Applications of Immunology!

1. **Vaccines**

Immunization using a closely related, less pathogenic organism to give protection against a more pathogenic one.

A-Provide an antigenic stimulus that does not cause disease

1-Attenuated strain

Tissue culture or unnatural / unusual host

Hypovirulent

2-Dead whole cells or inactivated viruses

Heat, formalin, UV irradiation

3-Purified antigen subunits from cells or viruses

4-Surface antigens produce via rDNA technology

5-DNA vaccines

B-Produces long lasting protective immunity

C-Edward Jenner (page 476)

www.sc.edu/library/spcoll/nathist/jenner2.html

Cowpox

Smallpox

Variola

Controlled experiments

D-Vaccinia virus

Cultured cow pox virus for many years. Small pox eradicated in 1973.

2. **Diagnostic tests**

A part of immunology that attempts to detect signs of infection in a patient's serum. Use Abs that specifically bind to Ag

Ag-Ab reactions are visible by

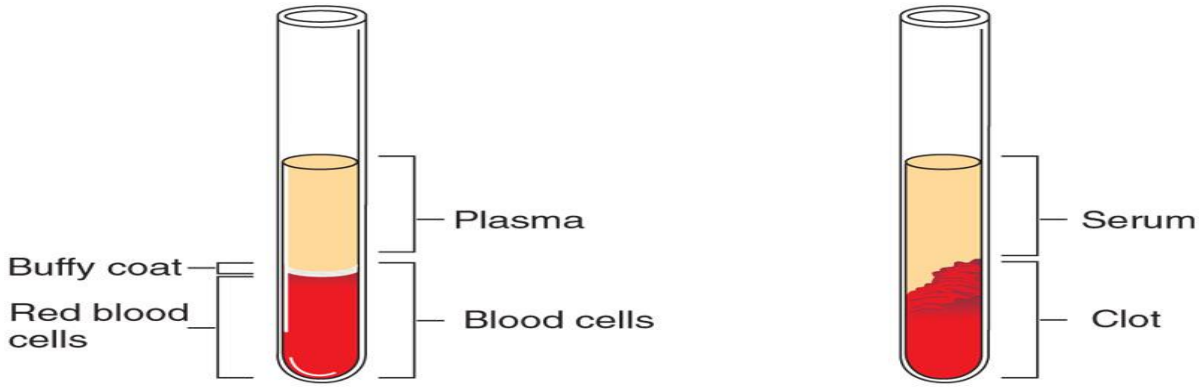
Clumps

Precipitates

Color changes

Release of radioactivity

The most effective tests have high specificity and sensitivity.



Diagnostic Immunology -use purified antibody solutions (antiserum) to diagnose disease! Diagnostic antibodies can be produced to detect particular microbes:

A. In animals (mixed antiserum)

1. Inject animal with microbe or antigenic fragments
2. Allow immune response (1-2 weeks)
3. Harvest blood
4. Purify antibodies from serum to make antiserum = purified antibody solution to one particular antigen-these preparations will produce multiple antibody types that recognize different epitopes on the antigen.

B. Monoclonal antibodies-isolate one immortalized B cell-clone in culture - produce cheap, pure, antiserum with one type of antibody that recognizes only one epitope on the antigen-requires cell culture, but no need for animal husbandry and blood purification.

-diagnostic immunology is the future of medical diagnosis for infectious disease.

Serum: The liquid part of blood, without the cells and the coagulating factors, but containing antigens and antibodies; it is the storehouse and means of transport of immunological components.

Antiserum: Blood serum containing antibodies arising out of immunisation or after an infectious disease.

The antigen-antibody recognition is a highly specific phenomenon of **bio-recognition** at the molecular level. Such a high degree of specificity is also found between the enzymes and their substrates, and lectins and their specific carbohydrates. Immune response is a selective reaction of a mammalian body to substances that are foreign (**exogenous**) to it or those that the immune system identifies as foreign. The three important aspects are:

a) **Memory**: the primary response of the formation of the memory template at the first encounter,

b) **Distinction between self- and non-self**: distinction between the organism's endogenous proteins and those that are foreign (exogenous), and

c) **Specificity**: the secondary response of production of antibodies very specific to each foreign agent.

Immunoglobulin (Ig): A protein molecule of the globulin-type, found in the serum or other body fluids and that possess antibody activity; there are five classes of immunoglobulins (IgA, IgD, IgE, IgG and IgM), based on antigenic and structural differences. In addition to these five classes, there are several subclasses (four in IgG) and other variants of Ig molecules.

