

Food allergy: predictive testing of food products

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Abstract

Food allergy is a substantial cause of distress in humans. Several biotechnological techniques can be applied to reduce the antigenicity of food proteins to produce for instance hypoallergenic infant formulas. Biotechnological techniques for synthesizing new proteins or new biological varieties for applications in food are also available. For such biotechnologically derived protein products (novel foods), allergenicity may also pose a major concern. For safety reasons, it is of importance to evaluate the residual antigenicity of modified protein products, to screen for possible cross-reactivity to prevent reactions in previously sensitized individuals, and to test for sensitizing properties of new and/or modified protein products. Besides physico-chemical and immunochemical analyses, several in vitro and in vivo bioassays may be applied in studying the antigenic or allergenic properties of (new or modified) food proteins. In this paper, an overview of several available assays and new developments for determining the antigenic or allergenic properties of dietary proteins, as well as their possible applications and limitations is presented. Special attention is paid to the role of the gastro-intestinal tract physiology in food allergy and in the evaluation of the allergenic potential of food proteins and to the possible applications of animal models in food allergy research and in the evaluation of the allergenicity of food proteins. © 1997 Elsevier Science B.V.

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1. Introduction: definition of food allergy

Adverse reactions to food can be separated into toxic and hypersensitivity reactions. Food hypersensitivity may be defined as a qualitatively and/or quantitatively different reaction to food, which is not so much caused by the food itself, but rather by a specific trait of the person who takes the food (National Organisation for Quality Assurance in Hospitals, 1993). The mechanisms underlying hypersensitivity reactions are used to distinguish between two different classes of food hypersensitivity: food allergy and food intolerance. Food allergy may be

defined as a food hypersensitivity in which the reactions are primarily immunologically-mediated, while non-immunological mechanisms play the major role in food intolerance. Often, a third class of food hypersensitivity is defined: the food aversion, in which food is not tolerated for psychological reasons. Based on the immunological mechanisms underlying allergic reactions, four different types of allergy are distinguished. Only IgE-mediated (type I) allergic reactions are for certain known to play a major and primary role in food allergy (National Organisation for Quality Assurance in Hospitals, 1993; Bruijnzeel-Koomen et al., 1995). IgE-mediated (food) allergy often occurs as a part of the so-called atopic syndrome. People with atopy are considered to have a hereditary trait (the atopic constitution) associated with a greater risk of development of IgE-mediated allergies.

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2. Current routine testing possibilities in the evaluation of the allergenicity of food proteins

Although well validated models to determine the allergenic potential of new dietary proteins are not available yet, currently several methods may be applied to generate some relevant information with respect to the antigenicity and allergenicity of food proteins.

2.1. *In vivo* antigenicity assays

To determine the antigenicity of proteins, several well validated assays are available. These assays are based on parenteral application of the test proteins to laboratory animals, in which the guinea pig is the most regularly used test species. Yet, these assays are also operational for other species, for example, the rat. Within these antigenicity assays, several well validated *in vitro* and *in vivo* tests and analyses can be applied to qualitatively and quantitatively determine specific immune responses and immune-mediated effects as a measure for the antigenicity of the test proteins. In general, these parenteral antigenicity assays demonstrate high sensitivity.

However, although the information from antigenicity assays may be of major relevance, it must always be recognised that such assays only provide information on the antigenicity of proteins. In general, any protein that may be recognized as an antigen (foreign protein) will induce humoral and cellular immune responses upon injection, and will most likely give a positive test result in such assays. Whether a protein has a high or low potency for inducing food allergic reactions in susceptible humans cannot be concluded or predicted only on the basis of the results of these parenteral antigenicity assays. Natural barriers such as the gastrointestinal acid-denaturation and digestion and the mucosal/epithelial layers, which are all known to prevent, reduce or in any other way influence the contact between food antigens and the local and systemic immune system, are not modelled or taken into account in such assays. Therefore the restriction or withholding of the application of new proteins based only on a positive testing result in a parenteral antigenicity assay appears not to be justified. More discriminating approaches that include an evaluation of the influence of other factors than only the antigenicity, are therefore of importance.

