IMMUNOLOGY

M.Sc. MICROBIOLOGY

SEMESTER-II, PAPER-III

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FOREWORD

Since its establishment in 1976, Acharya Nagarjuna University has been forging ahead in the path of progress and dynamism, offering a variety of courses and research contributions. I am extremely happy that by gaining 'A+' grade from the NAAC in the year 2024, Acharya Nagarjuna University is offering educational opportunities at the UG, PG levels apart from research degrees to students from over 221 affiliated colleges spread over the two districts of Guntur and Prakasam.

The University has also started the Centre for Distance Education in 2003-04 with the aim of taking higher education to the door step of all the sectors of the society. The centre will be a great help to those who cannot join in colleges, those who cannot afford the exorbitant fees as regular students, and even to housewives desirous of pursuing higher studies. Acharya Nagarjuna University has started offering B.Sc., B.A., B.B.A., and B.Com courses at the Degree level and M.A., M.Com., M.Sc., M.B.A., and L.L.M., courses at the PG level from the academic year 2003-2004 onwards.

To facilitate easier understanding by students studying through the distance mode, these self-instruction materials have been prepared by eminent and experienced teachers. The lessons have been drafted with great care and expertise in the stipulated time by these teachers. Constructive ideas and scholarly suggestions are welcome from students and teachers involved respectively. Such ideas will be incorporated for the greater efficacy of this distance mode of education. For clarification of doubts and feedback, weekly classes and contact classes will be arranged at the UG and PG levels respectively.

It is my aim that students getting higher education through the Centre for Distance Education should improve their qualification, have better employment opportunities and in turn be part of country's progress. It is my fond desire that in the years to come, the Centre for Distance Education will go from strength to strength in the form of new courses and by catering to larger number of people. My congratulations to all the Directors, Academic Coordinators, Editors and Lesson-writers of the Centre who have helped in these endeavors.

Prof. K. Gangadhara Rao
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M.Sc. MICROBIOLOGY
SEMESTER-II, PAPER-III
203MB24-IMMUNOLOGY
SYLLABUS

THEORY

UNIT-I

Structure, composition and functions of cells and organs involved in immune system- B-cells, T-cells, phagocytes, auxilliary cells, soluble mediators. Lymphokines and Cytokines, functions of important Interleukins; Lymphoid organs- primary (Thymus and bone marrow), secondary (spleen and lymph node); Types of immunity - Innate and acquired immunity; Humoral and cell mediated immunity; primary and secondary.

UNIT - II

Antigens - Nature and Properties;

Immunoglobulins - Structure, heterogenicity, types, sub-types; antibody production-hybridoma technique, catalytic enzymes.

Complement System - Structure, components, pathways and biological sequences of complement activation. Antigen - antibody reactions - agglutination, precipitation, complement fixation, Immuno fluorescence microscopy, ELISA, RIA.

UNIT - III

Hypersensitivity Reactions - Antibody mediated- Anaphylaxis; antibody dependent cell toxicity; immune complex mediated reactions; cell mediated hypersensitivity reactions. Brief account on the respective diseases.

UNIT - IV

Structure and Functions of MHC. Transplantation Immunology - concept, tissue typing methods, role of HLA, survival of allograft, graft versus host reaction.

Autoimmunity - General account of autoimmune diseases; mechanism and therapy of Rheumatoid arthritis. Tumor immunology - Tumor diagnosis by onco fetal antigens, effector mechanisms in tumor immunology.

UNIT-V

Immune Response to Infectious Diseases: Viral infections, bacterial infections, protozoan diseases. Vaccines - Designing vaccines for active immunization, whole organism vaccines, purified macromolecules as vaccines, recombinant vector vaccines, DNA vaccines, Synthetic peptide vaccines and multivalent subunit vaccines.

REFERENCE BOOKS:

1) ROITT, I.M. (1998). Essentials of Immunology. ELBS, Blackwell Scientific Publishers, London.
2) Kuby's Immunology. IV Edition. Freeman and Company, New York.
3) KLAUS DELGERT (1996) immunology- Understanding of immune system. Wiley-Liss. NY.
4) TOPLEY and WILLIAMS (1995). Text book on Principles of Bacteriology,
5) Virology and Immunology, Edward Arnold, London.

(203MB24)

MODEL QUESTION PAPER

M.Sc. DEGREE EXAMINATION,
MICROBIOLOGY - SECOND SEMESTER
IMMUNOLOGY

Time: Three hours **Maximum: 70 marks**

Answer All Questions **5 × 14 = 70M**

UNIT-I

1) a) Give an account on structure and functions of cells involved in immune system.

OR

b) Describe the primary lymphoid organs of immune system and their significance.

UNIT-II

2) a) Write an account on nature and properties of antigens.

OR

b) Describe the methodology of hybridoma technique for antibody production.

UNIT-III

3) a) Explain the antibody-mediated anaphylaxis reactions and its consequent diseases.

OR

b) Describe the immune complex mediated hypersensitivity reaction and its respective diseases

UNIT-IV

4) a) Write an account on structure and functions of MHC.

OR

b) Give a general account on autoimmune diseases.

UNIT-V

5) a) Explain the immune responses to bacterial infections

OR

b) Describe the characteristic features of whole organism and purified macromolecule vaccines and their uses.

CONTENTS

LESSON-1

CELLS OF IMMUNE SYSTEM

1. 0 OBJECTIVE:

- In this lesson, structure and functions of cellular components of immune system namely B cells, T cells, mononuclear phagocytic cells, granulocytes and other cells are described which will enlighten the students.

STRUCTURE:

1. 1 INTRODUCTION

Lymphocytes are the central cells of the immune systems, responsible for acquired immunity and the immunologic attributes of diversity, specificity, memory, and self/non self recognition. The other types of white blood cells play important but often ancillary roles, engulfing and destroying microorganisms, presenting antigens, and secreting cytokines. Lymphocytes constitute 20%-40% of the body's white blood cells and 99% of the cells in the lymph. There are approximately 10^{11} (range depending on body on body size and age: 10^{10}~10^{12}) Lymphocytes in the human body. These Lymphocytes continually circulate in the blood and lymph and are capable of migrating into the tissue spaces and lymphoid organs, thereby integrating the immune system to a high degree.

The Lymphocytes can be broadly subdivided into three population-B cells, T cells, and null cells-on the basis of function and cell-membrane components. The null population of lymphocytes does not express surface markers typical of B or T cells. It consists mostly of large, granular lymphocytes which are the natural killer cells (NK cells). Resting B and T lymphocytes are small, motile, non-phagocytic cells, which cannot be distinguished morphologically. B and T lymphocytes that have not interacted with antigen-referred to as naïve, or unprimed – are resting cells in the G_0 phase of the cell cycle (known as small lymphocytes). These cells are only about 6μm in diameter; their cytoplasm forms a barely

discernible rim around the nucleus. Small lymphocytes have densely packed chromatin, few mitochondria, and a poorly developed endoplasmic reticulum and Golgi apparatus. The naïve lymphocyte is generally thought to have a short life span. Interaction of small lymphocytes with antigen, in the presence of certain cytokines, induces these cells to enter the cell cycle by progressing from G_0 onto G_1 and subsequently into S, G_2, and M. As they progress through the cell cycle, lymphocytes enlarge into 15μm-diameter blast cells, called lymphoblasts; these cells have a higher cytoplasm: nucleus ratio and more organellar complexity than small lymphocytes.

Lympho blasts proliferate and eventually differentiate into effector cells or into memory cells. Effector cells function in various ways to eliminate the antigen. These cells have short life spans, generally ranging from a few days to a few weeks. Plasma cells-the antibody-secreting effector cells of the B-cell lineage-have a characteristic cytoplasm that contains abundant endoplasmic reticulum (to support their high rate of protein synthesis) arranged in concentric layers and also many Golgi vesicles. The effector cells of the T- cell lineage include the cytokine-secreting T helper cell TH cell) and the cytotoxic T lymphocyte Tc or, sometimes, CTL). Some of the progeny of B and T lympho blasts differentiate into memory cells. The persistence of this population of cells is responsible for life-long immunity to many pathogens. Memory cells look like small lymphocytes but can be distinguished from naïve cells by the presence or absence of certain cell- membrane molecules.

Different lineages or maturational stages of lymphocytes can be distinguished by their expression of membrane molecules recognized by particular monoclonal antibodies. All of the mono clonal antibodies that react with a particular membrane molecule are grouped together as a cluster of differentiation (CD). Each new monoclonal antibody that recognizes a leukocyte membrane molecule is analyzed for whether it falls within a recognized CD designation; if it does not, it is given a new CD designation reflecting a new membrane molecule. Although the CD nomenclature was originally developed for the membrane molecules of human leukocytes, the homologous membrane molecules of other species, such as mice, are also commonly referred to by the same CD designations.

1. 2. B LYMPHOCYTES (B CELLS)

The B lymphocyte derived its letter designation from its site of maturation, in the bursa of Fabricius in birds; the name turned out to be apt, for bone marrow is its major site of maturation in a number of mammalian species, including humans and mice. Mature B cells are definitively distinguished from other lymphocytes by their synthesis and display of membrane-bound immunoglobulin (antibody) molecules, which serve as receptors for antigen. Each of the approximately 1.5×10^5 molecules of antibody on the membrane of a single B cell has an identical binding site for antigen. Among the other molecules expressed on the membrane of mature B cells are -

B220 (a form of CD 45, CD45R) is frequently used as a marker for B cells and their precursors however, unlike antibody, it is not found uniquely on B-lineage cells.

- Class II MHC molecules permit the B cell to function as an antigen-presenting cell (APC).
- CRI (CD35) and CR2 (CD21) are receptors for certain complement products.
- Fcγ RII (CD32) is a receptor for IgG, a type of antibody.

- B7-1 (CD80) and B7-2 (CD86) are molecules that interact withCD28and CTLA-4, important regulatory molecules on the surface of different types of T cells, including TH cells.
- CD40 is a molecule that interacts with CD40 ligand on the surface of helper T cells. In most cases, this interaction is critical for the survival of antigen-stimulated B cells and for their development into antibody-secreting plasma cells or memory B cells.

Interaction between antigen and the membrane-bound antibody on a mature naïve B cell, as well as interactions with T-cells and macrophages, selectively induces the activation and differentiation of B-cells clones of corresponding specificity. In this process, the B cell divides repeatedly and differentiates over a4 to 5 day period, generating a population of plasma cells and memory cells. Plasma cells, which lack membrane-bound antibody, synthesize and secrete one of the five classes of antibody. All clonal progeny from a given B cell secrete antibody molecules with the same antigen-binding specificity. Plasma cells are terminally differentiated cells, and many die in 1 or 2 weeks.

Maturation of B Cells:

They originate from precursor cells from the yolk sac, foetal liver and bone marrow. During the maturation process, the pre B-cell is programmed to produce only one class or subclass of Ig after a switch from initial IgM production. On the basis of immunoglobulin, which is programmed to synthesize, B-lymphocytes can be subdivided into nine different subsets-IgM, IgD, IgG1, IgG2, IgG3, IgG4, IgA1, IgA2 and IgE.

The immature B-cells with IgM on the cell surface are known as virgin B-cells, which are immunologically competent but have not had contact with Ag. The virgin B- cells migrate to the periphery (spleen and lymphoid tissues) where they express IgD on their surface and one of the other Ig classes like, IgM, IgG, IgA or IgE. By reassortment of Ig genes, B-cells develop the capacity to produce Ig molecules.

On contact with specific Ag, the B-cell undergoes clonal proliferation. Some of the activated B-cells are converted into memory cells which are responsible for the recall phenomenon on subsequent contact with the same Ag and majority of the activated B- cells, are converted into Ab producing plasma cells.

Distribution of B-Cells:

Approximately 30% of the lymphocytes circulating in the blood are B-cells. Their distribution in various organ and tissues is: Peripheral blood--- 15-30%, Lymph nodes---30-35%, Bone marrow---75%, Spleen---55-60%, Tonsillar lymphocytes---50%.

Functions of B-Cells:

B-lymphocytes are the mediators of humoral immune response, which produce Abs.

Subsets of B-Lymphocytes:

B-cells also exist indifferent subpopulations (subsets) based on the type of immunoglobulin synthesized by them. Thus, the different subsets are those that selectively form the immunoglobulin classes, e. g. , IgM producing B-cells, IgG producing B-cells and so on.

Activation of B-cells:

Ig present on the surface of B-cell behaves as the specific receptor for Ag. When Ag enters into the body, it reacts with B-cell with appropriate specificity. This interaction

stimulates B-cell to undergo blastoid transformation and to get converted into plasma plasts (clone formation) and finally into plasma cells.

Each B-cell possesses the genetic instrument to produce Ab of unique Ag specificity as a membrane receptor. Once the signal is received, B-cells are differentiated into plasma cells, which produce and secrete Abs.

Plasma Cells:

Antigenically stimulated B-cells undergo blast transformation to form plasma cells. These are twice the size of small lymphocyte. The properties of mature plasma cells are: oval in shape; with small, eccentric, oval nucleus having radially arranged chromatin around the periphery that gives the appearance of a clock face or cart wheel. Cytoplasm contains a well-developed Golgiapparatus and abundant endoplasmic reticulum. The life-span of these cells is two or three days. The aggregates of Ig, called Russell bodies are seen sometimes in the endoplasmic reticulum. These cells are distributed in germinal centers of lymph nodes, spleen and diffused lymphoid tissue of alimentary and respiratory tracts. Plasma cells are the major Ab producing cells but lymphocytes and lymphoblastsmay also produce Abs to a certain extent.

Function:

Plasma cell is an antibody producing machinery. It can produce an Ab of a single specificity either IgM or IgG or IgA.

1. 3. T LYMPHOCYTES (T CELLS)

T lymphocytes derive their name from their site of maturation in the thymus. Like B lymphocytes, these cells have membrane receptors for antigen. Although the antigen- binding T-cell receptor is structurally distinct from immunoglobulin, it does share some common structural features with the immunoglobulin molecule, most notably in the structure of its antigen-binding site. Unlike the membrane-bound antibody; on B cells, the T-cell receptor (TCR) does not recognize free antigen. Instead the TCR recognizes only antigen that is bound to particular classes of self-molecules. Most T cells recognize antigen only when it is bound to a self-molecule encoded by genes within the major histocompatibility complex (MHC). Thus, a fundamental difference between the humoral and cell-mediated branches of the immune system is that the B cell is capable of binding soluble antigen, where as the T cell is restricted to binding antigen displayed on self-cells. To be recognized by most T cells, this antigen must be displayed together with MHC molecules on the surface of antigen-presenting cells or on virus-infected cells, cancer cells, and grafts. The T-cell system has developed to eliminate these altered self-cells, which pose a threat to the normal functioning of the body.

Maturation of T-lymphocytes:

T-cells originate from precursor cells from the yolk sac, foetal liver and bone marrow and migrate to the thymus and mature there. Several subsets of the T-cells arise during maturation process; each one is responsible for specific function. The earliest identifiable T-cells are the $CD7^+$ pro T cells, which acquire CD2 on entering the thymus. They synthesize CD3 in the cytoplasm and become preT-cells. They also synthesize T-cellreceptor (TCR). TCR is a heterodimer of lipoprotein chains, which along with CD3 acts as the Ag recognition unit like immunoglobulin on the surface of B-cells. TCR occurs as two pairs of glycoprotein chains either α βor γ δ. In majority of T-cells it is α β TCR. It is closely associated with CD3

Ag as transmembrane proteins. Both the TCR and CD3 molecules are analogous to immunoglobulins and each possesses variable and constant regions. The variable regions of α and β chains constitute the Ag binding site and non-covalently linked CD3 molecule is believed to act as a signal transducer that transduces Ag recognition signal to the interior of the cell. T-cells also develop MHC restriction, so that each T-cell after antigenic stimulation carries unique Ag specificity.

Distribution of T-lymphocytes:

T-lymphocytes are richly distributed in thymus, lymph nodes and peripheral blood. Their distribution in Peripheral blood is 55-75%, Lymph nodes - 60-75%, Spleen - 25-45%, Bone marrow - 10%, Thymus - more than 75%.

Functions of T-cells:

a) Mediate CMI through the production of cytokines.

b) Can directly act on and destroy virally infected host cells, tumour cells and foreign cells (cytotoxic effect).

c) Act as regulatory cells that modulate the activity of other T-cells, B- cells or macrophages.

d) Regulation can be in the form of help or suppression.

Types of T-Cells:

Like B cells, T cell expresses distinctive membrane molecules. All T-cell subpopulation express the T-cell receptor, a complex of polypeptides that includes CD3; and most can be distinguished by the presence of one or the other of two membrane molecules, CD4 and CD8. In addition, most mature T cells express the following membrane molecules:

- CD28, a receptor for the co-stimulatory B7 family of molecules present on B cells and other antigen-presenting cells.
- CD45, a signal-transduction molecule

T cells that express the membrane glycoprotein molecule CD4 are restricted to recognizing antigen bound to class IIMHC molecules, where as T cells expressing CD8, a dimeric membrane glycoprotein, are restricted to recognition of antigen bound to class I MHC molecules. Thus the expression of CD4 versus CD8 corresponds to the MHC restriction of the T cell. In general, expression of CD4 and of CD8 also defines two major functional subpopulations of T lymphocytes. $CD4^+$ T cells generally function as T helper (TH) cells and are class-II restricted; $CD8^+$ T cells generally function as T cytotoxic (TC) cells and are class-I restricted. Thus the ratio of TH to TC cells in a sample can be approximated by assaying the number of $CD4^+$ and $CD8^+$ T cells. This ratio is approximately 2: 1 in normal human peripheral blood, but it may be significantly altered by immunodeficiency diseases, autoimmune diseases, and other disorders.

TH cells are activated by recognition of an antigen-class II MHC complex on an antigen-presenting cell. After activation, the TH cell begins to divide and gives rise to a clone of effector cells, each specific for the same antigen-class II MHC complex. These TH cells

secrete various cytokines, which play a central role in the activation of B cells, T cells, and other cells that participate in the immune response Changes in the pattern of cytokines produced by TH cells can change the type of immune response that develops among other leukocytes. The TH 1 response produces a cytokine profile that supports inflammation and activates mainly certain T cells and macrophages, whereas the TH2 response activates mainly B cells and immune responses that depend upon antibodies. TC cells are activated when they interact with an antigen-class I MHC complex on the surface of an altered self-cell(e. g. , a virus-infected cellor a tumor cell) in the presence of appropriate cytokines. This activation, which results in proliferation, causes the TC cell to differentiate into an effector cell called a cytotoxic T lymphocyte (CTL). In contrast to TH cells, most CTLs secrete few cytokines. They cause destruction of target cells by releasing molecules known as lymphotoxins and perforin.

Another subpopulation of T lymphocytes-called T suppressor (TS) cells-has been postulated which constitute 25-40% of circulating T-lymphocytes. It is clear that some T cells help to suppress the humoral and the cell-mediated branches of the immune system by blocking Th activity or by acting directly on B-cells, but the actual isolation and cloning of normal TS cells is a matter of controversy and dispute among immunologists. For this reason, it is uncertain whether TS cell do indeed constitute a separate functional subpopulation of T cells. Some immunologists believe that the suppression mediated by T cells observed in some systems is simply the consequence of activities of TH or TC subpopulations whose end results are suppressive

Other Types:

T-regulator cells (Tr):

Tr cells are the regulator cells, which regulate the activity of Th and Ts cells.

Delayed hypersensitivity T-cells (TD-cells):

T-cells responsible for delayed type of hypersensitivity reactions are known as TD-cells. They are indistinguishable from Th- cells on the basis of surface markers. It is believed that TD-cells are one type of T-helper cells- Th1. They possess CD4 markers and secrete different lymphokines (e. g., γ interferon), which are responsible for inflammatory response of delayed hypersensitivity. These cells also secrete growth factors, which are believed to regulate lymphocyte activity.

1.4. NULL CELLS

A small group of lymphocytes, called null cells, in the peripheral blood do not express the membrane molecules and receptors that distinguish T- and B-cell lineages. These cells also fail to synthesize immunoglobulin and incorporate it into their plasma membrane. Because these cells do not produce antigen-binding receptor, they lackprecise immunologic specificity and memory. Most members of the null cell population are large, granular lymphocytes called natural killer (NK) cells: these cells constitute5%- 10% of the lymphocytes in human peripheral blood.

The natural killer cell was first described in 1976, when it was shown that certain null cells display cytotoxic activity against a wide range of tumor cells in the absence of any previous immunization with the tumor. NK cells were subsequently hown to play an

important role in host defense both against tumor cells and against cells infected with some, though not all, viruses. NK cells can recognize potential target cells in two different ways. In some cases, an NK cell employs NK cell receptors to distinguish abnormalities, notably a reduction in the display of class I MHC molecules and the unusual profile of surface antigens displayed by some tumor cells and cells infected by some viruses. Another way in which NK cells recognize potential target cells depends upon the fact that some tumor cells and cells infected by certain viruses display antigens against which the immune system has made and antibody response, so that antitumor or antiviral 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).

Several observations suggest that NK cells play an important role in host defense against tumors. For example, in humans the Chediak-Higashi syndrome- an autosomal recessive disorder-is associated with impairment in neutrophils, macrophages, and NK cells and an increased incidence of lymphomas. Likewise, mice with an autosomal mutation called beige lack NK cells; these mutants are more susceptible than normal mice to tumor growth following injection with live tumor cells.

There has been growing recognition of a special cell type, the NK1-T cell that has some of the characteristics of both T cells and NK cells. Like T cells, have T cell receptors (TCRs). Unlike most T cells, the TCRs of NK1-T cells interact with MHC –like molecules called Cd1 rather than with class I or class II MHC molecules. Like NK cells, they have variable levels of CD 16 and other receptors typical of NK cells, and they can kill cells. A population of triggered NK1-T cells can rapidly secrete large amounts of cytokines needed to support antibody production by B cells as well as inflammation; the development and expansion of cytotoxic T cells. Some immunologists view this cell type as a kind of rapid response system that has evolved to provide early help while conventional TH-Cell responses are still developing.

1.5. PHAGOCYTIC CELLS

Engulfment and digestion of foreign particle by a single cell is known as phagocytosis. The process of phagocytosis is the most important means of defense mechanism against microorganisms. It is a part of non-specific defense mechanism. The inactivation, removal, and disposal of microorganism is done by phagocytic cell, often with the help of Ab and complement. Phagocytic cells recognize foreign particles with the help of specific Abs and digest them with the help of complement factors. The phagocytic cells were first described by Metchnikoff (1883).

Role of Phagocytic Cells:

The primary role is the phagocytosis-engulfment and digestion of foreign particles. Also participates in the development of specific immune response, e. g. , trapping of Ag by macrophages and its presentation to lymphocytes in optimal concentration. Macrophages also participate in antitumor activity and graft rejection. Microphages do not play any significant role in specific immune response; however, they participate in inflammation, opsonisation, hypersensitivity reactions and immunity against parasitic infections.

Types of Phagocytic Cells:

In man, phagocytosis is carried out primarily by mononuclear macrophages of blood

and tissue, neutrophils and to a lesser extent eosinophils. The cells with less or no phagocyte activity but play an important role in immune reactions are basophils, mast cells and dendritic cells.

Mononuclear Macrophages:

The mononuclear phagocyte system consists of monocytes circulating in the blood and macrophages in the tissues. During hematopoiesis in the bone marrow, granulocyte-monocyte progenitor cells differentiate into promonocytes, which leave the bone marrow and enter the blood, where they further differentiate into mature monocytes.

Monocytes circulate in the bloodstream for about 8 h, during which time they enlarge; then they migrate into the tissues and differentiate into specific tissue macrophages. Differentiation of monocyte into a tissue macrophage involves a number of changes: The cell enlarges five-to ten-folds; its intracellular organelles increase in both number and complexity; and it acquires increased phagocytic ability, produces higher levels of hydrolytic enzymes, and begins to secrete a variety of soluble factors. Macrophages are dispersed throughout the body. Some take up residence in particular tissues, becoming fixed macrophages, whereas others remain motile and are called free, or wandering, macrophages. Free macrophages travel by amoeboid movement throughout the tissues. Macrophage-like cells serve different functions in different tissues and are named according to their tissue location: Alveolar macrophages in the lung; Histocytes in connective tissues; Kupffer cell in the liver; Mesangial cells in the kidney; Microglial cells in the brain ; and Osteoclasts in bone. Although normally in a resting state, macrophages are activated by a variety of stimuli in the course of an immune response. Phagocytosis of particulate antigens serves as an initial activating stimulus. However, macrophage activity TH cells, by further enhanced by cytokines secreted by activated TS 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 (IFNγ) secreted by activated TH cells.

Activated macrophages are more effective than resting ones in eliminating potential pathogens, because they exhibit greater phagocytic activity, an increased ability to kill ingested microbes, increased secretion of inflammatory mediators, and an increased ability to activate T cells. In addition activated macrophages, but not resting ones, secrete various cytotoxic protein that help them eliminate a broad range of pathogens, including virus-infected cells, tumor cells, and intracellular bacteria. Activated macrophages also express higher levels of class II MHC molecules, allowing them to function more effectively as antigen-presenting cells. Thus, macrophages and TH cells during the immune response facilitate each other's activation.

Granulocytic Cells

These are small non-dividing polymorpho nuclear leucocytes or granulocytes (Fig. 1.1) present in blood. Morphologically, there are three types of cells, which participate in immunological reactions. These include the neutrophils (45-60%), the eosinophils (1-3%) and the basophils (0. 3%) of the total leucocyte count. The neutrophils and to a lesser extent, the eosinophils are phagocytic. They contain granules and a wide range of bactericidal substances. They originate in the bone marrow from stem cells, undergo maturation and finally released into circulation. They are short lived cells with half-life of two days in circulation and few hours in tissue after penetration. The granulocytes are classified as neutrophils, eosinophils, or basophils on the basis of cellular morphology and cytoplasmic staining characteristics. The neutrophil has a multilobed nucleus and a granulated cytoplasm that stains with both acid and basic dyes; it is often called a polymorpho nuclear leukocyte

(PMN) for its multilobed nucleus. The eosinophil has a bilobed nucleus and a granulated cytoplasm that stains with the acid dye eosin red (hence its name). The basophil has a lobed nucleus and heavily granulated cytoplasm that stains with the basic dye methylene blue. Both neutrophils and eosinophils are phagocytic, whereas basophils are not. Neutrophils, which constitute 50%-70% of the circulating white blood cells, are much more numerous than eosinophils (1%-3%) or basophils (<1%).

Neutrophils:

Neutrophils are produced by hematopoies is in the none marrow. They are released into the peripheral blood and circulate for 7-10 h before migrating into the tissues, where they have a life span of only a few days. In response to many types of infections, the bone marrow releases more than the usual of infections, the neutrophils and these cells generally are the first to arrive at a site of inflammation. The resulting transient increase in the number of circulating neutrophils, called leukocytosis, is used medically as an indication of infection.

Movement of circulating neutrophils into tissues, called extravasation, takes several steps: the cell first adheres to the vascular endothelium, then penetrates the gap between adjacent endothelial cells lining the vessel wall, and finally penetrates the vascular basement membrane, moving out into the tissue spaces. A number of substances generated in an inflammatory reaction serve as chemotactic factors that promote accumulation of neutrophils at an inflammatory site. Among these chemotactic factors are some of the complement components of the blood-clotting system, the several cytokines secreted by activated TH cells and macrophages.

Like macrophages, neutrophils are active phagocytic cells. Phagocytosis by neutrophils is similar to that described for macrophages, except that the lytic enzymes and bactericidal substances in neutrophils are contained within primary and secondary granules. The larger, denser primary granules are a type of lyso some containing peroxidase, lysozyme, and various hydrolytic enzymes. The smaller secondary granules contain collagenase, lactoferrin, and lysozyme. Both primary and secondary granules fuse with phagosomes, whose contents are then digested and eliminated much as they are in macrophages.

Neutrophils also employ both oxygen-dependent and oxygen-independent pathway to generate antimicrobial substances. Neutrophils are in fact much more likely than macrophages to kill ingested microorganisms. Neutrophils exhibit a larger respiratory burst than macrophages and consequently are able to generate more reactive oxygen intermediates and reactive nitrogen intermediates. In addition, neutrophils express higher levels of defenses than macrophages do.

Eosinophils:

Eosinophils, like neutrophils, are motile phagocytic cells that can migrate from the blood into the tissue spaces. Their phagocytic role is significantly less important than that of neutrophils, and it is thought that they play a role in the defense against parasitic organisms. The secreted contents of eosinophilic granules may damage the parasite membrane.

Basophils:

Basophils are non-phagocytic granulocytes that function by releasing pharmacologically active substances from their cytoplasmic granules. These substances play a major role in certain allergic responses.

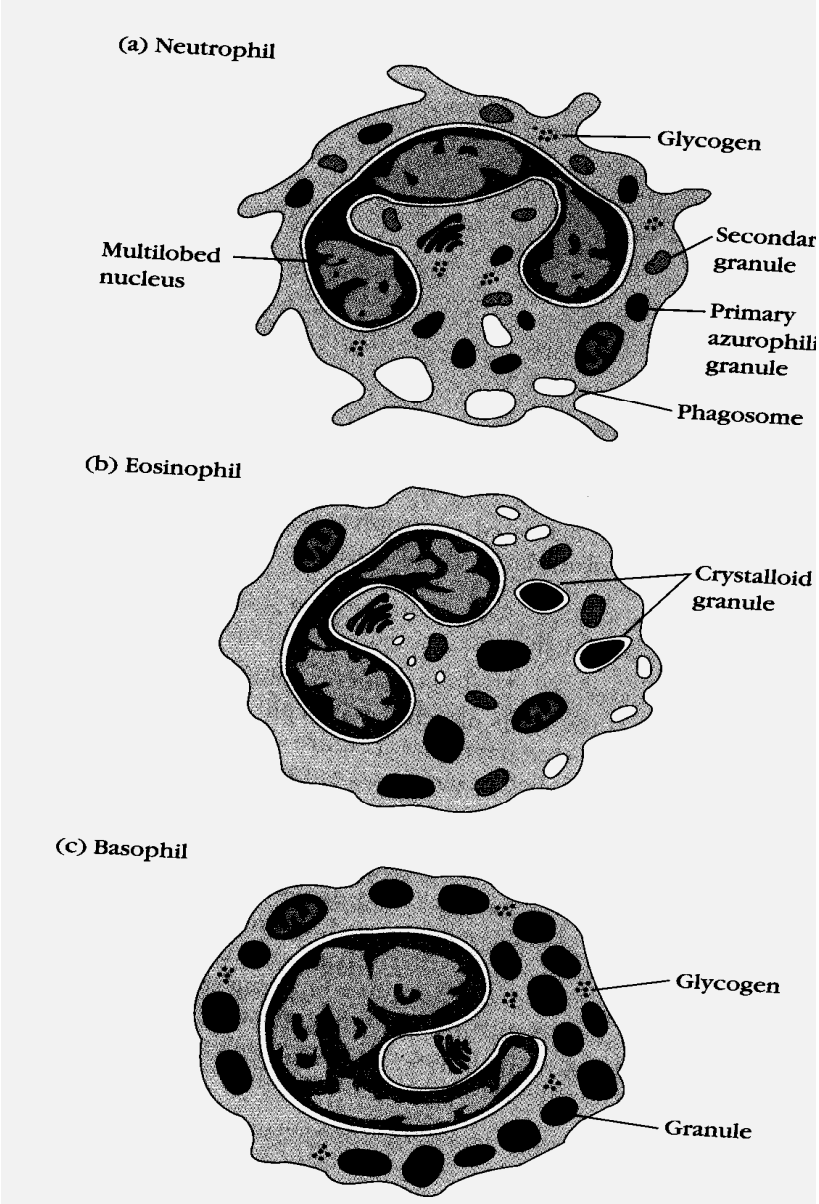


Fig-1. 1: Granulo Cytic Cells

Mechanism of Phagocytosis

The process of phagocytosis occurs in following steps.

The signal: The entry of foreign particles such as microorganisms gives signal to the phagocytic cells in following ways: The signal to phagocytic cells is given by chemotactic substances derived from the complement system and lymphocyte derived factors. Micro organisms react with their Absand then with C (complement). Some of the components of C diffuse out from the center of reaction and establish a concentration gradient of chemotactic factors (C3a, C5), which attract microphages. The sensitized lymphocytes stimulated by specific Ag produce lymphokines such as macrophage chemotactic factor or macrophage activating factor that are chemotactic for monocytes and macrophages. The signal no macrophages are also given by products of the complement system. Once the signal is noticed by phagocytic cell, it starts unidirectional movement towards an increasing concentration of chemotactic factors. The phagocytic cells move through the blood venules by a process called diapedesis.

Surface Recognition:

The engulfment of foreign particles largely depends upon surface properties of the particle to be phagocytosed, e.g., hydrophobicity and surface tension. Most of the nonpathogenic organisms are more hydrophobic than phagocytic cells, hence readily engulfed and destroyed. Organisms that are hydrophilic in nature, e.g., bacteria possessing hydrophilic capsule (pneumococci, *H. influenzae*), are difficult to engulf because of protective covering but the specific anticapsular Ab with or without complement neutralizes the charges on hydrophilic capsule, making it hydrophobic and increasing interfacial tension, so that it is readily engulfed by phagocytic cell.

Opsonisation:

It is done by opsonins. Opsonins are of two types: Heatstable opsonins- Abs. Heat labile opsonins - complement components. Opsonins increase interfacial tension and hydrophobicity by reacting with particle to be phagocytosed hence it is readily phagocytosed.

Ingestion and Digestion:

Once the contact is made with foreign particle, engulfment starts with a deep invagination of cell membrane, which forms a thin layer around the particle. This fuses to form a pouch called phagosome. Phagosome penetrates deep into cytoplasm and fuses with lysosomal granules to form phagolysosome. The granules rupture, discharging their enzymatic contents into the vacuole and come into contact with the ingested particle. The ingested particle is slaughtered by a battery of mechanisms. Lysosome contains a variety of hydrolyticenzyme such as glucuronidases, lipases, nucleases, peroxidases, phosphatases, lysozyme, phagocytin and other bactericidal substances, which quickly within 15 minutes kill most of the microorganisms

1. 6. Mast Cells:

Mast-cell precursors, which are formed in the bone marrow by hematopoiesis, are released into the blood as undifferentiated cells; they do not differentiate until they leave the blood and 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. Like circulating basophils, these cells have large numbers of cytoplasmic granules that contain histamine and other pharmacologically active substances. Mast cells, together with blood basophils, play an important role in the development of allergies.

1. 7. Dendritic Cells:

These are the Ag presenting cells like macrophages. They are derived from bone marrow and are different from the macrophages and T and B lymphocytes. They have little or no phagocytic activity. They are highly pleomorphic with a small central body and many long needle-like processes, resemble the dendrites of nerve cells They are present in peripheral blood and in the peripheral lymphoid organs, especially in the germinal centers of the spleen and lymph nodes. They are believed to play an important role in the presentation of antigens to T-cells during the primary immune response. Dendritic cells can be difficult to isolate because the conventional procedures for cell isolation tend to damage their long extensions. The development of isolation techniques that employ enzymes and gentler dispersion has

facilitated isolation of these cells for study in vitro. Most dendritic cells process and present antigen to TH cells. These cells can be classified by their location: Langerhans cells found in the epidermis (skin) and mucous membranes; Interstitial dendritic cells, which populate most organs (e. g. , heart, lungs, liver, kidney, gastro intestinal tract); Inter digitating dendritic cells present in T-cell areas of secondary lymphoid tissue and the thymic medulla; Circulating dendritic cells include those in the blood, which constitute0. 1% of the blood leukocytes, and those in the lymph (known as veiled cells).

The dendritic cells indifferent locations have different forms and functions. Despite their differences, all of these dendritic cells constitutively 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 Bcells, both of which need to be activated before they can function as antigen-presenting cells (APCs). After capturing antigen in the tissues by phagocytosis or by endocytosis, dendritic cells migrate into the blood or lymph and circulate to various lymphoid organs, where they present the antigen to T lymphocytes.

Dendritic cells descend from hematopoietic stem cells through the myeloid lineage. The exact developmental path or paths taken by these cells is still under investigation. The major issue is whether dendritic cells belong from an entirely separate lineage. Two possible developmental path ways. In one pathway, a dendritic cell develops from a myeloid precursor in the bone marrow, and then appears in the blood as an immature cell that completes its differentiation in the tissues. An alternative model proposes that a late monocytic-stage cell differentiates in the tissues to generate either a macrophage or dendritic cell. Some evidence suggests that mature macrophages and dendritic cells may interconvert, although this has yet to be confirmed. The morphologic and functional differences observed among Langerhans cells and interstitial, inter digitating, and circulating dendritic cells are thought to reflect different maturational states of the cells and the different microenvironments in which they reside.

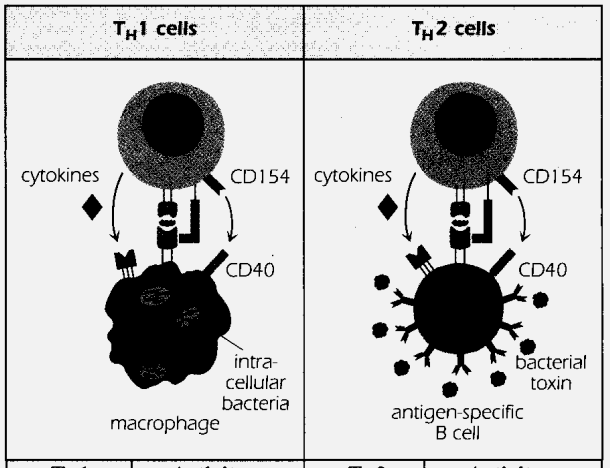
1. 8. CYTOKINES AND LYMPHOKINES:

As a result of appropriate stimulation, cells of the immune system secrete a wide variety of proteins that mediate signaling between cell and cell to control the immune responses. The generic term for these regulatory proteins secreted by cells is called Cytokines**.** Different terminology is used for cytokines based on the cells which secrete them and also on their functions. Cytokines secreted by lymphocytes are called Lymphokines and those produced by monocytes or macrophages are called Monokines**.** Most lymphokines exhibit multiple biological effects and the same effect may becaused by different lymphokines. The term interleukin is introduced for those products of leukocytes, which exert regulatory influence on other leukocyte cells. The lymphokines, monokines, interferons, growth factors and other factors have similar effects. Therefore, they are grouped as cytokines.

Cytokines are low molecular weight antigen-nonspecific proteins that mediate cellular interactions involving immuno, inflammatory and hematopoietic systems. Cytokines are short lived and may act locally either on the same cell that secreted it (autocrine) or on other cells (paracrine). Like hormones, they may act systemically (endocrine). Cytokine have a wide variety of functional activities as illustrated their ability to 1) Regulate specific immune responses 2) facilitate innate immuno responses, 3) activate inflammatory responses, 4) affect leukocyte movement, and 5) stimulate hematopoiesis.

Lymphokines are biologically active substances released by activated T-lymphocytes. These are regulatory proteins (mol. wt. 20,000-80,000) whose main function is regulation of immune response and growth and function of cells of reticuloendothelial system. They have several biological activities.

Cytokines are mainly produced by macrophages, while lymphokines are produced mainly by T cells. Macrophages produce four major classes of cytokines, namely IL-1, IL-6, IL- 12 and TNF-α. T cells produce many different lymphokines (Fig. 1. 2) that form two major groups- those produced by Th1 cells and those produced by Th2 cells. In general, the Th1 derived lymphokinestend to have biological activities that counter act the activities of the Th2 derived lymphokines. Lymphokines produced byTh1 cells are IL-2, INF-γ, and lymphotoxin (Tumor Necrosis Factor-β) and those produced byTh2 cells are IL-4, IL-5, IL-9, IL-10 andIL-13.



T_H1 Cytokines	Activity	T_H2 Cytokines	Activity
IFN-γ	Activates macrophages; Regulates cells	IL-4	Activates B cells
GM-CSF	Activates macrophages	IL-5	Activates B cells
TNF-α	Activates macrophages	IL-10	Inhibits macrophages
IL-3	Involved in hematopoiesis	IL-3	Involved in hematopoiesis
TNF-β	Involved in inflammatory responses	TGF-β	Inhibits monocyte activation

Fig-1.2: Cytokines produced by Th1 and Th2 cells

1. 9. INTER LEUKINS:

Interleukin-1 (IL-1):

It is a polypeptide, amonokine released by macrophages and other Ag presenting cells. It is heat labile, retains its activity up to 56ºC and pH 3-11. It occurs in two molecular forms as IL-1 α and IL-1β, which have exactly same function. Its production is stimulated by Ags, toxins, injury and inflammatory processes. Its production is inhibited

by cyclosporine - A, corticosteroids and prostaglandins. Immunological effects of IL-1 are - It stimulates activation of T-helper (Th) cells for the production of IL-2 and other lymphokines. Stimulation of B-cell proliferation and Ab synthesis; granulocytes (neutrophil chemotaxis) and phagocytosis. It mediates a wide range of metabolic, physiological, inflammatory, and haematological effects by acting on bone marrow, epithelial and synovial cells, fibroblasts, hepatocytes, vascular endothelium, and other targets. It is an endogenous pyrogen that induces fever by acting on hypothalamus. Along with tumour necrosis factor (TNF), it induces haematological changes in septic shock and also enhances initial meningeal inflammation in bacterial meningitis. It has a beneficial effect in severe infections in immune compromised hosts.

Interleukin- 2 (IL-2):

It is a cytokine produced by activated T-cells. It is a T-cell growth factor that induces proliferation of T-cell by binding to IL-2 receptor on the cells. It is a powerful modulator of the immune response. It promotes growth and differentiation of T and B cells. It stimulates cytotoxic T-cells and NK-cells. It converts large granular lymphocytes into lymphokine activated killer (LAK), cells which can destroy NK- resistant tumour cells. This property can be used in the treatment of certain types of cancers. Stimulates secretion of other lymphokines.

Interleukin- 3 (IL-3):

It is produced by T-cells. It acts as a growth factor for bone marrow stem cells. It stimulates multi lineage haematopoiesis and therefore also known as the multicolony stimulating factor (multi CSF).

Interleukin- 4 (IL-4):

It is produced by T-helper (Th) cells. It activates resting B-cells and acts a B-cell differentiating factor. It also acts as a growth factor for T-cells and mast cells. It enhances the activity of cytotoxic T-cells. It increase synthesis of IgG-1 and IgE and may play a role in atopic hypersensitivity.

Interleukin- 5(IL-5):

Produced by T-helper (Th2) cells. It causes proliferation of activated B-cells and eosinophils. Stimulates production of IgA and IgM. In conjunction with IL-2, IL5 induces cytotoxic T-cell activity

Interleukin- 6(IL-6):

Produced by stimulated T and B cells, macrophages and fibroblasts. It promotes terminal differentiation of B-cells into Ab producing plasma cells and encourages IgG production. It has properties in common with IL-1 can act on many cell types. Thus, it acts on B cells as a cofactor with IL-1 in IgM synthesis and IL-5 in IgA synthesis. It induces formationofIL-2 receptorson T-cells. It has a stimulatory effect on hepatocytes, nerve cells and haematopoietic cells. It acts as an inflammatory response mediator in host defense against infections.

Interleukin-7(IL-7):

It is derived from bone marrow and thymic stromal cell. It induced proliferation of pre-B cells, thymocytes and T cells and probably controls the lymphoid stem cells.

Interleukin-9(IL-9):

It is a single chain glycoprotein secreted by activated Th 2 cells. It induces antigen

independent growth of certain helper T cells clones but has no effect on Cytotoxic t cell clones. It enhances the proliferative response of bone marrow derived mast cells to IL-3. It also potentiates the effect of IL-4 on B cell Ig E production.

Interleukin-10(IL-10):

In mice IL-10 is immune supressive glycoprotein secreted by Th2 cells by some B-cells and by activated macrophages. It was originally called cytokine synthesis inhibiting factor (CSIF) since it appeared to down regulate cytokine production by Th1 cells in mice.

Interleukin-11(IL-11):

It is a protein secreted by bone marrow stromal cells (fibroblasts). It acts as a growth factor on certain B-cell lines in association with Il-6. It also stimulates megakaryocyte colony formation in association with IL-3 and so may have a role in stimulating platelet production.

Interleukin-12(IL-12):

It is produced by all the antigen processing cells (macrophages, dendritic cells, and B- cells), acts on Th1 cells and NK cells. It promotes Th1 cell differentiation from the Th0 stage. It is the co-stimulator of Th1 activity, inducing those cells to secrete IL-2 and IFN-γ and express IL-2R. It also inhibits some Th2 cell functions such as IgE formation.

Interleukin-13(IL-13):

It is a protein produced by activated Th2 cells. Its production is induced by ligation of CD 28 on the T cell surface with a B7 or related molecule on the surface of an activated antigen presenting cell. It effects similar to those of IL-4.

Interleukin-14(IL-14)

A B-cell growth factor produced by T-cells and some malignant B-cells has been called interleukin 14. It is a secreted protein that induces B-cell proliferation, inhibits immunoglobulin secretion and selectively expands some B-cell sub-populations.

Interleukin-15(IL-15)

It is produced by wide variety of cells especially peripheral blood mono neuclear cells and epithelial and fibroblast cell lines. It has biological activities that are similar to IL-2 and acts as a T-cell growth factor. It enhances proliferation of both helper (CD4) and cytotoxic (CD8) T-cell populations.

