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Sera and Vaccine

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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 zurHausen 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

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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 BaccilleCalmetteGuerin (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.

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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 *Haemophilusinfluenzae* 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.

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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).

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BacilleCalmette-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 anartificial 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 ofthe 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 Salkand 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 andto 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 vaccinecontains 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 immunitythan the Salk vaccine.

The trivalent Oral Polio Vaccine (Sabin) on very rare occasions has been associated withparalysis (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; *Corynebacteriumdiphtheriae*. 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

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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 rubellasyndrome 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.

Haemophilusinfluenzae 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.

***Haemophilusinfluenzae* type b**

Haemophilusinfluenzae is a Gram-negative coccobacillus. It is generally aerobic but can grow as a facultative anaerobe. It causes severe pneumonia, meningitis andother 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

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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.

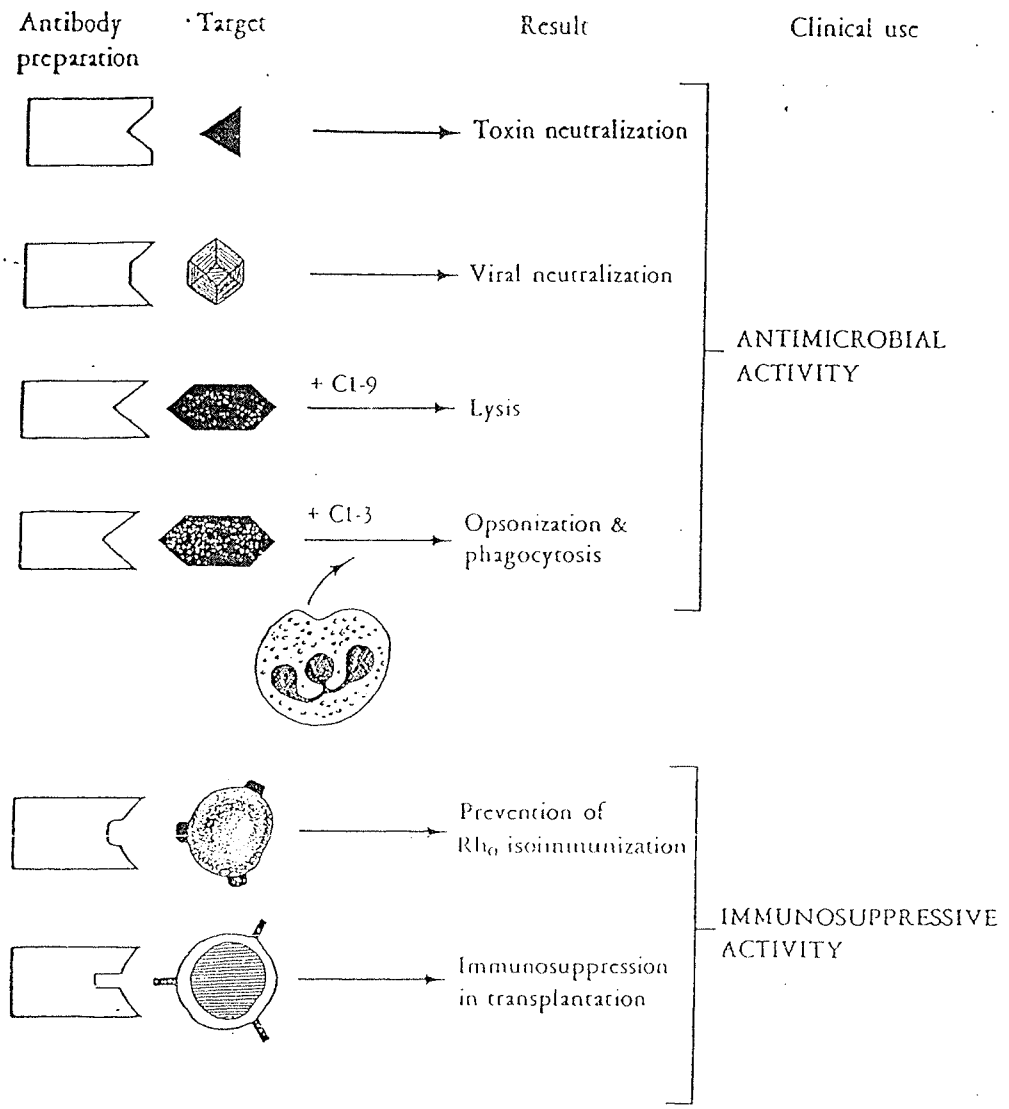

Figure 24–2. Schematic representation of the uses of antibody preparations in immunotherapy.