2.2. *Physico-chemical and immunochemical analyses*

In addition to *in vivo* antigenicity assays, several (combinations of) physico-chemical and immunochemical analyses are used routinely to determine antigenicity. In general, immunochemical methods also show high sensitivity in demonstrating the presence of antigens or in detecting antigen-specific antibodies. How-

ever, these *in vitro* analyses are not directly suitable in studying the allergenicity of new proteins, since antibodies or sera obtained from already sensitized subjects are needed. Yet, immunochemical analyses can be very useful in the control on hypoantigenicity of modified protein products or in the study of possible immunological cross reactivity. In addition, these analyses can be used to determine possible sensitization using sera collected from patients (diagnostic procedures) or test animals (*in vivo* antigenicity assays).

However, caution is needed in the interpretation of results from immunochemical analyses. For instance, monovalent antigenic peptides or small peptides carrying more antigenic determinants may be bound by IgE in immunochemical assays or by mast cell or basophil-bound IgE, yet are not necessarily able to cause bridging between the mast cell or basophil-bound IgE molecules. Although the immunochemical binding test was positive, activation of the mast cells or basophils and subsequent allergic reactions may therefore still be absent. For the detection of peptides carrying more antigenic determinants which can indeed induce IgE-mediated allergic reactions (bridging of antigen-specific IgE on mast cells or basophils), other assays have to be applied (see Section 2.3). With respect to relatively small peptides, it should be recognised that although these peptides may not be able to induce IgE-mediated reactions (mast cell or basophil activation due to bridging of IgE molecules), such peptides may still be able to cause immunological sensitization. Monovalent antigens can still be recognised by the immune system. The described *in vivo* antigenicity assays can be applied in studying possible T-cell sensitization to such small peptides.

2.3. *Mast cell and basophile degranulation tests*

Determination of allergenic proteins or fragments that are able to cause activation of mast cells and basophils is possible using *in vitro* mast cell or basophile degranulation tests. For these assays, mast cells or basophils are isolated and subsequently loaded with antigen-specific cytophilic antibodies (for instance using serum from sensitized humans or test animals). The sensitized cells are subsequently incubated with the antigen or test product. By means of various parameters, such as the measurement of histamine release, possible activation and degranulation of the cells can be determined. These *in vitro* assays can be conducted using antibodies (sera) and effector cells (basophils or mast cells) from various species. A well validated *in vivo* counterpart for the detection of mast cell activation is the passive cutaneous anaphylaxis (PCA) test. Sera from sensitized animals (for instance guinea pigs or rats) are injected subcutaneously into unsensitized animals. Possible cytophilic antibodies present in the

sera are bound by the receptors on the mast cells in the skin and as a consequence, the cells become sensitized. After a lag period, the antigen or a test substance is injected intravenously. Bridging of the cytophilic antigen-specific antibodies on the mast cells in the skin of the animals by injected antigen induces mast cell activation and release of mediators in the skin and the resulting anaphylactic reaction is detectable by various methods. In the active systemic anaphylaxis (ASA) test, which is also a validated anaphylaxis test, actively sensitized animals are injected with the antigen or test substance intravenously and several parameters are recorded to determine the systemic anaphylactic response as a measure for the degree of sensitization or the presence of antigen.

As in the case of immunochemical analysis methods, mast cell and basophile degranulation tests are not directly suitable in determining the allergenicity of new food proteins because sera from already sensitized subjects are needed. However, the assays are useful in the control of hypoallergenicity of modified protein products or in studying possible immunological and allergological cross reactivity. In addition, anaphylactic sensitization in *in vivo* assays can be determined using these *in vitro* and *in vivo* tests. The sensitivity of the *in vitro* and *in vivo* mast cell and basophile degranulation tests is acceptable.

2.4. Human studies

In the evaluation of the potential allergenicity of food products, patient assays such as skin prick tests or challenge procedures may also be used. For instance, these assays are applicable in the evaluation of the residual allergenicity of hypoallergenic products, in the evaluation of cross-allergenicity, or in the evaluation of the possible allergenicity of food products derived from biotechnologically derived crops in which a gene from a known allergenic source species has been introduced. However, the use of patients in such assays for non-diagnostic purposes requires careful ethical consideration. Since many of the questions may also be addressed by performing *in vitro* assays such as immunochemical analyses, the use of sera from patients should therefore always be preferred rather than performing patient assays. Human studies may also be conducted to investigate the sensitizing potential of new food products or new ingredients at a pre-market stage. Finally, a post-marketing surveillance will be of major importance in the evaluation of the consumers tolerance to a new product.

