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Antiviral Vaccines textbook

Intended for first-year Master's students in Microbial Biotechnology

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Introduction

Viruses are the smallest self-replicating organism in the nature, they replicates solely within the living cell of an organism. Historically characterized by their ability to bypass through fine filters that retain even smallest bacteria. Viruses are a small acellular infectious agent, generally less than 250nm diameter. Viruses particles consist of small genetic segment of DNA or RNA packaged within a protein coat. In some cases these particles are membranes enveloped derived from plasma membrane or other cellular organelles.

Viruses are obligate intracellular parasites with no metabolism of their own but rather they parasitize subcellular host cell machineries to ensure their multiplication through the expression of their genomes. Virus takes advantage from protein synthesis machinery including ribosomes, tRNA and enzymes to produce their own proteins. The host cell provide molecular building blocks such as amino acids, nucleotides, sugars, lipids and a source of energy. Viruses are the most abundant form of life on earth. They infect all kinds of organism, including microorganisms such as Bacteria, archaea, plants and animals. Viruses play an important role in the ecological equilibrium on earth, found in all the ecosystems that exist on the globe.

Some of these viruses interfere with normal cellular processes and cause tissue injuries and diseases (although many other viruses infect organisms without causing disease). Many viruses including Smallpox, Influenza, AIDS (acquired immunodeficiency syndrome), Ebola and COVID-19…etc. causes the most widespread and devastating human diseases. This shows the importance and emergency of the development of new vaccines to prevent and control the diseases spread. The development of new vaccines against these viruses is almost of importance to protect the global population from severe health consequences. The vaccine represent a biological preparation that induce active acquired immunity against a specific infectious disease. However, vaccine development can be challenging due to factors such as the evolution and mutations of some viruses as well as immune escape of some other viruses in infected organisms.

The purpose of this textbook is to provide a comprehensive overview of the different types of antiviral vaccine platforms, from standard live attenuated and inactivated vaccines to recombinant subunit, DNA and messenger RNA (m-RNA), and viral vector-based platforms, which led to enhanced vaccination effectiveness and improved production capabilities. Therefore, the present

work highlight the vaccines production and purification methods, current approved and future viral vaccines, the advantages and limitations of the current viral vaccine strategies, the difficulties encountered in vaccine development, and how these vaccines can be improved for future use.

Chapiter-1: Introduction to Vaccinology

Immunization is the process of protecting individuals from diseases by making them immunized. Immunity against microorganisms is achieved by two ways: active or passive immunization.

1. Passive immunity

Passive immunization is the process by which short-term protection from disease is conferred by the administration of antibodies coming from another source. This transfer of antibodies to unprotected individuals serve to prevent or treat a particular disease in immediate but temporary manner. This process occurs naturally during pregnancy, when large quantity of IgG antibodies are transferred across the placenta to the developing fetus and reaches serum concentration that are similar between mother and infant. It confer protection against many invasive microbes like *S. pneumonia, H. influenza* type b, rubella, mumps and poliovirus infections. Furthermore, maternal IgA isotype antibody present in colostrum and breast milk provide passive immunity to the infant. Passive immunization can also be achieved artificially by injecting a recipient performing antibodies. It is most frequently used in clinical situation for protection from rabies, Hepatitis, and SARS-CoV-2. The antibody preparations administered are called 'immune globulins'. Examples of commonly used immune globulins: anti- rabies, anti- hepatitis A, anti-hepatitis B and anti-VZV (chickenpox virus). Those preparations are necessary for people whose immune system does not respond adequately to an infection or for people who acquire an infection before vaccination (for example, after being bitten by an animal harboring rabies).

Antibodies for passive immunization are collected from several sources:

* Animal sera (usually horses) that have been developed immunity after exposure to a particular microbe or toxin.

* Blood collected from a large group of people regardless of whether or not they have had a disease (polyclonal human immune globulin). Polyclonal immune globulin to prevent specific infections such as measles and hepatitis A in vulnerable hosts, as the level of antibody against these viruses collected from blood donations is sufficiently high.

* People known to have higher blood levels of specific antibodies against a pathogen as they have received a vaccine or are recovering from the disease (hyper-immune globulin). Examples include varicella zoster immune globulin and hepatitis B immune globulin.

* Antibody-producing cells (usually taken from mice) and grown and immortalized as Hybridoma cultured cells in laboratory. These monoclonal antibodies are engineered for prevention of specific diseases, as in the case of the monoclonal antibody products against respiratory syncytial virus (RSV) and SARS-Cov2.

Before vaccines and antibiotics revolutionized modern medicine, antibody-based therapies represented the only effective medical treatment for many life-threatening infectious diseases. Today, most commercial forms of antibody-based immunotherapy for infectious disease still based on polyclonal antibodies from human or animal origin and monoclonal antibodies. The main advantage of passive immunity is to procure immediate protection against the microbe, for example, in the case of respiratory failure caused by RSV virus, the passive immunization with monoclonal antibody is the best preventative currently available. However, Passive immunity is short-lived because does not activate the immune system, so the host does not respond to the immunization and the relatively short half-life of the antibody proteins limits sustained efficacy. In addition, the protection provided is transient and lasts only as long as the received antibody persists. Passive immunization generates no memory response, so an immunized patient is not protected against subsequent exposure to the same toxin or microbe. In some recipients, passive immunization can develop responses to the foreign antibodies may lead to hypersensitive reactions.

2. Active immunity

Active immunization is achieved by a natural induction of immune response following infection by microorganisms, or artificial acquisition by administration of vaccines. The immune system plays an active role in this process, the induction of immune response leads to the stimulation and proliferation of the antigen-reactive T and B cells, enabling them to differentiate into T and B effector cells. Effector B cells provide a humoral immune response through antigen-specific antibodies synthesis and effector T cells provide the cellular immune response (lysis of the pathogen infected cells). This results in a formation of antigen-specific T and B memory

lymphocytes providing long-term protection against the infectious agent (over several years). The immunization is most often accomplished actively through vaccination, the delivery of antigens (substances that are foreign to the host) contained in vaccines for purposes of stimulating an acquired immune response. Vaccination remain for the body the best way to defend itself against viral infection and prevent disease as illustrated in Figure-1.

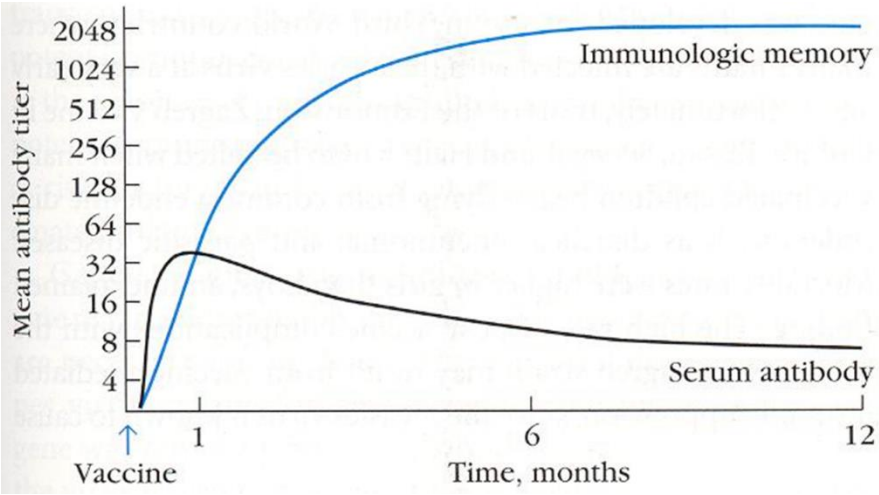

Figure-1: Comparison between the humoral immune response generated by passive immunity versus active immunity.

2.1. Definition

Vaccine is a suspension of live microorganisms (generally attenuated infectious agent), killed (inactivated) or fragment that does not cause disease thereof administered to a living organism (mostly given to healthy individuals) for the purposes to mobilize the immune system and induce a rapid and specific adaptive immune response, which is protective to a subsequent contact with the corresponding agent. This mimics the host's response to natural infection, but avoids the disease that is the harmful manifestation of infection.

The origin of the term vaccine is derived from the Latin word *vacca* which mean cow. This designation refer to cowpox virus, first vaccine against smallpox virus developed by Edward Jenner

in 1796. Cowpox virus causes usually a mild infection, but the administration of extracts of this virus provided humans immunization alive against smallpox virus.

2.2. The history of antiviral vaccines development

Viral pathogens have been serious threats to health and society throughout the human history. Variola disease caused by Smallpox virus is the most destructive disease in history, and has probably been part of human existence since 10,000 BC or before. It has been estimated that infection by smallpox virus killed in the 20th century alone, between 300 million and 500 million people died due to infection. Attempts to prevent this infectious diseases date to antiquity. The first partially successful prevention strategy was "Variolation": the deliberate injection or inhalation of dried pus from smallpox sufferers into healthy persons. This led to a milder form of the disease, the case fatality ratio was around 10 time lower than in people infected directly, but the resulting infection still resulted in a 2–3% case fatality rate. The practice of variolation was first introduced to Europe in 1700s after spreading throughout China, Turkey and Africa.

In 1796, British physician E. Jenner demonstrated that an infection with the relatively mild Cowpox virus conferred protection against the deadly Smallpox virus. He observed that women milkmaids infected with cowpox get a mild relative of the deadly smallpox virus. Jenner took infectious fluid from hand of milkmaid women and injected into the arm of a healthy local eight years old boy. Fifteen days later, Jenner challenged the young boy with deadly dose of smallpox virus.

The young boy survived to this potentially lethal challenge. He appeared to be protected from contracting the lethal virus. Jenner accomplished large-scale tests and found them to be successful in preventing the disease. It was the first-ever use of vaccination against any viral illness. Jenner promote the process of vaccination anti-variola and gained widespread support over the following years. 100 years later, Louis Pasteur Invented a rabies vaccine from dehydrated spinal cord of infected rabbits and introduced the term "vaccination" to honor the Jenner's pioneering work.

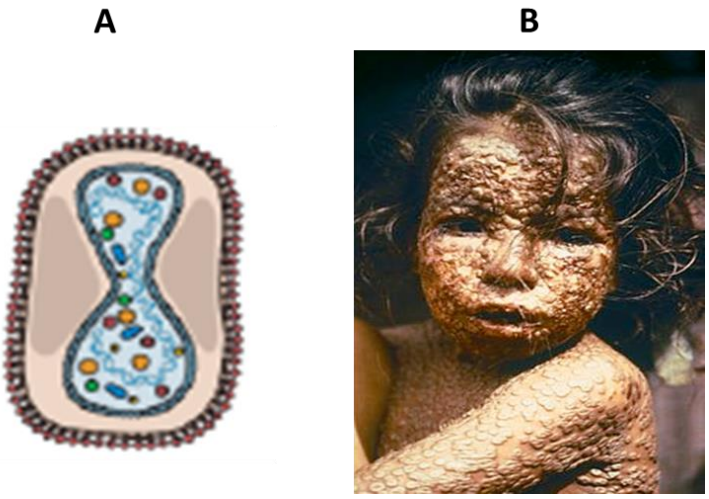

Figure-2: Characteristics of smallpox virus.

(A): Schematic representation of a smallpox virus particle. (**B**): Picture showing the characteristics of smallpox lesions on a young boy patient.

3. Basic elements of vaccines

3.1. Efficacy

A vaccine must induce protective immunity. The purpose of vaccination is to induce a sufficient level of immunity, which can effectively protect the host from infection. Following vaccination, the vaccine-induced antibody needs to be maintained at high level in the bloodstream as well as in the regions of our body in close contact with the pathogen. Importantly, an effective vaccine is expected to induce also high cellular immunity.

The vaccine stimulation of the immune system must induce immune response quite similar to those induced during infection by corresponding pathogens. This protection come from the induction of effector mechanisms capable of rapidly controlling the multiplication of pathogens and inactivating their toxins. These effector mechanisms are essentially higher affinity antibodies produced by B-lymphocytes enable to bind antigen and neutralize pathogens. Others effectors elements are cytotoxic CD8+ T cells by killing infected cells and secreting antiviral cytokines and CD4+ T helper cells (Th cells) important for the regulation of the immune response.

Another important characteristic of effective vaccines is that it should be able to induce long-term immunity that will persist for long period of time, a property called immune memory. Immune memory is maintained by the generation of a dedicated T and B-lymphocytes that remain long period after an infection has been resolved. These memory T and B cells are able to respond quickly to a subsequent exposure to the same virus.

3.2. Safety

Safety is the most important factor of an effective vaccine, because vaccines are administered to healthy people, including infants and youngsters, for preventive purposes, the vaccine must ovoid the exposition of the recipient to any kind of infection or other associated complications. Any undesirable side effects are intolerable from public health authorities. In addition, manufacturing processes should avoid any contamination of the virus preparations.

3.3. Feasibility

Several parameters that determine the feasibility of a vaccine. For example, the convenience in administration via liquid droplet or nasal spray is very useful for mass vaccination. In this case, medical staff is not required for administration. The storage conditions required are also important, in developing countries, vaccines with room temperature storage are more feasible than those requiring freeze.

4. Characteristics of an effective vaccine

4.1. Immunogenicity:

The vaccine must induce an appropriate T CD4+ immune response, Th1 versus Th2:

1-Induction of the protective cellular response (Th1 response):

Some pathogens are particularly intracellular, the elimination of pathogens bypass through killing the infected cells involving the activation of cytotoxic T cells (e.g. varicella virus).

2-Induction of the production of protective specific antibodies

Some pathogens, such as poliovirus, infect nerve cells (which do not regenerate), so the production of neutralizing antibodies are essential to neutralize the virus before reaching this site.

4.2. Antigenicity versus pathogenicity

A vaccine is antigenic but a non-pathogenic substance administered to a recipient organism. An antigenic vaccine contains the antigenic determinants from pathogens enable triggering and

stimulating an appropriate immune response within the host. A vaccine is non-pathogenic by lacking the viral determinants of pathogenesis responsible of the viral disease associated with the infection (Figure-3).

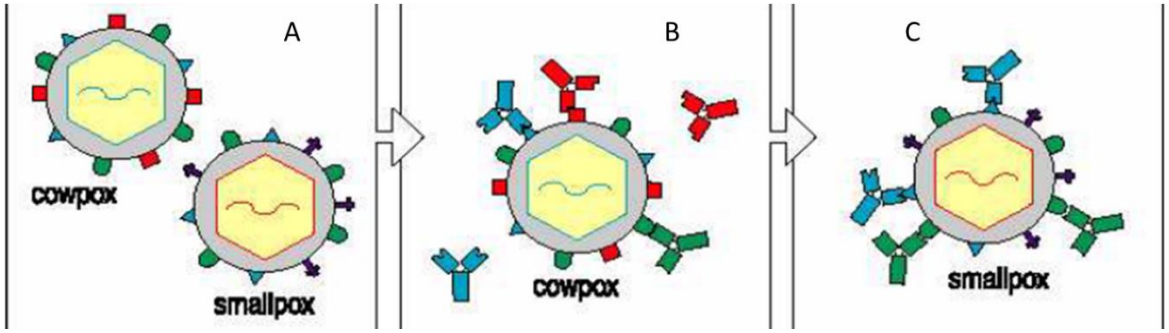

Figure-3: Representation of the concepts antigenicity vs pathogenicity of the cowpox vaccine against the smallpox vaccine developed by E. Jenner.

(A): Cowpox virus and smallpox virus express a number of common membrane antigens. (B): immunization with cowpox virus (non-pathogenic determinants) induces the production of antibodies directed against cowpox virus surface-antigens. (C): Antibodies directed against cowpox virus antigens recognize, bind and neutralize the smallpox virus antigens.

4.3. Other characteristics

- Longevity of immune response (several years).
- No risk of complications and minimal side effects.
- Stable under various conditions. Genetic stability, storage conditions.
- It is preferable to use combined-forms of vaccines.
- A single dose is better than multiple doses.
- Accessible to the public (in terms of price and availability).
- Oral administration preferred to injection with needle.

5. Factors contributing to the success of the first smallpox vaccine

Smallpox presented many advantages that made possible the invention of the first vaccine by E. Jenner:

- No animal reservoir for the smallpox virus.
- Lifelong immunity induced by the vaccine.
- Existence of a single smallpox virus serotype.
- Mutations are not common in this virus.
- Subclinical cases are very rare.

As a result, after worldwide effort, in 1978 the WHO has declared the complete eradication worldwide of the smallpox virus due to the efficiency of smallpox vaccine (vaccinia).

6. History of the development of vaccine discoveries

In the 18th century, Edward Jenner described the first vaccine developed in the human history. He inoculated an eight–year old boy with cowpox lesions from the hands of milkmaids to prevent smallpox infection. The young boy gained immunity against smallpox. After 80 years, Louis Pasteur developed the vaccine against rabies, which was highly successful. By the mid-20th century, after the invention of attenuated toxins (toxoids) the first generation of vaccines were developed. Through this development, it was possible to produce vaccines for diphtheria and tetanus. Science in the 20th century played a fantastic role in developing quick responses to viral attacks through the invention of various vaccinations. In the 1930s, with major advances in laboratories techniques allowed the cultivation of viruses on embryonated chicken eggs. This led to the development of influenza and yellow fever vaccines. The evolution of cell culture 15 years later led to the creation of the polio vaccine, and this marked the beginning of the golden age of vaccines. Salk introduced the inactivated polio-vaccine (IPV) in 1955, making it available for children. Sabin's oral vaccines appeared in 1960, the use of Sabin's Oral Polio Vaccine OPV brought polio incidence down through its direct reduction of polio-related mortality and disabilities. During this period a series of important vaccines like the measles, mumps, rubella, and varicella vaccines were developed. The introduction of recombinant DNA and molecular Biology techniques were major milestones in vaccine development since it allowed the generation of vaccinations that relied on particular viral proteins and not whole viruses. The first of these vaccines used was the hepatitis B vaccine developed in 1986, which made the hepatitis B surface antigen (HBsAg) using recombinant DNA technology. Next in the early 2000 were HPV vaccines designed to protect women from viruses that contribute to most cases of cervical cancers in women

see Table-1 and Figure-4. These vaccines laid the foundation of modern vaccines. It gave researchers the tools to develop new vaccines against pathogens, which was not possible before and many other are under construction.

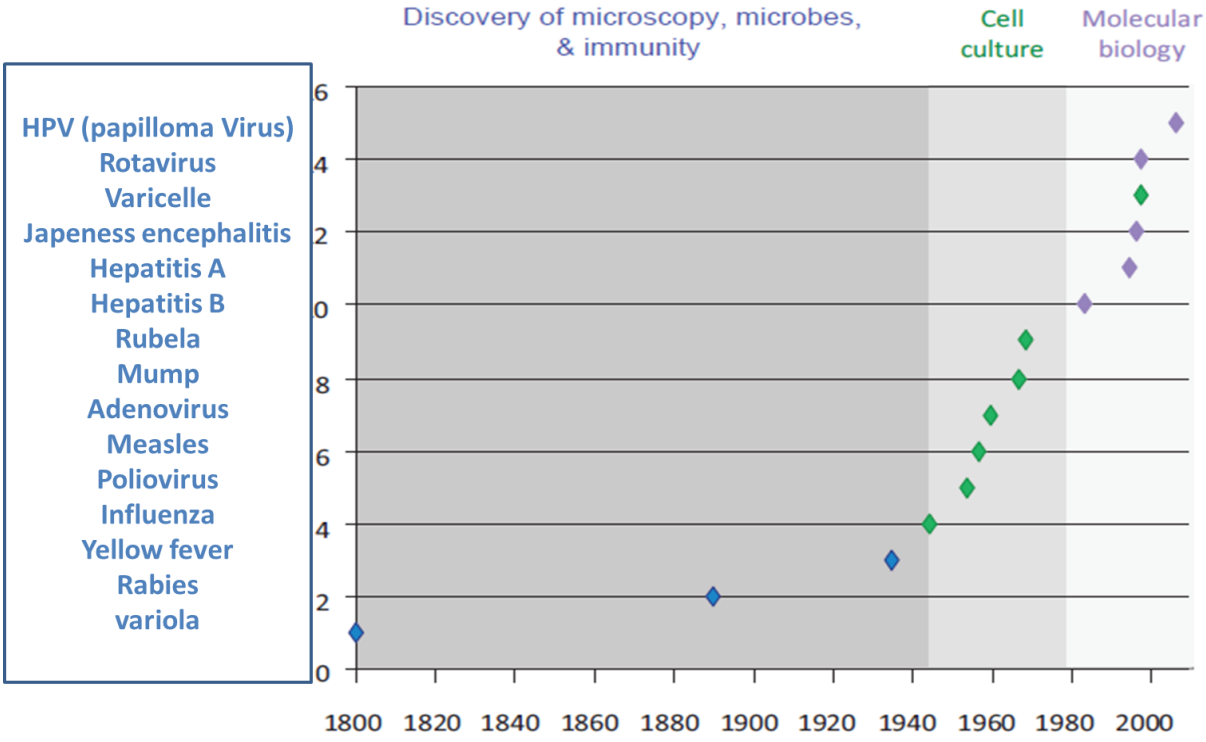

Figure-4: Diagram showing the history of the discovery of antiviral vaccines.

Table 1: Milestones in antiviral vaccines development:

10th Century- Practice of variolation to combat smallpox (China and the Ottoman Empire).
1700- Introduction of the concept of variolation into United Kingdom.
1796- Jenner's discovery of the smallpox vaccine.
1895-L. Pasteur and collaborators develop the rabies vaccine from dried rabbit spinal cord.
1910-30- first anti-bacterial vaccines, toxins and toxoids.
1930-50- first anti-viral vaccines, Japenese B encephalitis, yellow fever and flu vaccinse.
1950-1970- major advances in cell culture techniques, polio vaccines, mumps, measles and rubella vaccines.
1970-1990- Introduction of Hepatitis B and Hemophilus influenza B vaccines.
2000 -present-conjugate vaccines: rotavirus vaccine, papilloma virus vaccine, herpes zoster vaccine.

7. Aims of vaccination

. Vaccination primarily aims to prevent infectious diseases. This involves using vaccines to stimulate the body's immune system to create a protection against specific pathogens, preventing infection. Vaccination is important for public health, contributing to disease prevention, control, and in some cases, even eradication. Here is some of the major goals of vaccination:

7.1. Disease prevention

Vaccination is the most effective tool for primary prevention, it prevents diseases by introducing weakened or inactive pathogens (or parts of them) into the body, vaccine trigger an immune response that prepares the body to eliminate the real disease when encountered later. Vaccination help to protect individuals or a population against subsequent infection (systematic vaccination).

7.2. Reduce morbidity and mortality

Vaccines protect individuals from contracting diseases, reducing the overall burden of illness (morbidity) and preventing deaths (mortality) caused by infectious diseases.

7.3. Immunization of populations

High vaccination rates within a population create herd immunity, protecting even those persons who cannot receive vaccines (e.g., infants too young or individuals with certain health conditions).

7.4. Outbreak Control

Vaccinations are crucial in controlling and preventing the spread of infectious diseases during outbreaks, including those caused by new or emerging pathogens (e.g., Covid-19, Influenza).

7.5. Elimination

Some diseases, like Polio measles and rubella, have elimination goals where the aim is to stop the continuous transmission of the disease within a specific geographic region see figure-5.

7.6. Disease eradication

In some cases, vaccination is the key to eradicating a disease, meaning the complete and permanent worldwide cessation of disease transmission. Smallpox remain the only infectious human disease that has been eradicated through the WHO vaccination program.

7.7. Therapeutic effect

While primarily role of vaccines is a preventative measure, vaccines can also have a therapeutic effect, especially in cases where the immune system needs assistance in fighting off chronic infections. The goal is to stimulate the immune defenses against the pathogen and enable body to fight more effectively. For example, some vaccines are used in the treatments of Hepatitis B and HPV virus infections.

Nowadays, vaccines constitute an integral part of our lives. Immunizations are practiced on children, adults of all ages and animals.

