

IMMUNOTHERAPY FOR FOOD HYPERSENSITIVITY

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Foods are an important cause of severe acute hypersensitivity reactions including fatal anaphylaxis. Food allergy has been estimated to be the most frequent cause of anaphylaxis treated in emergency rooms.⁶⁹ Severe reactions to foods can occur at all ages, from infants receiving cow's milk or casein or whey hydrolysate formulas^{21, 57} to children, adolescents, and adults.^{56, 70} Although some reactions caused by formula proteins in infants may decrease in intensity with age,⁷ the risk for severe reactions to other foods persists for long periods of time, even in absence of exposure.⁸

The current management of patients with severe acute food reactions is based on avoidance of specific foods and on the availability of emergency medications to immediately treat an acute reaction. These severe reactions have been associated with fish, shellfish, nuts and seeds, legumes, celery, and to a lesser extent with grains, milk, and eggs.^{7, 8, 70} It is clear from looking at this list that even the most careful patients may not be safe from an accidental or inadvertent exposure. In most instances, individuals experiencing a food-induced anaphylactic reaction have inadvertently ingested the food that caused the allergic reaction.⁵⁶

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The unpredictability of accidental exposures and the long periods of time during which patients at risk may not come in contact with the offending foods makes it difficult to have acute treatment medications available at all times, which is necessary for the prevention of fatal food reactions.^{56, 70}

The vast majority of the acute, severe reactions to food appear to be IgE-mediated, although non-IgE-mediated reactions can also account for some severe food reactions.²⁸ The presence of IgE antibodies as the likely cause for severe acute food reactions suggests the possibility of changing this allergic reactivity to a less noxious or even protective form of immune response through immunotherapy or by altering the ability of major food allergens to interact with IgE antibodies through molecular biology methods.

Strategies for developing any potentially successful food allergy immunotherapy for patients with life-long sensitivities must satisfy several requirements. First, these methods must be safe and provide an optimal risk-to-benefit ratio for the patient. Procedures must be cost effective and relatively easy to perform so that patients can be treated on a routine basis. In the ensuing discussion, we explore the mucosal immune response as it relates to food allergy, potential treatments of food-allergic individuals through alteration of the host immune responses, use of mucosal vaccines, and traditional and novel approaches to immunotherapy. Finally, genetic engineering and its use to alter allergenic protein molecules to reduce or abolish their allergenicity is discussed.

OVERVIEW OF THE GUT MUCOSAL IMMUNE RESPONSE

Although it is clear that immune responses to most immunogens that pass through the gut are limited, allergic reactions occur in some individuals in response to certain ingested proteins. The mechanisms responsible for such allergic reactions occur in the context of what is known about normal immune responses in the gut mucosa, and as such, food allergies must be considered as mucosal immune responses.⁴³

The mucosal immune response in the gut may involve several different populations of leukocytes (for a review, see reference 48): (1) leukocytes within gut lymphoid aggregates (Peyer's patches); (2) leukocytes that are directly in contact with gut epithelial cells (intraepithelial leukocytes); and (3) leukocytes located in the lamina propria, a layer of connective tissue between the epithelium and muscularis mucosa. As would be expected, the location of each of these leukocyte populations dictates, in part, how they will contribute to mucosal immune responses in the gut. A review of the composition and function of these discrete leukocyte populations suggests localization and is an important factor for the inductive and effector phases of food allergies at mucosal sites (Fig. 1).

