Chapter# 1: Overview of the Immune System as the Body's main Defense Mechanism

Introduction to basic Terminology

Immunity

It is the resistance to disease specifically infectious disease or infections.

Immune system

The collection of cells, tissues and molecules that mediate resistance to infection is called as immune system

Immune response

The coordinated reaction of cells and molecules of immune system against infectious microbes is termed as immune response

Immunology

The study of immune system & immune response with physiological function to prevent infections and to eradicate established infections inside body

Infectious vs Non-infectious diseases

Infections

These are contagious, transmittable and communicable diseases which are caused by an invading or infectious agent. The infectious agent is a foreign particle to body's immune system. These can be microbes like bacteria, viruses, fungi and parasites. That's' why infections can be bacterial, viral, fungal and parasitic in nature for example typhoid is a bacterial infection while hepatitis is a viral infection. Infections can be transmitted from one individual to other through various ways like aerosols, blood and also from mother to fetus.

Non-infections

These are non-communicable and non-contagious diseases in contrast to infections. These are not caused by purely by an infectious or invading agents but these agents can be a secondary reasons for such kind diseases for example in case of most of cancers are caused by various infectious agents secondarily. Non-infectious diseases are generally considered as metabolic disorders in which there is any defect in the metabolism of any bio-molecule for example diabetes mellitus is a carbohydrate metabolic disorder. These disease also have the tendency to transmit genetically from one generation to other.

Role of Immune System

The importance of the immune system for health is evident by the conditions when individual suffering from various fetal diseases in case of defective immune response. The most important function of immune system is to protect the body from the attack of infectious or invading microbes. If the individual's immune system does not work properly then the chances of acquiring infections also increased for example in case of Acquired immunodeficiency syndrome (AIDS). This indicates the importance of immune system for defending against infections. Similarly, there is also very significant role of immune system in organ transplantation. Immune response act as a barrier for successful organ transplantation as immune system recognizes and responds to tissue graft and newly introduced proteins. Immune system also provides surveillance against tumors and cancers. Moreover, for treatment of cancers immunotherapy also have good implications. The products of immune system like antibodies are highly specific molecules for detecting any kind of molecules. Likewise, antibodies are widely used for developing immunologic approaches for laboratory testing in clinical medicine and research.

Types of Immunity

For providing defense against various infections, there are two important types of immunity

1) Innate or non-specific Immunity
2) Adaptive or specific immunity

Innate Immune System

It is a non-specific immune system which serve as the first line of defense against pathogens. Innate immune system is readily available all the time in order to fight against microbes. Moreover,

this form of immune system is also antigen independent and non-specific. There is immediate maximal response from innate immune system against attacking pathogens. Innate immune system lack in memory against the various forms of infections

Adaptive Immune System

It is considered as the second line of protection against various pathogen specifically. It needs activation after invasion by pathogen in a very specific manner. This system is antigen dependent with specificity. For the activation of adaptive immune system, it needs lag time for fully activation during which naive cells become active after differentiation and activation. Adaptive immune system has the unique attribute of memory against specific nature of pathogens.

Components of Innate Immune system

Innate immune system is the first line of defense against invading pathogens. It is comprised of following three important components or barriers

1) Anatomical barriers
2) Humoral (Secretory) barriers
3) Cellular barriers

Anatomical barriers

Those defensive mechanisms which are related to various structures of body are the part of anatomical barriers. These are composed of three major arms including

a) Mechanical arm
b) Chemical arm
c) Biological arm

Mechanical Arm

It is comprised of various mechanical defense by different structure of body for example like skin which is considered as a physical barrier for protection against invading agents. Similarly, the lining epithelium membrane of various body cavities like GIT & skin in the form of squamous epithelium which has the property of desquamation in order to get rid of infectious agent. Moreover, the peristalsis and cilliary movements of GIT also protect from the ingested

microorganisms Flushing actions of tears and saliva also make the mechanical arm of anatomical barriers. The mucus lining of GIT and respiratory tract also has the trapping action for providing protection against invading pathogens

Chemical arm

It is composed of various chemicals which effect on the invading pathogens for providing protection for example skin contains various fatty acids which don't allow the organisms to grow on it. Similarly, tears and nasal secretions carry various enzymes like lysozymes & phospholipases for degradation of invading pathogens by breaking their cell wall. Likewise, defensins also have antimicrobial actions against pathogens in GIT and lungs. Moreover, low pH of GIT & sweat secretions inhibit the growth of pathogenic microorganisms. However, lungs also contain surfactants which increase the process of phagocytosis for potential pathogen by the process of opsonization.

Biological Arm

It consist of normal flora or micro biota of various structures of body like skin, GIT, respiratory tract and genital tract. Microorganisms of micro biota prevents the colonization of potential pathogens in such structures of body. Similarly, the resident flora secrete various toxins for the potential invading agents. Furthermore, resident micro biota of body structures also has physical potential for utilization of nutrients by competing the pathogenic organisms.

Humoral (Secretory) barriers

After breaching the anatomical barriers, infectious agent penetrates the deeper tissue for inflammation which is the tissue response against infection. Humoral barriers in fact mediate that inflammatory process. These barriers consist of different molecules in the form of proteins, enzymes and cytokines which are present in the secretions of body particularly blood plasma including

Complement System

These are plasma proteins which are present in inactive form. These proteins become active after triggering by an infectious agent. Moreover, these proteins cause the lysis of potential invading

agent by forming membrane attack complex. Once activated complement can lead to increased vascular permeability, recruitment of phagocytic cells, and lysis and opsonization of bacteria.

Coagulation proteins

Depending on the severity of the tissue injury, the coagulation system may or may not be activated. Some products of the coagulation system can contribute to the non-specific defenses because of their ability to increase vascular permeability and act as chemotactic agents for phagocytic cells. In addition, some of the products of the coagulation system are directly antimicrobial. For example, beta-lysin, a protein produced by platelets during coagulation can lyse many Gram positive bacteria by acting as a cationic detergent.

Lactoferrin & Transferrins

These proteins limit bacterial growth, by binding iron which is an essential nutrient for bacteria.

Interferons

Interferons are the secretory molecules which are proteins in nature. These have ability to restrict virus replication in cells.

Cytokines

These are secretory molecules which act as mediators between immune cells. Il-1 induces fever and the production of acute phase proteins, some of which are antimicrobial because they can opsonize bacteria.

Cellular Barriers

These are the barriers which comprised on immune cells. As a result of the inflammatory response, there is the recruitment of the immune cells like polymorphonuclear leucocytes or neytrophils, eosinophiles and macrophages to sites of infection. These cells are the main line of defense in the non-specific immune system.

Neutrophils

Polymorphonuclear cells are recruited to the site of infection where they phagocytose invading organisms and kill them intracellularly. In addition, PMNs contribute to collateral tissue damage

that occurs during inflammation.

Macrophages

Tissue macrophages and newly recruited monocytes, which differentiate into macrophages, also function in phagocytosis and intracellular killing of microorganisms. In addition, macrophages are capable of extracellular killing of infected or altered self-target cells. Furthermore, macrophages contribute to tissue repair and act as antigen-presenting cells, which are required for the induction of specific immune responses.

Natural killer (NK) cells

NK cells can nonspecifically kill virus infected and tumor cells. These cells are not part of the inflammatory response but they are important in nonspecific immunity to viral infections and tumor surveillance.

Eosinophils

Eosinophils are the cells which have proteins in granules that are effective in killing certain parasites.

PHAGOCYTOSIS AND INTRACELLULAR KILLING

Phagocytic cells

Neutrophiles

Neutrophiles are motile phagocytic cells that have lobed nuclei. They contain two kinds of granules the contents of which are involved in the antimicrobial properties of these cells. The primary or azurophilic granules, which are abundant in young newly formed PMNs, contain cationic proteins and defensins that can kill bacteria, proteolytic enzymes like elastase, and cathepsin G to breakdown proteins, lysozyme to break down bacterial cell walls, and characteristically, myeloperoxidase, which is involved in the generation of bacteriocidal compounds. The second type of granule found in more mature PMNs is the secondary or specific granule. These contain lysozyme, NADPH oxidase components, which are involved in the generation of toxic oxygen products, and characteristically lactoferrin, an iron chelating protein

and B12-binding protein.

Monocytes/Macrophages

Macrophages are phagocytic cells that have a characteristic kidney-shaped nucleus. Unlike PMNs they do not contain granules but they have numerous lysosomes which have contents similar to the PNM granules.

Response of phagocytes to infection

Circulating PMNs and monocytes respond to danger (SOS) signals generated at the site of an infection. SOS signals include N-formyl-methionine containing peptides released by bacteria, clotting system peptides, complement products and cytokines released from tissue macrophages that have encountered bacteria in tissue. Some of the SOS signals stimulate endothelial cells near the site of the infection to express cell adhesion molecules such as Intracellular Adhesion Molecules-1 (ICAM-1) and selectins which bind to components on the surface of phagocytic cells and cause the phagocytes to adhere to the endothelium. Vasodilators produced at the site of infection cause the junctions between endothelial cells to loosen and the phagocytes then cross the endothelial barrier by “squeezing” between the endothelial cells in a process called diapedesis. Once in the tissue spaces some of the SOS signals attract phagocytes to the infection site by chemotaxis (movement toward an increasing chemical gradient). The SOS signals also activate the phagocytes, which results in increased phagocytosis and intracellular killing of the invading organisms.

Initiation of Phagocytosis

Phagocytic cells have a variety of receptors on their cell membranes through which infectious agents bind to the cells. These include:

Fc receptors

Bacteria with IgG antibody on their surface have the Fc region exposed and this part of the antibody molecule can bind to the receptor on phagocytes. Binding to the Fc receptor requires prior interaction of the antibody with an antigen. Binding of IgG-coated bacteria to Fc receptors

results in enhanced phagocytosis and activation of the metabolic activity of phagocyte.

Complement receptors

Phagocytic cells have a receptor for the 3rd component of complement, C3b. Binding of C3b-coated bacteria to this receptor also results in enhanced phagocytosis and stimulation of the respiratory burst.

Scavenger receptors

Scavenger receptors bind a wide variety of polyanions on bacterial surfaces resulting in phagocytosis of bacteria.

Toll-like receptors

Phagocytes have a variety of Toll-like receptors (Pattern Recognition Receptors or PRRs) which recognize broad molecular patterns called PAMPs (pathogen associated molecular patterns) on infectious agents. Binding of infectious agents via Toll-like receptors results in phagocytosis and the release of inflammatory cytokines (IL-1, TNF-alpha and IL-6) by the phagocytes.

Phagocytosis

After attachment of a bacterium, the phagocyte begins to extend pseudopods around the bacterium. The pseudopods eventually surround the bacterium and engulf it, and the bacterium is enclosed in a phagosome. During phagocytosis the granules or lysosomes of the phagocyte fuse with the phagosome and empty their contents. The result is a bacterium engulfed in a phagolysosome which contains the contents of the granules or lysosomes.

Respiratory burst and intracellular killing

During phagocytosis there is an increase in glucose and oxygen consumption which is referred to as the respiratory burst. The consequence of the respiratory burst is that a number of oxygen-containing compounds are produced which kill the bacteria being phagocytosed. This is referred to as oxygen-dependent intracellular killing. In addition, bacteria can be killed by pre-formed

substances released from granules or lysosomes when they fuse with the phagosome. This is referred to as oxygen-independent intracellular killing.

Oxygen-dependent myeloperoxidase-independent intracellular killing

During phagocytosis glucose is metabolized via the pentose monophosphate shunt and NADPH is formed. Cytochrome B which was part of the specific granule combines with the plasma membrane NADPH oxidase and activates it. The activated NADPH oxidase uses oxygen to oxidize the NADPH. The result is the production of superoxide anion. Some of the superoxide anion is converted to H_2O_2 and singlet oxygen by superoxide dismutase. In addition, superoxide anion can react with H_2O_2 resulting in the formation of hydroxyl radical and more singlet oxygen. The result of all of these reactions is the production of the toxic oxygen compounds superoxide anion (O_2-), H_2O_2, singlet oxygen (^{1}O2) and hydroxyl radical (OH•).

Oxygen-dependent myeloperoxidase-dependent intracellular killing

As the azurophilic granules fuse with the phagosome, myeloperoxidase is released into the phagolysosome. Myeloperoxidase utilizes H_2O_2 and halide ions (usually Cl-) to produce hypochlorite, a highly toxic substance. Some of the hypochlorite can spontaneously break down to yield singlet oxygen. The result of these reactions is the production of toxic hypochlorite (OCl-) and singlet oxygen (^{1}O2).

Detoxification reactions

PMNs and macrophages have means to protect themselves from the toxic oxygen intermediates. These reactions involve the dismutation of superoxide anion to hydrogen peroxide by superoxide dismutase and the conversion of hydrogen peroxide to water by catalase

Oxygen-independent intracellular killing

In addition to the oxygen-dependent mechanisms of killing there are also oxygen–independent killing mechanisms in phagocytes: cationic proteins (cathepsin) released into the phagolysosome

can damage bacterial membranes; lysozyme breaks down bacterial cell walls; lactoferrin chelates iron, which deprives bacteria of this required nutrient; hydrolytic enzymes break down bacterial proteins. Thus, even patients who have defects in the oxygen-dependent killing pathways are able to kill bacteria. However, since the oxygen-dependent mechanisms are much more efficient in killing, patients with defects in these pathways are more susceptible and get more serious infections.

Complement System

This system is composed of various serum proteins called as complement proteins. These are heat labile inactivated at 56°C for 30 minutes. These proteins have the ability to lyse the bacterial cell. However, these proteins exist as proenzymes which become activated after a cascade reaction

Functions of complement proteins

Complement proteins have very diverse functions for providing protection against invading pathogens. Complement proteins have following important functions. Complement proteins act as opsonin means facilitate the process of phagocytosis of pathogen by phagocytes. These are also act like chemoattractant for Polymorphonuclear Leucocytes (PMNs) to move towards the site of infection. Moreover, the role of complement proteins as Pro-Inflammatory molecules in order to facilitate the process of inflammation. As a result of inflammation, various undesirable effects are produced which are detrimental for host.

Activation of Complement

Complement proteins get activated by following three activation pathways

Classical Pathway of Complement Activation

In this pathway, the complement proteins become activated by antibodies attached on the surface of pathogen (bacteria). C1 protein is activated after interacting with Fc region of antibodies IgG

or IgM. After activation of C1 complex, C2 & C4 are activated. Finally C3 proteins are activated after the cleaving action of C2 & C4 in a cascade manner

Alternate Pathway of Complement Activation

In this pathway, there is no need of antibody for the activation of complement proteins. Rather C3 is directly converted into activated form. However, various co-factors of serum facilitate the activation of C3 sequentially. After fully activation of C3 proteins, finally C3 helps in the lysis of bacterial cell

Lectin Pathway of Complement Activation

Lectin pathway is considered very similar to classical pathway in context to activation of complement protein 1 (C1). In place of antibodies, mannose on the surface of specific pathogens are involved in activation of C1. Additionally, Mannose binding Lectins (MBL) in serum bind to surface of pathogen containing mannose in their cell wall e.g Fungus. Finally, C3 proteins are produced after interaction of mannose & Lectins which further make membrane attack complex for lysis of infectious agent.

Effector Functions of Complement Proteins

Complement proteins have following effector functions for providing immunity against infectious agents

- Opsonization (Opsonins)

Various complement proteins bind with the surface of pathogens which facilitate the process of phagocytosis by phagocytes. This enhanced process of phagocytosis is called as opsonization, while complement proteins involved in this process are called as opsonins.

- Chemoattraction (Chemoattractants)

Complement proteins also served as chemoattractants for the movement of phagocytic cells towards the site of infection. The chemoattractant function of complement proteins increased the defense mechanism against invading agents.

- Anaphylaxsis (Anaphylatoxins)

Complement proteins also involved in hypersensitivity (allergic) reactions which are also called as anaphylactic reactions in the form of exaggerated immune response. Such kind of complement proteins are called as anaphylatoxins.

- Pro-Inflammatory

The role of complement proteins in inflammatory process is well established. The tissue response against infectious agent is termed as inflammatory response and the complement proteins contributing towards such kind of response are considered as pro-inflammatory.

Pathology related to Complement Proteins

Complement proteins are considered as major humoral component of innate immune system. Once these proteins are activated lead the host towards following pathological conditions

- Acute Inflammatory response: Sudden inflammatory response is generated
- Capillary dilatation: Due to increase blood flow, there is capillary dilatation
- Exudation of plasma proteins & fluids (Edema): Accumulation of fluid in tissue spaces leading towards edema
- Bronchoconstriction: there is constriction of bronchioles leading towards respiratory distress
- Mast cells degranulation (Allergic reaction): Degranulation of mast cells leads towards the secretion of chemical mediators which finally become the cause of allergic reaction.

Chapter# 2: Cells & Organs of Immune System

Tissues of the Immune System

As immune system is the collection of cells, tissues and molecules that mediate resistance to infection. In order to generate an effective immune response there is a coordinated action of various cells of the immune system which are basically derived from the tissues of immune system. Based on the function of immune system, the tissues of immune system are classified into following two types

1) Primary Lymphoid tissues
2) Secondary Lymphoid tissues

Primary Lymphoid Tissues

These lymphoid tissues are involved in the generation of various lymphoid cells that's why these tissues are also termed as generative or central lymphoid tissues. On the basis of these lymphoid tissues various kinds of lymphocytes are matured. Primary lymphoid tissues comprised of following two important organs

1) Bone marrow
2) Thymus

Bone marrow is involved in the generation and maturation of B-lymphocytes while thymus in T-lymphocytes.

Secondary lymphoid Tissues

These are the lymphoid tissues which are involved in providing the specific immune response. These tissues are located distantly from central lymphoid tissues that's why are also called as peripheral lymphoid tissues. These tissues provide the specific site for interaction between immune cells like lymphocytes and the antigens. Such kind of tissues are located in various anatomical locations for providing continuous surveillance against the foreign agents. Secondary lymphoid tissues include lymph nodes in various parts of body like axillary & groin regions. Similarly, tonsils & adenoids are located in throat and larynx area. Spleen is located in the left hypochondrial

region. Moreover, appendix and peyer's patches are located in the gastrointestinal tract (GIT) for providing immune response against invading agents (Fig 2.1).

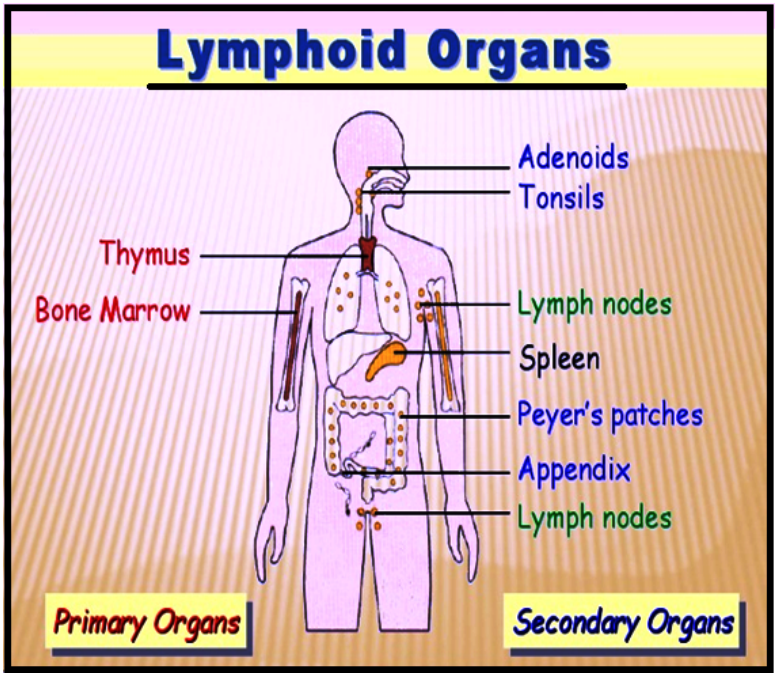

Fig 2.1: Anatomical location of various lymphoid tissues

Cells of Immune System

In order to provide an efficient immune response, immune tissues are made up of various immune cells. These cells are called as immune cells and originate from the hematopoietic stem cells. These stem cells are located in the primary lymphoid tissues like bone marrow. Hematopoietic stem cells differentiate into following two important cellular lineage according to their cellular source

1) Lymphoiod lineage
2) Myeloid Lineage

Lymphoid Lineage of Immune Cells

Lymphois lineage of immune cells give rise to various cells of specific immune systems like B-lymphocytes which differentiate into plasma cells or antibody forming cells (AFC). Similarly, T-

lymphocytes like cytotoxic and helper variety also originate from lymphoid lineage of immune cells. Moreover, the cells of non-specific immune system like natural killer (NK) cells also originate from lymphoid lineage (fig.2.2)

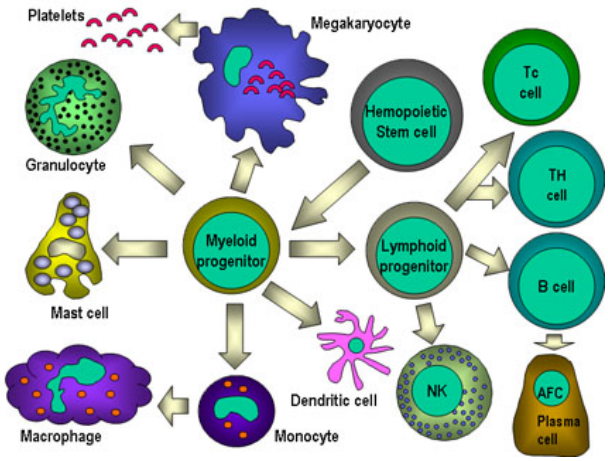

Figure 2.2: Development & maturation of Immune Cells

Myeloid lineage of Immune Cells

Myeloid lineage of immune cells from hematopoietic cells give rise to variety of immune cells like monocytes and macrophages which are phagocytic cells. In addition, other cells of innate immune cells like granulocytes which include neutrophils, basophils and mast cells also originate from this lineage of immune cells. However, another distinct variety of cells called megakaryocytes also originate from myeloid cells which are further source of platelets.

Lymphocyte Recirculation

Naive (virgin) lymphocytes enter the lymph nodes from the blood via High Endothelial Venules (HEVs). Homing receptors on the lymphocytes direct the cells to the HEVs. It is estimated that about 1-2% lymphocytes recirculate every hour. In the lymph nodes, lymphocytes with the appropriate antigen receptor encounter antigen, which has been transported to the lymph nodes by dendritic cells or macrophages. This process of activation of lymphocytes is called as priming and lymphocytes are called primed lymphocytes. After activation the lymphocytes express new receptors that allow the cells to leave the lymph node and reenter the circulation. Receptors on the activated lymphocytes recognize cell adhesion molecules expressed on endothelial cells near the

site of an infection and chemokines produced at the infection site help attract the activated cells (Figure.2.3)

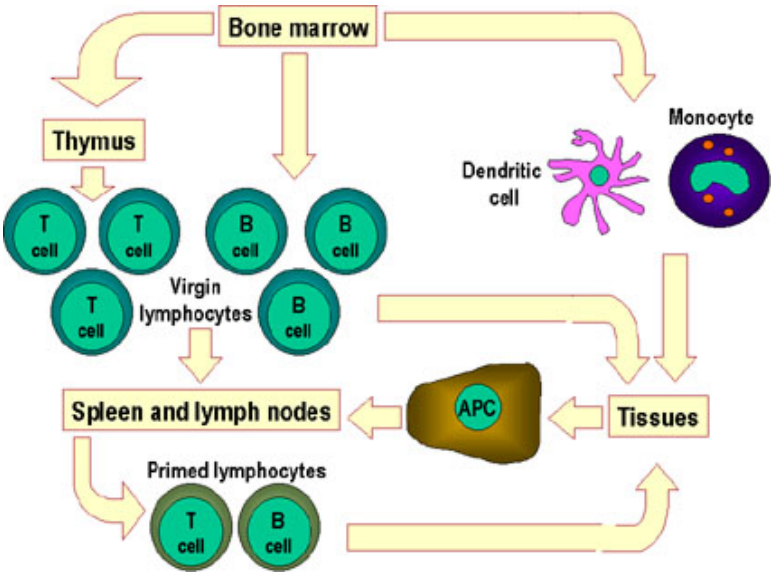

Figure 2.3: Lymphocytes recirculation between primary & secondary lymphoid tissues

Lymphocytes Migration & Localization

As relatively few T or B lymphocytes with a receptor for any particular antigen (1/10,000 – 1/100,000) are there, the chances for a successful encounter between an antigen and the appropriate lymphocyte are low. However, the chances for a successful encounter are greatly enhanced by the recirculation of lymphocytes through the secondary lymphoid organs. Lymphocytes in the blood enter the lymph nodes and percolate through the lymph nodes (Figure 2.4). If they do not encounter an antigen in the lymph node, they leave via the lymphatics and return to the blood via the thoracic duct. It is estimated that 1-2% of lymphocytes recirculate every hour. If the lymphocytes in the lymph nodes encounter an antigen, which has been transported to the lymph node via the lymphatics, the cells become activated, divide and differentiate to become a plasma cell, T-helper or Cytotoxic T-cell. After several days the effector cells can leave the lymph nodes via the lymphatics and return to the blood via the thoracic duct and then make their way to the infected tissue site.

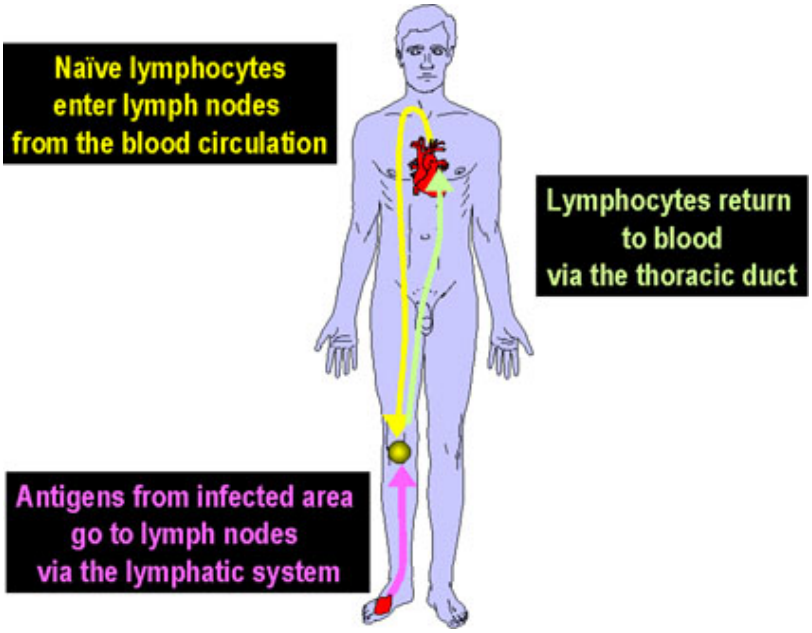

Figure 2.4: Lymphocytes migration & localization through secondary lymphoid tissues

Clonal Selection of Lymphocytes

Lymphocytes either B or T carry the unique specificity with their receptor. The specificity determines the nature of lymphocytes. There is a vast array of T & B lymphocytes with their unique receptor for particular antigenic determinants. The selection of each repertoire of lymphocytes with unique specificity occurs by selection of each specific clone of B or T-lymphocytes with unique BCR & TCR respectively which is termed as clonal selection theory. The clonal selection hypothesis states that the germline encodes many different antigen receptors - one for each antigenic determinant to which an individual will be capable of mounting an immune response. Antigen selects those clones of cells that have the appropriate receptor. There are following four basic principles of the clonal selection theory

1. Each lymphocyte bears a single type of receptor with a unique specificity.
2. Interaction between a foreign molecule and a lymphocyte receptor capable of binding that molecule with a high affinity leads to lymphocyte activation.
3. The differentiated effector cells derived from an activated lymphocyte will bear receptors of an identical specificity to those of the parental cell from which that lymphocyte was derived.

4. Lymphocytes bearing receptors for self-molecules are deleted at an early stage in lymphoid cell development and are therefore absent from the repertoire of mature lymphocytes.

Control of Extracellular Pathogens

Immune System provides protection against diverse pathogens which are highly contagious in nature. This kind of defense against pathogen is based upon the foreign nature of pathogen. These pathogens can attack the host either by residing outside of host cells. Such kind of pathogens are called as extracellular pathogens. The secretory products of immune system provide defense against extracellular pathogens particularly the antibodies. Antibodies control the extracellular pathogens by the following three important ways (Figure 2.5)

- Neutralization
  The pathogens or foreign substance are blocked to bind with their targets as antibodies bind with pathogens. For example, antibodies to bacterial toxins can prevent the binding of the toxin to host cells thereby rendering the toxin ineffective. Similarly, antibody binding to a virus or bacterial pathogen can block the attachment of the pathogen to its target cell thereby preventing infection or colonization.
- Opsonization
  Antibody binding to a pathogen or foreign substance can opsonize the material and facilitate its uptake and destruction by phagocytic cells. The Fc region of the antibody interacts with Fc receptors on phagocytic cells rendering the pathogen more readily phagocytosed.
- Complement activation
  Activation of the complement cascade by antibody can result in lysis of certain bacteria and viruses. In addition, some components of the complement cascade (*e.g.* C3b) opsonize pathogens and facilitate their uptake via complement receptors on phagocytic cells.

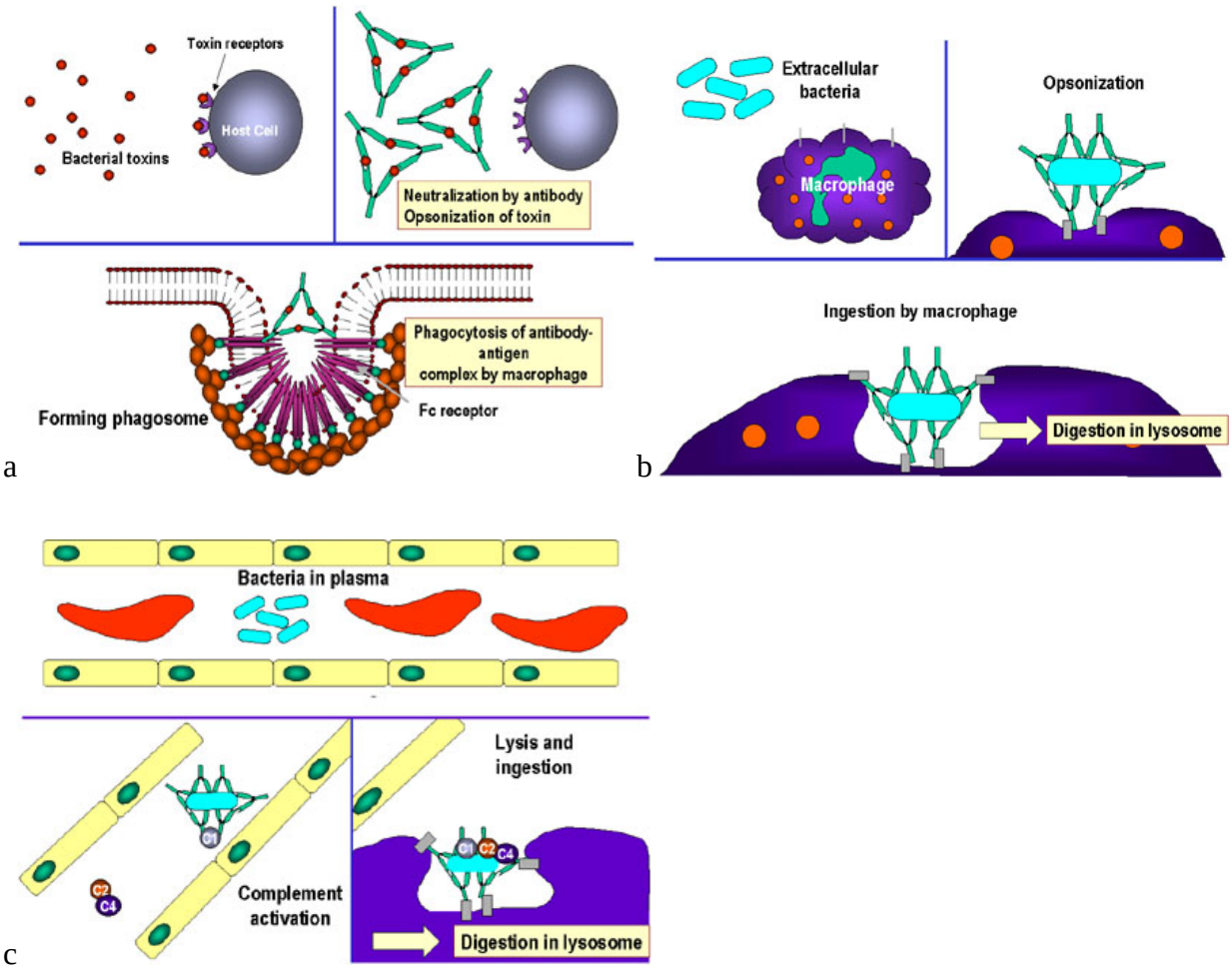

Figure 2.5: Ways of controlling Extracellular Pathogens a) Neutralization b) Opsonization c) Complement activation

Control of Intracellular Pathogens

Secretory molecules of immune system like antibodies are not able to provide effective immune response against those pathogens which attach the host cells by residing intracellularly. These pathogens are controlled by cell mediated immune response which is being provided by T-lymphocytes. These T-lymphocytes provide protection against intracellular pathogens according to the nature of pathogen location inside the cells. There are following two major kinds of T-lymphocytes which respond against intracellular pathogens according to the location of pathogen inside the cells (Figure 2.6)

1) Cytotoxic T-Lymphoctes: These are T-lymphocytes which provide cell mediated immune response against those intracellular pathogens which reside inside the cytosol of host cells e.g viruses are cytosolic in nature for causing infection.
2) Helper T-Lymphocytes: These T-Lymphocytes are effective against those pathogens which infect the host cells by residing inside the vesicles e.g *Mycobacterim tuberculosis*

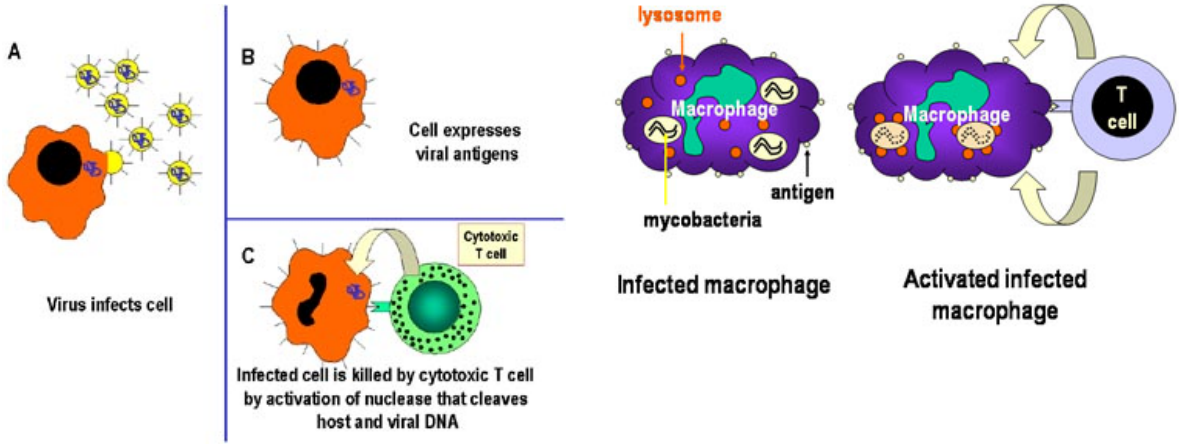

Figure 2.6: Control of Intracellular Pathogens a) Control of Cytosolic Pathogens b) Control of Vesicular Pathogens

Chapter# 3: Properties of antibodies and antigens together with their structure, functions & interactions

Comparison B/W Antigen, Immunogen & Hapten

Immunogen

A substance that is responsible for inducing specific immune response.

Antigen (Ag)

A substance that has the ability to reacts with the products of a specific immune response.

Hapten

A substance that is non-immunogenic but which can react with the products of a specific immune response. Haptens are small molecules which could never induce an immune response when administered by themselves but which can when coupled to a carrier molecule. However, free haptens can react with products of the immune. Haptens have the property of antigenicity but not immunogenicity.

Epitope or Antigenic Determinant

That portion of an antigen that combines with the products of a specific immune response.

Antibody (Ab)

A specific protein which is generated in response to an immunogen and which reacts with an antigen.

FACTORS INFLUENCING IMMUNOGENICITY

Contribution of the Immunogen

Foreignness

The immune system normally discriminates between self and non-self such that only foreign molecules are immunogenic.

Size

There is not absolute size above which a substance will be immunogenic. However, in general, the larger the molecule the more immunogenic it is likely to be.

Chemical Composition

Generally, the more complex the substance is chemically the more immunogenic it will be. The antigenic determinants are created by the primary sequence of residues in the polymer and/or by the secondary, tertiary or quaternary structure of the molecule.

Physical form

In general particulate antigens are more immunogenic than soluble ones and denatured antigens more immunogenic than the native form.

Degradability

Antigens that are easily phagocytosed are generally more immunogenic. This is because for most antigens (T-dependant antigens) the development of an immune response requires that the antigen be phagocytosed, processed and presented to helper T cells by an antigen presenting cell (APC).

Contribution of the Biological System

Genetic Factors

Some substances are immunogenic in one species but not in another. Similarly, some substances are immunogenic in one individual but not in others (*i.e.* responders and non-responders). The species or individuals may deficient or have altered genes that code for the receptors for antigen on B cells and T cells or they may not have the appropriate genes needed for the APC to present antigen to the helper T cells.

Age

Age is another biological factor which can also influence immunogenicity. Frequently the very young and the very old have a reduced ability to mount and immune response in response to an immunogen.

Method of Administration

Dose

The dose of administration of an immunogen can influence its immunogenicity. Generally the optimal dose of immunogen considered as having good immunogenicity. There is a dose of antigen above or below which the immune response will not be optimal.

Route

Generally the subcutaneous route which is underneath the skin is better than the intravenous or intragastric routes. The route of antigen administration can also alter the nature of the response

Adjuvants

Substances that can increase the immune response to an immunogen are called adjuvants. The use of adjuvants, however, is often hampered by undesirable side effects such as fever and inflammation.

CHEMICAL NATURE OF IMMUNOGENS (Figure 3.1)

Proteins

The majority of immunogens are proteins. These may be pure proteins or they may be glycoproteins or lipoproteins. In general, proteins are usually very good immunogens .

Polysaccharides

Pure polysaccharides and lipopolysaccharides are good immunogens.

Nucleic Acids

Nucleic acids are usually poorly immunogenic. However, they may become immunogenic when single stranded or when complexed with proteins.

Lipids

In general lipids are non-immunogenic, although they may be haptens.

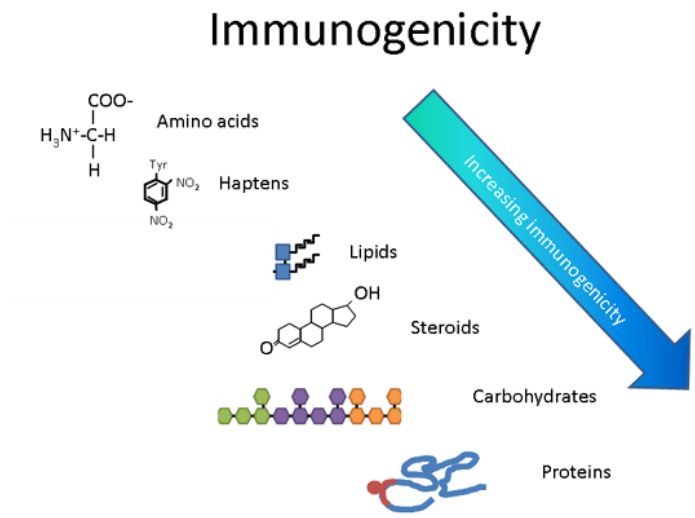

Figure 3.1: Immunogenicity of Bio-Molecules

Types of Antigens

The nature of antigen for mounting an immune response varies according to the requirement of T-cells. On this basis, antigens can be categorized into following two types

1) T-cell dependent antigens
2) T-cell independent antigens

T-cell dependent antigens

These are antigens which need the help of T-cells for mounting an immune response otherwise these cannot. T-dependent require the help of T-cells for activation of B-cells. Such kinds of antigens are protein in nature. Moreover, these antigens carry variety of antigenic determinants (epitopes) with few copies (Figure 3.2)

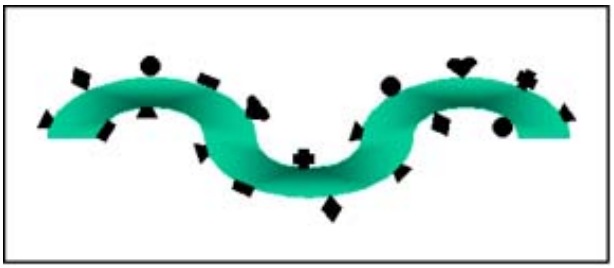

Figure 3.2: Shape of antigenic determinants on T-cell dependent antigen

T-Cell independent antigens

These antigens are those antigens which can directly activate B-cells without the help of T-cells. Such kind of antigens are non-protein and usually are polysaccharides in nature. These antigens carry same kind of epitopes in polymeric form (Figure 3.3). Moreover, such kind of antigens are most resistant to degradation.

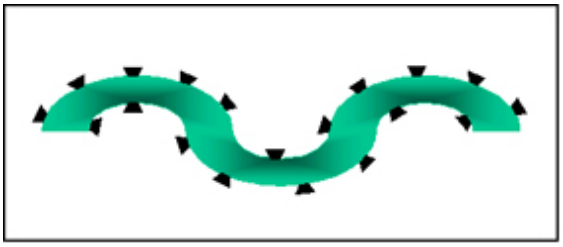

Figure 3.3: Shape of antigenic determinants on T-independent antigens

Superantigens

Superantigens are those antigens which can activate T-cells in extended or exaggerated form. Conventionally, T-dependent antigens have the ability to activate T-cells in few clones which is also called as oligoclonal response. But superantigens can activate T-cells in polyclonal fashion (Figure 3.4)

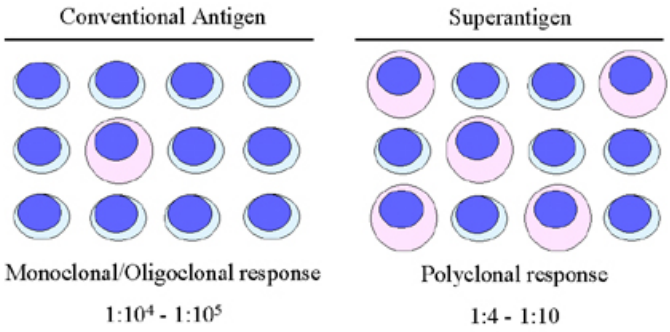

Figure 3.4: Comparison of Conventional & Superantigens for activation of T-cells

Superantigens include bacterial, viral and other microorganism's antigens. Among bacterial antigens various toxins like Staphylococcal enterotoxin which cause food poisoning, Staphloccoal exfoliatin toxin which is the causative agent for skin exfoliative syndrome and Staphlococcal toxic shock syndrome toxin (TSST) responsible for toxic shock syndrome.