Molecular structure of the antibodies: The conventional model of the Ig molecules is a 'Y' shaped configuration, with two heavy chains and two light chains, with two

open arms containing the antigen combining sites, which occur on both the light and the heavy chains. The two heavy chains are bound together by disulphide bonds. At any point, the molecule has two chain sections, parallel to each other.

The modern view of the structure of the Ig molecules is to look at them as containing series of regions activity called **domains.** Variable light, variable heavy, constant heavy and constant light are the domains recognised on Ig molecules. The constant domains provide for the identity of the molecules and the variable regions are responsible for the diversity in the specificity of the antibodies.

Ig molecules may occur as monomers (IgG and IgA), dimers (IgA) or pentamers (IgM) .Linking monomers by J chains forms higher configurations.

Immunoglobulin A (IgA): With a molecular weight of about 1,60,000, IgA molecules are only slightly heavier than the IgG molecules but they can form aggregates of higher molecular weights. IgA are about 13% of the total Ig with a concentration of 1.4 to 4 mg/ml in the normal serum. The IgA are the major Ig in the serum and mucous secretions, such as saliva, tears, nasal fluids, sweat, lung and the gastrointestinal tract. They defend the exposed external surfaces of the body against the attack of microorganisms. IgA antibodies seem to inhibit adherence of the microorganisms to the surface of the mucosal cells and thus prevent their entry into the body tissues. IgA molecules differ from the other Ig classes in having three disulphide bonds holding the two heavy chains, instead of two bonds in the others.

Immunoglobulin M (IgM): The IgM molecules are the heaviest of all Ig. They have a molecular weight of 900,000 and so are often known as the macroglobulins. They form about 6% of the total Ig and occur in a concentration of 0.5 to 2% of the normal serum. IgM are very efficient agglutinators of bacterial cells and are effective cytolytic agents. They form the most immediate and effective first line defence against bacteraemia. Since they appear in response to infection they are mostly confined to the blood stream. The anti-A and anti-B haemagglutinins and many antimicrobial antibodies as well as typoid exotoxin antibodies are all IgM. During the course of evolution of Ig, IgM seem to have appeared earliest.

Immunoglobulin G (IgG): IgG molecules are the lightest of all the Ig and have a molecular weight of about 1,50,000 and about 3% carbohydrate content. They form about 80% of the total Ig of the human body. In the normal serum their concentration ranges from 8 to 16 mg/ml. These are the most abundant component of Ig in the body fluids particularly the blood vessels where they combat microorganisms and their toxins. IgG are the only antibody that can get across the placenta and so provide the major line of defence during the first few weeks of the life of a foetus. IgG also diffuse very readily from the blood vessels into the body spaces. When IgG molecules attach to microorganisms, the susceptibility of the latter for phagocytosis increases. In a germ free environment, the IgG concentration of the serum is very

low and increases with infection. IgG are the major antibody synthesised during the secondary response, their synthesis being entirely governed by the antigenic situation.

All the IgG molecules are seemingly identical. The most fascinating thing is that there are an infinitesimal number of antigens, with each pathogenic organism producing several of them. During the course of our lifetime we develop immunity against a very large number of infections, some on a long-term basis and some ephemeral but repeated infection renewing our ability to combat the disease. The key to understanding this versatility of the IgG molecule lies in the fact that the IgG molecule has a part that is invariable and this gives the basic characteristics for it to function as an antibody. Another part of the IgG molecule is variable in its amino acid content and sequence and this gives the molecule the ability to be a specific antibody against a particular antigen. This is nothing surprising. Almost all proteins have variable and invariable regions.

Immunoglobulin E (IgE): The molecular weight of IgE is about 200,000 and they form only 0.002% of the total Ig with a serum concentration of 17 to 450 ng/ml. IgE protect the external mucosal surfaces of the body through plasma factors. Pathogens crossing the IgA line combine with IgE molecules specific to them. This results in the release of amines (eg. histamine) that increase the permeability of the blood vessels causing the symptoms of allergy. The release of amines is due to a degranulation of the mast cells. The level of IgE is raised during parasitic infections but the importance of IgE lies with atopic allergy.

Immunoglobulin D (IgD): IgD have a molecular weight of about 1,85,000 and form only about 1% of the total Ig. They occur at a concentration of 0 to 0.4% of the normal serum. They are present only on the surface of the lymphocytes along with IgM. The IgD are susceptible to enzyme degradation and so have a very short life span (2.8 days) in the plasma. IgD have the highest carbohydrate content (13%) of all Ig. The exact function of IgD is not understood.

