Novel Foods to Treat Food Allergy and Gastrointestinal Infection

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The gastrointestinal tract communicates directly with the external environment. Necessary nutrients must be absorbed and commensal bacteria tolerated, and foreign proteins, antigens, and pathogens must be simultaneously excluded or destroyed. Immaturity or disruption of the mucosal immune defenses increases vulnerability to food allergy, intolerance, and infectious disease. Diseases resulting from ingested foreign proteins and organisms are increasing and cause morbidity and mortality worldwide. There is no specific treatment for food allergy other than avoidance. Vaccination for infectious disease is limited by the cost and logistics of distribution and administration, particularly in developing countries. Novel strategies are being explored to modulate the gut mucosal immune system by altering protein expression in food. Crops are being developed to remove deleterious allergens to prevent immunogenic exposure while preserving nutritional quality. Local food plants that express protein fragments of pathogens might provide an effective means to stimulate gut mucosal immunity while increasing vaccine accessibility.

Introduction

Aberrant immune responses to ingested foreign proteins or pathogens may lead to the expression of allergic, autoimmune, or infectious disease in the gastrointestinal tract. Ideally, the mucosal immune response inactivates the foreign protein without injuring the host. Cellular and noncellular mechanisms participate [1]. Gastrointestinal proteolytic enzymes, extremes in pH, and emulsification by bile reduce antigenic potential. The mucin glycoprotein layer, secretory immunoglobulin A (sIgA), and epithelial tight junctions impede attachment and passage of foreign proteins. Junctional integrity may be compromised during the perinatal period, inflammation, and food allergy, permitting luminal antigens and pathogens to gain access to the lamina propria. Once the epithelial barrier is breeched, allergens may crosslink with IgE, thereby stimulating mast cell degranulation, epithelial fluid, and electrolyte secretion.

Food allergy arises when an oral allergen provokes an abnormal immune response. Responses occur by IgE-mediated or non-IgE cellular mechanisms. IgE causes hives, wheezing, and hypotension. A mixed pathology can manifest as gastrointestinal symptoms resulting from eosinophilic inflammation and increased vascular permeability. Clinical forms of delayed cell-mediated non-IgE pathology include celiac disease and protein enteropathy [2].

Luminal presentation of an antigen, in a food or vaccine, is an important means of stimulating mucosal immunity [3,4]. The antigen is taken up by specialized M cells within the intestinal lining and transferred to macrophages and B cells. Portions of the antigen displayed on macrophage membranes stimulate T-helper (Th) cells and activate B cells to produce neutralizing antibodies. Later ingestion of an intact pathogen elicits memory Th cells to produce cytotoxic T cells, which attack infected cells. Memory Th cells also induce a brisk secretion of antibodies by stimulated B cells. Therefore, oral vaccines can be a particularly effective first line of defense against many of the ingested pathogens responsible for gastrointestinal disease.

New strategies are being explored to modulate the gut mucosal immune system by altering protein expression in food plants. Food allergens can be removed from food plants by mutagenesis, gene silencing, or antisense oligonucleotides. Conversely, novel proteins can be expressed in plants to create edible vaccines. Recombinant DNA technology can be used to transfer genes from other organisms to plants. Similar technology has already been employed to make human insulin. One method of gene transfer involves coating microscopic metal particles with the desired DNA and accelerating the particles directly into plant cells with a particle gun. Alternatively, the gene of interest can be inserted into the DNA of a bacterial or yeast plasmid. Plasmid-bearing bacteria then transfer recombinant DNA into host plant cells, where the recombinant DNA incorporates into the plant genome. Transfer of the recombinant DNA into the plant cell by either method is followed by plant cell division, eventually yielding a plant with the transferred trait.

Hypoallergenic foods and edible vaccines show promise to address several public health issues on a very large scale. Allergic individuals could be insured adequate nutrition and avoid more devastating consequences, such as growth

impairment, anaphylaxis, and death. Populations could be immunized with locally grown, familiar food plants at increased efficiency and reduced cost. The following discussion reviews recent developments in potential treatments of food allergy (peanut, wheat, and soy), celiac disease, and gastrointestinal infection (hepatitis B, Rotavirus, *Escherichia coli*, *Vibrio cholerae, Helicobacter pylori*, and *Bacillus anthracis*).

