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REVIEW

Immunoglobulin E mediated food allergy. Modelling and application of diagnostic and predictive tests for existing and novel foods

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It is known that some foods cause an allergenic response in certain individuals. Clinical and immunological tests are available for the diagnosis of food allergy and identification of food allergens. However, there are no valid tests for the prediction of the allergenic potential of foods not normally recognized as allergenic. Such foods include: food products developed from foods which may not be recognizable as allergenic in their modified forms; foods produced using novel processes (novel foods), for example genetically modified foods; and foods not normally consumed but that are being used increasingly as alternatives to more traditional foods. Both the risks associated with food allergy and the fact that foods such as the ones described above will become available to the consumer, highlight the need for methods to screen for potential food allergens. This review provides a general overview of food allergy including mechanism, development and prevalence, but focuses on and discusses: 1) the possible risks (with specific reference to food allergy) associated with new and novel foods; and 2) the development/use of food allergy models (*in vivo* and *in vitro*) to assess the allergenic potential of new and novel foods.

Keywords: food allergy, IgE, novel food, GMO (genetically modified organism)

Introduction

A whole range of common foods are known to trigger an allergic response. These include milk, egg, peanuts, soya bean, tree nuts, fish, crustacea and wheat (Hefle *et al.* 1996). Foods that are allergenic contain specific proteins (allergens), parts of which are the initiators of the allergic reaction. These allergens have been identified as:

- glycoproteins which have a molecular weight of between 10 and 70 kDa (based on sodium dodecyl sulphate polyacrylamide gel electrophoresis)
- resistant to heat, proteolysis and digestion (with the exception of fruit and vegetable allergens).

(Hefle 1996, Hefle *et al.* 1996, Taylor and Lehrer 1996)

Food allergy can be divided into IgE-mediated reactions and non-IgE-mediated reactions. The IgE-mediated response, the immediate or Type I response, is the most common and frequently reported response to food allergens (Bruijnzeel-Kooman *et al.* 1995, Mekori, 1996) and is discussed in greater detail below.

There are three non-IgE-mediated (usually delayed) reactions. The cytotoxic (Type II) reaction involves the binding of antibodies to cell bound antigens/allergens and the subsequent destruction of the cell bound antigen/allergen either by phagocytosis, or cytolysis by complement proteins. The immune complex (Type

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III) reaction involves the generation of antigen or allergen/ antibody complexes. The cell mediated (Type IV) reaction involves the proliferation of sensitized T-lymphocytes in response to a specific antigen/allergen (Chandra and Shah 1984, Mekori 1996).

There is very little information on the role of the non-IgE-mediated mechanisms in food allergy. In a study of the types of allergic reactions that occur in children with known food allergies, Chandra *et al.* (1993) showed that 6 % had a Type II reaction, 10 % had a Type III reaction, 18 % had a type IV reaction and 28 % had more than one type of reaction. It is unclear which foods give rise to a Type II reaction, however it has been suggested that the Type II response may give rise to anaemia (Chandra *et al.* 1993). Both the Type III and Type IV reactions have been associated with gluten, cow's milk and soya allergies (Host 1994, Bock and Sampson 1994) and are said to be associated with the pathogenesis of gastrointestinal and skin reactions (Chandra *et al.* 1993, Host 1994, Bock and Sampson 1994).

Non-immunologically triggered adverse food reactions (termed food intolerances) are also linked to gastrointestinal disorders such as Celiac's and Crohn's diseases. These chronic autoimmune pathologies are exacerbated by specific food intolerances (Kitts *et al.* 1997; Ballegaard *et al.* 1997), and this is also true for individuals who suffer from ulcerative colitis and non-specific colitis (Ballegaard *et al.* 1997). Another manifestation of food intolerance is migraine headache, which is triggered by a range of stimuli including food high in tyramine or tryptophan. Finally there is a further group of adverse food reactions termed toxic or idiosyncratic reactions that are reported following ingestion of food additives or high levels of sucrose. Reactions in this category range from physical symptoms to behavioural changes (Kniker and Rodriguez, 1986, Boris and Mandel 1994,Wuthrich, 1993, Uhlig *et al.* 1997). Interpretation of oral challenge results in this area are often subjective given the wide range of symptoms that can occur. Also in this category are naturally occurring substances that can stimulate allergic effects; for example foods high in histamine such as fish of the *Scombridae* family, and ripening cheese, can produce a response similar to that mediated by IgE (Kniker and Rodriguez, 1986, Malone and Metcalfe, 1986).

However since the IgE-mediated reaction is the most common and the most extensively researched of the allergic responses, this review will focus on IgE-mediated food allergies.

