RECOMBINANT VACCINES

Dr.Shurooq Rayyis kadhim

Vaccine : are weakened, killed, or fragmented microorganisms or toxins, antibodies or lymphocytes that is administered primarily to prevent disease. A vaccine can confer active immunity against a specific harmful agent by stimulating the immune system to attack the agent. Once stimulated by a vaccine, the antibody-producing cells, called B cells , remain sensitized and ready to respond to the agent should it ever gain entry to the body. A vaccine may also confer passive immunity by providing antibodies or lymphocytes already made by an animal or human donor.
The original scientific strategy behind vaccinology has historically been to "isolate, inactivate, and inject," first invoked by Louis Pasteur.
Types of vaccines are currently available:

- live attenuated vaccines;
- Subunit vaccines;
- Toxoid vaccines;
- Conjugate vaccines

Live attenuated vaccines: Pathogens are attenuated to decrease their virulence using methods such as genetic manipulation (to eliminate virulence factors) or long-term culturing in an unnatural host or environment (to promote mutations and decrease virulence). The most common methods involve passing the disease-causing virus through a series of cell cultures or animal embryos (typically chick embryos). Therefore, when attenuated viruses are given to a human, they are not able to replicate enough to cause illness like they would naturally but will still provoke an immune response that can protect against future infection. They often require only a single immunization, eliminating the need for repeated boosters. However, there are potential safety risks to immune compromised recipients because the pathogen can revert back to a virulent state. The bacillus Calmette Guérin (BCG) vaccine against tuberculosis (TB), the measles, mumps and rubella vaccine (MMR), and the polio vaccine are examples of attenuated live vaccines.

Inactivated or Killed vaccines: contain whole pathogens that have been killed or inactivated with heat, chemicals, or radiation. For inactivated vaccines to be effective, the inactivation process must not affect the structure of antigens on the pathogen. Because the pathogen is killed or inactive, inactivated vaccines do not produce an active infection, and the resulting immune response is weaker and less comprehensive than that provoked by a live attenuated vaccine. In addition, inactivated vaccines usually require higher doses and multiple boosters. The advantages of long-term storage stability and ease of transport. Also, there is no risk of causing severe active infections. Chemical inactivation with formaldehyde or formalin has been successful. The Salk polio vaccine is produced by formaldehyde inactivation.

Subunit vaccines: Only expose the patient to the antigens of a pathogen not whole cells or viruses. Subunit vaccines can be produced either by chemically degrading a pathogen and isolating its antigens or by producing the antigens through genetic engineering. Subunit vaccines have substantial advantages because they made up of highly purified and well-defined components, and lack the ability to replicate. Hepatitis B vaccine contains the surface antigen (HBsAg) alone, which is sufficiently immunogenic. The killed virus, bacterial debris will not cause disease but will still cause immune reactions and prevent future infections. One limitation of these vaccines is that you may need booster shots to get ongoing protection against diseases.

Toxoid vaccines: They contain inactivated **bacterial toxins**, called toxoids. Toxoid vaccines are used to prevent diseases in which bacterial toxins play an important role in pathogenesis. These vaccines activate humoral immunity that neutralizes the toxins. For example, diphtheria and tetanus vaccines can be prepared by purifying bacterial toxins and then inactivating toxin with formaldehyde to form a toxoid. Inoculating with a toxoid induces an anti-toxoid antibody that is also capable of binding toxins and neutralizing their effects. Toxoid vaccines like some other types of vaccines, may need booster shots to get ongoing protection against diseases. Toxoid vaccines are used to protect against diphtheria and tetanus.

Conjugate vaccine is a type of subunit vaccine that consists of a protein conjugated to a capsule polysaccharide. The conjugated protein-polysaccharide antigen stimulates production of antibodies against both the protein and the capsule polysaccharide.

