

Review on Recombinant Vaccines

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ABSTRACT:

Vaccines were initially developed on experimental basis, depending mostly on attenuation or inactivation of pathogens. Advances in immunology, molecular biology, biochemistry, genomics and proteomics have added new outlook to the vaccinology field. The targeting of immune responses focused against few protective antigens is now possible by the use of the recombinant proteins. There are many expression systems with multiple advantages, allowing the production of large quantities of proteins depending on the required features. Live recombinant bacteria and viral vectors effectively stimulate the immune system as in natural long term cellular immune responses. In general, all these methods have shown advantages and disadvantages and their use will depend on the knowledge of the mechanisms of infection

of the target pathogen and of the immune responses needed for protection. In this review, I discuss some of major breakthroughs that have been achieved using recombinant vaccines technologies.

Introduction

A vaccine generally contains an agent that resembles a disease causing microorganism and is usually made of the microbe, its toxins or one of its surface proteins. Scientists adopt many strategies to produce vaccines against a pathogenic microbe. These choices are directed by nature of pathogen and infection and as well as considering natural body immune response. Most current vaccines have their success due to their ability to target pathogens that have low antigenic variability. This is the case for polio, tetanus, diphtheria, measles and hepatitis B, among others. As a result, vaccines that have capacity to neutralize antibodies against these pathogens were successful.

On the other hand, important cell mediated immunity against intracellular pathogens (which in most cases leads to chronic infection) is much more difficult using current vaccines strategies. In this regard, the recombinant vaccines are in use to produce immunity against pathogens. We can broadly classify the recombinant vaccines into Subunit recombinant vaccines, Attenuated recombinant vaccines and vector recombinant vaccines.

3. What is a vaccine?

In the most simplified terms, a vaccine is a biological preparation that provides active acquired immunity against a certain disease. Usually a vaccine consists of biological agent that represents the disease causing microorganism. It is generally made from a weakened or a killed form of the microorganism, its toxins or one of its surface protein antigens. The first successfully case of vaccination was performed by Edward Jenner in 1796. He noticed that individuals who had cowpox, did not touch smallpox even when coming in direct contact with disease.

4. What are the approaches for vaccines development?

Scientists take many approaches to make vaccines against a pathogenic microorganism. These choices are being selected by the nature of pathogen and infection as well as practical considerations about the use of the vaccines. Some of these options include live attenuated vaccines, inactivated vaccines, DNA vaccines and Recombinant subunit vaccines.

