

Edible vaccines: A new approach to oral immunization

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Edible vaccines offer exciting possibilities for significantly reducing the burden of diseases like hepatitis and diarrhoea, particularly in developing world where storing and administering vaccines are the major problems. Edible vaccines are prepared by molecular farming using the science of genetic engineering. Selected genes are introduced into the plants. The transgenic plant is then induced to manufacture the encoded protein. Owing to its low cost, it will be suitable for developing countries like India. Edible vaccines are mucosal-targeted vaccines, which cause stimulation of both systematic and mucosal immune response. Edible vaccines are being developed for various diseases, such as measles, cholera and hepatitis B, and many more are in the process of development. Thus, they may also help to suppress autoimmune disorders such as Type-I diabetes, diarrhoea, multiple sclerosis, rheumatoid arthritis, etc. Human trials conducted by the National Institute of Allergy and Infectious Diseases (NIAID), US Department of Health and Human Services, USA show that edible vaccines are feasible. ProdiGene, a biotech company, has a patent for vaccine against, viral diseases of hepatitis and transmissible gastroenteritis virus. This review comprises methods of preparation, mechanism of action, recent developments, clinical trials and therapeutic applications of edible vaccines.

Keywords: Transgenic plant, edible vaccine, oral immunization, mucosal immunity, autoimmunity

Introduction

Vaccines are designed to elicit an immune response without causing disease. Typical vaccines are composed of killed or attenuated disease-causing organisms. The administration of vaccines is the cost effective method of combating the spread of diseases. Successful vaccination programmes lead to far fewer individuals ever showing symptoms of diseases, thus reducing the need for costly treatment procedures¹. Vaccines that one can eat, called edible vaccines, are among the most unusual approaches for administering new vaccines. The idea of plant derived edible vaccines was first conceived and is now continuing to be developed, with the help of emerging innovations in medical sciences and plant biology, for the creation of efficacious and affordable pharmaceuticals²⁻⁴. Edible vaccines are like subunit preparation, in that they are engineered to contain antigen, but bear no genes that would enable whole pathogen to form⁵. Edible vaccines are currently being developed for a number of human and animal diseases, including measles, cholera, foot and mouse disease, and hepatitis B and C⁶. Many of these diseases require booster vaccination or

multiple antigens to induce and maintain protective immunity. Plants have capacity to express more than one transgene, allowing delivery of multiple antigens for repeated inoculations⁷.

A concern with oral vaccines is the degradation of protein components in stomach (due to low pH and astric enzymes) and gut before they can elicit immune responses, but the rigid plant cell wall could provide protection from intestinal degradation^{2,8}. It was, therefore, not surprising when Hiatt and co-workers attempted to produce antibodies in plants, which could serve the purpose of passive immunization⁹. The first report of edible vaccine (a surface protein from *Streptococcus*) in tobacco, at 0.02% of total leaf protein level, appeared in 1990 in the form of a patent application published under the international patent cooperation treaty¹⁰. The concept of edible vaccine got impetus after Arntzen and co-workers expressed hepatitis B surface antigen in tobacco plants¹¹.

Today's development of novel vaccines stresses the need for edible vaccines that are inexpensive, easily administered and capable of being stored and transported without refrigeration. Without these characteristics, developing countries find it difficult to adopt vaccination as the central strategy for preventing their most devastating diseases¹². With the advent of

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modern molecular biology techniques in the 1980s, new strategies were developed for the production of subunit vaccines. These are the vaccines comprising protein derived pathogenic viruses, bacteria or parasites; in general the proteins are produced not by the pathogens themselves, but by expression of genes encoding the protein in a surrogate organism¹³.

Second generation edible vaccines are also called as multicomponent vaccines that provide protection against several pathogens. Yu and Langridge demonstrated an elegant approach to achieve this goal, based on epitope fusion to both subunits of *Cholera toxin* (CT)¹⁴. CT provides a scaffold for presentation of protective epitopes of rotavirus and ETEC (Enterotoxigenic *Escherichia coli*) acts as a vaccine candidate by its own right and as a mucosal adjuvant devoid of toxicity. The trivalent edible vaccine elicited significant humoral responses, as well as immune memory B cells and T helper cell responses, important hallmarks of successful immunization. In the clinical trials, it has been described that 100 g of raw potato tubers expressing LT-B of ETEC in three doses had to be consumed in order to overcome digestive losses of the antigen and to elicit a significant immune response¹⁵. Some examples of edible vaccines are shown in Table 1^{11,16-25}.

Advantages of Edible Vaccines

Potential advantages of plant-based vaccines are:

- Edible means of administration.
- Reduced need for medical personnel and sterile injection conditions.
- Economical in mass production and transportation.
- Therapeutic proteins are free of pathogens and toxins.
- Storage near the site of use.