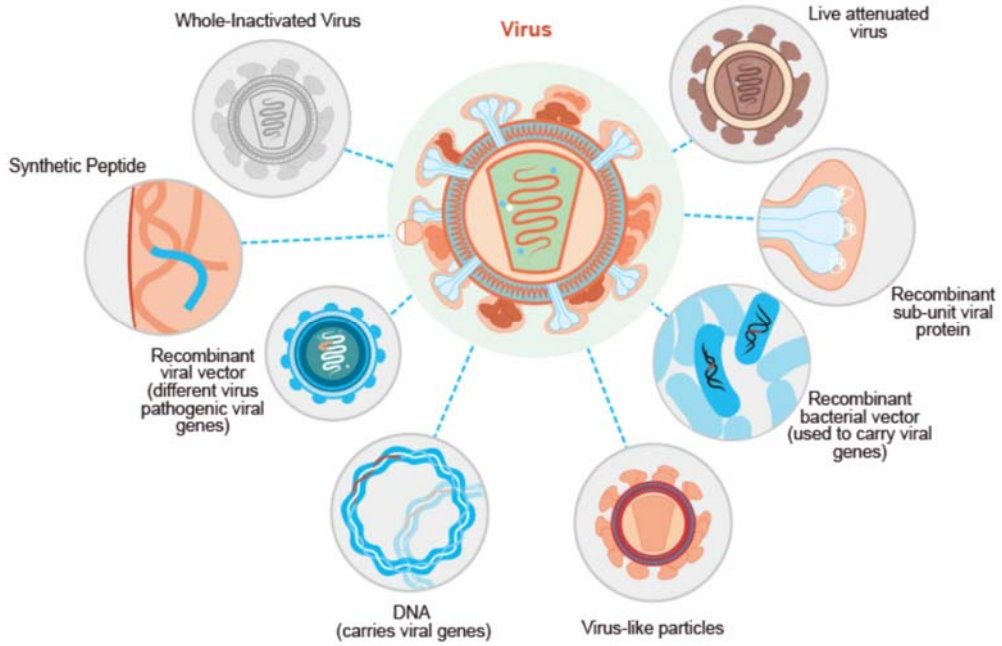
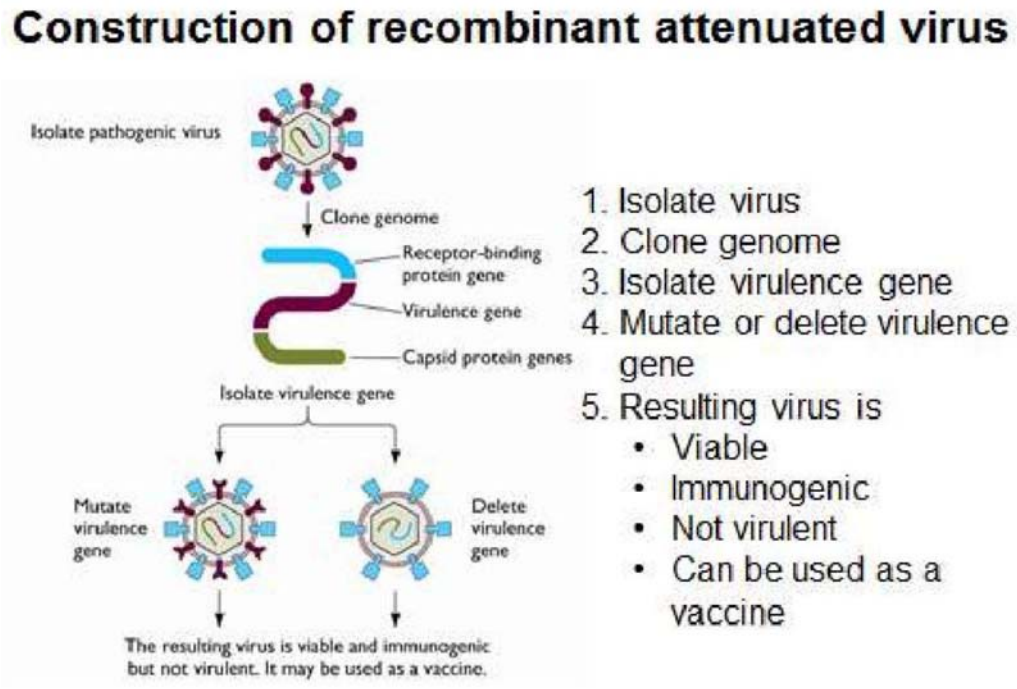


Figure :Various approaches for vaccine Development

Recombinant vaccine:

Vaccine produced by using recombinant DNA technology (i.e. mixing of two DNA from different sources) is called recombinant vaccine. This involves inserting the DNA encoding antigen (such as bacterial surface protein) that stimulates an immune response into bacterial cells, expressing the antigen in these cells and then purifying it from them. Recombinant vaccines are prepared with the help of expression system, such as bacteria and yeast cells in which the DNA encoding the genetic determinant can be inserted and expressed. However many factors must be checked before choosing the system for antigen expression. The level of expression we get by using each expression vectors and promoter , the selection marker of choice, the presence or absence of post-translational modifications by recombinant vector, besides other are important characteristics that hinders in quality production of recombinant antigens as vaccines.

Bacterial expression system is most common in use because they are easy to handle and their ability for high level expression.



