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Fergus M. Clydesdale
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Allergenicity of Foods Produced by Genetic Modification

International Food Biotechnology Council

IFBC

in collaboration with the

ILSI Allergy and Immunology Institute

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in collaboration with the

ILSI ALLERGY AND IMMUNOLOGY INSTITUTE

ALLERGENICITY OF FOODS PRODUCED BY GENETIC MODIFICATION

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ALLERGENICITY OF FOODS PRODUCED BY GENETIC MODIFICATION

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PREFACE

This report is the result of a collaborative effort of the **International Food Biotechnology Council (IFBC)** and the **International Life Sciences Institute (ILSI) Allergy and Immunology Institute (AII).** The **IFBC** was organized in 1988 to develop criteria and procedures to evaluate the safety of food or food ingredients derived from genetically modified plants and microorganisms. Members of the Council include companies in the food processing and food biotechnology industries. Its first major project was to identify and assemble a proposal for the evaluation of food products of biotechnology that government agencies, the food industry, and the public could use to evaluate the acceptability of foods and food ingredients rising from biotechnology. Published in December 1990, the initial **IFBC** report *"Biotechnologies and Food: Assuring the Safety of Foods Produced by Genetic Modification"* has been referenced extensively in subsequent discussions of the safety evaluation of such foods or food ingredients.

However, the 1990 report did not include a discussion of allergenicity as it relates to the safety evaluation of foods produced by biotechnology. The Council considered food allergy to be a complex and specialized issue that was beyond the scope of the first report. To address the allergenicity issue, the and **IFBC** and **AII** initiated efforts to develop a comprehensive science-based report, with extensive literature references, which includes a review of the current status of food allergies, discusses the potential for transferring allergens to new plant varieties, and presents technical approaches to assess this potential.

ILSI is a nonprofit, worldwide foundation established in 1978 to advance the understanding of scientific issues relating to nutrition, food safety, toxicology, risk assessment, and the environment by bringing together scientists from academia, government, industry, and the public sector to solve problems with broad implications for the well-being of the general public. The **AII** is that part of **ILSI** that sponsors research and educational programs to improve scientific understanding in allergy and immunology, particularly related to the diagnosis, treatment, prevention, and management of food allergies. **AII's** programs are guided by scientific advisors representing the fields of immunology, allergy, internal medicine, pediatrics, epidemiology, and food science.

The editors of the report appointed by **IFBC** and **AII** enlisted a number of recognized scientific experts to author the articles appearing here. The authors and editors met at significant points during the development process to plan the scope of the report, review progress, and offer constructive suggestions. To further help ensure that the report included the best scientific judgment available, drafts of the report were sent to approximately 80 scientists and health professionals working in academia, government, industry, and the public sector in many countries. More than 40 sets of substantive written comments were received, studied and, in large measure, incorporated by the authors. As a result, it is hoped this report will be viewed and accepted as a valuable scientific contribution.

ACKNOWLEDGMENTS

The **International Food Biotechnology Council (IFBC)** expresses its deep gratitude to all individuals who helped make this report possible. The involvement of the **ILSI Allergy and Immunology Institute** was a critical element in being able to address the multidisciplinary subject matter in a comprehensive and thoughtful manner. The editors and authors are commended for their outstanding contributions and their names are highlighted elsewhere within this publication. Additionally, completion of this document would not have been possible without the support of the **IFBC** members and the Board of Directors. The complex logistics required for the development, preparation, and review of this report were managed by **Ms. Sharon Weiss.** Other individuals who worked directly on important details of this endeavor include **Mr. Fred Degnan, Ms. Catherine Nnoka, Ms. Frances A. DeLuca,** and **Ms. Kemi Ilesanmi.** The **IFBC** also is grateful **to Dr. Fergus Clydesdale, Editor,** *Critical Reviews in Food Science and Nutrition,* for his interest in and enthusiasm for this project. At CRC Press, Inc., **Mr. Chris Richardson** and **Mr. Jim McGovern** provided invaluable assistance.

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EXECUTIVE SUMMARY

INTRODUCTION

This report is produced by the ILSI Allergy and Immunology Institute (AII), a scientific organization that sponsors research and educational programs to improve understanding in allergy and immunology, particularly related to the diagnosis, treatment, prevention, and management of food allergies, in collaboration with the International Food Biotechnology Council (IFBC). Since its inception in 1988, the IFBC has addressed scientific issues related to the safety of foods produced by genetic modification. In 1990, the IFBC published a report: *Biotechnologies and Food: Assuring the Safety of Foods Produced by Genetic Modification (Regulatory Toxicology and Pharmacology,* Vol. 12, No. 3, Part 2).

The 1990 IFBC report represented a consensus opinion on the scientific principles that should be employed in evaluating the safety of foods derived through the application of techniques of modern biotechnology, particularly recombinant DNA and gene transfer methods. The report has been referenced extensively in subsequent discussions of safety evaluations of these foods. However, the report did not include a discussion of allergenicity as it relates to the safety evaluation of foods produced by biotechnology. The Council considered food allergy to be a complex and specialized issue that was beyond the scope of the first report. In the current report the AII and the IFBC seek to address this issue by presenting a review of food allergy and food allergens, especially as they relate to the development of new plant varieties developed through the use of modern biotechnology. The report also proposes a scheme for assessing the allergic potential of gene products that may be introduced into plants that are destined for use as foods. The AII and IFB have not considered allergy issues related to microbial or animal foods that have been modified genetically. Furthermore, gluten-sensitive enteropathy (celiac disease), a distinct clinical pathologic entity that is observed in specific individuals sensitive to gluten in certain foods, is also not addressed specifically.

More than 80 copies of the draft report were distributed to reviewers with experience in allergenicity, food science, food safety policy, and biotechnology product development. Nearly 35 sets of substantive comments were received and the majority of suggested changes were incorporated into the report. The principal audiences for this report include the biotechnology and food industries, the general public, regulatory agencies, and officials at all levels of government. This summary describes the major issues and approaches suggested by the joint AII and IFB report, including specific decision criteria for the acceptance of modified foods and ingredients.

FOOD ALLERGIES

It is estimated that 1 to 2% of the adult population suffers from food allergies, which are defined for the purposes of this report as type I IgE-mediated immunologic reactions to specific food(s). The general consensus is that the most common allergenic foods, worldwide, are egg, milk, fish, crustacea, peanut, soybean, wheat and tree nuts, although the prevalence of food allergens may vary in other parts of the world according to dietary preferences. Allergies to foods such as milk and eggs are most prevalent among children and often disappear by adulthood. These commonly allergenic foods account for over 90% of food allergies among atopic individuals. However, an extensive search of the clinical literature has revealed a list of more than 160 other foods and food-related substances that have been associated with allergic reactions in individuals. This list includes most major grain, oilseed, and vegetable crops as well as processed products such as beer and chocolate.

In many cases food allergies are an inconvenience resulting in unpleasant reactions, such as tingling of the lips and mouth or diarrhea. However, for some individuals who are highly sensitive to particular foods (e.g., peanut), the results of consuming that food can be life threatening. These individuals exhibit severe anaphylactic reactions such as bronchospasm, choking, nausea, vomiting, and hypotension. There are many well-documented reports indicating that trace amounts of a food allergen may be sufficient to elicit a reaction in highly sensitive individuals. Such individuals typically take extreme precautions to avoid consumption of foods to which they are sensitive. Therefore, appropriate labeling of processed foods containing common food allergens is critical in helping allergic consumers make their dietary selections.

TRADITIONAL FOODS AND TRADITIONAL METHODS OF GENETIC MODIFICATION DEFINE THE ISSUE OF FOOD ALLERGIES

Almost all food allergens are proteins. The crops from which our staple foods are derived contain tens of thousands of different proteins. The distribution of those proteins varies markedly in different parts of the plant and can be profoundly influenced by environmental factors such as climate and disease pressure. Furthermore, in seeking to improve crops, plant breeders routinely access new genes from wild relatives that may introduce additional protein diversity into the food supply.

Despite the huge number and variation of proteins in our diet, it is apparent that very few are food allergens. Variations in the protein composition of our diet brought about through traditional crop-improvement practices have had little, if any, effect on the allergenic potential of our major foods. In contrast, changes in dietary preferences can have significant implications for the development of food allergies. For example, allergy to peanut occurs at a significant frequency in the U.S. and Western Europe but not in Japan, whereas allergy to rice occurs at a significant frequency in Japan but is rare in the U.S. and Western Europe. This reflects differences in dietary consumption. Also, recent food introductions, such as kiwifruit, have proven to be a significant new source of food allergens. These observations provide confidence that there are not a large number of potential allergens in the food supply but show that new allergenic foods are sometimes introduced into the marketplace.

THE APPLICATION OF MODERN BIOTECHNOLOGY

The modern techniques of biotechnology offer great potential to further improve the abundance, availability, and nutritional quality of our food supply. Potential products include maize that is naturally protected against insect pests and soybeans that yield healthier oils. Methods for the isolation and transfer of genes are highly specific and precise compared with the random reassortment that occurs during traditional crop improvement. However, the capacity to move genes from one food crop to another or to introduce genes from organisms that have never been part of our food supply raises concerns that food allergens might be moved between foods or that proteins with no significant history of consumption could prove to be allergenic when introduced into a major food crop. This report describes a safety assessment process that will help to identify allergenic proteins and minimize the risk that they will be introduced into foods derived by genetic modification.

SAFETY EVALUATION OF FOODS PRODUCED THROUGH THE APPLICATION OF MODERN BIOTECHNOLOGY

This report proposes that a rational assessment of the allergenic potential of foods derived from new plant varieties developed through genetic modification should be conducted in a careful stepwise process. A

decision tree strategy is recommended that takes account of the source from which a gene was obtained, amino acid sequence comparisons with known allergens, *in vitro* and *in vivo* immunologic analyses, as well as an assessment of physicochemical characteristics of the gene product. It is important to emphasize that it is the totality of these assessments that provides reasonable assurance that foods derived from new plant varieties will not introduce allergenic concerns beyond those that already exist relative to our current food supply, and that if an allergen is introduced it will be appropriately labeled so that it can be avoided by sensitive individuals.

The decision tree first addresses the source of the introduced gene and emphasizes the significance of determining whether the gene is from (1) a commonly allergenic food; (2) a less commonly allergenic food or other known allergen; or (3) a source with no allergenic history. As a first step in assessing allergenic concerns, the amino acid sequence of genes from all sources should be compared against a database of all known allergens, screening for immunologically significant sequence similarities. Based on the minimal peptide length for T-cell binding epitopes, the report concludes that any sequence identity comprising eight contiguous amino acids should be viewed as an indicator of potential allergenicity and would require the gene product to undergo further testing. The report recognizes the limitations of this analysis, but concludes that it is of sufficient predictive value to recommend that all gene products for food use should be screened using this approach. To facilitate that analysis, the report contains a list of 198 sequences of food and non-food proteins that are reported to be allergens, and that can be accessed from publicly available databases. It is anticipated that this database will quickly grow and yield more specific information as additional allergens are characterized.

Commonly allergenic foods

Most allergenic foods contain both major and minor allergens. The great majority of food allergic individuals are sensitive to one or more of the major allergens present in commonly allergenic foods. A major allergen is one to which >50% of sensitive individuals react; minor allergens elicit a response in a smaller percentage of sensitive individuals. Clinical reagents and test subjects should be readily available to conduct a statistically valid assessment of the potential of a gene product from a commonly allergenic food to be a major allergen for any individual sensitive to that food. The report recommends that foods containing new proteins produced from genes derived from commonly allergenic foods be subjected to a series of solid phase immunoassays (e.g., *in vitro* radioallergosorbent test (RAST™) or RAST™ inhibition assay or enzyme-linked immunosorbent assay (ELISA)). It is suggested that each food should be tested against immune sera from a minimum of 14 sensitive individuals with documented histories of sensitivity to the corresponding food. This will ensure a >99.9% probability of detecting the presence of a major food allergen and >95% probability of detecting a minor allergen to which >20% of the sensitive population reacts. A positive reaction in an *in vitro* test would raise concerns that the new protein might be allergenic. Unless this possibility can be discounted by additional *in vivo* testing, food containing the newly introduced gene should be clearly labeled as to the source of the gene, in compliance with the 1992 FDA policy (Statement of Policy; Foods Derived from New Plant Varieites, *Federal Register* 57: 22984–23005).

In the case of negative or equivocal results with the solid phase immunoassays the food should be investigated further using *in vivo* skin prick tests with a minimum of 14 sensitive test subjects. A positive skin prick test should raise the same concerns as a positive *in vitro* reaction requiring foods containing the newly introduced gene to be labeled. Foods that fail to elicit positive reactions in solid phase immunoassays or skin prick tests are unlikely to contain allergens, but this report recommends that the absence of allergens from commonly allergenic foods should be confirmed by a double-blind placebo controlled food challenge (DBPCFC) using a minimum of 14 sensitive subjects. A DBPCFC should only be conducted with the approval of an Institutional Review Board. Foods that fail to elicit a reaction in a DBPCFC are very unlikely to contain an allergen from a commonly allergenic food. For such foods

no meaningful scientific basis exists on which to require labeling concerning the source of the newly introduced gene.

Less commonly allergenic foods or other known allergens

The AII and IFB acknowledge that it will not always be reasonable to apply the same strict criteria to the evaluation of genes from less commonly allergenic foods. The report recommends that gene products from these sources should be subjected to immunological analysis of allergenic potential using sera from at least 14 sensitive individuals, wherever feasible. A positive result would provide a meaningful scientific basis for requiring that the food containing introduced protein bear labeling information regarding the source of the protein. However, sera from individuals who are sensitive to many of the less commonly allergenic foods listed in the report are extremely difficult to obtain. Therefore, if less than 5 sera are tested (i.e., there is <95% probability of detecting the presence of a major allergen) with negative results, then the gene product should be subject to physicochemical evaluation. Interest in the testing of gene products for potential allergenicity will likely stimulate the collection of libraries of sera representing many of the less commonly allergenic foods that will improve the availability of sera. No meaningful scientific basis exists for labeling the source of gene products derived from less commonly allergenic foods when the gene products fail to react with five or more sera. Accordingly, the report recommends that such foods be introduced without labeling the source at the gene product.

Many major food allergens are resistant to digestion and conditions commonly encountered during food processing. Gene products that prove sensitive to digestion and conditions commonly encountered during processing are therefore unlikely to be food allergens. Proteins that prove resistant to digestion or heat denaturation have a greater potential to be food allergens. Therefore, this report suggests that gene products derived from less commonly allergenic foods that were tested against less than 5 sera by solid phase immunoassay should be evaluated for their stability, *in vitro*, to gastric digestion and processing typical for that food. No meaningful scientific basis exists for labeling the presence of gene products in food if those gene products are senstive to digestion and/or processing. Accordingly, the report recommends that such foods be introduced without labeling the source of the gene product. For those gene products that are not sensitive to digestion and/or processing, the report recommends that developers should discuss the results with the appropriate regulatory authorities to determine how to proceed. A factor to be considered in those discussions may be the concentration of the gene product in foods. Most major food allergens are present in high concentrations in food.

Sources with no allergenic history

Assessment of gene products from sources with no allergenic history should begin with a comparison of the amino acid sequence against the database of all known allergens, screening for immunologically significant sequence similarities. For foods containing gene products with amino acid similarity to known allergens, assessment should proceed as with food containing a gene from a less commonly allergenic food or other known allergens. Gene products from sources with no allergenic history that lack immunologically significant sequence identity to known allergens should still be subjected to physicochemical evaluation before concluding that they are unlikely to be food allergens and, thus, present no basis for a labeling declaration. Developers should consult with the appropriate regulatory agency in cases where such gene products lack immunologically significant sequence identity to known allergens but show significant resistance to digestion and/or processing.

The AII and IFBC have considered the use of animal models to evaluate the potential allergenicity of gene products. The report acknowledges that animal models are valuable for mechanistic and

exploratory studies, but concludes that at the present time no reliable animal models are available that are generally predictive of the allergenic potential of foods for humans.

The science-based decision tree approach described to assess the allergenic concerns associated with the introduction of gene products into new plant varieties relies on a balanced judgment of all available data generated during the allergenicity assessment. Using this approach, food derived from these new plant varieties should be introduced into the marketplace with the same confidence that food derived from new plant varieties developed through traditional breeding has been introduced for centuries.

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Introduction to Allergic Diseases

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I. INTRODUCTION

An allergy is the immune-mediated state of hypersensitivity that results from exposure to an allergen. An allergen, in turn, is a biological or chemical substance that causes an allergic reaction.[1] The most common allergic reaction is mediated by immunoglobulin E (IgE) and involves activation of effector cells, mainly mast cells and basophils, which lead to an inflammatory response and clinical manifestations.

Allergic diseases affect a significant portion of the general population, estimated at 10 to 25% in developed countries, although food allergies are much less prevalent. Clinical disease requires both genetic predisposition and environmental allergen exposure. It is unclear why some people develop allergies and others do not. It is known, however, that people with a genetic predisposition to allergy (a condition called atopy) produce significantly greater quantities of total and specific IgE compared to nonallergic individuals.

An IgE-mediated response occurs in all normal individuals, but the presence of certain genes may be needed for antigen specificity and clinical manifestations of the atopic state. The genetic influence is evidenced by the known epidemiological findings which indicate that if one parent has allergies, the chances are 1:3 that each of their children will have allergies. If both parents have allergies, it is even more likely (60 to 70%) that their children will exhibit some allergic symptoms.

Allergic diseases result in major clinical and public health problems due to their high prevalence. For example, about 20% of Americans suffer from allergic rhinitis. Eight to 12% of the American population has asthma. Among chronic diseases, asthma is the leading cause of school absenteeism and is responsible for approximately 4000 deaths per year in the U.S. In 1990, the estimated cost of asthma-related illness in the U.S. was 6.2 billion dollars.[1] It is therefore obvious that the economic and social impact of allergic diseases in developed countries is significant.

II. CELLS OF THE IMMUNE RESPONSE

A normal immune system is essential for health; a dysfunctional system leads to disease. A deficiency in immune cell production or defective immune function may lead to a wide spectrum of immune-deficiency diseases. Overactivity of various components of the immune system leads to the development of allergic or autoimmune diseases. Multiple cells are included in the immune system, as is shown in Table 1. The cells, which are directly involved in the induction and/or elicitation of the allergic response, will be discussed in more detail below.

A. B Lymphocytes

B cells constitute one of the principal components of the immune system. They provide both cognitive and effector functions in the humoral immune response. Membrane Ig-expressing B cells are cognitive cells, because they recognize and respond to various antigens. Following antigenic stimulation, they differentiate into effector cells and secrete Ig. The vast array of antibodies produced by B cells belong to five Ig classes (isotypes), namely IgM, IgD, IgG, IgA, and IgE.[2] The basic structure of an Ig is shown in Figure 1.

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Table 1

CELLS OF THE IMMUNE RESPONSE

	Location	Function
LYMPHOCYTES	(% normal range)	
T	Blood (65-75), thymus (>95) lymph nodes (70-80), spleen (20-30) lymphoid organs (i.e. Peyer's patches, etc.)	Cell mediated cytotoxicity Cell mediated immunity Regulatory effects on other immune cells
B	Blood (10-20), (Thymus <1) lymph nodes (10-20) spleen (40-50)	Immunoglobulin production & secretion Antigen presentation
Natural Killer (NK)	Blood, bone marrow spleen	Killing of certain tumor cells; defense against viral infection; regulatory effects on other immune cells
Macrophages	Various tissues (i.e. lymph nodes, spleen, thymus, liver, lung, central nervous system, skin)	Phagocytosis and processing of antigens. Antigen presenting cell (APC)
GRANULOCYTES		
Neutrophils	Blood	Phagocytosis and bacterial killing; proinflammatory effects via extra-cellular degranulation (i.e. complement activation, degradation of connective elements), increased vascular permeability
Eosinophils	Blood	Parasite killing; effector cell in allergic inflammation (i.e. induces histamine release from mast cells, bronchospasm, damage to respiratory epithelia)
Basophils	Blood	Allergic/anaphylactic reactions; immune response against ticks
MAST CELLS	Tissues (connective and mucosal)	Major effector cell in the allergic response

The life history of the B-cell lineage in the bone marrow is divided into two main stages: an initial antigen-independent phase (pre-B cells to mature B lymphocytes) and an antigen-dependent phase (B lymphocytes to antibody-secreting plasma cells).[3] The pre-B-cell stage begins with a multipotent hemopoietic stem cell. Primitive B-cell precursors have μ chains (heavy chain of IgM) in their cytoplasm and no Ig on their surface. At the next identifiable stage in B-cell maturation, kappa or lambda light chains are also produced. These associate with the μ heavy chains and then assembled IgM molecules are expressed on the cell surface. Thus, more differentiated B cells (but still immature) have intact cytoplasmic and surface IgM. The cells in this stage of development do not proliferate and differentiate in response to antigens. In fact, their encounter with some antigens such as self antigens may lead to unresponsiveness (tolerance) rather than activation. Once a B cell expresses a complete heavy or light chain of the Ig molecule, it cannot produce another heavy or light chain containing a different variable region (antigen specificity). Mature B cells lose their cytoplasmic IgM and add surface IgD to the surface IgM. Both classes of membrane Ig have the same variable region and hence the same antigen specificity. Once the mature B cells are stimulated by antigens (or other signals) they proliferate and differentiate, producing an increasing proportion of their Ig in a secreted form and progressively less in a membrane-bound form. After activation, B cells in any given clone can switch Ig production from IgM to antibodies of different heavy-chain classes (isotypes), but with identical antigen-binding specificity. Thus, these cells begin to express Ig heavy-chain classes other than μ, e.g., γ, α, or ε. This crucial step of isotype switching facilitates the diversity of B-cell responses in Ig production and involves splicing

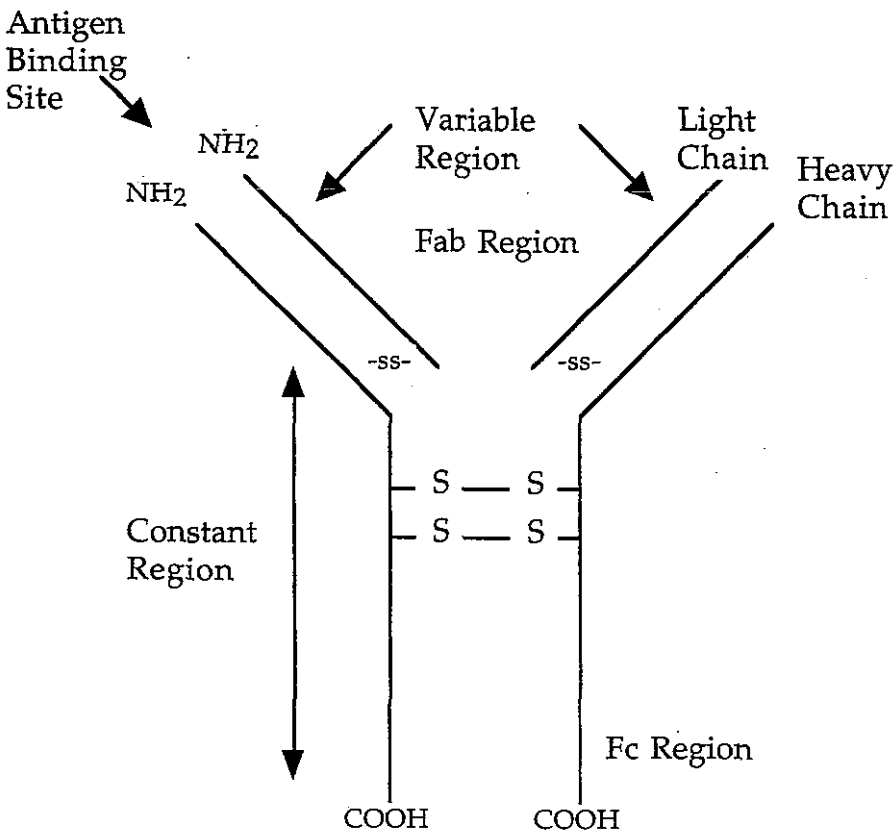

FIGURE 1. The basic structure of an Ig.

and deletion of exons in the Cμ gene (the gene for the constant region of IgM) located on chromosome 14. Other activated B cells may not secrete antibodies, but may instead persist as membrane Ig-expressing memory cells that survive for months without further antigenic stimulation and actively recirculate between the blood and lymphoid organs. Their stimulation by an antigen will lead to a secondary antibody response. Antigen-induced differentiation of mature or memory B lymphocytes culminates in the development of antibody-secreting cells, some of which can be morphologically identified as plasma cells. The primary function of an antibody is to bind specifically to an antigen and bring about inactivation or removal from the body, of the offending toxin, microbe, parasite, or other foreign substance. The biological characteristics of human Ig are presented in Table 2.

Surface-bound Ig may thus be perceived as antigen receptors. Antigens or other multivalent ligands that cross-link Ig molecules are able to induce the resting B cell to enter the cell cycle, enlarge, develop synthetic machinery, and begin DNA synthesis in preparation for division. Antigen molecules that bind to surface Ig receptors are internalized and partially digested. The antigen fragments are then recycled to the B-cell surface and expressed in association with class II molecules of the major histocompatibility complex (MHC). This combination of antigen fragments and the class II molecule on the B-cell surface may then be recognized by a T cell by means of its antigen receptor. In this way the B cell presents antigen to helper T cells and stimulates production of T-cell-secreted factors (lymphokines) that in turn will promote proliferation and differentiation of the antigen-presenting B cell. Other antigen-presenting cells (APC) of importance include macrophages and Langerhans cells. Lymphokines which are known to affect B-cell differentiation include interleukin-4 (IL-4), which controls the switch to IgE production,[4] interleukin-5 (IL-5) and the transforming growth

TABLE 2
Human Ig

	IgG	IgA	IgM	IgD	IgE
Mol wt (kDa)	150	160 (390)[a]	900[b]	175	190
Serum half-life (d)	23	6	5	3	2.5
Average serum concentrations (mg/dl)	1250 ± 300	200 ± 50	125 ± 50	3–4	0.03
Function	Secondary antibody response	Antibody in mucous secretions	Primary antibody response	Primary lymphocyte surface molecule	Mediates allergic responses
Antiviral activity	+	+++	+	?	?
Antibacterial activity	+	+	+++	?	?
Binding cells via Fc	Macrophages neutrophils, eosinophils, LGL[c]	Lymphocytes	Lymphocytes	None	Mast cells, basophils, B cells, eosinophils Langerhans

[a] Secretory IgA appears in saliva and GI secretion is a dimer.
[b] IgM circulates as a pentameric molecule.
[c] Large granular lymphocytes.

factor-β (TGF-β), which are responsible for IgA production, and interferon-γ (IFN-γ), which inhibits IgE synthesis.

B. T Lymphocytes

T lymphocytes (T cells) are critical components of the immune system. Unlike B cells, they predominantly recognize antigen when it is associated with membrane-bound products of the MHC. This dual recognition of antigen and MHC is important for activation of both effector T cells, including cytotoxic T cells, and immunoregulatory T cells.[5]

T cells arise from stem cells and are "educated" in the thymus, hence the name "T" cells. The central role of the thymus is related to the rearrangement and productive expression of the T-cell receptor (TCR) genes and the subsequent selection of the antigen receptor repertoire that enables mature T cells to recognize foreign but not self antigens. In the thymus, primitive T cells (pro-T cells) develop T-cell-specific surface antigens (or markers), such as CD2 and CD7.* Pro-T cells differentiate into pre-T cells, which begin to express CD1, CD4, and CD8 markers, usually within the thymic cortex. A proportion of the relatively immature $CD4^+8^+$ cells then lose expression of either CD4 or CD8 to become $CD4^+8^-$ or $CD4^-8^+$. The amount of CD3/TCR on the cell surface is increased, and the CD1 molecule is lost. T cells develop into a variety of clones, each bearing a different CD3/TCR molecule specific for a particular antigen-MHC complex. Only a minority of pre-T cells in the thymus successfully differentiate into mature T lymphocytes and migrate to the lymphoid organs and blood. In the peripheral blood, about 70% of lymphocytes are T cells.

T cells are heterogeneous with regards to their function. They initiate the immune response, mediate antigen-specific effector responses, and regulate the activity of other leukocytes by secreting soluble factors termed lymphokines. A correlation exists between the expression of membrane antigen markers and the functional activities of T cells. Effector functions of T cells include cell-mediated cytotoxicity where T cells recognize antigens associated with class I MHC or class 1

* CD-cluster of differentiation (nomenclature of cell surface molecules of human leukocytes).

MHC alone. These T cells destroy virally infected class I-bearing cells and are major effectors in allograft rejection. They bear the CD8 marker and appear to affect their targets directly.[6] Other T cells carry out delayed-type hypersensitivity reactions (DTH). These cells react with antigen-class II MHC on the APC and create effects mainly via lymphokine production and activation of other cells. These cells are usually $CD4^+$.

Regulator T cells augment or suppress immune responses through a variety of T cell-T cell and T cell-B cell interactions. T-helper cells (usually $CD4^+$) control many B-cell functions, including proliferation, maturation, and isotype switching, as well as proliferation and activation of other T cells. Most of these cells react with antigen-class II MHC. Two types of helper T cells have recently been identified according to their profile of cytokine production.[7] T-helper 1 (TH1) cells mainly produce IL-2 and IFN-γ and are active in DTH reactions. The T-helper 2 (TH2) cells produce IL-4, IL-5, IL-6, and IL-10, mainly affecting antibody production and allergic inflammatory responses. Suppressor T cells, most of which are $CD8^+$, down-regulate immune responses and are believed to be critical in various phases of immunoregulation, tolerance, and autoimmunity. They operate in part by secreting various mediators including free TCRs.

As mentioned above, T cells recognize antigens only when these are present on the cell surface of an APC, where they must be associated with polymorphic cell surface polypeptides encoded within the MHC. This dual specificity focuses the attention of T cells on cell-bound antigens. Hence, unlike B cells whose secreted antibodies act at a distance, T cells regulate or kill other cells through cell-cell contact. This contact is mediated through the TCR where its ligand is composed of a fragment of the foreign antigen complexed to some residues within an MHC molecule. The receptor appears to contact portions of both the antigen fragment and the MHC.

The TCR is a disulfide heterodimer composed of α and β glycoprotein chains with constant regions close to the cell surface that anchor the receptor to the T-cell membrane, and the polymorphic variable part (V region) away from the cell surface. It is the difference in the structure of the variable region (similar to antibodies) of the α and β chains that allows the development of different T-cell clones that will eventually recognize the different antigens. The complete molecule has a transmembrane region and a cytoplasmic tail that is responsible for transmitting signals to the cell interior. The TCR is noncovalently associated on the cell surface with CD3, a complex of at least five polypeptides that may participate in mediating activation signals.[8]

Like Ig genes, the genes that encode TCR α and β chains are formed from the joining of separate genetic elements. These gene segments rearrange during T-cell ontogeny within the thymus by deletion of intervening DNA to form a contiguous V gene. The rearrangement of the gene segments to form the TCR gene allows the generation of a diversity of receptors.

Recently, a smaller set of T cells were identified that express a receptor that is a disulfide-linked heterodimer composed of γ and δ chains (instead of α and β). These chains also consist of V and constant (C) regions and are encoded by rearranging gene segments. These cells resemble the regular α/β T cells, but are distinguished by having a smaller repertoire of V gene segments in their receptor. Their function and the ligand for the receptor are unknown, although many demonstrate non-MHC-restricted killing of target cells.

When an α/β-TCR on a CD4 lymphocyte binds to a specific peptide antigen-class II MHC molecule combination on an APC, a trimolecular complex is formed that transmits a signal to the cell. This signal initiates a series of biochemical reactions within the cell, resulting in subsequent biological responses such as proliferation and lymphokine production. The critical biochemical events are (1) hydrolysis of phosphatidyl inositol biphosphate to form inositoltriphosphate (IP3) and diacylglycerol (DAG); (2) influx of calcium into the cell cytoplasm; and (3) activation of protein kinase C (PKC). Calcium and PKC act as a second messenger, probably with other signals to induce transcription of IL-2 and other cytokine genes,[9] which in turn activate T cells and other immunocompetent cells.

C. Mast Cells and Basophils

In humans, mast cells are usually found in the loose connective tissues of all organs, notably

around blood vessels, nerves, and lymphatics. Basophils circulate in the blood stream. Both contain multiple prominent granules that stain from purple to red with blue aniline dyes, thus exhibiting metachromasia. This staining property is attributed to highly sulfated proteoglycans within mast cells and basophils. Both cells also synthesize and store histamine and proteases complexed to these proteoglycans. These substances are then released when mast cells or basophils are activated by an antigen that is recognized by antigen-specific IgE bound to high-affinity (FcεRI) receptors located on the surface of these cells.

All mast cells are derived from progenitor cells present in the bone marrow. Normally, mast cells are not found in the circulation. Progenitors are believed to migrate to the peripheral tissues as immature cells and undergo differentiation *in situ* under microenvironmenal influences. Four interleukins have been shown to sustain and promote the growth and development of murine mast cell populations, e.g., IL-3, IL-4, IL-9, and IL-10.[10] IL-3 is a necessary factor to maintain mast cell viability. IL-4 has little or no ability to sustain the proliferation of murine mast cells in the absence of IL-3. In the presence of IL-3, IL-4 promotes mouse mast cell maturation *in vitro*. Granulocyte macrophage-colony stimulating factor (GM-CSF), on the other hand, inhibits the growth of IL-3-dependent mast cells,[11] as does TGF-β.[12]

A significant advance in the understanding of mast cell biology is the appreciation that mast cells may assume one of two phenotypes.[13] Mucosal mast cells are found mainly in the mucosa of the gastrointestinal (GI) tract. Their main arachidonic acid metabolite is leukotriene (LT) C4 and their viability *in vivo* appears to depend on T cells, the presumed source of IL-3 and IL-4. The second phenotype of mast cells has been found in the skin, lungs, and serosal cavities. These connective tissue mast cells contain heparin as their main proteoglycan, produce prostaglandin (PG) D2, and depend on stromal cell-produced stem cell factor (SCF) for their maturation *in vivo*.[14,15] The gene encoding SCF has been cloned and SCF has been shown to be a product of the Sl locus in the mouse and the ligand for the proto-oncogene c-kit tyrosine kinase receptor (allelic with W locus).[15] However, mucosal and connective tissue phenotypes are not fixed, and bidirectional changes may be possible in suitable microenvironments. It is likely, however, that in normal development there is a maturational sequence of bone marrow precursors to mucosal type mast cells to connective tissue mast cells. Thus, the precise nature of the mast cell and its mediators varies with anatomic location.[13]

In humans, the factors that regulate mast cell growth and differentiation are less defined. In addition, human mast cells are not as clearly differentiated as mouse mast cells. Major differences between types of human mast cells reside in the composition of serine proteases found in the granule that are trypsin like or chymotrypsin-like in substrate specificity, and in the ultrastructural morphology of the granules. For example, in the skin, the predominant mast cell contains tryptase, chymase, and cathepsin G (MCtc mast cell type). The granules in these cells have a pattern described as lattice like or crystalline, and the cells degranulate in response to neuropeptides such as substance P or opiates. By contrast, GI mucosa mast cells (MC_t type) contain tryptase, but not other proteases, have a granule pattern described as scroll-like, and do not respond to substance P or opiates with degranulation. Nevertheless, it does appear that in humans as well as in mice, the pattern of mediators produced by mast cells may vary with anatomic location.[16]

Indeed, $CD34^+$ mononuclear cells that are cultured in the presence of IL-3 over agarose surfaces for 3 weeks yield cultures with 1 to 3% mast cells.[17] Mast cell granules in such cultures appear as homogeneous dense structures, but without the scroll, lattice, or reticular pattern seen in mature skin or lung mast cells. For human mast cells to express a more mature phenotype, coculture with fibroblasts is required. In these cocultures, $CD34^+$ progenitor cells give rise to mast cells that have IgE receptors and granules containing tryptase. More strikingly, cocultured mast cells have a variety of granule morphologies including scroll, reticular, and dense-core patterns found in mature human mast cells within tissues.

The human homolog of SCF has been cloned and its effects on human mast cells have been examined. Thus, mast cells cultured in IL-3 plus SCF, but not in IL-3 alone, are berberine sulfate positive, suggesting the presence of heparin proteoglycans within the granules.[10,17] Also, elec-

tron microscopic examination of cultures supplemented with IL-3 and SCF, but not with IL-3 alone, reveals that after 3 weeks in culture, mast cell granules contain tryptase and exhibit scroll and reticular patterns as seen previously in CD34/3T3 fibroblast cocultures. Thus, $CD34^+$ cells cultured in the presence of both IL-3 and SCF give rise to cultures containing mast cells (and basophils), with mast cells showing evidence of maturation. Bone marrow-derived IL-3-dependent murine mast cells undergo programmed cell death (apoptosis) upon removal of IL-3, an event that is prevented by the addition of SCF through its ligand c-kit. This demonstrates how these principal mast cell growth factors may act in concert to regulate mast cell number under physiologic or pathologic conditions.[18]

Activation of mast cells (or basophils) upon interaction of an antigen (allergen) with its specific membrane-bound IgE will result in an allergic response, to be discussed later in this article (Figures 2 and 3).

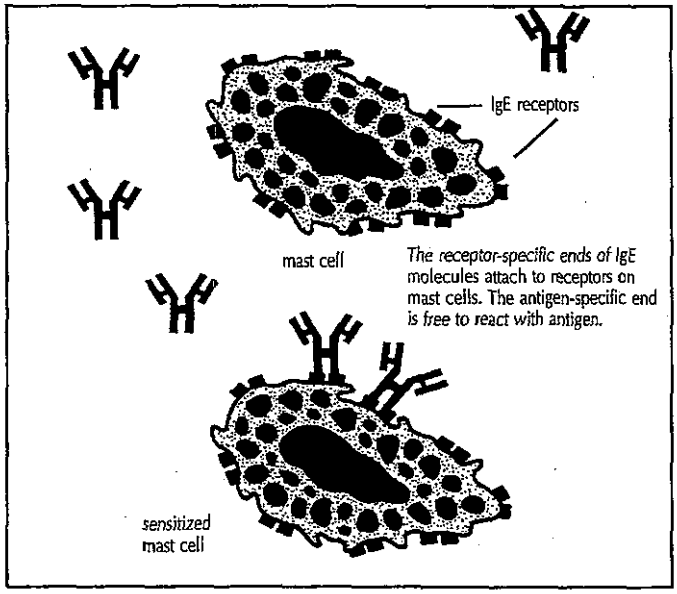

FIGURE 2. Sensitization of mast cells. (Reprinted from ILSI Europe Concise Monograph on Food Allergy. With permission from the International Life Sciences Institute.)

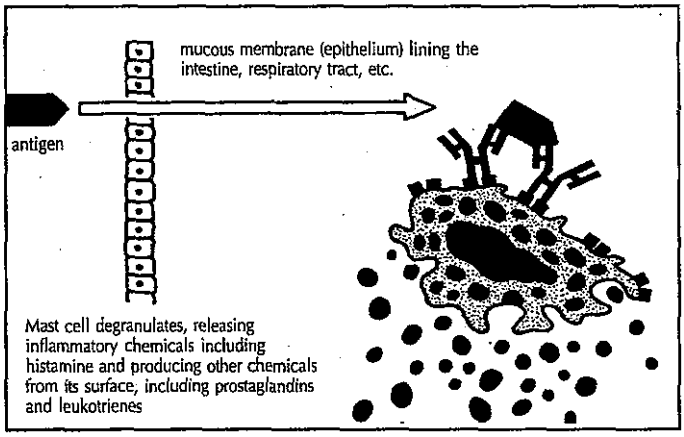

FIGURE 3. Inflammatory reaction initiated by mast cells. (Reprinted from ILSI Europe Concise Monograph on Food Allergy. With permission from the International Life Sciences Institute.)

III. IMMUNOLOGIC MECHANISMS OF TISSUE DAMAGE

When an adaptive immune response occurs in an exaggerated or inappropriate form causing tissue damage, the term hypersensitivity is applied.[19] Clinical allergy or hypersensitivity phenomena are associated with a wide range of processes. However, in each of these entities a common denominator of pathophysiology is either objectively demonstrable or reasonably inferred. Hypersensitivity is immunologically mediated through the interaction of an antigen of exogenous or endogenous origin with specific antibodies or lymphocytes. The consequence of this interaction may be quite diverse, and may account for a variety of clinical manifestations, including allergic reactions such as rhinitis or anaphylaxis; and autoimmune phenomena, including hemolytic anemia and connective tissue disorders, vasculitides, or graft rejection. As more basic immunologic data have been correlated with clinical manifestations, a workable system of general classification has been developed. Gell and Coombs[20] have classified the mechanisms of tissue injury due to hypersensitivity reactions into four distinct types. In practice these types do not necessarily occur in isolation from each other. These reactions are basically expressions of the beneficial immune responses acting inappropriately, and sometimes causing inflammatory reactions and tissue damage. The first three types are antibody mediated, whereas the fourth is mediated primarily by T cells and macrophages.

A. Type I or Immediate Hypersensitivity Reactions

These reactions occur when antigens such as certain proteins from pollens or foods bind to specific preformed IgE antibodies attached to the surface of basophils in the blood or mast cells in tissues. This interaction results in the release of various mediators such as histamine, PG, LT, and cytokines, which produce an acute inflammatory reaction with symptoms such as rhinitis, asthma, or anaphylaxis.

B. Type II or Antibody-Dependent Cytotoxic Hypersensitivity Reactions

These reactions involve the binding of antibodies to cell-bound antigen. This may lead to phagocytosis, cell destruction by killer cell activity, or complement-mediated cytolysis of the cells to which the antibody is bound. Examples of tissue injury by this mechanism include immune hemolytic anemia and Rh hemolytic disease of the newborn. It should be noted that antibody interaction with membrane-bound antigen may lead in certain cases not to cell destruction, but to inappropriate (exaggerated) function of the target cell as found in hyperthyroidism.

C. Type III or Immune Complex-Mediated Hypersensitivity Reactions

These reactions develop when antibody-antigen complexes are formed in large quantities or cannot be cleared adequately by the reticuloendothelial (or mononuclear phagocyte) system. Deposition of these complexes in tissues or on vascular endothelium results usually in complement activation, which leads to polymorphonuclear cell infiltration and activation. This cascade of events produces the tissue injury which is found in conditions such as serum sickness and vasculitis.

D. Type IV or DTH

DTH is manifested when antigen-sensitized T cells are stimulated with the specific antigen introduced to T cells in a specific manner by APCs. This interaction leads to the activation of the T cells and to lymphokine production that mediates a range of inflammatory responses due to the ability of lymphokines to attract and activate various cells with proinflammatory effects. The classic examples of this response are the tuberculin skin test reaction and contact dermatitis. DTH is particularly relevent in understanding the pathogenesis of gluten-sensitive enteropathy (see "Allergic Reactions to Foods" by J. Anderson in this issue).

IV. THE IMMEDIATE HYPERSENSITIVITY RESPONSE

The clinical and pathologic manifestations of immediate hypersensitivity (type I) reactions are the net result of the actions of the mediators released from sensitized mast cells upon their interaction with specific antigens. Thus, three essential components are involved in the elicitation of immediate hypersensitivity. These are the antigen (allergen), IgE antibodies directed at antigenic determinants on the allergen, and the effector cell, namely, the mast cell or basophil. Thus, the typical sequence of events in immediate hypersensitivity is as follows: (1) production of IgE by B cells in response to exposure to the antigen; (2) binding of IgE to its receptors on the surface of mast cells and basophils (Figure 2); (3) interaction of the reintroduced antigen with the bound-specific IgE; (4) activation of mast cells and basophils with the release of mediators that affect the various target organs resulting in the clinical and pathologic manifestations of disease (Figure 3).

A. The Antibody

IgE is central to the induction of immediate hypersensitivity through its binding to high-affinity receptors on mast cells and basophils. The critical difference between atopic and normal individuals is that the former produce high levels of IgE in response to particular antigens, whereas the latter generally synthesize other Ig isotypes and only small amounts of IgE. IgE is the least prevalent of the five antibody classes in terms of serum levels, which range in normal human subjects from 17 to 250 ng/ml and comprise approximately 0.002% of total serum Ig (Table 2). In pathologic conditions, such as severe atopy, this level can rise to over 1000 ng/ml. IgE circulates as a bivalent antibody and contains a relatively high proportion of carbohydrates (approximately 12%) giving it a mol wt of around 200,000. The IgE heavy-chain V (variable) regions and IgE light chains are products of the same genes as other Ig molecules. The heavy-chain C (constant) regions are encoded by the ε gene located in the heavy-chain gene cluster. Thus, IgE is produced as a result of heavy-chain isotype switching.

B. IgE Receptors

Despite the minuscule fraction that IgE constitutes in the serum, its action is amplified by the activities of the receptors to which it binds. The "high-affinity" receptor FcεRI on mast cells and basophils (affinity constant at about 10^{-9} to 10^{-10} *M*) binds the Fc region of the ε heavy chain of IgE and is responsible for sensitization. When a multivalent allergen associates with FcεRI-bound IgE, it cross-links these receptor molecules on the cell membrane. This triggers degranulation of the cell resulting in acute allergic inflammation in the surrounding tissues (Figure 3). FcεRI has been discovered recently on Langerhans cells (in the skin), eosinophils, and activated monocytes. Its biological functions in these cells have not been identified, except that in eosinophils FcεRI does mediate IgE-dependent killing of parasites *in vitro*.[21] B cells, eosinophils, and macrophages express various forms of a second lower affinity receptor for IgE termed FcεRII, and also known as CD23. This receptor mediates phagocytosis of immune complexes by monocytes *in vitro*, and on the surface of B cells it plays a part in IgE-dependent antigen presentation to T cells and in adhesion of B cells to each other.[21] It is also expressed on follicular dendritic cells in the lymph nodes and may thus be involved in the homing of B cells to the germinal centers in the lymphatic organs. Soluble fragments of FcεRII stimulate the growth and differentiation of the precursors of plasma cells, T cells, and basophils, thereby initiating both humoral and cellular immune responses. The soluble form of FcεRII has also been found to up-regulate IgE synthesis by B cells.[21] IgE itself closes a negative feedback loop by switching off its own synthesis through interaction with FcεRII. These observations are now interpreted in terms of a system of interacting proteins that are designated as the IgE network.[21]

C. Regulation of IgE Synthesis in Humans

Cytokines are essential for B-cell proliferation and differentiation. They not only determine Ig secretion quantitatively, they also direct Ig isotype switching. IL-4 is a crucial factor for

isotype switching to IgE. It provides a necessary but insufficient condition for the induction of IgE synthesis by B cells. In humans, a variety of second IgE-inducing signals synergize with IL-4 in the induction of IgE synthesis.[22] In addition to cytokines, contact-mediated signals delivered by $CD4^+$ T cells are required for B-cell proliferation and Ig production. Recently, the ligand for CD40, which is expressed on activated $CD4^+$ T cells, was shown to be one such membrane-associated molecule that acts as a stimulatory signal for IL-4-dependent IgE production by both murine and human B cells.[23] CD40 is a 50-kDa surface glycoprotein expressed on all human B cells. It is now clear that mast cells secrete IL-4[24] and express CD40-ligand,[25] allowing them to play a key role in allergy by producing inflammatory mediators, and by directly regulating IgE production. IL-4 up-regulates FcεRII on B and inflammatory cells, providing in turn a local source of contact stimulation and of soluble growth factors. Moreover, several cytokines, including IL-5 and IL-6, up-regulate the IgE response induced by IL-4 in mononuclear cells.[22] Recently, IL-13 induced IgE and IgG4 synthesis by human B cells, an effect that was found to be IL-4 independent.[26] Thus, IL-13 is another T-cell-derived cytokine that, in addition to IL-4, efficiently directs native human B cells to switch to IgE production. IFN-γ has been reported to inhibit IL-4-dependent IgE synthesis in both humans and mice.[22] Of particular interest is the finding that the expression of IFN-γ is down-regulated by IL-4, both at the protein and at the mRNA level. Induction of IgE synthesis by IL-4 may reflect not only the ability of this cytokine to induce isotype switching, but also its ability to simultaneously suppress a major functional IL-4 antagonist.

D. The Antigen

Antigens that elicit immediate hypersensitivity reactions are called allergens and are proteins or chemicals bound to proteins. Materials such as venoms, food, pollens, animal danders, and hormone extracts function as complete antigens and are capable of eliciting a complete IgE response. On the other hand, low-molecular-weight substances, including some drugs, function as haptens that by themselves cannot elicit an IgE response. These substances usually bind to self proteins (carriers), forming hapten-carrier conjugates that function as complete neoantigens. It is not known why some antigens cause strong type I responses, whereas other antigens, which may be encountered by the same route of administration, result in non-IgE humoral or cell-mediated immune responses. The property may reside in the antigen itself, perhaps in epitopes recognized by certain T cells. Some protein antigens are naturally encountered with adjuvant substances that favor IgE synthesis. The nature of the adjuvant may determine the type of APC that is activated or recruited and is likely to present the antigen to T cells. A particular type of APC may provide costimulators that favor the activation of helper T cells that are especially potent at stimulating IgE synthesis.[9] Also, genetic predisposition may determine the T-cell response to allergens. Atopic individuals produce larger numbers of allergen-specific IL-4-producing T cells than nonatopic persons. Moreover, allergen-specific CD4 T cells obtained from atopic patients produce high levels of IL-4 compared with CD4 T cells from the same patients not specific for this allergen or with CD4 cells from healthy individuals. These high IL-4-producing cells strongly support the induction of IgE synthesis.[27] These data suggest that chronic stimulation by allergens selects for allergen-specific T cells in individuals whose T cells are intrinsically prone to secrete large amounts of IL-4 on activation (TH2 cells).

In general, repeated exposure to a particular allergen is necessary to develop an atopic reaction to that allergen. For example, individuals with respiratory allergic reactions to pollens may benefit from a geographic change of residence with a change in indigenous plant pollens. Food allergies are common during childhood, perhaps because the mucosal barrier of the GI tract is not mature. A dramatic example of the influence of the natural history of exposure to allergen is seen in cases of bee sting anaphylaxis. The protein toxins in the venom are usually not of concern on the first exposure because the atopic individual has no preexisting specific IgE antibodies. However, a large IgE response may occur after a second sting.

Thus, to initiate a type I response, exposure to an appropriate antigen and a genetically determined capacity to respond with IgE production are required. Antigen presentation requires access of antigen to the mucus membranes, uptake by APC, antigen processing, and stimulation of local antibody production. IgE production occurs in the same local environment as antigen presentation, probably in the draining lymph nodes. As stated earlier, IgE production is regulated by locally produced cytokines including IL-4 secreted by local TH2 cells. The IgE that is produced sensitizes mast cells by binding to FcεRI on the cell surface (Figure 2).

E. Mast Cell Activation and Mediator Release

As mentioned, the event that initiates the immediate hypersensitivity reaction is the binding of allergen to IgE on the mast cell or basophil surface. Mast cells or basophils are activated by cross-linking of FcεRI molecules, which is thought to occur by binding of multivalent allergens to the attached IgE (Figure 3). Individuals subject to type I reactions are more likely to have greater amounts of IgE specific for a given allergen. There is a high probability that the allergen will bridge two adjacent IgE molecules specific for its antigenic determinants, and a cross-linking will occur.

Activation of mast cells (and basophils) results in three types of biological responses: secretion of preformed contents of their granules by exocytosis; synthesis of lipid mediators derived from precursors stored in the cell membrane and lipid bodies; and initiation of transcription, translation, and secretion of cytokines.[9]

Mast cells reach maturity within tissues and are the first cells activated in allergic inflammation. Basophils circulate in the blood as mature cells and are capable of migrating into tissues during an allergic inflammatory response. Through the release and production of a variety of proinflammatory molecules, both cells set in motion a series of events that result in both immediate and late-phase responses (LPR). Activation of mast cells and basophils involves a complex series of biochemical events that lead to degranulation and mediator release. Briefly, the cross-linking of FcεRI results in activation of a G protein that activates a membrane-bound phospholipase C to catalyze phosphatidyl inositol biphosphate to IP3 and DAG. IP3 causes elevation of cytoplasmic calcium and DAG activates PKC. These events are similar to those observed in T cell activation via the TCR. These events lead to phosphorylation of myosin chains, which is thought to result in disassembly of actin-myosin complexes beneath the plasma membrane, thus allowing fusion of the granules with the plasma membrane and resulting in exocytosis of the granule contents.[9]

In addition to the known ability of mast cells and basophils to degranulate in response to cross-linking of FcεRI, they may also be activated and degranulated by a variety of other stimuli. These include the complement-derived anaphylatoxins C3a and C5a produced by complement activation during humoral immune responses; IL-8 or other mononuclear-derived cytokines produced as part of cell-mediated immunity; neurotransmitters such as substance P, drugs such as codeine and drugs that create hyperosmolar conditions.

After mast cell and/or basophil activation, a number of chemical mediators are released from storage granules (preformed mediators) or newly generated. The best known mediator of the former group is histamine. Histamine is formed *in vivo* by the decarboxylation of the amino acid histidine, a process that takes place in the Golgi apparatus of mast cells and basophils. It is then stored as a complex with the highly charged sulfate groups of proteoglycan side chains in the secretory granules. Approximately 3 to 8 pg histamine per cell is found in mast cells isolated from human lungs, skin, and small intestine. Histamine exerts its biological effects on a variety of tissues in large part via two receptors, termed H-1 and H-2. H-1 receptors mediate such effects as bronchoconstriction, intestinal peristalsis, and pruritus. H-2 receptor-mediated effects include increased gastric acid secretion, increased mucous secretion, and increased gastic mucosal blood flow. H-1 and H-2 receptors act in concert to promote uterine contraction and vasodilation. Specific histamine-receptor blockers are now in use for the treatment of histamine-mediated effects. H-1 receptors are blocked by classic antihistamines such as chlorpheniramine and the newer nonsedative

antihistamines, including astemizole, loratadine, and terfenidine. H-2 receptor antagonists include cimetidine and ranitidine.

A second group of preformed mediators includes those that stay firmly associated with the granule after release. The proteoglycans comprise one such group of mediators. Heparin is the prototype proteoglycan mediator in mast cells, but it has become apparent that not all mast cells contain significant amounts of the compound.[13] Instead, mucosal mast cells are known to contain proteoglycans of the chondroitin sulfate class, which are less highly charged than the heparin molecule, or contain a mixture of both forms. Proteoglycans are a major granule constituent and may, by virtue of their charge density, provide a storage matrix for other mediators. After their release, proteoglycans may exert a variety of effects. Heparin binds to various enzymes as well as growth factors, thus influencing their function. Both heparin and oversulfated chondroitin sulfates inhibit lymphocyte blastogenesis. Heparin also acts as an anticoagulant.

A third group of preformed (granule-associated) mediators are the proteolytic enzymes. The most important enzymes in human mast cell granules are proteases. In humans, tryptase is found in both mucosal and connective tissue mast cells, with chymotryptase limited to connective tissue mast cells.[16] A variety of substrates for these proteases have been documented including collagen, bradykinin, and structural connective tissue proteoglycans. Tryptase isolated from human lung mast cells cleaves the complement component C3 to generate C3a anaphylatoxin *in vitro*. The long half-life of tryptase in the serum makes measurement of this mediator a reliable test to document the type I nature of a reaction such as anaphylaxis, but is less useful in food-induced reactions.[28,29]

The activation of mast cells not only causes the secretion of preformed granule-associated mediators, but initiates the *de novo* synthesis of certain lipid-derived substances.[10] Of particular importance are mediators derived from arachidonic acid, itself a cleavage product of membrane phospholipids. Arachidonic acid may be metabolized by two main enzymes: cyclooxygenase, leading to PG and thromboxanes (TX); and lipoxygenase, leading to hydroxyeicosatetraenoic acids (HETE) and LT. These lipid mediators exhibit potent effects on a variety of cells and tissues. For example, LTB4 has potent chemotactic activity for neutrophils, enhances lysosomal enzyme release, and augments superoxide anion production. It has also been suggested as a modifier of lymphocyte function by inducing specific suppressor lymphocytes and augmenting human natural cytotoxic cell activity. Other LT such as LTC4 are potent bronchoconstrictors and increase vascular permeability. PGD2 is generated within 15 s of immunologic activation of rat serosal mast cells and is the primary PG synthesized by mast cells. It is a potent inhibitor of platelet aggregation, causes bronchoconstriction, and induces chemokinesis of various cell types. In conjunction with LTD4, it will mediate accumulation of neutrophils in human skin. High levels of these mediators have been detected in the urine of mastocytosis patients, and contribute to hypotension observed in such patients. A final generated mediator is platelet-activating factor (PAF), which has been detected after activation of mouse bone marrow-derived mast cells, rabbit basophils, and human mast cells, but not following activation of human basophils. It is a potent mediator causing platelet aggregation and degranulation, eosinophil accumulation, bronchoconstriction, mucous secretion, and increased vascular permeability and vasodilation, leading to clinical manifestations such as systemic anaphylaxis.

Mast cells are a significant source of cytokines,[30] including tumor necrosis factor-α (TNF-α), IL-1, IL-4, IL-5, IL-6, GM-CSF, and IL-3. They may also produce chemokines such as T-cell activation gene 3 (TCA-3) and macrophage inflammatory protein α (MIP-1α) and MIP-1β. The relative contribution of mast cells vs. T cells or macrophages to the production of these cytokines *in vivo* is not yet clear. Nevertheless, it is obvious that this capability of mast cells enables them to play an important effector role in various inflammatory processes. These cytokines have multiple proinflammatory effects, including growth-promoting activity (GM-CSF; IL-5), stimulation of IgE synthesis (IL-4), chemotaxis (chemokines and TNF-α), and modulation of the biological functions of a number of immunologic effector cells, including neutrophils and lymphocytes. The biological functions affected by such

cytokines include cell adhesion to endothelial surfaces and to extracellular matrix components, cell migration to the site of inflammation, cellular activation, and cytokine release.[31]

The consequences of mediator release usually occur within minutes, thus the term "immediate" hypersensitivity. The mediators interact with the surrounding tissues and elicit the allergic responses, the nature of which is determined by the local environment. Many, but not all, hypersensitivity reactions result in a second LPR. These reactions begin 2 to 4 h after the original challenge and peak between 6 and 12 h afterward.

Unlike the immediate response, this late event is characterized by a local inflammatory process consisting of accumulation of cells including neutrophils, eosinophils, and in a later phase mononuclear cells, which are mainly lymphocytes. It now appears likely that cytokines, released after mast cell activation, contribute to the LPR. TNF-α in particular may account for the sequential polymorphonuclear and mononuclear cell infiltrates.[31] LPR is believed to be the major pathogenic mechanism that underlies chronic diseases such as asthma.

V. CLINICAL MANIFESTATIONS

The clinical and pathologic features of type I reactions are the result of the cumulative effects of mast cell mediators on the surrounding tissues. These features vary with the anatomic site because of various factors, including the nature and the point of contact with the antigen; the local mast cell phenotype; and the sensitivity of the target organs to the mast cell-derived mediators.[9] The clinical manifestations of food allergy are the focus of "Allergic Reactions to Foods."

The most common forms of type I disease are allergic rhinitis, asthma, atopic dermatitis (AD), and GI allergy. A diffuse multisystemic reaction is termed anaphylaxis.

A. Respiratory Manifestations

1. Rhinitis

Rhinitis is a manifestation of an immunologic response to allergens, such as pollen grains and mold spores, that are deposited on the nasal mucosa. Symptoms may include paroxysms of sneezing, itching of the nose, eyes and palate, and nasal stuffiness. Runny nose (rhinorrhea) with watery discharge is another typical feature of allergic rhinitis that is often accompanied by postnasal drip. A personal history of other atopic diseases, a strong family history of allergy, or a regular seasonal pattern of compatible symptoms is strongly suggestive of an allergic cause.[32] Most patients develop symptoms before the age of 30 years. Once symptoms have started, they can be exacerbated by various nonspecific irritants such as cigarette smoke, air pollution, strong odors, and climatic changes.

2. Asthma

Particles with an aerodynamic equivalent diameter of less that 4 μm are likely to reach the lower respiratory tract. Asthma is the clinical result of an immunologic response that takes place in the lower respiratory tract. It can be defined clinically as recurrent airflow obstruction causing intermittent wheezing, breathlessness, and sometimes cough with sputum production. Pathologically, the airflow obstruction of asthma is due to a combination of bronchial smooth muscle contraction, mucosal edema, inflammation, and mucus secretion. The disease involves large and small airways but not alveoli, and the processes leading to airflow obstruction are in general reversible.[32]

B. Dermatologic Manifestations

The two most common type I allergic diseases of the skin are urticaria and AD. These skin reactions may be provoked either directly by contact of the allergen with the skin surface or indirectly by mast cell activation by an allergen that is systemically spread via the circulation after ingestion or injection, such as allergic skin response to food, drugs, and stings.

