VACCINATION

Definition: It is injection of vaccines into the body to produce immunity and to protect against diseases (prophylaxis) is called vaccination.

Prophylaxis means prevention of diseases by immunization.

Immunization is rendering an individual resistant to infections.

Vaccines: It is an antigenic preparation of microorganisms, such as bacteria, viruses or rickettsiae administered for prevention or treatment of infectious diseases.

Examples of vaccines: Some commonly used vaccines are against- Diphtheria, Measles, Mumps, Pertusis, Poliomyelitis, Rubella, Tetanus, Diphtheria, Influenza, Typhoid etc.

Criteria for vaccine development and vaccination:

1) Identification of the causative organism.
2) It must be established that an Immune response can protect the disease. The time of administration and conditions of individual should be considered.
   Eg:Haemophilus indluenza typeB capsular polysaccharide vaccine protects children above the age of 18months. This is because young children do not develop IR against carbohydrates.
3) Vaccination is not without some disadvantages. Small pox vaccine rearly causes encephalitis and possibly death.

Types of immunization procedures:

Passive and active immunizations are the two methods by which an individual can be made resistant to an infectious agent.

Passive Immunization produces temporary resistance by transferring Abs from a resistant to a susceptible individual.

Active immunization has several advantages as it involves administering Ag to the individuals to induce IR to protect him. Reimmunization results in a secondary IR.

Advantage: Long lasting protection and capable of restimulation.

Disadvantage: Protection is not immediate as it is in passive immunization.

Passive Immunization: It requires Abs to be produced by a donor by active immunization and the resulting Abs must be given to susceptible animals, inorder to confer immediate protection. Abs can be produced in animals of any species and against a wide variety of pathogenic organisms.

Eg: Abs against measles and hepatitis can be produced in human and Abs against tetanus is raised in horse.

Antitetanus serum: Protecting human against tetanus by means of transfer of Igs raised in horse is the best example to be studied. Toxins of clostridia are made non-toxic by treating with formaldehyde, and the toxin converts o toxoid form. This tetanus toxoid is repeatedly injected into young horses.

The blood of the horses is collected from which serum is purified. Mainly the globulin portion of the plasma is prepared and then dialysed to get pure Abs. These Abs when administered give immediate protection but after some time they induce isotype Abs production by host and are eliminated from the blood.

To overcome this if Fc part of the Immunoglobulins is cleaved using pepsin, and only the 2F(ab) fragment is administered, they survive for a longer period.

Instead if antitetanus serum is collected from human donors, they survive for a longer period.

Disadv: Equine serum Abs are Ags to human and hence IR is generated against them and equine Abs are eliminated rapidly. If these are given to a horse they stay in their circulation for a longer period.

In human recipients immune complexes are formed which settle in the blood streams and cause hypersensitivity reaction which lead to serum sickness. Repeated injection of horse immunoglobulins can also lead to anaphylaxis.

Dosage: A unit of activity administered to an individual depends on the extent of damage caused. Around 250units of Ig are normally given for immediate protection and around 1000units are given in cases of severe tissue damage.

Examples of some sera: other diseases for which antisera are administered include:

Measles, Hepatitis A and B, Rubella, Varicella, Rabies, Diptheria, Group B sterptococci, Pseudomonas, Cytomegalovirus, Tetanus and Botulism.

Passive protection can also be done by administering monoclonal antibodies.

Active immunization: It offers long lasting protection. Recall of Immune response is exhibited and the extent of protection can be boosted up by injecting booster doses. This can be done by giving vaccines.

Ideal vaccine: That which can be used for immunization and should be cheap, stable and adaptable for mass vaccination. It should also be free of adverse side effects. Live organisms can also be used as vaccines but these must be treated in such a way that they loose their disease causing capacity but retain the capacity to stimulate IR. This is termed as loss of virulence and the phenomenon is called as attenuation. The most common method used for attenuation are as follows,

1) Adapting the organism to unusual environmental conditions so that they loose their ability to replicate uncontrollably in host.

Eg: i. Bacillus Calmette Guerin (BCG) strain of Mycobacterium is rendered avirulent by growing it for 13 years on a bile saturated medium. BCG is used as vaccine against tuberculosis.

ii. Vaccine for foul cholera is developed by growing organisms on medium with depleted nutrients.

2) Viruses can also be attenuated by growing them in abnormal culture conditions. Prolonged tissue culture, especially in cells of a species that the virus does not infect normally reduced virulence of the viruses.

Eg: Polio is attenuated by growing on monkey-tissue.

Attenuation of viruses is also done by growing the viruses in eggs. Like the influenza virus and yellow fever viruses.

3) Adapt viruses to grow at slightly low temperatures than the normal.

Eg: Herpes virus causes bovine rhinotracheitis. Temperature sensitive mutant are used in vaccine preparations.

Advantages of attenuation:

- Live organisms are effective and give prolonged and strong immunity.
- Few doses are required.
- No adjuvant is employed and therefore does not provoke hypersensitive reactions.
- Living organisms provoke interferon production and therefore better stimulation of immune system occurs.

