NATIONAL OPEN UNIVERSITY OF NIGERIA

SCHOOL OF SCIENCE AND TECHNOLOGY

COURSE CODE: BIO 318

COURSE TITLE: IMMUNOLOGY AND IMMUNOCHEMISTRY III

COURSE GUIDE

BIO 318
IMMUNOLOGY AND IMMUNOCHEMISTRY III

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INTRODUCTION

BIO 318: Immunology and Immunochemistry is a second semester, 3 credit units' course in Biology. It is a 300 level, second semester Biology course offered to students admitted in the School of Science and Technology

The course guide tells you briefly what the course is all about, what course materials you will be using and how you can work your way through these materials. It gives you some guidance on your Tutor-Marked Assignments.

There are Self-Assessment Exercise(s) within the body of a unit and/or at the end of each unit. The exercise(s) are an overview of the unit to help you assess yourself at the end of every unit.

WHAT YOU WILL LEARN FROM THIS COURSE

This course contains twenty units which cover general topics in Immunology, Immunochemistry and related topics.

Principles of immune response and how it works, autoimmunity, autopathology, immune response to tissue and organ transplant, chemotherapy and recent techniques utilised in immunology will be discussed.

COURSE AIMS

The aim of this course is to educate and introduce the students to Immunology, Immunochemistry and related topics.

COURSE OBJECTIVES

In addition to the aim of this course, the course sets an overall objective which must be achieved. In addition to the course objectives, each of the units has its own specific objectives. You are advised to read properly the specific objectives for each unit at the beginning of that unit. This will help you to ensure that you achieve the set objectives. As you go through each unit, you should from time to time go back to these objectives to ascertain the level at which you have progressed.

At the end of this course, you should be able to:

- discuss immune response in humans
- explain antigen-antibody interactions
- describe the causes of autoimmunity

- define the concept of chemotherapy
- discuss tissue and organ transplant tolerance in recipients
- examine the modes of action of antimicrobials.

WORKING THROUGH THIS COURSE

In this course, you are advised to devote your time in reading through the course materials. You would be required to do all that has been stipulated in the course: study the course units, read the recommended reference textbooks and do all the unit(s) self-assessment exercise(s) and at some point, you are required to submit your assignment (TMAs) for assessment purpose. You should therefore avail yourself of the opportunity of being present during the tutorial sessions so that you would be able to compare knowledge with your colleagues.

COURSE MATERIALS

You are to be provided with the two major course materials. These are:

- Course Guide
- Study Units

The course comes with a list of recommended textbooks. These textbooks are to complement the course materials so that you can avail yourself of reading further. Therefore, it is advisable you source for some of these textbooks and read them to broaden your scope of understanding.

STUDY UNITS

This course is divided into 4 modules with a total of 20 units, divided as follows;

Module 1

Unit 1 Basic Concepts in Immunology
Unit 2 Structure of Antigenic Determinants
Unit 3 Cellular Response
Unit 4 Genetics of Response to Antigenic Stimulation
Unit 5 Structure and Classification of Immunoglobulins

Module 2

Unit 1 Mechanism of Antibody Formation
Unit 2 Antigen/Antibody Interaction: Role of Lymphoid Tissues and Thymus in Immune Response
Unit 3 Hypersensitivity
Unit 4 Immunopathology
Unit 5 Auto-Pathology and Auto-Immunology

Module 3

Unit 1 Tissue and Transplantation Immunology
Unit 2 Immunoprophylaxis
Unit 3 Modern Techniques in Immunology and Immunochemistry
Unit 4 History of Chemotherapy
Unit 5 Principles of Chemotherapy

Module 4

Unit 1 Chemotherapeutic Agents
Unit 2 Modes of Action of Antimicrobials
Unit 3 Chemotherapy of Specific Diseases
Unit 4 Pharmaco-Dynamics and Pharmaco-Kinetics
Unit 5 Drug Bioassay and Sensitivity Tests

TEXT BOOKS AND REFERENCES

King, P. & Perry, M. (2001). "Hepatotoxicity of Chemotherapy". *Oncologist* 6:162–176.

Therasse, P. *et al*.(2000). "New Guidelines to Evaluate the Response to Treatment of Solid Tumors". *J Natl Cancer Inst* 92:205–216.

David, E. G. *et al*. (2005). *Principles of Pharmacology*. (1st ed.). Lippincott: Williams & Wilkins.

Charles, A.J. *et al*. (1999). *Immunobiology: The Immune System in Health and Disease*. Elsevier Science Ltd/ Garland Publishing.

Baron, S. (1996). *Medical Microbiology*.(4th ed.).

Murray, B. (1991). "New Aspects of Antimicrobial Resistance and the resulting Therapeutic dilemmas". *J Infect Dis*.163:1185.

Schaberg, D. (1994). "Resistant gram-positive organisms". *Ann Emergency Med*. 24(3):462

Julio,A.U. & Roberto, D. (2003). "Specific chemotherapy of Chagas disease: controversies and advances". *Trends in Parasitology*. .19:495-501.

Martin, W. *et al*. (2005). "Bleomycin pulmonary toxicity has a negative impact on the outcome of patients with Hodgkin's lymphoma". *J Clin Oncol* **23** (30): 7614–20.

Canellos, G. *et al*. (2004). "How important is bleomycin in the adriamycin + bleomycin + vinblastine + dacarbazine regimen?". *J Clin Oncol* **22** (8): 1532–3.

ASSESSMENT

There are two components of the assessment for this course:

- The Self-Assessment Exercise
- The Tutor-Marked Assignment (TMAs)
- The End of Course Examination

SELF-ASSESSMENT EXERCISE

The exercise within each unit is/are meant to probe your understanding of the concepts in the unit. It is non-grading and as such does not add up to your grade in the course.

TUTOR-MARKED ASSIGNMENT (TMA)

The TMA is the continuous assessment component of your course. It accounts for 30 percent of the total score you will obtain in this course.

FINAL EXAMINATION AND GRADING

The course is to be concluded by the final examination. The final examination constitutes 70 percent of the whole course. You will be adequately informed of the time of the examination. The examination will consist of questions which reflect all the basic concepts you would have learnt through the duration of the course.

SUMMARY

This is intended for you to have an underlying knowledge of the principles of Immunology & Immunochemistry and other related topics. By the time you complete this course, you should be able to answer conveniently questions on the following:

- immune response in humans
- antigen-antibody interactions
- the causes of autoimmunity
- the concept of chemotherapy
- tissue and organ transplant tolerance in recipients
- the modes of action of antimicrobials.

We wish you all the best in your study of this course.

Best wishes.

MAIN COURSE

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MODULE 1

UNIT 1 BASIC CONCEPTS IN IMMUNOLOGY

CONTENTS

1.0 INTRODUCTION

Immunology is a science that dates back to 1796 when Edward Jenner discovered that cow pox or vaccinia induced protection against human small pox is a fatal disease. This breakthrough led the World Health Organisation (WHO) to announce that small pox had been eradicated in 1979 and is regarded as one of the greatest achievements in modern medicine. The protection conferred against infectious diseases after an initial encounter with a pathogen, or through immunisation or other non-immunologic factors is termed *immunity*. The immune system first tries to deny access to invading microbes by using physical barriers such as skin and mucous membranes lining the respiratory, gastrointestinal and reproductive tracts. However, once the pathogen enters the body, the immune system goes straight into action by alerting the cells responsible for defending the body so as to offer protection against such intruders.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- *list the organs that make up the immune system*
- *have a general knowledge of the immune system and how it works*
- *discuss the different types of cells of the immune system.*

3.0 MAIN CONTENT

3.1 Organs of the Immune System

The organs of the immune system found throughout the body and are referred to as lymphoid organs include the tonsils and adenoids, thymus, lymph nodes, lymphatic vessels, Peyer's patches, bone marrow, spleen, and appendix. These organs take part in the growth, development and sending of lymphocytes to wherever the body's defence system is compromised. There are two classes of lymphocytes - B and T lymphocytes and this classification is based on where they mature. B lymphocytes complete their maturation in the bone marrow while the T lymphocytes migrate to the thymus where they multiply and mature into cells that become immunocompetent. The T cells become educated in order to be able to distinguish self cells from non-self cells.

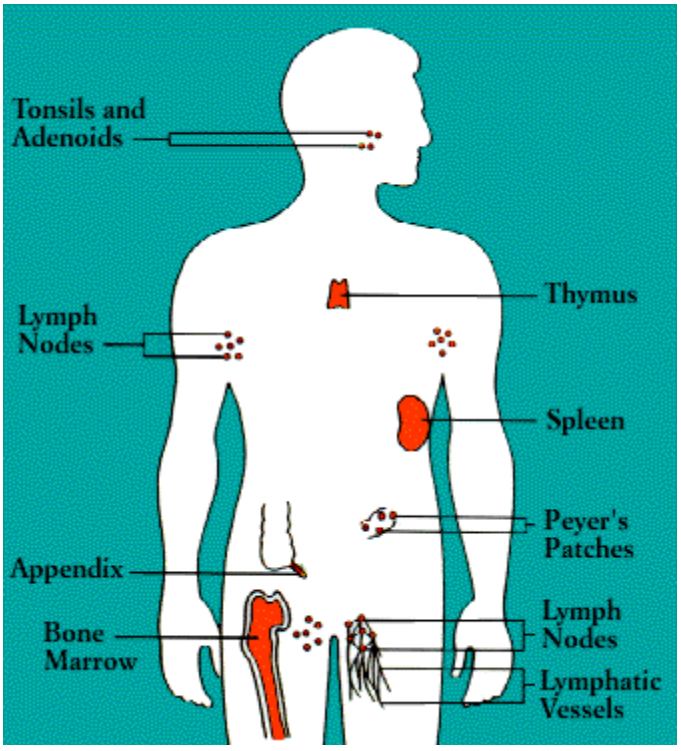

Fig. 1.1: Diagram of the Components of the Immune System

3.2 Components of the Immune System

The immune system provides defence against infectious micro organisms, and also non infectious foreign substances may also elicit an immune reaction. The cells of the immune system originate from the bone marrow and the cells remain there till maturity. The principal components of the immune system are lymphocytes, accessory cells and effector cells. They migrate to the tissues and circulate in the blood in a specialised system of vessels known as the lymphatic system. The red blood cells, platelets and white blood cells are derived from precursor cells called the ***hematopoietic stem cells*** *found in the bone marrow. Hematopoietic stem cells are said to be pluripotent because they give rise to different types of blood cells. The myeloid precursor is also important in immune response and they give rise to the granulocytes, macrophages, dendritic cells and mast cells.*

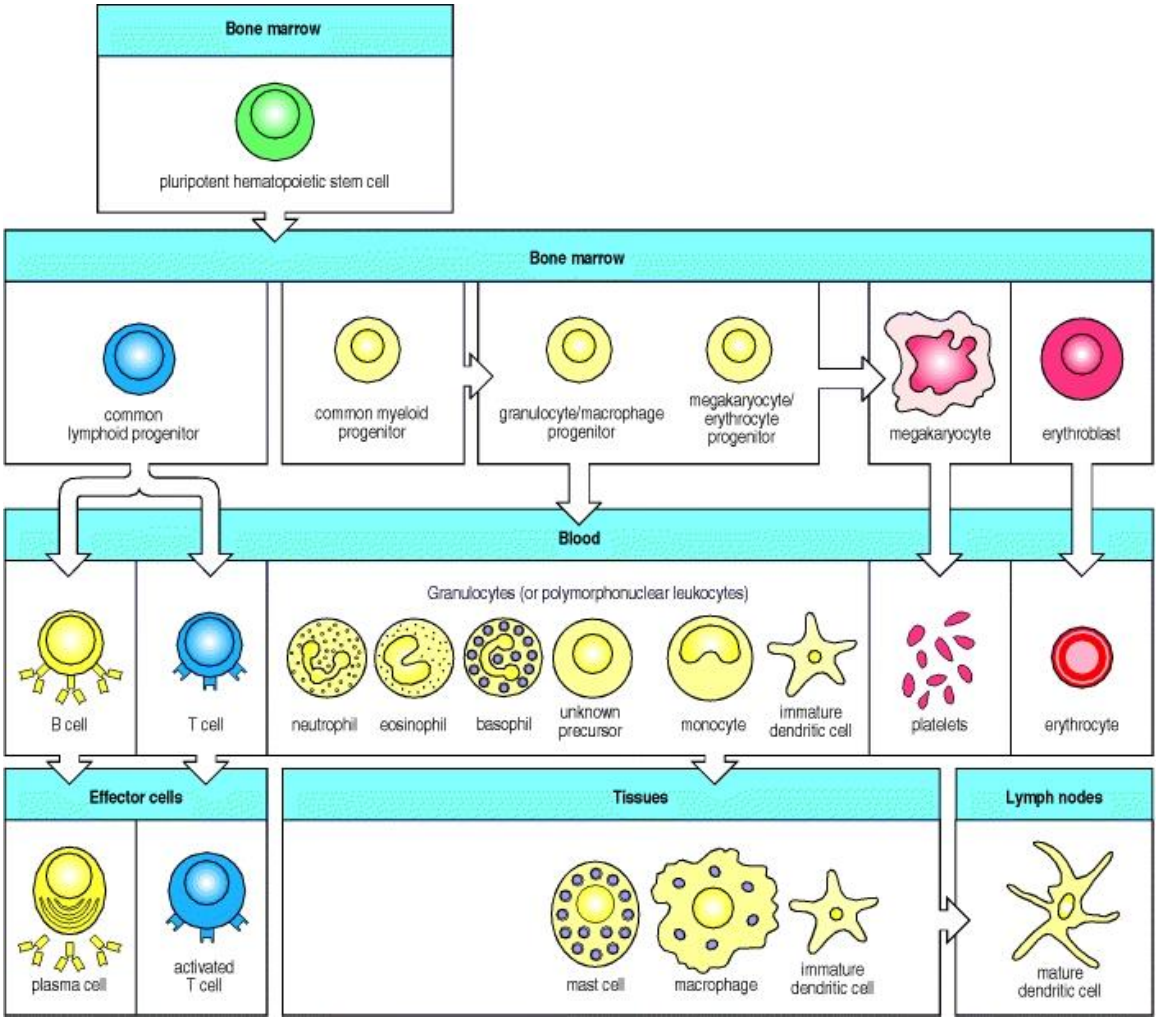

Fig.1. 2: Production and Development of the Lymphocytes

3.3 The Lymphoid Cell

Lymphocytes are the derived from the bone marrow like the blood cells and go through complex maturation process before they are able to acquire their characteristics. The lymphocytes are the only cells in the body that are able to recognise and distinguish different antigenic determinants. Small lymphocytes that have not encountered an antigen before are called naïve lymphocytes and on activation by an antigen they increase in size and are then called large lymphocytes or lymphoblasts. Lymphocytes are heterogeneous and their subsets have functions and protein products but they are the same in morphology. The B cells or lymphocytes are responsible for antibody production and are so called because they develop in the bone marrow of mammals whereas in birds they are present and reach maturity in an organ called bursa of Fabricus. The T cells or T lymphocytes though derived from the bone marrow migrate to the thymus where they mature. Two subsets of T cells exist, whic are the helper T cells and cytolytic or cytotoxic T lymphocytes (CTLs). Another type of lymphocyte cells called natural killer cells have receptors that are different from B and T lymphocytes, they are important in innate immunity. Functional characteristics of lymphocytes are identified using their membrane proteins such that most helper T cells are recognised by expressing a surface protein called CD4 while most cytotoxic T cells express CD8 surface proteins. The lymphoid marker CD, known as Cluster of Differentiation, is the accepted nomenclature to identify the lineage or stage of differentiation of the lymphocytes.

3.4 Myeloid Cells

These cells, as earlier stated consist of granulocytes, macrophages, dendritic and mast cells of the immune system. The granulocytes or polymorphs consisting of neutrophils, esinophils, basophils, monocytes etc are so called because they contain granules having acidic and alkaline phosphatases, defensins and peroxidases. These latter molecules are required for the clearing of the pathogen. Macrophages are long lived and function to engulf any pathogen by means of phagocytosis on passing the membrane barrier. Once the pathogen is engulfed, a phagosome is formed and the lysosomes secrete their enzyme leading to the destruction of the pathogen. Macrophages secrete cytokines whose function includes attracting other cells such as short lived neutrophils, as well as increasing the permeability of the endothelium and the production of neutrophils in the bone marrow. Dendritic cells take up antigens and display them for recognition by lymphocytes. The immature dendritic cells migrate from the blood to stay in tissues displaying both phagocytic and macropinocytic activities by taking up large quantities of fluid, but once they encounter a pathogen they rapidly

mature and migrate to lymph nodes. Mast cells contain electron dense granules in their cytoplasm and differentiate in the tissues. They stay near blood vessels and upon activation, release molecules which affect membrane permeability. They are also involved in eliciting allergic reactions.

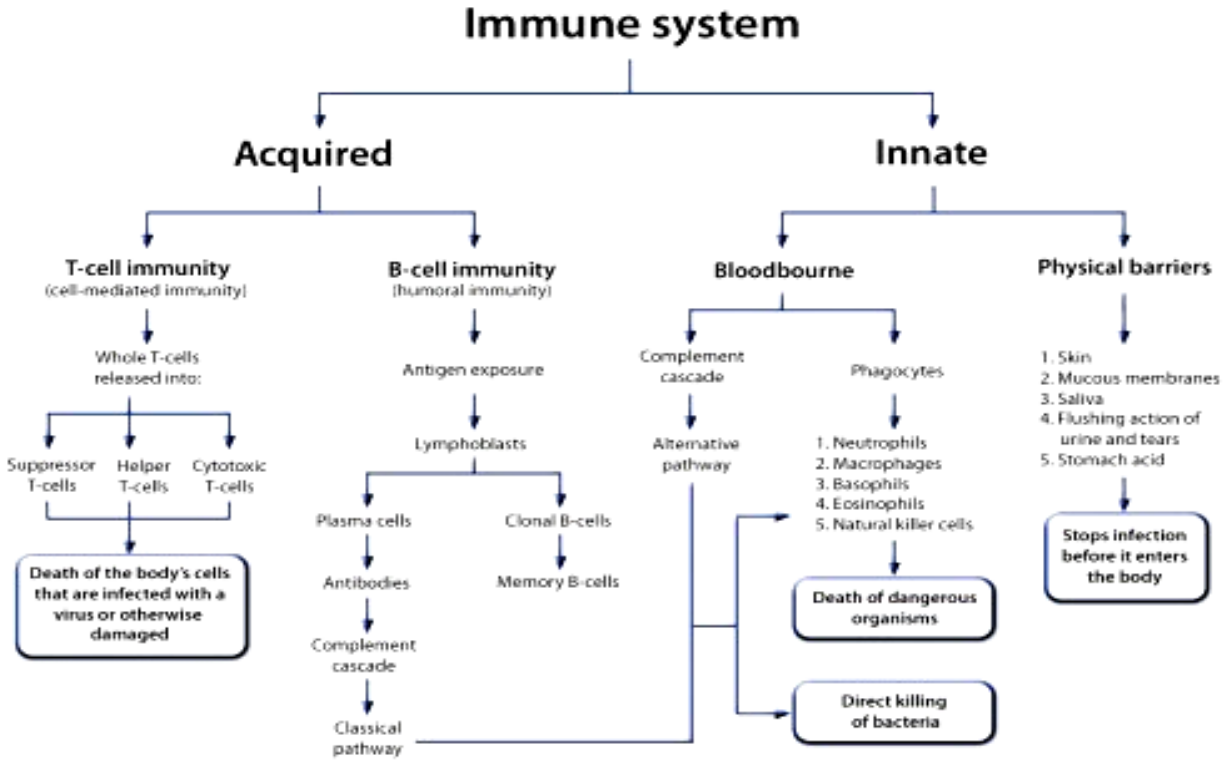

Fig.1.3: Classification of Immune Response

4.0 CONCLUSION

The immune system bears the responsibility of defending the body against foreign pathogen. Once the pathogen enters the body, the immune system goes into action by mobilising all the necessary cellular machinery to defend the body from such intruders. The principal components of the immune system are lymphocytes, accessory and effector cells. There are two types of lymphocytes; B and T lymphocytes classified on the basis of where they mature.

5.0 SUMMARY

In this unit, you have learnt that:

- the immune system defends the body against foreign intruders such as microbes
- there are different cells that make up the immune system
- the lymphocytes are the only cells able to recognise and distinguish different antigenic determinants

- macrophages live long in the body and engulf any pathogen that enters the body.

6.0 TUTOR-MARKED ASSIGNMENT

i. Why are hematopoietic cells said to be pluripotent?
ii. Which type of cell gives rise to granulocytes, macrophages, dendritic cells and mast cells?
iii. Distinguish between innate and acquired immunity.

7.0 REFERENCES/FURTHER READING

Abul Abbas *et al*. (N.D.). *Cellular and Molecular Immunology*. (4th ed.).

Taylor, D.J. (1997). *Biological Science*. (3rd ed.). Cambridge University.

UNIT 2 STRUCTURE OF ANTIGENIC DETERMINANTS

CONTENTS

1.0 INTRODUCTION

Antigens are substances capable of reacting with antibodies or T cell receptors. Before we discuss antigenic determinants we must first of all define terms that would enable us to understand the meaning of the term "Antigenic determinant". *Immunogens* are substances capable of eliciting immune reactions. There are some small molecules which are not capable of eliciting an immune response unless attached to a macromolecule called a carrier before immunisation. Such small molecules are called haptens and nitrophenol belongs to this group of small molecules. It is also possible for these small molecules to bind to an antibody without being immunogenic. Antigens bind only to a small portion of antibody molecule and since macromolecules are larger than the antigen binding region of the antibody, only a small portion of the macromolecule is bound to the antibody. This small portion of the macromolecule bound to antibody molecule is called *Epitope* or antigenic determinant but the portion of the epitope that recognises an antibody is called a *paratope*.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- understand the meaning of certain terms
- determine the features of antigenic determinants
- discuss structural basis of antigen recognition
- list types of antigens.

3.0 MAIN CONTENT

There is a wide range of biological molecules which are capable of binding to an antigen. The molecules include lipids, proteins, carbohydrates, sugars and nucleic acids that can bind to antibody unlike T cell receptors which can only bind to peptides antigens. There are multiple epitopes in macromolecules, and some of them may be repeated and can bind to an antibody. Polyvalency or multivalency is observed in cases where an antigen has multiple identical epitopes and this type of antigenic determinants are not commonly present in globular proteins except where aggregates of such proteins are found. However, polyvalency is observed in polysaccharides and nucleic acids where many identical epitopes are regularly spaced.

3.1 The Features of Antigenic Determinants

Epitopes may be formed depending on the protein folding as well as the covalent structure of the molecule. The antigenic determinants of phospholipids and complex carbohydrates are formed by covalent structures whereas those of some proteins depend on the covalent structure and others on the tertiary structure of the protein. In the case of nucleic acids and proteins, antigenic determinants depend on the non covalent folding of the molecule. Also, spatial arrangements may affect antigen binding such that when epitopes are well separated from each other two antibody molecules could bind to the same protein without having any effect on each other. Formation of epitopes may be linear, conformational or neo-antigenic. Linear epitopes are formed when there are several adjacent amino acid residues, while conformational determinants are formed when there is juxtapositioning of amino acid residues in a folded state. The neo-antgenic determinant may be formed when there is protein modification such as phosphorylation and proteolysis leading to a change in the covalent structure of the protein. New epitopes are subsequently produced which could be recognised by antibodies.

3.2 Structural Basis of Antigen Recognition

The study of the protein lysozyme showed that the structure of antigenic determinant recognised by antibody binding site has three principal characteristics namely: must be large about 750 $Å^2$ in area, formed by two segments of amino acids that are widely separated in primary structure but adjacent in its three dimensional structure and finally exposed on the surface of the antigen. The size of the antigenic determinants therefore would depend on the number of CDRs it has contact with. Two structural antigenic determinants have been proposed, continuous and non continuous determinants. The residues in contact

with antibody are all housed within a single segment of the amino acid sequence of the antigen in the continuous determinants while in the non continuous determinants, the amino acid residues are far apart but come together as a result of protein folding in its native conformation.

3.3 Types of Antigens

B cells respond to three types of antigens. Antigens could be T-independent or T- dependent. T-independent antigens can produce antibodies on stimulation by B cells without the involvement of T cells e.g. polysaccharides. When B cells are stimulated the responses received from these antigens are different from other antigens. T- Independent antigens are characterised by having polymeric structure, resist degradation and have the ability to polyclonally activate other cells. However, T-independent antigens may also be classified into Types 1 and 2 based on their ability to polyclonally activate B cells, the former activate B cell clones while the latter do not. The T dependent antigens require the help of helper T cells to stimulate antibody production by B cells.

4.0 CONCLUSION

Different types of biological molecules are capable of binding to an antigen to elicit an immune response. An epitope or antigenic determinant is a small portion of a macromolecule that can bind to an antibody molecule and multiple epitopes exist in macromolecules. The structure of antigenic determinants varies depending on the macromolecule. There are three structural characteristics of an epitope that are recognised by an antibody.

