

SOLMON

LECTURE I
IMMUNOLOGY
IMMUNOLOGY: THE HISTORICAL PERSPECTIVE

Immunology could be defined as the science that deals with the structure and functioning of the body's defense mechanisms. It involves the study of cells, organs and molecules responsible for surveillance, recognition and elimination of foreign substances or microbes. Thus ensuring resistance to sicknesses, or infection.

The study earlier emphasizes only on the body's defense mechanisms against foreign invaders. Later, it embraces the immune tolerance; the tendency for recognizing self. Currently, the concept include immune responses, interactions and other activities; that may modulate immunological responses and may become deleterious or fatal to the host. The deleterious conditions include allergy, autoimmune diseases, materno-foetal disorders and cancers.

The history of immunology dates back to 430BC in Athens during the Peloponnesian war II. There was a plague that period and people died. However, a Greek historian; Thucydides made some important observations. The major observation was that less attention was devoted to some sick persons as a result of an infection. Moreover, those that contacted the infection earlier and were severely affected also recovered fully. Again, those that were affected never experienced the attack for the second time. So by reason of interpretation, it means that the first attack of the disease conferred on the infected subjects, a level of resistance and prevented them from succumbing to the infection in subsequent times.

The Arabs and Chinese imbibed the concept and by 15th Century. Subsequently, they intentionally infected individuals with materials from postules of smallpox patients. The procedure gave the infected subjects a mild form of the disease as well as inducing resistance to the disease. The procedure was carried out by making incision with a pin (poking) on the skin which was referred to as VARIOLATION. In essence, Variolation could be defined as a form of immunization whereby healthy individuals were deliberately infected with live viral particles of small pox known as VARIOLA by inoculating the virus on the skin. This is different from Vaccination where the virulence of an organism has been removed and administered yet achieving resistance to an infection. Shortly, Variolation became a common practice and was also carried out in the Middle East. Variolation was condemned because it is unethical to administer an unattenuated organism; with its virulent factors into a subject.

By the 17th Century, Variolation was frequently carried out in Western Europe and in 1718 Lady Mary Wortley Montagu (the wife of the English Ambassador in Constantinople (former Istanbul)) popularized the practice. This she did when she permitted her son to be inoculated with smallpox by the surgeon of the Embassy. Similarly, the youngest son of Prince of Wales with the express permission of the King was inoculated with small pox materials. Shortly after the inoculation, the young boy fell sick and later recovered and the sickness was

attributed to variolation. Overall, the process was successfully carried out on the boy and he survived.

Subsequently, the same King of Wales was approached with a proposal to carry out the procedure on few condemned criminals, on the condition for them to be pardoned. The King accepted the proposal and in August, 1721 the inoculation was carried out on the criminals. In carrying out the procedure on the criminals (3 males and 3 females), incisions were made on their skin and pus from the smallpox lesion from sick individuals were inserted in them. After a brief illness, all the criminals recovered fully. Moreover, one of the female criminals was ordered to lie every night on the same bed with a smallpox patient and also attend to the person. At the end, the female criminal did not contract the disease. Similarly, 5 orphans were subjected to the same process; inoculated with smallpox material and all survived. Thereafter the Prince of Wales permitted her two daughters to be inoculated and they also survived. Notwithstanding that the inoculation with smallpox was successful; the procedure was severely opposed because the consent of the subjects were not obtained.

Considering the success recorded above, Edward Jenner; an English Physician in 1749-1823 in Gloucestershire noticed that farm workers frequently contacted pustular Hooves (called Grease) from Horses. The hooves disease is a Cowpox viral infectious disease. The milkmaids picked up the disease from the nipple of cows and developed sores on the skin. Thereafter in 14th May, 1796, Edward Jenner collected a substance from the sore on the hand of a dairy maid called Sarah Nelmes and by superficial incision inserted it into the arm of an 8 year old boy called James Philip.

On 1st July, 1796, Jenner inoculated the same boy with particulate matter from a smallpox patient. Resultantly, James Philip did not develop smallpox except for small sores at the site of inoculation (variolation). Thus the initial exposure to cowpox disease was seen to have resisted succumbing to the smallpox. Having seen that inoculating the individual with cowpox virus prevented the development of smallpox viral disease, it became necessary to explain the condition and it was ascertained that the two viruses (smallpox and cowpox) particles appear to be antigenically and immunogenically similar. Jenner subsequently conducted other experiments. In this manner, Edward Jenner initiated the science and birth of Immunology.

Furthermore, Immunology got developed when it was postulated that some unseen organisms may be responsible for causing disease. This postulation had existed for centuries ago however lacked scientific evidence. But success was achieved when Anton van Leeuwenhoek invented polished lenses of short focal length (microscope) and was able to describe Protozoans, Bacteria and Rotifers. In addition in 1677, Anton described the human sperm cells. Thereafter, discovering the microscope became a landmark in the development of Microbiology and Immunology.

Following the invention of microscope, Casmir Davaine in 1850 discovered the Anthrax bacillus in the blood of infected sheep. By 1864, a French chemist; Louis Pasteur proposed the Germ theory of diseases. The Germ theory of diseases emphasized that the air contains

microorganisms and the organisms are alive and the microbes in the air can cause decay of some substances. Emphatically, the microorganisms are responsible for the fermentation of some substances such as beer, wine and meat. In addition, they are also responsible in causing diseases. The Germ theory has helped in the growth of the agro-industrial sector.

In furtherance of his research, Louis Pasteur demonstrated that microorganisms could be weakened or killed by heat (Pasteurization). Therefore he posited that microbial virulence could be attenuated by temperature and by subculturing. Consequent upon these facts, he developed vaccines against Anthrax, Chicken cholera and Rabies using the various attenuated viral particles. Given the successes recorded, Louis Pasteur's works paved way for Modern Immunology and he is therefore regarded as the Father of Bacteriology.

Subsequently in 1882, Robert Koch expanded and applied the germ theory of diseases in immunology. Robert Koch indicated that germs (microorganisms) could cause a particular disease. Consequent upon the theory, he examined the blood of cows that died of anthrax using the microscope. He observed rod shaped bacteria and called them Anthrax. He also discovered and isolated the tubercle bacilli as the infective agent of tuberculosis. Again, He Kleb and Friedrich Loeffler discovered the Diphtheria bacillus whereas Joseph Lister discovered the Aseptic surgery in 1867. Lots of interests were captured in this area and scientists came up with discovering of vaccines for different diseases.

Progress was made again in Immunology when researchers showed enthusiasm in the production and administration of vaccines. As a matter of fact, Robert Koch produced the Anthrax vaccine whereas Theobold Smith in 1886 developed the Chicken cholera vaccine.

John Enders in 1949 succeeded in tissue culture (culturing human viruses outside a living host) and also produces vaccines against viruses.

By 1952, Dr. Jonas Salk had successfully cultured 3 strains of Polio viruses. He used formalin to inactivate the Polioviruses and produced the injectable polio vaccine. Later, Dr. Albert Sabin developed the attenuated poliovirus vaccine and produced Oral Polio vaccine. The vaccine was found to be effective and was licensed for use in 1960 and it is being used today.

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In advancing immunology, in 1890, von Behring and Kitasato introduced Serotherapy. In this procedure, toxoids that is; vaccines produced from toxins were used to treat patients. Toxins were contained in serum.

In 20th Century, landmarks were made in Immunology in an attempt to clearly establish the immune mechanisms. Concomitantly, two schools of thought were postulated and the postulations were of two different immune mechanisms. A school of thought postulated the cellular mechanism while the other postulated humoral mechanisms. The cellular immune mechanism was earlier proposed by a Russian scientist; Elie Metchnikoff in 1883 (19th Century) and that leukocytes were primarily responsible for immunity. From all indications,

Ellie Metchnikoff allied with Louis Pasteur and their supporters were known as strong proponents of cellular immune mechanism.

The other school of thought proposed the humoral immune mechanism. The Humoralists stipulated that antibodies (humoral substances) were saddled with the responsibility of inducing immune response. The postulation was spearheaded by Paul Ehrlich, a German Scientist who had earlier proposed the Side Chain Theory. In the Side Chain Theory, Paul Ehrlich proposed how antibodies were produced by B cells (Plasma cells). Again, he explained for the first time, the chemical structure of antibodies. Therefore, Paul Ehrlich contributed significantly in the development of the science of immunology when he showed the formation and structure of antibodies and that the interaction of the antigens with the antibodies that provoked their production, proffers resistance to diseases.

For every indication, the English were the proponent of the cellular arm of the immune response while the Germans were the proponents of the humoral arm of immune response. Overall, the cellularists and humoralists were partly correct as the cell mediated and humoral arms have been proved to mediate immune responses. The two arms interact with each other to consolidate immunity.

Another giant stride recorded in Immunology was in 1900 when Karl Landsteine discovered the ABO blood group system via the humoral concept of antibody production in immunity.

In 1903, Sir Almorth Wright discovered Opsonins (antibodies that assisted in phagocytosis), 1901, Bordet and Octave Gengon introduced the Complement fixation test. Complement fixation test was used as a procedure in diagnosing more viral and bacterial infections.

In 1939, Arne Withelm Tiselius and Elvinkabat discovered that antibodies belong to the family of immunoglobulin while in 1948, Atrid Fagraeus posited that antibodies are secreted by Plasma cells.

The later years of 1960s to early 1970s were regarded as the beginning of Modern Immunology. In 1960, the interactions between the humoral and cellular immune responses were appreciated and the functions of the B and T cells realized. Most importantly, in 1966, Henry Claman, E.A. Cheperon and R.F. Triplett discovered the cooperation between B and T lymphocytes.

In 1970s, the molecular and genetic techniques helped further to understand the immune system. As a matter of fact in 1982 and 1983, James Allison unassumingly discovered the T cell receptors.

Contrary to the benefit of immune response in protecting humans from microbes, the response was postulated in most cases to be harmful and life threatening to the host. Consequent upon that, Charles Richet and Paul Portier in 1902 discovered anaphylaxis while Clemens von Pirquet coined the name Allergic Reactions. Other recognizable adverse conditions that may be associated with immune responses are Autoimmune diseases such as Systemic Lupus Erythematosus, Rheumatoid arthritis, Hashimoto's disease, Graves' disease,

etc. Moreover Transplant rejection, Immunodeficiency and Cancers were much more recognized. It could be remembered that most cancer cases and transplant rejections were discovered firstly after the 1st and 2nd World Wars.

Between 1983 and 1990, the dreaded HIV/AIDS triggered further research and postulation, and responsible in transcribing the viral RNA into the host DNA. In addition, the activity of the macrophages, B and T cells to potentiate immunity and to eliminate the viral particles were also posited.

Currently, the COVID 19 pandemic of 2019 discovered in Wuhan, China has provoked more studies on the ways to combat the disease. The aetio-pathophysiology of the disorder; the invasion of the cells and the macrophages lining the respiratory tract and the consequent cytokine storm has been established. All these results in the acute respiratory distress and overall failure of the pulmonary system and the death of the infected persons. Omicron; the variant form of Covid-19, has been studied as occurring as a more fatal form of Corona virus. Currently, attention has been devoted to immunotherapy; strictly speaking with the vaccine production and Potentiating the immunity using zinc and administration of potent antioxidant as the most effective means to save human beings from the COVID-19 related death.

Conclusion: The history of immunology started when the terror unleashed on human beings by the smallpox virus. The disease was associated with frequent deaths. Subsequently efforts were made to produce a cure and that resulted in vaccine production and administration. It is not as if complete success has been recorded in the application of immunologic principles and practice. This is as a result of some observable limitations for instance, the cooperation between the T and B cells has not eliminated HIV virus completely besides some HIV drugs administered to patients are not without organ derangement such as chronic kidney failure, anaemia, cancer and other fatal consequences.

