EDIBLE VACCINES: CURRENT STATUS AND FUTURE

P Lal, VG Ramachandran, *R Goyal, R Sharma

Abstract

Edible vaccines hold great promise as a cost-effective, easy-to-administer, easy-to-store, fail-safe and socioculturally readily acceptable vaccine delivery system, especially for the poor developing countries. It involves introduction of selected desired genes into plants and then inducing these altered plants to manufacture the encoded proteins. Introduced as a concept about a decade ago, it has become a reality today. A variety of delivery systems have been developed. Initially thought to be useful only for preventing infectious diseases, it has also found application in prevention of autoimmune diseases, birth control, cancer therapy, etc. Edible vaccines are currently being developed for a number of human and animal diseases. There is growing acceptance of transgenic crops in both industrial and developing countries. Resistance to genetically modified foods may affect the future of edible vaccines. They have passed the major hurdles in the path of an emerging vaccine technology. Various technical obstacles, regulatory and non-scientific challenges, though all seem surmountable, need to be overcome. This review attempts to discuss the current status and future of this new preventive modality.

Key words: Chimeric, edible vaccines, transgenic

Vaccines have been revolutionary for the prevention of infectious diseases. Despite worldwide immunization of children against the six devastating diseases, 20% of infants are still left un-immunized; responsible for approximately two million unnecessary deaths every year, especially in the remote and impoverished parts of the globe. This is because of the constraints on vaccine production, distribution and delivery. One hundred percent coverage is desirable, because un-immunized populations in remote areas can spread infections and epidemics in the immunized “safe” areas, which have comparatively low herd immunity. For some infectious diseases, immunizations either do not exist or they are unreliable or very expensive. Immunization through DNA vaccines is an alternative but is an expensive approach, with disappointing immune response. Hence the search is on for cost-effective, easy-to-administer, easy-to-store, fail-safe and socioculturally readily acceptable vaccines and their delivery systems. As Hippocrates said, “Let thy food be thy medicine,” scientists suggest that plants and plant viruses can be genetically engineered to produce vaccines against diseases such as dental caries; and life-threatening infections like diarrhea, AIDS, etc. This is the concept of edible vaccines. The following discussion will address issues relating to their commercial development, especially their usefulness in preventing infectious diseases in developing countries.

Concept of Edible Vaccines

Creating edible vaccines involves introduction of selected desired genes into plants and then inducing these altered plants to manufacture the encoded proteins. This process is known as “transformation,” and the altered plants are called “transgenic plants.” Like conventional subunit vaccines, edible vaccines are composed of antigenic proteins and are devoid of pathogenic genes. Thus, they have no way of establishing infection, assuring its safety, especially in immunocompromised patients. Conventional subunit vaccines are expensive and technology-intensive, need purification, require refrigeration and produce poor mucosal response. In contrast, edible vaccines would enhance compliance, especially in children, and because of oral administration, would eliminate the need for trained medical personnel. Their production is highly efficient and can be easily scaled up. For example, hepatitis-B antigen required to vaccinate whole babies in the world each year on just 200 acres of land! They are cheaper, sidestepping demands for purification (single dose of hepatitis-B vaccine would cost approximately 23 paise), grown locally using standard methods and do not require capital-intensive pharmaceutical manufacturing facilities. Mass-indefinite production would also decrease dependence on foreign supply. They exhibit good genetic stability. They are heat-stable; do not require cold-chain maintenance; can be stored near the site of use, eliminating long-distance transportation. Non-requirement of syringes and needles also decreases chances of infection. Fear of contamination with animal viruses - like the mad cow disease, which is a threat in vaccines manufactured from cultured mammalian cells - is eliminated, because plant viruses do not infect humans.

Edible vaccines activate both mucosal and systemic immunity, as they come in contact with the digestive tract lining. This dual effect would provide first-line defense against pathogens invading through mucosa, like Mycobacterium
The antigens in transgenic plants are delivered through bio-encapsulation, i.e., the tough outer wall of plant cells, which protects them from gastric secretions, and finally break up in the intestines. The antigens are released, taken up by M cells in the intestinal lining that overlie Peyers patches and gut-associated lymphoid tissue (GALT), passed up by M cells in the intestinal lining that overlie peyer’s patches and gut-associated lymphoid tissue (GALT), passed on to macrophages, other antigen-presenting cells; and local lymphocyte populations, generating serum IgG, IgE responses, local IgA response and memory cells, which would promptly neutralize the attack by the real infectious agent.