Disadvantages:

- Live vaccines are difficult and expensive to produce.
- They may contain dangerous extraneous organisms.
- They may not be fully attenuated and therefore may retain virulence.
- These cannot be given to patients suffering from immunodeficiencies.

Eg: Poliomyelitis vaccine:

Sabine poliovaccine contains a mixture of 3strains of attenuated polioviruses (type1,2&3). These are attenuated by growing in renal epithelial cells of monkey kidney.

It is administered orally to children on sugar cube or in liquid sugar.

Viruses colonize in the intestine and stimulate IgA secretion. They also stimulate IgM and IgG production. It is usually given in 3 doses.

When the first dose of 3strains is given, Abs are raised against only one strain. Viruses interfere in each others growth and only one predominates against which Abs are raised.

When second dose is given, Abs produced earlier will limit the growth of first virus and one among the rest two will predominate and cause production of Abs.

When the third dose is given, the first two strains are rapidly eliminated and therefore the third will colonize and result in production of Abs. at the end of three doses memory is created against all he three strains of polioviruses.

Usage of dead organisms as vaccines: Heat killing is the crude method used for killing microorganisms. But has several disadvantages as it causes protein denaturation. Chemical inactivations are the best method. Some commonly inactivating agents include formaldehyde, ethylene oxide, ethylene oxide, ethylene imine, acetyl ethylene imine and beta propiolactone. These cause slight modification of the Ag.

Disadv: If dead poliovaccine is given it does not colonize and therefore cannot produce good immune response.

Therefore both dead and live vaccines have their own risks and benefits. Therefore several new methods were tried to prepare vaccines that are devoid of contaminations and have no side effects. These may be called **pure vaccines**.

Bacterial exotoxins as vaccines: bacterial exotins are purified and detoxified by treatment with formaldehyde. These act as excellent immunizing agents.

Even bacterial cell wall polysaccharides act as potent vaccines.Eg: Pneumococcla polysaccharides.

Some vaccines contain pilli of bacteria. Eg: Ecoli pili are used to prevent enteric diseases in animals. All these are studied under the heading purified macromolecules as vaccines.

Purified macromolecules as vaccines:

1) Capsular polysaccharides of bacteria: Virulance of some pathogenic bacteria is due to their hydrophilic polysaccharide capsule having antiphagocytic properties. If capsule is coated with Ab, it is lysed by complement or phagocytised.

Eg: Streptococcus pneumonia causes pneumonia. Its capsule has 23 antigenically different polysaccharides. Vaccines contain some of these polysaccharides and induce opsonising Ab formation.

Their mode of action:

a) If administered intravenously they directly induce Bcells as they act as T independent Ags. Therefore the class switching is less and no memory formation
b) If administered subcutaneously, they induce IgA secretion and memory is ceated.
c) If given as a conjugate with protein, they become more immunogenic and activate T_H cells. These enable class switching to IgG and therefore induce better memory formation. Eg: Vaccine for Haemophillus influenza type b(Hib) is prepared by

mixing typeB capsular polysaccharide along with tetanus toxoid as a carrier protein.

Some new approaches for vaccine production:

a) Genetically modified organisms as vaccines
b) Recombinant products as vaccines
c) DNA vaccines
d) Multiple subunit vaccines.

a) Genetically modified organisms as vaccines: Genetic engineering techniques provide a way to attenuate a virus irreversibly by selectively removing genes that are necessary for virulence. This has been done with a herpesvirus vaccine for pigs, in which the thymidine kinase gene was removed. Because thymidine kinase is required for the virus to grow in certain types of cells (e.g., neurons), removal of this gene rendered the virus incapable of causing disease. It is possible that similar genetic engineering techniques could eliminate the risk of reversion of the attenuated.

Recombinant vaccines

Recombinant Antigens: Techniques can be employed to isolate genetic material coding for a protein Ag of intrest. This can be placed in bacteria, yeast or other cells which can be express the protein.

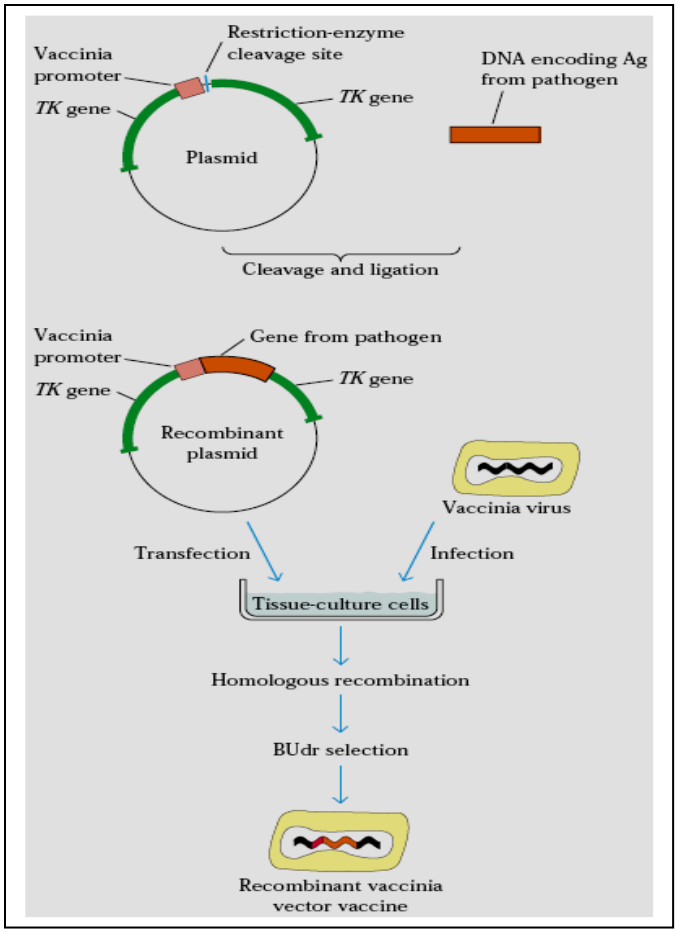
Eg: 1) HepatitisB vaccine is the first to be produced by this method. The surface Ag of Hepatitis B virus (HB'sAg) is expressed by using yeast cells. These are called recombinant Ags.

