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قسم علوم الحياة

Sera and Vaccines

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Syllabus

1- **Introduction of vaccines**
2- **Types of Immunity**
3- **Herd immunity**
4- **Type of vaccines**
5- **Production of vaccines and Adjuvant**
6- **Time of Vaccination**
7- **DNA vaccination**
8- **Introduction of Serology and Immunotherapy**
9- **Monoclonal production**

References:

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Introduction of vaccines & Sera

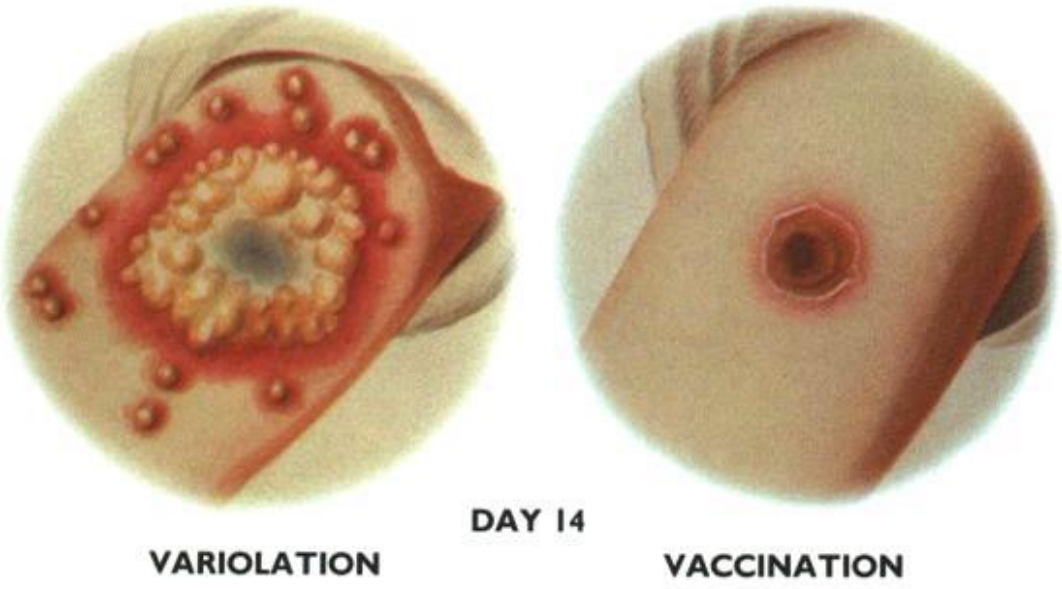
Vaccine: A vaccine is an antigenic material that stimulate adaptive immunity to a disease. Vaccines can prevent the effects of infection by many pathogens. Vaccine's are generally considered to be the most effective method of preventing infectious diseases. The material administered can either be live but weakened forms of either bacteria or viruses, killed or inactivated forms of these pathogens, or purified material such as proteins.

Variolation (inoculation) was the method first used to immunize an individual against smallpox (*Variola*) with material taken from a patient or a recently variolated individual, in the hope that a mild, but protective, infection would result. The procedure was most commonly carried out by inserting/rubbing powdered smallpox scabs or fluid from pustules into superficial scratches made in the skin.

Smallpox was the first disease people tried to prevent by purposely inoculating themselves with other types of infections. smallpox inoculation was started in India before 200 BC. In 1796 British physician **Edward Jenner** tested the possibility of using the cowpox vaccine as an immunization for smallpox in humans for the first time. The word vaccination was first used by Edward Jenner. **Louis Pasteur** furthered the concept through his pioneering work in microbiology.

Vaccination (Latin: *vacca* mean cow) is named because the first vaccine was derived from a virus affecting cows, the relatively benign cowpox virus, which provides a degree of immunity to smallpox, a contagious and deadly disease. Vaccination and immunization have the same meaning but is different from inoculation which uses un-weakened live pathogens. The word "vaccination" was originally used specifically to describe the injection of the smallpox vaccine.

Vaccination	**Variolation**
Vaccination is a method of immunization where administration of an attenuated virus takes place against a viral infectious agent	Variolation is a method of immunization where administration of live viruses takes place against a viral infectious agent
Form of immunization: attenuated viruses, DNA vaccine or edible vaccine	Form of immunization: live smallpox virus
Examples: Hepatitis, Malaria, Rubella, etc	Example: Smallpox vaccine



Immunization is the process whereby a person is made immune or resistant to an infectious disease, typically by the administration of a vaccine. Vaccines stimulate the body's own immune system to protect the person against subsequent infection or disease.

Immunization means to make someone immune to something. Vaccination just means to inject a suspension of attenuated or killed microorganisms administered for prevention or treatment of infectious disease.

No vaccine is 100% effective in preventing disease

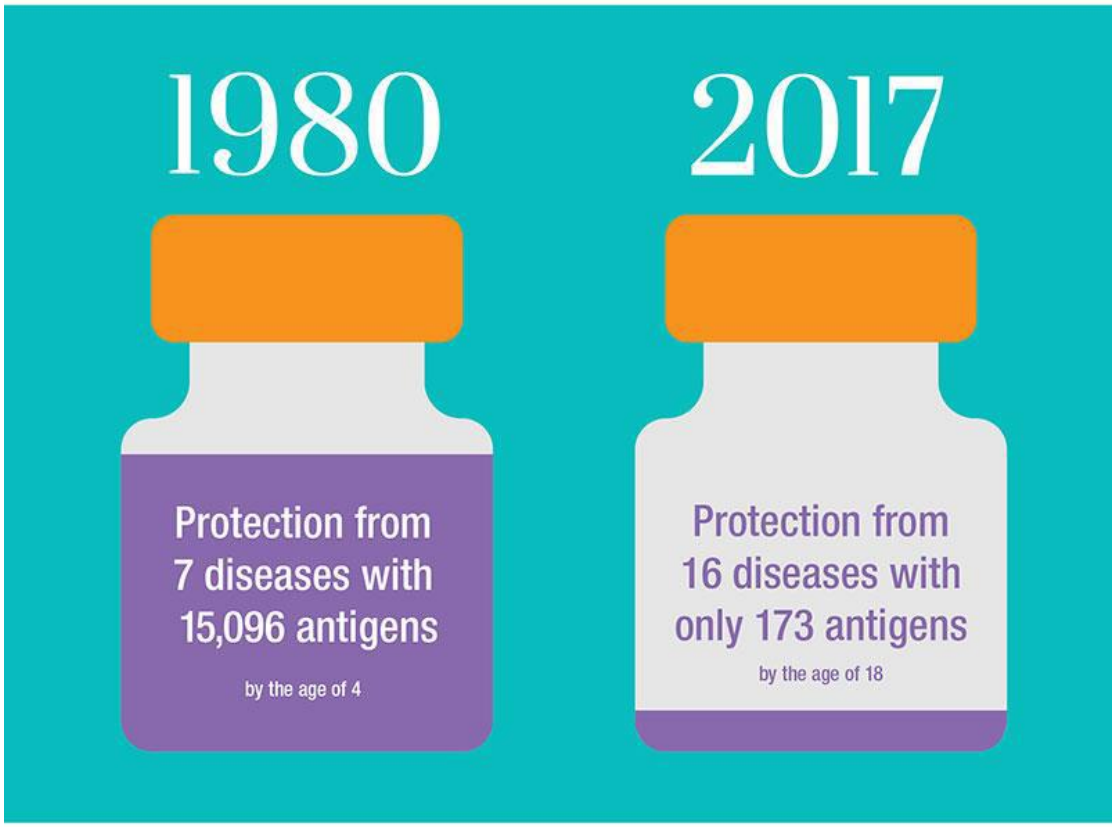
Since no vaccine is 100% effective, vaccination does not automatically mean the person is immunized against the disease.

vaccines have very high effectiveness rates, they are not completely effective for 100% of the people who receive them. For example, a full series of measles vaccine will protect 99 of 100 children from measles and polio vaccine will protect 99 of 100 children from polio. This means when there is a disease outbreak, the very small number of people for whom the vaccine did not work may still be able to catch the disease. Because almost all of our children are immunized and only few are not, it can be the case that during an epidemic the majority of cases occur among children who were immunized. However, the fact remains that those who have not received the vaccine are much more likely to catch the disease."


VACCINATION
VACCINATION IS THE ADMINISTRATION OF ANTIGENIC MATERIAL (A VACCINE) TO STIMULATE AN INDIVIDUAL'S IMMUNE SYSTEM TO DEVELOP ADAPTIVE IMMUNITY TO A PATHOGEN.
IMMUNIZATION
IMMUNIZATION IS THE PROCESS WHEREBY A PERSON IS MADE IMMUNE OR RESISTANT TO AN INFECTIOUS DISEASE, TYPICALLY BY THE ADMINISTRATION OF A VACCINE.

Vaccines Today Work Better Than Ever

Since 1980: More protection, fewer antigens.



Based on CDC Recommended Vaccine Schedule U.S. for children birth to 18 years.
Source: Plotkin's Vaccines (Seventh Edition)

Lec 2: TYPES OF IMMUNITY

Immunity divided into 2 types: Innate immunity and Acquired immunity figure 2.

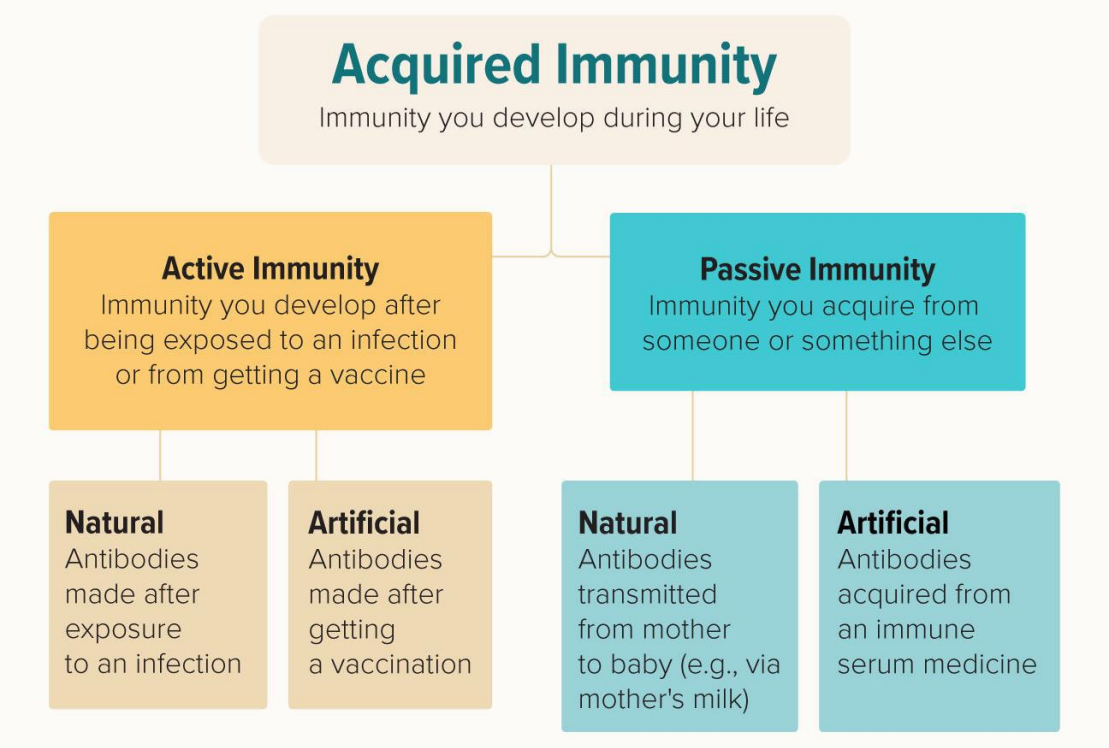


Figure 2. Types of Acquired immunity

Active Immunity

Active immunity refers to the process of exposing the body to an antigen to generate an adaptive immune response. The response takes days/weeks to develop but may be long lasting even lifelong. Wild infection for example with hepatitis A virus and subsequent recovery gives rise to a natural active immune response usually leading to lifelong protection. In a similar manner, administration of two doses of hepatitis A vaccine generates an acquired active immunity leading to long lasting protection.

Passive Immunity

Passive immunity refers to the process of providing IgG antibodies to protect against infection; it gives immediate, but short -lived protection- several weeks to 3 or 4 months at most. Passive immunity is usually classified as natural or acquired. The transfer of **maternal tetanus antibody** (mainly IgG) across the placenta provides natural acquired immunity for the newborn baby for several weeks/months until such antibodies is degraded and lost. **,** breast milk can also provide passive immunity to the infant in the form of maternally produced IgA.

The latter ,however, enters the baby's digestive tract and therefore has a different and complementary effect to maternal IgG circulating in the blood.

In contrast, acquired (artificial) passive immunity refers to the process of obtaining serum from immune individuals, pooling this, concentrating the immunoglobulin fraction and then injecting it to protect a susceptible person.

Passive immunization is used when:

1- There is high risk of infection and insufficient time for the body to develop its own immune response.

2- To reduce the symptoms of ongoing or immunosuppressive disease.

3- Can be provided when people cannot synthesize antibodies.

4- Used in the case of immunodeficiency disease (such as hypogammglobulinemia).

Immunization is often required shortly following birth to prevent disease in newborn such as tuberculosis, hepatitis B and pertussis, however, maternal antibodies can inhibit the induction of protective vaccine responses throughout the first year of the life, and this effect is usually overcome by secondary responses to booster immunization.

There is potential risk for hypersensitivity reactions, especially from gamma globulin of non-human origin. Passive immunity provide immediate protection, but does not develop memory, therefore the patient is at risk of being infected by the same pathogen later unless they acquire active immunity or vaccination.