Mechanism of Action

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Preparation of Edible Vaccines

Introduction of foreign DNA into plant’s genome can either be done by bombarding embryonic suspension cell cultures using gene-gun or more commonly through Agrobacterium tumefaciens, a naturally occurring soil bacterium, which has the ability to get into plants through some kind of wound (scratch, etc.). It possesses a circular “Ti plasmid” (tumor inducing), which enables it to infect plant cells, integrate into their genome and produce a hollow tumor (crown gall tumor), where it can live. This ability can be exploited to insert foreign DNA into plant genome. But prior to this, the plasmid needs to be disarmed by deleting the genes for auxin and cytokinin synthesis, so that it does not produce tumor. Genes for antibiotic resistance are used to select out the transformed cells and whole plants, which contain the foreign gene; and for expressing the desired product, which can then be regenerated from them.9 The DNA integrates randomly into plant genome, resulting in a different antigen expression level for each independent line,10 so that 50-100 plants are transformed together at a time, from which one can choose the plant expressing the highest levels of antigen and least number of adverse effects. Production of transgenic plants is species dependent and takes 3-9 months. Reducing this time to 6-8 weeks is currently under investigation. Some antigens, like viral capsid proteins, have to self-assemble into VLPs (virus-like particles). VLPs mimic the virus without carrying DNA or RNA and therefore are not infectious. Each single antigen expressed in plants must be tested for its proper assembly and can be verified by animal studies, Western blot; and quantified by enzyme-linked immunosorbent assay (ELISA).

“Second-Generation” Edible Vaccines

Successful expression of foreign genes in plant cells and/or its edible portions has given a potential to explore further and expand the possibility of developing plants expressing more than one antigenic protein. Multicomponent vaccines can be obtained by crossing two plant lines harboring different antigens. Adjuvants may also be co-expressed along with the antigen in the same plant. B subunit of Vibrio cholerae toxin (VC-B) tends to associate with copies of itself, forming a doughnut-shaped five-member ring with a hole in the middle. This feature can bring several different antigens to M cells at one time. For example, a trivalent edible vaccine against cholera, ET EC (Enterotoxigenic E. coli) and rotavirus could successfully elicit significant immune response to all three. Global alliance for vaccines and immunization (GAVI) accords very high priority to such combination vaccines for developing countries.

Various Strategies

Approaches to mucosal vaccine formulation include (i) gene fusion technology, creating non-toxic derivatives of mucosal adjuvants; (ii) genetically inactivating antigens by deleting an essential gene; (iii) co-expression of antigen and a cytokine, which modulates and controls mucosal immune response; and (iv) genetic material itself, which allows DNA/RNA uptake and its endogenous expression in the host cell. Various mucosal delivery systems include biodegradable micro- and nanoparticles, liposomes, live bacterial/viral vectors and mucosal adjuvants. “Prime-boost” strategy combines different routes of administration and vaccine types, especially where multiple antigens or doses are required. For example, a single parenteral dose of MV-H DNA (measles virus haemagglutinin) followed by multiple oral MV-H boosters could induce greater quantities of MV-neutralizing antibodies than with either vaccine alone.

Chimeric Viruses

Certain viruses can be redesigned to express fragments of antigenic proteins on their surface, such as CPMV (cowpea mosaic virus), alfalfa mosaic virus, TMV (tobacco mosaic virus), CaMV (cauliflower mosaic virus), potato virus X and tomato bushy stunt virus. Technologies involved are overcoat and epiccoat technology. Overcoat technology permits the plant to produce the entire protein, whereas epiccoat technology involves expression of only the foreign proteins. A plant-derived mink enteritis virus (MEV) injectable vaccine,
expressed on chimeric CPMV, was shown to protect minks against the clinical disease. Fragments of gp41 surface protein of HIV virus put into CPMV could evoke a strong neutralizing antibody response in mice. It may even be possible to present a cocktail of specific HIV epitopes on the surface of the plant virus. CPMV is genetically and thermally stable. It can survive acidity (pH 1) for one hour. Wide range of epitopes has been expressed in CPMV. They include HIV-1 (gp41), (gp120); human rhinoviruses; foot and mouth disease virus; canine parvovirus; mammalian epitopes from hormones or from colon cancer cells; fungal epitopes and protozoan epitopes from Plasmodium falciparum. Wide range of routes is available – parenteral and nasal (purified particles), oral (formulated leaf extracts), whole and homogenized leaves, fruits or vegetable tissues. In all these instances, plant viruses are engineered to carry the desired genes and used to infect their natural hosts such as the edible plants where the cloned genes are expressed to varying degrees in different parts of the plant, including their edible portions.