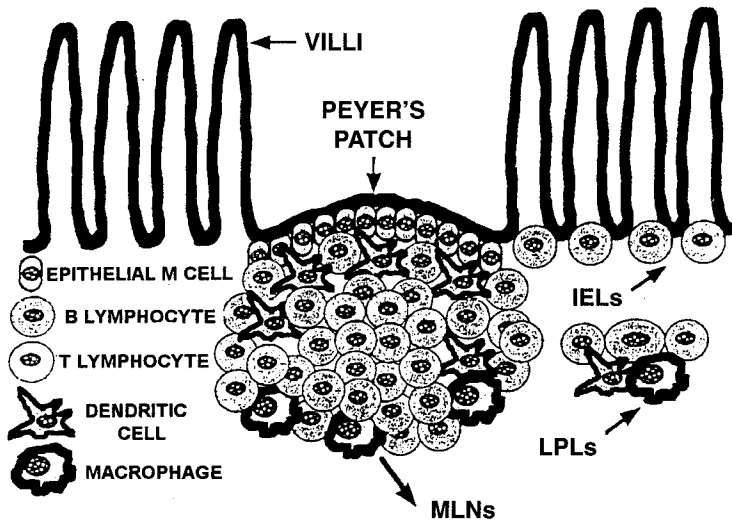


Figure 1. The mucosal immune system in the gut. On entering the lumen of the small intestines, immunogens present in the gut are sampled by way of specialized epithelial M cells that overlay the Peyer's patches. Immunogens then can interact directly with antigen-specific B lymphocytes or can be processed and presented to T lymphocytes by dendritic cells and macrophages in the Peyer's patches. Intraepithelial lymphocytes (IELs) also can interact with immunogens that can cross to the basolateral surfaces of epithelial cells that make up intestinal villi. Once activated by immunogens, leukocytes in the gut mucosa can drain to the mesenteric lymph nodes (MLNs), from which they can gain access to the peripheral circulation. Activated leukocytes also can recirculate back to the mucosa, and some lamina propria leukocytes (LPLs) represent populations of such cells that have relocated. Combined, the immune cells of the gut mucosa represent one of the largest populations of leukocytes in the body, and function to sample and respond to immunogens present in the lumen of the gut.

Peyer's patch lymphocytes are separated from the lumen of the gut by specialized epithelial cells, termed *M cells*, which are extremely efficient at sampling antigens present in the lumen of the small intestine.⁴⁵ Following entry of immunogens into the Peyer's patch via M cells, professional antigen-presenting cells (i.e., macrophages and dendritic cells) can then process and present antigenic peptides to T lymphocytes.³³ B lymphocytes can also interact with immunogens sampled into the Peyer's patches, and in this manner, T and B lymphocytes can be activated by antigens to proliferate and differentiate if the appropriate co-stimulatory molecules are also present. Such co-stimulatory signals include the interaction of cytokines with their receptors and the ligation of certain cell surface molecules (e.g., CD28 and B7). Thus Peyer's patches have an important "inductive" function in the gut that allows efficient initiation of an immune response. Allergens in the gut are presented within the Peyer's patches and activate allergen-specific lymphocytes that are found there. For food allergens, therefore, the Peyer's patch would also be an "inductive" site for lymphocyte activation.⁴⁸

Once activated, antigen-stimulated lymphocytes within the Peyer's patches can migrate into the lymphatics, through the draining lymph nodes, and eventually enter the peripheral circulation. It is in this manner that immunization at mucosal surfaces often leads to detectable immune responses in the periphery. Such migration of allergen-activated lymphocytes from mucosal sites would explain the presence of such allergen-specific lymphocytes in peripheral lymphoid organs or other areas distant from the gut. In addition, these antigen-activated lymphocytes recirculate back to the lamina propria of mucosal tissues.¹ Recirculation of mucosally activated lymphocytes back to mucosal sites is responsible for a significant proportion of the "effector" phase of the mucosal immune response. Here, these lymphocytes, along with macrophages and dendritic cells, can carry out effector functions, such as the secretion of dimeric IgA by plasma cells, or cytokines by CD4+ T lymphocytes. It would be expected that IgE-secreting plasma cells within the gut would be present in highest abundance in the lamina propria of allergic patients. Therefore, leukocytes within the lamina propria would represent an "effector" site for some of the mediators responsible for food allergies.^{1, 33}

In addition to leukocytes within the Peyer's patches, there are also significant populations of leukocytes closely associated with gut epithelial cells, called intraepithelial leukocytes. The lymphocytes present in this compartment are predominantly CD8+ T lymphocytes; however, there are also a significant number of gamma delta T lymphocytes, mast cells, and natural killer cells that also contribute to immune responses in the gut. These leukocytes could encounter antigens that are taken up by epithelial cells of the gut, and are then presented at the basolateral surface of the epithelial cells. Thus responses of intraepithelial leukocytes can represent both inductive and effector responses because epithelial cells can present antigens to initiate a response and because natural killer cells and mast cells can release mediators that would augment allergic reactions.

Complicating the interpretation of inductive and effector sites in the gut is the observation that leukocyte populations within the mucosa are significantly altered during immune responses. For example, increased infiltration of leukocyte populations into the lamina propria and intraepithelial cell compartments of the gut can occur during inflammatory responses. Furthermore, the type and duration of such infiltration depends in large part upon the particular immunologic insult. In an effort to understand the molecular and cellular basis of food allergies, future efforts need to establish appropriate models for type I- and type IV-mediated hypersensitivity disease.

