UNIT-I

CHAPTER I - HISTORY AND PERSPECTIVES OF IMMUNOLOGY

DEFINITIONS

- **Immunology** is the study of structure and functions of immune system that confers us immunity
- The Latin term *"IMMUNIS" means EXEMPT, referring to protection against foreign agents.*
- **Immune system**: The integrated body system of organs, tissues, cells and cell products that differentiates self from non – self, neutralizes and eliminates potentially pathogenic organisms, chemicals, altered self cells and thereby protects us from disease
- **Immunity:** the ability of an organism to resist a particular infection or toxin by the action of specific antibodies or sensitized white blood cells or simply protection from disease
- **Immune response:** An immune response is a reaction which occurs within an organism for the purpose of defending against foreign invaders or a bodily response to an antigen that occurs when lymphocytes identify the antigenic molecule as foreign and induce the formation of antibodies and lymphocytes capable of reacting with it and rendering it harmless

HISTORY

The first record crude attempts to deliberately induce immunity were performed by the Chinese and Turks in the 15th century. Chinese introduced dried crusts of small pox scabs into the nostrils of infants to make them immune to small pox infection.

In 1718 Lady Mary Wortley Montagu, observed that the insertion of dried crusts derived from smallpox pustules into small cuts in the skin (a technique called variolation), shows some positive effects against small pox. The technique was significantly improved by the English physician 'Edward Jenner 'in 1798. He injected the pustules of cowpox lesions (a mild infection related to small pox) into the persons infected with small pox. As predicted, the person did not develop small pox. Jenner's technique spread quickly throughout Europe but it was nearly a 100 years before the technique was applied to other diseases.

Then Pasteur discovered that aging has weakened the Virulence of the pathogen and that such an attenuated strain might be administered to protect against the disease. He called this attenuated strain a vaccine (from the Latin "Vacca", meaning "cow") in honor of Jemer's with cowpox inoculation. Thus he developed vaccine for chicken cholerae, anthrax and rabies.

Timeline of immunology:

- 1549 – The earliest account of inoculation of smallpox (variolation) occurs in Wan Quan's (1499–1582)
- 1718 – Smallpox inoculation in Ottoman Empire realized by West. Lady Mary Wortley Montagu, the wife of the British ambassador to Constantinople, observed the positive effects of variolation on the native population and had the technique performed on her own children.
- 1796 – First demonstration of smallpox vaccination (Edward Jenner)
- 1876 – Demonstration that microbes can cause disease-anthrax (Robert Koch)
- 1877 – Mast cells (Paul Ehrlich)
- 1880 – 1881 -Theory that bacterial virulence could be attenuated by culture in vitro and used as vaccines. Proposed that live attenuated microbes produced immunity by depleting host of vital trace nutrients. Used to make chicken cholera and anthrax "vaccines" (Louis Pasteur)
- 1883 – 1905 – Cellular theory of immunity via phagocytosis by macrophages and microphages (polymorhonuclear leukocytes) (Elie Metchnikoff)
- 1885 – Introduction of concept of a "therapeutic vaccination". Report of a live "attenuated" vaccine for rabies (Louis Pasteur and Pierre Paul Émile Roux).
- 1888 – Identification of bacterial toxins (diphtheria bacillus) (Pierre Roux and Alexandre Yersin)
- 1888 – Bactericidal action of blood (George Nuttall)
- 1890 – Demonstration of antibody activity against diphtheria and tetanus toxins. Beginning of humoral theory of immunity. (Emil von Behring) and (Kitasato Shibasaburō)
- 1891 – Demonstration of cutaneous (delayed type) hypersensitivity (Robert Koch)
- 1894 – Bacteriolysis (Richard Pfeiffer)
- 1896 – An antibacterial, heat-labile serum component (complement) is described (Jules Bordet)
- 1900 – Antibody formation theory (Paul Ehrlich)
- 1901 – Blood groups (Karl Landsteiner)
- 1902 – Immediate hypersensitivity anaphylaxis (Paul Portier) and (Charles Richet)
- 1903 – Opsonization
- 1905 – "Serum sickness" allergy (Clemens von Pirquet and (Bela Schick)
- 1909 – Paul Ehrlich proposes "immune surveillance" hypothesis of tumor recognition and eradication
- 1940 – Identification of the Rh antigens (Karl Landsteiner and Alexander Weiner)
- 1942 – Anaphylaxis (Karl Landsteiner and Merill Chase)
- 1945 – Coombs test a.k.a. antiglobulin test (AGT)
- 1946 – Identification of mouse MHC (H2) by George Snell and Peter A. Gorer
- 1948 – Antibody production in plasma B cells
- 1949 – Growth of polio virus in tissue culture, neutralization with immune sera, and demonstration of attenuation of neurovirulence with repetitive passage (John Enders) and (Thomas Weller) and (Frederick Robbins)
- 1951 – vaccine against yellow fever
- 1953 – Graft-versus-host disease
- 1953 – Validation of immunological tolerance hypothesis

- 1957 – Clonal selection theory (Frank Macfarlane Burnet)
- 1957 – Discovery of interferon by Alick Isaacs and Jean Lindenmann
- 1958–1962 – Discovery of human leukocyte antigens (Jean Dausset and others)
- 1959–1962 – Discovery of antibody structure (independently elucidated by Gerald Edelman and Rodney Porter)
- 1961–1962 Discovery of thymus involvement in cellular immunity (Jacques Miller)
- 1960 – Radioimmunoassay – (Rosalyn Sussman Yalow)
- 1963 – Gell and Coombs classification of hypersensitivity
- 1964–1968 – T and B cell cooperation in immune response
- 1965 – Discovery of "immune interferon" (gamma interferon) (E.F. Wheelock)
- 1965 – Secretory immunoglobulins
- 1967 – Identification of IgE as the reaginic antibody (Kimishige Ishizaka)
- 1974 – T-cell restriction to MHC (Rolf Zinkernagel and (Peter C. Doherty)
- 1975 – Generation of monoclonal antibodies (Georges Köhler) and (César Milstein)[4]
- 1975 – Discovery of Natural Killer cells (Rolf Kiessling, Eva Klein, Hans Wigzell)
- 1976 – Identification of somatic recombination of immunoglobulin genes (Susumu Tonegawa)
- 1983 – Discovery of the T cell antigen receptor TCR (Ellis Reinherz) (Philippa Marrack) and (John Kappler) (James Allison)
- 1986 – Hepatitis B vaccine produced by genetic engineering
- 1986 – Th1 vs Th2 model of T helper cell function (Timothy Mosmann)
- 2017 – The Indian government approves Apceden, the first potentially curative dendritic cell vaccine for the treatment of prostate, ovarian, colon, and lung cancers. It is developed by an Indian Biotechnology company, APAC Biotech.
- 2018 - James_P._Allison and Tasuku_Honjo won the Nobel Prize in Physiology or Medicine for their discovery of cancer therapy by inhibition of negative immune regulation

CHAPTER II – IMMUNITY

Immunity-the state of protection from infectious disease, has both specific & non specific components. The term immunity refers to the resistance exhibited by the host towards injury caused by microbes and their products. Immunity against infectious disease is of different types. Innate defence mechanisms provide the first line of host defence against invading pathogens until acquired immune response develops. Acquired immunity does not operate independently of inate immunity; rather, the specific response augments the non specific response, producing a more definative total response.

1. Innate immunity - species

a. Specific - individual

b. Non specific - racial.

2. Acquired immunity:

a. Active

(i) Natural
(ii) Artificial

b. Passive

(i) Natural
(ii) Artificial

Innate immunity:

Innate or native immunity is the resistance to an infection which individual posses by virtue of his genetic and constitutional make up. It is not affected by prior contact with microorganisms or immunization. It may be non – specific when it indicates the degree of resistance to infection in general, or specific when resistance to a particular pathogen is concerned. Innate immunity may be species, racial or individual.

Species immunity:

It refers to the total relative resistance to pathogens shown by all members of species. For e.g.: human beings are insusceptible to plant pathogen and some animal pathogens.

Racial immunity:

Within a species different races shows difference in susceptibility to infection. This is known as racial immunity. For e.g. high resistance of Algerian sheep to anthrax.

Individual immunity:

The difference in the innate immunity exhibited by different individuals in a race is known as individual immunity. Eg. Homozygous twins exhibit similar resistance or susceptibility to leprosy or TB while immunity differs in heterozygous twins.

Factors that influence innate immunity:

1. Age:

The two extremes of life carry high susceptibility to infectious disease. When compared to adults, the foetus is usually protected by the placental barriers from maternal immunity infection. Some organisms such as rubella and cytomegallo virus and *toxoplasma gondii* cross the placental barriers and cause infection leading to congenital malformation and some times death. New born are more susceptible to infection eg. Coxscakie virus cause fatal infection in suckling mice but not in adults.

2. Harmonal influence:

Increased susceptibility in the young may be due to hormonal influence. Ex. Tinea capitis caused by *Microsporum audounii* frequently undergoes spontaneous cure with outset of puberty. Endocrine disorder such as diabetes mellitus, hypothyroidism and adrenal dysfunction are associated with an enhanced susceptibility to infection. The high incidence of Staphylococcus sepsis in diabetes may be related to increase levels of carbohydrates.

3. Nutrition:

Malnutrition contributes to incidence and severity in several diseases. Cholera is usually fatal in malnourished persons. Measles is 100 –400 times as prevalent among under nourished African children.

4. Climate:

The seasonal incidence of influenza and cold justifies the role of climate and seasonal changes are causing epidemics of respiratory, diseases, cold. Dry winter conditions can dehydrate the mucous membrane and paralyze the ciliated mucosa. In hot humid climate the accumulation of moisture on the body encourage the growth of skin pathogens.

5. Sex:

Anatomical, hormonal and biochemical difference between men and women help to explain why some diseases are more common in one sex, than the other factor. For e.g.: women are much more likely to contract cystitis (urinary bladder infection) and subsequently upper urinary tract diseases because their shorter urethra makes the bladder more accessable to microbes.

6. Occupation:

Infectious disease is a persistant occupational hazard of certain professional, veterinarians and animal control offices.

7. Stress:

Prolonged (or) emotional crisis fatigue (or) depression affect hormonal balance and reduce resistance to disease. For e.g. French mouth (acute necrotising alterative gingivitis) is a stress associated disease caused by normal minor members of indigenous flora.

NON SPECIFIC RESISTANCE MECHANISM – OR INNATE IMMUNITY MECHANISM

The innate immunity mechanism includes:

1. Surface defences.

2. Cellular defences.

1. Surface defences:

Most infectious diseases are initiated by the microorganisms when it comes in contact with the surface of the body. The host uses mechanisms against these infectious agents.

Mechanical defence:

Epithelial surface:

a. Skin:

The intact skin and mucous membrane lining the respiratory, alimentary and genito urinary tract of the body gives protection against invasion by microorganisms. The skin is an effective barrier to the penetration of microorganisms. The healthy skin posses anti bacterial activity due to presence of high concentration of salt in the sweat.

b. Mucous membrane:

The mucous of respiratory tract have several innate mechanisms of defense. Microbes are constantly inhaled through the nose. Those that pass beyond are held by the mucous which is secreted by the epithelial cells of mucous membrane and are swept back to the pharynx where they tend to get swallowed or coughed out. Coughing and sneezing are important defence. The air ways of the respiratory tract are lined by cilia, hairs like process projecting from the epithelial cells. These cells secrete a layer of mucous. The sticky mucous efficiently traps particles that get in the respiratory tract. The mucous secreting cells possess cilia that move the mucous blanket, sweeping trapped microbes into the throat where they are swallowed and are killed by the acid of

the stomach. Nasal and respiratory secretions contain mucopolysaccharides capable of neutralizing influenza and other viruses.

The mouth is constantly bathed in saliva which has an inhibitory effect on many microorganisms. Particles deposited in the mouth are swallowed and subjected to the action of the digestive juices. Acidity of stomach also destroys most microorganisms. The intestinal mucosa is covered by a network of mucous. Particles enmeshed in the mucous and form small masses which are propelled by peristalsis.

Other mechanical defence:

Body had reduced the number of microbes settling onto the skin. The conjuctiva is freed of forcing particles by the flushing action of lacrymal secretions. In the eyes, tears are produced by lacrymal glands in the lower rid. These holes drain into the nasal chamber and then into the mouth, the microbes flushed from the eyes are swallowed by the stomach acid. The sloughing of the dead surface layer of our skin effectively discharges many microbes that have attached to these epithelial cells. The flushing action of urine, eliminates bacteria from the urethra.

Chemical Defence:

Various body fluids contain substances that resist against potential pathogens. Tears contain the antibacterial substance ensozyme flemming in 1922. This is a thermoriable substances causing lysis of bacteria. It is present in tissue and in mearly all body secretions except if CSF, sweat and urine. It acts by splitting certain PS components of cell walls of gram '+'ve bacteria. Fatty acid in sweat and ear wane have bacteriostatic and fungistatic effect. The activity of adult vagina due to fermentation of glycogen in epithelial cells by resident lactobacilli renders it inhospitable for many pathogens. There are several antibacterial substance in blood and tissues.

Interferon:

They are a group of proteins produced by host cells during virus infection. The function of the interferon is its ability to bind to the near by cells and induce a generalized antiviral state.

Lactic acid:

Acidic substance such as lactic acid are found in muscle tissue.

Lactoperoxidase:

It is present in milk and has antibacterial properties.

Spermine and Zinc:

Present in semen and has antibacterial activity.

Transferins:

These are iron binding proteins that reduce the level of free iron in blood. Lactoferins perform the same function in milk, respiratory, intestinal and genital secretion. Free iron is required for growth of many pathogens.

Interleukins:

It is a fever inducing protein in the phagocytic cells when stimulated by color substances such as bacterial endotoxins phagocytes discharges the content which circulate to the hypothalamic region of the brain and trigger and elevate body temperature. Although fever can itself damage human cells it has several protective effects.

i). It raises temperature above that which is not optimal for growth of some pathogens.

ii). It accelerates mobilization and protective efficiently of the body defence.

iii). It stimulates lymphocyte activity in immune response.

iv). It increases the rate of iron storage reducing its availability to the pathogens.

Complement:

It is a group of serum proteins that circulate in an inactive proenzyme state. These proteins can be activated by various specific and non specific immunological mechanisms that converts the inactive pro enzyme to active enzyme. The activated complement is a enzymatic cascade that results in the damage to the members of pathogen.

Microbial Defense:

The bodys normal flora provides another important line of defence. Normal flora continuously complete with pathogenic microbes for limited nutrients and space on the body epithelial surface. Since normal flora colonises first they usually win the competition. Normal flora also has the ability to alter their local environment so that it is generally unfavourable for the growth of most pathogens. For e.g., the lactobacilli normally inhabit the adult vagina produce lactic acid as a byproduct of growth. This lowers the PH of the vagina between 4-4.5 which is reliably acidic for most pathogens. E.g., Metabolic byproducts of skin flora create acidic condition on the epidermal surfaces (p^H 3-5) that oppose the growth of many potential pathogens.

Cellular Defense:

Once the invading microorganism has penetrated the various physiological and chemical barriers the next line of defence consists of various specialized cells whose purpose is to destroy the invador. There are several cell types that fulfill this function.

Endocytic and phagocytic barriers:

Another important innate defence mechanism is the ingestion of extra cellular molecules via endocytosis and if particulate material through phagocytosis.

Endocytosis:

In endocytosis, macromolecules with in the extra cellular tissue fluid are internalized by cells via the invagination and pinching off small regions of the plasma membrane. The resultant endocytic reside are small 0.1 mm in diameter. Endocytic occurs through one or two process.

(i). Pinocytosis or Receptor mediated Endocytosis:

In pinocytosis non-specific membrane invagination internalized macromolecules in proportion to the extracellular cone. In receptor mediated endocytosis, macromolecules are selectively interlised after binding to specific membrane receptor.

The endocytic reside formed by either process fuse with each other form endosomes, which are interacellular acidic compartments that is interior of endosomes facilitates disassociation of macromolecular ligands from their receptor and are recycled back to the errl surface. Free macromolecules contain within endosomes fuse with primary lysosomes to form structure known as secondary lysosemes. Primary lysosomes are derived from golgi complex and contain large numbers of degradatine enzymes including protrases, Lipases, nucleases and other hydrolytic enzymes with in secondary lysosomes. The ingested macromolecules are then digested into small breakdown products (peptides, nucleotides, sugars) which are eliminated from the cell.

Phagocytosis:

It involves the ingestion of particulate material including whole pathogenic micro organisms. In phagocytosis the PM expands around the particulate material to form large vesicle called phagosomes. These vesicles are 10-20 times larger than the endocytic vesicles the expansion of the membrane in phagolytosis requires the membrane in participation of microbial filaments which do not take part in the endocytosis. Only specialized cells are capable of phagocytosis, where as all cells are capable of endocytosis. The phagocytic cells include monocytes, neutrophils, and tissue macrophages. Once particulate material is injected into phagosome fuse with lysosomes and the injected material is digested and the process is similar to endocytosis.

Inflammatory Response:

Tissue damaged caused by a wound or by invasion by a sequence of events collecting known as inflammatory response. The signs of the inflammation include reduess, awelling, heat and pain. The events occurring during a inflammatory response are

(i). Vasodilation: A increase in the diameter of the blood vessels that carry blood always from all infected area constrict, resulting in enlargement of capillary network. The enlarged capillaries are responsible for the tissue redness (erythema) and an increase in tissue temperature.

(ii). An increase in capillary permeability facilitates an influx of fluid and ells from the enlarged capillary into the tissue. The fluid that accumulates (exudates) has a much higher content of exudates contributes to tissue swelling (odema)

(iii). Influx of phagocytosis from the capillary into the tissues facilitated by the increased capillary permeability. The emigration of events including adherence, of the cells to the endothelial wall (margination) followed by their emigration between the capillary endothelial cells into the tissues (diapedesis) or extravention and finally their migration through the tissue to the site of the inflammatory response (chemotaxis) As phagocytic cells accumulate at the site and begin to phagocytose bacteria, they can damage near by healthy cells. The accumulation of dead cells, digested materials and fluids from a substance is called as pus.

Mediators:

With the minutes after injury the first process begins with activation and increased cone of the pharmacologically powerful substances, such as group of proteins known as acute phase proteins. An X, membrane of the acute phase proteins. This protein binds to the membrane of certain micro-organisms and activate the complement system. This results in the lysis of the microorganisms cell wall component found on a variety of bacteria and fungi.

Histamine:

It is a mediator of inflammatory response. It is chemical released by the various number of cells in response to tissue injury. It binds to receptor on nearby capillaries, causing vasodilation and increased permeability.

Kinin:

It is a inflammatory mediator and causes vasodilation and increased permeability. These are small peptides which are present in all inactive form in blood plasma. Tissue injury induces activation of these peptides causing the particular kinin called bradikinin stimulates pain receptors in the skin.

Fibrin:

Vasodilation and increase in capillary permeability that occurs in an injured tissue also enables enzyme of the blood clotting system to enter the tissue. These enzymes activate an enzyme cascade that results in the deposition of insoluble strands of fibrins, which are the main component of a blood clot. The fibrin clot wall of the body and serve to prevent the spread of infection.

Cells Involved:

Most of the cells response are the phagocytic cells. First mainly consisting of poly morpho nuclear leukocytes (PMN's) which with in 30-60 mins, phagocytose the intrudes or damage tissue and release their lysosomal enzymes in. If it persists with in 5-6 hrs the area will be infiltered by mononuclear cells which include macrophage and lymphocytes. The macrophages do the phagocytic activity.

Once the inflammatory response has subsided and most of the debris has been cleared away by phagocytic cells, tissues repair and regeneration of new tissue occur. Tissues repair begins as capillaries grow into the fibrins of a blood clots, new connective tissue cells called fibroblast replace the fibrin as the clot dissolves. As fibroblast and capillaries accumulate scar tissue is formed.

Acquired immunity:

Specific immunity reflects the presence of a functional immune system that is capable of specifically recognizing and selectively eliminating foreign microorganisms and molecules. Acquired immunity is a consequences of an encounter with a foreign substance. Acquired immunity develops only after the given substance. Acquired immune response or adaptime and display and characteristics attributes.

i) Antigenic specificity:

The antigenic specificity of the immune system permits to distinguish differences among Ags. Abs can differentiate between two molecules that differ by only a single amino acid.

ii). Diversity:

The immune system is capable of generating tremendous diversity in its recognition molecules, allowing it to specifically recognize billions of different structure on foreign Ag's.

iii). Immunologic Memory:

Once the immune system have recognized and responded to an Ag it exhibits immunologic memory in the second encounter with the same Ag includes a highest state of immune reactivity.

iv). Self and non-self recognition:

The immune system normally response only to foreign ag's indicating that it is capable of self and non-self recognition which is essential for the outcome os an appropriate response.

Acquired immunity is of two types.

a. Active

b. Passive

a. Active:

It is the resistance developed by an individual as a result of an antigenic stimulus. This involves the active functioning of the persons immune apparatus leading to the synthesis of Ab's and or the production of immunologically active cells. Acquired sets is only after a latent period which is required for the immunological machinery to be set in motion. Once developed the acquired immunity is long lasting. If an individual who has been actively immunized against an Ag experiences the same Ag subsequently through immune response occurs more quickly and abundantly than during the first encounter. This is known as secondary response. Acquired immunity is associated with immunological memory. Acquired immunization is more effective and confers better protection than passive immunization. Active immunity may be natural or artificial.

Natural active immunity:

It results from either a clinical or inapparent infection by a parasite in a person who has recovered from an attack of measles develop natural acquired immunity. Majority of adults in the developing countries process Natural Acquired immunity (NAI) to polio due to repeated in apparent infection, with polio virus during child hood. NAI is long lasting but the duration varies with the type of pathogen.

Artificial active immunity:

It is the resistance induced by vaccines, are preparation of line or killed micro organisms or their pelts used for immunization e.g. (i) bacterial vaccines – line – BCG vaccine tuberculosis (ii) killed – TAB for enteric fever (iii) Sub unit – Bi polysaccharide for typhoid e.g., viral fever.

(i). Line –oral polio vaccine (saline)

(ii). Killed – injectable polio vaccine a salk.

(iii). Subunit – Hepatitis and vaccine.

Passive immunity:

The resistance that is transmitted to a recipient in a readymade form is known as passive immunity. There is no agic stimulus instead performed Ab's are administered. The protections is immediate and there is no latent period. The immunity is transient lasting usually for days or weeks only till the passively transmitted Ab's are metabolized and eliminated. There is no secondary response. Passive immunity may be natural or artificial.

Natural Passive Immunity:

It is the resistance passively transferred from the mother to the baby. Infants maternal Ab's are transmitted through the placenta, human colostrums which is rich in IgA Ab's resistant to intestinal digestion confess protection to the neonates.

Artificial passive immunity:

It is the resistance passively transferred to a recipient by administration of Ab's. The agents used for this purpose are hyper immune sera (ats) of animal or human origin. They are used for prophylaxis and theraphy e.g., anti-tetanus serum (ATS) and human hyper Ig, combined immunization.

