

## VACCINES |

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### 17.1 INTRODUCTION

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The number of infectious diseases that used to kill millions of people has drastically decreased due to “vaccinations”. Since 1997, no cases of spontaneously acquired smallpox have been documented, and the campaign to end polio has accelerated. However, polio has returned due to cultural and religious opposition, but many vaccination programs are back on track. Vaccines against AIDS, TB, malaria, and other diseases are still needed, and more work is required to increase efficacy, safety, cost, and administration. The process of creating novel, effective vaccines is complex and expensive, and rigorous testing is necessary to ensure the vaccine's effectiveness. Immunologists optimise immune responses by considering epitope variations, identifying antigen-processing pathways, and using techniques such as genetic engineering to enhance vaccination efficacy. You will study vaccines, their modes of action, and the distinctions between active and passive immunisation in this unit. You will also learn about the many types of immunisations, their benefits and drawbacks. The unit will also provide a summary of the immunisation programmes the Indian government has put in place.

## Objectives

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After studying this unit, you should be able to:

- ❖ define vaccines;
- ❖ describe how the immune system protects the body from infections;
- ❖ explain active and passive immunisation;
- ❖ discuss the mechanism of action of vaccines;
- ❖ explain how many generations of vaccines there are;
- ❖ enlist the different types of vaccines along with their formulations, benefits, and limitations; and
- ❖ explain the various vaccination programs implemented by the Government of India.

## 17.2 EVOLUTION OF VACCINES AND VACCINATION PRACTICES

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The discovery that people who had previously been exposed to certain infectious diseases were later protected against them led to the study of immunology.

The English word immunity, which refers to the condition of protection against infectious diseases, is derived from the Latin word 'immunis', which means exempt. Immunity was first described, at least in writing, by Thucydides, who is known for his history of the Peloponnesian War. According to his description of the plague that afflicted Athens in 430 BC, only those who had recovered from the illness could tend to the sick without worrying about contracting the illness again. While early societies noted the phenomenon of immunity, the idea was not translated to medical practice for nearly 2000 years.

The first recorded efforts to intentionally induce immunity began in the fifteenth century. They came from the Chinese and Turks in their ongoing efforts to combat smallpox, a disease that had a mortality rate of about 30% and left survivors permanently disfigured. It is believed that a method referred to as variolation was used to prevent smallpox through inhalation or the application of dried crusts from the smallpox pustules, either through minor cuts in the skin or the nostrils. Inspired by the finding that milkmaids who had the less severe cowpox were later protected against the disease, Jenner postulated in 1798 that persons who are resistant to severe smallpox could be protected from the sickness by giving fluid from a cowpox pustule.

Later, Edward Jenner, an English physician, made a significant advancement in the development of intentional immunity, specifically against smallpox. In 1798, Jenner proposed that those who were resistant to severe smallpox could be shielded from the illness by administering fluid from a cowpox pustule. This theory was prompted by the discovery that the milkmaids who contracted less severe cowpox were later protected. To prove his theory,

Jenner deliberately exposed an eight-year-old boy to smallpox after vaccinating him with cowpox fluid. The child did not get the illness, as was expected. Although Jenner's method of utilising cowpox as an inoculant to prevent smallpox was a major advancement, human experiments were unethical under prevailing medical guidelines. However, Jenner's approach quickly became popular in Europe. But it wasn't until almost a century later that this method was used for other illnesses. Astute observation and scientific serendipity led to the next significant advancement in immunology, the development of immunity against cholera.

Louis Pasteur achieved a crucial advancement by successfully culturing bacteria which cause fowl cholera in a laboratory. By injecting it into chickens, which later died of cholera, he was able to verify its virulence. Pasteur and his associates returned to their experiments after summer vacation. After administering an old bacterial culture to several hens, the birds became ill but unexpectedly recovered. After becoming intrigued, Pasteur created a new culture of bacteria to inject it into chickens that had never been exposed to it before. However, he did use a combination of previously exposed and unexposed birds, since fresh chickens were in short supply. Surprisingly, the chickens that had previously been exposed to older culture were immune to disease, while only fresh chickens died.

Pasteur postulated and subsequently verified that a pathogen's virulence had decreased with age. He proved that immunity against the illness could be conferred by administering such an attenuated strain. Pasteur named this attenuated strain "vacca," which translates to "cow," in recognition of Jenner's contributions to the development of the cowpox vaccine. Pasteur demonstrated the viability of attenuating pathogens and using them as vaccines by applying his revolutionary discoveries to other diseases beyond cholera.

In 1881, Pasteur carried out yet another ground-breaking vaccine investigation in the village of Pouilly-le-Fort. He first used heat treatment to attenuate the anthrax bacteria (*Bacillus anthracis*) in a single group of sheep. A strong culture of the anthrax bacillus was then given to the sheep, both vaccinated and unvaccinated. Interestingly, all the sheep that received the vaccination survived, whereas all the animals that did not receive the vaccination died from the infection.

Pasteur reached yet another significant milestone in 1885 when he gave his first vaccine to a human. The recipient was Joseph Meister, a little youngster who had suffered multiple bites from a rabid dog. Meister received a series of attenuated rabies virus inoculations. When administered shortly after exposure, the rabies vaccination is one of the few that can be effective as long as the virus has not yet entered the central nervous system and produced neurological symptoms. Following his recovery, Joseph Meister became a caregiver at the Pasteur Institute. In 1887, the Pasteur Institute was established as a facility for the treatment and prevention of infectious diseases. In keeping with its mission to fight contagious diseases, the Pasteur Institute remains a premier institution today.

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## 17.3 VACCINATION AND ITS ADVANCEMENT

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Many infectious illnesses that formerly claimed millions of lives have been brought under control or eradicated thanks to preventative vaccinations. There hasn't been a single documented case of spontaneous smallpox anywhere since October 1997. Following the worldwide triumph over smallpox, the campaign to end polio accelerated. By 2000, there were around 95% fewer cases of polio worldwide thanks to the efforts of the WHO and several significant charitable benefactors. Unfortunately, polio has returned recently due to cultural and religious opposition to immunisation; nevertheless, many vaccination programmes are again back on track. Additionally, at least 10 serious infectious diseases (measles, rubella, typhoid, mumps, tetanus, diphtheria, influenza, pertussis, and others) have been successfully controlled by global immunisation efforts.

Vaccines against AIDS, TB, malaria, and many other illnesses are still desperately needed. Furthermore, additional work is required on the current vaccines to either improve their safety and efficacy in certain situations or lower their cost and simplify their administration to reach as many people as possible, especially in developing countries. WHO research shows that millions of baby deaths are still attributable to diseases that may be avoided with current vaccinations.

The process of creating novel, effective vaccines is drawn out, complex, and expensive; Rarely does it progress to the extent that years of clinical trials are required. Numerous vaccine options that were promising in laboratory and animal trials end up causing undesirable side effects, worsening the illness or failing to prevent it. Since a significant number of individuals will receive authorised vaccines, rigorous testing is essential. Users must be given accurate information about adverse side effects, especially those that are uncommon, and they must carefully balance that information against the potential protection that the vaccine may offer.

Fundamental research is the first stepping stone toward developing a vaccine. To optimise the activation of humoral and cellular immune responses, immunologists can now consider variations in the epitopes recognised by T and B cells in vaccination candidates. Different antigen-processing routes have been identified, and design techniques and additives can be used to maximise antigen presentation and activate specific targeted immune pathways. There are also ongoing targeting initiatives to elicit protection at the most prevalent area of infection is the mucosal surfaces. Finally, vaccines that are easier to administer and that maximise the immune response to specific epitopes are being developed using techniques such as genetic engineering. Here, we outline the vaccines currently in use, the vaccination strategies, and novel approaches that may lead to future vaccines.

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## 17.4 IMMUNISATION

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Increasing or developing a person's resistance to an infectious disease is known as immunisation, and it typically involves administering a vaccine. Vaccines help the immune system recognise and combat pathogens (like bacteria or viruses) without causing the disease itself.

### 17.4.1 Classification of Immunisation

Immunisation can be classified into two categories based on its mechanism of action: active and passive immunisation. The distinct roles of each type influence the immune response.

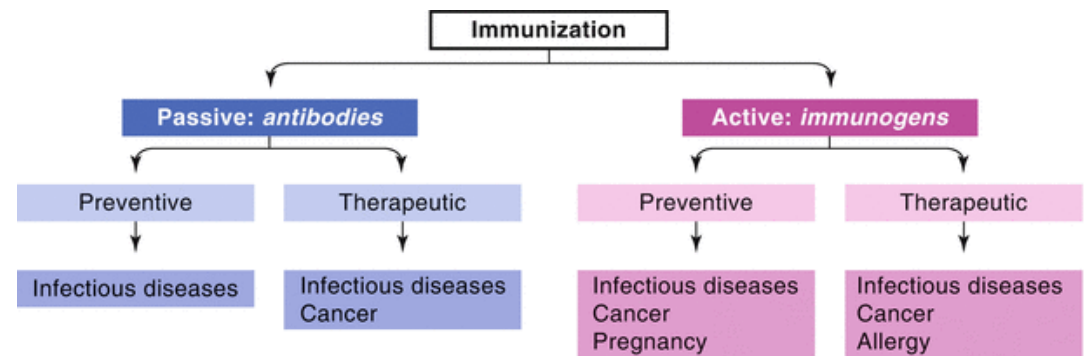


Fig. 17.1: Classification of Immunisation.

#### a) Passive Immunisation

Edward Jenner and Louis Pasteur were among the early pioneers of vaccination and are acknowledged as its founders. Hidesaburo Kitasato and Emil von Behring are also significant contributors. “Emil von Behring and Hidesaburo Kitasato” merit no less recognition for their significant contributions to passive immunity.

By injecting serum from the immune organism, these researchers were the first to show that immunity produced in one organism could be transmitted to another. When maternal IgG antibodies cross the placenta to the growing foetus during pregnancy, passive immunisation, which involves the transfer of prepared antibodies to a recipient, occurs spontaneously. Maternal antibodies passively protect the foetus against infections like poliovirus, rubella, mumps, tetanus, measles, streptococci, and diphtheria. Furthermore, the newborn can receive passive immunity from the mother's IgA antibodies present in breast milk.

Antiserum, or pre-made antibodies, from people immune to a certain disease can also be used to achieve passive immunisation. When vaccines and medicines were unavailable, passive immunisation provided vital humoral defence and has been the main effective treatment for some serious diseases, such as diphtheria. Passive immunisation is currently required for several conditions, including:

- Immunodeficiency, especially acquired or congenital B-cell abnormalities.
- Exposure to toxins or venoms that present an immediate life-threatening danger.
- Exposure to pathogens capable of causing death more rapidly than the host's immune system can mount an effective response.

Passive immunization is frequently given to infants with congenital immune deficits and children who have severe respiratory failure brought on by the respiratory syncytial virus (RSV). When unvaccinated people are exposed to




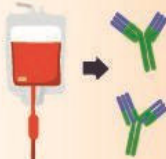
organisms which cause tetanus, botulism, hepatitis, measles, rabies, and diphtheria, they use passive immunity. It is also used to safeguard healthcare professionals and tourists who may be exposed to infections for which they lack protective immunity. Antiserum is utilised to protect against venomous snake and insect bites. It is important to note that the host's immune response is not activated by passive immunisation. As a result, it produces no memory response, and the security it offers is temporary.

While passive immunisation might be effective, it warrants cautious use due to the risks associated with injecting preformed antibodies. When antibodies originate from another species, such as horses (a common source), recipients may mount a robust response against the unique parts of the foreign antibody, known as isotypic determinants. There may be serious consequences from this anti-isotype reaction. Certain people produce IgE antibodies specific to horse-derived determinants. Increased concentrations of these IgE-horse antibody complexes have the potential to cause systemic anaphylaxis by causing extensive mast-cell degranulation. To create immune complexes that activate complement, others might generate IgG or IgM antibodies that bind the foreign antibody. When these compounds are deposited in tissues, type III hypersensitivity reactions may result. Even when human antiserum or gamma globulin is administered, recipients may generate an anti-allotype response against human immunoglobulins, recognising antigenic differences within the same species. However, this response typically has lower intensity than an anti-isotype response.

**b) Active Immunization**

Active vaccination aims to initiate an adaptive immune response that confers immunologic memory and protective immunity, whereas passive vaccination provides temporary protection or alleviates an existing ailment. Effective active vaccination eliminates the pathogen or prevents illness caused by its byproducts by triggering a secondary immune response when the patient is subsequently exposed to the pathogen. Active immunisation can happen spontaneously through microbial infection or synthetically through vaccination. The immune system actively participates in active immunisation by stimulating the growth of antigen-reactive B and T cells, which in turn produce memory cells that provide protection. This procedure is consistent with the main goal of immunisation. A comparison of active and passive immunisations is presented in Table 17.1.

**Table 17.1: Comparing Active and Passive Immunisation.**

ACTIVE IMMUNITY		PASSIVE IMMUNITY	
Natural	Artificial	Natural	Artificial
 Infection	 Vaccination	 Maternal antibodies	 Monoclonal antibodies

## SAQ 1

Answer the following questions in one word.