Given this brief overview of immune responses in the gut, it is clear that the cellular and molecular mechanisms that dictate reactions to food allergens in the gut are not well defined. In particular, it is not clear why some individuals have clinically significant allergic reactions whereas most do not. The same mechanisms that predispose patients to systemic allergies could explain this predisposition; however, as pointed

out previously immune responses within the gut mucosa are unique. Additional considerations for mucosally induced type I allergic reactions might include an increased number of IgE-producing cells in the lamina propria, an increased number of mast cells in the lamina propria, or an increased number of mast cells in the intraepithelial compartment. For type IV food allergies, the T lymphocyte phenotype and chemokine and cytokine profiles in the mucosa of patients might be important considerations. It would not be surprising if such differences were observed in patients with food allergies, which would result in a predisposition of these individuals for such diseases. However, there is yet no experimental or clinical evidence to support this possibility.

IMMUNOTHERAPY

Traditional and Novel Approaches

Immunotherapy of IgE-mediated diseases has been used since its first description by Noon in 1911.⁴⁶ Immunotherapy for food allergy was first described by John Freeman, a colleague of Noon, in 1930.²⁴ Freeman reported successful administration of a rush immunotherapy protocol with fish allergen to a 7-year-old boy with poorly controlled asthma. The child was exquisitely sensitive to fish. He developed urticaria, angioedema, vomiting, diarrhea, and worsening of his asthma with fish ingestion. The patient lost clinical sensitivity to fish as well as sensitivity on prick skin testing after rush desensitization. Interestingly, his desensitized state was maintained with daily ingestion of a fish-containing diet. Over the decades, however, injection immunotherapy has not been used in the treatment of food allergy; avoidance of the offending food(s) has been the standard recommended therapy. Until recently, few injection immunotherapy studies for food allergy had been performed; Nelson et al performed a double-blind, placebo-controlled trial of rush immunotherapy for the treatment of anaphylactic sensitivity to peanuts.^{44, 50} Although the patients in the treatment group developed increased tolerance to the oral ingestion of peanut allergen, an unacceptably high rate of systemic reactions accompanied not only the rush protocol but also the maintenance immunotherapy schedule.

Because of the high incidence of untoward reactions associated with this form of traditional immunotherapy, for both inhalant and food allergies, alternative methods are being developed to reduce the risk-to-benefit ratio in the treatment of allergic disease. Although these methods are being developed primarily for use in the treatment of inhalant allergies, some may potentially apply to treatment of food allergies.

Pharmacologic therapies for the treatment of respiratory allergic disease have markedly improved in the last decade, but treatment for food allergy remains strict avoidance and symptomatic relief of systemic reactions with antihistamines, vasoconstrictors, and volume expanders. Novel immunotherapy methods that are currently being developed and

Table 1. EMERGING IMMUNOTHERAPY METHODS

Therapy	Route	Mechanism	Safety
Oral immunotherapy	Oral	Tolerance	Appears safe
Immune complex therapy	Intradermal	Unknown	Appears safe
Peptide immunotherapy	Subcutaneous/oral	Diminish T-cell reactivity	Appears safe
Anti-IgE	Intravenous/subcutaneous	Deplete allergen-specific IgE	Appears safe
DNA immunization	Subcutaneous	Switch Th2 to Th1 response	Unknown

studied for IgE-mediated disease suggest promise for effective and safe future therapies. The methods currently being tested include oral, immune complex, and peptide immunotherapy, as well as anti-IgE therapy and DNA vaccine therapy (Table 1).

Oral Immunotherapy

Oral immunotherapy with large doses of allergen has been shown to induce immunologic tolerance in animal models,⁶⁵ but studies in humans have shown mixed results in efficacy.^{64, 66} These studies have been performed in patients with respiratory allergy associated with pollens. Taudorf et al⁶⁴ demonstrate a beneficial clinical effect of oral immunotherapy in birch pollen-allergic patients during birch pollinosis. Eye symptom scores and conjunctival sensitivity by conjunctival provocation test were decreased, whereas nasal symptom scores, nasal sensitivity by provocation testing, and allergy medication scores remained the same. Although effective birth pollen doses were up to 200 times those used in traditional subcutaneous immunotherapy, they were well tolerated, with minimal side effects.

More recently, Van Deusen et al⁶⁶ reported clinical efficacy and safety of oral immunotherapy with a new oral delivery system. A serologic response similar to that seen in conventional subcutaneous injection immunotherapy in the treatment group and increases in short ragweed-specific serum IgG and IgG₄ were noted; no blunting of the seasonal rise in ragweed-specific IgE was demonstrated. Increases in IgG and IgG₄ have been associated with clinical efficacy in conventional injection immunotherapy. Although decreases in nasal challenge responses and symptoms scores were not statistically significant, a trend downward in nasal symptoms and challenge scores was observed in the treatment group.

