



Review Article

EDIBLE VACCINES: FUTURE AND ITS PROSPECTS

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ABSTRACT

Vaccines were the result of trial and error research until molecular biology and genetic engineering made possible the creation of many new and improved vaccines. New vaccines need to be 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 diseases. Edible vaccines hold great promise as a cost-effective, easy-to-administer, easy-to-store, fail-safe and socio culturally 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. Plants are capable of producing recombinant antigens that undergo similar post translational modifications as their mammalian-derived counterparts and in contrast to bacterial expression systems.

KEYWORDS: Bacterial expression, Edible vaccines, Transgenic.

INTRODUCTION

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.^[3] 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.^[12] 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.^[11]

By the late 1990s an international campaign to immunize all the world's children against six devastating diseases was reportedly reaching 80 percent of infants (up from about 5 percent in the mid-1970s) and was reducing the annual death toll from those infections by roughly three million.^[15] Yet these victories mask tragic gaps in delivery. The 20 percent of infants still missed by the six vaccines against diphtheria, pertussis (whooping cough), polio, measles, tetanus and tuberculosis account for about two million unnecessary deaths each year, especially in the most remote and impoverished parts of the globe. Upheavals in many developing nations now threaten to erode the advances of the recent past, and millions still die from infectious diseases for which immunizations are nonexistent, unreliable or too costly.^[21]

When environmental or social disasters undermine sanitation systems or displace communities bringing people with little immunity into contact with carriers infections that have been long gone from a population can come roaring back. Further, as international travel and trade make the earth a smaller place, diseases that arise in one locale are increasingly

popping up continents away. Until everyone has routine access to vaccines, no one will be entirely safe.

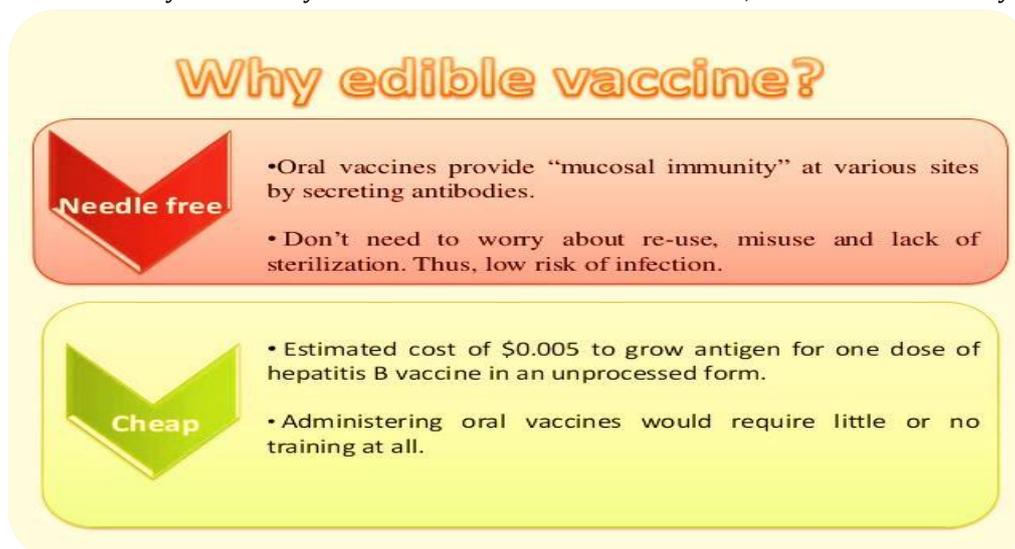


Figure 1: Need for Edible Vaccines

The Children's Vaccine Initiative

In 1990, translation of scientific advancements into the new vaccines needed by developing countries was lagging. At the World Summit for Children in New York that year, a consortium of philanthropic, health, and intergovernmental organizations urged the world to harness new technologies to advance the immunization of children. The resulting Children's Vaccine Initiative (CVI) recognized the role that new technologies might play in improving current vaccines and developing new ones.^[1]