IgE-mediated mechanism

The IgE-mediated response is divided into two stages, sensitization and allergic reaction. In allergic subjects, initial exposure to a food allergen causes production of IgE molecules by B lymphocytes, which specifically recognize the food allergen. The IgE molecules then bind to high affinity receptors on the surface of mast cells and basophils. Mast cells and basophils are types of white blood cells. Mast cells are present in tissues including skin and the gastrointestinal and respiratory tracts while basophils circulate in the blood. Once IgE molecules are bound to mast cells and basophils the subject is now sensitized to the food allergen. With repeated ingestion, the food allergen comes in contact with an IgE molecule bound to the surface of a mast cell or basophil via a high affinity IgE receptor. The IgE molecule recognizes the allergen and binds to it, becoming cross-linked with another IgE

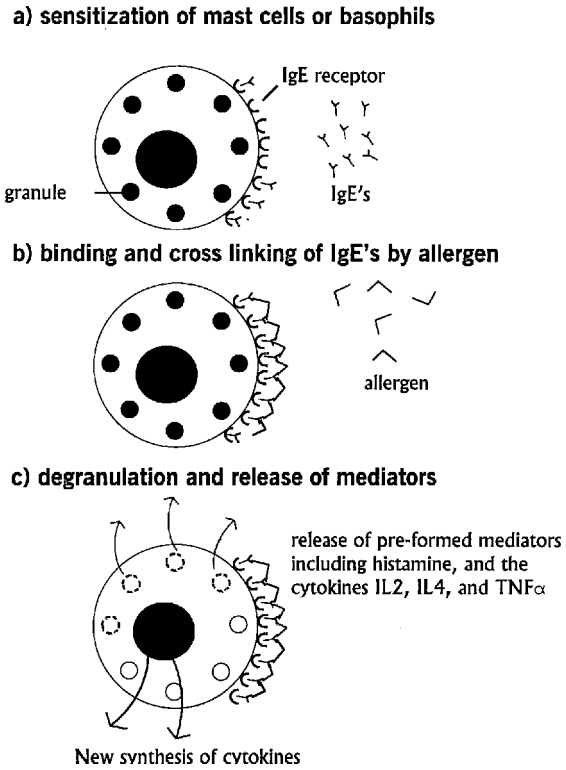

Figure 1. The mechanism of mediator release in sensitized basophils and mast cells.

molecule as a consequence. The cross-linking triggers degranulation of basophils/mast cells (figure 1) which leads to the release of preformed mediators such as histamine, and the synthesis and release of cytokines including IL-4 (interleukin 4) and TNFα (tumour necrosis factor alpha) (MacGlashan *et al.* 1994, Okayama *et al.* 1995, Schroeder *et al.* 1995, Mekori 1996). The allergen's ability to crosslink IgE molecules is key to the IgE-mediated response (Ishizaka and Ishizaka 1984). To achieve crosslinking it is crucial that the allergen is at least bivalent (as far as IgE binding is concerned) with the binding sites suitably/appropriately located (Hefle 1996, Taylor and Lehrer 1996).

As a result of an IgE-mediated response and the subsequent release of mediators, inflammatory reactions occur in the skin, gastrointestinal tract and/or respiratory tract. These reactions include vomiting, diarrhoea, asthma and eczema (atopic dermatitis). Severe reactions may lead to anaphylaxis which, if untreated, may lead to death (Bock and Sampson 1994, Bruijnzeel-Koomen *et al.* 1995, Hefle 1996). However, investigators have noted that the presence of allergen specific IgE does not always lead to the symptoms mentioned above. In some patients, especially children with atopic dermatitis, the presence of IgE specific to foods ingested does not lead to initiation of their symptoms (Bock and Sampson 1994). There are two explanations for what has been described as a distinct phenomenon. One is that the food allergens ingested do not reach the site of action and bind to the IgE sensitized mast cells and basophils in these patients. The other is that the allergen reaches the site of action and binds to IgE sensitized cells, but the

subsequent chain of events that take place following the IgE reaction, and which culminate in an inflammatory response (such as atopic dermatitis) do not occur.

Diagnosis of IgE mediated food allergy

There are a number of techniques available for the diagnosis of IgE-mediated food allergies (table 1). Tests *in vivo* include the Double Blind Placebo Control Food Challenge (DBPCFC) which involves orally giving the patient food containing the allergen. The smell and the taste of the food of interest are masked, although this can sometimes be difficult due to the quantity of food needed for the challenge and/or strong flavours or odours that the food of interest may possess (Bock 1991, Watson 1995). This test, although considered to be the gold standard for diagnosing food allergy, is not always practical, since severe reactions to the food can arise (Bock 1991). In addition, interpretation of results from DBPCFC is not always straight forward: a positive response to a food challenge indicates a cause and effect relationship but will not provide information on the underlying mechanisms that caused the response, that is whether or not the response is due to an IgE-mediated reaction (Watson 1995). This information may be obtained from the results of other diagnostic techniques which are discussed below. Another difficulty which may be encountered in interpreting the results of DBPCFC is that other factors in addition to the food of interest must be taken into account, such as food induced exercise associated anaphylaxis, which cannot be duplicated in a clinical setting due to ethical considerations (Kidd *et al.* 1983, Buchbinder *et al.* 1983, David 1989, Bock 1991, Watson 1995).