S.No	Active Immunity	Passive immunity
1.	Produced actively by host immune system	Received passively by the host. No participation by the immune system
2.	Induced by infection or by contact with immunogens.	Conferred by the introduction of readymade Abs.
3.	Durable and effective protection.	Protection transcient and less effective
4.	Immunity effective only after a lag period	Immediate immunity.
5.	Immunological memory present	No immunological memory
6.	negative phase may occur	No negative phase
7	Not applicable in immunodeficient host	Applicable in immuno deficient host

CHAPTER 2 CELLS OF THE IMMUNE SYSTEM

Introduction:-

The immuse system of the body includes a wide variety of organs, tissues an cells. The function of the immuse system is to protect the body from invading nuo's like bacteria fungi, and parasites etc., White blood cells or leucocytes are have a significant part in the development of the immune response of the different kinds of immune cells, only the lymphocytes possess the attributes of diversity specificity and self/non self recognition, the ball marks of immune response. All the other cells play accessory roles, serving to activate lymphocytes, to increase the effectiveness of ag clearance by phagocytosis, or to secrete various immune effector molecules. For the batter interaction between lymphocytes, antigen presenting cells (APC) and ag the lymphoid organs should provide a suitable microenvironment.

Haematopoiesis

It is the formation and development of red & white cells from stem cells. In human it begins in the yolk sac in the first week of embryonic development. Here yolk sack stem cells differentiate into primitive erythroid cells containing embryonic hemoglobin. In the 3rd month of gestation the factal liver & then to the spleen. These two organs have major role in hematopolesis from the 3rd to 7th month of gestation. As gestation continues the bone narrow becomes the major hematopoietic organ. By birth hematopolesis has caused within the liver & spleen.

A functionally specialized matured blood cell is derived from a common stem cell. A haematopoietic stem cell is "pluripotent" able to differentiate a long no. of pathways & thereby generate erthyeocytes, granulocytes, most cells lymphocytes and megakaryocytes. The stem cells are few in no. occurring with a frequency of one stem cell/CO4 bone narrow cells.

Early in hematopoiesis, pluripotent stem cells differentiates along one of the 2 pathways given rise to either,

- lymphoid stem cells
- Myeloid stem cells

Lymphoid & myeloid stem cells differentiate into progeniter cells.

- Lymphoid stem cells generate T4 & progenitor lympholytes
- The myeloid stem cells generate progenitor cells for RBC, the various WBCs (Neutrophils, eosinophils, basophils, monocytes, mast cells) and platelets.

In adult bone marrow, the marmatopoietic cells grow and mature on a mesh work of stromal cells, which are non haematopoietic cells that support the growth & differentiation of the hematopoietic cells. Stromal cells include for cells, endothelial cells, fibroblast etc.

Stromal cell influence haematopoietic stem cell differentiation by providing a haematopoietic inducing microenvironment consisting of a cellular matrix & either membrane bound or diffusable growth factors. As the hematrpoietic stem cells differentiate in this environment, they pass through the sinusoidial wall into the simsses of the bone marrow. Then they enter into the circulation.

Lymphoid Lineage:-

Lymohoid cell constitutes 20-40% of the body's WBC & 99% of the cells in lymph. They are ~ ie., 10^10 to 10^2 lymphocytes in the human body. The lymphocytes continuously circulate in blood and lymph. The lymphocytes can be broadly sub divided on the basis of functions into 3 populations, namely

- B Cells
- T Cells
- Null cells

All the 3 cell types are small, motile, non-phagocytic cells which cannot be distinguished morphologically. B an T cells which are not interacted with the ag are referred to as "native cells". They are district membrane glycoproteins, carryout different functions, and respond to different activation stimuli, for example different mitogens (substances that induce cell division or mitosis

B lymphocytes:-

The B lymphocyte derived its name from the site of maturation ie., the hursh of fabricius in birds, an outpouching of the cloacal epithelium, where the maturation occurs, in mammals the site of maturation is bone marrow. Mature B ells can be distinguished from other lymphocytes by the presence of membrane bound immunoglobulin (ad) molecules, which serve as receptor for eg(1.5 *10^5 molecules of Ab/B cell)

The naïve/unprimed B cells are called small lymphocytes.

Small Lymphocytes + Ag -> lymphoblasts -> effector cells /Plasma cells/As producing cells + Memory B cells

Differentiate of B cells occur by 2 pathways namely,

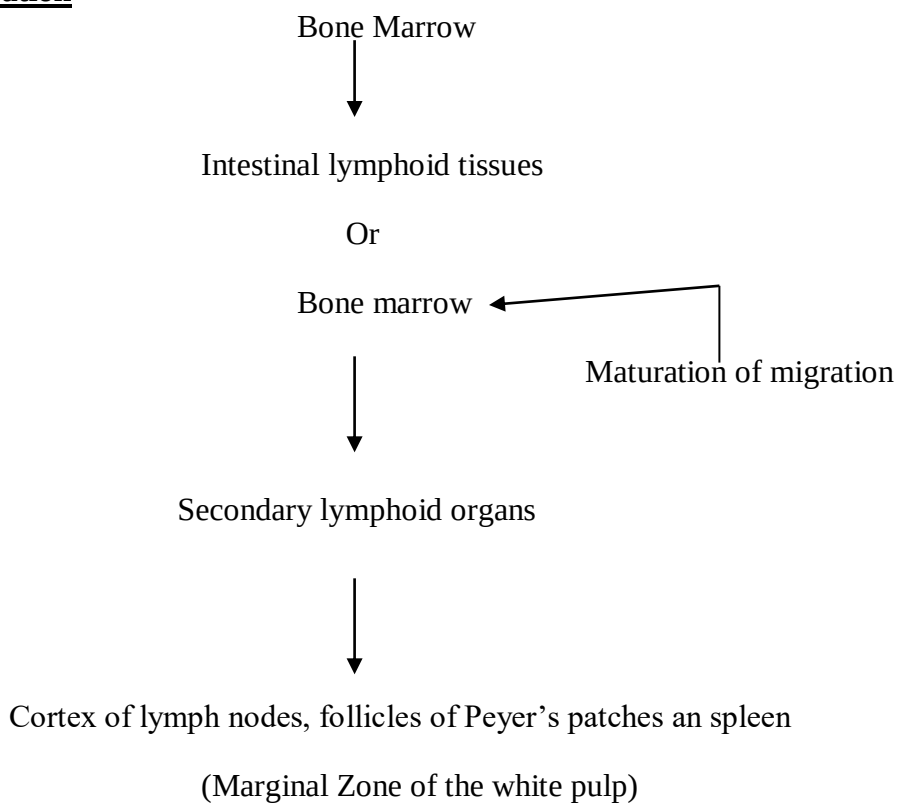
- Ag independent pathway
- Ag dependent pathway

B cell surface markers:-

Among the other molecules expressed on the memebrane of mature B cells are the following

- B220/CD45 –for signal transduction
- Class II MHC – Permits the B cell to function as APC
- CRI(CD35) & CR2(CD21) –complement receptors
- Fe gamma RII (CD 32) – Fc receptor
- B7 Constimulatory molecule

Maturation



T Cells:-

T lymphocytes derive their name from the site of maturation in the thymus. T cells also arise from haematopoietic stem cells in the bone marrow. Like B cells, these cells have membrane receptors for antigen, is structurally distinct from ab, it does share some common structural features with the same, called a "T cell Ag binding receptor" (TCR), can only recognize an ag with MHC.

- B cells are capable of binding soluble eg, whereas the T cell is restricted to binding ag displayed on self cells. Thus T cells system has developed to eliminate these cells altered self cells.

Like B cells, T cells express distinctive membrane molecules. All matured T cells express the following membrane molecules.

- Thy-1 -> earliest marker of the T cells lineage
- CD3 -> membrane complex associated with the TCR.
- CD28 -> Receptor for the costimulatory B7
- CD45 -> Signal Transduction molecule.

G cell Response:-

After Ag stimulation both B and T cells become activated, proliperate and may differentiate into memory cells. The ad product of B cell activation is, Ig secretion. But the T cell has 2 main functions:

- They produce a series of non-Ig soluble mediators such as cytokines, which influence many different cells.
- They may kill the cell that contains the foreign ag.
  When a naïve T cell encounters Ag associated with an MHC molecule on a cell. The t cell proliferates and differentiates into memory T cells and various effector cells.

There are 2 well defined sub population of T cells;

- T-Helper cells (TH)
- T-Cytotoxic cells(TC)
- And a least important another sub population is, T-suppressor cells(Ts).

TH cells:-

The ratio of TH & Tc cells in normal humal peripheral blood is 2:1, but it may be significantly altered in immunodeficiency diseases autoimmune disease and other disorders. T cells displaying CD4, generally function as TH cells. After the recognition of Ag with class I MHC complex, the cell is activated and produces increased amount of IL2, activates Tc cells, B cells and various other cells that participate in the immune response.

- TH 1 response, results in a cytokine profile that activates mainly t cytotoxic cells and macrophages, whereas the TH2 response activates mainly B cells

TC cells:-

The T cell expressing CD8 recognize Ag associated with class I MHC molecules function as T-cytotoxic cells(Tc) and are class I MHC restricted. Tc cell is activated by interaction with an Ag-class I MHC complex on the surface of an altered self cell9dry virus infected cells), in the presence of appropriate cytokines, generates, CTLS, mediates the destruction of altered self cells.

TS cells:-

Some T cells mediate suppression of humoral and cell mediated branches of the immune system, but no actual Ts cell has been isolated. It is unsute whether Ts cells constitute a separate sub population or whether the suppression is as a result of the activities by TH & TC cells.

Null cells:-

These are the small group of peripheral blood lymphocytes, which do not express membrane molecule, they lack immunologic specificity and memory.

Natural Killer cells:-

These are a functional population of null cells which are large granulated lymphocytes, they play important role in host defense against tumour cells in 2 different ways.

- An NK cells makes direct membrane contact with the tumour cell in on a non specific Ab dependent process.
- Some NK cells express CD16, a membrane receptor for the cooff-teminal and of the ab molecule. These NK cells can bind to antitumour abs found to the surface of tumour cells and subsequently destroy tumours. This specific process is called ADCC.
  Amount the receptors on the NK cell, LFA(lymphocyte function associated ag) & ICAM (intercellular adhesion molecule) play an important role in NK cell mediated response.

Myeloid lineage

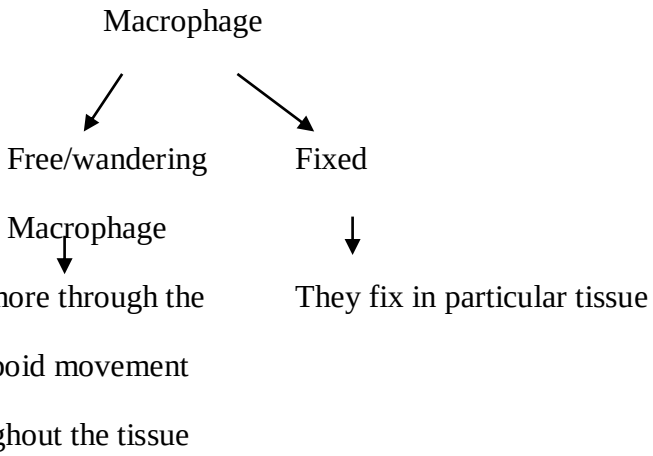
Mononuclear cells:-

The mononuclear phagocytic system consist of

- circulating monocytes in the blood
- Macrophage in the tissue
  Blood monocytes are differentiated into macrophages, involves a number of changes.

- The cell enlarge 5 to 10 fold.
- Its intracellular organells increase in both number and complexity
- It acquires increased phagocytic activity
- Produce high level of lytic enzyme
- Progressive maturation of mononuclear and phagocytic cells.

Macrophage:-



The name of the macrophage differs from where if resides:

* Kupffer cells- in liver

*Histiocytes- in connective tissues

*Alveolar macrophage - in lungs

*Mesaogial cells - in kidney

*Microglial cells - in brain

*Osteoclasts - in bone.

Macrophages are normally in resting stage, or antigenic stimulus the are activated.

Macrophage activity can be further enhances by,

- Cytokine secretion by TH cells
- Mediators of inflammatory response
- Bacterial cell wall
- INF gamma secreted by activated TH cells

  Activated macrophages secrete various cytotoxic proteins, that help them to eliminate a blood range of pathogens including virus infected cells, tumour cells and intracellular bacteria.

Phagocytosis:-

Macrophages are actively phagocytic cells capable of ingesting and digesting exogenous Ags such as whole Microorganisms, insoluble particules inures and dead lost cells and cellular debeis. The steps involved are,

- Chemotoxis
- Adherence and opspnigation
- Ingestion
- Destruction

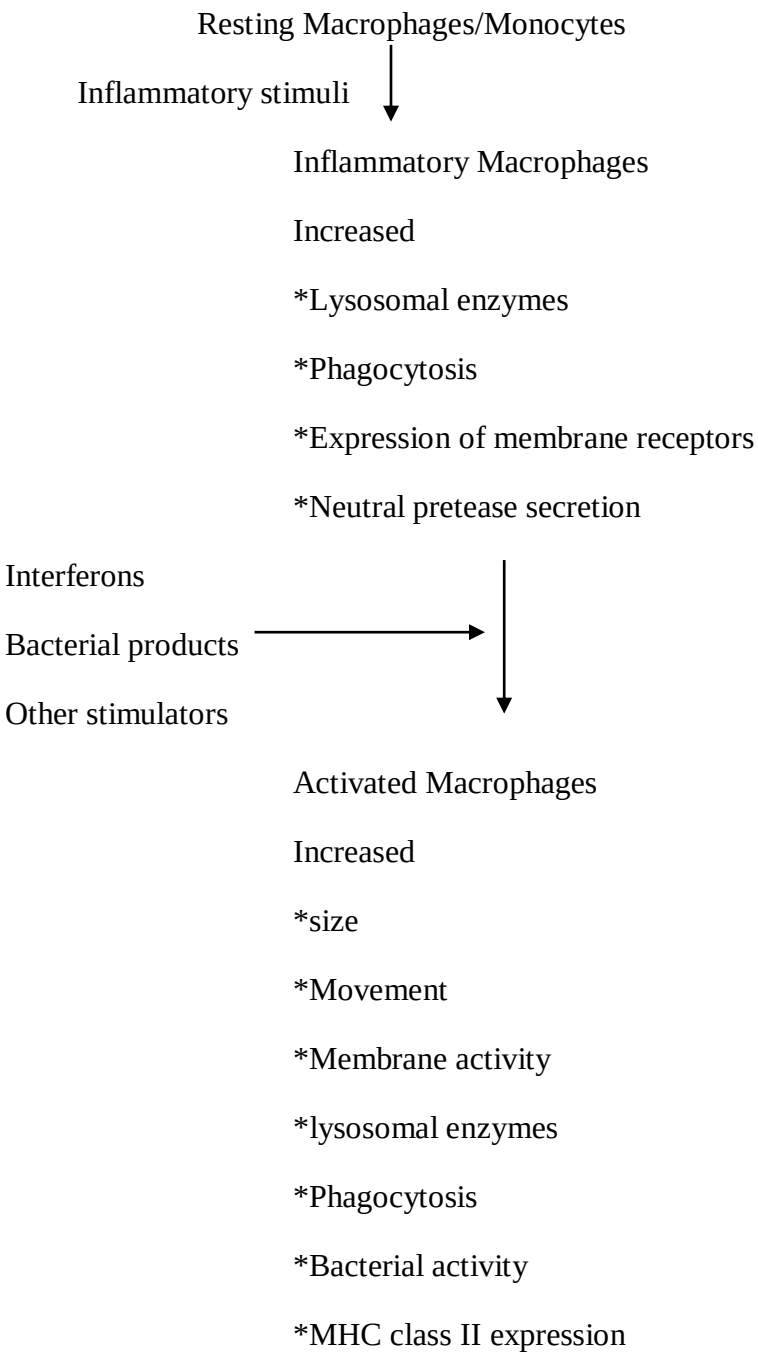


Fig: The activity of mononuclear phagocytes cells is a multistep process.

Chemotaxis:-

Macrophages are attracted not only to bacterial products such as the N- formylmethionyl peptides complements compentnet (c5a) and some cytokines but also to factor released by mamages cells and extra cellular matrix (fragments of collagen, elastic & fibrinagen). This process is called chemotaxis.

Neuutrophils on dying , release rlatase and collegenase and so generated monocyte chemotactic factors. Neutropils are thus the martyrs of the immune system: They reach and attack foreign mateials first, and in dying they attract macrophages to the site of microbial invasion.

Adherence & Opsonisation:-

Whole bacteria cells or virus particles adherse, whereas isolated proteins and encapsulates bacteria tend to adherse poorly. The hydrophobic nature of the ag induces adherence rapidly. For example My tuberculosis attaches strongly on a phagocyte than the capsulated bacteria.

The phagocyteic rate can be increased by the presence of opsonins, are molecules that bind to ag and to macrophages. The macrophage membrane possesses receptors for certain classes of abs and certain complement components. When an ag is coated with the appropriate ab complement component, it binds more readily to the macrophage, as a result. Phagocytosis is enhanced. Thus ab and complement are epsonins ad the process is called opsoniyation.

Ingestion:-

Adherence induces membrance protrusons called pseudopodia to extend around the ag & it fuse, enclosing the ag within a membrane bound structure called phagosome, which then enters the endocytic processing pathway.

Destruction:-

A No, of antimicrobial & Cytotoxic substances produced by activated marophages are responsible for the intracellular destruction of phagocytosed molecules. The toxic effect is produed by Oxidative and Non oxidative mechanism.

Oxidative Pathway:-

Activated macrophages produce a No. of reactive O2 intemediates(ROI) & Reactive N2 intermediates (RNI) that have otent antimicrobial activity. During Phagocytosis a process known as “respiratory birst” occurs in activated macrophages results in the activation of a amembrane bound oxidase that catalyses the reduction of O2 to super oxide anion(O2-) which is toxic to microbes. The superoxide also generates other oxidasing agent such as singlet O2(O2), H2O2 hydroxyl radical, halide ion etc.,

When macrophages are activated with bacterial cells wall lipopolysacchoride or muramyldipeptide, muramine dipetide together with a Tcell derived cytokine(INFgama). They egin to express high levels of Nitric oxide synthase, which oxidises the terminal guanido nitrogen atom of L-arginine, leading to the production of no. (nitric oxide) and Giteulline. Other reactive oxides of notrogen such as No2, No2-,N203 and No3- as well as nitrosamines and notrosothiols may also be produced. Those oxides of nitrogen are toxic. They form complexes

with iron containing enzymes and thus inhibit oxidative metabolism of ingested bacteria and intracellular parasites.

Macrophage derived No an inhabit lymohocyte and tumour cells DNA synthesis. Alternatively No can react with superoxide to form ONOO-, the peroxynitrite anion. This delayed to form the hightly toxic off radical

ONOO - + H4 --------> OH + NO2
< -------

When an ag persists for long period within the body, macrophages may accumulate in large nos around the ag, and look like epithelium on histiological examination. These cells are called epithelioid cells. They are usually packed closely together & hence are polygonal. They possess abundant cytoplasm containing many lysomes and much endoplasmic reticulum. Epithelioid cells may fuse to form multinucleated gaint cells when attempting to enclose particles too large to be ingested by a single cell. These cells are often found in the case of tubercle infection.

Non-oxidative pathway:-

Activated macrophages synthesize lysozyme and various hydrolytic enzymes have degaditive features which do not require the involvement of O2. In addition, activated macrophages produce a group of antimicrobial and cytotoxic peptides known as "defensins'. These molecules are cysteine rich, cationic peptides compsed of 29-35 aa residues. They can kill variety of bacteria including S-aureus, S-pneumoniae, E-coli, P-aeruginosa and H-influenyae. Activated macrophages also serete tumour necrosis factor alpha (TNFalpha), which is cytoxic for tumour cells but not to normal cells.

Pathogens resistant to phagocytosis:-

Some of the molecules can survive & multiply within the macrophages. These intercellular pathogens include Listeria monocytogenes, S-typhimurium, Neisseriae, M-tuberculosis. These organisms resist phagocytosis by:-

*Preventing lysosome - phagosome fusion & proliferate within the phagosomes.

*The cell wall components that sender them resistant to the content of lysosomes. And still others survive by escaping from phagosomes and proliferating within the cytoplasm of infected macrophages.

Granulocytic Cells:-

The granulocytic cells are classified based on their staining properties as;

- Neutrophils
- Eosinophils
- Basophils.

Neutrophils:-

The neutrophils has a multilobed nucleus and granulated cytoplasm that can be stained with both acid and basic dyes. It is often called polymerphonuclear leucocytes(PMN). Neutrophils are produced in the bone marrow during hematopoiesis. They are released into the peripheral blood and circulate for 7-10 hrs before migrating into the tissues, where they have a 3 days life span. In response to many types of infection, the bone marrow releases more than the usual no. of neutrophils. Neutrophils are,

- The first cells to arrive at a site of inflammation.
- Active phagocytic cells.
- They employ both oxidation and non oxidative mechanism to generate antimicrobial substances.
- They constitute about 50-70% of circulating WBCs.

Functions of Neutrophils:-

- Phagocytosis.

Chemotaxis:-

Chemotaxis is the directed movement of neturophils under the influence of external chemical gradients. Since neutrophils can crawl but cannot swim, they must attach to a surface before they can respond to chemotactic stimuli. Circulating neutrophils are triggered to leave the bloodstream as a result of local changes in blood vessel walls. In tissues where cells are damages as a result of bacterial invasion, there is an increase in adhesiveness of both neutrophils and vascular endothelial cells. In the vessel walls, the endothelial cells are stimulated to express surface proteins called integrins and selection. They stick to these endothelial cells before migrating into tissues.

Bacterial invasion and tissue destruction result in the production of many chemotatic factors, such as C5a, IL-8. IL8 is produced by many cells, including macrophages, fibroblasts, epithelial cells, and endothelial cells. Some lipid molecules, such as the leukotrienes released by blood platelets, can also attract neutrophils. Bacteria producing a peptide containing a unique amino acid, formylated methionine, strongly attracts neutrophils.

The chemo attractants trigger a change in cell surface electrical potential, and enhancement of plasma membrane fluidity, and a rapid increase in intracellular calcium levels. As a result the cells align themselves along the concentration gradient and crawl towards the source of the chemotactic materials.

Adherence and Opsonisation:-

If a bacterium is lodged in tissues, or trapped between a neutrophil and another cell surface and thus prevented from floating away, it can be readily ingested. This process is known as “surface phagocytosis” . firm attachment of an ag is required for a effective

phagocytosis. But adherence does not happen spontaneously, since both cells and bacteria have a negative charge called the Zeta potential and so repel each other.

The negative charge on the bacterium must be neutralized by coating it with a positively charges protein. For example ab molecules & C3b, bacteria coated with ab or C3b . have a reduced surface charge, enabling them to stick to negatively charges neutrophils, because they have membrane bound receptors for C3b and Fc protein of an ab. Molecules that coat bacteria in this way and so promote phagocytosis are called opsonins(Opsonins from Greek word for sauce , that they make the bacterium tastier for the necutrophil).

Ingestion:-

By pseudopodia, the neutrophil engulfs the attacked ag. Neutrophil cytoplasm readily flows over the hydrophodic surface as in the case of macrophages.

Destruction:-

Oxidative and Non-oxidative.

The Respiratory burst:-

- Within seconds of binding to a foreign particles neutrophils increase their O2 consumption nearly 100 fold, due to the rapid assembly and activation of a cell surface enzyme called NADPH oxidase.
- NADPH oxidase is a multicomponent enzyme that forms a transmembrane electron transport chain with cytosolic NADHP as the electron donor and O2 as the electron acceptor.
- When ab ag binds to neutrophil through ab on the Fc receptor, the oxidase is activated, thus converts, NADPH to NADO+ and donates O2 accepts a single donated electron, and converts into superoxide anion. NADPH oxidase

  NADPH + 202 ----------> NADP + + H+ + 2O2.