- Who is considered the pioneer of smallpox vaccination?
- Who gave the concept of an attenuated vaccine?
- Which immunisation involves the transfer of preformed antibodies?
- What type of antibody can cross the placenta during pregnancy?
- What type of immune cells form as a result of active immunisation?
- What process can render microorganisms non-pathogenic, while retaining their ability to grow transiently within a host?

## 17.5 GENERATION AND TYPES OF VACCINE

Vaccines are biological preparations that, without causing disease, promote the immune system—the body's natural defence mechanism—to identify and fight off specific invaders, typically bacteria or viruses.

They achieve this by injecting antigens, which are chemicals that mimic specific components of a disease and trigger an immune response in the body (Fig. 17.2). Antibodies and memory cells are produced as part of this reaction, helping the body fight off infections in the future. Here's an overview of how vaccines are manufactured and the various types available:

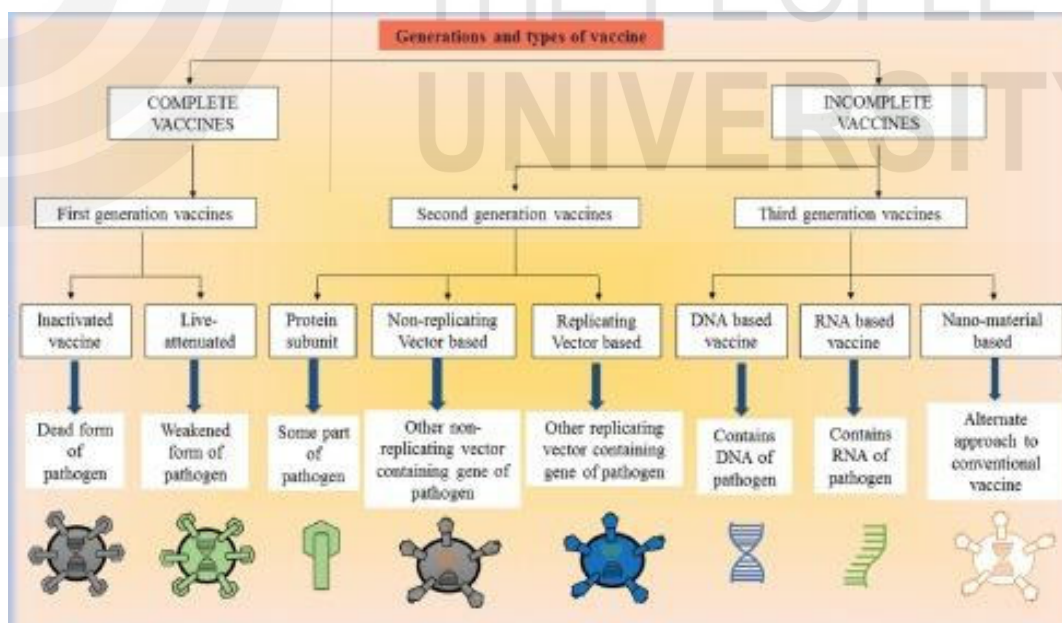


Fig. 17.2: Types of Vaccines and their generations.

### 17.5.1 Complete Vaccine

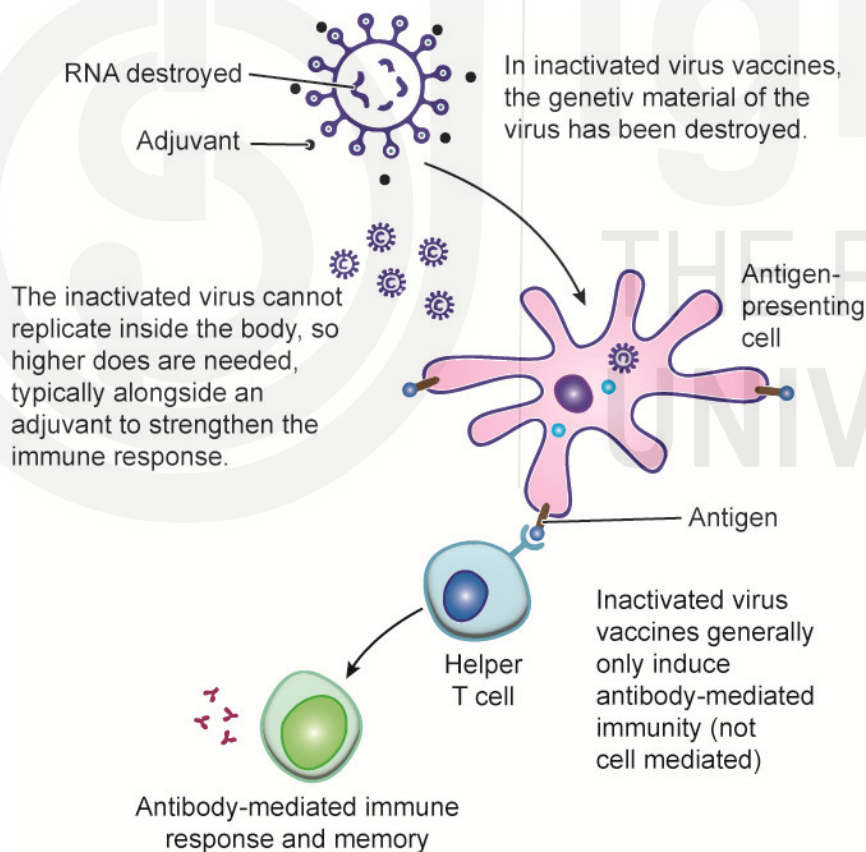
A complete vaccine refers to a fully formulated and functional immunisation product that contains all necessary components to safely and effectively stimulate a protective immune response against a specific pathogen.

## a) First-Generation Vaccine

First-generation vaccines use whole, inactivated, or attenuated pathogens to elicit an immune response without causing disease. Live-attenuated vaccines, such as smallpox, polio, and measles, rely on traditional methods and are highly effective. However, they do have limitations, such as reversion risks in live vaccines and the need for multiple doses with inactivated vaccines.

### i) Inactivated Vaccine

Another popular method for making pathogens suitable for utilisation in vaccines is to treat them using heat and chemicals. By efficiently eliminating the pathogen and stopping its replication, this technique preserves the pathogen's capacity to elicit an immune response to at least part of an organism's antigens. Maintaining the structure of surface antigen epitopes during the inactivation process is vital. Due to the tendency for extensive protein denaturation, the heat-inactivation method is often found to be unsatisfactory. Therefore, there is a good chance that epitopes based on higher levels of protein structure will change significantly. The use of formaldehyde or other alkylating chemicals for chemical inactivation has also been effective in making pathogens safe for use in vaccines.



**Fig.17.3: Inactivated vaccines that use viruses that have been killed or altered so they cannot replicate, which induce the production of antibodies.**

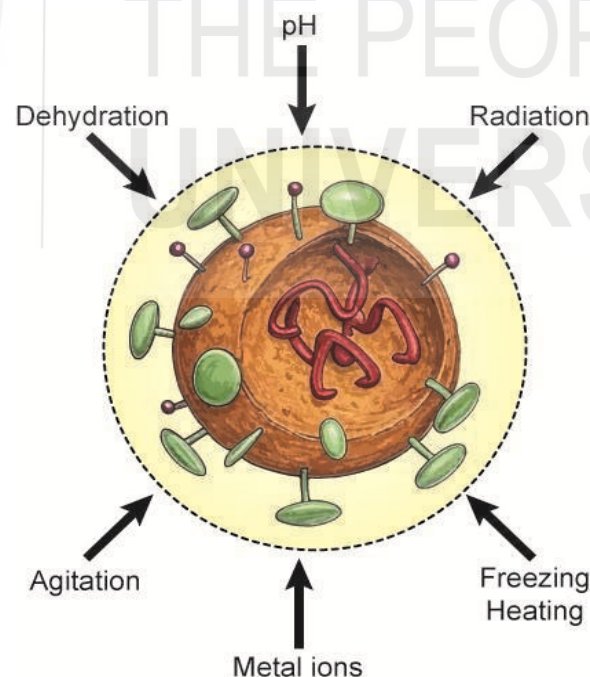
The poliovirus is inactivated with formaldehyde to produce the Salk polio vaccine. Killed vaccines often require multiple booster shots to induce protective immunity, whereas live attenuated vaccines usually provide lifelong immunity with a single dose. Typically, a predominantly humoral antibody response is elicited by killed vaccinations because they do not reproduce within the host. Generally speaking, they are less effective than attenuated

vaccines at generating a secretory IgA response or fostering cell-mediated immunity, two crucial components of an ideal mucosal-based and protective immune response.

There are still certain dangers associated with inactivated whole-organism vaccines even when they contain destroyed pathogens. In two vaccination lots, formaldehyde failed to destroy all of the virus, which led to a serious problem with the early Salk vaccines: a large proportion of recipients developed paralytic polio. Additionally, there are risks associated with the production of inactivated vaccines. People are at risk of infection if they handle substantial amounts of the infectious agent before it is inactivated. However, compared to live attenuated vaccinations, inactivated vaccines generally have a better safety profile. Hepatitis A and cholera vaccines, as well as the yearly flu shot, are examples of inactivated vaccines commonly used to prevent bacterial and viral illnesses. Apart from their relative safety, inactivated vaccines offer advantages including stability and ease of storage and transportation.

## ii) Live, Attenuated Vaccine

Sometimes bacteria can be attenuated or rendered non-pathogenic, meaning they can no longer cause serious illness (pathogenicity), but they can still temporarily flourish inside a host after inoculation. Some agents are naturally attenuated because they cannot cause disease in particular hosts, yet they can still confer immunity upon inoculation. The first vaccine developed by Jenner exemplifies the principle that inoculating humans with the vaccinia virus (cowpox) confers immunity to smallpox without causing the disease.



**Fig.17.4: Attenuation of live pathogens through different stressors.**

Attenuation is frequently achieved by cultivating a pathogen, such as a virus or bacterium, for prolonged periods under unusual culture conditions. Mutants that are more adapted to growing in these circumstances than in their native host are chosen through this process. For example, *Bacillus Calmette-Guérin* (BCG), an attenuated strain of *Mycobacterium bovis*, is produced by growing

*M. bovis* on a medium with elevated bile concentrations. After 13 years, this strain became sufficiently attenuated to serve as a TB vaccine upon adaptation to growth in high bile concentrations. However, BCG is not used in the US due to inconsistent efficacy and difficulties with monitoring. Attenuated viral strains are used in both the measles vaccination and the Sabin polio vaccine. There are benefits and drawbacks to attenuated vaccinations. Transient growth allows these vaccines to replicate the growth patterns of the real pathogen by exposing the immune system to specific epitopes on attenuated organisms for a prolonged period. This leads to greater immunogenicity and more effective memory cell formation. As a result, attenuated vaccines often require only one dose, which is advantageous in developing nations where booster shots can be challenging to obtain.

Many attenuated vaccines excel at inducing cell-mediated responses because they replicate within host cells. OPV (oral polio vaccine), developed by Albert Sabin, is one instance of this. OPV is given orally to children and consists of three attenuated poliovirus strains. Secretory IgA, a vital defense against normally acquired poliovirus, is produced when these attenuated viruses infiltrate the intestine. The vaccine also promotes the production of IgM and IgG antibodies, which ultimately confer protective immunity against all three deadly poliovirus strains.

Unlike most attenuated vaccines, OPV requires booster injections. The reason is that the three attenuated poliovirus strains interfere with one another's intestinal replication. One strain becomes more common after the initial inoculation, creating immunity to it. Subsequent immunisations face the challenge of overcoming the immunity generated by previous shots. Thus, tolerance to the once-dominant strain decreases with each new dose, allowing one of the other bacteria to colonise the intestines and confer immunity. In the end, immunity to all 3 strains is attained following the third vaccination. One of the main drawbacks of attenuated vaccinations is the potential for the living forms to transform and revert to virulent forms in the recipient, which might paralyse them and serve as a conduit for the transmission of infections. For instance, it is predicted that the OPV has a reversion rate of roughly 1 case per 2.4 million doses. In areas with poor sanitation or where wastewater is recycled, this reversion can potentially bring harmful viral types into the water supply. To reduce this risk, some regions have decided to use only the inactivated polio vaccine. The continued use of OPV worldwide may pose challenges in achieving the projected eradication of paralytic polio.

Additionally, attenuated vaccines may cause side effects similar to those seen in the underlying illness. A tiny proportion of measles vaccine recipients, for instance, may develop postvaccine encephalitis or other side effects; nonetheless, the risk of vaccine complications is still far lower than the risk of infection. According to studies, there were only about 1 case of vaccine-related encephalopathy for every 1.5 million doses administered between 1970 and 1993. This low incidence, compared with the rate of infection-related encephalopathy, highlights the effectiveness of immunisation. Additionally, even in affluent countries, measles infection is associated with a significant death rate, which emphasises the significance of vaccination efforts.

