**Ministry of Higher Education and Scientific Research**
**University of Baghdad**
**College of Science**
**Department of Biology**

# Theoretical Serology
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## المرحلة الثالثة - الدراستان الصباحية والمسائية
## الفصل الدراسي الثاني

**تدريسيو المادة:**

**أ.د. دنيا فريد سلوم**
**أ.م.د. هند حسين عبيد**
**أ.م.د. مي خليل إسماعيل**
**م.د. ياسر باسم عبدالوهاب**

# Lecture One

## Introduction

It was recognized long ago that individuals who survived smallpox, plague, and cholera rarely contracted the disease again, even when surrounded by others suffering from that particular disease.

Early forms of vaccinations developed as attempts to confer protection from these fortunate survivors to those who still faced the risk of severe illness or death. Among ancient cultures, the Egyptians and Chinese exposed individual to powders formed from the crusts and scales of pockmarks taken from individuals recovering from smallpox (*Variola major* virus). Sometimes individuals who were treated in this way developed mild forms of the disease; or they developed no apparent disease at all.

Edward Jenner demonstrated in 1794 that intentional inoculation with material from individuals with cowpox (*Variola minor*, a related virus that normally infects cattle, but only causes mild disease in human) protected against smallpox (caused by a more virulent type of *Vaccinia* virus). Jenner and his contemporaries**,** of course, did not know of microbes and their roles in disease.

## Historical overview

- 1870s: although vaccination was taken up eagerly by many, there was some violent opposition as it became more widespread. People found it hard to believe that it really worked. They also felt that it took away people's civil liberties, particularly when it was compulsory.
- 1880s: Louis Pasteur improved vaccination even more, and developed a rabies vaccine. As the science of immunology developed and scientists began to understand more about how diseases worked, other vaccines were created.

- 1890: Emil von Behring was awarded the first Nobel Prize. He discovered the basis of diphtheria and tetanus vaccines by demonstrated that animals injected with small amounts of the tetanus toxin became immune to the disease.
- By the end of the 1920s, vaccines for diphtheria, tetanus, whooping cough and tuberculosis (TB) were all available. Vaccination spread across the globe and although these early vaccines were crude, they worked. The first vaccination programme dramatically reduced the number of deaths from disease, and they were crucial in establishing the concept of preventive public health measures.
- 1955: Polio vaccination was introduced in the UK and it dramatically reduced the number of cases. Nowadays, polio is extremely rare and is close to being completely eliminated from the planet.
- 1956: The first attempt to use the smallpox vaccine on a global scale began when the World Health Organization (WHO) decided to try and eradicate smallpox across the world. Smallpox was declared as being eradicated in 1980. It was one of the most remarkable achievements in the history of medicine.
- 2008: Professor Harald zur Hausen discovered that cervical cancer was caused by a virus, making it possible to develop a vaccine for the disease. The scientist proved that a group of viruses called human papillomaviruses (HPV) caused cervical cancer. This discovery led to the development of the HPV vaccine, which protects against cervical cancer, and is now widely available.
- 2008: In England, the national health service (NHS) cervical cancer vaccination programme began, whereby all 12-13 year-old girls are offered HPV vaccination to protect them against cervical cancer. It is the

first time that a routine universal vaccine has been given to prevent a type of cancer.

- 2013: The NHS vaccination programme saw the introduction of rotavirus vaccination for babies and a shingles vaccine for over 70-year-old. A children's flu vaccine was launched. This is given as a nasal spray rather than an injection.
- 2015: The NHS vaccination programme saw the introduction of Men B vaccination for babies. The programme is the first national, routine, universal and publicly funded Men B vaccination programme in the world.

**Vaccination improves life**

The expanded use of vaccination led to an enormous improvement in human and animal health. For both children and adults, many of the most fearful diseases throughout human history have been practically eliminated in many parts of the world. The ability to vaccinate early in the life has dramatically reduced the burden of illness, crippling, and death that was once a routine part of childhood, resulting from diseases such as diphtheria, polio, and measles.

Childhood vaccination is usually provided as a routine service in maternal-child health clinics or other health facilities. Children should receive the vaccinations they need at the right age during scheduled or drop-in clinic visits. Most countries have a recommended vaccination schedule, that is, the ages at which children should receive each does of various vaccines.

## Terms and definitions

A vaccine is a suspension of whole (live or inactivated) or fractionated bacteria or viruses that have been rendered nonpathogenic, and is given to induce an immune response and prevent disease. Two points are considered in vaccine production 1- **specificity** 2- **memory.**

The term vaccine was derived from “vacca”, meaning cow, since Edward Jenner used cowpox virus (*Vaccinia*) to prevent smallpox infection. Vaccination is aimed to inducing active immunity in an individual. Even though both terms are frequently used interchangeably, the CDC defines them as follows:

**Immunization:** A process by which a person becomes protected against a disease through vaccination.

**Vaccination:** The act of introducing a vaccine into the body to produce immunity to a specific disease. Upon that, subsequent contact with the microorganism following natural infection induces strong protective immune response. The protective immunity may involve secretion of neutralizing antibodies or production of memory cytotoxic lymphocytes or Th1 cells. The use of vaccines is now being extended to immunize against tumors or to block fertilization (contraceptive vaccines).

## Herd immunity

Even though no vaccine is entirely safe or completely effective, their use is strongly supported by their benefit-to-risk ratio. Vaccination can provide excellent protection to a population, even if not every individual in a population is vaccinated, because of a phenomenon known as **herd immunity.** As the fraction of the population that is vaccinated increase, the chance of an infectious agent “finding” an unprotected individual becomes increasingly smaller, leaving the population resistant as a whole.

**Requirements 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**.

**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 does provide a complete immunization regimen.

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

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

**4-** It is heat stable, so that refrigeration ("cold chain") is not required during shipping and storage.

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

**6-** It can safely be administrated simultaneously with other vaccine either as a part of a specific combination vaccine (e.g. measles –mumps-rubella) or as separate individual vaccines.

**7-** adverse effects in vaccine recipients are few.

**8-** The microbe used to prepare the vaccine does not cause disease in recipients who have immune system weakened by 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 disease in vaccinated people or in their close contacts.

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

**Routes of Administration**

a- Subcutaneous or intramuscular route (most vaccines).

b- Oral routs (poliovirus).

c- Intradermal (BCG).

d- Scarification (smallpox).

e- Intranasal (live attenuated influenza vaccine).

**Characteristics of disease suitable for control by Vaccine and immunization:**

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

**2-** Disease is recognizable by health workers, (e.g. causes 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 (e.g., parents, health workers, and policy makers) support preventing its future occurrence.

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

**5-** Disease incubation period is not too short, so that vaccine still provide at least partial protection if given after exposure (e.g. measles vaccine give

early in the 10-14 day incubation period or rabies vaccine given soon after animal bite exposure).

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

**7-** Genetic mutations that results in biochemical changes to the microbe outer coat occur very slowly, if it at all, 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 feel ill or appear ill.

**Scheme of immunization:**

A. Primary vaccination: One dose vaccines (BCG, Variola, measles, Mumps, rubella, Yellow fever).

B. Multiple dose vaccines (polio, DPT, Hepatitis B): Booster vaccine to maintain immunity level after its declines after some time has elapsed.

# Lecture Two

## Vaccine composition

Generally, vaccines have several major components.

a) **Antigen** (active components): it 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 pathogen or toxin that causes the disease; however, the precise nature can vary between vaccines.

b) **Adjuvants**: Adjuvants are chemical compounds added to vaccines to help enhance the body's immune response. These aren't present in all vaccines.

c) **Preservative** (phenol, 2-phenoxyethanol, Thimerosal): 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 from the same rubber-capped vessel.

d) **Additives** confer stabilization of live attenuated virus: Stabilisers 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 stabilisers can be used; sugars (sucrose, lactose), amino acids and proteins (gelatin, Human serum albumin) can all be utilised for this purpose. They also prevent the vaccine components from adhering to any storage vessel. Many of the compounds used as stabilisers are found naturally in the body anyway, and so do not pose any risk.

e) **Buffers**

Buffers serve to resist changes in pH, adjust tonicity and maintain osmolarity. The most commonly used buffer is sodium chloride (table salt).

**f) Surfactants**

Surfactants are a type of emulsifier. They assist particles remain suspended in liquid, preventing settling and clumping, by lowering the surface tension of the liquid. An example is polysorbate 80 (Tween 80®), made from sorbitol (sugar alcohol) and oleic acid (omega-9 fatty acid), which is also used in foods such as ice cream. Surfactants are also used in shampoos, toothpastes, inks and fabric softeners.

**g) Solvents**

A solvent is a substance that dissolves another substance, creating a solution. The most common solvent used in everyday living, and vaccine manufacture, is water.

**h) Manufacturing residual**:

i. Inactivating 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 inactivate viruses, can be detected, but at levels far below that known to cause harm in humans.

ii. 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 reactions, such as penicillins, are avoided.

iii. Cellular residuals (e.g. egg protein, yeast proteins).

**f. Diluents**

Vaccines need to be diluted to their required concentration. Most often, this will be accomplished using either sterile water, or a saline solution.

## Vaccine classification

### 1-Live attenuated vaccines

These vaccines are composed of live, attenuated microorganisms that cause a limited infection in their hosts sufficient to induce an immune response, but insufficient to cause disease (table 1). Because a live, attenuated vaccine is the closest thing to a natural infection, these vaccines are good "teachers" of the immune system.

To make an attenuated vaccine, the pathogen is grown in foreign host such as animals, embryonated eggs or tissue culture, under conditions that make it less virulent. The strains are altered to a non-pathogenic form; for example, its tropism has been altered so that it no longer grows at a site that can cause disease. Some mutants will be selected that have a better ability to grow in the foreign host. These mutants tend to be less virulent for the original host.

The oldest and the most commonly used is the Baccille Calmette Guerin (BCG) vaccine, which was derived from a bovine strain of *Mycobacterium tuberculosis*. The efficacy of this vaccine varies in different population and although routinely given to children in some European countries, BCG is not used in the USA.

The influenza vaccine contains cold-adapted vaccine strains of the influenza virus that have been grown in tissue culture at progressively lower temperatures. After a dozen or more of these passages, the virus grows well only at around 25°C and *in vivo* growth is restricted to the upper respiratory tract.

Table 1: live attenuated vaccine.

| Bacteria/virus | Vaccine | Method | Rout |
|---|---|---|---|
| **Vibrio** | CVD103Hgr | Genetically modified | Oral |
| ***Salmonella*** | Ty21a | Genetically modified | Oral |
| ***Mycobacterium*** | BCG | Prolonged subculture | ID |
| **Polio** | Sabin | Passage in MK cells | Oral |
| **Yellow Fever** | 17D | Passage in chick embryo cells | SC |
| **Influenza** | | Temperature sensitive mutant | IN |
| **Measles, Mumps, Rubella** | MMR | Passage in fibroblasts cells | SC |
| **Rubella** | (Wistar RA 27/3) | Wistar Institute RA 27/3 strain of live attenuated rubella virus | SC |
| **Chickenpox** | Oka/Merck | Human diploid cell cultures | SC |
| **smallpox** | *Vaccinia* virus | naturally avirulent | ID |

**Advantages**

a. Infectious microbes can stimulate the generation of memory cellular as well as humoral immune responses.
b. It can multiply in the host; thereby, fewer quantities are injected to induce protection.
c. A single administration of vaccine often has a high efficacy in producing long-lived immunity. Multiple booster doses may not be required.
d. Whole microbes stimulate response to antigens in their natural conformation. They raise immune response to all protective antigens.

e. Some live vaccines can be given orally; such vaccines induce mucosal immunity and IgA synthesis, which gives more protection at the normal site of entry. Oral preparations are less expensive than giving injections. They can lead to elimination of wild type virus from the community.