3. The role of the gastro-intestinal tract physiology in food allergy and in the evaluation of allergenicity of food proteins

Despite the availability of several well validated methods in studying antigenic and allergenic properties

of protein products, current testing possibilities in investigating the allergenicity of food proteins at a pre-marketing stage are very poor. New, well validated methods with high sensitivity and high specificity cannot be expected in the near future. However, some strategies can be followed in obtaining additional relevant information apart from the information already obtained from the currently applied assays. For instance, the role of the gastro-intestinal tract physiology is hardly or not at all taken into account in the current approaches.

Many elements of the gastro-intestinal tract physiology influence the ultimate allergenicity of food proteins. These include the pH, digestive enzymes, bile, peristalsis, transit time, bacterial fermentation, and the intestinal barrier function, permeability and absorption. Based on human clinical observations, an important, yet poorly investigated and documented, role of digestion with respect to the allergenicity of food proteins is often suggested. Some attempts, however, have been made to correlate the susceptibility to enzymatic breakdown of cow's milk proteins and the intestinal permeability of these proteins with their allergenic properties (Taylor, 1986; Marcon-Genty et al., 1989; Savilahti and Kuitunen, 1992). Evidence of an important role of digestion with respect to food protein allergenicity comes also from animal studies. For instance, prefeeding of an endopeptidase inhibitor (aprotinin) to mice results in inhibition of oral tolerance induction by protein feeding (Hanson et al., 1993), while under normal conditions the feeding of protein antigens to mice is known to induce substantial systemic tolerance for specific antibody and cell mediated immune responses (Hanson et al., 1993). In our laboratory (Knippels et al., unpublished results), anaphylactic sensitization of rats can be achieved by means of an intra gastric dosing protocol, while *ad libitum* availability of protein antigens in drinking water or diet fails to induce IgE antibody production in the majority of rats. Moreover, specific immune responses can be induced in mice by intra gastric administration of proteins, yet *ad libitum* availability of proteins via drinking water or, in particular, via the diet generally results in tolerance induction in mice. Probably, intra-gastric dosing of proteins results in an abnormal digestive breakdown that is likely to account for these differences. These observations are indicative of the important role of gastro-intestinal tract physiology for the allergenicity of food proteins. In this respect, it should be recognised that primary, secondary, and tertiary structures of (glyco)proteins are affected to different degrees by digestion, indicating that B- and T-cell epitopes will be affected to different degrees by digestion. In addition, it should also be recognized that digestion of food proteins is part of the normal sequence of events following consumption of food and that food allergic patients may well have

become sensitized to digested allergens rather than to the native proteins. Indeed, *in vitro* enzymatic digestion of food allergens does not necessarily diminish patient IgE binding (Schwartz et al., 1980), and may even increase the IgE binding (Hadded et al., 1979).

3.1. *The intestinal barrier*

The intestinal barrier function, permeability, and absorption are rarely taken into account in the evaluation of the allergenicity of food proteins. Local damage may cause increased macromolecular absorption resulting in increased systemic food allergen load, particularly in patients suffering from gastro-intestinal pathology. However, although knowledge of the passage of specific protein antigens and their fragments may provide some additional information in the evaluation of the potential allergenicity of protein products, the macromolecular exclusion by the epithelial barrier is a rather ancient concept (Seifert et al., 1974, 1977; Gardner, 1988; Teichberg, 1988). This has become particularly evident from studies on the presence of food allergens and other food proteins in human breast milk (Kilshaw and Cant, 1984; Axelsson et al., 1986; Machtinger and Moss, 1986; Troncone et al., 1987; Høst et al., 1988, 1990; Clyne and Kulczycki, 1991). From these studies, it became clear that macromolecular passage from the intestinal lumen into the circulation should be regarded a normal phenomenon also in healthy individuals. Therefore, an impaired macromolecular exclusion by the epithelial barrier is not considered of primary importance in the development of food allergy and no specific attention is paid to gastro-intestinal absorption modelling in this paper.

