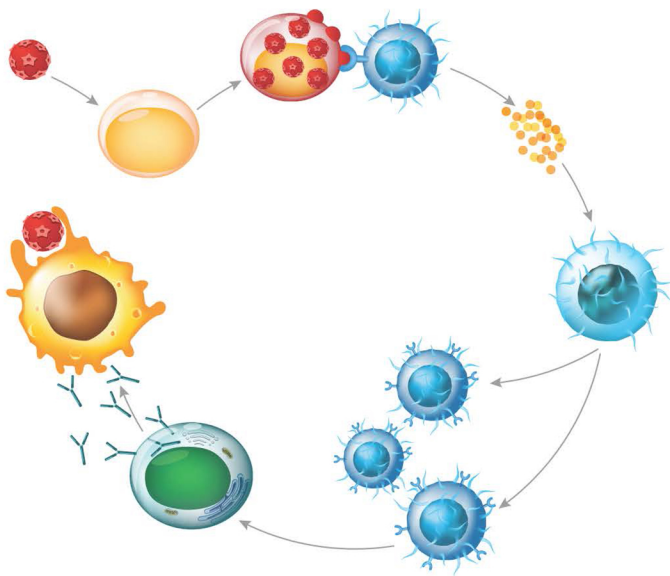
Basics and Fundamentals of Immunology



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Nova Medicine & Health

BASICS AND FUNDAMENTALS OF IMMUNOLOGY

IMMUNOLOGY AND IMMUNE SYSTEM DISORDERS

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PREFACE

Immunology is a distinctive subject that rose in the mid-20th century. The subject developed as scientists started to unravel the mysteries about the defense system against pathogens. Researchers started to understand the mechanisms employed by the innate and the adaptive immune system in defense against pathogens. During the last decade, the subject of immunology has been in sharp focus as the immunotherapies against diseases like cancer and AIDS seems last hope. Employing the body's own defense system against diseases like cancer and AIDS by activating specific cells of the immune system looks promising, and therapies like CAR-T cell therapy have been approved. In the first edition of the book "The Fundamentals of Immunology" we have explained the basics of the defense system of our body.

The book is organised into four volumes. The first volume comprises of ten chapters and it describes the rise, history and scope of immunology and the building blocks of the immune system *viz.*, cells, molecules and organs of the immune system. The second chapter describes the cells of the innate and the adaptive immune system and how the granulocytes and macrophages employ defense mechanisms to protect the body against pathogenic invasions. In the third chapter of this book, we have described the organs of the immune systems and how different organs are involved in the differentiation and maturation of immune cells. The chapter also

focused on the structure of lymph nodes and their function in concentrating the antigens. In chapter four of this book, we have described the terms like antigens, immunogens, antigenicity, immunogenicity and how immunogenicity of an antigen is affected and how antigenicity of an immunogens is related to the immune response. The innate and adaptive immune systems and the different types of cells and molecules employed by the two branches of immunity have been described in a separate chapter.

The structure and biology of immunoglobulins, their types and function in antigen binding and antibody dependent cellular cytotoxicity (ADCC) have been described well in chapter six. Focus has been laid on the distinction between an antibody and an immunoglobulin. The structure and function and major histocompatibility complex (MHC) has been described. The education of cells about self and non-self during their maturation and the processing and presentation of antigens by MHC bearing cells and how MHC coordinates both humoral and cell-mediated immune responses has been explained well throughout the book. The book has explained the complement system and its components, mechanisms and functions in a separate chapter. At the end of the book, we have given an insight about the vaccines, their history, development and how they are useful and helpful in the defense against diseases. The book also discusses the immune disfunction and diseases associated with the dysregulation of immune responses.

ACKNOWLEDGMENTS

First, I would like to thank Almighty for giving me strength, belief and good health. It is under his grace that we all live, learn and flourish.

Although it is difficult to thank everybody and interwove in words the genuine efforts made by the people directly or indirectly to make this book entitled “The Fundamentals of Immunology”. But I would like to take this opportunity to pen down my appreciation for a number of people whose contribution in numerous ways immensely helped me to move towards my destination.

It gives me immense pleasure to express my sense of gratitude and respect to my mentors Prof Talat Ahmad, Dr. Javed N Agrewala, Prof Mohammad Afzal Zargar, Prof Raid Saleem Albaradie, and Prof Farooq Ahmad Malik whose constant support, utmost patience, invaluable advice and guidance rekindled my interest for science from time to time. I will benefit for a long time to come from their sincerity, originality and truthfulness which has nourished my intellectual maturity. I admire and respect them all for their sincerity, dedication, devotion, amazing memory, thorough knowledge and constructive criticism.

I owe deepest gratitude to my parents. A seed of loyalty, hard work, dedication and sincerity sown in me by my parents played a crucial role in completion of this work. They have been a source of great strength throughout my life. I would like to thank other family members for

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Dr. Manzoor A Mir

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Chapter 1

INTRODUCTION TO IMMUNOLOGY

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ABSTRACT

The definition of immunity as a process by which body fight against infectious diseases. The concept of immune system emerged since 15th century, provides evidence how medical science has progressed through thick and thin. The fundamental scientific discoveries contribute too many human endeavours. The history of great scientific discoveries with the names of scientists ascribes their attribution in making science better each day. The immune system evolved as a result of natural selection pressure of organisms, which resulted in the development of defence mechanisms that have the capacity to protect the host organisms by destroying the invading micro-organisms and consequently eliminate their virulence effect.

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Keywords: immunity, harmful, recognition, response, infection, vaccine

OBJECTIVES

- Introduction to immunology.
- Understanding the concept of immunity.
- Historical perspective of the immune system.
- Scope of immunology

INTRODUCTION

A process by which body maintains a constancy of an in-built defence system against any unwanted physical, chemical and biological invasions. It's the capacity of body to resist against any harmful organism from entering into the body and help fight infections. It deals with the physiological functioning of immune system in both health and disease. Immunity, derived from a Latin word *immunis* meaning impunity/free is defined as the prodigiously protean defence system to resist infections by pathogens invading into the host vertebrates. The immune system is a wide spectrum complex network of cells, tissues, organs, molecules and their interactions, specifically designed to eliminate infection causing pathogens from the host vertebrates. Immunity is the capacity to perceive the infringement of materials foreign to the body and to deploy cells and cell products to help remove that particular foreign material with whizz and efficacy. The immune system has evolved as a powerful collection of defence mechanism to protect us from pathogens that take advantage of resources provided by the host vertebrate. Pathogens that are intracellular infect individual cells e.g., viruses while as the extracellular pathogens divide within the tissues or body cavities, constitute bacteria.

The function of immune system comprises of two steps as follows: 1) Antigen recognition and 2) Antigen eradication. In first step clonal

selection involves recognition of antigen by a particular clone of lymphocytes, leading to the clonal expansion of specific clones of T and B cells and differentiation to the effector and memory cells. In the effector step, these lymphocytes coordinate an immune response which eliminates the source of an antigen.

The immune system is capable to differentiate between body's own cells and the molecules from foreign cells. In addition, it is able to recognise subtle chemical differences that distinguish one foreign pathogen from other. Immunity is broadly divided in two types:

1) Innate or non-specific immunity and
2) Acquired or specific immunity.

Innate or Non-Specific Immunity

Innate also known as native or natural immunity is present since birth and consists of many factors that are relatively nonspecific-that is, it operates against almost any foreign molecules and pathogens. It is not specific to any one pathogen but rather acts against all foreign molecules and pathogens. It also does not rely on previous exposure to a pathogen and response is functional since birth and has no memory. Thus, providing first line of defence against pathogens. Innate immune response relies on different antimicrobial chemicals and phagocytic cells which provide protection against pathogens. Non-specific defence mechanism is similar for most type of infections. It resists infection by blocking the entry of pathogens into the body or by destroying the microbes through means other than antibodies. This type of immunity is inherited by the organisms from the parents and protects it from birth throughout life.

Acquired or Specific Immunity

Adaptive immunity is capable of recognising and eliminating specific pathogen antigens. If a pathogen is able to get pass the body's non-specific defence, the immune system reacts with a series of specific and selective defence that attacks the disease-causing agents. Response of the immune system to specific pathogens is called specific defence and it is the third line of defence developed by an animal in response to disease caused by infection of microbes. This occurs by making specialized cells and antibodies that make the pathogen useless.

Immunology has various applications in several disciplines of science.

Classical immunology, is the study of molecular and cellular components that comprise the immune system, including their function and interaction, and is the central science of immunology.

Clinical immunology is the study of diseases caused by disorders of the immune system (failure, aberrant action, and malignant growth of cellular elements of the system).

Evolutionary immunology is the study of the immune system in extant and extinct species and thus capable of giving us a key understanding of the evolution of species and the immune system.

Historical Perspective of Immunology

History of immunology dates back from ancient times to present day. The study of immunology goes as far back as 430 B.C (5th century) when Thucydides the great historian of Peloponnesian war, observed and wrote about the individuals who recovered from the plague which was raging in Athens, that only those who recovered from the plague could nurse the sick because they would not covenant the disease a second time. By 15th century, progress in the field of immunology enhanced. It recorded the earliest attempt to induce immunity intentionally to an infectious disease, performed by Chinese and Turks in form of dried crusts derived from smallpox papules and inhaled or applied into the wounds on skin, a

phenomenon called variolation. This phenomenon became quite common in the Middle East. In 1718, Lady Mary Wortley Montagu, the wife of the British ambassador to Constantinople observed the positive effects of variolation on the native population and had the technique performed on her own children. In 1721, Mary Montagu's daughter was the first person to be engrafted in Great Britain. By the year 1798, the field of immunology progressed much when Edward Jenner, known as the father of immunology, developed a vaccine for smallpox. Jenner inoculated an eight-year-boy with a fluid obtained from cowpox papules and later intentionally injected the child with smallpox and child did not developed smallpox.

Image source: https://www.sciencemag.org.

Figure 1.1. Edward Jenner who invented small pox vaccine.

Edward Jenner, who noticed that milkmaids, who had contracted cowpox disease were immune to smallpox, a very disastrous disease and thus rationalized the concept of introducing fluid from a cowpox lesion/papule into the people that might protect them from smallpox. In science serendipity and intuitive observation led to a major advancement in immunology, the induction of immunity to cholera. Louis Pasture, who successfully inoculated chickens with bacteria causing cholera and

observed that attenuated strain administered to chickens, protected them against the disease. He called this attenuated strain a vaccine, (from a Latin word *vacca* meaning cow) in honour of Edward Jenner. Pasture extended his work to other diseases and concluded that administering an attenuated or weakened pathogen as a vaccine is possible to protect against various diseases.

In 1881 Pasture first vaccinated sheep with heat-attenuated anthrax bacillus (Bacillus anthrax) and all vaccinated sheep lived. In 1885, Pasture first administered a young boy bitten repeatedly by a rabid dog. The boy was inoculated by a series of attenuated rabies virus preparations. The boy named Joseph Meister, survived and later became a custodian at the Pasture institute.

Figure 1.2. Louis Pasture.

EARLY STUDIES

Discovery of Phagocytosis and Cell-Mediated Immunity

In 1883, a Russian scientist Elie Metchnikoff while studding infection of the common water-flea (Daphnia) by a fungus demonstrated that certain white blood cells, which he termed phagocytes, were able to ingest microbial pathogens and other foreign substances. This was the first demonstration of the process of phagocytosis.

Noting that these phagocytic cells were more active in immunized animal and contribute to its immune state, Metchnikoff hypothesized that cells were the major effector of immunity and gave the concept of cell-mediated immunity.

Discovery of Humoral Immunity

Although Pasteur was the first to prove that vaccination worked, he didn't understand how. It was the experimental work of Emil von Behring and Shibasaburo Kitasato in 1890 that gave the first insights into the mechanism of immunity. Von Behring and Kitasato demonstrated that serum from animals previously immunized to diphtheria could transfer the immune state to unimmunized animals. Von Behring's work earned him a Nobel Prize in medicine in 1901.

During the next decade, numerous scientists demonstrated that an active component from immune serum could neutralize toxins, precipitate toxins and agglutinate bacteria, however, humoral immunity was fully confirmed only during 1930s mainly through the efforts of Elvin Kabat who demonstrated that a fraction of serum first called gamma-globulin (now called immunoglobulin or antibodies) was responsible for immunity acquired following immunization. The active molecules in the immunoglobulin fraction are called antibodies and because immunity was mediated by antibodies contained in body fluids, known as humors at that time, it was called humoral immunity.

Controversy over Cell-Mediated and Humoral Immunity

In due course of time, a controversy developed between those who held to the concept of humoral immunity and those who agreed with Metchnikoff's concept of cell-mediated immunity since it was difficult to study the activities of immune cells before the development of modern tissue culture techniques. The studies with serum could be readily done given the easy availability of blood and already established biochemical techniques. Although both studies were eventually proven to be correct, the lack of necessary technology required to study about cellular immunity is why information about it lagged behind the findings that concerned humoral immunity.

Cell-Mediated and Humoral Immunity Co-Exist

The controversy regarding the roles of cell-mediated and humoral immunities finally, came to an end in 1940s and 1950s when the two systems were found to be interrelated and necessary for the immune response. This became possible mainly by the contributions of Merill Chase, Bruce Glick and others. In a key experiment in the1940s, Merill Chase successfully transferred immunity against tuberculosis-organism by transferring white blood cells between guinea pigs. This demonstration helped rekindle the interests in cellular immunity.

The identification of lymphocytes as the cells responsible for both cell-mediated and humoral immunity during 1950s by using improved tissue culture techniques, and the existence of two types of lymphocytes (B lymphocytes and T lymphocytes) demonstrated by Bruce Glick during his experiments with chickens were the major breakthroughs in solving the controversy. B. Glick advocated that B lymphocytes are involved in humoral immunity, whereas T lymphocytes play role in cell-mediated immunity.

Immunity Also Reacts to Non-Pathogenic Substances

Around 1900, Jules Bordet, at the Pasteur Institute, demonstrated that the immunity also reacts to non-pathogenic substances such as red blood cells from other species. Serum taken from an animal inoculated previously with non-pathogenic material reacts with the latter in a specific manner, and this reactivity could be passed to other animals by transferring serum from the first.

Karl Landsteiner and others demonstrated that inoculating an animal with almost any organic chemical induces production of antibodies that would react specifically to the chemical. These demonstrations made clear that the antibodies possess ability for an almost unlimited range of reactivity. Also, Landsteiner discovered ABO blood groups which was used for successful blood transfusions in humans. This sensational discovery won Landsteiner a Nobel Prize in 1930.

Concept of Selective Theory

In 1900, Paul Ehrlich proposed the concept of selective theory. He demonstrated that cells in the blood expressed a variety of receptors on their surface, which he called “side-chain receptors.” Ehrlich explained that the receptors react specifically with pathogenic agents and inactivate them.

In addition, such an interaction induces the cell to produce and release more receptors with the same specificity. He also proposed that the specificity of the receptor was determined before its exposure to the antigen, and the antigen selected the appropriate receptor.

Clonal Selection Theory

The selective theory of Ehrlich was proved to be essentially correct by experimental data of Niels Jerne, David Talmadge, and E. Macfarlane

Burnet collected during 1950s. But these workers refined the selective theory and named it clonal selection theory.

Clonal selection theory explains that when an individual lymphocyte binds to an antigen on its specific receptor site, the binding activates the lymphocyte causing it to proliferate into a clone of cells that have the same immunologic specificity as the parent cell. The clonal selection theory has been further refined and is now accepted as the underlying paradigm of modern immunology.

Clonal selection theory has been widely accepted and in recognition Burnet was awarded Nobel Prize in 1960, which he shared with Sir Peter Medawar. Twenty-four years later in 1984, Niels Jerne was also awarded the Nobel Prize for his contributions, which led to the discovery of clonal expansion concept and evaluation of the idiotype network in the regulation of immune responses.

Structure of Antibody

In their pioneer work, Rodney R. Porter and Gerald M. Edelman demonstrated through enzyme cleavage experiments that the four polypeptide chains of antibody (immunoglobulin) molecule can be cleaved into three pieces, i.e., two antibody fragments (Fab) and one crystallisable fragment (Fc).

For this contribution. Porter and Edelman were awarded the Nobel Prize in 1972. Subsequently, Wu and Kabat demonstrated in 1970 that there occur hypervariable regions in the antibody molecule.

Major Histocompatibility Complex (MHC)

Peter Gorer in the mid-1930s developed the concept that the rejection of foreign tissue takes place due to an immune response to cell-surface molecules; the molecules now called histocompatibility antigens. During

these studies, Gorer identified four groups of genes, designated I through IV that encoded blood-cell antigens.

Gorer and Snell carried out the work in 1940s and 1950s and demonstrated that antigens encoded by the genes in the group designated II participated in the rejection of transplanted tumours and other tissue. Snell called these genes as histocompatibility genes. Although Gorer died before his contributions were recognised fully, Snell was awarded the Nobel Prize in 1980 for this landmark contribution along-with Dausset and Benacerraf who worked on human leucocyte antigen (HLA) complexes.

Technique of Somatic Hybridization

A major breakthrough was witnessed in 1975, when George Kohler and Ceaser Milstein demonstrated a technique of somatic hybridization and used it to produce immunologically homogenous monoclonal antibodies. Monoclonal antibody producing technique popularized by the name hybridoma technology. Kohler and Milstein were awarded the Nobel Prize in 1984 for their brilliant contribution.

The above mentioned important historical contributions led to rapid progress in immunology, which became instrumental in developing various fields of medical microbiology such as organ and tissue transplantation, vaccinology, and molecular biology. The table below lists the workers who have received Nobel Prize for their significant contributions to immunology.

MILESTONES IN THE HISTORY OF IMMUNOLOGY INCLUDE

- 1798 Edward Jenner initiates smallpox vaccination.
- 1877 Paul Erlich recognizes mast cells.
- 1879 Louis Pasteur develops an attenuated chicken cholera vaccine.

- 1883 Elie Metchnikoff develops cellular theory of vaccination.
- 1885 Louis Pasteur develops rabies vaccine.
- 1891 Robert Koch explored delayed type hypersensitivity.
- 1900 Paul Erlich theorizes specific antibody formation.
- 1906 Clemens von Pirquet coined the word allergy.
- 1938 John Marrack formulates antigen-antibody binding hypothesis.
- 1942 Jules Freund and Katherine McDermott research adjuvants.
- 1949 Macfarlane Burnet & Frank Fenner formulate immunological tolerance hypothesis.
- 1959 Niels Jerne, David Talmage, Macfarlane Burnet develop clonal selection theory.
- 1957 Alick Isaacs & Jean Lindemann discover interferon (cytokine).
- 1962 Rodney Porter and team discovery the structure of antibodies.
- 1962 Jaques Miller and team discover thymus involvement in cellular immunity.
- 1962 Noel Warner and team distinguish between cellular and humoral immune responses.
- 1968 Anthony Davis and team discover T cell and B cell cooperation in immune response.
- 1974 Rolf Zinkernagel and Peter Doherty explore major histocompatibility complex restriction.
- 1985 Susumu Tonegawa, Leroy Hood and team identify immunoglobulin genes.
- 1987 Leroy Hood and team identify genes for the T cell receptor.
- 1985 Scientists begin the rapid identification of genes for immune cells that continues to the present.

SCOPE OF IMMUNOLOGY

Our knowledge of the immunological processes underlying the reactions of the body to infectious agents, to tumours, and to transplanted tissues and organs has advanced remarkably by using modern techniques, including those developed by biochemists and molecular biologists. These techniques have enabled the identification of genes coding for molecules like the T-cell receptor and MHC molecules. The genes coding for immunologically important molecules have been cloned and relatively large amounts of pure recombinant proteins have been produced. It is now possible to culture many different cell types in vitro and to clone these cells so that a population with an identical genetic makeup is obtained.

Many different strains of inbred mice, including 'knock-out' and 'knock-in' mice, have been developed for the investigations of cellular interactions, gene inactivation's, etc. The role of a number of gene products has been elucidated by producing transgenic animals and studying the effect of the introduced genes.

Immunologists have developed many new techniques, including novel ways of producing a homogeneous immunoglobulin preparation, viz., monoclonal antibody, by using impure antigens. The development of these strictly defined reagents revolutionized immunoassays and detection systems that employ antibodies. Their potential in the treatment of infectious diseases, cancer patients, organ transplants, etc. is being actively investigated and a number of clinical trials have been performed. In addition to antibodies, other immunologically important molecules have been produced and are being developed as therapeutic agents.

The introduction of flow cytometry has revolutionized the analysis of cell populations and the use of polymerase chain reaction has increased the sensitivity of the detection of microorganisms. The interplay between cells and molecules of the immune system is extremely complex. We are only now beginning to understand the intricacies of immune recognition. Some molecules appear to have many different functions depending on their location or the presence of other molecules. The possibility of harnessing

these powerful reagents to aid the elimination of not only pathogenic microorganisms, but also cancer cells is being actively pursued.

The ability to predict the minimum structures that can induce protective immunity will allow the development of more effective and safer vaccines. It may also become possible to develop novel ways of treating autoimmune diseases, allergic conditions and tumours, and to develop new strategies to reduce transplant rejection. Thus, it can be seen that immunity in its original meaning, referring to resistance to infections by means of a specific immune response, is only one activity of a complex system in animals.

The total activity of the cellular system is concerned with mechanisms for preserving the integrity of the individual with far-reaching implications in embryology, genetics, cell biology, tumour biology and many non-infectious disease processes.

CONCLUSION

The word immunology owes its origin to the Latin words *Immunitas*and *Immunis* however neither of these words are even remotely related to our present day understanding of immunology. Initially, in Rome, these words implied "exemption of an individual from service or duty." Later, during the middle Ages, these words came to mean, "Exemption of the church and its properties." According to A.M Silverstein's book, The History of Immunology, it was the Roan poet, Marcus AnnaeusLucanus who first applied the word Immunes in the present-day biological context to describe the resistance to snake bite of the Psylii tribe of North Africa in his poem Pharsalia. Later, in the 14th Century, Colle used the word to describe his escape from a plague epidemic. The word immunology attained currency only in the 19th century, following the rapid spread of Edward Jenner's historic technique for small pox vaccination.

FREQUENTLY ASKED QUESTIONS (FAQS)

Q1. Where does the word immunology come from and by whom was it first used in its present day meaning?

Answer: The word immunology owes its origin to the Latin words Immunitas and Immunis which mean exemption from service. It was the Roan poet, Marcus Annaeus Lucanus who first applied the word Immunes in the present day biological context to describe the resistance to snake bite of the Psylii tribe of North Africa in his poem Pharsalia.

Q2. Briefly explain about Pasteur's contributions to immunology.

Answer: Louis Pasteur is considered as the progenitor of modern immunology because of his studies in the late nineteenth century that popularized the germ theory of disease, and that introduced the hope that all infectious diseases could be prevented by prophylactic vaccination, as well as also treated by therapeutic vaccination, if applied soon enough after infection.

Q3. How was the controversy regarding the roles of cell-mediated and humoral immunities solved and by whom?

Answer: The controversy regarding the roles of cell-mediated and humoral immunities came to an end in 1940s and 1950s when the two systems were found to be interrelated and necessary for the immune response. This became possible mainly by the contributions of Merill Chase, Bruce Glick and others. The identification of lymphocytes as the cells responsible for both cell-mediated and humoral immunity during 1950s by using improved tissue culture techniques, and the existence of two types of lymphocytes (B lymphocytes and T lymphocytes) demonstrated by Bruce Glick during his experiments with chickens were the major breakthroughs in solving the controversy.

Q4. How is adaptive immunity different from innate immunity?

Answer: Innate immunity refers to nonspecific defense mechanisms that come into play immediately or within hours of an antigen's appearance in the body. These mechanisms include physical barriers such as skin, chemicals in the blood, and immune system cells that attack foreign cells in the body. The innate immune response is activated by chemical properties of the antigen.

Adaptive immunity refers to antigen-specific immune response. The adaptive immune response is more complex than the innate. The antigen first must be processed and recognized. Once an antigen has been recognized, the adaptive immune system creates an army of immune cells specifically designed to attack that antigen. Adaptive immunity also includes a "memory" that makes future responses against a specific antigen more efficient.

Q5. What does the theory of clonal selection say?

Answer: The clonal selection theory is a hypothesis which states that individual B-cell lymphocytes express a receptor that is antigen-specific. This would be determined before the antibody ever encounters the antigen. Activation occurs within the lymph nodes, spleen, or similar lymphoid organs, which then encourages cloning, so that each individual cell is able to target an individual antigen with effectiveness.

It can be summarized through these four key points.

1. Every lymphocyte offers a single receptor that has an individualized unique specificity.
2. For cell activation to occur, receptor occupation is required.
3. Every differentiated effector cell that has been derived from a lymphocyte which has been activated will bear a receptor that is identical in specificity as its parent cell.
4. Lymphocytes that bear receptors for self-molecules are going to be deleted, primarily at an early stage.

This theory of antibody product was first proposed in 1957 by Dr. Frank Burnet, an Australian doctor who was attempting to explain how a diverse array of antibody was able to be produced during an immune response.

Q6. When was the antibody structure discovered? What is an antibody?

Answer: The earliest reference to antibodies came from Emil von Behring and Shibasabura Kitasato in 1890. In a landmark publication they showed that the transfer of serum from animals immunised against diptheria to animals suffering from it could cure the infected animals. In 1900 Paul Ehrlich, who is regarded as one of the fathers of modern immunology, proposed the side-chain theory, where he hypothesised that side chain receptors on cells bind to a given pathogen. Gerald Edelman and Rodney Porter independently published the molecular structure of antibodies in 1959.

Antibodies, also known as immunoglobulins, are Y-shaped proteins that are produced by the immune system to help stop pathogens from harming the body.

Q7. When can antibodies be referred to as monoclonal antibodies?

Answer: Originally discovered by Emil von Bering and Paul Erhlich, antibodies are proteins produced by the immune system that target a specific foreign object (antigen). They are called monoclonal Antibodies (mAbs) when they are produced by clones derived from a single parent cell. Monoclonal antibodies have a high affinity for their epitope, the specific site of the protein they bind to

Q8. What is the role of MHC?

Answer: Major histocompatibility complex (MHC), is a group of genes that code for proteins found on the surfaces of cells that help the immune system recognize foreign substances. MHC proteins are found in all higher vertebrates. MHC molecules were initially defined as antigens that stimulate an organism's immunologic response to transplanted organs and tissues. The 1950s skin graft experiments

carried out in mice showed that graft rejection was an immune reaction mounted by the host organism against foreign tissue. The host recognized the MHC molecules on cells of the graft tissue as foreign antigens and attacked them.

Q9. What is selective theory?

Answer: Selective theory stated that an antigen reacts with an already-existing antibody. It was proposed by Paul Ehrlich in 1900s. He demonstrated that cells in the blood expressed a variety of receptors on their surface, which he called "side-chain receptors."

Q10. What do you understand by immunologic tolerance?

Answer: immunological tolerance, or immunotolerance, is a state of unresponsiveness of the immune system to substances or tissue that have the capacity to elicit an immune response in given organism. It is induced by prior exposure to that specific antigen and contrasts with conventional immune-mediated elimination of foreign antigens.

Q11. What are antigens?

Answer: These are substances that provoke an immune response (they attack the immune system). Although a substance that induces a specific immune response is usually called an antigen, it is more appropriately called an immunogen. Antigens can be bacteria, viruses, or fungi that cause infection and disease. They can also be substances, called allergens that bring on an allergic reaction.

Q12. What is cellular immunity?

Answer: Cellular immunity is a protective immune process that involves the activation of phagocytes, antigen-sensitized cytotoxic T cells and the release of cytokines and chemokines in response to antigen. Cellular immunity is most effective against cells infected with viruses, intracellular bacteria, fungi and protozoans, and cancerous cells. It also mediates transplant rejection.

Q13. What do you understand by humoral immune response?

Answer: The immune response involving the transformation of B cells into plasma cells that produce and secrete antibodies to a specific antigen or a form of immunity mediated by circulating antibodies (immune-globulins IgA, IgB, and IgM), which coat the antigens and target them for destruction by polymorphonuclear neutrophils. Circulating antibodies are produced by plasma cells of the reticuloendothelial system.

Q14. What is immune response?

Answer: The immune response is the way in which your body recognizes and defends itself against bacteria, viruses, and other substances that are foreign and harmful. It is the job of the immune system to protect our bodies from harmful invaders by recognizing and responding to antigens. Typically, proteins, antigens reside of the surface of cells, viruses, fungi, or bacteria, but antigens also may be non-living substances including toxins, chemicals, drugs, and foreign particles like splinters. The immune system remembers, recognizes, and destroys antigen-containing substances.

Q15. What constitutes the second line of defence in an immune response?

Answer: When microbes enter the body, the immune response activates the body's second line of defence: phagocytes. Phagocytes, a type of white blood cell, move around and send out pseudopodia, allowing them to surround invading microbes and engulf them. Phagocytes also release digestive enzymes that break down trapped microbes, preventing them from doing harm. This part of the immune response is called phagocytosis.

MULTIPLE CHOICE QUESTIONS (MCQS)

Q1. Which of the following constitute the first Line of Defence?

A. Immune Response
B. Skin and Mucous membranes

C. Inflammatory response
D. Inflammatory response and skin and mucous membranes

Q2. The term vaccine was first used for:
A. Fowl cholera bacilli
B. Fluid from cowpox pustule
C. Crusts from smallpox vesicle
D. None of the above

Q3. The ancient practice of introducing pathogenic smallpox crusts into the host body is called as:
A. Vaccination
B. Attenuation
C. Activating immune system
D. Boosting immunity

Q4. Which of the following does not protect body surfaces?
A. Skin.
B. Mucus
C. Gastric acid
D. Salivary amylase

Q5. Which system of the body causes allergic reactions?
A. Circulatory
B. Immune
C. Lymph
D. Autoimmune

Q6. Innate immunity is present:
A. Naturally
B. Transferred from the mother to the baby
C. Adapted over time
D. Acquired due to an infection

Q7. Cells involved in secondary defence are:

A. Skin cells
B. Epithelial Cells
C. Phagocytes
D. Mucosal cells

Q8. In case of autoimmune diseases, the immune system:

A. Gets compromised
B. Isn't as efficient as a healthy immune system
C. Can't fight pathogens
D. Attacks your own body

Q9. MHC refers to:

A. Major histone complex
B. Minor histocompatibility complex
C. Major histocompatibility complex
D. Maximum histone concentration

Q10. Without an immune system to defend us,

A. We would fall sick often
B. Wouldn't be able to fight infections
C. Would be more proven to diseases
D. All of the above

Q11. The two types of immunity in humans are:

A. Innate and the acquired.
B. Overt and covert.
C. Internal and external.
D. Intrinsic and extrinsic.

Q12. Another name for innate immunity is:

A. immunity
B. explicit immunity
C. non-specific immunity.

D. specific immunity.

Q13. With acquired immunity the body fails to achieve specific immunity to a specific threat.

A. True
B. False

Q14. The acquired immune system is based upon the lymphocytes.

A. True
B. False

Q15. The two types of lymphocytes are:

A. B-cells and the T-cells.
B. Platelets and the T-cells.
C. T-cells and erythrocytes.
D. Platelets and erythrocytes.

Answers Key

1. B **2**. C **3**. A **4**. D **5**. B **6**. A **7**. C **8**. D **9**. C **10**. D **11**. A **12**. C **13**. B **14**. A **15**. A

ASSIGNMENTS

Long Answer Questions (500 Words)

Q1. Write a brief note on the scope of immunology and the current research in the field.

Q2. Specific immunity exhibits four characteristic attributes, which are mediated by lymphocytes. List these four attributes and briefly explain how they arise.

Short Answer Questions (200 Words)

Q1. Innate and adaptive immunities act in collaboration. Discuss in Brief.

Q2. Adaptive immunity has evolved in vertebrates but they have also retained innate immunity.

What would be the disadvantage of having only adaptive immunity?

Q3. Give examples of mild and severe consequences of immune dysfunction.

REFERENCES

Abbas, A. K., Lichtman, A. H., Pillai, S. *Basic Immunology E-Book: Functions and Disorders of the Immune System.* Elsevier Health Sciences; 2015 Oct 7.

Austyn, J. M., Wood, K. *Principles of cellular and molecular immunology.* Revista do Instituto de Medicina Tropical de São Paulo. 1994 Jun;36(3):224-.

Bellanti, J, editor. *Immunology.* Springer Science & Business Media; 2013 Nov 11.

Clark, W. R. *The experimental foundations of modern immunology.* Wiley; 1991 Jan 24.

Good, R. A. Cellular immunology in a historical perspective. *Immunological reviews.* 2002 Jul; 185(1):136-58.

Lamont, RJ, Burne R. A., Lantz, M. S., LeBlanc DJ. *Oral microbiology and immunology.* ASM press; 2006.

Mazumdar, PM. *Species and specificity: an interpretation of the history of immunology.* Cambridge University Press; 2002 Jul 18.

Nisonoff A. *Introduction to molecular immunology.* Sinauer; 1983.

Parish, C. R. Immune deviation: a historical perspective. *Immunology and cell biology.* 1996 Oct; 74(5):449-56.

Sakaguchi, S., Wing, K., Miyara, M. Regulatory T cells–a brief history and perspective. *European journal of immunology*. 2007 Nov; 37(S1): S116-23.

Silverstein, A. M. *A history of immunology*. Academic Press; 2009 May 30.

Simpson, E. A. historical perspective on immunological privilege. *Immunological reviews.* 2006 Oct; 213(1):12-22.

Stähelin, H. Historical background. In *Ciclosporin* 1986 (Vol. 38, pp. 19-27). Karger Publishers.

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Chapter 2

CELLS OF THE IMMUNE SYSTEM

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ABSTRACT

Immune system is a complex network of cells within our body that help fight against diseases and protect body from a number of unwanted microbial invasions. The Immune system comprises of different cells that have been found to play a very critical role in defense against pathogens. The Immune cells, primarily: neutrophils, basophils, eosinophils, macrophages, T-lymphocytes, B-lymphocytes, dendritic cells, NK cells, etc. are involved in fortifying our body against attack. Immune system is fundamental to host defense against pathogenic invaders. It's a complex system involving interaction amongst many different cell types dispersed throughout the body. The cells responsible for immune system are blood

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cells called leucocytes (WBC's) that originate from hematopoietic stem cell (HSC). The hematopoietic stem cell differentiates into two pathways giving rise to either: lymphoid progenitor cell or myeloid progenitor cell.

Keywords: immune cells, haematopoiesis, progenitor cell, lymphocytes, eosinophils, neutrophils

OBJECTIVES

- Different types of immune cells.
- Describe lymphoid progenitor cells and myeloid progenitor cells.
- Define B-lymphocytes and T-lymphocytes.
- Types of granulocytes.

INTRODUCTION

Animals have developed a remarkably versatile defense system that protects them from invading pathogenic microorganisms. The immune system consists of a variety of cells and molecules capable of specifically recognizing and eliminating an apparently limitless variety of foreign pathogens. The cells and molecules act together in a dynamic network. The immune system is the defensive system in a host consisting of widely distributed cells, tissues and organs found throughout the body that recognize foreign substances and pathogens and acts to neutralize or destroy them. The cells responsible for both specific and nonspecific immunity are the leukocytes or white blood cells. All blood cells arise from a pluripotent cell called the hematopoietic Stem cell (HSC). Stem cells are self-renewing cells, maintain their population by cell division and can differentiate into other cell types. In humans, haematopoiesis, the formation and development of all blood cells, begins in the embryonic yolk sac during the first weeks of development. Hematopoietic stem cells during the third month of gestation migrate from the yolk sac to the foetal liver

and then to the spleen. From the third to the seventh month of gestation liver and spleen have major roles in haematopoiesis. After the seventh month of gestation, differentiation of hematopoietic stem cells in the bone marrow becomes the major factor in haematopoiesis, and by birth there is little or no haematopoiesis in the liver and spleen. During haematopoiesis, the hematopoietic stem cell differentiates along two pathways, giving rise to either a common *lymphoid progenitor cell* or a common *myeloidprogenitor cell*. The myeloid and the lymphoid progenitor cells lose their capacity for self-renewal and are committed to a particular cell lineage.

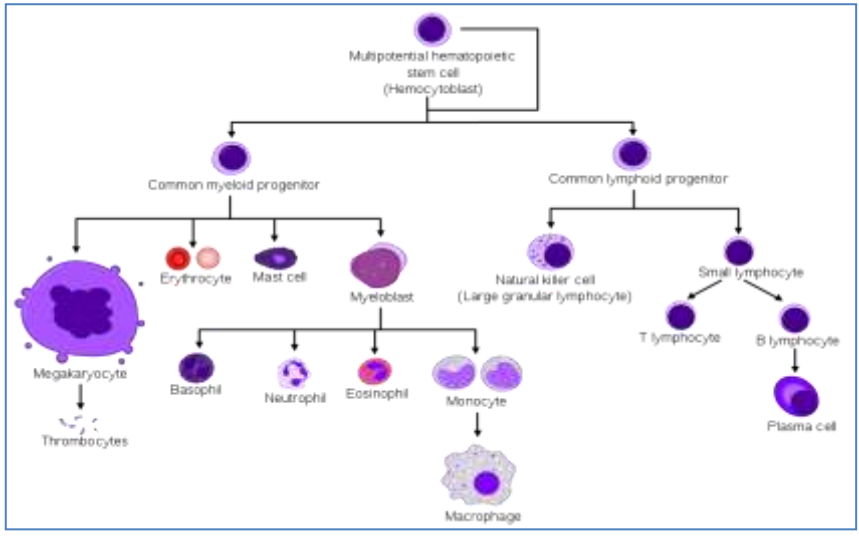


Figure 2. Schematic representation of Hematopoietic Stem Cells in Bone marrow.

Common lymphoid progenitor cells give rise to lymphocytes such as B-cells, T-cells, natural killer cells and some dendritic cells. The common myeloid progenitor cells generate progenitors of red blood cells, white blood cells (neutrophils, eosinophils, basophils, monocytes, mast cells, dendritic cells) and platelets. The commitment of the progenitor cells is dependent on the acquisition of responsiveness to particular growth factors and cytokines. Each of the leukocytes produced during haematopoiesis has

a characteristic life span and then undergo apoptosis. The life span varies from days like neutrophils to few months like red blood cells.

LYMPHOID PROGENITOR

The lymphoid progenitor cells give rise to mononuclear cells called lymphocytes which are the central cells of the immune system, responsible for the adaptive immunity and the immunological attributes of diversity, specificity, and memory and self/non-self-recognition. These cells constitute 20% - 40% of the body's white blood cells and 99% of the cells in the lymph. These cells circulate in blood and lymph and are capable of migrating into the tissue spaces and lymphoid organs, thereby integrating the immune system to a high degree. On the basis of function and cell-surface markers, lymphocytes can be broadly subdivided into three cell populations- B-lymphocytes or B-cells, T-lymphocytes or T-cells and Natural killer cells.

B Lymphocytes

B-cells derive its name from the site of its maturation. In humans and many other mammalian species bone marrow is the major site of B cell maturation, while in birds B cells mature in the Bursa of Fabricius. These are the only cells capable of producing antibody molecules and constitute the central cellular component of humoral immune response. After interacting with an antigen, B-cells differentiate into antibody secreting *Plasma cells* and *Memory cells.*

T Lymphocytes

T-cells arise in the bone marrow and derive their name from their site of maturation in the thymus. During its maturation in thymus, T-cells come

to express a unique antigen binding molecule, called the *T-cell receptor*. T-cell receptor shares some structural features with the immunoglobulin molecule but unlike B-cell receptor, T-cell receptor does not recognize free antigen. T-cell receptor recognizes only those antigens that are bound to a particular class of molecules expressed by self-cells. T-cells express distinctive membrane molecules. On the basis of one or the other of two membrane molecules, $CD4^+$ and $CD8^+$, there are two major sub-populations of T-cells: T-helper cells (T_H) carries$CD4^+$ membrane glycoprotein on their surfaces and T-cytotoxic cells (Tc) carries $CD8^+$ membrane glycoprotein on their surfaces.

The T_H cells can be activated and differentiate into a variety of effector T-cell subsets – T_{H1}, T_{H2}, T_{H17} and T_{FH}. T_{H1} cells regulate the immune responses to intracellular pathogens and T_H2 cells regulate the response to many extracellular pathogens. The T_{H17} secrete IL17, play an important role in *cell mediated immunity* and also act against fungal infections. T_{FH} (T follicular helper cells) play an important role in humoral immunity and regulate B-cell development in germinal centres.

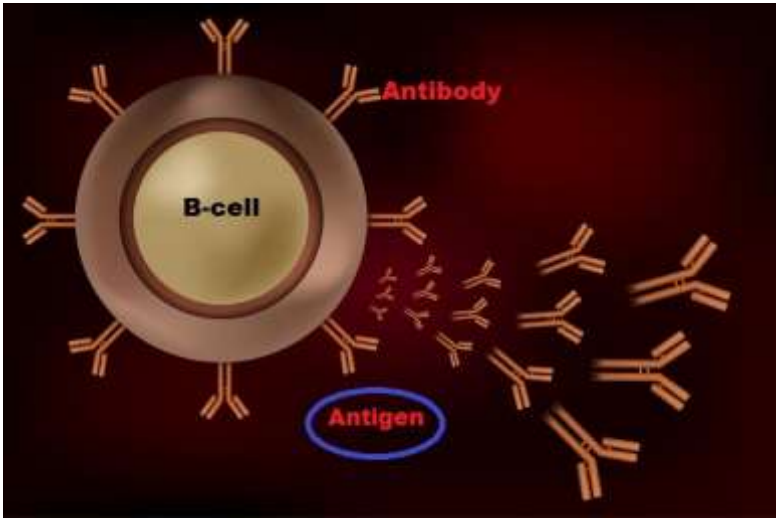


Figure 2.1. B-lymphocytes, antibodies can attach to B-cells, and serve to recognise foreign antigens.

Cytotoxic T-cells provide protection against intracellular pathogens such as viruses and some bacteria and parasites that multiply in the host-cell cytoplasm, where they are sheltered from attack by antibodies. These cells kill the infected cell before the microbes can proliferate and escape from the infected cell to infect neighbouring cells. When exposed to infected cells, cytotoxic T-cells release the cytokines, perforin, granzymes and granulysin. Through the action of perforin and granzymes enter the cytoplasm of the target cell and their serine protease function triggers apoptosis in the target cell.

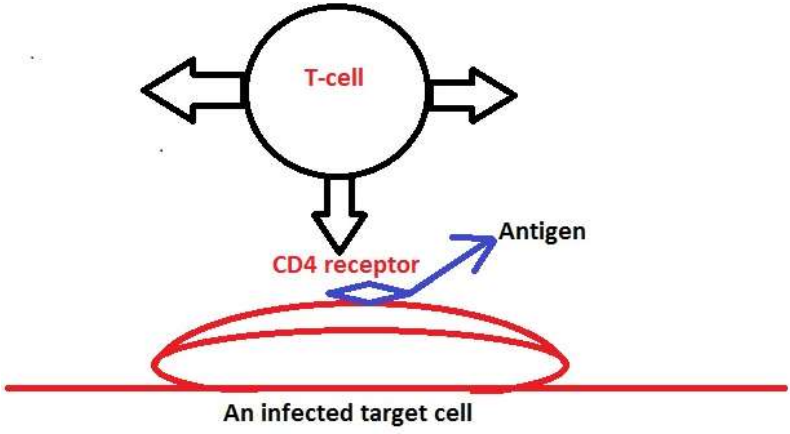


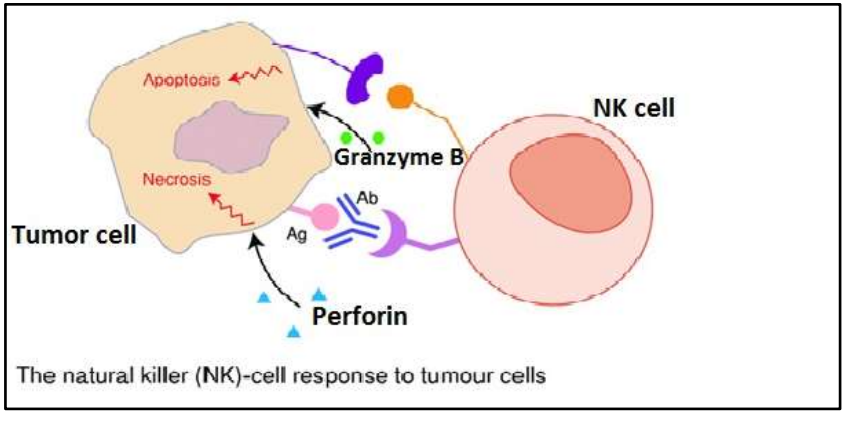
Figure 2.2. T-lymphocytes.

Natural Killer Cells

Natural killer cells (NK cells) constitute 5-10% of lymphocytes in human peripheral blood and do not express the membrane molecules and receptors that distinguish T- and B-cell lineages. NK cells are distinct from cytotoxic T-cells and play an important role in destroying cells infected with intracellular pathogens and also tumour cells in the absence of any previous immunization against the tumour. NK cells distinguish abnormalities like reduction in the display of class I MHC molecules and the unusual antigens displayed by some tumour cells and cells infected

with viruses. NK cells also recognize potential target cells which depends upon the fact that some tumour cells and cells infected by certain viruses display antigens against which the immune system has made an antibody response, so that anti-tumor or anti-viral antibodies are bound to their surfaces. Because NK cells express CD16, a membrane receptor for the carboxyl-terminal end of the IgG molecule, called the Fc region, they can attach to these antibodies and subsequently destroy the targeted cells. This is an example of a process known as *antibody-dependent cell mediated cytotoxicity (ADCC).*

NK cells destroy the target cells not by phagocytosis but by releasing biologically potent molecules and resemble T_C cells with their ability to destroy the infected cells. For example, NK cells also use perforin and granzymes to kill their targets. Chediak-Higashi syndrome- an autosomal recessive disorder- is associated with a lack of NK cells. People with this syndrome have abnormalities pigmentation, intravesicular movement and peripheral neuropathy. An autosomal mutation in mice called *beige* lack NK cells; rendering these mutants more susceptible to tumor growth.



(Image source: https://www.researchgate.net).

Figure 2.3. Natural Killer (Cell (The natural killer (NK)-cell response to tumour cell).

Dendritic Cells

Dendritic cell as its name resembles that of nerve-cells are bone marrow derived cells that arise from hematopoietic stem cells via different pathways and in different locations. These cells descend through both the myeloid and lymphoid lineages. There are many types of dendritic cells, although most mature dendritic cells have the same major function, acting as potent antigen-presenting cells. Dendritic cells are classified into four types:

- Langerhans cells
- Interstitial dendritic cells
- Myeloid cells
- Lymphoid dendritic cells

All of these cells express high levels of both class II MHC molecules and members of the co-stimulatory B7 family. For this reason, they are more potent antigen-presenting cells than macrophages and B cells, both of which need to be activated before they can function as antigen-presenting cells (APCs). Immature or precursor forms of dendritic cells acquire antigen by phagocytosis or endocytosis; the antigen is processed, and mature dendritic cells present it to $T_{H.}$

Follicular Dendritic Cell

It's another type of dendritic cell that does not arise from the bone marrow and has a different function from the antigen presenting dendritic cells. These cells do not express class II MHC molecules and therefore do not function as antigen presenting cells for T_H- cell activation. These dendritic cells were named for their exclusive location in organized structures of the lymph node called lymph follicles, which are rich in B-cells. Although they do not express class II molecules, follicular dendritic cells express high levels of membrane receptors for antibody, which allow binding of antigen-antibody complexes. And this interaction of B-cells with a bound antigen can have important effects on B cell responses.

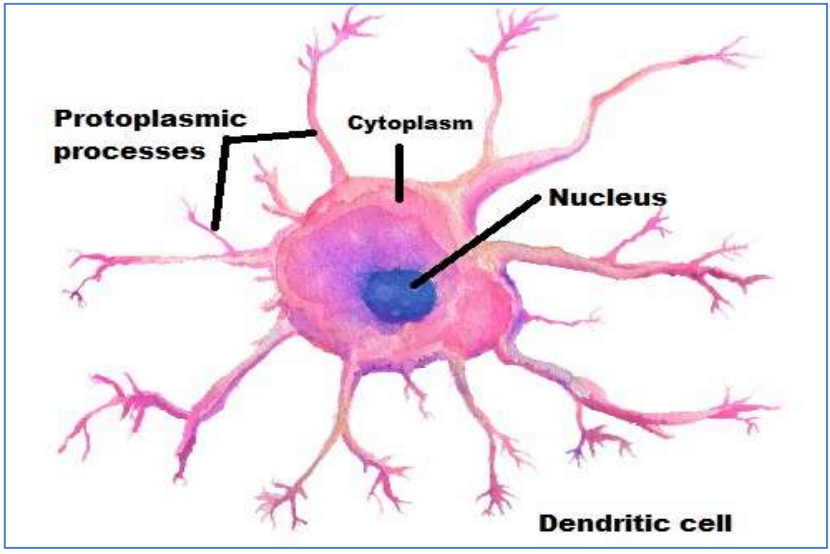


Figure 2.4. Dendritic cell.

MYELOID PROGENITOR

Cells that arise from the common myeloid progenitor include red blood cells (RBC's) as well as various types of white blood cells (WBC's) such as granulocytes, monocytes, macrophages and some dendritic cells.

Granulocytes

Granulocytic cells have irregular-shaped nuclei with two to five lobes and are often called as polymorphonuclear leukocytes. Some reactive molecules in their cytoplasmic matrix present inside these granules kill certain microorganism and enhance inflammation and allergic reactions. On the basis of cellular morphology these are classified as: neutrophils, eosinophils and basophils.

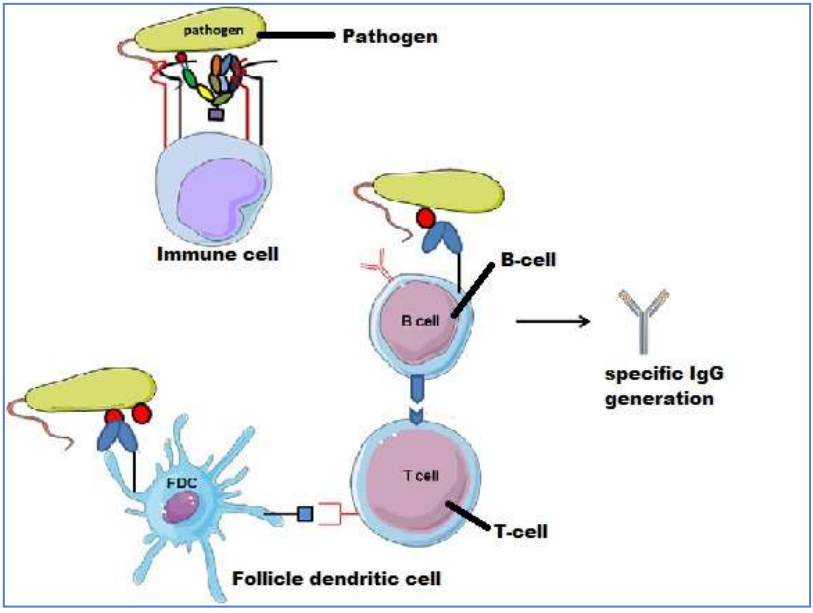


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Figure 2.5. Follicle dendritic cell.

Neutrophils

First responders at the site of infection or trauma, this abundant phagocytic cell represents 50 - 60% of leucocytes (WBC's) are produced in bone marrow and are released in the peripheral blood and circulate for 7-10 hours before migrating into the tissues, where they have a life span of only few days. In response to many types of infections, the bone marrow releases more than the usual number of neutrophils and these cells generally are the first to arrive at a site of inflammation. Releases toxins that kill or inhabit bacteria and fungi. It also recruits other immune cells to the site of infection. The neutrophils have a multi-lobed nucleus and a granulated cytoplasm that stains with both acidic and basic dyes. The resulting transient increase in the number of circulating neutrophils, called leukocytosis, is used medically as an indication of infection. Neutrophils accumulate at the site of inflammation in response to chemotactic factors

complement components of the blood-clotting system, and several cytokines secreted by activated T_H cells and macrophages.

Neutrophils are active phagocytic cells but unlike macrophages, the lytic enzymes and bactericidal substances in neutrophils are contained within primary and secondary granules. Neutrophils also employ both oxygen-dependent and oxygen-independent pathways to generate anti-microbial substances.

Eosinophils

Eosinophils have a distinct bilobed nucleus that stains with acidic dye eosin and express cell markers CD69 and CD44. These cells comprise 2% - 5% of WBCs and like neutrophils are motile phagocytic cells that can migrate from the blood into the tissue spaces. An increase in eosinophils, i.e., the presence of more than 500 eosinophils/microlitre of blood is called an eosinophilia, and is typically seen in people with a protozoan and helminth parasitic infection. Eosinophils persist in the circulation for 8 – 12 hours, and can survive in tissue for an additional 8 – 12 days in the absence of stimulation.

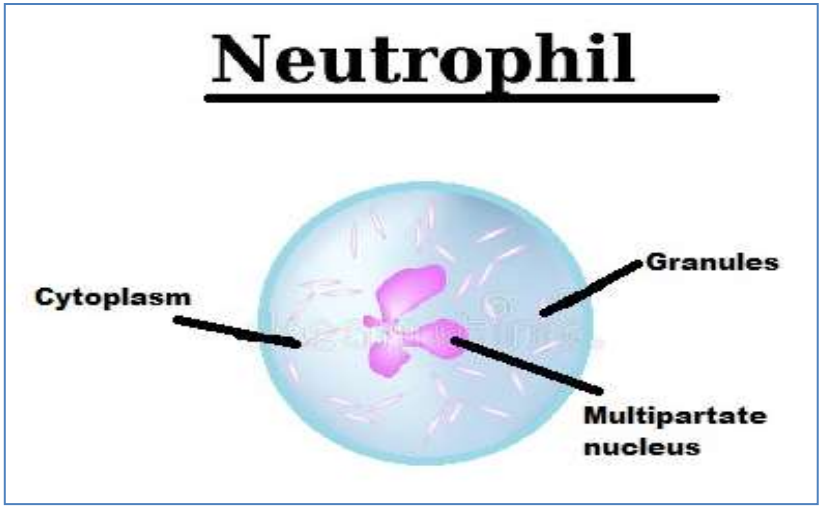

Figure 2.6. Neutrophil.

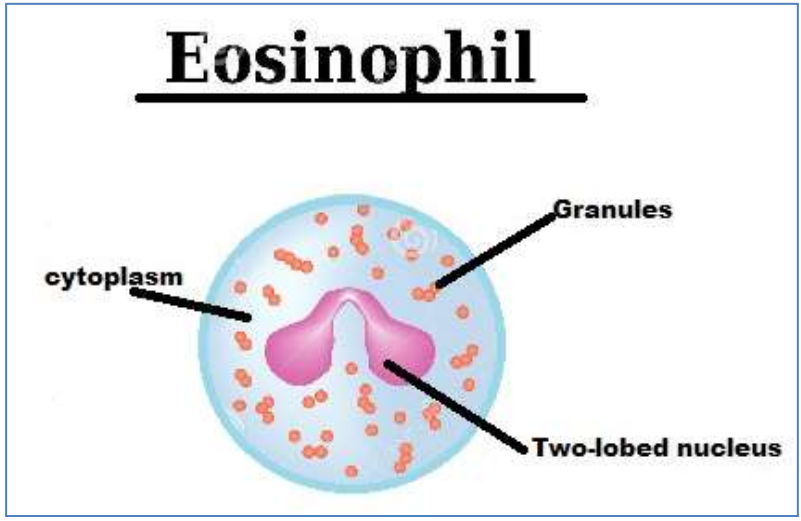

Figure 2.7. Eosinophil.

Following activation, eosinophils effector functions include production of:

- Cationic granule proteins and their release by degranulation.
- Reactive oxygen species such as hypobromite, superoxide, and peroxide (hypobromous acid, which is preferentially produced by eosinophil peroxidase).
- Enzymes, such as elastase.
- Growth factors such as TGFβ, VEGF, and PDGF.
- Cytokines such as IL-1, IL-2, IL-4, IL-5 and TNF alpha.

Basophils

Basophils have a lobed nucleus and stain with basic dye methylene blue and express cell surface marker CD164. Comprise less than 1% and are non-phagocytic granulocytes. They are responsible for inflammatory reactions during immune response, as well as in the formation of acute and chronic allergic diseases, including anaphylaxis, asthma; atopic dermatitis and hay fever. Basophils appear in many specific kinds of inflammatory

reactions, particularly those that cause allergic symptoms. Basophils contain anticoagulant heparin, which prevents blood from clotting too quickly. They also contain the vasodilator histamine, which promotes blood flow to tissues. They can be found in unusually high numbers at sites of ectoparasitic infection, e.g., ticks. Like eosinophils, basophils play a major role in both parasitic infections and allergies. They are found in tissues where allergic reactions are occurring and probably contribute to the severity of these reactions. Basophils have protein receptors on their cell surface that bind IgE, an immunoglobulin involved in macro parasite defense and allergy. A bound IgE antibody confers a selective response of these cells to environmental substances, for example, pollen proteins or helminth antigens. Recent studies in mice suggest that basophils may also regulate the behaviour of T cell and mediate the magnitude of the secondary immune response.

Basophils arise and mature in bone marrow. When activated, basophils degranulate to release histamine, proteoglycans (e.g., heparin and chondroitin), and proteolytic enzymes. They also secrete lipid mediators like leukotrienes (LTD-4), and several cytokines. Histamine and proteoglycans are pre-stored in the cell's granules while the other secreted substances are newly generated. Each of these substances contributes to inflammation. Recent evidence suggests that basophils are an important source of the cytokine, interleukin-4, perhaps more important than T cell. Interleukin-4 is considered one of the critical cytokines in the development of allergies and the production of IgE antibody by the immune system.

Mast Cells

Mast cell precursors are formed in the bone marrow by haematopoiesis and are released as undifferentiated cells into the blood circulation and differentiate once they enter the tissues. Mast cells can be found in a wide variety of tissues, including the skin, connective tissues of various organs, and mucosal epithelial tissue of the respiratory, genitourinary, and digestive tracts.

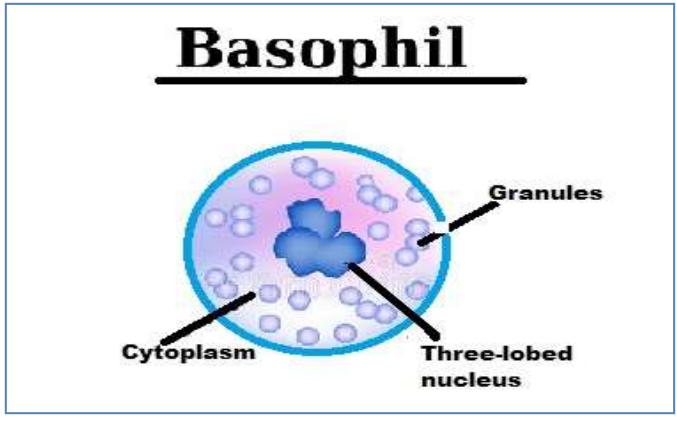

Figure 2.8. Basophils.

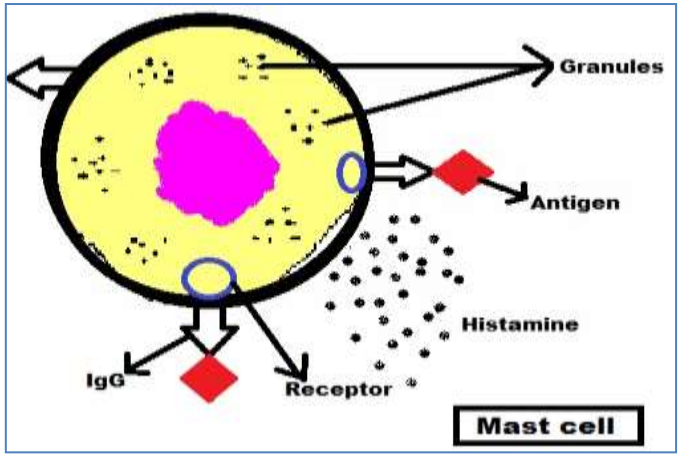

Figure 2.9. Mast cell.

Mast cells play a key role in the inflammatory process, dilation of blood vessels and induce inflammation through release of histamines and heparin. They are also involved in the recruitment of macrophages and neutrophils. When activated, a mast cell can either selectively release (piecemeal degranulation) or rapidly release (anaphylactic degranulation) "mediators," or compounds that induce inflammation, from storage granules into the local microenvironment. Mast cells are involved in

wound healing and defense against pathogens but can also be responsible for allergic reactions.