**Disadvantages**

a) May very rarely revert to its virulent form and cause disease.
b) Live vaccines cannot be given safely to immunosuppressed individuals.
c) Since they are live and because their activity depends on their viability, proper storage is critical.

**2-Killed /Inactivated vaccines**

These vaccines include organisms that are dead because of treatment with physical or chemical agents (table 2). 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. It must be understood, however, that it might be difficult to guarantee that every organism in a preparation is dead.

**Advantages:**

a) Safe to use and can be given to immunodeficient and pregnant individuals.
b) Cheaper than live attenuated vaccine
c) Storage not as critical as live vaccine

**Disadvantages**

a) Since the microorganisms cannot multiply, a large number are required to stimulate immunity.
b) Periodic boosters must be given to maintain immunity.
c) Only humoral immunity can be induced.

d) Most killed vaccines have to be injected.
e) Presence of some un inactivated microbes can lead to vaccine associated disease.
f) Inactivation such as formaldehyde in the case of the Salk polio may alter immunogenicity.

Table 2: Killed and inactivated vaccines.

| Microorganism | Vaccine | Method | Route |
|---|---|---|---|
| ***Salmonella enterica serovar* Typhi** | TAB | Heat, Phenol, Acetone | SC |
| ***Vibrio cholera*** | | Phenol | SC or ID |
| ***Yersinia pestis*** | Haffkine | Formalin | SC |
| ***Bordetella pertussis*** | | Merthiolate | IM |
| **Poliomyelitis** | Salk | Formalin | IM |
| **Rabies virus** | Semple | Phenol | SC |
| **Influenza virus** | | Formalin | IM |
| **Hepatitis A** | HM175 | Formalin | IM |

**3- Subunit or fractional vaccine**

Subunit vaccines contain purified antigens instead of whole organism. Furthermore, fractional vaccines are either protein-based or polysaccharide-based. Protein-based vaccines include toxoids (inactivated bacterial toxin) and subunit or subvirion products. Most polysaccharide-based vaccines are composed of pure cell wall polysaccharide from bacteria.

Polysaccharide vaccines are a unique type of subunit vaccine composed of long chains of sugar molecules that make up the surface capsule of certain bacteria. Pure polysaccharide vaccines are available for three diseases: pneumococcal, meningococcal, and *S.* Typhi.

The immune response to a pure polysaccharide vaccine is typically T-cell independent, which means that these vaccines are able to stimulate B cells without the assistance of T-helper cells. T-cell-independent antigens,

including polysaccharide vaccines, are not consistently immunogenic in children younger than 2 years of age. Young children do not respond consistently to polysaccharide antigens, probably because of immaturity of the immune system.

Repeated doses of most inactivated protein vaccines cause the antibody titer to go progressively higher, or "boost." This does not occur with polysaccharide antigens; repeat doses of polysaccharide vaccines usually do not cause a booster response. Antibody induced with polysaccharide vaccines has less functional activity than that induced by protein antigens. This is because the predominant antibody produced in response to most polysaccharide vaccines is IgM, and little IgG is produced.

The problems noted above could be overcome through a process called conjugation, in which the polysaccharide is chemically combined with a protein molecule. Conjugation changes the immune response from T-cell independent to T-cell dependent, leading to increased immunogenicity in infants and antibody booster response to multiple doses of vaccine. The first conjugated polysaccharide vaccine was for *Haemophilus influenzae* type b. A conjugate vaccine for pneumococcal disease was licensed in 2000. A meningococcal conjugate vaccine was licensed in 2005.

**Advantages:**

a) Subunit vaccines can be given to people with weakened immune systems.
b) These vaccines appear to give long-lived immunity
c) Since only parts of the virus are used for these vaccines, the risks of reactions are very low.

**Disadvantages:**

a) Less immunogenic than live attenuated vaccines.

b) Several doses must be given for proper life-long immunity.

## 4- Recombinant vaccine

Vaccine antigens may also be produced by genetic engineering technology. These products are sometimes referred to as recombinant vaccines. Recombinant vaccines can be classified into two major categories; Recombinant (protein subunit) vaccines and DNA vaccines.

### Recombinant (protein subunit) vaccines

Hepatitis B, human papillomavirus (HPV), and influenza vaccines are produced by insertion of a segment of the respective viral gene into the gene of a yeast cell or virus. The modified yeast cell or virus produces pure hepatitis B surface antigen, HPV capsid protein, or influenza hemagglutinin when it grows.

Live typhoid vaccine (Ty21a) is *Salmonella enterica* serovar Typhi bacteria that have been genetically modified to not cause illness. An attenuated strain of *S.* Typhi was made by mutagenesis and selection for loss of lipopolysaccharide necessary for pathogenesis. In this strain, an enzyme necessary for lipopolysaccharide synthesis is defect.

Live attenuated influenza vaccine has been engineered to replicate effectively in the mucosa of the nasopharynx but not in the lungs.

### DNA vaccines

These vaccines usually consist of synthetic DNA containing the gene that encodes the disease-agent protein. Usually, the plasmid DNA used as vaccine is propagated in bacteria such as *E. coli* and they are isolated and purified for injection. This "naked" DNA is usually injected intramuscularly or intradermally.

The principle behind a DNA vaccine is that the antigen can be expressed directly by host cells in a way that simulates viral infection and invokes an immune response from the host. DNA immunization technique allows antigen production to occur *in vivo*, bypassing the need to produce and purify protein antigen *in vitro*.

DNA vaccines have been used to express antigens from many different pathogens, such as influenza, HIV, malaria, TB, and leishmaniasis.

**ADVANTAGES**

a) In theory, the process should be extremely safe and protect against side effects of the foreign antigen(s)
b) DNA is relatively inexpensive to produce.
c) Serves as long term protection.
d) Better stability in comparison to traditional vaccines.
e) Storage conditions not critical.

**DISADVANTAGES**

a) Direct delivery of DNA into host cells are not optimal, especially not in large animals
b) Vaccine's DNA could potentially be integrated into the chromosome and may act as oncogens or possibly even turn on or off important genes
c) Long term effects are relatively unknown and not knowing such affects make such a technology dangerous
d) Extended stimulation from the antigen might provoke chronic inflammation or autoantibody production which results in a lot of wasted energy

# Lecture Three

## Vaccine production

The production of a vaccine can be divided into the following steps:

### 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:

a) Viruses are grown on primary cells such as cells from chicken embryos or using fertilised eggs (e.g. influenza vaccine) or cell lines that reproduce repeatedly (e.g. hepatitis A)
b) Bacteria are grown in bioreactors which are devices that use a particular growth medium that optimises the production of the antigen
c) Recombinant proteins derived from the pathogen can be generated either in yeast, bacteria or cell cultures.

### 2. Release and isolation of the antigen

The aim of this second step is to release as much virus or bacteria as possible. To achieve 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 a third step the antigen will need to be purified in order to produce a high purity/quality product. This will be accomplished using different techniques for protein purification.

### 4. Addition of other components

The fourth step may include the addition of an adjuvant. The vaccine is then formulated by adding stabilizers and preservatives. 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 labelled 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 interactions, by using pathogen-associated molecular patterns.

**Stages of vaccine development**

**New candidate vaccine**

A new candidate vaccine is a vaccine that is regarded in national regulations as separate and distinct from other candidate and licensed vaccines. Examples of new candidate vaccines include but are not limited to:

- a vaccine that contains a new antigenic component (that is, one not previously used in a licensed vaccine);

- a vaccine that contains a new adjuvant;
- a vaccine that contains antigen(s) ± adjuvant(s) not previously combined together in a vaccine;
- a vaccine with the same antigenic component(s) ± adjuvant as a licensed vaccine that is produced by a different manufacturer (including situations in which seed lots or bulk antigenic components used to make a licensed vaccine are supplied to other manufacturers for their own vaccine production).

Delivery of a vaccine in a programme such as Expanded Program on Immunization is the end result of years of discovery and development. Only a tiny percentage of candidate vaccines progress to licensing, making the costs of vaccine Research and Development extremely high. This fact also makes it essential to maintain a healthy product portfolio, with a range of vaccines at different stages in the pipeline.

Development of vaccines can be simplified into two broad stages:

1. **Pre-clinical development** is research carried out in lab assays and on animals. It includes:

    a) Identification (discovery) of relevant antigens (e.g. screening)
    b) Creation of the vaccine concept
    c) Evaluation of vaccine efficacy in test tubes and animals
    d) Manufacture of the vaccine to Good Manufacturing Practice standards

2. **Clinical development** is when the vaccine is first tested in humans. It covers four stages over several years, from initial clinical trials in humans (phase I) right through to introduction and beyond (phase IV).

Clinical development is built on rigorous ethical principles of informed consent from volunteers, with an emphasis on vaccine safety as well as efficacy.

## Preliminary trials (Phase I and II)

### Phase I

Clinical trials focus on safety and immunogenicity of a vaccine in about 10–100 healthy volunteers (subjects who have no history of previous exposure to the organism(s) against which the candidate vaccine is intended to protect). In Phase 1, scientists begin to learn how the size of the dose may be related to side effects. If possible at this early stage, scientists also try to learn how effective the vaccine may be. For diseases of poverty this covers trials in European volunteers (phase Ia) and then in populations in Developing Countries (phase Ib). If no serious side effects are found in Phase 1, then phase 2 is next

### Phase II

It involves 100 - 1000 volunteers. This phase looks mainly to assess the efficacy of the vaccine against artificial infection and clinical disease. Also includes studies that may provide additional information on common short-term side effects and how the optimum dose relates to immune response.

In most cases these trials are conducted in subjects who are representative of the intended target population for the vaccine at the time of licensure. For vaccines intended for a broad age range it may not be necessary in all instances to apply an age de-escalation approach (for example, to move from adults to adolescents, then to children aged 6–12 years, followed by younger children, toddlers and finally infants) to sequential trials or to groups within trials. For example, if a vaccine has negligible potential benefit for older children it may be acceptable in some

cases to proceed directly from trials in adults to trials in younger children, including infants and toddlers.

**Pivotal trials (Phase III)**

In this phase 1000 - 10000 of volunteers participate to evaluate efficacy under natural disease conditions. Vaccinated people are compared with people who have received a placebo or another vaccine so researchers can learn more about the test vaccine's safety and effectiveness and identify common side effects. If the vaccine retains safety and efficacy over a defined period, the manufacturer is able to apply to the regulatory authorities for a license to market the product for human use.

**Post licensure: Vaccine Safety Monitoring (Phase IV)**

The final phase IV happens after the vaccine has been licensed and introduced into use. Also called post-marketing surveillance, this stage aims to detect rare adverse effects as well as to assess long term efficacy.

After vaccines are licensed, they are monitored closely as people begin using them. The purpose of monitoring is to watch for adverse events (possible side effects). Monitoring a vaccine after it is licensed helps ensure that the benefits continue to outweigh the risks for people who receive the vaccine. Monitoring is essential for two reasons:

First, even large clinical trials may not be big enough to reveal side effects that do not happen very often. For example, some side effects may only happen in 1 in 100,000 or 1 in 500,000 people.

