TWO-YEAR
POST GRADUATE DEGREE PROGRAMME (CBCS)
IN
BOTANY

SEMESTER - IV

Course: BOTDSE T405.1

Advanced Immunology

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING
UNIVERSITY OF KALYANI
KALYANI – 741235, WEST BENGAL

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Director's Messeage

Satisfying the varied needs of distance learners, overcoming the obstacle of distance and reaching the unreached students are the threefold functions catered by Open and Distane Learning (ODL) systems. The onus lies on writers, editors, production professionals and other personal involved in the process to overcome the challenges inherent to curriculam design and production of relevant Self Learning Materials (SLMs). At the University of Kalyani a dedicated team under the able guidance of the Hon'ble Vice-Chancellor has invested its best efforts, professionally and in keeping with the demands of Post Graduate CBCS Programmes in Distance Mode to devise a self-sufficient curriculum for each course offered by the Directorate of Open and Distane Learning (DODL) University of Kalyani.

Development of printed SLMs for students admitted to the DODL within a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India under Open and Distance Mode UGC-DEB Regulations, 2020 had been our endeavour. We are happy to have achieved our goal.

Utmost care and precision have been ensured in the development of SLMs, making them useful to the lesrners, besides avoiding errors as far as practicable. Further, suggestions from the stakeholders in this would be welcome.

During the production-process of the SLMs, the team continuously received positive stimulations and feedback from **Professor (Dr.) Amalendu Bhunia**, Hon'ble Vice-Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticisms to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

Sincere gratitude is due to the respective chairpersons as well as each and every Members of PGBOS (DODL), University of Kalyani, Heartfelt thanks is also due to the Course Writers- faculty members at the DODL, subject-experts serving at University Post Graduate departments and also to the authors and academicians whose academic contributions have enriched the SLMs. We humbly acknowledge their valuable academic contributions. I would especially like to convey gratitude to all other University dignitaries and personnel involved either at the conceptual or operational level of the DODL of University of Kalyani.

Their persistent and co-ordinated efforts have resulted in the compilation of comprehensive, learners friendly, flexible text that meets curriculum requirements of the Post Graduate Programme through distance mode.

All the Self Learning Materials are self-writing and collected from e-Book, journals and websites.

Director
Directorate of Open & Distance Learning
University of Kalyani

SYLLABUS

COURSE – BOTDSE T405.1

Advanced Immunology

(Full Marks – 50)

Course	Group	Details Contents Structure		Study hour
BOTDSE T405.1	Advanced Immunology	**Unit 1. Modern Immunology**	1. Modern Immunology: Antigen presentation; Secondary signaling, co-stimulation, Cell signaling in immune response; DC activation, B cells as APC, experimental models in APC. Complements-Lectin pathway.	1
		Unit 2. Molecular immunology	2. Molecular immunology: Peptide epitopes, T cell B cell antigenic properties, prediction of T and B cell epitopes, Chimeric peptides, polytope vaccines, Major Histocompatibility Complex, Polymorphism transplantation.	1
		Unit 3. Clinical Immunology – I	3. Clinical Immunology: Cytokines: properties, receptor, antagonists, diseases, Therapeutic use of cytokines Experimental immunology: Vaccine development (Recombinant, Combined and polyvalent vaccines).	1
		Unit 4. Clinical Immunology – II	4. Clinical Immunology: Antigen Antibody reactions in diagnostics. Cancer Immunology, Transplantation immunology.	1
		Unit 5. Effector Mechanisms – I	5. Effector Mechanisms: Mucosal immunity, Peyer's patches, gut barriers, oral immunization, Oral tolerance, Cytotoxic response, ADCC, NK cells, CTL, Th, T regulation, Immunoregulation, anergy, tolerance, anti idiotype.	1
		Unit 6. Effector Mechanisms – II	6. Effector Mechanisms: Mechanisms of antiviral innate immune response.	1
		Unit 7. Immune Regulation Mechanisms – I	7. Immune Regulation Mechanisms: Brief account on immuno-induction.	1
		Unit 8. Immune Regulation Mechanisms – II	8. Immune Regulation Mechanisms: immunosuppression, immuno-tolerance, immuno-potentiation.	1

Content

COURSE – BOTDSE T405.1

Advanced Immunology

Soft Core Theory Paper **Credits = 2**

Content Structure

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1. Introduction
2. Course Objective
3. Modern Immunology: Antigen presentation; Secondary signaling, co-stimulation, Cell signaling in immune response; DC activation, B cells as APC, experimental models in APC. Complements-Lectin pathway.
4. Molecular immunology: Peptide epitopes, T cell B cell antigenic properties, prediction of T and B cell epitopes, Chimeric peptides, polytope vaccines, Major Histocompatibility Complex, Polymorphism transplantation.
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7. Immune Regulation Mechanisms: Brief account on immuno-induction, immuno□suppression, immuno-tolerance, immuno-potentiation.
8. Let's sum up
9. Suggested Readings
10. Assignments

1. Introduction

Advanced immunology is a branch of biology and medicine that covers the study of immune systems in all organisms. Immunology charts, measures, and contextualizes the physiological functioning of the immune system in states of both health and diseases; malfunctions of the immune system in immunological disorders (such as autoimmune diseases, hypersensitivities, immune deficiency, transplant rejection); and the physical, chemical, and physiological characteristics of the components of the immune system *in vitro*, *in situ*, and *in vivo*. This advanced immunology deals with Transplantation, Central & Peripheral Tolerance, Immune Regulation in Pregnancy, Breaking Tolerance: Autoimmunity & Dysregulation, Mucosal Immunity & Immunopathology, Regulation of Immunity & the Microbiome, Epigenetics & Modulation of Immunity, Inflammation and autoinflammation, T-cell mediated autoimmune diseases, Antibody-mediated autoimmune diseases. Advances in Immunology have provided students and researchers with the latest information in Immunology for over 50 years. You can continue to rely on Advances in Immunology to provide you with critical reviews that examine subjects of vital importance to the field through summary and evaluation of current knowledge and research.

2. Course Objectives

At the end of the course the learners will be able to:

- To understand the current status of knowledge within areas of cellular and molecular immunology
- To know about innate and acquired immune responses; cellular and molecular mechanisms of immunity; antigen processing and presentation; tissue-specific immune responses; immune-mediated pathologies; and vaccination
- To gather knowledge on normal regulation of immunity and ghow aberrations in the regulation can lead to immunological diseases.

- To assess the fundamental mechanisms underlying immunologic disease and associate these mechanisms with strategies for therapeutic modulation of the immune system.
- Perceive and understand the basic immunological principles underlying biotherapeutics, recognize the commonality among diverse organ-specific disease states, and infer the mechanisms of therapeutic effect.
- Analyze the medical literature reporting immunologic advances pertinent to their patients, cite the rationale for use of new immunodiagnostic and immunotherapeutic modalities in their patients, and serve as thought leaders within their medical communities.

3. Modern Immunology: Antigen presentation; Secondary signaling, co-stimulation, Cell signaling in immune response; DC activation, B cells as APC, experimental models in APC. Complements-Lectin pathway.

Objective: In this unit we will discuss about different aspects of antigen presentation, dendrite cell activation, How B cells acts as APC. E will discuss also about complement system.

Antigen Presentation:

Major histocompatibility complex (MHC) is a collection of genes coding for glycoprotein molecules expressed on the surface of all nucleated cells.

MHC I molecules are expressed on all nucleated cells and are essential for presentation of normal "self" antigens. Cells that become infected by intracellular pathogens can present foreign antigens on MHC I as well, marking the infected cell for destruction.

MHC II molecules are expressed only on the surface of antigen-presenting cells (macrophages, dendritic cells, and B cells). Antigen presentation with MHC II is essential for the activation of T cells. Antigen-presenting cells (APCs) primarily ingest pathogens by phagocytosis, destroy them in the phagolysosomes, process the protein antigens, and select the most antigenic/immunodominant epitopes with MHC II for presentation to T cells.

Cross-presentation is a mechanism of antigen presentation and T-cell activation used by dendritic cells not directly infected by the pathogen; it involves phagocytosis of the pathogen but presentation on MHC I rather than MHC II. T cells can only recognise antigens when they are displayed on cell surfaces. This is carried out by **Antigen-presenting cells (APCs)**, the most important of which are dendritic cells, B cells, and macrophages. APCs can digest proteins they encounter and display peptide fragments from them on their surfaces for other immune cells to recognise.

This process of antigen presentation allows T cells to "see" what proteins are present in the body and to form an adaptive immune response against them. In this article, we shall discuss antigen processing, presentation, and recognition by T cells.

Antigen Processing

Before an antigen can be presented, it must first be **processed**. Processing transforms proteins into antigenic peptides.

MHC Class I Molecules

Intracellular peptides for MHC class I presentation are made by proteases and the proteasome in the cytosol, then transported into the endoplasmic reticulum via TAP (Transporter associated with Antigen Processing) to be further processed.

They are then assembled together with MHC I molecules and travel to the cell surface ready for presentation.

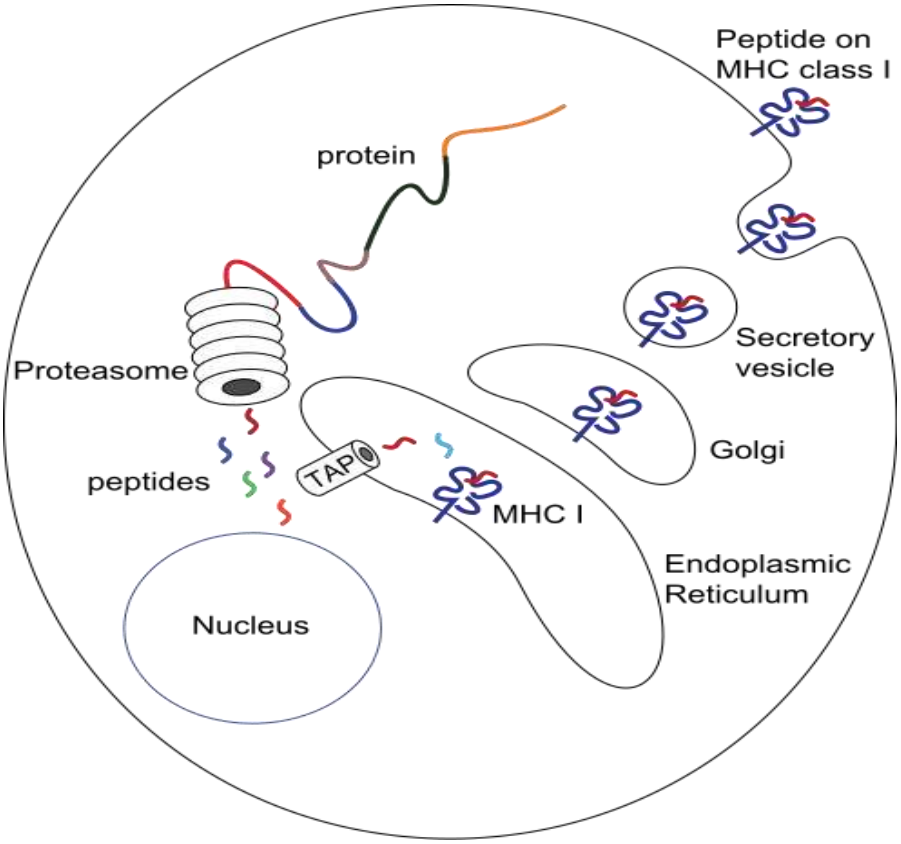


Fig.: – Diagram demonstrating the production of peptides for MHC class I presentation.

MHC Class II Molecules

The route of processing for **exogenous** antigens for MHC class II presentation begins with endocytosis of the antigen. Once inside the cell, they are encased within endosomes that acidify and activate proteases, to degrade the antigen.

MHC class II molecules are transported into **endocytic vesicles** where they bind peptide antigen and then travel to the cell surface.

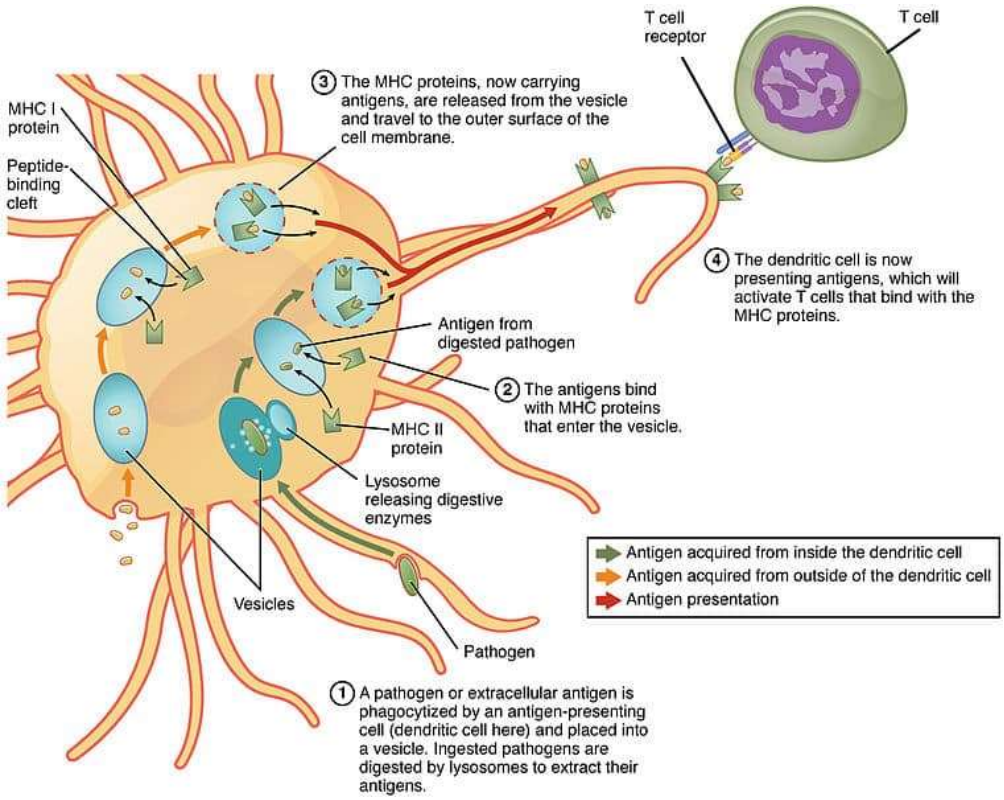


Fig.: – Diagram showing processing of antigens for MHC Class II presentation by a dendritic cell.

Antigen Presentation

The antigen presented on MHCs is recognised by T cells using a T cell receptor (TCR). These are antigen-specific.

T Cell Receptors

Each T cell has thousands of TCRs, each with a unique specificity that collectively allows our immune system to recognise a wide array of antigens.

This diversity in TCRs is achieved through a process called V(D)J recombination during development in the thymus. TCR chains have a variable region where gene segments are randomly rearranged, using the proteins RAG1 and RAG2 to initiate cleavage and non-homologous end joining to rejoin the chains.

The diversity of the TCRs can be further increased by inserting or deleting nucleotides at the junctions of gene segments; together forming the potential to create up to 1015 unique TCRs.

TCRs are specific not only for a particular antigen but also for a specific MHC molecule. T cells will only recognise an antigen if a specific antigen with a specific MHC molecule is present: this phenomenon is called MHC restriction.

Co-Receptors

As well as the TCR, another T cell molecule is required for antigen recognition and is known as a co-receptor. These are either a CD4 or CD8 molecule:

CD4 is present on T helper cells and only binds to antigen-MHC II complexes.

CD8 is present on cytotoxic T cells and only binds to antigen-MHC I complexes.

This, therefore, leads to very different effects. Antigens presented with MHC II will activate T helper cells and antigens presented with MHC I activate cytotoxic T cells. Cytotoxic T cells will kill the cells that they recognise, whereas T helper cells have a broader range of effects on the presenting cell such as activation to produce antibodies (in the case of B cells) or activation of macrophages to kill their intracellular pathogens.

Major Histocompatibility Complex Molecules

The major histocompatibility complex (MHC) is a collection of genes coding for MHC molecules found on the surface of all nucleated cells of the body. In humans, the MHC genes are also referred to as human leukocyte antigen (HLA) genes. Mature red blood cells, which lack a nucleus, are the only cells that do not express MHC molecules on their surface.

There are two classes of MHC molecules involved in adaptive immunity, MHC I and MHC II (Figure below). MHC I molecules are found on all nucleated cells; they present normal self-antigens as well as abnormal or nonself pathogens to the effector T cells involved in cellular immunity. In contrast, MHC II molecules are only found on macrophages, dendritic cells, and B cells; they present abnormal or nonself pathogen antigens for the initial activation of T cells.

Both types of MHC molecules are transmembrane glycoproteins that assemble as dimers in the cytoplasmic membrane of cells, but their structures are quite different. MHC I molecules are composed of a longer α protein chain coupled with a smaller β2 microglobulin protein, and only the α chain spans the cytoplasmic membrane. The α

chain of the MHC I molecule folds into three separate domains: α1, α2 and α3. MHC II molecules are composed of two protein chains (an α and a β chain) that are approximately similar in length. Both chains of the MHC II molecule possess portions that span the plasma membrane, and each chain folds into two separate domains: α1 and α2, and β1, and β2. In order to present abnormal or non-self-antigens to T cells, MHC molecules have a cleft that serves as the antigen-binding site near the "top" (or outermost) portion of the MHC-I or MHC-II dimer. For MHC I, the antigen-binding cleft is formed by the α1 and α2 domains, whereas for MHC II, the cleft is formed by the α1 and β1 domains (Figure below).

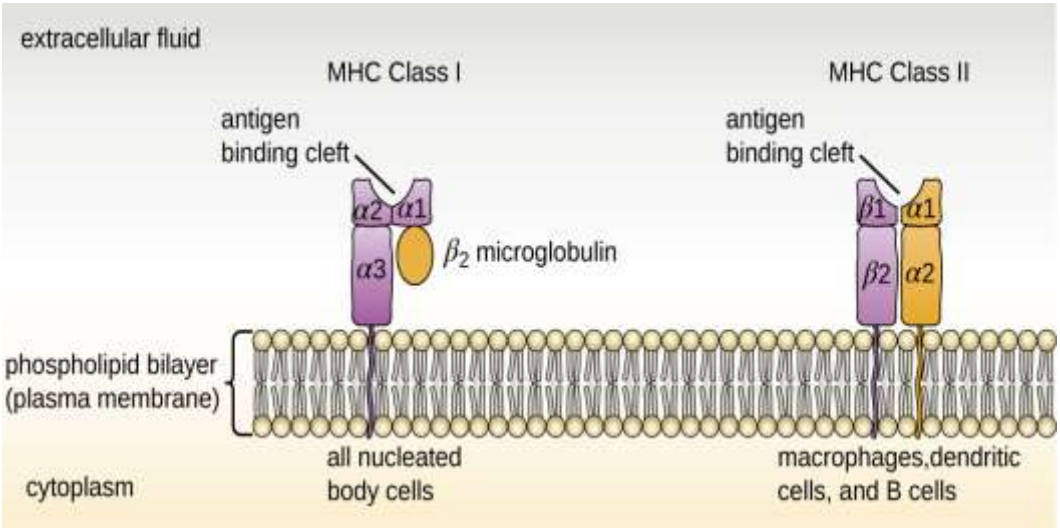


Fig.: – MHC I are found on all nucleated body cells, and MHC II are found on macrophages, dendritic cells, and B cells (along with MHC I). The antigen-binding cleft of MHC I is formed by domains α1 and α2.

Antigen-Presenting Cells (APCs)

All nucleated cells in the body have mechanisms for processing and presenting antigens in association with MHC molecules. This signals the immune system, indicating whether the cell is normal and healthy or infected with an intracellular pathogen. However, only macrophages, dendritic cells, and B cells have the ability to present antigens specifically for the purpose of activating T cells; for this reason, these types of cells are sometimes referred to as antigen-presenting cells (APCs).

While all APCs play a similar role in adaptive immunity, there are some important differences to consider. Macrophages and dendritic cells are phagocytes that ingest and kill pathogens that penetrate the first-line barriers (i.e., skin and mucous membranes). B cells, on the other hand, do not function as phagocytes but play a primary role in the production and secretion of antibodies. In addition, whereas macrophages and dendritic cells recognize pathogens through nonspecific receptor interactions (e.g., PAMPs, toll-like receptors, and receptors for opsonizing complement or antibody), B cells interact with foreign pathogens or their free antigens using antigen-specific immunoglobulin as receptors (monomeric IgD and IgM). When the immunoglobulin receptors bind to an antigen, the B cell internalizes the antigen by endocytosis before processing and presentting the antigen to T cells.

Antigen Presentation with MHC II Molecules

MHC II molecules are only found on the surface of APCs. Macrophages and dendritic cells use similar mechanisms for processing and presentation of antigens and their epitopes in association with MHC II; B cells use somewhat different mechanisms that will be described further in B Lymphocytes and Humoral Immunity. For now, we will focus on the steps of the process as they pertain to dendritic cells.

After a dendritic cell recognizes and attaches to a pathogen cell, the pathogen is internalized by phagocytosis and is initially contained within a phagosome. Lysosomes containing antimicrobial enzymes and chemicals fuse with the phagosome to create a phagolysosome, where degradation of the pathogen for antigen processing begins. Proteases (protein-degrading) are especially important in antigen processing because only protein antigen epitopes are presented to T cells by MHC II (Figure below)

APCs do not present all possible epitopes to T cells; only a selection of the most antigenic or immunodominantepitopes are presented. The mechanism by which epitopes are selected for processing and presentation by an APC is complicated and not well understood; however, once the most antigenic, immunodominant epitopes have been processed, they associate within the antigen-binding cleft of MHC II molecules and are translocated to the cell surface of the dendritic cell for presentation to T cells.