Monocytes

Monocytes are amoeboid mononuclear leucocytes (WBC's) having a granulated cytoplasm. Monocytes migrate into the tissues and differentiate into specific tissue macrophages and dendritic cells in response to inflammation. Monocytes are stored in spleen and move through blood vessels to infected tissues.

Macrophages

Macrophages are phagocytic cells derived from blood monocytes. Monocytes that migrate into tissues in response to infection consume foreign pathogens and cancer cells. Macrophages are dispersed throughout the body. They are of two types: fixed macrophages and wandering macrophages. Those take up residence in particular tissue, become fixed macrophages, while as others remain motile and become free, or wandering, macrophages. Free macrophages travel by amoeboid movement throughout the tissues. Macrophage-like cells serve different functions indifferent tissues and are named according to their tissue location. Example:

- Alveolar macrophages in the lung
- Histiocytesin connective tissues
- Kupffer cells in the liver
- Mesangial cells in the kidney
- Microglial cells in the brain
- Osteoclasts in bone

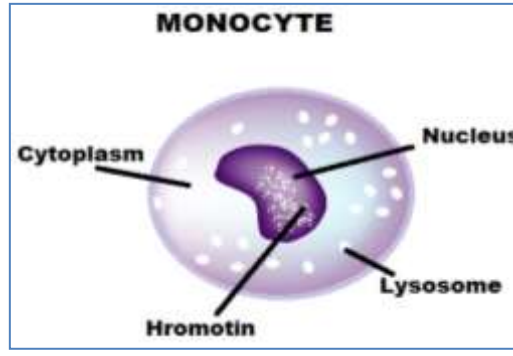

Figure 2.10. Monocyte.

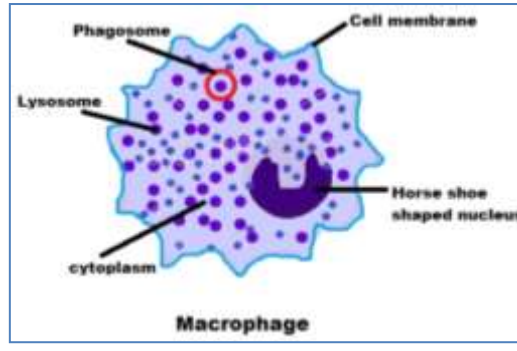

Figure 2.11. Macrophage.

Macrophage activity can be further enhanced by cytokines secreted from activated T_H cells, by mediators of the inflammatory response, and by components of bacterial cell walls. One of the most potent activators of macrophages is interferon gamma (IFN-γ) secreted by activated T_H cells. Activated macrophages have greater phagocytic activity, and secrete increased amounts of proteins central to the development of immune responses. These include a number of cytokines, such as interleukin 1 (IL-1), TNF-α and interleukin 6 (IL-6), that promote inflammatory responses. Typically, each of these agents has a variety of effects. For example, IL-1 activates lymphocytes; and IL-1, IL-6, and TNF-α promote fever by affecting the thermoregulatory centre in the hypothalamus. Cytotoxic molecules are also secreted by activated macrophages which eliminate a

broad range of pathogens, including virus- infected cells, tumor cells, and intracellular bacteria. Class II MHC expression also increases greatly after activation, allowing these cells to function more effectively as antigen presenting cells.

SUMMARY

- Most cells of the immune system derive from hemopoietic stem cells.
- Phagocytic cells are found in the circulation (monocytes and granulocytes).
- Development and differentiation of different cell lineages depend on cell interactions and cytokines.
- Each cell type expresses characteristic surface molecules (markers), which identify them.
- Eosinophils, basophils, mast cells, and platelets take part in the inflammatory response.
- NK cells recognize and kill virus-infected cells and certain tumor cells through apoptosis.
- B and - cells express antigen receptors, which are required for the antigen recognition.
- There are two major subpopulations of T cells, which have helper and cytotoxic activities.

FREQUENTLY ASKED QUESTIONS (FAQS)

Q1. Describe briefly the hematopoietic stem cell?

Answer. Multipotent hematopoietic cells in the bone marrow are a type of stem cells that can regenerate themselves. All the blood cells originate from the hematopoietic stem cell. These cells differentiate along two lineages; the common myeloid progenitor cell and the common lymphoid progenitor cell.

Q2. What types of cells originate from the common lymphoid progenitor cell?

Answer. The lymphoid progenitor cells give rise to mononuclear cells called lymphocytes which are the central cells of the immune system, responsible for the adaptive immunity and the immunological attributes of diversity, specificity and memory and self/nonself recognition. These cells constitute 20%-40% of the body's white blood cells and 99% of the cells in the lymph. Lymphocytes include B cells, T cells, NK cells and some dendritic cells.

Q3. What are the main features of B lymphocytes?

Answer. B cells derived its name from its site of maturation, in the bursa of Fabricius in birds. In humans and many other mammalian species bone marrow is the major site of B cell maturation. They are the only cells capable of producing antibody molecules and therefore the central cellular component of humoral immune response.

Q4. How do B cells recognize antigens?

Answer. The antibody molecules on the B cell membrane serve as receptors for antigens. This membrane bound immunoglobulin is also known as B cell receptor (BCR). B cells recognize both proteinaceous antigens and Lipopolysaccharide (LPS). The antigens recognized by these cells are soluble, unprocessed and in free form.

Q5. Describe briefly the function of B lymphocytes?

Answer. B cells constitute the humoral branch of adaptive immunity. These are the only cells which produce antibodies. These cells express a number of cell surface molecules like B220 frequently used as a marker for B cells. Class II MHC molecules are expressed by B cells which permit these cells to function as antigen presenting cells.

Q6. What are the main features of T lymphocytes?

Answer. T cells arise in the bone marrow and derive their name from their site of maturation in the thymus. During its maturation in the thymus, T cells comes to express a unique antigen binding molecular called the T- cell receptor.

Q7. Describe the types of T lymphocytes?

Answer. Two major subpopulations of T cells are defined by CD4 and CD8 expression. $CD4^+$T cells generally function as T helper (T_H) cells and are MHC class II restricted; $CD8^+$T cells function as cytotoxic T (T_C) cells and class-I restricted. Ratio of T_H to T_C cells in a sample can be approximated by assaying the number of $CD4^+$ and $CD8^+$ T cells, which is normally 2:1 in normal peripheral blood but is significantly altered during immune diseases.

Q8. Describe briefly the function of T_H lymphocytes?

Answer. The T_H cells can be activated and differentiate into a variety of effector T-cell subsets – T_H1, T_H2, T_H17 and TF_H. T_H1 cells regulate the immune responses to intracellular pathogens and T_H2 cells regulate the response to many extracellular pathogens. The T_H17 secrete IL17, play important role in cell mediated immunity and also act against fungal infections. T_{FH} (T follicular helper cells) play an important role in humoral immunity and regulate B-cell development in germinal centers.

Q9. Describe briefly the function of macrophages?

Answer. Macrophages are phagocytes derived from the blood monocytes. Monocytes that migrate into tissues in response to infection can differentiate into specific tissue macrophages. Macrophages are capable of ingesting and digesting exogenous antigens, such as whole microorganisms and insoluble particles and endogenous matter, such as injured or dead host cells, cellular debris and activated clotting factors. Macrophages are dispersed throughout the body.

Q10. How do T cells recognize antigens?

Answer. The antigen-recognition molecules of T cells are made solely as membrane-bound proteins and only function to signal T cells for activation. These T-cell receptors (TCRs) are related to immunoglobulin's both in their protein structure having both V and C regions and in the genetic mechanism that produces their great variability. However, the T-cell receptor differs from the B-cell receptor in an important way: it does not recognize and bind antigen directly, but instead recognizes short peptide fragments of pathogen protein antigens, which are bound to MHC molecules on the surfaces of other cells.

Q11. What are the distinct features of granulocytes?

Answer. The granulocytic cells have irregular-shaped nuclei with two to five lobes and are often called as polymorphonuclear leukocytes. These cells include neutrophils, basophils, eosinophils and mast cells. These cells contain secretory granules in their cytoplasm which contain pharma-cologically active components. Degranulation releases antimicrobial agents like defensins and cationic peptides, enzymes like acid hydrolases and lysozymes, NO, etc.

Q12. How are natural killer cells involved in immune response?

Answer. Natural killer cells are the differentiated cells of common lymphoid progenitor cell. NK cells constitute 5%-10% of lymphocytes in human peripheral blood, play role in destroying cells infected with intracellular pathogens and also tumor cells in the absence of any previous immunization against the tumor. NK cells distinguish abnormalities like reduction in the display of class I MHC molecules and the unusual antigens displayed by some tumor cells and cells infected with viruses.

Q13. How do cytotoxic T lymphocytes kill target cells?

Answer. Cytotoxic T cells provide protection against intracellular pathogens such as viruses, some bacteria and parasites that multiply in

the host-cell cytoplasm, where they are sheltered from attack by antibodies. When exposed to infected cells, TC cells release the cytotoxins, perforins, granzymes and granulysin. Through the action of perforin, granzymes enter the cytoplasm of the target cell and their serine protease function triggers apoptosis in the target cell.

Q14. What types of cells originate from the common myeloid progenitor cell?

Answer. Cells that arise from the common myeloid progenitor include red blood cells as well as various types of white blood cells such as granulocytes, monocytes, macrophages and some dendritic cells.

Q15. Describe briefly the role of neutrophils in immune system?

Answer. Neutrophils constitute 50% – 70% of the circulating white blood cells. In response to many types of infections, the bone marrow releases more than the usual number of neutrophils and these cells generally are the first to arrive at a site of inflammation. Neutrophils are active phagocytic cells but unlike macrophages, the lytic enzymes and bactericidal substances in neutrophils are contained within primary and secondary granules. Neutrophils also employ both oxygen-dependent and oxygen-independent pathways to generate antimicrobial substances. Neutrophils are in fact much more likely than macrophages to kill ingested microorganisms.

MULTIPLE CHOICE QUESTIONS (MCQS)

Q1.Which of the following cell has no role in immune defense?

A. B cells
B. T cells
C. RBC’s
D. Monocytes

Q2.Which of the following cells secrete antibodies?

A. Neutrophils
B. B cells
C. Monocytes
D. T cells

Q3. Class II MHC molecules expressed by B cells which permit these cells to function as

A. Neutralizing the antigen
B. Development of immune response by secretion of antibodies
C. Antigen presenting cells
D. Phagocytosis

Q4. B cells recognize the antigens

A. Processed proteinaceous antigens only
B. Lipopolysaccharide
C. Unprocessed soluble proteinaceous antigens and LPS
D. Unprocessed proteinaceous antigens only

Q5. Macrophages are formed from

A. Neutrophils
B. Lymphocytes
C. Monocytes
D. Basophils

Q6. Neutrophils, eosinophils and basophils are known as:

A. Platelets
B. Granulocytes
C. Buffers
D. Astocytomas

Q7. Cytotoxic T lymphocytes express

A. CD3 and CD8
B. CD3 and CD4

C. Only CD3
D. CD3, CD8 and CD4

Q8. Helper T cells express
A. CD3 and CD8
B. CD3 and CD4
C. Only CD3
D. CD3, CD8 and CD4

Q9. Chediak-Higashi syndrome- an autosomal recessive disorder- is associated with a lack of
A. Neutrophils
B. Natural killer cells
C. Monocytes
D. Basophils

Q10. Mast cells express a high-affinity receptor for the Fc region of
A. IgA
B. IgG
C. IgE
D. IgM

Q11. A cell with lobed nucleus and stain with basic dye methylene blue and express cell surface marker CD164.
A. Neutrophils
B. Eosinophils
C. Monocytes
D. Basophils

Q12. A cell with distinct bilobed nucleus that stains with acidic dyes and express cell markers CD69 and CD44.
A. Neutrophils
B. Eosinophils
C. Monocytes

D. Basophils

Q13. Macrophages in connective tissues are known as
A. Alveolar macrophages
B. Histiocytes
C. Kupffer cells
D. Mesangial cells

Q14. B cells mature at which site in mammals.
A. Bone marrow
B. Spleen
C. Thymus
D. Lymph nodes

Q15. T cells mature at which site in mammals.
A. Bone marrow
B. Spleen
C. Thymus
D. Lymph nodes

Answer Key

1. C **2**. B **3**. C **4**. C **5**. C **6**. B **7**. A **8**. B **9**. B **10**. C **11**. D **12**. B **13**. B 14. A **15**. C

ASSIGNMENTS

Long Answer Questions (500 Words)

Q1. Specific immunity exhibits four characteristic attributes, which are mediated by lymphocytes. List these four attributes and briefly explain how they arise.

Q2. What are the two primary characteristics that distinguish hematopoietic stem cells and progenitor cells?

Short Answer Questions (200 Words)

Q1. What effect would removal of the Bursa of Fabricius (bursectomy) have on chickens?

Q2. Some microorganisms (e.g., *Neisseria gonorrhoea*, *Mycobacterium tuberculosis*, and *Candida albicans*) are classified as intracellular pathogens. Define this term and explain why the immune response to these pathogens differs from that to other pathogens such as *Staphylococcus aureus* and *Streptococcus pneumoniae*.

Q3. What is the functional significance of the expression of MHC molecules on monocytes?

REFERENCES

Baeuerle, P. A.; Henkel, T. Function and activation of NF-kappaB in the immune system. *Annual review of immunology*., 1994 Apr, 12(1), 141-79.

Banchereau, J.; Paczesny, S.; Blanco, P.; Bennett, L.; Pascual, V.; Fay, J.; Palucka, A. K. Dendritic cells: controllers of the immune system and a new promise for immunotherapy. *Annals of the New York Academy of Sciences.*, 2003 Apr, 987(1), 180-7.

Banchereau, J.; Steinman, R. M. Dendritic cells and the control of immunity. *Nature*., 1998 Mar, 392(6673), 245.

Calder, P. C.; Kew, S. The immune system: a target for functional foods? *British Journal of Nutrition*., 2002 Nov, 88(S2), S165-76.

Clark, G. J.; Angel, N.; Kato, M.; López, J. A.; MacDonald, K.; Vuckovic, S.; Hart, D. N. The role of dendritic cells in the innate immune system. *Microbes and infection.*, 2000, Mar 1, 2(3), 257-72.

Czerkinsky, C.; Prince, S. J.; Michalek, S. M.; Jackson, S.; Russell, M. W.; Moldoveanu, Z.; McGhee, J. R.; Mestecky, J. IgA antibody-producing cells in peripheral blood after antigen ingestion: evidence for a common mucosal immune system in humans. *Proceedings of the National Academy of Sciences*, 1987, 84(8), pp. 2449-2453.

Dasgupta, D.; Ji, Z.; Gonzalez, F. Artificial immune system (AIS) research in the last five years. In *The 2003 Congress on Evolutionary Computation, 2003. CEC'03.*, 2003, December, (Vol. 1, pp. 123-130), IEEE.

Gabrilovich, D. I.; Nagaraj, S. Myeloid-derived suppressor cells as regulators of the immune system. *Nature reviews immunology*., 2009 Mar, 9(3), 162.

Goldsby, R. A.; Kindt, T. J.; Kuby, J; Osborne, B. A. Kuby Immunology, 5thedn. Oxford: WH Freeman, 2003.

Hansson, G. K.; Hermansson, A. The immune system in atherosclerosis. *Nature immunology*., 2011 Mar, 12(3), 204.

Hoffman-Goetz, L.; Pedersen, B. K. Exercise and the immune system: a model of the stress response? *Immunology today*., 1994, Aug 1, 15(8), 382-7.

Iwasaki, A.; Medzhitov, R. Control of adaptive immunity by the innate immune system. *Nature immunology*., 2015 Apr, 16(4), 343.

Jerne, N. K. The immune system. *Scientific American*., 1973, Jul 1, 229(1), 52-63.

Kono, H.; Rock, K. L. How dying cells alert the immune system to danger. *Nature Reviews Immunology*., 2008 Apr, 8(4), 279.

Liu, Y. J. Dendritic cell subsets and lineages, and their functions in innate and adaptive immunity. *Cell*, 2001, 106, 259.

Lubbers, R.; Van Essen, M. F.; Van Kooten, C.; Trouw, L. A. Production of complement components by cells of the immune system. *Clinical & Experimental Immunology*., 2017 May, 188(2), 183-94.

Makinodan, T.; Kay, M. M. Age influence on the immune system. *In Advances in immunology*, 1980, Jan 1, (Vol. 29, pp. 287-330). Academic Press.

Marchand, F.; Perretti, M.; McMahon, S. B. Role of the immune system in chronic pain. *Nature Reviews Neuroscience.*, 2005 Jul, 6(7), 521.

Medzhitov, R.; Janeway Jr., C. A. Innate immune recognition and control of adaptive immune responses. *In Seminars in immunology*, 1998, Oct 1 (Vol. 10, No. 5, pp. 351-353). Academic Press.

Melchers, F.; Rolink, A. B-lymphocyte development and biology. In *Fundamental Immunology*, 1999, 4th ed., W. E. Paul, ed., p. 183. Lippincott-Raven, Philadelphia.

Metcalfe, D. D.; Baram, D; Mekori, Y. A. Mast cells. *Physiological reviews*, 1997, 77(4), 1033-1079.

Nathan, C.; Shiloh, M. U. Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. *Proc. Natl. Acad. Sci.*, 2000, 97, 8841.

Parkin, J.; Cohen, B. An overview of the immune system. *The Lancet.*, 2001, Jun 2, 357(9270), 1777-89.

Picker, L. J.; Siegelman, M. H. (1999). Lymphoid tissues and organs. In *Fundamental Immunology*, 4th ed., W. E. Paul, ed., p. 145. Lippincott-Raven, Philadelphia.

Ricklin, D.; Hajishengallis, G.; Yang, K.; Lambris, J. D. Complement: a key system for immune surveillance and homeostasis. *Nature immunology*., 2010 Sep, 11(9), 785.

Rönnblom, L.; Pascual, V. The innate immune system in SLE: type I interferons and dendritic cells. *Lupus*., 2008 May, 17(5), 394-9.

Rothenberg, E. V. Stepwise specification of lymphocyte developmental lineages. *Current Opin. Gen. Dev.*, 2000, **10**, 370.

Solana, R.; Tarazona, R.; Gayoso, I.; Lesur, O.; Dupuis, G.; Fulop, T. Innate immunosenescence: effect of aging on cells and receptors of the innate immune system in humans. In *Seminars in immunology*, 2012, October, (Vol. 24, No. 5, pp. 331-341). Academic Press.

Vivier, E.; Raulet, D. H.; Moretta, A.; Caligiuri, M. A.; Zitvogel, L.; Lanier, L. L.; Yokoyama, W. M.; Ugolini, S. Innate or adaptive immunity? The example of natural killer cells. *science*., 2011, Jan 7, 331(6013), 44-9.

Weiskopf, D.; Weinberger, B.; Grubeck-Loebenstein, B. The aging of the immune system. *Transplant international.*, 2009 Nov, 22(11), 1041-50.

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Chapter 3

ORGANS OF THE IMMUNE SYSTEM

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ABSTRACT

The organs of immune system are important regulators of immune responses that protect our body against harmful micro-organisms. The immune system is a collection of lymphoid organs that are classified as primary and secondary lymphoid organs. These lymphoid organs defend gainst many dangerous pathogens that invade our body. The key primary lymphoid organs of the immune system are thymus and bone marrow, while that of secondary lymphoid organs include: are lymph nodes, spleen, tonsils, peyer's patches of the small intestine, which maintain gastro-intestinal homeostasis and mucosal associated lymphoid tissues (MALT). Lymphoid tissues are well supplied with different structural

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organisations in relation to a particular function of immune response. The most highly organised lymphoid tissue consists of thymus and lymph node. Lymphoid cells are a subset of immune cells that provide a barrier to tissues and help them in maintaining immune homeostasis.

Keywords: Haemopoietic stem cell, lymphoid organs, antibodies, spleen, thymus

OBJECTIVES

- Understanding the structure and function of diverse lymphoid organs in immune system.
- Distinguish between primary and secondary lymphoid organs.
- Understand the role of lymph node.

INTRODUCTION

A number of morphologically and functionally diverse organs and tissues are involved for the proper functioning and the development of immune responses. The evolution of these organs in mammals is a continual process that commences early in embryonic development, reaches a maximum at some point in early adulthood, maintains at a plateau for an indeterminate period, and then declines. At the time of birth, the immune system organs are at different stages of development, which is manifested as differences in morphology and function and which can further vary between different mammalian species. Cells of immune system are derived from haemopoietic stem cells in foetal liver and postnatally in the bone marrow. The human immune system comprises lymphoid organs, tissue cells and soluble molecules such as antibodies. Lymphoid organs are those organs where the maturation and proliferation and differentiation of lymphocytes takes place. The structurally and functionally diverse lymphoid organs and tissues are interconnected by the

blood vessels and lymphatic vessels through which lymphocytes circulate. There are two types of lymphoid organs.

Primary Lymphoid Organs

The primary lymphoid organs are those organs where T- lymphocytes and B-lymphocytes mature and acquire the antigen-specific receptors. After maturation, the lymphocytes migrate to secondary lymphoid organs. Primary lymphoid organs include bone marrow and thymus.

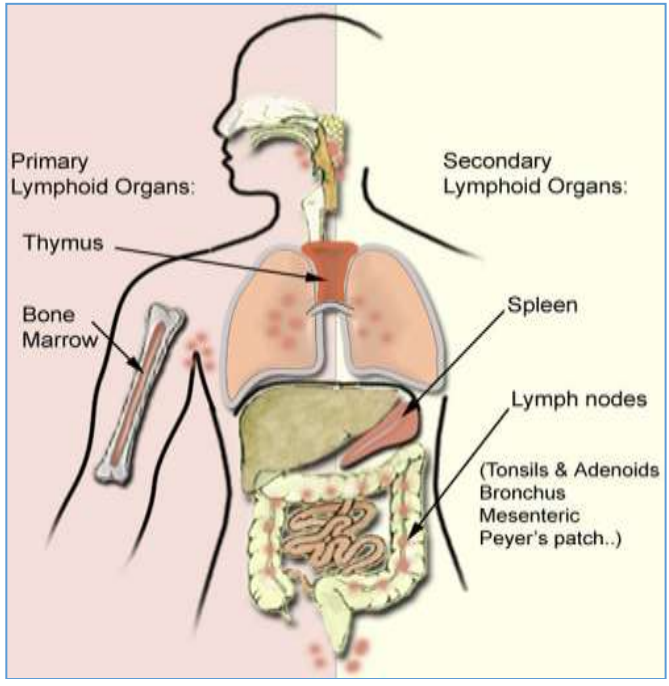
Secondary Lymphoid Organs

After maturation, T-lymphocytes and B-lymphocytes migrate through blood vascular and lymphatic system to the secondary lymphoid organs where they undergo proliferation and differentiation. The acquired immune response to antigens usually develops in these organs. The secondary lymphoid organs are lymph nodes, spleen, tonsils, peyer's patches of the small intestine and mucosal associated lymphoid tissues (MALT).

TONSILS AND ADENOIDS

Tonsils and adenoids are collections of lymphoid tissue that help the body fight infection. Both trap bacteria and viruses entering through the throat and produce antibodies. The tonsils and adenoids are largest in children who are 2 to 6 years of age.

The tonsils are located on either side of the back of the throat. The adenoids are located much higher and further back, where the nasal passages connect with the throat. The tonsils are visible through the mouth, but the adenoids are not. They act as filters to remove debris and antigens entering the respiratory track.



Source: The Mc Gill physiology virtual Lab.

Figure 3.1. Classification of Lymphoid organs.

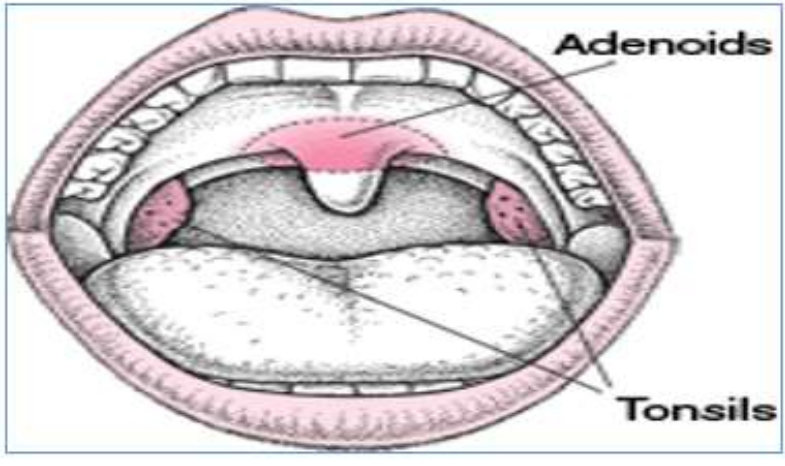


Image source: Enlarged tonsils and adenoids/

Figure 3.2. Tonsils and Adenoids.

THYMUS

The thymus is a lymphocyte-rich, bilobed, encapsulated organ situated above the heart and below the thymus gland. The activity of the thymus is maximum in early childhood and then grows slow at puberty although never totally disappearing. The thymus is derived from pharyngeal pouches during embryonic life. Thymus is very essential for the maturation of T cells and the development of cell-mediated immunity, so it is referred to as primary lymphoid organ. Thymus is composed of cortical and medullary epithelial cells, stromal cells, dendritic cells and macrophages. All these ells are important in the differentiation of T-cell precursors and their role prior to their migration into the secondary lymphoid tissues. The main *function* of the thymus *as a primary lymphoid organ* is to produce sufficient number of T-cells each with a unique T-cell receptor so that every individual T cell is potentially specific for each foreign antigen and to minimise auto-immune response. The thymus has an important role in the endocrine system.

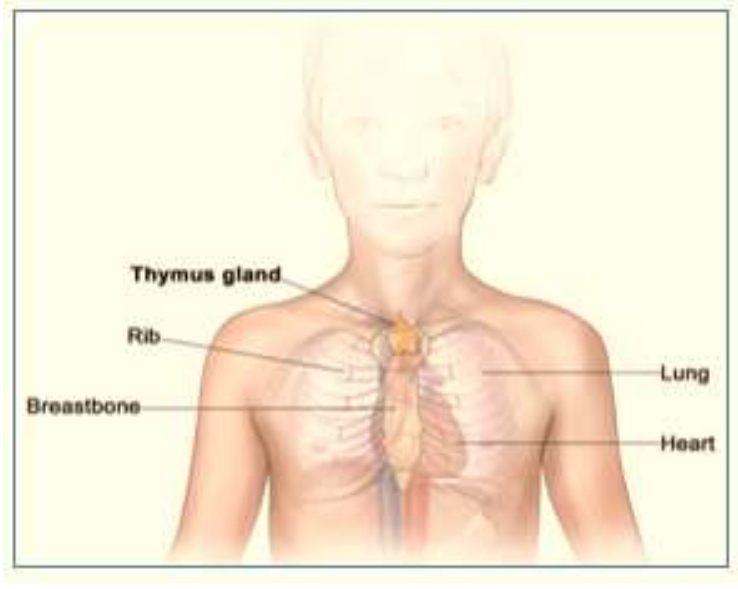


Figure 3.3. Diagram Showing location of Thymus (Image source: NCBI).

Thymus epithelial cells produce hormones: thymosin and thymopoietin and along with cytokines (IL-7) are important for the development and maturation of thymocytes into mature T cells.

SPLEEN

The spleen is the largest organ in the lymphatic system. It is an encapsulated, bean-shaped organ with a spongy interior (splenic pulp) and is situated on the left side of the body below the diaphragm. Like lymph nodes, the spleen is encapsulated by white connective tissue which is a few millimetres thick but unlike lymph nodes which are specialized to trap localized antigens from regional tissue spaces, the spleen filters blood and trap blood borne antigen and thus responds to systemic infections. It originates in the dorsal mesogastrum in the human embryo. In the first trimester, the human foetal spleen is erythropoietic and myelopoietic. The spleen is surrounded by a capsule that sends projections known as trabeculae into the compartments.

The compartment is of two types-red pulp and white pulp separated by diffused marginal zone. The red pulp consists of erythrocyte rich blood intermingled with many dendritic cells, macrophages, a few lymphocytes and plasma cells. It is the site where old and defective red blood cells are removed.

The white pulp surrounds the splenic arteries forming a peri arteriolar lymphoid sheath (PALS) populated chiefly by T lymphocytes. The marginal zone, located peripheral to PALS, is rich in B cells and macrophages which are organized into primary lymphoid follicles. It acts as a filter to remove debris and antigens and to foster contact with T-lymphocytes.

Spleen is most crucial organ of lymphatic system that protects body from a number of pathogens as well as from the effects of stress, toxins and harmful substances and even deficiencies. Apart from above, it keeps blood clean by removing wastes and maintains red blood cell and blood platelet count.

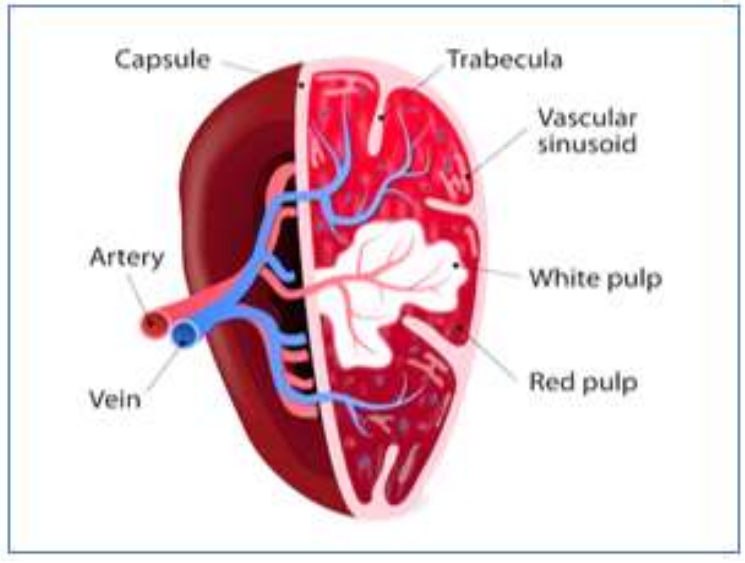


Figure 3.4. Diagram illustrating the anatomy of Spleen.

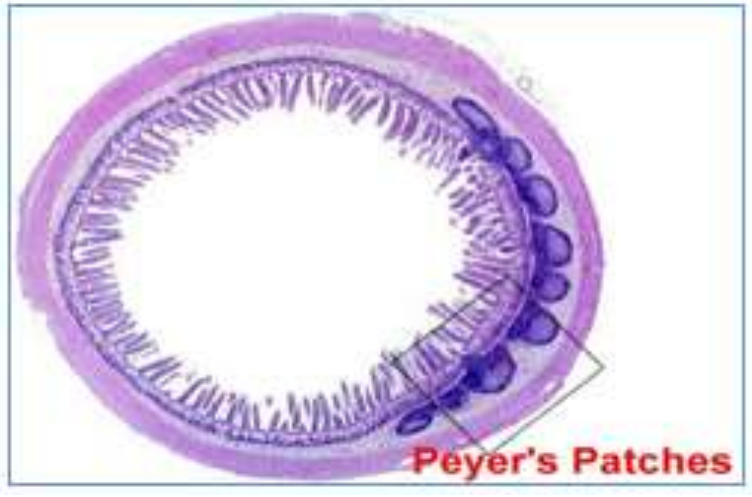


Figure 3.5. Showing a small section of payer's patches.

Peyer's patches: Peyer's patches, also known as the "Tonsils of the small intestine", are found associated with the epithelial wall of small intestines. They act as filters to capture, destroy and remove any unwanted debris foreign to the gastrointestinal track.

BONE MARROW

Bone marrow is a spongy tissue inside our bones. The new-born babies have active bone marrow, which means they are producing new marrow cells. Bone marrow is the source for all immune cells except during early foetal development when the cells are produced in the mesenchyme of the yolk sac. The bone marrow becomes a major haematopoietic tissue from fifth month onwards. Contains stem cells for B-lymphocytes which mature and become antibody-producing plasma cells that react against many bacteria, viruses, and other antigens; controls humoral immunity. About 90% of differentiating B cells are believed to have this fate. B cells that survive this selection process leave the bone marrow through efferent blood vessels. In birds, the 'Bursa of Fabricius', a gut associated lymphoid tissue, is the primary site of B cell maturation. The majority of mammals have bone marrow as the primary site of B cell maturation.

MUCUSA-ASSOCIATED LYMPHOID TISSUE (MALT)

The mucous membranes lining the digestive, respiratory and urogenital systems represent the main sites for the entry of microbes into the body. It is for this reason that almost 50 percent of the lymphoid tissue in the human body is located within the lining of the major tracts, defending these vulnerable membrane surfaces against microbial invasion. The lymphoid tissues are collectively called as mucosa-associated lymphoid tissue (MALT).

These tissues initiate immune responses to specific antigens encountered along all mucosal surfaces. MALT can be functionally divided into effector sites and inductive sites. Inductive sites contain secondary lymphoid tissues in which IgA class switching and clonal expansion of B-cells occurs in response to antigen specific T-cell activation. After activation and IgA class switching, T- and B-cells migrate from inductive sites to effector sites.

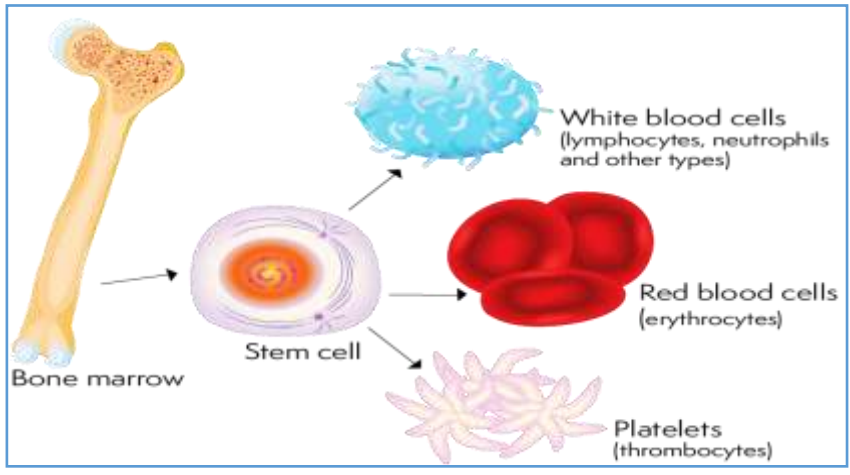


Figure 3.6. Illustrating the presence of Bone marrow and various components derived from it.

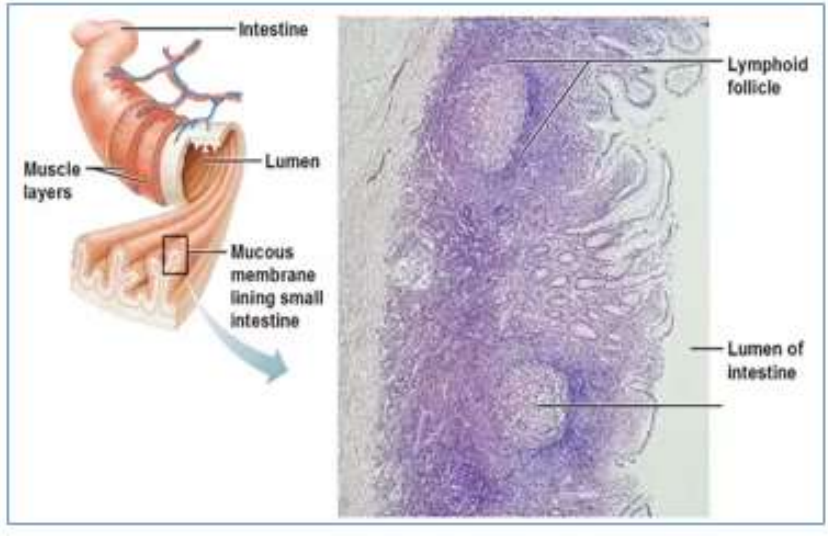


Figure 3.7. Diagram showing Mucosa-Associated lymphoid Tissue (MALT).

Effector sites are present in all mucosal tissues as disseminated lymphoid tissue diffusely distributed throughout the lamina.

LYMPH NODES

Human lymph nodes are encapsulated bean-shaped or ovoid structures ranging from a few millimetres to more than a centimetre in their largest dimension. Strategic sites rich in nodes are in the areas such as neck, retroperitoneum mediasternum, axillae, groin and abdominal cavity. The major function of the lymph nodes is to filter antigen from the lymph. The lymph, along with many cells and particles, drain out of tissues and seep across the tiny single-cell-walled lymphatic vessels. The vessels transport lymph to the nodes where antigens are filtered out. As the lymph is filtered, it is enriched with antibodies, cytokines and mainly T-lymphocytes. Morphologically, a lymph node can be divided into three regions: the cortex, paracortex and medulla, each of it providing a different microenvironment.

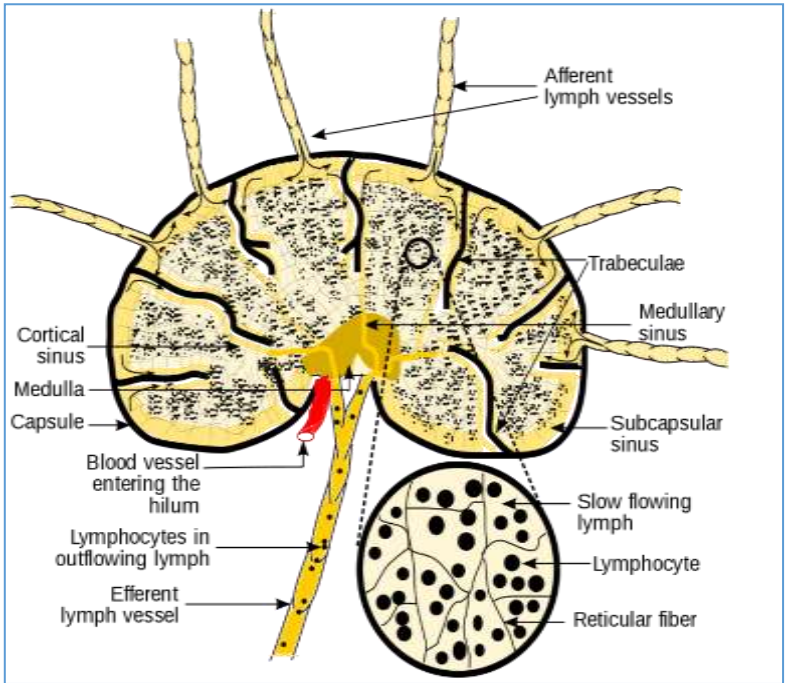


Figure 3.8. Diagram illustrating anatomy of lymph nodes.

Cortex is the outermost layer- contains mostly B lymphocytes, plus both follicular dendritic cells and macrophages all arranged in clusters called primary follicles. Following antigenic stimulation, primary follicles become secondary follicles consisting of concentric rings of densely packed lymphocytes and germinal central, macrophages, and dendritic cells. The germinal centres contain large proliferating B lymphocytes and plasma cells interspersed with macrophages and dendritic cells. It is a site of intense B-Cell activation and differentiation into plasma cells and memory cells. Paracortex is the region just beneath the cortex which is largely populated with T lymphocytes and also contains interdigitating dendritic cells.

These interdigitating dendritic cells express high levels of class II MHC molecules. Paracortex is a thymus dependent area as compared to the thymus independent cortex region. Medulla is the inner most region, more sparsely populated by cells. Many of the cells are plasma cells; activated T_H and T_C cells are also present. In addition, there is a high concentration of immunoglobulin in this region due to the large population of plasma cells.

SUMMARY

- Lymphoid organs and tissues are either primary or secondary.
- Lymphoid stem cells develop and mature within the Primary lymphoid organs – the thymus and bone marrow.
- Mammalian B cells develop mainly in the foetal liver and from birth onwards in the bone marrow.
- Lymphocytes migrate and function in the secondary lymphoid organs and tissues.
- Lymphoid organs and tissues protect different body sites – the spleen responds to blood-borne antigens; the lymph nodes respond to lymph-borne antigens; and MALT protects the mucosal surface.
- Systemic lymphoid organs include the spleen and lymph nodes.

- Mucosa-associated lymphoid tissue (MALT) includes all the lymphoid tissues associated with mucosal membranes. Peyer's patches are a major site of lymphocyte priming to antigens Crossing mucosal surfaces of the small intestine.

FREQUENTLY ASKED QUESTIONS (FAQS)

Q1. State the functions of primary and secondary lymphoid organs in humans.

Answer: Role of primary and secondary lymphoid organs in humans are:

Primary lymphoid organs: Here, immature lymphocytes are differentiated to form antigen-sensitive lymphocytes. Bone marrow and thymus are the examples of primary lymphoid organs.

Secondary lymphoid organs: Lymphocytes migrate here after attaining maturity. These organs provide an environment that enable mature lymphocytes to interact with each other, accessory cells and with antigens, resulting in the initiation of the antigen specific response. Spleen, lymph nodes tonsils, Peyer's patches and appendix are the examples of secondary lymphoid organs.

Q2. Name the two types of immune systems in a human body. Why is cell mediated and humoral immunities so called?

Answer: Human being are provided with two type of immune system-

1. Innate immunity
2. Acquired immunity

Innate immunity – is the type of immunity which is confer to a baby by birth, and is the primary defence system of our body.

Acquired immunity – it is acquired by a person during various stage of a person life, it involves B and T cells.

Humoral immunity is called so because blood is involved in developing defence in the body. It is supported by the presence of B cells. Cell mediated immunity is called so because of the involvement of different cell for developing immune response, it actively involves T cells.

Q3. What are the functions of the lymphatic system?

Answer: The lymphatic system drains excess interstitial fluid, transports dietary lipids, and carries out immune responses.

Q4. How are primary lymphatic organs different from secondary lymphatic organs?

Answer: Primary lymphatic organs are where cells mature while as secondary lymphatic organs are where the immune response occurs.

Q5. What is a splenic nodule? Where is it found?

Answer: The spleen has a connective tissue capsule. The invaginations of the capsule into the splenic parenchyma are trabeculae. The parenchyma of the spleen can be divided into the white pulp and the red pulp. The white pulp of the spleen is the lymphatic portion of the spleen. Within the white pulp, splenic nodules are found. Splenic nodules are also called Malpighian corpuscles. The red pulp is made up of the splenic sinuses and splenic cords. The splenic cords are also called the cords of Billroth.

Q6. What do you call the random distribution of lymphocytes that are found in the respiratory tract, genitourinary tract, and gastrointestinal tract?

Answer: The localized concentrations of lymphocytes that are seen in the respiratory tract, genitourinary tract, and gastrointestinal tract are lymph follicles. They are also called lymphatic nodules. The lymphatic tissue in the spleen is called white pulp. The large aggregates of lymphatic tissue in the ileum are called Peyer's patches.

Q7. What is the function of the thymic cortex and thymic medulla?

Answer: The thymus has two main cellular zones, clearly distinguishable histologically; the major outer zone, the cortex, and the smaller central zone, the medulla. The cortex and medulla both play a role in the development of T cells. The cortex stains more darkly (is more basophilic) than the medulla, because it contains more lymphocytes than the medulla.

Q8. What are Hassall's corpuscles?

Answer: Hassall's corpuscles were first described in the human thymus by the English microscopist Arthur Hill Hassall in 1846. The corpuscles, a distinctive feature of the medulla of the mammalian thymus, are rounded epithelial structures which vary in diameter from 30 μ to over 100 μ. They are composed of eosinophilic epithelial cells with pale, elongated nuclei. The cells are arranged concentrically, and the inner parts are often degenerate. It has been found that they have a role in the pathogenesis of diseases such as type 1 diabetes, rheumatoid arthritis, multiple sclerosis, autoimmune thyroiditis and others. They are also found to synthesise cytokines which affect the population of the other cells in the thymus medulla.

Q9. What makes up the stroma of the thymus? What happens to the thymus in adulthood?

Answer: Cells in the thymus can be divided into thymic stromal cells and cells of hematopoietic origin (derived from bone marrow resident hematopoietic stem cells). Developing T cells are referred to as thymocytes and are of hematopoietic origin. Stromal cells include epithelial cells of the thymic cortex and medulla, and dendritic cells.

Before birth and throughout childhood, the thymus is instrumental in the production and maturation of T-lymphocytes or T cells, a specific type of white blood cell that protects the body from certain threats, including viruses and infections. The thymus produces and secretes thymosin, a hormone necessary for T cell development and production. Once we reach

puberty, the thymus starts to slowly shrink and become replaced by fat. By age 75, the thymus is little more than fatty tissue.

Q10. What are the two primary characteristics that distinguish a haematopoietic stem cell from a progenitor cell?

Answer: Hematopoietic stem cells are one of the adult stem cells in our body and are found in the bone marrow. They have a big lineage generating around 11 different types of terminally differentiated cells which includes all blood cells.

They also have the capacity to self-renew. Progenitor cells are formed from HSCs during the pathway of differentiation. In the lineage the progenitors are restricted to differentiate in a few types of cells. E.g. lymphoid progenitor can only give rise to T cells, B cells and NK cells. Additionally, progenitor cells can divide only a limited number of times rather than indefinitely.

Q11. What do you understand by immune surveillance?

Answer: Immune surveillance is a theory that the immune system patrols the body not only to recognize and destroy invading pathogens but also host cells that become cancerous. Perhaps potential cancer cells arise frequently throughout life, but the immune system usually destroys them as fast as they appear.

Q12. Why is the immune system considered to be "layered?"

Answer: The immune system is considered to be layered because it has different layers of defences that are increasingly more specific for pathogens or cancerous cells. For example, the skin and mucous membranes are first layers of defence against pathogens. If pathogens penetrate this layer, immune cells generate a non-specific, innate response against them. If that is not sufficient, the adaptive immune response is activated which is tailored to the particular pathogen.

Q13. Describe the role of the lymphatic system in the absorption of nutrients from the digestive system.

Answer: Lymphatic vessels called lacteals, which are found in villi that line the small intestine, absorb fatty acids from the digestion of lipids in the digestive system.

The fatty acids are then transported through the network of lymphatic vessels to the bloodstream.

Q14. Summarize the immune function of the lymphatic system.

Answer: The immune function of the lymphatic system is producing mature lymphocytes and circulating them in lymph. Lymphocytes, which include B cells and T cells, are the subset of white blood cells that are involved in adaptive immune responses. They may create a lasting memory of and immunity to specific pathogens.

Q15. How is interstitial fluid related to lymph?

Answer: Interstitial fluid is fluid that is pushed out of capillaries into the tissue spaces between cells. Much of the water in this fluid is reabsorbed into blood capillaries, and then most of the remaining fluid is absorbed into lymph capillaries. Once it enters the lymph capillaries, the fluid is called lymph.

MULTIPLE CHOICE QUESTIONS (MCQS)

Q1. General functions of the lymphatic system include

A. Fluid and nutrient transport
B. Lymphocyte development
C. The immune response
D. all of the above

Q2. Which selection includes all the major components of the lymphatic system?

A. Lymphatic cells and structures, lymph, and lymph vessels

B. Thoracic duct, right lymphatic duct, and lymph
C. Lymphocytes, lymph, and lymph nodes
D. Spleen, thymus, and tonsils

Q3. A systematic defence against antigens, initiated by lymphatic cells, is called

A. A cross-reaction
B. Inflammation
C. Septicaemia
D. An immune response

Q4. T-lymphocytes mature in the _____, while B-lymphocytes mature in the _____.

A. Yellow bone marrow; red bone marrow
B. Thyroid follicles; bone marrow
C. Bone marrow; thymus
D. Thymus; bone marrow

Q5. Lymphopoiesis, the process of lymphocyte development and maturation, occurs in

A. The spleen and liver
B. Red bone marrow
C. The thymus
D. Both b and c

Q6. Oval clusters of lymphatic cells with some extracellular matrix but no connective tissue capsule is called

A. Lymph nodes
B. Lymphatic sinuses
C. Lymphatic nodules
D. Medullary sinuses

Q7. The large clusters of lymphatic nodules found in the throat are called

A. Peyer’s patches

B. Tonsils
C. Lymph nodes
D. Lymph glands

Q8. Large groups of lymphatic nodules in the mucosa of the gastrointestinal, respiratory, genital, and urinary tracts are collectively called

A. MALT
B. Peyer's patches
C. Germinal centres
D. Mucosal lymph nodes

Q9. The thymus is a lymphatic organ that

A. is divided into four lobes by its connective tissue capsule
B. reaches a maximum weight of 30-50 grams at puberty
C. is located in the posterior mediastinum, inferior to the heart
D. remains functional throughout adulthood, especially after age 40

Q10. What occurs in the thymus?

A. production of large quantities of antibodies
B. activation of the immune response by circulating antigens
C. T-lymphocyte maturation and differentiation
D. all of the above

Q11. Tissue fluid in the lymphatic system is called _____.

A. Plasma
B. Hemoplasma
C. Ringer's solution
D. Lymph

Q12. Some tissues have _____ that routinely act as scavengers, devouring old blood cells and other debris.

A. resident macrophages
B. suppressor T-cells

C. backup neutrophils
D. complimentary B-cells

Q13. Which of the following is NOT made of a framework of reticular fibers?

A. Bone marrow
B. Lymph node
C. Spleen
D. None of the above

Q14. A/an _____ is usually a protein or polysaccharide chain of a glycoprotein molecule that the body recognizes as "non-self."

A. allergen
B. antigen
C. antibody
D. complement

Q15. The main antibody type in circulation is _____.

A. IgG
B. IgM
C. IgD
D. IgA

Answer Key

1. D **2**. A **3**. D **4**. D **5.** D **6**. C **7**. B **8**. A **9**. B **10.** C **11**. D **12**. C **13**. D **14**. B **15**. A

ASSIGNMENTS

Long Answer Questions (500 Words)

Q1. Describe the circulation of the spleen.

1. How do interferons help your body fight viral infections?
2. What are the fundamental units of all antibodies?

Short Answer Questions (200 Words)

Q1. During maturation, what are the initial antibodies on a B-cell?

Q2. What is the usual process of a humoral immune response?

Q3. How are abnormal cells (including those infected by viruses) recognized?

REFERENCES

A Historical Perspective on Evidence-Based Immunology-Elsevier (2015) (by Moticka, Edward J.).

Aguzzi, A. Prions and the immune system: a journey through gut, spleen, and nerves. *Advances in immunology,* 2003 Jan. 1; 81: 123 - 72.

Banchereau, J., Steinman, R. M. Dendritic cells and the control of immunity. *Nature,* 1998 Mar.; 392(6673):245.

Björck, L., Grubb, A., Kjellén, L. Cystatin C, a human proteinase inhibitor, blocks replication of herpes simplex virus. *J. Virol.,* 1990 Feb.; 64(2):941 - 943.

Blankenvoorde, M. F., van't Hof, W., Walgreen-Weterings, E., van Steenbergen, T. J., Brand, H. S., Veerman, E. C., Nieuw Amerongen AV. Cystatin and cystatin-derived peptides have antibacterial activity against the pathogen Porphyromonas gingivalis. *Biol. Chem.,* 1998 Nov.; 379(11):1371 - 1375.

Boehm, T., Bleul, C. C. The evolutionary history of lymphoid organs. *Nature immunology*, 2007 Feb.; 8(2):131.

Elgert, K. D. *Immunology: understanding the immune system*. John Wiley & Sons; 2009 Sep. 8.

Felten, D. L., Felten, S. Y., Bellinger, D. L., Carlson, S. L., Ackerman, K. D., Madden, K. S., Olschowki, J. A., Livnat, S. Noradrenergic sympathetic neural interactions with the immune system: structure and function. *Immunological reviews*, 1987 Dec.; 100(1):225 - 60.

Goldman, M. J., Anderson, G. M., Stolzenberg, E. D., Kari, U. P., Zasloff, M., Wilson, J. M. Human beta-defensin-1 is a salt-sensitive antibiotic in lung that is inactivated in cystic fibrosis. *Cell*, 1997 Feb. 21; 88(4): 553 - 560.

Hall, Susan H., Hamil, Katherine G., French, Frank S. Host defense proteins of the male reproductive tract. *J. Androl.*, 2002 Sep. - Oct.; 23(5):585 - 597.

Immunology (by Barbara A. Osborne and Janis Kuby).

Janeway, Jr. C. A., Travers, P., Walport, M., Shlomchik, M. J. *The components of the immune system*.

Jin, Y. Z., Bannai, S., Dacheux, F., Dacheux, J. L., Okamura, N. Direct evidence for the secretion of lactoferrin and its binding to sperm in the porcine epididymis. *Mol. Reprod. Dev.*, 1997 Aug.; 47(4):490 - 496.

Larrick, J. W., Hirata, M., Balint, R. F., Lee, J., Zhong, J., Wright, S. C. Human CAP18: a novel antimicrobial lipopolysaccharide-binding protein. *Infect. Immun.*, 1995 Apr.; 63(4):1291 - 1297.

Lydyard, P. M., Porakishvili, N. *Cells, tissues and organs of the immune system*. Elsevier.

Macpherson, A. J., Smith, K. Mesenteric lymph nodes at the center of immune anatomy. *Journal of Experimental Medicine*, 2006 Mar. 20; 203(3):497 - 500.

Malm, J., Sørensen, O., Persson, T., Frohm-Nilsson, M., Johansson, B., Bjartell, A., Lilja, H., Ståhle-Bäckdahl, M., Borregaard, N., Egesten, A. The human cationic antimicrobial protein (hCAP-18) is expressed in the epithelium of human epididymis, is present in seminal plasma at

high concentrations, and is attached to spermatozoa. *Infect. Immun.,* 2000 Jul.; 68(7):4297 - 4302.

Nakanishi, T. Ontogeny of the immune system in Sebastiscus marmoratus: Histogenesis of the lymphoid organs and effects of thymectomy. *Environmental Biology of fishes*, 1991 Jan. 1; 30(1 - 2):135 - 46.

Nisapakultorn, K., Ross, K. F., Herzberg, M. C. Calprotectin expression in vitro by oral epithelial cells confers resistance to infection by Porphyromonas gingivalis. *Infect. Immun.,* 2001 Jul.; 69(7):4242 - 4247.

Parham, P. The immune system. *Garland Science*, 2014 Oct. 1.

Parker, G. A., Picut, C. A. Immune functioning in non-lymphoid organs: the liver. *Toxicologic pathology,* 2012 Feb.; 40(2):237 - 47.

Parkin, J., Cohen, B. An overview of the immune system. *The Lancet,* 2001 Jun. 2; 357(9270):1777 - 89.

Perdigon, G., Alvarez, S., Rachid, M., Agüero, G., Gobbato, N. Immune system stimulation by probiotics. *Journal of dairy science*, 1995 Jul. 1; 78(7):1597 - 606.

Reddy, E. S., Bhargava, P. M. Seminalplasmin--an antimicrobial protein from bovine seminal plasma which acts in E. coli by specific inhibition of rRNA synthesis. *Nature,* 1979 Jun. 21; 279(5715):725 - 728.

Robinovitch, M. R., Ashley, R. L., Iversen, J. M., Vigoren, E. M., Oppenheim, F. G., Lamkin, M. Parotid salivary basic proline-rich proteins inhibit HIV-I infectivity. *Oral Dis.,* 2001 Mar.; 7(2):86 - 93.

Selvan, R. S., Venkateswaran, K. S., Rao, A. R. Influence of arecoline on immune system: I. Short term effects on general parameters and on the adrenal and lymphoid organs. *Immunopharmacology and immunotoxicology,* 1989 Jan. 1; 11(2-3):347 - 77.

Sitaram, N., Nagaraj, R. A synthetic 13-residue peptide corresponding to the hydrophobic region of bovine seminalplasmin has antibacterial activity and also causes lysis of red blood cells. *J. Biol. Chem.,* 1990 Jun. 25; 265(18):10438 - 10442.

Spahn, T. W., Kucharzik, T. Modulating the intestinal immune system: the role of lymphotoxin and GALT organs. *Gut,* 2004 Mar. 1; 53(3): 456 - 65.

Straub, R. H., Westermann, J., Schölmerich, J., Falk, W. Dialogue between the CNS and the immune system in lymphoid organs. *Immunology today*, 1998 Sep. 1; 19(9):409 - 13.

The Elements of Immunology (by Fahim Halim Khan).

Travis, S. M., Anderson, N. N., Forsyth, W. R., Espiritu, C., Conway, B. D., Greenberg, E. P., McCray, P. B., Jr., Lehrer, R. I., Welsh, M. J., Tack, B. F. Bactericidal activity of mammalian cathelicidin-derived peptides. *Infect. Immun.*, 2000 May; 68(5):2748 - 2755.

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Chapter 4

ANTIGENS AND IMMUNOGENS

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ABSTRACT

Antigens are the substances that can be recognized by the surface antibody (B cells) or by the TCR when associated with MHC molecules. Antigens and immunogens are more or less similar in nature and differ only in their ability to elicit an immune response. All antigens and immunogens are antigenic and have the ability to bind to antibodies. All antigens are not immunogenic as all antigens are not able to elicit an immune response, whereas all immunogens are immunogenic. Non – immunogenic antigens can be made immunogenic by adhering them to a carrier molecule. This is the difference between antigen and immunogen. Immunogenicity is determined, in part, by various properties of the immunogen such as its foreignness, molecular size, chemical composition and complexity.

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Immunogen dosage and route of immunogen administration also plays a central role in the development of immunogenicity and hence immune response. Immunogens can be administered through number of common routes which include *Intravenous* (into a vein), *intradermal* (into the skin), *subcutaneous* (beneath the skin), *intramuscular* (into the muscle). Antigens administered via the most common route namely, *subcutaneously*, generally elicit the strongest immune responses. This is due to their uptake, processing, and presentation to effector cells by Langerhans cells present in the skin, which are among the most potent APCs.

Keywords: antigen, immunogen, epitopes, adjuvant, hapten, immunogenicity, affinity, avidity

OBJECTIVES

- Understand antigenicity and immunogenicity.
- Discuss antigenicity versus immunogenicity.
- Describe requirements for immunogenicity.
- Describe antigen-antibody interaction.
- The forces responsible for antigen- antibody binding.
- Describe adjuvants and immunogenicity.
- Describe Immunogen dosage and route of administration

INTRODUCTION

Antigen is any compound or substance that evokes the immune response. A term initially coined due to the ability of these compounds to cause antibody responses to be generated. Antigen may be any agent capable of binding specifically to T-cell receptor (TCR) or an antibody molecule (membrane bound or soluble). *Antigens* can be recognized by immunoglobulin receptor of B cells, or by the T cell receptor when complexed with major histocompatibility complex (MHC). The ability of a compound to bind with an antibody or a TCR is referred to as *antigenicity.*

Antigens are capable of stimulating an immune response, specifically activating lymphocytes, which are the body's infection-fighting white blood cells. Antigens may invade the body from outside or may be present within the body of an organism. In general, two main divisions of antigens are recognized i.e., foreign antigens (or heteroantigens) and autoantigens (or self-antigens). Foreign antigens originate from outside the body. Examples of distant or foreign antigens include: parts of or substances produced by viruses or microorganisms (such as bacteria and protozoa), as well as substances in snake venom, certain proteins in foods, and components of serum and red blood cells from other individuals. Autoantigens, on the other hand, originate within the body. Normally, the body is able to distinguish self from nonself (heteroantigen), but in persons with autoimmune disorders, normal bodily substances provoke an immune response, leading to the generation of autoantibodies. Antigenic molecules may be multivalent, having multiple epitope, or monovalent, having only one epitope. Generally, multivalent antigens produce a stronger immune response than monovalent antigens because wide arrays of antibody molecules are made against the multivalent antigens (Figure 4.1).

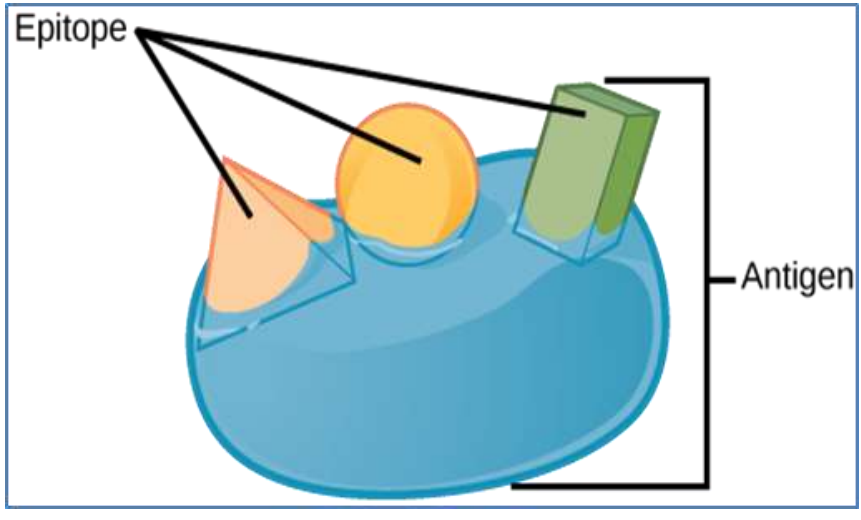


Figure 4.1. Showing structure of antigen.