Table 1—Examples of edible vaccines

S. No.	Vaccines	Vector used	Diseases/Condition it is used for	Ref.
1.	Hepatitis B virus	Tobacco Potato Lettuce	Hepatitis B	11,16
2.	Norwalk virus	Tobacco Potato	Diarrhoea Nausea Stomach cramps	17
3.	Rabies virus	Tobacco	Rabies	18,19
4.	Transmissible gastroenteritis Corona virus	Tobacco Maize	Gastroenteritis	20,21
5.	Rabbit hemorrhagic diseases virus	Potato	Hemorrhage	22
6.	HIV virus	Tomato	AIDS	23
7.	<i>Vibrio cholerae</i>	Potato	Cholera	24

- Heat stable, eliminating the need for refrigeration.
- Antigen protection through bioencapsulation.
- Subunit vaccine (not attenuated pathogens) means improved safety.
- Seroconversion in the presence of maternal antibodies.
- Generation of systemic and mucosal immunity.
- Enhanced compliance (especially in children).
- Delivery of multiple antigens.
- Integration with other vaccine approaches.
- Plant derived antigens assemble spontaneously into oligomers and into virus like particles.

Limitations

- Development of immunotolerance to vaccine peptide or protein.
- Consistency of dosage form fruit to fruit, plant-to-plant, and generation-to-generation is not similar.
- Stability of vaccine in fruit is not known.
- Evaluating dosage requirement is tedious.
- Selection of best plant is difficult.
- Certain foods like potato are not eaten raw, and cooking the food might weaken the medicine present in it^{7, 15, 16, 25}.

Mechanism of Action

Most pathogens enter at mucosal surfaces lining the digestive, respiratory, and urino-reproductive tracts, which are collectively the largest immunologically active tissue in body⁴. The mucosal immune system (MIS) is the first line of defense and the most effective site for vaccination against those pathogens, nasal and oral vaccine being the most effective^{3,26}. The goal of oral vaccine is to stimulate both mucosal and humoral immunity against pathogens. Edible vaccine when taken orally undergoes mastication process and the majority of the plant cell degradation occurs in the intestine as a result of action on digestive or bacterial enzymes on edible vaccines. Peyer's Patches (PP) are an enriched source of IgA producing plasma cells and have the potential to populate mucosal tissue and serves as mucosal immune effector sites. The breakdown of edible vaccine occurs near PP²⁷, consisting of 30-40 lymphoid nodules on the outer surface of the intestine and contain follicles from which germinal centre develops upon antigenic stimulation. These follicles act as the sites from which antigen penetrates the intestinal epithelium, thereby accumulating antigen within organized lymphoid structure. The antigen then

comes in contact with M-cells. It contacts with the lumen with broad membrane processes and contains a deep invagination in the basolateral plasma membrane. This pocket is filled with a cluster of B-cells, T-cells and macrophages. M-cells express class II MHC molecules and antigens transported across the mucous membrane by M-cells can activate B-cells within these lymphoid follicles. The activated B-cells leave the lymphoid follicles and migrate to diffuse mucosal associated lymphoid tissue (MALT) where they differentiate into plasma cells that secrete the IgA class of antibodies. These IgA antibodies are transported across the epithelial cells into secretions of the lumen where they can interact with antigens present in the lumen²⁸. The induction of mucosal immunity by edible vaccine is depicted in Fig. 1.

Developing an Edible Vaccine

Gene encoding antigen from pathogenic organisms (virus, bacteria or parasites) that have been characterized and for which antibodies are available, can be handled in two ways. In one case, the entire structural gene is inserted into a plant transformation vector between 5' and 3' regulatory elements; this will allow transcription and accumulation of coding sequence in plant. In second case, epitope within the antigen are identified, DNA fragment encoding these can be used to construct genes by fusion with a coat protein gene from plant virus e.g. TMV or CMV. The recombinant virus is then used to infect stabilized plants. The resultant edible plant vaccines are utilized for further immunological studies. Strategies for the production of candidate vaccine antigens in plant tissues are explained in Fig. 2.

Methods for Transformation of DNA/Gene into Plants

There are essentially two plant transformation methods:

- 1) Plasmid/Vector carrier system: *Agrobacterium tumefaciens* method.
- 2) Micro projectile bombardment (Biolistics) method.

A. tumefaciens, naturally occurring soil bacterium, is used to transfer a small segment of DNA into plant genome and this process is called transformation. Then whole plant can be regenerated from individual plant cell. It has been studied that genes successfully expressed in experimental model plants and when given orally to animals, the transgenic plants extract containing the antigen induced serum antibodies²⁹⁻³¹.