Mitogenicor Blastogenic Factor (MF orBF)

Released by the sensitized T-cells that are stimulated by specific antigen. It induces non- specific blast transformation of normal unsensitized T-lymphocytes. Along with transfer factor, it may be important in augmenting or amplifying the cell mediated response by recruiting uncommitted lymphocytes.

Transfer Factor (TF):

An extract from specific antigen sensitized lymphocytes that mediates passive transfer of CMI is known as transfer factor. Both dialyzable and non-dialyzable transfer

factors have been identified. The dialyzable transfer factor has low molecular weight (2000-4000). It is stable at 37ºC, at -20ºC and in the lyophilized from at 4ºC. It is inactivated at 56ºC in 30 minutes. It is resistant to treatment with DNAase, RNAase and trypsin and freeze thawing. Chemically, it is a polypeptide-polynucleotide. It is immunologically specific, i. e. it confers reactivity towards the antigen responsible for its generation. It is highly potent, an extract from0. 1ml of packed leucocytes is sufficient to transfer CMI. Applications: It is useful in immune compromised individuals to restore specific CMI, e. g., in T-cell deficiency (Wiskott-Aldrich syndrome, Nezel of syndrome, DiGeorge syndrome). Disseminated infections associated with deficient CMI (tuberculosis, lepromatous leprosy, mucocutaneous candidiasis, etc.). Cancer-melanoma, sarcoma, etc.

Cytokines Affecting Macrophages:

Antigenically stimulated T-lymphocytes produce certain biologically active soluble proteins (lymphokines), which recruit, activate, and regulate effector cells with the potential to fight with the infecting agent.

The Biological Activities of Lymphokines are as follows:

Macrophage Chemotactic Factor (MCF): It is chemotactic for mononuclear phagocytes. It causes accumulation of these cells at the site of Ag-mediated lymphokine release.

Migration Inhibiting Factor (MIF): It inhibits migration of phagocytic cells and localizes circulating and tissue monocytes at the site of infection. Once attracted or accumulated, cells are discouraged from leaving the site by this factor.

Interferon-γ (IFN-γ): It is also known as macrophage activating factor (MAF). It possesses powerful macrophage activating molecules. It produces significant morphological changes. It increases content of lysosomal enzymes and their activities, so that the macrophage kills ingested intracellular foreign particles. It also causes augmentation of neutrophil and monocyte functions and has anti tumour activity.

Interferon-α(IFN-α): It is produced by leucocytes. It has antiviral activity.

Interferon-β(IFN-β): It is produced by fibro blasts. It inhibits replication of virus in the host cell.

Cytotoxic Lymphokines:

Lympho Toxin (LT):

It is a cytotoxic protein released by activated Th-cells. It is also known as tumour necrosis factor-β (TNF-β). It is cytotoxic for foreign cells such as tumour cells, transplanted cells and microorganisms.

Tumour Necrosis Factor-α(TNF-α):

It is produced by macrophages and monocytes. It causes lysis of tumour cells. It also plays a role in elimination of certain bacteria and parasites. It is also known as

cachectin-causing cachexia-a wasting syndrome during chronic infections because of pronounced catabolic effects, e. g. , breakdown of muscle protein.

Other Cytokines:

Skin Reactive Factor (SRF):

It is produced by specifically sensitized lymphocytes with Ags and mitogens. When injected into the skin of normal guinea pig, it produces an indurated and erythematous lesion within 3 hr. It facilitates the movement of monocytes from blood vessels into extravascular spaces and induces skin hypersensitivity.

Lymphocyte Inhibitory Factor:

It has T-suppressor activity, suppresses Ab production.

Transforming Growth Factor-β (TGF-β):

It is produced by T and B-cells. It is a growth factor that transforms fibroblasts and promotes wound healing. It inhibits T and B cell proliferation and haematopoiesis.

Leukaemia Inhibitory Factor (LIF):

It is produced by T-cells. It helps in proliferation of stem cells and eosinophil chemotaxis.

Cytotoxic T-Cells:

Populations of killer (K) T-cells capable of killing or lysing target cells to which they bind are known as cytotoxic T-cells. They are formed in response to viral infection and graft from genetically dissimilar member. They are cytotoxic for host cells infected with virus, graft tissue and tumour cells. Mechanism of Action - The first stage is the binding of effector cell(K-cell) to target cell, through specific receptors. It is a calcium independent stage. A change occurs in the target cell which leads to cytolysis. This phase is calcium dependent.

1.10. SUMMARY:

The cells participate in the immune response are white blood, or leukocytes. All leukocytes develop from a common multipotent the matopoietic stem cell during hematopoiesis. There are three types of lymphocytes: B cells, T cells and null cells. Naïve Band T lymphocytes, after interacting with antigen, enlarge into lymphoblasts that proliferate and eventually differentiate into effector and memory cells. Macrophages and neutrophils are specialized for the phagocytosis and degradation of antigens. Basophils and mast cells are non-phagocytic cells that release a variety of pharmacologically active substances and play an important role in allergic reaction. Dendritic cells capture antigen. These cells along with macrophages and B cells play an important role in T_H cell activation.

1.11. TECHNICAL TERMS:

B cells, T cells, Phagocytes, Macrophages, Neutrophils, Eosinophils, Null cells, Mast cells, Dendritic cells, Lymphokines, Cytokines, Interleukines.

1.12. SELF ASSESSMENT QUESTIONS:

1) Write an essay on T cells and their role in Cell mediate immune reactions.
2) Give an account on types and subpopulations of B- lymphocytes and their functions.
3) Write an account on different types of mononuclear and granulocytic cells and their role in immune reactions.

1.13. SUGGESTED READINGS:

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Prof. A. Amruthavalli

LESSON-2

PRIMARY AND SECONDARY LYMPHOID ORGANS

2. 0 OBJECTIVE:

- To aware the students with the information on structure and functions of primary and secondary lymphoid organs and Mucosa associated lymphoid tissue.

STRUCTURE:

2.1. INTRODUCTION:

As blood circulate under pressure, its fluid component (Plasma) seeps through the thin wall of the capillaries into the surrounding tissue. Much of this fluid, called interstitial fluid, returns to the blood through the capillary membranes. The remainder of the interstitial fluid now called lymph, flows from the spaces in connective tissue into a network of tiny open lymphatic capillaries and then into a series of progressively larger collecting vessels called lymphatic vessels. The largest lymphatic vessel, the thoracic duct, empties in to the left sub-clavian vein near the heart. In this way, the lymphatic system captures fluid lost from the blood and returns it to the blood, thus ensuring steady-state levels of fluid within the circulatory system. The heart does not pump the lymph through the lymphatic system; instead the flow of lymph is achieved as the lymph vessels are squeezed by movements of the body's muscles. A series of one-way valves along the lymphatic vessels ensures that lymph flows only in one direction.

When a foreign antigen gains entrance to the tissues, it is picked up by the lymphatic system (which drains all the tissues of the body) and is carried to various organized lymphoid tissues such as lymph nodes, which trap the foreign antigen. As lymph passes from the tissues to lymphatic vessels, it becomes progressively enriched in lymphocytes. Thus, the lymphatic system also serves as a means of transporting lymphocytes and antigen from the connective tissues to organized lymphoid tissues where the lymphocytes may interact with the trapped antigen and undergo activation.

The immune system comprises of a variety of organs, which are responsible for growth and development of immunocompetent cells. These are lymphoid organs and reticuloendothelial system (lymphoreticular system).

Lymphoreticular System

Lymphoid system	Reticuloendothelial system
↓	↓
Lymphoid cells	Reticuloendothelial components
↓	↓
Lymphocytes and plasma cells	Phagocytic cells
↓	↓
Specific immune response	Nonspecific immunity (Phagocytosis)

The different categories of lymphocytes, plasma cells and macrophages (phagocytic cells) are the important cells, which participate in the different arms of the immune response to antigenic stimuli. Lymphocytes are the major immunological effector cells. They arise from a precursor or stem cell, which originates in fetal life in the yolk sac and is found subsequently in the liver and bone marrow. The stem cells are further differentiated in two different directions. Some of them migrate to thymus where they are processed to acquire certain markers and functional characteristics that separate them from other lymphocyte population. These are thymus derived or T-lymphocytes, which play an important role in cell-mediated immunity. Other stem cells are processed in the bone marrow of the bursa of fabricius (in chicken) to acquire surface markers that differentiate them from T-lymphocytes. These are B-lymphocytes, which play an important role in humoral or antibody mediated immunity. Both T and B cells, after maturation, migrate to spleen, lymph nodes and other organs where they initiate and participate in immune response to antigens.

The lymphoid system consists of - Lymphoid organs, Lymphoid cells (lymphocytes and plasma cells).The lymphoid organs are classified into the central (primary) and the peripheral (secondary) lymphoid organs based on different functions they perform.

2.2. PRIMARY LYMPHOID ORGANS:

These are the lymphoid organs in which proliferation and differentiation of lymphocytes takes place without antigenic stimulation (antigen independent maturation of lymphocytes). The primary lymphoid organs include thymus and bone marrow.

2.2.1 Thymus:

Thymus (Fig.2.1) is a lymphoepithelial bilobed structure located behind the upper part of the sternum (in the anterior mediastinum). It is derived from the third and fourth pharyngeal pouches and differentiated from these pouches at about the sixth week of fetal life. It acquires characteristic lymphoid appearance by the third month of gestation. It increases in

size during fetal development, reaches its maximum at birth and gradually decreases in size with age and finally atrophies. It is capsulated. The septa arising from the capsule divide the gland into lobules, which are differentiated into an outer cortex and an inner medulla. The cortex is composed mainly of epithelial cells and lymphocytes. The precursors of lymphocytes (stem cells-immature cells) from yolk sac, fetal liver and bone marrow reach the thymus and mature in the cortex, acquire surface characteristics of T-lymphocytes and then migrate into medulla. In the medulla, the lymphocytes complete their maturation process and exit into the blood as matured T- cells capable or responding to antigenic stimuli and seeded into the secondary lymphoid organs. Mature thymocytes in thymus are about 5-10% of the total population.

Functions of Thymus:

It is the center for development and function of the immune system, however, it does not participate in immune reaction. It is the major site for lymphocyte proliferation and production of T-lymphocytes. In the thymus, lymphocytes acquire new surface antigens. The thymus confers immunological competence on the lymphocyte. Pre thymic lymphocytes are not immunologically competent. In the thymus, they are educated by hormone like humoral factors--thymosin, thymopoietin, etc., produced by thymic epithelial cells, so that they become capable of mounting CMI.

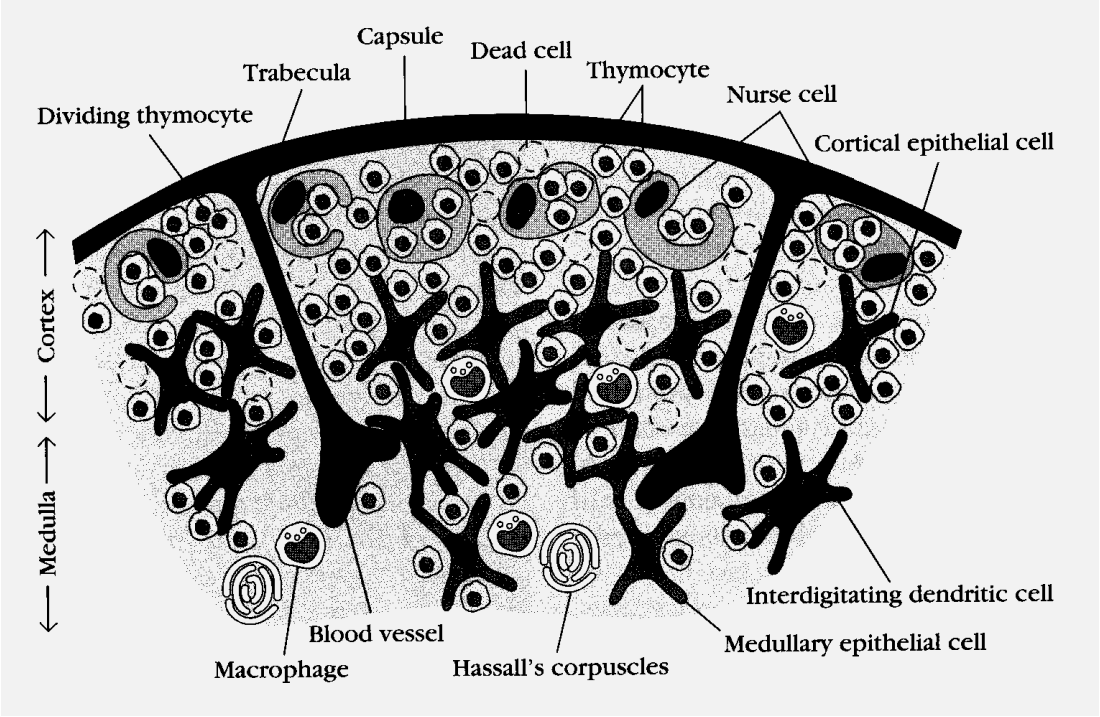

Fig-2.1: Diagrammatic Cross Section of A Portion of the Thymus

2.2.2 Bone Marrow

In birds, a lymphoid organ called the bursa of Fabricius, a gut-associated lymphoid tissue, is the primary site of B-cell maturation. In mammals such as primates and rodents, there is no bursa and no single counterpart to it as a primary lymphoid organ. Instead, in these animals, regions of bone marrow and possible of other lymphoid tissues serve as the 'bursal

equivalent', where B cell maturation occurs. Bone marrow is a spongy tissue found inside bone, particularly red marrow, which actively produces immune cells. Bone marrow is a crucial primary lymphoid organ where all blood cells, including lymphocytes (B cells and T cell precursors), originate through hematopoiesis. B cells born in bone marrow, complete their maturation there itself and develops the ability to recognize specific pathogens. However, immature T cells that born in the bone marrow migrate to the thymus for final maturation, further development and selection. In those animals whose immature B cells proliferate and differentiate within the bone marrow, stromal cells of bone marrow interact directly with the B cells and secrete various cytokines that are required for development.

2.3. PERIPHERAL (SECONDARY) LYMPHOID ORGANS:

These are the organs, which receive and maintain functional lymphocytes. The lymphocytes educated by central lymphoid organs are seeded into peripheral lymphoid organs where they initiate and participate in immune response to antigenic stimuli. The peripheral lymphoid organs include lymph nodes, spleen and mucosa associated lymphoid tissue.

Mechanism:

Various types of organized lymphoid tissues and diffuse collections of lymphocytes and macrophages are present in these organs. The lymphoid tissue is organized into structures called lymphoid follicles, which consist of aggregates of lymphoid and non-lymphoid cells surrounded by a network of draining lymphatic capillaries. Until it is activated by antigen, a lymphoid follicle-called a primary follicle- comprises a network of follicular dendritic cells and small resting B cells. After an antigenic challenge, a primary follicle becomes a larger secondary follicle-a ring of concentrically packed B lymphocytes surrounding a center (the germinal center) in which one finds a focus of proliferating B lymphocytes and an area that contains non-dividing B cells, and some helper T cells interspersed with macrophages and follicular dendritic cells.

Most antigen-activated B cells divide and differentiate into antibody-producing plasma cells in lymphoid follicles, but only a few B cells in the antigen-activated population find their way into germinal centers. Those that do enter a germinal center undergo one or more rounds of cell division, during which the genes that encode their antibodies mutate at an unusually high rate. Following the period of division and mutation, there is a rigorous selection process in which more than 90% of these B cells die by apoptosis. In general, those B cells producing antibodies that bind antigen more strongly have a much better chance of surviving than do their weaker companions. The small numbers of B cells that survive the germinal center's rigorous selection differentiate into plasma cells or memory cells and emerge.

2.3.1 Lymph Nodes:

These are small, round or oval shaped organs found in various parts of the body. They are generally located at major junctions of the network of lymphatic channels that connect to the thoracic duct, which passes lymphocytes and lymph to the large vein connected to the heart. They are differentiated into outer cortex and inner medulla. The cortex is further subdivided into the external cortex located just below the capsule and a deep cortex also known as the paracortical area. In the external cortex, there is accumulation of lymphocytes in regions called follicles (primary lymphoid follicles) within which there is development of germinal centers (secondary follicles) following antigenic stimulation. The follicles contain small lymphocytes and dendritic macrophages, which are responsible for trapping and

processing of antigen. In the medulla, lymphocytes are arranged as elongated branching bands (medullary cords). In activated lymph nodes, most of them are plasma cells secreting antibodies. The cortical follicles and medullary cords contain B-lymphocytes (bursa dependent area) while the T-cells are occupied in paracortical area (thymus dependent area) (Fig.2.2).

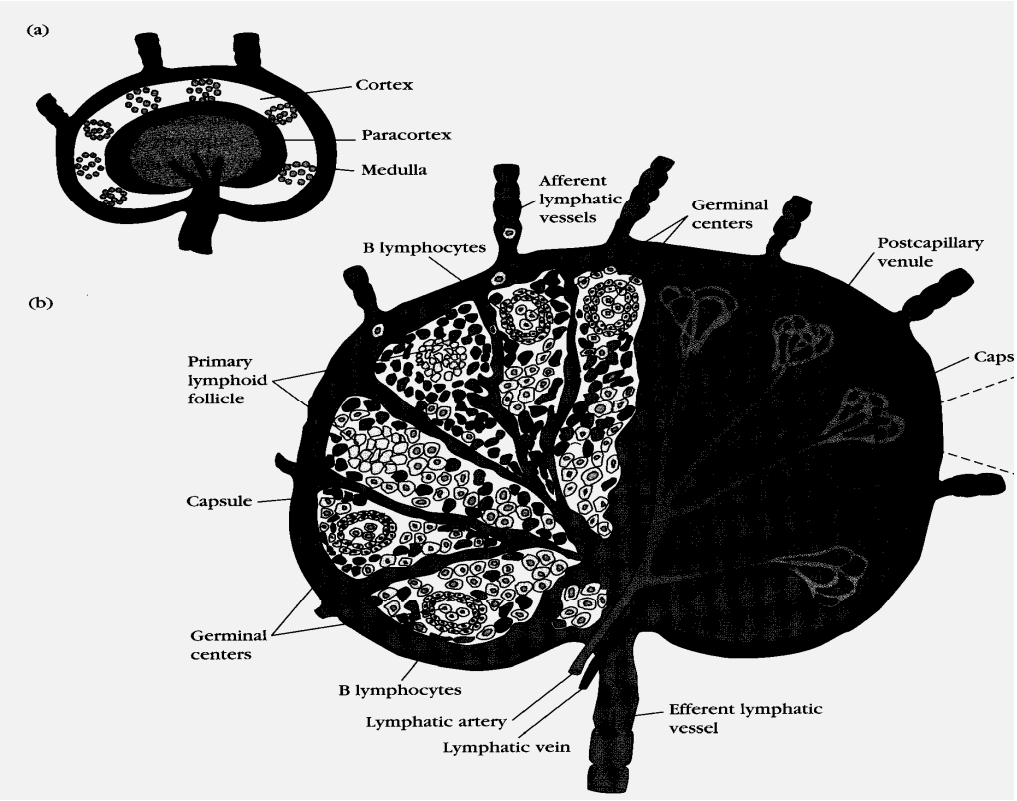

Fig-2.2: Structure of a Lymph Node

(a) The three layers of a lymph node (b) various regions within the lymph node.

Functions of Lymph Node:

They act as filters for lymph draining body tissues including foreign antigens. They act as junction between circulatory system and lymphatic system. Provide a site for phagocytosis and antibody production. Support the development of lymphocytes. Allow recirculation of lymphocytes.

2.3.2 Spleen

It is the largest lympho vascular organ about 250g in weight in adult man. The architecture is similar, but not identical to that of lymph node. It has two segregated area; the red pulp and while pulp, separated by marginal zone. The white pulp is rich in lymphoid tissue while the red pulp is abundant in sinuses and contains large quantities of RBCs. The white pulp is located mainly around small arteries and the peri arterial lymphatic sheath is composed primarily of T-lymphocytes and is called the thymus dependent area. The external lymphoid area surrounding the periarterial lymphatic sheath is a B-dependent area (peri-follicular region, germinal center and mantle layer). Approximately 30-40% of the cells in the

spleen are T-cells and 50% of the cells are B- cells. The periarterial lymphoid collections in the white pulp are called Malpighian corpuscles or follicles. Following antigenic stimulation, germinal centers are produced in the white pulp that are composed of large numbers of rapidly dividing cells, which differentiate into plasma B-cells and plasma cells and replace T-cells in the periarterial region (Fig. 2.3).

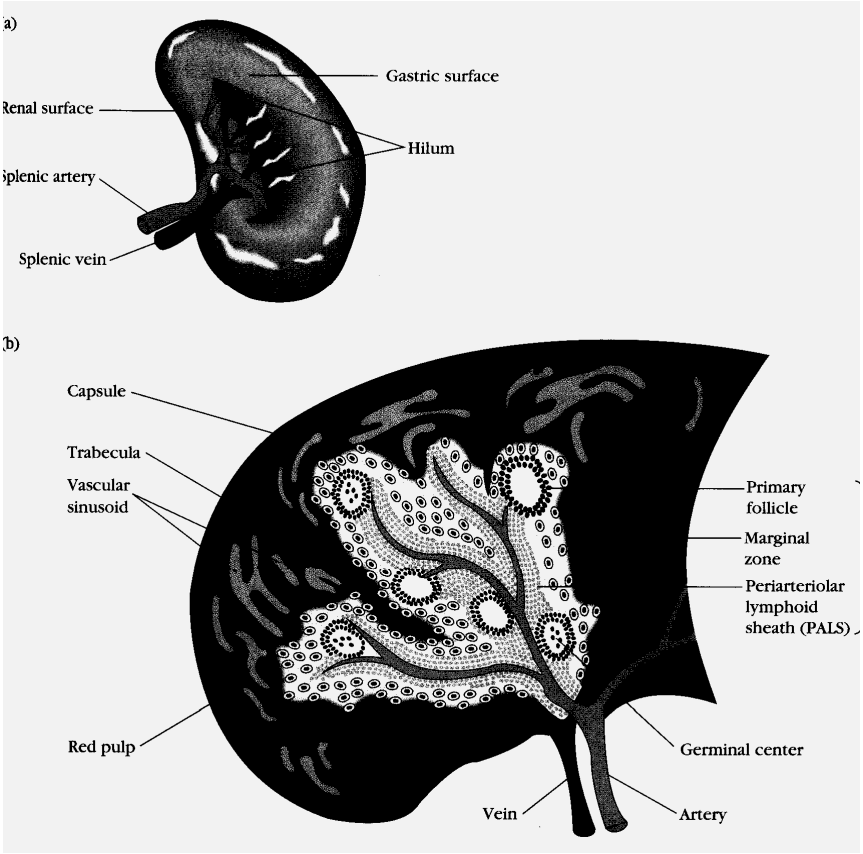

Fig-2.3: Structure of the Spleen

(a) The spleen whole organ (b) diagrammatic cross section of the part of the spleen

Functions of Spleen:

It is the sole lymphatic organ specialized to filter blood. Thus, it is a systemic filter to trap or concentrate circulating blood born particles. It is a major site for antibody synthesis against blood borne particles including microorganisms. Its immunological role is primarily directed against blood borne pathogens.

2.3.3 Mucosa Associated Lymphoid Tissue (MALT):

Lymph nodes and the spleen are the most highly organized of the secondary lymphoid organs; they comprise not only lymphoid follicles, but additional distinct regions of T-cell and B-cell activity, and they are surrounded by a fibrous capsule. Less- organized lymphoid tissue, collectively called mucosal-associated lymphoid tissue, (MALT), is found in various body sites. Potentially important collections of lymphocytes, mainly producing IgA are present throughout the mucosal lining of alimentary, respiratory, genitourinary and other surfaces. The MALT structure contains mixture of B- cells, T-cells as well as phagocytic cells. Secretory IgA is the main immunoglobulin produced by MALT, IgG, IgM and IgE are also produced locally.

MALT includes peyer's patches (in the small intestine), the tonsils, and the appendix, as well as numerous lymphoid follicles within the lamina propria of the intestines and in the mucous membranes living the upper airways, bronchi, and genital tract. However, these different lymphoid tissues can also be categorized according to their places of occurrence in the body. Like, lymphoid tissues of the gut are known as gut associated lymphoid tissue (GALT) and those in the respiratory tract are called the bronchus associated lymphoid tissue (BALT). The main GALT structures in man are: Tonsils (lingual, palatine and pharyngeal); Appendix (at the junction of small and large intestine) (Fig. 2.4.); Peyer's patches of the intestine; and Lamina propria of the intestine. BALT includes the tissues in the upper airways, bronchi, and genital tract.

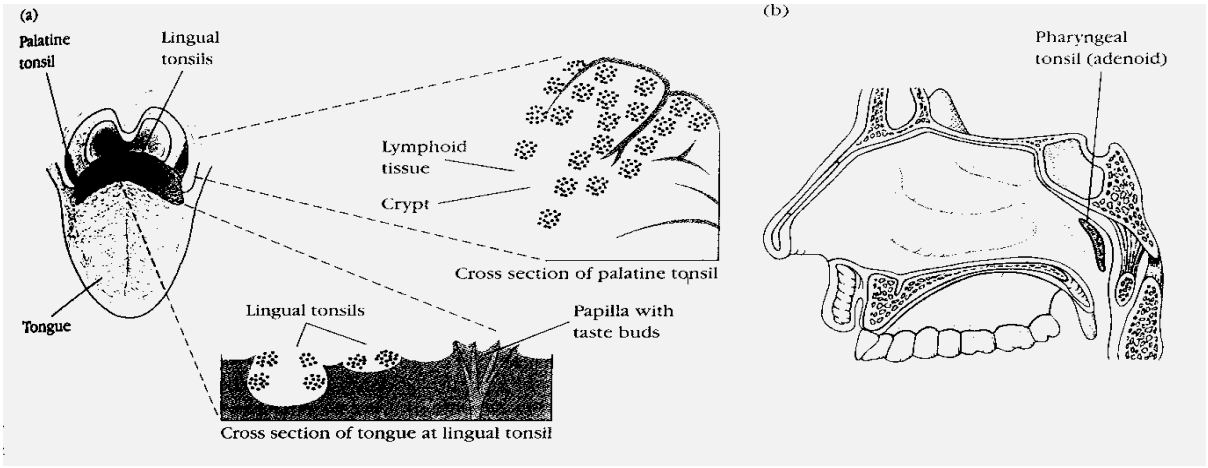

Fig-2.4: Three Types of Tonsils
a) The position and internal features of the palatine and lingual tonsils
b) a view of the position of the nasopharyngeal tonsils.

The mucous membranes lining the digestive, respiratory, and urino-genital systems have a combined surface area of about 400 m^2 and are the major sites of entry for most pathogens. These vulnerable membrane surfaces are defended by a group of organized lymphoid tissues known collectively as mucosal-associated lymphoid tissue (MALT). Structurally, these tissues range from loose, barely organized clusters of lymphoid cells in the lamina propria of intestinal villi to well-organized structures such as the familiar tonsils and appendix, as well as peyer's patches, which are found within the sub mucosal layer of the intestinal lining. The functional importance of MALT in the body's producing plasma cells, whose number far exceeds that of plasma cells in the spleen, lymph nodes, and bone marrow combined.

The tonsils are found in three locations: lingual at the base of the tongue; palatine at the sides of the back of the mouth; and nasopharyngeal (adenoids) in the roof of the nasopharynx. All three tonsil groups are nodular structures consisting of a meshwork of reticular cells and pulp surrounds the sinusoids. The white pulp forms a sleeve, the periarteriolar lymphoid sheath (PALS) around the arterioles; this sheath contains numerous T cells. Closely associated with the PALS is the marginal zone, an area rich in B cells that contains lymphoid follicles that can develop into secondary follicles containing germinal centers. Fibers interspersed with lymphocytes, macrophages, granulocytes, and mast cells. The B cells are organized into follicles and germinal centers; the latter are surrounded by regions showing T-cell activity. The tonsils defend against antigens entering though the nasal and oral epithelial routs.

The best studied of the mucous membranes is the one that lines the gastrointestinal tract. Lymphoid cells are found in various regions within this tissue. The outer mucosal epithelial layer contains so-called intraepithelial lymphocytes (IELs). The majority of these lymphocytes are T cell that express unusual receptors (γδ T-cell receptors, or γδ TCRs), which exhibit limited diversity for antigen. Although the population of T cells is well situated to encounter antigens that enter through the intestinal mucous epithelium, their actual function remains largely unknown. The lamina propria, which lies under the epithelial layer, contains large numbers of B cells, plasma cells, activated TH cells, and macrophages in loose clusters. Histological sections have revealed more than 15,000 lymphoid follicles within the intestinal lamina propria of a healthy child. The sub mucosal layer beneath the lamina propria contains peyer's patches, nodules of 30-40 lymphoid follicles. Like lymphoid follicles in other sites, those that compose peyer's patches can develop into secondary follicles with germinal centers.

The epithelial cell of mucous membranes play an important role in promoting the immune response by delivering small samples of foreign antigen from the lumina of the respiratory, digestive, and urino-genital tracts to the underlying mucosal-associated lymphoid tissue. This antigen transport is carried out by specialized cells, called M cells. The structure of the M cell is striking: these are flattened epithelial cells lacking the microvilli that characterize the rest of the mucous epithelium. In addition, M cells have a deep invagination, or pocket, in the basolateral plasma membrane; this pocket is filled with a cluster of B cells, T cells, and macrophages. Luminal antigens are endocytosed into vesicles that are transported from the luminal membrane to the underlying pocket membrane. The vesicles then fuse with the pocket membrane, delivering the potentially response-activating antigens to the clusters of lymphocytes contained within the pocket.

M cells are located in so-called inductive sites-small regions of a mucous membrane that lie over organized lymphoid follicles. Antigens transported across the mucous membrane by M cells can activate B cells within these lymphoid follicles. The activated B cells differentiate into plasma cells, which leave the follicles and secrete the IgA into the lumen, where they can interact with antigens. Mucous membranes are an effective barrier to the entrance of most pathogens, which thereby contributes to nonspecific immunity. One reason for this is that the mucosal epithelial cells are cemented to one another by tight junctions that make it difficult for pathogens to penetrate. Interestingly, some enteric pathogens, including both bacteria and viruses, have exploited the M cell as an entry route through the mucous-membrane barrier. In some cases, the pathogen is internalized by the M cell and transported into the pocket. In other cases, the pathogen binds to the M cell and disrupts the cell, thus allowing entry of the pathogen. Among the pathogens that use M cells in these ways are several invasive *Salmonella* species, *Vibrio cholerae*, and the *polio* virus.

Function of MALT:

Local immunity against pathogens invading local tissue.

2.4. SUMMARY:

The primary lymphoid organs provide site where lymphocytes mature and become antigenically committed. T lymphocytes mature within the thymus and B lymphocytes arise

and mature within the bone marrow of humans. Secondary lymphoid organs capture antigens and provide sites where lymphocytes become activated by interaction with antigens. Lymph nodes trap antigen from lymph, spleen traps blood borne antigens, intestinal associated lymphoid tissues interact with antigens that enter the body from the gastrointestinal tract. Lymphocytes present throughout the mucosal lining of alimentary, respiratory, genitourinary and other surfaces helps in local immunity against pathogens invading local tissue.

2.5. TECHNICAL TERMS:

Primary lymphoid organs, Secondary lymphoid organs, Thymus, Bone marrow, Lymph nodes, Spleen, MALT.

2.6. SELF ASSESSMENT QUESTIONS:

1) Write an essay on structure and functions of primary lymphoid organs.
2) Write in detail about structure and functions of secondary lymphoid organs.
3) Give an account on various tissues of MALT and its functions.

2.7. SUGGESTED READINGS:

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Prof. A. Amruthavalli

LESSON-3

TYPES OF IMMUNITY

3.0. OBJECTIVE:

- This lesson enlightens the students about different types of immune responses including innate immunity, acquired immunity, humoral immunity, cell mediated immunity and other types of immunity.

STRUCTURE:

3.1. INTRODUCTION:

In the beginning of eleventh century Chinese determined that those who survived an attack of small Pox disease would not get the disease second time. Then after, they practiced infecting young children with small pox by either inhalation or scratching of the dried pocks on the skin of the infected persons. This method of protecting children was spread to Westward and central Asia. In 1718 Lady Mary Montagu used the same technique, to save her children from smallpox. This technique was called as 'variolation' (variola' is the latin word for small pox). In 1774 Benjamin Jesty used dried material from cowpox to variolate his children and found successful. Later 1778 Edward Jenner, a physician, confirmed the same by variolating his parents and published the results. This Jenner's technique of variolation (vacca is the latin word for cow) replaced the variolation and popularized. By 1980 smallpox had become the first infectious disease to be eradiated from the earth by mass vaccination. In 1879 Louis Pasteur during his studies on chicken fowl cholera disease, found that aged cultures of *Pasterella multicida* , the causal agent of cholera, can even protect the chicken from disease. These aged cultures act as 'vaccine' against cholera. Pasteur, later studied the anthrax disease and found that heated cultures of *Bacillus anthrasis* can act as vaccines against the disease to protect the sheep, cattle and goat. In 1980, Emil von Behring and Shibasaburo Kitasato demonstrated that protection by vaccination is due to induced production of protective factors which they called as antibodies. Later Paul Ehrlich proved that antibodies could protect animals against toxins like recin. Similarly Pfeiffer showed that antibodies could clump and destroy *Vibrio cholerae*. Based on this, Isidone Widal

demonstrated that serum from typhoid patients would make the bacteria clump whereas serum from healthy individuals cannot clump- WIDAL TEST to *Salmonella typhi*. In 1894 Emile Roux demonstrated that by repeated injections of tetanus toxin to horse, antibodies against the toxin were developed in horse serum, this horse serum could be used to protect a human against tetanus for a few weeks after they have received a deep wound. This technique was known as passive immunization. In 1882 Eli Metchnikoff demonstrated that certain cells could eat foreign material, which he called phagocytes, that enter the body of invertebrates and mammals. In 1901 Emil von Behring was awarded nobel prize in medicine for his work on the production of antibodies against toxins. In 1905 Robert koch was awarded the nobel prize for his studies on tuberculosis, by the discovery of the tuberculin reaction. The nobel prize of 1908 was shared by Ehrlich and Metchnikoff for their contribution towards the development of concept that the immune system would not respond against normal body components. Karl Landsteiner was awarded a nobel prize in 1930 for his demonstration of blood groups, the complex carbohydrates found on the surface of red cells. In 1972 Rodney Porter and Gerald Edelman received the prize for demonstrating the chemical structure of antibody molecules and showed that they bound to foreign material. The 1984 nobel prize in medicine was awarded to Niels Jerne, Georges Kohler and Cesar Milstein for discovering the principles of production of monoclonal antibodies. In 1980 Donnall Thomas and Joseph Murray were awarded the prize for their pioneering work on organ transformation.

3.2. TYPES OF IMMUNE RESPONSES:

The latin term immunis meaning 'exempt' gave rise to the English word immunity, which refers to all the mechanisms used in the body as protection against environmental agents that are foreign to the body. Immunity may be innate or acquired.

3.3. INNATE IMMUNITY:

Innate immunity refers to the immunity response of the body due to physical and chemical barriers of the body, tissues, cells and all other mechanisms used as protection against environmental agents that are foreign to the body.

Mechanism of Immunity:

a) Physical and Chemical Barriers :

In order to produce disease, microorganisms must enter into the body. The simplest way to avoid infection is to prevent the entry of microorganisms. It is the first line of defense against infection (the external defense system). The entry of microbes can be prevented by surface defense.

The intact skin and mucous membrane confer protection against invasion by microbes.

i) Role of skin: The skin surface consists mainly of keratin, which is indigestible by most of the microbes. Thus, as long as it is intact, is impermeable to most of the infectious agents. Most bacteria fail to survive on skin for a long time because of the inhibitory effects of saturated and unsaturated fatty acids in sweat and sebum (oily secretion of skin). Sweat also contains high concentration of salts, which are inhibitory to bacteria and fungi. Acidic pH (5.2-5.9) of the skin due to lactic acid and other acids prevents the growth of bacteria.

ii) Role of mucous membrane: Mucous membrane secretes mucus, which is a protective barrier. Microbes trapped within the adhesive mucus are removed by ciliary movements, coughing and sneezing. Mucous secretion of the respiratory, alimentary and genitourinary tracts contains a bactericidal substance known as lysozyme, which acts on bacteria by hydrolyzing glycosidic linkages in the cell wall mucopeptides.

iii) Role of body secretion: Body secretes various fluids, which play an important role in defense mechanism. The skin secretions-sweat and sebum contain bactericidal substances. Tears contain lysozyme, which is bactericidal in nature; also the flushing action of tears makes conjuctiva free from microbes and dust particles. Saliva contains mucopolysaccharides, which inactivate bacteria and viruses. Gastric juice contains hydrochloric acid (HCl), which destroys most of the ingested bacteria and keeps stomach free from microbes. Gastric juice also contains an enzyme namely pepsin. Urine--the flushing action of urine eliminates bacteria from urethra. Semen is believed to contain antibacterial substances, for example, spermine.

Role of Commensal Microflora:

The microorganisms normally present in and on our body without causing any ill effects are known as commensals. Commensal microflora plays an indirect role in the defense mechanism. Some streptococci normally present in mouth produce hydrogen peroxide (H_2O_2), which is inhibitory to many other bacteria. Some of the coliform bacteria normally present in intestine produce colicins, which are inhibitory to other coliforms and shigellae. Lactobacilli normally present in vagina produce lactic acid. The resultant acidity gives protection against pyogenic infections. The propionibacteria normally present on skin produce propionic acid, which plays role in maintaining low pH. The anaerobic colon bacteria produce fatty acids with antibacterial activity. The intestinal anaerobes also prevent superinfection by coliforms during antibiotic therapy. It is also possible that the organisms of normal flora may tend to exclude pathogens by competing with them for nutrient material.

Tissue factors:

When the infective agent penetrates the body by passing the barriers of surface defense, the tissue factors come into operation. It is the second line of defense against infection (the internal defense systems).

a) Humoral Factors: Apart from specific antibodies, a variety of substances possessing antimicrobial activity are present in blood and tissue fluids. These include Lysozyme: It is a thermolabile, low molecular weight, basic protein found in high concentration in polymorphonuclear leucocytes and in most of the tissue fluids except sweat, urine, and cerebrospinal fluid (CSF). It is a mucolytic enzyme that acts on mucopeptide of the bacterial cell wall. Complement: It is a non-specific heat labile protein present in serum that has bactericidal activity. It plays an important role against pathogens invading blood and tissues. Properdin: It is a complement-like substance normally present in serum. It requires participation of components of complement and Mg++ ions for its bactericidal and antiviral activity. It is active against gram negative bacteria and shigellae in particular. Interferon: It is a non-specific antiviral agent that interferes with intracellular viral replication. It is synthesized in response to viral infections and is non-specifically active against other viruses. It increases the activity of non-specific killer cells. Phagocytin: It is a thermostable protein derived from

polymorphs. It is bactericidal for many gram positive and gram negative bacteria. Other antimicrobial substances: Betalysin in serum, plakins derived from platelets, leukin from leucocytes, etc., are also active against infectious agents.

b) Cellular Factors: Natural defense against microbes invading blood and tissues is medicated by phagocytic cells that engulf and digest them. Phagocytosis is the most important means of defense against microbes. There are two types of phagocytic cells - Microphages and - Macrophages. Their function is to remove the foreign particles. These cells, attracted by chemotactic mechanism, reach the sites of inflammation in large numbers and ingest the particulate material that is finally subjected to the action of the lytic enzymes present in lysosome and is digested. Microbes, for example, *Brucella* spp., lepra bacilli and tubercle bacilli which resist killing and digestion, may actively multiply inside the phagocytic cell. Phagocytic cells in such cases may actually help to disseminate infection to different parts of the body.

In addition to phagocytic cells, natural killer cells and large granular lymphocytes secrete several cytotoxic proteins that can destroy viral or tumour antigens.

a) Inflammation: Tissue injury or irritation due to the entry of pathogen results in a spectrum of cellular and systemic events that leads to inflammation which is an important non-specific defense mechanism. Inflammation occurs as a result of aggregation of macrophages and microphages by chemotactic mechanisms at the site of injury. The attracted phagocytic cells engulf and destroy the pathogens. The outpouring of plasma helps to dilute the toxic products and a fibrin barrier serves to wall off the site of infection.

b) Fever: Fever is a protective defense mechanism of the body. The thermo regulatory center in hypothalamus is sensitive to microbes and their products and reacts by increasing the body temperature, which increases circulation of blood and flushing of tissue that help to eliminate toxin through urine and sweat. Increase in body temperature may be harmful to invading microbes and in some instances may destroy the pathogens. It stimulates the production of interferon that helps in recovery of viral infection.

3.4. ACQUIRED IMMUNITY:

It is defined as an immunity specific for a particular disease, which an individual acquires during the course of his life. As it is specific for a particular disease, it is also known as specific immunity. It is of two types a) Active immunity b) Passive immunity

a) Active Immunity: It is the resistance developed by an individual in response to the microbes or their products (antigenic stimulus). The entry of antigen results in activation of immunocompetent cells producing antibodies (humoral/antibody mediated immunity) or activated T cells (cell mediated immunity). Active immunity requires a considerable time (latent period) for its development (weeks or months) but once developed, persists for long duration and may last for years. Active immunity is associated with immunological memory. The memory cells produced after the first entry of antigen retain the memory for long periods and give rapid and vigorous response when the same antigen enters subsequently (secondary response). Active immunity is of two types i) Naturally acquired active immunity. ii) Artificially acquired active immunity.

Naturally Acquired Active Immunity: Immunity that an individual develops as a result of natural contact with a pathogenic microbe. This contact may result in a major invasion with clinical disease are a minor invasion without clinical disease (in apparent or subclinical infection). Following infection, the patient, in most cases, will be resistant to further infection by the same pathogen for a period, which is different in different diseases. In some diseases like influenza, common cold, gonorrhoea and staphylococcal infection, the immunity lasts for short duration. While in other infections such as diphtheria, smallpox, measles, yellow fever, etc., it lasts for long duration and may persist for life. In general, immunity following bacterial infection is less permanent than immunity following viral infection. In syphilis, a special type of immunity known as 'premunition' or 'infection immunity' is seen, i.e., immunity to reinfection persists as long as the original infection remains active. Once the disease is cured and the organisms are eliminated from the body, the patient again becomes susceptible to the reinfection by *Treponema pallidum*. This accounts for the pingpong syphilis in sailors who acquire infection at one port, take treatment at next port, to get infection again at the next port.

Artificially Acquired Active Immunity:

Immunity, which an individual acquires as a result of artificial inoculation of microbes or their products (immunization with microbes or their products). The degree of immunity produced is same as that of natural infection. For immunization, vaccines or their products are used. Vaccines are preparations containing live or killed microbes.

Examples are:

Live attenuated vaccines

1) Bacterial - BCG for tuberculosis, Typhoral for typhoid.
2) Viral - Sabin oral vaccine for polio, MMR for mumps, measles and rubella.

Killed vaccines

1) Bacterial - TAB for enteric fever, Cholera vaccine.
2) Viral - Salk vaccine for polio, Rabies vaccine.

Microbial products, Diphtheria toxoid, Tetanus toxoid.

b) Passive Immunity: The resistance, which is induced by transfer of preformed antibodies against microbes or their products in another host, is known as passive immunity. Here, the immune system does not take any active part in the development of immunity. The passive immunity is rapidly established and the protective mechanisms come into force immediately. It is useful in certain situations like gas gangrene, tetanus, diphtheria and snake bite where the immediate protection is required. Passive immunity is of two types: i) Naturally acquired passive immunity and ii) Artificially acquired passive immunity.

i) Naturally acquired passive immunity: The newborn babies are normally devoid of acquired active immunity but are resistant to number of infections such as measles, chickenpox, diphtheria and scarlet fever. This resistance is due to passive transfer of antibody from mother to foetus and lasts for three to four months.

ii) Artificially acquired passive immunity: Passive immunity can be acquired artificially by injection of antibodies. The agents used for this purpose are hyperimmune sera of animal or human origin, convalescent sera and pooled human gammaglobulin. This immunity lasts for short duration, for example, the diphtheria antitoxin has a half-life of seven days. This procedure is used for: Treatment and also for prophylaxis and particularly indicated in clinical emergency for providing immediate and temporary protection in a non-immune host. The suppression of active immunity when it is injurious as in Rh negative women with Rh positive babies. For passive immunization, the hyperimmune sera of horse, sheep, goat, rabbit, guinea pigs or human beings are used. The serum may be antitoxic, antibacterial or antiviral.

3.5. HUMORAL IMMUNITY:

Humoral immunity is mediated by serum antibodies, which are the proteins secreted by the B cells. B cells are initially activated to secrete antibodies after the binding of antigens to specific membrane immunoglobulin (Ig) molecules (B cell receptors), which are expressed by these cells. Antibodies are a heterogenous mixture of serum globulins, all of which share the ability to bind individually to specific antigens.