In 1992, WHO estimated that three to five million children's lives could be saved each year if new vaccines were available to control or prevent commonly occurring infectious diseases. Diarrheal and respiratory infections are major causes of infant mortality in the developing world. CVI focused attention on the need to exploit technologies that would make vaccines-both their production and use-less expensive and more reliable, especially for the developing world. The Initiative emphasized the importance of oral vaccines because they eliminate the need for needles and syringes with their accompanying costs and risks.^[14] The pain of injections and the reaction of children is often a reason why subsequent vaccination visits are missed. (Oral vaccines may also induce mucosal immunity, creating a barrier in the gut, lungs, and urogenital tract that can block infection before the body must rely on a systemic response.) CVI also promoted development of multicomponent vaccines in which a antigens against several infectious agents could be delivered simultaneously, thus simplifying administration.^[16] Heat-stable vaccines, also advocated by CVI, would not need refrigeration-the traditional cold chain-which currently limits success and coverage in developing countries. Each of these desired features-low costs, improved reliability, elimination of injections, mucosal immunity, multicomponent vaccines, and heat-stability can potentially be found in plant-based vaccines.

Concept of Edible Vaccines

As our understanding grows of how the immune system recognizes individual proteins to which it has been exposed before, other recombinant subunit vaccines will be developed offering exciting disease prevention opportunities. But recombinant vaccine production is likely to remain dependent on costly fermentation and protein purification technology. Moreover, vaccines produced this way often need to be refrigerated and injected.^[11] Unfortunately, while research on which proteins make effective vaccines and on the genes that control their production proceeds in many laboratories, much less research has been devoted to the organisms in which to express these genes-microbes or plants that will produce the immunogenic protein in large quantities.^[21] The recombinant HBV vaccine produced by yeast cells grown in large fermenters provides a benchmark of efficacy and safety for vaccines produced in recombinant organisms. A transgenic plant, that is plants in which genes from other species have been introduced, may prove to be an equally useful way to produce proteins encoded by specific genes. ^[22]

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 immune compromised patients.^[23] 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. They are cheaper, sidestepping demands for purification (single dose of hepatitis-B vaccine would cost approximately 23 paisa), 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 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.^[7] 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 tuberculosis* and agents causing diarrhea, pneumonia, STDs, HIV, etc.. Edible

vaccines would also be suitable against neglected/rare diseases like dengue, hookworm, rabies, etc.^[10] They may be integrated with other vaccine approaches and multiple antigens may also be delivered. Various foods under study are banana, potato, tomato, lettuce, rice, etc. Edible vaccines are currently being developed for a number of human and animal diseases, including measles, cholera, foot and mouth disease and hepatitis B, C and E. ^[15]

Release Mechanism

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. ^[1] The antigens are released, taken 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. ^[2]

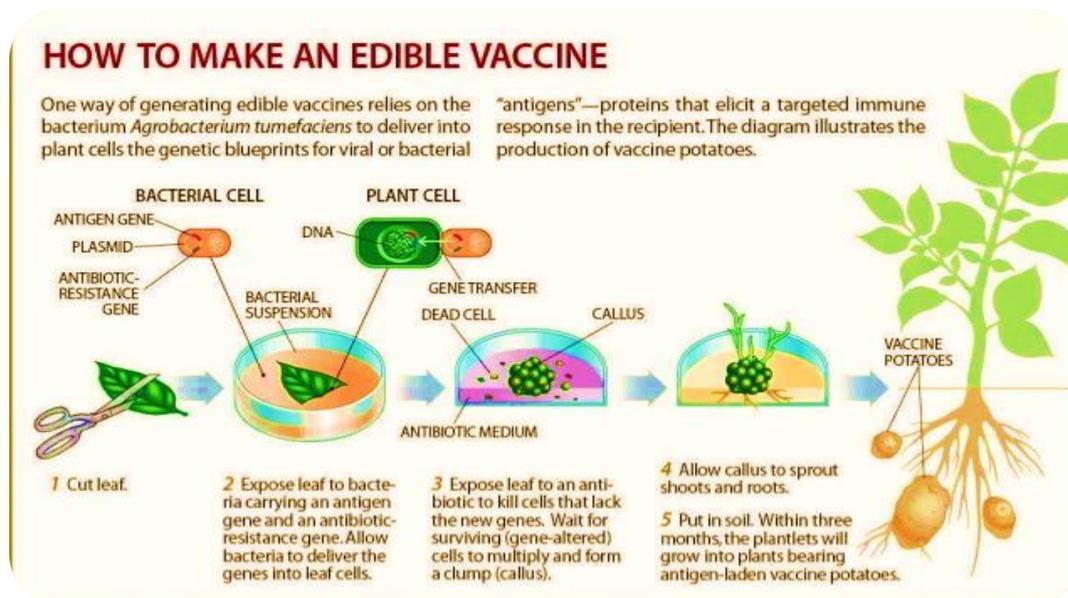


Figure 2: Process for preparation of Edible Vaccines ^[1]