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LECTURE 2

CLASSIFICATION OF IMMUNITY

Immunity refers to the processes or mechanisms whereby the body resists succumbing to infection. The process is divided into two broad arms. The arms are the innate and acquired immunity. Both arms are distinct entities that does not operate independent of the other rather interacts and function to keep the body in health. Dividing the arms into two categories however makes it easy to understand each component arm and the possible synergy existing between the two arms.

(Also NON-SPECIFIC IMMUNITY)
INNATE IMMUNITY (also known as the NATURAL IMMUNITY) involve the mechanisms that are already in place in the host before any challenge occurs (that is to say that they are preformed). In other words the mechanisms serve as the first line of defense to combat infection. The innate immunity has some characteristics in which it is identified.

The characteristics are;

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1. They are present at birth and do not require the entry of any microorganism for it to be formed.
2. The arm proffers the 1st line defense against any microbial invasion. And to achieve this, the mechanism respond immediately (does not wait for days) to combat any microbe.
3. The innate arm of immunity is non-specific in nature, This is to say that it responds to entering of any microbe irrespective of the nature of the microbe either as a pathogen or non-pathogen. In other words, it acts against any invader and does not differentiate whether it is harmful or not.
4. Does not recognize memory. The innate arm does not recognize whether the component cells had earlier been exposed to any invader. Therefore it is standardized in response. Being standardized in response mean that the magnitude of response is consistent and does not depend on dose effect.
5. The innate arm of immune response could be influenced by age, sex, race and some other anthropometric indices for instance there are some disorders in which their development is age dependent. In most cases is gender dependent and affects males more than females and vice versa. For race, the Caucasians may be vulnerable to a particular ailment than the other and vice versa and then could be genetically involving.

Classification of Innate (Natural) Immunity

External : Anatomical Barriers eg Skin, Mucous
① Salivary glands

Internal :

The natural arm of immunity is carried out in different ways and this culminates in classifying the arm based on the mechanism involved as EXTERNAL and INTERNAL MECHANISMS. The External mechanism proffers barriers that prevent microbes from entering into the body. The barriers acts on the external parts of the body and are said to be ANATOMICAL BARRIERS. The anatomical barriers may be found on the skin, the mucus

lining of the respiratory and digestive tract and the gastrointestinal tracts. The barriers include saliva, tears, and hair protrusions of the epithelial cells (cilia). As for the INTERNAL mechanisms of the innate arm, the mechanisms act within the body and exits as PHYSIOLOGIC, PHAGOCYTIC and INFLAMMATORY barriers as well as the ENZYME & SOLUBLE PROTEINS.

The Physiologic barriers could be provided in form of Temperature regulation and pH. As for temperature, it could be in febrile (feverish) condition which occurs as a result of activities in checkmating the spread of microbes. The pH of the body may be reduced as found in HCl content of the gastric juice and may prevent the spread of microbes.

The Phagocytic barriers are provided by the presence of phagocytes. The phagocytes are polymorphonuclear cells: the neutrophils, Eosinophils and Basophils as well as the mononuclear cells principally the monocytes. These cells engulf microbes and ward them off by various cooperative mechanisms.

Inflammatory Barriers: This involves the rapid response of phagocytic cells at the site of damage or microbial infection. It is associated with the accumulation of fluids and some serum proteins in eliminating the microbes. The serum protein include the Complement proteins e.g. C3b, C4, C5, and the acute phase reactant proteins such as the C-reactive protein and mannose binding protein, etc.

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The Enzyme & Soluble Proteins: These barriers are provided by the content of the granules and lysozymes of the cells as well as the barrier provided by acute phase protein. The acute phase proteins or acute phase reactants includes CRP, Mannose binding proteins and serum amyloid A, (SAA) and proteins of the complement pathways such as C3b, C5, C6, C7, C8 and C9. These proteins may help in Opsonization. Besides, with the aid of the enzymes, the body also generates Reactive Oxygen Species such as H_2O_2 , NO_3^- , OH^- , superoxide anions which are detrimental to the growth of microbes.

ACQUIRED IMMUNITY (ACTIVE)

The acquired immunity against microbe are responses induced in the host as a result of the entering of microbes. To be precise, the responses are not present at birth or before the entry of the microbe. The acquired immunity is considered as the most dependable form of immunity as a result of the strong responses mounted by the immune cells to killing and eliminating pathogen from the body. It has its characteristics,

1. **Inducibility:** It is provoked by the entrance of a microbe or substance and never occurs before birth.
2. **Specificity:** The immune responses are specific. It means that the response are strictly and only for that particular microbe for which the response is induced.
3. **Distinguishes self from non-self:** The responses elicited are directed against the offending microbe and not self. This is explained by the concept known as immune tolerance. In immune tolerance, the body recognizes microbes and raises responses against them but does not do so with self-cells.

4. **Memory:** Upon entering of microbe into the circulation, the major cells involved; the lymphocytes are activated. The activated cells later divide into two; the Effector cells and the Memory cells. The Effector cells eliminate the microbe while the Memory cells enter the tissues and may last for months and years. The importance of Memory cells is that they may be activated within a short time upon the re-entry of a particular microbe and mount strong immune response that results in the quick elimination of the microbe.

5. **Diversity:** Before the release of immune cells into the circulation, the immune cells are conferred with signals to enable them respond appropriately to a particular insult (or microbe). The signals could be the surface immunoglobulin for the B cells and the T cell receptors (TCR) for the thymus dependent lymphocytes. It can recognise a vast variety of foreign materials (antigens).

The acquired immune responses are the B and T lymphocytes and major cells of the communication molecules known as Cytokines. The secondary cells of the response are the Macrophages. The macrophages also secrete communication molecules (cytokines) which contributes in activation of other cells particularly the lymphocytes. The macrophages also eliminate intracellular organisms. Besides they act as Antigen Presenting Cells (APCs) by facilitating the removal of microbes to the T and B cells resulting in eliminating the organisms located intracellularly and extracellularly (on the surface of the cells).

CLASSIFICATION OF THE ACQUIRED IMMUNE RESPONSE

The acquired immune response is divided further into two components namely; the humoral immune response and the cell mediated immune response. The humoral and cell mediated immune response could be divided into active and passive immunity. Active immunity is gradually acquired within 5 to 14 days after antigen exposure and may last for years. It is markedly protective. The Passive immunity is immediate but lasts for days to months and has low to moderate protective effectiveness and may not develop memory in the recipients.

Both active and passive immunity may further be divided into Natural and Artificial and Formal or Adoptive transfers. However the sub-classification are less important and mere academic exercise.

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THE MECHANISM OF HUMORAL IMMUNE RESPONSE

The Humoral immunity is mediated by Antibodies. Antibodies are immunoglobulin molecules secreted by plasma cells. The plasma cells are progeny of activated B cells. On activation, the B cells transform into plasma cells and memory B cells. The plasma cells secrete immunoglobulin known as Antibodies. Antibodies could be defined as antigenic or immunologic activation. The antibodies protects against circulating extracellular antigens such as bacteria, microbial exotoxins and viruses. It means that antibodies are unable to penetrate living cells. The antibody secreted by plasma cells bind only to the antigens that provoked their production and this is the essence of the specificity of antibodies. As long as the antigen remains in the circulation, new plasma cells are being produced which also secrete more antibodies. Consequently, new plasma cells are produced with continuous

secretion of antibodies thus increasing the amount of antibody in the body. For example in infection such as *Mycobacterium tuberculosis* and leprosy, when the microorganisms tend to live in the host for a long time, the antibodies are continually produced and detected.

Plasma cells don't divide further after the secretion of antibodies and dies in few days or week. Consequent upon this, the elimination of the organism leads to the decreased production of plasma cell and antibodies are drastically and finally removed from the circulation by the macrophages.

CELL-MEDIATED IMMUNE RESPONSE (CMI)

The Cell-Mediated Immune response serves as another component of the acquired immune mechanism and it is mediated by the T lymphocytes (Thymus derived lymphocytes). The T lymphocytes are of two sub-populations: the helper T cells (T_H) and the Cytotoxic T cells (T_C). The CMI protects against intracellular microbes such as viruses and bacteria by the effector T cells. The effector T cells secrete cytokines such as interferon gamma which kill the microorganisms. Moreover, they are important in the rejection of organ transplants and tumor cells.

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LECTURE 3

CELLS OF THE IMMUNE RESPONSE

Introduction: Immunology could again be defined as the science or study that deals with the body's defense against infectious agents and other foreign substances. The infectious agents (pathogens) could be viruses, bacteria, fungi and parasites. Most foreign substances may not necessarily be infectious rather has the propensity to cause some disorders in the body. The disorders may even be of fatal or of terminal consequences. The cells of the body concerned to proffering resistance to infection are regarded as immune cells. The immune cells are majorly blood cells originates from the haemopoietic stem cells (HSC) through haemopoiesis

Haemopoiesis is the process whereby blood cells are synthesized; passes through regulated stages and proliferate, differentiate and mature into different cell lines. The HSC is small in size and arise in the mesoderm of the yolk sac during the first week of embryonic life. Within 2 months after conception about 5-7 weeks, the HSC migrates to the foetal liver where most of foetal haemopoiesis starts. Shortly after the liver, haematopoiesis continues in the spleen and kidney.

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About 9 weeks after conception, the HSC has migrated and colonizes the bone marrow cavities and continues with the production of blood cells. Under normal conditions, the commencement of haemopoiesis in the bone marrow marks the cessation of extramedullary haemopoiesis. Extramedullary haemopoiesis is the production of blood cells outside the bone marrow. At birth and throughout the person's life, the bone marrow is the normal site for haemopoiesis. However extramedullary haemopoiesis may occur later in life and may be considered abnormal.

The HSC has two unique characteristics which distinguishes it and away from other stem cells. These unique characteristics are;

- i. **It is pluripotent:** The haematopoietic stem cell being pluripotent means that it has the capacity to divide and generate different types of cells. On the first division of the HSC, two daughter cells are produced. Hence the name Pluripotent Haemopoietic Stem Cell. It is rare about 1:20 million and can repopulate to produce 10^6 blood cells after 70 mitotic divisions.
- ii. **Self-renewing:** For the HSC being self-renewing means that when it divides, one of the daughter cell retains the identical properties of the mother cell while the other daughter cell appears different from the mother cell. By extension, each of the daughter cell produced is different from the other.

With the above characteristics, the HSC under the influence of some Growth Factors and Haemopoietic Environment develops into various cell lineages and give rise to different progenitor or precursor stem cells.

The Growth factors are among the family of cytokines and those responsible for production of blood cells are known as Colony Stimulating Factors (CSF). The CSF are required to influence the growth and differentiation of blood cells from haemopoietic stem cells. Some

CSF that influences for the production of various or different progenitor stem cells are referred to as multilineage or multic colony stimulating factor e.g. the Granulocyte = Macrophage Colony Stimulating Factor (GM-CSF), Granulocyte Colony Stimulating Factor (G-CSF) and Macrophage Colony Stimulating Factor (M-CSF), IL-3, IL-17, etc.

While some CSF influences the production of only one cell line is known as Lineage Restricted Growth Factors e.g. Erythropoietin and thrombopoietin, IL-5, Monocyte Colony Stimulating Factors (M-CSF).

The haemopoietic environment, although poorly understood comprises of the endothelial cells, fibroblasts, adipocytes, macrophages and extracellular matrix. The stem cells and progenitors are bound to stromal cells or adhesion molecules within the matrix. The release of mature blood cells from the bone marrow into the circulation is regulated by the haemopoietic environment.

