DEPARTMENT OF BIOTECHNOLOGY

Recombinant Vaccines

Types

This contains notes on Recombinant Vaccines

RECOMBINANT VACCINES

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 a biological agent that represents the disease-causing microorganism. It is often made from a weakened or 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.

Recombinant Vaccines—

Recombinant DNA technology in recent years has become a boon to produce new generation vaccines. By this approach, some of the limitations (listed above) of traditional vaccine production could be overcome. In addition, several new strategies, involving gene manipulation are being tried to create novel recombinant vaccines.

Types of Recombinant Vaccines

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

1. Subunit Vaccines:

Subunit recombinant vaccines are the components (proteins, peptides, DNAs) of the pathogenic organisms.

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- The advantages of these vaccines include their purity in preparation, stability and safe use.
- The disadvantages are — high cost factor and possible alteration in native conformation. Scientists carefully evaluate the pros and cons of subunit vaccines for each disease, and proceed on the considered merits.

✓ Hepatitis B

- Hepatitis B is a widespread disease in man. It primarily affects liver causing chronic hepatitis, cirrhosis and liver cancer.
- Hepatitis B virus is a 42 nm particle, called Dane particle. It consists of a core containing a viral genome (DNA) surrounded by a phospholipid envelope carrying surface antigens (Fig.).
- Infection with hepatitis B virus produced Dane particles and 22 nm sized particles.
- The latter contain surface antigens which are more immunogenic. It is however, very difficult to grow hepatitis B virus in mammalian cell culture and produce surface antigens.
- The gene encoding for hepatitis B surface antigen (HBsAg) has been identified. Recombinant hepatitis B vaccine as a subunit vaccine, is produced by cloning HbsAg gene in yeast cells. *Saccharomyces cerevisiae*, a harmless baking and brewing yeast, is used for this purpose (Fig. 16.1B).
- The gene for HBsAg is inserted (pMA 56) which is linked to the alcohol dehydrogenase promoter. These plasmids are then transferred and cultured.
- The cells grown in tryptophan, free medium are selected and cloned. The yeast cells are cultured.
- The HBsAg gene is expressed to produce 2nm sized particles similar to those found in patients infected with hepatitis B. (These particles are immunoreactive with anti-HBsAg antibodies).
- The subunit HBsAg as 22 nm particles can be isolated and used to immunize individuals against hepatitis B.