All immunoglobulin molecules have common structural features, which enable them to do two things (1) recognize and bind specifically to a unique structural entity on an antigen (namely, the epitope) and (2) perform a common biologic function after combining with the antigen. Basically, each immunoglobulin molecule consists of two identical light (L) chains and two identical heavy chains (H), linked by disulfide bridges. On the basis of differences in their H chains, these molecules are divided into five major classes IgG, IgM, IgA, IgE and IgD each of which has several unique biological properties. It is important to note that antibodies in all five classes may possess precisely the same specificity against an antigen (antigen-binding sites), while at the same time having different functional (biological effector) properties.

Another important element involved in humoral immunity is the complement system. The reaction between antigen and antibody serves to activate this system, which consists of a series of serum enzymes, the end result of which is lysis of the target or enhanced phagocytosis (Ingestion of the antigen) by phagocytic cells. The activation of complement also results in the recruitment of highly phagotrophic polymorphonuclear (PMN) cells, which constitute part of the innate immune system. These activities maximize the effective response made by the humoral of immunity against invading antigens.

3.6. CELL MEDIATED IMMUNITY

CMI is the specific immune response mediated by sensitised T-cells independent of Ab. Certain microorganisms such as Bacteria-tubercle and leprosy bacilli. Viruses-small pox and measles. Parasites-*Toxoplasma* and *Leishmania*. Fungi-*Histoplasma* and *Blastomyces*. These organisms are intracellular pathogens, which have the ability to multiply within the host cells. Hence, Abs are not effective against such pathogens. Immunity against these pathogens is masterminded by the T-lymphocytes. Such an immune response, which involves the interaction of cells of the immune system with the Ag, is known as CMI.

Induction of CMI:

The nature of antigenic stimulus is important in the induction of CMI. The intracellular parasites are the best stimulators. The T-cells possess specific receptors (TCR) on their surface for Ag. The CMI is initiated by the binding of Ag with an Ag receptor on T-lymphocyte. The Ag may react directly or it may be presented by macrophage. When specific Ag reacts with specific receptor on T-cells, they undergo proliferation and a sequence of morphological and biochemical events occur. The cell membrane of T-cell becomes activated and the signal is transmitted to interior of the cell where the nucleus becomes depressed and the cell transforms into a large blast cell--blast transformation. DNA, RNA and protein synthesis is increased. This interaction finally results in blast transformation, clonal proliferation and differentiation. The result of these events are - The generation of Th and Ts cells for T-T and T-B interactions ; generation of cytotoxic T-cells ; generation of lymphokine producing T-cells ; generation of memory cells.

Proliferated T-lymphocytes provide two major effector mechanisms.

a) The release of biologically active soluble factors called lymphokines (cytokines).

b) The generation of cytotoxic T-cells of artificial active immunization, it may be possible to eradicate communicable diseases like diphtheria, polio, etc.

Detection of CMI:

Several tests are now available for detection of CMI. Skin test was the only method available till recently. A number of in vitro methods are now available.

These include Lymphocyte transformation test- in which there is a transformation of cultured sensitized T-lymphocytes on contact with specific Ag to blast cells-evidenced by enhanced DNA synthesis. Target cell destruction-in which there is a killing of cultured cells by lymphocytes sensitized against them. Migration inhibiting factor test-it is the most commonly used test for detection of CMI. In this test, macrophages packed in a capillary tube when placed in a tissue culture medium in a chamber, macrophages migrate out and spread over the galls walls of the chamber and form a lacy fan like appearance. If macrophages are from sensitized guinea pig, the addition of Ag to the culture chamber will inhibit the migration. The test has been adopted for clinical use by incubating human peripheral leucocytes in capillary tubes to culture chambers. When specific antigen is added, the migration of leucocytes is prevented. By comparing with the control test, a semi quantitative assessment of the migration inhibition is possible.

Other Types of Immunity:

Local Immunity:

Certain microbes infect only certain groups of cells and selective tissues. Resistance against such pathogens depends on the immunity of the corresponding cells and tissues. This is known as local immunity. For example, in poliomyelitis and influenza, systemic immunity developed as a result of active immunization with killed vaccines neutralizes the viruses when they enter the blood stream but the multiplication of viruses at the site of entry (gut mucosa in polio and respiratory tract in influenza) cannot be prevented. The local multiplication of the virus can be prevented by the local immunity acquired as a result of infection or

immunization with the live oral polio vaccine and intranasal influenza vaccine. A special type of antibody, IgA, plays an important role in local immunity. This IgA antibody, known as secretory IgA, is produced locally by plasma cells present on mucosal surfaces or in secretory glands. The IgA is the principal component in various body secretions such as mucus of respiratory, intestinal, urinary, and genital tracts, tears saliva, and milk.

Herd Immunity:

It is the overall level of immunity in a community and is important in the control of outbreaks. When herd immunity is low, an infectious disease may spread rapidly and may be severe in nature. When herd immunity is high, the infectious disease spreads less rapidly and is of mild form. By developing high level of herd immunity by means

3.7. PRIMARY IMMUNE RESPONSE:

When Ag enters for the first time (priming dose), the body gives primary response (Fig. 3.1). Immediately after the priming dose of an Ag, no Ab is detected in the serum. This period is known as latent period. After this, an active biosynthesis of Ab occurs during a log phase. The Ab concentration in serum remains constant during a plateau or steady state. Finally, a declined phase is observed in which catabolism is greater than synthesis. Thus, the primary response is S-Slow, S-sluggish, S-short lived ; with long lag phase of 5-7 days; low titres of Abs that persist for short duration and type of Ab - IgM of low affinity, low avidity and differ in specificity (Table 3.1).

Table-3.1: Difference between Primary and Secondary Response

Primary Response	Secondary Response
1. Slow, sluggish, short lived	Prompt, powerful, prolonged
2. Long latent phase	Short or negligible latent phase
3. Rate of Ab synthesis – low	Rate of Ab synthesis – high
4. Type of Ab – IgM	Type of Ab – IgG
5. Peak Ab titre – low	Peak Ab titre – high
6. Persistence of Ab titre – short period	Persistence of Ab titre – long period
7. Affinity of Ab – low	Affinity of Ab – high
8. Memory cells – few	Memory cells – many
9. Dose of Ag – high	Dose of Ag – low

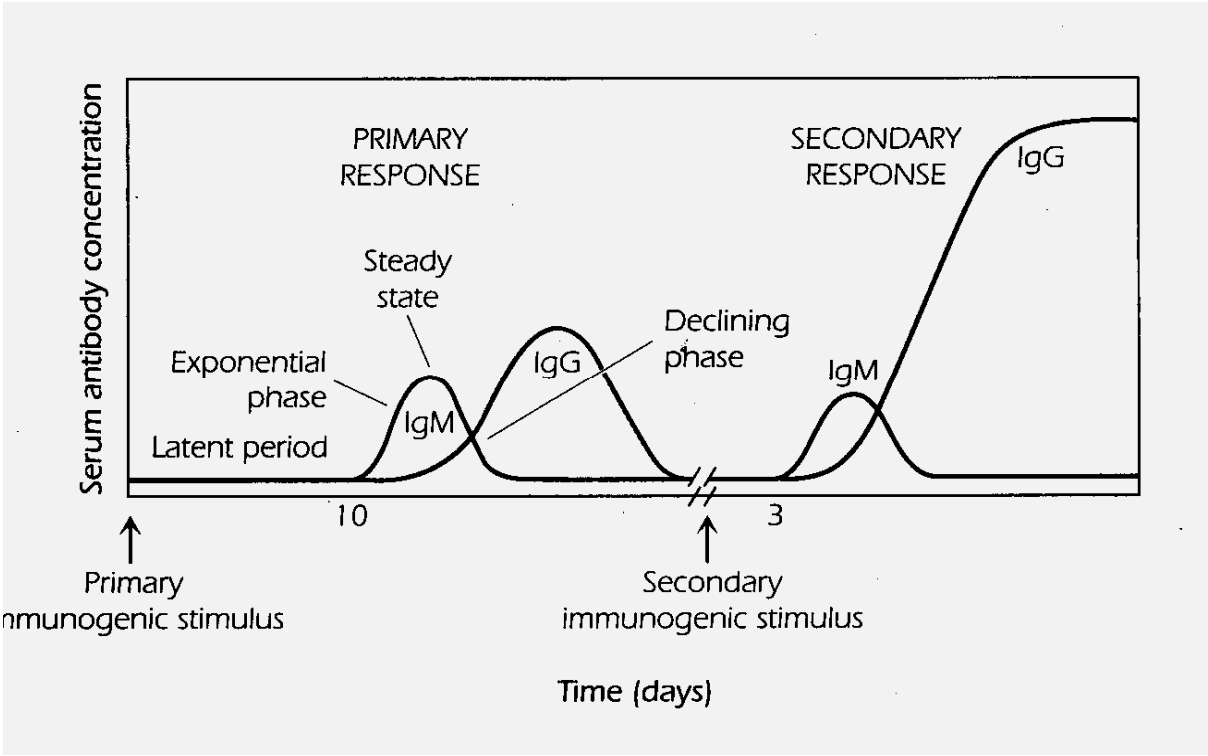

Fig-3.1: The Kinetics of an Antibody Response

3.8. SECONDARY IMMUNE RESPONSE:

When the same Ag enters for the second time (booster dose), after weeks, months or even after years, the body gives secondary response. Following the booster dose, there is a markedly enhanced response that is characterized by the accelerated appearance of immunocompetent cells and Ab. Thus, the secondary response (anamnestic or recall) is P-prompt, P-powerful, P- prolonged; with short or negligible latent phase of (2-3 days); high levels of Abs, lasting for long period and type of Ab involved is IgG of increased affinity, avidity and specificity.

If the specific Ab is present in the serum at the time of booster dose, the Ab disappears more rapidly than in the decline phase of the primary response. This is negative phase which is due to the combination of the Ab with newly injected Ag. If the dose of Ag is too small, no secondary response occur because of Ag-Ab complex formation. These differences in primary and secondary response are due to the number of responding B-cells. In the primary response, only few of the B-cells are converted into Ab producing B-cells and large number of B cells is converted into memory cells. In the secondary response, all B-cells are converted by Ag into Ab producing B-cells. Memory cells are longer lasting cells, which are able to respond same Ag when it enters for the second time and help to B-cells to produce Abs in high titre. The life of memory cells is three years or more, e.g., tetanus toxoid, can evoke a powerful secondary response after 20 years also.

IgM to IgG Switch:

Immune response is genetically controlled. An individual cell first produces IgM and

then IgG, IgA, IgD and IgE. This is because of genes and genetic control. At first, there is switching of μ gene so that IgM is produced, then there is looping out of μ gene, so that information is transmitted to γ gene to produce IgG. In this way, IgM antibodies are synthesized first and then IgG, IgA, IgD and IgE.

The capacity to make a secondary response may persist for a long time (years in humans), and it provides an obvious selective advantage for individuals who survive the first contact with an invading pathogen. Establishment of this memory for generating a specific response is, of course, the purpose of public health immunization programs.

3.9. SUMMARY:

Immunity refers to all the mechanisms used in the body as protection against environmental agents that are foreign to the body. Immunity may be innate or acquired. Innate immunity refers to the immunity response of the body due to physical and chemical barriers of the body, tissues, cells and all other mechanisms used as protection against environmental agents that are foreign to the body. Acquired immunity is the immunity that an individual acquires during the course of his life for a particular disease. As it is specific for a particular disease, it is also known as specific immunity. It is of two types a) Active immunity b) Passive immunity. Active immunity is the resistance developed by an individual in response to the microbes or their products (antigenic stimulus). Passive immunity is a type of immunity that is induced by transfer of preformed antibodies against microbes or their products in another host. Humoral immunity is mediated by serum antibodies, which are the proteins secreted by the B cells after the binding of antigens to specific membrane immunoglobulin. Immune response involving the interaction of cells of the immune system with the Ag is known as Cell mediated immunity. The response of the body for the first time entry of Ag is called primary immune response. When the same Ag enters for the second time (booster dose), after weeks, months or even after years, the body gives secondary response. In the primary response, only few of the B-cells are converted into Ab producing B-cells and large number of B cells is converted into memory cells. In the secondary response, all B-cells are converted by Ag into Ab producing B-cells.

3.10. TECHNICAL TERMS:

Innate immunity, Acquired immunity, Humoral immunity, Cell-mediated immunity, Active immunity, Passive immunity, Primary immune response, Secondary immune response.

3.11. SELF ASSESSMENT QUESTIONS:

1) Write an essay on innate and acquired immunity reactions.
2) Give an account on humoral and cell mediated immunity reactions.
3) Describe the primary and secondary immune responses.

3.12. SUGGESTED READINGS:

1) Nandini Shetty, 2001. IMMUNOLOGY Introductory Text Book., New Age International (P) Limited, publishers, New Delhi – 235 pp.

2) Richard Coico, G. Sunshine, & Eli Benjamini, 2003. IMMUNOLOGY. 5TH Ed. Wiley-liss, publication, California. 361pp.

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Prof. A. Amruthavalli

LESSON-4

ANTIGENS-NATURE AND PROPERTIES

4.0 OBJECTIVE:

- Students will get the knowledge on different types of antigens and their properties as well as functions.

STRUCTURE:

4.1. INTRODUCTION:

Antigens are the molecules or molecular structures that are foreign to the body and generally induce an immune reaction in the form of the production of antibodies against them. In simple words, antigens can be anything that doesn't belong to the body and are foreign. Even though antigens are usually defined by the induction of an immune response, all antigens might not induce an immune response. The antigens that induce a response are termed immunogens. The ability of antigens to elicit an immune response depends on the presence of specific regions on the antigens called antigenic determinants. The determinants bind to receptor molecules with the complementary structure on immune cells to elicit a response. Antigens are indicated by the term 'Ag', and these can occur in different forms like pollen, viruses, chemicals, or bacteria. The concept of antigen arose from the fact that our body can distinguish between the components of the body and foreign particles. In response to these antigens, the body induces the production of antibodies that act against the said antigens. Most antigens in humans are proteins, peptides, or polysaccharides; however, lipid and nucleic acids can also act as antigens when combined with proteins or polysaccharides. In addition, antigens might also be intentionally introduced into the body in the form of vaccines in order to induce the adaptive immune system of the body against the antigen.

4.2. PROPERTIES OF ANTIGENS

Antigens have different properties which determine the immunogenicity of the antigens and thus are essential in order to understand the immune reaction against them. Since these properties determine the immunogenicity, these are considered properties required to form a good antigen. The following are some of the properties of antigens;

1) Foreign Nature:

All antigens that induce an immune response in the host are foreign to the body of the recipient. The host body recognizes the antigen to be different from the normal body components. The immunogenicity of the antigen increases with the increase in the degree of foreignness. In the case of biological antigens, the foreignness increases with the increase in the phylogenetic gap between the two species. However, there are some exceptions in that some proteins occurring within the host might also induce an immune response, as in the case of auto antigens. Similarly, proteins and other molecules from other species might also not induce an immune response if they lack antigenic determinants or epitopes.

2) Chemical Nature:

The most potent and commonly encountered antigens are proteins followed by polysaccharides. However, other molecules like lipids and nucleic acids can also act as antigens when complex with proteins and polysaccharides. In the case of proteins, the antigen should contain immunogenic regions with at least 30% of amino acids like lysine, glutamine, arginine, glutamic acids, asparagine, and aspartic acid, along with a high number of hydrophilic or charged groups. The level of immunogenicity also increases with the heterogeneity of the molecules. Homopolymers are usually less immunogenic than heteropolymers.

3) Molecular Size:

The molecular size of the antigens is also crucial in the immunogenicity of the molecules. It has been established that antigens should have a minimum size of greater than 5000 Da before they can be considered immunogenic. However, low molecular weight substances can demonstrate immunogenicity when coupled with large-sized carriers. The low molecular weight substances are termed haptens that are considered ‘partial antigens’ with at least one antigenic determinant.

4) Molecular Rigidity and Complexity:

The rigidity and complexity of molecules are essential factors that determine immunogenicity. In general, rigid molecules are good antigens as they can raise antibodies to certain structures when compared to the less rigid ones. The complexity of the structure is also an essential factor as a peptide antigen with a repeating unit of a single amino acid is less immunogenic than a molecule with two or more repeating amino acids units.

5) Antigenic Determinants and Cross-reactivity:

Antigenic determinants are regions in an antigen molecule that is involved in the reaction with antibodies. Usually, antigens with two or more antigenic determinants can induce antibody production. Thus, a smaller antigen usually doesn’t induce antibody production as it is not possible for a small molecule to have more than one antigenic determinant. Cross-reactivity of antigens is also an essential factor where antibodies induced by a different antigen can interact with another antigen.

4.3. ANTIGEN STRUCTURE

The molecular structure of an antigen is characterized by its ability to bind to the antigen-binding site of an antibody. Antibodies differentiate between different antigens on the basis of the specific molecular structures present on the surface of the antigen. Most antigens are proteins or polysaccharides. These can include coats, capsules, flagella, toxins, and fimbriae of bacteria, viruses, or other microorganisms. Besides, secretions and other chemicals of the same nature can also act as antigens. Lipids and nucleic acids of these microorganisms are only antigenic when these are combined with proteins or polysaccharides. The structure of antigens might be different depending on the nature of the antigen, their size, and immunogenicity. All immunogenic antigens have a specific structural component called epitope or antigenic determinant. The number of epitopes differs in different antigens and determines the number of antibodies a single antigen can bound to. The structural components of interaction in antigens are different, which determines the classes of antibodies they bound to. The region on antibodies that interacts with antigens is called a paratope. It has been established that the structure of epitope and paratope can be defined with a lock and key metaphor as the structures are specific and fit with one another**.**

4.4. TYPES OF ANTIGENS:

Antigens can be grouped into different types based on different factors. Some of the common classifications are based on the origin of the antigen and its immunogenicity.

1. Types of Antigens-Based on their Origin

a) Exogenous Antigens:

Exogenous antigens are the antigens that are originated outside the body of the host and, thus, are foreign to the host. These antigens might enter the body through inhalation, ingestion, or injection and then circulate throughout the body via bodily fluids. The uptake of exogenous antigens is primarily mediated by phagocytosis via Antigen Processing Cells (APCs) like macrophages, dendritic cells, etc. Many antigens like intracellular viruses might begin as exogenous antigens and later become endogenous.

b) Endogenous Antigens:

Endogenous antigens are antigens that originate within the body of the host during metabolism or as a result of intracellular viral or bacterial infection. Endogenous antigens are usually the cells of the body or fragments, compounds, or antigenic products of metabolism.

These are usually processed in the macrophages and are later detected by cytotoxic T-cells of the immune system. Endogenous antigens include antigens that are xenogeneic or heterologous, autologous, and idiotype or allogeneic. Endogenous antigens might result in autoimmune diseases as the host immune system detects its own cells and particles as immunogenic.

c) Autoantigens:

Autoantigens are proteins or protein complexes of the host that are attacked by the host's immune system, resulting in autoimmune disease. Autoantigens can be deadly to the host as the body's own cells should not be targeted by the immune system. The immunological tolerance to such antigens is lost as a result of genetic and environmental factors.

d) **Tumor Antigens (Neoantigens):**

Tumor antigens or neoantigens are presented by Major Histocompatibility Complex (MHC) I and II on the surface of tumor cells. The antigens are produced as a result of a tumor-specific mutation during the malignant transformation of normal cells. These antigens usually do not induce an immune response as the tumor cells develop ways to evade antigen presentation and immune defense.

e) Native Antigens:

Native antigens are antigens that are not processed by any antigen-presenting cells (APC), and thus immune cells like T-cells cannot bind to these antigens. However, B-cells can be activated such antigens even without any processing.

2. Types of antigens on the basis of immune response

a) Complete antigens/ Immunogens:

Complete antigens or Immunogens are antigens that elicit a specific immune response. These antigens can induce an immune response by themselves without any carrier particles. These are usually proteins, peptides, or polysaccharides with high molecular weight (greater than 10,000 Da).

b) Incomplete antigens/ Haptens:

Incomplete antigens or haptens are antigens that cannot generate an immune response by themselves. These are usually non-protein substances that require a carrier molecule to form a complete antigen. Haptens have a low molecular weight (usually less than 10,000 Da) and fewer antigenic determinant sites. The carrier molecule bonded to the hapten is considered a non-antigenic component and is a protein or a polysaccharide molecule.

Antigen Processing and Presentation

Antigen processing and presentation is the process of digestion of antigens into smaller peptide fragments by an antigen-presenting cell (APCs) that are then displayed on the surface of the cells via antigen-presenting molecules like **MHC class I and II** for recognition by lymphocytes.

Antigen processing and presentation can occur via three different pathways -

1) Endogenous Pathway or Classical MHC class I Presentation

The endogenous pathway of antigen processing and presentation utilizes mechanisms similar to those involved in the normal turnover of intracellular proteins. The antigen-presenting cells degrade the protein antigen into short peptides by a specified cytosolic proteolytic system called the proteasome. The proteasome involved in the immune system is called the immunoproteasome, which has components induced by exposure to interferon-γ or TNF-α. The next step of the proteolytic mechanisms is the peptide trimming by amino peptidases in the ER lumen. The peptides formed after proteolysis are transported to the ER lumen by the Transporter associated with Antigen Processing (TAP). In addition to TAP, tapasin and calnexin-calreticulin system mediates the trimming and loading of peptides on MHC class I molecules. Depending on the affinity of

the loaded peptides, MHC class I molecules will either be transported to the cell membrane or recycled by a mechanism dependent on UDP-glucose glycoprotein transferase-1. In the case of high-affinity MHC class-1 complexes, the peptides are transported through the Golgi apparatus to the cell membrane in order to elicit antigen-specific CD8+ T cell responses.

2) Exogenous Pathway/ Classical MHC class II Presentation

In the case of the exogenous pathway, APCs internalize the antigen by simple phagocytosis, where the material first binds to specific surface receptors. The degradation of proteins into peptides occurs within the compartments of the cell by the endocytic processing pathway. The internalized antigen progresses through different acidic compartments encountering hydrolytic enzymes and low pH in each compartment. The APCs have a unique endosome in the MHC class II-containing compartment (MIIC), where the *final protein degradation* and peptide loading take place. Since APCs express both classes of MHC, there is a distinct mechanism to prevent the interaction of antigenic peptides for the class I molecules. When class II MHC molecules are synthesized, the class II αβ chains associate with a protein called the invariant chain. This protein interacts with the class II peptide-binding groove preventing the binding of endogenously derived peptides to the class II molecule. As the invariant chain moves through different compartments, it is degraded until it forms a short fragment termed CLIP (class II-associated invariant chain peptide). Later, a class II MHC molecule catalyzes the exchange of CLIP with antigenic peptide. The peptide binding is required to maintain the structure and stability of class II MHC molecules. After the binding of the peptide, the class II MHC –peptide complex is transported to the plasma membrane, where the neutral pH causes the complex to form a compact, stable form.

3) Cross-Presentation

Cross-presentation is the process where the APCs will divert antigen obtained by endocytosis (exogenous pathway) to a class I MHC loading and peptide presentation. The phenomenon of cross-presentation, however, requires the internalized antigens to be handled by the exogenous pathway leading to class II MHC presentation somehow are redirected to a class I loading pathway. Cross-presentation is primarily observed in the case of dendritic cells that accomplish cross-presentation by one of the two possible means. The first mechanism assumes that cross-presenting cells contain special antigen-processing machinery that enables the loading of exogenously derived peptides onto class I MHC molecules. The second mechanism hypothesizes that specialized endocytosis machinery is found in the cells that send internalized antigen directly to an organelle, where the peptides are then loaded onto class I MHC molecules. Cross-presentation of antigens has an advantage as APCs can capture viruses from extracellular environments, process them and activate cytotoxic T-cell lymphocytes that can attack virus-infected cells, which inhibit the further spread of the virus.

Antigen-Antibody Complex:

An antigen-antibody complex or immunogenic complex is a molecule formed by binding multiple antigens to antibodies. The binding of antibody and antigen is determined by the epitope and paratope present in the antigen and antibody, respectively. The ability of antibodies to fight against multiple pathogens is due to their ability to distinguish between different antigens. The interaction between antigens and antibodies is highly specific, and it is

determined by the amino acid sequence in the epitope and paratope of the species. The complex is formed by an antigen-antibody reaction which is then subject to a number of responses like complement deposition, opsonization, and phagocytosis**.** The shape and size of the immune complex are determined by the ratio of antigen to antibody. The size, in turn, determines the effect of the immune complex. Antigen-antibody complexes have become an important tool in understanding the antigen-antibody interaction and determining the basis of molecular recognition between an antibody and antigens. Immune complexes also play a role in regulating antibody production as the binding of antigen to cell receptors activates signaling cascade leading to the activation of antibodies. Even though immune complexes are essential for different immune functions, the deposition of the immune complex can lead to several autoimmune diseases like arthritis and scleroderma.

Antigen Examples:

1) **Blood group antigens:** Blood group antigens are proteins or sugars present on the surface of different components in the red blood cell membrane. The antigens in the ABO blood group are the sugar that is produced by a serried of reactions that catalyzes the transfer of sugar units. The type of sugar in the red blood cell is determined by the type of enzyme involved, which in turn is determined by the person's DNA. The antigens of the Rh blood group are proteins that are also determined by the host's DNA. The RhD gene encodes the D antigen, which occurs as a large protein on the red blood cell. These antigens can be distinguished by antigen-antibody reactions that help determine different blood groups in humans.

2) **Bacterial Capsule:** A bacterial capsule is a polysaccharide layer occurring outside the cell envelope that induces an immunogenic reaction in the host. The capsule is a well-organized layer that cannot be removed easily and thus is considered a possible cause of bacterial pathogenicity. In some bacteria, the capsule can also be involved in evading phagocytosis as a capsule-specific antibody is required to cause phagocytosis. Bacterial capsules are also used as antigens used in vaccines where the polysaccharide component of the capsule is conjugated with protein carriers. The exact structure, function, and involvement of capsules in bacteria differ in different bacterial species.

Applications of Antigens

1) The detection of different antigens in different species can be used to differentiate between bacteria species as antigens are usually very specific.

2) Antigens can also be used for diagnostic purposes to detect the presence of antibodies in a sample.

3) Antigens are an essential component of antigen-antibody complexes, which have forensic application in the identification of human blood and other samples.

4) These are also used in immunoassays for the quantification of various chemical and biological substances.

5) Autoantigens are responsible for autoimmune diseases, which in some cases can be lethal.

4.5. SUMMARY:

Antigens are the molecules which are foreign in nature to the body and generally induce an immune reaction and result in production of antibodies against them. Even though antigens are usually defined by the induction of an immune response, all antigens might not induce an immune response. The antigens that induce a response are termed immunogens. The ability of antigens to elicit an immune response depends on the presence of specific regions on the antigens called antigenic determinants. The determinants bind to receptor molecules with the complementary structure on immune cells to elicit a response. Antigens can be some pollen, viruses, chemicals, or bacteria. The concept of antigen arose from the fact that our body can distinguish between the components of the body and foreign particles. In response to these antigens, the body induces the production of antibodies that act against the said antigens. Most antigens in humans are proteins, peptides, or polysaccharides; however, lipid and nucleic acids can also act as antigens when combined with proteins or polysaccharides. Antigens are of various types with different properties and functions.

4.6. TECHNICAL TERMS:

Antigens, Haptens, Ag presentation, Exogenous antigens, Endogenous antigens, Native antigens, Autoantigens, Tumor antigens, Immunogens.

4.7. SELF ASSESSMENT QUESTIONS:

1) Write an account on definition, structure and functions of antigen.
2) Give a detail account on properties of antigens

4.8. SUGGESTED READINGS:

1) Nandini Shetty, 2001. IMMUNOLOGY Introductory Text Book., New Age International (P) Limited, publishers, New Delhi – 235 pp.
2) Richard Coico, G. Sunshine, & Eli Benjamini, 2003. IMMUNOLOGY. 5TH Ed. Wiley-liss, Publication, California. 361pp.
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6) Delgert, K 1996. Immunology-Understanding of immune system. Wiley-Liss New York.

Dr. J. Madhavi

LESSON-5

IMMUNOGLOBULINS

5.0 OBJECTIVE:

- Students will know about the different types, structures and properties of immunoglobulins or antibodies and also their properties.

STRUCTURE:

5.1. INTRODUCTION:

The immunoglobulins/antibodies are a group of glycoproteins present in the serum and tissue fluids of all mammals. With the possible exception of natural antibody, they are formed in response to foreign substance (antigen) administered into the body with which they react specifically and in an observable manner. Antibodies are globulins in nature and are known as immunoglobulins as they are involved in immune reactions. They contain sugar residues and hence are glycoproteins. Serum globulins can be separated into pseudoglobulins (water soluble) and euglobulins (water insoluble). Most antibodies are euglobulins. Immunoglobulins constitute 20-25% of the total serum proteins. When electrophoretically separated, most of the serum antibodies migrate in gamma region hence they are also termed as gamma globulins. Many immunoglobulins also migrate in beta region and even in alpha region. Sedimentation studies showed that most antibodies belong to 7S (Svedberg unit) class having molecular weight 1,50,000-1,80,000 and some belong to 19S having molecular weight 9,00,000 and are designed as M or macroglobulins. Of the different terms were used earlier, the generic term immunoglobulin is internationally accepted for 'proteins' of animal origin endowed with known antibody activity and for certain other related proteins. The term immunoglobulin denotes chemical structure of protein while antibody refers to biological activity and function of proteins. Accordingly, immunoglobulins include abnormal plasma proteins found in myeloma, macroglobulinemia, cryoglobulinemia and the naturally occurring subunits of immunoglobulins in addition to antibody globulins. Thus, all antibodies are immunoglobulins but all immunoglobulins may not be antibodies. Immunoglobulins are mainly synthesized by plasma cells and to some extent by lymphocytes.

5.2. STRUCTURE OF IMMUNOGLOBULIN (Fig. 5.1)

The detailed structure of immunoglobulin was studied by Porter *et. al.* (1962) by cleaving immunoglobulin molecule. They used rabbit IgG antibody to egg albumin for their

study. The IgG can be digested by papain in the presence of cysteine into three fragments. Out of three, two are identical having molecular weight 45,000 (sedimentation co-efficient 3.5S) and are able to combine with antigen but unable to precipitate the reaction. Therefore, these are univalent and are called Fab (fragment antigen binding). The third fragment has no power to combine with antigen (molecular weight 55,000) and is termed as Fc (fragment crystallizable). Fab contains a light chain and a part of heavy chain, and Fc contains part of heavy chain only. When treated with another proteolytic enzyme-pepsin, a 5S fragment composed of two Fab fragments held together in position is obtained. It is bivalent and precipitates with antigen. This fragment is known as $F(ab)_2$. The Fc portion is completely degraded by pepsin into smaller fragments. Immunoglobulins can also be broken down into peptide chains by reduction with sulfhydryl reagent such as 2-mercaptoethanol. Based on these findings, Porter *et al.* put forward a basic four-chain model for immunoglobulin containing two distinct types of polypeptide chains-two heavy chains and tow light chains linked together by disulfide bond (-s--s-) and non-covalent linkages.

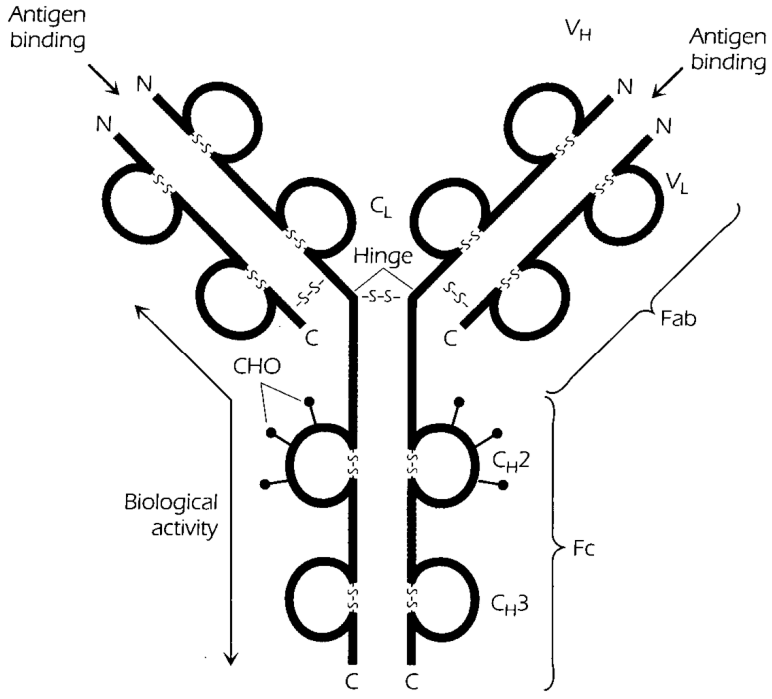


Fig-5.1: Structure of Immunoglobulin

a) Light Chain (L-Chain): These are smaller chains with molecular weight of 20,000--25,000 D, made up of 210 to 230 amino acids. This chain is attached to H-chain by disulfide bonds. L-chains are similar in all classes of immunoglobulins. They occur in two groups called *kappa* (κ) and *lambda* (λ). Immunoglobulin molecule may have either κ or λ, but never both together. κ and λ occur in a ratio of 2:1 in human sera. About 60% of molecules possess κ-type and about 30% of molecules possess λ-type.

b) Heavy Chain (H-Chain): These are large chains with molecular weight of 50,000 Daltons containing 420 to 460 amino acids. The two H-chains are joined together by one to five S--S bonds. H-chains are structurally and antigenically different for each class of immunoglobulin and are designated by Greek letters as follows.

Ig Class	Type of H-chain
Ig G	γ (Gamma)
IgM	μ (Mu)
IgA	α (Alpha)
IgD	δ (Delta)
IgE	ε (Epsilon)

c) Variable and Constant Region: Each polypeptide chain of immunoglobulin molecule contains an amino terminal end, which means there is a free amino group on the terminal amino acid. This is called variable region (V) which is different for each class and subclass. The first 110 amino acids from the amino terminal end are responsible for antibody reactivity. Each polypeptide chain contains carboxyl terminal end, which means there is a free carboxyl group on the terminal amino acid. This is called constant (C) region whose composition is constant in all immunoglobulin molecules from different animal species except for some minor genetic differences.

d) Functions of Fab and Fc: The antigen binding site (Fab) of the antibody molecule resides at amino terminal end. It is composed of both L and H chain. The portion of H-chain present in Fab fragment is known as Fc piece. The Fc fragment resides at carboxyl terminal end. It does not possess antigen binding site but determines the biological properties of the immunoglobulin molecule such as - Complement fixation, Placental transfer, Skin fixation, Catabolic rate, Secretion into body fluid, Binding to phagocytes, and Binding to mast cells. The antibody specificity of immunoglobulin molecule depends on the variability of the amino acid sequences at the variable region of the H and L chain, which form the antigen binding site. The antibody specificity is explained on the basis that the combining site of antibody molecule possesses a specific amino acid composition that is complementary fit for specific reactive area of the antigen molecule. Recently, hyper variable regions (paratopes) in variable portions of H and L chains have been identified. In L-chains these regions are L1 (residues 23-36), L2 (52-58), and L3 (91-99). In H-chains these are H1 (from 31 to 36), H2 (from 49 to 66), and H3 (from 99 to 104). These hyper variable regions (hot spots) are involved in the formation of antigen binding site.

e) Hinge Region: When antibody molecule is visualized under electron microscope, it appears as a Y-shaped structure whose arms can swing out to an angle of 180^{o} through the papain and pepsin sensitive region called hinge region. Hinge region consists of a large number of proline residues.

f) Immunoglobulin Domains: The polypeptide chains do not exist as a linear sequence of amino acid molecules but are folded by disulfide bonds into globular regions known as domains. The domains of H-chain are designated as C_H-1, C_H-2, C_H-3, C_H-4 (domains in constant region), and V_H (domain in variable region). The domains of L-chains are designated of C_L (Constant region) and V_L (Variable region).

Each domain serves a different function.

1) The V_L and V_H domains are responsible for the formation of specific antigen binding site.
2) The C_H-2 domain in IgG binds to Clq in classical complement pathway.
3) C_H-3 domain mediates adherence to the surface of monocytes.

5.3. CLASSES OF IMMUNOGLOBULINS

Human sera contain five different immunoglobulins-IgG, IgA, IgM, IgD, and IgE (Fig. 5.2) in the decreasing order of concentration.

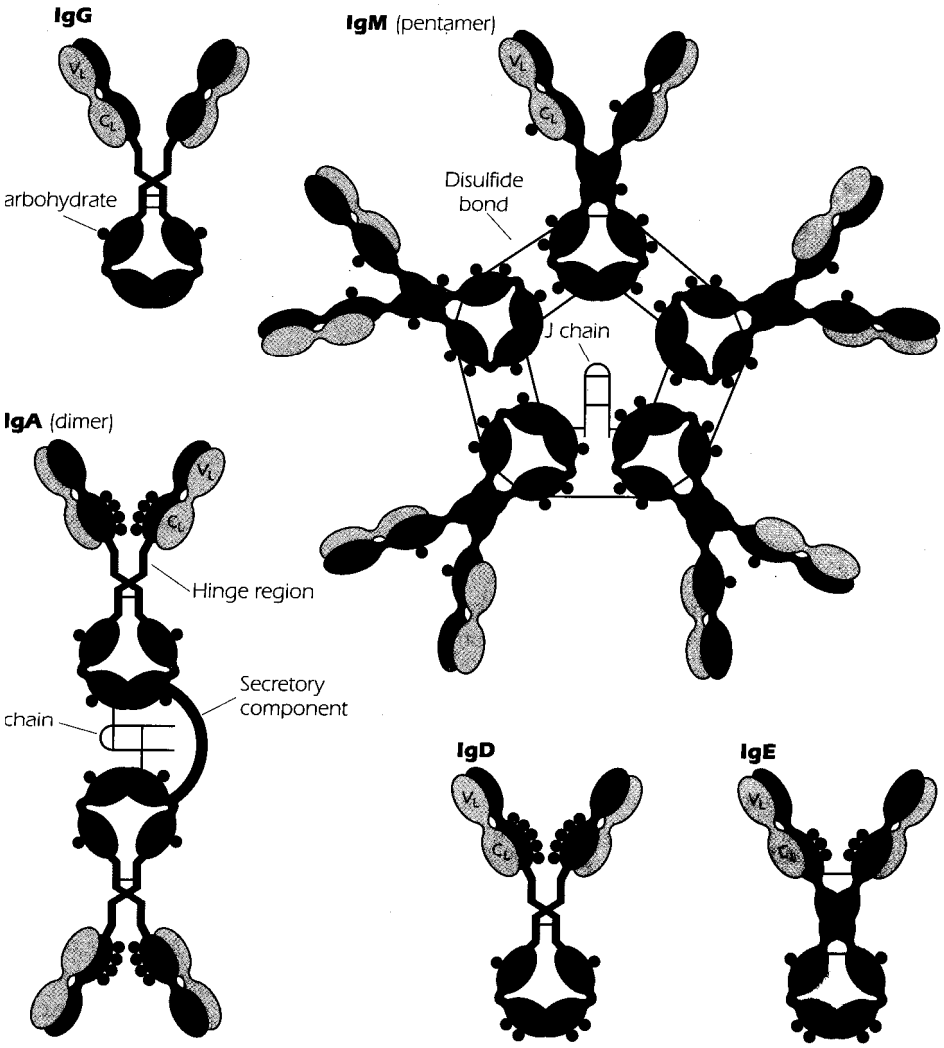


Fig-5.2: Structures of Five Different Immunoglobulin Molecules

1) Properties of Immunoglobulin-G (IgG)

1) It is the major immunoglobulin in normal serum accounting for 70---80% of the total.
2) It is a monomer consisting 2H and 2L chains.
3) Molecular weight ---1,50,000.
4) Sedimentation coefficient ---7S.
5) Half-life ---23 days.
6) Contains less carbohydrates ---3%.
7) Valency ---2.

8) Normal serum concentration---8 -16mg/ml.
9) IgG subclasses---four classes - IgG1(65%), IgG2(23%), IgG3(8%), and IgG4(4%). Each subclass possesses different type of H-chain designated as λ-1, λ-2, λ-3 and λ-4.
10) It is the major immunoglobulin synthesized during secondary response.
11) It is equally distributed between the intravascular and extra vascular compartments.
12) It possesses greater antigen binding affinity than IgM.
13) Catabolic rate---when its level in serum is raised, as in chronic malaria, kala-azar or myeloma, the IgG is rapidly catabolized; however, in hypogammaglobulinemia it is slowly catabolized.

Biological Activities:

1) Because of its ability to cross the placenta, it provides a major line of defense (naturally acquired passive immunity) against infection in newborn for the first few weeks.
2) In extravascular body spaces, it carries the major burden of toxin neutralization.
3) It binds to microorganisms and enhances their phagocytosis.
4) It is able to activate complement and thus helps to attract polymorphonuclear leucocytes (phagocytic cells) by chemotactic mechanism and stimulates ingestion and killing of microorganisms.
5) IgG with the help of Fab reacts with target cell and mediates extracellular killing by K-cells bearing the specific receptor, which react with Fc portion of IgG on target cell and kill it.
6) It participates in most of the immunological reactions such as complement fixation, precipitation and neutralization of toxins and viruses.
7) It participates in allergic reactions, e.g., Arthus reactions and also in autoimmune diseases.
8) When administered passively, it suppresses the homologous antibody synthesis by a feedback mechanism. This property is utilized in the isoimmunization of women during delivery by the administration of anti –Rh (D) IgG.

2) Properties of Immunoglobulin A (IgA)

1) It is the second most abundant immunoglobulin constituting 10--13% of the total.
2) It occurs in two forms--in human sera more than 80% of IgA occurs as monomer containing 2H and 2L chains but in most mammals it is polymeric.
3) Molecular weight--1,60,000.
4) Sedimentation coefficient---7S.
5) Half-life---6-8 days.
6) Carbohydrate content---11%.
7) Valency---2 or multiples of 2.

8) Normal serum concentration - 0.6 - 4.2 mg/ml.

9) IgA subclasses---2 classes --- IgA1 and IgA2. based on type of heavy chain (α-1 and α-2).

Secretory IgA

1) It is the predominant immunoglobulin present in the seromucous secretions such as saliva, tears, nasal fluids, sweat, colostrum and secretions of the lungs, genitourinary and gastrointestinal tract.

2) It is a dimer containing 4H and 4L chains.

3) Molecular weight---3,85,000.

4) Sedimentation coefficient---11S.

5) Possesses a cysteine rich polypeptide chain called J-chain (joining chain) of molecular weight 15,000 that joins two monomeric units of IgA. The J-chain is synthesized by same cells synthesizing dimeric S-IgA.

6) Possesses an additional structural unit - a glycine rich polypeptide called the T (transport) or S (secretory piece or secretory component) having molecular weight 60,000, synthesized by epithelial cells and is attached to the IgA molecule during transport across the cells. It appears to protect IgA from digestion by proteolytic enzymes (Fig. 5.3).

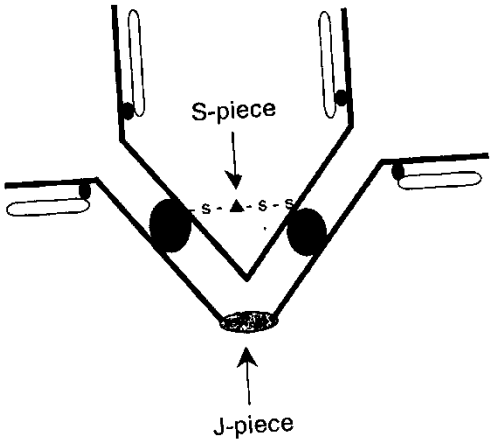


Fig-5.3: Secretory Ig A Molecule

Biological Activities:

1) IgA present in secretions is synthesized locally by plasma cells situated near the mucosal or glandular epithelium and selectively concentrated in secretions and on mucous surfaces forming an antibody paste. This IgA plays an important role in local immunity against respiratory, intestinal and urogenital pathogens by inhibiting the adherence of microorganisms to the surface of mucosal cells by coating them and thereby preventing their entry into the body tissue.

2) Can activate complement by alternate pathway and helps to kill certain coliforms with the help of lysozyme.

3) Promote phagocytosis and intracellular killing of microorganisms.
4) IgA has also been reported to mediate antibody dependent T-cell mediated cytotoxicity.
5) The potential functions of S-IgA are inhibition of bacterial adherence, virus and toxin neutralization and prevention of antigen uptake by epithelial cells.

3) Properties of Immunoglobulin M (IgM):

1) It is the first antibody formed in every antibody response.
2) Constitutes 5-8% of serum immunoglobulins.
3) It is a pentamer containing 10H and 10L chains--five subunits of monomer (Fig. 5.4).
4) Possesses J-chain (molecular weight 15,000) responsible for joining of subunits.
5) It bears an extra C_H domain.
6) Molecular weight 9,70,000, hence known as 'the millionaire molecule'.
7) Sedimentation coefficient--19S.
8) Half-life---5 days.
9) Carbohydrate content---10%.
10) Valency--theoretically---10 (observed with small haptens). The effective valency is five due to steric hindrance.
11) Normal serum concentration--- 0.5 - 2mg/ml.
12) IgM subclasses---two subclasses--IgM1 and IgM2, based on H-chains (μ-1 and μ-2).
13) Most of the IgM (80%) is intravascular in distribution.
14) Susceptible to mercaptoethanol---serum treatment with mercaptoethanol selectively destroys IgM.