Overall under the influence of various colony stimulating factor and haemopoietic environment, the HSC gives rise to myelo-erythroid progenitors and lymphoid stem cells. The myelo-erythroid progenitors divides to give rise to erythroid progenitors and myeloid progenitors. The myeloid progenitors subsequently give rise to Granulocytes, monocyte progenitors and other progenitors for other leucocyte lineages. The progenitor and precursor cell lines produces the erythrocytes, the leukocytes and the platelets (thrombocytes) of the blood cells. The leukocytes are the important and major cells involved in immune response or the body's defensive mechanism. The leukocytes are nucleated and some are capable of exhibiting amoeboid movement. The normal range of the total leucocyte in the peripheral blood is about $4 - 11 \times 10^9/L$. The leukocytes may be classified as polymorphonuclear cells, lymphocytes and monocytes.

The polymorphonuclear white blood cells are the Neutrophils, Eosinophils and Basophils. The mononuclear cells are the monocytes and Lymphocytes. Other cells of the body (away from blood cells) known to provide defense against pathogens are the Dendritic cells, Follicular dendritic cells and Mast cells.

Haemopoietic environment comprised of an admixture of several adherent cell types including fibroblast

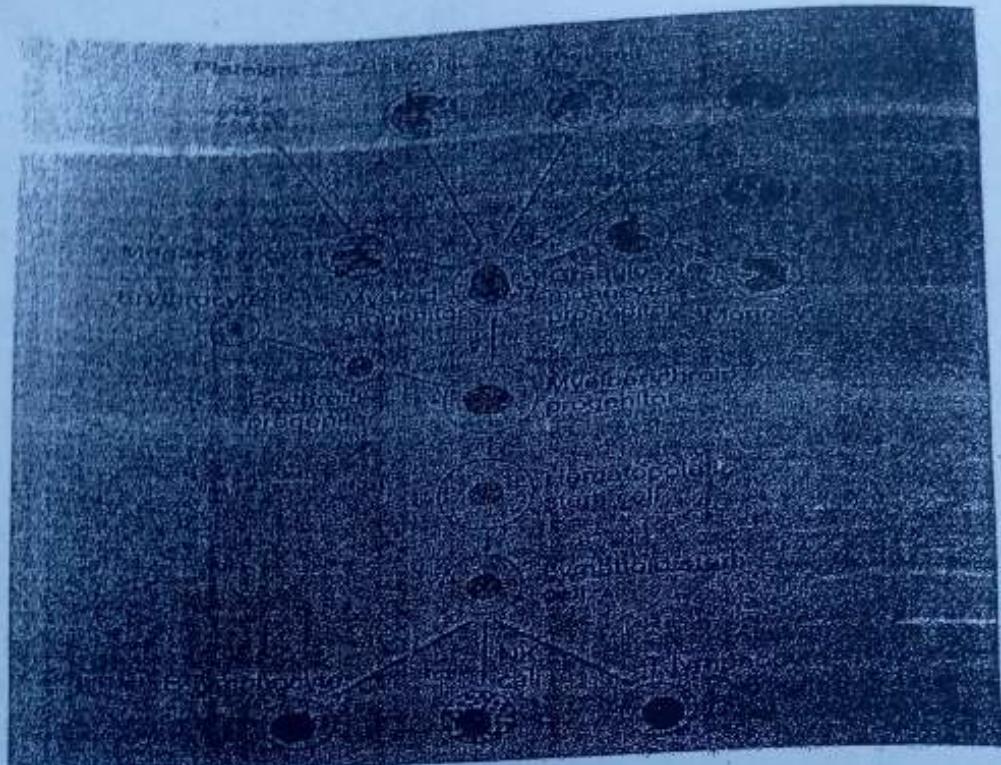


Figure illustrating the production of different cell lines by the Pleuripotent HSC

Functions of the cells of the Immune response

The leucocytes have numerous functions in the body and are linked to produce resistance to diseases and function depends on the particular white blood cells. The cells and their functions are as follows;

Lymphocytes constitute 20-30% of leukocytes population.

1. **Lymphocytes:** The lymphocytes in normal adult human being is about trillion (10^{12}). The cells are about $5-12\mu\text{m}$ in diameter and has a nucleus that occupies almost the entire cell, leaving scanty cytoplasm. The lymphocyte are classified as B and T lymphocytes and the Natural Killer (NK) cells. The relative proportions of T and B cells in the peripheral blood are about 75 and 10% respectively of all the lymphocytes while the remaining 15% are the NK cells.

Subgroups of T-cell exists; majorly the Helper T-cells and Cytotoxic T-cells. The T cells plays significant role in the elimination of pathogens through the Cell-Mediated Immune Response while the B lymphocytes functions in the Humoral Immune Response. The NK cell acts and eliminates the virus-infected cells, cancer cells and transplanted (such as kidney) foreign cells.

The B and T lymphocytes develop in the bone marrow however the T cells through the blood migrate to the thymus before entering again into the peripheral circulation. In the thymus, the immature T cells (known as progenitor T lymphocytes) develop unto maturity and leaves the thymus as mature T lymphocytes into the circulation. The T lymphocytes play important roles in Cell-Mediated immune response. It also interacts with the B cells before the B cell production of antibodies. Again, it functions as antigen presenting cells.

For the B cells, the cells mature in the bone marrow before entering the blood circulation. They enter the circulation as Virgin or resting B cells. The resting B cells do not secrete immunoglobulin molecules rather expresses molecules known as surface immunoglobulin (sIg) on the cell membrane. On activation some of the B cells become effector B cells and the memory B cells. It is the effector B cells that later becomes the Plasma cells which produces antibodies while the non-effector B cells become memory B cells. The Plasma cells secrete large amount of immunoglobulin before it dies usually in few days to few weeks.

- The Natural Killer (NK) cells are lymphocytes but do not require any previous exposure to the antigens hence are called Natural Killer cells. Also known as Null cells
2. **Monocytes/Macrophages:** Monocytes are the largest and mononuclear leucocytes in the peripheral blood. It is about $12\text{-}20\mu\text{m}$ in diameter and constitutes about 20-50% ($1.5 - 4.0 \times 10^9/\text{L}$) of the total white blood cell. The cells are motile and may migrate to the tissues where they are regarded as Macrophages or Histiocytes. The name designated to tissue monocyte (macrophage) sometimes depends on the tissue where the cell resides. In the blood, it is known as monocytes. In the bone marrow, it is seen as the monocyte and monocyte precursors (monoblasts, promonocytes). In the skin, it is known as Langerhans cells and in solid tissue, it is called Histiocytes. In the lungs, the macrophages are known as Alveolar macrophages, the Osteoclasts in the bone and as Type A Synovial Cells in the Synovial fluid. The macrophages in the central nervous system are called the microglia, the liver as Kupffer cells and in the Pleural cavity as Pleural macrophage. The Macrophages appear bigger than the Monocytes.

The monocytes and macrophage have numerous functions;

- ✓ i. They serve as scavenger cells because they engulf and digest microbes, foreign particles and debris from injured sites.
- ✓ ii. The cells act as phagocytes and eliminate phagocytized materials (phagosomes) after forming phagolysomes and subsequent degranulation.
- ✓ iii. The macrophages acts as Antigen Presenting Cells, presenting foreign materials to T and B cells. *UZOM Salomon*
- ✓ iv. Macrophages when activated may kill microbes through cytokines secretion. The cytokines; Interferon gamma is the principal cytokine for this activity.
- v. Macrophage secretes various cytokines which also performs different functions, e.g INF- γ kills tumor cells, and also activates other cells.
- vi. Macrophage has receptors for the Fc fragment of immunoglobulin as well as the complement protein C3b. With the receptors, the macrophage phagocytose bacteria, participate in antibody production, facilitates in Opsonization and in innate immune response by attaching to C3b, thus macrophage plays important role in the acquired immune response as well as the innate responses.
- ✓ vii. Macrophages help to prevent the spread of cancer cells from one site to another.
- viii. Macrophages mop and remove senescent or effete cells and smudge cells from the body,
- ✓ ix. Macrophages are required for tissue repairs and scar formation.

- X. The macrophages have lysozymes, hydrogen peroxide and nitric oxide and these have antibacterial activities and kills phagocytized bacteria.
3. **Neutrophils:** The neutrophils constitutes about 40-75% ($2.0 - 7.5 \times 10^9/L$) of the total white blood cell in the peripheral blood. The cells are between $10-12\mu m$ in diameter and are capable of amoeboid movement. Most of the cells have 2 or 3 lobed nucleus but it is possible to see up to 7 lobes. It contain significant amount of granules in the cytoplasm. The neutrophils are actively motile and migrate freely to sites of inflammation. At injured sites, neutrophils migrates in large numbers and accumulates at the site within few hours. The neutrophils may die at the site of injury and the enzymes released outside the cell. These enzymes liquefy the nearby host cells and the foreign substances to form a viscous semi fluid called PUS.
 The main function of neutrophils is to eliminate bacteria and other foreign substances by phagocytosis. In phagocytizing bacteria and other foreign substances, the polymorphis and monocytes engulf the substance (phagosomes) and allow it to fuse with their lysosomes to form Phagolysomes. The content of the granules are loaded with antimicrobial molecules and enzymes and are secreted. The secretions digest the phagolysomes resulting in the degranulation and killing the phagolysomes. The granular contents act on the phagocytized microbes and degrade them. This is the process in which neutrophils and other polymorphonuclear cells and monocytes phagocytose bacteria or other foreign substances. The Neutrophilic granules are classified as follows:
- The Primary (Azurophilic) granules; which contains mainly the myeloperoxidase, defensins and lysozymes. The myeloperoxidase catalyze the production of hypochlorite from chloride and hydrogen peroxide by the oxidative burst. The Defensins kill a variety of bacteria whereas the lysozymes degrade bacterial peptidoglycans.
 - The Secondary (Specific) granules: the granules also fuse with the phagosomes. It is suggested that the content of the specific granules are released to the exterior of the neutrophils and they modify the inflammatory responses.
 - The Tertiary (gelatinase) granules. The granules contain many membrane protein and are degraded and compositions of the inflammation.
 - Secretory vesicles
- 4. Eosinophil:** Eosinophil is about $12-17\mu m$ in diameter and commonly has a bilobed nucleus. The cytoplasm contain oval, deep and orange pink granules when stained with Romanosky stain. Eosinophil constitutes about 1-3% ($0.04 - 0.4 \times 10^9/L$). Eosinophils are amoeboid in movement similar to the neutrophils and are mostly helminthic parasitic infection. Eosinophil play significant roles in allergy and eosinophilia.
- 5. Basophils:** These are about $8-10\mu m$ in diameter. The cytoplasm contain a mass of large, deep purple staining granules when stained with Romanosky stain of which the circulation where it constitutes about less than 1% ($0.01 - 0.1 \times 10^9/L$) of the total

white blood cell, Basophils have receptors for Fc region of IgE (about 270,000 receptor are present in each cell). The cytoplasm contains granules rich in histamine. The granules also contain other constituent proteins such as Chondroitin sulphate, neutral proteinases and Charcot-Leyden protein. Basophils accumulate in the tissues during inflammatory response and plays important roles in IgE mediated reactions and in hypersensitivity.