- The NADP+ generated by the eny. Enters into PP pathway and generated energy for the cell.
- The produced 2 molecules of O2 – interact spontaneously (dismutation) to generate one molecules of H2O2 by the enzyme superoxide dismutase.

  Superoxide dismutase

  2O2 - + 2H+ ----------> H2O2 + O2

- H2O2 may then be converted to other bacterial compounds through the action of myeloperoxidase, the most significant respiratory burst enzyme in neutrophils found in large amounts in the primary granules.
- The H202 reacts with intracellular halide ions to produce hypohalides.

  Myeloperoxidase

  H2O2 + Cl - ----------> HG20 + OCl-

- Because of its reactivity OCl- does not accumulate but rapidly disappears in multiple reaction. OCl- is a powerful oxidant, kills bacteria by oxidizying their proteins and enhances the bactericidal activities of the lyosomal emzymes.
- OH radicals & singlet O2, both are very potent bactericidal agents, may also be generated by O2- , H2O2, and a metal catalyst, thus,

Fes +

$$H2O2 + O2 - \text{----------} > 2OH + O2$$

Non oxidative pathway:-

- Lysozyme can destroy the cell walls of some nonpathogenic G+ bacteria and other enzymes secreted in the phagolysosome destroys the ag.
- However some bacteria, Brucells abortus Listeria sp are resistant to phagocytosis.

Fate of neutrophils:-

Neutrophils possess a limited reserve of energy in the form of glycogen, They lack of stored energy sources, even inactive neutrophils survive for less than a day before dying in a proces called apoptosis. The dying neutrophil is usally ingested by macrophages before it can release its lysosomal enzymes and causes inflammation / tissue damage. These neutrophils may be considered a first like a desfence against an ag. Since neutrophils usually destroy all ingested ag they cannot prepare ag for presenation.

Eosinophils:-

They have a bilobed nucleus and granulated cytoplasm, which can be stained with the acid dye, “eosin y”. They are motile phagocytic cells, can migrate from the blood into the tissue spaces. They have a mahor role in defense against parasitic oganisms. The secretions of cosinophilic genules result in damage of the membrane of parasites. They constitue about 1-3% ciculatory WBCs.

- The granules of cosinophils contains acid phosphatase and peroxidase. The cosinophil peroxidase is chemically distinct from that found in neutrophils ad uses bromide ions in prefernce to chloride, so producing Obr-, This peroxide is more eficiently than neutrophil peroxide. In addition cosinophil granule contains a protein called major Basic protein (MBP) is highly toxic for invading parasitic worms.
- Phagocytosis by eosinophils is similar to the process in other cells.
- Specilized eosinophil chemotactic factor; for example 2 terapeptides (Val-Gly-Ser-Glu & Ala-Gly-Ser-Glu) secreted by most cells.
- Non Specific chemotactic factors for eosinohils include C5a,C567, histamine and some metabolites of arachidonic acid.
- Eosinophis are much more suited to extracellular destruction of large parasites. Since, they can extrude their granules into the surrounding gluid. Thus, when they stick to parasites, they extrude MBP and other toxic proteins, destroyed the worm’s cuticle.

Basophils:-

They have a lobed nucleus and heavily granulated cytoplasm, which can be stained with the basic dye, such as methylene blue, hematoxylin. They constitute less than 1% of circulating WBCs. They are non phagocytic cells, but contains pharmacologically & active substances, play a major role in certain allegic response(inflammatory response).

Mast Cells:-

Other tha the above meational 3 major cells, mast cells also a gramlocyte. During hematopoiesis mast cell precurssors are released from the bone marrow and don't differentiate until they minced with ciruclating blood & tissues. Mast cells found in a wide variety of tissues including the skin, connective tissues, and mucosal epithelical tissues of various orans and or respiratory genitourinary and digestive tract. And these cells have a significant part in the development of allergy.

Dendritic cells:-

The dendritic cells acquired its name because it is covered with a mass of long membrane resembling dendrites of nerve cells. Most dendritic cells process and present ag to TH cells. These cells can be classified based on their location.

- Non Lymphoid organs:-
  Skin – Langerhans cells
  Mucous membrane – Interstitial dendritic cells.
- Lymphoid organ:-
  Thymus (T cell area) – Interdigitating dendrific cells.
  Bone marrow(B cell area) – Follicular dendrific cells.
- Circulation:-
  Blood – Blood dentrific cells
  Lymph - veiled cells.

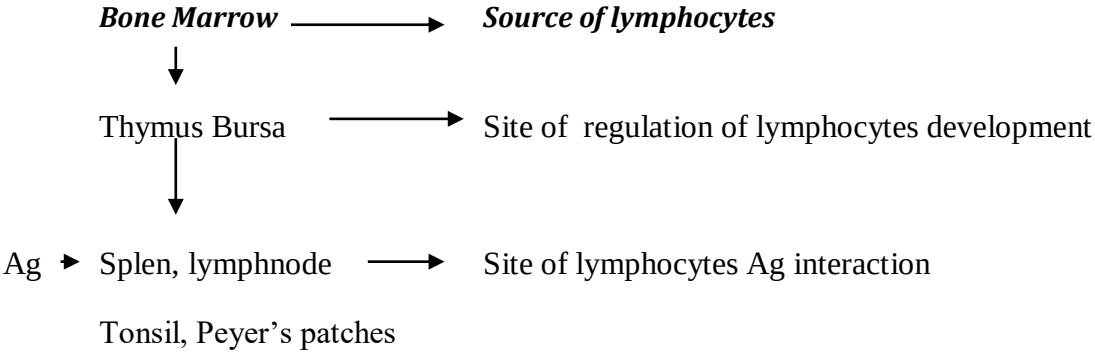
Funcitons:-

Most dendritic cells express high levels of both Class II MHC & constimulatory B-7 molecules. They are more potent APC than macrophages & B cells, both of them need to be activated.

CHAPTER 2 THE LYMPHOID ORGANS

The lymphoid organs are those organs in which lymphocyte maturation, differentiationa dn proliferation takes palce. Lymphocytes are the predominant cells in organs such as spleen, lymph node and thymus. So the major function of those organs is mount immune response following the centry of an ag. That is these response occurs within the lymphoid organs, which must therefore provide an microenvironment, in which efficient intraction between lymphocytes & Apcs may occur.

Lymphoid organs may be classified on the basis of their role in generating lymphocytes with specific regulations and in providin an environment for the optimal interaction between Ag & ag sensitive cells.



The lymphoid orans are generally classified into 3 categories.

- Primary lymphoid organs
- Secondary lymphoid organs
- Tertiary lymphoid organs (Normally contains few lysmphoid cells, which can import lymphoid cells during an inflammatory response).

Primary lymphoid orans:-

The thyms and bone marrow constiute the primary lymphoid organs where maturation of lymphocytes occurs. They are otherwise known as central lymphoid organs(bursa of fabrilicis is in bird instead of bone marrow). Immature lymphocystes generated during haematopoeisis, marured and become commited to a particular antigneic specificity within these organs.

These organs arise early in actal life from outgrowth at the ectodermal junction or from endoderm. Newly formed lymphoid stem cells from the yolk sac, the factal liver, first migrates to these orans via the blood stream. Then only the maturation of lymphocytes occurs ie., eneration of immuno competent cells, ie., capable of mounting an immune response. B & T cell maturation occurs in the bone marrow & thymus respectively.

Thymus:-

- The thymus is a pale tabulated oran located in the thoracic cavity between the lung.
- The size of the thymus varies considerably, relative large in the newborn and absolute size is greatest at puberty.
- After puberty the thymus atrophies & its cortex is gradually replaced by fatty tissue but remenants of thymus do presists until old age.
- In addition thymus shrinkage occurs rapidly in response to stress.

Structure:-

Thymus contain of cobules of loosely packed epitheloal cells, covered by a connective tissue capsule. Each lobule is divided into 2 compart ments, namely,

- The outer compartment/Cortex
- The inner compartment/Medulla.

Cortex:-

Which is densely infiltrated with rapidly dividing lymphocytes called thymocytes (immature T cells).

Medulla:-

Which is sparsely populated with thymocytes and the epithelial cells which are clearly visible. Within the medulla are sound bodies called thymic(Hassal's) carpuscles, whose function is not known.

Both the cortex and medulla of the Thymus are crisscrossed by a 3-dimensional stromal cell network composed of epithelial cells, interdigitating dendritic cells and macrophages, which make up the frame work of the organ and contribute to thymocyte maturation. Many of these stromal cells physically interact with the developing thymocytes. Some thymic epithelial cells in the outer cortex, called "nurse cells" have long membrane processes that susround as many as 50 thymocytes forming large multicellular complexes.

Functions:-

- The major function of the thymus is the maturation of thymocytes. The actual sequence of T cell maturation within the thymus is not completely understood.

Haematopoeisis → Progeniter T cells→ Enters Thymus→ Multiply rapidly within the cortex (This rapid multiplication of thymocytes is coupled to an enormous rate of cell death)
For this, a small subset of matured thymocytes migrates from the cortex to medulla ⟶ Maturation continues in medulla ⟶ Leave the thymus via postcapillary venules. But some studies shows that a small population of cortical thymocytes can mature and leave the thymus without ever entering the medulla..

Molecular mechanism of Maturation and selection of T cells:-

- Thymic epithelial cells secrete the hormonal factors.

➔ L1 thymosin
➔ B4 thymosin
➔ Thymopoietin
➔ Thymulin
➔ Thymostimulin

which are necessary for the differentiation and maturation of thymocytes, but the role each of these factors remain unknown.

- IL7 secreted by thymic stromal cells stimulates the growth of thymocytes.
- After developing thymocytes begin to express ag-binding receptors., ie., thymic stromal cells, express high levels of class I & class II MHC molecules, play a role in the selection process.
- During the selection process, any developing thymocytes that are unable to recognize self-MHC molecules or that have a high affinity for self ag plus self-MHC are eliminated by "Programmed cell death".
- Thus only those cells whose receptor recognizes a self-MHC molecules plus foreign ag are allowed to mature.

Thymus and immune response:-

The function of the thymus may be demonstrated by removing it and studying its consequences. Thymectomy in adults has no immediate obvious effect, but if the thymus is removed fom mewborn rodents (neonatal thymectomy), its consequence are immediately apparent. There is dramatic decrease in circulating lymphocytes of the T cell lineage and an absence of CMI, which results an increase in infectious disease.

* Digeorge's synclrome - in humans

The decline in immune response that accompanies

➔ aging
➔ leading to an increase infections
➔ Autoimmunity & cancer.

Bursa of fabricius:-

In birds a lymphoid organ called the bursa of fabricius, is the primary site of B cell maturation and it is found only in birds but not in mamals.

Structure:-

- It is a hallow sac about 1cm in diameter, located just above the cloacca and connected to it by a duct.
- The bursa reaches its greatest size in about 1-2weeks after the chick has hatched , and then it gradually atrophies.

- It develops from the hindgut apithelium at about 15th day of development. By the time of hatching it is fully functional. By 4 months it attains a size of 3 cms.
- Inside the bursa, folds of epithelium extend into the lumen and secreted through these folds are follicles of lymphoid cells.
- Each follicle is divided into a cortex & medulla.
- The cortex contains lymphocytes, plasma cells and macrophages. At the boundary between these regions (corticomedullary junction) there is a basement membrane and capillary network, on the inside of which are epithelial cells. These medullary epithelial cells are replaced by lymphocytes towards the center of the medulla, so that the center of the follicle appears to consist solely of lymphocytes. Each follicle is directly connected with the epithelium covering the surface of the fold.

Functions:-

- Provide the site for maturation and differentiation of B-cells.

Bursa & Immune response:-

Bursectomy:- slight drop in B lymphocytes, but they stop producing ab and lose their plasma cell. Bursectomized birds still reject foreign grafts, indicates the role of HMI & importance of CMI in transplantation. Several hormones secreted by bursa controls the maturation & differentiation of B cells. The most important of these is "Bussin" a tripeptide (lys-his-glycylamide).

Bone Marrow:-

There is no bursa in mammals; instead regions of bone marrow serve as the bursal equivalent where B cell maturation occurs. Bone marrow is the soft tissue within the cavity of the bones. In mammals it is the largest tissue with a total weight of about 3 Kgs in an average human adult.

Structure:-

Bone marrow can divided into 2 regions namely,

- vascular & adipose (tissue) region
- Portion ie., involved in haematopocisis or blood cell formation.

The vascular region is the circulating system that supplied nutrients and removes waste from the actively growing blood cells. This tissue forms about half the weight of marrow. The other half of the bone marrow which is actively involved in haematropoeisis is known as the "red bone marrow". This contains all the different cells of the blood with their precursors. In adult animals, most of the red bone marrow is replaced by a fatty tissue and become 'yellow marrow" during aging.

Functions:-

- immature B cells proliferate and differentiate within the microenvironment of the bone marrow.

- Stromal cells within the bone marrow interact directly with the B cells and secrete various cytokines that are required for the B cell maturation.
- Similar to T cell maturation, a selection process within the bone marrow eliminated B cells with self reactive ab receptors.
- About CO4 B cells are produced in the bone marrow in humans and they survive for several months.

Secondary lymphood Organs:-

The secondary lymphoid organs (SLO) arise from mosoderm, late in fetal life and presist through adult life. They grow in response to antigenic stimulus. The SLO consists of certain structure in which mature ag to undergo further division and differentiation. The major SLOA are

- Spleen
- Lymph node.
  In addition to this tonsils, appendix small intestine (Peyer's patches) as well as lymphoid aggregate spread through out the mucosal tissue are considered as SLOs. The later comprises of varibus areas o the body, such as the linings of the digestive tract, respiratory tract, the conjunction and the salivary glands. These SLOs, have been called as
- Mucosal associated lymphoid tissue-MALT
- GVT assocaited lymphoid tissue –GALT
- Bronchus associated lymphoid tissue-BACT.

Functions:-

- They are highly efficient in trapping & concentrating foreign substances.
- They are the main site of production of abs and the generation of ag specific T cells.

Lymphatic system:-

As blood circulates under pressure, the third component of the blood seeps through the thin wall of the capillaries into the surrounding tissue. Much of this fluid, called interstitial fluid, returns to the blood through the capillary membrane. The remainder of the intestitial fluid now called "lymph". The lymph flows from the connective tissue spaces into a network of the tiny open lymphatic capillaries and then into series of progressively larger collecting vessels called lymphatic vessels.

Functions:-

- Ag enters to the body

  ↓

  Trapped by the lymphatic system

  ↓

Through this ag, is carried to various organs which finally trap ag.

- Maintains the steady state level of the fluid within the circulatory system
- Transporting of lymphocytes.

Lymph node:-

Lymph modes are round or bean shaped structures (normally less than 1cm in diameter) placed on lymphatic vessels in such a way that they can filter any foreign materials carried by the lymph. Lymph node consists of fibrous reticular network filled with lymphocytes-Macrophages and dendritic cells. Lymphatic sinuses penetrate the node. The overall architecture of a lymph node provides an ideal microenvironment for lymphocytes to effectively encounter and respond to trapped ags. Morphologically a lymph node can be divided into 3 roughly concentric regions.

- Cortex
- Paracortex
- Medulla

Cortex:-

The outer layer, the cortex contains predominantly of the B cells & are arranges in nodules. Before exposure to ag, they are called as "primary follicles". In lymph node, that have been stimulated by ag, the cells within primary follicles expand to form structure known as "germinal centre". A follicle containing a g germinal centre is known as secondary follicle. Intense B cell activation and differentiation into plasma and memory cells occurs in the germinal centers of lymph nodes.

Paracortex:-

Beneath the cortex is the paracortex which is largely populated with T cells and also contain interdigitating dendrific cells though to have migrated from tissues to the node. These cells express high levels of Class II MHC, which are necessary for Ag presentation to T cells. The paracortex is referred to as Thymus dependent area, whereas the cortex is thymus independent area.

Medulla:-

The innermost layer of lymph node the medulla is more sparsely populated with lymphocytes, but many of these are plasma cells. The initial activation of B cells is also through to take place within the T cells rich paracortex.

Lymphocyte circulation:-

The thoracic duct is the major lymphatic vessels of the body. Lymph flows through the thoracic duct at about 500ml/hr and if contains about 1*10^8 lymphocytes/ml. As lymph leaves the nodes it is carried through large lympatic vessels which eventually drain into the circulatory

system at the thoracic duet. Lymphatics from the lower body join to form the duct, and it empties into the vena cave. The lymphocytes in the blood account only 2% of the total lymphocyte population of the body.

The increase of lymphocytes in lymph leaving the node is due in part to lymphocyte proliferation within the node in response to ag. However, blood borne lymphocytes that migrate into the node by passing between specialized endothetical cells lining the post capillary venules of the node. 25% of the lymphocytes leaving lymph node have migrated across this endothelial layer & entered the node from the circulation. Antigenic stimulation increases the concentration of lymphocytes in nodes resulting in visible swelling of the nodes. Factors released in lymph node during ag stimulation are through to facilitate this increased lymphocyte migration.

Functions:-

- Acts as efficient filter, trapping ag carried by the lymph.

Spleen:-

Just as lymph node filters lymph to the spleen filters blood, removes the ag & aged blood cells. The spleen stores RBC, platelets & it is involved in the formation of RBCs in the foetus. The spleen is the major size or the production of ab & effector T Cells.

Structure:-

The spleen which is about 5 inches long in adults is the largest lymphoid organ. The spleen is a large, avoid secondary lymphoid organ situated high in the left abdominal cavity. The spleen is surrounded by a capsule that sends a number of projections (trabecular) into the interior to form a compartmentalized structure. The components are 2 types, namely.,

*Red pulp –formation, storage of RBC & trapping ag.

*White pulp-immune response against the trapped ag.

These compartments are separated by a diffuse marginal zone of cells & marginal sinuses.

Red Pulp:-

The spleen in red pulp consists of a network of sinusoids populated with macrophages & numerous crythocytes. It is the site where old & defective RBCs are destroyed and removed. Many of the macrophages within the red pulp contains engulfed RBCS or iron pigments from degraded hemoglobin.

- **White pulp:-**

The white pulp of the spleen consists of masses of lymphocytes closely associated with the spleen, blood vessels. Vessels that enter the spleen travel through the muscular trabecular before entering its functional areas. Immediately on leaving the trabecular, each arteriole is surrounded by a thick layer of lymphocytes called Periarteriolar lymphoid sheath (PALS). The arterial capillaries eventually leave this sheath after branching opens either directly or immediately into venous capillaries. The PALS consist of T cells & is the depleted following neonatal

thymectomy. However, scattered through the sheath are primary follicles containing B cells. On antigenic stimulation these follicles develop germinal centre & so become secondary follicles. Each follicle is surrounded by a layer of T cells in what is known a marzinal zone.

Spleenic response:-

Blood borne ags carried into the spleen through the spleenic artery, which empties into the marginal zone, where they are taken up by macrophages and carry the ag to the primary follicles in the white pulp. After the few days, ab producing cells migrate from the primary follicle and colonize the marginal zone and the red pulp. So the ab production is first detected in these regions. Germinal centre formation occurs within the primary follicle within a few days. In animals already possessing circulating abs, the ag is trapped by dendritic cells within the secondary follicles. As in a primary immune response, the ab producing cells migrate from these follicles to the red pulp and to the marginal zone, where ab production largely occurs. Some ab may also be produced within the secondary follicles.

MALT:-

- The mucosal membranes lining the digestive, respiratory & urogenital systems are the major sites of entry for most of the pathogens. These tract are guarded immunologically by the subepithelial accumulation of lymphoid tissues.
- These may occur as diffuse connection of lymphocyte plasma cells and phagocyte throughout the lungs and lamine propria of the intestinal wall.
- Organized tissues with well formed follicles. In man it includes the lingual, palantine & Pharyngial tonsils, in the small intestine –Peyer's patches and the appendix.

Functions:-

- The malt has a large population of ab producing plasma cells.
- It forms a separate inter connected secretoary system within which cells connected to IgA or IgE synthesis.

Tonsils:-

The tonsils are found in 3 locations.

- Lingual at the base of the tongue.
- Palatine at the side of the back of the mouth
- Nasopharyngial (adenoids) in the roof of the pharynx.

All the 3 groups are nodular structure consisting of a meshwork of reticular cells & fibres interspersed with lymphocytes, macrophages, granulocytes & mast cells. The B cells are organised into the follicles & germinal centre. The later are surrounded by regions showing T cell activity. Play a role against the ags entering through the nasal & oral routes.

GALT:-

Lymphoid cells are found in 3 regions within the tissues.

- Intraepithelial lymphocytes(IELs):-

  The outer mucosal epithelial layer contains, so calle IELs. The majory of these lymphocytes are CD8+ cells that express unusual T cell recepter (gama8-TCTRs), are suited to encounter ags that enter through intestinal mucous membrane.

Lamina propria:-

The lamina propria which lies under the epithelial layer contains a large No. of B cells, plasma cells, activated TH cells & macrophages in loose clusters.

Peyer's patches:-

Within the submucosal layer of the intestinal tissues are nodular consisting of 3-40 organised lymphoid follicles called peyer's patches, and they can develop into secondary follicels with germinal centre.

M cells & transport of ag:-

The epithelial cells of mucous membrane plays an important role in promoting the immune response by delivering small samples of foreign ags from the lumen of the respiratory, digestive urogenital tract, to the underlying MALT. This ag transport is carried out by specialised cells called M Cells.

Structure:-

These are flattened epithelial cells, which contains a deep invagination or pocket in the basolateral plasma membrane, this pocket is filled with a cluster of B cells, T cells and macrophages.

Functions:-

Luminal ags are endocytosed into vesciles that are transported from the luminal membrane to the underlying pocket membrane. The vesicles then fuse with the pocket membrane, delivering the ags to the clusters of lymphocytes contained within the pocket

Inductive sites:-

M cells are located on inductive sites which are small regions of mucous membrane that lie over organised lymphoid follicles. Ags transported across the mucous membrane by M cells activate T cells within these follicles. The activated B cells differentiate into plasma cells, which lead follicle & secrete if A class of abs. These abs are then transported across the epithelial cells & are released as secretory IgA into the lumen, where they interact with the ag present in the lumen.

Tertiary lymphoid organs:-

Cutaneous Associated Lymphoid tissue:-

The epidermis consists of 3 types of cells, namely

- Keratinocytes
- Langerhans cells
- Intra epidermal lymphocyte.

Keratinocytes:-

The outer epidermal layer consist of specialized epithelial cells called keratinocytes, secrete a no. of cytokines that may induce a local inflammatory reactions. And these cells express class ii MHC & function as Apc.

Langerchans cells:-

The cells are scattered among the epithelial cell matrix of the epidermis are known a langerhans cells, a type of dendritic cell, which interacts with ag by phagocytosis. These cells, them migrate from the epidermis to regional lymph nodes, where they differentiate into inter digitating dendritic cells & express class II MHC & function as activator of naïve TH cells.

Intraepidermal lymphocytes:-

These are similar to intraepithelial lymphocytes of MALT in that they are largely CD8 + T cells.

UNIT II

CHAPTER 3 THE COMPLEMENT SYSTEM

Introduction:

"The complement system is a set of proteins that act together to destroy invading microorganisms". This system plays a critical sole in the defense of the body in association with abs and the cells of the immune system.

Complement:

This system consists of nearly 30 serum and membrane proteins. Following initial activation, the various complement components interact, in a highly regulated enzymatic cascade, to generate reaction products that facilitate ag destruction and generation of an inflammatory response.

