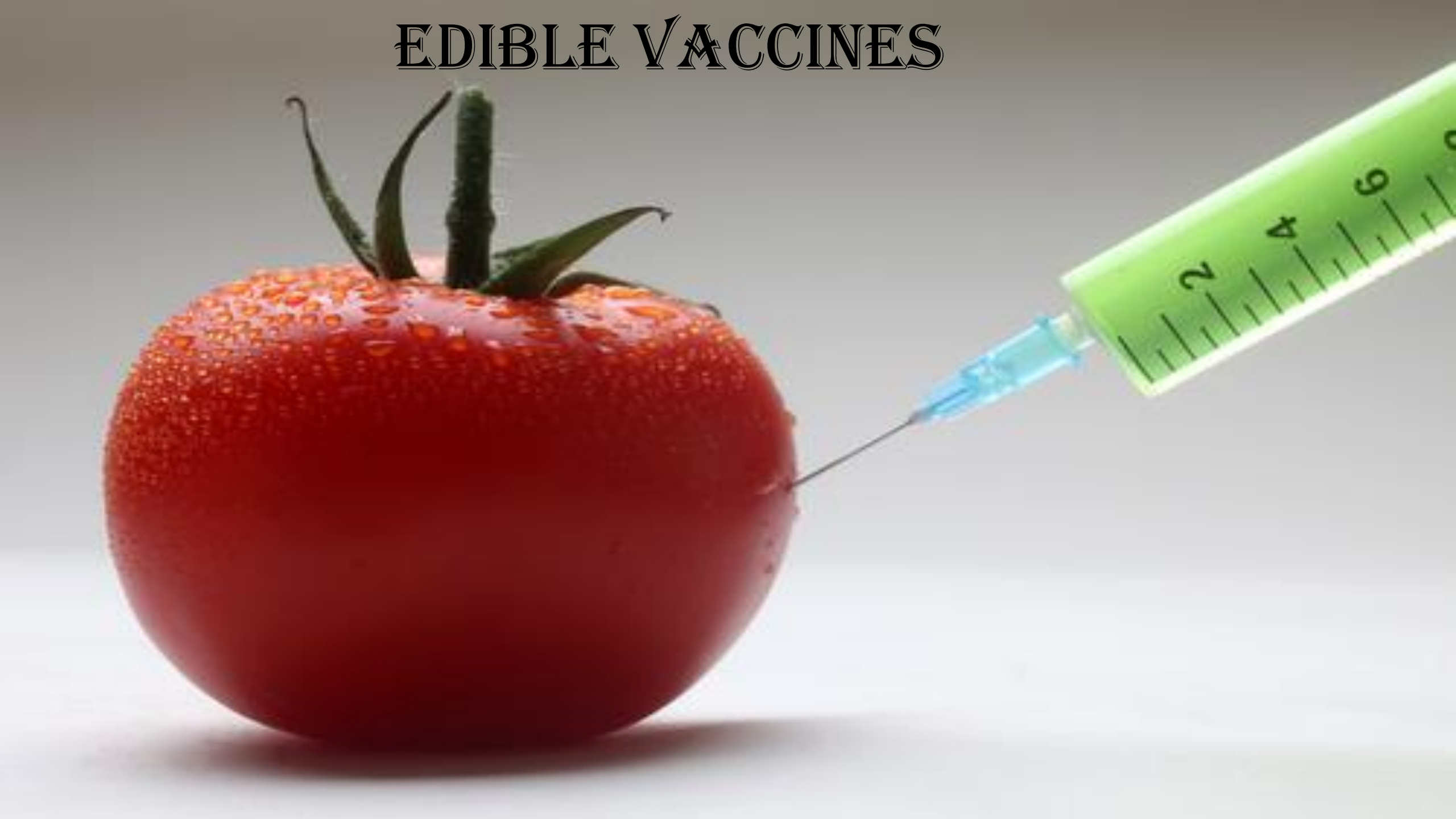
# EDIBLE VACCINES



# VACCINE

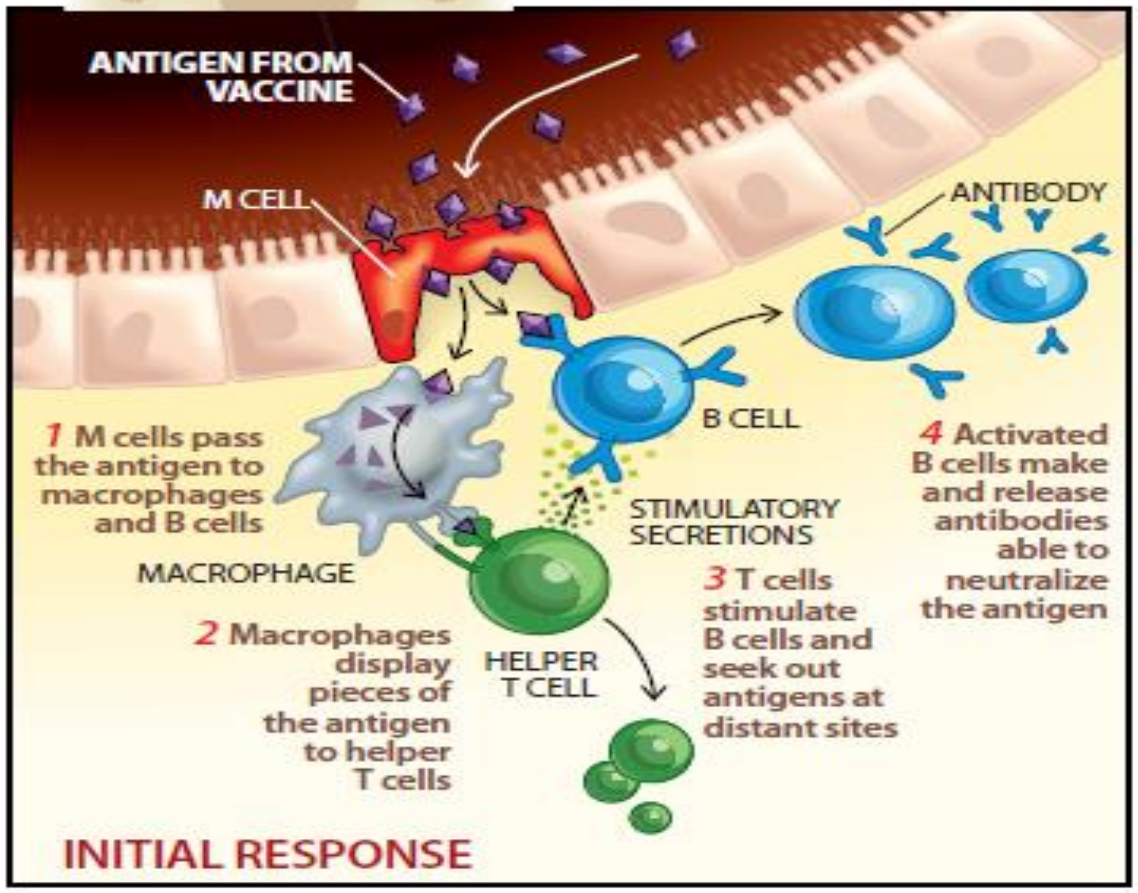
- According to Merriam-Webster, a vaccine is an antigenic preparation of a typically inactivated or attenuated pathogenic agent (such as a bacterium or virus) or one of its components or products (such as a protein or toxin)