5.0 SUMMARY

In this unit, you have learnt that:

- there are multiple epitopes in macromolecules
- these macromolecules could be proteins, complex carbohydrates, lipids or nucleic acids
- some structural features of an epitope are necessary in order to be recognised by an antibody
- B cells respond to three types of antigens.

6.0 TUTOR-MARKED ASSIGNMENT

i. Distinguish between epitope and paratope.
ii. What are the structural features that are important for antigen/ antibody binding?
iii. List the different ways in which epitopes may be formed.

7.0 REFERENCES/FURTHER READING

Gene, M. (N.D.). "The structural basis of antigen-antibody Immunology" (chapter three). In: *Antigens* by Dr. Gene Mayer.

Mariuzza ,R. A & Ann. Rev. (1987). "Recognition.Biophys". *Chem*.16:139-59.

UNIT 3 CELLULAR RESPONSE

CONTENTS

1.0 INTRODUCTION

The body responds to the introduction of foreign compounds by eliciting a cellular response provided by the immune system. There are two types of immune response namely; adaptive immune response which provides life-long protection against a particular pathogen and has memory and specificity, and the innate immune response, an inherent immunity, provides the first line of defence against pathogens and is non-specific. It is to be noted that the same innate immune mechanisms, providing the early lines of defence against infectious agents may be used by subsequent adaptive immune response to eliminate microbes.

A link exists between the innate and adaptive immune response in two ways. The first is that the innate immune response to pathogens is responsible for the nature of adaptive immune responses. The second is that many effector mechanisms of innate immunity are important to eliminate microbes in adaptive immunity. The host defence mechanisms employed by invertebrates against pathogens are mediated by innate immunity but in the course of evolution in vertebrates, adaptive immunity consisting of lymphocytes and antibodies became increasingly specialised. There is usually a delay of 4-7 days before adaptive immunity becomes operational; therefore the innate immune response plays a crucial role during this period.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- give the general properties of immune response
- list the types of immune response
- discuss adaptive immune response
- mention components of innate and adaptive immune response.

3.0 MAIN CONTENT

3.1 Properties of Innate Immune Response

The innate immune response which is the first line of defence against an intruding pathogen is very rapid and unable to memorise the intruder should it occur a second time. This type of immunity is inherent and exists before infection by the pathogen and will react in a similar manner if that same infection reoccurs. Innate immunity comprises of principal components such as the physical and chemical barriers, namely; epithelia and antimicrobial substances produced by epithelia surfaces, phagocytic cells mainly neutrophils, macrophages and natural killer cells, blood proteins including members of the complement system and mediators of inflammation and cytokines which are responsible for the regulation and coordination of the cells of the innate immunity. Innate immunity is affected by structures that the pathogens have in common and is unable to distinguish the differences between intruders. The ability of the microbe or pathogen to resist mechanisms of innate immunity shows the measure of its pathogenicity.

Table 3.1: Components of the Innate and Adaptive Immune Systems

	Innate system	Adaptive system
Cellular components	Monocytes/macrophages Neutrophils Eosinophils Basophils Mast cells Natural killer cells	B cells/plasma cells T cells
Secreted components	Complement Cytokines Lysozyme Acute phase proteins Interferons	Antibody Cytokines

3.2 Properties of Adaptive Immune Response

This type of immune response also called specific or acquired immune response is specific because the pathogen has been encountered before and the cells still have immunologic memory that make them respond vigorously on a second encounter with the same microbe. It is able to detect subtle differences even among closely related microbes and macromolecules. The major components of the adaptive immune response are lymphocytes and their products. This type of immune response has six features which are:

1) **Specificity:** It is specific for distinct antigen or different structural component of single complex proteins. The individual lymphocytes express membrane receptors that recognise minute differences in structure between antigens.
2) **Diversity:** The total number of lymphocytes repertoire in an individual is extremely large and this makes it possible for antigen binding sites of lymphocytes to be also diverse.
3) **Memory:** Once exposed to a particular antigen, the immune system reacts rapidly to a reoccurrence of the same infection. Immunologic memory results from the expansion of lymphocyte clones after exposure to the antigen, and memory cells are more efficient in destroying the antigen than naïve cells.
4) **Specialisation:** The immune system responds in a distinct manner and in special ways to different microbe.
5) **Self limitation:** The immune system after responding to antigenic stimulation return to basal state, a process called homeostasis that is they wane with time.
6) **Non-reactivity to self:** The immune system in a normal individual should be able to distinguish between foreign and self antigens, that is, they are should be tolerant to self antigens.

3.3 Types of Adaptive Immune Response

There are two classes of adaptive immune response carried out by lymphocytes. They are cell mediated and humoral (antibody) immune response. The cell mediated immune response is carried out by T lymphocytes and the B lymphocytes are involved in humoral immune response. Cell mediated or cellular immunity defends against intracellular microbes e.g. virus and bacteria which can survive and proliferate inside phagocytes and other host cells. When the microbes are intracellular, they become inaccessible to circulating antibodies and it is only cell mediated immunity that can eliminate microbes inside phagocytes as well as able to lyse the infected cell. The humoral immunity is mediated by molecules in the blood known as antibodies produced by B lymphocytes. It is the principal line of defense against extracellular microbes and their toxins. Antibodies evoke different

effector mechanisms to attack a pathogen because they are specialized. Some antibodies lead to phagoctosis of the pathogen while there are others that mediate the release of inflammatory substances from mast cells.

3.4 Active and Passive Immunity

There is also what is called active immunity where the individual is actively involved in responding to the antigen. Here, immunity is induced either from the host's response to the microbe, antibodies transferred to the host or lymphocytes specific to the host. It is also possible to have immunity conferred to an individual without encountering the pathogen. This is possible in cases where serum or lymphocytes from specially immunised individual is transferred to an unimmunised individual, without having to be challenged by the pathogen. This is known as passive immunity and it is the form of immunity conferred to an unborn baby by the mother. This helps the unborn baby to resist infections before the baby is able to produce antibodies.

4.0 CONCLUSION

There are two types of immune response namely: adaptive and innate immune response. The innate immune response provides the first line of defence against pathogens and does not have immunologic memory. The adaptive immune response is acquired and the body responses more vigorously when the same pathogen is encountered a second time. Its features include specificity, diversity, memory, specialisation and do not react to self antigens. Adaptive immunity has two classes which are cell mediated immunity and this type of immunity protects against intracellular microbes while humoral immunity is mediated by molecules in the blood called antibodies produced by B-lymphocytes.

5.0 SUMMARY

In this unit you have learnt that:

- there are two types of cellular response to pathogens
- innate immune response does not have immunologic memory, while adaptive immune response remembers the same pathogen when encountered a second time
- adaptive immune response comprises of cell mediated and humoral immunity
- the type of immunity conferred on the mother to her baby is called passive immunity.

6.0 TUTOR-MARKED ASSIGNMENT

i. Why does the immune system react vigorously when it encounters the same pathogen again?
ii. Why is innate immune response unable to distinguish between pathogens?

7.0 REFERENCES/FURTHER READING

Peter, J.D. *et al.* (N.D.).*Immunology: Roitt's Essential Immunology* Includes FREE Desktop Edition (Essentials).

David, M. *et al*. (N.D.). *Immunology: With STUDENT CONSULT Online Access* (Immunology Roitt).

Charles, A. J. *et al*. (2001). "Immunobiology'' In: *The Immune System in Health and Disease.* (5th ed.).

UNIT 4 GENETICS OF RESPONSE TO ANTIGENIC

STIMULATION

CONTENTS

1.0 INTRODUCTION

In response to antigenic invasion (antigenic stimulation), both the Innate and Adaptive Immune response are elicited. The adaptive immune response (comprising of the B & T lymphocytes) is the most diverse and specific, while innate immune response, serving as the first line of defence is unspecific usually reacting to all antigens the same way.
This property of the adaptive immune response, as shown by studies is due to the high level of genetic recombination that occurs during the course of the production of the antigen recognition sites of both the B& T lymphocytes as they mature in the bone marrow and thymus respectively.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- state the postulates of clonal selection theory
- give reasons for the specific and diverse nature of the adaptive immune response
- describe the mechanism of immune response to antigenic stimulation.

3.0 MAIN CONTENT

3.1 Recall: The Adaptive Immune Response

As earlier mentioned in the previous unit, the adaptive immune response is divided into humoral and cell-mediated immune response (table 1). The humoral immunity consists mainly of the B lymphocytes involved in the secretion of highly specific antibodies that bind and destroy the recognized antigen with great affinity.Antibodies eliminate soluble extracellular pathogens and their toxins found in the blood.The cell mediated adaptive immune response however mainly consist of the T lymphocytes which are needed to eliminate intracellular pathogens and the activation of other components of immune response in the elimination of antigens.There are two major types of T lymphocytes; the Helper T lymphocytes and the cytolytic/cytotoxic T lymphocytes (CTL). The actions of the CTL are the most direct. They recognize, cells infected with viruses, bind to them and initiate apoptosis in the infected cells. The Helper T lymphocytes on the other hand, play a major role in the activation of other components of the immune system, particularly the B lymphocytes (Figure 1).

The mechanisms of action of the adaptive immune response are mainly regulated by the activities of the T lymphocytes, particularly the Helper T cells.

Table 4.1: A Brief Comparison of the Humoral and Cell-mediated Adaptive Immune Response

Properties	Humoral Immunity	Cell- Mediated Immunity
Lymphocytes	B Lymphocytes	T Lymphocytes
Antigens Recognised	Protein, nucleotide, phospholipids, carbohydrates	Protein antigen mainly
Recognition Sensitivity	Detects only conformational Changes	Detects even a single amino acid residue change
Mode of Action	Secrete soluble antibodies that bind and destroy antigen	Helper T cell with the help of Antigen Presenting Cell (APC), detect antigen invasion and thus stimulate the proliferation and differentiation of other components of the immune response. While the cytolytic T Cell, in response to Helper T cell stimulation, bind and destroy virally infected cells.
Detection Ability	10^{11} different specific antibodies produced	Almost a limitless antigenic determinants can be detected
Antigen Binding sites	Found on the variable region of secreted Immunoglobulin molecules (antibodies)	Found on the surface of Cell receptors

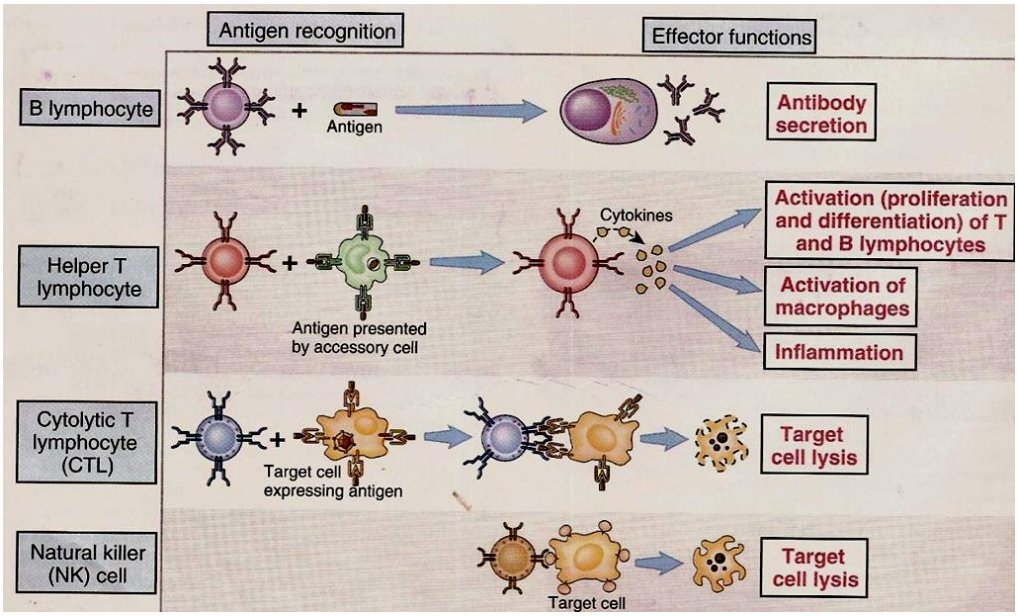

Fig.4 1: Diagrammatic representation of the Adaptive Immune Response. The B lymphocytes recognise soluble antigens and produce specific antibodies, while the T lymphocytes recognise both cellular and soluble antigens and help in the activation of immune response.

3.2 Clonal Selection Theory

Karl Landsteiner was the first to show that there was a repertoire of antibodies that could bind almost an infinite amount of pathogens in every individual. He proved this by demonstrating that virtually a limitless range of molecules can elicit antibody production even synthetic materials.

F McFarlane Burnet proposed the clonal selection theory in 1957 to explain the diverse specificity of adaptive immune response. The hypothesis states that the antigen specific clones of lymphocytes develop prior to exposure to an antigen. When an antigen enters the body, it selectively binds to a specific lymphocyte, thus activating it. Upon activation, a clonal proliferation of the bound lymphocyte occurs leading to the production of lymphocytes of identical specificity to the bound parent lymphocytes. Some of these newly produced clones differentiate into effector cells that help to eliminate the antigen, while some differentiate into the memory cells that help to keep and save that specific antigenic determinant. After elimination of the foreign antigen, the lymphocyte population returns to the normal state, i.e. normal cell count (Homeostasis).

Postulates of the clonal selection hypothesis:

- each lymphocytes bear a single type of receptor with a unique specificity
- interaction between a foreign peptide and the lymphocyte receptor cable of binding that molecule with high affinity usually leads to lymphocyte action
- the differentiated effector cells derived from an activated lymphocyte bear receptors of identical specificity to those of the parental cells which the lymphocyte was derived from
- lymphocytes bearing receptor specificity for ubiquitous self molecules are deleted early in the state of the development. They are usually absent in the repertoire of mature lymphocytes.

3.3 Generation of Diversity in Adaptive Immune Response: Genetics of Response

3.3.1 Generation of Diversity in Antibodies

The total collection of antibody specificity available in an individual is known as the antibody repertoire. During maturation of the B lymphocytes in the bone marrow, the genes responsible for the formation of the Immunoglobulin molecules (antibody) are expressed. Thus the specificity of each antibody in the repertoire of every individual is determined even before B lymphocyte maturation. The amino acid sequence of the variable region (V regions) of Immunoglobulin molecules is responsible for the specificity of each antibody.

Studies have revealed that although the genes coding for each variable region occurs in multiples, majority of them are deleted during B lymphocyte maturation. Thus, the diverse specificity of the antibody repertoire is achieved through the ***Somatic Recombination*** (random rearrangement of different genes) of the remaining variable genes. After this, ***point mutations*** (single nucleotide changes in the genes) are induced within the rearranged genes, further increasing the level of variability. Finally, the alternative trimming and splicing of the primary mRNA transcript before its translation into Immunoglobulin molecules leads to the specificity and diversity observed in antibodies. All these mechanisms, permits the response even to a single antigen diverse (Figure 4.2).

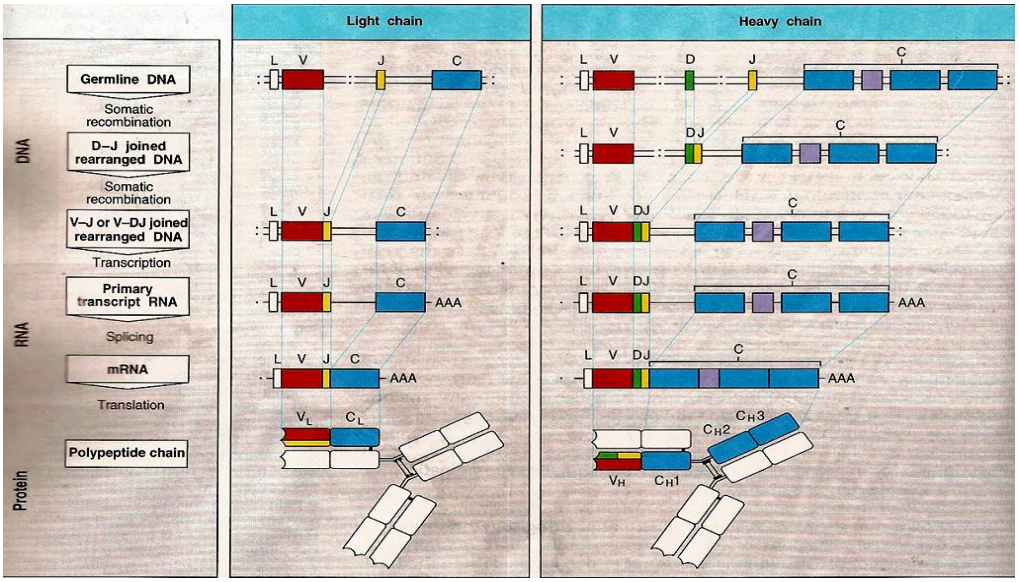

Fig.4.2: Illustrates the various processes by which variability is observed in the V regions of Antibodies

3.3.2 Diversity in the Tlymphocyte: MHC Molecules

T lymphocytes respond to peptide fragments of protein antigens that are displayed by Antigen presenting cells (APCs). The task of displaying the antigens of cell-associated microbes for recognition by T lymphocytes is performed by specialised proteins that are encoded by genes in the Major Histocompatibiliy Complex (MHC).

There are two different types of MHC gene products, called the class I MHC molecules and the class II MHC molecules, which sample different pools of protein antigens (extracellular antigens that have undergone endocytosis and cytosolic intracellular antigens). The human MHC, also referred to as the Human Leucocyte Antigen (HLA) locus is located on the short arm of chromosome 6 spanning about 4×10^6 base pairs. It encodes for about 200 genes that are highly polygenic (i.e. encodes different ranges of peptides binding specificity) and polymorphic (i.e. there are multiple alleles of each gene). The HLA locus and gene products are homologous to the mouse H2 locus and gene product (figure 3).

MHC molecules are transmembrane proteins (antigens) that have structure similar to the antibodies (immunoglobulin). They are ubiquitously expressed on all nucleated cell found in the body.The class I MHC molecules presents antigens to the $CD4^+$ helper T cells that help to activate other components of the immune response while the class II MHC molecules present antigens to the $CD8^+$cytolytic T lymphocytes that help to lyse virus infected cells (Figure 4).

T lymphocytes play a dual role of specificity in that they have the ability to recognize and differentiate self antigen from foreign antigens during immune response. Lymphocytes that actively react to self antigens are destroyed during maturation in the thymus.

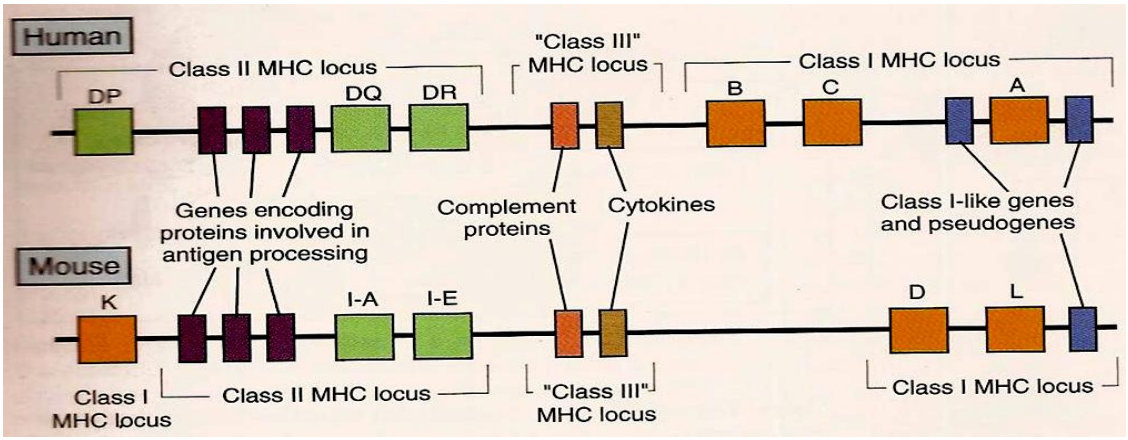

Fig.4. 3: Schematic maps of the human (HLA) and mouse (H2) MHC loci

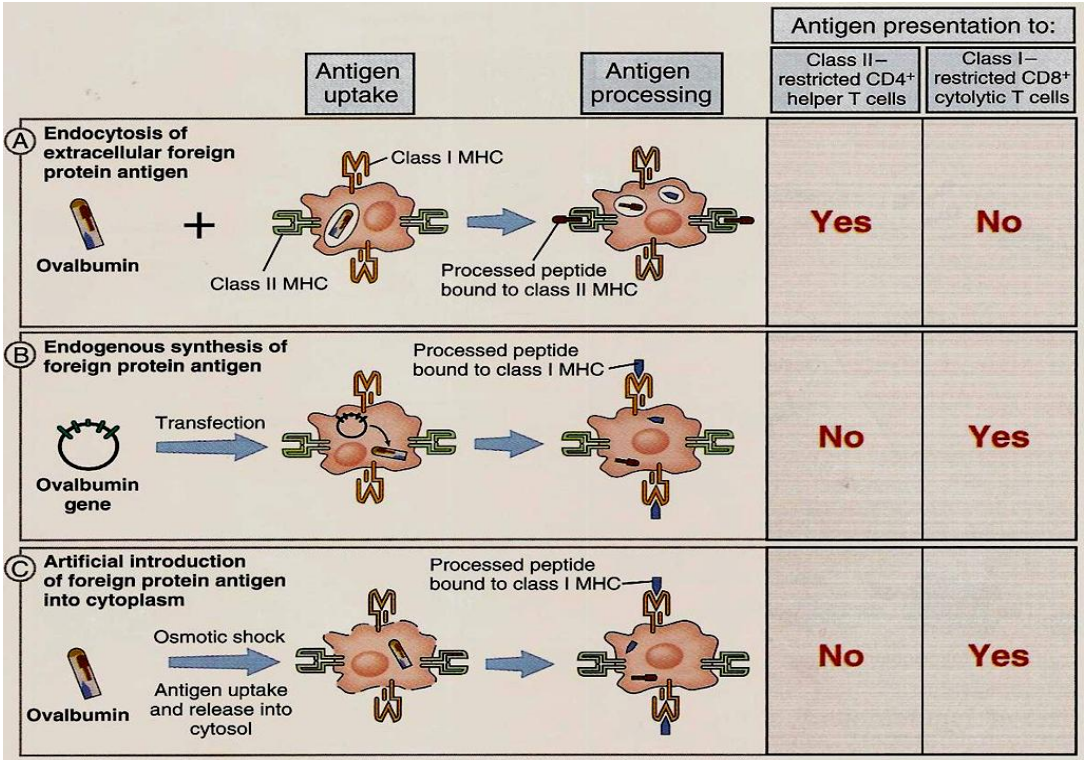

Fig. 4. 4: Antigen presentation by MHC molecules

3.4 Response to Antigenic Stimulation: A Practical Scenario

Let us consider a case study of a bacterial infection, after the first day of infection (i.e. the pathogenic escape of the innate immune response). As we know all microbes (as well as most other cell types) have surface proteins expressed on their membrane.

The B lymphocyte that recognises the bacterial epitope from the repertoire will selectively bind it (i.e. the antigenic determinant). Simultaneously, antigen presenting cells presents the extracellular antigens of the helper T lymphocytes or in some cases the B lymphocyte itself presents the antigen protein to the helper T cell (Figure 4.5). The helper T lymphocytes in turn recognises it has a foreign antigen, releases cytokines that activates the rapid proliferation of that particular Blymphocytes bound to the bacterial epitope to produce antibodies that collectively eliminate the bacterial and its toxins.