Cascade reactions:

The general principles of these reactions are that the products of one reaction catalyze a second reaction, whose products then catalyze a third reaction, and so on. This results in an expanding 'cascade' of reactions. Enzymatic chain reactions of this type are known as cascade reactions and usually require a 'trigger' to initiate the reaction chain, provided that many of the intermediate products either are,

- Present in limiting quantities.
- Have a very short half-life, or
- Easily inhibited

Then only it is possible to ensure that the reactions proceed very rapidly yet in a controlled fashion.

Complement is an essential part of the body's defense, but it must be carefully regulated since uncontrolled activation can lead to massive cell destruction. The complement system was discovered in 1893 by Jules Bordet, who called it "alexine". However, Paul Ehrlich later introduced the rival term "complement".

There are 3 pathways of complement activation:

- The classical pathway
- The alternative pathway
- Lectin pathway

The 3 pathways share a common terminal reaction sequence that generates a macromolecular "membrane.attack complex (MAC)", which lyses a variety of cells, bacteria, and viruses.

The Complement Components:

- The proteins and glycoproteins that form the complement system are either labeled numerically with the prefix 'C' – for e.g., c1,c2,c3-or designated by letters of the alphabet – B,D,P, and so forth.
- There are at least 19 of these components; they are all serum proteins and together they make up about 10% of the globulin fraction of serum.
- The molecular weights of the complement components vary between 24kDa for facture D and 460kDa for CLq.
- Serum concentrations in humans vary, b^+ 20μg/ml –C2 and 1300μg/ml – C3
- Complement components are synthesized at various sites throughout the body.
- Liver - C3,C6,C8 & B
- Macrophages- C2,C3,C4,C5,B,D,P & I

As a result, these components are readily available for defense at sites of inflammation where macrophages accumulate.

- Generally these components are in inactive forms in the serum, many of them as 'proenzymes' in which the enzymatically active site is marked. Each proenzyme is activated by cleavage of the molecule, there by removing the mask and exposing the active site.
- The complement components B, C4 and C2 are coded for by genes located in the MHC class III region.

Complement activation:

The effector functions of complement can be activated through 3 pathways.

- Classical pathway → activated by Ag-Ab complex
- Lectin pathway → activated by lectin binding to mannose. (Lectin-mannose complex)
- Alternative pathway → activated when a spontaneously activated complement binds to the surface of a pathogen.

Each pathway generates a protease called "C3 convertase' by a different route but the 2 convertases are identical and all are homologous and have the same activity and so the later events are the same for all the 3 pathways.

CLASSICAL PATHWAY

The components are designated as the letter 'c' followed by a number (C1,C4….) unfortunately the components were numbered in order of its discovery not the sequence which,

C1,C4,C2,C3,C5,C6,C7,C8,C9.

The products of cleavage reactions are designated by alphabets. The larger fragment is designated as 'b' and smaller as 'a'. For e.g., C4 cleaved into larger C4b and smaller C4a. Activated complement components are often designated by a horizontal line up on the letter. For e.g., C4bC2b – C3 convertase.

C1:

The first component of classical pathway of complement activation is C1, which is a complex of 3 proteins, C1q, C1r and C1s, held together by calcium-dependent bonds. That is two molecules of each of C1r and C1s [$C1r_2s_2$] being bound to each molecule of C1q [$C1q_2s_2$]. In the presence of calcium-chelating agents (such as EDTA) the C1 trimolecular complex falls apart.

C1q molecule has 6 globular head joined to a common stem by long filamentation domains like collagen molecule. It is composed of 18 polypeptide chains of 25kDa. The whole C1q complex along with Clr and Cls looks like a bunch of 6 tulips held together by the stems. Each globular head can bind to one Fc domain and binding of 2 or more globular heads activates the C1q molequle.

The binding of Clq to a single bound IgM or to two or more IgG molecules leads to the activation of an enzymatic activity in Clr followed by Cls to generate an active 'serine protease'.

Activation of Cl in normal serum is prevented by a protein called "Cl inhibtor" (CI-INH). Cl-INH binds to active Clr and Cls preventing their activation and removing active Clr and Cls from the complex. This inhibitory effect is overcome by complement activators such as immune complexes.

The binding of 2 or more Clq strands to an Ig results in a conformational change in the Clq that is transmitted to Clr. As a result, Clr changes to reveal an active protcolytic site, which cleaves a peptide, bend in Cls to convert that molecule to an enzymatically active form (serine protease). Cl is activated by Igs such as IgG and IgM. But IgG is much less effective than IgM,

since its structural configuration favours activation of C1. Cl may also be activated directly by the surface proteins of some viruses or by bacteria such as E-coli and K.pneumonia.

Cascade reaction:

Once bound ab has activated Cls. The Cls enzyme acts on the neat 2 components of the classical pathway clealing C4 and then C2 to generate two large fragments C4b and C2b, which together form C4bC2b, which is otherwise called as 'C3 convertase' of the classical pathway. Its most important function is to clear large number of C3 molecules into C3b, some of which bind to pathogen surface and C3a molecules triggers, mast cells and basophils to release histomine hence C3a is referred to as 'anaphylotoxin'.

Complement component C3 is a most abundant complement protein in serum. The activation of C3 by C4b2b (C4bC2b) is a major step since each C4b2b complex can activate as many as 200 C3 molecules.

Terminal complement pathway:

The next step in cascade is the generation of 'C5 convertase', by the binding of 'C3b to C4b2b' yields 'C4b2b3b'. This complex binds C5 and alters its conformation, so that the C4b2b3b component can cleave C5 into C5a, which diffuses, away, and C56 which attaches to the antigenic surface. The most dramatic effect of complement activation is the assembly of terminal components of complement to form a "membrane attack complex (MAC). The end result is a pore in the lipid – bilayer membrane of the ag, that destroys membrane integrity.

MAC:

The first step in the formation of MAC is the cleavage of C5 by a 'C5 convertase".

- One molecule of C5b then binds to C6 to form a complex C5b6, then it binds to one molecule of C7. C7, can bind to the C5b6 complex either in solution or bound to the cell membranes. When C5b67 is formed, it generates a meta stable membrane binding site. If it does not bind to a membrane, the complex self – aggregates and is inactivated.
- The next step is the binding of C8 to the membrane bound C5b67 complex, undergoes a conformational change, and penetrates the lipid – bilayer of the cell.
- The entire C5b678 complex acts as an initiator to induce the polymerization of C9, i.e., 10 to 18 molecules of C9 into the annular / ring structure called membrane attack complex ($C5B678\ (9)_n$).
- During polymerization the C9 molecules undergo a "hydrophilic amphiphilic transition . so that they also can insert into the membrane.
- This MAC has hydrophobic external phase and hydrophobic internal channel. The diameter of the channel is 100A; allowing free passage of solute and water across the lipid bilayer.
- Pores formed by MAC permits the passive exchange of small soluble molecules but not macromolecules. This situation results in influx of water into the cell and loss of electrolytes leading to "osmotic lysis". The MAC striking is similar to "perforin" pores generated by cytotoxic cells (Tc) and natural killer (NR) cells.

Alternative Pathway

Complement component C3 can also be activated by a second route called the alternative complement pathway. This pathway involves C3, Factor B, factor D and properdin (P). Unlike classical pathway which generally requires Ab for its initial activation. But the alternative pathway is initially activated in most cases by various cell surfaces constituents, that are foreign to the host.

- Serum C3, which contains an unstable thiorster bond, is subject to slow spontaneous hydrolysis (less than 1%) into C3b and C3a.
- This C3b comes in contact with bacterial cell wall. The cell wall of both G+ve and G-ve have constituents that can activate alternative pathway.
- This event occurs depends on the affinity of C3b for a protein called "Factor H' and this in turn depends on the nature of the surface.
- Lipopolysacchariode and pepitodolycon of bacterial membrane can induce alternative pathway.
- The membranes of most mammalian cells have high levels of "Silalic acid" which contributes to the rapid inactivation of bound C3b molecules on host cells. because many foreign antigenic surfaces have only low levels of sialic acid, C3b bound to these surface remains active for longer time.
- Surface bound C3b is able to bind a to a serum protein factor "B" to form C3bB6 complex, has C3 convertase activity and thus is analogous to the C4b2b complex in the classical pathway. The C3 convertise activity of C3bBb has a half-life of only "5 minutes" unless the serum protein properdin (p) binds to it, stabilizing it and extending the half-life of this convertase activity to 30 minutes (C3bB6P).
- The C3bBb generated can activate unhydrolyzed C3 to generate more C3b autocatalytically. As a result, the initial steps are repeated and amplified, so that more than 2×10^6 molecules of C3b can be deposited on an ag in less than 5 minutes.
- This C3 convertase cleares additional C3 to C3b and C3a results 'C3bBbPC3b complex' which is equal to 'C5 convertase' [C4bC2bC3b of classical pathway).
- This C5 convertase can activate the assembly of membrane attack complex (MAC) i.e., initiation of 'terminal complement pathway".

Classical pathway: Proteins that participate to formation of C5 convertase

Component	**Active product / split product**	**Immunologic function**
C1	C1q	Binds to F_c region of IgM and IgG
	C1r	Serine Protase: enzymatically activates C1s
	C1s	Serine protease: enzymatically activates C4 and C2
C4	C4a	Peptide mediator of inflammation (anaphylatoxin)
	C4b	Binds C2 forming complex that is cleared by C1s to yield C4b2b
C2	C2a	Serine protease: C4b2b acts as C3 convertase
	C2b	Unknown function
C3	C3a	Peptide mediate of inflammation (anaphylatoxin)
	C3b	Binds to C4b2b to form C5 convertase; 'major opsonin'

Alternative pathway : proteins that participate in formation of C5 convertase

Components	Active protein/ split product	Immunologic function
C3	C3a	Peptide mediator of inflammation (anaphylatoxin)
	C3b	Binds factor B, forming complex that is cleaved by factor D to yield C3bBb.
Factor B	Ba	Unknown function
	Bb	Serine protease : C3bBb acts as C3 comartase, which generates C3bIb3b (C5 convertase)
Factor D	D	Serine protease: cleaves factor B that is bound to C3b to form C3 convertase
Properdin		Binds to and stabilizes C3bBb.

Biological activities of Complement:

- Complement mediated cytolysis by MAC formation.
- Opsonization and promotion of phagocytosis of microbes.
- Anaphylatoxins (C3a, C4a and C5a etc); induces the release of vasso active substances like Histanine (nitrous oxide in rat prostaglandia). This results in contraction of smooth muscles (invaleatary muscles)
- Solubilization and phagocytic clearance of immune complexes. Immune complexes are formed by Ag binding to Fab and Fe of one Ab to Fe of another. These binding can be blocked by binding of complement to Fc.
- Diseases caused by complement:
    - Hereditary angioneurotic edema (HANE) caused by the deficiency of 'C1 inhibitor'.
    - C3 deficiency is associated with pyogenic bacterial infection that are fatal (pus formation)
    - Deficiencies of the early components $C1qr_2s_2$, C4 etc., result in Glomerulo nephritis and systemic Lupus erithematoses (SLE)

Defeciencies in terminal components like C5, C6, C7, C8 and C9 will result in non-formation of MAC.

CHAPTER 4. ANTIGEN

Introduction:

Substances capable of inducing a specific immune response commonly are referred to as ags i.e., antibody generators or immunogens.

Immunogenecity and Antigenicity:

- Immuno-genecity is the inherent ability of a substance to induce a specific immune response. The molecule induces such response is called as antigens and more appropriately immunogens.
- Antigenicity is the ability of a substance to combine specifically with the final products of the immune responses i.e., HMI and CMI.
- All of the immunogens are antigens but all antigens are not immunogens.

Factors that a influence immunogenicity:

The immune system actually recognizes particular macromolecules of an infections agent, generally either proteins or polysaccharides, the former are a potent ag than the later. In contrast nucleic acids and lipids do not serve as immunogens unless they are complexed to proteins / polysaccharides.

The degree of immunogenecity of a molecule is influenced by several factors, namely,

- Foreigness
- Molecular size
- Chemical composition and complexity
- Degradability
- Structural stability.

*** Foreigness:**

In order to elicit an immune response, a molecule must be recognised as nonself by the biological system. That is the degree of immunogenicity of an ag depends on the degree of its foreigness.

The greater phylogeneitc distance between 2 species,the greater the the immunogenicity.

For e.g., BSA is not immunogenic to cow and, but is an excellent immunogens when injected into rabbit and chicken because cow and goat are closely related to bovine than rabbit and chicken.

Some macromolecules like collagen and cytochrome C, were highly conserved throughout evolution and therefore display very little immunogenecity across diverse sp.

And some self components are known as immonlogically privileged sites such as corneal tissue and sperm, can elicit immune response in an animal from which they originated.

Then cellular organelles, mitochondria, are not normally exposed to immune system. The extensive cell destruction, mitochondria is exposed to the cell of the immune system and antimotochondrial antibody may develop.

Molecular size:

In general, large molecules are better args than small molecules. The size of the best immunogens ranges 100000 Da. Generally, substances with a Mu less than 5000-10000 Da are poor immunogens.

For e.g., hemocyanin – a large protein (6.7×10^3 Kda) is a potent ag. The hormone angiotensin – smaller mol (1031Da) is a poor ag. However, in some cases substances with a MW less than 1000 Da have proven to be immunogenic.

Chemical composition and Complexity:

The synthetic homopolymers tend to lack immunogenicity regardless of their size. But the lopolymers can elicit the better immune response than homopolymers.

The addition of aromatic amino acids, such as tyrosine / phenylalanine, has a major effect on the immunogenicity of the antigen. For e.g., a synthetic copolymer of glutamic acid and lysine requires a minimum molecular weight of 30000-40000 for immunogenicity. But the addition of tyrosin to the copolymer, the reduces the required MW for immunogenicity to 10000-20000. And the addition of both tyrosine and phenylalanine reduces the minimum for immunogenicity to 4000.

And the structural complexity of the molecule also affects immunogenicity. For e.g., primary, secondary, tertiary and quaternary structures of protein.

*** Degradability:**

The induction of CM1 requires the ag that has been processed and presented in association with class II, IMHC molecule on an APC; for T_H and T_c cells respectively.

'Macromolecules that cannot be degraded and presented with MHC molecules are poor immunogens'. For e.g., polymers of D-amino acids, which are sterioisomers of the naturally occurring L-aminoacids. Because the degradative enzymes within APC can only degrade proteins containing L-amino acids, polymers of D-amino acids cannot be processed and thus are poor immunogens.

Likewise plastics, steel are insoluble one and thus will not act as immunogens.

Contribution of the biological system to immunogenicity:

Other than those above mentioned factors, the immunogenicity of an ag, depend on certain properties of biological system that the ag encounters, namely,

- Genotype of the recipient animal.
- Dosage and route of administration of an ag.

Genotype of the recipient animal:

The genetic constitution (genotype) of an immunized animal influenzes the type of immune response as well as the degree of response. For e.g., 2 different inbred strains of mice exhibited very different responses to an immunogens, one strain produced high antibody titre and whereas the other strain produced low antibody titre. But the F1 generation these individuals produce an intermediate response to the immunogen. So the immune response is regulated by a genes in the MHC.

Dosage and route of Administration:

The immune response also depend on the dosage of administration of an immunogen. An insufficient dose will not stimulate an immune response either because it fails to activate enough lymphocytes or because it induces a non-responsive state (in the case of excessive high dose of most experimental immunogens will not induce a strong response; rather repeated administration over a period of weeks stimulate a strong immune response. Such boosters, increase the cloral proliferation of ag specific T or B cells.

Usually antigens are administered parenterally (around gut) and the following administration routes are common;

a. Intravenous : Into a vein.
b. Intradernal : Into the skin.
c. Subcutaneous : beneath the skin
d. Intramuscular : into a muscle
e. Intraperitoneal : into the peritoneal cavity

The immune response depend on the route of administration of an antigens. That is intravenous injection induces splenic response where as subcutaneous injection induces local lymph node response.

Adjuvants:

Adjuvants are substances, enhances the immunogenicity of an antigens when mixed with an ag. And are often used to boost the immune response when an antigens has low immunogenicity or when only small amounts of an ag are available. For e.g., BSA with an adjuvant produces five fold increase response than BSA alone.

Adjuvant enhances the immunogenicity of an ag by;

- Prolong ag persistence
- Enhance co-stimulatory signal
- Induce granuloma formation.
- Stimulate lymphocyte proliferation non specifically

→ Aluminium Potassium Sulfate increase antigens persistence by precipitation, results in a slower release of antigens from the injection site, so that the effective time of exposure to the antigens increase from a few days to several weeks. And it also increases the size of an antigens, thus enhances the phagocytosis.

→ Freund's water-in-oil adjuvants:

a. ag + H_2O + mineral oil + emulsifying agent (mannide monnoleate)
b. Freund's complete adjuvant
    - Heat killed mycobacteria + water + oil + emulsigying agen.

- Other adjuvants such as synthetic polyribonucleotides and bacteria lipopolysaccharides stimulates nonspecific lymphocyte prolification.

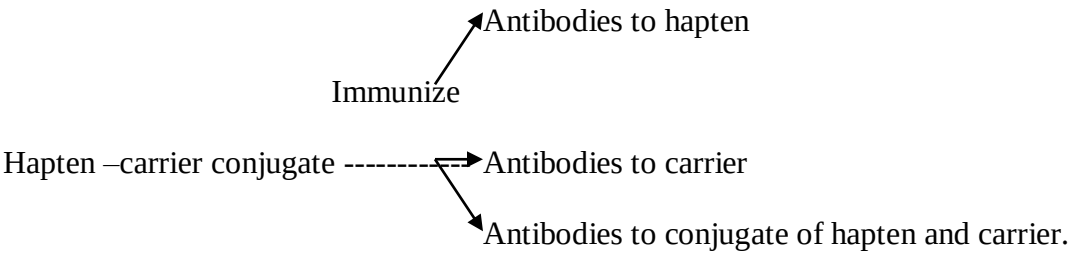
Haptens:

Haptens are small organic molecules that are antigenic but not immunogenic, the pioneering work of Karl Landsteiner created a simple, chemically defined system for studying the binding of an individual antibody to a unique epitope on a complex antigens. He coined those small molecules as Haptens.

Chemical coupling of a hapten to a large protein, called a carrier, yields an immunogenic hapten-carrier conjugate. Animals immunized with such a conjugate produce antobody specific for;

- The hapten determinant
- Unaltered epitopes on the carrier protein
- New epitopes formed by combined parts of both the hapten and carrier.

The beauty of the hapten-carrier system is that it provides the facts about the various chemical structures on immune specificity.



By observing which hapten modifications prevented or permitted cross reactions, landstriner was able to gain insight into the specificity of antigens -antobody interactions.

And using various derivatives of aminobenzene as haptens landstriner found that the over all configuration of a hapten plays a major role in determining whether it can react with a given ab.

Many biologically important substances, including drugs, peptide hormones, and steroid hormones, can function as haptens. So the hapten specific abs are useful for measuring the presence of various substances in the body. For e.g., β - HCG pregnancy test employed anti haptens antibodies.

Epitopes:

In an immunogen, lymphocytes recognize only discrete sites on the immunogen called epitopes / antigenic determinants.

"Epitopes are the immunological active regions of an immunogen that bind to antigens specific membrane receptors on lymphocytes or to secreted abs".

Ag recognition by B and T cells is fundamentally different when analysed with small ag. For e.g., when mice were immunize with glucagons, a small hormone of 29 amino acids ab was elicited to epitopes in theNH_3 terminal portion, where as the T cells responded only to epitopes in the COOH terminal portion.

Because T cells exhibit MHC – restricted antigens recognition, T-cell epitopes cannot be considered apart from their associated MHC molecues.

*** Comparison of antigens recognition by T and B cells:**

Characteristic	B cells	T cells
Interaction with ag	Involves binary complex of membrane Ig and Ag	Involves ternary complex of T cell receptor, Ag and MHC
Bindign of Solube Ag	Yes	No
Involvement of MHC molecules	None required	Required to display processed ag.
Chemical nature of ag	Protein, Polysaccharide, lupid	Only protein
Epitope Properties	Accessible, hydrophilic, mobile peptides containing sequential or non sequential amino acids.	Internal linear peptides produced by processing of ag and capable of binding to MHC.

Properties of B cell epitopes:

- The size of a B cell epitope is determined by the size of the ag binding site on the ab molecules displayed by B cells. In order for a strong interaction to occur, the ab's binding site and the epitope must have a complementory conformation. The studies revealed that 15-22 aas on the surface of the protein ag make contact with a similar number of residues in the ab's binding site. In the case of globular protein ags, the epitope is entirely dependent on the tertiary conformation of the native protein. Epitopes on globular protein ags appear to be considerably largely and occupy a more extemsive surface on the ab then do small ags.
- B cell epitopes in the native proteins generally are hydrophilic aminoacids on the protein surface that are topographically accessible to membrane bound or free antibody.
- B cell epitope must be accessible in order to be able to bind to an ab. Amino acid sequences that are hidden within the interior of a protein cannot function as B cell epitopes unless the protein is first denatured. For e.g., Abs elicited by (T,G)-A-L copolymer react largely with the A-(T,G)-L copolymer in which the tyrosine and glutamic acid residues are buried.
- B cell epitopes can contain sequential or non-sequential amino acids. The native conformation protein elicits ab response, because denaturation of ag usually results in loss of the topographical structure of their epitopes, abs to the native protein do not bind to the denatured protein.
- B cell epitopes tend to be locaed in flexible regions of an immunogen and display site mobility.
- Complex proteins contain multiple overlapping B cell epitopes, some of which are immunodominant, induce a mere pronounced immune response than other epitopes in a particular animal.

Properties of T cell epitopes:

In 1959 Gell and Barry suggested that there was a qualitative difference between the T and B cell response to protein ags. In 1980's, it become clear that T cells do not recognize

soluble native ag but rather recognize ag that has been processed into antigen peptides, which are presented in association with MHC molecules.

- Antingen peptides recognized by T cells from trimolecular complexes with a T cell receptor and MHC molecule.
    - Ags recognized by T cells must, therefore posses two distinct interaction sites.

→ Epitope – Interacts with the T cell receptor

→ Agretope – Interacts with an MHC molecule

- The ag binding cleft on an MHC molecule interacts with multiple oligomeric peptides, which function as T cell epitopes. Studies of the binding of peptides to class I MHC molecules have revealed that peptides of 9 aa bind most strongly, peoptides of 8-11 aa also bind but generally with lower affinity than nonamers. In the case of Class II MHC molecules, peptides of 12-25 amino acid residues are preferentially bound.
- Epitopes recognised by T cells are often internal.

Mitogens:

Mitogens are agents capable of inducing cell division in a high % of T and B cells. A mitogen can activate many clones of T and B cells irrespective of their ag specificity, hence they are known as polyclonal activators.

For e.g., Sugar binding protein – Lectin binds to membrane glycoproteins often leads to agglutination, or dustering, of the cells, which may trigger cellular activation and proliferation.

3 common lectins with mitogenic activity are; concanavalin A (conA), Phytohemagglutinin (PHA) and Pokewed mitogen (PWM); ConA and PHA activate T cells, and PWM activates both T and B cells.

Other than lectins, the Lipopolysaccharide (LPS) component of the G^{-ve} bacterial cell wall functions as a B-cell mitogen.

Superantigens:

An unusual group of substances, known as super ags, are among the most potent T cell mitogens known.

Super antigens bind to residues in the variable domain of the T cell receptor and to residues in class II MHC molecules outside of the ag binding cleft, thereby cross links a T cell to a class II MHC molecule even when the TCR does not recognize the bound agenic peptide, leading to activation of the T cell.

