities of antigen-specific IgE. Allergy, 44, 178-189.

25. Ewan, P.W., & Coote, D. (1990). Evaluation of a capsulated hydrophilic carrier polymer (the ImmunoCAP) for measurement of specific IgE antibodies. Allergy, 45, 22-29.

26. Bousquet, J., Chanez, P., Chanal, I., & Michel, F.B. (1990). Comparison between RAST and Pharmacia CAP system: A new automated specific IgE assay. J Allergy Clin Immunol, 85, 1039-1043.

27. Kleine-Tebbe, J., Eickholt, M., Gätjen, M., Brunnée, T., O'Connor, A., & Kunkel, G. (1992). Comparison between Magic Lite- and CAP-system: two automated specific antibody assays. Clin Exp.Allergy, 22, 475-484.

28. Matsson, P., Hamilton, R.G., Adkinson, N.F., Esch, R., Homburger, H.A., Maxim, P., & Williams, B. (1996). NCCLS. Evaluation methods and anlytical performance characteristics of immunological assays form human immunoglobulin E (IgE) antibodies of defined allergen specificities; Proposed guidelines (NCCLS document I/LA20-P). (1 ed.). Wayne, PA, USA: NCCLS.

29. Bindslev-Jensen, C., & Poulsen, L.K. (1997). In vitro diagnostic methods in the evaluation of food hypersensitivity. In D. D. Metcalfe, H. A. Sampson, & R. A. Simon (Eds.), Food allergy: Adverse reactions to food and food additives. (pp. 137-150). Cambridge, Massachusetts: Blackwell Scientific Publications.

30. Hlywka, J.J., Hefle, S.L., & Taylor, S.L. (2000). A sandwich enzyme-linked immunosorbent assay for the detection of almonds in foods. J Food Prot.2000.Feb.;63.(2.):252.-7., 63, 252-257.

31. Leduc, V., Demeulemester, C., Polack, B., Guizard, C., Le, G.L., & Peltre, G. (1999). Immunochemical detection of egg-white antigens and allergens in meat products. Allergy, 54, 464-472.

32. Jeoung, B.J., Reese, G., Hauck, P., Oliver, J.B., Daul, C.B., & Lehrer, S.B. (1997). Quantification of the major brown shrimp allergen Pen a 1 (tropomyosin) by a monoclonal antibody-based sandwich ELISA. J Allergy Clin Immunol, 100, 229-234.

33. Yeung, J.M., & Collins, P.G. (1996). Enzyme immunoassay for determination of peanut proteins in food products. J AOAC.Int, 79, 1411-1416.

Chapter 8, Risk assessment

At the FAO/WHO Consultations on Genetically modified foods in Rome, 1996, the following statements were launched (ref in[i]):

1 The transfer of genes from commonly allergenic foods should be discouraged unless it can be documented that the gene transferred does not code for an allergen.

2 Foods found to contain an allergen transferred from the organism which provided the DNA should not be considered for marketing approval unless such products can be clearly identified in the marketplace and this identity will not be lost through distribution and processing. Further, that labelling approaches may not be practical in these situations, and that particular problems exist for consumers who cannot read, or who may not be provided with labels.

3 Involved organizations should consider the appropriateness of, and/or actions to take, in respect to foods containing new protein(s) that are determined to have the characteristics of an allergen.

4 The identification of food allergens and the characteristics of these allergens that define their immunogenicity be encouraged.

The ILSI decision tree so far constitute the only guideline for assessment of potential change in allergenicity of genetically modified organisms[ii]. This decision tree divides GMO's into foods where the source of gene stems from a known allergenic source or a source not known to be allergenic. Furthermore, the allergenic source is further subdivided into sources from the commonly allergenic foods (the big eight) and less commonly allergenic foods, namely the remaining foods, only accounting for approx. 10 per cent of the clinical reactions. This latter statement is however, limited to the classical type I allergic reactions, elicited by e.g. peanut, milk, egg, soy, fish, crustaceans but does not take into account the much more abundant reactions to cross- or common reactivity between e.g. pollens and fruits/vegetables or latex and fruits[iii].