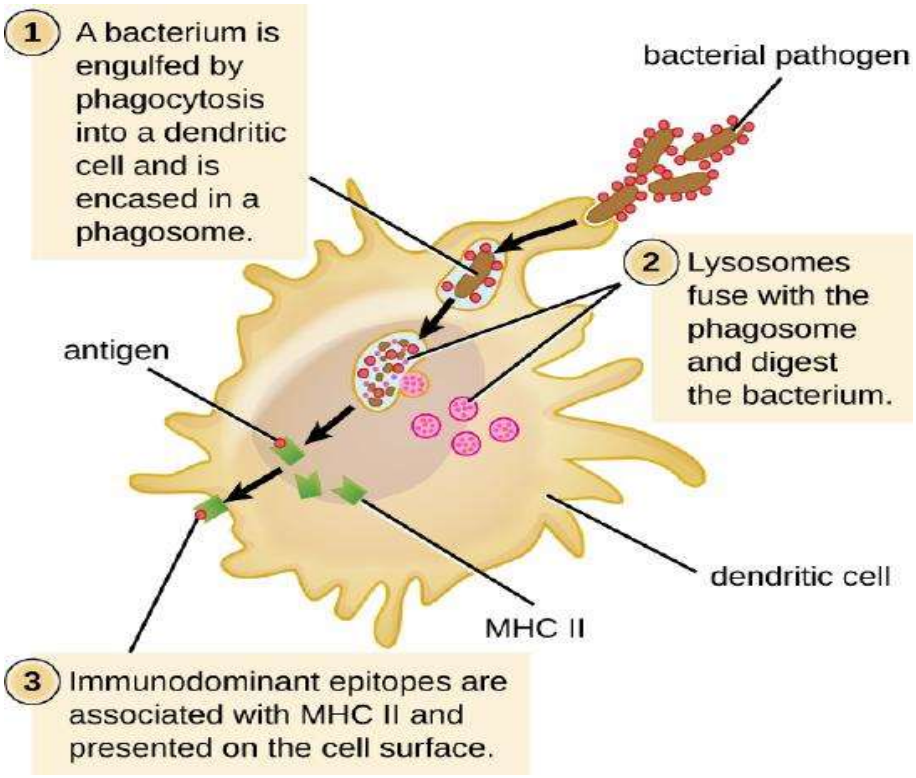


Fig.: – A dendritic cell phagocytoses a bacterial cell and brings it into a phagosome. Lysosomes fuse with the phagosome to create a phagolysosome, where antimicrobial chemicals and enzymes degrade the bacterial cell. Proteases process bacterial antigens, and the most antigenic epitopes are selected and presented on the cell's surface in conjunction with MHC II molecules. T cells recognize the presented antigens and are thus activatedThe antigen-binding cleft of MHC II is formed by domains α1 and β1.

Antigen Presentation with MHC I Molecules

MHC I molecules, found on all normal, healthy, nucleated cells, signal to the immune system that the cell is a normal "self" cell. In a healthy cell, proteins normally found in the cytoplasm are degraded by proteasomes (enzyme complexes responsible for degradation and processing of proteins) and processed into self-antigen epitopes; these self-antigen epitopes bind within the MHC I antigen-binding cleft and are then presented on the cell surface. Immune cells, such as NK cells, recognize these self-antigens and do not target the cell for destruction. However, if a cell becomes infected with an intracellular pathogen (e.g., a virus), protein antigens specific to the pathogen

are processed in the proteasomes and bind with MHC I molecules for presentation on the cell surface. This presentation of pathogen-specific antigens with MHC I signals that the infected cell must be targeted for destruction along with the pathogen.

Before elimination of infected cells can begin, APCs must first activate the T cells involved in cellular immunity. If an intracellular pathogen directly infects the cytoplasm of an APC, then the processing and presentation of antigens can occur as described (in proteasomes and on the cell surface with MHC I). However, if the intracellular pathogen does not directly infect APCs, an alternative strategy called cross-presentation is utilized. In cross-presentation, antigens are brought into the APC by mechanisms normally leading to presentation with MHC II (i.e., through phagocytosis), but the antigen is presented on an MHC I molecule for CD8 T cells. The exact mechanisms by which cross-presentation occur are not yet well understood, but it appears that cross-presentation is primarily a function of dendritic cells and not macrophages or B cells.

Complement System:

Definition:

The complement system consists of a series of heat-labile serum proteins that are activated in turn. The complements exist as soluble inactive precursors which once activated; a complement component may then act as an enzyme. Enzymatic chain reactions of this type are known as cascade reactions and usually require a “trigger” to initiate the reaction chain.

Complement is a chain of enzymes whose activation eventually results in the disruption of cell membranes and the destruction of cells or invading microorganisms. Complement is an essential part of the body defense system (Fig. below).

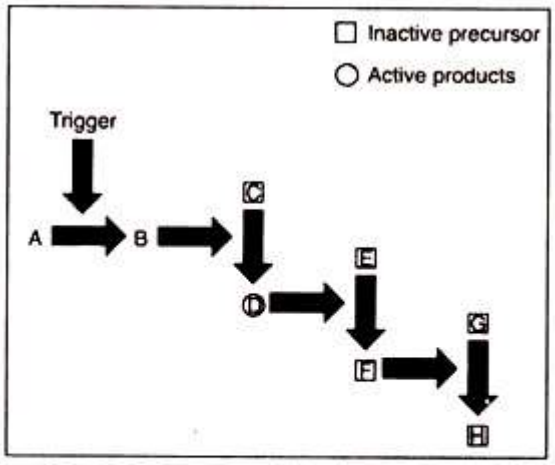


Fig. 7.1: Cascade reactions: The process is started by a trigger that initiates the conversion of an inactive proenzyme A to an active enzyme B. Enzyme B converts proenzyme C to active enzyme D. Enzyme D in turn converts pro-enzyme E to active enzyme F and so forth. The net effect is a very rapid acceleration in these reactions

History of Complement System:

The name "complement system" is derived from experiments performed by Jules Bordet.

Components of Complement System:

The complement system is made up of a number (mostly 30) of distinct serum (blood plasma) and membrane proteins which mostly assist the humoral branch of the immune system. As after initial activation, the various complement components interact sequentially to generate reaction products that facilitate antigen clearance and inflammatory response.

Different pathways of complement finally generate a macro-molecular membrane-attack complex (MAC) which helps to lyse a variety of cells, bacteria and viruses.

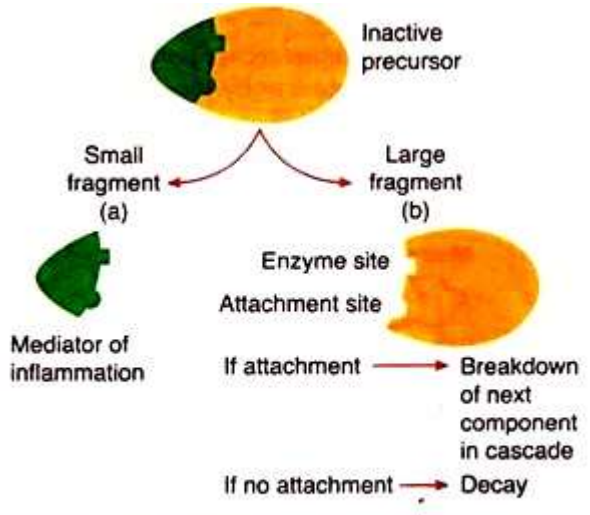


Fig. 7.7: Basic principle of underlying the cleavage of complement components

The complement products amplify the initial antigen-antibody reaction and convert that reaction into a more effective defense mechanism. Continuous proteolytic cleavage and activation of successive complement proteins lead to the covalent bonding or fixing of complement fragments to the pathogen surface. Each precursor of complement is cleaved into two major fragments- named as larger fragment (designated as 'b') and smaller fragment (designated as 'a').

The major or larger 'b' fragment has two biologically active sites—one binds to cell membranes to the target cell towards the site of activation and the other for enzymatic cleavage of the next complement component. The smaller 'a' fragments diffuse from the site and play a role in initiating a localized inflammatory response (chemotactic activity).

1. The proteins and glycoproteins composing the complement system are synthesized largely by liver hepatocytes, some by blood monocytes, tissue macrophages and epithelial cells of the gastro-intestinal and genitourinary tracts.

2. The proteins that form the complement system are labelled numerically with the prefix C (e.g., C_1–C_9).
3. Some complement components are designated by letter symbols (e.g., factor B, D, P) or by trivial names (e.g., homologous factor).

4. There are at least 19 of these components; they are all serum proteins and together they make up about 10% globulin fraction of serum.

5. The molecular weights of the complement components vary between 24 kDa for factor D and 460 kDa for C_{19}.

6. Serum concentration in humans varies between 20 µg/ml of C_2 and 1300 µg/ml of C_3.

7. Complement components are synthesized at various sites like C2, C3, C4, C5; B, D, P and I are from macrophages, C3, C6, C8 and B from liver

Table 7.2: The components of the complement system and their functions

Functionally distinct classes of complement protein	
Function	**Protein**
Binding to antigen; antibody complexes	C1q
Activating enzymes	C1r C1s C2b Bb D
Membrane-binding proteins and opsonins	C4b C3b
Peptide mediators of inflammation	C5a C3a C4a
Membrane attack proteins	C5b C6 C7 C8 C9

Pathways of Complement System:

1. The Classical Pathway of Complement:

The classical pathway of complement is initiated by the interaction of antibody with antigen directly (soluble antigen-antibody complexes or immune complexes).

The gradual progress of classical pathway can be mediated by these successive stages called:

(i) Activation of C1 component

(ii) Production of C3 convertase

(iii) Production of C5 convertase and

(iv) Action of membrane attack complex (MAC)

(i) Activation of CI component:

The initial stage of activation involves C1, C2, C3 and C4. The soluble antigen-antibody complex induces a conformational changes in the fragment crystalized (Fc) portion of the antibody molecule that exposes a binding site for the C1 component of the complement system.

(i) C1 is a complex macromolecular protein present in serum in inactive condition. It is a complex of three proteins named—C1q, C1r and C1s, out of which C1q recognizes and binds to the Fc region of the antibody and C1r and C1s remain as inactive proteases with their two subunits each. C1q and two molecules of each C1r and C1s held together is a complex called $C1qr_2s_2$ which is stabilized by Ca^{2+} ions.

(ii) The structure of C1 is mainly exhibited by C1q; a large molecule composed of 18 polypeptide chains that associate in such a way that forms six collagen-like triple helical arms. The amino-terminal two-thirds of the polypeptides form the stalk and the carboxy-terminal one-third of the polypeptides form the globular flower, which contains the binding site for antibody.

(iii) Normally, $C1r_2s_2$ complex remains in inactive form and never binds with C1q at that time and shows the configuration 'S'. Each C1r and C1s includes two

domains named catalytic domain and interaction domain. Due to action of interaction domain in presence of antigen- antibody complex in the serum it binds with C1q.

(iv) C1q binds to an antibody Fc region by its globular heads, in terms, activates serine proteases C1r and C1s which are proteolytic enzymes gives serine residues at the active site after being activated.

On binding to antibody, one molecule of C1r is induced to cleave itself, becomes enzymatically active. Gradually it cleaves and activates the second C1r and both C1s molecules. The activated serine protease C1s binds, cleaves and activates the next two components of the classical pathway i.e. serine protease C4 and C2. Ultimately active CI component is called $C1qr_2s_2$ (Fig. 7.9 and 10).

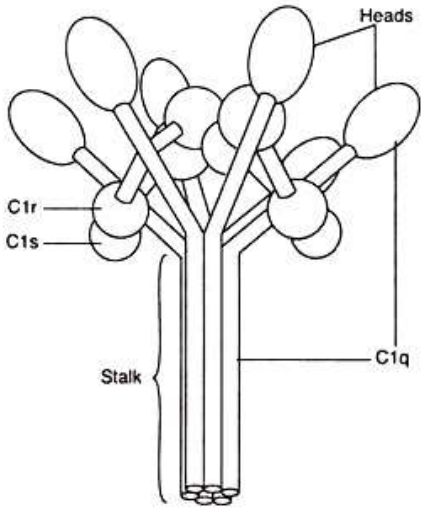


Fig. 7.9: Diagram of $C1qr_2s_2$ complex

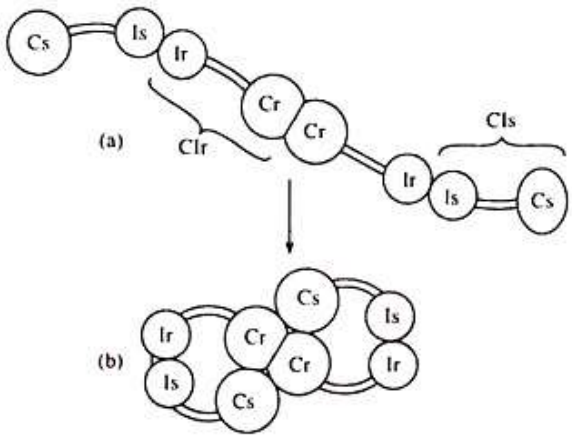


Fig. 7.10: Diagram of $C1qr_2s_2$ complex in relax state (a) and tense state (b)

Activation of classical pathway via IgM and IgG:

The cascade reaction of complement system is only initiated when antibody binds to multiple sites on a cell surface, normally that of a pathogen. When IgM (pentameric) is bound to antigen on a target surface, it requires at least three binding sites for C1q attachment.

In case of IgG molecule, it contains a single C1q binding site in the CH_2 domain of the Fc. As C1q globular head requires at least two Fc sites for a stable C1-antibody reaction, it indicates that two IgG are required to be present on a target surface.

The structural differences between IgM and IgG exert the effect on their activation level. At the activation of Clq binding, IgG requires less amount of time but a good number of IgG molecules are to be present. Whereas IgM activation is delayed one but it is more efficient, even a single IgM molecule can initiate the process (Fig. 7.11).

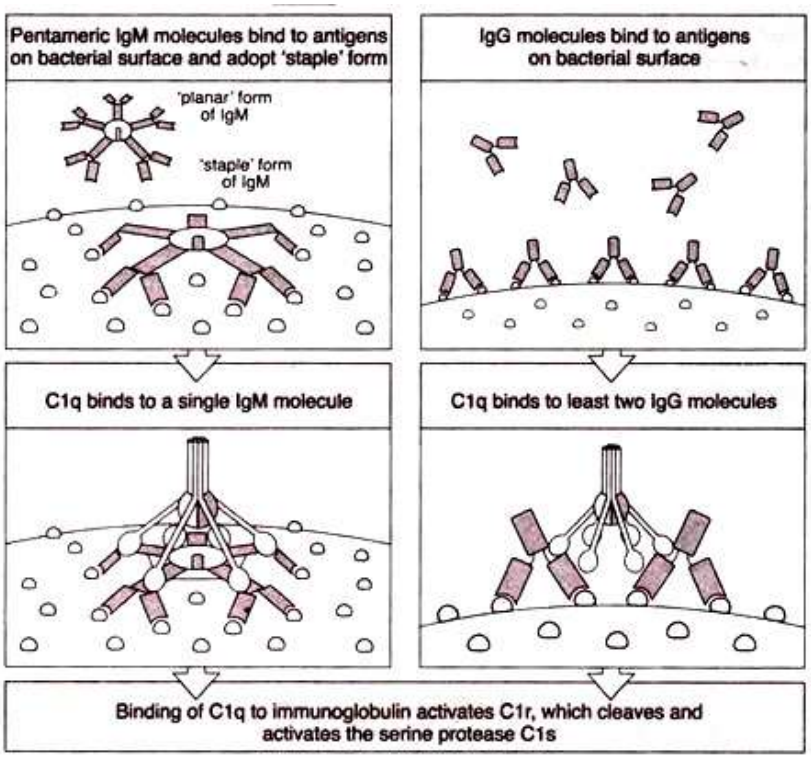


Fig. 7.11: **The classical pathway of complement activation is initiated by binding of C1q to antibody on a bacterial surface.** The binding of C1q to a molecule of pentameric IgM is shown in the left panels. On establishing multipoint binding to bacterial cell-surface antigens, the IgM molecule adopts a less planar conformation, the so-called staple conformation (upper panel). This distortion allows the C1q molecule to establish multipoint attachment to the Fc regions of a single IgM molecule, using the hinges in the C1q stalks to position the globular Fc-binding sites (lower panel). It also exposes binding sites for the C1q heads. The binding of C1q to IgG is shown in the right panels. The C1q molecule needs to find pathogen-bound IgG molecules that are close enough to each other for the C1q molecule to span between them. As a consequence, the activation of complement by IgG depends more on the amount and density of antibodies bound to a pathogen surface than does complement activation by IgM

(ii) Production of C3 convertase:

Active serine protease enzyme $C1qr_2s_2$ has two distinct substrates, C4 and C2. C4 component is a large globular glycoprotein containing three polypeptide chains named α, β and γ. C4 is activated when C1s hydrolyzes a small fragment C4a from the amino terminus of the chain, exposed a binding site on the larger fragment C4b. The C4b fragment attaches to the target surface of the C1 bound to antibody on the pathogen surface.

Besides, active C4 component, the activated C1s protease acts on C2 serine protease, as a result the smaller fragment C2b will be cleaved away from the site of action and C2a larger fragment will remain active at the active site. After that C4b2a active complex is formed which in turn act on the substrate C3 component. C4b2a is called C3 convertase of the classical pathway.

(iii) Production of C5 convertase:

C3 is almost very similar to C4. C3 component is with two types of polypeptide chains — α and β. C3 convertase (C4b2a) helps to cleave the smaller fragment C3a from the amino terminus of the a chain of C3 component.

Even a single C3 convertase molecule can accelerate the production of more than 200 molecules of C3b, and the result is amplification. In due course produced C3b binds with C4b2a to form a tri-molecular complex called C4b2a3b i.e. C5 convertase.

(iv) Action of membrane attack complex (MAC):

C5 convertase acts on C5 protein component, cleaves C5a from the action site and C5b attaches to the antigenic surface. This bound C5b initiates formation of membrane-attack complex (MAC) by taking participation of C6, C7, C8 and C9 components gradually and ultimately forms C5b6789 (MAC) which makes a large pore in the membrane of the antigen and accelerates lysis of it (Fig. 7.12).

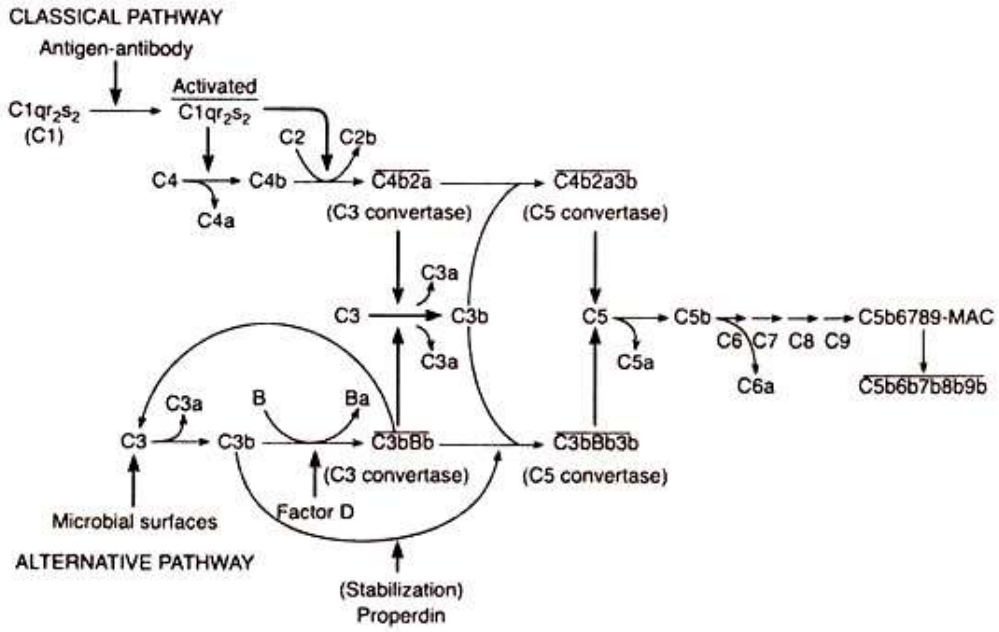


Fig. 7.12: Overview of the complement activation pathways (Classical and Alternative)

2. The Alternative Pathway:

Besides the classical pathway, complement system can be initiated by another method called alternative pathway. Unlike classical pathway the alternative pathway is initiated by the cell-wall constituents of both gram-positive and gram- negative bacteria as foreign particles.

Microbial surfaces directly affect the serine protease C3, gradually cleaving of C3 into C3a and C3b. This conformational change extends its effect on another factor i.e. factor B. In turn Ba removed from active site keeping Bb towards the C3b in presence of Mg^{++}; forms C3bBb, and considered as C3 convertase of alternative pathway.

Binding of C3b exposes a site on factor B that again serves as the substrate for an enzymatically active serum protein called factor D. Actually factor D cleaves the C3b bound factor B, and helps to form C3bBb. The action of C3bBb is very unstable, becomes stabilized by the presence of another exclusive serum protein properdin in this pathway, helps to increase the convertase activity period.

Formation of C3bBb accelerates the auto- catalyse of more C3 component and forms C3bBb3b as C5 convertase. Though structural basis of C3 and C5 convertase vary in these two pathways of complement system but their mode of action is alike.

Here, C3bBb3b subsequently hydropses the bound C5, C6, C7, C8 and C9 respectively, resulting in Membrane Attack Complex (MAC) formation which binds to the antigenic surfaces of microbes (antigen). MAC gradually displaces the membrane phospholipids, forms a large trans-membrane channel and gradually destroys the membrane and lysis of the antigen occurs.

The Lectin mediated pathway:

The third pathway of complement system is lectin-mediated pathway. Lectin-mediated pathway is activated by the binding of mannose-binding protein present in blood plasma to mannose containing proteoglycans on the surfaces of the bacteria and yeast, it forms MBP-MASP (Mannose-binding protein-mannose-associated serum protease). In lectin pathway MBP-MASP acts on the substrate C_4 and C_2 component protein. Three different pathways of complement activation is shown in the Fig. 7.13.

