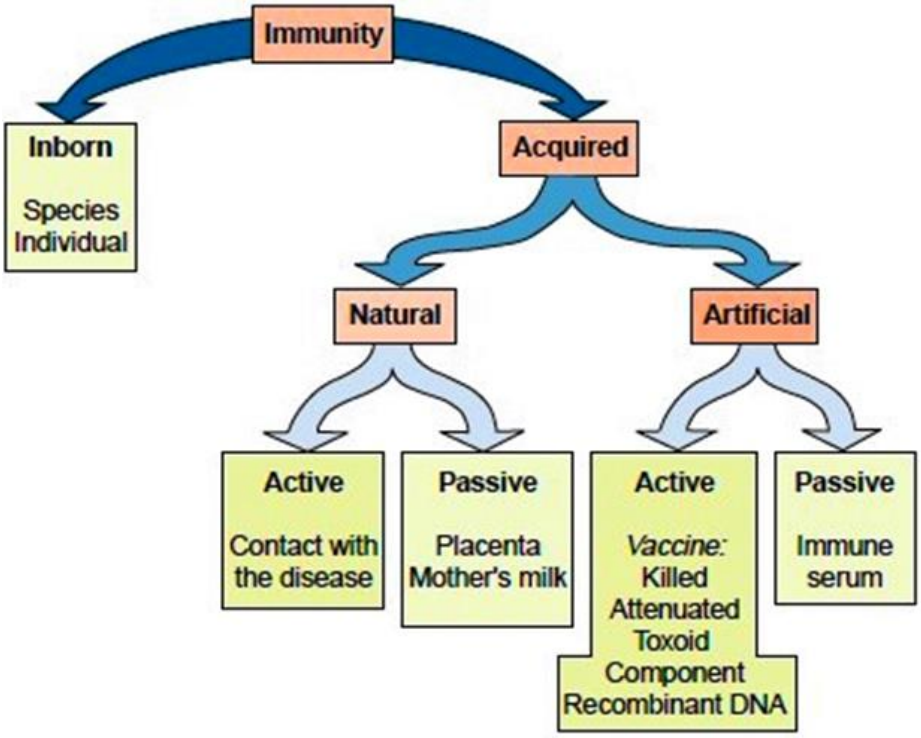
CHAPTER 6: DISEASE AND IMMUNITY

Immunization

Immunity is network of cells, tissues, and organs that work together to defend the body against attacks by "foreign" invaders. Immunity to infectious microorganisms can be achieved by active or passive immunization. Immunization is a means of providing specific protection against damaging pathogens by stimulating an organism's immune system. Immunity can be acquired either by natural processes or by artificial means.



Active immunity: immunity produced by the body following exposure to antigens.

- Naturally acquired active immunity is protective immune response against the pathogen.
- Artificially acquired active immunity: achieved by administering Vaccines of live (attenuated) organisms or dead pathogens or their components or secreted toxins (detoxified toxins).

Passive immunity: by transfer of serum or Ig (Ab) from immune donor to a non-immune individual

- Naturally acquired passive immunity: immunity is transferred from mother to fetus through placental transfer of IgG or IgA.
- Artificially acquired passive immunity: by transferring antibodies from immune individual to unimmunized one E.g., diphtheria, tetanus, measles, rabies, poisoning etc.

Immunodeficiency

Immunodeficiency is the failure of the immune system to protect against disease or malignancy. Individuals with immunodeficiency are susceptible to a variety of infections (especially by opportunistic microorganisms; bacterial, viral, and protozoan infections) and the type of infection depends on the nature of immunodeficiency. A defect in the early hematopoiesis leads to general immune defects and subsequent susceptibility to infections and often fatal but very rare. It can be treated successfully by bone marrow transplantation.

I. Primary Immunodeficiency: Are inherited defects of the immune system or caused by genetic or developmental defects in the immune system. These defects are present at birth but may show up later on in life. These defects may be in the specific or non-specific immune mechanisms. If the lymphoid progenitor cells are defective, then both the T and B cell lineages are affected and result in the severe combined immunodeficiency (SCID). Diagnosis is based on enumeration of T and B cells and immunoglobulin measurement. Severe combined immunodeficiency can be treated with a bone marrow transplant. T cell disorders affect both cell-mediated and humoral immunity making the patient susceptible to viral, protozoal and fungal infections. IgA deficiency (commonest): patients are very susceptible to gastrointestinal, eye, nasopharyngeal infections, high incidence of autoimmune diseases and lymphoid malignancies. Laboratory diagnosis is based on IgA measurement.

II. Secondary or acquired immunodeficiency (associated with infections): the loss of immune function as a result of exposure to disease agents, environmental factors, immunosuppression, or aging. Bacterial, viral (AIDS), protozoan, helminthic and fungal infections may lead to B cell, T cell and macrophage deficiencies. Immuno-deficiencies associated with aging include a progressive decrease in thymic cortex, reduction in the size of thymus, a decrease in suppressor cell function and increase in auto-reactivity, a decrease in CD4 cells functions. Other conditions in which secondary immuno-deficiencies occur are sickle cell anemia, diabetes mellitus, protein malnutrition, burns, alcoholic cirrhosis, renal malfunction, *etc*.

6.1. Immunity in parasitic infection and viral infections

1. Immunity against parasites infection

Evasion mechanisms of protozoan parasite

For the protozoans which live within the bloodstream, the humoral antibody is most effective. For intracellular protozoans, cell-mediated immune reactions are effective.