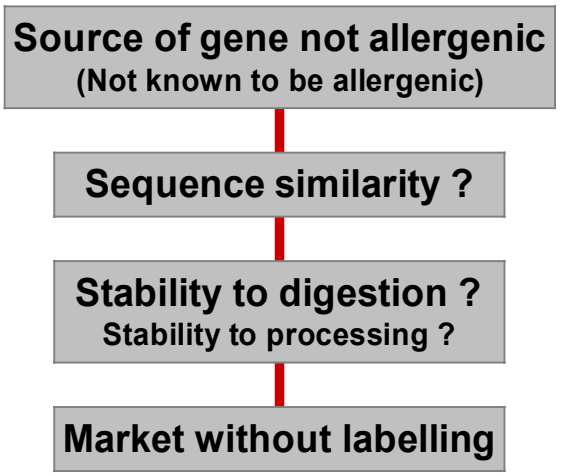

Fig. 2

The "left side" of the decision tree dealing with known allergenic proteins contains the test systems sufficient to rule out a potential risk to the allergic patient, provided the tests systems (both in vitro and in vivo) are used with material from high quality patients, fulfilling the EAACI guidelines[iv] and using validated test procedures[v],[vi]. The subdivision into common and less common foods is, however based on availability of test material rather than an actual risk assessment and should thus be left out - there are no data in literature supporting an increased risk for the actual patient to common food allergens than to less common food allergens.

The "right side" of the decision tree, fig 2, deals with inserted proteins, not known to be allergenic:

This aproach is based on the following assumptions:

1 The optimal peptide length for binding appears to be between 8 and 12 amino acids for T-cell epitopes and even longer for B-cell epitopes[vii]

2 All epitopes are sequential, and conformational epitopes are without significance

3 All relevant epitopes has already been sequenced.

4 The stability to digestion is a significant and valid parameter that distinguishes food allergens from non-allergens[viii].

None of these statements has been proven - there are examples of exceptions for all the above statements. Furthermore, the test is also likely to identify conserved sequences that are unrelated to the allergenic potential of the proteins. Furthermore, harmless proteins might also be excluded form market based on these tests.

The EAACI risk evaluation procedure

It therefore suggested to add a screening procedure to be applied to gene modified foods, not previously known to be allergenic (the right side), fig 2:

The various subsequent steps in the evaluation procedure is commented in the following (The numbers refer to the flow chart).