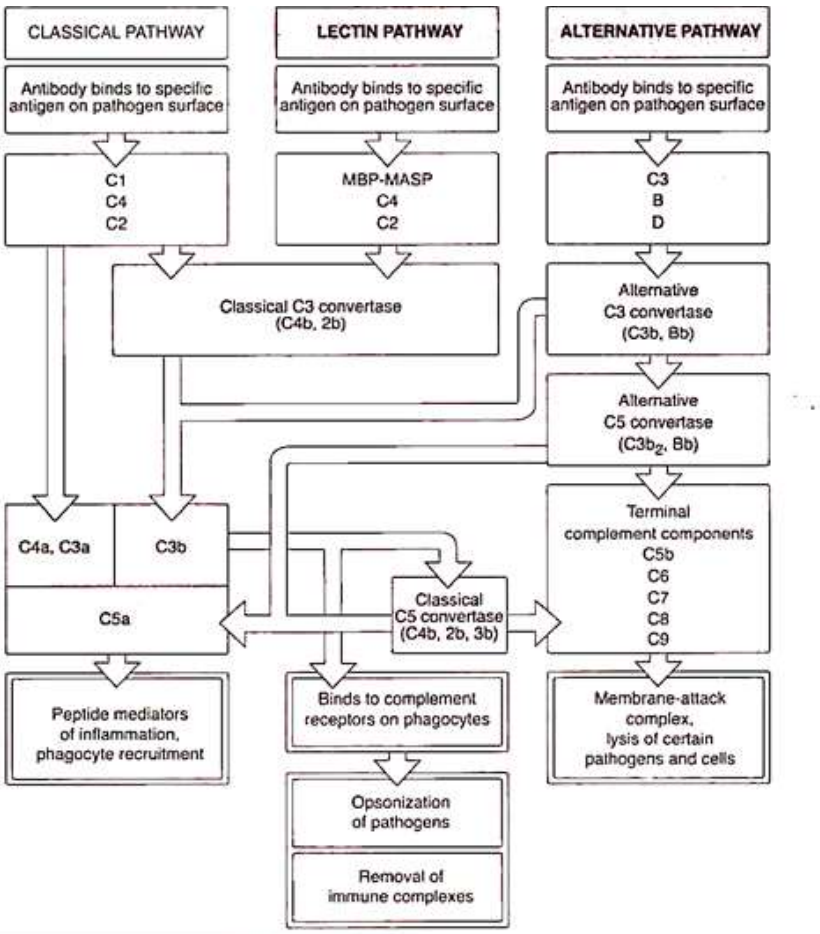


Fig. 7.13: Steps of the pathways of complement activation and action. The main difference between the three pathways is the recognition event that initiates activation and the steps that immediately succeed it. The function of the early part of all three pathways is to produce an enzyme called C3 convertase, which cleaves C3 into C3a and C3b fragments. The C3b fragment binds covalently to the pathogen's surface. C3b bound to microbial surfaces can either bind to complement receptors on phagocytic cells, which facilitates the phagocytosis of the pathgen, or can activate the terminal components of complement, which attack the integrity of the pathogen's cell membrane. The smaller C3a fragment, together with similar fragments cleaved from C4 and C5, induces inflammation by recruiting inflammatory cells into the area of complement activation. MBP, mannose-binding protein; MASP, MBP-associated serum protease

Biological Functions of Complement System:

Complements perform different biological functions like:

1. Cytolysis:

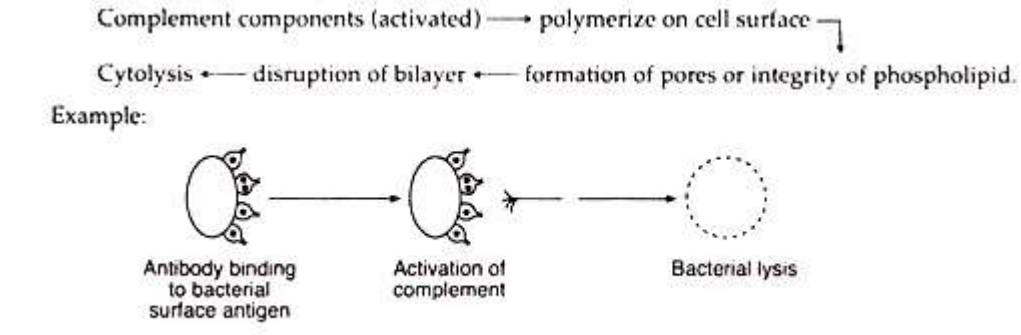


Fig. 7.3: Cytolysis of Bacteria

2. Opsonization:

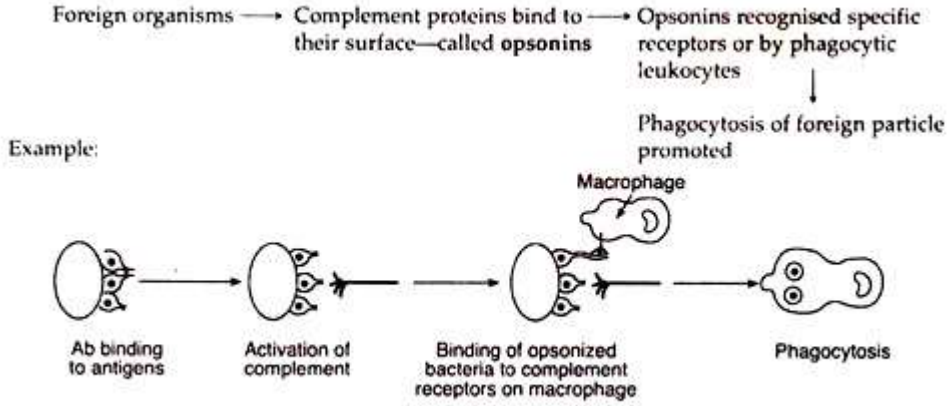


Fig. 7.4: Steps of opsonization

3. Activation of inflammation:

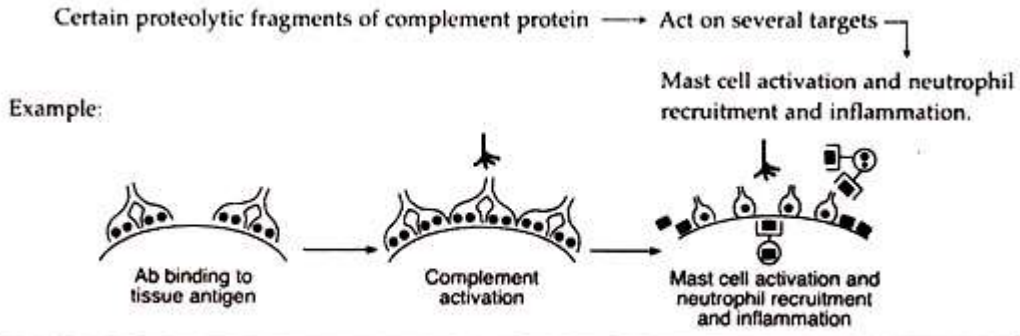


Fig. 7.5: Inflammatory response

4.

Solubilization and phagocytic clearance and immune complex:

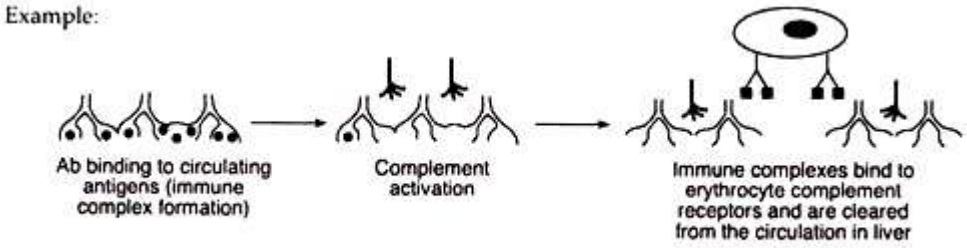


Fig. 7.6: Process of phagocytic clearance

4. Molecular immunology: Peptide epitopes, T cell B cell antigenic properties, prediction of T and B cell epitopes, Chimeric peptides, polytope vaccines, Major Histocompatibility Complex, Polymorphism transplantation.

Objective: In this unit we will discuss about different kinds of epitopes. We will also discuss about polytope vaccination process, MHC and polymorphism transplantation.

Antigen:

Definition:

Antigens are substances which, when introduced into the body, stimulate the production of antibodies.

Chemical Nature:

The antigens are mostly the conjugated proteins like lipoproteins, glycoproteins and nucleoproteins.

Structure:

Antigenic determinants or epitopes (Gk. epi – upon, topos- place) are components of antigen. Each antigen carries many epitopes. Each Y-shaped antibody molecule has atleast two binding sites that can attach to a specific epitope on an antigen. An antibody can also bind to identical epitopes of two different cells at the same time which can cause neighbouring cells to aggregate. Antigens combine with the antibody. The combination is very much like the lock and key analogy.

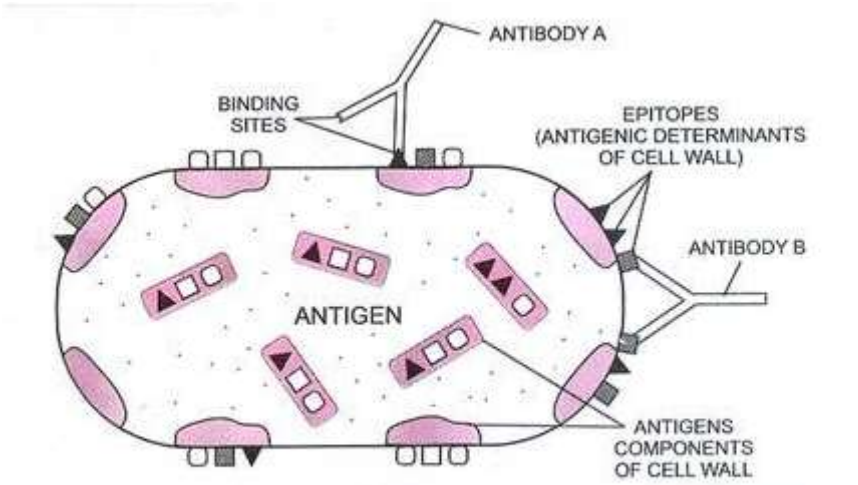


Diagram showing an antigen with epitopes (antigenic determinants). Two attached antibodies are also shown.\

Types:

Based upon the ability of antigens to carry out their functions, antigens are of two types: complete antigens and incomplete antigens (haptens). A complete antigen is able to induce antibody formation and produce a specific and observable reaction with the antibody so produced.

Haptens (Gr. hapten to grasp; partial antigens) are substances which are incapable of inducing antibody formation by themselves, but can be capable of inducing antibodies on combining with larger molecules (normally proteins) which serve as carriers.

Antigens which are present on the body's own cells are called the auto-antigens or self antigens. The antigens on the non-self cells are known as foreign antigens or non-self antigens.

H antigen:

Red blood corpuscles of all ABO blood groups possess a common antigen, the H antigen, which is a precursor for the formation of A and B antigens. Due to universal distribution, H antigen is not ordinarily important in grouping or blood transfusion.

However, Bhende et al (1952) from Mumbai reported a very rare example in which A and B antigens and H antigens were absent from the red blood corpuscles. This is known as Bombay or Oh blood group. Such individuals will have anti A, anti B and anti H antibodies. Therefore, they can accept the blood only from their own group.

Antigen Presenting Cells (APCs):

The cells that can engulf antigen and present fragments to T cells are called antigen presenting cells (APCs).

There are three types of antigen presenting cells in the body: macrophages, dendritic cells and B cells.

1. Macrophages:

Macrophages are usually found in a resting state. Their phagocytic capabilities are greatly increased when they are stimulated to become activated macrophages. The macrophages are present alongwith lymphocytes in almost all the lymphoid tissues, e.g., monocytes as blood macrophages and histocytes as tissue macrophages.

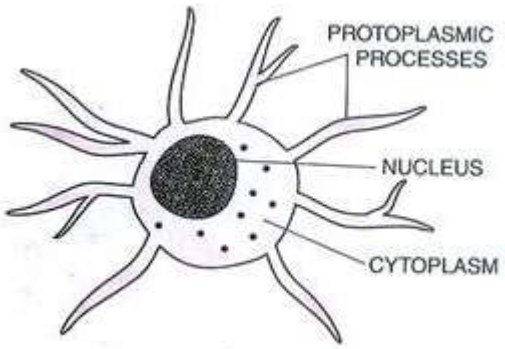
2. Dendritic Cells:

These cells are characterized by long cytoplasmic processes. Their primary role is to function as highly effective antigen-trapping and antigen presenting cells. These cells are nonphagocytic in nature. They are found in lymph nodes, spleen, thymus and skin. The different types of dendritic cells are:

a. Langerhan's dendritic cells in epidermis of skin which trap the organisms coming in contact with body surface.

b. Dendritic cells in spleen, which trap the antigen in blood.

c. Follicular dendritic cells in lymph nodes which trap the antigen in the lymph.



Dendritic Cell.

Thus macrophages and dendritic cells play an important role in the trapping and presentation of antigens to T and B cells to initiate the immune response.
Steinman was awarded Nobel Prize (2011) for his discovery of the dendritic cell and its role in adaptive immunity.

3. B-cells:

B-cells express on their surface intra-membrane immunoglobulin (Ig) molecules that function as B cell antigen receptors. Since all the receptors on a single B cell are identical, each B cell can bind only one antigen. This makes them much more efficient antigen-presenting cells than macrophages, which must ingest any foreign material that comes their way.
Descendants of B-cells (plasma cells) produce antibodies.

Meaning of Cell Surface Antigens:

Cells are antigenic. This means that when cells of one species are injected into another species, the recipient will first identify the injected cells as being of foreign origin and start producing antibodies to interact specifically with the alien cells. Therefore, the foreign cell that provokes antibody production in the recipient is called antigen.

If whole cells are injected, many cell surface components—like protein, carbohydrates or some combination of the two— may act as cellular antigen of the foreign cell. Several cell surface antigens have been studied in detail—the ABO blood group antigens, the MN blood group antigens, histocompatibility antigens etc. The most commonly studied cell surface antigens are discussed below.

Types of Surface Antigens:

(i) The ABO Blood Group Antigens:

The recognition of any blood grouping or specific type within it depends on the ability to detect the presence or absence of specific antigens on the red blood cells. Such antigens are found in the membrane of red cells or erythrocytes. In the human, classification of a person as blood type A, B, etc. is possible because of the presence of the detectable antigens.

If a certain antigen is present in the red blood cells, these may be clumped by the corresponding antibody when it is present. If the specific antigen is not present in the red blood cells, the corresponding antibody anti-A or anti-B is present in the blood serum.

A person of type AB blood is born with both A and B antigens in the red blood cells but no anti-A or anti-B antibody are found in the serum. The type O person lacks both A and B antigens into the membrane of the red blood cells, but the serum contains both antibodies anti-A and anti-B.

All these antigens are under genetic control so that an individual may possess either A or B antigen, or both, or neither. When neither antigen is present, the individual is called O type. However, individuals with O type erythrocytes possess an antigen called H which is the structural foundation on which A and B antigens are built. The antigens of ABO system are glycolipids, with the oligosaccharide portion of the glycolipid responsible for antigenic properties. The oligosaccharide antigen is covalently attached to sphingolipids which are immersed in the bi-molecular lipid leaflet of the plasma membrane. There are two types of oligosaccharide core chains which, ultimately, give rise to two subgroups of each antigenic type.

The difference is due to the type of linkage between galactose and N-acetylgalactosamine at the core of the oligosaccharide. In type I chain, galactose is attached with either N-acetyl glucosamine or N- acetylgalactosamine by β-1, 3 linkage, whereas in type II chain galactose is attached with N- acetyl glucosamine by β-1, 4 linkage. Erythrocytes that are O type, does not contain galactose or N-acetyl glucosamine or N- acetylgalactosamine as side chain and fucose is linked a-1, 2

galactose, a structure that constitutes the H antigen. From this unit the A and B antigens are enzymatically generated by glycosyltransferases that attach either on N-acetylgalactosamine unit (type A) or a galactose unit (type B).

The level of genetic control is exercised by presence or absence of glycosyl transferees that modify the H antigen; adding either an A or B determinant.

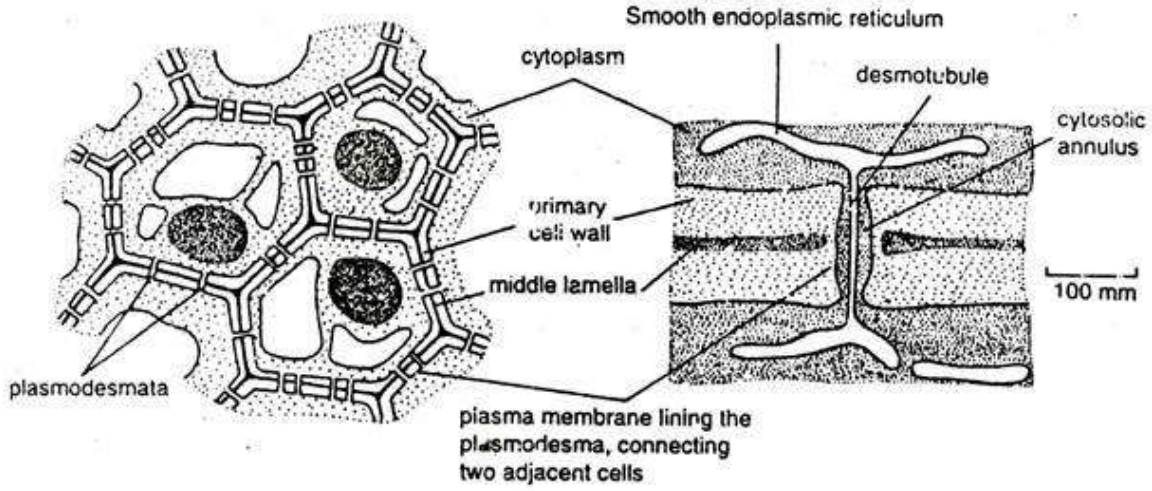


Fig. 4.26: Diagram of plasmodesmata.

(ii) The MN Blood Group Antigens:

The second major blood group system in human is MN system. In this case, the oligosaccharide is linked to a protein, glycophorin. It has two units. One "type of unit (A) is attached through asparagine, and the other unit (B) through serine or threonine. Variations on these structures exist.

They characteristically contain terminal sialic acid (N-acetylneuraminic acid). Both M and N determinants are destroyed when erythrocytes are treated with neuraminidase, an enzyme that removes sialic acid.

(iii) Histocompatibility Antigens:

Histocompatibility antigens are tissue cell surface proteins that differ from individual to individual. They are recognised by the mechanism of tissue graft rejection. There are different number of antigenic combinations possible on tissue surfaces and this is the

basis for the difficulties faced in getting a good tissue match for skin or organ transplantation surgery.

Some of the histocompatibility antigens are strong and others are weak. H-2 antigens in mice and HLA-antigen in humans have been studied in detail. Both these systems have many genetic variants. The H-2 antigen in mice is strongly antigenic. The intact H-2 antigen is made of two chains of which one is heavy or long and other is light or short.

The light claim is actually a protein and it is called β-2-micro-globulin. The heavy and light chains interact but not by covalent bonds. The proteolytic enzyme papain cleaves the heavy chain in such a way that a water-soluble portion called the F_s fragment, is released and a small piece, the F_m fragment is left in the membrane. Partial sequences have been worked out for the heavy chains of both H-2 and HLA antigens and similarities are seen between the two types of heavy chains.

Functions of Surface Antigens:

The chemical natures of several types of surface antigens are now known. Surface antigens have some biological importance and are involved in many functions. For examples, H-2 antigens may function during the mechanism of immunological surveillance.

If any change or modification in cell surface structures takes place—either by mutation or other means— the surface structures become abnormal. The abnormalities arising in cell surface are readily recognised by wandering lymphocytes in the immune system.

When a defective cell is found by the lymphocytes, a message is sent to the lymph modes where a class of lymphocytes called killer T cells are activated and, ultimately, destroy the abnormal target cell. The target cell must have an H-2 antigen plus an abnormal or modified antigen. The normal (H-2) and the abnormal antigens may be present independently, or they may form a hybrid surface structure. In any case they are then recognised or they may attract the lymphocytes without being physically attached to one another. In any event, in the absence of H-2 antigen, an abnormal cell will avoid destruction and proliferate.

Epitope:

1. An antibody that is specific for an antigen binds non-covalently to a region of the molecule surface known as epitope.

2. Naturally occurring epitopes are relatively small (either amino-acids or sugar residues).

3. Specific epitope should fit with the specific site present on antibody (antibody-binding site).

4. The site present on antibody called antigen- combining site or paratope is a cave pocket shaped one to match with the epitope having a convex site

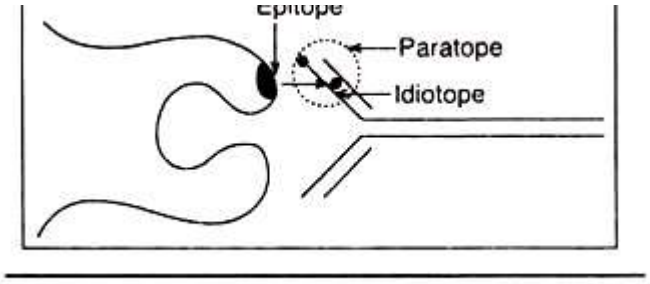


Fig. 4.8: Antigen recognition by antibody

5. Small antigens are mainly mono-epitopic where as large proteins and oligosaccharides can express many different and/or identical repeating multi-epitopes.

6. The forces responsible for binding include hydrophobic and Vander Waals forces, which are spherical, symmetrical and hydrogen bridges, which are directional and require matching of the reactants. Electrostatic forces might also contribute, but they act at distance.

7. Formation of stable immune complexes normally occur only when the epitope and paratope fit 'Jigsow Fashion'.

8. Not only the position of on epitope within a large molecule is important in determining its ability to induce an immune response, but the position of each subunit within the epitope may also be important. For e.g. each of the amino acid residues comprising a given accessible epitope might unequally contribute to bind with an

antibody paratope. Thus some components of an epitope are more immuno-dominant than others.

9. Any amino acid can contribute to a protein epitope. The residues that are not part of the epitope might not bind to antibody but might influence antigen conformation and affect epitope binding. Substitution of even a single amino acid in an epitope can affect binding of antibody.

Polytope Vaccines:

Vaccine-induced CD8 T cells directed to tumour-specific antigens are recognised as important components of protective and therapeutic immunity against tumours. Where tumour antigens have pathogenic potential or where immunogenic epitopes are lost from tumours, development of subunit vaccines consisting of multiple individual epitopes is an attractive alternative to immunising with whole tumour antigen. In the present study we investigate the efficacy of two DNA-based multiepitope ('polytope') vaccines containing murine (H-2b) and human (HLA-A*0201)-restricted epitopes of the E7 oncoprotein of human papillomavirus type 16, in eliciting tumour-protective cytotoxic T-lymphocyte (CTL) responses. We show that the first of these polytopes elicited powerful effector CTL responses (measured by IFN-gamma ELISpot) and long-lived memory CTL responses (measured by functional CTL assay and tetramers) in immunised mice. The responses could be boosted by immunisation with a recombinant vaccinia virus expressing the polytope. Responses induced by immunisation with polytope DNA alone partially protected against infection with recombinant vaccinia virus expressing the polytope. Complete protection was afforded against challenge with an E7-expressing tumour, and reduced growth of nascent tumours was observed. A second polytope differing in the exact composition and order of CTL epitopes, and lacking an inserted endoplasmic reticulum targeting sequence and T-helper epitope, induced much poorer CTL responses and failed to protect against tumour challenge. These observations indicate the validity of a DNA polytope vaccine approach to human papillomavirus E7-associated carcinoma, and underscore the importance of design in polytope vaccine construction.

Major Histocompatibility Compleex:

Definition of MHC Molecule:

The term histocompatible refers to the individuals who the same tissues i.e. identical twins. This term is used to determine how identical two unrelated individuals are. In case of two histocompatible individuals, a tissue or organ from a donor (the person giving the organ or tissue) that will not be rejected by the recipient (the patient in whom the tissue or organ is transplanted).