Antigenic masking: the parasite becomes coated with host components (antibody) and so fails to be recognized as foreign. E.g. trypanosomes

Antigen sheding: Some protozoans shed their antigen coats either spontaneously or after binding with specific antibodies. E.g. *E. histolytica*

Blocking: non-cytotoxic antibody combines with parasite antigens and inhibits the binding of cytotoxic antibodies or cells.

Intracellular Location: some parasite passes part of its life cycle in an intracellular location, for example, in erythrocytes or macrophages, in which it is sheltered from intracellular digestion and from the cytotoxic action of antibody and/or lymphocytes. *Plasmodium* grows in hepatocytes and then in RBCs, and *Leishmania* and *Toxoplasma* organisms are capable of growing in macrophages; all are protected from external immune threats.

Mimicry of Complement Structures: during evolution alongside their obligate hosts, some pathogens share structural or genetic similarities (antigenic cross-reactivity) with complement proteins or receptors. Such "molecular mimicry" enables the pathogens to avoid destruction by complement. E.g., *Schistosoma mansoni,* it produce mimicking protein and can bind and cleave C4 and C3; and it encodes a protein mimicking CD59 that inhibits membrane attack.

***Antigenic variation*:** alters their surface antigens during the course of infection and thus evading the host's immune responses. E.g., *African trypanosomes* have 1,000 different genes coding for surface antigens and *Giardia lamblia* have number of different gene coding for surface proteins.

***Immunosuppression*:** parasite may cause immunosuppression, reducing the host's immune response either to the parasite specifically or to foreign Ags in general. E.g., *Trypanosoma, Leishmania.*

2. Immunity against viral infection

The innate immune response: This is primarily through the induction of type I interferons and the activation of NK cells. Type I interferons induce an antiviral response or resistance (inhibit) to viral replication.

Antibodies against viruses: viruses initiate infection by binding to specific host-cell membrane molecules. If antibody to the viral receptor is produced, it can block infection altogether by preventing the binding of viral particles to host cells. E.g., IgA block viral attachment to mucosal epithelial cells.

***Cell-Mediated Immunity against viruses*:** Once an infection is established, cell-mediated immune mechanisms are most important in host defense. CD8 and CD4 cells are the main components, which produce cytokines to induce an antiviral state in cells and to eliminate virus-infected self-cells not to be potential sources of new virus.

Evasion mechanisms of viruses

Virus can evade immune by altering its antigenic type (surface structures), this is called Antigenic variation. E.g. Antigenic variation among rhinoviruses (common cold) is responsible for inability to produce an effective vaccine for colds. HIV undergoes a greater antigenic variation. Influenza virus constantly changes its antigens and cause repeated epidemics. Number of viruses encodes proteins which interfere at host defenses and enable viruses to replicate more effectively, E.g., *Hepatitis C virus*. Some viruses inhibit antigen presentation by infected host cells. These viruses synthesize a protein shortly after replication, which very effectively inhibits the human transporter molecule needed for Ag processing, then blocks Ag delivery to MHC receptors and T cells. E.g. *Herpes simplex viruses*

A large number of viruses evade the immune response by causing generalized immunosuppression. These viruses can either directly infect and destroy the immune cells or alter their function or imbalance production. E.g. *Measles virus*, HIV, etc.

Some viruses stop replication or hide not to cause immunity E.g., HIV, or initiate general immunity response.

6.2. Immune evasion mechanisms by pathogens

Infectious agents can cause recurrent or persistent disease by avoiding normal host defense mechanisms or by subverting them to promote their own replication. There are many different ways of evading or subverting the immune response.

i) Antigenic variation
ii)Latency
iii)Resistance to immune effector mechanisms, and
iv)Suppression of the immune response.

In some cases, the immune response is part of the problem; some pathogens use immune activation to spread infection, others would not cause disease if it were not for the immune response. Each of these mechanisms teaches us something about the nature of the immune response and its weaknesses, and each requires a different medical approach to prevent or to treat infection.

A) Antigenic variation- allows pathogens to escape from immunity

One way in which an infectious agent can evade immune surveillance is by altering its antigens; this is particularly important for extracellular pathogens, against which the principal defense is the production of antibody against their surface structures.

There are three ways in which antigenic variation can occur.

The virus might be in danger of running out of potential hosts if it had not evolved two distinct ways of changing its antigenic type; ***the first*** of these, antigenic drift, is caused by point mutations in the genes encoding hemagglutinin and a second surface protein, neuraminidase.

Major influenza pandemics resulting in widespread and often fatal disease occur as the result of ***the second*** process, which is termed antigenic shift. This happens when there is reassortment of the segmented RNA genome of the influenza virus and related animal influenza viruses in an animal host, leading to major changes in the hemagglutinin protein on the viral surface.

The third mechanism of antigenic variation involves programmed rearrangements in the DNA of the pathogen.

B) Latency- some viruses persist *in vivo* by ceasing to replicate until immunity wanes

Viruses usually betray their presence to the immune system once they have entered cells by directing the synthesis of viral proteins, fragments of which are displayed on the surface MHC molecules of the infected cell, where they are detected by T lymphocytes. To replicate, a virus must make viral proteins, and rapidly replicating viruses that produce acute viral illnesses are therefore readily detected by T cells, which normally control them. Some viruses, however, can enter a state known as latency in which the virus is not being replicated. In the latent state, the virus does not cause disease but, because there are no viral peptides to flag its presence, the virus cannot be eliminated. Such latent infections can be reactivated and this results in recurrent illness.