The findings in these two double-blind, placebo-controlled studies suggest that oral immunotherapy is safe, well tolerated, and may be effective clinically. Future studies with higher doses or longer duration

may prove beneficial as clinical and serologic parameters suggest immunologic alteration that has been associated with clinical improvement. Use of oral immunotherapy for foods, however, may not be practical since many food-allergic patients are in fact exposed orally to high allergen food doses prior to or following sensitization, without any documented diminution of their response.

Immune Complex Therapy

Immune complex therapy using autologous immune complexes has also been investigated. Machiels et al^{38, 39} studied the effects of antigen-antibody complex therapy for the treatment of immediate-type hypersensitivity disease. Patients suffering from allergic rhinitis and asthma associated with exposure to grass pollen were injected intradermally with antigen-antibody complexes prepared from grass pollen allergens and autologous-specific antibodies isolated from the sera of grass pollen-allergic patients. This form of therapy improves nasal and bronchial asthma symptom scores, and clinical benefit is achieved within weeks of treatment. In addition, this treatment prevents the expected seasonal rise in specific IgE antibodies without increasing specific IgG antibodies. Although many of these measures were generally subjective, there were statistically significant differences when compared with the placebo group, and this form of therapy appeared to be safe. In a second study,³⁹ asthmatics with sensitivity to the house dust mite *Dermatophagoides pteronyssinus* were treated with antigen-antibody complexes prepared from dust mite allergen and dust mite-specific IgG. After immunotherapy by the immune complex method, bronchial and skin reactivity to *D. pteronyssinus* were significantly improved when compared with that of placebo. Symptom scores and medication use were also significantly improved in the treated group. Again, this form of immunotherapy appeared safe, effective, and long lasting. Therefore, both oral immunotherapy and antigen-antibody complex forms of immunotherapy are reported to be safe and by some parameters beneficial^{38, 39, 64, 66}; however, as yet, such treatments have not been tested in food-allergic patients.

Peptide Immunotherapy

Peptide immunotherapy employs subcutaneous injection therapy with peptide fragments containing the allergenic epitope, rather than the complete protein, making the cross-linking of IgE molecules on the surface of mast cells and basophils unlikely. This form of immunotherapy would theoretically minimize the possibility of adverse effects commonly seen with traditional allergen immunotherapy, such as anaphylaxis. The rationale for this treatment, based on results of animal studies, is that peptides containing T-cell epitopes render T cells unresponsive to subsequent allergen exposure.¹⁰ Studies by Norman et al⁴⁷ show that cat-

allergic patients treated with subcutaneous injection of 750 g of T-cell-reactive peptides containing the dominant cat allergen epitopes had marginal yet statistically significantly improved nasal, lung, and total symptoms scores after exposure to a "cat room" when compared with those of placebo-treated patients. Allergic side effects were rare and required little treatment, making this form of therapy safe. However, such an approach is most successful with substances that contain only a single major allergen and is projected to be much more expensive compared with conventional methods.

The above novel approaches to the treatment of allergic disease have been allergen-specific, making treatment of multiple allergies potentially complicated. In allergic disease, a less specific form of therapy that could dampen or eliminate the allergic hypersensitivity state would be more desirable. Casale et al¹⁶ studied the use of an anti-IgE humanized monoclonal antibody in ragweed-induced allergic rhinitis. This humanized mouse monoclonal IgG anti-IgE binds to free IgE and not to IgG or IgA, blocks the binding of IgE to its specific high-affinity receptor (Fc RI) on mast cells and basophils, does not bind mast cell- or basophil-bound IgE, and inhibits the synthesis of IgE in cultured IgE-producing cells.^{55, 59} It recognizes IgE at the same site as the high-affinity receptor. These characteristics, like peptide immunotherapy, make anaphylaxis an unlikely event in this form of therapy. Casale et al¹⁶ showed that serum levels of free IgE decreased in a dose-dependent fashion with anti-IgE therapy. Although clinical efficacy was not demonstrated statistically, lower symptom scores would be expected in patients with lower levels of circulating IgE during the allergen season because symptom scores correlate with levels of antigen-specific IgE. The authors concluded that adjusting the dose of anti-IgE therapy according to the patient's baseline antigen-specific IgE level would likely lower free IgE levels to clinically insignificant levels. Adverse reactions were not different between treated and placebo patients. Recently, two studies were performed using anti-IgE therapy^{9, 23} in patients with mild asthma. Therapy with intravenous administration of recombinant humanized monoclonal anti-IgE (rh-MAb-E25) decreased both the early and late asthmatic response to allergen challenge, decreased airways hyperreactivity, and lowered serum-free IgE levels. MacGlashan et al⁴⁰ show that free IgE levels decrease to less than 1% of pretreatment levels, and the expression of Fc RI on basophils is downregulated in atopic patients treated with anti-IgE therapy. If successful for other allergies, immunotherapy with anti-IgE could be a potential treatment for food allergies; however, as yet, no such studies have been performed in food-allergic patients.

