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OUTLOOK OF VACCINE PREPARATION TECHNIQUES

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ABSTRACT

Immunization is the process of rendering man resistant to infections. It may be done by active or passive process. Inactive or non toxic part of the potion from live attenuated or killed virus or bacteria is used to design different vaccine. Hence, vaccine is antigenic preparations from microorganism (virus or bacteria or rickettsia) for protect and prevent the diseases from our body in long term after administered. After taking vaccine body system immunized and stimulate, and recognize the foreign particle then encounter them. Once specific vaccine is activated in our body means throughout life we saved from the definite diseases. This reviewed the recent literature on types of whole organism vaccines, subunit vaccine, recombinant vaccine, DNA vaccine, edible vaccine, synthetic peptide vaccine and multivalent subunit vaccine. Outlook of the designing vaccine have been studied extensively to help eradication the infectious disease development, and thereby decrease the need for drugs.

KEYWORDS: Immunization, passive, vaccine, rickettsia, recombinant, edible, synthetic peptide.

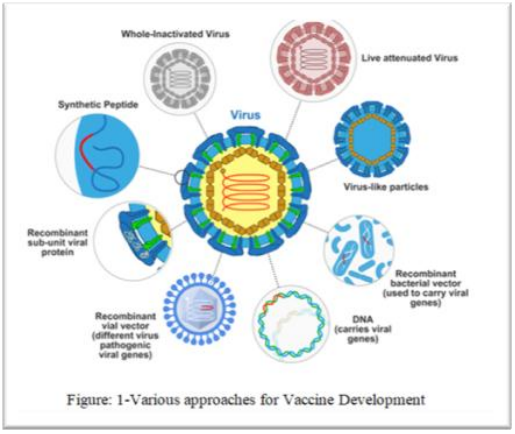


Figure: 1-Various approaches for Vaccine Development

Overview: In 1798, "An Inquiry into the Cause and Effects of Variolae" published by Edward Jenner which is the first evidence for the use of vaccination or immunization. The terms vaccine and vaccination are derived from Variolae vaccinae (smallpox of the cow), the term devised by Edward Jenner to denote cowpox.[1] Active immunization has brought direct benefit to human beings and domestic animal which refers to stimulate in specific antigen. Example: small pox. Whereas passive immunization is developed in a short term resistance to an infection in a non-individual by the administration of sensitized lymphocytes or serum antibodies. Example: snake venom. The administration of vaccines is called vaccination. Vaccination is the most effective method of preventing infectious diseases; widespread immunity due to vaccination is largely responsible for the worldwide eradication of smallpox and the restriction of diseases such as polio, measles, and tetanus from much of the world.[2] Several factors are considered to developing a successful vaccine. The first step is which branch of the immune system is activated, humoral or the cell mediated branches.[3] A second factor is the development of immunologic memory. For example, a vaccine that induces a protective primary response may fail to induce the formation of memory cells, leaving the host unprotected after the primary response to the vaccine subsides.[4] Vaccine is unlimitedly developed due to effectiveness of immune response of human being.

1. Whole organism vaccine: Whole organism vaccines contain multiple copies of each antigen, as well as other immunostimulatory molecules. Vaccine development has traditionally focused on whole organism vaccines, either live attenuated or inactivated vaccines. While successful for many different infectious diseases whole organisms are expensive to produce, require culture of the infectious agent, and have the potential to cause vaccine associated disease in hosts.[5] At present whole organism vaccine is moderated into two kinds of techniques such as (i) Live attenuated vaccines and (ii) Inactivated viral or bacterial vaccines.

(i) Live attenuated vaccines: An Attenuated Vaccine contain a group of microbes that had been weakened and decreased its virulent under laboratory conditions but it still alive therefore it will be less pathogenic but retain their capacity for transient growth within an inoculated host.[6,7,8] It t creates a strong and lasting immune but may not suitable for people whose immune system doesn't work, either due to drug treatment or underlying illness, due to the weakened viruses or bacteria could in some cases multiply too much and might cause disease in these people. For example: Rotavirus vaccine, MMR vaccine,