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.^[3] 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. The DNA integrates randomly into plant genome, resulting in a different antigen expression level for each independent line, 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.^[4] 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 Immune Sorbent Assay (ELISA).^[3]

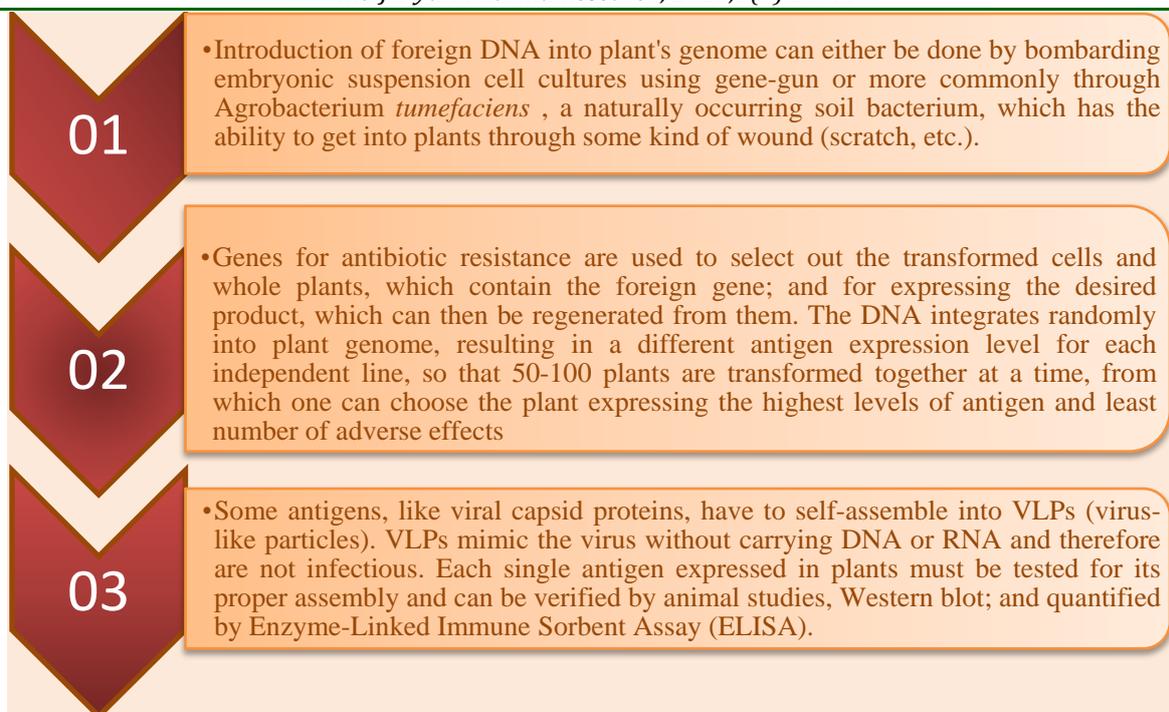


Figure 3: Flowchart depicting process of preparation of Edible Vaccines

Generations of 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.^[2] 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, ETEC (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.^[5]

In the course of evaluation of transgenic plants as a new means of vaccine production, three separate courses were pursued:

- First, experiment was done to determine the capacity of plants to produce foreign proteins that retain antigenic characteristics necessary to induce immunity.
- Second, evaluation of the immunogenicity of ingested plant-derived proteins was done.
- Third, an appropriate food crop that could be used for both production and distribution of vaccines, with attention to the needs of the developing world was sought.

