VACCINES FROM OUR GARDEN

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INTRODUCTION

There is a saying that, "Prevention is better than Cure". The best way to prevent a disease is to be immunized and the most effective way to get immunized is by vaccination. Thus, Vaccines can said to be a boon in the field of medical science. The world of vaccines can be classified mainly into two groups Classical Vaccines and New Generation Vaccines. Classical vaccines are made up of killed /inactivated or live attenuated microbial agents or bacterial toxoids. With the advancement in the field of biotechnology and molecular biology, we were able to introduce New Generation Vaccines like naked nucleic acid vaccines, vectored vaccines, anti-idiotypic vaccine, recombinant DNA vaccine etc. Still, according to WHO reports, the mortality rate due to illness are increasing every year especially in the third world countries. This may be due to constraints on vaccine production, distribution and delivery. The search by scientists to solve these limitations ultimately results in the development of "EDIBLE VACCINES".

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EDIBLE VACCINES

Edible vaccines are those vaccines based on genetically engineered expression of an antigenic protein by an edible plant. In simpler words, they are simply sub-unit vaccines that are edible in nature. Here, the gene of interest is introduced into plants and then these altered plants are induced to manufacture the corresponding proteins. This process is known as transformation and the altered plants are called transgenic plants. (Sibila, J. *et.al.* 2005*)*

The first report of edible vaccine appeared in 1990 in the form of patent application published under the international patent cooperation treaty. (Curtiss, R. I and Cardineau, C.A. 1990) It was regarding the successful expression of *Streptococcus mutans* surface protein A in tobacco. As this bacterium causes dental caries, it was envisaged that the stimulation of a mucosal immune response would prevent the bacteria from colonizing the teeth and there by protect against tooth decay. (Mason, H. S and Arntzen, C. J. 1995) Later in 1992, Charles Arntzen and his coworkers successfully expressed hepatitis B surface Antigen in tobacco plants in a cost effective manner. This paved the way for worldwide acceptance to the concept of edible

vaccine. (Mason, H. S. *et.al.*1992*)* Till then, various attempts have been made to develop edible vaccines against human and animal diseases including Norwalk virus particles, (Mason, H. S *et.al.*1996) Rabies (Hooper, D.C. *et.al.*, 1994) Gastroenteritis, Cholera (Arakawa, T. 1997).

PRODUCTION OF EDIBLE VACCINE

For producing Edible Vaccines, any of the two methods can be followed: (Shah, P.C *et.al.* 2011)

1. Appropriate plant virus is genetically engineered to express the desired peptides/ proteins. The recombinant virus is then inoculated into the plant. Large numbers of new plants are grown and chimeric virions are extracted and purified. The resultant plant edible vaccines are utilized for immunological purpose.

2. In another approach, the gene of interest is integrated with plant vector by transformation. A variety of techniques have been used to introduce transgene into plant cell; these could be grouped into following categories:

- Agrobacterium mediated gene transfer: The appropriate gene construct is inserted into the T-region of a disarmed Ti plasmid of Agrobacterium. The recombinant DNA is placed into Agrobacterium; a plant pathogen which is co-cultured with the plant cells or tissues to be transformed. The drawback of this method is that it gives low yield and the process is slow. This method worked especially well for dicotelydenous plants like potato, tomato and tobacco. Studies have also proved that the genes are expressed by this method in experimental animals and plants.

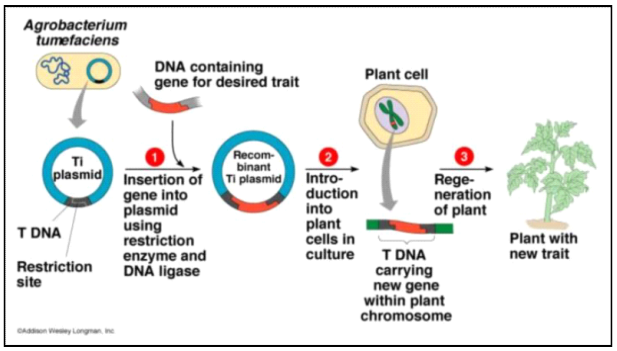
- Biolistic method: The gene containing DNA coated metal (e.g. gold, tungsten) particles are fired at the plant cells using gene gun. Those plant cells that take up the DNA are then allowed to grow in new plants, and are cloned to produce large number of genetically identical crop. This method is quite attractive because DNA can be delivered into cells of plant which makes gene transfer independent of regeneration ability of the species. But the chief limitation is that the gene gun is highly expensive.

- Electroporation: Here there is introduction of DNA into cells by exposing them for brief period to high voltage electrical pulse which is thought to induce transient pores in the plasma lemma. The cell wall presents an effective barrier to DNA therefore, it has to be weakened by mild enzymatic treatment so as to allow the entry of DNA into cell cytoplasm.