Nasal vaccine, BCG vaccine against TB(only special group), Chicken pox vaccine (only special group), Yellow fever vaccine etc. A new technique involves the removal of a gene from the virus or bacterial cell, this gene is responsible for a particular disease and virulence. Attenuated live vaccines is also carry a potential risk of contamination with adventitious viruses introduced during the attenuation process, from the cell lines used, and/or from the animal sera or other biologics often used in cell cultures. Very early Theiler's yellow fever attenuated virus was once "stabilized" with human plasma thought to contain hepatitis B virus, resulting in many cases of hepatitis.[9] Live attenuated vaccines can cause severe complications in immunocompromised patients due to HIV or from chemotherapy treatment.[10]

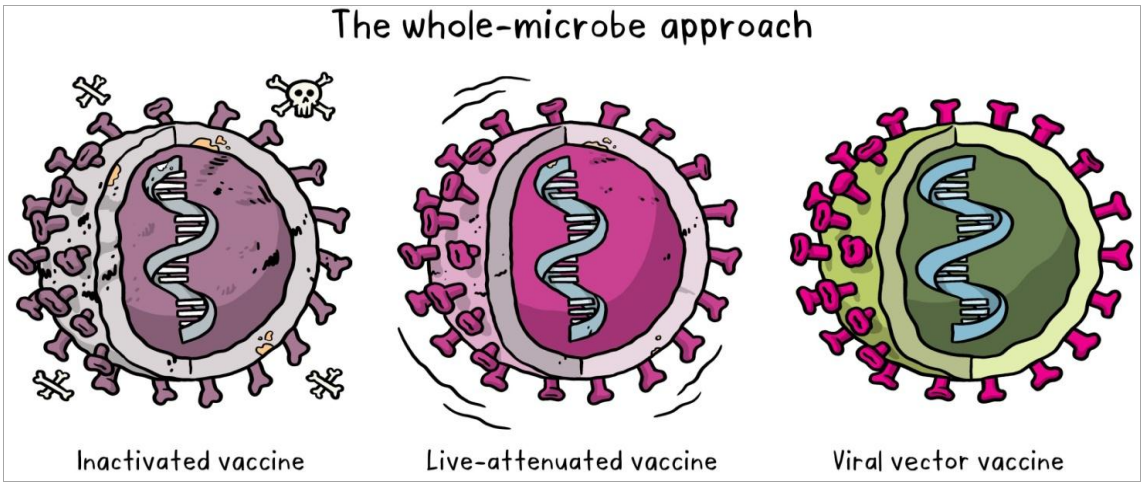


Figure 2: Whole Organism Vaccine.

(ii) Inactivated viral or bacterial vaccine: An Inactivated vaccines or killed vaccines is a vaccine consist from virus, bacterial or other pathogens that have been grown in a specialized culture and then completely killed by heat, radiation or chemicals so it is no longer capable of replication in the host and to be effective must contain much more antigen than live vaccines.[11] So they cannot response the diseases against which they protect, even in people with severely weakened immune systems. Excessive heat inactivation cause denaturation of protein therefore the epitope depend on structure of protein are altered or damaged. So the most successful methods to kill the pathogen depend on chemicals such as Formaldehyde, phenol, and binary ethylenimine (BEI).[12] Formaldehyde deactivates the virus or bacteria in traditionally. Excessive heat can destroy immunogenicity whereas insufficient heat can leave infectious virus capable of causing disease. During the period of 1956 to 1958, paralytic poliomyelitis used in USA to the distribution of inadequately inactivated polio vaccine and

demonstrated that the inactivation of this virus with formaldehyde. Study formaldehyde added during the BEIinactivation process strongly augments inactivation rates with a hundred to thousand-times (to 2.5-3.5 logs per hour).[3] The short inactivation times will limit proteolytic destruction of 146S antigen and increase antigen yields.[13] Likewise, the glutaraldehyde also fixes the antigen leading to alterations in the arrangement of the RNA and protein subunits of 146S.[14] Sodium thiosulfate 20% was added to the virus after the inactivation in a final concentration of 2% (up to 24 h) to neutralize the effect of BEI, also sodium bisulfite 20% was added after inactivation to neutralize the rest of formaldehyde.[15] For example: Rabies vaccine, Japanese encephalitis vaccine, Hepatitis A vaccine (special groups only), Inactivated polio vaccine or IPV (in the 6-in-1 vaccine, pre-school booster, teenage booster and pertussis vaccine in pregnancy), Some inactivated flu vaccines which are described as 'split virion'.