FDA Licensed immunoglobulin

Application of artificial passive immunity

Use	Source	Product	Disease
Treatment of wound and food borne forms of botulism, infant botulism is treated with human botulism immune globulin. The administration of horse antitoxin remains the only specific pharmacologic treatment available for botulism.	Horse	Specific equine IgG	**Botulism**
Prophylaxis, used most often in kidney transplant patients.	Human	Hyper immune IVIG	**CMV**

Prevention of hepatitis A and measles infection, treatment of congenital or acquired immunodeficiency (it is still indicated following exposure and prior to travel to areas of endemic infection	Human serum	Pooled human Ig	**Hepatitis A, Measles**
Post exposure prophylaxis, prevention in high risk infants (administration with hepatitis B vaccine)	Human	Hepatitis B Ig	**Hepatitis B**
Treatment of ITP and Kawiski disease, prevention and treatment of opportunistic infection with IgG deficiency	Human serum	Pooling human IgG	**ITP, Kawiski disease, IgG deficiency**
Post-exposure prophylaxis (administration with rabies virus)	Human	Rabies Ig	**Rabies**
Treatment of tetanus infection	Human	Tetanus Ig	**Tetanus**
Treatment of progressive vaccinia infection including eczema and ocular forms (usually resulting from smallpox vaccination in immunocopromised individual)	Human	Vaccinia Ig	**Vaccinia**
Post exposure prophylaxis in high risk individuals	Human	Varicella zoster Ig	**Varicella chickenpox**

Antitoxin: known as heterologous hyper-immune serum is often given prophylactically to individuals.

Advantages and disadvantages of passive immunization

Vaccines typically need time (weeks or months) to produce protective immunity in an individual and may require several doses over a certain period of time to achieve optimum protection. Passive immunization, however, has an **advantage** in that it is **quick acting**, producing an immune response within hours or days, faster than vaccine.

Passive immunization can override a deficient immune system, which is especially helpful in someone who **does not respond to immunization**.

Antibodies, however, have certain **disadvantages**. First, antibodies can be **difficult and costly to produce**. Although new technique can help produce antibodies in the laboratory, in most cases antibodies to infectious disease must be harvested from blood of hundreds or thousands of human donors, or, they must obtained from the

blood of immune animals (as with antibodies that neutralize snake venoms). In the case of antibodies harvested from animals, serious allergic reactions can develop in the recipient.

Another disadvantage is that many antibody treatments must be given via intravenous injection, which is a **more time-consuming** and **potentially complicated** procedure while the injection of a vaccine is less time consuming and less risk of complication.

Other individuals produce IgG or IgM antibodies specific for the foreign antibody, resulting in complement-activating immune complexes. The deposition of these complexes in the tissues can lead to type III hypersensitivity reactions. Even whenp urified human antiserum or human gammaglobulin is used (a mixture of IgG from many different human B cells), the recipient can generate an anti-allotype response.

Finally, the immunity conferred by passive immunization is short lived: it does not lead to the formation of long-lasting memory immune cells.

Active Immunization to Induce Immunity and Memory

Active immunization is to trigger the adaptive immune response in a way that will elicit protective immunity and long-lived immunologic memory. When active immunization is successful, a subsequent exposure to the infectious agent elicits a secondary immune response that successfully eliminates the pathogen or prevents disease mediated by its products.

Active immunization can be achieved by 1-**natural** :exposure to the infectious agent or a similar agent (e.g., cowpox exposure can protect against smallpox) or it can be **2-acquired artificially** by administration of a vaccine.

An example of the former might be the “chickenpox parties,Chickenpox in adults can be more serious with more complications, so **immunity**, as the name implies, the immune system plays an active role—proliferation of antigen-reactive T and B cells is induced and results in the formation of protective memory cells. This is the primary goal of vaccination.

Lec3

Herd Immunity

Vaccination can provide excellent protection to a population, even if not every individual in a population is vaccinated, because of phenomenon known as herd immunity. As the population that is vaccinated increase, the chance of an infectious agent becomes smaller.

There are limits to herd immunity, however, if a significant number of unprotected individuals become infected, infection could spread rapidly through the unprotected members of the population.

Herd immunity: is a form of immunity that occurs when the vaccination of significant portion of a population (or herd) provides a measure of protection for individuals who have not developed immunity.

The herd immunity arises when a high percentage of the population is protected through vaccination against a virus or bacteria, making it difficult for a disease to spread because there are so few susceptible people left to infect. This can effectively stop the spread of disease in the community, it is particularly crucial for protecting people who cannot be vaccinated, these include children who are too young to be vaccinated, people immune system problems, and those who are too ill to receive vaccines (such as cancer patients). See figure 1.

Requirments of herd immunity

1- Disease agents restricted to a single host species within which transmission occurs (e.g. Smallpox, no reservoir).

2- Direct transmission (direct contact).

3- Infection must induce solid immunity

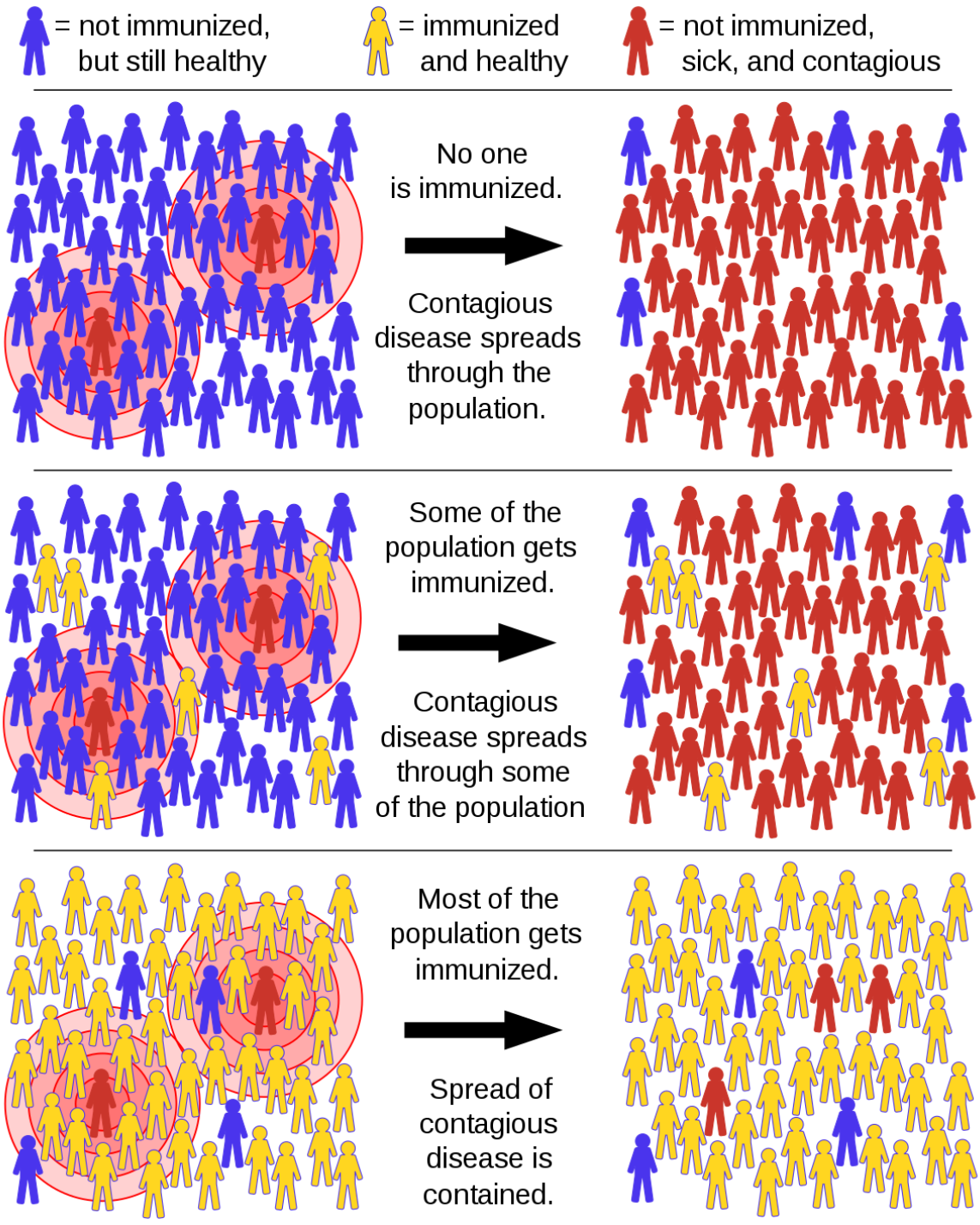


Figure 1. Herd immunity (with and without immunization)

The aim of immunization:

1- The prevention of disease in individuals or groups.

2- Protection of individuals against symptoms, ex: Diphtheria, Tetanus are for example anti-disease rather than antimicrobial vaccines.

Characters of vaccine (properties of ideal vaccine)

Vaccines must fulfill several, criteria to be effective in protecting large numbers of individuals:

1- It is **highly immunogenic**, so that a single vaccine dose provide a complete immunization regimen.

2- The recommended vaccine regimen is highly efficacious in **preventing disease** in individual vaccine recipients.

3- It has **long duration of immunity** so that frequent booster doses are not needed.

4- It **limits spread of infection**, because it prevents vaccine recipients from spreading infection to other people.

5- It is **heat stable**, so that refrigeration is not required during shipping and storage.

6- **Injection is not required** for administration, e.g. nasal spray of vaccine can be used.

7- It can safely be administrated simultaneously with other vaccine either as a part of specific combination vaccine (measles-mumps-rubella) or a separate individual vaccine.

8- **Adverse effect in vaccine recipients are few**, **non-sever, and temporary**, (the microbe used to prepare the vaccine does not cause disease in recipient who have weakened immune system from HIV infection, severe malnutrition, malignancies, or congenital immunodeficiency).

9- The **microbe** used to prepare the vaccine **never reverts to wild type** or otherwise.

mutates to cause dieses, new mutant forms might arise that could evade the immune system and produce disease, new vaccinated individuals.

10- It is technically **simple to manufacture**, so that it can be produced in less sophisticated settings.

11- It is **inexpensive** to manufacture, distribute and administer, so that it is affordable by the maximum number of people.

Routs of Administration

1- Subcutaneous or intramuscular rout (most vaccines).

2- Oral routs (Sabin, oral BCG).

3- Intradermal (BCG).

4- Scarification (Smallpox).

5- Intranasal (live attenuated influenza vaccine).

Characteristics of disease suitable for control by vaccine and immunization

1- Disease is well known by public and occurs commonly, so that many people are aware of its existence and importance.

2- Disease is recognizable by health workers, (cause rash) so that the consequences of the disease can be linked to a specific type of microbe and disease outbreaks can be recognized.

3- Disease short term or long term effects on individuals can sometimes be severe or permanent, so that the public (parents, health workers) support preventing its feature occurrence.

4- Disease is difficult to control at a population level without the use of immunization programs.

5- Disease incubation period (time between the exposure to the microbe and development of disease symptoms) is not too short, so that vaccine still provide at least partial protection if given often exposure (measles vaccine given soon after animal bite exposure).

6- Microbe has no nonhuman reservoir from which it can be reintroduced into the human population after adequate control has been achieved.

7- Genetic mutation that results in biochemical changes to the microbe outer coat occur very slowly so that the vaccine ability to prevent infection and disease is well maintained over time.

8- Infection with the microbe does not result in mild (subclinical) disease or in a prolonged "carrier state", so that there are no infected people who could easily spread the disease to susceptible contacts because they themselves do not feet ill or appear ill.

Scheme of immunization:

Primary vaccination:

- One dose vaccines (BCG, Variola, Measles, Mumps, Rubella, Yellow fever).

- Multiple dose vaccines (Polio, DPT, Hepatitis B).

Booster vaccine:

- To maintain immunity level after its declines after some time has elapsed.

Lec 4

Types of Vaccines

There are two basic types of vaccines: live attenuated and inactivated. The characteristics of live and inactivated vaccines are different, and these characteristics determine how the vaccine is used.

1- Live attenuated vaccines

Live attenuated vaccines are produced by modifying a disease-producing (wild) virus or bacterium in a laboratory. The resulting vaccine organism retains the ability to replicate (grow) and produce immunity, but usually does not cause illness. Attenuated vaccines can be composed of either whole viruses or bacteria, or fractions. Fractional vaccines are either protein-based or polysaccharide-based. Protein-based vaccine include toxoids (inactivated bacterial toxin) and subunit products. Most polysaccharide-based vaccine are composed of pure cell wall polysaccharide from bacteria. Conjugate polysaccharide vaccines contain polysaccharide that chemically linked to a protein, this linkage make the polysaccharide a more potent vaccine.

To produce an immune response, live attenuated vaccines must replicate in the vaccinated person. Small dose of virus or bacteria is administered, which replicates in the body and creates enough of the organism to stimulate an immune response. Damages the live organism in the vial (heat or light) or interferes with replication of the organism in the body (circulating antibody) can cause the vaccine to be ineffective. Although live attenuated vaccines replicate, they usually do not cause disease. When a live attenuated vaccine does cause disease it is usually much milder than natural disease.

The immune response to a live attenuated vaccine is virtually identical to that produced by a natural infection. Live attenuated vaccine produce immunity in most recipients with one dose, except those administered orally. However, a small percentage of recipients do not respond to the first dose of an injection live vaccine (MMR or Varicella) and a second dose is recommended to provide a very high level of immunity in the population. Live attenuated vaccines may cause severe or fatal reactions as a result of uncontrolled growth of the vaccine virus. This occurs in persons with immunodeficiency.