Second, vaccine trials may not include groups who might have different types of side effects or who might have a higher risk of side effects than the volunteers who got the vaccine during clinical trials. Examples of these groups include people with chronic medical conditions, pregnant women, and older adults.

If a link is found between a possible side effect and a vaccine, public health officials take appropriate action by first weighing the benefits of the vaccine against its risks to determine if recommendations for using the vaccine should change.

The Advisory Committee on Immunization Practices (ACIP), a group of medical and public health experts, carefully reviews all safety and effectiveness data on vaccines as a part of its work to make recommendations for the use of vaccines. The ACIP modifies recommendations, if needed, based on safety monitoring.

**VAERS**

Post licensure monitoring begins with the Vaccine Adverse Event Reporting System (VAERS), a national system used by scientists at FDA and the Centers for Disease Control and Prevention (CDC) to collect reports of adverse events (possible side effects) that happen after vaccination. Health care professionals, vaccine manufacturers, vaccine recipients, and parents or family members of people who have received a vaccine are encouraged to submit reports to VAERS if they experience any adverse events after getting any vaccine.

Scientists monitor VAERS reports to identify adverse events that need to be studied further. All serious reports are reviewed by medical professionals on a daily basis. VAERS data provide medical professionals at CDC and FDA with a signal of a potential adverse event. Experience has shown that VAERS is an excellent tool for detecting potential adverse events. Reports of adverse events that are unexpected, appear to happen more often than expected, or have unusual patterns are followed up with specific studies.

# Lecture Four

VAERS data alone usually cannot be used to answer the question, "Does a certain vaccine cause a certain side effect?" This is mainly because adverse events reported to VAERS may or may not be caused by vaccines. There are reports in VAERS of common conditions that may occur by chance alone that are found shortly after vaccination. Investigation may find no medical link between vaccination and these conditions.

To know if a vaccine causes a side effect, scientists must know whether the adverse event is occurring after vaccination with a particular vaccine more often than would be expected without vaccination. They also need to consider whether the association between the vaccine and the adverse event is consistent with existing medical knowledge about how vaccines work in the body.

**VSD**

Scientists use CDC's Vaccine Safety Datalink (VSD) to do studies that help determine if possible side effects identified using VAERS are actually related to vaccination. VSD is a network of eight managed care organizations across the United States. The combined population of these organizations is more than 9.2 million people.

Scientists can use VSD in two ways. First, scientists can look back in medical records to see if a particular adverse event is more common among people who have received a particular vaccine. Second, instead of looking back, scientists can use Rapid Cycle Analysis (RCA) to continuously look at information coming into VSD to see if the rate of certain health conditions is higher among vaccinated people. This second approach is new, and it allows results to be obtained much more quickly.

## Schedule of vaccination in Iraq

| Time of Vaccination | Vaccines |
|---|---|
| **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) |
| **4-6 years** | DTP, OPV (booster no.2) and MMR2 |

Administration of an OPV dose at birth (zero dose) serves as a 'priming dose' since it is not protective to the vaccine (*i.e.* it fails to induce protective levels of neutralizing antibodies owing to interfering maternal antibodies and secretary IgA in breast milk) but still manage to produce enough memory B cells that can be boosted to have improved serologic responses to future doses.

## Bacille Calmette-Guérin (BCG) vaccine:

The live attenuated strain of *Mycobacterium bovis* known as bacillus Calmette-Guérin (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 artificial medium for years.

BCG prevents dissemination of the bacterium or the development of other life-threatening complications such as meningitis. It is effective at reducing morbidity and mortality in children but is less useful in the prevention of adult respiratory disease (studies of the effectiveness of BCG

vaccine range from no protection to 70-80% protection. However, the vaccine is 70-80% effective against the most severe forms of the disease, such as TB meningitis in children. It is less effective in preventing respiratory disease, which is the more common form in adults).

**Route of administration**:

- BCG is given as a single intradermal 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 within 2 weeks.
- The lesion progresses to a papule or shallow ulcer of approximately 10 mm diameter and heals 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. Keloids; large, raised and ugly scars. The insertion of deltoid is most frequently used because the local complication rate is smallest when that site is used.
3. If BCG is accidentally given to an immunocompromised patient, it can cause disseminated or life threatening infection.

**Polio vaccines**

Polio, or poliomyelitis, is a crippling and potentially deadly disease. It is caused by the poliovirus. The virus spreads from person to person and can invade an infected person's brain and spinal cord, causing paralysis.

Two types are used: an inactivated poliovirus given by injection (IPV) developed by Jonas Salk and a weakened poliovirus given orally (OPV) developed by Albert Sabin.

The two vaccines have eradicated polio from most of the countries in the world and reduced the worldwide incidence from an estimated 350,000 cases in 1988 to less than 2000 cases in 2008 and to 2 in 2017.

**Inactivated Polio Vaccine**

Based on polio grown in a type of monkey kidney tissue culture, which is then inactivated with formalin. It contains three serotypes of vaccine virus.

The injected Salk's vaccine confers IgG-mediated immunity in the bloodstream, which prevents Polio infection from progress to viremia and protects the motor neurons, thus eliminating the risk of bulbar polio and post-polio syndrome.

It offers no protection to the mucosal lining of the intestine. 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.

**Oral live-attenuated vaccine**

Sabin's "Oral Polio Vaccine" is a live-attenuated vaccine contains 3 serotypes of vaccine virus. It replicates very efficiently in the gut, the primary site of infection and replication. Unable to replicate efficiently within nervous system tissue. Shed in stool for up to 6 weeks following vaccination.

The OPV proved to be superior in administration, and also provided longer lasting immunity than the Salk vaccine.

The trivalent Oral Polio Vaccine (Sabin) on very rare occasions has been associated with paralysis (vaccine-associated paralytic poliomyelitis, about 1 case per 750,000 vaccine recipients).

**DPT vaccine**

DPT: mixture of three vaccines, to immunize against Diphtheria, Pertussis, and Tetanus.

**Diphtheria**

Diphtheria caused by aerobic Gram-positive bacillus; *Corynebacterium diphtheriae*. Complications most attributable to toxin. Most common complications are myocarditis and neuritis, death occurs in 5%-10% for respiratory disease.

**Pertussis**

It is a highly contagious respiratory infection caused by *Bordetella pertussis.* Several complications are common; Pneumonia, Seizures, Encephalopathy.

**Tetanus**

It caused by anaerobic Gram-positive spore-forming bacteria; *Clostridium tetani*. Tetanus complications include Laryngospasm, Aspiration pneumonia and Death.

Minor reactions are quite frequent in 20–50% of vaccines. Local reactions include: Inflammation, induration or a painless nodule at the site of injection. These are progressively more common after the first injection. Moderate reaction occurs in 0.1% to 1.0% of children and include:

- ✓ ongoing crying (for three hours or more in the first 12 hours),
- ✓ a high fever (up to 40°C), and
- ✓ an unusual (screaming), high-pitched crying.

Severe problems happen very rarely (1 in 140,000 doses of DPT). Include;

- ✓ a serious allergic reaction,
- ✓ prolonged seizures,
- ✓ encephalopathy, or even death.

**MMR vaccine**

It composed of three live attenuated vaccines (Measles, Mumps & Rubella).

**Measles;** Caused by Paramyxoviridae (RNA). Complication: Diarrhea, Otitis media, Pneumonia, Encephalitis.

**Mumps;** caused by Paramyxoviridae (RNA). Complication: CNS involvement, Orchitis, Pancreatitis, Deafness.

**Rubella;** Caused by Togaviridae (RNA). Complication in children; rare; arthralgia or arthritis, thrombocytopenic purpura, Encephalitis, Neuritis, Orchitis. 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, Cataracts, Heart defects, Microcephaly, Mental retardation, Liver and spleen damage.

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% to 15% of recipients.
- ✓ Transient arthritis has been reported.
- ✓ Thrombocytopenia (rare).
- ✓ Encephalopathy (very rare).

A general rule of thumb is the "rule of 10" about 10% of children get a rash approximately 10 days after vaccine administration.

**Contraindications and Precautions**

1. Severe allergic reaction to vaccine component or following prior dose.
2. Pregnancy.
3. Immunosuppression
4. Moderate or severe acute illness.
5. Recent blood product.

**Hepatitis B vaccine**

Hepatitis B infection: Caused by Hepadnaviridae family (DNA). Hepatitis B vaccine consists of purified HBsAg particles produced through recombinant DNA technology in yeast. Vaccine usually is given intramuscularly as a three-dose series. Three doses induce seroconversion in 90-95% of healthy infants, children and adults.

**Rotavirus vaccine**

- In early childhood, the single most important cause of severe dehydrating diarrhea is rotavirus infection.
- Rotaviruses; Reoviridae family.
- The Pentavalent vaccine protects against rotavirus gastroenteritis.

- Oral route.
- Three doses; 2, 4, and 6 months.

**Haemophilus influenzae type b vaccine**

Type of vaccine: Conjugate.
Number of doses: three doses.
Adverse reactions: Mild local reaction
Injection site: Outer mid-thigh for infants.
Injection type: Intramuscular.
Given as quadruple or pentavalent vaccine.

***Haemophilus influenzae* type b**

*Haemophilus influenzae* is a Gram-negative coccobacillus. It is generally aerobic but can grow as a facultative anaerobe. It causes severe pneumonia, meningitis and other invasive diseases. 15% to 30% of children who survive Hib meningitis may develop permanent neurological disability, including brain damage, hearing loss, and mental retardation. 5% to 10% cases of Hib meningitis are at risk of dying.

**Vaccines Require More Than One Dose**

There are four reasons that babies—and even teens or adults for that matter—who receive a vaccine for the first time may need more than one dose:

• For some vaccines (primarily inactivated vaccines), the first dose does not provide as much immunity as possible. So, more than one dose is needed to

build more complete immunity. The vaccine that protects against the bacteria Hib, which causes meningitis, is a good example.

• In other cases, such as the DTaP vaccine, which protects against diphtheria, tetanus, and pertussis, the initial series of four shots that children receive as part of their infant immunizations helps them build immunity. After a while, however, that immunity begins to wear off. At that point, a “booster ” dose is needed to bring immunity levels back up. This booster dose is needed at 4 years through 6 years old for DTaP. Another booster against these diseases is needed at 11 years or 12 years of age. This booster for older children—and teens and adults, too—is called Tdap.

• For some vaccines (primarily live vaccines), studies have shown that more than one dose is needed for everyone to develop the best immune response. For example, after one dose of the MMR vaccine, some people may not develop enough antibodies to fight off infection. The second dose helps make sure that almost everyone is protected.

• Finally, in the case of the flu vaccine, adults and children (older than 6 months) need to get a dose every year. Children 6 months through 8 years old who have never gotten the flu vaccine in the past or have only gotten one dose in past years need two doses the first year they are vaccinated against flu for best protection. Then, annual flu shots are needed because the disease-causing viruses may be different from year to year. Every year, the flu vaccine is designed to prevent the specific viruses that experts predict will be circulating.

**Vaccinations delivery**

Childhood vaccination is usually provided as a routine service in maternal-child health clinics or other health facilities. Children should

receive the vaccinations they need at the right age during scheduled or drop-in clinic visits. Most countries have a recommended vaccination schedule, that is, the ages at which children should receive each does of various vaccines.