TYPES OF ANTIGENS

Based upon the Immune Response, Antigens Are of Two Types

Complete Antigens

A complete antigen is able to induce antibody release and produce a specific and observable reaction with the antibody so produced. These are generally molecules with high molecular weight (more than 10,000 Daltons). They possess antigenic properties and are usually proteinaceous in nature. Some of them can be polysaccharide in chemical nature.

Incomplete Antigens

Also known as haptens, incomplete antigens are substances which are incapable of inducing antibody formation by themselves, but can be capable of inducing antibodies on combining with larger molecules (normally proteins) which serve as carriers. They generally have low molecular weight (Less than 10,000 Daltons) and are usually non-protein substances. E.g., Capsular polysaccharide of pneumococcus, polysaccharide "C" of β-haemolytic streptococci, Cardiolipin antigens, etc.

Carrier molecule is a non-antigenic component and helps in provoking the immune response. Example: Serum Protein such as Albumin or Globulin.

According to Their Origin, Antigens Are of Four Types

Exogenous Antigens

These are antigens which are foreign to the host body hence are also called foreign antigens. These antigens enter the body from the outside through inhalation, ingestion, or injection. The immune system's response to exogenous antigens is often subclinical. By endocytosis or phagocytosis, exogenous antigens are taken into the antigen-presenting cells (APCs) and processed into fragments. APCs then present the fragments to T helper

cells (CD4+) by the use of class II histocompatibility molecules on their surface. Some antigens can be exogenous in origin but later can turn endogenous. E.g., Intracellular viruses. Intracellular antigens can be returned to circulation upon the destruction of the infected cell.

Endogenous Antigens: Also Called as Allo-Antigens

These are antigens which originate from own body of host organisms. These are body's own cells or sub fragments or compounds or the antigenic products that are produced. The endogenous antigens are processed by the macrophages which are later accepted by the cytotoxic T – cells. E.g., Blood group antigens, HLA (Histocompatibility Leukocyte antigens) etc. Endogenous antigens are generated within normal cells as a result of normal cell metabolism, or because of viral or intracellular bacterial infection.

Auto Antigens

These are usually normal proteins or protein complexes (and sometimes DNA or RNA) that is recognized by the immune system of patients suffering from a specific autoimmune disease. These are not immunogenic under normal condition however due to genetic and environmental changes or factors immunological tolerance is lost and immune response is generated. E.g., Nucleoproteins, Nucleic acids, etc.

Neoantigens

Neoantigens are those that are entirely absent from the normal human genome. As compared with non-mutated self-antigens, neoantigens are of relevance to tumour control, as the quality of the T cell pool that is available for these antigens is not affected by central T cell tolerance. Technology to systematically analyse T cell reactivity against neoantigens became available only recently.

SUPERANTIGENS

Superantigens (SAgs) are a class of antigens that cause non-specific activation of T-cells resulting in polyclonal T cell activation and massive cytokine release. Superantigens are produced by some pathogenic viruses and bacteria most likely as a defence mechanism against the immune system. Superantigens can interact with APCs and T lymphocytes in a nonspecific way. The superantigens act differently by interacting with MHC class II molecules of the APC and the Vb domain of the T-lymphocyte receptor. This interaction results in the activation of a larger number of T cells (10%) than conventional antigens (1%), leading to massive cytokine expression and immunomodulation.

When the immune system encounters a conventional T-dependent antigen, only a small fraction (1 in 104 -105) of the T cell population is able to recognize the antigen and become activated (monoclonal/oligoclonal response). However, superantigens polyclonally activate a large fraction of the T cells (up to 25%). Examples of superantigens include: Staphylococcal enterotoxins (food poisoning), Staphylococcal toxic shock toxin (toxic shock syndrome), Staphylococcal exfoliating toxins (scalded skin syndrome) and Streptococcal pyrogenic exotoxins (shock). The diseases associated with exposure to superantigens are, in part, due to hyper activation of the immune system and subsequent release of biologically active cytokines by activated T cells.

THE FORCES THAT BIND ANTIGEN TO ANTIBODY

The forces that bind antigen to antibody become larger as intercellular distances decrease.

These forces are, in essence, no different from the ‘non-specific’ interactions which occur between any two unrelated proteins. These intermolecular forces are:

- Electrostatic forces (attraction between oppositely charged ionic groups)
- Hydrogen bonding (reversible hydrogen bridges between hydrophilic groups)
- Hydrophobic bonding (similar to the manner in which oil droplets in water merge to form a single large drop, side chains of valine, leucine and phenylalanine tend to associate in an aqueous environment. This may account for over 50% of the strength in an antigen antibody bond).
- Van der Waals forces: interaction between the electrons in the external orbits of the two different macro molecules.

ANTIGEN-ANTIBODY INTERACTION

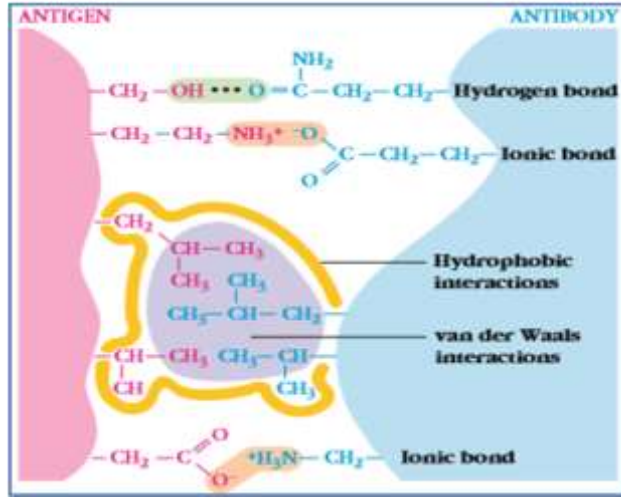


Figure 4.2. The interaction between an antibody and an antigen depends on four types of noncovalent forces: (1) hydrogen bonds, in which a hydrogen atom is shared between two electronegative atoms; (2) ionic bonds between oppositely charged residues; (3) hydrophobic interactions, in which water forces hydrophobic groups together; and (4) van der Waals interactions between the outer electron clouds of two or more atoms. In an aqueous environment, noncovalent interactions are extremely weak and depend upon close complementarity of the shapes of antibody and antigen (Adapted from kuby immunology, 6th edition).

Ag-Ab interaction is highly specific and occurs in a similar way as a bimolecular association of an enzyme-substrate. The binding between antigens and immune components involves weak non-covalent interactions. The binding forces are relatively weak and *reversible* and consist mainly of van der Waals forces, electrostatic forces, and hydrophobic forces, all of which require a very close proximity between the interacting moieties (Figure 4.2). The smallest unit of antigen that is capable of binding with antibodies is called an *antigenic determinant* (or epitope). The corresponding area on the Ab molecule combining with the epitope is called *paratope.* The number of epitopes on the surface of an antigen is its *valence*. The valence determines the number of antibody molecules that can combine with the antigen at one time. If one epitope is present, the antigen is *monovalent*. Most antigens, however, have more than one copy of the same epitope and are termed *polyvalent.*

AFFINITY, AVIDITY AND CROSS REACTIVITY

The intrinsic association constant that characterizes the noncovalent interaction between single antigen binding sites of an antibody (paratope) with an epitope is termed *affinity*. Low-affinity antibodies bind antigen weakly and tend to dissociate readily, whereas high-affinity antibodies bind antigen more tightly and remain bound longer.

When the antigen consists of many repeating identical epitopes or when antigens are multivalent, the association between the entire antigen molecules and antibodies depends not only on the affinity between each epitope and its corresponding antibody but also on the sum of the affinities of all the epitopes involved. The term *avidity* is used to denote the overall binding between antibodies and a multivalent antigen. So, when complex Ag having multiple repeating epitopes, are mixed with Ab having multiple binding sites, the interaction of such type between multivalent Ab and Ag is called, the avidity. Thus, in general, IgM antibodies with 10 antigen binding sites are of higher avidity than IgG antibodies, although the

binding of each Fab in the IgM antibody with ligand may be of the same affinity as that of the Fab from IgG.

Cross reactivity-Although Ag-Ab reaction is very specific; sometimes antibody elicited by one antigen can cross react with an unrelated antigen. An immunological reaction in which a particular antibody or T-cell receptor react with two or more antigens that possess a common epitope is called a *cross reaction*. Another form of the cross reactivity is seen when antibodies or cells with specificity to one epitope bind, usually weakly to another epitope that is not quite identical but has a structural resemblance to the first epitope.

Thus cross reactivity refers to the ability of an individual antibody binding site to react with more than one antigenic determinants or the ability of the population of antibody molecules to react with more than one antigen .Cross reactions arise because the cross reacting antigens shares an epitope in common with the immunizing antigen or because it has an epitope which is structurally similar to one on the immunizing antigen (multi-specificity).

IMMUNOGEN

An immunogen is an agent capable of *inducing* an immune response and is therefore immunogenic. There is a fractional distinction between the term antigen and immunogen. The distinction between the two terms i.e., antigen and immunogen is necessary because there are many compounds that are incapable of binding with components of immune system that have been induced specifically against them. Thus, all immunogens are antigens but all antigens are not immunogens. Immunogens stimulates the lymphocytes to produce antibody or to attack the antigen directly. The molecular properties of antigens and the way in which these properties ultimately contribute to immune activation are central to our understanding of the immune system. Fundamental differences in the way B and T lymphocytes recognize antigen determine which molecular features of an antigen are recognized by each branch of the immune system. On the

surface of antigens are regions, called *antigenic determinants or epitopes*, which fit and bind to receptor molecules of complementary structure on the surface of the lymphocytes. Antigenic determinant or epitope is the specific site to which a particular immunoglobulin or T-cell receptor binds. The binding of the lymphocytes' receptors to the antigen's surface molecules stimulates the lymphocytes to multiply and to initiate an immune response—including the production of antibody, the activation of cytotoxic cells, or both—against the antigen. The amount of antibody formed in response to stimulation depends on the kind and amount of antigen involved, the route of entry to the body, and individual characteristics of the host. Immune cells do not interact with, or recognize, an entire immunogen molecule; instead, lymphocytes recognize discrete sites on the macromolecule called epitopes, or antigenic determinants. Epitopes are the immunologically active regions of an immunogen (antigen) that bind to antigen-specific membrane receptors on lymphocytes or to secreted antibodies. Studies with small antigens have revealed that B and T cells recognize different epitopes on the same antigenic molecule. For example, when mice were immunized with glucagon, a small human hormone of 29 amino acids, antibody was elicited to epitopes in the amino terminal portion, whereas the T cells responded only to epitopes in the carboxyl-terminal portion. Lymphocytes may interact with a complex antigen on several levels of antigen structure. An epitope on a protein antigen may involve elements of the primary, secondary, tertiary, and even quaternary structure of the protein. In polysaccharides, branched chains are commonly present, and multiple branches may contribute to the conformation of epitopes. The recognition of antigens by T cells and B cells is fundamentally different. B cells recognize soluble antigen when it binds to their membrane-bound antibody. Because B cells bind antigen that is free in solution, the epitopes they recognize tend to be highly accessible sites on the exposed surface of the immunogen. Most T cells recognize only peptides combined with MHC molecules on the surface of antigen-presenting cells and altered self-cell; T-cell epitopes, as a rule, cannot be considered apart from their associated MHC molecules.

Adjuvants

The response to an immunogen is often enhanced if it is administered as a mixture with substances called adjuvants. Adjuvants function in one or more of the following ways;

- By prolonging retention of the immunogen,
- By increasing the effective size of the immunogen,
- By stimulating the local influx of macrophages and/or other immune cell types to the injection site and promoting their subsequent activities.

The most widely used adjuvant for humans is alum precipitate, a suspension of aluminium hydroxide on to which the immunogen is adsorbed. This adjuvant increases the effective size of the immunogen and so promotes its ingestion and presentation by macrophages. For a substance or molecule to be immunogenic certain conditions must be fulfilled; these are: chemical composition, molecular size, etc.

ANTIGENICITY VERSUS IMMUNOGENICITY

Antigenicity and Immunogenicity are related but distinct immunologic properties that sometimes are confused. Antigenicity – is the ability to combine with the final products of the humoral and/or cell mediated immune response. It is not activating the immune response rather it combines with the final products of the immune response. For example, when B cells get activated it secrets antibodies and the antigens which have the property of antigenicity will combine to these antibodies. Such antigens are called *haptens.* Haptens are small molecules with very low molecular weight and that is why they are not capable of inducing the immune response. An example of such hapten is drugs like penicillin. Haptens can be immunogenic if they bind with carrier proteins because once they bind

with carrier protein, it increases the molecular weight (becomes larger molecule) and thus it can induce the immune response (Figure 4.3).

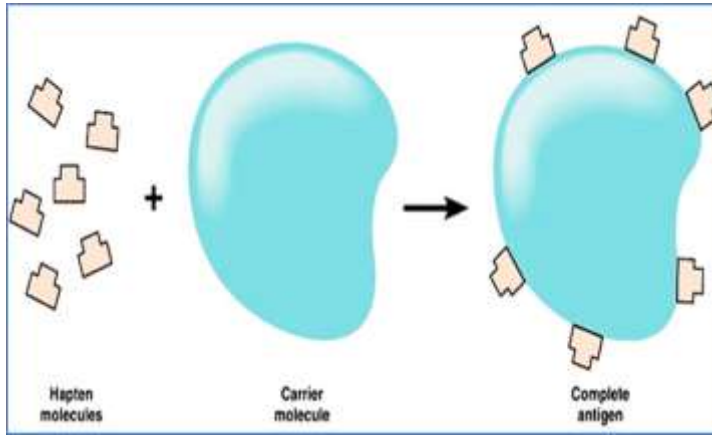


Figure 4.3. Diagram illustrating binding of hapten to carrier protein to elucidate immune response.

IMMUNOGENICITY

It is the ability to induce humoral and /or cell mediated immune response. We know when B and T cells get activated by antigen; it results in effector cells and memory cells. For example, if B cell gets activated, it will differentiate into plasma B cell which secrets antibodies. Such antigens are called *immunogen* more appropriately. These immunogens are large molecules with molecular weight at least >10,000 D and that is why they can induce the immune response. Although all molecules that have the property of immunogenicity also have the property of antigenicity, the reverse is not true. Some small molecules, called *haptens*, are antigenic but incapable by themselves of inducing a specific immune response. In other words, they lack immunogenicity. Immunogenicity is not an intrinsic property of an antigen but rather depends on a number of properties of the particular biological system that the antigen encounters. All immunogens are antigenic but all antigens are not immunogenic**.** This means all

immunogens are antigenic meaning it can react to the end product of immune response. It makes sense right that if immunogen can induce the immune response it can also bind with the final product of immune response.

Table 4.1. The difference between Antigen and immunogen

Antigen vs Immunogen	
An antigen is a foreign body or a molecule, which has the ability to bind to the antibody but does not necessarily initiate an immune response.	An Immunogen is a foreign molecule or a type of an antigen which can elicit an immune response by triggering the host immune system.
Immunogenic Property	
Immunogenic property is not found in all antigens; only some are immunogenic.	All immunogens are immunogenic.
Non immunogenic antigens can be made immunogenic by conjugating with a carrier.	

REQUIREMENTS FOR IMMUNOGENICITY

To invoke an immune response against the disease-causing invaders, the immune system must be able to recognize bacteria and their products, fungi, viruses and other pathogens as immunogens. The immune system actually recognizes particular macromolecules of a disease-causing agent. The molecules generally are proteins and polysaccharides of the infectious agent. Lipids and nucleic acids of the pathogen are not recognized as immunogens unless complexed with proteins or polysaccharides. Cell mediated immunity recognizes only immunogens that are proteins, lipids or glycolipids. The foreign proteins are processed first by the antigen presenting cells and then presented together with MHC molecules before they can be recognized as immunogens. Lipids and glycolipids that elicit cell mediated immunity must also be combined with MHC-like membrane

molecules called CD1. Immunogenicity is not an intrinsic property of an antigen but rather depends on a number of properties of the particular biological system that the antigen encounters. Immunogenicity is determined, in part, by four properties of the immunogen: its foreignness, molecular size, chemical composition and complexity, and ability to be processed and presented with an MHC molecule on the surface of an antigen-presenting cell or altered self-cell.

Foreigness

For the immune system to respond against a molecule, it must be recognized as non self by the immune cells. The capacity to recognize nonself is accompanied by tolerance of self, a specific unresponsiveness to self-antigens. The ability to differentiate between self and nonself arises during lymphocyte development, during which immature lymphocytes are exposed to self-components. The lymphocytes when exposed to molecules which it has not been exposed to during maturation are recognized as nonself, or foreign molecules. The degree of immunogenicity of an antigen depends on the degree of its foreignness. Generally, the greater the phylogenetic distance between two species, the greater the structural disparity between them. For example, the common experimental antigen bovine serum albumin (BSA) is not immunogenic when injected into a cow but is strongly immunogenic when injected into a rabbit. Moreover, BSA would be expected to exhibit greater immunogenicity in a chicken than in a goat, which is more closely related to bovines. Some macromolecules (e.g., collagen and cytochrome *c*) which have been highly conserved throughout evolution and therefore display very little immunogenicity. Conversely, some self-components (e.g., corneal tissue and sperm) are effectively sequestered from the immune system, so that if these tissues are injected even into the animal from which they originated, they will function as immunogens.

Molecular Size

The Immunogenicity of a macromolecule correlates with its size. The most potent immunogens tend to have a molecular mass of 100,000 daltons (da). In general, small molecules with a molecular weight <1000 Da (e.g., penicillin, aspirin) are not immunogenic. Molecules with molecular mass between 1000-6000 Da (e.g., albumin, tetanus toxin) are generally immunogenic. In short relatively small molecules have decreased immunogenicity, whereas, large substances have increased immunogenicity. Molecular mass of some common experimental agents used in immunology (Table 2).

Table 4.2. Molecular mass of some common antigens used in immunology

Antigen	Approximate molecular mass (Da)
Bovine gamma globulin (BGG)	150,000
Bovine serum albumin (BSA)	69,000
Flagellin (monomer)	40,000
Hen egg-white lysozyme (HEL)	15,000
Ovalbumin (OVA)	44,000
Sperm whale myoglobin (SWM)	17,000

Chemical Composition and Heterogeneity

The third characteristic necessary for a compound to be immunogenic is a certain degree of chemical complexity. Size and foreignness are not, by themselves, sufficient to make a molecule immunogenic; other properties are needed as well. For example, synthetic homopolymers (polymers composed of a single amino acid or sugar) tend to lack immunogenicity regardless of their size. A homopolymers of poly-γ-D-glutamic acid, capsular material of *Bacillus anthracis* with a molecular weight of 50,000 Da is not immunogenic. The absence of immunogenicity is because these compounds, although of high molecular weight, are not sufficiently chemically complex. Studies have shown that copolymers composed of

different amino acids or sugars are usually more immunogenic than homopolymers of their constituents. In this regard it is notable that all four levels of protein organization—primary, secondary, tertiary, and quaternary contribute to the structural complexity of a protein and hence affect its immunogenicity.

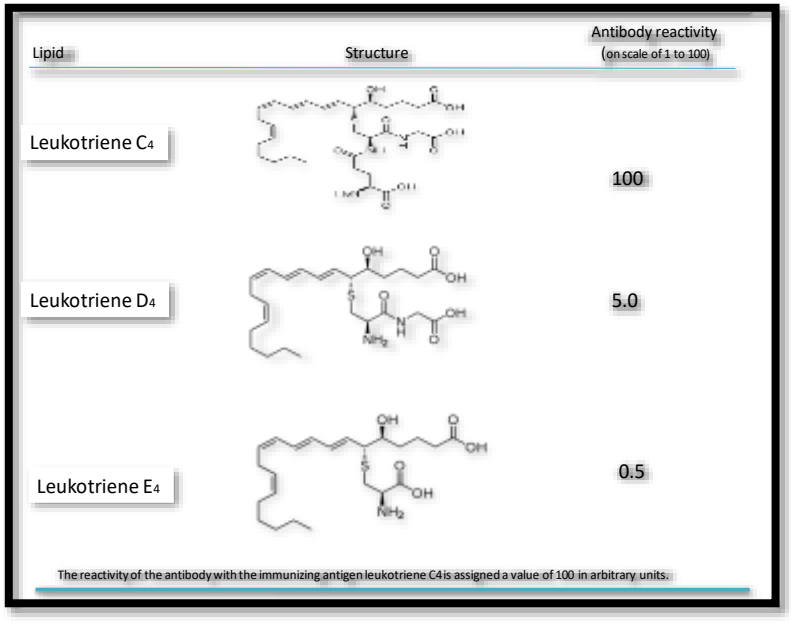
Carbohydrates are immunogenic only if they have a complex polysaccharide structure or part of complex molecules such as glycol-proteins. Carbohydrate antigens can induce antibody production without the help of T lymphocytes and are also recognized by cell surface lectins.

Lipids as Antigens

Presented lipoidal antigens can appropriately induce B- and T-cell responses. Lipids are used as haptens to stimulate B-cell responses. Haptens are organic molecules that are antigenic but not immunogenic. Chemical coupling of a hapten to a large protein such as keyhole limpet hemocyanin (KLH) or bovine serum albumin (BSA), called a carrier, yields an immunogenic hapten-carrier conjugate. By immunizing with these lipid-protein conjugates it is possible to obtain antibodies that are highly specific for the target lipids. Antibodies against a wide variety of lipid molecules have been raised while using this approach. Antibodies against steroids, complex fatty-acid derivatives, and fat-soluble vitamins such as vitamin E have been made which are of considerable medical importance. In clinical assays these antibodies are used to detect presence and amounts of medically important lipids. For example, determination of the levels of a complex group of lipids known as leukotrienes can be useful in evaluating asthma patients. During organ transplantation, an immunosuppressive steroid prednisone is often given as part of the effort to prevent rejection. The achievement and maintenance of adequate blood levels of this and other immunosuppressive drugs is important to a successful outcome of transplantation, and antibody-based immunoassays are routinely used to make these evaluations. The extraordinary sensitivity and specificity of assays based on the use of anti-lipid antibodies is

illustrated by Table 3, which shows the specificity of an antibody raised against leukotriene C4. This antibody allows the detection of as little as 16–32 picograms per ml of leukotriene C4. Because it has little or no reactivity with similar compounds, such as leukotriene D4 or leukotriene E4, it can be used to assay leukotriene C4 in samples that contain this compound and a variety of other structurally related lipids.

Table 4.3. Showing specificity of an antibody against a complex lipid. (Adapted from Kuby, immunology, 6th Ed.)



T lymphocytes recognize peptides derived from protein antigens when are presented as peptide-MHC complexes. Some lipids however can also be recognized by T cells. Complex lipids such as glycolipids and some phospholipids are recognized by T-cell receptors when presented as complexes with molecules that are very much like MHC molecules. These lipid-presenting molecules are members of the CD1 family are close structural relatives of class I MHC molecules. The lipid molecules

recognized by the CD1–T-cell receptor system all appear to share the common feature of a hydrophobic portion and a hydrophilic head group. The hydrophobic portion is a long-chain fatty acid or alcohol and the hydrophilic head group is composed of highly polar groups that often contain carbohydrates. Recognition of lipids is a part of the immune response to some pathogens, and T cells that recognize lipids arising from *Mycobacterium tuberculosis* and *Mycobacterium leprae,* which respectively cause tuberculosis and leprosy, have been isolated from humans infected by these mycobacteria.

IMMUNOGENICITY BY THE BIOLOGY OF THE ORGANISM

Even if a macromolecule has the properties that contribute to immunogenicity, its ability to induce an immune response will depend on certain properties of the biological system that the antigen encounters. These properties include the genotype of the recipient, the dose and route of antigen administration, and the administration of substances, called adjuvants, that increase immune responses.

GENOTYPE OF THE IMMUNIZED ANIMAL

The genetic constitution (genotype) of an immunized animal influences the type of immune response the animal manifests, as well as the degree of the response. For example, Hugh McDevitt showed that two different inbred strains of mice responded very differently to a synthetic polypeptide immunogen. After exposure to the immunogen, one strain produced high levels of serum antibody, whereas the other strain produced low levels. When the two strains were crossed, the F1 generation showed an intermediate response to the immunogen. By backcross analysis, the gene controlling immune responsiveness was mapped to a subregion of the major histocompatibility complex (MHC). Numerous experiments with

simple defined immunogens have demonstrated genetic control of immune responsiveness, largely confined to genes within the MHC. These data indicate that MHC gene products, which function to present processed antigen to T cells, play a central role in determining the degree to which an animal responds to an immunogen. The response of an animal to an antigen is also influenced by the genes that encode B-cell and T-cell receptors and by genes that encode various proteins involved in immune regulatory mechanisms. Genetic variability in all of these genes affects the immunogenicity of a given macromolecule in different animals.

IMMUNOGEN AND ROUTE OF ADMINITRATION

Each experimental immunogen exhibits a particular dose-response curve, which is determined by measuring the immune response to different doses and different administration routes. An antibody response is measured by determining the level of antibody present in the serum of immunized animals. Evaluating T-cell responses is less simple but may be determined by evaluating the increase in the number of T cells bearing TCRs that recognize the immunogen. Some combination of optimal dosage and route of administration will induce a peak immune response in a given animal. An insufficient dose will not stimulate an immune response either because it fails to activate enough lymphocytes or because, in some cases, certain ranges of low doses can induce a state of immunologic unresponsiveness, or tolerance. Conversely, an excessively high dose can also induce tolerance. The immune response of mice to the purified pneumococcal capsular polysaccharide illustrates the importance of dose. A 0.5 mg dose of antigen fails to induce an immune response in mice, whereas a thousand-fold lower dose of the same antigen (5×10^{-4} mg) induces a humoral antibody response. A single dose of most experimental immunogens will not induce a strong response; rather, repeated administration over a period of weeks is usually required. Such repeated administrations, or boosters, increase the clonal proliferation of antigen-

specific T cells or B cells and thus increase the lymphocyte population's specific for the immunogen.

Experimental immunogens are generally administered parenterally (*para,* around; *enteric*, gut)—that is, by routes other than the digestive tract. The following administration routes are common:

- Intravenous (iv): into a vein
- Intradermal (id): into the skin
- Subcutaneous (sc): beneath the skin
- Intramuscular (im): into a muscle
- Intraperitoneal (ip): into the peritoneal cavity

The administration route strongly influences which immune organs and cell populations will be involved in the response. Antigen administered intravenously is carried first to the spleen, whereas antigen administered subcutaneously moves first to local lymph nodes. Differences in the lymphoid cells that populate these organs may be reflected in the subsequent immune response.

SUSCEPTIBILITY TO ANTIGEN PROCESSNG AND PRESENTATION

Development of both humoral and cell mediated immune responses requires interaction of T cells with antigen T cells with antigen that has been processed and presented together with MHC molecules. Large, insoluble macromolecules generally are more immunogenic than small, soluble ones because the larger molecules are more readily phagocytosed and processed. Macromolecules that cannot be degraded and presented with MHC molecules are poor immunogens. This can be illustrated with polymers of D-amino acids, which are stereoisomers of the naturally occurring L-amino acids. Because the degradative enzymes within antigen-presenting cells can degrade only proteins containing L-amino acids,

polymers of D-amino acids cannot be processed and thus are poor immunogens.

HAPTENS

Haptens are organic molecules that are antigenic but not immunogenic. The term hapten (half antigen) was introduced in the 1920s by Karl Landsteiner to define low molecular weight chemical reagents which, as such, will not induce immune responses. However, immune responses to haptenated macromolecular carriers (proteins) lead to the production of hapten-specific antibodies and T lymphocytes. These molecules elicit an immune response only when attached to a large carrier such as a protein. Once the body has generated antibodies to a hapten-carrier adduct, the small-molecule hapten may also be able to bind to the antibody, but it will usually not initiate an immune response; usually only the hapten-carrier adduct can do this. Sometimes the small-molecule hapten can even block immune response to the hapten-carrier adduct by preventing the adduct from binding to the antibody, a process called *hapten inhibition*. Haptens have been used to study allergic contact dermatitis (ACD) and the mechanisms of inflammatory bowel disease (IBD) to induce autoimmune-like responses. The concept of haptens emerged from the work of Karl Landsteiner who also pioneered the use of synthetic haptens to study immunochemical phenomena.

Karl Landsteiner in the 1920s and 1930s created a simple, chemically defined system for studying the binding of an individual antibody to a unique epitope on a complex protein antigen. Landsteiner employed various haptens, small organic molecules that are antigenic but not immunogenic. Chemical coupling of a hapten to a large protein, called a carrier, yields an immunogenic hapten-carrier conjugate. Animals immunized with such a conjugate, produce antibodies specific for (1) the hapten determinant, (2) unaltered epitopes on the carrier protein, and (3) new epitopes formed by combined parts of both the hapten and carrier. By itself, a hapten cannot function as an immunogenic epitope. But when

multiple molecules of a single hapten are coupled to a carrier protein (or non-immunogenic homopolymer), the hapten becomes accessible to the immune system and can function as an immunogen.

There are three important characteristics of anti-hapten antibody responses to hapten-protein conjugates. First, such responses require both hapten-specific B cells and protein (carrier)–specific helper T cells. Second, to stimulate a response, the hapten and carrier portions have to be physically linked and cannot be administered separately. Third, the interaction is class II MHC restricted, that is, the helper T cells cooperate only with B lymphocytes that express class II MHC molecules that are identical to those that were involved in the initial activation of naïve T cells by dendritic cells. All of these features of antibody responses to hapten-protein conjugates can be explained by the antigen-presenting functions of B lymphocytes. Hapten-specific B cells bind the antigen through the hapten determinant, endocytose the hapten-carrier conjugate, and present peptides derived from the carrier protein to carrier-specific helper T lymphocytes (Figure 4.4). Thus, the two cooperating lymphocytes recognize different epitopes of the same complex antigen. The hapten is responsible for efficient internalization of the carrier protein into the B cell, which explains why hapten and carrier must be physically linked. The requirement for MHC-associated antigen presentation for T cell activation accounts for the MHC restriction of T cell–B cell interactions.

The characteristics of humoral responses elucidated for hapten-carrier conjugates apply to all protein antigens in which one intrinsic determinant, usually a native conformational determinant, is recognized by B cells (and is therefore analogous to the hapten) and another determinant in the form of a class II MHC–associated linear peptide, is recognized by helper T cells (and is analogous to the carrier that is the source of the peptide). The hapten-carrier effect is the basis for the development of conjugate vaccines, which contain carbohydrate epitopes recognized by B cells attached to proteins recognized by T cells.

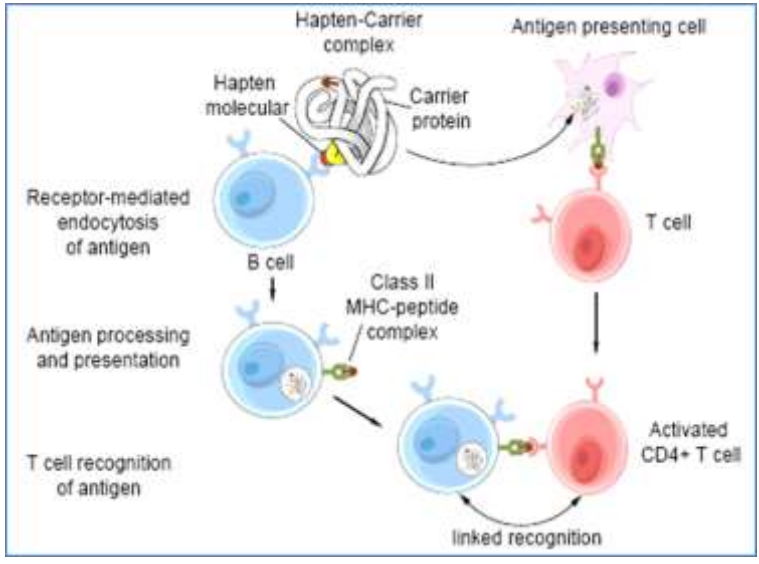


Figure 4.4. Diagram illustrating induction of immune response by Hapten-carrier complex.

EXAMPLES OF SOME HAPTENS

The first researched haptens were aniline and its carboxyl derivatives (o-, m-, and p-aminobenzoic acid). A well-known example of a hapten is urushiol, which is the toxin found in poison ivy. When absorbed through the skin from a poison ivy plant, urushiol undergoes oxidation in the skin cells to generate the actual hapten, a reactive quinone-type molecule, which then reacts with skin proteins to form hapten adducts. Usually, the first exposure causes only sensitization, in which there is a proliferation of effector T-cells. After a subsequent, second exposure, the proliferated T-cells can become activated, generating an immune reaction that produces typical blisters of a poison ivy exposure. Some haptens can induce autoimmune disease. An example is hydralazine, a blood pressure-lowering drug that occasionally can produce drug-induced lupus erythematosus in certain individuals. This also appears to be the mechanism by which the anaesthetic gas halothane can cause a life-

threatening hepatitis, as well as the mechanism by which penicillin-class drugs cause autoimmune haemolytic.

Other haptens that are commonly used in molecular biology applications include fluorescein, biotin, digoxigenin, and dinitrophenol. Lastly, nickel allergy is caused by nickel metal ions penetrating the skin and binding to skin proteins.

CARRIER PROTEIN

KLH (keyhole limpet hemocyanin) is a copper-containing protein that is found in arthropods and Mollusca. Therefore, it is an ideal carrier to use in mammalian hosts such as rabbits and mice. It is isolated from *Megathura crenulata*, and has a molecular weight that ranges from 4.5×10^5 to 1.3×10^7 Da. KLH is the carrier that is used most commonly because it has a higher immunogenicity than does BSA. However, its solubility in water is limited because of its size and structure, which results in a flocculent appearance. The turbidity does not affect immunogenicity, and the resulting solution can be used for successful immunizations.

BSA (bovine serum albumin) is a plasma protein in cattle that is one of the most stable and soluble albumins available. It has a molecular weight of 67 kDa, and includes 59 lysines. Of these, ~30–35 are accessible for use in linker conjugation, and so BSA is a popular carrier protein for weakly antigenic compounds. BSA is more water-soluble than KLH because it is smaller; therefore, it is used more commonly in immunoassays. However, because BSA is commonly used to block nonspecific binding sites in antibody-based assays, BSA conjugates should not be used for immunization if the end-point assay system uses BSA. This is because if anti-sera against peptide-BSA conjugates are used in these assays, false positives are common because the sera used also contain antibodies against BSA.

OVA (ovalbumin) is a protein isolated from hen egg whites. It has a molecular weight of 45 kDa. It is often used as a second carrier protein to

confirm that antibodies are specific for the Hapten rather than the carrier protein.

ADJUVANTS AND IMMUNOGENICITY

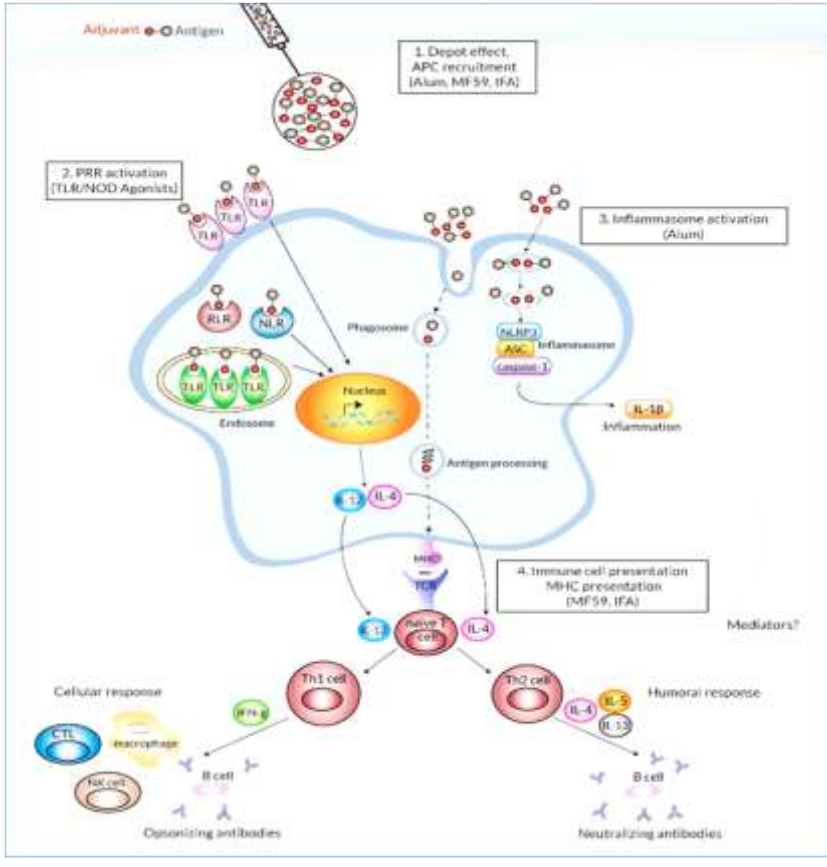


Figure 4.5. Mechanism of adjuvant action.

Adjuvants (from Latin ‘adjuvare’, ‘to help’) are substances that, when mixed with an antigen and injected with it, enhance the immunogenicity of that antigen. Adjuvants are often used to boost the immune response when an antigen has low immunogenicity or when only small amounts of an

antigen are available. For example, the antibody response of mice to immunization with BSA can be increased fivefold or more if the BSA is administered with an adjuvant. Precisely how adjuvants augment the immune response is not entirely known, but they appear to exert one or more of the following effects:

- Antigen persistence is prolonged.
- Co-stimulatory signals are enhanced.
- Local inflammation is increased.
- The nonspecific proliferation of lymphocytes is stimulated.

Aluminium potassium sulfate (alum) prolongs the persistence of antigen. When an antigen is mixed with alum, the salt precipitates the antigen. Injection of this alum precipitate results in a slower release of antigen from the injection site, so that the effective time of exposure to the antigen increases from a few days without adjuvant to several weeks with the adjuvant. The alum precipitate also increases the size of the antigen, thus increasing the likelihood of phagocytosis. Water-in-oil adjuvants also prolong the persistence of antigen. A preparation known as *Freund's incomplete adjuvant* contains antigen in aqueous solution, mineral oil, and an emulsifying agent such as mannide monooleate, which disperses the oil into small droplets surrounding the antigen; the antigen is then released very slowly from the site of injection. This preparation is based on *Freund's complete adjuvant*, the first deliberately formulated highly effective adjuvant, developed by Jules Freund many years ago and containing heat-killed *Mycobacteria* as an additional ingredient. Muramyl dipeptide, a component of the mycobacterial cell wall, activates macrophages, making Freund's complete adjuvant far more potent than the incomplete form. Activated macrophages are more phagocytic than inactivated macrophages and express higher levels of class II MHC molecules and the membrane molecules of the B7 family. The increased expression of class II MHC increases the ability of the antigen-presenting cell to present antigen to TH cells. B7 molecules on the antigen presenting cell bind to CD28, a cell-surface protein on TH cells, triggering co-

stimulation, an enhancement of the T cell immune response. Thus, antigen presentation and the requisite co-stimulatory signal usually are increased in the presence of adjuvant. Alum and Freund's adjuvants also stimulate a local, chronic inflammatory response that attracts both phagocytes and lymphocytes (Figure 4.5). This infiltration of cells at the site of the adjuvant injection often results in formation of a dense, macrophage-rich mass of cells called a *granuloma.* Because the macrophages in a granuloma are activated, this mechanism also enhances the activation of TH cells. Other adjuvants (e.g., synthetic polyribonucleotide and bacterial lipopolysaccharides) stimulate the nonspecific proliferation of lymphocytes and thus increase the likelihood of antigen-induced clonal selection of lymphocytes.

CONCLUSION

The terms *antigen* and *immunogen* are often used synonymously. However, these terms antigen and immunogen, imply two closely related entities. One which describes a molecule which reacts with the antibody produced or with the activated cellular constituents of cell mediated immunity, is referred to as an *antigen*. Other which describes a molecule that provokes an immune response is called an *immunogen*. Subsequently *antigenicity* is the ability to combine with the final products of the humoral and/or cell mediated immune response. It is not activating the immune response rather it combines with the final products of the immune response.

Antigens are recognised not only by antibodies but also by antigen specific T cell receptors. In contrast to immunoglobulin's, which usually recognize intact antigen, T cell surface receptors recognize processed antigen on the surface of antigen presenting cells, together with the major histocompatibility complex (MHC) Class I or Class II surface proteins.

Immunogenicity – is the ability to induce humoral and /or cell mediated immune response. In contrast haptens are small well-defined chemical groupings such as dinitrophenyl (DNP) which are not immunogenic on

their own but will react with preformed antibodies. To make a hapten immunogenic it must be linked to a carrier molecule which is itself immunogenic. Immunogenicity is not an intrinsic property of an antigen but rather depends on a number of properties of the particular biological system that the antigen encounters.

All immunogens are antigenic but all antigens are not immunogenic**.** A substance has to fulfil several requirements for immunogenicity as for example the nature of the Immunogen also contributes to Immunogenicity. Immunogenicity is determined, in part, by various properties of the immunogen such as its foreignness, molecular size, chemical composition and complexity. Immunogen dosage and route of immunogen administration also plays a central role in the development of immunogenicity and hence immune response.

The insufficient dose of immunogen may not stimulate an immune response either because the amount administered fails to activate enough lymphocytes or because such a dose renders the responding cells unresponsive. Besides the need to administer a threshold amount of immunogen to induce an immune response, the number of doses administered also affects the outcome of the immune response generated.

The route of administration also affects the outcome of the immunization because this determines which organs and cell populations will be involved in the response. Immunogens can be administered through number of common routes which include *Intravenous* (into a vein), *intradermal* (into the skin), *subcutaneous* (beneath the skin), *intramuscular* (into the muscle). Antigens administered via the most common route namely, *subcutaneously*, generally elicit the strongest immune responses. This is due to their uptake, processing, and presentation to effector cells by Langerhans cells present in the skin, which are among the most potent APCs. Immunogenicity is also manipulability through subtle structural modification. Learning about the structural basis of immunogenicity is critical for eliciting desired B cell antibody production through vaccination.

FREQUENTLY ASKED QUESTIONS (FAQS)

Q1. What is an immunogen?

Answer: An Immunogen is an antigen that induces a specific humoral and or cell mediated immune response and this ability is termed as immunogenicity.

Q2. What is the difference between an antigen and an immunogen?

Answer: All antigens are recognized by specific lymphocytes or by antibodies, but not every antigen can evoke an immune response. Those antigens that are capable of inducing an immune response are said to be immunogenic and are called immunogens. Thus all immunogens are antigens, but not all antigens are immunogenic.

Q3. What is immunogenicity and what are the various factors that affect immunogenicity.

Answer: Immunogenicity is the ability of an antigen to invoke immune responses. Immunogenicity is not an intrinsic property of an antigen but rather depends on a number of properties of the particular biological system that the antigen encounters. Immunogenicity is determined, in part, by four properties of the immunogen: its foreignness, molecular size, chemical composition and complexity, and ability to be processed and presented with an MHC molecule on the surface of an antigen-presenting cell or altered self-cell.

Q4. How foreignness of an antigen does affect immunogenicity?

Answer: For the immune system to respond against a molecule, it must be recognized as non self by the immune cells. The capacity to recognize nonself is accompanied by tolerance of self, a specific unresponsiveness to self-antigens. The ability to differentiate between self and non-self-arises during lymphocyte development during which immature lymphocytes are exposed to self-components. The lymphocytes when exposed to molecules which it has not been exposed to during maturation are recognized as nonself, or foreign molecules. The degree of

immunogenicity of an antigen depends on the degree of its foreignness. Generally, the greater the phylogenetic distance between two species, the greater the structural disparity between them. For example, the common experimental antigen bovine serum albumin (BSA) is not immunogenic when injected into a cow but is strongly immunogenic when injected into a rabbit. Moreover, BSA would be expected to exhibit greater immunogenicity in a chicken than in a goat, which is more closely related to bovines.

Q5. How is phylogeny related to immunogenicity?

Answer: Phylogeny refers to the evolutionary relationship between species. Generally, the greater the phylogenetic distance between two species, the greater the structural disparity between them. For example, the common experimental antigen bovine serum albumin (BSA) is not immunogenic when injected into a cow but is strongly immunogenic when injected into a rabbit. Moreover, BSA would be expected to exhibit greater immunogenicity in a chicken than in a goat, which is more closely related to bovines. Some macromolecules (e.g., collagen and cytochrome *c*) which have been highly conserved throughout evolution and therefore display very little immunogenicity.

Q6. How molecular mass of an antigen does affects immunogenicity?

Answer: The Immunogenicity of a macromolecule correlates with its size. The most potent immunogens tend to have a molecular mass of 100,000 daltons (da). In general, small molecules with a molecular weight <1000 Da (e.g., penicillin, aspirin) are not immunogenic. Molecules with molecular mass between 1000-6000 Da (e.g., albumin, tetanus toxin) are generally immunogenic.

Q7. How do lipid antigens evoke immune response?

Answer: Presented lipoidal antigens can appropriately induce B- and T-cell responses. Lipids are used as haptens to stimulate B-cell responses. Haptens are organic molecules that are antigenic but not immunogenic. Chemical coupling of a hapten to a large protein such as keyhole limpet

hemocyanin (KLH) or bovine serum albumin (BSA), called a carrier, yields an immunogenic hapten-carrier conjugate.

Q8. What is the chemical importance of antibodies against steroids, complex fatty-acid derivatives?

Answer: Antibodies against steroids, complex fatty-acid derivatives, and fat-soluble vitamins such as vitamin E have been made which are of considerable medical importance. In clinical assays these antibodies are used to detect presence and amounts of medically important lipids. For example, determination of the levels of a complex group of lipids known as leukotrienes can be useful in evaluating asthma patients.

Q9. How chemical complexity of an antigen does affects immunogenicity?

Answer: For a compound to be immunogenic, a certain degree of chemical complexity is necessary. Size and foreignness are not, by themselves, sufficient to make a molecule immunogenic; other properties are needed as well. For example, synthetic homopolymers (polymers composed of a single amino acid or sugar) tend to lack immunogenicity regardless of their size. A homopolymers of poly-γ-D-glutamic acid, capsular material of *Bacillus anthracis* with a molecular weight of 50,000 Da is not immunogenic. The absence of immunogenicity is because these compounds, although of high molecular weight, are not sufficiently chemically complex.

Q10. How genotype of the recipient hosts does affect immunogenicity?

Answer: The genetic constitution (genotype) of an immunized animal influences the type of immune response the animal manifests, as well as the degree of the response. For example, Hugh McDevitt showed that two different inbred strains of mice responded very differently to a synthetic polypeptide immunogen. After exposure to the immunogen, one strain produced high levels of serum antibody, whereas the other strain produced low levels. When the two strains were crossed, the F1 generation showed an intermediate response to the immunogen. By backcross analysis, the

gene controlling immune responsiveness was mapped to subregion of the major histocompatibility complex (MHC).

Q11. Define route of dosage of immunogen and how it affects immunogenicity?

Answer: The route of dosage of an immunogen is the path by which an immunogenic substance is taken into the body. The following administration routes are common: Intravenous (iv): into a vein, Intradermal (id): into the skin, Subcutaneous (sc): beneath the skin, Intramuscular (im): into a muscle, Intraperitoneal (ip): into the peritoneal cavity. The administration route strongly influences which immune organs and cell populations will be involved in the response. Antigen administered intravenously is carried first to the spleen, whereas antigen administered subcutaneously moves first to local lymph nodes. Differences in the lymphoid cells that populate these organs may be reflected in the subsequent immune response.

Q12. D-stereoisomers of polymers are poor immunogens, explain?

Answer: Macromolecules that cannot be degraded and presented with MHC molecules are poor immunogens. The polymers of D-amino acids, which are stereoisomers of the naturally occurring L-amino acids are very less immunogenic. Because the degradative enzymes within antigen-presenting cells can degrade only proteins containing L-amino acids, polymers of D-amino acids cannot be processed and thus are poor immunogens.

Q13. How does antigens presentation by MHC molecules affects antigenicity?

Answer: Development of both humoural and cell mediated immune responses requires interaction of T cells with antigen that has been processed and presented together with MHC molecules on antigen presenting cells like B cells, dendritic cells and macrophages. Large, insoluble macromolecules generally are more immunogenic than small, soluble ones because the larger molecules are more readily phagocytosed

and processed. Macromolecules that cannot be degraded and presented with MHC molecules are poor immunogens.

Q14. Do T cells recognize complex antigenic lipid molecules?

Answer: Complex lipids such as glycolipids and some phospholipids are recognized by T-cell receptors when presented as complexes with molecules that are very much like MHC molecules. These lipid-presenting molecules are members of the CD1 family are close structural relatives of class I MHC molecules. The lipid molecules recognized by the CD1–T-cell receptor system all appear to share the common feature of a hydrophobic portion and a hydrophilic head group. The hydrophobic portion is a long-chain fatty acid or alcohol and the hydrophilic head group is composed of highly polar groups that often contain carbohydrates.

Q15. What are the two main drawbacks of antibiotic penicillin?

Answer: One is the role of penicillin's in the evolution of antibiotic resistant bacterial strains. Another is their capacity to induce allergic reactions in some patients. Penicillin and its relatives are responsible for most of the recorded allergic reactions to drugs and 97% of the deaths caused each year by drug allergies.

MULTIPLE CHOICE QUESTIONS (MCQS)

Q1. Any molecule that induce or elicit an immune response are

A. Antigen
B. Antibodies
C. Epitope
D. Immunogens

Q2. Majority of antigens are

A. Proteins
B. Carbohydrates
C. Nucleic acids
D. Lipids

Q3. Materials that work well as immunogens include

A. proteins, lipoproteins, polysaccharides
B. glycoproteins, DNA, RNA
C. lipopolysaccharides, haptens, monosaccharides
D. small molecules, simple molecules, and repetitive molecules

Q4. Which among the following is poorly immunogenic?

A. Proteins
B. Carbohydrates
C. Nucleic acids
D. Lipids

Q5. The portion of an antigen which binds to the receptor molecules and develops an immune response is called

A. A mosaic
B. Hapten
C. Epitope
D. Alloantigen

Q6. Which among the following statements is NOT correct?

A. All antigens are also immunogens.
B. All immunogens are antigens
C. Processing is required to generate T cell antigens
D. Epitope is the portion of an antigen which is recognized and elicits an immune response

Q7. Which among the following statements is NOT correct?

A. Immunogenicity depends on the foreignness of an antigen
B. Proteins are more immunogenic than carbohydrates
C. Proteins are less immunogenic than carbohydrates
D. DNA and RNA are poorly immunogenic

Q8. Which one of the following will increase immunogenicity of a substance?

A. Making a molecule less foreign
B. Increasing chemical complexity
C. Making a molecule small
D. Increasing chemical complexity and decreasing molecular size

Q9. The antibiotic penicillin is a small molecule that does not induce antibody formation. However, penicillin binds to serum proteins and forms a complex that in some people induces antibody formation. Penicillin is therefore

A. An antigen
B. A hapten
C. An immunogen
D. Both an antigen and a hapten

Q10. Which one of the following is immunogenic?

A. Penicillin
B. Aspirin
C. Albumin
D. Poly-γ-D-glutamic acid

Q11. Which one among the following is poorly immunogenic?

A. Bovine serum albumin
B. D isomer of human albumin
C. L isomer of human albumin
D. D- glucose units coupled to a protein

Q12. Which among the following self-molecules if injected into self will generate an immune response?

A. corneal tissue
B. serum albumin
C. Self-liver tissue
D. Self-Kidney tissue

Q13. Which one of the following is not a route of administration?

A. Intravenous
B. Oral
C. Topical
D. Dissolution

Q14. Which among the following statement is true?

A. All immunogens are antigens but all antigens are not immunogens
B. All immunogens are antigens and all antigens are immunogens
C. All immunogens are not antigens but all antigens are immunogens
D. All immunogens are proteins and all proteins are immunogens

Q15. Which of the statements are true regarding antigen?

A. Generally self-molecules and molecules with low molecular weight are highly immunogenic
B. An antigen generally has only one epitope
C. Heteropolymers are more immunogenic than homopolymers
D. Foreignness of an antigen does not affect its immunogenicity

Answer Key

1. D **2**. A **3**. A **4**. C **5**. C **6**. A **7**. C **8**. D **9**. B **10**. C **11**. B **12**. A **13**. D **14**. A **15**. C

ASSIGNMENTS

Long Answer Questions (500 words)

Q1. Describe how you would set up an experiment to show grade school science students what you mean by "Immunogenicity." Do it by using antigen X and antigen Y injected into mice. X & Y have different molecular weights.

Q2. Why would removal of antigen lead to the decline in an immune response?

Short Answer Questions (200 words)

Q1. Many evolutionarily related proteins have conserved amino acid sequences. What consequences might this have in terms of the antigenicity of these proteins?

Q2. All immunogens are antigens but not all antigens are immunogens. Explain?

Q3. Explain why the self-molecules like corneal tissues and sperm will develop an immune response if they come in contact with immune cells.

REFERENCES

Arnon, R., Van Regenmortel, M. H. Structural basis of antigenic specificity and design of new vaccines. *The FASEB journal*. 1992 Nov; 6(14):3265-74.

Baker, M. P., Reynolds, H. M., Lumicisi, B.; Bryson, C. J., (2010). "Immunogenicity of protein therapeutics: The key causes, consequences and challenges." *Self/Nonself*. 1 (4): 314-322.

Barbosa, M. D., Celis E. Immunogenicity of protein therapeutics and the interplay between tolerance and antibody responses. *Drug discovery today*. 2007 Aug 1;12(15-16):674-81.

Bergmann, C. C., Yao, Q., Ho, C. K., Buckwold, S. L. Flanking residues alter antigenicity and immunogenicity of multi-unit CTL epitopes. *The Journal of Immunology*. 1996 Oct 15;157(8):3242-9.

Berzofsky, J. A., and Berkower, J. J.. 1999. Immunogenicity and antigen structure. In *Fundamental Immunology*, 4th ed., W. E. Paul, ed., Lippincott-Raven, Philadelphia.

CRUMPTON, M. J. Protein antigens: the molecular bases of antigenicity and immunogenicity. In *The antigens* 1974 Jan 1 (pp. 1-78). Academic Press.

De Groot AS, Scott DW, (2007). "Immunogenicity of protein therapeutics." *Trends in Immunology*. 28 (11): 482–490.

Feizi T, Childs RA. Carbohydrates as antigenic determinants of glycoproteins. *Biochemical Journal.* 1987 Jul 1;245(1):1.

Green DR, Ferguson T, Zitvogel L, Kroemer G. Immunogenic and tolerogenic cell death. *Nature Reviews Immunology.* 2009 May;9(5):353.

Heinz FX, Tuma WO, Kunz CH. Antigenic and immunogenic properties of defined physical forms of tick-borne encephalitis virus structural proteins. *Infection and immunity.* 1981 Jul 1;33(1):250-7.

Janeway Jr, C. A., Travers, P., Wolpert, M., Schlomchik, M. J. *Immunobiology.* London: Garland Publishing; 2004.

Jawa, Vibha; Degroot, A. S.; Cousens, L.; Awwad, M; Kropshofer, H.; Wakshull E. (December 2013). *"T-cell dependent immunogenicity of protein therapeutics: Preclinical assessment and mitigation."* Clinical Immunology. 149 (3): 534-555.

Lamm, M. E., Strober W., McGhee, J. R., Mayer, L., eds. Mestecky, J, Bienenstock, J. *Mucosal Immunology.* San Diego: Academic Press; 2005.

Lunde E, Bogen B, Sandlie I. Immunoglobulin as a vehicle for foreign antigenic peptides immunogenic to T cells. *Molecular immunology.* 1997 Nov 1;34(16-17):1167-76.

Lydyard PM, Whelan A, Fanger MW. Instant Notes in Immunology, 2nd edn. London: Garland Science/Bios Scientific Publishing, 2004.

Lynn A. K., Yannas, I. V., Bonfield W. Antigenicity and immunogenicity of collagen. *Journal of Biomedical Materials Research Part B: Applied Biomaterials: An Official Journal of The Society for Biomaterials,* The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials. 2004 Nov 15;71(2):343-54.

Mazor R, Onda M, Pastan I. Immunogenicity of therapeutic recombinant immunotoxins. *Immunological reviews.* 2016 Mar;270(1):152-64.

Mumford JA, Wilson H, Hannant D, Jessett DM. Antigenicity and immunogenicity of equine influenza vaccines containing a Carbomer adjuvant. *Epidemiology & Infection.* 1994 Apr;112(2):421-37.

Naisbitt DJ, Gordon SF, Pirmohamed M, Burkhart C, Cribb AE, Pichler WJ, Park BK. Antigenicity and immunogenicity of sulphamethoxazole: demonstration of metabolism-dependent haptenation and T-cell proliferation in vivo. *British journal of pharmacology.* 2001 May;133(2):295-305.

Novotný J, Handschumacher M, Bruccoleri RE. Protein antigenicity: a static surface property. *Immunology today.* 1987 Jan 1;8(1):26-31.

Playfair JHL, Chain BM. *Immunology at a Glance,* 7th edn. Oxford: Blackwell Scientific Publications; 2004.

Rajewsky KV, Schirrmacher V, Nase S, Jerne NK. The requirement of more than one antigenic determinant for immunogenicity. *Journal of Experimental Medicine.* 1969 Jun 1;129(6):1131-43.

Reddy KV, Yedery RD, Aranha C. Antimicrobial peptides: premises and promises. *Int J Antimicrob Agents* 2004; 24:536–547.

Roitt IM, Delves P. Essential Immunology, 10th edn. Oxford: Blackwell Scientific Publications; 2001.

Sable SB, Kalra M, Verma I, Khuller GK. Tuberculosis subunit vaccine design: the conflict of antigenicity and immunogenicity. *Clinical immunology.* 2007 Mar 1;122(3):239-51.

Sela M. Antigenicity: some molecular aspects. *Science.* 1969 Dec 12;166(3911):1365-74.

Selvarajah S, Puffer B, Pantophlet R, Law M, Doms RW, Burton DR. Comparing antigenicity and immunogenicity of engineered gp120. *Journal of virology.* 2005 Oct 1;79(19):12148-63.

Sette A, Vitiello A, Reherman B, Fowler P, Nayersina R, Kast WM, Melief CJ, Oseroff C, Yuan L, Ruppert J, Sidney J. The relationship between class I binding affinity and immunogenicity of potential cytotoxic T cell epitopes. *The Journal of Immunology.* 1994 Dec 15;153(12):5586-92.

Shinnick TM, Sutcliffe JG, Green N, Lerner RA. Synthetic peptide immunogens as vaccines. *Annual review of microbiology*. 1983 Oct;37(1):425-46.

Steinman RM. Dendritic cells: understanding immunogenicity. *European journal of immunology*. 2007 Nov;37(S1):S53-60.

Tao, X. and Xu, A., 2016. Basic Knowledge of Immunology. In *Amphioxus Immunity* .

Todd PE, East IJ, Leach SJ. The immunogenicity and antigenicity of proteins. *Trends in Biochemical Sciences*. 1982 Jun 1;7(6):212-6.

Wang W, Singh SK, Li N, Toler MR, King KR, Nema S. Immunogenicity of protein aggregates—concerns and realities. *International journal of pharmaceutics*. 2012 Jul 15;431(1-2):1-1.

Wilson IA, Niman HL, Houghten RA, Cherenson AR, Connolly ML, Lerner RA. The structure of an antigenic determinant in a protein. *Cell*. 1984 Jul 1;37(3):767-78.

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Chapter 5

THE INNATE AND ADAPTIVE IMMUNITY

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ABSTRACT

Immune system consists of a complex network of organisations and processes that have evolved to protect the host organisms against the attack of many unwanted foreign invaders. The function of these organisations or processes is divided into two mechanisms: 1. Innate or non-specific immune system and 2. Adaptive or specific immune system. Innate system being the first line of defence mechanism show same response to all pathogens.