Polyclonal Antibodies

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

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

The aim is to produce high titre, high affinity antibodies. Today these polyclonal antibodies are used extensively for research purposes in many areas of biology, such

as immunoprecipitation, histochemistry, enzyme linked immunosorbent assays (ELISA), diagnosis of disease, immunoturbidimetric methods, western blots and Biochip technology. Polyclonal antibodies are ideally suited for use in sandwich assays as second stage antigen detectors.

Often polyclonal antibodies will be tagged with reporter molecules such as horseradish peroxidase (HRP) or alkaline phosphatase (AP) so that under specific conditions the antibodies presence can be detected by light or colour changes.

Polyclonal antibodies

Facts:

- Recognize multiple epitopes on any one antigen. Serum obtained will contain a heterogenous complex mixture of antibodies of different affinity.
- Polyclonals are made up mainly of IgG subclass.
- Peptide immunogens are often used to generate polyclonal antibodies that target unique epitopes, especially for protein families of high homology.

Antibody production:

- Inexpensive and relatively quick to produce.
- Production is less complex compared with monoclonal antibodies.

Advantages:

Polyclonal antibodies recognize multiple epitopes on any one antigen. This has the following advantages:

- High affinity: polyclonals amplify signal from a target protein with low expression level, as the target protein will bind more than one antibody molecule on the multiple epitopes. However, this is disadvantageous for quantification experiments (eg in flow cytometry) as it generates inaccurate results.
- Recognize multiple epitopes to give better results in immunoprecipitation (IP) and chromatin immunoprecipitation (ChIP).
- More tolerant of minor antigen changes (eg polymorphism, heterogeneity of glycosylation or slight denaturation) than monoclonals.
- Can identify proteins of high homology to the immunogen protein, and can be used to screen for the target protein in species other than that of the immunogen.
- Often the preferred choice for detecting denatured proteins.
- Multiple epitopes generally provide more robust detection.

Disadvantages:

- Prone to batch-to-batch variability.
- Produce large amounts of non-specific antibodies, which can create background signal in some applications.
- Multiple epitopes make it important to check immunogen sequence for cross-reactivity.
- Not useful for probing specific domains of antigen because antiserum will usually recognize many domains.

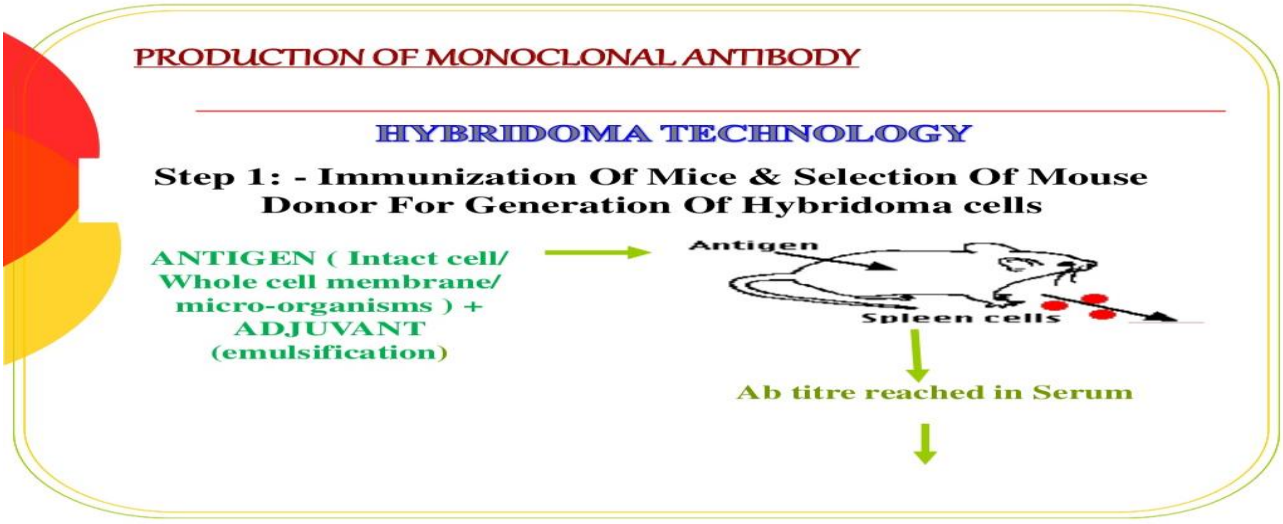
Monoclonal Antibodies

Monoclonal antibodies represent a single B lymphocyte generating antibodies to one specific epitope. B-cells can be isolated easily from the spleen and lymph nodes of immunised animals; however, these cells have a limited life span, and can only divide a limited number of times, coined the 'Hayflick limit'. This prohibits the culture of B-cells directly. For an antibody to be useful in research or industry, it must be readily available in large quantities. Due to the Hayflick limit, this would not be possible using B-cells cultured *in vitro* as they would eventually stop dividing and the population would die out.