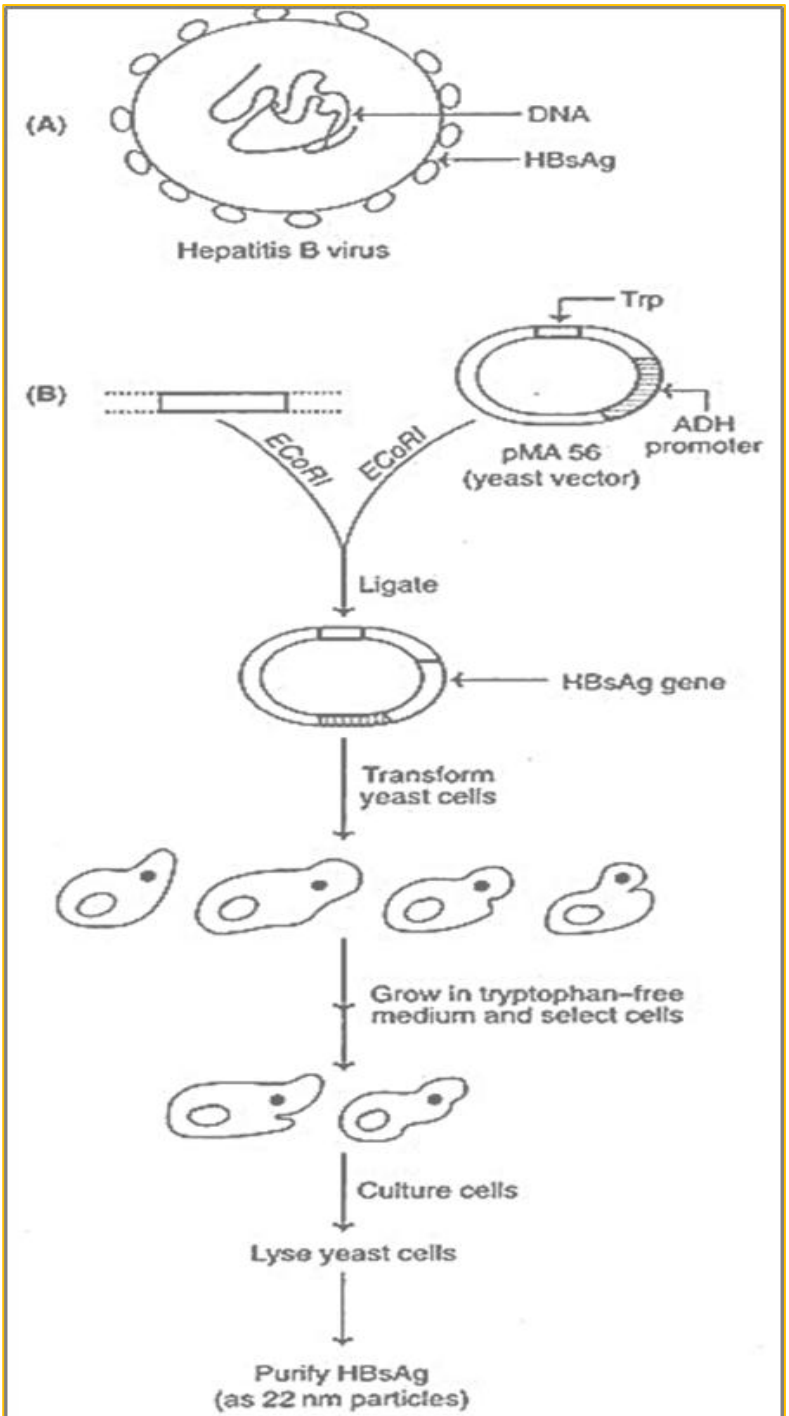

Fig. 16.1 : (A) Hepatitis B virus–Dane particle (42 nm particle); (B) Production of hepatitis B surface antigen (HBsAg) in yeast cells (Trp–Tryptophan, ADH–Alcohol dehydrogenase).

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2. Attenuated Recombinant Vaccines:

In the early years of vaccine research, attenuated strains of some pathogenic organisms were prepared by prolonged cultivation — weeks, months or even years. Although the reasons are not known, the infectious organism would lose its ability to cause disease but retains its capability to act as an immunizing agent. This type of approach 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 referred to as attenuated recombinant vaccines. The genetic manipulations for the production of these vaccines are broadly of two types:

1. Deletion or modification of virulence genes of pathogenic organisms.
2. Genetic manipulation of non-pathogenic organisms to carry and express antigen determinants from pathogenic organisms.

The advantage with attenuated vaccines is that the native conformation of the immunogenic determinants is preserved; hence the immune response is substantially high.

This is in contrast to purified antigens which often elicit poor immunological response.

Cholera:

- Cholera is an intestinal disease characterized by diarrhea, dehydration, abdominal pain and fever.
- It is caused by the bacterium, *Vibrio cholera.*
- This pathogenic organism is transmitted by drinking water contaminated with fecal matter.
- Cholera epidemics are frequently seen in developing countries where the water purification and sewage disposal systems are not well developed.
- On entering the small intestine, V. cholera colonizes and starts producing large amounts of a toxic protein, a hexameric enterotoxin.

- This enterotoxin stimulates the cells lining intestinal walls to release sodium, bicarbonate and other ions.
- Water accompanies these ions leading to severe diarrhea, dehydration, and even death.
- The currently used cholera vaccine is composed of phenol-killed V. cholera. The immuno-protection, lasting for 3-6 months is just moderate. Attempts are being made to develop better vaccines.
- The DNA technologists have identified the gene encoding enterotoxin (toxic protein). Enterotoxin, an hexamer, consists of one A subunit and five identical B subunits.
- The A subunit has two functional domains-the A1 peptide which possesses the toxic activity and A2 peptide that joins A subunit to B subunits.
- By genetic engineering, it was possible to delete the DNA sequence encoding A1 peptide and create a new strain of V. cholera.
- This strain is non-pathogenic, since it cannot produce enterotoxin. The genetically engineered *V. cholera* is a good candidate to serve as an attenuated vaccine.

RECOMBINANT VACCINES

Creating a new strain of V. cholera

The development of a new strain of Vibrio cholera that can effectively serve as an attenuated recombinant vaccine is depicted in Fig. 16.7, and briefly described below.