It includes physical barrier like skin, saliva, cells like macrophages, neutrophils, basophils, mast cells etc. This mechanism protects organisms for the first few days of infection and in certain cases when infection is very crucial, first line of defence is followed by second line of defence

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i.e., adaptive system of immunity, building up a memory of infection showing an enhanced response to each pathogen foreign to the body. The cells of the adaptive immune system are special types of leucocytes, called lymphocytes and antigen presenting cells, i.e., B-lymphocytes or B-cells and T-lymphocytes or T-cells, which are derived from hematopoietic stem cells in the bone marrow. B-cells are involved in the humoral immune response whereas T-cells are involved in cell-mediated immune response.

Keywords: innate, acquired, active, mast-cells, passive, cell-mediated, monocytes, humoral

OBJECTIVES

- Describe the concept of acquired immunity.
- Difference between active and passive immunity.
- Types of acquired immunity.

INTRODUCTION

Innate also known as native or natural immunity is present since birth and consists of many factors that are relatively nonspecific-that is, it operates against almost any foreign molecules and pathogens. It is not specific to any one pathogen but rather acts against all foreign molecules and pathogens. It also does not rely on previous exposure to a pathogen and response is functional since birth and has no memory. Thus, providing first line of defence against pathogens. Innate immune response relies on different antimicrobial chemicals and phagocytic cells which provide protection against pathogens. Non-specific defence mechanism is similar for most type of infections. It resists infection by blocking the entry of pathogens into the body or by destroying the microbes through means other than antibodies. This type of immunity is inherited by the organisms from the parents and protects it from birth throughout life.

It is further of two types: External and internal defence:

1) *External Defence or First Line of Defence*

It consists of physical and chemical barriers to the entry of pathogens into the body.

Table 5. 1. External defence mechanisms

Type	**Skin**	**Gastrointestinal tract**	**Respiratory tract**	**Urogenital tract**	**Eyes**
Physical barriers	Flow of fluid, perspiration, sloughing off of skin.	Flow of fluid, mucus, food and saliva	Flow of fluid and mucus, e.g., by cilia. Air flow	Flow of fluid, urine, mucus, sperm	Flow of fluid, tears.
Chemical barriers	Sebum (fatty-acids, lactic acid, lysozyme).	Acidity, enzymes (proteases)	Lysozyme in nasal secretions.	Acidity in vaginal secretions. Spermine and zinc in semen.	Lysozyme in tears.

2) *Internal Defence or Second Line of Defence*

This defence mechanism occurs when the first line of defence fails to prevent an access of pathogens to the tissues. The body's internal defence is carried out by white blood corpuscles, macrophages, natural killer cells (NK cells), inflammatory reactions, fever, interferon's and complements system.

Table 5.2. Cellular barriers in internal defence mechanism

Cell type	**Characteristics**	**Location**
Mast cells	Dilates blood vessels and induces inflammation through the release of histamine and heparin. Recruits macrophages and neutrophils.	Connective tissues, mucous membranes.
Macrophages	Phagocytic cells that consume foreign pathogens and cancer cells. These are of two types: Fixed and wandering macrophages.	Migrates from blood vessels into tissues.

Table 5.2. (Continued)

Cell type	Characteristics	Location
Natural killer cell	Kills tumor cells and virus-infected cells. They produce perforins that create pores in the membrane of target cells which then swell and burst	Circulates in blood and migrates into tissues.
Monocyte	Differentiates into macrophages and dendritic cells in response to inflammation.	Stored in spleen, moves through blood vessels to infected tissues.
Neutrophils	First responders at the site of infection or trauma. Represents 50-60% of all leucocytes.	Migrates from blood vessels into tissues.
Basophils	Responsible for defence against parasites. Releases histamine that cause inflammation and may be responsible for allergic reactions.	Circulates in blood and migrates to tissues.
Eosinophils	Releases toxins that kill bacteria and parasites.	Circulates in blood and migrates to tissues.

Inflammatory Reactions

Inflammation is a defensive response of the body to tissue damage. The conditions that may produce inflammation are pathogens, abrasions, chemical irritations and extreme temperature. Inflammatory reactions cause redness, heat, swelling, and pain in the area of infection. Redness and heat, both are due to capillary dilation resulting in increased blood flow. Swelling is caused by the passage of plasma from the blood stream into the damaged tissue. The pain is mainly due to the tissue destruction and to a lesser extent due to the swelling.

- *Fever*: It's often due to infection and is caused by the release of chemicals (pyrogens) from the damaged tissues and the cells involved in inflammation.
- *Interferons*: Interferon's are glycoprotein's released by living cells in response to viral attack and make the surrounding cells resistant to viral infection by inhabiting multiplication of viral particles.

They are divided into three groups based on the cell of origin, namely leukocyte (alpha interferon), fibroblast (beta interferon) and lymphocyte (gamma interferon).

Complement System

Complement system is a biochemical cascade consisting of 30 different serum proteins which participate in both innate and acquired immunities. It's of two types:

- *Classical pathway:* as seen in acquired immunity which becomes active in response to antigen-antibody complex; Alternative pathway or
- *Properdin system*: seen in innate immunity which is activated directly by the presence of bacterial endotoxins and other components of invading micro-organisms.

Acquired Immunity/Specific Defence Mechanism or Third Line of Defence

Adaptive immunity is capable of recognising and eliminating specific pathogen antigens. If a pathogen is able to get pass the body's non-specific defence, the immune system reacts with a series of specific and selective defence that attacks the disease-causing agents. Response of the immune system to specific pathogens is called specific defence and it is the third line of defence developed by an animal in response to disease caused by infection of microbes. This occurs by making specialized cells and antibodies that make the pathogen useless.

Adaptive Immunity Displays Four Characteristic Features

- *Antigenic specificity*: It's ability to discriminate among different epitopes/antigens.
- *Immunologic memory*: It's the ability to recall previous contact with a foreign molecule and respond to it in a learned manner, with a more rapid and large response.
- *Diversity*: The ability to respond to different epitopes even if the individual has not previously encountered them.
- *Self/non-self-recognition*: It's the ability to recognise and respond to molecules that are foreign and to avoid making a response to those molecules that are self (self-tolerance).

Cells Involved in Acquired/Adaptive Immunity

The cells of the adaptive immune system are special types of leucocytes, called lymphocytes and antigen presenting cells.

B-lymphocytes or B-cells and T-lymphocytes or T-cells: are the major types of lymphocytes that are derived from hematopoietic stem cells in the bone marrow. B-cells are involved in the humoral immune response whereas T-cells are involved in cell-mediated immune response.

Table 5.3. Cells involved in acquired/adaptive immunity

Types of Responses	Lymphocytes
B-lymphocytes (B-cells), arise from bone marrow, Bursa of Fabricus (in birds), peyer's patches.	T-lymphocytes (T-cells), arise from thymus.
Antibody mediated or humoral immune system (AMIS).	Cell mediated immune system (CMIS).

T-lymphocytes or T-cells

The maturation of T-lymphocytes occurs in thymus, hence the name. The T-cells can identify viruses and micro-organisms from the antigens displayed on their surfaces.

T-lymphocytes are responsible for the cell mediated immunity. There are four different types of T-cells:

1. *Inducer T-cells:* These mediate the development of T-cells in the thymus.
2. *Cytotoxic T-cells or Killer T-cells*: Capable of recognising and killing the infected or abnormal cells and destroying antigens by secreting a protein perforin.
3. *Helper T-cells*: These cells are numerous and stimulate the B-cells to produce antibodies. They help in overall regulation of immunity by forming a series of protein mediators called lymphokines.
1. *Suppressor T-cells:* They check on entire immune system from attacking the body's own cells and mediate the suppression of immune response. B-lymphocyte or B-cells: B-cells mature in bone marrow itself and produce specialized proteins called antibodies and therefore generate antibody mediated or humoral immunity. These B-cells give rise to following cells:
    - *Plasma Cells (Effector B-cells)*: The antigen specific T-lymphocytes stimulate specific B-cells to multiply rapidly forming clone of plasma cells. In presence of antigen the plasma cells form antibodies at the rate of 2000 molecules per cell per second, a total of some 20 trillion per day.
    - *Memory B-cells*: Activated B-cells that do not differentiate into plasma cells remain dormant as memory B-cells until activated by a new quantity of the same antigen.
    - *Antigen Presenting Cells (APCs)*: APCs are specialized cells which include macrophages (monocytes as blood macrophages and histocytes as tissue macrophages), B-lymphocytes and

dendritic cells (e.g., langerhans cells of epidermis of skin). These are distinguished by two properties:

- These cells express class II MHC molecules on the membrane.
- These cells are able to deliver a co-stimulatory signal that is necessary for helper T-cell activation.

Types of Acquired Immunity

Acquired immunity is of two types: natural or active and artificial or passive.

1. *Active immunity:* it is a resistance induced after contact with foreign antigens, e.g., person who has recovered from an attack of smallpox. Main advantage of this immunity is that resistance is long term and its major disadvantage is its slow onset especially the primary response. Active immunity is produced by clonal selection and expansion. Clonal selection leads to the eventual production of:
   - A pool of antibody-secreting plasma cells.
   - A pool of memory cells.
2. *Passive immunity***:** this type of immunity occurs when antibodies produced artificially are injected into a person to counteract antigens such as snake venom, rabies, tetanus toxin and *salmonella* infection etc. The yellowish fluid colostrum's secreted by mother during the initial days of lactation has abundant antibodies (IgA) to protect the infant. The foetus also receives some antibodies from its mother, through placenta during pregnancy. These are some examples of passive immunity. It has the advantage of providing immediate relief due to prompt availability of large number of antibodies.

Table 5.4. Difference between active immunity and passive immunity

Active immunity	**Passive immunity**
It is produced due to contact with pathogen or its antigen.	It is produced due to antibodies from outside.
Immunity is not immediate. A time laps occurs for its development.	Immunity develops immediately.
It lasts for a long period, may be for a lifelong.	It lasts only for few days.
Possibility of side effects is very few.	It may result in serum sickness, i.e., body reacts to the introduction of antiserum.

COMPONENTS OF ACQUIRED IMMUNITY

Acquired immunity has two components- humoral and cell mediated immunity:

1. *Humoral Immune Response or Antibody Mediated Immunity:* This immune system is associated with the appearance of antibodies, secreted by cells of the B-lymphocytes in the extracellular fluids such as plasma, lymph and external secretions. When an antigen enters body, some B-cells become plasma cells and other B-cells remember the antigen. Plasma cells produce antibodies, while memory cells cause the production of new plasma cells when antigen again enters the body and finally an antibody produced reacts with an antigen. It defends the body against: viruses, bacteria, toxins that enter the body fluids (blood and lymph) (Figure 5.1).
2. *Cell Mediated Immune Response or Cell Mediated Immunity*: This immunity is mediated by cells of the T-lymphocyte series with antigen-specific receptors on their surfaces. It defends the body against viruses, fungi and some bacteria which have entered the host's cell. When antigen enters body, T-cells produce many more T-cells that are sensitized to the antigen, and also stimulate the inflammatory response. Some T-cells attach to the cell that has

antigen and destroy it while other T-cells remember the antigen and such memory cells cause production of new T-cells when the antigen again enters the body and destroy it (Figure 5.2).

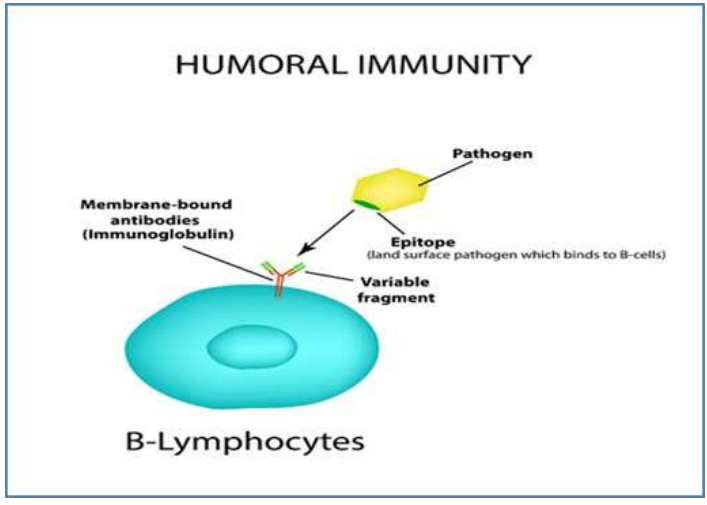

Figure 5.1. Humoral Immunity.

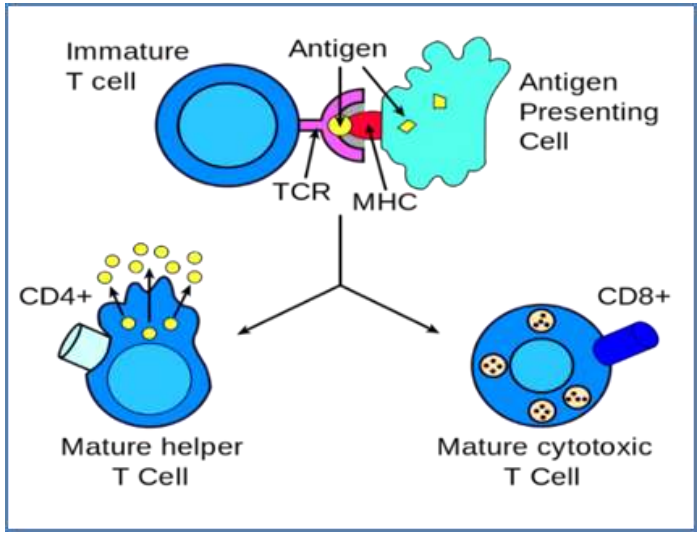

Figure 5.2. Cell-mediated immune system.

Table 5.5. Innate and Adaptive Immunity

Attribute	Innate immunity	Acquired immunity
Response time	Minutes/hours.	Days.
Specificity	Specific for molecules and molecular patterns associated with pathogens.	Highly specific; discriminates even minor differences in molecular structure.
Diversity	Limited number of germ line encoded receptors.	Highly diverse; very large number of receptors arising from genetic recombination of receptor genes.
Memory response	None.	Persistent memory, with fast response on subsequent infection.
Self/non-self-discrimination	Perfect; no microbe specific patterns in host.	Good; occasional failures of self/non-self-results in autoimmune diseases.
Soluble components of blood or tissue fluids	Anti-microbial peptides and proteins.	Antibodies.
Major cell types	Phagocytes (monocytes, macrophages, neutrophils), natural killer (NK) cells, dendritic cells.	T-cells, B-cells, Antigen presenting cells.

FREQUENTLY ASKED QUESTIONS (FAQS)

Q1. What is phagocytosis?

Answer: Phagocytosis is the ingestion of invading foreign particles, such as bacteria by individual cell. It is carried out by cell types such as neutrophils, macrophages, monocytes and dendritic cells.

Q2. What are the components of innate immunity?

Answer: Most components of innate immunity are present before the onset of infection and constitute a set of disease-resistance mechanisms that are not specific to a particular pathogen but include cellular and molecular components that recognize classes of molecules peculiar to frequently encountered pathogens. Phagocytic cells, such as macrophages and neutrophils, barriers such as skin, and a variety of antimicrobial

compounds synthesized by the host all play important roles in innate immunity.

Q3. Explain briefly the process of haematopoiesis?

Answer: Haematopoiesis is the process of blood cell production which comprises a complex sequence of events (including cell proliferation, differentiation and maturation) controlled by a variety of soluble secreted factors (known as cytokines or lymphokines) and hormones.

Q4. What are the properties of macrophages?

Answer: The properties of macrophage are as follows:

- Presence of membrane receptors such as complement receptors, cytokine receptors and TLRs.
- Production of cytokines such as TNF alpha, IL-2, IL-10, IL-6, fibroblast growth factor
- Antigen presentation
- Phagocytosis

Q5. What are the chemical mediators of innate immunity?

Answer: Diverse chemicals of innate immune system mediate protection against microbes during the period before adaptive immunity develops which includes complement proteins, cytokines, pattern recognition molecules, acute-phase proteins, cationic peptides, enzyme like lysozyme and many others. All these play an important role in providing chemical defence.

Q6. What are the cells involved in innate immunity?

Answer: Many specialized cell types like neutrophils, macrophages, monocytes, natural killer cells participate in innate host defence mechanisms. Once a pathogen evades the physical and chemical barriers, these specialized cells play a crucial role in protection. Phagocytosis is a fundamental protective mechanism carried out by these cell types.

Q7. What is the role of mast cells in innate immunity?

Answer: These are formed from the precursor cells in bone marrow and released into the blood in an undifferentiated state, until they reach the tissue. They contain large number of cytoplasmic granules containing histamine. Mast cells and basophils play role in allergic responses.

Q8. What is the difference between innate and adaptive immunity?

Answer: Innate or natural immunity is already present in a body since birth while as adaptive immunity is created in response to foreign substance. Once activated, adaptive immunity remains throughout the life.

Q9. What is the role MHC molecules in cell mediated immunity?

Answer: MHC molecules are important components of cell mediated immunity because they allow T lymphocytes to detect cells such as macrophages that have ingested infectious microorganisms.

Q10. What is the role of NK cells in cell mediated cytotoxicity?

Answer: NK cells lack antigen specific cell surface receptors. NK cells play an important role in lysis of tumor cells which involves exocytosis of cytoplasmic granules from NK cell. These granules contain number of proteins including perforins that induce formation of membrane lesions on the cancer cells resulting in NK cell mediated lysis of cancer cells.

Q11. What is the role of T cells in cell mediated immunity?

Answer: The cell-mediated response involves mostly T cells and responds to any cell that displays aberrant MHC markers, including cells invaded by pathogens, tumor cells, or transplanted cells.

Q12. What is cell mediated immunity?

Answer: The cell-mediated arm of the adaptive immune system driven by T lymphocytes is mainly concerned with cellular immune responses to intracellular pathogens, such as viruses, and also with regulation of B cell responses.

Q13. What is the consequence of cell mediated and humoral immunity?

Answer: Humoral immunity results in the activation and thus differentiation of plasma B-cells secreting antibodies. While as, In Cell mediated immunity, the end result of activation is secretion of cytokines by T cells.

Q14. What is the role of CD4+ cells?

Answer: CD4+ T cells comprise sub population of effector cells in cell mediated immunity that mediates delayed type hypersensitivity reactions.

Q15. Mention the types of cytotoxic effector cells? What is their role?

Answer: The various cytotoxic effector cells can be grouped into two general categories:

a) Antigen-specific cytotoxic T lymphocytes (CTLs) and

b) Nonspecific cells, such as natural killer (NK) cells and macrophages.

CTL or T killer cell helps in killing of cancer cells, cells infected with viruses and cells that are damaged. While as, NK cells play an important role in host rejection of both tumours and virally infected cells.

Q16. How T cells undergo clonal proliferation?

Answer: The recognition of an antigen-MHC complex by a specific mature T lymphocyte induces clonal proliferation into various T cells with effector functions (TH cells and CTLs) and into memory T cells. The T cell is restricted to binding antigen displayed on self-cells.

MULTIPLE CHOICE QUESTIONS (MCQS)

Q1. What are the main cells involved in humoral immunity?

a) T cells b) NK cells

c) Macrophages d) B- cells

Q2. If a person is infected with an extracellular pathogen, which immunity is elicited in his body?

a) Humoral immunity
b) cell mediated immunity
c) Cell mediated cytotoxicity
d) Hypersensitivity

Q3. Which of the following immunity shows delayed response?

a) Humoral immunity
b) Antibody dependant cellular toxicity
c) T cell mediated immunity
d) cell mediated immunity

Q4. The essential components of humoral immunity are?

a) Cytokines
b) Interferons
c) Anti bodies
d) NK cells

Q5. What are the non-specific cells in cell mediated immunity

a) B cells
b) NK cells
c) T_H cells
d) T_c cells

Q6. Helper functions of T cells is mediated by its contact with

a) T_C cells
b) B cells
c) T_H cells
d) Mast cells

Q7. In cell mediated immunity T cells can recognise antigens which are- bound with

a) MHC
b) glycolipid
c) T cell receptor
d) Antibody

Q8. Response of cell mediated immunity is

a) Fast
b) intermediate
c) Slow
d) very fast

Q9. Which cells among the following are precursor cells of macrophages.

a) Dendritic cells
b) monocytes
c) NK cells
d) eosinophils

Q10. Prostaglandins and leukotrienes are synthesized from lipids found in the cell membrane by the action of

a) Basophils
b) eosinophils
c) mast cells
d) phagocytes

Q11. Lysozymes and defensins play an important role in

a) antibody-dependent cellular cytotoxicity (ADCC)
b) inflammation
c) Oxygen dependant phagocytosis
d) oxygen independent phagocytosis

Q12. Kupffer cells are phagocytes present in

a) Liver
b) Spleen
c) Kidney
d) Lungs

Q13. Respiratory burst is a metabolic process which occurs in activated phagocytes resulting in

a) Constant oxygen use by a cell
b) increase in oxygen use by a cell
c) Decrease in oxygen use by a cell
d) absence of oxygen

Q14. Skin is a physical barrier which forms component of

a) Adaptive immunity
b) innate immunity
c) Cell mediated immunity
d) antibody dependant cellular cytotoxicity

Q15. Adhesion of eosinophils to parasites is mediated by molecules called

a) Integrin's b) lymphokines
c) Chemokine's d) Adhesins

Answer Key

1. D **2**. A **3**. D **4**. C **5**. A **6.** B **7**. A **8**. C **9**. B **10**. B **11**. D **12**. A **13**. B **14**. B **15**. A

ASSIGNMENT

Long Answer Questions (500 Words)

Q1. Give the description of innate immunity in detail?
Q2. Explain chemical mediators involved in innate immunity?

Short Answer Questions (200 Words)

Q1. Add a short note on the role of NK cells in cell mediated immunity?
Q2. List the differences between humoral and innate immunity?
Q3. Describe role of cytotoxic T lymphocytes in Immune Response?

REFERENCES

Abbas, A. K., Janeway, C. A.: Immunology: improving on nature in the twenty-first century, *Cell* 100:129–138, 2000.

Abbas, A. K., Lichtman, A. H., Pillai S: *Cellular and Molecular Immunology*, ed 6, Philadelphia, 2007, Saunders. Murphy K, Travers

P, Walport M.: *Janeway's Immunobiology*, ed 6, London, 2008, Garland Science. Staros EB:

Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. *Cell* 2006; 124:783–801.

Banchereau J, Steinman M. Dendritic cells and the control of immunity. *Nature* 1998;392:245–52.

Banchereau J., F. Briere, C. Caux, J. Davoust, S. Lebecque, Y. J. Liu, B. Pulendran, and K. Palucka. 2000. Immunobiology of dendritic cells. *Annu. Rev. Immunology.* 18: 767.

Beissert S, Schwarz A, Schwarz T. Regulatory T cells. *J Invest Dermatol* 2006; 126:15–24.

Bieber T. FcεRI-expressing antigen-presenting cells: new players in the atopic game. *Immunol Today* 1997; 18:311–3.

Bonish B, Jullien D, Dutronc Y, et al. Overexpression of CD1d by keratinocytes in psoriasis and CD1d-dependent IFN-γ production by NK-T cells. *J Immunol* 2000; 165:4076–85.

Bonizzi G, Karin M. The two NF-?B activation pathways and their role in innate and adaptive immunity. *Trends Immunol* 2004; 25:280–8.

Bos JD, De Rie MA, Teunissen MBM, et al. Psoriasis: activation of innate immunity. *Br J Dermatol* 2005; 152:1098–107.

Bos JD, ed. *Skin Immune System (SIS),* 1st ed. Boca Raton, FL: CRC Press, 1990.

Bos JD, ed. *Skin Immune System (SIS): Cutaneous Immunology and Clinical Immunodermatology*, 3rd ed. Boca Raton, FL: CRC Press, 2005.

Bos JD, Kapsenberg ML. The skin immune system (SIS): its cellular constituents and their interactions. *Immunol Today* 1986; 7: 235–40.

Bos JD, Zonneveld I, Das PK, et al. The skin immune system (SIS): distribution and immunophenotype of lymphocyte subpopulations in normal human skin. *J Invest Dermatol* 1987; 88:569–73.

Cameron AL, Kirby B, Fei W, et al. Natural killer and natural killer-T cells in psoriasis. *Arch Dermatol Res* 2002; 294:363–9.

Chu AC, Morris JF. The keratinocyte. In: Bos JD, ed. *Skin Immune System (SIS): Cutaneous Immunology and Clinical Immunodermatology*, 3rd edition. Boca Raton, FL: CRC Press, 2005: 77–99.

Clark RA, Chong B, Mirchandani N, et al. The vast majority of CLA+ T cells are resident in normal skin. *J Immunol* 2006; 176:4431–9.

De Boer OJ, Van der Loos CM, Teeling P, et al. Immunohistochemical analysis of regulatory T cell markers FOXP3 and GITR on CD4+CD25>+ T cells in normal skin and inflammatory dermatoses. *J Histochem Cytochem* 2007; 55:891–8.

De Vries HJC, Van Marle J, Teunissen MBM, et al. Lichen planus is associated with human herpesvirus type 7 replication and infiltration of plasmacytoid dendritic cells. *Br J Dermatol* 2006; 154:361–4.

Dudda JSSC, Simon JC, Martin S. Dendritic cell immunization route determines $CD8^+$ T cell trafficking to inflamed skin: role for tissue microenvironment and dendritic cells in establishment of T cell-homing subsets. *J Immunol* 2004; 172:857–63.

Esche C, Stellato C, Beck LA. Chemokines: key players in innate and adaptive immunity. *J Invest Dermatol* 2005; 125:615–28.

Furth R van, Cohn ZA, Hirsch JG, et al. The mono-nuclear phagocyte system: a new classification of macrophages, monocytes and their precursor cells. *Bull WHO* 1972; 46:845–52.

Godfrey DI, Kronenberg M. Going both ways: immune regulation via CD1d-dependent NKT cells. *J Clin Invest* 2004; 114:1379–88.

Godfrey DI, MacDonald RH, Kronenberg M, et al. NKT cells: what's in a name? *Nat Rev Immunol* 2004; 4:231–7.

Goldsby RA, Kindt TJ, Kuby J, Osborne BA. *Kuby Immunology*, 5th edn. Oxford: WH Freeman; 2003.

Gould HJ, Sutton BJ, Beavil AJ, et al. The biology of IgE and the basis of allergic disease. *Annu Rev Immunol* 2003; 21: 579–628.

Gutgesell C, Heise S, Seubert A, et al. Comparison of different activity parameters in atopic dermatitis: correlation with clinical scores. *Br J Dermatol* 2002; 147:914–9.

Innate immunity: new approaches to understanding its clinical significance, *American Journal of Clinical Pathology* 123:305–312, 2005.

Janeway CA. Approaching the asymptote? Evolution and revolution in immunology. *Cold Spring Harb Symp Quant Biol* 1989; 54:1–13.

Janeway Jr CA, Travers P, Wolpert M, Schlomchik MJ. *Immunobiology*. London: Garland Publishing; 2004.

Kapsenberg ML. Dendritic-cell control of pathogen-driven T-cell polarization. *Nat Rev Immunol* 2003; 3:984–93.

Karadimitris A, Patterson S, Spanoudakis E. Natural killer T cells and haemopoiesis. *Br J Haematol* 2006; 134:263–72.

Knol EF, Kuijpers TW, Roos D. Neutrophils, eosinophils and basophils in the skin immune system. In: Bos JD, ed. *Skin Immune System (SIS): Cutaneous Immunology and Clinical Immunodermatology*, 3rd edition. Boca Raton, FL: CRC Press, 2005:263–83.

Layfair JHL, Chain BM. *Immunology at a Glance*, 7th edn. Oxford: Blackwell Scientific Publications; 2004.

Lebre MC, Van der Aar AMG, Van Baarsen L, et al. Human keratinocytes express functional Toll-like receptor 3, 4, 5, and 9. *J Invest Dermatol* 2007; 127:331–41.

Liang SC, Tan XY, Luxenberg DP, et al. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J Exp Med* 2006; 203:2271–.

Lowes MA, Chamian F, Abello MV, et al. Increase in TNF-α and inducible nitric oxide synthase-express-ing dendritic cells in psoriasis and reduction with efalizumab (anti-CD11a). *Proc Natl Acad Sci USA* 2005; 102:19057–62.

Lu KQ, McCormick TS, Gilliam AC, et al. Monocytes and macrophages in human skin. In: Bos JD, ed. *Skin Immune System (SIS): Cutaneous Immunology and Clinical Immunodermatology*, 3rd edition. Boca Raton, FL: CRC Press, 2005:183–209.

Lydyard PM, Whelan A, Fanger MW. *Instant Notes in Immunology*, 2nd edn. London: Garland Science/Bios Scientific Publishing, 2004.

McGirt LY, Beck LA. Innate immune defects in atopic dermatitis. *J Allergy Clin Immunol* 2006; 118:202–8.

Medzhitov R, Janeway CA. Innate immunity. *N Engl J Med* 2000; 343:338–44

Nestle FO, Conrad C, Tun-Kyi A, et al. Plasmacytoid predendritic cells initiate psoriasis through inter-feron-α production. *J Exp Med* 2005; 202:135–43.

Orange JS. Human natural killer cell deficiencies. *Curr Opin Allergy Clin Immunol* 2006;6: 399–409.

Piskin G, Sylva-Steenland RMR, Bos JD, et al. *In vitro* and *in vivo* expression of IL-23 by kerati-nocytes in healthy skin and psoriasis lesions: enhanced expression in psoriatic skin. *J Immunol* 2006; 176:1908–15.

Robinson MJ, Sancho D, Slack EC, et al. Myeloid C-type lectins in innate immunity. *Nat Immunol* 2006;12: 1258–65.

Roitt IM, Delves P. *Essential Immunology*, 10th edn. Oxford: Blackwell Scientific Publications; 2001.

Rothenberg, E.V.2000. Stepwise specification of lymphocyte developmental lineages. *Current Opin. Gen. Dev*. 10:370.

Santamaria-Babi LF. CLA(+) T cells in cutaneous diseases. *Eur J Dermatol* 2004; 14:13–8.

Soumelis V, Reche PA, Kanzler H, et al. Human epithelial cells trigger dendritic cell-mediated allergic inflammation by producing TSLP. *Nat Immunol* 2002; 3:673–80.

Sugiyama H, Gyulai R, Toichi E, et al. Dysfunctional blood and target tissue CD4+CD25$_{>high}$ regulatory T cells in psoriasis: mechanism underlying unrestrained pathogenic effector T cell proliferation. *J Immunol* 2005; 174:164–73.

Tauber AI, Chernyak L. *Metchnikoff and the Origins of Immunology: From Metaphor to Theory*. New York: Oxford University Press, 1991.

Teunissen MBM, Koomen CW, de Waal Malefyt R, et al. Interleukin-17 and interferon-γ synergize in the enhancement of proinflammatory

cytokine production by human keratinocytes. *J Invest Dermatol* 1998; 111:645–9.

Teunissen MBM, Piskin G, di Nuzzo S, et al. Ultraviolet B radiation induces a transient appearance of IL-4+ neutrophils, which support the development of Th2 responses. *J Immunol* 2002; 168:3732–9.

Teunissen MBM. Langerhans cells and other skin dendritic cells. In: Bos JD, ed. *Skin Immune System (SIS): Cutaneous Immunology and Clinical Immunodermatology*, 3rd edition. Boca Raton, FL: CRC Press, 2005:123–82.

Ting JPY, Kastner DL, Hoffman HM. CATERPILLERs, pyrin and hereditary immunological disorders. *Nat Rev Immunol* 2006;6: 183–95.

Van der Aar AMG, Sylva-Steenland RMR, Bos JD, et al. Cutting edge: loss of TLR2, TLR4, and TLR5 on Langerhans cells abolishes bacterial recognition. *J Immunol* 2007; 178:1986–900.

Van der Vliet HJJ, Von Blomberg BME, Nishi N, et al. Circulating $Va24_{>+}$ $Vß11_{>+}$ NKT cell numbers are decreased in a wide variety of diseases that are characterized by autoreactive tissue damage. *Clin Immunol* 2001; 100:144–8.

Walport MJ. Complement: first of two parts. *N Engl J Med* 2001;344: 1058–1066.

Weaver CT, Harrington LE, Mangan PR, et al. Th17: an effector CD4 T cell lineage with regulatory T cell ties. *Immunity* 2006; 24:677–88.

Wollenberg A, Kraft S, Hanau D, et al. Immunological and ultrastructural characterization of Langerhans cells and a novel, inflammatory dendritic epidermal cell (IDEC) population in lesional skin of atopic eczema. *J Invest Dermatol* 1996; 106:446–53.

Zhang C, Zhang J, Tian Z. The regulatory effect of natural killer cells: do "NK-reg cells" exist? *Cell Mol Immunol* 2006; 3:241–54.

Zheng Y, Danilenko DM, Valdez P, et al. Interleukin-22, a TH17 cytokine, mediates IL-23—induced dermal inflammation and acanthosis. *Nature* 2007; 445:648–51.

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Chapter 6

IMMUNOGLOBULINS

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ABSTRACT

Immunoglobulins (antibodies) are glycoprotein molecules that play a key role in adaptive immunity. Antibody is a Y shaped molecule made up of four polpeptides-2 heavy chains and 2 light chains. They protect us in the hostile environment of bacteria, viruses, and parasites. There are five classes of human immunoglobulins: IgM, IgG, IgA, IgE, and IgD. Most IgM antibodies are "natural" i.e., they are produced even without an antigenic stimulus. Their antigen-binding polyspecificity ensures them a role as a first line of defence mechanism against invading pathogens. IgG is the most abundant immunoglobulin isotype in human plasma. The ability of IgG antibodies to bind with a high affinity and specificity to a remarkably large variety of antigens is their main feature. It combats

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microbes and their toxins. Serum IgA has effects similar to these of IgG, while secretory IgA antibodies are resistant to proteases and protect all mucosal surfaces. IgE is the class with the lowest plasma concentration. The contact of mast cell-bound IgE with the specific antigen results in an acute inflammatory reaction that might help to expel parasites from the gut. IgE antibodies are also believed to have a role in the host defense against noxious environmental substances, including venoms, environmental xenobiotics, and irritants IgD is primarily found on surface of mature B cells and is probably involved in lymphocyte activation. An immunoglobulin superfamily of which antibody is a large and diverse group of protein comprising antibodies. Antibodies can swiftly provide therapeutics to target disease-related molecules discovered in genomic research. Antibody engineering techniques have been actively developed and these technological innovations have intensified the development of therapeutic antibodies.

Keywords: antibody, isotype, allotype, idiotype, CDC, neutralization, Ig-SF

OBJECTIVES

- Understand antibody and immunoglobulin
- Understand structure of an antibody
- Explain the effector functions of an antibody
- Distinguish between the various types of antibodies
- Distinguish between isotype, allotype and idiotype

INTRODUCTION

Antibodies belong to a class of proteins called, “globulins” and can be defined as multifunctional glycoproteins produced in direct response against antigens by vertebrates and essential for the deterrence and resolution of infection by various pathogens. They initiate their biological effect by non-covalently binding to antigens but without modifying the covalent structure of antigens. Antibody molecules are referred to as

multifunctional molecules as they perform more than one function. One part of the immunoglobulin is involved in the binding of the antigen while the other part of the molecule may be involved in binding to receptors on phagocytes, the activation of complement pathways and the activation of the NK cells of the immune system.

IMMUNOGLOBULINS AND ANTIBODIES

Immunoglobulins and antibodies are terms used interchangeably however although all antibodies are immunoglobulin's, all immunoglobulins are not antibodies. Antibodies are immunoglobulin's (Igs) which are produced in the body in response to the antigen or foreign bodies. Immunoglobulin refers to any class of structurally related proteins in the serum and the cells of the immune system which functions as antibodies. It is produced in response to an antigen. The name immunoglobulin is derived from the fact that they migrate with globular proteins when antibody-containing serum is placed in an electrical field. The five classes of immunoglobulins are IgG, IgM, IgA, IgD, and IgE. Immunoglobulins are structurally similar to antibodies.

An antibody refers to a globin protein, which is produced by B cells in response to a particular antigen. The most characteristic feature of antibody is its specificity to a particular antigen. The main difference between immunoglobulin and antibody is that immunoglobulin has a transmembrane domain in order to be attached to the plasma membrane whereas antibody does not have a transmembrane domain.

Characteristics of Antibodies

- Antibodies are immunoglobulin (Ig) molecules
- Antibodies are antigen specific and bind to foreign molecules to host.
- They are produced by activated B-cells

- Antibodies are first molecules participating in specific immune response
- They mediate effector function to neutralize or eliminate foreign invaders.

ANTIBODY STRUCTURE

Antibodies are the globular proteins that belong to immunoglobulin family. These are synthesized by B-cells in billions of forms. Each antibody has different amino acid sequence and a different antigen binding site. Antibodies appear in circulation after infection and have ability to specifically react with epitopes of antigen introduced in the organism. There are five major classes of immunoglobulins: IgG, IgA, IgD, IgM and IgE.

Antibodies are generally found in blood stream, tissues and other bodily fluids. Antibodies with the help of antigens, can perform their function. Antibodies are the antigen binding proteins present on the B-cell membrane and secreted by plasma cells. Membrane-bound antibody confers antigenic specificity on B-cells; antigen specific proliferation of B-cell clones is elicited by the interaction of membrane antibody with antigens. Secreted antibodies circulate in the blood, where they serve as the effectors of humoral immunity by searching out and neutralizing antigens or marking them for elimination. All antibodies share structural features, bind to antigen, and participate in a limited number of effector functions.

The antibodies produced in response to a specific antigen are heterogeneous. Most antigens are complex and contain many different antigenic determinants, and the immune system usually responds by producing antibodies to several epitopes on the antigen. This response requires the recruitment of several clones of B-cells. Their outputs are monoclonal antibodies, each of which specifically binds a single antigenic determinant. Together, these monoclonal antibodies make up the polyclonal and heterogeneous serum antibody response to an immunizing agent.

Antibodies are Y shaped molecules. Antibody molecules have a common structure of four peptide chains. This structure consists of two identical light (l) chains, polypeptides of about 25,000 molecular weight, two identical heavy chains (H) chains, and larger polypeptides of molecular weight 50,000 molecular weight or more. Each light chain is bound to a heavy chain by a di-sulphide bond, and by such noncovalent interactions as salt linkages, hydrogen bonds and hydrophobic bonds to form a heterodimer (H-L). Similar non-covalent interactions and di-sulphide bridges link the two identical heavy and light chains (H-L) chain combinations to each other to form the basic four-chain $(H\text{-}L)_2$ antibody structure, a dimer of dimers (the exact number and precise positions of these interchain disulphide bonds differs among antibody classes and subclasses) (Figure 6.1).

The first 110 amino acids of the amino-terminal region of a light or heavy chain vary greatly among antibodies of different specificity. These segments of highly variable sequence are called V-regions: VL in light chains and V_H in heavy chains. All of the differences in specificity displayed by different antibodies traced to differences in the amino acid sequences of V regions. Infact, most of the differences among antibodies fall within areas of the V regions called complementary-determining regions (CDRs), and it is these CDRs, on both light and heavy chains, that constitute the antigen binding site of the antibody molecule. The regions of relatively constant sequence beyond the variable regions have been dubbed C regions, CL on the light chain and C_H on the heavy chain. L chains belong to one of two types, k (Kappa) or λ (lambda), on the basis of amino acid differences in their constant regions. Both types occur in all classes of immunoglobulins (IgG, IgM, etc.), but any one immunoglobulin molecule contains only one type of L-chain. The amino-terminal portion of each L chain participates in the antigen-binding site. H chains are distinct for each of the five immunoglobulin classes and are designated γ, α, μ, ε and δ. The amino-portion of each H chain participates in the antigen-binding site; carboxy terminal forms the Fc fragment, which has the biological activities as production of monoclonal antibodies.

The γ, δ and α heavy chains contains an extended peptide sequence between the C_H 1 and C_H 2 domains that has no homology with the other domains. This region is called Hinge region, is rich in proline residues and is flexible in giving IgG, IgD and IgA segmental flexibility.

Two prominent amino acids in the hinge region are proline and cysteine. The large number of proline residues in the hinge region gives it an extended polypeptide conformation, making it particularly vulnerable to cleavage by proteolytic enzymes. The cysteine residues form interchain disulphide bonds that hold the two heavy chains together. The number of interchain disulphide bonds in the hinge region varies considerably among different classes of antibodies and between species. Although μ and ε chains lack a hinge region, they instead have an additional "C_H 4domain".

If an antibody molecule is treated with a proteolytic enzyme such as papain, peptide bonds in the "hinge" region are broken, producing two identical Fab fragments, which carry the antigen-binding sites, and one Fc fragment, which is involved in placental transfer, complement fixation, attachment site for various cells and various other biological activities (Figure 6.2). The C_H 1 and C_L domains serve to extend Fab arms of the antibody molecule, thereby facilitating interaction with antigen and increasing the maximum rotation of the Fab arms. In addition, these constant-region domains help to hold the V_H and V_L domains together by virtue of the interchain disulphide bond between them.

The flexible hinge region is found in IgG, IgA and IgD. It is absent in IgM and IgE. It allows the distance between the two antigen binding sites to vary. These are small proteins that connect two or more basic units (that is Y-shaped molecules) in polymeric immunoglobulin. They are named as J (Joining) chains. The J chain is encoded by a separate gene and is a glycopeptide of approximately 15 kDa and is disulphide bonded to the carboxyl terminal portion of α and μ heavy chains. The structure of immunoglobulins includes several features which are necessary for their participation in the immune response. These features are:

- Specificity
- Biologic activity

Specificity is assigned to a defined region of antibody molecule containing the complementarity determining region (CDR). CDR limits the antibody the antibody to combine to only with those substances that contain a particular antigenic structure.

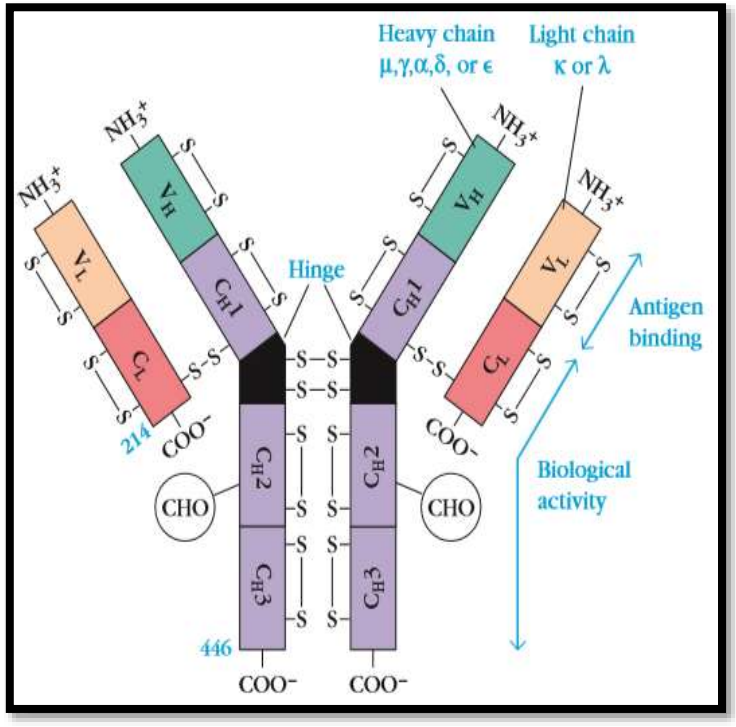

Figure 6.1. Schematic diagram of structure of immunoglobulins derived from amino acid sequencing studies**.** Each heavy and light chain in an immunoglobulin molecule contains an amino-terminal variable (V) region (aqua and tan, respectively) that consists of 100– 110 amino acids and differs from one antibody to the next. The remainder of each chain in the molecule the constant (C) regions (purple and red) exhibits limited variation that defines the two light-chain subtypes and the five heavy-chain subclasses. Some heavy chains (γ, δ and α) also contain a proline-rich hinge region (black). The amino-terminal portions, corresponding to the V regions, bind to antigen; effector functions are mediated by the other domains. The μ and ε heavy chains, which lack a hinge region, contain an additional domain in the middle of the molecule (Adapted from Kuby, immunology, 6th edition).

GENERAL FEATURES OF IMMUNOGLOBULINS

Antigen Binding

Immunoglobulins bind particularly to one or a few closely related antigens. Each immunoglobulin actually binds to a particular antigen determinant. Antigen binding by the antibodies is the primary function of antibodies and can result in the protection of host.

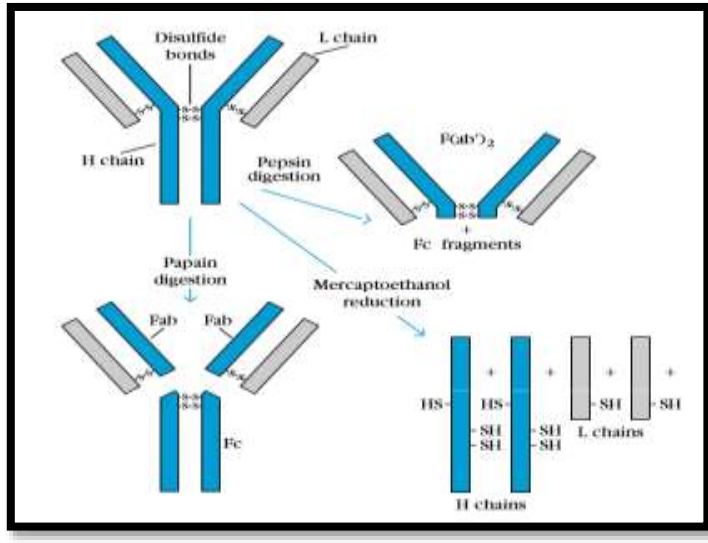

Figure 6.2. Prototype structure of IgG, showing chain structure and interchaindisulfide bonds. The fragments produced by various treatments are also indicated. Light (L) chains are in gray and heavy (H) chains in blue (Adapted from Kuby immunology, 6th edition).

Effector Functions

Frequently the binding of an antibody to an antigen has no direct biological effect. Rather, the significant biological effects are a consequence of secondary "effector functions" of antibodies. The

immunoglobulins mediate a variety of these effector functions. Usually the ability to carry out a particular effector function requires that the antibody bind to its antigen. Not every immunoglobulin will mediate all effector functions. Such effector functions include:

1. Fixation of complement - This result in lysis of cells and release of biologically active molecules
2. Binding to various cell types - Phagocytic cells, lymphocytes, platelets, mast cells, and basophils have receptors that bind immunoglobulins. This binding can activate the cells to perform some function. Some immunoglobulins also bind to receptors on placental trophoblasts, which results in transfer of the immunoglobulin across the placenta. As a result, the transferred maternal antibodies provide immunity to the foetus and new born.

ANTIBODY-MEDIATED EFFECTOR FUNCTIONS

Antibodies participate in a broad range of other biological activities, in addition to antigen binding. When considering the role of antibody in defending against disease, it is important to remember that antibodies generally do not kill or remove pathogens solely by binding to them. In order to be effective against pathogens, antibodies must not only recognize antigen, but also invoke responses effector functions that will result in removal of the antigen and death of the pathogen. While variable regions of antibody are the sole agents of binding to antigen, the heavy-chain constant region (CH) is responsible for a variety of collaborative interactions with other proteins, cells, and tissues that result in the effector functions of the humoral response. Because these effector functions result from interactions between heavy-chain constant regions and other serum proteins or cell-membrane receptors, not all classes of immunoglobulin have the same functional properties. An overview of four major effector functions mediated by domains of the constant region is presented here (Figure 4).

Opsonization is Promoted by Antibody

Opsonization, the promotion of phagocytosis of antigens by macrophages and neutrophils, is an important factor in antibacterial defenses. Protein molecules called Fc receptors (FcR), which can bind the constant region of Ig molecules, are present on the surfaces of macrophages and neutrophils.

The binding of phagocyte Fc receptors with several antibody molecules complexed with the same target, such as a bacterial cell, produces an interaction that results in the binding of the pathogen to the phagocyte membrane. This crosslinking of the FcR by binding to an array of antibody Fc regions initiates a signal-transduction pathway that results in the phagocytosis of the antigen-antibody complex.

Antibodies Activate Complement

IgM and, in humans, most IgG subclasses can activate a collection of serum glycoproteins called the complement system. Complement includes a collection of proteins that can perforate cell membranes. An important by-product of the complement activation pathway is a protein fragment called C3b, which binds non-specifically to cell- and antigen-antibody complexes near the site at which complement was activated. Many cell types for example, red blood cells and macrophages have receptors for C3b and so bind cells or complexes to which C3b has adhered. Binding of adherent C3b by macrophages leads to phagocytosis of the cells or molecular complexes attached to C3b. Binding of antigen antibody complexes by the C3b receptors of a red blood cell allows the erythrocyte to deliver the complexes to liver or spleen, where resident macrophages remove them without destroying the red cell. The collaboration between antibody and the complement system is important for the inactivation and removal of antigens and the killing of pathogens. The process of complement activation is described in detail in next Chapter.

ANTIBODY-DEPENDENT CELL-MEDIATED CYTOTOXICITY (ADCC) KILLS CELLS

The linking of antibody bound to target cells (virus infected cells of the host) with the Fc receptors of a number of cell types, particularly natural killer (NK) cells, can direct the cytotoxic activities of the effector cell against the target cell. In this process, called antibody-dependent cell-mediated cytotoxicity (ADCC), the antibody acts as a newly acquired receptor enabling the attacking cell to recognize and kill the target cell (Figure 3).

Some Antibodies Can Cross Epithelial Layers by Transcytosis

The delivery of antibody to the mucosal surfaces of the respiratory, gastrointestinal, and urogenital tracts, as well as its export to breast milk, requires the movement of immunoglobulin across epithelial layers, a process called transcytosis. The capacity to be transported depends on properties of the constant region. In humans and mice, IgA is the major antibody species that undergoes such transcytosis, although IgM can also be transported to mucosal surfaces. Some mammalian species, such as humans and mice, also transfer significant amounts of most subclasses of IgG from mother to foetus. Since maternal and foetal circulatory systems are separate, antibody must be transported across the placental tissue that separates mother and foetus. In humans, this transfer takes place during the third trimester of gestation. The important consequence is that the developing foetus receives a sample of the mother's repertoire of antibody as a protective endowment against pathogens. As with the other effector functions described here, the capacity to undergo transplacental transport depends upon properties of the constant region of the antibody molecule. The transfer of IgG from mother to foetus is a form of passive immunization, which is the acquisition of immunity by receipt of preformed antibodies rather than by active production of antibodies after

exposure to antigen. The ability to transfer immunity from one individual to another by the transfer of antibodies is the basis of passive antibody therapy, an important and widely practiced medical procedure. (Figure 6.4)

CLASSES OF IMMUNOGLOBULINS

Based on the amino acid differences in the carboxyl terminal of the heavy chain in humans and the higher mammals, five major groups termed as classes or isotypes have been defined. The five different classes are determined by the type of heavy chain involved (Figure 5).

1. IgG

- Molecular weight: 150,000 Da
- H-chain type: gamma (53,000 Da)
- IgG is the most abundant class of Immunoglobulin in serum and constitute of about 80% of total serum immunoglobulin.
- IgG molecule consists of two gamma (γ) heavy chains and two kappa (k) or two lambda (λ) light chains.
- There are four sub class of IgG (IgG1, IgG2, IgG3 and IgG4) on the basis of decreasing serum concentration.
- It has longest half-life among other antibodies. Half-life is about 23 days.
- IgG is the only antibody that can cross placenta. It cross placenta and provide immunity to foetus upto 6 month of age. The immunity is known as natural passive immunity.
- It can also activate complement.
- It crosses blood vessels.

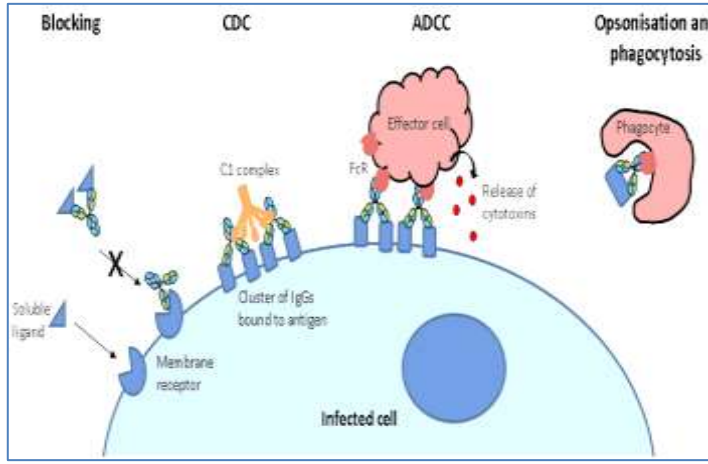

Figure 6.3. Antibody modes of action. Antibodies have several modes of action: (i) they can block ligand-receptor interactions; (ii) cause cell lysis through activation of complement dependant cytotoxicity (CDC); (iii) interact with Fc receptors on effector cells to engage antibody dependent cellular cytotoxicity; (iv) signal for ingestion of a pathogen by a phagocyte.

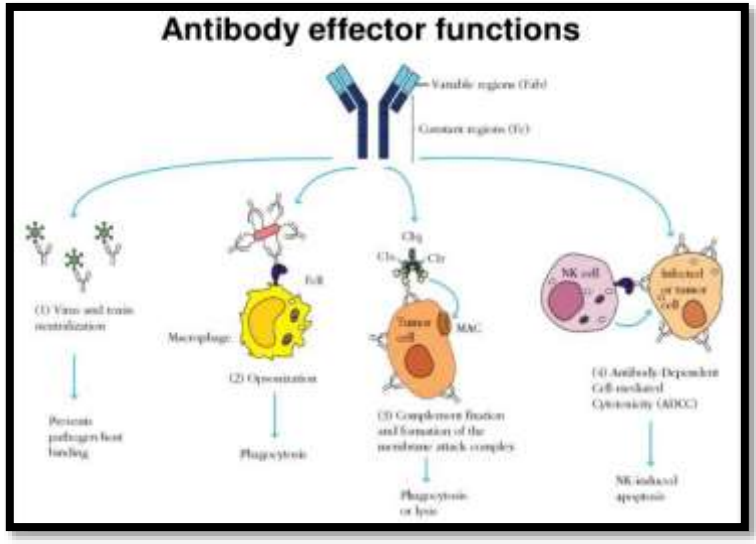

Figure 6.4. Overview of the effector functions of the antibody.

Biological Functions

- IgG is the major antibody produced in secondary immune response.
- Ig, IgG3 and IgG4 readily cross the placenta and play important role in protecting the fetus.
- IgG3 is the most effective complement activator followed by IgG1 and IgG2. IgG4 is not able to activate complement at all.
- IgG1 and IgG3 binds with high affinity to Fc receptor on phagocytic cell and thus mediate opsonisation.
- IgG helps in bacterial immobilization.
- IgG neutralize toxin and viruses.

2. IgM

- Molecular weight: 900,000 Da
- H-chain type: mue (μ) (65,000 Da)
- IgM accounts for 5-10% of total serum Immunoglobulin with an average serum concentration of 1.5mg/dl.
- IgM is secreted by plasma cell and it exists in pentameric form in which five IgM mononers are linked together by disulphide bond (J-chain).
- Due to large size, IgM is also known as millionare molecule.
- There are 10 antigen binding site (Fab) in pentameric IgM molecule but it cannot bind to 10 complete antigen due to steric hindrance.
- It is the major antibody produced during primary immune response.
- Monomeric form IgM (180000 Da) is also expressed as membrane bound receptor on B-cell.

Biological Functions

- IgM is the first antibody produced in primary immune response and it is also the first Ig produced by neonate.
- IgM has higher valency (antigen binding ste) due to its pentameric form.
- Due to pentameric form, IgG is very effective in agglutination reaction.
- IgM is more efficient than IgG in complement activation.
- IgM plays important accessory role as secretory immunoglobulin due to J-chain.

3. IgA

- Molecular weight: 320,000 Da
- H- chain type: Alpha (55000 Da)
- IgA constitute 10-15% of total serum immunoglobulin.
- It is the predominant Immunoglobulin n external secretions such as breast milk, saliva, tears and mucus of bronchial, genitourinary and digestive tracts.
- IgA primarily exists as monomeric form but dimeric, trimeric and some tetrameric form are also present.
- IgA in blood occurs in monomeric form whereas those in body secretion occurs in dimeric or multimeric forms.
- Dimeric form of IgA contains J-chain and secretory chain. Secretory chains helps in transcytosis.
- IgA can cross epithelial layer and enter into body secretion. The process of crossing epithelial layer by IgA is known as transcytosis.
- There are two sub-class of IgA i.e., IgA1 and IgA2.

Biological Functions

- IgA can cross the epithelial layer and enter into body secretion and provides local immunity in GI tracts, respiratory tract, genital tract, etc.
- In body secretion IgA neutralize viruses and prevent attachment on host surface.

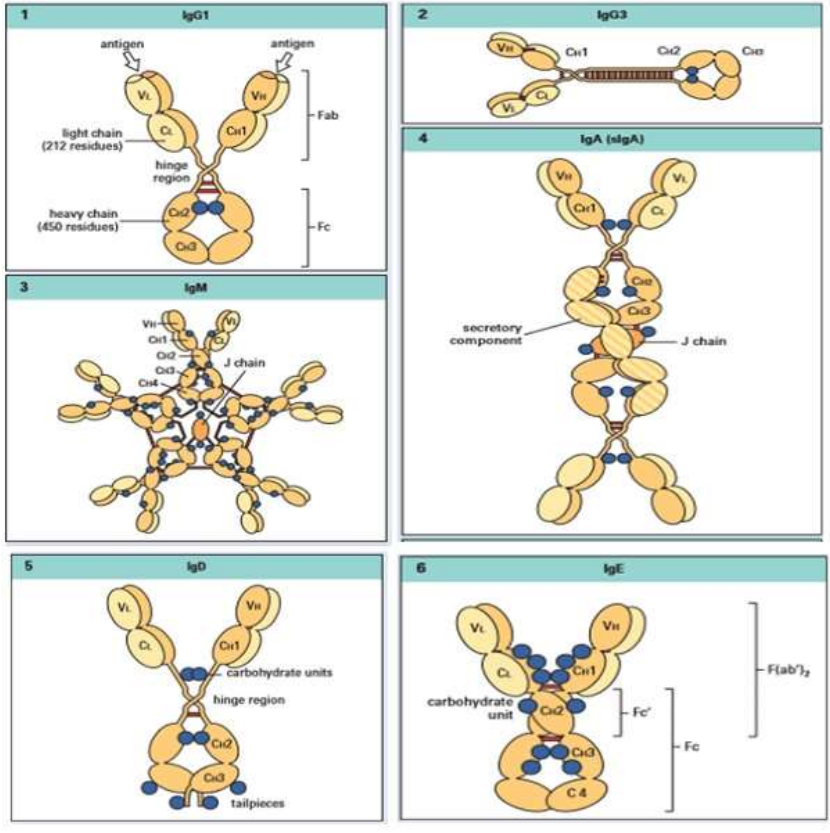

Figure 6.5. Carbohydrate side chains are shown in blue. Inter heavy (H) chain disulfide bonds are shown in red, but interchain bonds between H and L chains are omitted**.** (1) A model of IgG1 indicating the globular domains of H and L chains. Note the apposition of the CH3 domains and the separation of the CH2 domains. The carbohydrate units lie between the CH2 domains. (2) Polypeptide chain structure of

human IgG3. Note the elongated hinge region. (3) IgM H chains have five domains with disulfide bonds cross-linking adjacent CH3 and CH4 domains. The possible location of the J chain isshown. IgM does not have extended hinge regions, but flexion can occur about the CH2 domains. (4) The secretory component of sIgA is probably wound around the dimer and attached by two disulfide bonds to the CH2 domain of one IgA monomer. The J chain is required to join the two subunits. (5) This diagram of IgD shows the domain structure and a characteristically large number of oligosaccharide units. Note also the presence of a hinge region and short octapeptide tailpieces. (6) IgE can be cleaved by enzymes to give the fragments F(ab′)2, Fc, and Fc′. Note the absence of a hinge region (Adapted from David male, immunology, 7th edition).

4. IgD

- Molecular weight: 180,000 Da
- H-chain type: Delta (70000 Da)
- IgD is present in extremely low concentration and it constitute 0.2% of total serum immunoglobulin.
- IgD together with IgM is the major membrane bound immunoglobulin expressed on mature B-cell.
- There are two sub-classes of IgD (IgD1 and IgD2)
- IgD plays important role in maturation and proliferation of B-cell.