Many viral diseases have been eliminated as a public health threat through vaccinations programs:

Control - Elimination as a public health problem

- Hepatitis B

- Hepatitis A
- Yellow fever
- Rabies

Eradication in perspective

- Poliomyelitis
- Measles
- Rubella

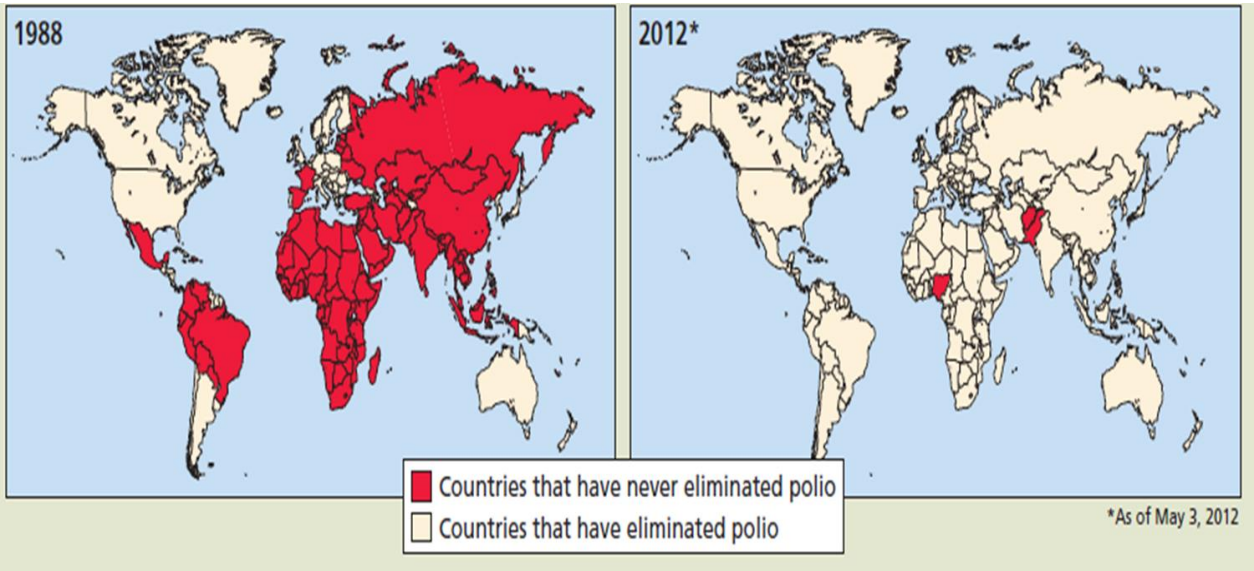

Figure-5: Globally reported incidence of poliomyelitis in 1988 and 2012.

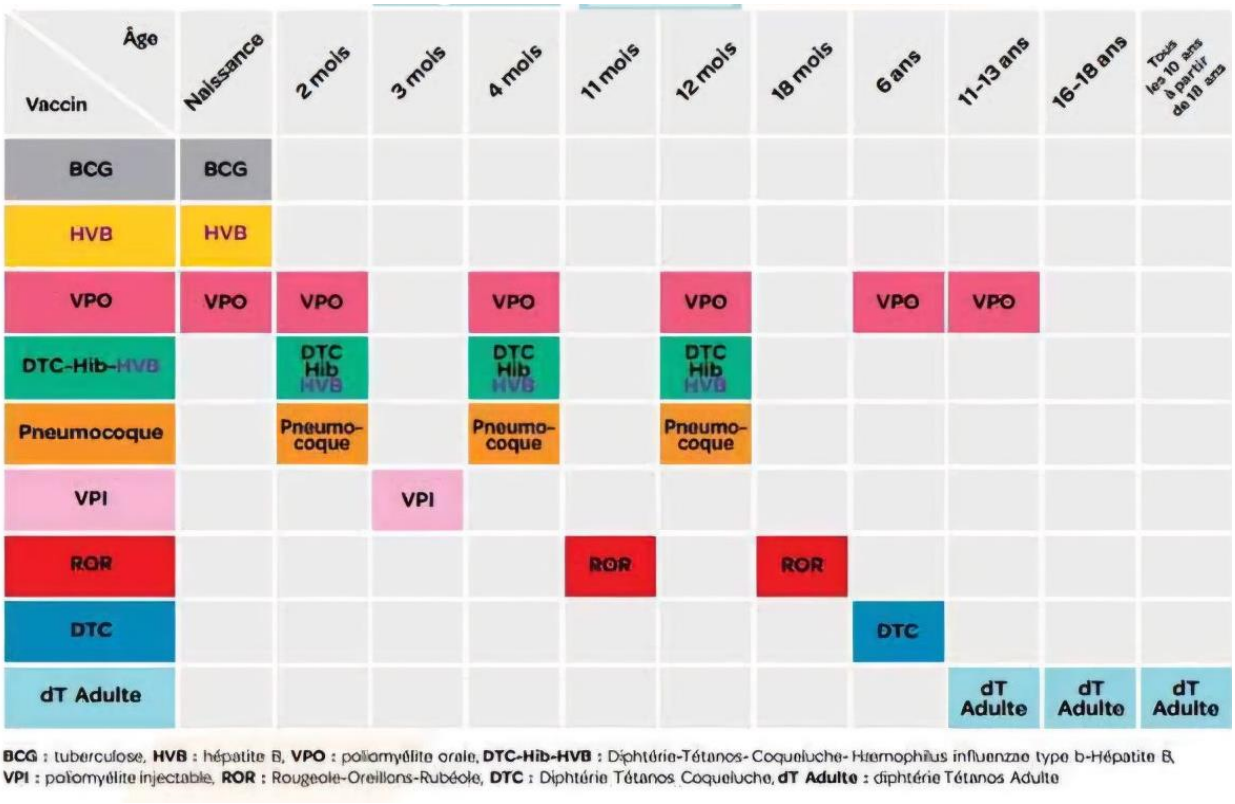

Figure-6: Official vaccination calendar of Algeria in 2023

8. Route of vaccine administration

Vaccines are administered through different routes (e.g., intramuscular, subcutaneous, intradermal, intranasal, and oral). These routes usually are determined during pre-licensure vaccines evaluation and based on the type of vaccine, immunogenicity, the pathogen. The route affects how the immune system recognizes and reacts to the vaccine.

8.1. Intramuscular injection

In this case, the vaccination is accomplished by injection into the muscle. The selection of the site of injection and needle size is based on the volume of vaccine to be inoculated, this is the most common route for many vaccines. The muscle has a good blood flow allowing a strong immune response. The slow absorption of the preparation induce a longer lasting immunity. Examples: Covid-19 (Pfizer, Moderna, J&J), Flu vaccines and Hepatitis-B…etc.

8.2. Subcutaneous injection

The vaccine is injected into the fat layer between skin and muscle. This route is recommended for live vaccines and some inactivated virus. Examples: MMR (measles, mumps and rubella), Varicella (chickenpox) and Yellow fever.

8.3. Intradermal injection

The vaccine is injected into the skin layer (dermis). Small dose is required in this case (cost effective). Administer should be trained to the process. Examples: Tuberculosis vaccine (BCG), influenza vaccine and smallpox.

8.4. Oral Administration

For vaccines given orally, vaccines are administered as liquid drops or as capsule, they must be swallowed to travel through gastrointestinal tract without being degraded or destroyed by gastric acids or enzymes. They are designed to survive during their route to the intestines, where they trigger the immune system, to stimulate gut immunity and ensure a mucosal response. Example: Oral Polio Vaccine OPV), rotavirus (Rotarix and Rotateq).

8.5. Nasal Spray (Intranasal)

Live attenuated influenza vaccine (LAIV) is licensed for healthy non-pregnant persons from 2 through 49 years of age and represent the only vaccine administered by the intranasal route by spraying the vaccine into the nose. This route is in favor to induce mucosal immunity, which is the first line of defense in respiratory infections.

9. Vaccines development

9.1. Pre-clinical trials

The starting point in the development of a candidate vaccine requires an understanding of the target disease, the infectious and the basis of the protective immunity against the pathogen as illustrated in Figure-7. These studies are initially performed in laboratory research. From these starting points, the vaccine researcher can begin to evaluate candidate for target immunogens and the possible incorporation of adjuvants and stabilizers into the final product. At this step, the selection of the vaccine platform is accomplished. For instance, live attenuated vaccines are often highly efficacious but carry risks associated with reversion to virulence or may cause disease. Whereas,

inactivated or subunit antigens are typically safer but less immunogenic, probably require multiple doses, and will require an adjuvant to boost the immune response. This basic research and development must also include assessment of the efficacy of the candidate vaccine. This will involve development of animal models of disease. A key component of the pre-clinical development of a candidate vaccine is the demonstration of safety and toxicology tests in animals. Safety and toxicology studies aim to assess any direct toxic effect of the vaccine or the induced immune response. Testing is often performed in two animal species, typically rodent and nonhuman primate. These studies allow assessment to both toxicological and pharmacodynamics parameters related to the direct effects of the vaccine and the induced host immune response as measures of safety.

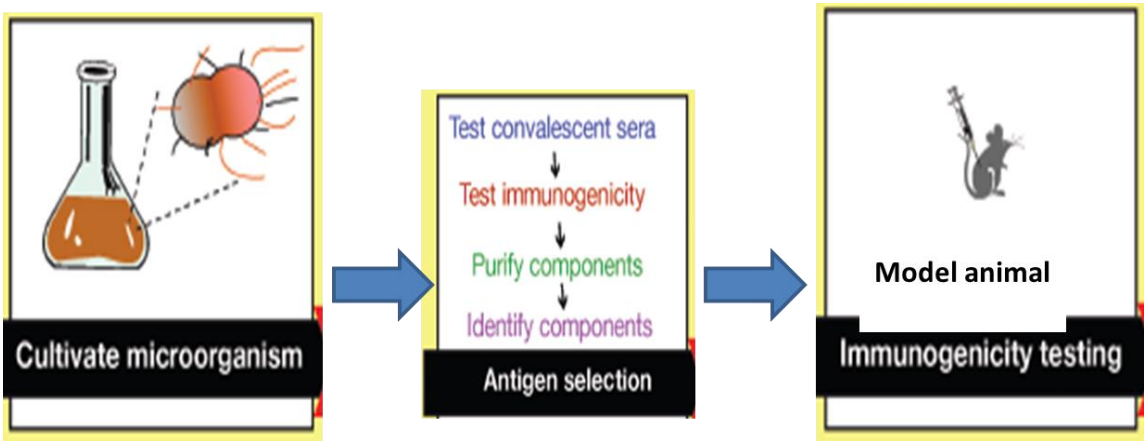

Figure-7: Schematic of preclinical studies in evaluating vaccine candidate.

9.2. Clinical trials:

The clinical tests of a new vaccine candidate help to assess to the safety, immunogenicity, and efficacy of that product in human recipients. Clinical trials occur in a stepwise fashion and include measurement of safety, immunogenicity, and efficacy.

Phase I: Trials are generally the "first carried on human", these trials usually involve <100 volunteers of healthy individuals and are intended to provide basic information on safety and tolerability, often will include some assessment of immunogenicity. Subjects are often drawn from the non-intended target population; for example, pediatric or geriatric vaccine candidates might

undergo initial testing in adults until basic safety is assured. Because of their small size, they can detect only common adverse events.

Phase II: Phase II trials are larger studies, usually involving several hundreds of subjects, and provide information about the vaccine's immunogenicity, dosing, and common side effects. These studies will provide a more extensive assessment of common adverse events. Dose ranging, the determination of an optimal dose for greatest safety and efficacy, will be assessed in phase II if not already included in phase I evaluation. There is often significant overlap between phase I and II studies during vaccine development. Studies performed on subject drawn from the target group.

Phase III: These trials represent the most significant investment of time and resources during the development process. These are large-scale studies involving thousands of subjects. Sample sizes large enough to ensure that questions about safety and efficacy will be assessed (these are often referred to as pivotal studies). They can also facilitate detection of lower frequency adverse events, intended to demonstrate clinical efficacy of the candidate vaccine. Subjects are meticulously observed for adverse events in the immediate post-vaccination period and sometimes as long as 45 days. Longer periods of observation allow for assessment of persistence of immune responses, protection from disease, side effects and adverse events of special interest. They are often multisite studies, sometimes including study sites in multiple countries, and are randomized, placebo controlled, and double blinded (i.e., study participants and investigators do not know who receives vaccine or who receives the control). Generally, two trials are required unless a single large trial generates clear evidence of efficacy.

Phase IV: In addition to very rare side effects, pre-licensure trials may not be able to detect adverse events with delayed onset and reactions in culturally or ethnically diverse populations. Formal post-marketing studies are designed to detect such rare events (pharmaco-surveillance).

10. Types of antiviral vaccines

There are four basic approaches to produce antiviral vaccines Figure-8. Each uses components of the parental pathogenic virus target. Vaccine developers may produce large quantities of the virus of interest and chemically inactivate it. (Inactivated vaccine), attenuate the pathogenicity through laboratory manipulation (replication-competent**,** Live attenuated vaccine), produce individual proteins free of the viral nucleic acid (Subunit vaccine), or molecularly clone all or portions of the viral genome for preparation of recombinant DNA vaccines (recombinant vaccine). The lasts are

also referred to as the modern vaccines. They can be produced by simply cloning one or more genes coding for immunogenic proteins of a pathogen and express it in a recombinant system (Recombinant subunit vaccines). Elsewhere, gene sequence such as a DNA or mRNA molecules are directly transferred to the host (DNA/mRNA vaccines). A chimeric virus produced after cloning the sequences of immunogenic genes of a targeted pathogen onto a virus vector like adenovirus (virus vector vaccines). The list in Table-2 illustrate some examples of these vaccines currently commercialized belonging to different types of vaccines.

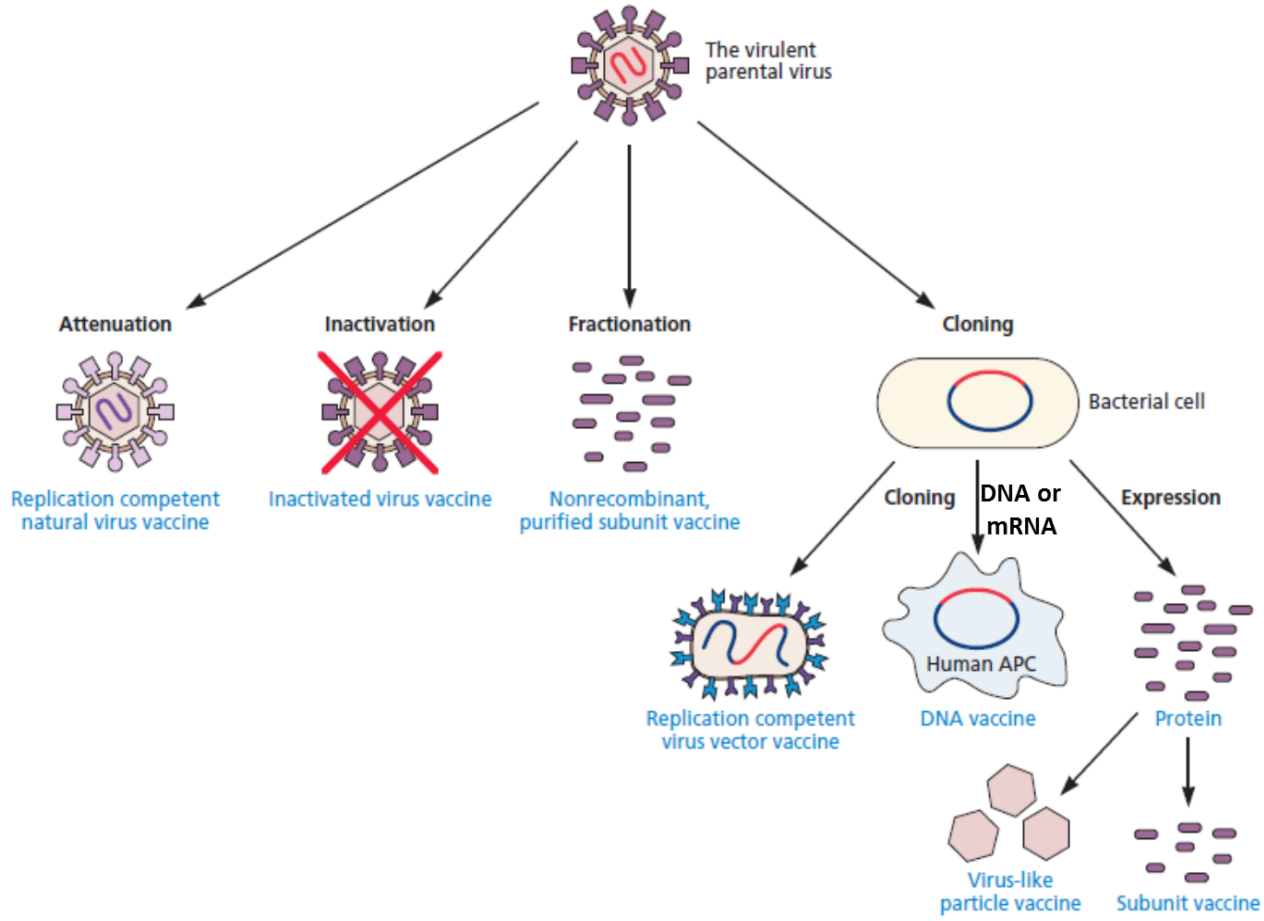

Figure-8: Schematic diagram showing the different strategies for generating different types of vaccines.

Table 2: Some currently licensed antiviral vaccines for human. Showcasing key targeted diseases, vaccine type and efficacy.

Pathology	Virus type	Type of vaccine	Efficacy
Variola	Variola virus	Vaccinia virus	100%
Polio	Picornavirus	Oral: Live Attenuated Injectable : inactivated	>95% >95%
Hépatitis A	Picornavirus	Inactivated virus	>90%
Hépatitis B	Hepadnavirus	Sub-unit	>80%
Influenza	Orthomyxovirus	Inactivated virus	50-70%
Measles	Paramyxovirus	Live Attenuated	>95%
Mump	Paramyxovirus	Live Attenuated	>90%
Rubella	Togavirus	Live Attenuated	>95%
Varicella	Varicella zoster	Live Attenuated	>80%
Rabies	Lyssavirus	Inactivated virus	100%
Yellow fever	Flavivirus	Live Attenuated	>90%
Japenese Encephalitis	Flavivirus	Inactivated virus	>90%

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Chapter-2: Live attenuated viral vaccines

1. Origin of the Live-antiviral vaccines

The origins of modern vaccination, particularly live viral vaccines, derive from the practice of variolation, which involves administering small doses of the smallpox virus to prevent subsequent infection. In 1796, E. Jenner developed the first live viral vaccine against smallpox infection (also known as variola). This was the first known vaccine used in the history of humanity. Jenner observed that milkmaids exposed to cowpox appeared to be "resistant" to acquisition of smallpox. Jenner tested whether inoculation of subjects with material from cowpox lesions protected individuals against smallpox upon direct exposure. Indeed, the approach was successful, and the term "vaccination" (derived from the Latin word *vacca*, meaning "cow") was introduced to describe the procedure. Smallpox vaccine was extraordinarily successful globally and, indeed, smallpox is today the only disease in the history of humanity eradicated by immunization.

2. Live wild-type antiviral vaccines

In some cases, a virus infecting one animal species can be used to infect another species in order to induce protection against a closely related target pathogenic virus. These vaccine viruses are generally adapted to replicate in their natural host. They are capable of infecting other organisms but replicate poorly and have limited or no pathogenic effects. However, these vaccine viruses may share common antigenic determinants with target pathogenic viruses. Resulting in an effective immune response against that virus. They are the simplest type of antiviral vaccine to produce and generally, the most effective in terms of the immune response induced. The only representative antiviral vaccine belonging to this group is the vaccinia virus, and in its present form, it is no longer being used, because smallpox has been eradicated. Although initial approaches to smallpox vaccination utilized cowpox virus, the smallpox vaccine historically employed in clinical practice was based on vaccinia, another related poxvirus, but one that has minimal potential for virulence in healthy, immune-competent individuals.

3. Live attenuated antiviral vaccines

Serial passage of pathogenic viruses in animals, eggs, or cells grown *in vitro* often results in the acquisition of a variety of mutations that reduce the pathogenic properties of the virus in its natural host. These are among the most successful vaccines currently used. These attenuated viruses replicate in humans and therefore can often induce protective immune responses as effectively as the parent viruses, while causing little or no disease.

3.1. Definition

Vaccines based on live attenuated viruses derives from the virulent pathogen. These vaccines consist of weakened viruses that multiply in the host and stimulate strong cellular and antibody responses similar to those induced by the natural disease. Attenuation of viruses in the laboratory proceed by their culture in animals or embryonated eggs, or by *in-vitro* cell culture in non-human cell lines. These vaccines correspond to live viral particles capable of replicating but lacking their virulence, generating microorganisms whose virulence for humans was reduced by adaptation to another different host,

Attenuated = rendered weakly virulent.

3.2. Manufacturing procedure

A standard procedure for developing live attenuated vaccine is to generate variants of attenuated strains that can grow well only under restricted conditions. One way to generate attenuated virus strains is by passage of a virulent strain in a cell line other than the host cell. For instance, cultivation of human viruses in a nonhuman cell line leads to the induction of mutations in the viral genome during adaptation. The accumulation of mutations often leads to the generation of a new virus strain that has lost virulence in a process termed "attenuation."

Live attenuated vaccines are easy to generate for certain viruses, particularly viruses with RNA genomes due to the higher mutation rates of their RNA-dependent RNA polymerase compared to viruses with DNA genomes and their DNA-dependent DNA polymerase (e.g., herpesviruses),

3.3. Cell culture

In 1940s, a revolution happened with the discovery of *in vitro* cell culture used as support for viral growth. Virologists demonstrate that many viruses could be grown in cell culture, including polio and measles viruses. Those methods were vigorously taken-up by vaccine developers. The oral

polio vaccine of Albert Sabin and the measles, rubella, mumps, and varicella vaccines were all developed *in vitro* through selection of clones by cell-culture passage. In essence, passage in cell culture induce an adaptation of virus to growth in that medium, and the best growing mutants have often lost or modified the genes harboring pathogeny and spread within human host. Examples of cell lines commonly used for the attenuation MRC-5 and Vero cells.

4. Cell culture attenuation steps in attenuated virus-based vaccines

(see Figure-9 and Table-3)

1- Firstly, the pathogenic virus is isolated from patients and grown in human cell culture in laboratory in order to propagate the virus.
2- The virus generated from human cell culture is used to infect other non-human cells (e.g. monkey cells).
3- Following numerous passages in non-human cells, the virus undergoes numerous mutations as consequence of the adaptation to the novel host.
4- Viral particles purified from non-human cells lose their pathogenic properties in human hosts and retain their immunogenic characteristics. (Used as a live attenuated vaccine).

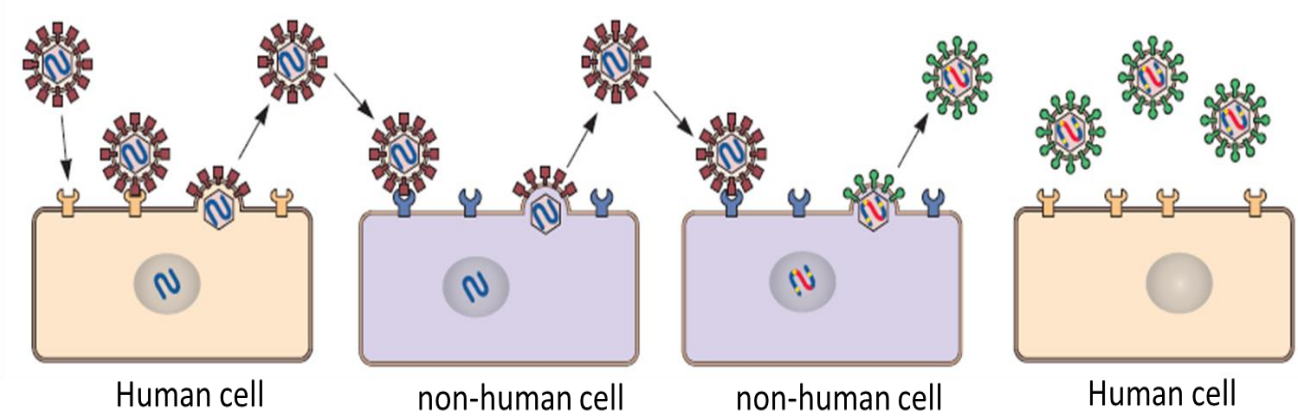

Figure-9: Illustration of human live virus attenuation process by serial passage in non-human cell lines culture.