Two principle methods are used for attenuation:

1- Serial passage in cell cultured in vitro.

2- Adaptation to low temperatures.

(With development of DNA technology it is now possible to induce the required genetic change).

Table 1. Live attenuated vaccines

Rout	Method	Vaccine	Bacteria/Virus
Oral	Generally modified	CVD103 hgr	***Vibrio cholera***
Oral	Generally modified	Ty21a	***Salmonella***
ID	Prolog subculture	BCG	***Mycobacterium***
Oral	Passage in monkey kidney cells	Sabin	**Polio**
SC	Passage in chick embryo cell	17D	**Yellow fever**
IN	Temperature sensitive mutant		**Influenza**
SC	Passage in fibroblast cells	MMR	**Measles**
SC	Wister institute (RA 27/3 strain of atten. Virus)	Wistar	**Rubella**
SC	Human diploid cell cultures	Oka/merck	**Chickenpox**
ID	Naturally a virulent	Vaccinia	**Smallpox**

Advantage

1- Infectious microbe can stimulate generation of memory cellular as well as humeral immune response.

2- Its can multiply in the host, fewer quantities must be injected to induce protection.

3- Multiple booster dose may not be required.

4- Some live vaccines can be given orally to induce mucosal immunity and IgA synthesis.

5- They can lead to elimination of wild type virus from the community.

Disadvantage

1- May very rarely revert to its virulent form and cause disease.

2- Live vaccines cannot be given safely to immunosuppressed individual.

3- Since they are live and because their activity depends on their viability, proper storage is critical.

2- Killed vaccines

Killed or inactivated organisms are used where attenuation has not been achieved, the reversion to wild type occurs too easily.

These vaccines include organisms that are dead because of the treatment with physical or chemical agents. In the case of toxins, they will have been inactivated (toxoid). They should be incapable of infection, replication, or function but still able to provoke immunity.

Table 2. Killed or inactivated vaccines

Rout	Method	Vaccine	Bacteria/virus
SC or ID	Phenol	CVD103 hgr	***Vibrio cholera***
SC	Heat, phenol, acetone	TAB	***Salmonella typhi***
SC	Formalin	Haffkine	***Yersinia pestis***
IM	Merthiolate	Sabin	***Bordetella pertussis***
IM	Formalin	Salk	**Poliomyelitis**
SC	Phenol	Semple	**Rabies virus**
IM	Formalin	MMR	**Influenza virus**
IM	Formalin	HM175	**Hepatitis A**

Advantage

1- Safe to use and can be given to immunodeficient and pregnant woman.

2- Cheaper than live attenuated vaccine.

3- Storage not are critical as live vaccine.

Disadvantage

1- Since the microorganism cannot multiply, a large number are required to stimulate immunity.

2- Periodic booster must be given to maintain immunity.

3- Only humoral immunity can be induced.

4- Most killed vaccines have to be injected.

5- Inactivated such as formaldehyde may alter immunogenicity

Vaccine type	Diseases	Advantages	Disadvantages
		WHOLE ORGANISMS	
Live attenuated	Measles Mumps Polio (Sabin vaccine) Rotavirus Rubella Tuberculosis Varicella Yellow fever	Strong immune response; often lifelong immunity with few doses	Requires refrigerated storage; may mutate to virulent form
Inactivated or killed	Cholera Influenza Hepatitis A Plague Polio (Salk vaccine) Rabies Zika	Stable; safer than live vaccines; refrigerated storage not required	Weaker immune response than live vaccines; booster shots usually required
		PURIFIED MACROMOLECULES	
Toxoid (inactivated	Diphtheria Tetanus	Immune system becomes primed to recognize	May require booster shots

Subunit	Hepatitis B Pertussis Streptococcal pneumonia	Specific antigens lower the chance of adverse reactions	Difficult to develop
Conjugate	*Haemophilus influenzae* type b Streptococcal pneumonia	Primes infant immune systems to recognize certain bacteria	
		OTHER	
Recombinant vector	Ebola (in clinical	Mimics natural infection, resulting in strong immune	Too early to tell
DNA	HPV (in clinical testing) Zika virus (in clinical testing)	Strong humoral and cellular immune response; relatively inexpensive to manufacture	Not yet available

mRNA Corona virus SARAS-2, Pfizer vaccine.

Compare between Live attenuated vaccines &Killed vaccines

Live attenuated vaccines	Killed vaccines
1-Attenuated vaccine has microbe with selective deletions of genes involved in Pathogenesis. Organisms replicate in the host, greatly increasing antigenic Stimulation.	Killed vaccines contain organisms inactivated By chemical or physical means.
2-A single inoculation may lead to lifelong immunity. Mucosal immunity possible with oral administration of some live-attenuated organisms. Increased potential for herd immunity compared with killed vaccines.	They are significantly safer than attenuated vaccines in immune compromised Hosts.
3-Reversion to wild-type is a rare but serious	Multiple doses must be given; immunity

complication, especially in immune compromised patients. Contamination by live organisms or toxins is also a rare But serious consequence. 4- Immune response ,IgG, IgA.	is not lifelong; and adjuvants are often required to further stimulate immune Response to the antigens. 4- Immune response mainly IgG.

3- Sub-cellular fraction

1- Polysaccharide capsule of pneumococci (Haemophilus and Meningitis).

2- Surface coat of hepatitis B virus (can be purified from the plasma carriers).

3- Pili of *E. coli* and *N. gonorrhoeae* removal of all infectious material is obvious a vital element is safety control and such vaccines.

4- Peptide vaccine consist of those peptide from the microbial antigen that stimulate protective immunity.

4- Toxoid

Bacterial toxins inactivated (usually by formaldehyde) so that they are no longer toxic but still induce protective antibodies.

For example, the tetanus toxoid is derived from the tetanospasmin produced by *Clostridium tetani*.

5- Microorganisms as a vector for cloned genes

The idea is the use of expression vector (M.O.) complete with inserted gene as a vaccine. Following, injection into the patient it would proliferate sufficiently to release an immunizing amount of foreign protein without inducing disease itself, Examples:

 Vaccinia virus in 1982 contain gene for hepatitis B surface Ag (HBsAg), influenza and herpes simplex. Their disadvantage is complication

Bacteria like:

A) Attenuated Salmonella typhi may act as a general vectors for vaccines against all enteric diseases.

B) BCG is the latest vector to be proposed its advantage is:

1- Large genome

2- The most widely used of all vaccines

3- It induce mediated immunity, both to itself and to other antigens.

4- It could be ideal vector for Ag from all intracellular organisms which include: Tuberculosis, Leprosy, Brucella, Leishmania, Toxoplasma and Listeria

6- Vaccine conjugate

Vaccine can produce humoral immunity through B-cell proliferation leading to antibody production, which may or may not involve helper T-cell, for example pneumococcal polysaccharide, and *H. influenza* type b have specific protective Ab without involvement of T-helper, these T-cell independent response are characterized by low Ab titers, particularly in children 3 to 18 months consequently. However by covalently conjugating the *Haemophilus* polysaccharide to protein Ag, such as diphtheria toxin protein, *Haemophilus* vaccine produced a robust T-cell dependent Ab response even in 3 months old infants.

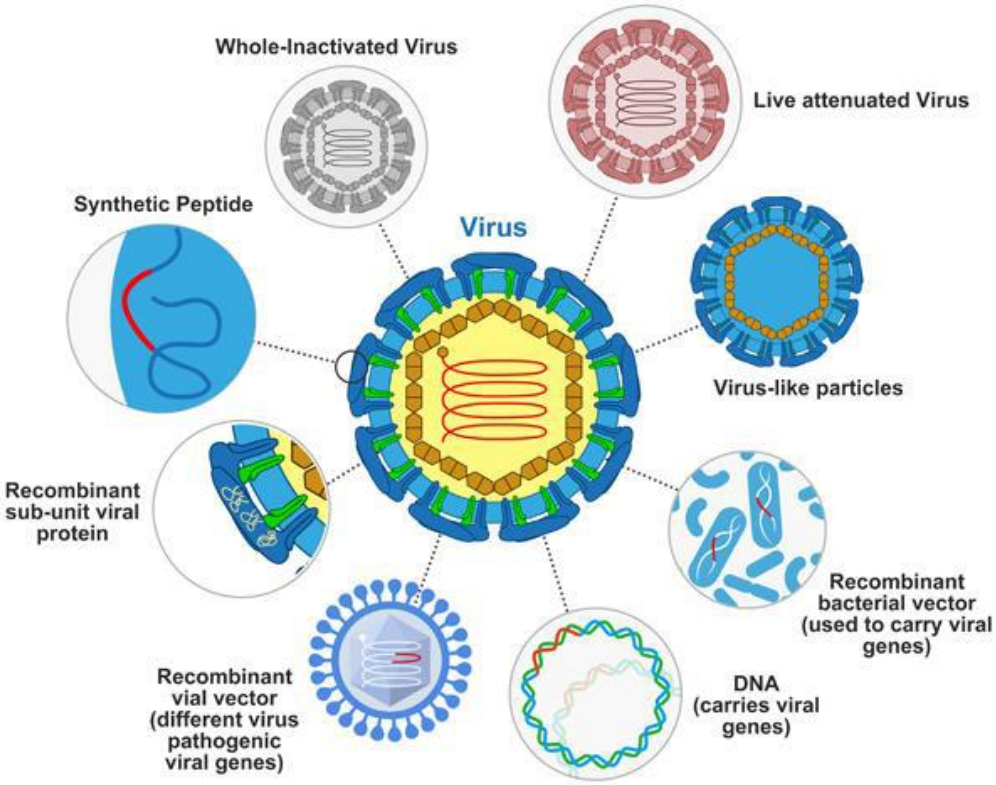


Figure 1. Various approaches for Vaccine Development.

Pathological consequences of vaccination

A) Extrinsic (element in vaccine)

- Contamination of attenuated viruses with other viruses.
- Hypersensitivity to egg albumin (vaccine grow in chicken embryo cells).

B) Intrinsic (pathological response induced from vaccine itself)

- Hypersensitivity ex: type III hypersensitivity to killed measles vaccine.
- Fever and malaise that follow vaccination with killed typhoid organisms is due to the endotoxin
- Autoimmunity as a result of antigenic similarity between host and microbe as in chagas disease (infection with *trypanosome*).
- Brain damage after vaccination for pertussis .

The immune-compromised host:
Living attenuated vaccine, in the immune-compromised patient is avoided

1-Vaccinia and BCG in patients with severe T-cell deficiency.

2-In less several T-cell deficiency live measles vaccine in now recommended.

3-In other T-cell deficiency of childhood including treatment with steroid or immunosuppressive drugs (Mumps, Measles, Rubella) is not advised.

4-Non-living vaccine is less dangerous

5-Vaccines for specific antibody induction e.g. capsular polysaccharide, hepatitis B are recommended in but the most sever B-cell deficiencies.

Lec 5 : Vaccine production &Adjuvants

Vaccine production

1-Generation of the antigen: the first step in order to produce a vaccine is generating the antigen that will trigger the immune response. For this purpose the pathogen's proteins or DNA need to be grown and harvested using the following mechanisms:

- Viruses are grown on primary cells such as cells from chicken embryos or using fertilized eggs (influenza) or cell lines that reproduce repeatedly (hepatitis A).

- Bacteria are grown in bioreactors which are devices that use a particular growth medium that optimizes the production of the antigen.

- Recombinant proteins derived from the pathogen can be generated either in yeast, bacteria or cell cultures.

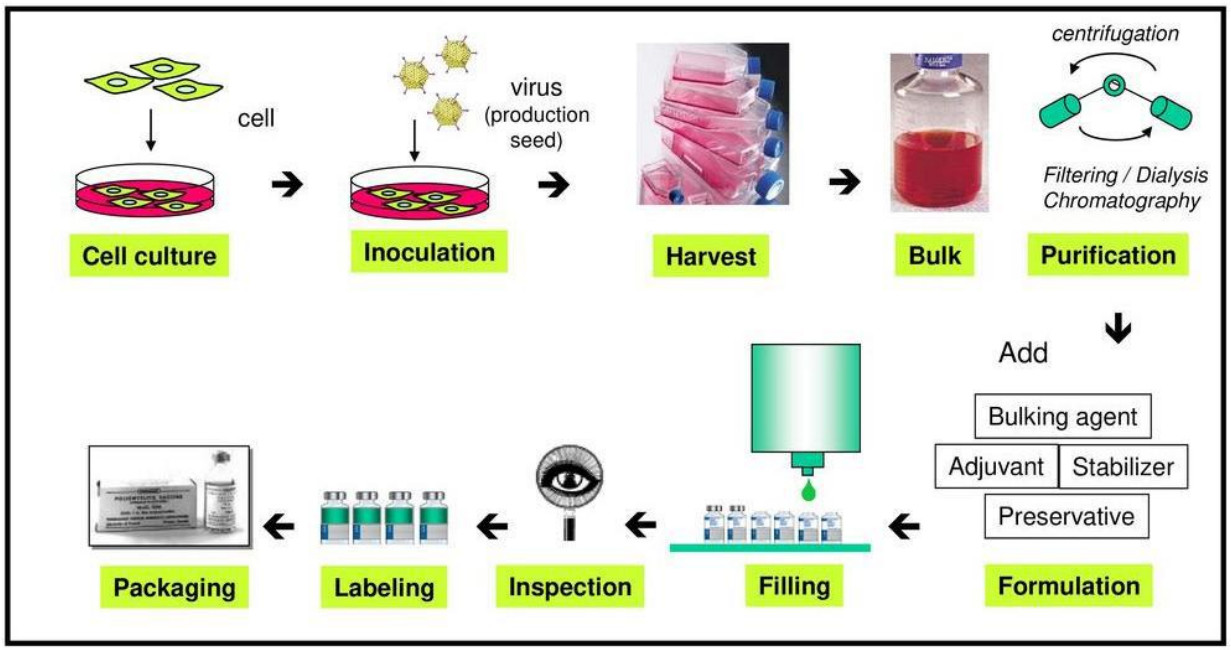
Release and isolation of the antigen: the aim of this second step is to release as much virus or bacteria as possible. To achieves this, the antigen will be separated from the cells and isolated from the proteins and other parts of the growth medium that are still present.