**Challenges**

There are many questions which need to be answered before developing a plant-based vaccine (Table 1). Three successful human clinical trials have shown that adequate doses of antigen can be achieved with plant-based vaccines. To determine the right dosage, one needs to consider the person’s weight, age; fruit/plant’s size, ripeness and protein content. The amount to be eaten is critical, especially in infants, who might spit it, eat a part or eat it all and throw it up later. Too low a dose would fail to induce antibodies, and too high a dose would instead cause tolerance. Concentrating the vaccine into a teaspoon of baby food may be more practical than administering it in a whole fruit. The transformed plants can also be processed into pills, puddings, chips, etc. Regulatory concerns would include lot-to-lot consistency, uniformity of dosage and purity.

Foreign proteins in plants accumulate in low amounts (0.01-2% of total protein) and are less immunogenic; therefore the oral dose far exceeds the intranasal/parenteral dose. For example, oral hepatitis-B dose is 10-100 times the parenteral dose; and 100 gm potato expressing B subunit of labile toxin of ETEC (LT-B) is required in three different doses, to be immunogenic. Attempts at boosting the amount of antigens often lead to stunted growth of plants and reduced tuber/fruit formation, as too much m-RNA from the transgene causes gene-silencing in plant genome. Some of the techniques to overcome these limitations are (i) optimization of coding sequence of bacterial/viral genes for expression as plant nuclear genes, (ii) expression in plastids, (iii) plant viruses expressing foreign genes, (iv) coat-protein fusions, (v) viral-assisted expression in transgenic plants and (vi) promoter elements of bean yellow dwarf virus with reporter genes GUS (β-glucuronidase) and GFP (green fluorescent protein), substituted later with target antigen genes. Antigen genes may be linked with regulatory elements which switch on the genes more readily; or do so only at selected times (after the plant is nearly fully grown); or only in its edible regions. Exposure to some outside activator molecule may also be tried.

To enhance immunogenicity, mucosal adjuvants, better
targeted to the immune system, may be used, like molecules that bind to M cells in the intestine lining and pass them to immune cells. These include CT-B (Cholera toxin - B subunit), LT-B (ETEC), mammalian/viral immunomodulators and plant-derived secondary metabolites.\(^1,2\) To decrease toxicity and allergic potential, mutant forms of \(E. \text{coli}\)-labile toxin, like LT-K63 and LT-R72 and hinge cleavage mutant LT-G192, are used.\(^26\) Another challenge would be in dealing with diseases caused by multiple serotypes (dengue) or by complex parts from different life cycles of parasites (malaria) or by rapidly mutating organisms (HIV, trypanosomes, influenza). Each plant possesses its set of advantages and disadvantages (Table 2).

**Nonscientific Challenges**

Presently, small technology companies are undertaking most research, as edible vaccines are targeted to markets of developing nations. Large companies are more interested in livestock market than human application. Only few international aid organizations and some national governments are rendering support, but the effort remains largely underfunded. Some of the companies funding edible vaccines research have failed to click due to lack of investors’ confidence in returns on investments in genetically-modified (GM) foods. There is also a lack of research and development (R&D) personnel in the pharmaceutical companies. In addition, the recombinant (injectable) vaccines against diphtheria, tetanus, etc., are so cheap now, that there would be little incentive to develop edible vaccines for them.