1. Urticaria (Hives)

Urticaria consists of blanchable, erythematous, edematous papules and plaques, usually

pruritic and ranging from 1 to 2 mm to several centimeters in diameter.[33] Individual lesions usually last several hours. Acute episodes of urticaria are commonly of brief duration, resolving within several days. Most cases of acute urticaria are IgE dependent, whereas an IgE-dependent mechanism can only rarely be implicated in chronic urticaria that is defined as urticaria that lasts more than 6 weeks. Cutaneous mast cells may also be activated and thus cause urticaria via local nonimmunologic mechanisms such as physical stimuli, including dermatographism, pressure, and cold.

2. AD

AD is a chronic inflammatory skin disease in which the role of activated mast cells has not yet been elucidated. It is characterized by pruritus, erythema, edema, and vesiculation. The chronic lesions become scaly and lichenified. The importance of IgE-mediated hypersensitivity is suggested by the clinical findings that 80% of patients with AD have significantly elevated IgE levels, allergens exacerbate AD, and a personal or family history of AD is usually present.[33] Recently, receptors for IgE have been found on the surface of Langerhans cells in the skin that serve as APC for T cells.

C. GI Manifestations

GI manifestations of IgE-mediated allergic responses (see "Allergic Reactions to Foods") are more frequent than the respiratory or cutaneous manifestations. These symptoms result from local effects of mast cell-derived mediators on the GI mucosa. Mast cells in the GI tract may be activated by either local direct stimuli such as foods or as a part of a generalized response to an antigen spread by the circulation. These GI symptoms usually include nausea, cramping, bloating, vomiting, and diarrhea.

D. Anaphylaxis

Anaphylaxis refers to the constellation of signs and symptoms that result from IgE-mediated mast cell- and basophil-activation and degranulation. The target tissues for the mast cell and basophil derived mediators are primarily blood vessels and smooth muscles.

The sites of responses to mediators are both local and remote from the site of the antigen deposition. This is a diffuse response and may be fatal if not treated. The clinical manifestations of anaphylactic reactions are specific, depending on the particular target systems affected. For example, reactions involving the skin are manifested by pruritus, flushing, urticaria, and angioedema. Involvement of the upper airways is exhibited by hoarseness, stridor, and laryngeal edema. The lower airway manifestations of anaphylaxis include dyspnea, tachypnea, wheezing, bronchorrhea, and ultimately respiratory arrest. Cardiac manifestations include tachycardia, arrhythmias, hypotension, and ultimately cardiac arrest. Finally, GI signs and symptoms include nausea, vomiting, cramping, and diarrhea.[34]

VI. DIAGNOSTIC APPROACH

The patient's medical history is the primary diagnostic procedure in allergy practice. Laboratory studies, including skin and *in vitro* tests for specific IgE antibodies, have relevance only when correlated with the patient's medical history.[1] The specific diagnostic approaches for the diagnosis of food allergies is detailed in "Allergic Reactions to Foods."

A. History

History of a possible allergic disease should include specific information such as: (1) seasonal, weekly, or diurnal variations of the symptoms; (2) relationship of symptoms to location (home, work); (3) exacerbating factors (e.g., dust, molds, animals); (4) medications and their efficacy in the treatment of the current symptoms; (5) occupational history; (6) family and past history of allergic diseases; (7) environmental survey that describes specific features of the patient's indoor or outdoor environment and the effects of specific environmental agents on symptoms.

B. Diagnostic Tests

The diagnostic tests currently employed verify the presence of specific IgE and point to the possibility of a type I allergic etiology to the patient's symptoms. These tests in and of themselves are

not diagnostic of IgE-mediated allergic disease, but provide evidence of immunologic sensitization.

1. Allergy Skin Testing (ST)

ST has been a primary diagnostic tool since the end of the nineteenth century. It is performed by introducing the allergen by prick or intradermal injection into the skin, although for foods, the prick skin test is preferred. For a skin test to be considered positive, a diluent control must be negative and a histamine control must be positive. A positive skin test is the culmination of a number of events that begin with the interaction of the allergen with IgE on the surface of cutaneous mast cells. This interaction is followed by mediator release and the subsequent biological effects on the surrounding tissues. These include vasodilation, increased vascular permeability, plasma leakage, and neuronal stimulation. These events lead to a "wheal" (leakage of fluid) and "flare" (vasodilation) response which is associated with local itching (neuronal response). The skin test indicates not only the presence of specific IgE, but also the sensitivity of mast cells and the biological effects of the mediators on tissues.

2. In Vitro *Testing*

In vitro tests detect the presence of specific IgE in the serum. A solid-phase immunoassay is widely used in which the allergen is bound to a solid phase and then incubated first with the patient serum and then with radiolabeled anti-human IgE antibody. In reports comparing *in vivo* and *in vitro* testing, *in vivo* testing (e.g., skin tests) is more specific for most antigens.[35]

3. Challenge Tests

This diagnostic procedure involves the introduction of the allergen in the "natural" way by inhalation or ingestion. Ingestion would be the most natural route for foods. Signs and symptoms are thereafter monitored. These tests diagnose disease, identify etiologic agents, and study pathogenesis. These challenge tests, including the double-blind placebo-controlled food challenge (DBPCFC), should be conducted by experienced medical personnel under careful conditions.

VII. PRINCIPLES OF THE THERAPEUTIC APPROACH

Three basic therapeutic techniques should be considered in treating an allergic disease: (1) avoidance of the offending allergens; (2) use of appropriate pharmacologic agents; and (3) allergy immunotherapy. Specific details on the therapeutic approach for food allergies are provided in "Allergic Reactions to Foods." (Also see International Consensus Report on the Diagnosis and Management of Asthma, U.S. Department of Health and Human Services publication 92-3091, June 1992.)

A. Allergen Avoidance

Whenever feasible, avoidance is the preferred form of treatment. It is the only treatment necessary in most cases of allergy to drugs, foods, animals, and some of the physical allergies.

B. Pharmacologic Therapy

Generally, the pharmacologic treatment of allergic diseases may be divided into two categories. The first is symptomatic control. Here, treatment mainly affects target organs involved in the clinical manifestations of the allergic response such as smooth muscles, blood vessel walls, and mucus glands. This category includes the use of H-1 and H-2 histamine receptor antagonists and the β2 adrenergic agents albuterol and salmeterol. The latter agents are specific for the β2 receptors that mediate a potent bronchodilating effect due to smooth muscle relaxation. These agents also reduce vascular permeability and plasma leakage. Also in this group are the anticholingeric drugs such as ipratropium bromide. Their anticholinergic effects include bronchodilation as well as inhibition of mucus secretion. Lastly, in this category of drugs are the methylxanthines such as

theophylline. Their major pharmacologic activity is relaxation of bronchial smooth muscles. Theophylline also improves contractility of the diaphragm, reverses mucosal edema, and accelerates mucociliary transport. Theophylline also antagonizes the effects of adenosine on mast cells and may thereby reduce mediator release.[36]

The second category of therapy affects the cellular components of the inflammation and may be referred to as anti-inflammatory. Drugs in this category include cromolyn sodium, an agent whose mode of action relates to its ability to reduce mast cell reactivity. Therefore, early administration may prevent allergen-induced early and late allergic responses in the lungs and upper airways. Nedocromil sodium, a pyranoquinoline, is another nonsteroid anti-inflammatory agent for the treatment of asthma. Its pharmacologic effects include inhibition of human mast cell and eosinophil activation, as well as inhibition of the mobilization of inflammatory cells such as eosinophils and neutrophils. Nedocromil also inhibits both immunologic and nonimmunologic activation of monocytes and macrophages.[37] These cumulative effects may be relevant to the treatment of allergic diseases such as asthma, given the role of airway inflammation in this disease process. Finally, corticosteroids are known for their impressive therapeutic effects on a variety of allergic diseases. They are useful in treating allergic diseases such as asthma, rhinitis, and AD. Among the most relevant effects are a reduction in microvascular permeability, mucus secretion, and PD production; inhibition of eosinophil migration and proliferation, cytokine production by inflammatory cells; an enhanced β-adrenergic receptor expression; and a reduction in mast cell number and reactivity *in vivo*.[36] These actions together lead to a range of therapeutic benefits including inhibition of late-phase allergic responses.

C. Immunotherapy

This is the process by which increasing doses of the specific allergens to which the patient has been found to be allergic are injected subcutaneously over time to prevent IgE-mediated allergic symptoms. This therapeutic mode affects several immunologic processes in the allergic responses and is the only treatment reported to have long-lasting preventive effects. Immunotherapy is effective in the treatment of allergy to Hymenoptera venom and in allergic rhinitis. The effectiveness of this treatment depends on several factors including delivery of an adequate dose of the respective allergens and the length of time of immunotherapy. Insufficient clinical data justify the use of immunotherapy in the treatment of food allergy.

The multiple effects of immunotherapy on cellular events, inflammatory responses, and immunologic changes that occur in IgE-mediated allergic processes have been reviewed.[38] Briefly, these effects include (1) an initial rise in specific IgE with subsequent significant decrease below treatment levels; (2) a drop in IgE that is inversely correlated with the rise of specific IgG antibodies capable of blocking the passive transfer of the allergic response, termed blocking antibodies; (3) an increase in IgG and IgA antibodies in the nasal secretions; (4) a decrease in basophil releasability where larger amounts of allergen are required for degranulation; (5) a decrease in antigen-induced eosinophil migration into the nasal cavity; (6) prevention of increased responsiveness of the nasal and bronchial mucosa to histamine in allergic patients during the season; and (7) effects on allergen-specific T cells such as a decrease in proliferation and suppression of cytokine production observed in response to the allergens. Recent works have demonstrated that the production of histamine-releasing factors by mononuclear cells is also decreased by immunotherapy.

VIII. SUMMARY

Allergic diseases result from IgE-mediated immune responses to foreign protein (allergens). The majority of such reactions are IgE-mediated (type I) reactions. Individuals who develop such reactions are allergic. Those predisposed on a genetic basis to synthesize IgE to environmental allergens are atopic. Most allergic reactions are precipitated when a specific allergen aggregates several IgE molecules attached to IgE receptors on the surfaces of mast cells and basophils. Chemical mediators are released which lead to the immediate signs and symptoms associated with

allergic diseases including hives, asthma, and anaphylaxis. More prolonged reactions follow if significant numbers of other cells including eosinophils, macrophages, and lymphocytes are drawn into sites of mast cell activation. Therefore, allergic diseases result from a complex interplay of immune cells, foreign porteins, and tissue inflammation.

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Allergic Reactions to Foods

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I. INTRODUCTION

The term *food allergy* is often overused by the public, as well as by some physicians and scientists, to describe any undesired or bothersome problem related to diet. To discuss allergic reactions to foods, it is important to establish a set of definitions to describe these types of reactions. Table 1 provides such a glossary of terms[1] that has evolved over the years from an earlier classification.[1] In this set of definitions, *food intolerance* is used to describe nontoxic, nonimmune-mediated reactions and *food allergy* relates to immunologic reactions (usually immunoglobulin E [IgE mediated]).

II. PREVALENCE OF ADVERSE FOOD REACTIONS

Although the best estimates suggest that less than 1 to 2% of the general population suffer from food allergies, the prevalence of adverse reactions to foods is unknown.[2,3] The public's perception of the importance of allergic reactions to food ingredients, however, substantially exceeds the prevalence of such reactions identified in limited clinical studies. In some surveys, one in four atopic adults believed they had experienced an adverse reaction following ingestion of a specific food.[4] The prevalence of adverse reactions to food confirmed by a double-blind, placebo-controlled food challenge (DBPCFC) during the first 3 years of life among 480 consecutively born infants in Denver, Colorado, was 8%.[5] Of these children, 25 (5.2%) were suspected to be milk allergic, but only 11 (2.3%) could be confirmed to be so by DBPCFC. In a prospective study among a cohort of 1749 newborns born in a single hospital from a municipality in Denmark during the calendar year 1985, 39 (2.2%) were found to have systemic adverse reactions after cow's milk protein challenge.[6]

The prevalence of food additive reactions in children is also unknown and a subject of debate. For example, a recent prevalence study among 4274 Danish school children age 5 to 16 years has been reported.[7] Following selection through a screening questionnaire and open challenges with mixes of food preservatives, dyes (colors), and flavors, 12 of 17 positive responders were challenged using the DBPCFC method and 6 (50%) were positive. Five of the six reacted to the coloring agent and one reacted to citric acid. (One had urticaria and five had exacerbation of atopic dermatitis [AD].) Based on this study, it was estimated that the incidence of adverse reactions to food additives among Danish children is 1 to 2%, although 6/4274 is 0.14%.[7]

The most common respiratory allergic manifestation in adults is allergic rhinitis due to pollens from trees, grasses, weeds, and, in North America, from ragweed. Although such respiratory symptoms are not commonly involved in food allergies, some individuals with pollen allergies experience mild adverse reactions confined to the oropharyngeal area after ingestion of certain fresh fruits and vegetables. In this oral allergy syndrome, localized itching and swelling of the oropharyngeal area occurs when exposed to such fresh fruits or vegetables.[8–10] In a series of 1447 ragweed hay fever patients, 6.2% were found to have local allergy symptoms to melons and bananas.[11,12] Of 274 patients allergic to one or more

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TABLE 1
Adverse Reactions to Food: Classification

I. Toxic reactions

These occur in any exposed individual if the dose is high enough. Toxic compounds may be naturally occurring or are induced during food processing or by contaminants. Symptoms of some toxic reactions may resemble those caused by allergy.

II. Nontoxic reactions

A. Immune-mediated
The term *food allergy* is recommended for immune-mediated reactions. Allergens are defined as the antigenic molecules giving rise to the immune response.
1. IgE-mediated
Symptoms include anaphylaxis and symptoms from the skin, respiratory, and GI tracts. None of these symptoms are specific.
2. Non-IgE-mediated
Diseases include protein-induced gastroenteropathy and celiac disease. The precise role of food in immune mechanisms involved in such disorders is not known.

B. Non-immune mediated
The term *food intolerance* is recommend for nonimmune-mediated reactions.
1. Enzymatic
Secondary lactase deficiency affects most of the world population, whereas most other enzyme deficiencies are rare inborn errors in metabolism.
2. Pharmacologic
This form of intolerance is present in individuals who are abnormally reactive to substances such as vasoactive amines normally present in some foods.
3. Undefined
This includes adverse reactions to food for which the offending mechanisms are unknown, including some additive intolerances.

Adapted from Bruijnzeel-Koomen et al.[125]

of three pollens (birch, grass, and/or mugwort), 111 (47%) had clinical symptoms or IgE antibodies to apples, potatoes, carrots, celery, peaches, or melons.[8]

Finally, an estimate of the prevalence of anaphylaxis and other systemic reactions to food and food additives (especially sulfites) was obtained through a prospective survey of 73 Colorado emergency departments over a 2-year period.[13] Twenty-five individuals ranging in age from 2 to 71 years (2/3 over 18 years) had a serious reaction. Two required cardiopulmonary resuscitation and one patient died. Based on the prevalence of food-associated reactions in comparison to overall emergency room admissions for life-threatening reactions, it is estimated that approximately 950 severe reactions to food and food additives requiring emergency intervention occur annually in the U.S., or 0.0004% for a population of 250 million. These observations in total present a variety of perspectives on the prevalence of adverse reactions to foods.

III. NATURAL HISTORY OF FOOD ALLERGY

Studies of the prevalence of food allergy have shown that the clinical manifestations of food reactions are most commonly observed in the first 3 years of life. In prospective studies, in 80 to 87% of situations, a child once proven reactive to a food can be shown to clinically tolerate that food by 3 years of age.[5] Clinical tolerance develops with soy, wheat, cow's milk, and egg allergy. The more serious the initial clinical reaction to food, however, the longer it takes the child to develop tolerance.[14] It has also been shown in

older children and adults that if the offending food can be identified and totally eliminated from the diet for a given period of time, clinical tolerance to that food develops in some patients.[15,16]

In patients who develop anaphylaxis to foods such as peanuts, tree nuts, fish, and crustacea such as shrimp, prawns, crab, lobster, and crayfish, the exact duration of the risk of repeat anaphylaxis upon further exposure is unknown, but may be lifelong.[4] In a group of peanut allergic individuals, the risk of repeat reactions to peanut lasts for at least 14 years.[17]

IV. EXPOSURE TO FOOD ALLERGENS

Most food exposure is initially through the gastrointestinal (GI) tract. The GI tract provides a barrier of immunologic and nonimmunologic defense mechanisms where unwanted, intact proteins are prevented from gaining ready access to the body.[4] Some intact food proteins do gain access. However, oral tolerance develops to most food proteins. The increased susceptibility of infants to food allergy is believed to result to some extent from the relative immunologic immaturity of the GI barrier at a young age. As a result, in familially predisposed infants, ingested and absorbed food protein may stimulate specific IgE antibody formation as well as other abnormal immune responses. Reexposure to these food proteins may result in clinical reactions (for pathophysiology, see "Introduction to Allergic Diseases" by Yoseph A. Mekori). Subsequent contact of the mucous membrane in the mouth or inflamed skin with food antigen may also be enough to bring on a reaction.[9] An example of the latter was an infant allergic to milk whose inflamed skin was exposed to a cow's milk protein that was an ingredient in a diaper rash ointment containing 5% calcium caseinate causing anaphylaxis.[18]

The major identified allergens responsible for IgE-mediated reactions to foods are detailed in "Allergenic Foods" by Susan L. Hefle et al. and "Principles and Characteristics of Food Allergens" by Steve L. Taylor and Samuel B. Lehrer in this issue. These food allergens are usually heat stable and resistant to proteolysis. Clinically, many patients react to only one or a few items from one food family. Peanut allergic individuals usually can be found to possess IgE antibody reactivity to other legumes (peas, beans, soy), yet can regularly ingest these foods without clinical reactions.[19] The major fish allergen is *Gad c* 1.[4] Although this allergen is found in a diversity of freshwater and seawater fish, some individuals only react clinically when challenged with specific fish types.[20]

In occupationally acquired food allergy in adults, the initial contact with food protein may be through inhalation or skin contact.[21] Probably the best known example is baker's asthma. Some bakers developed asthma and rhinitis after inhaling or being in skin contact with flour.[22] Most bakers, however, ingest bread without a reaction. Other workers, though, after being first sensitized through the respiratory or skin contact route, later become reactive when the same processed or cooked food is ingested. Such is the case with snow crab processors with crab;[23] bakery workers with egg;[24] and garlic spice processors with garlic.[25] IgE reactions may also occur through exposure to the aerosolized fish or seafood protein.[23] Aerosolization of food proteins during cooking may therefore pose a risk to certain highly food allergic individuals.

V. CLINICAL MANIFESTATION OF FOOD ALLERGY

There are a variety of clinical manifestations of food allergy (Table 2) and these are reviewed in this section.

TABLE 2
Clinical Manifistations of Food Allergy

I. Anaphylaxis
II. Oral allergy syndrome
III. Food-dependent, exercise-induced anaphylaxis (F-EIA)
IV. Atopic dermatitis (AD)
V. GI reactions
  A. IgE-mediated immediate reaction
  B. Food protein-induced enterocolitic syndromes
  C. Eosinophilic gastroenteritis
  D. Celiac disease (gluten-sensitivity enteropathy)
VI. Respiratory reactions
  A. Rhinitis
  B. Asthma
VII. Occupationally acquired food allergy
  A. Urticaria/angioedema/anaphylaxis
  B. Asthma
  C. Hypersensitivity pneumonitis

A. Anaphylaxis

Anaphylaxis is a systemic reaction characterized by urticaria, angioedema, laryngeal edema, bronchospasm, hypotension, diarrhea, cardiac arrhythmias, nausea, vomiting, abdominal cramping, and even death. The initial symptoms of anaphylaxis usually begin within a few minutes and almost always within 30 min after exposure.[26] Three clinical patterns of anaphylaxis have been described regardless of the initiating allergen: uniphasic, biphasic, and protracted.[27] In uniphasic reactions, symptoms appear shortly after food ingestion and last for several hours. In the biphasic reactions, an early and a late phase are separated by an interval of 1 to 8 h. In protracted anaphylactic reactions, symptoms persist for 5 to 32 h without remission.

Most individuals who experience life-threatening food-induced anaphylaxis have multiple clinical manifestations of allergy including asthma, and have demonstrable IgE antibodies to specfic incriminated allergens.[28,29] Foods incriminated in life-threatening reactions include, but are not limited to, peanuts, crustacea (crab, shrimp, lobster, and crayfish), tree nuts, milk, and egg. A more complete list of foods eliciting life-threatening reactions is found in "Allergenic Foods."[28,29] Most deaths occur in proven allergic individuals who inadvertently eat these foods away from home (e.g., restaurant or party) and in a disguised form (e.g., pastry, candy, salad, sandwich, or hors d'oeuvre).

The principal difference between death and near death in a group of known allergic children and adolescents who were exposed to a food to which they were sensitive was how rapidly epinephrine was given. Most children survive when epinephrine is given within 1 h.[29]

B. Oral Allergy Syndrome (OAS) (Fruit and Vegetable Syndrome)

In individuals allergic to trees (particularly birch), grasses and weeds (particularly mugwort), and ragweed pollens, symptoms may develop after contact exposure in the mouth with allergens in fresh fruits and vegetables.[8–11] The initial symptoms of OAS usually include a tingling oral sensation while eating the fresh fruit or vegetable, followed by itching of the lips, tongue, or buccal mucosa.[9] Swelling of the mouth parts occurs in about one half of the cases. Although most symptoms are localized to the mouth, in some cases the localized symptoms are also associated with systemic symptoms, particularly a stuffy or runny nose or conjunctivitis as well as, in a few cases, systemic anaphylaxis.[10]

In the majority of cases, the symptoms begin within 5 min of food contact.[30] Usually the localized symptoms abate within 30 min. Almost all symptoms disappear without therapy 90 min after discontinuation of eating the specific offending food, and after rinsing the mouth with water.

The foods associated with OAS include melons and bananas among ragweed allergic individuals; apples, pears, potatoes, hazelnuts, carrots, celery, and kiwi among birch pollen-sensitive patients; peach, tomatoes, and celery among grass allergic individuals; and celery in mugwort-sensitive individuals.[9,10,30]

Some individuals with documented latex allergy are also allergic to bananas, chestnuts, avocado, and kiwi.[31–33] Information points to the existence of cross-reactivity between the natural rubber latex allergens and both ragweed and blue grass pollens.[34]

C. Food-Dependent Exercise-Induced Anaphylaxis (F-EIA)

Exercise-induced anaphylaxis (EIA) is a form of physical allergy involving urticaria, angioedema, or shock after vigorous exercise. Almost any exercise may be involved, but jogging is the most frequent cause in the U.S.[35] This syndrome must be differentiated from exercise-induced asthma and from cholinergic urticaria induced by exercise. Although the exact pathophysiology of EIA is unknown, induced histamine release is one factor. An epidemiologic survey of 199 individuals with EIA showed that the process of eating and/or the ingestion of a specific food was an additional factor in the development of EIA in 54% of the cases.[35]

Foods associated with F-EIA include celery, shrimp, oyster, chicken, peach, and wheat. Some evidence exists for a late-onset F-EIA reaction,

presumably due to reactions to digestive breakdown products of wheat allergens.[36] Finally, in some individuals with F-EIA, the ingestion of any type of a meal, may precipitate symptoms within several hours of vigorous exercise.

D. Atopic Dermatitis (AD)

AD is a primary skin condition of childhood whose pathogenesis involves both immune and nonimmune factors. In a large series involving DBPCFC, approximately one-third of children with AD had food-related hypersensitivity.[37] In these cases, the eczema associated with AD may be at least partially explained by an IgE-mediated late-phase allergic food reaction. Studies using DBPCFC in AD children have shown that children are usually allergic to only one or a few foods such as eggs, cow's milk, peanuts, wheat, or soy. Data from *in vitro* studies have shown that basophils from patients with active AD will spontaneously release histamine when such patients routinely ingest the specific food to which they are sensitized.[38] Mediator release under these circumstances may be modulated by local, IgE-dependent, histamine-releasing factors. After eliminating the incriminated food from the AD patient's diet, the tendency for spontaneous *in vitro* basophil histamine release disappears with concomitant improvement in the skin condition.[38]

Children with AD and food allergy have been studied to assess the value of DBPCFC compared to skin testing (ST) and *in vitro* tests for food-specific IgE antibodies. For instance, the result of challenge testing correlated poorly with the mother's history of which food may be involved with the rash exacerbation.[37,39] Allergy ST and, to a lesser degree, *in vitro* testing to specific food allergens suggested which foods might be involved when subsequent DBPCFC testing was used for a final diagnosis.[39]

E. Gastrointestinal Reactions

GI signs and symptoms are the most common clinical manifestation of food allergy. These include itching and swelling of the mouth parts, nausea, vomiting, diarrhea, malabsorption, and blood and protein loss in the stool (in children).[40] These reactions may be immediate, IgE-mediated reactions, or delayed reactions such as observed in food-induced enterocolitis. In allergic children, symptoms may begin in infancy and be associated with either intolerance or allergy to cow's milk protein found in conventional baby formula.[6]

1. Food-Induced Enterocolitis

Several terms have been used to distinguish the GI food reaction in children, such as food-induced enterocolitis, protein intolerance, or milk-sensitive enteropathy. Both the small and large intestine may be involved. The small intestine may be damaged to varying degrees to include thinning of the mucosa, villous atrophy, crypt hyperplasia, and inflammatory infiltrates of lymphocytes, plasma cells, and eosinophils.[40] Prolonged diarrhea in some cases can lead to dehydration, malabsorption, and failure to thrive.

Milk-induced colitis is related to enterocolitic syndromes and may closely resemble ulcerative colitis. Biopsy is used to make the diagnosis and demonstrate eosinophilic inflammatory infiltrates in the bowel wall.[4] A friable mucous membrane may lead to either occult or gross blood loss.

In these syndromes, GI symptoms may occur late (after hours to days). They often can be proven to be related to cow's milk in the diet through challenge. However, these reactions cannot be categorically proven to be due to IgE-mediated reactions (allergy).[41] Such reactions may also occur to fish, soy, rice, and chicken.

2. Eosinophilic Gastroenteritis

Eosinophilic gastroenteritis affects both children and adults. This disorder is characterized by eosinophilic infiltrates of the bowel wall, peripheral blood eosinophilia, and GI symptoms.[4,40,42] The degree of clinical symptoms depends on the extent of disease involvement. Patients develop cramping abdominal pain, nausea and vomiting, diarrhea, and fecal blood loss. Disease may lead to either growth retardation (children) or weight loss (adults).

About one half of the 150 case reports of eosinophilic gastroenteritis have been in highly allergic individuals. In children, milk is a major allergen involved in this condition.[30] In adults with this condition, multiple food allergies are consistently demonstrated. Patients with the allergic form of eosinophilic gastroenteritis have high total IgE levels and have additional clinical manifestations of allergy including asthma and rhinitis. The disease is managed with diet and steroids.

F. Respiratory Reactions

Food allergy is not a major cause of either upper respiratory (rhinitis) or lower respiratory (asthma) symptoms.[43] However, during a systemic anaphylactic reaction to foods, it is common for individuals to have hay fever or asthma-like symptoms.[26] Most individuals who have had serious, life-threatening anaphylaxis to foods are highly allergic to many substances, and have both rhinitis and asthma.[28,29] Some of the more convincing evidence that food can cause rhinitis and asthma include reports that approximately one-third of children who manifest their allergy primarily with AD develop upper or lower respiratory symptoms during DBPCFC to foods to which they are sensitive.[37]

A syndrome has been described in infants characterized by cow's milk protein-induced recurrent pulmonary infiltrates associated with precipitins to cow's milk protein.[44] Some of these infants had pulmonary hemosiderosis with deposition of iron in the lung. This childhood syndrome is now rarely reported and there is no similar condition reported in adults. The pathogenesis is suspected to involve aspiration of milk-based formula in the first few days of birth, sequestration of these proteins in the lung, followed by a hyperimmune response with the production of large amounts of milk-specific IgG antibodies. Subsequent feeding of milk results in immune complex as well as cell-mediated tissue reactions.[45] Infiltrates resolve with milk elimination.

VI. GLUTEN-SENSITIVE ENTEROPATHY

Celiac disease or gluten-sensitive enteropathy is a permanent condition of wheat or gluten intolerance in children and adults.[46] This disease is more prevalent in the U.K. and Europe than in the U.S. Diagnosis depends on the results of a jejunal biopsy that demonstrates abnormal, short (flattened), or absent villi, crypt hyperplasia, and cellular inflammatory infiltrates of lymphocytes and plasma cells in the lamina propria. These bowel wall changes disappear after the patient is placed on a gluten-free diet, only to reappear after reinstitution of gluten. Clinically, the classic textbook description of an irritable "pot-bellied" growth-retarded child with chronic diarrhea, anemia, rickets, and muscle wasting is now rarely seen. Typically, the disease is less severe and single manifestations, such as growth retardation or anemia in a child or chronic weight loss and persistent diarrhea in an adult, will require investigation and intervention.

Although the pathogenesis of this condition is still being debated, and a toxic reaction to gluten is still possible, evidence points to a cell-mediated immune sensitivity to gluten as a principal pathologic event.[46] Some patients with celiac disease have dermatitis herpetiformis (DH), an associated skin condition. Most patients with DH have small bowel intestinal biopsies that are indistinguishable from those of celiac disease. Most DH patients respond to a gluten-free diet. Patients with celiac disease have a 50 to 100 times increased risk of developing a malignant lymphoma compared to the general population.

VII. OCCUPATIONALLY ACQUIRED FOOD ALLERGY

Reactions to food allergens in adults also occur in occupational settings (Table 3). Occupational exposure results in susceptible individuals developing IgE sensitization to food proteins, often through the respiratory or dermal route.[21,23,47] Continued exposure to these allergens results in rhinitis, bronchial asthma, conjunctivitis, contact and generalized urticaria, and in some instances, systemic anaphylaxis.[21]

The most important manifestation of occupationally acquired food allergy is occupational asthma. Approximately 2 to 15% of all adult-acquired asthma results from occupational exposure to chemicals, toxins, and natural proteins.

TABLE 3
Materials Used in Food or Related Industries That Are Known to Induce Occupational Asthma or Rhinitis

Agents	Occupational exposure
Animal products	
Sea animals	
Prawn	Seafood processing
Crab (including king and snow)	Seafood processing
Oyster	*Culture oyster workers*
Shrimpmeal	Aquaculture
Fishmeal	*Factory workers*
Mother of Pearl	Button factory workers
Seasquirt	Oyster shuckers
Seashells	Shell grinders
Farm Products	
Cows	Dairy farmers
Hogs	Hog farmers
Poultry	Poultry workers
Pheasants, quail, doves	Breeders
Eggs	Egg processors, bakery workers
Insects	
Poultry mites *(Ornithonyssus sylviarum)*	Poultry workers
Grain storage mites *(Glycyphagus destructor)*	Grain workers
Honey bees	Beekeepers, honey processors
Bee-moth	Fish bait breeders
Enzymes	
Pepsin	*Pharmaceutical workers*
Trypsin	Pharmaceutical workers
Pancreatic enzymes	*Pharmaceutical workers*
Miscellaneous	
Spiramycin	Chick breeders
Pyrolysis products of polyvinyl chloride or label adhesives	Meat wrappers
Plant/Fungi	
Grains/Flours	
Flour (wheat, rye)	*Bakers, millers*
Buckwheat	Food workers
Rice	Rice millers
Soybeans	Agricultural workers
Grain dust	Grain handlers
Spices/Herbs	
Garlic	Factory workers, farmers
Coriander, mace, ginger, paprika	Factory workers
Cinnamon	Spice workers
Paprika plants	Greenhouse workers
Enzymes	
Bromelin	Factory workers
Papain	Factory workers
Miscellaneous	
Coffee	*Coffee factory workers*
Tea	Tea factory workers, tea garden workers
Herbal tea	*Herbal tea workers*
Pollen	Sugarbeet, sunflower, grape workers
Alkaline hydrolysis derivative of gluten	*Bakers*
Alternaria/Aspergillus	
	Poultry vendors
Colophony	Poultry vendors
Hops	Brewery chemists
Devil's Tongue (*Amorphophallus konjac*)	Food workers
Mushrooms	Soup manufacturers, growers
Fungal amylase	Bakers
Verticillium albo-atrum	Greenhouse workers

Modified from O'Neil and Lehrer.[47]

For example, the prevalence of occupational asthma ranges from 10 to 30% in bakers,[48] 15% in snow crab workers,[23] and 3% among workers exposed to green coffee beans.[49]

Occupational asthma may be the result of irritation, pharmacologic, or IgE (allergic) mechanisms. Some of the clearest examples of IgE-mediated occupational food allergy are baker's asthma due to flour exposure,[48] snow crab processors exposed to water vapor from cooking snow crab,[23] spice workers exposed to garlic dust,[25] and bakery workers exposed to aerosolized egg protein.[24]

In baker's asthma, wheat and rye flour are the usual etiologic agents. Sensitization occurs through the respiratory route and correlates with a personal or family history of atopy, duration of exposure, and wheat and rye dust levels in the area of employment. Among apprentice bakers, the prevalence of positive immediate skin test reactions to wheat has increased from 8 to 30% over a 5-year period of exposure.[48] Bakers with asthma can usually eat the bread they bake. Snow crab processors and spice workers who become sensitized through the respiratory route, however, may eventually react when they ingest cooked crab or garlic in a meal.[23,25]

Hypersensitivity pneumonitis is another manifestation of occupational reactions to foods. In this condition, an intense, prolonged respiratory exposure to a number of different proteins from molds or fungus, insects, or foods (animal, fish, or avian sources) leads to a hyperimmune response and large amounts of IgG antibody. Reexposure on a continued basis to these causative proteins leads to an immune complex reaction characterized by diffuse mononuclear infiltrates in the lung that involve the interstitium and the alveoli.[47]

VIII. FOOD INTOLERANCE

Many adverse reactions to foods are mediated by mechanisms that do not involve the immune system. Some of these reactions may be confused with allergy. These reactions may be due to naturally occurring toxicants, microbial or chemical food contaminants, metabolic disorders, and idiosyncratic reactions.[50]

Scromboid fish poisoning is an example of a reaction occurring to a microbial-derived food contaminant. In this case, Scromboid fish (such as tuna, mackerel, and bonito) or other fish (such as mahi mahi or blue fish) have become spoiled but are cooked and eaten. Bacteria in the fish tissue, such as *Morganella morganii* or *Klebsiella pneumoniae*, can have decarboxylated histadine to create histamine. The individual who ingests such fish experiences a sharp peppery taste, and a burning sensation in the mouth, followed often by nausea, vomiting, abdominal cramps and diarrhea, as well as facial flushing and headache due to ingested histamine. Individuals may also develop urticaria, wheezing, or shock. Symptoms generally begin within 30 to 60 min of ingestion of the spoiled fish and last for 2 to 8 h.[51]

Other foods that contain histamine include cheese and some red wines.[51] Oriental food may contain histamine, particularly if its preparation involves fermentation.[52]

Endogenous pharmacologic agents in food that may mimic some symptoms of food allergy include tyramine, phenylethylamine, serotonin, caffeine, and theobromine.[52] These natural substances are aggravating factors in headaches, especially in patients with migraines, but frequently these reports are not well documented.

Commonly eaten foods may contain naturally occurring toxicants, but fortunately the dose consumed in most cases is so low that adverse reactions are few in number.[51] One example of an unsafe food, mistaken for a safe food, is the Amanita mushroom. When "poisoning" or toxic reactions do occur, however, rarely are allergic-like symptoms involved, except for the GI symptoms of nausea, vomiting, and diarrhea. A similar situation exists for foods contaminated with microbial toxins or bacterial or other infectious agents. These types of adverse reactions should be kept in mind when considering the possibility of an adverse reaction following a meal.[50,51,53]

A metabolic reaction after ingestion of a specific food is due to differences in individuals in the ability to digest a specific substance, or may be associated with concomitant medication, disease states, or malnutrition. For example, vasoactive amines are metabolized by a family of isoenzymes called monoamine oxidases (MAO). In patients treated with MAO inhibitors for depres-

sion, ingestion of foods rich in tyramine (e.g., cheese) can cause a rapid rise in blood pressure and severe headaches. This reaction is caused by either the uninhibited action of tyramine or the indirect action of the secretion of epinephrine or norepinephrine, following the lack of the metabolism of the pharmacologic agents by the adrenal MAO.[51] A similar reaction has occurred in patients taking the antituberculosis drug, isoniazid, while ingesting tuna. Reactions have occurred because isoniazid inhibits histaminases that normally degrade histamine present in the fish meat.[53,54]

Probably the most common problem induced by diet that is confused with an allergic reaction is lactose intolerance due to lactase deficiency in the GI tract.[56] Lactose is a water-soluble disaccharide found in dairy foods. Prior to absorption lactase enzymes in the intestinal brush border break down lactose to its monosaccharide components. When this enzyme is deficient or totally absent, the undigested lactose ferments in the bowel with resulting symptoms of gas and bloating, abdominal cramps, flatulence, and loose stools.

Congenital lack of lactase is rare. Primary lactase deficiency is usually manifested after the infant weaning period, but before puberty.[50] A GI infection in infancy or childhood may hasten the onset of this condition. Once lactase deficiency has been established, ingestion of a moderate amount of lactose sugar (5 to 12 g in the amount of 100 to 240 ml of whole cow's milk) may result in symptoms in susceptible individuals.[50] Secondary (acquired) lactose intolerance due to lactase deficiency may occur in a variety of clinical conditions including alcoholism, sprue, and cow's milk allergy. It may also occur transiently after a rotavirus or other GI infection.[56] Naturally fermented foods may sometimes be tolerated by lactase-deficient individuals.[57]

IX. ADVERSE REACTIONS TO FOOD ADDITIVES

Additives are often added to processed foods.[58] These agents are added to help preserve the food, stabilize the ingredients, or increase the appeal of the food through flavor or color. Food additives that have sometimes been associated with allergic-like reactions include the antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), aspartame, dyes, monosodium glutamate (MSG), and sodium benzoate. Because of their association with such reactions, some food additives (e.g., sulfites, Yellow no.5) must be affirmatively declared on food labels. Adverse reactions that are allergic-like may generally be either grouped as (1) urticaria, angioedema and generalized anaphylaxis or (2) asthma.[57,58]

A. Cutaneous/Anaphylactic Responses

Sporadic reports have linked urticaria with tartrazine (Yellow no. 5). The majority of these studies were not well controlled. Using the strict criteria of DBPCFC, only occasionally has an individual been proven to develop urticaria after the ingestion of tartrazine.[59] There is also a single report of two individuals who developed urticaria to sunset yellow (Yellow no. 6) when challenged using the DBPCFC technique.[60] Other food colors that have been implicated in anecdotal reports of urticaria are amaranth, erythrocin, and ponceau (Reds no. 2, 3, and 40, respectively) and brilliant blue and Indigotin (Blue no. 1 and 2, respectively).[57]

There are at least two documented reports of sulfite-induced allergic reactions.[58] Each patient developed positive immediate reacting IgE skin tests to sulfites, and passive transfer of IgE sulfite antibodies was possible.[61,62]

Reports of urticaria to BHA or BHT preservatives are rare. However, two patients who had chronic urticaria and who improved after eliminating these preservatives from their diet were challenge positive to these chemical agents using the DBPCFC technique.[63] There appears to be no proven case of urticaria involving sodium benzoate reported in the U.S.[58]

Reported cutaneous adverse reactions to MSG include sweating, facial flushing, tightness and burning of the face and chest, and a sensation of "crawling skin."[57] The relationship of these symptoms to MSG consumption has been the subject of much research and debate. Oriental food meals may sometimes contain several grams of MSG.

Some of the symptoms in this syndrome may be due to the high histamine content of some foods served in a Chinese restaurant.[52] There have been no proven (DBPCFC) cases of reproducible urticaria with MSG challenge.

Two cases of aspartame-induced urticaria/angioedema were reported to have been confirmed by DBPCFC.[64] Despite these two cases, when the allergic-like aspartame reactions were studied in the U.S. and Canada, the investigators were unable to substantiate any clear relationship between urticaria and angioedema and aspartame exposure using DBPCFC.[65] Furthermore, from the results of a combined single-blind, double-blind placebo-controlled study, other investigators reported that individuals who thought they were allergic to aspartame did not have reproducible reactions.[66]

B. Asthma

The principal agents associated with non-IgE-mediated asthmatic reaction are the sulfite preservatives (e.g., sodium metabisulfite [$Na_2S_2O_5$]) and related compounds, plus sulfur dioxide (SO_2). Common offending foods include sulfite-treated salads, avocado dishes, vinegars, sausages, dehydrated vegetables, fruit juices, cider, beer, and wine.[50]

Asthmatics may wheeze when exposed to less than 1.0 ppm of SO_2.[58] In line with this observation, there have been a series of reports of asthmatics, particularly in the early 1980s, who wheezed when eating restaurant foods, particularly from the salad bars, which had been treated with sulfiting agents.[67]

The principle behind the acute asthma exacerbation with sulfites is often the inhalation of SO_2 gas, which is released when the foods containing sulfite preservatives are chewed and/or swallowed.[68] In addition, in select individuals a sulfite oxidase enzyme deficiency of uncertain significance has been reported. In others, a rare IgE-mediated response to sulfites may be important.[58] Approximately 3.9% of asthmatics are at risk for an acute attack with sulfites or SO_2.[69] Of these, the risk is approximately 8.4% in serious or steroid-dependent asthmatics and 0.8% in mild to moderate asthmatics. Since U.S. federal guidelines were altered to require more labeling of sulfites on food packages and bar the use of sulfites on fresh fruits and vegetables other than potatoes, and since the food industry has adopted alternatives of sulfites for some applications, there have been fewer reports of sulfite-induced asthma.

A few patients have had an acute asthma attack after the ingestion of foods containing MSG in restaurants, similar to sulfite-induced asthma.[70] However, in DBPCFC studies, other investigators have been unable to substantiate claims that MSG in oral challenge up to 7.5 g causes significant asthma or alters bronchial reactivity to methacholine.[71,72] Another food additive that has been implicated in the past as provoking asthma is tartrazine (Yellow no. 5).[58] Numerous reports have claimed to link tartrazine ingestion with asthma exacerbation. However, especially in the aspirin-sensitive asthmatic, more recent, well-done DBPCFC studies have failed to confirm this association.[59,73]

X. OTHER CLINICAL REACTIONS INFLUENCED BY DIETARY FACTORS

A. Infantile Colic

There are a number of conditions in which claims have been made that food allergy plays a role when, in fact, there is little or no scientific confirmation. In each of these situations, diet may play an as yet undefined role. Such is the case in infant colic. Frequently, this syndrome is mentioned when discussing milk allergy or intolerance.[40] Approximately 20% of all infants develop colic. This condition is self-limited and occurs regardless of the type of diet fed to the infant or the breast-feeding mother.[74] In controlled studies, nondietary intervention such as increased motion or increased attention to the infant may be more helpful in controlling the colicky pain than any dietary manipulation.[75]

In one controlled study of infants who developed colic while on conventional cow's milk-based formula, approximately 25% improved spontaneously during the study period while on the same diet, 25% improved on a soy-based infant formula, and 50% improved on a casein hydrolysate infant formula.[76] There has been no

explanation for these dietary differences affecting the course of infant colic, but food allergy as a cause has been implicated in some cases.

B. Behavioral Changes

Food allergy or another type of adverse reaction to dietary components has been commonly cited as being related to behavioral problems such as hyperactivity, aggressiveness, misbehavior, or poor learning attributes.[77] Probably the most widely known theory linking behavior to adverse food reactions was that of Feingold[78] in the mid-1960s who proposed that food additives, especially colors, flavors, and preservatives, as well as natural salicylates, could cause hyperkinesis in children with attention deficit disorder (ADD).[78]

Although there was some initial enthusiasm about the use of the Feingold or the Kaiser Permenante (KP) diet, which is a diet devoid of colors, natural salicylates, and preservatives, subsequent analysis based on color challenge studies concluded that the KP diet is likely to help only in approximately 2% of children with hyperactive behavior.[79] One study found that children with ADD with hyperactivity, but not normal children who had active behavior, would perform progressively worse on a special laboratory learning test only when challenged with a color mixture, not with a placebo.[80] This study led to the conclusion that colors could act as pharmacologic agents, such as drugs, and affect behavior/learning. This problem was thought to be clinically important only in a small, select number of individuals with ADD with hyperkinesis. Although these studies seemed to stifle the enthusiasm surrounding the use of diets to help children with ADD, a Canadian report in 1989 fueled the flames of controversy again.[77] The behavior of 12 of 24 preschool hyperactive boys improved on a diet devoid of artificial colors, MSG, preservatives, caffeine, chocolate, and "any food" to which the family believed the child was allergic.[81] Still, the consensus today is that if hyperkinesis is affected by a diet, there is no evidence that the effect is immunologic or allergic in nature.[77]

Hyperactive and aggressive behavior has also been attributed to diets high in sugar content.[77] The term *sugar allergy* is a misnomer. This situation has no relationship to allergy nor is any immune function involved. Controlled studies have shown little or no evidence that sugar causes adverse behavioral effects in hyperactive children.[82] Some studies actually demonstrate a calming effect from sugar in the diet in these individuals. In addition, a prospective double-blind aspartame-controlled dietary study involving juvenile delinquents and normal individuals demonstrated no evidence of adverse reactions to sugar (sucrose) in the diet.[83]

C. Migraine Headache

Headache is a common complaint in the general population. The more serious migraine headache also is quite prevalent. Up to 25% of adult women, 15% of adult men, and 5% of children by the age of 15 may be afflicted by this condition.[77] Migraine headaches run in families; 63 to 88% of migraineurs have a close or distant relative who also suffers from migraines. Atopic diseases also run in families with approximately 20% of the population being involved. With these facts in mind, it is not unreasonable to hypothesize that some atopic individuals also have migraine headaches by chance. In fact, an association between migraine headache and food allergy has been suggested periodically since the nineteenth century, mostly in anecdotal reports.[77,84]

There have been several studies utilizing DBPCFC techniques in recent years that support the association between diet and migraine.[85–87] Among 104 adult migraine patients, 15% had reproducible double-blind, placebo-controlled, demonstrable food-associated migraine.[87] However, among children who have migraine, even those in whom diet improved migraine headache patterns, there was no correlation with immediate skin tests to foods.[85] In one study involving 36 children with migraine headaches, neither the food allergy skin test nor the history of an adverse reaction to a food could distinguish the patient who would react on DBPCFC with a headache.[88] In another study involving 43 adult migraineurs, the immediate skin test results seemed to be helpful in selecting the specific foods to which a patient would react with a headache on DBPCFC.[86] Most studies of migraine-prone individuals show

no immunologic differences between adults who have headaches and the normal population.[84]

There is some evidence involving chemical mediator release with specific food challenge in dietary-induced migraines. Three patients were reported with elevated plasma histamine levels following specific food challenges that correlated with onset of a migraine headache.[86] A patient was also reported to have reproducible headaches with the ingestion of beef in which threefold increases in blood histamine and $PGF_2\alpha$ were demonstrated.[89] Finally, five other patients with food-induced migraines had 3- to 38-fold increases in plasma histamine and increases in PGD_2 with headache symptom onset, followed by a second rise in PGD_2 4 to 6 h after food ingestion.[90]

To summarize, IgE-mediated food allergy probably plays little, if any, role in the pathogenesis of migraine headaches. Diet, apparently, does play a role in a subset of individuals. In select patients, migraine headache pathophysiology may include the release of chemical mediators.

XI. CONTROVERSIAL CONCEPTS

The allergic tension fatigue syndrome refers to children who demonstrate periods of hyperactivity, insomnia, and anxiety, alternating with periods of listlessness and fatigue.[77] This syndrome was once thought to be related to food allergy. However, there has never been a well-designed clinical study to prove such a relationship.

The diagnosis of chronic fatigue syndrome, particularly in adults, has become popular. A variety of explanations have surfaced to explain this symptom complex of long-standing or persistent fatigue associated with other constitutional symptoms, including myalgias, arthralgias, muscle weakness, sensations of being overheated or chilly, depression, apathy, headache, insomnia, soreness of the throat, and tenderness of the lymph nodes.[77] A variety of immune abnormalities have been reported in some of these patients. Although there is no proven relationship with food allergy or diet, one study showed that a high percentage of patients diagnosed with this disorder were also atopic as defined by skin tests.[91]

Since the early part of this century, foods and diet have been thought to play a role in the pathogenesis of rheumatoid arthritis.[77] Although arthritis has never been consistently related to food allergy, single- and double-blind randomized controlled studies have shown that diet manipulation may influence or exacerbate rheumatoid arthritis in a subset (5%) of patients with this condition.[77]

A. Pseudo-Food Allergy

A group of 23 adults who presented to the allergist with a series of complaints thought to be due to food allergy were examined in detail in a 1983 study.[92] A few of these patients had allergy-like symptoms. The rest presented with symptoms of depression, fatigue, headache, mental fuzziness, malaise, myalgia, arthritis, and listlessness. Many of these patients restricted their diet to a degree that they also had signs of malnutrition. All patients were independently evaluated by a psychologist after seeing an allergist. Each patient was evaluated for food allergy and had DBPCFC to foods they thought were important. Four patients were found to be allergic and these patients had classic allergic-type presenting symptoms. When examined by a psychologist, all had a normal psychological profile. In the remaining 19 patients, no adverse reactions to foods were found with DBPCFC. These 19 patients had presented with constitutional symptoms, usually not attributed to allergic disease. When examined by a psychologist, all were found to have psychological problems, especially depression. When the patients were confronted with the results of the food challenge, most of these patients could subsequently eat the foods to which they had once believed themselves to be sensitive. The remainder, over time and with psychological counseling, could eat a normal diet. Pearson[93] coined the term *pseudo-food allergy* to denote the false belief that one suffers from food allergy so as to unnecessarily restrict their diet to the degree that it becomes nutritionally unsound.

XII. THE DIAGNOSIS OF FOOD ALLERGY

A. History

A history for a patient suspected of having food allergy should include: (1) the description of

the problems and typical symptoms, (2) the timing of the onset and duration of the symptoms in relation to diet and specific foods ingested or other routes of food exposure, (3) frequency of symptoms, and (4) other circumstances involved with food exposure. In the majority of situations, when the symptoms about which the patient complained were caused by food allergy, the individual had a history of allergies (e.g., AD, asthma, allergic rhinitis). The presence of asthma in a food allergic individual is considered a risk factor for a subset of individuals who have serious, life-threatening food reactions.

If a food causes immediate symptoms such as acute urticaria or systemic anaphylaxis, and a specific food is commonly involved in this event and is eaten occasionally, the food may be easily identified as a likely cause of the patient's symptoms. On the other hand, if the patient has a long-standing or chronic problem such as AD or asthma, it is often difficult by history to pinpoint a component of the diet specifically involved. In fact, in studies involving children with AD who had food allergy confirmed by DBPCFC, the mother's identification of the likely food culprit poorly correlated with challenge testing.[37]

A food involved in provoking allergic symptoms may not be obvious and may require extensive detective work. Examples of unusual food allergies include a patient who had two systemic reactions while eating marinated chicken and was proven to be allergic by DBPCFC to coriander spice;[94] individuals known to be allergic to milk who reacted to cow's milk protein in "nondairy" products;[95,96] a patient allergic to house dust mite who had an anaphylactic reaction while eating a beignet baked with a commercial mix contaminated with mite protein;[97] and a nurse sensitized to psyllium from exposure to a stool bulking agent who developed urticaria after eating a breakfast cereal containing psyllium placed as an ingredient to help lower cholesterol.[98]

B. ST and *In Vitro* Assays

Allergy ST to detect IgE antibodies to food allergies is done almost exclusively by the epicutaneous route (prick or puncture technique), because the intradermal technique increases the frequency of nonspecific positive reactions using food extracts.[4] Food extracts are usually applied to the skin in a weight by volume concentration of 1:10 onto the back or forearm. The prick or puncture tests are read in 15 to 20 min. Resulting wheals are positive if they are larger than the negative diluent control by 3 mm or more. It has been verified that almost all subjects who have IgE-mediated allergic reactions to foods by DBPCFC will have a positive IgE skin test and *in vitro* test to that food.[5,9,15]

It is important to recognize that commercially available food allergen extracts are not always reliable as testing agents.[99] When investigating fruits and vegetables suspected of causing the oral allergy syndrome, epicutaneous skin tests utilizing the fresh juice of these items may be necessary, because the allergens responsible for IgE-mediated reactions are sometimes quite labile.[30]

ST with food extracts must be performed with caution in the evaluation of individuals who have histories of systemic anaphylaxis to a food. This is because minute quantities of food allergen introduced by ST may induce adverse symptoms.[4] An option is to use a food allergen IgE-specific *in vitro* assay even though these tests are somewhat less sensitive.[41] Food allergy ST is sometimes not possible in individuals with extensive skin disease (e.g., AD or urticaria) or in individuals on antihistamines that will block skin test reactivity (e.g., for rhinitis or urticaria). In these situations, *in vitro* antigen-specific IgE assays may be helpful. Basophil degranulation tests are occasionally used to evaluate IgE-mediated reactions to food allergens. The results of this type of test are comparable to those obtained by *in vitro* measures of allergen-specific IgE antibodies, but are not generally routinely available.[4]

The relationship between proven clinical reactions to food as measured by DBPCFC and positive allergy skin tests or *in vitro* tests has been well documented in AD.[39] If the allergy skin test to a major food (milk, eggs, peanut, wheat, soy) is positive, the chances are approximately 50% that the DBPCFC to that food will be positive. If an IgE skin or *in vitro* test to a specific food is negative, the probability is virtually 98% that a DBPCFC will be negative to that food.[4] For foods

that were shown positive on DBPCFC, either the IgE skin test or RAST™ was positive.[4,19,39]

XIII. DIARIES, DIETS, AND FOOD CHALLENGES

Diet diaries may be useful to record repeated episodes of isolated bouts of acute urticaria. The record of the foods consumed, as well as other events surrounding these specific episodes, may help narrow the variables and assist in identifying the probable offending food. Specific diets, made up of foods less likely to be involved in allergic reactions, may be helpful in nebulous situations in which food allergy is unlikely to be the cause of a problem (usually with negative food allergy skin tests) or in situations where there are positive allergy skin tests but no history of clinical activity.[53] In these situations, there are no suspected serious life-threatening reactions such as systemic food anaphylaxis. The specified diet is tried at home for 2 weeks. New foods are then added sequentially at 2- to 3-d intervals. The most common foods, such as milk, eggs, and wheat, are added first to quickly return the patient's usual diet to one that is nutritionally sound. The only way to be certain about a possible association between a given set of symptoms and exposure to a food, however, is to challenge the individual.[4] A positive DBPCFC under controlled conditions establishes the relationship.[100] Negative DBPCFCs need to be confirmed by follow-up ingestion of the food used in the challenge procedure.

DBPCFCs are important to research studies examining the pathogenesis of adverse food reactions. It is important to prove that a given group of individuals actually are reactive to the dietary item before proceeding with further research.[101–104] A positive food challenge does not establish the mechanism of the adverse event. Positive food challenges occur both in food allergy and in food intolerance.

Food challenges are not usually advocated in the clinical office in cases where the risk of further exposure to the food may be severe or life-threatening.[26] Usually, when a patient presents with clear-cut repeated reactions to a food known to be commonly associated with anaphylaxis (e.g., generalized urticaria, angioedema, and wheezing within minutes of eating a shrimp at a restaurant), only an *in vitro* assay to the probable offending food is performed. If this test is positive, a presumptive diagnosis of food allergy may be made and the patient is instructed to not eat the specific food.

In the case of inadvertent exposure to a food to which the patient has developed anaphylactic symptoms, the patient is instructed to use an epinephrine autoinjector syringe or a traditional epinephrine syringe. Three of these units are prescribed and positioned by the patient so that one unit is likely to be handy when needed. The patients are instructed to use epinephrine where there is proven or presumed systemic food anaphylaxis at the first sign of symptoms and then proceed to an emergency department.[26,29] They are cautioned against using an antihistamine first or "waiting and watching."

Sampson and Metcalfe[4] advocate the following steps in a DBPCFC technique.

1. The procedure should be done under controlled conditions, by personnel capable of recognizing and managing anaphylaxis, should this situation occur. DBPCFC can be done in the hospital, clinic, or office.[100]
2. The suspected food should be eliminated from the diet 10 to 14 d prior to challenge.
3. Antihistamine medication should be discontinued 12 h prior to challenge.
4. The individual to be challenged should be in a stable cardiovascular, pulmonary, and metabolic condition prior to challenge.
5. The food challenge should be done in a fasting state.
6. The challenge itself should involve gradually increasing doses of food, beginning either with the placebo or a dose low enough to be unlikely to provoke symptoms (such as 10 to 50 mg or less of lyophilized food).
7. The food challenge dose or the placebo dose is usually doubled every 15 to 20 min as tolerated.
8. The maximum food challenge dose approximates 10 g of lyophilized food.
9. Following completion of the DBPCFC procedure, a minimal recommended observa-

tion for the individual given the food challenge is 2 h for food allergic reactions and 4 to 8 h for food intolerances. All challenged individuals with GI symptoms as an end point should be observed for 4 to 8 h following completion of the challenge.

10. In the case of a negative food challenge, follow-up open feeding of the suspected food is recommended for the subsequent 24 to 48 h.

XIV. MANAGEMENT OF FOOD ALLERGY

A. Proven Food Allergy

Strict elimination of the offending food from the diet of the allergic individual is the only proven therapy once the diagnosis of food allergy has been established.[4] Long-term avoidance of certain foods may prove difficult, especially when dealing with processed foods and when eating away from home in restaurants, cafeterias, and at parties. Carrying a "lunch" prepared at home with known safe foods becomes important for some allergic individuals. Nutritional support may be necessary so as to maintain a sound diet.[4] Health professionals can obtain information concerning diets, recipes, and lists of processed foods containing the usual food allergens or food additives.[105] Patient support groups are helpful to patients or parents of patients afflicted with food allergy. Proper food labeling of ingredients in processed foods is extremely important for the patient when managing food allergy.

Patients who have had presumed or proven anaphylactic reactions to foods should be prepared at all times for inadvertent exposure to that food, especially when away from home.[28,29] The difference between life and death depends on how quickly epinephrine is administered after the anaphylactic symptoms begin.[28]

B. Hypoallergenic Cow's Milk Infant Formulas

Management of the infant who has a food allergy to cow's milk may be a particular problem. Often babies are switched from conventional milk-based infant formula to a soy-based formula because of problems of "fussiness" or true infant colic. Such switches have merit in some cases, but not because of food allergy.[76] Soy protein-based infant formula has caused the same spectrum of clinical reactions as those caused by cow's milk protein, especially GI reactions.[40,106–108] Often soy sensitivity quickly follows cow's milk sensitivity. Approximately 33 to 50% of all children with GI allergy or intolerance to conventional cow's milk infant formula cannot tolerate soy-based formula.[109–111] Therefore, the Nutrition Committee of the American Academy of Pediatrics recommends that conventional soy-based infant formula should not be used in the case of either milk or soy intolerance or in proven allergy.[109]

For the infant who has a documented allergic reaction or intolerance to cow's milk, an ideal substitute would be one that (1) has been proven safe by DBPCFC in infants who have documented cow's milk allergy or intolerance;[4] (2) is nutritious and allows for normal growth and development; and (3) is palatable for a long period of time.

Unfortunately, at present there is no perfect hypoallergenic formula for infants allergic or intolerant to milk.[106] Alternative formulas include those derived primarily from casein hydrolysate, whey hydrolysate, or crystalline amino acids. All marketed formulas have some reported, albeit apparently minor, instances of either intolerance or problems with taste.[110–116]

C. Prevention of Food Allergy

Manipulation of the mother's diet before delivery and the infant's diet following delivery for the child born to allergic parents has been a popular concept for many years because of the presumption that food allergy and perhaps other forms of allergy may be prevented. In infants at risk for allergic disease (born to parents with allergic disease) who were fed breast milk exclusively for as few as 4 months, it was found that the cumulative incidence and the severity of AD that developed by 18 months decreased.[15] In this particular study,

some mothers avoided cow's milk, eggs, fish, peanut, and soy during the lactation period. Others ate a normal diet. The incidence of AD was lower (22%) in the group of breast-fed infants whose mothers ate a special diet compared with breast-fed infants whose mothers ate an unrestricted diet (44%). In another study, breast feeding was also found to be superior.[117]

Several controlled, long-term studies have shown that soy-based formula does not influence the development of early food allergy symptoms or long-term development of inhalant allergy.[118–120] One well-controlled study assessing the role of diet in the prevention of allergic disease is worth reviewing.[120] In the prevention-treated group of 101 infants and mothers, the mothers avoided cow's milk, eggs, and peanuts during the last trimester and during lactation. The infants, who were not breast fed, were fed Nutramigen, a casein hydrolysate. All infants were kept away from cow's milk, corn, soy, citrus, and wheat for 12 months; and eggs, peanuts, and fish for 24 months. In the control group of 185 infants and mothers, the regular accepted American Academy of Pediatrics feeding practices were followed. The cumulative prevalence of allergy at 1 year was less in the prophylactically treated group (16 vs. 27%) as were food-related symptoms (5 vs. 16% control).[121] However, at 24 months, there were no differences in the incidences of rhinitis, asthma, skin, or GI allergy symptoms and the IgE skin test positivity to inhalants between the two groups. Thus, for the short term, diet may influence the incidence of food allergy symptoms in high-risk infants, but a special diet in the mother or infant has no long-term influence on the subsequent development of atopic disease.