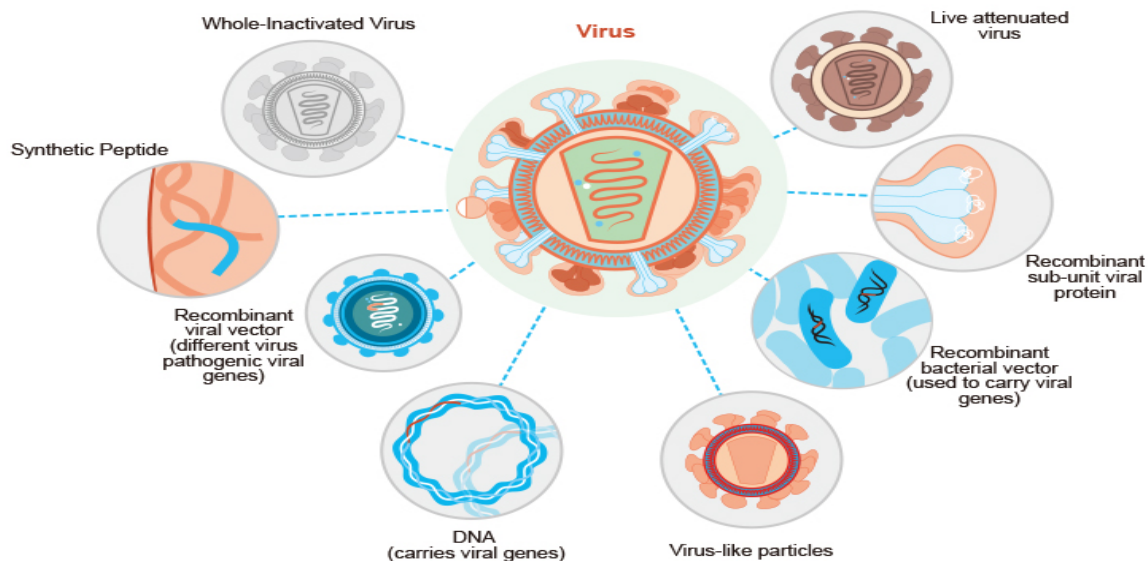


Figure 1: Various approaches for vaccine Development

5. What is a 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 or mammalian 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, insect, yeast, and mammalian 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(Initiator) ,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 are most common in use because they are easy to handle and their ability for high level expression. On the other hand, for antigens in which post-translational modifications (e.g. glycosylation) are necessary, the use of mammalian or insect cells should be preferred.

6.Types of Recombinant vaccines:

The recombinant vaccines can be broadly classified into three groups:

6.1. Subunit recombinant vaccines:

These are the components of the pathogenic organisms. Subunit vaccines are proteins, peptides and DNA.

6.2. Attenuated recombinant vaccines:

In this method, genetically modified organisms (bacteria or viruses) that are made non-pathogenic are used as vaccines.

6.3. Vector recombinant vaccines:

These are the genetically modified viral vectors that can be used as vaccines to protect from several pathogens. Some of the advancements made in the preparation of recombinant vaccines against certain diseases are shortly described.

Type # 6.1.Subunit vaccines:

The subunit vaccines contain only a fraction of the pathogenic organism. Usually these are synthetic peptides that show protein component that induces immune response. The benefits of these vaccines include their purity in manufacturing, stability and safe use. Following are the some of the examples of diseases in which scientists achieved to prepare vaccines by using subunits of pathogens.

a)Hepatitis B:

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).Recombinant hepatitis B vaccine as a subunit vaccine is produced by cloning(growing) HbsAg gene in yeast cells.*Saccharomyces cerevisiae*, a harmless baking and brewing 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-the first synthetic vaccine:

In 1987, the recombinant vaccine for hepatitis B (i.e.HBsAg) was the first synthetic vaccine for public use. Hepatitis B vaccine is safe to use, very accurate and produces no allergic reactions.

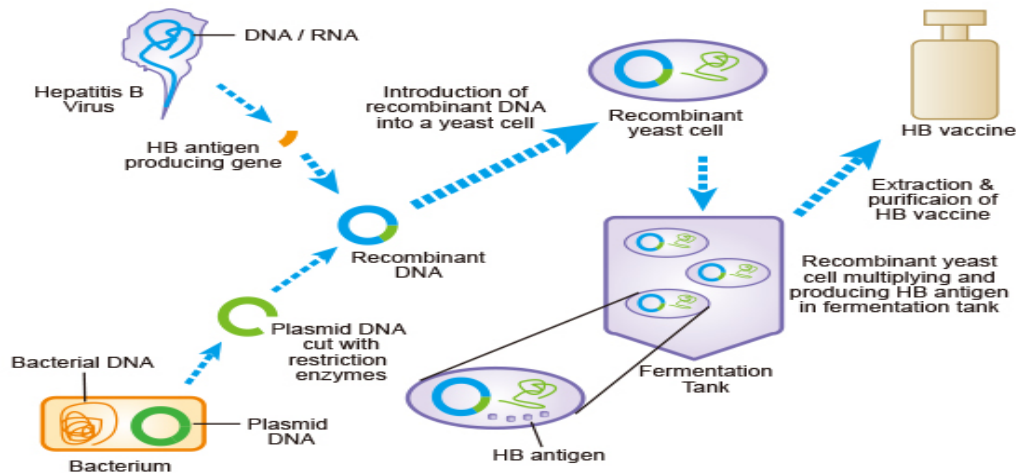


Figure 2: Hepatitis B vaccine production

Hepatitis B vaccine tomato?