Thus, histocompatibility is the property of having the same or mostly the same alleles of a set of genes called the 'major histocompatibihty complex'. These genes are expressed in most tissues as antigens to which the immune system makes antibodies.

Major histocompatibility complex (MHC) is a tightly linked cluster of genes present on chromosome 6 in humans (and chromosome 17 in mice) which encodes the MHC proteins. The MHC proteins are present on plasma membrane of almost all human tissue/cells. The MHC proteins participate in intercellular recognition and antigen presentation to T lymphocytes.

Generally, a group of linked MHC genes is inherited as a unit from parents. These linked groups are called haplotypes. MHC genes are polymorphic {i.e. there are a large number of alleles for each gene). Also they are polygenic (i.e. there are a number of different MHC genes). Human MHC molecules are also called human leucocyte antigens (HLA). In the mid 1930s Peter Gorer (England) established the concept of rejection of foreign tissue due to an immune response to cell surface molecules. This gave the birth to the study of histocompatibility antigens. He identified four types of genes (I to IV) which encode blood cell antigens. During 1950 George Snell (U.S.A.) pioneered the concept that antigens encoded by the genes took part in the rejection of transplanted tumours. He called these genes as histocompatibility genes. For this work Snell was awarded the Nobel Prize in 1980.

Classes of MHC Molecules:

The MHC genes are organized into three classes I, II and III which express three classes of molecules Classes I, II and III, respectively (Table 22.5). Classes I MHC genes consists of A, B and C gene loci. They secrete glycoproteins which are referred to as Class I MHC molecule. Glycoproteins are expressed on the surface of about all nucleated cells. Class I MHC molecules present the peptide antigens to T_C cells.

The human Class I MHC gene spans about 2,000 kb (about 20 genes) at the telomeric end of the HLA complex, whereas the Class II MHC genes (about 1,000 kb) are located at the centromeric end of HLA. Class III genes (flanked by about 10,000 kb long) located between the two genes.

The DP, DQ and DR region of Class II MHC genes in humans encode the Class II MHC molecules called glycoproteins. They are expressed on antigen presenting cells such as macrophages, dendric cells and B cells, and present the processed antigenic peptides to T_H cells. Class II molecules have specialised function in the immune response.

Both Class I and Class II molecules have common structural features. They have role in antigen processing. In addition, the Class III MHC gene is flanked by Class I and Class II regions and encodes molecules critical to immune function. Class III MHC molecules consist of complement components C4, C2, BF, inflammatory cytokines, including tumour necrosis factor (TNF) and heat shock proteins.

Structure of MHC Molecules:

The Class I molecule is a trans-membrane glycoprotein consisting of two chains: a-chain or heavy chain (of 42 KD molecular weight) non-covalently associated with a light chain called β_2-micro-globulin (molecular weight 12 KD).

The α-chain is organized into three extracellular domains (α_1, α_2, α_3) and a 'trans-membrane segment' (hydrophobic) followed by a short stretch of hydrophilic 'cytoplasmic tail' (Fig. 22.20A). These are encoded by A, B and C regions of HLA complex and expressed on the surface of plasma membrane of almost all cells except erythrocytes.

B_2-micro-globulin molecule is expressed by different chromosomes. Association of the α-chain with B_2-micro-globulin is must for expression of Class I molecules on cell membrane. The α_1 and α_2 form the antigenic-binding cleft located on top of surfaces of molecule.

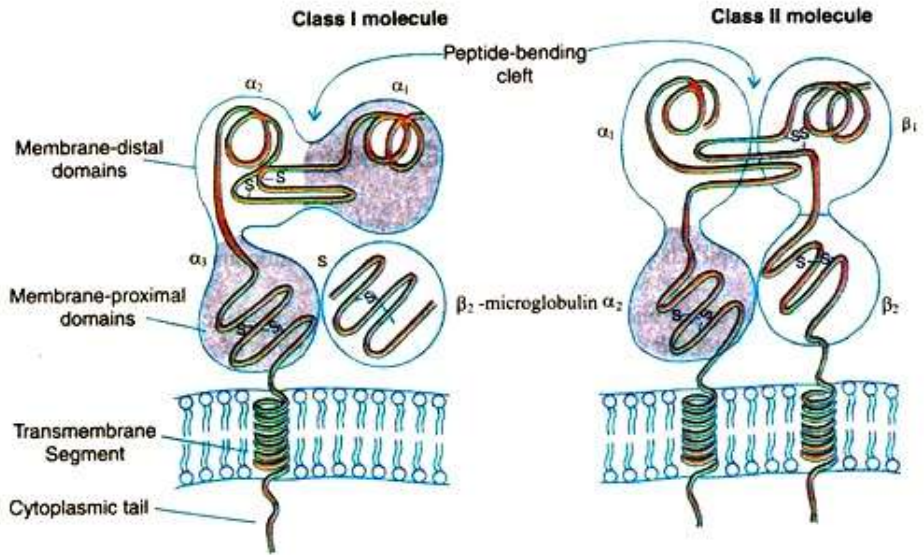


Fig. 22.20 : Structure of MHC molecules; A, Class I molecule; B-Class II molecule.

Class II MHC molecules are also trans-membrane glycoprotein encoded by separate MHC genes. They contain two different α and β chains of 33 and 28 KD, respectively. These two chains are associated non-covalently (Fig. 22.20B).

Further, both the chains fold to give two domains (β_1 and β_2 domains in other domain), one is membrane proximal domain and the second is membrane-distal domain. Like Class I MHC molecules, the class II molecules also contain trans-segment and a cytoplasmic anchor segment. Each chain of Class II molecule contains two external domains (α_1 and a_2 in one chain) and β_1 and β_2 domains in other chain.

Function of MHC Molecules:

MHC provides both cell mediated and humoral immune responses, while antibodies react only – with antigens, and most of the T cells recognise antigen only when it gets combined with an MHC molecule. Hence, MHC molecules act as antigen-presenting structure.

The MHC partly determines the response of an individual to antigens of infectious microorganisms. Therefore, it is implicated in susceptibility to disease and in the development of autoimmunity. Recently, it has been explained that the natural killer cells express receptors for MHC Class I antigens. The receptor-MHC interaction result in inhibition/activation.

Both Class I and Class II MHC molecules present the processed endogenous antigen to CD8 T cells. Class II molecules present the processed exogenous antigen to CD4 T cells. Class I molecules identifies mostly all the cells of the body as 'shelf. Also they induce the production of antibodies which introduced into host with different Class I molecules. This is the basis for MHC typing when a patient is to undergo for antigen transplantation.

Class II molecules comprise of the D group of MHC. They stimulate the production of antibodies. But they are required for T cell communication with macrophage and B cells. Part of T cells receptor recognises Class II molecules on the adjacent cell before cytokine secretion by T cells. This is necessary for immune response.

Both Class I and Class II molecules recognise the microorganisms. They are also involved in the susceptibility of an individual to a specific non-infectious diseases e.g. multiple sclerosis, acute glomerulonephritis, tuberculoid leprosy, paralytic poliomyelitis, etc. The Class III molecules (e.g. C_2, C_{4a} and C_{4b}) participate in the classical pathway and factor B in the alternate pathway of the immune responses.

Gene Regulation of MHC Expression:

Regulation of MHC genes has not been studied much. Understanding of complete genomic map of the MHC complex hopefully will accelerate the identification and coding, and regulatory sequences. Transcriptional regulation of the MHC is mediated by both positive and negative elements e.g. MHC II trans-activator (cll TA) and transcription factor (RFX) binds to promoter region of Class II MHC gene.

Any error in these transcription factors causes a type of disease in lymphocytes. Expression of MHC molecules is also regulated by many kinds of cytokines. Interferons and tumour necrosis factor increases the expression of Class I molecules on cells. Interferon-gamma induces the expression of cIITA.

Expression of MHC decreases after infection by certain viruses e.g. hepatitis B virus, and adenovirus 12, cytomegalovirus, etc. Adenovirus 12 causes a decrease in transcription of the transporter genes (TAP1 and TAP2). When these genes are blocked, class I molecules foil to assemble with β_2-micro-globulin. Decreased level of Class I molecules promotes viral infection. Expression of Class II molecules by B cells is down-regulated

by INF-gamma. Corticosteroids and rostaglandins decrease the expression of Class II molecules. The MHC has been divided into three regions on the basis of structure, function and alloreactivity of the gene products, which-are: 1. Class – I at the telomeric ends 2. Class – II at the centromeric ends 3. Class – III, which is located between class – I and II regions.

The classic class I and II genes code for the human leukocyte antigen (HLA), often referred to as histocompatibility or transplantation antigen (described above), while the non-classic genes encode non-HLA products with various immunologic functions. Other genes with no obvious functions in the immune system have also been mapped to the MHC because defects in such genes include the clinical syndromes of haemochromatosis, congenital adrenal hyperplasia and olivopontocerebellar ataxia.

Class – I genes:

Serologic HLA typing has permitted the recognition of three highly polymorphic class – I genes loci that encode the heavy chains of the HLA – A, B and Cw molecules. More recently, molecular analysis of this region has identified additional non classic Class -1 genes which are HLA – E, F and G, whose protein products have similar structures to HLA – A, B and C, but a more restricted tissue distribution.

Similarly the class – 1 pseudogenes like HLA – H, J, K and L are also identified. The HLA – A, B and C antigen's (glycoprotein) proton part (which is about 12 KD Molecular Weight) is encoded on chromosome 15. The nomenclature of individual class – I alleles - depend on whether they are identified and defined by serology or nucleotide sequence analysis.

Class – II genes:

The MHC class – II molecules are cell surface glycoproteins of which the protein part consists of two chain a and P, which are encoded by the class – II A and B genes in the MHC. There are at least 7 A genes and 16 B genes which can be divided into:

(a) Functional genes

(b) Pseudogenes

(c) Genes of unknown status.

The classic class – II molecules are HLA – DR, DQ and DP which are encoded by the DRA, DRB, DQA, DQB, DPA and DPB genes. The other expressed genes include DNA, DOB, DMA and DMB. The number of genes in the DR subregion varies depending on the haplotype.

All haplotype carry a single a-chain gene DRA and 2-5 β chain genes, but only one or two of these are expressed, while the others are pseudogenes. It has also been found that there are high polymorphism occurs in the class – II molecules and which is mainly due to 3 hypervariable regions in the α_1 and β_1 domains encoded by the second exons of the genes.

Class – III genes:

The MHC class – III regions contains genes that encode serum proteins involved in the effector functions of the immune system. These include components of the complement system that mediate cytotoxicity, phagocytosis and inflammation following exposure to antigen- antibody complexes and the inflammatory mediators, tumor necrosis factor (YNF) α and β.

Other immunoregulatory molecules encoded by the class – III regions of the MHC may include three proteins of the heat shock protein Hsp 70 family, Hsp 70-1, Hsp 70-2 and Hsp 70-Hom which have been predicted to serve a variety of immune functions, including protein folding and intracellular chapteroning and may play a part in antigen processing. Several novel expressed genes in the MHC, to which functions have not yet been attributed, may also ultimately be implicated in the immune system.

MHC and Disease Susceptibility:

Because of the important roles played by the products of the MHC genes in the immune system, it is not surprising that these genes, have an important influence on immune disorders.

These disorders can be divided mechanistically into two types:

1. Autosomal recessive immune deficiency caused by genetic defects that aborgate the synthesis of MHC gene products.

2. MHC associated disease concerns genetic susceptibility to a large number of disorders that involve MHC alleles which are also present in the general population.

The most commonly occurring MHC associated" diseases are:

(a) Autoimmune disease

(b) Insulin dependent Diabetes Mellitus

(c) Adult Rheumatoid arthritis

(d) Juvenile Rheumatoid arthritis

(e) Systemic Lupus Erythematosus

(f) Lupus Nephritis

(g) Autoimmune Hepatitis

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5. Clinical Immunology: Cytokines: properties, receptor, antagonists, diseases, Therapeutic use of cytokines Experimental immunology: Vaccine development (Recombinant, Combined and polyvalent vaccines), Antigen Antibody reactions in diagnostics. Cancer Immunology, Transplantation immunology.

Objective: In this unit we will discuss about properties and functions of cytokine molecules. We will also discuss about vaccination process . Antigen antibody interactions and cancer immunology will also be discussed along with transplantation immunology.

Cytokines:

1. Cytokines are a group of low-molecular- weight regulatory proteins secreted by WBC and other cells in the body.

2. Cytokine secretion is very specific and self- limited event as because they are not usually stored as performed molecules. Cytokine synthesis is initiated by new gene

transcription as a consequence of cellular activation. Once synthesized, cytokines are rapidly secreted, resulting in a burst of release when needed.

3. After secretion, cytokines (almost 60 different types of cytokines) regulate immune and inflammatory reactions (Fig. 8.1A, B).

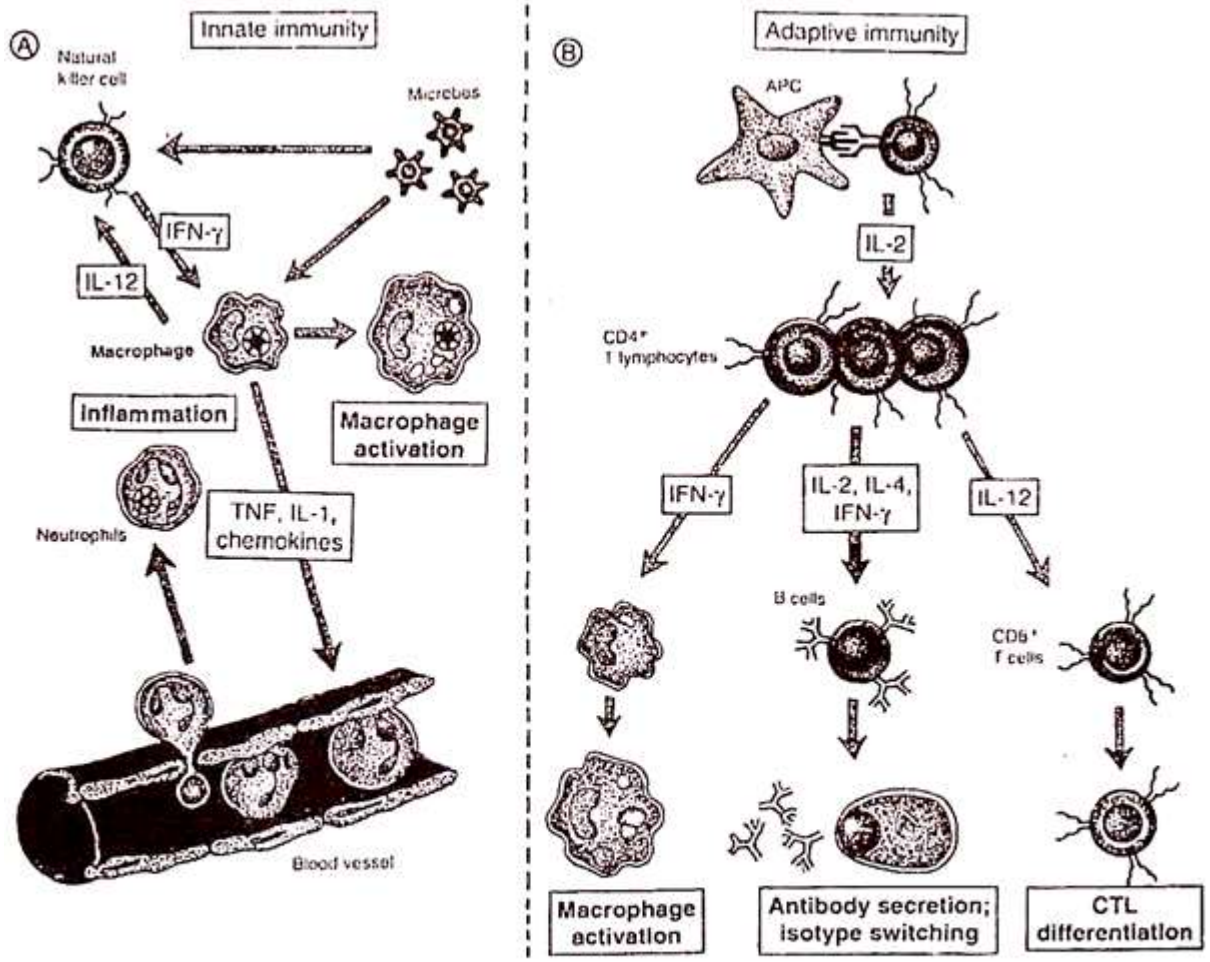

Fig. 8.1: Functions of selected cytokines in host defense. In innate immunity, cytokines produced by macrophages and NK cells mediate the early inflammatory reactions to microbes and promote the elimination of microbes. In adaptive immunity, cytokines stimulate proliferation and differentiation of antigen-stimulated lymphocytes and activate specialized effector cells, such as macrophages. The properties of the cytokines shown in this figure are discussed later in this chapter. APC, antigen-presenting cell

4. Cytokines bind to specific receptors on the membrane of target cells, triggering signal- transduction pathways that ultimately alter gene expression in the target cells (Fig. 8.2). The nature of the target cell for a particular cytokine is determined by the presence of specific membrane receptors. Cytokines are so specific due to their high affinity for which Pico molar concentrations of cytokines can mediate a biological effect.

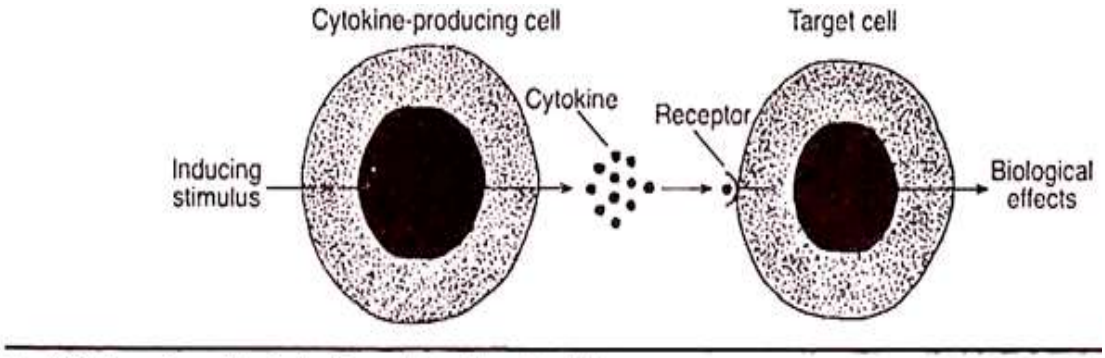

Fig. 8.2: Overview of the induction and function of cytokines

5. Cytokine actions may be local or systemic.

(i) Most of the cytokines act close to where they are produced, A particular cytokine when binds to receptors on the membrane of the same cell from where it has been secreted is called autocrine action (Fig. 8.3A).

(ii) When secreted cytokines bind to receptors on a target cell in close proximity to the producer cells, it is called paracrine action (Fig. 8.3B).

(ii) In most of the cases, cytokines act on cells that are in contact with the cytokine producers but when cytokines are produced in large amounts, it may enter the circulation and act at a distance from the site of production (Fig. 8.3C). This is called endocrine action.

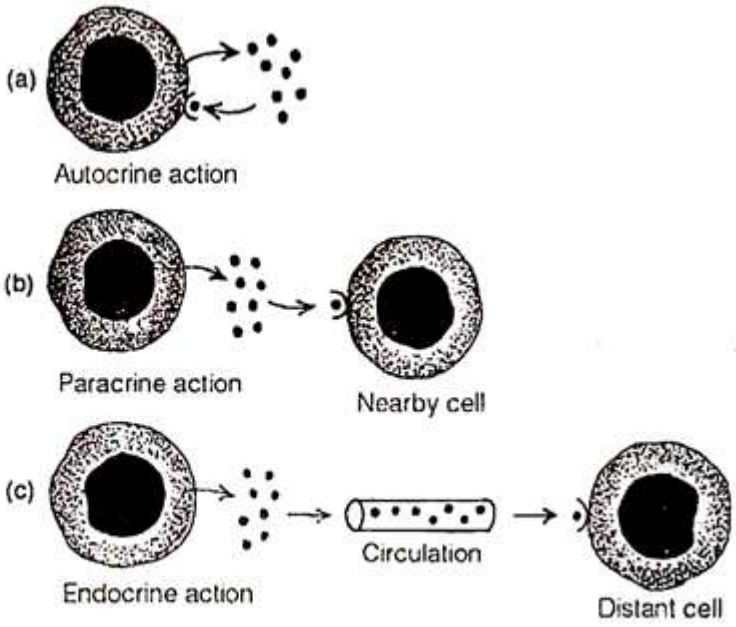

Fig. 8.3: Most cytobacteria exhibit autocrine and/or paracrine action; fewer exhibit endocrine action

6. Cytokines often influence the synthesis and actions of other cytokines and different immune cells. The ability of one cytokine to stimulate production of others, leads to cascade in which a second or third cytokine may mediate the biological effects of the first. Cytokines regulate the intensity and duration of the immune response by stimulating or inhibiting the activation, proliferation and differentiation of various cells.

7. The cytokines secreted by a single lymphocyte following antigen-specific activation can influence the activity of various cells involved in the immune response. For example, cytokines produced by activated T_H cells (T-helper cells) can influence the activity of B-cells, Tc cells, natural killer cells (NK cells), macrophages, granulocytes and haematopoietic stem cells. This means that it activates an entire network of interacting cells.

8. Cytokines exhibit the attributes of pleiotropy, redundancy, synergy and antagonism. Pleiotropism refers to the ability of one cytokine to act on different cell types which action; fewer exhibit endocrine action allows a cytokine to mediate diverse biological effects (Fig. 8.4A).

Redundancy refers to the property of multiple cytokines i.e. two or more cytokines having the same functional effects. This property of cytokine makes it difficult to explain a particular activity to a single cytokine (Fig. 8.4B).

Synergism exhibits to the phenomenon when the combined effect of two cytokines on cellular activity is greater than the additive effects of the individual cytokines (Fig. 8.4C).

Antagonism indicates the property that is just opposite to the synergism as in this case, the effects of one cytokine inhibit or offset the effects of another cytokine (Fig. 8.4D).