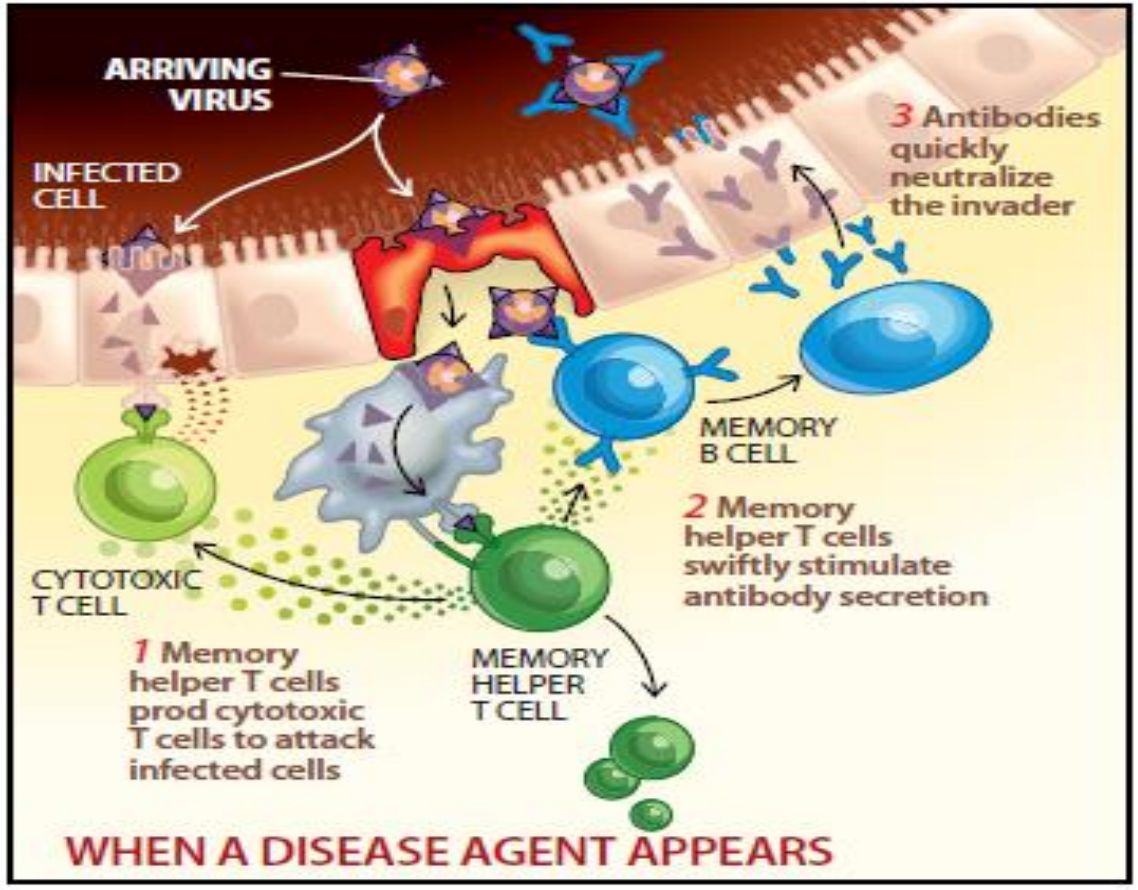
- It works by priming the immune system to swiftly destroy specific disease-causing agents, or pathogens, before the agents can multiply enough to cause symptoms.

- On detecting the presence of a foreign organism in a vaccine, the immune system behaves as if the body were under attack by a fully potent antagonist. It mobilizes its various forces to root out and destroy the apparent invader—targeting the campaign to specific antigens (proteins recognized as foreign). The acute response soon abates, but it leaves behind sentries, known as "memory" cells, that remain on alert, ready to fight the real pathogen if and when it enters the body.

- Vaccines have completely eradicated **smallpox** and are on the verge of eliminating **measles, mumps, rubella** and **polio.**