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.

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

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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.

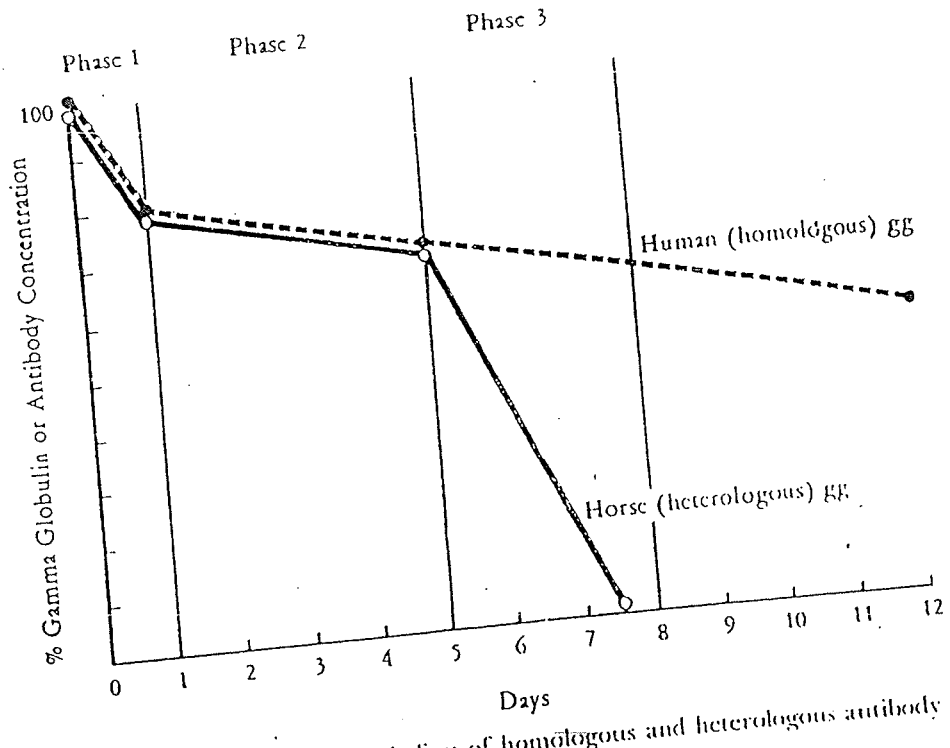


Figure 24–3. Schematic comparison of the catabolism of homologous and heterologous antibody (gg) in the human.

preparations .all preparations have **tendency to aggregates** into large IgG 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.

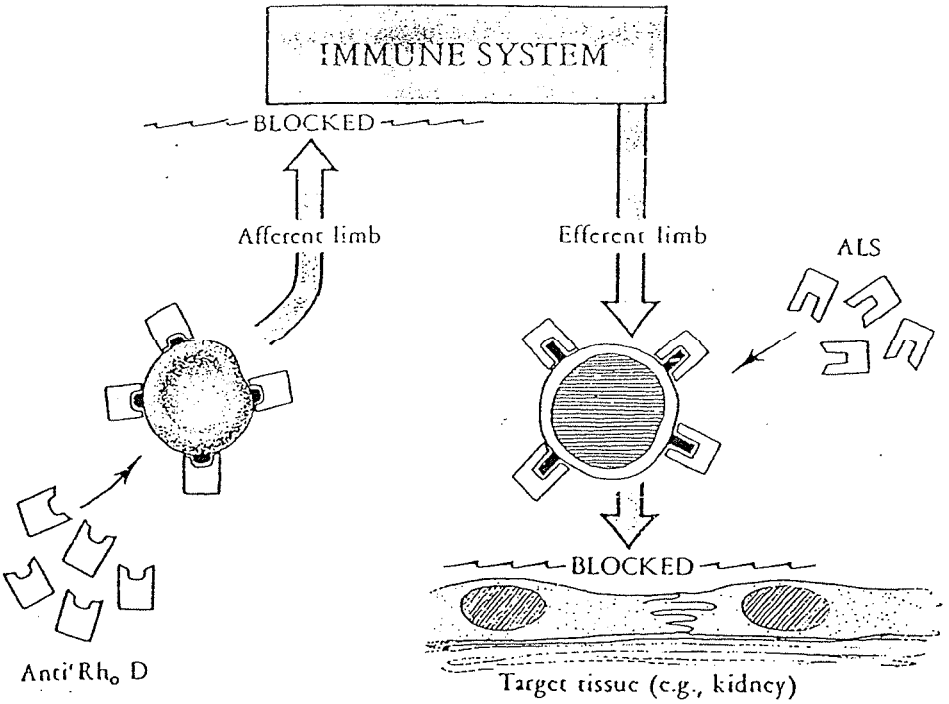


Figure 24–4. Schematic representation of the modes of immunosuppression by specific anti-Rh$_o$ antibody and by antilymphocyte sera (ALS).