Attenuated vaccines may also pose risks similar to those encountered in natural disease. For example, a small proportion of people who receive the measles vaccine may develop postvaccine encephalitis or other complications. Still, the risks associated with the vaccine are far lower than those of infection. In a separate investigation, 48 cases of vaccination-related encephalopathy were identified between 1970 and 1993, during which 75 million doses of the measles vaccine were administered. This is about 1 case per 1.5 million doses. This extremely low rate highlights the efficacy of vaccines when compared to the incidence of encephalopathy linked to measles infection. Furthermore, the high death rate related to measles infection, especially in affluent nations, provides an even stronger argument for vaccination. This reality underscores the importance of vaccination efforts in safeguarding public health and preventing potentially fatal outcomes associated with measles. Apart from conventional culturing techniques, genetic engineering can permanently weaken viruses by eliminating specific genes required for virulence or host growth. By removing the thymidine kinase gene, this method was used to create a pig herpesvirus vaccine. The elimination of thymidine kinase rendered the virus incapable of causing illness because it requires replication in specific cell types, such as neurons.

Apart from conventional culturing techniques, genetic engineering can permanently weaken a virus by eliminating specific genes required for virulence or host growth. By removing the thymidine kinase gene, this method was used to create a pig herpesvirus vaccine. The elimination of thymidine kinase rendered the virus incapable of causing illness because it requires replication in specific cell types, such as neurons. The rising popularity of cold-adapted, nasally administered flu vaccines can be attributed to their ease of administration and their ability to induce effective mucosal immunity.

### **17.5.2 Incomplete Vaccine**

A complete vaccine is not fully formulated. Subunit proteins, DNA or RNA sequences, and replicating or non-replicating vectors are all present, and they all cooperate to promote a defenceless immune response against a specific pathogen.

#### **Second-Generation Vaccine**

To enhance first-generation vaccines, such as live attenuated or inactivated vaccines, a second-generation vaccine has been developed using cutting-edge biotechnological processes. Second-generation vaccines include vector vaccines that proliferate or do not, as well as subunit (protein) vaccines. These vaccines are typically more precise and safer, allowing them to target specific pathogen components with greater effectiveness.

##### **i) Subunit vaccine**

To mitigate risks associated with attenuated or killed whole-organism vaccines, only specific, pure macromolecules derived from the pathogen should be used. The three primary uses of this method, referred to as a subunit vaccination, include essential recombinant protein antigens, capsular polysaccharides or surface glycoproteins, and inactivated exotoxins or toxoids.

Some subunit vaccines, particularly polysaccharide vaccines, have a serious disadvantage in that they cannot stimulate T cells. Rather, the thymus-independent type 2 (TI-2) pathway activates B cells that produce IgM but lead to little memory cell development, minimal class switching, and no affinity maturation. This problem can be reduced, though, by vaccinations that trigger TH cell responses by conjugating a polysaccharide antigen to the protein carrier.

Exotoxins are produced by a small number of bacterial pathogens, including those which cause tetanus or diphtheria, and they are responsible for most or all of the symptoms of infection-related sickness. Purified bacterial exotoxins are inactivated with formaldehyde to produce toxoids, which are used to make tetanus and diphtheria vaccines.

Immunisation with toxoid prompts the production of antitoxin antibodies that can bind to the toxin and neutralise its effects. It is crucial to tightly control and balance the conditions for producing toxoid vaccines to prevent excessive alteration of epitope structure while ensuring complete detoxification. As previously stated, unvaccinated people exposed to organisms that produce these exotoxins can also benefit temporarily from passive immunity. However, this method does not confer long-term protection. Certain pathogenic bacteria primarily rely on their hydrophilic polysaccharide capsule's antiphagocytic characteristics to determine their virulence. Macrophages and neutrophils are much more able to phagocytose such pathogens when the capsule is coated with complement and/or antibodies. Purified capsular polysaccharide vaccines are based on these discoveries.

For instance, the current vaccination, which contains 13 antigenically unique capsular polysaccharides (PCV13), is effective against Pneumococcal pneumonia caused by *Streptococcus pneumoniae*. All newborns are now advised to receive this vaccine, which promotes the development of opsonising antibodies. Purified capsular polysaccharides are also used in vaccines against *Neisseria meningitidis*, a prevalent cause of bacterial meningitis. Although they have had limited success, several viruses transmit surface glycoproteins, such as the envelope protein from HIV-1, which have been studied for use in antiviral vaccines. Moreover, in clinical trials of some vaccines, HSV-2 glycoprotein D is effective in preventing genital herpes. This implies that using glycoproteins could also be a viable strategy for some antiviral vaccinations.

Recombinant DNA technology may theoretically be used to clone and express any gene expressing an immunogenic protein in cultured cells. This approach is commonly applied in the development of subunit vaccines. For example, cloning the genes which encode exotoxins from pathogenic organisms into easily cultivated host cells is one of the safest ways to produce enough pure toxins for toxoid vaccines.

The development of vaccines has also involved the successful cloning of several genes encoding surface antigens from bacterial, viral, and protozoan illnesses into cellular expression systems. The hepatitis B vaccine, which was developed by cloning the gene encoding the main HBsAg (hepatitis B surface antigen) and expressing it in yeast cells, is the first recombinant vaccine

authorised for human use. This method allows HBsAg to accumulate in recombinant yeast cells by cultivating them in large fermenters. Following the extraction and disruption of the yeast cells, recombinant HBsAg is liberated and purified by standard biochemical methods. The recombinant hepatitis B vaccine has great potential to protect people worldwide against this infection by stimulating the production of protective antibodies.

### ii) **Replicating vector-based vaccine**

Replicating vector vaccines infect healthy cells, producing new viruses and vaccine antigens. This process can stimulate the immune system but also spread the infection. A viral vector vaccine that replicates inside cells is the newly authorised Ebola vaccine. These vaccines elicit a strong immune response and are usually safe. Pre-existing immunity to the vector could reduce vaccine effectiveness.

### iii) **Non-replicating vector-based vaccine**

Viral vectors that are modified so they cannot replicate are called non-replicating vectors. Over time, these viruses become weakened so they no longer multiply in human cells. However, they can still stimulate the immune system as intended. This makes them useful for vaccines and gene therapy.

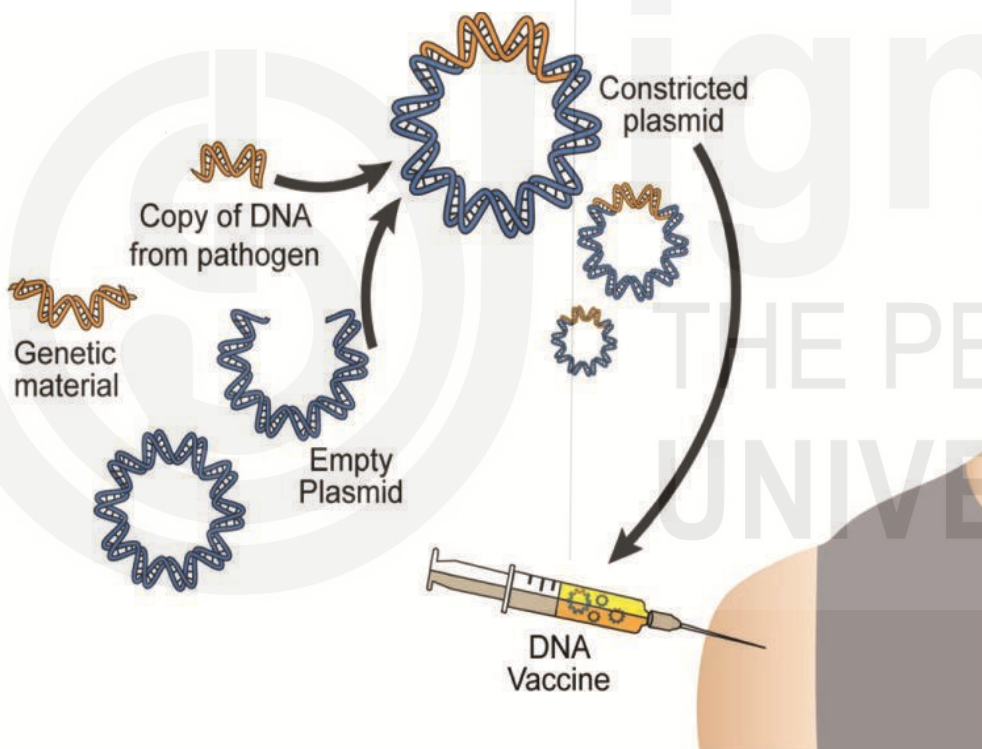
## **17.5.3 Third-Generation Vaccine**

These vaccines are more advanced and are made using synthetic methods. They often rely on recombinant DNA technology to produce antigens that trigger an immune response without using the actual pathogen. Third-generation vaccines have several features: they use synthetic antigens produced in labs, they include genetic material from viruses or bacteria via recombinant DNA, and they deliver this genetic material into the body using plasmids. These plasmids help the body recognise the pathogen and develop immunity. Third-generation vaccines are as follows:

### a) **DNA vaccine**

A relatively recent vaccination method, known as a DNA vaccine, involves injecting plasmid DNA containing antigenic proteins directly into the recipient's muscle. This technique relies on the DNA being absorbed by host cells, which then produce immunogenic protein *in vivo*. By directing antigen through endogenous MHC class I presentation pathways, it thereby improves CTL responses. It is observed that the DNA either remains episomal for a long time or integrates into the host chromosome. It is observed that the DNA either remains episomal for a long time or integrates into the host chromosome. DNA is usually carried up by muscle or dendritic cells (DCs) at the injection site. Delivery to local dendritic cells may be necessary for muscle cells to mount an antigenic response to DNA vaccines, given their low expression of class I MHC molecules and the lack of costimulatory molecules. DNA vaccines can elicit protective immunity against a variety of infections, including rabies and influenza viruses, according to experiments in animal models. Techniques such as adding more DNA sequences to the vector or giving follow-up booster shots with a protein antigen (sometimes referred to as the DNA prime-protein boost technique) can improve the immune response. Compared with many

current immunisation strategies, DNA vaccines offer several potential benefits. The immune response targets the antigen exactly as it manifests in the pathogen, causing the host to express the encoded protein in its native state, unaltered by denaturation or other alterations. This stimulation results in humoral and cell-mediated immunity. Non-DNA vaccines typically require inoculation with a live attenuated preparation to activate both arms of the adaptive immune response, which adds additional risks. Additionally, DNA vaccines enhance the establishment of immunological memory by prolonging antigen expression. Additionally, DNA vaccines offer several advantages, including eliminating the need to refrigerate plasmid DNA, thereby addressing storage issues over extended periods. In addition, many DNA vaccines for different infections can be produced simultaneously by customising a single plasmid vector to insert DNA encoding different proteins, saving time and resources. Plasmid DNA is coated onto tiny gold beads, which are then delivered by a gene gun through the skin into the underlying muscle as an improved DNA vaccine administration technique. This method improves safety and cost-effectiveness by allowing vaccines to be administered quickly to huge populations without requiring a large number of syringes and needles.



**Fig.17.5: Formulation and administration of DNA vaccine.**

Human trials are currently underway for various DNA vaccines targeting diseases involving HIV, malaria, Ebola, influenza, herpesvirus, and several cancers. Although there are presently no approved human DNA vaccines, 3 were approved for use in animals. A vaccine against the West Nile Virus (WNV) that has proven effective in protecting horses is one example. Humans have also been tested with this vaccine, and after three doses, the majority of subjects showed neutralising antibody titres similar to those observed in horses. Responses from CD4 T and CD8 cells against the virus were also noted. However, the hazards involved are still mostly unclear because the broad development of DNA vaccines for human utilisation is still in its early phases.

## b) Protein and peptide-based vaccine

Proteins or peptides are used as antigens in protein vaccines to elicit an immunological response. As mentioned earlier, the virus that causes the infectious disease can naturally produce these protein antigens either as whole proteins or as separable products. Although protein vaccines made from naturally occurring contagious agents are beneficial, they have several disadvantages. These vaccines are produced and purified using expensive, time-consuming procedures that require the development of several microorganisms. Furthermore, some viruses are either inefficient to grow or pose major risks when cultivated in large quantities, making it challenging to manufacture them from natural sources. Certain human proteins, such as protein factors, may occasionally be needed to treat non-infectious illnesses, including high blood pressure and cancer. Protein subunit vaccines can also be made as heterologous proteins in recombinant systems as an alternative to natural sources. It is thought to be safer and less costly to produce using well-established expression systems, such as yeast, recombinant bacteria, insect cells, and mammalian cells, rather than natural sources. Finding the ideal antigenic component to elicit a successful immune response is difficult, though. Although full-length proteins tend to retain their native shape and can elicit antibodies against distinct epitopes, they also increase the risk of nonspecific cross-reactivity.