XV. ROLE OF DRUGS AND ALLERGEN IMMUNOTHERAPY

Other preventive therapies have been tried in food allergies, such as the long-term use of antihistamines, corticosteroids, and oral cromolyn sodium. None has been proven to have more than minimal efficacy.[4] The unproven therapies of oral desensitization, including sublingual food drops or subcutaneous neutralization, have not been efficacious. If used at all, they should probably be restricted to research studies with appropriate informed consent.[77,101]

Conventional allergen immunotherapy is not an approved therapeutic option for food allergy.[4] This therapy has been tried experimentally, indirectly to help prevent the oral allergy syndrome (oral and injection birch tree pollen therapy) and directly with the use of a peanut extract to prevent peanut allergy.[122,123] Tree allergen immunotherapy was not helpful in the management of food symptoms in the oral allergy syndrome.[122] In the few patients treated with peanut extract, there was both clinical and immunologic evidence for some protection against peanut anaphylaxis. It was concluded from the latter study that more research involving immunotherapy for food allergy should be pursued because some patients with severe life-threatening symptoms to food are not always able to avoid these foods.[124] The potential risk of food allergen immunotherapy may be less than the real risk of inadvertent allergen exposure.[28,29]

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The Biology of Plant Proteins

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I. INTRODUCTION

This article summarizes some of the salient features of plant proteins and provides a basic foundation on which subsequent articles in this issue can expound. It discusses the source of proteins in plants, explains the role and function of proteins in food plants, their natural variation, and tells how microorganisms and pathogens can sometimes introduce other substances, including proteins, into food and produce. This is not a comprehensive treatment of plant protein chemistry. For more in-depth information, readers are referred to textbooks on plant and protein biochemistry.[1,2]

Proteins are naturally occurring polymers made up of constituent amino acids. Every cell of every organism, including those in humans, typically contains tens of thousands of proteins, each of which is structurally complex and distinct. These constituent proteins are encoded by the organism's genome. From estimates of genome size we can make rough estimates of the total numbers of proteins likely to occur in individual species. There are between 15,000 and 60,000 genes[3] in the small weed plant *Arabidopsis thaliana,* and just as many proteins.

There are 20 different amino acids in plant proteins. Of prime importance to a nutritionist is the balance of constituent amino acids in food plant proteins, and their availability during digestion in the alimentary tract. The human body efficiently breaks down food proteins into constituent amino acids during digestion. It then uses the amino acids as building blocks to synthesize other proteins needed for growth, development, and maintenance of normal functions. During digestion, proteins undergo partial disassembly into smaller molecules called peptides, which lack the functional activity of the original protein. These result from the enzymatic cleavage of the bonds linking specific pairs of amino acids.

In addition to their nutritional function, proteins contribute to food texture, flavor, aroma, feelings of satiety, ease of processing, and food quality. However, in sensitive people, proteins interact with immunoglobulin E (IgE), becoming allergenic or toxic to those who ingest them (see "Introduction to Allergic Diseases" by Yoseph A. Mekori and "Allergic Reactions to Food" by John A. Anderson in this issue). The process of dietary elimination and controlled food challenge reveals which, if any, food proteins individuals must avoid to remain healthy and free from food-induced problems. Catalogs of food allergies, food intolerances, and even poisonings have been assembled from such information.[4,5] They guide physicians in advising patients about problems that may be caused by their dietary intake of certain proteins.

II. PLANT PROTEINS: BIOLOGY OF OCCURRENCE

As a result of differentiation during development, plant cells may take a variety of forms which adapt them to their primary function in the plant. However, all plant cells have a number of features in common. Each has a cellulose wall enclosing a protoplast bounded by a protein-lipid membrane that is selectively permeable to ions in solution. The protoplast consists predominantly of a skein of protoplasm lining the wall which surrounds a fluid-filled central vacuole that con-

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tains various solutes. For example, the familiar leafy tissue of lettuce consists predominantly of the cell walls. The fluid in the cell vacuoles and the physical constraint imposed by the wall create turgid cells and the crisp texture that the consumer expects. The protein content of a lettuce leaf is low, about 1 to 3% of the dry weight of the leaf tissue, or less than 0.5% of the wet weight. The nucleus of the cell, located in the protoplasm, consists of various nucleoproteins and nucleic acids that, at cell division, resolve into the structures called chromosomes. The genes, carried on the chromosomes of the nucleus, direct the synthesis of proteins, many of which are enzymes. A gene is a unit of inherited information. It consists of a number of nucleotides arranged in a linear order like the words in a sentence. However, in the sentence represented by a gene there are only four letters (the nucleotides adenine, thymidine, guanine, and cytosine), and each word has only three letters. Each triplet represents an amino acid, so that the sequence of triplets encodes the linear sequence of amino acids that make up the polypeptide chain, or protein product of the gene. Gene expression is coordinated and regulated by elements within the nucleus that respond to environmental and developmental signals. It is the sum of the proteins produced in each type of cell that give that cell its properties. Major changes may be caused by the expression of groups of genes, resulting in the peculiarities of form and chemical composition that differentiate cell types from each other.

Although the principal location of the genetic determinants of each cell is in the nucleus, green plant cells have two other kinds of bodies, mitochondria and chloroplasts, that also carry genetic information. Chloroplasts are the site of photosynthesis, a process that uses the energy of sunlight to make carbohydrates like sugar and starch from carbon dioxide and water. During this process oxygen is formed and released. The products of photosynthesis are used as sources of energy by the mitochondria, which are the site for oxidative enzymes which create the energy currency the cell uses, and as raw materials for the synthesis of many other compounds. The enzyme ribulose bisphosphate carboxylase (rubisco), which occurs in the chloroplast and plays an important role in photosynthesis, is by far the most abundant protein on Earth. Its structure is encoded by the chloroplast DNA and by the DNA of the cell nucleus.[6] The cells of plant parts that are green may contain from 1 to as many as 50 or more chloroplasts. Plant parts that are not green (e.g., roots and tubers) normally do not contain chloroplasts. Mitochondria are smaller than chloroplasts and are found in all plant cells in numbers ranging into the hundreds. Because they are the site of oxidative enzymes, they are involved in the process of respiration. In this process, oxygen is used to accept electrons from donor molecules, which, as a result, carry charges that enable them to drive a series of other reactions in cell metabolism.

There are two centers of active cell division in a plant seedling, called meristems, that are responsible for the growth and development of a mature plant. The shoot apical meristem gives rise to leaves, stem, and eventually to flowers. The root apical meristem gives rise to root tissue. This may be simple and unbranched like that of a carrot, or highly branched and fibrous, like the roots of lawn grass. As the characteristic form of the mature plant develops, the immature cells undergo changes in shape, chemical composition, and their complement of proteins that are usually irreversible. Once they have assumed their mature form, these cells can no longer divide and many may die. For example, the xylem, or water-conducting tissue, in the roots and stems of plants consists of tubular elements made up of empty cells. The internal diameter of these cells ranges from 0.01 to 0.10 mm and they can be from 1 to 8 mm or more in length. The individual xylem cells (xylem elements) have reinforced walls. They are strung together to create a tubular network that carries water, and materials dissolved in it, from the roots to the leaves and other plant parts above the soil. As the xylem elements reach their maximal size and mature form, they undergo programmed cell death in preparation for their final function.[7] Other cells assume a long and narrow form and develop very thick walls and create bands or blocks of mechanically strong fibers that reinforce stems and leaves against the mechanical stresses imposed by wind and rain. In tree stems these two kinds of cells, water conducting and fiber, give rise to the wood and timber used in

commerce. In food plants fiber cells contribute important mechanical properties to the human diet.

The parts of plants commonly used as food by humans include storage organs such as roots (carrot, radish, turnip), tubers (potato, sweet potato), and stems (kohlrabi, kale, asparagus, celery), but also include a wide range of other plant parts such as leaves (cabbage, lettuce, spinach), bulbs (onion, garlic), flowers or inflorescences (broccoli, cauliflower, artichoke), fruits (apple, pear, tomato, berries, squash, melon, cucumber, banana), and seeds (corn, rice, peas, beans, wheat flour, nuts). In all of these plant parts the principal source of calories are the carbohydrates sugar and starch. Except in seeds, where they are often present in high concentrations, plant cells contain small quantities of protein even though there may be more than 1000 different proteins present.[8] Figure 1 shows a scanned image of cell proteins isolated from a newly germinated shoot of a corn seedling and revealed by two-dimensional gel electrophoresis. More than 1500 spots, each representing a different protein, are resolved. These likely represent only a fraction of the total proteins present in shoot tissue. Seeds contain relatively large amounts of a few special proteins and are also sources of fats and oils.[9]

It was believed for many years that some 15 major crops were largely responsible for feeding the world. These are the cereals: rice, maize, wheat, and sorghum; the legumes: beans, soybean and peas, and peanuts; the root crops: potato and cassava; and sugarcane, sugar beet, bananas, and coconuts. However, recent studies have shown the importance to individual groups and societies of more than 100 food plant species.[10] These include mangoes (28% by weight of the vegetable food consumed in St. Lucia), Chinese cabbage (12% in South Korea), taro (18% in Samoa), quinoa or grain amaranth (important in Bolivia), and cowpea (important in Niger).

III. THE ROLE OF PLANT PROTEINS

Proteins are an integral part of the chemical machinery for cell growth, division, and differentiation in young developing cells. The most important of the cell proteins are enzymes. These are catalysts responsible for the individual reaction steps in the biosynthetic pathways that produce the cell components. Other proteins function as signals that either turn on or turn off the expression of genes in the nucleus by binding to regions of nucleic acid that have a regulatory function. Yet other proteins have a mechanical role and provide a structural framework, or scaffold, for nucleic acids and other cell components.

The properties of proteins are a function of their structure. Built as a linear chain of 20 different amino acids, they are large molecules that are folded precisely into three-dimensional structures. Many plant proteins have attached to them small sugar residues and are said to be glycosylated, which may affect how they react with other cell components. The surface features of proteins, which may include pockets, grooves, and projections of various kinds, and that may carry different electric charges, determine how they interact with other molecules and function as catalysts. These surface features also influence how protein molecules are perceived by the human body either through external contact with the skin or through the mucosa of the mouth or intestinal tract.

IV. PROTEIN SYNTHESIS

The amino acid sequence of each protein is encoded in the nucleotide sequence of a region of the DNA that corresponds to a gene. A universal triplet code uses a sequence of three bases to specify each of the 20 amino acids commonly found in plant proteins. The DNA sequence of the gene is first transcribed to messenger RNA (mRNA), which is then processed by small particles called ribosomes. Protein assembly occurs as the ribosome reads the mRNA sequence and translates it into an amino acid sequence by selecting from a pool of transfer RNA molecules. Each transfer RNA molecule has bound to it a specific amino acid and functions as an adapter that presents the amino acids at the right time and place during the synthesis of the protein. As the ribosome moves along the mRNA the chain of ribonucleotides disintegrates, leaving in its place

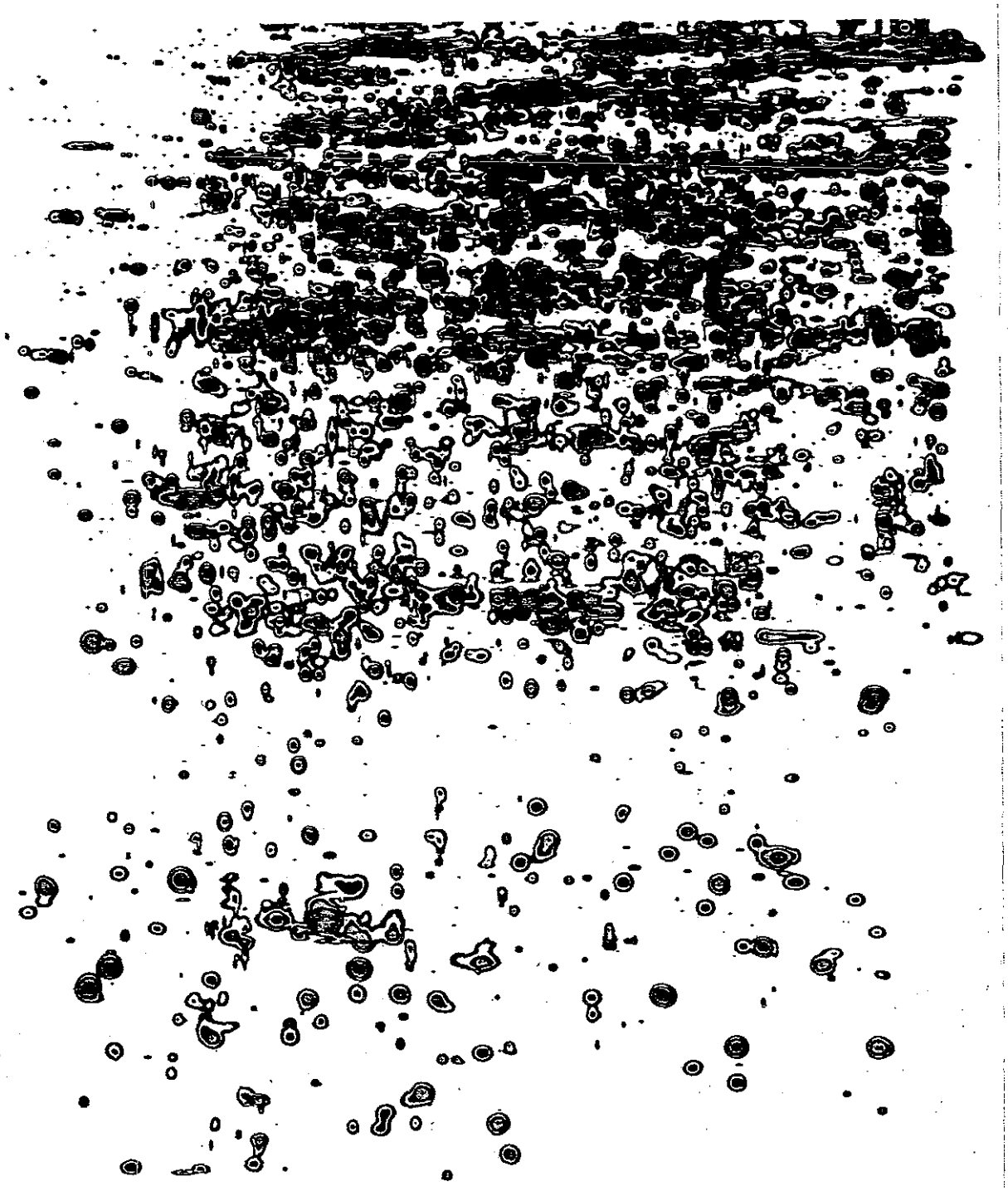

FIGURE 1. Scanned image of a two-dimensional gel electrophoretic separation of proteins from the newly germinated shoot of a corn seedling separated by isoelectric focusing in the first dimension (horizontal) and by molecular weight in the second dimension (vertical). (Reproduced, with permission, from Pioneer Hi-Bred International, Inc.)

a growing chain of amino acids. The secondary and tertiary structure imposed on this chain is in part the result of self-directed folding, often induced by the establishment of chemical cross-linkages, but also influenced by chaperone proteins whose function is to adjust the final form of

the protein molecule to the most stable functional configuration possible.

The processes of transcription to mRNA and translation of the nucleic acid sequence to an amino acid sequence do not occur continuously for all genes. The process, called gene expression, is controlled by promoters, chromosomal elements located near the gene, which may be activated or repressed. Promoters are generally repressed when a small protein molecule, called a repressor, is bound to them thereby preventing them from activating gene transcription. When the gene is induced, the inducing agent releases the promoter by interfering with the binding between the repressor and the binding site of the promoter. Constitutively expressed genes are expressed all the time. The signaling controls of gene expression are critically important for normal development. They ensure that the appropriate genes are activated and expressed when and where they are needed, and that expression is shut down when no longer required.

From studies of the controls of expression in transgenic plants there is evidence that developmental controls are very similar among different plants of different genera and families. For example, Altenbach et al.[11] used a promoter from a French bean to express the Brazil nut 2S seed storage protein in canola and tobacco. Similarly, a gene encoding a wheat seed endosperm gluten protein introduced into tobacco was only expressed in tobacco seed.[12,13] In each example, the cloned gene included a promoter that was able to respond to the signaling controls of entirely different plants.

Many of the larger protein molecules are composed of subunits. This presumably simplifies their assembly and synthesis, because different genes may be responsible for different subunits. For example, rubisco is composed of two kinds of subunits: a large one of ~55 kDa encoded by a chloroplast gene, and a small subunit of ~15 kDa encoded by a nuclear gene.[14] The complete structure also includes a metal atom or prosthetic group that is usually critical to the catalytic activity of the enzyme and is located in a position where the primary chemical reactions take place. The three-dimensional structure of protein molecules is established by X-ray crystallography. This technique uses diffraction patterns caused by a crystal of the protein placed in an X-ray beam to reconstruct a three-dimensional image of the molecule.

Many proteins are not used at the sites within the cell where they are synthesized. Some of these proteins may have, at one end of the amino acid chain, a so-called signal or targeting sequence that directs the protein to a specific location in the cell and is then removed.[15,16] This may be a site on, or within, a membrane where the properties of the protein are called into play.[17]

V. PROTEIN VARIATION

Broadly speaking, the more closely related organisms are the more similar are their constituent proteins. Many enzymes, such as rubisco, and other so-called house-keeping enzymes that perform basic cellular functions, are common to and conserved in most plants. A single change in the base sequence of the DNA that encodes a protein may lead to the substitution of an inappropriate amino acid at one point in the molecule. Depending on the site of this change, or mutation, it may have the effect of either abolishing or altering the function of the protein. Mutational changes over evolutionary time may also result in amino acid changes that for the most part have little effect on the way the protein molecules function. A common effect of these changes is to alter the mobility of the protein in electrophoresis, a technique that separates proteins on the basis of their size and charge. The altered forms of enzyme proteins are called isozymes. Different forms may occur within the same species or vary between species. In some respects these changes are evolutionary scars and almost certainly occurred as a result of spontaneous mutations at earlier stages of evolution. Changes that abolish important or vital functions, or substantially modify them, may be lethal or may reduce the fitness of the organism. In general, these changes would be selected against in nature because they would not survive and produce seeds in competition against unimpaired forms. Occasionally, mutations may be beneficial to the organism and confer competitive advantages over other types. This is the basis of evolution.

Naturally occurring variation in protein composition can be most easily detected by two-dimensional electrophoresis of plant extracts[8,18,19] (see Figure 1). However, this technique probably resolves only a fraction of the total number of proteins present in a particular tissue.

Changes that are beneficial or neutral with respect to protein function could be of significance if they created molecules that are allergenic or have enhanced allergenicity. However, in view of the very low frequency of IgE-mediated allergenic responses in humans to the very large numbers of proteins and naturally occurring protein variants they are normally exposed to in their diet, the probability of this occurring is almost certainly very low. It is also important to bear in mind that, except for seed storage proteins, the majority of individual plant proteins are present only in small amounts and at concentrations that may be below the threshold needed to stimulate an IgE-mediated response.

VI. PROTEINS INTRODUCED INTO THE DIET BY PLANT PRODUCTS

Some proteins have a mechanical or structural function contributing to the form of plant cells and their organelles. For example, the membrane systems within cells, which are the sites of various reactions inherent in cell metabolism, are proteinaceous. However, carbohydrate derivatives, often strengthened with polymers such as lignin, are far more important in giving rise to the gross rigid and fibrous structures we are familiar with in plants.

Enzymes are proteins that direct the many biochemical processes that accompany cell growth, cell division, and metabolism. There are large protein reserves in seeds that are used to sustain the growth and development of the seedling during and after germination. These often occur in cells as protein bodies and are broken down, or mobilized, by enzymes and transported and reassembled in regions undergoing rapid growth when the seed germinates. Unlike most other plant parts, seeds have large amounts of protein that may make up 10 to 50% or more of their dry weight.[9] This is why seeds and nuts are important protein sources in the human diet. Many of the stored proteins are present in relatively high concentrations. Numerous reports have suggested a correlation between the levels of protein and allergenicity (see "Principles and Characteristics of Food Allergens" by Steve L. Taylor and Samuel B. Lehrer and "Food Allergens" by Robert K. Bush and Susan L. Hefle in this issue). The high levels of storage proteins in seeds may explain, in part, why some people become sensitive to them after consuming them (e.g., peanuts) or processed seed products (e.g., wheat flour in bread).

In addition to the diversity of proteins that are normally present in plant tissues, other proteins are introduced by microorganisms associated with the plant, or by invading pathogens such as viruses, bacteria, and fungi. All fresh food and produce have a microbial flora. Fruits commonly carry yeasts that grow on their surfaces. All plant surfaces have soil organisms carried there on dust particles and other debris, or splashed on by rain or irrigation water. Airborne fungal spores are deposited on plants.

Some of the fruits and vegetables we purchase for consumption may carry plant disease organisms. Plant viruses increase to enormous numbers in infected cells such that the viral coat proteins, or other viral proteins that form inclusion bodies, compose a significant portion of the total cell protein. Data in Table 1 show the levels of coat protein from different viruses that were measured in four different fruits (cantaloupe, honey dew melon, yellow crook neck squash, and zucchini squash) obtained from a supermarket. These data illustrate the variability and extent of the different viral coat proteins that are components of our food supply.

In most cases the presence of organisms causing plant disease is revealed by the rots and discoloration (chlorosis and necrosis) that are associated with yet more changes in composition. These symptoms may appear in the field, before harvest, or after harvest when produce is in storage prior to distribution and sale. Soft rots are caused by the release of extracellular enzymes, formed by the invading microorganisms, that break down the pectin cement between cell walls and the cell walls themselves. In some cases host cell death is caused by toxins made by the pathogen

TABLE 1
Measurements of Viral Coat Protein in Supermarket Fruit as Measured by Enzyme-Linked Immunosorbent Assay

Fruit	CMV (μg/kg fruit)	PRV (μg/kg fruit)	ZYMV (μg/kg fruit)	WMV2 (μg/kg fruit)
YC77EZW20	ND	ND	68.4	430.6
C1	355,200	18,000	14,400	10,320
C2	130,464	5,472	10,944	115,488
C3	ND	252,000	28,800	720
C4	ND	ND	864	ND
C5	>2,400,000	1,200	8,400	ND
C6	>3,216,000	ND	14,000	ND
C7	>3,216,000	ND	12,864	ND
H1	ND	7,200	9,480	ND
H2	ND	6,840	1,800	ND
H3	ND	ND	2,200	ND
H4	359	4,752	3,888	173
H5	269	3,168	3,168	260
H6	238	ND	2,592	ND
H7	ND	5,928	1,824	137
H8	664	13,272	1,896	190
H9	82	960	24	24
H10	ND	ND	250	ND
H11	ND	ND	1,560	ND
H12	ND	ND	480	ND
H13	ND	ND	2,200	ND
H14	ND	3,120	720	ND
H15	ND	10,080	1,700	ND
H16	ND	ND	3,100	ND
Y1	ND	ND	11,424	ND
Y2	ND	ND	ND	ND
Y3	ND	ND	1,152	ND
Y4	ND	ND	13,056	ND
Z1	ND	ND	140	ND
Z2	ND	ND	ND	ND
Z3	ND	ND	454	ND
Z4	ND	ND	ND	ND
Z5	ND	ND	ND	ND
Z6	ND	ND	576	ND
Z7	43	ND	2,592	ND
Z8	14	ND	2,900	ND

Note: CMV, cucumber mosaic virus coat protein; PRV, papaya ringspot virus coat protein; ZYMV, zucchini yellow mosaic virus coat protein; WMV2, watermelon mosaic virus coat protein; ND, not detected; C1–7 are cantaloupes, H1–16 are honeydew melons, Y1–4 are yellow crookneck squash, and Z1–8 are zucchini squash. The top line is a transgenic line of squash being developed for market.

Reprinted with the permission of Hector Quemada, Asgrow Seed Company.

that then uses the killed host material as a substrate for further growth and sporulation.[20] Endogenous proteins may also be formed by plants in response to pathogen invasion or as a result of other environmental stresses.

VII. STRESS RESPONSE PROTEINS

When plants are invaded by pathogens they frequently respond by synthesizing the components (often proteins) of defense systems designed

to limit or contain the growth of the invading pathogen. In recent years, much research has focused on the so-called pathogenesis-related (PR) proteins that appear during the early stages of plant infection.[21] The amounts of these proteins are not large, less than 0.01% of the total cell protein. Their function is not understood in terms of specific resistance mechanisms and how these are controlled by PR proteins. Two PR proteins from kidney bean (*Phaseolus vulgaris*) have been identified as possible allergens.[22] There is no evidence that the proteins produced by pathogens cause such effects.

There is considerable information that consuming the chemical products formed by successful invading pathogens may have severe consequences. For example, stored peanuts may be invaded by molds (*Aspergillus* spp.) that produce nonproteinaceous compounds, aflatoxins, known to be carcinogenic.[23] There is also evidence that infected or contaminated feeding stuffs given to cattle may result in aflatoxins being present in their milk.[24,25]

A second example, which is not a problem in human nutrition, is encountered by livestock producers who may have problems with forage grasses that contain fungal endophytes.[26] These endophytic fungi live in the tissues of the grass leaves and stems without producing visible signs of disease until they reproduce sexually and form spores. However, certain chemical metabolites produced by the fungi are responsible for neurologic impairment and may cause a disorder known as staggers in grazing animals. Other compounds produced by the fungus are abortifacients and lead to stillborn calves. There is evidence that these compounds confer insect resistance and drought tolerance on the grasses, both of which are selective advantages. For this reason they are of interest to turf grass breeders.[27]

Both of these examples of potentially dangerous materials in food and fodder are produced by fungi. They are not proteins and result from somewhat complicated synthetic pathways that are controlled by the products (enzymes) of a number of genes. In the production of new crop cultivars, plant breeders typically screen much material collected from nature and from other breeders to look for certain characteristicss they need. Pest and disease resistance are important features of most such screening programs. Care must be taken to ensure that the resistant products do not contain unduly high concentrations of naturally occurring toxic compounds that may be partly responsible for their resistance.

Plant tissues that are stressed by high temperature or extreme drought form heat shock proteins.[28] These proteins are used by plant cells to help them recover their normal functions when the temperature falls, or more water becomes available.[29] Heat shock proteins play a role in ensuring that other important cell proteins have the correct three-dimensional configuration. Heat shock proteins are also called chaperones.

VIII. CONCLUSION

Proteins play important and intrinsic roles in plant structure, function, and development. Because humans cannot synthesize essential amino acids, plant and other sources of proteins are essential components of our diet. Each day we consume tens of thousands of different proteins from plants and plant-associated microorganisms. Most of these are present in the diet in small quantities, but some, such as the storage proteins in grains and seeds, may make up a substantial part of the plant proteins we consume. Although these proteins serve many different roles in the plant, from structural to enzymatic, they are typically rapidly degraded after consumption to individual amino acids, which are then used by our bodies to synthesize other proteins and nutrients.

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Genetic Modification of Proteins in Food

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I. INTRODUCTION

All cultivated plants grown for food have been improved by conscious selection and breeding. Yield, quality, and resistance to biotic and abiotic stresses have all been enhanced to improve agricultural productivity and satisfy mankind's growing needs for food and feed. Plant breeding is an exceptionally successful enterprise that has fashioned the raw material of unimproved germ plasm into the modern high-yielding crop and pasture varieties on which we now depend. Until recently, plant breeders had to depend on empirical methods to reach their goals. They intercross varieties with different characters expecting to recover progeny, in later segregating generations, that combine all of the desired features. After extensive testing these are introduced as new varieties. However, the discovery of plant transformation is changing the way that many breeders approach the challenge of creating new varieties to fulfill specific needs. Directed genetic change provides an important new tool and introduces the possibility of using genetic information from almost any life form, because the genetic code is universal.

Deliberate modification of the protein makeup of food through plant breeding had to await the discovery of the role of proteins and how they affect either the quality of the food material or their suitability for human consumption. Several examples of this in wheat, rice, and soybean are discussed below. Genetic theory implies that each time genetic changes are made in crop plants through plant breeding, there are associated changes in the proteins involved in the production of the new phenotypes.

The genetic controls of inheritance involve the transcription of DNA to messenger RNA (mRNA), which is then translated into the amino acid sequences of polypeptide chains and proteins. Many of the protein products of gene expression are enzymes that direct the synthesis of various cell components that collectively determine the characteristics of the cells, which in turn make up the tissues, organs, and the organism as a whole. The timing and coordination of the expression of these genes are governed by other genetic components called regulatory elements. The effect of this orchestration is the expression of cascades of different genes that, acting together, bring about the changes recognized as growth, development, and differentiation. These are observed as a plant passes from a seedling to a mature flowering structure, to produce a harvestable product that is consumed as food.

When breeding introduces changes in an organism's genetic constitution, usually subtle, but sometimes profound, variation is expected in its proteins. New proteins may be formed, or the balance of existing ones may be changed. A long history of producing new varieties of crop plants by introducing genetic information from a range of related wild species has very rarely resulted in forms that have had to be withdrawn from the market because of health concerns. Plant breeders have introduced thousands of new crop varieties that have had little, if any, effect on food safety.

The concern over genetic engineering stems from the fact that this new technology provides plant breeders access to genetic information from any living organism or indeed to synthetic DNA sequences. The developers of new plant varieties using genetic engineering have the responsibility

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of establishing that the newly introduced varieties, and the food products developed from these varieties, are as safe and nutritious as their traditional counterparts. This article compares the types of new plant varieties being developed by new technologies based on plant molecular biology with conventional plant breeding, and summarizes the directions that are being taken in crop plant development. This article focuses particularly on how genetic engineering could alter the allergenic potential of foods derived from major crops.

II. EARLY PLANT BREEDING

The practice of selecting superior parent plants from which to save seed had its origins during the transition that primitive humans made from hunter-gatherers to cultivators and keepers of livestock. There was a conscious selection of seed lots that gave good yields of easily harvested fruits, seeds, and other edible plant parts.

The first plants were chosen by hunter-gatherers through trial and error. We can assume they quickly learned to avoid plants that were poisonous, bitter tasting, made them sick, or that failed to satisfy their hunger. Seeds collected in times of plenty that were kept for the winter were probably planted in the spring or early summer. In this way we may suppose that the value of husbandry was learned and cultivation became a way of life for groups who were not content to depend entirely on chance in their foraging for food. As early agriculture developed, seeds and plants would have been exchanged and the better forms would have been prized for such qualities as yield, high rate of seed germination, and good flavor. The better farmers practiced selection, recorded their observations, and began to take the first steps in plant breeding by keeping seed lots from different sources separate. Such farmer's lines in Britain were given names that are recorded in early farming journals and diaries.

Modern plant breeding had its origins in the rediscovery of Mendel's observations from genetic experiments with peas. Mendel's factors, later called genes, showed that inheritance was particulate and did not result from the blending of characters, like mixing ink and water.

Biffen,[1] working in Cambridge, England, showed that resistance to yellow rust in wheat was inherited as a Mendelian character. Those who followed him began the process of deliberate selection of forms resistant to the diseases of our major food crops. If sufficient variation was not found among the varieties grown by farmers, then related unimproved and wild varieties and species were collected and screened to find the characters that were needed. Plant explorers and collectors sent and brought back seeds and live plants to be stored in germ plasm banks and botanical gardens. These stores of genetic variation came to be treasured as sources of genetic variation that could be found nowhere else short of returning to the original, often remote, collection sites. The best of modern germ plasm banks maintain detailed catalogs of their materials with computerized data banks showing the origins and characteristics of all the accessions. Because seeds deteriorate over time they must be periodically sown to regenerate fresh seeds for storage to maintain a supply that is available to plant breeders and others.[2]

Many other characters were included in the hybridization and selection programs as plant breeding gathered momentum in the early twentieth century. These programs all had the common objectives of improving the yield and quality of the harvested product and selecting for resistance to environmental stresses to maximize adaptation to the regions where the crops would be grown. The first consumers to test the products of plant breeding programs were the breeders themselves. By discarding forms with poor yield and low quality, the better ones were saved and compared with the best available materials. Improvements were kept and used for further hybridization. Ultimately, the market decides what has value. The crop plant varieties grown today are the result of the accumulation of many incremental steps in plant breeding. However, the early successes in breeding for disease resistance were followed by the discovery that the pathogens could also change.[3] Just as human disease organisms have evolved forms that are resistant to antibiotics, plant pathogens developed mutant forms that attacked the formerly resistant varieties. The Canadian plant pathologist Johnson,[4] who worked with the stem rust disease of wheat, described this process as human-guided evolution.

Most of the first modern plant breeding programs were carried out with public funds in universities and specialized institutions. Private plant breeders also existed, but at first there were no laws to protect the varieties that they developed. Hybrid maize, first introduced in the 1930s, began to change this dramatically.[5] Because the parentage of each hybrid was a trade secret, the knowledge of how to make it remained with the owner of the maize inbred lines used to prepare the F1 hybrid seed sown by farmers. Fresh seed had to be purchased each year for planting. If the farmer planted seed saved from his own crop it gave rise to a mixture of genetic forms caused by gene and chromosome segregation.

The introduction of plant breeder's rights in the 1950s in North America and Europe allowed the rapid growth of the private sector plant breeding industry by granting plant breeders rights and placing restrictions on the ability of farmers to sell seeds of nonhybrid crops to other farmers for planting.[6] The royalty income from new cultivars of crop plants financed the production of further improvements.

Improvements in agronomic traits such as yield and disease resistance continue to be driving forces behind today's seed industry, but, increasingly, attention is also focused on specialty traits, including high oilseed grains, low saturated fat oilseeds, and modified corn starches. Such traits command premium values in the marketplace and may offer unique opportunities for the application of new technologies.

III. PLANT BREEDING AND BIOTECHNOLOGY COMPARED

In conventional plant breeding, sexual crosses are made between different parents that may each have different characteristics that the breeder seeks to combine. The principal limit to the number and kinds of characters that can be introduced is the sexual compatibility between the plants that are being hybridized. If they are genetically distant, or unrelated, the likelihood of obtaining seeds from crosses is very low. Even when seeds are obtained they may fail to germinate or the plants they give rise to may be sterile. For example, in breeding tomatoes the principal sources of new genetic variation are other varieties of the same cultivated species *Lycopersicon esculentum*, or other species of *Lycopersicon* that are sexually compatible.[7] Table 1 shows a list of disease resistance traits that were introduced by traditional breeding. Although species in the genus *Solanum*, which includes the cultivated potato, are related they are not sufficiently closely related to produce seeds when crossed with tomato. If fertilization does take place following pollination, the development of the fertilized egg is often aborted and no viable seeds are formed. Sometimes hybrid embryos that would otherwise abort can be rescued by dissection and placing them on a nutrient medium for further development into seedlings.[8]

When crosses between parents produce fertile plants in the following generations, the progeny are selected for their conformation to the breeder's objectives. If the parents differ in many genes the precise combination of characters will occur rarely among the many different segregating forms. As a consequence, breeders must work with large segregating progeny families. These families are screened to select plants that approach the desired form, quality, yield, and resistance to pests and pathogens. These selection programs are rigorous and to be effective must be carried out over successive years and seasons in order to verify that the end products stably inherit the desired characteristics. For example, in winter wheat, a large breeding program may make up to 1000 crosses each year, but not produce new and proven finished varieties until some 8 to 10 years later.[9]

As a result of plant breeding, hundreds of new varieties of crop plants are introduced each year. There are accurate statistics on the numbers of new cultivars of each crop species introduced each year in countries that require variety registration and national trials. These trials test the distinctiveness, uniformity, stability, and the yield and quality of harvested product in order for new introductions to qualify for breeders royalties on seed sales (Table 2). The commercial lifetime of new plant varieties is often short, considering that it may take from 6 to 10 years to produce a new product. In the U.K., varieties of small grain cereals may have a commercial lifespan of 5 to 7 years before being replaced by other superior

TABLE 1
Resistance in Wild Species of *Lycopersicon* and *Solanum* of Some Economically Important Diseases of Tomato

Disease	Responsible organism	Source of resistance
	Bacteria	
Bacterial canker[a]	*Clavibacter michiganese*	*L. hirsutum, peruvianum, pimpinellifolium*
Bacterial speck[a]	*Pseudomonas tomato*	*L. pimpinellifolium*
Bacterial spot	*Xanthomonas vesicatoria*	*L. esculentum* var. *cerasiforme*
Bacterial wilt	*Pseudomonas solanacearum*	*L. pimpinellifolium*
	Fungi	
Collar rot	*Alternaria solani*	*L. hirsutum, peruvianum, pimpinellifolium*
Leaf mold[a]	*Cladosporium fulvum*	*L. esculentum* var. *cerasiforme*
Fruit anthracnose[a]	*Colletotrichum coccodes*	*L. esculentum* var. *cerasiforme*
Target leaf spot	*Corynespora cassiicola*	*L. pimpinellifolium*
Didymella canker	*Didymella lycopersici*	*L. hirsutum*
Fusarium wilt[a]	*Fusarium oxysporum* f. sp. *lycopersici*	*L. pimpinellifolium*
Phoma blight	*Phoma andina*	*L. hirsutum*
Late blight[a]	*Phytophthora infestans*	*L. pimpinellifolium*
Phytophthora fruit rot	*Phytophthora parasitica*	*L. pimpinellifolium*
Phytophthora root rot	*Phytophthora parasitica*	*L. esculentum* var. *cerasiforme*
Corky root[a]	*Pyrenochaeta lycopersici*	*L. peruvianum*
Septoria leaf spot[a]	*Septoria lycopersici*	*L. esculentum* var. *cerasiforme, hirsutum, pimpinellifolium*
Grey leaf[a]	*Stemphylium*	*L. pimpinellifolium*
Verticillium wilt[a]	*Verticillium albo-atrum*	*L. esculentum* var. *cerasiforme*
Dahlia wilt	*Verticillium dahliae*	*L. peruvianum*
	Nematodes	
Potato cyst	*Globodera pallida*	*L. hirsutum*
Sugarbeet	*Heterodera schactii*	*L. pimpinellifolium*
Root-knot[a]	*Meloidogyne incognita*	*L. peruvianum*
	Viruses	
Spotted wilt[a]	TSWV	*L. pimpinellifolium*
Tobacco mosaic[a]	TMV	*L. peruvianum*
Tomato yellow leaf curl	TYLCV	*L. cheesmanii*
		L. hirsutum, peruvianum, pimpinellifolium
Cucumber mosaic	CMV	*L. peruvianum, S. lycopersicoides*
Curly top[a]	CTV	*L. peruvianum*
Potato Y[a]	PYV	*L. esculentum* var. *cerasiforme*

[a] Resistance has been incorporated into cultivars.

Adapted from Rick et al.[75]

TABLE 2
Number of New Varieties of Major Agricultural Crops Registered in 1995 in the European Catalog

Crop	Number of new varieties
Alfalfa	26
Cotton	9
Flax	12
Maize	233
Pea	28
Rapeseed	43
Sorghum	25
Soybean	24
Sunflower	89
Wheat (soft and durum)	114
Vegetables (46 species)[a]	1760
Total	2363

[a] As of December 1, 1994.

varieties. Maize hybrids in the U.S. have a similar short commercial lifespan.

Most of the variation used by plant breeders has come from germ plasm collections that include cultivars and land races produced by earlier generations of farmers and breeders, related wild species collected from the crop's geographic center of origin, and material developed by today's successful seed companies. Much of the variation held in germ plasm banks arose from spontaneous mutation during evolution. Mutations are inherited changes in the DNA in which one or more nucleotide bases are deleted or are replaced with other different bases. If a mutation confers a selective advantage it survives and, in some areas, can replace its progenitor form. In most crops, breeders see spontaneous mutations arise and sometimes select them for propagation. For example, in fruit trees so-called bud sports are known in which the fruit has a different color or form. Pink-fleshed grapefruit and variants of dessert apple varieties have arisen in this way.[10]

Not surprisingly, there has been interest in increasing the normally low frequency of spontaneous mutation. One method uses the property of certain chemicals, called mutagens, and ionizing radiation (X-rays, gamma rays, neutrons) to cause changes in the DNA.[11] The majority of such induced mutations are deleterious. In crop plants where large numbers of seedlings resulting from mutagen treatments can be easily screened to detect new forms, there has been limited success in recovering disease- and herbicide-resistant forms. Another method makes use of the spontaneous variation observed when unorganized plant callus tissue, cultured on artificial media, is allowed to regenerate meristems and shoots (Figure 1). The plants that are recovered often show considerable variation in morphology and other characters. Although much of this somaclonal variation is of limited practical interest, there are claims that useful forms have been recovered which have given rise to new and superior varieties.

Tissue culture and mutagenesis have been combined, as in the case of chemically mutagenized *Brassica* pollen microspores that were cultured on medium containing herbicide from which fertile double-haploid herbicide-resistant plants were obtained.[12] Since 1964, at least 14 new plant varieties derived through induced mutation have been registered in Canada. These include Empress barley, Redwood 65 flax, Shamrock apple, Early Blenheim apricot, Sunburst sweet cherry, and Stellar canola.[11]

IV. PLANT TRANSFORMATION

Recombinant DNA technology overcomes the barriers imposed by sexual compatibility. Because

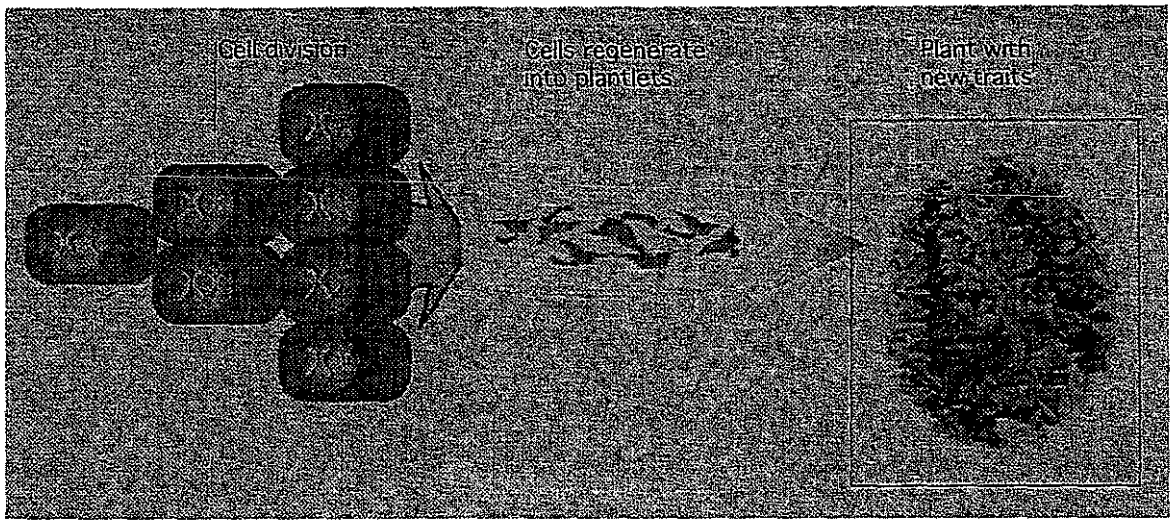

FIGURE 1. Plant regeneration. (Figure courtesy of Monsanto Company.)

of such technology, it is now technically possible to introduce into the tomato genetic information not only from the tomato, but from any other organism. Genes from viruses, bacteria, fungi, other green plants and other organisms can be introduced, although genes from nonplant sources may need to be substantially altered to allow them to be efficiently processed by plant cells. The process of introducing foreign DNA into plants is called plant transformation. The following is a brief description of the principal methods used in crop plant transformation intended to guide the unfamiliar reader. The earlier publication by the International Food Biotechnology Council[13] covers in more detail this and other aspects of genetically engineered foods and provides many more references to the literature.

The technology for carrying out genetic transformation was developed about 15 years ago.[14] Several different methods may be employed, but all depend on tissue culture on suitable media. Tissue cultures usually consist of unorganized cells (callus) that do not form recognizable organs like leaves, stems, or roots. However, under special culture conditions some are able to form small organized meristematic regions that develop shoots from which whole fertile plants can be regenerated.

A method of transformation commonly used for dicotyledonous plants (these generally have broad leaves and not the long narrow strap-shaped leaves of the grasses) depends on the crown gall bacterium, *Agrobacterium tumefaciens*.[15] This is a plant pathogen that invades wounds in the tissues of dicotyledonous plants. The bacterium introduces a small segment of its own DNA into some of the nuclei of the cells of its host where it becomes an integral part of one or more of the host chromosomes. In nature, this results in the formation of a tumor, or gall, at the site of infection which provides a refuge for the bacterium. In the laboratory, the tumor-inducing (Ti) DNA of the crown gall bacterium was disarmed so that it no longer causes plant disease. This was done by deleting the genes carried by the Ti plasmid that are responsible for tumor cell formation. After deletion of these genes all that is left are the left and right ends, or "borders", of the piece of DNA that is inserted in the host nucleus together with a few bases of DNA between them into which foreign DNA can be spliced. The disarmed Ti DNA, carrying the foreign DNA between the borders, is still inserted in the host nucleus, but no tumors are formed. The cells with introduced DNA are normal. In this way a desired gene can be spliced into the disarmed Ti DNA and, when carried into the host cell nucleus, becomes an integral part of the host chromosome (Figure 2). Provided the introduced genes have appropriate controlling elements, or promoters, attached to them, they will be ex-

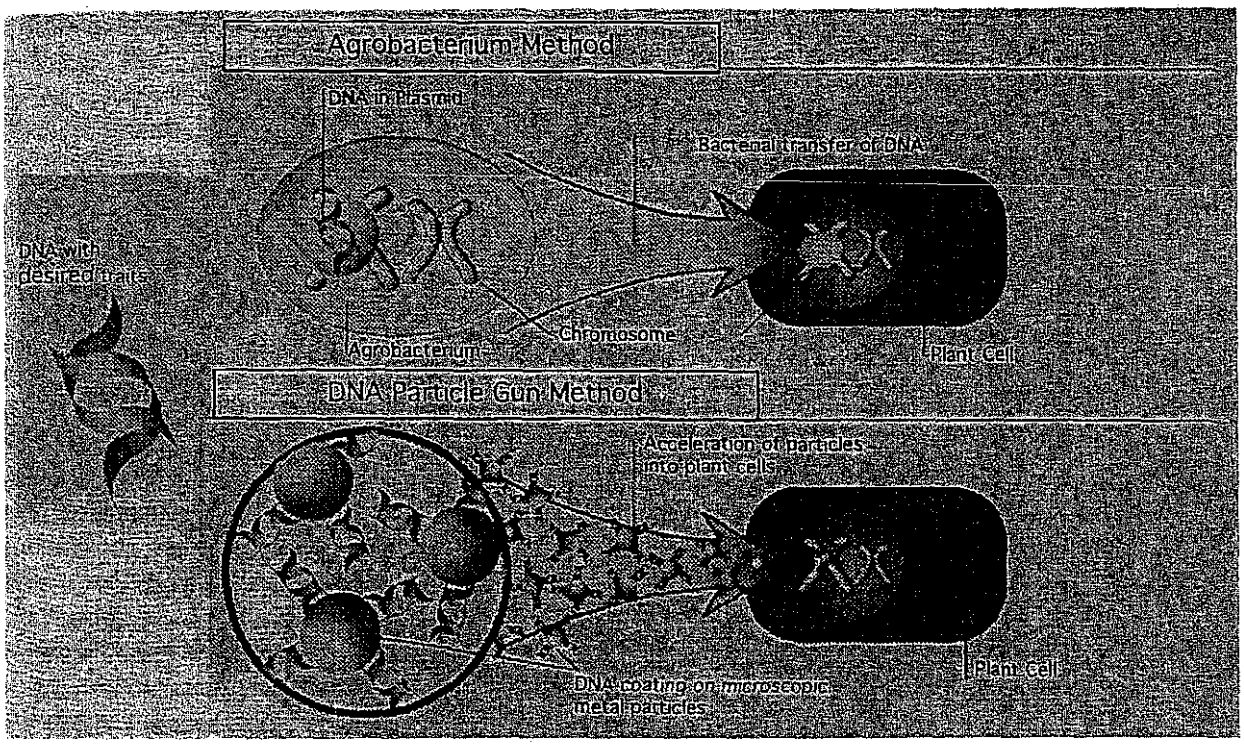

FIGURE 2. Plant transformation. (Figure courtesy of Monsanto Company.)

pressed at the correct stage of host plant development and in the appropriate tissues (e.g., leaves, flowers, fruits, or seeds).

The efficiency of transformation by the crown gall Ti DNA is low. Only 1 in 10,000 to 1 in 100,000 of the host cells exposed to the transforming bacteria are transformed.[16] If the untransformed cells are allowed to grow, they will crowd out the transformed cells. Therefore, a method of selecting host cells that have been transformed is needed.[17] The cells of many dicotyledonous plants in culture are sensitive to the antibiotic kanamycin. If the Ti DNA also carries a gene for kanamycin resistance (a gene that is commonly present in soil bacteria) with a constitutive plant promoter that causes the gene to be expressed all the time, only transformed cells that carry this gene will grow on tissue culture medium containing the antibiotic. The untransformed cells are killed. Antibiotic-resistant plants have no agronomic value. However, when a useful gene is linked to the kanamycin resistance gene, there is a high probability that the transformed tissue that expresses kanamycin resistance will also carry and express the other gene.

The "biolistic" method of transformation is an alternative to the *Agrobacterium* method and is often used for monocotyledonous plants that are not susceptible to the pathogen. The method uses microscopic gold or tungsten particles coated with transforming DNA that are accelerated, by explosive charge, high-pressure helium, or by electric discharge, toward tissue culture cells held in a partial vacuum[18] (Figure 2). A small proportion of the cells, penetrated by DNA-coated projectiles, become transformed. Provided they carry a suitable selectable marker, such as resistance to a herbicide that would normally kill the plant, they can be selected from the background of untransformed tissue and allowed to regenerate to recover whole plants. Variants of this method use carborundum particles or ground glass and a vortex mixer to carry DNA into host cells.

Yet another method uses protoplasts of cultured cells, from which the rigid cell walls are removed by enzyme treatment. DNA enters the cells, either by natural uptake of DNA or by electroporation when the protoplasts, suspended in a suitable osmoticum, are pulsed with an electric field.[19] The short current flow opens small

holes in the outer protoplast membrane greatly increasing the permeability of the cells to DNA molecules.

V. PLANT TRANSFORMATION AND CROP IMPROVEMENT

At first, the principal limitation to the use of genetic engineering in crop plant improvement was the difficulty in carrying out transformation at a sufficiently high frequency to recover useful numbers of plants. The development of reliable methods for culturing regenerable cell lines required lengthy experimentation. The methods often needed adjustment to suit the idiosyncracies of individual cultivars within a crop species. The cereals proved to be particularly difficult. The methods for rice[20] and wheat[21] have been developed only recently, aided by the use of herbicide resistance markers, and are not yet routine. Table 3 lists the plants that have been transformed successfully to date.

Another limitation lay in isolating and characterizing the genes to be used in transformation programs. Until the development of plant molecular biology, there was little progress toward understanding the biochemistry and genetic controls of the characters of most interest to plant breeders. Only with this detailed knowledge is it possible to plan and design changes that are likely to result in plant improvements.

TABLE 3
Plants That Have Been Modified Using Genetic Engineering Techniques

Alfalfa
Allocasuarina verticillata
Anagallis arvensis
Apple
Apricot
Arabidopsis thaliana
Asparagus
Aspen
Atropa belladonna
Barley
Black currant
Brassica carinata
Brassica juncea
Broccoli
Brown Sarson
Buckwheat
Cabbage
Canola
Carnation
Carrot
Cauliflower
Celery
Chicory
Chrysanthemum
Cotton
Cranberry
Cucumber Orchid
Eggplant
European larch
Fennel
Flax
Foxglove
Geranium
Grape
Horseradish
Kiwi
Lettuce
Lemon
Licorice
Lily
Lotus
Maize
Medicago truncatula
Morning Glory
Muskmelon
Nicotiana bigelovii
Nicotiana clevelandii
Nicotiana glauca
Nicotiana hesperis
Nicotiana plumbaginifolia
Nicotiana rustica
Nightshade
Oats
Oil Seed Rape
Onobrychis viciifolia
Orange
Orchardgrass
Papaya
Pea
Peach
Petunia
Plum
Poplar
Poppy
Potato
Raspberry
Rice
Rose
Rye
Snap Dragon
Solanum dulcamara
Solanum muricatum
Sorghum
Soybean
Spruce
Strawberry
Stylosanthes humilis
Sugarbeet
Sugar cane
Sunflower
Sweet potato
Tobacco
Tomato
Tulip
Walnut
Wheat
White clover
Yam

Adapted from Fisk and Dandekar.[76]

VI. GENE CLONING

Much of modern plant molecular biology involves the identification and isolation of genes involved in plant development and metabolism. Most of this is fundamental research to establish the nature and controls of the many pathways involved. Many of the characters of principal interest to breeders (e.g., yield, drought, and stress tolerance) are controlled by many genes. The analysis of how and where to make changes that would increase food production has barely begun. Some of the initial genes that have been isolated and reintroduced into plants are shown in Table 4. Some of these products are already commercially available, whereas others will likely reach the marketplace in the next few years. The following sections describe some of the characters introduced by transformation that are currently in use in plant breeding programs. Plants that have been modified by transformation are said to be transgenic or genetically engineered. The term *genetically modified* is sometimes used, although this could equally well apply to any organism that has been improved by breeding.

VII. GENE SUPPRESSION TECHNOLOGY

DNA transcription occurs in only one direction along the molecule, from the 5′ to the 3′ end. If a coding sequence is inserted in the DNA in an inverted order, transcription occurs from the antisense strand of the DNA molecule instead of from the sense strand. As a result, an antisense form of mRNA is produced, which may bind to the sense form of RNA produced by the normal gene in the cell. Binding is highly specific because it relies on accurate base pairing. If the two mRNA forms interfere with each other, the interference can prevent normal levels of translation into an amino acid sequence. As a consequence, the level of gene product may be greatly reduced, resulting in an altered phenotype.

Following review by the U.S. Department of Agriculture (USDA) and the Food and Drug

TABLE 4
Near-Term Products of Plant Biotechnology

Crop	Trait	Primary gene(s)	Organization
Tomato	Virus resistance	Viral coat protein	China
Tomato	Delayed softening	Anti-polygalacturonase	Calgene
Tobacco	Bromoxynil tolerance	Nitrilase	Rhône Poulenc
Tomato	Delayed ripening	Anti-ACCsynthase	DNA Plant Technology
Tomato	Delayed ripening	ACC deaminase	Monsanto
Tomato	Delayed softening	Anti-polygalacturonase	Zeneca
Squash	Virus resistance	Viral coat proteins	Asgrow
Cotton	Bromoxynil tolerance	Nitrilase	Calgene
Potato	Insect resistance	*cryIIIA*	Monsanto
Maize	Basta resistance	*bar*	AgrEvo
Maize	Insect resistance	*cryIA(b)*	Ciba Seeds
Flax	Sulfonylurea tolerance	Acetolactate synthase	University of Saskatchewan
Rapeseed	High laurate	Anti-12:0 thioesterase	Calgene
Rapeseed	Basta tolerance	*bar*	AgrEvo
Soybean	Glyphosate tolerance	EPSPS	Monsanto
Canola	Glyphosate tolerance	EPSPS/GOX	Monsanto
Rapeseed	Male sterility	Barnase/Barstar	PGS
Cotton	Insect resistance	cryIA(c)	Monsanto
Cotton	Glyphosate tolerance	EPSPS	Monsanto
Maize	Insect resistance	cryIA(b)	Monsanto
Maize	Glyphosate tolerance	EPSPS/GOX	Monsanto
Potato	Virus resistance (PVY)	Viral coat protein	Monsanto
Potato	Virus resistance (PLRV)	Viral replicase	Monsanto

Note: ACC, 1-amino-1-cyclopropane-carboxylic acid; EPSPS, 5-enolpyruvylshikimate-3-phosphate; GOX, glyphosate oxidoreductase; *cryIIIA*, the insecticidal gene from *B. thuringiensis* subsp. *tenebrionis; cryIA(b)*, the insecticidal gene from *B. thuringiensis* supsp. *kurstaki* strain HD-1; bar, phosphinothricin acetyltransferase; Barnase is the male sterility gene and barstar is the restorer gene.

Administration (FDA), a tomato carrying such an antisense construct was introduced in the U.S. in 1994. This tomato has an antisense form of the gene for the enzyme polygalacturonase (PG), in addition to the normal sense form of the gene.[22] PG is produced during the natural ripening of the fruit and is partly responsible for the softening that occurs once full ripeness is reached. The enzyme disrupts the materials that hold the cells of the fruit tissue together. Some tomato plants that carry the antisense PG gene had markedly reduced levels of PG during fruit ripening. Fruits borne by these plants show some delay in the softening process that normally accompanies fruit ripening. The antisense gene adds up to 5 d to the life of the fruit at the stage of full red ripeness before softening spoils it, allowing fruit to ripen on the vine before harvest.

These tomatoes were produced by transformation using a disarmed Ti plasmid vector that also carried the selectable marker gene for resistance to the antibiotic kanamycin. Detailed evidence was provided to the FDA to show that the levels of known toxic compounds normally found in the tomato, such as the alkaloid tomatine, as well as important nutrients, such as vitamins A and C, were no different than the cultivar from which it was derived. The engineered variety employed a selectable marker that causes the tomato to produce the enzyme neomycin phosphotransferase II in all tissues, including the fruit. This enzyme degrades kanamycin, rendering it inactive. Neomycin phosphotransferase is not known to be toxic to mammals, but attention was focused instead on its potential to interfere with the clinical use of the antibiotic even though kanamycin is only infrequently used in human medicine. It was shown that the enzyme was rapidly destroyed in the gut so destroying its activity. There is no evidence of transfer of genes from plant food materials in the gut to microorganisms that are part of the gut microflora.[23] The FDA concluded that there was no hazard presented by the tomato and imposed no restrictions on its sale or requirements that it be labeled as a product of genetic engineering. This tomato was marketed in 1994 as the first genetically engineered food in the U.S.

Since the discovery of antisense technology, it has been found that gene suppression may often occur in genetically engineered plants that carry sense constructs. Called trans- or co-suppression, the technique offers an alternative, and sometimes simpler, method of turning off the activities of particular genes.[24] Vine-ripened tomatoes that use co-suppression to reduce ethylene production have recently gone on sale in the U.S. after FDA review. In the context of allergenicity, because little or no foreign genetic information encoding protein is introduced by gene suppression technology, the only risk would appear to come from unspecific effects due to inadvertent disruption of other genes (see below) or from the products of marker genes used to select the transformants.

VIII. HERBICIDE TOLERANCE

In developed world agriculture, weed control is almost entirely carried out by using chemicals that do not harm the crop, but kill its principal weeds. Although some of the early herbicides had undesirable environmental effects, many have been replaced by newer chemicals that are used at very low doses and that are rapidly degraded by soil microorganisms and therefore do not persist in the environment. Most of these chemicals can only be used on the crops for which they were developed. They are not selective on other crops. For example, the widely used herbicide, glyphosate, cannot be used directly on any crops because it is a general herbicide designed to kill all unwanted vegetation. The analysis of the mode of action of herbicides has led to the discovery of genes for tolerance to them. These may, for example, involve the substitution of an altered form of a key enzyme that is no longer inactivated by the herbicide or the use of a gene that degrades the herbicide.[25] Because the mechanisms of resistance are known the nature of the determinative protein changes is also known, and with far greater precision than is possible with conventional plant breeding. An advantage of herbicide tolerance is that it can be selected for directly by incorporating the herbicide in the culture medium used after transformation to introduce the gene for tolerance. Herbicide-tolerant varieties will encourage more selective use of herbicides and will promote soil conservation practices such as reduced tillage.

IX. DISEASE RESISTANCE

In crop plant development, much effort is expended on breeding varieties that are innately resistant to diseases and pests. The growing disenchantment with chemical pesticides, their effects on the environment, and the perception that some may pose risks to human health has greatly enhanced interest in genetic resistance. There is much active research on the nature and mechanisms of plant disease resistance that will increasingly influence the ways in which we develop resistant crop plant varieties. The traditional method, which is still the most widely practiced, is to survey germ plasm to look for other cultivars, and related and wild species, to find individual plants or families that show high or useful levels of resistance when inoculated with the disease organism or when they are exposed to conditions that support heavy levels of disease infection. The most promising resistant accessions are crossed with one or more modern cultivars to begin a backcrossing program to isolate the genes for resistance while retaining a genetic background adapted for commercial use. Linkage to adverse traits, such as low yield, may make this a long and tedious process that may take up to 10 or more years. It is also a process that favors the selection of resistance genes that are dominant with major effects. Unfortunately, the usefulness of such genes has very often proven short-lived in agriculture, because they strongly select pathogen races that are not affected by them. Some authors advocate the selection of minor gene resistance on the grounds that it is more stable and long lasting.[26]

A variety of mechanisms are responsible for disease resistance. All plants are capable of defense-response reactions. The responses begin within minutes of wounding or injury, or invasion by an organism that is detected as a pathogen. One of the first responses is the appearance of a group of proteins, called pathogenesis-response (PR) proteins.[27] The role and function of the PR proteins are not fully understood. At later stages of response, a variety of compounds that inhibit the growth of invading microorganisms are formed, including products of phenol-propanoid metabolism. There is also strong evidence of the production of compounds that signal systemic responses. These reactions have effects at a distance from the original invasion site, not only elsewhere on the same leaf, but on other leaves and plant parts. This phenomenon is called systemic-acquired resistance, and appears to play an important role in protecting plants against disease organisms.[28–30]

Not surprisingly, an early goal of plant molecular biology was to isolate and clone genes for disease resistance. By the summer of 1994, a number of reports of the successful cloning of disease resistance genes in maize, *Arabidopsis*, tomato, flax, and tobacco had appeared.[31–34] These genes appear to be responsible for such components of the disease resistance reactions as a maize enzyme that inactivates a fungal pathotoxin,[35] and putative recognition proteins that detect the products of avirulence alleles carried by fungal or bacterial pathogens.