Biotechnologists have been successful in adding hepatitis B gene into the cells of tomato plant. These genetically engineered plants produce hepatitis B antigens. The day is no longer to get immunized against hepatitis B by having a tomato in lunch!

b)Foot and Mouth Disease:

Foot and mouth disease (FMD) is a highly contagious disorder of cattle and pigs. A formalin killed foot and mouth virus (FMDV) was recently used to vaccinate against this disease. Four viral proteins(Vp1,Vp2,Vp3 and Vp4) surrounds the genome of FMDV.From these,Vp1 is immunogenic. The sequence of nucleotides for Vp1 was discovered from the genome of FMDV.A double stranded complementary DNA (cDNA) was made from single-stranded viral RNA(genome).Restriction enzymes used to cut this cDNA and the fragments were cloned by using plasmid pBR322 in E.coli.In this way ,the recombinant vaccine for FMDV in the shape of viral protein 1 was used to vaccinate animals.

C)Human papillomaviruses viruses:

A recently developed example of recombinant vaccine is the vaccine against human papillomaviruses (HPVs) .HPV is a common sexually transmitted disease linked many kinds of mucocutaneous disorders in humans including cervical, vulva and vaginal cancer and genital warts.Currently,two vaccines are in use against HPV.These both vaccines have been developed based virus like particles (VLPs) obtained from HPV-6,-11,-16 and/or -18 subtypes.These vaccines use the L1 recombinant proteins of every subtype,produced either in yeast or an insect

cell system. The L1 is the major capsid protein that expresses in vitro causes the formation of VLPs.

DNA Vaccines (Genetic Immunization):

These vaccines usually consist of synthetic DNA containing the gene that encodes the disease agent protein. Normally, the plasmid DNA used as vaccine is cultivated in bacteria such as E. coli and they are separated and purified for injection. The concept behind a DNA vaccine is that the antigen can be expressed directly by host cells in a way that stimulates viral infection and starts an immune response from the host.

DNA vaccines—plasmids can be administered to the animals by one of the following delivery methods.

- i. Nasal spray
- ii. Intramuscular injection
- iii. Intravenous injection
- iv. Intradermal injection
- v. Gene gun or biolistic delivery (requires nanogram level of plasmids)

