AGRICULTURE & ENVIRONMENT
E-NEWSLETTER
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Article ID: AEN-2021-02-03-015

Production of Recombinant Hepatitis B Vaccine –An Insight

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In the past century rDNA technology was just an imagination with the objective to express desired characteristics in vectors and model organism. But in the recent era this field has proven to be a boon in medical sciences by improving quality of human life. By using rDNA technology we can now easily produce crucial proteins, metabolites affordably, safely and in sufficient amount. This technology has many applications and has the potential to improve the quality of human life by improving health and enhancing nutrition in food. Now a days RDNA technology is being widely used in all sectors of biological sciences. Now it is easier to produce both killed and live vaccines with high specificity using the recombinant DNA technology. recombinant vaccines is one of the gift that it has given and the most successful vaccine ever prepared is hepatitis B vaccine that is effectively been used for the prevention of disease. Based on the recent advancement and development in this field recently recombinant COVID-19 vaccine was also prepared. The recombinant vaccine was first used by the aid of yeast cells of saccharomyces cerevisiae where it was made by incorporation of silver. Later development includes use of other species of yeast as well. WHO recommends three of the organism to produce recombinant vaccines for hepatitis B virus. Future perspectives include the production of vaccine by viruses as well (vaccines) but it is a long mile still to achieve completely. There are safety guidelines to use, manufacture the vaccine which everyone must follow. Due to the tremendous advancement and discoveries in recombinant DNA technology, this review focuses on the importance of rDNA technology for the production vaccines

Introduction

Biotechnology involves combination of technology and biological sciences. It harness the biomolecule and cellular processes to develop advance technology and product that helps in improving the quality of time. With advancement in pharmaceutical and molecular biotechnology different therapeutic medicines and vaccines can be produced that help us in combating with rare disease, epidemics and pandemics. With an increase in outbreak of deadly diseases, epidemics and pandemics it is now very important to produce medicines and vaccines products at the faster rate. This global need can be surely achieved by biotechnology and advancement in genetic engineering. Genetic engineering involves techniques that alter the chemistry of genetic material and introduction of the genetically modified genes into host organism which ultimately changes the expression and phenotype of host organism. For production of vaccines, enzymes, antibiotics maintenance of sterile ambience is needed to enable growth of only desired microbe in large quantities (Zahry NR and Besley JC. 2019 ; Dubey. 2014).

Recombinant DNA technology is the technique of making novel genomes that are generally not present naturally in the host genomes by the aid of genetic recombination. It involving integration of genes isolated from various sources and formation of a new daughter DNA that contains additional expressional functions comparative to the parent DNA molecules. DNA sequences involving genes from various myriad of organisms and can be integrated between interspecies. DNA is considered to be recombinant when two or more than two strands of DNA is

being combined and ligated. It can be done by taking two strands from same organism or different organism, or different DNA from the same organism (Plasmid DNA and chromosomal DNA).Recombinant DNA is conceivable in light of the fact that DNA particles from all living beings share a similar synthetic structure, it is the sequence of nucleotide that adds to the diversity in the general structure of DNA which is generally indistinguishable (Knott & Doudna 2018; Micklos et al.1990). Recombinant DNA can be considered as chimeric as it is formed by the combination of two unique strands that differ on the basis of either sequence/ source organism/type of strand. R-DNA involves utilization of palindromic sequences so as to generate sticky and blunt ends (Hugenholtz et al.2003). The majorly it was used in medicine for the production of insulin. Hepatitis B infection is controlled using a recombinant hepatitis B antibody, which contains a type of the hepatitis B infection surface antigen that is created in yeast cells. The advancement of the recombinant subunit antibody was an imperative and fundamental improvement since hepatitis B infection, not at all like other regular infections, for example, polio infection, can't be developed in vitro (Lee and William. 1997).

Mechanism of vaccination and types of vaccines

The rule of immunization depends on the memory of the resistant framework. In immunization, antigenic proteins of pathogens or end of the weak pathogens (attenuated) is administered in the body .The antibodies injected in the body against these antigens will kill the pathogenic disease causing agent which can be an bacteria, virus or any other microorganism. The immunizations additionally create memory B cells and lymphocytes that identifies the pathogen rapidly when the pathogen invades for the second time. The memory B cell overpower the pathogen with a monstrous generation of antibodies. (Bloom et al .2002).

Traditional vaccines include attenuated vaccines (inactivated microorganism by irradiation, heat or chemical) inactivated vaccines (which include inactivated toxins which are real proteins) or subunit vaccines (Dubey & Uddin. 2020). The subunit vaccines have certain limitations as not all organism can be grown in culture, it also comes on the safety of the lab personnel, expensive, insufficient attenuation, moreover it doesn't works for all infectious agents, can be reversed to infectious state. This brings the need for creating something new that could overcome all limitations. The answer to this problem is recombinant vaccine (Yadav et al.2020; Pachuk et al .2000).