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.

Sera and Vaccine

Lec. 9

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.

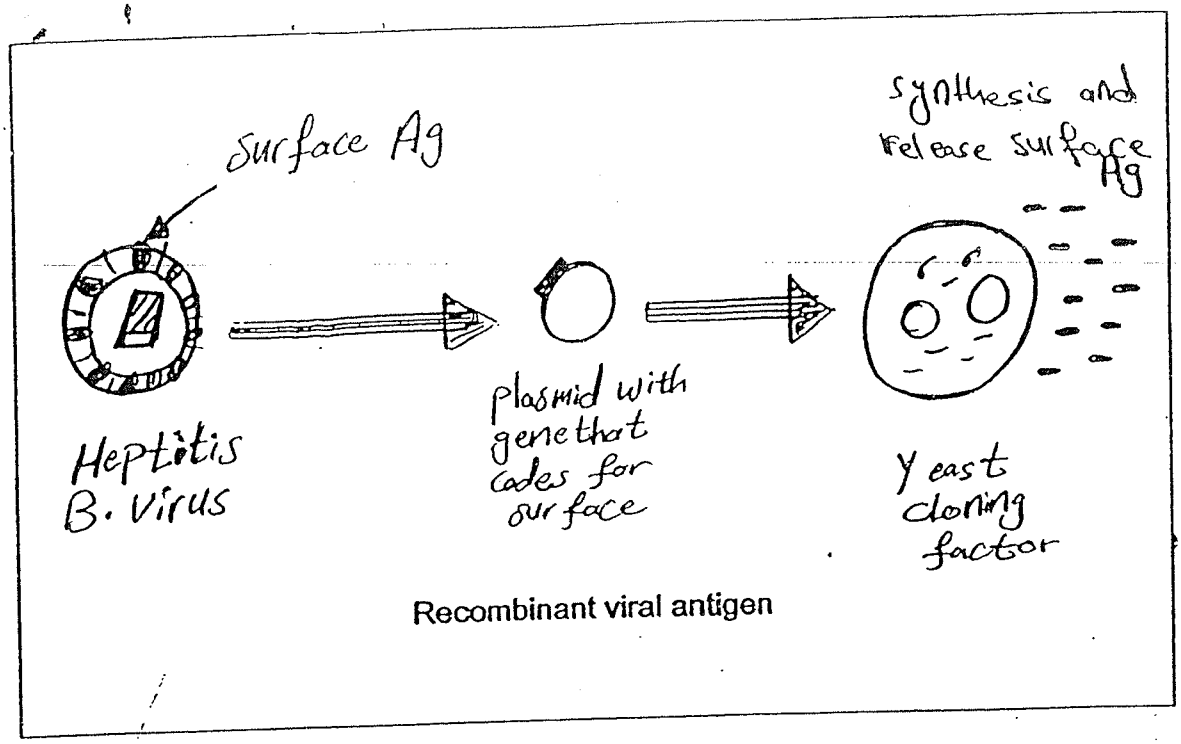
5- Edible Vaccin.

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.