DNA Immunization

DNA immunization is another novel approach under study for the treatment of allergic disease.²⁶ It was recently shown that naked plasmid DNA (pDNA) encoding an antigen is taken up *in vivo* by antigen-

presenting cells; it is suggested that an endogenously produced protein or peptide fragment is then presented on the surface of the cell, presumably inducing a Th1 phenotypic response, rather than the typical Th2 response with injection of exogenous protein antigens. Spiegelberg et al⁶¹ describes the latest findings in a review of animal studies using pDNA allergen vaccines. Immunization of mice with pDNA encoding human allergens,⁶⁰ such as Der p 5 (dust mite allergen) or Hev b 5 (latex allergen), induced a Th1 response, with the secretion of IgG2 and IFN and no IgE antibodies. Even more importantly, the pDNA-induced Th1 response dominated over a pre-existing Th2 profile and suppressed ongoing allergen-specific IgE production.⁵⁴ These findings, although in animal model stages at this time, suggest promise for this form of therapy for allergic disease in humans, including food allergies.

Novel approaches to therapy for allergic diseases are only in their infancy; more detailed investigations are required to better define the risk of adverse reactions, the duration of treatment effects, and the doses that will be tolerated or required for an appropriate long-lasting immunologic response. Peptide immunotherapy, anti-IgE therapy, and DNA vaccine therapy, used individually or in combination, may provide a cure for not only allergic disease such as rhinitis, asthma, and insect sting allergy, but also for the treatment of food allergy, which until now has essentially been impossible because of the incidence of severe untoward reactions associated with traditional injection immunotherapy.^{45, 50}

Peptide immunotherapy, anti-IgE therapy, and DNA vaccines have been studied using subcutaneous injection, like traditional immunotherapy. Immune complex therapy has been administered intradermally. The oral route of allergen therapy had been largely unsuccessful until recently. The reason is believed to be related to the pH of the gastric environment having the ability to denature proteins, altering the tertiary structure of the allergen or allergenic epitope. The most recent studies⁴⁶ showing a response to therapy with orally administered allergen have utilized a new microencapsulated, pH-sensitive, oral delivery system. This type of oral delivery system was first tested by Litwin³⁷ using short ragweed pollen extract. The pollen was microencapsulated with an aqueous coating system of a polymethacrylic acid co-polymer over a core of allergen. The microencapsulated pollen extract was resistant to dissolution in acid at pH 1.2 for at least 6 hours and dissociated rapidly at a pH greater than 6.0, releasing the pollen proteins. These properties allow the pollen extract protection during gastric transit with dissolution upon entering the duodenum; therefore, pollen extract proteins maintain their allergenic epitopes and are capable of possibly inducing the desired immunologic response.

MUCOSAL VACCINES

In the absence of clearly defined molecular and cellular mechanisms that mediate allergic reactions in the gut, it is difficult to speculate as to

Table 2. POTENTIAL STRATEGIES FOR FOOD ALLERGY IMMUNOTHERAPY THROUGH MUCOSAL IMMUNIZATION

Objective	Strategy	Example
Increase levels of allergen-specific secretory IgA and systemic IgG	Oral inoculation of immunogen plus mucosal adjuvant	<i>E. coli</i> lymphotoxin
Increase levels of allergen-specific secretory IgA and systemic IgG	Presentation of allergen in vaccine delivery vector	Attenuated <i>Salmonella</i> species
Tolerance	Oral immunization with high doses of immunogen	Autoimmunity

the most effective immunotherapeutic interventions. There are several possible therapeutic interventions that might prove successful for treatment of food allergies (Table 2). First, it may be possible to preferentially increase the amount of secretory IgA and systemic specific IgG against a particular allergen using certain oral immunization regimens. One such immunization scheme utilizes oral inoculation of immunogen along with a mucosal adjuvant, such as the heat-labile lymphotoxin from *Escherichia coli*.⁶³ Such an immunization scheme preferentially augments IgA and IgG antibody responses but does not significantly increase IgE levels. Although it is not clear how this adjuvant influences T lymphocyte to preferentially induce IgA and IgG secretion, the lack of a significant IgE response would conceivably benefit patients in the same manner as conventional systemic immunotherapy. Shifting the antibody response against a particular allergen away from IgE production would certainly be beneficial. Such a therapy might be effective for individuals who have significant type I mediated food allergies because the goal would be to alter the immunologic response of the patient to a particular allergen.