Another *in vivo* test is the skin prick test, which involves pricking an extract of the suspected allergen on to the patient's skin. If a bump surrounded by redness forms in 15 minutes then the test is positive and the patient is allergic to the food. The skin prick test is more practical than the DBPCFC and is commonly used in the diagnosis of food allergy. However, there are problems relating to variable responses to different forms of the same food (Bock *et al.* 1978, Sampson 1988, Ortolani *et al.* 1989, Norgaard *et al.* 1992). In addition, a positive skin prick test indicates only that the allergenic food tested and the patient's symptoms are related. Thus, the test cannot be used to determine whether a particular ingested food will give rise to clinical symptoms. In some situations a DBPCFC would need to be carried out, subsequent to the positive skin test to determine the positive predictive value of the test (Watson 1995).

The most common i*n vitro* food allergy diagnostic test is the radioallergosorbent test (RAST) which involves measuring the levels of allergen specific IgE in sera of

Table 1. Standard food allergy diagnostic tests

In vivo tests	*In vitro* tests	Double blind placebo control food challenge (DBPCFC)
Skin prick test prick food extract on to skin of subject; look for skin reaction – rash	Radioallergosorbent test (RAST) measures allergen specific IgE	subject given food, reactions to food observed, taste and smell are masked to prevent subjectivity

allergic patients. This test has been used successfully for egg, peanut and milk proteins. In general it is less sensitive than the skin prick test, more expensive and results are not immediately available (Sampson and Albergo 1984, Watson 1995).

Development and prevalence of IgE-mediated food allergies

As stated in the section on the IgE-mediated mechanism, prior exposure and sensitization to the food allergen must occur before the initial IgE-mediated allergic response. Factors that influence susceptibility to the development of food allergies include genetic predisposition, competence of systemic immunity and an immature mucosal barrier (Chandra *et al.* 1993, Hefle 1996). The latter factor is believed to be key in the development of food allergy in young children: the immature mucosal barrier allows more protein, including food allergens, through the barrier and into systemic circulation. As a result the child's immune system is exposed to these ingested allergens. In many cases of allergy to milk and egg, maturation of the mucosal barrier allows those foods to be eaten without provoking an allergic response (Hefle 1996, Bush and Hefle 1996). However allergic response to some foods, including peanuts and fish, still endure and are seldom outgrown (Bock 1982, Ford and Taylor 1982, Esteban *et al.* 1985, Bush and Hefle 1996). It is believed that the duration of food allergies does in part depend on the allergen (Kjellman 1991). Stability to digestion is a characteristic possessed by certain allergens which may influence the duration of the allergy. Both peanut and fish allergens are reported to be resistant to digestion (Burks *et al.* 1992, Taylor and Lehrer 1996), however, so are milk and egg allergens (Haddad *et al.* 1979, Schwartz *et al.* 1980, Elsayed *et al.* 1986, Honma *et al.* 1994). The age at which food allergy occurs is another factor that may influence duration. Bock (1982) reported that young children (3 years and younger) with food allergy seem to have at least a two-fold higher chance of outgrowing their food allergy than older children. Individual factors concerning the allergic person are also believed to influence duration of an allergy, for example the levels of serum IgE. If the serum IgE levels are abnormally high in children with positive food challenges, it appears that the level of IgE will remain elevated for years (May *et al.* 1980).

T he prevalence of IgE mediated food allergy is unclear. One reason is that many food allergies are perceived rather than real (Jansen *et al.* 1994, Burr and Merret 1983, Bender and Matthews 1981). Other problems are associated with the methods used to investigate the prevalence of food allergy. Methods differ in study design, techniques/procedures on which criteria for diagnosis are based, and criteria for diagnosis. For example, a number of retrospective and prospective studies on the prevalence of cow's milk allergy in children have estimated prevalence to be between 0·1 % and 7·5 % (Vendel 1948, Collins-Williams 1956, Bachman and Dees 1957, Johnstone and Dutton 1966, Halpern *et al.* 1973, Gerrard *et al.* 1973, Jakobsson and Lindberg 1979, Stintzing and Zetterstrom 1979, Hide and Guyer 1983, Bock 1987, Høst, *et al.* 1988, Schrander *et al.* 1993). These studies used diagnostic criteria based on patient history, controlled elimination diets, open milk challenges and/or blind milk challenges [DBPCFC]. Criteria of diagnosis differed in the period between milk challenge and occurrence of symptoms, protocols used for the disappearance of symptoms (e.g. either one cow's milk elimination or two dietary eliminations of cow's milk and cow's milk products) and the inclusion or exclusion of non-immunological intolerance. Additional

differences that may explain the wide variation in milk allergy prevalence reported in these studies include criteria for inclusion into a study, the number of intervals between clinical examination and geography (studies were carried out in Sweden, USA, Canada, England, Denmark and Holland, and possible differences in diet may have affected results).