3.2. *Acid-denaturation and digestion*

Because acid-denaturation and digestibility of a food constituent in the gastro-intestinal tract are likely to be important factors partly determining the allergenic potential of food proteins, knowledge of the acid-stability and digestibility of food proteins as well as of the residual antigenicity and allergenicity of absorbed fragments may be of major relevance in the evaluation of the allergenicity of new food proteins. Therefore, future strategies may involve *in vitro* gastro-intestinal digestion modelling in combination with parenteral antigenicity assays. Several food allergens or allergenic determinants were reported to be relatively resistant to acid-denaturation and proteolytic digestion (Elsayed and Apold, 1977; Schwartz et al., 1980; Kurisaki et al., 1981; Metcalfe, 1985; Taylor, 1986, 1992; Kortekangas-Savolainen et al., 1993; Honma et al., 1994). Unfortunately, insufficient information is available on possible differences in susceptibility to acid-denaturation and gastro-intestinal digestion between strongly allergenic

food proteins and proteins that possess weak or virtually no allergenic potential. Therefore, evaluation of acid-stability and digestibility of food proteins will, in most cases, not yet provide sufficient information regarding their allergenic potential upon ingestion, and additional research is needed in this respect. However, by evaluating the residual antigenicity of products in routine parenteral animal assays following digestive modelling, information regarding the sensitizing properties of potentially absorbed digestive fragments can be obtained. In addition, by testing the digested products on residual recognition by antibodies in sera and T-cells from already sensitized subjects (patients or test animals), additional information on the potential allergenicity of a product to already sensitized individuals can be obtained. Up to now, static *in vitro* models have been used in most studies performed on the acid-stability and digestibility of food proteins. However, with respect to the evaluation of the potential allergenicity of food proteins, the application of a more dynamic model (see Fig. 1) is preferred. Dynamic models particularly provide better opportunities in investigating possible complex consequences of differences or temporary disturbances in gastro-intestinal tract physiology.

4. **Animal models in food allergy research and research on allergenicity of food proteins**

4.1. *Introduction*

Several attempts at developing animal models for food allergy research have been conducted in the past. Although some of the attempts at developing enteral sensitization and/or challenge protocols for laboratory animals were rather successful or at least promising, these efforts hardly resulted in structured approaches aimed at the development of well validated enteral allergenicity models. One of the major factors that may have negatively affected the results of many oral sensitization studies may have been unscheduled dietary pre-exposure of the test animals or their parental generations to the antigen under investigation. For guinea pigs (Pahud et al., 1985) and rats (Knippels et al., unpublished results, see Fig. 2), it was demonstrated that dietary exposure of animals to the proteins under investigation affects the results of oral sensitization studies with the offspring. At least two generations of animals have to be bred on a specified antigen free diet to avoid any influence in this respect. In addition, since oral exposure to food antigens may easily induce immunological tolerance in mice and rats (Stokes et al., 1983b; Steinmann and Wottge, 1990), special attention is to be paid to the conduct of the applied protocol when the goal is the investigation of enteral sensitization in these species.

An *in vitro* gastro-intestinal model for microbiological and nutritional studies

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Introduction

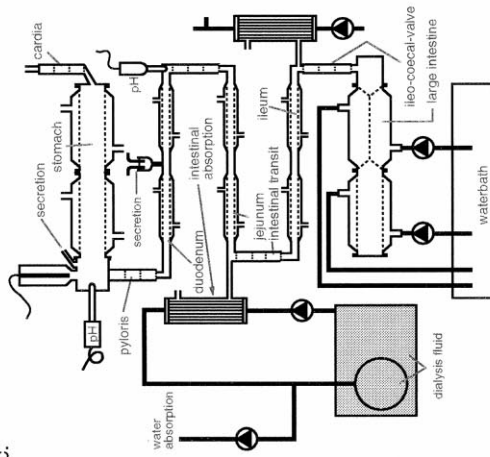
Studies in relation to the GI-tract performed in animals, humans and simplified laboratory models have its specific limitations: ethics, costs, extrapolation.