2. Subunit vaccine: A subunit vaccine contains a portion of bacteria or virus which is selected to produce a protective immune response. Antigens in subunit vaccines can be protein, polysaccharide, or a combination of polysaccharide and protein molecule (i.e., conjugate vaccine).[16] Advantages include being well-established technology and being suitable for immunocompromised individuals[17] Subunit vaccines do not always create such a strong or long-lasting immune response as live attenuated vaccines. Disadvantages include being relatively complex to manufacture compared to some vaccines, possibly requiring adjuvants and booster shots, and requiring time to examine which antigenic combinations may work best.[17] Adjuvants (the antigens alone in a subunit vaccine are insufficient to produce high immunogenicity, non-immunogenic materials known as adjuvants)[22] are often added to subunit vaccines. These are substances which help to strengthen and lengthen the immune response to the vaccine. It is four types. These are (a) protein subunit (contains isolated proteins from pathogens-virus or bacteria, exp: hepatitis B)[17,21] (b) polysaccharide subunit (contains chains of polysaccharides found in the pathogen's capsule such as cell walls of some bacteria, exp. pneumococcal polysaccharide vaccine, meningococcal vaccine, preventing diseases from Neisseria meningitidies group A, C, W-135, and Y)[17,21], (c) peptide subunit(employs a peptide instead of a full protein), and (iv) conjugate vaccine (contains polysaccharide chains bound to carrier proteins, such as diphtheria and tetanus toxoid, to boost the immune response, exp. Pneumococcal conjugate vaccine, haemophilus influenza type b conjugate vaccine, meningococcal conjugate vaccine)[17,21]

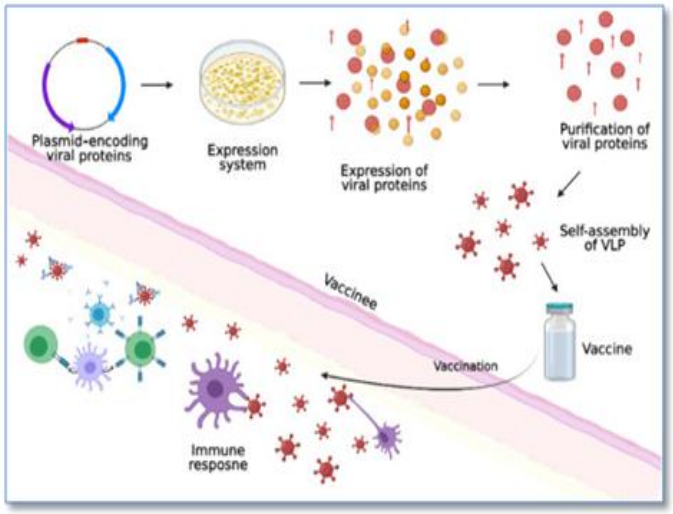


Figure-3- Subunit vaccine

The advantages of subunit vaccine are (i) Can not revert to virulence meaning they cannot cause the disease they aim to protect against.[18,19] (ii) Safe for immunocompromised patients.[20] (iii) Can withstand changes in conditions (e.g. temperature, light exposure, humidity).[18] The disadvantages are (i) Reduced immunogenicity compared to attenuated vaccines.[19,20] (ii) Require adjuvants to improve immunogenicity.[18,19] (iii)Often require multiple doses ("booster" doses) to provide long-term immunity.[18,19] (iv) Can be difficult to isolate the specific antigen(s) which will invoke the necessary immune response.[20]

3. Recombinant vaccine: Recombinant vaccines are products of genetic engineering, where a harmless agent such as yeast, is programmed to produce antigens of harmful pathogens.[23] A small piece of DNA is taken from the virus or bacterium against which we want to protect and inserted into the manufacturing cell.[24] Hepatitis B, human papillomavirus (HPV), and influenza (Flublok brand) vaccines are produced by insertion of a segment of the respective bacteria (E.coli expresses in a ‘work-horse’) or viral gene into DNA of yeast’s cell or virus. After modification (ease of bioprocessing and scale-up, strain engineering, lower cost, and shorter production times) of yeast cell or virus produces and grows pure Hepatitis B antigen, HPV capsid protein, or Influenza hemagglutinin. MenB vaccine contains protein from the surface of meningococcal bacteria and generated outer membrane. There is a disadvantages of using E.coli because sometimes codon usage bias and difficult to produce high molecular weight proteins. Then need to change and adjusting the growth, induction condition, changing media, buffers etc.

Recombinant DNA vaccines are immunogenic proteins or other biochemicals which initiate development of antibodies against some infectious diseases and are developed with the help of recombinant DNA. Examples: Vaccine against Hepatitis B, Influenza B and Meningitis.