For e.g., Staphylococcal enterotoxins (Ses)

Toxic shock syndrome Toxin1 (TSST1)

One out of every five T cells can be activated by a super ag, resulting in the release of abnormally high levels of cytokines, can lead to shock and death.

CHAPTER 5. IMMUNOGLOBULINS

Ig function as antibody, the ag binding protein present on B cell membrane and secreted in plasma cell. They slow diversity and heterogenity. There is evidence of extensive gene rearrangements which generate different type of Ig capable of many different antigens. They are a group of glycoproteins present in the serum and tissue fluid.

Igs – A family of proteins:

5 distinct classes of Igs are recognised IgG, IgA, IgM, IgD and IgE. They differ in size, change, aa composition and carbohydrate content. Igs within each class are heterogeneous. Electrophoretically they show a range of variations.

Igs – are bifunctional molecules:

It is each Ig is bifunctional. Its region is concern with the binding to ag while a different region mediate the effector function. Effect or function include binding of Ig to the host tissue, to various cells of the immune system, to some phagecytic cells and to the first component of Cg of the classical complement system.

Structure:

Ig is a unit consisting of 2 identical, light phypeptide chain with molecular weight 25,000 designated as L chains and 2 polypeptide chains are heavy chain m.w 50,000 designated as H chain. The class and subclass of Ign are determined by its heavy chain thus and human IgG subclasses have heavy chains called δ_1, δ_2, δ_3, δ_4 that differ slightly. When Iga is subjected to pepsin treatment (non specific protrolytic activity) digests the Ign into 2 fab like fragments that fragments retaining ag binds activity. And F_e fragment (fragment become crystal or crystallized during cold storage) this is not revered because it has been digested into fragments. The same digestion was done with papain produce 2 identical fab fragments and 1 F_c garment.

All Igns are glycoproteins but the carbohydrate content ranges from 2-3 for IgG, 12-14% for IgM, IgD, IgE.

Light chain sequencing:

The amino-terminal half of the chain consisting of 100-110 aas was found to vary, this region is variable region (V). The carboxyl terminal half of the molecule part is constant region

(C), had two basic aa sequences designated as Kappa (K) and Lambda(λ). In human 60% of light chains are kappa and 40% are Lambda whereas in mice 95% K, 5%λ.

Heavy chain sequencing:

The amino terminal half show great sequence variation from one H chain to the next-variable region. The remaining of the protein reveals 5 basic aa sequence corresponding to 5 different H-chain constant region. Each of the different H-chain constant regions is called Isotype. The heavy chains of a given ab determine the class of that ab.

Variable region domains:

V_L and V_H revealed that the sequence variability is concentrated in this part-hypervariable region. The remainder of the domain reveal less variable region – Frame work region (FRS). The HV region from he ag binding site because this is complementary to the structure of epitope of the ag. This region is called 'complementarity determining region'.

Antigenic determinants of Ig:

They fall into 3 major types.

1. Isotypic
2. Allotypic
3. Idiotypic that means sequence differenced between Ab may be of these types.

Isotypic Variation:

Genes for Isotypic variants are present in all healthy member of species. E.g., genes for δ_1, δ_2, δ_3, δ_4, α_1, 2, μ^1, δ, Σ, kappa and lambda chains all are present in the human genome and are therefore Isotypes.

Allotypic variations:

Although all members of a species inherit the same set of isotype genes – multiple alleles exists for some of the genes. These alleles encode aa differences in the constant region of the IgN. This occurs in the some members of the species. In human all type have been characterized for all 4 IgG subclasses, for one IgA subclass and for kappa hight chain. The δ chain allotypes referred to as Gm markers. There are 25 different Gm marker identified. E.g., variant of IgG3 is

characterised by a phenylalanine at position 436 of δ_3 H-chain. It is not found in all peoples. Allotypes occurs as variants of H-chain constant region.

Idiotypic Variations:

Variations in variable region that to in hyper variable region produces idiotypes. Each individual agenic determinant of the variable region is known as idiotope. In some cases it may be the actual ag binding site and in some users it consist of V-region sequences outside of ag binding site other than ag binding site. The ab present multiple idiotopes. Some of idiotopes called idiotype of the ab.

Ign Classes:

There are 5 differetn classes of Ign. They can be distinguished by unique aa sequces in the heavy chain constant region. There are IgG, IgA, IgM, IgD and IgE.

IgG:

It constitute about 80% of total serum Ign. The IgG is a monomer. There are 4 subclasses in human and their different from one another in the size of the number of disulfide linkeages. Min – 1,46,000 – distributed between vascular – intravascular – extravascular pool. These are the major Abs of secondary immune responses. In man they cross the placenta and give high degree of passive immunity to the new turn.

IgM:

IgM account fo 10% of the total serum Ign. Monomeric IgM has a molecular weight 180000 is expressed on membrane of the B cell. It is secreted by plasma cell as a pentamer in which 5 monomeric units can join together by disurphide bond. They are arranged with the F_c region and 10 ag binding site present in the perifuric of the molecule. It has a J chain which is disulphide bonded to the carboxyl terminal region. It is a firsts Ign produced in a primary response. Because of 10 ag binding site it has a higher valency than other ab molecules. It can bind either with 10 small lapten or 5 larger ag molecules. When RBC are incubated with specific ab they clump together in large aggreicantsin a process called agglutination. Because of its larger size it cannot diffuse into the tissue. They largely confined in the intravascular pool.

IgA:

IgA constitute 15% of total Ign. In man more than of serum IgA occurs as a monomer but in most mammals it is a polymer. The other types of secretary IgA present in enternal secretions. Such a milk, saliva, tears and mucus of brichi, genitourinary and digestive tract. Secretary IgA consist of timer, T chain and a polypeptide chain known as a secretary component. The J chain facilitate the polymerization of IgA molecule. The secretory component 17000 MW peptide produced by epithelial cells of mucus membrane. It consists of 5 domains that bind to FC region of the ab. They are linked.

IgE:

It is scans in serum – 0.3 μg/ml of blood. They mediate hypersensitivity reactions that are responsible of the symptoms of hay fever, asthma and some allergic diseases. It plays a role in immunity to helminthic parasite. IgE binds to F_c receptors on the membranes of blood basophils and tissue mast cell. Cross linkage of receptor bound IgE by ag (allergon) induces degranulation of basophil and most cell as a result a variety of pharmacellagically active mediators are released giving rise to allergic manifestations.

IgD:

Constitute about 0.2% serum Ign (30mg/ml). It is a major membrane bound Ign expressed by mature B cell. It plays a role in the activation of B cell by ag. It is more susceptible to proteolytic action and there is a single disulfide bond between the H-chain. Large amount of oligosaccharide distributed in multiple units.

UNIT IV

CHAPTER 6. ANTIGEN – ANTIBODY INTERACTIONS

Introduction:

The ag – ab interaction is a bimolecular association similar to an enzyme. Substrate interaction but it does not lead to an irreversible chemical alteration i.e the interaction is reversible. The specificity of ag – ab interactions has led to the development of a variety of immunologic assays. These assays can be used to detect the presence of either ab or ag and have played vital roles in diagnosing diseases, monitoring the level of the humoral immune response, and identifying molecules of biological or medical interest.

Strength of antigen – antibody interactions:

The interaction between an ab and an ag involves various non covalent interactions between the antigenic determinant, or epitope, of the ag and the variable region (V_H /V_i) domain of the ab molecule, particularly the hyper variable regions.

The non- covalent interactions: Includes ;

- Hydrogen bonding
- Ionic bonding
- Hydrophobic interactions and
- Vander waals interactions.

Because the strength of each of these interactions is weak, a large number of such interactions are required to form a strong Ag – Ab interactions.

A strong ag – ab interaction depends on a very close fit between the ag and ab ($1x10^{-7}$ mm = 1 A)

Antibody affinity:

- The strength of the total non-covalent interactions between a single ag – binding site on an ab and a single epitope is the affinity of the antibody for that epitope.
- Low affinity abs – binds weekly
- High affinity abs – binds stronger.

Antibody avidity:

- The affinity at one binding site does not always reflect the true strength of the ag – ab interaction.
- The strength of the multiple interactions between a multivalent ab and ag is called the avidity (in the case of abs with multiple binding sites).
- So the avidity of an ab is a better measure of its binding capacity with in biological systems than is the affinity of its individual biding sites.
- High avidity can compensate for low affinity. For eg. Igm often has a low affinity than Ig G but has a high avidity, enables it to bind ag effectively.

Cross reactivity:

Although Ag- Ab reactions are highly specific in some cases ab elicited by one ag can cross react with an unrelated ag. Such cross reactivity occurs if two different ags share a identical epitope or if abs specific for one epitope also bind to an unrelated epitope processing similar chemical properties.Cross reactivity is often observed among polysaccharide ags that contain similar oligosacharide residues. For eg. ABO blood – group ags.

Precipitation reactions:

- The interaction between an ab and a soluble ag in aqueous solution a lattice that eventually develops into a visible precipitate.
- Abs that thus aggregate soluble ags are called precipitins.
- The precipitate develops as neighbouring ab molecules within the lattice form ionic bonds with each other, causing the lattice to lose its charge and thus become insoluble.
- Formation of an Ag – Ab lattice depends on the valency of both the ab and ag:

  The ab must be bivalent.The ag must either be bivalent or polyvalent.

Several common immunologic assays are based on precipitation reactions; the sensitivity of these assays varies considerably.

Precipitation reactions in fluids:

- A quantitative precipitation reaction can be performed by placing a constant amount of ab in a series of tubes and adding increasing amounts of ag to the tubes.
- Excess of either ab or ag interferes with maximal precipitation which occurs in the so called equivalence zone, when the ration of ab to ag is optimal.
- As a large multi molecular lattice is formed at equivalence, the complex in size and precipitates out of solution.
- In the region of ab excess, unreacted ab is found in the supernatant and unreacted ags in a antigen excess region.
- The principles of Ag excess, Ab excess and equivalence apply to many Ag- Ab reactions.

Rapid test : (for the presence of Ab /Ag):

The interfacial (ring) precipitin test is preformed by adding antiserum to a small tube and layering ag on top. If the antiserum contains abs specific for the test ag, then the ab and ag diffuse toward each other and form a visible band of precipitation at the interface within a few minutes.

Precipitation reactions in gels:

Immune precipitates can form not only in solution but also in an agar matrix. When ag and ab diffuse toward one another in agar or ab is incorporated into the agar and ag diffuses into the ab-containing matrix, a visible line of precipitation will form.These immunodiffusion reactions can be used to determine relative concentrations of antibodies or ags, to compale ags, or to determine the relative purity of an ag preparation.

The frequently used immunodiffusion techniques are radial immunodiffusion (Mancini method) and double immunodiffusion (ouchterlony method)

Radial immunodiffusion:

- Concentration of an ag can be determined.
- Ag sample placed in a well and allowed to diffuse into agar containing a suitable dilution of an antiserum.
- At the zone of equivalence a ring of precipitation forms around the well.
- The area of the precipitin ring is proportional to the concentration of ag.

- By comparing the area of the precipitin ring with a standard curve, the concentration of ag can be determined.
- Generally used to quantitate serum levels of IgM, IgG and IgA. And also used to determine the concentrations of complement components in serum.
- A lot applicable for the samples with ag concentrations below 5-10mg/ml

Double Immunodiffusion (ouchterlony method)

- Both ag and ab diffuse readially from wells toward each other. As equivalence is reached, a visible line of precipitation forms.
- Effective qualitative tool for determining the relationship between ags and the number of different Ag-Ab systems present.

Identity:

- Occurs when 2 ags share identical epitopes

Nonidentity:

- Occurs when 2 ags are unrelated (i.e., share number common epitopes)

Partial identity:

- Occurs when 2 ags share some epitopes but one or other has a unique epitope(s). The antiserum forms a line of identity with the common epitope(s) and a curved spur with the unique epitope(s)

Immunoelectrophoresis:

- This technique combines separation by electrophoresis with identification by double immunodiffusion.
- An ag mixture is first electrophoresed and separated by charge.
- Troughs are then cut into the aga gel parallel to the direction of the electronic field, and antiserum is added to the troughs.
- The gel is then incubated in a humid chamber during which time ag and ab diffuse toward each other.
- The formation of precipitn bands with polyvalent or specific antiserum identifies individual ag components.

Merits:

- Widely used in clinical laboratories.
- Specific serum proteins can be identified.
- To determine whether a patient produces abnormally low amounts of one or more isotypes, characteristics of certain immunodeficiency diseases.
- Overproduction can also determined (albumin, Immuno-globulin and transferin)
- Is a strictly qualitative technique that can detect ab concentrations of 3-20 mg/ml
- Used to detect immunodeficiency and immunoproliferative disorders.

The related technique of rocket electrophoresis permits quantitation of ag levels as low as 0.2 mg/ml.

Agglutination Reactions:

The interaction between ab and a particulate ag results in visible clumping called agglutination.

Abs produce such reactions are called agglutinins.

Agglutination reactions are similar in principle to precipitation reactions. Just as ab excess inhibits precipitation reactions, an excess of ab inhibits agglutination reactions. The inhibition is called the prozone effect.

- The prozone effect can also occur at high concentrations of abs that bind to the ag but do not reduce agglutination; these abs, called incomplete abs, (IgG)

Hemagglutination:

- ABO blood grouping is basically depend on the agglutination reaction between the abs and RBCs.
- At neutral p^H RBCs are surrounded by a negative ion cloud that makes the cells repel one another; this repulsive force is called the Zeta potential.
- Because of its size and pentameric nature, IgM can overcome the Zeta potential and cross-link RBCs, leading to agglutination.

- The smaller size and bivalency of IgG makes it less able to overcome the Zeta potential. For this reason IgM is more effective than IgG in agglutinating RBCs.
- But Abs to some RBC ags (eg. the Rb eg) are of the IgG class exclusively.
- For this the zeta potential of the RBC must be reduced, by placing the RBCs in serum albumin, which has a high net negative charge that reduces the effect of the negative ion cloud surrounding the RBCs thus allowing anti-Rh ab to agglutinate Rh^+ cells.

Bacterial agglutination:

- The presence of abs complementary to bacterial ags can be detected by agglutination.
- The agglutination titer of an antiserum can be used to diagnose a bacterial infection. Patients with typhoid fever, for e.g., show a significant rise in the agglutination titer to S.typhi

Passive agglutination:

- A soluble ag is mixed with RBCs that have been treated with tannic acid or chromium chloride, both of which promote absorption of the ag to the surface of the cells.
- Serum containing ab is serially diluted into microtiter plate wells, and the ag-coated RBCs are added to each well, agglutination is assessed by the size of the characteristic spread pattern of agglutinated RBCs on the bottom of the well.
- It is far more sensitive than precipitation reactions and can detect ab concentrations as low as 0.001mg/ml

Agglutination inhibition:

- If free soluble ag is added to an agglutinating is inhibited.
- Their technique provides a highly sensitive assay to detect small quantities of an ag.
- Used to rapid diagnosis of pregnancy.
- Latex particles coated with human chorionic gonadotropin (HCG) and ab to HCG. The addition of urine from a pregnant woman, which contained HCG, inhibited agglutination of the latex particles when the anti-HCG ab was added; thus the absence of agglutination indicated pregnancy.
- Used to determine certain types of illegal drugs such as cocaine or heroin.

RADIOIMMUNOASSAY

Three groups of techniques are used to detect and measure ag-ab combination. The most sensitive technique is to directly measure or visualize the immune complexes formed in an invitro system. These tests are known as primary binding tests.

RIA is one of the most sensitive techniques of detecting ag/ab. The technique was first developed by 2 endocrinologists, S.A.Berson and Rosalyn yalow, in 1960, to determine levels of insulin-anti-insulin complexes in diabetics.

- Used to quantitate hormones, serum proteins, drugs and vitamins at concentrations of 0.001mg or less.
- The principle RIA involves competitive binding of radiolabelled ag and unlabelled ag to a high affinity ab.
- The ag is generally labeled with a ∂ emitting isotope such as 125I

RIA for antibody: (RAST – Radio Altergo sorbent test)

- In this technique, ag-impregnated cellulose disks are immersed in test serum so that specific ab binds to the ag.
- After washing, the disk is immersed in a solution containing radiolabelled antiglobulin.
- The antiglobulin binds to the disk only if abs have first bound to the ag.
- The level of ab activity in the test serum is measured by counting the radioactivity associated with the disk.
- The RAST is used to measure IgE ab levels in allergic individuals.

RIA for antigen:

- This is based on the principle that unlabelled antigen may displace radiolabelled ag from immuno complexes.
- The amount of unlabelled ag displaced is directly related to the amount of unlabelled ag added.
- An isotope such as tritium (^{3}H) ^{14}Carbon, or ^{125}I is used.
- When radiolabelled ag is mixed with its specific ab, the 2 combine to form immune complexes that may be precipitated out of solution with NH_2SO_4.
- The radioactivity of the supernatant fluid provides a measure of the amount of unbound labeled ag.

- If unlabelled ag is added to a mixture of labeled ag and unbound ab, the unlabelled ag competes with the labeled ag for ab-binding sites.
- As a result, some labeled ag is unable to bind ab, and the amount of radioactivity in the supernatant increases.
- If a standard curve is first constructed by using known amounts of unlabelled ag, then the amount of ag in a test simple may be measured by reference to this standard curve.
- Extremely sensitive test.
- For eg: morphine can be measured in urine at concentrations of 10^{-8} to 10^{-10}M.

ELISA

Enzyme – linked immunosorbent assay, commonly known as Elisa (or EIA), is similar in principle to RNA but depends on an enzyme rather than a radioactive label.

An enzyme conjugated to an ab reacts with a colorless substrate to generate a coloured reaction product. A number of enzymes have been employed for ELISA, including alkaline phosphate, horseradish peroxidase and P- nitrophenyl phosphatase.

This assay have more sensitive than RIA and have the advantage of being safer and less costly.

Based on this principle different kinds of methods are developed, by which we can type the ELISA. That is a number of variations of ELISA have been developed – allowing detection and quantitation of either ag or ab.

Indirect ELISA:

- Ab can be detected or quantitated.
- Cerum sample containing primary ab (Ab) is added to an ag- coated microtiter well and allowed to react with the bound ag.
- Any free Ab1 is washed away.
- The presence of Ab bound to the Ag is detected by adding an enzyme – conjugated secondary anti – isotype ab (Ab2), which binds to the primary ab.
- Any free Ab2 then is washed off.
- Then a substrate for the enzyme is added.

- The colored reaction product that forms is measured by specilaised spectrophotometric plate readers which can measure the absorbance of a 96 well plate is less than a minute.

Merits:

- Is the method of choice to detect the presence of serum abs against HIV.
- Generally, serum abs to HIV can be detected by this method within 6 weeks of infection.

Sandwich ELISA:

- Ag can be detected by this method.
- Ab is immobilized on a microtiter well rather than the ag.
- A sample containing Ag is added and allowed to react with the bound ab (capture ab)
- Well is washed to remove if any free Ag.
- A second enzyme – linked ab specific for a different epitope on the ag is added and allowed to react with the bound ag.
- The any free secondary ab is washed away, substrate is added and the colored reaction product is measured.
- Because this test involves the formation of an Ab- Ag- Ab layers, they are called sandwich Elisa.
- Used to detect the ag in too diluted samples.
- Thus this test employed to detect circulating virus in blood from patients with AIDS.

Competitive ELISA:

- In this technique Ab is first incubated in solution with a sample containing Ag.
- Then the Ag- Ab mixture is then added to an Ag- coated microtiter well.
- The more Ag present in the sample, the less free Ab will be available to bind the Ag-coated well.
- Addition of an enzyme conjugated secondary Ab specific for the isotype of primary Ab, can be used to quantitate the amount of primary Ab bound to the well as in an indirect Elisa.
- In this assay, however, the higher concentration of Ag in the original sample lowers, the absorbance.

Immunofluorescence

Antibodies / Antigens that are bound to cells or tissue reactions can be visualized by tagging the ab molecules with a fluorescent dye, or fluorochrome.

- The most commonly used fluorescent dyes are fluorescein and rhodamine.
- Dyes can be conjugated to the Pc region of an ab molecule without affecting the specificity of the Ab.
- Each of these days absorbs light at one wavelength and emits light at a longer wavelength.
- The emitted light is generally viewed with a fluorescence microscope.
- By conjugating both these dyes to separate / individual Abs, one can visualize two cell-membrane ags simultaneously on the same cell.
- FITC (Fluroescein isothicyanate) labeled abs can be used in several ways, the most important of which are the direct and indirect fluorescent antibody tests.

Direct fluorescent antibody test:

- Used to identify the presence of ag (bacterium / virus).
- Primary ab is directly conjugated with FITC.
- A tissue or smear containing the organism is fixed to a glass slide and incubated with the fluorescent antibody.
- Then it is washed to remove unbound Ab.
- The labeled antibodies bound on ag is can be seen under fluorescent micro scope as brightly fluorescing object.
- This test can be used to identify the bacteria in low concentrations. For eg sputum for M. tuberculosis and lesions for clostridial organisms.
- It may also be employed to detect viruses growing in tissue culture or tissue from infected animals. For eg Rabies and HIV.

Indirect fluorescent antibody test:

- Used to detect the abs in serum or the demonstration and identification of Ags in tissues or cell cultures.
- During test for ab, ag is employed in the form of a tissue smear, frozen section, or cell culture on a slide or cover slip.

- This is included in the serum sample and the specific Ab for the ag reacts with it.
- And the serum is then washed off, leaving only specific abs bound to the Ag.
- These bound abs may be visualized with secondary Abs labeled with FITC.
- The quantity of the Ab in the test serum may be estimated by examining increasing dilutions of serum on different ag preparations.

Merits:

- This test has several advantages over the direct technique.
- First, the primary ab does not need to be conjugated with label. Because the supply of primary Ab is often a limiting factor.
- Indirect methods avoid the loss of ab that usually occurs during radio labeling.
- Second, indirect methods increase the sensitivity of staining.
- Use of anitglobulin sera specific for each immunoglobulin class allows the class of the specific Ab present in the serum to be determined.

Particle fluorescence immunoassay:

- Automated and quantitated immunofluorescence assy.
- Ag is bound to polystyrene particles and mixed with test serum.
- After incubation, the particles are filtered out and exposed to fluorescent antiglobulin.
- The suspension is filtered again and washed to remove unbound reagents.
- Then the particle suspension is examined with fluorometer.

Flow cytometry:

- Cells labeled with fluorochrome conjugate ab can be analysed and sorted on the basis of the intensity of staining in a specialised flow cytometer called a “fluorescence activated cell sorter” (FACS).
- That is a cell suspension labeled with a fluorescent monoclonal Ab to a cell surface Ag.
- The cell suspension is then passed across a laser beam (FACS) and the characteristics of the labeled and unlabelled cells are determined.
- It provides a rapid quantitative technique for the analysis of complex cell mixtures.

Applications of immunofluorescence assays:

- Immunofluorescence has been applied.
- To identify a number of subpopulations of lymphocytes, notably the CD_4^+ and CD_8^+ T-cell subpopulations.
- To identify bacterial species.
- To detect Ag- ab complexes in autoimmune diseases.
- To detect complement components in tissues, and
- To localize the hormones and other cellular products in situ.

Direct and indirect immunofluorescent staining of membrane antigen (m Ag):

Notes:

In the indirect method, the secondary ab, is most commonly, fluorochrome – labeled anti – isotype antibody. Such as fluorescein, labeled goat anti-mouse immunoglobulin.