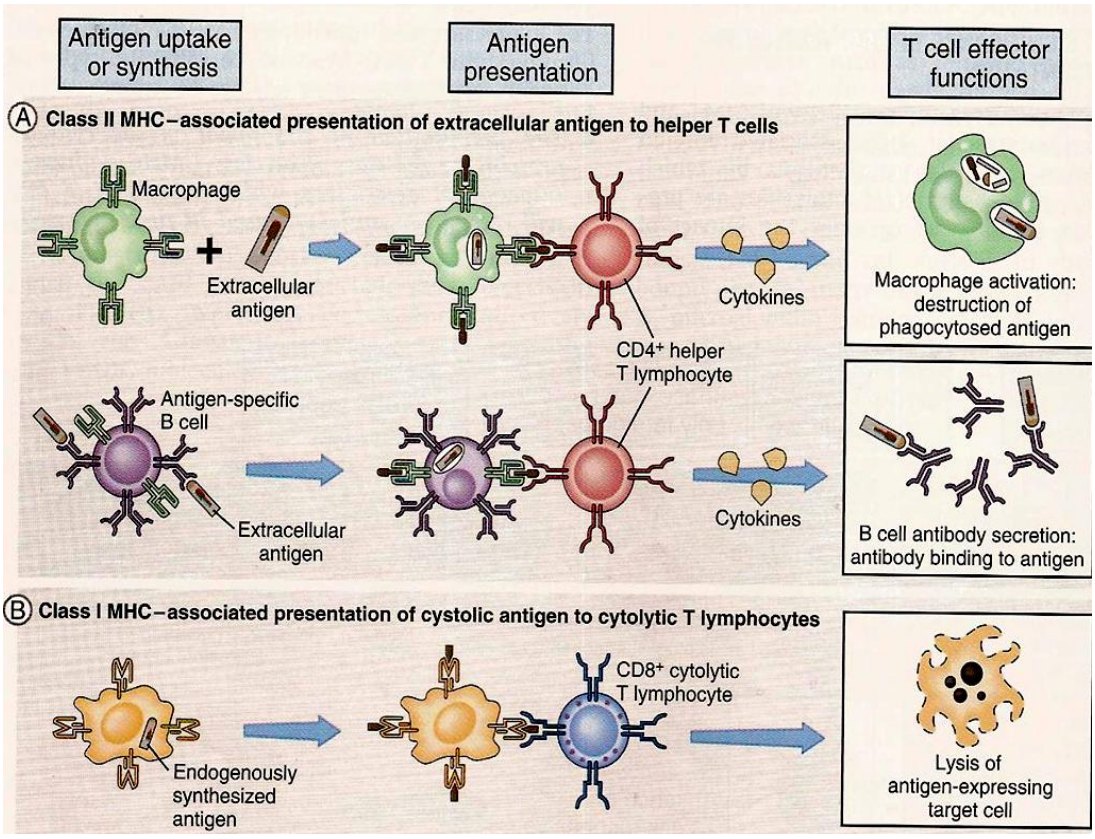

Fig.4.5: A- Extracellular antigens are presented by macrophages or B lymphocytes to CD4+ helper T lymphocytes which activates the macrophages and B lymphocytes to eliminate the extracellular antigen. B- Cytosolic antigens are presented nucleated cells to the CD8+ cytolytic T lymphocytes for elimination. MCH- Major Histocompatibility Complex

4.0 CONCLUSION

In terms of specificity and diversity, the adaptive immune response is more specific and diverse than the Innate Immunity. This specific and diverse nature of the adaptive immunity is accomplished through the genetic recombination of genes involved in the formation of antibody and receptor specificity during the development of B and T lymphocytes in the bone marrow and thymus respectively.

5.0 SUMMARY

In this unit, you have learnt that:

- the adaptive immunity are the most diverse and specific form of immune response
- through immense genetic recombination, the specificity and diversity of the adaptive immune response is obtained
- genetic diversity of the T lymphocyte receptor is controlled by the genes in the Major Histocompatibility (MHC) complex loci.

6.0 TUTOR-MARKED ASSIGNMENT

i. Briefly discuss the adaptive immune response.
ii. Discuss the Clonal Selection Theory.
iii. How is genetic diversity attained in the humoral immune response?

7.0 REFERENCES/FURTHER READING

Abbas, A.K. *et al*. (2000). *Cellular and Molecular Immunology*.(4th ed.). New York: W.B. Sanders Company.

Janeway, C.A. *et al*. (1999). *Immunology: The Immune System in Health and Disease.* (4thed.). London: Current Biology Publications.

UNIT 5 STRUCTURE AND CLASSIFICATION OF IMMUNOGLOBULINS

CONTENTS

1.0 INTRODUCTION

The term *immunoglobulin* was coined by Elvin Kabat and colleagues after they discovered that most antibodies after electrophoresis are detected in the third fastest migrating group named gamma globulins for the third letter of the Greek alphabet. Immunoglobulins (Ig) otherwise known as antibodies are soluble glycoproteins produced in a membrane bound form on B lymphocytes to recognise and bind to antigens. They are present in serum, tissue fluids, or on cell membranes. The B cells are the only cells that synthesise antibodies. Antibodies act as adaptors for immune system effector molecules by linking antigens to receptor molecules. So basically antibodies perform two main functions which are to recognise and bind to foreign material and to trigger the elimination of the foreign material through complex system involving different proteins. The study of antibodies and how they react with antigens is known as serology. When blood or plasma is made to clot, antibodies remain in the fluid called serum and a serum containing antibodies against a particular antigen is known as antiserum.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- understand the molecular structure of antibodies
- list the different classes of antibodies
- describe how immunoglobulins recognise and bind to antigens
- explain the structure-function relationships found in antibody molecules.

3.0 MAIN CONTENT

3.1 Molecular Structure of Antibodies

The antibody is a Y-shaped molecule consisting of three equal fragments connected to each other by a flexible hinge. Two of these fragments called Fab (fragment antigen binding) are identical to one another are involved in antigen binding and the other fragment known as Fc (fraction crystallisable) binds to effector molecules. These fragments were identified after proteolytic digestion with papain split the antibody molecule into two fragments in the hinge region before the hydrogen-hydrogen inter-chain disulfide bond. The antibody molecule is flexible thus allowing binding to different arrays of multivalent antigen.

The basic structure of immunoglobulins revealed a four chain unit polypeptide comprising of two identical light and heavy chains.

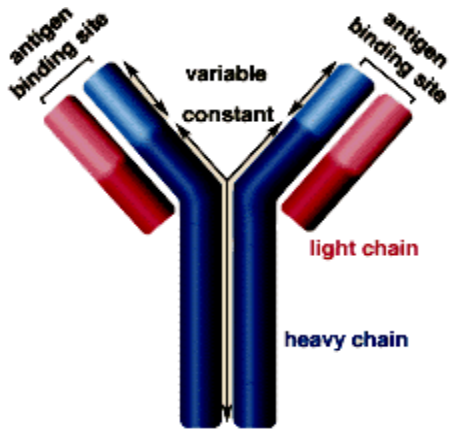

Fig.5.1: The structure of an antibody molecule

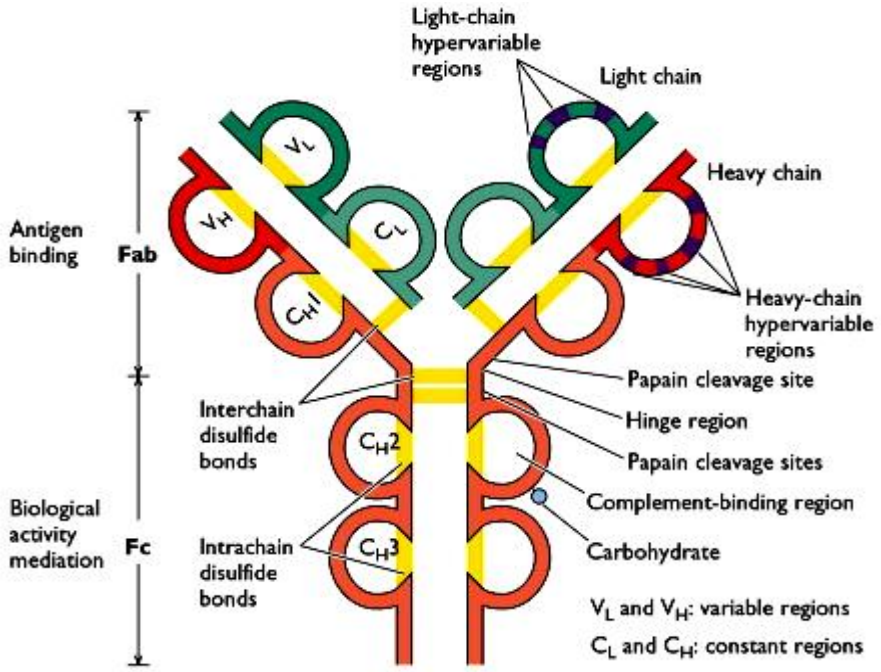

Fig.5. 2: Detailed structure of an antibody

The light chains (L) have a molecular weight of about 24 KD and the heavy chains (H) molecular weight of between 55 -70 KD. The heavy chains are connected to one another via disulphide bonds, while each heavy chain links a light chain by disulphide and non covalent bonds.

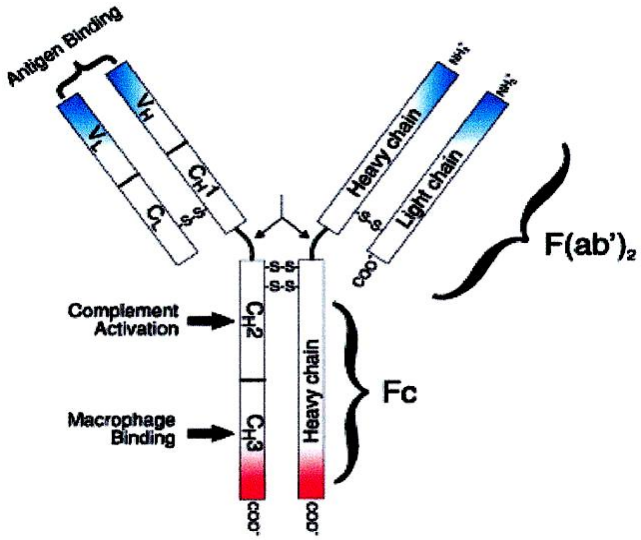

Fig.5. 3: Linear representation of an antibody molecule

The heavy and light chains consist of repeating, homologous units of 110 amino acid residues in length that fold independently to form an immunoglobulin domain. Both the heavy and light chains have regions called variable and the constant regions represented as C_H and C_L and V_H and V_L where the subscript denotes heavy or light chain. The amino

acid sequences of the variable region of both heavy and light chains consists of 110 amino acid residues whereas in the case of the heavy chain constant region, the amino acids vary , between 110 to 440 residues. The variable region consists of N-terminal of the amino acid sequence and the carboxy terminal, the constant region. The variable region defines the region where the amino acid sequence varies and this distinguishes antibodies of one clone from another clone. It has been observed that most of the variability in amno acid sequences occur in the three regions known as hypervariable or complementarity determining region (CDRs). Hypervariable or complementarity determining region are found in both the light and heavy chain regions and function in binding and recognition of an antigen

3.2 Classification of Antibodies

Immunoglobulins may be classified into five distinct classes based on differences in size, amino acid sequence, carbohydrate content and charge. The different classes are IgA, IgE, IgD, IgG and IgM. Some of these classes may be divided into subclasses egIgG (ϒ) has four subclasses namely IgG1, IgG2, IgG3 and IgG4 and IgA(α) has two subclasses –IgA1 and IgA2. A unique hinge region exists for each IgG subclass. The classes and the subdivisions represent what is referred to as isotypes and these nine isotypes are found in normal human beings. The other immunoglobulin types IgE, IgM and IgD do not have subclasses. The structure of the heavy chain of the Ig molecule is important in defining the isotype. Other terms used in classifying immunoglobulins are allotypes and idiotypes. The amino acid sequence and the three dimensional structure of the constant region of an Ig molecule determines the allotype , however, allotypes shows the genetic differences within the same species, consequently all members of the species will not possess any particular allotype. Idiotype defines the variation in amino acid sequence and three dimensional structure of the Ig molecule and the antigen binding specificity of any particular antibody.

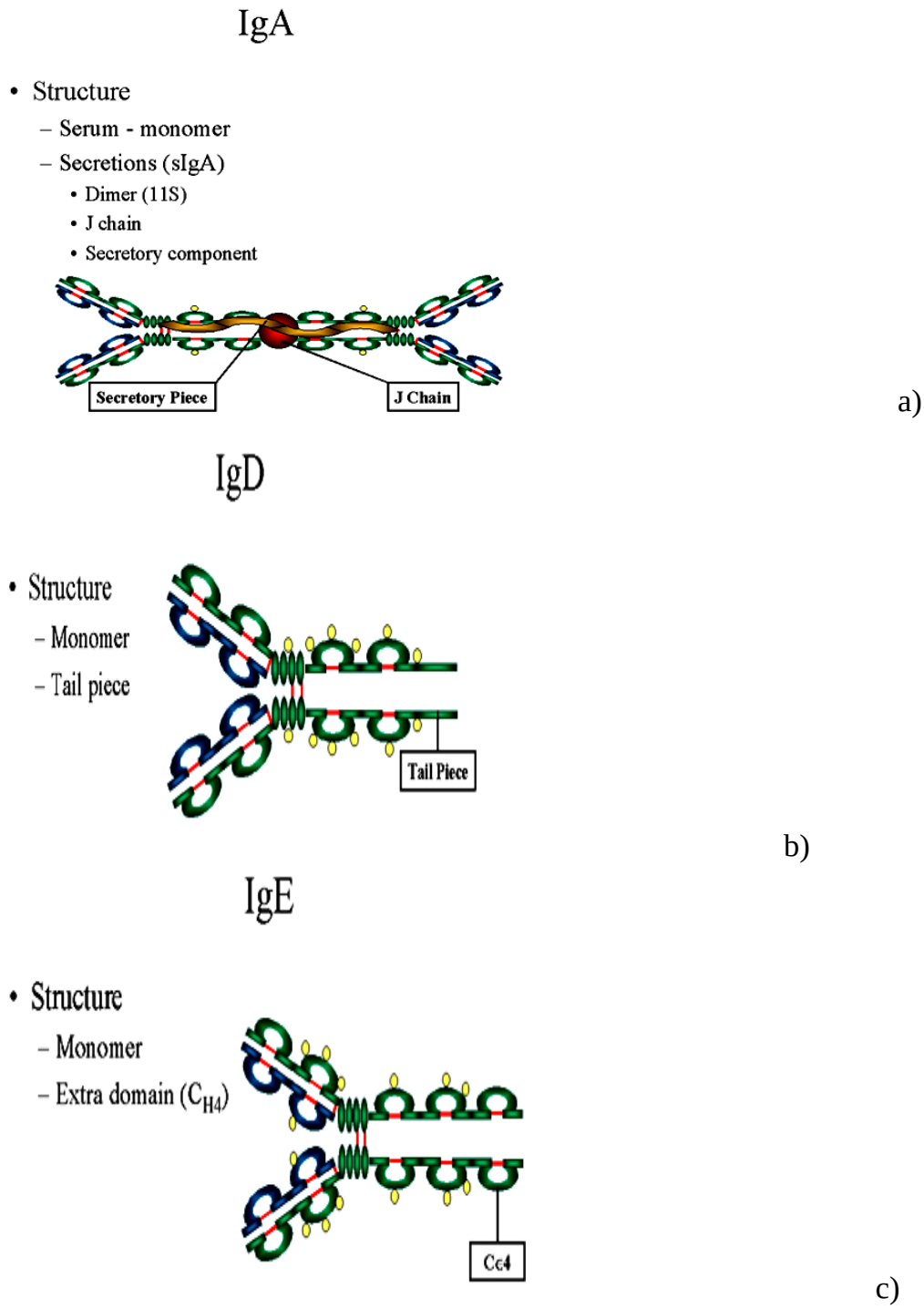

Fig.5. 4: a-Ig A, b)-Ig D , c)- Ig E

There are two classes or isotypes of light chains called Lambda (λ) and Kappa (k) after the Greek alphabet. Only one particular type of the light chain is present in an antibody molecule but never one of each. These two isotypes of light chains can be distinguished based on the carboxy terminal of the constant region.

3.3 Structure - Function Relationship of Antibodies

Different roles are played by different classes of immunoglobulins. Basically immunoglobulins recognise and bind to antigens and trigger off effector functions thus ensuring that the effector functions are specifically targeted towards elimination of that particular antigen. All immunoglobulin isotypes except IgD play a dual role which is to bind to antigen and exhibit one or more effector functions. There are certain features required in order for an antibody to be able to perform these roles. These features include specificity, diversity, affinity and avidity. Antibodies are able to distinguish subtle differences between antigens and these differences applies to recognition of all classes of molecules eg two linear protein determinants could be distinguished from each other based on the substitution of a single amino acid sequence that may or may not affect the secondary structure. The recognition of this fine specificity is important because biochemical constituents of all living organisms are basically related so that antibodies produced against microbial molecules do not react with structurally similar molecules. In some cases though, antibodies generated against a particular antigen reacts with a structurally similar antigen and this is what is regarded as cross-reaction. Diversity is generated from the large numbers of antibodies necessary to bind antigen as a result of genetic mutations that may bring about random variations in structure in the hypervariable regions of both the heavy and light chains. It is necessary that antibodies bind tightly to antigens to be able to eliminate a pathogen, therefore high affinity binding antibodies are generated by changes that occur during somatic mutation in antigen stimulated B lymphocytes. These B lymphocytes give rise to new V domain structures that bind antigens with greater affinity than the original V domains. The Fc fragment mediates the effector functions of an antibody and different domains of this fragment are responsible for carrying out different functions. During humoral immune response the isotype of the antibody influences the response of that antibody to an antigen, and this is because stimulation of B clone cells would produce different isotypes with identical V domains hence identical antigen binding sites. This is called isotype switching where naïve B cells would produce IgM and IgD with identical binding sites on activation. In isotype switching, there is a change in the type of the constant region heavy chain (C_H) or antibody isotype produced by the B cell whereas the V regions and antigen specificity remains the same. Different isotypes and subtypes may be produced from the original B cells as a result of isotype switching.

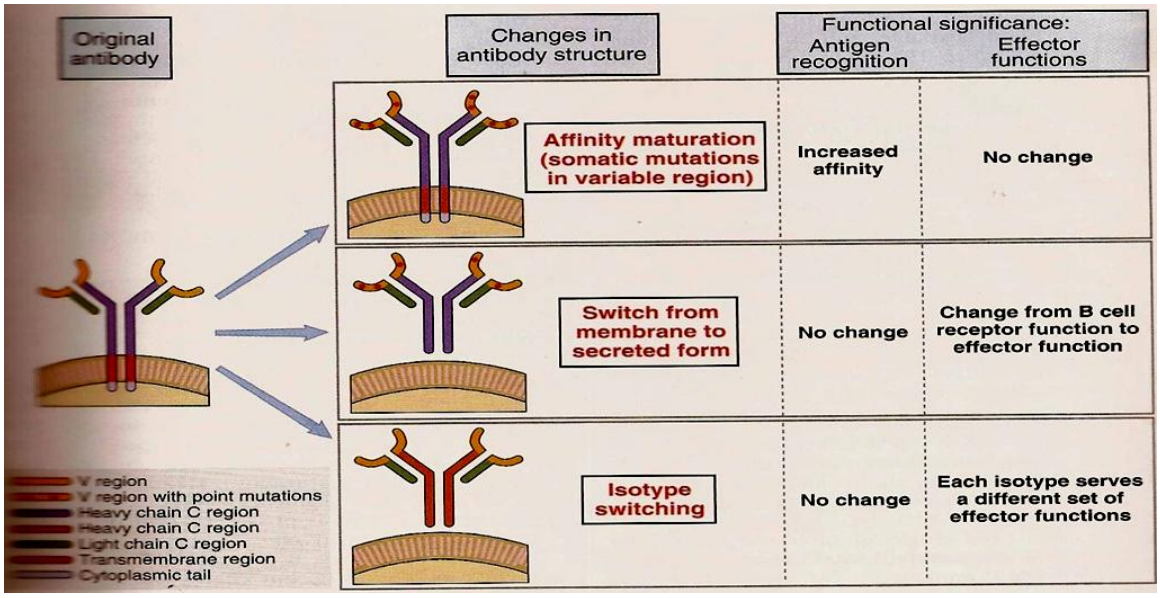

Fig.5.5: Changes in the antibody structure

4.0 CONCLUSION

The antibody molecule is Y – shaped consisting of two fragments called Fab (fragment antigen binding) and another fragment called Fc (fragment crystallisable). There are two identical heavy and light chains and both chains have regions called variable and the constant regions represented as C_H and C_L and V_H and V_L where the subscript denotes heavy or light chain. There are three regions called hypervariable or complementarity determining region (CDRs) found in both the light and heavy chain regions and function in binding and recognition of an antigen.

There are five classes of immunoglobulins known as IgA, IgE, IgD, IgG and IgM and these classes vary based on differences in size, amino acid sequence, carbohydrate content and charge. Structural and functional relationship exists among immunoglobulins which enables them to perform their roles. Features such as specificity, diversity, affinity and avidity are very important and again antibodies are able to distinguish subtle differences between antigens and these differences assist in the recognition of all classes of molecules.

5.0 SUMMARY

In this unit, you have learnt that:

- the antibody is a Y- shaped molecule
- there are two heavy and two light chains
- there are five classes of immunoglobulins

- Fc molecules mediate the effector functions of an antibody molecule
- isotype switching occurs in which naive B cells would produce IgM and IgE with identical binding sites on activation.

6.0 TUTOR-MARKED ASSIGNMENT

i. How do you distinguish antibodies of one clone from that of another?
ii. Distinguish between isotype, allotype and idiotype?
iii. Define isotype switching?

7.0 REFERENCES/FURTHER READING

Abbas, A.K. *et al*. (2000). *Cellular and Molecular Immunology*. (4th ed.). New York: W.B. Sanders Company.

Janeway, C.A. *et al*. (1999). *Immunobiology: The Immune System in Health & Disease.* (4thed.). London: Current Biology Publications.

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MODULE 2

UNIT 1 MECHANISM OF ANTIBODY FORMATION

CONTENTS

1.0 INTRODUCTION

Antibodies are present in biologic fluids throughout the body and also on the surface of a few cell types. The B cells are the only cells that synthesises antibody molecule. They are found in cytoplasmic membrane compartments such as endoplasmic reticulum and golgi complex and on the surface of B cells and are expressed as integral membrane proteins. There are also secreted forms of antibody found on the plasma, mucosal secretions and in the interstitial fluid of tissues in a small amount.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- discuss the natural distribution of antibodies
- describe the theories of antibody formation
- discuss the process of antibodies production
- differentiate between primary and secondary antibody response.

3.0 MAIN CONTENT

3.1 Theories of Antibody Formation

Two theories have been proposed to show how antibodies are produced. They are the selective and instructive theories. The first theory known as the instructive theory by Linus Pauling proposed that all antibodies had only one polypeptide structure which on induction by combining with an antigen folded in diverse ways. This proposal was rejected because it was observed that all antibodies still retain their specificity even after denaturation and renaturation in the presence of an antigen and moreover antigen specific antibodies were detected on lymphocyte surfaces before exposure to the antigen. The selective theory which replaced the instructive theory was put forward through the research efforts of these scientists: Jerne, Niels; Talmadge, David and Burnet MacFarlane (1955-1957). There were many versions of the theory that emerged but the clonal selection theory of antibody production was the most accepted. According to this theory antigen specific clones of lymphocytes develop prior to and it is independent of exposure to an antigen. The model of the clonal selection theory occurs in two phases, the first phase involves the generation of diverse immunoglobulins without being challenged by an antigen and subsequent incorporation into the cell surface. This stage is now followed when on exposure to an antigen, there is selection by B lymphocytes of antigens and subsequent proliferation and differentiation to become either memory or antibody plasma producing cells. The second stage occurs in the circulation and peripheral lymphoid tissues.

3.2 Production of Antibodies

When the body is exposed to an antigen on immunisation, a stimulation of antibody production follows but continued exposure to that same antigen would ensure the production of high affinity antibodies. B cells are activated to produce IgM antibody on initial exposure but 3-5 days later IgMisotype appears in the serum and reaches its peak at between 10- 14 days. Antibody levels begin to decline thereafter till it reaches the pre-immunisation or baseline levels. The figure below shows that this response goes through four phases namely the lag, log, and plateau and decline phases. In the lag phase also referred to as latent or inductive phase, on introduction of the antigen, it is seen as a foreign substance and the cells begin to proliferate and differentiate in response to the antigen usually 3-5 days. This is subsequently followed by the log or exponential phase in which the B cells different into plasma cells that produce antibody of the IgM type. In the third phase which is the plateau or steady state phase there is no net increase in antibody production because antibody synthesis is balanced by its decay and this happens

between 10-14 days. The final stage decline or decay, the rate at which antibodies are degraded outweighs the synthesis and this brings a decline in the level of antibody in the body till it reaches the baseline or pre-immunisation state. This is what happens during primary response to an antigen. However, a subsequent or secondary exposure to the same antigen results in a quicker response because of immunologic memory. The lag phase is shorter while the log phase responds quickly to the presence of an antigen. There seems to be no steady state and the response proceeds to the decline stage which is not as rapid and the antibody persists for months and even for years or a lifetime.

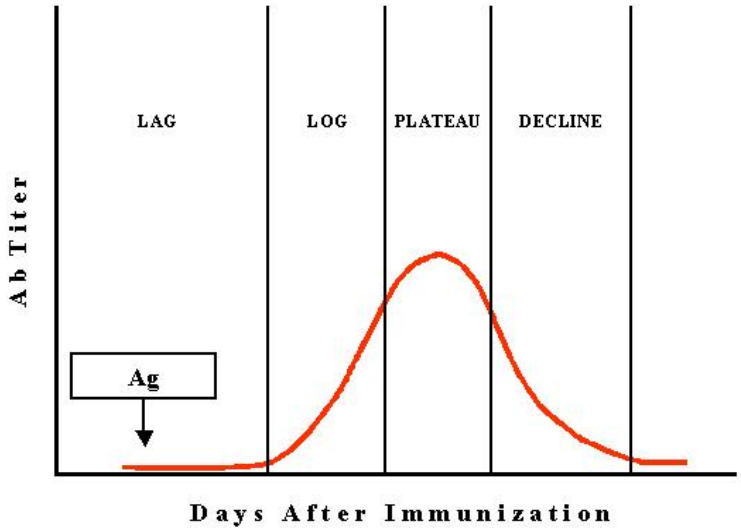

Fig. 1.1: Kinetics of Primary immune response to an antigen.