Food Allergy and Hypoallergenic Plants

Food allergy occurs in 6% to 8% of young children and 2% of adults in North America and Europe, and the prevalence is rising. Eight common foods cause more than 90% of allergic reactions: milk, egg, soy, wheat, peanut, tree nut, fish, and shellfish. However, some food allergies merit further concern either because of the severity and persistence of the allergic reaction (peanut) or the importance of the food as a fundamental dietary staple (soy, rice, and wheat) in specific geographic regions or during early stages of human development.

Peanuts

Peanuts are responsible for the greatest number of deaths due to food allergy. Fifty percent of peanut-allergic individuals experience moderate to severe symptoms, including compromised respiratory and cardiovascular function [5]. The prevalence of peanut allergy is now 0.6% to 1.0% in the United States, and prevalence in the European population is increasing. In most instances, peanut allergy lasts a lifetime.

The three major peanut allergens are Ara h1, a 64.5-kD vicilin family of seed storage proteins, Ara h2, a 17.5-kD conglutin family of seed storage proteins, and Ara h3, a 60-kD, glycinin-like seed storage protein (a preproglobulin). Most other peanut allergens are isoforms of Ara h1, Ara h2, or Ara h3. Prevention of IgE binding to these three antigens is the basis of the experimental hypoallergenic peanut. Linear epitopes predominate rather than conformational structures. This linearity is important because single amino acid substitutions within IgE-binding sites often lead to loss of binding and abrogate the allergic response [6]. In Ara h1, IgE-binding epitopes are near contact points critical to trimer formation. It is not yet known if amino acid substitution made within these epitopes could inadvertently disrupt trimer formation and alter protein function [6]. Ara h3 has been cloned and characterized. Single amino acid changes at critical residues diminished IgE binding [7]. Hypoallergenic peanuts should conserve the flavor, as different proteins confer taste. Effects on the peanut plant biology and use in food processing are under investigation.

Soybeans

Unlike peanut allergy, soy rarely results in severe or life-threatening reactions and is usually a transient allergy of infancy and childhood [5]. However, soy provides essential nutrition for many infants as well as for populations of all ages from Asia, where it is a food staple. The only treatment remains avoidance of dietary soy, which is challenging, and risks malnutrition. Soy is ubiquitous in food processing globally because of its high nutritional quality, dense protein content, and physical-chemical properties desirable in food preparation. Baby formulas, salad dressing, soy sauce, milk, flour, cereals, grits, and miso are popular soy-based products. Consequently, elimination of dietary soy risks malnutrition, especially in the very young.

As many as 15 protein components are recognized by the sera of soybean-sensitive patients [8,9]. The three principal allergens are Gly m Bd 30K, Gly m Bd 28K, and Gly m Bd 60K. The strongest allergen is Gly m Bd 30K/P34, a soybean oil–associated glycoprotein of MW 34,000. It is homologous to Der p (or f), the major allergen in house dust and a member of the papain superfamily. It causes allergy in 65% of soy-allergic individuals. Because all domestic and wild soybean varieties naturally contain Gly m Bd 30K/P34, there is no option to cultivate selectively a naturally occurring crop. Herman *et al.* [10••] have produced a soybean cultivar in which Gly m Bd 30K/P34 is silenced or not expressed [11]. Although the function of Gly m Bd 30K is unknown, the resulting soybean plants demonstrate normal phenotype, growth, development, and agronomics [10••]. No other proteins were induced or suppressed, compared with the wild type [10••]. Analysis with sera from soybean-sensitive individuals confirmed the loss of the Gly m Bd/P34 allergen without induction of new allergens. Alternatively, a mutant soybean line that lacks Gly m Bd 28K and 60K was created by irradiation. Gly m Bd 30K/P34 was then eliminated by physical-chemical separation and enzymatic digestion of soymilk from this line. In a preliminary trial, 80% of soybean-sensitive patients could ingest products prepared from the hypoallergenic soymilk [9].