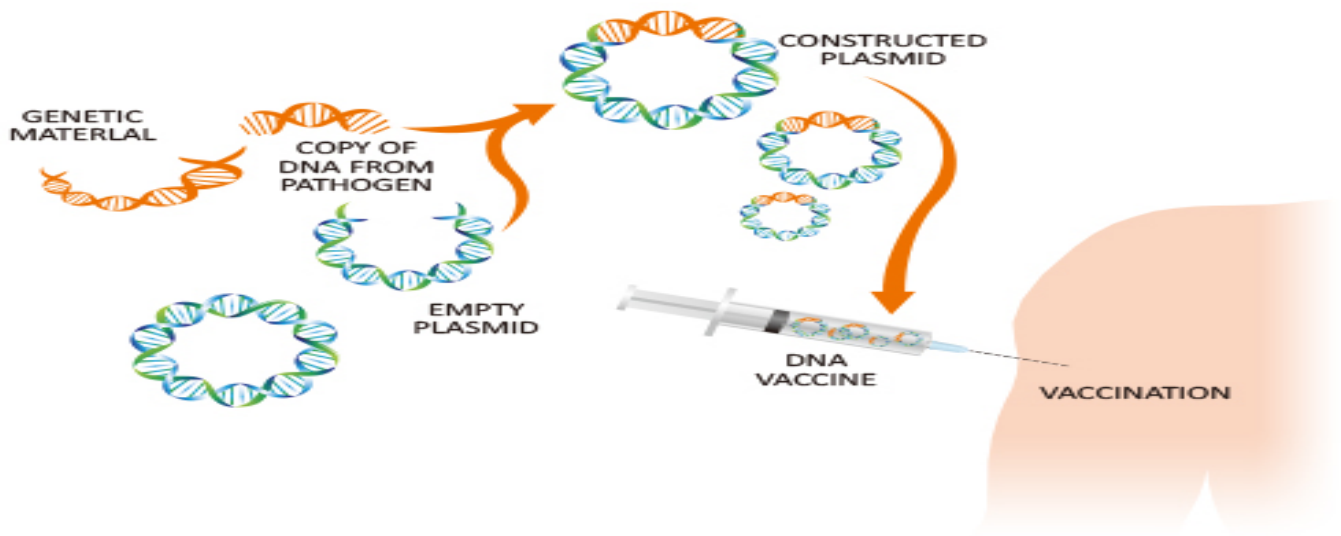


Figure 2: Principal of a DNA Vaccine

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 system for subunit vaccines. The edible vaccines can be easily ingested by eating plants. This removes the processing and purification methods that are otherwise needed. Transgenic plants (tomato, potato) have been developed for expressing antigens derived from animal viruses (rabies virus, herpes virus). A selected list of recombinant vaccines against animal viruses produced in plants is given below in table.

Table A selected list of plant edible subunit vaccines

edible subunit vaccines	
<i>Antigen</i>	<i>Host plant</i>
Rabies glycoprotein	Tomato
Foot and mouth virus (VPI)	<i>Arabidopsis</i>
Herpes virus B surface antigen	Tobacco
Cholera toxin B subunit	Potato
Human cytomegalovirus glycoprotein B	Tobacco

Type # 2 Attenuated Recombinant Vaccines

In the start of vaccine research ,attenuated strains of some pathogenic organisms were prepared by long growth—weeks,months or even years.Although the reasons are not known ,the infectious organisms would lose its ability to cause disease but retains its capacity to act as an immunizing agent.This type of method is almost outdated now.

It is now possible to genetically engineer the organisms (bacteria or viruses) and use them as live vaccines,and such vaccines are also named as attenuated recombinant vaccines.The genetic manipulations for the production of these vaccines are widely of two types:

- i. Deletion or modification of virulence genes (disease causing) of pathogenic organisms.
- ii. Genetic modification of non-pathogenic organisms to carry and express antigen determinants from pathogenic organisms.

Some of the important attenuated vaccines prepared by genetic modifications are briefly described.

a)Cholera:

Cholera is an intestinal disease.Its symptoms include diarrhea,dehydration,abdominal pain and fever.It is caused by the bacterium,*Vibro cholera*.On entering the small intestine,*V.cholera* grows and starts producing a toxic protein ,a hexameric enterotoxin.This enterotoxin causes the loss of ions from the cells and it leads to diarrhea,dehydration and even death.

The currently used cholera vaccine is made up of phenol-killed *V.cholera*.The immune-protection lasts for 3-6 months .The DNA techniques have discovered the gene of enterotoxin(toxic protein).Enterotoxin,a hexamer consists of one A subunit and five similar B subunits.The A subunit further has two functional domains-the A1 peptide which has the toxic activity and A2 peptide that links A subunits to B subunits.

By genetic engineering ,it was possible delete the DNA sequence encoding A1 peptide and produce a new strain of V.cholera.This is without pathogen and it cannot produce enterotoxin. The genetically engineered V.cholera is a good candidate to use as an attenuated vaccine.

b) Salmonella Species:

Typhoid,enteric fever,food poisoning and infant death are caused by different strains of Salmonella genus.Immunoprotection against Salmonella species is really necessary.Some scientists have been successful in deleting aro genes and pur genes in Salmonella.

Aro genes encode for the enzymes that are involved in biosynthesis of aromatic compounds,while pur genes carry information for enzymes of purine metabolism.The new strains of Salmonella can be grown in vitro on a complete medium.