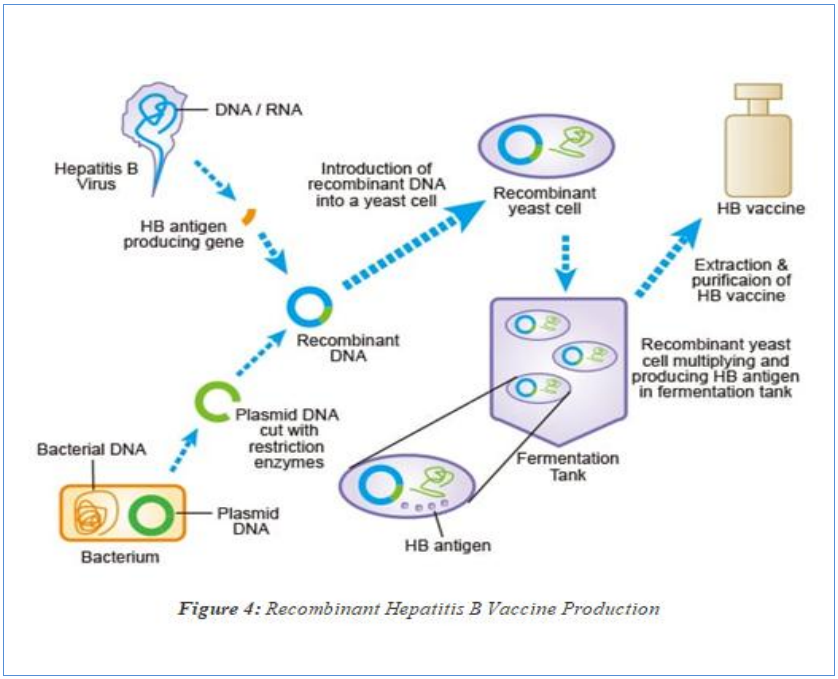


Figure 4: Recombinant Hepatitis B Vaccine Production

4. DNA vaccine: DNA vaccine is developed rapidly. It is directly coding DNA antigen(s) with plasmid (virus or bacteria) and relies on the in situ production of the target antigen. Including advantages of traditional approaches and stimulate both B- and T-cell responses to improved vaccine stability, the absence of any infectious agent and manufacture in large quantity.

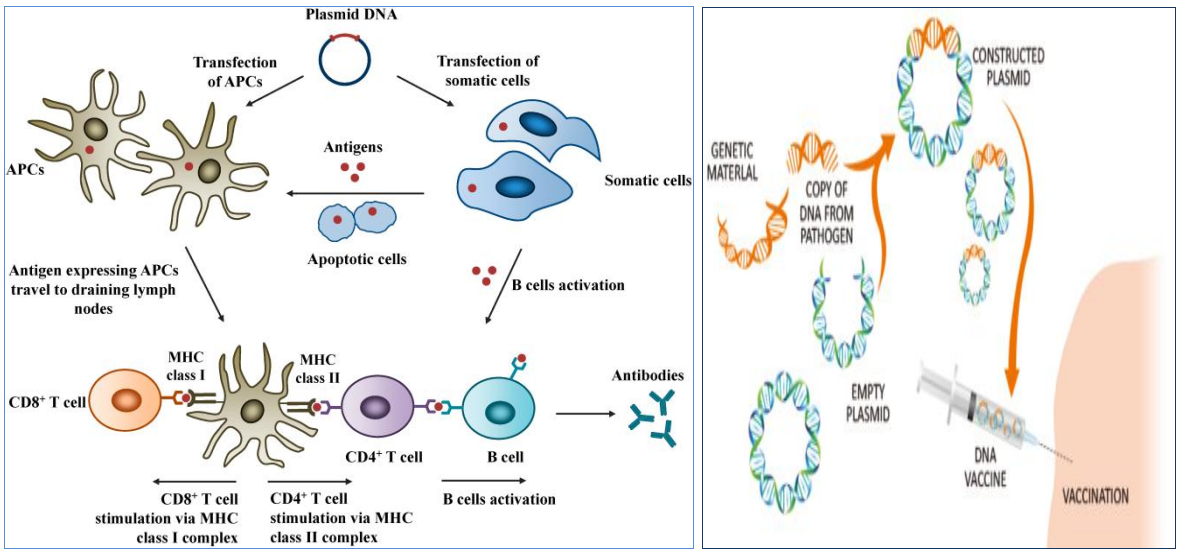


Figure 5: DNA vaccine.