C) <u>Resistance to immune effector mechanisms</u>- some pathogens resist destruction by host defense mechanisms or exploit them for their own purposes

Some pathogens induce a normal immune response but have evolved specialized mechanisms for resisting its effects. For instance, some bacteria that are engulfed in the normal way by macrophages have evolved means of avoiding destruction by these phagocytes; indeed, they use macrophages as their primary host.

D) <u>Suppression of the immune response</u>- immunosuppression or inappropriate immune responses can contribute to persistent disease

Many pathogens suppress immune responses in general. For example, staphylococci produce toxins, such as the staphylococcal enterotoxins and **toxic shock syndrome toxin-1** (Toxic Shock Syndrome, in *Case Studies in Immunology*), that act as super antigens. Super antigens are proteins that bind the antigen receptors of very large numbers of T cells, stimulating them to produce cytokines that cause significant suppression of all immune responses. The stimulated T cells proliferate and then rapidly undergo apoptosis, leaving a generalized immunosuppression together with the deletion of many peripheral T cells.

Many other pathogens cause mild or transient immunosuppression during acute infection. These forms of suppressed immunity are poorly understood but important, as they often make the host susceptible to secondary infections by common environmental microorganisms. A crucially important example of immune suppression follows trauma, burns, or even major surgery. The burned patient has a clearly diminished capability to respond to infection, and generalized infection is a common cause of death in these patients.

6.3. Application of immunology

1. Diagnostic
2. Vaccinations
3. Immunotherapy

6.3.1. Diagnosis

Diagnostic immunology involves using antibodies to acquire clinical data using procedures such as:

A. Precipitation Reactions

- ✓ The formation of insoluble Ab:Ag complexes

B. Agglutination

- ✓ The formation of visible Ab:Ag aggregates

C. Neutralization Reactions

- ✓ Inhibition of cytopathic effects due to antibody binding

D. Fluorescent Antibody Staining

- ✓ Reveal the presence of specific pathogens

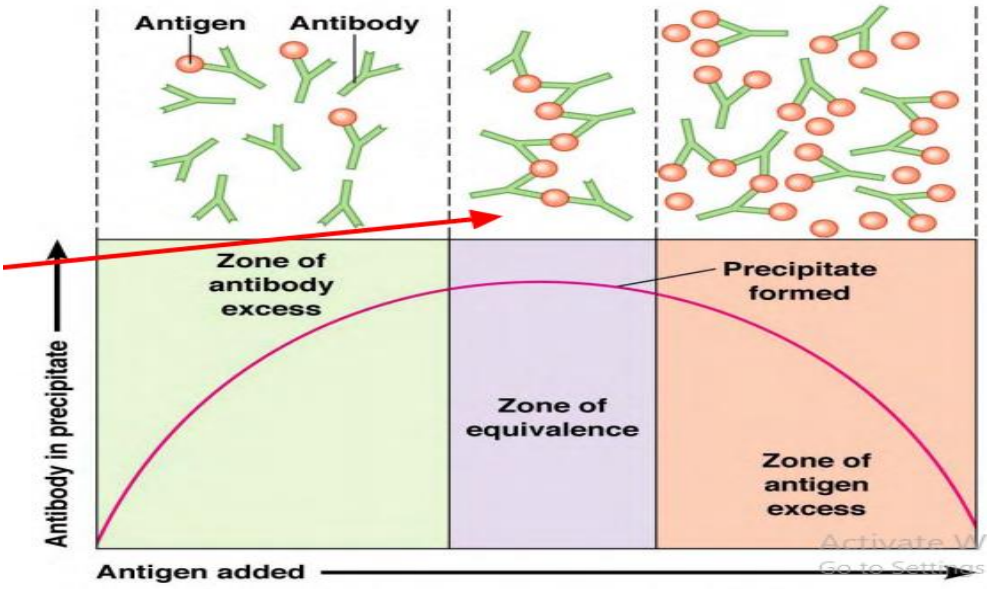
E. Enzyme-Linked Immunosorbent Assay (ELISA)

- ✓ Automated technique revealing presence of Ab or Ag

A. Precipitation Reactions - the Nature of Immunoprecipitation

Soluble protein antigen and antibody will form insoluble complexes when mixed in the right proportions:

- ✓ Excess antibody or antigen will result in no insoluble material
- ✓ ~equal proportions of antibody and antigen results in an insoluble complex (precipitate) of interconnected antibody complexes
- ✓ **usually works only with polyclonal antibody**