Table 3: Passage histories of the Oka varicella vaccine.

Oka-strain varicella virus
• Isolated by Takahashi et al. in 1974 from three-year-old Japanese boy (family name Oka) • Initial growth in human lung fibroblast cells • Twelve passages in primary guinea pig fibroblasts • Two passages in WI-38 cells (human diploid fibroblasts) • Three to six passages in MRC-5 cells (human diploid lung cells) • Licensed for use in Japan and Korea in late 1980s, and in USA in 1995

5. Live attenuated viral vaccines characteristics

The attenuated virus vaccines consist of weakened viruses that replicate in the host and stimulate strong cellular and antibody responses similar to those induced by the natural disease. They usually give protective immunity after one or a few doses. Examples include oral polio, rotavirus, influenza (intranasal), measles, mumps, rubella, smallpox, varicella, yellow fever and hepatitis A vaccines. These replication-competent, attenuated vaccines are effective for two reasons. Progeny virus particles are generally restricted to tissues around the site of inoculation, they do not spread to distant tissues, and this local restriction generally results in mild disease. However, the limited virus reproduction stimulates a potent and lasting immune response.

Usually the administration of a small dose is enough to replicate, proliferate and become able to activate the immune system. . It should be noted that in most situations the wild-type infectious agent will stimulate the immune system to induce lifelong immunity against the specific infectious disease while live vaccines stimulate long-term immunity that may be lifelong (e.g., yellow fever) or not (e.g., measles). Thus, for some live vaccines, booster doses help to maintain protective immunity years after the initial immunization, while other vaccines require two doses to provide an initial protective immune response (e.g., rotavirus). The nature of the immune response developed by live attenuated virus-based vaccines is similar to that triggered by infectious agents (cellular and humoral responses). The list in Table-4 shows approved live attenuated viral vaccines in the United States up to 2025.

6. Disadvantages of live attenuated viral vaccines

Despite the advantages of live attenuated vaccines, there are some downsides.

Risk of reversion into virulent state: All organisms observe mutations naturally, and the particles used as live attenuated vaccines are not different. There is a possibility that an attenuated particle in the vaccine could revert to a virulent form and cause disease. Nonetheless, continued testing of live vaccines for genetic stability and safety testing, is important for safety surveillance of live vaccines. Alternatives to the classical approach of attenuation can now be applied based on modern virology and recombinant DNA technology. For example, creating deletions/mutations with low probabilities of reversion are frequently practiced.

Immunocompromised individuals are not eligible for this type of vaccine, for their own protection, individuals who are immunocompromised or immunosuppressed should not normally receive live attenuated vaccines.

Storage conditions must be rigorous: Another limitation is that live attenuated vaccines usually need refrigeration to stay potent. A live vaccine may not be the best choice if the vaccine shipped overseas and stored by health care workers in developing countries where the ambient temperature is high and there is a lack of widespread refrigeration. Likewise, vaccines manufacturing cost can be high for those countries.

Potential contamination of vaccines with other laboratory materials: Ensuring purity and sterility of the vaccine is a problem inherent in the production of biological reagents on a large scale. If the cultured cells used for the propagation of attenuated viruses are contaminated with unknown viruses, or other contaminations coming from other laboratory materials.

Lack of capacity to activate the immune system after attenuation: due to the loss by mutations in the immunogenic motifs.

Not all pathogens can grow in cultured cells (lack of substrate).

Table 4: List of live attenuated viral vaccines licensed in the United States.

Vaccine name	Tradename	Manufacturer
Adenovirus Type 4 and Type 7 Vaccine	None	Barr Labs
Influenza Vaccine	FluMist Quadrivalent	MedImmune
Measles, Mumps and Rubella	PRIORIX	GlaxoSmithKline Biologicals
Rotavirus Vaccine, Oral	ROTARIX	GlaxoSmithKline Biologicals
Smallpox and Mpox (Vaccinia)	ACAM2000	Emergent Product Development Gaithersburg
Varicella Virus Vaccine	VARIVAX	Merck Sharp & Dohme
Zoster vaccine	Zostavax	Merck
Chikungunya Vaccine, Live	IXCHIQ	Valneva Austria GmbH
Yellow Fever Vaccine	YF-VAX	Sanofi Pasteur

7. Examples of live attenuated vaccines

7.1. Oral Polio vaccine (OPV)

The first live attenuated vaccine available was the OPV developed in 1961 by Albert Sabin. The Sabin's vaccine is made-up of three different serotypes of poliovirus (types1–3), all of which can cause paralytic polio. The vaccine originally generated by passage of poliovirus in monkey cells and human diploid cell cultures, using sub-physiological temperatures that allowed growth of virus, but facilitated the accumulation of attenuating mutations. Following tissue culture passage, mutations accumulate in the sequence of all three strains that lead to a loss of virulence and an inability of the viruses to cause paralysis. One of the features of these attenuating mutations is a reduction in the ability of translating the viral messenger RNA by the ribosome. The oral polio vaccine (OPV) is administered in four-dose series. The vaccine contains mixtures of all three attenuated polioviruses strains. These attenuated vaccine strains replicate very efficiently in the gastrointestinal tract, as does wild-type poliovirus, which probably contributes to an enhanced immune response compared to inactivated polio vaccine (IPV). Oral administration of the vaccine

not only provides convenience in administration but also represents an appropriate route for the induction of IgA antibodies, which provides protection in the mucosal site. In consequence, Sabin's OPV vaccine is mainly used for the poliovirus eradication program initiated by the WHO.

7.2. Live attenuated Influenza vaccine (FluMist)

Influenza is an RNA virus belonging to the Orthomyxoviridae family. There are two major subtypes of influenza virus: influenza A and influenza B. Both are important human pathogens. Influenza has a segmented RNA genome and influenza-A virus can undergo extensive recombination in nature, leading to the appearance of genetically variant strains not previously encountered in the human population. Universal annual influenza vaccination is eligible for every one over 6 months of age. Live attenuated influenza vaccines have only recently become available for clinical use. These vaccines are manufactured by passaging clinical isolates of influenza in cell culture using sub-physiological temperatures (25°C). This temperature incompatible with replication of the virus at 37°C, these vaccines are highly attenuated and extremely safe after cold adaptation process. The vaccine administration is by nasal drop or spray on an annual basis. The vaccine is in trivalent form, containing two strains of influenza A and a single strain of influenza B, or tetravalent, containing two strains of influenza A and two strains of influenza B, depending up on the manufacturer. The strains chosen to compose the vaccine each flu season depend upon what strains of influenza have recently circulated, or predicted by public health officials, to be circulating in the upcoming influenza "season". The mechanism of protection is mainly the induction of IgG antibody against influenza, particularly by production of hemagglutinin-specific antibodies.

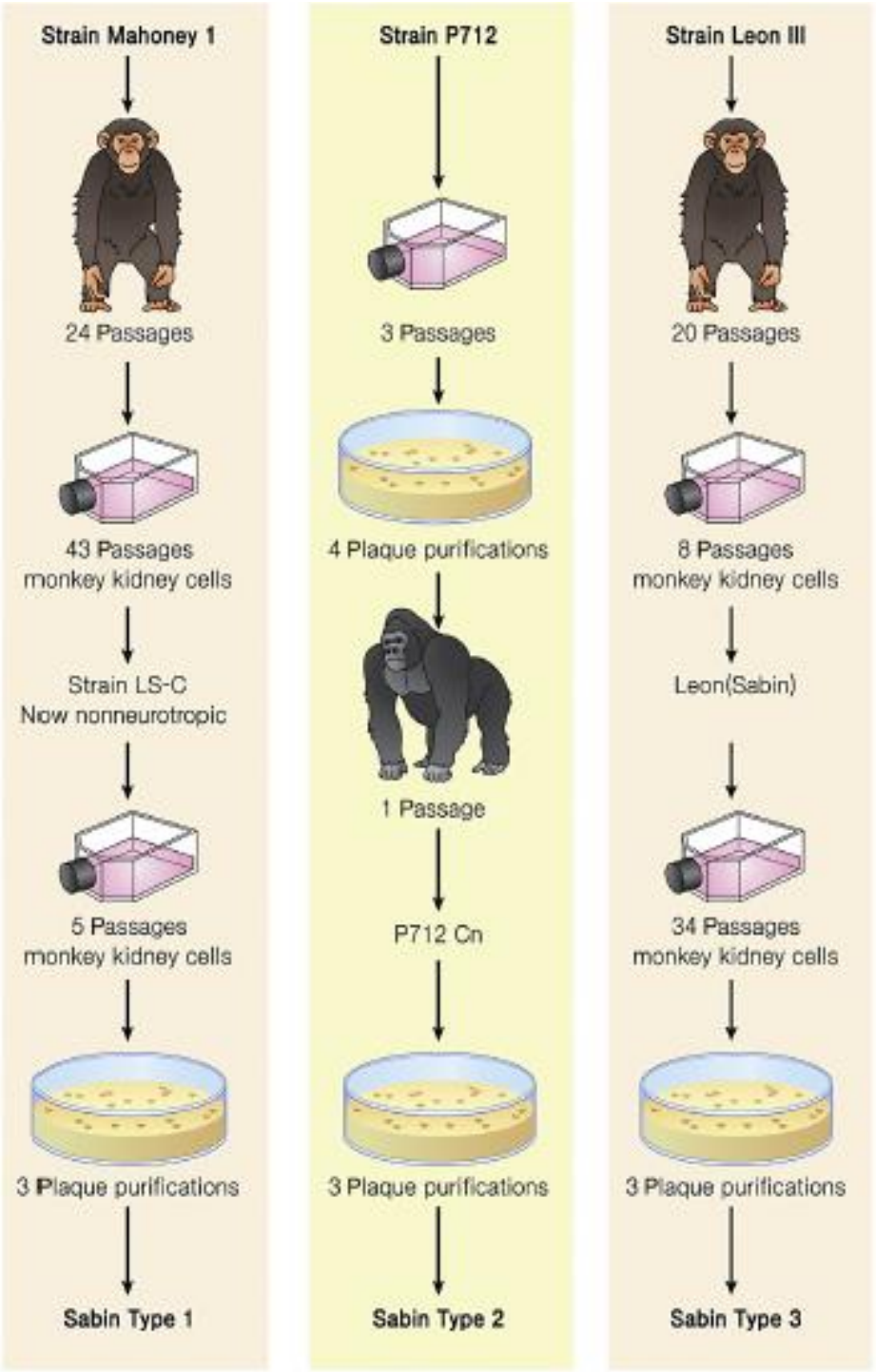

Figure-10: Production scheme of Sabin live attenuated poliovirus vaccine (OPV).

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Chapter-3: Inactivated viral vaccines

1. Origin of the inactivated (killed) antiviral vaccines

The foundations of immunization with inactivated virus preparations were laid at the end of the nineteenth century with L. Pasteur's partially inactivated rabies virus. In 1880, L. Pasteur and his colleagues attributed rabies disease to the replication of the rabies virus in the central nervous system (CNS). Then they demonstrated that drying at room temperature of desiccated spinal cords from rabid rabbit reduced their infectivity. Later they discovered that these dried rabbit spinal cord tissues infected with rabies virus are non-infectious. Moreover, inoculating dogs with these tissues gave them protection against lethal doses of rabies virus. In 1885, L. Pasteur and collaborators inoculated rabbit spinal cord tissues from rabid rabies and dried during 15 days at room temperature into a 9-year-old boy bitten by a rabid dog 2 days earlier. They carried out 13 inoculations over 10 days. Thereafter they challenged the vaccinated kid with lethal dose of rabies virus. In consequence, the child became resistant to rabies virus, the first partially inactivated anti rabies vaccine used on human was effective showing a clear therapeutic and protective effects.

2. Definition

Inactivated antiviral vaccines are a common way for vaccines production when a live vaccine is not available. Pathogenic virus is first cultivated on a substrate to produce large quantities of virus using primary cells, tissues, chicken fertilized eggs, and even whole organisms as substrates for virus propagation. Today, vaccine manufacturers are shifting toward virus growth on continuous cell lines. This reduce production costs and increased vaccine safety. Inactivation often involves purification and concentration of the virus after propagation. Inactivation is carried out by either chemical or physical methods. Among formaldehyde and β-Propiolactone (BPL) are widely used for inactivation of licensed human viral vaccines for decades. In the case of enveloped virus particles, nonionic detergents, such as Triton X-100, are preferred during inactivation. Inactivation leads to the death of the virus by degradation of its genome. In the absence of virus replication, the virus lacks infectivity, while retaining the antigenicity of surface viral proteins. Such inactivated vaccines can retain their ability to induce a strong immune response but are incapable of replication

and therefore do not cause disease. The best examples of inactivated virus vaccines include inactivated influenza vaccine, largely grown on chicken embryos but also in cell culture, where the virus inactivation is performed with formaldehyde or BPL (b-propiolactone). For example: inactivated poliovirus vaccine is grown in Vero cells in large bioreactors, then inactivated with formaldehyde; and hepatitis A vaccine, grown in MRC-5 cells on flat plate reactors and inactivated with formaldehyde. A list of licensed inactivated viral vaccines in the United States is shown in Table-5.

Table 5: List of inactivated viral vaccines licensed in the United States.

Vaccine name	Tradename	Manufacturer
Influenza Vaccine Trivalent	FLUARIX	GlaxoSmithKline Biologicals
Japanese Encephalitis Vaccine	IXIARO	Valneva Austria GmbH
Hepatitis A Vaccine	HAVRIX	GlaxoSmithKline Biologicals
Poliovirus Vaccine Inactivated	IPOL	Sanofi Pasteur
Rabies Vaccine	IMOVAX RABIES	Sanofi Pasteur
Tick-Borne Encephalitis	TICOVAC	Pfizer Ireland Pharmaceuticals

3. Inactivated vaccines characteristics

The inactivated vaccines are entire virus particles treated with chemicals such as formaldehyde) or physical methods like heat or radiation. Despite being dead, the inactivated virus vaccine retains its original antigenic structure, including surface proteins. When introduced to an individual, immune cells recognize these viral antigens, triggering a protective immune response. The key characteristics of these vaccines are:

Immune response: When introduced into the body, immune cells recognize these viral antigens, triggering a protective immune response, including antibody production and helper T cell activation directed against many antigens simultaneously. Generally, the immune response induced by inactivated vaccines tend to induce humoral response with antibodies secretion and weak cellular responses.

Reduced immunogenicity: The live vaccines induce superior immune responses compared to inactivated vaccines. The inactivated virus's inability to replicate means the immune system receives a relatively fixed amount of antigen, which may result in shorter-lasting immunity compared to live-attenuated vaccines more booster doses help to maintain a protective immune response as the first dose is generally insufficient to produce a protective response. The time length between prime and boosts shots is vaccine specific and depends on many criteria, including the age of the vaccinated person, the amount of vaccine in a dose, and the adjuvant used in the vaccine.

Safety: In the absence of replication, these vaccines are safe, there is no risk of reversion, especially for immunocompromised individuals, such inactivated vaccines are more stable and safer than live vaccines.

Furthermore, inactivated vaccines usually do not require refrigeration, and they can be easily stored and transported in a freeze-dried form, which makes them more accessible to people in developing countries. However, the costs to produce inactivated vaccines are often higher than live vaccines.

4. Disadvantages of inactivated viral vaccines

Incomplete inactivation: Contamination of vaccine stocks with potentially infectious viral nucleic acids form the major problems with this type of vaccine, though improved methods to detect residual infectious virus have reduced this risk substantially.

Sometimes, the production of this type of vaccine requires handling of large amount of high-risk pathogens.

Risk of denaturation of antigen epitopes during the inactivation step, this reduce the immunogenicity of the vaccine.

The production costs can be high.

5. Adjuvants

Latin *adjuvare*, to help. A substance added to vaccines that increases the magnitude and/or duration of the immune response to the vaccine antigen. Adjuvants are supplemented to vaccine antigens to enhance and modulate the immunogenicity of the antigen. Adjuvants can also modulate the type of immune response elicited by the vaccine. Actually more-specific and -powerful adjuvants are

being discovered and employed. Vaccines optimization involves the use of different combinations of adjuvant and immunogens. Adjuvants act by stimulating early intrinsic and innate defense signals, which then shape subsequent adaptive responses. These immunostimulators function in three distinct ways: by presenting antigens as particles, by sequestering antigen at the site of inoculation, and by directly stimulating the intrinsic and innate immune responses. Adjuvants are useful for antigens such as inactivated, subunit, and recombinant protein vaccines, which can lose, during the purification process some immunogenicity needed to trigger an adequate immune response. Adjuvants are included in vaccine candidates to enhance the efficacy of weak antigens to induce appropriate immune responses. Implementation of adjuvant use expected to reduce the amount of antigen needed by 5-10 fold.

Adjuvants vary in composition, from complex mixtures of killed mycobacteria and mineral oil (complete Freund's adjuvant) to lipid vesicles or mixtures of aluminum salts.

Examples of licensed adjuvants currently used in approved vaccines in the USA or European Union include alum, oil in water emulsions such as MF59 and AS03, and liposomes.

6. Examples of inactivated viral vaccines

6.1. Rabies inactivated vaccine

L. Pasteur introduced an experimental rabies vaccine in 1885 when he observed the rapid decrease of rabies virus virulence upon air-drying of rabies-infected rabbit spinal cords. Serially less dried rabies-infected rabbit spinal cords containing inactivated or at least partially inactivated rabies viruses, induced protection of dogs and later humans against challenge following inoculation. This kind of vaccination, although considered a treatment for infected people at the time, represent the foundation for rabies vaccines. This set up later a new chemically inactivation of rabies virus using phenol in 1908 leading to the first completely inactivated rabies vaccine, despite the disruptive action of the phenol solution on the antigenic sites on the proteins.

Due to the fact, that vaccines based on adult mammalian nerve tissue were associated with side effects such as encephalomyelitis and demyelination lesions in the CNS due to the presence of myelin, cell culture propagation of the virus are preferred to spinal cords. There are two primary avian cell lines used for rabies vaccine production; purified chick embryo cell vaccine (PCECV) and purified duck embryo rabies vaccine (PDEV) and multiple immortalized cell lines such as

MRC-5, Vero, and primary hamster kidney cells. Despite the variation in vaccine cell substrates, the majority of rabies vaccines are inactivated in a similar manner at a concentration 1:3,500 and up to 1:5,000 v/v of BPL at 2–8 °C for 24 h. After inactivation, different purification methods are used such as ultrafiltration, ultracentrifugation or chromatography. Once formulated, the vaccine efficacy for all of these vaccines is tested in mice animal model. Vaccination must be administrated prior to rabies virus exposure or within the latent period after exposure to prevent disease.

6.2. Inactivated Polio vaccine

The first licensed vaccine developed using the cell culture technique was the trivalent, formalin-inactivated polio vaccine (IPV) of J. Salk, licensed in 1955, and the impact was immediate, it lowers the risk of polio infection. Inactivation process is accomplished in formaldehyde solution at a dilution 1: 4000 in 37°C for 15-20 days.

Despite of this success, IPV was replaced by OPV in the early 1960s, due to the added benefits conferred by contact immunity, ease of administration (oral vs intramuscular), and lower cost. When it became clear that there was no longer any circulating wild-type poliovirus in the United States, and in fact that the only paralytic polio observed that time was vaccine associated paralytic polio (VAPP) caused by the neuro reverting OPV, IPV once again became the vaccine of choice. The IPV vaccine is administered by intramuscular or subcutaneous route. A four-dose series in childhood is recommended. There is no intestinal or mucosal immunity induced by IPV, in contrast to OPV. The mechanism of action is the production of IgG antibodies capable of neutralizing the virus and protecting the central nervous system against paralytic polio. Today, WHO .recommend the statistical sampling to control the completeness of inactivation of IPV on 1500 adult doses tested *in vitro* on a susceptible cell type using virus titration assay. Absence of Polio Virus infection in cell cultures inoculated with these doses for at least 3 weeks is required before the vaccine formulation.

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Chapter-4: Subunit viral vaccines

1. Introduction

The conventional antiviral vaccines have a little structural variation in antigenic motifs present on the target pathogen, as in the case of Polio, Measles, Rabies, etc. These vaccines are highly effective in preventing associated viral diseases. Moreover, the immune response generated by inactivated vaccines is mainly humoral. The synthesis of neutralizing antibodies is sufficient to eliminate the target pathogens from the body.

However, the use of attenuated antiviral vaccines capable of generating adequate cellular responses is ineligible in certain cases of virus infections because of their genetic instability and the risk of reversion to the pathogenic state. In consequence, conventional inactivated vaccines are not very effective in generating an adequate cellular immune response to many intracellular pathogens, which are responsible of many chronical infections. While, some viruses, such as HIV-1 and HCV, develop escape mechanisms from the immune system. These are difficult to counter with traditional vaccination strategies. In fact, there is an urgent need to develop new vaccines against many viral infections: such as respiratory syncytial virus (RSV), HIV, hepatitis C virus (HCV), Epstein-Barr virus and cytomegalovirus ... etc.

2. Modern vaccines

Innovative genetic engineering techniques have had a major impact on the development of novel research strategies in antiviral vaccinology field with the aim to produce non-virulent vaccines that generate effective immune responses against different viral infections. These systems allow the production of several vaccine antigens without use of the native infectious organism. Genetic engineering is exploited to produce many antigen candidates for vaccine systems by producing antigens in heterologous culture.

Since the 1980s, a large number of candidate molecules for vaccine purposes in recombinant form have been developed using various genetic engineering technologies. These exploit the properties

of heterologous production systems: bacteria, yeast, plant, insect and animal cells. Now, there are different types of vaccines available based on sophisticated genetic engineering methods (recombinant vaccines): Subunit, virus like particles (VLP), DNA, mRNA and virus vector vaccines.

Recombinant DNA techniques enable the selective cloning of specific viral genes in various non-pathogenic organisms such as viruses, bacteria, yeasts, plants, insect cells, etc. allowing the production of highly immunogenic viral proteins for subsequent use as vaccines. The first vaccine developed through genetic engineering tools was against the Hepatitis B virus. Innovative recombinant DNA techniques made it possible to clone the coding sequence of the HBV S antigen in yeast cells (*Saccharomyces cerevisiae*) and also in mammalian cells, enabling the production of large quantities of the recombinant protein (S antigen), used as a recombinant subunit vaccine, the production scheme is shown on Figure-11.