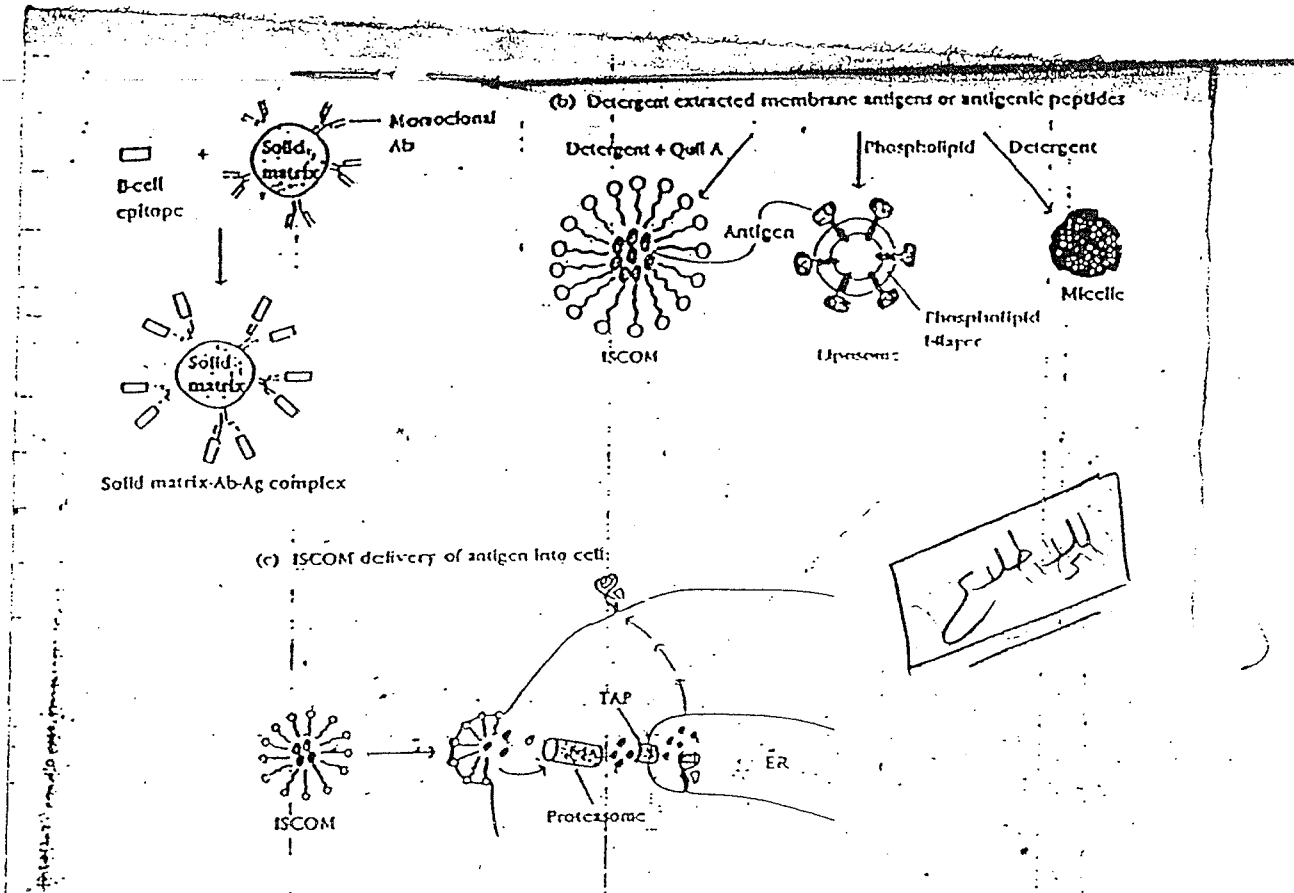


Fig 1 – Multivalent subunit vaccines. (a) Solid matrix-antibody-antigen complexes can be designed to contain peptides representing both T-cell epitopes and B-cell epitopes. (b) Protein micelles, liposomes, and immunostimulating complexes (ISCOMs) can all be prepared with extracted membrane or antigenic peptides. In micelles and liposomes, the hydrophilic 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.

4

Sera and Vaccine

Lec. 10

❖ 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.