Vaccine produced through recombinant technology is called recombinant vaccines. Recombinant vaccines are majorly divided into two categories, DNA vaccines and protein subunit vaccines (Bloom et al. 2002). They contain synthetic DNA containing the gene that encodes the diseased agent protein. The plasmid DNA will be used as vaccine is allowed to grow in bacteria that are host. this is based on the fact that antigen can be expressed directly by host cells so that it stimulates an immune response from the host as because the antigen introduced has created an environment of viral infection. It is a type of in vivo production of protein antigen (Robinson et al.1997).This contains only the fraction of pathogenic organism. These are synthetic peptides that express themselves and induce an immune response. They can also contain protein subunits expressed in a heterologous expression system. This is called recombinant protein expression technology. This is the type of recombinant vaccine that is used most widely in research area. The most successful example of recombinant vaccine is against hepatitis B virus (Liljeqvist & Ståhl 1999).

Hepatitis B and its causative agent Hepatitis B virus

Hepatitis B virus is an unusual virus that's features resemble similarity to retrovirus and it belongs to *hepadnaviridae* family. HBV virus is classified into eight different genotypes A to H. There are two types of strains that that doesn't have a viral nucleic acid composed of hepatitis B surface antigen and lipids that are derived from hosts. However the strains that cause viral disease

has doubled shelled structure instead of spherical structure. It is 42 nm in width that consists of lipid surface antigen that has core antigen inside it. This core antigen has viral DNA that encodes for viral disease and it is associated with viral polymerase. Its viral genome is partially double stranded that is also circular in nature. The polymerase binds to the DNA at the 5 end of the strand. The genome has divided into pre –S1, pre-S2 and S regions that have open reading frames. The core region gives the virus capability to self assembles into a capsid and has RNA binding activity**.** One of the ORF leads the translation product to endoplasmic reticulum where it is processed to secret antigens. These antigens provide the capability for persistent infection. The polymerase terminal domain leads to encapsidation, whereas it's another domains help in replication by degradation of pregenomic RNA. The part of genome also codes for some protein that regulates that the other proteins should not be degraded. This virus antigen has an oncogenic ability.

Viral infection cycle

1. The virus attaches to the host cell. Many receptors of the host cell helps in this but the carboxypeptidas D helps in this the most.
2. The gap between the genome of virus gets repaired and the viral genome gets converts into covalently closed circular DNA.
3. The genome has information for various pregenomic RNA and precore RNA that gets translated.
4. The translation starts from pregenomic RNA site and it codes for various proteins. The HBV is a major S gene product and L and M are minor. The glycoprotein layer contains amino acid and peptides.
5. Encapsidation leads to the replication.
6. The packaging signal gets interacted with polymerase and forms a nucleo-capsid
7. Reverse transcription of pre-genomic RNA
8. Formation of complete circular DNA
9. The nucleocapsid interacts with the envelope proteins and is spread to the host body (Karayiannis. 2017; Liaw et al. 2009).

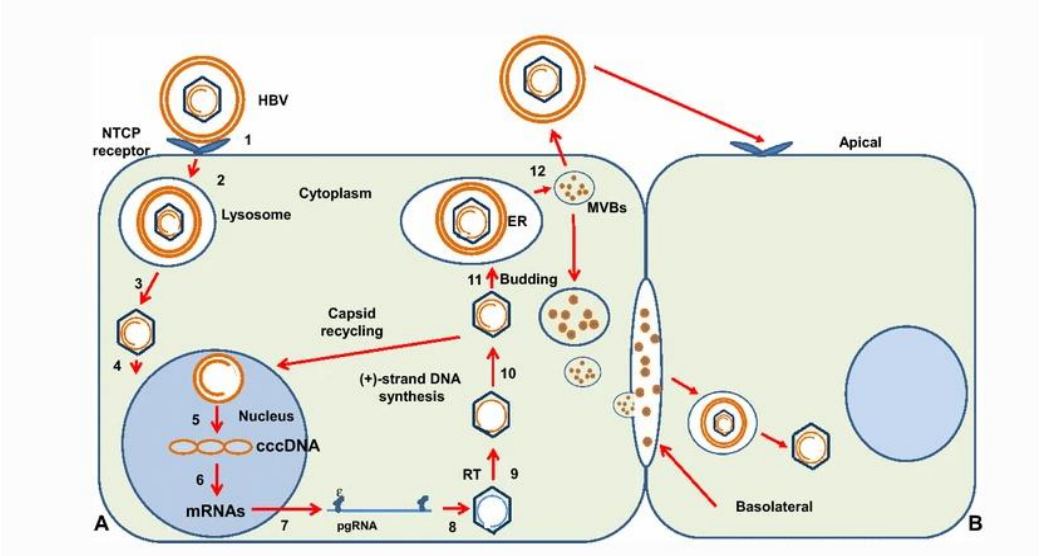

Figure 1: Infection cycle and mechanism of Hepatitis B virus (Karayiannis. 2017).