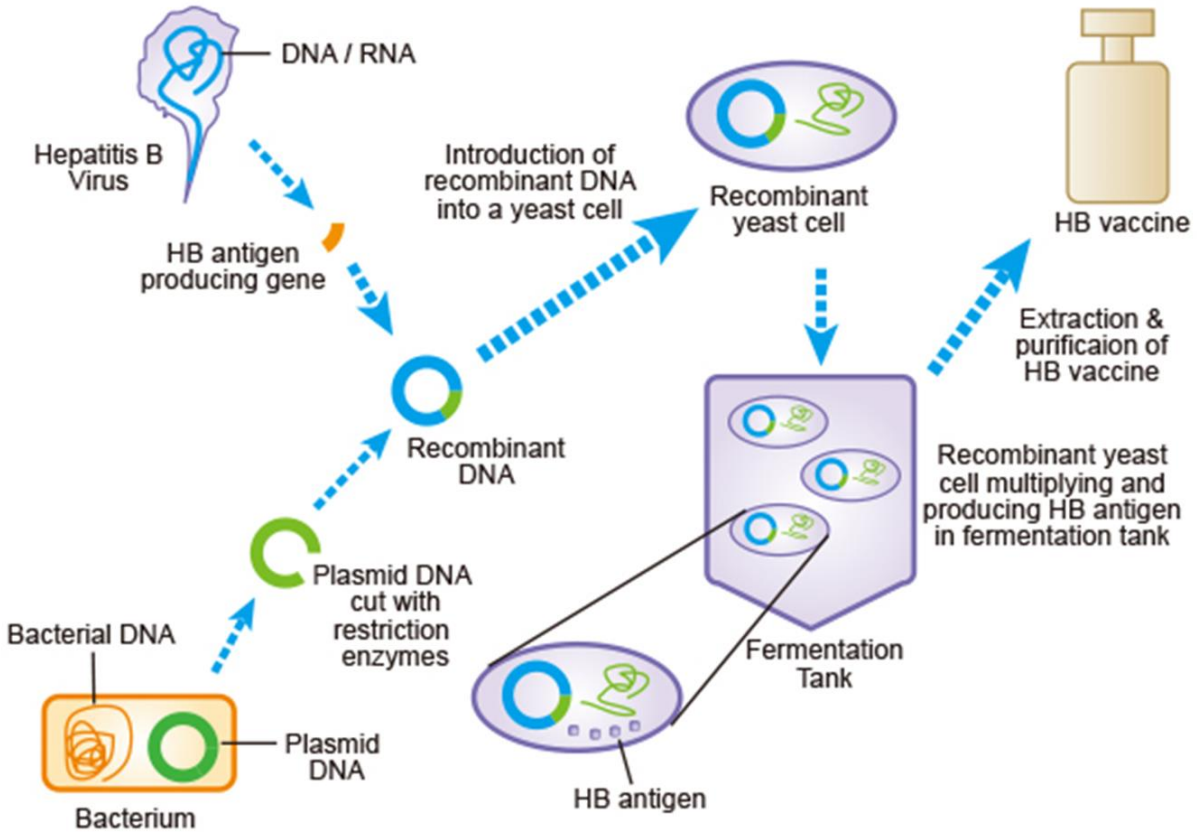

Figure-11: Schematic illustration of the production steps of a recombinant Hepatitis B vaccine.

3. Recombinant Subunit viral vaccines

The production of vaccine molecules is made-up by the expression and purification of higher immunogenic viral proteins expressed in heterologous recombinant systems. These recombinant viral proteins are capable of strongly stimulating the immune system. They are made-up of proteins containing the epitopes of the virus recognized by the immune system as shown in Figure-12. Only the antigenic substances of the pathogen that are necessary to elicit successful immune responses are included in subunit vaccination. The determination of which viral proteins to include in a subunit vaccine is accomplished by selecting those highly recognized by antibodies and cytotoxic T lymphocytes. This selection can be determined by assessing the immune responses of individuals who have recovered from the disease. There is no chance of disease development with subunit vaccines, because they do not include the entire pathogen solving a major safety problem inherent in inactivated virus vaccines. Viral structural proteins are important for development of this type of vaccine because of their high antigenicity and immunogenicity, of which the surface glycoprotein are the most important. Examples of licensed viral subunit vaccines are listed in Table-6.

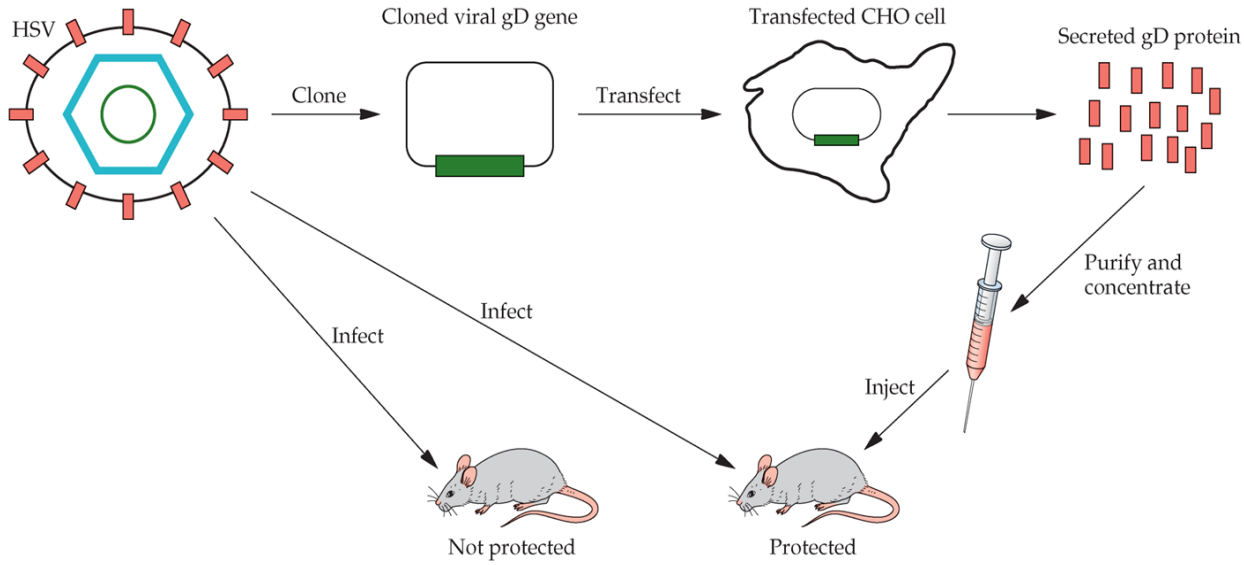

Figure-12: Outline of the strategy followed in the production of a recombinant subunit vaccine (glycoprotein gD) against the human herpes virus (HSV-1) in mice model.

4. Procedure for manufacturing subunit antiviral vaccines

Recombinant subunit vaccines are manufacturing involves several steps:

1- Identification and isolation of one or more viral genes encoding viral proteins containing the virus epitopes with the highest immunogenicity.
2- Transfer of the genetic material (genes) to a host cell system (heterologous expression systems) such as bacteria, yeast, insects, plants, etc.
3- Expression and production of viral antigenic proteins in those expressing systems.
4- Extraction and purification (high purity) of expressed recombinant viral proteins using a variety of chromatography techniques.
5- Purified recombinant viral proteins formulated as a recombinant subunit vaccine.

Table 6: List of inactivated viral vaccines licensed in the United States.

Vaccine name	Tradename	Manufacturer
Hepatitis A Vaccine	HAVRIX	GlaxoSmithKline Biologicals
Influenza Vaccine	FLUAD QUADRIVALI	Seqirus
Influenza Vaccine	Flublok	Protein Sciences Corporation
Respiratory Syncytial Virus	AREXVY	GlaxoSmithKline Biologicals
COVID-19 Vaccine	NUVAXOVID	Novavax
Zoster Vaccine Recombinant	SHINGRIX	GlaxoSmithKline Biologicals

5. Advantages:

Safety without risk of contamination: Subunit vaccines entails no risk of reversion or toxicity, even in immunodeficient subjects. For this reason, they are safer for immunocompromised individuals. As only a part of the viral material is used in the process, a portion of the viral genome is required for such production, there can be no contamination in the resulting vaccine with the original virus, solving a major safety problem inherent in inactivated virus vaccines. There is no chance of disease development because they do not employ the entire pathogen.

Specificity: Protein subunit vaccines also have high specificity for immunogenicity since they only recognize those proteins specific to the pathogen. These vaccines target specific proteins that are able to stimulate strong immune response with high capacity; they may be capsid proteins or surface glycoproteins. Subunit vaccines can provide a versatile approach to the protection from the infectious diseases owing to the possibility of combining large numbers of antigen at a time.

Possibility of producing large quantities of product with higher purity at very low cost (cost-effective). Viral proteins can be made in engineered organisms, under conditions that simplify their purification, in a relatively safer biological environment and quality control. They may also be significantly cheaper to manufacture, because they do not require expensive virus growth in cell culture systems and subsequent testing of the virus contamination. These viral proteins made under conditions that simplify purification and quality control.

For example: the appearance of complications in individuals taking the inactivated flu vaccine due to allergy to egg proteins can be bypassed by taking another recombinant Flu vaccine produced in *Escherichia coli* , insect cells or yeasts.

6. Disadvantages

Most of the subunit vaccine candidates induce partial to weak immune responses (absence of protective effect especially in the periphery (mucosal sites) and no longer IgA secretion.

Like inactivated virus vaccines, multiple doses of subunit vaccines are normally required for protection, and periodic boosting may be necessary to ensure long-term immunity. Like inactivated vaccines, subunit vaccines also typically include an adjuvant to enhance immunogenicity.

There are some subunit viral vaccines currently licensed and efforts to generate some other subunit vaccines were not successful. Numerous attempts to make HIV and HCV vaccines have failed. One of the reasons for the failure is that these viral antigens are not sufficiently immunogenic. Another issue is that IgA an immunoglobulin pertaining to mucosal defense against HIV and HCV infections is not sufficiently secreted by these vaccine candidates.

7. Subunit viral vaccines production in eukaryotic systems

7.1. Relevant criteria for the optimal production of recombinant subunit vaccines in eukaryotic systems

- Identification of the most immunogenic viral protein is crucial for the construction of a subunit vaccine.
- It is imperative to check that the eukaryotic system allows easy expression of the viral protein and that its production is not expensive.
- The coding gene of the viral protein must be associated with a strong promoter suited to the expressing system to allow a large amount production of the vaccine of interest.
- It is important to check that the expressed viral protein is completely separable from other contaminants; proteins, DNA and other cellular components.

Example: FDA standard for contaminating DNA in a vaccine must be < 10ng/dose.

Experimentally, there are Genetic Engineering tools that enable viral glycoproteins production in secreted form, making it easier to separate them from other cellular components (this involve the introduction of a secretory signal sequence within the gene encoding protein).

- Purified viral proteins must adopt the spatial conformations required to preserve their biological activities. For example, the inclusion of correct post-translational modifications characteristic of each viral protein is important.
- Viral protein purification methods must preserve the integrity of the protein epitopes capable of inducing an appropriate immune response.
- In cases of low immunogenicity of the vaccine, adjuvants are added to the formulation.
- The production of recombinant subunit vaccines in eukaryotic systems represented by yeast, insect or mammalian cells are commonly used in the production of viral proteins for the research and development of new viral vaccines.
- The development of immortalized mammalian cell lines provides optimal expression systems for the production of viral proteins. These systems provide an environment very close to the physiological conditions encountered during infection.

Examples of mammalian cell lines:

The CHO (Chinese Hamster Ovary) cell line is the preferred one for the production of viral proteins of therapeutic interest. It is successfully used for example, in the production of glycoproteins from HIV-1 and HSV-1 viruses, which are currently undergoing clinical trials.

- Cell lines of human origin: Characterization and development are in progress for the expression and production of viral proteins using human cell lines. Examples of cells being explored are the COS cell line derived from monkey kidney tissue (non-human primate origin), Human embryonic kidney (HEK) 293, etc.

7.2. Example of current subunit vaccines produced in eukaryotic systems

1- The recombinant hepatitis B subunit vaccine is produced in yeast cells (*Saccharomyces cerevisiae*).

There are a number of viral antigens difficult to purify from yeast cells (e.g. rabies virus glycoproteins).

2- The recombinant-subunit influenza vaccine production use the Baculovirus/insect cell system. This vaccine construct is produced by inserting the HA gene, hemagglutinin: membrane glycoprotein of the influenza virus, into Baculovirus viral vector, followed by infection of the insect cells with this recombinant virus, which enables these cells to produce large quantities of the HA viral protein (1 mg/1 million cells).

This recombinant influenza vaccine is highly immunogenic in adults and less active in children.

The insect cells used to produce influenza subunit vaccine are *Spodoptera frugiperda* (SF) -9 or 21 and *Trichoplusia ni* BTI 5B1-4.

8. Production of subunit viral vaccines in the prokaryotic system

The expression of recombinant protein is widely performed in bacterial hosts, with *E. coli* being the most prominent. Viral vaccine production in prokaryotic system uses bacteria to produce recombinant viral proteins. Prokaryotic systems are cost-effective for producing vaccines for global use. These systems allow for rapid production, which is crucial during a global pandemic. *E coli* system support high rates of protein vaccine production. In addition, *E. coli* is easy to handle in standard laboratories. However, the production of immunogenic viral proteins in a prokaryotic system is very difficult, due to some limitations, related to the existence of an adverse environment

for eukaryotic proteins. Expressed foreign proteins often form insoluble inclusion bodies due to the aggregation of the misfolded viral proteins within the bacterial cells.

This is also due to the absence of the processes required for protein maturation (glycosylation, acylation, phosphorylation, disulfide bonds formation etc.). The lack of correct posttranslational modifications and the proper eukaryotic chaperones can lead to the production of proteins in misfolded and aggregated states (inclusion bodies).

Prokaryotic systems cannot perform post-translational modifications that are essential for the proper folding and maturation of virus proteins particularly those infecting mammalian cells. This reflect the low immunogenicity observed on the produced viral proteins.

Bacteria have successfully made some viral proteins

1* Production of the ORF-2 protein of the hepatitis E virus, which has shown to be highly effective as a vaccine molecule according to the published results of a clinical study carried out on 100,000 people. Hecolin is an example of licensed vaccine against hepatitis B produced in *E coli* arguing the potential of bacterial systems in vaccines manufacturing.

2* Production of an HPV (Human papilloma virus) glycoprotein, although the associated clinical tests have not yet been published.

9. Subunit viral vaccines production in plants

There are currently genetically modified plants capable of expressing immunogenic viral proteins. Some of these viral proteins are currently undergoing clinical trials.

Advantage of the system: Plants have the advantage of expressing the human galactosidases that enable the fully glycosylation required for maturation of the viral proteins. They also have the advantage of being simple and cost-effective to grow up.

Examples:

*Expression of the VP1 protein of the Foot and Mouth Disease virus in the tobacco plant.

*Expression of rabies glycoproteins in plant system.

10. Methods of purification of viral recombinant proteins

The purification of viral proteins in their native and functional state is very important for applications in vaccinology.

Numerous purification methods are used:

1-Column chromatography

2-Exclusion chromatography

3-Ion exchange chromatography

4-Hydrophobic interaction chromatography

5-Affinity chromatography:

*Immune affinity

*Lectin affinity

*Immobilized metals

11. Examples of current subunit viral vaccines

11.1. Subunit Hepatitis A vaccine

Hepatitis A is an RNA virus classified in the Picornaviridae family. It is readily transmissible by the fecal–oral route and is highly contagious. Infection leads to liver inflammation "hepatitis". Although most infections are self-resolving, in some cases infection can lead to fulminant hepatic failure. Subunit vaccines based on formalin-inactivated hepatitis A virus, cultivated in MRC-5cells, are licensed and marketed (Havrix and Vaqta) by two manufacturers (GSK and Merck) in the United States. Hepatitis A vaccine is combined with hepatitis B vaccine in another combined form of vaccine licensed in the United States, a product known as "Twinrix". All vaccines are adjuvanted with aluminum hydroxide adjuvant. Two doses are given by intramuscular route in separate by at least 6 months. The mechanism of protection is mediated by the induction of hepatitisA-specific IgG antibody response. These vaccines work by presenting purified fragments to the immune system, which then produces antibodies to prevent future infection.

11.2. Recombinant subunit influenza vaccine Flublok

Flublok is a trivalent recombinant hemagglutinin (HA) vaccine, developed by Protein Sciences Corporation. Flublok contains HA protein antigens derived from three most prominent influenza

strains: influenza-A subtype H1N1, H3N2 and influenza B, which have been selected for inclusion in the annual influenza vaccine by the WHO and updated on an annual basis. The three proteins are produced in continuous insect cell line Sf9 cells (*Spodoptera frugiperda).* Each of the three recombinant HAs expressed separately in this insect cell line culture using a baculovirus vector. The individual HAs are extracted from the cells using extraction buffer and detergent, and further purified by column chromatography. Flublok provides an attractive alternative to the current egg-based trivalent inactivated influenza vaccine. Flublok does not contain egg-protein or preservatives. It is an alternative influenza vaccine for individuals developing allergies induced by egg products. Flublok contains three times more HA proteins than inactivated Flu vaccines. In fact, Flublok provide superior protection against influenza infection especially in at-risk populations (adults over 65 years, immuno-compromised, etc.). Flublok is recommended for people 65 years and older because this vaccine is potentially more effective than standard dose unadjuvanted-flu vaccines. Other benefits of recombinant flublok vaccine include that this vaccine technology is not dependent on an egg supply so the manufacturing process might be faster than that of egg-based vaccines during a pandemic event or shortage of the eggs needed to grow influenza viruses. It also avoids mutations that can occur when viruses are grown in eggs, which can sometimes affect the finished vaccine potency.

12. Synthetic peptide viral vaccines

Small fragments of viral proteins may be sufficient to induce protective immunity. Synthetic peptides of about 20 amino acids or more in length can induce specific antibody responses when chemically coupled to protein carriers, they are recognized, internalized, degraded, and presented by major histocompatibility complex (MHC) class II proteins within the cell presenting of antigens (CPA). Synthetic peptides have the basis for an extremely safe, well-defined vaccine, in which reversion or contamination with infectious virus is impossible. This vaccination strategy basic involves chemical synthesis of vaccine molecules from peptide motifs carrying the antigenic epitope of a viral protein. It is generally made-up of 20-30 amino acids. These peptide fragments are sufficient to trigger the stimulation of an appropriate immune response. This strategy avoids the side effects caused by the presence of numerous viral proteins that are not necessary for

activating the immune response. Many peptide vaccines are actually under clinical trials (see Table-7).

12.1. Disadvantages

To date, peptide vaccines have had little success, mainly because synthetic peptides are:

- Expensive to make in sufficient quantity,
- The antibody response they elicit is often weak and short-lived.
- Given the likely simplicity of host's "anti-peptide" response (usually a single epitope is contained within the peptide), selection of pathogen escape mutants is highly probable.
- Synthetic peptides are structurally unstable and poorly immunogenic.
- Requires the use of cargo molecules for stability and transport and the addition of adjuvants to trigger a robust immune response.
- Sometimes these cargo molecules and adjuvants are the cause of side effects and allergic reactions.

Table 7: List of synthetic peptide-based viral vaccines currently under clinical trials.

Peptide Vaccines Under Development		Clinical Indications for Candidate Peptide Vaccines Under Development
No. of Phase I Studies	270	Anticancer studies, Malaria, Falciparum Malaria, Anti-Plasmodium vivax, Influenza, Alzheimer's disease, Insulin dependent diabetes mellitus, Hand foot and mouth disease, anti HIV, HCV, HBV, CMV, Diabetes Mellitus, Type One, Cat allergy, Allergy
No. of Phase II Studies	224	Anticancer studies, anti HIV, HCV, HBV, CMV, Pneumococcal, genital Herpes—Herpes Simplex Type II, Tuberculosis, Diabetes, Diabetes Mellitus, Type One, Cat allergy, Ragweed allergy, Grass allergy, Ashtma, House dust mites - Rhinoconjuctivitis, maximum studies–anticancer
No. of Phase III Studies	12	Anti-cancer studies
No. of Phase IV Studies	NIL	No peptide vaccine reached market yet

12.2. Critical parameters for optimizing the construction of synthetic peptide for viral vaccine

- Choice of the most immunogenic epitope on the viral protein allowing the induction of strong immune response. Particularly in the case of intracellular pathogens, the design of the peptide candidate must focus on assessing its ability to generate a specific and robust cellular response.
- To generate a protective immune response, the peptide candidate must be effective to stimulate all together T lymphocytes (CTL), T helper lymphocytes and B lymphocytes.
- To provide more immunogenicity to the candidate peptides, compound long peptides have been designed, containing appropriate recognition motifs related to the different components of the immune system:

Linear motifs capable of being presented within MHC class I and class II molecules.

Linear motifs recognized by cytotoxic T lymphocytes (CTL).

Motifs adopting a spatial conformation recognized by B cells.

- Using cargo molecules and adjuvants to generate a protective immune response.

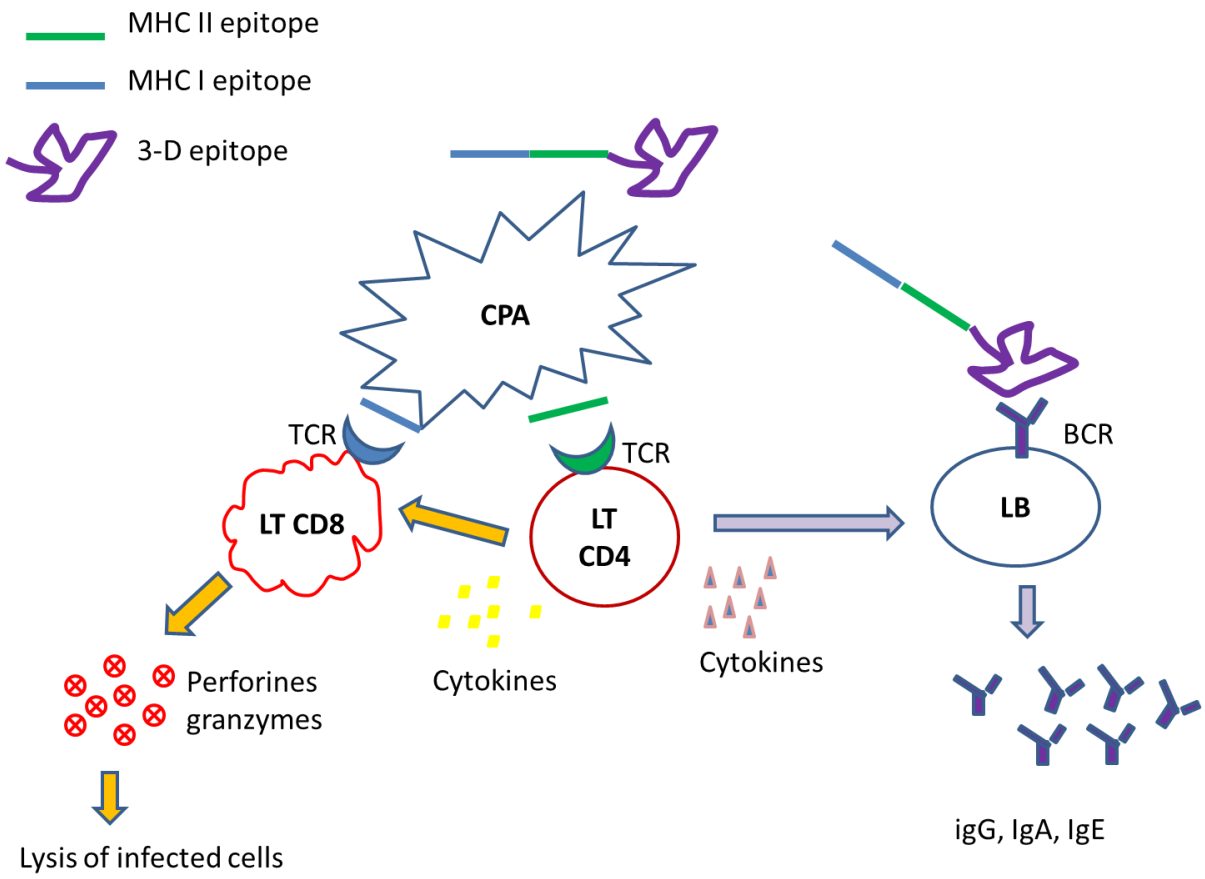

Figure-13: Schematic diagram illustrating the action of long synthetic peptide in activating various components of immune system.

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Chapter-5: Virus Like Particles (VLP) based vaccines

1. Definition

Vaccines based on virus-like particles (VLPs) are composed of one or more viral proteins with the ability to self-assemble after expression and purification of the protein(s) in a recombinant system. VLPs are protein multimers assembled from one or more structural proteins from the membrane of enveloped viruses or from one or more viral capsid proteins of naked viruses. These particles are organized into a multivalent array that resembles to the surface of geometrically rigid virions. Unlike virus particles, these capsids are empty: they contain no genetic material and they are not infectious. VLP are self-assembling, non-replicating, non-pathogenic, genomeless particles that are similar in size and conformation to infectious virions. However, these particles lack viral genetic material. As a result, they are unable to replicate in host cells. Thus, preserve the native antigenic conformation of the surface immunogenic proteins.

Other nanoparticle vaccines can be generated by mixing viral antigens with either lipids (e.g., liposomes, nano-emulsions) or proteins that tend to aggregate (e.g., proteosomes). Finally, VLPs that mimic the enveloped viruses can be generated in a number of prokaryotic and eukaryotic expression systems, including plants.