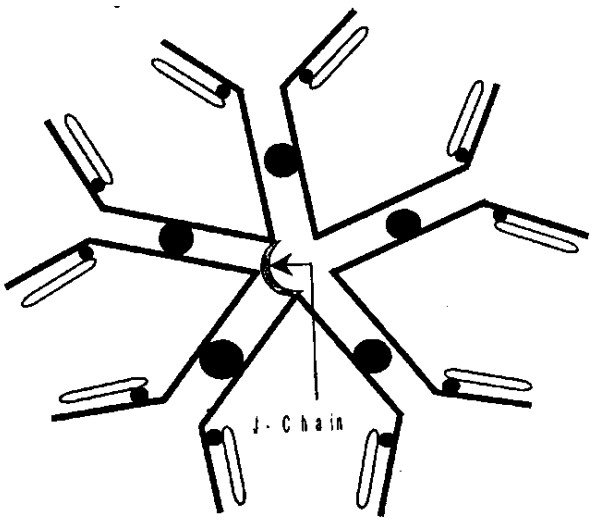


Fig-5.4: Ig M Molecule

Biological Activities :

1) Because of high valency, it is extremely efficient in agglutination and cytolytic activity.
2) As IgM is largely confined to blood stream, it offers protection against bacteremia.
3) As it is not transported across the placenta, its detection in fetus or new born indicated intrauterine infection, which is useful in the diagnosis of congenital syphilis, rubella, toxoplasmosis and HIV infection.
4) It fixes complement by classical pathway.
5) It is the most efficient antibody in agglutination, complement fixation and cytolytic reaction.
6) It is more effective than IgG in immune hemolysis, opsonization and bactericidal action.
7) It also neutralizes viruses and toxins but less efficient than IgG.
8) As it is a short lived immunoglobulin that disappears rapidly, its demonstration in serum indicates recent infection.
9) Monomeric IgM appears on the surface of unstimulated B-lymphocytes and acts as recognition receptor for antigens.

4) Properties of Immunoglobulin D (IgD)

1) Accounts for less than 1% of the total immunoglobulin.
2) Structurally, it resembles IgG--it is a monomer containing 2H and 2L chains.
3) Molecular weight---1,84,000.
4) Sedimentation coefficient--7S.
5) Half-life---2 - 8 days.
6) Carbohydrate content---13%.
7) Valency---2.
8) Normal serum concentration -- 0-0.04mg/ml.
9) Subclasses — two -- IgD1 and IgD2.
10) Susceptible to proteolytic degradation.

Biological Activities:

IgD together with IgM may function as antigen receptor on the surface of B-lymphocytes for recognition of antigens and for the control of lymphocyte activation and proliferation to produce antibodies or suppression.

5) Properties of Immunoglobulin E (IgE):

1) Occurs in very low concentration.
2) Structurally, it resembles IgG---it is a monomer containing 2H and 2L chains.

3) Molecular weight---1,88,000.
4) Sedimentation coefficient--8S.
5) Half-life---2 -3 days.
6) Carbohydrate content---12%.
7) Valency---2.
8) Normal serum concentration - occurs in very low concentration - 0.00003mg/ml but the level is greatly elevated in atopic conditions such as asthma, hay fever and eczema.
9) Heat labile---inactivated at 56^{o}C in one hour.
10) Susceptible to mercaptoethanol.
11) Mostly extravascular in distribution.

Biological Activities:

1) It has affinity towards the surface tissue cells, particularly mast cells and basophils and is responsible for degranulation of these cells, thereby releasing vasoactive amines and other mediators. Thus, it is responsible for Type-1 hypersensitivity reactions (hay fever, infantile eczema, asthma and atopic dermatitis).
2) The IgE on the mast cell surface triggers the release of vasoactive agents and factors chemotactic for granulocytes. The vasoactive agent causes the transudation of IgG and complement while chemotactic factors attract effector cells (neutrophils and eosinophils) needed to dispose the infectious agent coated with IgG and complement. Thus, it plays an important role in immunity against helminthic parasites.

5.4. ANTIBODY PRODUCTION BY HYBRIDOMA TECHNIQUE

Hybridoma technology is a cell-fusion-based method for producing monoclonal antibodies with uniform structure and specificity. In this technique, antibody-producing B lymphocytes are fused with immortal myeloma cells to generate hybrid cells, known as hybridomas that continuously secrete a single type of antibody. The development of this technology revolutionized immunology by enabling the production of highly specific, reproducible antibodies for diagnostics, therapeutics, and research applications.

Hybridoma technology was discovered in 1975 by two scientists, Georges Kohler and Cesar Milstein. They introduced a method to fuse murine B cells with myeloma cells, creating an immortal antibody-secreting cell line. Their pioneering work demonstrated, for the first time, that a single clone of fused cells could produce a uniform antibody indefinitely. This breakthrough resolved the long-standing challenge of obtaining pure antibodies of a single specificity, and the significance of their contribution was recognized with the Nobel Prize in Physiology or Medicine in 1984. For the past decades, hybridomas have fueled the discovery and production of antibodies for a multitude of applications.

Steps Involved in Hybridoma Technology

Hybridoma technology is composed of several technical procedures, including antigen preparation, animal immunization, cell fusion, hybridoma screening and sub-cloning, as well

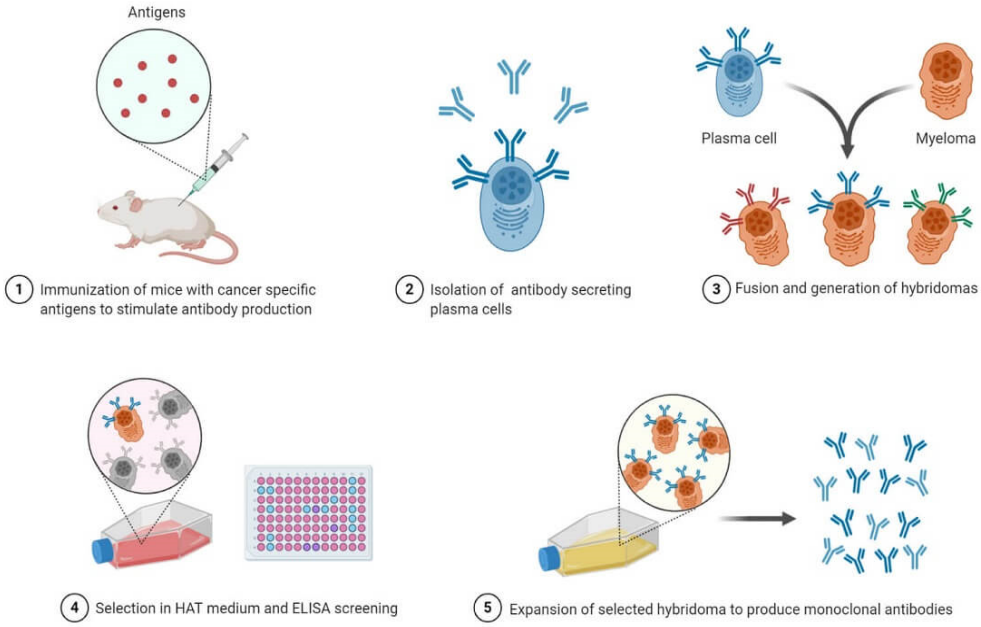
as characterization and production of specific antibodies. Monoclonal antibodies generation by the hybridoma approach requires knowledge of multiple disciplines and practice of versatile technical skills, ranging from animal handling, immunology to cellular and molecular biology. Generation and identification of high-quality hybridoma clones is a comprehensive and labor-intensive process, and requires months of work during the time frame from immunization to specific hybridoma identification.

1. Antigen Preparation:

The process begins with the careful preparation of the antigen against which monoclonal antibodies are desired. Antigens may be proteins, peptides, polysaccharides, or even whole cells. Their purity, stability, and immunogenicity determine the quality of the immune response. Adjuvants such as Freund's Complete or Incomplete Adjuvants are often mixed with the antigen to enhance the immune response, prolong antigen release, and stimulate strong B-cell activation. The prepared antigen is then ready for administration into the host animal.

2. Animal Immunization:

Laboratory mice are typically used as the immunization model. The antigen-adjuvant preparation is injected through intraperitoneal, subcutaneous, or intravenous routes according to the experimental design. Multiple booster doses are given at defined intervals to enhance the antibody response and ensure the generation of high-affinity B cells. After the final booster, the antibody titer in the serum is evaluated using ELISA to confirm that the mouse has mounted a strong and specific immune response. Once sufficient titers are achieved, the animal is sacrificed, and its spleen is collected for B-cell isolation.



Production of Monoclonal Antibodies

(Source: https://microbenotes.com/monoclonal-antibodies-types-uses-and-limitations/)

3. B-Cell Collection and Preparation:

The spleen is the preferred organ for isolating antibody-producing B lymphocytes, as it contains a large population of activated plasma cells following immunization. Under sterile conditions, the spleen is excised, and a single-cell suspension is prepared by gentle mechanical disruption. The suspension is then filtered to remove debris and washed to obtain viable B cells. These cells are short-lived and cannot proliferate indefinitely; hence, they are immediately prepared for fusion with myeloma cells. The quality of the B-cell population directly influences the efficiency of hybridoma formation.

4. Cell fusion:

Fusion of B lymphocytes with immortal myeloma cells is the central event in hybridoma technology. Myeloma cells are selected for their ability to grow indefinitely and for their deficiency in the HGPRT (hypoxanthine-guanine phosphoribosyl transferase) enzyme, which enables selective growth in HAT medium.

Two Main Techniques are used:

a) PEG-Mediated Fusion

Polyethylene glycol (PEG) induces fusion by dehydrating and bringing adjacent cell membranes into close contact, leading to their merger. This results in heterokaryons—cells with multiple nuclei derived from both parental cell types. Fusion efficiency depends on PEG concentration, exposure time, and temperature, requiring precise handling to minimize cytotoxicity.

b) Electrofusion

Electrofusion employs short electrical pulses to temporarily permeabilize cell membranes, allowing them to fuse more efficiently. This method offers higher reproducibility, less toxicity, and better control over fusion events.

The fusion mixture contains unfused B cells, unfused myeloma cells, fused myeloma cells, and the desired hybridoma cells.

5. Screening and Selection of Hybridoma Clones:

After cell fusion, the culture contains a complex mixture in which only a tiny proportion roughly 1% are successfully fused hybrids, and only about 1 in 10^5 of these will develop into viable hybridoma cells. To eliminate unwanted cells, the fusion mixture is grown in HAT medium. The unfused B lymphocytes from the immunized animal naturally survive only for a short period and therefore do not interfere with later steps. In contrast, the unfused myeloma cells are capable of continuous growth but are intentionally engineered to lack the enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT). This deficiency prevents them from using the salvage pathway for nucleotide synthesis, causing them to die in HAT medium. Hybridoma cells, however, inherit HGPRT from the B cell and immortality from the myeloma partner, enabling them to proliferate selectively under these conditions. Once the selection process enriches the hybridoma population, the surviving cells are distributed into microtiter plates so that each well supports the growth of a single hybrid line. As the clones expand, the culture supernatants from individual wells are screened to identify hybridomas producing antibodies of the desired specificity and affinity. The enzyme-linked

immunosorbent assay (ELISA) is most commonly used for this evaluation due to its sensitivity and precision. Positive wells are then subjected to repeated rounds of sub-cloning, typically through limiting dilution, to ensure that each selected hybridoma originates from a single parental cell. This step is crucial, as it guarantees the production of truly monoclonal antibodies that are homogeneous and identical in their antigen-binding characteristics.

6. Expansion and Long-Term Preservation:

After a stable monoclonal hybridoma clone is identified, it is expanded in larger culture vessels or bioreactors to increase antibody yield. The monoclonal antibodies are then collected from the culture supernatant and purified using chromatographic techniques. To guarantee long-term availability, the hybridoma cells are cryopreserved in liquid nitrogen, ensuring that the same monoclonal antibody can be produced consistently whenever required.

5.5. CATALYTIC ENZYMES (ABZYMES):

Abzymes are the catalytic antibodies that combine the specific binding properties of antibodies with catalytic activity of enzymes. They are created by immunizing animals with transition state analogs. Which are molecules that mimic the high energy intermediate of a chemical reaction. This makes immune system to produce antibodies that are structurally complementary to the transition state, allowing them to bind it tightly and lower the activation energy and there by accelerate the reaction. Key feature of abzymes is the dual function. Antibodies and enzymes share the ability to bind with compounds with great specificity and high affinity. This property has been exploited in the development of antibodies with catalytic activity. Antibodies have been 1st characterized as proteins produced by the IS for binding with molecules called antigens. One basic difference between antibodies and enzymes is that the former binds the complementary structure in its ground state, while enzymes bind in high energy state.

In 1986, the 1st monoclonal catalytic antibodies termed *abzymes* against a chemically stable analog of the transition state of a reaction were obtained. Abzymes are catalytic antibodies having structural complementarity for the transition state of an enzyme catalyzed reaction. They bind strongly to the transition state with high association constant, enhancing the reaction rate. Abzymes reduce rotational entropy.

Sources of Abzymes:

Abzymes are usually artificial constructs and are usually raised in lab animals immunized against synthetic haptens, but some natural abzymes can be found in normal humans (anti- vasoactive intestinal peptide autoantibodies) and in patients with autoimmune diseases such as systemic lupus erythematosus, where they can bind to and hydrolyze DNA. To date, abzymes display only weak, modest catalytic activity and have not proved to be of any practical use. They are, however, subjects of considerable academic interest. Studying them has yielded important insights into reaction mechanisms, enzyme structure and function, catalysis, and the immune system itself. They also obtained from human and animal serum. Found in normal humans and the patients with autoimmune diseases. These are capable of hydrolyzing proteins, DNA, RNA, polysaccharides etc.

Production of Abzymes:

Enzymes act by binding the transition state of a reactant better than the ground state. Antibodies which bind to specific small molecules can be produced by coupling this small

molecule to a protein carrier and using this protein for immunizing experimental animals. If this small molecule is a transition state analog, then the antibodies that are produced to bind to this molecule will function as enzyme towards the substrate of this reaction. Abzymes are selected from monoclonal antibodies produced by immunizing mice with haptens that mimic the transition state of enzyme catalyzed reactions.

For example, 28B4 abzyme catalyzes periodate oxidation of p-nitrotoulene methyl sulphide to sulphoxide, where electrons from the sulfur atom are transferred to the more electronegative oxygen atom. The rate of this reaction is promoted by enzyme catalysts that stabilize the transition state of this reaction, thereby decreasing the activation energy and allowing for more rapid conversion of substrate to product. In order to produce abzymes complementary in structure to this transition state, mice were immunized with an amino phosphonic acid hapten. Of the hapten-binding monoclonal antibodies produced with this hapten, many were found to catalyze sulphide oxidation but with a wide range of binding affinities and catalytic efficiencies. Abzyme 28B4 binds hapten with high affinity and exhibits a corresponding high degree of catalytic efficiency.

Examples of Abzymes:

1) Anti-vasoactive intestinal peptide (VIP) autoantibodies,
2) HIV-1-cleaving abzymes,
3) Antibodies that hydrolyze antibiotics,
4) Protabzymes,
5) Abzymes with Superoxide dismutase activity,
6) Abzymes with heme synthesis activity,
7) Pro-drug activating abzymes.

Applications:

Abzymes have proven to be very important tools in Biotechnology and Medical Pathology in combating dreaded human diseases like AIDS, Cancer etc. One of the promising directions in this field consists of the production of abzymes catalyzing rapid cleavage of hazardous compounds, including toxins and drugs such as cocaine. Treat viral and bacterial infections.

5.6. SUMMARY:

Antigen is defined as any substance, which, when introduced parenterally into the living animal body, evokes specific immune response either by producing specific antibody with which it reacts specifically and in an observable manner or by producing specifically sensitized T-cells or both. Immunogenicity and Immunological reactivity are the two attributes of Antigenicity based upon the ability of antigens to carry out the two functions, antigens are classified into two types a) complete antigen and b) Hapten.

The effective immunogens have molecular weight greater than 10,000. Immunoglobulins are glycoproteins. The basic unit of the molecule is a four chain monomer. These four polypeptide chains consist of two identical heavy chains and two identical light chains, designated as H and L chains respectively. Each polypeptide chain consists an amino terminal and a carboxyl terminal. The amino terminal of the molecule is part of the variable or 'V' regions (V_L and V_H) and carboxyl terminal portion the constant or C regions (C_H and

C_L). The C_H region is further divided into three areas CH_1, CH_2, and CH_3. There are mainly five classes of immunoglobulins based on the type of the heavy chains. They are IgG, IgD, Ig E, IgM and IgA named after the types of heavy chains gamma, delta, epsilon, mu, and alpha respectively. IgG protects the body fluids. IgM protects the blood stream. IgE mediates reagenic hypersensitivity. IgA protects the body surface. IgD together with IgM may function as antigen receptor on the surface of B-lymphocytes for recognition of antigens.

Natural antibody responses are diverse, with each B cell producing antibodies of varying specificity and affinity, which makes it difficult to obtain a uniform antibody. To overcome this, hybridoma technology was developed, in which antibody-producing B cells from immunized animals are fused with immortal myeloma cells to form hybridomas that can continuously secrete a single type of antibody. Abzymes are the catalytic antibodies engineered to act like enzymes. While normal antibodies bind antigens in their ground state, abzymes are designed to bind the transition state of a reaction, lowering activation energy and increasing reaction rate. Abzymes can be artificially created by immunizing animals with a transition-state analog linked to a carrier protein. Abzymes have applications in biotechnology and medicine, including degradation of toxic compounds, drug detoxification (e.g., cocaine), and potential treatment of viral and bacterial infections, cancer, and AIDS.

5.7. TECHNICAL TERMS:

Immunoglobulins, Antibodies, IgG, IgM, IgA, IgD, IgE, Hybridoma technique, Monoclonal antibodies, Constant region, Variable region, Hyper variable region.

5.8. SELF ASSESSMENT QUESTIONS:

1) Discuss the structure and types of immunoglobulins and add a note on their functions.
2) Give an account on antibody production by hybridoma technique.
3) Explain about Abzymes and their importance.

5.9. SUGGESTED READINGS:

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LESSON-6

COMPLEMENT SYSTEM

6.0 OBJECTIVE:

Students will understand the different complement pathways of immune system.

STRUCTURE:

6.1. INTRODUCTION:

The complement system is a part of the immune system that enhances (complements) the ability of antibodies and phagocytic cells to clear microbes and damaged cells from an organism, promotes inflammation, and attacks the pathogen's cell membrane. It is part of the innate immune system. The complement system can be recruited and brought into action by antibodies generated by the adaptive immune system. Ehrlich introduced the term "complement" as part of his larger theory of the immune system. According to this theory, the immune system consists of cells that have specific receptors on their surface to recognize antigens. Upon immunization with an antigen, more of these receptors are formed, and they are then shed from the cells to circulate in the blood. These receptors, which we now call "antibodies and he defined it as heat-labile component of serum that ***"complemented or augment"*** antibodies in the killing of bacteria

6.2. COMPLEMENT PROPERTIES AND COMPONENTS:

Properties:

1) Present in serum of all animals but its maximum concentration is in serum of guinea pig.
2) Complement of one species is able to react with antibodies of other species but not to the
3) same extent.
4) C- proteins constitute about 5% of normal serum protein all are glycoproteins.

5) Are synthesized rapidly in inflammatory responses hence are called acute phase proteins.
6) Heat labile and lost activity at 56º C for 30 minutes and inactivated. Immunoglobulins are
7) not inactivated at this temperature.
8) They bind with Fc potion of immunoglobulin.
9) Cause lysis of cells (bacteria, allografts, tumor cells).
10) Lead to generation of mediators of inflammation.
11) Involves in Opsonization – enhancement of phagocytosis

Complement Components:

1) Components are designated by numbers (E.g.; C1 – C9) or letters (E.g.: Factor D). These are present in serum as inactive ones, and activated sequentially as a cascade when pathogen enters in to the body.
2) Complement receptors are present on cell surface, recognize the activated components.
3) Regulatory proteins of complement are present both in serum and cell surface, inhibit activated components.
4) Complement proteins: are proenzymes – activation by cleavage.

   Eg. C4 --→ C4a and C4b.

   'a' fragment is small and diffuses out.

   'b' fragment is larger and remains bound to microbe.

   But exception is in the case of C2a that bounds to microbe and C2b diffuse out.

6.3. COMPLEMENT PATH WAYS

The complement system operates in three different pathways viz., Classical, Alternate and Lectin pathways (Fig. 6.1). These different pathways of body's immune system activate the complement system to fight against infection. All the three converge on a common step involving the C3 protein, which leads to the formation of the Membrane Attack Complex (MAC) to kill pathogens and enhances inflammation and other immune responses.

6.3.1. Classical Pathway:

This classical pathway is an antibody dependent pathway and triggered by the formation of soluble antigen-antibody complex or by binding of the antibody to the antigen present on the target cell surface. The classical pathway is a chain of events in which complement components react in specific sequences as a cascade resulting in cell lysis. It is activated by antibody bound to antigen (Antigen-Antibody complex) but never by native or free antibody. These complexes may be soluble, or they may be formed when an antibody binds to antigenic determinants, or epitopes, situated on viral, fungal, parasitic, or bacterial cell membranes. Soluble antibody-antigen complexes are often referred to as **immune complexes**, and only complexes formed by **IgM or certain subclasses of IgG** antibodies are capable of activating the classical complement pathway.

Activators of Classical Pathways:

1) Immunoglobulin IgM and IgG. IgG subclasses vary with regard to their efficiency in activating the complement. IgG3 are the most efficient, followed by IgG1 and IgG2. IgG4 do not activate the classical pathway.
2) Staphylococcal protein A
3) C-reactive protein
4) DNA

Steps of Classical Pathway:

The classical pathway of complement activation usually begins with the formation of soluble antigen–antibody complexes (immune complexes) or with the binding of antibody to antigen on a suitable target, such as a bacterial cell. Following are the sequential steps in the activation of classical pathway:

1) **Activation of C1:** The initial stage of activation involves the complement components C1, C2, C3, and C4, which are present in plasma as zymogens. The formation of an antigen-antibody complex induces conformational changes in the non-antigen binding (Fc) portion of the antibody molecule. This conformational change exposes a binding site for the C1 component of complement. This results in the sequential activation of C4, C2, and C3. In serum, C1 exists as a macromolecular complex consisting of one molecule of C1q and two molecules each of the serine proteases, C1r and C1s, held together in a Ca^{++} stabilized complex (C1qr2s2). The C1q molecule itself is composed of 18 polypeptide chains that associate to form six collagen-like triple helical arms, the tips of which bind the CH2 domain of the antigen-bound antibody molecule. Each C1 macromolecular complex must bind by its C1q globular heads to at least two Fc sites for a stable C1-antibody interaction to occur.
2) C1q binding in the presence of calcium ions leads to activation of C1r and C1s. Binding of C1q to the CH_2 domains of the Fc regions of the antigen-complexed antibody molecule induces a conformational change in one of the C1r molecules that converts it to an active serine protease enzyme. This C1r molecule then cleaves and activates its partner C1r molecule. The two C1r proteases then cleave and activate the two C1s molecules. Activated C1s is an esterase that splits C4 into two fragments: a small soluble fragment (C4a) and a larger fragment (C4b). C4a has anaphylatoxin activity, and C4b binds to cell membrane along with C1. C4b in the presence of Mg^{++} splits C2 into C2a and C2b. The smaller fragment (C2b) diffuses away, while the larger fragment (C2a) remains attached to C4b. The resulting C4b2a complex possesses enzymatic activity and is called C3 convertase, which converts C3 into an active form.
3) The C3 convertase activate thousands of C3 molecules and splits these molecules into C3a and C3b. A single C3 convertase molecule can generate over 200 molecules of C3b, resulting in tremendous amplification at this step of the sequence. The biological importance of activated C3b as well as C4b is that they are able to bind to C3b/C4b receptors (currently designated as CR1 receptors) present on almost all host cells, most notably phagocytes. The increased affinity of phagocytic cells for C3b (or iC3b)/C4b-coated particles is known as immune adherence. The latter is responsible for a significant enhancement of phagocytosis, which is one of the main defense mechanisms of the body.

4) Some of the C3b binds to C4b2a to form a trimolecular complex C4b2a3b called C5 convertase. The C5 convertase splits C5 into C5a and C5b. C5a diffuses away, while C5b attaches to C6 and initiates formation of C5b–9 complex otherwise known as membrane attack complex (MAC).

C4 is activated when C1s hydrolyzes a small fragment (C4a) from the amino terminus of one of its chains. The C4b fragment attaches covalently to the target membrane surface in the vicinity of C1, and then binds C2. C4b binding to the membrane occurs when an unstable, internal thioester on C4b, exposed upon C4 cleavage, reacts with hydroxyl or amino groups of proteins or carbohydrates on the cell membrane. This reaction must occur quickly, otherwise the thioester C4b is further hydrolyzed and can no longer make a covalent bond with the cell surface. Only antibodies bound to antigens, and not free circulating antibodies, can initiate classical pathway activation. The reason for this is that each C1q molecule must bind to at least two Ig heavy chains to be activated and each Ig Fc region has only a single C1q binding site. Therefore, two or more Fc regions have to be accessible to C1 in order to initiate classical pathway activation.

6.3.2. Alternative Pathway:

This is an antibody independent pathway stimulated by antigen directly e.g. Bacterial cell surface components. It was discovered after classical pathway but phylogenetically older than the classical pathway. This pathway is also part of the innate immune response and can be activated directly by pathogens without antibodies. It triggers when a complement protein called as C3 directly recognizes certain microbial surface structures, such as bacterial LPS. C3 is also constitutively activated in solution at a low level and binds to cell surfaces, but it is then inhibited by regulatory molecules present on mammalian cells. Because microbes lack these regulatory proteins, the spontaneous activation can be amplified on microbial surfaces. Thus, this pathway can distinguish normal self from foreign microbes on the basis of the presence or absence of the regulatory proteins.

6.3.3 Lectin Pathway:

It is an innate immune pathway and antibody independent but resembles classical pathway. It is triggered by a plasma protein called mannose-binding lectin (MBL), which recognizes terminal mannose residues on microbial glycoproteins and glycolipids, similar to the mannose receptor on phagocyte membranes. MBL is a member of the collectin family (discussed later) with a hexameric structure similar to the C1q component of the complement system. After MBL binds to microbes, two zymogens called MASP1 (mannose-associated serine protease 1, or mannan-binding lectin-associated serine protease) and MASP2, with similar functions to C1r and C1s, associate with MBL and initiate downstream proteolytic steps identical to the classical pathway.

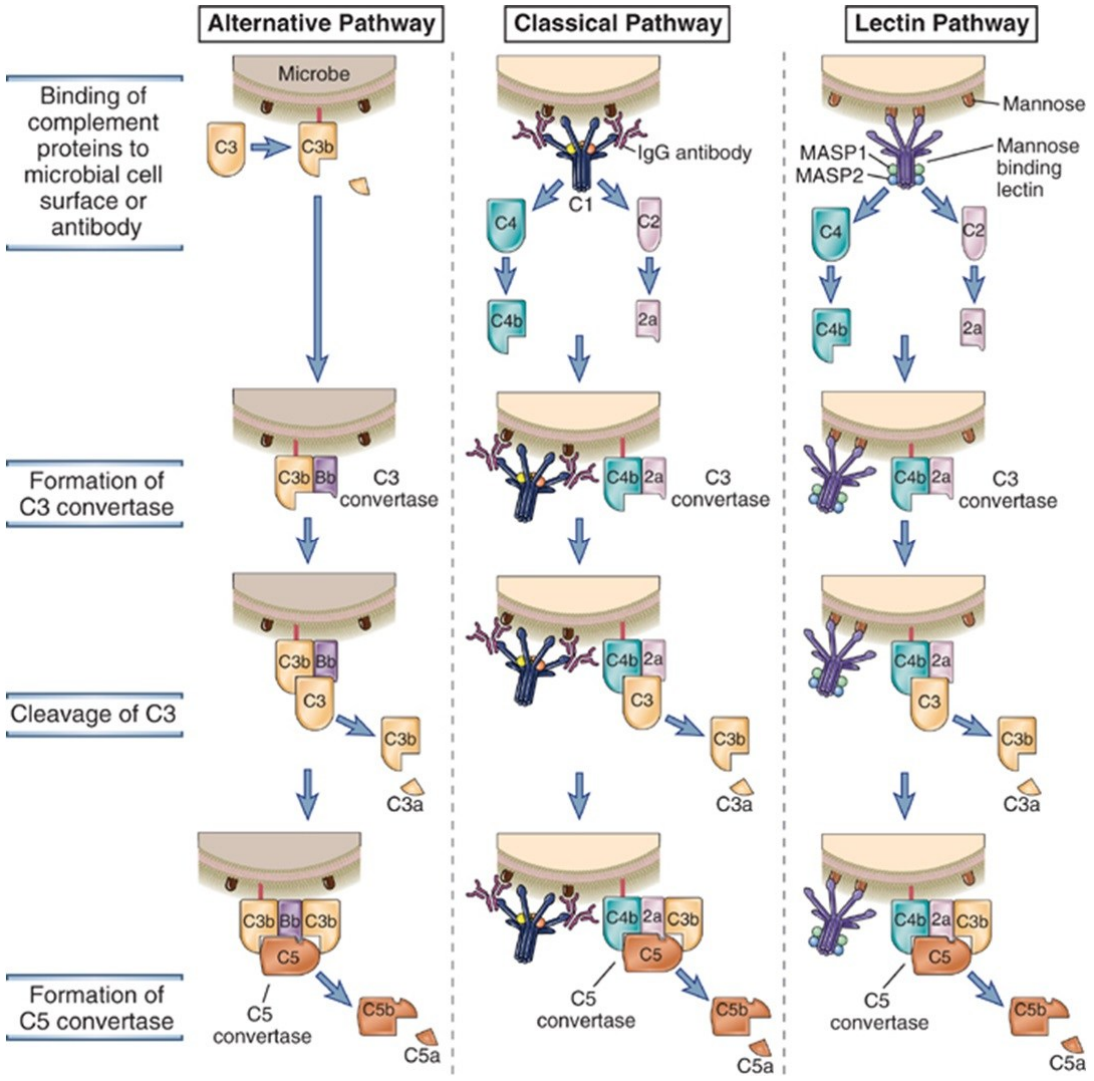

Fig-6.1: Types of Complement System

6.4. FUNCTIONS OF COMPLEMENT PATHWAY:

Some major functions of complements are -

1) **Opsonization and Phagocytosis**

   C3b, bound to immune complex or coated on the surface of pathogen, activate phagocytic cells. These proteins bind to specific receptors on the phagocytic cells to get engulfed.

2) **Cell Lysis**

   Membrane attack complex formed by C5b6789 components ruptures the microbial cell surface which kills the cell.

3) **Chemotaxis**

   Complement fragments attract neutrophils and macrophages to the area where the antigen is present. These cell surfaces have receptors for complements, like C5a, C3a, thus, run towards the site of inflammation, i.e. chemotaxis.

4) Activation of Mast Cells and Basophils and Enhancement of Inflammation

The proteolytic complement fragments, C5a, C4a, and C3a induce acute inflammation by activating mast cells and neutrophils. All three peptides bind to mast cells and induce degranulation, with the release of vasoactive mediators such as histamine. These peptides are also called anaphylatoxins because the mast cell reactions they trigger are characteristic of anaphylaxis. Binding to specific complement receptors on cells of the immune system, they trigger specific cell functions, inflammation, and secretion of immunoregulatory molecules.

5) Production of Antibodies

B cells have receptor for C3b. When C3b binds to B-cell, it secretes more antibodies. Thus C3b is also an antibody producing amplifiers which converts it into an effective defense mechanism to destroy invading microorganism.

6) Immune Clearance

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.

Complement Regulation:

The complement system has the potential to be extremely damaging to host tissues; hence regulatory mechanisms are required to restrict the complement pathway. Various plasma and cell membrane proteins regulate complement activation by inhibiting different steps in the cascade. The membrane of most mammalian cells has a high level of sialic acid, which contributes to the inactivation of complements.

Complement Related Diseases:

Diseases associated with complements can be due to the deficiencies in any of the protein components or in regulatory components.

1) 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 *Neisseria* infections like, gonorrhea and meningitis.
2) Deficiencies of regulatory proteins lead to too much activation of complements in wrong time and place which leads to unwanted inflammation and cell lysis. Pyogenic bacterial infection and glomerulonephritis are the results of such deficiencies.
3) Mutations in the complement regulators factors may lead to atypical hemolytic uremic syndrome, age-related macular degeneration, hereditary angioedema, etc.

6.5. SUMMARY:

The complement system is a part of the immune system that enhances (complements) the ability of antibodies and phagocytic cells to clear microbes and damaged cells from an organism, promotes inflammation, and attacks the pathogen's cell membrane. It is part of the innate immune system.

6.6. TECHNICAL TERMS:

Complement system, Classical pathway, Alternate pathway, Lectin pathway, C3 convertase.

6.7. SELF ASSESSMENT QUESTIONS:

1) Discuss in detail about Classical pathway of complement system.
2) Explain the Alternate and Lectin pathways of complement systems.
3) Describe the properties, components and functions of complement system.

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LESSON-7

ANTIGEN-ANTIBODY REACTIONS

7.0 OBJECTIVE:

- Students acquaint with the knowledge of different types of antibody and antigen reactions like precipitation, agglutination, neutralization and complement fixation.

STRUCTURE:

7.1. INTRODUCTION:

When an antigen solution is mixed in a correct proportion with a potent antiserum, an antigen antibody complex is formed. This complex formation in an observable manner due to combination of specific antigen with specific antibody is called antigen-antibody reaction.

Ag + Ab → Ag-Ab Complex

In the body, Ag-Ab reactions play an important role in Ab-mediated immunity against infectious diseases and tissue injury in hypersensitivity and autoimmune disease. In the laboratory, Ag-Ab reactions are useful - in the diagnosis of infections; in epidemiological surveys; in the identification of infectious agents; and in the identification of non-infectious agents such as enzymes. In general, these reactions can be used for the detection and quantitation of either antigens or antibodies. By definition the Ag-Ab reactions in vitro are known as serological reactions.

Characteristics of Ag-Ab Reactions:

The reaction between Ag and Ab is highly specific. This specificity, however, is not absolute and cross-reactions between related or similar Ags may occur. The antigenic determinant makes contact with an area on the hyper variable region called paratope of the

Ab. The molecules are held together in lock and key arrangement by spatial complementarity and not by covalent bonding. Entire molecules interact and not fragment. Though only antigenic determinant is involved in actual binding, the whole molecules or particles are agglutinated. The combination occurs at the surface, so only surface antigens participate during combination. The combination is firm but reversible. The firmness is affected by affinity and avidity of the reaction. Affinity is the intensity of attraction between antigen and antibody and avidity is the binding strength of the individual Ab with its specific antigenic determinant. Ags and Abs can combine in varying proportions due to their valences. Abs are generally bivalent and Ags may be multivalent.

7.2. TYPES OF ANTIGEN AND ANTIBODY REACTIONS:

7.2.1. Precipitation:

When a soluble Ag (precipitinogen) combines with its antibody (precipitin) in the presence of electrolytes (NaCl) at a suitable temperature (37^{o}C) and pH (7.4), the Ag-Ab complex is formed as an insoluble precipitate. This reaction is called as precipitation.

Precipitinogen + Precipitin $\rightarrow$ Insoluble precipitate

(Soluble Ag) (Ab) (Ag-Ab complex)

Principle:

Zone Phenomenon:

The amount of precipitate formed is greatly influenced by the relative proportions of Ags and Abs. When increasing quantities of Ag are added to the fixed amount of antiserum, precipitation occurs most rapidly and abundantly in one of the middle dilutions in which the Ag and Ab are present in optimum proportions. In the first few dilution in which the Ab is in excess and in the last few dilutions in which the Ag is in excess, the precipitation will be minimal or absent. If the amounts of precipitate at different dilutions are plotted, the resulting curve will have three different phases.

An Ascending Zone: A zone of Ab excess or prozone in which some uncombined Ab is present.

A Peak: A zone of equivalence in which Ag and Ab are completely precipitated.

A Descending Zone: A zone of Ag excess or postzone in which all Abs have combined with Ag but some uncombined Ag is present.

This is known as zone phenomenon. Zoning occurs in agglutination and some other reactions also. The prozone is very important, as sera rich in Ab may give a false negative precipitation or agglutination reactions.

Mechanism of Precipitation:

The first step is linking together of different antigens by Ab molecules that specifically attach to the antigenic determinants on the surface of Ag. Marrack (1934) proposed that each Ab which is divalent forms a bridge between two Ag molecules. Ag being multivalent can combine with a number of Ab molecules. This combination results in the

formation of multimolecular lattice, which makes the reaction visible. It is known as *lattice hypothesis.* This theory requires that - The Ab should be divalent at least and Ag and Ab in optimum proportion. Precipitation occurs when Ag and Ab react in equivalent proportion (zone of equivalence). The lattice formation does not occur in the zones of either Ag or Ab excess.

Applications:

Precipitation reaction is very sensitive for detection of Ag and can detect as little as one μg of protein antigen but comparatively less sensitive for the detection of Abs. The test may be carried out either as a qualitative test or quantitative test. The qualitative precipitation test is widely used for detection of antigens and is particularly valuable in - Identification of bacteria, Identification of bacterial components in infective tissues, Detection of unknown antibody, medico legal identification of human blood or seminal fluid and Standardization of toxins and antitoxins.

Techniques of Precipitation Reaction:

Precipitation reaction can take place in a liquid medium or in semisolid medium (gels) such as agar gels, agarose or polyacrylamide.

The following are the types of precipitation tests:

Ring Test:

In this test, antiserum is placed at the bottom of a narrow bottom tube and an antigen solution (an extract of the organism) is layered over it. A white ring of precipitate forms at the junction of two fluids. This is the simplest type of precipitation employed for the detection of Ag, for example, Ascoli's thermo precipitation test for anthrax; C-reactive protein test; the grouping of streptococci by Lancefield technique.

Slide Flocculation Test:

When a drop of antigen solution and patient's serum are placed on a slide and mixed well by shaking, the result is formation of floccules in positive cases. For example, VDRL test for diagnosis of syphilis.

Tube Flocculation Test:

Here, instead of slide, the test is performed in tube. An antigen and serum are placed in a tube and mixed by shaking. This results in formation of floccules. For example, Kahn test for diagnosis of syphilis. It is also used for the standardization of toxins and toxoids.

Immunodiffusion Test:

It is a precipitation reaction carried out in a gel. Precipitation reaction carried out in gel is more advantageous than precipitation in a liquid medium. The advantages are - The gel has got certain porosity through which antigens and antibodies migrate to form precipitation band where they meet in optimum proportion. The band is stable and can be preserved by staining, if necessary. Each antigen-antibody reaction results in the formation of a distinct band of precipitation, therefore the different antigens in the reacting mixture can be observed and detected. Immunodiffusion also indicates identity, cross-reaction and nonidentity between different antigens.

7.2.2. Agglutination Reaction:

When a particulate Ag (agglutinogen) combines with its Ab(agglutinin) in the presence of electrolytes at a suitable temperature and pH, the particles are clumped or agglutinated. Thus, agglutination is the aggregation of already insoluble particles or cells into larger clumps.

Agglutinogen + Agglutinin → Agglutination

(Particulate Ag) (Ab) (Ag-Ab complex)

Mechanism of agglutination is same as that of precipitation reaction. Among the immunoglobulins, IgM with 10 binding sites is a much more powerful agglutinator than IgG with only one binding site. These IgG antibodies are unable to agglutinate due to their monovalency and such antibodies are called incomplete antibodies. The incomplete Abs are able to combine with Ag but unable to cross-link the cells and thus may act as blocking Abs and inhibit agglutination by the complete Abs. This phenomenon is known as prozone phenomenon. Agglutination can be practiced in tubes or on slides and can be visualized with the naked eye or under the microscope.

Requirements: The particulate Ag, Serum containing agglutinin (Abs), Electrolytes in the form of normal saline.

Types of Agglutination Reactions:

Slide Agglutination Test:

In this test, a smooth and uniform suspension of particulate Ag is prepared in a drop of saline on a glass slide or a tile. Then a drop of appropriate antiserum is added and mixed with a wire loop. A positive result, in few seconds, is indicated by the clumping together of the particles and clearing of drop. Clumping is visible to the naked eye but sometimes requires confirmation under the microscope. On the same slide, control test is performed in which Ag suspension is taken in a drop of saline without antiserum. If no clumping occurs, it shows that antigen is not autoagglutinable. This test is used for identification of bacterial isolates from clinical specimens; blood grouping and cross matching. The advantage is only smaller quantities of reagents are required than tube agglutination method. And the disadvantage is - less quantitative than tube agglutination.

Tube Agglutination Test:

This is a standard quantitative test for determination of Ab titer. A fixed volume of particulate Ag suspension is added to the equal volume of serially diluted serum in test tubes and incubated. The highest dilution of serum that gives positive agglutination reaction is recorded as Ab titer. Mainly used for the diagnosis of Typhoid fever (Widal test), Brucellosis (*Brucella* agglutination test) and Typhus fever (Weil-Felix test). More quantitative than slide agglutination test, but requires larger quantities of reagents.

Haemagglutination:

Agglutination tests in which red blood cells are used are known as haemagglutination tests. The different types of haemagglutination tests are:

a) Direct active haemagglutination test:

In this test, RBCs are used as an Ag.

Examples:

Paul-Bunnel test for infectious mononucleosis in which sheep RBCs are used as Ag. Cold agglutination test for primary atypical pneumonia in which human 'O' group RBCs are used as an Ag.

b) Indirect Active Haemagglutination Test:

In this test, RBCs are coated with IgG Abs and the rabbit anti-IgG Abs are added which cause agglutination of RBCs. Here, the IgG form a bridge between two RBCs and anti-IgG Abs cross link the RBCs. Eg. Coomb's test. Some Abs fails to agglutinate corresponding Ag and also inhibit agglutination. These are known as incomplete or blocking Abs. For example, some anti - D Abs fail to agglutinate Rh-D positive red cells.

These incomplete Abs are detected by Coomb's test, which is of two types - direct and Indirect (antiglobulin test).

I. Direct Coomb's Test:

It is used to detect monovalent maternal Ab already present on RBCs. In this test, the sensitization of RBCs with incomplete Abs takes place in vivo. When such RBCs are treated with Coomb's serum (rabbit antiserum against human γ-globulin), agglutination occurs.

Uses: In the hemolytic diseases of newborn due to Rh incompatibility.

II. Indirect Coomb's Test:

The direct Coomb's test is frequently negative in hemolytic diseases due to A, B, O incompatibility. The amount of Ab bound to cell is too small for detection by direct Coomb's test. In such cases, indirect Coomb's test is used for detection of Abs in the patient's serum. In this test, sensitization of RBC with Ab is performed in vitro by incubating patient's serum with Rh positive RBCs (group 'O' or same group RBCs) and then Coomb's serum is added. This results into agglutination.

Uses: The test is used for detection of anti-Rh Ab (free) in the patient's serum. Also for demonstration of any type of incomplete or non-agglutinating Abs, for example, non-agglutinating Abs in brucellosis.

c) Direct Passive Haemagglutination Test:

In this text, the Ag is adsorbed or attached to the surface of RBC. Here, RBC acts as an inert carrier of Ag. These are agglutinated by Abs.

Uses: Tanned cell hemagglutination used for the demonstration of Abs to thyroglobulin. *Treponema pallidum* haemagglutination test used for the serodiagnosis of syphilis.

d) Indirect Passive Haemagglutination Test:

In this test, sheep RBCs are sensitized with rabbit anti-sheep erythrocyte Ab (amboceptor) and used as Ag. The example is the Rose-Waaler test for rheumatoid arthritis in which the autoantibody (RA-factor) that appears in the serum acts as an Ab and agglutinates SRBCs sensitized with amboceptor.

Passive Agglutination with Latex and Other Particles:

In this test, inert particles are used a carrier of Ag instead of RBCs. An Ag can be adsorbed on particles of bentonite or other particles of mineral origin such as polystyrene latex particles. Ag molecules are nonspecifically adsorbed to the surface of latex particles, which have uniform diameter of 0.8 - l μ. Addition of specific antibody transforms the latex (milk) from a milky white liquid to a coarse suspension of visible granules. The test can be performed in the test tube but more commonly on slides.

Examples:

RA factor test, Pregnancy test, CRP test (C-reactive protein), Antistreptolysin O test (ASO). The test can also be used for diagnosis of Cryptococcal meningitis, Amoebiasis, Meningococcal infections, Pneumococcal infections, *H. influenzae* infections, Hepatitis B virus infection and others.

7.2.3. Complement Fixation Test (CFT):

The ability of Ag-Ab complexes to fix complement is used to CFT. It is a versatile and sensitive test, applicable with a variety of Ags and Abs and capable of detecting as little as 0.04 μg of Ab nitrogen and 0.1 μg of Ag. Described by Bordet and Gengou in 1901.