Hapten-Carrier Conjugate

Hapten-carrier conjugate is an immunogenic molecule to which hapten is non-immunogenic while carrier is immunogenic in nature. The binding between hapten and carrier molecule is covalent in nature. Structurally, this conjugate is composed of haptenic & carrier determinants. The carrier determinants are native in nature as these have to generate immune response (Figure 3.5)

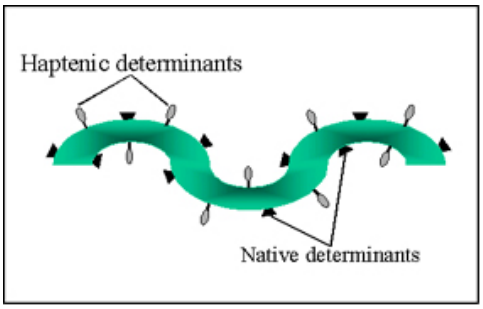

Figure 3.5: Structure of determinants on Hapten & Carrier conjugate

Antigenic determinants recognized by Innate Immune System

The antigenic determinants recognized by innate immune system is purely non-specific in nature due to non-specific attribute of innate immune system. These determinants are present on the surface of the pathogens in a form of various molecular configurations celled as pathogen associated molecular patterns (PAMPS). PAMPs include cell membrane proteins, lipopolysaccharide and flagellular proteins. For these antigenic determinants immune cells carry pattern recognition receptors (PRRs) which bind non-specifically with corresponding PAMPs in order to generate immune response (Table 3.1)

PAMPs	PRRs	Effector Function
LPS	Toll Like Receptors-4 (TLR-4)	Macrophage activation
Flagellin	TLR-5	Macrophage activation

Microbial cell wall components	Complement	Opsonization, Complement activation

Table 3.1: List of PAMPs, PRR & effector immune response

Characteristics of Immunoglobulins (Antibodies)

Immunoglobulins are also called as antibodies. These are the component of secretory or humoral part of specific immunity. These are glycoprotein molecules that are produced by plasma cells in response to an immunogen and which function as antibodies. The immunoglobulins derive their name from the finding that they migrate with globular proteins when antibody-containing serum is placed in an electrical field

Immunoglobulins have following important characteristics or functions

Primary Functions

The binding of specific antigen with its corresponding antibody is the primary function of immunoglobulin. This function determine the specific nature of antibodies. Moreover, the binding sites on antibodies for specific antigenic determinants is called as paratopes. Each antibody has its own specific paratope for its specific antigen. The number of available antigen binding sites on antibody are called as valency of antibody. Each antibody has minimum valency of two as two antigenic determinants can bind with a single antibody molecule.

Secondary or Effector Functions

After binding with its specific antigen, the antibody perform its secondary functions which are also called as biological or effector functions. These are as follows

1) Complement activation: complement proteins bind with antibody after binding with its corresponding antigen which leads towards complement activation for their effector function.

2) Binding to various cell receptors via specific receptors to Fc fragment of antibody also called as Fc receptors (FcR). These receptors present on the surface of phagocytic cells like neutrophils & macrophages for performing their corresponding effector functions.

Basic Structure of Immunoglobulin

Immunoglobulins composed of following important structures (Fig.3.6)

1) Heavy & Light chains:

Typically antibody composed of four chains, Two (02) identical light chains and two (02) identical heavy chains. Light chains are of two kinds

a) Kappa light Chains b) Lambda light chains

While heavy chains are of five following types

a) Gamma Heavy chain b) Mu Heavy chain c) Delta Heavy Chain d) epsilon heavy chain e) alpha heavy chain

2) Disulfide bonds: The heavy & light chains of immunoglobulin have peptide linkage in the form of disulfide bonds, These linkages are of Interchain which are in between light & heavy chain while Intrachain disulfide linkages are inside either heavy or light chains.

3) Variable & constant regions

   The regions in heavy& light chains which are conserved in nature are called as constant regions and represented as CH & CL respectively. On the other hand, the regions on heavy & light chains which are not conserved and variable in nature are called as variable regions and designated as VH & VL respectively.

4) Hinge region

   This region makes antibodies flexible to change its shape while performing function after binding with its corresponding antigens.

5) Domains

   These are folded regions which contain an intra-chain disulfide bond. Usually, heavy chains contain four domains while light chains composed on two domains.

6) Oligosaccharides

These are carbohydrates which are attached on CH2 domain in most of immunoglobulins

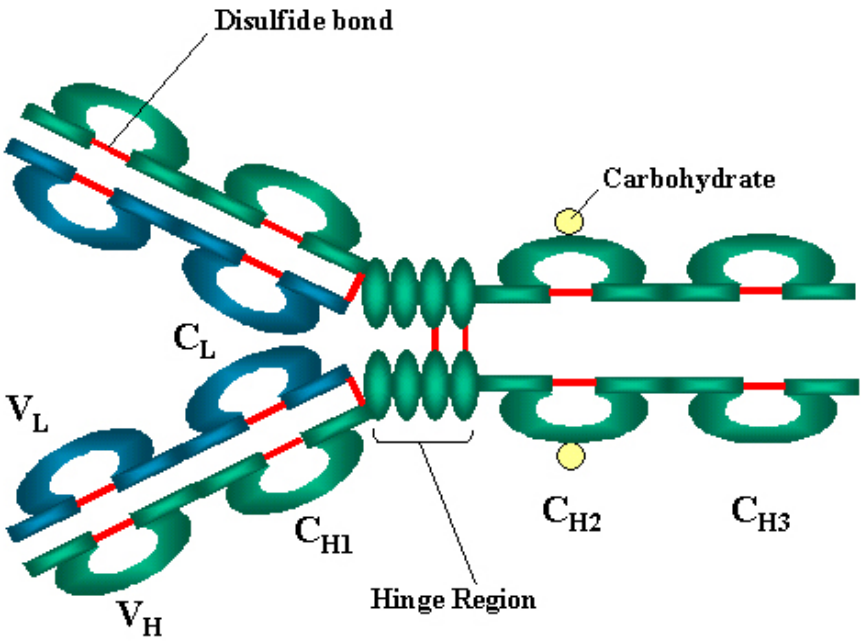

Figure 3.6: Structure of Antibody (Imunoglobulin): Chains in green colour represent heavy chains, chains in blue colour are light chains. Red lines indicate the disulfide linkage

Structure of the Variable Regions

Variable regions are present on heavy & light chains of Immunoglobulins. These regions are subdivided into two sub-regions

1) Hypervariable regions

These regions are present in the variable regions and are based on the variability in the amino acid sequences of heavy and light chains of immunoglobulis. There is high degree of variability among amino acid sequences (as shown in Figure 3.7). These regions are complementary to the epitopes of the antigens to which antibody is specific. These regions are also called as complementarity determining regions

2) Framework regions

These regions are located in between the hypervariable regions and abbreviated as FR (as shown in Figuer 3.7). These regions determine the groups and sub-groups of immunoglobulins. These regions are basically the product of various variable region genes.

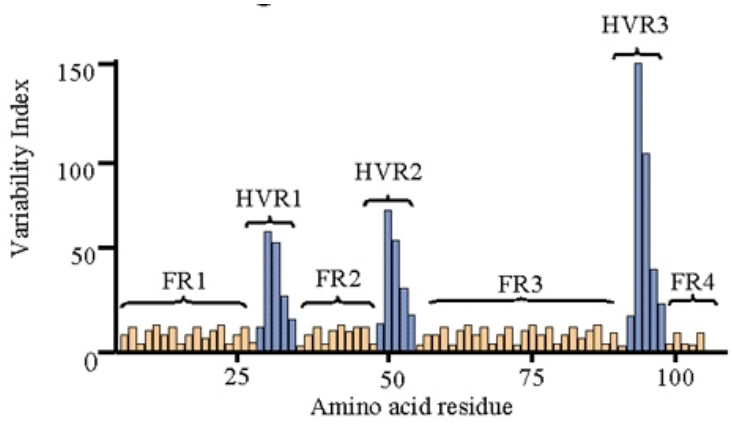

Figure 3.7: Structure of variable regions of Immunoglobulins

Antibodies Fragments Structure/Function

After treating with certain proteolytic enzyme like papain the immunoglobulins are hydrolyzed into two fragments. These fragments which are produced have functional and structural relationship. There are following two fragments

1) Fab Fragment

This fragment is called as antigen binding fragment. Papain causes the cleavage at hinge regions to produce two identical fragments containing one heavy & one light chain. Each fragment has monovalent valency for antigen binding (as shown in Figure 3.8)

2) Fc Fragment

This fragment is called as crystalizeable fragment. Structurally this fragment contains two heavy chains with CH2 & CH3 domains (Figure 3.8). Fc fragment has association with preformed effector functions of antibodies. These effector functions include

1) Complement fixation
2) Placental transfer
3) Binding to Fc receptors on various immune cells like phagocytes

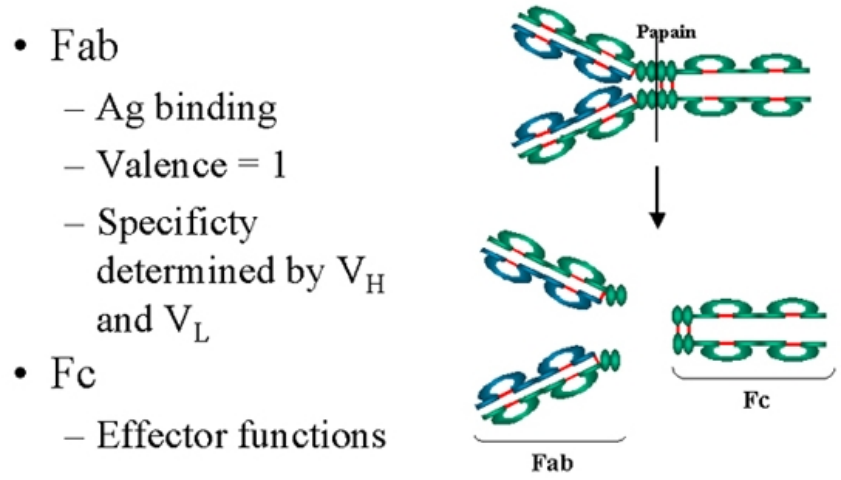

Figure 3.8: Structure of antibody fragments

Classes of Antibodies

Classes of antibodies are based on amino acid sequences in the constant region of heavy chains of antibody. There are five different heavy chains on which antibodies are classified into five different classes. These heavy chains are as follows with their corresponding class

1) Gamma (γ)heavy chain- IgG Class

2) Mu (μ) heavy chain- IgM

3) Alpha (α) heavy chain-IgA

4) Delta (δ) heavy chain-IgD

5) Epsilon (ε) heavy chain-IgE

IgM/IgG Properties & Functions

IgM

IgM contain Mu heavy chain in its structure. Structurally, IgM exist in pentamer generally as secretory form means five different molecules of IgM are connected together with J-chain (as shown in Fig 3.9). Moreover a monomeric form of IgM also exsist which is not secretory in nature but present on the surface of B-lymphocytes as a receptor.

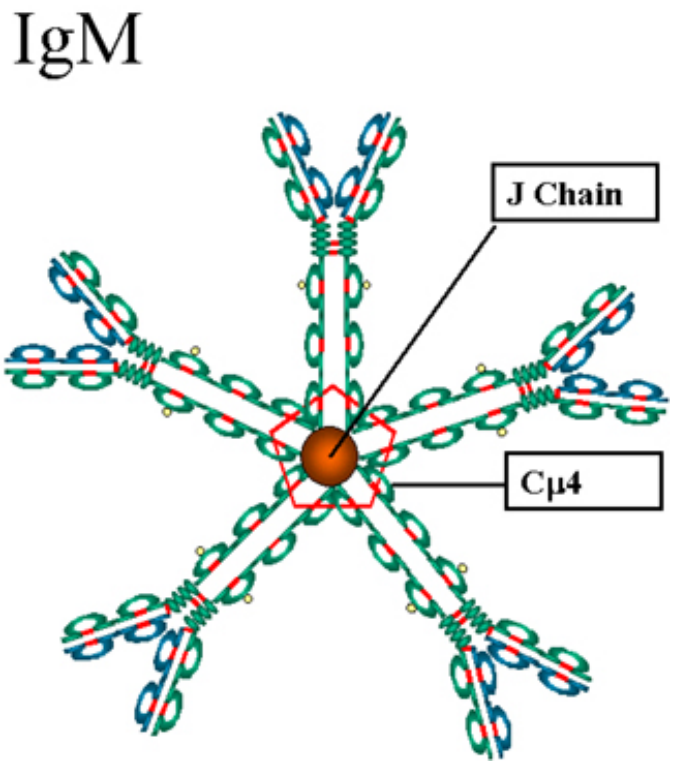

Fig 3.9: Structure of IgM antibody

Characteristics of IgM

IgM exsist as the third most common antibody which is present in serum. IgM serve as a first antibody made by virgin (naïve) B-cells in response to an antigen. IgM is considered a good complement fixing antibody due to its multivalent structure. IgM also has the ability to bind to surface of microorganisms as an opsonin molecule for removal by phagocytosis.

IgG

IgG carry gamma heavy chain in its structure. The molecule of IgG exist in monomeric (single) form. It has subclasses like IgG1,IgG2, IgG3 & IgG4 based on number of disulphide bonds & length of hinge region (as shown in Fig. 3.10).

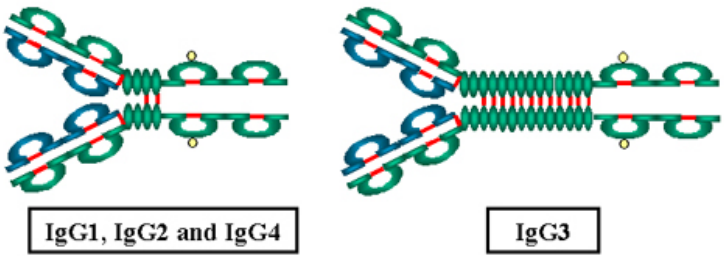

Fig 3.10: Structure of IgG subclasses

Characteristics of IgG

IgG is the major antibody of serum which is approximately 75% of total antibodies of the serum. It is also major one in extravascular space which is the space outside of the blood vessels. Functionally, IgG is considered as a good complement fixing antibody. It also has an important role in transfer via placenta by binding its specific receptor FcR on placental cells for providing maternal immunity. Moreover, IgG has good opsonization ability in order to remove the foreign microbes by phagocytes.

IgA/IgE/IgD Properties & Functions

IgA

IgA is the major antibody of body secretions like sweat, tears, mucosal secretions and breast milk. In secretions. IgA exist as dimeric molecule as two molecules of IgA are linked with Joining (J) chain (Fig.3.11 a). Secretory IgA contains secretory piece in its structure. The secretory piece is

added to IgA molecule in epithelial cells while transferring from basal surface to the apical surface of epithelial cell surface (Fig.3.11 b).

Fig 3.11 (a): Structure of IgA

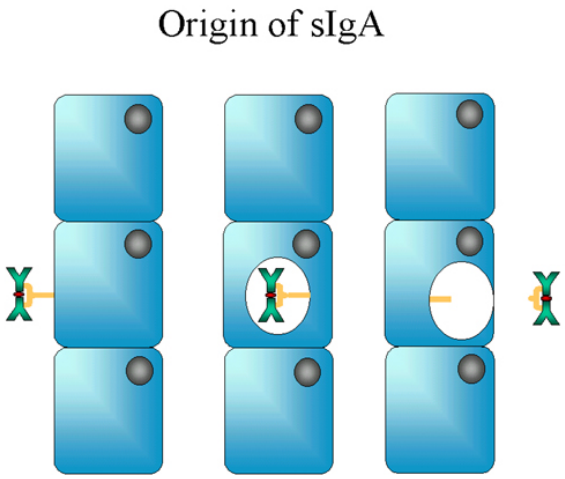

Fig 3.11b: Origin of secretory IgA during transfer from basal membrane to apical surface of epithelial cells

IgA does not have the ability to fix complement in normal structure. However, the aggregated form of IgA can fix the complement.

IgE

IgE is the least common antibody which is present in serum. Structurally, it exists as monomeric (single) form (Fig. 3.12). IgE has important role in allergic reactions which are also called as anaphylactic reactions or anaphylaxis. IgE binds with its corresponding receptors FcRε on the surface of eosinophils and basophils in allergic condition. Furthermore, the binding of specific allergen on the surface of IgE cause the cross-linking of IgE which further leads towards the degranulation of these cells. IgE is also responsible for parasite killing via IgE mediated cellular cytotoxicity. IgE cannot fix complement proteins.

IgE

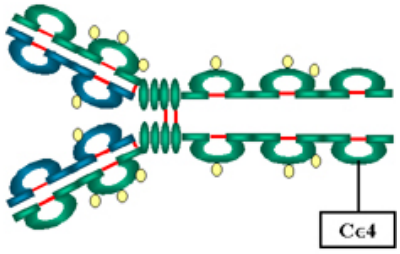

Fig 3.12: Structure of IgE

IgD

IgD is considered as very low level serum antibody due to its least concentration The role of IgD in serum is still unknown. Primarily, IgD found on B-cells surface as B-cell receptor (BCR) for binding with corresponding antigen with the help of tail piece. Structurally, IgD exist as monomer (Fig 3.13). IgD also cannot fix complement proteins.

IgD

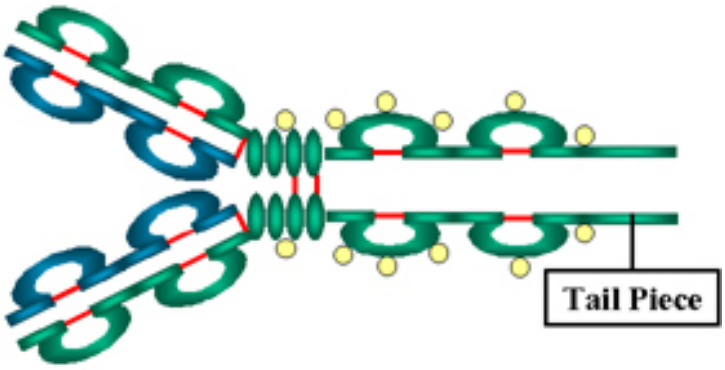

Figure 3.13: Structure of IgD

Types vs Sub-types of Antibodies

Types of Antibodies

Antibodies types are based on different kinds of light chains which are present in the structure of antibodies. There are following two light chains

1) Kappa (κ) light chains
2) Lambda (λ) light chains

Types of antibodies are based on differences in amino acid sequences in constant regions of light chains e.g in case of IgG there are two types IgG Kappa & IgG Lambda. In principle, both immunoglobulins belong to same class but due to presence of different light chains these belong to different types.

Sub-Types of Antibodies

The sub-types of antibodies are based on sub types of light chains which are present in the structure of antibodies. The light chains are divided into subtypes based on differences in amino acid sequences in constant regions of light chains. There are following subtypes of light chains

- Lambda chains have following four subtypes e.g L1, L2,L3 & L4
- Kappa chains have following two subtypes e.g K1 &K2

For example, the subtypes of IgG include IgG Kappa 1 & IgG Lambda 4. Similarly, the subtypes of IgM can be IgM Kappa2 & IgM lambda 3 respectively.

Antigen & Antibody Reactions

The reactions between specific antigens with its corresponding antibodies is called as antigen & antibody reactions. In these kind of reactions, either antigen is known for unknown antibody or vice versa. Such kind of reactions are between the antigenic determinants which are also called as epitopes with their corresponding binding sites on antibodies called are paratopes. As these antibodies and antigens are present in the serum of the patients that's why these reactions are also called as serological reactions. These reactions are based on lock & key concept because such kind of interactions between antigens & antibodies are very specific. Moreover, these interactions are like non-covalent bonding which include following forms of interactions or forces

- Hydrogen bonds

- Vander Wall forces
- Electrostatic forces

However, these reactions also have reversible nature due to non-covalent binding between antigen & antibody.

Factors Effecting on Antigen & Antibody Tests

The good antigen & antibody reactions are known due to high specific & sensitive nature. Various factors are involved which determine the nature of antigen and antibody reactions which are as follows

1) Affinity

It is the strength of binding between single antigenic determinant & single antibody site. These are the sum of attractive & repulsive forces which make the Ag/Ab reactions stronger. Higher the affinity of Ab for Ag, more stable interaction. Similarly, if the affinity would be weak between Ag & Ab then the reaction would be less stable (Fig.3.14).

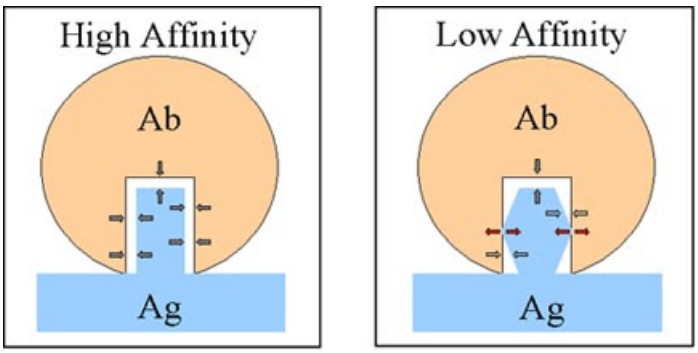

Fig 3.14: The binding interactions between antigen (Ag) and antibody (Ab).

2) Avidity

Avidity is the strength of binding of antigen with many antigenic determinant & multivalent antibody. It is also called as overall strength between multivalent antigens & antibodies. In contrast to affinity, avidity is influenced by valency of both Ag & Ab. Multivalent antibodies have more avidity as compare to univalent or oligovalent antibodies (Fig 3.15). That's why, the Ag/Ab reactions which have more avidity those are more stable & easy to detect.

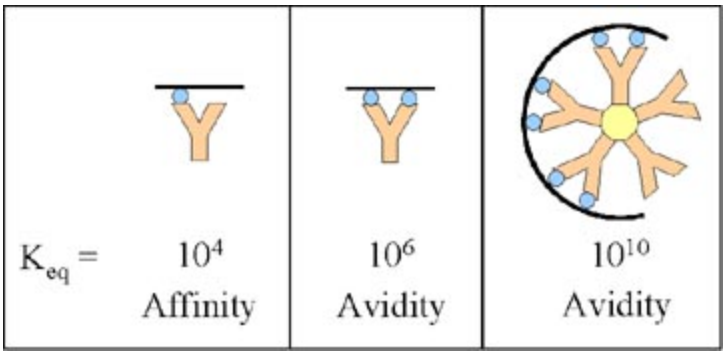

Fig 3.15: Effect of avidity on Ag (Blue colored) & antibody (orange colored) reactions

3) Antigen & Antibody Ratio

Ag/Ab ratio also considered as one of the important factor effecting on Ag/Ab reactions. It also determines the complex nature of Ag/Ab complex formation. For good Ag/Ab reaction, there would be the equal ratio between Ag/Ab and that point is also called as equivalence point for Ag/Ab reaction. As a result a complex structure develops which is called as lattice (Fig 3.16) which is easy to detect. However, in the case of unequal ratio between Ag/Ab doesn't form such kind of complex structure.

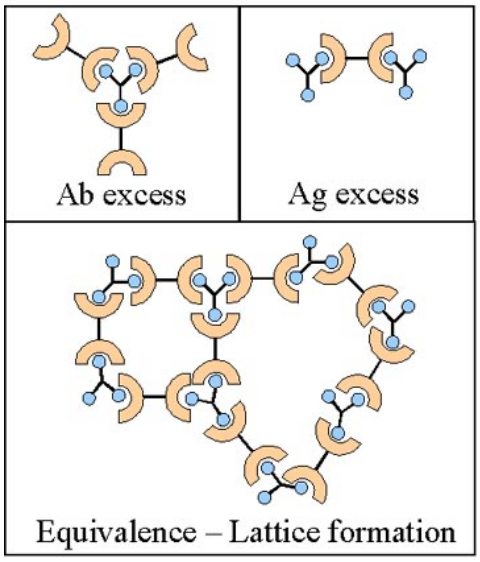

Fig 3.16: Ag/Ab ratio effects on Ag/Ab reactions

4) Physical Form

Physical form of antigen & antibody also determines the nature of Ag/Ab reaction. Antigen and antibody can exsist in the following two physical form

a) Particulate form
b) Soluble form

In case of particulate form i.e the presence of Ag or Ab on the surface of any particle like red blood cells, bacteria or latex particles lead a kind of Ag/Ab reaction called as agglutination reactions. However, in case of soluble form i.e in secretions or serum form precipitate form and these reaction are called as precipitation reaction.

Agglutination Reactions

These are antigen and antibody reactions in which the particulate nature of antigen causes the reaction with its specific antibody to form visible clumps. Such visible clumps are called as agglutinates. The antibodies which render such kind of reactions are called as “agglutinins”. As the antigens are particulate in nature and the nature of particle determines the kind of agglutination reactions. There are following three important kinds of agglutination reactions based upon the particle

1) Hemagglutination

In these reactions, the antigens are present on the surface of red blood cells (RBCs). That’s why these are called as hemagglutination reactions.

2) Bacterial agglutination

The bacterial antigens present on the surface of bacteria react with their corresponding specific antibodies and form visible agglutinates. Such kind of agglutination reactions are called as bacterial agglutinations.

3) Latex Agglutination

In this kind of agglutination reactions, the antigens are present on the surface of latex particles which form visible agglutinates after reacting with specific antibodies.

Hemagglutination

These are the reactions in which antigens are present on the surface of red blood cells (RBCs). That's why these are called as hemagglutination reactions (Fig 3.17).

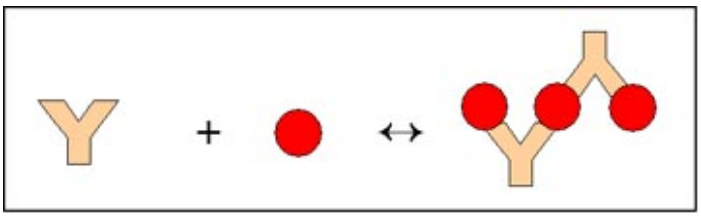

Figure 3.17: Hemagglutination reactions: Antibodies (Orange color) react with RBC (red color) carrying antigens

Hemagglutination reactions can be of two types based on the nature of reaction

A) Qualitative Hemagglutination:

These hemagglutination reactions determine the presence of antigen or antibody in the serum. For example the Blood group typing (ABO & Rh).

B) Quantitative Hemagglutination

This kind of heagglutination is used for determining the quantity of antibodies or antigens in the serum of the patient. In this kind of agglutination the titer of antibodies are determined which is the lowest concentration of Antibody which causes agglutination or the maximum dilution of serum which causes agglutination

Application of agglutination reactions

Agglutination reactions are used for following ways

- Blood group determination: ABO & Rh for both either on the basis of antigens of RBC or antibodies of serum
- Assessing bacterial infections e.g Widal test for diagnosis of Typhoid fever

Precipitation Reactions

These are the kind of antigen/antibody reactions in which soluble nature of antigen causes the reaction of antibody to form precipitate in a medium. These reactions are called as

immunoprecipitation due to involvement of antigen & antibody. The antibodies which cause such kind of reactions are called as precipitins. These reactions occur in a medium which is used for visualization of Ag & Ab reaction e.g Agar. Both Ag & Ab are soluble form in serum.

Types of Precipitaion reactions

On the basis of diffusion of each molecule i.e Ag & Ab, following two types of precipitation reactions are

1) Single immunodiffusion or precipitation
2) Double immunodiffusion or precipitation

Single Immunodiffusion

These kind of precipitation reactions are also called as Radial Immunodiffusion. In such kind of reactions, antibody is already incorporated in agar. While the patient's serum containing antigen is added in the agar well. Antigen diffuses in agar & react with corresponding antibody to form visible precipitate in the form of ring. The size of the ring determine the concentration of antigen in the patient's sera as there is a direct relationship between the diameter of precipitation ring and the antigen concentration (Fig 3.18) These kind of immunoassays are used for determining the level of serum immunoglobulins e.g IgA, IgG & IgM.

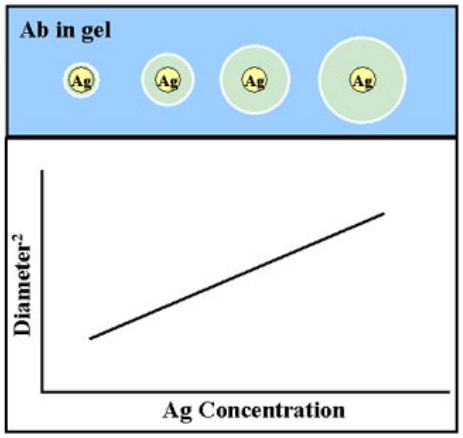

Figure 3.18: Appearance of precipitation ring in Single immunodiffusion

Double Immunodiffusion

These are the precipitation reactions in which both antigen & antibody is added in the medium separately in contrast to single immunodiffusion where antibody is already incorporated in the agar. Antigen & antibody are allowed to react together and reached at equivalence to form a visible precipitate. Such kind of reactions are used for qualitative analysis of complex mixture of antigens in a sample can be determined. Moreover, these reactions are also used for checking the purity of isolated serum proteins.

Complement Fixation Tests (CFTs)

These are the antigen & antibody reactions which can be detected on the basis of ability to fix or consume complement proteins by the antigen & antibody (immune) complex. These tests are usually used for determining the level of good complement fixing antibodies e.g IgG or IgM. Like a typical immune reaction, in these reactions also antigens are unknown from patient's serum while the antibodies are known. Complement proteins are used in order to carry on these reaction. Moreover, the pre-sensitized RBC with anti RBC antibodies are used as an indicator of these reactions (as shown in Fig 3.19)

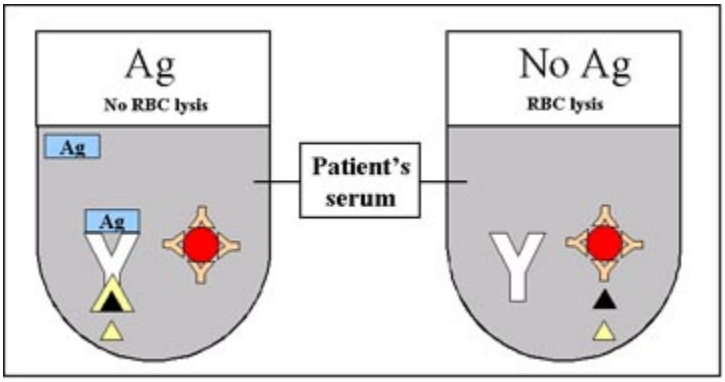

Fig.3.19: The principle of Complement Fixation test

Interpretation of CFT

- Lysis of RBC: No Complement fixation by Ag & Ab complex: No Ag: Negative CFT
- No Lysis: Complement fixation by AG & Ab complex: Ag is present: Positive CFT

Applications of Complement Fixation Test

Complement Fixation tests are used for determining Gonococcal Ab in serum.

Enzyme Linked Immunosorbent Assay (ELISA)

These are the kind of antigen & antibody reactions which can be detected on the basis of substrate utilization by enzyme. These reactions are also called as solid phase Immunoassay as these reactions occur on the solid surface e.g surface of plastic. Like other immune reactions, this is also used for determining either unknown antigen or antibody. Followings are the two types of ELISA based on whether antigen or antibody is determined

1) Direct ELISA
2) Indirect or Sandwich ELISA

Direct ELISA

In this type of ELISA, unknown antibody can be determined in patient's serum. Principally, known antigen is coated on solid phase which can be a Glass or plastic surface. Patient's serum containing unknown antibody is added for reacting with the specific coated antigen. Afterward, another antibody which is pre-coated with an enzyme e.g horseradish peroxidase is usually used. Then the activity of enzyme is determined by adding its substrate i.e hydrogen peroxide in the form of colour change in the reaction mixture (as shown in Fig.3.20). Moreover, the intensity of color determine the concentration of unknown antibody in the patient's serum indirectly.

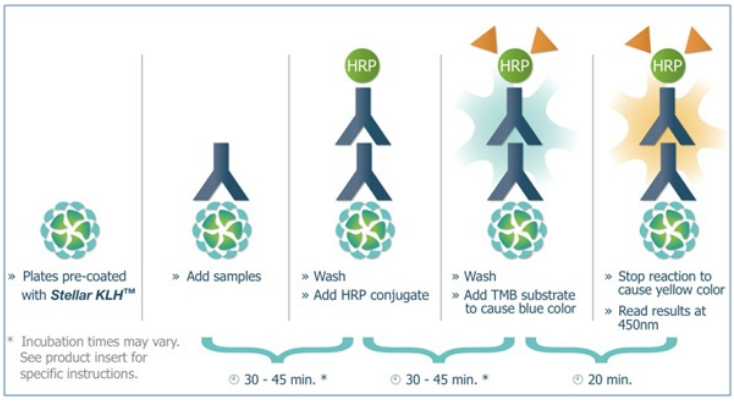

Figure 3.20: Principle of Direct ELISA

Indirect ELISA

In this type of ELISA, unknown antigen can be determined in patient's serum. Principally, known antibody against the unknown antigen is coated on solid phase which can be a Glass or plastic surface. Patient's serum containing unknown antigen is added for reacting with the specific coated antibody. Afterward, another antibody which is pre-coated with an enzyme e.g horseradish peroxidase is usually used, thus the unknown antigen get sandwiched between two antibodies, that's why it is also called as sandwiched ELISA. Then the activity of enzyme is determined by adding its substrate i.e hydrogen peroxide in the form of color change in the reaction mixture (as shown in Fig.3.21). Moreover, the intensity of color determine the concentration of unknown antibody in the patient's serum indirectly.

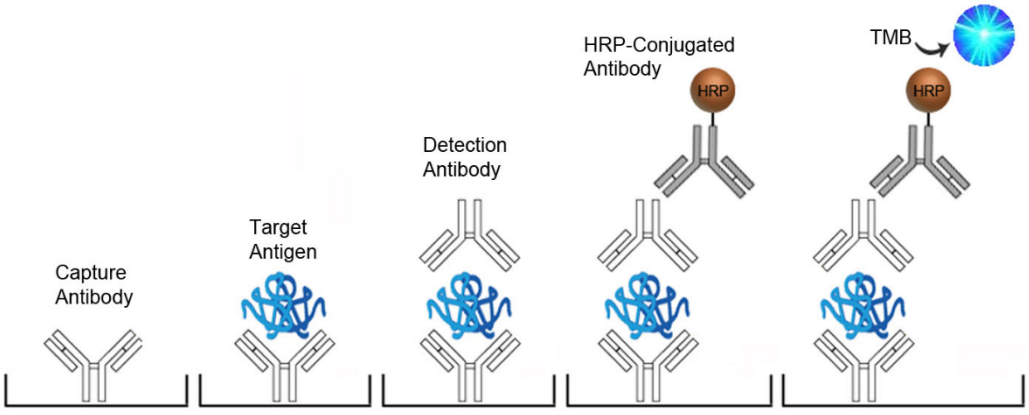

Figure 3.21: Principle of Indirect (Sandwiched) ELISA

Applications of ELISA

Followings are the significant applications of ELISA

- It is used for determining viral antigens in patient's serum e.g Hepatitis B surface Antigens (HbsAG)
- Also used for the determination of antibodies against viruses e.g Anti Hepatitis C Virus (HCV) antibodies
- Also used for estimating the levels of variou serum cytokine e.g Interleukin-1 (IL-1), Tumor necrotic factor alpha (TNF-a) etc.

Chapter# 4: Genetics of Antibody Structure & Diversity

Antibody Formation

It is a process of immune response against antigen or immunogen for producing their specific antibodies. This process can be T cell-independent or dependent based on the dependency of T-lymphocytes in this process. During the process of antibody formation, B-cells recognize the antigen with its specific receptor which is also called as B-cell receptor (BCR). After binding with specific antigen with BCR on B-cell, there is transformation of B-cells into a specialized variety of cells which are called as plasma cells. Plasma cells are also called as antibody secreting cells as these secrete antibodies (as shown in Fig 4.1).

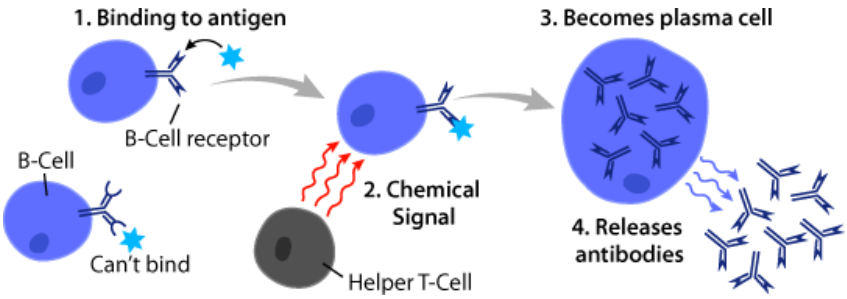

Figure 4.1: Events of antibody formation

General Characteristics of Antibody Response

Antibody formation process is well controlled mechanisms with following significant characteristics

1) Self/Non-Self Discrimination
2) Memory
3) Specificity

Self/Non-Self Discrimination

The antibodies which are generated during antibody formation process would be reactive only against non-self (foreign) antigens. If there is any reactivity against self-antigen then that is very important clinical condition which is called as autoimmunity meaning destruction of self-tissues by antibodies.

Memory

Remembering the nature of antigen is another important significant characteristic of antibody formation process. There is an illicit memory response against same antigen in case of again and again exposure. Memory response is also called as anamnestic response. This memory response facilitate the robust way of clearing non-self (foreign) antigen. There are specific cells for exhibiting the memory which are called as B-lymphocytes memory cells.

Specificity

The antibodies have high degree of specificity against specific antigens. This form of specificity lessen the chances of cross reactivity with irrelevant antigens. Moreover, this characteristic of specificity is the characteristic of adaptive immune response.

Fate of Immunogen

Immunogen enter in the body first time which is called as primary injection of immunogen while if there is second time exposure of body with the same immunogen is called as secondary injection of immunogen. The fate of immunogen is different in both cases

Primary Injection

Immunogen enters in the body first time (Primary Injection). There are the following four phases for kinetics of immunogen clearance

1) Equilibrium Phase

It is the rapid diffusion process. In this phase, an equilibrium develops between vascular & extravascular components by the process of diffusion (as shown in Fig 4.2)

2) Catabolic or Decay Phase

In this phase of immunogen fate, the host immune cells cause the decay of antigen by activating an effective immune response. There is active involvement of cells & enzymes in this phase. Usually, the macrophages & phagocytic cells are involved. The duration of this phase depends upon the nature of immunogen & the host immune status. It can be shorter in case of immunocompetent host or *vice versa*.

3) Immune Elimination phase

Newly synthesized antibodies combine with antigens to form Ag/Ab (Immune) complexes in this phase. Those immune complexes are eliminated by the process of phagocytosis that's why it is called as immune elimination phase. Antibodies appear in serum only after this phase get over as the concentration of immunogen get eliminated.

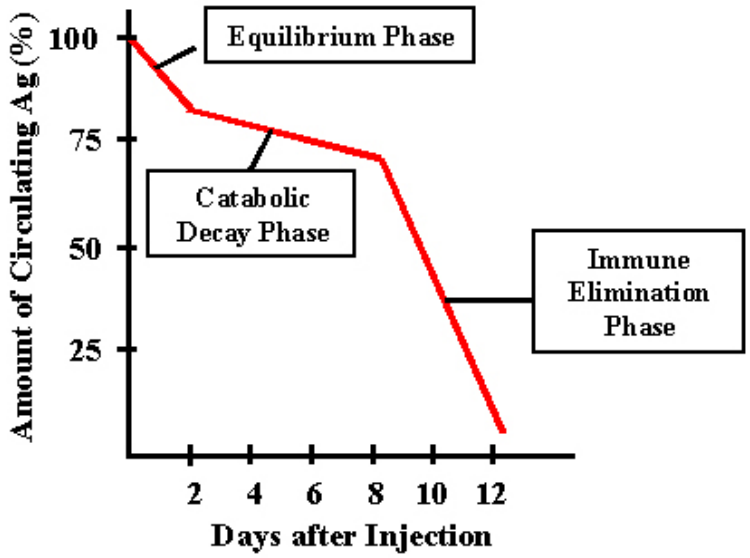

Figure 4.3: Various phases during fate of immunogen

Secondary Injection

During second time entry of immunogen inside the body, if there is circulating antibodies which were produced during primary injection then there is a rapid immune elimination of antigen. However, if there is no circulating antibodies then all three phases occur but immune elimination phase will be rapid due to memory response.

Primary & Secondary Antibody Response

Primary Antibody response

This is the first kind of antibody response against antigens which are entering inside the body. In this response major class of antibody belongs to IgM. Antibodies produced during this response are with less affinity and less persistent (Fig 4.4)

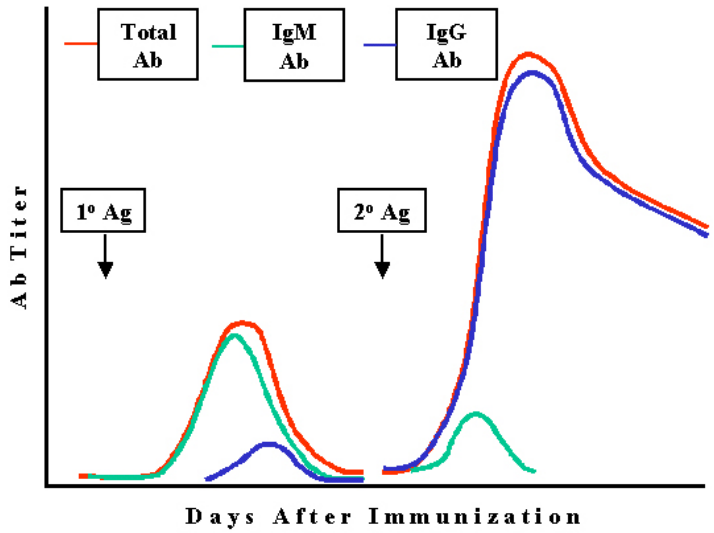

Figure 4.4: Antibody formation during primary & secondary response

Secondary Antibody Response

The second kind of antibody response against antigen is called as secondary antibody response. The major class is IgG but may IgE or IgA but less IgM (Fig 4.4). Antibodies produced in this response have high affinity. The affinity of antibodies depend upon the dosage of antigen, the low dosage of antigen causes increase in the affinity of antibodies which is called as affinity maturation (Fig 4.5)

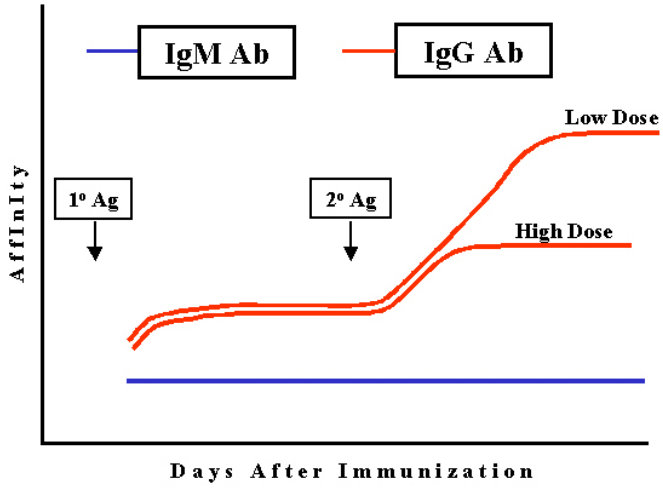

Figure 4.5: Affinity of antibodies during primary & secondary response

Kinetics of antibody response against T-dependent antigens

The antibody formation against T-dependent antigens also have following two impotent responses

1) Primary response
2) Secondary response

Primary Response

During primary antibody response, following four important phases are involved against T-dependent antigens

1) Inductive or Lag phase

In this phase, antigen is recognized as foreign particle for generation of immune response. Immune cells start to proliferate & differentiate in response to antigen. This phase usually last for 5-7 days (Fig 4.6)

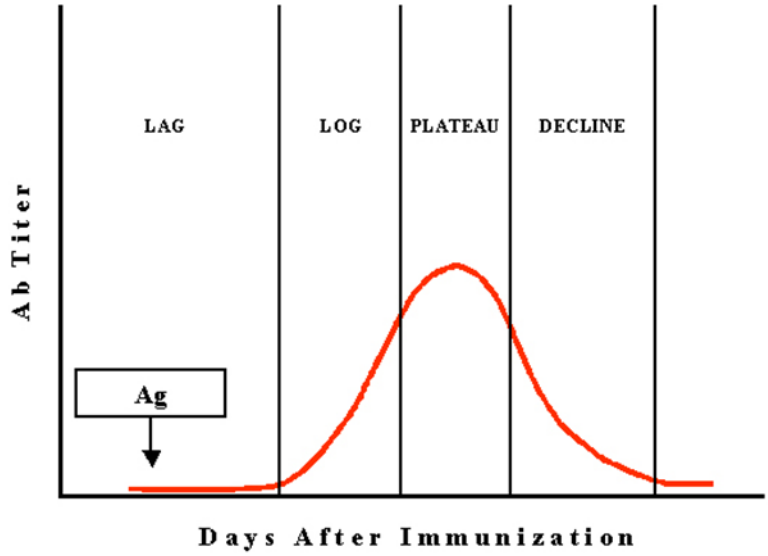

Figure 4.6: Kinetics of Primary response for T-dependent antigens

2) Log or Exponential phase

In this phase, there is rapid increase in serum antibody concentration as result of accelerated immune response. There is rapid activation & proliferation of B-cells in response to antigen. As a result, B-cells differentiation into plasma cells to secrete more antibodies

3) Plateau or Steady State phase

As the name suggests, in this phase there is a steady state between antibody synthesis & decay. That's why there is no further increase in serum antibody concentration.