Wheat

Wheat provides 20% of caloric intake [12] and half the world's supply of dietary protein [13]. After sugar, wheat gluten is the second most prevalent food substance in the Western diet [14]. In the United States, wheat gluten may be eaten as often as every meal due to its ubiquity in processed foods, including sauces, canned goods, soups, soy sauce, vinegar, beer, grain alcohols, pastas, and even over-the-counter medications [15]. Wheat proteins are introduced early in the human diet, through maternal breast milk in the newborn or in cereal at age 5 to 6 months.

Wheat causes significant pathology in individuals with wheat allergy and celiac disease. In wheat allergy, ingested wheat results in enteropathy (protein-sensitive and eosinophilic), atopic dermatitis, enterocolitis, vomiting, and even exercise-induced anaphylaxis [16–18]. In celiac disease, presentation ranges from asymptomatic latent states to malabsorption with varying degrees of diarrhea and growth impairment, to dermatitis herpetiformis, to end-stage intestinal failure with progressive lymphoma [19]. In the United States, one in 150, or 2 million people have celiac disease [14]. In Europe, the prevalence is one in 100, making it the

most common genetic disease there [20]. Gluten exposure triggers heightened T-lymphocyte and B-lymphocyte reactivity, resulting in variable mucosal damage, including jejunal atrophy with loss of villi. The extent of gut involvement determines whether individuals develop frank gastrointestinal symptoms [21]. Continued gluten exposure risks malignancy, short stature, seizures, miscarriage, congenital malformations, osteoporosis, and associated autoimmune disorders, whether gastrointestinal symptoms exist or not [22,23].

Individuals with wheat allergy are intolerant of wheat for different reasons than those with celiac disease. The toxicity of wheat derives from specific seed storage proteins that are classified based on solubility in water (albumins), dilute salt solutions (globulins), aqueous alcohol (gliadins), or dilute alkali or acid (glutenins). Gliadins and glutenins together are referred to as glutens or prolamins. Prolamins are rich in glutamine and proline. Proline confers resistance to proteolysis and results in presentation of intact antigens to the gut immune system. In wheat allergy, α-, β-, γ-, and ω-gliadins, low-molecular-weight glutenin subunits, albumins, and globulins elicit IgE reactivity, but gliadins and glutenins are the most clinically relevant [24]. Wheat allergy is generally transient. Celiac disease is the permanent intolerance to gluten occurring in genetically predisposed individuals. Here, proline not only protects against proteolysis but also enhances T-cell recognition of gluten through changes in conformation, deamidation, and charge favorable to binding. Susceptibility to celiac disease is linked to leukocyte antigens HLA-DQ2 and HLA-DQ8. HLA-DQ2/8 binds gluten, facilitated by tissue transglutaminase, and presents gluten to intestinal CD^{4+} T cells. The T cells proliferate and secrete interferon-γ, resulting in mucosal damage.

Under development are at least two food-based strategies to detoxify wheat. The first strategy relies on the premise that T cells mediate gluten toxicity and that T-cell recognition of gluten is enhanced by proline. Vader *et al.* [25••] recently used site-directed mutagenesis to abrogate the T-cell stimulatory response by substitution of a proline in glutenin and gliadin proteins. However, the response was not completely abolished in all T-cell clones tested, suggesting that additional substitutions might be needed or that only some subsets of celiac patients might benefit. Benahmed *et al.* [20] further note that young children with celiac disease possess more epitopes than adults, and possibly more clustered or repetitive epitopes. Removal of clustered or repetitive epitopes might reveal alternative competitive epitopes. Non–T-cell–mediated pathology must also be accounted for. One example is peptide 31-49 in the N-terminus of A-gliadins, which is inherently cytotoxic.