Requirements:

Complement – it is obtained from guinea pig serum. The recently obtained complement is not suitable for the test, so the blood from guinea pig should be collected 12-18 hrs. before the test. As it is heat labile and unstable, it deteriorates at ordinary temperature therefore it should be preserved either in lyophilized or freeze dried state or by adding preservatives (equal volume of 12% sodium acetate and 4% acetic acid). Patient's serum-five ml of blood is collected from a patient in a sterile container. Serum is separated and heated at 56^{o}C for 30 minutes to destroy the complement present. Ag - suitable, soluble or particulate Ag obtained from commercial source or prepared in laboratory. Sheep red blood cells - defibrinated sheep blood is collected. Blood is washed in saline till supernatant is colourless. Resuspended in saline. Amboceptor (Rabbit or horse anti-sheep RBC serum).

This Test include Two Steps:

Step 1: The inactivated patient's serum is incubated with its Ag in the presence of fixed amount of complement at 37^{o}C for 60 minutes.

Step 2: Sensitized RBCs (sheep erythrocytes coated with four MHD haemolysin) are added, mixed, and incubated at 37^{o}C for 30 minutes.

Results are interpreted on the basis of presence or absence of hemolysis. The test is considered as positive if there is no hemolysis. In positive test, the patient's serum contains Abs, which reacts with Ags and fix complement. Thus, complement is utilized in first step and there is no free complement present for lysis of SRBCs. The test is considered as

negative, if there is hemolysis. In negative test, the serum does not contain Abs hence there will be no Ag-Ab reaction and complement will not be utilized in first step. It will be left intact which reacts with SRBCs and causes hemolysis. The positive result (no hemolytic) indicates presence of Abs in patient's serum. The negative result (hemolytic of Serbs) indicates absence of Abs in patient's serum (Fig. 7.1)

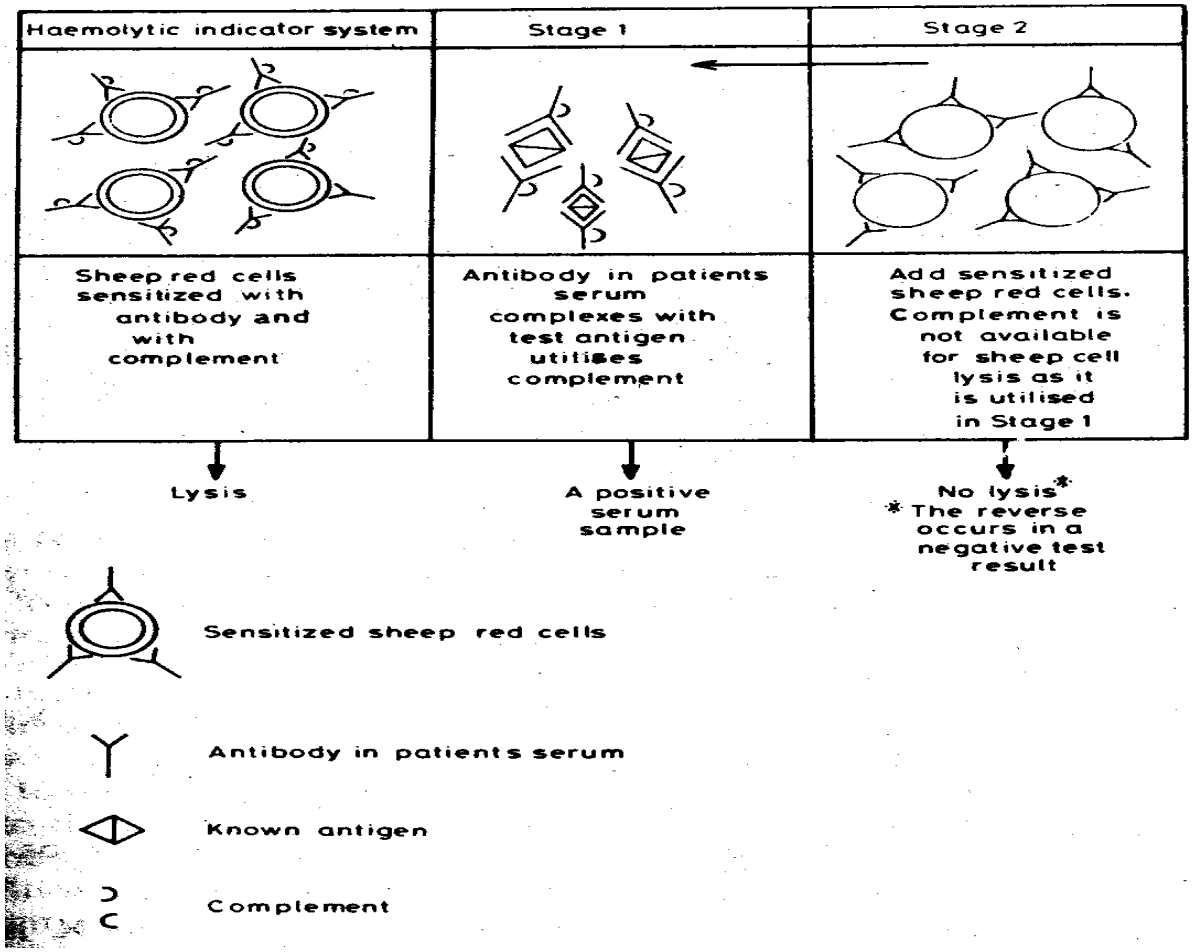


Fig-7.1: Complement fixation Test

a) Indirect complement fixation test (Indirect CFT):

Certain avian (duck, turkey, parrot) and mammalian (horse, cat) sera do not fix guinea pig complement. In such cases, indirect CFT is used. The test is carried out as follows - In first step, Ag, test sera and complement are added to each other. To this a standard antiserum known to fix complement is added after first step and finally sheep RBCs with Abs are added. In this test, if test serum contains Abs, they react with Ag hence standard antiserum will not react with Ag and complement will not be fixed. Thus, the complement causes hemolysis, which is considered as positive test. If test serum does not contain Abs, the standard antiserum will react with Ag and complement will be fixed, so that there will be no hemolysis which indicates negative test.

Ag + Test + C + Standard + SRBCs + Abs

Serum Antiserum

↓

Hemolysis - Positive Test

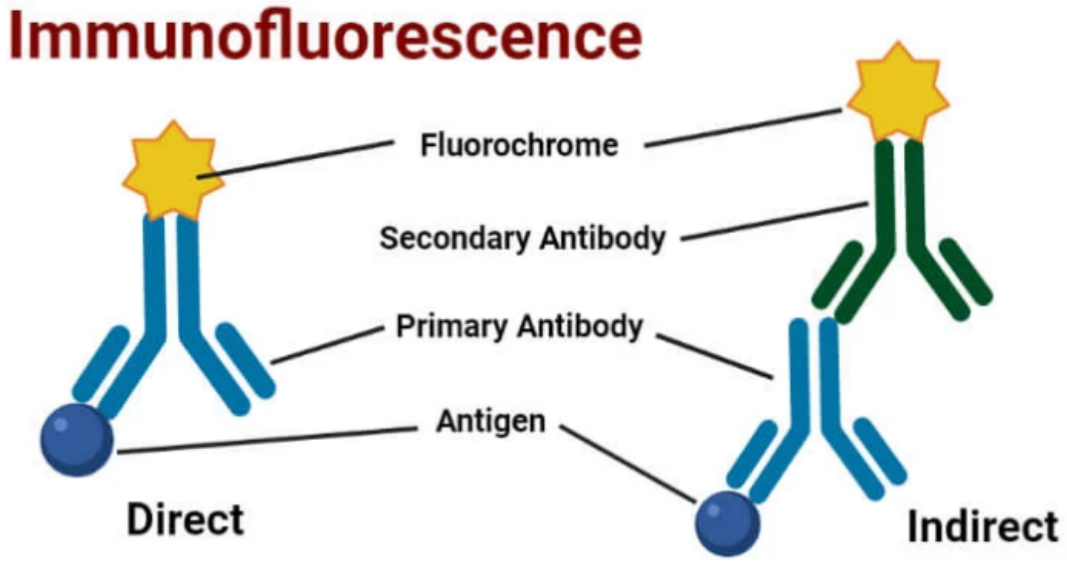
No hemolysis - Negative Test

7.2.4. Immunofluorescence Microscopy:

Immunofluorescence is a type of assay performed on biological samples to detect specific antigens in any biological specimen or sample and vice-versa. The specificity of antibodies to their antigen is the base for immunofluorescence. It was described in 1942 and refined by Coons in 1950, which used a fluorescence microscope able to read the specific immunological reaction and cellular slide preparations. It is an effective method for visualizing intracellular processes, structures, and conditions as well.

- In Vitro type of Ag-Ab Interaction.
- Detects surface antigens or antibodies.
- Fluorescent dyes are used for the visualization of Ag-Ab reactions.

The property of certain dyes absorbing light rays at one particular wavelength (ultraviolet light) and emitting them at a different wavelength (visible light) is known as fluorescence. In the immunofluorescence test, a fluorescent dye that illuminates in UV light is used to detect/show the specific combination of an antigen and antibody. The dye usually used is fluorescein isothiocyanate, which gives yellow-green fluorescence. Immunofluorescence tests are also termed fluorescent antibody tests (FAT).



Requirements of Immunofluorescence

The primary requirement is specific antibodies that can bind to the antigen of interest to form the Ag-Ab complex. They can be

a) **Primary Antibody:** The specific antibody which directly binds with antigen.
b) **Secondary Antibody:** The antibody which binds to the Fc region of a primary antibody hat is already bound with the specific antigen. It can be effectively used for different types of assays.

A secondary requirement is Fluorescent dye or Fluorchromes or Fluorophores which are conjugated to the antibody. Commonly used Fluorochromes are – Fluorescein, Rhodamine, Phycoerythrin

- Immunofluorescence microscope for visualization
- Wash buffers such as PBS (Phosphate Buffered Saline)- Helps to wash away unbound antibodies.

Principle of Immunofluorescence:

- Specific antibodies bind to the protein or antigen of interest.
- Antibodies could be labeled with molecules that have the property of fluorescence (fluorochromes)
- When light of one wavelength falls on fluorochrome, it absorbs that light to emit light of another wavelength.
- The emitted light can be viewed with a fluorescence microscope.

Types of Immmunofluroscence

- **Direct Immunofluorescence Test**
- **Indirect Immunofluorescence Test**

Direct Immunofluorescence Test:

Single antibody i.e. primary antibody is used that is chemically linked to a fluorochrome. If the antigen is present, the primary antibody directly reacts with it and fluorescence can be observed under the fluorescent microscope.

Procedure of Direct Immunofluorescence Test

1) Fixing of Specimen (Antigen) into the slide.
2) Fluorochrome labeled antibodies are then added to the slide.
3) Incubation and careful washing with wash buffers like PBS to remove other components except for the complex of antigen and fluorochrome-labeled antibody.
4) Observed under a fluorescence microscope.

Uses of Direct Immunofluorescence Test:

- For the detection of rabies virus antigen in the skin smear collected from the nape of the neck in humans and the saliva of dogs.

- For the detection of *N. gonorrhoeae, C. diphtheriae, T. pallidum,* etc. directly in appropriate clinical specimens.

Advantages of Direct Immunofluorescence Test

- Protocols for direct IF are usually shorter as they only require one labeling step.
- Species cross-reactivity is minimized indirect methods as the fluorophore is already conjugated to the primary antibody.

Disadvantages of Direct Immunofluorescence Test

- Separately labeled antibodies need to prepared for each pathogen.
- Requires the use of a much more primary antibody, which is extremely expensive.
- Less sensitive than indirect immunofluorescence.

Indirect Immunofluorescence Test:

Double antibodies are used i.e. primary and secondary antibodies. The primary antibody is not labeled and a fluorochrome-labeled secondary antibody is used for detection. The antigen used is known and it binds to the specific primary antibodies of interest in the sample. The secondary antibody then binds to the Fc region of the primary antibody.

Procedure of Indirect Immunofluorescence Test

1) Fixing of a known antigen on a slide.
2) The specimen to be tested is applied to the slide.
3) Incubation and careful washing with PBS.
4) A secondary antibody (e.g., fluorescently labeled anti-IgG) is added.
5) Incubation and careful washing again with PBS.
6) Observed under the fluorescence microscope.

Uses of Indirect Immunofluorescence Test

- In detection of specific antibodies for diagnosis of syphilis, amoebiasis, leptospirosis, toxoplasmosis, and other diseases.
- Also used in the detection of autoantibodies that causes auto immune disorders.
- Advantages of Indirect Immunofluorescence Test
- In case of secondary antibodies, a single fluorochrome-labeled antibody is used for detecting many Ag-Ab interactions.
- More sensitive than direct immunofluorescence test.
- Multiple secondary antibodies can bind to the Fc region of primary antibody which amplifies the fluorescence signal.

Disadvantages of Indirect Immunofluorescence Test

- It is more complex and time-consuming than the direct IF.
- Cross-reactivity of secondary antibody to other agents can be problmatic.

Result Interpretation of Immunofluorescence:

If there is the presence of a specific antigen or antibody of interest they would form an Ag-Ab complex. So, the fluorochrome-conjugated antibody will remain bound in the preparation even after washing and fluorescence of yellow-green or green or red (depending on the types of fluorochromes used) can be observed while visualizing through a fluorescent microscope. And the test can be considered positive.

If there is no presence of antigen or antibody of interest then Ag-Ab complex won't be formed and all the unbound antibodies would be washed away hence we cannot observe fluorescence if the test is negative.

Applications of Immunofluorescence:

1) Immunofluorescence can be used on tissues or cell sections to determine presence of different biological molecules which also includes proteins, carbohydrates, etc.
2) Also used in molecular biology for visualization of cytoskeletons such as intermediate filaments.
3) It also plays a key role in the detection of autoimmune disorders.
4) It can be used with some non-antibody methods of fluorescent staining, like the use of DAPI (4′,6-diamidino-2-phenylindole) to label DNA.

7.2.5. Enzyme Linked Immunosorbent Assay (ELISA):

It is a type of binding assay that depends on Ag - Ab reaction as base and enzyme reaction as marker. This is a simple, versatile and highly sensitive test and needs only micro-liter quantities of test reagents. It is widely used for detection of variety of Abs and Ags.

Requirements:

An absorbing material specific for the Ag or Ab, such as cellulose or agarose or a solid phase such as polystyrene, polyvinyl or polycarbonate tubes or micro wells or membranes or discs of polyacrylamide, paper or plastic is generally used as immunosorbent. Enzymes such as alkaline phosphatase or horse radish peroxidase. Substrates such as para-nitrophenyl phosphate for alkaline phosphatase or O-phenylene diamine dihydrochloride for peroxidase are generally used. Sodium hydroxide (3 M/l). Spectrophotometer, fluorometer or pH meter are the other requirements for this test.

There are two principal techniques:

1) Double Ab test for detection of Ag (sandwich ELISA).
2) Indirect method for detection of Ab.

The test may be performed in polystyrene tubes (macro ELISA) or in polyvinyl microtitre plates (micro ELISA)

a) Direct method (Double Ab sandwich ELISA):

It is used for assay of an Ag. In this test, the wells of microtitre plate are coated with specific Ab. The sample to be tested is added and incubated overnight at $4^{o}C$ or for 2 hr. at

37°C. The wells are washed and Ab labeled with an enzyme is added and incubated at 37°C for 1 hr. After washing a suitable substrate is added and held at room temperature. Reaction is stopped after a given time, i.e., 1 hr. or after the positive control shows the development of yellow colour. The reaction is stopped by adding sodium hydroxide. The enzyme activity is measured by spectrophotometric or fluorometric electrode method. If the test sample contains specific Ag, it is fixed to the Ab coating the wells. When the enzyme labeled Ab is added subsequently, it gets fixed on Ag and the presence of enzyme activity is indicated by the development of yellow colour which indicates positive test. If the sample is negative for Ag there will be no change in colour.

Uses: Used for detection of Ags such as Rota virus Ag, Hepatitis - B virus Ag, etc. Detection of hormones, Detection of toxins, Detection of HCG (Human chorionic gonadotrophin) - pregnancy test.

b) Indirect method:

It is used for detection of Abs. In this test, Ag is coated in well instead of Ab and treated with patient's serum and then antihuman immunoglobulin labeled with an enzyme is added. Rest of the procedure is almost similar to sandwich ELISA.

Uses: Used for detection of Abs in various infections such as *Salmonella, Haemophilus, V. cholerae, Brucella, Treponema,* Rubella, HIV, Hepatitis-B virus, Herpes simplex virus, Cytomegalovirus, To detect anti-DNA Abs in systemic lupus erythematosus, and detect human IgE, etc.

7.2.6. Radioimmuno Assay:

A technique in which a radioactive isotope is used to detect Ags or Abs is called radioimmunoassay (RIA) (Fig.7.2). It was introduced by Berson and Yallow (1960) for quantification of plasma insulin levels. Now it is used as sensitive and specific method of quantitation of any compound to which an antibody can be produced. RIA permits the measurement up to picogram (10^{-12} g) quantities.

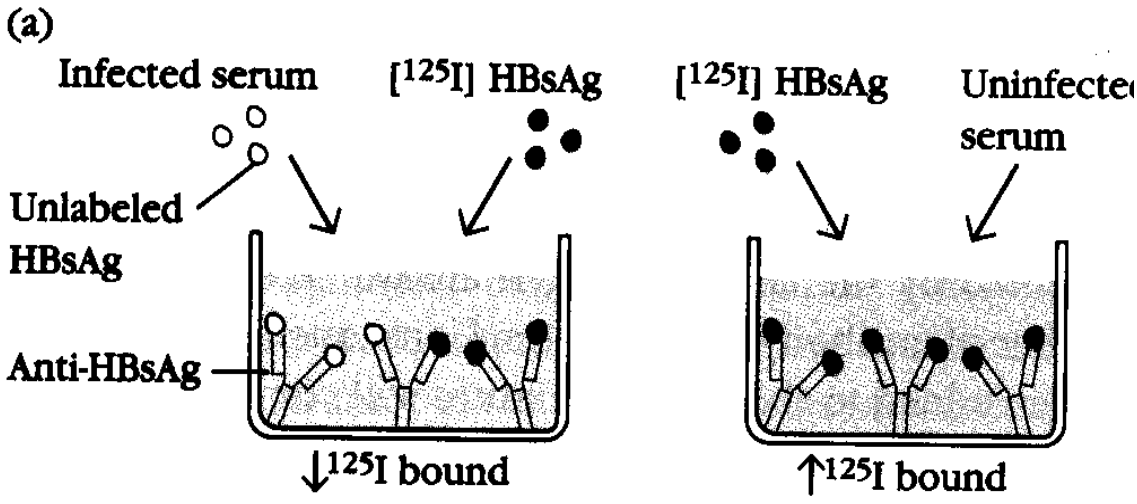


Fig-7.2: Radioimmuno Assay

Principle:

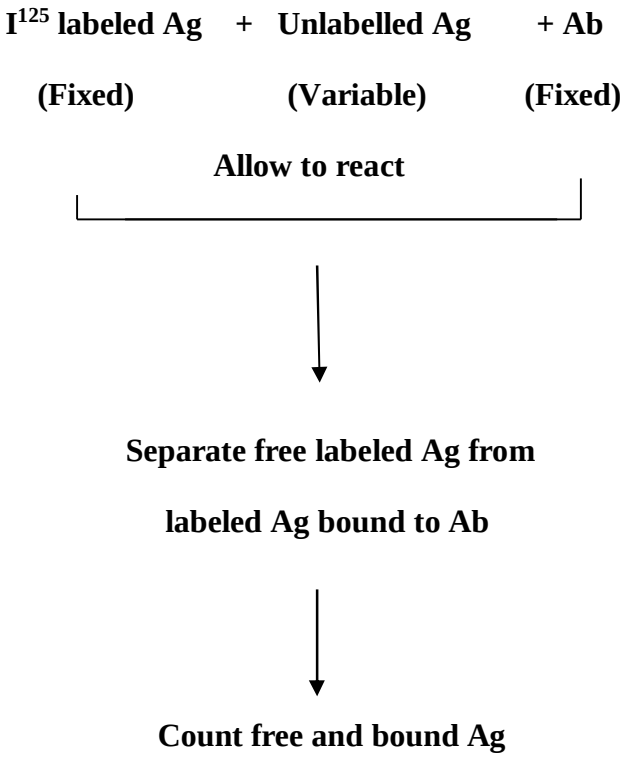
It is basically a competitive binding assay in which fixed amounts of Ab and radiolabeled Ag react in the presence of known (standard) or unknown (test) amounts of the Ag. The amounts by which the binding of labeled Ag to its Ab are competitively inhibited by increasing amounts of Ag. The amounts by which the binding of labeled Ag to its Ab are competitively inhibited by increasing amounts of standard Ag preparations are recorded. From this, a dose- response curve or standard curve is plotted. The amount of Ag present in unknown sample can be calculated from the standard curve by comparing the binding of labeled Ag.

Requirements:

Antisera-of high specificity, high titre and Abs having high avidity and affinity, Labeled Ag - of high specific radioactivity, Standard Ag, Buffer - pH 7.8, Test sample.

General Methodology:

First, antisera against Ag to be tested are raised in Guinea pig or rabbit. Then the Ag is radio-labeled. The labeled Ag is allowed to react with enough Ab to bind about 70% of it. Various known amounts of unlabeled Ag are added to allow competition for Ab. After an appropriate incubation, the labeled Ag bound to Ab is separated from unbound labeled Ag by electrophoresis, chromatography, gel diffusion or double Ab method. From amounts of labeled Ag bound at various concentrations of unlabeled Ag, a standard curve is constructed, which allows quantitation of unknown Ag in test sample.



Applications of RIA:

It can be used for quantitation for hormones, drugs, tumor markers, Ig E, viral Ags, autoimmune markers etc.

7.3. SUMMARY:

Immunological reactions are mainly between antibody and antigens. The reactions are mainly of five types. They are precipitation, agglutination, neutralization, complement fixation, ELISA and Radio immunoassay. In precipitation reactions the soluble antigens combine with its antibody and form an insoluble precipitate. Where as in agglutination reactions the particulate antigen reacts with its antibody and form into clumps (get agglutinated). In complement fixation reactions, fixation of complement occur during the interaction between Ag and Ab. Thus, in this teat, the consumption of complement in-vitro can be used as a test and measure Ab, Ag or both. In ELISA, the Abs linked with enzymes were used, which give characteristic colour on addition of suitable substrate, to react with Ags. RIA is the competitive binding assay where the amounts by which the binding of labeled Ag to its Ab are competitively inhibited by increasing amounts of standard Ag preparations are recorded.

7.4. TECHNICAL TERMS:

Ag-Ab reactions, Precipitation, Agglutination, Complement fixation, Immuno fluorescence microscopy, ELISA, RIA.

7.5. SELF ASSASSMENT QUESTIONS:

1) Write an essay on different types of precipitation and agglutination reactions.
2) Write in detail about the complement fixation and Radio immuno assay.
3) Describe in detail about ELISA and RIA.

7.6. SUGGESTED READINGS:

1) Nandini Shetty, 2001. IMMUNOLOGY Introductory Text Book., New Age International (P) Limited, Publishers, New Delhi – 235 pp.
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5) Roitt, I.M., 1988. Essentials of Immunology. ELBS, Blackwell Scientific Publ. London.

Dr. J. Madhavi

LESSON-8

HYPERSENSITIVITY REACTIONS

8.0 OBJECTIVE:

- To acquaint the students with the knowledge on different hypersensitivity reactions.

STRUCTURE:

8.1. INTRODUCTION:

Immune response, usually beneficial to the host, may sometimes become harmful also. Sensitized individuals may respond to subsequent antigenic stimuli in heightened or exaggerated manner leading to Tissue damage, Disease, or even death of the individual. This type of response is termed as hypersensitivity. The term 'allergy' was coined by Von Pirquet (1906) to cover any altered response to an antigen. Therefore, increased resistance is immunity and increased susceptibility is hypersensitivity. The terms allergy and hypersensitivity are now used synonymously for altered state of the body induced by an antigen. In allergic (hypersensitivity) reactions, an antigen is referred to as allergen or sensitizer. Immunization is referred to as sensitization.

Based on the time required for a sensitized host to develop clinical reactions upon re-exposure to the Ag, hypersensitivity reactions are classified into two types - Immediate hypersensitivity and Delayed hypersensitivity.

Immediate Type of Hypersensitivity:

Hypersensitive state in which an allergic reaction develops immediately within a short period after contact with an Ag (sometimes within a few seconds also) is called immediate type. It is a B-cell or Ab mediated reaction, examples are Anaphylaxis, Ab mediated cytotoxic hypersensitivity, and Ag-Ab complex mediated hypersensitivity.

Delayed Type of Hypersensitivity:

Hypersensitive state in which there is an appreciable delay between the exposure to the Ag and development of symptoms is referred as delayed type. It is a T-cell mediated reaction, e.g., Infection (tuberculin) type and Contact dermatitis type.

Differences between Immediate and delayed Types of Hypersensitivity

Immediate Type of Hypersensitivity	Delayed Type of Hypersensitivity
1. Appears immediately within short time and recedes rapidly.	Appears slowly in 24 – 72 hrs. and lasts longer
2. Induced by Ag or hapten by any route	Induced by infection, injection of Ag or hapten intradermally with Freund's adjuvant or by skin contact
3. Antibody mediated reaction	Cell mediated reaction
4. Passive transfer possible by serum	Passive transfer possible with lymphocyte or transfer factor
5. Desensitization easy but short lived	Desensitization difficult but longer lasting

Coombs and Gel Classification:

Coombs and Gel in 1963 classified hypersensitivity reactions into four types based on different mechanisms of pathogenesis. This classification is widely accepted. The types are: Type I - anaphylaxis (IgE or reagin dependent). Type II - Ab mediated cytotoxic hypersensitivity. Type III - immune complex mediated hypersensitivity. Type IV -Delayed or cell mediated hypersensitivity. One additional type –type V, has been recently proposed. Type I, II, and III are Ab mediated reactions. Type IV is cell mediated reaction.

8.2. TYPE I-ANTIBODY MEDIATED ANAPHYLAXIS (IgE OR REAGIN DEPENDENT)

It is an IgE mediated immediate type of hypersensitivity reaction. The term anaphylaxis (ana-without, phylaxis-protection) was coined by Richet in 1902 to describe his experiment on dog. Theobald Smith (1902) had noticed a similar phenomenon in guinea pigs following injections of toxin-antitoxin mixtures. When a guinea pig is injected with a small dose of an antigen (about 1 mg) such as egg albumin, no adverse effects are noticed. When a second dose of same antigen (i.e. egg albumin) is injected intravenously after an interval of 2-3 weeks, the sensitized guinea pig reacts very dramatically and a condition known as 'anaphylactic shock' is developed. The animal becomes restless, cyanosed, may develop convulsions and die. The initial injection of antigen is termed as sensitizing dose. It is more effective by parenteral route. The second injection of antigen is termed as shocking dose. It is more effective by intravenous route than intraperitoneal or subcutaneous route. During an interval between the two injections, the animal forms antibodies. Anaphylaxis is the result of interaction of the shocking dose of antigen with newly formed antibodies on the surface of tissue cells. This interaction triggers the release of pharmacologically active substances, which increase capillary permeability and cause smooth muscle contraction. Guinea pigs are highly susceptible to this reaction. Rabbit, dog and human beings show intermediate susceptibility while rats are resistant.

Mechanism:

The cell types of greatest importance in the production of anaphylactic reaction are the mast cells. They are normally present in submucosal layers of respiratory tract, gastrointestinal tract, skin and vascular endothelium. Basophils of blood also participate in this reaction.

Antibody Involved in Anaphylaxis:

Reaginic antibodies possessing specific configuration, which is complementary fit for specific receptor size on tissue cell and having a strong affinity for tissue cells are responsible for anaphylaxis. These antibodies belong to the class IgE. The IgG antibodies can also act as reagins in the guinea pig and mouse. The contribution of IgG in human anaphylaxis is not known.

Steps involved in the Mechanism of Anaphylaxis:

The first step is the synthesis of antibodies capable of binding to mast cells and basophils. For this, an antibody must possess a specific configuration, which is complementary fit for specific receptor site known a Fc ER (Fc receptor of IgE) on mast cells and basophils. The IgE antibodies coat the mast cells and basophils with the help of Fc region (CH-3 and CH-4 domains) and bind specifically to FcER sites on the mast cell surface. The second step in anaphylaxis is the combination of the cell fixed antibodies (Fab fractions) with specific antigen of shocking dose, bridging the gap between adjacent antibody molecules on cell. This interaction increases the permeability of the cells to calcium ions and cause degranulation through proteolytic enzymes, which leads to the release of pharmacologically active substances present in the granules (Fig. 8.1).

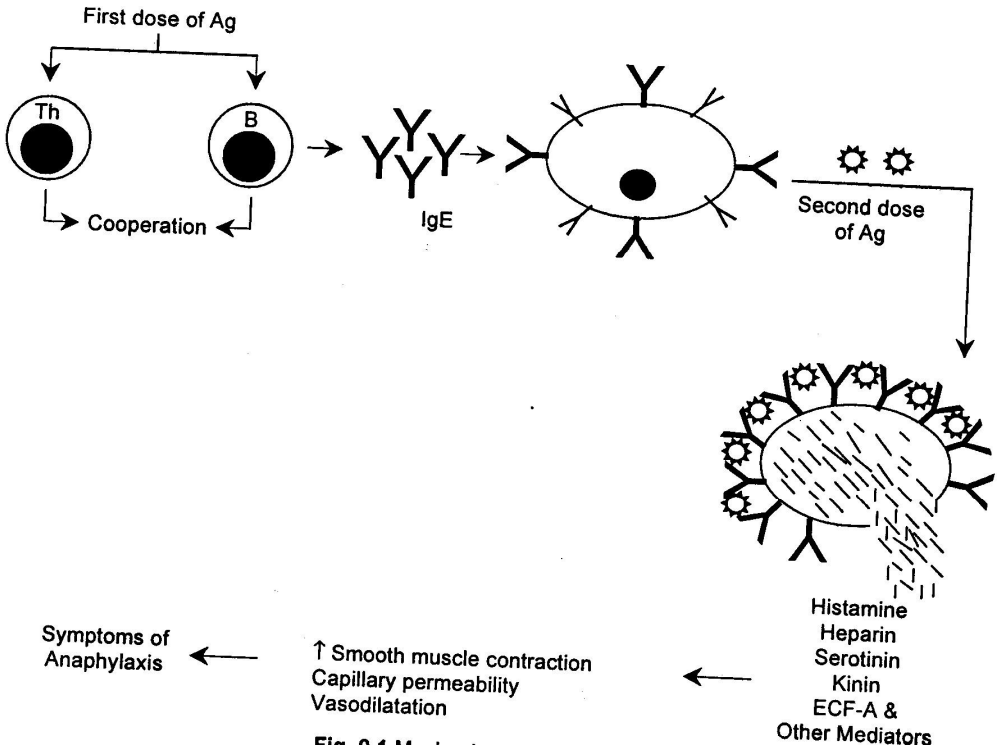

Fig-8.1: Mechanism of Anaphylaxis

Pharmacological Mediators of Anaphylaxis: There are two types of mediators

- Primary mediators are the preformed contents of mast cell and basophil granules. These include histamine, serotonin, eosinophil chemotactic factor of anaphylaxis, neutrophil chemotactic factor of anaphylaxis, heparin and various proteolytic enzymes.
- Secondary mediators are newly formed upon stimulation by mast cells, basophils and other leucocytes. These include slow reactive substance of anaphylaxis, prostaglandins and platelet activating factors.

Other Mediators: These include the anaphylatoxin released by complement activation and kinins formed from kininogen present in plasma (plasma globulins). These are other biologically active substances in addition to the mediators released by mast cells and other leucocytes.

Nature and Functions of Primary Mediators:

i) **Histamine:** Formed by decarboxylation of histidine present in the granules of mast cells, basophils and platelets. Induce smooth muscle contraction in tissues and organs such as intestine, uterus and especially the bronchioles in man and guinea pigs. Also cause a marked increase in capillary permeability and is a potent vasodilator.

ii) **Serotonin (5-hydroxy tryptamine):** Formed by decarboxylation of tryptophan. Causes bronchial and ileal smooth muscle contraction in guinea pig. Also increases capillary permeability and vasoconstriction. It is main mediator of anaphylaxis in rats and mice and does not play any significant role in human beings.

iii) **Heparin:** An acidic mucopolysaccharide stored in mast cells and basophils. It can block complement cascade, inhibit coagulation and fibrinolysis. Contribute to anaphylactic reaction in dogs, but apparently not in human beings.

iv) **Eosinophil chemotactic factors of anaphylaxis (ECFA):** Acidic tetra peptide released by mast cells and basophils. Chemotactic for eosinophils - probably contribute to the eosinophilia. Also enhance C3b activity and cause antibody and complement dependent damage to some parasites.

Nature and Functions of Secondary Mediators

i) **Slow Reacting Substance of Anaphylaxis (SRS-A):** An acidic lipid (leukotrienes). Acts on smooth muscles of large blood vessels and bronchi. It is more potent bronchoconstrictor than histamine. Its action is slow, prolonged and is not inhibited by antihistamines. Predominant pharmacological mediator in human asthma.

ii) **Prostaglandins and Thromboxanes:** Derived from arachidonic acid formed from disrupted cell membranes of mast cell and other leucocytes. Prostaglandin F2a and thromboxane are transient bronchoconstrictors. Prostaglandin E2 is a bronchodilator.

iii) **Platelet Activating Factor (PAF):** Lipid generated and released by basophils and mast cells. Cause aggregation of platelets and release of preformed serotonin (vasoactive amine).

Nature and Functions of Other Mediators:

Kinins: Polypeptides produced by the action of proteolytic enzymes on kininogen. Cause smooth muscle contraction, increased vascular permeability, vasodilatation and pain. The best known kinin is bradykinin. Its role in human anaphylaxis is not known.

Types of Anaphylaxis:

Systemic Anaphylaxis:

This is a condition of an acute shock usually terminating in death following the injection of an antigen into a previously sensitized animal. Death occurs due to suffocation from contraction of muscles in the walls of bronchioles.

Passive Systemic Anaphylaxis:

The animal can be passively sensitized to systemic anaphylaxis by injecting serum from another already sensitized animal and then the animal can be shocked by antigen that is used for sensitization. The symptoms are same like systemic anaphylaxis.

Systemic Anaphylaxis Is Man:

Systemic anaphylaxis in man is rare. Man develops systemic anaphylaxis following - Insect bite such as bee or wasp stings; antitoxic serum such as antitetanus (ATS), antidiphtheritic (ADS) or antigasgangrene serum (AGS) and Antibiotics such as penicillin. Clinical features - Lung is the principal shock organ in man hence the symptoms are - severe respiratory distress due to bronchiolar construction with vascular collapse; Laryngeal edema; Acute hypotension; Loss of consciousness and finally death.

Cutaneous Anaphylaxis (Local Anaphylaxis):

By intra-dermal injection of antigen into actively sensitized host, a local wheal and flare reaction may be produced. The wheal is a pale, central area of puffiness due to edema, which is surrounded by a flare caused by hyperemia and subsequent erythema. The reaction can be used as skin test for testing hypersensitivity and to identify the allergen responsible for atopy. (In highly sensitized individuals, the skin test may lead to systemic anaphylaxis terminating in death; hence the test should be carried out by taking all precautions).

Passive Cutaneous Anaphylaxis (PCA):

The cutaneous anaphylaxis may also be induced passively by the intra-dermal injection of antibody and the local wheal and flare reaction may then be demonstrated by intravenous injection of antigen with dye such as Evan's blue after 4-24 hr. Evan's blue is able to combine with serum protein. At the sensitized site, the combination of antigen with antibody increases capillary permeability and vasodilatation and permits the escape of protein dye complex at the site of intra-dermal injection that results in a immediate bluing. PCA can be used as an extremely sensitive in vivo method for detection of antibodies. PCA reaction conducted on human being is known as Prausnitz-Kustner (PK) reaction.

Local Anaphylaxis in Man (Atopy):

Hypersensitive states in which a person reacts with substances encountered during the course of everyday life are known as atopy (meaning out of place or strangeness) or idiosyncrasies. The term atopy was coined by Coca (1923). About 10% of the population suffer from atopy to allergens such as grass pollen, animal dander, mites in house, dust, food, etc. (allergens). Contact of allergen with cell bound IgE in the bronchial tree, the nasal mucosa, the conjunctival tissues, intestine or skin releases pharmacologically active

mediators and produces symptoms of Asthma, Hay fever (allergic rhinitis), Conjunctivitis, Gastrointestinal symptoms, Dermatitis, Urticaria in persons allergic to food such as strawberry.

Reactions occur at the site of entry of the Ag, e.g., inhalation of allergen affects lungs, leading to asthma but asthmatic symptoms may also be caused by the ingestion of the allergen, which is then carried to the respiratory tract via blood.

Mechanism:

The Abs responsible for atopy are known as reagins. These are IgE type of antibodies, heat labile, inactivated at 56^{o}C in 2-4 hr. Atopic sensitivity is due to excessive production of IgE antibodies and often associated with a deficiency of IgA. The lymphocytes responsible for synthesis of IgA and IgE are parallely distributed in the respiratory and intestinal sub mucosa. In normal individuals, the antigen interacts with IgA producing lymphocytes and hence the IgE producing lymphocytes do not come in contact of antigens. When IgA is deficient, an Ag causes massive stimulation of IgE producing lymphocytes, leading to overproduction of reagins responsible for the clinical expression of atopic reactions.

Acute or Temporary Desensitization:

It is a specific desensitization with the help of a series of carefully graded injections of the serum beginning with minute doses for 6-8 hrs. and then the full dose can be administrated without danger. This desensitization is short lasting.

Prolonged Desensitization:

Longer lasting desensitization can be achieved by giving repeated injections of antigen weekly or biweekly in increasing amounts. This results in production of blocking antibodies (IgG), which combine with allergens and prevent their contact with cell fixed IgE. An alternative method is an injection of allergen with oil adjuvant (depot therapy). In this case, desensitization occurs due to continuous production of IgG, which is the result of slow and continuous release of allergen from injection site.

Anaphylaxis in vitro:

Isolated tissues such as uterus or ileum from sensitized guinea pigs, held in a bath of ringer solution or isotonic fluid will contract vigorously on addition of the specific antigen. This is known as Shultz-Dale phenomenon. Tissues from normal animals can be passively sensitized by treatment with serum from sensitized animal.

Anaphylactoid Reaction:

It is a type of reaction that clinically resembles anaphylactic shock. It develops following the intravenous injection of peptone, trypsin, heavy metal salts, starch, or polysaccharide. Its clinical resemblance is due to the same chemical mediators participating in both the reactions. This reaction has no immunological basis and occurs non-specifically due to the activation of the alternative complement pathway and release of anaphylatoxin.

8.3. TYPE II-CYTO TOXIC REACTION

Reactions of this type are initiated by antigenic component, which is either part of tissue cell or closely associated with a cell, e.g., microbial product or a drug attached to cell

wall. Combination of this Ag with IgG or IgM Ab damages that cell by promoting contact with phagocytic cells either by – reduction in surface charges or by opsonic adherence directly through Fc or by immune adherence through bound C3. Cell death may also occur through activation of full complement pathway up to C8 and C9 (Fig. 8.2).

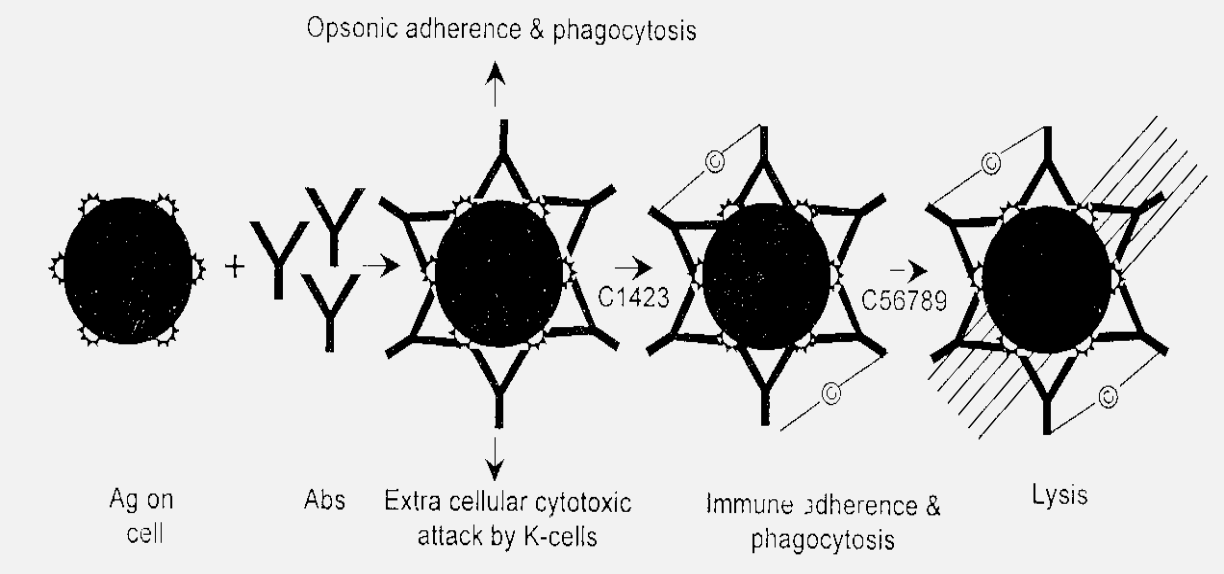

Fig-8.2: Mechanism of Cytotoxic Reaction

Examples:

Isoimmune reactions such as ABO transfusion reactions, erythroblastosis foetalis. Autoimmune Reactions, Lysis of RBCs caused by antierythrocyte Abs in autoimmune anaemias. Lysis of platelets by autoantibodies against platelets in autoimmune thrombocytopenia. Lysis of neutrophils by autoantibodies in agranulocytosis. Drug reactions, Drugs such as penicillin, phenacetin, quinidine, sedormid, sulfonamides, thiazide, chlorpropamide attach to the surface of cells such as RBCs, neutrophils or platelets causing alterations in the surface antigenicity that initiate Ab synthesis. Combination of these Abs leads to Cytotoxic or Cytolytic reactions. Classical example is thrombocytopenic purpura produced by sedormid (sedormid purpura). In certain bacterial reactions such as *Salmonella* and mycobacterial infections - their products coat the surface of RBCs and induce Ab synthesis and subsequent infection by same or related pathogen cause haemolysis.

8.4. TYPE III-IMMUNE COMPLEX MEDIATED HYPERSENSITIVITY:

It is a type of antibody mediated hypersensitivity reaction characterized by deposition of Ag-Ab complexes in tissues particularly on endothelial surfaces. The Ag-Ab complex formation in and around small blood vessels may result in acute inflammatory reactions and sometimes mechanical blockage of the vessels causing interference with blood supply to surrounding tissues. Ag-Ab complex may activate complement. If activated, complement will cause release of anaphylatoxin that causes histamine release with vascular permeability changes. Activation of complement also results in aggregation of polymorphonuclear leucocytes (PML), which start phagocytosis of the immune complexes, this in turn causes the release of proteolytic enzymes and polycationic proteins from the granules of PML, which

increase from the granules of PML, which increase capillary permeability (Flow chart). Activation of complement and massive infiltration by PML and attraction of platelets lead to inflammation and tissue injury.

There are mainly two types of type III reactions - Arthus reaction, and Serum sickness.

Arthus Reaction:

It is an inflammatory reaction with particular involvement of blood vessels, which may occur in the tissues of sensitized animals as a result of local Ag-Ab reaction. It is a type III reaction, first described by Arthus in 1903. He observed that repeated subcutaneous injections of soluble Ag (horse serum) into rabbits produced high level of precipitating Abs (hyper immunization). Injection of same Ag intradermally or subcutaneously into hyper immunized rabbit produced an erythema, edema, induration and haemorrhagic necrosis, which reaches a peak in 3-6 hr. This type of reaction is known as Arthus reaction.

Mechanism:

It is a local reaction that occurs at local site, i.e., in and around the walls of small blood vessels. Reaction occurs when an appreciable level of Abs, mainly IgG type, are produced by hyper immunization. IgG reacts with Ag - forms complex.

Ag-Ab complexes diffuse into vessel walls

↓

Activate complement

↓

Chemotactic complement components attract neutrophils and induce intravascular clumping of platelets.

↓

Neutrophils ingest complexes and release lysosomal enzymes, which damage neighboring cells and cause

necrosis and inflammatory reaction.

↓

Aggregation of platelets causes vascular occlusions

leading to ischemic necrosis of blood vessels.

Arthus Reaction-Special Feature:

Activation of Complement by classical or alternative pathway is must for Arthus

reaction. Arthus reaction can be passively transferred with sera containing Abs. In man, Arthus like reaction is produced in individuals who have received several injections of antitoxic sera and insulin.

Serum Sickness:

It is a systemic form of type III reaction. The term serum sickness was first applied by Von Pirquet and Schick in 1905 to a condition that may occur in man following the injection of horse serum and sometimes following the injection of drugs such as sulfonamide, penicillin, streptomycin and organic arsenicals. The drugs mentioned above are not antigenic but form antigenic complexes by combination with plasma or tissue proteins. A single dose of Ag is sufficient to produce the reaction. It can serve both as sensitizing dose as well as shocking dose. The symptoms appear after 7-14 days following a single injection of a high concentration of foreign serum such as diphtheria antitoxin or antitetanus serum.