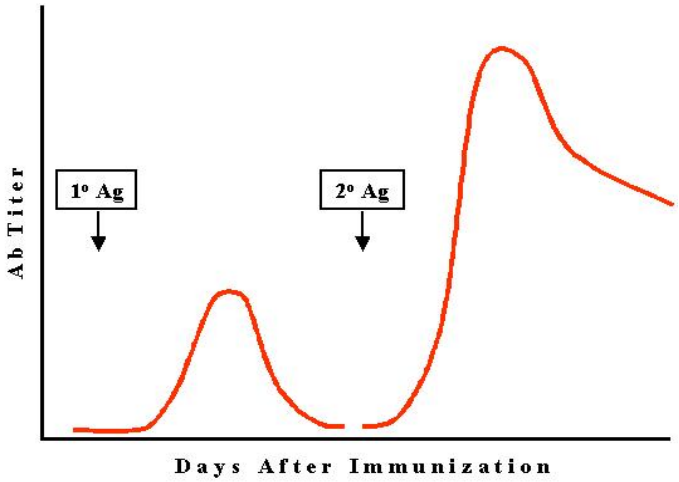

Fig.1. 2: Kinetics of Secondary immune response to an antigen

3.3 Qualitative Changes and Cellular Events during Primary and Secondary Response

The qualitative changes that occur during primary and secondary response involve the isotype of the antibody. The IgMisotypes are predominant antibodies produced but in secondary response, the isotypes present are the IgA, IgG and IgEisotypes and the IgGisotypes are the predominant antibodies. There is affinity maturation of the IgG antibody which implies that initial response to low doses of an antigen is low but it progresses with time. This explanation is explained by the clonal selection theory. Increased affinity leads to avidity and higher affinity is much likely to result in cross reactivity. Avidity refers to the strength with which an antibody binds to an antigen. Antibodies are multivalent in their reactions with multivalent antigens, thus creating a stronger bond. However, remember that a univalent antibody fragment could bind to a single antigenic determinant thus the avidity of an antibody for its antigen depends on the affinities of individual antigen binding sites o for the epitopes on the antigen. Cross reactivity may occur when an antibody/antigen reactions show a high level of specificity and in that case it is possible for an antibody to bind to an antigen that is structurally related though different.

The cellular events though earlier stated are seen in the phases of both primary and secondary immune response, where in the lag phase the B cells differentiate in plasma cells, followed by exponential increase in antibody concentration, then the plateau and decline phases. In the secondary response, all the differentiated B and T cells after encountering an antigen die off and some become memory cells. When challenged again by an antigen it is not only the naïve T and B cells that are activated but also the memory cells and that is the reason for a short lag period during secondary response.

4.0 CONCLUSION

The theories of antibody production were proposed and the clonal selection theory is the most acceptable. According to the theory, diverse immunoglobulins could be generated without being challenged by an antigen. Once challenged by an antigen, the B lymphocytes selects antigens, followed by proliferation and differentiation of cells that lead to such cells becoming either memory or antibody producing cells.

Antibodies are produced when the body is exposed to an antigen on immunisation, and high affinity antibodies are produced on continued exposure to the same antigen with a quicker response.

5.0 SUMMARY

In this unit, you have learnt that:

- antibodies are naturally distributed in biological fluids
- there are two theories of antibody production
- antibodies are produced when the body is challenged by a pathogen
- there are primary and secondary phases in immune response
- when the body is challenged again by the same antigen, the response period is shorter than the first time.

6.0 TUTOR-MARKED ASSIGNMENT

i. Why is the clonal selection theory the most acceptable of the theories of antibody production.
ii. Identify at least two cellular changes that occur during primary and secondary immune response.
iii. Compare and contrast primary and secondary antibody response.

7.0 REFERENCE/FURTHER READING

Janeway, C.A. *et al*. (1999). *Immunology: Then Immune System & Disease.* (4thed.). London: Current Biology Publications.

UNIT 2 ANTIGEN/ANTIBODY INTERACTIONS: ROLE OF LYMPHOID TISSUES AND THYMUS IN IMMUNE-RESPONSE

CONTENTS

1.0 INTRODUCTION

Immune responses are not carried out in any single organ, but in a wide variety of structures collectively known as lymphoid tissue. Lymphoid tissue can be generally categorised as central (or primary), and peripheral (or secondary). Central lymphoid tissues are those which act as a source of immunocompetent cells, these cells then migrate to the peripheral lymphoid tissues which are the sites of immune responses. Primary lymphoid tissues are the tissues in which lymphocytes are generated and differentiate into mature naïve lymphocytes; these are the bone marrow for B cells, and the bone marrow and the thymus for T cells. Secondary lymphoid tissues are the tissues in which immune responses are initiated, and the lymphatic vessels that connect them to the tissues and the bloodstream and thus to sites of infection.

The main function of the immune system is to defend the body against a wide variety of pathogenic infectious agents with vastly differing natures, i.e. viruses, bacteria, fungi, protozoa and parasitic worms. The complexity of this task requires a sophisticated repertoire of mechanisms for the recognition of, and defense of the body against, these pathogens. This is achieved by an array of cells (and molecules which they secrete) which are dispersed throughout the body and collectively constitute the immune system. Most of the major cell types of the immune system are derived from progenitors (stem cells) in the bone marrow.

Many of the mature cells circulate in the bloodstream and are dispersed throughout tissues of the body, while some also congregate in

specialised lymphoid tissues. Furthermore, in order to generate effective immunity, the various cell types cooperate with each other by means of direct interactions between cell surface molecules and via the molecules that they secrete.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- describe what lymphoid tissue and lymphocytes are
- discuss both B lymphocytes and T lymphocytes
- relate the function of B-lymphocytes with T-lymphocytes
- describe the development of T-lymphocytes in the thymus.

3.0 MAIN CONTENT

3.1 What are Lymphocytes?

They arise from lymphoid progenitors in the bone marrow mammalian B cells fully develop here, whereas T cell precursors migrate to the thymus for selection and maturation. The bone marrow and thymus are thus known as primary lymphoid organs. The three major types of lymphocytes are called B cells, T cells and NK (natural killer) cells. Mature B and T cells circulate in the bloodstream and lymphatic system, spending some time in the secondary lymphoid tissues, i.e. the spleen, lymph nodes and mucosa-associated lymphoid tissues (MALT). Two morphological types of resting lymphocytes can be distinguished: B cells and the majority of T cells are small lymphocytes with a thin rim of cytoplasm surrounding the nucleus, whereas natural killer cells and some T cells are larger, have more cytoplasm and distinct cytoplasmic granules, and are known as large granular lymphocytes. B and T lymphocytes are entirely responsible for adaptive or acquired immunity, i.e. the ability to recognise each pathogen in a specific way and to mount a faster and bigger response on repeated exposure to a particular pathogen (immunological memory). This is because B and T cells express surface receptors which specifically bind to materials that are foreign to the body (known as antigens). The receptors of a single lymphocyte are identical to each other and recognise a single antigen. However, millions of different antigen receptors are collectively expressed by the whole population of lymphocytes in the human body, thus conferring the ability to recognise many great foreign antigens.

3.2 What are B-Lymphocytes?

Cells of the immune system which are specialised to make antibodies are termed B-lymphocytes, they are produced in the bone marrow of

adult mammals. Without B-cells and the antibodies they produce, one can only survive if given frequent gamma-globulin injections. The B cells constitute 5–15% of human blood lymphocytes. The main function of a B cell is to secrete soluble recognition molecules called antibodies which specifically bind to an antigen recognised by that B cell. These antibodies (also known as immunoglobulins) are, in fact, the secreted form of a B cell's surface antigen receptors and bind to exactly the same antigen. A B cell will only produce antibodies when it has been activated by binding antigen; this activation process also usually requires help from T cells. The activated B cell undergoes multiple divisions and some of the resulting cells differentiate into antibody-secreting cells. These are known as plasma cells, and they possess copious rough endoplasmic reticulum involved in antibody synthesis.

The antibodies serves to neutralise toxins, prevents organisms adhering to mucosal surfaces, activates complement, opsonises bacteria for phagocytosis, and sensitisestumour and infected cells for antibody dependent cytotoxic attack by killer cells. Thus antibody acts to enhance elements of the innate system. Although ultimately antibody is the secreted product of activated B cells with the functions listed, early in B-cell development, it is a membrane bound molecule that acts as the B-cell receptor. In this role it internalises antigen and processes it to act as an antigen-presenting cell for T-cell responses.

Different classes of antibody predominate at different compartments of the body (IgM being intravascular, IgG the main antibody of the blood and tissues, IgA in secretions). Mucosa associated lymphoid tissue consists of lymphoid tissue at several mucosal sites (bronchus, gut, urogenital tract). However, these are all linked functionally as subpopulations of B cells home to these tissues specifically. A response generated at one site will induce immune responses to the same antigen at other sites.

The main structural difference between antibodies of different classes is in the non-antigen binding portion of the molecules, called the Fc region, which is constant in structure between antibodies of the same class produced by different B cells. An important aspect of antigen recognition by B cells is that these lymphocytes, and the antibodies they produce, bind to antigens in their natural or naive form, i.e. as they occur as constituents of pathogens.

3.3 What are T-Lymphocytes?

About 70% of human blood lymphocytes are T cells. The main functions of T lymphocytes are to exert effects on other cells, either regulating the activity of cells of the immune system or killing cells that

are infected or malignant. Like B lymphocytes, T cells have surface antigen receptors, but there is no secreted form of this equivalent to antibodies. Furthermore, T cells cannot recognise antigens in their native forms, but only when they are presented on the surface of antigen-presenting cells (APCs). The antigen receptors of most T cells (alpha-beta T cells) are composed of two polypeptides called alpha and beta chains, and they interact with peptides derived from the degradation (processing) of foreign antigenic proteins. These peptides are bound to molecules of the major histocompatibility complex (MHC) on the surface of APCs. The interaction between the T-cell antigen receptors and the peptide–MHC complexes binds a T cell to the surface of an APC, thus targeting the T cell to exert effects on the APC. There are two types of MHC molecules, called class I and class II, which present antigen peptides to alpha-beta T cells expressing the surface proteins CD8 or CD4, respectively. This is because CD8 binds to MHC class I and CD4 binds to MHC class II.

3.4 Development of T-Lymphocytes in Thymus

As soon as receptor rearrangement has occurred, T and B cells are able to respond to their antigen and induce an immune response. However, cell activation is tightly regulated to ensure that only damaging antigens elicit a reaction. Regulation particularly involves the initiation of T lymphocyte activation. This requires that antigen is presented to the T cell within the peptide binding groove of a self MHC molecule. This is because the T-cell receptor does not just recognise the antigenic epitope, but also the complex of the peptide in association with the self-MHC molecule. The delicate process of positive selection of T cells that can react with self-MHC and peptide adequately to induce immune responses occurs in the thymus. These cells could sometime not be excessively MHC-reactive to the extent which would cause self-tissue destruction.

The cells that emerge from the thymus and bone marrow having undergone gene rearrangement are naive i.e. they have not yet encountered their specific antigen within an immune response. These cells populate the secondary lymphoid tissues of the lymph nodes, spleen, tonsils, and mucosa associated lymphoid tissue. Because there are only a few naive T and B cells capable of reacting specifically with a foreign particle, in order for them to encounter their specific antigen, there has to be a system to bring them together. The lymphoid tissues provide the microenvironment for this process. In addition to T and B lymphocytes, they contain efficient antigen-presenting cells and are able to produce the cytokines necessary to maintain T and B lymphocytes. Lymphoid tissues express adhesion molecules in an ordered array, allowing cells to move through the tissue and increase the chance of

lymphocytes being brought into contact with antigen. The lymphoid organs communicate with the tissues using lymphatic and blood vessels.

4.0 CONCLUSION

B cells are generated throughout life in the specialised environment of the bone marrow and may require a second environment; the lymphoid tissue helps to maintain the existence as mature recirculation B cells. The thymus provides a specialised and architecturally organised microenvironment for the development of mature T cells.

5.0 SUMMARY

In this unit, you have learnt that:

- the bone marrow and thymus are the primary lymphoid organs involved in the production and differentiation of lymphocytes
- there are two major types of lymphocytes; the B-lymphocytes and the T-lymphocytes
- the development of T-lymphocytes occurs in the thymus.

6.0 TUTOR-MARKED ASSIGNMENT

1. Briefly describe different types of lymphocytes.
2. What are the importance of B cells and T cells?
3. Describe the development of T-Lymphocytes in the Thymus

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UNIT 3 HYPERSENSIVITY

CONTENTS

1.0 INTRODUCTION

Hypersensitivity may be defined as a process in which the adaptive immune response becomes over sensitive to a variety of infectious and innocuous antigens thereby inflicting injury to the host tissue. The disorders that result from such reactions are termed hypersensitivity diseases.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- understand the term hypersensitivity
- classify hypersensitivity reactions
- discuss some diseases associated with hypersensitivity reactions.

3.0 MAIN CONTENT

The most important function of adaptive immune response is in host defense against microbes. However, it could also be responsible for tissue injury and disease. This reaction could produce discomfort and sometimes may result in fatality. A common cause of hypersensitivity reaction is inability to tolerate self antigens, a property of the immune system whose responsibility it is to ensure that no reaction occurs between individuals and their own antigens. Inability to self tolerance and subsequent immune response against self or autologous antigens results in autoimmune diseases.

3.1 Types of Hypersensitivity Reactions

There are five types referred to as Type I, II, III, IV and V. Once contact is established with antigen a variation in timing exists for each of the hypersensitivity reactions (Table 3.1).

Table 3.1: Hypersensitivity Reactions

Hypersensitivity type	Appearance time	Mediators	Mechanism
i. Immediate	2-30 mins	IgE	Mast cell response (enhance acute inflammation)
ii. Cytotoxic	5-8 hours	IgM and IgG	Antibody and complement
iii.Immune complex	2-8 hours	IgM and IgG immune complexes	Antibody/antigen Complexes
iii. Delayed type	24-72 hours	CD4 and CD8 T cells, APCs	T cell mediated Macrophages activated Include granulomatous reactions
iv. Stimulatory		Autoantibodies	Autoantibodies against hormone

The Type 1 hypersensitivity reaction which is allergy affects about 17% of the population and it exists in two phases and mediated by IgE. It is an immediate hypersensitivity reaction because antibodies are involved and starts within minutes of contact with the antigen. The mechanism of action involves mast cells and basophils, both of which have receptors for IgE. After interaction of antibody/antigen some physiological substances such as histamine, serotonin and other mediators of inflammation are released thus inducing spasms of the smooth muscle.
The hypersensitivity Type II reaction also called cytotoxic reaction involves IgE and IgM antibodies. These antibodies interact with the antigen in the blood as well as analogous antigens on the surface of human body cells. This is done by opsonization and phagocytosis of cells. These mechanisms set off the complement system that lyses the cell.

Hypersensitivity type III mobilises not only IgE and IgM but also immune complexes. It can rise from soluble antigens. The immune complexes could vary in size, amount, affinity and the isotype of the responding antibody. Consequently, large immune complexes fix the complements and are disposed by mononuclear phagocytes from circulation, whereas the smaller complexes deposit in blood vessel walls

and can ligate Fc receptors on mast cells and other leucocytes which can lead to leucocyte activation and tissue injury.

Type IV hypersensitivity called delayed type hypersensitivity (DTH) reaction occurs within 24-72 hours and it is mediated by CD4, CD8 T cells as well as antigen peptide cells (APCs) eg Langerhans' cells. The activated T cells migrate to the site of antigenic entry where pro-inflammatory mediators such as Tumor necrosis factor (TNF) are released. The pro-inflammatory mediators facilitate blood flow and extravasation of plasma contents to the area. CD4 and CD8 cells are released resulting in the interferon gamma (IFN-γ) thus enhancing macrophage activity in the area.

The stimulatory or type V hypersensitivity are mediated by auto-antibodies. It is classified as a distinct type of hypersensitivity where the auto-antibodies bind to hormone receptors that imitate the hormone itself and this leads to stimulation of target cells. Autoimmune response defines the ability of an individual immune system to react with self antigens. There are some individuals who have auto-antibodies and will show no symptoms, but autoimmune disease results if the regulatory mechanisms breakdown. It is to be noted that the causes of autoimmune diseases are multi-factorial.

3.2 Diseases Associated With Hypersensitivity Reactions

Hypersensitivity diseases are clinically heterogeneous group of disorders whose manifestations are defined by the type of immune response that leads to cell and tissue injury. The mechanisms of immune response that leads to different types of hypersensitivity reactions have been stated above. There are some individuals prone to produce IgE in response to environmental allergens and show strong immediate hypersensitivity are said to be atopic and they suffer from allergy. This form of allergy may present in different forms such as hay fever, asthma, urticaria (hives) or chronic skin irritation. In extreme cases known as anaphylaxis, the mast cell and basophil derived mediators restrict the airways to the point of asphyxiation and produce cardiovascular collapse leading to death. Myasthenia gravis is an example of type II hypersensitivity reaction in which the antibody inhibits acetylcholine binding as well as down modulating receptors. Type III hypersensitivity reaction is presented in immune complexes mediated reactions such as Arthus reaction. The delayed type hypersensitivity results from tissue injury as a result of the production of reactive oxygen species, hydrolytic enzymes, nitric oxide and some pro-inflammatory mediators such cytokines. Insulin-dependent diabetes which is an organ specific auto immune disease is caused by DTH reactions induced by auto T cells. Diseases caused from

hypersensitivity reactions are shown in the Tables below.

Table 3. 2: Diseases caused by Cell- or Tissue Specific Antibodies

Disease	Target Antigen	Mechanisms of Disease	Clinicopathologic Manifestations
Autoimmune hemolytic anemia	Erythrocyte membrane proteins (Rh blood group antigens, I antigen)	Opsonization and phagocytosis of erythrocytes	Hemolysis, anemia
Autoimmune thrombocytopenic purpura	Platelet membrane proteins (gpIIb:IIIa integrin)	Opsonization and phagocytosis of platelets	Bleeding
Pemphigus vulgaris	Proteins in intercellular junctions of epidermal cells (epidermal cadherin)	Antibody-mediated activation of proteases, disruption of intercellular adhesions	Skin vesicles (bullae)
Vasculitis caused by ANCA	Neutrophil granule proteins, presumably released from activated neutrophils	Neutrophil degranulation and inflammation	Vasculitis
Goodpasture's syndrome	Noncollagenous protein in basement membranes of kidney glomeruli and lung alveoli	Complement- and Fc receptor–mediated inflammation	Nephritis, lung hemorrhage
Acute rheumatic fever	Streptococcal cell wall antigen; antibody cross-reacts with myocardial antigen	Inflammation, macrophage activation	Myocarditis, arthritis
Myasthenia gravis	Acetylcholine receptor	Antibody inhibits acetylcholine binding, down-modulates receptors	Muscle weakness, paralysis
Graves' disease (hyperthyroidism)	TSH receptor	Antibody-mediated stimulation of TSH receptors	Hyperthyroidism
Insulin-resistant diabetes	Insulin receptor	Antibody inhibits binding of insulin	Hyperglycemia, ketoacidosis
Pernicious anemia	Intrinsic factor of gastric parietal cells	Neutralization of intrinsic factor, decreased absorption of vitamin B_{12}	Abnormal erythropoiesis, anemia

Abbreviations: ANCA, antineutrophil cytoplasmic antibodies; TSH, thyroid-stimulating hormone.

Table 3. 3: Examples of Human Immune Complex-Mediated Diseases

Disease	Antigen Involved	Mechanisms of Disease	Clinicopathologic Manifestations
Systemic lupus erythematosus	DNA, nucleoproteins, others	Complement- and Fc receptor–mediated inflammation	Nephritis, arthritis, vasculitis
Polyarteritis nodosa	Hepatitis B virus surface antigen	Complement- and Fc receptor–mediated inflammation	Vasculitis
Poststreptococcal glomerulonephritis	Streptococcal cell wall antigen(s); may be "planted" in glomerular basement membrane	Complement- and Fc receptor–mediated inflammation	Nephritis

4.0 CONCLUSION

Hypersensitivity reactions are caused by disorders of immune response. The reactions are classified into five types based on the type of immune response and the effector mechanisms that are responsible for cell and tissue injury. The immune response could be autoimmune responses against self antigens or uncontrolled or over reaction to foreign antigens. The immediate hypersensitivity occurs within few minutes of being

challenged with an antigen and in extreme cases death may occur due to asphyxiation and circulatory collapse.

5.0 SUMMARY

In this unit, you have learnt that:

- hypersensitivity reaction is a disorder of the immune response
- there are five types of hypersensitivity reactions
- different types of hypersensitivity reactions are mediated by different effector mechanisms.
- some diseases are associated with hypersensitivity reactions.

6.0 TUTOR-MARKED ASSIGNMENT

i. Explain the differences between Type II and Type III hypersensitivity reactions.
ii. Why are some individuals hypersensitive to certain drugs or chemicals while some others are not?
iii. How does the mechanism of opsonization mediate in Type II hypersensitivity reaction?

7.0 REFERENCES/FURTHER READING

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UNIT 4 IMMUNOPATHOLOGY

CONTENTS

1.0 INTRODUCTION

Immunopathology is the branch of immunology that deals with the pathologies of the immune system. It can also be described as the branch of medicine that deals with immune responses associated with diseases. The immune system helps fight off infections but could also turn around to be harmful due to over activity and that is immunopathology. Early immunology studies looked at the ambivalence of the manifestations of allergy or hypersensitivity. This is because the immune system which was supposed to defend the body goes astray to create a variety of pathological conditions. However, with deeper understanding of the pathogenesis of some diseases such as tuberculosis and leprosy as well as the development of experimental models of some diseases like Masugi nephritis, and lymphocytic choriomeningities immunopathology was then viewed in a wider context of immunologic event. A few years after the development of the basic concepts of immunopathology, it was then divided into four classes viz anaphylaxis, delayed hypersensitivity, immune-complex disease and complement mediated lysis.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- understand the pathologies of the immune system
- relate immune responses to certain diseases
- assess the pathology of Immune responses.

3.0 MAIN CONTENT

3.1 Pathology of Immune Response

The reaction caused as a result of the response of the immune system to infections, could be beneficial, without effect or detrimental to the patient. When a reaction is beneficial, there are no signs or symptoms on the affected person, and a lifelong immunity is conferred on the individual. A role carried out effectively by the Adaptive immune response. If the effect on the patient is neutral, the individual experience symptoms, as a result of the response of the immune system to infections, mainly cytokines released from phagocytes and toxins released from dying bacteria. Cytokines affect the local blood vessels, causing inflammation; redness and swelling of the skin. Cytokines also play an imperative role in the adhesive properties of the endothelium, causing circulating leukocytes to stick to the endothelial cells of the blood vessel wall and migrate between them to the site of infection, to which they are attracted by other cytokines. This action accounts for the pain experienced during infection. A reaction is described harmful to an individual if it causes a variation of symptoms that are quite different from the normal symptoms associated with the infection. Symptoms of this kind of immune response are unique to the patient and are considered life threatening e.g. diarrhea, fatigue, muscle weakness, constipation, cough, depression, fever, flu e.t.c. The length or duration of immunopathological symptoms depends on the nature of infection and the defense system of the affected person. However, this does not mean that a rise in the intensity of symptoms is an indication of advancement in infection or disease, but in most cases suggests that the immune response system is effective in combating infectious pathogens e.g. diarrhea and vomiting associated with food poisoning are quite necessary in eliminating harmful toxins.