# How a vaccine works



# Understanding a virus

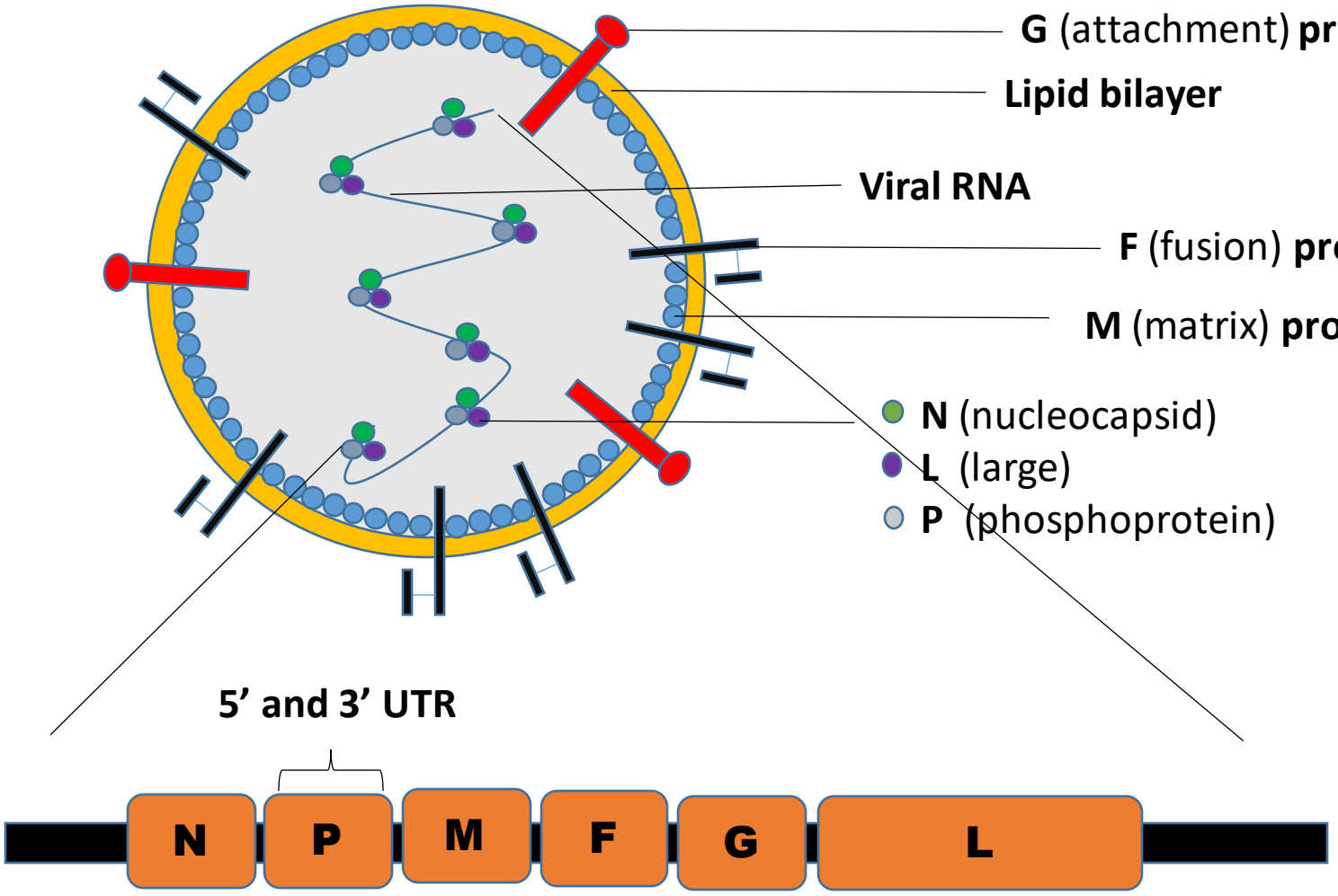



Fig:- Representation of a virus and viral genome

Classic vaccines pose a troubling risk that the vaccine microorganisms will somehow spring back to life, causing the diseases they were meant to forestall

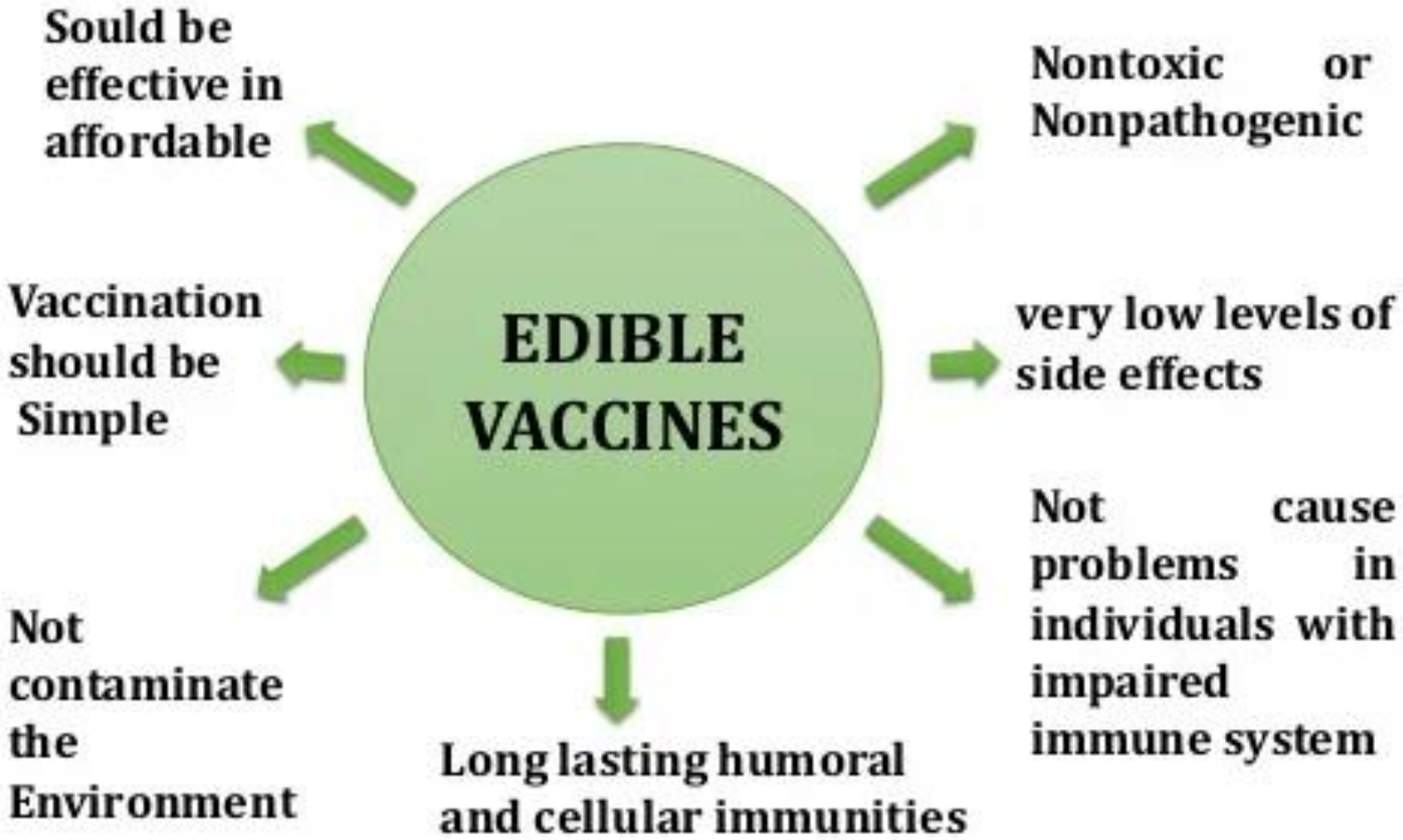
For that reason, vaccine makers today favor so-called subunit preparations, composed primarily of antigenic proteins separated from a pathogen’s genes. On their own, the proteins have no way of establishing an infection.