3- Purification: in third step the antigen will need to be purified in order to produce a high purity/quantity product. This will be accomplished using different techniques for protein purification. For this purpose several separation steps will be carried out using the differences in for instance protein size, physic-chemical properties, binding affinity or biological activity.

4-Addition of other components: the fourth step may include the addition of an adjuvant, which is a material that enhances the recipient's immune response to a supplied antigen. The vaccine is then formulated by adding stabilizers to prolong the storage life or preservatives to allow multi-dose vials to be used safely as needed. Due to potential incompatibilities and interactions between antigens and other ingredients,

combination vaccines will be more challenging to develop. Finally, all components that constitute the final vaccine are combined and mixed uniformly in a single vial or syringe.

5- Packaging: once the vaccine is put in recipient vessel (either a vial or a syringe), it is sealed with sterile stoppers. All the processes described above will have to comply with the standards defined for good manufacturing practices that will involve several quality controls and an adequate infrastructure and separation of activities to avoid cross-contamination. Finally, the vaccine is labeled and distributed worldwide.



Vaccine production techniques are evolving. Cultured mammalian cells are expected to become increasingly important, compared to conventional options such as chicken eggs, due to greater productivity and low incidence of problems with contamination. Recombination technology that produces genetically detoxified vaccine is expected to grow in popularity for the production of bacterial vaccines that use toxoids. Combination vaccines are expected to reduce the quantities of antigens they contain, and thereby decrease undesirable interaction, by using pathogen-associated molecular patterns.

Vaccine composition

Generally, vaccines have several major components.

1) **Antigen (active components):** the active component, or antigen, is the important part, responsible for inferring immunity to the disease or infection the vaccine is designed to guard against. It's composed of a modified form of the

virus, bacteria, or toxin that causes the disease; the precise nature can vary between vaccines.

2) **Adjuvant:** chemical compounds added to vaccines to help enhance the body's immune response, these aren't present in all vaccines.

3) **Preservative** (phenol, 2 phenoxyethanol, thimersa: DTap, polio, Hibl): preservatives are used to prevent bacterial and fungal contamination of the vaccine after its manufacture. This is particularly important for so-called "multi-dose" vaccines, where multiple injection doses are drawn for the same rubber-capped vessel.

4) **Additives confer stabilization of live attenuated virus:** stabilizers are added to the vaccine to protect it from adverse conditions which could impact its efficacy, allowing it to be stored for longer periods of time. a range of different possible stabilizers can be used; sugars (sucrose, lactose), amino acids and proteins (gelatin, human serum albumin) can all be utilized for this purpose. They also prevent the vaccine components

from adhering to any storage vessel. Many of the compounds used as stabilizers are found naturally in the body anyway, and so do not pose any risk.

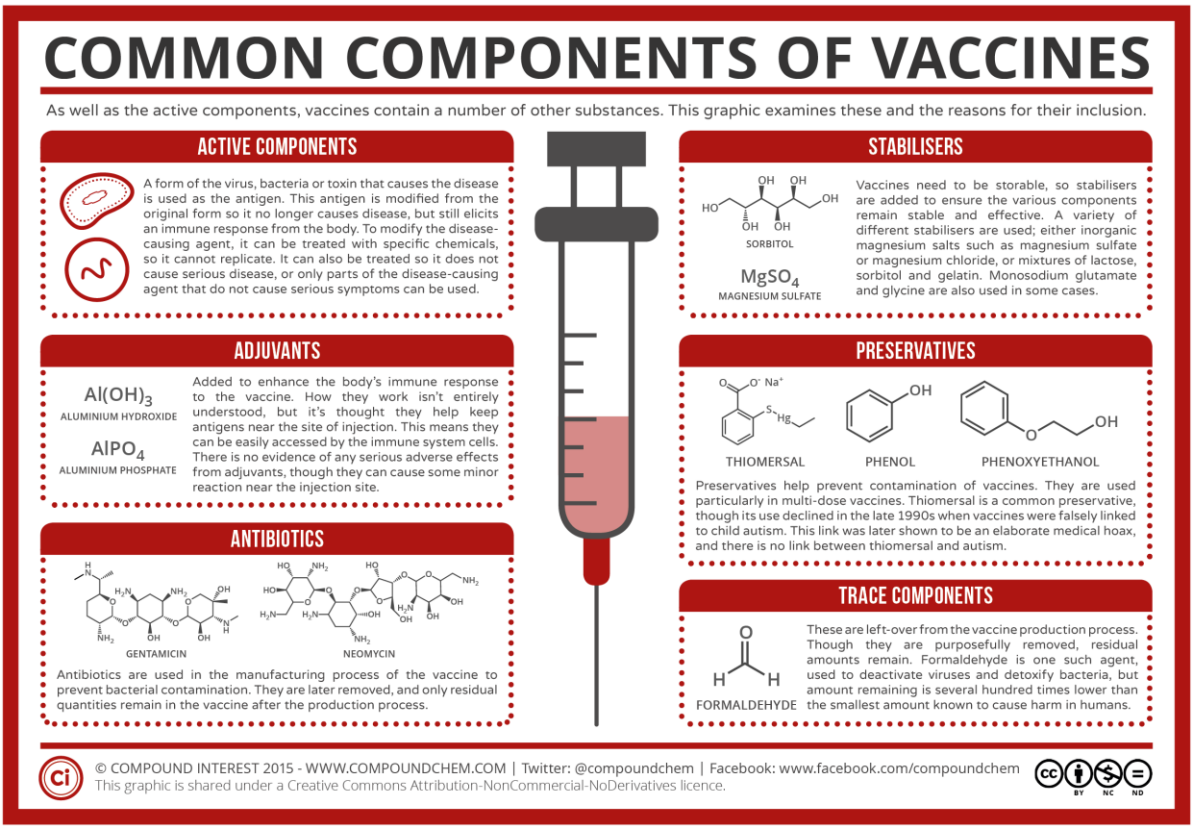
5) Manufacturing residual:

 In activating agent (formaldehyde, glutraldehyde): a number of trace components are left behind from the manufacturing process of the vaccine. The concentration of these components in the final vaccine is very low. Compounds such as formaldehyde, one of the agents that can be used to in activate viruses, can be detected, but at levels far below that known to cause harm in humans.

Antibiotics: in the manufacture of the vaccine, antibiotics will commonly be used to prevent bacterial contamination. Whilst these are removed after manufacture, trace amounts can still remain in the final vaccine. The antibiotics that commonly cause adverse allergic reaction, such as penicillin, are avoided

 Cellular residuals (egg protein, yeast proteins).

6) **Diluents:** vaccines need to be diluted to their required concentration. Most often, this will be accomplished using either sterile water, or a saline solution



Adjuvant

An adjuvant is an ingredient of a vaccine that helps create a stronger immune response in the patient's body. In other words, adjuvant help vaccines work better. Some vaccines made from weakened or dead germs contain naturally occurring adjuvant and help the body produce a strong protective immune response. However, most vaccines developed today include just small components of germs, such as their proteins, rather than the entire virus or bacteria. Adjuvant help the body to produce an immune response strong enough to protect the person from the disease he or she is being vaccinated against.

Adjuvanted vaccines can cause more local reactions (such as redness, swelling, and pain at the injection site) and more systemic reactions (such as fever, chills and body aches) than non-adjuvanted vaccines.

In some vaccines, the weakened or inactivated virus stimulates a strong immune response so no additional adjuvant is needed for it to be effective to protect against infections.

Factors affecting adjuvant selection:

1) Type of the antigen.

2) Species to be vaccinated: some adjuvant may

3) Rout of administration.

4) Side effects.

Classification of adjuvant: (according their mechanism of action)

1) Active immune-stimulants, being substances that increase the immune response to the antigen.

2) Carriers, being immunogenic proteins that provide T-cell help.

3) Vehicle adjuvant; being oil emulsions or liposome that serve as a matrix for antigens as well as stimulating the immune response.

Adjuvant challenge:

1- Toxicity

2- Stability

3- Bioavailability

4- Cost

5- Production difficulty

6- Epitope modification potential during formulation.

7- Pre-existing immunity to carrier protein

Types of adjuvant

1) Aluminum gels or aluminum salts are vaccine ingredients that have been used in vaccines since the 1930s. small amounts of aluminum are added to help the body build stronger immunity against the germ in the vaccine. Aluminum is one of the most common metals found in nature and is present in air, food, and water. The amount of aluminum present in vaccines is low and is regulated by the U.S. Food and Drug Adminstration (FDA).

2) Monophosphoryl lipid A is included in one human papillomavirus (HPV) vaccine. This immune-boosting substance was isolated from the surface of bacteria.

Lec (6) Time of Vaccination

Time of vaccination

Vaccines are designed to prevent diseases affect young children , bearing in mind certain considerations :-

1- The presence of maternally derived antibodies reduce the effectiveness of some vaccines, there for they usually delayed until the third month of life on later.

2- Live attenuated vaccines can cause severe disease in immunodeficiency states

3- Where the disease is mainly risk to the elderly e.g *pneumococcal pneumonia* vaccination is usually given at a late age.

According to the recommended immunization schedule for persons, children may receive up to **24 vaccinations** to protect them from up to 14 diseases by the time they are 2 years of age. Vaccines are recommended for very young children because their immune systems are not yet fully mature and also because their stomachs produce less acid, making it easier for ingested bacteria and viruses to multiply. These factors leave them the most vulnerable to the devastating effects of these serious diseases.

Some things should be note when scheduling vaccinations:

 allergic reaction to a previous vaccination or a vaccine ingredient, like eggs or gelatin.

 If a child has a high fever, or a history of fever after receiving a vaccination.

Vaccination schedule in Iraq

 At birth: BCG, OPV-0, HBV-1

 2 months completed: pentavalent vaccine (DTP-1, Hib1, and HBV-2), OPV1 and Rotavirus1.

 4 months completed: quadruple vaccine (DTP-2, and Hib2) OPV2 and Rotavirus2.

6 months completed: pentavalent vaccine (DTP-3, Hib3, and HBV-3), OPV3 and Rotavirus3.

 9 months completed: measles

 15 months completed: MMR1

 18 months completed: Quadruple vaccine (DTP, and Hib) OPV. (booster no.1)

 4-6years: DTP, OPV (poster no.2) and MMR2

1- Bacilli calmette-guerin (BCG) vaccine

The live attenuated strain of *mycobacterium bovis* known as bacillus Calmette-Guerin (BCG) uses shared antigens to stimulate the development of cross-immunity to *Mycobacterium tuberculosis*. It lost its virulence in humans by being specially cultured in an medium for years.

Benefit:

1- Prevention of *tuberculosis*.

2- BCG prevents dissemination of the bacterium or the development of other life-threatening complications such as meningitis.

3- BCG is effective at reducing morbidity and mortality in children but is less useful in the prevention of adult respiratory disease.

Route of administration:

BCG is given as a **single** intra-dermal injection at the insertion of the deltoid into the lateral aspect of the left upper arm.

 The insertion of deltoid is most frequently used because the local complication rate is smallest when that site is used

Successful BCG vaccination:

 A small bleb is raised and a successful vaccination leads to the development of a small local swelling with 2 weeks.

 The lesion progresses to a papule or shallow ulcer of approximately 10 mm diameter and within 12 weeks to form a small, flat scar.

Adverse effects:

1- Local ulceration and regional suppurative adenitis occur in 0.1-1 % of vaccine recipients.

2- If BCG is accidentally given to an immunocompromised patient, it can cause disseminated or life threatening infection

2- Polio vaccines

Poliovirus: Enterovirus (RNA), three serotypes: 1, 2, 3, Human is the reservoir, transmission by fecal-oral or possible oral-oral, communicability 7-10 days before onset, the virus present in stool for 3-6 weeks. viral spread along nerve fibers leads to destruction of motor neurons.

The two vaccines have eradicated polio from most of the countries in the world from an estimated 350,000 cases in 1988 to less than 2000 cases in 2008 and to 359 in 2014.

Salk's polio vaccine "inactivated polio vaccine" IPV
Based on polio grown in a type of monkey tissue culture, which is then inactivated with formalin. Contains 3 serotypes of vaccine virus. The injected Salk vaccine confers IgG-mediated immunity in the blood stream, which prevents polio infection from progress to viremia and protects the motor neurons. It offers no protection to the mucosal lining of the intestine, i.e. people vaccinated with salk's vaccine can still carry the disease and spread it
to unvaccinated individuals. IPV has essentially no adverse effects associated with it other than possible rare hypersensitivity reactions to trace quantities of antibiotics.
Sabin's polio vaccine "oral live-attenuated vaccine" OPV
Sabin's "oral polio vaccine" is a **live-attenuated** vaccine, Contains 3 serotypes of vaccine virus. It replication very efficiently in the gut, the primary site of infection and replication, unable to replicate efficiently within nervous system tissue. The OPV proved to be superior in administration, and also provided **longer lasting immunity than the Salk vaccine.** The trivalent OPV vaccine on very rare occasions has been associated with paralysis (vaccine-associated paralytic poliomyelitis, about 1 case per 750,000 vaccine recipients).