2) Production of VP1 Ag from foot and mouth disease virus. This is prepared by first isolating RNA genome from the viruses. Duplicated into DNA by reverse transcriptase. Cut the DNA by restriction endonucleases and the gene encoding VP1 is isolated. This is inserted into a suitable vector and then transformed into Ecoli. These bacteria when cultured in the presence of an inducer will express the product which can be harvested. This process is highly effective as around 4×10^7 doses of foot and mouth vaccine can be prepared from just 10litres of Ecoli having 10^{12} organisms per mL.

Live recombinant organisms: genes coding for protein Ags can be cloned into variety of organisms. Vaccinia virus is most widely used organisms. This virus has large genome and can be administered by dermal scratching. Large genome easily accommodates new gene inserts and promotes expression of new Ags. These proteins undergo appropriate processing steps, including glycoslation and membrane transport. Vaccinated individuals were reported to have increased serum Abs.

Other organism which can be used in vaccine preparations: Attenuted strains of Poliovirus, Salmonella, strains of streptococcus which normally exist in oral cavity can also be used.

Production of vaccinia vector vaccine: The gene that encodes the desired antigen (orange) is inserted into a plasmid vector adjacent to a vaccinia promoter (pink) and flanked on either side by the vaccinia thymidine kinase (*TK*) gene (green). When tissueculture cells are incubated simultaneously with vaccinia virus and the recombinant plasmid, the antigen gene and promoter are inserted into the vaccinia virus genome by homologous recombination at the site of the nonessential *TK* gene, resulting in a *TK*_recombinant virus. Cells containing the recombinant vaccinia virus are selected by addition of bromodeoxyuridine (BUdr), which kills TK_ cells.



DNA vaccines: In this plasmid DNA encoding antigenic proteins is injected directly into the muscle of recipient. Muscle cells take up DNA and encoded protein is expressed inducing both the Humoral immune response and cell mediated immune response. Injected DNA can either get integrated with chromosomal DNA or remain in episomal form. Muscle cells express viral Ag via their classI molecules. If DNA is injected into

dendritic cells it will result in a better response as it bring out Ag peptide via ClassII HLA molecules.

Advantages: DNA vaccines cause prolonged expression of the Ag, which generates significant immunological memory.
-protein is expressed in the host in its natural form.
-Induces both Humoral and cell mediated immune responses.
-Refrigeration is not required.
-Easy to handle and store.
-Can be produced at low cost.
-Can be custom tailored.
-If IL-12 gene is included along with Ag gene, it induces T_H1 type Immunity.
DisAdv: Can be used only for protein Ags only.

Multivalent subunit vaccines:

Synthetic peptide vaccines can stimulate only Humoral immune response. If syntheric peptides containing Bcell and T cell epitopes are synthesized they can stimulate both Humoral as well as cell mediated immune response. In this process multivalent subunit vaccines were developed.

a) Solid matrix Ag-Ab complexes(SMAA): monoclonal antibodies are attached to solid matrix and then saturated with peptides which consist of mostly T and B cell epitopes. Their particulate nature contributes to their increases immunogenicity by facilitating phagocytosis by phagocytic cells.

b) Micelles and liposomes: Detergent is used to incorporate protein Ags into micelles, lipid vesicles called liposomes or immune stimulating complexes. Mixing proteins in detergent and then removing the detergent forms micelles. The individual protein orient themselves with their hydrophilic residues at the centre so as to exclude their interaction with the aqueous environment.

Liposomes containing protein Ags are prepared by mixing the proteins with a suspension of phospholipids under conditions that form vesicles bounded by a bilayer. The proteins are incorporated into the bilayer with the hydrophilic residues exposed.

c) ISCOMS (Immune stimulating complexes): These are lipid carriers prepared by mixing proteins with detergent and a glycoside called quailA.

Eg: Membrane proteins of pathogenic viruses like influenza virus, measles virus, hepatitis B virus and HIV are prepared by this method.

Important questions:

1) Write an account on active immunization and passive immunization. -6

2) Give a detailed account on new generation vaccines. -14

3) What are DNA vaccines? Add a note on their advantages and disadvantages. -8