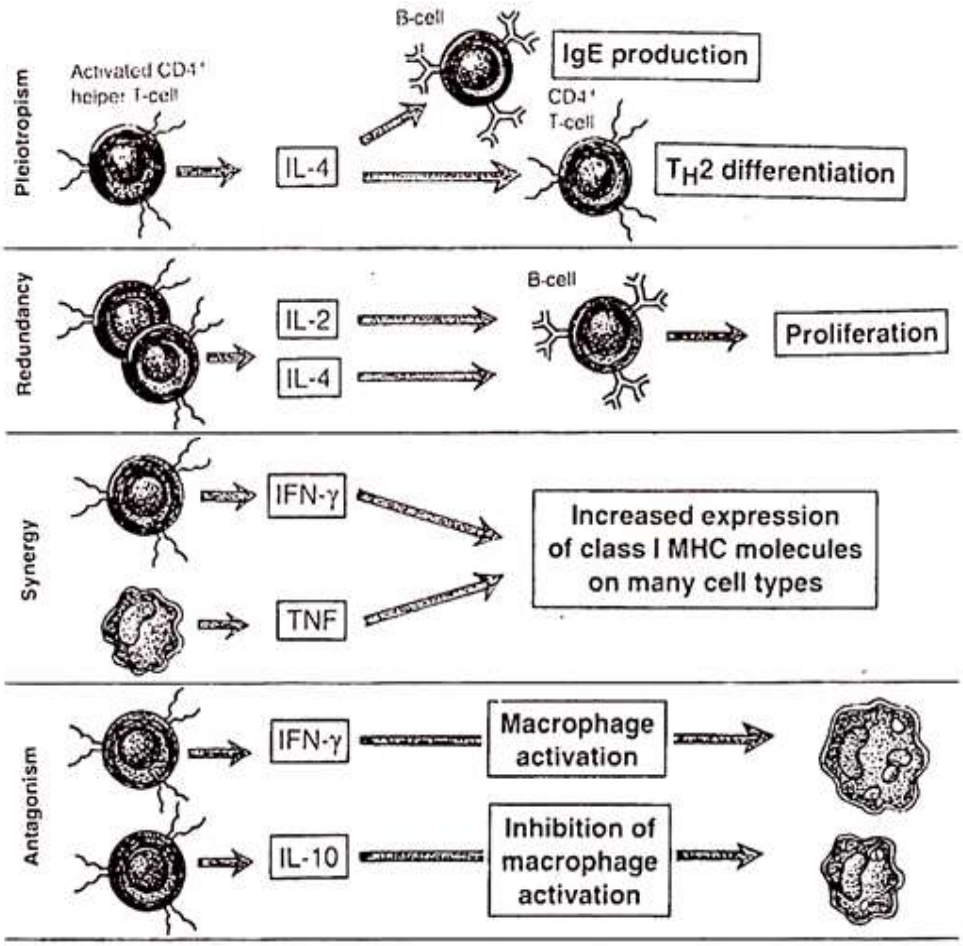

Fig. 8.4: Properties of cytokines. Selected examples are shown to illustrate the following properties of cytokines: **pleiotropism**, one cytokine having multiple effects on diverse cell types; **redundancy**, multiple cytokines having the same or overlapping actions; **synergy**, two or more cytokines having greater than additive effects; and **antagonism**, one cytokine inhibiting the action of another

9. Cytokine activity is also being regulated by external signals, the expression of cytokine receptors vary and also the responsiveness of cells to cytokines. As for

example, stimulation of T or B-lymphocytes by antigens leads to increased expression of cytokine receptors.

10. Many of the changes in gene expression induced by cytokines result in differentiation of T and B-lymphocytes and activation of effector cells such as macrophages.

11. Besides activation, cellular responses to cyto¬kines can also include feedback inhibitory signals to the cytokine activity. These mechanisms include cytokine induction of gene encoding inhibitors of the cytokine receptors. These inhibitors may inhibit the function of cytokine receptors expressed on the cell sur¬face, molecules that block interactions of signalling kinases, phosphatases.

On the basis of principal biologic actions; cytokines are grouped under three basic categories:

(i) Mediators and regulators of innate immunity:

These are produced mainly by mononuclear phagocytes in response to infectious agents: Pathogen-associated molecular patterns; like bacterial lipopolysaccharides (LPS) and viral double stranded RNA (dsRNA), also bind to Toll like receptors (TLRs) on the cell surface or in endosomes of macrophages and stimulate the synthesis of some important cytokines of innate of Innate immunity. They act on endothelial cells and leukocytes.

(ii) Mediators and regulators of adaptive immunity:

These are produced mainly by T-lymphocytes in response to specific recognition of foreign antigens. Some T-cell cytokines regulate the growth and differentiation of various lymphocyte populations and are related with T cell-dependent immune responses. These cytokines also regulate and activate mononuclear phagocytes, neutro-phils and eosinophils.

The comparative feature of cytokines of Innate and Adaptive immunity are placed in Table 8.3.

Table 8.3: Comparative Features of the Cytokines of Innate and Adaptive Immunity

Features	Innate immunity	Adaptive immunity
Examples	TNF, IL-1, IL-12, IFN-γ *	IL-2, IL-4, IL-5, IFN-γ *
Major cell source	Macrophages, NK cells	T-lymphocytes
Principal physiologic functions	Mediators of inflammation (local and systemic)	Regulation of lymphocyte growth and differentiation; activation of effector cells (macrophages, eosinophils, mast cells)
Stimuli	LPS (endotoxin), bacterial peptidoglycans, viral RNA, T cell-derived cytokines (IFN-γ)	Protein antigens
Amounts produced	May be high; detectable in serum	Generally low; usually undetectable in serum
Local or systemic effects	Both	Usually local only
Roles in disease	Systemic diseases (e.g. septic shock)	Local tissue inury (e.g., granulomatous inflammation)
Inhibitors	Corticosteroids	Cyclosporine, FK-506

* IFN-γ plays important roles in innate and adaptive immunity.

Abbreviations: IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; NK, natural killer; TNF, tumor necrosis factor

(iii) Stimulators of haematopoiesis:

These are produced by bone marrow stromal cells, leukocytes and other cells, and stimulate the growth and differentiation of immature leukocytes. Therefore, in general, the cytokines of innate and adaptive immunity are produced by different cell populations and act on different target cells. The functions of different cytokines are mentioned in Table 8.4.

Interleukin 5	T_H2 cells, mast cells	Activated B-cells	Stimulates proliferation and differentiation; induces class switch to IgA
		Eosinophils	Promotes growth and differentiation
Interleukin 6	Monocytes, macrophages, T_H2 cells, bone-marrow stromal cells	Proliferating B-cells	Promotes terminal differentiation into plasma cells
		Plasma cells	Stimulates antibody secretion
		Myeloid stem cells	Helps promote differentiation
		Hepatocytes	Induces synthesis of acute-phase proteins
Interleukin 7 (IL-7)	Bone-marrow, thymic stromal cells	Lymphoid stem cells	Induces differentiation into progenitor B and T-cells
		Resting T-cells	Increases expression of IL-2 and its receptor
Interleukin 8 (IL-8)	Macrophages, endothelial cells	Neutrophils	Chemokine; chemotactically attracts; induces adherence to vascular endothelium and extravasation into tissues
Interleukin 9 (IL-9)	T_H cells	Some T_H cells	Acts as mitogen, supporting proliferation in absence of antigen
Interleukin 10 (IL-10)	T_H2 cells	Macrophages	Suppresses cytokine production and thus indirectly reduces cytokine production by T_H1 cells
		Antigen-presenting cells	Down-regulates class II MHC expression
Interleukin 11 (IL-11)	Bone-marrow stromal cells	Plasmacytomas	Supports growth
		Progenitor B-cells	Promotes differentiation
		Megakaryocytes	Promotes differentiation
		Hepatocytes	Induces synthesis of acute-phase protiens
Inerleukin 12 (IL-12)	Macrophages, B-cells	Activated T_C cells	Acts synergistically with IL-2 to induce differentiation into CTLs
		NK and LAK cells and activated T_H1 cells	Stimulates proliferation
Interleukin 13 (IL-13)	T_H cells	Macrophages	Inhibits activation and release of inflammatory cytokines; important regulator of inflammatory response
Interleukin 15 (IL-15)	T-cells	T-cells, intestinal epithelium	Stimulates growth of intestinal epithelium, T-cell proliferation
		NK	Supports proliferation
		Activated B-cells	Co-mitogen for proliferation and differentiation
Interleukin 16 (IL-16)	T-cells (primarily $CD8^+$) Eosinophils	$CD4^+$ T-cells	Chemotaxis; induces expression of class II MHC; induces synthesis of cytokines; suppresses antigen-induced proliferation.

Vaccines:

The immune system is a complex system of interacting cells whose primary function is to identify foreign from self and eliminate it, usually referred to as “antigens”. The defense system of the body against this antigen usually involves the elicitation of both innate and adaptive branch of immunity. Acquired immunity concerns the production of protein molecules by B lymphocytes, called antibodies (or immunoglobulins), and of specific cells, including T-lymphocytes (also known as cell-mediated immunity) along

with the innate immunity involving the cells like macrophages, complement mediated lysis, production of pro-inflammatory cytokines etc. to eliminate the antigen. The most effective immune responses are generally produced in response to a live antigen. However, an antigen does not necessarily have to be alive, as occurs with infection with a virus or bacterium, to produce an immune response. Some proteins, such as hepatitis B surface antigen and other molecules like polysaccharide (long chains of sugar molecules that make up the cell wall of certain bacteria) are easily recognized by the immune system. Immunity can be achieved either by passive immunity or by active processes. Passive immunity is the transfer of preformed antibody produced by one human or other animal to another. Passive immunity provides protection against some infections, but this protection is temporary as the antibodies produced soon degrades during a period of weeks to months, and the recipient will no longer be protected. Active immunity is stimulation of the immune system to produce antigen-specific humoral (antibody) and cellular immunity. Unlike passive immunity, which is temporary, active immunity usually lasts for many years, often for a lifetime. The most tried and tested method to generate active immunity is through vaccines. Vaccines interact with the immune system and often produce an immune response similar to that produced by natural infection, but they do not subject the recipient to the disease and its potential complications. Many vaccines also produce immunologic memory similar to that acquired when infected by the natural disease. A vaccine is defined as a preparation of bacterial, viral or other pathogenic agents or their isolated peptides which is administered with the objective of eliciting the recipient's immunity. First ever encounter to the antigen elicits a primary immune response to the immune-compromised lymphocytes, which peaks at the fourteenth day of antigenic challenge.

This primary immune response leads to formation of IgM type of immunoglobulins, leading to activation of both B and T lymphocytes as well as memory cells are formed. Subsequent exposure to the same antigen leads to the secondary response due to memory cells, which is rapid and the response to the pathogen is by the high –affinity IgG type of immunoglobulins (Fig. 1). It is this rapidity towards secondary exposure to the antigen that protects the host against the potential threat by repeated attack by the same pathogen. Thus vaccine is basically an antigen or its component that can induce the secondary immune responses in the host. We can broadly say that a vaccine aims to introduce immunological memory against a pathogen in the host.

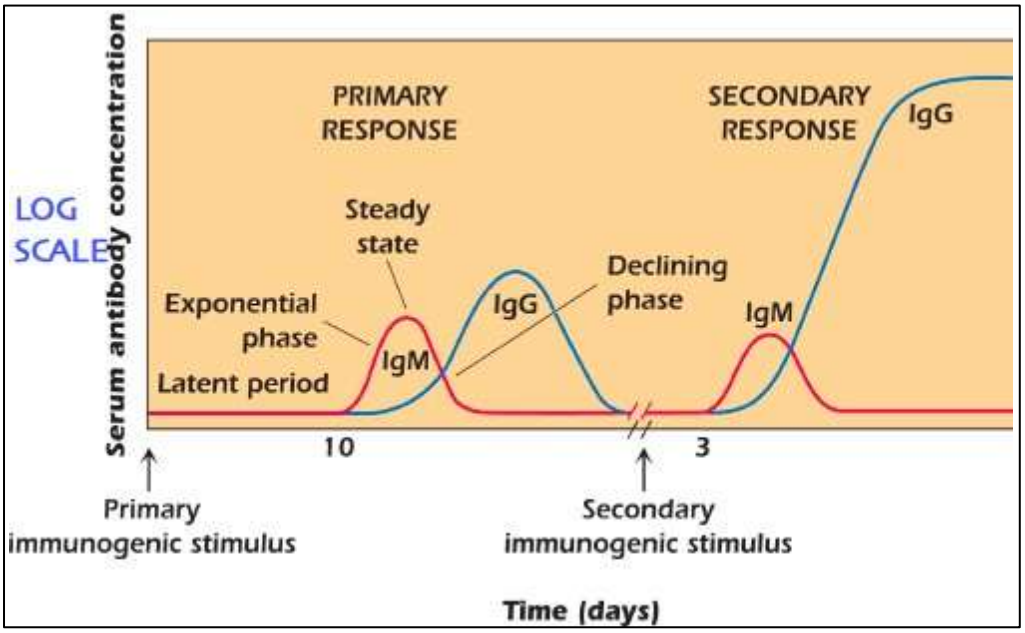

Fig. 1: Graph depicting the antibody titre for IgM and IgG during primary and secondary immune responses after an antigenic challenge

- ✓ A ***vaccine*** is a biological preparation that provides active acquired immunity against a particular disease. A *vaccine* typically contains an antigenic agent that is the disease-causing microorganism in inactivated form or in attenuated form, its toxins, or one of its surface proteins that would generate immune response and provide protection against the disease causing agent during its future attack.
- ✓ **Vaccination:** The act of introducing a vaccine into the body to produce protection from a specific disease. When a sufficiently large percentage of a population has been *vaccinated*, herd immunity results.
- ✓ **Immunization:** A process by which a person becomes protected against a disease through vaccination. This term is often used interchangeably with vaccination or inoculation.

Characteristics of a good vaccine:

1. A vaccine should be able to generate immunological memory. Both T- and Blymphocytes should be formed, hence should be able to generate both arms

of immunity i.e. humoral and cell – mediated.

2. It should have the ability to generate the appropriate immunity like cell-mediated immunity should be developed for tuberculosis and viral pathogens, while humoral immunity for all the other bacterial pathogens.
3. It should be able to provide lifelong immunity with a single dose.
4. It should be able to be introduced to the recipient probably through a non-invasive method like through oral administration or nasal spray.
5. Vaccines should be inexpensive, easily manufactured and stable in extreme temperatures or humidity.
6. It should be easy to store and transport

There are two types of immunity: active and passive.

1. **Active Immunity**

Active Immunity results when exposure to a disease organism triggers the immune system to produce antibodies to that disease. Active immunity can be acquired through natural immunity or vaccine-induced immunity.

- **Natural immunity** is acquired from exposure to the disease organism through infection with the actual disease.
- **Vaccine-induced immunity** is acquired through the introduction of a killed or weakened form of the disease organism through vaccination.

Either way, if an immune person comes into contact with that disease in the future; their immune system will recognize it and immediately produce the antibodies needed to fight it. Active immunity is long-lasting, and sometimes life-long.

2. **Passive Immunity**

Passive immunity is provided when a person is given antibodies to a disease rather than producing them through his or her own immune system.

- A newborn baby acquires passive immunity from its mother through the placenta.

- People can also get passive immunity through antibody-containing blood products such as immune globulin, which may be given when immediate protection from a specific disease is needed.

The major advantage to passive immunity is that protection is immediate, whereas active immunity takes time (usually several weeks) to develop. However, passive immunity lasts only for a few weeks or months. Only active immunity is long-lasting.

Active Immunity and Passive Immunity- Differences

Following are the important difference between active and passive immunity:

Active Immunity	Passive Immunity
Active immunity is usually permanent – it is produced by the antibodies of the host in response to direct contact of an antigen	Passive immunity lasts only for a few weeks or months. It is produced by the introduction of antibodies from outside to the host
It produces an immunological memory	It does not produce immunological memory
When the antigens enter the body, antibodies and other specialized lymphocytes are produced	Antibodies are introduced from an external source. For instance, a mother introduces antibodies to a fetus through the placenta and to an infant via mother's milk.
There are no side-effects	It may cause reactions
Immunity does not occur immediately	Immunity develops immediately

Active immunization:

Active immunization stimulates the immune system to produce antibodies against a particular infectious agent. Active immunity can arise naturally, as when someone is exposed to a pathogen. For example, an individual who recovers from a first case of the measles is immune to further infection by the measles-causing virus, because the

virus stimulates the immune system to produce antibodies that specifically recognize and neutralize the pathogen the next time it is encountered. Active immunization also can be artificially induced through vaccination. Vaccines are preparations containing antigens that stimulate an immune response without causing illness. The purpose of vaccination is to ensure that a large enough number of antibodies and lymphocytes capable of reacting against a specific pathogen or toxin are available before exposure to it occurs. Active immunization is often long-lasting and may be reactivated quickly by a recurrence of the infection or by revaccination.

How Vaccines Work?

Vaccines help develop immunity by imitating an infection. Vaccines contain weakened or inactive parts of a particular organism (antigen) that triggers an immune response within the body. This type of infection, however, does not cause illness, but it does cause the immune system to produce T-lymphocytes and antibodies. Sometimes, after getting a vaccine, the imitation infection can cause minor symptoms, such as fever. Such minor symptoms are normal and should be expected as the body builds immunity. Once the imitation infection goes away, the body is left with a supply of "memory" T-lymphocytes, as well as B-lymphocytes that will remember how to fight that disease in the future. However, it typically takes a few weeks for the body to produce T-lymphocytes and B- lymphocytes after vaccination. Therefore, it is possible that a person who was infected with a disease just before or just after vaccination could develop symptoms and get a disease, because the vaccine has not had enough time to provide protection.

Different Types of Vaccines

The first human vaccines against viruses were based using weaker or attenuated viruses to generate immunity. The smallpox vaccine used cowpox, a poxvirus that was similar enough to smallpox to protect against it but usually didn't cause serious illness. Rabies was the first virus attenuated in a lab to create a vaccine for humans.

Vaccines are made using several different processes. They may contain live viruses that have been attenuated (weakened or altered so as not to cause illness); inactivated or killed organisms or viruses; inactivated toxins (for bacterial diseases where toxins generated by the bacteria, and not the bacteria themselves, cause illness); or merely

segments of the pathogen (this includes both subunit and conjugate vaccines).

Sl. No.	Vaccine type	Vaccines of this type on U.S. Recommended Childhood (ages 0-6)
1.	Live, attenuated	Measles, mumps, rubella (MMR combined vaccine) Varicella (chickenpox), Rotavirus
2.	Inactivated/Killed	Polio (IPV), Hepatitis A, Rabies
3.	Toxoid (inactivatedtoxin)	Diphtheria, tetanus (part of DTaP combined immunization)
4.	Subunit/conjugate	*Haemophilus influenza* type b (Hib), Pertussis (part of DTaP combined immunization), Human papillomavirus (HPV), Pneumococcal Meningococcal
5.	Recombinant	Hepatitis B Vaccine
6.	DNA vaccine	Rabies vaccine, influenza vaccine

Live, attenuated vaccines currently recommended as part of the U.S. Childhood Immunization Schedule include those against measles, mumps, and rubella (via the combined MMR vaccine), varicella (chickenpox), and influenza (in the nasal spray version of the seasonal flu vaccine). In addition to live, attenuated vaccines, the immunization schedule includes vaccines of every other major type—see the table above for a breakdown of the vaccine types on the recommended childhood schedule.

The different vaccine types each require different development techniques are described as follows:

1. Live, Attenuated Vaccines

Attenuated vaccines can be made in several different ways. Some of the most common methods involve passing the disease-causing virus through a series of cell cultures or animal embryos (typically chick embryos). With each passage, the virus becomes better at replicating in chick cells, but loses its virulence. A virus targeted for use in a vaccine may be grown through— "passaged" through—upwards of 200 different embryos or

cell cultures. Eventually, the attenuated virus will be unable to cause disease in humans but will replicate well in human cells, and can be used in a vaccine. All of the methods produce an attenuated live vaccine where the pathogen loses its ability to cause disease in the host but possess all the characteristics to be usedas a vaccine.

When the resulting vaccine virus is given to a human, it will be unable to cause illness, but will still provoke an immune response that can protect against future infection.

One concern that must be considered is the potential for the vaccine virus to revert to a form capable of causing disease. Mutations that can occur when the vaccine virus replicates in the body may result in more a virulent strain. It is worth noting that mutations *are* somewhat common with the oral polio vaccine (OPV), a live vaccine that is ingested instead of injected. The vaccine virus can mutate into a virulent form and result in rare cases of paralytic polio. For this reason, OPV is no longer used in the United States, and has been replaced on the Recommended Childhood Immunization Schedule by the inactivated polio vaccine (IPV).

Protection from a live, attenuated vaccine typically outlasts that provided by a killed or inactivated vaccine.

The advantages of live attenuated vaccines are:

1. One single dose is capable in inducing long-term immunity
2. Provides wide spectrum of immunity
3. Rare incurrence of any allergic reactions or post vaccination lumps.
4. Cost-effective

The disadvantages are:

1. Potential to revert back to virulence form
2. Exacerbate diseased conditions in immune-compromised individuals.
3. In some rare case may lead to abortion or infertility.

2. Killed or Inactivated Vaccines

Inactivated vaccines generally termed as heat killed vaccines are created by inactivating a pathogen, typically using heat or chemicals such as formaldehyde or formalin. This destroys the pathogen's ability to replicate, but keeps it "intact" so that

the immune system can still recognize it. Because killed or inactivated pathogens can't replicate at all, they can't revert to a more virulent form capable of causing disease (as discussed above with live, attenuated vaccines). However, they tend to provide a shorter length of protection than live vaccines, and are more likely to require boosters to create long-term immunity.

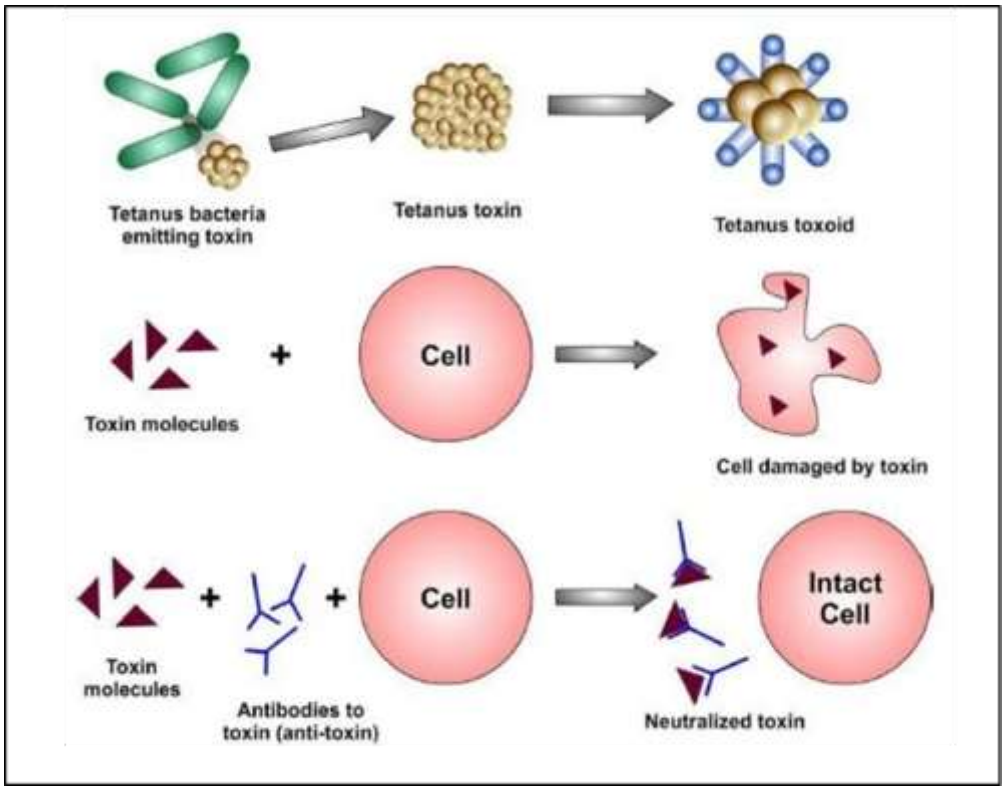

Fig 2: Toxin molecules destroy the cells but vaccinations by toxoids produce antibodies that neutralize the pathogens.