6. **Mast Cells:** The mast cells are similar to basophils however are larger; about 10-15 μm in diameter than the basophils. It is assumed to originate from the myeloid stem cell under the influence of a single lineage growth factor. It is predominantly located in the connective tissue. The coarse large purple granules contain histamine, chondroitin sulphate, neutral proteinases and Heparin. After activation, mast cells mediate the synthesis and secretion of TNF α , Platelet activating factor, leukotriene and Prostaglandin. Therefore, in immune response, mast cells play important role in hypersensitivity and in cytokine mediated activation of immune cells.
7. **Dendritic Cells:** Dendritic cells are assumed to develop from the bone marrow from a separate lineage away from the HSC or many develop from monocyte/macrophage lineage. For any reason, Dendritic cells have long membranous extensions resembling the Dendritic nerve. Hence the name dendritic cells. The role of Dendritic cell in immunity is that they act as Antigen Presenting Cells to the helper T cells. The Dendritic cells exist in different sites of the body and have different names as: Langerhans cells: Present in the skin and mucous membranes. Interstitial dendritic cells as seen in heart, lungs, kidneys and gastrointestinal tract. Others are Interdigitating dendritic cells; located in the T cell areas of secondary lymphoid follicle and thymic medulla and the circulating dendritic cells which are present in the blood and lymph. The circulating dendritic cells in the lymph are also known as veiled cells.

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ANTIGEN PRESENTING CELLS

The cells that process and present foreign molecules (antigens and immunogens) in the form that the immunogens can be recognized by T cells are called Antigen Presenting Cells. Virtually any cell may act as APC however conventionally the Macrophages, Monocytes, B cells and Dendritic cells that present foreign substances to T helper cells via Major Histocompatibility Complex (MHC) class II are called Antigen Presenting Cells.

Next organs of Immune system - Note

PHASES OF AN IMMUNE RESPONSE

As a matter of fact, the first time in life an antigen enters the body is called Priming. Shortly after the priming which may be few days or more, the process that stimulates the immune response is to begin. The immune response is divided into two phases; the primary and secondary immune responses.

First time / initial immune response
The Primary Immune response: This is the first phase in immune response. The phase is short-lived and relatively weak. The response is divided into four phases namely the Lag phase, Exponential phase, the Steady state phase and the decline phase.

The first time the human body is exposed to some particular type of pathogen, the immune system responds in a specific way; this response is called a primary immune response.

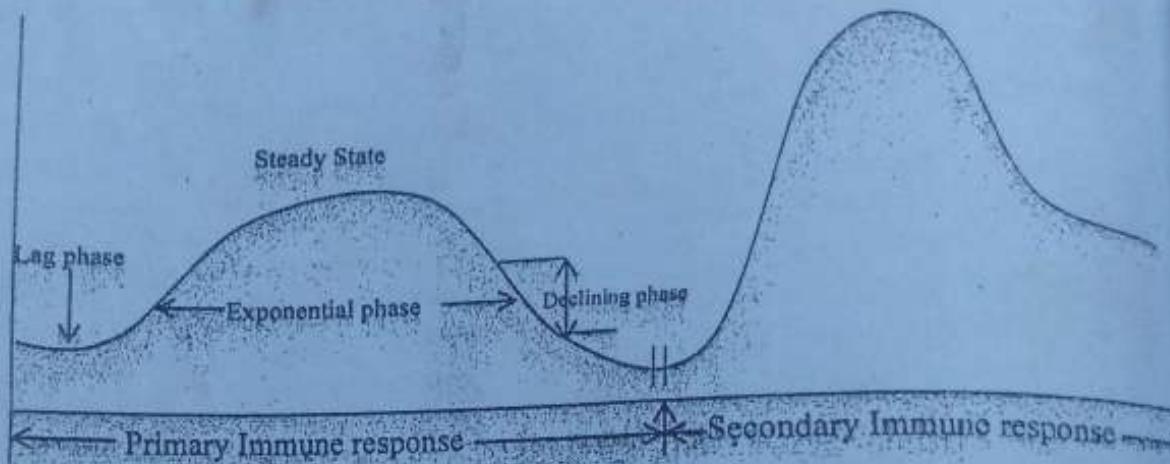


Figure illustrating the Primary and Secondary immune response

Latent phase

i. **Lag Phase:** The lag phase starts from the time of exposure of the immunogen to the time of detection of the antibodies. For the humoral mechanisms. In human being the lag phase is about 1 week although this may vary with an antigen. For instance the period is prolonged with HIV infection or may take weeks or months. It is commonly referred as the window period of HIV infection. During the lag phase, the specific T and B cells are activated by their contact with immunogen.

ii. **The Exponential Phase:** The exponential phase is a period when there is rapid production of antibodies. At this phase the plasma cells markedly secretes antibodies.

iii. **The Steady State Phase:** This phase is also known as the Plateau phase. During this time, the level of antibody production appears constant suggesting that the secretion and degradation of antibodies occur almost at equal rates.

iv. **The Declining Phase:** At this phase, the antibody level gradually declines because new plasma cells are no longer produced and the existing plasma cells are dying. This development indicates that the immunogens has been eliminated from the body of the immunocompetent patient and there is no further stimulation of immune cells for continued antibody production.

The Secondary Immune response: This phase starts when a similar antigen enters the immunocompetent host for the second time. The response induced at this time is said to be the Secondary immune response. The lag phase is shorter in the secondary immune response than that observed in the primary phase. Besides it does not take longer than to reach the plateau state. Again, the plateau state is markedly sustained resulting in the quick and

sustained elimination of the antigen in order to avoid causing a disease or disorder. The secondary immune response is consequent to the contributing actions of the memory cells.

In this response, we stipulate that the specific T and B cells have already been produced during the primary immune response and are activated. Therefore, secondary immune responses are also called Anamnestic Immune Responses.

IMMUNOGEN

An immunogen is defined as any molecule or substance that has the propensity to induce a specific response. The immune response could be either humoral or cell mediated or both. Most immunogen induces both humoral and cell mediated immune response. Similarly, an antigen is a molecule or substance which binds to an antibody or to the T cell receptor. It means that an antigen serves as the target of an immune response and that an antibody binds only to the antigen that invoked its production. Hence an antigen is also an immunogen. However there are some antigens that don't induce an immune response. So all antigen are not immunogens. The substance or molecule that has amino acid residue binding site but lacks the capacity to induce an immune response is known as a hapten. Some molecules are haptens because they may lack some factors that confer immunogenicity or antigenicity. In most cases, some alteration has been made and hapten has become immunogenic and this appears to be the basis for incorporating an adjuvant to some substance (hapten).

However an antigen is a more and commonly used term than an immunogen to denote a substance capable of inducing an immune response.

Properties of an Immunogen (Antigen)

There are some factors that influence immunogenicity or antigenicity of a substance. In other words, there are some properties that a molecule possess in order to serve as an antigen or immunogen thus being capable of inducing an immune response. The factors are as follows:

1. **Foreignness:** Foreignness refers to the tendency in which the body's immune cells recognize a substance because it does not share the same structure with the body cells. As a result of the difference in the structure of the immunogen and that of the body cells, the immunogen can provoke or stimulate immune response. Perhaps a substance is assembled to be an immunogen or antigen but shares the same structure as the host cells. The substance will not be recognized (as foreign or non self) and therefore will not stimulate an immune response.
2. **Molecular size:** Generally, being immunogenic or antigenic is influenced by the molecular size of the molecule. The most potent immunogen have greater molecular size above 10,000 daltons. However, the molecular size is not an absolute criterion because few substances with molecular size below 1,100 daltons have also been observed to induce strong immune response.
3. **Chemical Composition:** It is observed that proteins are usually potent immunogens than carbohydrates. However some polysaccharides and synthetic organic polymers such as Polyvinylpyrrolidone can be immunogenic. Lipids are not good inducers of

- immune response except for very few substances such as mycolic acid found in *Mycobacterium* that the lipids are antigenic
4. **Chemical Complexity:** The chemical complexity of a molecule is another factor that influences its immunogenicity. Molecules with more complex structures; contain more than two or three different amino acid residues are more immunogenic than molecules made up of simple amino acid residues and non-aromatic amino acids. Again polypeptides with amino acid tyrosine are better immunogens than polypeptides without tyrosine.
 5. **Dose of the Immunogen:** The amount or quantity of the substance may influence its immunogenicity granted that the host factor also contributes greatly. In most cases, if the amount of an immunogen used is small, a very poor immune response may be induced. On the contrary, if a two large amount of the immunogen is used, it may not induce any immune response at all; a condition called **Specific Unresponsiveness** or **Tolerance**.
 6. **Route of Entry:** The tendency for a substance to be immunogenic may be influenced by the route of entry or administration. The route of entry of a substance into the host may determine the type of and integrity of the immune response. For instance, the entry of a microbe through the gut may lead to the production of IgA type of antibody whereas the entry of the same microbe through the skin may result in the production of IgG type of antibody.
 7. **Epitope:** Epitope refers to the site on an antigen in which it binds to an antibody. It was formerly known as the antigenic determinant. It is more of the site on the immunogen that confers immunogenicity. In fact an immunogen may be made up of different type of molecules. However only few amino acid residues in the molecule may be recognized as epitope for the binding of an antibody or TCR and as such can stimulate an immune response.
 8. **Genetic makeup of the Host:** This is another factor that can influence immunogenicity however it is not strictly a property of the immunogen as it is more of the host animal. The host animal may be genetically programmed that an immunogen may be unresponsive in a particular animal but may elicit a response in another animal yet of the same specie as the former.

ROUTE OF ENTRY

Epitope
molecular size

foreign

Genetic make up

immunogenicity

fmc
etc
etc

etc

LECTURE 4

IMMUNOGLOBULINS

THE STRUCTURE AND FUNCTIONS OF ANTIBODIES

The activation of B cells result in the production of Plasma and Memory B cells. The Plasma cells are spherical, oval or elliptical in shape. The cytoplasm is abundant and may have a granular character. The nucleus is small in relation to the size of the cells. The nucleus is centrally located and contains dense mashes of chromatin often arranged in wheel-spoke fashion. Plasma cells don't replicate (divide) and does not express surface immunoglobulins and lives for a few days and later die by apoptosis. The Plasma cells secrete antibodies for about 2000 molecules per second. The antibodies or commonly regarded as immunoglobulins; is a name given to all globulins with antibody activity. When Plasma cell dies, further production of antibodies will cease.