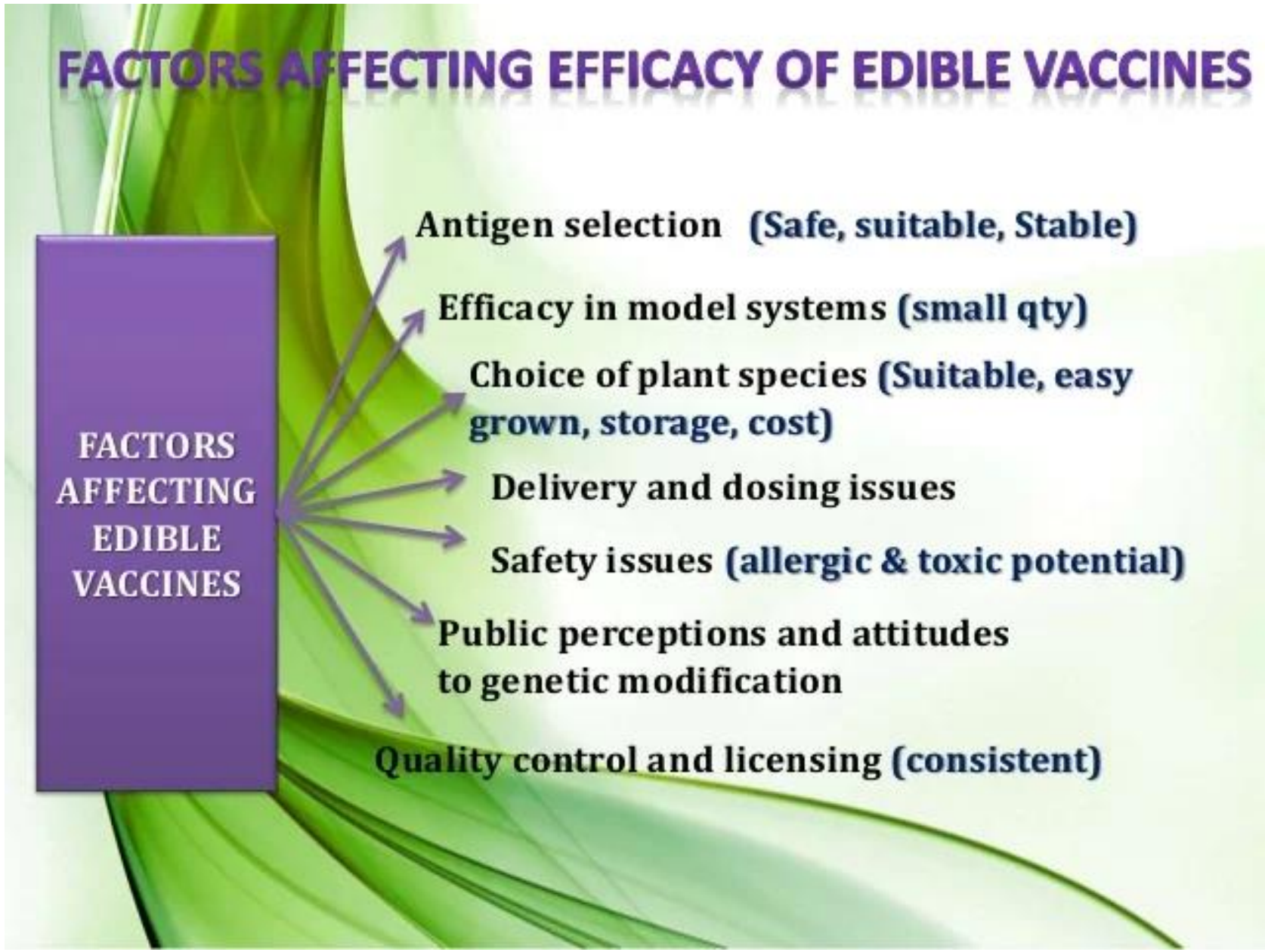
Subunit vaccines, however, are expensive, in part because they are produced in cultures of bacteria or animal cells and have to be purified out; they also need to be refrigerated

# Edible vaccines:

- The phrase edible vaccines was first used by Charles Arntzen in 1990 and refers to any foods; typically plants, that produce vitamins, proteins or other nourishment that act as a vaccine against a certain disease. Once the plant, fruit, or plant derived product is ingested orally, it stimulates the immune system.
- Presently, there are edible vaccines for **measles, cholera, foot and mouth disease, and hepatitis B, C, & E**. However, even though there are edible vaccines, they are predominately tested in the animal testing and in development phases, with some human clinical trials being conducted.
- The first example was the expression of a surface antigen from *Streptococcus mutans* in tobacco.
- Transgenic banana and tomato can be consumed to get protected from cholera and hepatitis B

# IDEAL PROPERTIES




FACTORS AFFECTING EFFICACY OF EDIBLE VACCINES
FACTORS AFFECTING EDIBLE VACCINES
Antigen selection (Safe, suitable, Stable)
Efficacy in model systems (small qty)
Choice of plant species (Suitable, easy grown, storage, cost)
Delivery and dosing issues
Safety issues (allergic & toxic potential)
Public perceptions and attitudes to genetic modification
Quality control and licensing (consistent)

# EDIBLE VACCINES

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 hence overcoming the threat of attenuated microorganisms springing back to life

Charles J. Arntzen was the pioneer scientist in the field of edible vaccines who responded to World Health Organization call for inexpensive, oral vaccines that needed no refrigeration.

While plant system may have the capability of producing any vaccine in large amounts and in a less expensive manner, purification of the product may be cumbersome.

Attention therefore has been paid to mainly those antigens that stimulate mucosal immune system to produce secretory IgA (S-IgA) at mucosal surfaces, such as gut and respiratory epithelia.

In general, a mucosal response is achieved more effectively by oral instead of parenteral delivery of the antigen. Thus, an antigen produced in the edible part of a plant can serve as a vaccine against several infectious agents which invade epithelial membranes.

# ❑ CANDIDATE PLANTS








**Lettuce**
Fast-growing But, Spoils readily

**Wheat**
Large number of seeds help in increased harvest. but, Need cooking

**Carrot**
Rich in β carotein, production of Insulin


- ❖Easily transformation
- ❖Stored for long period without refrigeration
- ❖No Cooking

- ❖2-3 years to mature&12 months to bear fruit
- ❖Spoils rapidly after ripening
- ❖Contains very little protein

- ❖Grow quickly
- ❖High content of vitamin A may boost immune response
- ❖Heat-stable
- ❖Do not need special facilities for storage and transportation.
- ❖They taste good.

- ❖Spoils easily

TOMATO

- Strategies for expression of antigens in plant
  1. Using a transformation vector (eg.- *Agrobacterium tumefacians*) carrying the antigen
  2. Infecting the plants with recombinant viruses carrying the desired antigen that is fused to viral coat protein

| Protein | Plant |
|---|---|
| Hepatitis B surface antigen | Tobacco |
| Rabies virus glycoprotein | Tomato |
| Norwalk virus capsid protein | Tobacco |
| *E. Coli* heat labile enterotoxin B subunit | Potato |
| Cholera toxin B subunit | Potato, Tobacco |
| Mouse glutamate D carboxylase | Potato |
| VP1 protein of foot and mouth disease virus | Arabidopsis |
| Insulin | Potato |
| Glycoprotein of swine-transmissible gastroenteritis coronavirus | Arabidopsis |

**Table1-** Antigens produced in transgenic plants

| Protein | Plant | Carrier |
|---|---|---|
| Influenza antigen | Tobacco | TMV |
| Murine zona pellucida antigen | Tobacco | TMV |
| Rabies antigen | Spinach | AIMV |
| HIV-1 antigen | Tobacco | AIMV |
| Mink enteritis virus antigen | Black eyed bean | CPMV |
| Colon cancer antigen | tobacco | TMV |

**Table2-** Transient production of antigens in plants after infection with plant viruses expressing a recombinant gene

- AIMV- Alfalfa Mosaic Virus; CMV- Cowpea Mosaic Virus; TMV- Tobacco Mosaic Virus