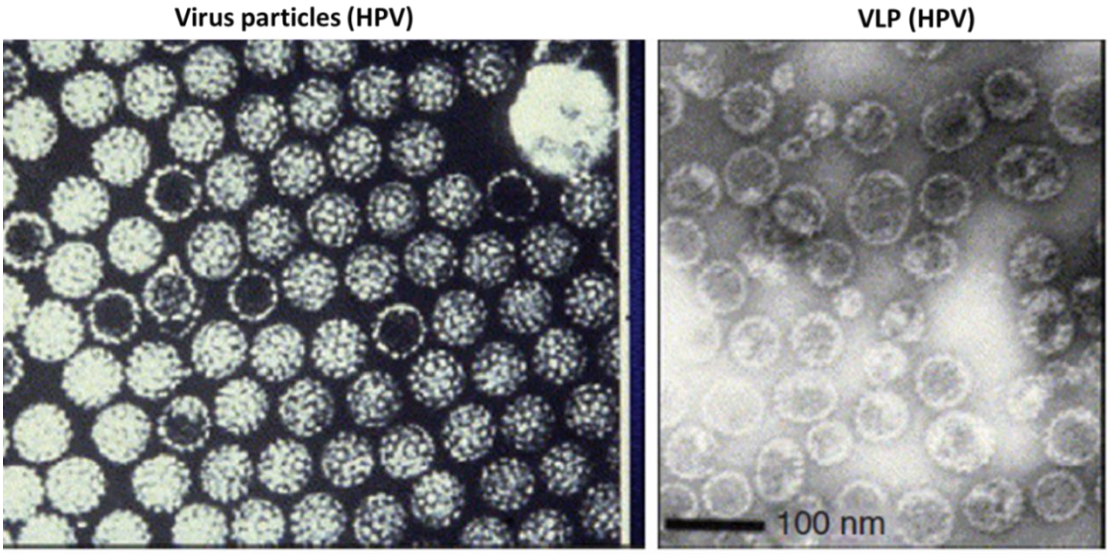

Figure-14: Electron microscope observations of HPV viral particles vs VLP.

2. Immunogenicity of VLP based vaccines

Virus-like particles are designed to mimic the overall structure of virus particles and, thus, preserve the native antigenic conformation of the immunogenic proteins. VLPs have many features, unlike conventional vaccines, which make them very attractive platform for vaccine design. VLP mimics the parental virus in terms of size (20–200 nm), geometry (i.e., icosahedral structures with multivalent epitopes) (Figure-15). VLP retain most of the conformational epitopes not found on purified or unstructured proteins, virus-like particle vaccines often induce durable neutralizing antibodies and other protective responses after injection. Furthermore, as the particles are completely noninfectious, inactivation with formalin or other agents is not required. This feature affords at least two additional advantages: immunogenicity is not compromised (formalin and other alkylating chemicals often alter the conformation of epitopes in inactivated vaccines), and help avoid concerns about efficiency of inactivation. The discovery of immunogenicity of VLP come from that the particles of hepatitis B surface antigen (HBsAg) found in HBV infected individuals are immunogenic and protective but noninfectious. These particles are purified from the blood of chronic carriers in 1981. The vaccine constructed from the purification of HBsAg particles purified from infected patients was note licensed because of the consideration that products derived from human blood considered potentially dangerous. These obstacles prompted the formulation of the first antiviral VLP vaccine (against hepatitis B)**,** HBsAg recombinant, which was licensed later in 1986. The Hepatitis B vaccine is based on the virus surface antigen or HBsAg, which upon expression in yeast forms spontaneously spherical VLPs that are then adsorbed onto aluminum as adjuvant. VLPs can induce potent, diverse, and durable serum antibody responses that are comparable to responses against live-attenuated pathogens. These empty viral particles can enter target cells by receptor-mediated and non-receptor-mediated mechanisms. Compared with soluble proteins/antigens, which often require multiple immunizations and the use of adjuvants to elicit protective immune responses, VLPs are capable of inducing strong cellular and humoral responses as direct immunogens. Table-8 illustrate a list of licensed VLP-based viral vaccines in the United States.

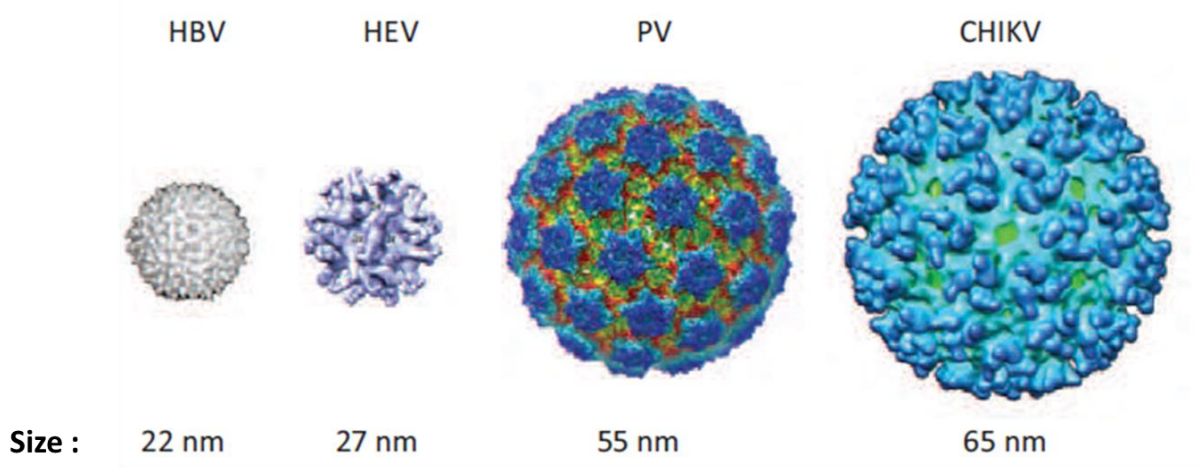

Figure-15: Examples of VLP particles observed with EM, the particle diameters are indicated on the bottom.

3. VLP based vaccines manufacturing procedure

VLPs are produced in various systems adapted to the expression of recombinant proteins. These production systems include bacteria (*E. coli*), yeasts (*S. cerevisae*), baculovirus/insect cells system, mammalian cell lines (CHO) and plant systems. The choice of expression system depends on the cost of vaccine production and the presence of various post-translational modifications required for the correct folding of the proteins, which can be essential in generating an optimal immune response. The generic manufacturing process for VLP-based vaccines generally consists of three main steps: production, purification, and formulation. The first step in VLP production is to clone the viral structural genes of interest. Thereafter, viral structural proteins with self-assembling ability are expressed in expression systems. After harvesting and lysing the cells, to ensure removal of contaminating cell debris and aggregates a clarification step is performed. Further purification steps such as ion-exchange chromatography and ultracentrifugation help generate intact and more purified VLPs. A final purification step allow remove the residual host cell proteins and nucleic acids. In the last step of manufacturing process of VLPs vaccine development, sterile filtration and formulation are accomplished, like addition of adjuvants, preservatives and stabilizers. To finally achieve a safe, efficient and effective product.

Table 8: List of VLP based viral vaccines licensed in the United States.

Vaccine name	Tradename	Manufacturer
Chikungunya Vaccine	VIMKUNYA	Bavarian Nordic
Hepatitis B Vaccine	RECOMBIVAX HB	Merck & Co
Hepatitis B Vaccine	ENGERIX-B	GlaxoSmithKline Biologicals
Human Papillomavirus 9-valent	GARDASIL 9	Merck Sharp & Dohme LLC
Human Papillomavirus Bivalent	Cervarix	GlaxoSmithKline Biologicals

4. Advantages of VLP based antiviral vaccines

- VLPs are safe products, since they do not contain the viral genome, and therefore, they cannot replicate in the host.
- Dendritic cells recognize easily the VLPs and their internalization is mediated by receptor-mediated endocytosis.

VLP proteins once processed inside the DCs, are presented in complex with MHC class I or class II to activate specific CD4+ or CD8+ T cells.

- VLP vaccines are capable of inducing robust cellular and humoral immune responses.
- VLP vaccines do not need to be attenuated or inactivated, which gives them the advantage of preserving the native conformations of antigenic epitopes. The virus inactivation process (in the case of inactivated vaccines) can induce denaturation of antigenic motifs.
- The antigenic epitopes of VLP vaccines are very similar to those found in native viruses, making it possible to generate a robust immune response adapted to the corresponding pathogens.
- Immune responses generated by VLP are much more effective compared to those generated by subunit vaccines based on protein monomers.
- The presence of antigens repeated motifs makes it possible to generate an optimal B lymphocyte response without the use of adjuvants (facilitating the phenomenon of BCR receptors cross-linking) as shown in figure-16.

- VLP vaccines can induce protective immune responses at low doses and often without the addition of adjuvants.
- The stability of VLPs simplifies the storage and distribution of vaccines.
- The VLP vaccines allow oral administration in certain cases of target viruses.

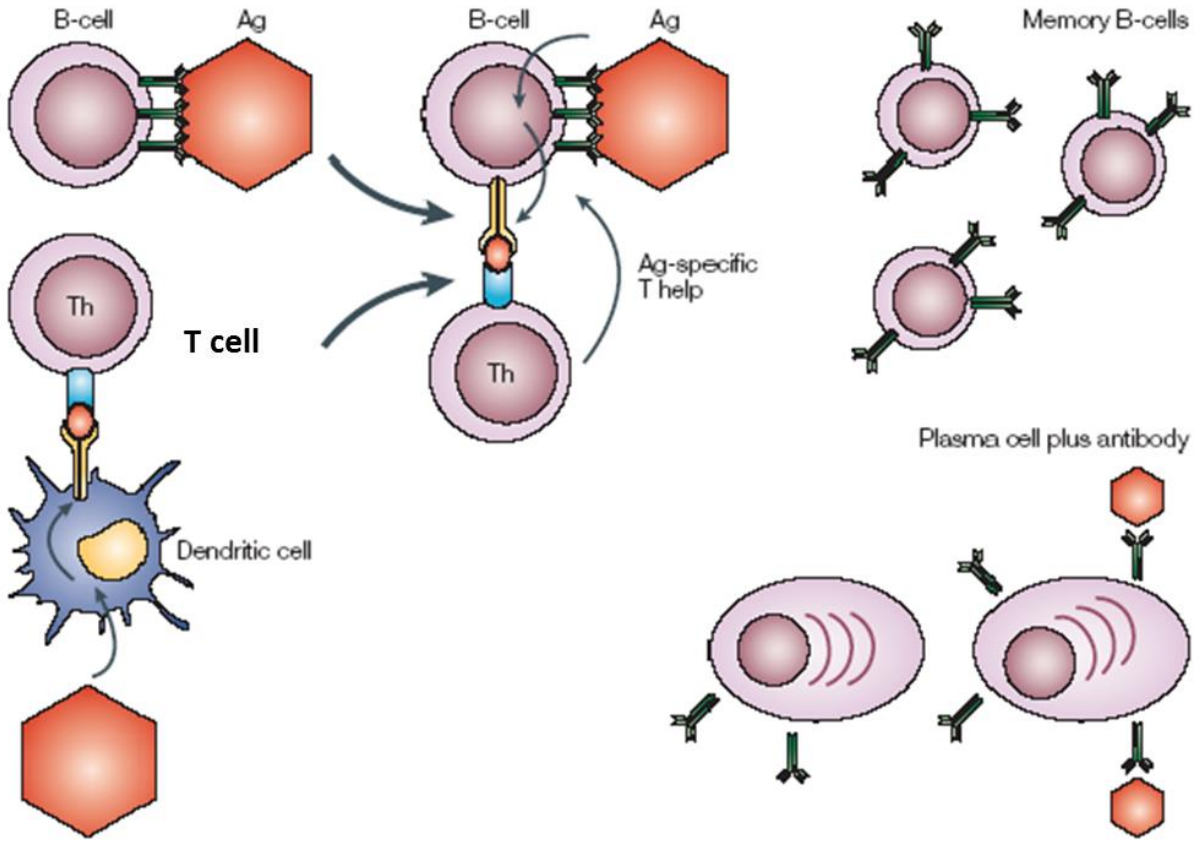

Figure-16: BCR crosslinking phenomenon and VLP associated activation of specific B cells.

5. Drawbacks of using VLP based antiviral vaccines

- VLP vaccines are not capable of generating a continuous secretion of antibodies, unlike live attenuated vaccines. However, VLP vaccines are highly antigenic and stable, and their immunogenicity allow enhancing through addition of adjuvants.
- VLP based vaccines construction cannot be applied to all enveloped viruses.

- VLP vaccines are relatively expensive, although advances in expression systems are lowering costs.

6. Current VLP-based vaccines

6.1. Hepatitis-B VLP-based vaccines

Hepatitis B Virus (HBV) is a double-stranded truncated DNA virus that is a member of the Hepadnaviridae family. The capsid is made-up of a viral protein called the viral body (C) or HBcAg. The viral membrane harbors three envelope proteins: S (Small), M (Middle) and L (Large) as shown in Figure-17. This virus is highly contagious, spread by exposure to infectious blood and body fluids. Infection leads to liver inflammation ("hepatitis"), vomiting, jaundice, and occasionally death. Hepatitis B infection is associated with cirrhosis of the liver and hepatocellular carcinoma. It is estimated that one-third of the world population has been infected with hepatitis B, and that over 400 million people are chronic carriers. Given the causal associations between hepatitis B and mortality, development of an antiviral vaccine was a major public health priority. HBV infections are considered as a global public health problem. Vaccination against the HBV virus is recommended by the WHO organization for children.

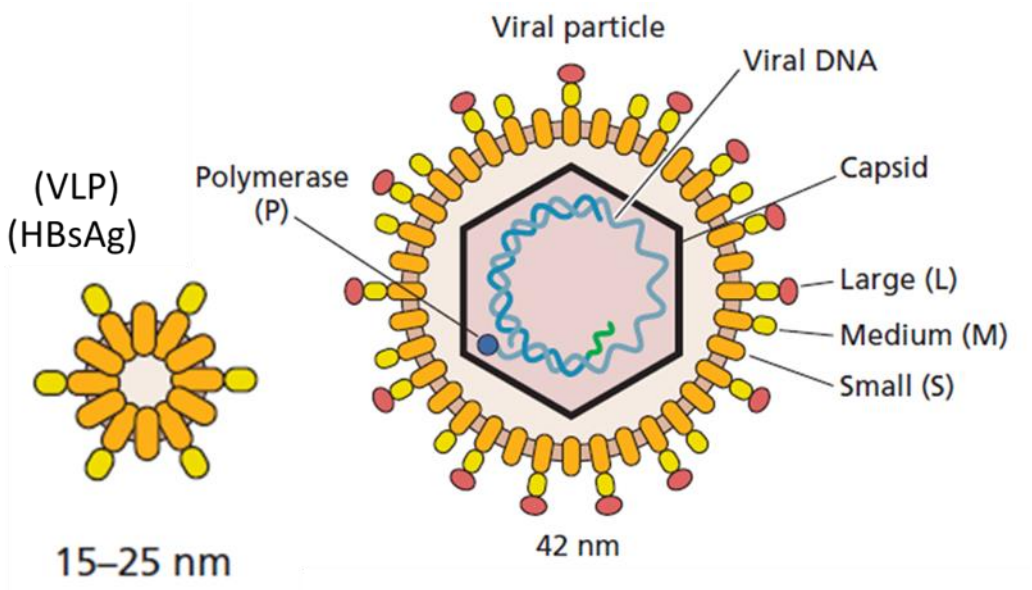

Figure-17: Schematic representation of the structure of an HBV viral particle and HBV VLPs.

In 1980s, the first generation HBsAg VLP-based vaccines was established by Blumberg using Hepatitis B surface antigen particles (HBsAg-VLPs) isolated from the blood of infected individuals. This concept worked very efficiently. Although this vaccine was safe and effective, and seemed free of any exogenous agents, the need to purify the vaccine proteins from the serum of hepatitis B-infected individuals was problematic. The advent of technologies to produce cloned, recombinant proteins for immunization purposes led to the discontinuation of this product in favor of a recombinant vaccine. The second generation of Hepatitis B vaccine is based on the expression of small HBV protein SHBs in cell cultures of *Saccharomyces cerevisiae* yeast. The subunit vaccine produced is much more effective than the first-generation HBV vaccine. It has led to a worldwide reduction in the prevalence of HBV infections. The third generation vaccine has been developed to mitigate some of the ineffectiveness of the second generation. This involves the production of HBV VLP vaccines using recombinant systems, such as yeasts (*Saccharomyces cerevisiae*, *Pichia pastoris* and *Hansenula polymorpha*) and also mammalian cells (Chinese hamster ovary [CHO] cell line) shown on Figure-18. More than 95% of VLP vaccine recipients develop protective immunity against HBV. The adjuvanted VLP HBV vaccine (exemple:. Alum) currently applied in routine pediatric vaccination. The TDA has approved this vaccine since 1986.(Recombivax HB).

A variety of manufacturers currently marketing licensed hepatitis B vaccines are shown in Table-9. These are recommended as universal vaccines for all newborns, administered by intramuscular route at 0, 1 and 6 months age. This third generation vaccine is used as therapeutic vaccine in chronical HBV infections.

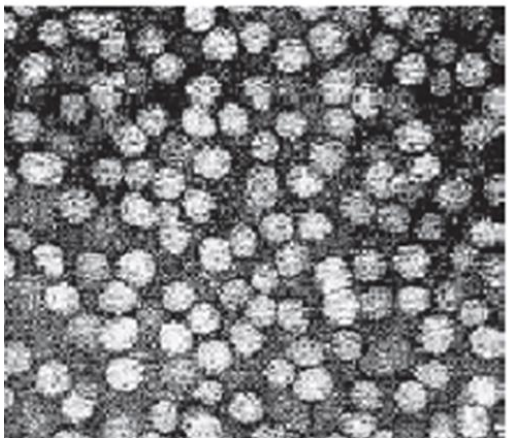

Figure-18: HBV virus like particles observed by electron microscopy.

Table 9: List of HBV VLP-based vaccines commercialized worldwide.

Nom commercial	Firme	Pays	Protéine recombinante	Syst d'expression	Aval FDA
DTP-Hep B	P.T. Bio Farma	Indonesia	HBsAg S protein	Yeast (*Pichia pastoris*)	
Engerix-B®	GlaxoSmithKline	Belgium	HBsAg S protein	Yeast (*Saccharomyces cerevisiae*)	1989
Enivac HB	Panacea Biotec Ltd.	India	HBsAg S protein	Yeast (*P. pastoris*)	
Euvax B	LG Life Sciences	South Korea	HBsAg S protein	Yeast (*S. cerevisiae*)	
Gene Vac-B®	Serum Institute of India Ltd.	India	HBsAg S protein	Yeast (*Hansenula polymorpha*)	
GenHevac B®	Pasteur-Mérieux Aventis	France	HBsAg S and M protein	Mammalian cells (CHO)	
Heberbiovac HB	CIGB – Heber Biotec	Cuba	HBsAg S protein	Yeast (*P. pastoris*)	
Hepavax-Gene®	Crucell	The Netherlands	HBsAg S protein	Yeast (*H. polymorpha*)	
Recombivax HB®	Merck and Co., Inc.	USA	HBsAg S protein	Yeast (*S. cerevisiae*)	1986
Revac-B+™	Bharat Biotech International Ltd.	India	HBsAg S protein	Yeast (*P. pastoris*)	
HeberNasvac	CIGB	Cuba	HBsAg S, HBcAg	*E. coli*	

6.2. Human papilloma (HPV) VLP-based vaccines

Viruses in the papillomaviridae family are non-enveloped double-stranded circular DNA particles of ~55 nm in diameter, present in the skin and mucous membranes of various animal species. More than 200 viral species have been described as being capable of infecting humans. Most species are asymptomatic. However, others can cause lesions and warts. Responsible for sexually transmitted infections. Although many serotypes are associated with benign epithelial proliferation leading to warts. The major medical significance of HPV virus, however, came from the fact that some subtypes are causally associated with malignancies of the urogenital tract, particularly cervical carcinoma. Head and neck malignancies, respiratory papillomatosis, and ano-genital warts are complications associated with HPV infection. Worldwide report estimates approximately 690,000 new cases of HPV-associated cervical cancer per year, and 423,000 deaths annually. Approximately fifteen HPV sub-types well establish to be associated with ano-genital malignancies, of these, HPV-16 and HPV-18 are the most prevalent, causing about 70% of cervical and anogenital cancer cases worldwide. HPV-6 and HPV-11 are the types most commonly associated with genital warts. The virus genome length is eight kilobases (kb), double-stranded circular DNA encoding 10 open reading frames. Which are divided into 8 early (E) and 2 late (L) proteins. In the 1990s, it was observed that recombinant HPV L1 protein is capable to self-assemble

in vitro into a particle containing 72 pentamers similar to the structure of the viral capsid (Figure-19). Interestingly, HPV L1 particles have the ability to induce a stronger immune response.

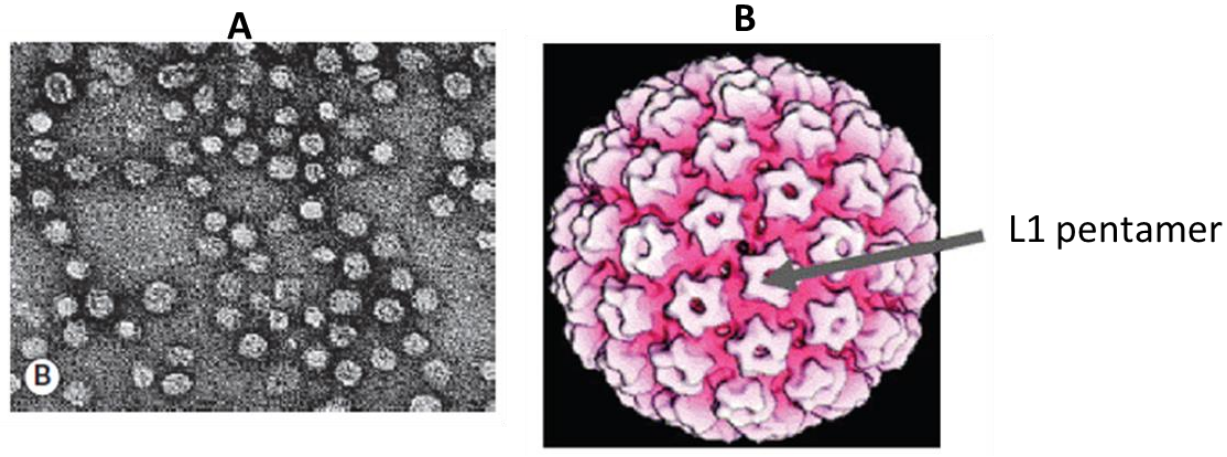

Figure-19: HPV capsid and VLP structures.

(**A**): HPV-16 L1 virus-like particles made by expressing HPV-16 L1 using baculovirus system.
(**B**): A model of the papillomavirus capsid.

Based on these observations, several VLP-based vaccines have been approved by the FDA agency for use in human to prevent certain types of HPV infections. Especially those implicating HPV types 6, 11, 16 and 18. For instance, Gardasil is the first HPV VLP-based vaccine approved in 2006 for use in USA. Gardasil (Merck), consists of recombinant VLPs self-assembled from major capsid protein L1 of HPV types 6, 11, 16 & 18 adjuvanted with neutral salt aluminum hydroxyl phosphate sulfate. Gardasil is expressed in yeast (*Saccharomyces cerevisiae*), each type of VLP is produced separately, these different isotypes are then combined together after a final purification of the particles. The adjuvant (aluminum hydroxyl-phosphate sulfate salt) is added at the end of the process. In 2014 Gardasil has been replaced by Gardasil-9 covering a wider range of HPV types additionally including types 31, 33, 45, 52 and 58. Indeed, these new types have been reported to be responsible for 20% of current cervical cancer cases. Furthermore, Gardasil-9 has higher L1 antigen load as well as more adjuvant than the first generation vaccine. Two other HPV vaccines with the potential to offer broader protections are in clinical trials; an 11-valent candidate vaccine with undisclosed HPV types and a 14-valent candidate vaccine are in phase III and phase I clinical

trials, respectively. The 14-valent candidate vaccine is expected to protect against HPV-6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59. The FDA granted approval to Cervarix vaccine from Glaxo Smith Kline Biologicals in 2007. It was made-up of recombinant VLP made from L1 protein multimers of HPV type 16 and 18 mixed with AS04 adjuvant. The vaccine expression process involves the use of the baculovirus/insect cells system (Trichoplusia ni (Hi-5)). Therefore, Cervarix contains HPV-VLPs belonging only to two serotypes compared to the nine serotypes covered by Gardasil-9. However, Cervarix immunogenicity is higher as it carries TLR4 agonist MPL (deacylated monophosphoryl lipid A) in AS04 aluminum hydroxide formulation, providing stronger and more long-lived responses (Table-10).