3. Toxoids

Some bacterial diseases are not directly caused by a bacterium itself, but by a toxin produced by the bacterium. One example is tetanus: its symptoms are not caused by the *Clostridium tetani* bacterium, but by a neurotoxin it produces (tetanospasmin). Immunizations for this type of pathogen can be made by inactivating the toxin that causes disease symptoms. As with organisms or viruses used in killed or inactivated vaccines, this can be done via treatment with a chemical such as formalin, or by using heat or other methods.

Immunizations created using inactivated toxins are called *toxoids*. Toxoids can actually be considered killed or inactivated vaccines, but are sometimes given their own

category to highlight the fact that they contain an inactivated toxin, and not an inactivated form of bacteria.

(i) Subunit and Conjugate Vaccines

Both subunit and conjugate vaccines contain only pieces of the pathogens they protect against. Subunit vaccines use only part of a target pathogen to provoke a response from the immune system. This may be done by isolating a specific protein from a pathogen and presenting it as an antigen on its own. The acellular pertussis vaccine and influenza vaccine (in shot form) areexamples of subunit vaccines.

Another type of subunit vaccine can be created via genetic engineering. A gene coding for a vaccine protein is inserted into another virus, or into producer cells in culture. When the carrier virus reproduces, or when the producer cell metabolizes, the vaccine protein is also created. The end result of this approach is a recombinant vaccine: the immune system will recognize the expressed protein and provide future protection against the target virus. The Hepatitis B vaccine currently used in the United States is a recombinant vaccine.

Another vaccine made using genetic engineering is the human papillomavirus (HPV) vaccine. Two types of HPV vaccine are available—one provides protection against two strains of HPV, the other four—but both are made in the same way: for each strain, a single viral protein is isolated. When these proteins are expressed, virus-like particles (VLPs) are created. These VLPs contain no genetic material from the viruses and can't cause illness, but prompt an immune response that provides future protection against HPV.

Conjugate vaccines are somewhat similar to recombinant vaccines: they're made using a combination of two different components. Conjugate vaccines, however, are made using pieces from the coats of bacteria. These coats are chemically linked to a carrier protein, and the combination is used as a vaccine. Conjugate vaccines are used to create a more powerful, combined immune response: typically the "piece" of bacteria being presented would not generate a strong immune response on its own, while the carrier protein would. The piece of bacteria can't cause illness, but combined with a carrier protein, it can generate immunity against future infection. The vaccines currently in use for children against pneumococcal bacterial infections are made using this technique.

(ii) Recombinant Vaccines

Today, the rise of genetic engineering and molecular biology has had great impact on development and manufacturing process of vaccines. Specific antigenic microbes have high power to arouse the immune response against pathogens. Currently, the sequence of the pathogenic protein antigens could be obtainable by sequencing genes of the main antigen, and producing them synthetically via recombinant DNA technology. Hepatitis B is the first and one of the most successful examples of synthetic vaccines. The surface antigen of this virus (HBsAg) is very immunogenic and effective, and able to produce high levels of antibody in the body. In the past, for providing hepatitis B vaccine, HBsAg was purified from the plasma of infection carriers and used for vaccination; of course there were some extensive restrictions in purification, such as difficult conditions and contaminated plasma. In order to make recombinant hepatitis B vaccine, recombinant HBsAg is expressed in cells that have a powerful expression system leading to the production of virus-like particles by HBsAg which are highly immunogenic. Other kinds of common vaccines are anti-herpes simplex virus, anti-rotavirus, and anti-HPV vaccines.

(iii) DNA Vaccine

DNA immunization is a novel technique by which direct injection of genetic material with the foreign gene into a living host leads to the production of that gene product and subsequent immune responses. Transfected muscle cells may produce antigen or foreign proteins resulting in production of B-cells as well they can also directly transfect APC's to elicit an MHC-dependent T- cell mediated response, thus mimicking the similar immune responses induced by pathogens(Fig 6). Production of DNA vaccines starts with E. coli cells which are transformed with the plasmid of interest. Growth of the E. coli is typically done via a fermentation process similar to that used in the manufacturing of certain alcoholic beverages. These cells are grown and stored frozen in a stock of vials called a Master Cell Bank. Cell lysis and release of the plasmid is attained, following the purification of the desired DNA by various chromatographic methods.

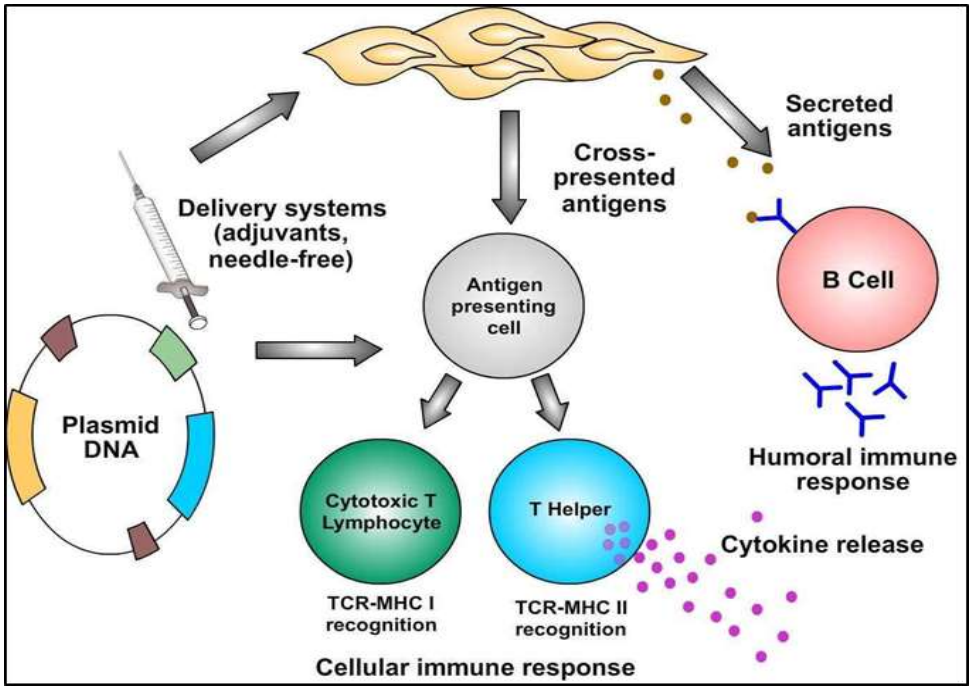

Fig 3: Master cell bank contains the cultures of E. coli cells with the plasmid of interest. Transfection of DNA into the myocytes results in both humoral and cell-mediated immune responses.

Advantages

DNA vaccination has many advantages compared to the conventional vaccine approaches particularly against potentially lethal emerging infectious diseases.

1. DNA vaccines can accommodate a combination of different genes that code for different antigens from one or more different pathogens. In addition they have a significantly shorter production time. This can result in the generation of broad immunity to multiple protein antigens in a short span of duration.
2. DNA vaccines have also been observed to stimulate both antibody and T cell arms of the immune system including those that are specialized to kill viruses or cancer cells (via cytotoxic or killer T cells).
3. The most significant advantage is that DNA vaccines do not require the handling of potentially deadly infectious agents, in light of fast emerging pathogens.

Antigen Antibody Interaction:

Immunotechnology focuses on the use of body defence system for the production of immunological agents and diagnosis of several diseases that protect living beings from these diseases. Immunological-based techniques have been extensively utilized for the detection and epidemiological studies of human viral infections. They can detect antiviral antibodies or viral antigens in clinical samples.

Antigen-antibody interaction, or **antigen-antibody reaction**, is a specific chemical interaction between antibodies produced by B cells of the white blood cells and antigens during immune reaction. The antigens and antibodies combine by a process called agglutination. It is the fundamental reaction in the body by which the body is protected from complex foreign molecules, such as pathogens and their chemical toxins. In the blood, the antigens are specifically and with high affinity bound by antibodies to form an antigen-antibody complex. The immune complex is then transported to cellular systems where it can be destroyed or deactivated. The types of antigen - antibody reactions are: Precipitation Reaction, Agglutination Reaction, and Complement Fixation.

Nature of Antigen-Antibody Reactions

a. Lock and Key Concept

An antibody molecule comprises of the Fab portion where the active site consisting the hyper-variable regions of the heavy and light chains is located. The antigenic determinant resides in a cleft formed by the active site of the immunoglobulin molecule as indicated by X-ray crystallography studies. Thus, the antigen-antibody interactions can be simulated by a key (the antigen) which fits into a lock (the antibody).

b. Non-covalent Bonds

The binding of an antibody and antigen is highly specific and involves weak and reversible non-covalent interactions comprising mainly of van der Waals forces, electrostatic forces, H-bonding and hydrophobic forces. The antigen combines to the antibody at the active site by non-covalent bonds. Multiple bond formation ensures that the antigen will be bound tightly to the antibody.

c. Reversible Nature

Antigen-antibody complexes are strengthened by non-covalent bonds, thus making their nature reversible.

- **Affinity and Avidity**

a. Affinity

The intensity of the reaction between an antigenic determinant and one active site on the antibody molecule defines the affinity of that antibody. Affinity is the equilibrium constant characteristic of a Ag-Ab interaction. It is the sum of the attractive and repulsive forces effective between the antigenic determinant and the active site of a specific antibody. Most antibodies have extremely high affinity and specificity for their antigens.

b. Avidity

Avidity amounts to the total strength by which an antigen binds to multiple antigenic determinants and multivalent antibodies. Avidity is affected by the valence of the antibody as well as that of the antigen and is therefore more than the sum of individual affinities. Hence, affinity is the strength of binding between a single antigenic determinant and its corresponding individual antibody combining site whereas avidity refers to the overall strength of binding between multivalent antigens and antibodies.

- **Specificity and Cross Reactivity**

a. Specificity

Specificity is defined as the ability of a particular antibody active site to recognize and interact with only a single antigenic determinant or the ability of a population of antibody molecules to react with only a single antigen. Antigen-antibody reactions possess extremely high degree of specificity. Antibodies are capable of discriminating between- a. the primary structure of an antigen b. isomeric forms of an antigen c. secondary and tertiary structure of an antigen.

b. Cross Reactivity

Cross reactivity is the capability of a particular antibody active site to react with more than a single antigenic determinant or that of a population of antibody molecules to react with multiple antigens.

Cross reactions are multi specific interactions arising due to sharing of an epitope by a cross reacting antigen and the immunizing antigen or because the epitope is structurally similar to one on the immunizing antigen Cross-reactivity usually occurs among polysaccharide antigens containing similar oligosaccharide residues. For instance, the

ABO blood-group antigens are glycoproteins expressed on the surface of erythrocytes. Factors distinguishing the blood-group antigens A and B include fine variations in the terminal sugar residues of these surface proteins. A person with type A blood has anti-B antibodies; a type B person has anti-A; and a type Operson therefore has both anti-A and anti-B antibodies. An individual lacking one or both of these antigens would generate serum antibodies to the missing antigen(s) (Table 1). The serum antibody response is not induced by exposure to erythrocyte antigens but by cross-reacting microbial antigens present on common intestinal bacteria. These antigens intern induce the production of antibodies in individuals lacking the similar blood-group antigens on their erythrocyte surfaces. These antibodies would cross-react with the oligosaccharides on foreign erythrocytes, forming the basis for blood typing and accounting for the essential compatible nature of blood types during blood transfusions.

Table 1: ABO blood type- Antigens present on surface of RBCs act as epitopes for generation of serum antibodies.

Blood type	**Antigens on RBCs**	**Serum antibodies**
A	A	Anti-B
B	B	Anti-A
AB	A and B	Neither
O	Neither	Anti-A and Anti-B

- **Factors Affecting Measurement of Antigen-Antibody Reactions**

Antigen-antibody complexes are formed under specific conditions of temperature and pH. In order to determine whether an antigen-antibody reaction has occurred,the Ag-Ab complexes formed have to be detected by direct or indirect means. A number of factors influence the detection of these complexes.

a. **Affinity** - Higher affinity of the antibody for the antigen ensures a stable reaction between the two thus facilitating the detection of the complex formed.

b. **Avidity** - Interactions between multivalent antigens and multivalent antibodies are very stable aiding in their detection.

c. **Antigen to antibody ratio** - The formation of Ag-Ab complexes is related to the concentration of the antigen and antibody, therefore its detection is directly dependent on the ratio in which they are present at a particular instance.

d. ***Physical form of the antigen*** **-** The physical form of the antigen plays an important role in detection of its interaction with an antibody. Particulate antigens result inagglutination on reaction with an antibody. However, precipitation of the antigen would occur in case it is soluble in nature when large insoluble Ag-Ab complexes are formed.

✓ **Antigen-Antibody Interactions**

- **Agglutination**

The word Agglutination comes from the Latin “agglutinare”, meaning "to glue,” referring to clumping of substances.Agglutination is defined as the visible clumping of a particulate antigen when mixed with antibodies specific for it, in the presence of electrolytes at an appropriate temperature and pH.Antibodiesare capable of binding multiple antigen molecules, linking themto create a large lattice like complex which is visible to the naked eye. Antibodies that generate such reactions are called **agglutinins**. All antibodies can theoretically agglutinate particulate antigens but IgM, due to its high valence, is particularly a good agglutinin. Large antigens with multiple epitopes easily adhere to particles such as animal cells or bacteria when combined with specific antibodies resulting in cross-linking. The process of agglutination involves two steps. First step is sensitization and second is lattice formation. Sensitization is the recognition and attachment of specific antibody to corresponding antigen. Temperature,pH and time of incubation influence the reaction. A Lattice is formed by cross linking between sensitized particles. Agglutination reaction used for diagnosis of diseases in lab either uses the particulate or soluble antigens. Example of agglutination reaction using particulate antigens is Salmonella typhi bacteria to detect specific antibody in serum from patient suffering from typhoid fever (Widal test).

Agglutination is a serological reaction similar to precipitation; with the exception of the antigen being large and particulate in case of agglutination. Both reactions are inhibited by antibody excess and this phenomenon is called the **prozone effect**, whereas in case of antigen excess **postzone effect** occurs. If the antigen is an integral part of the surface

of a cell or other insoluble particle, the agglutination reaction is known as **direct agglutination**. However, a cell or insoluble particle can be coated with a soluble antigen such as a viral antigen, a polysaccharide or a hapten and the coated cells can be used in an agglutination test for antibody to the soluble antigen in a reaction called **passive agglutination**.

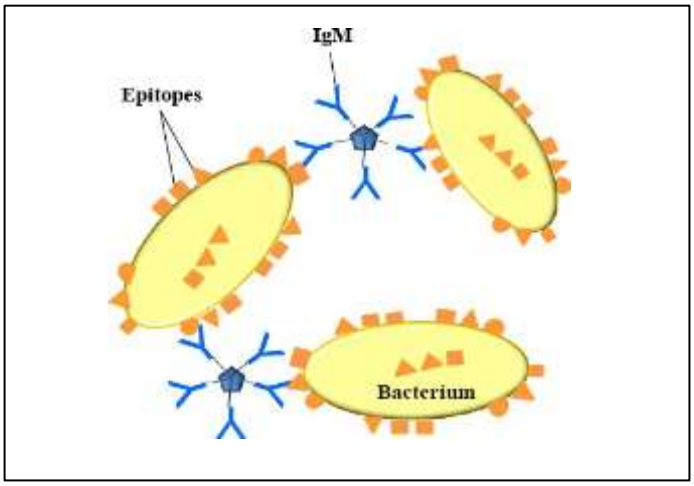

Fig.: Agglutination – Pentavalent Immunoglobulin IgM is shown to interact and bind with multiple antigenic epitopes on the surface of bacterial cells causing clumping or agglutination reaction.

	Red blood cells from individuals of type			
Serum from individuals of type	O	A	B	AB
O Anti-A & Anti-B antibodies	No agglutination	Agglutination	Agglutination	Agglutination
A Anti-B antibodies	No agglutination	No agglutination	Agglutination	Agglutination
B Anti-A antibodies	No agglutination	Agglutination	No agglutination	Agglutination
AB No antibodies to A or B	No agglutination	No agglutination	No agglutination	No agglutination

Fig.: Aggutination reaction in ABO blood typing- Serum of individuals would contain different types of antigens on surface of RBCs and corresponding antibodies to these antigens.

- ✓ **Applications of Agglutination Tests**

i. Determination of blood group types or antibodies to blood group antigens.

ii. Assessment of bacterial infections. eg. infection with a particular bacterium is indicated by the rise in titer of an antibody to this bacterium.

- **Precipitation**

The smallest unit of an antigen molecule that can bind with an antibody is known as antigenic determinant or epitope. The corresponding region on the antibody molecule that interacts with the epitope is called paratope. The number of epitopes on the surface of an antigen is known as its valence and it determines the number of antibody molecules that can combine with the antigen at one time. Monovalent antigens are those having a single epitope, however, more than one copy of the same epitope is present on most antigens called polyvalent antigens. Immuno precipitation involves interaction of a soluble antibody with a soluble antigen resulting in the formation of an insoluble product, the precipitate. These reactions consist of lattice (cross-links) formation when the corresponding antigen and antibody combine in optimal ratios. Lattice formation relies upon the valency of both antibody and antigen. Crosslinked complexes result when bi- or polyvalent antigens interact with more than one multivalent antibodies. If the Ag-Ab complexes formed are too large to stay in solution, visible precipitation results. Excess of either component reduces lattice formation and subsequent precipitation hence, these should occur at optimal concentrations. Antibodies that aggregate soluble antigens are called **precipitins.** Precipitation and agglutination reactions differ in size, solubility of the antigen and sensitivity. Antigens are soluble molecules and larger in size in precipitation reactions. Antigen-Antibody lattice formation is governed by the valence of both the antibody and antigen:

- The antibody should be polyvalent (Fab fragments) in order to form a precipitate.
- The antigen should be bi- or poly valent; i.e. it should possess at least two copies of an epitope, or have different epitopes that are capable of reacting with various antibodies in a polyclonal antisera.

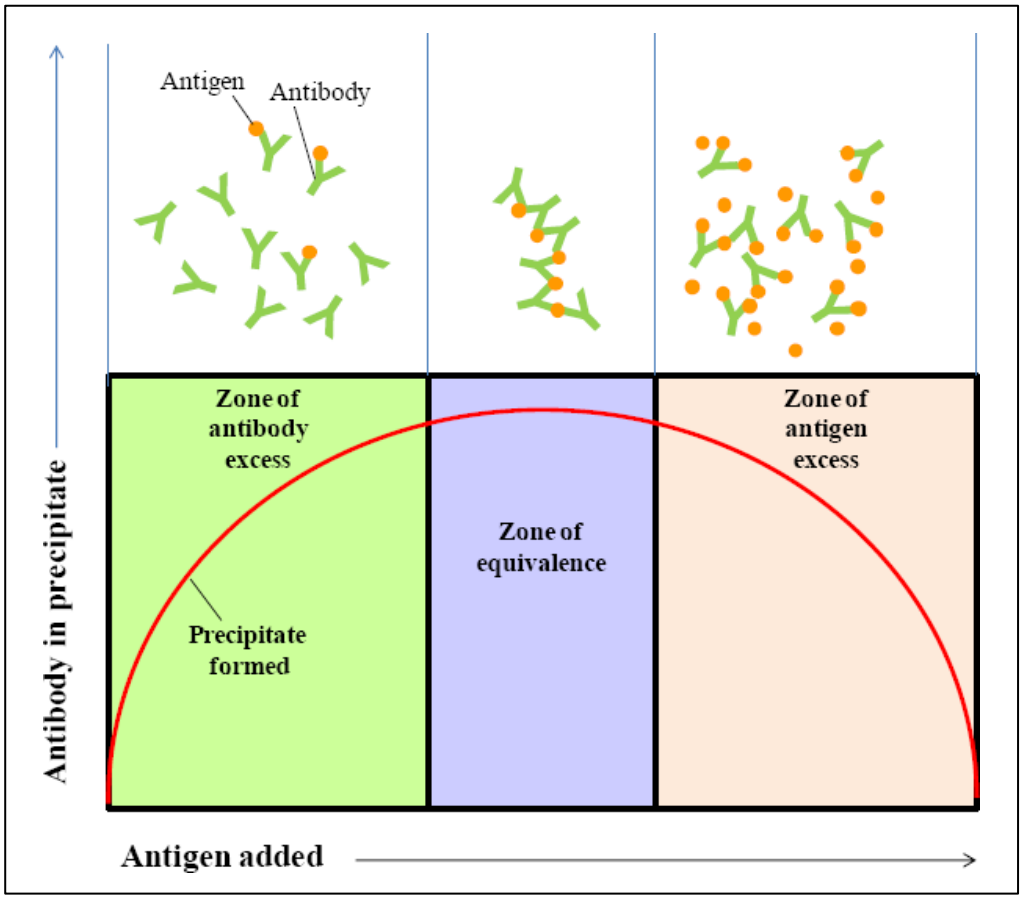

Fig.: Precipitation curve – Interaction of Antigens with specific antibodies generates the precipitate. Different zones are highlighted according to the concentration of differently distributed components.