From Traditional To Recombinant Vaccine

Development of hepatitis B virus is actually a kind of first anti-cancer vaccination as it helps in prevention of liver cancer. Dr. Baruch Blumberg was the first pioneer to identify this virus in 1956 and later he developed a vaccine against this infection. Earlier this infection was known as Australia antigen as it was identified by the aid of blood test performed on hemophilia patient of America as a result of immune response generated in his body. Irving Millman a microbiologist working with Dr Baruch Blumberg developed blood test to identify the infection. The widespread utilization and acceptance of this diagnostic test started in the year 1971 where blood donation bank started using it to check suitability of blood at this aspect of disease. This brought out the spread of infection and the chances as it reduced this by 25% due to cautionary blood transfusion. It was in 1975 when Dr. Baruch and his coworker Irving Millman came with the first vaccine which was prepared by heat killed /deactivated Virus.

The main shortcoming included that it doesn't become effective on commercial level and medical as well. Commercial Hepatitis B vaccine first came in the market in the year 1981. This vaccine was prepared by pasteurization, continuous heat treatment as well as usage of formaldehyde to deactivate the antigen present in the patient's blood isolated to create preventative vaccination for non-affected individuals. The vaccine was produced by Merck Pharmaceuticals and in market by the name of Hepatavax. After the introduction and production of recombinant vaccine the production of Hepatavax was terminated in the year 1990. The major shortcoming of Hepatavax was it usually contained blood products which used to result side effects. In 1986 when first recombinant and second generation vaccine started getting produced it answered the problem which is still being used in USA (Zaneeti et al. 2008).

Production of Recombinant Hepatitis B Vaccine

It is the worldwide need for continuous research for development of effective vaccination against the entire hepatitis virus as it causes Hepatocarcinoma, disorders related to liver. It is being produced by the aid of surface antigen utilization which can be isolated from the plasma of infected individuals. Sample from affected individuals bring problems as the plasma is contaminated most of the times and there is a need of procedures for purification purposes which will reduce the chances of contamination and remove all the infectious antigens. It also reduce the chances of causing infection to the recipient for possible other microbial antigens. First attempts include production of recombinant DNA vaccines in the *saccharomyces cerevisiae.* The antigen is introduced in the yeast by the aid of vector DNA. Earlier it was thought to introduce animals and other sources such as bacteria to create effective vaccine by transformed cell lines of animals and antigen cloning in bacteria which were not successful as cell lines may be neoplastic and no of antigen expressed in bacteria were low (M caller et al .1984). Later it was first reported that yeast has ability to produce antigen and also has the ability for assembling of polypeptides similar to humans (Valenzula et al. 1984).The vaccine was created in the following procedure-

- Introduction of antigen gene into the yeast cells
- Subsequent growth of antigen was observed in the cells
- The cells were collected and homogenized
- The antigens were separated by immune affinity chromatography
- Adsorption on alum for verification of antigenicity and immunogenicity
- Given to animals for testing

The antigens which were isolated were verified before adsorption by the method of electron microscopy where it showed similar structure compared with the case of humans. The UV adsorption was also similar when both the antigens; antigens of the human plasma and yeast cells were run in SDS PAGE electrophoresis. Incorporation of this confirmed the effectiveness in the four chimpanzees used as testing animal (Mc caller et al .1984).this method as well as production of recombinant vaccine is still widely used. Live attenuated vaccine can also prove them to as an alternative as well as vaccinia viruses but they have shortcomings of not be using in case of immune-compromised patients. Genetic engineering technology has enabled for the creation of subunit vaccines which results in generation of immune response.

Typical process of creation of recombinant gene for encoding hepatitis B gene includes separation of whole genome and isolation of desired gene and cloning in the plasmid. The entire genome of antigen is 2Kb, now without the aid of plasmid it cannot be incorporated directly into the yeast. Restriction endonuclease is used to cleave DNA which cut the DNA at desired positions. Creation of recombinant gene is not only sufficient expression vector is used which has alcohol dehydrogenase and leucin-2 as promoter and marker. In production of recombinant gene in *Saccharomyce cerevisiae* two kinds of vector were used.

Yeast fermentation technology is the technology which is used where recombinant yeast cells are used and later the vaccine is formed by aggregation with silver (Adkins et al .1998). The mode of vaccination is intramuscular and given as 3 doses and given during the infant period. According to the recommendations of world health organization apart from *sacharomyceas cervisiae* other yeast which are used are *pichia pastoris, Ogataea.polymorpha,* Chinese hamster ovary cells are also used for production. (World Health Organization, 1988). Recent developments include production of vaccine from the transgenic monocot banana (Kumar et al 2005).

There are safety guidelines and general recommendations for production, manufacturing and giving it to the patient. These guidelines are also given by world health organization which is being followed by most of the nations.

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