3- DPT vaccine

Diphtheria

Caused by aerobic gram-positive bacillus; *Corynebacterium diphtheria*

 complication are myocarditis and neuritis, death occurs in 5-10% for respiratory illness

Tetanus

- Caused by anaerobic gram-positive spore-forming bacteria; *Clostridium tetani*

- Complications:- laryngospasm, aspiration pneumonia, and death.

Pertussis

- Highly contagious respiratory infection caused by *Bordetella pertussis*

- Complication :- pneumonia, seizures, encephalopathy.

DPT: mixture of three vaccines, to immunize against diphtheria, pertussis and tetanus **Pertussis, whole heat or formalin killed vaccine with Diphtheria and Tetanus toxoid**
DPT administered in a dose of 0.5 ml intramuscularly five vaccinations before age 7 years (at 2,4,6, and 15-18 month and at 4-6 years)

Adverse effects

 Minor reaction:- inflammation, indurations or a painless nodule at the site of injection.

 Moderate reaction:- ongoing crying (for three hours or more in the first 12 hours), a high fever (up to 40 oC).

 Severe problems:- happen very rarely include, a serious allergic reaction.

4- MMR vaccine

Measles

caused by *paramyxovirus* (RNA); Complication: diarrhea, otitis media, pneumonia

Mumps

caused by *paramyxovirus* (RNA); Complication: CNS involvement, deafness

Rubella

caused by *togavirus* (RNA); Major concern is **congenital rubella syndrome** as up to 85% of infants affected during first trimester when placenta and fetus infected during viremia; infection may affect all organs, may lead to fetal death or premature delivery, deafness, liver and spleen damage.

MMR vaccine: composed of three live attenuated vaccines (Measles, Mumps & Rubella)

This highly effective vaccine is administered subcutaneously in two doses, the first MMR dose is recommended at age 12 to 15 months and the second at the child's entry into school (age 4 to 6 years), a dose given before 12 months of age will not be counted. The purpose of the rubella portion of this vaccine is to protect against congenital rubella syndrome by preventing the occurrence of rubella, which, by itself, is a mild disease. Because MMR is a live-attenuated vaccine, non allergy related side effects are noted 5 to 12 days following immunization. Fever and rash are relatively common, experienced by 5-15% of recipients.

Contraindications and precautions:

1- Severe allergic reaction to vaccine component or following prior dose

2- Pregnancy

3- Immunosuppression

5- Hepatitis B vaccine

Hepatitis B infection: caused by Hepadnaviridae fimily (DNA)

Hepatitis B vaccine consists of purified HBsAg particles produced through recombinant DNA technology in yeast. non living antigenic preparation can be derived from the blood of carriers, this is because the surface coat antigen (HBsAg) is over produce by the virus and circulate as free non infectious 22 nm spherical particles, these particles were shown be at least 95% protective. Vaccine usually is given intramuscularly as a three dose series, the second and

third doses given 1 and 6 months, respectively, after the first dose (0,1,6). Three doses induce seroconversion in 90-95% of healthy infants, children and adults.

Disadvantage:

1- Derived from human blood

2- Purified with exceptional care because the risks of transmitting live HBV or other

3- Antibody levels start to fall 1-2 years later so that boosting may be necessary.

6- Rotavirus vaccine

In early childhood, the single most important cause of severe dehydrating diarrhea is rotavirus infection.

 The pentavalent vaccine (attenuated virus) protect against rotavirus gastroenteritis.

 Oral route and three doses; 2,4 and 6 months.

7- *Haemophilus influenza* type b vaccine

Haemophilus influenza is a gram negative coccobacillus, cause severe pneumonia, meningitis and other invasive disease, 15-30% of children who survive (Hib) meningitis may develop permanent neurological disability, including brain damage, hearing loss, 5-10% cases of Hib meningitis are at risk of dying.

 Type of vaccine: conjugate

 Number of doses: three doses (2,4,6 months) and a booster shot at 18 months

 Injection site: outer mid-thigh for infants

 Injection type: intramuscular

 Given as quadruple or pentavalent vaccine.

8- Rabies vaccine

Killed preparation is used

 Post exposure case 5-6 injection intramuscularly

 Pre exposure 2-3 dose are usually sufficient for protection, with boost every few years.

9- Influenza vaccine

 The most widely used B-propiolactone killed viral vaccine

 Vaccine is offered to high risk groups such as nursing staff and patients with chronic respiratory cardiac disease.

 Revaccination in subsequent is required to maintain antibody level.

10- Chickenpox (*Varicella zoster*) vaccine

 Live attenuated vaccine is highly protective up to 95% protection

 Given to immunocompromised and neonates at risk.

11- Typhoid vaccine

Two vaccines have emerged

 Live attenuated by random chemical mutagenesis, they induce local immunity in the intestine when given orally.

 Polysaccharide vaccine is composed of purified virulence antigen; single dose has given protection into 70% range.

12- Cholera vaccine

 Heat killed – poor protection

 Toxoid using cholera toxin, some success

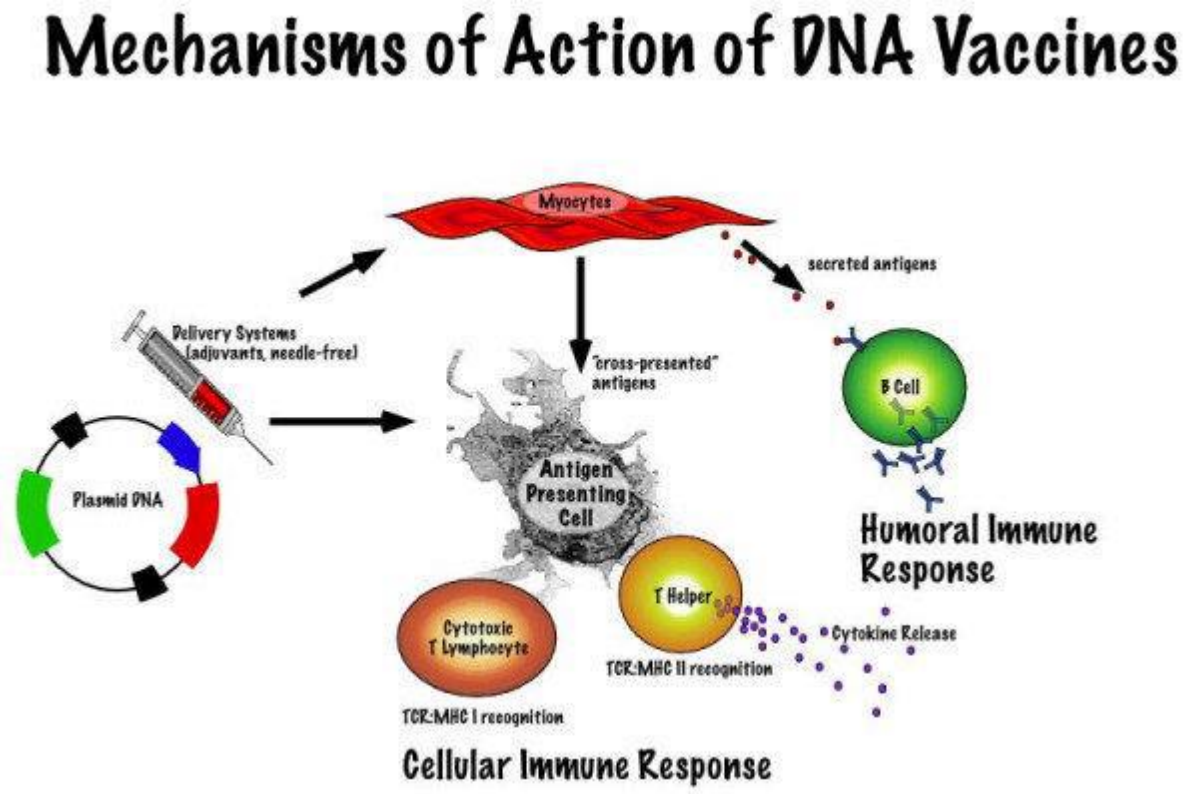
 Deletion mutant

 Expression cholera genes in an attenuated *Salmonella typhi* vaccine

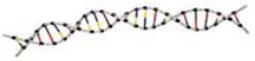
Lec 7 DNA Vaccination

DNA vaccination is a technique for protecting an organism against disease by injecting it with genetically engineered DNA to produce an immunological response. Nucleic acid vaccines are still experimental, and have been applied to number of viral, bacterial and parasitic models of disease, as well as to several tumor models. DNA vaccines have a number of advantages over conventional vaccines, including the ability to induce a wider range of immune response types.

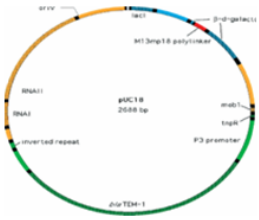
DNA vaccines are made up of small, circular piece of bacterial DNA called a plasmid that has been genetically engineered to produce one or two specific proteins (antigens) from a pathogen. The vaccine DNA is injected into the cells of the body, where the inner machinery of the host cells reads the DNA and converts it into pathogenic proteins, because these proteins are recognized as foreign, when they are processed by the host cells and displayed on their surface, the immune system is alerted, which then triggers a range of immune responses.



HOW DNA VACCINE IS MADE ?

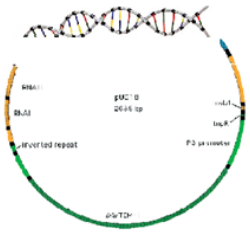


Viral gene



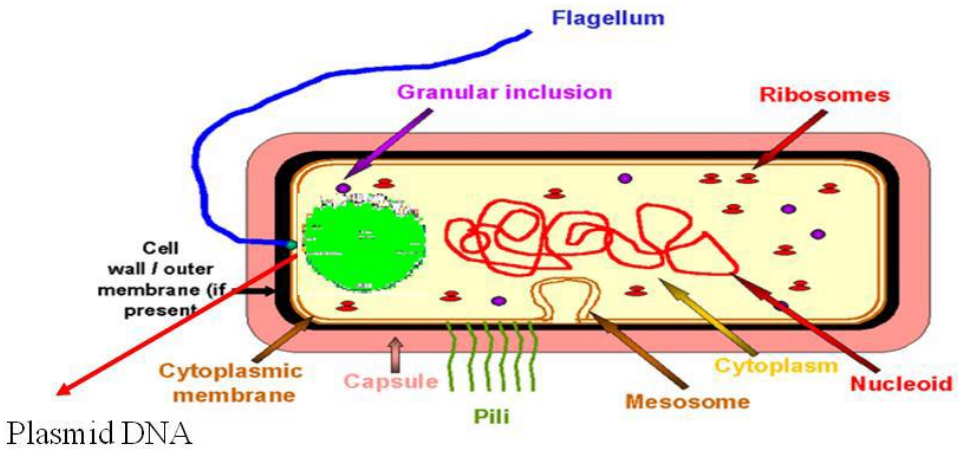
Expression plasmid

Recombinant DNA Technology



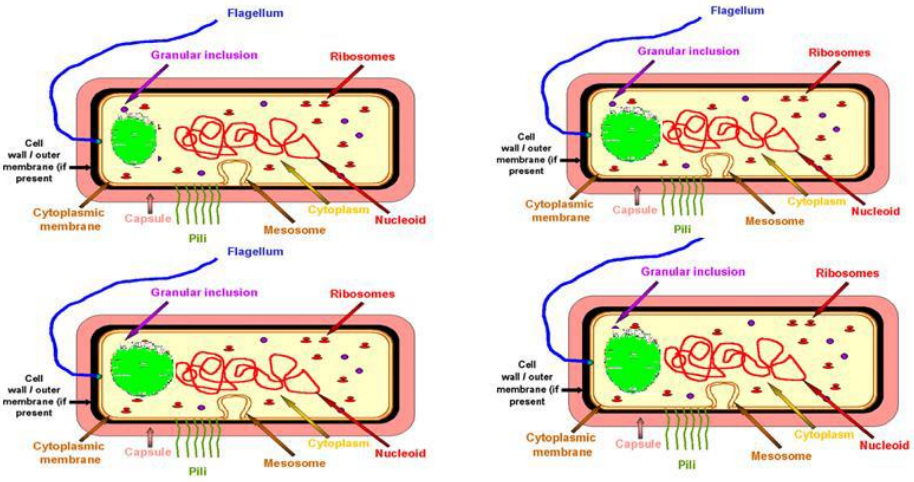
Plasmid with foreign gene

Transform into bacterial cell

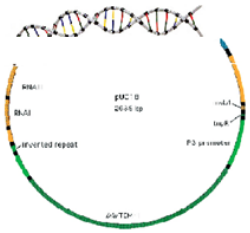


Bacterial cell

Plasmid DNA get Amplified



Plasmid DNA Purified



Ready to use

Pfizer-BioNTech Vaccine:

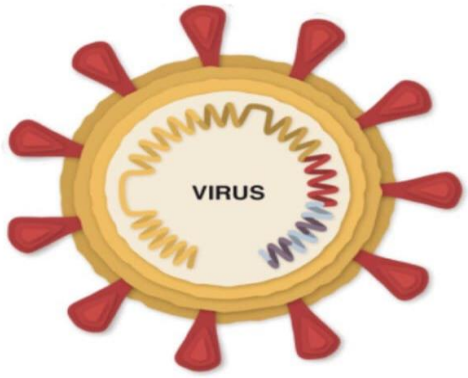
The German company BioNTech & Pfizer developed and tests a coronavirus vaccine known as BNT162b2. A clinical trial demonstrated that the vaccine has an efficacy rate of (95 %) in preventing Covid-19.

SARS-COV-2 virus uses many proteins to enter human cells. ACE2 serves as the entry point into cells for some coronaviruses, including HCoV- NL63, SARS-CoV, and SARS-CoV-2.