Table 10: List of HPV VLP-based vaccines commercialized worldwide.

Vaccin	Firme	Type de VLP	Dose de la protéine L1	Syst d'expression	adjuvant	Administration
Gardasil® 120 µg/dose	Merck & Co., Inc.	6/11/16/18	20 µg (types 6 and 18) and 40 µg (types 11 and 16)	*Saccharomyces cerevisiae* expressing L1	225 µg aluminum hydroxyphosphate sulphate	0, 2 and 6 months
Cervarix® 40 µg/dose	GlaxoSmithKline	16/18	20 µg (types 16 and 18)	*Trichoplusia ni* (Hi-5) insect cell line infected with L1 recombinant baculovirus	500 µg aluminum hydroxide, 50 µg 3-*O*-deacylated monophosphoryl lipid A	0, 1 and 6 months

TLR-4 Agonist

6.3. Hepatitis E and Chikungunya VLP-based vaccines

Hecolin is a VLP based vaccine used to protect against hepatitis E. (HEV). It gained approval in China in 2011, then licensed in Pakistan in 2020. The vaccine target the motif comprising amino acids 368–606, derived from the open reading frame 2 of the capsid protein of HEV genotype 1. This vaccine production is achieved in *E coli* recombinant system. Vaccine efficacy is 100% after 12 months with three doses in 16–64-year-old individuals. It is the first human licensed vaccine based on the expression of a recombinant protein in *E. coli.*

Ultimately, Vimkunya is a VLP-based vaccine indicated for prevention of Chikungunya virus infections in individuals 12 years of age and older. It is a virus like particle containing the viral proteins E1 and E2 envelope and capsid (C). The vaccine is expressed in human cell line HEK 293.

7. Evolution of VLP-based vaccine platforms

VLP-based viral vaccines first introduced in 1986, and since that date, there have been major improvements in the synthesis of VLP-based vaccine molecules.

7.1. 1st generation VLP vaccines

Viral antigens are produced in recombinant systems and undergo a spontaneous self-assembly process after purification. The structures of these VLP based vaccines are simple, containing repetitive antigenic motifs. They have a limited number of surface antigens. Many enveloped viruses are unlikely to form VLPs structures (e.g. HCV, CMV, etc.). Examples of vaccines marketed based on this platform: Gardasil, Cervarix, Engerix, Recombivax, etc.

7.2. 2nd generation VLP vaccines

The antigens of interest are covalently bound to a carrier protein providing the backbone for VLPs. These VLP molecules incorporate several types of viral antigens. This allows them to include a wide range of viral epitopes. The limitation of this strategy is that the antigens incorporated on the VLPs are attached in artificial ways, which can alter the structural conformation of the antigens. Thus may alter the native state. The Q Beta bacteriophage system is an example of this platform. The Q Beta bacteriophage consists of a 24 nm capsid containing a single protein called the coat protein. It is highly immunogenic with a high prevalence of repetitive motifs.

7.3. 3rd generation VLP-based vaccines (Enveloped VLP)

The backbone of the VLP particles is constructed by mixing proteins with lipid membranes, allowing the expression of antigens originating from the membrane of targeted virus.

Enveloped VLPs enable the presentation of viral membrane antigens in the same way as in viral particles as shown in Figure-20. They also offer the possibility of expressing several antigens on the VLP membrane. The main disadvantage of this platform is that these enveloped VLP particles still fail to meet FDA and EMA toxicity standards. There are several candidate vaccines under development following this strategy: CMV, HCV, Dengue, RSV, and West Nile.

Characteristics:

* They have the same size and structure as target viral particles.

* The presentation of viral antigens is similar to the native state condition.

* Enveloped VLP vaccines have demonstrated a high capacity to induce production of neutralizing antibodies against several viruses: CMV, HCV, Flu, West Nile, RSV and Dengue virus.

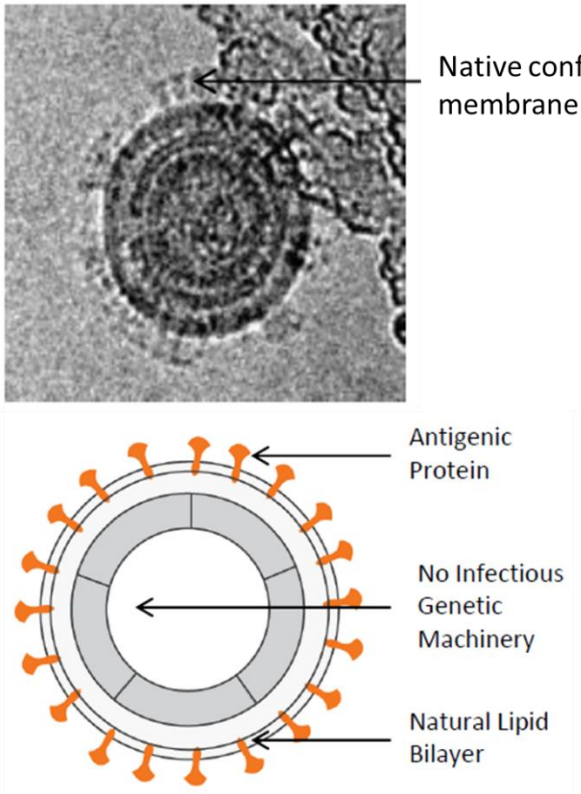

Figure-20: Structure of enveloped VLP particles.

Top: Electron microscopy image of a CMV virus-enveloped VLP particle. Bottom: Schematic representation of an enveloped VLP particle.

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Chapter-6: DNA-based viral vaccines

1. Definition

DNA vaccines represent a new platform for vaccine synthesis that is currently under development. They are considered as a third generation vaccine strategy (nucleic acid vaccine), offering new approaches for the prevention and therapy against several infectious diseases. DNA vaccines are manufactured by cloning one or more viral proteins onto a plasmid usually produced in bacteria. Once injected into a human or animal, the recombinant plasmid enables the transient expression of viral antigens in host cells (example: Macrophages, Dendritic cells, Myocytes…etc.). Such plasmids can effectively mimics the viral infection and can elicit broader immune responses than soluble proteins, because the viral proteins are synthesis occurs in host cells and thus efficiently recognized by the immune system. Recombinant plasmid has no capacity to replicate in the vaccinated host, but can be only the template for expression of the immunogenic protein. Remarkably, no adjuvants or special formulations are necessary to stimulate an immune response.

In the simplest case, the plasmid encodes only the immunogenic viral protein under the control of a strong eukaryotic promoter.

Immunogenicity of DNA fragment was first reported in the 1990s, studies showed that injection of plasmid DNA encoding for human growth hormone (HGH) into mice skin induced the production of specific antibodies. Since then, a number of observations have shown that this strategy is very promising in terms of generating vaccines capable to induce cellular and humoral responses against a wide range of infectious agents. DNA vaccines are effective in eliciting robust immune responses in a broad range of animal models, by targeting a large number of viruses (HIV, HCV, HSV-1 and -2, Influenza, Ebola, Zika, Rotavirus, etc.). This strategy is a new preventive and therapeutic vaccination approach targeting various viral or bacterial diseases.

2. DNA plasmid and its components

DNA-based vaccines are composed of purified closed-circular plasmid DNA, originally from bacteria. The naked DNA (plasmid) vaccines are vectors encoding viral antigens, once expressed in the immunized host they generate both cytotoxic (cellular) and humoral (antibody) responses.

A typical DNA plasmid or vector consists of several genetic elements required to drive intracellular expression of the foreign gene insert. (Figure 21) These include:

- An origin of replication allowing plasmid amplification in *E. coli.*
- A transcriptional promoter, which is the incorporation of a strong viral promoter to achieve optimal expression in mammalian cells of the cloned gene, such as cytomegalovirus (CMV) or simian virus 40 (SV40) which provide the greatest gene expression.
- An optional enhancer element to enhance gene expression.
- The foreign gene encoding an immunogenic viral gene product cloned at the multiple cloning site.
- RNA-processing elements, primarily a polyadenylation tail, transcription termination site and a ribosome-binding site.

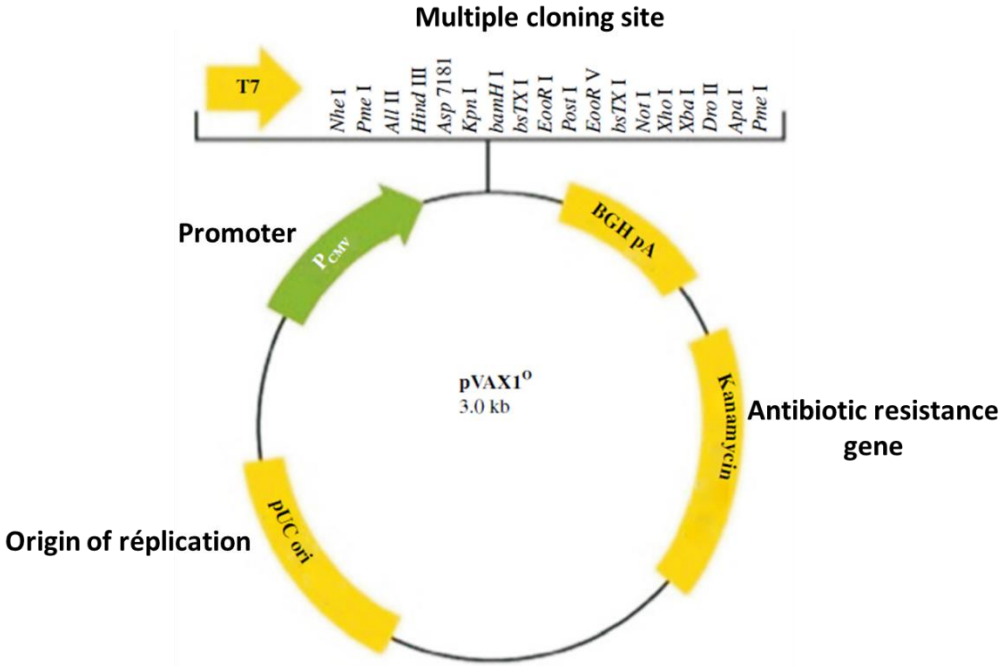

Figure-21: Structure and composition of the pVAX1 cloning plasmid for DNA vaccine purpose.

3. Mechanisms of DNA Vaccines

Immunization with DNA is a method widely used in laboratories to produce antibodies in different animal models. DNA vaccination follows this strategy to introduce nucleic acid into host cells, where it directs the synthesis of the encoded antigens to stimulate an appropriate immune response, such that construction of a DNA vaccine is achieved to permit localized, short-term expression of the target viral antigens. The host cell allow the transcription and translation of the DNA vaccines. The patient body then produces itself his own vaccine. This is allowed by transient expression and mass production of the antigenic proteins. Examples of target cells (myocytes, dendritic cells, etc.). The immunogenic proteins produced are endocytosed by antigen-presenting cells (APCs) thus present the antigenic motifs to the immune system:

*complexed to MHC-I molecules, for the activation of specific cytotoxic T cells.

*Coupled to MHC-II molecules, for the activation of specific T helper lymphocytes.

Recognition of soluble antigens by the BCR receptor leads to the activation of specific B lymphocytes enhancing the synthesis of specific high affinity antibodies. DNA vaccines have also demonstrated the ability to generate follicular T helper populations, which are critical for induction of high quality antigen-specific B cell responses. Numerous studies have shown that DNA vaccines are effective in inducing different types of adaptive immune response (cellular and humoral). DNA vaccination has proven successful in several animal models for preventing or treating infectious diseases, cancer and autoimmunity.

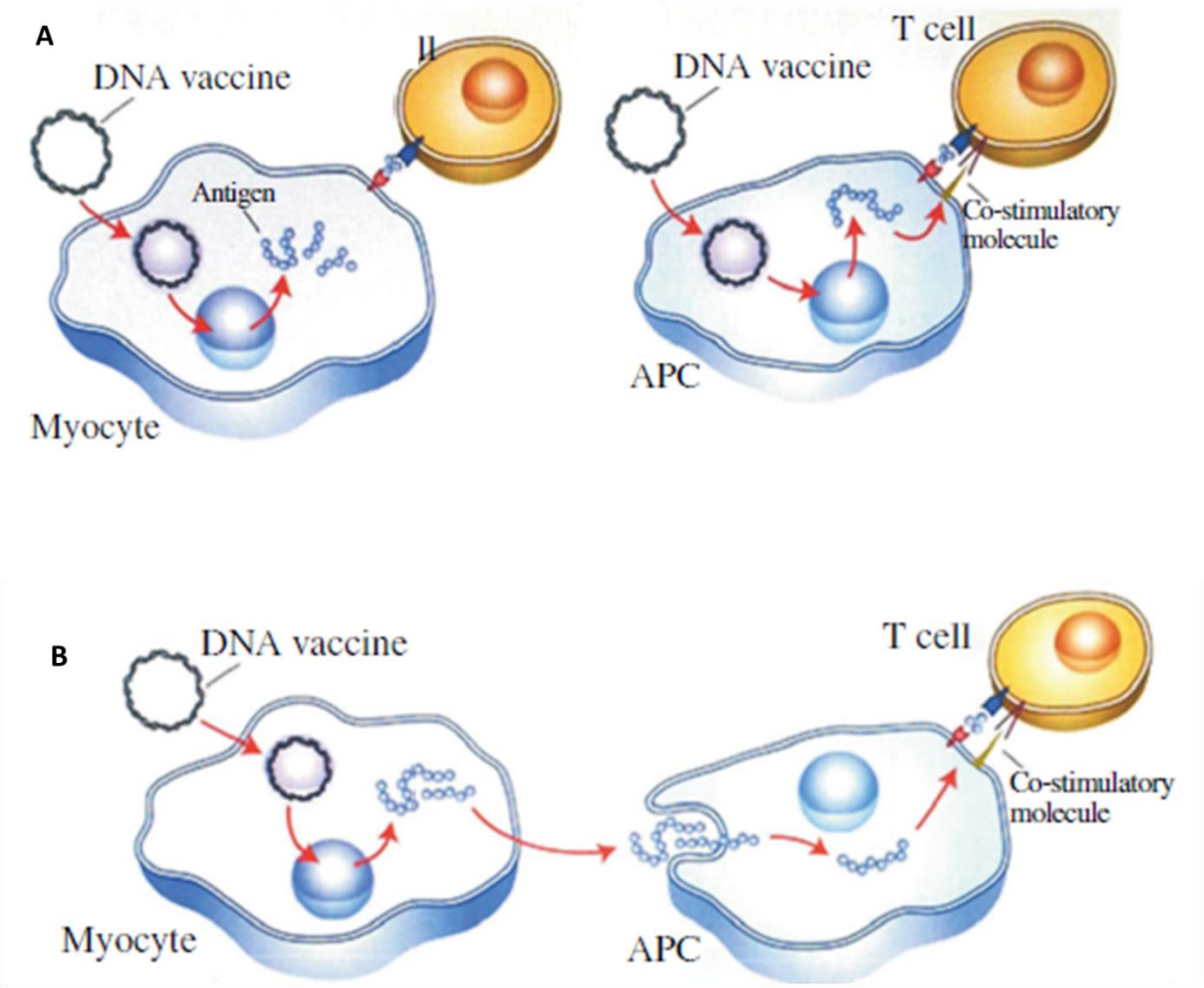

Figure-22: Mechanisms of T lymphocytes activation by DNA vaccines.

A: activation of CD4+ T cells using DNA vaccines. B: activation of CD8+ T cells using DNA vaccines.

4. Safety and tolerance of DNA-based vaccines

DNA vaccination is a riskless vaccination strategy. DNA vaccines seem to be more harmless and more stable than ordinary vaccines. Plasmids are non-viable and do not multiply, and therefore have a low risk of developing secondary disease and infection. The main concern about DNA vaccines is their potential of integration into the host genome. The risk of integration of plasmid DNA into the host chromosomes is minimal. Vaccination with DNA plasmid removes the necessity for protein purification from infectious pathogens, improving safety. Furthermore, DNA vaccination has an excellent safety profile in the clinic, with the most common side effect being

mild inflammation at the injection site. Importantly DNA vaccines provide a safe, non-live vaccine approach.

5. Advantages of DNA-based antiviral vaccines

Significant advantages of these vaccines include the cheapness, DNA vaccines are relatively easy and inexpensive to design and produce, as DNA is easier to purify than proteins or viral particles, transport and higher resistance (Table-11). DNA vaccines are less reactogenic then live or inactivated vaccines, since they do not contain any genetic material derived from the virus. The stability of DNA and its ability to withstand drying make this strategy particularly attractive for vaccine delivery in developing countries where refrigeration tool is limited. The other important feature of these vaccines is the ability to put combining several antigens in the plasmid, resulting in enhanced immunization against all of agent components. Furthermore, studies have shown that antagonisms made from vaccine plasmids in the host body for glycosylation and other post-translational modifications are the same as the main pathogenic protein. Plasmid components encodes for specific immunogens, usually surface proteins of viruses or bacteria capable to stimulate the major components of the immune response with better immunogenicity than inactivated vaccines. Viral proteins are produced inside the host cells and are better recognized by the cells of the immune system. They are expressed on the cell surface of complexed to the MHC molecules, ideally presented to the immune cells in their native conformation.

Table 11: Comparison of the immune response characteristics of three types of vaccines, DNA, live attenuated and subunit vaccines.

Immune response	DNA vaccine	attenuated vaccine	S/U vaccine
Humoral immune response	+++	+++	+++
Cellular response: CD4+ CD8+	Th2 ++	Th1 +++	Th1 -
Antigens presentation	MHC I et II	MHC I et II	MHC II
Memories: cellular response humoral response	++ +++	+++ +++	+/- +++
Chain production and easy development	++++	+	++
price	+++	+	+
Transportation and storage	+++	+	+

6. Disadvantages of DNA-based viral vaccines

- Possible development of anti-DNA antibodies eliciting the degradation of the vaccine.
- This approach remains risky, since it involves expressing foreign genes in the host organism.
- The major drawback of DNA vaccines is their low immunogenicity in humans. Contrary to what has been observed in many animal models. This is due to:

Small quantity of plasmid DNA transferred to the target cells.

Short duration of the expression of the introduced genes.

7. Currently licensed DNA-based viral vaccines

The first DNA vaccine approved for use in humans is the Indian ZyCov-D, which is a vaccine against SARS-CoV-2 manufactured by Cadila Healthcare pharmaceutical company India. This vaccine is composed of 2 mg of bacterial plasmid pVAX 1 expressing the SARS Cov 2 Wuhan Hu 1 Spike gene combined with an IgE signal peptide. Currently This vaccine is produced in bacteria *E. coli*. The administration of the vaccine is accomplished using the Gene gun tool. In addition to ZyCov-D there are several DNA vaccines approved for veterinary use as listed on Table-12.

Table 12: List of licensed DNA-based viral vaccines applied for veterinary purposes.

Vaccine name	species	Target Disease
Oncept	Dog	melanoma
Apex-IHN	Fish	Infectious Hematopoietic Necrosis Virus
West Nile Innovator	Equine	West Nile Virus

The clinicaltrials.gov database enumerate more than 1000 ongoing clinical trials and pre-clinical trials related to DNA vaccines studies (Table-13).

Table 13: List of human infectious diseases targeted by DNA vaccines currently under clinical trials.

Infectious Diseases
Human immunodeficiency virus
Influenza (Seasonal, Pandemic)
Malaria
Hepatitis B
Seasonal Acute Respiratory Syndrome
Marburg
Ebola
Human Papilloma Virus (see Cancer)
West Nile Virus
Dengue
Herpes Simplex Virus
Measles
Cytomegalovirus

8. Improvements in DNA-based vaccines design

DNA vaccination is an interesting method of immunization due its ability to induce CTL stronger responses it can also activate other immune systems. The DNA vaccine achieves this goal by mimicking natural viral infections. Current research and development on DNA vaccines focus in improving the efficacy of the immune responses of DNA vaccines in humans to reproduce their efficacy observed in animal models (small animals and primates).

8.1. Improvements in plasmid design

- Modifications in promoter/enhancer regions, by inserting stronger promoters derived from viruses, such as the SV40 and CMV promoters. As well as the use of transcription regulatory elements like Transcription activating sequence ‘Long Terminal Repeat: LTR’ from HTLV-1 Virus.

- Addition of polyadenylation sequences allowing mRNA stability, and proper transcription termination of the plasmid.
- Introduction of ribosome binding sequence.

  Example: Kozac sequence: gccRccAUGG where R represents a purine (adenine or guanine).

  It enables the mRNA to recognize the ribosome start codon.
- Incorporation of secretion signal sequences allowing the easy cell secretion of the expressed protein.

8.2. Improvements in cell transfection methods

- Electroporation: transient rupture of the plasma membrane due to the effect of an electrical shock.
- Gene Gun: plasmid DNA is complexed to gold beads. The vaccine administration to the patient using a particle accelerator that enable the vaccine to bypass through the skin and therefore deliver the vaccine to the target cells often in the nuclei location.
- Polyethylenimine (PEI) and Vaxfectin: These cationic polymers are capable of transporting DNA vaccine inside cells by direct crossing through lipid bilayer of the plasma membrane.
- DNA tattooing: consist on intradermal introduction of the DNA vaccine.

8.3. Using adjuvants

- Use of non-methylated CpG DNA motifs.
- Use of TLR agonists.
- Co-administration of plasmids encoding for cytokines (IL-12, IL-2), chemokines and co-stimulatory molecules.

9. Examples of DNA vaccines under development

Phase II /III study of **COVID-19** DNA vaccine. (**AnGes, TAKARA Osaka univ)**

Safety and efficacy study of Herpes Simplex Virus Type 2 **(HSV-2)** Therapeutic DNA vaccine.

Efficacy and Tolerane of nacked DNA vaccine in patients with **Chronic B Hepatitis**

Safety of and immune response to a DNA **HIV-1** vaccine in HIV infected individuals with acute HIV infection.

A **Zika virus** DNA vaccine in healthy adults and adolescents.

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Chapter-7: mRNA-based viral vaccines

1. Definition

mRNA vaccines are based on the administration of synthetic messenger RNA designed to mimic natural mRNA. These molecules are engineered to encode one or more specific immunogenic proteins from the target virus, typically viral surface proteins. Once in the host cell, these mRNAs are directly translated into proteins within the cytoplasmic ribosomes, These antigens are addressed to the cellular membrane, or secreted outside the cell. Some of these proteins has an intracellular localization. The development of mRNA vaccines is the culmination of extensive research spanning several decades. The discovery of the immunogenicity of mRNA molecules dates back 1990s. Subsequently, mRNA vaccine is able to elicit both innate and adaptive immunity. For example in 1995, a study showed that synthetic mRNA encoding the influenza virus hemagglutinin (HA) protein could be translated into the HA protein *in vivo*, triggering a specific immune response in mice.

The mRNA vaccines represent a new class of vaccines that harness the body's cellular machinery to produce specific antigens, which will trigger the immune responses against the produced antigens. This innovative approach offers numerous advantages over traditional vaccine platforms. It has garnered significant attention, especially in the context of recent successes, such as the rapid development and deployment of mRNA-based vaccines against the SARS-CoV-2 virus with the Pfizer–BioNTech and Moderna mRNA vaccines.

The structure of mRNA vaccines closely resembles that of eukaryotic mRNA, consisting of a single-stranded molecule featuring a 5′cap, a 3′poly(A) tail, and an open reading frame (ORF) flanked by untranslated regions (5′ and 3′ UTRs) as illustrated in Figure-24. Structurally, mRNA vaccines are optimize to ensure stability and efficient translation.