Another reagent is fluorochrome labeled protein A from S. aureus.

The use of secondary biotin- conjugated anti-isotype antibody followed by addition of fluorochrome – conjugated avidin (a protein that binds to biotin with extremely high affinity), is also applicable for this type of assay.

Complement Fixation Test

The activation of the complement system by immune complexes results in the generation of the MAC, which can disrupt all members. If the immune complexes are generated on RBC surfaces, the RBC membranes are lysed and hemolysis occurs. It is possible to use this reaction to measure serum ab. The most important test of this type is the hemolytic complement fixation test (CFT).

The CFT is one of the most widely applicable of all immunological techniques. The CFT may be used to detect many immune reactions if the required reagents are standardized. The test is easily read and does not depend on the availability of purified suspensions of ags. It is therefore commonly used in the diagnosis of viral diseases.

The most important disadvantage of the CFT is its complexity, (regard to standardization and preparation of the reagents required).

Protocol:

- The hemolytic CFT is performed in two parts. First, ag and the serum under test are incubated in the presence of normal guinea pig serum, which provides a source of complement, because its complement has a high hemolytic activity.
- After the short incubation the amount of free complement remaining is measured by adding an "indicator system" consisting of "ab – coated sheep RBCs".
- Lysis of these RBCs seen as the development of a transparent red solution, is a negative result, since it indicates that complement was not activated or fixed. And ab was therefore absent from the serum under test.
- Absence of lysis, seen as the cloudy RBC suspension, is a positive result.
- In titration, the reaction in each tube will depend on the dilution of serum.
- The titer may be considered to be the reciprocal of the highest dilution of serum in which no more than 50% of the RBCs are lysed.

Disadvantage:

- Improper standardizaton and preparation of reagents required for the test, affects the accuracy of the test.
- The quantity of complement is critical, since too little complement results in incomplete lysis, where as excessive complement is not completely activated by immune complexes and therefore leads to false – negative results.
- The above mentioned problem is also associated with an ag
- The hemolysin should be heated at 56C for 30' before being used in order to destroy the content of complement.
- Complement, if fixed (activated) by ag and ab is unavoidable to lyse the indicator system.
- In the absence of ab, the complement will remain unfixed and available for lysis of the indicator system.

Cytotoxicity Test

Complement can cause membrane damage to nucleated cells and protozoans also. Abs against cell surface ags may therefore be measured by mixing a cell suspension with complement and the serum under test.

- If abs are present in the serum, they bind to the cells, complement is activated, and the cells are lysed. The presence of dead cells can be measured by adding a dye such as trypan blue or easin Y to the cell suspension.
- Living cells do not take up these dyes, and dead ones stain intensely.
- This form of test is employed in the identification of MHC class I molecules and in the diagnosis of "toxoplasmosis".

UNIT V

CHAPTER 7. HYPERSENSITIVITY

Introduction:

Generally an immune response induces an inflammatory reaction that eliminates ag without extensive tissue damage to the host. Under certain conditions however this inflammatory response can have deleterious effects resulting in tissue damage or even death. These reactions have been turned hypersensitive or allergic reactions.

Hypersensitive reactions may develop in the course of either humeral or cell – mediated responses. Reactions within the humoral branch are initiated by Ab or Ag- Ab complexes, and are termed "immediate hypersensitivity" reactions. Because the symptoms manifest within a minutes or hours following an encounter with ag by a sensitized recipient. Three types of such reactions are commonly recognized. They are

- Ig E mediated hypersensitivity
- Ig G mediated hypersensitivity / ADCC
- Immune complex mediated hypersensitivity (Anaphylatoxin).

Reactions within the cell-mediated branch are initiated by TDTH cells and are referred to as "delayed type hypersensitivity" in reference to the delay of symptoms for days following Ag exposure.

Gell and coombs classification:

Gell and cooms proposed a classification scheme in which hypersensitive reactions are divided into 4 types, namely,

- Type I – Ig E mediated hypersensitivity
- Type II – Ig G mediated hypersensitivity
- Type III – Immune complex mediated hypersensitivity.
- Type IV – Cell mediated hypersensitivity.

Each involving distinct mechanisms, cells and mediator molecules. This classification scheme has served an important function in identifying the mechanistic differences among various hypersensitive reactions.

Gell and coombs classification of hypersensitive reactions

Type	Descriptive name	Initiation time	Mechanism	Typical manifestations
Immediate reactions				
Type I	Ig E mediated hypersensitivity	2-30’	Ag induces cross – linkage of Ig E bound to mast cells and basophils with release of vasoactive mediators	Systemic anaphylaxis Localised anaphylaxis: Hay fever Asthma Food allergies Exzema
Type II	Antibody mediated cytotoxic hypersensitivity (Ig & mediated)	5 –8 h	Ab directed against cell surface ags mediates cell destruction via complement activation or ADCC.	Blood transfusion reactions. Erythroblastosis tetalis auto immune hemolytic anemia.
Type III	Immune complex mediated hypersensitivity	2 – 8 h	Ag – Ab complexes deposited in various tissues induce complement activation and an ensuring inflammatory response.	Localized arthus reaction. Generalized reactions: Serum sickness Glomerulonephritis. Rheumatoid arhteitis Sle

Delayed reactions				
Type IV	Cell mediated hypersensitivity	24 – 72 h	Sensitized TDTH cells release cytokines that activate macrophages or TC cells, which mediate direct cellular damage.	Contact dermatitis tubercular lesions graft rejection.

Ig E – mediated (Type I) Hypersenstivity:

A type I hypersensitive reaction is induced by certain types of ags, referred to as "allergens', induces a humoral ab response. But the plasma cells secretes Ig E which differentiates this reaction from the normal humoral responses.

"Type I hypersensitivities are inflammatory reactions mediated mainly by Ig E, bound to mast cells and basophils ; the reactions result from the release of pharmacologically active molecules from these cells."

IgE: (Sensitization and Response)

This class of ab binds with high affinity to Fe receptors on the surface of tissue mast cells and blood basophils. Such IgE, coated mast cells and basophils are said to be 'sensitized'.

- A later exposure to the same allargen cross links the membrane-bound IgE on sensitized mast cells and basophils, causing 'degranulation' of these cells.
- The pharmacologically active mediators released from the granules eaert biological effects on the surrounding tissues.
- The principle effects, vasodilation and smooth muscle contraction may be either systemic or localized depending on the extent of mediator release.

Components of Type I Reactions:

The are several components have a critical role in the reaction. They are namely,

- allergens
- Reagin Antibody (IgE)
- Mast cells and Basophils
- IgE binding F_c receptors

*** Allergens:**

IgE reactions are mount in human only as a defense against parasitic infections. The term allergen refers specifically to non parasitic ags capable of stimulating type hypersensitive responses in allergic individuals.

- Allergens enter the body either by inhalation or ingestion.
- Proteins, plant pollens, drugs, foods insect products, mold spores, animal hair and dander – these are all common ags associated with type V hypersensitivity.
- Rather, allergens as a group appear to possess diverse properties. Some allergens, including foreign serum and egg albumin, are potent ags, others such as plant pollens, are weak ags

IgE:

The local wheal and flare response that occurs when an allergen is injected into a sensitized individual is referred to as the 'P- reation' (Pransnit2 and kustner in 1921). Because the serum components responsible for the P-K reaction displayed specificity for allergen, they were assumed to be abs, but the nature of these P-K abs, or reagin was not demonstrated for may years.

- Normal IgE level in serum → 0.1 – 0.4 μg/ml
- Half life → 2-3 days

Mechanism of IgE – Mediated degranulation:

The biochemical events mediating degraulation of mast cell and blood basophils have many features in common. The degranulation reaction between basophil and mast cells has slight biochemical difference.

Although mast cell degranulation generally is initiated by allergen cross-linkage of bound IgE. Otherthan this, a number of diffeent stimuli can also initate the proless, including the,

- Anaphylatoxins → C3a, C4a, and C5a
- Drugs → synthetic ACTH codeine, morphine and calcium ionophore.
- Ig E mediated degranulation begins when an allergen cross links receptor bound IgE on the surface of a mast cell or basophil.
- In itself the binding of IgE to its receptor has no effect on a target cell. It in only after cross linkage by allergen of the fixed IgE-receptor complex, that proceeds degranulation.
- Although cross linkage is normally effected by the interaction of fixed IgE with di or multivalent allergen, it can be effected by anti-IgE ab, chemicals or abs to the receptor.

I Cross linkage of IgE receptors is thought to activate serine esterase, that converts phosphatidyl serine into phosphatidyl ethanolomine (cephaline)

II The cross linkage of IgE receptors also activates 2 other membrane bound enzymes namely, phospholipid methyl transferease I and II. [PMT I AND PMT II]

- PMT I faces the cytoplasmic side of the plasma membrane (PM) and PMT II faces the exterior side. These 2 enzymes, by methylation converts phosphatidyl ethanolamine to phosphatidly choline (lecithin)
- Accumulation of phosphatidyl choline on the exterior surfaces of PH causes an increase in membrane fluidity and it though to facilitate the formation and opening of calcium

chanells. Through these chanells calcium from outside enters the cell which activates phospholiphase A_2, which promotes breakdown of phosphatidyl choline to form lysophosphatidyl choline and arachidonic acid.

- The lysophosphatidyl choline further increase membrane fluidity facilitating further calcium influx.
- The arachidonic acid is further converted into 2 classes of mediators.
- Prostagludin
- Leukotrienes, which play a vital role in allergic manifetation.

II At the time of phospholipid methylation and calcium influx, there is an increase in membrane bound Adenylete cyclase activity with a rapid production of C-AMP. The C-AMP activates protein kinase, which phosphorylate the granule membrane proteins (myosin – muscle protein), thereby charging the granule permeability to H_2O and Ca^{2+}.

The consequent swelling of the granules appears to facilitate their fusion to the PM in degranulation. This fusion results in the release of the mediators from the granule.

Mediators:

The mediators are of two types

- Primary mediators – are produced before degranulation and stored in the granules.
  E.g., Histamine, protease, eosinophil chemotactic factor, neutrophil chenutactic factor.

- Secondary mediators are synthesized after the target cell activation.
  E.g., Leukotrienes, Prostaglandins, cytokines (IC1-IC6, TNFα etc)

Consequences of type I reactions:

The clinical manipulations of type I reactions can range from serious life-threatening conditions, systemic an dlocalized anaphylaxis etc.

Systemic Anaphylaxis: (PORTIER and RICHET – 1902)

While attempting to immunized dogs with sublethal extract of seaanimone tentagles. They observed that a secondary challenge, several weeks later with the same sublethal extract brought on rapid sequence of symptoms including,

→ Vomiting

→ Diarrhoea

→ Asphyxia

→ Unconciousness and death

They called the response anaphyaxis (against to protection) to denote that this is an inappropriate response counter to counter host protective mechanisms. A wide range of ags had been shown to trigger this reaction susceptible humans including Bewast and antistriking, penicillin, insulin, antitoxin and sea food.

Localised anaphylaxis:

The reaction in this case limited to a specific target often involving epithelial surfaces at the site of allergen entry. They tendancy to manifest localized anaphylactic reactions in inherited and is referred to as 'atopic reaction'.

Allergic rhinitis hay fever, asthma, eczema (atrophic dermatitis) and food allergies are some examples.

Therapy for anaphylactic reaction:

- Repeated injections of increasing doses of allergens subcutaneously results in the production of Ig G instead of Ig E. This Ig G is called 'blocking antibody", which joins with allergen and later phagocytosed. Xlow the allergen is not available for receptor bound Ig E on the mast cell hence no de-granulation occurs.
- Anti – listamines block attachment of histamines to target cell.
- Mast cell degranulation is blocked by theophylline (foe – asthma), adrenaline, cortisox etc.

Diagnosis:

- Radio immunosorbent test (RIST)
- Radio allergosorbent test (RAST)

RIST:

Anti Ig E coated to solid phase + patient Ig E (Sample)

↓

Anti – Ig E – Ig E complex

↓

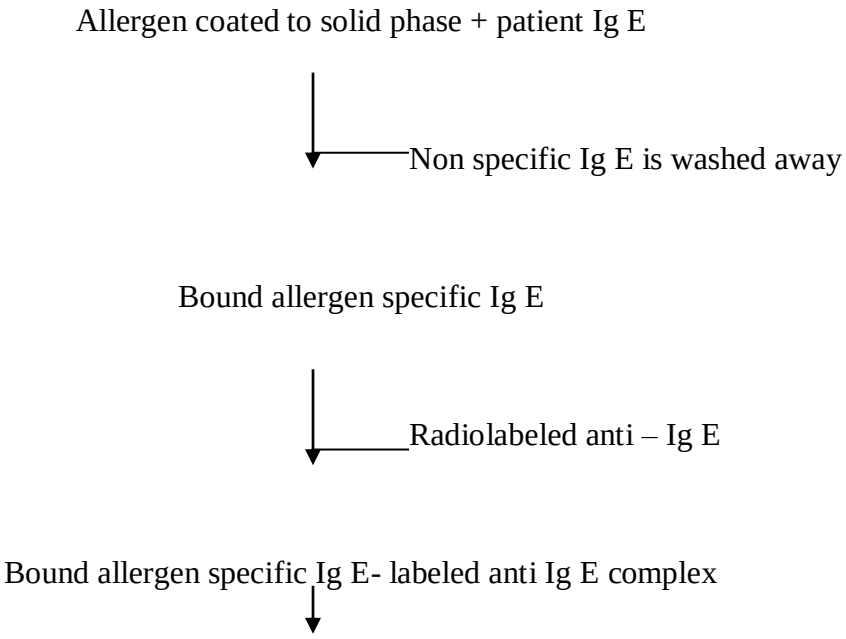
Radiolabelled anti – Ig E

Anti – Ig E – Ig E complex + labeled anti Ig E

↓

Count bound label

RAST:



Antibody mediated cytotoxic (Type II) hypersensitivity:

Type II hypersensitive reactions involve antibody mediated destruction of cells. Ab can mediate cell destruction by activating the complement system to create pores in the membrane of the foreign cells. For eg. Incompatible blood transfusion, results the proliferation of Ig G directed against cell surface ag (RBC and Ag)

The binding of ab to the cell surface usually activates the complement system. Cell destruction of 4 modes are follows:

- The binding of ab to the cell surface leads to the cytotoxic attack by killer cell. This is Ab dependent cell mediated cytotoxicity.
- Ab coated cell binds to macrophages through their receptors for the Fc portion of Ig G and initiates phagocytosis.
- Activation of the complement system leads to the deposition of C3b on the cell surface. C3b coated cells are bound by phagocytic cells through their receptors for C3b and phagocytosis follows.
- If complement fixation continues to the terminal stages of biding C8 and C9 (MAL formation) and the cell is lysed.

Some of the examples for type II hypersensitivity:

Generally incompatible transfusion reactions and autoimmune diseases are fall into this category.

- Haemolytic disease of the new born.
- Immune thrombo cytopenic purpura.
- Good Pasteur's syndrome.
- Myasthenia grvis (MG).
- Graves disease

Ig G and Ig M are abs commonly involving in this type of reaction.

*** Haemolytic disease of the new born:**

Ig G abs synthesized by the mother who is Rh negative, are directed against RBC Rh^{+ve} ags of their foetus, who has inherited the ag from its father. With subsequent Rh^{+ve} foetus the abs (Ig G) produced by the mother cross the placenta and kills the foetus.

An ABO incompatibility between mother and foetus generally does not present any problem since the abs are Ig M and they cannot cross the placenta. But Ig M is the ab ever first produced after an of exposure, then 'class switching' to Ig G/ any ab occurs.

* **Immune thrombo cytopenic purpura:**

The ags in this disorder on platelets and the ab is coat the platelets and opsonized them for phagocytosis. This results in reduction in platelets, internal bleeding into the skin causing 'purple patches' (Purpura).

*** Good pasteure's syndrome:**

In this reaction the abs are directed against ags on the basement membrane of glomerular of kidney resulting in cell damage and leakage of blood and protein into the urine.

*** Myasthenia gravis (MG):**

The abs are formed against acetyl chlorine receptor resulting in axon end plate potential (EPP). The reduction in EPP leads to non contraction of skeletal muscle.

*** Graves disease:**

(Thyrotoxicosis – hyperthyrodism)

The auto antibodies binds to TSH receptor on the thyroid cell membrane and stimulate the thyroid cell to secrete T3 or T4 or thyroxine. These auto abs act similar to TSH. The continuous stimulation result in hyperthyroidism. In grave's disease, the negative feed back to stop thyroid cell stimulation is broken.

Immune complex mediated (type III) hypersensitivity:

In general ag – ab complex leads to the destruction of that particular ag. In some cases, however, large amounts of immune complexes can lead to tissue damage type III hypersensitive

reactions. The magnitude of the reaction depends on the quantity of immune complexes as well as their distribution within the body.

- When the complexes are deposited in tissue vary near the site of ag entry, a localized reaction develops.
- When the complexes are in the blood, a reaction can develop wherever the complexes are deposited.

Mechanism:

The immune complexes activates / fixes the complement, with the resulting production of anaphylatoxins such as C3a, C4a and C5a. These complement split products are act on mast cells and basophils eliciting the release ofhistamine and leuckotrienes i.e., localized degranulation. These mediators produce "vasodilation", increased capillary permeability and thus inflammation.

The inflammatory effect of mast cell is magnified by the release of "platelet activating factor (PAF)', which in turn triggers the release of histamine and seratonin from platelets. C5a is also a chemotactic for neutrophol.

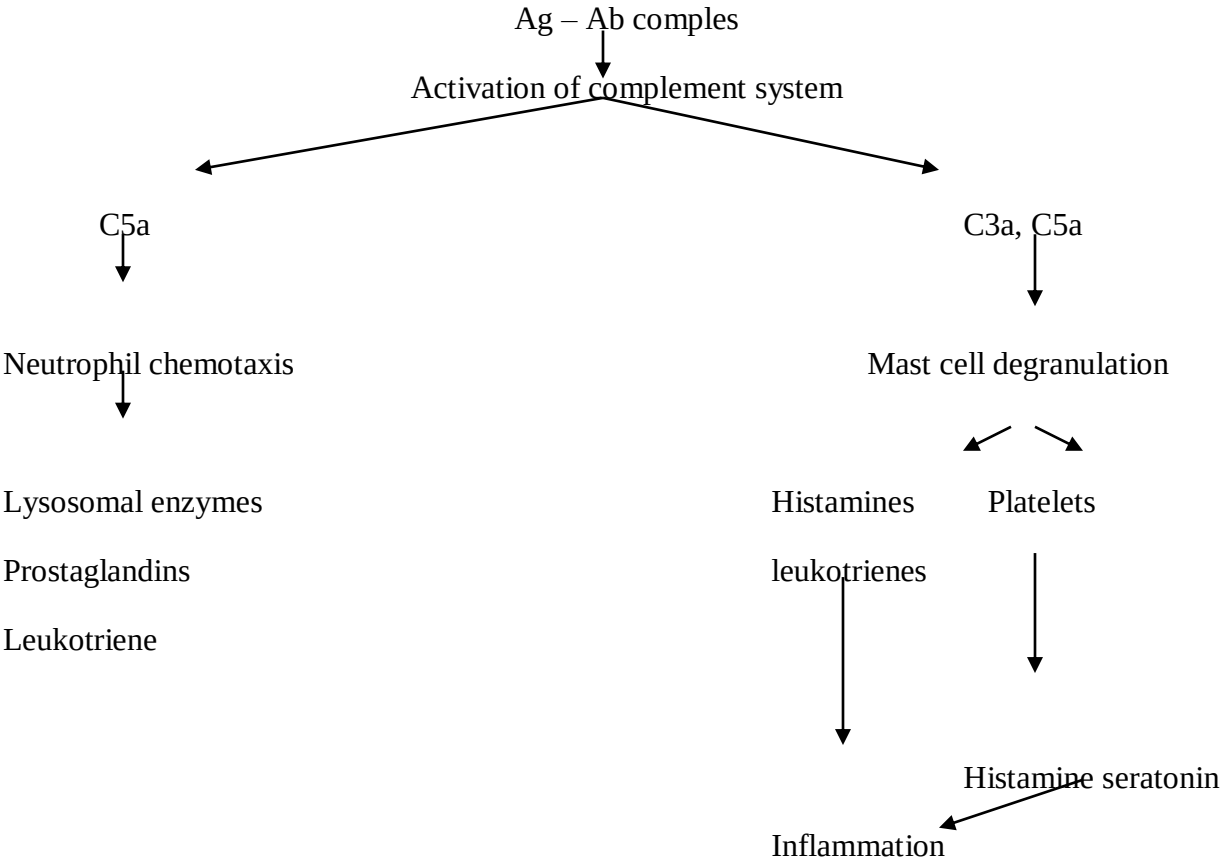


Fig: complement mediated hypersensitivity or immune complex mediated hypersensitivity.

Localized type III reactions:

Injection of an ag intradermally or subcutaneoulsy into an animal that has high levels of circulating ab specific for that ag leads to the formation of localized immune complexes, which mediate an acute "Arthus reaction", with in 4-8h. This reaction is slower than type II but faster than type IV.

As the reaction develops, localized tissue and vascular damage results in an accumulation of fluid (edema) and RBCs (erythema) at the site. The severity of the reaction can vary from mild swelling and redness to tissue necrosis.

- Following insect bite → type I reaction after 4 –8 hr → type III reaction develops.
- Intrapulmonary arthus – type reactions induced by bacterial spores, fungi or dried fecal proteins (preumonitis / alveolitis).
- "Farmer's lung" → thermophilic actinomycetes.
- Pigeon fancier's disease → inhalation of a serum protein in dust derived from dried pigeon feces.

Generalized type III reactions:

Ags -> blood stream -> binds to Abs.

If Ag is in excess, small complexes form; because these are not easily cleared by the phagocytic cells, they can cause tissue – damaging type III reactions.

Eg: administration of antitoxins can cause this type of reaction. So the abs for that particular foreign antiserum, circulates throughout the body and causes damage to the cells, by within days / weeks. An individual begins to manifest a combinations of symptoms that are called 'serum sickness'.

The symptoms include,

- Fever
- Weakness
- Rashes with edema and erythema
- Lymphadenopathy
- Arthritis and (sometimes)
- Glomerulonephritis.

The formation of circulating immune complexes contributes also,

- Autoimmune diseases (SLE, RA, GS)
- Drug reactions (penicillin and sulfonamides)
- Infectious diseases (poststreptococcal, glomerulonephritis, meningitis, malaria, hepatitis etc).

T_{DTH} – mediated (type – IV) hypersensitivity:

This reaction develop when ag activates sensitized T_{DTH} cells; these cells generally appear to be a T_{H1} subpopulation although sometimes Tc cells are involved.

DTH is a form of CM1 reaction in which the ultimate effector cell is the activated macrophage. If the ag is not a microbe, DTH reaction produce tissue injury without providing a protective function hence the term hypersensitivity.

Mechanism:

There are 2 phases of DTH response,

- Sensitization / cognity phase.
- Activation phase.

Sensitization phase:

During this phase T_H cells are activated and clonely expanded by ag presented together with class II MHC molecule on an APC.

Langerhans cells are thought to pick up ag that enters through skin, and transport it to the regional lymph nodes, where T cells are activated.

Activation phase:

In this phase the activated T cells secretes a variety of cytokines, including IC-2, IFN - γ, Macrophage inhibition factor (MCF) and TNF- β, which are responsible to draw macrophages into the area and activate them, promoting increased phagocytic activity and increased concentrations of lytic enzymes for more effective killing.