As proof of the principle of DNA vaccination, immune responses in animals have been obtained using genes from a variety of infectious agents, including influenza virus, hepatitis B virus, human immunodeficiency virus, rabies virus, lymphocytic chorio-meningitis virus, malarial parasites and mycoplasms.[25] The value of DNA vaccine is increased due to nature of immunized agents. RNA is also used in vaccine preparation. To appropriate methods for the production and control of plasmid DNA vaccines and specific information will help to develop DNA vaccine preparation.

DNA vaccines have theoretical advantages over conventional vaccines, including the "ability to induce a wider range of types of immune response".[27] Several DNA vaccines have been tested for veterinary use.[26] In some cases, protection from disease in animals has been obtained, in others not[26] Research is ongoing over the approach for viral, bacterial and parasitic diseases in humans, as well as for cancers.[26] In August 2021, Indian authorities gave emergency approval to ZyCoV-D. Developed by Cadila Healthcare it is the first DNA vaccine approved for humans.[28]

During high expression vector DNA vaccine is evoked best immune response. The plasmid carries small genetic code which is promoting a vehicle to express optimizing protein expression in vector designing. The SV40 promoter was conventionally used until research showed that vectors driven by the Rous Sarcoma Virus (RSV) promoter had much higher expression rates.[29]

Advantages and disadvantages of DNA vaccine are shown in the table (1) format.

Table 1: Advantages and disadvantages of DNA vaccine.[29,30,31,32]

Method of delivery	Advantage	Disadvantage
Intramuscular or Intradermal injection	• No special delivery mechanism • Permanent or semi-permanent expression • pDNA spreads rapidly throughout the body	• Inefficient site for uptake due to morphology of muscle tissue • Relatively large amounts of DNA used • Th1 response may not be the response required
Gene gun	• DNA bombarded directly into cells • Small amounts DNA	• Th2 response may not be the response required • Requires inert particles as carrier
Jet injection	No particles required DNA can be delivered to cells mm to cm below skin surface	• Significant shearing of DNA after high-pressure expulsion • 10-fold lower expression, and lower immune response

		• Requires large amounts of DNA (up to 300 μg)
Liposome-mediated delivery	• High levels of immune response can be generated • Can increase transfection of intravenously delivered pDNA • Intravenously delivered liposome-DNA complexes can potentially transfect all tissues • Intranasally delivered liposome-DNA complexes can result in expression in distal mucosa as well as nasal muscosa and the generation of IgA antibodies.	• Toxicity • Ineffectiveness in serum • Risk of disease or immune reactions

5. Edible vaccine: Conventional vaccines were 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.[33] Edible vaccines are nothing but transgenic plant and animal-based production of or those contain agents that trigger an animal's immune response. In simple, plant or animal-made pharmaceuticals are edible vaccines. In 1989, the effort to produce a plant-based vaccine was formulated by Hiatt and co-workers.[34] Causing by microbes, to activate the systematic and mucosal immunity response to the foreign particles is main focused for edible vaccine. Compared to other traditional vaccine, it is accepted easily by the society people for cost effective, efficient and safe using, needless, convenient, better prevention option from diseases. Most of the plant-based vaccines were against viruses and bacteria that infect human, animals as well as poultry which cause fatal illness.[33] So far, there is no edible vaccine that was approved by USFDA because, this type of vaccines were characterized under genetically modified crops.[35,36]

Secretory immunoglobin A (SIgA) and systemic immunoglobin A (IgA) is the key to improve vaccine efficacy.[37,38] Microfold (M) cells are found in gastrointestinal tract which capture a wide range of macromolecules from lumens in the small intestines to antigen submucosal cells (APCs) on Peyer's patches effectively.[39] The transgene can be expressed in the plant by direct or indirect gene delivery. In biolistic method, DNA or RNA is directly introduce from plant cell is known as gene gun or micro-projectile bombardment method. This vector independent method is applicable when gene transfer through agrobacterium species-mediated not transformation.[40,41,42] It is costly method because gold or tungsten used as a micro-carrier and high pressure in Helium gas is used in coated DNA placed in gene gun.