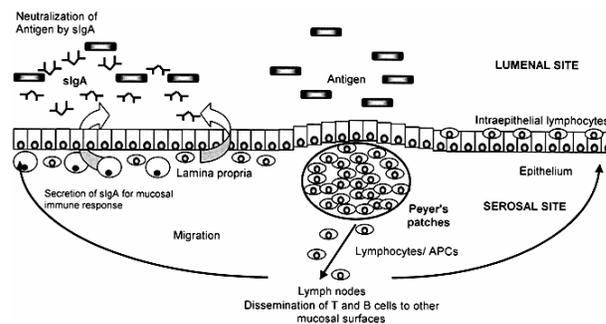


Fig. 1—Schematic representation of induction of immune response by edible vaccine.

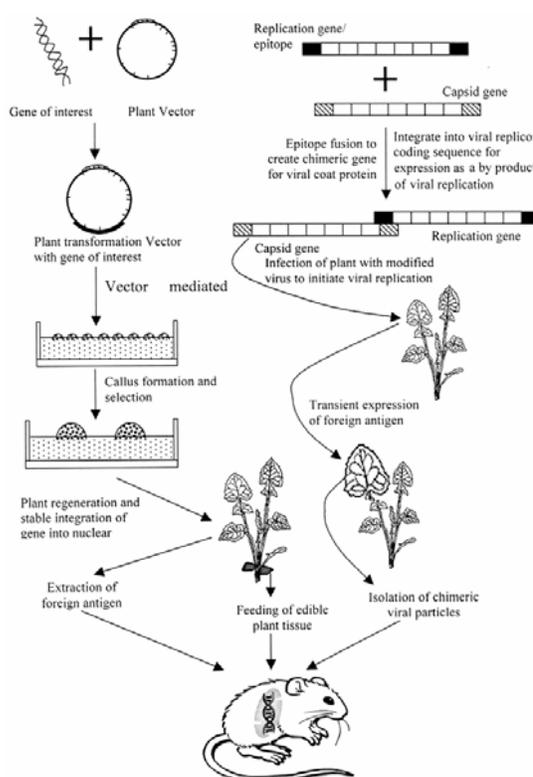


Fig. 2—Strategies for the production of candidate vaccine antigens in plant tissues.

The study revealed that *A. tumefaciens* is used to produce the edible vaccine³²⁻³³. It is based on vegetable pathogens *A. tumefaciens* and *A. rhizogenes*, property to integrate their DNA (T-DNA) with infected cells nuclear genome³⁴. The introduction of exogenous genes into the adequately modified T-DNA of *Agrobacterium* cells and following infection of a vegetable tissue led to the study of gene's stable integration in the plant's genome and production of a transgenic protein. The application of *Agrobacterium*-mediated transformation, first limited to tobacco and few other species, which are the infection's natural targets, has now been extended to most

vegetable species of agronomic interest, including Graminae and Leguminosae^{31,35}. This opens interesting new prospects for the development of edible vaccines for both human and veterinary use.

The second approach is based on the microprojectile bombardment method³⁶. Selected DNA sequences are precipitated onto metal microparticles and bombarded against the vegetable tissue with a particle gun at an accelerated speed. Microparticles penetrate the walls and release the exogenous DNA inside the cell where it will be integrated in the nuclear genome through mechanisms that have yet to be entirely understood. Vegetable cells have cytoplasmic organelles called chloroplasts, which contain chlorophyll, generally known for their photosynthetic function. These organelles, which, like mitochondria, are supposed to derive from ancient bacterial predecessors and which have penetrated a larger cell as symbionts, have an independent chromosome complement, but their characteristics are typical of prokaryotic cells. The biolistic particle delivery system “shoots” adequately processed DNA particles, which penetrate into the chloroplast and integrate with its genome. The chloroplast’s transformation is an interesting alternative to nuclear transformation³⁷⁻³⁸.

Candidates for Edible Vaccines

Foods under study as alternatives to injectable vaccines include bananas, potatoes and tomatoes as well as lettuce, rice, wheat, soybean and corn³⁹. When choosing a plant to be used as a vaccine it is important that it is a hardy, palatable plant with high nutritive and protein content. The plant is also one that would best be indigenous to the country in which it is to be used and should be able to be transformed with relative ease. Tobacco plants have been used extensively in this research but new work is being done in potatoes, tomatoes, lettuce, corn and other crop plants. Little is known about the optimum dosage needed to confer immunity or how long this immunity lasts so there is still a lot of research to be done. Lettuce might replace booster shots in the next generation of vaccines. Researchers have raised the immunity of mice to measles by feeding them a booster vaccine derived from plants⁴⁰. Potatoes are a good system to test the idea of edible vaccines⁴¹. Bananas are also good candidates for edible vaccines as they are eaten raw, inexpensive to produce and native to many developing countries⁴².