"SECOND GENERATION"EDIBLE VACCINES

Scientific community developed the second generation edible vaccine which provides protection against several pathogens. These multicomponent edible vaccines can be obtained by crossing two plant lines harboring different antigens. Yu and Langridge (Jie, Yu and William H.R. Langridge. 2001) fused Cholera toxin (CT) B and A2 subunit complementary DNAs (cDNAs) to a rotavirus enterotoxin and enterotoxigenic

Escherichia coli fimbrial antigen genes and transferred into potato. It was found that this trivalent edible vaccine could elicit humoral responses, as well as immune memory B cells and T helper cell responses which are hallmarks of successful immunization.



MECHANISM OF ACTION

Mucosal Immune System (MIS) is the first line of defense mechanism of body and thereby the most effective site for vaccination. The most effective route of mucosal immunization is oral route. (Korban, S. S. et.al. 2002). The transgenic plant parts with desired gene are fed directly since the outer tough wall of plant cells acts to protect the antigens against attack by enzymes, gastric and intestinal secretions. This method is known as bio-encapsulation. The plant cell wall breaks in the intestines near payer's patches and antigens are released. (Rudzik, R. *et.al.*1975).These released antigens are taken up by the M-cells and is presented to B cells with the help of antigen presenting cells (APC). The activated B-cells get differentiated into plasma cells and secrete IgA class of antibody and elicit mucosal immunity and humoral immunity. Another important component of mucosal immunity is T-cell mediated immune response where the T-cells specifically recognize pathogens and directly kill the invader themselves. It also helps indirectly to the antibodies to clear infection. T-cells produced in the mucous are capable of travelling the mucosal tissues through special 'homing' receptors on their membranes. This means that if an immune response is generated in gastrointestinal lining, T-cells produced there can travel to other mucosal sites, (e.g. the lungs, nasal cavity) providing protection over a large surface area

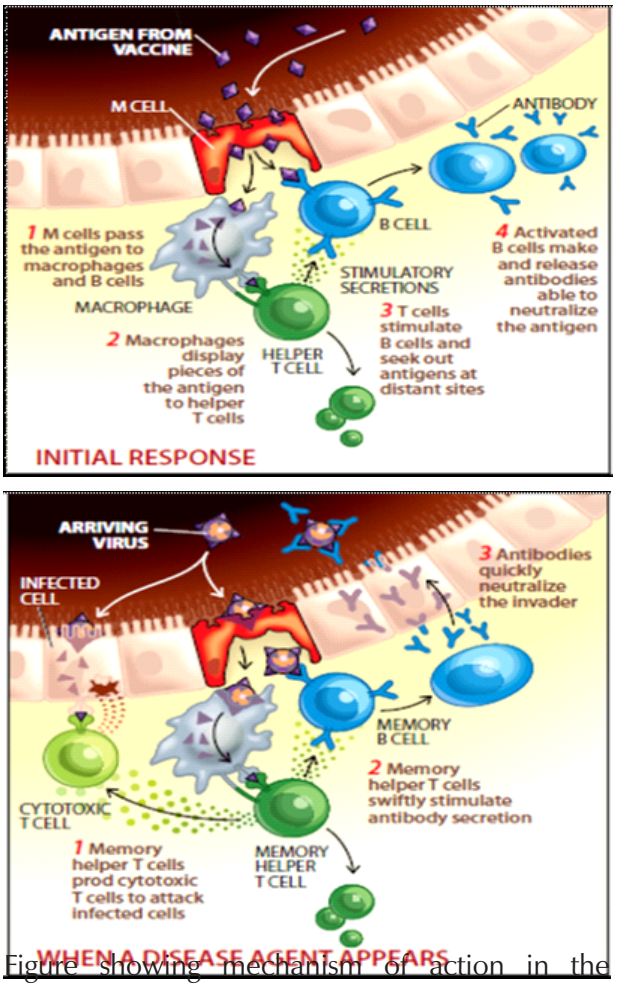


Figure showing mechanism of action in the intestinal mucosa

CLINICAL TRIALS

Edible vaccines have been checked for their efficacy in humans. The results of human trials that were tested for different transgenic plants

have showed positive responses and no major safety concerns. (Streatfield, S.J. 2001). Transgenic plant-made vaccines are also being used in animals. The first edible vaccine to demonstrate efficacy in animal trials was against Transmissible gastroenteritis virus (TGEV) in pigs. Livestock animals are fed with transgenic plants, like *Arabidopsis thaliana*, alfalfa and potato with antigens to protect them from pathogens; including FMDV, BRV and bovine viral diarrhea virus (BVDV)(Streatfield, S.J. 2001)

ADVANTAGES

1. Trigger mucosal immunity (traditional vaccines may bypass this) which is the body's first line of defense.

2. Adjuvant to enhance immune response is not necessary.

3. Cost-effective in terms of manufacturing, storage and transportation. Edible vaccines are made in molecular farms, and not in multimillion dollar machines. Since most countries have soil-rich land, it provides them the convenience in obtaining edible vaccines, and long distance transportation is not required. Maintenance of cold chain not required because plant tissues can be dried or, as when the seeds are targeted, have low moisture content. (Pascual, D. W.2007). Thus the amount spent yearly to preserve vaccine can be saved.