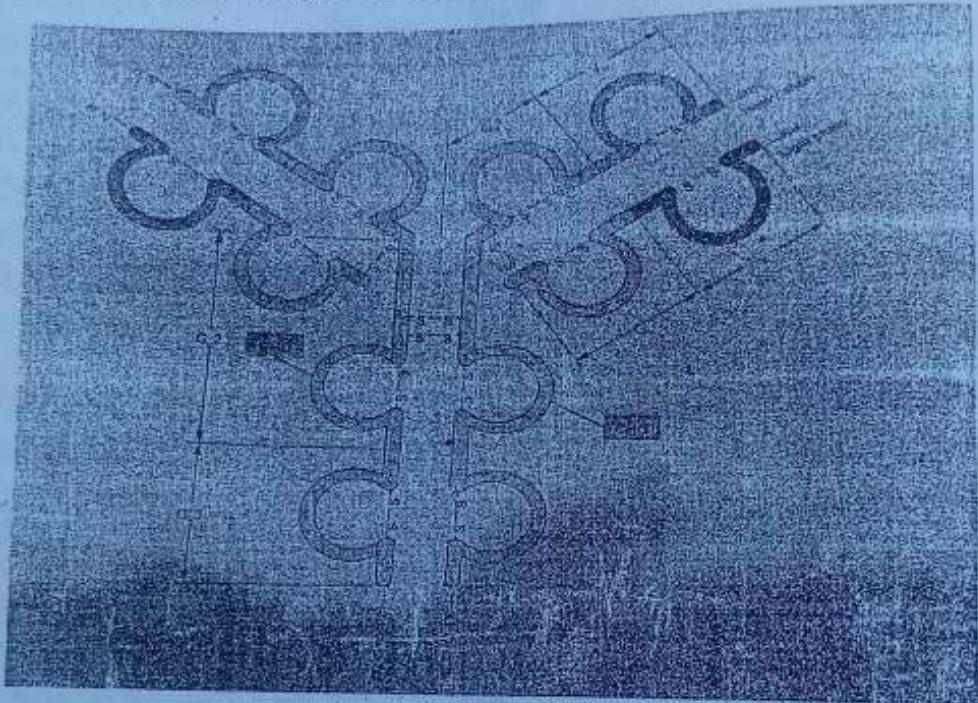
Antibodies are a group of glycoproteins present in the serum or body fluids of mammals that react specifically with the antigens that induced their formation. Antibodies are heterogeneous and differ from each other in molecular size, structure, amino acid composition, charge and carbohydrate content. Antibodies can be separated according to their net charges in any electric field or by ultracentrifugation. Antibodies are classified into five, according to the nature and properties of the heavy chain. The five classes are gamma (γ), alpha (α), mu (μ), delta (δ) and epsilon (ϵ) and these are designated as IgA, IgG, IgM, IgD and IgE respectively. Each immunoglobulin class differs in its general properties, half-life, and distribution in the body, concentration in the blood and in interaction with other components of host defensive systems.

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The IgG is used as the prototype antibody to explain the basic structure of the other antibodies. The choice of IgG in describing the structure of antibodies could be that it has the highest number of sub classes: about 4 and its concentration in the serum is highest in comparison with other antibodies. Most importantly, the amino acid sequencing of the antibody is similar to that of myeloma proteins. Recall that Myeloma proteins are molecules that have the characteristic structure of antibodies but originate from a disease state: Multiple Myeloma. The Myeloma pathologic immunoglobulins; Multiple Myeloma are formed by individual with a cancer of antibody Plasma (B) cells. This is contrary to antibodies which originate usually from immune response.

y a u 8 e

The basic functional unit of each antibody is an immunoglobulin (Ig) monomer. The Basic Unit: The basic unit of antibody shows that the molecule is bivalent and symmetrical in shape. The molecule is made up of four polypeptide chains (2 pairs each). Two of the polypeptide chains are called the Heavy chains whereas the other two are called the light chains. The polypeptide chains are identical and the two pairs are regarded as monomers. Some antibodies only consist of two monomers (two basic unit). Each monomer is connected to the other monomer by the J chain and when it occurs in this form, it is called a dimer. Furthermore, some Immunoglobulins such as IgM may consist of five basic units (five monomers) are called a pentamer.



The bivalent symmetrical Y shaped form of the antibody and the distinct regions or portions mentioned are related to the functions of the Ig molecule

Along the chains and within a short distance are amino acids folded into globular regions or loops. The globular loops are called domains. Each domain may have about 100-110 amino acids and are held together by an intrachain disulfide bonds (bridges). The heavy chain has 4 or 5 domains while the light chain has 2 domains. Each light chain has about 220 amino acids and a molecular weight of approximately 23,000 - 25,000 daltons whereas each heavy chain has about 440 amino acids and a molecular weight of approximately 50,000-70,000 daltons.

Sulfide bonds hold
① Light & heavy chain together
② two domain together.

The light chain is made of kappa (κ) and lambda (λ). Classifying the light chain into κ and λ depends on the amino acid sequence in the constant region (C_{H1}) of the light chain, however, the differences has been proved to be functionally not significant. Furthermore, an antibody molecule has either kappa or lambda chain only and never a mixture of both.

Fab and Fc regions: The schematic view of the immunoglobulin (antibody) molecule show that it is Y shaped. Moreover, fragmentation study divides the antibodies into two fragments. The fragments are the Antibody binding Fragment (Fab) and the Crystallizable fragment (Fc). The fragmentation is observed when the fragmentation site of an antibody occurs at the Hinge region. The Fab region is the two branched corners of a Y and consists of the variable portion V_L and a constant C_H portion. The Fab region precisely the variable portion is considered as the antigen binding site of the antibody. The Antigen Binding Site of the antibody ends at the N or NH₂ terminal of the variable region.

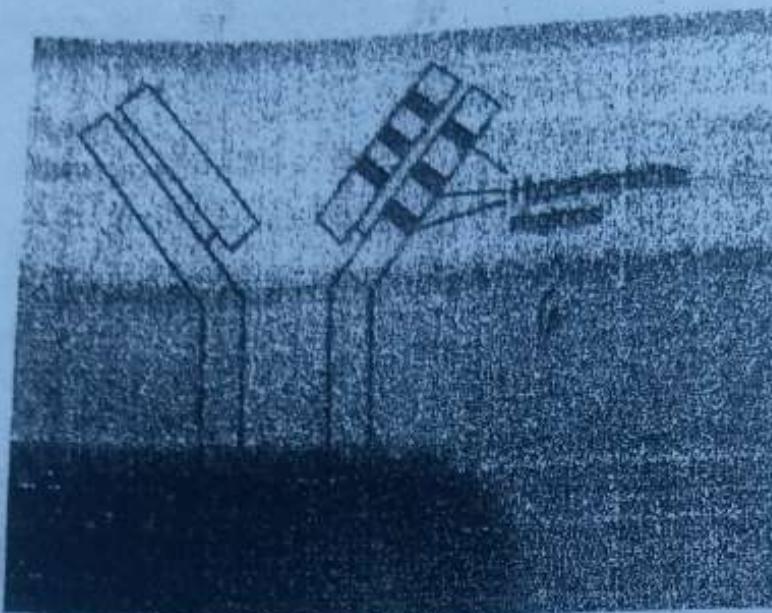
The amino acid sequence in the tips of the "Y" varies greatly among different antibodies.

Variable and Constant Regions: Between different antibody molecules, the amino acid sequence of the N terminal domain vary widely, hence N terminal domain is called the variable region abbreviated V_{H1} and V_L (V_{H1} for variable region in heavy chain and V_L for variable region in the light chain). On the other hand, the amino acid sequence in the remaining domains are relatively constant, hence the domains are called the constant region abbreviated C_H and C_L representing the constant region in the heavy and light chain respectively. The constant regions are numbered C_{H1} , C_{H2} , C_{H3} and sometimes C_{H4} . The numbering of constant region starts with the domain next to the variable domain and moves along the Fc region. The amino acid sequence of constant domain of the heavy chain determines the classes of heavy chain, an

UZOM SOLOMON CDR or part of Variable Regions
Complementarity Determining Region: Although the variable regions of both heavy and light chains are designated as mentioned however the amino acid sequences throughout the domains are not variable. Rather the extreme variability of the sequence occurs only at certain stretches called the HYPERVARIABLE REGIONS or COMPLEMENTARITY DETERMINING REGIONS (CDRS). Each variable region has 3CDRS namely CDR1, CDR2 and CDR3. The CDR contains about 9-12 amino acids. Along the V region, the CDRs are separated from each other by invariant stretches of sequences called FRAMEWORK REGIONS (about 15-30 amino acids long). The antigen binding site of the antibody primarily involves the hypervariable regions and the

Hypervariable region

sequencing of the region determines the antigen specificity. A single antigen binding site consists of hypervariable loops (3 from V_L and another 3 from V_H chains,



The other fragment i.e., the Fc is shown as the stalk of the Y. It is made up of the other constant regions of the Ig molecule. The constant regions may range beyond $C_H 2$ (up to 3 or 4) in most classes of Ig molecule. The Fc region does not bind to antigens rather exerts some biological function of the Ig molecule and it is the region that recognizes the different types of cells. The Fc fragment crystallizes during cold storage and is therefore known as Fc region.

The division of the Ig into fragments is best studied by the enzymatic cleavage of the hinge region. Two enzymes have demonstrated the cleavage of Ig and these are Papain and Pepsin. Papain cleaves at the NH_2 or N terminal side of the intersulfide bridge of the Hinge region resulting in the formation of three fragments (two fragments of Fab) and one fragment (Fc) consists of the carboxyl terminal portions of the heavy chain. Papain acts on the exposed hinge region.

Similarly Pepsin cleaves Immunoglobulins but on the carboxyl terminal of the Hinge region resulting in one large fragment regarded as E(ab)2. The E(ab)2 has two fragments linked by disulfide bonds. The FC region does exist when Immunoglobulins are treated with Pepsin because the enzyme degrades completely the FC regions, and as a result of loss of disulfide bonds.



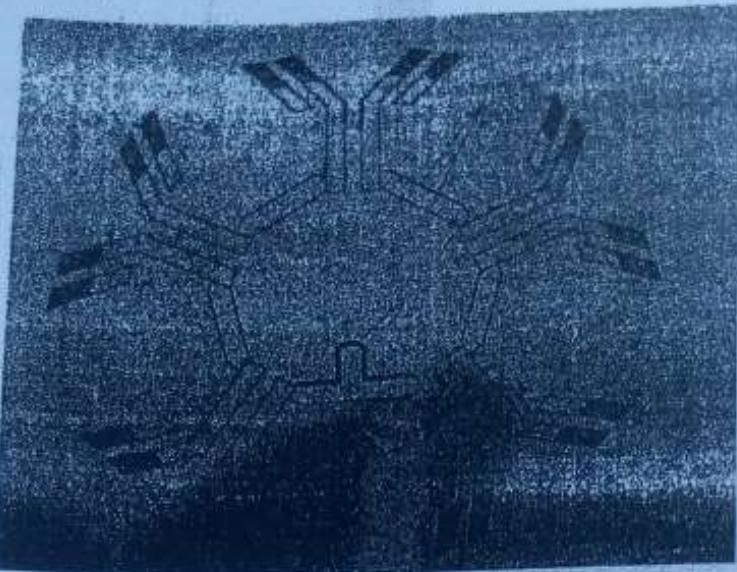
Hinge Region: Hinge region is a short segment situated between C_{H1} and C_{H2} domains of the heavy chains. Hinge region consists mainly of cysteine and proline. Inter-heavy chain disulfide bonds are formed between the cysteine residues of the two heavy chains. The Hinge region is more susceptible to enzymatic attack (Papain and Pepsin). The bonding at the hinge region is loosely strong to permit the relative movement of antibodies with respect to each other.

Disulfide Bridge: Disulfide bonds or disulfide bridges of Ig molecules are required to maintain the association of the polypeptide chains. The H and L chains are connected to each other by Interchain disulfide bonds. The interchain disulfide bonds hold together the H and L chains. Again, it also holds together the two H chains.

Uzom : Solomon

In addition to inter-disulfide bridges are other disulphide bonds or bridge which exist within the domain of the H chain and within the domains of the light chain and these are called **Intrasulfide bonds**. The sulfide bonds contain amino acids.

The Joining (J) chain: It has earlier being emphasized that the Four-chain basic unit of immunoglobulin is called a monomer. If an Ig has more than one basic unit, it is called a Polymer. Two basic units are referred to as a dimer whereas five basic units are referred to as a Pentamer. The IgM and IgA generally exist as polymers of basic five and 2 units respectively. Nevertheless, 3 or 4 units have been identified with IgA. The Joining (J) chain is the link or the bridge between two or more monomers. The J chain is about 15,000 daltons and helps in polymerization of IgM and IgA.