Precipitation Reactions in Fluids Yield a Precipitin Curve

When a constant amount of antibody is taken in a series of tubes and increasing amounts of antigen is added to these, variable amounts of precipitates form resulting in a quantitative reaction. Initially, this method was used to determine the amount of antigen or antibody present in a particular sample. After precipitation, the tubes are centrifuged to pellet the precipitate and the amount of precipitate is measured on removing the supernatant. The amount of precipitate when plotted against increasing antigen concentrations yields a **precipitin curve**. Excess of either antibody orantigen interferes with maximal precipitation. Hence, formation of an insoluble antigen-antibody complex occurs within a narrow optimal concentration range known as the **zone of equivalence**. This zone represents the conditions under which antigen-ntibody complexes formed are sufficiently large to be precipitated. At equivalence, a

large multi-molecular complex is formed which increases in size and precipitates out of solution. On the other hand, outside this zone antigen or antibody excess occurs resulting in the formation of small soluble complexes.

Immunoassay

An immunoassay is a biochemical test used to identify the presence or amount of a particular molecule referred to as an "analyte", in a solution by combining it with an antibody or an antigen. The principal of immunoassays is formation of an immune complex involving the recognition and binding of an antibody to a specific molecule among a mixture of molecules. A key feature of all immunoassays is generation of a measurable signal in response to the binding. Immunoassays utilize a wide range of labels; some emit radiation, result in a visible colour change, fluoresce under light, or could be induced to emit light.

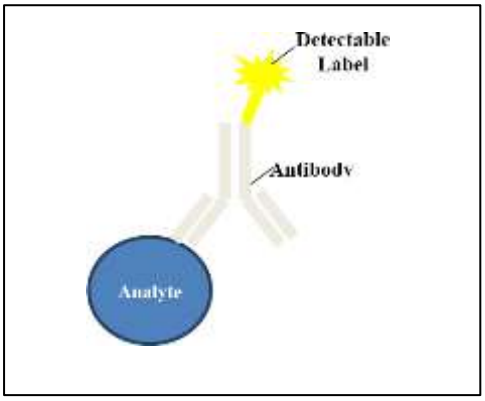

Fig.: Basic components of an Immunoassay. The analyte specifically binds to the antibody labeled with detectable label.

Examples of the application of immunoassay include:

i. Drug testing

ii. Hormone testing (insulin in diabetic patients)

iii. Bacterial or viral testing (AIDS, hepatitis)

iv. Environmental testing (herbicides, pesticides)

Advantages of immunoassays are:

- ✓ Inexpensive

- ✓ Highly selective
- ✓ Low limits of detection
- ✓ High-throughput usually
- ✓ Applicable to the determination of a wide-range of compounds

Categories of Immunoassays

Competitive –A competitive assay or limited reagent assay involves measurement of an unlabeled analyte or antigen by its ability to compete with the labelled antigen in the immunoassay. The assay mixture consists of antibodies saturated by labeled antibodies, hence higher the reduction in label at the end of assay, greater is the amount of antigen in the test sample.

Non-Competitive

One site Non-competitive - The unknown analyte in the sample are allowed to react with labelled antibodies. After the binding reaction is complete, unbound antibodies are washed away, and the bound labelled antibodies are measured as signals for the complexes formed. Therefore, intensity of the signal is directly proportional to the concentration of unknown antigen.

Two site Non-competitive - An antibody adsorbed on the solid phase surface is allowed to interact with the test sample. The labeled antibodies are in excess in this system and bind specifically to their respective analyte. Subsequently, a second labeled antibody is added causing sandwiching of the target analyte. The quantitation of the labelled antibody helps in determining the concentration of the antigen since the two are directly proportional. The technique is also known as sandwich assay because the analyte is "sandwiched" between two antibodies.

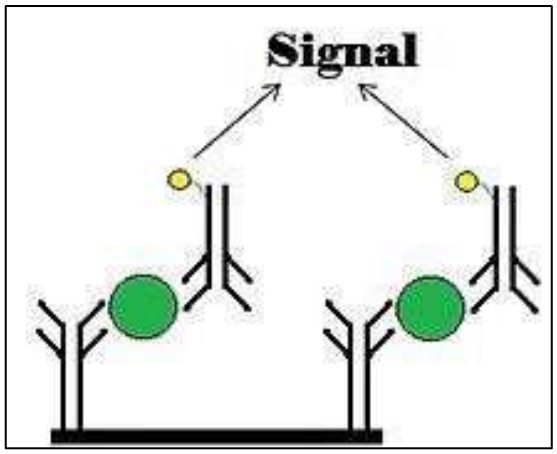

Fig.: Two site Non competitive interatction between antigen and antibody.

Enzyme-Linked Immunosorbent Assay Enzyme-linked immunosorbent assay (ELISA)

Enzyme-Linked Immunosorbent Assay Enzyme-linked immunosorbent assay, commonly known as **ELISA** or EIA was first developed by Avramais (1966, 1969) and Pierce (1967).In this assay, an antibody coupled enzyme reacts with a colorless substrate called a **chromogenic substrate** to generate a visible coloured reaction product. The enzymes commonly employed for ELISA, include alkaline phosphatase, horseradish peroxidase, and galactosidase. These assays possess high sensitivity and are safe and cost effective. The result generated from an ELISA assay could be qualitative identifying the presence or absence of a particular antigen molecule; semi-quantitative, analyzing relative antigen amounts in assay samples or quantitative, defining precise antigen concentrations with respect to a standard curve. ***There are Numerous Variants of ELISA.***

ELISA assays can be performed in a variety of ways which allow both qualitative and quantitative analysis of either antigen or the antibody. ELISA can be used to identify the presence of antibody or antigen qualitatively. The unknown concentration of a sample can alternatively be determined by a curve based on known concentrations of antibody or antigen. The assay variants are described below –

I. **Indirect ELISA** Indirect ELISA is used for both qualitative and quantitative measurements of antibodies. The procedure includes addition of the sample solution

containing primary antibody (Ab1) to a microtiter well pre-coated with the antigen, such that the antibody would react with this well bound antigen. Unbound antibody is washed away and this is followed by detection of the antibody bound to the antigen with the help of an enzyme-conjugated secondary anti-isotype antibody (Ab2) which specifically binds to the primary antibody Ab1.Unbound secondary antibody is also washed away followed by addition of substrate for the enzyme. The coloured reaction product is analyzed spectrophotometrically by plate readers.

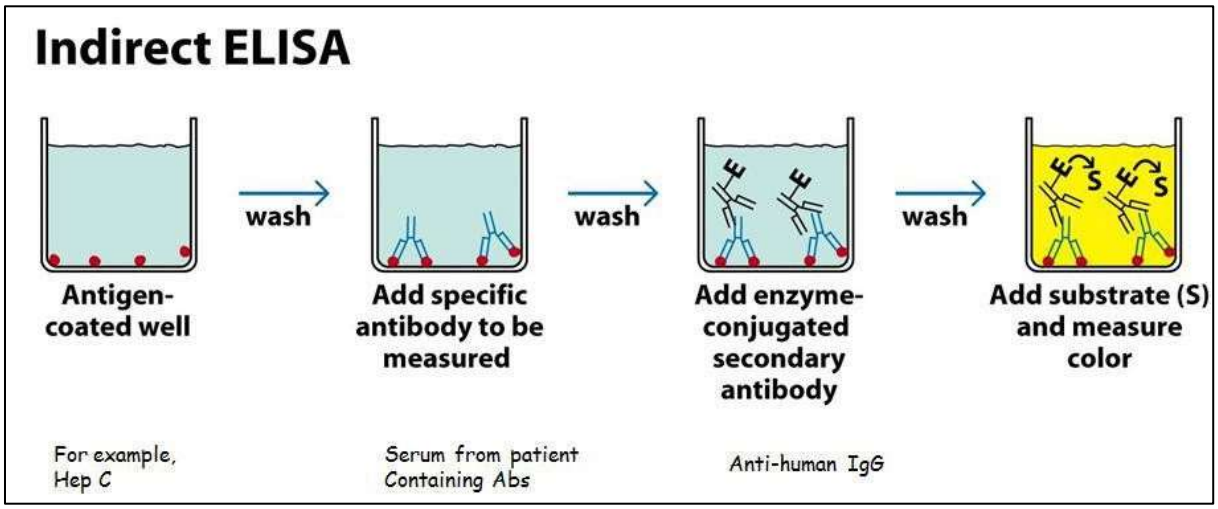

Fig.: Indirect ELISA - Antigen is immobilized on the surface and sample is added, if antibodies specific to the antigen of interest is present binding would occur and visualized with the help of enzyme conjugated secondary antibody.

Indirect ELISA is preferred for detecting the presence of serum antibodies against human immunodeficiency virus (HIV), the causative agent of AIDS. The recombinantenvelope and core proteins of HIV are used as antigens plated on to microtiter wells.

II. Sandwich ELISA Sandwich ELISA is used for quantitative or qualitative analysis of antigens. The basis of this technique remains the antigen-antibody interaction however; the antibody instead of the antigen is immobilized on the microtiter well. An antigen sample is then added to the well pre-coated with theimmobilized antibody. The excess or unbound antigen is washed off using buffers, followed by addition of a second enzyme-linked antibody specific to a second epitope on

thebound antigen. Unbound secondary antibody is then washed off and a substrate corresponding to the enzyme on the secondary antibody this added, and the coloured reaction product analyzed spectrophotometrically.

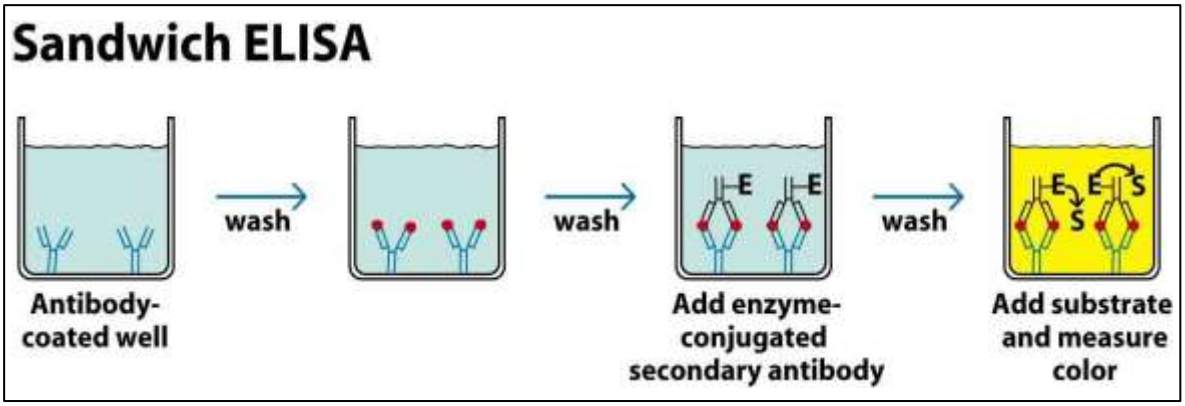

Fig.: Sandwich ELISA – the antigen of interest is sandwiched between primary antibody immobilized on solid surface and enzyme conjugated secondary antibody.

III. ***Competitive ELISA*** Competitive ELISA is also a variant technique for the quantitation of antigen. The procedure consists of a pretreatment step where the antibody isincubated in solution with a sample containing the antigen. A microtiter plate coated with the same antigen is then incubated with the previously procured antigen-antibody mixture. Greater the amount of antigen in the sample, lesser would be the amount of free antibody available to bind to the antigen-coated well. On addition of an enzyme-conjugated secondary antibody (Ab2) specific for the isotype of the primary antibody, the amount of primary antibody immobilized on the well can be determined like in an indirect ELISA. This competitive interaction suggests that higher the amount of antigen in the original sample, the lower would be the value of absorbance.

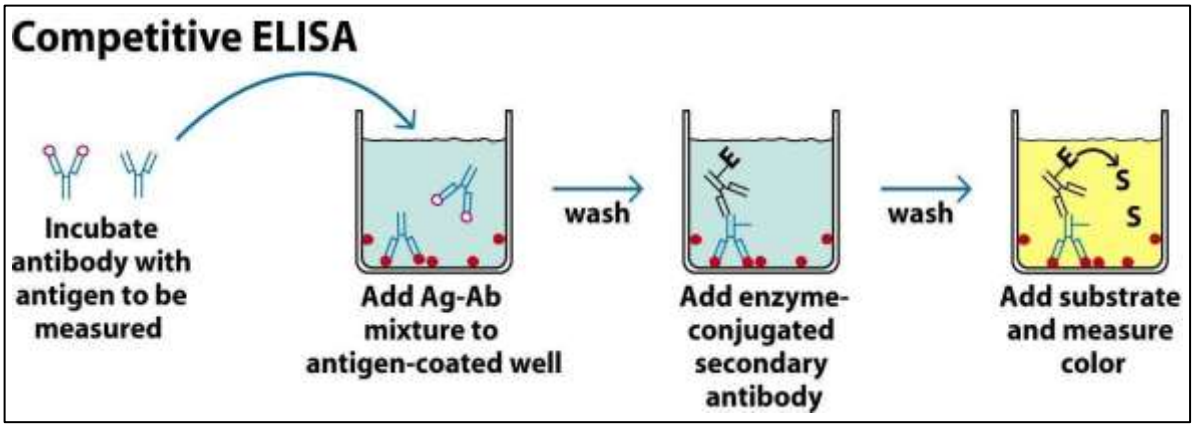

Fig.: Competitive ELISA - Antigen-antibody mixture is added in addition to the free antibodies and incubated with antigen coated wells. Enzyme conjugated secondary antibodies when allowed to react with substrate generate coloured product which is quantitated by measuring absorbance.

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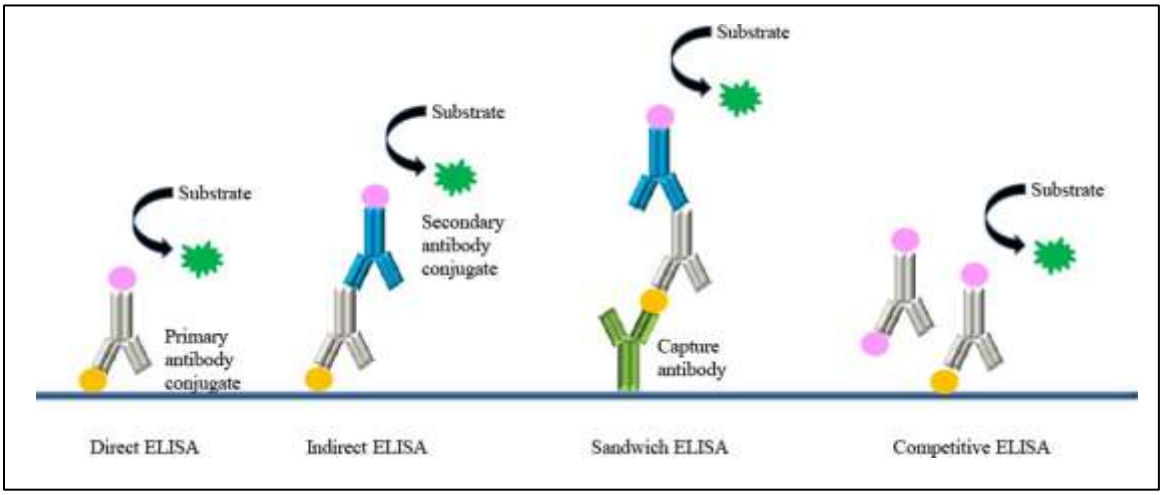

Fig.: Comparison between different types of ELISA. This overview helps to visualize the differences between different ELISA variants. A particular type of assay can be selected depending upon specific interests.

Radioimmunoassay – RIA

Radioimmunoassay (RIA) one of the most sensitive techniques for detecting antigen orantibody was first reported by S. A. Berson and Rosalyn Yalow in 1960, in order to analyze the levels of insulin - anti-insulin complexes in diabetics. This was the first attempt for detection of blood hormones by an *in-vitro* assay. The technique

demonstrates high sensitivity and is capable of quantitating hormones, serum proteins, drugs, and vitamins at concentrations as low as 0.001 *micrograms* per milliliter. The basic principle of this technique is competitive binding between the radiolabeled and unlabeled antigen to a high-affinity antibody. First the antibody is allowed to interact with the radio labeled antigen saturating the antigen-binding sites of the antibody. This is followed by addition of large amounts of sample containing unknown amount of unlabeled antigen. The available binding sites on the antibodies are available to both the labeled and unlabeled antigens as the antibody is unable to distinguish between the two. The labeled antigen is progressively displaced from the antibody binding sites with increasing amount of the unlabeled antigen. This reduction in the amount of radio labeled antigen bound to the specific antibody on increasing antigen concentration in the unknown sample is measured in order to quantitate antigen concentrations in the test sample. The primary step for this assay is to ascertain the amount of antibody needed to saturate 50% - 70% of a specific quantity of radioactive antigen in the test mixture. The antibody to antigen ratio is taken such that the labeled antigen displays more number of epitopes than the total number of antibody binding sites. This ensures competitive binding between unlabeled antigen added to the mixture and the radio labeled antigen against the limited supply of antibody. The bound labeled antigen is quantitated by precipitating the Ag-Ab complex and segregating it from free antigen; and eventually the radio activity of the precipitate is measured.

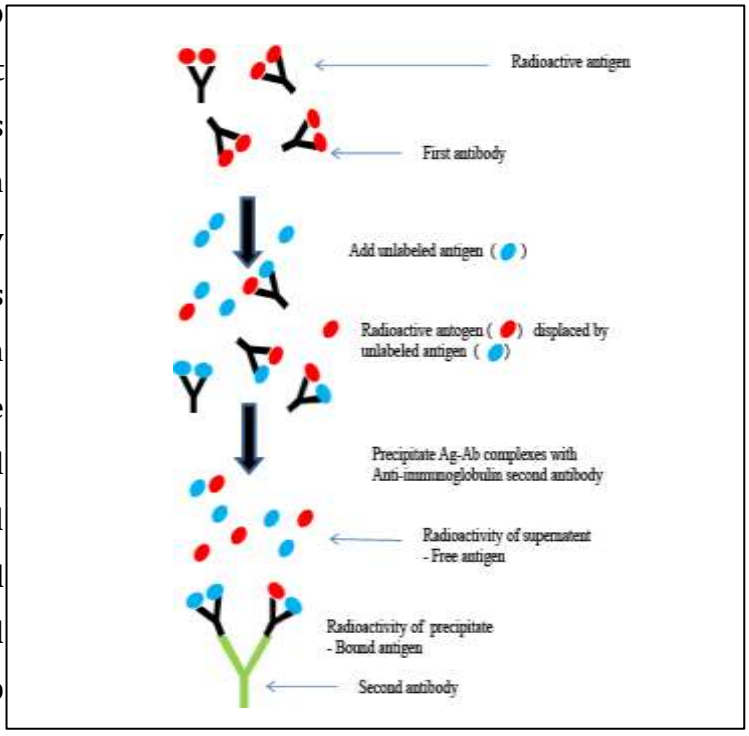

Fig.: Radioimmunoassay (RIA): Based on competitive binding of radiolabeled and unlabeled antigen to a high-affinity antibody particular type of assay can be selected depending upon specific interests.

Immunofluorescence: Albert Coons first demonstrated the labeling of antibodies with fluorescent molecules in 1944. These molecules possess the inherent property of absorbing light of a particular wavelength (excitation) and emitting light of another wavelength. Antibodies tagged with a fluorescent dye, or fluorochrome, can be identified by emission of colored light when excited by light of a specific wavelength**,** when these are a part of immune complexes. This technique also allows detection of antibodies bound to antigen epitopes in cell cultures or tissue sections. Molecules with luminescent properties emit light of a different wavelength on absorbing light of a particular wavelength. Fluorescent materials give off light very promptly due to their atomic structure. The light emitted from luminescent objects can be visualized using a fluorescence microscope equipped with a UV light source. These fluorescent probes are conjugated to the Fc arm of an antibody molecule ensuring that its specificity is not affected. Commonly used fluorochromes are:

i. **Fluorescein** is the most frequently used organic label dye for immunofluorescence. It absorbs blue light (490 nm) and emits a higher wavelength intense yellow-green fluorescence (517 nm).

ii. **Rhodamine** is another organic dye, absorbing light in the yellow-green range (515 nm) and emitting a deep red fluorescence (546 nm). Two-color immunofluorescence assays can be performed using a combination of these two dyes simultaneously as rhodamine emits fluorescence at a longer wavelength than fluorescein. Spatial distribution and comparative assays for two antigens are performed in a single experiment where an antibody specific to one determinant is tagged with fluorescein, and a second antibody recognizing another antigen is labeled with rhodamine. The co-localization of the fluorescein- tagged antibody visualized by its yellow green color, is discretely distinguishable from the red color emitted where the rhodamine-tagged antibody is bound.

iii. **Phycoerythrin** an efficient absorber of light (~30-fold greater than fluorescein) and a brilliant emitter of red fluorescence, is also widely used as an immunofluorescence label.

Applications of immunofluorescence can be wide range, starting with identification of a number of subpopulations of cells in culture, identifying bacterial species, detecting Ag-

Ab complexes in disease conditions, detection of complement components, as well as localizing and staining of hormones and other subcellular molecules in situ. It also finds use in analysis of cells in suspension, cultured cells, tissue, beads and microarrays for the detection of specific proteins. A very important application of immunofluorescence is tissue or cell specific antigen localization. The target antigens can be localized in cells or tissues andvisualized by fluorescence microscopy thus, making it a potent tool for associating the molecular architecture of tissues and organs to gross anatomy and physiology.

Fluorescent In Situ Hybridization (FISH):

Fluorescence *in situ* hybridization (FISH) is a kind of cytogenetic technique which uses fluorescent probes binding parts of the chromosome to show a high degree of sequence complementarity. Fluorescence microscopy can be used to find out where the fluorescent probe bound to the chromosome. This technique provides a novel way for researchers to visualize and map the genetic material in an individual cell, including specific genes or portions of genes. It is an important tool for understanding a variety of chromosomal abnormalities and other genetic mutations. Different from most other techniques used for chromosomes study, FISH has no need to be performed on cells that are actively dividing, which makes it a very versatile procedure.

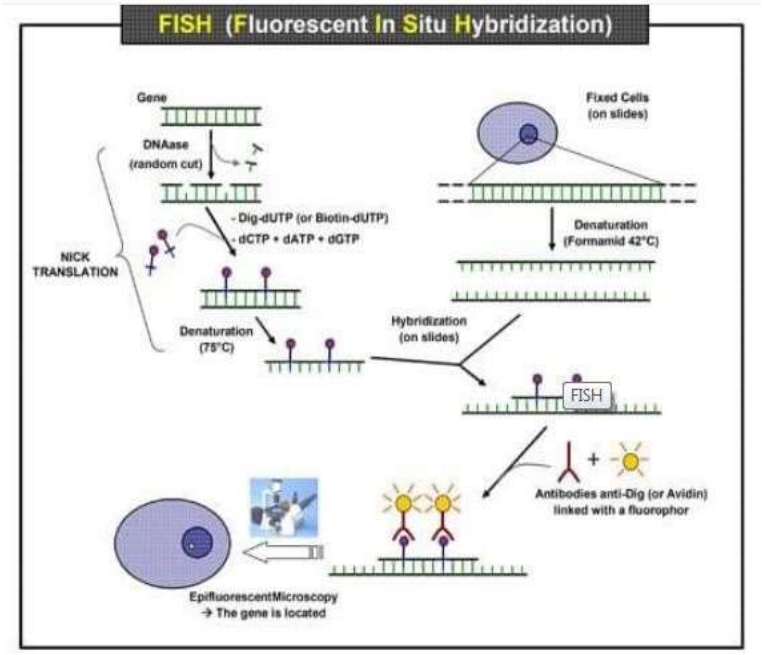

Fig.: Scheme of the principle of the FISH experiment to localize a gene in the nucleus.