Since effective immune responses can be elicited by specific amino acid sequences within the full-length antigen, some immunogenic peptides that mimic T- and B-cell epitopes have been proposed as possible vaccine candidates. Therefore, it is crucial to optimise the amino acid sequence to decrease the number of epitopes recognised by non-neutralising antibodies and increase those recognised by neutralising antibodies, which block pathogens and provide protection. Although the antigens or epitopes in subunit vaccines are relatively small and have low valence, they often exhibit lower immunogenicity than traditional vaccines.

Furthermore, unlike subunit protein vaccines, whole-organism vaccines don't expose a single copy of an antigen, as they contain multiple copies of each antigen along with other immunostimulatory molecules. This holds whether the vaccine comes from a closely related species, is inactivated, or is attenuated. Numerous techniques, including the use of adjuvants to enhance immunity and the development of effective antigen display systems via nanotechnology, have been used to create subunit vaccines that work. Adjuvants can increase the vaccine's immunogenicity, fortify the humoral or cellular immune response, enhance the APC's ability to digest the antigen, lower the overall dosage required, or aid in the development of long-term memory response.

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### *SAQ 2*

Read the following statements and write True (T) or False (F).

- a) One of the drawbacks of attenuated vaccines is the potential of live forms to mutate and revert to virulent forms within vaccinated individuals.
- b) Killed vaccines, since they do not reproduce within the host and typically require multiple booster shots to elicit a protective immune response.

- c) Recombinant DNA technology cannot be used to create a subunit vaccinees.
- d) The primary HBsAg gene was cloned and expressed in yeast cells to create the Hepatitis B vaccine, the first subunit vaccine authorised for human use. The Hepatitis B vaccine was the 1<sup>st</sup> subunit vaccine approved for human use, developed by cloning the gene for the major hepatitis B surface antigen (HBsAg) and expressing it in yeast cells.
- e) By using techniques like administering follow-up booster shots with a protein antigen or adding more DNA sequence to the vector, DNA vaccines can improve the immune response.
- f) The DNA vaccine led to prolonged expression of the antigen.
- g) Adjuvant cannot improve the immunogenicity of the protein vaccines.
- h) Full-length proteins in vaccines may raise the risk of non-specific cross-reactivity, due to their tendency to retain natural shape.

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### c) Plant vaccine

In the field of molecular farming, the primary objective is to generate recombinant proteins in plants for direct utilisation, rather than for any inherent trait or functionality they might confer on the plant. In this procedure, functional recombinant proteins are expressed using either whole plants or plant cells/tissues cultivated under controlled conditions. A practical and affordable alternative to traditional production techniques, including culturing microorganisms or mammalian cells in large-scale bioreactors, is molecular farming. Numerous studies have supported this strategy. The first attempts to create recombinant proteins in plants were performed in 1986 and 1989. At first, biopharmaceutical proteins were the main focus of plant-based protein production. Notably, several studies have shown that the commercialisation of plant-derived human enzymes has been underway since 2012. A multitude of preclinical and clinical investigations of plant-derived recombinant pharmaceutical proteins for both human and animal applications have underscored the viability, effectiveness, and safety of plant-based production systems, particularly in vaccine manufacturing.

Numerous pre-clinical and clinical research employing plant-derived human and animal recombinant pharmaceutical proteins have demonstrated the feasibility, efficacy, and safety of using plant factories to produce vaccines. For recombinant subunit vaccines, a plant-based approach has substantial benefits over expression systems employing microorganisms or mammalian cells.

- i) **Environmental Sustainability:** The plant-based vaccine production platform utilises freely available solar energy and facilitates the capture of CO<sub>2</sub>, resulting in lower energy requirements or minimal greenhouse gas emissions, thereby decreasing vaccine production costs.
- ii) **Ease of Oral Vaccine Production:** Vaccines that can be administered orally can be produced using plant-based technologies. This makes it

more affordable than traditional expression methods such as *E. coli* and baculovirus-infected insect cells, as it eliminates the need for complex bioreactors and downstream processing. Consequently, scaling up production is more economical, with scalability not constrained by bioreactor size or number.

- iii) **Safety Profile:** Plant systems are inherently safe for vaccine production. Plant-made subunit vaccines alleviate safety concerns associated with live vaccines. Furthermore, unlike mammalian-based production systems, plant-based systems do not contain undesirable or hazardous components such as bacterial endotoxins or yeast hyperglycosylated proteins.
- iv) **Enhanced Immunogenicity:** Similar to naturally occurring systems, plant-based systems have a strong potential for biosynthesis, enabling intricate folding and assembly as well as post-translational modifications such as glycosylation. These processes enhance vaccine immunogenicity.

On the other hand, the unpredictability of changes in polymerisation, methylation, glycosylation, the amount and quality of recombinant proteins, and vaccination dose in tissues still poses problems for plant-based platforms. Three different plant biotechnology platforms have been used to generate a large number of vaccine antigens, mAbs (monoclonal antibodies), or other biopharmaceuticals: steady nuclear expression of transgenes in the nuclear genome of transgenic plants or cell cultures, transient expression of transgenes in plants via viral vectors, and stable expression of transgenes in the plastid (chloroplast) genome of transplastomic plants. Every system has pros and cons, and the necessary production primarily determines which technique is best. Furthermore, extensive research has been conducted on the regulatory aspects of plant-derived recombinant proteins and on the development of excellent manufacturing procedures. Many plant species are accessible for plant molecular farming. To create a variety of foreign proteins in vegetables like tomatoes, lettuce, cabbage, and potatoes, plastid genome engineering has been used.

Chloroplast-based expression systems have been proven to be a means of producing oral vaccines. Genetically modified plants were utilised for vaccine production targeting viruses, bacteria, and parasites. Algae-based systems also serve as an alternative for the transient expression of vaccines, with notable instances of algal-derived biopharmaceuticals. Microalgae provide nutritional benefits as a feed supplement due to their digestibility, high protein and lipid content, essential nutrients, and potential immunogenic properties. A viable method for producing proteins and delivering them orally is microalgae, especially for vaccinating fish. Developments in microalgae genetic engineering promise to create "functional feed additives" without requiring intricate post-expression procedures, such as cold-chain maintenance, purification, and injection. *Chlamydomonas reinhardtii*, *Dunaliella salina*, *Cyanobacteria*, and other microalgae were used to express antigen genes in chloroplasts to control or prevent infectious illnesses. Despite the widespread use of several microalgae species in the aquaculture sector, many have yet to establish themselves as effective and fully developed genetic manipulation

systems. Several reviews have extensively covered plant-based vaccine development. Commercialisation of human plant-based immunisations has not yet occurred, despite multiple attempts to produce subunit vaccines in transgenic plants. A transient gene expression method has been used to produce hepatitis C virus E1E2 and its variant E1E2ΔN6 in lettuce, resulting in proper glycosylation and processing. Vaccine-stimulated IgA (Immunoglobulin A) was secreted by mice given lettuce orally, suggesting that complex viral antigens can be produced, digested, and functionally changed in edible plants. Numerous studies have examined the efficacy of plant-based vaccinations against rabies, the porcine reproductive and respiratory syndrome virus, and post-weaning diarrhoea in piglets.

To date, the US Department of Agriculture has authorised only one plant-derived vaccine for chickens, a tobacco-based vaccine against Newcastle disease. Research has also explored plant-based veterinary vaccines for mink, cats, and dogs. Vaccines made from plants offer benefits for oral delivery, including a simple manufacturing process and no need for additional injection equipment. High purity of these vaccines enhances convenience and elicits robust immune responses. When taken orally, vaccination antigens pass through the stomach, enter the intestine, and are taken up by M cells in the follicle-associated epithelium, triggering systemic and mucosal immune responses. A needle-free, convenient way to administer vaccines is also provided by those made from edible plants, involving lettuce, corn, rice, tomato, and potato. Due to the successful high-level expression of many human therapeutic proteins in lettuce chloroplasts, lettuce shows potential as a plant substrate for oral vaccinations. One instance is the dengue fever oral vaccination made from lettuce chloroplasts.

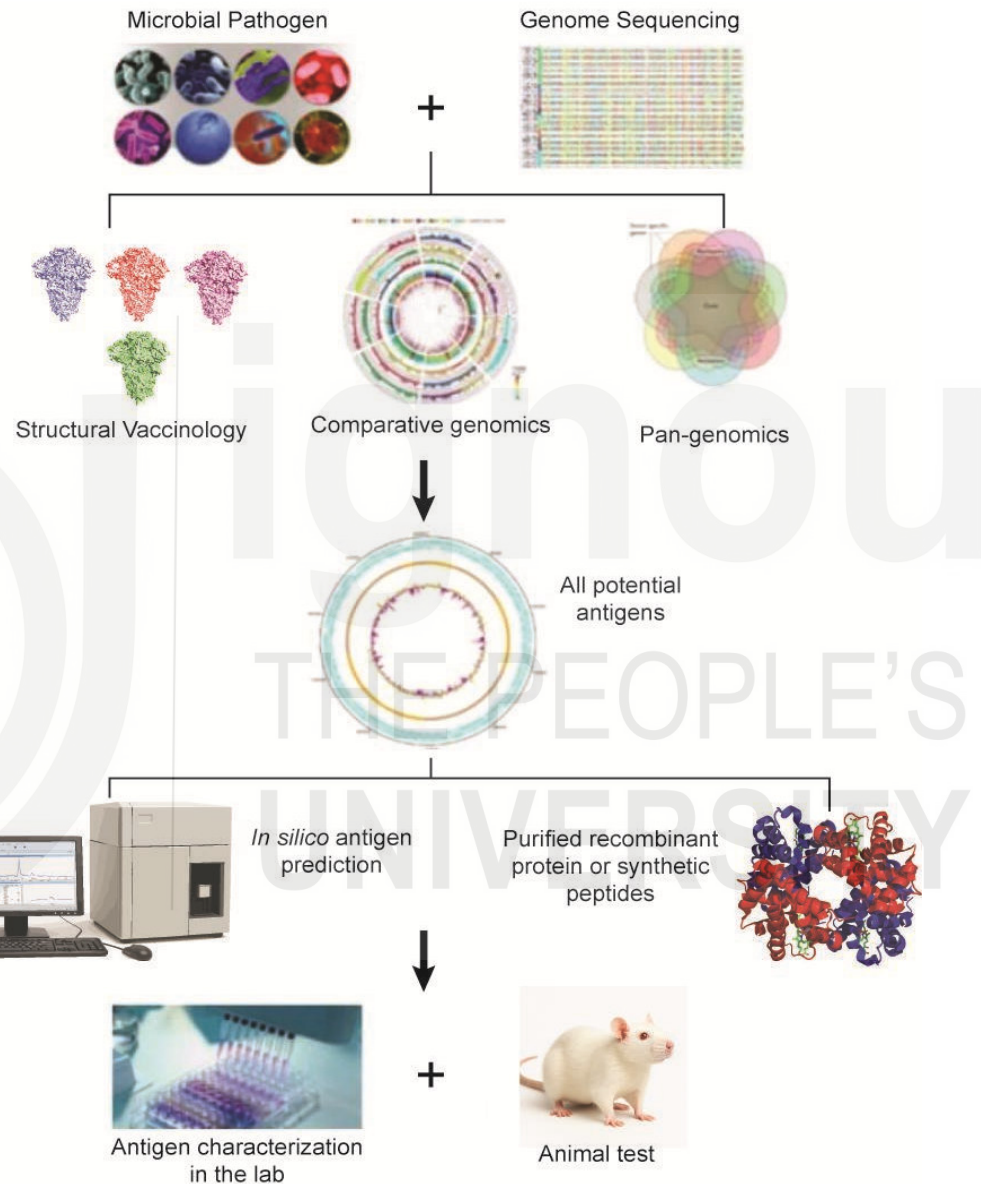
Regulatory limitations are another obstacle that commercial GM plants face globally, mainly in Europe. Regulation and production costs increase when *Agrobacterium* is used for plant antigen transformation, transgene containment, and long-term stability at room temperature. While production costs are reduced under an open-field system, large-scale field research shows considerable regulatory expenditures. An excellent way to reduce regulatory costs is to employ stable expression of viral components in chloroplasts without using *Agrobacterium* or plant pathogenic sequences. In the meantime, using a greenhouse and eliminating seed production might significantly reduce the cost of regulatory approval.

#### **d) Reverse vaccinology**

Rino Rappuoli coined the term "reverse vaccinology," which refers to the study of microbial genomes — particularly bacterial ones—to identify proteins that may be used as vaccines. Following genome sequencing, DNA open reading frames (ORFs) are computationally evaluated to determine which ones are capable of producing secreted or surface antigens.

To induce the expression of the corresponding proteins, identified ORFs are then inserted into the bacterium, *Escherichia coli*. Serum samples are obtained from mice that have been immunised with these produced proteins to evaluate their surface location and bactericidal activity. Serum samples may be considered for inclusion in vaccine development if they show that the

protein is conserved across several bacterial strains. Five proteins from group B *Neisseria meningitidis* have been identified via reverse vaccinology and incorporated into a vaccine currently undergoing clinical testing. Due to the interaction between capsular antigens and neural cell adhesion molecules in the developing brain, which impeded its development, there was no group B vaccine. Reverse vaccinology has also helped develop a number of additional bacterial vaccines, such as experimental vaccines against *Porphyromonas gingivalis*, the leading cause of periodontitis, *Chlamydia pneumoniae*, *P. pneumococci*, *Bacillus anthracis*, and group B streptococci.