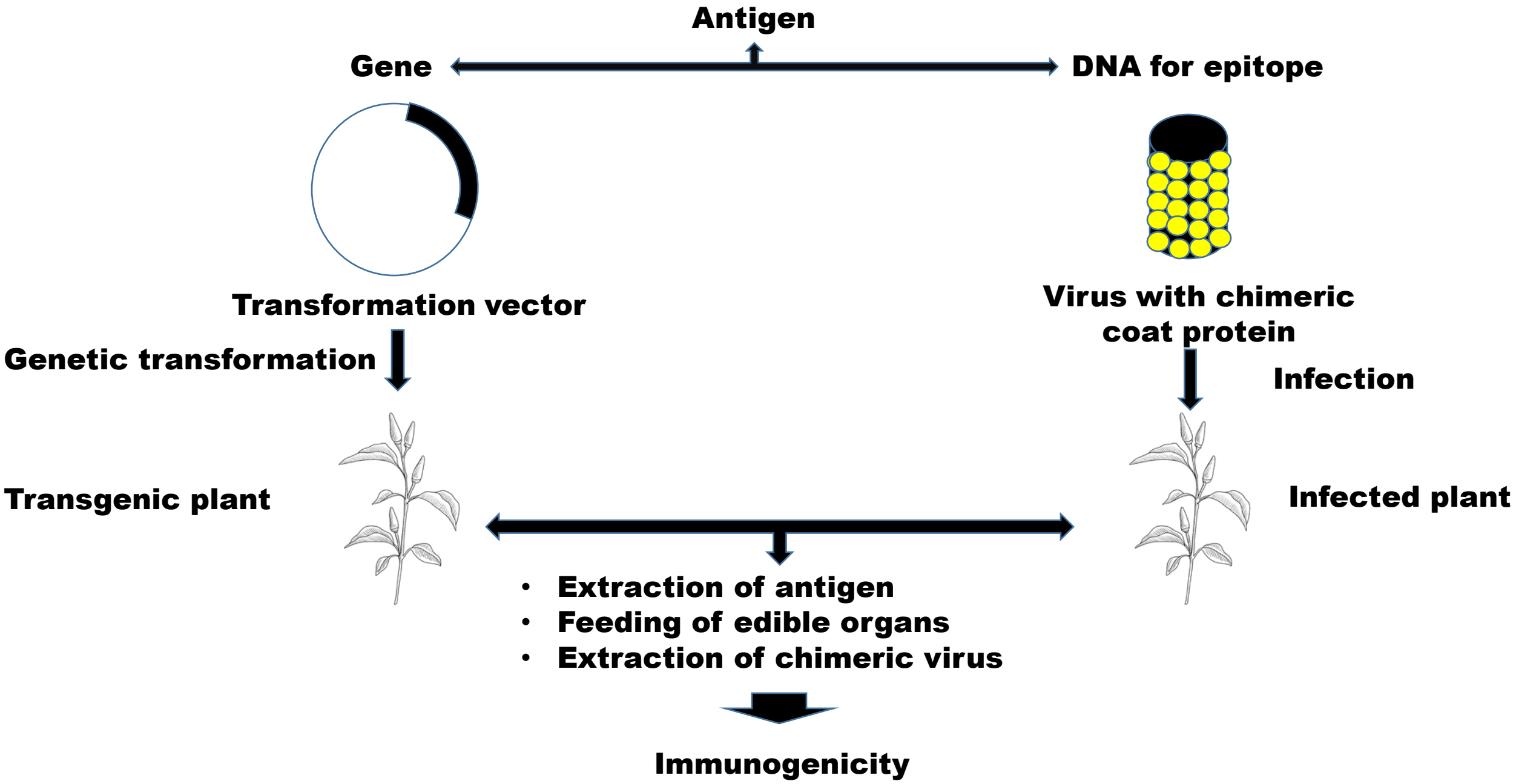
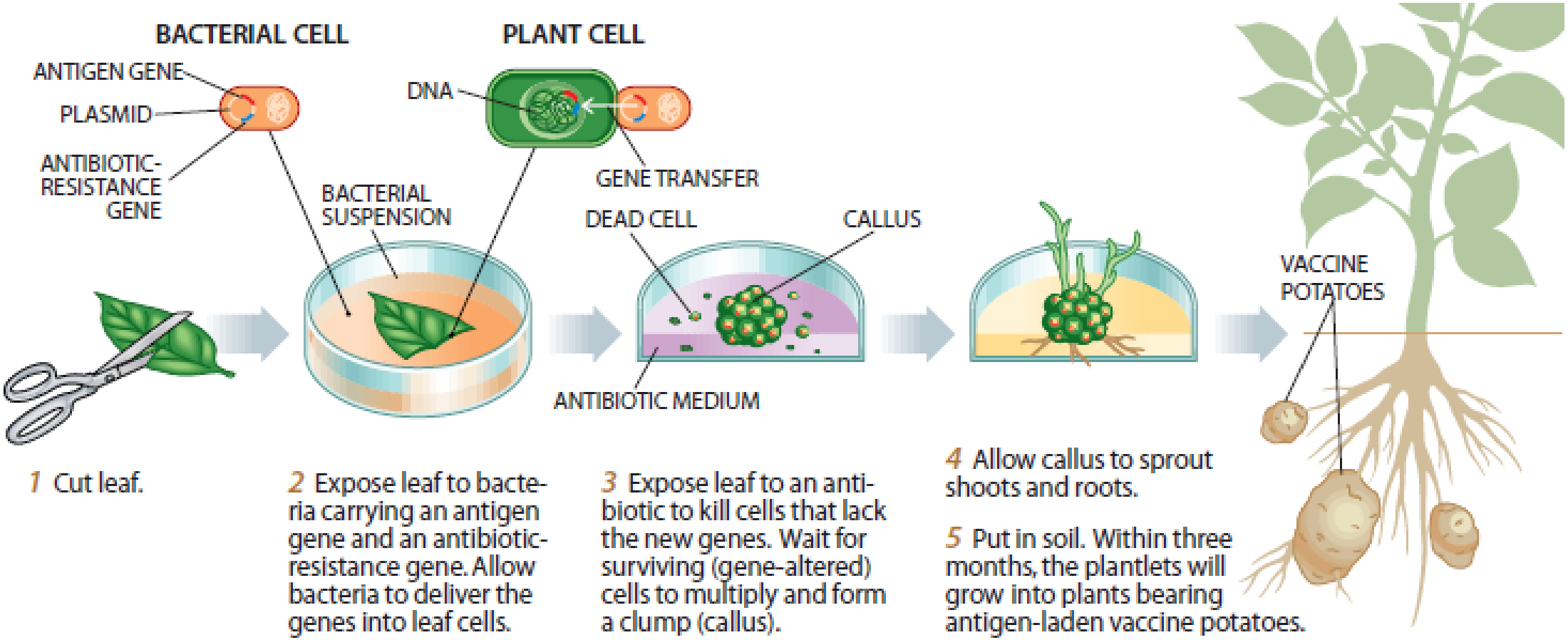



**Fig:-** Strategies for expression of antigens in plants

- **Strategy 1-** Using a transformation vector carrying the antigen



- Following are examples of transgenic plants carrying antigens

1. Acute watery diarrhea is caused by enterotoxigenic *Escherichia coli* and *Vibrio cholera* that colonize the small intestine and produce one or more enterotoxin, an attempt was made towards the production of edible vaccine by expressing heat-labile enterotoxin (LT-B) in tobacco and potato.

   The enterotoxin (LT) from *E. coli* is a multimeric protein, quite similar to cholera toxin (CT) structurally, functionally and antigenically. LT has one A subunit (27 kDa) and a pentamer of B subunits (11.6 kDa). LT-B and CT-B are both potent oral immunogens.

   An oral vaccine composed of the cholera toxin-B subunit (CT-B) with killed *V. cholerae* cells has been reported to give significant level of protection against cholera.

   But the cost of production of CT-B by conventional methods is too high to allow distribution of this vaccine.