The extent to which naturally occurring genes for resistance will be isolated for use in genetically engineered plants as a means of speeding up breeding for disease resistance remains to be seen. However, because this approach directly parallels the very successful and, so far, very safe conventional breeding approach, the risk of allergenicity seems low. Even if a gene for resistance were obtained from a plant known to be allergenic, e.g., a legume, and was introduced into food plants belonging to other genera and families, the gene product could be evaluated for potential allergenicity in premarket testing (for details see example with soybean protein below and "Assessment of the Allergenic Potential of Foods Derived from Genetically Engineered Crop Plants" by Dean D. Metcalfe et al. in this issue).

In conventional plant breeding, disease and pest-resistant cultivars have not been singled out for special testing. The reason for this is that there has been little evidence that hazards have been introduced in this way. The products of conventional plant breeding programs are thus covered by the safety standard of not requiring premarket approval if the product is generally recognized as safe (GRAS). In the debates over the dangers from pesticide residues in food crops, some have argued that the levels of naturally occurring toxicants in our daily diet are very much higher than the trace amounts of pesticides that are the cause of so much concern. It has also been suggested that naturally occurring antioxidants in the same

plant foods, such as ascorbic acid (vitamin C) and riboflavin, may protect humans from the effects of these toxicants.

X. NOVEL MECHANISMS FOR DISEASE AND PEST RESISTANCE

A. Virus Resistance

A number of important and very damaging plant diseases are caused by viruses. Many of these are transmitted by insects, such as aphids, and plant hoppers that feed by sucking plant sap. In 1986, Powell-Abel et al.[37] discovered that if the coat protein gene for the virus causing tobacco mosaic (TMV) was introduced into the host plant, its expression interfered with the replication and systemic spread of the virus. This made the plant resistant to virus infection. Others soon showed that this was a general phenomenon that applied to many other plant viruses and their hosts.[38,39] Because effective virus resistance was uncommon up to that time, the discovery was seen as the harbinger of a new way to control virus diseases. One of the first potential commercial applications has been the introduction of viral coat protein genes of the cucumber mosaic virus, watermelon mosaic virus, and zucchini yellow mosaic virus into squash.[40] The engineered squash is resistant to all three viruses but contains viral coat proteins that constitute less than 0.1% of the total protein of the squash fruit. This compares favorably with the somewhat greater virus content of fruit from plants naturally infected with the viruses that are commonly consumed because these viruses are widespread (see Table 1). These products do not pose a new allergenic risk.

Another approach to virus resistance involves cloning the viral gene that encodes a replicase. This is an enzyme that the virus needs in order to replicate its own genomic RNA. Somewhat surprisingly, plants that express viral replicase show resistance to the virus concerned and it is assumed that this is because the excess replicase interferes with orderly replication of the viral genome.[41] Viruses also produce certain proteins that appear to be responsible for enlarging the lumen of the plasmodesmata that connect adjacent host cells. This enlargement is necessary to allow the virus particle to move from cell to cell to establish systemic infection. Genes for viral movement proteins have been cloned and expressed in host plants and can interfere with viral movement, thereby conferring resistance.[42]

B. Fungal Disease Resistance

The observation that the cell walls of many fungal pathogens are made of chitin, and that some host plants respond to fungal infection by producing chitinase, has encouraged the production of novel transgenic plants that express this enzyme constitutively. Various sources of chitinase genes have been employed including other plants (bean), and bacteria (*Serratia marcescens*). Although interesting, and potentially promising, results have been reported,[43] there are not yet any cultivars in use that employ this as a primary defense against fungal infection.

C. Insect Resistance

Bt is a successful commercial biological insecticide based on the crystal-protein endotoxin produced by some strains of the soil bacterium *Bacillus thuringiensis*. When ingested by larvae of sensitive insects, the insecticidal protein is cleaved and an active part of the molecule attaches to the brush border membrane of the larval intestine.[44] As a result, the intestinal wall loses its property of semipermeability and the insect leaks hemolymph into the intestinal cavity, becoming dehydrated and eventually dies. There are a number of different *B. thuringiensis* endotoxin proteins identified that are specifically active against different groups of insects.[45] For example, the cryI proteins are active against many lepidopteran larvae, whereas the cryIII proteins are active against coleopteran larvae. Extensive use of Bt insecticides containing the cryI proteins has not shown any evidence of human toxicity or allergenicity.[46,47] Consequently, biotechnologists were anxious to use *B. thuringiensis* genes encoding various insecticidal proteins, coupled to constitutive promoters, to protect transgenic plants

from insect attack. This strategy is currently in use to protect cotton plants from attack by larvae of the boll worm, potato plants from attack by the Colorado potato beetle,[48] and maize plants from attack by the European corn borer.[49]

The use of Bt toxins in the tissues of crop plants used for food raises issues that must be addressed. Clearly in each case there must be satisfactory evidence that the form of the protein used poses no risk to humans or livestock, and that the protein is not an allergen. Several Bt-based transgenic plant products, including potato, maize, and cotton, are nearing market introduction (see Table 4). Of these Bt-based products, the insect-resistant potato product has completed USDA review, FDA consultation, and has been approved by the U.S. Environmental Protection Agency (EPA).[50] The EPA specifically assessed the safety of the insecticidal Bt protein, from *B. thuringiensis* subsp. *tenebrionis*, including its allergenic potential (based on a history of safe use and its susceptibility to digestion), and identified no significant concerns. The Bt-based insect-resistant cotton and maize products also completed the USDA, FDA, and EPA review processes in 1995. In some cases, the expression of insecticidal proteins can be restricted to certain tissues that are not consumed through the use of tissue-specific promoters. Bt could be expressed in the leaves and stems of potatoes, but it would not be produced in tubers. It is also possible to design the expression of the insecticidal gene so that it only occurs in response to injury such as that caused by a chewing insect. Any such system must provide economically useful control.

An important issue associated with the use of any pesticide is that its effectiveness can be compromised if pests develop resistance. For example, large acreages of transgenic crops expressing Bt will likely exert a strong selection pressure for any insects that become less sensitive to the insecticidal protein. Resistance to Bt has been observed in the diamondback moth from extensive use of Bt insecticide.[51] This is in spite of the fact that selection is potentially less intense because the insecticide is relatively short-lived in nature; it is degraded by ultraviolet (UV) light and washed off the foliage by rain. In a transgenic plant the expression of Bt, under the control of a constitutive promoter, will be constant throughout the life of the plant. Developers of Bt crops are aware of the risk of resistance and have recommended special management practices that may need to be implemented to conserve Bt as an efficient biological insecticide.

Another strategy employed against insects is the use of a trypsin inhibitor gene isolated from an exotic collection of cowpea germ plasm, the seeds of which are unusually resistant to the larvae of moths that attack the grain in storage.[52] By interfering with the digestion of the plant proteins ingested by feeding larvae, transgenic plants that express this gene show moderately high levels of resistance. Trypsin and other protease inhibitors are common components of many of our major food and feed crops. However, any transgenic food crop containing a new trypsin inhibitor would need to be tested to assure safety, especially because the Kunitz trypsin inhibitor is known to be an allergen in soybeans. Appropriate resistance management strategies would need to be developed for any transgenic crop.

Both Bt and trypsin inhibitors are examples of biological pesticides that the EPA proposed to regulate as "plant-pesticides" under the provisions of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and the Federal Food Drug and Cosmetic Act.[53]

XI. QUALITY TRAITS: ENHANCED PROTEIN CONTENT AND ALTERED PROTEIN EXPRESSION

A. Maize and Soybean

Some deficiencies are identified when the amino acid composition of plant proteins is compared with the ideal composition for human and animal nutrition. For example, legume proteins have a less than ideal content of methionine and cysteine, both of which are important sulfur-containing amino acids. Livestock feed based on soybean meal must be supplemented with methionine to provide a nutritionally adequate diet. Maize meal fed to livestock has limiting amounts of the amino acid lysine. Many research groups have addressed these problems. For example, in maize

grain, the lysine content is increased in a mutant called opaque-2.[54] Much effort has been expended in using this gene in breeding programs to produce feed with a higher lysine content. Because of other effects on grain storage and agronomic characters that appear to be closely linked to the high lysine gene(s), these efforts have not been very successful in competition with manufactured lysine feed supplements of microbial origin. Messing and his colleagues[55] used another approach to improve the grain methionine content by selectively increasing the amount of specific methionine-rich proteins in maize endosperm.

In soybean, Townsend et al.[56] used a cloned gene from a Brazil nut that encodes a 2S storage protein found in the nut meat and introduced this gene by transformation. The Brazil nut 2S protein has a methionine content of 18%. The protein was expressed in the seeds of transgenic soybean, resulting in a significant improvement in nutritional quality. The same gene has also been expressed in canola (oilseed rape) where it increases the methionine content of seed meal by more than 30%.[57] However, because Brazil nuts are allergenic for some sensitive individuals, the developers investigated the allergenic potential of the 2S protein. The expressed 2S protein from the soybean was tested against immunoglobulin E (IgE) from the sera of eight Brazil nut-sensitive individuals using the RAST inhibition test and immunoblotting.[58] Seven of the eight sera detected the 2S protein, suggesting that it is likely to be a major allergen in Brazil nuts and would therefore need to be labeled if used in fresh or processed foods in compliance with the 1992 FDA policy[59] on foods derived from new plant varieties. In view of this restriction, development of this product has been discontinued.

B. Rice Proteins

Some Japanese children suffer from an atopic dermatitis (AD) that is caused by a 16-kDa globulin protein in the rice they consume. This protein is heat stable and resistant to proteolytic enzymes in the gut. Although the allergen can be destroyed by enzyme treatment, the cost of producing hypoallergenic rice in this way is excessive. Japanese scientists set out to produce mutants in which the amount of allergenic protein was reduced. Several such mutants were isolated by Nishio and Iida[60] following treatment of rice seeds with a chemical mutagen. In one mutant, with good agronomic qualities, the amount of the allergenic protein was reduced by about 50%. However, two others, each with only trace amounts of the allergen, were almost sterile and therefore of little use in agriculture. Izumi et al[61] characterized the allergen and sequenced the gene that encodes it. Matsuda et al.[62] reported that they have succeeded in significantly reducing the amount of the 16-kDa allergen protein in rice by introducing an antisense RNA. Further studies are underway to try to further reduce or eliminate this major allergen in rice. Similar antisense approaches could be used to decrease the levels of other allergenic proteins in food crops, for example, peanuts, soybeans, and Brazil nuts.

C. Wheat Endosperm Proteins

Wheat breeders have for some time been aware of the incidence of celiac disease, a gluten-sensitive enteropathy that affects between 1 and 16 people per 8000 in Europe. People with celiac disease have to avoid consuming wheat and all products made with wheat flour. Although not an IgE-mediated allergic response, celiac disease could, in theory, be alleviated by breeding wheat varieties that lack the offending gliadin proteins responsible. These are relatively small molecules that range from 10 to 50 kDa in size. Recent information[63] shows that several molecules are reactive and carry and share repeated sequences of nucleotides. The genetic control of the rice allergen is relatively simple and lends itself to the mutational or genetic engineering approaches described. In wheat, the situation is more complex. Unlike rice, which has a single genome, wheat is an allohexaploid with three ancestral genomes. This means that there is likely to be redundancy in the genetic controls with up to three sets of genes for each developmental and metabolic pathway. Also, the offending molecules carry shared repeated amino acid sequences. If these are responsible for the enteropathy, the task

of changing them to reduce significantly their impact on sensitive individuals will be difficult, if not impossible. However, there is a considerable amount of research on the composition, structure, and molecular biology of wheat endosperm proteins in general because the gliadins, together with larger molecular weight glutenins, determine the baking quality of wheat flours.[64]

D. Modified Oil and Starch

Vegetable oils derived from the seeds of crops such as maize, soybean, and sunflower represent an important component of the human diet in many countries, and have achieved new prominence as consumers have sought to reduce dietary intake of animal fats. Oil composition can be affected by climatic conditions, as well as by the genetics of each species. Oil profiles have been significantly changed through traditional breeding and selection. The level of erucic acid in wild *Brassica napus* has been reduced from more than 50% to less than 5% to yield low erucic acid rapeseed oil, otherwise known as canola oil.[65] Canola oil has established a reputation as an especially healthy oil because it has the lowest levels of saturated fatty acids of all oilseed crops. Sunflower varieties have been selected that have a high concentration of the polyunsaturated fatty acid, linoleic acid, that makes the oil particularly suitable for dressings and salad oil. Other sunflower varieties have been bred to have a high concentration of oleic acid that improves the oil quality for frying.[66] Hot-processed vegetable oils contain very little, if any, protein and are unlikely to elicit allergenic reactions. Studies with a limited number of subjects have shown that individuals who are allergic to soybeans, peanuts, or sunflowers can consume soybean, peanut, or sunflower oil without ill effects.[67–69]

More recently the 12:0 ACP thioesterase gene from the California bay (*Umbellaria californica*) was introduced into canola to generate a line that produces a laurate-rich oil.[70] High laurate canola oil can replace tropical oils in nondairy coffee whiteners and whipped toppings, as well as in confectionery products. After consultation with the FDA, the first crop of laurate canola was grown and harvested in 1995.[71] Other oil profiles (e.g., high oleic canola and high stearate, low polystearate soybean) altered through genetic engineering will likely reach the market in the late 1990s.[71]

There are numerous other examples of enhancing or modifying the quality aspects of crop plants. Considerable research and success have been reported in modifying the starch content and/or structure of starch in several food crops. The overall amount of starch has been increased significantly in potato and other crops by expressing a mutant of the *Escherichia coli* ADP glucose pyrophosphorylase protein selectively in the potato tuber.[72] Starch is typically composed of a combination of a linear polymer of glucose called amylose and a branched glucose polymer called amylopectin. Amylose-free starch was produced in transgenic potatoes by inhibiting the amount of granule-bound starch synthase.[73] The degree of branching may also be altered by modifying the levels or activities of other enzymes in starch metabolism. The ability to specifically modify and design new starches will enhance the use of starches as thickeners, bulking agents, sources of calories, and stabilizers in foods. Because starches are not involved in allergenic responses, and the enzymes being engineered are already present in food, these applications are unlikely to raise significant allergenic concerns.

XII. COINCIDENTAL GENETIC CHANGES

Although the predicted or intended changes that result from plant transformation can be described and confirmed, there is concern that other random changes may have unpredictable effects. There is no control over the site of DNA integration, or of the number of copies of foreign DNA introduced into the recipient genome. Therefore, it is usual to make a number of different primary transformants to select those that are most vigorous and best express the new characters in a manner similar to the selection approach used in traditional breeding. If the integrated DNA disrupts an important coding sequence of an existing gene, this could have undesirable effects on the fitness of the resulting plant after regeneration,

which will cause the material to be rejected during field screening. The site of integration of the foreign DNA may also affect the expression of neighboring genes, and may turn them off or even turn on genes that are normally silent. Again, this may have effects on plant fitness that will lead to those lines being discarded.

It is possible that either kind of effect may alter the protein balance or constitution of the transgenic food plant and raises questions about its safety. However, there are natural processes leading to protein variation that occur in conventional plant breeding. These include genome mixing, transposition due to chromosome breakage and reunion, and loss or gain of whole chromosomes as well as mutations caused by the insertion of transposons; the so-called jumping genes.[13] Despite these significant genomic disruptions, traditional plant breeding has a long history of safe product development. New plant varieties that have been developed by sexual crossing or transformation undergo several generations of further breeding and evaluation to test the products for field performance. This is to establish that they have value to the end user or processor and do not carry deleterious traits. In addition to extensive scientific investigation conducted by industry into the safety of transgenic varieties of plants, such plants are also subject to consideration by FDA under the agency's "consultation process."[58] Under this mechanism, industry has the opportunity to provide science-based assurance that transgenic varieties of plants pose no safety concern to the health of consumers.

A considerable data base has accumulated since the first products of plant biotechnology were engineered (Table 4). The data show that the composition of the food and feed products derived from transgenic plants is equivalent to that of their traditionally bred counterparts. At a recent World Health Organization workshop, Fuchs et al.[74] presented compositional data on 20 independently transformed lines including five crops and six products. No meaningful differences were observed after analyzing over 450 components of these plants. These results establish that the introduction of new traits by genetic engineering, coupled with the traditional plant breeding methods used to select specific lines for marketing, produce food products that are equivalent to those derived from traditional breeding.

XIII. SUMMARY

Plant breeders have been extremely successful in improving the quality and yield of the major crops, while maintaining the safety of the food supply. This success has been achieved with very little understanding of the biochemical mechanisms that determine the selected traits. Each time a cross is made, tens of thousands of genes are mixed and reassorted, largely at random. The skill of the breeder lies in selecting the lines to be crossed and recognizing the preferred progeny, discarding those that lack the desirable trait or exhibit undesirable properties.

With the advent of recombinant DNA technology, breeders have not only extended the range of biological materials from which genes can be accessed, but have also gained new insights into genome organization and gene structure as well as the nature and function of the proteins that those genes encode. Such knowledge affords exquisite specificity in altering the genetic makeup of new crop varieties. For example, resistance to insect pests can now be achieved through the addition of a single well-characterized gene, instead of introducing thousands of unwanted genes from a wild relative that code for uncharacterized and possibly toxic proteins that must be eliminated by generations of backcrossing and screening to recover a commercially acceptable insect-resistant line. The technology also affords unique opportunities to identify the individual components of foods that may cause allergies, and to remove them from food, or change them, so that the food can be consumed safely.

A number of commercial products derived through genetic engineering have been approved through regulatory processes that address environmental and food safety concerns. These products are available, or will shortly be available, to growers, producers, and consumers. They will provide foods and feeds that are produced with fewer chemical inputs and have improved nutritional composition and quality.

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Allergenic Foods

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I. INTRODUCTION

Virtually all food allergens are proteins, although only a small percentage of the many proteins in foods are allergens.[1] Any food that contains protein has the potential to cause allergic reactions in some individuals. However, a few foods or food groups are known to cause allergies on a more frequent basis than other foods. At a 1995 consultation on food allergies sponsored by the Food and Agriculture Organization (FAO), a group of international experts confirmed that peanuts, soybeans, crustacea, fish, cow's milk, eggs, tree nuts, and wheat are the most common allergenic foods.[2] These foods are responsible for over 90% of serious allergic reactions to foods. Allergies to certain fresh fruits and vegetables are also rather common, but the allergens tend to be labile to processing and cooking and the symptoms are mild and confined primarily to the oropharyngeal area.[3] The prevalence of allergic sensitivities to specific foods varies from one country to another depending on the frequency with which the food is eaten in that country and the typical age at its introduction into the diet. For example, peanuts are a much more frequent cause of food allergies in the U.S. than in most other countries. Americans eat peanuts more often and introduce peanut butter into the diet of children at an early age. The Japanese probably experience more soybean and rice allergies than some other cultures because of the frequency of these two foods in the Japanese diet.[2] Scandinavians have a high incidence of codfish allergy[4] for similar reasons.

Table 1 provides a listing of the most common allergenic foods and food groups compiled from a thorough search of the medical literature. Too many studies have been conducted to allow citation of the entire body of medical literature; citations reflect the most relevant studies documenting the allergenicity of those foods. Such hallmark studies have comparatively large groups of patients and use the most objective diagnostic criteria, such as double-blind, placebo-controlled food challenge trials (DBPCFC). For some of the individual foods within an allergenic food group, only a few published accounts of allergic reactions can be found. For example, allergic reactions to certain tree nuts, such as pistachio or macadamia, are rarely reported, probably due to less frequent consumption.

Table 2 provides a listing of the less common allergenic foods. Only some of the foods listed in this table have been documented to cause severe, life-threatening allergic reactions. Citations are provided to studies and/or case reports that document the allergenicity of those particular foods. The absence of a particular food on this list may not mean that it is nonallergenic, but may indicate that its allergenicity has not been documented. Conversely, the presence of a specific food on the list merely indicates that it has been listed in one or more reports as a cause of food allergy and does not indicate the prevalence or potential as an allergenic food.

Obviously, considerable differences exist in the quality of the information used to establish the allergenicity of each specific food appearing in

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TABLE 1
Common Allergenic Foods and Food Groups

Food	Ref.
Crustacea (shrimp, lobster, crab)	26, 39, 45, 46, 51, 143, 145, 156, 199, 253
Egg	23, 26, 27, 35, 36, 67, 101, 172, 173, 199, 200, 201, 202, 203
Fish	1, 35, 36, 78, 84, 86, 101, 126, 128, 161, 198, 199, 200, 201, 202, 253
Milk	23, 26, 27, 35, 36, 79, 101, 172, 173, 175, 198, 199, 200, 201, 202, 203, 211
Peanuts	17, 25, 26, 27, 35, 101, 117, 198, 199, 200, 201, 202, 203, 254, 255
Soybeans	17, 23, 25, 27, 101, 198, 199, 200, 201, 202, 203, 255
Tree nuts	3, 5, 7, 8, 13, 14, 16, 18, 26, 27, 30, 62, 63, 65, 77, 93, 94, 102, 103, 141, 155, 165, 173, 181, 190, 204, 214, 237
Wheat	4, 11, 12, 26, 35, 36, 51, 101, 117, 120, 198, 199, 200, 201, 202, 203

Note: This table was compiled using literature searches of the Agricola (1972 to July 1994) and Medline (1966 to July 1994) databases.

Table 2. Supporting data range from highly objective DBPCFCs to anecdotal reports based primarily on clinical histories. Reports were not included in the summary if they were based on histories of controversial symptoms not widely acknowledged as being caused by allergic reactions to foods, or if the supporting clinical data were based solely on controversial diagnostic techniques. Table 2 provides information on symptoms, age of patients, and the supportive diagnostic data provided in those reports.

The variability in symptoms is quite large both between individual patients and between different studies (groups of patients). Even individual patients display variable responses depending on such factors as the exposure dose to the offending food. Certainly, some symptoms are more serious than others. Systemic anaphylaxis, asthma, and laryngeal edema are potentially life-threatening. Some foods are primarily associated with mild adverse reactions, such as the so-called oral allergy syndrome, which is associated with itching, hives, and other mild reactions in the oropharyngeal area only after ingestion of fresh fruits, and only rarely with systemic reactions.[3]

DBPCFCs represent the most objective diagnostic approach to establish a cause-and-effect relationship between ingestion of a food and an allergic reaction.[5] Other types of challenge studies, such as the single-blind and open, are also useful, but are somewhat less objective than the DBPCFC. Challenge trials establish a cause-and-effect relationship between ingestion of the food and the onset of symptoms in a sensitized individual. However, they do not establish an allergic mechanism behind the illness.

Table 2 also provides information relating to results of skin tests, immunoassays, and histamine release tests that do provide evidence of an IgE-mediated allergic mechanism for the illness. However, these tests alone are insufficient to establish a cause-and-effect relationship because false positives, and to a lesser extent, false negatives do occur.[6]

Please note that the bibliography for this section of text follows the list of references and appears on page S89.

TABLE 2
Less Common Allergenic Foods and Food Groups[a]

Food	Symptoms (see end of table for list)	Age Years	Oral challenge	Skin Test	RAST	Other assays	Ref.
Abalone	A, CO, DY, FL, PR (with exercise)	19		1+	0+	PH 1+ (after exercise)	51
		16–42		5+	7+		39
Acacia gum	I			1+			196
	TT, U, W	39		1+			252
Allspice	CD, DM, EX, PM	11–87		26+			166
Amaranth	A, AE, BR, H, U			1+	1+		147
Amaranth dye	AE *or UC*		Single; 2+				148
	AS		Double; 0+				245
	AE, UC		Open; 5+				150
Anise		48			1+		88
						IB 1+	129
				46+	23+		213
	AE, DY	26	*Open; 1+*	1+			214
						IB 1+	231
Annatto	AE, UC		Open; 15+				150
	AE, UC	8–72	*Single; 10+*				106
	A, AE, H, PR, U	62					169
Apple	AE, CJ, I (hands), RN			8+			5
				20+	18+		20
		4–18		157+			55
	AE, I (oral, palms), RN			39+			83
	I (eyes, nasal), LE, W	48			1+		88
	AE, BR, D, H, RN, U, V	>10	Double; 2+	9+			117
		6–41		51+ ("Apple")			121
				32+ (Granny Smith)			
				34+ (Sturmer)			
				16+ ("Punkanoli")			
	AE, I (oral)	10-61		36+	32+		177
	D, DI, H, N, V	13		1+	0+	PK 1+	187
	I (hands), OI, SW (hands)	24		1+			228
	A, AE, AS	25		1+	1+		237
	AE	28		1+			247
	AE, DY, PR (palms), U (with exercise)	12	Open; 1+	1+	1+		9
Aspergillus niger	HA, N, V	28	Open; 1+				92
	HA	50					92
	AS	27	Open; 1+				92
Avocado	AC, BR, U				1+		42
	BR			1+	1+		125
Balsam of Peru	EX(hands)			1+ (patch)			116
				1+ (patch)			132
	AE, AS, D, U, V	24–62		6+ (patch)			222
	EX		Open; 9+				232

TABLE 2 (continued)
Less Common Allergenic Foods and Food Groups[a]

Food	Symptoms (see end of table for list)	Age Years	Oral challenge	Skin Test	RAST	Other assays	Ref.
Banana	AE, D, I (throat), U, V, W	5–75		6+			6
	CU, GI, RC		Double; 1+				26
	AC, BR, U	30			1+		42
	A, AE, I (mouth), D, RN, S, U	17–32		3+	0+	HR 2+	63
	AE	28		1+			66
	A, AE, DY, U	53, 56		2+	2+		72
	A, U	67		1+	1+		125
	A, AE, I (throat), U, V, W	15		1+	1+	PK 1+	126
	AE, LV, PP, RN, W	56	Open; 1+	1+	1+	IB 1+	155
	AE, I (oral)	10-61		2+			177
	AE	44	Open; 1+	1+			158
	AE, LV	28, 30		2+	2+	HR 2+	190
	A, AE, DY, U	32		1+	1+		205
	I (pharyngeal), RN	33				EL 1+	239
Barley				3+	3+		53
	A, EX, W	10	Double; 1+				101
	AS, GI, U				8+		217
	AE, PR, VC (with exercise)	16		1+	1+	BC 1–	12
Beans	AS	20-22		2+	0		73
Garbanzo	A, AE, W	39		1+			78
	AS, CO, DY, RN	20		1+	1+	BC 1+	140
Green	AD, AS, RN		Double; 0+	9+			17
		20		1+	1+	HR 1+	140
	I (eyes), U	42	Double; 1+	1+			180
	AC, AD, AS, N, RN, V	0.33–24		7+			202
	I (eyes, nasal), RN, S	46		1+	1+	BC 1+; HR 1+	182
Kidney		23			1+		113
Lima	H, LE, W		Double; 0+	13+			17
Pinto	A, AE, U	37		1+		HR 1+	78
Sprouts — taugeh	AE, U	22		1+	1+	HR 1+	229
Beef	DY, H, LE, W	3–27		2+			199
	AD, CU, GI, RC	0.5–25	Double; 9+				200
	AD, AP, AS, N, RN, V	0.33–24	Double; 2+	18+			202
	AD, CU, GI, RC	0.75–24	Double; 0+	4+			35
Cooked	CMA		Double; 3+				246
Less cooked	CMA		Double; 3+				246
Beer	I (facial), U			1+			227
Beta-carotene	AE, UC	8–72	Single; 10+				106
Broccoli		21		1+			21
Buckwheat	A, I (throat), TP, U	38		1+	1+		48
	A			1+		EL 1+, IB 1+	162

Cabbage	A, AE, DY	21		1+	1+		21
				1+			178
Caraway	AD, GP, RN	1–47		31+	5+		167
Cardamom	CD, DM, EX, PM	11–87	Single; 1+	4+			166
Carrot		4–18		150+			55
	AE			20+			83
	I, TS, W	48		1+	1+		88
				2+	2+		90
	LE, OAS	19–20		2+		EL 2+	105
	I, SW	24		1+			228
		34		1+			115
	AE, BR, D, H, N, RN, U, V	>10	Double; 3+	9+	1+		117
		6–41		46+			121
	BR, LE					IB 1+	129
	A, RCJ, U	41		1+	1+		159
	AE, I (oral)	10-61		13+	6+		177
		11–50		28+			250
	A			1+			250
Cassia				10+ *(patch)*			132
		52		1+ (patch)			54
Cassia oil	OI, PU (lips), ST	39		1+ (patch)			209
Cauliflower		21		1+			21
		child		1+			178
Celery	AE	50		1+			19
	C, H, TN, U	55	Open; 1+	1+			66
	I, LE, TN, W	48			1+		88
	A, GI, LE, OAS	19–53		4+		EL 4+	105
	A, AE, U	18–55		14+		PK 2+	114
	AE, P	22			1+		124
	BR, LE					IB 1+	129
	A, AE, RC, U	14–49		20+	17+		183
	A, AE, RCJ, U	14–49		9+	9+		184
	A, H	66		1+			193
				70+			213
	AE, DY, H, LE, U	34	Open; 1+	1+			214
	A, EX, U				7+		230
	AE, DY, H, LE, U	23	Open; 1+	1+			214
				36+	36+	IB 21+	224
	A	50		1+	1+		234
	A, AE, AC, RN, U	27–53		70+	70+		251
	AC, AE, U	28		1+	1+		103
	AC, AE, DI, I, PR, U, W, WE (with exercise)	20-39		3+			115
	AE, H, PR (with exercise)	23		1+			210

TABLE 2 (continued)
Less Common Allergenic Foods and Food Groups[a]

Food	Symptoms (see end of table for list)	Age Years	Oral challenge	Skin Test	RAST	Other assays	Ref.
Celeriac (celery root)				31+			250
Raw		27–53		66+			251
Cooked		27–53		25+			251
Celery salt				25+			250
Chamomile (tea)	A, CO, I (skin), V	8		1+		PK 1+; EL 1+	216
Cherry	AE, I (oral)	10–61		13+			177
Chicken	CU, GI, RC	0.25–19	Double; 2+				26
	AD, CU, GI, RC	0.75–19	Double; 1+	4+			35
	AD, AP, D, N, S, V, W	0.6–19	Double; 1+	1+			36
	A, EX	2.5	Open; 1+		1+		47
	AD, EX	4	Open; 1+				47
					3+		91
	A, DY, U	29		1+	1+	HR 1+	189
	AD, AP, CU, D, N, RN, S, V	1.3–19	Double; 2+	6+			198
	AD, CU, GI, RC, W	0.5–25	Double; 6+				200
	AD		Double; 2+	1+	2+		201
	D	0.11	Open; 1+				235
	DY, H, LE, W	3–27	Double; 2+				199
	AD, AP, AS, N, RN, V	0.33–24	Double; 3+	19+			202
	AE	39		1+	1+		238
Chocolate	CO, FL, HA, NS, U, V	17–58	Double; 5+;	3+			56
	AD		Open; 0+	0+			60
	AC, I (throat, nasal), P, S, U, V, W	4–20		18+			69
	AE, BR, D, H, RN, U, V	>10	Double; 2+	17+			117
	AE, N, S, U, W	4–60	Double; 3+	3+			142
	AP, CU, D, N, RN, S, V, W	1.33–19	Double; 1+	0+			198
	AS, AP, AS, N, RN, V	0.33–24		4+			202
			Double; 4+	0+	0+	HR 1+	256
Cocoa	CU, GI, RN	0.75–19	Double; 0+	1+			35
	AE, AS, D, DM, RN	1–80	Open; 14+	12+			168
	AD, CU, GI, RC	1.33–19	Double; 10+	13+			198
	DY, H, LE, W	3–27	Double; 42+				199
	AD		Double; 15+	11+	12+		201
	AD, AP, AS, N, RN, V	0.33–24	Double; 44+	55+			202
	AD, AS, RN	3–18	Double; 45+	59+			203
	AD, CU, GI, RC, W	0.5–25	Double; 106+				200
Cinnamate — methyl				6+ (patch)			152
Cinnamate — benzyl				11+ (patch)			152
Cinnamic acetate				3+ (patch)			132

Cinnamic acid				26+ (patch)			152
Cinnamic aldehyde	ST	52		1+ (patch)			54
	EX(hands), CD			3+ (patch)			116
				15+ (patch)			132
				14+ (patch)			152
Cinnamon	CD			117+ (patch)			166
Cinnamon leaf oil				1+ (patch)			132
Cinnamon oil				2+ (patch)			132
	ST	22		1+ (patch)			123
	BU, G, OD			3+ (patch)			151
	EX(hands), CD			*2+ (patch)*			*116*
Cinnamyl alcohol				29+ (patch)			152
Citral	DM	52		1+ (patch)			38
Clams	A, AE, FL, PR, U (with exercise)				1+		143
	AC, CJ, N, U	33	Double; 1+	1+	1+		180
Clove	CD, DM, EX, PM	11–87		36+			166
Clove oil	OI, PU (lips), ST	39		1+ (patch)			209
Coconut				14+			71
Coffee (instant)	FC, OI	47		1+			212
Coriander	A, SW, U, W	14	Double; 1+	1+			24
		48			1+		88
	AD, GP, RN			29+	5+		167
				26+	19+		213
	AS, EX				12+	HR 9+	230
					4+	IB 4+	231
Corn (see maize)							
Cottonseed	AE, BR, D, H, N, U, V	9–79	Double; 2+	6+			15
	A			1+			134
	AE, I, OE, U	29		1+	1+		133
	A	58		1+	1+		176
Cucumber	AE, I (lips, tongue, throat)	12–71				IB 43+	59
	A, GI, LE, OAS	19–53		3+		EL 3+	105
	AE, BR, D, H, RN, U, V	>10	Double; 0+	32+			117
Pickles	*PR*	*1.5*		*1+*			*57*
Cumin					1+		88
				24+	11+		213
Curry	CD, DM, EX, PM	11–87	Single; 3+	6+			166
	EX, U				12+	HR 9+	230
					4+	IB 4+	231
Cuttlefish	A, AE, GI, TN, U		Double; 1+	1+	1+		208
Dill				1+			115
Ethanol	PU	60	Open; 1+	0+			2
	U	28	Open; 1+	0+			112
Fennel	AE, I (oral)	10-61	Open; 4+				177
				28+	24+		213
	A, AE, U (with exercise)	19		1+	0+		192
Flax seed	AE, H, U	43	Open; 1+	1+			214
Garlic	D, FL, N, OA, W	30	Double; 1+	1+	1+	BC 1+	130
Gelatin	AD, U	32		1+	1+	IB 1+	240
Ginger	CD, DM, EX, PM	11–87		7+			166
				3+	13+		213
					1+	IB 1+	231

TABLE 2 (continued)
Less Common Allergenic Foods and Food Groups[a]

Food	Symptoms (see end of table for list)	Age Years	Oral challenge	Skin Test	RAST	Other assays	Ref.
Grapes	A, CO, FL, NS, PR (with exercise)	15		1+	0+	PH 1+ (after exercise)	51
	A, U, W (with exercise)	24		0+			33
Red	A, EX	4	Open; 1+				47
Grapefruit seeds				2+			188
Guava	I (*pharyngeal*), *RN*	33				EL 1+; IB 1+	239
Honey	D, DY, U	35		1+	0+		128
Forest		16–78		10+	7+		89
Rape		16–78		10+	6+		89
Dandelion		16–78		17+	14+		89
Sunflower	AP, D, V	24		1+	1+		29
	A	50		1+	1+		19
Royal jelly	A, BR, D	11					34
Hops	AE, DY, H, LE, U	23	Open; 1+	1+			214
Karaya gum				1+ (erythema only)			252
Kiwi	A, AE, I (eyes, throat)	26		1+			61
	OAS	32		1+			205
	I (ears), eyes), RC, U	53		1+		PK 1–	64
	AE, D, PS			10+	10+		74
	OPP			12+	0+		74
	OAS, PR, U, V	26		1+		EL 1+, HR 1–	75
	U			1+	1+		76
	A			3+	3+		104
	B, CL, PR	30	Open; 1+	1+			233
				3+			236
	AD, AE	26		1+	1+		237
	AE, GP, I (tongue), U	20	Open; 1+	1+			258
Lemon	DM	52		1+ (patch)			38
Lettuce	A, AE, U	20		1+			192
	A			2+	2+		90
Eruca sative	I (OM), WH	43		1+	1+		186
Lime	DM	52		1+ (patch)			38
Limpet	A, AE, CH, U	3–27		5+	5+		157
Lentils	DY, V	10		1+			70
	AS	20-22		2+	0+		73
	CO, DY, RN			1+	1+	HR 1+, BC 1+	140
	AS, RN	20				BC 1+	139
		46		1+	1+	HR 1+	182
Lupine				5+	4+		87
	AE, U	5	Open; 1+	1+	1+		87
Mace	EX			12+		HR 8+	230

Maize	CU, GI, RC	0.75–19	Double; 0+	2+			35
	AE				1+		95
						PK 9+	96
	AD, AP, N, RN, V	0.33–24		7+			202
	A	0.71	Open; 1+		0+		47
Maize syrup						PK 3+	96
Maize dextrimaltose						PK 4+	96
Maize invert sugar	U	30	Open; 1+				171
Maize GF sugar		30	Open; 1+	1+		HR 1+	171
Maize isomerized dextrose	U	30		1+			171
Maize o-psicose	*U*	*30*		*1+*		*HR 1+*	170
Malt	I (facial), U			1+			227
Maple syrup	ER, PR, U			1+			18
Mango	AC, AE, SW (face), U	28		2+	2+		103
	A, AE, W	24					43
	A, I (eyes), I (mouth), PR, RC	32					195
	A, DY, ER, U	32		1+	0+		149
Melon	AE, D, I (throat), N, U, V	5–75		6+			6
	AE, I (oral)	10-61		3+	0+		177
Watermelon	AD, DY, LE, OAS, U	20-36		3+		EL 3+	105
	OAS	14–67				EL 6+, IB 6+	58
Millet seed	A, AE	25		1+	1+	HR 1+	179
Mushrooms	AE, H, LE, U	31	Open; 1+	1+			214
Ramaria flave	A(1)				1+		118
Shiitake	ER, F	41		0			218
	DM	15–76					163
Mycoprotein ("Quorn")	D, V			2+	2+		221
Mussel		16–42		2+	1+		39
Mustard		21		1+			21
					10+	HR 4+, EL 4+	80
		39		1+			115
	EX	40		1+			146
	A, AE, RC	17		1+	1+		154
	A, AE, N, RC	14		1+	1+		154
	AD, GP, RN	1–47		29+	5+		167
	SW (glottis), U	Child		1+	1+		178
	AE, H, LE	43	Open; 1+	1+			214
	A, AE, U	25			1+		248
Black		Child			1+		178
White		Child			1+		178
Seed	A, AE, U				7+	HR 2+	52
	AE, AS, DY, I (nasal), S, U	31		1+	1+	HR 1+	135
	AE, AS, I (scalp, genitals), U, V	32		1+	1+		135

TABLE 2 (continued)
Less Common Allergenic Foods and Food Groups[a]

Food	Symptoms (see end of table for list)	Age Years	Oral challenge	Skin Test	RAST	Other assays	Ref.
Nutmeg				3+	1+, 14+ flower		213
Oats				3+	2+		53
	AE, U				0+		95
	AD, AP, AS, N, RN, V	0.33–24		7+			202
	AS, GI, U				9+		217
Orange	DM	52		1+ (patch)			38
	AE, BR, D, H, RN, U, V	>10	Double; 6+	21+			117
	AE, I (oral)	10–61		11+	3+		177
	A, AE, DY, I, U	49					249
	A, AE, AS, GI, LE, N, RC, U, V	25–61	Open; 0+ (juice)	0+ (juice)		PK 1+ (seed)	257
			Open; 3+ (seed)	3+ (seed)			257
				2+ (seed)			188
Mandarin	I (pharyngeal), RN	33				EL 1+, IB 1+	239
Orange juice	*AC, AE, DY, N, U*	*33*		*0+*			*78*
						PK 20+	188
Oysters	AE, FL, PR, U (with exercise)				1+		143
		16–42		2+			39
Papain	AE, E, DY, I, W	31	Single; 1+	1+		PK 1+	137
	AP, CJ, D, U		Double; 5+	5+	5+		138
Chymopapain					6+		197
Paprika	CD, DM, EX, PM	11–87		4+			166
	AD, GP, RN			22+	6+		167
Parsley	I (nasal, eye), LE, TS, W	48		1+			88
	A, AE, U	18–55		12+			114
Pea	AD, AS, RN		Double; 2+	18+			17
		0.25–19	Double; 5+				26
	DY, V	10		1+			70
	AS	20-22		2+	2+		73
		20		1+	1+	HR 1+	139
	AE, AS, D, DM, RN	1–80		42+			168
	AE, I (oral)	10–61		5+	4+		177
	AC, AD, AS, N, RN, V	0.33–24		19+			202
Chickling		39		1+			98
Peach	AE, AS, GI, RN, U			69+		HR 58+	3
	DY, N	30			1+		42
	AC, U	28		1+	1+		103
	AE, BR, D, H, RN, U, V	*>10*	*Open; 6+, Double; 43+*	*65+*			117
	CU, OAS	16–27		25+ (flesh)			127
				22+ (skin)			127
	AE, U	14	Double; 0+	1+	0+		128
				8+	8+	IB 3+	220

	A, I (pharyngeal), RN	33				EL 1+	239
	A, AE, D, GI, U (with exercise)	24		1+			33
Peanut oil	AD, AE (with peanuts)	0.5	Open; 1+				153
	AE, AS, D, H, LE, N, V (with peanuts)	17–45	Double; 0+	0+			219
	AD, AE, ER (with peanut oil)	0.5	Open; 1+	1+	1+		153
	AD (with peanut oil)	0.33	Open; 1+	1+	1+		153
	EX (with peanut oil)	"infant"	Open; 1+	1+			85
Pear	AE, I (oral)	10-61		6+			177
	I (hands), OI, SW (hands)	24		1+ raw; 0+ cooked			228
Pepper		48		1+			88
Cayenne	AD, GP, RN	1–47		20+	7+		167
Red				11+	16+		213
White	CD, DM, EX, PM	11–87		4+			166
	EX				12+	HR 2+	230
	AD, GP, RN	1–47		4+	3+		167
				5+	15+		213
Pineapple	A, D, I, V	5–70					107
Plum	A, EX	4	Open; 1+				47
Pomegranate	AE, TS	85	Double; 1+	0	0	HR 1–	97
Poppy seed	AE, RC, U, V	20		1+	1+		31
	AE, AS	27		1+	1+		110
	A, AD, AE, AS, D, P, U, V	11–33		5+	5+		237
Pork	AD, CU, GI, RC	0.75–19	Double; 0+	3+			35
	AD, DY, H, LE, W	3–27	Double; 1+				199
	AD, AP, CU, D, N, RN, S, V	1.33–19	Double; 0+	7+			198
	AD, CU, GI, RC		Double; 3+				200
	AD, AP, AS, N, RN, V	0.33–24	Double; 2+	32+			202
Potato	AE, RN			24+			5
	A, AE, AS, H, S, U, V	11		1+	1+	HR 1+; PK 1+; IB 1+	40
		4–18		139+			55
	AE, DY, W	17		1+		HR 1+	78
	AE			2+			83
	RN, S, TP	24		1+		PK 1+	164
	AE, OAS	10-61		7+	3+		177
	AE, AS, U	21		1+		PK 1+	185
	CU, GI, LE, NS	0.5–25	Double; 4+				200
	AP, D, N, NS, RN, S, V, W	1.33–19	Double; 1+	2+			198
	DY, H, LE, W	3–27	Double; 1+				199
	AD, AP, AS, N, RN, V	0.33–24	Double; 2+	7+			202
	GI, U	1–16			12+	IB 5+; HR 8+	241
	EX	0.71	Open; 1+				47
Psyllium	A, I (eyes), V	43		1+			10
	A, AC, CO, D, H, I (eyes), N, V, W	27–73			18+	IB 20+	102
	A, AE, CO, I (mouth), V	43		1+			111
	A, AE, TC, U	60			1+	HR 1+	122
	AS, RN	28		1+			194

TABLE 2 (continued)
Less Common Allergenic Foods and Food Groups[a]

Food	Symptoms (see end of table for list)	Age Years	Oral challenge	Skin Test	RAST	Other assays	Ref.
Rape		40		1+			146
Rice	A, D, V	0.75	Single; 1+				28
	A, AC, AE, DY, PR	21		1+		HR 1+	78
	GI, NS	55	Open; 1+	1+ raw, 1+ cooked	1+ raw, 0+ cooked	HR 1+, IB 1+	160
	AD, AP, AS, N, RN, V	0.33–24		8+			202
	AS, EX	5–21		6+	6+		207
	D, FL, V	2			1+	IB 1+, PK 1+	215
	AD, AS					HR 17+, IB 32+	223
	QE	25		1+			226
	D, V	0.5	Open; 1+				235
					4+		242
	DM				10+		243
Rye	CU, GI, RC	0.25–19	Double; 1+				26
				3+	3+		53
	AD, AP, CU, D, N, RN, S, V, W	1.25–19	Double; 1+	2+			198
	AD, AP, AS, N, RN, V	0.33–24		8+			202
	AS, GI, U				10+		217
	AS, DM, RN	12–46		2+	8+		237
	AE, PR, VC (with exercise)	16		1+	0+		12
Sesame seed	A, AE, B, FL, I (general), RD, TN, U, W	30			1+		99
	A, AE, D, E, N, U, V	20-38		5+	5+		108
	A, AE, H, PP, U	31–72			3+		136
	AE	45		1+	1+		237
	AS	6–17					244
Single cell protein	D, N, V		Open; 7+		0+		206
	D, N, V		Open; 2+		0+		206
Soybean oil	AE, AP, LE, RC, RN, U, W (with soybeans)	18–63	Double; 0+	0+			37
Spinach		20		1+	1+	HR 1–	49
Squash	CU, GI, RC	0.25–19	Double; 1+				26
Squid — Raw		16–42		0+	1+	BC 1+	39
Boiled	AE, AS, CJ, D, N, U, V	16–42	Double; 0+	7+	7+		39
Strawberry	AC, AD, AS, N, RN, V	0.33–2		1+			202
	I (pharyngeal), RN	33				EL 1+	239
Sugar beet		20		1+	1+	HR 1–	49
Sunflower seed	AE, BR, D, H, RN, U, V	>10	Double; 25+	52+			117
	A, AE, DY, N, PR, U, V, W	29–37	Open; 0+ (oil)	2+	2+	PK 1+ (seed)	82
	A, AE, GP, I (lips)	11–58		3+	3+		174
	AE, H, LE	34	Open; 1+	1+			214
		13	Open; 0+ (w/exercise)	1+			141

Sunflower oil	A, AE, DY, N, PR, U, V, W (with sunflower seeds)	29–37	Open; 0+ (oil)	2+	2+	PK 1+ (seed)	82
Swiss chard	AS, RCJ	20	Open; 1+	1+	1+	HR 1+	49
	I (eyes, nasal), RN, S	46		1+	1+	BC 1+; HR 1+	182
Tangerine	AC, AE, DY, N, U	33		1+			78
Tangerine seeds				2+			188
Tomato	AE, AS, DM, U	12–45		9+			22
	DM, I (lips)			7+			83
	AE, BR, D, H, RN, U, V	>10	Double; 2+	18+			117
	EX, U			1+			144
	AE, I (oral)	10-61		12+	5+		177
	AD, AP, AS, N, RN, V	0.33–24		5+			202
	DM, I (throat, lips)			4+	9+		50
	A (with exercise)	20–39		2+			115
Tragacanth gum	AS, U	33	Open; 1+	1+		PK 1+	32
	AE, AP, DY, PR	35		1+			44
	I			1+			196
		39		1+ (erythema only)			252
Turkey	CU, GI, RC	0.25–19	Double; 1+				26
Turnip				1+			178
Vanillin				3+			166
				8+ (patch)			152
Vitamin A	AD, V	0.75	Double; 1+	0+			81
Vitamin E	DM	21		1+ (patch)			191
Wine	PU	60	Open; 1+				2
	A, AE, U	31		1+	0+	PK 1–	41
Yeast	U		Double; 5+				100
	AE, U	34	Open; 1+ (wine)	1+			112
	U	33	Open; 1+ (wine)	1+			112
	AS, EX, RN	6–51		39+			119
	AE, NS, PR, U	15–66		7+			131
	AS	15		1+			225
Saccharomyces cerevisae	AD, EX			28+			109
Zucchini		12–71				EL 62+	59

[a] This table was compiled using literature searches of the Agricola (1972 to July 1994) and Medline (1966 to July 1994) databases.

Abbreviations: **A**, anaphylaxis; **AC**, abdominal cramping; **AD**, atopic dermatitis; **AE**, angioedema; **AP**, abdominal pain; **AS**, asthma; **B**, burning mouth or lips; **BC**, bronchial challenge; **BR**, bronchospasm; **BU**, buccal ulceration; **C**, chills; **CD**, contact dermatitis; **CH**, choking; **CJ**, conjunctivitis; **CL**, Colic; **CMA**, cows' milk allergy; **CO**, cough; **CU**, cutaneous complaint; (see banana) **CY**, cyanosis; **D**, diarrhea; **DI**, dizziness; **DM**, dermatitis; **DS**, difficult swallowing; **DY**, dyspnea; **E**, edema; **EL**, enzyme linked immunosorbent assay; **ER**, erythema; **EX**, eczema; **F**, fever; **FC**, flatulence; **FL**, flushing; **G**, gingivostomatitis; **GI**, gastrointestinal symptoms; **GP**, gastric pain; **H**, hypotension; **HA**, headache; **HR**, histamine release assay; **I**, itching; **IB**, immunoblotting; **LE**, laryngeal edema; **LV**, lost voice; **M**, migraine; **N**, nausea; **NS**, nasal symptoms; **OA**, occupational asthma; **OAS**, oral allergy syndrome; **OD**, oral dermatitis; **OE**, oral edema; **OI**, oral irritation; **OM**, oral mucosa; **OPP**, oropharyngeal pruritus; **P**, palpebrae (eyelids); **PH**, plasma histamine; **PK**, Prausnitz-Kustner test; **PM**, pompholyx; **PP**, pharyngeal pruritus; **PR**, pruritus; **PS**, pharyngeal swelling; **PU**, purpura; **QE**, Quincke's edema; (see rice) **RAST**, radioallergosorbant test; **RC**, respiratory complaints; **RCJ**, rhinoconjunctivitis; **RD**, respiratory distress; **RN**, rhinitis; **S**, sneezing; **ST**, stomatitis; **SW**, swelling; **T**, elevated tryptase; **TC**, tachycardia; **TN**, tongue swelling; **TP**, throat pain; **TS**, throat swelling; **TT**, throat tightness; **U**, urticaria; **UC**, chronic urticaria; **V**, vomiting; **VC**, vascular collapse; **W**, wheezing; **WE**, weakness; **WH**, wheal.

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Principles and Characteristics of Food Allergens

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I. INTRODUCTION

A. Difficulties Investigating Food Allergens

Much remains to be learned about food allergy. The frequencies, severity, and variety of symptoms caused by foods are controversial, and a precise definition of food allergy is still lacking. Many food-induced sensitivities persist for years, whereas others do not. The target organ specificity of many food allergies is poorly understood and variable from patient to patient. Some foods are more common in provoking allergic reactions than others that are very similar. Still unresolved is whether other mechanisms, in addition to immunoglobulin E (IgE) antibodies, are important in food allergy. The diagnosis of food allergy is still difficult because many food allergen extracts used for skin testing and IgE antibody determinations are neither well characterized nor well standardized. Even the gold standard for the diagnosis of adverse reactions to foods, the double-blind placebo-controlled food challenge (DBPCFC), is only as accurate as the activity of the food material used and the circumstances of the challenge test.[1,2]

What are food allergens? There have been several immunologic mechanisms implicated as pathophysiologic mechanisms in food allergy. However, IgE-mediated reactions are believed to be responsible for most, if not all, food-induced allergic reactions of the immediate hypersensitivity type.[2] Food allergens are defined as those food component(s) that induce the production of, and react with, IgE antibodies to cause mediator release from mast cells and basophils resulting in immediate hypersensitive reactions. This is a very broad definition that includes not only ingested food allergens, but also food aeroallergens and contact food allergens.

Through application of both classical immunochemical methods and recently developed recombinant DNA technologies, considerable knowledge has been obtained about allergen structure and identification of allergenic determinants.[3,4] Our knowledge of the structure of allergens will continue to increase rapidly primarily due to the cloning and sequencing of allergens. These methods will also provide large amounts of allergenic materials for clinical use.[3–5]

B. Special Challenges That Food Allergens Present

Although most food allergens are similar to other allergens that are present in our environment, there are a number of unique features that may make them somewhat different from other allergens. First is exposure. Clearly the majority of exposures to food allergens occur through the gut whose primary function is to process ingested food into a form that can be absorbed for energy and cell growth. This organ presents a rather unusual exposure environment containing a variety of hydrolytic enzymes, acids, and bile salts that can substantially affect the structure of most molecules.[6] However, some food proteins remain immunologically active, pass through the epithelium, enter the circulatory system, and are distributed throughout the body.[7–14] The factors that affect food digestion and result in absorption of intact allergens require better definition. Further-

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more, the digested allergen may be different from the form that was originally ingested. This further complicates establishment of a cause-and-effect relationship necessary for allergen identification.

The gut-associated lymphoid tissue (GALT) is unique in that it must mount a rapid response to harmful foreign substances while remaining unresponsive to enormous quantities of nutrient antigens. GALT is composed of Peyer's patches and the appendix, lymphocytes and plasma cells of the lamina propria, intraepithelial lymphocytes, and mesenteric lymph nodes.[15] It may respond differently from peripheral lymphoid tissue in the spleen and lymph nodes from which most of our information concerning the immune response is obtained. Food antigens in such an environment may have different properties, compared with conventional antigens.

Exposure to foods also occurs through other routes such as through contact (skin) and inhalation (lungs). This has been well described in occupational reactions in food industry workers.[16] The relationship of food allergic responses induced through inhalation to those stimulated through the gut and the similarities and/or differences of allergens that induce such responses need to be studied. There is also a need to establish the relationship of inhaled allergens to ingested allergens with regard to food allergy pathogenesis, and to determine other significant factors about food allergies such as threshold levels of sensitization for provocation, important in terms of establishing parameters for regulation.[17]

C. Complexity of Foods

Food is a complex mixture containing a variety of allergenic and nonallergenic components. Identifying, purifying, and characterizing the allergens in food can be a major challenge. Most known food allergens are soluble in aqueous solvents; there is essentially no information on allergenic components that are not soluble in aqueous solvents. As noted above, digestive processes can also affect food allergens. The nature of food allergens that actually interact with the GALT may be quite different from the form that was ingested with foods.

Some food allergens may be present in trace amounts, yet may be major allergens. Purifying and characterizing such trace allergens can be especially difficult. Because of the exquisite sensitivity of some allergic consumers, allergens may be present in harmful amounts even as trace contaminants. This situation can easily lead to mistaken conclusions regarding the allergenicity of specific foods.

Significant allergenic cross-reactivity has been described among foods from closely related sources as well as between foods and seemingly unrelated or remotely related materials with essentially no phylogenetic relationship.[18,19] Legumes[19,20] and crustacea[19] are examples of foods that cross-react within the same family. However, the mere fact that members of these food families cross-react does not necessarily mean that these reactions are clinically significant. The substantial cross-allergenicity for crustacea appears to be clinically important,[21,22] but cross-reactivity among the legumes does not appear to be clinically significant.[23] These observations further complicate our understanding of food allergen reactivity.

In an attempt to organize allergens with regard to patient reactivity, the following classification system has been developed to better describe allergens. Allergens to which the majority of patients react are described as "major allergens," compared to other allergens, or "minor allergens," to which a minority of patients react[24] (see Article 7). Generally, although not always, major allergens tend to be predominant components present in allergen extracts. There have been several attempts to define major allergens based on the number of individuals who react with these materials, or on the intensity of the biological reaction.[25–27] Although major allergens are generally of greatest interest, we cannot ignore minor allergens, which also sometimes cause serious reactions in sensitized subjects (see Article 7).

Some extracts contain allergens, which although having similar physical, chemical, and immunochemical structures, differ slightly from one another in isoelectric point.[24] The term *isoallergens* has been used to denote such molecules isolated from the same source. The slight differences observed in these allergens are due to minor structural differences in the carbohydrate

moiety of the molecule (degree of glycosylation), differences in degree of protein amidation, or genetic variation.[28]

II. PROPERTIES OF FOOD ALLERGENS

A. General Characteristics of Allergenic Food Proteins

Foods contain a variety of different proteins, only a few of which are known allergens. In plant foods such as soybeans and peanuts, many of the allergenic proteins are storage proteins[29-31] that exist in large amounts in some food. It might be tempting to conclude that the likelihood of allergenicity is correlated with the extent of exposure to a particular protein. However, proteins present in less significant or even minor amounts also serve as major food allergens. For example, *Gad c* 1, the major allergen in codfish, is not a particularly prominent protein.[32] Also, many major proteins such as actin and myosin in beef, pork, and chicken are not allergenic. Although the dose of exposure is likely a critical factor, the immunogenicity of the protein is clearly more important.

As with all antigens, to stimulate a significant immune response, food allergens must be foreign molecules to the host, because molecules of host origin are not normally recognized by the immune system. Most food allergens are highly stable molecules that are resistant to food processing and cooking, and the digestive processes.[33] There are, however, exceptions such as the labile allergens present in apple and other fresh fruits and vegetables.[34,35] Generally, such labile allergens cause reactions that are restricted to the oral mucosa, as they lose allergenic activity after degradation. Conversely, digestion may alter antigens by releasing or uncovering neoepitopes that induce allergic reactions, although this possibility has not been well investigated.

Historically, mucosal permeability has been thought to be a major factor in food allergy. Many food proteins cross the gut mucosal membranes, prompting immune responses even in normal individuals.[13,36] However, differences in mucosal absorption of allergens have not been well studied in food-allergic individuals, compared with normal individuals; the relationship of the phenomenon of gut permeability to food allergy has not been resolved either. This is a particularly important issue in infants prior to mucosal gut barrier maturation when large molecules more easily pass through the gut epithelium and induce allergic sensitization.[6]

Finally, there is the nature of the food protein being ingested. Some foods that are common components in our diets are not very allergenic. For example, beef and pork are important foods in the American diet that have a high protein content. Yet, beef and pork are not common causes of food allergy, compared with fish and shellfish, which are major allergenic foods. This is particularly intriguing because beef and pork also contain the muscle protein tropomyosin, a molecule identified as the major allergen in shrimp.[37-40] Although chicken and shrimp tropomyosin share significant (60%) amino acid sequence homology, the allergenic reactivity differs considerably. Shrimp tropomyosin and presumably tropomyosins present in other crustacea are very potent allergens, whereas tropomyosins present in beef, pork, and chicken are not. Structural differences that contribute to or diminish the allergenicity of a molecule are important topics for further research.

B. Biochemical and Immunochemical Characteristics of Allergenic Food Proteins

Because all allergens must be able to bridge IgE molecules on the surface of mast cells to cause degranulation, they are somewhat constrained in their molecular dimensions. Thus, these allergens must contain at least two IgE antibody-reactive sites (B-cell epitopes) to trigger mediator release. However, it is noted that some monovalent allergens, such as the 21-residue venom peptide mellitin,[41] still elicit histamine release from basophils or mast cells or generate anaphylaxis in mice. These molecules have the ability to bind to IgE antibodies on the surface of basophils/mast cells. They aggregate, or aggregate and then bind, converting the monovalent allergens to polyligands

that can cause allergic reactions. They also act as haptens by binding to macromolecules and subsequently as complexes that cause IgE bridging. It has not yet been determined that this occurs *in vivo* in allergic disease and is a substantial factor in allergic reactions.