2. Types of mRNA-based vaccines

There are two types of mRNA vaccines: conventional mRNA and self-amplifying mRNA (Figure-23).

2.1. Non-replicating mRNA-based vaccine

The conventional mRNA vaccine contains untranslated regions (5′UTR, 3′UTR) flanking the coding region of mRNA that can be transcribed into one copy of immunogenic protein; therefore, it is called non-amplifying or non-replicating mRNA. The immune response is directly proportional to the number of transcribed mRNAs, which may need a high dose of mRNA due to its unamplified behavior that could require repeated administration of mRNA vaccine.

2.2. Self-amplifying mRNA-based vaccine

Self-amplifying or self-replicating mRNA vaccines are genetically modified mRNA by adding replicons derived from the genome backbone of self-replicating RNA virus (e.g. alphavirus family), generally Venezuelan equine encephalitis complex (VEEV), in which the genes encoding the viral RNA replication machinery are intact but those encoding viral structural proteins are replaced with a sequence encoding target viral antigen. A self-amplifying RNA vaccine can elicit substantially stronger immune responses than conventional mRNA. The amplification of encoded protein expression will require significantly a lower dosage than most conventional mRNA.

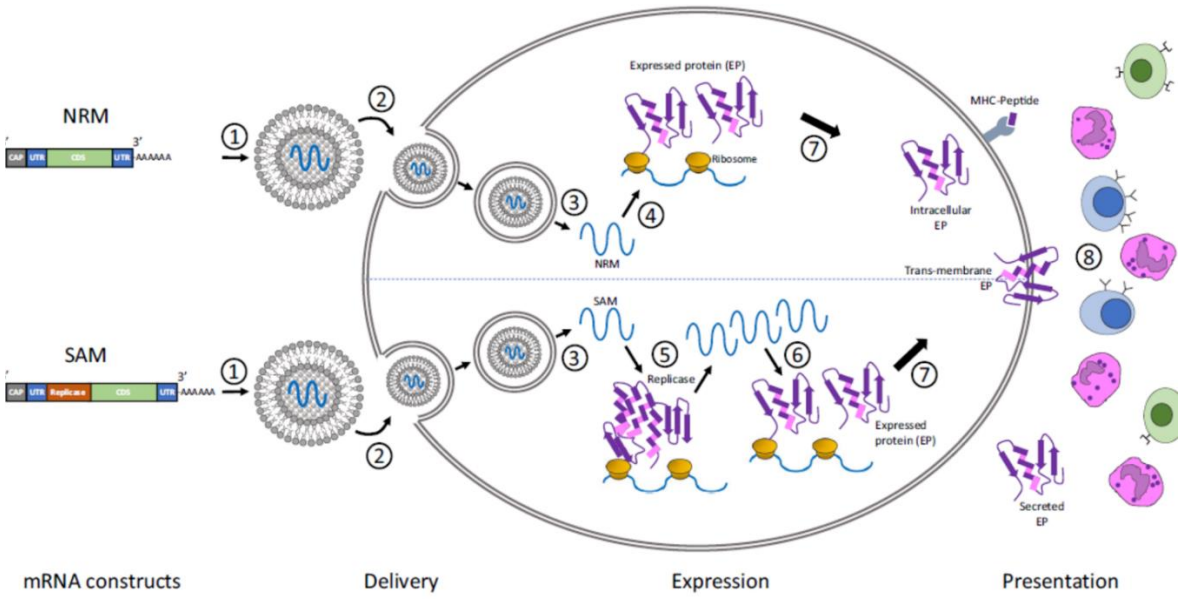

Figure-23: Mechanisms of action of non-replicating mRNA vaccines vs replicating mRNA vaccines.

2. Composition of the mRNA-based vaccine molecules

As shown in Figure-24, mRNA vaccines contains several regions:

5′ Cap

The 5′ end of the mRNA contains a 7-methylguanosine (m7G) moiety, followed by a triphosphate moiety to the first nucleotide (m7GpppN). 5′ cap has a protective structure to protect mRNA from exonuclease cleavage and initiates mRNA translation.

5′ and 3′ UTRs

UTRs ends are non-translated sequence into the desired antigen or protein, their role involve in regulating mRNA expression. These regions are located between the ORF and the 5′ and 3′ ends, in the upstream and the downstream of the mRNA. These UTRs contain regulatory sequences associated with the stability of mRNA and the efficient and correct translation of the messenger RNA within the ribosome. They also help in the recognition of mRNA by ribosomes.

ORF

Open reading frame, contains the encoding sequence of the viral antigen. It is a critical determinant of the immunogenicity and translational efficiency of the mRNA vaccines. The coding sequence for the target antigen is meticulously optimized to facilitate optimum protein folding and translation efficiency regulation, codon optimization plays a pivotal role. This process introduces functional peptides and ensures compatibility with the human translation machinery, enhancing the translational process.

Poly(A) Tail

mRNA has a polyadenylated region located at the 3′ end known as poly(A) tail. This polyadenylated tail is essential to the determination of the lifespan of the mRNA. The poly(A) tails is naturally incorporated to the mRNA molecules in mammalian cells. The incorporation of poly(A) tails permit the production of mRNA vaccines with longer half-life. Recognition of Poly(A) tail on mRNA fragment by ribosome is essential for a successful translation process to generate the corresponding antigen.

Replicon complex

The self-amplifying mRNA vaccines contains additional genetic sequences responsible for the mRNA replication before the translation and production of the antigenic proteins. An RNA-dependent RNA-polymerase (RdRp) that catalyzes the replication of RNA and other regulatory elements derived from alphavirus.

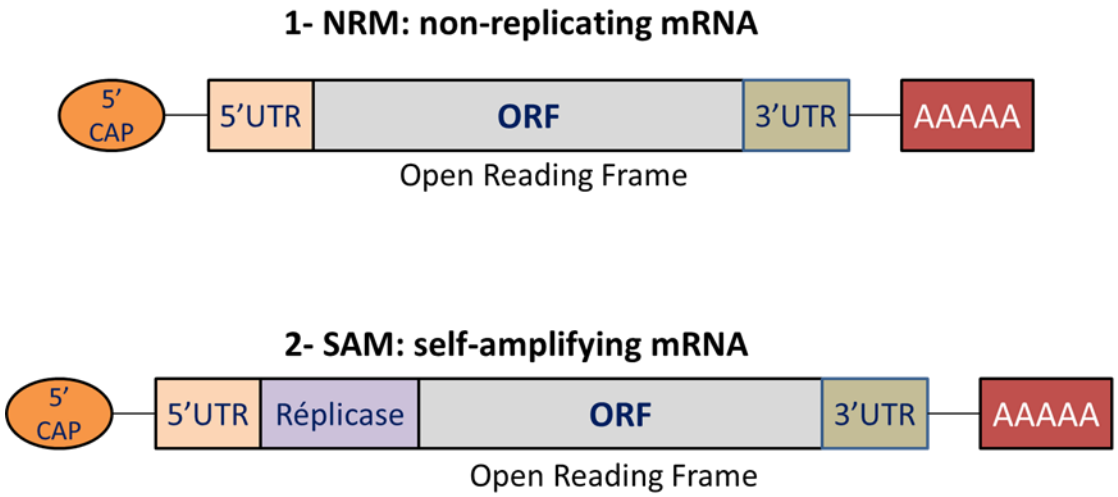

Figure-24: schematic representation of the structure of the two types of mRNA vaccines: conventional non-replicating mRNA and self-replicating mRNA vaccines.

3. mRNA-based vaccines manufacturing steps

- Generation of a plasmid containing a T7 promoter close to the mRNA encoding the antigen sequence. The constructed plasmid is therefore linearised.
- *In-vitro* transcription of the plasmid DNA catalysed by the T7 DNA-dependent RNA polymerase enzyme. This reaction is called *in-vitro* transcription reaction (IVT).
- The plasmid DNA template is degraded at the end of this step by adding DNaseI.
- The 5' cap part and the poly(A) region are added enzymatically to the linearized mRNA.
- The mRNA produced by the IVT reaction is isolated and purified by multiple purification steps. The removal of all impurities is essential and critical to obtain a pure mRNA product harboring efficacy and safety. Chromatography method is a commonly and widely used purification strategy.

- Formulation: mRNA are negatively charged molecules, they are formulated with a lipid-based drug delivery system to avoid its degradation and to improve the transfection efficiency and again increase their half-life. LNPs are the most trustworthy, reliable, and FDA-approved lipid-based non-viral carrier system for delivering mRNA vaccine drug substances.

Up to 2025, there are 3 mRNA licensed vaccines by the US-FDA as shown n Table-14.

Table 14: List of licensed mRNA vaccines in the United States.

Vaccine name	Tradename	Manufacturer
COVID-19 Vaccine	MNEXSPIKE	ModernaTX
COVID-19 Vaccine	COMIRNATY	Pfiezer-BioNTech Manufacturing GmbH
Respiratory Syncytial Virus	MRESVIA	ModernaTX

4. mRNA-based Vaccine delivery systems

4.1. Polymeric nanoparticles

The conjugation of polyethylenimine (PEI), polyethylene glycol (PEG) and binding it to the cyclodextrin is an effective and safer approach for mRNA delivery, and it can be administered through different routes, which may lead to the production of distinct antibodies isotypes. Generally, high molecular weight polymers, such as PEI, need modification to improve the transfection efficacy and durability. At the same time, the mRNA affinity to the vesicles can be influenced by the polymer's length, charge density, and mixture concentration.

4.2. Peptides and proteins nanoparticles

The protein nanoparticles typically have 20 to 200 nm diameters, ideal for effective lymph node targeting. A reliable and secure method of administering vaccines is using self-assembled protein carriers as vaccine delivery systems. For example, it was reported that a hepatitis B virus (HBV) vaccination with both therapeutic and preventative properties was achieved using the conjugation of Pre-S1 HBV surface antigen and self-assembled ferritin nanoparticles (NP-PreS1).

Amphipathic peptides such as cell-penetrating peptide (CPP) can help deliver mRNA into cells due to their cationic or amphipathic amine groups, such as arginine, which can electrostatically bind to the mRNA, creating nano-complexes. The endosome's pH level can alter the fusion cell-penetrating peptide (CPP) structure with repeated arginine-alanine-leucine-alanine (RALA) motifs.

It can thereby, facilitate the creation of pores within membranes of endosomes, enabling the delivery of mRNA into cytoplasm of target cells. Additionally, RALA motifs can activate T-cell-mediated immunity and transport mRNA inside the dendritic cells.

4.3. Protamine nanoparticles

Protamine is a cationic protein that is composed mainly of positively charged amino acids. The positive charge of protamine allow its binding to nucleic acids, such as RNAs, Due to the versatile features and clinical safety of protamine, it has multiple applications in biomedical research as a drug delivery nano-carrier. The most important feature of the protamine delivery system is the cationic properties due to an arginine-rich sequence, which enhances the binding of protamine with negatively charged molecules, such as mRNA, which is exploited in the design of delivery system of mRNA-based vaccines.

4.4. Lipid nanoparticles (LNPs)

The LNPs are nanoscale lipid-based carriers for mRNA transport into the cytosol. These particulate nano-carriers can effectively deliver mRNA intracellularly by fusing with the lipid bilayer of the early endosomes (endosomal escape) or by fusion with the plasma membrane, providing the mRNA into the cytoplasm, and safeguarding the mRNA against RNase hydrolysis inside systemic circulation. The LNPs have been employed in developing SARS-CoV-2 mRNA vaccines that have received clinical approval. For mRNA administration, LNPs provide substantial advantages, such as simplicity of formulation, modularity, biocompatibility, and high mRNA load capacity. The LNPs typically consist of three main components: an ionizable lipid (40–50%); cholesterol (38–45%); and a helper phospholipid (10–12%); in some cases, a fourth component is added, such as PEG lipid (1–2%), as illustrated in figure-25. These components function together to enclose and safeguard the naked mRNA vaccine.

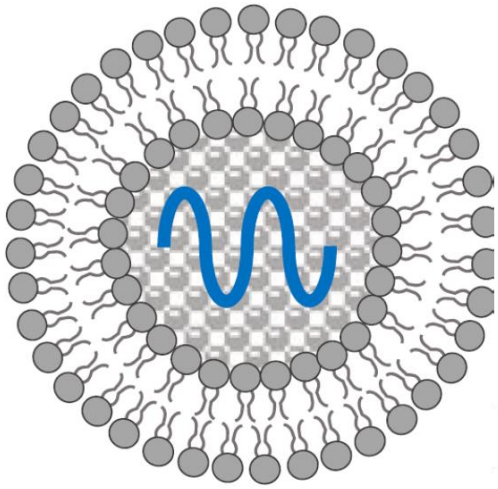

Figure-25: Structure of a lipid nanoparticle (LNP) used for mRNA vaccine delivery.

5. Advantages and disadvantages of mRNA-based vaccines

One of the key advantages of mRNA vaccines is their rapid development and production capabilities. Unlike traditional vaccines, which often time-consuming and require costly manufacturing processes, mRNA vaccines can be designed and synthesized quickly, offering flexibility and speed to fight against emerging infectious diseases or evolving variants. Additionally, the modular nature of mRNA vaccines enables easy adaptation to different pathogens by simply modifying the mRNA sequence encoding the desired antigen. The absence of the risk of integration into the host genome is considered as an advantage of mRNA vaccines (with respect to DNA vaccines). A clear advantage of mRNA vaccines is that, unlike DNA vaccines, they do not need to reach the nucleus to express the antigen. Instead, once inside the nucleus, mRNA vaccine will produce many copies of mRNA molecules, resulting in the production of more antigen of interest per transfected cell, these are self-amplifying RNA vaccines. However, the efficient delivery of mRNA molecules into target cells poses a significant challenge, as they are susceptible to degradation, have limited stability, and face barriers in reaching the desired sites of action. Efficient delivery systems capable of targeting specific cells or tissues are still under development, Most Approved mRNA vaccines require storing mRNA vaccines at low

(− 20 °C) or ultra-low temperatures (− 60 °C), complicating the distribution of mRNA vaccines in resource-limited countries. As a result, the stringent requirements for cold chain logistics and storage of these vaccines significantly limit the clinical application and distribution of mRNA vaccines, due to the lack of transport and refrigeration facilities.

Many mRNA vaccines have entered into clinical trials, including those that prevent influenza virus, foot-and-mouth disease virus, and rabies, some examples are summarized in Table-15.

Table 15: mRNA vaccines under clinical trials (up to June 2023).

Name of Product	ClinicalTrials.Gov Number	Payload	Disease	Phase
			Infectious diseases	
mRNA-1893	NCT04917861	Structural proteins of the Zika virus	Zika virus	2
mRNA-1647	NCT04232280	Six mRNA codings for pentamer viral antigen and gB protein of Cytomegalovirus	Cytomegalovirus infection	2
mRNA-1345	NCT05127434	The stabilized prefusion F protein	Respiratory syncytial virus	2–3
CVnCOV	NCT04652102	SARS-CoV-2	SARS-CoV-2	2–3
ARCT-021	NCT04668339	SARS-CoV-2	SARS-CoV-2	2
BNT162b2	NCT04380701	SARS-CoV-2	SARS-CoV-2	1–2
mRNA-1273	NCT04785144	Codes for the full-length prefusion stabilized S protein of the SARS-CoV-2 B.1.351 variant.	SARS-CoV-2 B.1.351 variant	2

6. mRNA-based vaccines against Severe Acute Respiratory Syndrome Coronavirus 2

Since the beginning of 2021, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected a billions of confirmed cases as well as caused millions of deaths worldwide. The majority of SARS-CoV-2 infections do not pose a life -threatening risk to individuals without preexisting diseases, however, in cases of severe infection, uncontrolled immune responses can be triggered in the lungs, destroying epithelial cells and alveoli, causing pulmonary edema, a dangerous increase in vascular permeability and death. Therefore, the spike protein represents a prime target for SARS-CoV-2 mRNA vaccines encoding either the receptor-binding domain or the full-length spike

protein. To date, two mRNA vaccines designed to target the full-length spike protein of the coronavirus disease 2019 (COVID-19) have gained approval and widespread usage globally.

The most extensively utilized mRNA vaccines Pfizer-BioNTech (BNT162b2) and Moderna (mRNA-1273) COVID-19 vaccines that encode the spike protein of SARS-CoV-2 stimulate an immune response against the virus, therefore protecting the infected subject from this life-threatening infection. Both vaccines were formulated as LNPs encapsulating the mRNA. Two doses of BNT162b2 proved to be 94% effective at preventing symptomatic COVID-19, and 95% overall effective at preventing COVID-19. Primary and booster injections of both BNT162b2 and mRNA-1273 shows to be 90% effective against SARS-CoV-2. It should be noted that the Moderna vaccine showed good safety and effectiveness following its storage for a month at 4–8 °C, while the Pfizer–BioNTech vaccine needs −60 °C storage conditions.

7. mRNA-based vaccine against Respiratory Syncytial Virus (RSV)

Respiratory syncytial virus (RSV), a negative-sense single-stranded RNA virus, is one of the main cause of serious respiratory disease in young infants and the elderly. Nearly all children experience an infection by 2 years of age. It is estimated that nearly 32 million children under 5 years of age are annually infected by RSV worldwide, causing acute lower respiratory tract infection and about 60,000 deaths. Elderly and immunocompromised individuals are at higher risk of experiencing a symptomatic RSV infection, with higher mortality. However, natural infections by RSV do not induce persistent immune protection. Although vaccination is commonly considered as one of the most cost-effective strategies for preventing infectious diseases. Currently, two recombinant protein vaccines (Arexvy and Abrysvo) and one mRNA vaccine (mRESVIA), have been approved for use in adults aged 60 and older, demonstrating both efficacy and safety. mRESVIA is Moderna's mRNA vaccine approved in the U.S. to protect adults aged 60 years and over from severe lower respiratory tract disease (LRTD) caused by respiratory syncytial virus (RSV) infection. The vaccine contains 50 μg of mRNA-1345, it encodes for a stabilized pre-fusion form of the RSV fusion -F (RSV-F) glycoprotein derived from RSV strain-A. The RSV pre-fusion F glycoprotein mediates viral fusion and host-cell entry and elicits neutralizing antibodies. The same antigen is used in the subunit vaccines Arexvy and Abrysvo. mResvia mRNA vaccine is a lipid nanoparticle-encapsulated.

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Chapter-8: Viral vector based vaccines

1. Definition

Vectored vaccines use a modified version of harmless virus as a vector to deliver genetic material coding for a desired antigen (for example the COVID-19 spike protein). These vaccines have two key components: 1) the viral vector, generally originated from different organism than the organism to treat, which correspond to a vehicle to deliver 2) the DNA "cargo" that enables production of the antigen of interest. One or more genes encoding immunogenic proteins of the target virus inserted into this vector (Figures-26 and 27). The viral vector used as the delivery vehicle is genetically altered, so it cannot cause illness and the genetic material does not integrate into the host genome. The DNA cargo contains the coding sequences for the desired antigen(s), which are expressed once inside the cells, allowing to trigger immune response in the body. Like DNA vaccines, viral vector vaccines transfer the DNA encoding viral immunogenic proteins to the treated organism. Once the viral DNA has been transferred into the host, antigenic proteins are produced in the target tissues. They are responsible for the development of a protective immune response. This approach often yields potent T-cell and B-cell responses that can exceed those elicited by protein-based or inactivated vaccines.

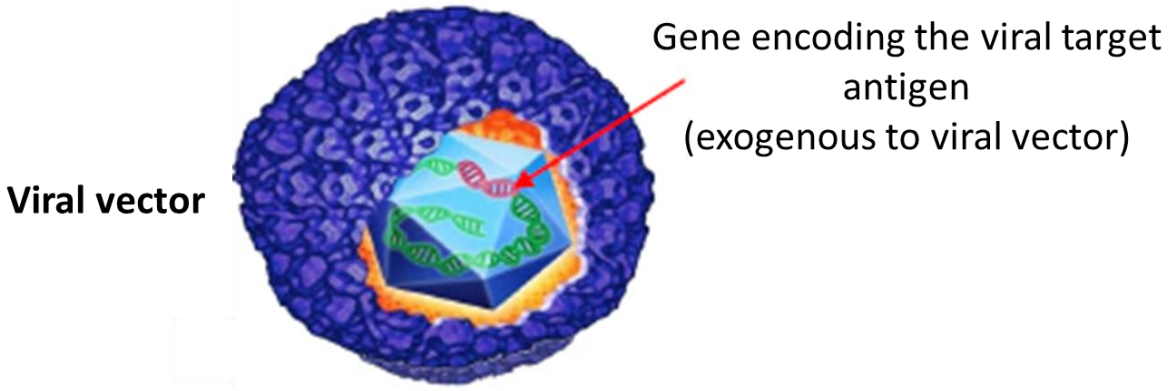

Figure-26: Schematic representation of viral vector vaccine structure.

2. Discovery

The first report describing the use of recombinant viruses as vectors for transporting viral antigens derived from another virus for vaccination purposes was published in 1984. This study described the use of vaccinia virus, an attenuated virus used for vaccination against smallpox, as a recombinant vector expressing hepatitis B surface antigen HBsAg. Vaccinia virus expressing the HBsAg antigen have shown to be effective in inducing a protective response against HBV infection in Chimpanzees.

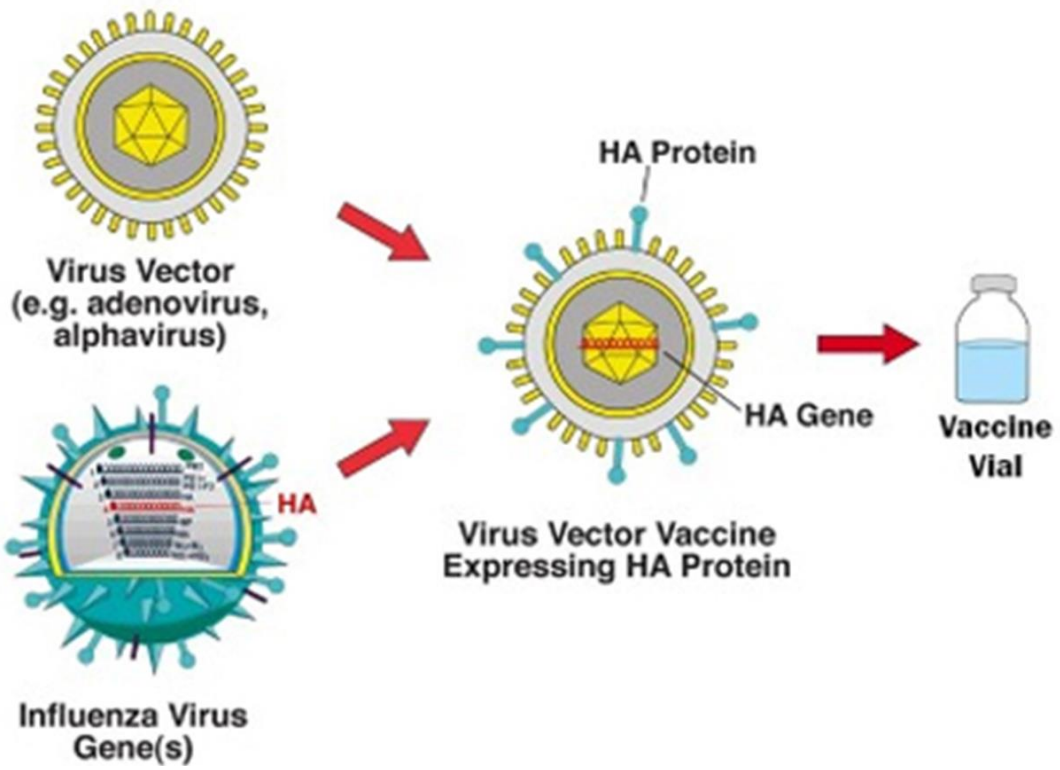

Figure-27: Diagram describing the stages involved in producing a vaccine based viral vector.