   The recombinant LT-B (rLT-B) produced in tobacco and potato showed partial pentamerization after the engineering of subunit gene in a way that allowed retention of the protein in microsomal vesicles. On testing immunogenicity of rLT-B by feeding potato tubers to mice, both humoral and mucosal immune responses were reported to be stimulated

2. Cholera toxin, which is very similar to *E. coli* LT, has also been expressed in plants. Hein *et al*. generated tobacco plants expressing CT-A or CT-B subunits of the toxin. Cholera toxin-B subunit, when expressed in potato was processed in a natural way: the pentameric form. Even after boiling transgenic potato tubers till they became soft, approximately 50% of the CT-B was present in the pentameric GM1 ganglioside-binding form.
3. A rabies virus coat glycoprotein gene has been expressed in tomato plants.
4. The Hepatitis B surface antigen (HBsAG) has been reported to accumulate to 0.01% of soluble protein level in transgenic tobacco. The antigens, delivered in a macromolecular form, are known to survive the gut atmosphere and perform better. A crude extract from plants was used for parenteral immunization in mice. The immune response included all IgG subclasses as well as IgM against hepatitis B.
5. Carrillo *et al*. expressed structural protein, VP1, of foot-and-mouth disease virus in *Arabidopsis*. The mouse that was immunized intraperitoneally with a leaf extract elicited immune response to synthetic peptides carrying various epitopes of VP1, or to complete VP1.

- **Strategy 2-** The technique involves either placing the gene downstream a subgenomic promoter, or fusing the gene with capsid protein that coats the virus.
- Examples of plants infected with recombinant viruses carrying the desired genes

1. Modelska *et al*. have shown that immunization of mice intraperitoneally or orally by gastric incubation or by feeding of plants infected with the recombinant alfalfa mosaic virus (AIMV) carrying rabies peptide CPDrg 24 mounted local as well as systemic immune response. Oral administration could stimulate both serum IgG as well as IgA synthesis. After immunization, 40% of the mice were protected against the challenge with a lethal dose of the virus.
2. A 13-amino-acid epitope of zona pellucida, ZP3, protein and another epitope from malarial sporozoites have been expressed as fusion proteins with TMV capsid protein with the idea of developing anti-fertility and anti-malarial vaccines.
3. Scientists at Axis Genetics, Cambridge, have shown that injecting mink with extracts of plants infected with a cowpea mosaic virus, that expresses a mink enteritis antigen gene, protects the animal against subsequent virus challenge.

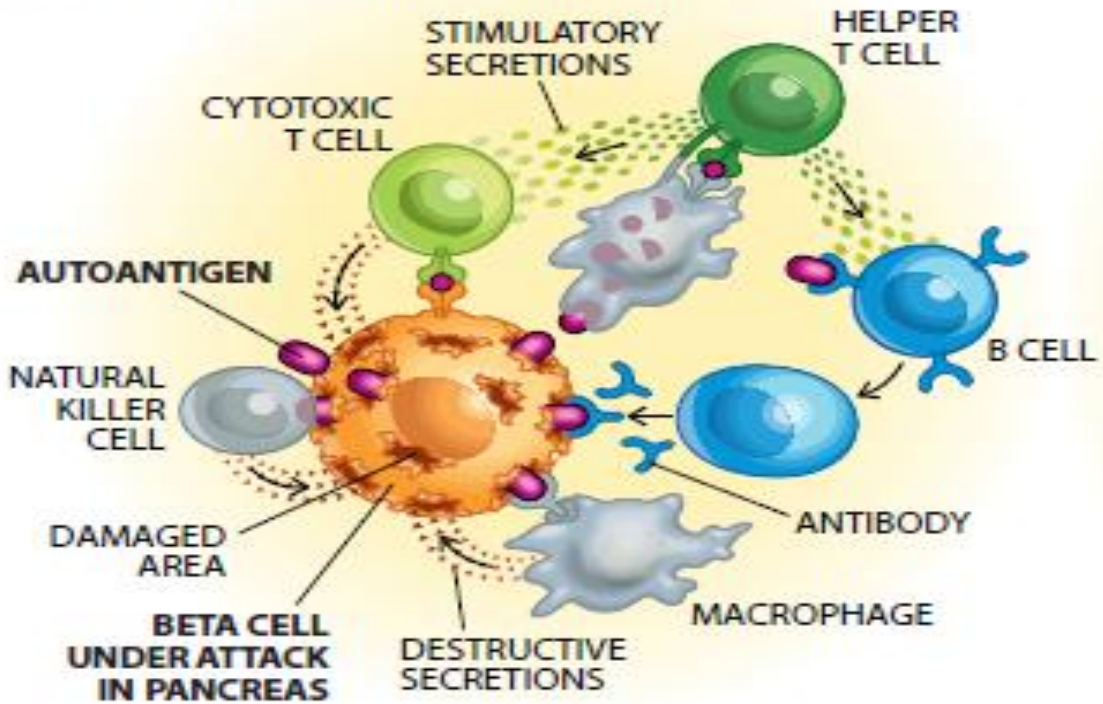
# OTHER ASPECTS

## Fighting Autoimmunity

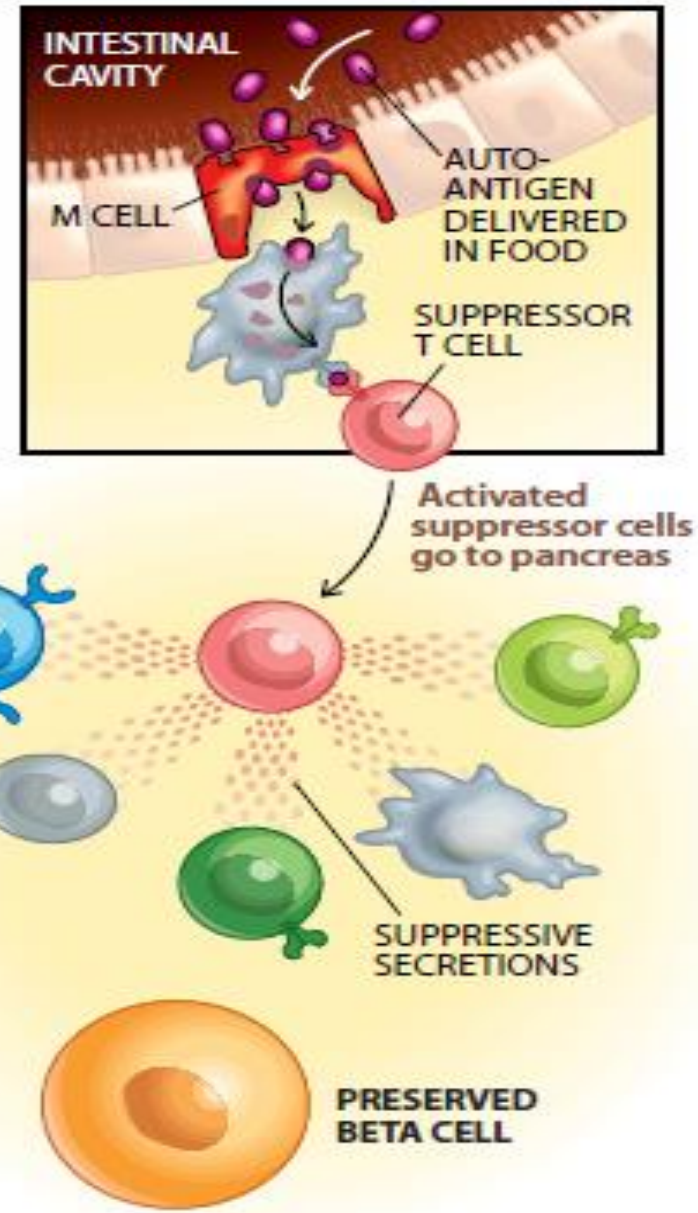
- One of the utilities of producing antigens in plants in large amount is in treatment of autoimmune diseases like diabetes mellitus which involve production of antibodies against glutamic acid decarboxylase (GAD) and insulin, leading to destruction of insulin-producing pancreatic cells.
- Insulin and GAD have been produced in potato and tobacco, respectively. To direct the delivery of plant-synthesized insulin to the gut associated lymphoid tissue, insulin was linked to cholera-toxin B subunit. Non-obese diabetic mice which were fed with the transformed potato tuber tissue containing microgram level of the recombinant insulin delayed the progression of clinical diabetes.
- Similarly, GAD-producing tobacco plants, given as a dietary supplement, inhibited the development of diabetes in the non-obese diabetic mouse.