The human version of the enzyme is hACE2.

Angiotensin-converting enzyme 2 (ACE2) is an enzyme attached to the membranes of cells located in the lungs, arteries, heart, kidney, and intestines.

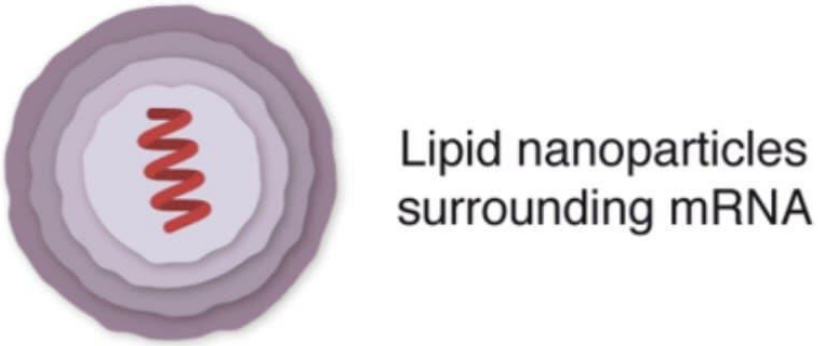
Spike proteins & Spike protein gene are the best targets for potential vaccines and treatments.



Like the Moderna vaccine, the Pfizer-BioNTech vaccine is based on the virus's genetic instructions for building the spike protein. (mRNA Inside an Oily Shell)

The vaccine uses messenger RNA, genetic material that our cells read to make proteins. The molecule MRNA is fragile and would be chopped to pieces by our natural enzymes if it were injected directly into the body. To protect their

vaccine, Pfizer and BioNTech wrap the mRNA in oily bubbles made of lipid nanoparticles.



Because of their fragility, the mRNA molecules will quickly fall apart at room temperature. Pfizer is building special containers with dry ice, thermal sensors and GPS trackers to ensure the vaccines can be transported at -94°F (-70°C) to stay intact.

Entering a Cell

After injection, the vaccine particles bump into cells and fuse to them, releasing mRNA. The cell's molecules read its sequence and build and display spikes and spike proteins. The mRNA from the vaccine is eventually destroyed by the cell, leaving no permanent trace.

Some of the spike proteins form spikes that migrate to the surface of the cell and stick out their tips. The vaccinated cells also break up some of the proteins into fragments, which they present on their surface. These protruding spikes and spike protein fragments can then be recognized by the immune system.

When a vaccinated cell dies, the debris will contain many spike proteins and protein fragments, which can then be taken up by antigen-presenting cells which digest the proteins and Present spike protein fragments. The cell presents fragments of the spike protein on its surface. When helper T cells detect these fragments, the helper T cells can raise the alarm and help other immune cells to fight the infection.

Other immune cells, B cells, encounter the coronavirus spikes on the surface of vaccinated cells, or free spike protein fragments. A few of the B cells may be specific to the spike proteins. If these B cells are then activated by helper T cells, they will start to proliferate and differentiate into antibody-producing plasma cells. The antibodies can bind onto coronavirus spikes, mark the virus for destruction and prevent infection by blocking the spikes from attaching to other cells. The antigen-presenting cells can also activate another type of immune cell called a killer T cell to seek out and destroy any coronavirus-infected cells that display the spike protein fragments on their surfaces.

The Pfizer-BioNTech vaccine requires two injections (each 0.3ml), given 21 days apart, to prime the immune system well enough to fight off the coronavirus. But because the vaccine is so new, researchers don't know exactly how long its protection might last. A preliminary study found that the vaccine seems to offer protection about 10 days after the first dose, compared with people taking a placebo:

Preparation and Injection of Pfizer-BioNTech Vaccine

Each vial of the vaccine contains 5 doses of 0.3 milliliters. The vaccine must be thawed before injection and diluted with saline. After dilution, the vial must be used within six hours.

Studies point to the Pfizer-BioNTech and Moderna vaccines as effective against variants of the SARS-CoV-2 virus that causes COVID-19. They suggest that the antibodies stimulated by the vaccines are only slightly less potent against the variants than against the Wuhan wildtype strain.

The Pfizer and Moderna vaccines are made using messenger RNA. The proteins made with the mRNA instructions activate the immune system and develop antibodies and other immunity weapons to fight it.

Johnson & Johnson's Covid-19 vaccine

The J&J vaccine uses a different approach to instruct human cells to make the SARS-2 spike protein, which then triggers an immune response. It is what's known as a viral vectored vaccine. A harmless adenovirus – from a large family of viruses, some of which cause common colds - has been engineered to carry the genetic code for the SARS-2 spike protein. Once the adenovirus enters cells, they use that code to make spike proteins.

J&J employed this same approach to make an Ebola vaccine that has been authorized for use by the European Medicines Agency.

Johnson & Johnson's Covid-19 vaccine could prevent 66% of symptomatic Covid- 19 cases. Both mRNA vaccines from Pfizer-BioNTech and Moderna showed they could stop over 90% of symptomatic Covid-19 cases in their late-stage clinical trials. But Janssen's vaccine reducing the severity of the disease, potentially freeing up hospital beds.

Janssen's vaccine prevents 85% of severe Covid-19 cases, defined as a positive Covid-19 test, plus elevated breathing, heart rates, or low oxygen saturation.

Data from the Moderna and Pfizer-BioNTech vaccine trials showed nearly complete protection from severe cases, with zero and 10 severe cases total in the vaccinated groups, respectively.

While the Janssen vaccine may not prevent as many Covid-19 cases compared to other vaccines, it could keep hospitalization rates low, similar to the benefit of the annual flu shot. Flu shots don't offer absolute protection, but they make flu cases less lethal. (Janssen's vaccine efficacy rate of 66%

Janssen's shot offers other advantages: It only needs to be refrigerated, instead of deep-frozen, and it only requires one jab. These factors could ease logistics, partially responsible for distribution delays, and accelerate immunity across the population.

The Pfizer vaccine has been authorized for use for people aged 16 and older, though the company has recently asked the FDA to change the label to allow children 12 and older to be vaccinated. Moderna's has been cleared for use in people 18 and older, though the company is now testing its vaccine in 12- to 17-year-olds. J&J's vaccine has been tested in people 18 and older, and that's who it was authorized for. Until testing in children and younger teens is conducted, this vaccine won't be available for use to anyone under 18 years old either.

Vaccine efficacy

The Pfizer vaccine showed an efficacy of 95% at preventing symptomatic Covid infection after two doses. The vaccine is more or less equally protective across age groups and racial and ethnic groups.

The Moderna vaccine was 94.1% effective at preventing symptomatic Covid-19 after the second dose. The vaccine is equally effective across different ethnic and racial groups.

Comparing the Covid-19 vaccines developed by Pfizer, Moderna, and Johnson & Johnson

In the United States so far, three vaccines have been issued emergency use authorizations - green lights from the Food and Drug Administration to be put into use, even though they have not yet been fully licensed.

A vaccine developed by the partnership of Pfizer and German-manufacturer BioNTech came first in mid-December, followed closely by one developed by Moderna with assistance from the National Institute of Allergy and Infectious Diseases.

Johnson & Johnson vaccine doesn't require the cold-chain needed to keep the Pfizer and Moderna vaccines from spoiling. It is cheaper because only one dose is needed... Some people couldn't easily get two doses.

Potential advantage and disadvantage of nucleic acid based immunization:
Advantage

1. Subunit vaccination with no risk for infection.

2. Antigen presentation by both MHC class I and class II molecules.

3. Able to polarize T-cell help toward type 1 or type 2.

4. Immune response focused only on antigen of interest.

5. Ease of development and production.

6. Cost-effectiveness

7. Stability of vaccine for storage and shipping.

8. Long term persistence of immunogen.

9. Obviate need for peptide synthesis, expression and purification of recombinant proteins and the use of toxic adjuvant.

10. In vivo expression ensure protein more closely resembles normal Eukaryotic structure, with accompanying post-translational modifications.

Disadvantage

1. Limited to protein immunogens (not useful for non-protein based antigens such as bacterial polysaccharides).

2. Risk of affecting genes controlling cell growth.

3. Possibility of inducing antibody production against DNA.

4. Possibility of tolerance to the antigen (protein) produced.

5. Potential for atypical processing of bacterial and parasite proteins.

Delivery methods:

DNA vaccines have been introduced into animal tissues by a number of different methods. The two most popular approaches are:

1- Injection of DNA in saline using a standard hypodermic needle

Conducted intramuscular (IM) in skeletal muscle or intradermally (ID), with DNA being delivered to the extracellular spaces.

Immune responses to this method of delivery can be affected by many factors including:

- Needle type
- Needle alignment
- Speed of injection
- Volume of injection
- Muscle type and age
- Sex and physiological condition of the animal being injected.

2- Gene-gun delivery

The other commonly used method of delivery ballistically accelerates plasmid DNA (pDNA) that has been adsorbed onto gold or tungsten micro particles into the target cells, using compressed helium as an accelerant.

Alternative delivery methods have included aerosol instillation of naked DNA on mucosal surfaces, such as the nasal and lung mucosa.

The method of delivery determines the dose of DNA required raising an effective immune response, saline injection require variable amounts of DNA from 10 μg -1 mg, whereas gene gun deliveries require 100 to 1000 times less DNA than intramuscular saline injection to raise an effective immune response.

Alternative approaches for vaccine production:

1- Recombinant viral antigen subunit vaccines

Virus proteins or genetic material from a selected infectious agent is inserted into live microbe that is non-pathogenic, the recombinant microbe will multiply and express the foreign gene, and the vaccine recipient will be immunized against microbial Ag. *E. coli* cell were first to be used for this purpose, vaccinia the virus originally used to vaccinated for smallpox and adenoviruses have proved practical agents for this technique.

These methods are particularly effective in designing vaccines for obligate parasite that are difficult or expensive to culture syphilis spirochete or malaria parasite. Ex: HB virus.

This technology provides mean of isolating the gene that encode various microbial Ag, inserting them into plasmid vectors, and cloning them in appropriate host.

2- Synthetic peptide

Identification of the peptide sequences that trigger a protective immune response (immunogenic site) and to use completely synthetic versions of these as the vaccine substance.

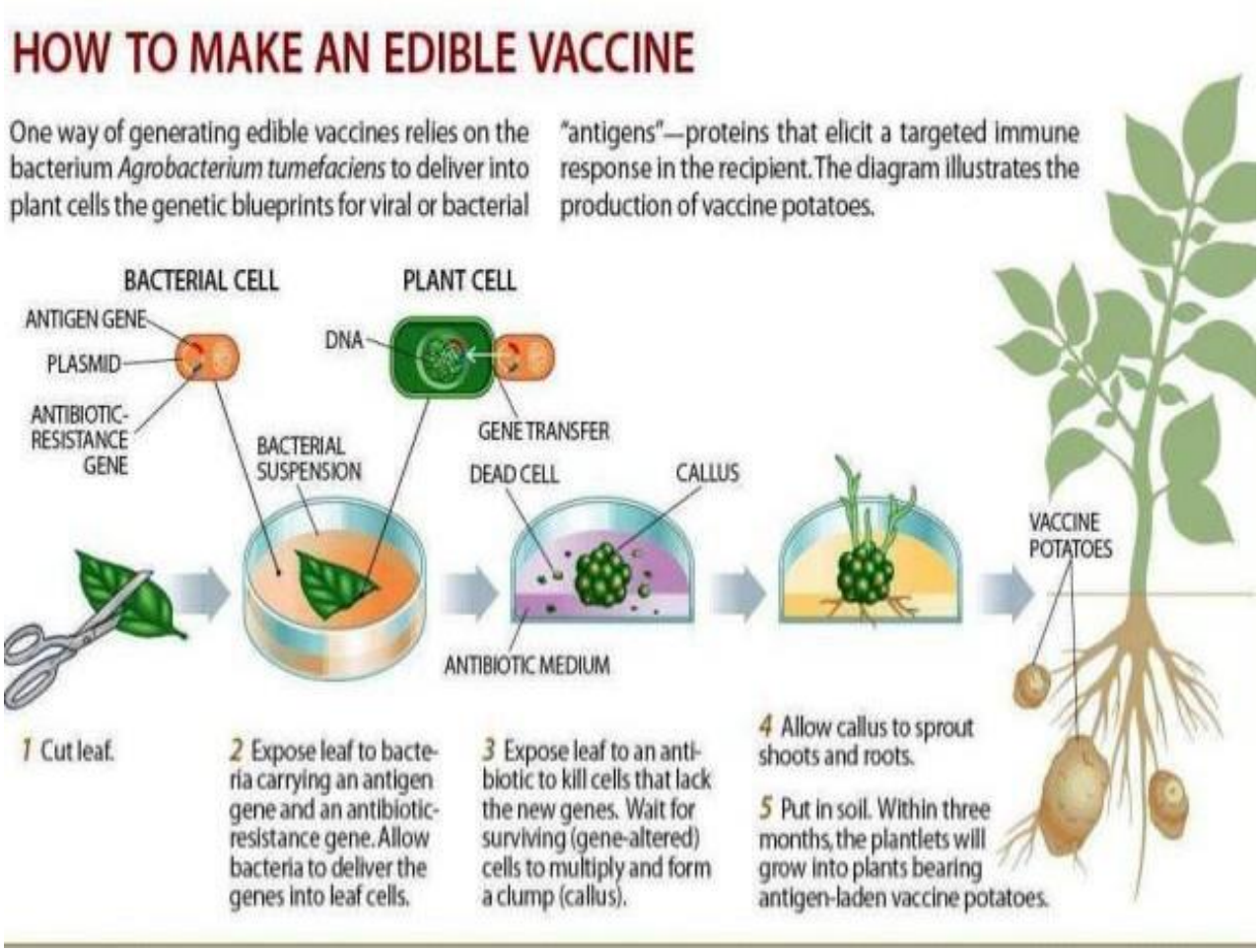
Cowpea mosaic virus was genetically engineered to include: surface antigen from foot and mouth disease virus (pathogenic to human and animal). This virus was used to infect its natural host (black-eyed pea plant), and introduced genes from the foot and mouth disease virus was expressed handsomely in the plant, and the plant needs to be sacrificed a few week after infection. One leaf from the infected plant produced enough surface Ag to serve as a vaccine for 200 dose.