Tomatoes serve as ideal candidates for HIV antigen because unlike other transgenic plants that carry the

protein, tomatoes are edible and immune to any thermal process, which helps retain its healing process. Tobacco is good model system for evaluating the production of recombinant proteins; however, it produces toxic compounds, which make it unsuitable for vaccine delivery. Clinical trails have shown the induction of immune responses with antigen expressed in potato and lettuce^{16,43}. Lettuce is a fast growing species suitable for direct consumption and experimental studies. Recent studies have shown that mammalian proteins can be expressed in high levels in transgenic rice⁴⁴. Potato was the first major system to be used for vaccine production, and transgenic potato tubers have been administered to humans in at least three clinical trails to date. During the last few years, potatoes have been evaluated for the production of human serum albumin⁴⁵, novel vaccine candidate^{46,47}, tumor necrosis factor α (TNF- α)⁴⁸ and antibodies⁴⁹.

Factors Affecting Efficacy of Edible Vaccines

The enterotoxin in *Vibrio cholerae*, the causative agent of cholera, includes a nontoxic subunit B (CTB) that helps the toxin bind to gut cells. CTB is also immunogenic as it stimulates an antibody response in humans and animals. Researchers introduced the CTB gene into potato and developed transgenic plants. Mice were fed with raw potato tubers every week for a month with a final booster feed after another 40 d. Mice fed transgenic potatoes produced cholera-specific antibodies in their serum and intestine; IgA and IgG antibodies reached their highest levels after the fourth feeding⁵⁰. *Cholera toxin* and *Escherichia coli*, heat-labile enterotoxins (LT), are potent oral antigens but when administered with other antigens they provide adjuvantcity to that antigen as shown in Fig. 3. The approach is to enhance the immunogenicity of the

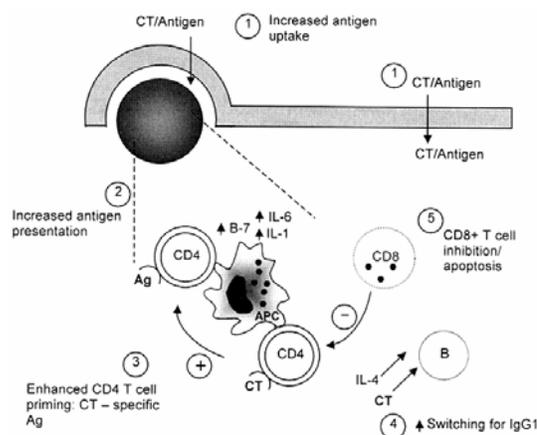


Fig. 3—Mechanism of adjuvant effect of CTB.

orally delivered antigens by using mucosal adjuvants. One such approach is the making use of bacterial enterotoxin such as CT or LT, mammalian and viral immunomodulator as well as plant-derived secondary metabolites¹⁴. Other factors, which are important in determining the efficacy of the vaccine, are the delivery vehicle. Thus the delivery vehicle for antigen should be such that it retains the immunogenicity of the delivered antigen in case it is processed⁵¹. Further studies revealed that IgA antibody response could be improved by increasing the immunization frequencies and use of appropriate adjuvant in primary and booster immunization²⁸.

Recent Developments

Edible plants could be used to deliver antigens for active immunization or to deliver cloned monoclonal secretory antibodies to provide passive immunotherapy^{52,53}. Passive antibodies topically applied to mucosal surfaces have been shown to offer protection against colonization of the human mouth with streptococcus mutants, a cause of dental caries⁵³. The use of alfalfa mosaic virus coat protein fusion vectors to produce HIV and rabies vaccines has been studied¹⁷. A study conducted by Mason *et al* looked at the immunogenic effect of transgenic potato tubers and tobacco leaves carrying a Norwalk virus capsid protein (NVCP) in mice¹⁸. Successful expression of antigens in plants was achieved for rabies virus G-protein in tomato⁵⁴. Wu *et al* expressed rotavirus VP7 in transgenic potatoes and the oral immunization of the transgenic tubers to the mice successfully elicited serum IgG and mucosal IgA specific for VP7. The mucosal IgA titre was as high as 1000, while serum IgG titre was only 600⁵⁵. Systemic and mucosal antibody response in to LT-B in young and aged mice vaccinated with corn-derived antigen against *E. coli* heat labile enterotoxin has been studied⁵⁶. Ma *et al* studied the expression of ORF2 partial gene of hepatitis E virus in tomatoes and immunoactivity of recombinant protein extracted from transgenic plants was examined by enzyme-linked immunosorbent assay (ELISA) using a monoclonal antibody specifically against HEV (hepatitis E virus)⁵⁷. Mason *et al* developed edible transgenic potato vaccine by expressing synthetic LT-B gene into potato plants, which protects mice against *E. coli* heat-labile enterotoxin (LT)⁵⁸. A positive response was observed when gene of protective antigen (PA) of anthrax expressed in plant system and thus the possibilities of development of edible anthrax vaccine has been