**Regulatory Issues**

It is still unclear whether the edible vaccines would be

<table>
<thead>
<tr>
<th>Plant/fruit</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
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<tbody>
<tr>
<td>Tobacco</td>
<td>Good model for evaluating recombinant proteins</td>
<td>Produces toxic compounds*</td>
</tr>
<tr>
<td></td>
<td>Low-cost preserving system (numerous seeds, stored for long time)</td>
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<tr>
<td></td>
<td>Easy purification of antibodies stored in the seeds, at any location</td>
<td></td>
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<tr>
<td></td>
<td>Large harvests, number of times/year</td>
<td></td>
</tr>
<tr>
<td>Potato</td>
<td>Dominated clinical trials</td>
<td>Needs cooking, which can denature the antigens and decrease immunogenicity**</td>
</tr>
<tr>
<td></td>
<td>Easily manipulated/transformed</td>
<td></td>
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<tr>
<td></td>
<td>Easily propagated from its “eyes”</td>
<td></td>
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<tr>
<td></td>
<td>Stored for long periods without refrigeration</td>
<td></td>
</tr>
<tr>
<td>Banana</td>
<td>Do not need cooking</td>
<td>Trees take 2-3 years to mature</td>
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<tr>
<td></td>
<td>Proteins not destroyed even if cooked</td>
<td>Transformed trees take about 12 months to bear fruit</td>
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<tr>
<td></td>
<td>Inexpensive</td>
<td>Spoils rapidly after ripening</td>
</tr>
<tr>
<td></td>
<td>Grown widely in developing countries</td>
<td>Contains very little protein, so unlikely to produce large amounts of recombinant proteins</td>
</tr>
<tr>
<td>Tomato</td>
<td>Grow quickly</td>
<td>Spoils readily</td>
</tr>
<tr>
<td></td>
<td>Cultivated broadly</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High content of vitamin A may boost immune response</td>
<td>Grows slowly</td>
</tr>
<tr>
<td>Rice</td>
<td>Commonly used in baby food because of low allergenic potential</td>
<td>Requires specialized glasshouse conditions</td>
</tr>
<tr>
<td></td>
<td>High expression of proteins/antigens</td>
<td></td>
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<tr>
<td></td>
<td>Easy storage/transportation</td>
<td></td>
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<tr>
<td></td>
<td>Expressed protein is heat-stable</td>
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<tr>
<td>Lettuce</td>
<td>Fast-growing</td>
<td>Spoils rapidly</td>
</tr>
<tr>
<td></td>
<td>Direct consumption</td>
<td></td>
</tr>
<tr>
<td>Soybean</td>
<td>Large harvests, number of times/year</td>
<td></td>
</tr>
<tr>
<td>and Alfalfa</td>
<td>Muskmelon (cantaloupe)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fast growing</td>
<td></td>
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<tr>
<td></td>
<td>Easily propagated by seed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Easily transformed</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>Carrots, peanuts, wheat, corn</td>
<td></td>
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</tbody>
</table>

*Currently, therapeutic proteins in tobacco are being produced. **Some kinds of South American potatoes can be eaten raw. Although some studies show that cooking does not destroy full complement of antigen in potatoes.\(^3\) ***Freeze-dried tomato powder containing NV capsid and LT-B was found immunogenic. Same technology is also used for potatoes and carrots.
regulated under food, drugs or agricultural products and what vaccine component would be licensed - antigen itself, genetically engineered fruit or transgenic seeds. They would be subjected to a very close scrutiny by the regulatory bodies in order to ensure that they never enter the food supply. This would include greenhouse segregation of medicinal plants from food crops to prevent out-crossing and would necessitate separate storage and processing facilities. Although edible vaccines fall under “GM” plants, it is hoped that these vaccines will avoid serious controversy, because they are intended to save lives.

Clinical Trials

Antigen expression in plants has been successfully shown in the past, like LT-B (ETEC) in tobacco and potato, rabies virus-G protein in tomato,7 HBsAg in tobacco,7 and potato, norwalk virus in tobacco and potato; CT-B (Vibrio cholerae) in potato.1 Ethical considerations usually preclude clinical trials from directly assessing protection, except in a few cases.29 In contrast, veterinary researchers can assess immune protection more directly.

Norwalk virus

Nineteen (95%) out of 20 people fed with transgenic potato expressing norwalk virus antigen showed seroconversion.31 Attempts are underway to engineer bananas and powdered tomatoes expressing norwalk virus.