A second possible mode of immunization that might have therapeutic value involves the presentation of allergen in a vaccine delivery vector. For example, by expressing an immunogen in an attenuated bacterial strain with affinity for invading the gut, it is possible to mount an enhanced mucosal and systemic immune response. One example of such a delivery system that has been used successfully to deliver immunogens to mucosal surfaces is by expressing the protein in attenuated *Salmonella* strains.¹⁸ These attenuated *Salmonella* species have a limited ability to cause disease but effectively invade the gut and induce cellular and humoral immune responses against the expressed immunogen. With the goal of altering the atopic state of the patient, such an altered presentation of allergen might direct the immune response away from a pathologic one to one with more benign symptoms.

The gut-associated lymphoid tissue is unique in that most immunogens that are presented in this compartment result in tolerance. The mechanisms responsible for this suppression are still being defined;

however, the delivery of immunogens at this site has been utilized as a method for inducing local and systemic tolerance, especially for altering autoimmune diseases.⁶⁸ Upon oral inoculation with high doses of immunogen, reduced immune responses to these immunogens have been documented. This has resulted, for example, in decreased autoimmune disease, presumably by inducing tolerance or suppression, or by sufficiently altering the immune response so that the disease state is limited.²⁵ Such therapy could also be applied to the induction of oral tolerance to food allergens.³¹ Oral tolerance is most easily induced in most systems by the administration of high doses of immunogen; however, this could present some problems because such treatment reflects exposure to most food allergens and most likely may cause some level of allergy as discussed in the previous section. Such a problem might be circumvented by the oral administration of selected peptides that represent immunodominant T lymphocyte epitopes. By selecting "optimal" peptides derived from a protein allergen, it is possible to suppress the immune response. Clearly, one problem associated with such a treatment would be the design of delivery methods to mucosal surfaces that would permit peptides to survive digestive enzymes and acids. Such technology is currently available using, as discussed, microencapsulation of the peptides for delivery.^{17, 34} For such a therapy to be successful, however, identification of immunodominant epitopes that induce suppression is needed. This undertaking could be a considerable one because the few selected food allergens studied have multiple T-cell and B-cell epitopes, and most foods contain many allergens.

There are a variety of other possible oral therapies that may prove useful in the treatment of food allergies in addition to the ones noted previously. Each therapy has its limitations, however, and it is not possible to predict the efficacy of any one such treatment presently. A more thorough characterization of the cellular and molecular mechanisms responsible for stimulation of allergic reactions at mucosal surfaces would contribute greatly to an understanding of which therapies might be most successful. In the final analysis, however, efficacy of oral immunotherapies must be evaluated using appropriate model systems or using clinical trials.

DEVELOPMENT OF HYPOALLERGENIC FOODS THROUGH BIOTECHNOLOGY

Ideally, the preferred treatment for food-allergic individuals is avoidance of exposure to the food that stimulates an allergic reaction. Avoidance is not always possible, however, particularly as new foods are developed that may contain proteins of unique or exotic origin. Indeed, the manipulation of plants through biotechnology has yielded a number of transgenic crops that are resistant to chemical herbicides and insect pests and have enhanced nutritional quality, increased shelf life,

and other traits desired by consumers.^{4, 27, 30, 49} These new properties have all resulted from molecular biology techniques that have altered the genes governing the protein content of individual foods to improve the quality of the product.³⁰ The application of these same molecular methods to improve the quality of foods by reducing their allergenicity is possible and currently being tested for some food allergens.^{41, 42}

As discussed previously, the preferred treatment for food-allergic individuals, if avoidance is not possible, is the development of hypoallergenic foods with reduced or no allergen content that allergy patients can tolerate.⁵ In the past, physical and chemical treatments (i.e., the chemical or enzymatic hydrolysis of proteins such as those present in milk or rice) have been used to reduce allergen content.^{15, 67} Based on genetic engineering, however, it may now be possible to suppress or delete the gene coding for a particular allergen or induce more subtle changes by altering allergen protein structure⁶ resulting in a food with little or no allergenic activity.