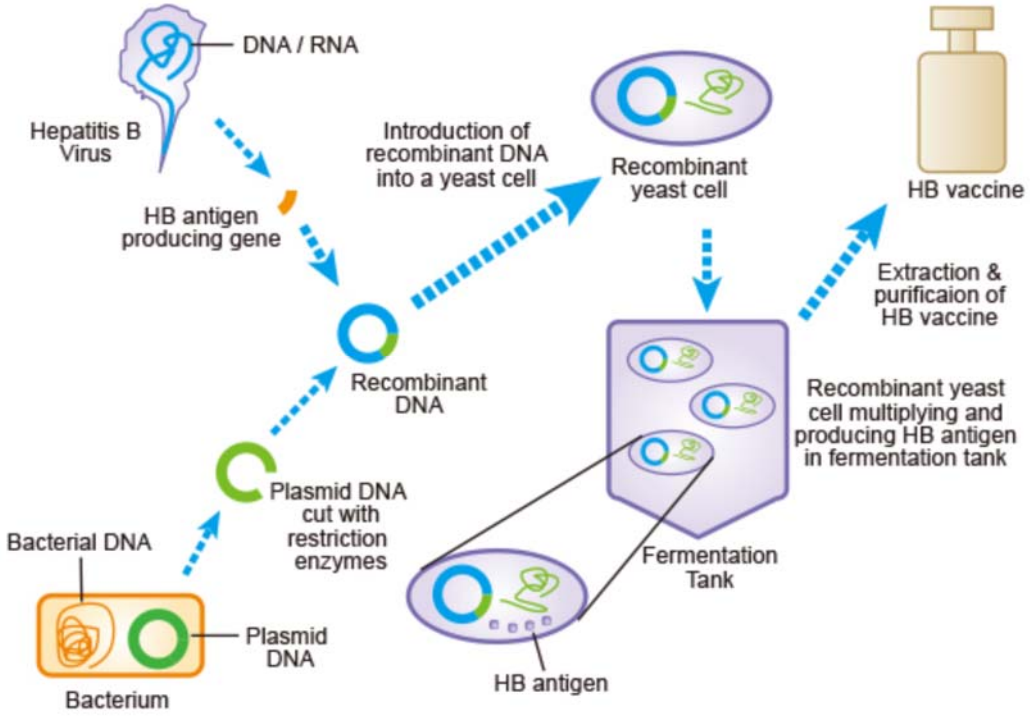
Types of Recombinant vaccines:

The recombinant vaccines can be classified into three groups:

1. Subunit recombinant vaccines: These are the components of the pathogenic organisms. Subunit vaccines are proteins, peptides and DNA.
2. Attenuated recombinant vaccines: In this method, genetically modified organisms (bacteria or viruses) that are made non-pathogenic are used as vaccines.
3. Vector recombinant vaccines: These are the genetically modified viral vectors that can be used as vaccines to protect from several pathogens.

Example :Recombinant hepatitis B vaccine:

Hepatitis B is a common viral disease in man. It basically affects liver causing chronic hepatitis and liver cancer. It contains a core having a viral genome (DNA) surrounded by phospholipids envelop carrying surface antigens. Scientists has identified the gene encoding for hepatitis B surface antigen (HBsAg) as a subunit and produced by cloning HbsAg gene in yeast cells. *Saccharomyces cerevisiae,* a harmless baking yeast, is used in this purpose. The HBsAg assembles into virus like particles (VLPs), which are highly immunogenic, making the HBV vaccine, a very good vaccine. After expression in yeast system, it is purified. Hepatitis B vaccine is safe to use, very accurate and produces no allergic reactions.



Hepatitis B vaccine production

DNA Vaccines

The sequence of a pathogenic protein antigen is cloned in plasmid. The constructed plasmids are then subsequently grown in bacteria like *E. coli* and highly purified via chromatographic methods.

After purification the circular double-stranded DNA plasmids are ready for vaccination. The de novo production of the encoded antigens in the host results in the elicitation of both the antibody and the cellular response by activating cytotoxic T lymphocytes (CTLs). Vaccine proteins made by the host are natural proteins and contain important posttranslational modifications such as the correct glycosylation. But like subunit vaccines, DNA vaccines must be adjuvanted. Naked DNA does not work.

The unique advantage of DNA vaccines is their ability to mimic the effects of live attenuated vaccines without the risk associated with the administration of infectious attenuated material. DNA vaccines are able to stimulate a complete, humoral and cellular immune response and also is very stable. Therefore, the storage, transportation, and distribution of DNA vaccines are more practical.