Description of the model

The TNO gastro-intestinal Model (TIM) comprises glass modules with a flexible wall inside kept at 37°C. By connecting the modules with peristaltic valves and ultra-filter systems the model simulates the cardia, controlling the volume and duration of the meal as well as eating patterns; the stomach in which the food is mixed by peristaltic movements with secreted HCl, gastric enzymes and electrolytes; the pylorus, which regulate the kinetics of gastric emptying dependant on the type of food; the duodenum in which the content is mixed with secreted NaHCO₃, bile and pancreatic juice containing natural enzyme activities; the jejunum and intestinal transit, regulated by pump-valves between the small intestinal units; intestinal absorption of digested food and water through hollow fibre systems; ileum and ileo-coecal-valve, which regulate delivery from the ileum to the large intestine (in development).

Aim

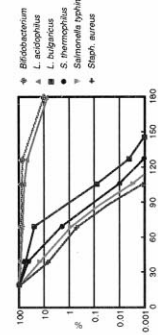
Development of an *in vitro* model which simulates in high degree the dynamic physiological processes of the stomach and intestine for research in relation to nutrition, micro-ecology, medicines, etc.



Application in:

micro-ecological studies

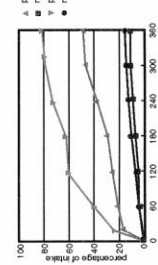
- kinetics of probiotic microorganisms
- kinetics of food borne pathogens
- interaction with food and food additives
- risk assessment of new (genetically modified) organisms



Survival of micro organisms in the stomach

nutritional studies

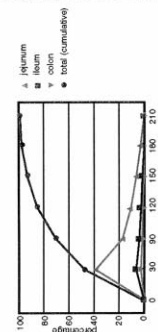
- digestibility of proteins, carbohydrates
- digestibility and chemical stability of novel foods
- slow-release characteristics
- bio-availability of minerals and trace elements
- efficiency of added enzymes



Cumulative recovery of (non-)protein nitrogen from skim milk and calve milk in TIM

pharmacological studies

- kinetics of medicines
- controlled-release from matrix
- drug-nutrient interactions



Absorption of medicine in jejunum and ileum after oral uptake in TIM

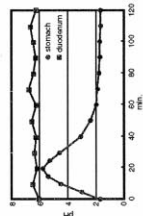
Computer control

All set points with respect to pH values, concentrations of chemicals and bio-active compounds, gastric emptying and intestinal transit times are controlled by the computer using specifically developed soft-ware.

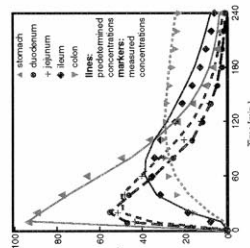
In this way TIM simulates the most important conditions in the gastro-intestinal tract as described for the *in vivo* situation.

Results

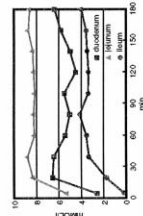
The TNO model permits to mimic accurately and reproducibly the kinetics of physiological events in the GI-tract. Survival kinetics of microorganisms in the stomach and small intestine of this model fitted the *in vivo* data. Results of digestion and absorption studies showed good similarity with *in vivo* data.



pH curves in stomach and small intestine after a meal



Kinetics of gastric emptying and intestinal transit



Concentrations of bile salts in the different compartments of the small intestine

Conclusions

The TNO model has unique properties for research related to the GI-tract under controlled and validated conditions. This model can contribute to reduction of the use of (fistulated) animals in microbiological, nutritional and pharmacological research.

Fig. 1. An *in vitro* gastro-intestinal model for microbiological and nutritional studies.