Biological Function

- Not established
- Probably involved in lymphocyte activation and suppression
- Cannot activate compliment, cross placenta or cause mast cell degranulation.

5. IgE

- Molecular weight: 200,000 Da
- H-chain type: epsilon (73,000Da)
- IgE accounts for 0.3% of total serum Immunoglobulin.
- IgE is also known as reagenic antibody due to its involvement in allergic reaction. IgE mediate immediate hypersensitivity reaction and responsible for symptoms like hay fever, asthma, anaphylactic shocks, etc.
- Fc region of IgE binds on blood basophils and tissue mast cells. The cross reaction with antigen to Fc region bound IgE causes degranulation of mast cell and basophils releasing histamine. Histamine is responsible for symptoms of allergy.

Biological Functions

- IgE provides immunity against parasite by Antibody dependent cell mediated cytotoxicity (ADCC).
- Level of IgE antibody in blood of normal individual is very low and its level increases during parasitic infection and in allergic reactions.

ANTIBODIES AS ANTIGENS

Immunoglobulins are protein molecules and therefore can be Immunogens too. Immunologists have exploited this property to generate anti-immunoglobulins and this anti-antibody is used to characterize epitopes present on antibody. The antigenic determinants or epitopes on immunoglobulins have been classified into three major types (Figure 6).

- Isotypic
- Allotypic
- Idiotypic

Isotypic

The prefix ‘Iso’ means same in all members of the same species. Isotypic determi-nant is present in the constant region of the heavy chain. Isotypic determinant is characteristic for a particular species and is present in all members of the same species. Therefore, all individuals of the same species have the same isotype. But the isotypic determinants between different species are not the same. Consequently, when an antibody from one species is injected into another species, the injected antibodies are recognized as foreign, resulting in the induction of antibodies (anti-antibodies) against the isotypic determinants of the injected antibodies. The antibodies react with isotypic determinants in the constant region of the injected antibodies.

Allotypic

The prefix ‘Alio’ means that different in individuals of the same species. Within a species multiple allele exists for certain isotypic genes; consequently, some members within the same species have different antigenic determinants called allotypic determinants. Therefore, injection of antibodies from one animal to another animal of the same species induces antiantibodies (anti-allotypic antibodies) formation against the allotypic determinants of the injected antibodies, provided that the two animals differ in their allotypic determinants. Genes in the constant region of immunoglobulin code the allotypic determinants. In humans allotypic determinants are found in the four IgG subclasses, IgA2 subclass, and k light chain.

Idiotypic

Antigen-binding site in antibody molecule is formed by the amino acids of the VH and VL chains. The amino acids of the antigen binding site

also act as immunogen. The antigenic determinants of the VH and VL region are called idiotypic determinants or idiotopes. One antibody molecule has many idiotopes in the antigen-binding site. The sum of the individual idiotopes in an antibody molecule is called the idiotype of the antibody. The idiotopes are further designated alpha, beta, and gamma idiotopes.

IMMUNOGLOBULINS SUPERFAMILY (IGSF)

The immunoglobulin superfamily (IgSF) is a class of proteins that are associated with the adhesion, binding and recognition processes of cells. The term "immunoglobulin superfamily" (IgSF) initially referred to Immunoglobulins and other proteins involved in the immune response and sharing the same 3D topology. The subsequent discovery of the Ig fold in proteins not functionally related to Igs led to the definition of new functional families, structurally similar to the Igs, such as that of the cytokine receptors or of the bacterial proteins containing the fibronectin type III module.

Proteins which have similar structure to immunoglobulins are classified into IgSF. In recent years, increasing numbers of novel members of the IgSF have been identified. Membership of the IgSF of a newly identified protein is usually based on the conservation in folding and in sequence of specific features found within the Ig molecules. These criteria include domain size (-100 amino acids), the number of strands, and the general topology of the Ig domain or Ig fold. All IgSFs possess at least one immunoglobulin domain or fold. The IgSF superfamily is composed of surface antigen receptors, co-receptors of the immune system, cell adhesion molecules, some cytokine receptors and molecules involved in antigen presentation to lymphocytes. Most of the IgSF members play roles in the immune system. But the sperm-specific protein Izumo is essential for sperm-egg fusion.

Therapeutic Antibodies

Antibodies can swiftly provide therapeutics to target the disease-related molecules that have been discovered in genomic research because:

- The high level of specificity and affinity to the target molecule or antigen achieves a high level of efficacy and fewer adverse events,
- Their ability to target diverse molecules and the modes of action of the antibodies allow them to be applied to a wide range of therapeutic targets,
- Modification and refinement by genetic engineering technology and the establishment of recombinant manufacturing technology has made industrial manufacturing possible.

Development of therapeutic antibodies boomed in the 1980's, and the first therapeutic antibody, a mouse antibody, was launched in 1986 as an immunosuppressive agent used during organ transplantation. Although problems, such as mouse antibodies expressing antigenicity in humans, prevented any therapeutic antibodies being launched in the next 10 years, antibody engineering techniques continued to be actively developed and resulted in techniques to produce chimeric antibodies and humanized antibodies from mouse antibodies. In chimeric antibodies, 33% of the structure originates from mouse, with variable regions from mouse and constant regions from human, and in humanized antibodies, up to 90% of the structure originates from human, with only the antigen binding site in the variable region (complementarity-determining region) originating from mouse. Furthermore, new techniques made it possible to obtain human antibodies from human antibody phage libraries and human antibody–producing mice. These technological innovations intensified the development of therapeutic antibodies, and from the mid-1990's, a series of therapeutic antibodies were launched that are now being used in clinic. The disease areas that therapeutic antibodies can target have subsequently expanded, and antibodies are currently utilized as pharmaceuticals for

cancer, inflammatory disease, organ transplantation, cardiovascular disease, infection, respiratory disease, ophthalmologic disease.

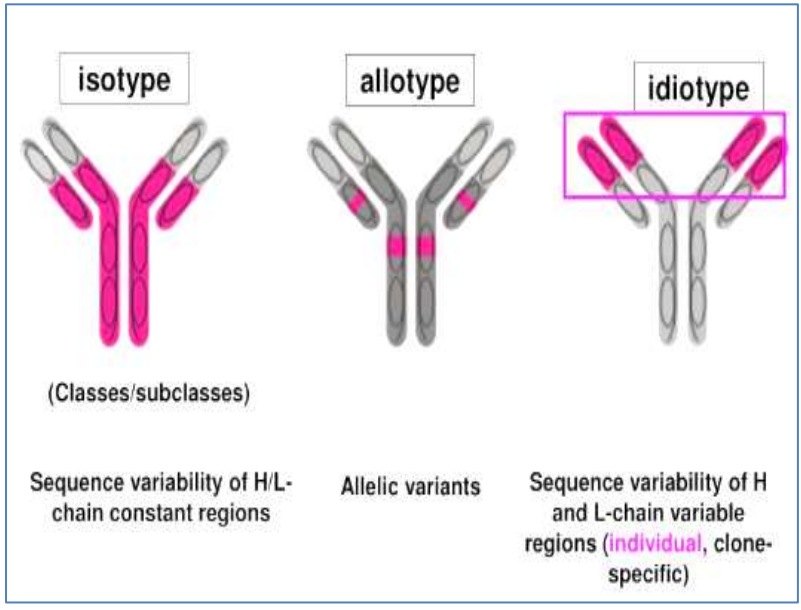

Figure 6.6. All immunoglobulins have the basic four-chain structure. There are three types of immunoglobulin variability. (1) Isotypic variation is present in the germline of all members of a species, producing the heavy (μ, δ, γ, ε, α) and light chains (Igκ, λ) and the V region frameworks (subgroups). (2) Allotypic variation is intraspecies allelic variability. (3) Idiotypic variation refers to the diversity at the antigen binding site (paratope) and in particular relates to the hypervariable segments.

MECHANISMS OF ACTION OF THERAPEUTIC ANTIBODIES

The efficacy of therapeutic antibodies stems from various natural functions of antibodies neutralization, antibody-dependent cell-mediated cytotoxic (ADCC) activity, or complement-dependent cytotoxic (CDC) activity or the antibody can be utilized as a drug delivery carrier.

- **Neutralization:** Many therapeutic antibodies utilize neutralization to block the pathophysiological function of their target molecules. In this case, antibodies bind to the ligand or receptor that is expressed on the cell surface and block the target signalling pathway. When the signalling in the tumour through these ligands or receptors is diminished, it can result in cellular activity being lost, proliferation being inhibited, pro-apoptotic programs being activated, or cells being re-sensitized to cytotoxic agents.
- **Antibody-dependent cell-mediated cytotoxic:** To trigger ADCC, the Fv binding domain of an antibody binds to a specific antigen expressed on the surface of a target cell. The antibody is then able to recruit immune-effector cells (such as macrophages and NK cells) that express various receptors able to bind to the Fc and thus activate the immune-effector cells to lysis of the target cell.
- **Complement-dependent cytotoxic (CDC) activity:** CDC is triggered when the C1 complex binds the antibody–antigen complex, activates a cascade of complement proteins, and causes a complex to form that attacks the membrane. This results in lysis of the target cell. Both ADCC and CDC are interactions that involve components of the host immune system and, among the therapeutic antibodies being developed for cancer, there are presumably products that utilize more than one mechanism (ADCC, CDC, and neutralizing functions) in their pharmacological actions.
- **Drug delivery carrier:** Antibodies can be applied as drug delivery carriers when conjugated to radioisotopes, toxins, drugs or cytokines. The advantage of these conjugates over conventional drugs is that cytotoxic agents can be delivered directly and at higher local concentrations to tumour tissues, without causing damage to normal cells.

SUMMARY

The term antibody signifies the existence of a discrete body that can act against a pathogen or its product. Antibodies are defence proteins produced by the adaptive immune system of vertebrates to combat invading pathogens. Antibody is a Y shaped molecule made up of four polpeptides-2 heavy chains and 2 light chains. Antibodies have been divided into different classes based on difference in the constant region. The classes IgG, IgA, IgM, IgD and IgE have different functional properties. IgG is the most abundant antibody of internal fluids. It combats microbes and their toxins. IgA is predominant antibody in external secretions where it defends the surface against viral and bacterial assault. IgM is a very effective agglutinator, while IgE is the major antibody of allergic reactions. IgD is primarily found on surface of mature B cells and is probably involved in lymphocyte activation. An immunoglobulin superfamily of which antibody is a large and diverse group of protein comprising antibodies. Antibodies can swiftly provide therapeutics to target disease-related molecules discovered in genomic research. Antibody engineering techniques have been actively developed and these technological innovations have intensified the development of therapeutic antibodies.

FREQUENTLY ASKED QUESTIONS (FAQS)

Q1. What are antibodies?

Answer: Antibody, also called immunoglobulin, a protective protein produced by the immune system in response to the presence of a foreign substance, called an antigen. Antibodies recognize and latch onto antigens in order to remove them from the body. A wide range of substances are regarded by the body as antigens, including disease-causing organisms and toxic materials such as insect venom.

Q2. Are antibodies and immunoglobulins the same?

Answer: Immunoglobulin and antibody are disease-fighting proteins developed by most vertebrates in response to a particular antigen. Both immunoglobulin and antibody are glycoproteins. Both of them contain similar regions in their molecules. The main difference between immunoglobulin and antibody is that immunoglobulin has a transmembrane domain in order to be attached to the plasma membrane whereas antibody does not have a transmembrane domain.

Q3. What is an immunoglobulin superfamily?

Answer: A superfamily is a group of proteins that share similar amino acid sequences where sequences are less than 50% identical. The immunoglobulin superfamily is a large and diverse group of proteins comprising more than 100 different polypeptides that contain segments of conserved sequence of 100 amino acids, an Ig homology unit which have significant similarities to those of immunoglobulin domains.

Q4. What do you understand by antibody dependent cell mediated cytotoxicity?

Answer: Antibody dependent cell mediated cytotoxicity is a lytic process in which antibody coated target cells are killed by the cells of the immune system such as NK cells and macrophages.

Q5. What does the term opsonisation mean?

Answer: Opsonisation is a process whereby a particle often a bacteria is rendered more attractive for ingestion by phagocytes by coating it with a specific antibody or by complement components.

Q6. What is a complement system?

Answer: The activation of the complement system is one of the most important effector mechanisms of immunoglobulins. The complement system is a group of approximately 20 proteins/enzymes present in plasma.

Q7. What are agglutinins?

Answer: Antibodies that cause agglutination or aggregation of cells or bacteria are called as agglutinins.

Q8. Define the framework region.

Answer: The invariant peptide sequence within the variable region that supports the CDRs is termed as the framework region. It acts as the scaffolding to which CDRs are fixed.

Q9. What are CDRs?

Answer: The region within the variable region that generates the antigen binding site are called the complementarity determining regions or CDRs.

Q10. What do you understand by an immunoglobulin fold?

Answer: Two β sheets linked by a disulphide bond and tightly packed against each other comprise the immunoglobulin fold. This motif is found in the immunoglobulin domain.

Q11. How many types of antibodies are there and how do they differ from one another?

Answer: There are five immunoglobulin classes (isotypes) of antibody molecules found in serum: IgG, IgM, IgA, IgE and IgD. They are distinguished by the type of heavy chain they contain. IgG molecules possess heavy chains known as γ-chains; IgM have μ-chains; IgAs have α-chains; IgEs have ε-chains; and IgDs have δ-chains. The variation in heavy chain polypeptides allows each immunoglobulin class to function in a different type of immune response or during a different stage of the body's defence.

Q12. What is meant by therapeutic antibodies?

Answer: Development of therapeutic antibodies boomed in the 1980's, and the first therapeutic antibody, a mouse antibody, was launched in 1986 as an immunosuppressive agent used during organ transplantation.

Antibodies can swiftly provide therapeutics to target the disease-related molecules that have been discovered in genomic research because 1) the high level of specificity and affinity to the target molecule or antigen achieves a high level of efficacy and fewer adverse events, 2) their ability to target diverse molecules and the modes of action of the antibodies allow them to be applied to a wide range of therapeutic targets, and 3) modification and refinement by genetic engineering technology and the establishment of recombinant manufacturing technology has made industrial manufacturing possible.

Q13. What are monoclonal antibodies?

Answer: Monoclonal antibodies are laboratory produced antibodies designed to recognise and bind to specific receptors found on the surface of cells. They are derived from natural antibodies; complex proteins derived from a single B cell made by the body's immunological defence system to recognise and fight foreign invaders such as bacteria and viruses.

Q14. How are MAbs produced?

Answer: MAbs are produced through hybridoma technology. The production of monoclonal antibodies involves several steps. In the first instance a laboratory animal is injected with a desired target to stimulate their immune system. Following this, antibody producing cells, B lymphocytes, are harvested from the animal's spleen and fused with an immortal myeloma cell line to create hybrid cells, or hybridoma. The hybrid cells are then screened to find those that secrete antibodies with the desired specificity for a particular target. Once identified the hybrid cell is cloned to establish a hybridoma colony. This colony is then maintained in a culture medium to provide a continual supply of monoclonal antibodies. Each of the antibodies secreted by the hybrid cell is identical (monoclonal) and has the capacity to bind to a specific receptor found on the surface of a cell.

Q15. How are MAbs useful?

Answer: Monoclonal antibodies (mAbs) are important reagents used in biomedical research, in diagnosis of diseases, and in treatment of such diseases as infections and cancer. The use of monoclonal antibodies (mAbs) in biomedical research has been and will continue to be important for the identification of proteins, carbohydrates, and nucleic acids. Their use has led to the elucidation of many molecules that control cell replication and differentiation, advancing our knowledge of the relationship between molecular structure and function. These advances is basic biologic sciences have improved our understanding of the host response to infectious-disease agents and toxins produced by these agents, to transplanted organs and tissues, to spontaneously transformed cells (tumours), and to endogenous antigens (involved in autoimmunity). In addition, the exquisite specificity of mAbs allows them to be used in humans and animals for disease diagnosis and treatment. Under the appropriate conditions, mAbs-producing hybridoma survive indefinitely, so continued production of mAbs is associated with the use of fewer animals, especially when production involves the use of in vitro methods.

MULTIPLE CHOICE QUESTIONS (MCQS)

Q1. Antibodies are produced by

A. lymphocytes
B. phagocytes
C. monocytes
D. thrombocytes

Q2. Antigen binding sites of an immunoglobulin are located in

A. light chain alone
B. heavy chain alone
C. Fc region of the antibody
D. Fab regions of the antibody

Q3. The class of immunoglobulins which can cross the placenta is

A. IgM
B. IgG
C. IgA
D. IgD

Q4. Self-reactive antibodies are not found in significant concentration in normal serum because

A. it is impossible to generate a self-reactive antibody
B. self-reactive B cells are killed by CD8 T cells
C. B cells that are stimulated via their surface bound antibody in the absence of T cell help to commit suicide
D. self-reactive B cells switch to IgA which is all secreted and not present in serum

Q5. Simulation of which of the following will lead to appearance of first antibody to appear following stimulation by an antigen?

A. IgM
B. IgA
C. IgE
D. IgG

Q6. Which is the most efficient complement fixing class of antibody?

A. IgE
B. IgA
C. IgM
D. IgG

Q7. The class of immunoglobulin, most abundant in body is

A. IgD
B. IgE
C. IgG
D. IgM

Q8. J chain is present in

A. IgA
B. IgM
C. Both (a) and (b)
D. IgE

Q9. Which is not a function of IgG?

A. Major antibody in serum
B. First antibody type produced against an antigen during the primary antibody response
C. Activates or fixes complement
D. Involved in opsonisation

Q10. Which of the following (s) is/are function (s) of Fc fragment of IgG?

A. It determines catabolic rate
B. It binds complement
C. It is related to passage of IgG across the placental barrier
D. All of these

Q11.Which is not a function of IgA?

A. Protect mucosal surfaces
B. Fix complement
C. Protect eyes
D. Agglutinate antigen

Q12. Which of the following is not true about antibody structure?

A. Antibodies have multiple identical antigen binding sites.
B. Antibodies are built from equal numbers of large (heavy) and small (light) peptide chains.
C. Antibodies are secreted and function away from the cell. They are not attached to the cell membrane.
D. The class of the antibody molecule is determined solely by its heavy chain.

Q13. Where do precursor T-lymphocytes develop into fully competent but not yet activated T-cells?

A. The thymus gland
B. The bone marrow
C. The lymph nodes
D. The spleen

Q14. Which of the following is not a feature of a secondary immune response to an antigen, when compared to the first response to the same antigen?

A. The antibody is generated faster.
B. More antibody is produced.
C. The antibody produced has greater affinity for the antigen.
D. Antibody is generated without T-cell help.

Q15. Cancer cells often have reduced amounts of cell surface proteins, including class I MHC antigens. Which of the following cells of the immune system can exploit this property to kill cancer cells?

A. Cytotoxic T-cells
B. Natural killer cells
C. Helper T-cells
D. Macrophages

Answer Key

1. A **2**. D **3**. B **4**. C **5**. A **6**. C **7**. C **8**. C **9**. B **10**. D **11**. B **12**. C **13**. A **14**. D **15**. B

ASSIGNMENTS

Long Answer Questions (500 Words)

Q1. If the variable region of the antibody is localised towards the c terminal region, do you think it is going to affect antibody function in any way?

Q2. Do you think having two dissimilar antigen binding sites on antibody molecules will be advantageous to human beings or not?

Short Answer Questions (200 Words)

Q1. Why do you think the body needs five different classes of antibodies?

Q2. How do you believe that monoclonal antibodies will change the map of treatment and diagnostics in future?

Q3. What are few of the areas of current research in therapeutic antibodies?

REFERENCES

Alejandra, MM; Lansang, MA; Dans, LF; et al. Intravenous immunoglobulin for treating sepsis an septic shock. *Cochrane Database Syst Rev*., 2002, (1), CD001090.

Annane, D; Bellssant, E; Cavaillon, JM. Septic shock. *Lancet*., 2005, 365, 63–78.

Borghesi, L; Milcarek, C. "From B cell to plasma cell: regulation of V (D)J recombination and antibody secretion". *Immunologic Research*., (2006), 36 (1–3), 27–32. doi :10.1385/IR:36:1:27. PMID 17337763.

Cafiero, F; Gipponi, M; Bonalimi, U; et al. Prophylaxis of infection with intravenous immunoglobulins plus antibiotics for patients at risk for

sepsis undergoing surgery for colorectal cancer: results of a randomized, multicentre clinical trial. *Surgery*., 1992, 112, 24–31.

Cohen, J. The immunopathogeneis of sepsis. *Nature*., 2002, 420, 885–91.

Darabi, K; Abdel-Wahab, O; Dzik, WH. Current usage of intravenous immune globulins and the rationale behind it. The Massachusetts General Hospital data and a review of the literature. *Transfusion*., 2006, 47, 741–53.

Dellinger, P; Levy, MM; Rhodes, A; et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock, 2012. *Intens Care Med.*, 2013, 39, 165–228.

Diaz, M; Casali, P. "Somatic immunoglobulin hypermutation". *Current Opinion in Immunology*., (April 2002), 14 (2), 235–40. doi:10.1016/S0952-7915(02)00327-8. PMC 4621002. PMID 11869898.

Dominioni, L; Dionigi, R; Zanello, M; et al. Effects of high-dose IgG on survival of surgical patients with sepsis score of 20 or greater. *Arch Surg*., 1991, 126, 236–40.

Ehrenstein, MR; Notley, CA. The importance of natural IgM: scavenger, protector and regulator. *Nat Rev Immunol*., 2010, 10, 778–86.

Fanning, LJ; Connor, AM; Wu, GE. "Development of the immunoglobulin repertoire". *Clinical Immunology and Immunopathology*., (April 1996), **79** (1). doi:10.1006/clin.1996.0044. PMID 8612345.

Giamarellos-Bourboulis, E; Apostolidou, E; Lada, M; et al. Kinetics of circulating immunoglobulin M in sepsis: relationship with final outcome. *Critical Care*., 2013, 17, R 247.

Hotchkiss, RS; Karl, IE. The pathophysiology and treatment of sepsis. *N Engl J Med*., 2003, 348, 138–50.

Hotchkiss, RS; Monneret, G; Payen, D. Immunosuppression in sepsis: a novel understanding of the disorder and a new therapeutic approach. *Lancet Infec Dis*., 2013, 13, 260–268.

Jump up to: Pier, GB; Lyczak, JB; Wetzler, LM. Immunology, Infection, and Immunity. ASM Press., (2004). ISBN 978-1-55581-246-1.

Jump up to: Janeway, C. Immunobiology (5th Ed.). Garland Publishing., (2001). ISBN 978-0-8153-3642-6.

Lindenmann, J. "Origin of the terms 'antibody' and 'antigen'". *Scandinavian Journal of Immunology*., (April 1984), **19** (4), 281–5. doi:10.1111/j. 3083.1984.tb00931.x. PMID 6374880.

Litman, GW; Rast, JP; Shamblott, MJ; Haire, RN; Hulst, M; Roess, W; Litman, RT; Hinds-Frey, KR; Zilch, A; Amemiya, CT. "Phylogenetic diversification of immunoglobulin genes and the antibody repertoire". *Molecular Biology and Evolution*., (January 1993), **10** (1), 6072. doi: 10.1093/oxfordjournals.molbev.a040000. PMID 8450761.

Market, E; Papavasiliou, FN. "V (D)J recombination and the evolution of the adaptive immune system". *PLoS Biology*., (October 2003), **1** (1), E16. doi:10.1371/journal.pbio.0000016. PMC 212695. PMID 14551913.

Maverakis, E; Kim, K; Shimoda, M; Gershwin, ME; Patel, F; Wilken, R; Raychaudhuri, S; Ruhaak, LR; Lebrilla, CB. "Glycans in the immune system and The Altered Glycan Theory of Autoimmunity: a critical review". *Journal of Autoimmunity*., (February 2015), **57** (6), 1–13. doi :10.1016/j.jaut.2014.12.002. PMC 4340844. PMID 25578468.

Medzhitov, R; Janeway, C. Advances in immunology: innate immunity. *N Engl J Med*., 2000, 343, 337–44.

Mian, IS; Bradwell, AR; Olson, AJ. "Structure, function and properties of antibody binding sites". *Journal of Molecular Biology*., (January 1991), **217** (1), 133–51. doi:10.1016/0022-2836(91)90617-F. PMID 1988675.

Milland, J; Sandrin, MS. "ABO blood group and related antigens, natural antibodies and transplantation". *Tissue Antigens*., (December 2006), **68** (6), 459–66. doi:10.1111/j.1399-0039.2006.00721 .x. PMID 17176435.

Norby-Teglund, A; Haque, KN; Hammarström, L. Intravenous polyclonal IgM-enriched immunoglobulin therapy in sepsis: a review of clinical efficacy in relation to microbiological aetiology and severity of sepsis. *J Intern Med*., 2006, 260, 509–16.

Ojeda-Andaluz, D; Iglesias, V; Bobillo, F; et al. Early levels of immunoglobulin M and natural killer cells predict outcome in

nonseptic critically ill patients. *J Crit Care*., 2013, 28, 1110e7–1110e10.

Parker, DC. “T cell-dependent B cell activation”. *Annual Review of Immunology*, (1993), 33160. doi:10.1146/ annurev.iy.11.040193. 001555. PMID 8476565.

Pildal, J; Goetzshe, PC. Polyclonal immunoglobulins for the treatment of bacterial sepsis: a systematic review. *Clin Infect Dis*., 2004, 39, 38–46.

Racaniello, Vincent. “Natural antibody protects against viral infection”. Virology Blog. Archived from the original on 20 February 2010. Retrieved 22 January 2010., (6 October 2009).

Ravetch, JV; Bolland, S. “IgG Fc receptors”. *Annual Review of Immunology*., (2001), **19** (1), 275-90. doi:10.1146/ annurev. immunol.19.1.275. PMID 11244038.

Rhoades, RA; Pflanzer, RG. (2002). *Human Physiology* (4th Ed.). Thomson Learning. p. 584. ISBN 978-0-534-42174-8.

Rodriguez, A; Rello, J; Neira, J; et al. Effects of intravenous immunoglobulin and antibiotics on survival for severe sepsis undergoing surgery. *Shock*., 2005, 23, 298–304.

Rus, H; Cudrici, C; Niculescu, F. “The role of the complement system in innate immunity”. *Immunologic Research*., (2005), **33**(2), 103–12. doi:10.1385/IR:33:2:103. PMID 16234578.

Schedel, I; Dreikhausen, U; Newtig, B; et al. Treatment of gram negative septic shock with immunoglobulin preparation: a prospective, randomized clinical trial. *Crit Care Med*., 1991, 19, 1104.

Späth, PJ. Structure and function of immunoglobulins. *Sepsis*., 1999, 3, 197–218.

Turgeon, AF; Hutton, B; Fergusson, DA; et al. Meta-analysis: intravenous immunoglobulin in critically ill adult patients with sepsis. *Ann Intern Med*., 2007, 146, 193–203.

Venet, F; Gebeile, R; Bancel, J; et al. Assessment of plasmatic immunoglobulin G, a and M levels in septic shock patients. *Internat Immunoprharmacol*., 2011, 11, 2086–2090.

Werdan, K. Intravenous immunoglobulin for prophylaxis and therapy of sepsis. *Curr Opin Crit Care*., 2001, 7, 354–61.

Werdan, K. Immunoglobulins in sepsis: therapeutic use of immunoglobulins. *Sepsis*., 1999, 3, 239–46, 13.

Williams, CM; Galli, SJ. "The diverse potential effector and immunoregulatory roles of mast cells in allergic disease". *The Journal Allergy and Clinical Immunology.*, (May 2000), **105**, 84759. doi:10.1067/mai.2000.106485. PMID 10808163.

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Chapter 7

THE MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)

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ABSTRACT

Tightly linked cluster of gene products of the Major histocompatibility complex (MHC), play roles in intercellular recognition and in discrimination between self and nonself. The MHC participates in the development of both humoral and cell mediated immune responses. While antibodies may react with antigens alone, most T cells recognize antigen only when it is combined with an MHC molecule. Furthermore, because MHC molecules act as antigen-presenting structures, the particular set of MHC molecules expressed by an individual, influences the repertoire of antigens to which that individual's TH and TC cells can

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respond. For this reason, the MHC partly determines the response of an individual to antigens of infectious organisms, and it has therefore been implicated in the susceptibility to disease and in the development of autoimmunity. The recent understanding that natural killer cells express receptors for MHC class I antigens and the fact that the receptor–MHC interaction may lead to inhibition or activation expands the known role of this gene family. The present chapter examines the organization and inheritance of MHC genes, the structure of the MHC molecules, and the central function that these molecules play in producing an immune response.

Keywords: MHC, humoral and cell mediated immunity, APCs, HLA and H2-complex

OBJECTIVES

- To learn the role of MHC in self/non-self-recognition.
- To recognise various haplotypes of MHC and their structures.
- To differentiate between Class I and Class II MHC antigen presentation.
- To understand the general organisation and inheritance of the MHC.

INTRODUCTION

The first descriptions of the MHC were made by British immunologist *Peter Gorer* in 1936. MHC genes were first identified in inbred mice strains. *Clarence Little* transplanted tumors across differing strains and found rejection of transplanted tumors according to strains of host versus donor. George Snell selectively bred two mouse strains, attained a new strain nearly identical to one of the progenitor strains, but differing crucially in histocompatibility - that is, tissue compatibility upon transplantation—and thereupon identified an MHC locus. For this work,

Snell was awarded the 1980 Nobel Prize in Physiology or Medicine, together with Baruj Benacerraf and Jean Dausset.

The major histocompatibility complex is a set of genes arrayed within a long continuous stretch of DNA on chromosome 6 in humans and on chromosome 17 in mice. The MHC is referred to as the human leukocyte antigen (HLA) complex in humans and as H-2 complex in mice. Both B and T cells use surface molecules to recognise antigen and they do so in different ways. The B-cell receptors can recognise the antigen on their own but the T-cell receptors only recognise pieces of antigens that are processed and expressed on the surface of other cells. These antigen pieces are held within a binding groove of the cell surface protein called the Major Histocompatibility Complex (MHC) molecule. These fragments are generated inside the cell following digestion. The complex of antigenic peptide and the MHC molecule then appears on the cell surface. MHC molecules thus act as cell surface vessel for holding and displaying fragments of antigen so that the T cells can engage with the peptide-MHC complex via their T cell receptors. The MHC got its name from the fact that the genes in this region encode protein determine whether a tissue transplanted between two individuals will be accepted or not.

TYPES OF MHC

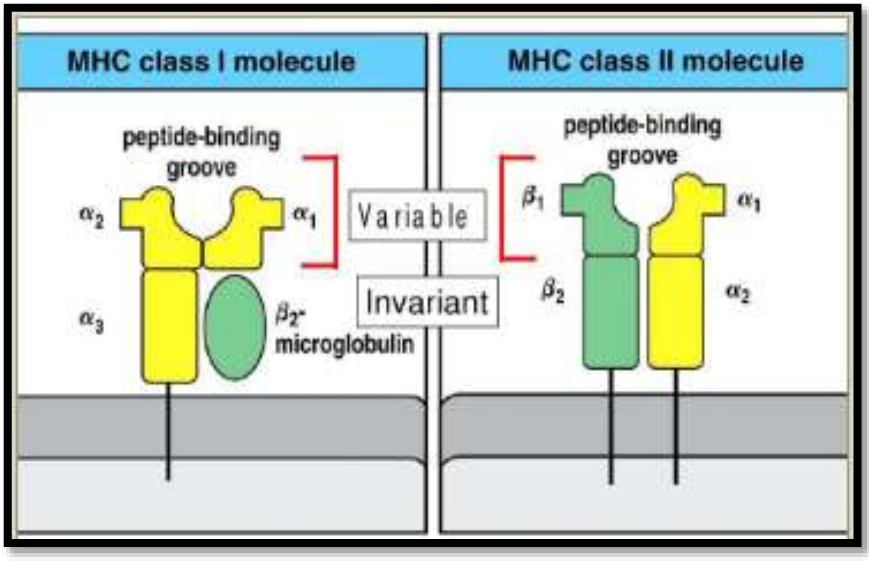
The MHC genes encode three classes of molecules: Class I, class II and class III MHC molecules.

1) Class I MHC genes encode glycoproteins expressed on the surface of nearly all nucleated cells of the body. These specialise in presenting antigens that originate in the cytosol such as viral proteins. The major function of class I gene products is presentation of endogenous peptide antigens to CD8+ T cells.
2) Class II MHC genes encode glycoproteins expressed predominantly on APC's (macrophages, dendritic cells and B

cells) where they primarily present exogenous antigenic peptides to CD4+ T cells.

3) Class III MHC genes encode a group of unrelated proteins that do not share structural similarity or function with class I and II molecules, although many of them do participate in various aspects of immune response.

Class I and class II molecules are similar in their quaternary structure (Figure 7.1).



Source: Google.com.

Figure 7.1. Showing Structure of Class I and Class II MHC molecules.

CLASS I MHC

Structure

Two polypeptides assemble to form a single molecule – a 45-kilodalton (kDa) α chain and a 12 kDa β_2-microglobulin molecule. The α

chain is organised into three external domains (α_1, α_2, α_3), each approximately 90 amino acids long; a transmembrane domain of about 25 hydrophobic amino acids followed by a short stretch of charged (hydrophilic) amino acids and a cytoplasmic anchor segment of 30 amino acids. β_2-microglobulin does not contain a transmembrane region and is bound non-covalently to the MHC class I α chain.

The α_1 and α_2 domains interact to form a platform of eight antiparallel β strands spanned by two long α-helical regions. The structure forms a deep groove or cleft with long α helices as sides and β strands of the β sheet as the bottom. This groove serves as the site for peptide binding and is large enough to bind a peptide of 8-10 amino acids.

The α_3 domain appears to be highly conserved among class I MHC molecules and contains a sequence that interacts strongly with the CD8 cell surface molecule found on the T_c cells.

All the three molecules i.e., α chain, β_2-microglobulin and a peptide are essential to proper folding and expression of MHC-peptide complex on the cell surface.

Presence

Class I MHC molecules are expressed constitutively on nearly are nucleated cells of the body. However, the level of expression differs among different cell types. Highest levels of class I MHC molecules are found on the surface of lymphocytes. MHC class I molecules usually constitute approximately 1% of the total plasma membrane proteins per lymphocyte. In contrast some other cells such as fibroblasts, muscle cells, hepatocytes and some neural cells express very low or negligible levels of class I MHC molecules. This low-level expression on liver cells may contribute to relative success of liver transplant by reducing the likelihood of graft rejection by T_C cells of the recipient. A few cell types (e.g., subsets of neurons and sperm cells at certain stages of differentiation) appear to lack class I MHC molecules altogether. Nucleated cells without class I

expression are quite rare. Non-nucleated cells such as red blood cells in mammals do not typically express any MHC molecule.

Class I MHC-Peptide Interaction

Class I MHC molecules bind peptides and present them to $CD8^+$ T cells. These peptides are often derived from endogenous intracellular proteins that are digested in the cytosol and the ER, where the interact with class I MHC molecules. This process is known as cytosolic or endogenous processing pathway. These peptide fragments are generated via a giant cytoplasmic protein complex known as the *proteasome*. The proteasome is involved in the normal turnover of cellular proteins and breaks them down into about 15 amino acids in length. Cytosolic enzymes (aminopeptidases) remove more amino acids from the peptides. Some peptides are destroyed and others are selectively transported into the endoplasmic reticulum by a two-chain peptide transporter (TAP). The peptides transported from the cytoplasm to the ER bind to the newly synthesized MHC class I molecules.

The normal fate of the peptides that reach the endoplasmic reticulum is degradation by an aminopeptidase which removes amino acids one at a time and the peptides are completely degraded. Some peptides with appropriate binding characteristics (i.e., 8-10 amino acids in length and with sufficient affinity to bind to the MHC class I binding groove) are rescued from this fate by binding to a newly synthesized MHC class I molecule. The peptide that binds to the newly synthesized class I MHC molecule in the endoplasmic reticulum moves via the Golgi apparatus to the cell surface, where it is displayed and presented to a $CD8^+$ T cell expressing an appropriate antigen receptor (Figure 7.2).

Since MHC class I molecules are expressed on all nucleated cells, the processing and presentation of endogenous antigens can occur in every nucleated cell of the body. Pathogens can infect any cell in the body, therefore, $CD8^+$ T cells scan the MHC class I and peptide combinations expressed on any nucleated host cell to identify whether it has been infected.

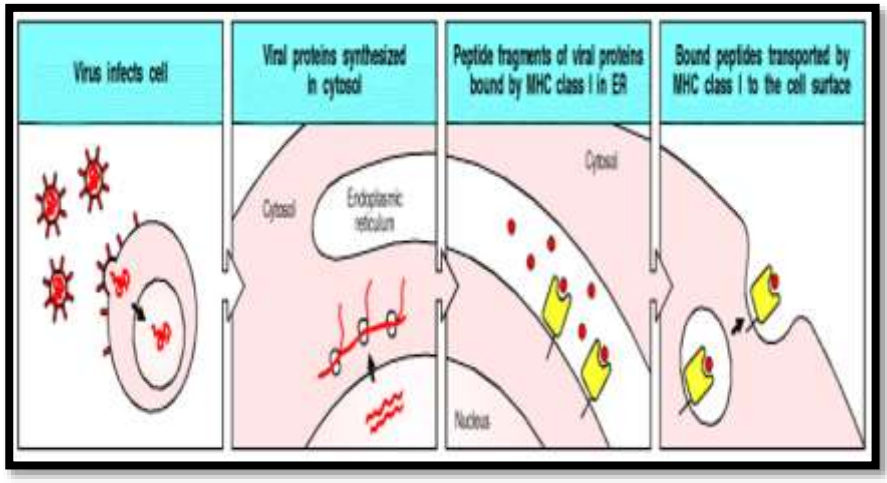


Figure 7.2. MHC class I molecules present antigen derived from proteins in the cytosol. In cells infected with viruses, viral proteins are synthesized in the cytosol. Peptide fragments of viral proteins are transported into the endoplasmic reticulum (ER) where they are bound by MHC class I molecules, which then deliver the peptides to the cell surface (Adapted from walport, immunology, 2001).

CLASS II MHC

Structure

Class II MHC molecules contain two polypeptide chains, a 33-kDa α chain and a 28-kDa β chain which are linked together by non-covalent interactions. Like class I MHC molecules, class II MHC molecules are membrane bound glycoproteins that contain external domains, a transmembrane segment and a cytoplasmic anchor segment. The α chain is organised into two domains: α_1 and α_2. The β chain is also organised into 2 domains: β_1 and β_2. The α_1 and β_2 domains form the peptide-binding groove which is composed of a floor of eight antiparallel β strands and the sides of antiparallel α helices where peptides normally containing 13-18 amino acids can bind. The class II MHC molecules lack the conserved residues and thus forms an open-ended groove.

Presence

The expression of MHC molecules is restricted to antigen-presenting cells. APC's are specialised for their ability to alert the immune system to the presence of an invader and drive the activation of T cell responses.

Among the various APC's, marked differences in the level of MHC class II expression is seen. In few cases, class II expression depends on cells differentiation stage or the level of activation. APC activation usually occurs following interaction with a pathogen and via cytokine signalling, which in turn induces significant increases in MHC class II expression.

A variety of cells can function as APC's. Their distinguishing feature is their ability to express class II MHC molecules and to deliver a costimulatory signal to T cells. Three types of cells are known to have these features and are thus often referred to as professional APC's: dendritic cells, macrophages, B cells. These cells differ from one another in their mechanism of antigen uptake, in whether they constitutively express class II MHC molecules and in their costimulatory activity.

- Dendritic cells are generally viewed as the most effective of the APC's. Because these cells constitutively express high levels of class II MHC molecules and have inherent costimulatory activity, they can activate naïve T_H cells.
- Macrophages need to be activated before the express class II MHC molecules or costimulatory membrane molecules.
- B cells constitutively express class II MHC molecules and possess antigen-specific surface receptors, making them particularly efficient at capturing and presenting their cognate antigen. However, they must be activated by, e.g., antigen, cytokines or pathogen associated molecular patterns (PAMP's) before they express the costimulatory molecules required for activating naïve T_H cells.

Other cell types known as non-professional APC's can be induced to express class II MHC molecules and costimulatory signal under certain

conditions. These cells can be recruited or professional antigen presentation for short periods and in certain situations, such as during sustained inflammatory response.

Class II MHC-Peptide Interaction

Class II MHC molecules bind and present peptides to $CD4^+$ T cells. These molecules can bind to a wide range of peptides. These peptides are derived from exogenous proteins. Most of the peptides associated with MHC class II molecules are derived from self-membrane-bound proteins or foreign proteins internalised by phagocytosis or receptor mediated endocytosis and then processed through the exogenous pathway.

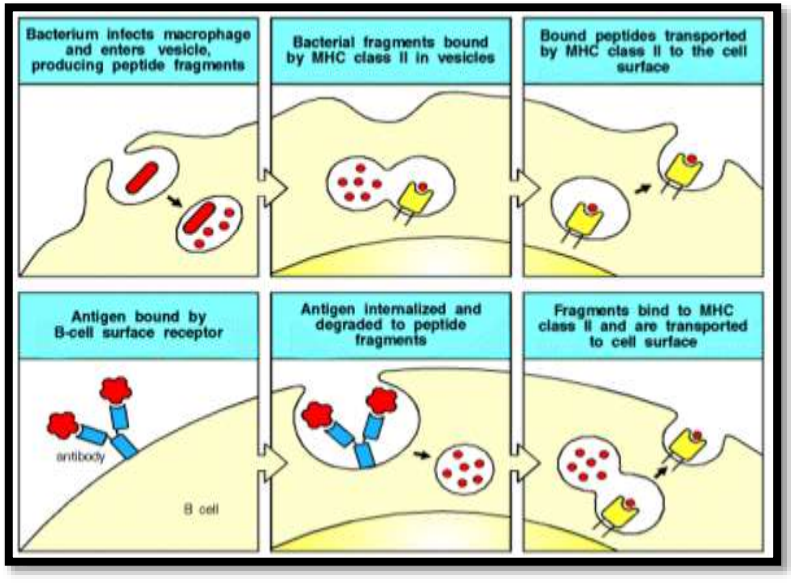


Figure 7.3. *MHC class II molecules present antigen originating in intracellular vesicles.* Some bacteria infect cells and grow in intracellular vesicles. Peptides derived from such bacteria are bound by MHC class II molecules and transported to the cell surface (top row). MHC class II molecules also bind and transport peptides derived from antigen that has been bound and internalized by B-cell antigen receptor-mediated uptake into intracellular vesicles (bottom row) (Adapted from walport, immunology).

The bound peptides isolated from MHC class II molecules are usually 13-18 amino acids long. The peptide binding groove in MHC class II molecule is open at both ends, thereby, allowing longer peptides to extend beyond the ends. Studies have shown that a central core of almost 13 amino acids determines the ability of a peptide to bind to class II molecule. Unlike class I-binding peptides, class II-binding peptides lack conserved anchor residues. Instead, hydrogen bonds are present between the peptide and the class II molecule throughout the binding site. Peptides that bind to class I MHC molecules contain an internal sequence of 7-10 amino acids that provide the main contact points. Usually, this sequence has an aromatic or hydrophobic residue at the amino terminus and three additional hydrophobic residues in the middle and C-terminus (Figure 7.3).

Table 7.1. Feature of Class I MHC and Class II MHC

Feature	Class I MHC	Class II MHC
Structure	α-chain and β_2 m	α- and β-chain
Polymorphic domain	α_1 and α_2 domains	α_1 and β_2 domains
Binding site for T-cell co-receptor	α_3 region binds to CD8	β_2 region binds CD4
Constitutive cellular expression	Nearly all nucleated cells	APCs
Peptides derived from	Endogenous antigens	Exogenous antigens
Peptide presented to	CD^+8 T-cells	CD^+4 T-cells
Nomenclature		
Human	HLA-A, HLA-B, HLA-C	HLA-DR, HLA-DQ, HLA-DP
Mouse	H-2K, H-2D, H-2L	I-A, I-E

PATHWAYS FOR ANTIGEN PROCESSING AND PRESENTATION

In MHC class I, any nucleated cell normally presents cytosolic peptides, mostly self peptides derived from protein turnover and defective ribosomal products. During viral infection, intracellular microorganism infection, or cancerous transformation, such proteins degraded in the proteosome are as well loaded onto MHC class I molecules and displayed

on the cell surface. T lymphocytes can detect a peptide displayed at 0.1%-1% of the MHC molecules.

In MHC class II, phagocytes such as macrophages and immature dendritic cells take up entities by phagocytosis into phagosomes—though B cells exhibit the more general endocytosis into endosomes—which fuse with lysosomes whose acidic enzymes cleave the uptaken protein into many different peptides. Via physicochemical dynamics in molecular interaction with the particular MHC class II variants borne by the host, encoded in the host's genome, a particular peptide exhibits immunodominance and loads onto MHC class II molecules. These are trafficked to and externalized on the cell surface.

ORGANISATION AND INHERITANCE OF MHC MOLECULES

The genes that reside within the MHC region are highly polymorphic, i.e., various alternative forms of each gene exist in a population. The genes of MHC loci lie so close to one another that their inheritance is linked. Hence, there is a very low chance of crossing over between these genes. Because of this reason, most individuals inherit these genes as a single set which is known as a haplotype**.** An individual receives one haplotype from the father and one from the mother. Also, the MHC genes exhibit codominance i.e., both paternal and maternal gene products are expressed at the same time.

Yet another feature of MHC region is that it is polygenic as it contains multiple genes with the same function but with slightly different structure. Since the MHC alleles are co-dominantly expressed, heterozygotes express the gene products encoded by both the alleles at each MHC locus.

ROLE OF MHC MOLECULES

MHC is the tissue-antigen that allows the immune system (more specifically T cells) to bind to, recognize, and tolerate itself (autorecognition). MHC is also the chaperone for intracellular peptides that are complexed with MHCs and presented to T cell receptors (TCRs) as potential foreign antigens. MHC interacts with TCR and its co-receptors to optimize binding conditions for the TCR-antigen interaction, in terms of antigen binding affinity and specificity, and signal transduction effectiveness.

Essentially, the MHC-peptide complex is a complex of autoantigen/ alloantigen. Upon binding, T cells should in principle tolerate the auto-antigen, but activate when exposed to the allo-antigen. Disease states occur when this principle is disrupted.

Antigen Presentation

MHC molecules bind to both T cell receptor and CD4/CD8 co-receptors on T lymphocytes, and the antigen epitope held in the peptide-binding groove of the MHC molecule interacts with the variable Ig-Like domain of the TCR to trigger T-cell activation.

Autoimmune Reaction

Having some MHC molecules increases the risk of autoimmune diseases more than having others. HLA-B27 is an example. It is unclear how exactly having the HLA-B27 tissue type increases the risk of ankylosing spondylitis and other associated inflammatory diseases, but mechanisms involving aberrant antigen presentation or T cell activation have been hypothesized.

Tissue Allorecognition

MHC molecules in complex with peptide epitopes are essentially ligands for TCR. T cells become activated by binding to the peptide-binding grooves of any MHC molecule that T cells were not trained to recognize during thymus positive selection.

Transplant Rejection

In a transplant procedure, as of an organ or stem cells, MHC molecules act themselves as antigens and can provoke immune response in the recipient, thus causing transplant rejection. MHC molecules were identified and named after their role in transplant rejection between mice of different strains, though it took over 20 years to clarify MHC's role in presenting peptide antigens to cytotoxic T lymphocytes (CTLs). Each human cell expresses six MHC class I alleles (one HLA-A, -B, and -C allele from each parent) and six to eight MHC class II alleles (one HLA-DP and -DQ, and one or two HLA-DR from each parent, and combinations of these). The MHC variation in the human population is high, at least 350 alleles for HLA-A genes, 620 alleles for HLA-B, 400 alleles for DR, and 90 alleles for DQ. Any two individuals who are not identical twins will express differing MHC molecules. All MHC molecules can mediate transplant rejection, but HLA-C and HLA-DP, showing low polymorphism, seem least important. When maturing in the thymus, T lymphocytes are selected for their TCR incapacity to recognize self-antigens, yet T lymphocytes can react against the donor MHC's peptide-binding groove, the variable region of MHC holding the presented antigen's epitope for recognition by TCR, the matching paratope. T lymphocytes of the recipient take the incompatible peptide-binding groove as nonself antigen. The T lymphocytes' recognition of the foreign MHC as self is allorecognition.

Transplant Rejection Has Various Types Known to Be Mediated by MHC (HLA)

Hyperacute rejection occurs when, before the transplantation, the recipient has preformed anti-HLA antibodies, perhaps by previous blood transfusions (donor tissue that includes lymphocytes expressing HLA molecules), by anti-HLA generated during pregnancy (directed at the father's HLA displayed by the foetus), or by previous transplantation.

Acute cellular rejection occurs when the recipient's T lymphocytes are activated by the donor tissue, causing damage via mechanisms such as direct cytotoxicity from CD8 cells.

Acute humoral rejection and chronic disfunction occurs when the recipient's anti-HLA antibodies form directed at HLA molecules present on endothelial cells of the transplanted tissue.

In all of the above situations, immunity is directed at the transplanted organ, sustaining lesions. A cross-reaction test between potential donor cells and recipient serum seeks to detect presence of preformed anti-HLA antibodies in the potential recipient that recognize donor HLA molecules, so as to prevent hyperacute rejection. In normal circumstances, compatibility between HLA-A, HLA-B, and HLA-DR molecules is assessed. The higher the number of incompatibilities, the lower the five-year survival rate. Global databases of donor information enhance the search for compatible donors.

CONCLUSION

MHC molecules play a crucial role in response of T cells to antigens that are taken in by the cells of the body. These molecules selectively bind peptides derived from antigens and present them to T cells with an appropriate receptor. The MHC is a complex set of genes that are generally inherited as a unit from parents. These linked units are called haplotypes. The MHC codes for two major categories of cell surface membrane molecules, MHC class I and MHC class II.

MHC class I is expressed in all nucleated cells of the body. It consists of a large glycoprotein α chain encoded by an MHC gene, and β_2-microglobulin, a protein with a single domain.

MHC class II molecules are expressed specifically on APCs such as dendritic cells, macrophages and B cells. Each molecule is composed of two non-covalently associated glycoproteins, α and β chain, encoded by separate MHC genes. Within one individual, the same MHC class I and class II molecules are expressed in all cells of the body. Different individuals express different MHC class I and class II molecules.

This diversity arises because individuals within a species have a huge range of inheritable forms of MHC class I and class II genes (genetic polymorphism). Because of this extensive polymorphism of MHC gene, every individual has an almost unique array of MHC genes. In most cases, class I molecules present processed endogenous antigen to $CD8^+$ T cells and class II molecules present processed antigen to $CD4^+$ T cells.

The outer region of every MHC class I and II molecule comprise a deep cleft called the peptide-binding groove that binds a peptide derived from the catabolism of the protein antigens. The binding of peptides to MHC molecules is selective i.e., each MHC molecule binds a range of peptides but favours the binding of peptides with particular motifs. MHC class I molecules bind peptides 8-9 amino acids long derived from proteins catabolised in the cytoplasm (endogenous antigen). MHC class II molecules bind peptides 12-17 amino acids long derived from proteins taken into cells (exogenous antigens). Susceptibility and resistance to many autoimmune diseases and inflammatory conditions are associated with the expression of a particular MHC molecule.

FREQUENTLY ASKED QUESTIONS (FAQS)

Q1. Define antigen presenting cells?

Answer: These are those cells that present processed antigenic peptide and MHC class II molecules to the T-cell receptor on $CD4^+$ T-cells.

Q2. Explain the difference between antigen presenting cell and the target cell.

Answer: Antigen presenting cells are those cells that present processed antigenic peptide and MHC class II molecules to the T-cell receptor on $CD4^+$ T-cells. whereas, target cells are any cells that present antigenic peptide and MHC class I molecule to the $CD8^+$ T-cells

Q3. What is antigen presentation?

Answer: Antigen presentation is the process by which protein antigen is presented to lymphocytes in the form of short peptide fragments.

Q4. What is meant by antigen processing?

Answer: Antigen processing involves cellular degradation of antigens into peptides that associate with class I and class II MHC molecules.

Q5. Name three types of professional APC's.

Answer: Dendritic cells, macrophages and B cells are professional APC's. Dendritic cells are generally viewed as the most effective of the APC's.

Q6. On what type of cells are MHC class I molecules expressed?

Answer: Class I MHC molecules are present on almost all nucleated cells of the body. The level of expression differs among different cell types. Highest levels of class I MHC molecules are found on the surface of lymphocytes. MHC class I molecules usually constitute approximately 1% of the total plasma membrane proteins per lymphocyte. in contrast some other cells such as fibroblasts, muscle cells, hepatocytes and some neural cells express very low or negligible levels of class I MHC molecules.

Q7. Human RBC's are enucleated and do not express any MHC molecules. How is this property considered to be fortuitous for blood transfusions?

Answer: If RBC's would express MHC molecules, extensive tissue typing would be required while carrying out transfusions and only few

individuals could be donors. The whole process of blood transfusion would hence, be as complex as organ transplants.

Q8. What is the function of MHC molecules?

Answer: The major function of MHC molecule is to bind peptide fragments derived from pathogens and display them on the cell surface for recognition by the appropriate T-cell.

Q9. Define professional APC's?

Answer: Professional APCs specialize in presenting antigen to T cells. Professional APC's specialize in presenting antigen to T cells, processing the antigen into peptide fragments and then displaying those peptides, bound to a class II MHC molecule, on their membrane.

Q10. What is human leukocyte antigen?

Answer: Human leukocyte antigen or HLA system refers to the major histocompatibility complex in humans.

Q11. How many polypeptides make up class I MHC molecule?

Answer: Two polypeptides assemble to form a single class I MHC molecule, a 45-kDa α chain and a 12-kDa β_2-microglobulin molecule.

Q12. What holds the peptide into the peptide binding groove?

Answer: Hydrogen bonds link the peptide and MHC class II molecule. These bonds are present between the peptide and the class II molecule throughout the binding site.

Q13. What is the nature of peptides that bind to MHC class I molecules?

Answer: The bound peptide is an extended structure in which both the ends interact with the MHC groove but the middle portion arches up away from the MHC molecule.

Q14. Which class of MHC binds to longer peptides?

Answer: Peptides isolated from class II MHC-peptide complexes generally contain 13-18 amino acid residues, somewhat longer than the class-II MHC peptides. The peptide binding groove is open at both the ends, thus, allowing longer peptides to extend beyond the ends.

Q15. What is a peptide binding cleft and what makes up the peptide binding domain in class I and class II MHC molecules?

Answer: The peptide binding cleft is the region formed by the MHC molecule which acts as a site of interaction for antigenic peptides. In class I MHC, the α_1 and α_2 domains interact to form a platform of eight antiparallel β strands spanned by two long α helical regions. This structure forms a deep grove – the peptide binding groove. In class II MHC, the α_1 and β_1 domains form the peptide binding groove.

MULTIPLE CHOICE QUESTIONS (MCQS)

Q1. All the following are characteristics of MHC class I and class II molecules except:

A. They are expressed co-dominantly.
B. They are expressed constitutively on all nucleated cells.
C. They are involved in presentation of antigen fragments to T cells.
D. They are polypeptides with domain structures.

Q2. MHC class I molecules are important for which of the following?

A. Binding to CD8 molecules on T cells.
B. Presenting exogenous antigen to B cells.
C. Binding to CD4 molecules on T cells.
D. Binding to Ig on B cells.

Q3. Which of the following is incorrect regarding MHC class II molecules?

A. B cells may express different allelic forms of MHC class molecules on their surface.
B. MHC class II molecules are synthesized in the endoplasmic reticulum of APC's.
C. MHC class II molecules are associated with β_2-microglobulin on the cell surface.
D. Genetically different individuals express different MHC class II alleles.

Q4. Which of the following is found on surface of every B cell, T cell and pancreatic cell?

A. MHC class II molecules.
B. Immunoglobulin.
C. CD19
D. MHC class I molecules.

Q5. The peptide transporter TAP:

A. Binds β_2-microglobulin
B. Is a part of proteasome
C. Transports peptides into the endoplasmic reticulum for binding MHC class I
D. Transports peptides into the endoplasmic reticulum for binding MHC class II

Q6. Which of the following statements about HLA genes is incorrect?

A. They code for complement proteins.
B. They code for both chains of every HLA class I molecule expressed.
C. They code for both chains of every HLA class II molecule expressed.
D. They are associated with susceptibility and resistance to different diseases.

Q7. The T cells recognise antigen:

A. In a three-dimensional form
B. In solution in the plasma
C. When presented on the surface of antigen presenting cells
D. Following presentation by pattern recognition receptors

Q8. $CD4^+$ T cells are generally restricted by:

A. CD-1
B. MHC class -I
C. MHC class-II
D. β2-microglobulin

Q9. Which of the following statements is NOT true regarding major histocompatibility complex?

A. MHC class I is present on all cells.
B. MHC class II is present on T cells, activated B cells, and macrophages.
C. They are cell surface glycoproteins.
D. They are present on chromosome 6.

Q10. Which of the following is NOT the structural component of MHC class II molecule?

A. α_1
B. α_2
C. α_3
D. β_1

Q11. Which of the following statements is the most accurate regarding the MHC molecules and the genes that encode them?

A. The genes encoding class II MHC proteins are highly polymorphic whereas the genes encoding class MHC I proteins are not.
B. The genes are codominant and each individual express class I and class II MHC genes inherited from both the parents.

C. The genes encoding class I and class II MHC molecules are located on different chromosomes.
D. The class I MHC molecules are found on the surface of all cells while as class II MHC molecules are expressed only on phagocytes.

Q12. Proteasomes play a key role in processing:
A. Exogenous antigen
B. Bacterial antigen
C. Endogenous antigen
D. Class II MHC peptides

Q13. Class I MHC molecules contain:
A. One γ chain and one β chain
B. One α and one β chain
C. One α chain and a β_2-microglobulin
D. One heavy chain and one light chain

Q14. Helper T-cells recognise the antigen on antigen presenting cell as:
A. Antigen alone
B. Antigen complexed with class I MHC molecule
C. Antigen in association with the complement
D. Antigen complexed with class II MHC molecule

Q15. MHC molecules are membrane bound:
A. Glycoproteins
B. Lipids
C. Carbohydrates
D. glycolipids

Answer Key

1. B **2**. A **3**. C **4**. D **5**. C **6**. B **7**. C **8**. C **9**. A **10**. C **11**. B **12**. C **13**. C **14**. D **15**. A

ASSIGNMENTS

Long Answer Questions (500 words)

Q1. Draw diagrams illustrating the general structure of class I and class II MHC molecules.

Q2. Write a note on class II MHC-peptide interaction.

Short Answer Questions (200 words)

Q1. What are the major structural differences between class I and class II MHC molecules?

Q2. Compare and contrast the functions of MHC class I and class II molecules.

Q3. Define the following terms and give examples.

a. Polygeny
b. Polymorphism
c. Codominant expression.

REFERENCES

Abbas Abul K, Andrew H. Litchman, Shiv Pillai. (2017). Hypersensitivity disorders. *Cellular and molecular immunology* 9:117-130.

Anders AK, Call MJ, Schulze MS, Fowler KD, Schubert DA, Seth NP, et al. HLA-DM captures partially empty HLA-DR molecules for

catalyzed removal of peptide. *Nat Immunol* (2011) 12:54–61.10.1038/ni.1967.

Bade-Doding C, Theodossis A, Gras S, Kjer-Nielsen L, Eiz-Vesper B, Seltsam A, et al. The impact of human leukocyte antigen (HLA) micro-polymorphism on ligand specificity within the HLA-B*41 allotypic family. *Haematologica* (2011) 96:110–8.10.3324/haematol.2010.030924.

Bouvier M, Wiley DC. Structural characterization of a soluble and partially folded class I major histocompatibility heavy chain/beta 2m heterodimer. *Nat Struct Biol* (1998) 5:377–84.10.1038/nsb0598-37.

Brown JH, Jardetzky TS, Gorga JC, Stern LJ, Urban RG, Strominger JL, et al. Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. *Nature* (1993) 364:33–9.10.1038/364033.

Chen Y, Sidney J, Southwood S, Cox AL, Sakaguchi K, Henderson RA, et al. Naturally processed peptides longer than nine amino acid residues bind to the class I MHC molecule HLA-A2.1 with high affinity and in different conformations. *J Immunol* (1994) 152:2874–81.

Chicz RM, Urban RG, Lane WS, Gorga JC, Stern LJ, Vignali DA, et al. Predominant naturally processed peptides bound to HLA-DR1 are derived from MHC-related molecules and are heterogeneous in size. *Nature* (1992) 358:764–8.10.1038/358764a0.