The overall objective is to produce a food that either no longer contains the offending allergen(s) or whose proteins have been modified in such a way so that they are no longer allergenic. The chances for success of this approach are greatest for foods that contain a limited number of major allergens. The allergens in any potential candidate food must be either nonessential structural or functional proteins (because suppression or alteration of such proteins could be a lethal mutation) or the alteration of allergen structure must not significantly affect its function.³⁴

Table 3 lists several gene therapies that could be used for suppression of plant or animal allergens. These molecular biology methods have been used for alteration or suppression of protein synthesis. The most drastic approach is gene deletion, which can be achieved through a number of techniques such as chemical mutagenesis or treatment with x-rays or gamma radiation. The objective, of course, is to remove or inactivate the gene that encodes the offending allergen. The advantages of these methods are that they are rapid, simple, and relatively easy to perform. The disadvantage is that they are a "shotgun" type of approach

Table 3. POTENTIAL GENE THERAPY FOR PLANT AND ANIMAL ALLERGENS

Gene Deletion
Chemical mutagenesis
Radiographs
Gamma radiation
Gene Suppression
Antisense DNA technology
Gene Alteration
Site-directed mutagenesis
Homologous recombination
Chimeric oligonucleotide gene targeting

and as such lack specificity and can induce undesirable effects as well. For example, plant breeders need to perform many backcrosses; even so, the possibility that a gene has been damaged that is completely unimportant until a particular plant disease or environmental stress comes up during growing season will continue to exist. In addition, many proteins in plants are encoded by multigene families, and one would virtually never knock out all such genes.

Gene suppression is a method to suppress the translation of the gene into protein. Antisense DNA technology has been applied to suppression of a major rice allergen.⁴¹ This method reduces the levels of specific allergens in a food by the introduction of genes in the antisense (opposite direction required to produce a protein) orientation. Thus antisense mRNA is complimentary to normal (sense) mRNA; theoretically sense and antisense mRNA will bind, thus inhibiting mRNA translation and reducing protein production. The advantages of this approach are that it is proven technology and is reasonably precise. The disadvantages are that it eliminates the entire gene (a lethal change for an essential protein) and the system may be leaky, that is, there is not complete suppression of the expression of the protein.

Matsuda et al cloned a gene that encoded the 16 kD rice allergen.² Using antisense DNA technology, they introduced another gene encoding the protein in the antisense orientation into rice. The level of the 16 kD protein was significantly reduced in the rice seed from 312 to 60 g per seed (Fig. 2). Further studies are under way to achieve an even greater reduction in this allergen and apply the antisense approach to other food crops such as peanuts and soybeans to selectively reduce the levels of specific allergens.⁵ Multi-allergen systems and multi-gene families make this approach more complex. In addition, if any of these allergens are essential proteins, such a change could be lethal to the plant.

A more subtle change in structure can be achieved by a site-directed mutagenesis, an approach in which specific changes can be made in the nucleotide sequence of the gene governing a major allergen. These changes are made in the genome and are carried on from one generation to the next. Because this method is very specific, conceivably one can render highly allergenic proteins nonallergenic without substantially altering the protein's function. Such an approach would be important for allergens that are essential structural or functional proteins and thus allow only minimal changes in their structure. Regrettably, site-specific mutagenesis or gene replacement has not been demonstrated in plants.

Site-directed mutagenesis can be used to reduce or abolish IgE-binding epitopes of specific food allergens. Because many food protein genes are being cloned, their nucleotide sequence is determined, and a number of major IgE antibody-binding epitopes have been identified,³⁵ as shown in Table 4. One of the best and earliest examples of a food allergen studied is the major allergen of cod fish, Gad c 1.²² Five IgE-binding epitopes were reported for this molecule.⁵⁸ Other major allergens studied are those in egg, shrimp, and peanut.^{22, 29, 36, 54, 58, 62}

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Figure 2. Suppression of 16 kD rice allergen with antisehse technology. Rice allergen levels were quantified by ELISA for each genetically engineered rice variety (clones 17-2, 17-5, 17-6, and 17-9) compared with wild-type rice seeds. (*From Astwood JD, Fuchs RL, Lavrik PB, et al: Food biotechnology and genetic engineering. In Metcalfe DD, Sampson HA, Simon RA (eds): Food Allergy: Adverse Reactions to Foods and Food Additives. Cambridge, Blackwell Science, 1997, pp 65–92; with permission.*)