4) Decline or Decay Phase

In this phase, there is increase in the decaying rate of antibodies which results in fall in antibody level. This fall leads the antibody to the base level.

Secondary Antibody Response

The second time entry of T-dependent antigen in the body also lead towards the following four phases with different kinetics. The secondary antibody response is also called as memory or anamnestic response

1) Lag phase

During secondary response against T-dependent antigen, the lag phase is comparatively shorter as compare to primary response due to memory response (Fig 4.7).

2) Log Phase

In case of T-dependent antigen this phase is more rapid and sharp. There is generation of higher levels of antibodies in short time due to memory response (Fig 4.7).

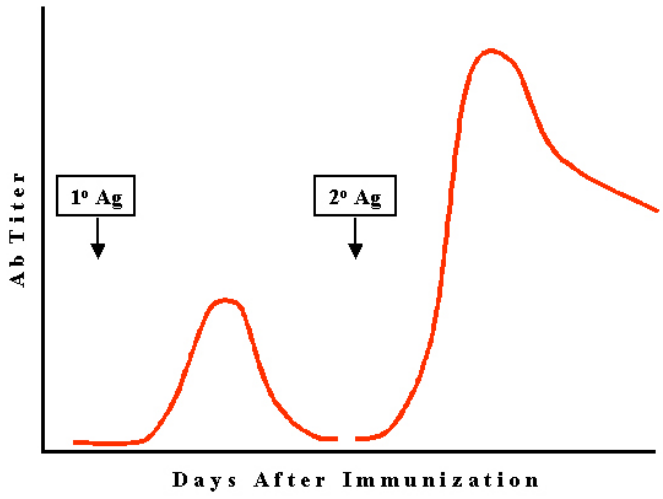

Fig 4.7: Kinetics of secondary response for T- dependent antigens

3) Plateau or Steady state phase

There is no plateau or steady state during secondary response due to less decaying of antibodies (Fig 4.7).

4) Decline phase

This phase is less rapid and sharp. Antibodies may persist for days, months or even yeas due to memory response (Fig 4.7).

Kinetics of antibody response against T-independent antigens

In case of T-independent antigens, mainly IgM class of antibodies are produced in contrast with T-dependent antigens. Likewise T-dependent antigens, there are all those four phases in primary response. However, secondary response also has same phases in second time exposure to antigens. Moreover, there is no memory response in case of T-independent antigens (Fig 4.8)

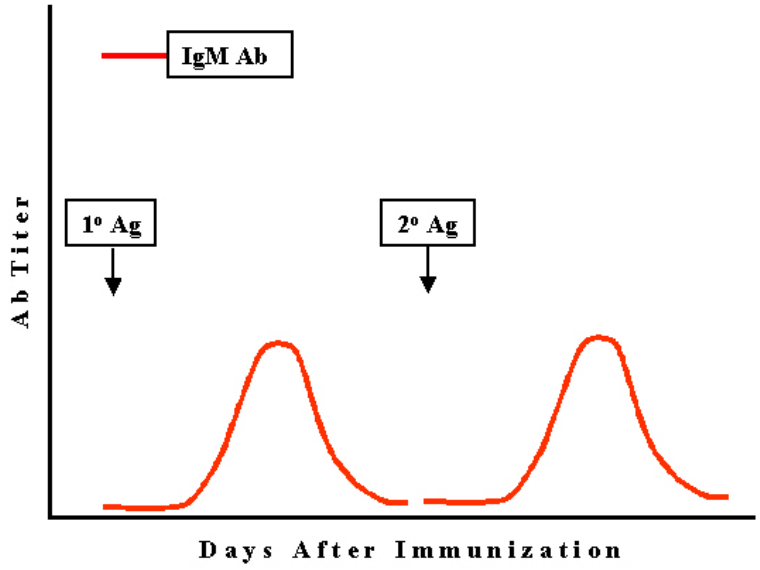

Figure 4.8: Kinetics of antibody formation against T-independent antigens

Cellular Events during Antibody Response

As far concern of cellular events during antibody formation against antigens also varies on the phase of antibody formation

Primary Response

In primary response, the cellular events also depend upon the phase as followings

1) Log phase

In this phase, the clones of B & T-lymphocytes bind to antigens with their receptors. As a result, B-Cells differentiate into plasma cells. These plasma cells start to secrete antibody as these are antibody secreting cells (Fig 4.9).

2) Log phase

During this phase, plasma cells initially secrete IgM as Mu (μ) chains gene is close to rearranged VDJ regions (Fig 4.9).

3) Plateau phase

In this phase, due to antigen depletion the T-cells are no longer active which leads towards the death of Plasma cells. As a result, newly synthesized antibody equilibrates (Fig 4.9).

4) Decline Phase

As there is no new antibody formation in this phase due to the death of most of plasma cells (Fig 4.9).

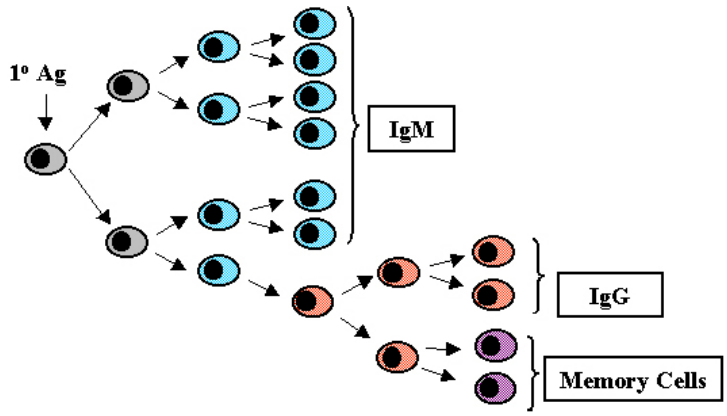

Figure 4.9: Cellular events during Primary response of antibody formation

Secondary Response

During secondary response, there is a Memory cells pool which is mainly comprised of T & B lymphocytes. These cells are basically belong to the activated T& B cells which are activated during primary response Mostly memory pool cells are activated in this response of antibody generation. Moreover, there is antibody class switching from IgM to IgG. There is mostly IgG in secondary response. Some plasma cells also differentiate into memory cells (Fig 4.10)

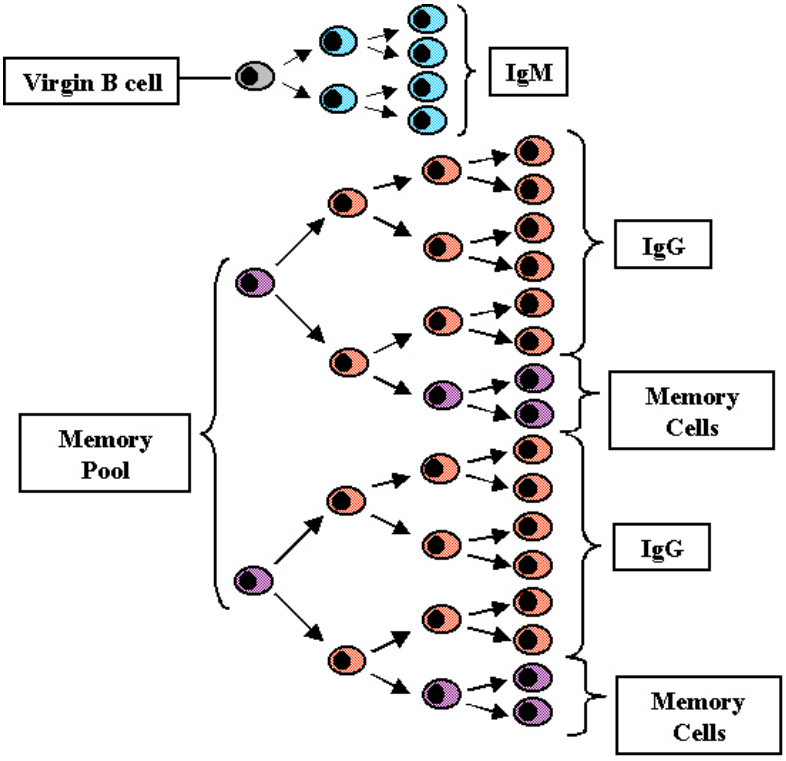

Figure 4.10: Cellular events during Primary response of antibody formation

Chapter# 05: Expression of Immunoglobulin Genes & V (D) J Recombination

Structure of Human Antibody Gene (Loci)

Antibody Gene

As one Gene encode for one Protein, similarly the immunoglobulin which is glycoproteins are encoded by a single gene. The Immunoglobulin repertoire is encoded by multiple germ line gene segments. These segments undergo somatic recombination during development of B-Cells. The basic component of gene is inherited but alteration occur during lifetime for diversification of antibodies. Immunoglobulin gene is divided into following two families based upon the structure of antibody

1) Light chain gene family
2) Heavy chain gene family

Light Chain Gene Family

This family encode for following light chains of immunoglobulin

1) Lambda Light Chains

This gene is located on chromosome 22. Lambda light chain gene is composed of 4 constant (C) region genes. Among these everyone encodes for each subtype of Lambda. It also contain 30 Variable (V) genes. Each of V region gene composed of two exons, one L that encodes for Ladder exons and the other V that encodes for variable region. Upstream of each of the C genes there is and additional exon called J (joining). The L, V, J and C exons are separated by introns (intervening non-coding sequences) (Fig 5.1).

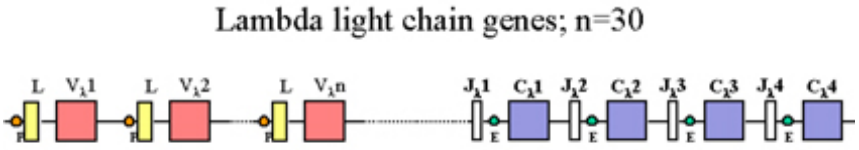

Fig 5.1: Structure of Lambda Light Chain gene

2) Kappa Light Chains

The kappa light chain gene family contains only one C region gene, since there is only one type of kappa light chain. There are many V region genes (approximately 250) each of which has a leader exon and a V exon. In the κ gene family there are several J exons located between the V

and C genes. All of the exons are separated by introns.

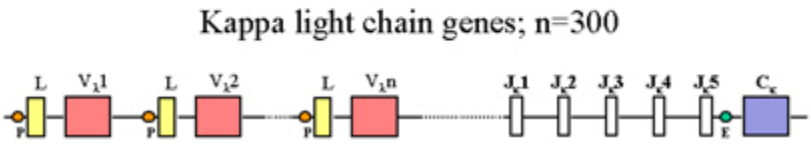

Figure 5.2: Structure of Kappa light chain gene

Heavy Chain Gene Family

Heavy chain gene is located at chromosome 14. It is composed of many constant © region gene for a each class & sub class. Each of C contain exons for hinge region & domains. There are approx.1000 variable genes. Each of V gene composed of L (Ladder) exons. Also contain V (variable) exons. In contrast to light chain gene family, it also carry additional D (diversity) exons which determines the diversity of immunoglobulin. Moreover, J (Joining) exons also exist. There are also introns in between exons (Fig 5.3)

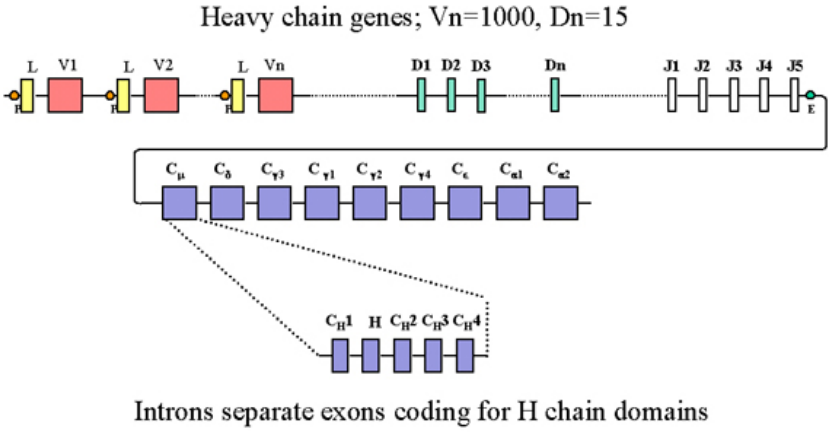

Fig 5.3: Structure of heavy chain gene

Somatic Recombination

Recombination involves rearrangement of DNA in somatic cells. The newly recombined genes are not inherited in contrast to germ cells. Primary Ig repertoire differ slightly from one individual to next one. Also differ in individual's lifetime by their exposure to different antigens. As B-cell differentiate into mature one, it make light chain (Kappa). There is rearrangement of genes (exons) as introns are removed and genes start to express. In this recombination, V genes become close to J genes by removing introns. Recombined DNA processed into mature RNA by splicing. As B-cell differentiate into mature one, it make heavy chains. There is rearrangement of genes (exons) as introns are removed and genes start to express. In this recombination, D genes become close to

J & then V recombines with DJ. Finally recombined DNA processed into mature RNA by splicing (fig 5.4)

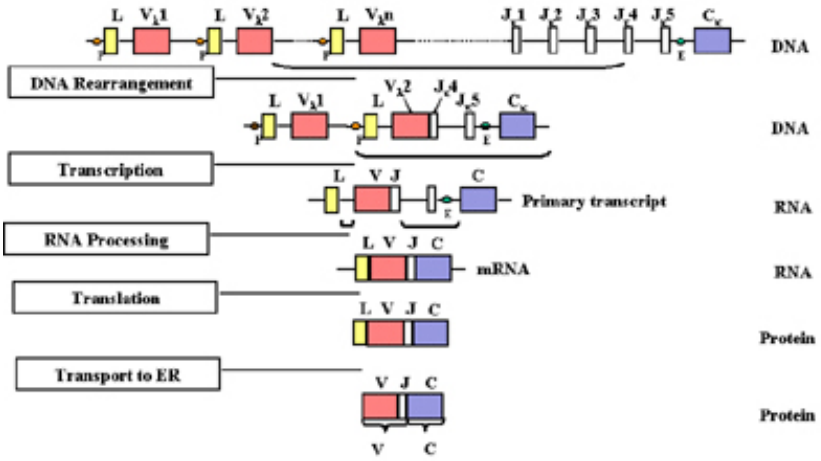

Fig 5.4: The process of somatic recombination

Somatic Hyper mutation

The phenomenon of enhanced rate of point mutation in the immunoglobulin V region genes is called as Somatic hyper mutation. It occurs particularly in V gene which codes for 2nd hypervariable region. As a result, there is an increase in immunoglobulin diversity after various antigenic stimulation. Moreover, it also causes increase the affinity of immunoglobulin in order to compete for limited amount of available antigens.

Role of Somatic Hyper mutation in diversity of antibodies

Somatic hyper mutation has very significant role in the diversity of antibody. As a result, there is generation of sum of all the possible antibody specificities that an organism can produce. Normally, humans can make 10^7-10^8 different antibody molecules. Immunoglobulin diversity increase after various antigenic stimulation. So increase in antibody specificities due to this phenomenon of somatic mutation. It occurs at high rate approximately at 10^6 times higher. The exact mechanism by which mutation occurs in V region gene without effecting the C region is still under research. However, there is some role of activation induced cytidine deaminase (AID) which is considered essential in DNA deamination for somatic hyper mutation.

V (D) J combinational Diversity

The component of antibody diversity that is generated by joining of various antibody gene segments. As in case of light chain genes, the V & J region genes combination occurs, while for heavy chain, all V, D & J region genes combination occurs. The D region combines first with J region and then V to form a complete heavy chain.

Regulation of V (D) J Recombination

For the regulation of V (D) J recombination, there is some regions exist called as recombination signal sequence (RSS). These are flanked between V, D & J exons. These regions are composed of conserved regions in the form of haptamer & nonamer(based on number of basepairs) separated by two & one turn signals (as shown in Fig 5.5). The process recombination occurs between two & one turn

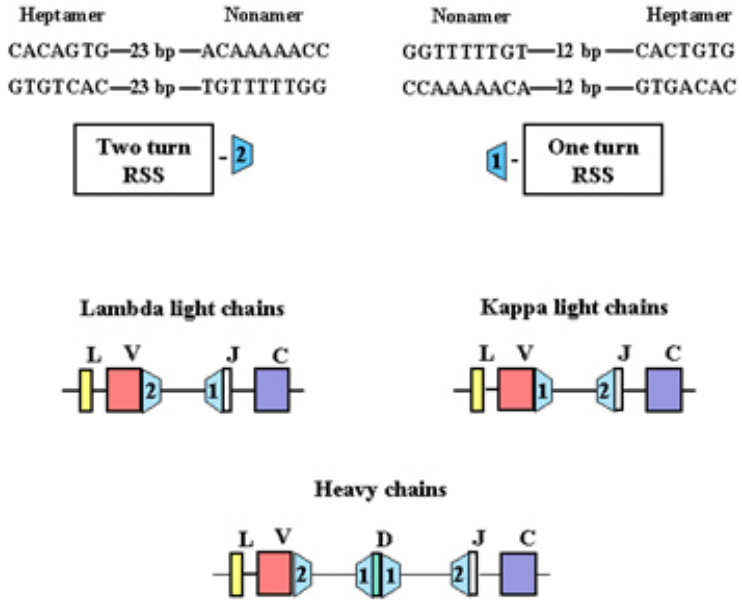

Figure 5.5: Regulation of V (D) J recombination

Recombination occurs after the removal of introns between V & J in case of light chain like lambda & kappa chains. However, it occurs after the removal of introns between, D & J in case of heavy chain (as shown in Fig.5.5). Furthermore, there is involvement of certain enzymes which are called as recombination associated genes (RAG) which are responsible for this recombination. There are two types of RAG, RGA1 & RAG2 respectively. Clinically, the absence of RAG enzymes leads towards an immunodeficiency state called as severe combined immunodeficiency (SCID).

Chapter# 6: Antigen Processing & Presentation

Review of B & T-Cell Receptors for Antigens

B-Cell Receptors (BCR)

B & T lymphocytes recognize various form of substances as antigens in different & unique ways. The role of receptors is very significant in such form of antigen recognition. B cell receptor (BCR) is composed of surface immunoglobulin molecule with a certain specificity for antigen. BCR can recognize following antigens in soluble form

- Proteins (Both Native & Denatured determinants)
- Nucleic acids
- Polysaccharides
- Some lipids
- Small molecules i.e haptens

T-Cell Receptors (TCR)

T cell receptor (TCR) recognize mainly those antigens which are protein in nature. Those antigens are processed in fragmented form before recognition by TCR. Unlike BCR, TCR cannot recognize soluble form of antigens. The processed proteins (antigens) are recognized by TCR after association with a complex called as major histocompatibility complex (MHC). The MHC expressed on all nucleated cells of body. T-cells are grouped functionally on the basis of associated MHC with fragmented protein fragments

1) Cytotoxic T-Cells which recognize antigens in association with class I MHC
2) Helper T-Cells which recognize antigens in association with class II MHC

Introduction to Antigen Processing & Presentation

All the processes that occur within the cell for fragmentation of antigens also called as proteolysis. The process of association of fragmented peptide with Major Histocompatibility Complex (MHC) molecule expressed on the surface of antigen presenting Cells (APC) e.g Macrophages. The

presentation of processed antigen with MHC molecule to T-cell determine the function of T-cell too which is as follows

- Cytotoxic T-Cells: MHC I
- Helper T-Cells- MHC II

Antigen Processing & Presentation of Endogenous antigens

The process in which the antigen is being processed is called as antigen processing. This process varies according to the source of antigen. The intracellular source of antigen which is also called as Endogenous antigen reside in the cytosol of antigen presenting cell like Viruses. For presenting such kind of endogenous antigens MHC I is required which is present in antigen presenting cells. MHC I express on all nucleated cells of body. Furthermore, antigens (endogenous) are processed in a cellular organelle called as proteasome which is a complex having proteolytic activity. After proteolytic activity, the fragmented proteins move across the membrane of endoplasmic reticulum (ER) using transporter membrane. Likewise the synthesis & assembly of MHC I complex occur inside ER. Within ER MHC I form a stable complex with fragmented peptide & express on the surface of cell membrane for presenting to T-cells (as shown in Fig. 6.1).

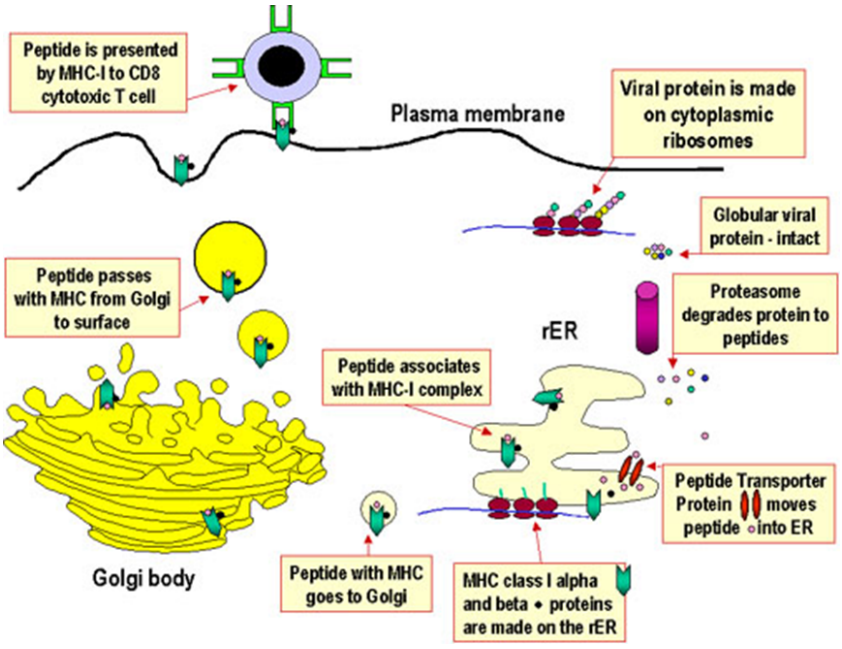

Fig 6.1: Events of processing & presentation for endogenous antigens

Antigen Processing & Presentation of Exogenous antigens

Unlike the endogenous antigens, exogenous antigens are not cytosolic in nature while reside in the endosome e.g bacteria which are taken up by the process of endocytosis in endosomes.Class II MHC is required for presenting such kind of antigens. MHC II express on limited number of cells like antigen presenting cells (APC) like macrophages, dendritic cells & B-cells. In contrast to endogenous antigens, exogenous proteins are processed in endosomes by proteolytic proteins like proteases. The synthesis & assembly of MHC II complex occur inside ER and then transported across golgi complex. Moreover, Tran Golgi complex combines with endosomes containing fragmented peptides. The fragmented peptides complexed with MHC II present on the surface of antigen presenting cell (as shown in Fig 6.2)

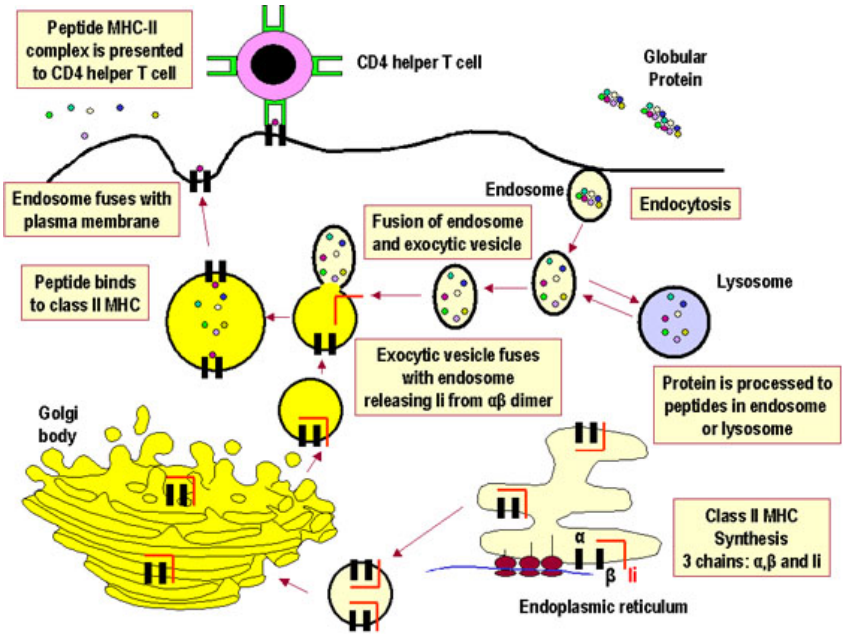

Figure 6.2: Events of processing & presentation for exogenous antigens

Chapter # 07: Major Histocompatibility Complex (MHC)

Introduction to MHC

In adaptive immunity, cell to cell interaction or cell mediated immunity is one of the important mechanism for providing defense against pathogens. In cell mediated immunity, cell to cell interaction is being orchestrated by a structure called as immunological synapses. This synapse is generated during interaction between antigen presenting cells (APC) and T lymphocytes. From T-cells the T-cell receptor (TCR) contributes for this synapse. While from antigen processing cells (APC), MHC with processed peptides contribute for this synapse. There are following two classes of major histocompatibility complex (MHC)

1) Class I MHC
2) Class II MHC

The MHC is being encoded by genes which are highly polymorphic in nature. Those genes were first identified in case of tissue transplant rejection. The Class I MHC is expressed on all nucleate cells of body. While Class II MHC expression is limited to antigen presenting cells (APC) like macrophages, dendritic cells & Langerhans cells. (Figure 7.1)

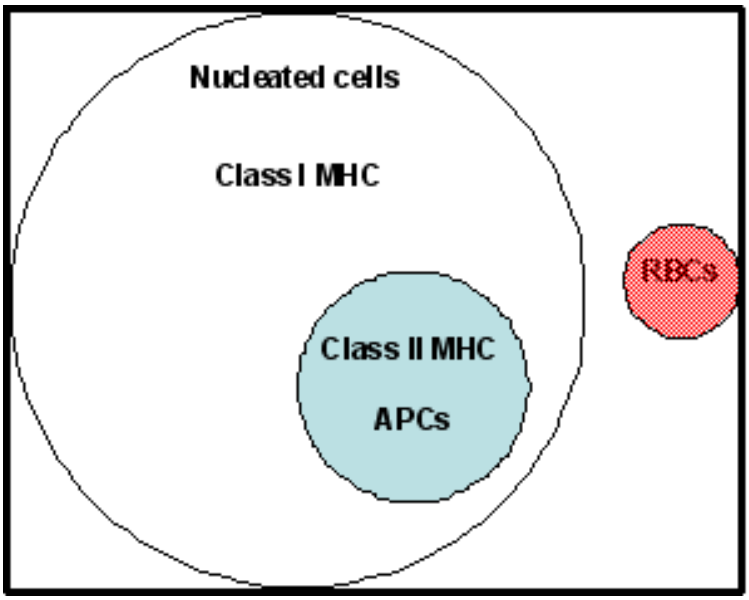

Figure 7.1: Distribution of MHC among various kind of cells

Structure of Class I MHC molecules

The class I MHC is composed of following two polypeptide chains

1) A long alpha chain
2) A short beta chain

These chains are anchored in the cell membrane of cells (as shown in figure 7.2). There is also a cytoplasmic region which reach inside the cytoplasm of cell. Similarly, in between the membrane a Trans membrane region exist which connect the cytoplasmic region and membrane anchored region of peptide. Another peptide chain exist in the structure of Class I MHC which is highly conserved region and is different from α1 & α2 region is known as α3 region (shown in blue color in Fig 7.2). There is also a highly polymorphic peptide binding region with binding ability of various peptides.

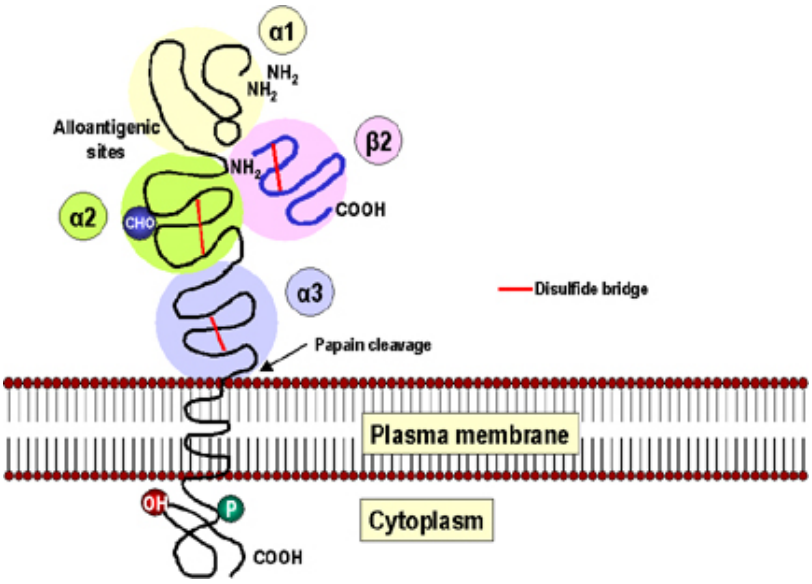

Figure 7.2: Structure of Class I MHC

Antigen binding Groove of Class I MHC

Antigen binding groove of class I MHC is composed of α1 & α 2 domain. The antigenic peptides reside within the antigen binding groove. Within that groove peptide make contact with residue peptide that's why this region is highly polymorphic in nature. In this groove, up to 8-10 amino acids can accommodate (as shown in figure 7.3)

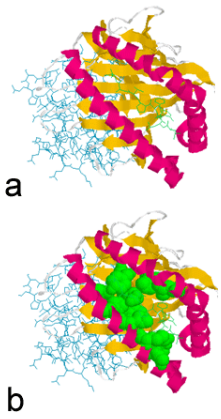

Figure 7.3: Structure of antigen binding groove a) Structure of α1 & α 2 domain without antigenic peptides b) Structure of α1 & α 2 domain with antigenic peptides (green in color).

Structure of Class II MHC molecules

Class II MHC is composed of two polypeptide chains α & β chains of equal length. These chains are anchored in the plasma membrane through trans membrane. Both chains also have a cytoplasmic region for phosphorylation & binding to cytoskeleton of cell (as shown in Figure 7.4). The α & β chains contain a highly conserved α2 & β2 domain for binding to one of the accessory molecule of T-cell known as CD4. Similarly, a highly polymorphic peptide binding region formed by α1 & β1 domains where antigen binds. This antigen binding groove accommodates antigen peptides. This antigen peptide also remain in contact with residues which are highly polymorphic in nature. As groove is open it can accommodate approximately 13-25 amino acids in length in contrast to class I MHC which can accommodate only 8-10 peptides. The polymorphic nature of this groove has ability to bind different amino acids.

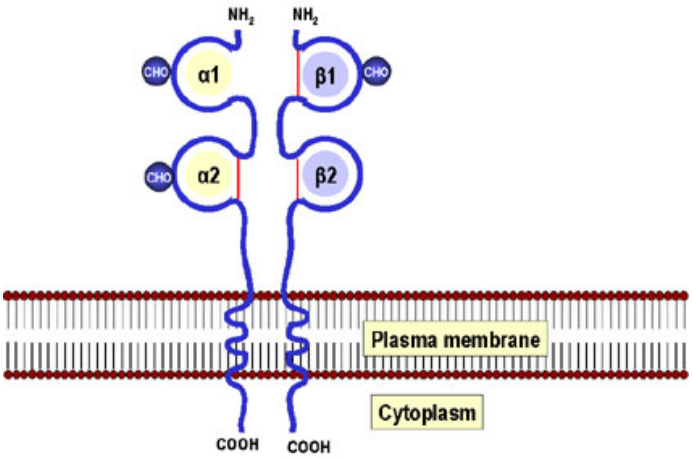

Figure 7.4: Structure of Class II MHC molecule

Important Aspects of MHC

Major Histocompatability complex (MHC) have following significant aspects

- MHC molecules are membrane bounded
- These molecules are recognized by T-cells with antigen as a result of cell to cell interaction
- For an immune response, peptide must be bounded with MHC, this kind of immune response is called as one level control.
- Mature T-cell must have a TCR which recognize MHC bounded peptide, this kind of immune response is called as second level control.
- The expression of MHC is increased under the action of Cytokines
- Class I MHC recognize peptides from cytosol
- Class II MHC recognize peptides from vesicle
- The phenomenon of Polymorphism in MHC determines the specificity of immune response

Role of MHC in Tissue Matching

MHC has very important role in tissue transplantation from one individual to other. The individual who donates the tissue is called as donor while the individual who receive the tissue is called as

recipient. The prospective donor & recipient are tested for compatibility prior to transplantation. That's why MHC is also called as Human leucocyte antigen (HLA). These molecules are the primary target of immune responses against allogeneic transplants Moreover, HLA molecules are highly diverse in human population. HLA typing or tissue matching detects & classifies this diversity. There is extensive polymorphism in HLA that's why there is less chances of HLA matched donors for transplants. Approximately 25% of siblings inherit same HLA type

Chapter # 08:

Monoclonal & Polyclonal Antibodies

Differences B/W Monoclonal & Polyclonal Antibodies

Monoclonal Antibodies

The antibodies which are produced from a single antibody producing B-cell are called as monoclonal antibodies. These antibodies also able to bind with single and unique antigen binding sit (epitope). Monoclonal antibodies mainly consist of single subtype of IgG for example IgG1, IgG2 & IgG3.

Polyclonal Antibodies

The collection of antibodies from a different antibody producing B lymphocytes are called as polyclonal antibodies. These antibodies also able to bind with multiple epitopes on a same antigen. These antibodies are obtained from serum which has antibodies having different affinities. Polycolonal antibodies mainly belong to IgG class.

Generation of Polyclonal Antibodies

The general procedure for generation of polyclonal antibodies include following phases

1. Antigen preparation/Antigen & Adjuvant conjugate

In this phase, the peptides of antigens are synthesized. The peptides of various length are designed for effective immune response generation. Moreover, the peptides are also conjugated with other molecules like carbohydrate & lipids in order to produce more diverse immune response.

2. Antigen Immunization

After peptide synthesis, these are administered in animal like rabbit for generating immune response. The antigen immunization can last for 6-8 rounds depending upon the levels of generated antibodies which are detected through ELISA.

3. Antibody Purification from animal’s serum

As immune response is generated in the form of antibodies after challenging with antigens, those antibodies are purified from animal's serum through various methods. These methods usually based on antigen affinity or specific affinity. This phase lasts from 1-2 weeks.

4. Antibody Validation

In this phase, antibodies are validated for their specificity and function. Moreover, the support and services for the purpose of commercialization are being done.

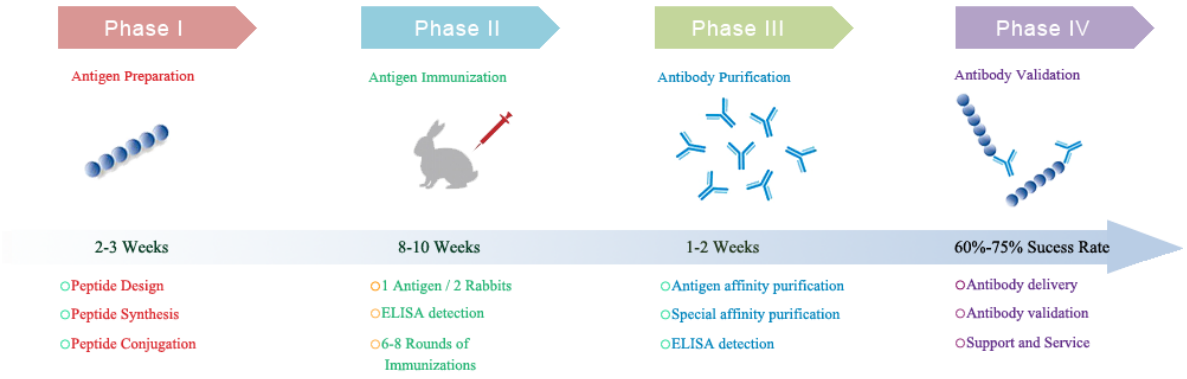

Figure 8.1: Phases of generation of polyclonal antibodies

Role of Adjuvants in Polyclonal Antibodies Production

Adjuvants are the substances which potentiates immune response against antigens. These have the ability to modulate towards desired immune response. However, with adjuvants there are mostly undesirable effects like cellular toxicity. That's why the selection of adjuvant is being made in order to get maximum immunostimulation. For the production of polyclonal antibodies following adjuvants are used

1. Freund's Complete Adjuvants
2. Freund's Incomplete Adjuvants

Freund's Complete Adjuvants (FCA)

Freund's complete adjuvant (FCA) are water-in-oil emulsion containing antigens with heat killed *Mycobacterium tuberculosis*. These adjuvants have the ability to stimulate both humoral & cell mediated immune response for antibody production.

Freund's Incomplete Adjuvants

These adjuvants are also water-in-oil emulsion containing antigens without heat killed *Mycobacterium tuberculosis.* These are used as booster antigen dose. These are used by mixing equal parts of the antigens.

Production of Monoclonal Antibodies

The production of monoclonal antibodies is done using immortal clone of cell with single antibody specificity. The immortal cells are those cells which don't have the ability to proliferate indefinitely. For this purpose, the normal antibody producing cell is being fused with an appropriate B-cell tumor line which is called as hybrid cell. Such kind of hybrid cells are for production of large amount of antibodies.

Hybridoma Formation

The fusion of lymphoid tumor cell with normal B-Lymphocyte with single specificity hybrid cell line is called as hybrid cell line. This hybrid cell line has dual function.

1) Immortality of tumor cell
2) The production of antibody with single specificity

In hybridoma formation, following principle involves

Animal (mouse) is being injected with a specific antigen for a specific time point. After that, B-lymphocytes are isolated and sorted from the spleen of mouse. In the next phase, the B-lymphocytes are fused with lymphocyte immortal cell which are myeloma cell. These cells provide the immortality to fused B-cells. The fusion between these two cells leads towards the generation of fused cell called as hybridoma. After fusion, the selection Hybridoma cell is usually done by using HAT medium (Hypoxanthine, aminopterin, thymidine) which allows the growth of hybridoma cells. While unfused cells undergo programmed cell death (apoptosis).

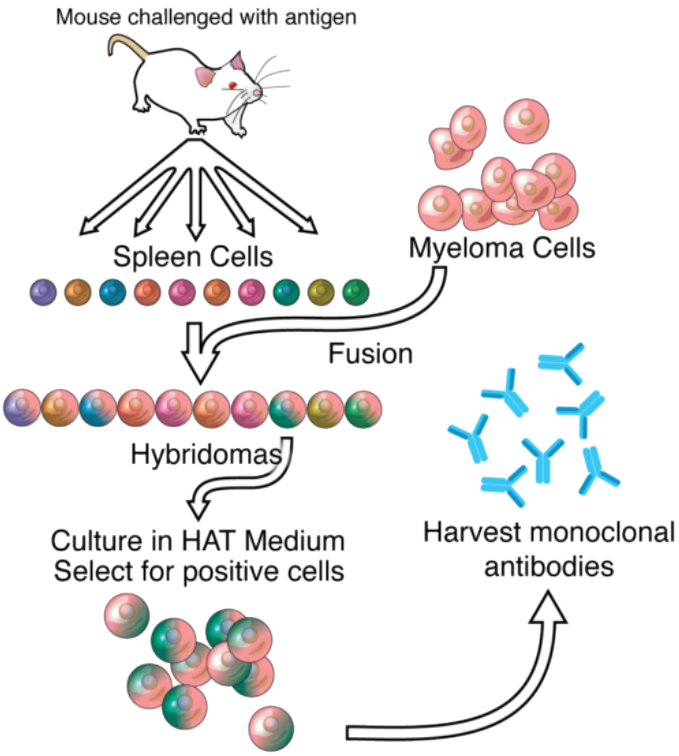

Figure 8.2: The process of hybridoma generation

The selected hybridoma cells are harvested for production of monoclonal antibodies. For large amount of monoclonal antibodies, hybridoma cells are propagated (as shown in Fig.8.2).

Usage of Monoclonal Antibodies

Monoclonal antibodies have very significant applications in clinical and basic medical sciences which are as follows

1. Usage in prevention, diagnosis & treatment of disease
2. Immunophenotyping of Immune cells, Monoclonal antibodies are used against cell surface molecules like Cluster of Differentiation (CD)
3. Typing of various tumors e.g Leukemia

4. Immunohistchemistry of solid tumors
5. Use in Immunotherapy: Against various tumor marker e.g CD20 B-Cell lymphoma
6. Use in Fluorescence Activated cell sorting (FACS)

Chapter# 9: T-Lymphocyte Receptors, Maturation, Activation & Differentiation

Structure of T-Cell Receptor (TCR)

The surface receptor which is present on T-lymphocytes for binding with antigen bounded with major histocompatibility complex (MHC) is called as T-cell receptor (TCR). This receptor is just like immunoglobulin in its structure, all those structures which resemble with immunoglobulin structures are called as immunoglobulin superfamily. That's why TCR is the part of immunoglobulin superfamily. TCR is a heterodimer surface receptor

TCR is composed of two following chains of equal length

1. A (α) Chain
2. B (β) Chain

These two chains are anchored through the cell membrane of T- cells through transmembrane region which has hydrophobic amino acids. A short cytoplasmic tail also exist at the end of transmembrane region which is not sufficient for signal transduction. These two chains of TCR also contain disulfide bridge between them. Both of these chains also contain carbohydrate moiety. Structurally, both chains contain constant (C) & variable (V) regions like immunoglobulin molecule (as show in Figure. 9.1). The variable regions of both chains contain hyper variable regions which determine the specificity of TCR. Likewise immunoglobulin, each TCR carry single specificity for a single antigen.