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.^[25] 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.^[26]

Requirements of "Vector" plants

Some of the reasons for the requirements of vector plants are:

- Able to produce functional copies of the specified protein(s).
- Able to produce a substantial amount per cell (up to 0.05% of total soluble proteins) of the specified proteins.
- Able to grow in volume (plants sometimes grow poorly when they start producing large amounts of foreign proteins).

- Able to produce a defined amount of the specified protein(s) to ensure that any given amount of vaccine food provides a predictable dose of antigen. Regulatory agencies will not approve vaccines with variable dosing.
- Able to be grown locally.
- Able to be regenerated readily without the growers having to purchase more seeds or plants year after year.
- Other components of transgenic plants may have inhibiting or promoting effects on immunogenicity.
- Allergy to antigens that until now only have been applied parenterally.
- Possible recombination events of viral sequences of plant and animal pathogenic viruses.

Advantages

- Safer than those produced in animal tissues because the chances of unknown human pathogens hitching a ride would be extremely small.
- Economical advantages (obstacle to interests of vaccine industry).
- Cheap to produce. Cost per dose would be low.
- No refrigeration required by vaccine-containing seeds or dried leaves, a significant advantage in developing countries.
- No injection required and there is no risk of accidental parenteral infections from contaminated needles.
- Elicit mucosal immunity in addition to systemic immunity: anyway nasal immunization is more efficient than oral immunization at stimulating effector immunity in the reproductive tract.

Scientific Challenges

There are many questions which need to be answered before developing a plant-based vaccine. Three successful human clinical trials have shown that adequate doses of antigen can be achieved with plant-based vaccines. [1] To determine the right dosage, one need 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. [21] 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,

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. uniformity of dosage and purity. [23]

Some of the techniques to overcome these limitations are:

- Optimization of coding sequence of bacterial/viral genes for expression as plant nuclear genes.
- Expression in plastids.
- Plant viruses expressing foreign genes.
- Coat-protein fusions.
- Viral-assisted expression in transgenic plants and promoter elements of bean yellow dwarf virus with reporter genes GUS (β -glucuronidase) and GFP (green fluorescent protein), substituted later with target antigen genes. [18]

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 (EPEC), mammalian/viral immunomodulators and plant-derived secondary metabolites. To decrease toxicity and allergic potential, mutant forms of E. coli -labile toxin, like LT-K63 and LT-R72 and hinge cleavage mutant LT-G192, are used. 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).

Non-Scientific 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. [13] 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. [19]

Clinical Trials

Antigen expression in plants has been successfully shown in the past, like LT-B (EPEC) in tobacco and potato, rabies virus-G protein in tomato, HBsAg in tobacco and potato, norwalk virus in tobacco and potato; CT-B (*Vibrio cholerae*) in potato. Ethical considerations usually preclude clinical trials from directly assessing protection, except in a few cases. In contrast, veterinary researchers can assess immune protection more directly. [8]

Examples

Recombinant protein (vaccine)	Transgenic plant	Protection against
Rabies glycoprotein	Tomato	Rabies virus
Foot and mouth virus (VPI)	Arabidopsis	Foot and mouth virus
Herpes virus B surface antigen	Tobacco	Herpes simplex virus
Cholera toxin B subunit	Potato	<i>Vibrio cholerae</i>
Human cytomegalovirus glycoprotein B	Tobacco	Human cytomegalovirus

Table 1: Examples of Recombinant vaccines from different plants [29]

CLOSTRIDIUM TETANI VACCINE

Transgenic plants: stable integration of a gene into the plant nuclear or chloroplast genome can transform higher plants (e.g. tobacco, potato, tomato, banana) into bioreactors for the production of subunit vaccines for oral (but also for parental) administration. This can also be achieved by using recombinant plant viruses as transient expression vectors in infected plant. [15]

Tobacco (*Nicotiana spp.*): easy to manipulate, but high levels of toxic alkaloids in the leaves don't allow studies on animal feeding. For experimental purposes antigens have to be substantially purified and then tested for their immunogenicity. [17]

Potato (*Solanum tuberosum*): easily propagated from "eyes", generated within a few months, stored for long periods without refrigeration, but they have to be cooked to be palatable (apart from toleration by volunteers. Anyway mice accept raw potato tubers and further some kinds of potatoes are actually eaten raw in South America populations), and heating can sometimes (but not always) denature antigens. [18]

Banana (*Musa acuminata*): need no cooking, but banana trees take at least 2 years to mature, the fruit has low protein content and spoils fairly rapidly after ripening.