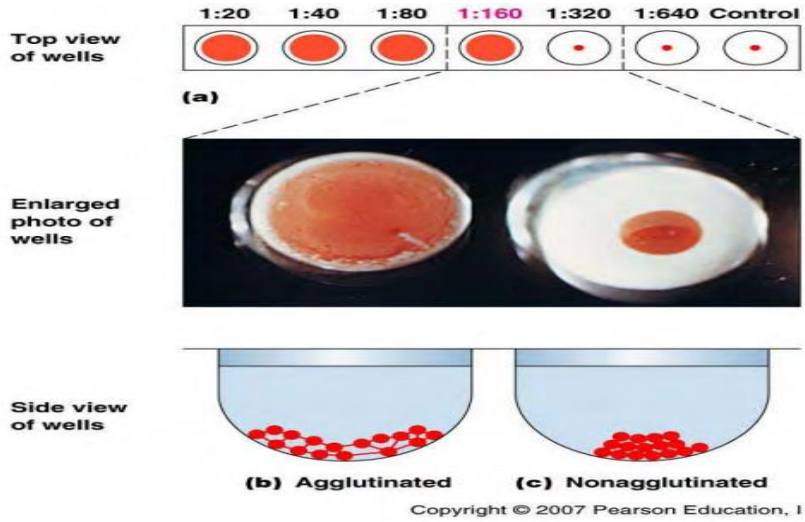


B. Agglutination

a) Direct Agglutination: Test Large, complex antigens (e.g, viruses or bacteria) can be agglutinated by specific antibody:

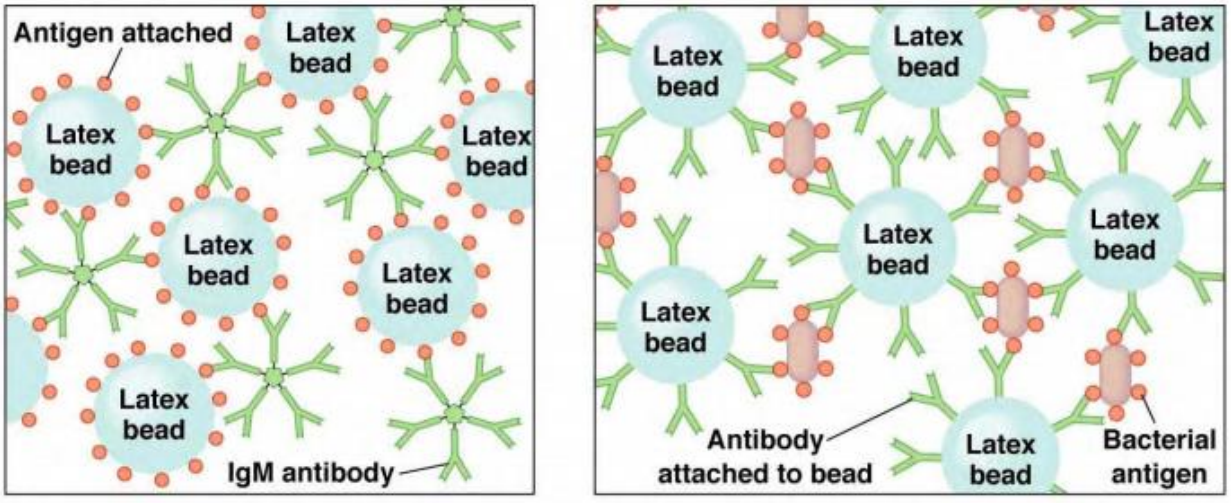
- ✓ Does not require precise proportions as with immuno-precipitation.

- ✓ Can use polyclonal or monoclonal antibody.
- ✓ Allows detection of antibody to specific antigens as well as the determination of "antibody titer" by serial dilution.



b) Indirect Agglutination involves the same basic principles as with the direct agglutination, except uses multiple copies of specific protein antigen or antibody attached to a synthetic particle (i.e., a "bead"):

- ✓ Agglutination occurs due to multiple copies of epitope or Ab on same particle.



(a) Reaction in a positive indirect test for antibodies. When particles are coated with antigens, agglutination indicates the presence of antibodies, such as the IgM shown here.

(b) Reaction in a positive indirect test for antigens. When particles are coated with monoclonal antibodies, agglutination indicates the presence of antigens.

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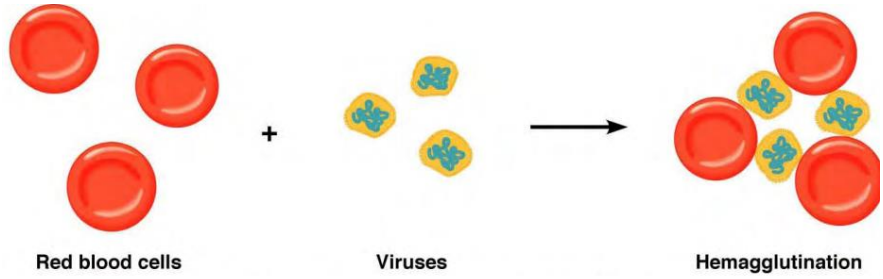
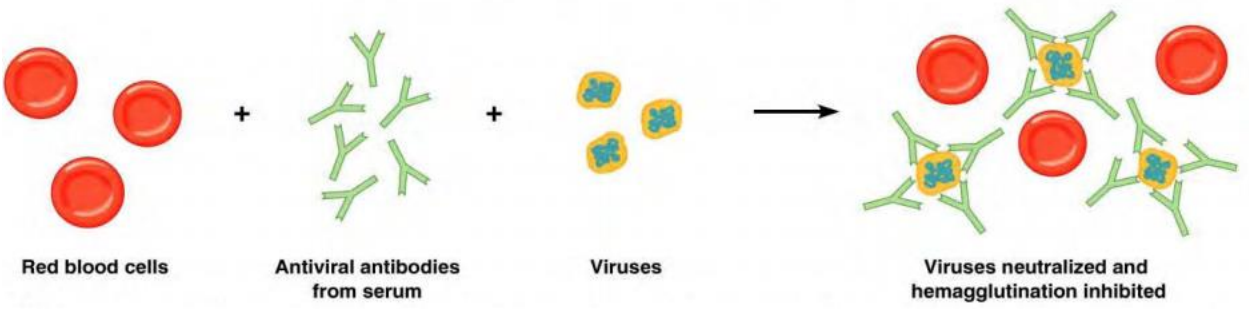
C. Neutralization Reactions