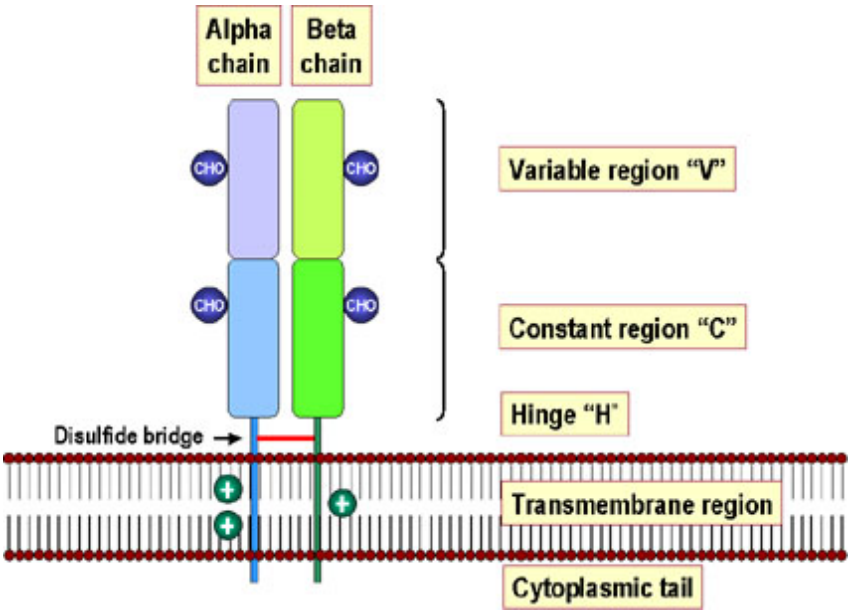

Figure 9.1: The structure of T-cell receptor (TCR)

Diversity of T-Cell Receptor (TCR)

The diverse array of TCR is based on the gene which encodes for TCR. Structurally, the TCR gene is composed of various gene segments which are joined together. T-cell maturation occur in an organ which is called as thymus where the expression of the gene for TCR occur. The germ line gene for α chain contain only two segments which are called as V (Variable) & J (Joining) segments. However, the germ line gene for αβ chain is composed of three segments called as V (variable), D (diversity) & J (Joining) segments. The diversity for specificity of TCR is based on combinational diversity which in fact is based on for various combinations of V, D & J segments for TCR. In majority, the specificity of TCR is based on the combination of α & β chains which constitutes the major T-cell population. However, the small population T-cells also exist which carry the TCR with γ (Gamma) & δ (Delta) chains. This subset of T-cells is called as γδ T-cells. Usually the T-cells predominates in mucosal epithelium for elimination of certain bacterial & viral antigens in MHC dependent antigen processing. This predominant subset of T-cells belong to αβ variety.

γδ T-cells also contain the repertoire which is also based on combinational diversity of various gene segments. The Gamma (γ) chain gene contain V & J segment, while the Delta (δ)chain germline gene contain V, D & J segments. The γδ T-cells recognize antigens independent of MHC association in contrast with αβ variety.

CD3 complex Structure & Function

The CD3 stands for Cluster of differentiation 3. This structure is present adjacent to TCR. This is basically the transducing element of TCR. It exists as a complex form which is composed of five following proteins (as shown in fig.9.2), that's why it is also called as CD3 complex

1. One Gamma(γ)
2. One delta(δ)
3. Two Epsilon (ε)
4. Two Zeta (**ζ)**

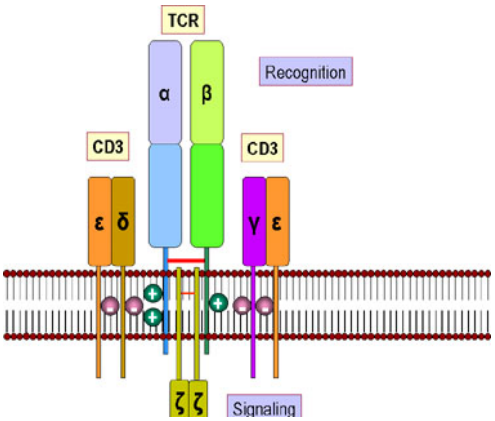

Figure 9.2: The structure of CD3 complex

The CD3 complex is considered as invariant proteins means with conserved regions without variability. It has no role in determining the specificity of T-Cell. However, it is necessary for surface expression of TCR during development of T-cell. This has very important role in transducing signals from T-cell surface receptor to interior of cell which is called as Signal transduction. This transduction signals arise after engaging of antigen with TCR in association with MHC complex.

Cell Surface Molecules involved in T-Cell & Other Interaction

The interface between antigen presenting cell (Target Cell) and T-lymphocyte occur for mounting a strong immune response against antigen. This interaction between TCR & MHC molecules are not so strong and called as immunological synapse. In this immunological synapse, the cell surface molecules on T-cells & their interacting molecule on antigen presenting cells are involved. T-cells also express co-receptor for MHC molecule (e.g CD8 & CD4). Moreover, the expression of supporting molecules are increased by the action of cytokines released from either T-cells or antigen presenting cells. The increased expression of supporting molecules for making immunological synapse strong are called as modulators of immune system. In addition to these molecules, some molecules are also required for T-cell activation. For the activation of T-cells following two signals are required

1. Signal 1 is generated after engagement of TCR with Antigen/MHC complex
2. Signal 2 is generated after engagement of supporting molecules with their corresponding ligands

For signal 2, the co-stimulatory molecules are also required for activation of T-cells e.g C28 is a co-stimulatory molecule on T-cell surface & its ligand exists on APC which is known as B7-1. If there is lack of co-stimulation, then there is no activation of T-cells occur and this process is called as cellular anergy. For the full activation of T-cells, the engagement of Immunological synapse is mandatory. As a result the antigen presenting cells (APC) must possess & present peptides to T-cells.

Accessory Molecules

The interaction between TCR on T-cells & antigen/MHC complex on APC is considered as weak for mounting a strong immune response. In order to stabilize this interaction, there is the need of additional molecules in addition to TCR & antigen/MHC which are called as accessory molecules. These molecules are invariant molecules and have no role in determining the specificity of T-cells. However, these accessory molecules can modulate the immune system in either positive or negative manner. These molecules are critical to success or failure of controlling the immune response to foreign antigen like infectious agents. Moreover, these are also involved in aberrant response to self-antigens like in autoimmune diseases and response to tumors or cancerous cells.

These molecules mainly promote or suppress the immune response. The nomenclature of these molecules are according to their function.

Followings are the important accessory molecules (Fig 9.3 & 9.4)

1. CD4

   This molecule of T-cell is for binding to class II MHC which ensures the binding of T-helper (Th) cells to antigen presenting cells

2. CD8

   This accessory molecule is for binding to Class I MHC which ensures the binding of cytotoxic T (Tc) cells to target cell

3. CD2

   This accessory molecule on the surface of T-cell has a ligand known as Leucocyte Function Antigen 3 (LFA-3) on antigen presenting cells (APC)

4. Leucocyte Function Antigen 1 (LFA-1)
   This molecule has ligand known as Intracellular Adhesion Molecule-1 (ICAM-1)

5. CD28

   CD28 is the accessory molecule on T-cell and has ligand called as B7-1/B7-2

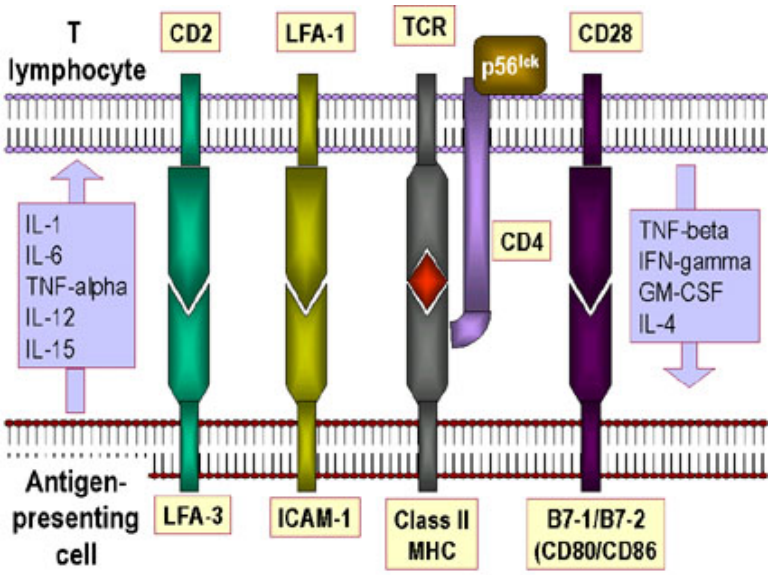

Figure 9.3: Accessory molecules of Helper T-cells & their specific ligands on APC

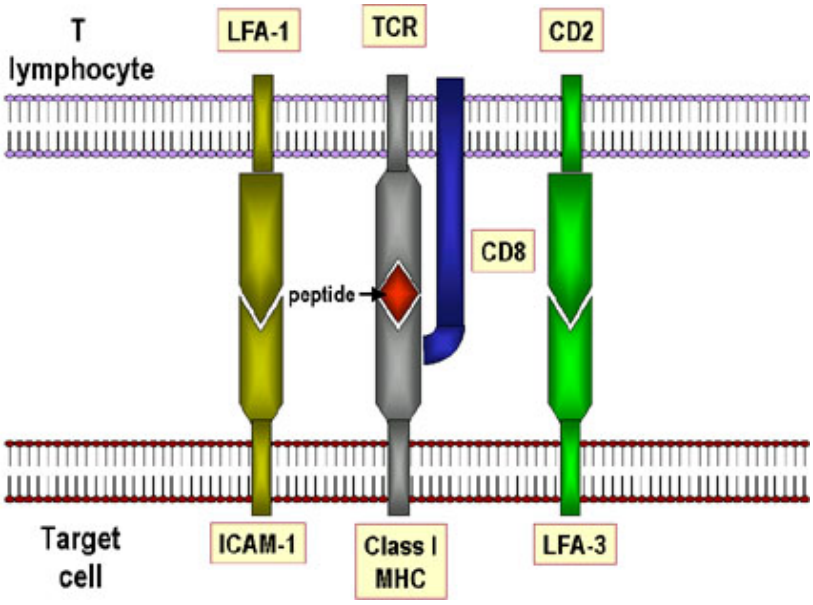

Figure 9.4: Accessory molecules of Cytotoxic T-cells & their specific ligands on APC

Co-Stimulatory Molecules for Activation & Maturation of T-Cells

The molecules required for activation of T-Cells after binding to its ligands on antigen presenting cells. The following two signals required for T-cells activation

1. Engagement of TCR with antigen which is associated with MHC
2. Engagement of co-stimulatory molecules with their ligands

The molecules which transmit the signals to a cell to enhance the response of that cell in positive manner are called as co-stimulatory molecules. CD 28 is the co-stimulatory molecule of both types of T-cells which has ligand known as B7-1 or B7-2 on the surface of APC (Fig 9.5). The multiple interaction of TCR, MHC, accessory & co-stimulatory molecules constitute a structure called as immunological synapse. These co-stimulatory molecules are also invariant with no involvement in determining the specificity of interaction.

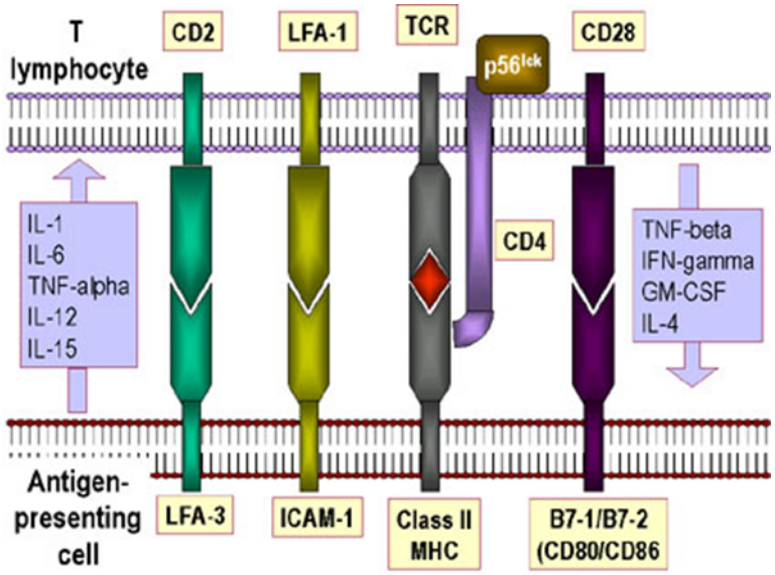

Figure 9.5: The structure of co-stimulatory molecule of T-cell & its ligand

T-cell activation

The engagement of TCR with MHC containing antigen on antigen presenting cell. Furthermore, the engagement of co-stimulatory & accessory molecules leads towards the secretion of cytokines after T-cell activation. Theses cytokines causes the maturation & differentiation of T-Cells (as shown in fig 9.6)

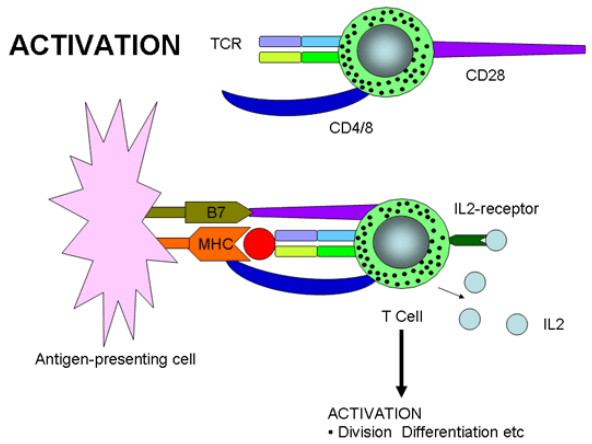

Figure 9.6: The process of T-cell activation

The lack of co-stimulation even after engagement of TCR & antigen/MHC molecule leads the T-cell towards a state called as anergy. This is non-responsiveness of T-cells towards antigen as a result no T-Cell activation occur (Fig 9.7)

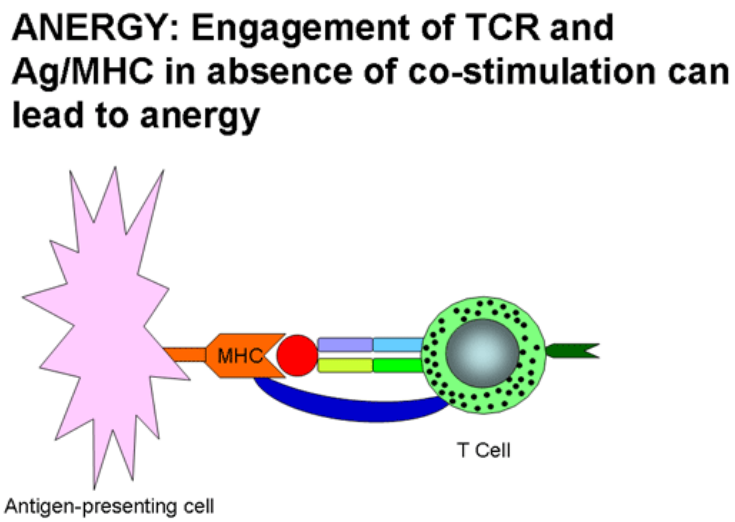

Figure 9.7: Anergy of T-Cells

The lack of co-stimulation also leads to down regulation of T-Cell activation which is due to absence of CD28. There is expression of CTLA-4 which engages CD28 molecule. This cause no responsiveness of T-cell to antigen. Finally, there is no T-Cell activation (Fig 9.8)

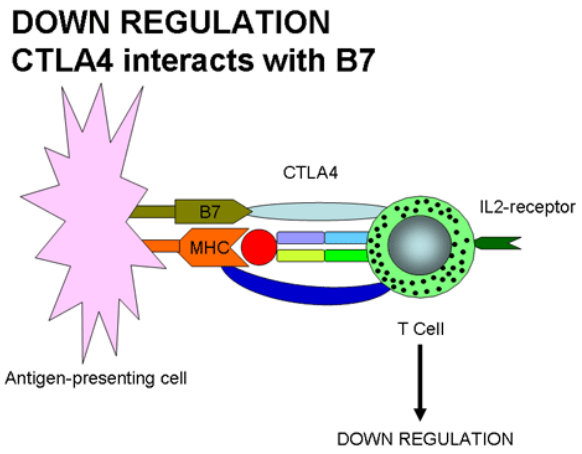

Figure 9.8: Inactivation of T-cell due to down regulation of accessory molecule CD28

No Engagement of TCR, MHC with antigen, interaction of CD28 with B7. As a result, there is no signal for activation of T-cells (Fig.9.9)

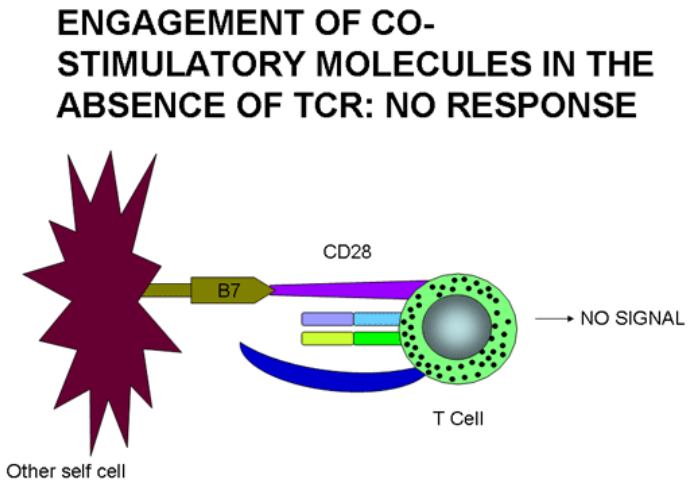

Figure 9.9: Inactivation of T-cells due to no engagement of MHC

Chapter # 10: B-Lymphocytes Receptors, Maturation, Activation & Differentiation

Antigens Responding to B-Cells

B-cells are stimulated only by antigens which are called as T-Independent Antigens because these antigens have the ability to stimulate the B-cells directly without the help of T-lymphocytes. Chemically, these antigens are polysaccharides in nature for example the antigens of *Streptococcus Pneumococci*. These antigens exist in a polymeric structure which mean that antigens are characterized by the same antigenic determinants (epitopes). These antigenic determinates are repeated many times on a single antigen (Fig.10.1)

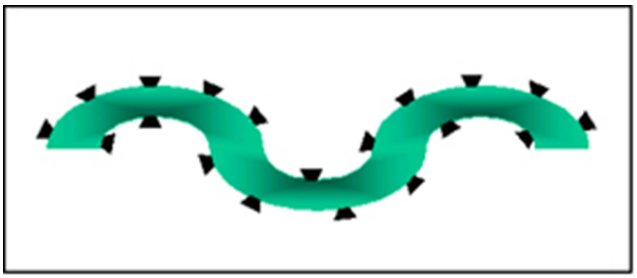

Figure 10.1: Structure of T-independent antigen with antigenic determinants (black in color)

T-independent antigens have the ability to activate the various clones of B-Cells at a same time. This kind of antigenic activation is called as polyclonal activation. Most of these antigens can activate B-cell clones specific for other antigens. T-Independent antigens can be classified broadly in to two main following types based on their ability to activate B-cells

1. Type 1 T-independent antigens

These antigens are polyclonal activator as these have the ability to activate a large number of B-cell clones

2. Type 2 T-independent antigens

These antigens are not polyclonal activator of B-cells in contrast to type-1 antigens. These can activate usually few clones of B-lymphocytes.

T-independent antigens are more resistant to degradation by phagocytic cells. That's why such antigens can persist for longer time in the body for providing effective immune response for longer time points. Moreover, these antigens are also involved in consistent stimulation of immune system. These antigens also have the attribute of mitogenicity for various immune cells in order to increase the number of cells accordingly.

Like T-independent antigens, T-dependent antigens can activate the B-cells not directly but indirectly with the help of T-cells for B-cell activation. T-dependent antigens didn't fulfil the molecular requirements for direct stimulation of B-cells that's why help of T-cells is required. These T-dependent antigens readily degraded by phagocytes afterward these are presented to T-cells with class II MHC for B-cells activation. These antigens are protein in nature unlike T-independent antigens. Moreover, these antigens contain variety of epitopes with few copies on the surface of antigen (Fig.10.2).

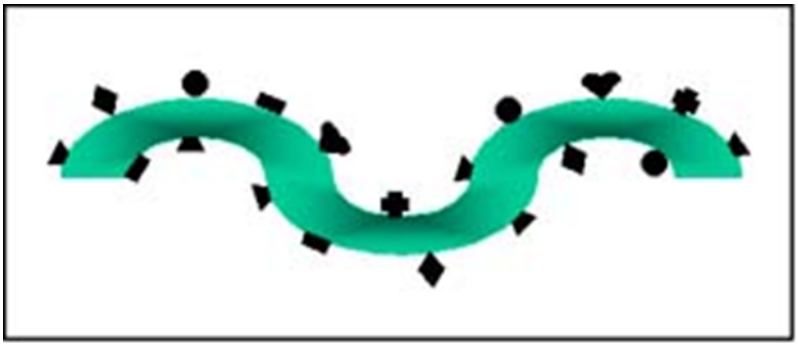

Figure 10.2: Structure of T-dependent antigen with antigenic determinants (in black color)

Antigens Processing by B-Cells

For T-independent antigens

These antigens have the ability to stimulate the B-cells directly. These also have the ability to activate a substantial proportion of the B-cell pool. This kind of B-cell activation is polyclonal activation that is without reference to antigen specificity of the surface receptor hypervariable regions for example the type I T-independent antigens. B-cells are activated when there is high concentration of antigen for example in case of bacteria the lipopolysacchrides antigens exist in greater quantity. B-cell activation occur after binding of antigen to a surface molecule which is

known as B-cell receptor. Afterward there is bypassing of the early part of the biochemical pathway mediated by specific antigen receptor. Cross linking of immunoglobulin receptor occur due to repeating determinants on antigenic structure. As a result the transformation of B-cells occur into plasma cell for immunoglobulin secretion (Fig.10.3)

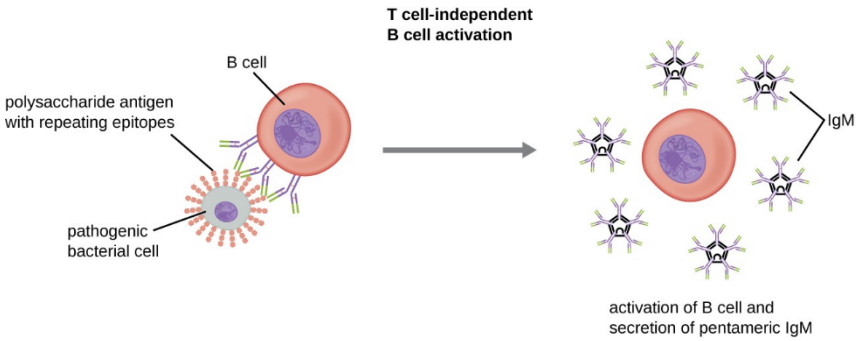

Figure 10.3: Antigen processing by B-cells for T-independent antigens

T-dependent antigens

In case of T-dependent antigens, there is no direct stimulation of B-cells without the help of T-cells. The T-dependent antigens are recognized by surface immunoglobulin receptor known as BCR. These antigens are univalent with respect to specificity of each determinant on the surface of antigen. The antigens are internalized within endosomes of B-cells where those are processed by fusion with lysosomes into simple peptides. Afterward, the processed peptides are expressed on the surface of B-cells with Class II MHC molecule to Helper T-cells with co-stimulatory molecules. As a result, the clonal expansion & differentiation of B-cells into plasma cells occur (Fig.10.4).

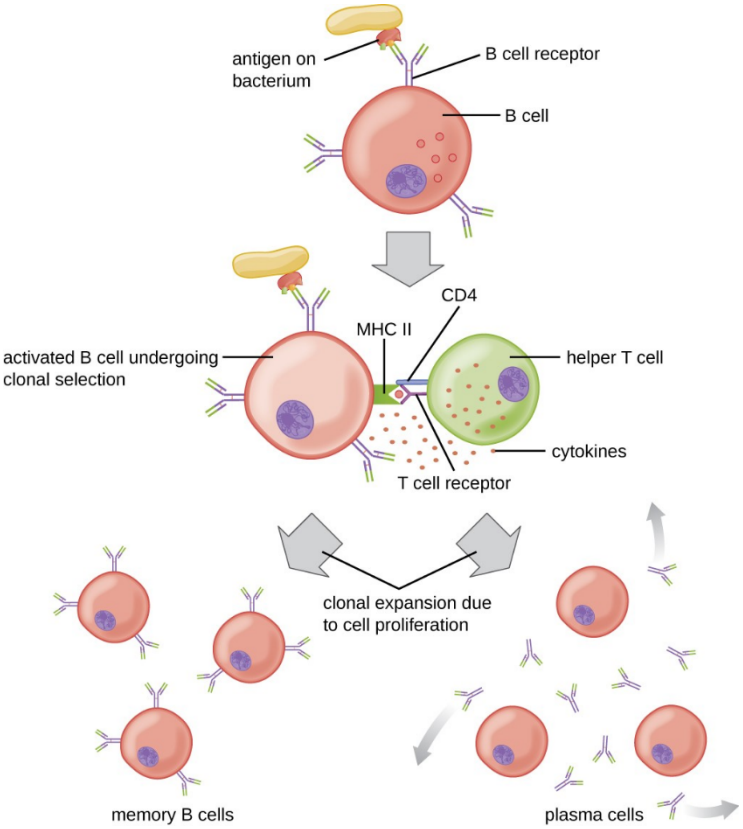

Figure 10.4: Antigen processing by B-cells for T-dependent antigens

Nature of B-Cell Activation

The naïve or resting B-cells are non- dividing as like to T-cells. The B-cells Activation occur through BCR which is a surface molecule of B-cells. Like T-cell, BCR (surface immunoglobulin) doesn't process any intrinsic enzymatic activity which means there is need of certain other molecules for full activation of B-cells. These molecules are called as accessory molecules associated with antigen receptor. These molecules propagate activation signals into B-cells for differentiation into plasma cells for antibody secretion

Structure of B-Cell Receptor (BCR)

BCR exist as a complex structure. It is composed of membrane anchored immunoglobulin (IgM) in the cell surface of B-cell. Additionally, Igα & Igβ heterodimer exist in the chain forms which are associated with disulfide linkage between these two chains. The cytoplasmic tail of Igα & Igβ contain a single Immune receptor tyrosine based activation motif (ITAM). The cross linking of

BCR through antigen results in the initiation of phosphotyrosine kinase (PTK) driven signals which are seeded by ITAM (Fig.10.5)

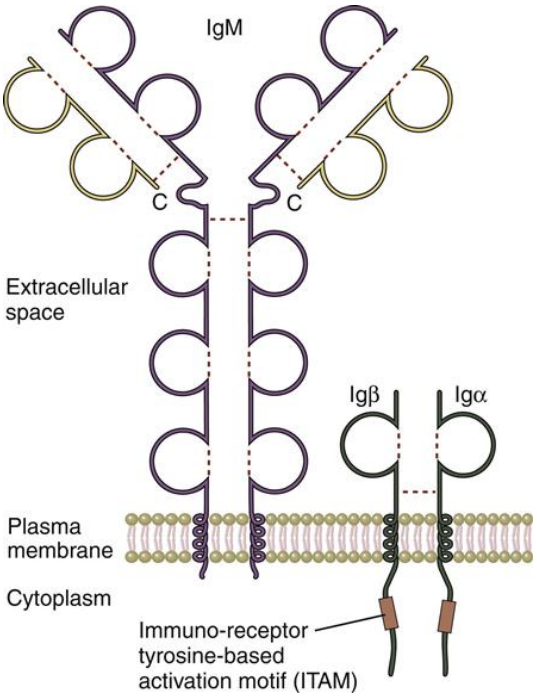

Figure 10.5: The structure of BCR

Like T-cells activation, the B-Cells also require co-stimulation to mount efficient effector responses. As like C28 in case of T-cells, a complex of co-stimulatory molecules perform this function. This complex of co-stimulatory molecules composed of following molecules (Fig.10.6)

1. CD81
2. CD21
3. CD19

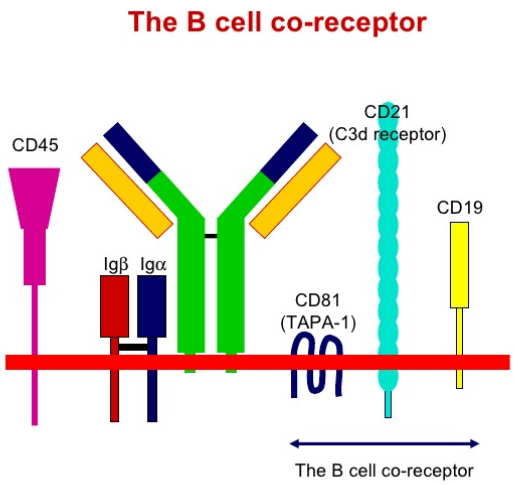

Figure 10.6: The structure of BCR with co-stimulatory complex

B-Cell Receptor & Co-stimulation for Maturation

The process of activation of B-cells occur after cross linking of BCR through antigen. As the cross linking initiates, there is activation of a molecule called as Src for cellular signaling. The Src phosphorylates tyrosine on ITAM of Igα & β. Then the phosphorylated ITAM act as docking site for Syk protein. After that, the downstream signaling for antibody synthesis occur. There are following two biochemical processes are activated during antibody synthesis

1. The Activation of Phospholipase C (PLCγ)
2. The Phosphorylastion of adapter protein called as SLP which in turn cause the activation of Ras & Rac proteins

In turn, the mitogen activation proteins (MAP) for example JNK are activated. Afterward, the AP-1 moves to nucleus for B-cell proliferation & differentiation into plasma cells (Fig.10.7)

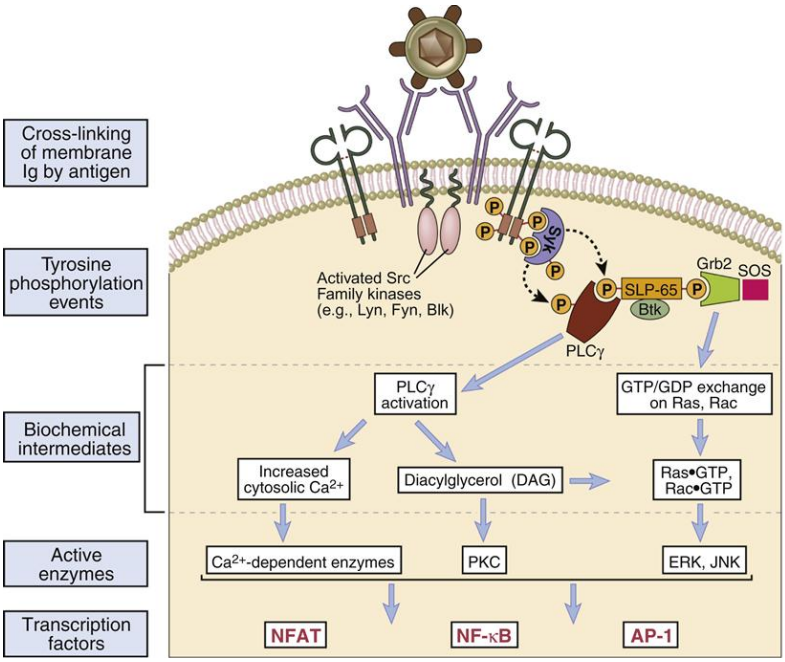

Figure 10.7: The signaling process of B-cell after antigenic stimulation

A second signal is also required after BCR engagement during which the accessory molecules are involved for amplification of B-cell signals. In this case, the antigen molecule is bounded with complement protein like C3d. The amplification process occur when multivalent antigen with C3d molecule engage the complement recptor-2 (CR2) with BCR. The Signals from immunoglobulin & CR2 cause the phosphorylation of Syk protein. Moreover, another accessory molecule called as CD19 also reorients for PI-3 kinase for B-cell activation & differentiation (Fig.10.8).

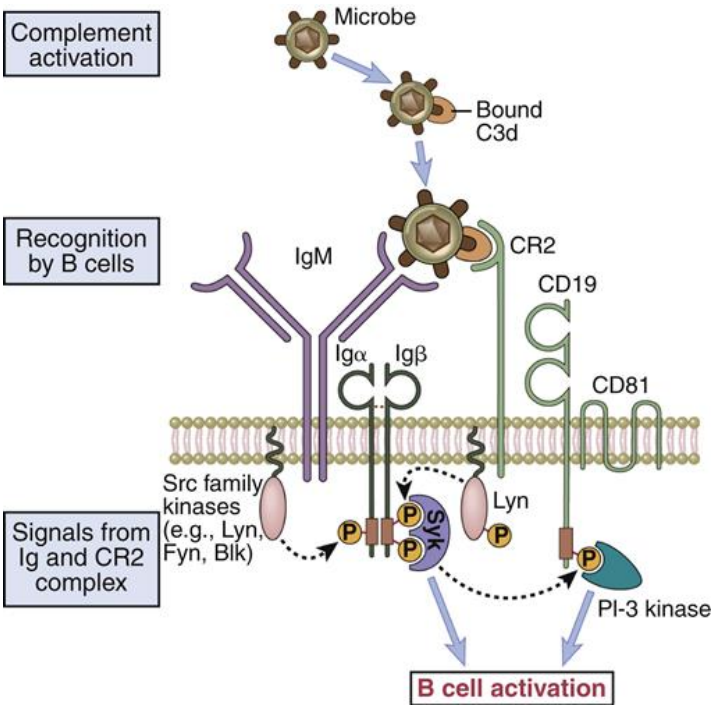

Figure 10.8: Activation of B-cell through accessory molecules like complement

Role of CD40 in B-Cell Activation

The T-dependent antigens are univalent in nature with single type of antigenic determinant on it. For such antigens, the B-cells process & present such antigens. There is the process of up regulation of co-stimulatory molecule on B-cell which are known as CD40. The engagement of CD 40 occur with its ligand on T-cells which is called as CD40 ligand. For fully activation of B-cells there is also ensured co-stimulatory signaling in B-cells. In this case of T-dependent antigens, the co-stimulatory signal is the CD40 & CD40 ligand interaction. After that the cytokines are released from T-cell like IL-2/4/5 for acting on B-cell for activation (fig.10.9). These cytokines are involved in B-cell proliferation & differentiation into plasma cells. Moreover, the process of class switching and affinity maturation also occur for an effective humoral response. Clinically, the absence of CD40L due to defect in the gene of CD40 ligand on T-cells as a result there is absence of class switching process. This defect manifest the hyper IgM globunemia with no IgG due to absence of class switching process. Consequently, the patients having this defect present with recurrent infections and immunodeficiency.

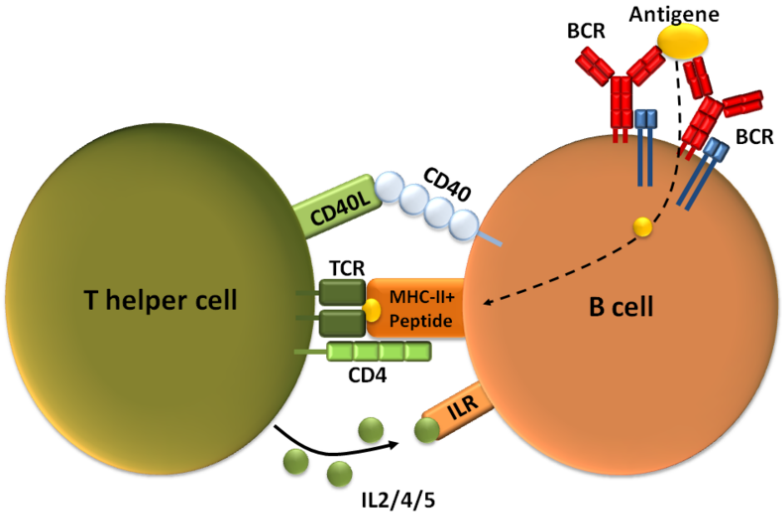

Figure 10.9: Activation of B-Cells for T-dependent antigen with CD40/CD40 ligands

Chapter # 11: Complement System

Introduction to Complement System

Complement system is comprised of heat labile serum proteins which are able to destroy or lyse pathogens. This form of host defense is termed as one way of host defense against potential pathogens. Complement proteins become inactive by heating serum at 56°C for 30 minutes. This system is composed of more than twenty (20) proteins. These proteins are produced by variety of body's cells including hepatocytes (Liver cells), macrophages & gut epithelial cells. Among complement proteins, some proteins has the ability to bind with immunoglobulin molecules while some are involved in binding on the membrane component of various immune cells. Most of complement proteins exist in inactive form which are also called as Proenzymes. Such proteins need activation into active form for proper functioning. These proteins when activated can cleave one or more other complement proteins. Such kind of activation process is called as cascading for complement activation.

Functions of Complement Proteins

Complement system provides specific as well as non-specific resistance against infections. This kind of resistance is exhibited with following two important functions

- Primary function

  This function of complement proteins is considered primary as these activated proteins used to kill or lyse infectious agents like bacteria. For this complement proteins get deposited on the surface of infectious agents which make hole in those cells for their lysis or killing.

- Secondary or Effector functions

  These functions of complement proteins effect on other functions of immune system. These functions of complement proteins are as follows

1) Opsonization

   It is the process by which phagocytosis of microbes by immune cells get enhanced. Those proteins which undertake this process are called as opsonins. For example, some

complement proteins like C3b, iC3b and C4b are good opsonins. These proteins attach to microorganism first making a complex. This complex of complement protein & microbe bind with complement receptor on the surface of phagocytic cell as a result phagocytosis of microbe occur by phagocytes (Fig.11.1)

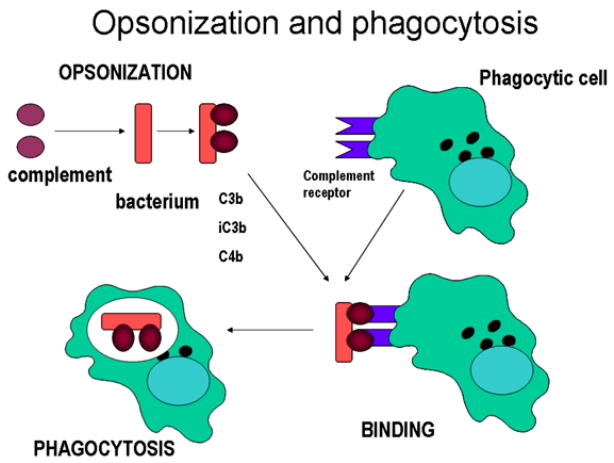

Figure 11.1: The opsonization function of complement proteins

2) Chemotaxsis

The phenomenon by which phagocytic cells move towards the site of infection is called as chemotaxsis, and the molecules involved in this process are called as chemotactic factors. The complement proteins are considered as good chemotactic factors like C5a. These act as potent activator of neutrophils, basophils & macrophages. These cause the induction of adhesion molecules on surface of blood vessel's endothelial cells, which bind the phagocytic cells on it (Fig.11.2). One of the important complex of complement proteins called as membrane attack complex (MAC) composed of C5b, C6 and C7 also act as good chemotactic factor.

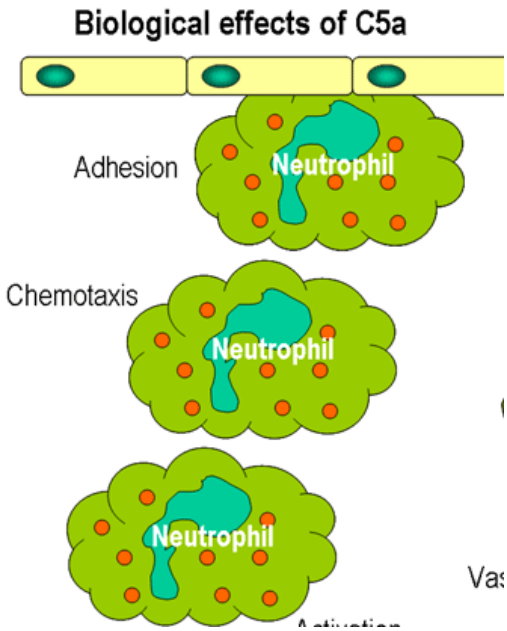

Figure 11.2: The chemotactic function of complement proteins

3) Anaphylaxsis

Anaphylaxsis is the form of immune response in which exaggerated response is generated against allergen. The molecules which produced such response are called as anaphylatoxins. The complement proteins like C4a, C3a & C5a act as potent anaphylatoxins. These molecules cause basophils and mast cells degranulation as a result various mediators or cytokines are generated (Fig 11.3). Those mediators involved in smooth muscles contraction, vasodilatation and bronchoconstriction.

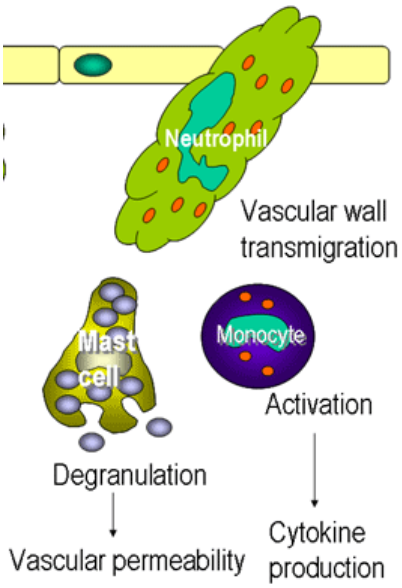

Figure 11.3: The anphylatoxsis function of complement proteins

Complement Activation

Complement proteins are activated through a series of cascading reactions which are called as complement activation. Followings are the important pathways for complement activation

1) Classical Pathway
2) Alternate Pathway
3) Mannose Bindning Lectin (MBL) Pathway

These pathways generate the activated form of complement like C5 which leads towards a pathway called as common pathway as a result the membrane attack complex is generated for the lysis of microorganism (Fig 11.4)

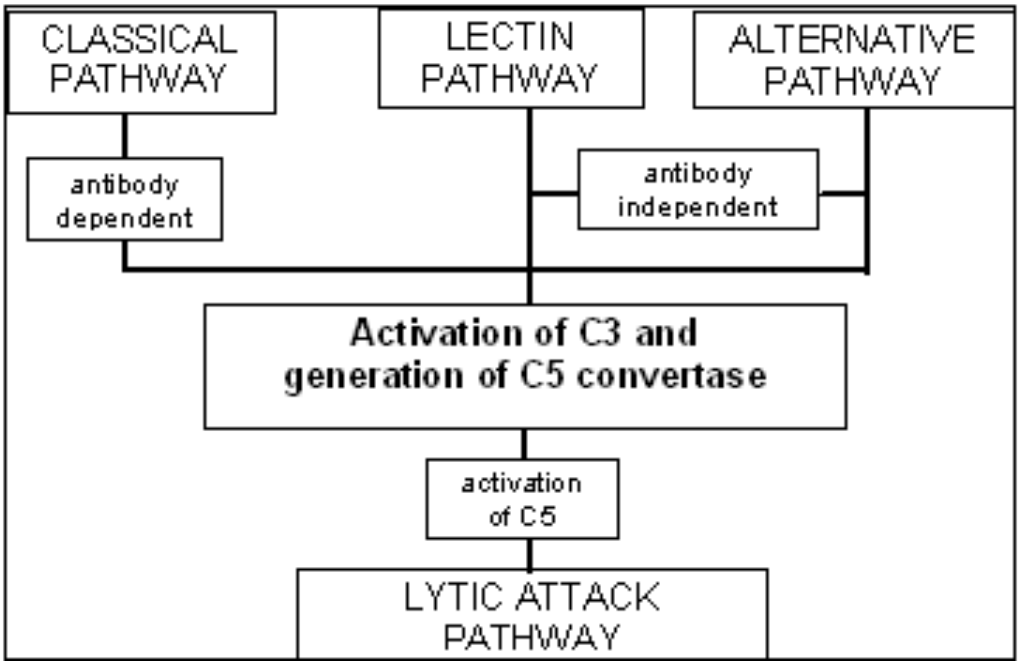

Figure 11.4: The pathways of complement activation

Classical Pathway

This pathway of complement activation is also called as antibody dependent complement activation. As in this pathway, antibody bind to microbe which links as the first molecule of classical pathway. This pathway begins with C1 activation which is a multi subunit protein containing three sub proteins

i) C1q,
ii) C1r
iii) C1s

C1 protein binds with antibody on the surface of microorganism

Followings are important events of this pathway

1) Activation of C1

In this event, there is binding of C1q to Fc portion of Immunoglobulin (IgM& IgG) which have bounded with antigens on bacterial surface. Binding of C1q in turn activates another subunit C1r and ultimately C1s (Fig 11.5). The activated C1qrs act as enzyme for C4 to cleave it in C4a & C4b

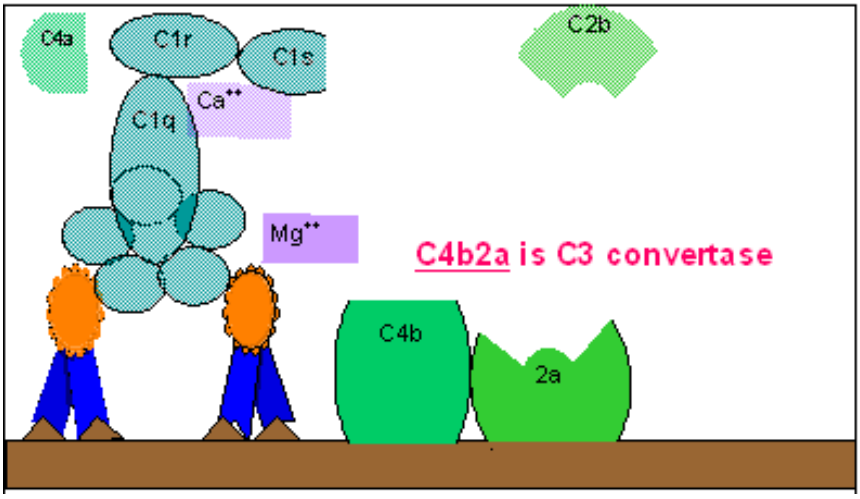

Figure 11.5: Initiation of classical pathway with C3 convertase generation

2) Generation of C3 convertase

As C1qrs is activated, this complex also act on C2. As a result, C2 cleaves into two subunits C2a & C2b. C2a binds on bacterial surface with C4b which is also a subunit of C4 generated after its cleavage. This complex of C4b & C2a on bacterial surface is called as C3 convertase which further acts on C3 to c3a and c3b (as shown in Fig. 11.5).