The differences in the study design and criteria of diagnosis, and the consequent differences in the results obtained from the studies mentioned above indicate that a more co-ordinated approach is needed for the systematic investigation and reporting of food allergy prevalence. Factors considered important in minimizing the differences reported for the prevalence of cow's milk allergy in children include: (i) strict criteria for diagnosis and inclusion; (ii) a well defined and controlled procedure/technique for diagnosis of food allergy; (iii) clinical examination to be carried out by the same physician(s); (iv) close professional observation and (v) use of a standard food in diagnosis (Høst 1994).

Such factors could be used across the board for assessing the prevalence of other food allergies.

Factors that influence the prevalence of food allergy

Genetics: People may be genetically predisposed to developing food allergy. Studies on children have shown that the risk of developing a food allergy is approximately 50 % when one parent is allergic and 67–100 % when both parents are allergic (Schatz *et al.* 1983).

Crossreactivity: Different allergens may share the same epitope structure (the part of an allergen that binds to the allergen's specific IgE) and will therefore cross react. Patients sensitized to pollen, e.g. birch pollen and mugwort pollen, may also suffer from adverse reactions to certain fruits and vegetables including apple, pear, hazelnuts, kiwi carrots and celery (Eriksson *et al.* 1982, Pauli *et al.* 1985, D'Amato 1991, Ebner *et al.* 1991, Hirschwehr *et al.* 1992, Gall, *et al.* 1994).

Geography: In areas where allergy to birch pollen and mugwort pollen is prevalent 30–50 % of these people present symptoms when ingesting the fruits and vegetables listed above (Dreborg and Roucard 1983, Ortolani *et al.* 1989, De Blay *et al.* 1991).

High consumption of certain foods: Food allergy prevalence may also be influenced by the large consumption of certain foods. Soya bean and rice allergies are more prevalent in Japan, probably because of the high consumption of both these foods (Food and Agriculture Organisation of the United Nations 1995). The frequent consumption of peanuts and the early age at which children have peanuts (in the form of peanut butter) introduced into their diets are probably the two major factors underlying the high prevalence of peanut allergy in the US (Hefle *et al.* 1996). In addition, since peanut products have been widely marketed throughout Europe, it is now a major allergen in Europe (Report of the Scientific Committee for Food on Adverse Reactions to Food and Food Ingredients). The prevalence of fish allergy in Scandinavia has been attributed to similar causes, namely exposure at a young age and frequent ingestion as part of the normal diet (Aas 1966).

Introduction of new foods to the diet: Not only is the prevalence of food allergy influenced in part by the frequency with which particular foods deemed common to a particular country are eaten, but the introduction of new (uncommon or non-

indigenous) foods can also influence the incidence of food allergy. For example, the introduction of soybean to the French diet in the mid 1980s, led to an increased incidence of soya bean allergy (Moneret-Vautrin 1986). In the US, the introduction of kiwi fruit in the 1980s was followed by reports of allergic reactions to kiwi in the literature (Fine 1981, Fallier 1981).

The introduction of new foods, and specifically the allergenic potential of such foods, has generated a lot of attention. Recently, much has been written on the subject of assessing the allergenic potential of new and novel foods. The second part of this article will assess the current thinking on determining the allergenic potential of new and novel foods and discuss the possible use of standard IgE models/tests in the prediction of allergenicity.

The potential allergenicity of new and novel foods

New and novel foods, and their allergenic potential have attracted a lot of attention and concern from a number of food safety organizations/committees (Jonas *et al.* 1996, Metcalfe *et al.* 1996, Nestle 1996, Nordlee *et al.* 1996). In a report of the Scientific Committee for Food on Adverse Reactions to Food and Food Ingredients it was noted that with the development of new technologies the food industry is able to develop products "which may not be recognized as allergenic in their modified form... or may become allergenic in previously non-allergic patients."

Examples of allergenicity

Foods that are produced using novel processes including genetic modification, for example transgenic soya beans. Transgenic soya beans were developed in an attempt to increase the levels of sulphur rich amino acids (methionine and cysteine) in soya based animal feed so as to promote growth, (Nestle 1996, Nordlee *et al.* 1996). A transgene for the protein 2S albumin, which is rich in methionine and cysteine, was introduced into soya bean. However, this gene was isolated from Brazil nut which is known to elicit an IgE-mediated response in certain individuals. Taking the allergenicity of Brazil nut into consideration, Nordlee *et al.* (1996) assessed the allergenicity of 2S albumin. Results from their study showed that 2S albumin is probably a major Brazil nut allergen. In addition, their study showed that 7 out of 9 people allergic to Brazil nuts but not to soya beans, were allergic to transgenic soya beans, and thus provided an example of genetic manipulation leading to the transfer of an allergen to a new food.