The symptoms are:

Fever, Lymphadenopathy, Splenomegaly, Arthritis, Glomerulonephritis, Endocarditis, Vasculitis, Urticaria, Abdominal pain, Nausea and vomiting.

The Pathogenesis - is the formation of immune complexes, which get deposited on endothelial lining of blood vessels at the site of lesions in various parts of the body causing inflammatory infiltration. The damage to tissue produced is same as in Arthus reaction. The disease is self-limited. With continued rise in Ab production, Ag-Ab complexes become larger in size and more susceptible to phagocytosis and immune elimination.

8.5. TYPE IV-DELAYED HYPERSENSITIVITY:

This term is applied to a group of hypersensitivities in which there is an appreciable delay between the exposure to Ag and the development of symptoms. It may be defined as an increased reactivity to specific Ags mediated by T-cells and not by Abs. Delayed hypersensitivity is demonstrated by a cutaneous reactivity, which usually becomes visible after 24-48 hrs following introduction of Ag. The cutaneous reactions are inflammatory and indurated type involving lymphocytes and macrophages and not wheal and flare type as seen in anaphylaxis. The reaction is inducted by sensitized T-cells which, on contact with the specific Ag, release lymphokines that cause biological effects on leucocytes, macrophages, and tissue cells. Passive transfer-delayed hypersensitivity is a cellular phenomenon hence cannot be transferred passively by serum. Passive transfer to a normal recipient is possible with the help of sensitized T-lymphocytes from a sensitized donor or with the help of transfer factor. It occurs in two main clinical forms- tuberculin (infection) type and contact dermatitis type.

Tuberculin (Infection) Type:

It develops as a result of infection with tubercle bacillus and is demonstrated by tuberculin reaction. When a small dose of Ag (tuberculin) 1-5 TU (1 TU, equivalent to 0.01 mg OT or 0.00002 mg PPD) is injected intradermally in an individual sensitized to tubercular protein by previous infection or immunization, an erythema and indurated swelling develop gradually, which reach maximal intensity and size after 24-72 hrs and then slowly regress.

Histologically, the reaction is characterized by an infiltration of injection site with mononuclear cells, mainly lymphocytes and 10-20% macrophages. These inflammatory cells (macrophages and lymphocytes) may be seen around blood vessels and nerves. Tuberculin type of hypersensitivity is also developed in many other infections such as leprosy, brucellosis, lymph granuloma venereum and in most fungal, viral, and parasitic infections, especially when infection is sub-acute or chronic and pathogen is intracellular.

Contact Dermatitis Type:

Delayed hypersensitivity, sometimes, develops as a result of exposure of skin to a variety of substances such as: Drugs like penicillin, sulfonamide, organic arsenicals, etc. Metals like nickel, cobalt, chromium, etc. Chemicals like picryl chloride, dinitrochlorobenzene, formaldehyde, iodine, dyes, cosmetics, soaps, etc. These substances are not antigens but act as haptens. After absorption in skin these substances covalently combine with skin proteins and become antigenic. Contact of these substances in a sensitized individual leads to contact dermatitis. The lesions vary from macules and papules to vesicles that breakdown leaving behind raw weeping areas typical of acute eczematous dermatitis. The cutaneous inflammation is similar to tuberculin type. Histologically, there is a mononuclear cell infiltration in the upper layers of the skin and around hair follicles. In this, it may be possible to identify the substance responsible for sensitivity by patch test in which the suspected substance is applied to skin under an adherent dressing and observed for itching (4-5 hr) and local reaction (erythema to vesicle or blister formation in 24-28 hr).

8.6. SUMMARY:

Hypersensitivity reactions are broadly categorized into two types depending on the time taken for the reaction – namely early and delayed types. Coombs and gel classified them in to four types. Type 1 - This type of reactions mediated by Ig E molecules are referred to as allergic reactions. Type I is an immediate type of hypersensitivity reaction, involving IgE antibodies. In this type, the synthesized IgE will bind over to the mast cell and basophils and result in degranulation leading to the release of pharmacologically active substances. In type II cytotoxic reactions, IgG or IgM antibodies mediated cell death occurs through the activation of complement pathway. In type III reactions, Ag-Ab complexes will get deposited in the tissues, particularly on the endothelial surfaces. In delayed type reactions, the activated T-cells release lymphokine which activates the macrophages and results in inflammation.

8.7. TECHNICAL TERMS:

Antibody mediated - Anaphylaxis, Cell toxicity, Immune complex mediated reaction, Cell mediated hypersensitivity, Serum sickness.

8.8 SELF ASSESSMENT QUESTIONS:

1) Write in detail about types of anaphylactic reactions.
2) Give an account on type II and III hypersensitive reactions.
3) Write an essay on type IV type of hypersensitive reactions.

8.9. SUGGESTED READINGS:

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LESSON-9

BRIEF ACCOUNT ON HYPERSENSITIVITY DISEASES

9.0 OBJECTIVE:

- To enrich the students' knowledge on the diseases caused due to hypersensitivity reactions.

STRUCTURE:

9.1. INTRODUCTION:

Hypersensitivity type 1-related diseases include common allergic conditions like allergic rhinitis, allergic asthma, anaphylaxis, food allergies, and urticaria (hives) and angioedema. These conditions are mediated by the IgE antibody and mast cells, and are also referred to as atopic disorders. Type 2 hypersensitivity is related to autoimmune diseases like myasthenia gravis, Graves' disease, and autoimmune hemolytic anemia, as well as non-autoimmune conditions such as mismatched blood transfusion and hemolytic disease of the newborn. These conditions involve antibodies that target specific cells or tissues, leading to their destruction. Type III hypersensitivity creates diseases by forming damaging immune complexes, which can lead to conditions like systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), serum sickness, and several types of glomerulonephritis. These reactions occur when antibodies bind to antigens, forming complexes that deposit in tissues and trigger an inflammatory response, causing damage to organs like the skin, joints, and kidneys. Type IV hypersensitivity is related to autoimmune diseases like Type 1 diabetes and multiple sclerosis, contact dermatitis from things like poison ivy, transplant rejection, and certain severe drug reactions such as Stevens-Johnson syndrome and Toxic Epidermal Necrolysis. These are cell-mediated, delayed-type reactions that involve T-cells and are not antibody-driven.

9.2. HYPERSENSITIVITY TYPE I DISEASES

Hypersensitivity type1-related diseases include common allergic conditions like **allergic rhinitis**, **allergic asthma**, **anaphylaxis**, **food allergies**, and **urticaria (hives) and angioedema**. These conditions are mediated by the IgE antibody and mast cells, and are also referred to as atopic disorders.

9.2.1 Allergic Rhinitis

Allergic rhinitis, also known as hay fever, is an allergic reaction causing inflammation of the nasal passages due to an overreaction to allergens like pollen, dust mites, and animal dander. Symptoms include sneezing, a runny or stuffy nose, itchy eyes and throat, and watery eyes. Post-nasal drip (mucus dripping down the back of the throat), headache, sinus pressure, fatigue, difficulty in sleeping, and poor concentration in severe cases. Allergens like pollen, mold spores, dust mites, animal dander, cockroach waste etc. triggers and cause the disease.

Diagnosis:

Diagnosis is mainly based on the review of the symptoms, clinical history and potential triggers by physician. A physical examination is performed. Allergen skin testing can help to identify specific allergens that trigger the symptoms.

Treatment and Management:

The first step in treatment focuses on identification and avoiding allergens or triggers avoidance. Use of corticosteroid nasal sprays is often the most effective first-line treatment for reducing inflammation and congestion. Oral antihistamines can help manage symptoms like sneezing and itching. Nasal rinsing with saline water can provide temporary relief and help in clearing the nasal passages. Allergy shots (allergen immunotherapy) can be an effective treatment for people whose symptoms are not controlled by other methods or who prefer it. Depending on the severity, other medications may be considered, including leukotriene receptor antagonists.

Complications:

Poorly controlled allergic rhinitis can lead to chronic inflammation and complications such as: Sinusitis (sinus infections), Otitis media (ear infections), Obstructive sleep apnea, Upper respiratory tract infections.

9.2.2 Allergic Asthma:

Allergic asthma is a chronic condition where breathing in an allergen triggers an allergic reaction where the airways become inflamed and narrow and causing symptoms like wheezing, coughing, and shortness of breath. It is the most common type of asthma, and common indoor triggers viz., dust mites, animal dander, and mold and outdoor allergens including pollen and mold spores. The body mistakenly identifies these harmless substances as threats, leading to the release of chemicals like IgE, which causes the airways to swell and produce excess mucus. Other irritants like respiratory infections, cigarette smoke, cold air, strong scents, and physical activity can also trigger attacks in people with allergic asthma.

Symptoms:

- Asthma symptoms: Wheezing, coughing, shortness of breath, and chest tightness.
- Allergic symptoms: These can occur alongside asthma symptoms and may include a runny or stuffy nose and itchy eyes.

Diagnosis:

A doctor will review the medical history and may perform a skin prick test to identify specific allergies. Lung function tests and measuring inflammation in the airways (e.g., exhaled nitric oxide) are also used for diagnosis.

Management and Treatment:

Management involves avoiding known triggers, using a quick-relief inhaler for acute symptoms, and taking daily medication to reduce inflammation, with potential options like allergy immunotherapy for long-term tolerance. Stay indoors with windows closed when pollen counts are high, use air conditioners with clean filters, and use allergen-proof covers on mattresses and pillows are the measures to avoid the triggers.

Medications:

Long-Term Control: Daily maintenance medications, such as corticosteroids, help reduce inflammation and prevent symptoms.

Quick-Relief (Rescue) Inhaler: Used to quickly open airways during an asthma attack.

Allergy Immunotherapy: In some cases, this treatment can help the body become more tolerant of specific allergens over time.

Asthma Action Plan: Create a personalized plan with the doctor that outlines your triggers, daily medication, and what to do if your symptoms worsen. If the symptoms are severe, breathing worsens rapidly, or quick-relief inhaler isn't working effectively, and then seeks immediate medical treatment.

9.2.3 Anaphylaxis:

Anaphylaxis is a severe, rapid, life-threatening and generalized allergic reaction where the immune system releases a flood of chemicals. Anaphylaxis can occur rapidly after exposure to triggers like food, medication, or insect venom. It can affect multiple body systems, causing a drop in blood pressure and narrowing of the airways. The most common and main triggers are certain foods (e.g., peanuts, nuts, shellfish, milk, eggs), some medications (e.g., antibiotics), insect venom (e.g., stings from bees or wasps), latex, exercise (sometimes in combination with food).

Symptoms:

Symptoms include breathing difficulties, wheezing, tightness in the throat, swelling, hives, rash, itching, nausea, vomiting, diarrhea, abdominal cramps, anxiety, confusion, rapid or weak pulse, drop in blood pressure and fainting.

The immediate treatment is an epinephrine injection, followed by emergency medical care, and long-term management involves identifying triggers, carrying an epinephrine auto-injector, and having an action plan. Seek emergency medical assistance immediately.

Administer Epinephrine:

If available, use an epinephrine auto-injector. It is the first-line treatment and can be repeated if necessary. The injection should be given into the outer mid-thigh for fast absorption.

Lie the Person Down:

Have the person lie flat to improve blood flow to the brain. If they are having breathing difficulties, they can sit up, but they should lie down if they feel faint.

Oxygen and Other Medications:

The person should receive oxygen, and other medications like antihistamines or corticosteroids may be given by medical professionals.

Long-Term Management:

Avoid Triggers: Identifying and avoiding known triggers is crucial.

Carry Epinephrine: Always carry at least two epinephrine auto-injectors and know how to use them.

Anaphylaxis Action Plan: Develop an individualized action plan with a healthcare provider and share it with family, friends, and school personnel.

See an Allergist: Referrals to an allergist are recommended to help confirm diagnoses and develop long-term strategies.

9.2.4 Urticaria and Angioedema:

Urticaria, or hives, is raised, itchy skin swelling, while angioedema is deeper swelling in the skin and tissues, often involving the face, lips, and throat. Both are often caused by a histamine release and can occur together, although urticaria is typically itchy and transient, and angioedema is often painful and slower to resolve. Treatment mainly focuses on relieving symptoms with antihistamines for urticaria, but life-threatening swelling requires immediate medical attention.

Urticaria (Hives):

Raised, red, itchy welts on the skin caused by swelling in the superficial dermis. Can be discrete or merge into large patches, with central clearing to form a "wheal and flare" pattern. They can have serpiginous (wavy) borders. Typically very itchy, but can sometimes have a burning sensation. Individual lesions usually resolve within 24 hours and leave no bruising or scarring.

Angioedema:

Swelling that occurs in the deeper layers of the skin and subcutaneous tissues. Localized, soft tissue swelling, often in the face, around the eyes and mouth, hands, feet, or throat. Less itchy and more often described as a burning or painful sensation. Can take longer to resolve, sometimes up to 72 hours.

Key Differences and Similarities:

Feature	Urticaria (Hives)	Angioedema
Level	Superficial dermis	Deep dermis and subcutaneous tissue
Appearance	Raised, red, itchy welts	Deeper, localized swelling
Sensation	Intense itching	Burning or pain, not typically itchy
Duration	Individual lesions < 24 hours	Up to 72 hours

Urticaria and angioedema often appear together, affecting about 40% of patients. Can be classified as acute (less than 6 weeks) or chronic (more than 6 weeks). Can be triggered by allergies, infections, or even physical factors like pressure or heat. Swelling of the throat or tongue can block the airway and is a medical emergency requiring immediate attention. The primary treatment for the itch and swelling is often a non-sedating antihistamine. Avoidance of triggers like NSAIDs (aspirin, ibuprofen) is also recommended, as they can worsen symptoms.

Other Causes:

Angioedema can have other causes, such as certain medications (e.g., ACE inhibitors) and genetic conditions, which require different treatments.

Atopic Dermatitis:

A topic dermatitis is a chronic inflammatory skin condition causing dry, itchy, and red skin that can also appear brown, purple, or gray. It often begins in childhood, is not contagious, and is associated with other allergic conditions like asthma and hay fever. Management involves frequent moisturizing, gentle bathing, identifying and avoiding triggers, and may include topical corticosteroids, and in severe cases, other medications or phototherapy.

Symptoms and Appearance:

Intense itchiness ("the itch that rashes"), dry skin, redness, and scales. Can present as red patches on lighter skin tones or darker brown, purple, or gray patches on darker skin tones. Small, fluid-filled bumps may appear on skin, which can leak fluid and then crust over. In chronic cases, the skin can become thickened and leathery (lichenification). Distribution is age-dependent. In infants, it often affects the face and scalp, while in older children and adults, it commonly appears in the folds of the elbows and knees.

Causes and Triggers:

A defect in the skin barrier that makes the skin more susceptible to inflammation from irritants and allergens. Can vary but may include certain foods, dust mites, pollen, weather changes, sweat, stress, and harsh soaps.

Management and Treatment

Daily Care:

- Moisturize frequently to repair the skin barrier and lock in moisture.
- Bath with gentle cleansers and avoid very hot water.
- Wear soft, cotton clothing to minimize irritation.

Medical Treatments:

Topical Corticosteroids: A first-line treatment for flare-ups to reduce inflammation.

Topical Immunomodulators: Alternative for sensitive areas like the face.

Antihistamines: Can help with itching.

Antibiotics: May be prescribed if a bacterial infection develops due to scratching.

Phototherapy: Used for disease unresponsive to topical therapies.

Systemic Treatments: For severe, refractory cases.

Associated Conditions:

Atopic dermatitis is often part of a larger group of allergic conditions known as the "atopic march." People with atopic dermatitis are more likely to develop other atopic diseases, including:

- Food allergies
- Asthma
- Allergic rhinitis (hay fever)

9.2.5 Allergic Conjunctivitis

Allergic conjunctivitis is an inflammation of the eye's lining caused by an allergic reaction to substances like pollen, dust mites, or pet dander. Key symptoms include intense itching, redness, watery or stringy discharge, and eyelid swelling. It is not contagious and can be categorized into types such as seasonal, perennial, and the more severe vernal keratoconjunctivitis, and is typically managed with cold compresses, lubricants, and anti-allergy eye drops.

It is an inflammatory eye condition caused by an allergic reaction, not an infection. Occurs when the conjunctiva (the clear membrane covering the white of the eye) reacts to allergens.

Symptoms may resemble an eye infection but are not contagious.

Common Allergens:

- **Seasonal:** Pollen from trees, grasses, and weeds (causing symptoms in spring and summer).
- **Perennial:** Year-round allergens such as dust mites, pet dander, and mold spores.

Symptoms:

Intense itching or burning; Redness; Watery or stringy discharge; Swollen, puffy eyelids; A gritty feeling in the eyes; Sensitivity to light; Blurred vision.

Types:

- **Seasonal Allergic Conjunctivitis:** Symptoms occur during specific seasons, usually from airborne allergens like pollen.
- **Perennial Allergic Conjunctivitis:** Symptoms are present year-round, often triggered by indoor allergens like dust mites or pet dander.
- **Vernal Kerato Conjunctivitis:** A more severe type that reappears seasonally, often in males aged 5-20, and is associated with other allergic conditions like eczema and asthma.

Management and Relief:

Avoid Rubbing the Eyes: This can worsen symptoms.

Cold Compresses: Apply cold packs to the eyes to relieve swelling and discomfort.

Lubricating Eye Drops: Can help wash out allergens from the eye.

Prescription Medication: A doctor may prescribe anti-allergy eye drops or oral medications for more severe cases.

Address Systemic Allergies: Treating coexisting nasal or other allergies can help reduce eye symptoms.

9.3. HYPERSENSITIVITY TYPE II DISEASES:

Type 2 hypersensitivity is related to autoimmune diseases like **myasthenia gravis, Graves' disease, and autoimmune hemolytic anemia**, as well as non-autoimmune conditions such as **mismatched blood transfusion** and **hemolytic disease of the newborn**. These conditions involve antibodies that target specific cells or tissues, leading to their destruction.

9.3.1 Myasthenia Gravis:

Myasthenia gravis (MG) is a chronic autoimmune neuromuscular disorder that causes fluctuating weakness in voluntary muscles. It's caused by the immune system mistakenly attacking and blocking acetylcholine receptors at the neuromuscular junction, disrupting communication between nerves and muscles. Symptoms include drooping eyelids, double vision, and weakness in the face, throat, limbs, and respiratory muscles, which worsen with activity and improve with rest. Treatment involves medications, and in severe cases, thymectomy, plasma exchange, or intravenous immune globulin.

Symptoms and Signs:

- Fluctuating weakness and muscles get weaker during activity and improve with rest.
- Drooping of eyelids in one or both eyes.
- Double or blurry vision due to weakness in eye muscles.
- Difficulty in speaking, chewing, and swallowing.
- Weakness in the neck and limbs.
- Shortness of breath in severe cases, affecting the respiratory muscles.

Pathophysiology:

- **Autoimmune attack:** The immune system produces antibodies that attack acetylcholine receptors at the neuromuscular junction.
- **Impaired nerve-to-muscle communication:** This attack prevents the neurotransmitter acetylcholine from binding to the receptors, which stops the muscle from being signaled to contract.
- **Muscle weakness:** The result is weakness and fatigue of the voluntary muscles.

Diagnosis and Treatment

Diagnosis:

Usually diagnosed through clinical examination, blood tests to look for antibodies and tests that measure nerve and muscle function.

Medication

- Acetylcholinesterase inhibitors (e.g., pyridostigmine) can help improve muscle strength.
- Immunosuppressants (e.g., prednisone) may be needed for more severe cases.

Surgery

- Thymectomy (surgical removal of the thymus gland) can improve symptoms in many patients.

Other Therapies

- Plasma exchange (plasmapheresis): Removes harmful antibodies from the blood.
- Intravenous immune globulin (IVIG): An infusion of antibodies that temporarily improves symptoms.

Emergency Treatment

- A myasthenic crisis (severe breathing or swallowing problems) requires urgent hospital care, sometimes with a ventilator.

Risk Factors

- **Age:** Most commonly diagnosed in women under 40 and men over 60.
- **Genetics:** Certain human leukocyte antigens (HLAs) are associated with an increased risk.
- **Other Autoimmune Diseases:** MG is more common in people with other autoimmune conditions like Graves' disease or rheumatoid arthritis.

9.3.2 Autoimmune Hemolytic Anemia

Autoimmune hemolytic anemia (AIHA) is a rare disorder where the immune system mistakenly attacks and destroys the body's own red blood cells, leading to anemia. It is characterized by a shortened red blood cell lifespan and can be caused by an underlying illness or occur on its own. Symptoms often include fatigue, shortness of breath, and a rapid heartbeat, and it is diagnosed using blood tests and a positive Coombs test. It is a condition where the body produces autoantibodies that target and destroy its own red blood cells. This leads to a reduced number of red blood cells, which are responsible for carrying oxygen throughout the body. The red blood cell lifespan can drop from the normal 100–120 days to just a few days.

Causes:

Primary AIHA: Occurs with no known underlying cause.

Secondary AIHA: Caused by an underlying condition, such as: Infections (e.g., Epstein-Barr virus, HIV), Autoimmune disorders (e.g., lupus, rheumatoid arthritis), Lymphoproliferative disorders and blood cancers, Certain medications

Types:

- **Warm AIHA:** Autoantibodies react to red blood cells at body temperature.
- **Cold AIHA (Cold Agglutinin Disease):** Autoantibodies react to red blood cells in colder temperatures. Symptoms may include bruising, redness, and pain in the hands and feet upon exposure to cold.

Symptoms: Fatigue and weakness; Shortness of breath; Rapid heart rate; Pale or yellowish skin (jaundice); Dizziness; Dark-colored urine; Enlarged spleen and liver; Chest or back pain.

Diagnosis:

Diagnosis involves laboratory tests that show anemia and reticulocytosis (a high number of immature red blood cells).

A positive direct antiglobulin test (or Coombs test) is key, which detects antibodies and/or complement proteins on the red blood cells.

The pattern of the reaction on the Coombs test can help determine the type of AIHA.

Treatment:

Treatment is aimed at the underlying cause if there is one (e.g., stopping a medication, treating an infection or cancer).

For warm AIHA, first-line treatment is often corticosteroids, such as prednisone, which slowly tapered over several months.

Avoiding cold is important for cold AIHA.

Blood transfusions may be needed to treat the anemia, but finding compatible blood can be challenging.

9.3.3 Goodpasture Syndrome

Goodpasture syndrome is a rare, life-threatening autoimmune disorder where the body's immune system attacks the lungs and kidneys by producing antibodies against the glomerular basement membrane (GBM). It causes kidney damage and inflammation (glomerulonephritis) and lung bleeding (pulmonary hemorrhage). Symptoms include shortness of breath, coughing up blood, and fatigue, and if untreated, it can lead to kidney failure and death. Treatment is urgent and involves medications and a form of blood transfusion to remove the harmful antibodies.

Characteristics:

Autoimmune Disorder: The immune system mistakenly creates antibodies that attack the body's own tissues, specifically the collagen in the GBM of the lungs and kidneys.

Pulmonary-Renal Syndrome: This term describes the simultaneous involvement of both the lungs and kidneys.

Prognosis: It is considered a serious condition with a poor prognosis if not treated promptly.

Symptoms

Lung Symptoms - Coughing up blood (hemoptysis); Cough; Shortness of breath; Fatigue and weakness.

Kidney Symptoms - Proteinuria (protein in the urine); Edema (swelling); Loss of appetite.

Causes

Autoimmune Reaction: The exact cause is unknown, but it is triggered by an autoimmune response.

Potential Triggers

Environmental factors, such as exposure to tobacco smoke, certain chemicals (like Paraquat or dry cleaning solvents), or viral respiratory infections

Genetics and Certain medications

Diagnosis:

Blood and Urine Tests: To detect the presence of antibodies and signs of kidney damage.

Immunofluorescence: This is a crucial test that can visualize a linear pattern of IgG antibodies along the glomerular basement membrane, confirming the diagnosis.

Imaging: X-rays or CT scans of the lungs to check for bleeding.

Treatment

Plasma Exchange (Plasmapheresis): A procedure to remove the harmful antibodies from the blood.

Immunosuppressive Medications: Drugs to suppress the immune system's attack on the lungs and kidneys.

Supportive Care: Oxygen therapy and medication to manage symptoms. Kidney failure may require long-term dialysis or a kidney transplant.

9.3.4 Pernicious Anemia: Pernicious anemia is an autoimmune condition causing vitamin B12 deficiency, leading to megaloblastic anemia (large red blood cells) and potential neurological damage. It occurs because the body's immune system attacks the cells that produce intrinsic factor (IF), a protein needed for vitamin B12 absorption, or attacks IF itself. Treatment involves lifelong vitamin B12 supplementation, often through injections, to bypass the absorption problem. It is an autoimmune disorder that impairs the body's ability to absorb vitamin B12 from food. Causes a deficiency in vitamin B12, which is essential for making red blood cells. Results in megaloblastic anemia, characterized by large, immature red blood cells.

Causes:

Autoimmune attack: The body's immune system mistakenly attacks the gastric parietal cells that produce intrinsic factor (IF), or it attacks the IF protein directly.

Lack of intrinsic factor: Without enough intrinsic factor, the body cannot absorb vitamin B12 from the small intestine.

Atrophic gastritis: A condition marked by chronic inflammation and thinning of the stomach lining, which can impair IF production.

Symptoms:

Anemia-related: Fatigue, weakness, and lack of energy; Shortness of breath, especially with exertion; Pale or yellowish skin; Lightheadedness

Neurological: Numbness, tingling, or a "pins and needles" sensation; Difficulty in balance or walking; Memory problems, confusion, or cognitive changes; Depression or mood swings;

Other symptoms: Sore, swollen, or red tongue; Heartburn; Diarrhea or constipation.

Diagnosis:

Blood tests to check for low vitamin B12, low hemoglobin, and large red blood cells (macrocytosis).

Testing for anti-intrinsic factor antibodies or anti-parietal cell antibodies is highly specific for pernicious anemia.

Other blood tests may include homocysteine and methylmalonic acid levels, which are elevated in vitamin B12 deficiency.

Treatment and Management:

Lifelong vitamin B12 supplementation: Because the root cause is malabsorption, lifelong treatment is necessary.

Injections: Intramuscular injections are common to bypass the digestive system entirely.

High-dose oral supplements: Oral pills or nasal sprays at high enough doses can be effective for some people by forcing enough B12 into the bloodstream.

Monitoring: Regular monitoring is essential to ensure B12 levels remain adequate and to prevent complications.

9.4. HYPERSENSITIVITY TYPE III DISEASES:

Type III hypersensitivity creates diseases by forming damaging immune complexes, which can lead to conditions like systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), serum sickness, and several types of glomerulonephritis. These reactions occur when antibodies bind to antigens, forming complexes that deposit in tissues and trigger an inflammatory response, causing damage to organs like the skin, joints, and kidneys.

9.4.1 Graves' Disease:

Graves' disease is an autoimmune disorder that causes an overactive thyroid (hyperthyroidism), where the immune system attacks the thyroid gland, leading to an overproduction of thyroid hormones. Key symptoms include a rapid heartbeat, weight loss, anxiety, heat intolerance, excessive sweating, and an enlarged thyroid (goiter). It can also cause specific symptoms affecting the eyes (Graves' ophthalmopathy) and skin. Treatment options include anti-thyroid medications, radioactive iodine therapy, or surgery.

It is an autoimmune disorder where the body mistakenly attacks its own thyroid gland. It is the most common cause of hyperthyroidism. Thyroid hormones control your body's energy use, so an overactive thyroid speeds up many body functions. Graves' disease can affect more than just the thyroid, leading to eye and skin issues.

Symptoms:

Metabolic and Systemic: Rapid or irregular heartbeat (tachycardia); Unintentional weight loss, despite an increased appetite; Heat intolerance and excessive sweating; Anxiety, irritability, and nervousness; Tremors (shakiness), especially in the hands; Fatigue; Increased bowel movements or diarrhea; Sleeping difficulties.

Physical: Enlarged thyroid gland (goiter); Thinning of skin; Hair loss.

Specific to Graves' eye disease (Graves' ophthalmopathy): Bulging or protruding eyeballs; Irritation, redness, or a gritty feeling in the eyes; Watery eyes or pressure behind the eyes; Double vision, blurred vision, or vision loss in severe cases.

Specific to men: Enlarged breasts (gynecomastia).

Specific to women: Irregular or missed menstrual periods

Risk factors:

More common in women, especially those over age 30.

Family history of Graves' disease or other autoimmune disorders increases risk.

Other genetic and environmental factors are involved.

Complications:

Thyroid storm: A rare, life-threatening condition caused by a sudden worsening of hyperthyroidism.

Heart problems: Atrial fibrillation (irregular heart rhythm) or heart failure.

Bone loss: Leading to osteoporosis.

Eye problems: Damage to the optic nerve or cornea, which can cause vision loss.

Pregnancy complications: Including miscarriage or birth defects.

Treatment:

Anti-thyroid medications: Control overactive thyroid, often used for 18 months. Can be a first-line treatment, but the disease may return after stopping.

Radioactive iodine therapy: Uses a radioactive iodine dose to shrink or destroy the thyroid gland.

Surgery: Removal of all or part of the thyroid gland (thyroidectomy).

Long-term medicine: May be needed for those who cannot tolerate other treatments or have relapses.

Management during pregnancy: Requires careful monitoring and adjustment of treatment to protect both the mother and fetus.

9.4.2 Serum Sickness:

Serum sickness is a delayed immune-mediated reaction to foreign proteins, such as those in certain medications (especially antibiotics, sulfonamides, and monoclonal antibodies) or animal-derived antiserums. It causes fever, rash, and joint pain, appearing 7-21 days after exposure. Treatment involves discontinuing the offending agent, with symptom relief from anti-inflammatory drugs, corticosteroids for severe cases, and antihistamines for itching.

Causes:

Antibiotics (like penicillin, cefaclor, and sulfonamides), bupropion, barbiturates, and some monoclonal antibodies are common culprits.

Antiserums / Biological Products:

Injected animal or foreign proteins, including antithymocyte globulin and certain blood products, can trigger the reaction.

Symptoms:

Symptoms typically appear 7 to 21 days after first exposure, though they can appear sooner with a later exposure. Fever, rash (hives), and joint pain (arthralgia) are classic signs. General feeling of illness or malaise, Joint swelling, Swollen lymph nodes, Itching.

Diagnosis:

- Based on a patient's history of exposure to a potential trigger.
- Blood tests may show low platelets, low white blood cells, or high inflammatory markers.

Treatment:

Primary treatment: Stop the medication or product causing the reaction.

Symptom management:

Anti-inflammatories/NSAIDs: To help with joint pain.

Corticosteroids: Used for more severe cases to reduce inflammation.

Antihistamines: To relieve itching and rash.

9.4.3 Systemic Lupus Erythematosus (SLE)

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease where the immune system attacks the body's own tissues, causing inflammation and potential damage to almost any organ system, including joints, skin, kidneys, and the nervous system. It can range from mild to severe and is characterized by flares, with common symptoms like fatigue, joint pain, and rash (including the classic "butterfly" rash). Diagnosis involves a combination of symptoms, physical exam, and blood tests, with treatment focusing on managing symptoms and preventing organ damage, often using medications like hydroxychloroquine, corticosteroids, and other immune-suppressing drugs.

Description and Pathophysiology:

The body's immune system mistakenly attacks its own healthy cells and tissues.Can affect various parts of the body, including joints, skin, kidneys, brain, lungs, and blood cells. Manifestations range from mild to severe and life-threatening, with a relapsing and remitting course (flares and periods of stability).

Common Signs and Symptoms:

Fatigue: Extreme tiredness is a very common symptom.

Joint pain, stiffness, and swelling: Often affects the small joints of the hands, feet, and knees.

Skin rashes: A common manifestation, such as the characteristic butterfly or malar rash across the cheeks and nose, or discoid rashes that cause scarring.

Photosensitivity: Skin rash triggered by sunlight exposure.

Oral or nasal ulcers: Sores in the mouth or nose.

Other symptoms: May include hair loss, fever, chest pain, shortness of breath, headaches, seizures, or kidney problems.

Diagnosis:

Combination of factors: Diagnosis relies on a combination of symptoms, physical exam, and blood tests.

Blood tests: A key diagnostic tool includes checking for antinuclear antibodies (ANA), which are present in over 95% of people with SLE. Other tests look for specific antibodies (like anti-dsDNA, anti-SM) and markers of inflammation (like complement levels).

Tissue biopsy: In some cases, a biopsy of affected tissue (such as the kidney) may be used to confirm the diagnosis.

Treatment:

To manage flares, prevent organ damage, and improve quality of life.

Medications:

Hydroxychloroquine: A cornerstone of treatment for all patients.

Corticosteroids: Used for more active or severe disease to control inflammation.

Immunosuppressants: Other drugs that suppress the immune system are used for major organ involvement or when other treatments are not effective.

Other therapies: Newer treatments, such as anti-CD20 monoclonal antibodies, are available for severe, refractory cases.

Risk Factors and Genetics:

Genetics: There is a strong genetic component, although most people with a genetic predisposition do not develop the disease.

Environmental factors: Environmental and other triggers are thought to be necessary to start the disease process in genetically susceptible individuals.

Demographics: SLE is more common in women, particularly during childbearing years, and affects certain ethnic groups more frequently (e.g., African Americans, Hispanics, and Asian Americans).

9.4.4 Rheumatoid Arthritis (RA):

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disease where the immune system mistakenly attacks the lining of the joints (synovial membrane), causing inflammation, pain, swelling, and stiffness. The condition is progressive and can lead to joint and tissue damage, reduced mobility, and extra-articular manifestations affecting other organs like the heart, lungs, and nerves. Early and appropriate treatment with a combination of medication, physical therapy, and lifestyle management is crucial to controlling symptoms and preventing severe disability. The body's immune system attacks the synovium, the tissue lining the joints, causing inflammation. RA is not just limited to the joints; it can affect other parts of the body and lead to complications. If left untreated, it can lead to progressive joint and bone destruction, deformity, and disability.

Symptoms:

Joint Symptoms: Pain, swelling, tenderness, and warmth in the joints: Stiffness that is typically worse in the morning and lasts for more than an hour; Usually symmetrical, affecting the same joints on both sides of the body; Most often starts in the small joints of the hands and feet, Fatigue, Low-grade fever, Weight loss, General malaise.

Causes and Risk Factors:

The exact cause is not fully understood, but it's believed to be a combination of genetic and environmental factors. There is a genetic component, but genetics alone are not the sole cause. Smoking is a major environmental trigger, especially for those with a genetic predisposition. Other risk factors include obesity and exposure to air pollution. RA affects women more frequently than men, and onset is most common between the ages of 30 and 60.

Diagnosis:

It can be difficult to diagnose in the early stages as symptoms can mimic other conditions.

Diagnosis methods: A comprehensive approach is required, which includes Physical examination of affected joints, Blood tests (e.g., for rheumatoid factor and anti-CCP antibodies), Imaging tests like X-rays.

Medications:

NSAIDs and Steroids: To help with symptoms

Disease-modifying antirheumatic drugs (DMARDs): Medications like methotrexate and hydroxychloroquine can slow the progression of the disease

Biologic DMARDs: May be used when other treatments are not effective

Therapy and Life Style:

Physical and occupational therapy: To maintain range of motion and function

Exercise: Low-impact aerobic exercise like walking or swimming can help with pain and flexibility

Rest: Balancing activity with rest is important

Diet: An anti-inflammatory diet may be beneficial

Surgery: In severe cases with extensive joint damage, surgical procedures like joint replacement may be an option.

Complications:

Cardiovascular disease: Increased risk of heart disease

Lung damage: Can affect lung tissue

Rheumatoid vasculitis: Inflammation of blood vessels that can affect various organs

Spinal injury: Damage to the bones in the neck

9.5. HYPERSENSITIVITY TYPE IV DISEASES:

Type IV hypersensitivity is related to autoimmune diseases like Type 1 diabetes and multiple sclerosis, contact dermatitis from things like poison ivy, transplant rejection, and certain severe drug reactions such as Stevens-Johnson syndrome and Toxic Epidermal Necrolysis. These are cell-mediated, delayed-type reactions that involve T-cells and are not antibody-driven. Specific Type 4 hypersensitivity diseases are Type 1 diabetes, Multiple sclerosis, Hashimoto's thyroiditis, Inflammatory bowel disease (e.g., Crohn's disease) etc.

9.5.1 Type 1 Diabetes:

Type 1 diabetes is an autoimmune disease where the body's immune system destroys insulin-producing cells in the pancreas, resulting in little to no insulin production. This leads to high blood sugar because glucose cannot enter cells for energy. Management requires daily insulin therapy, either via injections or a pump, along with regular blood sugar monitoring and a healthy lifestyle. Symptoms often appear suddenly and include increased thirst, frequent urination, and blurred vision. An autoimmune condition where the immune system attacks and destroys the insulin-making beta cells in the pancreas. Results in an absolute or near-total lack of insulin, a hormone needed to move glucose from the blood into cells for energy. Characterized by high blood sugar (hyperglycemia) because glucose builds up in the bloodstream. It can develop at any age, but is most often diagnosed in children, adolescents, and young adults. Genetics play a role, and there is an increased risk if a close family member has it.

Symptoms: Abnormal thirst (polydipsia); Frequent urination (polyuria); Blurred vision; Nausea and vomiting.

Diagnosis:

Based on symptoms and age, blood tests such as fasting plasma glucose or an oral glucose tolerance test.

Management:

Insulin therapy: Daily insulin injections or an insulin pump are required to survive and manage blood sugar.

Blood glucose monitoring: Regular monitoring using finger-prick tests or a continuous glucose monitor is crucial for managing insulin doses and balancing carbs.

Healthy lifestyle: Following a doctor's recommendations for a healthy lifestyle is essential.

Regular checkups: Regular doctor visits are necessary to monitor the condition and manage potential complications.

Self-management education: Diabetes self-management education and support are important.

Important Considerations:

Diabetic ketoacidosis (DKA): A serious, life-threatening complication of type 1 diabetes that can occur when the body doesn't have enough insulin. It requires immediate medical care.

Hypoglycemia: Taking too much insulin can cause dangerously low blood sugar. Some people experience "hypoglycemia unawareness," where they don't feel the warning signs of low blood sugar.

Long-term health: Regular eye, foot, and kidney function checks are important to monitor for complications from high blood sugar over time.

9.5.2 Multiple Sclerosis:

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) where the immune system attacks the myelin sheath, a protective covering for nerve fibers. This damage disrupts communication between the brain and body, leading to symptoms like numbness, weakness, vision problems, fatigue, and balance issues. There is no cure, but treatments can help manage relapses, slow disease progression, and alleviate symptoms through medications, physical therapy, and other lifestyle changes.

Symptoms:

Sensory: Numbness, tingling, and "electric-shock" sensations.

Motor: Weakness, tremors, lack of coordination, and unsteady gait.

Vision: Blurred vision, double vision, and pain during eye movement.

Fatigue: Chronic and often debilitating tiredness.

Other: Speech difficulties, dizziness, and problems with bladder or bowel control.

Diagnosis and Treatment:

Diagnosis: Involves neurological exams, and an MRI to look for lesions in the brain and spinal cord.

Treatment include:

- Medications like disease-modifying therapies to reduce relapses and inflammation.
- Physical therapy to help with mobility and other symptoms.
- Treatments for specific symptoms like spasticity, pain, and bladder issues.

9.5.3 Hashimoto's Thyroiditis:

Hashimoto's thyroiditis is an autoimmune disease where the immune system attacks the thyroid gland, leading to chronic inflammation and, over time, hypothyroidism (underactive thyroid). Symptoms can be mild or absent initially, progressing to fatigue, weight gain, cold intolerance, dry skin, joint pain, and depression. Diagnosis typically involves blood tests, and treatment for hypothyroidism is usually thyroid hormone replacement with levothyroxine. The disease develops slowly over many years, and early symptoms may not be present. The immune attack progressively damages thyroid cells, causing inflammation and fibrosis. As damage increases, the thyroid produces less thyroid hormone, leading to hypothyroidism. The thyroid can become enlarged (goiter) because the body tries to stimulate it to produce more hormones, or due to the swelling from the immune attack.

Symptoms - Fatigue, lethargy, Weight gain, Increased sensitivity to cold, Dry skin, brittle hair, Joint and muscle pain, Depression, mood disturbances, Constipation, Heavy or irregular menstrual cycles, Memory or concentration problems.

Diagnosis and Treatment:

Diagnosis: Blood tests are used to measure thyroid hormone levels and antibodies like anti-TPO and anti-Tg.

Treatment: If hypothyroidism develops, the standard treatment is daily replacement therapy with a synthetic thyroid hormone called levothyroxine.

Dosage: The dosage of levothyroxine is personalized based on age, weight, and severity of the condition.

9.5.4 Inflammatory Bowel Disease (e.g., Crohn's disease):

Inflammatory bowel disease (IBD) is a chronic condition affecting the gastrointestinal tract, with the two main types being Crohn's disease and ulcerative colitis. Crohn's disease can affect any part of the GI tract from the mouth to the anus, while ulcerative colitis specifically targets the large intestine and rectum. Both cause chronic inflammation that leads to symptoms like diarrhea, abdominal pain, and fatigue, though IBD can also have extraintestinal manifestations in the eyes, skin, or joints.

Crohn's Disease:

Can affect any part of the digestive tract, from mouth to anus. Causes transmural inflammation (inflammation through the entire thickness of the bowel wall), leading to patches of damage.

Symptoms: Common symptoms include diarrhea, cramping, abdominal pain, and fatigue. Mouth sores and malnutrition can also occur.

Complications: Can lead to complications such as strictures, fistulas, and abscesses.

Types: Common forms include ileocolitis (small and large intestine), colitis (large intestine), and ileitis (small intestine).

Ulcerative Colitis

Affects only the large intestine and rectum. Causes inflammation and ulcers in the innermost lining of the large intestine.

Symptoms: Common symptoms for both include diarrhea, stomach pain, and changes in bowel movements. Symptoms can be mild to severe and may come and go.

Cause: The exact cause is unknown, but it is believed to be a combination of genetic, environmental, and immune factors. An overactive immune response to gut bacteria is a key factor, along with breaches in the intestinal barrier.

Diagnosis: Diagnosed through a combination of symptom review, physical exams, blood and stool tests, endoscopy, biopsies, and imaging.

Treatment: There is no cure, but treatments aim to reduce inflammation, control symptoms, and achieve remission. Treatment may include medications, dietary adjustments, and, in some cases, surgery.

Lifestyle: Smoking cessation is recommended for those with Crohn's disease, as smoking increases the risk of developing it.

Genetic Link: Crohn's has a strong genetic component, with multiple genes increasing the risk.

Extraintestinal Manifestations: IBD can also involve other parts of the body, such as the eyes, skin, joints, and liver.

9.6. SUMMARY:

Hypersensitivity type 1-related diseases include common allergic conditions like allergic rhinitis, allergic asthma, anaphylaxis, urticaria (hives) and angioedema. These are mediated by the IgE antibody and mast cells. Type 2 hypersensitivity diseases include myasthenia gravis, Graves' disease, and autoimmune hemolytic anemia etc. These conditions involve antibodies that target specific cells or tissues, leading to their destruction. Type III hypersensitivity causes diseases by forming damaging immune complexes, which can lead to conditions like systemic Lupus Erythematosus (SLE), rheumatoid arthritis (RA), serum sickness, and several types of glomerulonephritis. These reactions occur when antibodies bind to antigens, forming complexes that deposit in tissues and trigger an inflammatory response, causing damage to organs like the skin, joints, and kidneys. Type IV hypersensitivity is related to autoimmune diseases like Type 1 diabetes and multiple sclerosis, These are cell-mediated, delayed-type reactions that involve T-cells and are not antibody-driven.

9.7. TECHNICAL TERMS:

Rheumatoid arthritis, Type I Diabetes, Crohn’s disease, SLE, Allergic asthma, Anaphylaxis, Myasthenia gravis, Serum sickness, Multiple sclerosis.

9.8. SELF ASSESSMENT QUESTIONS:

1) Write an account on the important diseases of hypersensitivity type I diseases.
2) Give an account on the important diseases of hypersensitivity type II diseases.
3) Write an account on the important diseases of hypersensitivity type III diseases.
4) Give an account on the important diseases of hypersensitivity type IV diseases.

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LESSON-10

MAJOR HISTOCOMPATIBILITY COMPLEX AND TRANSPLANTATION IMMUNOLOGY

10.0 OBJECTIVE:

- This lesson mainly deals with structure and overall functions of Major histocompatibility molecules and transplantation immunology.

STRUCTURE:

10.1. INTRODUCTION:

The major histocompatibility complex (MHC) is a region on chromosome consisting of a closely linked cluster of genes that code for a number of cell surface and plasma protein antigens. The MHC in mammals also consists of a region where the histocompatibility linked immune response genes (IR) are located. This chromosomal segment controls: synthesis of transplantation antigens and graft rejection; immune response to infection; and susceptibility to the development of immunologically mediated diseases. The two MHC systems that have been most extensively studied are: The H2 antigen system in the mouse. The HLA (Human Leukocyte Antigen) system in man. The major antigens determining histocompatibility in human beings are alloantigens found on the surface of leucocytes (hence are known as HLA Ags) and the MHC system is known as the HLA system.