Cholera

Transgenic potato with CT-B gene of Vibrio cholerae was shown to be efficacious in mice. Eating one potato a week for a month with periodic boosters was said to provide immunity.32 Co-expression of mutant cholera toxin subunit A (mCT-A) and LT-B in crop seed has been shown to be effective by nasal administration and is extremely practical.33

Measles

Mice fed with tobacco expressing MV-H (measles virus haemagglutinin from Edmonston strain) could attain antibody titers five times the level considered protective for humans, and they also demonstrated secretory IgA in their faeces.34 Prime boost strategy by combining parenteral and subsequent oral MV-H boosters could induce titers 20 times the human protective levels. These titers were significantly greater than with either vaccine administered alone.5,15 MV-H edible vaccine does not cause atypical measles, which may be occasionally seen with the current vaccine.35 Thus it may prove better for achieving its eradication. The success in mice has prompted similar experiments in primates. Transgenic rice, lettuce and baby food against measles are also being developed. When given with CT-B (adjuvant), 35-50 gm MV-H lettuce is enough; however, an increased dose would be required if given alone.7

Hepatitis B

For hepatitis B, parenteral VLPs could invoke specific antibodies in mice.36 First human trials of a potato-based vaccine against hepatitis B have reported encouraging results. The amount of HBsAg needed for one dose could be achieved in a single potato. Levels of specific antibodies significantly exceeded the protective level of 10 mIU/mL in humans. When cloned into CaMV (cauliﬂower mosaic virus), plasmid HBsAg subtype ayw showed higher expression in roots as compared to leaf tissue of the transgenic potato. Further studies are required to increase the production of antigen by using different promoters, like patatin promoter. The resulting plant material proved superior to the yeast-derived antigen in both priming and boosting immunity in mice. Prime boost strategy in mice with a single sub-immunogenic parenteral dose of yeast-derived recombinant HBsAg and subsequent oral transgenic potatoes led to the development of antibodies that immediately peaked at >1,000 mIU/mL and were maintained at >200 mIU/mL for five months. This could be a useful immunization strategy for developing countries.37 Tomatoes expressing hepatitis B are being grown in guarded greenhouses. Enough antigens for 4,000 vaccine doses were obtained from just 30 tomato plants.38 Transgenic lettuce is also being developed.

Rabies

Tomato plants expressing rabies antigens could induce antibodies in mice.39 Alternatively, TMV may also be used.40 Transformed tomato plants using CaMV with the glycoprotein (G-protein) gene of rabies virus (ERA strain) was shown to be immunogenic in animals.41

HIV

Initial success in splicing HIV protein into CPMV has been achieved.39 Two HIV protein genes and CaMV as promoter were successfully injected into tomatoes with a needle, and the expressed protein was demonstrable by polymerase chain reaction (PCR) in different parts of the plant, including the ripe fruit, as well as in the second-generation plant.42 Recently, spinach has been successfully inoculated for Tat protein expression cloned into TMV. Each gram of leaf tissue of spinach was shown to contain up to 300-500 μg of Tat antigen.43 Mice fed with this spinach followed by DNA vaccinations resulted in higher antibody titers than the controls, with the levels peaking at four weeks post-vaccination.

STDs

Human papilloma virus type-11 (HPV-11) recombinant VLPs produced in insect cells are immunogenic when given...
orally to BALB/c mice. The response is dose-dependent, conformationally-dependent and genotype-restricted. Thus, VLPs may be effective oral immunogens for the prevention of anogenital HPV disease.\(^{41}\)

**Anthrax**

Tobacco leaves bombarded with pag gene (anthrax protective antigen - PA) using a gene gun could express a protein structurally identical to the major antigen present in existing vaccine. Billions of units of anthrax antigen could be produced. In addition, this vaccine was devoid of edema factor and lethal factor, responsible for the toxic side effects. The same anthrax antigen is now being put in tomato plants.\(^{44}\) Scientists are also trying to transform spinach by inoculating it with TMV-expressing PA, as spinach might be a safer vaccine.\(^{45}\)

**Others**

Transgenic *Arabidopsis thaliana* plants are able to produce a fusion protein consisting of LT-B and early secretory antigen ESAT-6 of *Mycobacterium tuberculosis*, demonstrating both the antigens by ELISA.\(^{46}\) Respiratory syncytial virus (RSV) pneumonia takes a heavy toll of life of children below two years of age, each year. RSV expressed in tomato and potato plants has shown encouraging results in mice. Further clinical trials are being planned. ‘Apple juice’-based RSV vaccine is also being developed.\(^{47}\) Programs for development of new vaccines against rotavirus and *Streptococcus pneumoniae* have been instituted by program for appropriate technology in health and GAVI, with work originating in developing countries. For rotavirus, transgenic potatoes expressing VP7 could induce high titers of IgG and mucosal IgA in mice.\(^{48}\) For parasites like malaria, advances in vaccine have been hindered by the complex multistage life cycle of the parasite, its inaccessibility to study and by its large genome. Nevertheless, chimeric coat proteins of CPMV expressing malarial and foot-and-mouth disease epitopes have been reported.\(^{39}\)