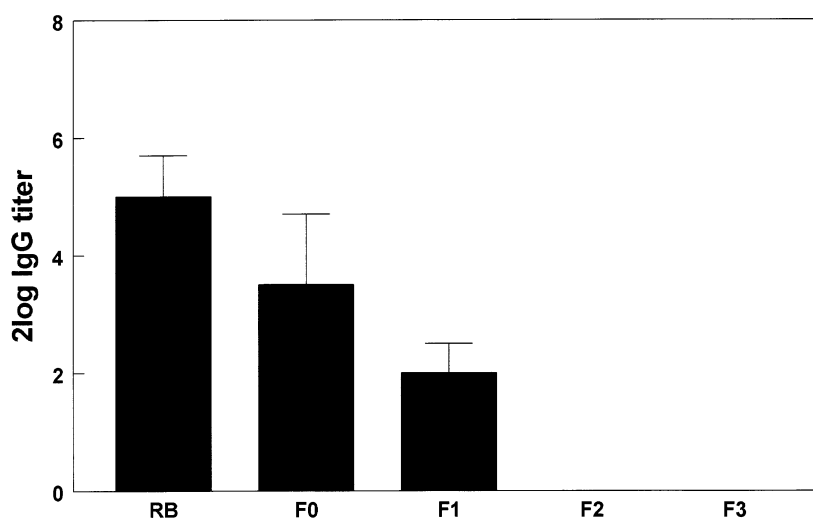


Fig. 2. Soy-specific IgG titers in rats either bred or not bred on a soy protein-free diet: RB, routine bred animals (animals from routine breeding fed a soy protein-containing diet); F0, parent animals (animals bred on a soy protein-containing diet yet fed a soy protein-free diet for at least 12 months); F1, first generation of offspring bred on a soy protein-free diet (blood sampled at approximately 1 year of age while animals were kept on the soy protein-free diet from weaning from F0 until blood sampling); F2, second generation of offspring bred on a soy protein-free diet; F3, third generation of offspring bred on a soy protein-free diet.

For food allergy research, three rodent species have frequently been used: the mouse, the guinea pig and the rat. In many studies, sensitization was performed parenterally or passively and effects of enteral challenges were subsequently studied (Byars and Ferraresi, 1976; Bloch and Walker, 1981; Freier et al., 1985; Granato and Piguet, 1986; Miller and Nicklin, 1988; Pahud et al., 1988; Turner et al., 1988; Curtis et al., 1990; Turner et al., 1990a,b). In addition, the effects of challenges have frequently been investigated in *in vitro* studies with intestinal tissue or ligated gut (Roberts et al., 1981; Baird et al., 1984; Lake et al., 1984; Catto-Smith et al., 1989a,b). However, with respect to the scope of this paper, i.e. possible strategies in the evaluation of the allergenicity of food proteins, possible applications of rodent models for investigating enteral sensitization need closer consideration.

4.2. Mouse

Mice have frequently been used to investigate humoral and cellular immune responses and immune-mediated effects following enteral protein exposure. However, although enteral protein feeding to mice may result in immune priming or sensitization when adjuvants are used (Challacombe and Tomasi, 1980; Bozelka et al., 1987) or when enteral protein exposure is performed at early stages of life (Hanson, 1981; Strobel and Ferguson, 1985; Strobel, 1986; Strobel and Ferguson, 1986), oral protein exposure of mice through both gavage, as well as via the drinking water or diet easily results in tolerance induction (Ngan and Kind, 1978; Stokes et al., 1983b; Strobel et al., 1985; Strobel

and Ferguson, 1986; Mowat et al., 1987a,b). Repeated exposure was particularly demonstrated in resulting in systemic tolerance rather than priming or humoral and cellular immune responses (Stokes et al., 1983a; Strobel and Ferguson, 1984). The easy induction of immunological tolerance upon enteral protein exposure makes the mouse an inappropriate species for the development of an enteral sensitization model for food allergy research and research on the allergenicity of food proteins. On the other hand, the mouse may be most useful in oral tolerance induction research.

4.3. Guinea pig

For the guinea pig, oral sensitization to food proteins has repeatedly been investigated and documented (Devey et al., 1976; Coombs et al., 1978; Andersson et al., 1979; McLaughlan et al., 1981a,b; Baird et al., 1984; Pahud et al., 1985, 1988). Sensitization usually was demonstrated using passive cutaneous anaphylaxis (PCA) and/or active systemic anaphylaxis (ASA), or by an enzyme-linked immunosorbent assay (ELISA). Oral guinea pig sensitization assays are quite sensitive (Pahud et al., 1988; Knippels et al., unpublished results) and proved of value in studies on differences in sensitizing properties of 'classical' infant formulas, as well as formulas based on modified protein products (hypoallergenic infant formulas) (McLaughlan et al., 1981a,b; Pahud and Schwarz, 1985; Pahud et al., 1985; Piacentini et al., 1994). Although the guinea pig is generally used in sensitization studies on chemicals, it is not an ideal species for studying the effects of substances on the immune system. Major reasons are the poor knowl-

edge of the guinea pig's immune system and the limited availability of tools for more detailed analysis of the effects on the guinea pig immune system. Although the guinea pig proved very sensitive in studying oral sensitization to proteins and a reduced antigenic sensitizing potential of hypoallergenic products could be demonstrated, it remains questionable whether a guinea pig assay will demonstrate any specificity in investigating differences in allergenic sensitizing potentials of food proteins.