explored⁵⁹. Recently, the researchers used green microalgae as a novel source for the production of recombinant proteins⁶⁰. Papillomavirus L1 protein (for development of vaccine against cervical cancer) expressed in transgenic plant could potentially activate the humoral immune response⁶¹. Efforts are also being made on the development of edible vaccines against neurocysticercosis, which occurs due to *Taenia solium*⁶². Current researches in edible vaccines have also indicated the delivery of antigen Tet C in plants⁶³. A team of scientists in Singapore working for three years developed edible vaccine for SARS virus, by immunizing mice against a SARS-like virus by feeding them the genetically modified lactic acid bacteria. The mice that consumed it developed antibodies and protected them from infections with the virus⁶⁴.

In Canada, a genetically engineered tobacco plant made to produce Interleukin ten will be tested to treat Crohn's disease, an intestinal disorder. The research has also fueled speculation that certain food vaccines might help suppress autoimmunity, in which the body's defenses mistakenly attack normal, uninfected tissues. Among the autoimmune disorders that might be prevented or eased is type I diabetes, multiple sclerosis and rheumatoid arthritis^{15, 65}.

Molecular farming is used to produce medicinal or industrially significant compounds in plants, traditionally used in agriculture. These super plants include corn, soybean, alfalfa, potatoes and tobacco. Crop plants are ideal bioreactors for the production of the prized proteins. These plants are able to produce large amount of the proteins in seed and leaves and hence lend themselves to easy harvesting. Molecular farming uses the science of genetic engineering to turn ordinary plants into factories for the production of inexpensive vaccines for diseases like Crohn's disease, diabetes mellitus, rheumatoid arthritis and multiple sclerosis. Carrillo *et al* demonstrated that foot and mouse disease virus protein produced in plants could be used to protect against FMDV infection^{66,67}.

Applications of Edible Vaccines

Malaria

Malaria remains one of the most significant causes of human morbidity and mortality worldwide, with 300 to 500 million new cases of infection annually resulting in 1.5 to 2.7 million deaths. The world malaria situation has become significantly worse in recent years as the main forms of malaria control,

spraying programmes and chemotherapy, becoming less effective in the development of vector and parasite resistance. Three antigens are currently being investigated for the development of a plant-based malaria vaccine, merozoite surface protein (MSP) 4 and MSP 5 from *Plasmodium falciparum*, and MSP 4/5 from *P. yoelli*. Wang *et al* have demonstrated that oral immunization of mice with recombinant MSP 4, MSP 4/5 and MSP1, co-administered with CTB as a mucosal adjuvant, induced antibody responses effective against blood-stage parasite. For those studies, however, proteins were expressed in *E. coli* and protection was only evident when high dose antigen was administered. Whether oral delivery of a plant-derived malaria vaccine would induce significant immune responses in humans is uncertain. It has been suggested that antigen expression levels in plants are so low that an unrealistic quantity of plant material would have to be consumed to achieve meaningful immunity. For this approach to bear fruits, transgenic technology has to improve antigenic expression to induce responses in susceptible populations like children with moderate food intake. Moreover, due to the high levels of antigen anticipated to be necessary, it is likely that strong adjuvants will also be required⁶⁸. Hence, appropriate adjuvants have to be identified and tested. Finally, in the face of reports showing induction of tolerance or immunity through comparable oral immunizations vaccination regimens must be rigorously tested in preclinical studies^{14,69}.

Measles

Globally, measles cause over 800000 deaths every year. Many other affected people become deaf or develop encephalitis. The vaccine currently in use produces 95% seroconversion in individuals who are over the age of 18 months at the time of vaccination⁷⁰⁻⁷². Measles live-attenuated vaccine does not produce oral immunization effect and destroyed by heat. Hence, refrigeration is the prerequisite for its storage. Maternal antibodies also reduce the immunization response of vaccine. MV-H antigen was selected for the development of edible vaccine, which can be transformed in tobacco plant by plasmid/vector *A. tumefaciens*^{73,74}. It has been observed that upon oral administration, MV-H encapsulated transgenic plant extract induced serum antibodies, which were able to neutralize wild type MV and retained its immunogenicity. Results indicated that IgA

antibodies were found in the faecal samples of animals immunized orally with plant derived MV-H⁷⁵⁻⁷⁷. It has also been studied that transgenic carrot plant could be used to deliver viral antigens for the development of measles vaccine⁷⁸. Serum samples from healthy experimental animals, fed with transgenic banana, were analyzed for the presence of anti-hemagglutination-specific antibodies. The results showed that the banana plant can produce the antigenic hemagglutination protein immune responses in experimental animals⁸.