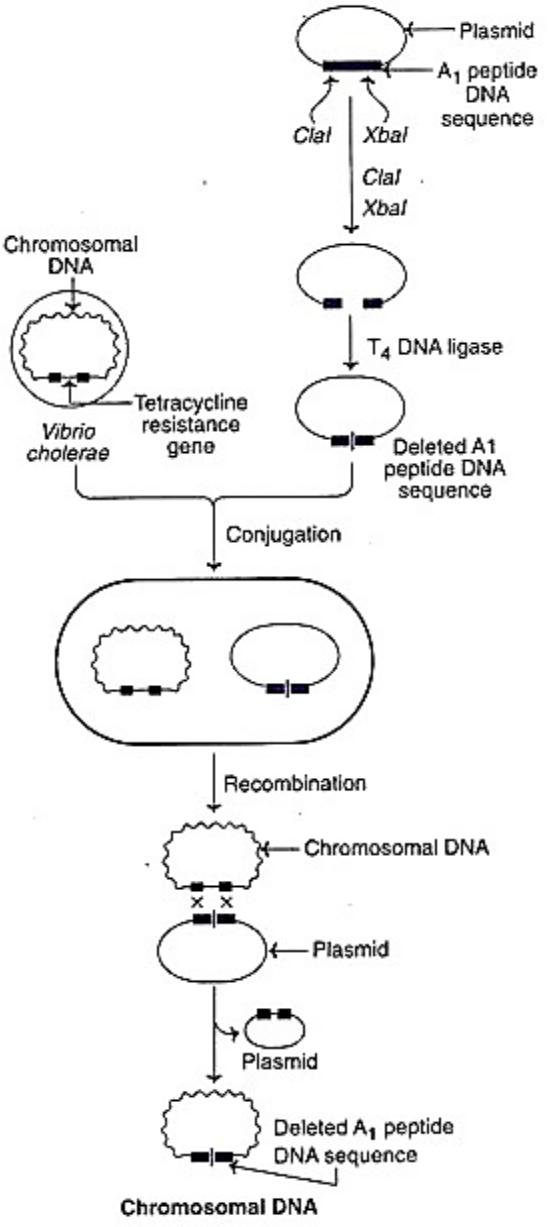

Fig. 16.7 : Development of a new strain of V. cholerae *as an attenuated recombinant vaccine (*ClaI *and* XbaI *are restriction endonucleases).*

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3. Vector Recombinant Vaccines:

Some of vectors can be genetically modified and employed as vaccines against pathogens.

Vaccines against Viruses- Vaccinia Virus:

- Vaccinia viruses is basically the vaccine that was originally used by Jenner for the eradication of smallpox. The molecular biology of this virus has been clearly worked out. Vaccinia virus contains a double-stranded DNA (187 kb) that encodes about 200 different proteins. The genome of this virus can accommodate stretches of foreign DNA which can be expressed along with the viral genes.
- The vaccinia virus can replicate in the host cell cytoplasm (of the infected cells) rather than the nucleus. This is possible since the vaccinia virus possesses the machinery for DNA replication, transcription-DNA polymerase, RNA polymerase etc. The foreign genes inserted into the vaccinia virus can also be expressed along with the viral genome. Thus, the foreign DNA is under the control of the virus, and is expressed independently from the host cell genome.
- The vaccinia viruses are generally harmless, relatively easy to cultivate and stable for years after lyophilization (freeze-drying). All these features make the vaccinia virus strong candidates for vector vaccine. The cloned foreign genes (from a pathogenic organism) can be inserted into vaccinia virus genome for encoding antigens which in turn produces antibodies against the specific disease- causing agent.
- The advantage with vector vaccine is that it stimulates B-lymphocytes (to produce antibodies) and T-lymphocytes (to kill virus infected cells). This is in contrast to a subunit vaccine which can stimulate only B-lymphocytes. Thus, vaccinia virus can provide a high level of

immunoprotection against pathogenic organisms. Another advantage of vaccinia virus is the possibility of vaccinating individuals against different diseases simultaneously. This can be done by a recombinant vaccinia viruses which carries genes encoding different antigens.

- Antigen genes for certain diseases have been successfully incorporated into vaccinia virus genome and expressed. Thus, vector vaccines have been developed against hepatitis, influenza, herpes simplex virus, rabies, angular stomatitis virus and malaria. However, none of these vaccines has been licensed for human use due to fear of safety. It is argued that recombinant vaccinia virus might create life threatening complications in humans.

Production of recombinant vaccinia viruses:

The development of recombinant vaccinia virus is carried out by a two-step procedure (Fig.).

1. Assembly of plasmid insertion vector:

- Fresh vaccinia (cow pox) viruses are processed to release their DNAs. Now genes from hepatitis B virus, herpes simplex virus and influenza virus are added one after another and inserted into vaccinia virus genome. These DNA clusters are cloned in E. coli for increasing their number and to produce plasmid insertion vectors. The plasmid contains the foreign subunit genes, the natural vaccinia genes, including the promoter genes. The recombinant plasmids are isolated and purified and serve as plasmid insertion vectors.

2. Production of recombinant vaccinia viruses:

- The animal cells are infected with plasmid insertion vectors and normal vaccinia viruses. As the viral replication occurs, the plasmids are taken up to produce recombinant vaccinia viruses. The plasmid insertion

vector incorporates its genes into vaccinia virus genome at a place that encodes for the enzyme thymidine kinase (TK).