Tomato (*Lycopersicon esculentum*): grow more quickly, but their fruit too has low protein content.

Lettuce (*Lactuca sp.*)

Carrot (*Daucus carota*)

Peanut (*Arachis hypogaea*)

Rice (*Oryza sativa*)

Wheat (*Triticum aestivum*)

Corn/Maize (*Zea mays subsp. mays*) (if for animal vaccines: alfalfa, grains and beans). Transgenic corn is particularly attractive for this purpose since the

recombinant antigen is stable and homogeneous, and corn can be formulated in several edible forms without destroying the cloned antigen. [25]

Lupine (*Lupinus sp.*)

Thale cress (*Arabidopsis thaliana*)

Soybean seeds (*Glycine max*)

Corn / Maize seeds (*Zea mays subsp. mays*)

Other Examples

Human pathogens:

Viruses:

Anti-Norwalk virus vaccine: capsid protein in GM tobacco and potatoes.

Anti-rotaviruses vaccine

VP7 DNA vaccine can induce high levels of IgG in mice but cannot protect mice against challenge. Mice immunized with transgenic potato successfully elicited serum IgG and mucosal IgA specific for VP7. The mucosal IgA titer was as high as 1000, while serum IgG titer was only 600. Neutralizing assays indicate that IgA could neutralize rotavirus. [13]

Cholera toxin (CT) B and A2 subunit complementary DNAs (cDNAs) fused to a rotavirus enterotoxin and enterotoxigenic *Escherichia coli* fimbrial antigen genes and transferred into potato. Fusion antigens are synthesized in transformed tuber tissues and assembled into cholera holotoxin-like structures that retained enterocyte-binding affinity. [27]

Anti-HAV vaccine: the construction of the plant expression vector pBI121-A was reported, which contained a fusion gene encoding hepatitis A capsid proteins. The gene was located between the left and right Ti border sequences under the control of CaMV35S promoter. The vector was identified via PCR and restriction enzyme analysis and was introduced into *Agrobacterium tumefaciens* LBA4404. The

transgenic Citrus plants were produced by Agrobacterium-mediated transformation of epicotyl segments. [28]

Anti-HBV vaccine: HBsAg using Agrobacterium mediated transformation in transgenic

Potatoes accumulate intracellular as tubular structures, with a complex size distribution, differing substantially from the virus-like particle (VLP) preparations of the current commercial vaccines: natural bio encapsulation of the antigen may provide protection from degradation in the digestive tract until plant cell degradation occurs near an immune effector site in the gut. Extensive disulfide-bond cross-linking, which is important for immunogenicity, was evident and 21-37% of total HBsAg protein displayed epitopes which correlate with vaccine potency. [26]

Tomatoes: Arntzen and his colleagues are focusing on creating them and converting them into pills. [3]

Banana cv. Rasthali (AAB): 4 different expression cassettes (pHBS, pHER, pEFEHBS and pEFEHER) were utilized to optimize the expression of HBsAg in banana. The transgenic nature of the plants and expression of the antigen was confirmed by PCR, Southern hybridization and RT-PCR. The expression levels of the antigen in the plants grown under in vitro conditions as well as the green house hardened plants were estimated by ELISA for all the four constructs. Maximum expression level of 38 ng/g F.W. of leaves was noted in plants transformed with pEFEHBS grown under in vitro conditions, whereas pHER transformed plants grown in the green house showed the maximum expression level of 19.92 ng/g F.W. of leaves. Higher mAb binding of 67.87% of the antigen was observed when it was expressed with a C-terminal ER retention signal. [27]

Anti-HPVs vaccine: Transgenic tobacco and potato plants carrying the HPV type 16 major structural gene L1 under the control of the cauliflower mosaic virus 35S promoter. The plant-derived L1 protein displayed conformation-specific epitopes and assembled into virus-like particles. Furthermore, we did not find any indications of protein modification of the L1 protein produced in plants. Plant-derived L1 was as immunogenic as L1 expressed in baculovirus-infected insect cells. Feeding of tubers from transgenic potatoes to mice induced an anti-L1 antibody response in 3 out of 24 mice, although this response was only transient in two of the mice. However an anti-L1 response was primed in about half of the 24 animals. [19]