3) Generation of C5 convertase

In this stage, the activated C3 convertase (C4b&C2a) act on C3 to convert into C3a & C3b. C3a moves into microenvironment for other immunological functions while C3b binds with C4b &C2a. As a result, a complex of C4bC2aC3b is formed. This complex is called as C5 convertase for the conversion of C5 into C5a & C5b.

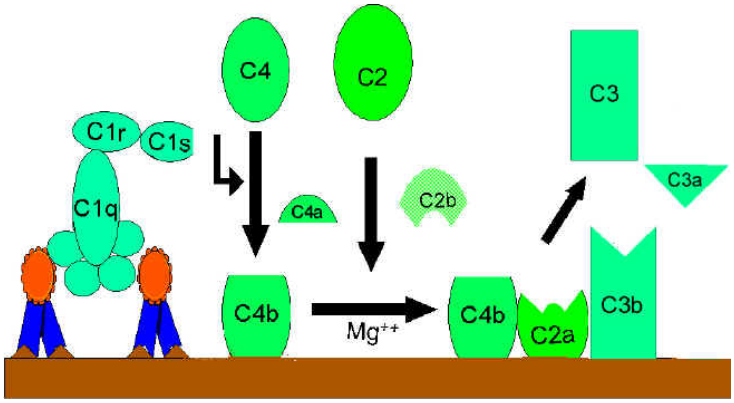

Figure 11.6: An overview of events during classical pathway of complement activation

Alternative Pathway of Complement Activation

This pathway of complement activation is antibody independent complement activation as there is no need of antibodies for this. This is initiated by direct conversion of C3 into C3a & C3b. Moreover, there is need of various serum proteins & factors like Factor B, D & Mg++ ions. Generally, in serum there is low level spontaneous hydrolysis of C3 to produce C3i. In serum, then factor B binds with C3i to form a complex of C3iB. This complex become susceptible to another serum factor called as factor D which cleaves B into Bb. As a result, a complex C3iBb is formed which acts as C3 convertase for conversion of C3 into C3a & C3b. The resulting C3b reacts again with factor B and become susceptible to factor D, There is continuous formation of C3bBb which acts as C3 convertase for cleavage of C3 (as shown in fig.11.7).

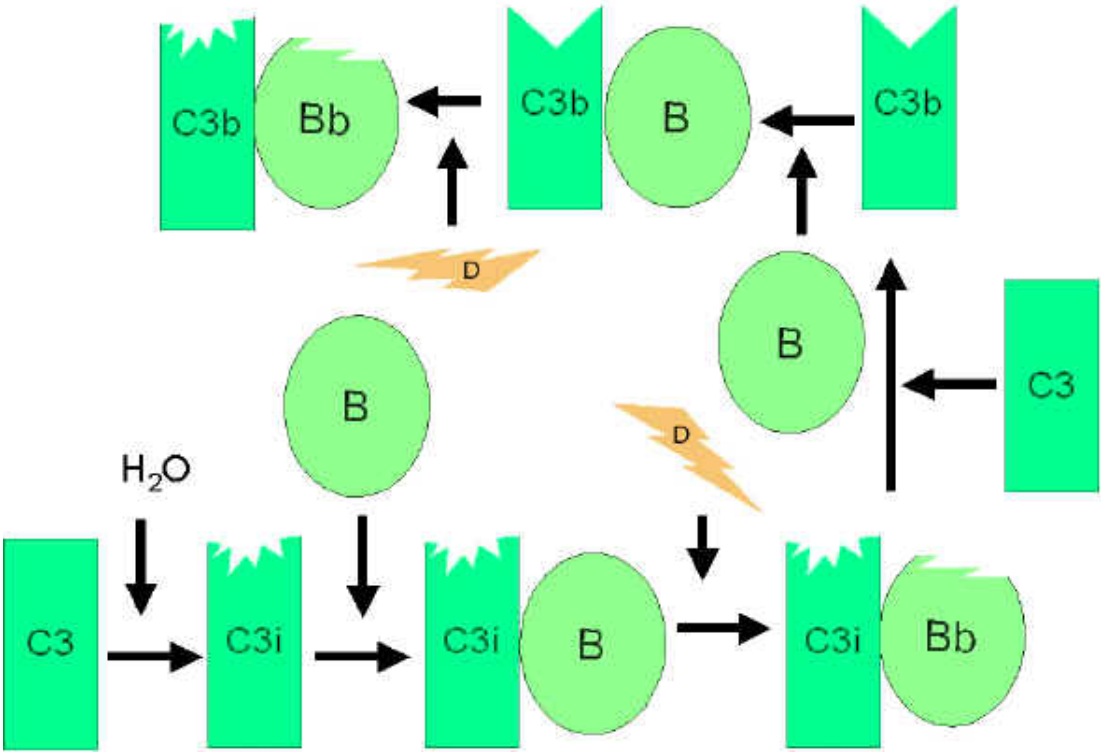

Figure 11.7: Conversion of C3 into C3b during alternative pathway

As a result of this C3b amplification loop, there is more production of C3b. This amplification loop of C3b is triggered by various molecules of microorganisms like lipopolysaccgrides (LPS) of Gram negative bacteria, cell wall of bacteria & yeasts. These are also called as activator of alternative pathway. Physiologically, the autonomus activation of C3 is controlled by following mechanisms

1) By Decay accelerating factor (DAF)
2) Control of C3b amplification is done by a molecule called as Decay accelerating factor (DAF), as a result the formation of C3 convertase is blocked (Figure 11.8)

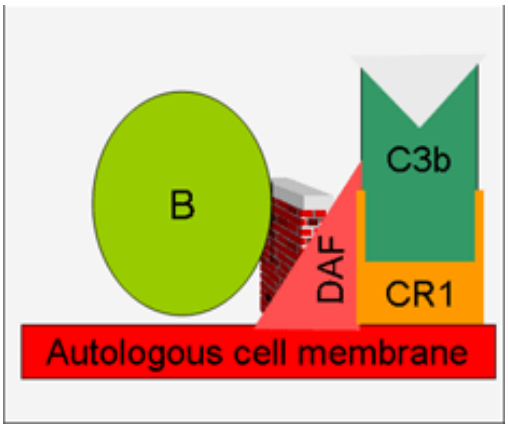

Figure 11.8: Control of spontaneous activation of C3 via DAF

2) By dissociating C3 convertase after cleavage of Bb from C3b

In this control mechanism, the spontaneous amplification of C3b loop is controlled by enzymatic degradation of C3b by serum factor i.e factor H & I (Figure 11.9)

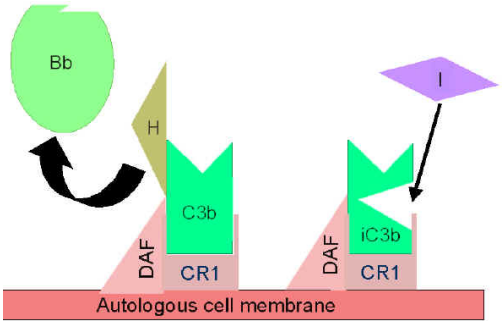

Figure 11.9: Dissociation of C3 convertase after cleavage of Bb from C3b

Clinically, the deficiency of Factor H & I in serum leads to increased susceptibility of patients to various infections due to non-availability of active C3.

In alternative pathway, the activated C3b is stabilized on the surface of infectious agent which is also called as activation surface by a protein called as protector (P) protein. This protector protein is absent on homologous cells of body (Fig. 11.10)

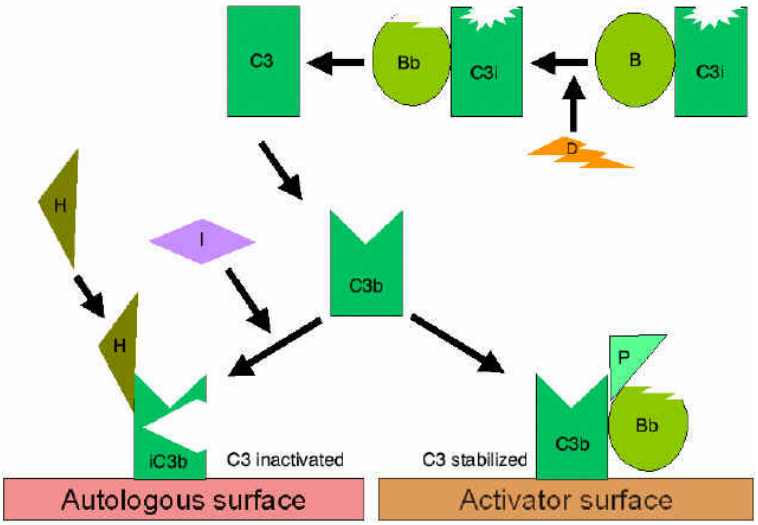

Figure 11.10: Stabilization of C3 on Activator surface by Protector Protein

As C3b is stabilized on activator surface, two molecules of C3b, Bb and protector protein (P) form a complex called as C5 convertase which is the fate of alternative pathway (Fig.11.11).

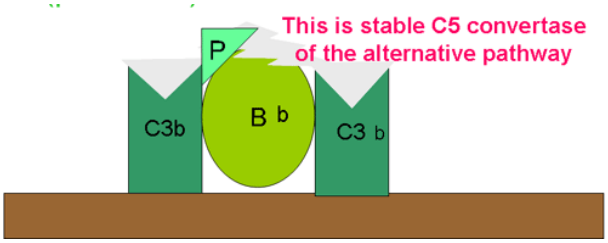

Figure 11.11: Structure of C5 convertase of alternative pathway

Lectin Pathway of Complement Activation

This pathway is also called as antibody independent complement activation like alternative pathway. However, it is initiated by mannose binding Lectins (MBL) on bacterial surface with mannsoe containing polysaccharides (Mannans). After binding of MBL on bacterial surface, there is association of two serine proteases called mannose-associated serine proteases (MASP). There are following two types of MASPs

1. MASP-1
2. MASP-2

In this pathway, MASP-1 corresponds like C1r & MASP-2 like C1s of classical pathway respectively. The MBL acts like C1q of classical pathway. The activation of MASPs happen after the formation of MBL/MASP-1 & MASP-2 tri molecular complex. This complex cleaves C4 & C2 into C4b & C2a respectively for the formation of C3 convertase (Fig.11.12)

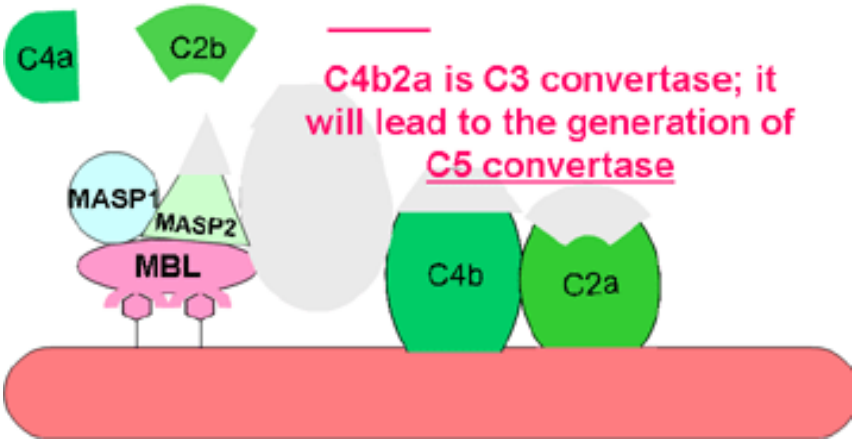

Figure 11.12: Mannose binding Lectin (MBL) pathway for complement activation

This C3 convertase cleaves of C3 into C3a &C3b. The C3b binds with C4b & C2a for the generation of C5 convertase (C4b, C2a & C3b complex). While C3a move to microenvironment for other immunological functions. The biological activities of C4a, C2b & C3a & regulatory proteins of MBL pathway are same like classical pathway.

Lytic (Common) Pathway

This pathway is also called as Membrane attack complex (MAC) pathway as it is involved in the lysis of infectious agents. C5 convertase is generated from all three following pathways

- Classical:C4b2a3b
- Alternative:C3bBb3b
- Lectin:C4b2a3b

This C5 convertase converts C5 into C5b &C5a. This C5b rapidly associates with other complement proteins like C6 &C7 and insert into membrane. Subsequently, another protein called C8 binds with these membrane inserted proteins followed by several molecules of C9. These C9 proteins make pore in the membrane which leads towards leakage of cellular contents for cytolysis. The C5bC6C7C8C9 is called as membrane attack complex (MAC) (Fig 11.13)

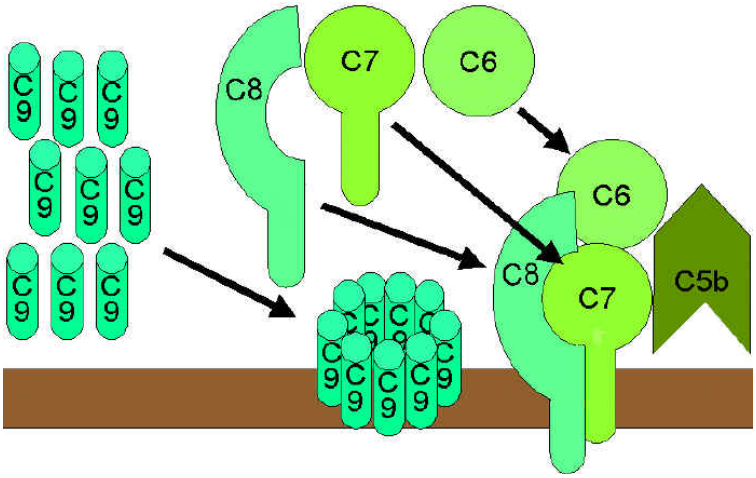

Figure 11.13: The lytic (common) pathway of complement activation

Biological Active Products of Complement

The complement system provides specific & non-specific resistance against infections. As as result of complement activation, there is production of various biological active molecules which are involved in resistance, anaphylaxis & inflammation. Followings are the important biological active products of complement

Kinnin production

C2b which is produced in classical pathway is called as Pro-Kinnin. This Pro-Kinnin is activated by serum factor called as Plasmin in to Kinnin which is a potent biological active compound. The excess C2b production cause undesirable effects like smooth muscles contraction and vasodilatation

Anaphylatoxins

Anaphylaxsis is the form of immune response in which exaggerated response is generated against allergen. The molecules which produced such response are called as anaphylatoxins. The complement proteins like C4a, C3a & C5a act as potent anaphylatoxins. These molecules cause basophils and mast cells degranulation as a result various mediators or cytokines are generated (Fig 11.3). Those mediators involved in smooth muscles contraction, vasodilatation and bronchoconstriction.

Chemotactic factors

The phenomenon by which phagocytic cells move towards the site of infection is called as chemptaxsis, and the molecules involved in this process are called as chemotactic factors. The complement proteins are considered as good chemotactic factors like C5a. These act as potent activator of neutrophils, basophils & macrophages. These cause the induction of adhesion molecules on surface of blood vessel's endothelial cells, which bind the phagocytic cells on it (Fig.11.2). One of the important complex of complement proteins called as membrane attack complex (MAC) composed of C5b, C6 and C7 also act as good chemotactic factor.

Opsonins

It is the process by which phagocytosis of microbes by immune cells get enhanced. Those proteins which undertake this process are called as opsonins. For example, some complement proteins like C3b, iC3b and C4b are good opsonins. These proteins attach to microorganism first making a complex. This complex of complement protein & microbe bind with complement receptor on the surface of phagocytic cell as a result phagocytosis of microbe occur by phagocytes (Fig.11.1)

Chapter #12: Hypersensitivity

Introduction to Hypersensitivity

It is the state of undesirable reaction by normal immune system, in which immune system over-reacts against various stimuli. These are also known as hypersensitivity reaction or intolerance. This state of body is considered as damaging, uncomfortable and fetal. This clinical state requires pre-sensitize state of host, in which the host is already exposed to stimulus in the form of allergens. In hypersensitivity, the protective role of immune system become harmful for its host. This condition is also termed as Allergy which is an abnormal response against the otherwise harmless environment stimulus e.g food, pollen & animal dander. The hypersensitive state of immune response also exhibits in the form of autoimmune disorders which are the abnormal immune response against host's self- tissues.

Classification of Hypersensitivity

Hypersensitivity reactions are classified on the basis of following two factors

1) Nature of immune response involved
2) The time required for immune response

Followings are the important types of hypersensitivity reactions

i) Type I
ii) Type II
iii) Type III
iv) Type IV

Type I Hypersensitivity Reactions

These reactions are also known as Immediate or Anaphylactic reactions which involve various tissues of the body with following clinical conditions

- Skin: Urticaria & Eczema
- Eyes: Conjunctivitis
- Nose: Rhinorrhea, Rhinitis
- Lungs: Asthma
- Gastro-Intestinal Tract (GIT): Gastroenteritis

These reactions manifest various undesirable symptoms from minor inconveniences to fetal death. In these reactions, usually reaction is quick & immediate ranging15to 30 minutes, however, sometimes can be 10-12 hours. These are mediated by IgE antibodies. The primary cells involved in these reactions include basophils and mast cells. The reaction is amplified by involvement of other cells like platelets, neutrophils & eiosinophils. The allergens of type I reactions have preferential involvement of IgE antibodies which are produced as a result of class switching

phenomenon. During these reactions, there is sensitization of mast cells with IgE via a specific FcR receptor. The fixed IgE is cross linked with allergen (Fig. 12.1). As a result, degranulation of mast cells occur which release various mediators of significant clinical featuers of type I reactions as shown in Table 12.1

Preformed mediators in the granules of Mast cells	
Histamine	Bronchoconstriction, mucus secretion, vasodilatation, vascular permeability
Tryptase	Proteolysis
Kininogenase	Kinins and vasodilatation, vascular permeability, edema
ECF-A (tetrapeptides)	Attract eosinophil and neutrophils

Table 12.1: Various mediators of Mast cell granules with their functions

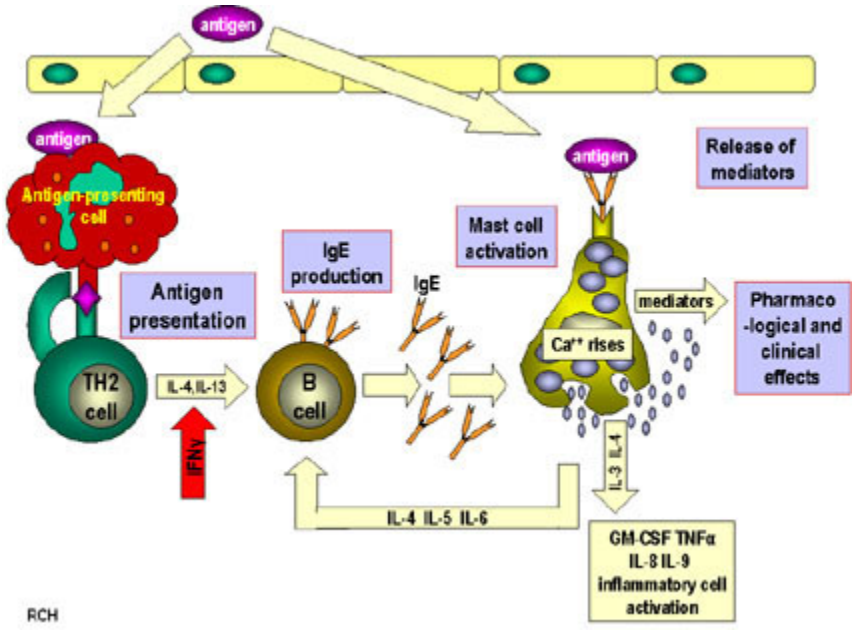

Figure 12.1: Mechanism of Type I hypersensitivity reactions

Diagnosis of Type I Hypersensitivity

Type I hypersensitivity reactions are diagnosed in the following important ways

- By Skin (Prick & Intradermal) test

In this test, the allergen is administered through skin and the reaction is observed in the form of redness, itching and swelling of the skin

- Measurement of total IgE levels

In patient's serum, the measurement of total IgE also help in the diagnosis of type I hypersensitivity reactions.

- Allergen specific IgE levels

In addition to total IgE levels, the allergen specific IgE levels also help in the diagnosis of immediate hypersensitivity reactions.

Type II Hypersensitivity

This type of hypersensitivity is also known as cytotoxic hypersensitivity. This condition effects various organs & tissues of body which determines the tissue specific nature of hypersensitivity. For such reactions there is the involvement of various antigens. These antigens are usually either endogenous (from inside of body) like antigens of body's self-tissues or exogenous (from outside of body) like various chemicals in the form of haptens. These exogenous antigens bind to the surface of cell membrane. There is generation of antibodies against these antigens which are IgM or IgG in nature. Antigen and antibody reaction occur on the surface of the target cells. The complement proteins get fixed on immune complex on the surface of target cell like RBC surface As a result, lysis of target cell occur (Fig.12.2). These kind of reactions have reaction time from minutes to hour. The examples of exogenous antigens include Drug induced hemolytic anemia, granulocytopenia & thrombocytopenia.

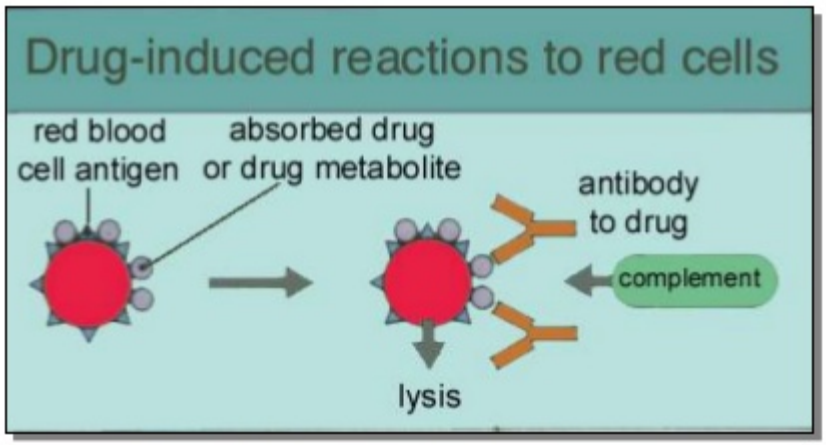

Figure 12.2: Drug (exogenous antigen) induced hemolytic anemia

Similarly endogenous antigens also induce immune response in the form of generation of antibodies. The example of endogenous antigens induced cytotoxic hypersensitivity include erythroblastosis fetalis which is also called as hemolytic disease of newborns (HDN). This condition occur due to Rh incompatibility between mother & fetus. As a result, there is the development of anti-Rh antibodies in maternal serum (IgG) which has the ability of crossing placenta in subsequent pregnancy (Fig. 12.3)

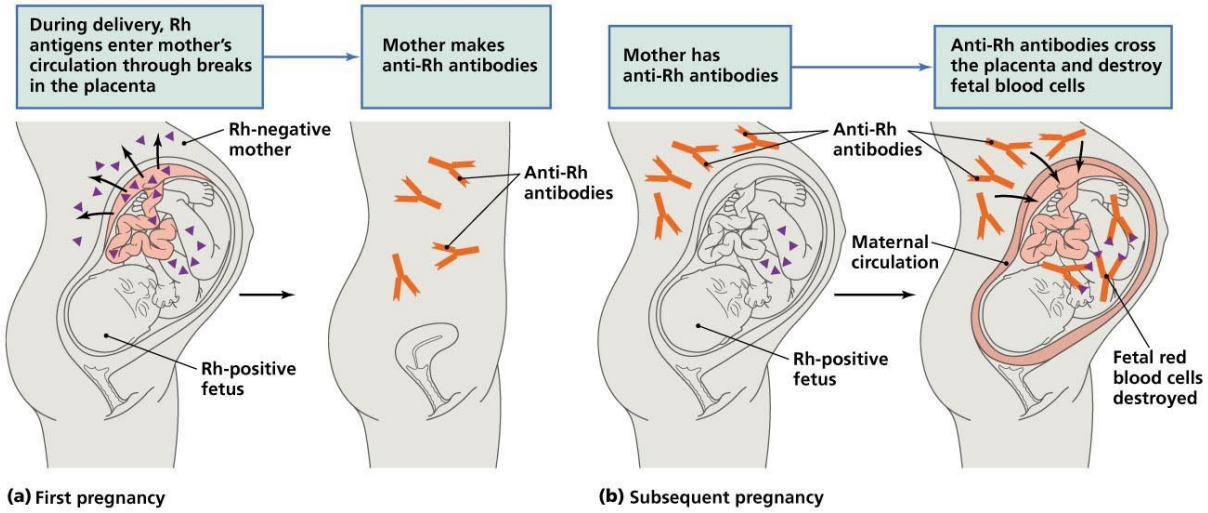

Figure 12.3: Events of Endogenous antigen induced cytotoxicity in erythroblastosis feralis or hemolytic disease of newborns (HDN)

Likewise, other examples of endogenous antigen induced cytotoxic hypersensitivity include following autoimmune diseases like

Goodpasture's Syndrome in which there is generation of Autoantibodies against glomerulus basement membrane

Hashimoto's thyroiditis in which Autoantibodies are generated against thyroid

Antibody dependent Cellular Cytotoxicity (ADCC) is also another form of Type II hypersensitivity. This type hypersensitivity is independent of complement system. However, the effector immune cell lyse the target cell coated with antibody. These effector cells can be the primary effector cells like natural killer (NK) cells while neutrophils, macrophages and eiosinophils are also included in such kind of hypersensitivity reactions. Examples of such kind of reactions include the cytotoxicity of tumorous cells by NK cells (Fig. 12.4)

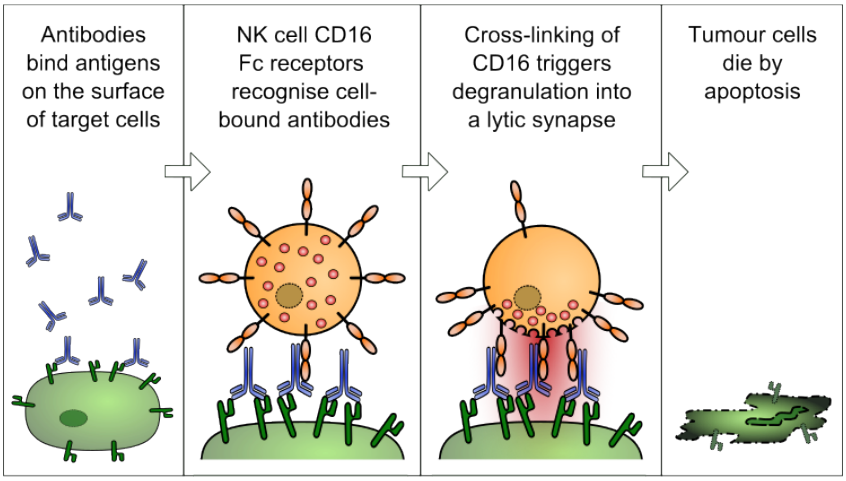

Figure 12.4: Cytotoxicity of tumor cell through cytotoxic hypersensitivity

Type III Hypersensitivity

These reactions are also known as immune complex mediated hypersensitivity. The reaction time required for such reactions is 3-10 hours after exposure to antigen. There is generation of antibodies which bind with corresponding antigens as a result soluble immune complex formation occur. These immune complex effect generally the host and that condition is called as serum sickness. Otherwise, these reactions are specific to various organs and cause the following conditions

- Skin: Systemic Lupus erythematous (SLE), Arthus reaction
- Kidney: Lupus nephritis
- Lung: Aspergillosis
- Blood vessels: Polyarteritis
- Joints: Rheumatoid Arthritis

For the pathogenesis of these reactions there can be the involvement of microorganisms

Figure 12.5: Clinical picture of SLE & Rheumatoid arthritis patients

The antigens involved in these reactions are soluble in its state which can be of two types

1. Exogenous

These include various microbial infections like chronic bacterial, viral & parasitic infections

2. Endogenous

Non-organ specific autoimmunity e.g SLE

Antibodies involved in these reactions are mostly IgG but IgM are also involved. However, the primary components of these reactions are soluble immune complexes with complement proteins like (C3a & C5a). Moreover, platelets and neutrophils are also involved in this damage. The lesion of these reactions contain primarily neutrophils with immune complex and complement. Furthermore, infiltrating macrophages also contribute later in healing process

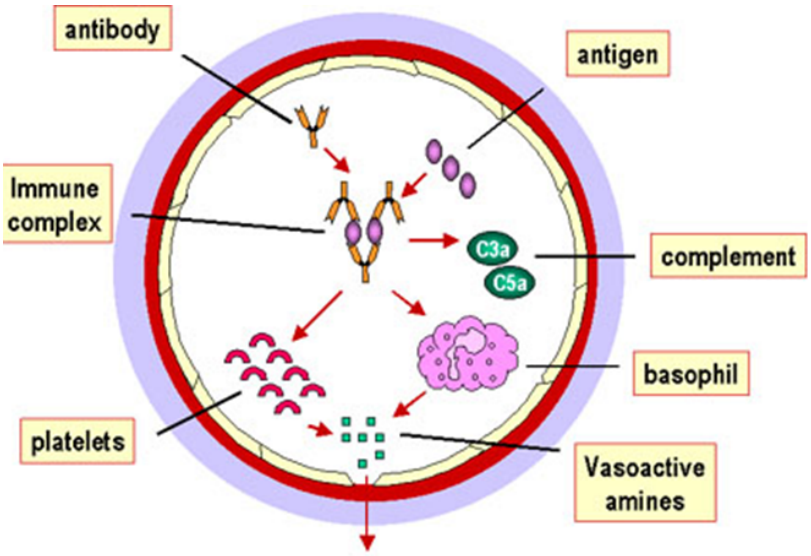

Figure 12.6: Components involved in Type III hypersensitivity reactions

The severity of these reactions also based on affinity of antibodies, the Size of immune complex and the type of tissue involved. In the lesion of these reactions, aggregated platelets form micro thrombus which increase the vascular permeability (Fig. 12.7). The diagnosis of these reactions is done by presence of immune complex with complement in tissue biopsies through immunofluorescence.

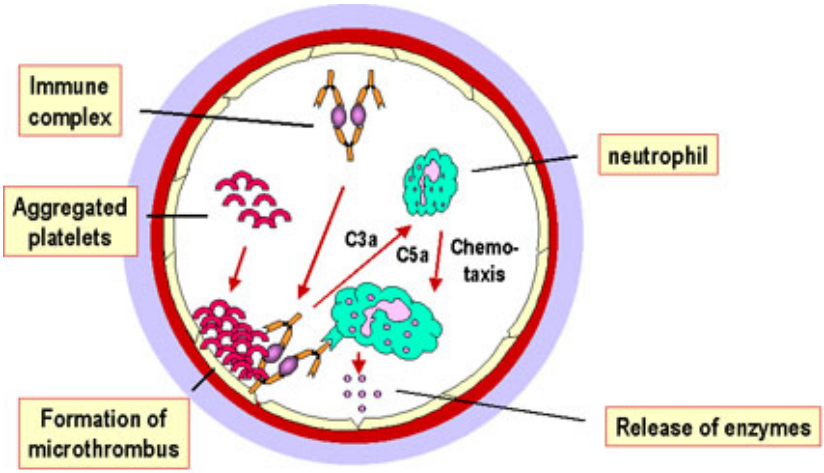

Figure 12.7: Mechanisms of tissue destruction in type III hypersensitivity reactions

Type IV Hypersensitivity

These reactions are also termed as cell mediated hypersensitivity. Due to more time requirement like 48-72 hours, these reactions are also called as delayed type of hypersensitivity. Immunologically, T-cells, Macrophages and monocytes are involved in these reactions. In these

reactions, the response is against intracellular pathogens that's why these reactions are antibody independent in nature. Lymphocytes like CD8 (Cytotoxic) T-cells cause direct damage to target cells. Likewise, CD4 (Helper) T-cells for example Th1 cells recognize foreign antigen with MHC class II. Moreover, the secretion of Cytokines, activation of macrophages and Inflammation increase the magnitude of tissue injury & damage.

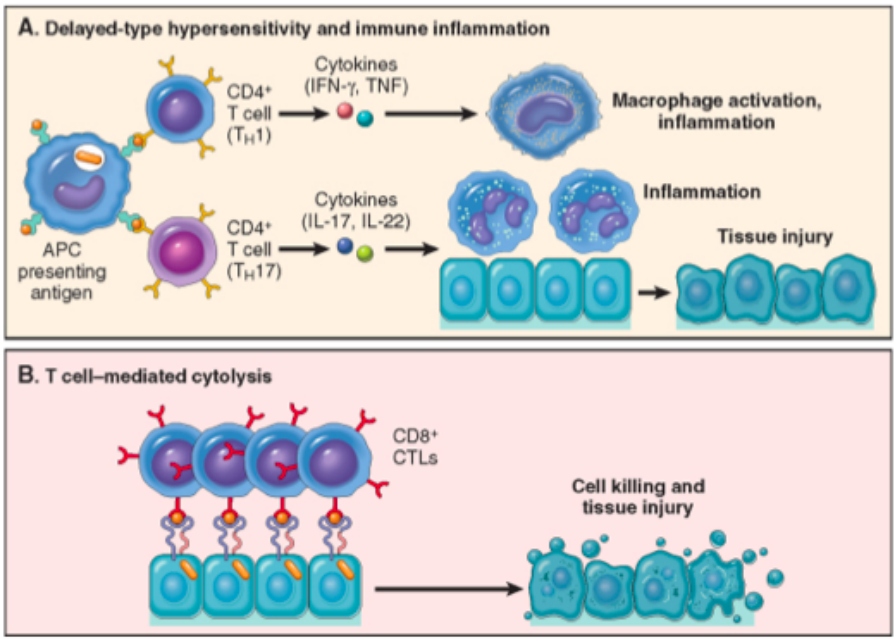

Figure 12.8: Involvement of immune mechanisms in Type IV hypersensitivity reactions

Chapter # 13: Cytokines

Overview about Cytokines

Cytokines belong to diverse group of non-antibody proteins which act as mediators between cells. Initially, these proteins were considered as products of immune cells. More importantly, these were also considered to act as mediators for immune cells. Later on, it was also found that these are also produced from non-immune cells and served as mediators too. Cytokines are used as biological response modifier for treatment of various diseases. In general, cytokine is a general term but it can be more specific term which is used according to their cell of origin as follows

- Monokines

These are produced by mononuclear phagocytic cells.

- Lymphokines

These are produced by activated lymphocytes.

- Interleukins

These are the mediators between leucocytes

- Chemokines

These are responsible for leucocytes migration and inflammatory process

Cytokines usually perform their functions as cascade signaling in the form of a network which is termed as cytokine network. Their functions are for enhancing their effects of other cytokines in an additive or synergistic manner. Moreover, these act for suppressing the effects of other cytokines in an opposing or antagonistic way. Cytokines are not as preformed proteins like antibodies. These are produced by gene transcription as needed because mRNA for cytokines are short lived in nature. Furthermore, the individual cytokine can act on many cells of the host which is termed as pleotropic response of cytokines.

Mechanism of acting of Cytokines

Cytokines act through specific receptors present on various cells. These are considered as redundant in nature. The redundancy of cytokines is due to the nature of cytokine receptors. The complex interaction between host's cells and cytokines occur through cytokine network. Different cells can respond to same cytokine due to structural similarities among cytokine receptors (Fig.13.1). The cytokine signaling is flexible which can have both protective and damaging effects. Similarly, thes can also influence the synthesis of other cytokines.

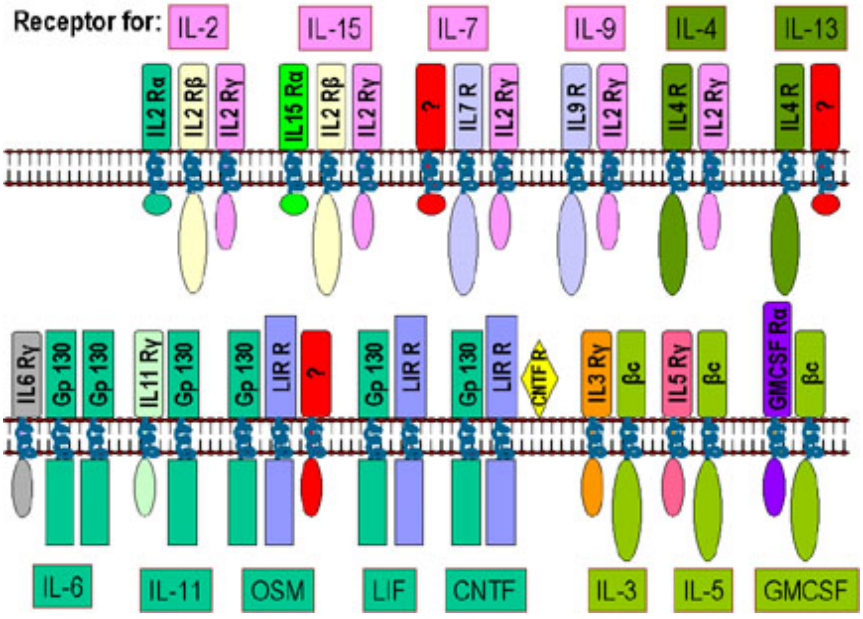

Figure 13.1: The structure of various cytokine receptors

Cytokines bind to specific receptors with high affinity and respond in following three ways

1. Autocrine

These effect on same cells which secrete the cytokine

2. Paracrine

In this way, the cytokines effect on nearby cells of cytokine secreted cells.

3. Endocrine

Through this way, the cytokines effect on distant cells through circulation.

Categories of Cytokines

Cytokines are categorized on following two basis

I. Their source of origin

II. Their functions

I. Categories based on source cells

Followings are the categories of cytokines on the basis of their source of origin

i) Monokines

As the name indicates, these are produced by mononuclear phagocytic cells for example Interferon gamma and Interleukin (IL-1) from macrophages & Monocytes

ii) Lymphokines

These are produced by activated lymphocytes for example IL-3, IL-4 & IL-5.

iii) Interleukins

These are mediators between leucocytes for example IL-1, IL-10& IL-18.

iv) Chemokines

These are responsible for leucocytes migration for example IL-8.

II. Based on functions

On the basis of functions, cytokines are categorized into following two categoryies

a) Cytokines which act for Innate (non-Specific) immune system e.g TNF-α, IL-1, Il-10 etc

b) Cytokines which act for Adaptive (Specific) immune system e.g Il-2,IL-4 & IL-5

Cytokines of Innate Immune System

These cytokines which act primarily for innate (non-Specific) immune system. These are as follows

1) Tumor Necrosis Factor-Alpha (TNF-α)

It is produced by activated macrophages in response to microbes or their products for example lipopolysaccharides (LPS) of gram negative bacteria. It acts a mediator of acute inflammation. It recruits neutrophils and macrophages at the site of infection either by stimulating endothelial cells to secrete adhesion proteins or by secreting chemokines. Moreover, TNF-a induces fever and acute phase proteins in host.

2) IL-1

This is also an inflammatory cytokine as like TNF-a. It is produced by activated macrophages. Furthermore, it also help to activate T-cells.

3) IL-10

It is an inhibitory cytokine which inhibits the cytokine production of cytokines from macrophages.

4) IL-12

This cytokine is produced by dendritic cells and macrophages. It enhances the cytolytic activity of cytotoxic T-cells

5) Type I interferon

This class of interferon include Interferon α & β. These inhibit viral replication in cells by increasing the expression of class I MHC which in turn make them susceptible to cytotoxic T-lymphocytes (CTL).

6) IFN-γ

It is produced primarily by Th1 cells. It enhances the cytolytic activity of NK cells by induction of class I & II MHC molecules.

7) Chemokines

These are chemotactic cytokines produced by many leucocytes. These recruits inflammatory cells at site of infection for an inflammatory response.

Cytokines of Adaptive Immune System

Followings are the cytokines which act primarily for Adaptive (Specific) immune system

1) IL-2

It is produced by T-helper (Th) cells but Cytotoxic T-cells (Tc) also produced it in lesser extent. It stimulate B-cell division. It can also activate NK cells and monocytes. Moreover, IL-2 acts on T-cells in autocrine manner.

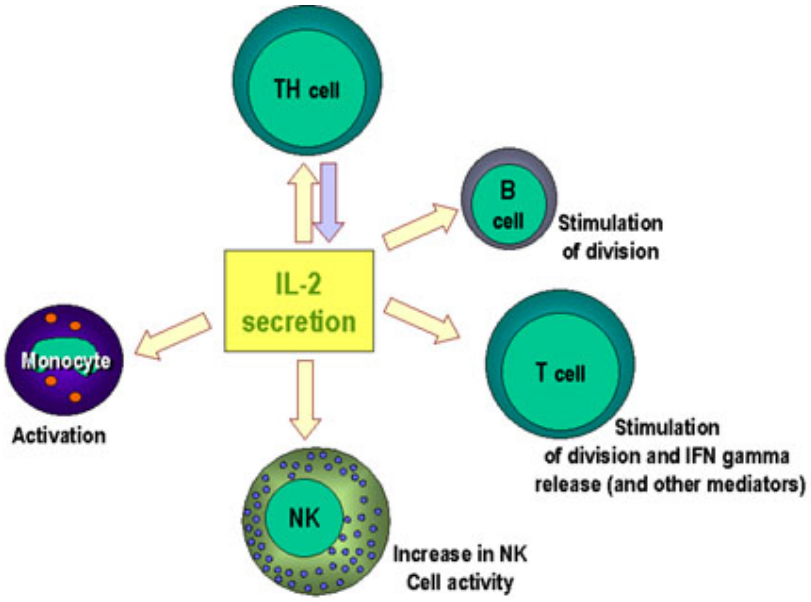

Figure 13.2: Functions of IL-2 on various other immune cells

Activation of T-cells result in expression of IL-2R receptors and also production of IL-2. IL-2 binds to IL-2R and promotes cell division. However, IL-2 receptor decay if T-cells are not activated and eventually proliferative phase ends (Figure 13.3).