**Veterinary Sciences**

The first patented edible vaccine to demonstrate efficacy in animal trials was against the transmissible gastroenteritis virus (TGEV) in pigs and was under planning to be made commercially available.\(^{49}\) Vaccines against porcine reproductive and respiratory syndrome (PRRS) and other diseases like parvovirus are being investigated. Various transgenic animal feeds are currently undergoing clinical trials in pigs.\(^{50}\) Other candidates are diseases of pets, animals in swine and poultry industries, draft and wild animals. Different plant viral system patents exist (Table 3).

**Passive Immunization**

Just as any antigenic protein can be expressed in plants, antibody molecules too can be expressed in plants. Protective antibodies produced in plants are sometimes referred to as “plantibodies.” To date, only four antibodies produced in plants have the potential to be used as therapeutic agents, and only IgG-IgA against *Streptococcus mutans* has been tested on humans. Attempts are being made to formulate these secretory antibodies into toothpaste to protect against tooth decay. Human trials are also being planned. The other antibodies have been tested on animals and also show great promise. Producing complete secretory antibodies was quite difficult.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Target host</th>
<th>Plant</th>
<th>Mode of administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterotoxigenic <em>E. coli</em> (ETEC)</td>
<td>Humans</td>
<td>Tobacco</td>
<td>Oral</td>
</tr>
<tr>
<td>ETEC</td>
<td>Humans</td>
<td>Potato</td>
<td>Oral</td>
</tr>
<tr>
<td>ETEC</td>
<td>Humans</td>
<td>Maize</td>
<td>Oral</td>
</tr>
<tr>
<td><em>Vibrio cholerae</em></td>
<td>Humans</td>
<td>Potato</td>
<td>Oral</td>
</tr>
<tr>
<td>Hepatitis B virus</td>
<td>Humans</td>
<td>Tobacco</td>
<td>Injection (extracted protein)</td>
</tr>
<tr>
<td>Hepatitis B virus</td>
<td>Humans</td>
<td>Potato</td>
<td>Oral</td>
</tr>
<tr>
<td>Hepatitis B virus</td>
<td>Humans</td>
<td>Lettuce</td>
<td>Oral</td>
</tr>
<tr>
<td>Norwalk virus</td>
<td>Humans</td>
<td>Tobacco</td>
<td>Oral</td>
</tr>
<tr>
<td>Norwalk virus</td>
<td>Humans</td>
<td>Potato</td>
<td>Oral (VLPs)</td>
</tr>
<tr>
<td>Rabies virus</td>
<td>Humans</td>
<td>Tomato</td>
<td>Intact glycoprotein</td>
</tr>
<tr>
<td>CMV Cytomegalovirus protein</td>
<td>Humans</td>
<td>Tobacco</td>
<td>Immunologically related</td>
</tr>
<tr>
<td>Rabbit hemorrhagic disease virus</td>
<td>Rabbits</td>
<td>Potato</td>
<td>Injection</td>
</tr>
<tr>
<td>Foot-and-mouth disease virus</td>
<td>Agricultural domestic animals</td>
<td>Arabidopsis</td>
<td>Injection</td>
</tr>
<tr>
<td>Foot-and-mouth disease virus</td>
<td>Agricultural domestic animals</td>
<td>Alfalfa</td>
<td>Injection or oral</td>
</tr>
<tr>
<td>Transmissible gastroenteritis virus (TGEV)</td>
<td>Pigs</td>
<td>Arabidopsis</td>
<td>Injection</td>
</tr>
<tr>
<td>TGEV</td>
<td>Pigs</td>
<td>Tobacco</td>
<td>Injection</td>
</tr>
<tr>
<td>TGEV</td>
<td>Pigs</td>
<td>Maize</td>
<td>Oral</td>
</tr>
</tbody>
</table>