Anti-HIV-1 vaccine: chimeric virus particles (CVPs) in which gp41 are expressed as N-terminal translational fusion with the potato virus X (PVX) coat protein. Transgenic tomatoes expressing the Tat protein. Two independent plants testing positives to transgene detection analysis were selected and grown to maturity. Monoclonal antibodies against tat recognized a protein of the expected size. Interestingly, expression of Tat seemed to be toxic to the plant as in all cases the fruit

presented underdeveloped reproductive structures and no seeds. 9 groups of ten pathogen-free male mice were primed either, orally, intraperitoneally (i.p.) or intramuscularly (i.m.) with 10 mg of tomato fruit extract derived from transgenic or wild type plants and with 10 mg of Tat86 recombinant protein. [27]

Non-human pathogens: Anti-foot-and-mouth disease virus (FMDV) vaccine: transgenic potatoes plants containing the VP1 gene cloned under the regulatory activity of either a single (pRok2) copy of the S35 cauliflower mosaic virus (CaMV 35S) promoter: use of double CaMV 35S promoter does not cause a significant increase in the level of the VP1 expressed.

Anti-infectious bronchitis virus (IBV) Vaccine: S1 glycoprotein in potatoes. [25]

Anti-rabbit hemorrhagic disease virus (RHDV)

Vaccine: major structural protein VP60 in transgenic tubers of potatoes plants.

Anti-SIV Vaccine: cholera toxin B subunit (CTB) was linked 5' to the simian immunodeficiency virus (SIVmac) Gag p27 capsid gene (CTB-Gag). The fusion gene was transferred into potato cells by Agrobacterium tumefaciens-mediated transformation methods and transformed plants regenerated. The CTB-Gag gene fusion was detected in transformed potato leaf genomic DNA by polymerase chain reaction-mediated DNA amplification. The results of immunoblot analysis with anti-CTB and anti-Gag antibodies verified the synthesis of biologically active CTB-Gag fusion protein in transformed leaf and tuber tissues. Synthesis and assembly of the CTB-Gag fusion protein into oligomeric structures of pentamer size was confirmed by GM1-ganglioside-enzyme-linked immunosorbent assay (GM1-ELISA) of transformed potato tuber tissue extracts.

The Future of Edible Vaccines

Vaccines have been one of the most far-reaching and important public health initiatives of the 20th century. The future of edible vaccines is bright as it holds great promise as a cost-effective, easy-to-administer, easy-to-store, fail-safe and socio-culturally 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. [26] 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. [27,28] The

studies completed so far in animals and people have provided a proof of principle; they indicate that the strategy is feasible. Yet many issues must still be addressed. Researchers are also grappling with the reality that plants sometimes grow poorly when they start producing large amounts of a foreign protein.^[28] One solution would be to equip plants with regulatory elements that cause antigen genes to turn on--that is, give rise to the encoded antigens--only at selected times (such as after a plant is nearly fully grown or is exposed to some outside activator molecule) or only in its edible regions. This work is progressing.

Few credible studies are there on the safety of GM foods. By facilitating horizontal gene transfer/recombination, genetic engineering may contribute to emergence and re-emergence of infectious, drug-resistant diseases, rise of autoimmune diseases, cancers and reactivation of dormant viruses. Bacteria may take up transgenic DNA in food in human gut.^[15] 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.^[26] This may be misused for the intentional creation of bio-weapons.^[28] 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.

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.).

Future Plans

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 immunization plans.^[22] 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.

CONCLUSION

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. Plants are capable of producing recombinant antigens that undergo similar post translational modifications as their mammalian-derived counterparts and in contrast to bacterial expression systems. Yet many issues must still be addressed. Researchers are also grappling with the reality that plants sometimes grow poorly when they start producing large amounts of a foreign protein. The studies completed so far in animals and people have provided a proof of principle; they indicate that the strategy is feasible and beneficial for future use in treatment of various diseases.

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