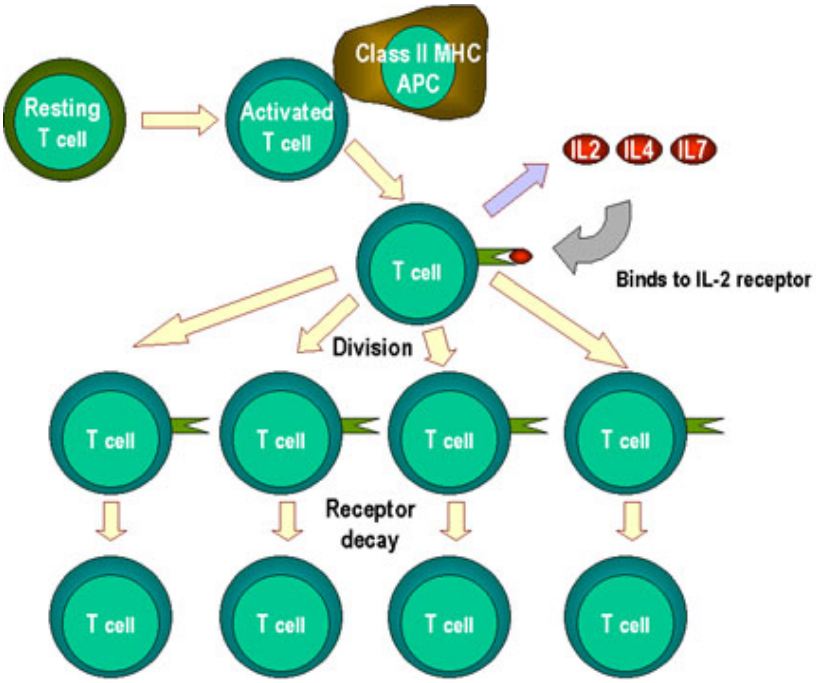

Figure 13.3: Activation of T-cells by IL-2

2) IL-4

IL-4 is produced by macrophages and Th2 cells. It promotes the development of Th2 from naïve T-helper (Th) cells. The Differentiated Th2 cells result in the production of antibodies. IL-4 also involves in the class switching of antibody into IgE

3) IL-5

IL-5 is produced by Th2 cells. It promotes the development of B-cells and eosinophils. It also activates mature eosinophils.

4) Tumor Growth Factor (TGF)-β

TGF- β is produced by T cells and many other cells types. It acts as inhibitory cytokine which inhibits the proliferation of T-cells and activation of macrophages. It also acts on neutrophils and endothelial cells to block the effects of pro-inflammatory cytokines.

Cytokine Network

A complex series of overlapping and inter-related connections among cytokines. As cytokines are secreted from one kind of cells and have effect on other type of cells & organs. Within this network, some cytokines have synergistic effects on other cytokines while some have antagonistic effects. Through this network, lymphocytes, macrophages and other tissues are regulated for their functions (Fig. 13.4)

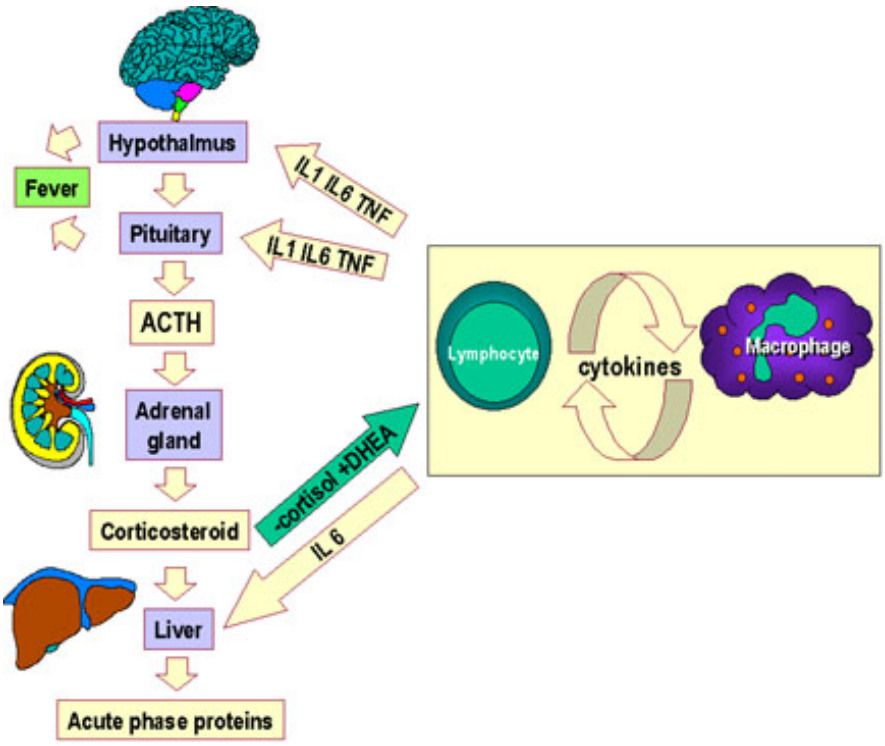

Figure 13.4: Communication B/W Lymphocytes, Macrophages & Other Tissues

Similarly, monocytes, lymphocytes and other components are also communicated through this network (Fig. 13.5)

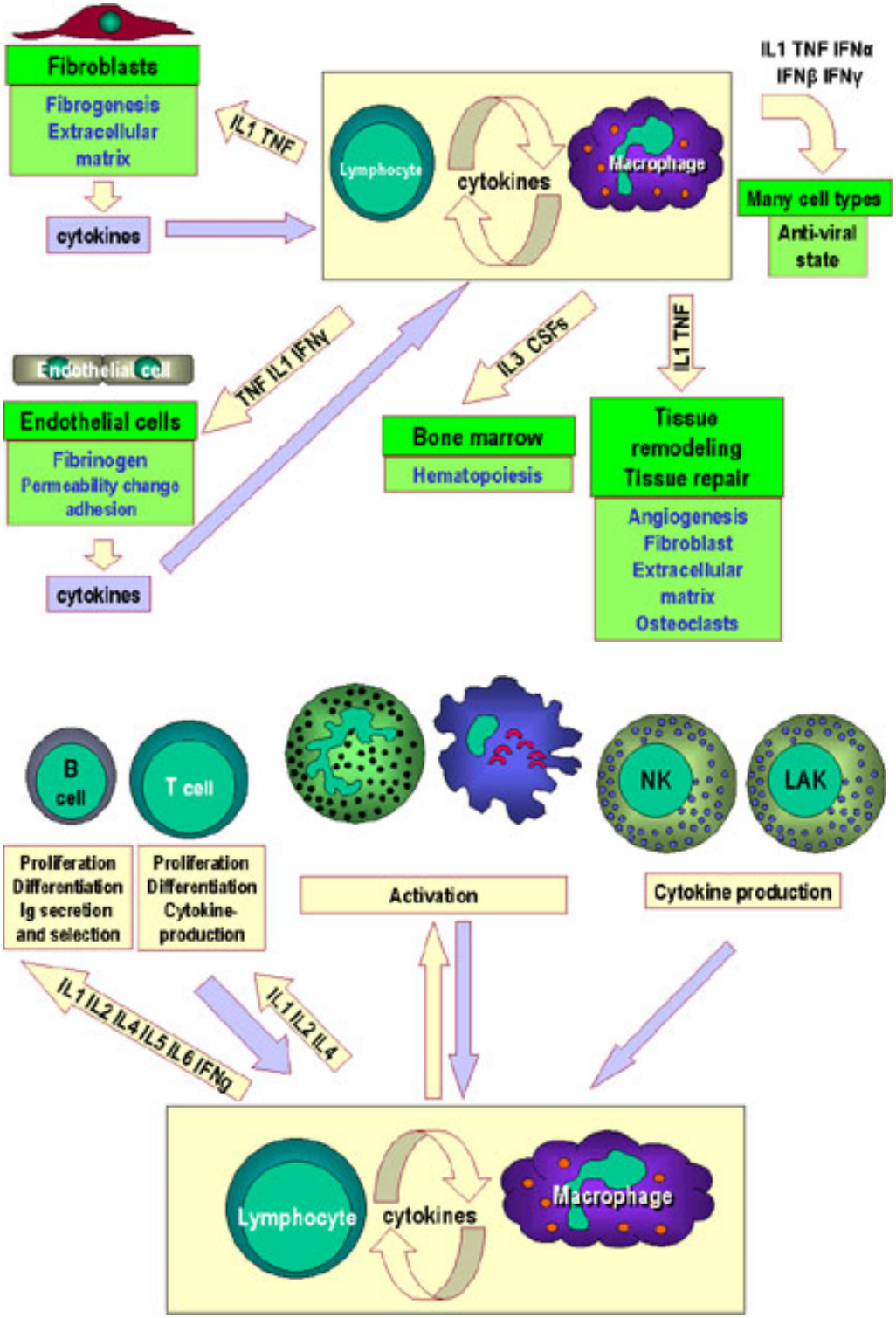

Figure 13.5: Communication B/W Lymphocytes, Macrophages & Other Components of Immune Syste+m

Immuno-Regulation by Cytokine

The control of immune response between lymphocytes and macrophages. The balance is required between antigen driven activation of lymphocytes and regulatory influences which is called as immune-regulation. Immuno-regulation occur at following three phases of immune responses

1) Recognition phase
2) Activation Phase
3) Effector Phase

The cytokines are considered as positive or negative regulator of immune response. The cytokines can act on many stages of immune responses. Their activity is dependent on presence of other cytokines in the tissue's microenvironment. It also depends upon receptor expression on effector cells. This kind of regulation regulates the nature and extent of generated immune response.

Chapter#14: Resistance and Immune Response to Infectious diseases

What is Immune Evasion?

All the strategies used by pathogenic organisms and tumor cells to evade host immune's system are called as immune evasion mechanisms. Immune system has one of the important characteristics of immune surveillance for protection against pathogenic organisms and transformed cells. However, pathogens maximize their ability to flourish and develop infection. Likewise, tumor cells also have the potential to evade the host's immune surveillance and sustain inside the body. Immune-evasion occurs either due to weak host immune response or by strategies devised by pathogens. In immune evasion, immune system can be bypassed by evading humoral immunity. Moreover, cell mediated immunity can also be evaded by pathogenic microbes and transformed cells.

Mechanisms of Immune Evasion

The strategy to escape from immune system is caused by different microbes differently as follows

a) Extracellular organisms

These organisms usually inactivate the humoral components of immune system

b) Intracellular organisms

These organisms usually bypass the host immune system by inactivating intracellular killing and other cell mediated immune mechanisms which are as follows

Antigenic Variation

Microorganisms mutate their antigenic surface molecules for immune-evasion which is called as antigenic variation. As a result, there is not available longer protection by antibodies produced under previous exposure of antigenic forms. There are following two forms of antigenic variations

- Antigenic shift

  This is the major form of change in antigenic structure of antigen as a result the new strains of microbes are generated (Fig.14.1)

- Antigenic drift

  This is the minor form of antigenic variation within the same strain for example influenza (Flu) virus exhibits both antigenic shift and drift for pandemic and seasonal flu respectively. Similarly, bacteria like *E.coli*, *Neisseria gonorrhoeae* and *Salmonella typhimurium* also exhibit antigenic drift (Fig. 14.1).

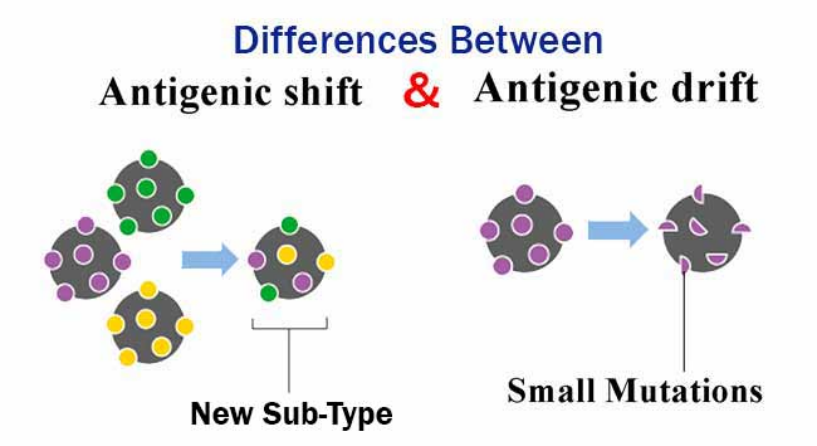

Figure 14.1: Comparison between antigenic shift and drift

Inhibition of Complement activation

The degradation of complement proteins through complement deviation which occurs by deviation of the complement activation site on bacterial cell. Moreover, the resistance to insertion of Membrane Attack Complex (MAC) in bacterial due to thick bacterial cell wall and capsule also responsible for inhibition of complement activation.

Resistance to Phagocytosis

The inhibition of phagocytosis process of bacteria due to cell surface molecules of bacteria for example capsule of bacteria like pneumococcus. Similarly, the trapping of bacteria by bacterial enzymes for example coagulase of *S. aureus*. Moreover, the killing of phagocytes due to various secreted toxins from different bacteria for example Leucocidin & Lysins from *S. aureus* and Streptococci respectively. The different immune-evasion mechanisms of *S. aureus* (Fig.14.2)

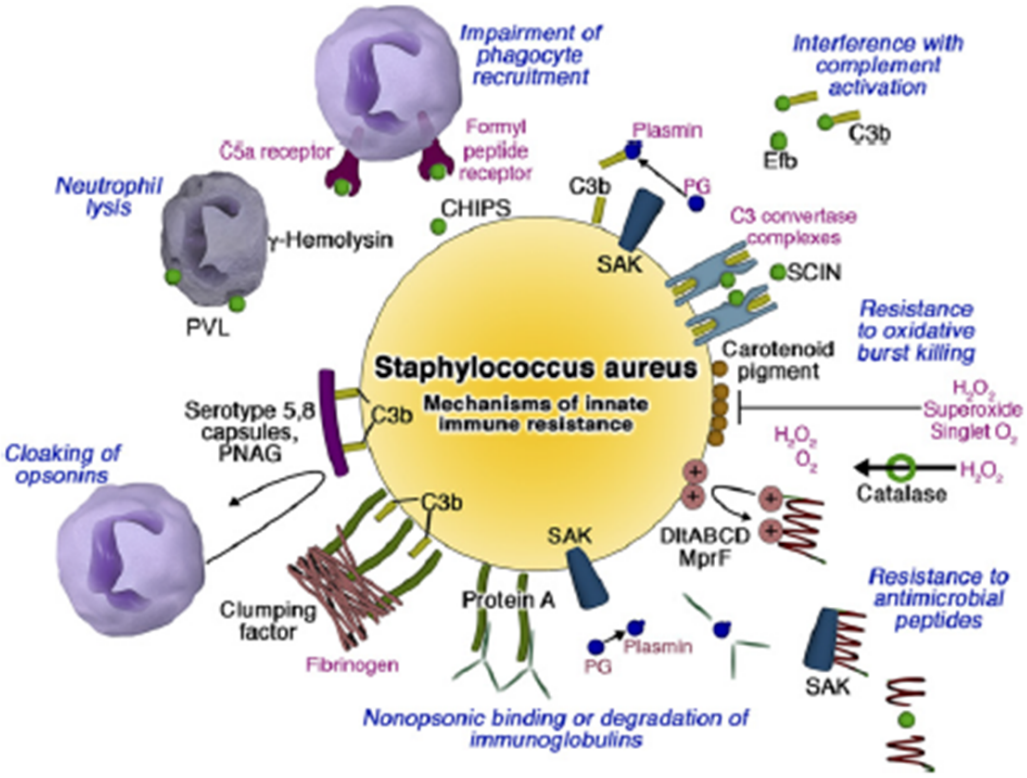

Figure 14.2: Strategies of Immune evasion by *S. aureus*

Immune Response against Extracellular Pathogens

The secretory or humoral immune molecules are effective against extracellular pathogens. Various secretory molecules like antibodies and complement proteins contribute in such kind of protection

There are three ways of controlling

1) Neutralization
2) Opsonization
3) Complement activation
1) Neutralization

The pathogenicity of bacteria is being determined by secreted molecules of bacteria like toxins. These toxins are neutralized by specific antibodies against toxins in a form of antitoxins by the process of neutralization. The binding of antibodies with toxins leads towards immune complex formation. Furthermore, the clearance of immune complex is done by the process of phagocytosis.

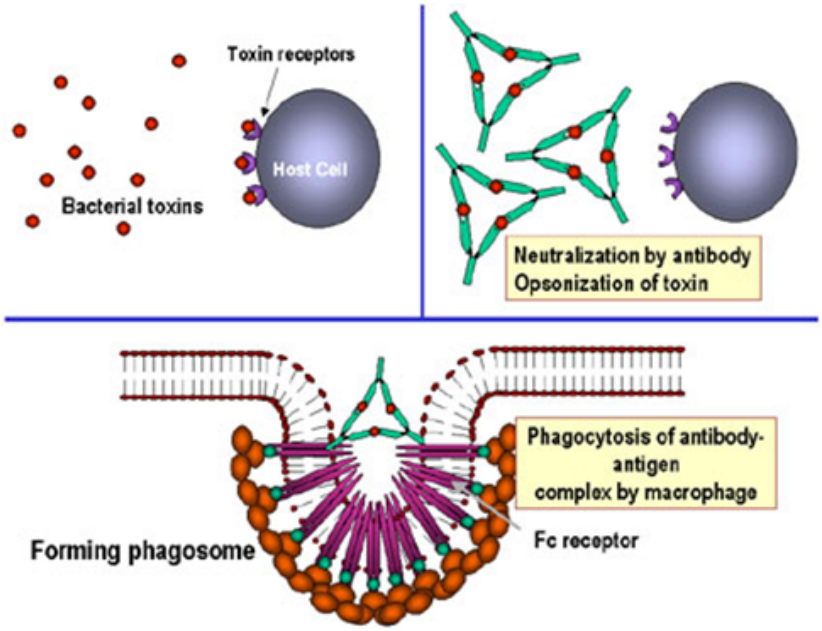

Figure 14.3: The process of neutralization

2) Opsonization

The enhancement of the process of phagycytosis by phagocytes is called as opsonization. The antibodies which bind specifically with extracellular bacteria e.g IgG or IgM are called as opsonins. This result in the formation of immune complex. Moreover, the binding of immune complex occur with specific receptors against Fc fragment of antibodies (Fig. 14.4).

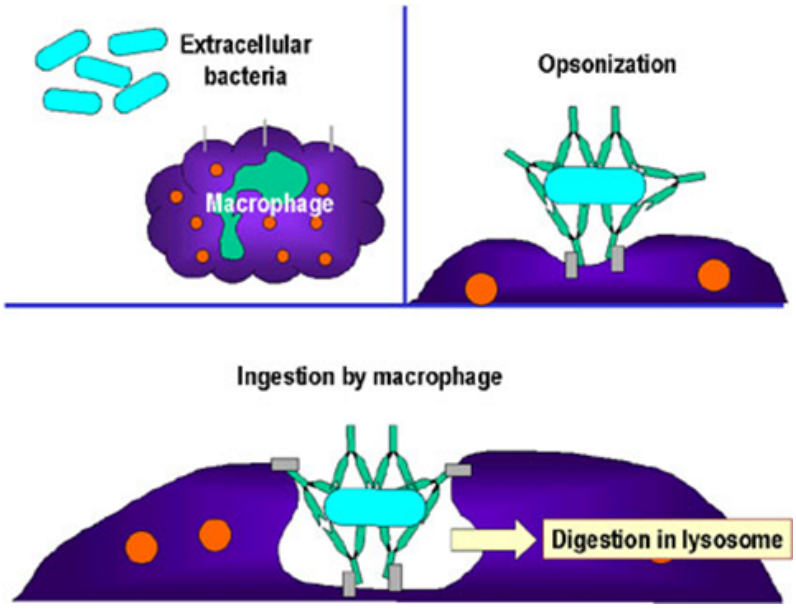

Figure 14.4: The process of opsonization

Complement activation

The inactive complement proteins are activated by extracellular bacteria in combination with specific antibody. Further, the complement proteins are fixed on antibody which is already bounded with bacterial antigen on bacterial surface. This cause the lysis of bacteria which is the primary function of complement proteins by forming membrane attack complex (MAC) (Fig. 14.5).

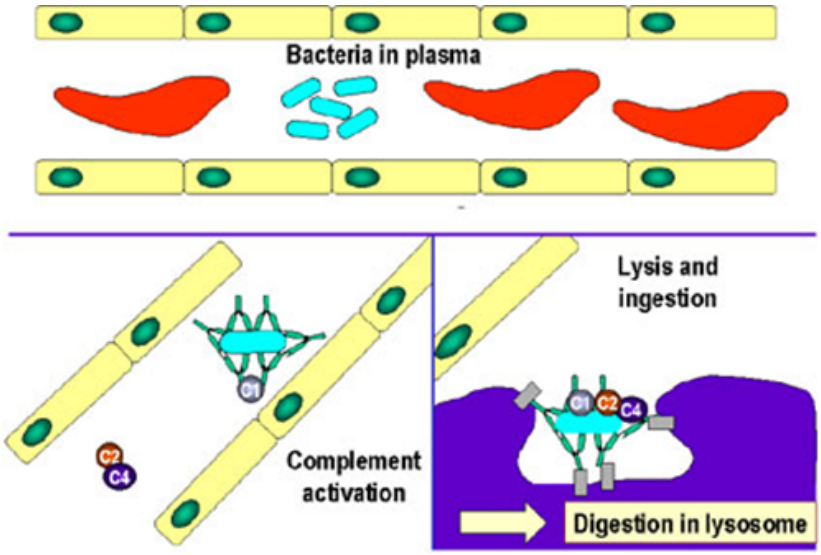

Figure 14.5: The process of complement activation

Immune Response against Intracellular Pathogens

The secretory immune molecules are ineffective against intracellular pathogens like viruses and intracellular bacteria. The cell mediated immune response is the primary defense against intracellular pathogens. The T-Lymphocytes play vital role in cell mediated immunity. Cell mediated immune response varies according to the residing site of the pathogen

1) Cytosolic site
2) Vesicular site

Control of Cytosolic Pathogens

The exogenous pathogens are the pathogens which used to reside in the cytosol of infected cell. Their antigens are presented in combination with class I MHC to Cytotoxic T-Lymphocytes: which recognize such antigens .Viruses are controlled through such mechanism (Fig 14.6)

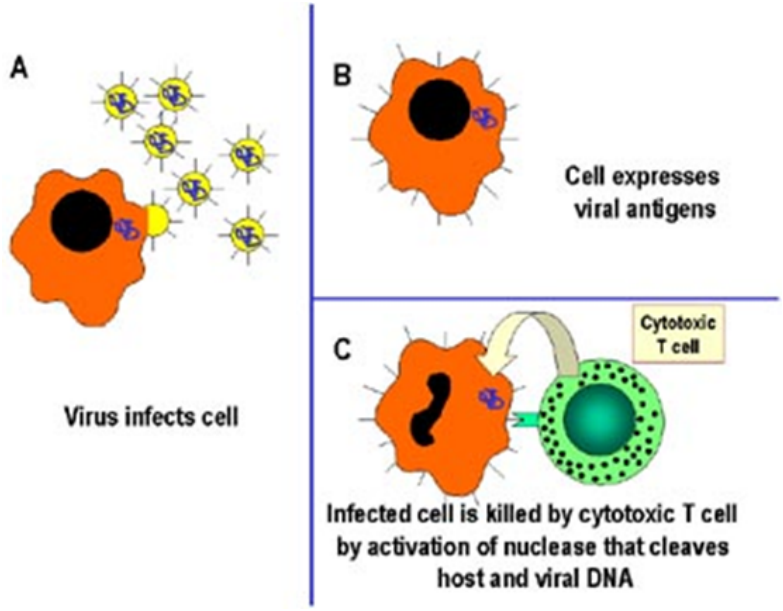

Figure 14.6: The control of cytosolic pathogens

Control of Vesicular Pathogens

The endogenous pathogens are those pathogens which used to reside in the vesicle like phagosomes of infected cell. The antigens of such pathogens are presented in combination with class I MHC to Helper T-Lymphocytes which recognize such antigens (Fig. 14.7) .Intracellular bacteria e.g *Mycobacterium, Liesteria*

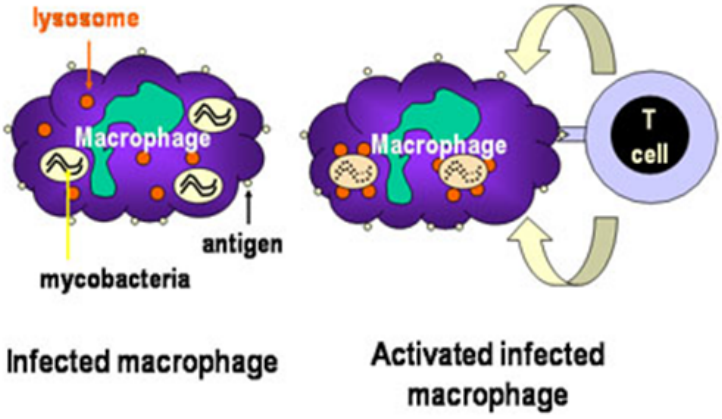

Figure 14.7: The control of vesicular pathogens

Followings are the ways by which intracellular bacteria evade the host immune mechanisms (Table 14.1)

Mechanisms of Immune Evasion by intercellular Bacteria

Mechanism of Immune Evasion	Examples
Inhibition of phagolysosome formation	*Mycobacterium tuberculosis, Legionella pneumophila*
Inactivation of reactive oxygen and nitrogen species	*Mycobacterium leprae* (phenolic glycolipid)
Disruption of phagosome membrane, escape into cytoplasm	*Listeria monocytogenes* (hemolysin protein)

9

Table 14.1: Mechanisms of Immune Evasion by Intracellular Bacteria

Chapter # 15: Cell Mediated Effector Response

Cell Mediated Immunity

The immune response which is independent of antibodies and based on cell to cell interaction is called as cell mediated immune response or immunity. In this kind of immunity, there is activation of phagocytes and antigen specific cytotoxic T-lymphocytes which further release various cytokines from activated cells in response to various antigen. Moreover, naïve & mature T-cells are differentiated into effector T-cells after interacting with antigens via antigen presenting cells (APC). On contrary, the humoral immunity is based on cell free body fluid like serum which contain various protective molecules like antibodies. However, in cellular immunity is associated with cells for protective functions of immunization. Both innate and adaptive immune systems have humoral & cellular immunity

Cellular mediated immunity provides protection by following ways

- T-cell immunity

In this form of protection, cytotoxic T-cells (CTL) are activated for the killing of infected and transformed cells

- By activating macrophages

By this way, macrophages are activated for destroying various pathogens

- By activating natural killer (NK) cells

In this way of protection, NK cells are activated for killing of transformed and viral infected cells

Role of Cytotoxic T-Lymphocytes in Cell Mediated Immunity

The cytotoxic T-Lymphocytes are also called as CTLs and CD8+ve. These cells are not mature after exiting thymus where these have functional TCR which can recognize antigens but cannot kill the target cells. There is needs of differentiation for fully activation of CTLs. CTL differentiates from Pre-CTLs in response to following two signals

1. The specific antigen in context of Class I MHC
2. The cytokines produced from T-helper 1 (Th1) cells especially IL-2 & INF-γ (Fig.15.1)

CTLs cause the killing of target cells. This of killing is antigen specific where target cell must bear same antigen with class I MHC molecule

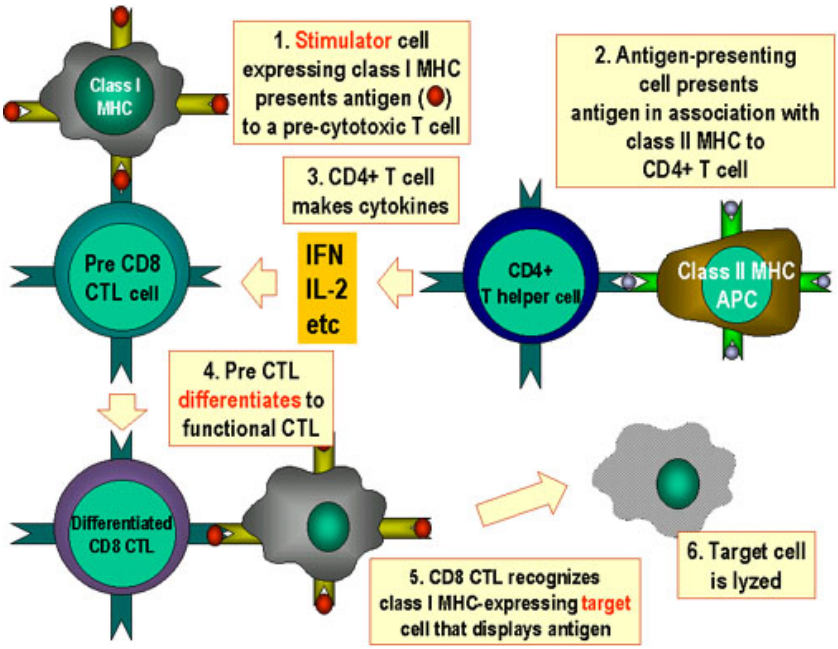

Figure 15.1: Maturation & Differentiation of cytotoxic T-cells (CTLs)

Moreover, CTL killing also requires cell to cell contact in which target cell should contain cell surface MHC molecule. However, CTLs are not injured while targeting the target cell. One CTL can kill sequentially numerous target cells.

Mechanisms of CTL killing

Mechanistically, CTLs rely on granule-mediated killing. In this form of killing, fully differentiated CTL have numerous granules which contain perforin & graenzymes in its cytoplasm. Upon CTL degranulation, perforin monomers are polymerized to produce polyperforin molecules. These polyperforin molecules are assembled in the form of channels in the presence of calcium ions (Fig.15.2)

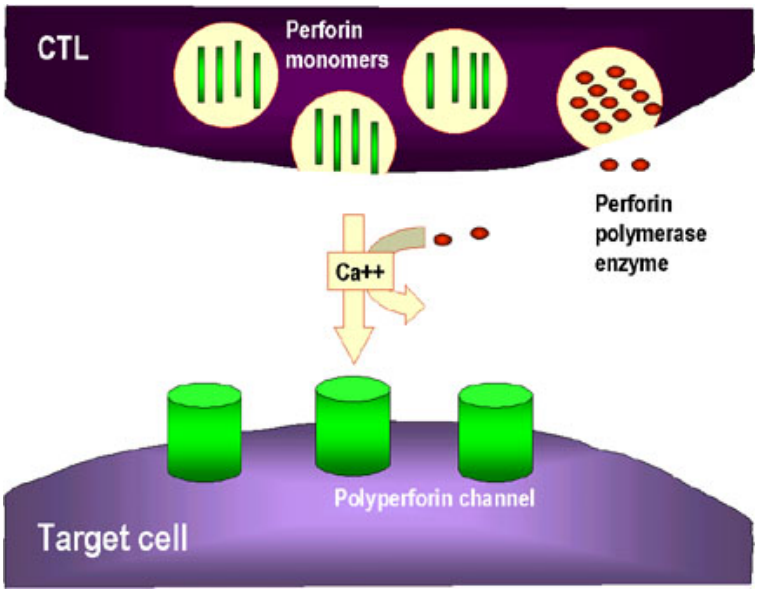

Figure 15.2: Release of perforin molecules from CTL and formation of polyperforin channel in the membrane of target cell

After developing polyperforin channels in the membrane of target cell, the CTL release degradative enzyme or toxins which travel through perforin channels towards target cells (Fig. 15.3). The killing of target cell occur with the help of degradative enzymes. Furthermore, the cytokines like TNF-a & INF-γ from CTL bind to target cells and induce programmed cell death or apoptosis of target cell

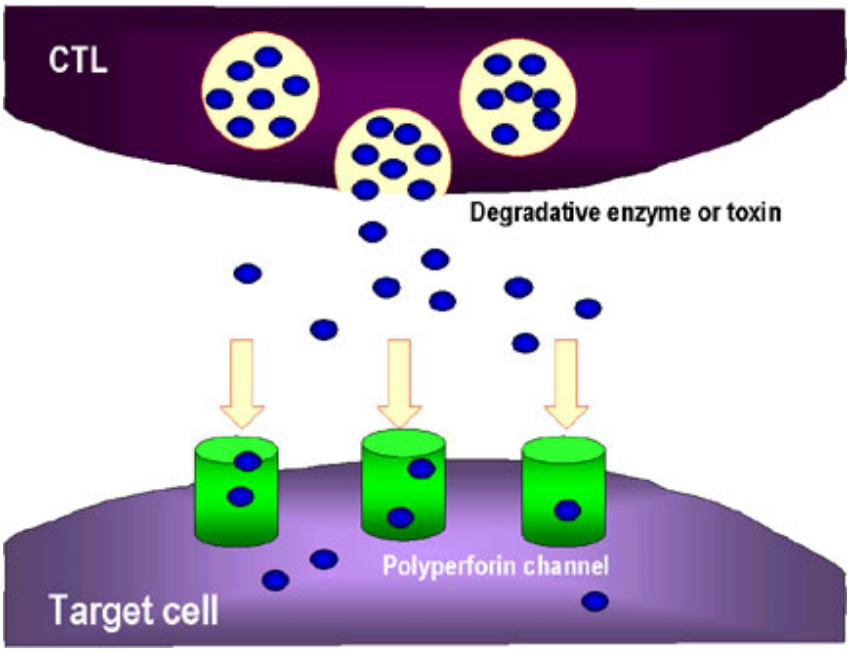

Figure 15.3: Killing of target cell after releasing of degradative enzyme or toxin

Role of Helper T-Lymphocytes in Cell-Mediated Immunity

Helper T (Th)-Lymphocytes which are also called as CD4+ve cells. These cells are at the center of cell mediated immunity. Helper T-lymphocytes recognize specific antigens presented with class II MHC. Moreover, these cells are also involved in B-lymphocytes activation. Helper T-cells also release various cytokines which further activate other immune cells (Fig.15.4)

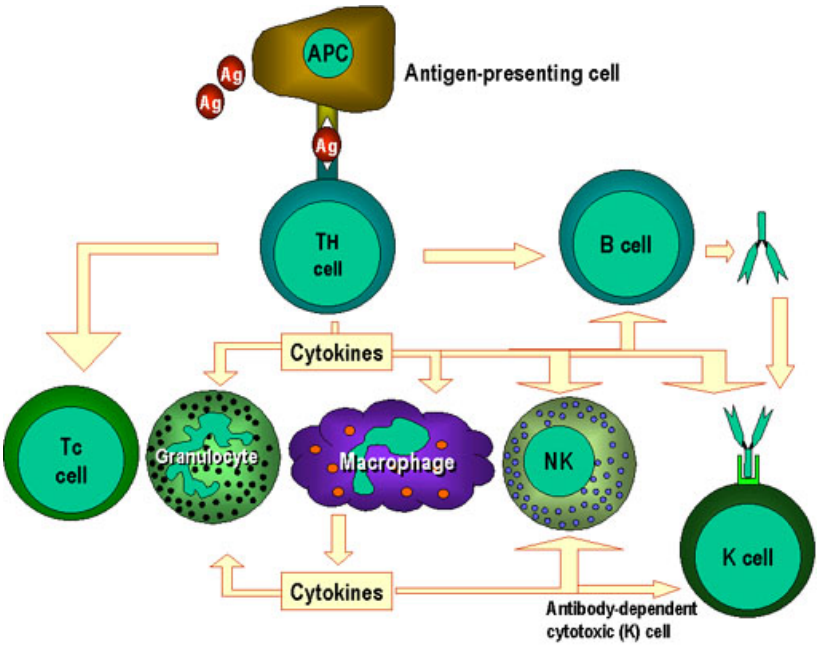

Figure 15.4: Functions of Helper T-lymphocytes

There are two major subtypes of Th cells

1) Th1 cells
2) Th2 cells

- Th1 cells

These cells release cytokine like IFNγ upon activation which then activate macrophages. In turn, macrophages participate in the generation & differentiation of CTL for cell mediated immunity. However, IFNγ acts as inhibitory cytokine for Th2 (Fig.15.5)

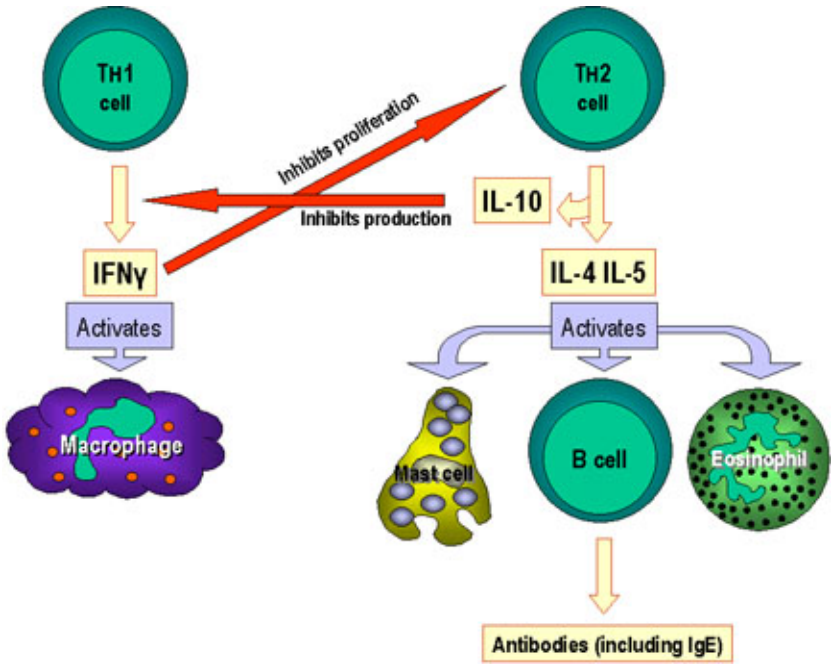

Figure 15.5: Function of Th1 & Th2 cells

- Th2 cells

Upon activation, Th2 cells secrete cytokines like IL-4 & IL-5 which further activate B-cells for humoral immunity. This kind of humoral response is also involved in the generation of IgE antibodies through the process of class switching. Moreover, Th2 cells are also involved in the activation of mast cells and eosinophils for providing immunity against allergen and parasitic infections. Th2 cell also secrete IL-10 which is inhibitory for IFNγ production from Th1 cells (Fig.15.5).

Role of Natural Killer (NK) cells in Cell Mediated Immunity

NK cells are also called as Large Granular Lymphocytes (LGL) as these resemble with lymphocytes except that they are larger and have numerous granules. These have surface molecules like C16 and CD56 but lack in CD3 molecule. NK cells have the ability of killing virus infected and malignant cells after activation. These are activated upon exposure to cytokines like IL-2 & IFNγ and then recognize the infected and malignant cells (Fig.15.6). The recognition is based on the absence of class I MHC molecule on the surface of infected cells in contrast to normal cells. Afterward, NK cells kill the target cell like CTL using perforin & graenzymes

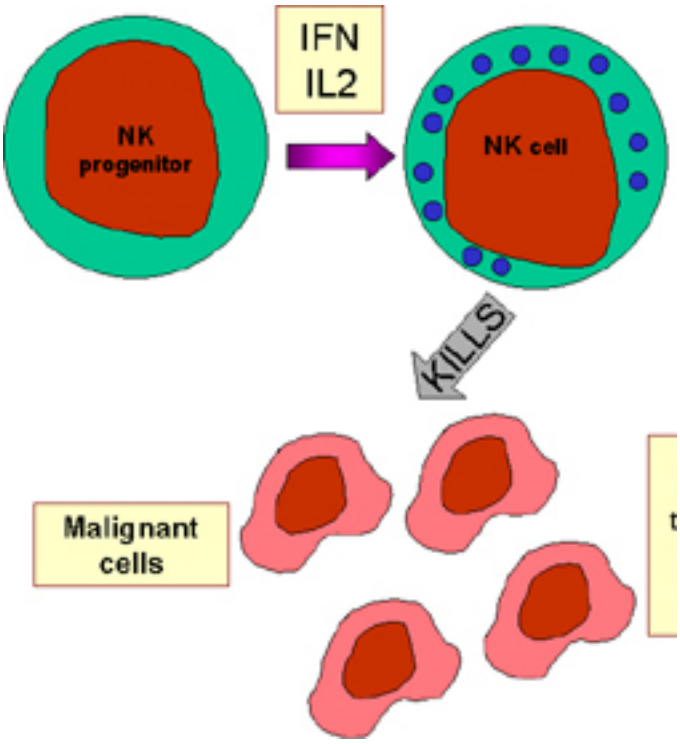

Figure 15.6: Activation & killing of malignant cells by NK cells

NK cells need cell to cell interaction for its effector function to kill malignant cells. NK cell cannot kill normal cells which are self cells due to normal expression of Class I MHC. However, the viral infected and malignant cells are recognized due to absence of Class I MHC (Fig.15.7).In this way viral infected & tumor cells are rejected

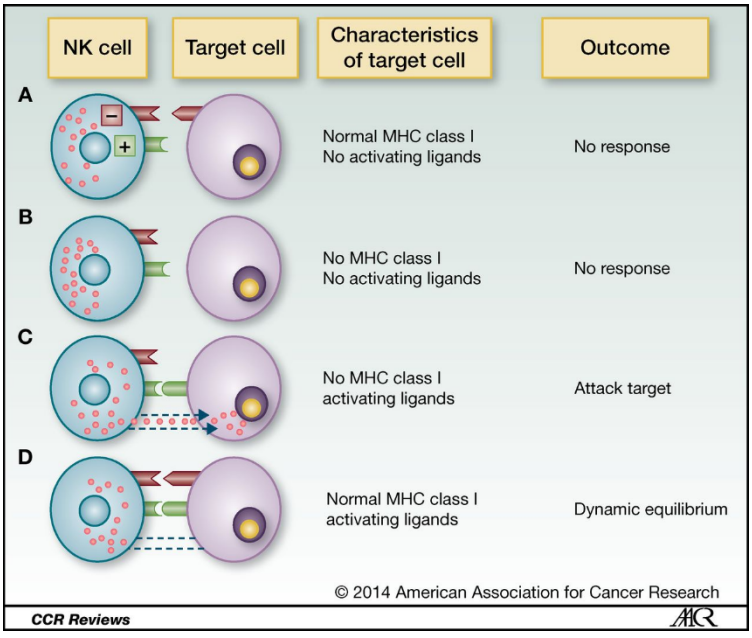

Figure 15.7: Functioning of NK cells based on characteristics of target cells

Antibody dependent Cellular Toxicity by Killer Cells

Antibody dependent Cellular Cytotoxicity or (ADCC) is a kind of cellular killing which is based on antibody and killer (K) cells. K cells are not morphologically distinct type of cells. These are any cell which can mediate ADCC like NK cell, macrophages & PMNLs. Antibody act as to bring K cell close to target cell leading towards cell to cell interaction. K cells have Fc receptors (FcR) on their surface. For such kind of killing firstly antibodies like IgG which are specific to antigens of target cell bind to target cell. The coated target cell binds to Fc receptor for IgG present on NK, LAK cells and macrophages. Moreover, the killing of the target cell is being done through perforin/graenzyme mediated mechanisms (Fig.15.8).