Vaccinations can also be delivered in campaigns where vaccine teams try to vaccinate all eligible persons within a few days or weeks in the targeted area. Such campaigns sometimes deliver only one vaccine, but sometimes include other vaccinations and/or other preventive health interventions, such as vitamin A supplementation. These campaigns usually send health workers to the community to vaccinate children at local places, such as school, churches, or mosques, and do not rely on parents to bring their children to a health facility.

Vaccinations in emergencies are often delivered both ways, in routine clinic visits as part of primary health care and in campaigns meant to rapidly provide maximum protection to the population. In most emergencies, the vaccine-preventable disease of greatest concern is measles. Large outbreaks of measles have occurred in displaced populations. Such outbreaks can have a very high case-fatality rate, as high as 10-20%, in malnourished populations of children.

# Lecture Five

## Immunotherapy definition and its application

**Immunotherapy** is the "treatment of disease by inducing, enhancing, or suppressing an immune response". Immunotherapies designed to elicit or amplify an immune response are classified **as activation immunotherapies**, while immunotherapies that reduce or suppress are classified as **suppression immunotherapies.**

Immunotherapy, also called **biologic therapy**, is a type of cancer treatment designed to boost the body's natural defenses to fight the cancer. It uses materials either made by the body or in a laboratory to improve, target, or restore immune system function. It is not entirely clear how immunotherapy treats cancer. However, it may work in the following ways:

- Stopping or slowing the growth of cancer cells.
- Stopping cancer from spreading to other parts of the body.
- Helping the immune system work better at destroying cancer cells.

Immunotherapy includes:

### a- Immunomodulators

The active agents of immunotherapy are collectively called **immunomodulators** (table 6). They are a diverse array of recombinant, synthetic and natural preparations, often cytokines. Some of these substances, such as granulocyte colony-stimulating factor (G-CSF), interferons, imiquimod and cellular membrane fractions from bacteria are already licensed for use in patients. Others including IL-2, IL-7, IL-12, various chemokines, synthetic cytosine phosphate-guanosine (CpG) oligodeoxynucleotides and glucans are currently being investigated

extensively in clinical and preclinical studies. Immunomodulatory regimens offer an attractive approach as they often have fewer side effects than existing drugs, including less potential for creating resistance in microbial diseases.

Table 6: Immunomodulators.

| Agent | Example |
|---|---|
| Interleukins | IL-2, IL-7, IL-12 |
| Cytokines | Interferons, G-CSF, Imiquimod |
| Chemokines | CCL3, CCL26, CXCL7 |
| Other | cytosine phosphate-guanosine, oligodeoxynucleotides, glucans |

**Cell-based immunotherapies**

**Cell-based immunotherapies** are proven to be effective for some cancers. Immune effector cells such as lymphocytes, macrophages, dendritic cells, natural killer cells (NK Cell), cytotoxic T lymphocytes (CTL), etc., work together to defend the body against cancer by targeting abnormal antigens expressed on the surface of the tumor due to mutation.

**b- Activation immunotherapies**

Activation immunotherapies mostly used to treated cancer. Cancer immunotherapy attempts to stimulate the immune system to reject and destroy tumors. Immuno cell therapy for cancer was first introduced by Rosenberg and his colleagues of the National Institutes of Health in the United States. In the late 1980s, they published an article in which they reported a low tumor regression rate (2.6–3.3%) in 1205 patients

with metastatic cancer who underwent different types of active specific immunotherapy (ASI), and suggested that immuno cell therapy along with specific chemotherapy is the future of cancer immunotherapy. Initially Immunotherapy treatments involved administration of **cytokines** such as Interleukin. Thereafter the adverse effects of such intravenously administered cytokines lead to the extraction of the lymphocytes from the blood and expanding *in vitro* against tumour antigen before injecting the cells with appropriate stimulatory cytokines. The cells will then specifically target and destroy the tumor expressing antigen against which they have been raised.

**BCG** immunotherapy for early stage (non-invasive) bladder cancer utilizes instillation of attenuated live bacteria into the bladder, and is effective in preventing recurrence in up to two thirds of cases. **Topical immunotherapy** utilizes an immune enhancement cream (imiquimod) which is an interferon producer causing the patient's own killer T cells to destroy warts, actinic keratoses, basal cell cancer, vaginal intraepithelial neoplasia, squamous cell cancer, cutaneous lymphoma, and superficial malignant melanoma. **Injection immunotherapy** ("intralesional" or "intratumoral") uses mumps, candida, the HPVvaccine or trichophytin antigen injections to treat warts (HPV induced tumors). Lung cancer has been demonstrated to potentially respond to immunotherapy.

### 1- Autologous dendritic cell-based immunotherapy

Dendritic cells can be stimulated to activate a cytotoxic response towards an antigen. Dendritic cells, a type of antigen presenting cell, are harvested from a patient. These cells are then either pulsed with an antigen or transfected with a viral vector. Upon transfusion back into the patient these

activated cells present tumour antigen to effector lymphocytes (CD4+ T cells, CD8+ T cells, and B cells). This initiates a cytotoxic response to occur against cells expressing tumour antigens (against which the adaptive response has now been primed). The cancer vaccine Sipuleucel-T is one example of this approach.

### 2- T-cell adoptive transfer

Adoptive cell transfer uses T cell-based cytotoxic responses to attack cancer cells. T cells that have a natural or genetically engineered reactivity to a patient's cancer are generated *in vitro* and then transferred back into the cancer patient. One study using autologous tumor-infiltrating lymphocytes was an effective treatment for patients with metastatic melanoma. This can be achieved by taking T cells that are found with the tumor of the patient, which are trained to attack the cancerous cells. These T cells, referred to as tumor-infiltrating lymphocytes (TIL), are then encouraged to multiply *in vitro* using high concentrations of IL-2, anti-CD3 and allo-reactive feeder cells. These T cells are then transferred back into the patient along with exogenous administration of IL-2 to further boost their anti-cancer activity.

Thus far, a 51% objective response rate has been observed; and in some patients, tumors shrank to undetectable size.

## Autologous immune enhancement therapy

The Autologous immune enhancement therapy (AIET) is an autologous immune cell-based therapy wherein the patient's own peripheral blood-derived NK cells, Cytotoxic T Lymphocytes and other relevant immune cells are expanded *in vitro* and then reinfused to attack cancer. There are also studies proving their efficacy against Hepatitis C Viral infection, Chronic fatigue Syndrome and HHV6 infection, include:

# Lecture Six

**1- Genetically engineered T cells**

Genetically engineered T cells are created by infecting patient's cells with a virus that contain a copy of a T cell receptor (TCR) gene that is specialised to recognise tumour antigens. The virus is not able to reproduce within the cell however integrates into the human genome. This is beneficial as new TCR gene remains stable in the T-cell. A patient's own T cells are exposed to these viruses and then expanded non-specifically or stimulated using the genetically engineered TCR. The cells are then transferred back into the patient and ready to have an immune response against the tumor (aggressive melanomas, advanced skin cancer).

**2- Immune recovery**

The potential use of immunotherapy is to restore the immune system of patients with immune deficiencies as result of infection or chemotherapy. For example, cytokines have been tested in clinical trials. Interleukin-7 has been in clinical trials for HIV and cancer patients. Interleukin-2 has also been tested in HIV patients.

**3- Vaccination**

Anti-microbial immunotherapy, which includes vaccination, involves activating the immune system to respond to an infectious agent.

a- **Suppression immunotherapies**

Immune suppression dampens an abnormal immune response in autoimmune diseases or reduces a normal immune response to prevent rejection of transplanted organs or cells, include:

**1- Immunosuppressive drugs**

Immunosuppressive drugs are important tools in the management of organ transplantation and autoimmune disease. Immune responses depend on lymphocyte proliferation, and cytostatic drugs are immunosuppressive. Glucocorticoids are somewhat more specific inhibitors of lymphocyte activation, whereas inhibitors of immunophilins more specifically target T lymphocyte activation. Immunosuppressive antibodies target an increasingly broad array of steps in the immune response, and there are still other drugs that modulate immune responses.

**2- Immune tolerance**

Immune tolerance is the process by which the body naturally does not launch an immune system attack on its own tissues. An immune tolerance therapy seeks to reset the immune system so that the body stops mistakenly attacking its own organs or cells in autoimmune disease or accepts foreign tissue in organ transplantation. A brief treatment should then reduce or eliminate the need for lifelong immunosuppression and the chances of attendant side effects, in the case of transplantation, or preserve the body's own function, at least in part, in cases of type 1 diabetes or other autoimmune disorders.

**3- Allergies**

Immunotherapy is also used to treat allergies. While other allergy treatments (such as antihistamines or corticosteroids) treat only the symptoms of allergic disease, immunotherapy is the only available treatment

that can modify the natural course of the allergic disease, by reducing sensitivity to allergens.

A one-to-five-year individually tailored regimen of injections may result in long-term benefits. Immunotherapy does not work for everyone and is only partly effective in some people, but it offers allergy sufferers the chance to eventually reduce or stop symptomatic/rescue medication.

The therapy is indicated for people who are extremely allergic or who cannot avoid specific allergens. For example, they may not be able to live a normal life and completely avoid pollen, dust mites, mold spores, pet dander, insect venom, and certain other common triggers of allergic reactions. Immunotherapy is generally not indicated for food or medicinal allergies. This therapy is particularly useful for people with allergic rhinitis or asthma.

The therapy is particularly likely to be successful if it begins early in life or soon after the allergy develops for the first time. Immunotherapy involves a series of injections (shots) given regularly for several years. The first shots contain very tiny amounts of the allergen or antigen to which one is allergic. With progressively increasing dosages over time, one's body adjusts to the allergen and becomes less sensitive to it, in a process known as desensitization.

## 4- Helminthic therapies

**Helminthic therapy**, an experimental type of immunotherapy, is the treatment of autoimmune diseases and immune disorders by means of deliberate infestation with a helminth or with the eggs of a helminth. Helminths are parasitic worms such as hookworms, whipworms, and threadworms that have evolved to live within a host organism on which they

rely for nutrients (side effect) and these organisms must be classified as immuno-therapeutic agents.

Helminthic therapy is being investigated as a potentially highly effective treatment for the symptoms and or disease process in disorders such as relapsing remitting multiple sclerosis, Crohn's, allergies and asthma.

The precise mechanism of how the helminths modulate the immune response, is currently unknown. However, several broad mechanisms have been postulated, such as a **re-polarisation of the Th1 / Th2 response**, **and modulation of dendritic cell function.** The helminths down regulate the pro-inflammatory Th1 cytokines, Interleukin-12 (IL-12), Interferon-Gamma (IFN-γ) and Tumour Necrosis Factor-Alpha (TNF-ά), while promoting the production of regulatory Th2 cytokines such as IL-10, IL-4, IL-5 and IL-13.

# Lecture Seven

## Principles of Passive Immunization

General principles

The basic principle of passive immunization is **the injection of antibodies from an immunize host into a nonimmune host** to achieve a desired prophylactic or therapeutic effect.