The adaptive immune response involves production of antibodies against a particular pathogen. These antibodies continually provide an adaptation to infection from that pathogen, as a result may confer a life-long protective immunity to re-infection with the same pathogen. Antibodies are produced only in response to specific infections, hence antibodies present in an individual directly represents the number of pathogens the individual has been exposed to. Adaptive immune responses depend on the activities of lymphocytes. Lymphocytes help to identify and defend against pathogenic molecules not recognised by the phagocytes and macrophages e.g. Viruses and bacteria with protective capsules. All lymphocytes require two signals in order to be activated: one that results from antigen binding and a second signal from a T-cell if the lymphocyte is a B-cell, or from macrophages, dendritic cell or B-cell if it is a T-lymphocyte. Mature dendritic cells are found extensively

in the T-cell. The surface receptors of B cells and T cells are adapted to recognise antigen in two different ways. B cells of the lymphocytes recognize antigen that is present outside the cells of the body, where most bacteria are found; T cells by contrast can detect antigen generated inside host cells such as Viruses. Antibodies produced by the lymphocytes are found in the plasma or extracellular fluid, and they protect from pathogens by either binding to them, thereby blocking their access to cells that they may infect or destroy, a process known as **Neutralisation**, or through **Opsonization**. Opsonization involves the coating of pathogens by antibodies, enabling ingestion and digestion by phagocytes. Another form of protection by antibodies is through activation of a system of plasma proteins known as **Complement.** The function of the complement is similar to Opsonization. T-cells are very crucial in detecting pathogens that replicate inside cells. As earlier stated innate immune response, involves ingestion and digestion of pathogens by phagocytic cells called macrophages. These phagocytic cells are always available to combat a wide range of microorganisms, without requiring prior exposure, and they act in the same way in all normal individuals. The innate responses largely involve granulocytes and macrophages. The defence system of the innate immunity is effective in combating many pathogens. However, they cannot recognise novel pathogens that lack surface molecules, common to many pathogens. The adaptive and innate immune responses work together to provide an effective defensive system, ensuring successful destruction of all forms of pathogens that in most instances cause no pathology.

3.2 Immunopathology of Infectious Diseases:

Immunopathology captures both hypersensitivity and autoimmunity as a result of overactive immune system and immunodifficiency, which is the inability of an individual to fight infection. If a virus infects a host, an immune response is generated which could determine the survival or the death of the host in the case of acute infection or persistent/chronic infection. This type of infection could result in tissue damage or pathology and it is different from direct virus attack on cells resulting in tissue damage or apoptosis. Thus there must be a balance between viral clearance and immunopathology (Fig 1).Several factors determine immunopathology which include age of the host, genetic makeup of the genes, route and /or site of infection, as well as whether the immune system is compromised and the strain or type and the size of the inoculums/dose.

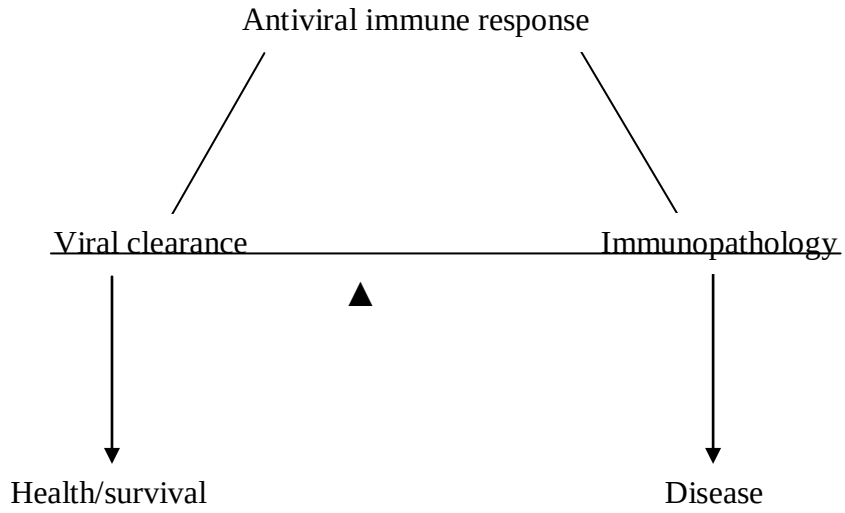

Fig.4.1: The antiviralimmune response is a balance between viral clearance and immunopathology. One direction leads to survival of the host, whereas the other can lead to disease.

3.3 Immunopathology of Parasites

Tissue damaging reactions occur when there is persistent infection of parasites e.g. *Plasmodium malariae, Schistosomajaponicum*. This is because the persistence of the parasite antigen in the blood leads to immunopathological disorders such as immune complex nephrotic syndrome, liver granuloma and autoimmune lesions of the heart. It is believed that cross reactions between parasite and self could result in autoimmunity and is the basis for the cardiomyopathy in Chagas disease. Generalised immunosupression increases susceptibility to bacterial and viral infections.

3.4 Diseases of the Blood

There are some immunological diseases known as cytopenia are blood diseases eg autoimmune neutropenia, immune thrombocytopenic purpurae.t.c. These are believed to be autoimmune diseases caused by the destruction of blood components by autoantibodies.

4.0 CONCLUSION

Understanding Immunopathology is very critical in the treatment of diseases and infections. Since the symptoms associated with infections are as a result of the combat effect of the Adaptive and Innate immune responses to the causative pathogens, assessment of immunopathological symptoms, through visual analysis of patients or laboratory test helps to determine the pace of therapy. Accurate analysis

of Immunopathology is sometimes complicated by chance events and environmental factors. In most cases, the goal of a treatment is to generate a tolerable immunopathology. In other words, reducing the symptoms associated with an infection to a less harmful level in the patient.

5.0 SUMMARY

In this unit, you have learnt that:

- the immune response to infections is carried out effectively by the combination of the Adaptive and Innate immune response system
- tissue damage to host due to immunological event could be as a result of hypersensitivity reaction to innococus materials, vigorous rejection to foreign grafts and the loss of self tolerance consequently leads to autoimmune disease
- sometimes, the immune response to these pathogens could be beneficial, or lead to symptoms in infected areas; inflammation or pain, or cause other symptoms not usually associated with the infection
- tissue damaging reactions occur when there is persistent infection of parasites.

6.0 TUTOR-MARKED ASSIGNMENT

i. What is the difference between immunopathology and auto-pathology?
ii. Categorise immunopathology into two broad groups based on reaction of the cells or tissues to antibody reaction with foreign material.
iii. Under what condition could the immune mechanism be disadvantageous to the host?

7.0 REFERENCES/FURTHER READING

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UNIT 5 AUTO-IMMUNOLOGY AND AUTO-PATHOLOGY

CONTENTS

1.0 INTRODUCTION

While Auto-immunology is the branch of immunology that deals with Autoimmunity, Auto-pathology is the pathology that describes the autoimmune system. The immune system of an individual is well equipped to protect it against invasion by harmful microorganisms, but sometimes the immune system fails to recognise its own constituent parts, thereby attacking its own cells and tissues. This process of allowing immune responses against itself is termed Autoimmunity. Autoimmunity also referred to as 'Self Immunity' is known to be present in everyone, and diseases resulting from it are termed autoimmune diseases.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- explain the possible causes of autoimmunity
- describe the pathology of autoimmunity.

3.0 MAIN CONTENT

3.1 Possible Causes of Autoimmunity

The adaptive immune responses are responsible for autoimmunity, and its actions are similar to the way it acts against foreign antigens. However, in the case of self antigens, it is almost impossible for the immune response mechanism to eliminate the antigen completely, thereby prolonging the response, which could be detrimental to the

affected tissues in most cases. There is no specific explanation to why the adaptive immune response attacks its own self; rather susceptibility to autoimmune diseases seems to be caused by a combination of factors; genetics, sex and the environment.

3.1.1 Genetic factors

Certain individuals that have a family or personal history of autoimmune diseases are likely to be susceptible to autoimmunity. This susceptibility is discovered to be associated with a combination or group of genes. Based on twin and family studies carried out for several autoimmune diseases e.g. insulin-dependent diabetes mellitus, genes related to immunoglobulins, T-cell receptors, and the major histocompatibility complexes (MHC) have been suspected to be mainly responsible for autoimmunity. However, the most consistent association for susceptibility to autoimmunity has been linked to the MHC genotype. This correlation of MHC genotype with autoimmune diseases is not surprising, because autoimmune responses is linked to the adaptive immune response mechanism which involves T cells, and the ability of T cells to respond to a particular antigen depends on MHC genotype. Various studies also carried out on affected patients suggested that siblings affected with the same autoimmune disease are far more likely than expected to share the same MHC type. However the MHC genotype alone does not determine susceptibility, in fact monozygotic twins are far more susceptible to autoimmunity than MHC- identical siblings, which is a confirmation to the fact that genetic factors besides MHC affect susceptibility. Genes linked to immunoglobulins and T-cell receptors, involved in antigen recognition, are inherently variable and susceptible to recombination. This attribute enables the immune system to respond to a wide variety of harmful pathogens, and as a result may give rise to lymphocytes capable of self- immunity.

3.1.2 Sex

It has been observed that the frequency of autoimmune diseases is higher in females than males, although studies also show that the autoimmune diseases present in males tend to be severe. However, the reasons for this anomaly are unclear, but there are few explanations to this wide range in frequency between the two sexes. Compared to males, females are shown to exhibit larger inflammatory responses, when their immune system is triggered, hence increasing the risk of autoimmunity. Hormonal levels also tend to play a role in autoimmunity, based on indications that autoimmune diseases fluctuate with hormonal changes during menstrual cycle or pregnancy. Some studies carried on autoimmune thyroiditis, suggests that the high frequency of the females to autoimmunity is as a result of the imbalanced X chromosome inactivation.

3.1.3 Environmental Factors

Various studies have shown that the expression of a disease could be influenced by environmental factors. Since the presence of an autoantibody requires an autoantigen to cause autoimmunity, certain factors have been observed to inhibit or affect the binding of the autoantibody to the autoantigen, thereby modulating the expression of these diseases among different individuals. For example, in the Goodpasture's disease, it was observed that cigarette smokers exclusively exhibited pulmonary haemorrhage while non cigarette smokers showed no sign of pulmonary haemorrhage. This is because cigarette smoke stimulates an inflammatory response in the lungs, which damages alveolar capillaries, as a result exposing autoantigen to autoantibody. Some studies also show the existence of an inverse relationship between infectious diseases and autoimmune diseases. It was observed that autoimmunity seems to prevail in areas where there are no infectious diseases, while autoimmune diseases are very rare in areas where infectious diseases are endemic. Various hypotheses have tried to explain this correlation, but the details of the mechanism are not fully known. Certain chemicals and drugs have also been implicated in the expression of autoimmunity e.g. in the drug- induced lupus erythematosus, withdrawal of the drug removes the symptoms automatically. Exposure to heavy metals such as mercury and lead, have been shown to induce autoantibodies, hence causing autoimmunity.

3.2 Pathology of Autoimmunity

The pathology and clinical expression of an autoimmune disease is determined by the antigens or group of antigens, together with the mechanism by which the antigen-bearing tissue is damaged. Antigens responsible for autoimmunity are known to be few in number (DNA, receptor), and several have been observed to play signaling roles in the immune response, while the mechanisms operative in the pathology of an autoimmune disease are numerous, and are mostly mediated by the actions of autoantibodies and T cells. Studies carried out on autoimmune haemolyticanaemia, showed that responses of immunoglobulin G (IgG) or immunoglobulin M (IgM) autoantibodies to autoantigens on cell surfaces of red blood cells triggered the destruction of red blood cells. It has also been observed that IgG or IgM may bind to cells in tissues for a prolong period of time, causing inflammatory damage or may bind to receptors on cell surfaces, stimulating receptor or blocking the binding of cell surface receptors to its natural ligand. Tissue damage can also be due to the binding of autoantibodies to autoantigens in the extracellular matrix or the binding of autoantibodies to soluble autoantigens. T cell responses have been directly implicated in causing tissue damage or indirectly by activation of macrophages. It was observed in the

autoimmune disease insulin-dependent diabetes mellitus, that specifically reactive T cells selectively destroyed the insulin-producing β cells of the pancreatic islets. Further studies on the immune response also showed that the requirement for the activation of the B cell by the T cell, in order for the B cell to produce antibodies maybe sometimes bypassed, hence causing the continual production of antibodies that may damage tissues. Molecular mimicry, an important mechanism discovered to be important in autoimmunity, involves the production of antibodies to an antigen that mimics a self antigen, thereby causing the binding of the antibodies to the self antigens. There are still more research going on the mechanisms associated with the pathology of autoimmunity, especially the roles of T cells.

4.0 CONCLUSION

In some studies, conclusion that various diseases had an autoimmune basis was as a result of the fact that autoantibodies were present in the serum of affected patients. However, further studies revealed that similar autoantibodies were present in the serum of patients, if the tissue damage was caused by trauma or infection. Thus, suggesting that autoantibodies may be as a result of tissue damage, rather than be the cause of it. Research is still going on to further understand autoimmunity and the mechanism of pathology involved, since a disease cannot be classified as autoimmune unless the autoantibody isolated is proven to be pathogenic.

5.0 SUMMARY

In this unit, you have learnt that:

- a disease can only be classified as autoimmune, if it is shown clearly that the response of the adaptive immune system to the autoantigen causes the pathology observed
- various factors such as genetic, sex and environmental are known to modulate autoimmunity
- the pathology of autoimmunity is determined by the antigens and mechanism of the tissue damage, and autoimmune diseases are observed to be mediated mainly by auto-antibodies and T cells
- the MHC genotype of individuals have been linked to the susceptibility of individuals to autoimmunity

6.0 TUTOR-MARKED ASSIGNMENT

i. List the factors that influence autoimmunity
ii. Discuss the pathology of autoimmunity

7.0 REFERENCES/FURTHER READING

Smith, G., Hall, L**.** & Walker. M. (2002). *Handbook of Toxicologic Pathology*. (2nd ed.). *Volume 1.* Academic Press. *p156*

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MODULE 3

UNIT 1 TISSUE AND TRANSPLANTATION IMMUNOLOGY

CONTENTS

1.0 INTRODUCTION

Transplantation can be defined as the process or act of transferring cells, tissues or organs from one site to another. Tissue transplantation as a form of medical therapy has grown to be very important and preferred form of treatment in a range of life threatening clinical conditions such as liver, kidney or heart failures. However, the major obstacle to the use of tissue transplantation as a routine medical treatment is the adaptive immune response. Blood transfusion, which is the earliest and commonest form of tissue transplantation involves only four major ABO blood types and two Rhesus blood types, hence matching between individuals to avoid destruction by antibodies is relatively easy. When tissues containing nucleated cells are transferred from one individual to another, the responses of T cells to the Major Histocompatibility Complex molecules, triggers an elaborate response against the grafted

organ, causing destruction. For a successful tissue transplant to take place there must be a perfect match of the MHC molecules of the donor with that of the recipient, which is only possible amongst related individuals. In spite of that, genetic differences at other loci can still trigger tissue rejection.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- discuss the immune responses to tissue grafts
- explain transplant tolerance
- describe immunosuppressive therapy.

3.0 MAIN CONTENT

3.1 Immune Responses to Tissue Grafts

The extent to which the immune system responds to a tissue graft will depend on the level of genetic differences between the graft and the host. A number of terms have been used to classify a graft, according to its origin and possible outcome in the host.

- **Autograft**: Transplantation of tissues or organs from the same individual e.g. skin. Transplantation is always 100% successful, since they do not elicit rejection.

- **Syngeneic graft**: Transplantation of tissues or organs between genetically identical animals or individuals. Similar to Autograft, Syngeneic graft undergoes no rejection.

- **Xenograft**: Transplantation of tissues or organs from one species to another. This elicits the maximum immune response.

- **Allograft**: Transplantation of tissues or organs between genetically unrelated individuals. This is the most common form of transplantation, and the graft is initially accepted but it is then rejected after some days. The degree of rejection is dependent on the level of disparity in the MHC molecules between the donor and the recipient. MHC molecules present endogenous and exogenous peptides to T lymphocytes, which decide whether the peptide-MHC complex is a potentially threatening antigen, thereby triggering an immune response. In humans, the MHC is known as the human leukocyte antigen (HLA), located on the short arm of chromosome 6, near the complement genes. HLA matching has been shown to improve the success rate of tissue or organ

transplant, but in itself does not prevent the risk of rejection. This is due to the imprecise nature of HLA matching, as unrelated individuals who possess identical HLA type with antibodies against MHC proteins rarely have similar MHC genotype. However, this is not the case with HLA identical siblings because the siblings inherit their MHC gene as a haplotype; that is one sibling in four is expected to be HLA identical. In spite of this, graft rejection is still possible although less rapid. The minor H antigens also known as minor histocompatibility antigens are responsible for the rejection of HLA identical grafts between donor and recipient. Two mechanisms are involved in the recognition of alloantigens in grafted organs, the direct recognition; whereby T cells bind directly to donor MHC-peptide complex, causing graft rejection, and the Indirect Recognition; is similar to the process whereby T cells become activated in response to a pathogen. In contrast to direct recognition pathway, T cells bind to allogeneic peptides presented by the recipient antigen presenting cells and not MHC molecules expressed on the donor graft itself. The contribution of direct and indirect recognition pathway to the graft rejection response is still unknown. Rejection of transplant is divided into stages, Hyperacute rejection; this is the most aggressive form of graft rejection, as it occurs within minutes or hours. This stage of rejection is mediated by antibodies that are thought to be induced by prior blood transfusion, prior transplants, xenografts or even multiple pregnancies. Damage to the transplant is as a result of these antibodies binding to the target antigens in the graft, activating the complement and blood clotting cascades and blocking the vessels of the graft, which leads to the immediate death of the transplant. Kidney are observed to be more susceptible to hyperacute rejection, because preservation time is longer when compared to the heart and liver, due to pretransplant cross match test often required in kidney transplant. Acute rejection; direct recognition is thought to be responsible for acute rejection. This is as a result of the immune system recognising new foreign antigens, and is mediated either by antibodies or T lymphocytes. Acute rejection usually occurs within the first few weeks after transplantation and may be triggered at a much later date mostly by infection. Chronic rejection; this is the major form of graft rejection, and it takes place months to years after transplant. This is because tissue typing methods and immunosuppressive drugs have assisted in the survival of allografts within the first year of transplant. However, chronic rejection is yet to benefit from these recent technological advancements. Chronic rejection is mostly likely caused by antigraft immune response, as supported by the fact that previous episodes of acute rejection determine the degree of chronic rejection. It is also associated with the level of HLA

mismatch between donor and recipient, and is characterised by gradual decline in transplant functions. Antibodies are observed to play a major role in chronic rejection, although studies have also implicated T lymphocytes.

The response of the immune system to transplants also varies with the type or source of grafts. Some parts of the body are deemed to be immunologically privileged sites, since they rarely evoke any immune response e.g. eyes, the brain and the testis. Similarly, the developing foetus, which expresses both maternal MHC antigen and the foreign paternal MHC antigen, is protected from the mother's immune system. Transplants at this site relatively require no tissue matching and they are useful sites for the study of experimental transplantations and immunological tolerance mechanisms.

3.2 Transplant Tolerance

Studies have shown that even though tissue or organ rejection cannot be completely prevented, immunological tolerance can be achieved. In a group of liver transplant patients, it was observed that stopping the use of immunosuppressive therapy did not result to rejection of the transplant. Several mechanisms have been postulated to explain transplant tolerance; clonal deletion, suppression and anergy. Clonal deletion involves the removal of all T cells that can aggressively respond to self MHC molecules. In suppression, T regulatory cells which are known to maintain the state of immunological unresponsiveness in individuals have been observed to prevent allograft rejection when infused into transplanted mice. Allograft tolerance can also be achieved by altering the balance of local and circulating cytokines in favour of those that maintain tolerance, through gene therapy and antibody treatment. Anergy also known as unresponsiveness, results in transplant tolerance when T cells are not completely activated, hence causing the generation of T regulatory cells. Despite the reduction in drug treatment for maintenance therapy after transplant, due to the use of modern immunosuppressive induction techniques, immunological or transplant tolerance is still a field that requires intense research as most patients are yet to benefit from these techniques.

3.3 Immunosuppressive Therapy

There are various immunosuppressive agents utilised in the reduction of transplant rejection, and they are grouped according to their mode of actions.

3.3.1 CalcineurinBlockers (Ciclosporin and Tacrolimus)

Calcineurin is a protein phosphatase that helps to activate transcription factors necessary for the initiation of transcription of a number of genes. Ciclosporin and tacrolimus are calcineurin inhibitors that bind to immunophilins within the lymphocyte, as a result suppressing activation of T lymphocytes through the inhibition of cytokines. Although tacrolimus have similar side effects with ciclosporin, such as nephrotoxicity, hypertension, and diabetes, its use is associated with a limited number of acute rejection episodes compared to ciclosporin. Ciclosporin is secreted by the fungus *Tolyplocadiuminflatum,* while tacrolimus is an antibiotic derived from the soil fungus *Streptomycestsukubaensis.*

3.3.2 AntiproliferativeAgents (Azathioprine and MycophenolateMofetil)

Azathioprine and mycophenolatemofetil are the most commonly used in this category, and they act by inhibiting DNA replication through the limitation of the availability of purines, thereby suppressing the proliferation of T and B lymphocytes. Both have similar side effects, which is nausea and diarrhea. They are usually used in conjunction with calcineurin blockers, since their normal dose ranges results in suppression of the bone marrow.

3.3.3 Corticosteroids

Corticosteroids are thought to be the cornerstone of immunosuppression, because they have a wide range of immunosuppressive activities. Corticosteroids are mostly used for autoimmune diseases and inflammatory conditions. The numerous side effects associated with corticosteroids have caused many to deviate from its use. However, they are still very imperative in the treatment of acute rejection episodes. Mostly commonly used corticosteroids in transplantation are oral prednisolone and intravenous methylprednisolone.

3.3.4 m-TOR Inhibitors (Sirolimus and Everolimus)

Sirolimus and its derivatives are similar to tacrolimus in structure, and also bind to immunophilins but they do not inhibit calcineurin but m-TOR. m-TOR, which is the mammalian target for rapamycin, regulates gene translation and protein synthesis. The binding of sirolimus to m-TOR inhibits lymphocyte activation. Due to its numerous side effects, especially delayed wound healing and delayed graft function, the use of sirolimus is often delayed until several weeks after transplantation. Sirolimus is derived from the soil bacterium *Streptomyceshygroscopicus.*

3.3.5 Antibodies

Approval of antibodies in the therapeutic use in transplantation is simply based on the rationale that removal of the target cell will diminish an immune response against an allograft. Antibodies react with lymphocyte surface antigens depleting lymphocytes and interfering with immune responses. Common adverse effects associated with the use of antibodies are fevers, chills and headaches.

Apart from the groups of immunosuppressive agents highlighted above, several agents are still being developed to help counter acute rejection episodes and maintain transplant tolerance.

4.0 CONCLUSION

Over the years, tremendous progress has been made in tissue or organ transplantation, especially in the areas of abating transplant rejections, treatment of acute rejection episodes, and tissue transplant tolerance. However, much work still needs to be done in areas of understanding the contribution of allograft recognition pathways to the immune response, and also elimination or further reduction of the adverse effects caused by the use of the various immunosuppressive agents.

5.0 SUMMARY

In this unit, you have learnt that:

- based on the genetic differences between the graft and the host, tissue transplants are divided into sygeneic grafts, autografts, xenografts and allografts
- allografts are the major forms of transplants, and rejection is often as a result of differences in MHC molecules between the donor and the recipient
- some sites on the body are known to exhibit little or no immune responses and are termed immunological privileged sites and these sites are useful in the study of transplantation mechanisms and transplant tolerance
- tolerance of grafts by the host is attributed to three mechanisms; suppression, clonal deletion and anergy
- various immunosuppressive agents are utilised in transplantation which are very useful in the treatment of acute rejection episodes in tissue or organ transplant, they also help to reduce graft rejection.

6.0 TUTOR-MARKED ASSIGNMENT

i. Discuss the immune responses to tissue grafts.
ii. Discuss the process of transplant tolerance.
iii. List and discuss the various immunosuppressive agents utilised in transplant rejection.

7.0 REFERENCES/FURTHER READING

Smith, G., Hall, L**.** & Walker. M. (2002). *Handbook of Toxicologic Pathology*. (2nd ed.). *Volume 1.* Academic Press. p156

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UNIT 2 IMMUNO-PROPHYLAXIS

CONTENTS

1.0 INTRODUCTION

Immunoprophylaxis is a branch of immunology that deals with the prevention of infectious diseases, through the administration of immunological preparations, such as vaccines, gamma globulins and hyperimmunesera, so as to create immunity. Immunoprophylaxis has helped in the eradication of many diseases such as smallpox, tetanus and poliomyelitis in some parts of the world, and its study is considered to be important in the prevention and further eradication of infectious and parasitic diseases.

2.0 OBJECTIVES

At the end of this unit, you should be able to;

- discuss the two major types of immunisation
- elaborate on the roles of vaccines in Immunoprophylaxis.

3.0 MAIN CONTENT

3.1 Types of Immunisation

Immunisation is simply the process of rendering an individual immune against infectious diseases. Immunisation can be passive or active, depending on the source of immune response.

3.1.1 Passive Immunisation

This involves the administration of sensitised lymphoid cells or serum from immune individuals to non-immune individuals, to create immunity. In passive immunity, recipients do not produce antibodies to the infectious disease; rather the immune response is acquired. Passive immunity can also be natural e.g. the transfer of antibodies of a mother to the foetus, and recently the cure of HIV through bone marrow transplant from immune donors. In most cases, especially in the artificial form of passive immunity, the conferred immunity is short-lived, usually 4-6 weeks. Examples of artificial passive immunisations are the injection of serum for the treatment of tetanus or diphtheria, and the administration of gamma globulin to hypogammaglobulin children.