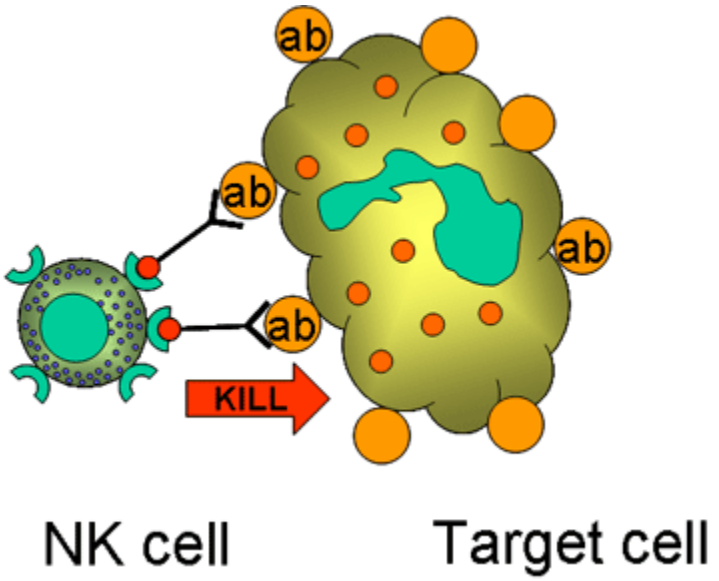

Figure 15.8: The process of Antibdy dependent cellular cytotoxicity (ADCC)

Furthermore, ADCC can also be mediated through IgE. For this the mast cells and eosinophils are involved which have Fc receptors for IgE. Firstly, IgE coat the parasite surface which then bring coated parasitic cell to mast cells. Mast cells or eosinophils get degranulated for killing of parasite (Fig.15.9)

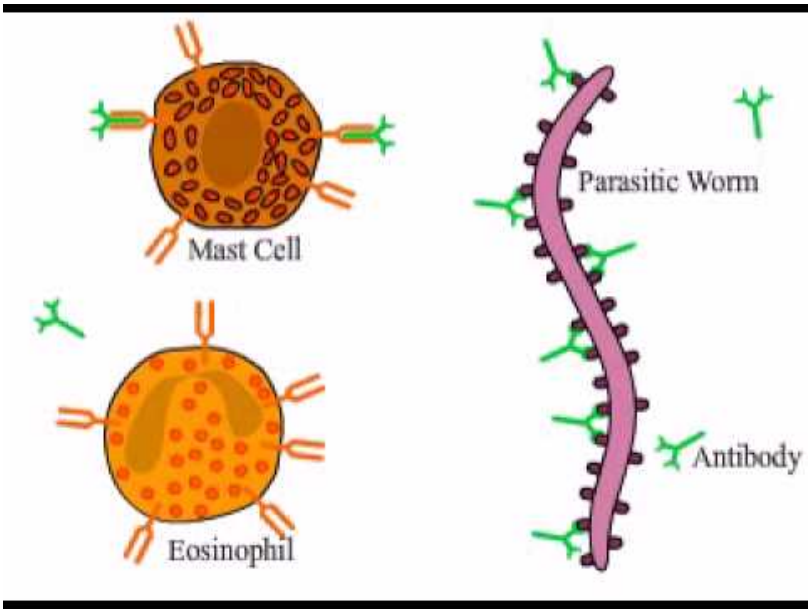

Figure 15.9: IgE mediated killing of parasite via ADCC

Lymphokine Activated Killer Cells in Cell Mediated Immunity

Lymphokine activated killer (LAK) cell are NK cells which are differentiated by continuous exposure to IL-2 and interferon (Fig.15.10). Upon activation, LAK cells kill the transformed and malignant cells. This form killing process is selective for sparing normal cells but need cell to cell interaction

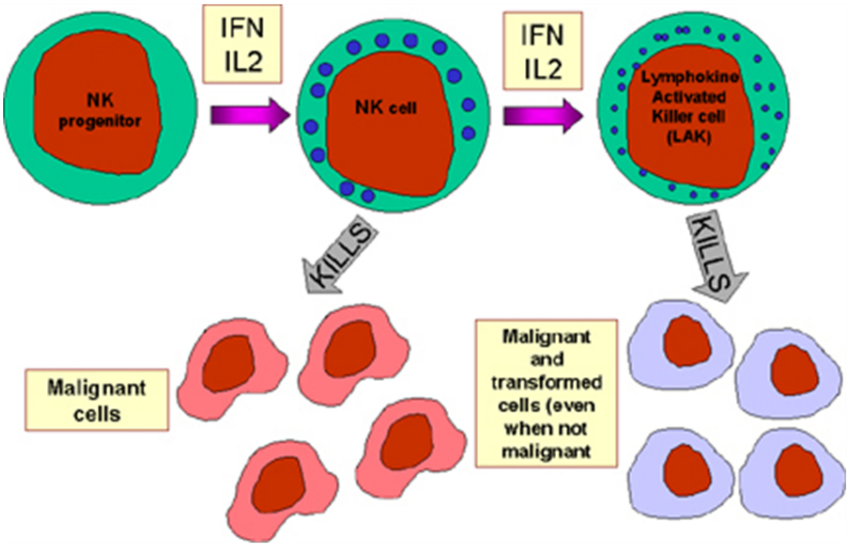

Figure 15.10: Activation of LAK cells

The killing is executed as tumor cells lack class I MHC, LAK cells are specific for tumor cells. LAK cells respond to those tumor cells which are resistant to NK cells killing. LAK cell therapy is used for treating various tumors.

Chapter # 16: Leucocytes Migration and Inflammation

Inflammation vs Infection

Inflammation

It is the body's attempt in order to protect from harmful stimulus. It is one of the complex tissue response which is in fact the part of host immune system. Inflammation is exhibited in a series of events which is also termed as inflammatory response. This is for wound healing as well as for clearance of infections. Inflammation can be beneficial for host in order to remold or restore the damaged tissue. Inflammatory response is exhibited in the form of undesirable effects which are in the form of following signs of inflammation (Fig.16.1)

1. Dolor (Pain)
2. Calor (Heat)
3. Rubor (Redness)
4. Tumor (Swelling)
5. Functio laesa (Loss of function)

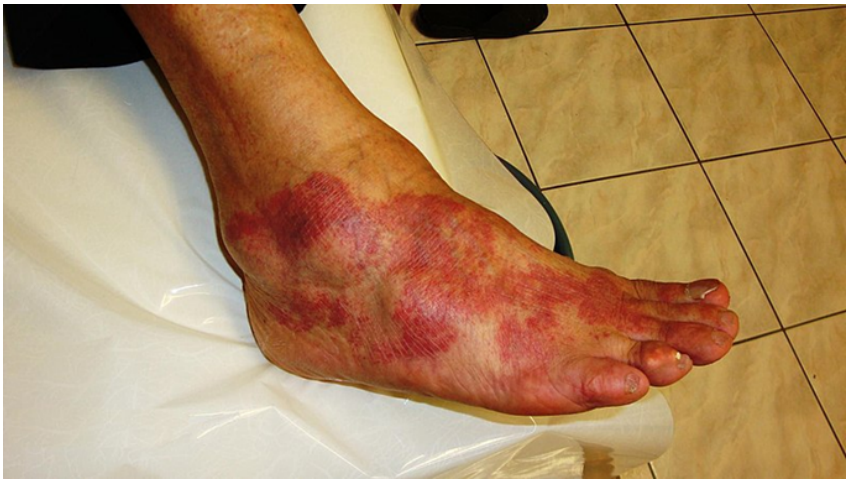

Figure 16.1: Signs of Inflammation

Inflammation is indicated with a suffix "itis" for example appendicitis is the inflammation of appendix.

Outcomes of Inflammation

In response of inflammatory process, following outcomes occur

- ✓ There is restoration of normal tissue
- ✓ Large amount of tissue is destroyed which is called as fibrosis
- ✓ There is also pus formation which is also termed as abscess formation

- ✓ Inflammation can be prolonged for longer time points which is also called as chronic inflammation

Infection

Infections is not synonym to inflammation. It is the invasion of host tissue by pathogen which is the disease causing microbes for example bacteria, viruses, parasites, and fungi etc. It is also termed as infectious disease which is transmittable or communicable. Infection should comply the following Koch's postulates

I. Infectious agent is present in patients suffering from infection however, absent in healthy individuals
II. Infectious agent can grow as pure culture

Infection can be f the following nature based on its outbreak

a) Epidemic: which is the sudden outbreak of infection

b) Endemic: which is the constant occurrence of a particular infection in a community or population

c) Pandemic: it is the global occurrence of an infection

Infections also describe the action of body after invasion in the form of inflammatory response. Infectious diseases can be various kinds according to anatomical locations like

- Respiratory tract infections
- Gastrointestinal (GIT) infections
- Skin infections
- Genital tract infections

Role of Phagocytes in Inflammation

The phagocytes are inflammatory cells in nature like macrophages and neutrophils. They have the ability of recognition of infectious agent via receptors on them which are termed as pattern recognition receptors (PRRs). Followings are the different kinds of PRRs

- Toll like receptors (TLRs)
- Fc receptors
- Complement receptors
- Scavenger receptors

PRRs recognize various molecular patterns on the surface of pathogens which are called as pattern associated molecular patterns (PAMPs). These are in the following forms

- Lipopolysacchrides (LPS)

These are recognized by TLRs

- Flagellins

These are also recognized by TLRs

- Cell wall component

These are recognized through complement receptor after binding with complement

Phagocytes ingest infectious agent by the process of phagocytosis (Fig.16.2)

Figure 16.2: The process of phagocytosis of bacteria by phagocytes

Neutrophils ingest the pathogen first and then kill them intra-cellularly. During inflammation there is collateral tissue damage. Macrophages also have the ability to ingest and kill the infectious agent. Macrophages are Tissue macrophages which generate inflammatory response in tissue. They also contribute in tissue repair and the process of antigen presentation.

Chemotaxsis & Diapedesis of Leucocytes in Inflammation

Leucocytes provide response against infectious agents by

- Chemotaxsis

This is the movement of circulating phagocytes towards the site of infection by various chemicals also called as chemotactic agents for example save our soul (SOS) signals from bacteria like N-formylmethionine containing peptides. The clotting system peptides and complement proteins also serve as chemotactic agents. For macrophages, cytokines are considered as good chemotactic agents (Fig. 16.3).

- Adherence of phagocytes on endothelial surface

SOS signals also activate endothelial cells of blood vessels. As a result, there is an increased expression of adhesion molecules like ICAM-1 and selectins. This increased expression facilitate the binding of phagocyte to adhesion molecules (Fig. 16.3).

- Rolling of phagocytes

The release of vasodilators cause the loosening of the gap between endothelial cells. After loosening of endothelial cells there is squeezing of phagocytes occur between endothelial cells (Fig. 16.3).

- Diapedesis

This is the process of crossing the endothelial barrier after squeezing & movement extravascularly towards the site of infection. Infected tissues sites cause the attraction of phagocytes called as chemotaxsis and the activation of phagocytes (Fig. 16.3).

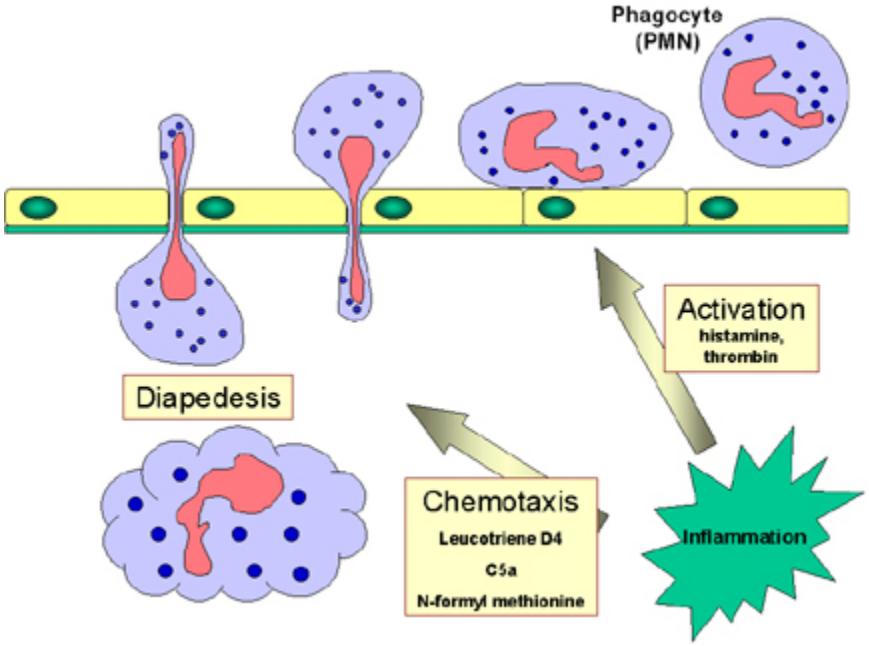

Figure 16.3: The events for the process of phagocytosis

Opsonization of Bacteria

The enhancement of phagocytic process is called as opsonization. In this process, the pathogen is marked for ingestion by phagocytes. The marking of bacteria by a molecules which enhance the phagocytosis is termed as opsonins like immunoglobulin (IgG & IgM) and complement proteins. In this process of opsonization, the opsonins bind with bacterial surface. However, under normal inflammatory circumstances, PAMPs of bacteria bind with PRRs of phagocytes which mediate neutrophil or macrophage phagocytosis. PRRs also cause the expression of opsonin receptor on phagocytes like Fc receptors and complement receptors

There is interaction of opsonin receptor of phagocytes with opsonin on bacterial surface which cause the binding. After binding, the bacteria cell is internalized in the vesicle called as phagosome where killing of bacteria occur through intracellular killing.

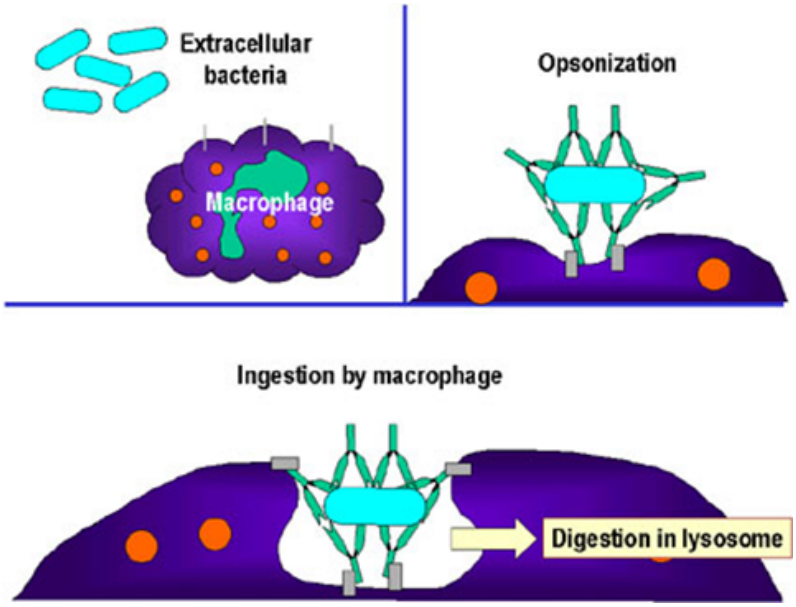

Figure 16.4: Opsonization of bacteria by opsonins

Intracellular Killing by Leucocytes during Inflammation

During inflammation, the ingested bacteria are killed by phagocytes. After phagocytosis, the ingested bacteria is being killed by a process called as intracellular killing

There are following two ways of intracellular killing

1) Oxygen independent
2) Oxygen dependent

1) Oxygen Independent

In this process of intracellular killing of bacteria, there is no need of oxygen. The granules and vesicles of phagocytes secrete various enzymes which are hydrolytic proteins .Those proteins are bacteriocidal in nature according to their modes of action (Table 16.1)

Table 16.1: List of hydrolytic proteins with their functions

Effector Molecule	Function
Cationic proteins (including cathepsin)	Damage to microbial membranes
Lysozyme	Splits mucopeptide in bacterial cell wall

Lactoferrin	Deprives proliferating bacteria of iron
Proteolytic and hydrolytic enzymes	Digestion of killed organisms

2) Oxygen Dependent

There is requirement of oxygen for such kind of intracellular killing of bacteria. It is also termed as “Respiratory Burst” as requirement of glucose and oxygen get increased after phagocytosis. As a result, oxygen containing bacteriocidal radicals are produced. This process can be of two types

Oxygen dependent Myeloperoxidase (MPO) dependent

MPO from granules of phagocytes are involved in this process as a result halide ions (OCl^-) are formed as a result of the following reactions (Fig. 16.5). These are bacteriocidal in nature

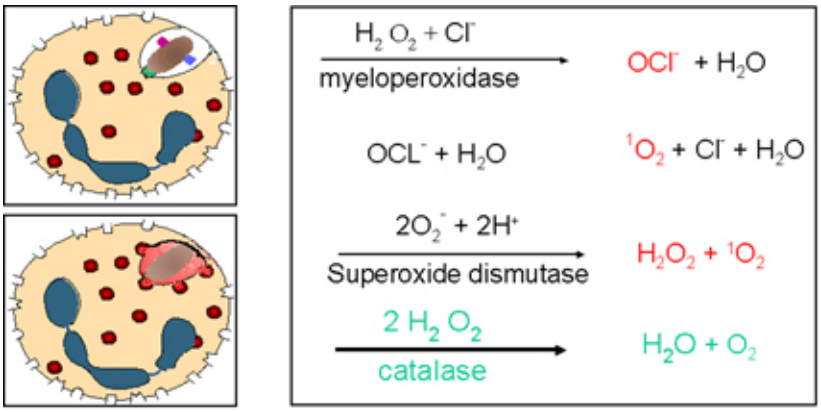

Figure 16.5: Oxygen dependent Myeloperoxidase (MPO) dependent

Oxygen Dependent Myeloperoxidase (MPO) independent

It is myeloperoxidase independent in which there is involvement of Hexose monophosphate shunt. The reactive oxygen species (ROS) for example superoxide radicals, hydrogen peroxide and singlet oxygen as a result of following reactions (Fig.16.6)

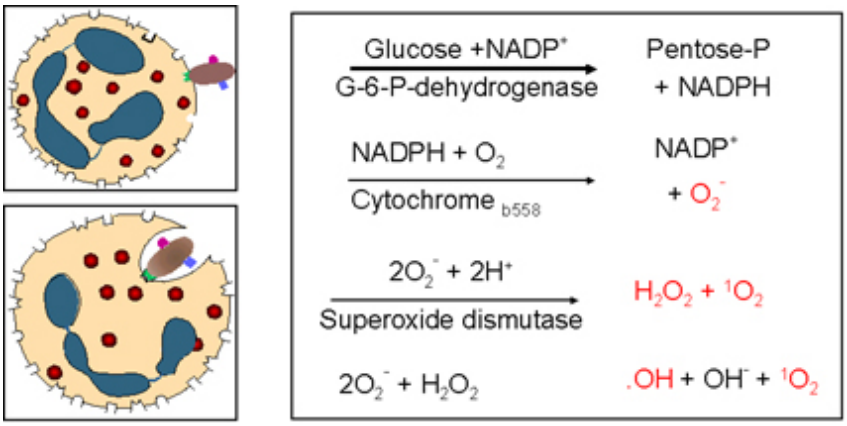

Figure 16.6: The process of Oxygen dependent myeloperoxidase (MPO) independent

Role of Cytokines in Inflammation

Cytokines are considered as mediators of inflammation. A complex variety of mediators are involved in acute inflammatory response. Some of cytokines directly act on smooth muscles wall surrounding the arterioles in order to alter the blood flow. On the other hand, some act on venules to cause contraction of endothelial cells for the opening of junctions as a result there is migration of leucocytes from bloodstream.

Moreover, cytokines also up regulate the expression of adherence molecules on endothelial cells. Likewise, the adherence molecules on the surface of leucocytes are also up regulated. Cytokines also lead the leucocytes towards the inflamed site through chemotaxsis.

For inflammation cytokines can be grouped into two groups

- Pro-Inflammatory cytokines

These are involved in up regulation of inflammatory process. These are predominantly produced from activated macrophages and Th cells like IL-1β, IL-6 & TNF-α, IL-12 and INF-γ. These have role in mediating innate immune response. Furthermore, various inflammatory diseases like atherosclerosis, cancer and depression also occur due to these cytokines.

- Anti-Inflammatory cytokines

These cytokines are involved in down regulation of inflammatory process. These also control pro-inflammatory cytokine response. In this category of cytokines IL-4, IL-10, IL-11 and IL-13 are involved. These have pathological role in systemic inflammatory states. A balance between Pro & anti-inflammatory cytokines is required.

Inflammosomes in Inflammation

A multi-protein oligomer which is responsible for activation of inflammatory responses is called as inflammosomes. These promote the maturation and secretion of pro-inflammatory cytokines like IL-1β and IL-18. Inflammosomes are expressed in myeloid cells which is the important component of innate immune system. There is secretion of cytokines upon inflammasomes activation cells leading towards death process. The kind of cell death induced after inflammosomes

is called as pyroptosis. It mediates the inflammatory process by killing infected cells in physiological state while pathologically increased pyroptosis leads towards inflammatory diseases like Deregulated Inflammosomes activity leads to pathological states like autoimmune disorders for eaxmple rheumatoid arthritis, inflammatory bowel disease, Inflammatory disorder, metabolic disorders and cancer. The germline encoded PRRs of immune cells like TLRs, Nod like receptors (NLRs) recognize PAMPs on pathogens lead the assembling of inflammosomes. As a results, caspase-1 get matured which further cleaves the pro or inactive forms of pro-inflammatory cytokines like IL-1β & IL-18 into mature ones (Fig.16.7)

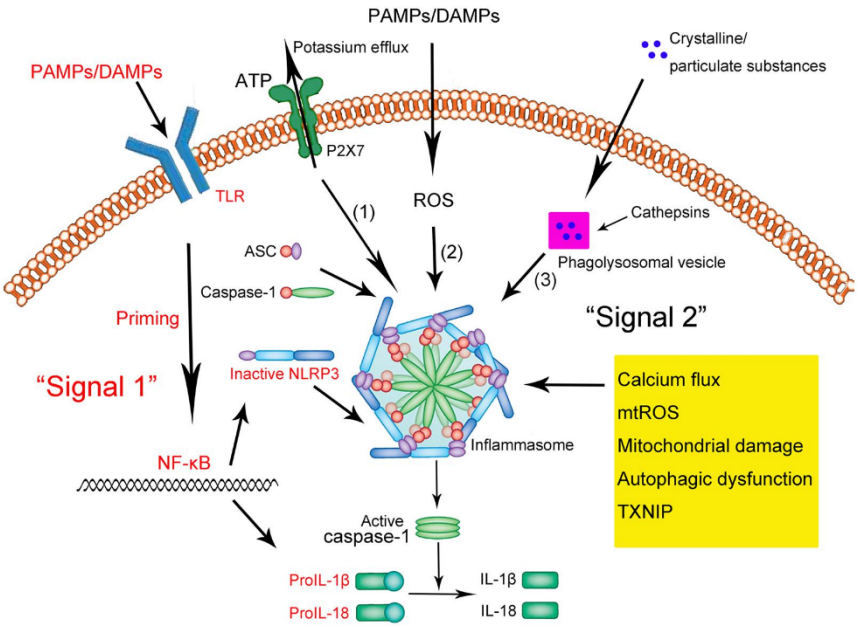

Figure 16.7: Activation of inflammosomes by pathogens

Inflammosomes are activated either by microbial attack or sterile attack (Fig. 16.8)

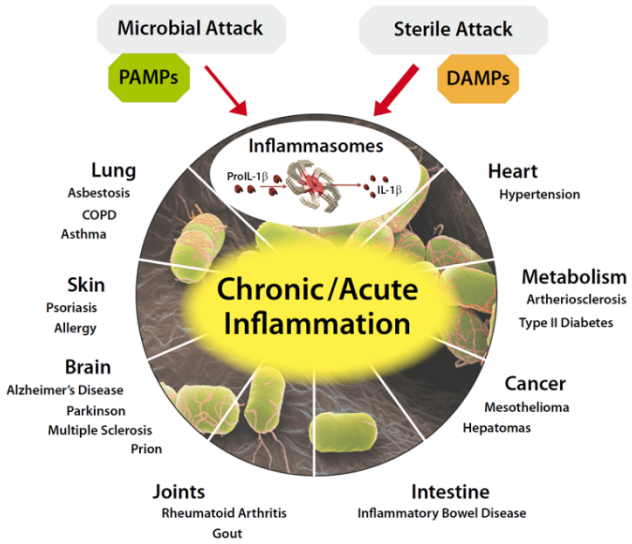

Figure 16.8: Stimulus for inflammosome activation in various tissues of host

Acute vs Chronic Inflammation

Acute Inflammation

This form of inflammation is a short term process occurring in response to tissue injury. It appears in minutes to hours. It is also involved purely in physical damage and then healing process. There is also activation of immune system in this sort of inflammation.

Three main processes involved in acute inflammation which are as follows

1) Increased blood flow
2) Increased permeability
3) Migration of Leucocytes

Increased blood flow

It happens due to dilatation of blood vessels in the effected tissue. Redness appears in the affected area with increase in temperature of effected area (Fig.16.8).

Increased permeability

There is also increase in vascular permeability which causes the leakage of plasma into tissue interstetium. As a result, there is accumulation of fluid in tissue leading towards edema which is the swelling of the affected area due to additional accumulation of fluid into interstitial space of the region. The endothelial cells also separate due to increased vascular permeability (Fig.16.9).

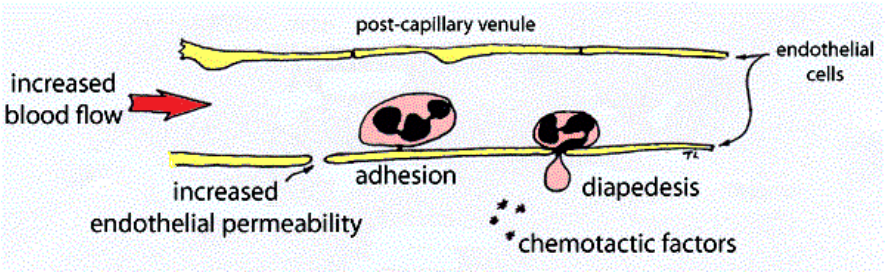

Figure 16.9: The process of acute inflammation

Migration of Leucocytes

The increased movement of leucocytes towards the site of infection is called as chemotaxsis. Cytokines also play their role in migration of leucocytes from circulation to site of infection. Moreover, the expression of adhesion molecules on vessels and leucocytes occur (Fig.16.8).

Chronic Inflammation

This form of inflammation persists for long time. It is a slow process occurring in response to tissue injury. This process lasts for months to even years. The hallmark of chronic inflammation is macrophages and lymphocytes as neutrophils are short lived so those are replaced by lymphocytes and macrophages.

There are two types of chronic inflammation

1) Non-Specific proliferative inflammation
2) Granulamatous inflammation

Non-Specific Proliferative Inflammation

It is characterized by the presence of non-specific granulation tissue. There is also infiltration of mononuclear cells like Lymphocytes, monocytes and plasma cells. In this process, proliferation of fibroblasts also occur which is called as fibrosis. Moreover, the proliferation of connective tissues and vessels occur for example in case of lung abscess (fig. 16.10).

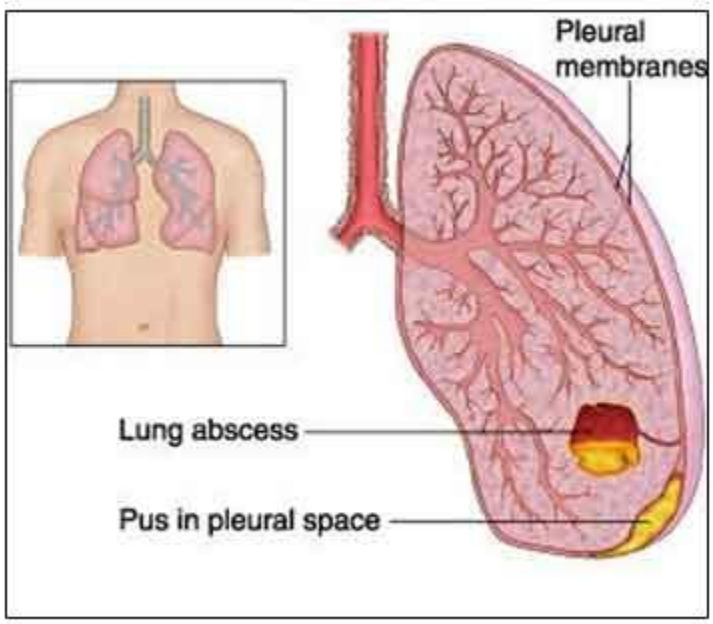

Figure 16.10: Non-Specific Proliferative Inflammation of lung

Granulamatous Inflammation

It is characterized by the presence of distinct nodular tissue called as granuloma which is the aggregation of activated macrophages with their derived cells called epitheloid cells. Infectious granuloma occur due to infections for example tuberculosis and leprosy (Fig. 16.11).

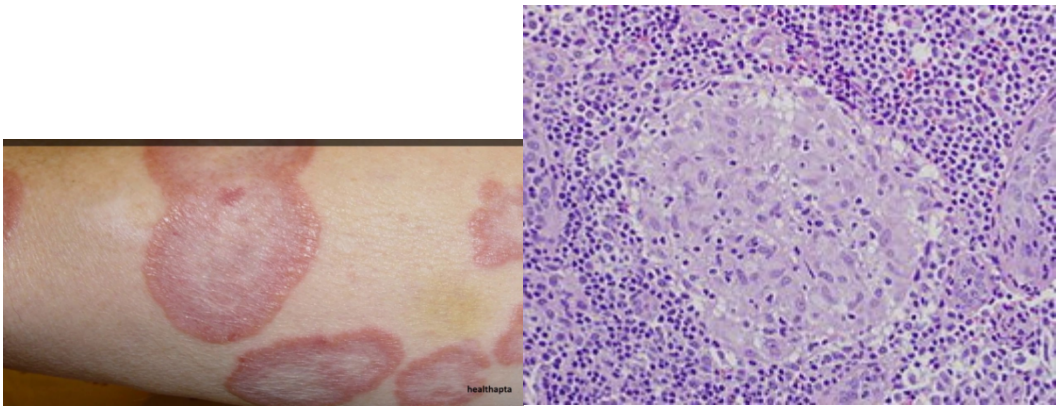

Figure 16.11: The Granulamatous Inflammation

Adverse Effects of Inflammation

Inflammation is associated with general flu like symptoms including

- Fever
- Chills
- Fatigue/Lethargy/Loss of energy
- Headaches
- Loss of appetite
- Body pains
- Muscle stiffness

Chapter # 17: Vaccines

Introduction to Vaccination (Immunization)

The ways of providing specific protection against many common and damaging pathogens after stimulation of organism's or individual's immune system is called as vaccination or immunization. For vaccination, there is stimulation of humoral immunity for production of antibodies against pathogen. These antibodies has the ability to neutralize specific antigens of pathogens. This is depending upon the nature of pathogenesis and the site of infection by pathogen. These antibodies are also called as Antitoxin which neutralize the toxin production from pathogens. Those toxins get masked by antitoxins and not able to bind its specific receptor on target cell. Moreover, antibodies against pathogens also bind complement and lead to lysis of pathogen which is also termed as complement mediated intracellular killing of pathogen. However, intracellular pathogens cannot be neutralized by antibodies for which cell mediated immunity is activated by providing specific T-cells to host. Immunization against intracellular pathogen is provided through cell-mediated immunity

Types of Vaccination

There are two modes of providing specific protection against mostly common & damaging pathogens. These modes of immunization can be natural or artificial in their nature. Following are the types of immunization

1) Active Immunization
2) Passive Immunization

- Active Immunization

It is the induction of immunity after exposure of host to antigen. In this case, antigenic exposure is mandatory. Active immunization can occur naturally by exposing to microbe or other antigen when there is no prior exposure before the entry of antigen. Similarly there is also no pre made antibodies in the host's serum. In this form of immunization, immune system develops antibodies against the microbe after its exposure. This process of antibodies generation is considered slow. Moreover a memory response is also generated in which antibodies remained in use for longer time. This form of immunization can be artificial in nature which is achieved by either injection of microbe before natural exposure or treated microbes or toxins.

- Passive Immunization

It is the induction of active humoral immunity. In this kind of immunization, antibodies are mandatory. Passive immunization can occur naturally by transfer of maternal antibodies to fetus through placenta. However, the artificial induction can be done by injecting gamma globulins from other individuals or animals to host.

Natural vs Artificial Immunization

Natural active immunization

Natural immunization is acquired immunization which is achieved by natural exposure to antigen or acquiring infection by an infectious agent or microorganism e.g measles virus. This is also termed as natural active immunization. This form of natural exposure to antigen stimulate the humoral immunity as a result specific antibodies are generated against exposed microbe. Moreover, there is also activation of cell mediated immunity for intracellular pathogen.

Artificial Active Immunization

Artificial immunization is the kind of immunization which is acquired by artificial exposure to antigen or microorganism which is administrated to body in less virulent or attenuated form like heat killed forms of microbes. This artificial exposure with antigen stimulate the humoral immunity in the form antibodies generation. Moreover, artificial activation of cell mediated immunity for intracellular pathogen for example BCG for *Mycobacterium tuberculosis.* Artificial immunization: acquired immunization by artificial exposure. This artificial exposure to antigen or microbe which is by administration of less virulent (attenuated), heat killed microbes to organism. Artificial exposure to antigen stimulate the humoral immunity for antibodies

Natural Passive Immunization

On the other hand, natural immunization can be acquired immunization which can be obtained by isolating gamma globulins (antibodies) from other's individual. This is called as natural passive immunization. Furthermore, the transfer of gamma globulins (IgG) from mother's blood to fetus via placenta. The placental cells contains FcRγ for IgG transfer to fetal circulation. Similarly, the natural transfer of IgA can be occur through breast milk to new-born baby.

Artificial Passive immunization

This form of artificial immunization is the acquired immunization which is achieved by administrating gamma globulins or antibodies from others individual's or immune animal's serum for example antibodies against tetanus

Artificial Passive Immunization

This form immunization is done by transferring or injecting of specific gamma-globulins from other individual or from other immune animals .Artificial passive immunity is used against various following acute bacterial infections

- Diphtheria
- Tetanus

Moreover, artificial passive immunity is also used against various poisoning conditions like

- Insects or sting biting e.g immunization against various strains of Malaria producing Plasmodium

- Reptiles e.g anti-venom administration in case of snake biting
- Food poisoning (Botulism)

Artificial passive immunity is also used as prophylactic (preventive) measure against various viral infections like Influenza, Poliomyelitis, Measeles and Rabies.

For artificial passive immunization, antibodies which are used usually from human origin which are also termed as homologous antisera. Homologous antisera has potential risk of transmitting HIV and Hepatitis among individuals. However, these are also raised in other species or animals after immunization called as Heterologous antisera. Heterologous antibodies provide immediate protection but also a source of allergies like serum sickness and anaphylaxis.

Likewise, passive transfer of cell mediated immunity is also used for treating cancer and immunodeficiency

Artificial Active Immunization

This form of immunization is achieved by transferring or injecting live, dead or other components of microbes

Followings are the important vaccines which are used as artificial active immunization

1) Live (attenuated) organisms
2) Killed organisms
3) Sub-unit vaccines

1) Live (attenuated) Vaccines

These vaccines are produced after inactivation of organism by heat for the purpose of loss of pathogen's virulence. These are used against various viral infections like Smallpox, measles, mumps, hepatitis A virus Moreover, these are used as live bacterial vaccine against tuberculosis: for example Bacille Calmette-Guerin vaccine (BCG)

2) Killed Vaccines

Such vaccines are produced after killing of organism by heat, chemical treatment or UV irradiations. These are used against various viral infections like Polio, rabies & influenza. Also, most bacterial vaccines are also killed like typhoid fever, cholera, plague and pertussis.

3) Subunit Vaccines

These vaccines consist of various components of microbes like polysaccharides from capsule and surface proteins. The polysaccharides are T-independent antigens while proteins areT-dependent antigens. These vaccines are used for reducing the toxicity for example Pneumococcus vaccines.

Novel Vaccines

There are also various novel ways of designing vaccines in order to reduce in toxicity of vaccines. These have the potential to provoke both humoral & cell-mediated immunity. These are mainly used in experimentally for research purpose. However, these would be available for clinical use in future

1) DNA vaccines

These are cloned viral peptides which are transfected into host cell. These vaccines can generate both humoral and cell-mediated response like live attenuated vaccines. Similarly, Anti HIV-DNA vaccines are used in experimental stage which have very low efficacy so far.

2) Immunodominant Peptides

These are simple and easy to prepare. Moreover, these peptides are incorporated with MHC to induce both humoral & cell-mediated responses.

3) Anti-Idiotype antibodies

These antibodies are used against polysaccharide antibodies. These have long lasting immune response with memory

Role of Adjuvant in Vaccination

These are the substances which increase the antigenicity of weak antigens. These can be of two forms

1) Chemicals
2) Biological

1) Chemical

These are basically aluminum salt or Alum in its structure. This is the only chemical suitable for human use. These are used in DTP (Diphtheria, Tetanus & Pertussis) vaccine. Alum causes slow release of antigen in order to increase in TLR interaction, activation of mononuclear phagocytes. As a result, increased cytokine secretion is induced for appropriate immune response generation

2) Biological adjuvants

Various bacteria are used as biological adjuvants. These are basically the bacterial products

3) *B. pertussis* used as adjuvant
4) *M. bovis* (BCG and others)

These are used in combination with oil and detergent for better activation of mononuclear phagocytes and cytokine secretion.

Adverse Effects of Vaccination

As live microbe are used for vaccination so these have some adverse effects on host. These effects can be sever, rare, mild and moderate in its nature. For example, active immunization causes fever, malaise and discomfort. Followings are the adverse effect in respective kind of vaccination

- Joint pains (Arthritis): Rubella vaccination
- Can be fetal: in case of Pertussis
- Neurological disorders: Influenza vaccination
- DPT vaccination has local effects like Redness, swelling and pain
- Mild/Moderate effects: fever, drowsiness. Vomiting and anorexia
- Severe effects: persistent crying, fever, convulsions, collapse, acute encephalopathy, permanent neurological deficit due to pertussis components

Chapter # 18: Tolerance, Diseases of Immune System-Autoimmunity

Introduction to Tolerance

The specific immunological unresponsiveness or non-reactivity to an antigen resulting from a previous exposure to same antigen is called as immunological tolerance. This kind of unresponsiveness is mostly against self-antigens. This form of non-reactivity can be induced for non-self (foreign) antigen. The antigens which induce tolerance are called as tolerogen. Physiologically, there is no immune response against self-antigens which is also termed as self-tolerance. On the other hand, if immune response is generated against self-antigens is called as autoimmunity (Pathology). Immune system recognize self-antigens and mount strong immune response. The discrimination between self and non-self-antigens is achieved by a process called as self MHC recognition. The tolerance can be generated against non-self-antigens. However, immune response should be against non-self-antigens. The modification of antigens leads towards immune tolerance. Most bacteria and viruses develop tolerance which is the way to exploit or evading host immune system for example in Lepromatus type of leprosy there is tolerance against *M. leprae* as a result no immune response is generated. Tolerance to tissue and cell antigens can be induced artificially for example by injecting hemopoietic stem cells in neonatal or severely immunocompromised animals. Likewise, the generation of chimeras animals through transferring of allogeneic primary lymphoid tissues at early developmental life of animal. These are the ways of inducing tolerance against allogeneic tissues.

Immunological features of Tolerance

Tolerance is a phenomenon of specific immune unresponsiveness which is different from non-specific immunosuppression & immunodeficiency. This is an active antigen dependent process in response to antigen. Like specific immune response, tolerance is also specific and has immunological memory. Tolerance to T-cells is longer as compare to B-cells. Similarly, the induction of tolerance to T-cells is easier and require small amount of antigen (Tolerogen). However, for B-cell tolerance requires larger amount of tolerogen. For the maintenance of tolerance the persistence of antigen in the body is necessary. The lack of persistence leads to breach of tolerance. Immuno-Tolerance can be break in following two ways

1) Naturally

In case of autoimmune disorders e.g Rheumatoid Arthritis, SLE etc.

2) Artificially

Exposure to immunosuppressive drugs or X-ray irradiation e.g in case of experimental animals for bone marrow transplantation

Mechanisms of Tolerance Induction

There are following two forms of immunological tolerance

1) Central Tolerance
2) Peripheral Tolerance

Central Tolerance

It occurs in primary lymphoid organs e.g bone marrow & thymus

Peripheral tolerance

It occurs at secondary lymphoid organs e.g spleen, lymph nodes, tonsils etc

Central Tolerance

In this kind of tolerance the mechanism involved is Clonal deletion. It is also called as negative selection by which Self-reactive B & T-lymphocytes are deleted in bone marrow & thymus respectively. The Clones of auto reactive cells are deleted by programmed cell death is called as apoptosis. B-cells during development in bone marrow encounter with self-soluble or cell surface associated antigen. Such kind of self-reactive B-cells are deleted from bone marrow through negative selection via apoptosis process (Fig. 18.1)

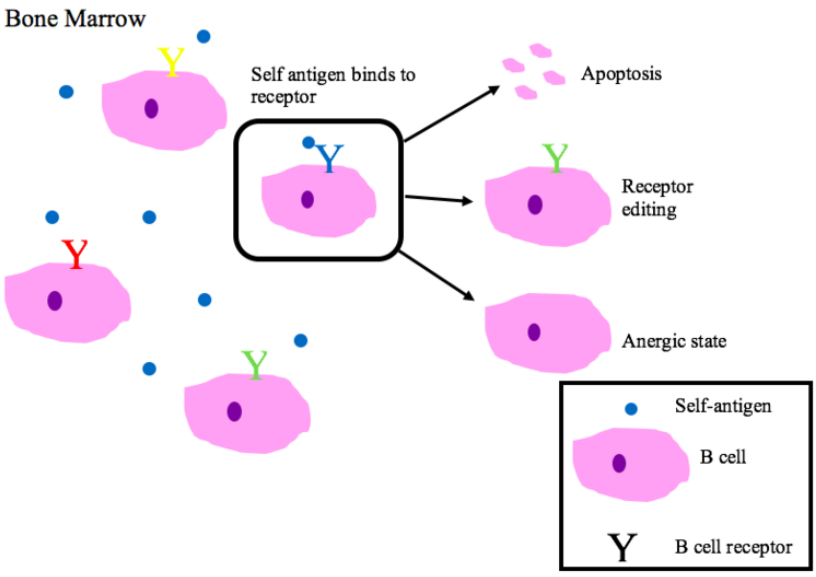

Figure 18.1: The process of central tolerance for B-lymphocytes in bone marrow

Similarly, T-cells develop in thymus after expressing CD8 & CD4 molecules on its surface. These cells also acquire αβ TCR. The positive selection of T-cells occur after interacting with self MHC molecule. However, self-reactive T-cells with either class I or II are negatively selected. In this way auto-reactive T-cells are controlled to escape from central lymphoid tissues

Peripheral Tolerance

In central tolerance, the clonal deletion is considered as one of the major mechanism for controlling auto reactive T-cells. Sometimes it is not considered as fool proof system as B & T cells fail to

undergo deletion and escape from central tolerance and auto-reactive immune cells reach peripheral lymphoid organs. In this case specific un-responsiveness occur in peripheral lymphoid tissues which is called as peripheral tolerance. Followings are the important mechanisms of peripheral tolerance

Activation induced cell death

This is the process by which death of auto reactive T-cell occur upon their activation. Various secretory cytokines from activated T-cells cause the expression of molecules like Fas ligand on T-cells. The self-reactive T-cells in periphery are deleted by the process of apoptosis after engagement of Fas Ligand (FasL) with Fas

Clonal Anergy

Exposure of T-cells to self-antigens lead to functional inactivation which is termed as anergy. The loss of co-stimulation upon interacting with self-antigen on antigen presenting cells (APC). There is no interaction of C28 on T-cells with CD80 (B7-1) or CD86 (B7-2) on APC which lead towards functional unresponsiveness of auto-reactive T-cells (Fig. 18.2)

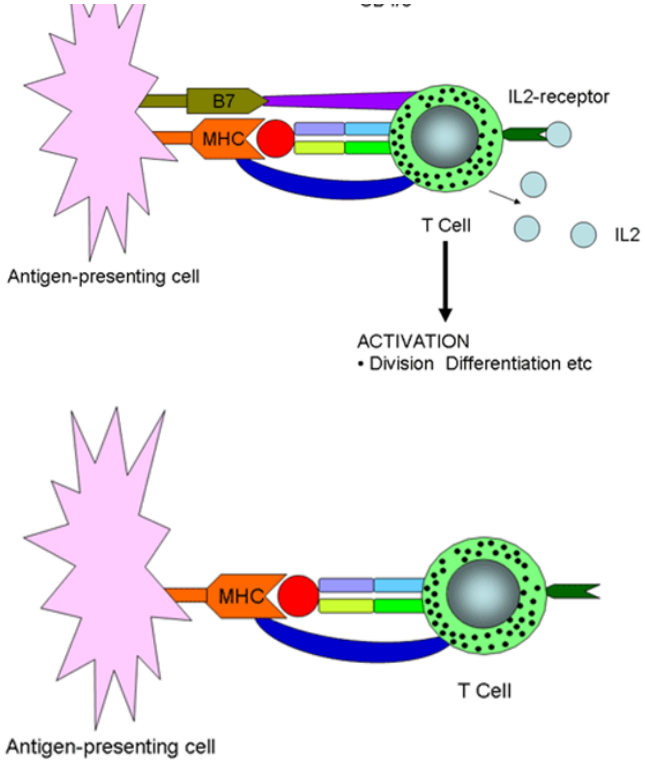

Figure 18.2: The colnal anergy of auto-reactive T-cells

Clonal Ignorance

In this mechanism, there is lack of interaction of T-cells with appropriate antigen. After maturation in thymus, auto reactive T-cells reach the periphery where there is sequestration of these self-

reactive T-cells in inaccessible tissues for ignorance. The death of such clones happen due to continuous ignorance termed as clonal ignorance.