3- Edible vaccine

Edible vaccine are mucosal-targeted vaccines, which cause stimulation of both systemic and mucosal immune response. Edible vaccines are being developed for various diseases, such as measles, cholera and hepatitis B, and many more are in the process of development.

Edible vaccine would not need purification, refrigeration and injection, this made the vaccine cheep. Tomatoes and lettuce have been transformed to produce HBs Ag.

Conventional vaccination programmes in many countries are seriously handicapped due to a lack of equipment for storage and transport of the vaccines and the shortage of paramedical This essay highlights the importance staff to administer the vaccines .produced in plants.



4- Anti-idiotypic vaccine

 This unique amino acid structure in the antibody is known as the idiotype, which can be considered as a mirror of the epitope in the Ag.

 Antibodies can be raised against the idiotype by injecting the antibody into another animal.

 This anti-idiotype antibody mimics part of the three dimensional structure of the Ag. This can be used as a vaccine.

 When the anti0ideotye antibody is injected into a vaccine, antibodies (anti-idiotype antibodies) are formed that recognize a structure similar to part of the virus and might potentially neutralize the virus.

Lec 8: Introduction of serology and immunotherapy

Serology: is the scientific study of blood serum in practice, the term usually refers to the diagnostic identification of antibodies in the serum. This antibodies are typically formed in response to:

1- Infection (microorganisms like bacteria, virus, fungi and parasite)

2- Foreign proteins (mismatched blood transfusion)

3- Owen proteins (autoimmune disease)

Serological tests:

Serological tests are diagnostic methods carried out on a sample of blood serum, the clear liquid that separates from the blood when it is allowed to clot. The purpose of such a test is to identify antibodies and antigens in a patient's sample. Serological tests used to diagnose infections and autoimmune diseases and blood groups. Serological tests may also be used in forensic serology to investigate crime scene evidence.

Several methods can be used to detect antibodies and antigens, including ELISA, agglutination, precipitation, complement-fixation, and fluorescent antibodies.

The study of serum is serology

Serum is the fluid and solute component of blood which does not play a role in clotting. It may be defined as blood plasma without fibrinogens. Serum contain proteins, electrolytes, antibodies, antigens, hormones, and any exogenous substances (drugs or microorganisms). Serum does not contain leukocytes, erythrocytes, platelets, or clotting factors. Serum is used in numerous diagnostic tests as well as blood typing. Measuring the concentration of various molecules can be useful for many applications, such as determining the therapeutic index of a drug candidate in a clinical trial.

The serum of convalescent patients successfully recovering (or already recovered) from an infectious disease can be used as a biopharmaceutical in the treatment of other people with that disease, because the antibodies generated by the successful recovery are potent fighters of the pathogen. Such convalescent serum antiserum is a form of immunotherapy.

Serum therapy, also known as serotherapy, describes the treatment of infectious disease using the serum of animals that have been immunized against the specific organisms or their product

Antiserum:

Antiserum is a blood serum that contains specific antibodies against an infective organism or poisonous substance. Antiserums are produced in animals (horse, sheep, ox, rabbit) and humans in response to infection, intoxication, or vaccination and may be used in another individual to confer immunity to a specific disease or to treat bites or stings of venomous animals. Antiserums from animals are most often used, but in persons allergic to animals, human antiserums have proved valuable.

In 1891 **Emil Behring** saved the life of a young girl with diphtheria by injecting antiserum for the first time in history. Serum horses proved to be saviors of diphtheria-infected people. Based on his observation that people who survived infection with the diphtheria bacterium never became infected again, he discovered that the body continually produces an antitoxin, which prevents survivors of infections from being infected again with the same agent. Subsequently, treatment of tetanus, rabies, and snake venom developed, and proactive protective vaccination against diphtheria and other microbial diseases began.

In 1901, Behring won the first Nobel Prize in Medicine for his work in the study of diphtheria.

IMMUNOTHERAPY

Immunotherapy : the another type for treatment of tumor immunology.

The possibility of stimulating the patient's own immune system to respond to tumor-associated antigens has long intrigued scientists. Immunotherapeutic methods used can be separated into two types: passive or active immunotherapy.

Passive immunotherapy involves transfer of antibody, cytokines, or cells to patients who may not be able to mount an immune response. With active immunotherapy, patients are treated in a manner that stimulates them to mount immune responses to their tumors.

Applications of immunotherapy

Q/ Enumerate the applications of Immunotherapy

1- Protection and prevention(prophylaxis) against infectious disease e.g diphtheria, tetanus,rabies and treatment for snake bite.

2- immunosuppression this include prevention of maternal isoimmunization Rho sensitization and immunosuppression during tissue transplantation.
3- Antibody preparation (serum,antitoxin and gamma globulin). The use of Ab preparation in immunotherapy are shown in fig I and include such antimicrobial activity as toxin neutralization ,viral nuntralization and antibacterial effects due to lysis or opsonization and phagocytosis.In addition,immuosuppresisive activity with antibody preparations has been successful in the prevention of maternal Rho isoimmunization and in immunosuppression during tissue transplantation.
4- Today in most case, passive immunization is achieved with immunoglobulin derived from pooled human plasma or serum but it such product are not available,gloulins sera from animal source,mostly equine are also used.Antibody of human origin are preferred.because these proteins do not elicite an immune response that could have an adverse effect e.g. serum sickness(as seen following the use of gamma globulins of animal origin.

Passive Immunotherapy

Passive transfer of allogeneic cellular immunity from one person to another to fight cancer has many barriers because of possible recipient rejection of foreign cells, graft-versus host disease (GVHD), and the fragility of live cells, Inducing a patient's own cellular immunity is far more likely to be successful, However, a form of GVHD called **graft versus leukemia** has been demonstrated with transfer

of allogeneic T cells and is associated with improved patient prognosis. Therefore, successful passive transfer of anticancer T cells is theoretically possible .Adaptive T-cell therapy has been attempted using several models. For example, T cells from allogeneic donors can be immunized against tumors. After recipients are immunosuppressed to prevent rejection and to eliminate T suppressor mechanisms, they receive the T cells. One strategy in this model to treat GVHD is to genetically engineer the allogeneic T cells

3-Passive transfer of antibody to treat cancer almost always employs monoclonal antibodies. "Naked" monoclonal antibodies

against cancer could induce antibody-dependent cell-mediated cytolysis (ADCC), complement-mediated lysis, or opsonization. If the antibodies are directed toward

particular receptors, they could trigger a desirable action in the cell such as inducing apoptosis or inhibiting growth signals

4. **Antibody conjugates,** or **immunotoxins,** are antibodies conjugated to toxins or radioisotopes on the premise that they can kill cancer cells while leaving adjacent cells intact.

Active Immunotherapy

The goal of active immunotherapy is to have the patient develop an immune response that will help eliminate the tumor. Nonspecific stimulation by adjuvants such as Bacillus Calmette Guerin (BCG) was first attempted, and superficial

bladder cancer is still treated with BCG. Improved technology has allowed the production of novel adjuvants and selective use of stimulatory cytokines (TNF-α, IFN-γ,IL-1, IL-2, and so on) in immune competent patients to enhance the natural antitumor response and the artificial vaccine-induced response.

Other attempts at stimulating host immune systems have involved transfection of normal cells or isolated tumor cells with genes for cytokine production and injection of the modified cells into or around the tumor. This has been

done with many cytokines, including TNF-α, interferons,IL-2, and granulocyte monocyte–colony stimulating factor (GM-CSF). Of the cytokines transfected, GM-CSF has shown the most promise.

Cancer vaccines have been of great interest to researchers. When specific viruses are associated with a cancer, vaccine construction is relatively straight forward, since viral antigens are obviously foreign. The vaccine for human papillomavirus

(HPV) to prevent cervical cancer is an excellent example. It is important to note that many viruses have several serotypes ,not all of which may be associated with cancer, so vaccines must be protective against the appropriate epitopes. HPV vaccines, for example, are directed epitopes that prevent initial infection with carcinogenic serotypes but do not help treat established cervical cancer, as these epitopes are down regulated in cancer cells. Therefore, a distinction exists between prophylactic vaccines and therapeutic ones.

These protocols will be important adjuncts to traditional therapies in which tumors will first be de bulked and then the immune system will eradicate residual tumor and micro metastases. The increased understanding of tumor immunology in recent years has made this a field of active study and increased optimism.

Q1/ Compare and contrast passive versus active immunotherapy, describing common techniques used in each.

Hypo sensitization :Hypo sensitization or allergy desensitization is an allergy treatment that can help decrease long-term sensitivities to allergens. Also known as immunotherapy, in this the patient is gradually vaccinated sub-cutaneous against increasingly larger doses of the allergen. The purpose is to reduce the severity of the disease and/or eliminate hypersensitivity altogether. Hypo sensitization is generally recommended for people who have selective sensitivity to specific allergens. How do hypo sensitization injections work? The injection shot formulated consists of a mixture of the various pollens, fungi spores, animal dander and dust mites to which a person is allergic. This mixture which is called an allergy extract contains no medication such as antihistamines or corticosteroids. Using allergy test results and the patient's history, the allergist prepares a customized extract, made of one or many allergens. Since most allergic people react adversely to more than one allergen, several extracts may be prepared. Some people need only one shot each time, while others may need more than one extract mixture. Small doses of an allergen or allergens are regularly introduced into the body so that one can develop a resistance to it. The injections lead to the development of a **protective response** both by increasing the suppressor T cells and by increasing the protective, or "blocking" Ig_G (Immunoglobin G) antibodies. The more tolerant the body becomes, the fewer symptoms one will show. Immunotherapy injections work well in both children and adults. Are hypo sensitization injections safe for pregnant injections? Immunization injections are usually safe, and can be administered to Pregnant women. However, in some cases doctors advise stoppage of this course of treatment during pregnancy. Although immunotherapy does not cause malformations in the developing baby, but in the case of a rare adverse reaction to the treatment, the fetus might suffer from oxygen deprivation. When are hypo sensitization shots usually administered? Hypo sensitization injections are prescribed when: There is zero response to allergy medications. Side effects from allergy medications. To allergic children with hay fever and/or asthma. In case of allergic reactions to insect stings - immunotherapy may be given regardless of age. There is a need to decrease long-term use of medications, such as in a child or in a woman wanting to get pregnant. In older patients, hypo sensitization is usually not recommended as they may have a reduced capacity to cope with side-effects. Hypo sensitization is effective if started at an early age, soon after the development of allergies. How effective is hypo sensitization? About 80 to 90 percent of children improve with hypo sensitization, if started at

an early age. It usually takes atleast 12-18 months before concrete reduction in allergy symptoms can be noticed.

Hypo sensitization treatment needs to be continued along with other allergy medications. It is also important to continue eliminating triggering allergens from the environment. What are the advantages? Hypo sensitization injections, taken on a regular basis help relieve asthma symptoms, especially for those prone to airborne allergies. Hypo sensitization reduces asthma symptom severity, reduce medication use over time, improve quality of life, and even reduce the risk of developing new allergies in the future.

Serum Sickness

Serum sickness is a generalized type III reaction that is seen in humans, although not as frequently as it used to be.

Serum sickness results from passive immunization with

1-animal serum, usually horse or bovine, used to treat such infections as diphtheria, tetanus, and gangrene.

2-Vaccines and bee stings may also trigger this type of reaction.1 Generalized symptoms appear in 7 to 21 days after injection of the animal serum

Local reaction :

In the form of redness, itching and swelling at the injection site might occur. If this condition occurs repeatedly, then the strength or timing of a shot is changed.

A systemic reaction:

Can also involve not the place of injection, but a different site. Symptoms include nasal congestion, swollen lips, loss of bladder and/or bowel control, sneezing, swelling, wheezing, and low blood pressure. Such reactions can at times be serious and life threatening. However, deaths related to immunotherapy are rare.

Symptoms:

include headache, fever, nausea, vomiting, joint pain, rashes, and lymphadenopathy. Recovery:takes between 7 and 30 days.

In this disease, the sensitizing and the shocking dose of antigen are one and the same, because antibodies develop while antigen is still present. High levels of antibody form immune complexes that deposit in the tissues. Usually this is a benign and self-limiting disease, but previous exposure to animal serum can cause cardiovascular collapse on re exposure.

Diagnosis

Is made by observing the symptoms and reviewing the patient's medical and medication history. Although the symptoms of serum sickness may be similar to other conditions, patients who present with symptoms of serum sickness and who have a recent history of exposure to a drug or other product which may cause this type of reaction should be suspected of having serum sickness.

Age

Individuals older than 15 years may experience more frequent and more severe disease because they receive larger volumes of antitoxin.

Treatment

The first step in treatment of serum sickness is always to discontinue the drug or other substance which is suspected of causing the reaction. After that, all treatment is symptomatic. Antihistamines, pain relievers, and corticosteroids may be given to relieve the symptoms. The choice of treatment depends on the severity of the reaction.

Monoclonal antibody treatment using mouse antibodies to human cells.

Now, however, monoclonal antibodies are genetically engineered human antibodies, and such reactions do not occur.