A second food-based strategy to detoxify wheat involves elimination of disulfide bridges. This approach may apply to other food allergies as well. Disulfide bridges render many allergens impervious to digestion, resulting in the presentation of intact proteins to the distal gut with subsequent immune stimulation. Glutenins and gliadins are sulfur-rich proteins. Thioredoxins occur in animals, plants, and bacteria. In plants, thioredoxin reduces disulfide bonds in small proteins (such as gliadins and glutenins in wheat) to mobilize starch and protein reserves to provide carbon and nitrogen to germinating seeds. In sensitized atopic dogs, thioredoxin mitigates wheat allergy, as determined by skin testing [26••]. In some patient subsets, however, thioredoxin alone may not completely abrogate wheat allergy, because some significant peptides, such as ω-gliadin, are not reduced due to lack of disulfide bonds [18,26••]. Even if possible, elimination of all toxic wheat peptides might not be adequate in the event of environmental exposure to (suspected) molecular mimics such as Ad12 adenovirus, which contains an E1b protein that shares homology with α-gliadin [27]. Lemaux [12] targeted gene expression in grain endosperm, resulting in a 30-fold increase in thioredoxin expression. Further study is needed to determine the degree to which overexpression of thioredoxin alone could alleviate celiac or allergic symptoms, to identify key allergens and patient subpopulations, and to elucidate the role of molecular mimics.

Gastrointestinal Infection and Edible Vaccines

Novel proteins can be expressed in plants to prevent the spread of global infection. In contrast to the deletion of endogenous antigens to create hypoallergenic foods, novel proteins can be expressed to act as vaccine antigens. Dietary crops can be used as a vehicle for mass immunization. Global spread of infection threatens developing and developed nations. Overcrowding, contaminated water, poor sanitation, and lack of access to vaccines increase susceptibility in developing countries. Developed nations are vulnerable due to local spread through day care and through compromise of sanitation by environmental disaster or political and social de-stabilization. Severe acute respiratory syndrome (SARS) has heightened awareness that infections originating overseas rapidly spread through international trade, travel, and adoption. All nations are threatened by bioterrorism.

High costs of production, packaging, and delivery undermine the feasibility of current vaccines, particularly in developing nations. Injectable vaccines not only are expensive but also require refrigeration for shipping and storage, trained personnel for administration, disposal of needles and syringes, or sterilization. People are more apt to accept oral vaccines. Oral vaccines stimulate mucosal immunity more effectively than injectable vaccines.

Candidate foods as vehicles for edible vaccines

Recombinant vaccines contained in food have been in development for over a decade [28]. Recombinant vaccines may be safer, as they do not contain intact pathogens. Edible plants containing vaccines can be fed directly to individuals and do not require purification. Transformations have already been reported in a variety of crops, such as tobacco, tomato, and potato [29–31], but bananas possess several advantages. Bananas provide one fourth of all food calories

in western and central Africa and feed tens of millions in Central America and Asia [32]. As a local crop, bananas incur no cost of foreign production and transport. Bananas are eaten raw, thereby avoiding denaturation of recombinant protein by cooking. Even infants can eat bananas. Ripening bananas contain several upregulated genes that may later prove useful for expression of edible vaccines [33]. Because banana trees require years to grow mature fruit, other plant models are being studied first to determine how best to maximize expression of a vaccine antigen in plant tissue. Eventually, a single banana could yield up to 10 vaccine doses, reducing the cost of one dose to less than one cent [34]. In contrast, one dose of hepatitis B surface antigen (HBsAg) now costs 90 cents, which is more than the daily income of nearly 1 billion people [35].

Candidate pathogens for edible vaccines

Initial work in edible vaccines has focused on hepatitis B virus (HBV) and enteric infections, and is being explored in *H. pylori* and *B. anthracis*. Over 2 billion individuals are infected with HBV, contributing to chronic liver disease and hepatocellular carcinoma, with 1 million deaths annually [36]. Carriers continue to transmit infection laterally and vertically. Enteric infection with Norwalk virus, Rotavirus, *V. cholera*, and enterotoxigenic *E. coli* causes diarrhea that kills 3 million infants each year, especially in poor or remote areas [37,38].