Falk K, Rotzschke O, Stevanovic S, Jung G, Rammensee HG. Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. *Nature* (1991) 351:290–6.10.1038/351290a0.

Gunther S, Schlundt A, Sticht J, Roske Y, Heinemann U, Wiesmuller KH, et al. Bidirectional binding of invariant chain peptides to an MHC class II molecule. *Proc Natl Acad Sci USA* (2010) 107:22219–24.10.1073/pnas.1014708107.

Guo HC, Jardetzky TS, Garrett TP, Lane WS, Strominger JL, Wiley DC. Different length peptides bind to HLA-Aw68 similarly at their ends but bulge out in the middle. *Nature* (1992) 360:364–6.10.1038/360364a0.

Hansen TH, Lybarger L, Yu L, Mitaksov V, Fremont DH. Recognition of open conformers of classical MHC by chaperones and monoclonal antibodies. *Immunol Rev* (2005) 207:100–11.10.1111/j.0105-2896.2005.00315.

Hassan C, Chabrol E, Jahn L, Kester MG, De Ru AH, Drijfhout JW, et al. Naturally processed non-canonical HLA-A*02:01 presented peptides. *J Biol Chem* (2015) 290:2593–603.10.1074/jbc.M114.607028.

Helen Chapel, Mansel Haeney, Siraj Misbah, Neil Snowden. (2014). Histocompatibility genetics in humans. *Essentials of clinical immunology* **6**:158-162.

Hunt DF, Henderson RA, Shabanowitz J, Sakaguchi K, Michel H, Sevilir N, et al. Characterization of peptides bound to the class I MHC molecule HLA-A2.1 by mass spectrometry. *Science* (1992) 255:1261–3.10.1126/science.1546328.

Janeway CA Jr, Travers P, Walport M, et al. Immunobiology: The Immune System in Health and Disease. 5th edition. New York: Garland Science; 2001 *The Major Histocompatibility Complex and Its Functions.*

Janeway CA Jr, Travers P, Walport M, et al, Immunobiology: The Immune System in Health and Disease, 5th edn (New York: Garland Science, 2001), *"The major histocompatibility complex and its functions."*

Judith A. Owen, Jenni Punt, Sharon A Strandford, Patricia P. Jones. (2013). The major histocompatibility complex and antigen presentation. *Kuby immunology* **7**:261-293.

Kimball's Biology Histocompatibility Molecules.

Klein J (1986). "Seeds of time: Fifty years ago Peter A. Gorer discovered the H-2 complex." *Immunogenetics.* 24 (6): 331–338. doi:10.1007/bf00377947.

Little CC 1941, "The genetics of tumor transplantation," pp 279–309, in *Biology of the Laboratory Mouse*, ed. by Snell GD, New York: Dover.

Lyngaa R, Pedersen NW, Schrama D, Thrue CA, Ibrani D, Met O, et al. T-cell responses to oncogenic Merkel cell polyomavirus proteins distinguish patients with Merkel cell carcinoma from healthy donors.

Clin Cancer Res (2014) 20:1768–78.10.1158/1078-0432.CCR-13-2697.

Madden DR, Garboczi DN, Wiley DC. The antigenic identity of peptide-MHC complexes: a comparison of the conformations of five viral peptides presented by HLA-A2. *Cell* (1993) 75:693–708.10.1016/0092-8674(93)90490-H.

McFarland BJ, Katz JF, Beeson C, Sant AJ. Energetic asymmetry among hydrogen bonds in MHC class II*peptide complexes. *Proc Natl Acad Sci USA* (2001) 98:9231–6.10.1073/pnas.151131498.

Mohan JF, Unanue ER. A novel pathway of presentation by class II-MHC molecules involving peptides or denatured proteins important in autoimmunity. *Mol Immunol* (2013) 55:166–8.10.1016/j.molimm.2012.10.024.

Natarajan SK, Stern LJ, Sadegh-Nasseri S. Sodium dodecyl sulfate stability of HLA-DR1 complexes correlates with burial of hydrophobic residues in pocket 1. *J Immunol* (1999) 162:3463–70.

Painter CA, Stern LJ. Conformational variation in structures of classical and non-classical MHCII proteins and functional implications. *Immunol Rev* (2012) 250:144–57.10.1111/imr.12003.

Paul S, Kolla RV, Sidney J, Weiskopf D, Fleri W, Kim Y, et al. Evaluating the immunogenicity of protein drugs by applying in vitro MHC binding data and the immune epitope database and analysis resource. *Clin Dev Immuno*l (2013) 2013:467852.10.1155/2013/467852.

Peter J. Delves, Seamus J. Martin, Dennis R. Burton, Ivan M. Roitt. (2017). Membrane receptors for antigen. *Roitt's essential immunology* 13:119-127.

Richard Coico, Geoffrey Sunshine. (2015). The role of major histocompatibility complex. *Immunology: a short course* **7**:117-133.

Schlundt A, Gunther S, Sticht J, Wieczorek M, Roske Y, Heinemann U, et al. Peptide linkage to the alpha-subunit of MHCII creates a stably inverted antigen presentation complex. *J Mol Biol* (2012) 423:294–302.10.1016/j.jmb.2012.07.008.

Schmitt L, Boniface JJ, Davis MM, Mcconnell HM. Conformational isomers of a class II MHC-peptide complex in solution. *J Mol Biol* (1999) 286:207–18.10.1006/jmbi.1998.2463

Schmitt L, Boniface JJ, Davis MM, Mcconnell HM. Kinetic isomers of a class II MHC-peptide complex. *Biochemistry* (1998) 37:17371–80.10.1021/bi9815593.

Smith KJ, Reid SW, Stuart DI, Mcmichael AJ, Jones EY, Bell JI. An altered position of the alpha 2 helix of MHC class I is revealed by the crystal structure of HLA-B*3501. *Immunity* (1996) 4:203–13.10.1016/S1074-7613(00)80429-X.

Snell GD, Higgins GF (1951). "Alleles at the histocompatibility-2 locus in the mouse as determined by tumor transplantation." *Genetics*. 36: 306–310.

Springer S, Doring K, Skipper JC, Townsend AR, Cerundolo V. Fast association rates suggest a conformational change in the MHC class I molecule H-2Db upon peptide binding. *Biochemistry* (1998) 37:3001–12.10.1021/bi9717441 [PubMed] [CrossRef] [Google Scholar].

Stadinski BD, Zhang L, Crawford F, Marrack P, Eisenbarth GS, Kappler JW. Diabetogenic T cells recognize insulin bound to IAg7 in an unexpected, weakly binding register. *Proc Natl Acad Sci USA* (2010) 107:10978–83.10.1073/pnas.1006545107.

Stern LJ, Brown JH, Jardetzky TS, Gorga JC, Urban RG, Strominger JL, et al. Crystal structure of the human class II MHC protein HLA-DR1 complexed with an influenza virus peptide. *Nature* (1994) 368:215–21.10.1038/368215a0.

Thomas J. Kindt; Richard A. Goldsby; Barbara Anne Osborne; Janis Kuby (2007). *Kuby immunology*. Macmillan. ISBN 978-1-4292-0211-4. Retrieved 28 November 2010.

Tynan FE, Borg NA, Miles JJ, Beddoe T, El-Hassen D, Silins SL, et al. High-resolution structures of highly bulged viral epitopes bound to major histocompatibility complex class I. Implications for T-cell receptor engagement and T-cell immunodominance. *J Biol Chem* (2005) 280:23900–9.10.1074/jbc.M503060200.

Urban RG, Chicz RM, Lane WS, Strominger JL, Rehm A, Kenter MJ, et al. A subset of HLA-B27 molecules contains peptides much longer than nonamers. *Proc Natl Acad Sci USA* (1994) 91:1534–8.10.1073/pnas.91.4.1534.

Vigneron, Nathalie; Stroobant, Vincent; Chapiro, Jacques; Ooms, Annie; Degiovanni, Gérard; Morel, Sandra; Bruggen, Pierre van der; Boon, Thierry; Eynde, Benoît J. Van den (2004-04-23). "An Antigenic Peptide Produced by Peptide Splicing in the Proteasome." *Science.* 304 (5670): 587–590. doi:10.1126/science.1095522. ISSN 0036-8075. PMID 15001714.

Wieczorek M, Sticht J, Stolzenberg S, Gunther S, Wehmeyer C, El Habre Z, et al. MHC class II complexes sample intermediate states along the peptide exchange pathway. *Nat Commun* (2016) 7:13224.10.1038/ncomms13224.

Wilson IA, Fremont DH. Structural analysis of MHC class I molecules with bound peptide antigens. *Semin Immunol* (1993) 5:75–80.10.1006/smim.1993.1011.

Yamazaki, K, Boyse, EA, Miké, V, Thaler, HT, Mathieson, BJ, Abbott, J, Boyse, J, Zayas, ZA, Thomas, L. (1976-11-02). "Control of mating preferences in mice by genes in the major histocompatibility complex." *Journal of Experimental Medicine.* 144 (5): 1324–1335. doi:10.1084/jem.144.5.1324. ISSN 0022-1007. PMC 2190468. PMID 1032893.

Zacharias M, Springer S. Conformational flexibility of the MHC class I alpha1-alpha2 domain in peptide bound and free states: a molecular dynamics simulation study. *Biophys J* (2004) 87:2203–14.10.1529/biophysj.104.044743.

Zarutskie JA, Sato AK, Rushe MM, Chan IC, Lomakin A, Benedek GB, et al. A conformational change in the human major histocompatibility complex protein HLA-DR1 induced by peptide binding. *Biochemistry* (1999) 38:5878–87.10.1021/bi983048m.

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Chapter 8

THE COMPLEMENT SYSTEM

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ABSTRACT

Complement is a system of plasma proteins that can be activated directly by pathogens or indirectly by pathogen-bound antibody, leading to a cascade of reactions that occurs on the surface of pathogens and generates active components with various effector functions. Various complement components bind and opsonize bacteria, rendering them susceptible to receptor-mediated phagocytosis by macrophages, which express membrane receptors for complement protein. Complement components constitute approximately 1.5% of the globulin protein fraction in plasma and their combined concentration can be as high as 3

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mg/ml. Some complement components are found in high concentrations (e.g., C3 at about 1 mg/ml), while others (such as factor D and C2) are found in only trace amounts. Complement components are designated by numerals (C1-C9) by letter symbols (e.g., factor D), or by trivial names (e.g., homologous restriction factor). Complement activation pathways involve the activation of one component that triggers the activation of the next component in the sequence. The complement can act through three different pathways called classical, lectin and alternative pathway, respectively. Deficiencies of complement components, regulators of complement pathways, or receptors for complement components may result in increased susceptibility to infection or the development of inflammatory conditions.

Keywords: complement system, classic, lectin, alternate pathway, opsonin, anaphylatoxin, CIINH, factor I, factor H.

OBJECTIVES

- Complement components.
- Complement functions.
- Complement activation
- Regulation of complement system
- Deficiencies of complement system

INTRODUCTION

Complement was discovered many years ago as a heat-labile component of normal plasma that elevates the opsonisation of bacteria by antibodies and allows antibodies to kill some bacteria.

The term “complement,” was coined by “Paul Ehrlich,” which is made up of approximately 30 circulating and membrane expressed proteins/glycoproteins. It plays an important as an arm of both the innate and antibody-mediated acquired immune responses. The complement system is one of the major mechanisms by which pathogen recognition is

converted into an effective host defense against initial infection. This activity was said to 'complement' the antibacterial activity of antibody.

Complement is a system of plasma proteins that can be activated directly by pathogens or indirectly by pathogen-bound antibody, leading to a cascade of reactions that occurs on the surface of pathogens and generates active components with various effector functions. Various complement components bind and opsonize bacteria, rendering them susceptible to receptor-mediated phagocytosis by macrophages, which express membrane receptors for complement protein. There are many other complement proteins, which elicit inflammatory response interface with components of adaptive immune system and clear immune complexes from the serum and/or banish apoptotic cells. Ultimately, a membrane attack complex (MAC) assembled from complement proteins directly kills some pathogens by creating pores in microbial membranes.

Jules Bordet began working on complement in the 1890's at the institute Pasture in Paris and demonstrated that the sheep antiserum to the bacterium Vibrio cholera caused the lysis of the bacteria and that heating the antiserum destroyed its bacteriolytic activity. Surprisingly, the ability to lyse the bacteria was restored to the heated serum that contained no antibodies directed against the bacterium and was unable to kill the bacterium by itself. Bordet then reasoned that bacteriolytic activity requires, two different substances:

First, specific antibacterial antibodies which survive the heating process, and second, heat-sensitive component responsible for the lytic activity. He devised a simple test for the lytic activity that easily detected lysis of antibody –coated red blood cells, called haemolysis.

Independently in Berlin, Paul Ehlrich carried out similar experiments and, defined it as "the activity of blood serum that completes the action of antibody." In fortifying years, researchers discovered that the action of complement was the result of interaction of a large and complex group of protein.

COMPLEMENT COMPONENTS

Most complement components are synthesized in the liver by hepatocytes, although some are produced by other cell types, including blood monocytes, tissue macrophages, fibroblasts, and epithelial cells of the gastrointestinal and genitourinary tracts. Complement components constitute approximately 1.5% of the globulin protein fraction in plasma and their combined concentration can be as high as 3 mg/ml. Some complement components are found in high concentrations (e.g., C3 at about 1 mg/ml), while others (such as factor D and C2) are found in only trace amounts. In addition, several of the regulatory components of the system exist on cell membrane, as the term therefore now embrace glycoproteins distributed among the plasma and cell membrane. Complement components are designated by numerals (C1-C9) by letter symbols (e.g., factor D), or by trivial names (e.g., homologous restriction factor). Designation C1, C2, C9 denote the order in which the components were discovered, rather than their position in the activation sequence.

Complement component can be classified into seven functional categories;

a) **Initiator complement components:** These proteins initiate their respective complement cascades by binding to particular soluble or membrane bound molecules. Once bound to their activating ligand, they undergo conformational alterations resulting in their biological activity. The C1q complex, mannose binding lectin (MBL) and the ficolins are examples of initiator complement components.
b) **Enzymatic mediators:** Several complement components, (e.g., C1r, C1s, MASP2, and factor B) are proteolytic enzymes that cleave and activate other members of complement cascade. Some of these proteases are activated by binding to other macromolecules and undergoing conformational change. Others are inactive until cleaved by another protease enzyme and are thus termed zymogens: proteins that are activated by proteolytic

cleavage. The complement that cleave C3 and C5 components are called as C3 convertase and C5 convertase.

c) **Membrane-binding components or Opsonins:** Upon activation of complement cascade, several proteins are cleaved into two fragments, each of which then takes on a particular role. For C3 and C4, the larger fragments, C3b or C4b, serve as opsonins, enhancing phagocytosis by binding to microbial cells and serving as a binding tags for phagocytic cells bearing receptors for C3b or C4b.

d) **Inflammatory mediators:** Some small complement fragments act as inflammatory mediators. Which enhance the blood supply to the area in which they are released, by binding to receptors on endothelial cells lining the small blood vessels and inducing an increase in capillary diameter. They also attract immune cells to the site of tissue damage because such effects can be harmful in excess, these fragments are called ac anaphylatoxin, meaning substances that cause anaphylaxis.

e) **Membrane attack proteins**: The proteins of the membrane attack complex (MAC) insert into the cell membranes of invading microorganisms and punch holes that result in the lysis of pathogens.

f) **Complement receptor proteins:** Receptor molecules on the cell surfaces bind complement proteins and signal specific cell functions. For example, some complement receptors such as CR1 bind to complement components such as C3b on the surface of pathogens, triggering phagocytosis of the C3-bound pathogen. Binding of the complement component C5a to C5aR receptors on neutrophils stimulates neutrophil degranulation and inflammation.

g) **Regulatory complement components:** Host cells are protected from unintended complement-mediated lysis by the presence of membrane-bond as well as soluble regulatory proteins. The regulatory protein includes factor I, which degrades C3b.

COMPLEMENT ACTIVATION

The biological activities triggered by complement activation enhance pathways that remove microbial pathogens and they also directly attack the pathogen itself. Because these attacks are so powerful, and however, may also damage the host. Thus, under normal conditions, complement activation is tightly regulated. Most complement components are present in the serum in functionally inactive forms as proenzymes or zymogens. Complement activation pathways involve the activation of one component that triggers the activation of the next component in the sequence. The complement can act through three different pathways called classical, lectin and alternative pathway, respectively. The activity of each respective pathway is triggered by different mechanisms/components.

Classical Pathways

The classical pathway was so named because it was the first complement pathway to be worked out. The classical pathway commonly begins with the formation of antigen-antibody complexes. Thus, this pathway is a major effector pathway of the humoral adaptive immune response. Other activators include some viruses, necrotic cells and subcellular membranes (e.g., from mitochondria), aggregated immunoglobulin's, and beta-amyloid, found in Alzheimer disease plaques.

The classical pathway is initiated when C1 binds to the antibody in an antigen-antibody complex. C1 is a complex of three different proteins: C1q (made up of six identical subunits) combined with two molecules each of C1r andC1s. IgM and certain subclasses of IgG (human IgG1, IgG2, and IgG3) are efficient complement-activating antibodies. Among the human immunoglobulins, the ability to bind and activate C1 is, in decreasing order, IgM> IgG3 > IgG1>> IgG2. IgG4, IgD, IgA, and IgE do not have C1q receptors; these antibodies do not bind or activate C1 and thus do not activate the classical complement pathway. C1q of C1 binds to the Fc region of antibodies. This activates C1r, a *serine protease* that initiates the

cascade of the classic pathway. First, C4 is proteolytically activated into C4b, which in turn cleaves C2 into C2a and C2b. C4b and C2a together form *C3 convertase.*

Lectin Pathway

Lectins are proteins that recognize and bind to specific carbohydrate target. The lectin pathway is activated by terminal mannose residues of glycoprotein or polysaccharides found on the surface of bacteria. This pathway does not depend on (antibodies) Ab for activation. It is activated by binding of mannose binding lectin (an acute phase protein) to mannose residues. These terminal mannose residues are not found on the surface of mammalian cell, and so the lectin pathway of complement activation may be responsible for discrimination between self and non-self. Because this pathway is activated in absence of antibody, it is a part of innate immune defense.

Alternative or Properdin Pathway

The alternate pathway is antibody-independent. This pathway of complement activation is triggered by almost any foreign substance. The most widely studied include lipopolysaccharides from the outer membrane of gram-negative bacteria, the cell walls of some yeast, and a protein present in cobra venom known as cobra venom factor. Some agents that activate the classical pathway-viruses, aggregated immunoglobulins, and necrotic cells also trigger the alternative pathway. Activation of the alternative pathway occurs in the absence of specific antibody. Thus the alternative pathway of the complement activation is an effector arm of the innate immune defenses. This major pathway of complement activation involves four serum proteins: C3, factor B, factor D, and properdin, also known as factor P.

In the alternate pathway, serum C3is subject to spontaneous hydrolysis to yield C3a and C3b. The C3b component can bind to the foreign surface antigens. The C3b present on the surface of the foreign cells can bind another serum protein called factor B to form a complex. Binding to C3b serves as the substrate for an enzymatically active serum protein called factor D. Factor D cleaves the C3b –bound factor B. releasing a small fragment (Bb) that diffuses away and generating C3 convertase. Properdin binds with C3 convertase and stabilize it.

Activation of C3 and C5

Table 8.1. Summary of the activators of the classical, lectin, and alternative complement activation pathways (Adapted from David Male, immunology 7th edition)

Summary of the activators of the classical, lectin, and alternative pathways

	immunoglobulins	microorganisms			other
		viruses	bacteria	other	
classical pathway	immune complexes containing IgM, IgG1, IgG2, or IgG3	HIV and other retroviruses, vesicular stomatitis virus		*Mycoplasma* spp.	polyanions, especially when bound to cations PO_4^{3-} (DNA, lipid A, cardiolipin) SO_4^{2-} (dextran sulfate, heparin, chondroitin sulfate)
lectin pathway		HIV and other retroviruses	many Gram-positive and Gram-negative organisms		arrays of terminal mannose groups
alternative pathway	immune complexes containing IgG, IgA, or IgE (less efficient than the classical pathway)	some virus-infected cells (e.g. by Epstein –Barr virus)	many Gram-positive and Gram-negative organisms	trypanosomes, *Leishmania* spp., many fungi	dextran sulfate, heterologous erythrocytes, complex carbohydrates (e.g. zymosan)

C3 convertase formation is a critical step in all three complement pathways. The C3 cleaves C3 into two fragments: the smaller, C3a and large fragment, C3b. C3b continues the complement activation cascade by binding to the cells surface around the site of complementation activation. C3a is a fluid-phase anaphylatoxin. Anaphylatoxin bind to receptors on mast cells and blood basophils and induce degranulation, with release of

histamine and other active mediators. Anaphylatoxins also induce smooth muscle contraction and increased vascular permeability. C3 binding to all three complement pathways allows the next component in the sequence, C5, to bind and be cleaved. For this reason, the C3 convertase with bound C3b are referred to as C5 convertase. For the classical and lectin pathways, the C5 convertase has the composition C4b2a3b; for the alternative pathway, the C5 convertase has the formulation C3bBbC3b. The cleavage of C5 produces two fragments; C5a is released into the fluid phase and acts as protein anaphylatoxin. C5b binds to the cell surface and forms the nucleus for the binding of terminal complement components.

The Terminal Pathway

The terminal components of the complement cascades are C5b, C6, C7, C8, and C9. These components bind to each other sequentially to form a macro molecular structure complex called the membrane attack complex (MAC) that results in cell lysis. The three complement pathways converge at the membrane attack complex. The complex forms a large channel through the membrane of the target cell, enabling ions and small molecules to diffuse freely across the membrane. The first step in membrane attack complex (MAC) formation is C6 binding to C5b on a cell surface. C7 then binds to C5b and C6 and inserts into the outer membrane of the cell. These subsequent binding of C8 to C5b67 results in the complex penetrating deeper into the cell's membrane. The final step in the formation of the membrane attack complex (MAC) is the binding and polymerization of C9, a perforin, like molecule, to the C5b678complex. C5b678 on the cell membrane acts as a receptor for C9 that binds to C8. Additional C9 molecules interact with the C9 molecules in the complex to form polymerized C9 (poly-C9). This poly-C9 forms a transmembrane channel that disturbs the cell's osmotic equilibrium; Ions pass through the channel, and water enters the cell. The cell swells, and the membrane becomes permeable to macromolecules, which then escape from the cell. The result is cell lysis.

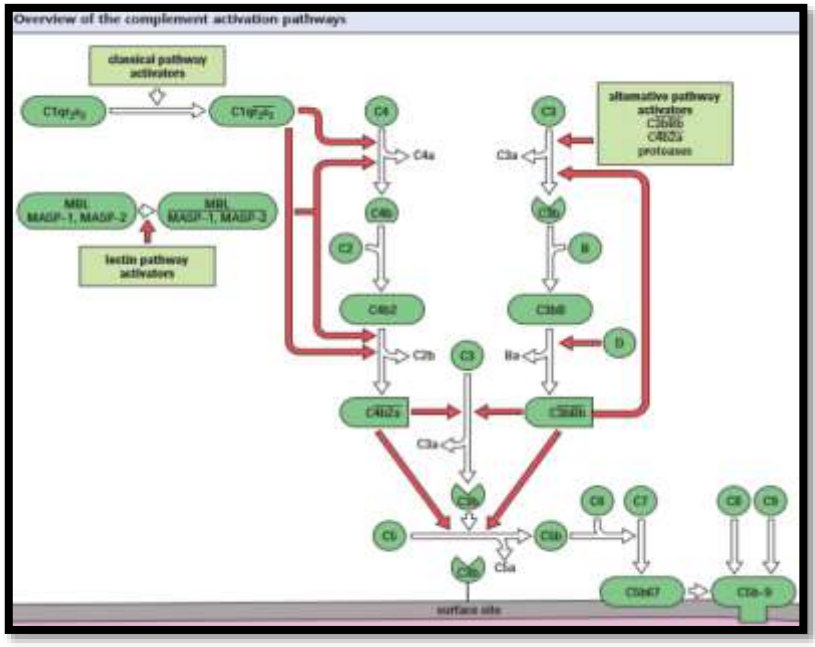

Figure 8. The proteins of the classical and alternative pathways are assigned numbers (e.g., C1, C2). Many of these are zymogens (i.e., pro-enzymes that require proteolytic cleavage to become active). The cleavage products of complement proteins are distinguished from parent molecules by suffix letters (e.g., C3a, C3b). The proteins of the alternative pathway are called 'factors' and are identified by single letters (e.g., ,factor B, which may be abbreviated to fB or just 'B'). Components are shown in green, conversion steps as white arrows, and activation/cleavage steps as red arrows. The classical pathway is activated by the cleavage of C1r and C1s following association of C1qr2s2 with classical pathway activators, including immune complexes. Activated C1s cleaves C4 and C2 to form the classical pathway C3 convertase C4b2a. Cleavage of C4 and C2 can also be effected via MASP-1 and MASP-2 of the lectin pathway, which are associated with mannan-binding lectin (MBL). The alternative pathway is activated by the cleavage of C3 to C3b, which associates with factor B and is cleaved by factor D to generate the alternative pathway C3 convertase C3bBb. The initial activation of C3 happens to some extent spontaneously, but this step can also be effected by classical or alternative pathway C3 convertases or a number of other serum or microbial proteases. Note that C3b generated in the alternative pathway can bind more factor B and generate a positive feedback loop to amplify activation on the surface. Note also that the activation pathways are functionally analogous, and the diagram emphasizes these similarities. For example, C3 and C4 are homologous, as are C2 and factor B. MASP-1 and MASP-2 are homologous to C1r and C1s, respectively. Either the classical or alternative pathway C3 convertases may associate with C3b bound on a cell surface to form C5 convertases, C4b2a3b or C3bBb3b, which split C5. The larger fragment C5b associates with C6 and C7, which can then bind to plasma membranes. The complex of C5b67 assembles C8 and a number of molecules of C9 to form a membrane attack complex (MAC), C5b–9 (Adapted from immunology David male, 7 ed.).

FUNCTIONS OF COMPLEMENT SYSTEM

The biological activities of complement system affect both innate and acquired immunity and reach for beyond the original observation of antibody-mediated lysis of bacteria and red blood cells.

The functions of the complement system include: the triggering and amplification of inflammatory reactions; attraction of phagocytes by chemotaxis; clearance of immune complexes; cellular activation; direct microbial killing; and an important role in the development of antibody responses.

Structural comparisons of the proteins involved in complement pathways place the origin of this system in primitive organisms processing the most rudimentary innate immune system. By contrast the realization that interaction of cellular receptors with complement proteins controls B-cell activities gives this system, a role in the highly developed acquired immune system. Thus, we have a system that straddles innate and acquired immunity, contributing to each in a variety of ways. Complement components interact with wide range of cells that express specific receptors. The receptors fall into two broad categories:

1) Receptors that binds to (complement) C3b or (complement) C4b and their breakdown products, and
2) Receptors for the anaphylatoxins (complement) C3a and (complement) C5a.

The receptors that bind (complement) C3b, (complement) C4b and their breakdown products are involved in response that enhances phagocytosis of pathogens. And the receptors and cells that bind the anaphylatoxins are involved in inflammation responses. Besides its role in antibody-induced lysis of microbes, complement also has important functions in innate immunity, many of which are mediated by the soluble innate immune receptors such as (mannan binding lectin) Bl and the Ficolins. Complement also plays an important role in the contraction phase of the adaptive immune responses, and recent work has even suggested that

it is important in the elimination of excess synapses during the development of the nervous system. The three main functions of complement are:

Production of Opsonin

The most important function of complement in host defense is considered to be the generation of fragments with opsonic activity that deposit on the surface of pathogens. (Complement) C3b and (complement) C4b are the major opsonins generated, but iC3b, a fragment of (complement) C3b that does not activate complement, also has opsonic activity. Bacteria coated by opsonins are rapidly taken up and destroyed by phagocytic cells such as macrophages and neutrophils. These cells express the receptors (complement receptor) CR1, (complement receptor) CR3, and (complement receptor) CR4, which have broad specificities for complement pathway-generated opsonins and for other complement components. Complement receptor of the immunoglobulin family (CRIg) is expressed on tissue resident macrophages, including those found in liver (Kupffer cells). It interacts with (complement) C3b and iC3b. (Complement receptor) CR1 is also a regulator of complement activation.

Production of Anaphylatoxin

The second major function associated with complement activation is the action of anaphylatoxins. (Complement) C5a is the most potent, followed by (complement) C3a; (complement) C4a is much less potent. The name “anaphylatoxin” derives from the earliest recognition of their function: the ability to induce the shock like characteristics of the systemic allergic or anaphylactic response. We now recognize that these small peptides play key roles in inducing inflammatory responses, which form part of the body’s defenses in removing an infectious agent that has penetrated the tissues. The anaphylatoxins interact with receptors

expressed on many different cell types. They activate vascular endothelial cells (lining the walls of blood vessels), increasing the vascular permeability and leading to local accumulation of fluid (edema) in the tissue. The influx into the tissue of fluid containing phagocytic cells (macrophages and neutrophils), antibodies, and complement components enhances the response to the pathogen. The anaphylatoxins are also chemotactic for neutrophils; that is, the cells migrate from an area of lesser concentration to an area of higher concentration. As a result, neutrophils circulating in the blood are activated, leave the circulation at the site of inflammation, and destroy the foreign material. The anaphylatoxins also induce smooth muscle contraction. Interaction of the anaphylatoxins with basophils or mast cells in tissues results in the release of many inflammatory mediators, including histamine. The effects of histamine (discussed in and the anaphylatoxins on vascular permeability and smooth muscle contraction are similar.

Lysis

The third major function of complement is the lysis of pathogens. The terminal step in the three complement activation pathways is the formation of a (membrane attack complex) MAC on the surface of a cell. This results in the lysis of the cell, particularly of microbial pathogens.

OTHER IMPORTANT COMPLEMENT FUNCTIONS

Complement and Antigen Presenting Cells

Dendritic cells (DCs), follicular dendritic cells (FDCs), and macrophages express all of the known complement receptors. When bound to antigens, (Mannan binding lectin) MBL, (complement) C1q, (complement) C3b and (complement) C4b are each capable of engaging their respective receptors on antigen presenting cells during the process of

antigen recognition, and signaling through their respective receptors acts to enhance antigen uptake.in addition, signals of antigen presenting cells through the (complement) C5Ar anaphylatoxin in receptor has been shown to modulate their migration and effect interleukin production, particularly that of the cytokine (interleukin) IL-12. Production of (interleukin) IL-12 by an antigen presenting cell normally skews the (thymus) T-cell response towards the (thymus helper) Th1 phenotype. Since both induction and suppression of (interleukin) IL-12 production have been detected after activation of the anaphylatoxin receptors, depending upon the root of the antigen, and maturation status of the antigen-presenting cell, we must infer that many signaling pathways are being integrated to arrive at the eventual biological response to antigen and complement components.

Enhancing B-Cell Responses to Antigens

The binding of complement component C3d or of the final breakdown product of (complement) C3, (complement) C3dg, to (complement receptor) CR2 (CD21) enhances antibody responses in several ways. First, (complement) C3dg binds to antigen that is also bound to B-cell surface (immunoglobulin) Ig. (Complement) C3dg can bind simultaneously to (complement receptor) CR2, which is part of the B-cell co-receptor. Signaling through both the surface (immunoglobulin)Ig and the co-receptor augments activation of the B cell. Thus, (complement) C3dg binding to antigen and the B-cell surface lowers the threshold for B-cell activation by as much as 1000-fold compared to binding in the absence of (complement) C3dg. Second, the follicular dendritic cells in the germinal centre bind antigen–antibody complexes and present antigen to proliferating B cells. This interaction is critical for the eventual development of memory cells. Follicular dendritic cells express the (complement receptors) CR2, which binds C3dg, and (complement receptor) CR1, which binds iC3b. Thus, follicular dendritic cells can present antigen–antibody complexes bound to one of these complement components to germinal centre B cells. In this way, complement components also play a role in the induction of B-cell

memory. In addition, B-cell processing of T-dependent antigens is more rapid when the antigen is bound to C3dg than when it is not; presumably the binding of (complement) C3dg to (complement receptor) CR2 on the B-cell surface enhances uptake and processing of the antigen. This may be another way in which complement enhances B-cell responses to T-cell-dependent antigens.

Complement and (Thymus) T Cell-Mediated Immunity

The mechanism by which complement affects (thymus) T-cell responses are not as well characterized as those for (bone marrow) B-cells and antigen-presenting cells. However, some recent, interesting results have been generated by the study of mice either genetically deficient in one or more complement components or following treatment with complement inhibitors.

Controlling Formation and Clearance of Immune Complexes

When antibodies bind to multivalent antigens, cross-linking between the molecules tends to produce large antigen–antibody complexes that increase in size until they become insoluble. Although this precipitation of complexes has proved useful for identifying antigens and antibodies in vitro, the formation of large insoluble complexes in vivo can be detrimental to the host. The individuals deficient in early components of the classical pathway components and in some autoimmune conditions such as systemic lupus erythematosus (SLE) may show large insoluble immune complexes in tissues such as the skin and kidneys, inducing inflammation and damaging surrounding cells. Complement plays a role in clearing these complexes. Deposition of C3b on a large antigen– antibody complex interferes with the bonds that keep the complex together. As a result, it breaks up into smaller pieces that can be cleared by macrophages. Deposition of C3b on the antigen–antibody complex also allows binding to

erythrocytes, which express the (complement receptor) CR1 on their surface. Erythrocytes clear the complexes from the circulation by transporting them to the liver and spleen. In these organs, the complexes are transferred from the erythrocyte CR1 to macrophage CR3 and Fc receptors. Macrophages phagocytose the complexes and destroy them.

Removing Dead or Dying Cells

Cells dying by necrosis can activate complement, leading to C4b and C3b deposition on the cell surface. The cell is then cleared by interacting with CR1 or (complement receptor) CR3 on phagocytic cells. Subcellular membranes, from organelles such as mitochondria and endoplasmic reticulum, also directly activate both classical and alternative pathways and are cleared in a similar way. (Complement receptor protein) CRP, the acute-phase protein and component of the inflammatory response, also binds to damaged and necrotic cells and activates the classical complement pathway. The same structure that (Complement receptor protein) CRP binds to on bacterial cell walls the polysaccharide phosphocholine is also exposed on damaged and necrotic mammalian cells. Recent evidence also indicates that cells dying as a result of apoptosis may trigger complement activation. In all these situations, complement removes dead or dying cells from the tissues and contributes to homeostasis. In some conditions, however, complement activation by dead or dying cells may have clinical consequences. Notable examples include complement activation by ischemia and reperfusion. In ischemia, an area of tissue dies after blood and oxygen supplies have been cut (important examples include cardiac muscle after a myocardial infarction or brain tissue following a stroke). Reperfusion is the attempt to restore blood supply to the affected tissue. Complement activation is considered a major contributor to the inflammatory responses associated with both of these states, which damages healthy tissue. Complement-based therapies are currently being tested to reduce the deleterious effects of the inflammatory response.

Responses to Viruses

Complement plays a role in defense against viral infection. C1 can bind directly to and become activated by the surface of several viruses, including the type C retroviruses, lentiviruses, HIV-1, and HTLV-1. In addition, MBL of the lectin pathway binds to and is activated by mannose residues on the surfaces of HIV-1, HIV-2, and influenza virus. Antibodies generated in the response against these viruses mediate further binding and activation of the classical pathway on the surface of the virus. Repeating subunits on the viral capsid or membrane surfaces activate the alternative pathway. Binding of complement proteins leads to opsonization and lysis of the virus by phagocytic cells. Complement binding also interferes with the ability of the virus to interact with the membrane of its target cells and thus blocks viral entry into the cell, viruses subvert the immune response by synthesizing proteins that bind to molecules in antigen-processing pathways. Many viruses also use mechanisms that subvert the action of complement proteins. For example, some viruses produce proteins that mimic complement inhibitor function: the herpes viruses make proteins that have (complement decay-accelerating factor) DAF-like and/or MCP-like activities and others that block C5b-9 formation. In addition, vaccinia virus produces a protein that binds to C3b and C4b and inhibits complement activation. The protein has both decay accelerating activity and acts as a cofactor for factor I (human immune deficiency virus) HIV-1, (human thymus-cell lymphotropic virus) HTLV-1, simian immunodeficiency virus (SIV), and cytomegalovirus (CMV) capture the complement control proteins (complement decay-accelerating factor) DAF, (membrane cofactor protein) MCP, and CD59 when the virions bud from host cell membranes. As a consequence of these strategies, the viruses are protected from complement-mediated responses. Some viruses even use complement components to promote infection, for example, by binding to complement receptors and gaining entry into cells. One of the most studied interactions is the Epstein–Barr virus infection of human B lymphocytes: The virus's membrane glycoprotein, gp350/220, binds to (complement receptor) CR2 (CD21) expressed on the B-cell surface, allowing the virus

to be taken into the cell. Some viruses activate complement and use the C3b deposited on them to bind to host cell complement receptors; in this way, HIV-1 uses (complement receptor) CR1, (complement receptor) CR2, and (complement receptor) CR3 to infect T cells, B cells, and monocytes. Other viruses bind to membrane-expressed complement regulators: Paramyxovirus (measles virus) uses (membrane cofactor protein) MCP and viruses of the picornavirus family use (complement decay-accelerating factor) DAF to infect epithelial cells.

REGULATION OF COMPLEMENT SYSTEM

The Uncontrolled complement activation can result in depleting complement components, leaving the host unable to defend against subsequent invasion by infectious agents. In addition, the fragments generated by complement activation (especially the cleavage products of C3, C4, and C5) induce potent inflammatory responses, which may damage the host. Indeed, complement activation is believed to play an adverse role in auto inflammatory conditions such as rheumatoid arthritis and in myocardial infarctions (heart attacks) in which complement is activated by necrotic tissue. In addition, dysregulation of complement function in the eye has been suggested as playing a major role in age-related macular degeneration, the leading cause of visual impairment and blindness in the USA among individuals over 60 years of age. Normally, inappropriate activation of complement does not occur, because many steps in the complement pathways are negatively regulated by specific inhibitors. Some of these negative regulators are specific for one complement activation pathway, but many inhibit all the pathways. The importance of these complement regulators is underscored by the clinical conditions that arise when regulatory molecules are lacking: The individual may be either damaged by inflammatory responses or become susceptible to infectious diseases. Many of the molecules that regulate complement activation are expressed on the surface of mammalian cells but not

microbial cells. Consequently, damage to the host by complement activation is generally limited compared to damage to the pathogen.

Table 8.2. Proteins that regulate the complement system

Classical pathway component deficiencies

Protein	Type of protein	Pathway affected	Immunologic function
C1 inhibitor (C1Inh)	Soluble	Classical	Serine protease inhibitor: causes $C1r_2s_2$ to dissociate from C1q
C4b-binding protein (C4bBP)*	Soluble	Classical and lectin	Blocks formation of C3 convertase by binding C4b; cofactor for cleavage of C4b by factor I
Factor H*	Soluble	Alternative	Blocks formation of C3 convertase by binding C3b; cofactor for cleavage of C3b by factor I
Complement-receptor type 1 (CR1)* Membrane-cofactor protein (MCP)*	Membrane bound	Classical, alternative, and lectin	Block formation of C3 convertase by binding C4b or C3b; cofactor for factor I-catalyzed cleavage of C4b or C3b C3bBb
Decay-accelerating factor (DAE or CD55)*	Membrane bound	Classical, alternative, and lectin	Accelerates dissociation of $C4\overline{b2a}$ and $C3\overline{bBb}$ (classical and alternative C3 convertases)
Factor-I	Soluble	Classical, alternative, and lectin	Serine protease: cleaves C4b or C3b using C4bBP, CR1, factor H, DAE, or MCP as cofactor
S protein	Soluble	Terminal	Binds soluble C5b67 and prevents its insertion into cell membrane
Homologous restriction factor (HRF) Membrane inhibitor of reactive lysis (MIRL or CD59)*	Membrane bound	Terminal	Bind to C5b678 on autologous cells, blocking binding of C9
Anaphylatoxin inactivator	Soluble	Effector	Inactivates anaphylatoxin activity of C3a, C4a, and C5a by carboxypeptidase N removal of C-terminal Arg

*An RCA (regulator of complement activation) protein. In humans, all RCA proteins are encoded on chromosome 1 and contain short consensus repeats.

Adapted from Kuby, immunology 6th ed.

The major regulators of complement activation are:

- **Complement activity is passively regulated by protein stability and cell-surface composition:** Protection of vertebrate host cells against complement mediated damage is achieved by both general, passive and specific, active regulatory mechanisms. The relative instability of many complement components is the first means by which the host protects itself against inadvertent complement activation. For example, the C3 convertase of the alternative

pathways, C3bBbC3b, has a half-life of only 5 minutes before it breaks down, unless it is stabilized by reaction with properdin. A second passive regulatory mechanism depends upon the difference in the cell surface carbohydrate composition of host versus microbial cells. For example, fluid phase protease that destroy C3b bind much more effectively to host cells, which bear high levels of sialic acid, than to microbes that have significantly lower levels of this sugar. Hence, any C3b that happens to alight on a host cell is likely to be destroyed before it can effect significant damage. In addition to these more passive environmental brakes on inappropriate complement activation, a series of active regulatory proteins act to inhibit, destroy, or tune down the activity of complement proteins and their active fragments.

- **Factor I:** The serum protein, cleaves C4b on the cell surface after C2a. Because factor I cleavage of C4b requires the presence of one or more of C4BP, MCP, and CR1, these molecules are referred to as cofactors for Factor-I-mediated cleavage. Note that DAF dissociates the C4b2a complex but does not act as a cofactor for factor I. Factor I cleaves C4b into two fragments: C4c, released into the fluid phase, and C4d, which remains attached to the cell surface. C4c and C4d do not continue the complement cascade and have no known biologic activity. C4BP regulates the classical (and lectin) pathways, which use C4bC2a as the C3 convertase. CR1, MCP, and DAF regulate the classical, lectin, and alternative pathways.
- **C1 esterase inhibitor (C1INH):** C1INH, the C1 inhibitor, is a plasma protein that binds in the active site of proteins called serine protease inhibitors (serpins), and it acts by forming a complex with the C1 protease, C1r2s2, causing them to dissociate from C1q and preventing further activation of C4 or C2. C1INH inhibits both C3b and serine protease MASP2, it is the only plasma protease capable of inhibiting the initiation of both the classical and lectin complement pathways.

- **Factor H:** a serum protein, has two important regulatory functions in the alternative pathway. First, factor H competes with the previously described factor B for binding to C3b on a cell surface. Factor B binding to C3b continues the alternative pathway, but if factor H binds to C3b, the pathway stops. The nature of the surface to which the C3b is bound is important in determining which factor binds to C3b: The sialic acid coating of mammalian cells favours the binding of factor H, but bacterial cells lack sialic acid, so they favour the binding of factor B to C3b. As a result, mammalian cells are protected by the regulatory function of factor H, but bacterial cells are targeted for further activation of the complement pathway.

DEFICIENCIES OF COMPLIMENT COMPONENTS

Mutations in complement proteins are rare events that can be catastrophic and cause partial or total deficiency of the protein, or more subtle, altering the expression level or functionally efficiency of the protein. Polymorphisms are common variations in protein compositions that may or may not have functional consequences. The most dramatic outcome of a mutation of a complement protein is deficiency, which may involve a loss of protein product (Type I deficiency) or the production of the functionally inactive protein (Type II deficiency).

Deficiencies of CP component proteins (C1, C4, C2) remove the capacity for efficient activation of complement on ICs and are strongly associated with lupus- like IC disease and infections. And these deficiencies in any of the early components of the classical pathway like (C1q, C1r, C1s, C4, C2) result in increase in immune-complex diseases such as SLE, glomerulonephritis, and vasculitis.

Lectin Pathway Component Deficiencies

A deficiency in mannon binding lectin (MBL), the first component of the lectin pathway, has been shown to be relatively common, and results in serious pyrogenic (fever-inducing) infections in babies and children. Children with MBL deficiency suffer from respiratory tract infections. MBL deficiency is also found with a frequency two to three times higher in systemic lupus erythematosus (SLE) patients than in normal subjects, and certain mutant forms of MBP are found to be prevalent in chronic carriers of hepatitis B.

Alternative Pathway Component Deficiencies

Deficiency of the alternative pathway component properdin, or of factors B or D, is associated with pyogenic, particularly Neisseria, infections. Deficiency of MBL can be a major problem in early life, manifesting as severe recurrent infections. Individuals who lack components of the MAC, C5b-C9, tend to get recurrent Neisseria infections.

Deficiencies of the Terminal Pathway

Deficiencies of the terminal pathway predispose to both autoimmune disease and infections (particularly *Neisseria meningitidis*, due to the role that the membrane attack complex ("MAC") plays in attacking Gram-negative bacteria. Infections with *N. meningitidis* and *N. gonorrhoeae* are the only conditions known to be associated with deficiencies in the MAC components of complement.40-50% of those with MAC deficiencies experience recurrent infections with *N. meningitidis*.

Deficiencies of Complement Receptors or Complement Regulatory Proteins

Deficiencies or disorders of complement receptors or complement regulatory proteins may also have serious consequences. Patients with a defect in CR3 (CD11a/CD18) may have a disorder known as leukocyte adhesion deficiency I in which adhesion and migration of all leukocytes is impaired. These patients suffer from recurrent pyogenic bacterial infections, but pus is not formed. The regulatory protein C1INH is the only control protein for classical pathway components C1r and C1s, and deficiency results in uncontrolled cleavage of C2 and C4. Genetic deficiency of C1INH results in hereditary angioedema. The condition is characterized by localized edemas in the skin and mucosa resulting from dilatation and increased permeability of the capillaries. The symptoms are recurrent attacks of swelling, such as of the face and limbs, pain in the abdomen, and swelling of the larynx, which can compromise breathing. This condition is thought to be due to the lack of inhibition by C1INH of enzymatic activity in serum cascades other than the complement cascade; one of these pathways forms kinins, including bradykinin, which are potent vasodilators and inducers of vascular permeability and smooth muscle contraction. Deficiency of C1INH is thought to lead to increased production of these vascular mediators. In the USA, injectable C1INH prepared from human plasma has been approved as a treatment for this condition.

Deficiency of Factor H or Factor I

Deficiency of factor H or factor I results in uncontrolled activation of the alternative pathway. One outcome is membrane proliferative glomerulonephritis, inflammation of the capillary loops in the glomeruli of the kidney, characterized by increased cell number and thickening of capillary walls. Factor H deficiency is also associated with atypical hemolytic-uremic syndrome (10% of all hemolytic-uremic syndrome

cases). Usually resulting from bacterial infection, it is characterized by destruction of red blood cells, damage to endothelial cells, and in severe cases kidney failure. A monoclonal antibody that blocks C5 cleavage is being evaluated to treat this syndrome.

CONCLUSION

The Complement system is an integral part of immune response which acts as a bridge between innate and acquired immunity. It consists of a series of proteins that are mostly (although not exclusive) synthesized in the liver and exists in the plasma and on the cell surfaces as in active precursors (zymogens).

Complement mediates responses to inflammatory triggers through a coordinated sequential enzyme cascade leading to clearance of foreign cells through pathogen recognition, opsonization and lysis. Complement also possesses anti-inflammatory functions: it binds to immune complexes and apoptotic cells and assists in their removal from the circulation and damaged tissue. The complement proteins are activated by, and work with immunoglobulin G (IgG) immunoglobulin M (IgM) antibodies, hence the name “complement.” Many complement proteins exist in a precursor form and are activated at the site of inflammation. The complement system is more complex than many enzymatic cascade as it requires the formation of sequential non-covalently associated activated protein fragments. These in turn become convertase and cleave components for the next enzymatic complexes in the cascade, and the rapid dissociation of these complexes (and loss of enzymatic activity) forms an integral part of the elegant regulation of complement activity.

Complement proteins include initiator molecule, enzymatic mediators, membrane-bonding components or opsonins, inflammatory mediators, membrane attack proteins, complement receptor proteins, and regulatory components. Complementation activation occurs by three pathways---a) Classic pathway, b) Lectin pathway, and c) Alternative pathway, which converge in a common sequence of events leading to membrane lysis.

Deficiencies of complement components, regulators of complement pathways, or receptors for complement components may result in increased susceptibility to infection or the development of inflammatory conditions.

FREQUENTLY ASKED QUESTIONS (FAQS)

Q1. What is the role of complement system in immunity?

Answer: The complement system plays a major role in defense against many infectious organisms as part of both the innate and antibody-mediated adaptive immune responses. Complement is a system of plasma proteins that can be activated directly by pathogens or indirectly by pathogen-bound antibody, leading to a cascade of reactions that occurs on the surface of pathogens and generates active components with various effector functions.

Q2. What are the important functions of complement?

Answer: The major functions of the complement system include lysis of cells, bacteria and viruses, opsonization; which promotes the phagocytosis of particulate antigens, binding to specific complement receptors on cells of the immune system triggering specific cell functions, inflammations and secretion of immunoregulatory molecules, immune clearance, which removes immune complexes from the circulation and deposits them in the spleen and liver.

Q3. How is membrane attack complex formed?

Answer: Membrane attack complex: An abnormal activation of the complement (protein) portion of the blood that forms a cascade reaction and brings blood proteins together, binds them to the cell wall, and then inserts them through the cell membrane. Abbreviated MAC. MAC allows water, ions, and other small molecules to move freely into and out of a cell, and it quickly results in cell death.

Q4. What are the functions of inflammatory mediators?

Answer: Some small complement fragments act as inflammatory mediators. Which enhance the blood supply to the area in which they are released, by binding to receptors on endothelial cells lining the small blood vessels and inducing an increase in capillary diameter. They also attract other cells to the site of tissue damage because such effects can be harmful in excess, these fragments are called ac anaphylatoxin, meaning substances that cause anaphylaxis.

Q5. What is the role of C5a?

Answer: C5a is an anaphylatoxin, which induces degranulation of mast cells, resulting in the release of histamine, causing vasodilation and contraction of smooth muscles. C5a is also chemotactic, attracting leukocytes to the area of its release where an antigen is reacting with antibodies and activates the complement system; this is a part of the inflammatory response to an infection.

Q6. What is complement decay-accelerating factor (DAF)?

Answer: DAF is a cell surface regulator of complement activation that destabilizes the C3 convertases of the alternate and classical pathways (C3bBb and C4b2a, respectively). Like other regulators of complement activation—including CR1, factor H, and C4bBP— these proteins accelerate decay (dissociation) of the C3 convertase, releasing the component with enzymatic activity (Bb or C2a) from the component bound to the cell membrane (C3b or C4b).

Q7. What are the main causes responsible for the development of systemic lupus erythematosus (SLE)?

Answer: Inherited homozygous deficiency of the early proteins of the classical complement pathway (C1, C4, or C2) are strongly associated with the development of systemic lupus erythematosus (SLE). Such deficiencies result in abnormal processing of immune complexes in the absence of a functional classical pathway of complement fixation. Serum levels of C3

or C4 decrease in SLE due to the large number of immune complexes that bind to them.

Q8. What is the role of properdin?

Answer: Properdin is essential for the activation through the alternative pathway, since it stabilizes the complex (C3bBb) formed between C3b and activated serum factor B, which acts as a C3 convertase and activates C During the activation of the alternative pathway both C3a and C5a are generated; both are anaphylatoxins and cause degranulation of mast cells.

Q9. Explain the classical pathway deficiency?

Answer: Deficiencies of component proteins (C1, C4, C2) of classical pathway remove the capacity for efficient activation of complement on ICs and are strongly associated with lupus- like IC disease and infections. And these deficiencies in any of the early components of the classical pathway like (C1q, C1r, C1s, C4, C2) result in increase in immune-complex diseases such as SLE, glomerulonephritis, and vasculitis.

Q10. What do you mean by anaphylatoxin?

Answer: Anaphylatoxin is the ability to induce the shock like characteristics of the systemic allergic or anaphylactic response. The anaphylatoxins interact with receptors expressed on many different cell types. They activate vascular endothelial cells (lining the walls of blood vessels), increasing the vascular permeability and leading to local accumulation of fluid (edema) in the tissue.

Q11. How a pathogen can protect it from pathogen attack?

Answer: Binding of host complement regulators on their membrane allows pathogens to inactivate complement. Bacteria, viruses, fungi, and parasites have been shown to bind high levels of efficient complement regulators such as FH, factor H-like 1 (CFHL-1), and C4 binding protein (C4BP). Recruitment of these regulators can accelerate the decay of the C3

convertase and provide cofactors for FI, which cleaves C4b and C3b, thus protecting the pathogen against complement attack.

Q12. What is the role of C5convertase in complement system?

Answer: C5 convertase, generated by the alternative, classical, or lectin pathway, initiates the activation of late components of the complement system to form membrane attack complex (MAC) and ultimately kills the pathogen. This occurs through three pathways; Classical pathways, activated by antigen-antibody reaction, Alternative pathways, activated on microbial cell surfaces, and Mannose binding lectin pathways, activated by a plasma lectin that binds to mannose residues on microbes.

Q13. What is the role of C1INH inhibitor in complement system?

Answer: C1 esterase inhibitor (C1INH) is a plasma protein that binds in the active site of proteins called serine protease inhibitors (serpins), and it acts by forming a complex with the C1 protease, C1r2s2, causing them to dissociate from C1q and preventing further activation of C4 or C2. C1INH inhibits both C3b and serine protease MASP2, it is the only plasma protease capable of inhibiting the initiation of both the classical and lectin complement pathways.

Q14. How does the deficiency of factor H affect the body?

Answer: Deficiency of factor H or factor I results in uncontrolled activation of the alternative pathway. One outcome is membrane proliferative glomerulonephritis, inflammation of the capillary loops in the glomeruli of the kidney, characterized by increased cell number and thickening of capillary walls. Factor H deficiency is also associated with a typical hemolytic-uremic syndrome which results in destruction of red blood cells, damage to endothelial cells, and in severe cases kidney failure.

Q15. What is complementary deficiency. Illustrate with an examples?

Answer: Complementary deficiency is an immunodeficiency of absent or suboptimal functioning of one of the complement system proteins.

Deficiency of C2 and C4 can cause systemic lupus erythematosus; deficiency of C3 and factor D can cause pyogenic bacterial infection; and deficiency of C5-C9 (or MAC deficiency) may lead to the Neisserial infections like, gonorrhea and meningitis.

Q16. What do you understand by immune clearance?

Answer: The complement system removes immune complexes from the circulation and deposits them in the spleen and liver. Thus it acts as anti-inflammatory function. Complement proteins promote the solubilization of these complexes and their clearance by phagocytes.

MULTIPLE CHOICE QUESTIONS (MCQS)

Q1. Complement component C3 is cleaved by:

A. C3b
B. C3bBb
C. Factor B
D. Factor D

Q2. The membrane attack complex in the complement pathway consists of:

A. C3b3b, Bb
B. C5b,6,7,8,9
C. Colicins
D. Properdin

Q3. Of the following complement components, which one is the most important opsonin:

A. C1
B. C3a
C. C3b
D. C5b

Q4. Complement component C3b:

A. Directly injures bacteria.
B. Is an anaphylatoxin.
C. Is the inactive form of C3.
D. Opsonizes bacteria

Q5. A complement component which is strongly chemotactic for neutrophils is:

A. C3
B. C3b
C. C5a
D. C5b

Q6. Several of the complement components are:

A. Antibodies
B. Cytokines
C. Enzymes
D. Glycolipids

Q7. The classical and alternative pathways meet at complement component:

A. C3
B. C4
C. C4b
D. C5
E. Factor D

Q8. One principal function of complement is to:

A. Bind antibodies attached to cell surfaces and to lyse these cells
B. Inactivate perforins
C. Mediate the release of histamine
D. Phagocytize antigens

Q9. The major role of the complement system is to work in conjunction with

A. Antibodies to lyse cells via the C8 and C9 components
B. Antibodies to lyse cells via the perforin molecules
C. Antibodies to opsonize cells
D. The major histocompatibility complex for cell recognition

Q10. An antigen—antibody immune complex in a C3-deficient individual will still result in

A. Anaphylatoxin production.
B. Depression of factor b.
C. Production of chemotactic factors.
D. Activation of c

Q11. Which of the following is associated with the development of systemic lupus erythematosus (sle)?

A. Deficiencies in C1, C4, or C2
B. Deficiencies in C5, C6, or C7
C. Deficiencies in the late components of complement
D. Increases in the serum C3 level

Q12. Which component(s) of complement could be missing and still leave the remainder of the complement system capable of activation by the alternative pathway?

A. C1, C2, and C3
B. C3 only
C. C2, C3, and C4
D. C1, C2, and C4
C1, C3, and C4

Q13. Active fragments of C5 can lead to the following, except

A. Contraction of smooth muscle.
B. Vasodilation.
C. Attraction of leukocytes.

D. Attachment of lymphocytes to macrophages.

Q14. Decay-accelerating factor (DAF) regulates the complement system to prevent complement-mediated lysis of cells. This involves

A. Dissociation of C4b2a or the C3bBb enzyme complex.
B. Blocking the binding of C3 convertase to the surface of bacterial cells.
C. Inhibiting the membrane attack complex from binding to bacterial membranes.
D. Acting as a cofactor for the cleavage of C3b.

Q15. Of the following, which one is the most important function of the complex formed by complement components C5b, 6, 7, 8, 9?

A. To inhibit immune complex formation
B. To opsonize virus
C. To perforate bacterial cell membrane
D. To release histamine from mast cells

Answer Key

1. B **2**. B **3**. C **4**. D **5**. C **6**. C **7**. A **8**. A **9**. A **10**. D **11**. A **12**. D **13**. D **14**. A **15**. C

ASSIGNMENTS

Long Answer Question (500 words)

Q1. What is complement system? Write down the functions of complement system?

Q2. How does complement activation takes place?

Q3. Identify complement regulatory proteins and their effect on specific complement components?

Short Answer Questions (200 Words)

Q1. Describe the consequence of complement deficiency.
Q2. Describe the three pathways for complement system?

REFERENCES

Abbas AK, Lichtman AH, Cellular and Molecular Immunology (6th ed.).

Ammitzboll, Christian Gytz; Kjær, Troels Rønn; Steffensen, Rudi; Stengaard-Pedersen, Kristian; Nielsen, Hans Jørgen; Thiel, Steffen; Bøgsted, Martin; Jensenius, Jens Christian (2012). "Non-Synonymous Polymorphisms in the FCN1 Gene Determine Ligand-Binding Ability and Serum Levels of M-Ficolin." *PLoS ONE*. 7(11): e50585. Bibcode: 2012PLoSO...750585A. doi: 10.1371/journal.pone.0050585. PMC 3509001. PMID 23209787.

Anderson DM (2003) *Dorland's Illustrated Medical Dictionary* (30th ed.), Philadelphia: W.B. Saunders, ISBN 0-7216-0146-4.

Arumugam, Thiruma V; Shiels, Ian A; Woodruff, Trent M; Granger, D Neil; Taylor, Stephen M (2004). "The Role of the Complement System in Ischemia-Reperfusion Injury." Shock. 21 (5):401–9. doi:10.1097/00024382-200405000-00002. PMID 15087815.

Atkinson JP (2009). "Complement system," In Firestein GS, Budd RC, Harris ED Jr, McInnes IB, Ruddy S, Sergent JS, eds. (2009). *Kelley's Textbook of Rheumatology*, pp. 323–336, Philadelphia, PA: Saunders/Elsevier, ISBN 978-1-4160-3285-4.

Chaplin H (2005). "Review: the burgeoning history of the complement system 1888–2005."

De Franco AL, Locksley RM, Robertson M (2007). *Immunity: The Immune Response in Infectious and Inflammatory Disease*. London; Sunderland, MA: New Science Press; Sinauer Associates, ISBN 978-0-9539181-0-2.

Doan T, Melvold R, Viselli S, Waltenbaugh C (2007). *Lippincott's Illustrated Reviews: Immunology*, 320p. Lippincott Williams & Wilkins.

Frank K, Atkinson JP (2001) Complement system. In Austen KF, Frank K, Atkinson JP, Cantor H. (ed.) Samter's Immunologic Diseases (6th ed.) Vol. 1, p. 281–298, Philadelphia: Lippincott Williams & Wilkins, ISBN 0-7817-2120-2.

Goldman AS, Prabhakar BS (1996). "The Complement System." In Baron S, et al. (eds.). *Baron's Medical Microbiology* (4th ed.). Univ of Texas Medical Branch. ISBN 978-0-9631172-1-2.