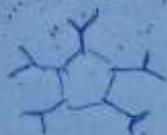
Overall, the immunoglobulins have unique distinct parts that have extensively been studied and are composed of amino acids. The parts essentially important and requires mentioning are the heavy and light chains, the domains or amino acid globular folding and the variable and constant regions. Others are the Fab and Fc portion, hypervariable complementarity determining regions (CDRs), the disulfide bonds, and the J chain and the Hinge region.

CLASSIFICATION OF IMMUNOGLOBULINS

Immunoglobins exist in various classes. The various classes occur and are attributed to the differences in the amino acid sequences of the constant region of the Heavy chain. The differences are also linked to different physical properties of the antibody. The physical properties include the molecular weight, serological reactivities and biological activities of the antibodies,

Considering amino acid sequencing of the heavy chains, the antibodies are divided into 5 classes designated as IgM, IgG, IgA and IgE and IgD.

① **IgM:** IgM is a pentameric Ig and consists of five basic immunoglobulin units i.e. five monomers. The monomers are joined to each other by J chain. The J chain appears to be required for the polymerization of the five monomers. As a pentamer, IgM has 10 identical antigen combining sites. It has also been reported that about 5% of IgM may exist in a hexameric form. The hexameric molecule means that it contains 6 monomers. Hexameric form like the pentamers have complement activities and is said to be about 20 fold more efficient than the pentameric form. IgM constitutes about 10% of normal serum.



level of Ig, IgM is predominantly the first Ig class produced by plasma cell during primary immune response and tends to decrease in the serum subsequently during the secondary immune response. The detection of IgM is important in diagnostic immunology; it is an indication of current microbial infection.

Given that IgM is a pentamer and the heaviest of all Ig classes and therefore does not cross the placenta barrier to reach the foetal circulation. The detection of presence of IgM in the umbilical cord blood of the new born should have been produced during foetal life and suggests that the foetus is infected during pregnancy.

Again, IgM fixes or activates complements via the classical pathway of complement activation. Besides IgM agglutinates bacteria.

② IgG: About 70% - 75% of the total serum immunoglobulin in the adult is IgG. IgG occurs in monomeric form. It is further classified into 4 subgroup namely IgG1, IgG2, IgG3 and IgG4. The classification depends on concentration and the number of C_H in the serum. IgG is the most predominant antibody produced during 2^o immune response and suggest past infection. IgG is the only class of Ig that crosses the human placenta barrier and reaches the foetal circulation. The main reason apart from the molecular weight why IgG passes into the foetal circulation is that the placental cells have receptors for the Fc portion of IgG which does not seem to be present for other Igs. The IgG from the maternal subjects may be found in the infant blood and may remain in the circulation to protect the infant in the first few months of birth. On the contrary, its presence may be harmful because it is associated with haemolytic disease of the newborn (HDN).

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③ IgA: IgA is about 13% of the antibodies found in the serum. It is predominantly found in body secretions such as saliva, tears, milk, intestinal, urogenital and bronchial secretion. IgA is dimeric but may also occur as trimers and tetramers. IgA may bind to microbes and ensures their elimination from the mucosal surfaces and subsequent entry into the host. IgA also may prevent microbes in the lumen of the gut.

④ IgE: IgE is a monomer. A trace amount of IgE is present in the blood. IgE has 4 constant regions namely; C_H1, C_H2, C_H3 and C_H4. The C_H4 may bind to Fc receptors on Mast cells (in the tissues) and Basophils (in the blood). Upon first exposure of IgE to the specific antigen (allergen), the FAB region binds to an

allergen and the binding may result in sensitization. On the second exposure and having receptors linked to the mast cells or/and basophils, the cell will degranulate and releases inflammatory mediators such as histamine and other pharmacological mediators of anaphylaxis and the interaction manifests as allergic reaction and immediate hypersensitive reactions. On the other hand, IgE protects the host against helminthic and multicellular parasitic infections. It does this by releasing mediators that firstly attracts eosinophil (which is known to fight parasitic infection) and secondly by stimulating gut hypermotility. The eosinophil combat the parasites and therefore migrates in the blood and are commonly seen in the peripheral blood film. This is the reason for seeing more eosinophils in the peripheral blood during parasitic and allergic reaction. Similarly, the gut hypermotility is associated with increased rate of movement of the intestinal content which is required in the elimination of helminthic parasites.

5 IgD: IgD is a monomer. IgD is present in the blood in trace amount and does not cross the placenta. The function of IgD is not yet known.

TABLE SHOWING THE Ig CLASSES, THE DIFFERENT BIOLOGICAL AND PHYSICAL PROPERTIES.

Uzom Solomon

Mechanism for the elimination of microbes

The overall function of the antibodies is to eliminate and kill microbes and foreign bodies, thus protecting the host. The antibodies by itself does not kill and/or eliminate the antigen rather achieves its function through different ways; firstly by binding an antigen. The purpose of the binding is to elicit subsequent effector mechanisms. Consequent upon these facts after binding, the antibody initiates some activities through the Fc region. The nature and composition of the Fc region allows the antibodies to exert the subsequent immune-biological effector functions geared to protect the host.

The process is as follows; The antibody binds to the microbes, and may activate the classical complement pathway through the Fc region. Complements have receptors for IgM and IgG and activation leads to the formation of Membrane Attack Complex (MAC) (formation of pores) on cell wall of microbes. This is the mechanism whereby bacteria and viruses in the extracellular environment are killed or eliminated by the activation of the classical complement pathway.

2 In addition the cell membrane of some immune cells such as macrophages and NK cells have receptors for the Fc region of the antibodies and form complexes and remove microbes by lysosomal enzymes during Phagocytosis or by Opsonisation.

3 Furthermore, antibodies may remove a microbe by the antibody-dependent Cell-Mediated Cytotoxicity (ADCC). ADCC

4 Moreover, antibodies also neutralizes toxin. Toxins produced by *C. tetani* and *C. diphtheriae* are neutralized by antibodies.

5 Again, Ig prevents infection by secreting IgA in the mucosal secretion of GIT, Saliva, genitourinary tract and respiratory tracts. The antibodies in the secretion combat and checkmates the microbial invasion. Moreso, the IgE initiates allergic and hypersensitivity reaction and also ensures the elimination of parasitic infection.

Finally the IgG crossing of the placenta confers temporary resistance to the foetus and new born.

In general, the immunologic functions of the antibodies are itemized as follows;

- i. The binding of the antigens at the Fab region
- ii. The removal of the antigens by phagocytic macrophages by Opsonization
- iii. Removal of the antigen by complement activation
- iv. Antibody dependent Cell-Mediated Cytotoxicity (ADCC).
- v. Triggering of allergic and immediate hypersensitivity by IgE binding on the Fc receptor of basophils and mast cells
- vi. The protection of the foetus/newborn by the IgG crossing of the placental barrier
- vii. The warding off of microbes by the IgA molecules predominantly in the secretion.

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SOURCES OF ANTIBODIES

Some molecules such as Lipopolysaccharides, mannose and Phosphotidylcholine are present on the surface of many microorganisms and have the propensity to activate the B cells resulting in the production of antibodies. The antibodies produced in their manner are expressed without specific antigenic stimulation or immunization and called Natural Occurring Antibodies. The natural occurring

antibodies play specific role in innate immunity and are capable of activating complements.

On the other hand, some antibodies are produced by immunization of domestic animal or by human volunteers. Whether humans or animals, purified antigen are injected into the host. The host immune system recognizes and responds to the antigen and the B cells proliferate and differentiate to produce specific antibodies. To promote the efficacy of the antigenic stimulation of antibody production, the antigen is mixed with an adjuvant. Following repeated injection of antigens at regular intervals, blood is withdrawn from the host and allowed to clot. The fluid that remains after blood has clotted is known as Serum. Considering that the serum contains desired (specific) antibodies, it is called Antiserum.

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(2)

Antibodies produced and obtained by immunization are known as Polyclonal Antibodies. Polyclonal antibodies are therefore not naturally occurring antibodies and are produced by several B cell clones and have different sensitivities and are also prone to cross reactivity with closely related antigen molecules. Therefore, it is relatively good for Serological testing.

(3)

HYBRIDOMA ANTIBODIES: The use of polyclonal antiserum has lots of limitations such as in the cross reactivity. Such limitations of the antiserum have been overcome by the development of hybridoma techniques. Hybridoma techniques produce antibodies by *in vitro* culturing of mammalian cells or bacterial colonies. The culturing of the cells synthesizes antibody. The antibodies are of a single specificity and considered as **Monoclonal Antibodies**.

In one of the methods for producing monoclonal antibodies, an animal usually mice or rats are immunized with an antigen or doses of antigens. Once the animals have produced antibodies, their spleens are removed. The splenic cells are separated from each other and fused with myeloma cells by the addition of polyethylene glycol which promotes membrane fusion. Myeloma cells are cancerous Plasma cells that can be readily cultivated.

The fusion mixture is transferred to a culture medium containing a combination of hypoxanthine, aminopterin and thymidine (HAT). Aminopterin is a poison that blocks a specific metabolic pathway in cells: preventing the proliferation of other cell lines.

Recombinant antibody: These monoclonal antibodies produced by recombinant DNA technology. They are generated *in vitro* using synthetic genes and antibody fragments.

Myeloma cells lack an enzyme that allows their growth in the presence of aminopterin. However, the pathway is by-passed in spleen cells provided with intermediate metabolites hypoxanthine and thymidine. Consequently, hybridoma cells grow in HAT whereas Myeloma cells die.

The hybridoma cells are randomly placed on culture well. The wells are individually tested for the production of the desired antibodies and if positive, the wells are cloned. The clone is immortal and produces monoclonal antibodies.

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ANTIGEN-ANTIBODY INTERACTION REACTION

Antigen/Antibody interaction may be recognized *in vitro* by several immunological methods. Some methods such as Enzyme Linked Immunosorbent Assay are preferred to some other methods such as agglutination and immuno precipitation due to the increased specificity and sensitivity. Sensitivity in quantitative immunoassay refers to the lowest amount of the antibody or antigen that the system can detect. For the antigen or antibody detection, the corresponding known antibody or the antigen is used and vice versa. The methods that are commonly used include: Agglutination, immunoprecipitation, immunodiffusion, immune-turbidometry, Radioimmunoassay and Nephelometry. Other methods are immunoelectrophoresis, immunofixation, Enzyme-Linked Immunosorbent Assay (ELISA) and Chemiluminescence. Other methods are immunohistochemistry, Western Blotting or Immunoblotting, Immunochromatography, Complement fixation test, Lymphocyte activation assays, Enzyme-Linked Immunospot assay (ELISPOT), Neutrophil function assays and Flow cytometry.

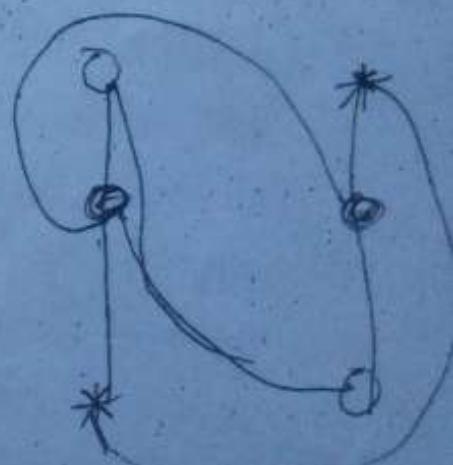
Note: Good numbers of the above mentioned techniques have been modified and details are seen in textbooks.

PC

294 boxer

1154 ocher

[Handwritten signature]



THE COMPLEMENT SYSTEM:

Complements are plasma proteins and the majority of the proteins are beta globulins. Under normal condition, in the circulation complement proteins are functionally inactive (pro-enzymes) and on becoming zymogens, potentiates the host defense mechanism. The activation of the proteins may result in irreversible membrane damage and death of the targets cell.