Neutralization: is an antigen-antibody reaction in which the harmful effects of a bacterial exotoxin or a virus are blocked by specific antibodies.

a) Viral Hemagglutination: Many viruses such as influenza virus can stick to and agglutinate red blood cells in a process called viral hemagglutination:

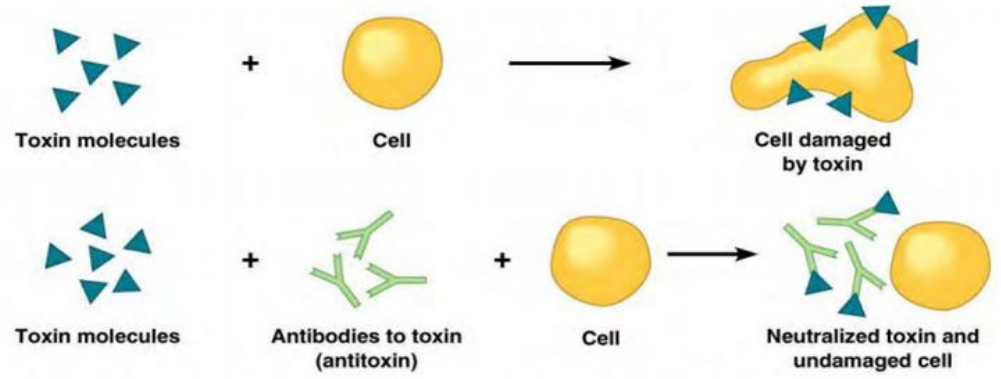


b) Neutralization of Viral Hemagglutination: This type of diagnostic test reveals the presence of specific viral antibodies in serum (i.e., due to exposure to the virus) due to the prevention of viral hemagglutination. If the person's serum contains antibodies against these viruses, these antibodies will react with the viruses and neutralize them. For example, if hemagglutination occurs in a mixture of measles virus and red blood cells but does not occur when the patient's serum is added to the mixture, this result indicates that the serum contains antibodies that have bound to and neutralized the measles virus.



c) Neutralization of Bacterial Toxins: Bacterial toxins can also be effectively neutralized by specific antibodies: For example, when the serum antibody neutralizes the toxic substances produced by the diphtheria pathogen, *Corynebacterium diphtheriae*. Such neutralizing substance is called an "Antitoxin".

Antitoxin: is a specific antibody produced by a host as it responding to a bacterial exotoxin or its corresponding toxoid (inactivated). So the antitoxin combines with the exotoxin to neutralize it.

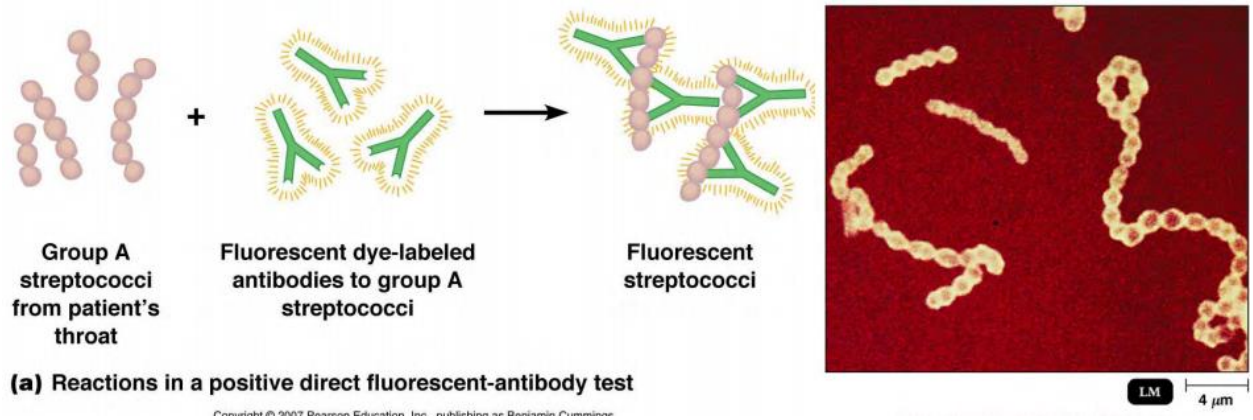


D. Fluorescent Antibody Staining

These are immunological tests that use antibodies labeled with fluorescent dyes for identify microorganisms in clinical specimens and detect the presence of a specific antibody in serum. These techniques combine fluorescent dyes such as fluorescein isothiocyanate (FITC) with antibodies to make them fluoresce when exposed to ultraviolet light by using (fluorescent microscope).

a) Direct Fluorescent Antibody Labeling: Tests are used to identify specific microorganisms (Ag+ Labeled Ab). Antibodies labeled with a fluorescent dye are useful for identifying pathogens in a tissue sample for example:

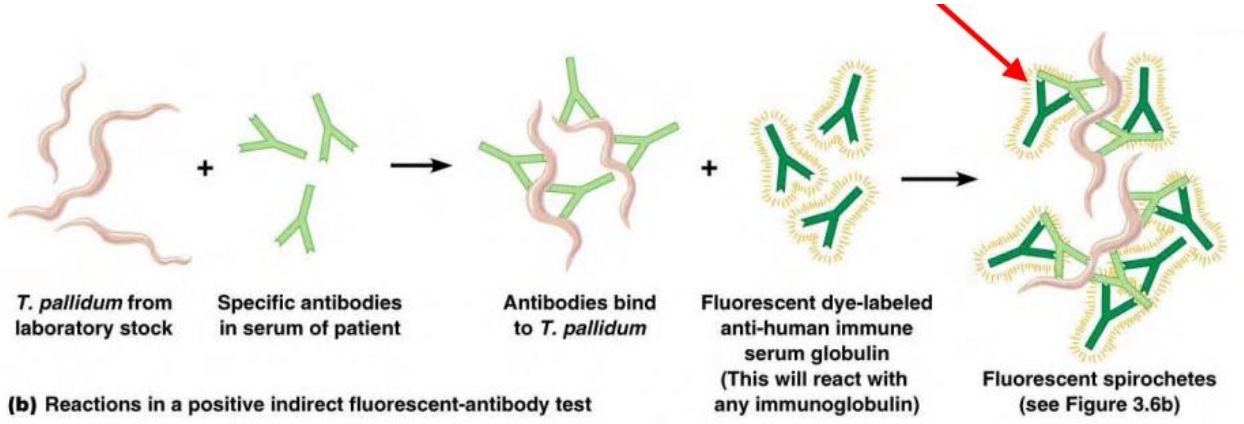
- ✓ With direct labeling the primary antibody (antibody that binds to the pathogen) itself is labeled



(a) Reactions in a positive direct fluorescent-antibody test

b) Indirect Fluorescent Antibody Labeling: Tests are used to demonstrate the presence of antibody in serum (Ag + Ab + Lebeled Anti-Ab). It is frequently more practical to label a secondary antibody (2 ^{o} Ab) to reveal the binding of unlabeled primary antibody (1^{o}Ab).

- ✓ This indirect method is useful for detection the presence of specific antibody in clinical samples
- ✓ 2^{o}Ab is specific for constant region of 1^{o}Ab



E. Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA is technique that use antibody linked to an enzyme (conjugate). So that, antigen – antibody reactions are detected by enzyme activity leading to color change in microtiter plate well that absorbent with Ab or Ag.

- ❖ Main components of ELISA test:

1) Samples such as serum for (Ab detection) or urine for (Ag detection).

2) Microtiter plate wells (U-shape) that absorbents with Ab (Direct ELISA) or with Ag (Indirect ELISA).

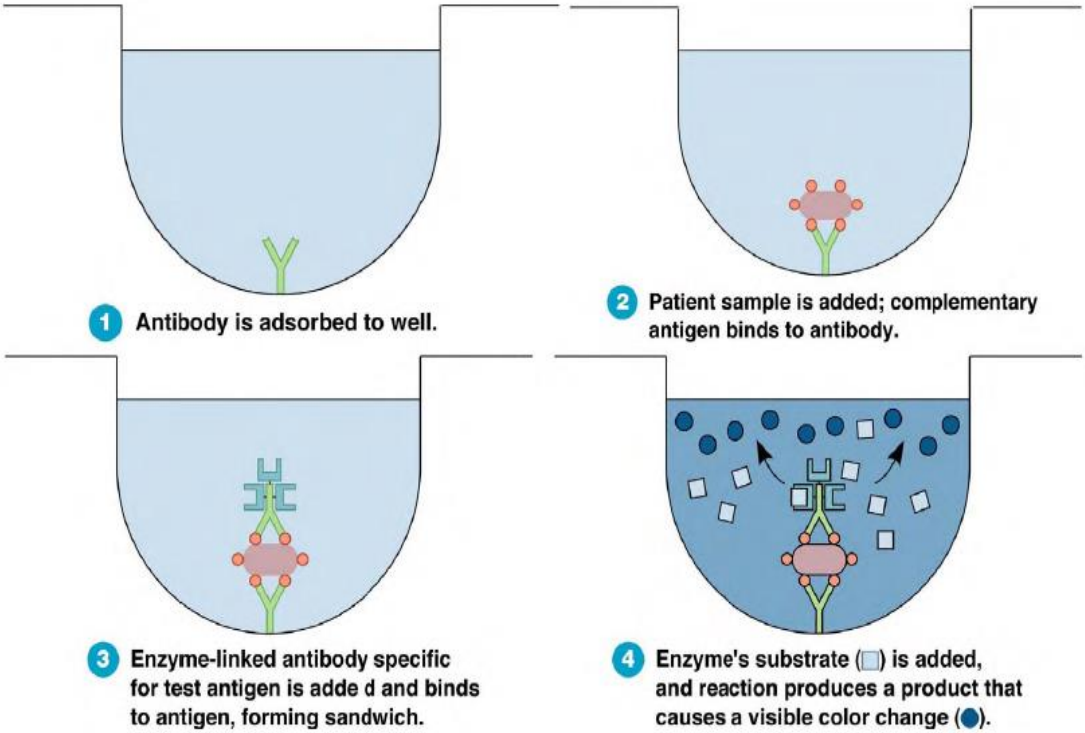
3) Enzyme-linked antibody (conjugate) such as (horseradish peroxidase or alkaline phosphatase). It can be found as Ab-conjugate for direct ELISA or Anti-Ab-conjugate for Indirect ELISA.