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because apart from four protein chains (two heavy, two light chains), they contain two additional proteins, one of which is added in vivo during secretion. This was finally achieved by crossing four separate lines of transgenic tobacco plants. This process occurring in plants can be exploited to great advantage. Complex and ‘high molecular weight’ antigens can be expressed in different lines of the same plant, optimally as component parts, and cross-pollination between these lines of plants may yield a progeny producing the complex antigen in its native configuration, obviating the need for any in vitro manipulation. This would also make enormous economic sense. Another advantage of IgA plantibodies is that major proportion (57%) exists in the dimerized form, in contrast to IgA produced in insect cells using Baculovirus, which is mostly monomeric.\(^3\) Antibodies expressed in seeds are preferred over antibodies in leaves, which risk early decay, thus requiring immediate extraction. In contrast, antibodies in seeds can be preserved for long at room temperature until their extraction or consumption. This has been achieved by linking antibody genes to a genetic “switch.” Injectable materials are now being targeted. They are further associated with challenges of purification.\(^3\) HBV antibodies in tobacco plants are being investigated. A number of other plantibodies like anti-HIV antibodies are under development. A cream containing anti-HIV antibodies, which might help reduce the risk of HIV transmission during sexual intercourse, has been proposed. Monoclonal antibodies (Mabs) against STDs like genital herpes have been expressed in soybean. When compared with humanized anti-HSV-2 Mab expressed in mammalian cell culture, they were found to be similar in efficacy in preventing vaginal HSV-2 infection in mice.\(^3\)

**Other Potential Domains**

**Cancer therapy**

Plants can make monoclonal antibodies for cancer therapy in sufficient quantities. Soybean has been genetically engineered to make monoclonal antibody (BR-96) as a vehicle for targeting doxorubicin for breast, ovarian, colon and lung tumors.\(^3,39\) Non-Hodgkin’s lymphoma is also being investigated.

**Birth control**

TMV has been designed to express protein found in mouse zona pellucida (ZB3 protein).\(^3,39\) When injected, resulting antibodies were able to prevent fertilization of eggs in mice.

**Chloroplast transformation**

The chloroplast genome is maternally inherited; so following chloroplast transformation, the protein would not be present in pollen, thereby reducing the risk of transmission of the transgene to neighboring crops or weed species by cross-pollination.\(^52\) It may also result in accumulation of significantly greater quantities of transgenic protein.

**Role in autoimmune diseases**

For unknown reasons, oral delivery of auto-antigens can suppress immune activity by switching on suppressor cells of the immune system, producing immunological tolerance. For example, intake of collagen in arthritis patients resulted in relief. This phenomenon is frequently seen in test animals and is time- and dose-dependent. To induce tolerance, antigen is required to be given repeatedly or continuously in doses larger than the immunogenic dose. Misidentification and misadministration of plants/fruits may unknowingly induce tolerance. Hence it becomes essential to identify the transgenic plant/fruit and to determine safe, effective doses and feeding schedules, which would determine whether an antigen would stimulate or depress immunity. Among the autoimmune disorders that might be prevented or eased are type 1 diabetes, multiple sclerosis, rheumatoid arthritis, transplant rejection, etc.\(^1,53\) Potatoes expressing insulin and a protein called GAD (glutamic acid decarboxylase), linked to CT-B subunit, were able to suppress immune attack in a mouse strain that would become diabetic and could delay the onset of high blood sugar.\(^54\) Another diabetes-related protein is also being investigated. Work on multiple sclerosis, rheumatoid arthritis, lupus and transplant rejection is under progress. For autoimmune diseases, production of increased amounts of self-antigen in plants is still required.

**Recombinant drugs/proteins**

Apart from vaccines and antibodies, plants are also being modified by engineered virus inoculation to produce enzymes; drugs such as albumin, serum protease and interferon, which are otherwise difficult or expensive to produce - for example, glucocerebrosidase (hGC) production in tobacco plants for treating Gaucher’s disease. This development would bring down its cost thousand-fold.\(^39\) Transgenic tobacco plant producing Interleukin-10 is being tested to treat Crohn’s disease. Industrial processes for the large-scale production of recombinant therapeutic proteins in plants have been developed. Other novel compounds include an anti-viral protein that inhibits the HIV virus in vitro, trichosanthin (ribosome inactivator) and angiotensin-I (antihypertensive drug).\(^39\) Transgenic plant-derived biopharmaceutical hirudin (an antithrombin) is now being commercially produced. Food crops are being increasingly used to produce pharmaceuticals and drugs.