Foods may contain unfamiliar forms of ingredients that are known to elicit an allergic response, for example Simplesse®, a fat substitute. Simplesse® is a product formed by the microparticulation (heating and shearing of food proteins to cause coagulation) of egg white and cow's milk protein. It has been used in cream and oil based foods including sour cream, soft cheese, salad dressing and mayonnaise, and is available in the US and the UK. Since processing of food proteins may lead to alterations in the allergenicity of the natural food (Bleumik 1970), investigators studied the effect of microparticulation on the allergenicity of egg white and milk proteins (Sampson and Cooke 1992). The study found that the major egg and milk allergens in the microparticulated product, Simplesse®, were the same as those found in the starting materials, egg white or milk, and thus concluded that microparticulation had no effect on the allergenicity of egg and milk. However, the

study did highlight that presentation of egg and milk proteins in an unfamiliar form by microparticulation could be a potential danger to persons allergic to either egg or milk, in that they may unknowingly ingest egg and milk allergens. Therefore clear labelling of Simplesse® and other food products containing microparticulated proteins is required since other proteins, e.g soya beans, can be used in this process.

Foods that are not normally consumed but are gathering prominence as alternatives to traditional foods, for example lupin seed, also come under the category of new foods. Lupin seed comes from the same family as soya bean, peanut and pea which are all known to elicit IgE-mediated allergic responses. Hefle *et al.* (1994) reported the reaction of subjects, known to be allergic to green peas, to pasta that contained lupin seed flour. Their study also suggested that peanut-sensitive subjects may also be allergic to lupin seed flour.

Methods used to assess the allergenicity of the above foods were all standard diagnostic and immunologic techniques (skin prick test, RAST, SDS PAGE) since all the foods either contained or were associated with allergenic foods. However such tests are of little use in the initial allergy assessment of new or novel foods whose components are not known to be associated with allergenicity. Nordlee *et al.* (1996) in their paper on transgenic soya beans pointed out that genetically modified foods for which the 'allergenicity of the donor genetic material is not known' were being used increasingly in food biotechnology. It is therefore essential that predictive tests for such foods are available. However, in a review on the assessment of safety of novel foods the International Life Sciences Institute (ILSI) European Novel Food Task Force (Jonas *et al.* 1996) noted that " there are no validated predictive tests for assessing the potential allergenicity of proteins from sources that are not commonly recognized allergens."

Assessing the potential allergenicity of new and novel foods: suggested approaches

Due to the lack of predictive tests for assessing the potential allergenicity of new and novel foods, alternative approaches for screening for allergenic potential have been put forward. Investigators have suggested following criteria based on specific characteristics of known food allergens to identify novel foods and proteins as potential allergens (Jonas *et al.* 1996, Metcalfe *et al.* 1996). This approach involves comparing properties – physical, chemical and biological – of novel foods/proteins with those of known food allergens, i.e. using the properties of known allergens as indicators of allergenicity.

Properties of allergens

Molecular weight and possession of carbohydrate moieties. Most known food allergens have a molecular weight of between 10–70 kD and are glycosylated (Taylor and Lehrer 1996). However, these properties are possessed by many proteins that are not allergens, therefore molecular weight and glycosylation are not useful indicators of allergenicity (Fuch and Astwood 1996, Hefle 1996).

Heat stability, resistance to digestion and low pH environment. Most food allergens are resistant to high temperatures, low pH, and proteolysis and digestion (Taylor and Lehrer 1996). Exceptions include fruit and vegetable allergens. Reactions to fruit and vegetable allergens occur primarily in the mouth (oral allergy syndrome – OAS; Amlot *et al.* 1987, Ortolani *et al.* 1988, Hefle 1996, Vieths 1997). By the time

the fruit or vegetable reaches the gastrointestinal system, specifically the stomach, symptoms recede, indicating that the allergens are not resistant to the process of digestion.