- Thus the recombinant viruses have lost their ability to produce TK. There are two advantages of loss of TK gene. One is that it is easy to select recombined vaccinia viruses that lack TK gene and the second is that these viruses are less infectious than the normal viruses. The recombinant vaccinia viruses, released from the cultured animal cells, can be successfully used as vaccines. These live viral vaccines have some advantages over the killed or subunit vaccines.

Advantages:

1. Authenticated antigens that closely resemble natural antigens can be produced.
2. The virus can replicate in the host cells. This enables the amplification of the antigens for their action on B-lymphocytes and T-lymphocytes.
3. There is a possibility of vaccinating several diseases with one recombinant vaccinia virus.

Disadvantages:

1. The most important limitation is the yet unknown risks of using these vaccines in humans.
2. There may be serious complications of using recombinant viral vaccines in immunosuppressed individuals such as AIDS patients.

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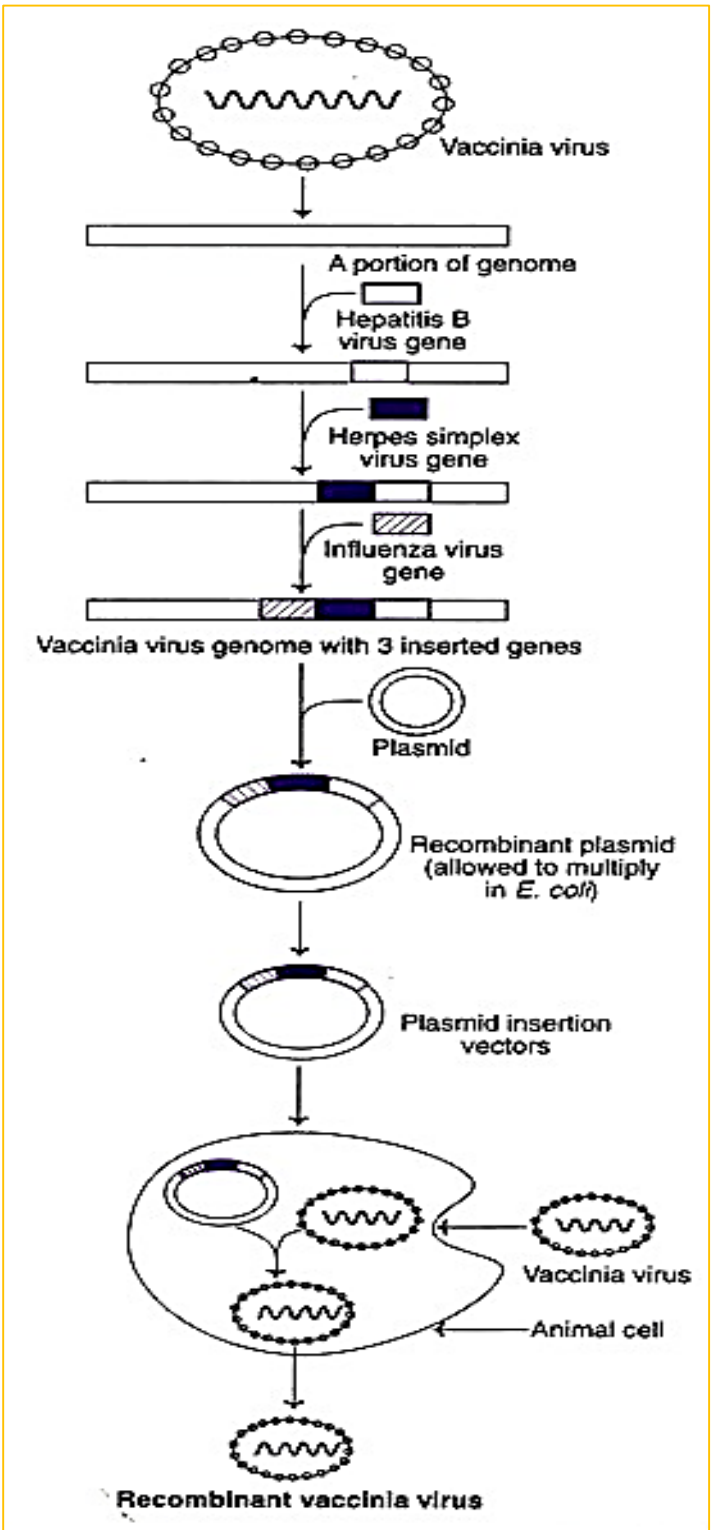

Fig. 16.8 : Production of recombinant vaccinia virus.

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