Consequently, in 1975 Kohler and Milstein developed a technology to fuse immortal heteromyleoma cells with lymphocytes, using poly ethylglycol (PEG) to break down cell membranes and allow mixing of the genetic material from both cell types. The resulting cell type is called a hybridoma. This hybridoma takes on the characteristics of both the lymphocyte and heteromyeloma cell, creating an immortal cell with the ability to produce antibody. As the new cell line hybridoma is a product of the fusion of one heteromyeloma cell with one B-cell, the culture only ever has one antibody within the supernatant which, when purified, is called a Monoclonal antibody. This technology allows scientists to extract and purify one antibody from the complex mixture of antibodies present in the *in vivo*polyclonal response. This cell line, once stabilised via single cell cloning, can be frozen and stored indefinitely under liquid nitrogen, allowing the antibody to be produced *in vitro*, in large quantities when required.

Monoclonal antibodies can be raised against many targets. Specific antibody characteristics can be identified and selected e.g. sensitivity requirements and cross reactivity levels can be specified and monoclonal antibodies screened to identify any cell lines exhibiting the required characteristics.

Monoclonals can also be generated to cross react with a group of molecules, for example the tricyclic anti-depressants have a similar overall structure with substitutions of differing atoms into the cyclic structure. This is very useful in drug detection when many possible combinations of the drug are to be tested in a patient.



Step 2: - Screening Of Mice For Antibody Production

After several weeks of immunization

Serum Antibody Titre Determined
(Technique: - ELISA / Flow cytometery)

Titre too low

BOOST
(Pure antigen)

Titre High

Cell fusion performed

Step 3: - Preparation of Myeloma Cells

Myeloma Cells
Immortal Tumor Of Lymphocytes

+ HAT Medium

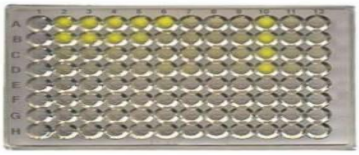
Myeloma Cells
HGPRT-
High Viability & Rapid Growth

Step 5: - Cloning of Hybridoma Cell Lines by " Limiting Dilution" or soft agar.