Sequence homology (comparison of amino acid sequences with those of known food allergens and linear allergenic epitopes). From epitope mapping studies done on both food and non-food allergens including fish, egg, yellow mustard seed, house dust mite and grass and tree pollen (Elsayed and Apold 1983, Menendez *et al.* 1990, Johnson and Elsayed 1990, Elsayed *et al.* 1991, Greene *et al.* 1991, Zhang *et al.* 1992, van Milligen *et al.* 1994, Shimojo *et al.* 1994, Aki *et al.* 1994) investigators have recommended that an immunologically significant sequence identity requires at least eight contiguous identical amino acids (Rothbard and Gefter 1990). Such a technique may be helpful in identifying potential continuous IgE-binding epitopes (i.e. epitopes composed of a series of covalently linked amino acids, also known as linear epitopes, Taylor and Lehrer 1996). If a protein under investigation matches the above criteria, IgE binding capabilities can be investigated using either RAST, ELISA, or Western blotting techniques (Metcalfe *et al.* 1996). However, this technique cannot be used to identify conformational epitopes (i.e. epitopes that depend on the tertiary structure of the allergen or specific amino acid sequences present on the surface of the allergen, also known as discontinuous epitopes) because they are made up of two different amino acids sequences which, *via* their tertiary structure, form one epitope (Seiberler *et al.* 1994, Taylor and Lehrer 1996). To facilitate identification of such epitopes, 3-D structures of known allergens would need to be compared with those of proteins under investigation. However, construction of these 3-D structures may prove long and difficult. An additional drawback of using the comparison of linear amino acid sequences to identify potential linear epitopes is that conserved sequences that are not related to the allergenic potential of the proteins are likely to be identified (Metcalfe *et al.* 1996).

It is possible that with more research into the physico-chemical properties and primary and tertiary structures of food allergens and their epitopes, criteria for selection may change. However, in the meantime some of the properties listed above may be useful in providing an indication of potential allergenicity. Beyond the use of these properties as indicators of allergenicity, further assessment of allergenic potential is possible with the use of standard diagnostic and immunological techniques (Metcalfe *et al.* 1996) discussed earlier. Alternatively a novel predictive model based on the IgE mediated response could be used.

Possible models for determining allergenic potential of novel foods/proteins

Currently the majority of IgE models have been used to further characterise and understand the IgE mediated mechanism and conditions associated with food allergy. The potential of some of these models to assess and investigate food allergenicity will be discussed below.

Animal models and adaptations of existing in vivo and in vitro models

A number of animal models have been used to investigate and understand the IgE mediated mechanism in humans. These models include rat and guinea pig anaphylaxis models (Perdue and Gall 1985, Granti *et al.* 1985, Becker 1995), dog models to study asthma (Ebbeling and Buckley 1989, Frick *et al.* 1995), and mouse IgE models (Sehon and Mohapatra 1992). One animal IgE model that has been

modified for use in the assessment of allergenic potential is the Brown Norway (BN) rat, which in the past has been used to study allergic responses (Atkinson and Miller 1994, Eidleman *et al.* 1988, McMenanmin and Holt 1993, Miller and Nicklin 1988). The BN rat model has been successfully modified/adapted to investigate food allergy. Studies by Atkinson and Miller (1994) identified optimum conditions for inducing sensitivity to milk proteins using the BN rat model, and showed that the IgE specificity against milk proteins present in rat sera resembled IgE responses to milk found in the sera of milk-allergic patients. The presence of IgE in rat sera was assessed by passive cutaneous anaphylaxis (PCA), an *in vivo* allergy assay which involves the release of a dye (Evans blue) from blood vessels into the skin following an IgE response (Ovary 1964). A further study by Atkinson *et al.* (1996) successfully showed that the BN rat could be used to investigate the influence of dietary factors on the development of food allergy, specifically the development of oral sensitization by food allergens. Thus, the BN model's capacity to mimic the IgE response in humans and to demonstrate the influence of other factors on the development of food allergy suggest that it can be used to investigate food allergies and their reactions, and to assess the allergenic potential of new/novel foods.

The need to understand further the immune responses in humans using animal models has led to the development of a 'humanized' mouse IgE model (and 'humanized' mouse and rat mast cells and basophils) expressing IgE receptors that are able to bind human IgE. (IgE molecules are species specific therefore human IgE cannot bind to murine high affinity IgE receptors.) The process of 'humanization' involves genetic manipulation of the rodent high-affinity IgE receptor. The high-affinity IgE receptor has four subunits: one alpha (to which IgE binds directly), one beta and two gamma. Incorporation of the human alpha subunit gene into the rodent high affinity IgE receptor has been achieved by transfecting appropriate cDNA into rat basophilic cells (Miller *et al.* 1989, Wilson *et al.* 1993, Taudou *et al.* 1993, Lowe *et al.* 1995). It has also been accomplished by transfecting genomic DNA that encoded the human alpha subunit into embryonic stem cells which were then used to generate transgenic mice whose mast cells expressed the human alpha subunit (Wai-Ping *et al.* 1996, Dombrowicz *et al.* 1996).