Example of vaccines produced by biolistic methods are Cholera, Lyme disease, Anthrax, Tetanus, Plague, Rota virus and Canine parvovirus.[43]

In vector-mediated gene delivery or indirect gene delivery method, the desired plant cells were infected with plant bacteria or plant virus to produce the protein of interest.[44] *Agrobacterium tumefacians* and *Agrobacterium rhizogenes* are commonly used indirect gene delivery method. These gram negative bacteria attack the plants and transfer their genes to plant nucleus. *Agrobacterium tumefaciens* carries tumour-inducing Ti plasmid and *Agrobacterium rhizogenes* carries root-inducing plasmid Ri plasmid.[45] Examples for vaccines produced by this method were diarrhoea, TB, dengue, avian flu virus, ebola.[46]

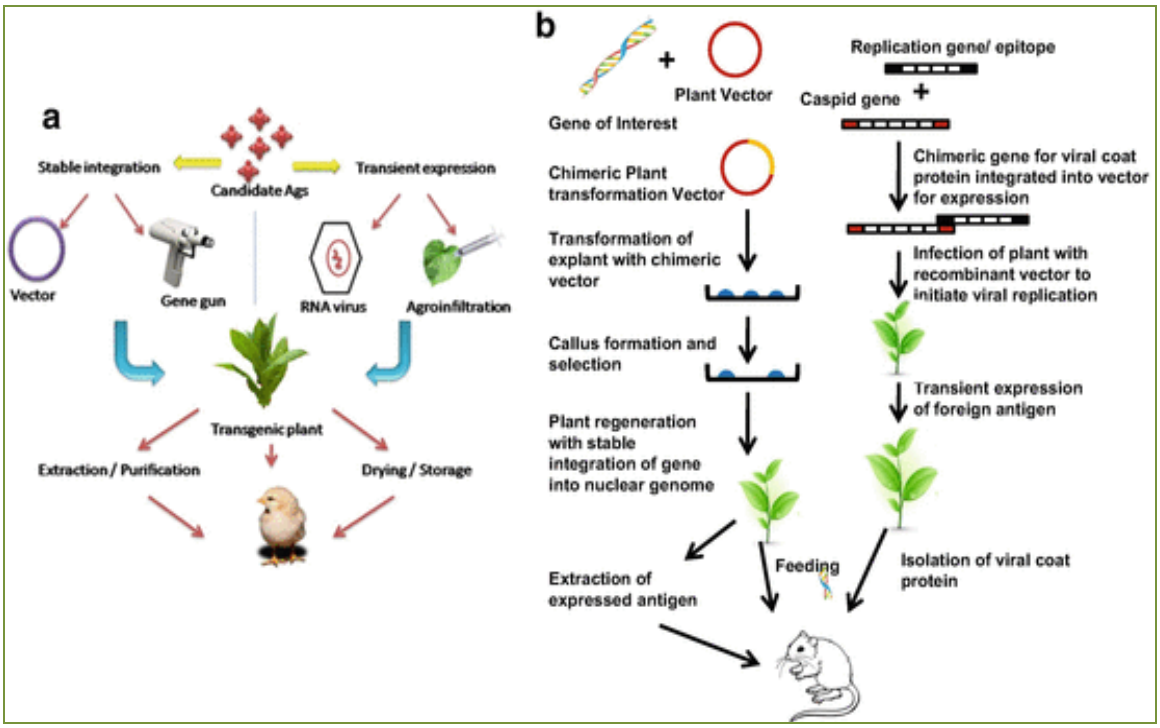
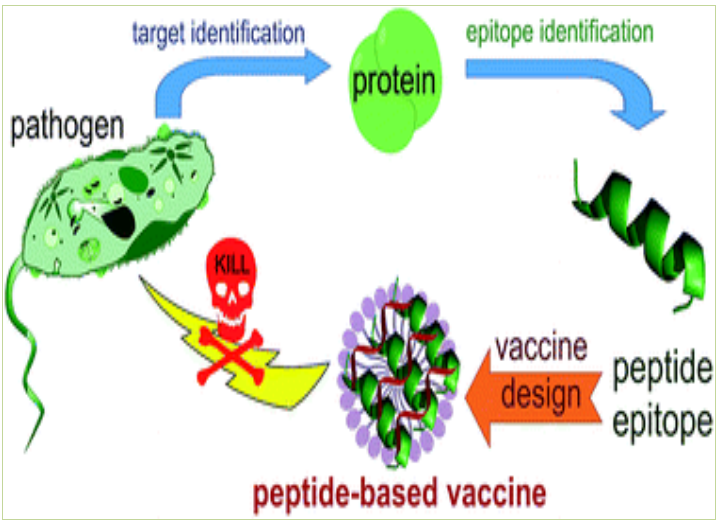


Figure 6: Edible vaccine.