Most known food allergens have mol wt between 10,000 and 70,000 Da.[19] Although smaller molecules may be immunogenic, the mol wt of 10,000 Da probably represents the lower limit for the allergenic response. The upper limit is probably a result of restricted mucosal absorption of larger molecules.[19] However, some allergens such as the peanut allergens *Ara h* 1 (mol wt 63.5 kDa) and *Ara h* 2 (mol wt 17 kDa),[42,43] exist in native form as large protein polymers that are 200 to 300 kDa in size. It is not clear if such large molecules act as allergens or are disassociated during the digestive process. Most food allergens are glycoproteins with acidic isoelectric points. However, this is a property of most antigens and does not necessarily represent a unique property for food allergens. All allergens are proteins, but not all proteins are allergens. Many allergens contain posttranslational modifications, such as the addition of oliogosaccharides.

The biochemical structure of a few food allergens has been determined (see "Food Allergens" by Robert K. Bush and Susan L. Hefle in this issue). When the biochemical structure of various allergenic proteins is compared, there does not appear to be any consistent pattern that is representative of allergens in general, or food allergens specifically. Comparisons of primary amino acid sequences of allergenic proteins have not revealed unique or typical patterns. There also does not appear to be any particular pattern to the protein tertiary structure, such as an alpha helix, beta strand, or loops.[44] When the primary structure of an allergen is compared to other proteins, amino acid sequence similarities occur with a number of proteins in our environment. In light of the evolution of all living organisms, this should not be surprising, but illustrates that there may be certain as yet undetected structural features of allergens that render them different from other proteins.

The surface of an antigen/allergen that interacts with cells of the immune system or specific antibodies are defined as epitopes. Epitopes that react with T cells are called T-cell epitopes.[45] Those that react with antibody or antibody-producing B cells are called B-cell epitopes.[44]

Epitopes are either conformational or linear. Conformational epitopes depend on the tertiary structure of the protein or several amino acid sequences on the protein surface. Linear epitopes depend on the linear sequence of amino acids in a protein. The general belief is that T-cell epitopes are linear, whereas B-cell epitopes are conformational. However, there are also exceptions to this rule.[44] By definition, all B-cell epitopes must be on an outer surface of the molecule (Figure 1). If an epitope is composed of a series of covalently linked amino acids it is called a continuous epitope. An epitope that is composed of two different amino acid sequences which through their tertiary structure form one epitope is called a discontinuous epitope. Because investigating discontinuous epitopes is technically more demanding, there is generally much more information available on continuous epitopes. The minimum number of amino acid residues is 8 for continuous epitopes,[46] whereas discontinuous conformational epitopes have 16 or more.[44]

T cells exclusively recognize protein antigens that are processed by proteolysis into short peptides. They are recognized by the T-cell receptor (TCR) only after the allergen has been phagocytized by an antigen-presenting cell (APC).[46] By structural analysis with X-ray crystallography, it has been shown that the peptides are set in an extended form in a cleft of the presenting molecules between the α-helices of the α_1 and the α_2 domain.[46] Antigen processing by an APC includes proteolytic digestion of the antigen and presentation of the peptide fragments in the peptide binding groove of the HLA class II molecule on the APC surface. The TCR then responds specifically to the epitope in the major histocompatibility complex (MHC) II groove. Chemical analysis of the peptides eluted from MHC molecules has established that they are usually eight to nine amino acids long.[46] Determination of the complete repertoire of T-cell epitopes of allergens remains a formidable task due to their number and the variability in recognition by different individuals.

Although our knowledge of T-cell epitopes of food allergens is largely extrapolated from

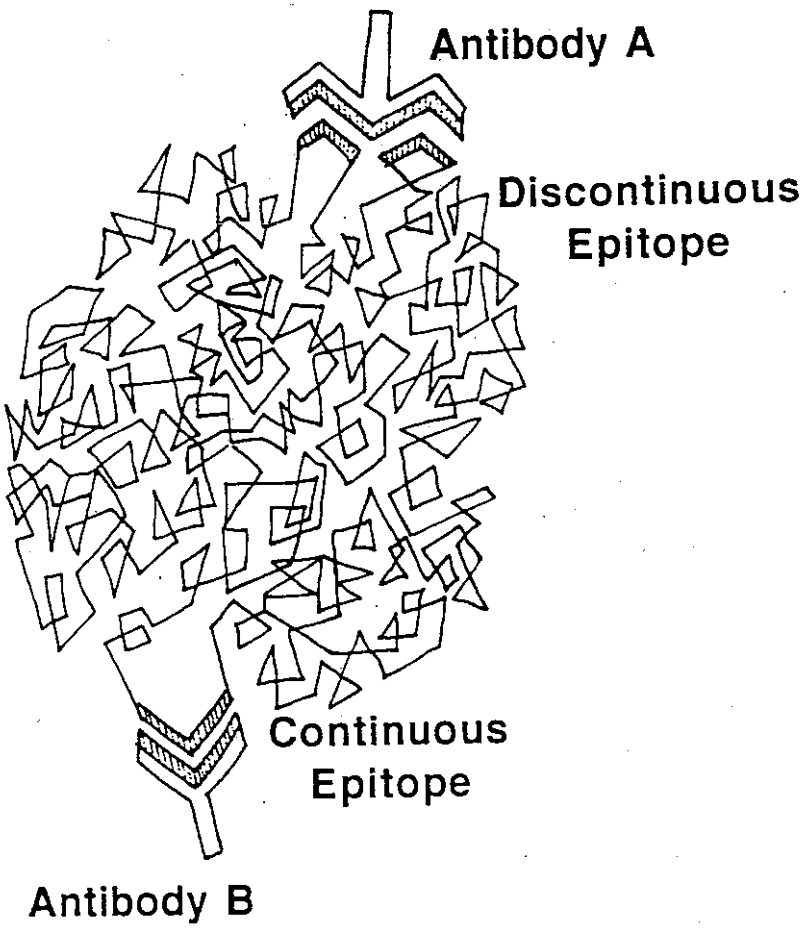

FIGURE 1. The epitope structures of allergens.

nonfood sources, such as pollens, food allergens may have unique properties. Food allergens are recognized by GALT or other lymphatic cells in the body. The immunoregulatory mechanisms of T-cell responses of these systems have not been well defined; thus, there may be unique properties to T-cell epitopes in food allergens. Identification of T-cell epitopes may be achieved using overlapping peptides based on their ability to induce responses in T cells present in peripheral blood mononuclear cells or in T-cell clones derived from individuals sensitized to the native allergen. However, because a substantial amount of blood must be readily available from donors, these data are restricted by the availability of source material. On the basis of inducing nonresponsiveness and in the down-regulation of established immune responses in animals, there is considerable interest in peptides containing T-cell epitopes as a basis for immunotherapy.[47] Immunotherapy with T-cell-reactive peptides is under intense study.

B-cell epitope peptides do not appear to have any unique or common patterns in amino acid sequences (Table 1). Antibodies specific for continuous epitopes invariably have a lower affinity than those for discontinuous epitopes.[44] From the point of view of antibody affinity, most antibodies produced by an allergic person appear to be specific for discontinuous (high affinity) epitopes.[44] Whether this generalization applies to food allergens is not yet known (Table 2). Although there are approximately 8 to 16 residues in an epitope, not every single residue contributes equally to the total binding energy.[44,46]

The entire surface of a protein antigen is covered with potential B-cell epitopes; however, they are all not as equally reactive. In fact, many are not recognized. There are methods to predict B-cell

TABLE 1
Sequence of Some B-Cell Epitopes of Five Allergens

Source	Allergen/residue	AA sequence	Ref.
Ragweed pollen	*Amb a* 3 residue 53–63	TrpArgGluGluValArgAsnGluGluAlaTyr	47a
Mite	*Der p* 1 residue 117–133	CysGlnIleProProAsnAlaAsnLysIleArg GluAlaLeuAlaGln	47b
Bee venom	*Api m* 4 residue 20–26	IleLysArgLysArgGlnGln	47c
Milk	Lactoglobulin residue 124–134	ArgThrProGluValAspAspGluAlaLeuGlu Ala	47d
Shrimp	Tropomyosin residue 153–161	PheLeuAlaGluGluAlaAspArgLys	39

TABLE 2
Continuous and Discontinous B-Cell Epitopes of Some Food Allergens

Source	Name	Antibodies tested	Continuous epitopes	Discontinuous epitopes	Ref.
Egg	*Gal d* 2	IgE + IgG	7		47e
	Gal d 4	IgG		3	47f
Milk	β-lactoglobulin	IgE	1		47d
	α-lactalbumin	IgE	1		
Wheat	Amylase inhibitor	IgE	5		47g
Fish	*Gad c* 1	IgE + IgG	3		47e
Shrimp	*Pen a* 1	IgE	2		39

epitopes, although they are not totally successful. For example, algorithms based on the calculations of polar and nonpolar amino acid residues have been used to predict those residues located on the surface of the molecule. However, this information is incomplete because host responsiveness determines those epitopes to which an individual reacts.

C. Agronomic Considerations

Variations may occur in the type and amount of individual proteins in foods as a result of agronomic conditions. For example, the glycinin content of soybeans[48] and the arachin content of peanuts[49] are affected by agronomic conditions. However, no studies have been done to determine if such conditions have any effect on the allergenicity of specific foods.

The type and amount of individual proteins in foods may also vary from one variety or cultivar to another. Very limited studies have been conducted to determine if such conditions have any effect on the allergenicity of specific foods. The IgE-binding proteins of almonds are similar for the three major varieties: Nonpareil, Mission, and Carmel.[50] Florunner and Virginia peanuts are reasonably equivalent in their abilities to bind peanut-specific IgE in inhibition immunoassays.[51] Some individuals with allergies to fresh avocados react to only one of two common varieties, although most react to both varieties.[52] The amount of a specific 27-kDa albumin in wheat, which is a major occupational allergen in baker's asthma, was variable among seven wheat cultivars used commonly in Germany.[53]

The stage of development of a plant or animal also affects the nature and amount of specific proteins and perhaps allergens. Again, this possi-

bility has received little study. Soybean sprouts are equivalent to soybean seeds in their ability to bind IgE from soy-allergic patients in inhibition immunoassays.[54]

D. Physical and Chemical Characteristics of Allergenic Proteins

Food allergens tend to be resistant to typical processing and preparation conditions. These proteins are comparatively resistant to heat and acid treatment, proteolysis, and digestion. However, important exceptions do exist. Although these resistance properties may be useful in the assessment of the potential allergenicity of a specific protein of unknown allergenic potential that has been transferred or developed through genetic engineering, a combination of tests should be used to increase the reliability of such evaluations.

1. Resistance to Heat

Many allergenic food proteins are resistant to heat. However, some heat-labile food allergens have been identified. The application of heat promotes protein denaturation and the loss of conformational IgE-binding epitopes. The resistance of some food allergens to heat denaturation suggests that conformational epitopes are not always critical for IgE binding in the case of food allergens. Cow's milk proteins have been the most extensively studied in this regard. Heat treatment can reduce the antigenicity of whey proteins, but has virtually no effect on the antigenicity of casein.[55] However, whey proteins retain some allergenicity even when subjected to severe heat.[55–57] The IgE-binding capacities of β-lactoglobulin and bovine serum albumin (BSA) are diminished by heating at 80 to 100°C for 15 min. In contrast, little change is observed in the IgE-binding capacities of α-lactalbumin and the caseins after heat treatment.[57] The IgE antibodies in the sera of some patients still react significantly with milk proteins, even after severe heating at 100°C for 3 h.[57] This heterogeneous response of patients with cow's milk allergy to heat-treated milk proteins illustrates the difficulties of using heat treatment as a hypoallergenic process.[57]

Most other food allergens are also resistant to heat. The primary codfish allergen, *Gad c* 1, is resistant to heat.[58] The major allergens in shrimp are heat stable[59–61] and have been isolated from the cooking water.[60,61] The IgE-binding ability of the rice glutelin and globulin fractions as assessed by immunoassay was reduced by 40 to 70% by heating at 60°C for 1 h or 100°C for 2 to 10 min.[62] The 7S- and 11S-globulin fractions of soybean bind soy-specific IgE, but that binding activity can be decreased but not eliminated by heating at 80°C for 30 min.[63] Later, Burks et al.[30] demonstrated that the IgE titers from the sera of patients with soy allergy were reduced when examined using the 7S and 11S fractions of soy protein that had been heated at 80°C for 60 min by comparison to unheated soybeans. No change was observed in the IgE titer using the whey fraction of soy protein under those conditions. Substantial decreases in the IgE titers were observed when examined with the three soy protein fractions after heating at 120°C for 60 min.[30] In further experiments, the IgE-binding capabilities of the three major soy protein fractions were only minimally affected by heating at 37°C for 60 min, 56°C for 60 min, 100°C for 5 min, 100°C for 20 min, or 100°C for 60 min.[31]

The IgE-binding capabilities of a crude peanut extract and two major peanut allergens, *Ara h* 1 and *Ara h* 2, were unaffected by heating under similar conditions.[31] The IgE-binding ability of one of the major peanut allergens, the concanavalin A-reactive glycoprotein, is stable to temperatures of up to 100°C over a pH range of 2.8 to 10.[64] Raw peanuts also appear to contain several heat-labile allergens.[65] The allergenic activity of the albumin fraction of peas retained all of its activity when heated or boiled.[66] The allergens in cottonseed are quite heat stable.[18] Ovalbumin and ovomucoid, two of the major allergens from egg whites, are relatively heat stable and can be immunologically detected in cooked eggs, while conalbumin is less stable.[67] Ovomucoid retains its ability to bind to IgE from the serum of egg-allergic patients even after prolonged heating at 100°C, although the ovomucoid from eggs stored for 12 d at 4°C was slightly less stable to heating

at 100°C than ovomucoid from fresh eggs.[68] Thermal denaturation of ovalbumin had little effect on its allergenicity[69] in one study, but heat-denatured ovalbumin had substantially less binding to IgE when compared with native ovalbumin in another study.[70]

Some food allergens are quite sensitive to heat denaturation. The allergens in fresh fruits and many vegetables are a good example.[35] With apples, skin prick tests were positive with extracts of fresh apples, but were negative with extracts of apples heated at 175°C for 30 min.[34] Other foods also contain a mixture of heat-stable and heat-labile allergens; peanuts as discussed are a good example.[65]

Although food allergens tend to be resistant to heat denaturation, this property is obviously not universal. The use of heat denaturation in the assessment of the potential allergenicity of a specific protein obtained through genetic engineering is problematic. The ability of the heat-treated protein to induce immunogenic responses should be evaluated in IgE-responsive systems. Assessment of the heat stability of other biological activities of specific proteins cannot be used to predict the heat stability of immunogenic or allergenic responses.

2. Resistance to Digestion, Proteolysis, and Hydrolysis

Most food allergens are resistant to proteolysis or hydrolysis.[71] Although heat treatment leads to protein denaturation and loss of conformational epitopes, enzymatic or acidic cleavage of the polypeptide chains may destroy both conformational and linear epitopes. However, the amount of information on the stability of food allergens to digestion, proteolysis, and hydrolysis is relatively limited.

A few studies have even suggested that digestion might enhance the allergenicity of food proteins. Haddad et al.[72] reported that the IgE from the serum of some patients with cow's milk allergy is actually more reactive with proteolytic digests of β-lactoglobulin than to its undigested form. Schwartz et al.[73] reported that pepsin digestion did not diminish the IgE-binding ability of β-lactoglobulin.

Limited proteolytic digestion of cow's milk proteins does not diminish their ability to bind to IgE, although complete hydrolysis does result in a substantial loss of allergenicity.[74,75] The hydrolysis of whey proteins with trypsin resulted in a partial hydrolysate that had no sensitizing capacity in guinea pigs.[76,77] A commercial infant formula made with this hydrolysate was determined to contain less than 20% free amino acids, detectable peptides with chain lengths of up to 10 to 15 amino acids, and a small amount (about 1%) of proteins with mol wt up to 3000 indicating the presence of proteins of 27 amino acids in length.[78] These proteins are apparently reactive with cow's milk specific IgE based on the occurrence of adverse reactions to this formula among milk-sensitized infants[79,80] and the demonstration of significant IgE binding to these partial whey hydrolysates using the sera of certain cow's milk-allergic subjects.[81]

The combined effects of pepsin and trypsin on whey proteins resulted in a greater degree of hydrolysis and abolished the binding to IgE.[82] The hydrolysis of whey proteins has also been conducted with an alkaline protease from *Bacillus lichenformis* and the resulting peptides separated according to their mol wt by gel filtration.[83] The peptides having mol wt above 6 kDa were capable of eliciting local hypersensitivity reactions in mice while the smaller peptides did not,[83] suggesting that extensive hydrolysis may be necessary to eliminate allergenic peptides derived from whey proteins. The partial hydrolysis of whey proteins with a mixture of commercial proteases produced two hydrolysates with 20.8 and 12.4% degrees of hydrolysis, respectively.[84] Although these hydrolysates displayed substantially reduced IgE binding, their IgE reactivity was not completely destroyed.[84] Ultrafiltration of the hydrolysates did produce a hydrolyzed whey permeate with no detectable IgE-binding activity.[84]

The effect of digestion and partial hydrolysis of casein on its allergenicity in humans has not been investigated. In rabbits, casein peptides with mol wt between 3500 and 5000 were as immunogenic as the intact protein, whereas smaller peptides were 1000-fold less immunogenic.[85] While partial casein hydrolysates tend to retain some immunogenicity, the specificity of the proteolytic enzymes used in the generation of these hydroly-

sates has some effect on residual immunogenicity.[86] The extensive hydrolysis of casein does yield a product with greatly diminished allergenicity that is commonly used to produce a hypoallergenic infant formula.[75,87] Careful examination of these casein hydrolysates revealed that they contained 70% free amino acids and detectable peptides with chain lengths up to five to eight amino acids.[78] IgE binding to casein hydrolysates is also detectable in some patients.[81]

Other food allergens are also resistant to proteolysis and digestion. The codfish allergen, *Gad c* 1, is resistant to proteolysis; small peptide fragments of *Gad c* 1 retain their ability to bind to IgE.[88–90] However, extensive hydrolysis of a codfish allergen extract with trypsin, pepsin, subtilisin, and pronase resulted in the destruction of its IgE-binding ability. Elastase hydrolysis and simulated digestive proteolysis were only partially effective.[91] Similarly, the IgE-binding abilities of ovomucoid and ovalbumin, the major allergens in egg white, were also unaffected by proteolysis and resulted in the isolation of a peptide fragment with IgE-binding activity.[69,70,92]

The IgE-binding capability of a soybean extract was reduced tenfold when subject to pepsin hydrolysis followed by hydrolysis with trypsin, chymotrypsin, and a mixture of intestinal peptidases.[31] When subjected to similar proteolytic treatment, the IgE-binding capability of a peanut extract was reduced 100-fold.[31] The extensive hydrolysis of soy protein yielded a hydrolysate that was not immunogenic to rabbits.[93] Similar results were obtained with guinea pigs using a collagen/soy protein hydrolysate.[94]

The skin test reactivity of wheat extracts in a group of patients with IgE-mediated, exercise-induced anaphylaxis was destroyed by treatment with trypsin, but not with pepsin.[95] The sizes of the peptides generated by trypsin and pepsin hydrolysis were similar. However, partial hydrolysis with trypsin destroyed the IgE-binding epitopes on wheat proteins, whereas pepsin hydrolysis did not materially affect these same epitopes.[95] A hypoallergenic wheat flour has been developed by treating solubilized wheat proteins with actinase, collagenase, and transglutaminase.[96]

The major cottonseed allergens are unaffected by pepsin hydrolysis.[97] The rice allergen could be largely inactivated by hydrolysis with actinase, while papain caused a decrease in the IgE-binding activity of the rice allergen. Pepsin, trypsin, chymotrypsin, and pancreatin had no effect on the activity of this allergen.[98]

Proteins may also be hydrolyzed by chemical means, either through specific agents such as cyanogen bromide or nonspecific methods such as treatment with concentrated hydrochloric acid. Chemical modifications of allergens may offer another means for the reduction of the allergenicity of these proteins. Cyanogen bromide cleavage of ovomucoid had no effect on its IgE-binding ability.[92,99] However, this treatment reduced the allergenicity of ovalbumin.[69,99] The denaturation of ovalbumin with urea, hydrochloric acid (pH 3.0), and dithiothreitol did not diminish its IgE-binding activity, but treatment with sodium hydroxide (pH 11.0) did destroy its IgE-binding activity.[70]

Some food allergens are more sensitive to proteolysis and digestion. Fresh fruits commonly cause oral allergy syndrome (OAS) in affected individuals.[100,101] Apparently, such allergens in fresh fruit are easily digested in the gastrointestinal (GI) tract and do not cause systemic effects. This theory, however, has not been carefully evaluated.

Most food allergens are resistant to digestion, proteolysis, and other forms of hydrolysis. However, the findings depend on the nature of the enzymatic or chemical treatment used in the experiments, the choice of method for assessing the immunogenicity of the hydrolysis products, and, to a lesser extent, the specific food allergen being evaluated. Although the assessment of the resistance to hydrolysis of proteins could offer valuable information regarding the potential allergenicity of specific proteins, a rigorous protocol for such experiments has not been established. Ideally, this protocol would mimic digestive proteolysis and include tests on the isolated protein and the protein in the appropriate food matrix. The experience with the evaluation of the immunogenicity of partial whey hydrolysates in animal models dictates that extreme caution be used in the evaluation of results obtained from such animal models. The development of further data on the comparative stability of food allergens vs. other food proteins to digestion, proteolysis, and hydrolysis would be highly desirable to deter-

mine the utility of this tool for the assessment of the potential allergenicity of specific proteins.

3. Solubility

Most food allergens are soluble in water and/or saline solutions, thus belonging to the classes known as albumins (water soluble) and globulins (salt soluble).[102] However, many food proteins fall into these two solubility categories, so these features are not particularly distinguishing. Food proteins may also be either alcohol soluble (prolamines such as gliadin from wheat) or alcohol insoluble (such as glutelin from wheat). Proteins in these two categories have not been frequently classified as allergens. However, these two categories of proteins have been less frequently examined for their allergenic potential. Occasionally, these proteins have been implicated in IgE-mediated allergies. For example, IgE antibodies to wheat gluten have been identified in a few wheat-allergic patients.[103] Also, celiac disease, which involves a cell-mediated immune reaction associated with an intolerance to wheat, rye, barley, and oats, is caused by certain gliadin peptides.[104] Recently, a codfish allergen was identified in codfish surimi from which the soluble proteins, including *Gad c* 1, had been removed by extensive washing.[105]

4. Acid Stability

Food allergens are usually quite stable to moderate acid treatments.[33] The treatment of food allergens with acid concentrations simulating stomach acid conditions typically has little effect. The lability of the allergens in fresh fruit to such acidic conditions has already been noted as an exception to this statement. More typically, one of the major peanut allergens, the 65-kDa concanavalin A-reactive glycoprotein, is stable at pH 2.8.[64] Ovalbumin, one of the major eggwhite allergens, is stable at pH 3.0.[70] A demonstration of a lack of immunogenic stability under simulated stomach acid conditions could be an additional criterion to apply in the assessment of the potential allergenicity of a given protein. Again, rather limited information exists on the comparative acid stability of food allergens. Gathering additional data would be an important consideration.

5. Effect of Chemical Modification of Amino Acids on Allergenicity

Chemical modification may also involve the modification of the amino acids in the IgE-binding epitopes, decreasing binding to allergen-specific IgE. These approaches occasionally have value in identifying the amino acids involved in the IgE-binding epitopes, but do not generally have much practical value in the processing of foods. In one of the earliest attempts to use this strategy, Lee and Sehon[106] observed a decrease in the IgE binding of ovalbumin after treatment with polyethylene glycol. The chemical modification of the lysyl, tyrosyl, tryptophyl, or arginyl side chains of *Gad c* 1, or the release of the two calcium ions associated with this protein also resulted in a significant reduction in IgE binding.[107] The polymerization of *Gad c* 1 similarly resulted in diminished allergenic activity.[107,108]

Other approaches also diminish the IgE binding of some food allergens, but these are the exception rather than the rule. In contrast, the reaction of lactose with β-lactoglobulin increased the skin reactivity of this milk protein in cow's milk-allergic individuals,[109] suggesting that the browning reaction may enhance allergenicity in some situations.

The effect of chemical modifications on the allergenicity of various food proteins is likely to be highly specific and related to the amino acid sequence in the epitope of the allergen. The use of such approaches in the general assessment of the potential allergenicity of genetically engineered proteins would be impossible.

III. EFFECT OF PROCESSING ON FOOD ALLERGENS

A. Thermal Processing

Because food allergens are generally resistant to heat and proteolytic enzymes, food processing

is not particularly effective in eliminating the allergenicity of various foods. Some notable exceptions do exist. For example, the allergenicity of fresh fruits is rather easily eliminated by heating,[101] such as would occur in the canning of fruits or the making of jam. The allergenicity of sliced apples is diminished by storage at room temperature,[34,110–112] probably another indication of the ease of denaturation of some of the allergens found in fresh fruits. However, the allergens in freshly picked apples increase during storage if the apples are not sliced.[112] The allergens present in salmon seem to be destroyed by the thermal processes used in the commercial canning of salmon because salmon-allergic patients in one study could tolerate canned salmon.[113] However, fish-allergic individuals are often allergic to cooked fish, so the extent of the heat process is an important variable.

Some occupational food allergens are heat labile. Green coffee beans were found to possess allergenic activity, but roasted coffee beans did not, which suggests the presence of heat-labile allergens.[114] The cocoa bean allergen is destroyed by processing into chocolate, but the responsible processing operation has not been identified.[115] Because cocoa beans undergo substantial heat processing, the possibility of heat denaturation seems likely, perhaps in concert with chemical modifications.

Most allergenic foods retain their allergenicity during the typical heat processes used in manufacturing or preparation. Cow's milk and its two principal protein fractions, casein and whey, retain their allergenicity even when subjected to severe heat treatments.[55,116–119] These severe heat treatments may adversely affect the nutritional quality of the milk.[116] Cow's milk reportedly retains its allergenicity after such common heat-processing treatments as condensation, evaporation, and drying.[119]

Both raw and roasted peanuts are allergenic and bind to peanut-specific IgE.[51,65] Most other commercially processed peanut products, including peanut butter and various peanut flours, retain their allergenicity.[51] Raw and roasted soybeans contain similar IgE-binding proteins.[29] Soy protein isolate, which is prepared by acid and alkaline extraction of defatted soybeans, has diminished IgE-binding capability, although some residual allergenicity remains.[29] Other soy products, including soy flours and soy protein concentrate, which were also subjected to drying, retained most of their IgE-binding proteins.[29] Boiled eggs retain their ability to bind to IgE.[68]

B. Enzymatic Proteolysis

Enzymatic proteolysis is not typically effective in eliminating the allergenicity of proteins in various allergenic foods. The degree of proteolysis is very likely an important variable, but rather extensive proteolysis may be necessary to eliminate all of the allergenicity of an allergenic food protein. Regulatory agencies typically do not have regulations that differentiate between extensively and partially hydrolyzed proteins. Cow's milk is the best studied example by far. An infant formula was developed based on the partial hydrolysis of whey proteins,[120] as described by Jost and co-workers.[76,77] This product was less sensitizing than a typical cow's milk formula when fed to newborn infants in some studies,[121–123] but no difference was noted in other studies.[124,125] This partial whey hydrolysate formula triggered allergic reactions when fed to certain infants already sensitized to cow's milk.[79,80,126] Complete hydrolysis of an allergenic protein to its constituent amino acids would be predicted to destroy the IgE-binding capability of the protein. In fact, the most widely marketed hypoallergenic infant formulas are based on casein hydrolysates that were subjected to extensive hydrolysis. However, occasional allergic reactions are even reported to casein hydrolysate formula in highly sensitive infants,[126–128] which is notable because the degree of hydrolysis in such formulae is greater than 85%.[93,129] Wahn et al.[87] determined that the casein hydrolysate formulas indeed had little residual allergenic activity. However, casein hydrolysates can, at least occasionally, contain peptide fragments large enough to be immunogenic.[83,130]

An extensively hydrolyzed peanut protein sold as a flavor enhancer did not bind to peanut-specific IgE.[51] However, both a commercial- and an acid-hydrolyzed vegetable protein (HVP) made from soybeans bound to serum IgE from soy-

bean-allergic individuals,[54] probably because the degree of hydrolysis was insufficient to destroy the IgE-binding epitopes on the soybean allergens. Fermented soy products, such as mold-hydrolyzed soy sauce, tempeh, tofu, and miso, also retained their allergenicity, although the level of IgE-binding ability was diminished by comparison to extracts of raw soybeans.[54]

A hypoallergenic wheat flour has been developed through the treatment of solubilized wheat proteins with actinase, collagenase, and transglutaminase.[96] Similarly, a hypoallergenic rice has been developed through the treatment of rice with proteolytic enzymes.[98] This preparation was quite effective in the treatment of rice-allergic patients.[131,132] The selective digestion of β-lactoglobulin from a whey protein concentrate was accomplished by using thermolysin under high hydrostatic pressure (1000 to 3000 kg/cm^2). The resultant product was devoid of β-lactoglobulin, but was enriched in α-lactalbumin, another common cow's milk allergen.[133]

C. Oil Extraction

Removal or exclusion of either all proteins or only the allergenic proteins during processing would obviously eliminate the allergenicity of a resulting food. The extraction of edible oils from oilseeds, such as peanut, soybean, and sunflower seeds, by the hot solvent extraction procedure eliminates all traces of protein. This makes the resultant oils safe for consumption by allergic individuals.[134–136] However, Porras et al.[137] identified soy protein in some, but not all, samples of soybean oil from Europe, indicating that oil extraction may not always eliminate the proteins or the allergens entirely. Soy protein was also detected frequently in samples of soya lecithin, a common food ingredient.[137] Similarly, nonprotein ingredients from cow's milk may contain traces of the cow's milk allergens. For example, lactose can contain allergenic contaminants.[138]

D. Other Processes

Other approaches may diminish the IgE binding of some food allergens, but these tend to be the exception rather than the rule. The apple allergen is inactivated by phenolic browning.[110] Lyophilization may diminish the allergenicity of certain fish extracts. Fish-allergic patients reacted positively to open challenges with nonlyophilized, cooked fish but negatively to double-blind, placebo-controlled challenges with lyophilized cooked fish.[32] However, lyophilization is a fairly unusual food processing operation, and freeze-dried fish are seldom marketed. *Gad c* 1 appears to be removed from codfish along with other soluble sarcoplasmic proteins in the production of surimi.[105] (Surimi is minced and extensively washed fish which has a gelatinous nature.) However, another codfish allergen remains in the surimi, making this product nonhypoallergenic.[105]

The effect on food allergenicity of other processes used in food manufacturing has not been evaluated. Homogenization has no noticeable affect on the allergenicity of cow's milk.[117] This is despite theories that homogenization results in an enhancement of availability of allergenic epitopes on cow's milk proteins. Most allergenic food proteins likely retain their allergenicity during food processing and preparation.

E. Approaches to Hypoallergenic Processing of Foods

Because of the lack of research and developmental interest, little progress has been made in formulating hypoallergenic foods. The only widely commercialized, hypoallergenic foods are certain infant formulas intended for babies who develop allergies to cow's milk and soy proteins used in formulas. These hypoallergenic infant formulas are based on extensively hydrolyzed casein and/or whey. Because the proteinaceous allergens are hydrolyzed to amino acids and very small peptides, these formulas are relatively safe for ingestion by most cow's milk-allergic infants.

A few other foods, such as peanut oil, could be characterized as hypoallergenic because they lack the allergens from the source foods. However, these products are not marketed in this manner, and it might be unwise to take this approach unless each preparation is verified to be reproducibly free of allergens.

IV. DOSE-RESPONSE RELATIONSHIPS

A. Sensitivity

Although the level of exposure to a specific protein necessary to sensitize an individual is unknown, individuals with preexisting IgE-mediated food allergies can respond adversely to extremely low levels of the offending food. The interaction of food allergens with the IgE antibodies on the surface of the mast cell or basophil membrane leads to the release of comparatively large quantities of biologically active mediators of the allergic response including histamine and the leukotrienes. A small amount of allergen interacting with IgE may elicit clinically significant adverse reactions. The lowest dose of a food allergen that is likely to elicit an adverse reaction cannot be calculated with any degree of certainty for all individuals. This is because the tolerance for an offending food differs from one food to another and one individual to another.

The best estimates of the lowest tolerated dose in most individuals for various allergenic foods can probably be derived, however, from carefully taken histories of actual allergic reactions and extrapolations based on experiments with carefully controlled double-blind food challenges. Anecdotal reports indicate reactions from such incidental contacts as opening packages of the offending food,[139] wiping counters or tables containing peanut residues,[140] inhaling vapors of the cooking food,[141] and kissing the lips of someone who has eaten the offending food.[141] Occasionally, severe reactions can occur from contamination with trace amounts of the offending food. For example, the ingestion of french fries that were fried in the same oil used for the deep-fat frying of fish likely caused a fatal reaction in a fish-allergic patient.[142] Exclusively breast-fed infants have also developed food allergies to foods such as peanuts ostensibly through exposure to food allergens in the breast milk as a result of ingestion of the offending food by nursing mothers.[143–145] The extent of exposure to the allergenic food proteins via breast milk must be quite small. However, in most of these cases, the infants become sensitized, but do not react until exposure to a larger quantity of the allergen during the weaning period.[144] Although these experiences and anecdotes confirm that very small amounts of food allergens will provoke allergic responses in some sensitive individuals, they do not provide quantitative estimates of the lowest tolerated dose. The severity of the allergic reactions is also likely to be directly related to the dose of exposure. Therefore, exposure to very small amounts of an allergenic food is not likely to elicit severe reactions in most cases.

Quantitative estimates of the lowest tolerated dose can be derived from actual allergic reactions in patients from whom careful histories are taken regarding the amount of the offending food ingested. Settipane and Settipane[146] estimated that ingestion of 1 to 2 g of shrimp and 25 mg of peanut in allergic individuals will provoke adverse reactions. Gern et al.[147] investigated several cases of allergic reactions to milk that occurred with food products that did not declare the presence of milk on the label. Some of the cases resulted from ingestion of a frozen dessert made from soybeans that were probably contaminated from the use of equipment that was also used to process dairy products. Here, a single serving contained the equivalent of approximately 2.5 ml of cow's milk.[147] This amount of milk would provide about 80 to 100 mg of milk proteins, 80% of that being caseins and 20% being whey proteins. Several cases involved the ingestion of hot dogs that contained partially hydrolyzed casein as a natural flavoring. Each hot dog contained an amount of casein found in approximately 0.3 ml of cow's milk,[147] which would provide about 10 to 12 mg of milk proteins. Jones et al.[148] investigated an allergic reaction to milk from a raspberry sorbet product that had become contaminated with milk through the use of shared equipment in the processing plant. The milk-allergic child reacted to the ingestion of an unknown amount of the sorbet containing 0.52% milk protein.[148] Sunflower butter processed on equipment used to manufacture peanut butter was responsible for an allergic reaction in a peanut-allergic individual who ate less than 0.25 teaspoonful of sunflower butter contaminated with approximately 1% peanut.[149] Yman et al.[150] reported a series on incidents related to inadvertent or unexpected exposure to allergenic foods that resulted in allergic reactions among sensitive individuals. Many of these incidents involved exposure to small quantities of the

offending food including milk in nine incidents at levels ranging from 0.04 to 1.1%, egg in three incidents at levels ranging from 0.003 to 0.16%, wheat gluten in three incidents at levels ranging from 0.3 to 11.9%, soy protein in three incidents at levels ranging from 0.5 to 7.0%, and hazelnut in one incident at a level of 0.2%. Quantitative exposure information was available in several of these cases. Fatal anaphylaxis occurred in a milk-allergic consumer after eating approximately 100 g of a sausage product containing 0.06% undeclared casein that equated to 60 mg of casein.[150] An asthmatic reaction occurred in a hazelnut-allergic consumer following the ingestion of about 3 to 6 g of a chocolate confectionery product containing 0.2% of undeclared hazelnut that equated to 6 to 12 mg of hazelnut.[150]

Perhaps the best estimates of the lowest tolerated dose can be obtained from DBPCFC. These challenges have been conducted for diagnostic purposes rather than for determining the lowest tolerated dose. The typical protocol involves starting at a dose that is one half or less than the amount of the offending food estimated by the patient to provoke symptoms.[151] A typical starting dose is often 400 to 500 mg,[152] although considerable physician judgement is always involved in the selection of the starting dose. In fact, some physicians choose not to perform blinded food challenges on patients with histories of life-threatening anaphylactic reactions.[152] Therefore, these estimates of the lowest tolerated dose may exclude some of the most sensitive patients. Atkins et al.[153] demonstrated that cottonseed meal protein elicited adverse reactions in sensitive individuals at doses ranging from 100 to 350 mg. Sampson[140] indicated that 50 to 100 mg of peanut protein elicited adverse reactions in some allergic children in DBPCFC. In their pioneering studies on the use of rush immunotherapy to decrease the severity of peanut allergy, Oppenheimer et al.[154] elicited adverse reactions in peanut-allergic patients at doses as low as 30 mg before immunotherapy, although the provoking doses ranged from 30 mg to 8 g with an average of 4 g. In patients with histories of life-threatening symptoms, Oppenheimer et al.[154] initiated DBPCFC at 1 mg of defatted peanut. Other peanut-allergic patients were started at a dose of 100 mg of defatted peanut. The upper limit in DBPCFC is typically 8 g of the offending food.[151] In the studies of Oppenheimer et al.,[154] a cumulative dose of 15.8 to 15.9 g was reached, although the maximum single dose was 8 g. Certainly, many food-allergic individuals do not react until several grams of the offending food are ingested. These experiences demonstrate convincingly that very small doses of food allergens can elicit allergic reactions. Milligram quantities of allergenic foods have elicited allergic reactions in sensitive individuals. Although a precise estimate of the lowest tolerated dose cannot be ascertained reliably, considerable caution should be exercised to limit consumer exposure to known allergenic proteins.

B. Severity as a Function of Dose

As with all toxic responses, allergic reactions display a dose-response relationship and become more severe as a function of dose. This is clearly the case in dose-response relationships observed in intradermal skin testing (ST). The evidence supporting this statement with food allergies is mostly observational and anecdotal. Again, the experience from DBPCFC demonstrates that a threshold exists at a given time in each patient below which no clinically significant reaction is evident. As noted above, the thresholds are quite low for many patients. The DBPCFC is stopped at the lowest dose eliciting adverse reactions. Because human subjects are involved, it would be unethical to determine in any controlled way that a higher dose would provoke a more serious reaction. However, most patients experience rather mild reactions in DBPCFCs, and describe much more severe symptoms occurring during actual allergic reactions where more of the offending food was likely eaten.

C. Sources and Detection of Trace Exposures

The primary means of preventing food-allergic reactions is to follow a specific food avoidance diet.[141,155] Because allergic individuals can react adversely to ingestion of trace quantities of

the offending food, the success of this preventive measure can be compromised by the presence of small amounts of one food contaminating another. Trace quantities of the offending food can be obtained from a number of personal practices, in foodservice facilities, and in processed foods facilities, although a number of these situations have only been described anecdotally.[156]

At the personal level, trace elements may be obtained when attempting to remove the offending food from a mixture before eating; using shared serving utensils, containers, cooking vessels; touching utensils, containers, countertops; handling the offending food; kissing the lips of someone who has just eaten the offending food; opening packages of the offending food; inhaling vapors from cooking of the offending food; and transferring food allergens from mother to infant via breast milk. In foodservice facilities, exposure to the offending food occurs primarily because the foods are not labeled. Allergic individuals must rely on information provided by waitstaff and cooks, which can be erroneous. Creative formulation of restaurant foods, such as the use of peanut butter in chili, can also lead to unexpected exposure to an offending food.[142] In such situations, allergic consumers can inadvertently ingest rather significant quantities of the offending foods. Foodservice facilities can also be sources of exposure to trace residues of the offending foods through shared use of serving utensils, containers, griddles, cooking pots; use of the same frying oil for more than one type of food; and inhalation of cooking vapors from tableside food preparation. Food-processing practices can also lead to the contamination of one food with another. In many of these situations, the labels of the packaged foods do not acknowledge such contamination. Common practices in the processed foods industry that can lead to such contamination include the use of shared processing equipment; use of rework (leftovers from one formulation added to another); presence of allergenic proteins in other ingredients such as natural flavors and starches; mistakes in formulation; use of the wrong packaging material with resultant inaccurate labeling; and switching ingredients without altering labels.

Although these situations may occur fairly frequently, only a few such incidents have been well described. The use of shared equipment has resulted in product contamination and allergic reactions in several situations.[147,149,157] Switching natural flavoring ingredients in hot dogs from a yeast autolysate to a partially hydrolyzed casein product resulted in allergic reactions.[147] Porras et al.[137] detected soybean protein in several soy-based ingredients, including lecithin and oil. Yman et al.[150] have documented numerous incidents in Sweden involving the undeclared use of allergenic ingredients in packaged foods, the use of shared equipment in ice cream, pasta, and chocolate processing, the labeling of spelt pasta as wheat free (when spelt is actually a type of wheat), the changing of formulations without changing labels, and the presence of allergenic proteins in ingredients such as flavorings and binders. Occasionally, more bizarre forms of contamination can occur such as the contamination of beignet mix with *Dermatophagoides farinae* mites, which are highly allergenic when present in house dust.[158]

Immunoassays can detect residues of allergenic food proteins in other foods.[33] These immunoassays, which can involve the use of serum from allergic individuals that contains allergen-specific IgE antibodies,[137,147–149,159] are quite reliable. They detect the actual allergen, but are not commonly available because they rely on serum from allergic individuals. Animal antisera and monoclonal antibodies have also been be used effectively in such analyses.[150,160,161] These antibodies are not always directed specifically at the allergenic proteins, but can be quite specific for detection of proteins from a particular food.

V. TESTING FOR FOOD ALLERGENS AND THE ALLERGENICITY OF FOOD PROTEINS

The *in vitro* and *in vivo* tests described below, which use human subjects or samples, are only suitable to investigate allergenicity to already-sensitized individuals. These tests cannot be used to predict the sensitizing potential of novel proteins. Animal models, although not yet fully developed, offer the only future hope for improvement of such assessments.

A. *In Vitro* Tests

1. Immunoassays and Inhibition Immunoassays

The radioimmunoassay (RIA) and RIA inhibition procedures have been described in some detail by Yunginger and Adolphson.[162] In the RIA (Figure 2), an extract of the food is attached to a suitable solid phase. The serum from individuals with allergies to that food is incubated with the solid phase allowing the allergen-specific IgE antibodies to bind to the solid-phase allergen. The bound IgE antibodies are then detected with the aid of ^{125}I-labeled anti-human IgE. In an alternative enzyme-linked immunosorbent assay (ELISA) procedure, enzyme-labeled anti-human IgE is used. These immunoassays rely on the availability of blood serum from food-allergic individuals. Unlike *in vivo* tests, these individuals do not have to be physically present during the test.

In the inhibition format, an extract of the food is bound to the solid phase just as in an immunoassay. The IgE-binding ability of a test extract (e.g., a processed form of the food) is assessed by mixing increasing concentrations of the test extract with human sera from food-allergic individuals and allowing the mixtures to incubate with the solid phase. A competition occurs for IgE binding to the solid phase vs. the test extract. The amount of IgE bound to the solid phase is determined using ^{125}I-labeled anti-human IgE. An inhibition curve is developed which plots the counts bound to the solid phase vs. the protein concentration in the free phase or test extract. The inhibition immunoassay has been used successfully to detect trace residues of allergenic proteins contaminating other foods,[147–149,157,159] and to assess the effect of processing on the allergenicity of various products made from peanuts and soybeans.[51,54]

2. Crossed Immunoelectrophoresis and Radioimmunoelectrophoresis

CIE and CRIE have been used to separate and detect the multiple allergens that exist in most foods.[74,163,164] Although these procedures have some limitations and have been largely supplanted by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), these procedures could still be useful in the detection of specific allergens. The general procedures for CIE and CRIE were described in some detail by Yunginger and Adolphson.[162] The major limitation by comparison to SDS-PAGE is the comparative lack of resolution of individual proteins in the agarose gels used in CIE and CRIE.

3. SDS-PAGE and Immunoblotting

The separation and detection of allergens is perhaps best accomplished by SDS-PAGE with immunoblotting. This procedure has been frequently applied to the identification of multiple allergens existing in many foods.[29,43,50,60,61] However, the clinical significance of the individual IgE-binding proteins revealed by SDS-PAGE has not been determined, especially with regard to minor allergens (see "Food Allergens"). In SDS-PAGE, a food protein extract is treated with SDS to disrupt polymeric proteins into their simplest monomeric subunits.[165] These proteins are then separated by PAGE by a variety of slightly different procedures. Immunoblotting techniques are thoroughly described elsewhere.[166,167] The separated proteins are electrotransferred to nitrocellulose; the nitrocellulose is overlaid and incubated with human sera containing IgE antibodies to the particular food extract; and the IgE-binding proteins are detected using either enzyme-labeled or radiolabeled anti-human IgE antibodies and a subsequent colorimetric enzymatic reaction or autoradiography as appropriate.

B. *In Vivo* Tests

1. Skin Tests

The skin prick test is probably the most frequently used test for the diagnosis of allergies including food allergies. It is typically conducted by the prick/puncture method with diluted (1:10 or 1:20) extracts of the specific food. A positive skin test is indicated by the development of a

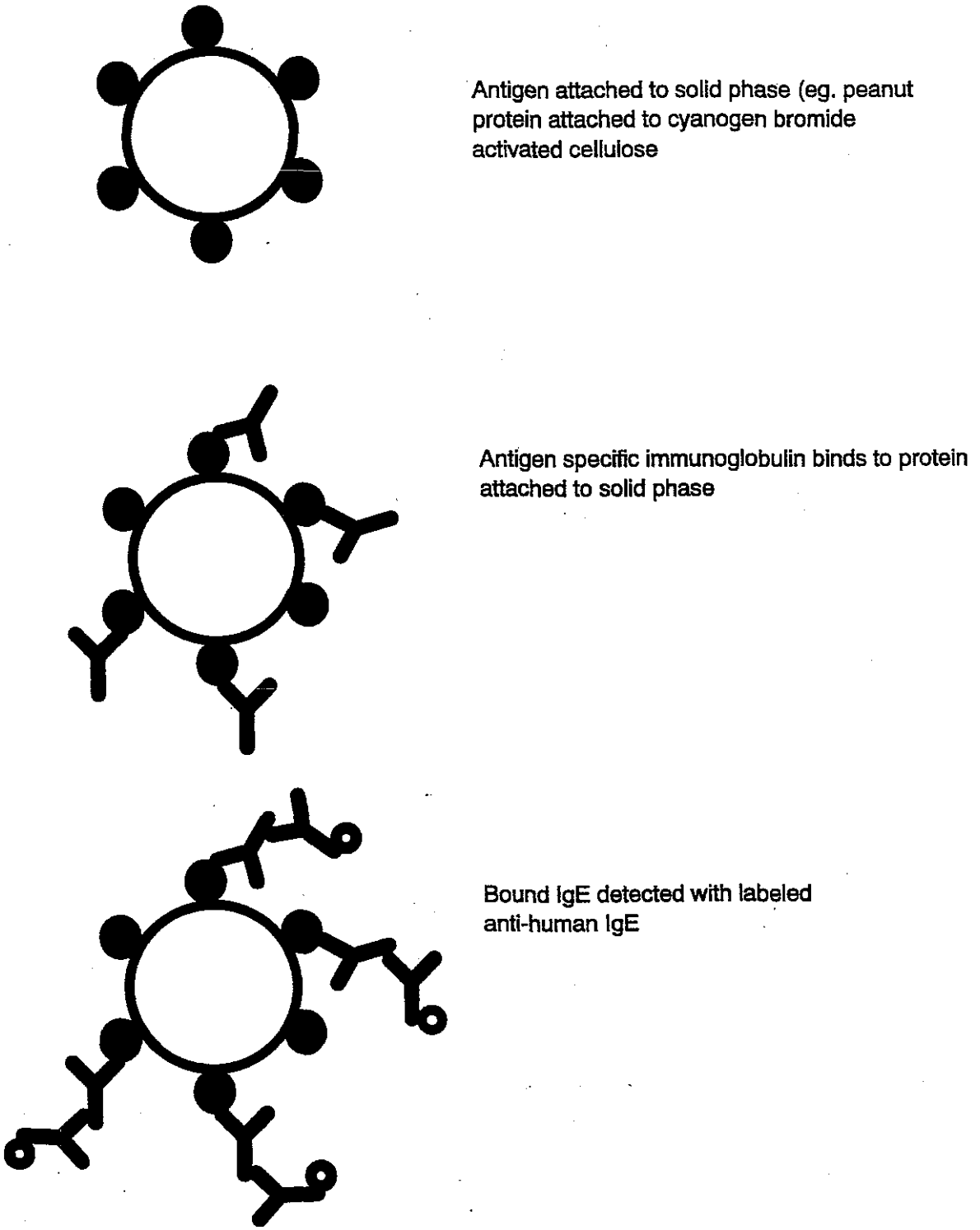

FIGURE 2. Radioimmunoassay (RIA) for food-specific IgE antibodies.

wheal 3 mm or larger in diameter at the puncture site than by a diluent control wheal measured within 10 to 30 min of antigen treatment. The proper application of the skin prick test in the evaluation of food allergies was described by Bock et al.[168] When used in the evaluation of the

allergenicity of specific proteins, the skin test extract is typically prepared from purified or semipurified material. The standardization of allergen extracts would be desirable to enhance the reliability of skin tests (see "Allergic Reactions to Foods" by John A. Anderson in this issue).

2. Challenge Tests

The DBPCFC is considered to be the gold standard for the diagnosis of adverse reactions to foods.[151] The DBPCFC is often used in the diagnosis of adverse reactions to foods (see "Allergic Reactions to Foods"). For purposes of testing the allergenicity of food proteins, a group of patients with well-documented histories of allergic reactions to the food under study, who also have positive skin tests or immunoassays, are selected. Patients with severe reactions would not participate in DBPCFC.

For the DBPCFC, the food must be disguised in some fashion. Usually, the food is dried in some way, and the dried product is encapsulated in opaque, tinted gelatin capsules.[151] The amount of product that can be placed in one capsule usually ranges from 400 to 500 mg, although some exceptions may exist. Alternatively, the food may be given in a liquid vehicle such as Vivonex™ or a milk shake; the food may also be disguised in another solid food such as a hamburger or apple sauce.[151] The taste of the food must be thoroughly disguised in whatever vehicle is used. Often, capsules are wiped clean of any excess food and then dipped in powdered sugar to provide a uniform and sweet taste.[151] In liquid or solid vehicles, the taste of the vehicle must effectively disguise any taste conveyed by the test substances. Strong flavors such as grape or chocolate for liquid vehicles are often preferred.

The DBPCFC is typically performed over a range of doses. The starting dose should provide no more than one half of the food that was present in the minimally provoking dose by history. The maximum dose should be 8 g of the dried food product in a capsule challenge, although larger quantities might be used with liquid or solid vehicles in adults.

A variety of placebos can be considered.[151] Obviously, with liquid or solid vehicles, the placebo is the vehicle without the test substance. Placebo capsules are often filled with sugar or some other readily metabolized ingredient. DBPCFCs are strongly preferred over either open challenges or single-blind challenges. Open challenges should be considered as a follow-up to negative DBPCFC to assure that reactions would not occur under typical preparation practices.

VI. UTILIZATION OF TEST METHODS IN THE EVALUATION OF THE ALLERGENICITY OF GENETICALLY ENGINEERED FOODS

A. *In Vitro* Tests

1. Immunoassays and Inhibition Immunoassays

In immunoassays with genetically engineered foods, the newly introduced or expressed protein could be attached directly to the solid phase if available in purified form. Alternatively, an extract of the genetically engineered food could be attached to the solid phase. In this case, the amount of the newly introduced or expressed protein in the extract actually attached to the solid phase should be quantified. The extract must be prepared in a fashion to assure extraction of the newly introduced or expressed protein as discussed below for skin test extracts. Ideally, the concentration of the newly introduced or expressed protein attached to the solid phase should be equivalent to that found with a solid phase prepared with the donor food material.

The immunoassays can be more easily used in the detection of allergens from genetically engineered foods than the skin test (see Section VI.B.1) because the patients do not need to be assembled in one location. This is particularly advantageous for less commonly allergenic donor foods. Of course, the serum must be drawn from individuals with well-documented allergies to the donor food in question.

Although the immunoassays will be useful in the evaluation of the allergenicity of genetically

engineered foods, the inhibition immunoassays should be even more useful. In the inhibition immunoassay, a protein extract of the donor food material would be attached to the solid phase. An extract would be made of the genetically engineered food with all of the caveats as noted for skin test extracts (see Section VI.B.1). Increasing concentrations of this genetically engineered food extract would be mixed with human serum from allergic individuals with the appropriate allergy to the donor food and allowed to incubate with the solid phase. A competition would occur between the donor food allergen bound to the solid phase and any allergen from the genetically engineered food contained in the free phase. Again, the amount of IgE bound to the solid phase would be determined using ^{125}I-labeled anti-human IgE. An inhibition curve plotting percent counts bound to the solid phase vs. the protein concentration in the free phase would be prepared.

The inhibition immunoassay method was used successfully to identify the allergenicity of a methionine-rich Brazil nut protein that has been cloned into soybeans to enhance their nutritional value.[169]

2. SDS-PAGE and Immunoblotting

Immunoblotting following SDS-PAGE could be easily adapted to the analysis of genetically engineered foods containing new proteins derived from known allergenic foods. In these experiments, the genetically engineered food can be compared to the food from the host plant and the food from the donor. A comparison of the IgE-binding proteins among these three foods would reveal if the genetically engineered food contains any allergens derived from the donor. SDS-PAGE with immunoblotting is quite sensitive especially when radiolabeled antibodies and autoradiography are employed. Thus, the allergens would likely be detected even if the amounts expressed in the genetically engineered food were rather small. This technique has been used to determine that a high-methionine protein from Brazil nuts, which was transferred into soybeans to correct the sulfur amino acid deficiency, was the most prominent IgE-binding protein in Brazil nuts.[169]

B. *In Vivo* Tests

Certain ethical issues must be considered in the use of human subjects in the evaluation of the potential allergenicity of genetically engineered foods. Institutional Review Board (IRB) approval must be obtained.

1. Skin Tests

The skin test for IgE antibody reactivity could be useful in the assessment of the allergenicity of genetically engineered foods in situations where the donor material is derived from known allergenic foods. Any assessment would require the identification of a group of individuals with a well-documented allergy to the donor food material (see "Assessment of the Allergic Potential of Foods Derived From Genetically Engineered Crop Plants" by Dean D. Metcalfe et al. in this issue).

The preparation of the extract from the genetically engineered food for use in the skin prick test is critical. Fresh foods should be used for the preparation of the extract in most cases, although the comparison of fresh and processed foods might be useful on occasion. Extracts of the donor food and the nonengineered host food could be prepared and included for comparative purposes. Most food extracts for skin prick testing are prepared by extracting the pulverized fresh food in saline. Some foods are defatted first, but only if enough fat exists in the food to interfere with extraction. The saline extract should contain the soluble albumin and globulins from the particular food. If the novel protein is known to be insoluble in saline, then some alternative extraction procedure might be devised. In a few cases, it may be unnecessary and unwise to make an extract. Extracts prepared from fresh fruits, are notoriously unstable. In these situations, the preferred method would be to use a drop of the fresh fruit juice on the skin or to puncture the skin by inserting the needle through the fruit or other food after placing it on the surface of the skin. The presence of the novel protein in the skin test extract should be confirmed and quantitated. The extent of the dilution of the saline extract can be adjusted, as necessary, to achieve a proper concentration. If the

saline extract does not contain sufficient amounts of the novel protein, some concentration step should be considered. The stability of the novel protein in the saline extract should also be determined. If this protein is unstable then extracts should be freshly prepared before each test.

2. Challenge Tests

The DBPCFC would be applied in the evaluation of individual(s) who have a well-documented allergic reaction to the food that is the source of the inserted genetic material in genetically engineered foods (see "Assessment of the Allergenic Potential of Foods Derived From Genetically Engineered Crop Plants"). The documentation of the allergic status of the individuals in the test group should be based on significant clinical histories of adverse reactions and positive DBPCFC to the source food, as well as positive skin tests and immunoassays for specific IgE antibodies.

ST and immunoassays with extracts of the genetically engineered food should be conducted before the DBPCFC. If positive skin tests were obtained with food extracts, extra precautions would need to be exercised in the DBPCFC.

The food used for DBPCFC challenge should be harvested, cleaned, and processed in a manner that would be typical for that food. The challenge food should contain as many of the components of that food as might be usually expected. For example, if evaluating a wheat, the wheat grains might be converted into a whole-wheat flour. The form of the product should be that suspected of causing a reaction. With oilseeds, the meal and the oil might be tested separately because the oiliness of whole peanuts could create difficulties in some administration vehicles. Although most foods would be dried for convenience before administration, fresh foods might be needed for some DBPCFCs. For example, allergic reactions to fruits usually only occur to fresh fruits so that fresh fruit would be the most appropriate test substance in that case. The dosage recommendations would be similar to those suggested for any DBPCFC. Alternatively, the purified recombinant protein could be used in DBPCFC, although IRB approval for human challenges might prove difficult.

When conducting DBPCFCs of genetically engineered foods, the placebo would be the food from the nonengineered variety. This placebo should add value to these tests because it would be analogous in taste, texture, and composition to the genetically modified food, except for the newly introduced proteins.

C. Animal Models

New animal models could also be evaluated for their potential to assess the allergenicity of foods derived from genetically modeled plants. There are several criteria that, in theory, such animal models of food allergy must satisfy. First, the test animal must tolerate the majority of food proteins to which it is naturally exposed while mounting a significant IgE antibody response to only a few allergenic ones. Second, exposure and challenge with an allergen must occur orally. Third, this model must reflect organ sensitivities similar to those seen in humans during a food-induced allergic reaction such as GI reactivity, dermatologic, and/or respiratory hypersensitivity. Finally, such a model must be relatively easy to induce and consistently reproducible, from one time interval to another within the same species and different laboratories. Unfortunately, such an animal model is not available now, and may not be in the near future. The only alternatives are animals that produce allergen-specific IgE antibodies in response to parenteral allergen immunization plus adjuvant.[170] Although these models are useful in food allergy research and allergenicity determinations, they are not adequate food allergy models.

Unfortunately, at this time there are no animal models that can accurately predict the allergenicity of food proteins or replicate a food-induced allergic response. The major problem is that the requirements for such a model are inherently strict and it is difficult to answer complex questions with only animal models. Another difficulty with animal models is that all IgE antibody responses are not the same. Mice and rats differ in IgE antibody responsiveness,[171] and even different strains of mice can differ from one another.[172,173] This, however, may not necessarily be a deficiency, because it does reflect the variety of

IgE responsiveness seen in humans. Another problem is that the responsiveness of experimental animals does not always remain the same. Kemeny[171] has reported that changes in an animal's IgE responsiveness were observed from 1 year to the next in his own laboratory under conditions using identical allergen and immunization methods.

It is very difficult to get significant IgE antibody responses in animals such as mice (the best studied) without the use of adjuvants such as aluminum hydroxide[172] or *Bordetella pertussis*.[173] Perhaps the best investigated is the *B. pertussis* vaccine; yet the component inducing this response has not been fully characterized. More recently, Kemeny used the castor toxin, ricin, to induce IgE antibody responses. Using this substance he demonstrated that $CD8^+$ T cells were involved in the regulation of IgE antibody responses.[174]

A final consideration for animal models is that animals must respond to ingested or gavaged allergens. Unfortunately, there is very little information on responses of animals exposed to allergens in this fashion, particularly in the absence of adjuvants. Over the years a number of investigations have used animals to study food-induced allergic responses. Jarrett[175] studied rats responding to antigens administered by mouth or by injection, and reported that by either route, antigen diminished rather than heightened IgE responses to a subsequent allergen exposure. These animals have a highly developed capacity to suppress the production of IgE by immunoregulatory mechanisms. An interesting intestinal anaphylaxis model in rats was developed by Byars and Ferraresi.[176] Changes in permeability of the intestine after oral challenges of sensitized rats was the parameter measured through uptake of ^{125}I-labeled BSA. Other models used intestinal anaphylactic reactions in sensitized mice[177] and a rabbit hyperimmunization model[178] to assess the potentially reduced allergenicity of treated, compared with untreated cow's milk. Although the authors claim some promising results from these models, this is in contrast to what has been reported for assessment of other hypoallergenic formulas.

Bozelka and colleagues[179] attempted to develop a food allergen model in C_3H/HeJ mice immunized parentally or intragastrically with two food allergens, soy and shrimp, without significant success. Although both preparations elicited specific IgE antibody responses to allergen when given intraperitoneally, soy or shrimp administered intragastrically with or without pertussis adjuvant did not induce detectible IgE antibody responses.