4) Substrate which act as co-enzyme for conjugate.

a) Direct ELISA

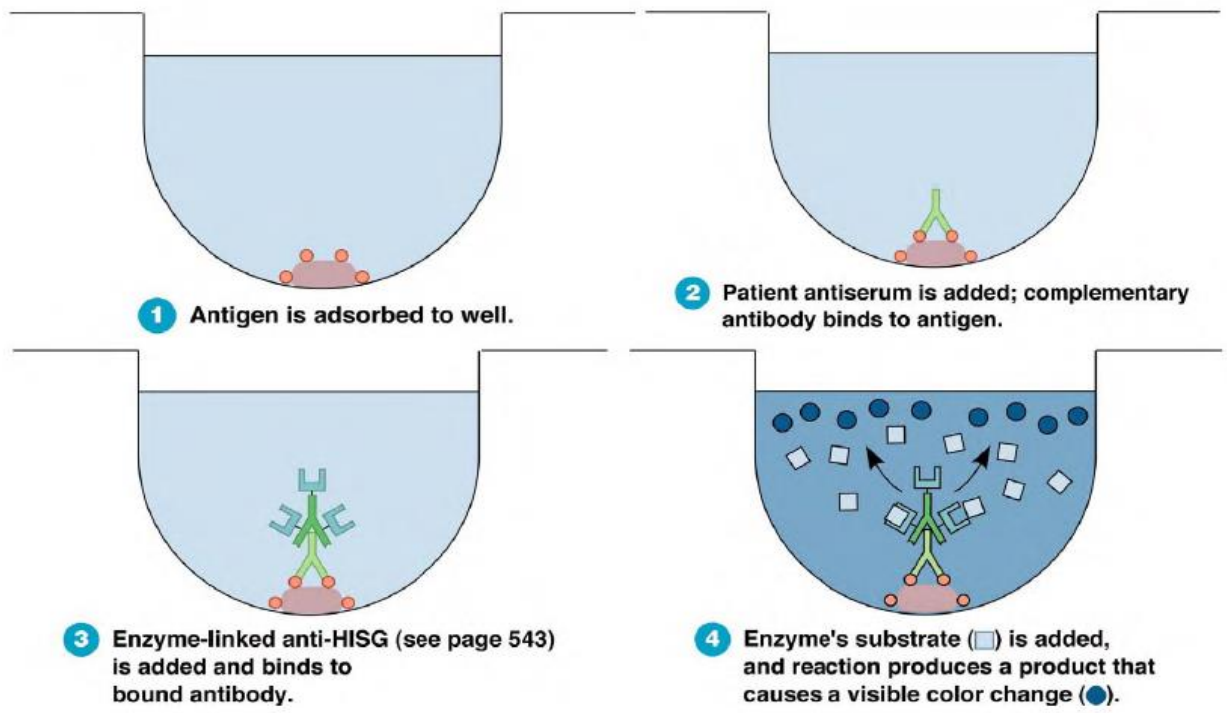
In the first step, the antibody specific for the antigen to be detected is absorbed to the surface of the wells of the microtiter plate. A patient's serum containing unidentified antigen is then added to each well. If the antigen reacts specifically with the antibodies adsorbed to the well, the antigen (a drug in urine test, for example) will be retained there when the well is washed free of unbound antigen. A second antibody specific for the antigen is then added. If both the antibody adsorbed to the wall of the well and the antibody known to be specific for the antigen have

reacted with the antigen, a "Sandwich" will be formed, with the antigen between two antibody molecules. This reaction is visible only because the second added antibody is linked to an enzyme, such as horseradish peroxidase or alkaline phosphatase. Unbounded enzyme-linked antibody is washed from the well. Then, the enzyme's substrate is add to it. Enzymatic activity is indicated by a color change that can be visually detected. The test will be positive if the antigen has reacted with absorbed antibodies in the first step. If the test antigen was not specific for the antibody absorbed to the wall of the well, the test will be negative because the unbounded antigen will have been washed away. A common use of the direct ELISA test is to detect the antigen in sample such as for diagnosis of causative agents of diseases.



b) Indirect ELISA

In the first step, a known antigen is absorbed to of the wells of the microtiter plate. The antibody (antiserum) is added to the well. If the serum contains antibody specific to the antigen all unreached antiserum is washed from the well. The anti-antibody conjugate such as (anti-HISG), reacts with the antibodies that are bound to the antigens in the wells. Finally, all unbounded anti-HISG is rinsed away, and the correct substrate for the enzyme is added. A colored enzymatic reaction occurs in the wells in which bound antigen has combined with antibody in serum sample. The type of ELISA can be widely used for screen of Ab in serum samples.



6.3.2. Vaccines and immunotherapy

A) Vaccines:

A vaccine is a biological preparation that improves immunity to a particular disease. A vaccine typically contains an agent that resembles a disease-causing pathogen and is often made from weakened or killed forms of the pathogen, its toxins or one of its surface proteins. The agent stimulates the body's immune system to recognize the agent as foreign, destroy it, and keep a record of it, so that the immune system can more easily recognize and destroy any of these microorganisms that it later encounters.