Hepatitis B

The hepatitis B virus is estimated to have infected 400 million people throughout the globe, making it one of the most common human pathogen. Since immunization is the only known method to prevent the diseases of the hepatitis B virus, any attempt to reduce its infection requires the availability of large quantities of vaccine, hepatitis A surface antigen (HBsAg). The HBsAg subtype ayw was cloned into CaMv plasmid and the regenerated plants from the transformed cells were shown to produce HbsAg. Furthermore, expression of the antigen was found to be higher in roots of transgenic potato than in leaf tissues⁷⁹. However, the expression of HbsAg in transgenic potatoes is not sufficient for using as oral vaccine. Further studies are underway to increase the level of HBsAg by using different promoters such as patatin promoter, and different transcription regulating elements.

In an attempt to increase the expression of HbsAg in potato, Arntzen and his colleagues tested the introduction of a number of signaling peptides and 5'- and 3' - untranslated regions (UTRs) in constructs driven by normally constitutive cauliflower mosaic virus (CaMV) 35S promoter. Sequences tested included 5'- UTRs from tobacco etch virus and tobacco mosaic virus, and 3'- UTRs from soybean vspB and potato pinII genes. After normalizing to transcript levels as described above, it has been found that the use of different 5'- UTRs had a little effect on expression levels, but the introduction of vspB and pinII 3' UTRs increased the amount of HBsAg protein significantly⁸⁰.

HBsAg expressed in tobacco plant and administered to animals as parenteral vaccine. It elicited primary response as those obtained against conventional vaccine. Investigation on experimental animals revealed that they would reliably eat up to 5 g of raw tuber overnight, once per week (42 µg HBsAg/dose) and increase in serum antibody titre could be detected after

two doses of potatoes whereas two doses of purified yeast derived HBsAg gave no detectable serum antibody increase, even though the dosage of yeast derived material was four fold higher. Memory response is a critical factor in vaccine design and implementation and upon introduction of fresh raw HBsAg potatoes to animals stimulated a primary immune response, following a time interval of several weeks, booster immunization by parental route produced a rapid recall response that persisted for at least 150 days. HBsAg produced and delivered in transgenic potatoes produced as effective systemic and mucosal immune response. Oral adjuvants such as cholera toxin and *E.coli* heat liable enterotoxin increase the immunogenicity of HBsAg³⁸.

Stopping Autoimmunity

In the past 15 years, investigators have identified several data cell proteins that can elicit autoimmunity in people predisposed to Type I diabetes. The development of plant based diabetes vaccine in potatoes and tobacco containing insulin or GAD linked to the innocuous B subunits of the *V. cholerae* toxin to enhance the uptake of antigens by M-cells was attempted. The development of transgenic potato and tobacco plants when fed to nonobese diabetic mice showed increased levels of IgG, an antibody associated with cytokines that suppress harmful immune response. Feeding of the vaccines to mouse strain that becomes diabetic helped to suppress the autoimmune attack and to prevent the delay of high blood sugar^{52, 81}.

Bovine Pneumonia Pasteurellosis

Mannheimia haemolytica A, leukotoxin (LkT) was the antigen selected for the development of edible vaccine against Bovine Pneumonia Pasteurellosis and removal of transmembrane domains from mannheimia haemolytica retained its immunogenicity. It is coded as LkT66; whereas LkT50 is subjected to fusion with modified green fluorescence protein (mGFPs). The complex so formed is transcribed by cauliflower mosaic virus 35S promoter and *A. tumefaciens* used for plant transformation. Studies on rabbits showed the positive response upon oral immunization of this edible vaccine⁷⁷.

Cholera

Cholera and other diarrhoeal diseases cause up to ten million deaths per year in the developing world, primarily among children. Relatively little work on

vaccines, among children to prevent these diseases is underway, as they represent more of a nuisance than a severe problem developed in developed countries. Studies supported by WHO have demonstrated possibility of an effective vaccine for cholera, which provide cross-protection against enterotoxic *E. coli*. To address this limitation, plants were transformed with the gene encoding B subunit of the *E. coli* heat liable enterotoxin (LT-B). Transgenic potatoes expressing LT-B were found to induce both serum and secretory antibodies when fed to mice; these antibodies were protective in bacterial toxin assay *in vitro*. This is the first “proof of concept” for the edible vaccine. Since people eat only cooked potatoes, the effect of boiling on the properties of CTB expressed in transgenic potatoes was examined. After boiling for five minutes, over half of the vaccine protein survived in its biologically active form, providing evidence that cooking does not always inactivate edible vaccines. Thus, the spectrum of plants for producing edible vaccines may be expanded beyond raw food plants such as fruits^{58, 82, 83}.

Diabetes

More than 100 million people are affected with diabetes worldwide. Type I diabetes, also known as insulin dependent diabetes mellitus (IDDM) or juvenile-onset diabetes, primarily affects children and young adults and accounts for 5-10% of the diagnosed diabetes in North America. It is an autoimmune disease where the pancreatic beta cells which produce insulin are destroyed by the body's own immune system.