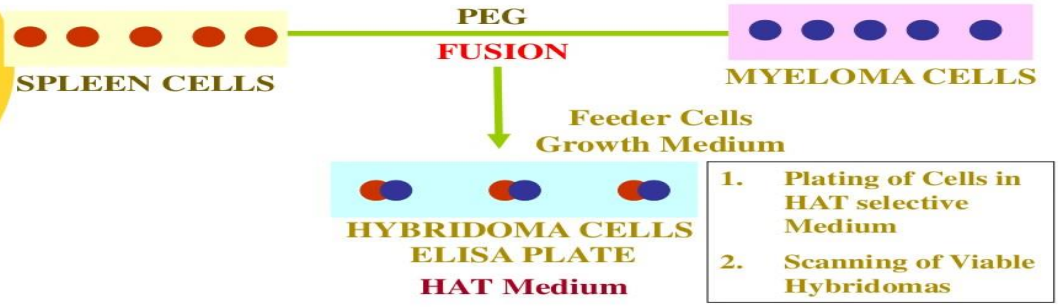
A. Clone Each +ve Culture

B. Test Each Supernatant for Antibodies

C. Expand +ve Clones

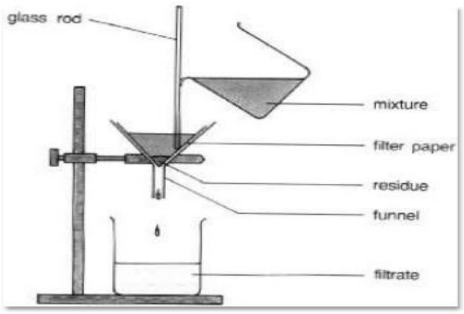


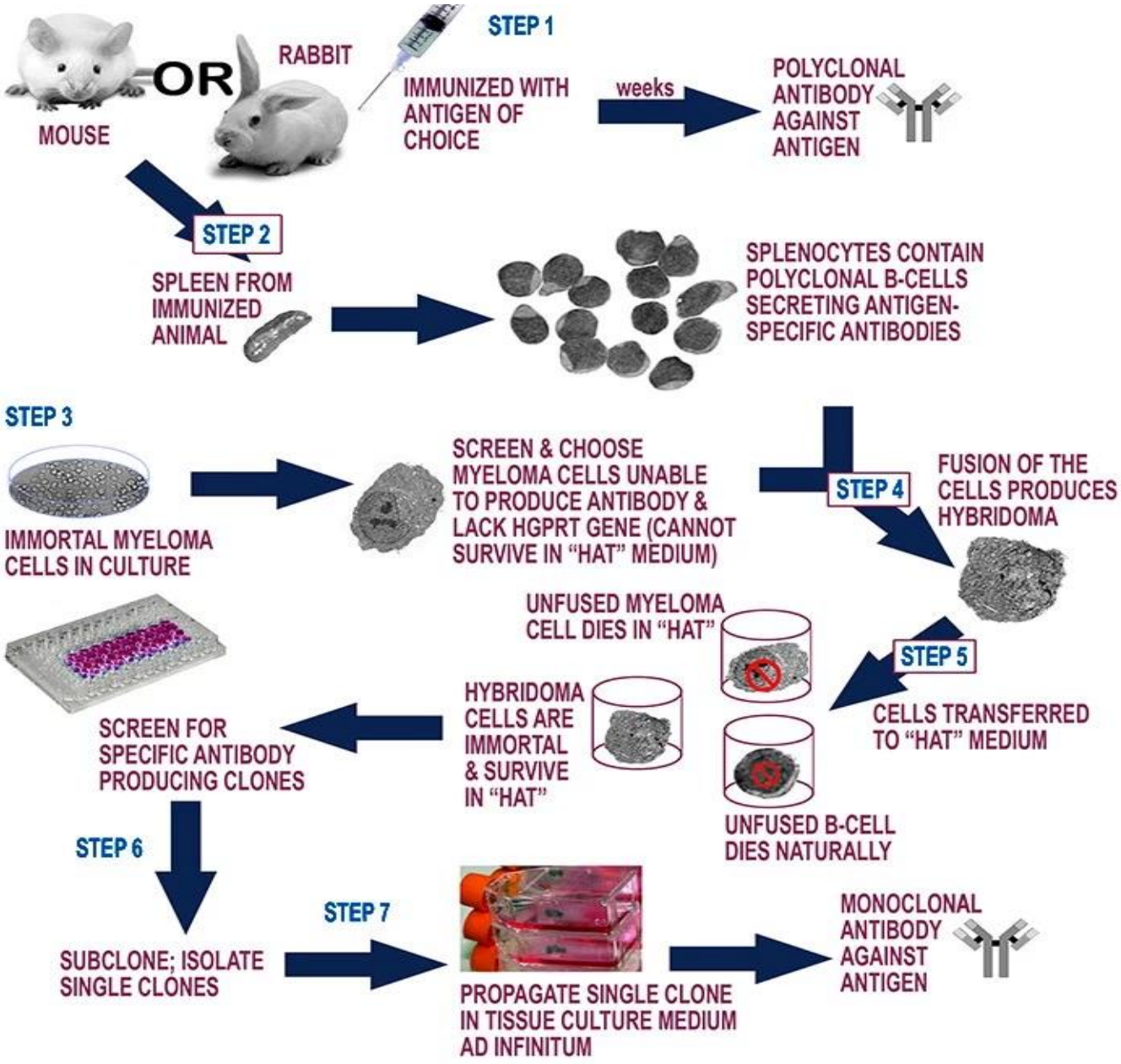
Step 4: - Fusion of Myeloma Cells with Immune Spleen Cells & Selection of Hybridoma Cells



PURIFICATION TECHNIQUES

- Cells, cell debris, lipids, and clotted material are first removed, typically by filtration with a 0.45 um filter.
- Chromatography
  - Affinity chromatography: IgG antibodies using protein A agarose
  - Anion exchange chromatography: Endotoxins and DNA
  - Gel filtration:high and low molecular wt MABs such as aggregates and small fragments





Monoclonal antibodies

Facts:

- High specificity. detect only one epitope on the antigen.
- Consist of only one antibody subtype (eg IgG1, IgG2, IgG3). When a secondary antibody is required for detection an antibody against the correct subclass should be chosen.

Antibody production:

- High technology required.
- Training is required for the technology used.
- Long timeframe for hybridoma production.

Advantages: Hybridomas are a constant and renewable source once created, and all batches will be identical, increasing consistency and standardization of experimental procedures and results.

Monoclonals detect one epitope per antigen. This has the following advantages:

- Less background from staining of sections and cells. Specifically detecting one target epitope means they are less likely to cross-react with other proteins.
- Due to their high specificity, monoclonal antibodies are excellent as the primary antibody in an assay and will often give significantly less background staining than polyclonal antibodies.
- Homogeneity of monoclonal antibodies is very high relative to polyclonals. If experimental conditions are kept constant, results from monoclonal antibodies will be highly reproducible between experiments.
- High specificity makes them extremely efficient for the binding of an antigen within a mixture of related molecules, such as during affinity purification.