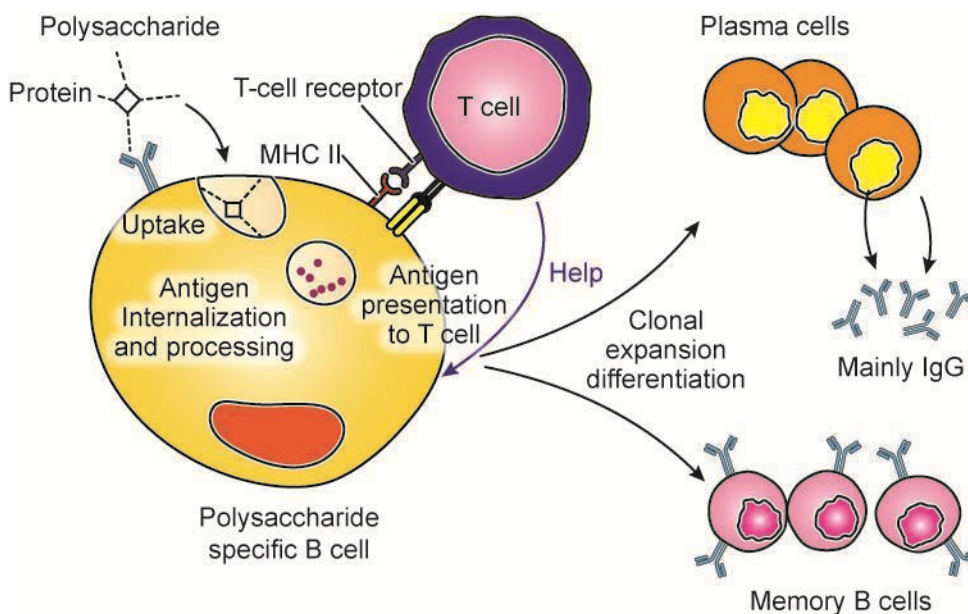
**Fig.17.6: Schematic workflow for reverse vaccinology.**

Reverse vaccinology has also been further refined more recently through pan-genome reverse vaccinology and comparative genomic analysis. In pan-genome reverse vaccinology, genomes of numerous strains of a particular organism are sequenced to find common antigens. These antigens are then incorporated into a vaccine intended to provide broad coverage across all or nearly all strains. This method has proven successful when used against group B streptococci. Comparative genome analysis aims to identify antigens exclusive to pathogenic strains, a task that is crucial for organisms that can exist in both pathogenic and non-pathogenic forms.

### e) Conjugate vaccine

The potential for poor or limited adaptive responses is a notable drawback of methods that do not use a live vaccination approach. To overcome this, strategies that combine a weak vaccine immunogen with a highly immunogenic protein (known as conjugation) or include exogenous proteins (multivalent approach) have been developed to boost or complement immunity against the pathogen. A conjugate formulation of vaccine against *Haemophilus influenzae* type b (Hib) is part of the recommended paediatric schedule. Hib frequently causes bacterial meningitis and infection-induced deafness in children. Tetanus toxoid, a protein carrier, is covalently bonded to type B capsular polysaccharide. The US and other nations that have utilised conjugate Hib vaccination have seen a sharp decrease in Hib cases since its introduction. In addition to being far more immunogenic than polysaccharide alone, the polysaccharide-protein combination enables class switching from IgM to IgG via  $T_H$  cell activation.

Such vaccinations cannot stimulate memory T cells specific to the pathogen, although they can trigger memory B cells. But in the case of Hib vaccination, it shows that memory B cells can still be partially activated even in the absence of a memory TH cell population, which enhances the vaccine's potency. MCV4, another conjugate vaccine that protects young infants from meningitis, employs a similar approach to Hib. The multivalent vaccine MCV4 consists of individual capsular *Neisseria* polysaccharide antigens linked to the highly immunogenic diphtheria toxoid. In a recent study, mice and rats were immunised with  $\beta$ -glucan extracted from brown algae and conjugated to diphtheria toxoid. As a result of this vaccination, these animals developed antibodies that protected them against the threats posed by *Aspergillus fumigatus* and *Candida albicans*. It is noteworthy that immunity is antibody-mediated chiefly, as the protection provided by antibodies was transmitted through the serum or vaginal fluid of the inoculated animals. For those with compromised immune systems, fungal infections pose a significant risk. The challenges posed by antifungal drug toxicity and the emergence of resistant strains may be addressed through vaccination or antibody-based therapies, particularly in hospital settings where these problems are severe.



**Fig.17.7: The mechanism of action for conjugate vaccines.**

Given that subunit polysaccharide or protein vaccines typically elicit humoral responses rather than cell-mediated responses, there is a need to develop methods for creating vaccines that stimulate both T- and B-cell immune responses. Furthermore, the vaccine must be given intracellularly to enable peptide digestion and presentation via class I MHC molecules if a cytotoxic T lymphocyte (CTL) response is intended. One innovative technique for creating multivalent vaccines that deliver multiple copies of the antigen into cells is to integrate antigens into protein micelles, lipid vesicles (also known as liposomes), and immunostimulant complexes. Micelles form when proteins are mixed with a detergent, and the detergent is then removed. Hydrophilic residues of individual proteins are oriented towards the surrounding aqueous environment within these micelles. In contrast, the hydrophobic residues are positioned at the centre to avoid contact with the aqueous environment.

Proteins are combined with phospholipid solution to form lipid bilayer vesicles, which is how protein-containing liposomes are formed; the hydrophilic residues of the proteins are exposed and integrated into the bilayer. Protein, detergent, and an adjuvant glycoside called Quil A are combined to create lipid carriers called immune-stimulating complexes (ISCOMs). As potential vaccinations, membrane proteins from a variety of illnesses, including hepatitis B, HIV, measles, and influenza, were incorporated into liposomes, micelles, and ISCOMs. Liposomes and ISCOMs appear to merge with the plasma membrane and are more immunogenic. This enables them to deliver antigen into cells, where it can be processed by an endogenous pathway and elicit CTL responses.

#### f) **Idiotypic vaccines and Marker vaccines**

In 1963, two research laboratories presented findings indicating the presence of a novel marker on antibodies, different from allotypes. For determinants recognised by antibodies, the term "IDIOTYPE" was adopted. Considering the large number of B cells that produce antibodies and the fact that antibodies can bind to antibodies, Niels Jerne deduced the presence of functional networks involving Id (idiotypes) and anti-Id (anti-idiotypes). The Idiotype network hypothesis has therefore been developed. Previously, there was insufficient evidence to support the idea that an anti-Id response may regulate network interactions and immunological activity during an induced immune response.

Several studies demonstrating the ability of anti-Id antibodies to inhibit particular immune responses surfaced by 1972. These results did not prove that immunological modulation was a normal component of an antigen-induced immune response, even though they did imply that anti-Ids might affect immune responses. Later findings supported this idea. The idiotypic cascade model,  $Ab1 > Ab2\beta > Ab3$ , was proposed.  $Ab3$  was designated  $Ab1'$  since it resembled  $Ab1$ . Jerne distinguished 2 types of anti-Ids based on this notion. According to this framework,  $Ab2\beta$  molecules structurally resembled the antigen, leading to the term "Internal Image of antigen" to describe this mimicry.

Several labs explored the feasibility of this concept shortly after it was first proposed by utilising  $Ab2\beta$  as an antigen to trigger target-specific immune

responses. Depending on the IgG class, Ab2's dual functional nature was established, allowing it to either suppress or stimulate a certain reaction. According to the idiotypic cascade, therapeutically administered Ab1 may cause an antibody response specific to the antigen. Patients who produced low-level HAMA (Human Anti-Mouse Antibody) responses to GD2-reactive Ab1 had better long-term survival than those who didn't, supporting the idiotypic cascade in the clinic. The disialoganglioside GD2, which is expressed in neuroectodermal malignancies such as melanoma and human neuroblastoma, is expressed at very low levels in normal tissues, primarily in peripheral nerves and the cerebellum. GD2 is an excellent target for monoclonal antibody therapy because of its tumour-specific expression. It may also offer an opportunity to investigate and understand network interactions. It has been proposed that the idiotype cascade is a functional component of Dinutuximab, a monoclonal antibody targeting the GD2 antigen approved by the US FDA. FDA-approved monoclonal antibodies dinutuximab (sold as Unituxin) and dinutuximab beta (marketed as Isquette) are utilised as second-line treatment for children with high-risk neuroblastoma. Furthermore, differences in the germline origins of the chosen monoclonal Ab1 used in therapeutic applications may account for variability in immune responses to Ab1.

In one clinical trial, IL-2 combined with the chimeric antibody Ch14.18 demonstrated robust activation of antibody effector functions, but outcomes were not improved. When human anti-chimeric antibodies (HACA) developed in 21% of patients, complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity were eliminated, leading to a significant drop in Ch14.18 levels. IGHV2-9\*02 germline is the source of one of the monoclonal antibodies, whereas the IGHV1S135\*01 germline is the source of the variable area of Ch14.18. Despite these distinctions, they have received minimal attention, even though immunologically, no two antibodies necessarily exhibit identical characteristics.

### **g) Virus-like particles (VLPs)**

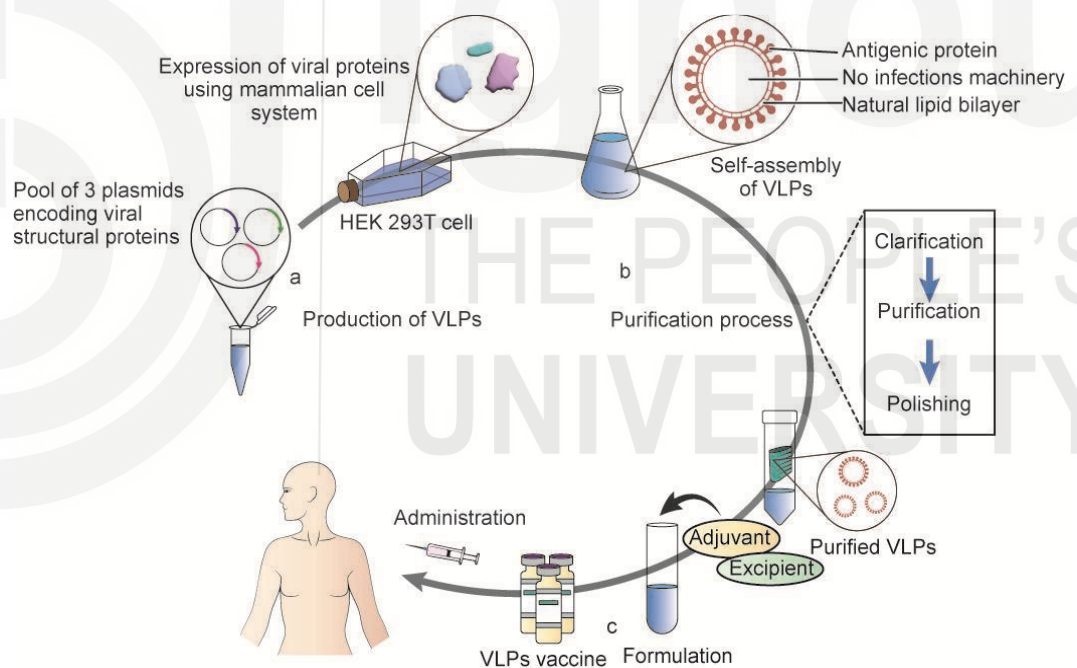
Some viral proteins can self-assemble without assistance from other viral proteins. In addition to protecting against the strains of the human papillomavirus (HPV) that cause genital warts, there are currently two vaccines that are effective against the types of HPV that cause cervical and other malignancies. L1 proteins from viruses, generated by introducing corresponding genes into either baculoviruses or *Saccharomyces cerevisiae*, are used in these vaccinations. Using this method, virus-like particles (VLPs) composed entirely of a single protein are produced. However, producing these vaccines is costly, potentially delaying their deployment in developing nations.

Recently, a novel method for developing an oral papillomavirus vaccine has been proposed. With this approach, an existing typhoid fever vaccine, Ty21a, utilising the auxotrophic strain of *Salmonella enterica* serovar typhi, is used. The high-risk oncogenic HPV type 16 (HPV16) is introduced into *Salmonella* by expressing its L1 protein. When oral delivery of modified Ty21a was used in mice tests, intestinal cells invaded, or VLPs were produced locally. The mice also produced antibodies against HPV16 in their vaginal secretions and sera.

The utilisation of virus-like particle (VLP) production has become increasingly prevalent due to the increased immunogenicity of proteins presented in a structured form rather than in solution. VLPs have therefore become attractive options for vaccine development, as they target a wide range of viruses, from influenza to Ebola. Replicon strategy, depending on flaviviruses and alphaviruses, is a novel way to combine vectors with VLPs. It is possible to modify these viruses so that a section of their genome required for replication is removed and inserted into a producer cell. When the producer cell is transfected with a genomic region encoding viral non-structural and structural proteins, along with an additional gene of interest, a particle containing both viral and foreign proteins is produced.