Janeway CA Jr., Travers P, Walport M, Shlomchik MJ. (2001). "The complement system and innate immunity." *Immunobiology* (5th ed.). Garland Publishing. ISBN 978-0-8153-3642-6.

Janeway, CA Jr., Travers P, Walport M. et al. (2001). "The complement system and innate immunity." *Immunobiology: The Immune System in Health and Disease*.

Murphy, Kenneth; Weaver, Casey (2017). "Innate Immunity: the First Lines of Defense." *Janeway's Immunobiology* (9th ed.). Garland Science. p. 49. ISBN 978-0-8153-4505-3.

Naesens, M, Li, L, Ying, L, Sansanwal, P, Sigdel, TK Hsieh, SC, Kambham, N, Lerut, E, Salvatierra, O, Butte, AJ, Sarwal, MM. (2009). "Expression of Complement Components Differs Between Kidney Allografts from Living and Deceased Donors." *Journal of the American Society of Nephrology*. 20 (8): 1839–51. doi: 10.1681/ASN.2008111145. PMC 2723986. PMID 19443638.

Nesargikar PN, Spiller B, Chavez R. *"The complement system: history pathways, cascade and inhibitors."*

Parham P, (2005). *The Immune System.* New York: Garland, ISBN 0-8153-4093-1.

Paul WE, (ed.) (1999). *Fundamental Immunology* (4th ed.), 1589p. Philadelphia: Lippincott-Raven, ISBN 0-7817-1412-5.

Peakman M, Vergani D. (1997). *Basic and Clinical Immunology*. New York: Churchill Livingstone, ISBN 0-443-04672-7.

Pranav Kumar, Usha Mina; *life sciences fundamental and practice-1* (fifth edition).

Richard Coico, Geoffery Sunshine; *immunology short course* (seventh edition).

Sacks, Steven H, Chowdhury, Paramit, Zhou, Wuding. (2003). "Role of the complement system in rejection." *Current Opinion in Immunology.* 15 (5): 487–92. doi:10.1016/S0952-7915(03)00100-6. PMID 14499254.

Sims PJ, Wiedmer T. (2000). "Complement biology," In Hoffman R, Benz EJ, Shattil SJ, Furie B, Cohen HJ, Silberstein LE, Mc Glave P, (eds.) (2000). *Hematology: Basic Principles and Practice,* 3rd ed.pp. 651–667, New York; Edinburgh: Churchill-Livingstone, ISBN 0-443-07954-4.

Stoermer, Kristina A., Morrison, Thomas E. (15 March 2011). "Complement and Viral Pathogenesis." *Virology.* 411 (2):362–373 Central. doi:10.1016/j.virol.2010.12.045. PMC 3073741. PMID 21292294.

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Chapter 9

VACCINES AND THEIR PROPERTIES

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ABSTRACT

Vaccines represent one of the greatest triumphs of modern medicine. Despite the common origins of vaccinology and immunology more than 200 years ago, the two disciplines have evolved along such different trajectories that most of the highly successful vaccines have been made empirically, with little or no immunological insight. Vaccine can be defined as a preparation of bacterial, viral or other pathogenic agents or of their isolated antigens which is administered with the objective of stimulating a recipient's protective immunity. There are several basic types of vaccines that are currently and conventionally used. These are

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divided into 3 main generations namely: first generation vaccines, second generation vaccines and third generation vaccines. An effective vaccine could have an immense impact on the control of the tragic spread of death and disaster. In addition to the challenges presented by diseases for which no vaccines exist, there remains the need to improve the safety and efficacy of present vaccines and to find ways to lower their cost and deliver them efficiently to all who need them.

Keywords: vaccination, attenuated, inactivated, subunit, toxoid, conjugate, DNA vaccines

OBJECTIVES

- Understand the concept of vaccines
- To learn about the historical background of Vaccines
- To study the types of vaccines, describe the working of vaccines and their properties
- To study the few examples of vaccines
- To be able to understand the latest advancements in vaccines

INTRODUCTION

An individual may be exposed to an antigen to induce formation of antibodies, a type of immunity known as "artificial active immunity". The material used to induce artificial active immunity, the antigen or a mixture of antigens, is known as a vaccine (or an immunogen), and the process of generating such an immune response is immunization**.** Immunization is commonly known as vaccination.

Vaccine can be defined as a preparation of bacterial, viral or other pathogenic agents or of their isolated antigens which is administered with the objective of stimulating a recipient's protective immunity. The first vaccine was named after vaccinia, the cowpox virus. It can be defined as a nontoxic or non-virulent preparation of antigenic material that can be used

to induce long term humoral as well as cell mediated immune response against pathogens. Its use was pioneered 200 years ago by Edward Jenner.

UNDERLYING CONCEPT BEHIND VACCINES

In any immune response, the antigen(s) induces clonal expansion in specific T and/or B cells, leaving behind a population of memory cells. These enable the next encounter with the same antigen(s) to induce a secondary response, which is more rapid and effective than the normal primary response. While for many infections the primary response may be too slow to prevent serious disease, if the individual has been exposed to antigens from the organism in a vaccine before encountering the pathogenic organism, the expanded population of memory cells and raised levels of specific antibody are able to protect against disease.

After primary exposure of antigen to immunocompetent lymphocytes, there occurs an initial but slightly delayed immune response called primary response. Primary immune response peaks on approximately the 14^{th} day of antigen exposure. There are two main outcomes of primary response: First, specific immunocompetent cells (B and T cells) are activated; and second and more important one, memory cells are formed. Subsequent exposure to the same antigen stimulates these memory cells which results in a rapid and more heightened immune response. Secondary response usually occurs within two to three days. Primary response also elicits the formation of IgM antibodies while a secondary response protects the host with higher affinity IgG antibodies. It is the rapidity with which secondary response occurs upon secondary exposure to antigen that protects the host against any potential threat by repeated exposure to pathogen.

Thus, a vaccine is basically an antigen or its component that can induce secondary or adaptive immunity in the host. A vaccine aims to prevent severe complications of infections by reinforcing or broadening the defenses by introducing immunological memory.

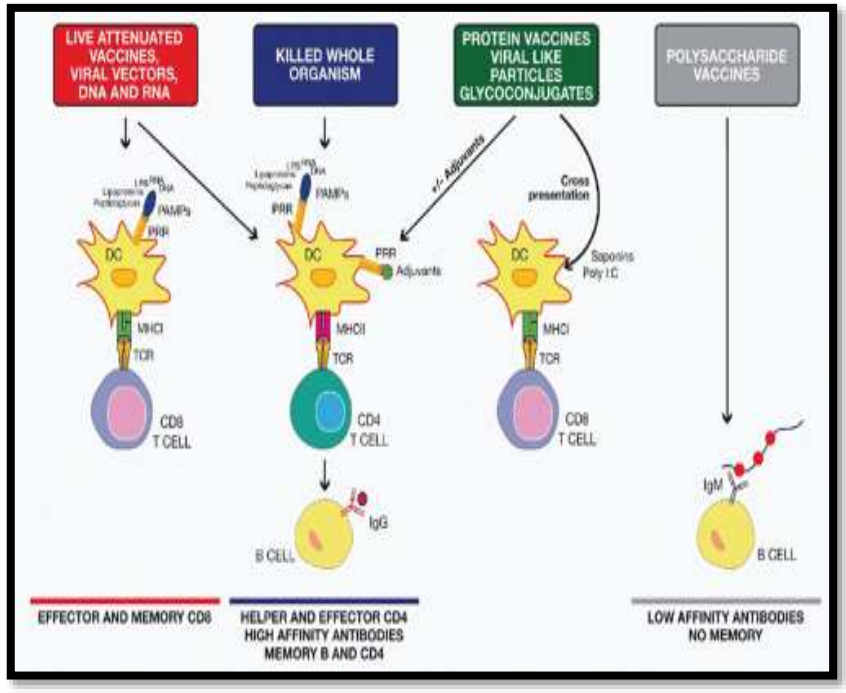

Figure 9.1. Mode of Action of Different Classes of Vaccines. Live attenuated, viral vectors and nucleic acid vaccines induce both major histocompatibility complex (MHC) class I and MHC class II antigen presentation and activate pattern recognition receptors (PRRs) expressed by dendritic cells (DCs) through several pathogen-associated molecular patterns (PAMPs) including peptidoglycan, lipopolysaccharide, and bacterial or viral ribonucleic acid and deoxyribonucleic acid. They generally induce robust cluster of differentiation (CD)$^{8+}$ and CD4 T-cell responses, high antibody titers, and good memory. Killed whole-organism vaccines activate DCs through the same PAMPs and induce good CD4 and B-cell responses. Protein vaccines, viral-like particles, and glycoconjugate vaccines may need an adjuvant for optimal DC activation and CD4 T-cell priming. Some adjuvants such as polyinosinic: polycytidylic acid or saponins can also induce CD8 T-cell responses to subunit vaccines through cross-presentation mechanisms. Finally, polysaccharide vaccines induce a T-cell-independent antibody response.

So, to be efficacious, a vaccine needs to generate an immunologic status that is sufficient to block infection by the pathogen or at least to inhibit the establishment of disease. Depending on the type of infection to be prevented, an effective vaccine may require the induction of different humoral and cellular immune effector mechanisms. Protective humoral

responses include neutralizing antibodies, opsonizing antibodies, or antibodies able to induce complement-mediated killing. Protective cellular mechanisms include different classes of cluster of differentiation $(CD)^{4+}$ T cells and CD^{8+} cytotoxic T cells (CTLs). Based on the type of material from which they are made, licensed vaccines can be broadly classified into three general categories: live-attenuated vaccines, whole-killed vaccines, and subunit vaccines. Different types of vaccines target diverse adaptive immune response (Figure 9.1). For example, glycoconjugate vaccines elicit high-avidity bactericidal antibodies but do not trigger any antigen-specific effector T-cell responses. Protein based vaccines can induce bactericidal and neutralizing antibodies and can also trigger robust CD4+ T-cell responses; however, they are not efficient in inducing CTLs. Finally, live-attenuated vaccines are able to generate all humoral and cellular responses, including CTLs.

HISTORY BEHIND VACCINES

Immunology has its genesis in the early vaccination trials of Edward Jenner and Louis Pasteur. Since those pioneering efforts, vaccines have been developed for many diseases that were once major afflictions of mankind. The incidence of diseases such as diphtheria, measles, mumps, pertussis etc. have declined dramatically since the introduction of vaccinations.

It was the first deliberate scientific attempt to prevent an infectious disease (smallpox), but it was done in complete ignorance of viruses (or indeed any kind of microbe) and immunology. It was not until the work of Pasteur 100 years later that the general principle governing vaccination emerged – altered preparations of microbes could be used to generate enhanced immunity against the fully virulent organism.

Edward Jenner is considered the founder of vaccinology since he demonstrated immunity to smallpox, the practice of immunisation dates back hundreds of years. Buddhist monks drank snake venom to confer immunity to snake bites. In 1798, the first smallpox vaccine was

developed. Over the 18th and 19th centuries, systematic implementation of mass smallpox immunisation culminated in its global eradication in 1979. Louis Pasteur's experiments spearheaded the development of live attenuated cholera vaccine and inactivated anthrax vaccine in humans. Plague vaccine was also invented in the late 19th Century. Between 1890 and 1950, bacterial vaccine development proliferated, including the Bacillis-Calmette-Guerin (BCG) vaccination, which is still in use today.

Alexander Glenny in 1923, refined a method to inactivate tetanus toxin with formaldehyde. The same method was used to develop a vaccine against diphtheria in 1926. Pertussis vaccine development took considerably longer, with a whole cell vaccine first licensed for use in the US in 1948. Viral tissue culture methods developed from 1950-1985, and led to the advent of the Salk (inactivated) polio vaccine and the Sabin (live attenuated oral) polio vaccine.

The middle of the 20th century was an active time for vaccine research and development. Methods for growing viruses in the laboratory led to rapid discoveries and innovations. During years 1930 to 1950, especially during World War II, military targets created high motivation in the development of vaccines. Also, from that period onwards, the support of foundations and public agencies, such as WHO and research institutes increased significantly. Vaccines, such as Polio, Influenza were produced at this time.

After World War II, targeting vaccination programs became a ubiquitous tool for improving public health. In the wake of the global vaccination program and the supply of vaccines around the world, related mortality was significantly reduced, and Smallpox and Polio were eradicated. In the second half of the 20th century, scientific findings, which are associated with screening and industrial production of vaccines, led to the development of a new generation of vaccines and this period became known as the, "golden age of vaccine science". This period began by producing vaccines against Measles, Rubella, and Mumps in the 1960s, and continued with the production of Chickenpox vaccine, in the 1970s. After this period, in the 1980s, the conjugated vaccine of capsular polysaccharide and proteins were developed. Progress in the field of

genetic engineering led to the development of recombinant vaccines. These vaccines enhanced the effectiveness of the immune response and safe use of antigens for immunogenicity in a group of pathogens like hepatitis B. Ultimately, in the 1990, vaccines evolution entered a bold and surprising final phase with the development of vaccines, called DNA vaccines.

VACCINE DEVELOPMENT

The way to successful development of a vaccine that can be approved for human use, manufactured at a reasonable cost and efficiently delivered to at-risk populations is costly, long and tedious. Stringent testing is an absolute necessity when it comes to development of vaccines before approving them for use by masses.

Recent advances in immunology and molecular biology have led to effective new vaccines and to promising strategies for finding new vaccine candidates. Knowledge of the differences in epitopes recognized by T cells and B cells has enabled immunologists to begin to design vaccine candidates to maximize activation of both arms of the immune system.

The production and evaluation of vaccines includes 3 steps:

- Identify and producing antigens.
- Tests on animal models.
- Clinical trials.

In the first phase of the clinical trial, safety and tolerability of the vaccine is evaluated and the range of doses or repeated doses of the vaccine is also measured. In the second phase, the vaccine safety and immunogenicity of active ingredient is evaluated in the target population and the proper dose of vaccine is identified. In the third and final phase, necessary measures are taken for obtaining the license and testing is done on the target population for a long time. On an average, all vaccine development processes take 16 years and cost about a billion dollars.

TYPES OF VACCINES

There are several basic types of vaccines that are currently and conventionally used. These are divided into 3 main generations namely: first generation vaccines, second generation vaccines and third generation vaccines.

The first generation of vaccines includes vaccines prepared by conventional methods, e.g., the small pox vaccine, Salk's polio vaccine etc. Attenuated pathogens, full organisms or inactivated bacterial toxins, which are effectively immunogenic, are used in making these vaccines. The first generation of vaccines is further classified into attenuated vaccines and inactivated vaccines. While the attenuated vaccines use a weakened live pathogen as antigen, the inactivated vaccines use a killed pathogen.

Since the risk of the pathogen reverting back to a virulent form made first generation vaccines risky, the second generation of vaccines were created in order to minimize that risk. The way these vaccines work is that they do not contain the whole organism, but rather subunits. Subunits may consist of the toxins that the pathogen create (if they are bacterial) or only contain protein sections of the pathogen, such as an acellular form as such they can only be prepared through genetic engineering. A great example of a 2nd generation vaccine is DTP. The vaccine contains diptheria toxoid, tetanus toxoid, pertussis toxoid, as well as the acellular version of pertussis.

The third generation of vaccines is also artificially produced. This generation of vaccines includes recombinant vector vaccines where the antigen is a sub unit of pathogen and is integrated into another virus against which the human body is known to mount a decent response like the adenovirus. In a DNA vaccine (which form a big part of this generation), for example, DNA based antigen is incorporated into a plasmid vector which is directly given to us and our body absorbs the DNA and replicates it so we have many copies of the antigen. Examples of third generation vaccines are leukaemia and Ebola vaccines. Now let us take a detailed look at these vaccines:

FIRST GENERATION VACCINES (A TRADITIONAL VACCINE)

These are produced by conventional methods, e.g., small pox vaccine, Salk's polio vaccine. Attenuated and inactivated vaccines are identified in the first generation. Attenuated pathogens, full organisms or inactivated bacterial toxin, which are effectively immunogenic, are used in making these vaccines. There are some advantages in these kinds of vaccines due to their high ability to stimulate innate immunity, induction of long-term protection, easy production, and low production costs. However, there are some disadvantages in this generation, such as inducing disease due to the use of complete pathogen (live or inactivated) and virulence recursively of the pathogen in the host body.

Attenuated Vaccines

Attenuated refers to the weakening of the pathogenic bacteria or virus by making it less virulent without altering its immunogenicity. Microorganisms are attenuated or weakened so that they do not cause any disease. Attenuation can be achieved by growing pathogenic microorganisms (bacteria or virus) for a long period of time in a foreign host such as an embryonated eggs or tissue culture cells. After Louis Pasteur, the second and more famous attenuation was successfully carried out by two scientists Albert Calmette and Camille Guerin in 1921. They grew the bovine strain of *Mycobacterium tuberculosis* called *Mycobacterium bovis* for several years on a medium containing increasing concentration of bile. This in vitro cell culture of pathogenic *Mycobacterium bovis* changed it into a less virulent and more suitable form of bacilli known as BCG (Bacillus Calmette-Guerin), a commonly used vaccine against tuberculosis.

The triple vaccine of Measles, Mumps and Rubella (MMR) is a kind of prominent attenuated vaccine, which was supplied in the United States in 1971. This vaccine contains all 3 types of viruses, which have been attenuated separately. The Polio (OPV), Influenza, Yellow fever, and Hepatitis A are the other known vaccines in this group of vaccines (Figure 9.2).

These kinds of vaccines, which are known as live attenuated vaccines, contain the major types of pathogens, which have all the pathogenic features of the main organisms but have been attenuated (weakened) in laboratory conditions. These vaccines are useful as they have the ability to emulate the infection process, which could lead to induction of potent antibody responses and cellular immunity.

The process of attenuation involves growing microbes under abnormal in vitro conditions, be it high bile concentration or passage through foreign cell/tissue such as embryonated eggs. These abnormal environmental conditions select those mutant cells that are able to survive and multiply under these conditions. These microbes are then harvested and used as vaccine. In a normal host, these "pathogenic" microorganisms either fail to multiply or multiply very slowly because these microbes are now used to be grown under abnormal conditions. The reason for the loss of pathogenicity achieved by the "foreign cell passage" method is difficult to determine. However, it is quite clear now that growing microbes in the unnatural host induces a purely random series of mutations in their genome. This mutation can result in the change of surface proteins of the virus as in type 1 polio virus or can make them temperature sensitive or sometimes even cold adapted.

Advantages of Attenuated Vaccines

Due to the slow growth of attenuated viruses under normal body conditions, attenuated vaccines provide for prolonged exposure of viral antigen to the immune system resulting in the production of a large number of B and T cells and more importantly memory cells. As a consequence,

most of these vaccines are administered only once in a lifetime and do not require repeated boosters.

The attenuated vaccines that are given orally to children. e.g., Sabin Polio vaccine type I are given on sugar cubes or drops. The attenuated virus enters the gastrointestinal tract and induces the production of secretory IgA as well as humoral IgG. These antibodies serve as an important defence against naturally occurring poliovirus.

Disadvantages of Attenuated Vaccines

The principle limitation of these vaccines relates to concerns about their safety. A major disadvantage of using live attenuated vaccines is the possibility of their reversion to the virulent form. Type II and type III Sabin Polio vaccine has been shown to revert frequently to their wild form. This has led to the outbreak of paralytic poliomyelitis and countries such as Sweden have discontinued the use of Sabin polio vaccine. Another disadvantage of live attenuated vaccines is that they cannot be given to people having immunodeficiency diseases as the immune system of such patients is severely compromised and can be attacked by even attenuated pathogens. Besides these, since attenuation is done by growing in tissue cultures, sometimes these culture cells can become contaminated with other viruses also. These kinds of vaccines, which are known as live attenuated vaccines, contain the major types of pathogens, which have all the pathogenic features of the main organisms but have been attenuated (weakened) in laboratory conditions. These vaccines are useful as they have the ability to emulate the infection process, which could lead to induction of potent antibody responses and cellular immunity.

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Diseases		Remarks
Viruses	Polio	Type 2 and 3 may revert; also killed vaccine
	Measles	80 % effective
	Mumps	
	Rubella	Not given to both sexes
	Yellow fever	Stable since 1937
	Vericella zoster	Mainly in leukemia
	Hepatitis A	Also killed vaccine
Bacteria	Tuberculosis	Stable since 1921; also some protection against leprosy.

Figure 9.2. Attenuated vaccines are available for many, but not all, infections. In general, it has proved easier to attenuate viruses than bacteria (Adapted from immunology David male, 7th edition, immunology).

Inactivated Vaccines

An inactivated/killed vaccine is a vaccine consisting of either whole viruses or bacteria, or fractions of either, that have been grown in culture and then killed using various physical and chemical methods. The pathogen particles are destroyed and cannot divide, but maintain some of their integrity to be recognized by the immune system and evoke an adaptive immune response. In case of the fractional vaccines, the organism is further treated to obtain only those components that are to be included in the vaccine (e.g., the polysaccharide capsule of pneumococcus). In comparison with attenuated vaccines, the main advantage of inactivated vaccines is their usage of inactivated or killed pathogens, which cause an increase in safety and immunity level. However, there are some concerns, such as return of the virulence characteristics of the pathogen, which lead to host disease and because of non-proliferation, pathogens clear rapidly from the body, which could decrease the efficiency and effectiveness of

vaccines in comparison with attenuated vaccines. Hepatitis A is an inactivated vaccine that was produced by using hepatitis A virus, which is inactivated by formalin.

Inactivated vaccines are not active and cannot replicate, so there is always a need for multiple doses. In general, the immune response does not occur after the first dose, but usually after the second or third dose. The immune response is very similar to the natural infection, mostly humoral immunity, with little or no cellular immunity. The titers of antibody against the inactivated antigens decrease with time. Therefore, some inactivated vaccines require regular supplemental doses to increase efficacy.

Advantages

- Inactivated vaccines are more stable and safer than live vaccines. There is absolutely no chance the bacteria or virus in the vaccine can cause disease. The killed viral particles or bacteria are not able to come back to life.
- The virus or bacteria is still recognized as an invader by the body even though it does not cause disease, so the immune system still makes protective antibodies against it.
- The vaccine can be given to people with weakened immune systems.
- These vaccines do not usually need to be refrigerated. They can be stored easily and transported when freeze-dried. This makes them great vaccines to use in developing countries.

Disadvantages

- Inactivated vaccines often stimulate a much weaker immune response than live vaccines. Therefore, there are typically several doses needed before gaining immunity to a disease. In addition,

booster shots are sometimes needed to keep immunity to the disease.

- Without regular access to healthcare, many people are not aware of boosters, or given the opportunity to have the boosters. These people may have a false sense of immunity to these diseases.

Examples of Inactivated Vaccines

- Viral Vaccines include inactivated Polio Vaccine (Salk vaccine), Hepatitis A Vaccine, Influenza Vaccine. Rabies Vaccine etc.
- Bacterial vaccines include inactivated Typhoid Vaccine, Inactivated Cholera Vaccine, Plague Vaccine, Diptheria, Tetanus and Pertussis Vaccine or the DPT (Figure 9.3).

disease		remarks
viruses	polio	preferred in Scandinavia; safe in immunocompromised
	rabies	can be given post-exposure, with passive antiserum
	influenza	strain-specific
	hepatitis A	also attenuated vaccine
bacteria	pertussis	potential to cause brain damage (controversial)
	typhoid	about 70% protection
	cholera	protection dubious; may be combined with toxin subunit
	plague	short-term protection only
	Q fever	good protection

Figure 9.3. The principal vaccines using killed whole organisms. (Adapted from immunology David male, 7th edition, immunology).

SECOND GENERATION VACCINES

The first generation vaccines, made from live attenuated pathogens had the possibility of returning to infectious forms and cause disease. To solve this problem, scientists offered second-generation vaccines. The basis of this generation was subunit elements, recombinant or synthetic proteins, non-protein antigens, and expressed bacterial immunogen or viruses, which include numerous molecules and epitopes of different species and strains of pathogens. The second generation vaccines include subunit, conjugated, and recombinant vaccines.

Subunit Vaccines

A vaccine can be made of single or multiple antigenic components of a microorganism that are capable of stimulating a specific immune response sufficient to protect from the relevant pathogen infection or from the clinical manifestation of the disease. Depending on the molecular composition of the purified antigen used to prepare the vaccine, and on the techniques applied to obtain the final material used as a vaccine, different types of subunit vaccines can be defined. Instead of the complete pathogen, parts are used for the production of these kinds of vaccines. Subunit vaccines have one or more protein-peptide or polysaccharide, which are naturally found in the pathogenic structure. Due to partial use of pathogen in subunit vaccines, they do not have the ability to proliferate and thereby make unintended response. Because of their immunity and low production costs, they are viable alternatives to the traditional vaccines.

Toxoids

Toxoids are toxins that are inactivated by chemical or physical treatment, but that nonetheless preserve their antigenic structure. This approach allows the formation of a protein that has completely lost toxic

activity but is still capable of inducing the production of antibodies that will recognize and block the native toxin. This approach can be applied in case of infections in which the pathology exclusively depends on the damage induced by a single toxin produced by a pathogen, and, therefore, production of antibodies neutralizing the activity of the toxin or rapidly causing its elimination will be enough to avoid any disease. Vaccines against tetanus and diphtheria are based on toxoids; as in both cases, the pathology is caused entirely by a single toxic protein secreted by *Clostridium tetani* or *Corynebacterium diphtheriae*, respectively. Another commonly used toxoid is the inactivated pertussis toxin from *Bordetella pertussis*, which is one of the components of the acellular pertussis vaccine.

Conjugated Vaccines

Polysaccharide antigens are large molecules with repetitive epitopes, and antigen presenting cells are not enable to process them, thus the antibody response occurs without the participation of T cells against them and causes a rise of antibody response at a small dose and short-time period. These responses cannot make immune memory and affinity maturation for these kinds of infections. Unlike polysaccharide antigens, proteins can be processed well by antigen presenting cells, and make long-term response and immunological memory. In 1929, Avery and Goebel used protein to increase the immunogenicity of polysaccharide antigens. They observed that weak immunogenicity of polysaccharide antigen of *Streptococcus pneumonia* type 3 increased by its connection to the carrier protein in rabbit. These observations led to a foundation for producing modern conjugate vaccines. In 1987, haemophilus influenza B (HIB) was the first conjugate vaccine, which could obtain license for medical usage and was later used for immunization of infants.

Chemical conjugation of polysaccharides with protein carriers allows the processing of protein carriers by polysaccharide-specific B cells and presentation of the resulting peptides or glycopeptides associated with

MHC class II on their surface. Further interaction with carrier-specific T cells then induces polysaccharide-specific B cell differentiation. Therefore, the conjugate vaccine has induced a T cell-dependent response early in life, leading to immune memory and promoting response through further doses of the vaccine (Figure 9.4).

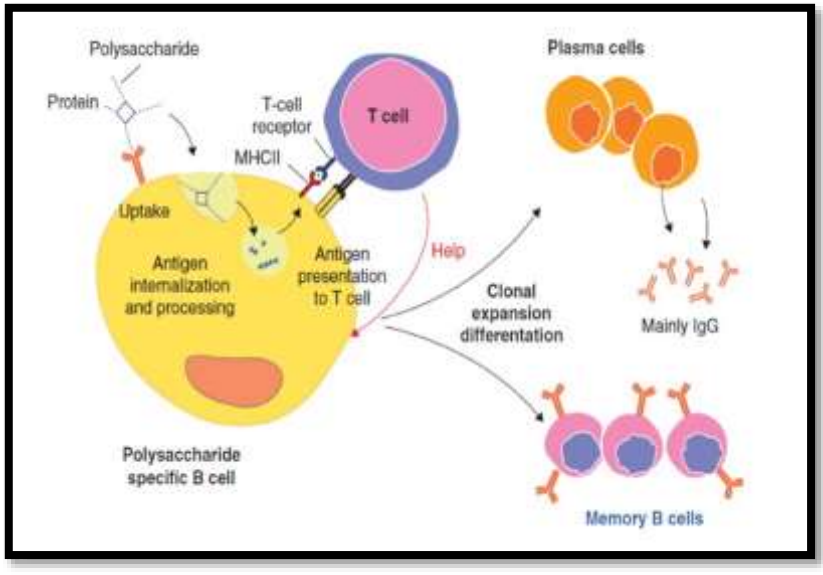

Figure 9.4. Schematic diagram of mechanism of action for conjugate vaccines (source: Creative biolabs vaccine).

Recombinant Vaccines

The rise of genetic engineering and molecular biology has had great impact on development and manufacturing process of vaccines. Specific antigenic microbes have high power to arouse the immune response against pathogens. Currently, the sequence of the pathogenic protein antigens could be obtainable by sequencing genes of the main antigen, and producing them synthetically via recombinant DNA technology (Figure 9.5).

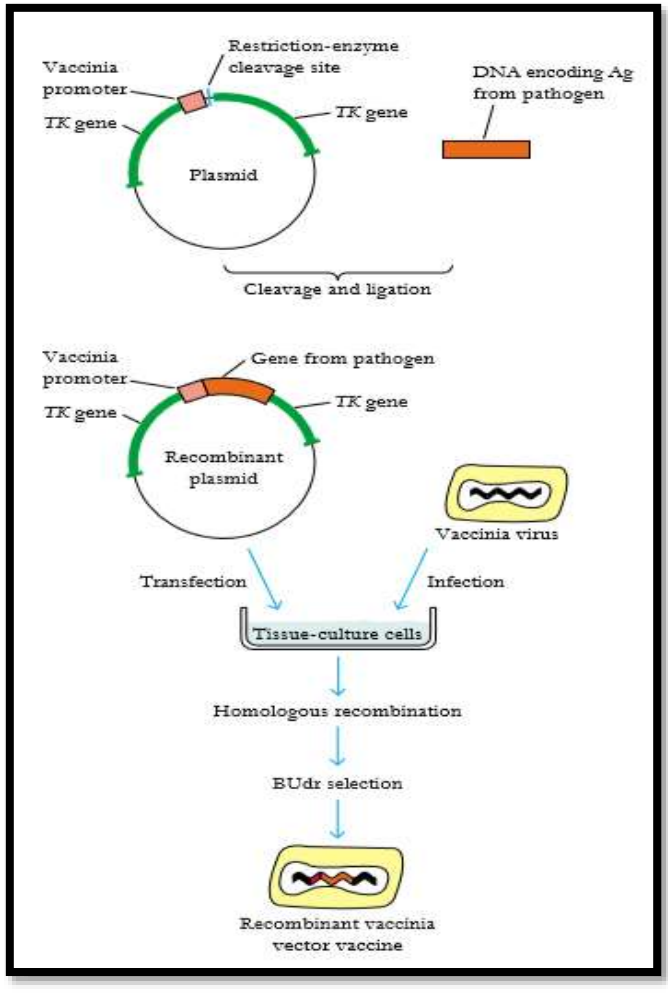

Figure 9.5. Production of vaccinia vector vaccine. The gene that encodes the desired antigen (orange) is inserted into a plasmid vector adjacent to a vaccinia promoter (pink) and flanked on either side by the vaccinia thymidine kinase (TK) gene (green). When tissue culture cells are incubated simultaneously with vaccinia virus and the recombinant plasmid, the antigen gene and promoter are inserted into the vaccinia virus genome by homologous recombination at the site of the nonessential TK gene, resulting in a TK' recombinant virus. Cells containing the recombinant vaccinia virus are selected by addition of bromodeoxyuridine (BUdr), which kills TK'cells. [Adapted from B. Moss, 1985, Immunology]

Hepatitis B is the first and one of the most successful examples of synthetic vaccines. The surface antigen of this virus (HBsAg) is very immunogenic and effective, and able to produce high levels of antibody in the body. In the past, for providing hepatitis B vaccine, HBsAg was purified from the plasma of infection carriers and used for vaccination but there were some extensive restrictions in purification, such as difficult conditions and contaminated plasma. In order to make recombinant hepatitis B vaccine, recombinant HBsAg is expressed in cells that have a powerful expression system (such as yeast) leading to the production of virus-like particles by HBsAgs, which are highly immunogenic. Since these particles have no genome, they do not create disease and lead to effective and powerful response against the main pathogen. Other kinds of common vaccines in this type are anti-herpes simplex virus, anti-rotavirus, and anti-HPV vaccines.

THIRD-GENERATION VACCINES

Immunogenic potential administration of a plasmid containing a gene encoding the antigen, known as genetic vaccines, is categorized as third generation vaccines, and is a valuable method, which has been considered by researchers since the beginning of 1990s. Different names have been given for this kind of vaccine, such as DNA vaccines, RNA vaccines, and plasmid vaccines.

DNA vaccines include direct injection of plasmid containing the encoding gene of the considered antigen, which is expressed in the cells with the aid of specific promoter that causes induction of the immune system. Therefore, instead of prescribing recombinant protein needed to stimulate the immune system (such as hepatitis B), it will be produced in the body. The expressed protein that are in the natural form stimulate cellular and humeral immunity during different stages. The dendritic cells play an essential role in providing antigen to immune cells (Figure 9.6).

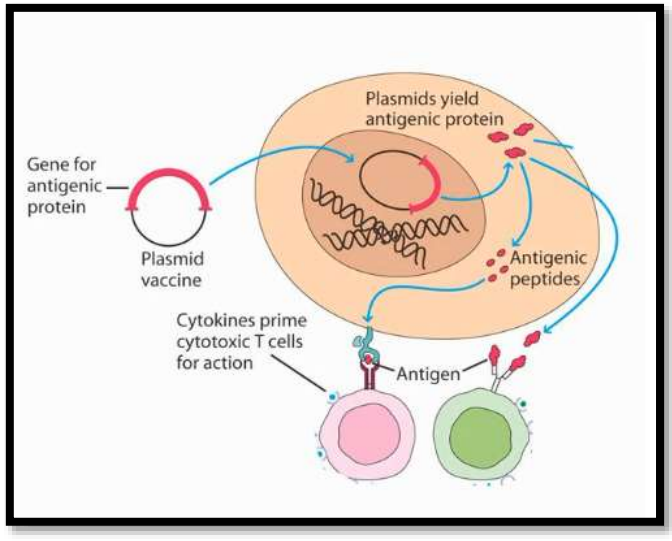

Figure 9.6. Diagram illustrates the procedure for DNA vaccine pathway

(Adapted from Kuby immunology, 6th ed).

MECHANISM OF ACTION OF DNA VACCINES

An antigenic gene in the DNA plasmid is expressed using a host (human) expression system. This antigen is presented by APC through MHC class I (for endogenous antigens) or MHC class II (for exogenous antigens). APCs containing antigens drains into the lymph nodes through afferent lymphatic vessels. In the lymph nodes, the antigen is presented to the naïve T cells and T cell receptor (TCR) along with co-stimulatory molecule, resulting in the immune responses initiation and T cells expansion. T cells response through T helper cells CD^{4+} activation. This activation stimulates the cytokines production that plays roles in the B cells and cytotoxic T cells activation. CD^{4+} T cells can differentiate into various types of T helper cells, depending on the cytokines, such as IL-6 and IL-21 triggers the Th17 cells formation while IL-2 and IL-4 promote the Th2 cells formation. In addition, the antigen is also captured by the B cell receptor then it is processed and presented to CD4 T helper cells. Later on,

the T helper cells facilitate an effective B cell response with the help of IL-21. Cytotoxic T lymphocytes CD^{8+} (CD^{8+} CTLs) are activated when the antigen endogenously presented by MHC class I. Activated T cells and B cells are returned to circulation via the lymphatic vessels efferent and provide defense against antigens. The immune system induction by the DNA vaccine is presented in (Figure 9.7). Activated $CD8^{+}$ CTLs react to antigen in peripheral tissues. CTLs bind to their antigens through complex bonds that is mediated by antigen receptors, co-receptors ($CD8^{+}$), and adhesion molecules (e.g., ICAM-1). The bond is very specific, thus it only recognizes cells that express the target antigen. It is formed between CTLs and target cells in an area which is called synapse. Furthermore, CTLs secrete granules (granzymes and perforins) proteins that induce target cell apoptosis. Perforin polymerizes and forms pores on the target cell membrane. Granzyme cuts substrates in cells and induces caspase activation. CTLs also induce cell apoptosis without granule secretion, but through Fas-ligand (FasL) secretions that induce extracellular apoptosis mechanism through its association with Fas death receptor. After delivering the granules and FasL, CTLs discharge from the target cells. CTLS also expresses cathepsin B, a protein that plays a role in the CTLs protection against perforin.

Apoptotic cells release apoptotic cell-associated molecular patterns (ACAMPs) or damage associated molecular patterns (DAMPs) that are being recognized by pattern-recognition receptors (PRRs) expressed by macrophage cells. Although they have similar structures with pathogen associated molecular patterns (PAMPs), ACAMPs induce different macrophage responses. The PAMPs presentation is followed by a proinflammatory response, whereas the ACAMPs presentation does not induce a pro-inflammatory response. In addition, ACAMPs induce a response that is TLR-independent while PAMPs response is TLR-dependent. After the apoptotic molecules recognition, the next step is the binding of apoptosis cells with various receptors on the macrophage cell surface, e.g., lectin, avb3, CD36, SR-A, MER, CD14, ABCA1, PSR, CR3, CR4, CD91 /calreticulin, CD31, FccR, SHPS-1, or oxidation specific receptors. The binding process is followed by the signalling process within

the macrophage cells that induces cytokine production, initiation of the engulfment process, and phagocytosis B cell receptors on the B cells surface capture the antigens expressed as extracellular proteins. B cells require $CD4^+$ T cells for the activation process. T cells helper expresses CD40L, which plays role in the cytokines secretion, such as IFN. Type I CD40L and IFN binding stimulates cell proliferation and differentiation.

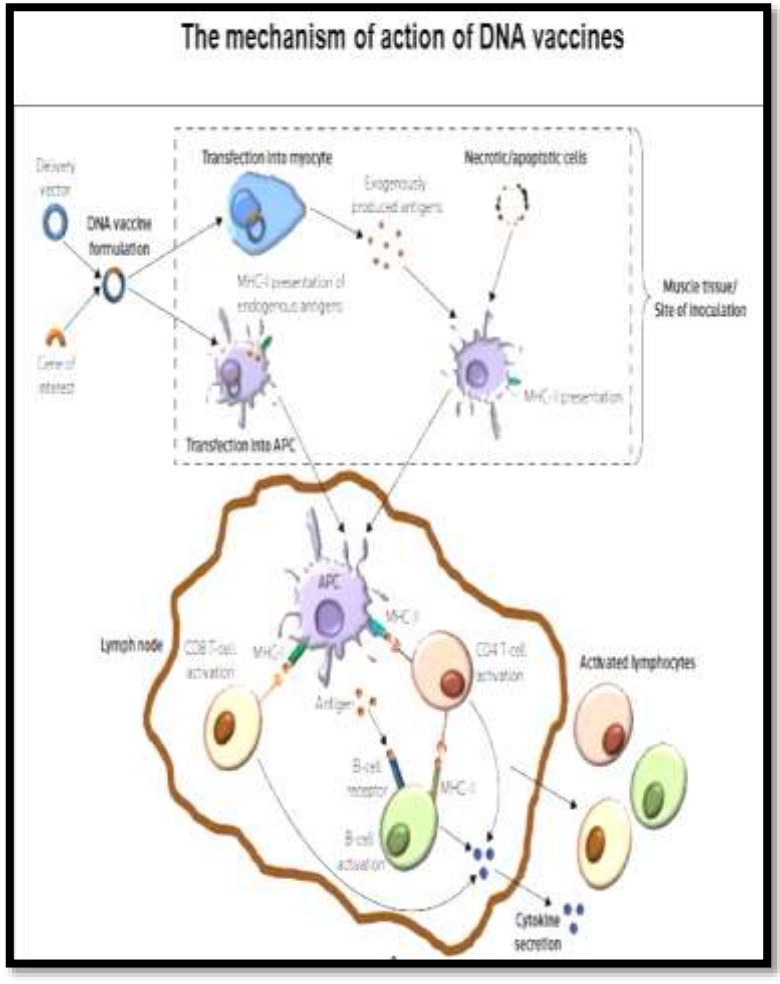

Figure 9.7. Showing the mechanism of action of DNA vaccines.

With the help of cytokines and follicular helper T cells (TFH), the affinity maturation of B cell affinity occurs in the germinal center. Affinity maturation occurs through a somatic mutation process in IgG heavy chain and light chain. The result of this affinity maturation is B cells that can produce antibodies with high affinity. In addition, with the T cells helper assistance, isotype switching process occurs through recombination switching, this process leads to various Ig isotope production. B cells differentiate into plasma cells and memory cells. Plasma cells migrate to the spinal cord while the memory cells circulate between the lymph nodes and the spleen, and respond rapidly when encountering antigens in the circulation. The humoral immune response is primarily mediated by antibodies produced by plasma cells. The antibody-mediated reactions include neutralization of the antigen, opsonization assistance and antigen phagocytosis, complement activation, and cellular cytotoxic induction. The neutralization process of toxins and antigens is completed by blocking antigen or toxin binding with cells. IgG facilitate the opsonization through the antigen binding to the Fc receptors (FcγRI) that are presented on the phagocyte surface. Opsonized particles is internalized in vesicles called phagosomes and then undergo fusion with lysosomes. Furthermore, the antigens are degraded within phagolysosomes.

The various advantages of DNA vaccines are:

- They do not have the limitation of common recombinant protein and peptide vaccines.
- They are able to immunize against several different strains of qualified antigen diversity.
- They produce antigen in a natural form and can be properly deliberated to the immune system.
- They are able to stimulate the humeral, cellular, and mucosal immunity.
- They cause a lack of immune response to the injected vector and do not generate antigen against transfected cells.

- There is no possibility to activate attenuated vaccines and infection risk.
- It has been possible to produce multiple vaccines.
- They have ease, speed of mass production and similar stages of vaccine production.
- The quality control is an easy process.
- They remain stable in various temperatures and the cold chain is not required for maintenance.

But DNA vaccines have some serious limitations as well. Their limitations include:

- The ability to be only used for protein antigens.
- They effect cells growth by controlling genes.
- They are capable of joining the host genome and inducing tumours.
- They can cause autoimmune diseases.

In the recent years, extensive research projects have been done on the use of this generation of vaccines for hepatitis, HIV, influenza, and Ebola. They have, however, not received indispensable license for human usage.

EXAMPLES OF A FEW VACCINES

1. BCG Vaccine

A bacterium (bacillus) named after Calmette and Guérin. This is a live, freeze-dried vaccine prepared from an attenuated strain of bovine (cattle) TB. This vaccine protects children against TB meningitis and miliary TB, both more common among the developing part of our population, and both potentially fatal.

2. Polio Vaccine

There are two vaccines that protect against poliomyelitis, namely the inactivated poliovirus vaccine (IPV), known as the Salk vaccine, which is given by injection and the oral, live attenuated vaccine (OPV) which is administered orally. This is also called the Sabin vaccine.

3. Hepatitis B Vaccine

Hepatitis B is endemic and can cause a serious disease of the liver, for which there is no cure, and may lead to death or chronic liver problems which will persist into adult life. The vaccine contains only the coat protein (surface antigen) which covers the surface of the virus. It is prepared from the plasma of human carriers, or produced by recombinant DNA technology in yeasts. Both types have been shown to be equally safe and effective.

4. Measles Vaccine

Measles causes an acute febrile illness with a rash which can be life threatening in young children. The vaccine consists of a weakened strain of the measles virus. It is given to children over the age of nine months.

LATEST ADVANCEMENTS

Preventing diseases is much more effective and less costly than treatment after obtaining the disease; therefore, vaccine is the most important and powerful instrument for humans in order to prevent diseases and the development of vaccination is very important. The aim of new strategies is increased safety, efficiency, and stability of vaccines in the

future. By expanding our knowledge in microbiology, immunology, molecular biology, genetic engineering, and manufacturing techniques for vaccine, the path of advanced generation of vaccines, which are able to prevent and treat such diseases, becomes easier than before. The biotechnology revolution, culminating in the sequencing of the genome of a great many pathogens, together with increased knowledge of the immune responses to infections, has allowed the unprecedented rational development of new recombinant vaccines that will hopefully help control infectious diseases, including those that appear most complex, such as HIV/AIDS, tuberculosis, and malaria. However, despite these new tools, the challenges remain formidable.

It is vital to continuously develop new vaccines and to improve existing ones if we are to combat diseases and preserve human life.

CURRENT VACCINE DEVELOPMENT

1. Diarrheal Diseases

Conservative estimates place the death toll from diarrheal diseases at 4 million to 6 million per year, with most of these deaths occurring in young children. In the long term, access to clean water, better hygiene, and improvement of sanitation would have the greatest impact on diarrheal diseases, but immunization against specific pathogens is the best hope for the short term and medium term. The burden of diarrhoea among children aged younger than 5 years in the developing world is estimated to be 1.5 billion episodes per year, leading to 3 million deaths. The development of new vaccines against viral diarrhoea caused by rotavirus, present in countries with high and low levels of hygiene, is the focus of intense international efforts.

Rotavirus is a double-stranded RNA virus belonging to the Reoviridae family. It is the leading cause of severe diarrheal disease and dehydration of infants in both industrialized and developing countries.

2. Acute Respiratory Infections

Both viruses and bacteria are a common cause of acute lower respiratory infection (LRI) in children worldwide.

The sudden emergence in early 2003 of an epidemic of atypical pneumonia originating in China led to the identification of the severe acute respiratory syndrome (SARS) virus, a coronavirus unrelated to previously known coronaviruses. The development of a vaccine against SARS has been judged a global priority, but it is still only at the early clinical stage.

3. HIV/AIDS

More than 40 million adults and children are living with HIV/AIDS worldwide and close to 5 million people become infected each year. Asia currently experiences the world's fastest-growing HIV/AIDS epidemic. Highly active antiretroviral therapy has reduced progression to AIDS, deaths, and HIV transmission from mother to child in North America and Western Europe. However, success with treatment has not been matched by progress toward prevention. Despite the great efforts to make antiretroviral drugs available to the underprivileged populations, a preventive vaccine is needed more than ever, particularly in developing countries to prevent this disease from taking more lives.

CONCLUSION

The discipline of immunology has its genesis in the early vaccination trails of Edward Jenner and Louis Pasteur. Since their pioneering efforts, vaccines have been developed for many diseases that were once major afflictions to the mankind. The incidence of diseases such as diphtheria, measles, mumps, pertussis (whooping cough), rubella (German measles), poliomyelitis, and tetanus has declined dramatically as vaccination has become more common. Perhaps in no other case have the benefits of

vaccination been as dramatically evident as in the eradication of smallpox. Since October 1977, not a single naturally acquired smallpox case has been reported anywhere in the world. Equally encouraging is the predicted eradication of polio. The last recorded case of naturally acquired polio in the Western Hemisphere occurred in Peru in 1991. Vaccination is a cost-effective weapon for disease prevention. But even today, millions throughout the world die every year from diseases for which there are no effective vaccines. It is estimated by the World Health Organization that 16,000 individuals a day become infected with HIV-1, the virus that causes AIDS. An effective vaccine could have an immense impact on the control of this tragic spread of death and disaster. In addition to the challenges presented by diseases for which no vaccines exist, there remains the need to improve the safety and efficacy of present vaccines and to find ways to lower their cost and deliver them efficiently to all who need them, especially in developing countries of the world. The WHO estimates that millions of infant deaths in the world are due to diseases that could be prevented by existing vaccines.

The way to successful development of a vaccine that can be approved for human use, manufactured at reasonable cost, and efficiently delivered to at-risk populations is costly, long, and tedious. Experience has shown that not every vaccine candidate that was successful in laboratory and animal studies prevents disease in humans. Some potential vaccines cause unacceptable side effects, and some may even worsen the disease they were meant to prevent thus stringent testing is an absolute necessity. Recent advances in immunology and molecular biology have led to effective new vaccines and to promising strategies for finding new vaccine candidates.

FREQUENTLY ASKED QUESTIONS (FAQS)

Q1.What are Vaccines?

Answer: Vaccines are products that produce immunity to a specific disease. Most vaccines are given by injection (needle) but some are given orally (by mouth) or nasally (sprayed into the nose).

Q2. How do vaccines work?

Answer: Vaccines work to prime your immune system against future "attacks" by a particular disease. When a pathogen enters the body, the immune system generates antibodies to try to fight it off. Some of the antibodies that were created to fight off the pathogen remain in the body playing watchdog even after the pathogen attack has been neutralised. On exposure to the same pathogen in the future, these antibodies "recognize" it and fight it off. Vaccines work because of this function of the immune system. They're made from a killed, weakened, or partial version of a pathogen. When we get a vaccine, whatever version of the pathogen it contains isn't strong or plentiful enough to make us sick, but it's enough for our immune system to generate antibodies against it. As a result, we gain future immunity against the disease without having gotten sick.

Q3. Why aren't all vaccines 100% effective?

Answer: Vaccines are designed to generate an immune response that will protect the vaccinated individual during future exposures to the disease. Individual immune systems, however, are different enough that in some cases, a person's immune system will not generate an adequate response. As a result, he or she will not be effectively protected even after immunization.

Q4. Do vaccines provide better immunity than natural infections?

Answer: Vaccines interact with the immune system to produce an immune response similar to that produced by the natural infection, but they do not cause the disease or put the immunized person at risk of its potential complications. In contrast, the price paid for getting immunity through natural infection might be

cognitive impairments from Haemophilus influenzae type b (Hib), birth defects from rubella, liver cancer from hepatitis B virus, or death from complication due to measles.

Q5. Why do some vaccines require boosters?

Answer: It's not completely understood why the length of acquired immunity varies with different vaccines. Some offer lifelong immunity with only one dose, while others require boosters in order to maintain immunity. Recent research has suggested that the persistence of immunity against a particular disease may depend on the speed with which that disease typically progresses through the body. If a disease progresses very rapidly, the immune system's memory response (that is, the "watchdog antibodies" generated after a previous infection or vaccination) may not be able to respond quickly enough to prevent infection unless they've been "reminded" about the disease fairly recently and are already watching for it. Boosters serve as a "reminder" to your immune system.

Q6. What is immunisation?

Answer: Immunisation describes the process whereby people are protected against illness caused by infection with micro-organisms (formally called pathogens). Immunization is done through various techniques, most commonly vaccination. Vaccines against microorganisms that causes diseases can prepare the body's immune system, thus helping to fight or prevent an infection.

Q7. What is the difference between vaccination and immunization?

Answer: Vaccination is the act of introducing a vaccine into the body to produce immunity to a specific disease while as Immunization is the process by which a person or animal becomes protected against a disease. However, the terms are often used interchangeably.

Q8. What is herd immunity? Is it real? Does it work?

Answer: Herd immunity, also known as community immunity, refers to the protection offered to everyone in a community by high vaccination rates. With enough people immunized against a given disease, it's difficult for the disease to gain a foothold in the community. This offers some protection to those who are unable to receive vaccinations including newborns and individuals with chronic illnesses by reducing the likelihood of an outbreak that could expose them to the disease.

Q9. Do vaccines cause autism?

Answer: Vaccines do not cause autism. This possibility was publicized after a 1998 paper by a British physician who claimed to have found evidence that the MMR (measles, mumps and rubella) vaccine was linked to autism. The potential link has been thoroughly explored; study after study has found no such link, and the original 1998 study has been formally withdrawn by The Lancet, which had originally published it. Studies were also done regarding the possibility of a link between the preservative thimerosal, which is used in some vaccines, and autism; again, no such link was found. It's likely that this misconception persists because of the coincidence of timing between early childhood vaccinations and the first appearance of symptoms of autism.

Q10. How long does immunity last after getting a vaccine?

Answer: A few vaccines, like the two for measles or the series for hepatitis B, may make you immune for your entire life. Others, like tetanus, last for many years but require periodic shots (boosters) for continued protection against the disease.

Q11. Do vaccines have side effects?

Answer: Vaccines are among the safest medicines available. Some common side effects are a sore arm or fever. There is a very

small risk that a serious problem could occur after getting a vaccine. However, the potential risks from the disease's vaccines prevent are much greater than the potential risks associated with the vaccines themselves.

Q12. What are toxoid vaccines?

Answer: Toxoid vaccines are vaccines that are made from the toxins (harmful chemicals) from bacteria. There are some bacteria that cause disease through releasing a protein called a toxin. Scientists can inactivate these toxins in the lab using a chemical called formalin (a solution of formaldehyde) and sterilized water, which are completely safe to use in small quantities in the human body. Once the toxin is inactivated, it's called a toxoid, and it can no longer cause harm. The body learns how to fight off the bacteria's natural toxin once exposed to the toxoid through producing antibodies that bind into the toxin like keys into a lock.

Q13. Describe Conjugated vaccines in detail.

Answer: Conjugate vaccines or immunogen conjugates are advanced forms of immune system modulators that are used extensively to generate protection against invading pathogens or to produce specific antibodies against a target molecule. These vaccines consist of antigens (polysaccharides or oligosaccharides) that are chemically coupled to a protein carrier (PC). Coupling of the saccharides to protein converts polysaccharides to T-dependent antigens, which elicit robust immune responses in infants and adults. These "glycoconjugate" vaccines elicit T-cell help for B-cells that produce IgG antibodies to the conjugated polysaccharide.

Q14. List some of the other ingredients found in commercial vaccines.

Answer: Commercial vaccines, besides the strain preparation, may also contain:

- Fluid, such as sterile water, saline or a protein-containing fluid, to suspend the contents.

- Stabilisers, such as albumin, gelatine or sugars, to help the active ingredients and prevent them from spoilage when exposed to adverse conditions, such as extreme temperatures or changes in light, humidity or acidity.
- Preservatives, such as phenols or antibiotics, to prevent the growth of dangerous bacteria and fungi in the vaccine.
- Some vaccines also contain adjuvants: substances such as aluminium compounds that strengthen the immune response to the vaccine's antigens. These are commonly used in subunit vaccines.
- Vaccines may also contain trace elements of substances used during their manufacture: for example, some vaccines are grown using fertilised hens' eggs and so may contain traces of egg protein, and inactivated vaccines may contain extremely small traces of formaldehyde.

Q15. Why are vaccines kept cold?

Answer: Keeping vaccines cold right up until they are administered (known as 'maintaining the cold chain') is important because the antigens in a vaccine are biological matter, and so will break down if exposed to high temperatures. This can reduce or even entirely destroy a vaccine's potency. Biological molecules also degrade naturally over time, and reducing their temperature slows the rate of degradation. However, vaccines containing aluminium-based adjuvants cannot be frozen, as sub-zero temperatures upset the adjuvants' structures, rendering them ineffective. World Health Organization (WHO) recommends keeping most vaccines between 2°C and 8°C, though there are some exceptions. The oral polio vaccine, for instance, is notoriously unstable, and so WHO recommends storing it between –25°C and –15°C ahead of it being distributed for administration.

MULTIPLE CHOICE QUESTIONS (MCQS)

Q1. Vaccines protect from infections by:

a. Destroying the infective agent in the environment
b. Killing the insect vectors responsible for the spread of infection
c. Increasing the immunity of the host
d. None of the above

Q2. Vaccines usually provide passive immunity to the host.

a. True
b. False

Q3. Which of these vaccines is used to prevent tuberculosis?

a. BCG
b. HCG
c. MMR
d. DPT

Q4. A booster dose is an additional dose of a vaccine given to enhance the immunity against the particular infection.

a. True
b. False

Q5. A vaccine administered to young girls to prevent cervical cancer is:

a. OPV
b. HPV
c. TAB
d. CCV

Q6. Which of these vaccines is available for oral administration?

a. Measles
b. Rabies

c. Hepatitis B
d. Typhoid

Q7. The biggest challenges to improving global vaccine coverage are:
a. Limited resources
b. Competing health priorities
c. poor management of health systems
d. All of the above

Q8. Which of the following is NOT a vaccine-preventable disease?
a. Cervical cancer
b. Polio
c. Hepatitis B
d. Asthma

Q 9. An attenuated vaccine is composed of
a. killed microorganisms.
b. living, weakened microorganisms.
c. inactivated bacterial toxins.
d. purified macromolecules.

Q10. Passive immunization is routinely administered to individuals exposed to certain microbial pathogens that cause diseases, EXCEPT:
a. Botulism
b. Diphtheria
c. Hepatitis
d. Chicken pox

Q11. An immunogenic yet non-toxic derivative of toxin is called?
a. Vaccine
b. Toxoid
c. Endotoxin
d. Exotoxin

Q12. Vaccination induces:

a. Naturally acquired active immunity
b. Artificially acquired active immunity
c. Naturally acquired passive immunity
d. d. Artificially acquired passive immunity

Q13. Immunosuppressive measures are most effective when administered:

a. just prior to antigen exposure
b. one week before antigen exposure
c. at the time of antigen exposure
d. following antigen exposure

Q14. The tuberculin skin test is an example of:

a. type IV delayed hypersensitivity
b. allergy reaction
c. serum sickness
d. precipitation reaction

Q15. Which of the following is/are true about conjugated vaccines:

a. Conjugated vaccines are those in which there is more than one vaccine antigen, e.g., MMR.
b. Conjugation involves attaching a polysaccharide antigen to a carbohydrate carrier.
c. Meningitis C vaccine is not available in a conjugated form.
d. Hib vaccine is an example of a conjugated vaccine.

Answers Key

1. C **2**. B **3**. A **4**. A **5**. B **6**. D **7**. D **8**. D **9**. B **10**. D **11**. B **12**. A **13**. C **14**. A **15**. C

ASSIGNMENTS

Long Answer Questions (500 words)

Q1. Explain the relationship between the incubation period of a pathogen and the approach needed to achieve effective active immunization.

Q2. A young girl who had never been immunized to tetanus was injured deeply by a rusty nail. The doctor after cleaning out the wound gave her an injection of tetanus antitoxin;

a. Why was antitoxin given instead of a booster shot of tetanus toxoid?
b. If she receives no further treatment and is again injured by a rusty nail 3 years later, what shot will she be given?

Short Answer Questions (200 words)

Q1. What are the advantages and disadvantages of using attenuated organisms as vaccines?

Q2. List the three types of purified macromolecules that are currently used as vaccines.

Q3. Explain the phenomenon of herd immunity. How does this phenomenon relate to the occurrence of epidemics?

REFERENCES

Afzal, M. F., et al. 2000. Clinical safety issues of measles, mumps, and rubella vaccines. WHO Bull. 78:199.

Alireza Tahamtan. *An Overview of History, Evolution, and Manufacturing of Various Generations of Vaccines.*

Bloom, D. E., Canningm, D., Weston, M. *The value of vaccination* 2005; Clem AS. Fundamentals of vaccine immunology. *J Glob Infect Dis.* 2011; **3**(1): 73-8.

Bloom, B. R. 1998. The highest attainable standard: ethical issues in AIDS vaccines. *Science* 279:186.

Brentjens R. J., et al. CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. *Sci Transl Med*. 2013;5(177):177ra38.

Centers for Disease Control and Prevention. 2009. *Immunity Types.* [Last cited on 2009 Dec 20].

Coler, R. N., Reed, S. G. Second-generation vaccines against leishmaniasis. *Trends Parasitol.* 2005; 21(5): 244-9.

Dadar, M., Chakraborty, S., Dhama, K., Prasad, M., Khandia, R, Hassan, S, … Chaicumpa, W. (2018). Advances in Designing and Developing Vaccines, Drugs and Therapeutic Approaches to Counter Human Papilloma Virus. *Frontiers in immunology*, *9*, 2478. doi:10.3389/ fimmu.2018.02478.

Delany I, Rappuoli R, & De Gregorio E. (2014). Vaccines for the 21st century. *EMBO molecular medicine*, *6*(6), 708–720. doi:10.1002/ emmm.201403876.

Di Tommaso A, et al. 1997. Acellular pertussis vaccines containing genetically detoxified pertussis toxin induce long-lasting humoral and cellular responses in adults. *Vaccine* 15:1218.

Dittmann, S, 2000. Sucessful control of epidemic diphtheria in the states of the former Union of Soviet Socialist Republics: lessons learned. *J. Inf. Dis.* 181 (Suppl. 1):S10.

Donnelly JJ, Ulmer JB, Shiver JW, Liu MA. DNA vaccines. *Annual review of immunology.* 1997 Apr; 15(1):617-48.

Ehreth J. The global value of vaccination. *Vaccine.* 2003; 21(7-8): 596-600.

Fahim Halim Khan. *The Elements of Immunology.*

Goldsby RA, Kindt TJ, Osborne BA, Kuby J. New York: Freeman. 2003

Grandi, G. 2001. Antibacterial design using genomics and proteomics. *Trends in Biotech.* 19:181.