The uses of antibodies preparations in immunotherapy are shown in fig.1 and include such antimicrobial activities as toxin neutralization, viral neutralization, and antibacterial effects due to lyses or opsonization and phagocytes. In addition, immunosuppressive activity with antibody preparations has been successful in the prevention of maternal RH0 and in immunosuppression during tissue transplantation. Today, in most cases, passive immunization is achieved with immunoglobulin derived from pooled human plasma. In industrialized countries, vertually all such material is derived from human plasma or serum. In the other parts of the world where the cost of such material is prohibitive or where the facilities for their production are not yet available, globulins or sera from animal sources, mostly equine, are used **Antibodies of human origin are Preferable because this proteins do not elicit an immune response** that could have an adverse effect,e.g. serum sickness, as in following the use of gamma globulins of animal origin.

### Immediacy of action

The most important reason for the use of passive **immunization is its immediacy of action** -- the ability of preformed antibody to exert its effect immediately on interaction with an antigen. The delay of the **latent period required by active immunization response is thereby avoided. The obvious advantage** therefor, is the passive immunization procedures can be used in **emergency** situations when there insufficient time to achieve an active immune response or when vaccine **is unavailable**. in general, the efficacy of passive immunization is e related to the length of time between exposure to the pathogen and administration of the an antibody, i.e., the shorter the interval, the greater of prevention of the disease or its successful teratment.in some instance, the antibody may given prior to exposure as use gg for prevention of Hep.A in individual traveling to high-risk areas. Passive immunization has important advantages for individual who have primary or acquired (secondary) deficiency of antibody synthesis.

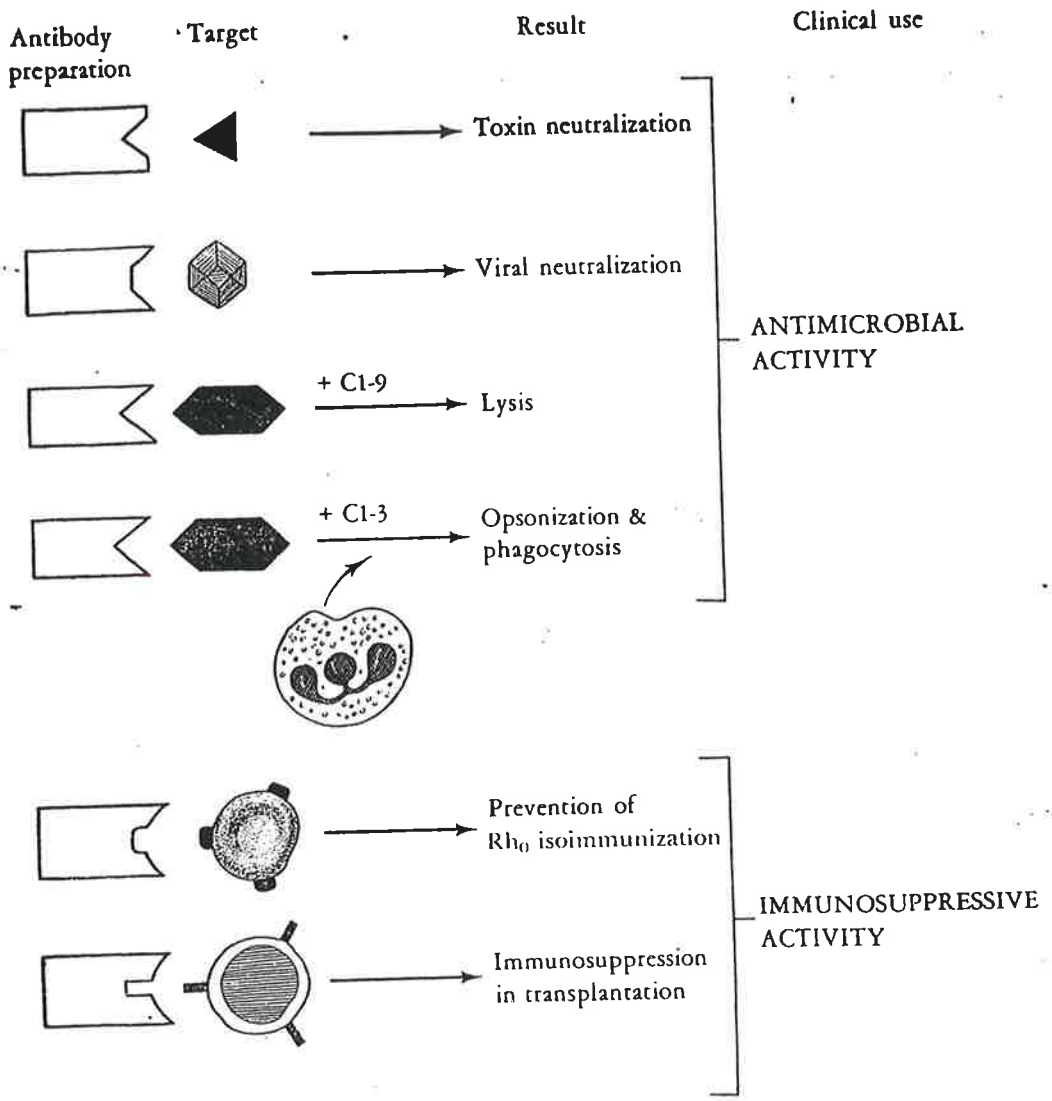



**Figure 24–2.** Schematic representation of the uses of antibody preparations in immunotherapy.

## Metabolism of Gamma Globulin

Antibodies like all other protein molecules have a limited biologic half-life. There is continual loss and replacement of these molecules in dynamic state that is referred to as turnover. For example if human (homologous) IgG immunoglobulin is administrated to a healthy human, the biological half-life (t1/2) will be 20-30 days. More rapid rates of degradation are observed with other isotype, e.g., IgA t1/2= 4-8 days and IgM t1/2 = 2-4 days. The half-life of horse (heterologous) immunoglobulin administrated to human is considerably shorter. The recognition of horse gg by human results in active immune response leading to antibody- mediated enhanced catabolism or immune elimination.

Fig.2.for Ig of animal origin. This degradation process may vary. Antitoxins of equine origin, such as diphtheria or tetanus globulins, have treated with pepsin to remove as much of fc fragment as possible. The pepsin –digested antibodies are catabolised quite rapidly, with an approximate half-life of 2-8 days. The duration of protection is finite and determined by both the amount and the type of Ig. the pattern of elimination of gg (Ab) administrated intravenously occurs in 3 phases fig -2 **phase 1** is a period **of redistribution or equalization** between vascular and extravascular spaces that result in a striking drop in peak titer shortly after administration of antibody. **Phase 2** is **a slower, stady drop in serum levels** due to metabolic (catabolic) half-life of the gg. These diminutions in serum level apply to both homologous & heterologous gg.**phase 3** is an **accelerated period** of degradation, ocurring only in the case of heterologous gg.that takes place simultaneously with the development of Ab of the foreign gg. This third phase is referred to as **immune elimination**. These metabolic properties of gg also have relevance to the passive immunization that occurs as natural event in every human –the transplacental transfer of homologous IgG Ig from mother to fetus. There is a gradual degradation and diminished concentration of IgG following birth.



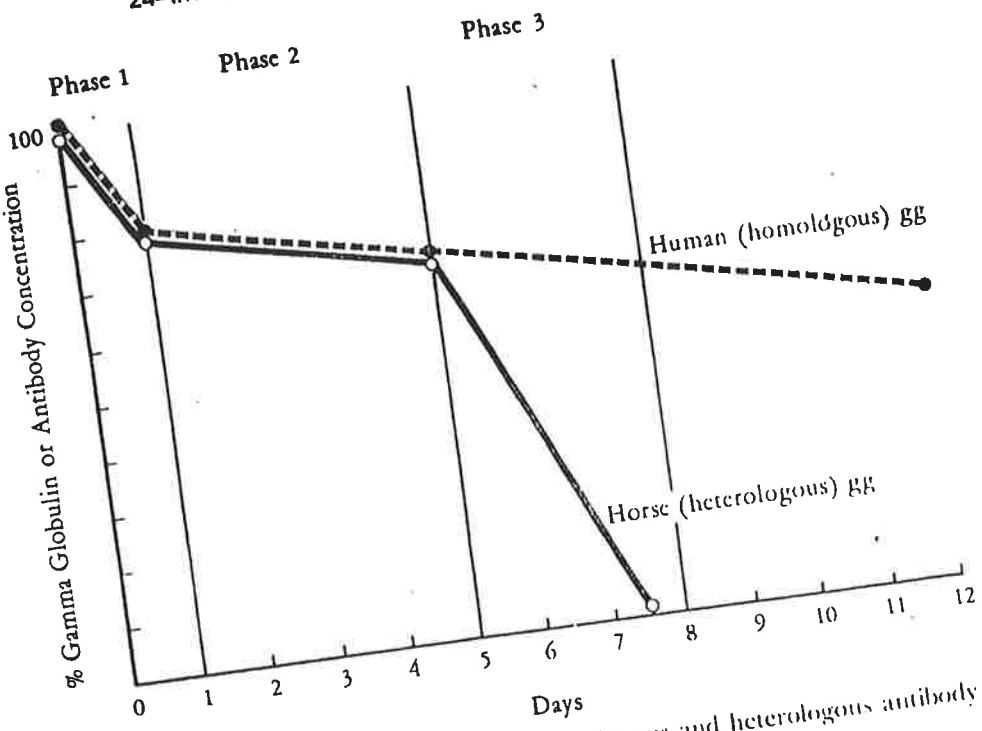



**Figure 24–3.** Schematic comparison of the catabolism of homologous and heterologous antibody (gg) in the human.

## Variability of Preparation

A third principle governing the use of passive immunization is the great effectiveness seen with different preparations of gamma globulin .some time high effective for some diseases, particularly when antibody is used to neutralize the effect of extracellular e.g. tetanus toxin, or in certain viral disease e.g. measles other instances ,the effectiveness of immunotherapy is less than optimal, but its use continues nevertheless. For example, the use of immunotherapy in hepatitis A dose not necessarily prevents disease, but it may convert a clinical disease into a subclinical one. Other situation the value of passive immunization is uncertain, higly questionable, and often controversial for example administering gamma globulin to a pregnant female exposed to rubella in the first trimester may prevent clinical rubella in the mother, but a subclinical disease may occur. This may followed by the full-blown congenital rubella syndrome in the infant. The **following factors affecting the efficacy (action) of gamma globulin preparation used in immunotherapy** 1-the time of administration of preparation.optimally, an antibody preparation should be given immediately after exposure to an infectious agent 2-the differences in pathogenesis of the various disease entities. Those infectious agents that have a blood –borne phase will be more effectively neutralized by the use of gamma globulin than those that localized.3- the content of specific antibody in any given preparation. Because of variations in antibody content in serum and to insure an adequate level of antibody, gamma globulin preparations ere optimized preferably from hyperimmune sera.

## Inhibition of Primary immune response

The fourth important principle is the application of the immune therapy is the suppression of the immune response. The passive administration of specific antibody will inhibit active production of antibody by means of negative feedback inhibition.