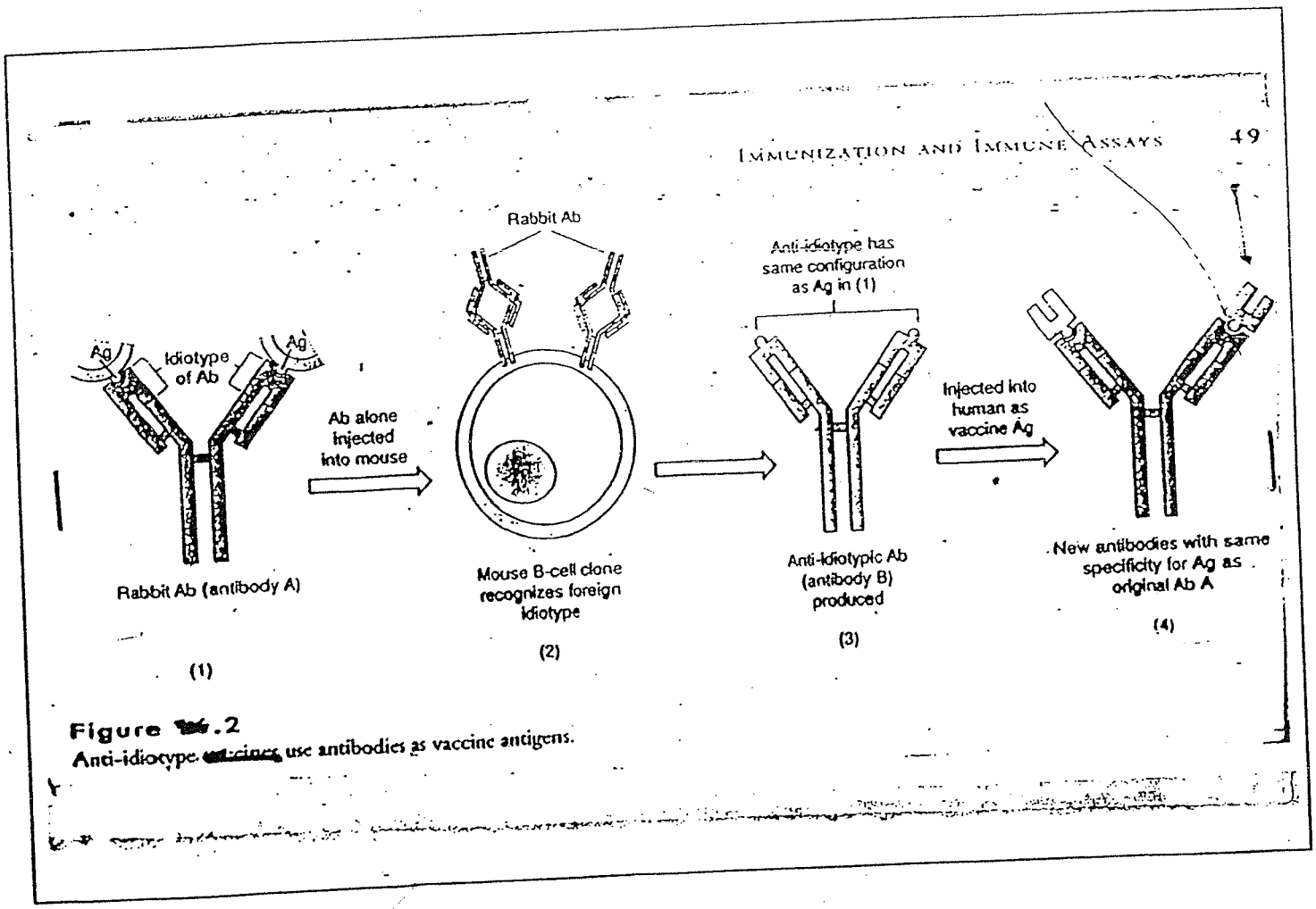
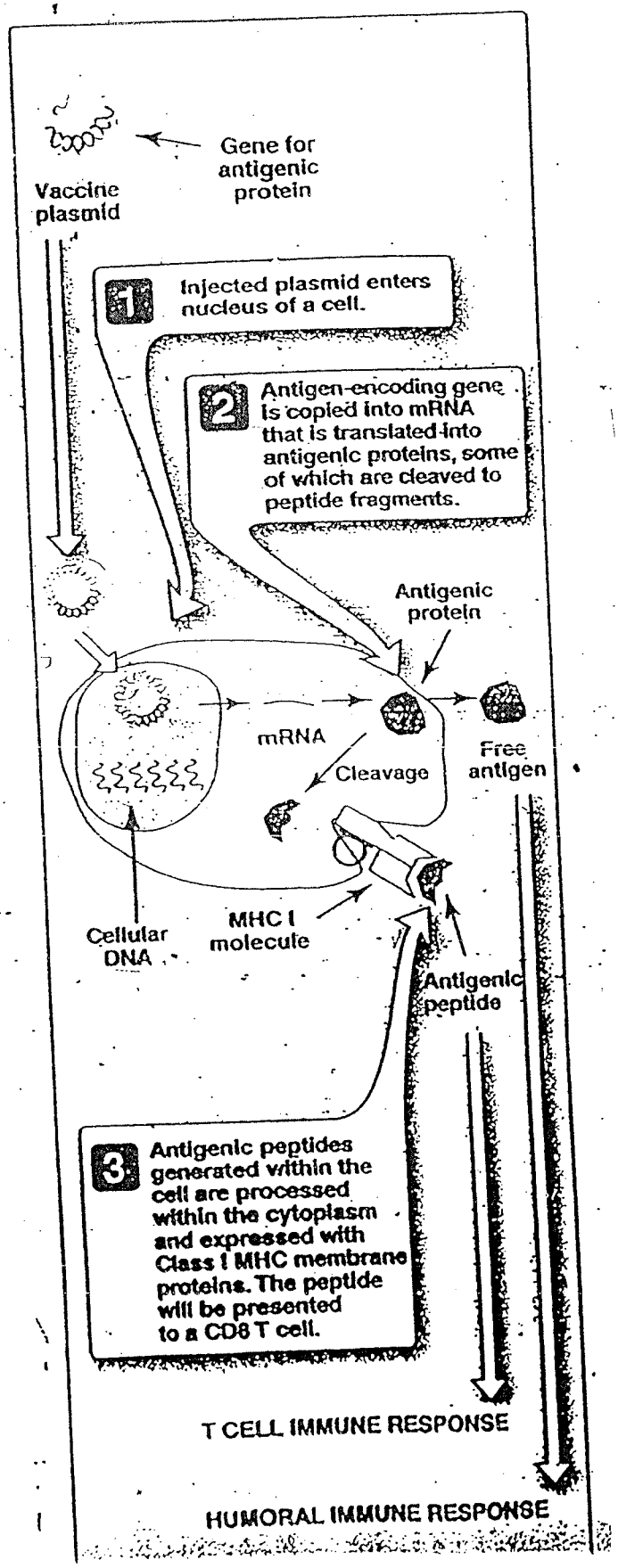