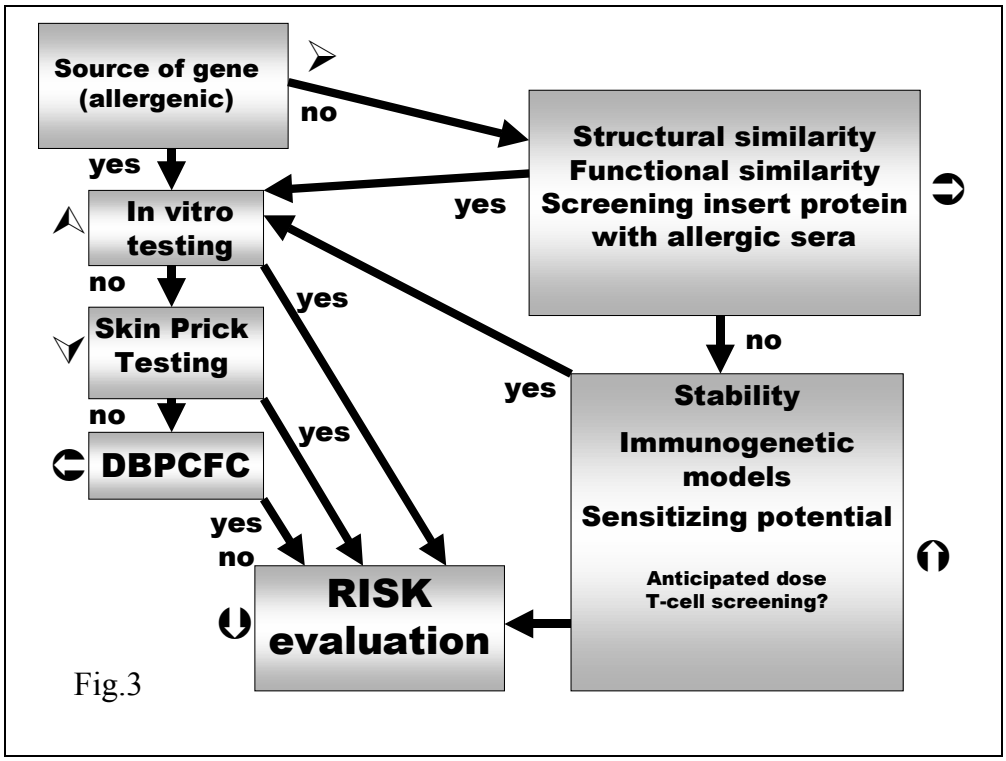

Fig.3

The "left side" of the decision tree dealing with known allergenic proteins contains the test systems sufficient to rule out a potential risk to the allergic patient, provided the tests systems (both in vitro and in vivo) are used with material from high quality patients, fulfilling the EAACI guidelines and using validated test procedures.

After a negative outcome of testing for sequence similarity to known allergens, the food is subjected to solid phase immunoassays screening for allergenicity using sera from patients with established allergy to major allergens, especially allergens, where cross-reactivity to foods are abundant (pollen allergics).

It is suggested to use as a minimum 3 * 10 patients allergic to birch, grass, artemisia respectively or to other relevant allergens. There are major regional differences between reaction severity and plants in question within Europe. Allergens should therefore be included according to origin e.g. ragweed, Parietaria or other types of food or inhalant allergens according to type of GM-food in question.

Positive results in these tests transfer the evaluation of the food to the left side of the flow chart.

Serological cross-reactions should also be dealt with by transferral to the left side of the decision tree (see 2-4).

This step constitute considerations on the more uncertain aspects of a novel food. In this phase of evaluation, aspects like e.g. models for evaluation of a possible immunogenetic role as weel as considerations on a possible sensitizing potential, possibl estimulation of TH-2 system or creation of Neo-allergens. Also, the anticipated dose of intake and other aspects may be included.

Finally, the natural variability of allergens in wild type foods must be taken into account when assessing quantitative aspects of measurement of allergens in GMO's.

At present, the methods for such evaluation procedures are not fully developed. The upcome of animal models, which at present are not sufficiently developed for use may elucidate these aspects in the future.

After evaluation of the above parameters, it will be possible for the authorities to perform a proper risk assessment of the GMO in question.

The left side of the flow chart contains various levels of safety. A positive outcome in step 4 (DBPCFC) of course constitutes the highest possible risk, whereas previous experience with safe ingestion diminishes the absolute risk.

The in vivo and in vitro investigations (2-5) thus results in data concerning the absolute risk of introduction of the GMO in question to the market, whereas data from 6 enables a calculation on the relative risk at a certain level (determined in 2-5).

References to this chapter.

i.Bindslev-Jensen C: Allergy risks of genetically engineered foods. Allergy 1998;53:58-61

ii. Metcalfe DD, Astwood JD, Townsend R, Sampson HA, Taylor SL, Fuchs RL: Assessment of the allergenic potential of foods derived from genetically engineered crop plants. Crit Rev Food Sci Nutr 1996;36; Suppl:S165-86

iii. Pastorello EA, Incorvaia C, Pravettoni V, Ortolani C: Crossreactions in food allergy Clin Rev Allergy Immunol 1997;15(4):415-27.

iv.Bruinjzeel-Koomen C, Ortolani C, Aas K, Bindslev-Jensen C, Bj□ksten B, Moneret-Vautrin D, W□rich B: Adverse reactions to food. Position Paper. Allergy 1995, 50: 623-635.

v.Bindslev-Jensen C, Poulsen LK: In vitro diagnostic tests. Chapter 7 In: Sampson HA, Simons E, Metcalfe DD: Food Allergy 2nd edition. Blackwell Scientific Publications, 1996, 137-150.

vi.C.Ortolani, C.Bruijnzeel-Koomen, U.Bengtson, C.Bindslev-Jensen, B.Bj□ksten, A.H□t, M.Ispano, R.Jarish, C.Madsen, K.Nekam, R.Paganelli, LK.Poulsen & B.W□rich: Controversial aspects of adverse reactions to food. Allergy 1999, 54: 27-46.

vii.(Rothbard Jb and Gefter ML: Interactions between immunogenic peptides and MHC proteins. Annu. Rev. Immunol. 9:527-xxx, 1991).

viii. Astwood JD, Leach JN, Fuchs RL: Stability of food allergens to digestion in vitro. Nat Biotechnol 1996;14:1269-1273