3.1.2 Active Immunisation

This is the treatment that provides immunity to an individual, through the administration of a specific antigen, hence stimulating the recipient's immune system to produce antibodies against the organism. Immunity from this treatment develops slowly, but it's long lasting, sometimes over the course of the entire life of the recipient. Active immunisation can also be natural, through previous exposure to live pathogen. Active immunisation is also referred to as inoculation or vaccination, since the artificial form of active immunisation is through the administration of vaccines.

3.2 Vaccine

Vaccine is an immunological preparation that provides immunity to a specific disease. Vaccines are made to resemble a pathogen or disease causing microorganism, hence stimulating the immune system to initiate a response against it. As a result, the immune system remembers 'it' and provides an attack towards 'it' or any similar pathogen the immune system encounters in future. In as much as the immune system generates a response against the vaccine, the response does not result in symptoms associated with the disease. Vaccines are mostly made from weakened or killed form of microbe or its toxins, and they can be therapeutic or prophylactic. Vaccines help to prevent diseases such as cholera,

diphtheria, rabies, poliomyelitis, tetanus and measles. There are different types of vaccines, based on the strategy to try to reduce risks while retaining the ability to generate a beneficial immune response.

3.2.1 Dead/Killed

These are vaccines that are made from the destruction of previously virulent microbes. The microorganisms are destroyed either through heat (60°C), radiation, chemicals (formalin or phenol) or antibiotics. Killed vaccines most times do not provide long lasting immunity, they do not stimulate cytotoxic T cell response, they are safe and can be given to pregnant women, and they are stable to heat. Examples are influenza vaccine, polio vaccine, cholera vaccine and rabies vaccine.

3.2.2 Live/Attenuated

These are vaccines made from living microorganisms that have lost their virulent properties, but are still able to generate an immune response when administered to a recipient. Many of the microorganisms used are viruses, while some are bacteria and they are known to provoke a durable immune response, hence making them more preferable for healthy adults to other forms of vaccines. Live vaccines are not stable to heat and are not safe for pregnant women. Examples are vaccines developed against the viral diseases; measles, rubella and yellow fever, and the bacterial disease; typhoid fever.

3.2.3 Toxoid

These vaccines are made from the detoxification of bacteria toxins, rather than the bacteria themselves. The toxins from bacteria are treated with formalin to destroy toxicity and retain antigenic properties. Vaccines made from toxins are known to be efficient, but not all Toxoid vaccines are made from microorganisms. Examples are vaccines for tetanus and diphtheria.

3.2.4 Subunit

These are vaccines made from part or fragment of a microorganism. Examples are the vaccine against Hepatitis B virus, which is composed only of cell surface proteins of the virus, the vaccine against Human papillomavirus, composed mainly of the major capsid proteins of the virus.

3.2.5 Conjugate

These are vaccines created by linking the polysaccharide outer coat of bacteria with a protein or toxin, so as to enable recognition of the bacteria by the immune response team. Since the outer polysaccharide coat on its own generates little or no immune response. An example is *Haemophilusinfluenzae* type B virus vaccine.

3.2.6 Experimental

These are vaccines created from innovations, such as DNA recombination technology. They are also known as synthetic vaccines, and they are very easy to produce and store. Experimental or synthetic vaccines are composed mainly of carbohydrates, antigens or synthetic proteins.

3.2.7 Valence

Sometimes vaccines are designed to provide immunity to more than one microorganism or more than one strain of a microorganism, and such are termed polyvalent vaccines. While those that provide immunity to just one microorganism is termed monovalent vaccine.

In spite of the numerous types of vaccines, and the success achieved so far in the eradication of infectious diseases such as small pox through the administration of vaccines, vaccination does not guarantee protection from diseases. The immune response triggered by the administration of vaccines, can be affected by various factors such as the use of steroid by the recipient, age of the recipient or inability of the recipient immune system to trigger production of antibodies to the specific antigen. It has been observed that the more vaccines administered to an individual, the more the likelihood of that individual being affected by the adverse effects of the vaccine, which is rarely more harmful than the intended disease the vaccine, was meant to protect against. Nevertheless, the administration of vaccines is known to reduce the risk of individuals to specific diseases.

4.0 CONCLUSION

Conferment of immunity through passive or active immunisation helps to prevent and reduce the spread of infectious diseases. However, the immune response to active immunisation depends partly on the recipient immune system, which may not produce antibodies to the given immunological preparation. As a result, immunisation may produce different observable result in genetically different individuals; providing immunity against a disease in some, while exposing others to the same

disease. There are various economic, social and ethnic challenges associated with vaccination, therefore the adverse effects of vaccine administration is still a subject of much debate.

5.0 SUMMARY

In this unit, you have learnt that:

- immunoprophylaxis is the prevention of infectious diseases through immunisation by the administration of vaccines and hyperimmune sera
- immunisation could either be active (administration of vaccines) or passive (administration of hyperimmune sera
- in passive immunisation, immune response is acquired and short-lived while active immunisation stimulates the recipient immune response to produce antibodies against the administered antigen and similar pathogens in future, hence conferring a long lasting immunity to the recipient
- Vaccines are important in the prevention of diseases.

6.0 TUTOR-MARKED ASSIGNMENT

i. Define immunoprophylaxis.
ii. List and discuss the two major types of immunisation.
iii. List the different types of vaccines.

7.0 REFERENCES/FURTHER READING

Smith, G., Hall, L**.** & Walker. M. (2002). *Handbook of Toxicologic Pathology*. (2nd ed.). *Volume 1.* Academic Press. p156

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UNIT 3 MODERN TECHNIQUES IN IMMUNOLOGY AND IMMUNOCHEMISTRY

CONTENTS

1.0 INTRODUCTION

The development of new techniques in the field of immunology and immunochemistry has led to the detailed and critical understanding of the immune system and their response to pathogens. Many years back, scientists' comprehension of the immune response system was confined to visual examination of living cells of blood samples through the microscope. Now it is possible to observe whole organisms, cells, tissues and organs and view the response of the immune system to different pathogenic diseases. Through present day immunological techniques, the mechanisms of the immune response system have been made easier to understand and various approaches are being made through this knowledge to prevent and control the spread of many diseases, as well as their treatment.

2.0 OBJECTIVE

At the end of this unit, you should be able to

- discuss the recent techniques in immunology and immunochemistry.

3.0 MAIN CONTENT

3.1 Recent Techniques in Immunology

The reliance of scientists on new and improved tools and techniques to aid their proper understanding of the immune system cannot be overstated, and in fact it is attributed to be the main cause for the advancements in technology. **Cell based assays** have been improved

tremendously to be able to measure multiple cytokines level simultaneously, and also cytotoxic T cells against infected targets in humans. This improvement enables researchers to know if the T cells recognize the epitopes to activate them. The development of **tetramer technology**, which consists of four HLA molecules of the same kind bound to a peptide, has helped to provide reagents necessary to identify specific T cells. Hence, no need for the use of antigen stimulation to detect antigen specific T cells. **Monoclonal and polyclonal antibodies** are also being improved to enhance the detection of antibodies, especially in combination with western blotting. Culture systems needed to generate large quantities of specific antibodies are also being improved. This advancement has made it easier for researchers to get specific polyclonal antibodies, since they are cheap and come in large amounts; hence there is no need to raise antibodies. However, creation of monoclonal antibodies is a more complex process, but once completed, there is an unlimited generation of specific antibodies needed for research. **Separation techniques** needed to separate a required cell from a mixture have also benefited from these advancements. Antibodies can be attached to a chromatography column and then used to bind a cell carrying the antigen recognised by the specific antibody. Another separation technique, known as magnetic attraction, involves bonding antibodies specific for a particular cell target to magnetic particles. Flow cytometers which is a form of cell separation techniques, involves the tagging of antibodies with fluorescent labels, which are then identified and sorted out with the aid of a flow cytometer. This technique helps to eliminate several separate runs needed to measure more than one parameter, since it's a parametric method. **ELISA**, which is a common technique for immunological research, and used in detection of antibody-antigen complex, is being modified such that researchers can now obtain antibodies conjugated to a variety of labels, unlike the past when a researcher would have to undertake a complicated task of having to conjugate their own antibodies and label.

3.2 Recent Techniques in Immunochemistry

Immunochemistry is a branch of chemistry that studies the reactions and components of the immune system, hence any advancements in immunology definitely impacts immunochemistry. Various techniques in immunochemistry are being refined, developed and used in scientific study. Similar to immunology, **immunoassays** are being modified to help quantify specific substances through the formation of antigen-antibody complex. The antibody is labeled with enzyme, radioisotope or fluorescent dye to enable detection and separation. Another technique is **western blotting**, also known as **immunoblotting**, which helps to analyse soluble antigens in a mixture, through the resolution of gel

electrophoresis. Immunochemistry is also studied from the aspect of using antibodies to label epitopes of interest in cells and tissues. The use of enzyme labels instead of dyes opened the door to technological advancements in immunochemistry. The development of **EPOS (Enhanced polymer one step)** system, enables the entire immunochemical staining procedure, from primary antibody to enzyme to be accomplished within a single step, compared to the widely use of streptavidin-biotin method, which is complex and associated with frequent background staining. **Rolling circle amplification**, a signal amplification system, that generates a local signal via extension and amplification of an oligonucleotide tail, was developed for the detection of nucleic acid. However, its application in immunochemistry is very useful in the detection of a variety of cell surface and intracellular molecules.

The various techniques highlighted are not without their weaknesses, and as a result much study is still being carried out to tackle these differences and also to delve into more complicated areas, that are yet to achieve any significant improvements. The use of vaccines to counter the effect of HIV is of much interest to the scientific society, especially since the human genome project has been successfully completed. Also of importance in immunology is the identification of the epitopes that are recognised by the T cells, the portions of specified proteins bound by HLA molecules presented to the T cells and ultimately recognised by the lymphocytes. In fact, the identification, optimisation and analysis of epitopes are areas of intense study in immunology.

4.0 CONCLUSION

Advancements in immunology and immunochemistry techniques serves as the backbone of success recorded by researchers in the areas of disease prevention, understanding the mechanisms of pathogen infections and also in the study of immune system response to microorganisms. However, as new technologies are being unveiled, it is expected that areas yet to be covered or understood by scientists will become clearer.

5.0 SUMMARY

In this unit, you have learnt that:

aspects of immunology and immunochemistry such as detection, isolation and analysis of antibodies, improvements in immunoassays and cultures have benefited immensely from modern techniques.

6.0 TUTOR-MARKED ASSIGNMENT

Briefly discuss the recent techniques in immunology and immunochemistry.

7.0 REFERENCES/FURTHER READING

Smith, G., Hall, L. & Walker. M. (2002). *Handbook of Toxicologic Pathology*. (2nd ed.). *Volume 1.* Academic Press. p156

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UNIT 4 HISTORY OF CHEMOTHERAPY

CONTENTS

1.0 INTRODUCTION

Chemotherapy is simply the treatment of diseases with chemicals, but it is commonly referred to as cancer treatment. The use of antibiotics in treatment of bacteria is referred to as antibacterial chemotherapy, thus the first chemotherapeutic agent used is arsephenamine used to treat syphilis. It was discovered in 1909. Other forms of chemotherapy are the treatment of autoimmune diseases and transplant suppressions.

2.0 OBJECTIVE

At the end of this unit, you should be able to:

- highlight the history of chemotherapy.

3.0 MAIN CONTENT

3.1 History of Chemotherapy

Paul Ehrlich considered to be the father of immunology and chemotherapy is reported to have laid down the principles on which chemotherapy is based. The first use of drugs to treat cancer was in the early 20^{th} century, when mustard gas was used as a chemical warfare agent in World War 1. It was observed that individuals exposed to mustard gas had low white blood cell count, and similar compound nitrogen mustard was studied during World War II. Based on the hematopoeisis properties of mustard gas, in December 1942 several patients with lymphomas were given intravenous injection of mustard gas, and there was a temporary remarkable improvement in the illness. It was also observed during a military operation in World War II, survivors of the hundreds of people exposed to mustard gas after an air raid by Germany on the Italian harbor of Bari, had low white blood cell counts. This incidence led scientists to start thinking of other substances

that would be of help in the treatment of cancer, and the first drug produced from this line of research is known as mustine. Also after World War II, folic acid was also discovered to be effective against the treatment of leukaemia in children, by blocking certain chemical agents such as aminopterin and amethopterin. In 1965, POMP regimen, which is a combination of different drugs to fight against cancer, was discovered. Through this combination, long term remission of leukaemia was made possible in children. This discovery is suggested to be responsible for the improvements made so far in the field of chemotherapy and nearly all successful chemotherapies are known to use this combination approach. Recently is the discovery of adjuvant therapy, whereby chemotherapy is applied to tumor at a very early stage or the application of chemotherapy to remaining cancer cells after the removal of tumor by surgery. It was discovered that high doses of chemotherapy after surgery prevented the cancer cells from returning. Another chemical agent 5- fluorouracil, a DNA inhibitor was discovered to improve the rate of colon cancer when combined with surgery. A lot of chemicals were tested during the 1970s, to fight against cancer and even till present moment, drugs used to treat malaria, plant extracts have all been used in the treatment of cancer, and improvements have been recorded in certain places and areas. Nevertheless the use of chemotherapy in the fight against cancer is losing grounds based on the couple of side effects attached to it. Most of the chemical agents used are poisons and are known to suppress bone marrow function, hence exposing the patients to risk of infections.

4.0 CONCLUSION

Chemotherapy is many decades old, and a lot of improvements and achievements have been recorded within this time frame, especially in areas of drug improvements to help reduce side effects of chemotherapy. However, there is still a lot to be done in this field as the cure for cancer is yet to be discovered, even though it has been known to be suppressed by the use of a couple of chemical agents. The history of chemotherapy shows that researchers are drawing closer to the cure of cancer.

5.0 SUMMARY

In this unit, you have learnt that:

- the first use of chemotherapy was by coincidence, through the discovery of mustard gas as a hematopoietic agent
- achievements have been recorded in areas of cancer growth suppression through the use of drugs like folic acid, but a lot is still required to be done to reduce the adverse effects associated with chemotherapy as well to eradicate cancer.

6.0 TUTOR-MARKED ASSIGNMENT

Briefly discuss the history of chemotherapy.

7.0 REFERENCES/FURTHER READING

Smith, G., Hall, L**.** & Walker. M. (2002). *Handbook of Toxicologic Pathology*. (2nd ed.). *Volume 1.* Academic Press. p156

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UNIT 5 PRINCIPLES OF CHEMOTHERAPY

CONTENTS

1.0 INTRODUCTION

Cancer is uncontrolled growth of cells coupled with invasion, and is believed to be caused by genetic and environmental factors. The successful use of chemotherapy in treatment of cancer is based on the mode of action of the chemical agents used, and the genetic constituents of the patients.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- explain the cell division process in relation to cancer growth
- describe the principles of chemotherapy.

3.0 MAIN CONTENT

3.1 Process of Cell Division

Numerous studies have shown that kinetics of cell growth determines tumor growth characteristics, and also partly explains the biological behavior and heterogeneity of tumors. In cell cycle, mitosis results in three subpopulations of daughter cells; non-dividing and terminally differentiated cells, continually proliferating cells and stem cells, which are resting cells and may be recruited in the cell cycle. These cells are also observed in tumors. There are four distinct phases in the cell cycle division process, and they are the **G-phase**, where preliminary synthetic process occurs that prepare the cells for the DNA synthetic phase, known as the **S-phase**. In the S-phase, specific protein signals regulate the cell cycle and allow replication of the genome where the DNA contents become tetraploid. **G2-phase** is another resting stage for cells, before heading on to mitotic phase, which is the **M-phase.** M-phase is

characterised with chromosome condensation and separation, as well as cell division into two daughter cells. Studies reveal that the variations in the growth of tumor or cancer reflect the proportion of actively dividing cells, the length of the cell cycle and the rate of cell loss. Tumors have been observed to have a sigmoid shaped growth curve, which suggests that tumors rate of growth increases at a faster rate when the size is small, and growth reduces as the size increases. This slow pace in the rate of growth of cancer cells at large sizes is as a result of the complex process that results from cell loss and tumor blood and oxygen supply. To increase the chances of cancer cure using chemotherapy, researchers have shown that chemical agents must be able to achieve a fractional cell kill in logarithmic fashion. It is from this concept that chemotherapy models are designed and fashioned.

3.2 Principles of Chemotherapy

Cells targeted by chemical agents in chemotherapy are cells that have the indefinite capacity to reproduce themselves, which are stem cells. Most chemical agents act by impairing mitosis or altering the delicate mechanisms of cell division, effectively targeting fast dividing cells, while ignoring slowly developing tumor cells. Some agents cause the tumor cells to undergo programmed cell death, known as apoptosis. Since most chemical agents target cell division process, tumors with high growth fraction such as aggressive lymphomas, acute myeloid leukaemia and Hodgkin's disease are more likely to be affected by chemotherapy as a larger part of the cells are undergoing cell division at any point in time. Indolent lymphomas tend to respond to chemotherapy at a very slow and modest pace. Tumors still at a young stage at affected more effectively by chemotherapy, because the mechanisms regulating cell growth are still very much preserved, since they are more differentiated. As tumor or cancer cells ages, they become less differentiated and growth becomes less regulated. Hence tumor cells response to chemical agents or chemotherapy is low, and at this stage surgery or radiation becomes more imperative than chemotherapy. Near the centre of some solid tumors, there is absolute cessation of cell division, and in most cases chemical agents do not have access to core of the tumor, so they are highly insensitive to chemotherapy. The targeting of rapidly dividing cells in the cell division cycle by chemical agents, also leads to destruction of cells responsible for hair growth and replacement of intestinal epithelium. These side effects have pressured researchers to find ways to identify malignant cells, so that they can be uniquely targeted without causing further damage to the patients. Imatinib, a monoclonal antibody drug was suggested to have been developed to target the Philadelphia chromosome, mostly seen in patients with specific forms of tumors. Efforts are on to identify much more features in order to develop efficient treatment modalities. Small

pumps have been identified on the surface of cancer cells that actively move chemotherapy from inside the cell to the outside. Also, medications to alter the effect of p-glycoprotein are being tested to enhance the efficacy of chemotherapy.

4.0 CONCLUSION

The principles on which chemical agents work to inhibit the growth of cancer cells are similar, and as a result, cancer cells have developed high resistance to chemotherapy treatments. Chemical agents are now being used in conjunction with other methods, such as radiation to effectively curb growth of tumor cells.

5.0 SUMMARY

In this unit, you have learnt that:

- understanding the process of cell division is very critical in chemotherapy treatments
- variations in the growth of cancer cells have been attributed to three different factors; proportion of actively dividing cells, length of the cell cycle and the rate of cell loss
- chemotherapeutic models are designed on the basis that, chemical agents must be able to kill tumor cells in logarithmic fashion
- chemical agents act by altering or impairing the cell division process, and as a result tumor cells undergoing rapid cell division are easily affected by chemotherapy.

6.0 TUTOR-MARKED ASSIGNMENT

i. Explain the process of cell division.
ii. Describe the principles involved in chemotherapy treatment.

7.0 REFERENCES/FURTHER READING

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MODULE 4

UNIT 1 CHEMOTHERAPEUTIC AGENTS: ANTIBACTERIAL, ANTIFUNGAL, ANTIVIRAL, ANTIPROTOZOAN, AND ANTI-HELMINTH

CONTENTS

1.0 INTRODUCTION

Antimicrobial chemotherapeutic agents are the oldest known form of chemotherapy, commonly referred to as antibiotics. Antibiotics refer to substances of biological origin, while chemotherapeutic agents are substances derived from chemical components. However, most new antibiotics are chemically modified or chemically synthesised biological products, so the unique differences have been lost over time. The common name for antibiotics and chemotherapeutic agents is antimicrobial agents. Typically, chemotherapeutic agents work in two different ways; they either kill the microbes or interfere with its growth. An effective chemotherapeutic agent is one that poisons the pathogens, with little side effects to the host. Antimicrobial chemotherapeutic agents come in different forms, and are differentiated by their source, mode of action, toxicity and spectrum of activity.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- discuss the various antimicrobial chemotherapeutic agents
- list the different types of antimicrobial chemotherapeutic agents.

3.0 MAIN CONTENT

3.1 Antibacterial Chemotherapeutic Agents

The known various antibacterial chemotherapeutic agents are derived directly or indirectly mostly from microbes and plants. Antibacterial chemotherapeutic agents can be bactericidal or bacteriostatic. A bactericidal effect occurs when a chemotherapeutic agent binds irreversibly to bacteria, while a bacteriostatic effect occurs when the chemotherapeutic agent poorly binds to the bacteria, hence binding is reversible. Bactericidal agents destroy the bacteria to which they bind, while bacteriostatic agents inhibit the growth of the bacteria to which they bind. In other words, bactericidal agents are more effective, while bacteriostatic agents permit the host defense mechanism to eliminate the bacteria. Regardless of the method of binding, antibacterial chemotherapeutic agents act against the bacteria in different ways.

3.1.1 Inhibition of Bacteria Cell Wall Synthesis

Inhibition alters bacteria growth because most bacteria require peptidoglycan cell wall to avoid osmotic lysis. **Bacitracin,** an antibiotic inhibits the second stage of cell wall synthesis in eubacteria. β lactam antibiotics such as **penicillin, cephalosporins, thienamycins** and **aztreonam**, inhibit the attachment of peptidoglycan to the bacteria cell wall. **Vancomycin** interrupts cell wall synthesis, and is useful in treatment of bacteria that have grown resistance to β lactam antibiotics.

3.1.2 Disruption of Cell Membrane Functions

Polymyxin B and **polymixin E** are known chemotherapeutic agents that disorganise the cell membranes of gram negative bacteria.

3.1.3 Protein Synthesis Inhibition

The ability of these chemotherapeutic agents to inhibit protein synthesis is based on the structural difference between bacteria ribosome and eukaryotic ribosome. Aminoglycosides such as **gentamicin** and **streptomycin** bind irreversibly to the 30S ribosome. While, **tetracycline** bind irreversibly to the 30S ribosome.

3.1.4 Nucleic Acid Synthesis Inhibition

Antibacterial chemotherapeutic agents that inhibit nucleic acid synthesis act at different levels. **Rifampin** inhibits bacteria RNA synthesis.

3.1.5 Antimetabolites

Bacteria cannot use preformed folic acid for growth; hence they have to synthesise their own. Chemotherapeutic agents such as **sulfonamides** inhibit the synthesis of folic acid.

3.2 Antifungal Chemotherapeutic Agents

Over time, many antifungal chemotherapeutic agents have been developed, but only a few are active. Some cannot be used in humans because they do not diffuse into tissues, are inactivated by induced enzymes, or are too toxic. Factors that affect the permeability of fungi to chemotherapeutic agents are; the structure of the fungal cell wall and the cytoplasmic membranes. As a result, most antifungal chemotherapeutic agents are developed to break the cell wall of fungi, by inhibiting the synthesis of chitin which is component of fungi cell wall, or inhibiting the synthesis of ergosterol which is a major component of the fungi cell membranes. The three major categories of antifungal chemotherapeutic agents are; azoles, polyenes and allylamine/ thiocarbamates.

- **Azoles**: This group of chemotherapeutic agents inhibits the synthesis of ergosterol and is active against dermatophytes group of fungi. Examples are **clotrimazole, ketoconazole, miconazole** and **tioconazole**.
- **Polyenes**: This group binds to fungal membrane sterols, causing it to leak, and as a result tiny molecules are lost. Examples are **nystatin**, and **amphotericin B.**
- **Allylamines**: This group inhibits fungal sterol synthesis, by obstructing a major part of ergosterol production pathway. Examples are **Terbinafine**, **butenafine, naftifine** and **amorolfine.**

Another important chemotherapeutic agent is **flucytosine**, which is very important in the treatment of yeast, by interfering with nucleic acid synthesis.

3.3 Antiviral Chemotherapeutic Agents

Most antiviral chemotherapeutic agents have been proven to have little or no use, as this virus they intend to eradicate uses the host cell metabolic reactions, thus these agents in the long run attack the cells they are developed to protect. However few have been proven to be of clinical useful;

- **Receptor Binding**: **AMD-3100** and **maraviroc** disrupts HIV- co receptor interactions by binding to the receptors.

- **Fusion of viral and host cell membrane**: **Enfuvirtide** is a chemotherapeutic agent also necessary in treatment of HIV infection, by blocking the conformational change of some of the peptides required in interaction.

- **Uncoating**: Some antiviral chemotherapeutic agents act by inhibiting uncoating of the virus. Examples are **arildone** and **pleconaril.**Other very important antiviral chemotherapeutic agents are **Amantadine** and its derivative **rimantadine**. They act by blocking the protein that functions as ion channels in the disease influenza. **Acyclovir** inhibits the herpes DNA polymerase in herpes simplex virus infections.