As lytic enzymes leak out of the activated macrophages into the surrounding tissue, localized tissue destruction can occur. These reactions typically take 48 – 72 h to develop, the time required for initial T_{DTH}- cell activation of cytokine secretion to mediate accumulation of macrophages and subsequent reactions.

T_{DTH} cells:

The activated T cell (CD_4^+) are often designated as T_{DTH} to denote their function in DTH although in reality they are simply a subset of T_H cells (T_{H1}).

Cytokines involved in DTH:

I. Sensitization:

APC ----------→ CD_4^+ cell ------→ T_{DTH}

activation

II. Activation:

1. T_{DTH} --------→ induce haemotapoiesis

IL-3

GM- CSF monocyte neutrophil

IFN - γ

2. T_{DTH} ----------→ facilitate extravasation of monocytes and other cells from blood to

TNF-β, IC-1 tissue

MIF

3. T_{DTH} --------→ inhibits migration of macrophages from the area of DTH reaction.

IL – 4

IFN

4. T_{DTH} --------→ activating macrophage.

This type of response is non-specific and often results in significant damage to healthy tissue. This is the prize of the body for successful elimination of cells harboring intracellular bacteria and fungal pathogens.

Chronic – DTH reactions:

When the phagocytosis is not effective the continued presence of the pathogen's ags can provoke a chronic DTH reaction, which is characterized by excessive numbers of macrophages, continual release of lytic enzymes and consequent tissue destruction.

For eg:

Granulomatous skin lesions → M. leprae

Lung cantiation → M.tuberculosis

ID – reaction:

The reaction to an intradermal (ID) injection of an ag can serve as a test for the presence of T_{DTH} cells previously sensitized by that ag.

To use of 'purified protein derivative' (PPD) ag to detect previous exposure to M.tuberculosis. After the injection of PDD, the characteristic response of DTH evolves over 24-48 hr. about 4 hrs after injection of an ag.

- Neutrophil infitrate through high endothelial venules (HEV) at the injection site.
- By about 12 h, the site become infiltrated by T cells and monocytes.
- Vasodilation occurs, fibrinogen escapes from the blood vessels into the surrounding tissue, where it is converted to 'fibrin'.
- Deposition of fibrin and accumulation of T-cells and monocytes at the injection site cause the tissue to swell and become hard (indurated).
- Induration is a hall mark of DTH, is detectable about 18 h and is maximal by 24/48h.

Merits:

Similar skin tests to detect previous exposure to leprosy and coccidiomycosis utilize, lepromin and coccidiodin respectively as test ags.

AIDS can be monitored by repeated skink testing with any of the various ags that induce a type IV DTH response. As AIDS progresses, the decline in T_{DTH} cells is reflected in decreased skin reactivity to such ags.

Some of the antigens elicits DTH:

- Formaldehyde.
- Trinitrophenol.
- Nickel
- Turpentine.
- Various cosmetics.
- Hair dyes.
- Poison oak -> pentadecacatechol from the leaves.
- Poison ivy.

All these ags complex with skin, proteins, exposed to langerhans cells leads to DTH.

CHAPTER 9. TRANSPLANTATION IMMUNOLOGY

Introduction:

Transplantation, as the term is used in immunology, refers to the act of transferring cells, tissues, or organs from one site to another. The surgical procedures for many kinds of transplantation were developed by turn of the century.

In the early 1900s a Viennese surgeon observed that he could surgically remove a kidney from an animal and then transplant it back into the same animal and restore kidney function. When the kidney was transplanted to a different animal, however, it soon ceased to function.

Several observations by P.B.Medawar in the early 1940's provided insight into the nature of graft rejection.

- The grafts of skin from one site to another on the same patient were readily accepted, whereas grafts from relatives were rejected.
- The second graft from the same different donor, the rejection reaction occurred much faster and with much greater intensity.

In subsequent experiments, he discovered that prior sensitization with donor cells led to heightened rejection of a subsequent graft. And the graft rejection resulted from an immunologic response to the donor graft.

"The Principle of graft rejection is, that the immune system that evolved to recognize and destroy altered self-cells is simply performing the function by recognizing and destroying the foreign cells of a graft".

Today, kidney, heart,lung liver, bone marrow and cornea transplantations are performed with ever increasing frequency and success.

Immunologic basis of graft rejection:

The Degree of immune response to a graft varies with the type of graft. Such grafts are,

- Autograft – is self-tissue transferred from one body site to another in the same individual. For example skin grafts of patients with burns.
- Isograft – is tissue transferred bether genetically identical individuals. For e.g., grafts between inbred mice and monozygotic twins in humans.
- Allograft – is tissue transferred between genetically different members of the same species. In humans, unless an identical twin is available, most organ grafts from one individual to another are allografts.
- Xenografts – is tissue transferred between different species. E.g, the graft of a baboon heart into a human.

Autografts,
Isografts } usually accepted.

Allograft - often recognised as foreign, since it will be rejected.
Xenografts - vigorous rejection.

Specificity and memory of the rejection response:

The time sequence of allograft rejection varies according to the tissue involved. In general, skin grafts are rejected faster than more vascularized tissues such as kidney or heart.

Despite these time differences, graft rejection always displays the specificity and memory.

*** Specificity:**

Strain – A inbred mouse

Grafted with skin from Strain B

Primary Graft rejection
(first-set rejection)

- The vascularized transplant becomes infiltrated with lymphocytes, monocytes and other inflammatory cells.
- There is decrease vascularization of the transplanted tissue by 6-9 days.
- Visible necrosis by 10 days.
- And complete rejection by 14 days.

Memory:

Previously transplanted strain-A→ Again transplanted with strain B→Immediate Graft rejection

(second-set rejection)

- Complete rejection occurring within 5-6 days.
- The specificity of second-set rejection can be demonstrated by grafting an unrelated 'strain-c' graft at the same time as the second strain B graft.
- Rejection of the strain-c graft proceeds according to first-set rejection kinetics, whereas the strain-B graft is rejected in an accelerated second-set rejection.

Role of cell mediated response:

- T lymphocytes (CD_4 and CD_8) have a important role in the graft rejection.
- The removal of CD_4^+ T- cells from the recipient leads the prolonged graft survival from 15 to 30 days.
- The removal of CD_8^+ T-cells alone had no effect on graft survival
- But the removal of both of the CD_4^+ and CD^+_8 T cells resulted in longterm survival (up to 60 days) of the allongrafts.

This study indicated that both CD_4^+ and CD_8^+ T cells participated in rejection.

Transplantation antigens:

Tissues that are antigenically similar are said to be 'histocompatible' such tissues donot induce and immunologic response (no rejection).

Tissues displaying antigenic differences are histoincompatible, such tissues induce an immune response leading to tissue rejection.

MHC: (MAJOR)

The ags that determine histoincompatibility are encoded by more than 40 different loci, but the loci responsible for the most vigorous allograft rejection are located within the major histocompatibility complex (MHC)

The organisation of the MHC,

H-2 complex – mice, HLA complex – humans.

MHC: (MINOR)

MHC identity of donor and host is not the sole factor determining the tissue acceptance. When tissue is transplanted between genetically different individuals, even if their MHC ags are identical, the transplanted tissue is likely to be rejected because of differences at various 'minor histocompatibility loci' (MHC)

- Injection – usually less vigorous than that induced by major histocompatibility differences.
- But still, reaction to this MHC differences often results in graft rejection.
- For this reason, transplantation even between HCA – identical individuals requires some degree of immune suppression.

Mechanism of graft rejection:

Graft rejection is caused principally by a cell-mediated immune response to alloantigens expressed on cells of the graft. Both delayed type hypersensitive and cell-mediated cytotoxicity reactions have been implicated.

Generally graft rejection process result in damage to vascular endothelium and other cells in the graft that can be reached by the host's T lymphocyte.

The process of first-set rejection can be divided into two stages:

*** Sensitization phase:**

In which ag-ractive lymphocytes of the recipient proliferate in response to alloantigens on the graft.

*** An effector phase:**

In which immune destruction of the graft takes place.

*** Sensitization phase (of the recipient):**

- Graft allvantigens may reach the ag-sensitive cells of the recipient by several routes.
- Thus graft cells may release soluble MHC molecules that enter the host, and graft dendritic cells may emigrate into host lymphoid tissue.
- More importantly, circulating host T cels (CD_4^+ and CD_4^+) encounter foreign MHC molecules either naturally on cell surfaces or on presentation by host dendritic cells (APCs) that also invade the graft.
- These T cell populations recognise both of the MHCs on the APCs.
- Activation of host T_H cells requires the interation with an APC expressing an appropriate ag-MHC complex and providing the requisite 'co-stimulatory signal'.

APC:

- Depending on the tissue, different populations of cells within a graft may functions as APCs.
- Generally dendritic cells are acts as APCs , because of its abundant presence in most of the tissues.

Passenger leukocytes:

In some organ and tissue grafts (eg: kidney, thymus and pancreatic islets) a population of donar APCs called "passenger leutocytes", migrate from the graft to the regional lymph node as a result of the lymphocyte "Trap".

- At least in skin grafts, the lymphatic route is probably of major importance since rejection is considerably delayed if a graft is prevented from developing lymphatic connections with the host.
- The CD_4^+ cells / passenger leucocytes that recognise the MHC class II molecules on the graft cells move to the draining lymph node and activate $CD8^+$ effector T-cells, by secreting CC-2
- These effectors cells are the transported back to the graft blood or lymph vessels.
- Recognition of the alloags expressed on the cells of a graft induces vigorous T-cell proliferation in the host.
- This amplified population of activated TH cells is thought to play a central role in inducing the various effector mechanisms of allograft rejection.

Effective phase (destruction of the graft):

The variety of effector mechanims participate in allograft rejection. The most common are cell –mediated reactions involving,

- Delayed type hypersensitivity.

- CTL- mediated cytotoxicity.

Less common mechanisms are,

- Antibody – plus- complement lysis.
- Antibody dependent cell mediated cytotoxicity (ADCC).
- $CD8^+$ Tcell (effector cells) leave the node

In the efferent lymph and reach the graft through the blood.

- When these cells enters the graft, they recognise its MHC class I and bind and destroy the vascular endothelium and other accessible cells by direct cytotoxicity.
- As a result, hemorrhage, platlet aggregation, thrombosis and stoppage of blood flow occur.
- The grafted organ dies because of the failure of the blood supply.

(participation of CD_8^+ cells -> CTL mediated killing
participation of CD_4^+ cells -> activation of effector cells).

Cytokines:

The CD4+ cells secrets different kinds of cytokines, plays a central role in the graft rejection.

For eg. IL – 2 -> Promotes T-cell proliferation and necessary for the generation of effector CTLs.

IFNγ- development of DTH response, promoting the influx of macrophages into the graft and their subsequent activation into more destructive cells.

TNF- β -> have direct cytotoxicity on the cells of a graft.

A number of cytokines promote graft rejection by inducing expression of class I and II MHC molecules on the graft cells.

IFN (α, β & γ) TNF-α, TNF- β -→ increases class I MHC expression.

IFN - γ → increases class II MHC expression.

During a graft rejection, the levels of these cytokines increases, inducing a variety of cell types within the graft to express class I and II MHC.

ADCC:

Although CTLs are most important in graft rejection, abs also play a significant role in the same. Abs directed against graft MHC class I molecules act with complement and neutrophils, or through ADCC, to cause vascular endothelial cell destruction. Abs seem to be of greatest importance in second – set and xenograft reactions.

Graft – versus – host disease:

If healthy lymphocytes are injected into the skin of an allogenic recipient, local inflammation occurs as a result of the lymphocytes attacking the host cells – the graft attacks the host.

Provided the recipient has a functioning immune system, this graft –vs-host (GVH) reaction is not serious. If however, the recipient cannot reject the grafted lymphocytes then these cells may cause uncontrolled destruction of the host's tissues, disease and eventually death.

MHC-class I:

The lesions generated in GVH disease depend on the MHC disparity between graft and host. When the difference is in the MHC class I molecules, then the invading lymphocytes attack all the nucleated cells of the host. The result is a wasting syndrome characterized by bone marrow destruction, leading to total loss of blood cells (pancytopenia) and a plastic anemia, loss of T and B cells, and hypogammaglobulinemia.

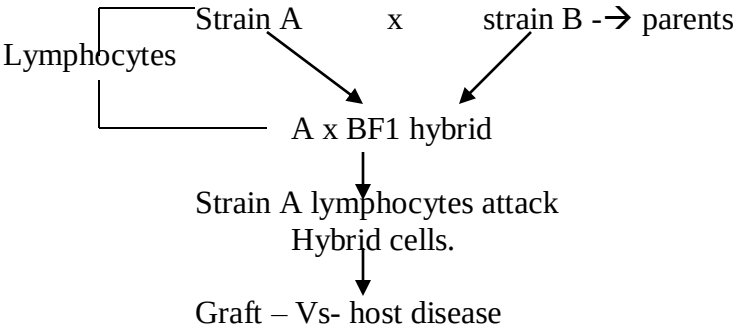


Fig graft – Versus – host disease

Individuals suffering from this type of GVH disease develop a lymphocyte infilteration of the intestine, skin and liver. These lymphocytes secrete TNF- α, which causes,

*Mucosal destruction and diarrhea
* Skin and mouth ulcers.
* Liver destruction.
* Jaundice.

Affected persons stop growing and may die if untreated.

MHC Class II:

If, there is a MHC class II displarity between the graft and host, then the graft and host lymphocytes will interact. This may lead to,

- Immunostimulation.
- Auto antibody formation and
- Clinical signs resembling systemic lupus erythematous (SLE) and polyarthritis.

Treatment:

Is similar to immunosuppressive therapy used to prevent graft rejection.

Grafts that are not rejected:

Previleged sites:

Anterior chamber of the eye, cornea, and brain – lack effective lymphatic drainage.

Sperm:

Seminal plasma is immuno suppressive and sperm exposed to this fluid are non immunogenic.

Prostatic fluid – inhibits complement.

Foetus:

Is prevented by the combined activities of several immunosuppressive mechanisms.

Phospholipids – surrounding the foetus is a major immunosuppressive.

CD 55 – blocks complement activity.

IL-3, GM- CSF – stimulates placental growth.

IFN, progesterone etc – immunosuppressive.

Cultured or stored organs:

Due to the loss of dendritic cells from the thyroid tissue during culture.

Immunologically favoured organs:

If the recipients are first sensitized and then given liver allografts, subsequent grafts are retained, the implication being that liver grafting also blocks immunological memory.

This is because, the release of immunosuppressive molecules, such as glycoterroprotein bilisubin.

Clinical manifestations of graft rejection:

Graft rejection reactions; have various time courses depending upon the type of tissue or organ grafted and the immune response involved.

- Hyperacute rejection – occur within the 1st 24 hour after transplantation.
- Acute rejection – usually begin in the first few weeks after transplantation.
- Chronic rejection – occur from months to years after transplantation.

Prevention of graft rejection:

Graft rejection may be prevented by,

- Immune suppression with drugs like cyclosporin, corticosteroid and irradiation. Development of immunological tolerance to the foreign tissue ag (use of monoclonal abs to induce tolerance).

CHAPTER 10. AUTOIMMUNE DISEASES

Introduction:

Autoimmune responses are immune responses of an individual to antigens presents in the individual's own tissue. Autoimmune diseases affect more than 20 million Americans. These disorders are characterized by chronicity and usually are nonreversible (i.e., patients never fully recover and must learn to live with their disease.

Etiology:

1. A defect in the mechanisms underlying self-recognition (autotolerance, self-tolerance can result in autoimmune disease.
2. Autoimmune responses can be mediated by humoral factors (circulating antibodies, immune complexes) or cellular factors (delayed hypersensitivity, cytotoxic T (Tc) cell mediated)

a. Most autoimmune diseases have associated autoantibodies.
    i. These antibodies are believed to be etiologically involved and to cause the resultant damage, namely Gell and Coombs types II and III hypersensitivity reactions.
    ii. The autoantibodies, however, may merely reflect the damage done to an organ. As such, they are of diagnostic but not etiologic interest.

b. The autoimmune diseases that involve nervours tissue (the encephalomyelitides and Guillain-Barre syndrome) are though to result from Gell and Coombs type IV hypersensitivity reactions. Guillain – Barre syndrome also may have an antibody component.
c. Some autoimmune disease (dermatomyositis, Hashimoto's thyroiditis, diabetes mellitus) are "mixed" – that is, they have both an antibody and a T-cell component.
d. The pathogenesis of multiple sclerosis is still unclear; however, the finding that patients have a defect in suppressor T (Ts) cells and excessive numbers of activated T cells in the blood and spinal fluid supports the theory of a defect in immune regulation.

3. **Possible Mechanisms:** The basic process most likely involves recruitment of a helper / inducer T cell that cooperates with preexisting autoreactive B cells or Tc-cell precursors to induce a self-destructive immune response. The immunologic imbalance may arise from one or more of the following.

a. An Excess of Self – reactive helper T (Th) – cell activity:
    i. The Th-cell hyperactivity may be induced, for example by,

1. Aletred froms of self-antigen
2. Antigens that cross-react with self-antigens as a result of epitope similarity
3. Molecular Mimicry, in which self-antigens and foreign materials such as viruses or bacteria share identical epitopes.

    ii. Altered forms of cross-reacting antigens may be produced by coupling of a virus or a chemical or drug (e.g., hydralazine) to self-antigen.

b. A bypass of the requisite self-reactive Th- cell activity:

i. Such a bypass may occur via polyclonal B-cell activation by materials such as bacterial lipopolysaccharide or Epstein-Barr virus.
ii. Modified chemical-host or viral-host antigens could recruit a cross-reactive Th cell and elicit autoantibody production.

c. **A deficiency of Ts cells,** which normally down-regulate the immune response to self-antigen.
d. **A release of sequestered antigen** (e.g., from the lens of the eye or from sperm)
    i. Sequestered antigens are not ordinarily available for recognition by the immune system.
    ii. Release may occur by such means as trauma or infection.

4. **Genetic Predisposition:** Genetic factors clearly play a role in autoimmune disease.
a. The abserved familial incidence is believed to be largely genetic rather than environmental.
b. This association is thought to relate to those major histocompatibility complex (MHC) genes that code for the class II antigens that are so important in the presentation of antigens during the induction of an immune response.
    i. Most autoimmune diseases appear to be associated with MHC class II molecules (also called human leukocyte antigens (HLS's) DR2, DR3, DR4, and DR5.
    ii. Over 90% of patients with ankylosing spondylitis have the HLA-B27 antigen, compared to only 8% of the otherwise healthy population.

5. **Other factors affecting incidence:**
a. Sex: Autoimmune diseases tend to occur at **higher frequencies in women than in men.**
    i. For example, the incidence of systemic lupus erythematosus (SLE) in women is four to six times that in men, and rheumatoid arthritis is three to four times more common in women.
    ii. The reason for this still uncertain, but sex hormones may play a role.

b. **Age:** The frequency of autoimmune diseases **increases with age.** Very few autoimmune diseases occur in children and adolescents; most of these disorders first appear in the 20 to 40 year age group.

B. Clinical Categories:
1. Autoimmune diseases have been divided into two clinical types, **organ-specific and systemic,** based on the distribution of lesions.
2. Systemic autoimmune diseases are sometimes called **collagen vascular diseases**, indicating the widespread distribution of lesions in connective tissues and the vascular system.

C. Diagnosis:
1. **General Signs** of autoimmune disease that may have diagnostic value include the following.
    a. Elevated serum gamma-globulin levels.
    b. Presence of diverse autoantibodies

c. Depressed levels of serum complement.
d. Immune complexes in serum.
e. Depressed levels of Ts cells.
f. Lesions detected on biopsy (e.g., glomerular lesions) resulting from deposition of immune complexes.

2. Diagnostic Tests are designed to detect antibodies specific to the particular antigen involved in the autoimmune disease. Certainfacts should be notd.

a. Patients may have more than one antoantibody and, in fact, may suffer from **multiple autoimmune diseases.**

1. Appoximately 10% of patients with autoimmune thyroiditis also suffer from pernicious anemia, and 30% have antibodies to gastric patietal cells. Fully 50% of people with pernicious anemia have antibodies reactive with thyroid antigens.
2. Nearly 50% of patients with Sjogen's syndrome suffer from rheumatoid arthritis or other autoimmune arthritic diseases
3. Patients with SLE may have 10 to 15 autoantibodies of differing specificities.

Tissue Distribution and Persumed Immune Mediation in Representative Autoimmune Disease:

Disease	Persumed Mediation	Tissue Involvement
Systemic		
Rheumatoid arthritis	H	Joints and vascular bed
Systemic lupus erythematosus (SLE)	H	Kidney, skin, central nervous system, cardiovascular system.
Organ – or tissue – specific		
Sjogren's syndrome	H	Lacrimal and salivary glands
Addison's disease	H	Adrenal glands
Acute disseminated encephalomyelitis	T	Nervous system
Guillain – Barre syndrome	T	Nervous system
Multiple sclerosis	T	Nervous system
Myasthenia gravis	H	Nervous system
Hashmoto's thyroiditis	H,T	Thyroid
Primary myxedema	H	Thyroid
Graves disease	H	Thyroid
Diabetes mellitus	H,T	Pancreas
Goodpasture's syndrome	H	Lung and kidney
Pernicious anemia	H	Stomach
Atrophic gastritis	H	Stomach
Crohn's disease	H	Ileum and colon
Autoimmune hemolytic diseases	H	Red blood cells
Idiopathic thrombocytopenic purpura	H	Platelets
(ITP)	H	Skin
Pemphigus	H	Skin
Pemphigoid	H,T	Skin and muscle
Dermatomyositis		

Representative Examples of Organ-Specific Antigens:

Disease	Antigen
Addison's disease	Microsomal proteins of adrenal cells
Acute disseminated encephalomyelitis	Basic protein of myelin
Diabetes Mellitus	Islet cell antigens Insulin
Goodpasture's syndrome	Type IV collagen of basement membranes
Graves disease	Thyroid-stimulating hormone receptors
Hashimoto's thyroiditis	Thyroglobulin
Myasthenia gravis	Acetylcholine receptors
Pernicious anemia	Gastric parietal cell antigens Intrinsic factor
Sjogren's syndrome	Salivary gland nucleolar antigens

c. **Multiple Antibodies:** Although SLE is associated primarily with antinuclear antibodies, and rheumatoid arthritis primarily with rheumatoid factor, both types of antibodies may be found in both diseases.
d. **Autoantibodies are not unique to autoimmune disease.** Antinuclear antibodies may be found in tuberculosis, in histoplasmosis, and in malignant lymphoma and other neoplasia.

D. Treatment:

Therapy for autoimmune disease involves several approaches.

1. Metabolic control:

May be effective in certain organ – specific diseases.

a. In thyrotoxiocis (Graves disease), antithyroid drugs such as propylthiouracil or methimazole may be prescribed. Surgical or radionuclide (iodine 131) ablation of the gland also is effective.

b. Viatamin B_{12} is given to patients with pernicious anemia.

2. Some non steroidal anti – inflammatory drugs (eg aspirin, indomethacin), steroidal anti inflammatory drugs (eg cortisone, which also may be immunosuppressive), and immunosuppressive cytotoix drugs (eg cyclophosphamide, azathioprine) are useful in treating disease symptoms. The later agents are used only in advanced disease.

3. Anticholinesterase drugs and thymectomy are of value in myasthemia gravis.

4. Plasmapheresis, or plasma exchange therapy, appears to be useful in the treatment of certain diseases (eg Guillain – barre disease, SLE, goodpastures syndrome). The removal of the offending antibodies and immune complexes is beneficial. The plasma that is removed is replaced with normal serum albumin or fresh frozen plasma.