**The Future of Edible Vaccines**

The future of edible vaccines may be affected by resistance to GM foods, which was reflected when Zambia refused GM maize in food aid from the United States despite the threat of famine.\(^55\) Transgenic contamination is unavoidable. Besides pollen, transgenes may spread horizontally by sucking insects, transfer to soil microbes during plant wounding/breakdown of roots/rootlets and may pollute surface and ground water.\(^56\)
Recently, a GM corn approved only for animal consumption appeared in human foods. It was found growing from seeds left behind from the previous plantings. Quarantine done to prevent any further spread caused monetary loss, penalties and possible criminal charges for alleged violation of the permit to grow the gene-altered crops. This incident shows failure at an elementary level. Overall, transgenic contamination has cost the United States approximately $12 billion. Before endorsing such vaccines for human use, the WHO’s concerns of quality assurance, efficacy and environmental impact need to be addressed and GM crops in greenhouse facilities should be rigidly controlled, and they need to be surrounded by protective buffer crops. GM crops have not been able to significantly increase yield or reduce herbicide/pesticide use, as proposed. In a few cases, the instability of transgenic lines and transgene inactivation have led to major crop failures. Dangerous/harmful gene products or potent immunogens/allergens may also be incorporated. Some are reported to produce side effects - for example, cytokines induce sickness and CNS toxicity; α-interferon causes dementia, neurotoxicity, mood/cognitive changes, etc. Terminator crops with “suicide” genes for male sterility, as a means of “containing” the spread of transgenes, actually spread both male sterile suicide genes as well as herbicide tolerance genes via pollen. Emerging multiple herbicide-tolerant volunteers and weeds (super weeds) and biotech-resistant pests demand the use of highly toxic herbicides (atrazine, glufosinate ammonium, glyphosate). Few credible studies are there on the safety of GM foods. An investigation on GM foods showing ‘growth factor’- engineering may contribute to emergence and re-emergence of pathogenic bacteria, making infections very difficult to treat. Minor genetic changes in pathogens can result in dramatic changes in host spectrum and disease-causing potentials, and inadvertently plants may become their unintentional reservoirs. There is also the risk of creating altogether new strains of infectious agents, like super viruses. By DNA shuffling, geneticists can create in a matter of minutes in the laboratory, millions of recombinant viruses that have never existed in billions of years of evolution. This may be misused for the intentional creation of bio-weapons. Genetic engineering is inherently hazardous because it involves creating vectors/carriers specifically designed to cross wide species barriers, like between plant and animal kingdoms, and transferring genes by overcoming their defense mechanisms which are physiologically operative against such genetic assaults. Inadvertent birth of a Frankenstein would result in unmitigated disaster. Naked DNA vaccines are perhaps the riskiest, as these short pieces of DNA are readily taken up by cells of all species and may become integrated into the cell’s genome material. Unlike chemical pollutants, these small DNA fragments can multiply and mutate indefinitely. Feeding GM products like maize to animals may also carry risks, not just for the animals but also for human beings consuming the animal products. The ecological and environmental risks of edible vaccines need to be considered. It is still a very crude science and has a long way to go before it will be ready for large-scale testing in people for combating infectious diseases and for autoimmunity.

Increase in global area utilized in cultivating transgenic crops from 1.7 to 44.2 million hectares from 1996 to 2000 and the number of countries growing them from 6 to 13 reflects the growing acceptance of transgenic crops in both industrial and developing countries. At least 350 genetically engineered pharmaceutical products are currently under development in the United States and Canada. Edible vaccines offer major economic and technical benefits in the event of bioterrorism, as their production can be easily scaled up for millions of doses within a limited period of time (smallpox, anthrax, plague, etc.).

Conclusion
Edible plant-derived vaccine may lead to a future of safer and more effective immunization. They would overcome some of the difficulties associated with traditional vaccines, like production, distribution and delivery, and they can be incorporated into the immunisation plans. They have passed the major hurdles in the path of an emerging vaccine technology. Before becoming a reality, the technical obstacles, though all seem surmountable, need to be overcome. However, with limited access to essential health care in much of the world and with the scientific community still struggling with complex diseases like HIV, malaria, etc., a cost-effective, safe and efficacious delivery system in the form of edible vaccines will become an essential component in our disease-prevention arsenal.

References


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