3. Types of Viral Vector based Vaccines

Viral vectors are commonly divided into two broad classes, replication-deficient and replication-competent. The viral vector can be rendered replication-competent or replication-deficient to increase their safety and reduce their reactogenicity. In both cases, vectors will act as genetic vaccines, since they are able to either infect and undergo viral replication or introduce genetic material into a host cell through transduction. Even though viruses are not living organisms, both types are generally considered "live" vaccines due to the principles behind viral vector vaccines acting as live viruses to deliver genetic material to cells using the same mechanisms as wild-type virus.

3.1. Replication-Deficient

Replication-deficient (RD) vectors are those that are missing one or several essential genes required for virus replication and thus cannot replicate outside of specific cells that have been engineered to stably express the deleted genes. Production of RD vectors often involves the usage of a specific cell lines such as HEK-293 or Vero cells. These cells express genes necessary for viral replication that are not normally present in human cells, and thus allow *in vitro* propagation of the viruses until they reach suitable titers. Since the cells of the vaccinated host lack the essential genes that the modified viruses need for propagation, these vectors are unable to propagate in host cells. Some vectors, such as the recombinant modified vaccinia virus Ankara (MVA), are not totally replication deficient, as they can replicate normally in embryonic fibroblasts of chickens and are only RD in mammalian cells. Since these vectors are unable to replicate and produce infectious progeny in human cells, the safety profile for them is generally high. Because of this high safety profile, a majority of viral vector vaccine candidates use RD vectors. Currently, there are seven RD viral vectored vaccines that have gained worldwide approval for use: Oxford-AstraZeneca (ChAdOx1 nCoV-19), Janssen (Ad26.COV2.5), Convidecia 20 (AD5-nCOV), and Sputnik V (Gam-COVID-Vac) vaccines for SARS-CoV-2, the Zabdeno (Ad26.ZEBOV)/Mvabea (MVA-BN-Filo) prime-boost regimen vaccine for Ebola, Dengvaxia (YFV-17D) for Dengue virus and IMOJEV (YFV-17D) for Japanese encephalitis virus (JEV).

3.2. Replication-Competent

Replication-competent (RC) vectors work similarly to RD vectors in a way that they contain genetic material allowing the expression of a target protein antigen, and require specific cell components for its production. However, RC vectors do not rely on proliferation in cell lines or transient transfection with plasmids to propagate the virus vector. RC vectors are usually cultured in eukaryotic cell lines, similar to currently available for live-attenuated vaccines. The primary concern of all viral vector vaccines in their usage is safety, specifically when using RC vectors, due to high risk of recombination, which could reintroduce a level of pathogenicity to the vector. RC vectors are typically more immunogenic than RD vectors due to the strong pro-inflammatory signal generated that is effective in inducing T cell responses against the target virus. Additionally, since the vector will undergo multiple replication cycles, the presence of viral antigen is sustained for a longer duration in the body, causing a prolonged immune response and increased antibody amount. Currently, there is only one RC vector approved for human usage in the prevention of Ebola virus Ervebo (rVSV-ZEBOV).

4. Viral vector based vaccines manufacturing

The main idea of viral vector vaccines is to use a harmless virus (the vector) as a delivery tool to carry a gene encoding immunogenic protein of the target pathogen (e.g., SARS-CoV-2 spike protein gene) into human cells. The cells then will express this gene to produce the foreign protein, training the immune system to recognize the real pathogen and develop robust immune response (Figure-27). The production process is complex and occurs through multiple stages.

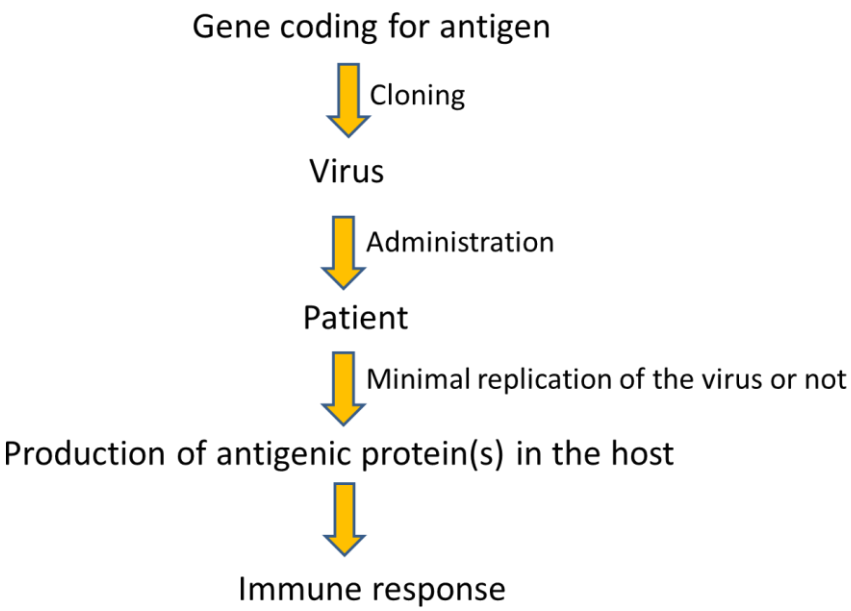

Figure-28: Schematic diagram of the production and application steps used in viral vector vaccination.

4.1. Step 1: Upstream process "Manufacturing"

Design and creation of the viral vector

The production of viral vector vaccines begins with creation of the delivering vector that will express the desired antigen. Production methods for vectors will vary based on the type of virus used. This research step is completed long before large-scale production of the desired vaccine.

Vector selection: The virus vector is chosen by taking account to the safety and efficiency considerations. Adenoviruses (e.g., ChAdOx1 from AstraZeneca) are commonly used because they are well-characterized, no risk of integration into human chromosomes, and they can hold large foreign genetic sequences.

Engineering of the vector for delivering the desired antigens: Developers remove key genes from the virus genome that are essential for its replication. This makes the vector "replication-deficient" for safety. Then, they insert the gene of interest by cloning the encoding sequence of the target antigen (e.g., the COVID-19 spike protein) into the vector virus's genome.

The result is a recombinant plasmid (a small circular extrachromosomal DNA) containing the entire sequence of the engineered viral vector.

Cell culture

The viral vectors for vaccine are especially engineered in cell lines**.** The most commonly exploited cell line for production of viral vector vaccines is human embryonic kidney (HEK) 293 cells. HEK293 cells are convenient to this purpose due to their ease of culturing, rapid reproduction, tolerance for a variety of transfection methods, and efficient proteins production. These cells are grown in massive, sterile bioreactors (ranging from 200 to 2000 liters) filled with culture medium. The environment (temperature, pH, oxygen) is tightly controlled to help cell growth. Another commonly used cell line for viral vector production are Vero cells.

Transfection and infection (production)

The engineered plasmid DNA containing the viral vector is introduced into the HEK 293 cells. This process is called transfection. Once inside the cells, the cellular machinery translate the recombinant viral DNA and starts assembling thousands of copies of the viral vector particles. Each vector genome includes the gene encoding the target antigen.

Harvesting (vector particles collection)

After several days, the viral vectors have multiplied inside the cells, eventually causing cell lysis and release of the neo-vector particles into the culture media. The entire contents of the bioreactor (a mixture of culture medium, cell debris, and the valuable viral vectors) are collected. This mixture is commonly called the harvest**.**

4.2. Step 2: Downstream process "purification"

The goal of this step is to isolate the viral vectors from all the impurities found in the harvest.

Initial clean-up

First, the harvest is filtered through a series of filters to remove large cell debris and other impurities, leaving a clarified liquid containing the viral vectors.

Purification (chromatography)

This is a critical step. The clarified solution is loaded through a series of chromatography columns. These columns contain resins with special chemical properties that bind only to the viral vectors, removing all other contaminants (host cell proteins, DNA, etc.) to wash through. Finally, the viral

vectors are eluted for release from the resin in a highly purified form. Multiple different types of chromatography are used to obtain a high purity.

4.3. Formulation

The purified viral vector solution is concentrated to the exact viral titer required for the vaccine dose. It is then formulated with a buffer solution to stabilize it and ensure the vaccine remains effective during long storage periods.

5. Advantages and challenges of viral vector based vaccines

Viral vector vaccines consist of viral particles whose genomes have been modified to include one or more foreign genes encoding the targeted antigens. The advantages of viral vector vaccines combine between those of live attenuated and nucleic acid vaccines. Viral vectored vaccines are safe and induce both arm of innate and adaptive immunity. These responses are induced without involvement of the complete hazardous pathogen. Moreover, viral vectors have intrinsic adjuvant properties due to the engineered expression of diverse pathogen-associated molecular patterns (PAMPs) allowing the activation of innate immunity. In addition, viral vectors can be manufactured to deliver antigens to specific cells or tissues. Similarly, they can be engineered as replication-competent or replication-deficient to increase their safety and reduce the reactogenicity. Notably, the viral vector vaccine can recapitulate the natural infection process of specific pathogens, thus triggering classical acute inflammation and immune detection through the natural production of PAMPs, enabling mucosal delivery therefore the induction of local-mucosal and systemic immunity. Phase III clinical trials have been conducted for several viral vector-based prophylactic vaccine candidates or their approval has been already obtained for other.

The challenges of viral vector vaccines include pre-existing immunity against the viral vector backbone such as adenovirus-5, limit in the number of antigens incorporated to the particles, risk of persistence of viral vector replication and pathogenesis especially for immunocompromised individuals, risk of recombination during replication cycle. If the proper mutations occur, these vectors have potential to regain some pathological functions that were lost in the process of attenuation of the vector. Manufacturing scale-up constraints, distribution issues, and the broader

social-ethical complexities to ensure equitable access to novel vaccines. Generation of recombinant vectors remains time-consuming and relatively labor-intense due to the complexity of the process.

6. Current vector platforms

Many viruses have the intrinsic ability to be used as vectors and each family has its own advantages and disadvantages. Some of the most commonly used viruses belong to the adenovirus (Ad), poxvirus, lentivirus and rhabdovirus families.

6.1. Adenovirus

Adenoviruses (Ad) are double-stranded DNA viruses, their genome size is between 26-45 kb, non-enveloped viruses of 90-100 nm diameter see example in Figure-29. They commonly infect humans, characterized by mild clinical manifestations (respiratory symptoms). Ad vectors have a broad tropism, with strong gene expression capacity, and are generally quite safe due to low pathogenicity, but the sero-prevalence of existing antibodies against human Ad strains is quite high. Currently efforts are being redirected towards the use of Chimpanzee adenoviruses (ChAd) vector, which have a high degree of sequence homology with human Ad. The advantage of these adenoviruses is that they are not recognized by human antibodies. The best isotypes characterized for clinical studies are ChAd-3 and ChAd-63. Current viral vectored SARS-CoV-2 vaccines and Zabdeno, the first dose of the Zabdeno/Mvabea Ebola vaccine use the Ad platform.

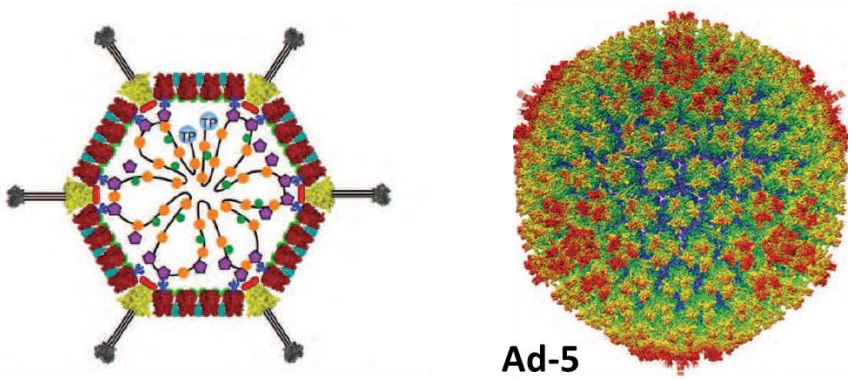

Figure-29: Molecular structure of adenovirus particle.

6.2. Poxvirus

The eradication of the smallpox virus is a good indication of the great potential for immunization by viruses belonging to the poxvirus family (Figure-30).

Poxvirus vectors show high immunogenicity and are easy to produce in cultures, but sero-prevalence of preexisting specific antibodies is high. Poxviruses have large genomes, which offer the capacity to incorporate multiple or complexed antigens. This makes them attractive for delivering multivalent vaccines. Example of derived vectors: There are several vectors developed from live attenuated poxviruses. For examples Modified Vaccinia Ankara (MVA), New York vaccinia (NYVAC) and Canarypox etc. The attenuated derivative MVA (Modified Vaccinia Ankara) is particularly noteworthy for its excellent safety profile: thus replicates poorly in most human cells but retains a robust immunogenic capacity. Nevertheless, manufacturing poxviruses can be more complex than adenovirus, partly due to their large size and cytoplasmic replication cycle mode.

Mvabea vaccine, the second dose of the Zabdeno/Mvabea Ebola vaccine uses a modified vaccinia Ankara vector, which is a type of poxvirus.

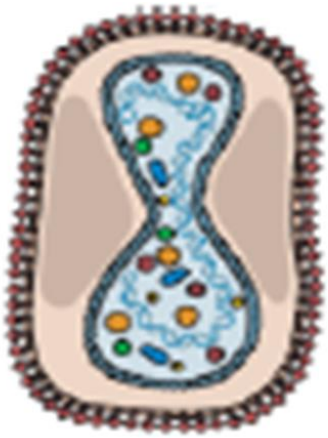

Figure-30: Schematic representation of the structure of poxvirus particle.

6.3. Yellow Fever Virus (YFV-17D)

The live attenuated Yellow Fever Virus YFV strain 17D is a viral vector belonging to the Flavivirus family (Figure-31). YFV-17D was developed in the 1930s as the first ever empirically derived human vaccine. The live attenuated vaccine targeting the Yellow Fever Virus YFV-17D is capable of inducing a very robust and effective immune response over an extended duration. YFV-17D is

used as a viral vector in the development of vectored vaccines especially targeting a number of viruses belonging to the Flavivirus family (JEV, WNV, Dengue virus).

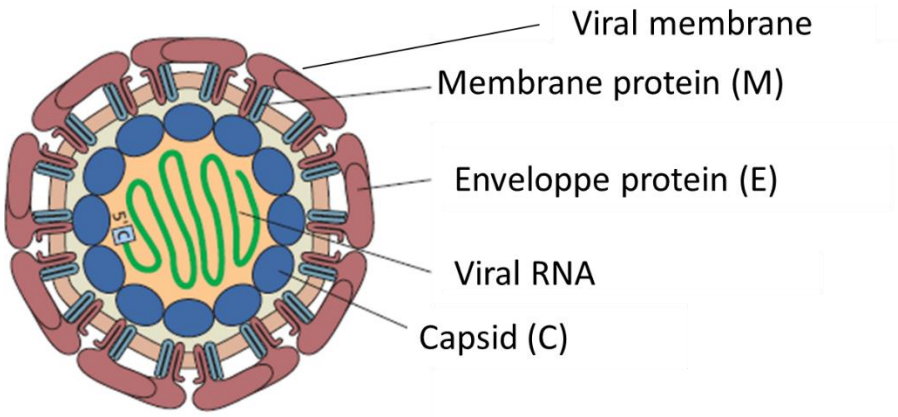

Figure-31: Structural organization of YFV viral particle.

7. Current viral vector based vaccines

7.1. Ebolavirus viral vector vaccines

Ebola virus is a single-stranded RNA virus with negative polarity. Its genome codes for nine functional proteins (Figure-32). Since their discovery in 1976, Ebola viruses have posed a persistent threat to human health. The risk of a resurgence of Zaire Ebola virus disease (EVD) is high, as shown by its 2014–2016 reemergence in West Africa, resulting in over 28000 cases and 11000 deaths. The frequent Ebola outbreaks highlight the need for persistent prevention and disease control activities. To date, two vaccines have been pre-qualified by the World Health Organization (WHO) and have received marketing authorization by the European Medicine Agency (EMA). Merck's Ervebo (rVSV-ZEBOV) is a recombinant vesicular stomatitis Indiana (VSV) virus expressing Zaire Ebola Glycoprotein (GP). The VSV vector is the only approved replication-competent viral vector vaccine valuable to this day.

The Johnson & Johnson vaccine is a combination that has two doses, one with the adenovirus-based vaccine Zabdeno (Ad26.ZEBOV), which expresses Zaire Ebola virus glycoprotein (GP) from Mayinga strain. The second dose Mvabea boost (MVA-BN-Filo), is made of Modified poxvirus Ankara (MVA) vector encoding GP from Zaire Ebola virus (Mayinga strain) Sudan virus

(Gulu strain) and Marburg virus (Musoke strain), along with the nucleoprotein from the Tai Forest virus.

) from Mayinga strain, combined to encoding GP from Zaire Ebola virus (Mayinga strain) Sudan virus (Gulu strain) and Marburg virus (Musoke strain), along with the nucleoprotein from the Tai Forest virus expressed on.

Additionally, the Russian vaccine GamEvac Combi consists of two live attenuated viruses: Adenovirus type-5 (Ad-5) and the replicative-competent Vesicular Stomatis Virus (VSV) both expressing the envelope glycoprotein (GP) of the Ebola virus Makona-C15 strain.

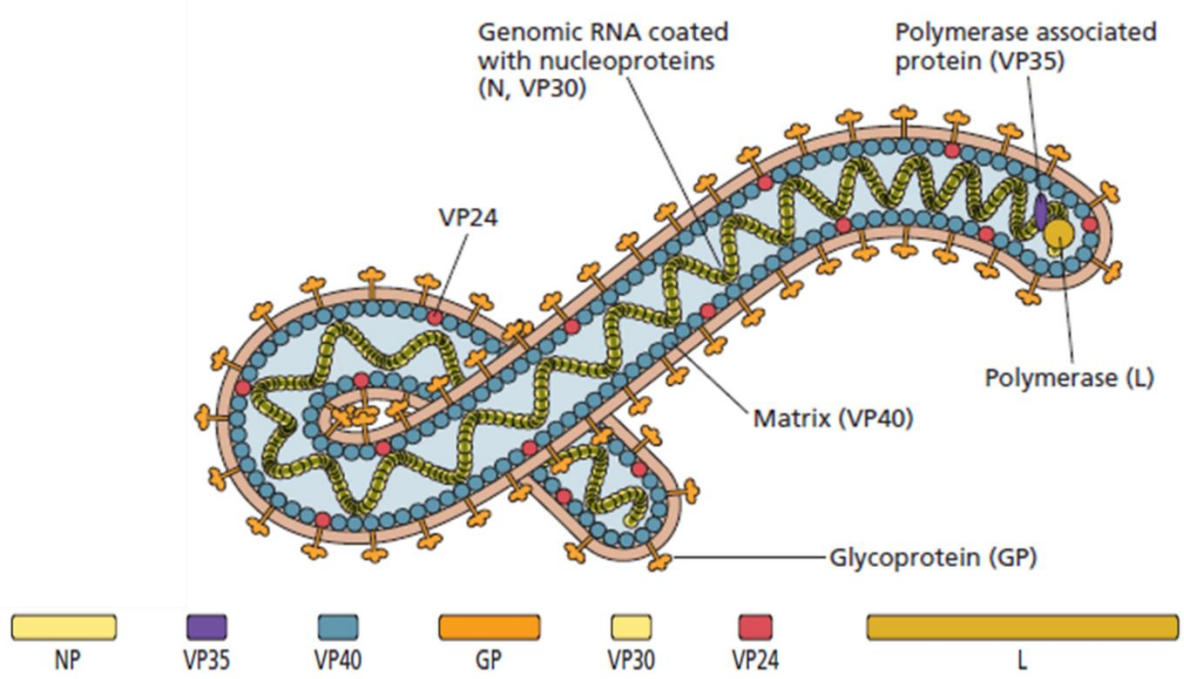

Figure-32: Illustration of Ebolavirus particle structure and highlight of the genes encoding its functional proteins.

7.2. SARS-Cov2 viral vector based vaccines

Currently four replication-deficient viral vectored vaccines against SARS-Cov2 that have gained approval for use in at least one country in the world: Oxford-AstraZeneca (ChAdOx1 nCoV-19), Janssen (Ad26.COV2.5), Convidecia (AD5-nCOV), and Sputnik V (Gam-COVID-Vac), the vaccines against SARS-CoV-2 (Table-16).

Table 16: List of approved viral vector vaccines against Sars-Cov2 worldwide.

Campany	Vector	Country
Oxford-Astra Zeneca	ChAdOx1	UK
Johnson and Johnson	Ad-26	USA
Sputnik V Gamaleya	Ad-26, Ad-5	Russia
Convidecia	Ad-5	China

7.3. IMOJEV vaccine against Japanese Encephalitis Virus (Sanofi Pasteur)

YFV-17D as a powerful vector and promising platform for the development of novel recombinant live vaccines, including two licensed vaccines against Japanese Encephalitis and Dengue viruses, even in pediatric use.

IMOJEV: also known as ChimeriVax™-JE represents the first viral vector-based one dose vaccine licensed for use in humans produced by Sanofi-Pasteur. This vaccine is based on the YFV-17D (yellow fever virus-17D) vector and directed against the Japanese Encephalitis Virus (JEV). The cDNA encoding the envelope proteins: (PrM (pr-membrane) and E (envelope) of the YFV17D vector are replaced by those belonging to the attenuated strain of JEV virus (strain SA14-2) (Figure-33).

Figure-33: Genome structure and design of YFV-17D viral vector vaccines against JEV and Dengue viruses.

PrM and E proteins on the YFV17D vector mutated to those belonging to attenuated JEV virus or to Dengue virus serotypes (CYD-1 to 4).

7.4. Dengvaxia against Dengue virus (Sanofi Pasteur)

Dengvaxia is a vaccine used to help protect against Dengue hemorrhagic fever in people aged 6 to 45 years who have had a previous Dengue virus infection. The vaccine is administrated through

intracutaneous route in three doses. Dengue disease is a mosquito-borne tropical disease caused by the Dengue virus, leading to mild, flu-like symptoms in most people. However, a small number of patients develop severe disease, with potentially fatal bleeding and organ damage. The risk of severe disease is higher in people who have been infected a second time. The tetravalent Dengue vaccine technology is based on the construction of a chimeric virus formed by the YFV-17D vector backbone carrying modifications in PrM (pr-membrane) and E (envelope) structural genes, which are mutated with the prM and E structural genes belonging to Dengue virus named Dengue virus serotypes (CYD-1 to 4) (Figure-33). There are several serotypes of Dengue virus and Dengvaxia protects against serotypes 1, 2, 3 and 4.

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Conclusion

Human diseases, particularly infectious diseases pose unprecedented challenges to public health and the global economy. The development of novel prophylactic and therapeutic vaccines are the prioritized countermeasures of human disease. Conventional vaccine platforms, such as inactivated or live attenuated vaccines, have proven highly effective against many pathogens. Currently, most approved vaccines are live attenuated, inactivated, and provide immunizations against a wide range of pathogens. Since Edward Jenner's discovery of the first vaccine in 1796, humanity has made great progress in the field of vaccinology. Despite these advances, the rate of vaccine development over the past 40 years has slowed drastically. Many infectious diseases still do not have approved-vaccines, even after decades of rigorous research. Some of the major factors for this decline in vaccine development include the individual properties of various viruses (genetic instability, hosts variety, lack of animal models). Additionally, traditional vaccination methods are unable to produce results with some of these more difficult pathogens such as HIV-1, Zika virus, HCV, CMV, HSV, EBV, Nipah virus etc. Thus, new sophisticated methods must be developed. Nucleic acid vaccine approaches are known as a possible solution for a rapid pandemic response. The need for only the sequence of pathogen in order to generate the vaccine and this simplicity in manufacture have long been recognized as superpowers in nucleic acid vaccines with regard to the delivery of a rapid response to an emerging epidemic. In addition, to the safety and high immunogenicity of these vaccines. Among vaccine platforms, viral vectors, the genetically engineered viruses that can deliver foreign genes into host cells, have emerged as a powerful strategy for prophylactic and therapeutic vaccine development. Currently, viral vector vaccines remain one of the best strategies for induction of robust humoral and cellular immunity, often superior to what traditional inactivated or subunit vaccines can achieve, against human diseases without use of adjuvants, this platform represent prominent choices for pathogens that have failed control efforts based on conventional vaccine strategies.