5. Splenectomy is of value in hemolytic diseases and idiopathic thromobocytopenic purpura.

Representative autoimmune diseses:

I. Rheumatoid arthritis is a chronic, inflammatory joint disease with systemic involvement.

1. Clinicopathologic features:

a. A unknown etiologic agent initiates a non specific immune response. An inflammatory join lesion that begins in the synovial membrane can become proliferative, destroy adjacent cartilage and bone, and result in joint deformity, rheurhatoid arthritis, primarily affects women and is associated with HLA – DR4 which may confer genetic susceptibility.

b. General symptoms include weight loss, malaise fever, fatigue, and weakness.
c. Serum protein electrophoresis may show hypergammaglobulinemia and hypoalbuminemia.

2. Immunologic findings:
a. Rheumatoid factor:

A hall makr of rheumatoid arthritis is the presence of rheumatoid factor, an anti IgG immunoglobulin (Classically igM, but also IgG and IgA) that is produced by B cells and plasma cells in the synovial membrane, IgM and IgG rheumatoid factors are the primary components of immunoglobulin production in the rheumatoid synovial membrane.

1. Most patients (≥ 90%) with established rheumatoid arthritis have rheumatoid factor in their serum. In these patients, the disease is generally more servere than in patients without rheumatoid factor.
2. Rheumatoid factor has antibody specificity for the Fc fragment of IgG, IgG is apparently altered in some way to appear as "nonself", perhaps by binding to another antigen.

b. Immune complexes:

The synovial fluid of patients with rheumatoid arthritis contains immune complexes consisting of rheumatoid factor, IgG, and complement. These complexes can trigger an immune complexes consisting of rheumatoid factor, IgG and complement. These complexes can trigger an immune response in two ways.

1. Immune complex activation of complement:
a. Rheumatoid factor combines with IgG away from its antibody combining site, which leaves the IgG molecule free combine with its homologous antigen or with more antibodies to form large complexes.

b. These aggregates can become large enough to activate both the classic and alternative complement pathways, with production of eutrophil chemotactic factors (C5a).

2. Immune complex ingestion:
a. An inflammatory response is amplified as aggregates of IgG rheumatoid factor are phagocytized by macrophages, which release cytokines, and by neutrophis, which release digestive enzymes. Neutrophils that have engulfed immune complexes are called rheumatoid arthritis (RA) cells.

b. Synovial levels of IgG but not IgM rheumatoid factor are relatively reduced compared to serum levels. This suggests that phagocytosis of IgG rheumatoid factor may play a specific role in inflammatory response.

c. Antinuclear antibodies:

These also are present in some patients with rheumatoid arthirits

II. Systemic lupus erythematosus (SLE):

Is a chronic, inflammatory, systemic (multiorgan) disorder that predominantly affects young women of child bearing age. At least 5000 Americans die of SLE each year, but most cases are controlled by medical treatment. Death usually results from renal failure or from infection brought on by immuno suppressive therapy.

1. Clinicopathologic features:

a. General symptoms:

Include malaise, fever, lethargy, and weight loss. Multiple tissues are involved including the skin, mucosa, kidney, brain, and cardiovascular system.

1. The most characteristic feature is the butterfly rash, an erythematous rash that maculopapular, bullous) also have been described.
2. **Renal involvement** occurs in 50% of patients with SLE. Diffuse proliferative glomerulonephriits and membranous glomerulonephritis are common.
3. **Central connective tissue disease,** or overlap syndrome, occurs primarily in female patients who have features of rheumatoid arthritis, SLE, polymyositis dermatomyosists, or scleroderma.

2. Immunologic findings:

a. Lupus erythematosus (LE) cell phenomenon:

This discovery of this phenomenon led to the current understanding of the immunologic basis of SLE.

1. When peripheral blood from a patient is incubated at 37C for 30-60 minutes, the lymphocytes well and extrude their nuclear material.
2. This nuclear material is opsonized by anti – DNA antibody and complement and is then phagocytized by neutrophils, which in this situation are called LE cells.

b. Anti nuclear antibodies:

The detection of antinuclear antibodies by indirect immunofluorescence is of dianostic importance in SLE. Three primary types of antibodies either IgG or IgM to DNA can be detected:

1. Antibodies to single – stranded DNA (ssDNA).
2. Antibodies to double stranded DNA (dsDNA) which are complement fixing antibodies. These are most closely associated with active SLE and the glomerulonephritis that is triggered by the deposition of immune complexes (consisting of DNA, antibody, and complement on the basement membrane of blood vessels of renal glomeruli.
3. Antibodies that react with both ssDNA and dsDNA.

c. Other autoantibodies:

1. Some patients with SLE also form antibodies against RNA, red blood cells, platelets, mitochondria, ribosomes, lysosomes, thromboplastin, or thrombin.
2. Some patients (30%) demonstrate a positive test for rheumatoid factor.

d. Genetic aspects:

Autoantibody formation is in part genetically determined.

1. Patients with the HLA-DR2 gene are more likely to produce anti – dsDNA antibodies.
2. Those with HLA – DR3 produce anti – SS A (R0) and anti SS-B (La) autoantibodies. (SS= sjogrens syndrome, Ro, La and Sm = patients in whom the antibodies were first identified).
3. Those with HLA – DR4 or HLA – DR5 produce anti Sm and anti RNP (ribonucleo protein) autoantibodies.

e. Altered serum levels:

Most patients show hypergammaglobulinemia and reduced serum complement levels (particularly C3 and C4) as a result of increased complement consumption caused by immune complex formation.

f. T- cell changes:

The number of T cell decreases and thus the ability to develop delayed hypersensitivity is impaired. Antibodies cytotoxic for T cells correlate with this T- cell deficiency.

g. Animal studies:

1. Mice of the NZB / NZW strain spontaneously develop SLE with essentially all of the immunologic features of the disease (eg LE cells, antinuclear antibodies) immune complex glomeulonephritis, T- cell deficiency). The deficiency of Ts cells is associated with the presence of thymocytotoxic antibodies in the serum.
2. Estrognes enhance anti – DNA antibody formation and increase the severity of renal disease in experimental animals, where androgens have the opposite effect. These findings suggest that hormones may be involved in the predominance of SLE in women.

III. Encephalomyelitis:

1. Acute disseminated encephalomyelitis:

May occur after vaccination (eg. Rabies immunization or vial infection (eg measles, influenza).

a. Clinicopathologic features:

1. Symptoms:

Include headache, backache, stiff neck, nausea, low – grade fever, malaise and weakness or paralysis of extremities. Vaccination with attenuated viruses (eg. Measles rubella) may produce symptoms such as elevated body temperature, convulsion, and drowsiness that may progress to a comatose state, and paralysis of textremities (particularly the legs).

2. Pathologic examination reveals perivsacular accumulation of macrophages, lymphocytes, and some neutrophils through out the gray or white matter of the brain with variable demyelination.

3. Although survivors of the acute stages of disease usually experience no permanent neuroloigc sequelae, mental retardation, epileptic seizures, or even death can result.

b. Immunologic findings:

These suggest that the disease represents a cell mediated allergic response to myelin basic proteins, similar to that seen in experimental allergic encephalomyelitis. Antibodies to myelin basic protein do not appear to be involved.

2. Experimental allergic encephalomyelitis:

Is the experimental model for postvaccinal and postinfectious encephalomyelitis in humans. This disease can be induced in animals (eg guinea pigs) by the injection of either homologous orheterologous brain or spinal cord extracts emulsified in complete friends adjuvant.

a. Clinicopathologic feature:

1. Two to three weeks after sensitization, animals begin to lose weight and then develop impaired righting reflex, ataxia, flaccid paralysis of the hind legs, urinary incontinence, and fecal impaction. Although most animals ultimately die, some recover completely.
2. histologic lesions consists of demyeliantion and perivascular accumulation of lymphocytes, mononuclear cells and plasma cells throughout the brain and spinal cord.

b. Immunologic findings:

1. The encephalitogenic factor appears to be certain polypetide sequences of myelin basic protein.
2. Although both B cells and T cells respond to basic protein, T cells evidently are responsible for lesions idemyelination.
    a. Animals demonstrate classic cutaneous delayed hypersensitivity to basic protein, and lymphoid cells can passively transfer the disease.
    b. Antibodies to basic protein are formed, but they correlate poorly with disease and cannot passively transfer it.

IV. Multiple sclerosis:

Is a relapsing neuromuscular disease with periods of exacebation and remission.

1. Clinicopathologic features:

a. Symptoms:

Include motor weakness, ataxia, impaired vision, urinary bladder dys- function, paresthesia, and mental aberrations.

b. Inflammatory lesions (sclerotic plaques), consisting of mononuclear cell infiltrates and demyelination, are confined to the myelin in the central nervous system.

2. Immunologic findings:

a. The cause of multiple sclerosis remains unknown, but the clustering of cases suggests an infectious agent.

1. Epidemiologic studies have revealed high risk and low risk areas of the world, indicating that a transmissible agent may play a role.
2. Patients with this condition tend to have elevated levels of antibodies to measles virus in their serum and spinal fluid: however, the exact role of measles virus is unknown. Evidence indicates that other viral agents may be involved.

b. A close associated with HLA – DR2 and HLA – DRwl suggests that susceptibility may be genetically determined.

c. Most patients show increased concentration of IgG in the spinal fluid and elevations in the spinal fluid – serum immunoglobulin ratio. The oligoclonal pattern seen on electrophores indicates that local IgG synthesis occurs in nerve tissue.

d. Patients tend to have suppressed levels of circulating T cells and increased levels of B cells. This may explain the decrease in the delayed hypersensitivity response to measles observed in patients.

V. Myasthenia gravis:

Is a chronic disease resulting from faulty neuro muscular transmission.

1. Clinicopathologic features:

a. The disease is characterized by muscle weakness and fatigue, particularly of the orcular, pharyngeal, facial, laryngeal and skeletal muscles.
b. Muscle weakness and neuromuscular dysfunction result from depletion of acetyl choline receptors at the myoneural junction.

2. Immunologic findings:

a. Experimental model:

1. Injection of experimental animals with purified acetylcholine receptor incorporated in complete Freunds adjuvant induces experimental myasthenia gravis that closely minics human disease.
2. Serum form animals with experimentally induced disease, as well as serum from human patients, passively transfers the condition to normal animals.

b. Human disease:

Approximately 60% to 80% of patients have an enlarged thymus, and 80% to 90% have antibodies (Frequently an IgG3 isotype) against the acetylchoneural junction, causing endocytosis of the receptors. Although evidence indicates that cellular immunity also may play a role (eg. Lymphocyte stimulation by purified acetylcholine receptor and passive transfer with lymphoid cells), the antibody response appears to be more intimately involved.

c. Complement – binding immune complexes may cause further problems at the myoneural junction.

VI. Chronic thyroiditis (Hashimoto's thyrodiditis):

Is a thyroid disease that mainly affects women between 30 and 50 years of age.

1. Clinicopathologic features:

a. The thyroid gland may be enlarged (goiter) and a firm or hard. Histologic examination reveals a lymphocyte and plasma cell infiltrate, disapperance of colloid, and varying amounts of fibrosis.
b. As the disease progresses, signs of hypothyroidism (eg low values of circulating thyroid hormones, decreased thyroidal uptake of radioiodine) may be seen.

2. Immunologic findings:

a. Various antibodies to thyroid specific antigens can be demonstrated.

1. Antibody to thyroglobulin precipitates this antigen and aggluntinates, tanned red blood cells coated with thyroglobulin (passive hemagglutination). Antithyroglobulin antibodies also may be detected via radioimmunossay (RIA) immunofluorescence, and enzyme linked immunosorbent assay (ELISA).
2. Antibody to thyroid microsomal antigen can be detected by complement fixation and hemagglutinaiton tests.
3. Antibody to a second colloid antigen has been detected but is not well defined.

b. Experimental model:

1. Animals (eg. Rabbit, guinea pig, rat, mouse) injected with homologous or autologous thyroid extract incorporated in complete Fruends adjuvant develop lesions and other features similar to those seen in human disease.
2. A delayed hypersensitivity reaction to thyroid extract also can be demonstrated.

c. The relative importance of T cells and B cells in the pathogenesis of diseases is not yet clearly resolved. Both cell types may be essential or maximum effect. Antibody dependent cell – mediated cytoxoicity also may be involved.

VII. Graves disease (thyrotoxicosis, hyperthroidism):

Results from the overproduction of thyroid hormone (throxine).

1. Clinicopathologic features:

Patients exhibit fatigue, nervousness, increased sweating, palpitations, weight loss and heat intolerance.

2. Immunologic findings:

a. An increase in the number of peripheral B cells correlates with the severity of the disease.

b. Patients also show a decrease in the percentage of T cells in blood, mainly Ts cells.

c. Current evidence suggests that patients produce several antibodies to thyrotropin receptors.

1. One type of antibody blocks the binding of thyroid stimulating hormone (TASH) to thyroid epithelial cells.
2. A second antithyroid antibody causes thyroid cells to proliferate.
3. A third type, referred to as thyroid stimulating antibody, reacts with TSH receptors on the thyroid cell membrane and minics the action of the pituitary horomoe thyrotropin. The result of this interaction is overproduction of thyroid hormone and hyperthyroidism.

d. Because thyroid stimulating antibodies are IgG, they can cross the placenta and cause hyperthyroidism in the new born. The condition resolves spontaneously as the maternal IgG is catabolised by the infant over a period of several weeks.

e. Increased frequencies of HLA – Bw 35 and HLA – DR3 have been found.

VIII. Insulin dependent diabetes mellitus (IDDM : Type 1 diabetes, juvenile diabetes):

Results from immunologic destruction of the insulin producing beta cells of the islets of langerhans in the pancreas. This disease affects approximately 1 of every 500 people in the United States. Its onset peaks between the ages of 10 and 15 years.

1. Clinicopathologic features:

a. An inability to synthesize insulin makes the patient susceptible to wide fluctuation in blood glucose levels. Acute manifestations of insulin insufficiency include ketoacidosis, polyuria, polydipaisa, and polyphagia.

b. Chronic hyperglycemia and associated abnormal metabolic events lead to cardiovascular disease, neuropathies, kidney problems and cataracts.

c. Treatment:

Patients with IDDM are refractory to dietary control and oral hypoglycemic drugs, and require daily insulin injections.

2. Immunologic findings:

a. Affected people produce antibodies to insulin and to a membrane and cytoplasmic constituents of beta cells:

1. Anti – islet cell antibodies of the IgG or IgG4 subsclases are observed in most patients (> 90degree) with type 1 diabetes.
2. Anti – insulin antibodies are produced by most patients with diabetes on insulin therapy. These are most likely induced by the hormone injections; however, these same antibodies also are seen in patients with IDDM at the time of diagnosis before any insulin therapy.

b. Cytotoxicity of T cells for islet cells:

Also has been reported. The islets are infiltrated with B and T cells and are eventually destroyed.

c. The onset of IDDM is often preceded by a viral infection, mumps, cytomegalovirus, influenza, rubella, and coxsackievirus have been implicated.

1. Mumps and coxsackievirus can destroy islet cells into vitro. A B4 coxsackievirus, isolated from the pancreas of a diabetic child, has produced a similar disease in experimental animals.
2. IDDM develops in 15% of children with congential rubella.
3. reports have indicated that antigenic mimicry between insulin and a retroviral antigen is responsible for the emergence of anti – insulin antibodies in a strain of diabetes – prone mice. This further supports the notion that IDDM may be triggered by infection with an agent that expresses cross reactive antigens.

d. People with HLA – DR3 and HLA – DR4 haplotypes have an increase risk for development of IDDM.

e. Genetics:

Variations in B – chain alleles of an HLA – DQ antigen distinguish susceptible from resistance people.

1. Serine, valine, or alanine at amino acid position 57 appear to confer susceptiability.

2. Aspartic acid at position 57 (asps57) is correlated with protection. The mechanism of this effect is obscure. Suggested theories have included the following:
    a. The epitope with the asp57 resembles the antigen (self or viral) that triggers, the autoimmune response and thus induces autotolerance.
    b. Asp 57 adversely affects epitope presentation to T cells because it lies in the peptide binding groove of the class II MHC molecule.

IX. Goodpasture's syndrome:

Is a rare, progressive disease of the lungs and kidneys. This disorder occurs in all age groups, but unlike most other autoimmune diseases, it mainly affects young men. The prognosis is poor.

1. Clinicopathologic features:

Classic symptoms include pulmonary hemorrhage, hemoptysis, hematuria, and glomerulonephritis. Pulmonary infiltrates may show on radiographs.

2. Immunologic findings:

a. Immunofluorescence reveals linear deposits of immunoglobulin (usually IgG) and complement on alveolar and glomerular basement membranes. The IgG appears to represent an antibody specific for an antigen shared by these kidney and lung membranes.
b. Some cases have characteristics suggestive of an immune complex phenomenon.

X. Pernicious anemia:

Results from defective red blood cell maturation caused by faulty absorption of vitamin B_{12}. Normally, dietary Vitamin B_{12}, is transported across the small intestine into the body as a complex with intrinsic factor, which is synthesized by parietal cells in the gastric mucosa, in patients with pernicious anemia, this process is blocked.

1. Clinicopathologic features:

a. The hallmark of the disease is the progressive destruction of gastric glands and the associated loss of parietal cells. The consequent lack of intrinsic factor leads to failure of vitamin B_{12} absorption.
b. Mononuclear leukocytes and neutrophils infiltrate the gastric mucosa. Defective red blood cell maturation leads to megaloblastic animea, with attendant weakness, loss of appetie, fatigue, pallor and weight loss.
c. Neurologic damage can occur as a result of vitamin B_{12} deficiency and may be irreversible. Patients also may show an increased incidence of stomach cancer.

2. Immunologic findings:

Patients produce antibodies (mainly IgG) to three different gastric parietal cell antigens, all of which are cell specific. In addition, antibody to intrinsic factor is produced.
a. Antibody to intrinsic factor may block the attachment of vitamin B_{12} to intrinsic factory or may bind to intrinsic factor or the intrinsic factor – B_{12} complex.
b. Some patients also show cell mediated immunity to intrinsic factor and parietal cell antigens.

XI. Autoimmune hemolytic disease:

These include warm – antibody hemolytic anemia (the most common type of autoimmune hemolytic disease) cold antibody hemolytic anemia, and paroxysmal cold hemoglobinuria.

1. Clinicopathologic features:

Classic symptoms include fatigue, fever, jaundice and splenomegaly relating to the presence of antibodies directed against self red blood cell antigens and the resultant anemia.

2. Immunologic findings:

a. Warm – antibody hemolytic anemia:

1. So called "warm" antibodies show optimum reactivity at 37C. they are primarily IgG, are poor at complement fixing, and can be detected on the red blood cell surface by the antiglobulin (coombs) test. Warm antibodies are directed primarily against Rh determinants.
2. Various patterns of red blood coating can be detected in different patients. Cells can be coated with IgG alone, IgG plus complement, or complement alone. Antibody coated red cells appear to be opsonized for phagocytosis primarily in the spleen.

b. Cold antibody hemolytic anemia:

Hemolysis is usually mild and the symptoms are confined to cold exposed extremities.

1. So called cold antibodies show optimum reactivity at 4 C. they are primarily of the IgM class, fix complement, and agglutinate red blood cells directly, with out the requirement for Coombs antiglobulin.
2. The IgM is specific against red blood cell antigen I.
3. Cold agglutinins also may be detected secondary to infections (eg mycoplasmal pneumonia).

C. paroxysmal cold hemoglobinuria:

This rare syndrome is associated with Donath Landsteiner antibody , a cold antibody of the IgG class directed against red blood cell antigen P.

1. The antibody is biphasic in that it sensitizes cells in the cold, usually below 15 C and then hemolyzes them when the temperature is elevated to 37C.
2. Patients demonstrate symptoms (eg fever, pain in extremities, jaundice, hemoglobinuria) after exposure to cold.

XII. Idiopathic thrombocytopenic purpura (ITP):

Which, may be either acute or chronic, results from antibody mediated platelet destruction. In children, this disease is sometimes preceded by a viral infection.

1. Clinicopathologic features:

a. Patients demostrate petechiae and various bleeding problems in the gums, gastrointestinal tract, and genitourinary tract.
b. Peripheral blood platelet counts are profoundly suppressed (i.e < 100,000 ml). Platelet survival time is also low.

c. Immune complex induced thrombocytopenia can be a significant component of serum sickness brought on by therapeutic administration of murine monoclonal antibodies.

2. Immunolgoic findings:

a. IgG antibodies specific for platelets can be demonstrated. Antibody coated platelets are sequestered and destroyed by macrophages of the spleen and liver primarily. Routine, simple laboratory techniques are not sensitive enough to detect the antibodies and more sophisticated tests must be used (eg. ELISA or radiolabeled coomsb antiglobulin test).

b. Thrombocytopenia sometimes may be drug – induced.

1. Causative drugs include sulfonamide, antihistamines, quinidine, and quinine.
2. Drug antibody complexes are adsorbed on to the platelet surface, resulting in complement activation. Treatment consists of withholding the drug.

XIII. Bullous (Vesicular disease):

Are chronic dermatolgoic problems that result when destruction of intercellular bridges (desmosomes) interferes with cohesion of the epidermis. This process leads to the formationof blisters (bullae).

1. Clinicopathologic features:

a. Pemphigus vulgaris:

An erosive disease of the skin and mucous membranes, is characteriszed by intraepidermal blisters.

b. Bullous pemphigoid:

A bullous disease of the skin and mucosa, is usually seen in middle aged and older people. The blisters form beneath the epidermis at the dermal epidermal junction.

2. Immunologic findings:

a. Pemphigus vulgaris:

Skin lesions show antibody (mainly IgG) deposition and complement components in squamous intercellular spaces when examined by immunofluoresence.

b. Bullous pemphigoid:

Lesions demonstrate deposition of antibody and complement along skin basement membrane. Circulating antibasement membrane antibodies also can be detected.

XIV. Polymyositis – dermatomyositis:

Is an acute or chronic inflammatory disease of the muscles and skin.

1. Clinicopathlogic feature:

Patients demonstrate weakness of striated muscle, with some muscle pain and tenderness, and a characteristic skin rash.

2. Immunologic findings:
a. Hypergammaglobulinemia is common, along with deposition of immunoglobulin and complement in the vessel walls of the skin and muscle.
b. Studies showing cellular passive transfer and lymphokine release by T cells indicate that cellular immunity also may play a role in pathogenesis.

XIIV. Scleroderma:

Is a slowly progressive, chronic, disabling disease characterized by abnormally increased collagen deposition in the skin and occasionally in the internal organs. This disorder commonly begins in the third or fourth decade of life and affects women twice as often as men.

1. Clinicopathologic features, Raynaud's phenonomenon:

Intermittent bilateral attacks of ischemia of the fingers to toes, or sometimes the ears or nose, marked by severe pallor and accompanied by paresthesia and pain) frequently is the first symptom noted. Skin involvement may be limited to the hands, forearms, feet or face.

a. The unique pentad of symptoms, the so called CREST syndrome (Calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyl, telangiectases may remain stable for years.
b. Progressive systemic sclerosis usually begins with polyarthritis, but symptoms vary depending on the organs involved in the abnormal collagen deposition.

2. Immunologic findings:
a. Antinuclear antibodies having reactivity with nucleolar antigens are commonly found.
b. Patients with CREST syndrome have antibodies of diagnostic importance that react with centromeres.