Hyper sensitization: A state of increased reactivity or sensitivity to a stimulus**.**

Lec 9: Monoclonal Production

MONOCLONAL ANTIBODY

The knowledge that B cells are genetically preprogrammed to synthesize very specific antibody has been used in developing antibodies for diagnostic testing known as **monoclonal antibodies.** Normally, the response to an antigen is heterogeneous,because even a purified antigen has multiple epitopes that stimulate a variety of B-cell clones. In 1975,Georges Kohler and Cesar Milstein discovered a technique to produce antibody arising from a single B cell, developed a technology to fuse immortal hetero myleoma cells with lymphocytes,

Q1/ How do PEG play role in monoclonal antibody production?

Answer: using poly ethylglycol (PEG) to break down cell membranes and allow mixing of the genetic material from both cell types.

The resulting cell type is called a hybridoma. This hybridoma takes on the characteristics of both the lymphocyte and heteromyeloma cell, creating an immortal cell with the ability to produce antibody. which has evolutionized serological testing. For their pioneering research, they were awarded the Nobel Prize in 1984.Kohler and Milstein's technique fuses an activated B cell with a myeloma cell that can be grown indefinitely in the laboratory. Myeloma cells are cancerous plasma cells.Normally, plasma cells produce antibody,

Q/ Why myeloma cells unable to produce antibody?

so a particular cell line that is not capable of producing antibody is chosen. In addition, this cell line has a deficiency of the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT) that renders it incapable of synthesizing nucleotides from hypoxanthine and thymidine, which are needed for DNA synthesis.

Hybridoma Production

A mouse is immunized with a certain antigen, and after a time, spleen cells are harvested. Spleen cells are combined with myeloma cells in the presence of polyethylene glycol(PEG), a surfactant. The PEG brings about fusion of plasma cells with myeloma cells, producing a **hybridoma.** Only a small percentage of cells actually fuse, and some of these are like cells—that is, two myeloma cells or two spleen cells.

After fusion, cells are placed in culture using a selective medium containing

Q/ What is the purpose of the use HAT media in monoclonal antibody preparation?

Answer: hypoxanthine, amino pterin, and thymidine (HAT). Culture in this medium is used to separate the hybridoma cells by allowing them to grow selectively .Myeloma cells are normally able to grow indefinitely in tissue culture, but in this case they cannot, because both pathways for the synthesis of nucleotides are blocked. One pathway, which builds DNA from degradation of old nucleic acids, is blocked, because the myeloma cell line employed is deficient in the required enzymes HGPRT and thymidine kinase. The other pathway, which makes DNA from new nucleotides, is blocked by the presence of amino pterin.Consequently, the myeloma cells die out. Normal B cells cannot be maintained continuously in cell culture, so these die out as well. This leaves only the fused hybridoma cells,which have the ability (acquired from the myeloma cell) to reproduce indefinitely in culture and the ability (acquired from the normal B cell) to synthesize nucleotides by the HGPRT and thymidine kinase pathway **(Fig. 1).**

Selection of Specific Antibody-Producing Clones

The remaining hybridoma cells are diluted out and placed in microtiter wells, where they are allowed to grow. Each well, containing one clone, is then screened for the presence of the desired antibody by removing the supernatant.

Once identified, a hybridoma is capable of being maintained in cell culture indefinitely, and it produces a permanent and uniform supply of monoclonal antibody that reacts with a single epitope.

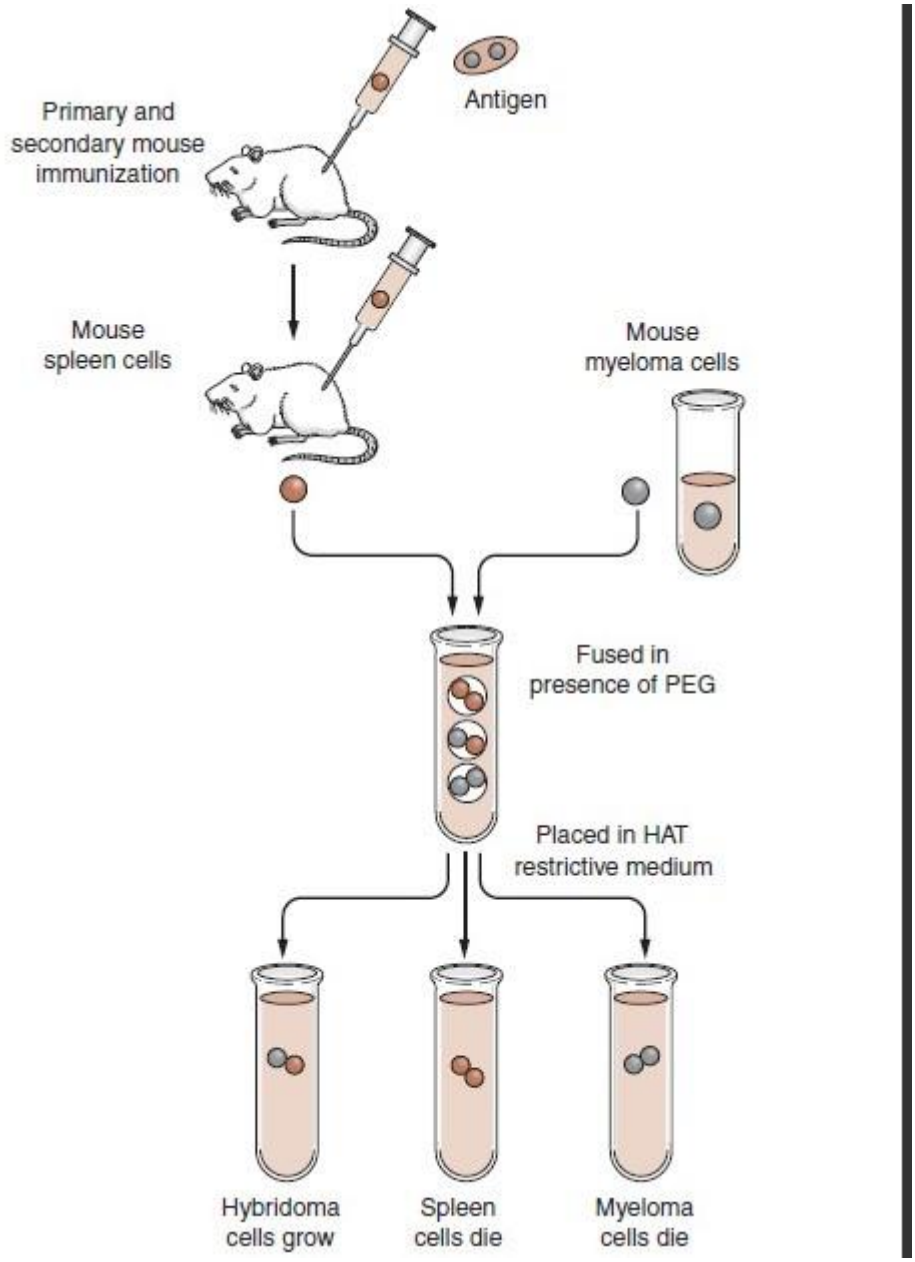
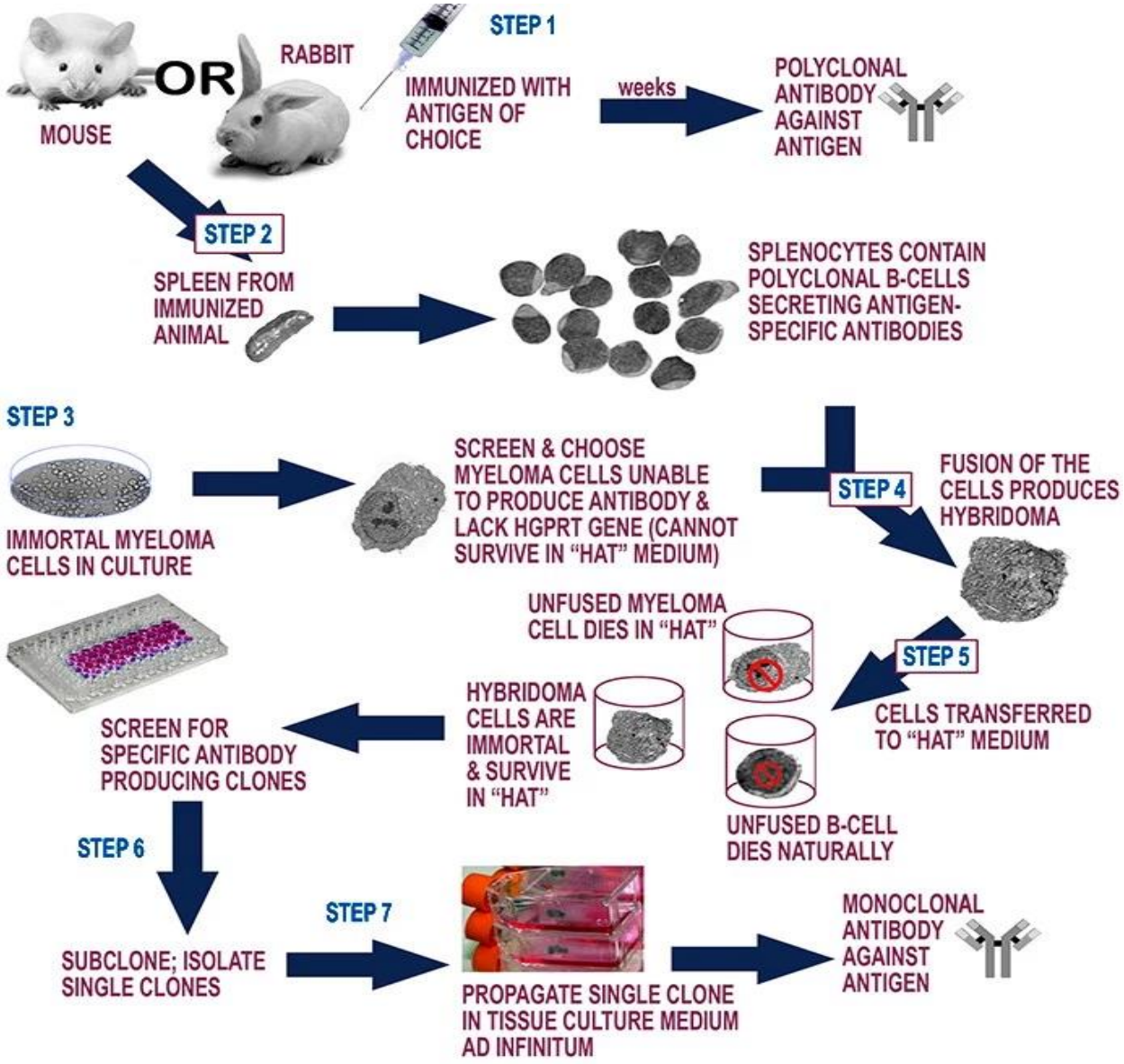


Fig1: Formation of a hybridoma in monoclonal antibody production. A mouse is immunized, and spleen cells are removed. These cells are fused with non secreting myeloma cells and then plated in a restrictive medium. Only the hybridoma cells will grow in this medium,where they synthesize and secrete a monoclonal immunoglobulin specific for a single determinant on an antigen.



Q/ Describe the method of production of a monoclonal antibody?

Clinical Applications

Monoclonal antibodies were initially used for in vitro diagnostic testing. A familiar example is pregnancy testing, which uses antibody specific for the chain of human chorionic gonadotropin, thereby eliminating many false-positive reactions.

Other examples include detection of tumor antigens and measurement of hormone levels .Recently, however, there has been an emphasis on the use of monoclonal antibodies as therapeutic agents. One of the biggest success stories is in the treatment of two autoimmune diseases: rheumatoid arthritis and Crohn's disease (a progressive inflammatory colitis).

Monoclonal antibodies have also been used to treat various types of cancers. In the case of metastatic breast cancer ,

Uses of Monoclonal Antibodies

The greatest impact of MAbs in immunology has been on the analysis of cell membrane antigens. Because they have a single specificity rather than the range of antibody molecules present in the serum, MAbs have multiple clinical applications, including the following:

• Identifying and quantifying hormones
• Typing tissue and blood
• Identifying infectious agents
• Identifying clusters of differentiation for the classification of leukemia's and lymphomas and follow-up therapy
• Identifying tumor antigens and autoantibodies
• Delivering immunotherapy .

Polyclonal Antibodies

The immune response to an antigen generally involves the activation of multiple B-cells all of which target a specific epitope on that antigen. As a result a large number of antibodies are produced with different specificities and epitope affinities these are known as polyclonal antibodies.

For production purposes these antibodies are generally purified from the serum of immunised animals were the antigen of interest stimulates the B-lymphocytes to produce a diverse range of immunoglobulin's specific to that antigen.

The aim is to produce high titre, high affinity antibodies. Today these polyclonal antibodies are used extensively for research purposes in many areas of biology, such as immune precipitation, histochemistry, enzyme linked immunosorbent assays (ELISA), diagnosis of disease, immunoturbidimetric methods, western blots and Biochip technology. Polyclonal antibodies are ideally suited for use in sandwich assays as second stage antigen detectors.

Polyclonal antibodies	**Monoclonal antibodies**
Inexpensive to produce	Expensive to produce
Skills required for production	Training is required for the technology

are low	used
Relatively quick to produce	Hybridomas take a relatively long time to produce
Generate large amounts of non-specific antibodies	Generate large amounts of specific antibodies
Recognize multiple epitopes on any one antigen	Recognize only one epitope on an antigen
Can have batch-to-batch variability	Once a hybridoma is made, it is a constant and renewable source
	No or low batch-to-batch variability