Disadvantages:

- May be too specific to detect across a range of species.

More vulnerable to the loss of epitope through chemical treatment of the antigen than polyclonal antibodies. This can be offset by pooling two or more monoclonal antibodies to the same antigen (eg cocktail antibodies).

Polyclonal antibodies	**Monoclonal antibodies**
Inexpensive to produce	Expensive to produce
Skills required for production are low	Training is required for the technology used
Relatively quick to produce	Hybridomas take a relatively long time to produce
Generate large amounts of non-specific antibodies	Generate large amounts of specific antibodies
Recognize multiple epitopes on any one antigen	Recognize only one epitope on an antigen
Can have batch-to-batch variability	Once a hybridoma is made, it is a constant and renewable source
	No or low batch-to-batch variability

Antigen: a substance, usually a protein, that stimulates the immune system to produce a set of specific antibodies and that combines with an antibody specific to itself, at a

specific binding site; differs from immunogen in that it is not involved in eliciting cellular response and in that it can complex with antibodies.

Immunogen: a substance, usually a protein, that elicits a cellular immune response, and/or antibody production; differs from antigen in that it mainly elicits cellular response but does not complex with an antibody.

Hapten: a low-molecular weight non-protein molecule which contains an antigenic determinant but which is not itself antigenic unless it complexes with an antigenic carrier, such as a protein; once an antibody is available, it can readily recognise the hapten, even without the carrier, and bind with it. To be antigenic, the hapten must bind to an exogenous protein carrier.

Epitope: a part of a protein molecule that acts as an immunogenic/antigenic determinant, and so determines specificities; a macromolecule, such as a protein, may contain many different epitopes, each capable of stimulating the production of specific antibodies, each with a correspondingly specific binding site.

Vaccines

A vaccine is an agent, sourced from the pathogen, and is deliberately introduced into the mammalian system in order to impart a 'memory' of the pathogen or its pathogenic component. The memory is imparted on the first contact of the vaccine with the mammalian immune system. Vaccine usually contains the modified pathogenic organism or a protein or a low molecular weight non-protein compound (hapten) conjugated with the protein, obtained from the pathogen.

Vaccines contain antigens (that elicit the production of antibodies), or immunogens (that trigger thecellular component of immune response). In the event of an encounter with the corresponding antibodies, only the antigens can bind with the antibodies, and form an antigen-antibody complex that neutralises the harmful effects of the antigens or the organisms that produce them.

Since vaccines employ a part of the chemical machinery of the organisms themselves, pathogens cannot easily acquire resistance to vaccines, as they do for antibiotics and chemical therapeuticagents.

Vaccination/Immunisation

The process of the deliberate introduction of a vaccine into the organism is vaccination, for which the term inoculation is also often used. Since vaccination immunises the organism, the process is also called immunisation.

When an organism is vaccinated, the immune system is readied to show an immune response by way producing antibodies against the pathogen, in the event of a second encounter with the pathogen, basing on the memory imparted by the vaccine used for the first encounter.

Immunotherapy

Immunotherapy differs from vaccination in that in the former antibodies isolated from an immunised organism (polyclonal or monoclonal immunoglobulin antibodies or cytokines) is used to cure the patient. Immunotherapy becomes essential when there is no time to prepare the patient through vaccination or when the patient is physically and/or physiologically not competent to respond to vaccination.

Composition of vaccines

Vaccines are suspensions, in saline or buffered saline, of weakened pathogenic organisms or their fractions or the proteins they secrete, which have the potential to cause a disease. A virulent organism cannot be used as a vaccine.

Adjuvants

Antigens often need to be coupled with an adjuvant, which is a compound that holds the antigen and releases it slowly over a longer period of time. The most commonly used immunological adjuvant in experimental systems is Freund's Complete Adjuvant, which contains mineral oil and heat killed mycobacteria. The bacteria are intended to heighten immunological response, but may produce hypersensitivity in many patients. The mineral oil also may prove to be harmful. Hence FCA is not normally used in human immunisation schedules. Aluminium hydroxide is human safe but is a pooradjuvant. Some plant saponins are now projected as efficient and human safe adjuvants. There are some effective and safe synthetic adjuvants, but their composition is a trade secret.