10.2. MHC COMPLEX:

Originally the concept of histocompatibility arises from transplantation rejections by the body. In tissue transplantation rejections, products of the major histocompatibility complex genes and T cells play an important role. Every vertebrate species has MHC genes and products but most detailed information is known about the human and mouse systems. The concept that the rejection of foreign tissue is the result of an immune response to cell-surface molecules now called histocompatibility antigens. The major histocompatibility complex is a collection 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 HLA complex in humans and as the H-2 complex in mice. The MHC genes are organized into regions encoding three classes of molecules.

Class I MHC genes encode glycoproteins expressed on the surface of nearly all nucleated cells; the major function of the class I gene products is presentation of peptide antigens to Tc cells.

Class II MHC genes encode glycoproteins expressed primarily on antigen presenting cells (macrophages, dendritic cells and B cells) where they present processed antigenic peptides to T_H cells.

Class III MHC genes encode in addition to other products, various secreted proteins that have immune functions, including components of the complex system and molecules involved in inflammation. Eg: C2 and C4 of the classical pathway, properdin factor of the alternative pathway and tumor necrosis factor **α** and **β (TNF)**.

The major function of MHC is antigen presentation and processing. Pathogens such as bacteria and viruses can penetrate and infect the cells of the body and T cells mount an immune response against the cells harboring the invading organism. The foreign antigens that trigger an immune response are of two types. First, when the pathogens enter the body, the antigens derived from these organisms are called exogenous antigens. Secondly, the antigens made inside the body. For example, the new viral proteins synthesized in the virus infected cells. If all foreign material that gets into the body were totally ingested, digested by phagocytic cell, there would be no need of stimulus for an immune response. This may in fact be a common occurrence. Nevertheless, some antigens must persist to stimulate antigen-sensitive cells and initiate an immune response. These cells are called antigen presenting cells. In side these cells, the proteins are broken- down in to smaller fragments, peptides. Some of these peptides associate inside the cell with MHC class I or class II molecules, and the resulted peptide-MHC complex moves to the surface of the cell where it can be recognize by a T cell with an appropriate receptor. The events involved in the generation of peptides from proteins inside cells, the binding of peptides to MHC molecules and the display of peptide-MHC complexes at the cell surfaces for the T cell recognition are known collectively as **antigen processing and presentation**.

MHC class I and II molecules have different functions in T cell responses. The function of MHC class I molecules is to present the peptides derived from protein antigens to CD8+ T cells. By contrast, the function of MHC class II molecules is to present peptides to CD4+ T cells. Exogenous antigens are those antigens taken in to cells, normally by endocytosis or phagocytosis. Exogenous antigens can be derived from pathogens (example bacteria and viruses) and from foreign proteins (example ovalbumin and sheep red blood cells) that do not injure the host but activate an immune response. Endogenous antigens are synthesized inside a cell; typically, they are derived from pathogens that have infected the cells. Processing of endogenous antigens occurs in the cytoplasm rather than in the acid vesicles in which exogenous antigens are processed. The major mechanism for generating peptide fragments in the cytoplasm is via a giant protein complex known as the proteasome. This cleaves into peptides about 15 amino acids in length. Cytosolic enzymes (aminopeptidases) remove even more amino acids from the peptides.

Histocompatibility Molecules (Antigens):

a) Class I molecules (antigens) (Fig. 10.1.):

The class I antigen is - a transmembrane (**α**-chain) glycoprotein having about 350 amino acids. Molecular weight is 44 kD. Non-covalently associated with **β**-2 macroglobulin

(**β**-chain) having 100 amino acids of an molecular weight of12kD. The **β** chain (**β**-2 macroglobulin) is a plasma protein, encoded by a gene outside the MHC and located on chromosome -15. The **α**-chain is encoded by three structural genes in the HLA- A, B and C regions and its products are the HLA - A, B and C antigens. The MHC class I products (antigens) are found on the surface of most nucleated cells and most abundantly on lymphoid cells. They are absent on sperm and trophoblastic cells. They are detected by their reactivity with human or mouse alloantisera.

Other important functions of class I antigens:

Essential for immune T-cell recognition of specific target antigens. Lymphocytes can only bind to antigens that are associated with these molecules. Class I antigens are strong transplant antigens responsible for graft rejection and elimination of virus infected cells (cell mediated cytolysis). They may function as components of hormone receptors. The CD-8 cells are specific for MHC- class I antigens.

b) Class II molecules (antigens) (Fig. 10.2.):

It is an heterodimer consisting of two non- covalently bound glycoprotein chains called α and β chains of about 34 kDa and 29 kDa molecular weight, respectively. They are anchored in the cell surface membrane. Each chain has two extracellular domains, transmembrane portion and short intra cytoplasmic region. The proximal domain is the constant region and the distal domain is variable α1 and β1 (the two distal domains) constitute the Ag-binding site for recognition by CD-4 lymphocytes. The class II antigens are distributed on limited cell types and are found on macrophages, dendritic cells, activated T-lymphocytes (CD-4) and B-lymphocytes. These antigens are encoded by genes of HLA - DR, DQ and DP regions.

Additional Functions of Class II Antigens:

They play a major role in Graft versus host response, Immune responsiveness, Immune suppression, Cellular recognition, Cellular interactions, and mixed lymphocyte reaction. The functions of domains are- recognition of Ig, cell surface Ags (Thy 1, T-cell surface antigens, T4 and T8) and the T-cell antigen receptor.

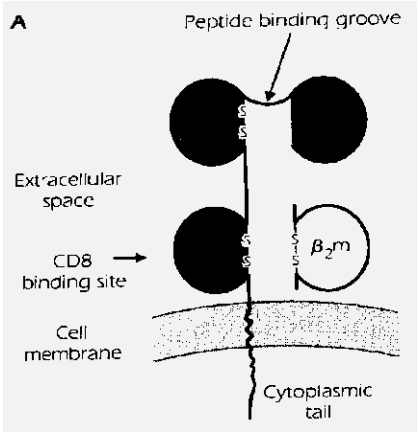

Fig-10.1: Structure of MHC Class I Molecule

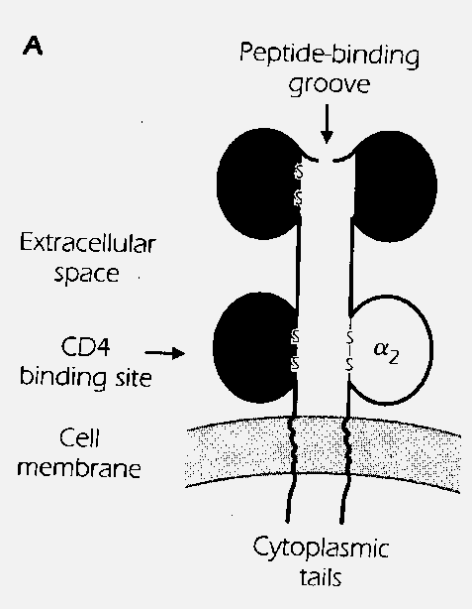

Fig-10.2: Structure of MHC class II Molecule

c) Class III antigens: These are heterogeneous molecules. Include complement components C2, C4 and factor B (components responsible for the formation of C3 convertase). They also include heat shock proteins and tumor necrosis factor. These molecules are encoded by class III MHC genes.

Overall function of MHC: MHC class I and class II genes and their products provide a system for intracellular communication. MHC antigens are essential for recognition of antigen by T-cells. T-cells recognize antigen when it is presented in association with class I and class II histocompatibility antigens. T-helper cells with CD4 molecules recognize foreign antigen in association with class II antigen whereas cytotoxic T-cells with CD8 molecules are restricted by Class-I antigens. MHC class III products are the important components of complement system and are responsible for various cellular events.

10.3. TRANSPLANTATION IMMUNOLOGY

Transplantation, as the term is used in immunology, refers to the act of transferring cells, tissues, or organs from one site to another. The desire to accomplish transplants stems from the realization that many diseases can be cured by implantation of a healthy organ, tissue, or cells (a graft) from one individual (the donor) to another in need of the transplant (the recipient). The development of surgical techniques that allow the facile reimplantation of organs has removed one barrier to successful transplantation, but others remain. One barrier is the lack of organs for transplantation and another one is the immune system. The first systematic study of transplantation was reported in 1908 by Alexis Carrel, who interchanged both kidneys in a series of nine cats. The first successful human kidney transplant, which was between identical twins, was accomplished in Boston in 1954. Today, kidney, pancreas, heart, lung, liver, bone-marrow, and cornea transplantations are performed among non-identical individuals with ever increasing frequency and success using techniques that have been developed to dampen the intensity of the immune response against the foreign organ.

Tissue typing and role of HLA:

Since differences in blood group and major histocompatibility antigens are responsible for the most intense graft rejection reactions, various tissue-typing procedures to identify these antigens have been developed to screen potential donor and recipient cells and assess the likelihood of tissue compatibility. Initially, donor and recipient are screened for ABO blood-group compatibility. The blood-group antigens are expressed on RBCs, epithelial cells, and endothelial cells. Antibodies produced in the recipient to any of these antigens that are present on transplanted tissue will induce antibody-mediated plus complement lysis of the incompatible donor cells.

HLA typing of potential donors and a recipient can be accomplished with a micro cytotoxicity test. In this test, white blood cells from the potential donors and recipient are distributed into a series of wells on a microtiter plate, and then antibodies specific for various class I and class II MHC alleles are added to different wells. After incubation, complement is added to the wells, and cytotoxicity is assessed by the uptake or exclusion of various dyes by the cells. If the white blood cells express the MHC allele for which a particular monoclonal antibody is specific, then the cells will be lysed upon addition of complement, and these dead cells will take up a dye such as trypan blue. HLA typing based on antibody-mediated micro cytotoxicity can thus indicate the presence or absence of various MHC alleles. Even when a fully HLA-compatible donor is not available, transplantation may be successful. In this situation, a one way mixed-lymphocyte reaction (MLR) can be used to quantify the degree of class II MHC compatibility between potential donors and a recipient. Lymphocytes from a potential donor that have been X-irradiated or treated with mitomycin C serve as the stimulator cells and lymphocytes from the recipient serve as responder cells. Proliferation of the recipient T cells, which indicates T cell activation, is measured by the uptake of [^{3}H] thymidine into cell DNA. The greater the class II MHC differences between the donor and recipient cells, the more [^{3}H] thymidine uptake will be observed in an MLR assay. Intense proliferation of the recipient lymphocytes indicates a poor prognosis for graft survival. The advantage of the MLR over microcytotoxicity typing is that it gives a better indication of the degree of T_H-cell activation generated in response to the class II MHC antigens of the potential graft. The disadvantage of the MLR is that it takes several days to run the assay. If the potential donor is a cadaver, for example, it is not possible to wait too long for the results of the MLR, because the organ must be used soon after removal from the cadaver. In such case, the microcytotoxicity test, which can be performed within a few hours, must be relied on.

Survival of kidney grafts depends primarily on donor-recipient matching of the HLA class II antigens. Matching or mismatching of the class I antigens has a lesser effect on graft survival unless there also is mismatching of the class II antigens. HLA matching is most important for kidney and bone-marrow transplants; liver and heart transplants may survive with greater mismatching. MHC identity of donor and host is not the sole factor determining tissue acceptance. When tissue is transplanted between genetically different individuals, even if their MHC antigens are identical, the transplanted tissue can be rejected because of differences at various minor histocompatibility loci. The tissue rejection induced by minor histocompatibility differences is usually less vigorous than that induced by major histocompatibility differences. Still, reaction to these minor tissue differences often results in graft rejection. For this reason, successful transplantation even between HLA-identical individuals requires some degree of immune suppression.

Mechanism of Allograft Reaction:

The general rules of transplantation actually transpired form the study of skin and malignant tumour grafts in mouse led to the development of inbred strains of mouse. Successful transfer of tumours was possible between the individuals of a strain, but not of different strains, suggesting that rejection of a graft is one the basis of genetic differences between the host and donor. Thus, autografts and isografts are found to endure, whereas allografts and heterografts are rejected. The mechanisms of the allograft reaction have been mostly studied with skin grafts because they can be prepared easily, their rejection is readily detected, and their median survival times provide a quantitative estimate of the degree of the host's immune response. Rejection of an allograft is probably due, in part, to a local delayed-type hypersensitivity reaction, and in part to killer cell-mediated cytotoxicity. Thus, lymphoid cells sensitized to a graft have been found in vitro to release macrophage migration inhibition factor when challenged with the appropriate histocompatibility Ags, and the site of an allograft undergoing rejection is intensely infiltrated with macrophages and lymphocytes, whereas granulocytes and plasma cells are much less conspicuous. Tissue culture studies have also shown that lymphoid cells taken from animals sensitized by a graft which they have rejected are able to kill target cells possessing the same transplantation Ags as the original graft. The MIF test may give an early indication of sensitization in a grafted individual.

Acceptance of Allografts:

In certain exceptional circumstances allografts are not rejected. There are some "Privileged sites" like the anterior chamber of the eye and the meninges of the brain for allografts to flourish without inducing immunity in host. Blood vascular and lymphatic drainage are lacking at these sites and so stimulation of the host's lymphocytes is minimal. Pregnancy is another situation where the embryo, bearing the Ags determined by the father's genome and alien to the mother, usually does not evoke an allograft rejection, even when the mother has been previously immunized against the father's histocompatibility Ags. The histocompatibility Ags express on cell surface early in embryonic life, as early as seven days of gestation in mouse. The mechanism of the mother's tolerance to the alloantigen bearing embryo is not totally understood yet. There are several suggestions to explain it; one explanation is that the histocompatibility Ags is masked by mucilaginous secretions, especially at the placental interface between the foetus and its mother.

Graft-Versus-Host (GvH) Reaction:

When competent allogeneic lymphoid cells are transferred to an immunologically incompetent recipient, the host is incapable of reacting against them; rather the grafted cells are free to react immunologically against the Ags on the host's cells which they recognize as foreign. Instead of the normal transplantation reaction of host against graft, the reverse reaction, graft versus host reaction occurs. The ensuing reaction may be fatal. The newborn mouse, an adult recipient depleted of its own lymphocytes by X-irradiation or cytotoxic drugs, as often done in human bone marrow transplantation, are found to be immunologically incompetent and become victims of GvH reaction. In the newborn mouse there can inhibition of growth (runting), spleen enlargement, diarrhea, skin lesions and often haemolytic anaemia and death may occur after a few weeks. All these symptoms are collectively known as the runting syndrome. In humans, fever, anaemia, weight loss, rash, diarrhoea and splenomegaly are observed. The 'stronger' the transplantation antigen difference, the more severe is the reaction. Competent lymphoid cells in blood or grafted organs given to immunosuppressed patients may give rise to GvH reactions.

10.4. SUMMARY:

MHC class I and class II genes and their products provide a system for intracellular communication. The major function of MHC is antigen presentation and processing. MHC antigens are essential for recognition of antigen by T-cells. T-cells recognize antigen when it is presented in association with class I and class II histocompatibility antigens. T-helper cells with CD4 molecules recognize foreign antigen in association with class II antigen whereas cytotoxic T-cells with CD8 molecules are restricted by Class-I antigens. MHC class III products are the important components of complement system and are responsible for various cellular events. Cytokines are low molecular weight antigen-nonspecific proteins that mediate cellular interactions involving immune-inflammatory and hematopoietic systems. The major functions of cytokines are to 1) regulate specific immune responses 2) facilitate innate immune-responses, 3) activate inflammatory responses, 4) affect leukocyte movement, and 5) stimulate hematopoiesis.

10.5. TECHNICAL TERMS:

HLA, Class I MHC molecules, Class II MHC molecules, Transplantation, Allograft, GvH reaction, Graft rejection.

10.6. SELF ASSESSMENT QUESTIONS:

1) Write an essay on major histocompatibility complex and its functions.
2) Give an account on transplant immunology.
3) Explain the mechanism of allograft and also graft-versus-host reactions.

10.7. SUGGESTED READINGS:

1) Nandini Shetty, 2001. IMMUNOLOGY Introductory Text Book., New Age International (P) Limited, Publishers, New Delhi – 235 pp.
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Dr. K. Nagaraju

LESSON-11

AUTOIMMUNITY

11.0 OBJECTIVE:

- This lesson enrich the students' understanding nature of different types of autoimmune diseases were described in detail and the therapeutic measures for these diseases was added.

STRUCTURE:

11.1. INTRODUCTION:

The recognition of self and non-self is an important function of immune system. Response to self as antigen is prevented by appropriate mechanisms. This is known as 'tolerance to self'. Under some circumstances, these mechanisms must break down and self antigens become recognized as foreign antigens. When this occurs, auto antibodies or sensitized lymphocytes capable of reacting with self components are produced. This abnormal response is known as **autoimmunity**. The autoimmunity may lead to autoimmune diseases. Thus, autoimmune disease is a condition in which the natural unresponsiveness or tolerance to self terminates, as a result of which antibody or sensitized lymphocytes reacts with self antigen causing disease.

11.2. AUTOIMMUNITY:

Criteria for Autoimmune Diseases

The criteria are known as Witbsky's postulates.

1) The autoimmune response must be regularly associated with disease.

2) The antigen responsible for the immune response must be identified, isolated and characterized.
3) A replica of the disease must be inducible in laboratory animals and immuno-pathological changes in the natural and experimental diseases should parallel to each other.
4) Transferring autoantibody or self –reactive lymphocytes to a host should reproduce the disease.

Causes of Autoimmune Disease

Genetic Susceptibility:

The most common evidence for the existence of a genetic predisposition to autoimmune disease s in the higher incidence of the disease in monozygotic twins, with a lower but still increased incidence in dizygotic twins and family members compared to an unrelated population.

Hidden or Sequestered Antigen Theory:

According to this theory, during embryonic development, tissues that are exposed to the lymphoreticular system are recognized as self antigens and hence are unable to induce immune response. However, many auto antigens e.g., eye lens protein, brain tissue protein, thyroglobulin, have no opportunity to develop tolerance because they are anatomically confined to sites that prevent their access to lymphoreticular system. Though these antigens are self antigens, they are recognized as non-self. Contact of these antigens with immunocompetent cells results in immune response leading to trauma or injury. Spermatozoa have no opportunity to develop tolerance because they develop with puberty, hence treated as non-self. Exposure of the sequestered tissue antigens through trauma or infection in later life leads to autoimmune disease.

Altered Forms of Self Antigens or Neoantigens:

In certain circumstances, the native tissue antigens may undergo antigenic alteration by physical (irradiation), chemical (drugs and other chemicals) or biological (viral infections, microbial enzymes) means and thus assumes a new antigenic specificity. The antibodies formed against such antigens react with native antigens and injure the cell. Antigens may also arise by mutations.

Shared or Cross Reacting Antigens:

Some organisms carry antigenic determinants that resemble host cell components. These are the cross reacting antigens, which may induce an immune response damaging the particular organ or tissue in the host. For example, in rheumatic fever, antibodies produced against group A Streptococcus react with human heart tissue and produce injury. Another example is the neurological injury that occur sometimes following anti- rabies immunization in human being. Anti-rabies vaccine prepared by using sheep brain tissue when injected, elicits an immune response against sheep brain antigens, which damage the host's nervous tissue due to the cross reaction between human and sheep brain antigens.

Loss of Immunoregulation:

Functional loss of activity of T helper cells (enhanced Th activity) and T- suppressor cells (decreased Ts activity) results in heightened antibody or T cell response. This loss is synonymous with the loss of self tolerance and self antigens behave like foreign antigens leading to autoimmune disease. Defects in the thymus in stem cell development and macrophage function have also been considered as causes. Another hypothesis is non-specific polyclonal B-cell activation by certain stimuli such as 2-mercatoethanol, lipopolysaccharide, trypsin, nystatin and infections with some organisms like mycoplasma, EB virus (Epstein-Barr virus) and malarial parasites.

Genetic Abnormalities:

The mutations in immunocompetent cells to antigenic responsiveness towards self antigens result in autoimmune disease. Defects in immune response genes or immunoglobulin genes may also be the cause of autoimmune disease. A disordered immune regulation based upon genetically immune regulation based upon genetically determined imbalances of the T-helper inducer and T suppressor/cytotoxic is an important determinant in the development of autoimmune disease as per recent evidence.

11.3. AUTOIMMUNE DISEASES:

Traditionally, autoimmune diseases have been classified as B-cell or T-cell mediated diseases. Autoimmune diseases are classified into four types based on the site of involvement and nature of lesions.

11.3.1 Hemolytic Anemia:

Hemolytic anemia is autoimmune when antibodies react with self red blood cells (RBCs). In this connection, the number of RBCs in the circulation is decreased because antibody directed against an antigen on the surface of the blood cell destroys of removes the cells. The destruction of the RBCs can be attributed to two mechanisms. The destruction of the RBCs can be attributed to two mechanisms. One involves the activation of the complement cascade and eventual lysis of the cells. The resultant release of hemoglobin may lead to its appearance in the urine – that is **hemoglobinuria**. The second is by the opsonization of RBCs facilitated by antibody and the C3b components of component. In the latter case, the RBCs are bound to and engulfed by macrophages whose receptors for Fc and C3b attach to the antibody –coated RBCs.

It is customary to divide the antibodies responsible for autoimmune hemolytic anemia into two groups on the basis of their physical properties. The first group consists of the warm autoantibodies, so-called since they react optimally with RBCs at 37^{o}C. The warm autoantibodies belong primarily to the IgG class, and some react with rhesus (Rh) antigens on the surface of the blood cells. Because activation of the complement cascade requires the close alignment of at least two molecules of IgG and Rh antigens sparsely distributed on the surface of the erythrocyte, complement-mediated lysis does not occur. On the other hand, IgG antibodies to these antigens are effective in inducing immune adherence and phagocytosis. Individuals with autoimmune hemolytic anemia can be identified by a coombs test, which is designed to detect bound IgG on the surface of RBCs.

A second kind of antibody, the cold agglutinins, and attaches to RBC's only when the temperature is below 37ºC and dissociates from the cells, when the temperature rises above 37ºC. Cold agglutinins belong primarily to the IgM class and are specific for I or i antigens present on the surface of RBCs. Since the cold agglutinins belong to the IgM class, they are highly efficient at activating the complement cascade and causing lysis of the erythrocytes to which they attach. Nevertheless, hemolysis is severe in patients with autoimmune hemolytic anemia due to cold agglutinins, as long as their body temperature is maintained at 37ºC. When arms, legs, or skin are exposed to cold and the temperature of circulating blood is allowed to drop, severe attacks of hemolysis may occur.

Although the cause of autoantibody formation is often not known, some clues are offered by drug-induced anemia. A drug like penicillin, which behaves as hapten, may bind to some protein on the surface of RBCs, and this entire complex may then act as an antigen eliciting antibodies to the surface of the cell, causing lysis or phagocytosis. In such case, however, the disease is self-limited and disappears when the drug use is discontinued. Another example of drug-induced anemia occurs in a small minority of patients using α-methyldopa, an anti-hypersensitive drug. It leads to a disorder that is almost identical to that characterized by warm autoantibodies. Sometimes cold agglutinins appear after infection by *Mycoplasma pneumoniae* or viruses, implicating a role of an infectious disease trigger in genetically susceptible individuals.

11.3.2 Myasthenia Gravis:

Another autoimmune disease in which antibodies to a well-defined target antigen are implicated is Myasthenia Gravis (Fig. 11.1). The target self-antigen in this disease is the acetylcholine receptor at the neuromuscular junctions. The autoantibody acts as an antagonist that blocks the binding of acetylcholine (ACh) to the receptor. This inhibits the nerve impulse from being transmitted across the neuromuscular junction, resulting in severe muscle weakness, manifested by difficulty in chewing, swallowing and breathing, and eventual death from respiratory failure. It affects individuals of any age, but the peak incidence is women in their late 20s and men in their 50s and 60s. The female to male ratio is approximately 3:2. Some babies of myasthenia mothers have transient muscle weakness, presumably because they received sufficient amounts of pathogenic IgG by trans-placental passage.

The disease can be experimentally induced in animals by immunization with ACh receptors purified from torpedo fish or electrical eel, which demonstrate significant cross-reactivity with mammalian receptors. In the experimental disease, resulting from the formation of antibodies against the foreign receptors, the antibodies bind to the mammalian receptors and mimic almost exactly the natural form of the disease. The disease may be passively transferred with antibody.

The development of myasthenia gravis appears to be linked to the thymus, since many patients have concurrent thymoma, or hypertrophy of the thymus, and removal of thymus sometimes leads to regression of the disease. Molecules cross-reacting with the ACh receptor have been found on various cells in the thymus, such as thymocytes and the epithelial cells, but whether these molecules are the primary stimulus for the development of the disease is unknown. There is a genetic component to the disease as myasthenia gravis is associated with HLA – DR3 alleles.

11.3.3 Graves' Disease:

One of the main manifestations of the Graves' disease (Fig. 11.1) is a hyperactive thyroid gland (Hyperthyroidism). This aspect of disease serves as an example in which antibodies directed against a hormone receptor may activate the receptor rather than interferes with its activity. For reasons not yet understood, in Graves' disease, patients develop autoantibodies against thyroid cell - surface receptors for thyroid-stimulating hormone (TSH). The interaction of these antibodies with receptor activates the cell in a manner similar to activation by TSH. Hence, the autoantibody behaves as an agonist. The long lasting stimulation by these antibodies causes hyperthyroidism due to the continuous stimulation of the thyroid gland.

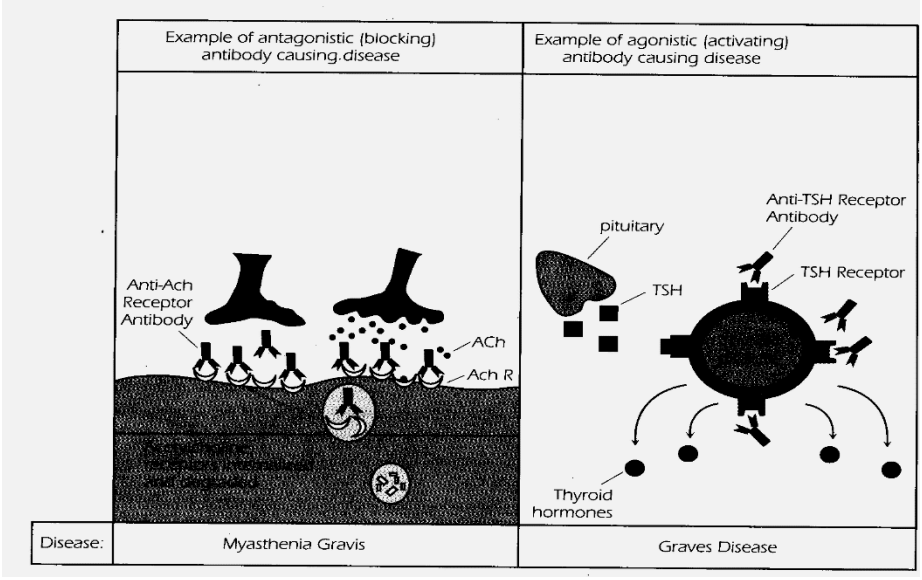

Fig-11.1: The antibody to the acetylcholine receptor in Myasthenia Gravis and Graves' diseases.

11.3.4 Systemic Lupus Erythematosus:

SLE gets its name (literally, 'red wolf') from a reddish facial rash on the cheek, which is a frequent early symptom. However, the distribution of the rash resembles the wings of butterfly rather than the face of a wolf. The designation Wolf-like is thus far-fetched, but the term *systemic* is quite appropriate since the disease attacks many organs of the body and causes fever, join pain and damage to central nervous system, heart, and kidneys. The pathophysiology of the kidney lesions, which cause the most mortality from SLE, is the most clearly understood.

Despite the mystery concerning the origin of this disease, details of the immunologic mechanisms responsible for the pathology are partially known. Patients with SLE produce antibody against several nuclear components of the body (antinuclear antibodies- ANA), notably against native ds-DNA. Occasionally, antibodies are also produced against denature, single-stranded DNA and against nucleohistones; but clinically, the presence of anti-ds DNA correlates best with the pathology of renal involvement in SLE. Antibodies to ssDNA are produced in normal individuals, but they are generally low- affinity IgM antibodies. They

can, however, undergo isotype switching and somatic mutation to result in the production of high affinity IgG antibodies to both single- and double- stranded DNA, provided the B cells are given appropriate T cell help.

Double stranded DNA may become trapped in the glomerular basement membrane through electrostatic interactions with a constituent of the membrane such as collagen, fibronectin, or laminin. The bound ds DNA may then trap circulating IgG anti-ds –DNA antibodies and lead to the formation of immune complexes. These complexes may activate the complement cascade and attract granulocytes. Alternatively, anti- ds- DNA antibodies may cross-react with glomerular antigens. Deposition of IgG antibodies in the kidney of Lupus patients can be demonstrated by immunostaining a tissue section from the kidney with a fluorescently labeled antibody to human IgG. In the kidney, the extent of inflammatory reaction forms the basis of classifying the kidney pathology. The resulting damage to the kidneys (glomerulonephritis) leads to the leakage of protein (proteinuria) and sometimes hemorrhage (Hematuria), with symptoms waxing and waning as a rate of formation of immune complexes rises and falls. As the condition becomes chronic, the inflammatory $CD4^+$ T_H1 cells enter the site and attract monocytes, which further contribute to the pathologic lesions.

11.3.5 Type 1 Insulin-Dependent Diabetes Mellitus:

Insulin dependent diabetes mellitus (IDDM) is a form of diabetes that involves chronic *inflammatory destruction of the insulin-producing β-islet cells of the pancreas*. In IDDM, the major contributors to *β*-cell destructors are *cytotoxic T cells* and cytokines followed by autoantibodies. Genetic factors include several genes in the MHC class II regions, the insulin gene on the chromosome 11, and at least 11 other non-HLA linked diabetes susceptibility genes. Some HLA class II haplotypes predispose for the disease, and others are protective. For example, approximately 50% of IDDM patients are HLA-DR3/DR4 heterozygotes in contrast to 5% of the normal population. On the other hand, individuals with HLA-DQB1*0602 rarely develop the disease.

An experimental animal model, the NOD mouse, shares many key features with the human disease, including the destruction of pancreatic *β*-islet cells by infiltrating lymphocytes, the association with MHC susceptibility genes, and the transmission by T cells. At least 14 genes contribute to diabetes found in NOD mice. There are, however, notable differences between the human disease and the mouse model. These include the predominance of T cells in NOD mice compared to IDDM in humans and a greater bias to incidence of disease in female mice compared to human disease.

11.3.6. Rheumatoid Arthritis:

Rheumatoid arthritis (RA) is characterized by chronically inflamed synovium, densely crowded with lymphocytes, which results in the destruction of cartilage and bone. The inflamed synovial membrane, usually one cell thick, becomes so cellular that it mimics lymphoid tissue and forms new blood vessels. The synovium is densely packed with dendritic cells, macrophages, T, B, and NK cells, and clumps of plasma cells; in some cases, the synovium develops secondary follicles. The pathology in its most intense form is probably the consequence of a mixture of immunopathologic mechanisms, specially, antigen-antibody

complexes, component, polymorphonuclear neutrophils, inflammatory $CD4^+$ T cells, $CD8^+$ cytotoxic T cells, activated macrophages and NK cells. This "angry mix" releases a variety of cytokines (of which TNF α and IL1 are among the earliest), degradative enzymes, and mediators that destroy the integrity of the cartilage. Chondrocytes, the cells of the cartilage become exposed to the immune system and perpetuate the damage not only by serving as potential targets but also by releasing cytokines and growth factors. Synovial fluid often accumulates in the joints of RA patients and contains large number of polymorphonuclear neutrophils. After repeated bouts of inflammatory insults, *fibrin is deposited*; cartilage is replaced by fibrous tissue, and the joint fuses (*ankylosis*).

It has been suggested that inflammatory processes are initiated by abnormally produced antibody, generally IgM—called *Rheumatoid factor* (RF)—*which is specific for a determinant on the Fc portion of the patient's own IgG molecules.* However, it is unlikely that RF is the common initiator of the disease, since 30% of RA patients do not have detectable levels of the factor. The group of patients with RF tends to develop a more aggressive disease. RF serves as a useful marker of disease activity, since reduced levels of serum RF are found during remission. The presence of RF contributes to the pathology of RA but probably does not account for the T cell response. The initial insulting trigger may be diverse. A high portion of RF patients have elevated number of B cells infected with Epstein-Barr virus; γδ T cells from RA patients recognize heat-shock proteins; and bacteria have been associated with RA. Women are affected three times more often than men, and the age of onset is usually during the 40s and 50s of age.

11.3.7 Multiple Sclerosis:

Multiple sclerosis (MS), an autoimmune disease that affects the central nervous system, is the most common cause of neurological disability associated with disease in western countries. The symptoms may be mild, such as numbness in the limbs, or severe, such as paralysis or loss of vision. Most people with MS are diagnosed between the age of 20 and 40 years. Individuals with this disease produce auto reactive T cells that participate in the formation of inflammatory lesions along the myelin sheath of nerve fibers. The cerebrospinal fluid of patients with active MS contains activated T lymphocytes, which infiltrate the brain tissue and cause characteristic inflammatory lesions, destroying the myelin. Since myelin functions to insulate the nerve fibers, a breakdown in the myelin sheath leads to numerous neurologic dysfunctions. Epidemiological studies indicate that MS is more common in the Northern hemisphere, which suggests that there is an environmental component of the risk of contracting MS. It has been suggested that infection by certain viruses may predispose one to MS.

11.4. CONTROL MEASURES OF AUTOIMMUNE DISEASES:

For many years, the major approach to the treatment of most autoimmune disease has been to eliminate auto reactive cells. Because it is not routinely possible to distinguish an auto reactive B or T cell from one that will protect against microbial infection, broadly ablative therapies have been used. Therapeutic agents are often *cytotoxic drugs*, such as *cyclophosphamide* and *azathioprine* that interfere with DNA replication and indiscriminately destroy the body's WBC. In addition, drugs like *cyclosporine A* and *FK506* block intracellular signaling pathways and prevent cellular activation.

More recently, *anticytokine therapies* have proven to be very successful in several diseases. Blockade of TNFα by antibody or soluble receptor is an important therapeutic option in rheumatoid arthritis and inflammatory bowel disease. Inhibition of IL-1β by soluble receptor also seems a useful strategy in rheumatoid arthritis. These immunomodulatory agents prevent an inflammatory response. While they appear to curtail the disease process, they also render the host immunosuppressed. Thus infections represent a major complication of the treatment of many autoimmune diseases. Some autoimmune diseases may be treated by removing or administering a cytokine—for example, interferon-β (INFβ) is used in the treatment of multiple sclerosis. How the cytokine exerts a therapeutic effect is not understood.

Recently, more targeted approaches to therapy have been explored. A non-depleting *monoclonal antibody* to CD3 is being tested in new-onset autoimmune diabetes. Costimulatory blockade, to prevent the interaction of B7 molecules with CD28, appears promising in RA and psoriasis. These new approaches have demonstrated efficacy, but it is likely that they will interfere with protective as well as pathogenic immune responses and thus be immunosuppressive. There are some antigen-specific approaches to therapy that may lead eliminate auto reactivity without causing global immunosuppression. Altered peptide ligands, peptides that bind to the MHC groove but are not capable of activating a given T cell, have been used to induce tolerance in rodent models of disease but have not demonstrated efficiency in humans. Oral antigen has also been used to induce tolerance in animal models, but clinical trials with oral collagen and myelin basic protein in RA and MS, respectively, have not demonstrated efficacy. T cell receptors have been administered to patients as an immunogen in an effort to raise clonotype-specific cytolytic T cells.

The recent recognition of multiple populations of regulatory T cells has led to yet another therapeutic strategy. Several studies suggest an absence or a decrease in numbers of T suppressor cells in autoimmune individuals. Investigators are beginning to learn how to generate regulatory cells in autoimmune individuals. There are as yet no clinical trials that are attempting to activate suppressor cells, but in mice this strategy appears quite effective.

11.5. SUMMARY:

Autoimmunity is a condition in which the body mounts an immune response to one or more of its own constituents. Establishing a disease as auto immune rests on several types of evidence: 1) direct proof made by transferring autoantibodies or self-reactive lymphocytes and reproducing the disease in an otherwise healthy individual; 2) Indirect proof, which requires finding an experimental animal model to mimic the disease; and 3) circumstantial evidence based on familial tendency, involvement of immune cells and antibodies, and clinical improvement with immunosuppressive drugs. Initiation of autoimmune diseases usually requires a combination of genetic and environmental events. It is believed that many auto reactive clones of T and B cells exist normally but are held in check by homeostatic mechanisms. It is the breakdown of these controls, by various mechanisms, that lead to the activation of auto reactive clones and autoimmune disease. Many of organs and tissues are involved in autoimmune disease, and the effecter mechanisms of tissue damage may involve antibody, complement, T-cells, and macrophages.

11.6. TECHNICAL TERMS:

Autoimmunity, Graves' Disease, Systemic Lupus Erythematosus, Rheumatoid Arthritis, Myasthenia Gravis, Hemolytic anemia, Multiple Sclerosis.

11.7. SELF ASSESSMENT QUESTIONS:

1) Write an essay on different types auto immunity diseases.
2) Write in detail about autoimmunity diseases and their control measures
3) Describe the Graves' disease and Systemic Lupus Erythematosus
4) Explain in detail about Rheumatoid Arthritis and Myasthenia Gravis along with control measures.

11.8. SUGGESTED READINGS:

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Dr. K. Nagaraju

LESSON-12
TUMOR IMMUNOLOGY

12.0 OBJECTIVE:

- To acquaint the students about the tumor immunology and tumor cells.

STRUCTURE:

12.1. INTRODUCTION:

The proliferation of normal cells is carefully regulated. However, such cells when exposed to chemical carcinogens, irradiation and certain viruses may undergo mutations leading to their transformation into cells that are capable of uncontrolled growth, producing a tumor or neoplasm. Tumor immunology has been part of immunology that deals with the relationship between the immune system and cancer cells, focusing on how the immune system can both fight and fail to stop tumor growth. It explores the mechanisms by which immune cells recognize and destroy cancer cells, as well as how tumors evade immune surveillance. This knowledge is critical for developing new immunotherapies, which harness the body's own immune system to treat cancer.

12.2. TUMOR ANTIGENS:

Tumor cells, despite their development from self – cells in the body, express tumor antigens. These could be useful tumor markers in identifying tumor cell with diagnostic tests and potential candidates for use in cancer therapy. Some immune responses promote cancer growth. Most tumor antigens that elicit immune responses are neo antigens generated by random passenger mutations.

The following are the Tumor Antigens:

1) Products of mutated oncogenes and tumor suppressor genes.
2) Over expressed or aberrantly expressed cellular proteins.
3) Tumor antigens produced by oncogenic viruses
4) Oncofetal antigens.
5) Altered cell surface glycolipids and glycoproteins.
6) Cell- type specific differentiation antigens.

12.3. TUMOR DIAGNOSIS BY ONCOFETAL ANTIGENS:

Oncofetal antigens are proteins that are present during fetal development but typically disappear or are found at very low levels after birth. They can reappear in adults with certain cancers, as well as in normal tissues during wound healing or tissue repair. Examples include alpha-fetoprotein (AFP) and carcino embryonic antigen (CEA), which are used as tumor markers to help diagnose and monitor certain cancers like liver cancer and colorectal cancer.

Characteristics:

- **Fetal Expression**

  Oncofetal antigens are expressed during embryonic and fetal development and are often found in high levels in the fetus.

- **Postnatal Suppression**

  After birth, their expression in healthy adults decreases significantly or is suppressed, though some may remain at low levels in normal adult tissues.

- **Reactivation in Cancer**

  They are re-expressed in cancer cells, sometimes due to a reactivation of developmental genes during malignant transformation.

- **Reactivation in Repair**

  These antigens can also be found in areas of tissue regeneration or wound healing in non-cancerous conditions.

Examples of Oncofetal Antigens:

- **Alpha-fetoprotein (AFP)**

  Highly expressed in fetal serum, it is a key tumor marker for hepatocellular carcinoma (liver cancer).

- **Carcino embryonic antigen (CEA)**

  Highly expressed in colorectal cancer and other epithelial tumors, but also present in lower levels in normal epithelial cells of the intestine.

- **Human Chorionic Gonadotropin (hCG)**

  Expressed in trophoblast cells during embryogenesis and can be re-expressed in several cancer types.

- **Immature laminin receptor protein (iLRP)**

  Expressed in embryonic and early fetal cells, it is re-expressed as a monomer in tumor cells after it is suppressed in the full-term fetus and adult tissues.

Cancer Diagnosis and Monitoring:

Some onco fetal antigens, like AFP and CEA, can be measured in the blood. Elevated levels can indicate the presence of certain cancers.

1. Alpha-Feto Protein (AFP):

Alpha-fetoprotein is a protein that can be used as a tumor marker to help detect, diagnose, and monitor certain cancers, primarily liver (hepatocellular carcinoma), germ cell (testicular and ovarian), and some gastrointestinal and urological cancers. Elevated levels of AFP in an adult's blood suggest an underlying health issue, but it is not a definitive cancer diagnosis on its own and is used in conjunction with other tests. Especially when combined with imaging like an ultrasound, and can also be used to screen high-risk individuals.

Prognosis:

The level of AFP can help predict how a cancer might behave over time.

Treatment monitoring

It is used to assess the effectiveness of cancer treatment and check for recurrence.

Potential therapeutic targets

Because they are specifically expressed on cancer cells, they are targets for cancer therapies, such as immunotherapy and antibody-drug conjugates.

Role in tumor biology

Recent research shows that AFP isn't just a marker; it can also play an active role in the development and progression of some tumors by affecting the immune system and promoting cell survival.

2. Carcino Embryonic Antigen (CEA):

The test measures the level of a protein in the blood that can be elevated in certain cancers, but it is not used to screen for or diagnose cancer itself. Instead, the test is a valuable tool for monitoring certain types of cancer, such as colorectal, breast, and lung cancer, after an initial diagnosis. CEA levels can indicate how well cancer treatment is working or whether the cancer has returned.

Diagnosis: An elevated CEA level does not automatically mean a person has cancer, as other conditions can also cause it to rise. Conversely, low or normal levels do not guarantee a person is cancer-free.

Normal levels: In healthy adults, CEA levels are typically very low. A common cut off is less than 2.5 ng/mL. Rising levels May indicate the cancer is growing or has returned. Stable or high levels can suggest the cancer is still present or recurring, but further testing is needed to confirm. The other onco fetal antigens are also helpful in diagnosis of various cancers.

12.4. EFFECTOR MECHANISMS IN TUMOR IMMUNOLOGY:

Tumor immunology refers to the relationship between immune function and tumor cells, which is crucial for our understanding of the mechanisms of both tumor rejection and tumor progression. The immunological mechanisms involved in cancer growth are highly complex, including tissue-resident and blood-derived cells. The human immune system mounts natural endogenous response to highly immunogenic tumor cells through a series of steps, including the presenting of tumor antigens to T cells via antigen-presenting cells (APCs), priming and activation of T cells in the lymph nodes, trafficking and infiltration of T cells into tumor beds, recognition of cancer cells by T cells, development of antigen-specific effector and memory T cells, and humoral immunity, allowing effector T cells and other endogenous immune cells, as well as tumor-effective antibodies to tumor to eliminate cancer cells.

Innate Immune Response to Tumor Cells

Cancer cells can alter the steady-state activity of all myeloid cells present in the tumor microenvironment by secreting factors such as interleukin (IL)-6 or granulocyte-macrophage colony-stimulating factor (GM-CSF), that induce the recruitment of immature myeloid cells to tumor cells, as well as cell proliferation.

Natural killer (NK) cells can kill target cells without the need for prior activation, especially in conditions that major histocompatibility complex (MHC) class I molecules (which at normal levels inhibit NK cells) are absent or under expressed in target cells. As many neoplastic cells lose the expression of MHC-I during malignant transformation, they continue to express ligands (e.g. glycolipid) that activate NK cells. This recognition mechanism further leads to the progression of antitumor immune response through the production of interferon-γ (IFN-γ). IFN-γ can activate a number of IFN-γ dependent signaling pathways which enhance the killing of a proportion of the tumor. In addition, IFN-γ, released at a tumor site, induces the production of chemokines that further recruit more cells of the innate immune system to the tumor. Macrophages are recruited into tumors following activation of colony-stimulating factor 1 receptor (CSF1R) by either CSF1 or IL-34. Besides, the chemokine CCL2 may also facilitate the recruitment of macrophage. IFN-γ activates macrophages that express reactive oxygen and nitrogen metabolites which are tumoricidal products. Macrophages can also secret tumor necrosis factor (TNF) that activates endothelial cells and causes coagulation, leading to tumor necrosis and directly stimulating apoptosis. Moreover, cytokines such as IL-12, IL-15, and the type I interferons stimulate NK cells, which leads to proliferation and increased cytotoxic activity.