The most fascinating benefit of the complement activation is in the induction of cell lysis or death of microbes by forming the Membrane Attack Complex (MAC). On the contrary, if complement activation is not regulated, it may exert some deleterious effect on the host. The role of complement in defense response is classified into five groups; the induction of cell lysis through membrane attack complex, and Opsonization and phagocytosis by splenic and liver macrophages; other role of the complement protein include stimulation of inflammatory activities via chemoattractants and anaphylatoxins, removing of immune complexes by reducing the complexes into small soluble aggregates, that will enhance clearance by macrophages. Complement also play significant roles in the activation of memory B-cells. The memory B cells are activated by antigens and may result in the production of antibodies.

Considering the above mention roles of complements on defense response, it shows that the proteins on activation induces specific and nonspecific immune response. In fact, it could be said that complement activity is a bridge between humoral and cellular immune responses. This is to say that, complement participates in the humoral as well as in the innate immune response, it attaches to IgG, IgM, polysaccharides and erythrocytes. Granted that complement participates in specific immune reaction however it differs from Ag/Ab interaction because it

disperse protective mechanism invivo to the host unlike in the later (Ag/Ab) reaction such as agglutination or precipitation that may have no protective function invivo to the host.

About 30 protein components participate in the complement system. Some are recognized by the letter C and numeral as attached to the Cs, for example C1, C2, C3 etc. Some of the proteins are represented using symbols for instance, Factor B, Factor D, Factor I and Factor H while others such as in Properdin is known by their names. The complement proteins are sensitized in the liver, macrophages, monocytes, epithelial cells of the gastrointestinal and genitourinary tract. Complement activation is in sequence and produces a cascade reaction similar to coagulation factor cascade. A horizontal bar ^{to add} annexed or placed over a complement, components eg. C1 in most cases is used to designate an activated component. Upon activation, the complement proteins are fragmented into two and a lower case letter word "a" or "b" is annexed after figure eg. C3a and C3b. Again two or more component with enzymatic activity may be joined together eg. C4bC2a3b. Fragment that have lost biologic activity sometimes maybe represented using a lower case letter placed in the front the abbreviation, eg. iC3b. In some complexes, no letter is used before the Inactive component eg. C3dg and C3c.

Complement Activation

Having mentioned earlier, a complement protein is cleaved into two fragments: designated as "a and b". The fragment with enzymatic activity cleaves other components and the complex interaction continues further in the activation of next component resulting in a cascade of reaction. On the contrast, the fragment with no enzymatic activity diffuses away from the cascade into the systemic and may act as chemoattractants and anaphylatoxins. Currently complement activation is posited to occur

in 3 pathways as against the earlier recognized 2 pathways. The complement pathways are the classical, alternative and lectin pathways and each pathway is divided into 3 phases:-

- (i) The Initiation
- (ii) Amplification
- (iii) Membrane attack phase.

The difference in each pathway occurs only at the Initiation phase but is same during the amplification and membrane attack phases.

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CLASSICAL PATHWAY:

The classical pathway is initiated on fixing complement in an Ag/Ab interaction. It occurs only between Antigens and Antibodies (those capable of fixing complements such as IgM and IgG). C1 comprises of 3 similar proteins namely C1q, C1r and C1s. If an Ag/Ab interaction occurs, in most interactions complement is fixed. In such interaction, complement C1 will be fixed. Binding of C1 results in a conformational change in which C1q activates C1r. Activated C1r activates C1s. C1s later activates C4 cleaving the protein into C4a and C4b. C4b subsequently activates and cleaves C2 into two fragments; C2a and C2b. C4a and C2b have no activity in activation cascade and diffuses away from the complex whereas C4b and C2a remains bound to the Ag/Ab complex.

Besides the activation of C4 by C1s, more C1s components also activate C2 and cleaves the components into C2a and C2b. It is important to note that this reaction is facilitated by Mg⁺⁺ ion. The formation of C4bC2a marks the end of the initiation phase. C4bC2a complex is called C3 convertase (because it has the propensity of activate C3) and activates C3 into C3a and C3b. The enzymatic activity of C4bC2a lies on the C2a residue of the complex because C4b is more of a carrier molecule, to make the complex accessible to C3. C3b cleaves to

C4bC2a to form C4bC2aC3b. This is the commencement of the amplification phase. It is the amplification phase because as hundreds of molecules are generated in a reaction that started with one molecules of C1q and a single C3 convertase can act on a C3 molecule and generates about 200 C3b fragments. This manner of activation produces effective complement mediated defense mechanism.

C3b potentiates immune response because some of the molecule acts as opsonins, the most important function of complement activation and enhances phagocytosis.

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Furthermore, C3b contributes to remove immune complexes by digesting the complexes in small soluble aggregates, facilitating clearance of the C3b coated complexes that binds via receptors to phagocytes, and macrophages in the liver and spleen. ^{C4bC2aC3b} Ca2aC3b has the capacity to cleave C5 to C5a and C5b and is therefore known as C5 convertase. C4b C2a C3b complex continues the activation cascade by cleaving C5. C5b binds to target or microbial cells and activates C6. C5a diffuses away from the complex into the fluid and act as chemoattractant. C5a is the major chemoattractant, as well as an anaphylatoxin. Activation of C5 marks the end of amplification phase and the commence of the common pathway of complement activation. The common pathway is so-called because at this phase, all the 3 pathways take the same route of the activation cascade, leading to membrane attack phase.

C5b activating C6 starts the Membrane attack phase. Activated C6 later activate C7. C5C6C7 exposes hydrophobic binding region of cell wall of the target microbes subsequently and the complex is inserted into the phospholipids of membrane of the target cells. C5bC6C7 also may be deposited on Innocent-by stander cells inducing reactive lysis.

C8 is joined to C5C6C7 and a complex is formed which creates small pores of about 10 Angstrom (\AA) in diameter on the membrane of target cells. Subsequently, about 10-17 molecules of C9 binds to one to about 70-100 \AA . This is known as the membrane attack complex (MAC) or terminal Complement Complex. During complement activation, lots of MAC are formed on the cell membrane of microbes or other target cells. Consequent upon high osmotic pressure, from the exterior, water enters the target cells through the pores. The cell swells and bursts leading to death of the microbe or whatever target cells as it occurs in the haemolysis of erythrocytes.

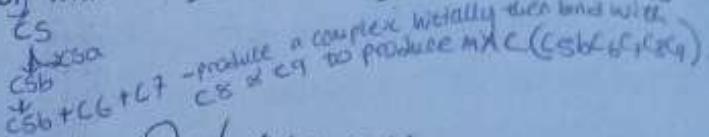
UZOM · SOLOMON

Overall the classical pathway of complement activation is initiated by fixing C1 to Fc portion of IgG or IgM. By this pathway, complement plays a role in humoral immune response.

ALTERNATIVE PATHWAY:

As for the alternative pathway of complement activation, antibodies are not required in the fixing of complement contrary to classical pathway. In the pathway, complement is activated by polysaccharides on bacterial cell walls, proteolytic enzymes, endotoxin, erythrocytes and Cobra venom. The major complement components involved in the initiation of the pathway are: C3, Factor B, Factor D and Properdin. For the pathway to be activated, C3 is directly activated. C3 has a thio-ester bond and the bond being unstable is spontaneously hydrolysed into C3a and C3b. Following the spontaneous activation of C3 into C3a and C3b, target cells nearest to the vicinity of C3b, attaches to the component and subsequently Factor B, resulting in C3bB. Simply C3b binds the surface of target cell and subsequently Factor B Factor D enzymatically binds to C3bB fragmenting B to Ba and Bb. Usually Ba diffuses away leaving the complex as C3bBb. C3bBb has a

short shelf life of about 5mins and binding to Properdin extends the half-life to about 30 minutes. The C3bBb is the C3 convertase of the alternative pathway. Binding of more C3b to C3bBb results in C3bBb3b complex (also known as the C5 convertase of the alternative pathway). More C3a and C3b fragments are produced when other C3 bind to C3bBb. C3bBb C3b cleaves C5 and the cascade continues progresses to common pathway and later with attachment of C6 until MAC is formed.



LECITIN PATHWAY: UZOM SOLOMON

Lecitins are protein molecules. They bind to carbohydrate and activates Complement activation. Examples of lecithins are: Mannose binding lecithin (MBL) and Mannose binding – associated serine protease, 1 and 2 (MASP-1 and MASP-2). Lecithins activate complement cascade in a process similar to event in the Classical pathway except that antibodies do not participate to initiate the activation. In the lecithin pathway, MBL, MAS P-1 and MASP-2 acts on C1q, C1r and C1s. MBL may also bind to carbohydrates on bacterial cell surface. MASP – 1 and MASP -2 may also activate C4 and C2. The subsequent steps are similar to that of Classical pathway and leads to MAC formation.

Overall the lecithin pathway and alternative pathways of complement activation appear to be the participation of the complement proteins in innate defense mechanism,

finz - chemotaxis

Other Roles of Complement:

- **Chemoattractant:-** Are molecules that have receptor for monocytes and neutrophils and attract the phagocytic cells to inflammatory sites. They include C5a, C4a, C3a etc.
- **Anaphylatoxin:-** are group of mediators, peptides or hormone like molecules involved in inflammation. They causes degranulation of

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mast cells with consequent release of histamine, vasodilators and smooth muscle contractors and enhance vascular permeability. The effect of anaphylatoxin appears similar to that obtained in immediate hypersensitivity reaction.

- Innocent by Standers:

Innocent by Standers, are host cells that are close to the vicinity of complement mediated destruction of microbes. They are destroyed alongside or together with microbes in a complement mediated response. The destruction extended to the cells are as a result of staying closely in the area or environment where the microbes are destroyed.

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REGULATION OF COMPLEMENT ACTIVATION

Complement activation is aimed at eliminating microbes however may cause untoward effect or damage to host if unchecked. This may cause Innocent by Stander cell lysis, autoimmune haemolytic anaemia (AIHA), drug mediated immune haemolytic anaemia and other immune mediated processes. It may also cause thrombocytopenia and other harmful consequences. Therefore, complement activation should be controlled or regulated. Given that complement activation occurs at various steps (not only a step). It could be reasoned that, regulation of complement activity and progression will occur at different steps; such that regulation of a particular step will prevent subsequent activation. The control or regulation of activation may occur in the blood or on formed complexes attached to the target cells.

Generally, there are two ways whereby complement activation is controlled. These are by spontaneous decay and inhibition of various regulatory proteins.

For the spontaneous decay process, some component proteins after activation rapidly decays or loses activity unless they bind or attaches to

sites, and become membrane bound and stabilizes. Therefore when the proteins are prevented from binding to molecules and are no longer available to continue and sustain the activation, the process will cease. e.g. The plasma half-life of C5, C3 and C4 are about 2 mins, 30 mins and 60 minutes respectively. The activity are lost if it does not bind to activated membranes. Similarly, C3b forms a complex with C4bC2a within a short time, if the C3b with C4bC3a is delayed it may react with water to form a conformationally altered inactive molecules known as C3 (H₂O). In addition, C3bBb has a half-life of about 5min. Its half-life cannot be extended to about 30 mins., unless it binds to Properdin. Without binding to Properdin, its activity is lost and the complement activation is controlled.

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Moreover, C5b is extremely labile and becomes inactive after 2 minutes unless it binds to C6. Furthermore, Factor I cleaves C3b into iC3b and C3f and these complexes have no complement activity.

Similarly, Factor I cleaves C4b into C4c and C4d and the resulting complexes have no complement activity. With these examples complement activation is regulated.