## Type of preparations

### Immune serum globulin (ISG)

Immune serum globulin (human), also called gamma globulin, is derived from the blood, serum, or plasma, or serum of human donors and contains most of the antibody found in whole blood. The amounts of specific antibody vary in different preparations. The ISG preparation (usually derived from placental blood) contains a concentration of antibodies approximately 25 times that found in blood. Final concentration of the preparation contains 165 mg of gg /ml each lot of immune serum globulin represent a pooling of not fewer than 1000donors,which provides a wide spectrum of antibody but also increase the risk os sensitization after prolonged usage. These preparations contain primarily IgG Ig with lesser amounts of IgM which important in bacterial defense. Although there are IgA Ig in commercial gg, these proteins are poorly transmitted to mucosal surface site where the secretory IgA globulins normally provide defense. The **advantages** of gamma globulin preparation s over whole serum are 1-gg is free from hep.A virus 2- its concentrated. Perrmitting the administration of large amount p of Ab in a small amount volume 3- its stable during long- term storage. There are **disadvantages** to the use of concentrated ISG

# Lecture Eight

preparations .all preparations have **tendency to aggregates** into large [redacted]S polymer. These aggregates account for the occasional anaphylactic reactions seen following the prolonged usage. The frequency of these reactions is far too high for ISG to be administrated via the intravenous rout; therefore, since each lot represents a pool of many donors, the sensitization is increased with continual usage. The intact preparation should not be given intravenously. The value of ISG has been demonstrated clearly in only three situations; 1- prevention of hep.A 2- prevention of rubella 3-replacement therapy in congenital agammaglobulinemia of the Burton type. Although it has not been proved, ISG may be useful in prevention three other infectious disease 1- rubella in the first trimester pregnancy 2- varicella in exposed patients on immune suppressive therapy. 3-post transfusion hepatitis. One reason for the lack of effectiveness in these latter diseases may be the lack of sufficient specific antibody in ordinary ISG preparations. Recently, gamma globulin preparations for intravenous use have been developed. These material are prepared by enzymatic or chemical treatment of intact gamma globulin in order them suitable for intravenous use .although the inductions for the use of these preparations are essentially similar to those of intramuscular ISG preparations, they have the advantages of the obviating the local reactions that follow intramuscular injection in the older child or adult.

**Specific immune Serum Globulin (SIG)**

Specific immune serum globulins (SIG) are prepared from the sera of convalescent those who are hyperimmunized to given material. Since such preparations contain a higher content of specific antibody to the agent in question than that found in ISG they are preferable to the letter.

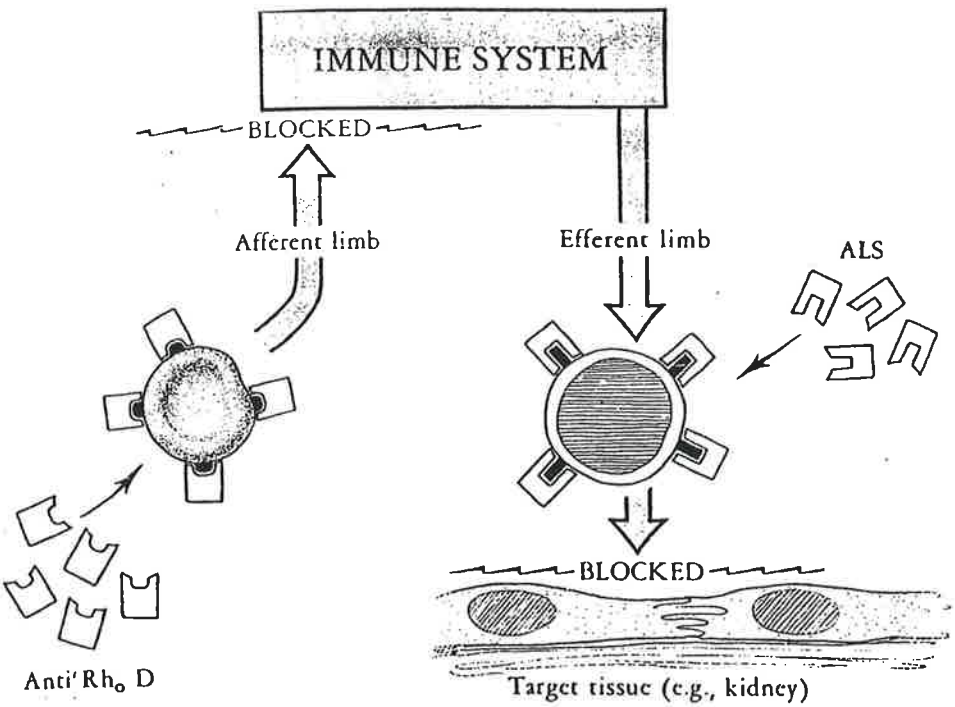



**Figure 24–4.** Schematic representation of the modes of immunosuppression by specific anti-Rh$_o$ antibody and by antilymphocyte sera (ALS).

**Therapeutic Immune sera and antitoxin**

The antisera and antitoxins which are produced in animal should be used only in clinical situations in which human sources of immune globulin are not available, because they present the problem of possible hypersensitivity reaction (serum sickness).

RH0 immune globulin

This material consist of IgG-anti-RH0 (D) prepared from pooled human sera this preparation has been used in the prevention of RH0 isosensitization.

Antilymphocyte sera (horse)

This material is produced by active immunization of horses with human thymocytes for use in immunosuppression in transplantation. although its a licensed product, the use of this material in humans is confined primarily to the pretreatment of transplant recipients .

Uses of Passive immunization

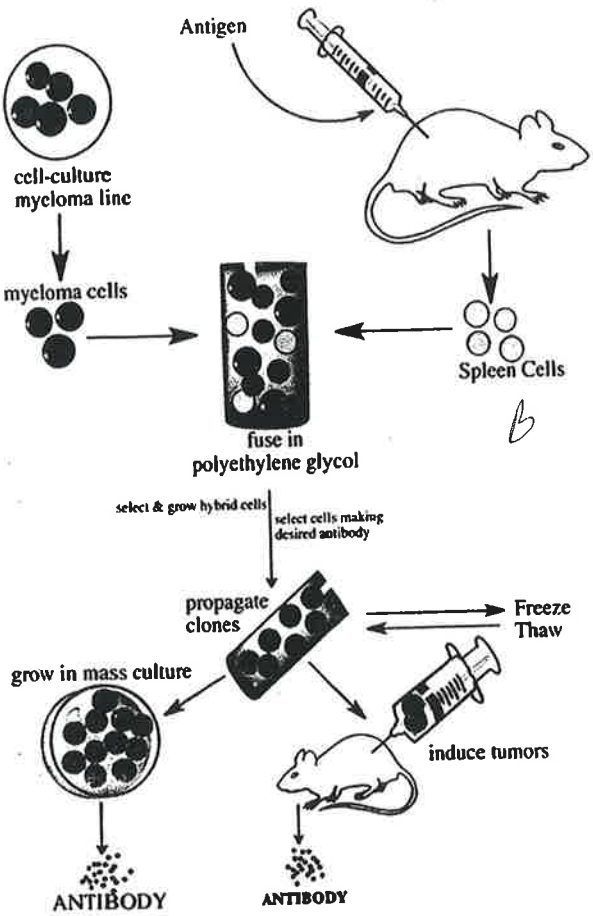
The classical use of passive immunization has been used in prevention or treatment of infectious disease, including those associated with bacteria or their products (toxins), viruses, and certain protozoa (malaria).the new applications of passive immunization form tow major categories: replacement therapy and suppression of the primary immune response. replacement therapy is used in cases in which gg is congenitally deficient,e.g. agammaglobulinemia,or in which there is an absence of specific antibody, e.g. unimmunized individuals. The use of passive immunization in suppressing the immune response includes the prevention of Rh0 isoimmunization and immunosuppression in tissue transplantation.

# ((---Monoclonal Antibody Production---))

The importance of antibodies is well documented, but any antibody that is prepared or isolated by conventional methods is a group of different antibodies that target various agents, known as polyclonal antibodies.

In 1975, Köhler and Milstein were able to devise a method to isolate **monoclonal antibodies** that would target only one antigen.

It is based on injecting an animal with the antigen, collecting the B-lymphocytes from the serum or spleen (would give rise to polyclonal antibodies). Fusing the isolated B-lymphocytes with a cancerous Melanoma cell then creates a hybridoma. This hybrid cell can be cultured and cloned. Each of these daughter clones will secrete a single specific antibody (Monoclonal Antibodies).




One limitation for Mab is a the risk of an immune reaction to mouse proteins that may result in destroying the antibodies. This can be addressed by using **Chimeric** [using the human constant region] or **humanized** [using the constant plus some of the variable region] antibodies. This is achieved via splicing the mouse genes for the highly specific antigen-recognizing portion of the antibody and combining it with the human genes that encode the rest of the antibody

## Passive transfer of cell-mediated immunity

Passive or "adoptive transfer" of cell-mediated immunity, is conferred by the transfer of "sensitized" or activated T-cells from one individual into another. It is rarely used in humans because it requires histocompatible (matched) donors, which are often difficult to find. In unmatched donors this type of transfer carries severe risks of graft versus host disease.[7] It has, however, been used to treat certain diseases including some types of cancer and immunodeficiency. This type of transfer differs from a bone marrow transplant, in which (undifferentiated) hematopoietic stem cells are transferred

## The hazards of immunoglobulin therapy

## ((~The hazards of Immunoglobulin therapy ...)) ز

The majority of side effects are mild, transient, and self-limited and do not require discontinuation of therapy. The most common side effects are[1]:

- Headache , fever, chills, chest pain, nausea or/and vomiting.

The clinical reaction to gamma globulin could be

| Reaction | mechanisms |
|---|---|
| Cardiovascular shock, fever | Aggregates |
| Lose antibody activity<br>Sensitization | Fragmentation<br>sensitization due to<br>1- anti-Gm determinant<br>2-antibody to aggregates<br>3- anti-IgA antibody |
| Development of anti-A and Anti-B | A and B contamination of placental sources |

Standard gg cannot given intravenously, since sever pyrogenic and cardiovascular reaction have occurred that are believed to be related to the presence of aggregates in the preparation. The reactions are believed to be nonimmunogenic in nature and can occur in individuals' receiving intravenous gg for first time. The use of plasmin digests of gg has been shown to be clinically effective, and plasmin digest Appear to be eliminated the problem of aggregates these preparations are still biologically active and can given intravenously.

In addition to this type of reaction, the repeated use of gg can lead to sensitization in normal persons and even in patients with various types of immunogenic deficiencies. At least three types of sensitization have been described previous.

# Lecture Nine

# New Strategies for Vaccine Preparation

## Alternative approaches for vaccines production :

1- Recombinant viral antigen subunit vaccines.

2- Synthetic peptides.

3- Recombinant whole virus vaccines.

4- Anti – idiotype antibodies.