Edible recombinant vaccines are a form of subunit vaccine [28]. This strategy induces a host immune response via protein fragments, rather than intact pathogens. HBsAg uses the S protein of the viral capsid, which self-assembles into virus-like particles (VLPs). Recombinant VLPs include hepatitis B [39,40], hepatitis E [41], Norwalk virus [42], and Rotavirus [43]. The first model of a vaccine grown in plants used HBV, which was also the first recombinant vaccine. HBsAg has been expressed in tobacco and potato plants, with formation of VLPs [40,44]. Current studies seek to increase expression in plants. The Norwalk virus capsid protein (NVCP) has also been expressed in tobacco and potato plants [42]. Oral immunogenicity has been demonstrated in mice fed tubers containing recombinant NVCP [45]. The ideal oral dose is still to be determined.

Edible vaccines are feasible and may protect against multiple pathogens

Preliminary studies of NVCP in mice demonstrate that oral vaccines can survive gastric protease digestion and stimulate a gut immune response. Phase I trials in human volunteers fed tubers containing heat-labile enterotoxin from enterotoxigenic *E. coli* demonstrated successful delivery of recombinant antigens via plant ingestion by humans [46••]. Candidate antigens are protected from denaturation by encapsulation within plant cell walls and membranes. Antigen dosing must be adequate and predictable, but current plant models produce only small, variable amounts of vaccine. Adjuvants may serve to enhance uptake of plant vaccines and stimulate the immune response. The B subunit of *V. cholera* toxin binds well to M cells. When coupled with other antigens, it can stimulate protection against multiple diseases simultaneously [47]. Therefore, the concept of an edible vaccine is feasible.

Future edible vaccines: *H. pylori* and *B. anthracis*

H. pylori and *B. anthracis* are being investigated as potential edible vaccines. Treatment of both organisms is cumbersome. Each organism presents unique technical challenges due to prevalence, microecology, or unpredictable sudden involvement of large populations.

More than 50% of the world's population, or over 3 billion people, are infected with *H. pylori*. Even asymptomatic people remain at risk for complications, which include duodenal ulcers, gastric carcinoma, pangastritis, atrophic gastritis, and mucosa-associated lymphoid tissue lymphomas. In developing countries, *H. pylori* infection produces associated chronic diarrhea, hypochloridria, malnutrition, predisposition to other enteric infections such as typhoid fever or cholera, and impaired growth. This complex clinical scenario reflects the higher frequency and earlier age of infection in these countries, resulting from environmental factors and poverty. Infection usually persists for a lifetime. Longer duration of colonization correlates with greater risk of complications.

Side effects, poor patient compliance, bacterial resistance, and reinfection limit efficacy of treatment with a proton pump inhibitor and two antibiotics. This regimen is particularly unfeasible in developing nations, where some areas have infection rates exceeding 90% and reinfection rates as high as 13%, and costs are prohibitive [48]. It is likely that an effective vaccine could be developed sooner than the changes in public health and standard of living needed to control the infection.

H. pylori occupies a unique ecologic niche in the extracellular environment of the stomach and the duodenum. Therefore, mucosal immunization might be the best route to induce local protection against the organism where it resides. Mounting evidence demonstrates that, in the gastrointestinal tract, B cells preferentially home back to the original site of antigen exposure [49]. Further, unlike animals, humans fail to demonstrate adequate IgA antibody secretion after intranasal or rectal administration of *H. pylori* antigen [49]. These preliminary data suggest that oral or intraintestinal routes might optimize mucosal induction of B-cell responses in the stomach and proximal gut. Another model is a non–*H. pylori* typhoid oral vaccine (Ty21) used in *H. pylori*–infected subjects to study T-cell responses. These studies demonstrated that greater than 95% of circulating T cells possess the gastrointestinal mucosal homing receptor $\alpha 4\beta 7$, suggesting that T-cell responses can also be expressed in gastric mucosa, as cited by Svennerholm [50]. Gastric and jejunal immunization in *H. pylori*–infected humans produced antibody-specific cells that also expressed the gastrointestinal mucosal homing receptor integrin $\alpha 4\beta 7$. Oral immunization induced signifi-