- ② As for the plasma regulatory proteins, complement control activation is regulated by binding/and forming complexes in the blood or on the membrane of target cells e.g C1 inhibitor (C1INH) binds to C1r prevents the progression of the pathway. In similarly, related activity of C1-INH, the complex acts as an inhibitor on activated Hageman factor (Factor XII) and on all the other enzyme activated by Hageman factor. To buttress this point, C1INH regulates enzymes of the kinin - generating system, clotting system and fibrinolytic system as well as the classical pathway of complement activation. The activity of C1-INH in regulating the complement activation is an intriguing inter-relationship between complement system and the haemostatic system.

*C1 inhibitor regulates classical pathway
factor Hb1 regulates alternative pathway*

Note: Plasma regulatory proteins circulate in blood. They regulate complement activation by forming complexes with activated complement components.

Another example of the plasma proteins regulating complement activation is in the activity of Vitronectin (Protein S). Protein S binds to C5C6C7 on the cell membrane and prevents the insertion of the complex into the cell resulting in the prevention of cell lysis, haemostasis.

In addition, plasma protein regulation of complement activation is in preventing the activity of CD59. CD59 act as membrane inhibitor preventing MAC function. It binds to C567 complex and prevents the insertion of the complex on the membrane and finally, prevents the formation of MAC. The list of regulatory protein is endless. Details of plasma protein regulation of complement activation is found in the text book of immunology.

CD59 inhibits the binding of C9 to C6C7

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COMPLEMENT AND HAEMOLYSIS:

Activation of complements results in the destruction of erythrocytes. The haemolysis may occur by: Phagocytosis and MAC formation. The formation of MAC results in intravascular haemolysis while that of phagocytosis in extravascular haemolysis. Most often, the C3 fragments formed during activation adheres to the surface of red cells. The macrophages in spleen and liver have receptors for complement fragment. The coated red cells bind to the complement receptors on macrophages and are destroyed extravascularly in the reticuloendothelial cells. Clinically, complement mediated intravascular lysis of red blood cells occur rarely because of complement regulatory proteins are commonly expressed on the surface of red blood cells and prevents the formation of MAC. But with massive complement activation, protective effect of regulatory proteins are overwhelmed and red blood cells are lysed. This condition occurs in major transfusion reactions and

In Paroxysmal cold haemoglobinuria, Complement activation leading to haemolysis may also occur in; Autoantibodies or alloantibodies mediated haemolytic reaction. In those conditions, it also occurs as Carrier protein/hapten reaction with antibodies eg. Penicillin-erythrocyte complexes reacts with penicillin antibodies. It also occurs in certain drugs that may attach to cascade proteins or erythrocyte and react non-specifically to cause in vivo haemolysis.

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IMMUNE MEDIATED DESTRUCTION OF RED BLOOD CELLS

Normal red blood cell destruction (haemolysis) occurs after a mean life span of 120 days. Given that the red cells are not nucleated and the enzyme system deteriorating systematically and are not replaced, the cell become non viable with reduced metabolic activity. The red cells become senescent or effete and are removed or destroyed by reticuloendothelial system particularly in the bone marrow, spleen and liver. Then the haem portion is broken down. The iron is stored in the macrophage and are recycled and transported by transferring to marrow erythroblast for erythropoiesis. The protoporphyrin is broken down to biliverdin and by the action of the biliverdin reductase, bilirubin is produced. The bilirubin is taken to the liver where it is conjugated with glucoronides and transported to the bile ducts. From the bile, the bilirubin enters duodenum and is converted to stercobilinogen. Some amount of bilirubin in the duodenum is reabsorbed to the circulation and excreted in the urine as urobilinogen. As for the globin portion, the non-prosthetic complex is broken down to amino acid which are re-utilized in the synthesis of protein. Normal destruction of the red cells and some of non immune haemolysis occur in the phagocytic cells of reticuloendothelial cells and are known as extravascular haemolysis. It result in anaemia when the rate of destruction exceeds the

Autoantibodies - They are antibodies that react with 'self' antigen (ie one or more of the individual protein). It occurs in certain disease or treatment.

Alloantibodies - They are antibodies that react with foreign antigen introduced to the body via pregnancy, transfusion, transplantation.

compensatory mechanisms in the production and synthesis of red blood cells.

As for immune mediated lysis of red cells increased destruction of red cells occurs by immunologic factors such as antibody, complement and drug induce immune complexes. Offending drugs when introduced to the body may serve as haptens and bind to plasma proteins. The complexes formed may be IgM or IgG. On re-exposure to the drugs, the antibody-antigen complex non specifically bind to red cell membrane, activate complement resulting in destruction of red cell and innocent bystander red cells. Immune mediated haemolysis occurs by two mechanisms. The mechanisms are intravascular and extravascular haemolysis.

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INTRAVASCULAR HAEMOLYSIS:

This occurs when red blood cells are destroyed within the blood vessels (Vascular walls) by activation of complements protein (C5-C9) resulting in the formation of MAC. It may also occur by complement induced membrane peroxidation. Haemoglobin binds to haptoglobin and the complex haemoglobin-haptoglobin complex is removed by reticuloendothelial cells.

Haptoglobin are acute phase proteins. It is present in normal levels and are capable of binding haemoglobin. In intravascular haemolysis, free haemoglobin is released which rapidly saturates plasma haptoglobins, resulting in reduced or absent haptoglobin in the plasma. Excess free haemoglobin is filtered by the glomerulus and if the rate of haemolysis saturates the renal tubular reabsorption, free haemoglobins will enter the urine. If iron is released, the renal tubules may become loaded with

haemosiderin. Methaemoglobin (Hb) is also formed in the intravascular haemolysis.

EXTRAVASCULAR HAEMOLYSIS:

Immune mediated haemolysis may also occur extravascularly. The majority of extravascular haemolysis occurs by the phagocytic removal of antibody and/or complement coated red blood cells by macrophages in the spleen or liver. In some specific reaction C3b formed during complement activation may bind on the red cells and in the presence of factor I and factor H may be degraded to iC3b. iC3b is further degraded into G3c and G3dg by factor I. The RBCs coated with C3b/iC3b binds to complement receptors on macrophages in the spleen and liver are removed by phagocytosis. Red blood cell coated with IgG and complement fragments tend to show accelerated removal by the macrophages of the spleen and liver.

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DESTRUCTION OF COMPLEMENT

The action of complement can be destroyed or inhibited in vitro in the following ways:

1. The addition of chelating agents prevents complement activity, as C1 activation requires Ca⁺⁺. For example, the anticoagulant sodium (or potassium) ethylenediaminetetraacetic acid (EDTA) prevents complement activity by removing (chelating) the ionized calcium (Ca⁺⁺) that holds C1 together. In the absence of Ca⁺⁺, C1 is irreversibly inactivated. Inactivation of serum at room temperature with EDTA destroys all C1. Heparin is also anti-complementary, but very large amounts are needed to inhibit complement activity completely. CPD (citrate phosphate dextrose) is anti-complementary ~~as well~~ as the citrate chelates calcium and

- ~~thus~~ thus interferes with the binding of C1. Most (if not all) anticoagulants can be considered anti-complementary.
2. Heating serum to 56°C

COMPLEMENT FIXATION TEST

This is one of the applications of complement activity in diagnosis and is exemplified in diagnosis of microbial infections such as in the diagnosis of HIV, T. pallidum and Toxoplasma gondii infection etc.

In complement fixation tests, (TPHA) or other relate texts, complement induces haemolysis of red cells. A measured amount of complement is added to a reaction. If the complement is fixed, there will be no indication of lysis and the test is considered positive but if there is no Ab production and complement fixing consequent to absence of microbial infection, complement will be readily available to lyse an indicator cell. (The indicator cell often used is the red cell). It shows shows evidence of lytic activity and the test is considered Negative.

~~Complement~~ Complement fixation test principle: When antigen and antibody interact with each other, they form a complex called antigen-antibody (Ag-Ab) complex. This complex then interacts with complement protein and gets fixed with it.

This test is based on the principle that Ag-Ab complex can only fix the complement and its effect on the hemolysis of RBC (used in the indicator system)

TITLE:

BLOOD AND MICROBIAL INFECTION

Preamble:

Invasion of the blood by pathogenic microorganism tends to spell deleterious consequences on the host. To this effect, the host use to mount different defensive mechanisms to checkmate or ward-off the microbes. The defense mechanisms could be humoral, cell mediated and innate immune responses and complement activation. The defense mechanisms mentioned above have been discussed in previous lectures. The study will focus on haematologic abnormalities associated with microbial infections.

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HAEMATOLOGICAL CHANGES ASSOCIATED WITH MICROBIAL INFECTIONS.

Infections are caused by pathogens of different species of bacteria, fungi, viruses, parasites and prions. The microbes includes malaria parasite, Babesia, Clostridium perfringens, Bortonella specie, Tubercole bacillus, Escherichia coli, Mycoplasma specie, Haemophilus and Salmonellae specie. Others are Cytomegalovirus, HIV, HSV, Epstein-Barr virus and influenza virus. The list is inexhaustible and comprises of emerging microbes and diseases. Blood disorders associated with mention microbes causes red cell destruction, anaemia, varied leucocyte and platelet counts, acquired platelet abnormalities and prothrombotic states. The mechanism whereby these disorders occur have been captured in previous studies eg as AgAb, reaction and complement activity.

Anaemia: Anaemia may be caused by malaria parasite, Babesia microti, Babesia divergens, Bortonella bacilliformis and Clostridium perfringens etc.

The organism have different ways or mechanisms whereby they cause anaemia whereas some organism may combine different mechanism to perpetuate anaemia. The mechanisms are by direct lysis of red cells and by immune complexes. Others are diversion of iron and perturbation as microbial infiltration of the marrow. Clostridium causes anaemia by elaboration of haemolytic toxins. *C. perfringens* or *C. welchii* produces a -erythrocyte membrane and liberate potent haemolytic substance known as lysolecithins. Anaemia resulting from immune complexes either by phagocytosis, complement activation or antibody formation have been discussed earlier.

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Leucocyte count: Varied leucocyte count are obtained in microbial infection. This sometimes depend on the severity and duration of the infection. Leucocytosis may occur with infectious mononucleosis, toxoplasmosis, cytomegalovirus, viral hepatitis, brucellosis and rubella and other acute and severe bacterial infection. Leucopenia may occur in HIV and legionella pneumoniae.

Thrombocytopenia: Thrombocytopenia may occur as a result of megakaryocytic depression, immune complex mediated and direct interaction with platelet. It could be obtained in acute viral infection, measles and malaria.

Prothrombotic states: Prothrombotic states occurs with chronic inflammation and consumption coagulopathy obtained with HIV infection.

In conclusion, haematologic disorders with microbial infection could be as a result of direct activity of microbes or the defensive response of the

blood consequent to the presence of microorganism. Whatever activity that is in place, the detection of the loss of C1 and Membrane attack complex thus interferes with the binding of C1 and Membrane attack complex. Thus anticoagulants can be considered anti-complement drugs such as anaemia, leucocytosis, leukocytosis, thrombocytopenia etc.

2. Heating serum to 56°C and immunological factors could be diagnostic.

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COMPLEMENT FIXATION TEST

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In complement fixation tests, (TPHA) or other relate texts, complement induces haemolysis of red cells. A measured amount of complement is added to a reaction. If the complement is fixed, there will be no indication of lysis and the test is considered positive but if there is no Ab production and complement fixing consequent to absence of microbial infection, complement will be readily available to lyse an indicator cell. (The indicator cell often used is the red cell). It shows evidence of lytic activity and the test is considered Negative.