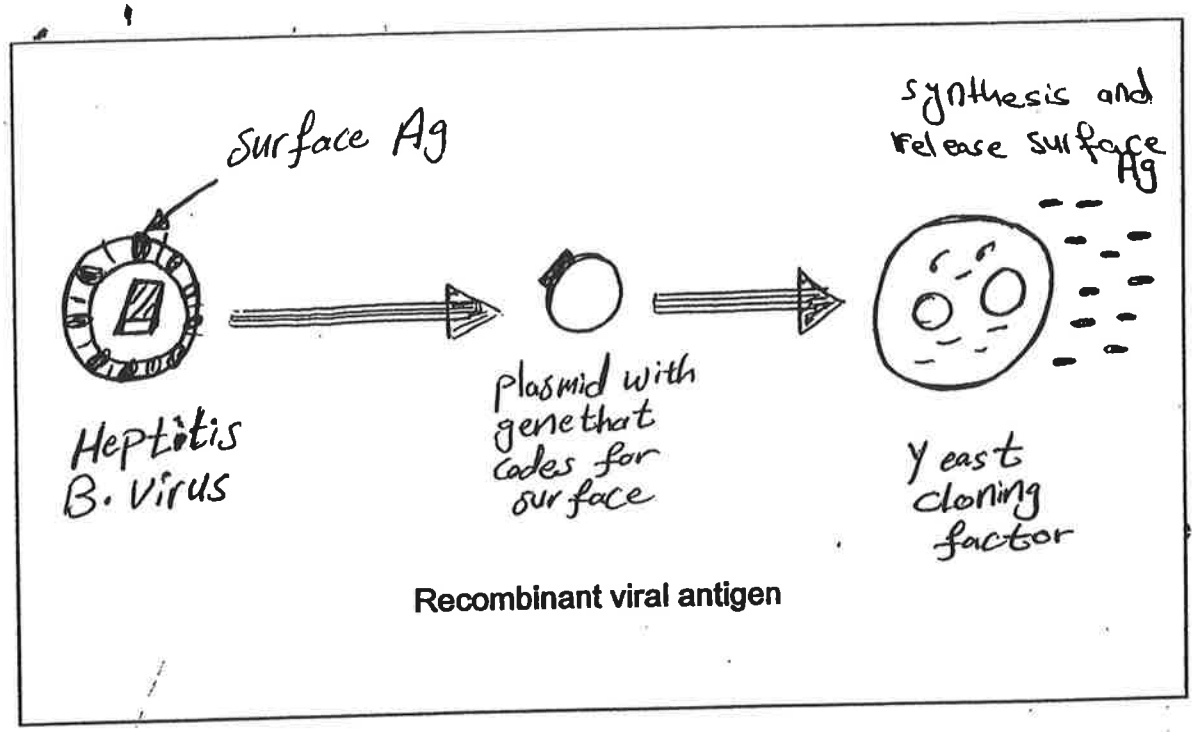
5- Edible Vaccin.

6- DNA Vaccin.

### ❖ Recombinant viral antigen subunit vaccines.

Virus proteins have been expressed in bacteria, yeast , mammalian cells and viruses . *E.coli* cells were first to be used for this purpose. These methods are particularly effective in designing vaccines for obligate parasite that are difficult or expensive to culture such as syphilis spirochete or malaria parasite.

This technology provides means of isolating the gene that encode various microbial Ags , inserting them into plasmid vectors , and cloning them in appropriates host . The outcome of recombinants can be varied as desired , for instance . The cloning host can be stimulated to synthesize and secrete a protein production Ags , which is then harvested and purified (HB vaccine , AIDS vaccines undergoing clinical trials Ags from syphilis , schistosoma and influenza.




Recombinant viral antigen

❖ **Synthetic peptides :**

The development of synthetic peptides that might be useful as vaccines depends on the identification of immunogenic site . The best known example is food and mouth disease , where protection was achieved by immunizing animals with a linear sequence of 20 a. a.

Cowpea mosaic virus was genetically engineered to include : a surface Ag from food –and-mouth disease virus (pathogenic to animals or human). This virus was used to infect its natural host ,black – eyed pea , and introduced gens from the food and mouth disease virus was expressed handsomely in the plant .The cowpeas mosaic virus eventually kill the plant , and therefore the plant needs to be sacrificed a few week after infection . one leaf from the infected pea plant produce enough surface Ag to serve as vaccine for 200 dose . Synthetic peptide vaccines would have many advantages .Their Ags are defined and free from unnecessary components which may be associated with side effects . They are stable and relatively

cheap to manufacture furthermore , less quality assurance is required synthetic peptides are not applicable to all viruses .

Advantages of defined viral Ags or synthetic peptide :

1- Production and quality control simpler.
2- No nucleic acids or other viral external proteins , there less toxic .
3- Safer in cases where viruses are oncogenic or establish a persistent infection .
4- Feasible even if virus cannot be cultivated.

Disadvantages :

1- May be less immunogenic than conventional inactivated whole –virus vaccines.
2- Requires adjuvant .
3- Fail to elicit CMI .

Recombinant viral proteins and synthetic peptides Ags are usually less immunogenic than convential inactivated whole –virus vaccines . This problem may be circumvented to some extent by the use of ISComs (Immuno Stimulating Complex ), where the Ag is presented in an accessible , multimeric . ISComs are composed of adjuvant and antigen held in a cage like structure by lipid . Such a multimeric presentation mimic the natural situation of the antigen on the virus.

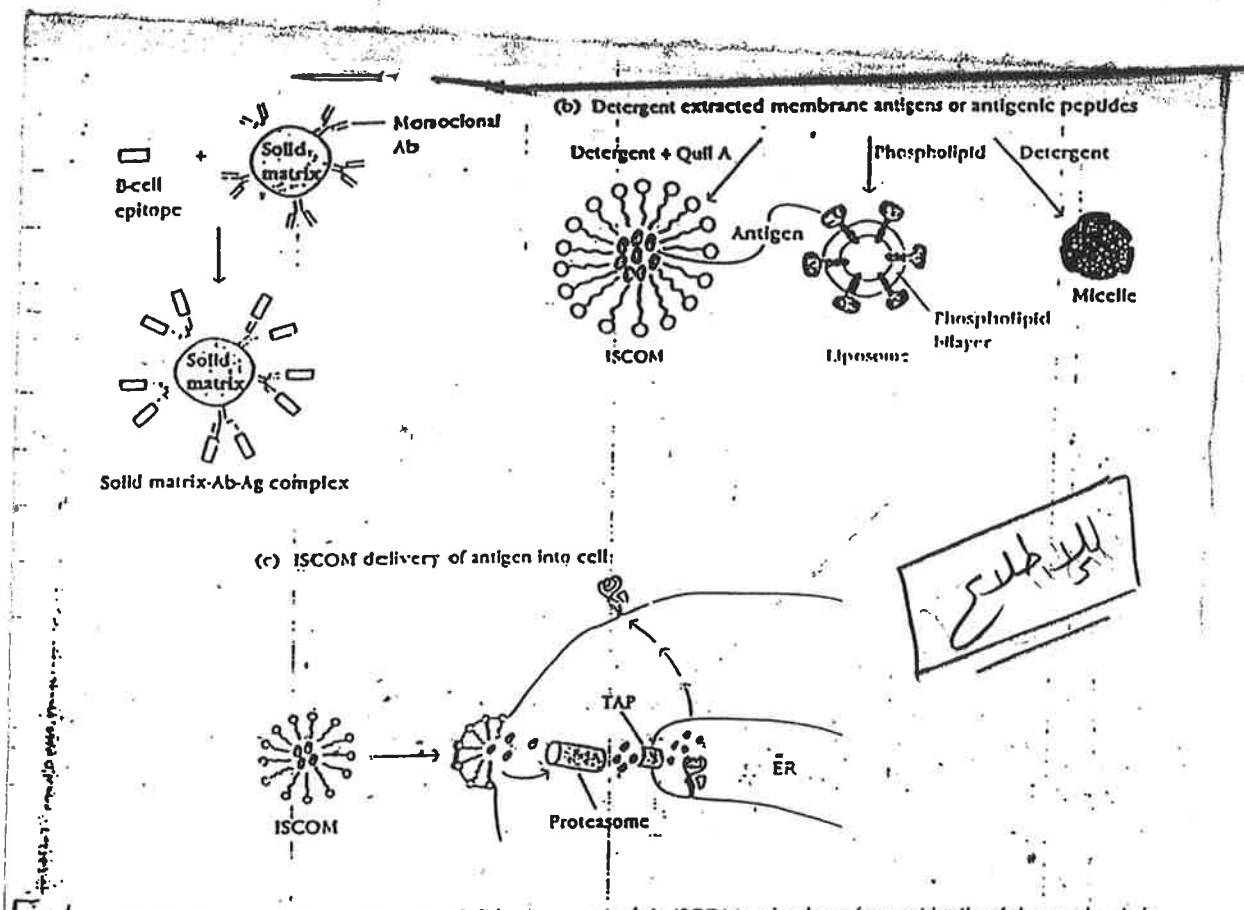



Fig. 1– Multivalent subunit vaccines. (a) Solid matrix-antibody-antigen complexes can be designed to contain peptides representing both T-cell epitopes and B-cell epitopes. (b) Protein micelles, liposomes, and immunostimulating complexes (ISCOMs) can all be prepared with extracted membrane proteins or antigenic peptides. In micelles and liposomes, the hydrophobic residues of the antigen molecules are oriented outward. In ISCOMs, the long fatty-acid tails of the external detergent layer are adjacent to the hydrophobic residues of the centrally located antigen molecules. (c) ISCOMs and liposomes can deliver antigens inside cells, so they mimic endogenous antigens. Subsequent processing by the cytosolic pathway and presentation with class I MHC molecules induces a cell-mediated response.

# Lecture Ten

❖ **Edible vaccine :**

where the vaccine is eaten as a part of the plant . Edible vaccines would not need the purification , strict refrigeration and injection , this make this vaccine cheap . Only certain antigens can trigger a normal immune response before the enzymes and acid in the gastrointestinal system destroy them , Tomatoes and Lettuce have been transformed to produce HBs Ag . *E. coli* entrotoxin was produced in Potato and Tobacco plants . Mice can be immunized against.

This pathogen by munching on the Tobacco leaves or the Potato tuber .This genetically modified plant (GM) might have environmental and health risk .

❖ **Recombinant virus vaccines :**

Genetic material from a selected infectious agent is inserted into alive microbe (virus) that is nonpathogenic . In theory , the recombinant microbe (or virus) will multiply and express the foreign gene , and the vaccine recipient will be immunized against microbial Ags . Vaccinia the virus originally used to vaccinate for smallpox and adenoviruses have proved practical agents for this technique.

Vaccinia is used as the carrier in one of the experimental vaccines for AIDS,Herpes, Simplex 2 , Leprosy and tuberculosis . The genes of several viruses can be inserted so the potential exists for producing polyvalent live vaccines . HBsAg ,rabies Hsv and other viruses have been expressed in vaccinia .

Hybrid virus vaccines have all the advantages of live viral vaccines . They are stable and stimulate both cellular and humoral immunity . They are relatively cheap and simple to produce . Being live vaccines smaller quantities are required for immunization .

❖ **Anti –idiotype vaccines :**

Anti – idiotype vaccine is based on principle that the Ag binding (variable) region or idiotype of a given Ab (A) can be antigenic to a genetically different recipient and can cause that recipient immune system to produce (Ab)(B) also called anti–idiotyic Ab , special for the variable region on Ab(A) . The purpose for making identical configuration as the desired Ag and can used in vaccines . The Ab it stimulates in the vaccine recipient will be able to react with the natural Ag . This method avoid giving a microbial Ag thus reducing the potential for dangerous side effect . In addition the exact nature of microbial Ag need not be known.