Even if such a particle can reach a vaccinated person's cells, its lack of the replication-permitting gene prevents it from replicating more than once. Nevertheless, during this singular replication cycle, it produces VLPs that contain both foreign and vector proteins, thereby stimulating the immune system.

DNA plasmids can also be used to create replicons, and plasmids containing the replicon's genetic material likewise exhibit immunogenicity, but with a lower induction of anti-vector immunity.



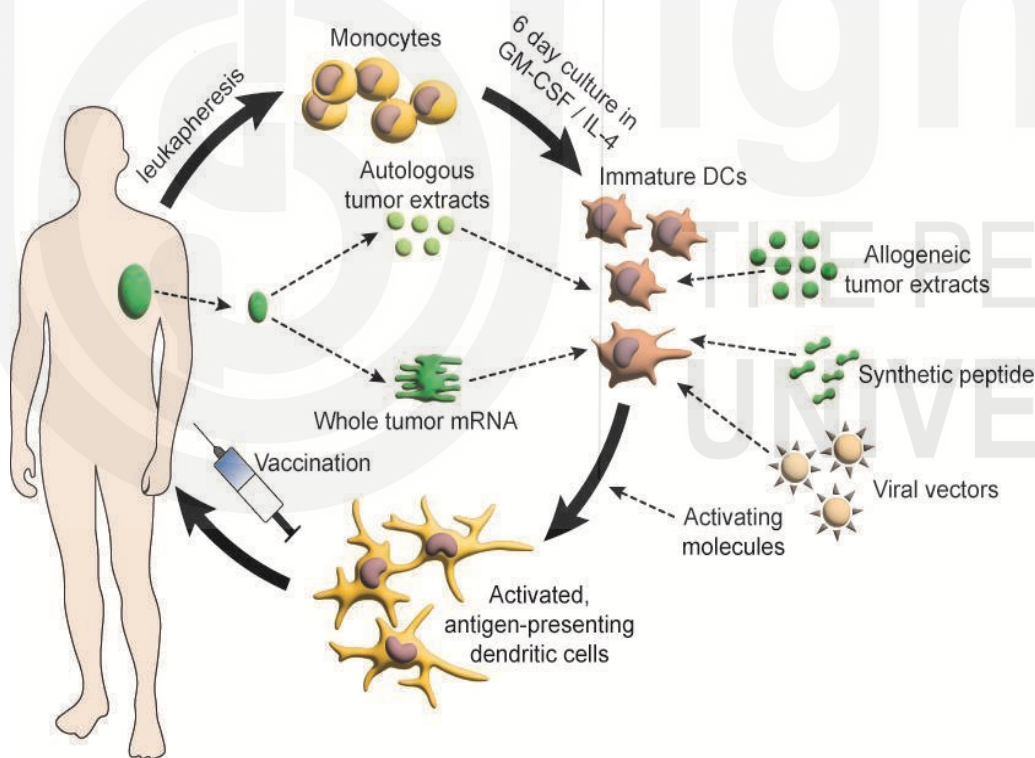
**Fig.17.8: Schematic representation of the production, purification, and formulation process for virus-like particle (VLP) vaccines.**

The overlap between VLP and vector technologies is significant, as some vectors can also be used to generate VLPs.

### h) Dendritic cell-based vaccines

The most significant cells in the immune system for delivering antigens are DCs. They can stimulate B cells, CD4<sup>+</sup> helper T cells, and both naive and memory CD8<sup>+</sup> T cells. In their immature form, DCs are present in tissues and the circulation, where they prepare foreign antigens for the immune system to encounter. When DCs encounter an appropriate antigen, they mature and migrate to lymph nodes, where they can interact directly with immune effector

cells. Mature DCs are adept at producing antigen-specific CD8<sup>+</sup> cytotoxic T-lymphocytes (CTLs) and initiating T helper type-1 immunological responses. Nonetheless, DCs tend to promote tumour tolerance within the tumour microenvironment, which, in turn, stimulates T helper type-2 responses. Consequently, dendritic cells (DCs) can significantly influence the development of tumour-specific cellular immune responses, both favourably and adversely. A person's own monocytes are usually used in DC vaccinations; these are developed in a lab and loaded with antigen before administration. These vaccines were administered to thousands of patients across various age groups and with different tumour types, generally causing minimal toxicity aside from mild local skin reactions. Despite reports of antigen-specific immune responses in several of these trials, these responses usually exhibit limited duration and magnitude, and observable clinical improvements have been scarce. In a Phase III clinical study, the FDA approved Sipuleucel-T, an autologous DC immunisation primed with a recombinant antigen of prostatic acid phosphatase linked to GM-CSF as an adjuvant, as the only dendritic cell vaccine that was proven to be sufficiently efficacious. Despite this vaccine being intended to treat adult cancers, its effectiveness suggests the development of a DC vaccine for paediatric conditions.



**Fig.17.9: A workflow for dendritic cell vaccine production for cancer.**

In children with malignant solid tumours, DC vaccines have so far demonstrated encouraging safety but subpar efficacy in treating high-grade CNS tumours and a broader range of recurrent solid tumours. The DC vaccine manufacturing process offers opportunities to improve efficacy at each step, including DC creation, antigen loading, in vitro maturation, and injection with or without adjuvant. Therefore, research on DC vaccines has focused on increasing the number of DC available and enhancing their immunogenicity, creating new immune adjuvants to enhance the source and presentation of antigen, and exploring concurrent immunomodulation or chemotherapy.

### i) T-cell-based vaccine

Authorised vaccines against bacterial and viral diseases increased dramatically over the 20th century. "Decade of Vaccines" (<http://www.gatesfoundation.org/vaccines/Pages/decade-of-vaccines.aspx>) is the moniker given to the second decade of the twenty-first century in response to requests for increased research and better access to already available vaccines. Development of T-cell-inducing vaccines is a new field in vaccination. These vaccines are designed to activate CD4<sup>+</sup> and/or CD8<sup>+</sup> T lymphocytes with specific properties, such as adequate numbers, the desired phenotype, or effector function. Instead of only assisting B cells via CD4<sup>+</sup> T cells, which generate protective antibodies, these activated T cells directly contribute to pathogen clearance through cell-mediated effector mechanisms. Although current vaccines successfully increase antibody production against bacterial and viral infections, fighting more complex pathogens would require activating T cells, another part of the adaptive immune system. DCs and T cells may have short, antigen-driven interactions at first, but these connections can stabilise and last for up to 12 hours. Activating signals are vital for T cells during this critical time. To properly activate naïve CD8<sup>+</sup> and CD4<sup>+</sup> T cells, three distinct signals are required: costimulatory signals (signal 2), antigenic signals via the T cell receptor (signal 1), and signals from inflammatory cytokines (signal 3). Specific chemokine receptors, such as CCL19 and CCL21, enhance immune responses by promoting T-DC contacts during antigen presentation. Activated DCs and CD4<sup>+</sup> T cells also release chemokines that draw in rare antigen-specific T lymphocytes and promote the growth of CD8<sup>+</sup> T cells. T cell activation changes the expression of a number of molecules, including integrins, selectins, and chemokine receptors. Key intracellular signalling pathways are modulated by these alterations, thereby promoting T cell migration, differentiation, and proliferation in inflammatory tissues. Only a little but varied pool of memory cells remains once the infection is over, while the majority of effector T cells (90–95%) are destroyed by programmed cell death. Memory T cells were formerly divided into two main categories according to their proliferative potential, morphological characteristics, and migratory ability. Effector-memory T (TEM) cells are identified by their expression or lack of expression of cell-surface markers, including KLRG1<sup>hi</sup>/CD44<sup>hi</sup>/CD127<sup>lo</sup>/CD62L<sup>lo</sup>. Although these cells can quickly release cytokines such as IFN- $\gamma$  and TNF, as well as effector molecules, when triggered by TCRs, their ability to multiply is constrained. TCM (Central-memory T) cells are characterised by expression of surface markers such as KLRG1<sup>lo</sup>/CD44<sup>hi</sup>/CD127<sup>hi</sup>/CD62L<sup>hi</sup>, greater potential for proliferation, and cytokine production, such as interleukin (IL)-2, which are directly linked to improved secondary expansion.

TEM and TCM cells can circulate, but the newly identified tissue-resident T cell class cannot migrate. These cells, called TRM (tissue-resident memory T) cells, persist in peripheral tissues indefinitely, even after infection has been eliminated. The expression of CD69<sup>hi</sup>/CD62L<sup>lo</sup>/CD44<sup>hi</sup> and additional surface markers (e.g., CD11a, CD38, CD49a, CD103, and CXCR3) indicates that

TRM cells are present in most organs and tissues. While TEM cells are primarily located in non-lymphoid tissues, TCM cells primarily dominate in secondary lymphoid organs. Tissue-specific signals shape the composition of these markers, leading to differences in expression levels across tissues. In addition to the three primary subsets of memory T cells, there is a minor subset that exhibits stem cell-like characteristics and proliferates more rapidly than other T cell subsets. Often called stem cell memory T cells (TSCM cells), these memory T cells share several traits with naive T cells (TN cells), including the KLRG1<sup>lo</sup>/CD44<sup>lo</sup>/CD127<sup>hi</sup>/CD62L<sup>hi</sup>/CD69<sup>lo</sup> phenotype. They also co-express the chemokine receptor CXCR3, the stem cell antigen Sca-1, and the  $\beta$  chains of the IL-15 and IL-2 receptors (CD122 and IL-2R $\beta$ ). According to several studies, vaccinations may stimulate T cells to begin differentiating.

However, it remains unclear whether this induction significantly contributes to vaccine effectiveness. Therefore, further investigation is needed to determine whether vaccines can generate enough TSCM-like T cells capable of providing protection. It is important to remember that mice and humans share similar T cell biology, and the aforementioned subsets (TCM, TEM, TRM, TSCM) exhibit identical traits in both species.

One important mechanism in T cell-mediated immunity is cytokine production. CD4<sup>+</sup> and CD8<sup>+</sup> T cells with a "Th1" cytokine profile are involved in protective T cell responses in the majority of bacterial and viral illnesses. The (co-) production of IFN- $\gamma$ , IL-2, and TNF defines this profile. T cell production of IFN- $\gamma$  is a frequently utilised biomarker to assess vaccine-induced responses. It has been demonstrated that IFN- $\gamma$  plays a useful part in the elimination of some viral infections.

Nonetheless, numerous instances highlight that the magnitude of T cell response producing IFN- $\gamma$  alone may not adequately correlate with immune protection. IFN- $\gamma$ -producing single-positive T cells can make up a significant fraction of the overall population of cytokine-producing CD8<sup>+</sup> and CD4<sup>+</sup> T cells after immunisation; nevertheless, they frequently do not have the capacity to remain as memory T cells.

Consequently, prophylactic vaccinations, which activate a sizable percentage of individual T cells that produce IFN- $\gamma$ , might not provide adequate protection. This emphasises the importance of measuring the quality of the immune response rather than simply counting the number of T cells that produce IFN- $\gamma$  to determine long-term protection. A strong correlation has been shown between the level of protection and the production of higher frequencies of polyfunctional T cells, which release TNF, IFN- $\gamma$ , and IL-2 simultaneously, in studies of T cell responses elicited by vaccinations against HCV, HBV, HIV, CMV, influenza, and Leishmania. According to some studies, determining the number of CD8<sup>+</sup> and CD4<sup>+</sup> T cells that produce IFN- $\gamma$  alone is not sufficient to predict protection. Rather, they stress the importance of evaluating the quality of CD8<sup>+</sup> and CD4<sup>+</sup> T cell responses, especially regarding polyfunctional T cells.