By Regulatory T-cells (Suppressor T-cells)

These are T-lymphocytes which are CD4 and CD25 +ve. These cells cause the secretion of immunosuppressive cytokines like TGF-β & IL-10. These cytokines cause the inactivation or suppression of auto-reactive cells

Tolerance to Tissues & Cells

This specific unresponsiveness against various cells & tissues cause inhibition of immune response against antigens of cells and tissues. These antigens are foreign to immune system which are also called allogeneic antigens. It occurs in tissue graft like allogenic graft. In this case tolerance is induced against tissues and cells in the following ways

By injecting hematopoietic stem cells at neonatal stage as during early development stage there is no development of fully matured lymphoid tissues.

By immunosuppression e.g lethal irradiation for killing of host's own stem cells in primary lymphoid organs.

By using immunosuppressive drugs in order to inactivate immune cells of host

By Chimeras

These are animals with hybrid nature of immune cells with host's own cells and donor's cells. In this case both transplantation of donor's bone marrow and thymus in early age or by immunosuppression which leads towards functional inactivation of host immune cells.

Introduction to Autoimmunity

All mechanisms which are responsible for breakdown of self-tolerance against self-antigens are called as autoimmunity or auto immune reactions. In this condition, there is generation of immune responses against components of self-tissues. These are harmful or aberrant immune responses against self-tissues. Various products of immune system damage the host tissues like both antibodies and T-cells are involved in autoimmunity. Moreover, genetic predisposition like certain genes of immunoglobulins, TCR & MHC are also associated with various autoimmune diseases. Various environmental factors are also responsible for autoimmunity e.g drugs & infections.

Etiology of Autoimmunity

The exact mechanism for autoimmunity is still unknown. However, various theories have been proposed for better understanding the mechanism of autoimmunity. These are as follows

- Sequestered antigens
- Escape of auto reactive cellular clones
- Lack of regulatory T-cells

- Cross reactive antigens

Sequestered antigens

In this mechanism, lymphoid (immune) cells may not be exposed to certain self-antigens during development and differentiation. Certain self-antigens are confined to specialized organs for example testis, brain & eyes etc. The release of such antigens from tissues due to any injury or accident initiate various autoimmune diseases of these organs.

Escape of auto reactive clones

The loss of central tolerance leads auto-reactive T-cells to escape from thymus to periphery. As not all of self-antigens are presented to T-cells in thymus for clonal deletion which make them auto-reactive in peripheral lymphoid tissues. Likewise, auto-reactive B-cells also escape from clonal deletion or negative selection

Lack of regulatory T-cells

There are few regulatory T-cells in autoimmune diseases which leads towards absence of effective Suppressive against T-cells. Furthermore, absence of immunosuppressive cytokines like TGF-β & IL-10 also augment this condition.

Cross Reactive antigens

Antigens on certain pathogens have determinants which can cross react with self-antigens. There is generation of antibodies against those determinants which can also cross react with self-antigens. This happens during Post-streptococcal nephritis & carditis where M-proteins of Streptococcus are involved in this mechanism.

General Classification of Autoimmunity

Autoimmunity is classified based on the type of tissues or organs involved

There are following two categories of autoimmunity

- Organ-specific autoimmunity
- Non-organ Specific autoimmunity

Organ Specific autoimmunity

In this kind of autoimmunity, the immune response is generated against organ associated specific antigens in the form of auto-antibodies. These antibodies are against organ associated antigens which damage the organ. For organ specific autoimmunity, following organs can be target organs with particular auto-immune disease and the antibody involved (Table.18.1)

- Skin
- Thyroid gland
- Muscles

Table 18.1: Various Diseases of Organ Specific Autoimmunity

Disease	Organs	Antibody to
Hashimoto's thyroiditis	Thyroid	Thyroglobulin, thyroid peroxidase (microsomal)
Primary Myxedema	Thyroid	Cytoplasmic TSH receptors
All hemolytic anemia	RBC	RBC antigens
Good Pasteur's Syndrome	Kidney, Lung	Renal & Lung basement membrane
Ulcerative colitis	Colon	Colon Lipopolysaccharide

Non-Organ Specific autoimmunity

In this kind of autoimmunity, the immune response is not against organ associated specific antigens. Similarly, the auto-antibodies which are generated in response to immune response are not against organ associated antigens. This kind auto-immunity effect mainly all those tissues which are present throughout the body like skin, joints, soft tissues etc. That's why these disease are also termed as systemic autoimmune diseases (Table.18.2)

Table 18.2: Various diseases of Non-organ Specific Autoimmunity

Disease	Organs	Antibody to
Rheumatoid Arthritis	Skin, kidney, Joints etc	IgG

Systemic Lupus Erythematous (SLE)	Skin, joints	DNA, RNA, Nucleoproteins
Sjogren's syndrome	Moister-producing glands	Basement membrane
Scleroderma	Skin, blood vessels, muscles & internal organs	DNA, RNA, Nucleoproteins
Sarcodosis	Lung, skin, hear, nervous system	Auto reactive T-cells

Diagnosis of Autoimmune Diseases

The autoimmune diseases are diagnosed based on symptoms and the nature of autoantibodies produced. Autoantibodies can be against two kinds of antigens

1) Self-cell or cell associated antigens
2) Soluble antigens
3) Biochemical test

Autoantibodies against self-cell/Cell associated antigens

These auto-antibodies are detected using tissues section like kidney, skin etc through immunofluorescence technique. These auto-antibodies can produce following two kind of patterns in immunofluorescence based on the kind of auto-immunity

- Linear Pattern (Goodpastuer Syndrome)
- Granular Pattern (Systemic Lupus Erythramatous)

Autoantibodies against Soluble Antigens

Autoantibodies against soluble antigens are detected using patient's serum for auto-antibodies by following techniques

- Agglutination e.g Anti-nuclear antibodies (ANA) for SLE & RA
- Enzyme linked Immunosorbent Assays (ELISA) e.g Rheumatoid factor for Rheumatoid Arthritis

- Radioimmunoassay (RIA) for detection of anti-thyroid antibodies

Biochemical Assays

Following biochemical assays in the serum of autoimmune patients can be performed for their diagnosis of disease

a) For Intrinsic factor (IF) in case of Pernicious anemia

b) Competition for TSH receptors in case of Grave's disease

c) Auto-reactive T-cells can also be detected by flow-cytometry

Treatment of Autoimmune Diseases

Autoimmune diseases are treated with the goal of reducing symptoms or the better control of autoimmune response in order to increase the quality life of patient. Moreover, treatment strategy is for increasing the ability of immune system to fight against infections. Treatment varies based on specific disease and symptoms for reducing inflammatory process & immune response. Followings are the drugs used for treating auto-immune diseases

- Anti-Inflammatory drug therapy

  Corticosteroid is used which particularly inhibit the cellular signaling for production of pro-inflammatory cytokines. Moreover, it relieves from the symptoms of inflammatory process like pain, redness and fever and reduce inflammatory process

- Immunosuppressive drug therapy

Following immune-suppressive drugs can be used for treating auto-immune diseases

i. Cyclosporine

ii. Cyclophosphamide

iii. Azathioprine

These drugs reduce immune response against self-antigens. Also provide relieving from the symptoms of immune response

- Specific approaches

These kind of strategies using specific antibodies against receptor blocking the effects of immune response generated during auto-immunity. Currently, these approaches are used mainly in research. Followings are the antibodies which are used against their corresponding antigens on the surface of immune cells

- Anti-TNFα receptor antibodies
- AntiIL-2 receptor antibodies

- Anti CD4 antibodies
- Anti TCR antibodies
- Anti-Idiotype antibodies against autoantibodies

Chapter # 19: Transplantation Immunology

Immune Response against Transplants

The process of moving cells, tissues and organs from one site to another among individuals is termed as transplantation**.** In this process of transplantation, one person called as donor who donates tissue to other person termed as recipient. Followings are the important kinds of tissue transplantation based on the source of donated tissue

Isograft

This is a form of tissue which is transplanted between individuals of same genetic makeup like identical twins.

Autograft

It may be from the same person tissue to another site within same individual.

Allograft

This form of transplantation which can be from one person to other non-identical person

Xenograft

This kind of transplantation is across the species for example from animal to human (Fig 19.1).

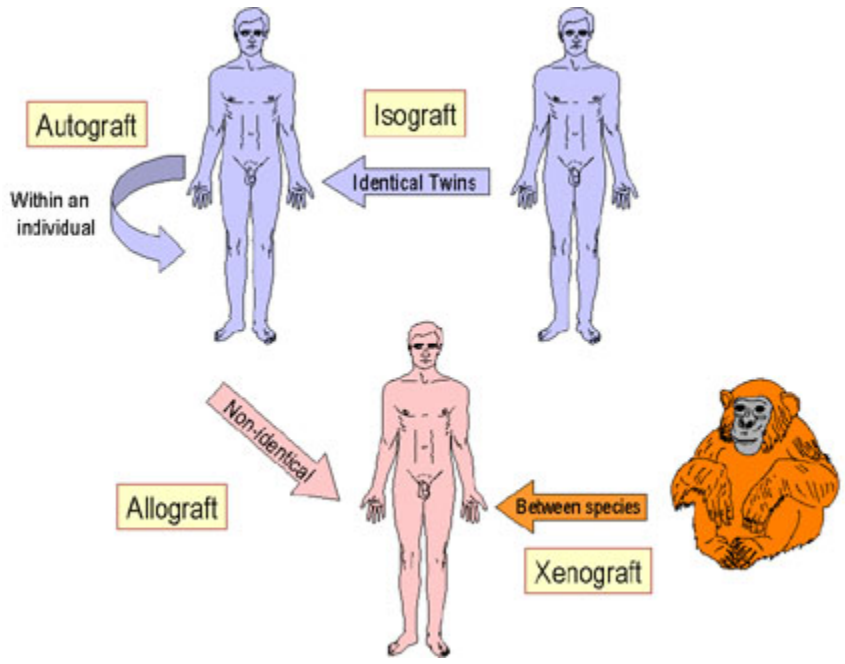

Figure 19.1: Kinds of tissue transplants

Immune system plays an important role in tissue transplantation as it acts as a barrier for transplantation. Immune system identifies tissue transplant as foreign and mount an immune response leading destruction or damage of transplanted tissue. This process through which recipient's immune system activation occur against donor's tissue is termed as transplantation rejection. The immunocompetent host recognize foreign antigens on grafted tissues as a result immune response is generated against grafted tissue is called as host vs graft rejection. Similarly, tissue transplants in immunocompromised individuals lead to immune response by immunocompetent cells in graft is termed as graft vs host rejection. In this case, host antigens serve as foreign. However, if there is no immune response against donated tissue by the recipient's immune system due to similar genetic makeup then it is called as graft acceptance.

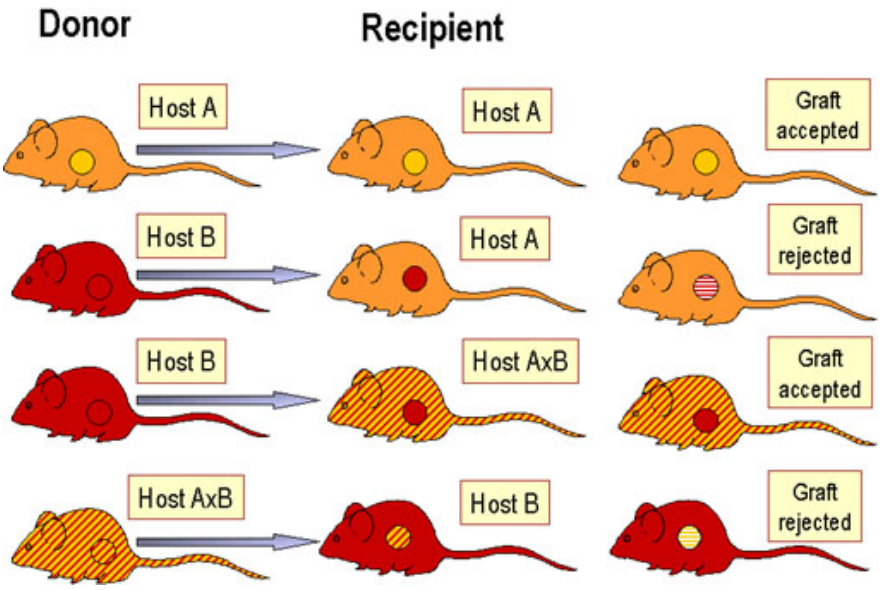

Figure 19.2: Tissue transplantation acceptance and rejection by host

Transplantation Antigens

The transplantation antigens which are also termed as Major Histocompatibility Complex (MHC) or Human Leucocytes Antigens (HLA). The MHC exist as a complex which is encoded by group of genes on a same chromosome called as haplotype. These genes are in fact responsible to influence allograft rejection

There are following two major types of MHC

1) Class I MHC
2) Class II MHC

Class I MHC

These genes are for Human MHC which are located at chromosome no. 6. These contain following three major loci

1) Locus B

2) Locus C

3) Locus A

Each major locus encode for polypeptide for MHC structure. In which there is a α-chain that contains antigenic determinants

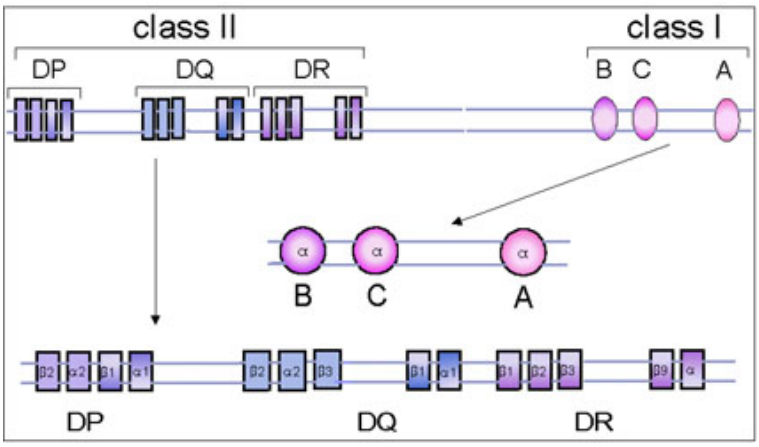

Figure 19.3: Structure of MHC locus

There are many alleles of α-chains which make it polymorphic in nature (Fig.19.2). Another chain of MHC is β2-microglobulin (β-chain) which is encoded by outside of MHC I haplotype. This β-2 chain has role in expression of class I MHC on cell surface (Fig.19.3). If there is any defect in β-2 chain then there is no expression of Class I MHC on cell surface. This condition leads towards the deficiency of cytotoxic T-lymphocytes (CTLs) which have to interact with class I MHC on target cell

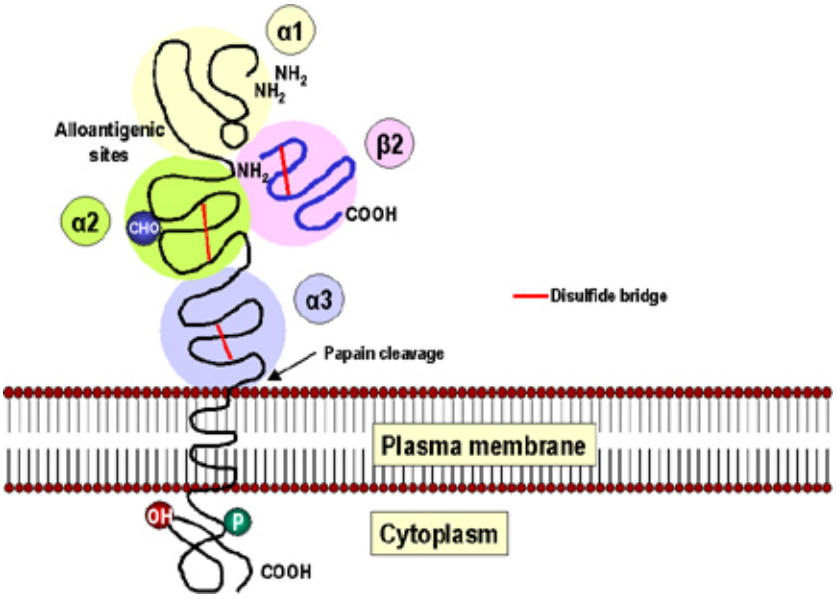

Figure 19.4: Structure of Class I MHC molecule on cell surface

Class II MHC

The class II MHC is encoded by genes for Human MHC which are located at chromosome 6. Structurally, the Class II MHC complex is also composed of three following major loci (Fig. 19.3)

1) DP
2) DQ
3) DR

Each of these loci code foe one alpha & one beta chain. Both of alpha and beta chains associate together to form class II MHC. Likewise to class I, class II antigens are also polymorphic in nature which means these contain many alleles of α-chains. The DR locus contain more than one beta chain genes and possibly four in various combinations. The Class II MHC is expressed on B-lymphocytes and antigen presenting cells (APC).

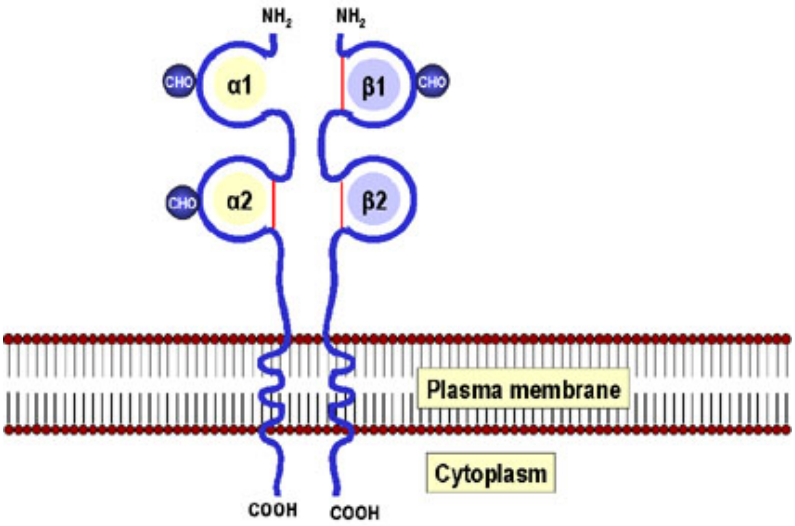

Figure 19.5: Structure of class II molecule on cell surface

Induction of Immune Responses against Transplants

Clinically the significance of MHC is in tissue transplantation. The cells and tissues are transplanted for the treatment of various diseases. The immune response is generated against transplanted tissue which is called as rejection or destruction of transplant. Immune response can be of following two types based on the kind of tissue rejection

1) Host vs Graft Rejection

In this kind of transplant rejection, there are antigens on the surface of graft which are being recognized as foreign by host immune system

2) Graft vs Host Rejection

In this form of transplant rejection, the lymphoid (immune) tissues in graft recognize host immune system as foreign as a result immune response is mounted

Both of these immune responses lead towards graft rejection which is in fact the loss of grafted tissue. The rejection is based on antigenic nature of graft and host immune status. Induction of immune response is mediated by following ways

I. Inflammatory process through inflammatory cells

II. Antibodies against antigens of graft which lead towards complement mediated lysis of graft

III. Antibody mediated Cellular cytotoxicity (ADCC) of graft tissue

IV. T-lymphocytes mediated lysis of graft tissue

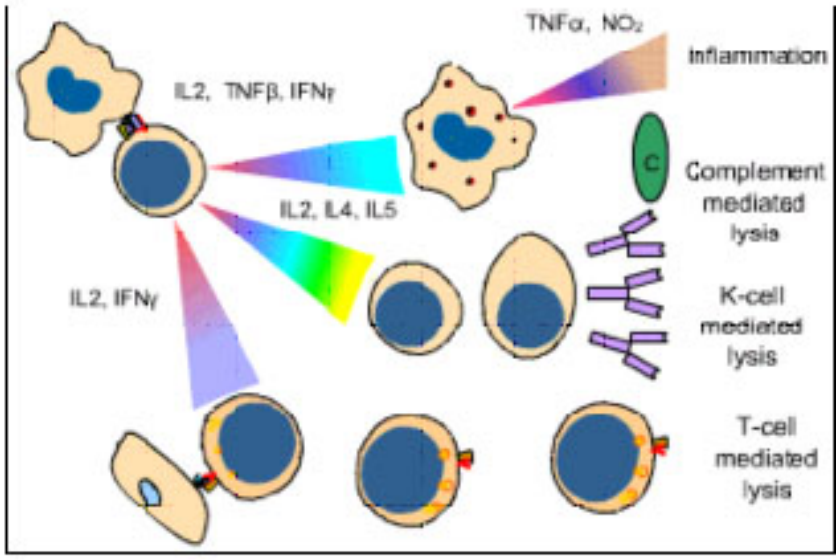

Figure 19.6: Mechanisms of destruction of transplanted tissues

Immune Mechanisms of Graft Rejection

The reaction of host against allo-antigens of graft which is also called as host vs graft (HVG) rejection. This considered as a main obstacle in organ transplantation. The involvement of immune mechanisms in graft rejection is based on

a) Time of rejection

b) Nature of allo-antigens of graft

c) Immune status of host

According to the time of rejection, the graft rejection can be of following types with distinct immune mechanisms

1) Hyper acute rejection

2) Accelerated rejection

3) Acute Rejection
4) Chronic Rejection

Hyper-acute Rejection

This kind of transplant rejection has very quick onset for tissue rejection. It occurs within minutes to hours. The high titer of pre-formed antibodies against antigens of graft are responsible for such form of reactions. Antigen/antibody (immune) reaction occurs on the tissue surface. Afterward the fixation of complement leads towards graft destruction

Accelerated Rejection

This kind of rejection is also called as secondary or 2^{nd} set rejection. It occurs after transplantation of second graft. It is due to sharing of antigenic determinants with the first transplant. Time required for this form of rejection is within 2-5 days. The sensitized T-cells during first graft are responsible for this kind of rejection. Moreover, the Lymphokines and cytotoxic T-lymphocytes (CTLs) augment it.

Acute Rejection

It is also called as primary or 1^{st} set of tissue rejection. It occurs during first graft with allo-antigen on grafted tissue. The time span required for such form of rejection is 1-3 weeks. These reactions are mediated by sensitized T-cells to class I & II of allo-graft. Furthermore the secretion of Lymphokines leads towards the activation of monocyte and macrophages.

Chronic Rejection

This form of transplantation rejection is called as delayed rejection which occur within months to years. After transplantation the graft remains normal for months to years but sudden rejection occur due to still unknown mechanisms. These are many hypotheses reading such form of rejection like the involvement of various infections which are responsible for loss of immunological tolerance by grafted tissue in host.

Prevention & Treatment of Graft Rejection

If there is decrease in tissue rejection then there is relative increase in survival of graft. There are successful grafts occur for example mostly in case of kidney & cornea. For successful transplantation there is need of better understanding of immune response and MHC

The success of tissue graft is based on following important factors

1) Donor selection
2) Recipient preparation
3) Immunosuppression

Donor Selection

For appropriate donor selection, there should be MHC compatibility with recipient. Identical twins are considered as the ideal donor which are also termed as Isograft. HLA matched siblings have 95-100% chance of graft success. One haplotype of parent or sibling must be HLA-D matched for successful graft. Moreover, ABO compatibility is also essential for high success rate of transplant

Recipient Preparation

For successful tissue graft, the recipient should be in good health with no current infection. Similarly the absence of active malignancy would also increase the success rate. Moreover, in recipient the absence of any systemic diseases would lead towards better rehabilitation. Likewise, the recipient should not be hypertensive. One to five transfusions of 100-200 ml of donor's blood at 1-2 weeks interval would also increase the compatibility level with donor.

Immunosuppression

This is the way through which host immune system can be suppressed and is most essential component of allo-transplantation. For this there is usage of following immunosuppressive drugs with their specific mode of actions

- Cyclosporin A

It inhibit IL-2 synthesis following antigen exposure to host as a result no immune response is generated for allo-antigens.

- Rapamycin

It inhibits signal transduction for the activation and differentiation of immune cells. For instance inhibition of T-cells proliferation and activation is achieved by this.

Transplantation of Blood Cells & Bone Marrow

Clinically, transplantation of blood and bone marrow is used for treating various hematological disorders. The success of these graft is achieved by having good compatibility between donor and recipient. For this purpose following procedures are used

1. ABO blood grouping
2. Cross matching
3. HLA typing

Chapter # 20: Tumor Immunology

Evidence of Immune Reactivity to Tumor

The mass containing un-controlled proliferating cells is called as tumor. There are a lot of evidences which indicate that tumors elicit immune response. Like in case of age, the young & old populations have increased incidence of tumors because due to insufficiency of immune response at these ages. Similarly, tumors having mononuclear infiltration have better recovery as compare to those which lack mononuclear cells which indicate the involvement of immune cells during clearance of tumors. Tumor regression also occur due to appropriate immune response for example certain tumors regress spontaneously like melanomas (tumor of melanin producing cells of skin), neuroblastomas (tumor of nerve cells). Moreover, immune system facilitate the regression of metastatic tumor which has the tendency to move from tissue of origin to other tissues. For example in case of metastatic tumors, the removal of primary tumor regress metastatic tumor due to decrease in tumor load. Additionally, there is increased incidence of tumor in immune deficient patients like patients suffering from AIDS are susceptible to Kaposi Sarcoma. Likewise, the patients receiving transplants also get Epstein-Barr virus (EBV) induced lymphoma due to immune suppression. The presence of tumor specific antibodies & T-lymphocytes in patient's serum is also the hallmark of immune reactivity against tumor.

Tumor Associated Antigens

In order to generate effective immune response by immune system, tumor must have certain antigens on its surface. These antigens are generated through alteration in number of gene expression by a process called as tumorigenesis. The expression of new antigens on the surface of tumor are called as neo-antigens. Moreover, there is also alteration in existing antigens which are present on normal cells. These antigens are membrane bounded receptors, regulators of cell cycle & apoptosis and molecules of signal transduction

There are following two main types of tumor associated antigens

1) Tumor specific transplantation antigens
2) Tumor Associated transplantation antigens

1) Tumor specific transplantation antigens

These antigens are unique to tumor cells. These are not expressed on normal cells. These antigens are responsible for rejection of tumor by host immune system. In most cases, these antigens cannot be easily identified

2) Tumor Associated transplantation antigens

These antigens are expressed by both tumor & normal cells in contrast to tumor specific transplantation antigens. Various chemicals, UV radiations & viruses are responsible for

expression of these neo-antigens. Majority of these tumors are weakly immunogenic or on-immunogenic

Tumor Associated Transplantation Antigens

The tumor antigens which are also expressed by normal cells. These are expressed in high levels by tumor cells as compare to normal cells. These kind of antigens are also called as onco-fetal antigens as these are expressed during early development & lost during adult life. However these antigens re-expressed on tumor cells. These antigens also serve as tumor markers due to their importance in the diagnosis & prognosis of cancers

- There are following two types of onco-fetal antigens

1) Alpha-fetoproteins (AFP)
2) Carcino-embryonic antigens (CEA)

1) Alpha-fetoproteins (AFP)

These antigens are found as secretory protein in serum. Their level get raised during hepatocellular carcinoma.

2) Carcino-embryonic antigens (CEA)

These antigens are found both in secretory & cell associated form. Their level get raised in colon cancer

Immunity against Tumors

The immune system provides anti-tumor activity in humans, however, the evidence for immunity against malignancy mainly come from experimental studies with animals. Laboratory animals like mice can be immunized with irradiated tumor cells for providing defense against tumors.. Similarly, after removal of primary tumor if mice are challenged with the same live tumor then there would be resistance against same tumor upon re-challenge. Moreover, antibodies also play important role against various cancers for their neutralization. Likewise, the cell-mediated immunity also play pivotal role in tumor rejection by host immune system. Helper T-lymphocyte process the tumor antigen like shed from tumor and present with Class II MHC. Additionally, Helper T-lymphocyte also help B-cells to produce antibodies against tumor antigens. The role of CTL is also very critical for tumor regression. Helper T-lymphocyte also help in activation & differentiation of cytotoxic T-lymphocytes (CTLs). Cells of innate immune system like natural killer (NK) cells also kill the tumor cells due to lack of class I MHC. Furthermore, the cytokines like Interferon -gamma (IFN-γ) for tumorocidal activity.

Escape of Tumor from Immuno -Surveillance

The mechanisms which is responsible for tumor rejection in host is termed as Immuno-surveillance mechanisms**.** Tumor cells develop strategies for evading immune surveillance

Tumors escape from immune response by following mechanisms

I. Tumor cells may not express neo-antigens which are immunogenic in nature as a result no immune response is generated against such tumors
II. Tumor cells may fail to express co-stimulatory molecules for activation of T-lymphocytes for generating effective cell mediated immune response against tumors
III. Certain tumors may lack or poor expression of MHC for proper immune response generation
IV. At early development of tumor, there is low level of antigens which are in sufficient for activation of immune system which is termed as low dose tolerance
V. Overwhelming of immune system, after sudden maturation & expression of neo-antigens on tumor cells which is called as high dose tolerance
VI. Certain tumors secrete immunosuppressive molecules for inactivation of immune cells
VII. Some tumors shed their antigens for neutralization of antibodies

Use of Tumor Neo-antigens in Immuno-diagnosis & Immunotherapy

Tumor neo-antigens on tumor cells can be used for both immune-diagnosis & immunotherapy

Immune-diagnosis

Tumor neo-antigens are used for *In-vivo* detection of relatively small tumor foci (masses) through radiolabelled monoclonal antibodies against tumor antigens. Likewise the *In-vitro* use for finding the cell origin of undifferentiated tumor particularly lymphocytic origin in the blood & bone-marrow. Furthermore, Immuno-histochemical use of monoclonal antibodies against metastatic foci of tumors can help in the diagnosis of such tumors (Fig. 20.1)

Immunotherapy

The tumor neo-antigens can also be used for Immunotherapy for treating various tumors in different following ways

1) Active immunotherapy

In this form of immunotherapy, host actively participates against tumors. Active immunotherapy is achieved by inducing immune response by injecting irradiated tumor cells having neo-antigens on them. The active form of immunotherapy has following two forms

a) Non-specific active immunotherapy

This is generated for activation of immune response against neo-antigens using various vaccines e.g BCG for activation of macrophages against tumors

b) Specific active immunotherapy

In this form, the killed tumor cells or their antigens are used for killing of tumor cells

2) Passive immunotherapy

This form of immune-therapy uses the pre-formed antibodies against specific neo-antigens of tumor through following ways. The specific monoclonal antibodies can be used against tumor antigen can be served for

a) As a vehicle for delivering anti-cancer drug to tumor or cancer

b) Activation of components of innate immune system like complement proteins

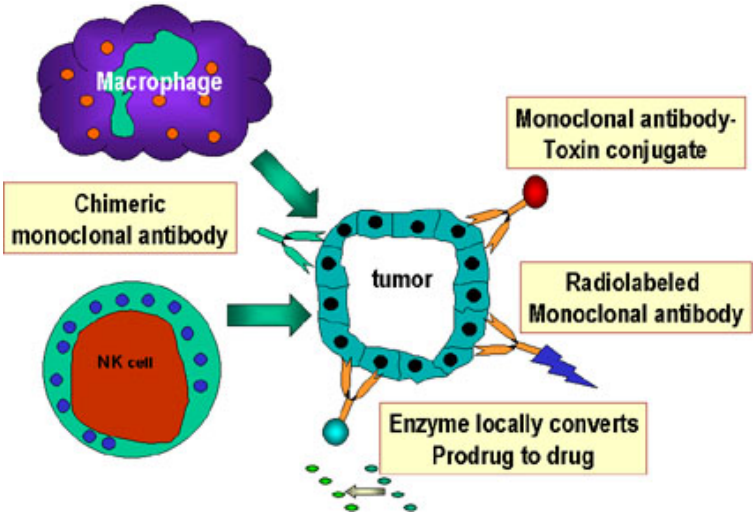

Figure 20.1: Use of Tumor neo-antigens for immuno-diagnosis & immunotherapy

Chapter # 21: Immunodeficiency

Introduction to Immunodeficiency

The deficiency or failure of immune system is called as immunodeficiency. It is a state of complete absence of immunity or effective immune response. There are defects regarding generation of immune reactivity against infectious agents like bacteria & virus etc. Furthermore, there is no immune response against transformed cells like tumors or malignancy. The persons having immunodeficiency are also called as immunocompromised patients because there is increased vulnerability for opportunistic infections in addition to other forms of infections. As there is decrease surveillance against tumors so more chances of getting tumors are there in immunodeficient patients.

Classification of Immunodeficiency

Immunodeficiency is classified into following two main categories based on mode of its acquisition

1) Primary Immunodeficiency
2) Secondary Immunodeficiency

Primary Immunodeficiency

Primary immunodeficiency is basically the inherited or congenital defects of the immune system. These defects can be either in specific or non-specific immune systems. These are classified on the basis of the site of lesion in the developmental or differential pathway of immune system. The chances of susceptibility to variety of infections get increased in this kind of immunodeficiency. The nature of infection depends on the kind of immunodeficiency

Primary immunodeficiency can be of two kinds

1. Immunodeficiency in specific immune system
2. Immunodeficiency in non-specific immune system

Immunodeficiency in specific immune system

This kind of immunodeficiency is due to defects in stem cell differentiation. Followings are the important disease of immunodeficiency in specific immune system

Reticular dysgenesis

It is either complete absence or severe deficiency of lymphocytes & granulocytes in blood due to decrease differentiation of bone marrow stem cells.

Severe combined immunodeficiency (SCID)

This disorder is due to the involvement of lymphoid stem cells. There is complete absence of T & B cell immunity. The patients suffering from SCID are susceptible to variety of bacterial, viral, mycotic & protozoan infections.

DiGeorge Syndrome

This is the disorder of T-lymphocytes due to congenital thymic aplasia or hypoplasia. This condition is highly immunodeficient as live vaccines also cause infections in the patients suffering from DiGeorge syndrome.

X-linked hypo gammaglobulinemia

This kind of immunodeficiency is due to functional disorder of B-Lymphocytes. Such patients are highly susceptible to recurrent bacterial infections.

IgA deficiency

There is deficiency of IgA in this disorder as a result individuals are susceptible to mucosal surface infections like GIT, eye & nasopharynx.

Selective IgG deficiency

In this kind of immunodeficiency, there is selective deficiency of IgG which leads towards hyper IgM immunodeficiency.

Immunodeficiency in Non-Specific immune system

Followings are the important conditions which are linked with immunodeficiency in non-specific immune system

Cyclic Neutropenia

There is low number of circulating neutrophils in the blood which cause recurrent bacterial infections among such patients.

Chronic Granulomatous disease (CGD)

This kind of immunodeficiency is due to defects in phagocyte functions.

Complement deficiency

There is deficiency of complement proteins in this kind of immunodeficiency. Patients suffering from such disorder are susceptible to infections particularly *Neisseria*

Secondary Immunodeficiency

This kind of immunodeficiency is not congenital in nature in contract to primary immunodeficiency. This state of immunodeficiency is associated with infections for example

AIDS. There is also association of aging factor like hypo-cellularity in bone marrow. Malignancies for example Leukemia and Myeloma are also linked with secondary immunodeficiency. Furthermore, other metabolic disorder like diabetes and renal malfunction etc are also linked with secondary immunodeficiency.

Acquired Immunodeficiency Syndrome (AIDS)

This condition is caused by a virus called as human immunodeficiency virus (HIV). This condition is initiated through an initial infections with influenza like illness. Further, the disease progress with other defects in immune system leading towards severe immunodeficiency. The patients get severe opportunistic infections like tuberculosis. Moreover, the chance of getting tumor also increased. AIDS is linked with abnormalities in circular lymphocytes. There is reduction in the number of helper-T cells which are also called as $CD4^+$ve cells which consequently cause reversal in $CD4^+/CD8^+$ T-cell ratio. Furthermore, there is normal NK cells number but with reduced activity to kill virally infected as well as transformed cells. AIDS patients have increased susceptibility to infections with opportunistic pathogens like Cryptococcus, herpes simplex, herpes zoster, and mycobacterium etc. During initial or primary infection there is systemic fever, lethargy & malaise with localized tissue involvement (Fig. 21.1). AIDS patients have also increased susceptibility to various following tumors

- Kaposi sarcoma
- Burkitt's lymphoma
- Primary central nervous system lymphoma

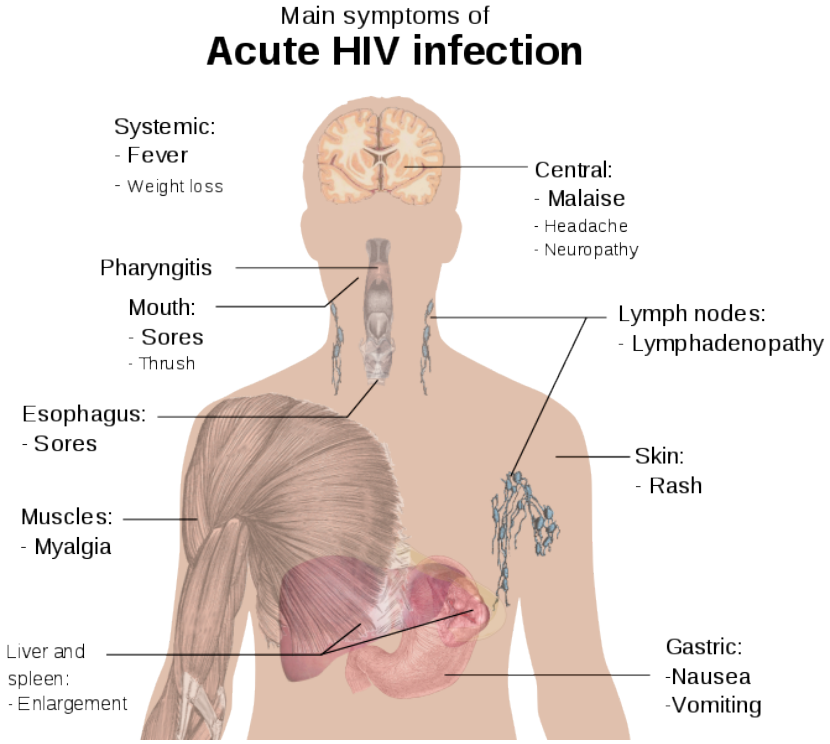

Figure 21.1: Involvement of various tissues during AIDS

For AIDS, HIV infection is transmitted by following two modes

1) Horizontal transmission

This kind of transmission occur usually by person to person contact either sexually or through body fluids like blood and semen

2) Vertical transmission

This form of transmission is from mother to fetus during the process of pregnancy, delivery & breast feeding

Immunodeficiency: Disorders of T- Cells

As T-lymphocytes have very significant role in providing cell mediated and humoral immunity, that's why immunodeficiency associated with T-cells disorders effects both cell mediated & humoral immunity. There is complete absence & functional abnormality in T-cells which increase the chances of getting multiple infections like viral, protozoal & fungal infections. In immunodeficiency associated with T-cells the incidence of viral infections like cytomegalovirus and measles get increased.

DiGeorge Syndrome

It is one of the primary immunodeficiency in which there is complete absence of T-lymphocytes. This condition is also called as thymic aplasia or hypoplasia. This form of immunodeficiency is also linked with hypoparathyroidism due to abnormal development of fetus. There is poor development of heart, thymus & parathyroid due to which there is abnormal development of heart, thymus & parathyroid. All patients suffer from DiGeorge syndrome have thymic aplasia. This disease is autosomal dominant caused by deletion in chromosome number 22. This deletion is of variable size which doesn't correlate with severity of disease. The treatment of DiGeorge syndrome occur through thymic graft only.

T-cells deficiency with variable degree of B-cells deficiency

This form of immunodeficiency is linked with T-lymphocytes deficiency which also effect the number of B-lymphocytes with a variable degree. In this condition, both cell mediated as well as humoral immunity get effected leading to multiple form of infections.

Ataxia-telangiectasia

This form of T-cells associated immunodeficiency has deficient T-cells with less movement of blood vessels which also effects the function of T-lymphocytes.

Wiskott-Aldrich Syndrome

In this condition, there is normal number of T-cells with reduced functions as a result chances of getting multiple infections become increased.

Immunodeficiency: Disorders of B- Cells

This form immunodeficiency is associated with normal T-cells, however number of B-lymphocytes may be low or normal. As a result, the serum Immunoglobulin levels are low. There is more chances of getting pyogenic infections like bacterial infections

X-linked hypo-gammaglobulinemia

In this form of B-cells associated immunodeficiency, the numbers of B-cells are very low. Due to which the immunoglobulin levels are also very low. This condition is linked with defect in B-cell maturation. Patients suffer from this immunodeficiency are prone to recurrent bacterial infections

IgA deficiency

This is the commonest form of immunoglobulin deficiency. There is defect in class switching phenomenon of immunoglobulin synthesis. These patients are prone to mucosal surface infections like GIT, eye & nasopharyngeal infections. This form of immunodeficiency is diagnosed through measurement of serum IgA levels by various immunological methods

X-linked hyper IgM -Immunodeficiency

This form of B-cell associated immunodeficiency has very high levels of IgM. As a result, the levels of IgG & IgA get reduced. The underlying defect in this condition is in class switching due to defect in CD40 ligand on CD4 T-cells. The patients are susceptible to pyogenic infections like bacterial infections. This condition can be treated with intravenous gamma globulins injections.

Immunodeficiency: Defects of Phagocytic Cells

The primary immunodeficiency associated with cells of non-specific immune system including phagocytic cells like neutrophils, monocytes and macrophages. There is also defect in killer cells like NK cells

Congenital Agranulomatosis

This form of congenital immunodeficiency has decreased neutrophil count. There is defect in myeloid progenitor differentiation into neutrophils. These patients are prone to pyogenic infections like bacterial.

Chronic Granulomatous disease (CGD)

This form of primary immunodeficiency has decreased neutrophil function while their number remains normal. The underlying defect is due to poor intracellular killing ability of neutrophils. This defect of neutrophils is due to deficiency of NADPH oxidase & other co-factors which are required for respiratory burst. CGD patients have increased susceptibility to bacterial infections.

Leukocyte Adhesion deficiency

As the name suggests this form of immunodeficiency is linked with defect in integrin molecules as a result there is decreased process of diapediesis & defective neutrophils movement towards

chemotactic signals. This defective phagocytic function leading towards recurrent bacterial infections among such patients.

Immunodeficiency: Defects of Complement System

In this form of immunodeficiency of non-specific immune system, there are abnormalities in complement proteins which are also called as Hypo-complementemia. These defects are genetic in nature due to inherited defects in the synthesis of various complement proteins. There is major defect in the synthesis of C3 due to defective C3 synthase. Moreover, defects are also due to various regulatory proteins like Factor H & I. Majority of complement deficiency is autosomal recessive in nature except properdin deficiency which has X-linked inheritance. However, MBL deficiency can be both. The patients suffering from such disorders have recurrent bacterial infections particularly Neisseria & Streptococcus infections. Furthermore, these conditions also cause autoimmune disorders like SLE, vasculitis etc.