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Review

Green revolution vaccines, edible vaccines

Swamy Krishna Tripurani, N. S. Reddy and K. R. S. Sambasiva Rao*

Centre for Biotechnology, Nagarjuna university, Nagarjuna Nagar, Guntur-522510, A.P., India.

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Edible vaccines are sub-unit vaccines where the selected genes are introduced into the plants and the transgenic plant is then induced to manufacture the encoded protein. Edible vaccines are mucosal targeted vaccines where stimulation of both systematic and mucosal immune network takes place. Foods under study include potatoes, bananas, lettuce, rice, wheat, soybean, corn and legumes. Edible vaccines for various diseases such as measles, cholera, hepatitis-B, and many more are in the process of development. Food vaccines may also help to suppress autoimmunity disorders such as Type-1 Diabetes.

Key words: Edible vaccines, oral vaccines, antigen expression, food vaccines.

INTRODUCTION

Vaccination involves the stimulation of the immune system to prepare it for the event of an invasion from a particular pathogen for which the immune system has been primed (Arntzen, 1997). The release of vaccine is practiced so that T and B cells specific for the pathogen vaccinated against, or specific for part of it, will be ready to proliferate and differentiate a lot faster in the event of a natural challenge by a pathogen. Construction of vaccine in several cases has been hampered because of varying strains of the pathogen, antigen drift, antigenic shift and other unrevealed mechanisms. These phenomenons make it hard to determine which peptide sequence to prime the immune system since the peptide sequences of the individual strains will all, be different. Also using live, attenuated pathogens to prime the immune system has resulted in a few people actually acquiring the disease being vaccinated against.

The process of using DNA vaccines to prevent or slow down the spread of disease is also known as polynucleotide immunization. DNA that is injected into the subject undergoes the transcription and translation which yield protein, making the specific T and B cells to differentiate and proliferate. By vaccination, therefore, the immune system is ready to combat invading pathogens very quickly, before they get the chance to spread throughout the body while causing discomfort to the host. Though most of the above mentioned methods of vaccination have been effective against diseases for which vaccines can be produced, the introduction of oral vaccines was made to ease the discomfort associated with the mode of introduction of conventional vaccines into the body.

Oral vaccines are more affordable and accessible to the inhabitants of developing countries, who needlessly die, in the thousands, from diseases, which can easily be

*Corresponding author. Phone: 91-863-2293400 (O), 91-863-2233433 (R). Fax: 91-863-2293378. Email: krssrao@yahoo.com.

prevented by vaccination. Food vaccines are like subunit preparations in that they are engineered to contain antigens but bear no genes that would enable whole pathogens to form. These vaccines basically work in the same way as the injected DNA vaccine, since a peptide sequence similar to an infectious part of a pathogen is synthesized, by itself, and is used to prime T and B cells in the body. The big difference in this case is that the protein sequences are encoded in a plant to form the desired protein. This protein is then ingested, as the plant or its fruit is eaten. One becomes immune against the ingested protein, as T and B cells become stimulated to proliferate and differentiate.

Successful expression of antigens in plants was achieved for Rabies virus G-protein in tomato (McGarvey et al., 1995), Norwalk virus capsid protein in tobacco and potato (Mason et al., 1996), Hepatitis B virus surface antigen in tobacco and potato (Thanavala et al., 1995), *E. coli* heat-labile enterotoxin B subunit (LT-B) in tobacco & potato (Hirst and Holmgren, 1987), Cholera toxin B subunit (CT-B) in potato (Arakawa, 1997). Food vaccines are also used to suppress autoimmune disorders like type-1 diabetes, multiple sclerosis, rheumatoid arthritis etc (Prakash, 1996). Foods under study include potatoes, bananas, lettuce, rice, wheat, soybean, corn and legumes.

Potatoes are a good system in which to test the idea of edible vaccines (Steinberg, 1996). Bananas (Hassler, 1995) is a good candidate for edible vaccines since they were eaten raw, appealing to children, inexpensive to produce, native to many developing countries. But the only limitation is the time from transformation to evaluation of fruit is 2 years or more.