cantly greater expression of α4β7 by B cells than did subcutaneous immunization. A plant vaccine might further enhance *H. pylori* antigen delivery compared with current vaccines, which are compromised by denaturation in gastric acid and by inadequate immune response to single-antigen vaccines. A plant vaccine might protect antigens by encapsulation within cell walls or membranes and could serve as a vehicle for delivery of multiple *H. pylori* antigens.

Anthrax is caused by the spore-forming bacterium *B. anthracis* introduced through cutaneous, gastrointestinal, or inhalation exposure [51]. Treatment requires 60 days of antibiotics because germination can occur up to 60 days after exposure. Without early intervention, mortality is high. Although anthrax is rare in humans naturally, the threat of biologic warfare warrants mass vaccination. Current vaccines work, but utility is limited by lack of standardization, high cost, repeat dosing, and side effects. Protective antigen (PA) is the primary immunogenic component. Once bound to mammalian cell surface receptors, PA undergoes cleavage and activation, which facilitates binding to edema factor or lethal factor to form edema toxin and lethal toxin, which are then transported into the host cytoplasm. Cell lysis, toxic shock, and death ensue. Vaccination would obviate disease expression by interfering with PA binding to mammalian cells [52]. Only a fragment of the PA protein is needed to elicit a protective immune response. Recombinant PA has been expressed within spinach plants. Currently, the plants are used as production vehicles to make a safer vaccine. Plants are devoid of human diseases and do not require screening for bacterial toxins or viruses, thus reducing costs. The ideal route of delivery is under investigation (Personal communication, Alexander Karasev, PhD, Thomas Jefferson University) [52,53].

Oral tolerance and autoimmunity

One concern regarding the use of edible vaccines is the possible development of oral tolerance. Oral tolerance occurs when ingestion of an antigen suppresses, rather than stimulates, systemic humoral and cell-mediated immune response [4]. In fact, the lack of immune response to commensal bacteria and to food antigens in the gut is thought to be due to oral tolerance. This phenomenon would be counterproductive in an edible plant vaccine intended to provide immunity. However, oral tolerance may provide a means to suppress autoimmunity. Ongoing studies are investigating the technical aspects of creating transgenic plants that express adequate autoantigens to produce vaccines against various autoimmune human diseases and food allergy, as well as insuring that immunity is stimulated by oral plant-based vaccines intended to combat infectious disease.

Conclusions

This paper reviews trends in biotechnology in the creation of novel foods to treat food allergy and gastrointestinal infections. Before implementation, several issues must be clarified: 1) safe use in humans must be established [54••]; 2) patient subsets for whom such treatment is appropriate must be identified; 3) all relevant allergens/toxins must be characterized; 4) nutritional quality must be preserved; 5) taste, texture, and temperature properties necessary to food preparation must be retained; and 6) normal agronomics must be maintained. There is much to gain, particularly for highly atopic individuals, who bear the greatest risk of malnutrition due to multiple food allergies or severe reactions. Determination of what components render foods allergenic will contribute to eventual effective therapy. Edible vaccines demonstrate obvious advantages in administration, accessibility, and cost, particularly on a large scale and in developing nations. The key here is to assure antigenic protein expression that is both adequate and predictable in order to elicit an effective protective immune response. The eventual elucidation of the molecular biology of humans, food allergens, and pathogens will clarify key interactions and appropriate therapeutic modalities—some of which may be the food we eat.

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This is the first comprehensive summary of risks and benefits of genetically engineered foods to human health with particular emphasis on children. Benefits discussed include nutritionally enhanced crops, designer oils from oilseed crops to decrease disease risk, and edible vaccines. Safety issues, such as regulation, allergenicity, and altered gene expression in plants, bacteria, and humans, are addressed.