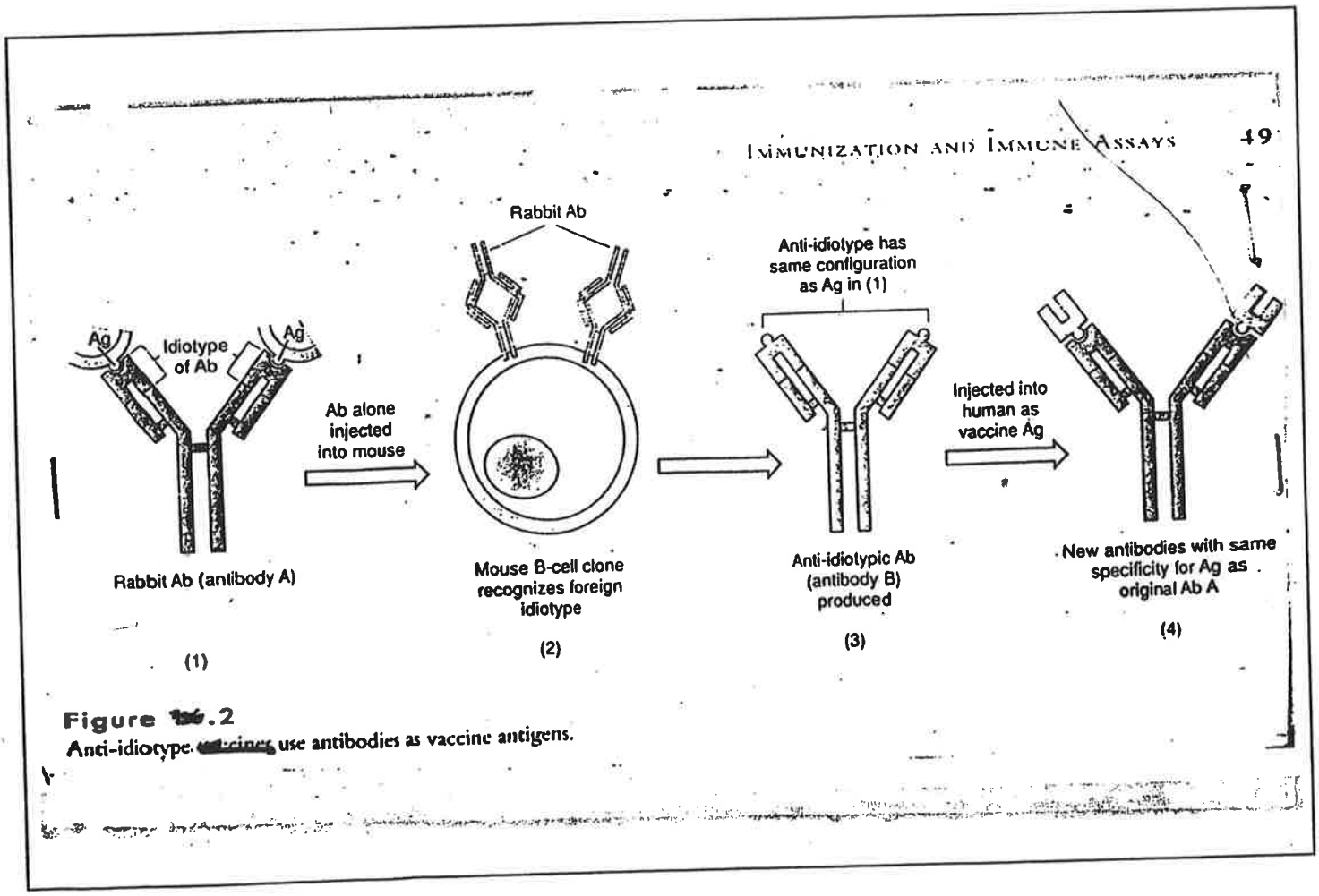
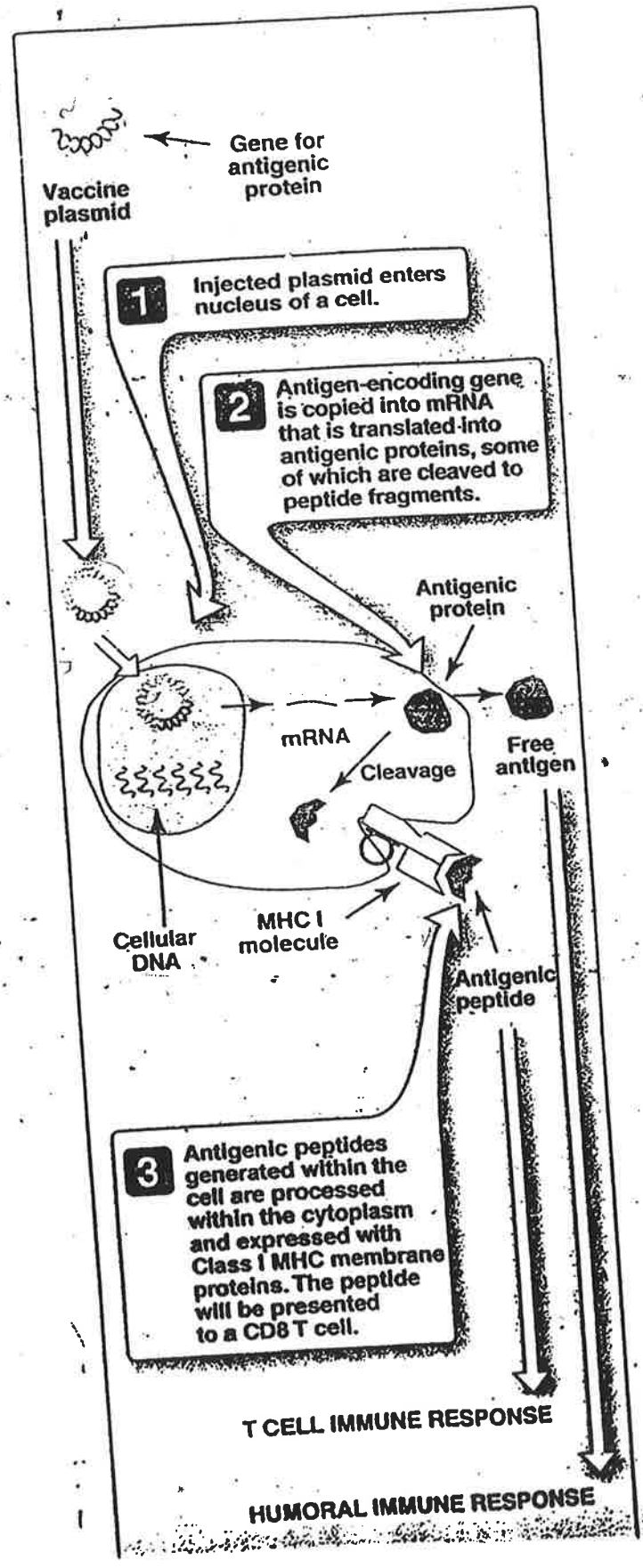



**Figure 16.2**
Anti-idiotype vaccines use antibodies as vaccine antigens.

❖ DNA Vaccines :

DNA Vaccines represent a new approach to vaccination . The propose mechanism for these vaccines can be summarized as follows: The gene for the antigen of interest is cloned into a bacterial plasmid that is engineered to increase the expression of the inserted gene in mammalian cell.

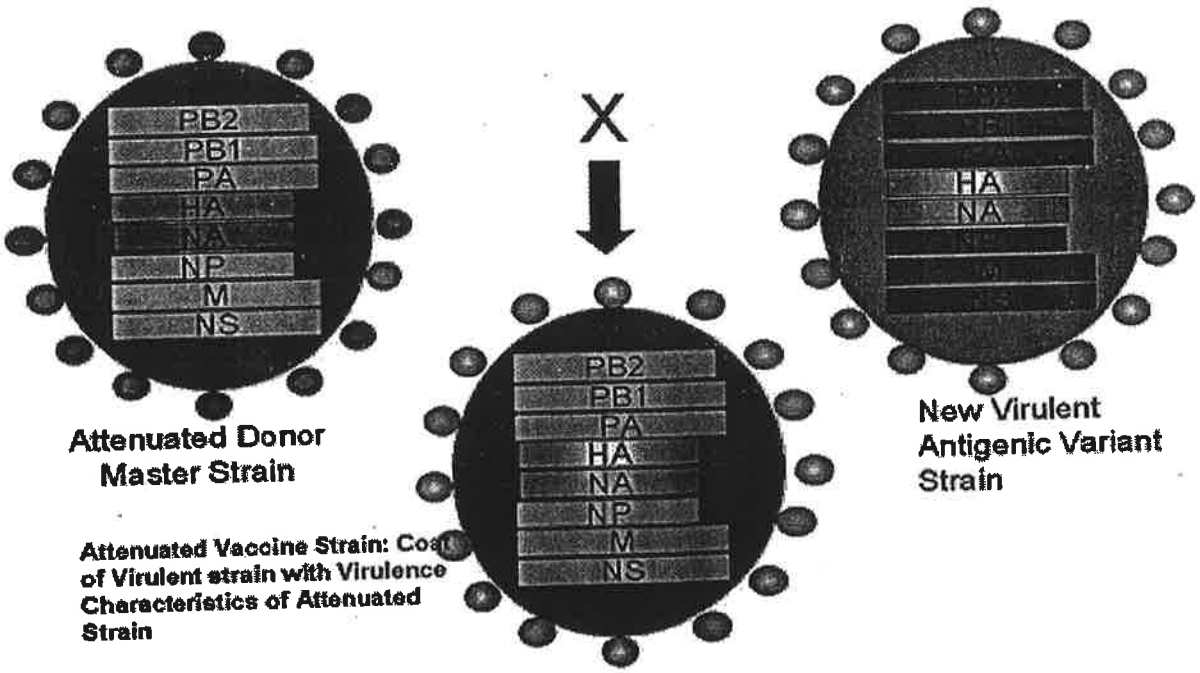
After being injected ,the plasmid enter a host cell where it remains in the nucleus as an episome (that is: it is not integrated into the cell's DNA) using the host cell's protein synthesis machinery ,the plasmid DNA in the episome directs the synthesis of the protein it encodes . This antigenic microbial protein may leave the cells and interact with T helper and B cells , or it may be cleaved into fragment and presented as MHC I antigen complex on the cell surface ,resulting in activation of Killer T – cells . To date , the potency of DNA vaccines human has been disappointing.


Gene for antigenic protein
Vaccine plasmid
1 Injected plasmid enters nucleus of a cell.
2 Antigen-encoding gene is copied into mRNA that is translated into antigenic proteins, some of which are cleaved to peptide fragments.
Antigenic protein
mRNA
Cleavage
Free antigen
Cellular DNA
MHC I molecule
Antigenic peptide
3 Antigenic peptides generated within the cell are processed within the cytoplasm and expressed with Class I MHC membrane proteins. The peptide will be presented to a CD8 T cell.
T CELL IMMUNE RESPONSE
HUMORAL IMMUNE RESPONSE

❖ Selection for mis-sense

Conditional lethal mutants. Temperature-sensitive mutants in influenza A and RSV have been made by mutation with 5-fluorouracil and then selected for temperature sensitivity. In the case of influenza, the temperature-sensitive gene can be reassorted in the laboratory to yield a virus strain with the coat of the strains circulating in the population and the inner proteins of the attenuated strain. Cold adapted mutants can also be produced in this way. It has been possible to obtain mis-sense mutations in all six genes for non-surface proteins.

The attenuated influenza vaccine, called FluMist, uses a cold-sensitive mutant that can be reassorted with any new virulent influenza strain that appears (figure 9). The reassorted virus will have the genes for the internal proteins from the attenuated virus (and hence will be attenuated) but will display the surface proteins of the new virulent antigenic variant. Because this is based on a live, attenuated virus, the customization of the vaccine to each year's new flu variants is much more rapid than the process of predicting what influenza strains will be important for the coming flu season and combining these in a killed vaccine.




Attenuated influenza vaccine strain using a could sense mutation that can be restorted with new virulent strain