These doubly deleted strains have very restricted growth in vivo but they can stimulate immunological response.The genetically changed attenuated vaccine of Salmonella have been very good as oral vaccines in experimental animals(mice,cattle,sheep and chickens).Some scientists claim that new strains of Salmonella has immunoprotection in humans also.

Type # 6.3 Vector recombinant vaccines:

Some of vectors (carriers) that may be bacteria or virus can be used as vaccines after their genetic modification. Recombinant vector vaccines use an attenuated virus or bacterium to introduce microbial DNA to cells of the body.Following are some of the uses of this kind of approach:

Live recombinant vaccines using bacterial or viral vectors:

As a result of advancements in the fields of molecular biology and genetic engineering it is now possible to create live recombinant vectors that have ability to deliver heterologous antigens by introduction of antigen encoding gene.

The idea behind this method is to use the ability of infection and immunological properties of the live vector to produce an immune response against its own pathogenic proteins, as well as heterologous proteins being presented. A number of bacteria (such as *Salmonella typhi*, *Bacille Calmette-Guerin* (BCG) and viruses (such as vaccinia and adenovirus) have been tested as live recombinant vector vaccines.

Delivery of Antigens by Bacteria:

After several studies, it is observed that antigens are located on the surface of bacterial cell are more immunogenic than antigens in cytoplasm. On this observation, many scientists have developed an approach to coat surface of non-pathogenic organisms with antigens of pathogenic bacteria.

The flagella of *Salmonella* contain the flagellin protein. A synthetic oligonucleotide having the epitope of Cholera toxin B subunit was injected into *Salmonella* flagellin gene. This epitope was found on the surface of the flagellum. These flagella-modified bacteria, when inserted to mice, produced antibodies against the Cholera toxin B subunit peptide.

Future prospective:

It can be possible in future to deal with multiple epitopes (2,3) into the flagellin gene to produce multivalent bacterial vaccines.

Conclusions

Vaccines induce an immune response in the animal host that subsequently recognizes infectious agents and helps fight off the disease; vaccines must do this without causing the disease. Using recombinant DNA technologies, scientists have been able to develop live genetically modified organisms, recombinant killed vaccines, and genetic vaccines that no longer cause disease yet induce a strong immune response. Developing vaccines using rDNA technologies requires a thorough understanding of the disease agent, particularly the antigens critical for inducing protection and the factors involved in causing disease. In addition, it is important to understand the immune response of the host to ensure that the vaccine induces the appropriate immunological reaction.

Paralleling the development of new, more efficacious, stable, and safe recombinant vaccines is the study of vaccine delivery methods. In addition to using conventional delivery routes such as oral, intranasal, intradermal, transcutaneous, intramuscular, and IP, scientists are experimenting with needle-free systems and vaccine strategies that allow mass vaccination of many individuals simultaneously.

Another active area of research is the study of compounds with the potential to enhance the immune response to vaccines. These approaches include incorporating immunomodulating compounds into vaccines that can affect the type of immune response directly and immunopotentiating compounds that strengthen the immune response. The antigenic pathway can thus be modulated to stimulate the appropriate arm of the immune response to provide solid protection. Also, new compounds that indirectly stimulate the immune response (such as microparticles and adjuvants) are being studied. These compounds are designed to present antigens to the immune system in such a way that optimal stimulation is achieved.

The promise of better vaccines to benefit animal agriculture and companion animals through rDNA technology is becoming a reality. A number of recombinant vaccines are available commercially, and many more are projected to be available soon, so the future of recombinant vaccines is bright. New efforts need to focus on delivery methodology as well as development of vaccines for economically important diseases for which no currently available vaccines exist or for diseases where poorly effective vaccines are currently in use. Advances in rDNA technology,

in knowledge of the host immune response, and in the genetic makeup of disease agents will lead to new vaccines against diseases for which no control measures currently exist.

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