Gurunathan S, Klinman DM, Seder RA. DNA vaccines: immunology, application, and optimization. *Annual review of immunology*. 2000 Apr; 18(1):927-74.

Gurunathan, S, et al. 2000. DNA vaccines: immunology, application and optimization. *Ann. Rev. Immunol.* 18:927.

Han S. Clinical vaccine development. *Clin Exp Vaccine Res.* 2015; 4(1): 46-53.

Hilleman MR. Vaccines in historic evolution and perspective: a narrative of vaccine discoveries. *Vaccine*. 2000; 18(15): 1436-47.

Huu Thuy Trang Duong, Thavasyappan Thambi, Yue Yin, Jung Eun Lee, Young Kyu Seo, Ji Hoon Jeong, Doo Sung Lee. Smart pH-Responsive Nanocube-Controlled Delivery of DNA Vaccine and Chemo-therapeutic Drugs for Chemoimmunotherapy. *ACS Applied Materials & Interfaces* 2019, *11*(14), 13058-13068. DOI: 10.1021/ acsami.8b21185.

Leung AKC. *History of Vaccine Development.* 2011; 5-12.

MacLennan Calman A, and Allan Saul. "Vaccines against poverty." *Proceedings of the National Academy of Sciences of the United States of America,* vol. 111, 34 (2014): 12307-12. doi:10.1073/pnas. 1400473111.

Marie Paule Kieny 2004 November. *Research and Development of New Vaccines against Infectious Diseases.*

Melero I, et al. Therapeutic vaccines for cancer: an overview of clinical trials. *Nat Rev Clin Oncol*. 2014; 11(9):509–524.

Moticka Edward J. *A Historical Perspective on Evidence-Based Immunology*-Elsevier (2015).

Nagill R, Kaur S. Vaccine candidates for leishmaniasis: a review. *Int Immunopharmacol.* 2011; 11(10): 1464-88.

Osborne Barbara A, and Kuby Janis. *Immunology.*

Pagliusi S., Dennehy M., Kim, H, & DCVMN AGM Organizing Committee (2018). Vaccines, inspiring innovation in health. *Vaccine, 36*(48), 7430–7437. doi:10.1016/j.vaccine.2018.05.035.

Pastan I, Hassan R. Discovery of mesothelin and exploiting it as a target for immunotherapy. *Cancer Res*. 2014;74(11):2907–2912.

Plotkin SA. *History of vaccine development.* 2011.

Plotkin SA, Plotkin SL. (2011). The development of vaccines: How the past led to the future. *Nat Rev Microbiol* 9(12):889–893.

Rappuoli R, Aderem A. (2011). A 2020 vision for vaccines against HIV, tuberculosis and malaria. *Nature* 473(7348):463–469.

Rappuoli, R, Pizza M, Del Giudice G, & De Gregorio E. (2014). Vaccines, new opportunities for a new society. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(34), 12288–12293. doi:10.1073/pnas.1402981111.

Reardon DA, et al. An update on vaccine therapy and other immunotherapeutic approaches for glioblastoma. *Expert Rev Vaccines.* 2013;12(6):597–615.

Riedel S. Edward Jenner and the history of smallpox and vaccination. *Proc (Bayl Univ Med Cent).* 2005; 18(1): 21-5.

Sette A, Rappuoli R. (2010). Reverse vaccinology: Developing vaccines in the era of genomics. *Immunity* 33(4):530–541.

Stover CK, De La Cruz VF, Fuerst TR, Burlein JE, Benson LA, Bennett LT, Bansal GP, Young JF, Lee MH, Hatfull GF, Snapper SB. New use of BCG for recombinant vaccines. *Nature.* 1991 Jun; 351(6326):456.

Tahamtan A, Charostad J, Shokouh H, Javad S, Barati M. An overview of different types of immune stimulating adjuvants and their application. *Paramedical Sci Mil Health.* 2016; 11(3): 43-51

Tahamtan A, Ghaemi A, Gorji A, Kalhor HR, Sajadian A, Tabarraei A, et al. Antitumor effect of therapeutic HPV DNA vaccines with chitosan-based nanodelivery systems. *J Biomed Sci.* 2014; 21: 69.

Teimourpour R, Meshkat Z, Arzanlou M, Peeridogaheh H. DNA vaccine: The third generation vaccine [In Persian]. *Qom Univ Med Sci J.* 2016; 10(10): 86-99.

Walter S, et al. Multipeptide immune response to cancer vaccine IMA901 after single-dose cyclophosphamide associates with longer patient survival. *Nat Med*. 2012;18(8):1254–1261.

Wheeler AW, Woroniecki SR. Immunological adjuvants in allergy vaccines: past, present future. *Allergology International.* 2001 Jan 1;50(4):295-301.

Yang Shi, Twan Lammers. Combining Nanomedicine and Immunotherapy. *Accounts of Chemical Research* 2019, *52* (6), 1543-1554. DOI: 10.1021/acs.accounts.9b00148.

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Chapter 10

IMMUNE DYSFUNCTION: AIDS AND RHEUMATOID ARTHRITIS

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ABSTRACT

Immune dysfunction is the disorder of the immune system. The disorders can be characterized in several ways like the component(s) of the immune system affected or whether the immune system is overactive or underactive and whether the condition is congenital or acquired. An autoimmune disease is a condition arising from an abnormal immune response to a normal body part. Autoimmune mechanisms underlie many diseases, some organ-specific, others systemic in distribution, and these autoimmune disorders can overlap an individual may have more than one organ specific disorder, or more than one systemic disease. Genetic

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factors play a role in the development of autoimmune diseases. Factors such as HLA type are important. The most significant global cause of immunodeficiency is HIV infection. Over 25 million people have died from AIDS since the first cases were described in 1981. Acute HIV infection is associated with a transient depletion of peripheral CD4T cells. Rheumatoid arthritis (RA) is an autoimmune disease that causes pain and swelling of the joints. The normal role of your body's immune system is to fight off infections to keep you healthy. In an autoimmune disease, your immune system starts attacking your own healthy tissues. In RA, the immune system targets the lining of the joints, causing inflammation and joint damage.

Keywords: immune dysfunction, hashimoto disease, autoreactive cells, HIV, CD4 T-cells, HLA, rheumatoid arthritis

OBJECTIVES

- To discuss the immune dysfunction
- To highlight the effects of autoimmunity
- To discuss immunodeficiency
- To describe AIDS and HIV
- To discuss rheumatoid arthritis and its management

INTRODUCTION

The immune system has tremendous diversity and, because the repertoire of specificities expressed by the B and T cell populations is generated randomly, it is bound to include many that are specific for self-components. The body must therefore establish self-tolerance mechanisms to distinguish between self and non-self-determinants to avoid auto-reactivity. However, all mechanisms have a risk of breakdown. The self-recognition mechanisms are no exception, and a number of diseases have been identified in which there is autoimmunity, due to bountiful production of auto-antibodies and autoreactive T cells. One of the earliest examples in

which the production of auto-antibodies was associated with disease in a given organ is Hashimoto's thyroiditis.

Hashimoto's thyroiditis has been particularly well studied, among the autoimmune diseases. It is a disease of the thyroid that is most common in middle-aged women and often leads to formation of a goitre and hypothyroidism. The gland is infiltrated, sometimes to an extraordinary extent, with inflammatory lymphoid cells. These are predominantly mononuclear phagocytes, lymphocytes and plasma cells, and secondary lymphoid follicles are common.

In Hashimoto's disease, the gland often shows regenerating thyroid follicles, but this is not a feature of the thyroid in the related condition, primary myxedema, in which comparable immunological features are seen and where the gland undergoes almost complete destruction and shrinks. The serum of patients with Hashimoto's disease usually contains antibodies to thyroglobulin. These antibodies are demonstrable by agglutination and by precipitin reactions when present in high titer. Most patients also have antibodies directed against a cytoplasmic or microsomal antigen, also present on the apical surface of the follicular epithelial cells and now known to be thyroid peroxidase, the enzyme that iodinates thyroglobulin (Figure 10.1).

An autoimmune disease is a condition arising from an abnormal immune response to a normal body part. Autoimmunity is associated with disease. Autoimmune mechanisms underlie many diseases, some organ-specific, others systemic in distribution, and these autoimmune disorders can overlap an individual may have more than one organ specific disorder, or more than one systemic disease. Genetic factors play a role in the development of autoimmune diseases. Factors such as HLA type are important, and it is probable that each disease involves several factors. Self-reactive B and T cells persist even in normal subjects. Autoreactive B and T cells persist in normal subjects, but in disease are selected by autoantigen in the production of autoimmune responses. Controls on the development of autoimmunity can be bypassed. Microbial cross-reacting antigens and cytokine dysregulation can lead to autoimmunity.

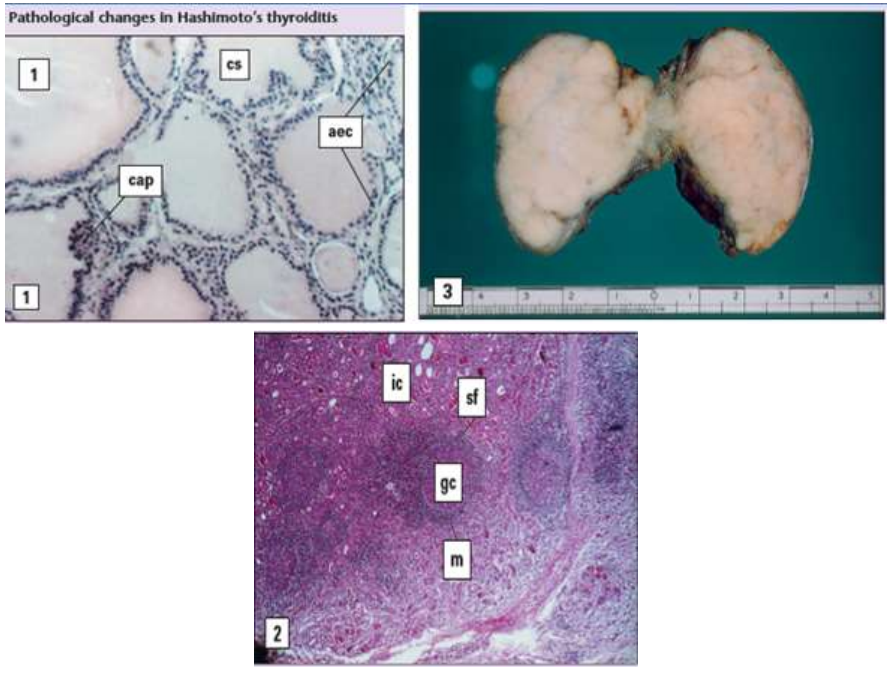

Figure 10.1. (1) The normal thyroid gland, the aciner epithelial cells (aee) line the colloid space (es) into which they secrete thyroglobulin, which is broken down on demand to provide thyroid hormones (cap, capillaries containing red blood cells). In the Hashimoto gland (2), the normal architecture is virtually destroyed and replaced by invading cells (ie), which consist essentially of lymphocytes, macrophages, and plasma cells. A secondary lymphoid follicle (sf), with a germinal center (gc) and a mantle of small lymphocytes (m), is present. H&E stain. X80. (Reproduced from Woolf N. Pathology: basic and systemic. London: WB Saunders; 1998) (3) In contrast to the red color and soft texture of the normal thyroid, the pale and firm gross appearance of the Hashimoto gland reflects the loss of colloid and heavy infiltration with inflammatory cells (Adapted from David male, immunology 7th edition).

In most diseases associated with autoimmunity, the autoimmune process produces the lesions. The pathogenic role of autoimmunity can be demonstrated in experimental models. Human autoantibodies can be directly pathogenic. Immune complexes are often associated with systemic autoimmune disease. Autoantibody tests are valuable for diagnosis and sometimes for prognosis. Treatment of autoimmune disease has a variety of aims. Treatment of organ-specific diseases usually involves metabolic control. Treatment of systemic diseases includes the use of anti-

inflammatory and immunosuppressive drugs. Future treatment will probably focus on manipulation of the pivotal autoreactive T cells by antigens or peptides, by anti-CD4, and possibly by T cell vaccination.

Some drugs selectively alter immune function. Immunomodulatory drugs can severely depress immune functions. Steroids affect cell traffic, induce leukocytopenia, and inhibit cytokine synthesis. Cyclophosphamide, azathioprine, and mycophenolate mofetil act directly on DNA or its synthesis. Nutrient deficiencies are generally associated with impaired immune responses. Malnutrition increases the risk of infant mortality from infection through reduction in cell-mediated immunity, reduced CD4 helper cells, reduced T cell help, and a reduction of secretory IgA. Trace elements, iron, selenium, copper, and zinc are important in immunity. Lack of these elements can lead to diminished neutrophil killing of bacteria and fungi, susceptibility to viral infections, and diminished antibody responses. Vitamins A, B6, C, E, and folic acid are important in overall resistance to infection. Carotenoids are antioxidants like vitamin C and E and can enhance NK cell activity, stimulate the production of cytokines and increase the activity of phagocytic cells. Diet and nutrition are powerful innovative tools to reduce illness and death caused by infection. The most significant global cause of immunodeficiency is HIV infection. AIDS is caused by HIV, which is a double stranded RNA retrovirus that infects CD4 T cells. Severe CD4 depletion results from a variety of mechanisms, with drastic functional impairment of cell-mediated immunity and death from opportunistic infections. Combination therapy for AIDS with inhibitors of reverse transcriptase, protease, and viral entry are reasonably successful, but associated with long-term toxicities in almost 50% of persons. An effective vaccine remains an elusive goal.

ACQUIRED IMMUNE DEFICIENCY SYNDROME

The most significant global cause of immunodeficiency is HIV infection. HIV is a retrovirus that is transmitted sexually, in blood or blood products, and perinatally. There are two main variants, HIV-1 and HIV-2:

- HIV-2 is endemic in West Africa and appears to be less pathogenic;
- HIV-1 has several subtypes (or clades), which are designated by the letters A through K, and the prevalence of the different clades varies by geographical region – over 90% of people infected with HIV-1 live in developing countries and spread is 80% by the sexual route.

Over 25 million people have died from AIDS since the first cases were described in 1981. Although sub-Saharan Africa has the highest prevalence of HIV-1 infection in the world (7.4% for the region), the largest increase of new infections is occurring in East Asia, where the number of HIV-positive people increased by 50% between 2002 and 2004. As of the end of 2004, The World Health Organization (WHO) estimates that, approximately 40 million people are living with HIV infection worldwide, with approximately five million new infections and three million deaths due to AIDS each year.

CD4 Antigen Is the Main Receptor for HIV Entry

HIV is an enveloped retrovirus that contains two copies of a single-stranded RNA (ssRNA) genome (fig). Upon entry into a cell, the genome is reverse transcribed into complementary DNA (cDNA), which is integrated into the host cell genome (provirus). The 10-kilobase genome encodes nine genes flanked at each end by long terminal repeat (LTR) sequences, which are:

- Essential for integration of viral DNA into the host cell DNA; and
- contain binding sites for regulatory proteins involved in viral replication.

The basic gene structure contains *gag* (core protein), *pol* (reverse transcriptase, protease, and integrase enzymes), and *env* (envelope protein)

genes. In addition to these three main gene products, the virus encodes six regulatory and accessory proteins (Tat, Rev, Vpr,Vpu, Vif, and Nef), which regulate viral protein synthesis (Figure 10.2). The viral genes overlap in different reading frames and the proteins are generated by alternative splicing mechanisms. CD4 antigen is the main receptor for viral entry it is present on $CD4^+$ T lymphocytes, monocytes, dendritic cells (DCs), and brain microglia. The envelope glycoprotein gp120 is also capable of binding DC-SIGN. Although HIV-1 does not use DC-SIGN to infect DCs, this interaction allows DCs to internalize HIV-1 at mucosal sites and then migrate to lymph nodes where they deliver HIV-1 to CD4 T cells (Figure 10.3).

HIV Infection Induces Strong Immune Responses

Both humoral and cellular immune responses develop after infection. Detectable HIV-specific antibodies are evident in the first few weeks of infection. Production of neutralizing antibodies (which prevent virus-cell fusion and correlate with protection) does not occur until at least 12 weeks after infection. Therefore, although antibodies are sufficient to drive evolution of viral epitopes, they are insufficient to prevent disease progression.

Evading the Immune Response

HIV is a rapidly mutating virus. Both error-prone reverse transcription and high recombination frequencies during reverse transcription combine with an extremely high virus production rate to generate genetic diversity. Particularly during acute infection, immune clearance via antibody and CTL recognition favor survival of virions with envelope or peptide sequence changes in regions targeted by host-protective epitopes. Additionally, the HIV protein Nef limits CTL detection of infected cells via selective downregulation of both HLA-A and HLA-B expression,

reducing the surface display of viral epitopes. Viral escape, coupled with the existence of latent viral reservoirs undetectable by HIV-specific immune responses, prevents the immune system from eliminating HIV infected cells. Exacerbating this issue, the immune responses become progressively weaker over time.

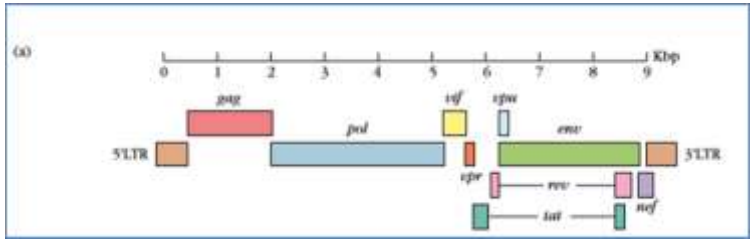

Figure 10.2. Genetic organization of HIV-1 (a) and functions of encoded proteins

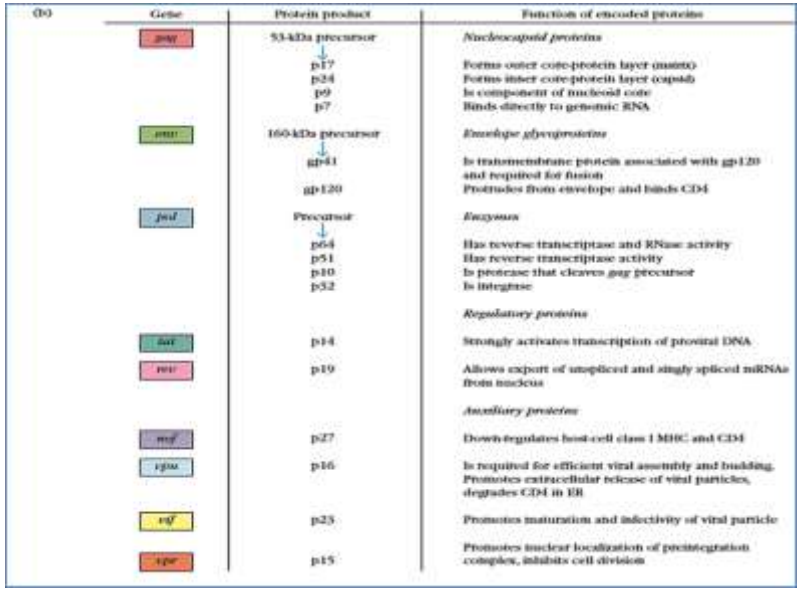

Figure 10.2. Genetic organization of HIV-1 (a) and functions of encoded proteins (b). The three major genes—gag, pol, and env—encode polypeptide precursors that are cleaved to yield the nucleocapsid core proteins, enzymes required for replication, and to envelop core proteins. Of the remaining six genes, three (tat, rev, and nef) encode regulatory proteins that play a major role in controlling expression, two (vif and vpu) encode proteins required for virion maturation, and one (vpr) encodes a weak transcriptional activator. The 5' long terminal repeat (LTR) contains sequences to which various regulatory proteins bind. The organization of the HIV-2 and SIV genomes is very similar except that the vpu gene is replaced by vpx in both of them (Adapted from Kuby, 7th edition).

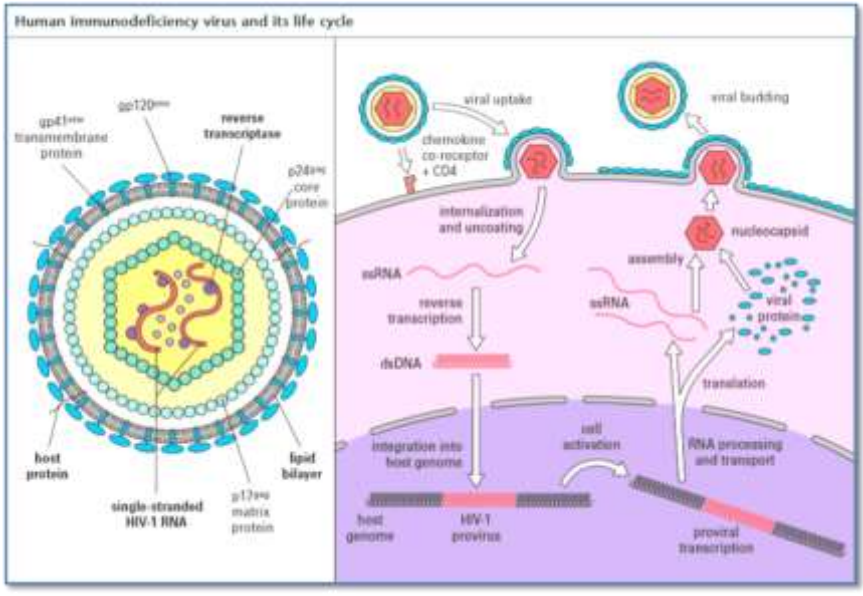

Figure 10.3. After attachment to CD4 and a chemokine coreceptor (usually CCR5), the virus membrane fuses with the cellular membrane to allow entry into the cell. Following uncoating, reverse transcription of viral RNA results in the production of double-stranded DNA (dsDNA). This is inserted into the host genome as the HIV provirus, by a virally coded integrase enzyme, which is a target for new antiviral medications currently in development. Cell activation leads to transcription and the production of viral mRNAs. Structural proteins are produced and assembled. Free HIV viruses are produced by viral budding from the host cell, after which further internal assembly occurs with the cleavage of a large precursor core protein into the small core protein components by a virally coded protease enzyme, producing mature virus particles, which are released and can go on to infect additional cells bearing CD4 and the chemokine co-receptor. (Adapted from David male immunology 7th edition).

IMMUNE DYSFUNCTION IN HIV

The chronic phase of HIV infection is marked by persistent generalized immune activation. Infected persons present with B cell polyclonal activation and hypergammaglobulinemia. Attachment of gp120 to mannose binding lectin and to subsets of Ig+ B cells contributes to this activation. Inflammatory cytokines such as IFNα, IFNγ, IL-18, IL-15, and TNFα are elevated during acute infection. Finally, increased translocation

of bacteria through the gut barrier, due to extensive HIV infection within the GALT and lamina propria, increases circulating LPS levels, activating many immune effectors via Toll-like receptors. In patients with high viral load, HIV-specific CTL typically contain low levels of intracellular perforin and have a poor proliferative capacity. During this chronic phase, CD8 T cells often express PD-1, a receptor associated with programmed cell death. The points above are all consistent with persistent activation and eventual exhaustion of the supply of anti-HIV CD8 T cells that worsens over time.

Of equal if not greater impact on overall immune dysfunction from HIV infection, however, is the loss of the CD4 T cells. Though circulating CD4 T cell counts often rebound (at least quantitatively if not qualitatively) to pre-infection levels after the acute phase, they do not recover within the mucosal associated lymphoid tissues. Furthermore, in untreated patients, CD4 T cell levels eventually decline though they may remain above critical levels for a period of 6 months to 10 years.

CD4 T cell loss is due to direct killing by HIV and activation-induced apoptosis. Additionally, infected CD4 T cells present both gp120 and HIV peptide: HLA complexes on the cell surface leaving them subject to anti-HIV B and CTL clearance. Ongoing loss of CD4 T cell help ultimately contributes to the collapse of the CD8 T cell response as demonstrated experimentally by the ability to rescue anti-HIV CD8 T cell functionality by replacing the lost CD4 T cell population. However, progressive immunodeficiency is a hallmark of HIV and the eventual diminished CD4 T cell levels correlate tightly with the subsequent progression to advanced disease and death.

AIDS is the Final Stage of HIV Infection and Disease

Progression to this clinical stage includes a CD4 T cell count of <200/μL. As blood CD4 T cell counts gradually decline during the chronic phase, patients become susceptible to opportunistic infection and malignancies. Below 500 CD4 cells/μL, less severe conditions such as oral

candidiasis, recurrent herpes virus outbreaks (e.g., shingles from varicella zoster virus and anogenital herpes from herpes simplex virus), and pneumococcal infections occur. CD4 T cell levels below 200/μL are associated with increased risk of life-threatening infections and malignancies including *Pneumocystis jirovecii* pneumonia and Kaposi's sarcoma, respectively. With CD4 levels below 50/μL, patients become vulnerable to additional systemic infection with organisms such as *Mycobacterium avium* complex. The three main organ systems affected are the respiratory system, gastrointestinal tract, and central nervous system. Neurological complications in AIDS are due to direct effects of HIV infection, opportunistic infections, or lymphoma. AIDS-related dementia once affected between 10–40% of patients with other manifestations of AIDS, but with more effective antiviral treatment has become less common. Kaposi's sarcoma (KS), caused by infection with KS-associated herpes virus (KSHV), is the most common AIDS-associated malignancy. KSHV infections, similar to CMV herpes virus infections, are often asymptomatic in individuals with competent T cell immunity. With HIV co-infection, however, KSHV titers increase and KS emerges with multifocal lesions of mixed cellularity often resulting in widespread involvement of skin, mucous membranes, viscera (gut and lungs) and lymph nodes.

Most of the opportunistic infections as well as malignancies, such as KS and Epstein–Barr virus-associated non-Hodgkin's lymphomas, are due to the inability of the immune response to suppress baseline levels of reactivation of latent organisms in the host and in some cases, to ubiquitous organisms to which we are continually exposed. They are difficult to diagnose and treatment often suppresses rather than eradicates them. Relapses are common and continuous suppressive or maintenance treatment is necessary.

AN EFFECTIVE VACCINE REMAINS AN ELUSIVE GOAL

Currently, treatment for HIV focuses on anti-viral drug cocktails that significantly reduce the patient's viral load. Due to the rapid rate of mutation in the viral genome during replication, single drug therapy nearly always leads to rapid drug resistance. However, by providing the patient with a cocktail of anti-viral drugs, each targeting a different aspect of the viral life cycle, it is possible to prolong the period of time before plasma viral load increases and T cell counts drop. Anti-retroviral therapies, unfortunately, are not a cure, nor can they prevent transmission of the virus.

Despite increasing characterization of adaptive immune responses, the correlates of protection remain to be fully defined, and this has left the field with mostly empiric approaches. Ideally, investigators will develop a vaccine that provides sufficient protection to prevent viral transmission. Encouraging early reports of persons repeatedly exposed to HIV who never became infected suggested that adaptive immune responses, particularly HIV-specific CD8 T cell responses, might be responsible for apparent protection, but this remains somewhat controversial. Such a vaccine will almost certainly also require the induction of broadly neutralizing antibody responses; something that candidate vaccines have yet to achieve. To date there have been clinical trials of numerous candidate HIV vaccines, but most would agree that an effective vaccine remains an elusive goal. As no cure or vaccine is currently available, our main weapon is prevention through health education and control of infection.

RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is an autoimmune disease that causes pain and swelling of the joints. The normal role of your body's immune system is to fight off infections to keep you healthy. In an autoimmune disease, your immune system starts attacking your own healthy tissues. In RA, the

immune system targets the lining of the joints, causing inflammation and joint damage. Most commonly, the wrist and hands are involved, with the same joints typically involved on both sides of the body. The disease may also affect other parts of the body. This may result in a low red blood cell count, inflammation around the lungs, and inflammation around the heart. Fever and low energy may also be present. Often, symptoms come on gradually over weeks to months. The erosions of cartilage and bone in rheumatoid arthritis are mediated by macrophages and fibroblasts, which become stimulated by cytokines from activated T cells and immune complexes generated by a vigorous immunological reaction within the synovial tissue. The course of the disease varies greatly. Some people have mild short-term symptoms, but in most the disease is progressive for life. Around 20%–30% will have subcutaneous nodules (known as rheumatoid nodules); this is associated with a poor prognosis.

SIGNS AND SYMPTOMS

The Signs and Symptoms of RA Include

- Pain and stiffness lasting for more than 30 minutes in the morning or after a long rest.
- Tender, warm, swollen joints. Joint inflammation often affecting the wrist and finger joints closest to the hand; other affected joints can include those of the neck, shoulders, elbows, hips, knees, ankles, and feet. Rheumatoid nodules are sometimes present.
- Symmetrical pattern. For example, if one knee is affected, the other one is also.
- Fatigue, occasional fever, a general sense of not feeling well (malaise).
- Symptoms affecting other parts of the body besides the joints.

Etiology

The etiology of RA is not fully understood despite extensive study of metabolic and nutritional factors, the endocrine system, and geographic, psychological, and occupational data. It now appears that an unknown antigen initiates the autoimmune response resulting in RA. This response supports the suspicion of an infectious origin of the disease process, which includes various bacteria and viruses, but without evidence of precipitating events. Even without this specific knowledge, treatment modalities have

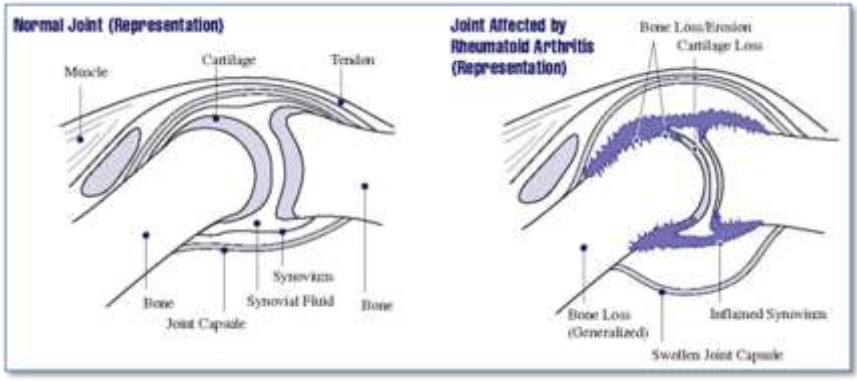

Figure 10.4. Diagram illustrating the comparison and etiology of rheumatoid affected joint.

been developed that, while not curing the disease, can provide relief from the symptoms of the disease. Evidence points to a complex interplay between environmental and genetic factors. In monozygotic twins, there is a more than 30 percent concordance rate for rheumatoid arthritis development, and 80 percent of whites with rheumatoid arthritis express the HLA-DR1 or -DR4 subtypes. These and other regions of the Major Histocompatibility Complex may confer susceptibility to more severe disease by causing a specific arthrogenic peptide to be presented to CD4+ T cells. Scientists are now focusing on the idea that it is a T-cell-mediated autosomal disease precipitated by both genetic and environmental factors (Figure 10.4).

PATHOPHYSIOLOGY

The joint capsule is lined with a type of tissue called synovium, which produces synovial fluid. The synovial fluid secreted by the synovium is thought to serve two main purposes, lubrication of the joint and provision of nutrients to the avascular articular cartilage. The attack on a joint by the disease usually begins with the synovium. Joint damage in rheumatoid arthritis begins with the proliferation of synovial macrophages and fibroblasts after a triggering incident, either autoimmune or infectious. White blood cells that are part of the normal immune system travel to the synovium and cause a reaction. This reaction, or inflammation, is called synovitis, and it results in warmth, redness, swelling, and pain that are typical symptoms of RA. Lymphocytes infiltrate the perivascular regions, endothelial cells proliferate and these result in neovascularization. Thus, early in the disease edema begins to be seen in cells in the synovium and multiplication of synovial lining cells occur. During the inflammation process, the cells of the synovium grow and divide abnormally, making the normally thin synovium thick and resulting in a joint that is swollen and puffy to the touch. Blood vessels in the affected joint become occluded with small clots or inflammatory cells. As the disease progresses, inflamed synovial tissue begins to grow considerably and irregularly, forming invasive pannus tissue. The pannus is a sheet of inflammatory granulation tissue that spreads from the synovial membrane and invades the joint in rheumatoid arthritis and destroys cartilage and bone ultimately leading to fibrous ankylosis. Pannus can be considered the most destructive element affecting joints in the patient with rheumatoid arthritis. Pannus can attack articular cartilage and destroy it. Further, pannus can destroy the soft subchondral bone once the protective articular cartilage is gone. There is chronic inflammation with lymphocytes and plasma cells that produce the blue areas beneath the nodular proliferations. Multiple cytokines, interleukins, proteinases, and growth factors are released, causing further joint destruction and the development of systemic complications (Figure 10.5).

Pathology of Rheumatoid Arthritis

In this disease process, an interaction between antibodies and antigens occurs, and causes alterations in the composition of the synovial fluid. Ultimately, digestants are formed in the fluid that attacks the surrounding tissue. Once the composition of this fluid is altered, it is less able to perform the normal functions noted above, and more likely to become destructive. The changes in the synovium and synovial fluid are responsible for a large amount of joint and soft tissue destruction. The destruction of bone eventually leads to laxity in tendons and ligaments. Under the strain of daily activities and other forces, these alterations in bone and joint structure result in the deformities frequently seen in patients with rheumatoid arthritis. Considerable destruction of the joint can occur with pannus invading the subchondral bone.

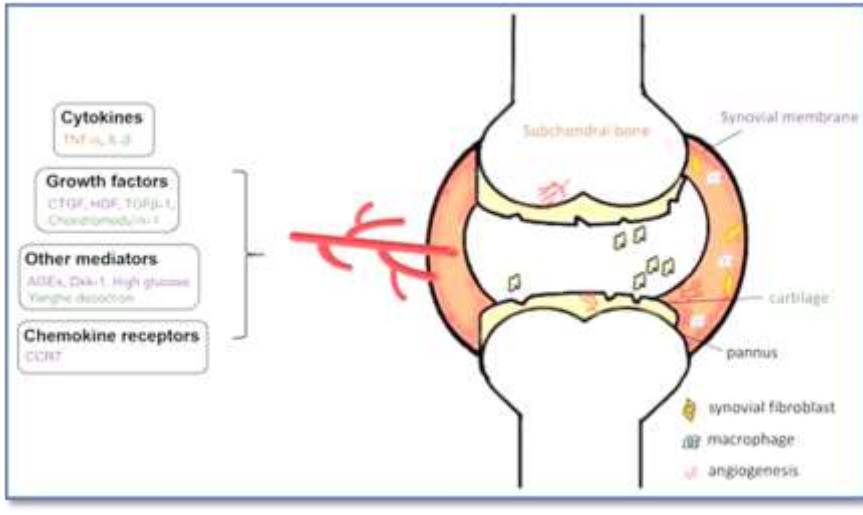

Figure 10.5. In the rheumatoid arthritis joint an inflammatory infiltrate is found in the synovial membrane, which hypertrophies forming a 'pannus'. This covers and eventually erodes the synovial cartilage and bone. Immune complexes and neutrophils (PMNs) are detectable in the joint space and in the extra-articular tissues where they may give rise to vasculitic lesions and subcutaneous nodules (Implications of Angiogenesis involvement in Arthritis, *Int. J. Mol. Sci.* 2018, *19*(7), 2012; https://doi.org/10.3390/ijms19072012).

Bone destruction occurs at areas where the hyaline cartilage and the synovial lining do not adequately cover the bone. If the disease progresses to a more advanced stage, the articular cartilage may lose its structure and density resulting in an inability to withstand the normal forces placed on the joint. In such advanced cases, muscle activity causes the involved ends of the bones to be compressed together causing further bone destruction. Further, the disease can irreversibly change the structure and function of a joint to a degree that other degenerative changes may occur, especially in the weight bearing joints of the body. Thus, joint destruction can progress to the degree that joint motion is significantly limited and joints can become markedly unstable.

While rheumatoid arthritis (RA) primarily affects joints, problems involving other organs of the body are known to occur. Extra-articular (outside the joints) manifestations other than anemia (very common) are clinically evident in about 15–25% of individuals with rheumatoid arthritis. It is difficult to determine whether disease manifestations are directly caused by the rheumatoid process itself, or from side effects of the medications commonly used to treat it for example, lung fibrosis from methotrexate or osteoporosis from corticosteroids.

Management of Rheumatoid Arthritis

There is no cure for RA, but treatments can improve symptoms and slow the progress of the disease. Disease-modifying treatment has the best results when it is started early and aggressively. The goals of treatment are to minimize symptoms such as pain and swelling, to prevent bone deformity (for example, bone erosions visible in X-rays), and to maintain day-to-day functioning. This is primarily addressed with disease-modifying antirheumatic drugs (DMARDs); analgesics may be used to help manage pain. RA should generally be treated with at least one specific anti-rheumatic medication. The use of benzodiazepines (such as diazepam) to treat the pain is not recommended as it does not appear to help and is associated with risks.

Lifestyle

Regular exercise is recommended as both safe and useful to maintain muscles strength and overall physical function. It is uncertain if specific dietary measures have an effect. Physical activity is beneficial for persons with rheumatoid arthritis complaining of fatigue. Occupational therapy has a positive role to play in improving functional ability of persons with rheumatoid arthritis.

Disease Modifying Agents

Disease-modifying anti-rheumatic drugs (DMARDs) are the primary treatment for RA. They are a diverse collection of drugs, grouped by use and convention. They have been found to improve symptoms, decrease joint damage, and improve overall functional abilities. DMARDs should be started early in the disease as they result in disease remission in approximately half of people and improved outcomes overall. The following drugs are considered as DMARDs: methotrexate, hydroxyl-chloroquine, sulfasalazine, leflunomide, TNF-alpha inhibitors (certolizumab, infliximab and etanercept), abatacept, and anakinra. Rituximab and tocilizumab are monoclonal antibodies and are also DMARDs.

The most commonly used agent is “methotrexate” with other frequently used agents including sulfasalazine and leflunomide. Leflunomide is effective when used from 6-12 months, with similar effectiveness to “methotrexate” when used for 2 years. Methotrexate is the most important and useful DMARD and is usually the first treatment. Adverse effects should be monitored regularly with toxicity including gastrointestinal, hematologic, pulmonary, and hepatic. Side effects such as nausea, vomiting or abdominal pain can be reduced by taking folic acid.

Biological agents should generally only be used if methotrexate and other conventional agents are not effective after a trial of three months. They are associated with a higher rate of serious infections as compared to

other DMARDs. Biological DMARD agents used to treat rheumatoid arthritis include: tumor necrosis factor alpha (TNFα) blockers such as infliximab; interleukin 1 blockers such as anakinra, monoclonal antibodies against B cells such as rituximab, and tocilizumab T cell co-stimulation blocker such as abatacept. They are often used in combination with either methotrexate or leflunomide. Abatacept should not be used at the same time as other biologics. In those who are well controlled on TNF blockers decreasing the dose does not appear to affect overall function. Persons should be screened for latent tuberculosis before starting any TNF blockers therapy to avoid reactivation.

TNF blockers and methotrexate appear to have similar effectiveness when used alone and better results are obtained when used together. TNF blockers appear to have equivalent effectiveness with etanercept appearing to be the safest. Abatacept appears effective for RA with 20% more people improving with treatment than without but long term safety studies are yet unavailable. However, there is a lack of evidence to distinguish between the biologics available for RA. Issues with the biologics include their high cost and association with infections including tuberculosis.

Anti-Inflammatory and Analgesic Agents

Glucocorticoids can be used in the short term and at the lowest dose possible for flare-ups and while waiting for slow-onset drugs to take effect. Non-NSAID drugs to relieve pain, like paracetamol may be used to help relieve the pain symptoms; they do not change the underlying disease.

NSAIDs reduce both pain and stiffness in those with RA but do not affect the underlying disease and appear to have no effect on people's long term disease course and thus are no longer first line agent. NSAIDs should be used with caution in those with gastrointestinal, cardiovascular, or kidney problems. Use of methotrexate together with NSAIDS is safe, if adequate monitoring is done. COX-2 inhibitors, such as celecoxib, and NSAIDs are equally effective.

The neuromodulator agents topical capsaicin may be reasonable to use in an attempt to reduce pain. Nefopam by mouth and cannabis are not recommended as of 2012 as the risks of use appear to be greater than the benefits.

Surgery

Especially for affected fingers, hands, and wrists, synovectomy may be needed to prevent pain or tendon rupture when drug treatment has failed. Severely affected joints may require joint replacement surgery, such as knee replacement. Postoperatively, physiotherapy is always necessary.

Scientists are making rapid progress towards understanding the complexities of rheumatoid arthritis: how and why - it develops, some people get it and others do not, some people get it more severely than others? Results from research are having an impact today, enabling people with rheumatoid arthritis to remain active in life, family, and work far longer than was possible 20 years ago. In the future hope lies in the fact as researchers begin to apply new technologies such as stem cell transplantation and novel imaging techniques. These and other advances will lead to an improved quality of life for people with rheumatoid arthritis and also provide for a holistic approach and cure for the same.

CONCLUSION

Immune dysfunction is the disorder of the immune system. The disorders can be characterized in several ways like the component(s) of the immune system affected or whether the immune system is overactive or underactive and whether the condition is congenital or acquired. An autoimmune disease is a condition arising from an abnormal immune response to a normal body part. There are at least 80 types of autoimmune diseases. Autoimmune mechanisms underlie many diseases, some organ-specific, others systemic in distribution, and autoimmune disorders can

overlap an individual may have more than one organ specific disorder, or more than one systemic disease. In most diseases associated with autoimmunity, the autoimmune process produces the lesions. The pathogenic role of autoimmunity can be demonstrated in experimental models. Human autoantibodies can be directly pathogenic. Immune complexes are often associated with systemic autoimmune disease. Autoantibody tests are valuable for diagnosis and sometimes for prognosis.

Rheumatoid arthritis (RA) is a long-term autoimmune disorder that primarily affects joints. It typically results in warm, swollen, and painful joints. Pain and stiffness often worsen following rest. Most commonly, the wrist and hands are involved, with the same joints typically involved on both sides of the body. The disease may also affect other parts of the body. This may result in a low red blood cell count, inflammation around the lungs, and inflammation around the heart. Fever and low energy may also be present. Often, symptoms come on gradually over weeks to months. The erosions of cartilage and bone in rheumatoid arthritis are mediated by macrophages and fibroblasts, which become stimulated by cytokines from activated T cells and immune complexes generated by a vigorous immunological reaction within the synovial tissue.

Immunodeficiency (or immune deficiency) is a state in which the immune system's ability to fight infectious disease and cancer is compromised or entirely absent. Primary immune deficiency diseases are those caused by inherited genetic mutations. Secondary or acquired immune deficiencies are caused by something outside the body such as a virus or immune suppressing drugs. Human immunodeficiency virus infection and acquired immune deficiency syndrome (HIV/AIDS), a secondary immune deficiency is a spectrum of conditions caused by infection with the human immunodeficiency virus (HIV). HIV is an enveloped retrovirus that contains two copies of a single-stranded RNA (ssRNA) genome. Upon entry into a cell, the genome is reverse transcribed into complementary DNA (cDNA), which is integrated into the host cell genome (provirus). Acute HIV infection is associated with a transient depletion of peripheral CD4T cells.

FREQUENTLY ASKED QUESTIONS (FAQS)

Q1. What is an immune dysfunction?

Answer: Immune dysfunction is the disorder of the immune system. The disorders can be characterized in several ways like the component(s) of the immune system affected or whether the immune system is overactive or underactive and whether the condition is congenital or acquired.

Q2. Why is it difficult to identify suitable antigens that could be used in a neutralizing vaccine?

Answer: The very rapid rate of HIV mutation and the high rate of virus production mean that the virus can readily mutate to evade a specific immune response, and yet may develop variants that retain infectivity.

Q3. What is the functional significance of the observation that Nef does not decrease HLA-C and HLA-E?

Answer: HLA-C and HLA-E are inhibitory signals for natural killer cells. Therefore, Nef can decrease cell recognition by CTLs while not increasing susceptibility to NK cells

Q4. What is likely to be the effect on the virus of antibodies to V2 and V3 during an HIV infection?

Answer: Specific antibodies will act as selective pressure on the virus to mutate these segments of the antigen to avoid immune recognition.

Q5. How will the switch in the viral phenotype affect which cells are infected?

Answer: CCR5 is selectively expressed on mononuclear phagocytes. CXCR4 is present on a number of cell types, including naive T cells, B cells, and monocytes; consequently, there is a shift towards T cell infectivity.

Q6. What is rheumatoid arthritis?

Answer: Rheumatoid arthritis (RA) is an autoimmune disease that causes pain and swelling of the joints. The normal role of your body's immune system is to fight off infections to keep you healthy. In an autoimmune disease, your immune system starts attacking your own healthy tissues. In RA, the immune system targets the lining of the joints, causing inflammation and joint damage.

Q7. What are the symptoms of rheumatoid arthritis?

Answer: The signs and symptoms of RA include:

- Pain and stiffness lasting for more than 30 minutes in the morning or after a long rest.
- Tender, warm, swollen joints. Joint inflammation often affecting the wrist and finger joints closest to the hand; other affected joints can include those of the neck, shoulders, elbows, hips, knees, ankles, and feet. Rheumatoid nodules are sometimes present.
- Symmetrical pattern. For example, if one knee is affected, the other one is also.
- Fatigue, occasional fever, a general sense of not feeling well (malaise).
- Symptoms affecting other parts of the body besides the joints.

Q8. What do u understand by AIDS?

Answer: AIDS is the final stage of HIV infection and disease. Progression to this clinical stage includes a CD4 T cell count of <200/μL. As blood CD4 T cell counts gradually decline during the chronic phase, patients become susceptible to opportunistic infection and malignancies. Below 500 CD4 cells/μL, less severe conditions such as oral candidiasis, recurrent herpes virus, and pneumococcal infections occur. CD4 T cell levels below 200/μL are associated with increased risk of life-threatening infections and malignancies including *Pneumocystis jirovecii* pneumonia and Kaposi's sarcoma.

Q9. What do you understand by HIV?

Answer: HIV is an enveloped retrovirus that contains two copies of a single-stranded RNA (ssRNA) genome (Figure 1). Upon entry into a cell, the genome is reverse transcribed into complementary DNA (cDNA), which is integrated into the host cell genome (provirus).

Q10. How does HIV evade the immune responses?

Answer: HIV is a rapidly mutating virus. Both error-prone reverse transcription and high recombination frequencies during reverse transcription combine with an extremely high virus production rate to generate genetic diversity. Particularly during acute infection, immune clearance via antibody and CTL recognition favor survival of virions with envelope or peptide sequence changes in regions targeted by host-protective epitopes. Additionally, the HIV protein Nef limits CTL detection of infected cells via selective downregulation of both HLA-A and HLA-B expression, reducing the surface display of viral epitopes.

Q11. How are joints affected in rheumatoid arthritis?

Answer: The attack on a joint by the disease usually begins with the synovium. Joint damage in rheumatoid arthritis begins with the proliferation of synovial macrophages and fibroblasts after a triggering incident, either autoimmune or infectious. White blood cells that are part of the normal immune system travel to the synovium and cause a reaction. This reaction, or inflammation, is called synovitis, and it results in warmth, redness, swelling, and pain that are typical symptoms of RA. Lymphocytes infiltrate the perivascular regions, endothelial cells proliferate and these result in neovascularization. Thus early in the disease, edema begins to be seen in cells in the synovium and multiplication of synovial lining cells occur. During the inflammation process, the cells of the synovium grow and divide abnormally, making the normally thin synovium thick and resulting in a joint that is swollen and puffy to the touch.

Q12. What do u understand by autoimmune diseases?

Answer: An autoimmune disease is a condition arising from an abnormal immune response to a normal body part. There are at least 80 types of autoimmune diseases. Autoimmune mechanisms underlie many diseases, some organ-specific, others systemic in distribution, and autoimmune disorders can overlap an individual may have more than one organ specific disorder, or more than one systemic disease. In most diseases associated with autoimmunity, the autoimmune process produces the lesions. The pathogenic role of autoimmunity can be demonstrated in experimental models. Human autoantibodies can be directly pathogenic. Immune complexes are often associated with systemic autoimmune disease. Autoantibody tests are valuable for diagnosis and sometimes for prognosis.

Q13. What are the different types of HIV?

Answer: HIV is a retrovirus that is transmitted sexually, in blood or blood products, and perinatally. There are two main variants, HIV-1 and HIV-2: HIV-2 is endemic in West Africa and appears to be less pathogenic. HIV-1 has several subtypes (or clades), which are designated by the letters A through K, and the prevalence of the different clades varies by geographical region over 90% of people infected with HIV-1 live in developing countries and spread is 80% by the sexual route.

Q14. What are DMARDs?

Answer: Disease-modifying antirheumatic drugs (DMARDs) are the primary treatment for RA. They are a diverse collection of drugs, grouped by use and convention. They have been found to improve symptoms, decrease joint damage, and improve overall functional abilities. DMARDs should be started early in the disease as they result in disease remission in approximately half of people and improved outcomes overall.

Q15. What are the effects of HIV infection on CD4 T cells?

Answer: CD4 T cell loss is due to direct killing by HIV and activation-induced apoptosis. Additionally, infected CD4 T cells present both gp120

and HIV peptide: HLA complexes on the cell surface leaving them subject to anti-HIV B and CTL clearance. Ongoing loss of CD4 T cell help ultimately contributes to the collapse of the CD8 T cell response as demonstrated experimentally by the ability to rescue anti-HIV CD8 T cell functionality by replacing the lost CD4 T cell population.

MULTIPLE CHOICE QUESTIONS (MCQS)

Q1. The highest number of HIV infections are found in
A. The Philippines
B. Sub-Saharan Africa
C. The united states
D. Indonesia

Q2. In which body fluid is the HIV virus not present
A. Semen
B. Breast milk
C. Sweat
D. Blood

Q3. Your body's T cell count refers to
A. The number of opportunistic infections you have had
B. Your body's CD4 cell count, which help your body fight infections
C. The viral load in your blood stream
D. The time it takes for HIV to develop AIDS

Q4. HIV virus has a protein coat and a genetic material which is
A. Double stranded DNA
B. Double stranded RNA
C. Single stranded DNA
D. Single stranded RNA

Q5. Which of the following could be called immune disorder?

A. AIDS and cholera
B. SCID and AIDS
C. AIDS and cancer
D. Hepatitis and leukemia

Q6. Which one among the following the following molecules is the main entry site for HIV virus?

A. CD1
B. CD3
C. CD4
D. CD8

Q7. Development of vaccine for AIDS is difficult because *env* gene

A. Undergoes mutation at rapid rate
B. Undergoes reverse transcription
C. Integrates into large number of host genes
D. Integrates its genome into that of the helper T cells

Q8. HIV belongs to which of the following families of virus?

A. Reovirus
B. Lentivirus
C. Togavirus
D. Adenovirus

Q9. Which of the following is not a feature of rheumatoid arthritis?

A. Swollen joints
B. Painful joints
C. Morning stiffness
D. Headache

Q10. The exact etiology of rheumatoid arthritis is not known but the following have been implicated as having a role in its causation, except

A. Genetic factors
B. Autoimmunity
C. Microbial infection
D. Low levels of calcium in the body

Q11. NSAIDs are used as the first line of treatment for rheumatoid arthritis because of their

A. Anti-inflammatory properties
B. Immunosuppressive properties
C. Cytotoxic properties
D. TNF inhibitor properties

Q12. Epiphyseal enlargement is seen in

A. Down's syndrome
B. Hypothyroidism
C. Rheumatoid arthritis
D. Still's disease

Q13. Rheumatoid arthritis is a multisystem disorder; it can involve any of the following except

A. Eyes
B. Lungs
C. Heart
D. Ear

Q14. Biologic response modifiers are drugs that target the immune factors, particularly tumor necrosis factor TNF which plays role in the destructive process during rheumatoid arthritis. Which of the following belong to this class of drugs?

A. Etanercept
B. Celecoxib
C. Rofecoxib
D. Penicillamine

Q15. Corticosteroids used for management of rheumatoid arthritis have all the properties, except

A. Reduce inflammation and swelling
B. Beneficial for systemic involvement in rheumatoid arthritis
C. Can be taken orally or as an injection
D. Delayed onset of action

Answer Key

1. B **2**. C **3**. B **4**. D **5**. B **6**. C **7**. A **8**. B **9**. D **10**. D **11**. A **12**. C **13**. D **14**. A **15**. D

ASSIGNMENTS

Q1. Briefly describe autoimmunity
Q2. What is the difference between HIV and AIDS?
Q3. Discuss the etiology of rheumatoid arthritis
Q4. Describe the management of rheumatoid arthritis
Q5. Describe the opportunistic infections affecting AIDS patients?

REFERENCES

Alamanos, Y., Voulgari, P. V., Drosos, A. A. (2006). Incidence and prevalence of rheumatoid arthritis. Based on the 1987 American College of Rheumatology criteria: a systematic review. *Semin Arthritis Rheum*, 2006; 36(3):182–8.

Altfeld, M., Allen, T. M., Yu, X. G., et al. HIV-1 superinfection despite broad CD8+ T cell responses containing replication of the primary virus. *Nature* 2002;420:434–439.

Arpaia, N. et al. A Distinct function of regulatory T cells in tissue protection. *Cell* **162**, 1078–1089 (2015).

Baum R, Gravallese EM (2014) Impact of inflammation on the osteoblast in rheumatic diseases. *Curr Osteoporos Rep* 12:9–16.

Brenchley JM, Schacker TW, Ruff LE, et al. $CD4^+$ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. *J Exp Med* 2004;200:749–759.

Brennan P, Bankhead C, Silman A, Symmons D. Oral contraceptives and rheumatoid arthritis: Results from a primary care-based incident case–control study. *Semin Arthritis Rheum*, 1997; 26: 817–23.

British Society for Rheumatology. A patient and public guide to the National Clinical Audit for Rheumatoid and Early Inflammatory Arthritis. 1st Annual Report 2015. Jan 2016. Available from: http://www.hqip.org.uk/resources/a-patient-and-public-guide-to-the-national-clinicalaudit-for-rheumatoid-and-early-inflammatory-arthritis.

Carette S, Surtees PG, Wainwright NW, Khaw KT, Symmons DP, Silman AJ. The role of life events and childhood experiences in the development of rheumatoid arthritis. *J Rheumatol*, 2000; 27: 2123–30.

Churchill, M. J., Deeks, S. G., Margolis, D. M., Siliciano, R. F. & Swanstrom, R. HIV reservoirs: what, where and how to target them. *Nat. Rev. Microbiol*. **14**, 55–60 (2016).

Cohn, L. B. et al. Clonal $CD4^+$ T cells in the HIV-1 latent reservoir display a distinct gene profile upon reactivation. *Nat. Med*. 24, 604–609 (2018).

Cooper S (2016) Sarilumab for the treatment of rheumatoid arthritis. *Immunotherapy* 8:249–250.

Crooks, A. M. et al. Precise quantitation of the latent HIV-1 reservoir: implications for eradication strategies. *J. Infect. Dis*. 212, 1361–1365 (2015).

Day CL, Walker BD. Progress in defining CD4 helper cell responses in chronic viral infections. *J Exp Med* 2003;198:1773–1777.

Doitsh, G. & Greene, W. C. Dissecting how CD4 T cells are lost during HIV infection. *Cell Host Microbe* **19**, 280–291 (2016).

Elshabrawy HA, Essani AE, Szekanecz Z, Fox DA, Shahrara S (2017) TLRs, future potential therapeutic targets for RA. *Autoimmun Rev* 16:103–113.

Firestein GS. *Etiology and pathogenesis of rheumatoid arthritis: Kelley's Textbook of rheumatology*. 7th ed. Philadelphia; WB Saunders: 2005, pp. 996–1042.

Furst DE, et al. Overview of biologic agents and kinase inhibitors in the rheumatic diseases. UpToDate. February 2017. Available from: https://www.uptodate.com/contents/overview-of-biologicagents-and-kinase-inhibitors-in-the-rheumatic-diseases.

Gravallese EM, Goldring SR, Schett G (2014) *The role of the immune system in the local and systemic bone loss of inflammatory arthritis*. In: Lorenzo J, Horowitz M, Choi Y, Takayanagi H, Schett G (eds) Osteoimmunology, 2nd edn. Elsevier, Amsterdam.

Koepsell TD, Dugowson CE, Nelson JL, Voigt LF, Daling JR. Non-contraceptive hormones and the risk of rheumatoid arthritis in menopausal women. *Int J Epidemiol*, 1994; 23:1248–55.

Kremer JM, et al. *Use of methotrexate in the treatment of rheumatoid arthritis*. UpToDate. June 2016. Available from: https://www.uptodate.com/contents/use-of-methotrexate-in-the-treatment-ofrheumatoid-arthritis.

Kwong, P. D. & Mascola, J. R. HIV-1 vaccines based on antibody identification, B Cell ontogeny, and epitope structure. *Immunity* 48, 855–871 (2018).

Li, J. Z. et al. The size of the expressed HIV reservoir predicts timing of viral rebound after treatment interruption. *AIDS* 30, 343–353 (2016).

Lozada CJ, Firestein GS. *Management of osteoarthritis: Kelley's Textbook of Rheumatology.* 8th ed., Vol. 2, Philadelphia; Saunders Elsevier: 2009, pp. 1563-77.

Martin, G. E. et al. Post-treatment control or treated controllers? Viral remission in treated and untreated primary HIV infection. *AIDS* 31, 477–484 (2017).

McInnes IB, Schett G (2011) The pathogenesis of rheumatoid arthritis. *N Engl J Med* 365:2205–2219.

Migueles SA, Laborico AC, Shupert WL, et al. HIV-specific CD8+ T cell proliferation is coupled to perforin expression and is maintained in nonprogressors. *Nat Immunol* 2002;3:1061–1068.

National Institute for Health and Care Excellence. Adalimumab, etanercept, infliximab, certolizumab pegol, golimumab, tocilizumab and abatacept for the treatment of rheumatoid arthritis not previously treated with DMARDs or after conventional DMARDs only have failed. TA375. January 2016. Available from: https://www.nice.org.uk/ guidance/ ta375.

Piot P, Feachem RG, Lee JW, Wolfensohn JD. Public health. A global response to AIDS: lessons learned, next steps. *Science* 2004; 304:1909–1910.

Poignard, P. et al. Neutralizing antibodies have limited effects on the control of established HIV-1 infection *in vivo*. *Immunity* 10, 431–438 (1999).

Rothschild BM, Rothschild C, Helbling M. Unified theory of the origins of erosive arthritis: conditioning as a protective/directing mechanism. *J. Rheumatol*, 2003; 30(10): 2095–102.

Sakaguchi, S., Miyara, M., Costantino, C. M. & Hafler, D. A. FOXP3^{+}regulatory T cells in the human immune system. *Nat. Rev. Immunol.* **10**, 490–500 (2010).

Schett G, Gravallese E (2012) Bone erosion in rheumatoid arthritis: mechanisms, diagnosis and treatment. *Nat Rev Rheumatol* 8:656–664.

Schoofs, T. et al. HIV-1 therapy with monoclonal antibody 3BNC117 elicits host immune responses against HIV-1. *Science* **352**, 997–1001 (2016).

van Steenbergen HW, Ajeganova S, Forslind K, Svensson B, vander Helm-van AHM (2015) The effects of rheumatoid factor and anticitrullinated peptide antibodies on bone erosions in rheumatoid arthritis. *Ann Rheum Dis* 74:e3.

Wagner, T. A. et al. Proliferation of cells with HIV integrated into cancer genes contributes to persistent infection. *Science* 345, 570–573 (2014).

Zhang F, Ding R, Li P, Ma C, Song D, Wang X, Ma T, Bi L (2015) Interleukin-34 in rheumatoid arthritis: potential role in clinical therapy. *Int J Clin Exp Med* 8:7809–7815.

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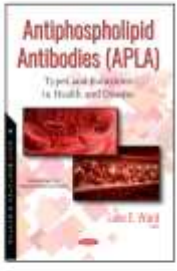

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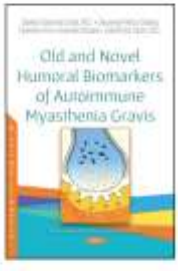

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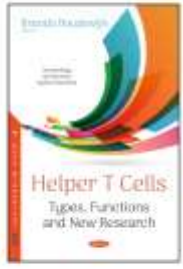

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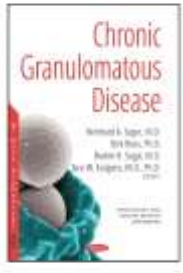

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