Research by Ma *et al* at the University of Western Ontario showed that diabetes can be prevented in mice by feeding them with plants engineered to produce a diabetes-related protein. The idea is based on ‘oral tolerance’ where the autoimmune system is selectively turned off early by teaching the body to tolerate the “antigenic proteins”. The pancreatic protein, glutamic acid decarboxylase (GAD67), is linked to the onset of IDDM, and when injected into mice it is known to prevent diabetes. The Canadian group developed transgenic potato and tobacco plants with the gene for GAD67, fed them to non-obese diabetic mice, which developed insulin-dependent diabetes spontaneously. The results were intriguing: only 20% of the pre-diabetic mice feed with transgenic plants developed the diabetes while 70% non-treated mice developed the disease. The treated mice also showed increased levels of IG1, an antibody

associated with cytokines, which suppresses harmful immune responses. Thus, the antigen produced in plants appears to retain immunogenicity and prevent diabetes in an animal model. According to Ma, this is the first proof of principle for the use of edible vaccines in the treatment of autoimmune diseases^{65,84}.

Human Trials

In the first human study of transgenic plant vaccine designed to induce active immunity, 14 adult volunteers were given either 100 g of transgenic potato, 50 g of transgenic potato or 50 g of wild type potato, each transgenic potatoes containing from 3.7 to 15.7 µg/g of LT-B¹⁵. The variable dose per gram of potato was due to the tissue specificity of the promoter, therefore, that LT-B was expressed to a different degree in the different tissues of the potatoes. The potatoes in this study were ingested raw; however, subsequent studies have shown that transgenic potatoes expressing the B subunit of cholera toxin could be boiled for 3 min until the tissue becomes soft with loss of only about 50% of the CT-B pentameric GM1-binding form⁶⁸. Serologic responses were also detected after vaccination. Ten (91%) out of the 11 volunteers who ingested transgenic potatoes developed IgG anti-LT, and in half of them responses occurred after the first dose. Six (55%) of the 11 volunteers developed four-fold rise in serum IgA anti-LT¹⁵.

Opening a new era in vaccine delivery, researchers supported by the National Institute of Allergy and Infectious Diseases (NIAID) have shown for the first time that an edible vaccine can safely trigger significant immune responses in people. The goal of the Phase 1 proof-of-concept trial study was to demonstrate that an edible vaccine could stimulate an immune response in humans. Volunteers ate bite-sized pieces of raw potato that had been genetically engineered to produce part of the toxin secreted by the *E. coli*, which causes diarrhoea⁸⁵. Previously, NIAID supported *in vitro* and preclinical studies showed that transgenic potatoes containing this segment of the toxin stimulated strong immune responses in animals. The transgenic potatoes were created and grown scientifically. The trial enrolled 14 healthy adults; 11 were chosen at random to receive the genetically engineered potatoes and 3 received pieces of ordinary potatoes. The investigators periodically collected blood and stool samples from the volunteers to evaluate the vaccine's ability to stimulate both systemic and intestinal immune responses. Ten of the

11 volunteers (91 per cent) who ingested the transgenic potatoes had four-fold rise in serum antibodies at some point after immunization, and 6 of the 11 (55 per cent) developed fourfold increase in the intestinal antibodies. The potatoes were well tolerated and no one experienced serious adverse side effects. Encouraged by the results of this study, NIAID-supported scientists are exploring the use of this technique for administering other antigens. Edible vaccines for other intestinal pathogens are already in the pipeline. Potatoes and bananas that might protect against Norwalk virus, a common cause of diarrhoea, and potatoes and tomatoes that might protect against hepatitis B are being developed^{4,53,86}. Thanavala's group has developed a potato vaccine booster for use in conjunction with injected hepatitis B vaccine. It is currently in phase II clinical trials and I for patients who have previously been vaccinated⁸⁷. Tacket *et al* studied the human immune response to the NVCP expressed in potatoes¹⁶. Overall, 95%, 19 out of 20 volunteers developed some kind of immune response, although the antibody increase in some cases was modest. Some applications of edible vaccines are given in Table 2.