There is no adequate animal model to measure food allergens in terms of a full range of their activities. There may be some hope for an animal model to assess a protein's ability to induce IgE antibody response, but this requires further research. One needs to be careful about over reliance on any animal model in terms of its ability to predict the allergenic potential of a particular food substance in humans. An unfortunate example of this is efforts to develop hypoallergenic infant formulas based on a partial hydrolysis of whey.[76] These products were screened for immunogenicity in rabbit animal models measuring IgG antibody responses. These models predicted decreased immunogenic activity, and the products were marketed as hypoallergenic infant formula. In reality, these partial whey hydrolysates still contained substantial allergenic activity that triggered allergic reactions when fed to cow's milk-allergic infants.[79,80,126] One must view with caution interpretation of any studies based solely on such animal models.

VII. SUMMARY

Food allergens are typically proteins that are resistant to digestion, acid, and heat treatments. However, some notable exceptions exist. The amount of these proteins needed to elicit an allergic response in previously sensitized individuals is quite low (milligram amounts). The allergenicity of specific proteins derived from known allergenic sources can be determined, whereas the potential allergenicity of proteins derived from sources of unknown allergenicity is much harder to assess.

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Food Allergens

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I. INTRODUCTION

This article reviews the biochemistry of common and less commonly allergenic foods and the allergenic proteins that have been identified. The techniques utilized to document the allergenicity of various foods and allergenic proteins are presented. Often, particularly in the case of anaphylaxis, oral challenges have not been conducted. Rather, the allergenic material in question has been implicated presumptively based on the demonstration of a positive skin test or an *in vitro* assay for specific immunoglobulin E (IgE) antibodies.

A. Allergen Nomenclature

In response to the heightened progress in recombinant technology, an allergen nomenclature system has been adopted. This system was described recently in detail by King et al.[1] Allergens are designated according to the accepted taxonomic name of their source. The first three letters of the genus are used, followed by the first letter of the species and then an Arabic number. The numbers are assigned to these allergens according to the order of their identification and generally the same number is usually used to designate homologous allergens of related species. For example, the first allergen described in brown shrimp, *Penaeus aztecus*, is designated *Pen a* 1 and the homologous molecule from Indian shrimp, *Penaeus indicus*, is *Pen i* 1.

Members of an allergen group that have more than 67% amino acid sequence homology are called isoallergens.[1] Each isoallergen may have multiple forms of closely similar sequences that are designated as variants. The system can describe polymorphic forms that differ slightly from each other in their amino acid sequences or degree of glycosylation. Furthermore, the nomenclature system provides rules for describing allergen genes, messenger RNAs (mRNAs), cDNAs, and recombinant and synthetic peptides of allergenic interest.[1] Examples of the use of this nomenclature system for describing several of the major food allergens are presented in Table 1.

In addition, researchers customarily describe allergens as *major* or *minor*. Major allergens are generally defined as proteins for which 50% or more of the allergic patients studied have specific IgE.[1,2] Examples of major allergens are *Ara h* 1 from peanuts,[3] ovalbumin, ovomucoid, and ovotransferrin from eggs,[4] and *Pen a* 1 from shrimp.[5] The significance of minor allergens has been debated. Minor allergens may be the result of experimental artifact or may contain similarities in structure to major allergens that allow for IgE binding, but do not have the conformation necessary to elicit histamine release. For example, research has shown that peanut-allergic patients possess IgE that can bind to proteins from many other legumes, resulting in positive

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TABLE 1
Major Food Allergens Identified

Allergen source	Allergens (systematic and original names)	Mol wt (kDa)	Sequence data[a]	Ref.[b]
Arachis hypogea (peanut)	*Ara h* 1	63.5	C	138
Bertholletia excelsa (Brazil nut)	*Ber e* 1; 2S albumin	12	C	194
Brassica juncea (oriental mustard)	*Bra j* 1; 2S albumin	14	C	349
Gadus callarias (cod)	*Gad c* 1; allergen M	12	C	69
Gallus domesticus (chicken — eggs)	*Gal d* 1; ovomucoid	28	C	31
	Gal d 2; ovalbumin	44	C	35
	Gal d 3; ovotransferrin	78	C	37
	Gal d 4; lysozyme	14	C	42
Glycine max (soybean)	*Gly m* 1	34	P	174
Penaeus aztecus (brown shrimp)	*Pen a* 1; tropomyosin	36	P	5
Penaeus indicus (indian shrimp)	*Pen i* 1; tropomyosin	34	P	92
Metapenaeus enis (greasyback shrimp)	*Met e* 1; tropomyosin	34	C	94
Sinapis alba (yellow mustard)	*Sin a* 1; 2S albumin	14	C	268

[a] Amino acid sequence obtained directly or deduced from cDNA sequence.
[b] References refer to those where partial (P) or complete (C) sequence data are available.

skin test and RAST results. However, the clinical manifestations of such cross-reactivity are rare, as the patients are only documented to be allergic to peanut and perhaps one other leguminous food.[6] On the other hand, given that the most popular technique used for identifying allergens is a process that causes proteins to be broken down into subunits (SDS-PAGE, see "Principles and Characteristics of Food Allergens" by Steve L. Taylor and Sam B. Lehrer in this issue), minor allergens could actually be parts of larger major allergens. Therefore, they may have the potential to cause significant reactions in some individuals.

The major allergens are often found in abundance in a food. *Ara h* 1 is part of a peanut storage protein.[7] This is not always the case, however; for example, the major codfish allergen, *Gad c* 1, comprises a small fraction of the total protein of the codfish, yet is the major allergen.[8]

II. COMMONLY ALLERGENIC FOODS OF ANIMAL ORIGIN

A. Cow's Milk

Throughout the world, cow's milk (*Bos taurus*) is used for the majority of human milk consumption. IgE-mediated sensitivity to cow's milk is one of the most common food allergies. The overall prevalence of cow's milk allergy in infants and children worldwide is estimated to be between approximately 2.5% in the first 3 years of life.[9–11] Symptoms often begin at age 3 months or younger, but many children lose sensitivity by age 3. Cow's milk allergy in adults is rather unusual. It is often manifested in children by vomiting and diarrhea, with approximately one-third to one-half of those affected experiencing skin manifestations such as atopic

dermatitis (AD), urticaria, and angioedema, or erythematous rashes.

Cow's milk comprises a number of proteins. Traditionally, two major groups of cow's milk proteins have been identified: caseins, which comprise 80% of the total protein, and whey proteins, which contribute 20% of the total. Caseins are phosphoproteins that precipitate from raw skim milk after acidification to pH 4.6 at 20°C, whereas whey proteins remain in the fluid ("serum") after the precipitation of caseins.[12] The nomenclature of specific milk proteins utilizes a Greek letter with or without a subscript preceding the class name to identify the family of proteins. The genetic variant of the protein is indicated by an upper case Arabic letter with or without a numerical superscript following the class name. Post-translational modifications are added in sequence. Many milk proteins are heterogeneous.[12] Characteristics of the major milk proteins are found in Table 2.

1. Major Allergens

A number of milk proteins have been identified as allergenic or immunogenic in humans. Many patients are allergic to more than one milk protein as identified by skin-test reactivity or by oral challenge. Individuals allergic to cow's milk will often exhibit the presence of IgE antibodies in their serum to goat or sheep's milk.[13,14] Caseins and β-lactoglobulin are the major allergens in cow's milk.[10,14,15] The percentage of persons allergic to individual cow's milk proteins or protein families will vary depending on the method used to define the response. By oral challenge, β-lactoglobulin produces more positive responses than does casein.[14,16] When skin tests were employed, casein was slightly more prevalent (63%) than β-lactoglobulin (62%).[10] Although these individual studies show one group of proteins more reactive than the other by the methods employed, the general consensus is that caseins and β-lactoglobulin are most frequently the cause of allergic reactions to cow's milk.

Caseins — The caseins are a family of chemically related proteins. The frequency of reactivity to individual casein proteins has not been systemically evaluated. α_{s1}-casein has at least five genetic variants. It is a 23-kDa phosphoprotein with sequential mouse epitopes.[17] The amino acid sequence of the protein has been established.[12,18] Based on the predicated structure, hydrophobic and hydrophilic domains connected by a segment of α-helix have been identified.[12] α_{s2}-caseins have

TABLE 2
Major Milk Proteins

Protein	Concentration (g/l)	Percentage of total protein	Mol wt (kDa)
Caseins	24–28		
α-caseins	15–19		23.6–25.2
α_{s1}	12–15	34	
α_{s2}	3–4	8	
β-caseins	9–11	25	23.9
κ-caseins	3–4	9	19
γ-caseins	1–2		11.5–20.5
Whey proteins	5–7		
β-lactoglobulin	2–4	9	18.2
α-lactalbumin	1–1.5	4	14.1
Proteose-peptones	0.6–1.8	4	
Blood proteins			
Albumin	0.1–0.4	1	67
Ig	0.6–1.0	2	160–200

Adapted from Yunginger.[199]

varying degrees of posttranslational phosphorylation. Four genetic variants have been identified.[12] β-caseins have one major component with seven genetic variants and eight minor components that are proteolytic fragments of the major component. The molecular weight of the major component is 23,980.[12,18] Modeling studies indicated that the protein has hydrophobic side chains dispersed over the C-terminal end and center surface of the structure, with a hydrophilic N-terminus. κ-caseins comprise two genetic variants. This protein is cleaved at residue 105 to 106 by rennin (chymosin) into two domains. The hydrophobic domain (para-κ-casein) is not soluble, whereas the polar domain (macropeptide) is extremely soluble.[12]

β-lactoglobulin — Whey proteins comprise approximately 20% of milk proteins. The most abundant whey protein is β-lactoglobulin, an 18-kDa protein belonging to the lipocalin family. There are at least six genetic variants of this protein. The primary structure has been obtained.[12,18] The complete DNA sequence encoding β-lactoglobulin has been reported and shows 91% sequence homology with ovine β-lactoglobulin.[19]

2. Minor Allergens

To a lesser degree, the whey proteins α-lactalbumin and bovine serum albumin (BSA) have been implicated in allergic reactions to cow's milk.[16]

α-lactalbumin — α-Lactalbumin consists of two genetic variants and its molecular weight is approximately 14 kDa. The protein has been cloned and the nucleotide sequence identified.[20] The primary amino acid structure has been determined.[12,18] The two genetic variants differ in only one amino acid sequence. Sequence analysis indicates homology to lysozyme. This protein apparently promotes transfer of galactose to glucose to form lactose, the major milk sugar. α-Lactalbumin produces both positive skin tests and positive oral challenges in a significant number of cow's milk-allergic individuals.[14]

BSA — BSA can be detected in milk and is identical in characteristics to bovine blood serum albumin. BSA has produced both positive skin tests and oral challenges in some cow's milk-allergic individuals.[16] BSA is a heterogeneous protein with a molecular weight of 67 kDa comprising approximately 1% of the total milk protein.

Other proteins — Other milk components may occasionally be allergenic. These include bovine immunoglobulins (Ig), which comprise less than 2% of the total milk protein, β_2-microglobulin, transferrin, lactoferrin,[14] lactoperoxidase, alkaline phosphatase, and catalase.[21] Maillard reaction adducts, which are lactose-protein conjugates, may occasionally act as allergens. A β-lactoglobulin lactose conjugate was shown in one study to be 10 to 100 times more potent in intradermal skin tests than native β-lactoglobulin.[14]

3. Structure-Epitopes

Although caseins are significant allergens, no data are available regarding their human T- or B-cell epitopes. For α_{s1}-casein, the mouse T- and B-cell epitopes have been identified as sequential epitopes.[17] Because α_{s1}-casein is the casein subunit that most often interacts with IgE from infants and children with clinically allergic symptoms,[15] it would be helpful to have information regarding its epitopes in human systems. Baldo[21] found that the glycomacropeptide from κ-casein (amino acid residues 106 to 167) and the polypeptide fragment encompassing amino acid residues 99 to 167 reacted with most of the serum in his IgE-blotting study; the latter peptide proved more reactive, and might indicate the presence of an IgE epitope in amino acid sequence 99 to 105.

4. Dose Response

The exact quantity of milk protein required to produce sensitization (IgE antibody production) or clinical symptoms is not known. Trace quantities of milk proteins in processed foods may produce symptoms in sensitive individuals.[22] Some infants may develop cow's milk allergy despite breastfeeding. β-Lactoglobulin levels in women's breast milk increased from a basal level of 0.0 to 3.5 μg/l (after abstinence from cow's milk ingestion for at least 24 h) to as much as 0.01 to

2.34 μg/l after administration of 400 ml of cow's milk.[23] This may explain the source of sensitization in some situations. Because β-lactoglobulin could not be detected in all samples, it may not be the only explanation. Intact proteins, such as BSA, have also been measured in peripheral blood from individuals who have eaten these proteins.[24]

B. Egg

Eggs from chickens (*Gallus domesticus*) are widely used for human consumption. Egg allergy is one of the most frequently implicated causes of immediate food-allergic reactions in children in the U.S. and Europe.[25] Frequently, egg sensitivity disappears by the fourth or fifth year of life or no later than the first decade of life; however, one third of individuals have clinical sensitivity that lasts over 6 years.[25] Although there is extensive cross-reactivity among the proteins from various birds, hen eggs tend to be slightly more allergenic than duck eggs.[26] The egg white (albumin) is more allergenic than the yolk. Egg white proteins have been studied extensively, and most have been purified and their amino acid sequences determined.

Eggs are composed of 56 to 61% egg white and 27 to 32% egg yolk. The egg white is approximately 87 to 89% water and 9 to 11% protein, whereas the egg yolk contains 50% water, 32 to 35% lipid, and 16% protein.[27] The predominant protein in the egg white is ovalbumin, comprising 54% of the protein present. Other major proteins in the egg white are ovotransferrin (12%), ovomucoid (11%), ovomucin (3.5%), and lysozyme (3.4%). In addition to these proteins, a variety of other proteins have been identified. Ovomucin is a complex glycoprotein with two subunits of 180 and 400 kDa. Ovoinhibitor is a 44-kDa protein whose amino acid sequence is known and whose gene sequence has been obtained by cDNA cloning.[28] Ovoflavoprotein has been found in both egg white and egg yolk, and is known to be a riboflavin-binding protein. Avidin composes 0.5% of egg white protein. It is a heterogenous tetrameric glycoprotein with a molecular weight between 66 and 69 kDa, and acts as a biotin-binding protein. Other proteins include ovomacroglobulin, the G_2, G_3 globulins (which are not well characterized), and cystatin, an inhibitor of cysteine proteases. The characteristics of egg white proteins are summarized in Table 3.

Egg yolks can be separated into two fractions using ultracentrifugation. The sedimented fraction is called the granule fraction, containing 60% protein and 35% lipid. The other fraction, the clear supernatant solution, is called the plasma fraction, containing 18% protein and 80% lipid.[27] The granule fraction contains lipovitellin (high-density lipoprotein), phosvitin, and low-density lipoprotein. The low-density lipoprotein in the egg yolk exhibits a much higher molecular weight (3 to 10 × 10^6 Da) than that present in the egg white (160 to 190 kDa). Phosvitin comprises 16% of total egg yolk protein and is the iron-carrying molecule.[29,30] The characteristics of egg yolk proteins are summarized in Table 4.

1. Major Allergens

Ovomucoid — Ovomucoid (*Gal d* 1) is a glycoprotein with a molecular weight of 28 kDa, a pI of 4.1, and exhibits trypsin inhibitor activity. It contains no tryptophan, which is found in most other egg white proteins. Its amino acid sequence contains 186 residues.[31] There are three tandem domains, each of which is homologous to pancreatic secretory trypsin inhibitor, and each acts as a native globular protein. Its predicted secondary structure indicates some α-helical and β-structural elements, and polymorphism of the protein exists. Ovomucoid has also been documented in several studies as a major allergen. For example, in one study, 48 of 68 sera samples from egg-sensitive patients had positive crossed radioimmunoelectrophoresis (CRIE) to ovomucoid,[32] whereas in a similar study, 21 of 34 sera samples from egg-allergic patients had positive RAST and CRIE to ovomucoid.[33] However, Bernhisel-Broadbent et al.[34] suggested that the use of commercially purified ovalbumin has led to the erroneous concept of ovalbumin as a major egg allergen, because ovomucoid was found to have contaminated commercial ovalbumin preparations (less than 1%). In this study, ovomucoid was found to be a more potent allergen than purified ovalbumin by skin testing (ST) and RAST in 18 children with egg allergy.

TABLE 3
Characteristics of Egg White Proteins

Protein	Percentage total protein	Mol wt (kDa)	pI	Carbohydrate moiety
Allergens				
Ovalbumin	54	45	4.5	Y
Ovotransferrin	12–13	77.7	6.0	Y
Ovomucoid	11	28	4.1	Y
Ovomucin	1.5–3.5	0.23–8.3 × 10^6	4.5–5.0	Y
Lysozyme	3.4–3.5	14.3	10.7	N
Unproven allergenicity				
G2 Globulin	4.0	49	5.5	Y
G3 Globulin	4.0	49	5.8	Y
Ovoinhibitor	0.1–1.5	49	5.1	Y
Ovoglycoprotein	0.5–1.0	24.4		
Ovoflavoprotein	0.8	32	4.0	Y
Ovomacroglobulin	0.5	0.76–0.9 × 10^6	4.5–4.7	Y
Cystatin	0.05	12.7	5.1	N
Avidin	0.05	68.3	10	Y

Adapted from Li-Chan and Naka.[51]

TABLE 4
Characteristics of Major Egg Yolk Proteins

Protein	Percentage of total protein		Mol wt (kDa)
	Granule	Plasma	
Granule			
Lipovitellin	70		400
Phosvitin	16		*160–190*
Low-density lipoprotein	12		—
Plasma			
Low-density lipoprotein		64	3–10 × 10^6
Livetin		14	45–150

Adapted from Yunginger.[199]

Ovalbumin — Ovalbumin (*Gal d* 2) is a monomeric phosphoglycoprotein with a molecular weight of 43 to 45 kDa and a pI of 4.5. The 385 amino acid sequence has been established.[35] Purified ovalbumin has three variants, A_1, A_2, and A_3, which contain two, one, and no phosphate groups per molecule, respectively. The susceptibility to denaturation increases in order of decreasing degree of phosphorylation. The mRNA nucleotide sequence of ovalbumin has been reported.[36]

Ovalbumin has been documented in several studies as a major allergen. For example, 68 of 68 sera from egg-sensitive patients were positive in CRIE to ovalbumin in one study,[32] whereas in a similar study,[33] 34 of 34 sera from egg-allergic patients were positive in RAST and CRIE to ovalbumin.

Ovotransferrin (conalbumin) — Ovotransferrin (*Gal d* 3) has a molecular weight of 77 kDa, and a pI of 6.0. Its 686 amino acid residues and sequence have been identified directly[37] and de-

duced indirectly by mRNA sequencing.[38] It has antimicrobial activity and iron-binding properties. Ovotransferrin has also been documented in several studies as a major egg allergen. For example, in one study, 35 of 68 sera from egg-sensitive patients had positive CRIE to ovotransferrin,[32] whereas in a similar study, 20 of 34 sera from egg-allergic patients had positive RAST and CRIE to ovotransferrin.[33]

Apovitellins — Apoproteins derived from the low-density lipoprotein fraction of the egg yolk are major allergens for some egg-sensitive individuals. Apovitellin I has been shown to be a major allergen in RAST studies using sera from egg-sensitive individuals.[39,40] Apovitellin VI was found to be a major allergen in RAST analysis in a study by Walsh et al.[40] Anet et al.[39] discovered that the apovitellins III, V, and VI were minor allergens for the individuals in their study.

2. Minor Allergens

Lysozyme — Lysozyme (*Gal d* 4) is a 14.3-kDa protein with a pI of 10.7. Its 129 amino acids have been sequenced.[41] It is a single polypeptide chain cross-linked by four disulfide bridges. The chain is folded upon itself so that the first 40 residues from the N-terminal end form a compact globular domain.[42] There is a second hydrophilic domain (residues 40 to 85) that forms one site of the active site cleft. Its mRNA with exons and flanking introns have also been identified.[43] The role of lysozyme in egg allergy has not been established. Miller and Campbell[44] found lysozyme to be a major allergen by ST, but Langeland[32] found that 0 of 68 sera from egg-allergic patients had a positive CRIE to lysozyme. Anet et al.[39] found that 4 of 9 sera from egg-sensitive patients were positive for lysozyme using RAST.

Ovomucin — Walsh et al.[40] found that ovomucin was a minor allergen in RAST studies using sera from egg-allergic subjects.

3. Structure — Epitopes

Phosvitin — Walsh et al.[40] also concluded that phosvitin was a minor allergen in RAST analyses using sera from egg-allergic subjects.

Some progress has been made in determining the T- and B-cell epitopes of ovalbumin. Shinoda et al.[45] examined the T-cell proliferative response to ovalbumin in children with AD who were sensitive to egg protein. The proliferating cell population appears to be $CD4^+$ $CD45RA^+$ T cells. The epitopes responsible for this proliferative response were not examined. However, a synthetic peptide prepared from ovalbumin demonstrates that sequences that are recognized by human IgE antibodies may also stimulate rabbit T cells.[46] This peptide comprises amino acid sequences 323 to 339. Renz et al.[47] found this same sequence to be important in the generation of immediate hypersensitivity responses in Balb/c mice exposed via the respiratory route. T-cell clones for ovalbumin have been established.[48] These cell lines appear to be $CD4^+$ cell lines that secrete interleukin 4 (IL-4), suggesting that these cells are of the T-helper 2 cell type (TH2).

B-cell epitopes for ovalbumin have been more clearly established. Johnsen and Elsayed[46] demonstrated that IgE binding occurs with a peptide of amino acid sequences 323 to 339. Kahlert et al.,[49] using cyanogen bromide cleavage of a commercial ovalbumin preparation, demonstrated IgE binding to peptide sequences 41 to 172 and 301 to 385. Data obtained from studies of ovotransferrin (*Gal d* 3) show seven continuous epitopes.[50] Ovomucoid has prominent carbohydrate-containing domains.[51] IgE binds to the glycosylated domains but not to the nonglycosylated domains, although it is questioned if the carbohydrate moiety acts as an IgE-binding epitope.[52] The lysozyme IgG-binding epitope has been studied, and three discontinuous epitopes have been identified[53] (Table 5). It is of note that lysozyme and bovine lactalbumin have approximately 43% sequence identity.[52]

Homology in the DNA sequence of egg white proteins and egg yolk apovitellin II has been reported.[54] It is possible that the yolk and white could have some IgE-epitope homology. Interestingly, Anet et al.[39] found some cross-reactivity between egg yolk and egg white in RAST inhibition studies.

Little is known about the dose of egg proteins required to elicit an immune response. Ovalbumin is not easily degraded. It is hypothesized to

TABLE 5
Amino Acid Sequences of Three Discontinuous Epitopes of Lysozyme

Epitope D1.3		
Residues	18–27	D-N-Y-R-G-Y-S-L-G-N
	116–129	K-G-T-D-V-Q-a-w-l-r-g-c-r-L
Epitope HEL-5		
Residues	41–53	Q-a-T-N-R-T-D-G-s-t-d-Y
	67–70	G-R-T-P
	84	L
Epitope HEL-10		
Residues	15–21	H-G-l-d-n-Y-R
	63	W
	73–75	R-w-L
	89–102	T-a-s-v-N-c-a-K-K-l-v-S-D-G

Note: Residues denoted by lower case letters are not in contact with the antibody combining site.

Adapted from Davies et al.[53]

persist in the body and may stimulate memory B cells, possibly due to follicular dendritic cells retaining antigen-antibody complexes,[34] although this theory has not been proven.

C. Fish

The consumption of fish and inhalation of cooking vapors are causes of IgE-mediated reactions. There have been no published reports on the prevalence of IgE-mediated reactions to a particular species of fish, as most studies refer only to cod or to "fish" in general. However, fish is one among the most commonly implicated allergenic foods, and has been incriminated in fatal anaphylactic reactions.[55] Although the true prevalence of fish allergy is unknown, incidence of fish hypersensitivity is observed to be higher in countries where fish consumption is above average. For example, codfish allergy may be the most common food allergy in Scandinavian countries.[56]

Most edible fishes belong to class Osteichthyes. Sharks are not in this class, as they are cartilaginous fish (order Squaliformes). The most commonly consumed fishes in the U.S. belong to only a few orders: Clupeiformes (salmons, trouts, whitefishes, smelts, pikes, herrings, sardines, anchovies, shad, menhadens, and alewives), Perciformes (basses, perches, dolphins, snappers, groupers, orange roughy, redfishes, rockfishes, mackerels, swordfishes, and tunas), Gadiformes (codfishes, pollocks, haddocks, and hakes), Pleuronectiformes (flounders, halibuts, and soles), and Cypriniformes (carps and catfishes).[57]

1. Major Allergens

Gad c 1 — The most comprehensive analysis of a food allergen was done by Aas and Elsayed and colleagues, which resulted in the purification and characterization of the major codfish allergen, *Gad c* 1 (originally designated Allergen M). Several studies have documented that *Gad c* 1 is the major codfish allergen,[58–60] belonging to a group of muscle tissue proteins known as parvalbumins.[8] Parvalbumins control the flow of calcium in and out of cells and are only found in the muscles of amphibians and fish. The existence of structurally related parvalbumins in different fish species may explain cross-reactivity in fish-allergic individuals, because *Gad c* 1 shares approximately 34% homology with similar proteins from hake, carp, pike, and whiting.[8] The proportion of *Gad c* 1 in fresh white cod muscle tissue is 0.05 to 0.1%.

2. Minor Allergens

Ag-17-cod — CRIE studies indicate that there are other minor codfish allergens distinct from *Gad c* 1,[58,61] but these have not been characterized. One of these minor allergens was designated Ag-17-cod, and two of eight serum samples from cod-allergic subjects demonstrated IgE binding to this allergen.[58] It has also been reported that approximately 10% of cod-allergic individuals react to a distinct protein found in cod blood serum, but none appear to react exclusively to it.[62]

Protamine sulfate — A low-molecular-weight protein widely used as a heparin antagonist, is a sperm protein of salmon and related fish species belonging to the families Salmonidae and Clupeidae (herring, sardines, trout).[63] One study revealed the presence of antiprotamine sulfate IgE in the serum of one fish-allergic subject. This subject also exhibited a positive skin test against protamine sulfate.[64] However, another study found no cross-reactivity between IgE to salmon and protamine sulfate in two fish-allergic subjects.[65] Although protamine sulfate has been identified as an allergen in some reports of individuals with fish hypersensitivity,[66] one study showed that protamine administration did not cause any adverse reaction in 16 fish-allergic subjects.[67] Therefore, it can be concluded that protamine sulfate is rarely allergenic for individuals with fish hypersensitivity.

Surimi 63-kDa protein — Mata et al.[68] found that surimi, a collection of one or many different varieties of small fish that are minced and washed extensively, gave a single protein band of 63.5 kDa in SDS-PAGE. Using RAST, they showed that six of six sera from fish-allergic patients showed slightly positive responses to surimi, although skin-prick tests were only positive in two of six patients. No further conclusions can be reached on the significance of this allergen until more research is completed.

3. Structure-Epitopes

a. Gad c 1

Gad c 1 is an acidic protein (pI 4.75) with a molecular weight 12,328 Da, and is composed of 113 amino acids and one glucose molecule.[69] As a parvalbumin, the tertiary structure of *Gad c* 1 comprises three domains: AB, CD, and EF. The CD and EF domains coordinate one Ca^{+2}-binding site each, whereas the AB domain does not have this property (Figure 1).

Gad c 1 contains at least five IgE-binding sites.[70] The single arginine at position 75 in *Gad c* 1 plays a major role in the tertiary structure of the allergen, but modification of the arginine residue resulted in no difference in IgE reactivity.[71] Tryptic cleavage at the arginine residue resulted in two allergenic fragments, TM1 and TM2, which were equally active in skin-prick tests, Prausnitz-

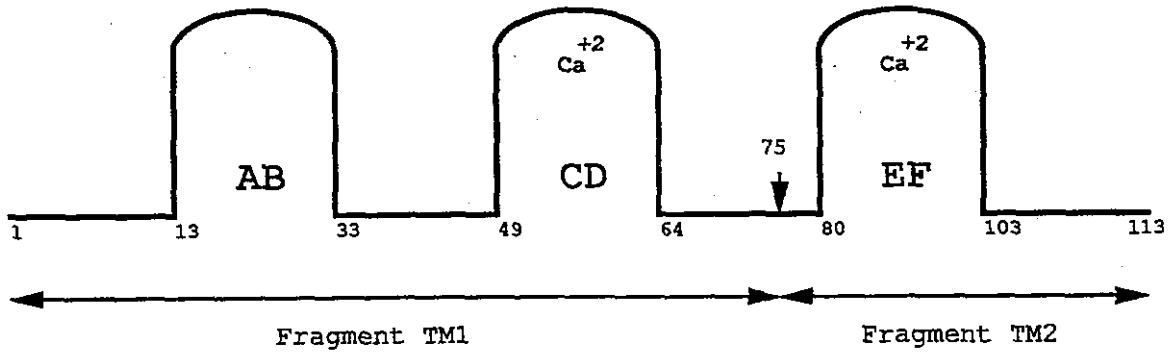

FIGURE 1. The *Gad c* 1 molecule. Calcium is bound by loops CD and EF, while loop AB lacks this property. Fragment TM1 contains amino acid residues 1 through 75; fragment TM2 contains amino acids 76 through 113. (Adapted from Elsayed and Apold.[70])

Kustner (passive sensitization) tests, and RAST inhibition studies.[72] TM1 comprises amino acid residues 1 to 75 and envelopes the AB and CD domains. The acidic residues 59 to 62 are associated with the Ca^{+2} binding of the CD domain.[8] The single glucose molecule present in *Gad c* 1 is located at the Cys-18 position.[73] This carbohydrate moiety does not appear to be involved in allergenicity, as the allergenic activity of TM1 without the carbohydrate was equal to that of TM2.[72] Fragment TM2 comprises amino acid residues 76 to 113 and envelopes the EF domain; residues 90 to 101 constitute the Ca^{+2}-binding region of the EF domain.[74] Fragment TM2 also contains one residue of tryptophan, which seems to have no bearing on allergenicity.[75] Further tryptic hydrolysis studies of fragment TM1 followed by skin tests and Prausnitz-Kustner tests showed that region 33 to 44 was important for allergenicity.[76] Tryptic hydrolysis studies of TM2 showed that region 88 to 96 is partially responsible for the allergenicity.[77]

Studies using synthetic peptides established that region 49 to 64 encircled two repetitive sequences (Asp-Glu-Asp-Lys and Asp-Glu-Leu-Lys). These two tetrapeptides are mutually important for antibody binding, as region 49 to 64 showed relatively high RAST inhibition (39%) compared with *Gad c* 1 (68%), and produced positive Prausnitz-Kustner tests. Region 57 to 64 did not show any allergenic activity.[78] In a subsequent article, it was shown that region 41 to 64 contained three homologous tetrapeptides, repeated in three sites, interspaced by six amino acids in a segment of 24 residues. A series of synthetic peptides of this region showed that at least two of the tetrapeptides were necessary for interaction with antibody, as all peptides encompassing a minimum of two of the tetrapeptides produced positive RAST inhibition and Prausnitz-Kustner tests.[79] IgE-binding capability is independent of both the constitution and sequence of the spacer amino acids.

Studies of synthetic peptides also showed that residues 88 to 103 in the EF domain have 37.5% amino acid sequence homology with the CD domain peptide, but lack the essential terminally located tetrapeptides responsible for antibody binding in region 41 to 64. However, this residue bound specifically to IgE both *in vivo* (Prausnitz-Kustner tests) and *in vitro* (RAST inhibition tests).[80] Therefore, region 88 to 103 was proposed to have a monovalent binding function that can consequently block, but not elicit allergic reactions.

The AB domain, which does not bind calcium, nonetheless shares more than 30% amino acid sequence homology with the CD and EF domains, and comprises residues 13 to 32. Synthetic peptides of this region showed that the AB domain is functionally divalent in Prausnitz-Kustner and RAST inhibition analyses. It reacts in RAST inhibition in equimolar concentrations at a ratio of 6:1 in comparison with *Gad c* 1.[81]

Regions 13 to 32 and 49 to 64 possess the divalent determinants necessary to elicit an allergic reaction. Repeating amino acid sequences are abundant in *Gad c* 1.[8] In regions 35 to 41 and 67 to 73, five of the seven residues are identical (Leu-X-Ala-Phe-X-Ala-Asp), suggesting repeating IgE-binding sites. Region 65 to 83 is found between the CD and EF domains. A similar region joins the AB and CD domains and embraces section 33 to 44. These two regions possess a high degree of homology.[71] This, plus the high degree of immunologic cross-reactivity between TM1 and TM2, all point to IgE-binding sites distributed in a repetitive manner along the polypeptide chain.

b. Protamine Sulfate

Salmine AI, a protamine from salmon, has the amino acid sequence

10 20 30

H-P-R-R-R-R-S-S-S-R-P-V-R-R-R-R-R-P-R-V-S-R-R-R-R-R-R-G-G-R-R-R-R-OH.[63]

Iridine Ia, a protamine from rainbow trout, differs from salmine AI only in that there is an insertion of an additional Arg at position 19 (underlined).

Although there is not much information available on the amount of fish required to produce an allergic response in fish-allergic individuals, it was reported that cod-allergic patients reacted to less than 1 mg of purified *Gad c* 1 disguised in a 50-g meatball.[59] O'Neil et al.[82] found that 1 g or less of catfish and 4 g or less of other species tested (codfish or snapper) elicited allergic reactions in fish-sensitive individuals in double-blind placebo-controlled food challenges (DBPCFC). Positive reactions were obtained in DBPCFC using 2 oz (uncooked weight) of fish.[83] Another study reported that 8 g dehydrated fish administered over 1 h could elicit reactions.[84] However, these last two studies were not done in subjects with a history of anaphylaxis to fish.

D. Crustacea

At least 30 edible species of crustacea are commonly consumed in the U.S. The crustacean family (phylum Arthropoda, class Crustacea) includes shrimp, prawns, crabs, lobsters, and crayfish, and is a common cause of food hypersensitivity.[85] Although the prevalence is not known, researchers have estimated that more than 250,000 people in the U.S. have potential for developing allergic reactions to shellfish.[86] Like fish, a higher incidence of allergy to shellfish would be expected in geographic areas where more is consumed on a regular basis.

1. Shrimp: Major Allergens

a. Antigens I and II

Shrimp is the most studied of the crustacea allergens. Hoffman et al.[87] were the first to partially characterize allergens from shrimp. Two allergenic proteins were found in the body and shell extracts of raw shrimp, and were called antigen I and II. In a study of 11 shrimp-sensitive subjects, 7 of 11 serum samples bound to antigen I. As only a trace of antigen I was found in raw shrimp and shell extracts, it was thought to be a heat-labile protein composed of two noncovalently bound polypeptide chains with a molecular weight of 21 kDa. Purification by gel filtration of antigen I resulted in a molecular weight of 45 kDa, suggesting it was a dimer. Antigen I had an pI of 4.75 to 5 and contained 189 amino acid residues and 0.5% carbohydrate.

Antigen II, isolated easily from boiled shrimp, was an acidic, heat-stable glycoprotein with a molecular weight of 38 kDa and a pI of 5.4 to 5.8, composed of 341 amino acid residues and 4% carbohydrate. It appeared to be the major allergen for the subjects in this study, as it bound IgE in all of the 11 shrimp-allergic serum samples. Antigen II gave a correlation coefficient of 0.98 with cooked shrimp in RAST inhibition studies. The allergens were not evaluated using skin tests, so divalent binding ability was not assessed. Antigens I and II were considered to be unrelated, based on amino acid composition and immunologic studies.

b. SA-I and SA-II

Nagpal et al.[88] described two allergenic polypeptides isolated from boiled shrimp. Allergen SA-I had a molecular weight of 8.2 kDa and was not analyzed further. The second allergen, SA-II, was composed of 301 amino acid residues, had a molecular weight of 34 kDa, and appeared to be similar to antigen I isolated by Hoffman and colleagues,[87] but was reported not to possess any carbohydrate.

Nagpal et al. stated that approximately 54% of the allergenic epitopes of SA-I and SA-II were shared, suggesting that SA-I was a fragment of SA-II. Taking this into consideration, SA-I contributed approximately 33% and SA-II approximately 56% of the total IgE-binding activity of crude boiled shrimp extract. The authors suggested that the remaining IgE-binding activity (11%) was contained in the shrimp tRNA allergen discussed below. These allergens were not evaluated using skin-test methods.

c. Pen a 1 and Pen i 1

Daul et al.[5,89,90] isolated a major shrimp allergen, *Pen a* 1, from boiled brown shrimp (*P. aztecus*) and reported that its sequence was similar to fruit fly tropomyosin. *Pen a* 1 has a molecular weight of 36 kDa, is readily isolated from the boiling water[91] and meat of cooked shrimp, and is similar to SA-II.[88] It constitutes 20% of the soluble protein in crude cooked shrimp extract and inhibited the RAST reactivity of pooled shrimp-sensitive subjects' serum to whole body shrimp meat extract by 75%. The allergen bound IgE in 28 of 34 (82%) sera from shrimp-sensitive individuals.

Pen a 1 comprises 312 amino acid residues and 2.9% carbohydrate and has a pI of 5.2. It is referred to as *Pen i* 1 if isolated from a different species of shrimp, *P. indicus*.[92]

Endoproteinase Lys-C studies of *Pen a* 1 resulted in protein sequencing of a 21-residue peptide that demonstrated significant homology (60 to 85%) with tropomyosin from various species, consistent with the conclusion that *Pen a* 1 was a shrimp tropomyosin.[5] The greatest homology occurred in region 129 to 149; 72 to 87% with fruit fly tropomyosin, and 60 to 62% with tropomyosin from various mammalian species. The higher homology seen with *Drosophila* tropomyosin can be construed as being indicative of the phylogenic connection between shrimp and insects. The amino acid sequence of the 21 residue peptide is

V-L-E-N-R-S-L-S-D-E-E-R-M-D-A-L-E-N-Q-L-K

Shanti et al.[92] also reported that sequenced tryptic digests of *Pen i* 1 were similar to fruitfly tropomyosin, and that two tryptically derived peptide sequences from shrimp tropomyosin bound shrimp-specific IgE. These were regions 50 to 66 and region 153 to 161: 50-66 is M-Q-Q-L-E-N-D-L-D-Q-V-Q-E-S-L-L-K and 153-161 is F-L-A-E-E-A-D-R-K. Both peptides 50 to 66 and 153 to 161 inhibited binding of SA-II-specific IgE to shrimp tropomyosin and 50% inhibition was attained at 100 pmol/ml for both peptides. Other tryptically derived peptides (some less than 2 kDa in molecular weight) inhibited IgE binding to a lesser extent, but these peptides may not have been free of minor IgE-binding components.

Corresponding regions of tropomyosins from different vertebrates showed little cross-reactivity in region 50 to 66, but demonstrated significant allergenic cross-reactivity with tropomyosins from mammalian species in region 153 to 160: seven of nine amino acids for chicken, rabbit, and humans, and six of nine for rat tropomyosin. Fruit fly tropomyosin was identical to the SA-II allergen in region 153 to 161. Many tropomyosins have homology in the 155 to 161 region; the authors (Shanti et al.[95]) suggested that lack of homology in residues 153 (Leu) and 154 (Ala) between other tropomyosins and shrimp tropomyosin implies that they may be crucial for IgE binding.

The amino acid composition of shrimp allergens *Pen a* 1, antigen II, and SA-II is similar (Table 6). This further indicates that these three allergens are the same protein, shrimp tropomyosin,[93] although both antigen II and *Pen a* 1 have associated carbohydrate moieties.

TABLE 6
Amino Acid Composition of Shrimp Allergens *Pen a* 1, Antigen II, and Sa-II

	Pen a 1	Antigen II	SA-II
Mol wt (kDa)	36	38	34
Alanine	33	31	21
Arginine	26	19	30
Aspartic acid	40	58	39
Cysteine	ND	2	3
Glutamic acid	80	61	75
Glycine	10	20	6
Histidine	1	4	3
Isoleucine	35	30	30
Leucine	6	12	6
Lysine	26	27	27
Methionine	8	9	6
Phenylalanine	4	9	6
Proline	2	6	3
Serine	14	15	12
Threonine	12	12	9
Tryptophan	ND	ND	4
Tyrosine	4	7	6
Valine	13	19	15
Total	312	341	301

Adapted from Daul et al.[5]

d. Met e 1

Leung et al.[94] produced a recombinant shrimp allergen from a cDNA library of the greaseyback shrimp, *Metapenaeus enis*. The allergen has 281 amino acid residues, is similar in amino acid composition to *Pen a* 1 and *Pen i* 1, and has a molecular weight of 34 kDa in SDS-PAGE. In immunoblotting studies, the recombinant allergen bound IgE in serum samples from all eight individuals in the study with histories of anaphylactic reactions to shrimp. Leung et al.[94] confirmed the observations of other groups identifying the 34-kDa allergen as shrimp tropomyosin. They also found that the recombinant shrimp allergen *Met e* 1 possessed an IgE-binding sequence identical to the 50 to 66 region of Shanti et al.[92] shown above, and another small IgE-binding sequence of F-L-A-E-E-A-D-R-K, similar to the 153 to 161 region.

2. Shrimp: Minor allegens

a. Transfer RNA

A minor allergenic tRNA moiety from boiled shrimp (*P. indicus*) has been described.[95] The "purified" RNA allergen possessed 11% of its dry weight as amino acids. After enzyme treatment, 84% of the amino acids were lost, but allergenicity was retained. Approximately 1 μg shrimp RNA caused 89% inhibition of a solid-phase shrimp RNA RAST. However, Nagpal et al.[95] only used one patient's sera in their studies, and so their results may not reflect normal clinical reactivity. It is possible that the allergenicity was due to RNA-associated proteins/peptides, as the RNA was not totally barren of amino acid residues. The RNA allergen was not analyzed in skin tests, so its divalent binding ability was not assessed. This remains the only documented example of a nucleic acid from food implicated in inducing an IgE response.

b. Dose Response

Daul et al.[96] found that six subjects reacted positively in a total of seven double blind challenges in 30 subjects with shrimp hypersensitivity. Four positive reactions were to a dose of four shrimp equivalents (one shrimp equivalent is approximately 8 mg, or the amount of protein extract obtained from a standard 4-g medium-sized shrimp) and three positive reactions occurred to a dose of 16 shrimp equivalents. It appears that only 1 to 2 g of shrimp is needed to elicit an anaphylactic reaction in sensitive individuals.[97]

Oropharyngeal pruritus and occasional subjective throat-pharyngeal swelling was related by most of the individuals experiencing positive challenges at a lower dose of shrimp than that inducing their objective positive symptoms.[96] In a group of subjects having a history consistent with immediate type I hypersensitivity reactions to shrimp, only atopic patients reported anaphylaxis after ingestion; 30 nonatopic patients reported generalized pruritus as their only symptom.[98]

3. Crab

Snow crab has been shown to cause allergic sensitization in occupational settings.[99,100] Heat-labile and -stable allergens have been found in snow crab extracts, and snow crab-specific IgE bound more to boiled snow crab than to raw crab.[100]

The most prominent bands in SDS-PAGE gels were 37-42 kDa in crab cooking water and in extracts of cooked crab meat. Immunoblotting of these SDS-PAGE-separated proteins showed that the majority of snow crab-allergic serum samples displayed IgE binding to the 37- to 42-kDa bands, but a majority also demonstrated heavy radiostaining to bands at or near 14 kDa (Hefle, Bush, Cartier, Malo, Lehrer, personal communication).

4. Lobster

The IgE-binding ability of distinct spiny lobster precipitins resolved in crossed immunoelectrophoresis (CIE) was demonstrated in CRIE using 14 crustacea-sensitive sera.[101] Thirteen crustacea-allergic serum samples reacted in CRIE to these precipitins. Spiny lobster extract contained four IgE-binding precipitins; antigens 8 (positive in ten sera) and 13 (positive in five sera)

are the major allergens, giving the most radiostaining. Antigens 3 and 6 gave weak radiostaining in eight and two sera, respectively.

5. Crawfish (Crayfish)

In the study discussed above,[101] crawfish precipitins were also evaluated for their ability to bind crustacea-specific IgE. Six crawfish antigens produced positive radiostaining using CRIE. Antigen 11 was the main allergenic component (positive in nine sera); antigen 12 (also positive in nine sera) may also be a major allergen, but it was situated under the antigen 11 arc and, therefore, could have been an artifact of coprecipitation. Antigens 6 (positive in one sera), 8 (positive in six), 10 (positive in two), and 13 (positive in seven) exhibited radiostaining to varying degrees.

III. COMMON ALLERGENIC FOODS OF PLANT ORIGIN

A. Peanuts

The peanut is an annual plant belonging to the family Leguminosae and is native to South America. In the U.S., several types are grown, although the three most popular are the Virginia, Spanish, and runner varieties. Virginia peanuts are used primarily for whole kernel consumption and confections. Runner types are used most frequently for oil production and peanut butter.[102] Most of the peanut crop in the U.S. (63%) is used for production of peanut butter.[103] Children are often exposed to peanuts at an early age, usually in the form of peanut butter. Although a popular food, the peanut may be the most common allergenic food known. Allergic reactions to peanuts are often acute and severe. Peanut allergy is seldom outgrown.

Peanut proteins have been customarily classified as albumins (water soluble) or globulins (saline soluble). Most of the storage proteins are globulins, which make up 87% of the total protein.[104] Over the years, peanut proteins have been further fractionated, and classified as albumins, arachin, and conarachin or nonarachin.[105-109] The globulins are made up of two major proteins, arachin and conarachin, which correspond to legumin and vicilin, respectively. The former are primarily composed of large molecular weight globulins known as α-arachin and α-conarachin.[106]

Arachin and conarachin readily associate and dissociate under different conditions of ionic strength and pH, making exact classification of individual components difficult.[110-112] In addition, arachin and conarachin have similar amino acid compositions and comparable electrophoretic mobilities, suggesting structural similarities. Both arachin and conarachin components are glycoproteins containing neutral and amino sugars.[108,113] Immunochemical and electrophoretic analysis of polymorphism in peanuts show varietal differences,[114,115] but most wild varieties contain much less arachin than do cultivated strains. A 45-kDa polypeptide is found in almost all genotypes,[116] whereas an arachin subunit of 36 kDa is only found in certain cultivars.[117]

Arachin — In its native state, arachin exists as a molecule of at least 600 kDa and readily dissociates into a 340- to 360-kDa dimer and a monomer of approximately 170 to 180 kDa.[118,119] Arachin contains approximately six subunits ranging in size from 19 to 42 kDa in SDS-PAGE,[119,120] with pI ranging from 5.8 to 8.3.[119] The globulin ranges from 0.6 to 0.3% in carbohydrates.[108,113,120] A subunit of arachin has been isolated that comprises 201 amino acid residues.[121] Although basic amino acids dominate the structure, there is no distinct basic linear region.

Conarachin — Johnson and Naismith[111] showed by ultracentrifugation that conarachin could be further divided into two fractions, 2S and 8.4S. Later, these were designated conarachin I and conarachin II (or α-conarachin), respectively. Although conarachin I (molecular weight 142 kDa) constitutes nearly 30% of total peanut protein,[122] it has not been examined to any great extent. Conarachin II has a molecular weight of 290 kDa[109] and represents 15 to 25% of the total peanut protein.[122] Various studies have reported from six to eight subunits within conarachin II.[109,111,123,124] Basha and Cherry,[125] using SDS-PAGE, found that conarachin II dissociates into seven subunits, with molecular weights of 84, 46, 34, 31, 26, and 23 kDa. Shetty and Rao[124] found only one major subunit at 64 kDa, and minor ones of 60, 32, and 21 kDa. Conarachin II contains no carbohydrate, and has a pI of 3.9.[124]

1. Peanut Allergens

A multiplicity of peanut allergens have been reported.[3,126–132] These have yet to be fully identified and characterized. Part of the problem lies in the large number of allergenic peanut proteins; investigators have reported more than 20.[126,129,130] Barnett et al.[126] found 16 IgE-binding protein bands in raw peanut extracts and 7 in roasted peanut extracts. Bush et al.,[129] using two-dimensional PAGE, found 11 IgE-binding proteins in extracts from raw peanuts. Studies using RAST inhibition[126,127,131] and IgE enzyme-linked immunosorbent assay (ELISA)[128] have shown that no single protein is solely responsible for all of the peanut allergenicity. The peanut constituents relevant to allergy are either proteins or glycoproteins. ST and DBPCFC studies have shown that peanut oil is not allergenic.[133]

Barnett et al.[126] examined the allergenicity of various peanut constituents by RAST and CRIE using sera from peanut-sensitive patients. They found that the allergenicity of the peanut is spread throughout the arachin and conarachin fractions. Taylor et al.[132] also found that the arachin and conarachin fractions are allergenic in RAST inhibition assays.

Using the RAST technique, Heiner and Neucere[134] tested the allergenicity of different peanut preparations, including extracts of cotyledons, hypocotyls, and other parts of the peanut kernel. They found that the cotyledons, the hearts (axial germ tissue), and the skins were allergenic. The cotyledons (kernels) are probably the major source of allergen for most individuals, as the skins and hearts are often removed during processing. This is because the hearts contain saponins that impart a bitter flavor, and the skin contains catechol tannins and related compounds, which give finished products an undesirable color.[135]

a. Major Allergens

Peanut-1 —Sachs et al.[131] isolated and partially purified a peanut allergen, named "Peanut-1", from raw peanuts. From SDS-PAGE analysis, Peanut-1 was determined to contain two major bands, with molecular weights of 20 and 30 kDa. There were also several minor bands whose molecular weights were reported to be above and below these two, but were not identified. Thin-layer isoelectric focusing gave a pI of 5.25 to 5.75 for Peanut-1. The authors concluded that Peanut-1 was a major acidic glycoprotein with non-identical subunits, but was not the only allergenic moiety present in peanuts.

Concanavalin A-reactive glycoprotein (CARG) — Gleeson and Jermyn[136] first described the isolation of a CARG from raw peanuts. The isolated protein had a molecular weight of 69 kDa and contained 12% carbohydrate. Barnett and Howden[127] later identified and purified a 65- kDa CARG peanut allergen. Based on results obtained with RAST inhibition studies, the authors characterized CARG as a major allergen, because approximately 50% of serum samples from peanut-sensitive patients in this study demonstrated IgE binding to this protein. CARG constitutes approximately 1% of the total peanut protein,[136] has pI of 4.6, contains 2.4% carbohydrate, and is stable at and above 100°C and over the pH range of 2.8 to 10.0. Removal of the carbohydrate moiety of the CARG slightly decreased, but did not completely eliminate the allergenic activity.[127] Meier-Davis et al.[130] using SDS-PAGE and immunoblotting of crude roasted peanut extract, identified three IgE-binding bands at 15, 20, and 66 kDa molecular weight that appeared to be major allergens, but did no further characterization of these bands.

***Ara h* 1** — Burks et al.[3] identified a 63.5-kDa molecular weight glycoprotein peanut allergen using immunoblotting and ELISA methods with sera from peanut-sensitive AD patients. This allergen, *Ara h* 1, was found to have a pI of 4.55. Although it appears that CARG and *Ara h* 1 are perhaps the same protein, *Ara h* 1 does not bind to concanavalin A.

***Ara h* 2** — In a later report, Burks et al.[128] identified and purified another peanut allergen, *Ara h* 2, with a molecular weight of 17 kDa in SDS-PAGE and pI of 5.2. The physicochemical characteristics of the above peanut allergens are listed in Table 7.

2. Structure-Epitopes

Ara h 1 has been cloned and its amino acid sequence deduced.[137] It contains multiple IgE-

TABLE 7
Characteristics of Isolated Peanut Allergens

Name	Mol wt (kDa)	pI	Carbohydrate moiety	Ref.
Ara h 1	63.5	4.55	Yes	3
Ara h 2	17	5.2	Yes	128
CARG	65	4.6	Yes	127
Peanut-1	20	5.25–5.75	Yes	131
	30			

binding epitopes[138] and has significant sequence homology with the vicilin seed storage proteins.[7]

3. Dose Response

A research team at the Mayo Clinic reported four cases of death due to peanut-induced anaphylactic shock in a period of 16 months.[55] In this and other reports,[139,140] victims unknowingly ingested food containing peanut protein. Yunginger et al.[55] point out that in the cases they examined, the amount of peanut protein ingested was probably in the range of milligram to gram quantities. Using DBPCFC, 50 to 100 mg of peanut protein has elicited allergic symptoms in some children.[141] However, acutely sensitive patients are not orally challenged, due to the threat of anaphylaxis. In a study using rush immunotherapy for treating peanut allergy, Oppenheimer et al.[142] reported that 30 mg to 8 mg of peanut (mean approximately 4 g) administered in a double-blind fashion elicited reactions in peanut-sensitive subjects.

B. Soybeans

Soybean globulins are the major proteins. By adjusting the pH of the saline-soluble soybean protein fraction to 4.5, the globulins precipitate, leaving a resulting whey fraction (constituting 6 to 8% of the protein).[143] The whey fraction contains hemagglutinin, trypsin inhibitors, and urease,[144] ranging from 1S to 6S.[145]

When subjected to ultracentrifugation, the globulins separate into 2S, 7S, 11S, and 15S fractions, which have been used to classify the various soybean protein components.[146] Because of the wide range of different analytical methods used in the study of these proteins, Catsimpoolas et al.[147] proposed the following system: the 2S component different from soybean trypsin inhibitor is referred to as α-conglycinin and the 7S component isolated by the method of Robert and Briggs[148] is referred to as β-conglycinin (vicilin). Glycinin and β-conglycinin comprise 70 to 80% of the protein fraction of soybeans.[149,150] The 7S component isolated by the method of Koshiyama and Iguchi[151] is referred to as γ-conglycinin and the 11S component is referred to as glycinin. The 15S fraction consists mainly of polymers of glycinin.[152]

α-Conglycinin (2S) — α-Conglycinin is one of the major fractions of soybean. The 2S fraction possesses heat-stable components of 18.2 and 32.6 kDa, but also contains trypsin inhibitor and cytochrome c activity.[144] Vaintraub and Shutov[153] found that the 2S fraction could be resolved into two components, 2.8S and 2.3S. The 2.8S component gave a 36-kDa band in PAGE and had a pI of 4.4. All of the trypsin inhibitor activity of the soybean was thought to be contained in the 2S fraction, including the Bowman-Birk- (6 to 10 kDa) and Kunitz-type trypsin inhibitors (KSTI) (20 to 25 kDa).[154] However, it was later found that the activity was due to coprecipitation of the inhibitors during the isolation of the globulins.[155]

β-Conglycinin (7S) — This glycoprotein exists as a trimer and/or hexamer in solution, and probably in seed.[154] The monomeric form is 150 to 170 kDa, and the dimer approximately 370 kDa; the pI has been determined to be 4.9. β-Conglycinin is made up of three subunits denoted α, α′, and β, and at least seven different forms can exist (B_0 to B_6) as a result of different combinations of subunits.[156,157] The α and α′ subunits have a molecular weight of 54 kDa, and the β subunit has a mol wt of 42 kDa.[154] All three subunits contain

4.5% carbohydrate.[156] Sato et al.[158] described a "basic" 7S globulin (pI = 9.1 to 9.3) that had two kinds of subunits of mol wt 16 and 26 kDa; the native molecule gave a 42-kDa band in the absence of reduction in SDS-PAGE. The pIs for the 16- and 26-kDa polypeptides were 6.5 to 7.0 and 7.7 to 7.9, respectively. Coates et al.[159] investigated the electrophoretic profiles of the predominant subunits after cyanogen bromide treatment. The α′ subunit was composed of major bands at 47, 19.5, and 15.5 kDa. The 47- and 19.5-kDa bands were glycoproteins. The α subunit gave major bands at 54 and 19.5 kDa, and both were glycosylated. The β-subunit had no methionine residues, and therefore, no resulting bands were observed; the β′-subunit gave four fragments, although their molecular weights were not described. This study also described the derived amino acid sequence from the sequence of a cDNA of an α-subunit clone.

γ-Conglycinin (7S) — γ-Conglycinin is a 7S fraction of soybean protein and is a glycoprotein with a molecular weight of 154 to 177 kDa.[160–162] The pI is 5.4.[160] One report indicates that γ-conglycinin has nine subunits of 22 kDa,[163] another describes the subunits as glycopeptides of 38 kDa and simple peptides of 32 kDa, respectively.[164] Yamauchi et al.[162] described γ-conglycinin as having three subunits of 50 kDa each. In this study, cross-linked subunits subjected to SDS-urea-PAGE gave a 54.4-kDa monomer, a 109-kDa dimer, and a 154-kDa trimer.

Glycinin (11S) — Of the globulins, the 11S fraction, or glycinin (legumin), is the most studied. It has a molecular weight of 320 to 360 kDa and is composed of 12 subunits,[165] which range from 10 to 45 kDa. The acidic subunits range from 37 to 45 kDa and the basic subunits are 20 kDa in molecular weight,[166,167] although Nielsen et al.[168] found subunits of 54.3 to 63.7 kDa. The acidic subunits, A_1 to A_4, and the basic subunits, B_1 to B_4, are present in equimolar amounts in the glycinin molecule.[169] The acidic subunits share some sequence homology and antigenic determinants, suggesting conserved sequences.[170] The basic subunits share considerable sequence homology with each other, but are distinct from the acidic subunits.[171] In the native glycinin, the subunits are packed into two identical hexagons, one on top of the other, forming a cylinder shape.[165] The amino acid sequence of one of the subunits of glycinin has been elucidated.[167]

1. Soybean Allergens

Soybeans contain multiple allergens. Shibasaki et al.[144] studied soybean globulin fractions 11S, 7S, and 2S by RAST and RAST inhibition using sera from three AD and one asthmatic soy-allergic patients. Specific IgE reactivity and considerable cross-reactivity were found in all the fractions. The 2S fraction had the highest potency in inhibiting RASTs for all fractions; furthermore, 50 μg of 2S could inhibit 90% of the IgE binding for all other fractions in RAST studies. When the fractions were heated to 80°C for 30 min, the inhibitory activity of the 2S fraction was enhanced, whereas the others decreased to 39 to 75% of that of the native globulin. However, the potency of the 2S fraction decreased at temperatures beyond 80°C.

In a study using sera from eight AD pediatric patients with positive DBPCFC to soy, significant levels of specific IgE for the 7S fraction and IgG for the 11S fraction were found.[172] Most antigens bound IgE, but the 7S fraction seemed to be more allergenic. Variable IgE-binding patterns on immunoblots suggested that no one component of the soy fractions bound more IgE. Specific IgE antibody was found to both the 7S and 11S fractions in most serum samples. IgE binding was demonstrated to the α-, α′-, and β-subunits of the 7S fraction and the A and B subunits of the 11S fraction.

In another study by Ogawa et al.,[173] most IgE-binding bands were assigned to protein components of the 7S fraction, although binding was also observed in bands from the whey and 2S fractions. Sixteen soybean proteins were observed in SDS-PAGE, ranging from 14 to 70 kDa. After immunoblotting with serum from 10 AD subjects, major IgE-binding bands were observed in the 7S fraction. The 11S fraction was scarcely recognized. Therefore, in this study it was not important for soy-allergic individuals with AD, although their hypersensitivities to soy were not verified by DBPCFC. IgE-binding bands occurred in the 7S fraction at 40 to 70 kDa, with major

binding to a 30-kDa fraction, a minor component of the fraction. The 30-kDa band has been designated *Gly m* 1. This 30-kDa band could not be detected in the 2S, 11S, or whey fractions. Specific IgE binding was observed to an acidic subunit of the 11S fraction. In the 7S fraction, ten major IgE-binding bands were observed, including the α- and β-subunits of β-conglycinin. IgE also bound to the 2S fraction. Ogawa et al.[173] suggest that the 20-kDa band is KSTI, but IgE-reactive bands occur at 17 and 15 kDa in this fraction also. They said that the data show that allergenic cross-reactivity does not exist between the 7S and the other fractions; however, cross-reaction was found between the 20-kDa band in the 2S fraction and the 18- to 21-kDa bands in the whey fraction. In this study, in contrast to other reports, the 11S fraction was less allergenic than the other fractions, even though it comprises much of the total storage protein in the soybean.

a. Major Allergens

***Gly m* 1** — *Gly m* 1 is described by Ogawa et al.[173] as a 30-kDa mol wt protein, a minor constituent of the 7S globulin fraction. Sixty-five percent of the subjects in this study had specific IgE for *Gly m* 1; however, these individuals were AD patients who did not experience severe or anaphylactic reactions to soy. In a later article, this same research group[174] found that the native allergen had a molecular weight of more than 300 kDa by gel permeation chromatography. The monomeric form had a molecular weight of 32 kDa, and pI of 4.5, in two-dimensional electrophoresis. The first 15 amino acid residues of *Gly m* 1 are identical to those of soybean seed 34-kDa oil body-associated protein (also called the soybean vacuolar protein P34). In addition, the 34-kDa oil body-associated protein bound strongly to IgE from sera and monoclonal antibodies that were made against *Gly m* 1 in immunoblotting studies. The 34-kDa oil body-associated protein has been assumed to comprise approximately 5% of the total seed cotyledon protein in the Miyagisiro variety of soybean, although amounts may vary depending on the content of lipid in soybean seeds.[175] The presence of *Gly m* 1 as a minor component of the 7S fraction could have been the result of elimination of the majority of the allergen during preparation of the crude 7S globulin fractions from defatted soybean flakes.

b. Minor Allergens

68-kDa allergen — Ogawa et al.[176] found that 25% of IgE from sera of soybean-allergic individuals with AD recognized a 68-kDa protein of the 7S globulin fraction in immunoblotting studies. The protein was the α-subunit of β-conglycinin, with a pI of 5.0 to 5.2. The sera recognized the α-subunit, but did not recognize the α′- or β-subunits, even though they have a high degree of homology with the α-subunit.