Mostly all plasmid DNA constructs used for vaccination share five main characteristics:

- Strong promoter/enhancer sequence for driving the incorporated foreign gene
- Convenient cloning site for insertion of foreign genes
- Origin of replication for initiation of plasmid replication
- Polyadenylation /termination sequence for production of mature mRNA
- Resistance/antibiotic marker for selection

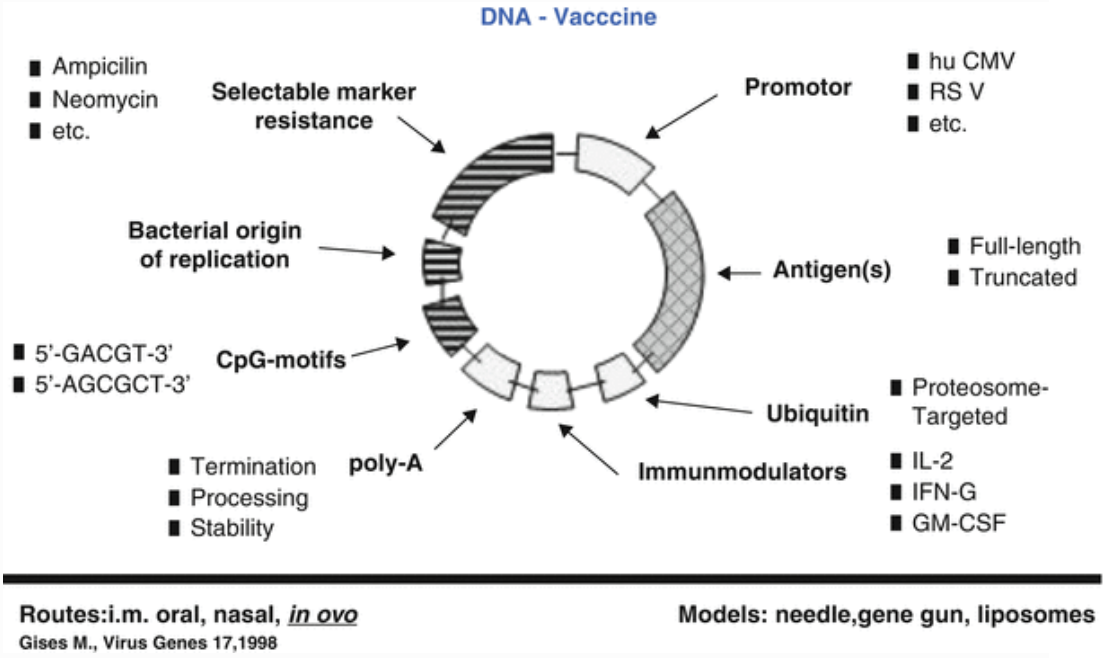


Fig. Model of an expression plasmid used for DNA vaccination. Individual elements comprising functional expression cassettes. The encoded antigen, as full-length or truncated cDNA, is under control of strong promoter/enhancer and polyadenylation sequences. Co-expression of cytokines will specifically enhance the immune response. Unspecific activation of the immune system can be initiated by CpGs. Vaccines focusing on a strong cellular response can be enhanced by co-expression of ubiquitin targeting the proteasome pathway. Various application routes and modes of administrations are possible.

Uptake of Plasmid DNA. Some biological barriers have to be overcome by DNA vaccines on the way to the cell nucleus where the plasmid DNA is translated into cellular mRNA. After delivery of plasmid DNA to the target tissue, e.g., skeletal muscle or skin, lots of tissue nucleases attack and degrade a large amount of DNA. Also the extracellular matrix with collagen and hyaluronic acid influences the passage from the application site to the cell membrane.

Only a small portion (1 % estimated) of the still intact plasmid DNA will cross the cell membrane by phagocytosis or pinocytosis. Inside the cell the route toward the nucleus is also spiked with exo- and endonucleases so that probably only 0.1 % (estimated) is successfully and actively transported through the nucleus pore membrane (NPC). Small particles (<~40 kDa) are able to pass through the nuclear pore complex (NPC) by passive diffusion; larger particles need the support of carrier proteins for efficient passage through the complex.

Because of this enormous loss of plasmid DNA , various tools were developed to protect the plasmid DNA and thus increase the efficacy such as encapsulation into liposomes or binding of DNA to dendrimers.