Plant Virus such as Tobacco Mosaic Virus (TMV), Powder Virus (PVX), Alfalfa Mosaic Virus (AIMV), Cytomegalovirus (CMV) expression system mainly includes engineered viruses.[47] As per this, 200 acres of plot is required for the production of edible vaccine for all infants in the world.[48] Various edible products like plants, algae, insect cells, whole yeast and lactic acid bacteria are used as alternative agents for parenteral vaccines.[48]

Table 2: Development status of edible vaccines in clinical trials.[49,50,51,52,53,54,55,56,57,58]

Pathogen	Antigen	Host	Use	Clinical trial status
Enterotoxigenic E. coli	LT- B	Potato	Diarrhoea	Early phase 1
Enterotoxigenic E. coli	LT- B	Maize	Diarrhoea	Early phase 1
Norwalk Virus	CP	Potato	Diarrhoea	Early phase 1
Rabies Virus	GP/ NP	Spinach	Rabies	Early phase 1
HBV	HBsAg	Lettuce	Hepatitis B	Early phase 1
HBV	HBsAg	Potato	Hepatitis B	Phase 1
Vibrio cholerae	CTB	Rice	Cholera	Phase 1
HBV	HBV	*Saccharomyces cerevisiae*	Chronic HBV	Phase 2
HCV	HCV	*Saccharomyces Cerevisiae*	Chronic HCV	Phase 2



6. Synthetic peptide vaccine: Synthetic peptide vaccines represent fragments of protein antigen sequences, which are synthesized from amino acids and assembled into a single molecule or a supramolecular complex or just mechanically mixed; they are recognized by the immune system and induce the immune response.[59] This immune response may involve either cytotoxic T-cells or B-cells (i.e. directed to elaboration of specific antibodies), or combine both possible pathways.[60] Fragments of protein molecules exhibiting B and/or T epitope activity, which determine the direction and specificity of the immune response, are the main components of peptide vaccines.[61] In addition, such vaccines may also contain some individual compounds or supramolecular complexes (e.g. micelles, liposomes, polymer particles, etc.), which can nonspecifically or specifically activate certain stages of the immune response to peptides and therefore potentiate it.[62] An increase in chemical stability of peptides is achieved by their attachment to carriers, which simultaneously act as activators of the immune response.[63] This relative inexpensive,