Effectiveness of vaccine: Vaccines do not guarantee complete protection from a disease. Sometimes, this is because the host's immune system simply does not respond adequately or at all. This may be due to a lowered immunity in general or because the host's immune system does not have a B cell capable of generating antibodies to that antigen. Adjuvants are typically used to boost immune response and combined with vaccine agents to enhance immunity response and contribute for the effectiveness of the vaccine. Vaccines may be monovalent (univalent) to immunize against a single antigen (pathogen) or multivalent (polyvalent) to immunize against two or more strains (same or different pathogen)

Types of vaccine

Killed: Some vaccines contain killed, but previously virulent, that has been destroyed with chemicals, heat, radioactivity or antibiotics. E.g., influenza vaccine, cholera vaccine, bubonic plagues vaccine, polio vaccine, hepatitis A vaccine, and rabies vaccine.

Attenuated: Some vaccines contain live, attenuated pathogen. Many of these are live viruses that have been cultivated under conditions that disable their virulent properties, or which use closely related but less dangerous organisms to produce a broad immune response. Although most attenuated vaccines are viral, some are bacterial in nature. Attenuated vaccines have some advantages and disadvantages. They typically provoke more durable immunological responses and are the preferred type for healthy adults. But they may not be safe for use in immuno-compromised individuals, and may rarely mutate to a virulent form and cause disease. The advantage of the attenuated oral polio vaccine is that it induces production of secretory IgA, which effectively blocks attachment of poliovirus along the gastrointestinal tract

Toxoid vaccines: are made from inactivated toxic compounds that cause illness rather than the pathogen. Toxoid vaccines are known for their efficacy. E.g. Tetanus and Diphtheria.

Subunit (**Protein subunit**): rather than introducing an inactivated or attenuated pathogen to an immune system, a fragment of it can create an immune response. E.g., *Hepatitis B virus* vaccine composed of only the surface proteins of the virus.

Conjugate: certain bacteria have polysaccharide outer coats that are poorly immunogenic. By linking these outer coats to proteins (e.g. toxins), the immune system can be led to recognize the polysaccharide as if it were a protein antigen. This approach is used in the Haemophilus influenzae type B vaccine.

Dendritic cell vaccines: combine dendritic cells with antigens in order to present the antigens to the body's white blood cells, thus stimulating an immune reaction.

Recombinant Vector: by combining the physiology of one pathogen and the DNA of the other, immunity can be created against diseases that have complex infection processes.

DNA vaccine: created from an infectious agent's DNA. It works by insertion of the DNA into host cells. The immune system that recognizes the proteins expressed will mount an attack against these proteins and cells expressing them. Because these cells live for a very long time, if the pathogen that normally expresses these proteins is encountered at a later time, they will be

attacked instantly by the immune system. One advantage of DNA vaccines is that they are very easy to produce and store but is still experimental.

T-cell receptor peptide vaccines: these peptides able to modulate cytokine production and improve cell mediated immunity.

B) Immunotherapy:

Immunotherapy refers to the use of cells, molecules, and genes of the immune system for the therapy of infectious disease, autoimmunity, neoplastic diseases, and graft-versus-host disease (GVHD), and to prevent the rejection of organ transplants. Immunotherapy can be passive, involving the administration of immunoglobulin (Ig), antibodies, cells, and immunoconjugates (ICs), for example, which home to the target tissue and generate a therapeutic effect in situ. Immunotherapy can also be active, involving mobilization of the immune system by the administration of vaccines or immunomodulators, which increase the immunogenicity of endogenous or exogenous antigens. Both passive and active immunotherapies are interrelated because the administration of any agent will have downstream effects on various immunologic circuits. Some immunotherapies have achieved success in humans and several immunopharmaceuticals have been approved or are being evaluated in advanced clinical trials.

Immunotherapy of Cancer

It has long been postulated that the use of cells or molecules of the immune system or the active mobilization of the immune system should provide specific clinical benefits that would be difficult to achieve by the administration of broadly reactive cytotoxic agents. In the case of cancer, this goal has been difficult to attain because of the impediments to treating cancer per second. For a long time, there was significant controversy about whether there was any immune response against cancer cells or whether the immune system played any role in either preventing or containing neoplasia. More recently, cancer has been viewed as the failure of the immune system either to respond robustly enough or to keep up with the extraordinary rates of tumor cell growth and mutation.

The general problems that make cancer difficult to treat by immunologic approaches are as follows:

(a) The similarity in antigens between tumor cells and normal cells and the finding that most tumors are not strongly immunogenic because they are recognized as self-tissue;

(b) The finding that antigens or tumor cells mutate at a high rate and hence circumvent ongoing immune responses; and

(c) The finding that many tumors down-regulate major histocompatibility (MHC) antigens and make poor targets for cytotoxic T cells (CTLs); moreover, many tumors interfere with the immune system and are able to dampen or avoid the immune response by suppressing it or by killing effector cells.

Immunomodulators

Agents that enhance the immune response of the host against cancer, infectious diseases, or immunologic disorders are referred to as "immunomodulators." Immunomodulators belong to a highly heterogeneous group of molecules with different mechanisms of action. Immunomodulators can be co-administrated with antigens to increase their local retention (depot effect) and thereby facilitate their slow release into the body. Immunomodulators can also alert the immune system to "danger" by inducing changes in antigen-presenting cells (APCs), particularly dendritic cells (DCs).