# STOPPING AUTOIMMUNITY

The autoimmune reaction responsible for type I diabetes arises when the immune system mistakes proteins that are made by pancreatic beta cells (the insulin producers) for foreign invaders. The resulting attack, targeted to the offending proteins, or "autoantigens," destroys the beta cells (*below, left*). Eating small amounts of autoantigens quiets the process in diabetic mice, for unclear reasons. The autoantigens might act in part by switching on "suppressor" cells of the immune system (*inset*), which then block the destructive activities of their cousins (*below, right*).




BEFORE TREATMENT

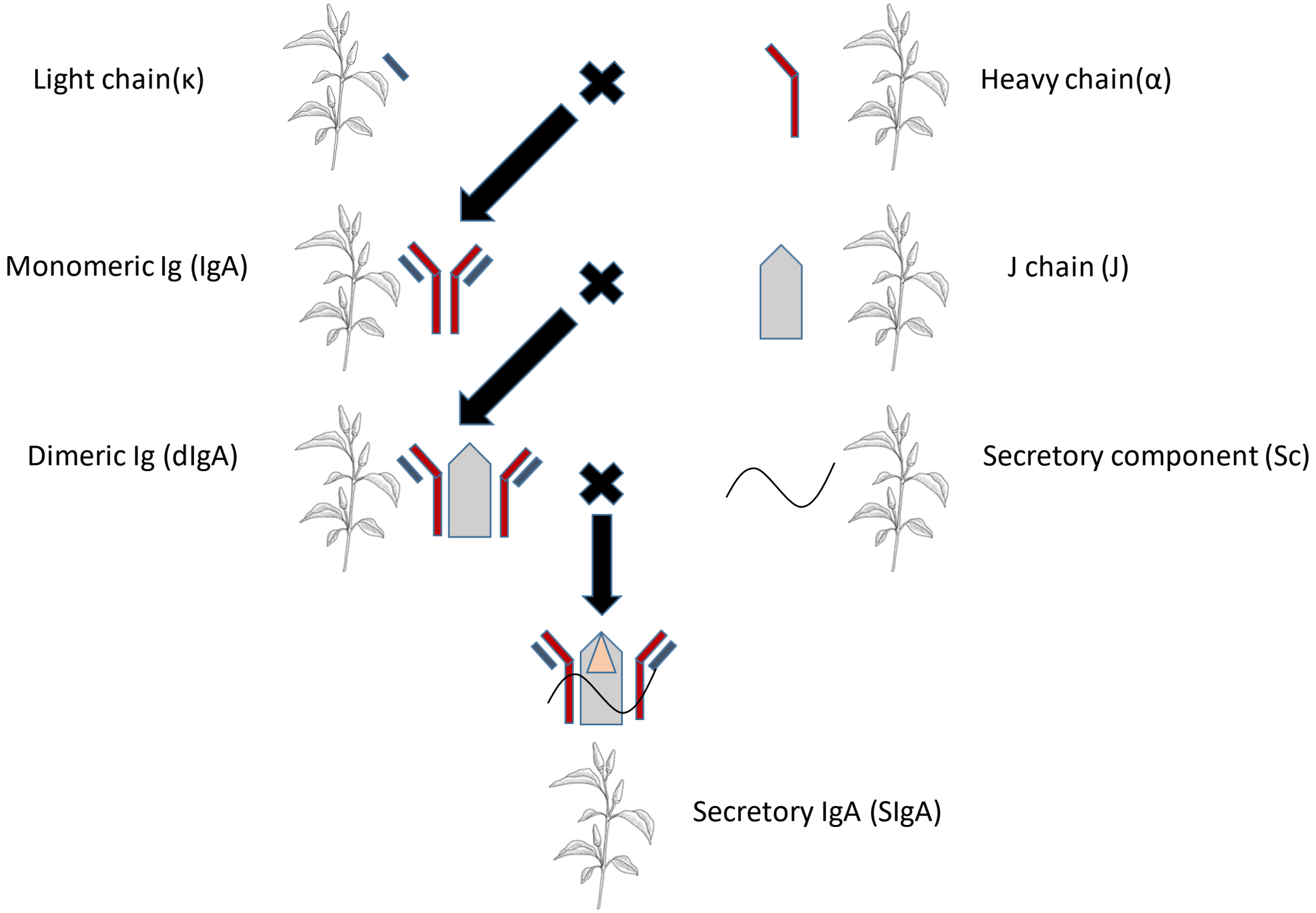



AFTER TREATMENT

# Expression and assembly of antibodies in plants

- Transgenic plants are also being looked upon as a source for producing large-scale antibodies which can serve the purpose of passive immunization by direct application, in addition to providing a tool for drug targeting or interactive inactivation of undesirable molecules.
- Using gene technology, not only genes coding for both the light and heavy chains have been expressed, but modified genes capable of expressing only Fab fragments (assembled light chains and shortened heavy chains) or scFV (single peptide chains where variable domains of heavy and light chains are covalently linked by a short flexible peptide) have also been expressed in bacteria and mammalian cells.
- Transgenic plants not only provide the means to express antibodies but also enable the glycosylation and entry into secretory pathway which allow assembly of complete antibodies and Fab fragments.
- In plants, antibody production has been achieved by cross-pollination of individually transformed plants expressing light or heavy chains. Other approaches involve double transformation, or transformation by constructs having genes for both light and heavy chains on the same vector.