Figure .2
Anti-idiotype vaccines use antibodies as vaccine antigens.

❖ 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.

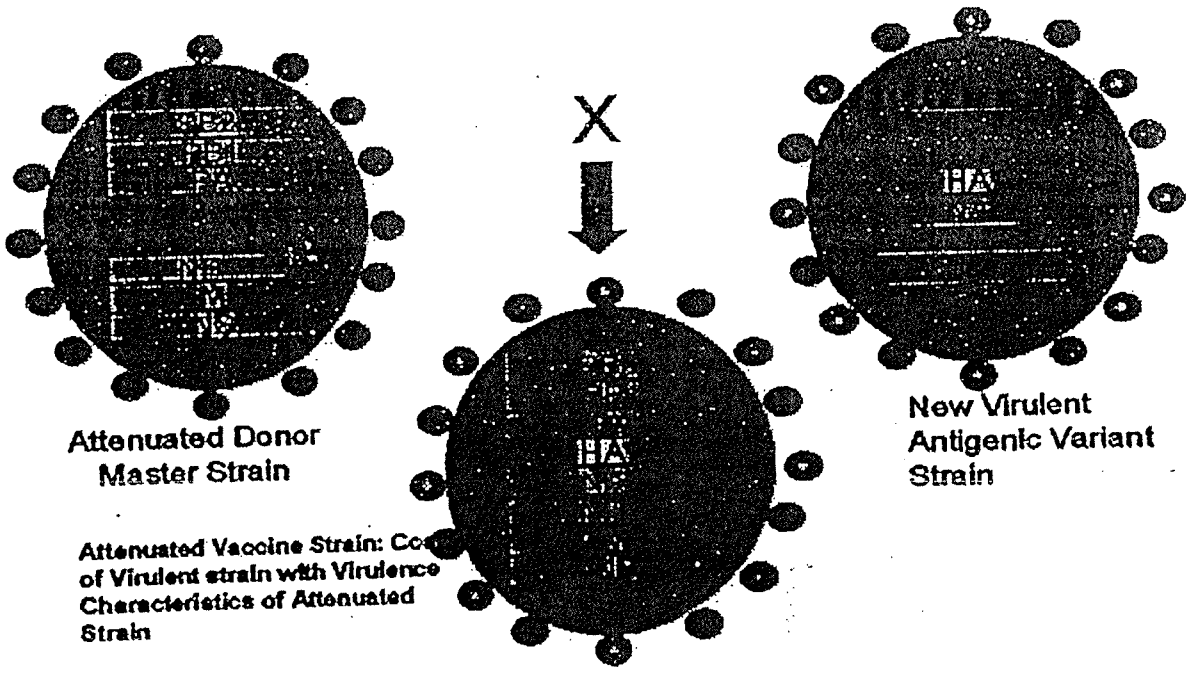
After being injected ,the plasmid enter a host cell where it remains in the nucleus as an episome (that is: it is not integrated into the cell's DNA) using the host cell's protein synthesis machinery ,the plasmid DNA in the episome directs the synthesis of the protein it encodes . This antigenic microbial 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