KSTI — A study of KSTI as an allergenic protein was prompted by a soy-allergic woman working with KSTI in an occupational setting.[177] The patient was skin test and RAST positive to KSTI and whole soybean; there was no IgE reactivity with other trypsin inhibitors or to peanut extract. The whole soybean RAST was inhibited completely by KSTI. Of the two other sera from soy-allergic individuals utilized in the study, both were negative by RAST for KSTI, and only one was positive for whole soybean RAST. KSTI could not inhibit the IgE binding to the whole soybean extract. Therefore, KSTI appears to be a relatively minor allergen. Certain soybean cultivars have been produced with reduced or zero levels of KSTI to reduce its antinutritional effects.[178] KSTI has been sequenced.[179,180] The inhibitor is made up of 181 amino acid residues. This group also found sequence variants of the inhibitor, deviating in nine sites in the molecule, with only a single amino acid substitution at each site. The KSTI has a molecular weight of 20 kDa. Brandon et al.[181] found that KSTI has as least two distinct antigenic sites, one of which is retained under denaturing conditions, and may be linear.

S-II (20-kDa protein) — Herian et al.[182] described a 20-kDa IgE-binding protein from soybean, designating it S-II. Two serum samples from subjects allergic to soy showed IgE-binding to a 20 kDa band. No IgE binding was observed to pure KSTI. Roasting seemed to enhance IgE binding to the 20-kDa allergen. Preliminary work indicated that S-II is not a basic subunit of glycinin. One serum sample from a soy-allergic subject

showed binding only to a 14 kDa band. Serum samples from subjects who were both allergic to soybeans and peanuts showed IgE binding to several bands in the range of 50 to 70 kDa, apparently to β-conglycinin subunits. No varietal differences in IgE binding of raw soybeans were observed. The IgE-binding proteins were not characterized further.

2. Structure-Epitopes

Glycinin — ELISA studies[183,184] of the antigenic and allergenic properties of the subunits of glycinin showed that all the acidic chains reacted similarly to a rabbit antiserum raised against glycinin. The basic subunits had no reactivity. Nielsen et al.[168] theorized that these subunits are situated on the inside of molecule. IgG-ELISA using serum from ten soy-allergic adults showed that six samples had IgG-binding activity to glycinin preferably, and none seemed specific for a single subunit. In IgE-binding studies, four sera showed the most IgE binding to subunit A4, whereas another four had the most IgE binding to native glycinin. One sample had no IgE binding to glycinin, but significant binding to the subunits. Specific IgG is directed toward the native glycinin molecule, whereas IgE is directed toward the subunits. The authors suggested that the IgE might be directed toward more fragmented fractions, reflecting the possibility that a certain measure of antigenic processing is required for IgE formation. However, only 80% of the IgE-binding inhibition could be attained by the subunits; therefore, not all of the allergenicity of glycinin is due to the subunits. Antigenicity of glycinin was more conformation dependent than that of β-conglycinin.[185]

***Gly m* 1** — *Gly m* 1 has an N-terminal sequence and an amino acid composition identical to that of soybean seed 34-kDa oil body-associated protein, and close homology to papain-like thiol proteinases.[186] It is interesting to note that *Gly m* 1 possesses 30% sequence homology to *Der p* 1, the major dust mite allergen, which is also a thiol protease.[174] The N-terminal sequence for *Gly m* 1 is

10
K-K-M-K-K-E-Q-Y-S-C-D-H-P-P-A

and is identical to the sequence of the first 15 residues from the N-terminus of the 34-kDa oil body-associated soybean protein.

68-kDa allergen — The IgE-binding site in the 68-kDa minor allergen of soybeans was judged to be located in the residue sequence 232 to 383. IgE in soy-allergic sera recognized the α subunit, but did not recognize the α′- or β-subunits of β-conglycinin, even though they have a high degree of homology with the α-subunit. The α- and α′-subunits share over 90% homology.[187] The predicted IgE-binding region on the α-subunit, residues 232 to 383, corresponds to residues 258 to 417 on the α′-subunit.[188] Therefore, further investigation is necessary to determine whether structural differences between the two subunits can account for the difference in allergenicity for this select population of soy-allergic individuals.

3. Dose Response

There is little information available on the threshold dose of soybean protein required to elicit an allergic reaction. Double-blind challenge studies have been done with children suffering from AD and are, therefore, not exquisitely sensitive to soybean. James et al.[189] found that 250 to 500 mg soy could elicit reactions in their population of AD patients. However, soybean is capable of severe reactions in some individuals, as evidenced by the death of one child from eating pizza containing soybean-fortified sausage.[190] As with all commonly allergenic foods, the smallest amount of food required to elicit an allergic reaction from sensitive individuals is unknown.

C. Tree Nuts

1. Almond

Bargman et al.[191] used immunoblotting techniques to detect IgE-binding proteins in almond extracts, using sera from seven almond-allergic individuals. Two major allergens were identified. One was a 70-kDa heat-labile protein; the other was a 45-50 kDa heat-stable protein. An extensive number of proteins with molecular weights ranging from 38 to 70 kDa bound IgE.

2. Brazil Nuts

Brazil nuts cause systemic anaphylaxis in some individuals. Using immunoblotting to detect Brazil nut peanut allergens, Arshad et al.[192] found several allergenic fractions in the serum from allergic individuals. The major allergen from Brazil nuts, *Ber e* 1, is a high-methionine, 2S protein[193] that is composed of two subunits. The 9-kDa subunit of the protein contained 77 amino acids, and a 3-kDa subunit has also been reported. The cDNA sequence for *Ber e* 1 has been established.[194] *Ber e* 1 has 44% and 21% homology with the castor bean and rapeseed high-methionine proteins, respectively.

3. Hazelnuts

Hazelnut allergy is prominent in Europe among individuals with tree pollen allergies (see Section VII.F). Hirschwehr et al.,[195] using sera from 25 birch pollen-allergic subjects who reported adverse reactions to hazelnuts, found IgE binding to the 17-kDa major hazel pollen allergen, *Cor a* 1 (100%), and to the 14-kDa hazel pollen protein profilin (16%). In addition, IgE bound to proteins of comparable molecular weights in hazelnut extract (18 and 14 kDa), suggesting that proteins similar to *Cor a* 1 and to the 14-kDa hazel profilin might also be expressed in hazelnuts. In contrast, only four sera (22%) of 18 subjects with tree pollen allergy, but without any history of intolerance to hazelnuts, showed IgE binding to the 18-kDa protein of hazelnut extract, and none exhibited IgE reactivity to hazelnut profilin. Immunoblotting inhibition studies revealed that the 18-kDa protein shares IgE-binding similarities with *Cor a* 1, the major of hazel pollen, *Bet v* 1 (the major allergen of birch pollen),[195] and *Bet v* 2 (birch pollen profilin).[196] However, this protein has not yet been sequenced.

4. Pistachio

Pistachio, which is a member of the cashew and mango family (Anacardiacea), has a prominent IgE-binding protein of 34 kDa.[197] Other allergens ranged in molecular weight from 41 to 60 kDa. Some cross-reactivity was demonstrated against peanut, walnut, and sunflower seeds.

D. Wheat

In the U.S., wheat is a dietary staple. The most frequent allergic complaint with cereal grains is occupational asthma from exposure to dusty work surroundings[198] (see Section VI). The wheat proteins include the water-soluble albumins, the saline-soluble globulins, the 70% aqueous ethanol-soluble prolamins, and the acid- or alkali-soluble glutelins.[199]

Using serum from one subject who suffered asthma after ingestion of wheat, Hoffman[200] found that the wheat globulin and albumin fractions were most reactive in a RAST. Sutton et al.[201] also observed that the highest IgE-binding activity was associated with the globulin fraction in a RAST study using the serum of 20 children with high RAST scores to wheat.

In six individuals with food-dependent exercise-induced anaphylaxis (F-EIA), ingestion of wheat 30 min before exercise was associated with a subsequent anaphylactic event and all demonstrated immediate positive skin-test reactions to wheat extracts.[202] Many of these subjects also had skin-test reactivity to trypsin and pepsin digests of wheat. The authors hypothesized that "neoantigens" or novel allergens developed from digestion. However, because the individuals reacted to the native proteins, polypeptides removed from the intact proteins may be a more likely explanation.

IV. LESS COMMONLY ALLERGENIC FOODS OF ANIMAL ORIGIN

A. Mollusks

The phylum Mollusca is made up of class Pelecypoda (bivalves), mussels, clams, cockles, oysters, and scallops; the class Gastropoda, consisting of abalones, conches, limpets, snails, and whelks; and the class Cephalopoda, the octopuses and squids.[57] Mollusk allergens have not been well studied, although they are known to cause IgE-mediated reactions.[203–206]

1. Oyster (Bivalve)

Oysters have been frequently known to induce adverse reactions in sensitive subjects.[207] Lehrer and McCants[207] tested six oyster-sensitive subjects (who exhibited only GI manifestations after ingestion) and found that skin tests and RAST did not appear to correlate with oyster sensitivity.[207]

2. Squid (Cephalopod)

Squids precipitate IgE-mediated reactions in sensitive subjects after ingesting or inhaling cooking vapors. All seven squid-allergic patients in one study demonstrated strong positive skin-test reactions to boiled squid extracts and various commercial crustacea extracts. In addition, serum samples from squid-allergic individuals were positive in specific IgE immunoassays for boiled squid extract.[205]

3. Limpet/Abalone (Gastropods)

Anaphylactic reactions have been reported following ingestion of grand keyhole limpets and abalones. Sensitive subjects had positive skin tests and RASTs to extracts of the offending shellfish.[204,208] In one study, limpet-allergic subjects demonstrated positive skin tests and basophil histamine release in response to a cooked limpet extract, but not to a raw extract.[204] By immunoblotting, the major IgE-binding proteins of grand keyhole limpets appeared to have molecular weights of 38 and 80 kDa, but further characterization has not been done.[209]

4. Snail (Gastropod)

In a study of ten subjects allergic to snails, eight experienced bronchial symptoms, whereas six reported no skin or GI symptoms. All subjects could ingest cephalopods and bivalves without adverse reaction,[206] and all ten had positive basophil histamine release and skin tests for snail extracts.

In another study,[203] 61% of 70 atopic subjects were positive for boiled snail extract in skin tests and 19% showed RAST reactivity for snail antigens. Asthma symptoms were reported after ingestion of snail by 15% of the subjects. Six different IgE-binding protein bands resulted from SDS-PAGE and immunoblotting of the boiled snail extract, ranging in molecular weight from 12 to over 66 kDa. One microgram of snail extract used in basophil histamine release studies in skin-test- and RAST-positive subjects was positive. Keyhole limpet hemocyanin did not provoke cross-reactive basophil histamine release or skin-test responses in these subjects. Specific IgE binding was exhibited to a 66-kDa band (two of ten sera), a 24-kDa band (nine of ten), a 15-kDa band (three of ten), and a 12-kDa band (six of ten); however, these bands were not characterized further.

V. LESS COMMONLY ALLERGENIC FOODS OF PLANT ORIGIN

A. Buckwheat

Buckwheat is a member of the Polygonaceae group of weeds and is not related to the cereals.[210] Ingestion of buckwheat has been associated with GI symptoms, urticaria, angioedema, and anaphylaxis.[211,212] Occupational exposure to buckwheat has been documented to cause occupational allergic reactions. Immunoblotting using sera from a patient who suffered repeated episodes of anaphylaxis following buckwheat ingestion revealed four IgE-binding bands in the molecular weight range 9 to 40 kDa, all of which were glycoproteins.[213] Yano et al.[214] found three proteins of molecular weight of 8 to 9 kDa that bound IgE from serum of patients with high RAST scores to buckwheat. One of the proteins was a trypsin inhibitor.

B. Lupin (*Lupinus albus*)

Lupin is a member of the legume family. It is a pea-like plant cultivated worldwide, primarily for use as a feed or to be plowed under for its nutrients.[215] However, this legume has also been evaluated over the years for use in foods for human consumption.

Hefle and Bush[216] reported the adverse reaction of a peanut-allergic child to a lupin-fortified pasta product. They investigated the lupin proteins further using ST and *in vitro* analysis of IgE binding from six peanut-allergic adults. The IgE-binding proteins of lupin have molecular weights of 21 kDa and from 35 to 55 kDa in SDS-PAGE, and are heat stable. Three of the six sera bound only weakly to the 21-kDa band, whereas this band appeared to be a prominent IgE-binding protein for the other three individual sera. The subjects experiencing a positive skin-test reaction to lupin extract also reported a history of adverse reactions to green peas.[216]

C. Peas

Although peas are part of the legume family, the frequency of allergic sensitivity to peas is considerably less than to peanuts or soybeans. However, this may be related to the degree of exposure to pea proteins in human diets. Addition of leguminous proteins, such as pea and lupin, into the diet in increasing amounts could increase the prevalence of pea allergy.

The globulin fraction accounts for 75 to 80% of the total seed protein, whereas the albumin fraction constitutes most of the remainder, depending on the cultivar and the isolation methods used.[217] The green pea (*Pisum sativum* L.) legumin is approximately 256 kDa and is composed of six pairs of 20- and 40-kDa subunits. Each subunit is made up of a 60-kDa polypeptide chain.[218] Green pea vicilin is a trimer composed of 50-kDa subunits,[219,220] and a vicilin-like pea protein called convicilin consists of four monomers of 71-kDa.[221]

Crude pea and pea albumin extracts produced positive skin tests. However, the major pea globulins legumin (11S) and vicilin (7S) did not produce positive reactions in skin tests in a study of ten green pea-sensitive subjects.[222] The albumin fraction retained all of its allergenic activity when heated or boiled.

A 53-kDa major pea albumin designated "PMA-L" had two subunits of approximately 25 kDa, Another component, "PMA-S," had a molecular weight of 48 kDa and possessed two 24-kDa subunits. Neither PMA-L nor PMA-S was significantly degraded during germination, indicating that they are probably not seed storage proteins.[223] Later work on a pea albumin designated "Psa MA" (*P. sativum* major albumin), a homodimer with subunits of 24 or 25 kDa, led to the discovery and sequencing of "Psa LA," a low-molecular-weight albumin component (11 kDa) with 54 amino acid residues. The protein is most probably a dimer of two 6-kDa polypeptides,[224] and does not possess protease inhibitor activity. Psa LA did not react to antibodies raised against the pea storage proteins, Psa MA, or pea lectin by Ochterlony or immunoblotting techniques. A green pea allergen with an approximate molecular weight of 1.8 kDa and a carbohydrate content of 30% in SDS-PAGE was purified from pea dialysate,[225] but was not characterized further.

D. Psyllium

Psyllium mucilloid is obtained from the seed husk of plants in the *Plantago* genus. It has been used in bulk laxatives since the 1500s, and its allergenicity in occupational settings is well documented.[226,227] Psyllium was added to cereal products after observations that it was useful in lowering serum cholesterol levels in hypercholesterolemic patients.[228] Ingestion of these psyllium-fortified cereal products has caused significant anaphylactic reactions;[229] most of the affected individuals had been sensitized through occupational exposure, but some patients were not sensitized via this route. In one study,[229] 20 patients who were either inhalation- or ingestion-sensitized possessed IgE to six psyllium protein bands in the molecular weight range of 20 to 36 kDa.[229] These IgE-binding proteins were not characterized further.

E. Rice

Rice (*Oryza sativa*) is a dietary staple for approximately one-half of the world population. In Japan, rice frequently aggravates AD through IgE-dependent mechanisms. There is one report of two protein fractions of rice, glutelin and globu-

lin, being reactive with specific IgE from rice-allergic individuals as demonstrated by RAST.[230] The major rice allergens consist of micro-heterogeneous albumin proteins, with molecular weights ranging from 14 to 16 kDa with pI of 6 to 8,[17] encoded in a multigene family.[231] The nucleotide sequence of cDNA coding for the major rice allergen has been determined,[232] a 486-nucleotide sequence with an open reading frame that encodes for a 162 amino acid residue. The mature protein has a molecular weight of approximately 14.7 kDa. The deduced amino acid sequence has homology to barley trypsin inhibitor (20%) and wheat α-amylase inhibitor (40%).[232] The allergenic rice protein is heat stable and resistant to proteolysis. Because a single protein accounts for much of the allergenic reactivity, attempts have been made to reduce rice allergenicity by selecting strains induced by chemical mutation to produce hypoallergenic cultivars.[233] A second approach is to use the nucleotide sequence of the gene to prepare an anti-sense gene to reduce the amount of allergenic protein formed in the grain.[233] Watanabe[234] employed a process that involved the use of a protease to reduce the allergenicity of the rice grains. This was somewhat successful, but the amount of enzyme required was large.

F. Apples

Fresh apples may produce localized oral allergy syndrome (OAS). This syndrome seems much more common in Europe than in other parts of the world (see "Allergenic Foods" by Susan L. Hefle et al. in this issue). In Europe, apple is a common allergenic food. Ebner et al.[235] showed immunologic cross-reactivity between *Bet v* 1 from birch pollen and an apple allergen with a molecular weight of 17 to 18 kDa. They were also able to hybridize RNA from birch pollen to apple-derived transcripts that approximated 800 base pairs. Vieths et al.[236] linked allergenicity to the 18-kDa protein in apple by immunoblotting and also found allergens at 13 kDa and at 30 to 50 kDa. They compared various apple varieties as to the concentration of the 18-kDa protein and noted that Golden Delicious and Granny Smith apples contained higher concentrations of this protein than other varieties. Gloster and Jamba varieties were low in the 18-kDa protein. Vieths et al.[237] have conducted amino acid sequencing of 26 N-terminal residues of the 18 kDa protein and found significant sequence homology (62%) between this protein and that of the major birch pollen allergen *Bet v* 1. The 18-kDa allergen may be related to disease resistance. Hsieh et al.[238] found that of 34 sera of patients with tree pollen allergies in immunoblotting studies, 37.5% demonstrated IgE binding to an 18-kDa protein and 75% to a 31-kDa protein. In addition, IgE binding was observed to proteins of 12, 14, 16, 38, and 50 kDa mol wt (in SDS-PAGE). The amino-terminal amino acid sequences of the 18- and 31-kDa proteins share about 50% sequence identity with *Bet v* 1 and other disease resistance proteins of various plants.[238] Although Vieths et al.[236] found that storage increased the levels of the 18-kDa allergen, this study found no observed increase in 18-kDa allergen content resulting directly from ripening and maturation. Therefore, it was concluded that the increased levels of the allergenic protein might be produced by factors related to disease resistance.[238]

G. Cabbage

Cabbage (*Brassica oleracea*) is a member of the Brassicacea (mustard family). Using gel filtration techniques, allergenic fractions with molecular weights ranging from 20 to 67 kDa have been identified.[239]

H. Celery

Celery has been reported to be a cause of OAS symptoms in individuals with mugwort and birch pollen allergy (see Section VII.F). Occasionally, celery can also cause more serious manifestations. Vallier et al.[240] identified a 15-kDa profilin protein from celery. This was a heterogeneous protein that showed two bands on SDS-PAGE. It was shown to be cross-reactive with birch and mugwort pollens using IgE antibodies from subjects with these allergies.[240,241] As with apple, celery allergy is quite common in Europe

(see "Allergenic Foods" by Susan L. Hefle et al. in this issue).

I. Chocolate

Chocolate is reported by many patients to cause allergic reactions, but such sensitivity is frequently not reproducible. There are a few documented cases of actual sensitivity by DBPCFC. Skin-test reactivity as assessed with intradermal testing, however, is frequently positive.[242,243] Because ST with chocolate produces many false-positive reactions, the use of these reagents as diagnostic tests should be avoided. Because it is questionable whether chocolate acts as a true IgE-binding allergen, no work to characterize its constituents has been done.

J. Melons

Watermelon, cantaloupe, honeydew melon, and banana occasionally cause OAS in ragweed pollen-allergic individuals. Occasionally, more severe systemic reactions can occur. Enberg et al.[244] separated a protein in a watermelon extract by isoelectrofocusing at a pH range of 4 to 6, transferred the protein to a nitrocellulose membrane, and probed the membranes for IgE binding using serum from watermelon-sensitive subjects. No consistent IgE binding was observed. However, by using SDS-PAGE and immunoblotting, Jordan-Wagner et al.[245] found a 15-kDa protein in watermelon extracts that bound IgE from watermelon-sensitive subjects. The protein was cross-reactive with a similar protein found in celery, cucumber, and carrot, but has not been further characterized.

K. Papain

Papain is a proteolytic enzyme derived from papaya. It is used as a meat tenderizer, for clarifying beer, and as a reagent in the biochemical, immunochemical, and pharmaceutical industries. Several reports have implicated papain as a cause of occupational asthma.[246,247] Some individuals have developed sensitivity as a result of ingestion of papaya,[248] or meat tenderizer,[249] or from the injection of chymopapain used to treat herniated invertebral discs.[248] Positive skin and RAST tests and oral challenges have confirmed the presence of IgE-mediated sensitivity to this enzyme.

L. Peach

Peach can produce allergic reactions ranging from OAS to anaphylaxis.[250] Several studies of peach proteins analyzed by immunoblotting have been reported with variable results. Wadee et al.[251] detected a 30-kDa allergenic protein in peach extracts, but not in pear or apple extracts. Taylor et al.[252] found several labile allergenic proteins or glycoproteins with molecular weights of 41, 67, and 72 kDa in fresh peach pulp. Lleonart et al.[253] described a peach skin protein doublet with a molecular weight of 8 to 10 kDa that bound IgE from peach-sensitive subjects. Pastorello et al.[250] identified a 13-kDa allergenic protein common to several Prunoideae (apricot, cherry, peach, and plum). They also found a 14- and a 30-kDa allergen in the peach and cherry, respectively. None of the peach IgE-binding proteins have been purified or sequenced to date.

M. Potato

Sensitivity to potato is fairly uncommon. Immunoblotting studies using serum from subjects with sensitivity to potato have shown IgE binding to raw potato proteins with molecular weights ranging from 16 to 65 kDa with pI of 4.5 to 5.2.[254,255] Some individuals with birch pollen allergy report lip and mouth itching from ingestion of raw potatoes. Often these individuals tolerate cooked potato without difficulty. The putative allergen is theorized to be profilin (see Section VII.F).

N. Tomato

Tomato (*Lycopersicon esculentum*) is a member of the Solanaceae family. Bleumink et al.[256] fractionated tomato proteins using ion-exchange chromatography. An allergenic glycoprotein frac-

tion was isolated. However, allergens were polydispersed throughout a number of fractions. Allergenicity also depended on the state of ripening of the fruit. Skin-test reactivity was highest in red-ripe fruit stored for 14 d at room temperature. It was hypothesized that the allergenic fractions were produced by nonenzymatic browning (Maillard) reactions between proteins and reducing sugars during the ripening process.

O. Miscellaneous Food Allergens

1. Cottonseed

Cottonseed (*Gossypium* species) can be used as a source of edible oil and protein. The presence of a toxic pigment called gossypol limited its use in human foodstuffs until traditional breeding techniques resulted in gossypol-free cultivars.[257] This development led to the use of cottonseed protein and oil in food products. Anaphylactic reactions have been documented after consumption of food supplements, candy, and bread containing cottonseed protein or flour.[258–260] The source of the allergen appears to be the 2S proteins, which are water-soluble albumins.

2. Sesame Seed

Sesame seed (*Sesam indicum*) is an East Indian herb of the Pedaliacea family. Both the seed and oil obtained from sesame seeds can produce anaphylaxis.[261] Using ultracentrifugation and immunoblotting techniques, multiple allergenic components have been noted with molecular weights ranging from 8 to 84 kDa.[199,262] Using immunoblotting techniques and RAST inhibition, several cross-reactive bands have been identified among a variety of foods, including sesame seed, hazelnut, rye grain, kiwi, and poppy seed.[263]

3. Poppy Seed

Poppy seed produces systemic reactions on occasion.[264] Other than possessing cross-reactivity as mentioned above, little work on its allergenic components has been done.

4. Spices

Several members of the Apiacea family, which includes celery, anise seed, fennel, coriander, and cumin, elicit positive skin tests in celery-allergic individuals, especially those with mugwort pollen and birch pollen sensitivity[265](see Section VII.F). Cross-reactivity between mugwort pollen and coriander has also been demonstrated using RAST inhibition techniques.[266] Helbling et al.[267] found some cross-reactivity between raw carrots and the apiaceous spices, anise, cumin, and coriander. Immunoblotting studies by this group also demonstrated IgE binding to 17-, 21-, and 23-kDa proteins in anise. IgE binding could only be detected to a 17-kDa protein in cumin and coriander.

5. Mustard

Yellow mustard (*Sinapis alba*) and oriental mustard (*Brassica juncea*) are members of the Brassicacea family. Allergens from both of these plants have been cloned. *Sin a* 1 is a 2S albumin from yellow mustard seed and is the major yellow mustard allergen. It is a seed storage protein consisting of two disulfide-linked polypeptide chains, each with 39 and 88 amino acid residues, respectively.[268] The amino acid sequences of both chains have been established. This protein has also been isolated from rapeseed, castor bean, and Brazil nut.[268] Further studies have suggested that IgE binding to the protein is conformational, because reduction and carboxyamidomethylation of both polypeptide chains produced a substantial decrease in IgE binding. Specific IgE binding was also decreased when the only tyrosine residue of the protein underwent nitration. This tyrosine residue is located at the 60th position on the 88 amino acid chain. A murine monoclonal antibody which also binds to this site decreased IgE binding by 50%. This role for tyrosine is similar to the importance of tyrosine and IgE binding to the codfish allergen, *Gad c* 1.[268]

Further studies of the genetics of the *Sin a* 1 allergen were conducted using polymerase chain reaction (PCR) technology using nondegenerative oligo primers encoding for both the N- and C-terminal regions. Two nucleotide sequences were identified indicating polymorphism of the

gene. The 2S protein is a member of the Napins family, which is encoded by genes without introns, synthesized as polypeptide precursors, and processed by specific maturation proteases to render the two chains of the mature protein.[269]

Isolation of the major allergen of the oriental mustard seed, *Bra j* 1, has been reported.[270] The oriental mustard is more commonly used in the U.S. and Japan, the yellow mustard being used predominantly in Europe. Table mustard is often a mixture made from the flour obtained from the seeds of both species. The *Bra j* 1 allergen showed microheterogeneity and was immunologically cross-reactive with the major yellow mustard allergen *Sin a* 1. Its molecular weight ranges between 16 and 16.4 kDa. Both of these proteins appear to have an α-helical structure that is resistant to proteolytic and thermal degradation, and are storage proteins with a high glutamine content. Similar proteins have been identified in cabbage and turnip, both of which are members of the same family of plants.

VI. FOODS AS INHALANT ALLERGENS

The ingestion of food proteins with sensitization occurring through the GI tract is the principal cause of food allergic reactions. In some cases, however, individuals become sensitized through a respiratory route, particularly following occupational exposure in the food-processing industry or perhaps as a consequence of sensitization to various pollens that share cross-reactive allergens to foods. It is also hypothesized that food allergens may be incorporated into house dust and become airborne.[271]

Several allergens cause occupational asthma. Milk proteins have been implicated in the development of occupational asthma in two individuals. In one case, inhalation of powdered milk led to the development of nasal symptoms and wheezing.[272] The patient also experienced oral itching and burning upon ingestion of milk products. The reactive protein was identified as sodium caseinate. In a second case, Bernaola et al.[273] reported an individual who developed occupational asthma as a result of exposure to α-lactalbumin.

Egg proteins have been associated with the "bird-egg syndrome." Individuals with this syndrome generally become sensitized through the respiratory route after being exposed to bird serum antigens. Subsequently, they react to ingestion of chicken egg yolk. Although the syndrome is primarily identified in adults,[274] it has also been described in children.[275] The allergen responsible for this reaction is α-livetin, a serum albumin of 70 kDa. This protein is also present in the yolk of the chicken egg.[276]

van Toorenenbergen et al.[277] found a 60-kDa protein in egg yolk to be allergenic in adults who were exposed to bird serum antigens. In contrast, children without bird exposure, but with egg allergy by ingestion, reacted to a 35-kDa protein. These egg yolk proteins were not further characterized.

Organic dusts from legumes and other plants sensitize through the respiratory route. Inhalation of soybean flour has been implicated as a cause of inhalant asthma in a number of individuals.[278] Evaluation of the soybean proteins involved in soy bean flour asthma has identified nine proteins with molecular weights ranging from 14.9 to 54.5 kDa.[279] Soybean lecithin has also produced occupational asthma in a sensitive individual.[280] Epidemic outbreaks of asthma have been attributed to airborne soybean proteins (Barcelona, Spain)[281] and airborne castor bean dust.[282–284] The green bean has been implicated as a cause of occupational asthma in a homemaker preparing and cooking raw green beans.[285] Green coffee bean dust has caused occupational asthma[286] in coffee processors.

Fish and crustacea are responsible for occupational reactions, particularly in the seafood industry.[287] A number of occupational asthma cases have been attributed to the inhalation of airborne proteins from seafood such as Norway lobster (*Nephrops norvegicus*), king crab (*Paralithodes camshaticus*), snow crab (*Chinoecetes opilio*), and squid,[204] among others. Sensitization is believed to follow inhalation of proteins in steam generated in processing.

Baker's asthma is observed in individuals working in the baking industry. Proteins of wheat, rye, and barley flour are the most commonly implicated sources of allergens. A number of proteins from these cereal grain flours have been purified,[288] and allergens from wheat barley flour[289] have been cloned. Blands et al.[290] found 53% of

163 bakers examined had allergy to wheat flour, 25% to rye flour and 23% to both. The highest skin-test reactivity was induced by the water-soluble fractions. Forty antigenic components were identified by CIE, some of which may share partial identity due to proteolytic degradation. Franken et al.[291] demonstrated IgE binding to a 14-kDa protein using immunoblotting techniques. A similar protein was found in rye flour, although there was less IgE binding to the rye than to the wheat material. Pfeil et al.[292] found three major wheat allergens in immunoblotting studies with molecular weights of 47, 17, and 15 kDa. Gomez et al.,[293] using immunoblotting techniques, identified the major allergens associated with baker's asthma as members of the α-amylase inhibitor family from wheat endosperm. Consequently, several trypsin α-amylase inhibitor proteins from wheat species (*Triticum duram* Desf cv Agathe,[294] *T. aestivum* L. genomes AABBDD cv Chinese Spring,[295] *T. turgidum* L. genomes AABB cv Senatoree-Caplelli,[295] *T. aestivum* cv Timgalen[296]) have been molecularly cloned and their DNA sequences reported.

Armentia et al.[288] purified 11 proteins from wheat and barley flour that comprised a family of α-amylase/trypsin inhibitors. Most of the flour allergens were found in the albumin and globulin fractions. Subsequently, the prominent allergens have been identified, including a monomeric form from wheat and a dimeric form from barley. Mena et al.[289] cloned the major barley allergen, a 14.5-kDa barley endosperm protein. This is a glycosylated monomeric member of a multigene family of inhibitors of α-amylase/tryptase from cereal grains. Its deduced amino acid sequence contains 132 residues. Five continuous IgE-binding epitopes have been identified on the wheat amylase inhibitor.[50]

VII. CROSS-REACTIVITY

A. Cow's Milk

Individuals allergic to cow's milk will often have serum IgE antibodies to goat or sheep's milk.[13,14,21] For the most part this has not been confirmed by DBPCFC, but nine of ten cow's milk-allergic children ingesting goat's milk had reactions similar to those provoked by cows' milk (H. A. Sampson, personal communication).

B. Fish

The degree of allergic cross-reactivity between different fish species varies widely between individuals.[297] Several studies have attempted to assess the reactivity of fish-allergic subjects to different species of fish. de Martino and associates[298] performed skin tests in 20 cod-allergic children with 17 different species of fish. Eel extract was the most reactive (85%), although parents in every case indicated that their children had never eaten eel. Pascual et al.[299] studied sole, whiff, witch, hake, cod, and albacore skin-test reactivity in 79 children with fish hypersensitivity. All of the subjects reacted to the six types of fish. It is apparent that skin testing alone cannot adequately predict clinical cross-reactivity for fish species.

Research using DBPCFC and other tests[56,298] in fish-allergic children has shown that subjects are not uniformly sensitive to all species. Bernhisel-Broadbent et al.[83] found variable reactions to DBPCFC in that positive oral challenges resulted with only one fish species in seven subjects, two fish species in one subject, and three fish species in two subjects. Skin tests in these subjects were positive to all ten fish species evaluated in 8 of the 11 subjects. The three remaining subjects had positive skin tests to at least two species. However, among nine fish-allergic subjects in one study,[60] only one did not react to oral challenge to the type of fish they reacted to in ST.

The *in vitro* cross-reactivity of fish extracts has also been investigated using SDS-PAGE and immunoblotting. One study[83] examined extracts from nine species of raw and cooked fish. With the exception of raw and cooked tuna, all extracts had a prominent band in SDS-PAGE at 13 kDa that appeared to be analogous to the major codfish allergen, *Gad c* 1. Immunoblotting results using serum from fish-allergic individuals showed the most pronounced IgE binding was to this 13-kDa band. Tuna did not appear to contain this 13-kDa protein, possibly explaining why tuna does not cross-react extensively with other species.

Immunoblotting further indicated IgE binding to extracts of fish to which the subjects had no

clinical sensitivity as determined by oral challenge. In ELISA inhibition assays, the concentration of fish extract required to achieve 50% inhibition of specific IgE binding was similar for fish to which patients were clinically allergic and those to which they were not clinically allergic.[83]

RAST inhibition studies have shown a variable amount of cross-reactivity among species of fish. Tuna or albacore has been the least effective inhibitor in RAST studies,[298,300] showing the fewest reactions in skin tests.[299] Helbling et al.[300] found that tuna extract inhibited a trout RAST by 45% and a mackerel RAST by only 26%. When RAST inhibition was performed with *Gad c* 1 against hake, whiff, sole, witch, and albacore, it was observed that although parvalbumin is important as an allergen in cod, it is not as important in another gadiform, hake, and even less so for other types of fish.[299]

Helbling et al.[300] found that many individuals with a history of adverse reactions to fish also report adverse reactions to crustacea, mainly shrimp. However, RAST inhibition studies showed that fish extracts (salmon, anchovy, tuna, trout, pollock, and mackerel) could not inhibit a shrimp RAST, indicating that the individuals probably had multiple food allergies.

C. Crustacea and Mollusks

1. Crustacea

Histories of IgE hypersensitivity to multiple types of shellfish are frequently reported. Shrimp-allergic individuals react to other species of crustacea. They exhibit positive skin tests and RAST to other crustaceans.[98,301] Studies using RAST inhibition assays and additional immunochemical techniques indicate common antigenic/allergenic epitopes in shrimp and other crustacea.[101,302,303] Halmepuro et al.[101] found that five of six IgE-binding CIE precipitins from crawfish share partial immunologic identity to precipitins in spiny lobster, white shrimp, and blue crab extracts, and that three of four spiny lobster IgE-binding CIE precipitins had partial immunological identity with crawfish, white shrimp, and blue crab extracts. In addition, extracts from shrimp, blue crab, and crawfish all inhibit *Pen a* 1 RAST to a similar extent.[5] *Pen a* 1-reactive IgE as well as *Pen a* 1-specific monoclonal antibodies can detect a 36-kDa protein present in crawfish, blue crab, and spiny lobster, perhaps indicating the presence of common IgE-binding epitopes.[93] The presence of IgE to unique and shared class allergens may explain an individual's clinical sensitivity to one or more members of the crustacea.

2. Mollusks

Although mollusks are much less allergenic than crustacea, a study of cross-reactivity of oyster and crustacea extracts indicated some common antigenic/allergenic epitopes based on RAST inhibition studies. Shrimp, blue crab, spiny lobster, and crawfish were all highly cross-reactive with oyster.[207]

Squid-allergic patients in one study also experienced symptoms after ingesting shrimp, and demonstrated strong positive skin-test reactions to boiled squid extracts and various commercial crustacea extracts. Specific IgE-binding inhibition studies showed cross-reactivity between shrimp, lobster, crab, oyster extracts, and boiled squid extract, although cross-reactivity was not demonstrated between squid and octopus (another cephalopod), nor squid and round clam, mussel, or other mollusks.[205]

Limpet-sensitive subjects in one study could consume squid, clam, cockle, octopus, snail, oyster, shrimp, lobster, and crawfish without adverse reaction.[204] Cross-allergenicity between grand keyhole limpet, abalone, and keyhole limpet hemocyanin was demonstrated using sera from limpet-sensitive subjects in a raw limpet RAST.[208]

3. Seafood/Insect Cross-Reactions

Crustacea and mollusks may share allergenic determinants with some species of arthropods, as phylogenic conservation may occur. Shared allergenic determinants have been demonstrated between the major shrimp allergen and fruit fly (*Drosophila*) antigens. *Pen a* 1 and *Pen i 1* share 86 to 87% sequence similarity with fruit fly tropomyosin.[5,92] Patients allergic to Chironomids (nonbiting midges) often demonstrate positive skin

tests to crustacea. Chironomid extracts inhibit shrimp RAST, and vice versa,[304] although other researchers report low cross-reactivity using this assay method.[305]

Witteman et al.[306] found that a monoclonal antibody raised against *Dermatophagoides pteronyssinus* (dust mite) extract cross-reacted with a shrimp allergen, presumably *Pen a* 1, and also reacted with extracts of chironomids, mosquitos, and cockroaches. The authors concluded that tropomyosin is involved in cross-reactions between mite, shrimp, and insects in shrimp-allergic individuals. Aki et al.[307] recently cloned a recombinant mite (*Dermatophagoides farinae*) protein that is a major allergen for the 31 mite-allergic subjects in the study. The deduced amino acid sequence from a cDNA fragment had 76% homology to fruit fly tropomyosin and was coincident with partial sequence fragments of purified native mite tropomyosin. The deduced amino acid sequence had 11 of 17 and 6 of 9 identical amino acids, respectively, to two IgE-binding segments of shrimp tropomyosin (shrimp residues 50 to 66 and 163 to 161). It was concluded that this new allergen was mite tropomyosin. Some snail-sensitive serum samples show positive IgE binding to mite, and inhibition of snail RAST was elicited by *Dermatophagoides* extract (14 to 66% inhibition).[203] Specific IgE binding for another gastropod, limpet, was significantly inhibited by *D. pteronyssinus*.[205] However, this same group[204] reported no significant inhibition of squid-specific IgE by *D. pteronyssinus* or cockroach extracts.

It is believed that the invertebrate hemoglobin (erythrocruorin) molecule might be involved in the reported cross-reactivity of caddis fly and mollusk.[308] It is a potent allergen for chironomid-sensitive individuals, and serum from caddis-sensitive patients in one study reacted with a component of similar molecular weight in mollusk and bee venom extracts.[308]

D. Legumes

Extensive *in vitro* allergenic cross-reactivity in the legume family has been documented. For example, Barnett et al.[309] found that 25% of sera from legume-sensitive patients reacted strongly with peanut, garden pea, soybean, and chick pea extracts in RAST studies. Bernhisel-Broadbent et al.,[310] using immunoblotting and dot-blotting methods, found extensive *in vitro* cross-allergenicity with peanuts, soybeans, peas, and lima beans in 57 of 62 patients with legume sensitivity. However, an earlier study by the same researchers found that clinical and *in vitro* results did not correlate in evaluating allergenic cross-reactivity in the legume family; 59% of skin-test-positive patients reacted to oral challenge, and only 2.8% reacted in oral challenge to more than one legume. Peanut hypersensitivity accounted for 31% of the positive reactions, soybean 23%, and pea 5%. Green and lima beans gave no positive oral challenge reactions.[6]

The interrelationships of proteins in each legume as subunits or degradation products of one another, or as a result of influence of posttranslational glycosylation on molecular weight, are not understood. The cross-reacting sequences or epitopes in these legumes which cause clinical and *in vitro* reactions are also not known.

The studies reviewed above reveal that although IgE antibodies can cross-react with proteins in related foods and cause positive skin-test and RAST results, the clinical manifestations of such cross-reactivity are apparently rare. However, peanut-allergic individuals can develop severe IgE-mediated reactions to taugeh (sprouted small green beans), a typical component of egg rolls.[311] A case of lupin-fortified pasta causing a reaction in a peanut-sensitive child has recently been reported.[216] The subjects experiencing a positive skin-test reaction to the lupin extract also reported a history of adverse reactions to green peas. Therefore, while it appears from the literature that clinical sensitivity to one legume does not always warrant elimination of all legumes from the diet in most cases, this should be evaluated on an individual basis.

E. Cereal Grains

Molecular techniques have led to increasing insights into the cross-reactivity of cereal grain proteins. The major allergens in baker's asthma have been identified as a group of α-amylase inhibitor proteins. α-Amylase inhibitors from

barley and wheat share 37% amino acid sequence homology.[294] The deduced amino acid sequence homology of rice α-amylase/trypsin inhibitor and wheat is 40%, and between rice and barley it is 20%.[232] A monoclonal antibody directed against a 14-kDa wheat α-amylase inhibitor also recognizes a similar component in rye flour,[291] suggesting shared epitopes. Franken et al.[312] confirmed the importance of α-amylase inhibitor as a major allergen of wheat. The extent of IgE cross-reactivity and clinical sensitivity to cereal grains has been explored recently.[313] Approximately 25% of wheat-allergic children reacted to another cereal grain (barley, oat, or rye).[313]

F. Tree Nuts, Vegetables, Fruits, and Pollen Allergy

It is well documented that individuals with tree pollen allergies (birch, alder, hazel, hornbeam, and oak) also frequently suffer intolerance to nuts, fruits, and vegetables.[314–318] In Northern Europe, up to 70% of patients with birch pollen allergy demonstrate intolerance to these foods, in contrast to 19% of patients not allergic to birch pollen.[314] Apples[314,319] and hazelnuts[315,316] are the most common offenders, although reactions to botanically unrelated fruits, such as kiwi, have been reported.[263,320] Individuals who suffer from grass or weed pollen allergies often show sensitivity to carrot, celery, potato, and some spices,[266,321,322] and many individuals with ragweed allergy report intolerance to fruits in the gourd family, and banana, a nongourd.[244,323] Most of these cross-reactions manifest as OAS, but a certain percentage of individuals experience systemic symptoms.[324] Although many of these cross-reacting allergens are inactivated by cooking, the cross-reacting allergens of tree nuts are heat stable.

T-cell epitope mapping has been investigated for birch, hazel nut, and alder pollens. The specific amino acid sequences resulting in sensitivity, however, have not been identified. Exposure to tree pollens can lead to the development of IgE antibodies that recognize epitopes on a variety of food proteins containing similar amino acid sequences. The primary sensitization is to the pollen, and not to the food.

1. Bet v *1*

Bet v 1 is the major birch pollen allergen and is the most important allergen for birch pollen-food cross-reactions. It is a 17-kDa cytosolic protein whose cDNA sequence[325] is highly conserved in dicotyledonous plants,[326] and has homology to a family of mRNAs that are induced in somatic tissues of some higher plants by pathogen infection.[327]

Using immunoblotting techniques, Ebner et al.[235] found that serum from 81 of 83 birch pollen-allergic subjects showed IgE-binding to *Bet v* 1 and also apple (double bands) in the molecular weight range of 17 to 18 kDa. Complete inhibition of IgE binding to these apple allergens was observed after preincubation of the sera with *Bet v* 1.[235,324] In addition, birch pollen and apple allergen-encoding nucleic acids cross-hybridized in Northern blots.[235] Other researchers have found that the 18- and 31-kDa apple allergens share about 50% identity with *Bet v* 1, in addition to disease resistance genes in other plants.[238] Immunoblotting studies have also revealed that the 18-kDa hazelnut allergen shares IgE-binding similarities with *Bet v* 1, as binding of IgE from patients with hazelnut allergy to the 18 kDa allergen could be blocked by preincubation of sera with recombinant *Bet v* 1.[195]

In a study of 43 patients with birch pollen allergy and a history of intolerance to fruits, two groups were discovered; those whose serum reacted with a 20-kDa protein in birch pollen and fruits, and those who reacted to an 18-kDa protein in birch pollen, fruits, grass pollen, and potato.[315] RAST inhibition and immunoblot analysis indicated that there was antigenic similarity between the allergens of birch pollen and fruits, in particular apple, cherry, peach, and pear, which are all members of the Rosaceae family.

No biological properties of *Bet v* 1 are yet known. It is considered to be a B-cell epitope *in toto*, as only cDNAs encoding the entire open reading frame could be isolated by screening expression libraries with birch-allergic patient serum.[328]

*2. Profilin (*Bet v *2)*

Profilin is involved in birch pollen-fruit sensitivity, but also plays a wider role in cross-

reactivity with other foods.[329] In contrast to other major allergens in botanically related species, profilins represent important cross-sensitizing allergens responsible for allergic symptoms,[326] as approximately 20% of pollen-allergic patients show IgE binding to allergen.[330] *Bet v* 2, a birch pollen protein identified as a profilin with a molecular weight of 14 kDa, has also been identified as a cross-reactive allergen in a variety of fruits and vegetables.[329] Profilins, which are highly conserved ubiquitous proteins found in almost all eukaryotic organisms, control actin polymerization. They have been isolated from a variety of pollens.

Pollen profilins appear to share cross-reactivity with a number of foods. Ebner et al.[324] found in immunoblotting studies that preincubation of birch pollen-allergic serum with recombinant *Bet v* 2 reduced cross-reactive IgE binding to pear, celery, carrot, and potato proteins. Vallier et al.[241] found that of 63 mugwort-allergic subjects, 18 had specific IgE to a 15-kDa protein in mugwort and to two birch pollen proteins at 14- and 16-kDa. Of the 36 that were positive for celery RAST, 18 had specific IgE binding to two celery proteins at approximately 15 kDa. These 18 sera also showed IgE-binding to the 15-kDa protein in mugwort and the 14- and 16-kDa birch pollen proteins. In a later study, the same researchers purified the 15-kDa celery allergen and showed that preincubation with it prevented IgE binding to the 15-kDa allergen present in mugwort and birch pollen. In addition, IgE from three *Bet v* 2-allergic subjects bound to the purified 15 kDa celery allergen, and this binding could be prevented by preincubation with recombinant *Bet v* 2.[240]

Raw maple syrup induced angioedema of the tongue in an individual who had tree pollen sensitivity.[331] It was theorized that a heat-labile allergen, possibly profilin, accounted for this reaction.

3. Cross-Reacting Carbohydrate Determinants

Besides *Bet v* 1 and profilin, carbohydrate structures on glycoproteins are also involved in pollen-vegetable cross-reactivity, but their clinical relevance is doubtful.[321,332]

G. Cross-Reactions between Latex and Foods

With the increase in latex IgE-mediated allergy noted in the last 10 years, cross-reactions of latex with various foods have been reported. Blanco et al.[333] reported that among 25 patients with latex sensitivity, 7 experienced systemic reactions to avocado, 4 to chestnut, 5 to bananas, and 2 each to kiwi, papaya, and figs. RAST inhibition analyses showed cross-reactivity with latex and avocado, chestnut, and banana; therefore, latex shows shared antigenic determinants with other fruits that are not botanically related. Papaya, fig, and kiwi showed a lower association in the RAST inhibition tests.

Anibarro et al.[334] reported a latex-allergic subject who reacted to an oral challenge of chestnut, manifested by urticaria and angioedema. Immunoblotting using this patient's serum showed strong IgE binding to a band at 14 kDa and several in the range of 25-30 kDa less strongly in addition to binding to latex proteins in these same molecular weight ranges.[335] In another study, 7 out of 10 latex-allergic subjects experienced anaphylaxis to avocado. These individuals also reported allergies to banana, chestnut, kiwi, and papaya. RAST inhibition suggested common epitopes among avocado, latex, chestnut, and banana.[335] Latex-allergic subjects have also reported anaphylaxis to banana or chestnuts.[336] In addition to the cross-reactivities noted above, latex cross-reaction has been associated with celery, passion fruit, and peach.[337]

VIII. RECOMBINANT ALLERGENS AND IMPLICATIONS FOR FOOD TECHNOLOGY

A. Introduction: Advantages and Disadvantages of Recombinant Allergens

The dramatic increase in knowledge of allergen structure since 1983 is due primarily to the successful application of recombinant DNA technologies. The molecular techniques have helped define primary antigenic structure, as well as B- and T-cell epitopes from a variety of allergenic proteins.[338]

These technologies have revolutionized efforts to develop reliable sources of allergenic material for laboratory study and clinical use.

It is now possible to create a reliable, reproducible allergen source that has batch-to-batch consistency and is available in stable, large quantities, a remarkable achievement.[339] This should result in improved allergenic extracts for diagnosis and immunotherapy. Recombinant allergens can be produced at very high purity, an advantage over extracts from natural sources that may contain a number of other allergens and nonallergenic material. An additional advantage of recombinant allergens is that they may be added to natural allergenic extracts to improve the quality of current extracts.[340] However, another method would be to use a few (two to four) recombinant allergens cloned from a given source for diagnostic purposes.

Perhaps the greatest value of biotechnology, however, will be to provide recombinant allergens to be used instead of allergens that are present in natural products in limited quantities, such as vespid venoms. Recombinant allergens will provide better tools for allergen standardization, structural investigation, and T- and B-cell epitope identification. Such studies could yield new concepts in immunotherapeutic approaches in the treatment of allergic diseases. Recombinant DNA technology will also be useful in analyzing cross-reactivity between allergens. Such techniques have demonstrated that a number of pollen allergens have inter- and intraspecies and intergenic variability and cross-reactivity.[341]

A major potential disadvantage of recombinant allergens is that their IgE binding affinity may be less than their native counterparts. Also, because many allergenic extracts contain multiple allergens, substantial resources could be required to prepare all recombinant allergens in a specific food, if that was necessary. Finally, the safety and efficacy of recombinant allergens are major concerns and are issues that must be considered prior to use in humans.

B. Requirements of Recombinant Allergens

A major objective for any recombinant allergen produced is that it be comparable to its natural counterpart. Recombinant allergens used *in vivo* should be adequately analyzed for the following properties: the purity of the protein must be defined as described in the World Health Organization (WHO) document on recombinant proteins.[342] Recombinant allergen samples must be demonstrated to lack toxicity using animal model systems (see "Principles and Characteristics of Food Allergens" by Taylor and Lehrer in this issue). These molecules should be stable and the half-life should be assessed with *in vitro* assays normally used in allergen standardization.

Because a number of allergens possess inherent biological activity, such as enzymes or lectins, their recombinant counterpart may possess these activities as well. If there exists undesired traits, such as toxicity, these traits may have to be removed. Site-directed mutagenesis could be used to accomplish this goal. Finally, it is critical that recombinant allergens have similar IgE-binding activity to their natural counterparts.[340]

C. Molecularly Cloned Allergens

A host of allergens have been cloned in the past few years. Emphasis has been directed at inhalant allergens (for review, see Stewart[343] and Scheiner[344]). Among the first allergens to be cloned was the major dust mite allergen, *Der p* 1.[345] Clones were detected by rabbit anti-sera and oligonucleotide probes. In the expression system used, however, IgE binding to the expressed protein could not be readily demonstrated. This suggested that posttranslational events of conformational structure are necessary for IgE binding. Other systems have shown that recombinant allergens can be detected using IgE binding for identification of relevant clones.

A number of nonfood inhalant allergens have been identified including numerous grass pollen antigens.[346] The sequence homology of birch pollen allergens with food such as hazelnut and the profilins was discussed in Section VII.F. Several ragweed pollen allergens have also been cloned. The use of PCR-based technology has led to the identification of a full-length gene that encodes for the major cat allergen, *Fel d* 1. The information derived from these studies has led to the identification of T-cell epitopes in the *Fel d* 1

molecule. Currently, synthetic peptides based on these amino acid sequences are under evaluation for immunotherapy to reduce allergic reactions in cat-allergic individuals. Fungal allergens have also been cloned from *Alternaria* and *Aspergillus*. The *Aspergillus* protein is one of the few in which the recombinant allergen has been shown to produce a positive skin test in allergic subjects.[347] Molecular cloning of stinging insect venom proteins has also been reported. The sequence homologies of some of these proteins with plant-derived proteins, and cockroach allergens with food proteins, have been discussed.

Recombinant food allergens have also been produced. One peanut allergen, *Ara h* 2, has been produced using recombinant technology.[348] Genes coding for major allergenic proteins from rice seeds have been reported.[231,232] Several cDNA and genomic clones were prepared. The sequence analysis of these indicated a deduced amino acid sequence that has homology to barley trypsin inhibitor and wheat α-amylase inhibitor. The clones were detected by oligonucleotide probes based on the amino acid sequence of the purified 16-kDa major rice allergen. However, neither of the studies examined the ability of the molecularly cloned protein to bind IgE from human serum. Gonzalez de la Pena et al.[269] described the cloning and expression of a major allergen from yellow mustard seed (*Sin a* I). Cloning was carried out by PCR using nondegenerate oligo primers and coding for the N- and C-terminal regions of the mature protein. Nucleotide sequence analysis indicated that there was polymorphism, suggesting the existence of multiple isoforms of the allergen. However, IgE-binding experiments were not conducted.

A list of allergens cloned and sequenced to date is shown in "Assessment of the Allergenic Potential of Foods Derived from Genetically Engineered Crop Plants" by Dean D. Metcalfe et al. in this issue (see Tables 1 and 2). This list will undoubtedly increase with time and will provide invaluable information on allergens and facilitate new therapies for the treatment of allergic diseases.

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Assessment of the Allergenic Potential of Foods Derived from Genetically Engineered Crop Plants*

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I. INTRODUCTION

Over 60 different plant species, including the most economically important crops, have been successfully genetically engineered; and the list is growing[1] (see Table 2 in "Genetic Modification of Proteins in Food" by Peter R. Day in this issue). Traits being introduced into these crops include insect protection, delayed ripening, virus resistance, modified starch, herbicide tolerance, modified oils, disease resistance, male sterility, and many others[2] (refer to article cited above). More than 20 different genetically engineered plant products are predicted to be in the marketplace within the next 4 or 5 years[3] (see Table 3 in the article cited above).

Prior to market introduction, each of these products is subjected to extensive food, feed, and environmental safety assessments. This article presents a consensus and a science-based approach to assess the allergenic potential of foods derived from genetically engineered crops as one component of that food safety evaluation. Most traits introduced into crops result from the expression of one or a few new proteins. In some cases the desired trait will be produced by introduction of a gene that turns off another gene (e.g., antisense or cosuppression). Therefore, there may be no new protein introduced into the crop, with the exception of a marker gene protein. Typically, these proteins are expressed at low levels and represent a minor percentage of the total plant protein. In contrast, a specific plant may express tens of thousands of discrete proteins, some of which are present in high concentrations (see "Genetic Modification of Proteins in Food"). Despite this enormous variety, allergy to food proteins occurs in less than 1 to 2% of the population.[4,5] Where food allergy is confirmed, patients are usually allergic to only a few specific proteins within one or two specific foods.

Eight foods or food groups (peanuts, soybeans, tree nuts, milk, eggs, fish, crustacea, wheat) account for over 90% of the documented food allergies worldwide (see Table 1 in "Allergenic Foods" by Steven L. Taylor and Samuel B. Lehrer in this issue). This list of foods is based on discussions at the recent expert consultation on food allergies sponsored by the Food and Agriculture Organization (FAO).[6] The majority of individuals with documented immunologic reactions to foods exhibit immunoglobulin E (IgE)-mediated immediate hypersensitivity reactions that can be sudden, severe, and life-threatening;[7,8] and that are thus the focus of general concern. Although other forms of food protein-induced hypersensitivity exist (see "Allergic Reactions to Foods" by John A. Anderson in this issue), the IgE-mediated form provides the most sensitive indicator for the transfer of a protein that induces an immunologic response. It will be the only immune response specifically addressed in this article. Gluten-sensitive enteropathy (celiac disease), a distinct clinical pathologic entity that is observed in specific individuals sensitive to gluten in certain foods, is not specifically addressed in this article. The assessment approach suggested in this article is not appropriate for celiac disease.

* Please note: The content of this article was developed through a series of joint meetings and discussions involving all authors.

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Assessment of the allergenic potential of foods derived from genetically engineered plants should focus on a multilevel analytical process. This approach takes into account the source from which the gene is obtained, amino acid sequence comparisons with known allergens, *in vitro* and *in vivo* immunologic analyses, and an assessment of key physicochemical characteristics.

A rational assessment of allergenic potential should be conducted in a careful step-wise process, using a decision tree strategy (Figure 1). The totality of these assessments provides reasonable assurance that foods derived from new plant varieties will not introduce allergenic concerns beyond those that exist relative to the current food supply. If allergens are introduced, the foods will be appropriately labeled so that they can be avoided by susceptible individuals. This decision tree approach will be illustrated, as appropriate, with examples of proteins introduced into plants by genetic modification.

II. THE SOURCE

The Food and Drug Administration (FDA), in their Policy on "Foods Derived from New Plant Varieties,"[9] recognized the need to address the potential transfer of food allergens. The FDA stated that if a gene was obtained from an allergenic source "FDA considers it prudent practice for the producer initially to assume that the transferred protein is the allergen. Appropriate *in vitro* or *in vivo* allergenicity testing may reveal whether food from the new variety elicits an allergenic response in the potentially sensitive population (i.e., people sensitive to the food in which the protein is ordinarily found)." The FDA further stated that "labeling of foods newly containing a known or suspect allergen may be needed to inform consumers of such potential." The label should disclose the source from which the gene was transferred.*

The source from which the gene is derived is the critical parameter in the assessment of potential allergenicity. It dictates the need to verify whether a gene encoding an allergenic protein was transferred and expressed in a food component, and mandates labeling should that be the case.

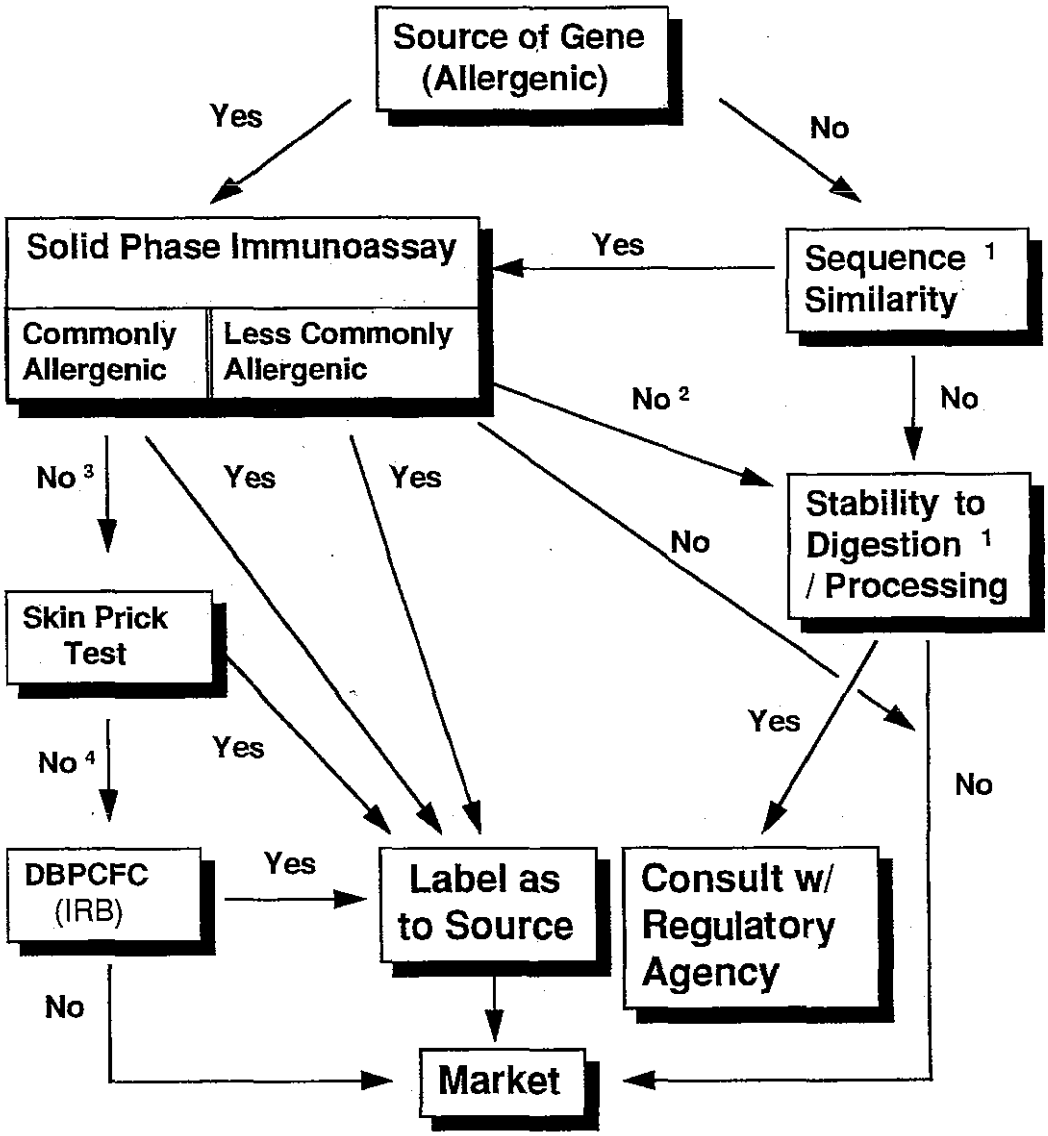
If a gene is obtained from a known allergenic source and the protein encoded is expressed in a food component of the new plant variety, data should be generated to assure that the gene does not encode an allergen. If the gene from an allergenic source is not normally expressed in those

* The FDA has long recognized that certain foods or food ingredients may present risks for some consumers that can be averted. In these cases, the FDA has consistently relied on affirmative labeling declarations identifying the presence of ingredients that possess the potential to trigger allergic reactions in people with sensitivities to such foods or ingredients. Examples include sulfiting agents (21 C.F.R. § 101.100(a)(4)) and color agents like D&C Yellow No. 5 (21 C.F.R. § 74.705 (d)(2)). Another good example, one involving a natural component of food, is gluten. The FDA has required the identification of the source of gluten as either "corn gluten" (21 C.F.R. § 184.1321) or "wheat gluten" (21 C.F.R. § 184.1322) because persons with celiac disease are unable to ingest gluten from wheat without intestinal upset but are able to ingest gluten from corn without side effects. In each case, the required label disclosure alerts consumers to the presence of the ingredient and provides consumers an opportunity to avoid exposure. This use of food labeling ensures that food that is safe for the general population will not provide a risk of harm to comparatively small subpopulations. It is important to note that the FDA has used this labeling authority only when sound science establishes that risk is, in fact, presented.