- A group of scientists have succeeded in producing multimeric secretory IgA (SIgA) molecules in plants which represent the predominant form of immunoglobulin in mucosal secretions. SIgA not only contains heavy and light chains but it is also dimerized by a J chain, and protected from proteolysis by a fourth polypeptide, the SC. Thus, four transgenic tobacco plants were produced by genetic engineering which produced a murine monoclonal antibody light k chain, the hybrid IgA-G antibody heavy chain, murine J chain and rabbit secretory component. A series of sexual crosses was carried out to allow expression of all the four proteins simultaneously. The progeny produced a functional secretory immunoglobulin very efficiently (figure in following slide).
- A humanized monoclonal antibody against glycoprotein B of herpes simplex virus 2 (HSV-2) has been expressed in soybean. This antibody was found to possess the same efficacy for prevention of vaginal HSV-2 infection in mice and similar stability in human semen as the antibody expressed in human cell culture.
- A hybrid monoclonal antibody (IgA/G), having constant regions of IgG and IgA fused, has been used successfully against human dental caries caused by the bacterium *Streptococcus mutans*. Ma *et al*. compared the secretory antibody generated in transgenic tobacco (SIgA/G) and the original mouse IgG. Though both had similar binding affinity to surface adhesion protein of *S. mutans*, SIgA/G survived for 3 days in the oral cavity, whereas IgG could survive for just one day. The plant antibody provided protection against the colonization of the *S. mutans* for at least four months.


Light chain(κ)
Heavy chain(α)
Monomeric Ig (IgA)
J chain (J)
Dimeric Ig (dIgA)
Secretory component (Sc)
Secretory IgA (SIgA)

| Antibody | Antigen | Plant |
|---|---|---|
| IgG (κ) | Transition state analog | Tobacco |
| IgM (λ) | NP (4-hydroxyl-3-nitrophenyl) acetyl hapten | Tobacco |
| Single domain (dAb) | Substance P | Tobacco |
| Single chain Fv | Phytochrome | Tobacco |
| Single chain Fv | Artichoke mottled crinkle virus coat protein | Tobacco |
| Fab; IgG (κ) | Human creatine kinase | *Arabidopsis* |
| IgG (κ) | Fungal cutinase | Tobacco |
| IgG (κ) and SIgG/A hybrid | *S. mutans* adhesion | Tobacco |
| Single chain Fv | Abscisic acid | Tobacco |
| Single chain Fv | Nematode antigen | Tobacco |
| Single chain Fv | B-glucuronidase β-1,4-endoglucanase | Tobacco |
| Single chain antibody fragment | Atrazine, Paraquat | Tobacco |
| IgG | Glycoprotein B of Herpes Simplex virus | Soybean |

**Table3- Antibodies and antibody fragments produced in transgenic plants**

# Advantages and Disadvantages

- Advantages
  - Plants can be grown locally and cheaply
  - Due the use of antigens only, it eliminates the risk of resurgence of the attenuated microorganism
  - Elicits mucosal immunity
  - The tough outer wall of plant cells serves as temporary armor for the antigens, keeping them relatively safe from gastric secretions
  - Helps in tackling autoimmune disease such as diabetes mellitus
- Disadvantages
  - Amount of vaccine produced is low
  - Much of the dose is lost during cooking
  - Reduced shelf life
  - Plants sometimes grow poorly when they start producing large amounts of a foreign protein
  - Social stigma considering genetically modified plants

## Regulatory Issues

- It is still unclear whether the edible vaccines would be 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.

# 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 immunization 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

**Transgenic plants for the production of edible vaccines and antibodies for immunotherapy**

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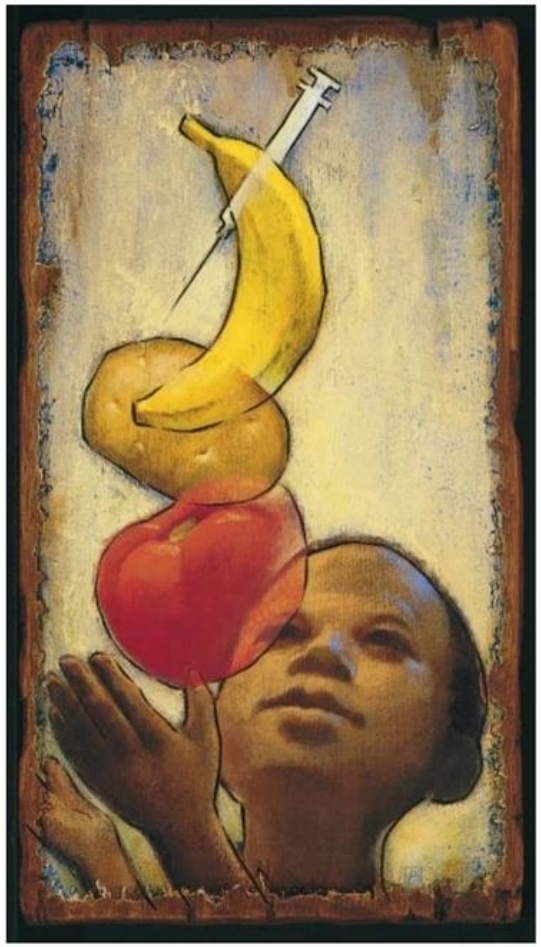
**Edible Vaccines**

by William H. R. Langridge

One day children may get immunized by munching on foods instead of enduring shots. More important, food vaccines might save millions who now die for lack of access to traditional inoculants

# Edible Vaccines

by William H. R. Langridge



Vaccines have accomplished near miracles in the fight against infectious disease. They have consigned smallpox to history and should soon do the same for polio. 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.

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.

This situation is worrisome not only for the places that lack health care but for the entire world. Regions harboring infections that have faded from other areas are like bombs ready to explode. 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.

In the early 1990s Charles J. Arntzen, then at Texas A&M University, conceived of a way to solve many of the problems that bar vaccines from reaching all too many children in developing nations. Soon after learning of a World Health Organization call for inexpensive, oral vaccines that needed no refrigeration, Arntzen visited Bangkok, where he saw a mother soothe a crying

FOODS UNDER STUDY as alternatives to injectable vaccines include bananas, potatoes and tomatoes, as well as lettuce, rice, wheat, soybeans and corn.







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## Edible Vaccines: Promises and Challenges

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### Abstract

Go to:

Vaccines are biological preparations that improve immunity to particular diseases and form an important innovation of 19th century research. It contains a protein that resembles a disease-causing microorganism and is often made from weak or killed forms of the microbe. Vaccines are agents that stimulate the body's immune system to recognize the antigen. Now, a new form of vaccine was introduced which will have the power to mask the risk side of conventional vaccines. This type of vaccine was produced from plants which are genetically modified. In the production of edible vaccines, the gene-encoding bacterial or viral disease-causing agent can be incorporated in plants without losing its immunogenic property. The main mechanism of action of edible vaccines is to activate the systemic and mucosal immunity responses against a foreign disease-causing organism. Edible vaccines can be produced by incorporating transgene in to the selected plant cell. At present edible vaccine are developed for veterinary and human use. But the main challenge faced by edible vaccine is its acceptance by the population so that it is necessary to make aware the society about its use and benefits. When compared to other traditional vaccines, edible vaccines are cost effective, efficient and safe. It promises a better prevention option from diseases.