- **Antiprotozoan chemotherapeutic agents**: There has been little progress made in the search for new chemotherapeutic drugs to combat protozoan. However due to the number of deaths caused by AIDS, it has become urgent to develop drugs with novel characteristics, since most AIDS patients die as a result of other opportunistic diseases which are majorly protozoan in origin. Some examples of antiprotozoan chemotherapeutic agents are **chloroquine** against malaria, **metronidazole** and **iodoquinol** against amebiasis.

- **ntihelminth chemotherapeutic agents**: These are agents used to treat infections with parasitic worms. Despite the high prevalence of parasitic worms, the rate of drug discovery or therapy is low, because most countries affected by these infections have little or no money to fund research in these areas. As a result, the number of antihelminth chemotherapeutic agents available for human treatment is very small and most originates from veterinary medicine. It was also observed that the success associated with the development of the drug **ivermectin** over the last 20 years, further decreased the motivation in the search for antihelminth chemotherapeutic agents. Other antihelmint chemotherapeutic agents are **praziquantel,mebendazole** and **albendazole**.

Over time, microbes have developed resistant mechanisms against the chemotherapeutic agents, and as a result drug development is an ongoing research. Also research on drug discovery is going on to reduce the adverse effects of these agents on the host.

4.0 CONCLUSION

The various antimicrobial chemotherapeutic agents have played a major role over the years in controlling the spread of diseases. However some microbes such as viruses still require a great deal of study, in order to develop drugs with potent characteristics.

5.0 SUMMARY

In this unit, you have learnt that:

- antimicrobial chemotherapeutic agents are used to fight and cure most of the various diseases
- these agents are known to have detrimental effects on the host after use
- antimicrobial chemotherapeutic agents could either be antibacterial, antifungal, antiviral and so on.

6.0 TUTOR-MARKED ASSIGNMENT

List three antibacterial, antiviral, antiprotozoan, antifungal and antihelminth chemotherapeutic agents, and explain their mode of action.

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UNIT 2 MODES OF ACTION OF ANTIMICROBIALS

CONTENTS

1.0 INTRODUCTION

Different antimicrobial agents act by interfering with synthesis of biological molecules essential for life (nucleic acids), synthesis of microbial cell wall, folate synthesis, integrity of the plasma membrane and ribosomal function.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- discuss what antimicrobial agents are
- list the different types of antimicrobial agents
- discuss their modes of action.

3.0 MAIN CONTENT

3.1 Antibacterial Agents

- **Inhibitors of cell wall synthesis**: Cell wall synthesis inhibitors are bactericidal and cell wall synthesis can be inhibited by ß-lactams e.g. Penicillins and Cephalosporins, which inhibit peptidoglycan polymerization and transpeptidation reactionwith concomitant release of C-terminal D-alanine. They act as artificial substrate for D-alanyl-D-alanyltranspeptidases. Cycloserine and Vancomycin binds to precursors of the peptidoglycan layer in bacterial cell walls resulting in the inhibition of the synthesis of peptidoglycan. Cycloserine, resembles alanine and inhibits addition of alanine into peptide chain while Vancomycin combines with cell wall

substrates, inhibits transglycosidase enzyme and prevents peptidoglycan chain elongation. Ethambutol, Isoniazid and pyrazinamide inhibit mycobacterial cell wall. Ethambutol inhibits arabinosyl-transferase enzyme involved in addition of arabinose in arabino-galactan chains while Isoniazid and pyrazinamide inhibit enoylreductase, involved in fatty acid (mycolic acid) synthesis for mycobacterial cell wall by inhibiting fatty-acid synthase enzyme.

- **Inhibitors of protein synthesis**: Different steps in protein synthesis are susceptible to inhibition by different groups of antibiotics .Inhibitors may inhibit protein synthesis in prokaryotes, eukaryotes or both e.g. the aminoglycosides permanently bind to several sites at 30S and 50S subunits and freeze the 30S initiation complex (30S-mRNA-tRNA), so initiation can no longer occur.

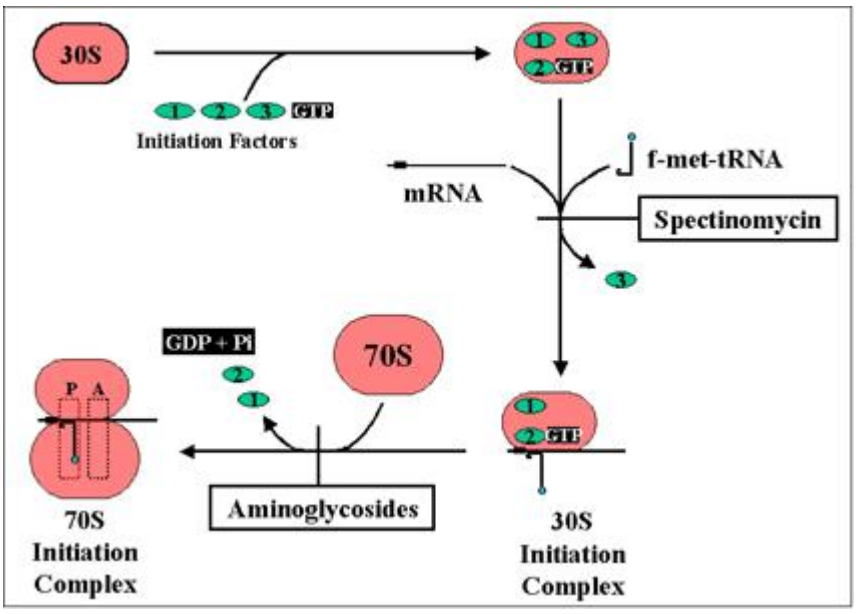

Fig. 2.1: Antibiotics that act at the level of protein synthesis initiation

They slow down protein synthesis that was already initiated and impair translational accuracy leading to the misreading of the mRNA sequence and/or premature termination of protein synthesis e.g. Streptomycin interferes with the formation of the 30S initiation complex. The irregular proteins produced as a result of misreading may be inserted into the cell membrane, changing permeability and enhancing the entry of aminoglycoside into bacterial cells. It induces binding of wrong t-RNA-AA complexes resulting in false proteins and also interferes with polysome formation.

Tetracyclines inhibit bacterial protein synthesis by preventing the association of aminoacyl-tRNA to the acceptor site on the mRNA-ribosome complex thereby blocking the A (aminoacyl) site of the 30S ribosome while chloramphenicol attaches to 50 S subunit of the 70 S ribosome and inhibits peptide synthetase or peptidyltransferase activities. It also prevents the binding of aminoacyl-tRNA to the active site of peptidyltransferase. Macrolides bind to 50 S subunit of the ribosome and interferes with translocation process by blocking peptide bond formation and peptidyltRNA translocation from A to P site leading to premature termination of the peptide chain. Tetracycline, macrolides and chloramphenicol are bacterostatic while aminoglycosides are bacteriocidal.Lincosamides prevent protein synthesis by binding to the 50 S subunit of bacterial ribosomes.

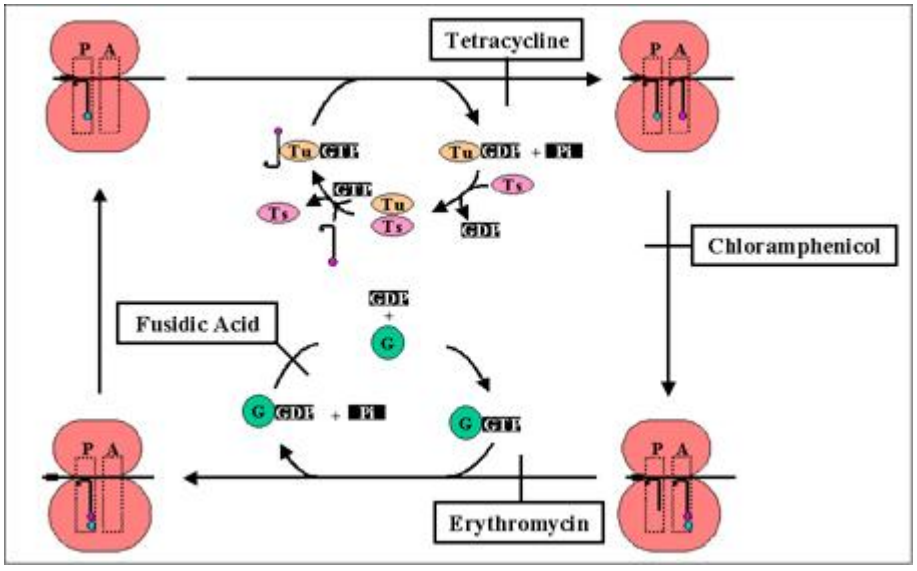

Fig.2. 2: Antibiotics that act at the level of protein synthesis elongation phase

Someantimicrobials interfere with elongation factors e.g. Fusidic acid binds to elongation factor G (EF-G) and inhibits release of EF-G from the EF-G/GDP complex.

- **Inhibitors of nucleic acid synthesis and cell division**: Rifampicin blocks RNA synthesis and functions by binding to DNA-dependent RNA polymerase thereby inhibiting the initiation of RNA synthesis while the quinolonesbind to the A subunit of DNA gyrase and inhibits bacterial DNA gyrase (topoisomerase II and IV). It crosses the outer membrane of gram negative bacteria via porins and prevents super coiling of DNA. This inhibits DNA

synthesis and consequently disrupting the spatial arrangement of DNA.

- **Inhibitors of other metabolic processes**: The sulphonamides are analogues of para-aminobenzoic acid and competitively inhibit the formation of dihydropteric acid by dihydropteroate synthase during which para-aminobenzoic acid is incorporated into the synthesis of folic acid while Trimethoprim binds to dihydrofolatereductase and inhibits the formation of tetrahydrofolic acid therebyinhibiting the synthesis of folic acid. Sulfonamides and trimethoprim block the synthesis of the folate needed for DNA replication. Isoniazid inhibits synthesis of mycolic acids. These are called antimetabolite antimicrobials. Polymyxinsbind with anionic lipopolysaccharide molecules by displacing calcium and magnesium from outer cell membrane of gram negative bacteria. This results in leakage of cell contents and cell lysis.

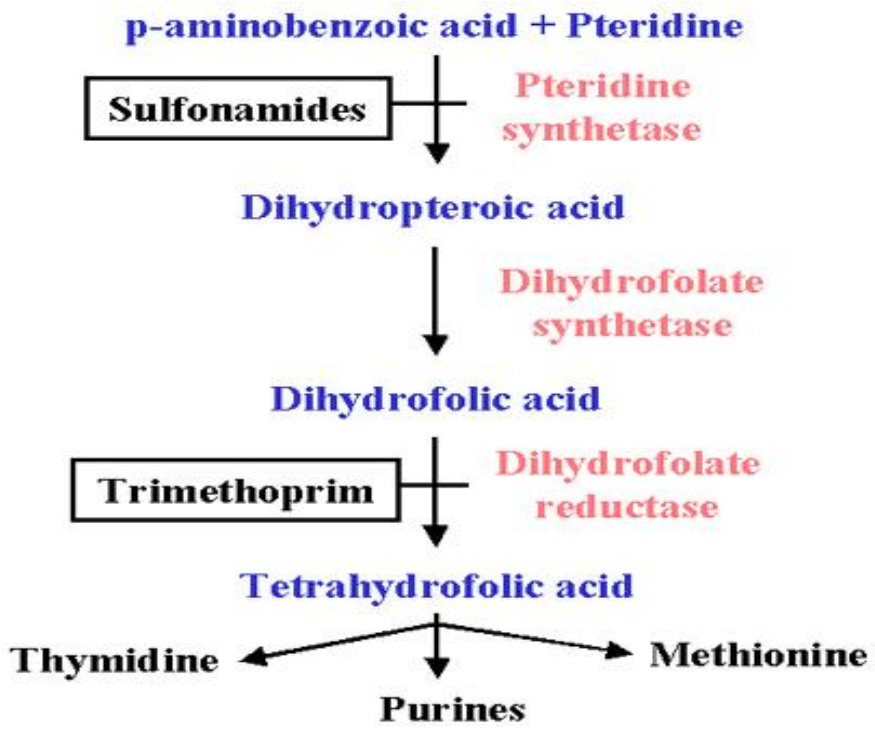

Fig.2. 3: Folic acid metabolism and points of inhibition by antimetabolites

3.2 Antifungal Agents

Different classes of antifungal agents target the plasma membrane, sterol biosynthesis, DNA biosynthesis and β glucan biosynthesis. Antifungals can be classified into three classes based on their mode of action.

- **Inhibitors of fungal cell membranes**: The antifungal activities of Azoles, polyenes, and allylamine/thiocarbamates, can be attributed to their ability to inhibit the synthesis of ergosterol or their directinteraction with ergosterol. Antifungal agents can inhibit synthesis of ergosterol or form ergosterol binding compounds. Azole antifungals such as miconazole, ketoconazole, and itraconazole target the heme protein. They block the synthesis of ergosterol by co-catalyzingcytochrome P-450-dependent 14 α - demethylation of lanosterol causing inhibition of C14 α - demethylase. This leads to depletion of ergosteroland accumulation of sterol precursors in the membranes of fungi exposed to imidazoles, including 14-methylatedsterols,resulting in the formation of a plasma membrane with altered structure (holes in the cell membrane)and function. Polyene antifungals physicochemically interact with plasma membrane sterols e.g. Amphotericin B binds to ergosterol in the cell membrane and increases the permeability of the membrane resulting in the production of aqueous pores. These pores consist of a ring-like structuremade up of eight amphotericin B molecules coupled hydrophobically to themembrane sterols leading to leakage of essential elements and loss of macromolecules then followed by cell lysis. Terbinafine selectively obstructs the biosynthesis of ergosterol thus synthesis of fatty acids and phospholipids are affected, most probably because of the abnormal intracellular buildup of squalene and decrease in erogsterol levels in cellular membranes and consequentlydisrupting the cellularstructure.

- **Inhibitors of DNA synthesis**: Flucytosine a cytosine analogue inhibits synthesis of the macromolecule thymidylate. It can act directly on fungal organisms by aggressively inhibiting uptake of nucleic acids and indirectly by intracellular metabolism to 5-fluorouracil. The antifungal activity of 5- Fluorouracil is exerted through its conversion into several active metabolites e.g. 5-fluorodeoxyuridine monophosphate (DNA synthesis inhibitor) and fluorouridine triphosphate (RNA synthesis inhibitor), whichinhibits protein synthesis by being falsely incorporated into fungal RNA or inhibits thymidylate synthase and interfers with the biosynthesis of fungal DNA. This leads to unbalanced cell growth and lysis. The sordarins selectively inhibit fungal protein synthesis which consequently impairs the function of fungal translation elongation factor 2 (EF2).

- **Inhibitors of fungal cell wall**: The echinocandins are cyclic hexapeptides linked to a long chain fatty acid e.g. Caspofungin. They are noncompetitive inhibitors of the biosynthesis of 1, 3- β-glucansynthetase and prevents more than 90 % of glucose

integration into glucan. This results in the disruption of fungal cell wall and subsequent death. Griseofulvin is concentrated by dermatophytes by an energy-dependent process. It interacts with and disrupts the mitotic spindle formation in dividing cells by interacting with the polymerized microtubules in susceptible fungi. This impedes the transport of material through the cytoplasm to the periphery resulting in inhibition of hyphal cell wall synthesis, followed by distortion, irregular swelling, and spiral curling of the hyphae cell wall.

Fig.2. 4: Mechanisms of Action of Antimicrobial Agents

3.3 Antiviral Agents

Antiviral agents act directly on viruses to prevent them from multiplying thus able to hinder a virus-specific function or interfere with a cellular function so that the virus cannot reproduce. They are grouped based on their target sites.

- **Fusion inhibitors**: Agents that inhibit penetration of host cell interfere with the receptor-mediated entry of the virus into a cell. They obstruct the binding, fusion and or penetration of HIV into a human cell e.g. Enfuvirtide mimics the structure of the heptad repeat 2 (HR2) region of the 41 kDa glycoprotein (gp41) that binds

to the heptad repeat 1 (HR1) region and facilitates the fusion of the viral envelope with the cell membrane. Binding of Enfuvirtide to the HR1 region prevents the HR2 region from access to HR1 resulting in inhibition of the fusion process. Amantadin is a tricyclic amine that inhibits penetration anduncoating, targeting matrix protein and haemagglutinin. It blocks cellular membrane ion channels and is effective against influenza A viruses.Pleconaril a small cyclic drug binds to a canyon pore of the picorna virus thereby blocking attachment and uncoating of the viral particle. It fits into a hydrophobic compartment in the nucleocapsid and disrupts the replication of the virus stopping the shedding of nucleocapsid proteins from the RNA.

- **Inhibitors of Nucleic acid synthesis**: Nucleoside and non-nucleoside reverse-transcriptase inhibitors are two classes of antiretroviral drugs that suppress HIV replication by affecting the action of reverse transcriptase.Nucleoside-Analog Reverse Transcriptase Inhibitors (NRTI) inhibit viral RNA-dependent DNA polymerase (reverse transcription) and are incorporated into viral DNA nucleoside chain to prevent ongoing viral DNA synthesis causing chain-termination e.g. Zidovudine. Zidovudine, an analogue of thymidine is phosphorylated by cellular enzymes to the triphosphate form. Thereafter it competes with equivalent cellular triphosphates which are essential substrates for the formation of proviral DNA by viral RNA dependent DNA polymerase. It terminates the chain when it gets inserted into the growing viral DNA strand. Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) are not incorporated into viral DNA but inhibit HIV replication directly by binding non-competitively to reverse transcriptase retarding its function. They attach to the reverse transcriptase and affect the activity of the enzyme by restricting its mobility and making it incapable to function. Delavirdine a non-nucleoside reverse transcriptase inhibitor (NNRTI) of HIV-1 binds directly to reverse transcriptase and obstructs RNA-dependent and DNA-dependent DNA polymerase activities. It does not compete with primer or deoxynucleoside triphosphates.

- **Protein processing inhibitors**: Protease Inhibitors are substrate analogues for the HIV aspartyl protease enzyme, involved in the processing of viral proteins. They bind to the HIV protease active site and block it from further activity thereby preventing the enzyme from cutting the viral protein molecules to their correct sizes. This hinders the viral maturation process leading to lack of functional virion formation. This protease does not occur in humans because it is a virus specific protease. The

viral particles cannot make copies capable of infecting other cells. Protease inhibitors inhibit viral production in cell that have been infected for a long time and newly infected cells compared to NRTIs and NNRTIs that are active against only newly infected cells e.g. Saquinavir. All Protease inhibitors inhibit the cytochrome P450 enzymes.

- **Thymidine kinase substrates:** Acyclovir (acycloguanosine) a chain terminator inhibits viral DNA polymerase. It enters the cell through the plasma membrane in form of a nucleoside and is then distinctively phosphorylated inside the cell by herpes virus thymidine kinase to an active form. It subsequently obstructs DNA synthesis by hindering polymerisation.

- **DNA integration:**Integrase inhibitors target the HIV enzyme responsible for the integration of viral genetic material into human DNA (integrase). Raltegravira 1-N-alkyl-5-hydroxypyrimidinone inhibits the insertion of HIV-1 viral genomic DNA into the host chromosome. It extends first phase viral decay and alters viral decay kinetics of HIV by significantly reducing the second phase and challenging current hypotheses of viral replication.Elvitegravir is a specific inhibitor of the strand-transfer step of HIV integration and is active against HIV-1 and HIV-2. It is also active against organisms resistant to nucleoside reverse-transcriptase inhibitors (NRTIs), non-nucleoside reverse-transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs).

- **RNA synthesis inhibitors:** Ribavirin interferes with viral DNA synthesis and can inhibit both DNA and RNA viruses. It introduces multiple mutations into viral RNA making it unable to infect new cells. Neplanocin A is an effective inhibitor of S-adenosylhomocysteine hydrolase, and may also inhibit capping of mRNA. They also hinder HIV replication by inhibiting the trans-activation process.

- **Protein modification inhibitors**: Castanospermine apotent inhibitor of lysosomal alpha- and beta-glucosidasesmay interrupt folding of some viral proteins by preventing the elimination of the terminal glucose residue on N-linked glycans.Neuramidase inhibitors such as Zanamivir and Oseltamivir inhibit neuraminidase that is essential for viral replication and are effective against influenza A and B virus.

- **Protein synthesis inhibitors**: e.g.Fomivirsen a phosphorothioateoligonucleotide inhibits the replication of human cytomegalovirus (HCMV) by binding to the corresponding

sequence of the mRNA transcribed from the major immediate-early transcriptional unit of CMV. Fomivirsen binds to the target mRNA resulting in inhibition of IE2 protein synthesis, which then inhibits virus replication.

- **Immunomodulators**: Interferons induce in the host cells ribosomes enzymes which inhibit viral mRNA e.g. hepatitis B virus. Pavilizumab is a mononclonal antibody against infusion proteins of respiratory syncytial virus.

3.4 Antiparasitic Agents

Antiparasitic agents are a class of drugs which are used for the treatment of parasitic diseases e.g. Helminthiasis, amebiasis, malaria. Parasitic infections belong to a class of infectious diseases that have protozoa or helminths as the infectious agents.

- **Antihelmintic Drugs:** Antihelmintics are divided into classes based on the similarity of chemical structure and mode of action e.ginhibitors of tubulin polymerisation, potentiation of inhibitory transmitters, inhibitors of glucose uptake, uncouplers of oxidative phosphorylation, muscle hyperpolarization, cholinesterase inhibitors, cholinergic agonists and inhibitors of enzymes in the glycolytic pathway.

- **Inhibitors of tubulin polymerization**: Mebendazole binds to beta-tubulin, interfers with beta-tubulin dependent glucose uptake preventing microtubule formation and effects glycogen depletion. It blocks the uptake of glucose leading to depletion of the helminth's own glycogen with a resultant decrease in adenosine triphosphate formation. It causes degenerative alterations in the outer body covering among members of the phylum Platyhelminthes and intestinal cells of these worms by binding to the colchicine-sensitive site of tubulin, thereby inhibiting its polymerisation or assembly into microtubules. This leads to immobilisation of the parasite and ultimately death. However, mebendazole does not affect serum glucose concentrations in humans and used in treatment of Ascariasis and Enterobiasis.

  Albendazole binds to beta-tubulin and prevents microtubule assembly thus inhibiting beta-tubulin dependent glucose uptake. It also inhibits fumaratereductase, decreases levels of NADH causing degradation of endoplasmic reticulum and mitochondria with decreased production of ATP.

- **Potentiation of inhibitory transmitters**: Macrocyclic lactones are a group of hydrophobic compounds with broad spectrum antinematodal and antiarthropodal properties derived from soil microorganisms belonging to the genus *Streptomyces*. Inhibitors likeivermectineprinomectin, selamectin. ivermectin activate the opening of gated chloride channels. They are potent against many immature nematodes and arthropods by binding to glutamate-gated chloride channel receptors in nematode and arthropod nerve cells. This results in the opening of the gated channel leading to an influx of chloride ions with subsequent paralysis of the pharyngeal pumping mechanism, body wall and uterine muscles of helminths. Paralysis of uterine muscles results in disruption of reproduction.

- **Inhibition of glucose uptake:** Blocking the uptake of glucose, the primary source of worms' energy would lead to starvation and ultimate death of the parasite. This may be achieved by modulating the cytosolic or mitochondrial enzymes. Praziqunatelan isoquinolone analog increases cell membrane permeability in susceptible worms and flukes leading to loss of intracellular calcium, paralysis and contractions of the parasite's muscles and subsequently expulsion of the parasite. It is active against Tapeworms. Inhibition of glucose uptake by Praziquantel in *Hymenolepisdiminuta* may be mediated through modulation of mitochondrial enzymes. Mebendazole is vermicidal in action and causes degeneration of parasite's cytoplasmic microtubules and thereby carefully and permanently blocks glucose uptake in susceptible adult intestine-dwelling helminths and their tissue-dwelling larvae leading to depletion of the parasite's store of glycogen.

- **Uncouplers of oxidative phosphorylation in mitochondria** e.g. fasciolicides mainly salicylanilides and substituted phenols inhibit the coupling between the electron transport and phosphorylation reactions and thus inhibit Adenosine Triphosphate (ATP) synthesis without affecting the respiratory chain and ATP synthase (H+-ATPase). They exhibit protonophoric action in the H^+-impermeable mitochondrial membrane, permitting hydrogen ions to leak through the inner mitochondrial membrane. They are active against the hematophagous nematodes, eg, *Haemonchus* and *Bunostomum.* Niclosamide inhibits oxidative phosphorylation in the mitochondria of cestodes. When Clorsulon is ingested by *Fasciolahepatica* they are killed since glycolysis is inhibited and cellular energy production is disturbed.