Under these circumstances, reliance on product-labeling declarations has proven to be an effective and valuable public health mechanism for enabling consumers to choose wisely among foods. Administered in a science-based manner, labeling serves the central purposes of informing, instructing, and warning the consumer.

Moreover, the FDA has also consistently recognized that these goals can only be achieved if consumers can understand and use the information on food labels. For this reason, the agency has limited labeling information to that which is essential about the identity and quality of the subject food or food ingredient.

In addition to being sound as a matter of law and science, this practice of carefully considering whether labeling information is, indeed, justified is reinforced by the very real difficulties that would accompany any attempt to label the plant products of biotechnology. For the most part, these products are fungible crop commodities. Differentiating in the marketplace among such commodities on the basis of biotechnology and traditional breeding techniques would be costly, burdensome, and largely unworkable in light of harvesting, shipping, storage, and processing practices. Moreover, no definitive methodology would be available for distinquishing one type of product from another.



1. It is recommended that an assessment for amino acid sequence similarity to all known allergens and an assessment of stability to digestion be performed for all gene products.
2. Solid phase immunoassay tests depend on availability of sera. Ideally, 14 sera should be used. However, if less than 5 sera are used, then proceed to stability box if results are negative and consult with the appropriate regulatory agency.
3. In the case of equivocal results or suspected false postives, proceed to skin prick tests.
4. DBPCFC's are performed on food products in which there is no evidence of allergenicity based upon solid phase immunoassays and skin prick tests. To assure lack of allergenicity, DBPCFC's should be performed following IRB approval.

FIGURE 1. Assessment of the allergenic potential of foods derived from genetically engineered food crops.

parts of the source organism to which humans are exposed through oral or respiratory routes (e.g., soybean roots or peanut leaves), then the gene need not be considered as coming from an allergenic source. If a known allergen is expressed and restricted to plant parts that are not normally consumed as food, then it should be documented that gene expression and accumulation of the protein

product are limited to the nonfood plant parts before concluding there is no allergenic risk.

In assessing the source from which genes are derived, it is important to distinguish: (a) common allergenic foods; (b) less common allergenic foods and other known allergen sources; and (c) sources with no allergenic histories. For this article, common allergenic foods are defined as the eight food categories mentioned above (also see those listed in Table 1 in "Allergenic Foods"). These foods account for over 90% of the reported food allergies and are those for which clinical reagents, such as patient sera, are likely to be available for the assessments proposed below. This may not be the case for individual species within a food group, such as fish or tree nuts (e.g., swordfish or Brazil nut). Other allergenic sources include the less common allergenic foods listed in Table 2 in "Allergenic Foods" for which clinical reagents may not be readily available, as well as other allergens, for example, pollens, molds, danders, and venoms.

Both common and less common allergenic foods and food groups contain both major and minor allergens. Major and minor allergens are classified according to the frequency with which the allergen is associated with clinical reactions to a specific food. A major allergen is defined as one to which more than 50% of individuals sensitive to that substance react by skin testing (ST) or solid-phase immunoassays. Sensitivity by history or challenge requires signs and symptoms of classic immediate hypersentivity reactions. For example, all individuals with peanut sensitivity react to one or both major allergens in peanuts, whereas the clinical significance of the minor allergens is largely unknown. For these reasons, the majority of concern focuses on the major allergens from both common and less common allergenic foods and food groups.

III. AMINO ACID SEQUENCE SIMILARITY TO KNOWN ALLERGENIC PROTEINS

Assessing the allergenic concerns of foods containing genes from any source should begin with an examination of the amino acid sequence similarity to known allergens. Allergen sources include certain plant- and animal-derived foods (see Tables 1 and 2 in "Allergenic Foods"). Nonfood allergens, such as pollens, fungal spores, insect venoms, and feces, and animal dander and urine,[10] should also be considered. Individuals may experience adverse reactions if they have become sensitized to a protein through the oral, epidermal, or respiratory route, and, subsequently, consume that protein after it has been introduced into a food through recombinant DNA technology. Many respiratory or dermal allergens are labile in the environment or the gastrointestinal tract and are, thus, unlikely to induce sensitivity via that route. However, the oral allergy syndrome (OAS) suggests that even limited exposure in the buccal cavity can induce localized symptoms.

The functions of allergenic proteins *in vivo* are diverse, ranging from enzymes[11] to regulators of the cell cytoskeleton[12] and are of no predictive value in assessing allergenicity. The allergenic proteins of many major sources of allergens, including food allergens, have been characterized by molecular methods (see "Principles and Characteristics of Food Allergens" by Steve L. Taylor and Samuel B. Lehrer in this issue). The important IgE, T-, and B-cell epitopes of some allergens have been mapped.[13–24] The immunologic mechanisms that distinguish atopy from nonpathogenic responses remain obscure.[25] However, from these mapping studies it can be generalized that the optimal peptide length for binding appears to be between 8 and 12 amino acids for T-cell epitopes, and even longer for B-cell epitopes.[26]

Based on this information, it is possible to define a sequence test for comparing the amino acid sequence of an introduced protein with that of known allergens: an immunologically significant sequence identity requires a match of at least eight contiguous identical amino acids. This is well below the level of similarity expected between biologically related (homologous) proteins.[27] The criteria for comparing amino acid sequences may change or evolve over time with additional research and insight into the molecular structure of allergens. The amino acid sequences of allergenic epitopes are known for relatively few allergens, especially food allergens. However, this approach is reasonable in the absence of comprehensive epitope data for allergens in that

no attempt is made to identify matches with known epitopes per se. Instead, the emphasis is on identifying a potential match: failure to find a match of eight contiguous residues anywhere among known allergen amino acid sequences suggests that there is little probability that the introduced protein could possess a shared linear epitope with known allergens. Exact conservation of amino acid sequences of epitopes in homologous pollen allergens of disparate species is occasionally observed. This can explain the IgE cross-reactivity among allergens.[22,28] Furthermore, conservative amino acid substitutions that were introduced into synthetic epitopes[29] or introduced by site-directed mutagenesis[30] reduced IgE-binding efficiency, further supporting the importance of the amino acid sequence of the epitope.

Clearly, this approach is limited in that it cannot identify discontinuous conformational epitopes (see "Food Allergens" by Robert K. Bush and Susan L. Hefle in this issue) that depend on the tertiary structure of the allergen. For example, IgE binding of patient sera to the birch pollen allergen, *Bet v* 3, depends on calcium-regulated conformational changes;[31] *Bet v* 3 contains EF hand motifs, which are calcium-binding domains. IgE binding of *Bet v* 3 depends on the presence of calcium, which presumably changes the conformation so as to allow for recognition of a conformational epitope. The test is also likely to identify conserved sequences that are unrelated to the allergenic potential of the proteins. Therefore, it is used as an indicator and not a determiner of allergenic potential.

Using the FASTA[32] or a similar computer program and the test criteria mentioned above, the amino acid sequences of allergens present in the public domain genetic databases (GenBank, EMBL, PIR, and SwissProt) should be searched for matches to allergenic protein sequences from genetically engineered plants. It is necessary to obtain and retrieve amino acid sequences of allergenic proteins to perform this test. This retrieval method is critical in ensuring that relevant sequences are obtained. A search strategy that only seeks allergen entries on the basis of a key word like "allergen" will not retrieve all relevant food allergens, and may also retrieve nonallergens. For example, many food allergens, such as casein, β-lactoglobulin, and ovomucoid, are not recovered using the key word "allergen." This database should also contain amino acid sequences for all allergenic proteins, not just food allergens. Tables 1 and 2 contain the most comprehensive list of reported amino acid sequences for allergens to date. These tables include food (Table 1) and nonfood allergens (Table 2). Accession numbers are included to facilitate its use. These tables were constructed in May 1995[33] and should be continuously updated to reflect reports of additional amino acid or nucleotide sequences of allergenic proteins.

Searches of this allergen sequence database (or other similar databases) have shown no significant matches for the proteins listed in Table 3, which are examples of proteins expressed in genetically engineered plants. It was concluded from these data that none of these introduced proteins share linear IgE epitopes with known allergenic proteins. Using the information generated from amino acid sequence homology analysis, a different path is taken on the decision tree based on the source from which the gene was derived (Figure 1).

IV. FOOD CONTAINING A GENE DERIVED FROM A COMMONLY ALLERGENIC FOOD

Foods containing a gene derived from an allergenic food, irrespective of the information obtained from the amino acid sequence analysis, must be subjected to immunologic analysis of allergenic potential. Both *in vitro* and *in vivo* assays should be employed. These assays may include the various solid-phase immunoassays such as the RAST or RAST inhibition assay[34,35] or the enzyme-linked immunosorbent assay (ELISA).[36] A positive immunoblot could substitute for a positive solid-phase immunoassay result, but a negative immunoblot would require that a solid-phase immunoassay be performed because of its increased sensitivity. The solid-phase immunoassay employed should allow a calculation of at least a 95% confidence

TABLE 1
Food Allergen Sequences Retrieved from the Public Domain Databases for Proteins and Nucleic Acids*

Species	Common name	Allergen	Synonym/function	Accession
			Food (plant)	
Arachis hypogea	Peanuts	*Ara h* 1	*Clone P41b*	L34402
			Clone 5A1	L33402
			Clone P17	L38853
		Peanut lectin	Agglutinin	S14765
Bertholletia excelsa	Brazil nut	*Ber e* 1	2S albumin (BE2S1 gene)	X54490
Brassica juncea	Leaf mustard	*Bra j* IE-L	*2S albumin large chain*	S35592
		Bra j IE-S	2S albumin small chain	S35591
Carica papaya	Papaya	Papain		M15203
Glycine max	Soybean	Glycinin	A1aBx subunit	X02985
			A2B1a subunit	Y00398
			A3B4 subunit	M10962
			A5A4B3 subunit	X02626
			G1 subunit	X15121
			G2 subunit	X15122
			G3 subunit	X15123
		β-Conglycinin	α-Subunit	X17698
			CG4 subunit	S44893
		Soy lectin	Soy agglutinin	K00821
		Kunitz trypsin inhibitor	KTi-s subtype	X80039
			KTi-a subtype	X64447
			KTi-b subtype	X64448
Hordeum vulgare	Barley	*Hor v* 1	α-Amylase/trypsin inhibitor	S26197
		Hor v 1	α-Amylase/trypsin inhibitor	P32360
Malus domestica	Apple	*Mal d* 1	Profilin	X83672
Oryza sativa	Rice	RAP	Rice allergenic protein	X66257
		RAG1	Rice allergen 1	D11433
		RAG2	Rice allergen 2	D11434
		RAG5	Rice allergen 3	D11430
		RAG14	Rice allergen 14	D11432
		RAG17	Rice allergen 17	D11431
Phaseolus vulgaris	Kidney bean	PR-1	Pathogenesis-related protein 1	S11929
		PR-2	Pathogenesis-related protein 2	S11930
Sinapis alba	White mustard	*Sin a* 1.1	2S albumin/amylase inhibitor	S54101
		Sin a 1.2	2S albumin/amylase inhibitor	PC1247
Triticum aestivum		WGA	Wheat germ agglutinin A	M25536
		WGA	Wheat germ agglutinin D	M25537
Triticum durum	Pasta wheat	WGA	Wheat germ agglutinin	J02961
Triticum turgidum	Poulard wheat	16K allergen	α-Amylase inhibitor	S19296
			Food (animal)	
Bos taurus	Cow	BSA	Serum albumin	M73993
		β-Lactoglobulin	Milk globulin (whey)	X14712
		α-Lactalbumin	Milk albumin (whey)	J05147
		Casein	Type α-S1	M33123
			Type α-S1	M38641
			Type α-S2	M16644
			Type β	M15132
			Type κ	M36641
Gadus callarias	Cod fish	*Gad c* 1	β-Parvalbumin, allergen M	A94236
Gallus domesticus	Chicken	*Gal d* 1	*Ovomucoid*	M10639
		Gal d 2	Ovalbumin Y gene	J00922
		Gal d 2	Ovalbumin	M34352
		Gal d 3	Conalbumin (ovotransferrin)	Y00407
		Gal d 4	Lysozyme	J00885
		Gal d 4	*Isolysozyme*	X61001
		Vitellogenin II	Lipovitellin/phosvitin	A92941
		Apovitellenin I	Low density lipoprotein II	A91484
Metapenaeus ensis	Shrimp	*Met e* 1	Tropomyosin	U08008

Note: Some of these allergens may be airborne or associated with occupational allergies rather than directly ingested.

* Public domain databases: GenBank/EMBL/Genpept ver 86.0, SWISSPROT ver 30, PIR ver 41.

TABLE 2
Nonfood Allergen Sequences Retrieved from the Public Domain Databases for Proteins and Nucleic Acids*

Species	Common name	Allergen	Synonym/function	Accession
		Pollen		
Agrostis alba	Bent grass	*Agr a* 1	Group I	E37396
Alnus glutinosa	Alder tree	*Aln g* 1	*Bet v* 1 homolog	S50892
Ambrosia artemisiifloia	Ragweed (short)	*Amb a* 1.1	Antigen E	A39099
		Amb a 1.2	Antigen E	B39099
		Amb a 1.3	Antigen E	C39099
		Amb a 1.4	Antigen E	D53240
		Amb a 2	Antigen K	E53240
		Amb a 3	Ra3	P00304
		Amb a 5	Ra5	A03371
Ambrosia trifida	Ragweed (tall)	*Amb t* 5	Ra5 homolog	S39336
		Amb t 5	Ra5 homolog	A23859
Ambrosia psilostachya	Weed	*Amb p* 5 (A2)	Ra5 homolog	L24465
		Amb p 5 (A3)	Ra5 homolog	L24466
		Amb p 5 (B1)	Ra5 homolog	L24467
		Amb p 5 (B2)	Ra5 homolog	L24468
		Amb p 5 (B3)	Ra5 homolog	L24469
Anthoxanthum odoratum	Sweet vernal grass	*Ant o* I	Group I	G37396
Artemisia vulgaris	Mugwort	*Art v* 2	Glycoprotein allergen	A38624
Betula verrucosa	Birch tree	*Bet v* 1	Pathogenesis related (PR)	S05376
		Bet v 1N	*Bet v* 1 isoform	X82028
		Bet v 2	Profilin	B45786
		Bet v 3	Profilin	X79267
Carpinus betulus	Hornbeam tree	*Car b* 1	*Bet v* 1 homolog	C53288
Castanea sativa	European chestnut	*Cas s* 1	*Bet v* 1 homolog	PC2001
Corylus avellana	Hazel tree	*Cor a* 1-5	*Bet v* 1 homolog	S30053
		Cor a 1-6	*Bet v* 1 homolog	S30054
		Cor a 1-11	*Bet v* 1 homolog	S30055
		Cor a 1-16		S30056
Cryptomeria japonica	Japanese cedar	*Cry j* 1-A		D26544
		Cry j 1-B		D26545
		Cry j 2		D29772
Cynodon dactylon	Bermuda grass	*Cyn d* 1		A61226
Dactylis glomerata	Orchard grass	*Dac g* 2		S45354
		Dac g 3		A60359
Festuca elator	Reed fescue	*Fes e* 1-A		C37396
		Fes e 2-B		D37396
Glycine max	Soybean	*Gly m* cim1	Cytokinin-inducible protein	U03860
Holocus lanatus	Meadow velvet	*Hol l* 1	30K allergen	Z27084
Hordeum vulgare	Barley	*Hor v* 9	Group IX	U06640
Lolium perenne	Ryegrass	*Lol p* 1	Group I	M57476
		Lol p 1	Group I	M57474
		Lol p 1b	Group I	M59163
		Lol p 2-A	Group II	A34291
		Lol p 2	Group II	A48595
		Lol p 3	Group III	A33422
		Lol p 4	Group IV	A60737
		Lol p 9	Group IX	L13083
		Lol p 30K	30K group V allergen	S38290
		Lol p 34K	34K group V allergen	S38289
		Lol p 50K	50K allergen	S38288
Lycopersicon esculatum	Tomato	LAT52	*Ole e* 1 homolog	P13447
Olea europea	Olive tree	*Ole e* 1		S36872
Parietaria judaica	Parietaria	*Par j* 1		X77414
Parietaria officinalis	Parietaria	*Par o* 1		A53252

TABLE 2 (continued)
Nonfood Allergen Sequences Retrieved from the Public Domain Databases for Proteins and Nucleic Acids*

Species	Common name	Allergen	Synonym/function	Accession
		Pollen		
Phleum pratense	Timothy grass	*Phl p* 1	Group I	X78813
		Phl p 1		Z27090
		Phl p 2	Group II	X75925
		Phl p 5a	Group V, group IX	X70942
		Phl p 5b	Group V	Z27083
		Phl p 6		Z27082
		Phl p 32K	Group V-like	S38294
		Phl p 38K	Group V-like	S38293
		Phl p 11	Group XI/profilin	P35079
Poa pratensis	Kentucky blue-grass	*Poa p* 1	Group I	F37396
		Poa p 1	Group I	A60372
		Poa p 9 (KBG31)	Group IX	M38342
		Poa p 9 (KBG41)		M38343
		Poa p 9 (KBG60)		M38344
Quercus alba	Oak tree	*Que a* 1	*Bet v* 1 homolog	D53288
Secale cereale	Cultivated rye	*Sec c* 30K	30K group Vallergen	S38292
Triticum aestivum	Bread wheat	*Tri a* 2.1	Profilin	S72384
		Tri a 2.2	Profilin	S72374
		Tri a 2.3	Profilin	S72375
Zea mays	Maize	*Zea m* 1	*Lol p* I homolog	JC1524
		Clone c13	*Ole e I homolog*	P33050
		Mites		
Euroglyphus maynei	House mite	*Eur m* 1	Group I, thiol protease	S21864
Dermatophagoides farinae	House mite	*Der f* 1	Thiol protease	X65196
		Der f 2.1	Antigen 2	D10447
		Der f 2.1	Antigen 2	A61241
		Der f 2.2	Antigen 2	D10448
		Der f 2.2	Antigen 2	B61241
		Der f 2.3	Antigen 2	D10449
		Der f 2.3	Antigen 2	PS0417
Dermatophagoides microceras	House mite	*Der m* 1	Thiol-protease	B27634
Dermatophagoides pteronyssinus	House mite	*Der p* 1	Antigen P_1	U11695
		Der p 1	Antigen P_1	JQ0337
		Der p 2		A60381
		Der p 3	Trypsin	U11719
		Der p 4	Amylase	A61242
		Der p 5	14K allergen	S06734
		Der p 7		X17699
Lepidoglyphus destructor	Feces mite	*Lep d* 1		X81399
		Insect venoms		
Apis mellifera	Honeybee	*Api m* 1	Phospholipase A2	P00630
		Api m 3	Melittin	P01501
Dolichovespula arenaria	Yellow hornet	*Dol a* 5	Antigen 5	M98859
Dolichovespula maculata	Whiteface hornet	*Dol m* 1.02	Phospholipase A1	A44563
		Dol m 2	*Hyaluronidase*	L34548
		Dol m 5	Antigen 5 clone f5	J03602
		Dol m 5	Antigen 5 clone f10	J03601
Myrmecia pilosula	Bulldog ant	*Myr p* 1		X70256
Polestes annularis	Wasp	*Pol a* 5	Antigen 5	M98857
Polestes exclamans	Paper wasp	*Pol e* 5	Antigen 5	P35759
Polestes fascatus	Paper wasp	*Pol f* 5	Antigen 5	F44522

TABLE 2 (continued)
Nonfood Allergen Sequences Retrieved from the Public Domain Databases for Proteins and Nucleic Acids*

Species	Common name	Allergen	Synonym/function	Accession
		Insect venoms		
Solenopsis invicta	Red fire ant	*Sol i* 2	Phospholipase	A37330
		Sol i 3		B37330
		Sol i 4		C37330
Solenopsis richteri	Black fire ant	*Sol r* 2	Phospholipase	E60727
		Sol r 3		D60727
Vespa crabro	European hornet	*Ves c* 5.0001	Antigen 5	G44522
		Ves c 5.0002	Antigen 5	H44522
Vespula flavopilosa	Yellow jacket	*Ves f* 5	Antigen 5	B44522
V. germanica	German yellowjack	*Ves g* 5	Antigen 5	A44522
V. maculifrons	Eastern yellowjack	*Ves m* 1	Phospholipase A1	A44564
		Ves m 5	Antigen 5	M35760
V. pensylvanica	Western yellowjack	*Ves p* 5	Antigen 5	C44522
V. squamosa	Southern yellowjack	*Ves s* 5	Antigen 5	D44522
V. vidua	Yellow jacket	*Ves vi* 5	Antigen 5	E44522
V. vulgaris	Yellow jacket	*Ves v* 5	Antigen 5	M98858
		Parasitic Nematodes		
Loa loa	Filarial worm	LL20	15K ladder protein	U03103
		Segmented Worms		
Ascaris lumbricoides	Common roundworm	*Asc l* 1	Aba-1	B37188
A. suum	Earthworm	*Asc s* 1	Aba-1	A37188
		Asc s 1	Aba-1	L03211
		Animals		
Felis domesticus	Cat saliva	*Fel d* 1.1	Antigen 4	M74952
		Fel d 1.2	Antigen 4	M74953
		Fel d 1.3	Antigen 4	M77341
Mus masculus	Mouse urine	*Mus m* 1	Major urinary protein (MUP)	M27608
			MUP I	M16355
			MUP II	M16356
			MUP III	M16359
			MUP IV	M16358
			MUP V	M16360
Rattus norvegicus	Rat urine	*Rat n* 1	Hepatic α-2u globulin	J00737
		Fungi (spores)		
Alternaria alternata		*Alt a* 2	Aldehyde dehydrogenase	X78227
		Alt a 6	Ribosomal protein	X78222
		Alt a 7		X78225
Aspergillus fumigatus		*Asp f* 1	Mitogillin toxin/ ribonuclease	M83781
		Asp f 1-A		S39330
Cladosporium herberum		*Cla h* 2	Enolase	X78226
		Cla h 3	Aldehyde dehydrogenase	X78228
		Cla h 4	Ribosomal P2	X78223
		Cla h 5		X78224

* Public domain databases: GenBank/EMBL/Genpept ver 86.0, SWISSPROT ver 30, PIR ver 41.

TABLE 3
Summary of Proteins Introduced Into Crops by Genetic Engineering

Introduced protein[a]	Crop products[b]
ACC deaminase (ACCd)	Delayed ripening tomato
B.t.t. insecticidal protein (*B.t.t.*)	Insect-protected potato
B.t.k. HD-1 insecticidal protein (*B.t.k.* HD-1)	Insect-protected corn and tomato
B.t.k. HD-73 insecticidal protein (*B.t.k.* HD-73)	Insect-protected cotton
CP4 EPSP synthase (CP4 EPSPS)	Herbicide-tolerant canola, cotton, corn, soybean, and sugarbeet
Glyphosate oxidoreductase (GOX)	Herbicide-tolerant canola and corn
β-D-Glucuronidase (GUS)	Herbicide-tolerant soybean
Neomycin phosphotransferase II (NPTII)	Delayed ripening tomato, insect-protected cotton and potato, Flavr Savr™ tomato
Phosphinothricin acetyltransferase (PAT)	Herbicide-tolerant corn

Note: The specific proteins included in this table were based on those proteins for which digestive stability data were available (see Table 4).

[a] ACC, 1-amino-1-cyclopropane-carboxylic acid; *B.t.t.*, *Bacillus thuringiensis* subsp. *tenebrionis*; *B.t.k.*, *Bacillus thuringiensis* subsp. *kurstaki* proteins from strains HD-73 and HD-1, corresponding to the [(Cryla(c)] and [CrylA(b)] proteins according to the nomenclature of Höfte and Whiteley;[103] CP4 EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4.

[b] Adapted from Fuchs et al.,[104] Ciba Geigy,[105] and Noteborn and Kuiper.[85]

limit from the examination of at least 14 sera* from confirmed allergic patients.

The solid-phase immunoassays (described in detail in "Principles and Characteristics of Food Allergens") use IgE fractions of sera from individuals who are confirmed allergic to the food from which the gene was derived. Serum donors should meet rigid clinical criteria, including testing positive in double-blind, placebo-controlled food challenges (DBPCFC),[34,37] or must have a convincing history of severe, IgE-mediated, systemic reactions.[38] A convincing history would consist of an immediate hypersensitivity response following an isolated ingestion/exposure that required medical management, and that was documented in the medical records. Data from one or more of these *in vitro* assays provide strong evidence as to whether the transferred gene encodes an allergen. Sera from at least 14 documented reactors should be tested separately. Any positive result (outside the 95% confidence limit) from the *in vitro* tests should require that any food contain-

* Binomial probability theory (i.e., that a subject in a population will either pass or fail a test) can be used to determine the sample size (N) needed to predict, with a particular level of confidence, the likelihood that a single individual, from a population composed only of individuals sensitive to the food from which a protein is isolated (i.e., the at-risk population, not the general population), will react to a specific protein. To apply the theory, it is necessary to make certain assumptions about the probability (p) that a random subject in the test population is allergic to that particular protein and will test positive (i.e., fail the test).

To be conservative, a predictive incidence of 20% and a 95% level of confidence was used to determine the number of sera to be recommended. These criteria would provide a 95% probability that an allergen to which at least 20% of the sensitive individuals have IgE would be detected if transferred. Using these assumptions and binomial probabilities, a sample of 14 sera (N = 14) would need to be tested and to show no positive reaction with any of the sera (i.e., passed the test) to conclude that an allergen had not been transferred, under the given assumptions. Using the criteria of 14 sera and a predictive incidence of 50% (instead of 20%) would increase the probability of detecting the transfer of a major allergen to >99.9%, compared with a >95% for a minor allergen present at 20% of the sensitive population. This assessment does not exclude the possibility that the transferred protein could represent an allergen that elicits an IgE response from an even less frequent portion of the sensitive population. However, these values provide reasonable assurance to, and protection for, the public.

ing the gene product be labeled as to the source of the transferred gene, as per FDA guidance[9] (Figure 1).

If the *in vitro* test results are negative or equivocal or if a nonspecific cross-reaction is suspected, then the *in vivo* skin prick test[34] should be employed as a further screen for allergenicity. Details on the types of skin tests and the need to verify the quality of the extracts and standards used for these types of tests are critical and are described in this issue (see "Principles and Characteristics of Food Allergens"). At least 14 patients, skin test positive to the source food in question, with a convincing history of sensitivity should be tested. A positive result from this *in vivo* test would raise the same concerns as a positive *in vitro* test and should require labeling as to the source of the transferred gene (Figure 1).

If no positive response is observed in either the *in vitro* or prick/puncture skin tests, a final test could consist of performing double-blind, placebo-controlled food challenges (DBPCFCs) under controlled clinical conditions with patients sensitive to the food in question. The ethical considerations for this type of assessment would include, but not be limited to, factors such as the likelihood of inducing anaphylactic shock in test subjects and the availability of appropriate clinical safety data and procedures. Most, if not all, institutions that perform DBPCFC studies have Institutional Review Boards (IRBs) that review all DBPCFC studies for ethical considerations. This includes a judgement as to whether the risk of performing the procedure places the volunteer at any increased risk over what should take place in the usual assessment of sensitivity in that subject. Obtaining data from an *in vitro* assay and ST should be prerequisites for requesting DBPCFC studies, to minimize any risks to the participants. An earlier article (see "Principles and Characteristics of Food Allergens") describes details for performing DBPCFC studies, including suggested doses and precautions. If there is a positive reaction in one sensitive patient, of a total of at least 14 sensitive individuals tested in the DBPCFC studies, food derived from crops containing the protein should be labeled as to the source of the transferred gene.

If no positive reactions are observed in these three levels of assessments, it can be concluded that the gene obtained from this allergenic food source does not encode one of the allergenic proteins.

An example that illustrates the effectiveness of this assessment approach is the Brazil nut 2S storage protein. The protein was engineered into soybean to increase sulfur-containing amino acid levels to improve the quality of soybean meal as an animal feed. The expression of the 2S protein in soybean represented a significant fraction of total transgenic soybean seed protein.[39,40] The Brazil nut is known to cause anaphylactic reactions in a small number of sensitive individuals.[41,42] A solid-phase immunoassay was used in conjuntion with immunoblotting to assess whether an allergenic protein from Brazil nut had been transferred to soybean.[40,43] A positive response in the immunoassay was observed with sera for eight of the nine Brazil nut-sensitive individuals. The results showed that the gene obtained from Brazil nut probably encoded the major Brazil nut allergen. If a soybean product containing this protein were to be commercialized, any foods containing soybean products derived from this variety should be labeled as containing protein from Brazil nut. This example demonstrates the value and effectiveness of using *in vitro* assays to identify the transfer of known allergenic proteins by genetic modification.

V. FOOD CONTAINING A GENE DERIVED FROM A LESS COMMONLY ALLERGENIC FOOD OR OTHER KNOWN ALLERGEN SOURCE

In the case of a food containing a gene derived from a less common allergenic food or other known allergen source, irrespective of the information obtained from the amino acid sequence analysis, the food should be subjected to immunologic analysis of allergenic potential wherever feasible. *In vitro* assays should be employed. Sera from at least 14 documented reactors should be tested, if reasonably available (e.g., ragweed). In cases where sera are not reasonably available (e.g., maize), the maximum number of sera obtainable

should be used. However, if fewer than five sera* are obtained, the appropriate regulatory agency should be consulted. An examination of the physicochemical properties (see below) should also be undertaken. Any positive result (outside the 95% confidence limit) from the *in vitro* immunologic assays should require that any food containing the gene product be labeled as to the source of the transferred gene, as per FDA guidance[9] (Figure 1). If no positive result is obtained in the *in vitro* immunoassays and at least five sera are analyzed, the product can be marketed without labeling.

In evaluating a gene product transferred from a less commonly known allergenic food or other known allergen source, the gene product should be subjected to physicochemical analysis if less than five sera are tested using a solid-phase immunoassay and all results are negative. The physicochemical and biological characteristics of the gene product can be compared to the characteristics of known allergenic proteins, as a means of predicting allergenic potential. However, at this time, with the exception of identifying known allergens transferred from allergenic sources, there appears to be no single predictive property that can conclusively determine the allergenic potential of a gene product.[9,44,45]

A key prerequisite for food protein allergenicity is its resistance to digestion (e.g., the stability of the protein to proteolytic and acid conditions of the human digestive system). The relative stability of an allergen to conditions encountered during processing operations used for specific food products (e.g., heat denaturation) is also an important property of most food allergens. For example, peanut and soybean allergens retain their allergenic potential through the steps used in processing food products such as peanut butter[46] and soy flour.[47] For these reasons, digestibility and stability during processing should be considered when assessing the potential allergenicity of a protein introduced into a given food. However, an exception to the observation that food proteins tend to resist digestion is seen in oral allergy syndrome (OAS); labile proteins cause the disease but infrequently cause systemic reactions.

Allergenic proteins are also typically 10 to 70 kDa in molecular weight and are often glycosylated. However, these properties are shared by many nonallergenic proteins, and many allergens themselves are exceptions to this generalization. Therefore, these criteria are not included in the decision process.

A. Digestibility

The ability of food allergens to reach and cross the mucosal membrane of the intestinal tract is likely a prerequisite to allergenicity. Intact proteins are capable of crossing the mucosal membrane of the gut and entering the circulatory system.[48] Clearly, a protein that is largely stable to the proteolytic and acidic conditions of the digestive tract has an increased probability of reaching the intestinal mucosa. Many allergens exhibit proteolytic stability,[49–56] although the majority remain directly untested (see "Principles and Characteristics of Food Allergens").

Simulated gastric and intestinal digestive models of mammalian digestion, as described in the U.S. Pharmacopeia,[57] have been employed to compare the relative stability of the proteins engineered into plants with a number of the commonly known allergenic food proteins.[58] These digestion models have also been used to investigate the digestibility of plant[59,60] and animal[61] proteins, and food additives.[62] A similar model has also been used to examine the stability of milk allergens.[63,64]

One study that looked at this issue examined the common food allergens shown in Table 4. Without exception, these food allergens were stable to digestion in the gastrointestinal (GI) digestive model.[58] For the allergens shown in Table 4, either the allergen or a proteolytic fragment of the allergen was stable for at least 2 min in simulated gastric fluid. The major allergens were typically stable for more than 1 h. Similar stability data were reported by other investigators; however, relative stability was not as well defined.[65,66] In contrast to these allergenic food

* Using five sera, there is a ≥67% probability that an allergen present in the sensitive population at a frequency of ≥20% would be detected, if transferred, and there is a ≥95% probability that a major allergen (an allergen present in the sensitive population at a frequency of ≥50%) would be detected, if transferred.

TABLE 4
Summary of Allergen and Protein Stability in a Gastric Model

Protein	% Total protein	Stability (min)[a,b]	
		Whole protein	Fragments
	Egg white allergens[106]		
Ovalbumin (*Gal d* 2)	54	60	—
Ovomucoid (*Gal d* 1)	11	8	—
Conalbumin (*Gal d* 3)	12	0	15
	Milk allergens[106]		
β-Lactoglobulin	9	60	—
Casein	80	2	15
Bovine serum albumin	1	0.5	15
α-Lactalbumin	4	0.5	2
	Soybean allergens		
β-Conglycinin (β-subunit)[107]	18.5[c]	60	—
Kunitz trypsin inhibitor[108]	2–4	60	—
Soy lectin[109]	1–2	15	—
β-Conglycinin (α-subunit)[107]	18.5[c]	2	60
Glycinin[107]	51	0.5	15
Gly m Bd 30K[110]	2–3	0	8
	Peanut allergens		
Ara h II[111]	6[d]	60	—
Peanut lectin[112]	1.3	8	—
	Mustard allergens[113]		
Sin a I	20	60	—
Bra j IE	20	60	—
	Common plant proteins		
Rubisco LSU (spinach leaf)[114]	25[c]	0 (<15 s)	—
Rubisco SSU (spinach leaf)[114]	25[c]	0 (<15 s)	—
Lipoxygenase (soybean seed)[115]	<1	0 (<15 s)	—
Glycolate reductase (spinach leaf)[e]	<1	0 (<15 s)	—
PEP carboxylase (corn kernel)[e]	<1	0 (<15 s)	—
Acid phosphatase (potato tuber)[e]	<1	0 (<15 s)	—
Sucrose synthetase (wheat kernel)[e]	<1	0 (<15 s)	—
β-Amylase (barley kernel)[e]	<1	0 (<15 s)	—
	Introduced proteins[58,100]		
B.t.t. insecticidal protein	<0.01	0 (<30 s)	—
B.t.k. HD-73 insecticidal protein	<0.01	0.5	—
B.t.k. HD-1 insecticidal protein	<0.01	0.5	—
CP4 EPSP synthase	<0.1	0 (<15 s)	—
Glyphosate oxidoreductase	<0.01	0 (<15 s)	—
ACC deaminase	0.4	0 (<15 s)	—

TABLE 4 (continued)
Summary of Allergen and Protein Stability in a Gastric Model

Protein	% Total protein	Stability (min)[a,b] Whole protein	Fragments
	Introduced proteins[68,100]		
β-D-glucuronidase	0.01	0 (<15 s)	—
Neomycin phosphotransferase II	<0.01	0 (<10 s)	—
Phosphinothricin acetyltransferase	n.d.	0	—

[a] After Astwood and Fuchs et al.[116]
[b] Gastric digests were perfomed as described previously[80] with the following modifications: 170 ng/μl of protein was digested in 200-μl aliquots of simulated gastric fluid composed of 0.3% (w/v) pepsin, 0.03 *M* NaCl, pH 1.2. Digests were quenched by neutralization with 75 μl 0.16 *M* Na_2CO_3 at the following times: 0, 15, and 30 s; and 1, 2, 4, 8, 15, and 60 min. Digestion of proteins was evaluated by SDS-PAGE (10 to 20% acrylamide with tricine buffers[117]) and visualized by Coomassie Brilliant Blue colloidal staining.[118] 500 ng protein was loaded per lane. Stability represents the last time point at which the protein or a proteolytic fragment was observed; (n.d.) = not detectable; (—) = no fragments.
[c] Total amount of protein for combined subunits.
[d] Reported as % crude extract.
[e] Values estimated from the literature.

proteins, common food proteins with no allergenic history rapidly degraded under similar conditions. All eight of the common food proteins shown in Table 4 rapidly degraded within 15 s, the first time point analyzed.

Rapid proteolytic degradation of proteins greatly minimizes the likelihood that proteins could be absorbed by the intestinal mucosa, and should limit the opportunity for sensitization. The human digestive system provides an effective mechanism to remove these proteins before they have the opportunity to reach the intestinal mucosa. Therefore, the simulated gastric model provides a method to assess allergenic potential of proteins introduced into food plants.

B. Stability to Processing

The stability of a protein to various food-processing activities is also an important factor when assessing the allergenic potential of an introduced protein. Food allergens, particularly those present in processed food products like peanuts and soybeans, tend to be stable to processing conditions (see "Principles and Characteristics of Food Allergens"). This is expected because the processed food products derived from these foods maintain their allergenicity. If a protein is being engineered into fresh market products such as tomatoes, squash, or lettuce, processing stability is irrelevant because the product will be consumed fresh. If, on the other hand, a protein is engineered into soybeans, wheat, or rice, which are processed in one or more ways prior to consumption, the stability of the protein to processing conditions should be taken into account and suitable tests to investigate stability should be designed and conducted. If the product used for human consumption is free of protein (e.g., oils or carbohydrates), there is no significant human exposure and the allergenic potential of the expressed protein is greatly minimized or eliminated. Studies using direct food challenges with a limited number of subjects, with oils derived from several different crops, including soybean, peanut, and sunflower, showed no allergic reaction in patients who are allergic to these foods.[67–69] This is not surprising because there is an extremely low or negligible level of protein in hot-processed crop-derived oils.[70]

If a protein derived from a less commonly allergenic food or other allergenic source is highly

susceptible to digestion and/or is otherwise extensively degraded or removed by processing (if all food products derived from that plant are processed), no labeling should be required. If the protein is resistant to processing and/or digestion, the appropriate regulatory agency should be consulted.

VI. FOOD CONTAINING A GENE DERIVED FROM A SOURCE THAT HAS NO HISTORY OF ALLERGENICITY

If the gene is derived from a source that has no history of allergenicity, a comparison of the amino acid sequence identity between the gene product and known allergens should be conducted as described in Section III. If significant amino acid similarity exists between the gene product and a known allergen and sera from individuals that are sensitive to that allergen are reasonably available, the gene product should be assessed for immunologic reactivity using a solid-phase immunoassay. This should be performed as described for genes encoding proteins derived from less common allergenic foods or other known allergenic sources.

If there is no significant amino acid similarity, the digestibility and stability of the gene product should be assessed as described in Sections V.A and V.B. If the protein shows no significant amino acid similarity and is rapidly degraded or removed by processing, the product should be marketed without labeling. For example, comparison of the amino acid sequence of the nine proteins introduced into the number of different genetically modified plant products listed in Table 3 demonstrated that these proteins did not show any significant amino acid similarity to known allergens. These same nine proteins were rapidly degraded in the digestibility assay described above (Table 4). Based on these data, the products containing these proteins should be marketed without labeling.

VII. ADDITIONAL CONSIDERATIONS

A. Prevalence in Food

Many food allergens, especially those in the common allergenic foods, are present as major protein components, typically ranging between 1.0 and 80% of total protein. Examples of highly abundant allergens (Table 4) include those in milk,[54,55,71,72] soybean,[73,74] and peanuts.[75–78] Therefore, if a protein is expressed in the food at a level exceeding 1% of the total protein, this should also be taken into account in the allergenicity assessment. In contrast to the food allergens shown in Table 4, which are typically present at less than 1% of the total protein, the proteins expressed in the initial genetically engineered plants targeted for market introduction (Table 3) range from approximately <0.001 to 0.03% of the raw product on a fresh weight basis or <0.01 to 0.4% of the protein content,[79–85] and therefore do not trigger this concern.

B. The Host

Patients who are already sensitized to foods derived from hosts that contain endogenous allergens will likely still avoid the food derived from genetically engineered varieties of the same host (e.g., soybean or peanut). However, significant increases in the level of an endogenous allergen(s) that inadvertently resulted from the genetic modification could result in more individuals becoming sensitized to the allergen(s). Therefore, if the host being genetically modified is known to contain specific endogenous allergenic proteins, and sera from sensitive patients are readily available (e.g., for commonly allergenic foods), the food derived from the new plant variety should be analyzed to assure that the level of endogenous allergens was not increased during the modification process beyond natural differences that occur in the plant. It may not be necessary to evaluate the levels of endogenous allergens in all genetically modified, common allergenic host plants such as soybeans. The nature of the desired change should be determinant. If an antisense gene is introduced to turn off the production of a key allergen in soybeans, the levels of all endogenous soybean allergens in the resultant recombinant crop should be checked. However, these levels would not need to be checked if there were no reason to expect the introduced genetic material would influence the level of endogenous soybean allergens.

When an analysis of endogenous proteins is desired and feasible, immunoblotting and/or ELISA methods could be implemented for this assessment.[86] However, this assessment cannot be performed if the plant has no history of causing allergy or a limited history that precludes the availability of sera.

C. Animal Models

Although animal models provide important information for understanding the mechanisms of allergenicity, these models have not been validated for assessing the allergenic potential of specific proteins in humans. Examples of animal models include (1) mouse models to evaluate IgE responses to modified recombinant allergens;[87] (2) IgE-mediated rat anaphylaxis models;[88] (3) guinea pig models of anaphylaxis;[89–91] (4) dog models to study asthma[92,93] and food allergy;[93] and (5) mouse models to study possible immunotherapeutic peptide epitopes[24] and immunoprophylactic strategies.[94]

Animal models provide opportunities to study fundamental questions and mechanisms of allergenicity. However, none of these models have been shown to predict the allergenic potential of introduced proteins.[95,96] In all cases, these models have been used to study the biological or molecular mechanisms of immunopathogenesis of established allergenic responses. In the absence of data, these models cannot be extrapolated to humans. Variable responses from allergen to allergen, animal to animal, species to species, and even within the same animal over time[95,96] suggest that it will be extremely difficult to develop a reliable animal model that will be predictive for human allergenicity.

An example of an instance in which one animal model did not predict allergenicity is the Brazil nut 2S globulin protein, which was assessed by passive cutaneous anaphylaxis in mice fed the antigen orally.[97] This study reported that the 2S albumin protein did not elicit an IgE response in the mouse strains used under specific conditions. The authors came to the conclusion that the 2S gene was a strong candidate for genetic engineering into crop plants to enhance the nutritional quality of derived foods.[97] This does not mean that a more appropriate animal model would not have predicted allergenicity.

VIII. NATIONAL AND INTERNATIONAL CONSENSUS

The recommendations in this article are consistent with and expand other suggested approaches. The FDA provided guidance for allergenicity assessment in their 1992 "Food Policy" document.[9] The Environmental Protection Agency (EPA) provided some guidance in their November 1994 draft guidelines for pesticidal plants.[98] The FDA, EPA, and the U.S. Department of Agriculture (USDA) cosponsored a symposium in April 1994[97] focused specifically on assessing the allergenic potential of foods derived from genetically engineered plants. Recent workshops sponsored by the Organization for Economic Cooperation and Development (OECD)[99] and the World Health Organization (WHO)[100] also provided guidance on allergenicity assessment.

IX. FUTURE PROSPECTS

Genetic engineering can also provide an important tool to reduce the levels of specific allergenic proteins in the food supply. By suppressing gene expression, for example, by introducing genes in the antisense orientation (the opposite orientation required to produce a protein), the levels of specific proteins can be dramatically reduced. This is the technique used to produce the delayed-softening, Flav-Savr™ tomato. Inhibiting the production of the polygalacturonase enzyme, which causes the tomato to soften, extended the shelf life of the tomato.[101] This same approach has been used to significantly reduce the primary allergen in rice. Tada et al.[102] cloned the gene encoding the 16-kDa allergenic protein from rice and introduced the gene encoding this protein in the antisense orientation. The levels of the 16-kDa protein were significantly reduced in the rice seed in a number of the progeny. However, this protein was not completely eliminated in these plants. Further studies are underway to achieve greater reductions in this allergenic protein.

This approach could be used in other crops containing known allergens, such as peanuts and soybeans, to selectively reduce or eliminate the levels of specific allergenic proteins. The presence of multiple allergens in foods like peanuts and soybeans, however, greatly complicates this challenge. Furthermore, a protein that is an allergen and which also serves a critical structural or functional role cannot be removed without a negative impact on the plant.

X. FUTURE RESEARCH NEEDS

The cornerstone of allergenicity assessment is the accumulation of physicochemical, immunologic, and biochemical knowledge concerning food allergens. Assessment depends on validated assays (simulated digestion models), the availability of immunologic reagents (patient sera for assessing proteins from allergenic sources), and information on newly characterized allergens (i.e., an evolving database of allergen amino acid sequences). A reliable animal model may also be desirable.

The amino acid and/or nucleotide sequences of additional allergenic proteins, especially food allergens, and the mapping of the major B- and T-cell epitopes on known allergens would provide valuable information to expand the existing data base. Generating the physicochemical and biological data that are recommended in this article on a much larger collection of allergenic and nonallergenic proteins, as well as proteins introduced into genetically engineered plants, would help to validate the use of these criteria in the decision tree approach. The generation of easily accessible serum banks would greatly facilitate the assessments described.

A greater understanding of the molecular basis of immunopathogenesis (what makes certain food proteins allergenic) and the requirements for sensitization and elicitation of allergenic reactions is needed. Such research not only facilitates the assessment of the allergenic potential of foods derived by genetic engineering, it also serves as a basis for the development of new approaches to treat or prevent the development of allergies.

XI. SUMMARY

This article provides a science-based, decision tree approach to assess the allergenic concerns associated with the introduction of gene products into new plant varieties. The assessment focuses on the source from which the transferred gene was derived. Sources fall into three general categories: common allergenic food proteins; less common allergenic foods or other known allergen sources; and sources with no history of allergenicity. Information concerning the amino acid sequence identity to known allergenic proteins, *in vitro* and/or *in vivo* immunologic assays, and assessment of key physiochemical properties are included in reaching a recommendation on whether food derived from the genetically modified plant variety should be labeled as to the source of the transferred gene.

In the end, a balanced judgement of all the available data generated during allergenicity assessment will assure the safety of foods derived from genetically engineered crops. Using the approaches described here, new plant varieties generated by genetic modification should be introduced into the marketplace with the same confidence that new plant varieties developed by traditional breeding have been introduced for decades.

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GLOSSARY OF TERMS

Agronomic Trait—a genetically determined attribute of the plant that confers an observable, beneficial characteristic to the plant, e.g., insect, viral, disease protection, herbicide selectivity.

Allergen—biological or chemical substance that induces an allergic reaction or response; sometimes defined strictly as an immunoglobulin E-mediated response.

Allergenic—ability of a substance to bind immunoglobulin E and elicit allergic symptoms.

Allergic Rhinitis—seasonal and/or perennial symptoms of nasal congestion, sneezing, and runny nose usually due to inhaled plant pollens, mold spores, animal allergens, or mite allergens. It is an allergic indication that occurs in approximately 10% of the general population.

Allergy—the immune-mediated state of hypersensitivity that results from exposure to an allergen.

Anaphylatoxin—a molecule with the ability to release histamine and basic peptides from mast cells, especially complement peptides C3a, C4a, and C5a.

Anaphylaxis—a generalized inflammatory immune reaction to a foreign protein in a sensitized individual that may be severe enough to be life threatening.

Angioedema—a skin eruption similar to hives, but affects larger areas and extends deeper into the tissue.

Antibody—a protein molecule produced and secreted by B lymphocytes in response to an antigen that is capable of binding to that specific antigen.

Antigen—a foreign substance or protein that, when introduced into the body, is recognized by the immune system.

Antigen-Processing Cell (APC)—immune response cells that display peptide fragments of phagocytozed antigens, including B lymphocytes and macrophages.

Antigenic Determinant—see epitope.

Asthma—reversible airway obstruction that occurs in approximately 5% of the general population, demonstrated by wheezing and shortness of breath resulting from a number of factors, including reactions to allergens.

Atopic Dermatitis—a chronic (long-lasting) skin disorder that occurs primarily in children characterized by an eczematoid rash resulting from exposure to both food allergens and nonallergic factors.

Atopy—the genetic predisposition to allergy. Such individuals produce greater quantities of total and specific immunoglobulin E compared with healthy individuals.

B cells (B lymphocytes)—white blood cells derived from stem cells in bone marrow and elsewhere that produce antibodies.

Basophils—white blood cells that release histamine or other substances causing allergic symptoms.

Biotechnology—use of biological processes to produce products.

Celiac Disease—a chronic gastrointestinal disorder resulting from immune and nonimmune reactions in certain genetically susceptible humans sensitized to wheat gluten.

Complement—proteins and peptides of the immune system responsible for cytolytic activity.

Complementary DNA (cDNA)—the complementary DNA sequence to the primary amino acid sequence.

Crossed Radioimmunoelectrophoresis (CRIE)—electrophoresis of antigens through a neutral gel followed by electrophoresis at 90° to the first axis and into a radiolabeled antibody-containing gel.

Cultivar—a specific crop variety within a species with distinct features introduced by breeding or other genetic modification.

Cytokine—hormone-like protein secreted by cells, such as lymphocytes or macrophages, that act as molecular signals for communication between different cells of the immune system.

Dander—small scales from the hair or feathers of animals, which may be the cause of allergy in sensitive persons.

Deoxyribonucleic acid (DNA)—complex biochemical substance of which genes are made and which carries hereditary information in most living systems. DNA is composed of alternating phosphate groups and deoxyribose with one of four attached nucleotide bases: adenine, thymine, cytosine, and guanine. The sequence of bases in the DNA determines what expression product, if any, will be derived from the DNA.

Domain—region of a protein that has a definite tertiary structure.

Double-Blind, Placebo-Controlled Food Challenge (DBPCFC)—a standardized, oral feeding technique that includes sequential challenge of increasing amounts of foods or appropriate placebo to humans under controlled conditions.

Eczema—a skin rash, often on the face, hands, or skin folds.

Electrophoresis—biochemical method by which molecules are separated according to their charge and molecular weight.

Endosperm—a storage tissue that provides nutrition for the embryo of a seed during development (dicotyledonous plants). A starchy seed storage tissue of cereals such as rice or wheat.

Enzyme—a protein catalyst that facilitates specific chemical or metabolic reactions necessary for digestion of nutrients, cell growth, and proliferation.

Enzyme-Linked Immunosorbent Assay (ELISA)—an *in vitro* antibody assay that can be used to measure food, allergen-specific IGE antibody involving an enzyme activation of a human anti-human immunoglobulin E identification step.

Eosinophil—a white blood cell that contains cytoplasmic granules with an affinity for acid dyes, and induces hitamine release from mast cells during allergic inflammation.

Eosinophilic Gastroenteritis—an uncommon chronic gastrointestinal disorder that occurs in both food-allergic and nonallergic humans characterized by many eosinophils in the intestinal biopsy.

Epitope—region on the antigen that interacts with the cells of the immune system; if the portion reacts with T cells, it is called a T-cell epitope; if it reacts with either B cells or antibody, it is called a B-cell epitope.

Exercise-Induced Anaphylaxis (Food Dependent; F-EIA)—a form of anaphylaxis in which urticaria or shock develops after the combination of both eating a meal (or ingesting a specific food) and performing vigorous exercise within 2 h of eating.

Food—defined in the U.S. Food, Drug and Cosmetic Act as articles used for "food or drink for man or animals, chewing gum, and articles used for components of any other such article." In a broader context, food should be considered as anything sold or consumed as such. Under U.S. law any person who introduces food into commerce is responsible for ensuring that it complies with all applicable safety standards.

Food-Induced (e.g., milk) enterocolitis—a chronic gastrointestinal food allergy syndrome that may occur in infants.

Food Intolerance—a nonimmune adverse reaction to foods due to dietary or other factors.

Gene—smallest portion of a DNA molecule that contains sufficient heritable information to direct the production of a protein or a molecule of transfer or ribosomal mRNA, or to perform a regulatory function.

Gene Cloning—the use of recombinant DNA technology to identify, isolate, and characterise genes or cDNAs encoding potentially desirable traits.

Gene Suppression—the use of recombinant DNA technology to ablate the expression of a specific gene.

Genetic Engineering—directed modification of the genome to produce desired changes in the characteristics of an organism.

Genetic modification—addition, deletion, substitution, rearrangement, or recombination of heritable genetic material. Processes for achieving genetic modification include plant and animal breeding, cell and tissue culture, cell and protoplast fusion, mutagenesis, and recombinant DNA with transformation.

Genome—the total hereditary material of a cell, comprising the entire chromosomal set found in each nucleus of a given species.

Gluten—a general term for the association of wheat endosperm components, gliaden proteins and starch, into an insoluble elastic dough.

Glycoprotein—protein that has attached sugar molecules.

Glycosylation—the attachment of sugar chains to a protein.

Hapten—a low molecular weight nonimmunogenic substance (including some drugs) that can bind to a protein resulting in an immunogen.

Histamine—an amine found in all tissues of the body. Large amounts are released by mast cells when the body encounters a substance to which it is sensitive, thereby triggering symptoms of allergy.

Homology—similarity in amino acid sequence between different proteins.

Hydrolysis—chemical process by which a protein is broken down into smaller peptides.

Hypoallergenic—the modification of a food by chemical or genetic means so as to reduce the expression or quantity of endogenous allergens, possessing less-than-normal allergenicity.

IgE (Immunoglobulin E)—a specific class of immunoglobulin secreted by B cells. It binds to specific receptors on mast cells. Interaction of an allergen with mast cell-bound IgE can trigger allergic symptoms.

IgE binding—ability to bind to IgE.

IgE Receptor—surface proteins of mast cells or basophils that bind circulating IgE-antigen complexes and triggers degranulation.

Immunoblotting—biochemical technique by which proteins bound to a nitrocellulose or nylon membrane are detected using immunochemical techniques.

Immunochemical—techniques that utilize antibody as a specific detection system for substances.

Immunoglobulin Isotype—synonym of class when referring to immunoglobulin variants, including IgG, IgA, IgM, IgD, and IgE.

Immunotherapy—any type of treatment that targets the immune system. In allergy it is treatment in which a progressively increasing dose of an allergen is given in order to induce an immune response characterized by tolerance or "desensitization."

Interferon (IFN)—a cell growth-retarding class of proteins secreted by T cells and macrophages, which are down-regulated by IL-4.

Interleukin (IL)—cytokine secreted mainly by mononuclear cells that induces growth, activation, and differentiation of lymphocytes and pluripotential hemopoietic stem cells.

Intron—nonrelevant polynucleotide sequence; does not code for information for protein synthesis.

In vitro—outside of the living body, for example, in a test tube or in laboratory tissue culture.

In vivo—within the living body.

Isoallergen—allergen proteins that belong to a gene superfamily sharing more than two-thirds amino acid sequence identity; a synonym of allergen isoform.

Isoelectric Point—pH at which all electrical charges on a protein are equal to zero.

Macrophage—one of three principal cell types that respond to antigens, are derived from blood monocytes, reside in tissues, perform phagocytosis of cells and particles, and are APCs.

Major Histocompatibility Complex (MHC)—a region of a chromosome that contains the genes encoding the antigenic determinants of nucleated cells and that includes the genes that influence helper, suppressor, and other T-cell subsets.

Marker Gene—gene with a detectable or selectable phenotype that is engineered into a vector to allow detection of neighboring sequences (a gene or genes of interest) in a new genetic element.

Mast Cells—tissue cells that contain granules containing histamine. During an allergic response, degranulation releases histamine-causing allergic symptoms.

Messenger RNA (mRNA)—nucleic acid that carries instruction to a ribosome for the synthesis of a particular protein; important molecule in the production of protein from DNA.

Nucleotide—basic structural units of RNA and DNA.

Oral Allergy Syndrome (Fruit and Vegetable Syndrome; OAS)—a form of allergic reaction in which humans who are allergic to inhaled pollen, react, usually, with localized symptoms of itching and swelling of the mouth parts when eating certain fruits and vegetables.

Oropharyngeal—involving the mouth and throat.

PCR—polymerase chain reaction.

Passive Cutaneous Anaphylaxis (PCA) and Prausnitz-Küstner Reaction—a test for immediate hypersensitivity performed in a normal subject who has been passively sensitized by IgE from an allergic individual.

Pathogen—any virus or microorganism that causes disease.

Peptide—two or more amino acids joined by a linkage called a peptide bond. Peptides result from proteolysis or the digestive process that breaks down proteins into smaller molecules.

Phenotype—observable characteristics, resulting from an interaction between an organism's genetic makeup and the environment.

Pollen—the mass of microspores (male-fertilizing elements of flowering plants). Many pollens, especially the airborne pollens, are allergens.

Proteins—the principal constituents of the protoplasm of all cells, they are high in molecular weight and consist essentially of combinations of α-amino acids in peptide linkage. Twenty different amino acids are commonly found in proteins, and each protein has a unique, genetically defined amino acid sequence that determines its specific shape and function.

Proteolysis—a form of hydrolysis.

Quality Trait—a genetically determined attribute of the plant that effects a compositional component of the food or feed product, e.g., delayed softening or ripening, increased solids content, and increased nutritional value.

Radioallergosorbant Tests (RAST)—an *in vitro* IgE food allergen-specific antibody assay involving an anti-human IgE identification step.

Recombinant DNA Technology (rDNA)—processes of cutting and recombining DNA molecules to remove segments from or otherwise modify an organism's genetic material, or to combine segments of DNA from different types of organisms.

Ribonucleic acid (RNA)—nucleic acid composed of alternating phosphate groups and ribose with one of four attached nucleotide bases: adenine, guanine, cytosine, and uracil.

Simulated Digestion—an *in vitro* model of gastrointestinal proteolysis.

Skin Test—intradermal injection of allergen extracts and assessment of local edema.

Somaclonal Variation—phenotypic expression of genetic changes observable after growth of plant tissue in cell or tissue culture.

Spore—the reproductive element, produced sexually or asexually, of one of the lower organisms, such as protozoa, fungi, algae.

T cells (T lymphocytes)—white blood cells originating from stem cells in the thymus that are involved in the immune response.

T cell Receptor—surface proteins of T cells that recognize and bind antigens displayed by APCs resulting in T-cell proliferation and lymphokine production.

Ti Plasmid—a naturally occurring tumor-inducing plasmid of *Agrobacterium tumafaciens* that is capable of transfection and transformation of plants. Used as a vector to shuttle desirable traits into agricultural crops.

Transfer RNA (tRNA)—important molecule in the production of protein from DNA.

Transformation—introducing the DNA encoding a specific gene(s) into the genome by direct methods.

Transgenic Plant—a plant derived from rDNA technology that contains genetic material from outside the particular plant species.

Translation—the process by which DNA is transformed into protein.

Urticaria—an acute (immediate) or chronic (repetitive or long lasting) skin disorder in humans characterized by itching and hives resulting from many factors of both allergic and nonallergic nature.

Venom—a toxic substance normally secreted by a serpent, insect, or other animal.

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