The recognition of human IgE by these modified rodent mast cells and basophils was assessed by the release of IgE mediators (histamine, β-hexoaminidase and serotonin; Wilson *et al.* 1993, Lowe *et al.* 1995 Wai-Ping *et al.* 1996, Dombrowicz *et al.* 1996), increase in intracellular (mast cell or basophil) calcium and the activation of tyrosine kinases (early intracellular signalling events triggered by cross-linking of IgE molecules; Taudou *et al.* 1993, Drombrowicz *et al.* 1996, Wai-Ping *et al.* 1996), and PCA (Wai-Ping *et al.* 1996, Dromdrowicz *et al.* 1996). It is hoped that this type of model will facilitate the design of drugs that target the binding of IgE to the high affinity receptor. However, some investigators have used 'humanized' rodent basophil cell lines to confirm the presence of allergen specific IgE in blood of allergic patients: Lowe *et al.* (1995) developed a rat basophil cell histamine assay using rat basophilic leukaemic cells transfected with the human alpha subunit. Cells were pre-sensitized with IgE present in plasma from human subjects allergic to common allergens, including house mite and ragweed. A comparison of allergen induced histamine release showed a good correlation between the rat basophil cell histamine assay and its human equivalent.

Thus, the 'humanized' model certainly has the potential for screening new/novel foods associated with possibly allergenic foods/food components, using the sera of sensitized humans.

Rodent and human cell/organ based models

The use of human and rodent cell models in investigating the IgE response has focused on IgE mediators, mainly cytokines, and their involvement in antigen/allergen specific IgE-associated inflammatory responses. The models themselves all involve the sensitization of mast cells or basophils with IgE, after which the cells are challenged either with a specific antigen/allergen or anti-IgE (for a non-specific response). Production of cytokines from the IgE-activated cells is then assessed by determining cytokine-mRNA levels and/or release. Numerous studies using freshly prepared mouse peritoneal mast cells, cloned growth factor independent/dependent mast cell lines derived from mouse spleen cells, mouse bone marrow, or mouse foetal liver, have been used to demonstrate IgE-dependent cytokine (TNFα, IL-1 IL-3, IL-4, IL-5 and IL-6) synthesis and release (Richards *et al.* 1988, Plaut *et al.* 1989, Wodnar-Filipowicz *et al.* 1989, Burd *et al.* 1989, Gordon and Galli 1990, Tsai *et al.* 1990, Gurish *et al.* 1991, Galli *et al.* 1991).

Freshly prepared human mast and basophilic cells have also been used to study IgE dependent cytokine release. Gordon *et al.* (1991) reported that purified human lung cells release TNFα following IgE dependent activation. In addition, Bradding *et al.* (1992) reported an increase in IL-4 release from purified human lung mast cells following IgE-dependent activation. Okayama *et al.* (1995) showed that the mRNA levels of cytokines TNFα, IL-4 and IL-5 could be increased in IgE sensitized cells following non-specific activation with anti-IgE in purified human lung mast cells. Work by Brunner *et al.* (1993) using human leukaemic basophils, showed that, following IgE dependent activation, there was an increase in the production and release of IL-4. MacGlashan *et al.* (1993) and Schroeder *et al.* (1994), using human basophils, reported that IgE sensitized human basophils produce and release IL-4 following IgE mediated activation. In addition, to using human cell systems, investigators have also utilized human organ culture systems to investigate the production and release of cytokines from mast cells. Investigators have used human foreskin and lung culture systems to show increased production and release of TNFα from human skin and lung mast cells, respectively (Klein *et al.* 1989, Ohkawara *et al.* 1992) following IgE dependent activation of these systems.

As an alternative to using freshly prepared human mast cells and basophils, cells developed *in vitro* may be used. Human mast cells have been derived *in vitro* from nucleated cells of human umbilical cord blood co-cultured with mouse skin derived 3T3 fibroblasts (Furitsu *et al.* 1989). After approximately 12 weeks in culture, mature human mast cells had developed. The presence of mast cells was confirmed by electron microscopy and by the presence of tryptase and chymase, proteases which are found exclusively in mast cells. In a further study, nucleated cells of human umbilical cord blood differentiated into human mast cells following 14 weeks culture (Mitsui *et al.* 1993). Differentiation of the blood cord cells was induced by the mast cell growth factor c-kit ligand. The mast cells were found to be immature but they expressed high affinity IgE receptors and could be sensitized with human IgE for anti-IgE activated histamine release. Human mast cells have

also been developed *in vitro* from human foetal liver cells grown with the mast cell growth factor c-kit ligand or co-cultured with 3T3 fibroblasts.

Human basophils have also been derived *in vitro*. Razin *et al.* (1981) reported that basophilic leukocytes were derived from human foetal liver cells grown in culture for approximately 6 days. Characterization of the cells included histamine content and the presence of IgE receptors. Human basophils have been developed from umbilical cord blood cells (Ogawa *et al.* 1983, Dvorak *et al.* 1985), human bone marrow cells (Denburg 1990) and leukaemic cells (Fishman *et al.* 1985). Characterization of these *in vitro*-developed basophils included determining histamine content, identification of surface IgE receptors, and stimulation of histamine release following non-specific activation using anti-IgE. In addition to characterization, such *in vitro*-derived basophils have been used successfully to investigate IgE mediators. Arock *et al.* (1993) have reported that IL-4 release was significantly increased following IgE dependent activation of human normal basophils derived from bone marrow cells.