4. Their production is highly efficient and can be easily scaled up. For example, hepatitis-B antigen required to vaccinate whole of China annually, could be grown on a 40-acre plot and all babies in the world each year on just 200 acres of land!

5. Easily available since produced from plants. If we run out, we can simply plant more. Moreover, we can select the local/native crop of a particular area and can engineered it to produce the vaccine.

6. Giving an oral vaccine would require little or no training at all, which reduces the requirement of trained professionals.(Streatfield, S.J. 2001)

7. Eliminates the need of syringes & needles for administration. Needle administered vaccines are plagued with problems of re-use, mis-use and an occasional lack of sterilization.

8. Plants cannot host most human pathogens, so the vaccines will not pose a danger to humans.

9. Fear of contamination with animal viruses like the mad cow disease, which is a threat in vaccines manufactured from cultured mammalian cells, can be eliminated.

10. They are subunit preparations containing only the antigen and not any pathogenic genes. Thus it enhances the safety of individual.

11. Multi-component ability of vaccine allows to harbour more than one antigen to prevent many diseases in same individual. (Jie, Yu and William H.R. Langridge. 2001)

12. Antigen can be protected by means of bioencapsulation.

13. They can seroconvert even in presence of maternal antibodies, thus having a potential role in protecting infants against like Group-B Streptococcus, Respiratory Syncytial Virus etc. which are under investigation.

FUTURE CHALLENGES/CONCERNS

1. There is need of proper distinguishing characters to identify between 'vaccine fruit' and 'normal fruit' to avoid misadministration of vaccine

which could lead to tolerance.

2. Antigen selection involves safety concerns of whether or not selected antigens are compatible enough with the selected plant type to be expressed (Lal, P. *et.al*. 2007)

3. How can the vaccine dose be controlled? This remains the most difficult task. Consistency of dosage form differs from fruit to fruit, plant to plant and generation to generation. It is determined by protein content, patient's age, weight, ripeness of the fruit and quantity of the food eaten. If low doses are consumed then the production of antibodies are less likely to occur, and if high doses are consumed it may cause tolerance. (Lal, P. *et.al*. 2007)

4. Glycosylation patterns in plants differ from those in humans and could affect the functionality of the vaccines.

5. Allergic reactions to the fruit or vegetable expressing the foreign antigen may be an issue.

6. The doubt still exists about whether the antigens be able to survive the hostile, acidic conditions of the stomach and even if they did, will they be able to trigger the immune system in the right way. Although initial trials have shown promising results in human subjects, it is not clear what will happen when the person comes in contact with the actual virus.

7. Certain foods are not eaten raw (e.g. potato) and needs cooking which will denature or weaken the protein present in it. (Moss, W. J. *et.al*.1999)

8. Variable storage conditions for edible vaccine are also a major problem. Potatoes containing vaccine could be stored for longer time while a tomato does not last long. Thus these vaccines need to be properly stored to avoid infection through microbial spoilage.

9. Another concern is if the transgenic plants are mass produced, they may have an inconsistent expression caused by the small interfering RNAS. (Tonks, A. 2007)

10. There is chance of plant/crops (food) contamination through cross pollination and of vaccine itself in plant debris spreading dust and other pollutant in surfaces and ground waters. The vaccine antigen may affect browsing animals and humans living in the area drinking vaccine polluted water or breathing vaccine polluted dust. The cultivation and production should be limited to facilities like greenhouse, or in plant tissue culture that prevent their environmental release.

CONCLUSION

Transforming plants to carry vaccines is one of the latest innovations of medical technology and promises greatest hope for the floundering biotech industry. It can prove to be very effective, if rightly implemented, in providing accessibility to developing and underdeveloped countries where rates of diseases are relatively high. Edible vaccines are prominent over typical traditional vaccines due to its positive aspects like they are cost-effective, safe, easy to administer and can store at the site of production. But it has to overcome the above mentioned concerns and technical obstacles to become a reality. Future research is also required to demonstrate whether these vaccines meet the standards of quality (purity, potency, safety, efficacy and durability) defined for vaccines by the World Health Organization. It can give us new and dramatic hope for improved life. Although in the initial stages of development, a day is not away

when we will be able to pluck a fruit from the garden, munch on it and get protected from diseases… making needles needless…

As Hippocrates said, "Let thy food be thy medicine"

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