**SAQ 3**

Match the items given under Column 'A' with those given under Column 'B' :

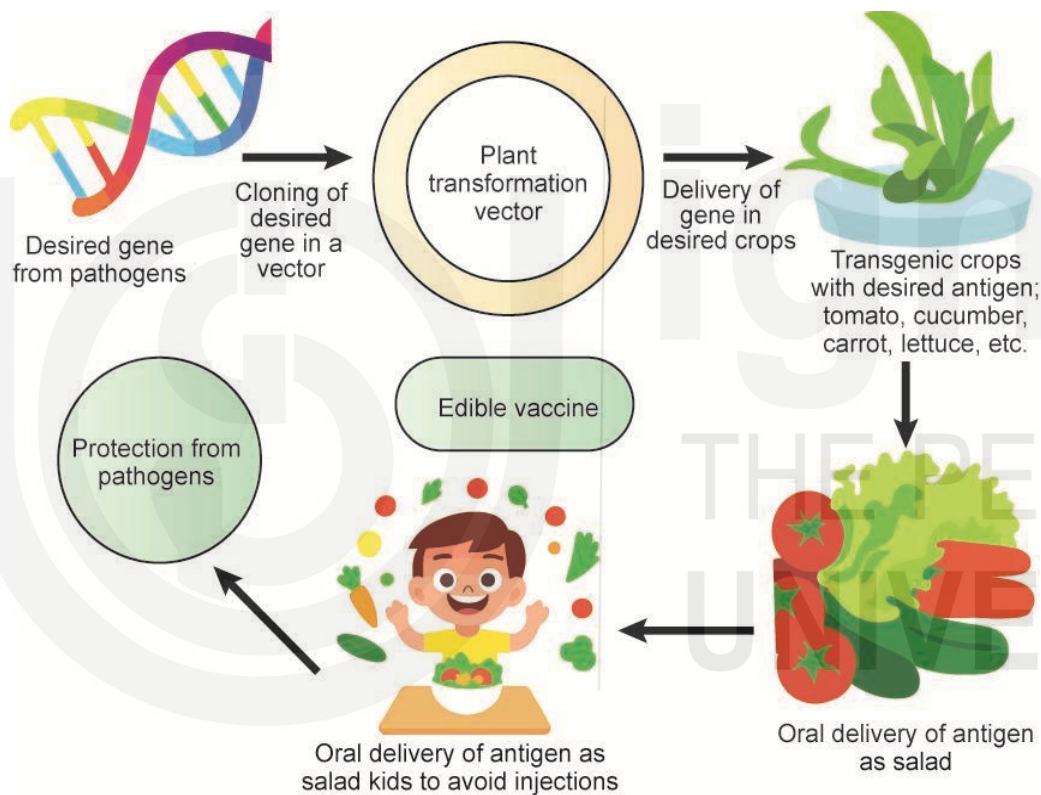
Column 'A'	Column 'B'
a) Molecular farming	i) Identification of vaccine candidates through genomic analysis and prediction of antigenic proteins.
b) Reverse vaccinology	ii) Using the immune system's most effective antigen-presenting cells to stimulate specific immune responses.
c) Conjugate vaccine	iii) Combining a weak immunogen with a highly immunogenic protein to enhance the immune response.
d) Idiotypic vaccine	iv) Production of recombinant proteins in plants for vaccine development.
e) Virus-like particles (VLPs)	v) Using proteins that self-assemble into structures mimicking viruses to enhance immunogenicity.
f) Dendritic cell-based vaccines	vi) Utilising antibodies against antibodies to regulate immune functions.
g) T-cell-based vaccines	vii) Developing vaccines that directly activate CD8+ T and/or CD4+ cells for pathogen elimination.

**j) Edible vaccine**

Edible vaccines refer to vaccines that are produced using transgenic plants or animals, or that contain components capable of triggering an animal's immune response. Essentially, they are pharmaceuticals made from plants or animals that can be consumed. Hiatt and associates first proposed creating a plant-based vaccine in 1989. In 1990, Dr. Arntzen further developed the idea, suggesting that subunit vaccines be produced and administered using transgenic plants. Arntzen's suggestion demonstrated how edible vaccines could overcome some of the drawbacks of conventional vaccine manufacturing techniques. Mason and associates produced a hepatitis B surface antigen on tobacco plants (*Streptococcus* mutants), marking a major advancement in the field of edible vaccine manufacturing. This discovery marked an important turning point in the development of edible vaccines. The discovery of edible vaccines in tobacco was accompanied by attempts to manufacture heat-labile toxin B and hepatitis B in potatoes. These vaccines offer obvious advantages over traditional vaccines, especially in regions with limited resources. Edible vaccines offer promising prospects for reducing the prevalence of illnesses such as hepatitis B and diarrhoea in developing

nations, where vaccine administration and storage pose major challenges. Selecting appropriate plants, yeast, algae, insect cells, and lactic acid bacteria whose products are ingested fresh to avoid degradation is preferred when producing edible vaccines or antibodies.

An edible vaccine was licensed by “National Institute of Allergy and Infectious Diseases” in 1998 due to its exceptional immunogenicity. As an alternative to conventional vaccine production techniques, this edible vaccine is needle-free, affordable, safe, practical, and simple to give. Numerous plant-based vaccines have been developed, with many currently undergoing clinical trials. These vaccines primarily target viruses and bacteria that infect animals, humans, and poultry, causing severe illnesses. However, despite their potential, no edible vaccine has yet been approved by the US Food and Drug Administration (USFDA) due to the regulatory challenges associated with genetically modified crops.



**Fig.17.10: Fundamental concept of edible vaccines.**

We need to focus on the uses, potential, and challenges of edible vaccines from a long-term perspective. The innate and adaptive arms of the immune system (B and T cells) are involved in the primary activation of mucosal immunity by edible vaccines. These vaccines' composition is specifically designed to target tissues associated with lymphoid mucosa (MALT). Moreover, secretory IgA (SIgA) is essential for protecting mucosal surfaces because it inhibits bacterial adherence and the action of toxins. To increase vaccine effectiveness, novel delivery methods that can trigger systemic IgG and SIgA responses specific to pathogens or toxins must be developed. A critical mechanism for intestinal antigen uptake is the microfold (M) cells. Mostly found in the gastrointestinal tract, these cells are subgroups of follicular-associated enterocytes (FAE). “Antigen-presenting cells (APCs)” in Peyer's patches receive a variety of macromolecules that M cells transport

from the lumen of the small intestine. Among the many APCs, DCs seem to be especially good at priming naive T cells to start an adaptive immunological response. DCs are initially observed in a stable condition with strong endocytic activity but no ability to engage with primary naive T lymphocytes.

DCs, however, mature, move to T-cell zones within lymph nodes, and upregulate co-stimulatory chemicals in inflammatory situations. Antigens are presented, and cytokines are released during this process, helping naive antigen-specific T cells differentiate into effector cells that migrate to specific inflammatory sites. Intestinal DCs support naive T cell stimulation and follicular Tfh (T-helper cell) differentiation, either directly by promoting Tfh differentiation or indirectly by facilitating the conversion of T-17 cells into Tfh. Activated B cells subsequently depart from follicles and migrate to mucosal-associated lymphoid tissues (MALT), where plasma cells produce IgA and other antibodies. DCs possess the ability to specifically capture antigens from the lumen through the epithelial cell layer as well as extend dendritic projections into the lumen for this purpose. Goblet cells, which play a role in mucin production, have also recently been identified as a small-intestine antigen-capture mechanism. Studies using intravenous microscopy have demonstrated that intestinal antigens can be directly captured and delivered by goblet cells. An effective edible vaccine would elicit specific T and B cell responses, promoting the development of durable memory cells that can provide defence against future infections. Even as our understanding of "oral tolerance," a T-cell-mediated process that reduces immune responses to previously encountered antigens via the oral route, has advanced, it has sparked debate about the use of oral vaccines. The intestinal immune system releases antigens without causing inflammation, enabling immature dendritic cells to present antigens to T cells and induce tolerance. Cell-to-cell contact between regulatory T cells and the release of cytokines, such as IL-10, prevent dendritic cells from maturing and functioning, thereby altering their to lerogetic mechanisms. Moreover, systemic suppression of immune responses may result from repeated injections of mucosal antigens, making it difficult to develop vaccines with reliable efficacy.

#### **k) Therapeutic vaccine**

Typically, vaccines are formulated to activate the immune system, enabling it to control disease-causing organisms (pathogens) from initiating infections. On the other hand, there is a class of vaccines called therapeutic vaccines.

Unlike conventional vaccines that aim to prevent illness, therapeutic vaccines are designed to activate the immune system to combat existing diseases or impede their progression, thereby serving as a treatment measure. While the field of therapeutic vaccines remains mainly in the experimental stage, the Food and Drug Administration (FDA) has granted 3 of these vaccines licenses for cancer treatment. The current research predominantly emphasises the development of therapeutic vaccines for cancer. However, many scientists are also directing their efforts towards creating therapeutic vaccines for treating cholera, viral hepatitis, human papillomavirus (HPV), HIV, and various other potentially severe diseases. Therapeutic vaccines are given after a person has already developed an illness, whereas regular vaccines are given before an infection occurs to prevent it. Their purpose is to stimulate a stronger, disease-specific immune response to combat the existing illness.

When creating therapeutic vaccines, there are two primary methods:

- a) Autologous vaccines: These are a type of personalised medicine in which immune or cancer cells are taken from the patient's body and utilised to make a vaccine specifically for that patient.
- b) Allogeneic vaccines: These are made from cells that have been generated in a lab or taken from other people. This method is frequently applied to the development of cancer therapeutic vaccines. These cells can be used to create a variety of therapeutic vaccines with distinct mechanisms of action. This category comprises DNA, dendritic, and antigenic vaccines.

The field of cancer treatment has seen the most significant breakthroughs in therapeutic vaccination research. In the US, 3 vaccines were approved for use: one to prevent the development of precancerous carcinoma *in-situ*, and two to treat advanced cancer that has spread to other parts of the body. The following is a list of the approved vaccinations, arranged chronologically by approval date:

- a) Tice (Bacillus Calmette-Guerin): In 1990, this live-attenuated vaccine was approved for the treatment of bladder cancer *in situ*.
- b) Provenge (sipuleucel-T): Provenge is a vaccine dependent on dendritic cells, which was approved in 2010 to treat hormone-resistant, metastatic prostate cancer.
- c) Imlygic (talimogenelaherparepvec): a live-attenuated vaccine approved in 2015, is used to treat advanced oncolytic melanoma, a form of skin cancer.

Other cancer therapeutic vaccines in development include an allogeneic vaccination against invasive bladder cancer called Canvaxin, an antigenic vaccination for kidney cancer (renal cell carcinoma) called TroVax1, and GVAX, a pancreatic ductal adenocarcinoma whole-tumour cell vaccination.

Therapeutic vaccination research is also examining the herpes simplex virus (HSV). The goal has been to create a vaccine that can constantly suppress the virus without the use of antiviral drugs, as our understanding of the causes of the virus's sudden reactivation after dormancy and the ensuing herpes outbreaks has expanded. Therapeutic HSV immunisations may reduce viral shedding, a condition in which the virus multiplies unpredictably and levels up in bodily fluids and tissues. During an acute herpes outbreak, shedding not only worsens but also increases the risk of spreading to others. Early research on therapeutic vaccination candidates has shown promise in reducing HSV shedding and lesions. These candidates include the HSV529 vaccine, which utilises a virus with replication defects to induce a persistent immune response without causing illness, and Delta gD-2, a genetically modified herpes virus used in this vaccination approach. The mRNA-based technology used in the development of Moderna and Pfizer COVID-19 vaccines has also facilitated the creation of GSK4108771A, another innovative vaccine.

Therapeutic vaccinations are considered a strategy for impeding the progression of diseases such as hepatitis B, which currently lacks a defined

treatment, unlike hepatitis C. Hepatitis B can lead to severe complications like cirrhosis, liver failure, and liver cancer. The goal of therapeutic vaccination is to reduce hepatitis B viral load, a measure of viral activity, by inducing a robust immune response. The rate of illness progression is correlated with a larger viral load. GS-4774, an antigen vaccine with an adjuvant made from yeast, HBsAg-HBIG, another antigen vaccine with an adjuvant made of aluminium, and HBcAg/HBsAg, an antigen vaccination that combines two different hepatitis antigens (one from the virus's surface and one from its core), are some of the promising therapeutic vaccination options for hepatitis B.

The study of therapeutic vaccines is a fascinating area of vaccine science. As more is discovered about the processes behind the course of disease, the area is expected to grow, with three FDA-approved vaccines validating the concept. Treatment of a disease doesn't always necessitate complete eradication; instead, it may focus on reducing its spread. Despite the enthusiasm surrounding therapeutic vaccinations, the field remains relatively nascent. Years may pass before an efficient treatment for viral illnesses like HPV, hepatitis B, or HIV becomes accessible. In the meantime, it's critical to prioritise prevention to avoid these potentially dangerous illnesses.