Types of vaccines

a) Inactivated vaccines: The pathogen is killed using heat or formalin, as for example, typhoid or Salk poliomyelitis vaccines.

b) Attenuated vaccines: The pathogen is weakened (attenuated) by aging or altering growthconditions, but is alive, as in the case of measles, mumps and rubella vaccines. There is some risk of the concerned virus becoming virulent.

c) Avirulent organisms: A non-pathogenic strain of a pathogenic organism is used as a vaccine, as in BCG (Bacillus Calmette Guerin) vaccine against *Mycobacterium tuberculosis,* the tuberculosis bacterium.

d) Toxoids: The toxin from the pathogen is used as an antigen to produce the vaccine. The severity of the toxicity of the antigen is reduced by treating it with aluminium salts while preparing the 'toxoid', as in the case of diphtheria and tetanus.

In the case of allergy, the allergenic proteins from pollen and other allergenic material are isolated and used to immunise the patient.

e) Acellular vaccines: Only the antigenic component of the organism is used instead of the whole organism, as in Haemophilus influenza B vaccine.

f) Subunit vaccines: Genetic engineering techniques have now made it possible to use as a vaccine only a part of an organism that is adequate to stimulate the immune response. An appropriate segment of genetic material is isolated from the pathogens and introduced into bacteria or yeasts, to transcribe and translate the inserted foreign DNA. The product is used as a vaccine, as in the case of Hepatitis B vaccine. These vaccines cannot cause the disease even in patients whose immunological system is impaired (immunocompromised) patients.

g) DNA vaccines: Described as the third vaccine revolution, DNA vaccines are an offshoot of gene therapy. Selected segments of DNA, when introduced into the patients system synthesise and deliver proteins that are needed to replace the defective enzyme system or tag a cell for destruction. Viruses or lipid vehicles are used to

deliver the DNA into the cells. This recent technology is being tried to produce vaccines against HIV, by a direct injection of plasmid borne DNA.

Herd immunity

Use of vaccines to prevent disease in communities is herd immunity, which affords protection by decreasing the number of susceptible people in a community, with time. This basically constitutes mass immunisation. Polio vaccination programmes now target an enormous number of children throughout the world, to eradicate polio, as was done for smallpox earlier.

Booster doses

The effectiveness of certain vaccines is life long as for example of smallpox, measles, mumps and rubella. Attenuated vaccines normally afford life long immunity. But in the case of certain others, the effectiveness is short lived and the immune system needs to be re-educated through periodicalbooster doses. The vaccine is administered one or more times, with appropriate time gaps, after the initial vaccination, to boost to the immune system to produce adequate quantities of antibodies against the intended pathogen. Toxoid vaccines require a booster every ten years or so. Boosterdoses are also needed in case of inactivated or acellular vaccines, which are very safe, as they cannot cause the disease.

Multiple vaccines

While most vaccines contain antigens of a single pathogen, there is a practice of multiple vaccines, which combine antigens of more than one pathogen. For example, diphtheria, tetanus and pertussis are administered together as DTP vaccination.

Vaccine administration

Vaccines are administered, as injections (DTP), or dermally (smallpox, anthrax), or orally (polio) or as a nasal spray (influenza virus).

Edible vaccines

Now transgenic plants are being developed through genetic engineering techniques, where the vaccine is synthesised in the edible part of a food plant (edible vaccines). Transgenic bananas, melons, and tomatoes are choice candidates for carrying edible subunit vaccines, as for example against rabies. The obvious advantage is the ease of transportation and storage of the vaccine bearing material and administration without technical support. Conventional vaccination programmes in many countries are seriously handicapped due to a lack of equipment for storage and transport of the vaccines and the shortage of paramedical staff to administer the vaccines.

Recombinant Vaccines

Vaccine antigens may also be produced by genetic engineering technology. These products are sometimes referred to as recombinant vaccines. Four genetically engineered vaccines are currently available in the United States. Hepatitis B and human papillomavirus (HPV) 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 produces pure hepatitis B surface antigen or HPV capsid protein when it grows. Live typhoid vaccine (Ty21a) is Salmonella Typhi bacteria that have been genetically

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

Polysaccharide Vaccines

Polysaccharide vaccines are a unique type of inactivated 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 disease, meningococcal disease, and Salmonella Typhi. A pure polysaccharide vaccine for Haemophilus influenzae type b (Hib) is no longer available in the United States.

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, includingpolysaccharide 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.

In the late 1980s, it was discovered that 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 Hib. A conjugate vaccine for pneumococcal disease was licensed in 2000. A meningococcal conjugate vaccine was licensed in 2005.