Edible vaccines reduce the need for skilled personnel to administer injections and negate the concerns regarding the reuse of needles (Webster et al., 2002). A measles vaccine that can be directly consumed would significantly increase the availability in places where maintenance of a cold-chain during storage and transport is difficult.

EDIBLE VACCINES

A concern with oral vaccines is the degradation of protein components in the stomach (due to low pH and gastric enzymes) and gut before they can elicit immune responses (Daniell et al., 2001) but the rigid plant cell walls could provide protection from intestinal degradation (Webster et al., 2002). The degradation can be compensated by repeating the exposure of the antigen until immunological tolerance is accomplished (Mason et al., 2002).The M cells lining the small intestine take in the components that have entered the small intestine (including pathogens) and pass them to other cells of the immune system, such as antigen presenting cells and macrophages. These cells chop up their acquisitions and display the resulting protein fragments on the cell surface. Helper T lymphocytes recognize the displayed fragments as foreign, induce B lymphocytes to secrete neutralizing antibodies and also help to initiate a broader attack on the perceived enemy. Mucosal immune responses represent a first line of defense against most pathogens.

Second generation edible vaccines are also called as multicomponent vaccines that provide protection against several pathogens. An elegant approach to achieve this goal, based on epitope fusion to both subunits of the cholera toxin (CT), was recently demonstrated by Yu and Langridge (2001). CT provides a scaffold for presentation of protective epitopes of rotavirus and ETEC (Enterotoxigenic E.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 described 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 response (Tacket et al., 1998).

The techniques to enhance antigen accumulation in plant tissues are being explored and include optimization of the coding sequence of bacterial or viral genes for expression as plant nuclear genes, and defining the sub cellular compartment in which to accumulate the product for optimal quantity and quality. Several laboratories are also developing alternative expression systems to improve accumulation. Systems involve plant viruses for expression of foreign genes (Nemchinov et al., 2000) or coat-protein fusions (Modelska et al., 1998) and even viral assisted expression in transgenic plants (Mor et al., 2002). The expression in plastids is advocated by some (Daniell et al., 2001; Ruf et al., 2001). The other approach is to enhance the immunogenicity of the orally delivered antigens by using mucosal adjuvants. One such approach is making use of bacterial entertoxins such as CT or LT (Yu and Langridge, 2001), mammalian and viral immunomodulators as well as plant-derived secondary metabolites.

Enterotoxigenic *Escherichia coli* and *Vibrio cholerae*

Toxins produced by *E. coli* and *V. cholerae* can cause acute watery diarrhoea. Infection by these microorganisms is common in places where there is poor sanitation and untreated water supplies. If allowed to go untreated, there are fatal consequences of exposure to these microorganisms, especially in children.

The synthetic genes of *E.coli* heat-labile enterotoxin B subunit (LT-B) and cholera toxin B subunit (CT-B) are related proteins with similar structure, function and immunochemistry to the actual toxins found in each

species (Jobling et al., 1991). The production of these recombinant proteins in yeast or bacteria is expensive compared to the production of a recombinant potato that produces modified LT-B. It has been shown that mice which eat the transgenic potato raise antibodies in response to the potato LT-B that were effective in inhibiting LT activity on mammalian cells.

Synthetic LT-B coding sequence (sLT-B) was modified for cloning in plants. The potato plant cells were transformed by leaf disc co-cultivation the transformants were selected and are regenerated as plantlets on a selective media. Tubers from a mature plant were used as seed for the next production cycle. Later the mice were fed with transformed potato and it was found that the mice had shown resistance to the pathogen. The transgenic potato is useful as a vaccine component or as a booster vaccine (Mason et al., 1998).

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 pathogens. Since immunization is the only known method to prevent the disease of the Hepatitis B virus, any attempt to reduce its infection requires the availability of large quantities of vaccine, hepatitis B 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 the transgenic potato than in leaf tissues (Domansky, 1995). 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 the HbsAg by using different promoters such as the patatin promoter, and different transcription regulating elements.