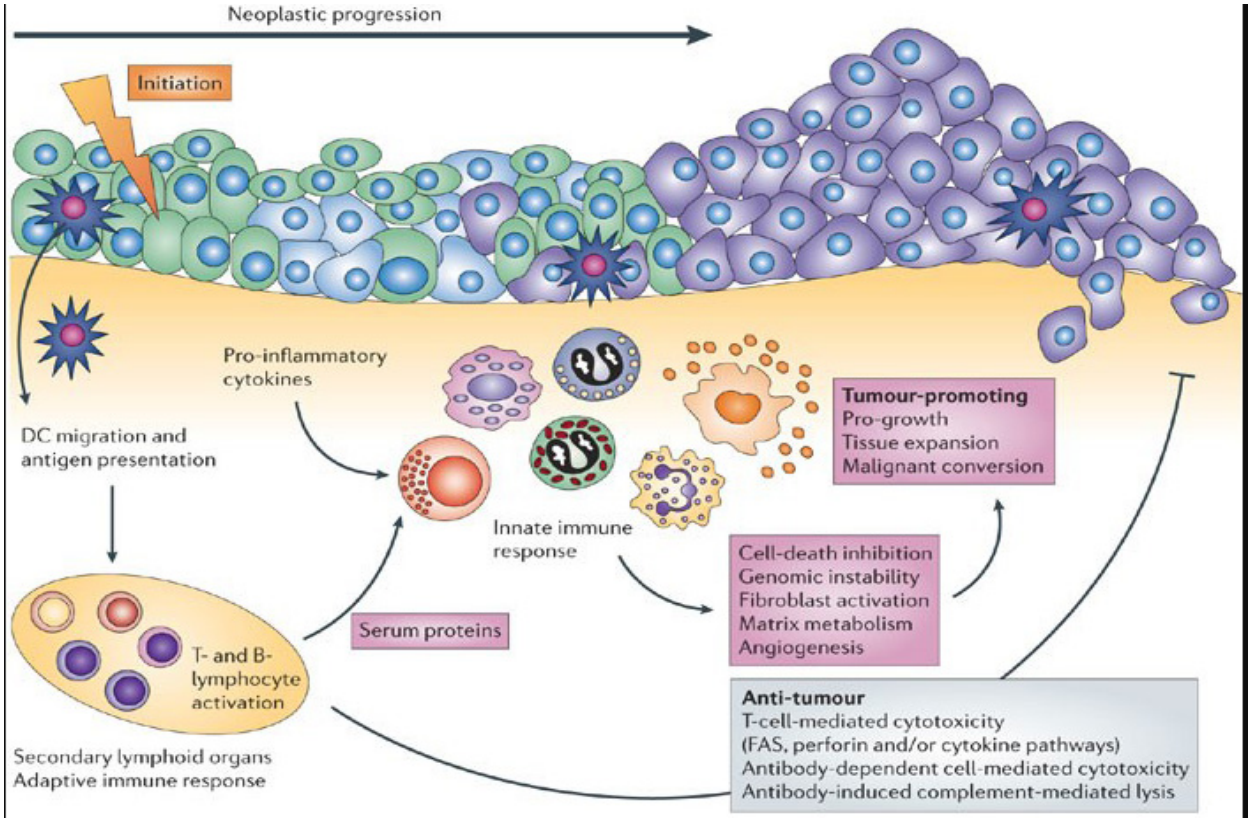

Fig. 12.1. A model of innate and adaptive immune responses to tumor cells.

Adaptive Immune Response to Tumor Cells

Cytotoxic T lymphocytes (CTLs) are the primary mechanism of tumor cell killing in adaptive immune response, which, in many cases, requires the participation of APCs to present the relevant tumor antigen to the CTLs in the appropriate conditions for an antigen-specific immune response. MHC-I and -II molecules must be present to stimulate the production of CTLs. Typically, MHC-I APCs, such as dendritic cells (DC), present antigen (tumor-derived peptides) to CD8+ T cells in the context of co-stimulation through CD80, CD70, and 4-1BB, as well as through DC-derived cytokines such as IL-12, type I interferon, and IL-15. CD8+ CTLs have been demonstrated in numerous different types of solid tumors *in vivo* and have been shown to cause tumor cell destruction *in vitro*. CD4+ T helper cells release cytokines, leading to the anti-tumor immune reaction. The Th1-polarized CD4+ T cells secrete IL-2, TNF-α, and IFN-γ, which promote the development of CD8+ CTLs and the activation of macrophage cytotoxic activity. In addition, they can up regulate antigen processing and the expression of MHC-I and -II molecules in professional APCs such as macrophages and DCs. In contrast, Th2 polarized CD4+ T cells release cytokines IL-4, -5, -6, -10, and -13, resulting in T-cell energy and loss of T-cell-mediated cytotoxicity, enhance humoral immunity, and regulate the tumor-promoting activities of macrophages.

In addition to the effector mechanism mediated by CTLs, the host immune system can generate specific antibodies against cancer antigens, which exert cytotoxic effect against the antigen-bearing tumor cells. Rather than recognizing only protein-derived antigens by T cell antigen receptors, antibodies can bind to multiple types of tumor antigens including polysaccharides, lipids, and proteins. This enhances the anti-tumor ability of the host immune system by broadening the number of tumor antigens that can be exploited for cytotoxic reactions. Mechanisms of antibodies mediated tumor cytotoxicity include antibody-dependent cell-mediated cytotoxicity and complement-mediated cytotoxicity.

12.5. SUMMARY:

Oncofetal antigens are substances which are produced by tumors and also by fetal tissues but they are produced in much lower concentration by adult tissues. The oncofetal antigens which have been identified are reviewed. The relevance of alpha - 1 - fetoprotein (AFP) and carcinoembryonic antigen (CEA) act as diagnostic markers in diagnosis of cancer. The effector mechanisms of different cells like, Natural killer cells, **IFN-γ has a major role in innate immune response of cancer and cytotoxic** T- cells like CD-4 and CD-8 humoral immunity, and regulate the tumor-promoting activities of macrophages.

12.6. TECHNICAL TERMS:

Macrophages, Tumor, TNF, Cytotoxic T-cells, CEA, Oncofetal antigens, Tumor antigens.

12.7. SELF ASSESSMENT QUESTIONS:

1) Explain the role of oncofetal antigens in diagnosis of tumors.
2) Give a detailed account on effector mechanisms in tumor immunology.

12.8. SUGGESTED READINGS:

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LESSON-13

IMMUNE RESPONSE TO INFECTIOUS DISEASES

13.0 OBJECTIVE:

- Students will get a clear understanding about the immune response to some infectious diseases.

STRUCTURE:

13.1. INTRODUCTION:

Despite innate and adaptive immune responses to pathogens, infectious diseases which have plagued human populations throughout history still cause millions of deaths per year. There are 4 main types of pathogens that cause infectious diseases: ¬ Viruses ¬ Bacteria ¬ Protozoa ¬ Helminths.

13.2. VIRAL INFECTION:

The immune response to viral infections involve both Humoral immunity and Cell-mediated components. Antibody to a viral receptor can block viral infections of host cells. However, a number of viruses, including influenza, are able to mutate their receptor molecules and thus evade the humoral antibody response. Once a viral infection has been established, cell-mediated immumity appears to be more important than humoral.CD8 Tc and CD4 TH cells are the main components of cell-mediated antiviral defense.

Induction of antiviral activity by IFN-α and IFN-β - These interferons bind to the IFN receptor, which in turn induces the synthesis of both 2- 5(A) synthetase and protein kinase (PKR). The action of 2-5(A) synthetase results in the activation of RNAse L, which can degrade messenger RNA. PKR inactivates the translation initiation factor elF-2 by phosphorylating it. Both pathways thus result in the inhibition of protein synthesis and thereby effectively block viral replication. Here is the one example of viral infection which is caused by Influenza virus.

Influenza Virus:

Influenza virions are surrounded by an outer envelope (Fig.13.1). A lipid bilayer derived from the plasma membrane of the infected cell, plus various virus- specific proteins.

Imbedded in this envelope are two key viral glycoproteins, hemagglutinin (HA) and neuraminidase (NA) HA trimers are responsible for the attachment of the virus to host cells, binding to the sialic acid groups on host-cell glycoproteins and glycolipids. NA is an enzyme that cleaves *N*-acetyl neuraminic (sialic) acid from nascent viral glyco- proteins and host-cell membrane glycoproteins, facilitating viral budding from the infected host cell. Thus these two structures are essential for viral attachment and for exit of new virus from infected cells—so important in fact that we track new strains of influenza based on their antigenic sub- types of HA and NA (e.g., H1N1versusH5N1virus). Within the envelope, an inner layer of matrix protein surrounds the nucleocapsid, which consists of eight different strands of single-stranded *RNA* (ssRNA) associated with protein and RNA polymerase. Each RNA strand can encode.

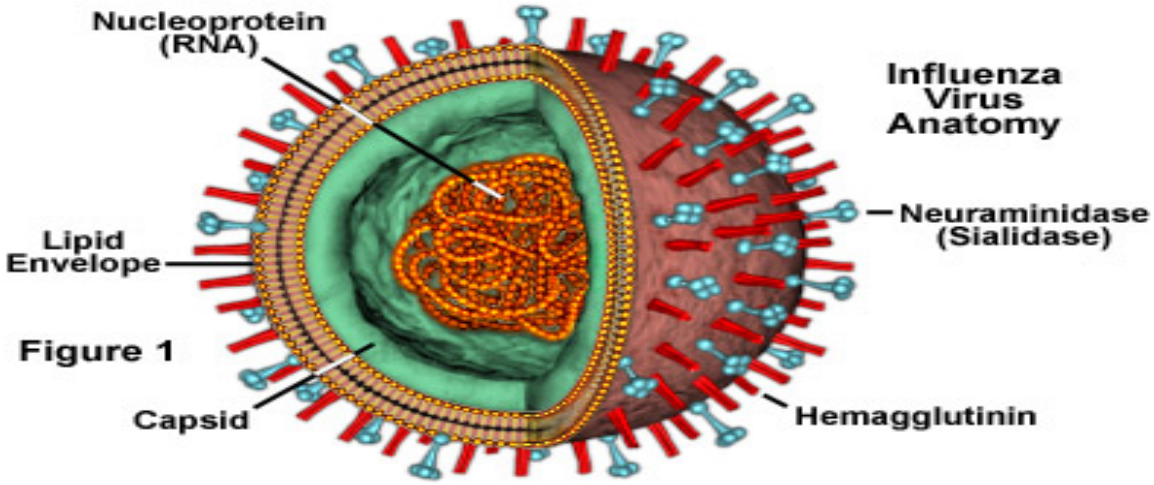

Fig.13.1: Influenza Virus

Influenza viruses are mainly 3types – A, B & C. These three viruses are distinguished by differences in their nucleoprotein and matrix proteins. Distinguishing feature of influenza virus is its variability - Antigenic drift and antigenic shift.

Antigenic Drift: Involves a series of point mutations that occur gradually, result changes in HA and NA over time.

Antigenic Shift: Results in sudden emergence of a new subtype of influenza, where the structure of HA and NA are considerably different from the virus present in a preceding year.

Symptoms of Influenza (flu) Disease: Fever, Muscle ache and pain, Headache, Fatigue, Dry cough, Sore throat and runny nose.

Influenza virus infects the upper respiratory tract and major central airways in humans, horses, birds etc. The envelope is covered with neuraminidase and hemagglutinin spikes,

which are responsible for the attachment of the virus to the host cells. Inside is an inner layer of matrix proteins surrounding the nucleocapsid, which consists of eight ssRNA molecules associated with nucleoprotein. The eight RNA strands encode ten proteins: PB1, PB2, PA, HA (hemagglutinin), NP (nucleoprotein), NA (neuraminidase), M1, M2, NS1 and NS2.

Host response to influenza infection:

The immune response to influenza virus involves a rapid innate defense (mucus, interferons) followed by a strong adaptive response (B cells, T cells, antibodies) to clear infection, generate memory, and prevent future disease, though the virus actively tries to evade these defenses. But Influenza viruses have proteins (like NS1) that suppress IFN production and innate immune signaling to establish infection. So, development of Flu vaccines stimulates a controlled adaptive response (antibody production) to provide protection without causing illness.

13.3. BACTERIAL INFECTIONS:

Immunity to bacterial infections is achieved by means of antibody unless the bacterium is capable of intracellular growth, in which the delayed type hypersensitivity (DTH) has an important role, bacteria enter the body enter through a number of natural routes.

There are two types of bacterial infections -Extracellular and Intracellular.

Extracellular Bacteria:

The bacteria replicate outside the host cells and they cause disease by two principle mechanisms

a) By inducing inflammation.

b) By producing toxins-Endotoxins or/and Exotoxins.

The immune responses against extra-cellular bacteria are aimed at eliminating the bacteria and neutralizing the effects of their toxins.

Innate Immunity to Extracellular Bacteria:

Extra-cellular bacteria can be eliminated through phagocytosis by neutrophils, monocytes, and the tissue macrophages and activation of the compliment system, in the absence of antibody. Humoral immunity is the principle specific immune response against extra-cellular bacteria which includes strong IgM responses are caused by polysaccharides.

Antibodies IgM and IgG against bacterial surface antigens and toxins stimulate three types of effector mechanisms –

1) IgG antibodies opsonize bacteria and enhance phagocytosis.
2) Antibodies neutralize bacterial toxins.
3) IgM and IgG antibodies activate the complement system (Fig. 13.2).

Evasion of Immune Mechanisms by Extra-Cellular Bacteria:

1. Genetic variation of the surface antigen is one of the mechanisms used by bacteria to evade specific immunity. The capsule of many gram-negative and gram positive bacteria contain one or more sialic acid residues that inhibit complement activation by the alternative pathway.

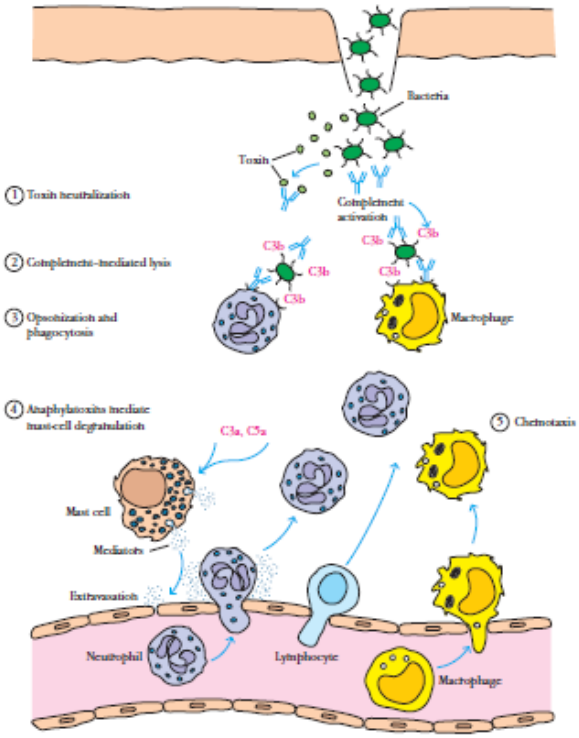

Fig.13.2: Antibody-mediated mechanisms against infection by extracellular bacteria.

Innate Immunity to Intracellular Bacteria:

During the innate immune response, the intracellular bacteria phagocytes ingest and attempt to destroy. Intracellular bacteria are resistant to degradation within phagocytes. Intracellular bacteria also activate NK cells, either directly or indirectly by stimulating macrophages production of IL-12 (a powerful NK cell – activating cytokine).

Specific Immunity to Intracellular Bacteria:

Cell-mediated immune response is the major specific immune response against intracellular bacteria.

There are two types of Cell-Mediated Reactions:

1) Killing of phagocytosed intracellular bacteria as a result of macrophage activation by T cell – derived cytokines, particularly IFN-g.
2) Lysis of infected cells by CTLs.

Tissue Injury:

Tissue damage can be caused by macrophage activation that occurs in response to intracellular bacteria and the macrophages accumulate and result in the formation of a granuloma.

Evasion of Immune Mechanisms by Intracellular Bacteria:

Ability of intracellular bacteria to resist elimination by phagocytes is an important mechanism for survival in evasion of the immune response.

Some Intracellular Bacteria:

(a) Inhibit phagolysosome fusion.

(b) Produce hemolysin that blocks bacterial killing in macrophages.

A number of bacteria escape host – defense mechanism through their ability to survive with in phagocytic cells. Eg: *Mycobacterium* species blocks lysosomal fusion with the phagolysosome or resist the oxidative attack that typically takes place in the phagolysosome.

Tuberculosis:

Tuberculosis is caused by *Mycobacterium tuberculosis*, a leading cause of death in the world from a single infectious agent. *Mycobacterium* spreads easily and pulmonary infection results from inhalation of small droplets of respiratory secretion containing a few bacilli. These are ingested by alveolar macrophages in the lung and are able to survive and multiply intracellularly by inhibiting formation of phagolysosme. In this pattern, CD4 T cells are activated within 2 to 6 weeks after infection and secrete cytokines that induce the infiltration of large numbers of activated macrophages. These cells wall off the organism inside a granuloma called a tubercle. A cluster of small lymphocytes surrounding infected macrophages. The localized concentrations of lysosomal enzymes in these granulomas can cause extensive tissue necrosis and massive enzymes form a lesion. Much of the tissue damage was seen with *M. tuberculosis.*

In cell-mediated immune response, activated macrophages suppress proliferation of the phagocytosed bacilli. Cytokines produced by CD4 T CELLS (TH subsets) play an important role in response by activating macrophages. They are able to kill the bacilli or inhibit their growth. The role of IFN- gamma in the immune response to mycobacteria with knockout mice lacking IFN- gamma, these mice dead when they were infected with attenuated strain of mycobacteria, whereas IFN- gam wild type mice survived.

13.4. PROTOZOAN INFECTIONS:

The term parasite encompasses a vast number of protozoan and helminthic organisms. Protozoans are unicellular eukaryotes that usually live and multiply with in host cells for at least part of their life cycle, whereas helminthes are multicellular organisms that can be quite large and have ability to live and reproduce outside their human host. Parasites can evade the immune system, allowing them to chronically infect

their human host and exact a lifelong toll. E.g.: Malaria, African sleeping sickness, Leishmaniasis and Toxoplasmosis are the most common parasitic diseases.

The type and effectiveness of immune response to protozoan infection depends in part on the location of the parasite with in the host and the life cycle stage of the parasite. Many protozoans spend part of their time free with in the blood stream: Humoral antibody is the most effective during these stages. At other stage they may grow intracellularly, making cell mediated immune reactions the most effective host defense.

Malaria:

Malaria is number one parasitic cause of death worldwide. Half of the world population lives in malaria endemic zone. The causative agent of Malaria: one of the several species of the genus *Plasmodium* of which *P. falciparum* is the most virulent. *Plasmodium* has an extremely complex life cycle, female Anopheles mosquitoes serve as the vector and host for part of the parasites life cycle.. Human infection begins when sporozoites, enter the blood stream, when an infected female mosquito takes blood meal. This sporozoite antigen has 45KDa weight. These are mediate in hepatocytes. In hepatocytes, the parasite differentiates into merozoites, which infect red blood cells, initiating the major symptoms of Malaria. The symptoms of malaria include chills, fever and sweating that peak roughly every 48hours. Due to successive generation of merozoites are release from infected red blood cells, individual eventually became weak and anemic. The merozoites cause heart failure, head ache and renal failure due to excessive production of cytokines,

In regions where malaria is endemic the immune response is poor. In younger children, less than 14 years, has weakest immune response and consequently are most likely to develop malaria. Most people living in endemic regions have lifelong low – level plasmodium infections. A number of factors will contribute for low level of immune response due to maturational changes allow the organisms to keep changing its surface molecule resulting in continual changes in the antigens seen by the immune system. Even when an antibody response does develop to sporozoites, plasmodium overcomes that response by sloughing off the CS surface antigens, thus rendering the antibodies ineffective. So, the search for vaccine is so important.

African Sleeping Sickness:

Two species of African trypanosomes cause African sleeping sickness, a chronic disease transmitted to humans and cattle by the bite the tsetse fly. In the blood stream, the trypanosome (a flagellated protozoan) differentiates into a long slender form that continues to divide every 4 to 6 hours. In progression of disease they multiply in neurologic stage in which parasite infect cells of the central nervous system, leading to meningoencephalitis and eventual loss of consciousness. Thus it is named as sleeping sickness.

The surface of *Trypanosoma* parasite is covered with variable surface glycoproteins (VSG), several unusual genetic processes generate extensive variation in the surface structure, enabling the organism to escape immunologic clearance.

As parasite numbers increases after infection, an effective humoral response develops to VSG covering the surface of the parasite. The antibodies eliminate most of the parasites from the blood stream, both by complement- mediated lysis and by opsonization and subsequent phagocytosis. If the parasite escape the initial antibody response, begin to proliferate in the blood stream and go on to populate the next wave of parasitemia in the host. The successive waves of parasitemia reflect a unique mechanism of antigenic shift by which the trypanosomes evade the immune response to their surface antigens. So, the continual shifting of surface antigens has made vaccine development extremely difficult. So, prevention is much better than cure.

Leishmaniasis:

Leshmania is a flagellated protozoan that lives in the phagosomes of macrophages and transmitted by sand flies. It is usually results in one of two syndromes:

1) Localized cutaneous lesion formation that is generally painless and self-resolving.
2) Systemic form of the disease, called visceral Leishmaniasis, which is nearly always fatal without treatment.

Immune response to Leishmaniasis is well with the production of IFN- gamma and the development of a TH1 response. If animals mount a TH2 type response to *Leshmania* infection producing high levels of IL-4 and essentially no IFN-gamma, studies have shown that a small subset of CD4 cells in the susceptible animals recognize a particular epitope on *L. major* and produce high levels of IL-4 early in the response to the parasite. Understanding how different T- helper responses affect the outcome of infection could contribute to the rational design of effective treatments and vaccine against this and other pathogens.

13.5. SUMMARY:

The immune response to infectious diseases caused by Viruses, Bacteria, Fungi and Protozoa involve two main parts: the innate immune system, which is an immediate and general defense, and the adaptive immune system, a slower but highly specific defense. Innate immunity uses physical and chemical barriers like skin and stomach acid, as well as cells like neutrophils, to provide an immediate but non-specific response. Adaptive immunity, involving B and T cells, targets specific pathogens (antigens) and creates a "memory" of them for faster future responses.

13.6. TECHNICAL TERMS:

Immune response, humoral immunity, Cell-mediated immunity, Viral infections, Bacterial infections, Protozoan infections, Influenza, *Mycobacterium*, *Leishmania*.

13.7. SELF ASSESSMENT QUESTIONS:

1) Discuss in detail about immune response to bacterial infections.
2) Explain the immune response to Protozoan infections.
3) Describe the immune response to viral infections.

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Prof. V. Umamaheswara Rao

LESSON-14
VACCINES

14.0 OBJECTIVE:

- To make the students to understand the different types of vaccines and their development.

STRUCTURE:

14.1. INTRODUCTION:

The discipline of immunology has its roots 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, rubella, poliomyelitis, and tetanus has declined dramatically as vaccination has become more common. Vaccination is a cost-effective weapon for disease prevention. The vaccination benefit is so evident in eradication of smallpox, one of the mankind's' long standing and most terrible scourges. Equally encouraging is the predicted eradication of polio. Another important vaccine is that works against rotavirus which cause diarrhea in infants.

The control of a number of diseases that cause significant mortality and morbidity has mad outstanding progress, but there remains a crying need for vaccines against others. The beginnings of any vaccine development pathway are in basic research. 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 the immunologists to begin the designing of vaccine candidates to maximize the activation of either the humoral or the cell-mediated branch of the immune system. Genetic engineering techniques can be used to develop vaccines to maximize the immune response to selected epitopes and to simplify the delivery of vaccines.

14.2. DESIGNING VACCINES FOR ACTIVE IMMUNIZATION:

For developing a successful vaccine, the first and foremost important factor is to recognize the important differences between activation of the humoral and the cell mediated branches. The second factor is the development of immunologic memory. For example, a vaccine that induces a protective primary response may fail to induce the formation of memory cells, leaving the host unprotected after the primary response to the vaccine subsides. The role of memory cells in immunity depends, in part, on the incubation period of the pathogen. In the case of influenza virus, which has a very short incubation period, disease symptoms are already under way by the time memory cells are activated. Therefore, the effective protection against influenza depends on maintaining high levels of neutralizing antibody by repeated reimmunizations. For the pathogens with a longer incubation period, demonstrable neutralizing antibody at the time of infection is not necessary. The poliovirus, for example, requires more than 3 days to begin to infect the central nervous system. An incubation period of this length gives the memory B cells time to respond by producing high levels of serum antibody. Thus, the vaccine for polio is designed to induce high levels of immunologic memory. After immunization with the Salk vaccine, serum antibody levels peak within 2 weeks and then decline, but the memory response continues to climb, reaching the maximal levels at 6 months and persisting for years. If an immunized individual is later exposed to the poliovirus, these memory cells will respond by differentiating into plasma cells that produce high levels of serum antibody, which protect the individual from infection.

14.3. WHOLE ORGANISM VACCINES:

Many of the common vaccines currently in use consist of inactivated (killed) or live but attenuated (avirulent) bacterial cells or viral particles.

Inactivated Viral or Bacterial Vaccines:

A common approach in vaccine production is to inactivate the pathogen by heat or by chemical means, so that it is no longer capable of undergoing replication in the host. It is critically important to maintain the structure of epitopes on surface antigens during inactivation. Heat inactivation is generally unsatisfactory because it causes extensive denaturation of proteins; thus, any epitopes that depend on higher orders of protein structure are likely to be altered significantly. Chemical inactivation with formaldehyde or various alkylating agents has been successful. The Salk polio vaccine and the pertussis (whooping cough) vaccine are produced by formaldehyde inactivation.

Unlike immunization with attenuated vaccines, which generally requires only one dose to induce long-lasting immunity, repeated boosters of killed vaccines are often needed to maintain the immune status of the host. In addition, killed vaccines induce a predominantly humoral antibody response; they are less effective than attenuated vaccines in inducing cell mediated immunity and in eliciting a secretory IgA response. Though they contain killed pathogens, inactivated whole-organism vaccines are still associated with certain risks. A serious complication with the first Salk vaccines arose when formaldehyde failed to kill all the viruses in two vaccine lots, which caused paralytic polio in a high percentage of recipients. The pertussis vaccine highlights another problem that can arise with a complex whole organism vaccine. The encephalitis-type reactions that occurred in a small percentage

of infants receiving this vaccine have led to the development of a new acellular vaccine for pertussis.

Attenuated Viral or Bacterial Vaccines

In some cases, microorganisms can be attenuated so that they lose their ability to cause significant disease (pathogenicity) but retain their capacity for transient growth within an inoculated host. Attenuation often can be achieved by growing a pathogenic bacterium or virus for prolonged periods under abnormal culture conditions. This procedure selects mutants that are better suited to growth in the abnormal culture conditions and are therefore less capable of growth in the natural host. For example, an attenuated strain of *Mycrobacterium bovis* called **Bacillus Calmette-Guerin (BCG)** was developed by growing *M. bovis* on a medium containing increasing concentrations of bile. After 13 years, this strain had adapted to growth with increased bile and had become sufficiently attenuated that it was suitable as a vaccine for tuberculosis. The Sabin polio vaccine and the rubella vaccine consist viral strains that have been successfully attenuated and now serve as successful vaccines. The poliovirus used in Sabin vaccine was attenuated by growing in monkey kidney epithelial cells. The rubella vaccine contains a strain of rubella virus that was grown in duck embryo cells and later in human cell lines.

Attenuated vaccines have some advantages and some disadvantages. Because of their capacity for transient growth, such vaccines provide prolonged immune system exposure to the individual epitopes on the attenuated organisms, resulting in increased immunogenicity and production of memory cells. As a consequence, these vaccines often require only a single immunization, eliminating the need for repeated boosters. This property is a major advantage in Third World countries, where epidemiologic studies have shown that roughly 20% of individuals fail to return for each subsequent booster. The ability of many attenuated vaccines to replicate within host cells makes them particularly suitable for inducing a cell-mediated response.

The Sabin polio vaccine, consisting of three attenuated strains of poliovirus, is administered orally to children on a sugar cube or in sugar liquid. The attenuated viruses colonize the intestine and induce protective immunity to all three strains of virulent poliovirus. The ability of the attenuated Sabin vaccine to colonize the intestines enables it to induce production of secretory IgA, which serves as an important defense against naturally acquired poliovirus. The vaccine also induces IgM and IgG classes of antibody. Unlike most other attenuated vaccines, which require a single immunizing dose, the Sabin polio vaccine requires boosters, because the three strains of attenuated poliovirus in the vaccine interfere with each other’s replication in the intestine. With the first immunization, one strain will predominate in its growth, inducing immunity to that strain. With the second immunization, the immunity generated by the previous predominant strain in the vaccine, enabling one of the two remaining strains to predominate and induce immunity. Finally, with the third immunization, immunity to all three strains is achieved.

A major disadvantage of attenuated vaccines is the possibility of their reversion to a virulent form. The rate of reversion of the Sabin polio vaccine (OPV) leading to subsequent paralytic disease is about one case in four million doses of vaccine. This reversion implies

that pathogenic forms of the virus are being passed by a few immunized individuals and can find their way into the water supply, especially in areas where sanitation standards are not rigorous or where waste water must be recycled. Another concern with attenuated vaccines is the presence of other viruses as contaminants. In 1960, it was discovered that the oncogenic virus SV40 had contaminated some monkey kidney cultures used in the production of the Sabin vaccine. Although no adverse reactions caused by this contaminant have been recorded, more stringent vaccine testing was required to eliminate SV40 from the oral polio vaccine. Attenuated vaccines also may be associated with complications similar to those seen in the natural disease. A small percentage of recipients of the measles vaccine, for example, develop post vaccine encephalitis. Although these complications are undesirable, the risk of such complications is far less than that observed in a naturally acquired measles infection.

In some cases, however, post vaccine complications may render a potential vaccine unacceptable. This situation illustrated by trials conducted several years ago with an experimental measles vaccine. This vaccine-an attenuated strain of the virus called the Edmonston-Zagreb strain-is immunogenic in infants as young as 4-6 months old. Maternal antibodies render the standard measles vaccine ineffective when it is given before 9 months of age. The new vaccine was developed for use in Third World countries, where many infants are infected with the measles virus at a very early age. Unfortunately, trails of the Edmonston-Zagreb vaccine in Guinea-Bissau, Senegal, and Haiti had to be halted when many vaccinated children began dying from common endemic disorders such as diarrhea, pneumonia, and parasitic diseases. Mortality rates were higher in girls than boys, an unexplained finding. The high rate of post vaccine complications with the Edmonston-Zagreb strain may result from vaccine-mediated immunosuppression, since the measles virus is known to cause transient immunosuppression.

Genetic engineering techniques provide a way to attenuate a virus irreversibly by selectively removing genes that are necessary for virulence. This has been done with a herpes virus vaccine for pigs, in which the thymidine kinase gene was removed. Because thymidine kinase is required for the virus to grow in certain types of cells, removal of this gene rendered the virus incapable of causing disease. It is possible that similar genetic engineering techniques could eliminate the risk of reversion of the attenuated polio vaccine. More recently, a vaccine against rotavirus, a major cause of infant diarrhea, was developed using genetic engineering techniques to modify an animal rotavirus to contain antigens present on the human viruses.

14.4. PURIFIED MACROMOLECULES AS VACCINES:

Some of the risks associated with attenuated or killed whole organism vaccines can be avoided with vaccines that consist of specific, purified macromolecules derived from pathogens. Three general forms of such vaccines are in current use: inactivated exotoxins, capsular polysaccharides, and recombinant surface antigens.

Polysaccharide Vaccines:

The virulence of some pathogenic bacteria depends primarily on the antiphagocytic properties of their hydrophilic polysaccharide capsule. Coating of the capsule with antibodies and/or complement greatly increases the ability of macrophages and neutrophils to phagocytose such pathogens. These findings provide the rationale for vaccines consisting of purified capsular polysaccharides. The current vaccine for *Streptococcus pneumonia*, which causes pneumococcal pneumonia, consists of 23 antigenically different capsular

polysaccharides. It is marketed as Pneumovax 23 by Merck and as Pnu-Immune 23 by Lederle Laboratories. The vaccine induces formation of opsonizing antibodies and is administered to high-risk groups such as infants, splenectomized patients, other immune-suppressed individuals, and the elderly. The vaccine for *Neisseria meningitides*, a common cause of bacterial meningitis, also consists of purified capsular polysaccharides.

One limitation of polysaccharide vaccines is their inability to activate T_H cells. They activate B cells in a thymus-independent type 2 manner, resulting in IgM production but little class switching, no affinity maturation, and little, if any, development of memory cells. Several investigators have reported the induction of IgA secreting plasma cells in humans receiving subcutaneous immunization with the pneumococcal polysaccharide vaccine. In this case, since T_H cells are not involved in the response, the vaccine may activate IgA-specific memory B cells previously generated by naturally occurring bacterial antigens at mucosal surfaces. Because these bacteria have both polysaccharide and protein epitopes, they would activate T_H cells, which in turn could mediated class switching and memory-cell formation. One way to involve T_H cells directly in the response to a polysaccharide antigen is to conjugate the antigen to some sort of protein carrier. For example, the vaccine for *Hemophilus influenza* type b (Hib), the major cause of bacterial meningitis in children under 5 years of age, consists of type b capsular polysaccharide covalently linked to a protein carrier, tetanus toxoid. The polysaccharide-protein conjugate is considerably more immunogenic than the polysaccharide alone, and because it activates T_H cells, it enables class switching from IgM to IgG. Although this type of vaccine can induce memory B cells, it cannot induce memory T cells specific for the pathogen. In the case of the Hib vaccine, it appears that the memory B cells can be activated to some degree in the absence of a population of memory T_H cells, thus accounting for the efficacy of this vaccine.

Toxoid Vaccines:

Some bacterial pathogens, including those that cause diphtheria and tetanus, produce exotoxins. These exotoxins produce many of the disease symptoms that result from infection. Diphtheria and tetanus vaccines, for example, can be made by purifying the bacterial exotoxin and then inactivating the toxin with formaldehyde to form a toxoid. Vaccination with the toxoids induces antitoxoid antibodies, which are also capable of binding to the toxin and neutralizing its effects. In production of toxoid vaccines, the conditions must be closely controlled to achieve detoxification without excessive modification of the epitope structure. One of the problems with vaccines consisting of purified macromolecules is the difficulty of obtaining sufficient quantities of the purified starting materials. In the case of diphtheria and tetanus toxoid vaccines, this limitation has been overcome by cloning the exotoxin genes and then expressing them in easily grown host cells. In this way, large quantities of the exotoxin can be produced, purified, and subsequently inactivated.

Recombinant Antigen Vaccines:

The gene encoding any immunogenic protein can be cloned and expressed in bacterial, yeast, of mammalian cells using recombinant DNA technology. A number of genes encoding surface antigens from viral, bacterial, and protozoan pathogens have been successfully cloned into bacterial, yeast, insect, or mammalian expression systems, and the expressed antigens used for vaccine development. The first such recombinant antigen vaccine approved for human use is the hepatitis B vaccine. This vaccine was developed by cloning the gene for the major surface antigen of hepatitis B virus (HBsAg) in yeast cells. The yeast

cells are harvested and disrupted by high pressure, releasing the recombinant HBsAg, which is then purified by conventional biochemical techniques. This recombinant hepatitis B vaccine has been shown to induce the production of protective antibodies.

14.5. RECOMBINANT VECTOR VACCINES:

Introduction of genes that encode major antigens of especially virulent pathogens into attenuated viruses or bacteria is possible. The attenuated organism serves as a vector, replicating within the host and expressing the gene product of the pathogen. A number of organisms have been used for vector vaccines, including vaccinia virus, attenuated poliovirus, adenoviruses, attenuated strains of *Salmonella*, and the BCG strain of *Mycobacterium bovis*. Vaccinia virus, the attenuated vaccine used to eradicate smallpox, has been widely employed as vector vaccine. This large, complex virus with a genome of about 200 genes can be engineered to carry several dozen foreign genes without impairing its capacity to infect host cells and replicate. The genetically engineered vaccinia expresses high levels of the inserted gene product, which can then serve as a potent immunogen in an inoculated host. Like the smallpox vaccine, genetically engineered vaccinia vector vaccine can be administered simply by scratching the skin, causing a localized infection in host cells. If the foreign gene product expressed by the vaccinia is a viral envelope protein, it is inserted into the membrane of the infected host cell, inducing development of cell-mediated immunity as well as antibody-mediated immunity.

Other attenuated vector vaccines may prove to be safer than the vaccinia vaccine. The canarypox virus has recently been tried as a vector vaccine. Like its relative vaccinia, the canarypox virus is a large virus that can easily be engineered to carry multiple genes. Unlike vaccinia, the canarypox virus does not appear to be virulent even in individuals with severe immune suppression. Another possible vector is an attenuated strain of *Salmonella typhimurium*, which has been engineered with genes from the bacterium that causes cholera. The advantage of this vector vaccine is that Salmonella infects cells of the mucosal lining of the gut and therefore will induce secretory IgA production. Effective immunity against a number of diseases, including cholera and gonorrhea, depends on increased production of secretory IgA at mucous membrane surfaces. One of the poliovirus strains used in the Sabin vaccine is another candidate for a safe and effective vector vaccine. In this case, the poliovirus vector is engineered so that a portion of the gene that encodes the outer capsid protein of poliovirus is replaced by DNA that encodes the epitope of choice. The resulting poliovirus chimera will express the desired epitope in a highly accessible presentation that protrudes from the nucleocapsid.

14.6. DNA VACCINES:

In a recently developed vaccination strategy, plasmid DNA encoding antigenic proteins are injected directly into the muscle of the recipient. The DNA is taken up by muscle cells and the encoded protein antigen is expressed, leading to both a humoral antibody response and a cell-mediated response. The DNA appears either to integrate into the chromosomal DNA or to be maintained for long periods in an episomal form. The vital antigen is expressed not only by the muscle cells but also by dendritic cells in the area that take up the plasmid DNA and express the viral antigen. The fact that the muscle cells express rather low levels of class I MHC molecules and do not express co-stimulatory molecules suggests that dendritic cells in the area may be crucial to the development of antigenic responses to DNA vaccines.

DNA vaccines offer advantages over many of the existing vaccines. The encoded protein is expressed in the host in its natural form – there is no denaturation or modification. The immune response will be directed to the antigen exactly as it is expressed by the pathogen. DNA vaccines induce both humoral and cell-mediated immunity. This stimulation of both arms of the immune response normally requires immunization with a live attenuated vaccine. DNA vaccines cause prolonged expression of the antigen, which generates significant immunological memory. The practical aspects of DNA vaccines are also very promising. Refrigeration is not required for the handling and storage of the plasmid DNA, a feature that greatly lowers the cost and complexity of delivery. The same plasmid vector can be custom tailored to make a variety of proteins, so that the same manufacturing techniques can be used for different DNA vaccines, each encoding an antigen from a different pathogen. An improved method for administering these vaccines is to coat microscopic gold beads with the plasmid DNA and then deliver the coated particles through the skin into the underlying muscle with an air gun called as gene gun. This will allow rapid delivery of a vaccine to large populations without the requirement for massive quantities of needles and syringes.

Tests of DNA vaccines in animal models have shown that these vaccines are able to induce protective immunity against a number of pathogens, including the influenza virus. At present, there are human trials underway with several different DNA vaccines, including those for malaria, AIDS, influenza, and herpesvirus. Future experimental trials of DNA vaccines will mix genes for antigenic proteins with those for cytokines or chemokines that direct the immune response to the optimum pathway. For example, the IL-12 gene may be included in a DNA vaccine. The expression of IL-12 at the site of immunization will stimulate TH_1 type immunity induced by the vaccine.

14.7. SYNTHETIC PEPTIDES VACCINES:

The use of synthetic peptides as vaccines has not progressed as quickly as originally projected. Peptides are not as immunogenic as proteins, and it is difficult to elicit both humoral and cellular immunity to them. The use of conjugates and adjuvants can assist in raising protective immunity to peptides, but barriers to the widespread use of peptide vaccines remain and pose an interesting problem for immunologists. Construction of synthetic peptides for use as vaccines to induce either humoral or cell-mediated immunity requires an understanding of the nature of T-cell and B-cell epitopes. Although the amino acid sequence of many important antigens from pathogens is known, few have been subjected to X-ray crystallographic analysis, so their three-dimensional structure is unknown. However, because B-cell epitopes must constitute accessible surface regions, vaccine designers commonly analyze the primary structure of an antigen to identify strongly hydrophilic sequences, which most likely correspond to such surface regions. Synthetic peptides corresponding to these potential B-cell epitopes are then prepared. Ideally, vaccines for inducing humoral immunity should include peptides that compose immunodominant B-cell epitopes. Such epitopes can be identified by determining the dominant antibody in the sera of individuals who are recovering from a disease and then testing various synthetic peptides for their ability to react with that antibody with a high affinity.

An effective memory response for both humoral and cell-mediated immunity requires generation of a population of memory T_H cells. A successful vaccine must therefore include immunodominant T-cell epitopes. Since MHC molecules differ in their ability to present peptides to T cells, MHC polymorphism within a species influences the level of T-cell response by different individuals to different peptides. Moreover, different subpopulations of T cells probably recognize different epitopes. Experiments by E. Sercarz have identified

some peptides that induce a strong helper response to an antigen and other peptides that induce immunogenic suppression. These helper and suppressor peptides generally represent different, non-overlapping amino acid sequences within an antigen. For example, immunization with the amino-terminal residues 1-17 of hen egg-white lysozyme suppressed the response to native lysozyme. By identifying suppressor peptides and eliminating them from synthetic vaccines, it might be possible to generate enhanced immunity.

14.8. MULTIVALENT SUBUNIT VACCINES:

One of the limitations of synthetic peptide vaccines and recombinant protein vaccines is that these vaccines tend to be poorly immunogenic; in addition, they tend to induce a humoral antibody response but are less likely to induce a cell-mediated response. Construction of a synthetic peptide vaccine, by some method, that contains both immunodominant B-cell and T-cell epitopes is needed. Furthermore, if a CTL response is desired, the vaccine must be delivered intracellularly so that the peptides can be processed and presented together with class I MHC molecules. A number of innovative techniques are being applied to develop multivalent vaccines that can present multiple copies of a given peptide or a mixture of peptides to the immune system.

One approach is to prepare solid matrix-antibody-antigen (SMAA) complexes by attaching monoclonal antibodies to particulate solid matrices and then saturating the antibody with the desired antigen. The resulting complexes are then used as vaccines. By attaching different monoclonal antibodies to the solid matrix, it is possible to bind a mixture of peptides or proteins, composing immunodominant epitopes for both T cells and B cells, to the solid matrix. These multivalent complexes have been shown to induce vigorous humoral and cell-mediated responses. Their particulate nature contributes to their increased immunogenicity by facilitating phagocytosis by phagocytic cells. Another means of producing a multivalent vaccine is to use detergent to incorporate protein antigens or synthetic antigenic peptides into protein micelles, into lipid vesicles, or into immunostimulating complexes. Micelles are formed by mixing proteins in detergent and then removing the detergent. The individual proteins orient themselves with their hydrophilic residues toward the aqueous environment and the hydrophobic residues at the center so as to exclude their interaction with the aqueous environment. Liposomes containing protein antigens are prepared by mixing the proteins with suspension of phospholipids under conditions that form vesicles bounded by a bilayer. The proteins are incorporated into the bilayer with the hydrophilic residues exposed. Immunostimulating complexes (ISCOMs) are lipid carriers prepared by mixing protein or peptide antigens with detergent and a glycoside called Quil A.

Membrane proteins from various pathogens, including influenza virus, measles virus, hepatitis B virus, and HIV have been incorporated into micelles, liposomes, and ISCOMs and are currently being assessed as potential vaccines. In addition to their increased immunogenicity, liposomes and ISCOMs appear to fuse with the plasma membrane to deliver the antigen intracellularly, where it can be processed by the cytosolic pathway and thus induce a cell-mediated response.

14.9. SUMMARY:

Three types of vaccines are currently used in humans: attenuated (avirulent) microorganisms, inactivated (killed) microorganisms, or purified macromolecules. Attenuated vaccines undergo transient growth in the recipient and therefore stimulate a more

pronounced immune response and memory-cell production without the requirement for additional boosters. However, they pose the risk of reversion to a pathogenic state. In activated vaccines require repeated boosters, but pose no risk of reversion to a pathogenic state. The use of purified macromolecules, which are less complex than whole organism vaccines, avoids certain complications due to unknown side effects. By applying recombinant DNA techniques, it is possible to produce large quantities of a defined antigen for immunization. Recombinant vectors, including vaccinia virus, canarypox virus, and attenuated S. *typhimurium*, can be engineered to carry multiple genes form infectious microorganisms. DNA vaccines induce expression of the antigen in its native state and induce both humoral and cell-mediated immunity. Synthetic peptides that represent immunodominant T-or B-cell epitopes are being evaluated as vaccines for several diseases. Various types of multivalent vaccines have been devised to induce both humoral and cell-mediated immunity.

14.10. TECHNICAL TERMS:

Attenuated vaccines, Killed vaccines, DNA vaccines, Recombinant vaccines, Synthetic peptide vaccines, Multivalent vaccines, B-cells, T-cells, Humoral immunity, Cell-mediated immunity.

14.11. SELF ASSESSMENT QUESTIONS:

1) Explain the attenuated or avirulent vaccines and their production with suitable examples.
2) Describe the use of inactivated or killed vaccines with suitable examples.
3) Give an account on recombinant vector vaccines, synthetic peptide vaccines and multivalent vaccines.
4) Explain the DNA and purified macromolecule vaccines.

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