Patents on Edible Vaccines

ProdiGene, a biotechnology company, has announced receipt of patent (US Patent # 6, 136, 320) for its process that uses plants to develop oral vaccines to immunize humans and animals against viral diseases. It has a patent that covers viral disease vaccines, for hepatitis and Transmissible Gastroenteritis virus, produced in genetically enhanced plants. The vaccines produced using this technology can be marketed either in edible form, made from parts of a fruit, vegetable or grain plant, or in injectable form⁹². Clinical trials for the Transmissible Gastroenteritis Virus vaccines are in progress now, and ProdiGene research utilizing plant

Table 2—Therapeutic & diagnostic application of edible vaccines

S. No.	Application	Plants	Ref.
1.	Dental caries	<i>Nicotiana tabacum</i>	52,53
2.	Cancer treatment	Wheat Rice <i>N. tabacum</i>	44,88,89
3.	B- Cell lymphoma treatment	<i>N. benthamiana</i>	90
4.	Colon cancer	<i>N. benthamiana</i>	91
5.	Herpes simplex virus-2	Soybean	92
6.	Diagnosis	Alfalfa	93

Table 3—Selected patents on edible vaccine technologies

S. No.	Patent holder	Claim	Ref.
1	Prodigene	Recombinant antigen production and transfer to plants cells using plasmid vector system; Vaccine produced in genetically engineered plants for hepatitis and transmissible gastroenteritis virus	94
2	Found Advan Mil Med (USA)	Antibacterial vaccine expressed in plant cells, particularly useful against shigellosis	93
3	Ribozyme-Pharm	Nucleic acid vaccine used to treat or prevent viral infections in plants, animals or bacteria	94
4	Rubicon-Lab	Retrovirus expressed in animal or plant cells useful as virus and cancer vaccine	93,95
5	Applied Phytologics	Gene constructs for disease resistance, vaccine production in rice, barley, wheat, corn	94,96
6	Biosource (now Large Scale Biology)	Plant viral vector with potential as anti-AIDS vaccine; recombinant proteins for use in vaccines to protect against parasitic infection, eg malaria	94,97
7	University of Yale	Vaccine against invertebrates (insects, arachnids, helminthes, etc)	93
8	University of Texas	Hepatitis B virus core antigen recombinant vaccine	95,98
9	Biocem; Rhone-Merieux	Rabies vaccine in transgenic plants	
10	Institute Pasteur	Attenuated <i>E coli</i> vaccine for use in gene therapy	94
11	University of Texas A&M/Tulane University	Transgenic plants containing <i>E coli</i> enterotoxin B for edible vaccine application in animals	15,93
12	USDA/Univ. Philadelphia	Rabies vaccine expressed in tomato plant	94
13	Scripps Research Institute	Recombinant antigen production in lettuce, spinach, tobacco, kidney bean, or <i>Chenopodium amaranticolor</i>	94
14	Cornell University	Increasing foreign protein expression	93
15	University Loma Linda	Gene constructs used to produce edible vaccines to treat autoimmune diseases	94
16	Agr Genet/ Purdue Research Foundation	Modified viruses used for vaccine production in plants, esp. against food and mouth disease, HIV and human rhino virus	94,96

technology to develop an edible vaccine that protects against HIV infection by stimulating mucosal immunity. ProdiGene is developing numerous industrial and pharmaceutical proteins from plants^{65,94}. Some selected patents on edible vaccine technologies are given in Table 3.

Future Research

Edible vaccines hold great potential, especially in Third World countries where transportation costs; poor refrigeration and needle use complicate vaccine administration. While research is also being conducted with laboratory animals, diabetics may some day benefit from an edible form of insulin. NSF and other Government-agency and industry-funded researchers have developed technologies that permit the introduction of a hybrid gene, which produces human insulin in potatoes. For diabetics, insulin-bearing potatoes may help train the body's defenses to stop reacting to insulin as if they were a foreign material.

Edible vaccines might overcome some of the difficulties of production, distribution and delivery associated with traditional vaccines. Significant

challenges are still to be overcome before vaccine crops can become a reality. However, while access to essential healthcare remains limited in most of the world and the scientific community is struggling with complex diseases such as HIV and malaria, plant-derived vaccines represent an appetizing prospect. Strategies to improve the recombinant protein yield in plants include the development of novel promoters, the improvement of protein stability and accumulation through the use of signals that target the protein to intracellular compartments, and the improvement of downstream processing technologies⁹⁹.

Conclusions

Edible vaccines might be a solution that will enable the positive effects of vaccines for reaching and to decrease some potential hazards associated with parenteral vaccines such as toxic compounds, allergic responses and risk of attenuated strains reverting to pathogenic strains. Edible vaccines offer a way to deliver a vaccine orally, without the need for the cold chain, decreasing the cost of production and shipping and may be ideal for facing bio-weapons and veterinary use among other benefits.

Plant-based vaccines are an exciting and novel new strategy for the development of oral vaccines. Edible vaccines is a milestone on the road to creating inexpensive vaccines that might be particularly useful in immunizing people in developing countries, where high cost and logistical issues, such as transportation and need for certain vaccines to be refrigerated, can obstruct effective vaccination programme. The hope is that the edible vaccines could be grown in many of the developing countries where they would actually be used. Edible vaccines for combating autoimmunity and infectious diseases have a long way to go before they will be ready for large-scale testing in humans. The technical obstacles, though many, seem surmountable.

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