Measles

Measles is a highly contagious viral disease caused by the Paramyxovirus spread by air and includes symptoms such as high fever, skin rash and spots and it can lead to many different complications which can be even more severe than the disease itself. Each year, almost one million children die from the measles and many of the survivors are weakened by pneumonia or encephalitis or become deaf. Recent studies report expression of the Paramyxovirus surface protein hemagglutinin in tobacco, potato, rice and lettuce with satisfying results (Webster et al., 2002).

Serum samples from healthy experimental animals, fed with transgenic banana, were analyzed for the presence of anti-hemagglutinin-specific antibodies. The results are highly significant and demonstrate that the banana plant can produce the antigenic hemagglutinin protein of the measles virus and elicit immune responses in experimental animals (Webster et al., 2002).

Stopping autoimmunity

In the past 15 years, investigators have identified several beta cell proteins that can elicit autoimmunity in people predisposed to Type I diabetes. The development of plant based diabetes vaccines in potatoes and tobacco containing insulin or GAD linked to the innocuous B subunit of the V. cholerae toxin (to enhance uptake of the antigens by M cells) was attempted. The developed transgenic potato and tobacco plants when fed to non-obese diabetic mice, showed increased levels of IgG1, an antibody associated with cytokines that suppress harmful immune responses. "Molecular Pharming" to produce autoantigens in plants targeting other autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, systemic lupus and even transplant rejection is under way.

LIMITATIONS OF EDIBLE VACCINES

Even though edible vaccines are stable and easily accessible there are some limitations which restricts its development. For example, one could develop immunotolerance to the vaccine peptide or protein. Little research has been done on this (Richter and Kipp, 1999). Another concern with whole fruit or vegetable vaccines is the consistency of dosage from fruit to fruit, plant to plant, and generation to generation. Another limitation is storage of edible vaccines. Potatoes containing vaccine proteins seem to store well at 40^0C but tomatoes will not last very long. Using potatoes or bananas may require some processing such as smashing and a liquoting as in baby food jars of bananas. Since these fruits are being used as vectors for the vaccines in question, they have to be properly stored to avoid infection or disease through spoilage of these fruits (Richter and Kipp, 1999). Other concerns are about transgene escape and identification of "vaccine" fruit vs a normal fruit. Fruit vaccines should be easily identifiable to avoid the misadministration of the vaccine, which may lead to complications such as immunotolerance.

Nevertheless, plant-based vaccines are an exciting and novel new strategy for the development of oral vaccines. Edible vaccine 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 the need for certain vaccines to be refrigerated, can thwart effective vaccination programs. The hope is that edible vaccines could be grown in many of the developing countries where they would actually be used.

REFERENCES

Arakawa T (1997). Expression of cholera toxin B subunit oligomers in transgenic potato plants. Transgenic Res. 6:403-413.

Arakawa T, Chong D, Langridge W (1998). Efficacy of a food plant-based oral cholera toxin B subunit vaccine. Nature Biotechnol. 16:292-297.

Arntzen CJ (1997). Edible Vaccines. Public Health Rep. 112:190- 197.

Daniell H, Streatfield J, Wycoff K (2001). Medical Molecular farming: production of antibodies, biopharmaceuticals and edible vaccines in plants. Trends in Plant Sci. 6: 219-226.

Domansky N (1995). Organ-Specific Expression of Hepatitis B Surface Antigen in Potato. Biotechnol. Lett. 17:863-866.

Hassler S (1995). Bananas and Biotech Consumers. Bio/Technology 13: 417.

Hirst RT, Holmgren J (1987). Conformation of Protein Secreted Across Bacterial Outer Membranes: A Study of Enterotoxin translocation from *Vibrio cholerae*. Proc. Nat. Acad. Sci. USA. 84: 7418-7422.

Jobling MG, Holmes RK (1991) . Analysis of Structure and Function of the B subunit of Cholera Toxin by the use of Site-directed Mutagenesis. Mol. Microbiol. 5: 1755-1767.

Ma SW, Zhao DL, Yin ZQ, Mukherjee R, Singh B, Qin HY, Stiller CR, Jevnikar AM (1997). Transgenic plants expressing autoantigens fed to mice to induce oral immune tolerance. Nature Med. 7:793-796.