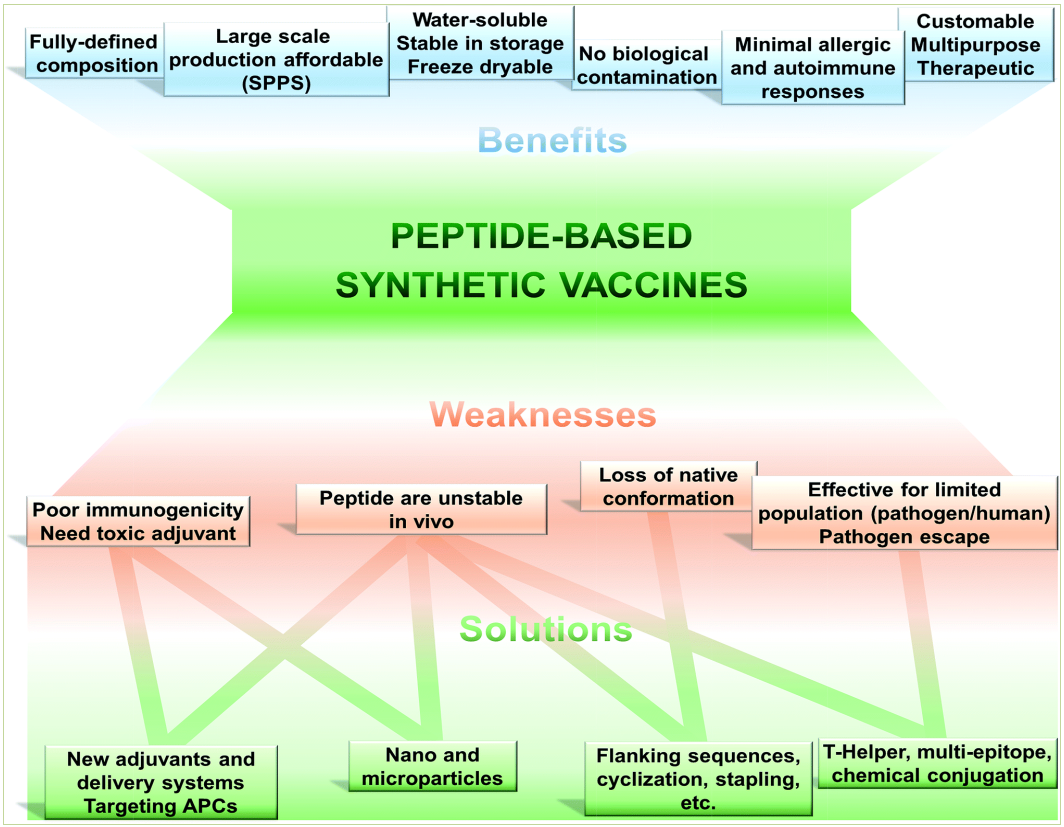


Figure 7: Synthetic Peptide Vaccine.

high standardization and safe production technology vaccine induces the immune response to those structural elements of a protein antigen which exhibt weak immunogenicity within the whole antigen molecule.

Development of peptide vaccines against malaria started in 1970th and in spite of the first unsuccessful attempt.[64] EpiVacCorona, a peptide- base vaccine against COVID-19. IC41is a peptide vaccine candidate against the Hepatitis C virus. It conists of five synthetic peptides along with the synthetic adjuvantcalled ply-I-arginine. Multimeric-001 is the most efficient peptide vaccine candidate against influenza.

7. Multivalent subunit vaccine: The term ‘multivalent / polyvalent vaccine’ is ambiguously used to describe either a vaccine candidate with the ability to project against several diseases or a vaccine candidate that can protect against several stains of a single pathogen.[65] The multivalent subunit vaccine was formulated to contain a combination of F. tularensis protective antigens: OmpA-like protein (OmpA), chaperone protein DnaK and lipoprotein Tul4 from the highly virulent F. tularensisSchuS4 strain. Two different vaccine formulations and immunization schedules were used.[66]

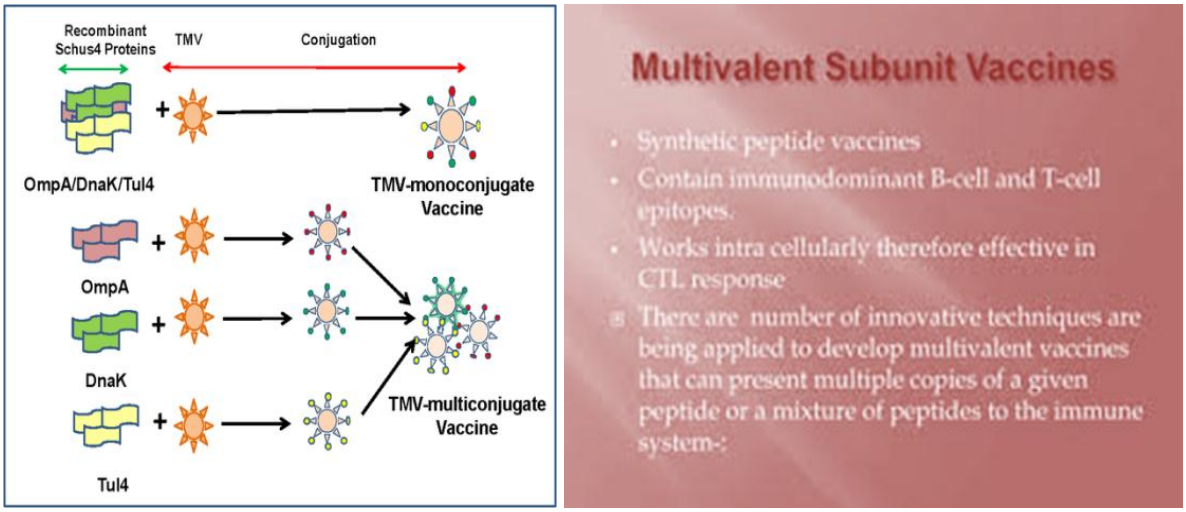


Figure 8: Multiple Subunit Vaccine.

CONCLUSION

This review article gives a conceptual idea of different kinds of vaccine and their working principle. The agent stimulates body's immune system to against foreign particle and gives healthy and wealthy life.

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