DNA vaccines—plasmids can be administered by one of the following delivery method.
i. Nasal spray
ii. Intramuscular injection
iii. Intravenous injection
iv. Intradermal injection
v. Gene gun or biolistic delivery (requires nanogram level of plasmids)

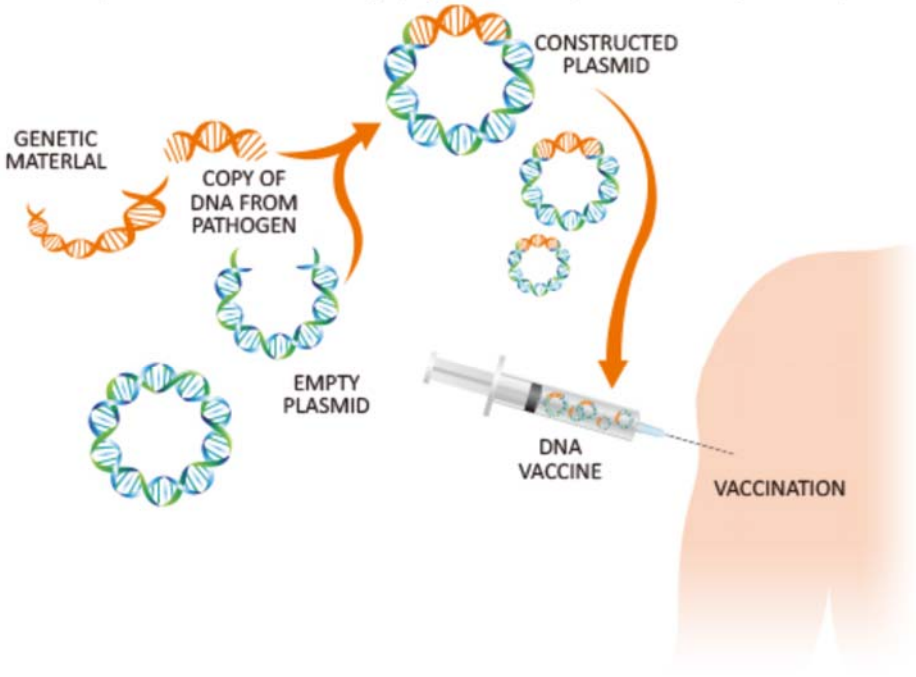


Fig. Principal of a DNA Vaccine

Present status of DNA Vaccines:
After 1990, many groups of workers World-wide have been trying to develop DNA vaccines against several diseases. Genetic immunization has been done against a number of pathogenic organisms. These include influenza A virus, rabies virus, hepatitis B virus, herpes virus, HIV and plasmodium species (malarial parasite).It must be noted that DNA vaccines have not been tried in humans for unknown risks of these foreign DNAs.
Plants as Edible Subunit Vaccines: Plants serve as a cheap and safe production systems for subunit vaccines. Grains and potato were used as a good plants for production of vaccines .

Recombinant vaccines for COVID-19
SARS-CoV-2, the causative agent of COVID-19, has imposed a major public health threat, which needs effective therapeutics and vaccination strategies. Several potential candidate vaccines being rapidly developed are in clinical evaluation. Considering the crucial role of SARS-CoV-2 spike (S) glycoprotein in virus attachment, entry, and induction of neutralizing antibodies, S protein is being widely used as a target for vaccine development. Based on advances in techniques for vaccine design, inactivated, live-vectored, nucleic acid, and recombinant COVID-19 vaccines are being developed and tested for their efficacy.

Recombinant COVID-19 vaccines

Nucleic acid-based coronavirus vaccine

The greatest advantage of DNA- and RNA-based vaccines is their potential for rapid development and reduced side effects. DNA vaccines have shown strong potential to trigger immune responses against CoVs in animal models. However, clinical data on the efficacy of DNA vaccines in humans remain limited. In a previous study on mice, a DNA vaccine encoding the S protein of SARS-CoV was found to induce T cells, a neutralizing antibody response, and protective immunity. A group of prototype DNA vaccines expressing various SARS-CoV-2 S proteins has been developed and tested in 35 rhesus macaques. The vaccinated macaques demonstrated specific humoral and cellular immune responses. Further upon being challenged with SARS CoV, the animals showed a remarkable reduction of viral replication in the upper and lower respiratory tract. The data displayed the significant role of DNA vaccine against SARS-CoV infection.

Vectored vaccines against coronavirus

Viral vectors represent one of the prospective strategies for the CoV vaccine platform and their utility depends on their ability to infect cells. The main advantage of this platform is its efficient and gene-specific delivery as well as its initiation of healthy immune responses. A recombinant adenovirus type-5 (Ad5) vectored COVID-19 vaccine expressing S protein of SARSCoV-2, was assessed for phase 1 trial at Wuhan, China. The increase in specific neutralizing antibodies and T cell response were observed on day 14 after vaccination. The results remained promising and expect further evaluation.