Mason HS, Ball JM, Jian-Jian Shi, Xi Jiang, Estes MK, Charles J. Arntzen CJ (1996). Expression of Norwalk Virus Capsid Protein in Transgenic Tobacco and Potato and its Oral Immunogenicity in Mice. Proc. Nat. Acad. Sci USA. 93: 5335-5340.

Mason HS, Haq TA, Clements JD, Arntzen CJ (1998). Edible vaccine protects mice against E. coli heat-labile Enterotoxin (LT): Potatoes expressing a synthetic LT-B gene. Vaccine 16:1336-1343.

Mason HS, Warzecha H, Tsafir MS, Arntzen CJ (2002). Edible plant vaccines: applications for prophylactic and therapeutic molecular medicine. Trends Mol. Med. 8:324-329.

McGarvey PB, Hammond J, Dienelt M M, Hooper DC, Fu ZF, Dietzschold B, Koprowski H, Michaels FH (1995). Expression of the Rabies Virus lycoprotein in Transgenic Tomatoes. Bio/Technology 13: 1484-1487.

Modelska A, Dietzschold B, Sleysh N, Fu FZ, Steplewski K, Hooper DC, Koprowski H, Yusibov V (1998). Immunization against rabies with plant-derived antigen. Proc. Natl. Acad. Sci. U.S.A. 95:2481-2485.

Mor TS, Gómez-Lim MA, Palmer KE (1998). Edible vaccines: a concept comes of age. Trends Microbiol. 6:449-453.

Mor TS, Moon YS, Palmer KE, Mason HS (2003). Geminivirus vectors for high level expression of foreign proteins in plant cells. Biotechnol. Bioeng. 81:430-437.

Nemchinov LG, Liang TJ, Rifaat MM, Mazyad HM, Hadidi A, Keith JM (2000). Development of a plantderived subunit vaccine candidate against hepatitis C virus. Arch. Virol. 145:2557-2573.

Prakash CS (1996). Edible Vaccines and Antibody Producing Plants. Biotechnol. Dev. Monitor 27: 11-13.

Richter L, Kipp PB (1999). Topics in Microbiology and Immunology. Plant Biotechnology: New Products and Applications. ed. Hammond J, McGarvey P, Yusibov V (Springer-Verlag, Heidelberg), pp. 159-176.

Ruf S, Hermann M, Berger IJ, Carrer H, Bock R (2001). Stable genetic transformation of tomato plastids and expression of a foreign protein in fruit. Nat. Biotechnol. 19:870-875.

Sági L, Panis B, DeSnet K, Remy S, Swennwn R, Cammue BPA (1995). Genetic Transformation of Banana & Plantain (Musa spp.) via Particle Bombardment. Bio/Technology. 13: 481-485.

Steinberg J (1996). Edible-Vaccine Trial: No Small Potatoes. NIH Res. 8: 26.

Thanavala Y, Yang Y, Lyons P, Mason HS, Arntzen C (1995). Immunogenicity of Transgenic Plant- derived Hepatitis B Surface Antigen. Proc. Nat. Acad. Sci. USA. 92: 3358-3361.

Tacket CO, Mason HS, Losonsky G, Clements JD, Wasserman SS, Levine MM, Arntzen CJ (1998). Immunogenicity in humans of a recombinant bacterialantigen delivered in transgenic potato. Nat. Med. 4:607- 609.

Tacket CO, Mason HS (1999). A review of oral vaccination with transgenic vegetables. Microbes Infect. 1:777-783.

Webster DE, Cooney ML, Huang Z, Drew DR, Ramshaw IA, Dry IB, Strugnell RA, Martin JL, Wesselingh SL (2002). Successful boosting of a dna measles immunization with an oral plant-derived measles virus vaccine. J. Virol. 76: 7910-7912.

Webster DE, Thomas MC, Strugnell RA, Dry IB, Wesselingh SL (2002). Appetising solutions: an edible vaccine for measles. Med. J. Aust.176: 434-437.

Yu J, Langridge WH (2001). A plant -based multicomponent vaccine protects mice from enteric diseases. Nat. Biotechnol. 19:548-552.