## **17.6 GOVERNMENT SCHEMES FOR VACCINATION**

- a) As an expansion of the EPI offered in 1978, UIP (Universal Immunisation Programme) was introduced in 1985. All Indian children and pregnant women are to receive free vaccines under the UIP, one of the most extensive public health programs in the world. UIP immunises against a series of preventable diseases, involving: tuberculosis, polio, pertussis, diphtheria, tetanus, measles, hepatitis B, rubella and others. As a part of the national immunisation schedule, there is a series of vaccines provided free of cost, including: BCG for tuberculosis, D-T-P immunisation for diphtheria, pertussis and tetanus, Hepatitis B, Hib (Haemophilus influenzae type B), oral and inactivated polio vaccines, Measles-Rubella vaccine, Rotavirus vaccine, PCV (Pneumococcal conjugate vaccine), Japanese Encephalitis Vaccine (in endemic regions only); Td (Tetanus and diphtheria) is provided to adolescents and pregnant women.
- b) Mission Indra Dhanush: Launched in 2014 to rapidly elevate full immunisation coverage to 90%, i.e. vaccination among all children and pregnant women. Mission Indra Dhanush targeted areas with the lowest immunisation rates, particularly those with remote, underserved populations. The campaign involved special vaccination drives in specific districts in conjunction with urban areas that met the designated high-risk criteria due to poor coverage.
- c) The government initiated Intensified Mission Indra Dhanush (IMI) in 2017 to expedite progress. IMI targets districts with low persistent immunisation coverage by implementing 4 rounds of immunisation in quick succession.
- d) National Vaccine Policy: India introduced the National Vaccine Policy in 2011 to direct the planning and implementation of immunisation

- strategies. The National Vaccine Policy focuses on introducing new vaccines based on disease burden and cost-effectiveness, strengthening vaccine production in the country, ensuring a robust supply chain, and improving vaccine monitoring and safety systems.
- e) Pulse Polio Immunisation Programme: The Pulse Polio Immunisation Programme was initiated in 1995, as a mass campaign to eradicate poliomyelitis from India. The campaign consisted of giving the OPV (Oral Polio Vaccine) to all children < 5 years of age on designated National Immunisation Days. Due to the implemented programme, India was declared polio-free by the WHO in 2014. However, continuing regular campaigns is warranted to prevent re-emergence.
- f) Immunisation Programs for Adolescents: Adolescents are incorporated into immunisation programs through tetanus and diphtheria (Td) booster doses; in selected areas, introduction of the HPV vaccine to protect against cervical cancer. In addition, older children are the target of measles-rubella (MR) vaccine catch-up campaigns.
- g) COVID-19 Vaccine Initiative: On January 16, 2021, India launched its COVID-19 vaccination campaign. Initially intended for healthcare and frontline workers, it was later made available to older adults and, ultimately, to children aged 12 and older. Their use included Covishield, Covaxin, Sputnik V, and others. A digital platform called Co-WIN was launched for registration, tracking and certification.
- h) Electronic Vaccine Intelligence Network (eVIN): The eVIN system represents an electronic innovation for vaccine logistics, including vaccine proportion inventory tracking, actual storage temperature, and distribution at cold chain points, with real-time reporting. The information provided by the eVIN system helps reduce vaccine waste, enables timely replenishment of stock, and makes vaccine supply efficient and accountable.

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### SAQ 4

Choose the correct option:

- a) What are edible vaccines?
- Edible Vaccines.
  - Vaccines injected into plants and animals.
  - Vaccines produced using transgenic plants or animals.
  - Vaccines that require refrigeration.
- b) Who first proposed the idea of plant-based vaccines?
- Dr. Arntzen
  - Hiatt and colleagues
  - Mason and colleagues
  - National Institute of Allergy and Infectious Diseases

- c) What role do Microfold (M) cells play in the context of edible vaccines?
- Producing antibodies
  - Delivering vaccines to the bloodstream
  - Activating B cells directly
  - Capturing antigens at the intestinal level
- d) What distinguishes therapeutic vaccines from traditional vaccines?
- They treat existing diseases or slow their progression.
  - They prevent diseases before infection.
  - They are only used in animals.
  - They are administered orally.
- e) What is a key focus of therapeutic vaccines for hepatitis B?
- Complete eradication of the virus
  - Reducing the hepatitis B viral load
  - Inducing immediate immune response
  - Preventing hepatitis C infection

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## 17.7 SUMMARY

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Let us summarise what you have learnt so far:

- Biological medicines called vaccines help the immune system identify and eliminate dangerous conditions like bacteria or viruses. Vaccines were ranked among the top health interventions for preventing infectious diseases and have reduced disease and death worldwide.
- The ancient Greeks first described immunity. Immunisation using science began with Edward Jenner in 1798, who explored the use of cowpox for immunity against smallpox. With Louis Pasteur's establishment of an attenuated or weakened vaccine to immunise against rabies and anthrax, this heralded the beginnings of modern immunology.
- Initially, immunisation was classified as either passive or active. Active immunisation uses the stimulatory properties of the agents to elicit an immune response that produces memory cells. Passive immunisation is the transfer of pre-formed antibodies to a person; immunity is immediate but short-lived, and the individual has no immunological protection against the agents.
- Inactivated (killed) and live attenuated vaccines were among the first generation of vaccines. This is the whole pathogen, in either its live (infected) state or killed form, to induce a particular level of immunity,

such as the Salk polio vaccine or the BCG vaccine for TB. The benefits of a live vaccine create anxiety about possible adverse events, like the change from live agents' virulence back to a more virulent state.

- Second-generation vaccines use only parts of the pathogen itself, such as proteins and sugars. These vaccines, also called subunit and conjugate vaccines, are both safer and more selective. The Hib conjugate vaccine and the hepatitis B vaccine are two examples. Second-generation vaccines usually require both an adjuvant and multiple doses to be effective.
- VLPs are entities that resemble viruses and are composed entirely of viral proteins rather than genetic material. Because VLPs are non-infectious and immunogenic, they are used for vaccines, such as the HPV vaccine. VLPs, in general, may stimulate strong antibody responses due to their morphology.
- Plants produced using genetic modification have been used to create edible vaccines. Cereals, potatoes, tomatoes, lettuce, and other foods can be used to make edible vaccinations. Edible vaccines are designed to be inexpensive, painless, and suitable for mass immunisation with minimal health infrastructure support, especially in underdeveloped countries. The oral administration of palatable vaccines enhances mucosal immunity.
- The ability to modify DCs to show a particular tumour antigen outside makes them a particularly effective sort of antigen-presenting cell. The DCs are then reintroduced into the patient to stimulate a potent anti-cancer immune response. The use of these cells is fascinating, particularly in cancer immunotherapy.
- T-cell-based vaccinations, as opposed to conventional immunisations, stimulate CD4+ and CD8+ T cells to kill diseased (or malignant) cells rather than producing antibodies. These vaccines are extremely important in cases where antibody responses are ineffective or require the formation of memory T Cells for long-term protection.
- Idiomatic vaccines utilise antibodies produced as a response to a pathogen antigen in a way that triggers the immune response, like the patient was directly exposed to the actual pathogen antigen. The use of idiomatic vaccines in cancer immunotherapy may be especially relevant, where the immune system must recognise the specific markers of a tumour.
- Reverse vaccinology is a modern approach that employs bioinformatics and genetic sequencing to identify potential antigens directly from the genome of the pathogen. This approach allows scientists to identify vaccine candidates from available data rapidly and can also be used to target known pathogens such as *Neisseria meningitidis*.
- Instead of preventing diseases, therapeutic vaccinations are used to treat those that already exist. They are designed to enhance the immunosystem's ability to fight chronic infections or cancers. Examples include prophylaxis for prostate cancer and therapeutic vaccines in development for HIV and hepatitis B.

- Despite progress, challenges remain in developing vaccines for diseases like HIV, TB, and malaria. Future efforts aim to build more effective, affordable, and easier-to-administer vaccines using advanced technologies such as gene guns, nanotechnology, and mucosal delivery systems.

## 17.8 TERMINAL QUESTIONS

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1. What are some challenges in developing vaccines for diseases like AIDS, TB, and malaria?
2. How does vaccine development progress over time?
3. Who were the pioneers of passive immunity, and what is passive immunisation?
4. What are the goals and outcomes of active immunisation?
5. Explain how attenuated vaccines differ from killed vaccines in terms of immune response.
6. How do DNA vaccines work, and what are their potential advantages over traditional vaccines?
7. Describe the process of reverse vaccinology.
8. What are the newer variations of reverse vaccinology, and how do they differ?
9. Explain the concept of conjugate vaccines and provide an example.
10. What is the idiotypic network hypothesis, and how does it relate to idiotypic vaccines?
11. What innovative approach combines vectors with VLPs in vaccine development?
12. How are dendritic cell (DC) vaccines typically produced, and what has been their effectiveness in clinical trials?
13. What are the primary approaches to developing therapeutic vaccines, and what diseases are they primarily targeting?

## 17.9 ANSWERS

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### Self-Assessment Questions

1.
  - a) Edward Jenner
  - b) Louis Pasteur
  - c) Passive immunisation
  - d) IgG
  - e) Memory cells
  - f) Attenuation

2. a) True      b) True      c) False      d) True  
 e) True      f) True      g) False      h) True
3. a) Molecular farming –(iv). Production of recombinant proteins in plants for vaccine development.  
 b) Reverse vaccinology –(i). Identification of vaccine candidates through genomic analysis and prediction of antigenic proteins.  
 c) Conjugate vaccine –(iii). Combining a weak immunogen with a highly immunogenic protein to enhance the immune response.  
 d) Idiotypic vaccine –(vi). Utilising antibodies against antibodies to regulate immune functions.  
 e) Virus-like particles (VLPs) –(v). Using proteins that self-assemble into structures mimicking viruses to enhance immunogenicity.  
 f) Dendritic cell-based vaccines –(ii). Using the immune system's most effective antigen-presenting cells to stimulate specific immune responses.  
 g) T-cell-based vaccines –(vii). Developing vaccines that directly activate CD8+ and/or CD4+ T cells for pathogen elimination.
4. a) Vaccines produced using transgenic plants or animals  
 b) Hiatt and colleagues  
 c) Capturing antigens at the intestinal level  
 d) They treat existing diseases or slow their progression  
 e) Reducing the hepatitis B viral load

### Terminal Questions

- Challenges in developing vaccines for diseases like AIDS, TB, and malaria include the need for improved efficacy, safety, cost reduction, and easier administration methods to reach the largest number of people, especially in poor nations. The process is complex, expensive, and lengthy, often requiring years of clinical trials and rigorous testing to ensure safety and effectiveness.
- Initially, vaccine development primarily relied on fundamental research and basic understanding of the immune system. The first step in developing a vaccine is fundamental research. Given differences in the epitopes recognised by T and B cells, immunologists are now optimising the humoral and cellular immune responses in vaccine candidates. Advances include identifying different antigen-processing routes, using design techniques and additives to maximise antigen presentation, targeting mucosal surfaces to protect against infection, and employing genetic engineering to enhance vaccine administration and immune response.

3. Emil von Behring and Hidesaburo Kitasato were the pioneers of passive immunity. In passive immunisation, an immunised person transfers pre-made antibodies to a recipient, providing short-term protection without activating the recipient's immune system. It occurs naturally during pregnancy and through breast milk, and it can be administered via antiserum for conditions such as immune deficiencies, toxin exposure, and rapid-onset infections.
4. By triggering the adaptive immune response, active immunisation seeks to produce immunologic memory and protective immunity. After being exposed to the pathogen again, this triggers a secondary immune response that effectively eradicates the virus or prevents the disease. Active immunisation occurs naturally through infection or artificially through vaccination, playing a crucial role in reducing deaths from infectious diseases.
5. Attenuated vaccines replicate within the host, mimicking the growth patterns of the actual pathogen, and typically induce humoral immune and cell-mediated responses. In contrast, killed vaccines do not replicate within the host and usually trigger a primarily humoral antibody response.
6. DNA vaccines include injecting plasmid DNA containing antigenic proteins directly into the recipient's muscle. The immunogenic protein encoded by the DNA and delivered to host cells elicits humoral and cell-mediated immune responses. Potential advantages include precise targeting of antigens, prolonged antigen expression, and ease of storage and transportation.
7. Microbial pathogen genome sequencing is the first step in reverse vaccinology. Next, computer analysis is used to identify DNA ORFs (open reading frames) that encode surface or secreted antigens. To produce proteins, discovered ORFs are subsequently expressed in bacterial hosts. These proteins are used for immunisation studies in mice, and serum samples are evaluated for bactericidal activity and surface localisation.
8. Two of the more modern types of reverse vaccinology include pan-genome reverse vaccinology and comparative genomic analysis. In pan-genome reverse vaccinology, several strains of an organism are sequenced to find common antigens for vaccines with broad coverage. Comparative genome analysis aims to identify antigens produced exclusively by dangerous strains of organisms that exist in both pathogenic and non-pathogenic forms.
9. Conjugate vaccines combine weak vaccine immunogens with highly immunogenic proteins to enhance immune responses. For example, the Hib conjugate vaccine combines tetanus toxoid with type B capsular polysaccharide to protect against *Haemophilus influenzae* type B infections.

10. The idiotypic network hypothesis suggests a functional network involving idiotypes and anti-idiotypes in the regulation of immune responses. Idiotypic vaccines leverage antibodies against antibodies, using anti-idiotypes to modulate immune responses.
11. The replicon strategy combines vectors with VLPs by modifying alphaviruses and flaviviruses to produce VLPs containing foreign and viral proteins. This approach stimulates the immune system and holds promise for combating a wide range of viral diseases.
12. DC vaccines typically involve the use of a person's own monocytes, which are matured in a laboratory setting and loaded with antigen before being administered. While some trials have reported antigen-specific immune responses, these responses often exhibit limited duration and magnitude, with few observable clinical improvements.
13. The primary approaches to developing therapeutic vaccines are autologous vaccines, which use cells from the patient's own body, and allogeneic vaccines, which use cells harvested from others or engineered in the laboratory. Therapeutic vaccines primarily target diseases such as cancer, hepatitis B, herpes simplex virus (HSV), and HIV.

### **Suggested Readings**

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