- **Cholinesterase Inhibitors**: Organophosphates bind to the enzyme usually in charge for breaking down acetylcholine after it has

passed its message through the synapse. It makes the cholinesterase unavailable for breaking down the acetylcholine so the neurotransmitter continues to cause the neuron to transmit electrical charges leading to overstimulation of the nervous system causing death.Trichlorfon irreversibly binds to the active site of acetylcholine esterase, thereby inactivating it. This leads to loss of control by the parasite over its nervous system causing paralysis.

- **Cholinergic agonists or parasympathomimetics:** are nicotinic receptor agonists that combine with acetylcholine receptors and imitate the effects of parasympathetic stimulation by causing spastic muscle paralysis due to persistent activation of the excitatory nicotinic acetylcholine receptors on the body wall muscle of nematodese.g. imidazothiazoles and pyrimidines.The antihelmintic activity of imidazothiazoles is attributed mainly to their cholinomimetic activity, whereby they stimulate ganglion-like structures in somatic muscle cells of nematodes. This results in continued muscular contractions leading to neuromuscular depolarising blockade causing paralysis. levamisole'sagnositic activity as an antiparasitic agent is of the ganglionic nicotinic type. It acts against the L-subtype nicotinic acetylcholine receptors in nematode muscles thereby reducing the capacity of the males to control their reproductive muscles and limit their ability to copulate.Levamisole is highly effective against both adult and immature stages of *Ascarissuum*.

- **Muscle hyperpolarisation**: Drugs like Piperazine targets nervous synaptic transmission. Piperazine, a pharmacological analogue of a natural inhibitory transmitter blocks neuromuscular transmission in the parasite by hyperpolarising the nerve membrane leading to flaccid reversible paralysis of body wall muscle. The extent of muscle hyperpolarisation by piperazine depends on the concentration of extracellular chloride decreasing once a fraction of the chloride ions is replaced by the sulphate anions. The parasite is removed by peristalsis once it is paralysed and depleted of energy. It also blocks succinate production by the parasite and is active against ascarids.

3.5 Antiprotozoal Agents

Anti-protozoal drugs are used to treat infections or diseases caused by Protozoa. The commonest diseases caused by protozoa are Malaria, Trichomoniasis, Amoebiasis, Trypanosomiasis or sleeping sickness, Toxoplasmosis e.t.c. These drugs destroy protozoa or prevent their growth and ability to reproduce. The anti protozoal agent can target either the enzymes found in both host and parasite but are indispensable

only for the parasite, enzymes found exclusively in the parasites, common biochemical functions found in both the parasite and the host but with dissimilar pharmacological properties or exhibit a selective toxicity for the parasite as compared to the host.

Enzymes found only in parasites:

- **Enzymes for dihydropteroate synthesis**: 6-hydroxymethyl-7,8-dihydropteroate synthase (DHPS) is an essential protein in lower eukaryotes, prokaryotes and plants used in the synthesis of folate. Folates are essential cofactors in the production of methionine, thymidylate, glycine, purines etc for the organism. Therefore in order to survive, intracellular sporozoan parasites e.g. Eimeriaspp, Plasmodium and Toxoplasma must synthesize their own folate.

- *Plasmodia* generate majority of their folates from the beginning, and hence inhibition of dihydropteroate synthase by these drugs leads to depletion of deoxythymidine triphosphate and decreased DNA synthesis. In the treatment of human malaria caused by *P. falciparum*, sulfadoxine has been used in combination with pyrimethamine. Pyrimethamine is an antifolate drug that inhibits dihydrofolatereductase. The sulfonamide group of compounds such as sulfadoxine and dapsone, inhibit dihydropteroate synthase which catalyzes the condensation of paraaminobenzoic acid (PABA) with 6-hydroxymethyldihydropterin pyrophosphate to yield 7,8-dihydropteroate. These drugs inhibit cell growth and cause cell death through folate cofactor depletion. Sulfadoxine and pyrimethamine are folic acid antagonists that sequentially inhibit the activity of two enzymes involved in the biosynthesis of folinic acid within the parasites dihydropteroate synthase and dihydrofolatereductase respectively. The asexual erythrocytic stages of *Plasmodium falciparum* are sensitive to sulfadoxine and pyrimethamine.

- **Pyruvate:ferredoxinoxidoreductase** are present in anaerobic protozoa e.g. *Entamoeba* spp, *Giardia* and *Trichomonas*. This enzyme can reduce the nitro group of metronidazole to form cytotoxic reduced products that bind to DNA and proteins. Nitazoxanide and Tenonitrozole are antiprotozoal agent that interfere with the action of Pyruvate:ferredoxinoxidoreductase. Nitazoxanide interferes with the pyruvate:ferredoxin 2-oxidoreductase enzyme-dependent electron transfer reaction that is vital to anaerobic energy metabolism in susceptible organisms.Sporozoites and oocysts of *Cryptosporidium parvum* and trophozoites of *Giardia lamblia* are sensitive to nitazoxanide

- **Nucleoside phosphotransferase:** All of the protozoan parasites studied thus far are deficient in de novo synthesis of purine nucleotides. The purine nucleoside phosphotransferase found in Leishmania can phosphorylate purine nucleoside analogs such as allopurinol riboside, formycin B, 9-deazainosine, and thiopurinolriboside converting them to the corresponding nucleotides. These nucleotides are either further converted to triphosphates or eventually incorporated into nucleic acids or become inhibitors of other essential enzymes in purine metabolism. *T. vaginalis*, *T. foetus*, and *Giardia lamblia* appear to be deficient in de novo synthesis of both purines and pyrimidines. Pyrimidine and purine salvage pathway becomes indispensable for these parasites.

4.0 CONCLUSION

Bacterial cells have to replicate often in order to reach large numbers required during an infection, therefore a large amount of biomolecules are required to enable them grow and divide. There are some specific processes essential for growth and or division of bacteria and these processes are inhibited by antibacterial agents. These antimicrobial agents are classified into groups based on their mode of action. Antimicrobial agents may kill the target bacteria or fungus and are said to be bactericidal or inhibit its growth (bacteriostatic).

5.0 SUMMARY

In this unit, you have learnt that:

- antimicrobial agents inhibit specific cellular processes
- some antimicrobial agents are bactericidal and others bacterioststic
- antifungal agents target the plasma membrane, sterol, DNA and β glucan biosynthesis
- antiviral agents act directly on viruses to prevent them from multiplying and inhibit a virus-specific function or interfere with a cellular function to prevent them from replication
- antiprotozoal agent can target either the enzymes found in both host and parasite but are indispensable only for the parasite.

6.0 TUTOR-MARKED ASSIGNMENT

i. Why is folate very important in microbial metabolism?
ii. Could antimicrobial agents be both bactericidal and bacteriostatic?
iii. Why does the activity of salicylanilides not affect the respiratory chain and ATP synthase?

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UNIT 3 CHEMOTHERAPY OF SPECIFIC DISEASES

CONTENTS

1.0 INTRODUCTION

A majority of infectious diseases can be treated with antibiotic or chemotherapeutic agents. It is important to establish the diagnosis of the kind of infection, prepare cultures to isolate the microorganism and carry out sensitivity tests before commencement of chemotherapy.

2.0 OBJECTIVE

At the end of this unit, you should be able to:

- describe the chemotherapy of Chagas and Hodgkin's diseases.

3.0 MAIN CONTENT

3.1 Chemotherapy of Chagas Disease

- Chagas disease is caused by the protozoan *Trypanosomacruzi*, and it is prevalent in Latin America. The mode of transmission is through hematophagousreduviid vectors such as *Triatomainfestans*, which establish a habitat in human dwellings. Over a long period, the pathogenesis of Chagas disease was unclear and it was thought to be an autoimmune condition. However, current knowledge indicates that parasitic invasion coupled with an unbalanced immune response plays an important role in the characteristic pathology observed in both acute and chronic human chagas disease. Chemotherapeutic drugs most frequently used in the treatment of chagas disease are nitroheterocyclic compounds such as **nitrofuran**, **nifurtimox** and**benznidazole** a nitroimidazole derivative, **radanil**. *Trypanosomacruzi* lacks the mechanisms to detoxify oxygen metabolites especially hydrogen peroxides, and are thus more

sensitive to oxidative stress than vertebrates. Nifurtimox thus acts by reducing nitro groups to unstable nitroanion radicals, which in turn react to produce toxic reduced oxygen metabolites. Benznidazole acts through a different mechanism, by a covalent modification of macromolecules by nitroreduction intermediates. Nifurtimox and benznidazole have been observed to be very active in the acute stage of chagas disease, while they are inactive at the chronic stage. Due to their oxidative and reductive properties, they are also associated with a lot of side effects such as anorexia, vomiting, and allergies, and in some cases this usually leads to discontinuation of the drugs. The major limitations of nitroheterocyclic compounds is the difference in the level of efficacy at the acute and chronic stages of the disease, even though the reason for this disparity is yet unknown. Studies have also shown that specific sterol inhibitors such as **itraconazole**, used in the treatment of fungal diseases, inhibit sterols required by Trypanosomacruzi for cell viability and proliferation at all stages of cell division. Thus, important in the reduction of parasitic load.**Posaconazole** and **ravuconazole**, triazole derivatives were the first chemotherapeutic agents found to induce parasitological cure in both acute and chronic stage of chagas disease. They were also found to be able to eradicate nitrofuran and nitroimidazole resistant strains of *T. cruzi*. A chemotherapeutic agent **N-methyl-piperazine-urea-F-HF-vinyl-sulphone-phenyl** inhibits cruzain, responsible for the major proteolytic activity of all stages of the parasites life cycle, and as a result prolongs survival and induces parasitological cure in acute and chronic stages of human chagas disease with minimal toxicity. Inhibition of cruzain seems to be an effective anti-*T. cruzi* target and so many current researches are being modeled towards this mechanism.

3.2 Chemotherapy of Hodgkin's Disease

Hodgkin's disease is a lymphoma, a cancer of the lymphatic system. Hodgkin's disease is distinguished from non Hodgkin's lymphoma by the presence of large abnormal cells, known as Reed-Sternberg cells. Hodgkin's disease is classified into two types, classical Hodgkin's lymphoma which is more common and the less common nodular lymphocyte-predominant Hodgkin's disease. Hodgkin's disease is considered one of the most curable forms of cancer, if diagnosed and treated early. The main types of treatments for Hodgkin's disease are radiation and chemotherapy. The standard chemotherapy regimens for Hodgkin's disease are ABVD and standard V. ABVD is a four drug combination; **doxorubicin, bleomycin, vinblastine** and **dacarbazine**, while standard V is a seven drug combination; **etoposide, prednisone, vincristine, mechlorethamine**, **doxorubicin, bleomycin,** and

vinblastine. BEACOPP (**doxorubicin, bleomycin, vinblastine,cyclophosphamide**,**etoposide, prednisone,** and **precarbazine)** is a chemotherapy regimen reserved for high risk patients, and has been proven to be very effective especially at the advanced stage of the disease. Although it has been shown, that treatment with this combination increases the risk of leukaemia in patients. There are numerous side effects associated with the use of chemotherapeutic regimens, and these effects increases with higher doses and over the course of treatment of the disease. Common mild and temporary side effects are vomiting, nausea, hair loss, weight loss, diarrhea and depression. Serious side effects that may occur are; neutropenia, which is a severe drop in white blood cells, anaemia, a drop in red blood cells, liver and kidney damage, allergic reaction and abnormal blood clotting. However, drugs have been developed to reduce the intensity of the adverse effects of these combinations, but the duration of the combat actions of these drugs are short. Long term implications associated with the use of these combinations are infertility, fatigue, menopause, heart failure, and bone thinning. Bleomycin has been associated with lung toxicity, while vinblastine is observed to be toxic when used in combination with radiation. The combination of chemotherapy and radiation are used in the treatment of the advanced stages of Hodgkin's disease.

4.0 CONCLUSION

Chemotherapy has proved effective in the treatment of infectious diseases, and is used as a major form of cancer treatment or in combinations with other forms of therapy in cancer treatment. However, the adverse effects associated with the use of chemotherapy is a cause of concern, as it has been reported that sometimes most patients die as a result of the effects of the drugs used.

5.0 SUMMARY

In this unit, you have learnt that;

- the effective chemotherapeutic agents developed towards the treatment of Chagas disease are modeled towards the inhibition of cruzain
- the effective chemotherapeutic agents used in the treatment of Hodgkin's disease are used in combination with radiation
- the side effects of chemotherapy have become a subject of recent research.

6.0 TUTOR-MARKED ASSIGNMENT

Differentiate between the chemotherapy of Chagas disease and that of Hodgkin's disease.

7.0 REFERENCES/FURTHER READING

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UNIT 4 PHARMACO-DYNAMICS AND PHARMCO-KINETICS

CONTENTS

1.0 INTRODUCTION

Pharmacodynamics is study of the biochemical and physiological effects of drugs on the body or on microorganisms or parasites within or on the body and the mechanisms of drug action and the relationship between drug concentration and effect. While pharamacokinetics involves the determination of the fate of substances administered externally to a living organism. Pharmacodynamics can be simply referred to as what the drug does to the body, while pharmacokinetics is what the body does to the drug.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- describe pharmacodynamics and pharmacokinetics
- list the processes of pharmacokinetics involved in drug action.

3.0 MAIN CONTENT

3.1 Pharmacodynamics

The mode of most drug actions is in form of mimicry of major vital processes of parasites and microorganisms, or rather through inhibition of these vital processes. Mode of drug actions, under these common methods are; stimulating or depressing actions by directly binding to receptors to trigger response, blocking action by directly binding to

receptor without activating it, stabilizing action by acting neither as a stimulant or depressant, replacing or accumulating substances to form a reserve, scavenging free radicals obtained from oxidation, and direct harmful chemical reaction that may result in the damage or destruction of the cells through induced toxic or lethal damage. Only few drugs are specific in the choice of receptor binding, but most have relative specificity. The ability of a drug to affect a given receptor is determined by its affinity and intrinsic efficacies, which are further determined by its chemical composition. However, there are some drugs that induce effects without binding to receptors or altering cellular functions, e.g. antacids act by just decreasing gastric acidity through chemical reactions with acids to produce salts. The desired effect of a drug on the body is observed mainly due to one of the following reasons; interaction with structural or carrier proteins, interaction with ion channels, or disruption of the cell membranes. For example, aspirin acts by irreversibly inhibiting the enzyme prostaglandin synthetase, as a result preventing inflammatory response, and colchicine interferes with the structural protein tubulin. Pharmacodynamics is not restricted to benefits alone, there are also adverse effects associated with the use of drugs, e.g. induced physiological damage or abnormal chronic conditions, or increasing the probability of cell mutation. It has been observed that a drug's pharmacodynamics can be affected by physiological changes due to disorders, aging or the effects of other drugs. Disorders such as genetic mutations, malnutrition, Parkinson's disease or insulin-resistant diabetes mellitus are affected. These disorders could act by changing receptor binding such as aging, alter the level of binding proteins or decrease receptor sensitivity. Other drugs affect drug pharmacodynamics by competing with receptor binding sites or alteration of post-receptor responses.

3.2 Pharmacokinetics

This is often studied in association with pharmacodynamics. Pharmacokinetics includes the study of the mechanisms of absorption and distribution of an administered drug, the rate at which a drug action begins and the duration of the effect, the chemical changes of the substance in the body and the effects and routes of excretion of the metabolites of the drug. Drug pharmacokinetics determines the onset, duration and intensity of a drug's effects. The processes of pharmacokinetics can be explained and summarised by absorption, distribution, metabolism and excretion.

3.2.1 Absorption

Drug absorption is determined by the drug's physiochemical properties, formulation and route of administration. Most drugs are weak organic

acid or bases, existing in ionized or non-ionised forms. The non-ionised forms are lipid soluble, and readily diffuse across membranes, while the ionized forms are water soluble. Drugs are mostly administered orally, and must be in a solution in order to be absorbed. Drugs administered orally, must cross several semi-permeable membranes before they reach the systemic circulation. Hence mode of drug transport across membranes is through passive diffusion, pinocytosis or active transport. Other factors affecting absorption of drugs are differences in luminal ρH, presence of bile and mucus, and nature of epithelial membranes. However, drugs administered through IV injection enter the systemic circulation immediately. Perfusion, which is blood flow per gram of tissue, also affects absorption of drugs. In other words, site of injection affects absorption rate. The rate and extent to which a drug enters systemic circulation to gain access to the site of action, known as bioavailability is dependent on properties of the dosage form, age, sex, physical activity, stress, disorders e.t.c.

3.2.2 Distribution

Drugs are distributed to tissues after they gain entry into the systemic circulation. Distribution is affected by differences in the regional ρH, cell membrane permeability, tissue binding and blood perfusion, and these results to an uneven distribution of drugs to tissues. The rate of blood flow to the tissue, tissue mass, and partition characteristics between blood and tissue determine the rate of entry of drug into the tissue.

3.2.3 Metabolism

This is the irreversible transformation of a compound into daughter metabolites, and the principal site for this is in the liver. Drugs can be metabolised by oxidation, reduction, isomerisation, conjugation, hydrolysis, condensation or hydration, mainly for easier excretion by the body. Rate of drug metabolism is dependent on the individual genetic components, disorders or drug effects affecting metabolic rates, and the age of the patients.

3.2.4 Excretion

This is simply the removal or elimination of broken down metabolites from the body, although rarely, these substances may accumulate in the body tissues. Most drugs and their metabolites are excreted from the body through renal and biliary excretion. Renal excretion provides the most common form of mechanism of drug excretion.

Patients- related factors such as genetic properties, sex and age can be used to predict the pharmacological response of populations. For example, the half-life of some drugs is remarkably longer in the elderly.

4.0 CONCLUSION

As a result of individual differences, administration of drugs should be based on individual needs, adjusting dosage levels continually till the target therapeutic condition is met. Although this approach can lead to adverse effects, but knowledge of pharmacokinetics helps to ensure dosages are adjusted appropriately and accurately.

5.0 SUMMARY

In this unit, you have learnt that:

- Pharmacodynamics and pharmacokinetics are interrelated studies
- Drugs act by either acting similarly to microorganisms or through inhibition of their properties
- Drugs that selectively bind to receptors, stimulate, depress or block the actions of these receptors.

6.0 TUTOR-MARKED ASSIGNMENT

i. What is pharmacodynamics?
ii. Explain the processes of pharmacokinetics.

7.0 REFERENCES/ FURTHER READING

Lees, P, Cunningham, F.M. & Elliott, J (2004). "Principles of pharmacodynamics and their applications in veterinary pharmacology". *J. Vet. Pharmacol.Ther.* **27** (6): 397–414.

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UNIT 5 DRUG BIOASSAYS AND SENSITIVITY TESTS

CONTENTS

1.0 INTRODUCTION

Biological assays or bioassays are scientific experiments used to test the effects of a compound on a living organism, and are essential in drug developments and monitoring of environmental pollutants. Sensitivity tests are laboratory methods used to determine the susceptibility of microorganisms to drug therapy.

2.0 OBJECTIVE

At the end of this unit, you should be able to:

- describe drug bioassays and explain various drug sensitivity tests.

3.0 MAIN CONTENT

3.1 Drug Bioassays

Several drugs are isolated from natural products, and as a result pharmacological screening or evaluation is necessary to guide the isolation process towards the production of a pure active bio-component. Bioassays determine the concentration of purity or biological activity of substances such as hormones and plant extracts, while measuring the effects on an organism or tissue cells compared to a standard preparation. The purposes of drug bioassays are; measurement of the pharmacological activities of new or chemically undefined compounds, investigating the functions of endogenous mediators, to understand the side effects of the drug to be used, and concentration measurement of known substances. Drug bioassays are usually quantitative, in that they measure the biological response evoked by the use of such drugs, and they are usually analysed using biostatistics. A drug bioassay may be primary or secondary assay depending on specific criteria such as cost

and duration of results. A primary bioassay may not be quantitative, and is applied to a large number of samples; it is cheap and produces results quickly. While secondary bioassays are applied to more detailed samples, are slow and costly. A primary bioassay should meet certain criteria such as:

- its results should be able to predict therapeutic potential of the substance being tested either directly or indirectly by comparing with other known effective drugs that have been screened through the same process
- a potentially useful pharmacological activity should not go undetected even though the activity may be unexpected or unique
- the probable nature of the activity should be highlighted such that subsequent research can be carried out intelligently
- the bioassay should be tolerant of many impurities present in the crude extract, but also be able to detect the presence any interesting substances in low concentration
- the bioassay procedure should be unbiased and it should allow for coding of all samples
- results obtained should be reproducible
- the screen should allow the use of both crude materials and pure isolates so that the procedures can be used to direct the extraction, isolation and purification work of the natural product chemist
- the procedure should not require expensive equipments or sophisticated laboratory environment so that the primary level of screening experiment can be conducted synchronously with the fractionation process
- the procedure should be simple enough to be taught to laboratory technicians so that there would not be a need to require highly trained and qualified researchers to run the routine operation of bioassay program
- test animals, if required for the bioassays should be easily obtainable, easily bred, easily handled and resistant to the infection
- most importantly the bioassay should be economical to conduct over a long period of time.

There are four approaches that serve as a bioassay guide in drug discovery research, and the choice of screening approach depends on the target disease and available information about the target organism to be studied. The approaches are:

a. the use of a single bioassay technique to search for a specific type of pharmacological activity e.g. anti-inflammatory activity
b. the use of specific bioassay technique, with each procedure designed to capture specific useful properties
c. the use of single bioassay technique to discover multiple activities

d. the use of a variety of combination of bioassays to detect specific and multiple activities.

There are basically two types of bioassays; Quantal and Graded. A quantal bioassay requires all or none response, which has no room for intermediate results. In the case of toxicity studies, the animal receiving the drug either dies or not. Graded bioassay is based on the observation that, proportionate increase in dose levels causes a similar increase in response. Techniques involved in the determination of bioassays are:

a. Matching bioassays: The simplest type of bioassay and it involves taking the response of the first substance and then matching it with the standard response. This method is applied to small sample sizes and the major limitation is that it does not take into consideration sensitivity of the drug; hence it is not precise or reliable.
b. Interpolation method: This is conducted by determining the amount of preparation of unknown potency required to produce a definite effect on suitable tissues or test animals under a standard condition, and the effect is compared to the standard.

Other techniques are bracketing method and multiple point assays. Statistical methods are more reliable method of calculating concentration of drugs in bioassay techniques.

3.2 Drug Sensitivity Tests

When a microorganism has been recovered from a clinical specimen, it is cultured and tested against a variety of drugs. If the growth of the microbe is inhibited by the action of the drug, it is reported as being sensitive to the drug, but if the actions of the drug have little or no effect on the growth of the microbe, the microbe is reported to be resistant to the drug. This process is known as drug sensitivity test, and culture used in this test is dependent on the type and kind of microorganism. Drug sensitivity tests help to provide information about the potency of certain drugs, if they are still effective and to what extent if they are. Without drug sensitivity tests, drugs are prescribed on a trial and error basis, which could take many days for an observed improvement and sometimes may lead to several complications during the course of illness such as kidney impairment, or even lead to death of the patients. Culturing microorganisms before testing for drug sensitivity is done to enable reproduction and growth of the microbe for easy identification of results. It usually takes up to 18 hours to obtain results for bacteria, but it could take several weeks to observe colonies in the bacterium that causes tuberculosis. Culture mediums are usually sterile plastic dishes containing nutrient gel on which they feed, and sometimes substances are added to the medium to suppress the growth of other bacteria not

needed. However, viruses require living cells to grow. After culturing, a small portion of the colony is removed and stained for proper identification under the microscope. Once properly identified, drug sensitivity test is carried out by inserting paper discs containing the drugs of specification, into the bacteria culture medium. The medium is checked after 24 hours to observe the drug which the bacterium is most sensitive to. An effective drug sensitivity test does not depend solely on the kind of drugs used, but also on the use of proper culture medium, careful extraction of bacteria from colony, and adequate storage facilities for the culture medium to avoid contamination from outer source.

4.0 CONCLUSION

Drug bioassays are necessary in drug discovery research, while drug sensitivity tests ensure the success of treatment of infectious diseases.

5.0 SUMMARY

In this unit, you have learnt that:

- pharmacological screening of compounds or drugs is necessary in order to determine its beneficial and detrimental effects, as well as its potential for future drug therapy
- screening of drugs could be primary or secondary, depending on costs and specificity required
- drug bioassay may be quant or graded, and techniques used in bioassays are matching bioassay, interpolation method, bracketing method and multiple point bioassay
- drug sensitivity testing is an important process in the successful treatment of diseases, and method of propagation of microbe growth is a relevant factor in this process.

6.0 TUTOR-MARKED ASSIGNMENT

i. Mention 5 criteria that should be met by a primary bioassay, and differentiate between a primary bioassay and a secondary bioassay.
ii. Explain the process of drug sensitivity test.

7.0 REFERENCES/FURTHER READING

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Kubo, I. (1991). "Method in Plant Biochemistry" In: Dey, P.M. and Harborne ,J. B.. (Eds). London: Hostettman ,K. Academic Press.