4.4. Rat

As for the rat, immune responses to enterally administered food proteins have also been the subject of investigation. In several studies, adjuvants such as *B. pertussis* were used to facilitate immune responses (Bazin and Platteau, 1976; Jarrett et al., 1976). Both sensitization to food antigens administered through the diet (Steinmann and Wottge, 1990; Dahlgren, 1991; Dahlman et al., 1994) and sensitization upon intra-gastric dosing (Bazin and Platteau, 1976; Jarrett et al., 1976) were documented. Studies investigating dose-response relationships and effects of multiple repeated doses demonstrated that larger doses at priming and frequent dosing may not always result in more marked immune responses and may even suppress IgE responses upon booster (Jarrett, 1984). This would indicate that caution is needed in the conduct of oral sensitization studies with rats. Nevertheless, the rat may provide several advantages as a species for oral sensitization research.

First, although immunological tolerance induction may occur in rats (Steinmann and Wottge, 1990), tolerance induction was not observed to be the general response upon oral antigen feeding of rats. In contrast, in rats, as in healthy humans, induction of cell mediated immunity and antigen-specific IgG responses were demonstrated to be characteristic responses to protein antigen feeding, while the induction of IgE responses and anaphylactic sensitization of rats requires specific prerequisites with respect to the study protocol (Knippels et al., unpublished results). In an enteral rat IgE-induction model currently under development (Knippels et al., unpublished results), the percentage of IgE responders, the time of onset of sensitization, as well as the titers of induced antigen specific IgE are being evaluated as possible endpoints in a rat food allergy research model and a model for food protein allergenicity research. A second advantage of the rat as a species for research on effects of substances on, or interactions with, the immune system is the rather broad knowledge of the rat immune system and the availability of the many tools for studies in this field. Moreover, since the rat is commonly used in routine toxicity testing as well as in immunotoxicity studies,

possibilities to investigate oral sensitizing properties of food proteins in the rat will be advantageous and facilitate the evaluation of the knowledge on sensitizing properties of food proteins in a proper perspective with respect to the total findings in rat safety studies. Finally, recent studies demonstrated that upon intra-peritoneal injection of food antigens, specificities of induced antibodies were similar to those in man (Atkinson and Miller, 1994), but comparable studies based on oral sensitization of rats have not been documented as yet. However, rat studies are currently in progress aimed at investigating the differences in oral sensitizing potentials of various food proteins and comparing the results with human clinical data (Knippels et al., unpublished results).

5. Conclusion

Since new, well validated methods for food allergenicity testing with high sensitivity and high specificity can not be expected in the near future, the most benefit is currently expected from a combination of assays and model systems. By using models for human gastro-intestinal digestion in combination with operational *in vivo* antigenicity assays to investigate the residual antigenicity of absorbed fragments, it is possible to study several aspects which are of major relevance in terms of the allergenic potency of dietary proteins. This approach provides the possibility of taking into account the influence of the gastro-intestinal digestion. The combination of these procedures with the routine antigenicity assays may provide an important refinement of the currently available antigenicity assays. Current activities in the field of animal food allergy model development may also prove of value in the near future of the evaluation of allergenicity of food proteins. In addition, the application of combinations of *in vitro* gastro-intestinal model systems under both human and laboratory animal conditions extended with *in vivo* animal studies, may be very helpful in the extrapolation of animal data to man. On a case-to-case basis, evaluation of the antigenicity, process and acid stability, digestibility, residual antigenicity of digested and absorbed protein fragments, and sensitizing properties in animal feeding studies may be performed, thus finally enabling an optimal, yet probably still not perfect, evaluation of the allergenic potency of dietary protein products.

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