Inactivated Vaccines

Inactivated vaccines are produced by growing the bacterium or virus in culture media, then inactivating it with heat and/ or chemicals (usually formalin). In the case of fractional vaccines, the organism is further treated to purify only those components to be included in the vaccine (e.g., the polysaccharide capsule of pneumococcus.)

Inactivated vaccines are not alive and cannot replicate. The entire dose of antigen is administered in the injection. These vaccines cannot cause disease from infection, even in an immunodeficient person. Inactivated antigens are less affected by circulating antibody than are live agents, so they may be given when antibody is present in the blood (e.g., in infancy or following receipt of antibody-containing blood products.)

Inactivated vaccines always require multiple doses. In general, the first dose does not produce protective immunity, but "primes" the immune system. A protective immune response develops after the second or third dose. In contrast to live vaccines, in which the immune response closely resembles natural infection, the immune response to an inactivated vaccine is mostly humoral. Little or no cellular immunity results. Antibody titers against inactivated antigens diminish with time. As a result, some inactivated vaccines may require periodic supplemental doses to increase, or "boost," antibody titers.

Currently available whole-cell inactivated vaccines are limited to inactivated whole viral vaccines (polio, hepatitis A, and rabies). Inactivated whole virus influenza vaccine and whole inactivated bacterial vaccines (pertussis, typhoid, cholera, and plague) are no longer available in the United States. Fractional vaccines include subunits (hepatitis B, influenza, acellular pertussis, human papillomavirus, anthrax) and toxoids (diphtheria, tetanus.) A subunit vaccine for Lyme disease is no longer available in the United States.

Live Attenuated Vaccines

Live vaccines are derived from "wild," or disease-causing, viruses or bacteria. These wild viruses or bacteria are attenuated, or weakened, in a laboratory, usually by repeated culturing. For example, the measles virus used as a vaccine today was isolated from a child with measles disease in 1954. Almost 10 years of serial passage using tissue culture media was required to transform the wild virus into attenuated vaccine virus.

To produce an immune response, live attenuated vaccines must replicate (grow) in the vaccinated person. A relativelysmall dose of virus or bacteria is administered, which replicates in the body and creates enough of the organism to stimulate an immune response. Anything that either damages the live organism in the vial (e.g., heat, light) or interferes with replication of the organism in the body (circulating antibody) can cause the vaccine to be ineffective.

Although live attenuated vaccines replicate, they usually do not cause disease such as may occur with the "wild" form of the organism. When a live attenuated vaccine does cause "disease," it is usually much milder than the natural disease and is referred to as an adverse reaction.

The immune response to a live attenuated vaccine is virtually identical to that produced by a natural infection. The immune system does not differentiate between an infection with a weakened vaccine virus and an infection with a wild virus. Live attenuated vaccines produce immunity in most recipients with one dose, except those administered orally. However, a small percentage of recipients do not respond to the first dose of an injected live vaccine (such as MMR or varicella) and a second dose is recommended to provide a very high level of immunity in the population.

Live attenuated vaccines may cause severe or fatal reactions as a result of uncontrolled replication (growth) of the vaccine virus. This only occurs in persons with immunodeficiency (e.g., from leukemia, treatment with certain drugs, or human immunodeficiency virus (HIV) infection).

A live attenuated vaccine virus could theoretically revert to its original pathogenic (disease-causing) form. This is known to happen only with live (oral) polio vaccine.

Active immunity from a live attenuated vaccine may not develop because of interference from circulating antibody to the vaccine virus. Antibody from any source (e.g., transplacental, transfusion) can interfere with replication of the vaccine organism and lead to poor response or no response to the vaccine (also known as vaccine failure). Measles vaccine virus seems to be most sensitive to circulating antibody. Polio and rotavirus vaccine viruses are least affected.

Live attenuated vaccines are fragile and can be damaged or destroyed by heat and light. They must be handled and stored carefully.

Currently available live attenuated viral vaccines are measles, mumps, rubella, vaccinia, varicella, zoster (which contains the same virus as varicella vaccine but in much higher amount), yellow fever, rotavirus, and influenza (intranasal). Oral polio vaccine is a live viral vaccine but is no longer available in the United States. Live attenuated bacterial vaccines are bacille Calmette-Guérin (BCG—not currently available in the U.S.) and oral typhoid vaccine.

Safety of vaccines

By and large vaccination programmes have proven to be reasonably safe for the human populations. However, at certain times complications may arise mostly due to an incorrect handling of the vaccines and/or vaccination or due to individual metabolic deficiencies. In spite of all that is adverse in vaccination, immunisation is one of the most efficient means of disease prevention, particularly in large sections of the human population. In the case of HIV and epidemic diseases and even cancer, immunisation is probably the only hope.