One objective for using human mast cells and basophils derived *in vitro* was to try to define the role of mast cells and basophils in IgE-mediated responses *in vivo*, however investigators recognize that there are drawbacks to this approach, specifically for *in vitro* derived mast cells. In the case of mast cells, their populations (lung, skin, gastrointestinal tract) in both humans and rodents are phenotypically distinct, suggesting that these mast cell populations may exhibit different functions (Kitamura 1989, Stevens and Austen 1989, Furitsu *et al.* 1989, Galli 1990, Mitsui *et al.* 1993). This means that interpretation of data from an *in vitro*-derived-mast cell IgE-mediated model applied to IgE-stimulated mast cells *in vivo*, as far as defining the roles of specific mast cell populations during an immunologic response, needs to be cautious. In spite of these problems such models may still prove useful in the screening of potential food allergens since the focus would be on the food and its allergenicity rather than on defining the immunological roles of different mast cell populations. A preliminary study done in the authors' laboratory to investigate the usefulness of an *in vitro* model in screening for potential allergens has suggested that such models could be used to predict allergenicity (unpublished observations). The study involved the screening of antigens using a cloned growth factor-dependent murine mast cell line (MC/9) sensitized with mouse IgE (monoclonal anti-zearalenone). Following the exposure of a series of antigens/allergens (zearalenone-BSA, anti-IgE and peanut lectin) to the IgE-sensitized mast cells, the release of IgE mediators histamine and TNFα was measured. Of the two mediators TNFα proved to be the better marker of IgE specificity, suggesting that TNFα could be used as a marker of allergenicity in a mast cell *in vitro* based system. However, one drawback of any *in vitro* model is the inability to generate allergen specific IgE. One would need to rely upon an *in vivo* model such as the BN rat model to generate allergen specific IgE if using a rodent cell line, or be able to access a wide range of human sera (for example the Janus serum bank, Jellum *et al.* 1995) if using human or humanized rodent cell lines.

The leukocyte (basophil) histamine release test is one *in vitro* test that does not require the separate generation of allergen specific IgE, since the basophils used are obtained from sensitized subjects. Significant correlation between basophil histamine release and inhalant allergy has been reported (DuBuske 1993). The role of the basophil histamine test as a diagnostic tool in food allergic individuals has been investigated (Kleine-Tebbe 1993). However, due to conflicting results which

include false positives, and the spontaneous release of histamine from basophils (minus allergen challenge) obtained from food allergic individuals (May 1976, Sampson *et al.* 1989) there have been recommendations that the basophil histamine release is not a useful diagnostic tool (Kleine-Tebbe 1993). However, studies suggest that the basophil histamine release test may be used to determine the immunological activity of food allergens. Studies using milk, potato and fruit allergens, have shown that the pattern of response of basophil histamine release to these allergens was indicative of the immunological activity of these allergens (Kleine-Tebbe 1993, Wahl *et al.* 1990, Kleine-Tebbe *et al.* 1992). With further development a basophil system might be useful for predicting the immunological activity of foods suspected of containing allergenic components or altered allergenic components.

Conclusion

With the introduction of new and novel foods to the consumer and the increased awareness of the dangers of food allergy to certain individuals, the determination of the allergenic potential of such foods is essential. With this need to assess food allergenicity comes the need for predictive tests. Most food allergies are mediated via the Type I (IgE-mediated mechanism) and there are *in vivo* and *in vitro* models (some of which have been discussed in this review) that have been used to study the IgE-mediated response extensively. It is therefore reasonable to suggest that such models, including those discussed in this review, could be used as viable alternatives to the standard food allergy diagnostic techniques (skin prick test and RAST) which have been used to screen new and novel foods known to contain possibly allergenic components (e.g lupin seed flour and transgenic soya beans).

Concerning novel foods where there appears to be no association with allergenicity, the use of criteria based on the physical, chemical and immunologic properties of known food allergens, such as those suggested by ILSI, may be used as a preliminary screen to indicate allergenicity. Furthermore, such criteria used in conjunction with a viable predictive IgE model/test could facilitate the assessment of allergenic potential. An IgE based model such as the BN rat could be used to identify a novel food as allergenic *via* the production of allergen specific IgE. This could be assessed using immunoblotting, ELISA or PCA. Alternatively, allergen specific IgE could be used together with an *in vitro* cell based model (for example, a rodent mast cell model similar to the one developed in the authors' laboratory) to confirm the allergenicity of a novel food/protein, by measuring the release of mediators following IgE-mediated mast cell degranulation. Overall it is clear that any approach or system used in determining the potential allergenicity of novel foods/proteins requires further development, and a more co-ordinated approach to draw together the epidemiology, diagnosis, and research of food allergy. This includes setting firm criteria for food allergic reactions, the establishment of IgE serum banks and developing guidelines and tests for assessing allergenic potential of novel foods.

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