One of the best studied allergenic foods in recent years is the peanut. Two major peanut allergens, Ara h 1 and Ara h 2 with molecular weights of 63.5 and 17 kD, respectively, have been demonstrated.^{13, 14} Through the use of overlapping synthetic peptides and pooled sera of peanut-allergic subjects, 10 IgE-binding epitopes were identified in Ara h 2. Using amino acid substitution, amino acids essential for IgE binding

Table 4. IgE BINDING EPITOPES ON MAJOR FOOD ALLERGENS

Food	Allergen	Protein	Molecular Weight (kD)	Epitopes	Reference
Cod fish Egg Shrimp	Gad c 1	Paralbumin	12.3	3	Elsayed et al ²²
	Gal d 2	Ovalbumin	43–45	7	Elsayed et al ²²
	Pen I 1	Tropomyosin	34–36	5	Shanti et al ⁵⁸
Peanut	Pen a 1				Daul et al ²⁰
	Ara h 1	Vicilin*	63.5	23	Burks et al ¹²
	Ara h 2	Conglutin*	17.0	10	Stanley et al ⁶²

*Seed storage proteins.

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Figure 3. Reduction of IgE binding capacity of peptide 7, which contains a major Ara h 2 IgE binding epitope from amino acid substitution. Results indicate that some amino acid substitutions abolish IgE binding capacity. (From Stanley JS, King N, Burks AW, et al: Identification and mutational analysis of the immunodominant IgE binding epitopes of the major peanut allergen Ara h 2. *Arch Biochem Biophys* 342:249, 1997; with permission.)

were identified in each peptide; thus IgE binding can be altered for a particular epitope by amino acid substitution with neutral amino acid alanine (Fig. 3).⁶² Although several amino acid substitutions resulted in some increased IgE binding, substitution of other amino acid residues reduced or completely abolished IgE binding. Current studies are directed at investigating the effects of such amino acid substitutions on the allergenicity of the entire molecule.¹¹ This approach is very exciting because it demonstrates that biotechnology may be used successfully to alter major food allergens, as well as suppress their production.

Our group as well as others have investigated IgE binding epitopes for the major shrimp allergen, shrimp tropomyosin.^{20, 36, 54, 58} Based on the results from several laboratories, there appears to be at least 10 IgE binding regions in shrimp tropomyosin. Tropomyosins provide a natural experiment because those present in foods of vertebrate origin, although very similar structurally, are not allergenic.⁵³ Thus by comparison of homologous amino acid sequences of allergenic and nonallergenic tropomyosins, nonallergic amino acid sequences can be identified and replicated in tropomyosin allergens. Although shrimp allergen affords the advantage that only one major allergen accounts for most (85%) of the shrimp allergenic activity, genetic modifications may be more complicated in animal (shrimp), than in plant (peanut) species.

SUMMARY

The future certainly holds promise for the treatment of food allergies. Generally future treatments can be divided into immunologic manipulation of the food-allergic patient (mucosal vaccines, new immunotherapies, cytokine level alterations) or manipulation of the food through genetic engineering to diminish or abolish its allergenic activity.

A review of the strategies that may allow desensitization of IgE antibodies to food allergens reveals that they are almost invariable based on a change in the chemical format or the route of administration of the offending allergen. Another possibility that needs to be explored is the

modification of the host in such a way that the development of IgG or cellular immunity predominates over the production of IgE. Immune responses are regulated by two subsets of T-helper cells. The Th1 subset produces IL-2, IL-12, and IFN- γ , promoting delayed-type hypersensitivity and protection against intracellular pathogens,⁶ whereas the Th2 subset produces IL-4 and IL-5, which in combination with the interaction of CD40 with the CD40 ligand shifts the immune response to IgG or IgE antibody production.^{3, 19} It is possible that future approaches to alter IgE reactivity to food allergens may include strategies such as DNA vaccines to change the Th1 or Th2 balance of the host as a means to effect a faster and less risky change to nonpathogenic forms of immune reactions to food allergens.

Genetic engineering has the potential to substantially reduce the allergenicity of our food supply. There are a number of novel approaches in immunotherapy based on biotechnology developed methods used primarily in the treatment of inhaled or ingested allergens and allergen delivery systems, which have been proposed for vaccines that may be applicable for food allergy treatment in the future. One of the most exciting developments, based on our current level of knowledge of the molecular structure of allergens such as those in rice, peanuts, and shrimp, is that we may be able to actually abolish the allergenic activity of foods by either suppressing production of the allergen or altering allergenic epitopes. These are truly exciting developments for the study and treatment of food allergy, and significant advances in food allergy treatment should be made within the next several years that will improve the quality of life of patients with food-induced allergic reactions.

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