How does FISH work?

FISH is useful, for example, to help a researcher identify where a particular gene falls within an individual's chromosomes. Here's how it works:

- Make a probe complementary to the known sequence. When making the probe, label it with a fluorescent marker, e.g. fluorescein, by incorporating nucleotides that have the marker attached to them.
- Put the chromosomes on a microscope slide and denature them.
- Denature the probe and add it to the microscope slide, allowing the probe hybridize to its complementary site.
- Wash off the excess probe and observe the chromosomes under a fluorescent microscope. The probe will show as one or more fluorescent signals in the microscope, depending on how many sites it can hybridize to.

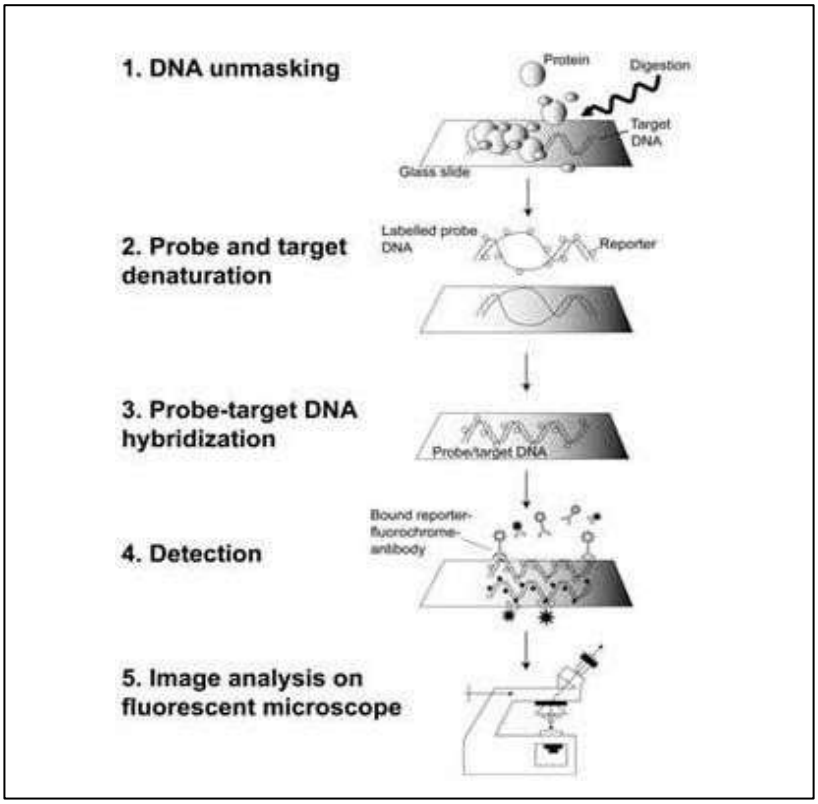

Fig.: The five basic steps of FISH.

What is FISH used for?

FISH is widely used for several diagnostic applications: identification of numerical and structural abnormalities, characterization of marker chromosomes, monitoring the effects of therapy, detection of minimal residual disease, tracking the origin of cells after bone marrow transplantation, identification of regions of deletion or amplification, detection of chromosome abnormalities in non-dividing or terminally differentiated cells, determination of lineage involvement of clonal cells, etc. Moreover it has many applications in research: identification of non-random chromosome rearrangements, identification of translocation molecular breakpoint, identification of commonly deleted regions, gene mapping, characterization of somatic cells hybrids, identification of amplified genes, study the mechanism of rearrangements. FISH is also used to compare the genomes of two biological species to deduce evolutionary relationships.

How many types of probes for FISH?

Generally, researchers use three different types of FISH probes, each of which has a different application:

Locus specific probes bind to a particular region of a chromosome. This type of probe is useful when researchers have isolated a small portion of a gene and want to determine on which chromosome the gene is located.

Alphoid or centromeric repeat probes are generated from repetitive sequences found in the middle of each chromosome. Researchers use these probes to determine whether an individual has the correct number of chromosomes. These probes can also be used in combination with "locus specific probes" to determine whether an individual is missing genetic material from a particular chromosome.

Whole chromosome probes are actually collections of smaller probes, each of which binds to a different sequence along the length of a given chromosome. Using multiple probes labeled with a mixture of different fluorescent dyes, scientists are able to label each chromosome in its own unique color. The resulting full-color map of the chromosome is known as a spectral karyotype. Whole chromosome probes are particularly useful for examining chromosomal abnormalities, for example, when a piece of one chromosome is attached to the end of another chromosome.

Genomic in situ hybridization (GISH)

Genomic in situ hybridization (GISH) is an efficacious technique, that is, used for genome differentiation of one parent from the other by utilizing special chromosome-labeling techniques. GISH has a gratuity role in cytogenetics for investigation of evolutionary relationship of crops and identification of inserted region in the parent from the alien species. GISH technique follows the same protocol as in the fluorescent in situ hybridization (FISH) technique. However, genomic and blocking DNA utilization in GISH differentiate it from FISH analysis.

Main steps of the genomic in situ hybridization (GISH) are discussed below.

- Direct and indirect probe labeling.
- Fragmentation of the blocking DNA.
- Slide preparation.
- Probe and blocking DNA denaturation in a hybridization mixture.
- Addition of the hybridization mixture with the probe and the blocking DNA.
- Denaturation of the chromosome DNA.
- In situ hybridization of probe and blocking DNA in the target sequence of the chromosome.
- Detection of the probe in the chromosome DNA of one parent, in an indirect labeling.
- Chromosome DNA molecule of the second parent associated to the unlabeled blocking DNA.
- Visualization of hybridization signals associated to a probe (green) in a fluorescence microscope.
- Unmarked chromosomes are visualized with a counter-staining (blue). When the probe labeling is direct, the detection step of the GISH can be excluded.

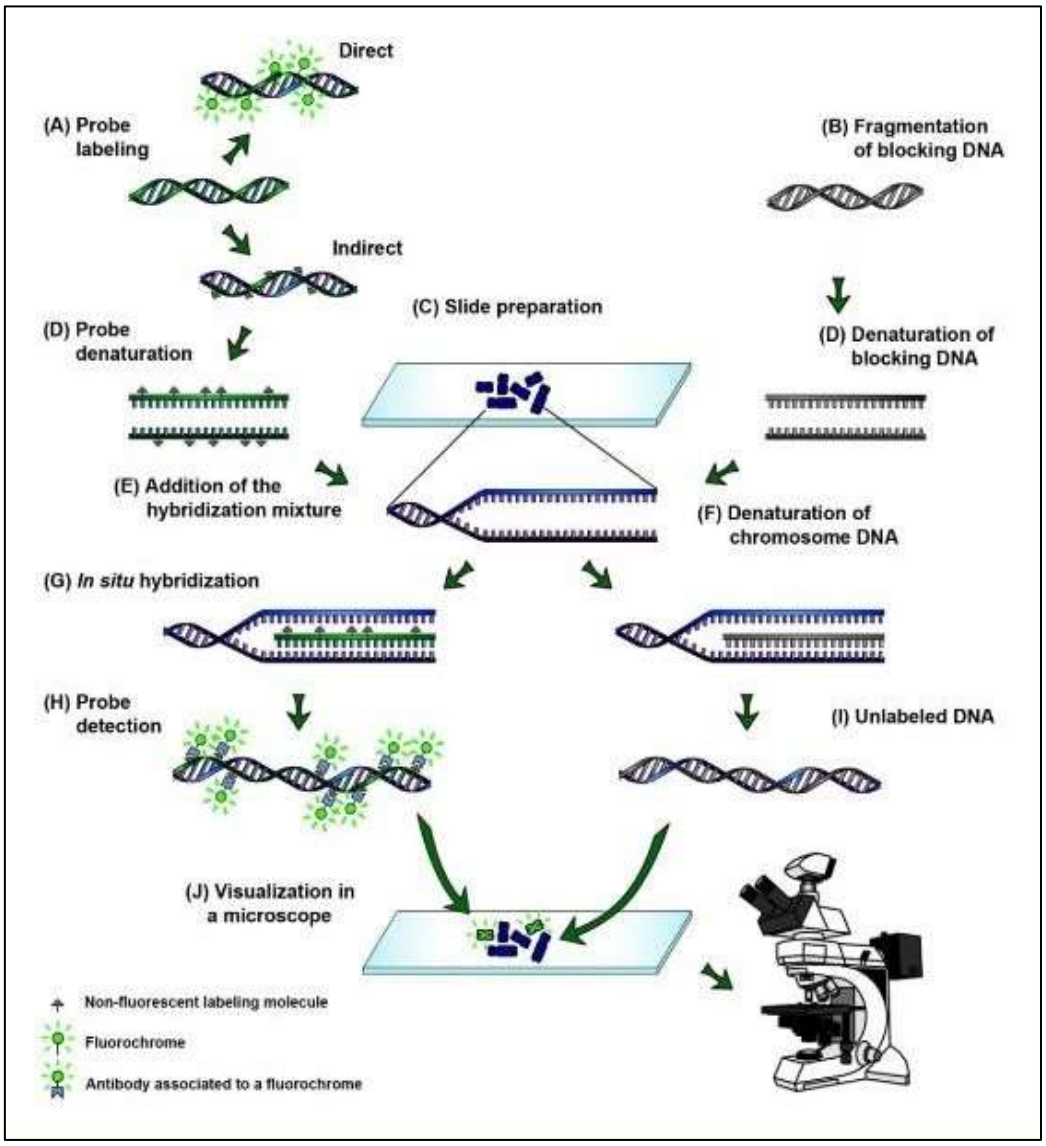

Fig.: Main steps of the GISH.

Immunohistochemistry (IHC):- a technique used for localizing the proteins in cells of a tissue section.

Immunohistochemistry or IHC refers to the process of localizing proteins in cells of a tissue section exploiting the principle of antibodies binding specifically to antigens in biological tissues. It takes its name from the roots "immuno," in reference to antibodies used in the procedure, and "histo," meaning tissue.

Immunohistochemical staining is widely used in the diagnosis and treatment of cancer. Specific molecular markers are characteristic of particular cancer types. IHC is also widely used in basic research to understand the distribution and localization of

biomarkers in different parts of a tissue. Visualizing an antibody-antigen interaction can be accomplished in a number of ways. In the most common instance, an antibody is conjugated to an enzyme, such as peroxidase, that can catalyse a colour-producing reaction.

Alternatively, the antibody can also be tagged to a fluorophore, such as FITC, rhodamine, or Texas Red. The latter method is of great use in confocal laser scanning microscopy, which is highly sensitive and can also be used to visualize interactions between multiple proteins.

Antibody Types:

The antibodies used for specific detection can be polyclonal or monoclonal. Monoclonal antibodies are generally considered to exhibit greater specificity. Polyclonal antibodies are made by injecting animals with peptide antigens and then after a secondary immune response is stimulated, isolating antibodies from whole serum. Thus, polyclonal antibodies are a heterogeneous mix of antibodies that recognize several epitopes.

Antibodies can also be classified as primary or secondary reagents. Primary antibodies are raised against an antigen of interest and are typically unconjugated (un-labelled), while secondary antibodies are raised against primary antibodies. Hence, secondary antibodies recognize immunoglobulin's of a particular species and are conjugated to either biotin or a reporter enzyme such as alkaline phosphatase or horseradish peroxidase.

Some secondary antibodies are conjugated to fluorescent agents, such as the Alexa-Fluor family, are also frequently used for detection of proteins in IHC procedures. Protein concentration is generally measured by densitometry analysis, where the intensity of staining correlates with the amount of the protein of interest.

Sample Preparation:

In the procedure, depending on the purpose and the thickness of the experimental sample, either thin (about 4-40 μm) slices are taken of the tissue of interest, or if the tissue is not very thick and is penetrable it is used whole. The slicing is usually accomplished through the use of a microtome, and slices are mounted on slides. "Free-floating IHC" uses slices that are not mounted; these slices are normally produced using a vibrating microtome.

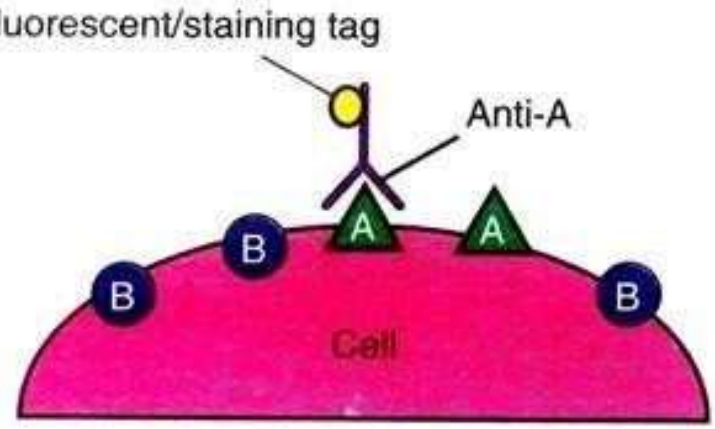

Fig. 6.13: The direct method of immunohistochemical staining uses one labelled antibody, which binds directly to the antigen being stained for.

Diagnostic IHC Markers:

IHC is an excellent detection technique and has the tremendous advantage of being able to show exactly where a given protein is located within the tissue examined. This has made it a widely-used technique in the neurosciences, enabling researchers to examine protein expression within specific brain structures.

Its major disadvantage is that, unlike immuno-blotting techniques where staining is checked against a molecular weight ladder, it is impossible to show in IHC that the staining corresponds with the protein of interest. For this reason, primary antibodies must be well-validated in a Western Blot or similar procedure. The technique is even more widely used in diagnostic surgical pathology for typing tumours (e.g., carcinoma vs. melanoma).

i. Carcinoembryonic antigen (CEA): used for identification of adenocarcinomas. Not spe- cific for site.

ii. CD 15 and CD30 : used for Hodgkin's disease.

iii. Alpha fetoprotein: for yolk sac tumours and hepatocellular carcinoma.

iv. CD117: for gastrointestinal stromal tumours (GIST).

v. Prostate specific antigen (PSA): for prostate cancer.

vi. Estrogens and progesterone staining for tumour identification.

vii. Identification of B-cell lymphomas using CD20.

Transplantaion Immunology

1. Meaning of Transplantation:

Transplantation is a useful procedure in surgical repair or replacement of diseased tissues or organs. It has been well-established that an animal accepts grafts of its own tissue or that of an identical twin, while a graft from another animal of the same species is rejected. The immune response induced by the transplantation (HLA) antigens present in all mammalian cells is the reason for the rejection of exogenous grafts.

The first record of plastic surgery is found in Sushrut Sanhita (800 BC). The first Indian surgeon Sushruta reconstructed a severed nose of a patient with the patient's own skin flap.

Transplantation may be fresh organs or stored organs in orthotropic grafts, the transplant is applied in anastomosed normal site (e.g. skin graft), whereas heterotopic grafts are placed in anatomically abnormal sites (e.g.. thyroid transplanted in subcutaneous tissue).

2. Types of Transplant:

- Auto-graft (Autogenic graft). It is a tissue of one site engrafted to another site in the same individual.
- Isograft (Syngenetic graft). It is a graft placed in another individual of the same genetic constitution (e.g. monozygous twins or members of inbred strains).
- Allograft (Homograft or allogeneic graft). It is a graft transfer between two genetically different members of the same species.
- Xeno graft (Xenogeneic graft). Grafts between members of different species are called xeno-graft. It was formerly known as heterograft.
- Auto-graft and isograft are usually accepted and survives causing a minimum inflammatory reaction. Allografts and xenografts usually undergo necrosis and are rejected due to genetic and antigenic incompatibility.

Allograft Reaction:

Rejection of the graft by recipient is called allograft reaction. The most successful organ transplant is that of kidney. Other organ transplants such as bone marrow has been tried but with little success.

3. Mechanism of Transplant Rejection:

Changes observed in human renal allograft rejection is almost similar to that observed in mice:

a. The graft becomes vascularized within a few days and appears to be accepted initially.

b. Between 3-9 days, there is increasing infiltration of the graft by lymphocytes and monocytes with marked reduction in circulation. At this stage, very few plasma cells invade the graft.

c. Between 10-11 days, the necrosis begins to be visible to the naked eye.

d. On 12th or 13th day, the graft sloughs off completely.

Cell mediated reaction is almost responsible for the rejection. Rejection is brought by helper T cells which activate cytotoxic T cells, macrophages and B cells when second allograft from the same donor is applied on a sensitized recipient, it will be rejected in 5-6 days. In this accelerated rejection of second graft second set reaction, antibodies play an important role along with cell-mediated immunity.

Antibodies are formed abundantly from the 11th day onward of transplantation. Thus a graft is rejected either by sensitized T cells or by circulating antibodies. Antibody induces platelet aggregation or type II hypersensitivity reaction (ADCC), Antibody Dependent cell mediated cytotoxicity.

4. Tissue Typing and Matching in Transplantation:

ABO compatibility is essential in all tissue transplantation. Survival of an allograft depends on HLA compatibility. HLA antigens are expressed on the surface of leucocytes (especially lymphoid cells). A full HLA type of an individual contains two haplotypes (haplotype—one strand of chromosome pair), inherited from each parent.

There is reasonable opportunity for genetic matching of siblings of a patient, 25% chance for both haplotypes and 50% chance of one haplotype. With many HLA antigens discovered, there is possibility of thousand halo-types. There is a remote chance that two random individuals will have completely identical haplotypes. The best HLA compatible donors are selected from the family members.

i. Leucocytes Grouping:

The HLA antigens of class I type on leucocytes are identified by sera obtained from:

(i) Multiparous women who usually possess antibodies to HLA antigens of their husbands

(ii) Recipient of multiple blood transfusion

(iii) Volunteers who are repeatedly skin grafted

(iv) Monoclonal antibodies prepared against HLA antigens.

Cytotoxic Test: A purified suspension of blood lymphocytes of donor is mixed with the recipient's serum and a panel of standard sera for HLA antigens in presence of complement. When lymphocytes carry the appropriate antigens, the cytotoxic antiserum will combine with the lymphocytes (target cell).

The antigen-antibody complex will activate the complement and damage the cell membrane and the permeability of cell membrane is increased and the same is detected by dye (trypan blue) uptake into the cell, i.e. the dead cells are stained by the dye.

ii. Mixed Leucocyte Culture Test:

The test detects class II antigens of HLA complex and cell mediated immunity. Lymphocytes from both donors and recipients are cultured together (co-cultivation). The test is based on the principle that T-lymphocytes—when exposed to incompatible HLA antigen—undergo blastoid transformation, take up thymidine and divide.

iii. Lymphocyte Transfer Test:

Peripheral blood lymphocytes of recipient is injected intradermally into several prospective donors. Delayed hypersensitivity reaction at the injection site is indicative of immune response of recipient leucocytes against the donor tissue. Persons showing no response against recipient's leucocytes is chosen as donor.

iv. Detection of Preformed Antibody:

Donor's lymphocytes are matched with recipient's serum. If preformed antibodies are present in patient's serum, the lymphocytes of the donor undergo lysis.

5. Prevention of Graft Rejection in Transplantation:

1. Immunosuppression:

When HLA typing of the recipient and donor is well-matched, a state of immuno-suppression is produced in the recipient so that the transplant tissue survives for a long time. By irradiation, corticosteroids and anti-lymphocytic serum (ALS) the immunological reactivity may be non-specifically depressed. Such patients are susceptible to infections and prone to development of lymphomas.

2. Transplantation in Anatomically Protected Sites:

There are certain anatomically protected sites where allografts are permitted to survive, e.g. cornea, cartilage and testicle grafting. The avascularity of cornea limits the entry of lymphocytes into the graft.

3. Immunological Enhancement:

The circulating antibody produced against graft antigens can—under circumstances—protect the graft from cell mediated immune response. This phenomenon is known as "**Immunological enhancement**". If the recipient animal is previously immunized with one or more injections of tissue from the prospective donor and transplant applied subsequently, the transplant survives for longer duration.

Following transplantation of kidney in rats by this method, the graft maintains normal function for a year or more. The immunological enhancement phenomenon can be passively transferred from immunized animal to a normal animal. In certain human kidney transplantations, the method has been used with some success.

Cancer Immunobiology and Immunotherapy:

Cancer involves a loss of the ability of cells to control their cell cycle, the stages each eukaryotic cell goes through as it grows and then divides. When this control is lost, the affected cells rapidly divide and often lose the ability to differentiate into the cell type appropriate for their location in the body. In addition, they lose contact inhibition and

can start to grow on top of each other. This can result in formation of a tumor. It is important to make a distinction here: The term “cancer” is used to describe the diseases resulting from loss of cell-cycle regulation and subsequent cell proliferation. But the term “tumor” is more general. A “tumor” is an abnormal mass of cells, and a tumor can be benign (not cancerous) or malignant (cancerous).

Traditional cancer treatment uses radiation and/or chemotherapy to destroy cancer cells; however, these treatments can have unwanted side effects because they harm normal cells as well as cancer cells. Newer, promising therapies attempt to enlist the patient’s immune system to target cancer cells specifically. It is known that the immune system can recognize and destroy cancerous cells, and some researchers and immunologists also believe, based on the results of their experiments, that many cancers are eliminated by the body’s own defenses before they can become a health problem. This idea is not universally accepted by researchers, however, and needs further investigation for verification.

Cell-Mediated Response to Tumors

Cell-mediated immune responses can be directed against cancer cells, many of which do not have the normal complement of self-proteins, making them a target for elimination. Abnormal cancer cells may also present tumor antigens. These tumor antigens are not a part of the screening process used to eliminate lymphocytes during development; thus, even though they are self-antigens, they can stimulate and drive adaptive immune responses against abnormal cells.

Presentation of tumor antigens can stimulate naïve helper T cells to become activated by cytokines such as IL-12 and differentiate into TH1 cells. TH1 cells release cytokines that can activate natural killer (NK) cells and enhance the killing of activated cytotoxic T cells. Both NK cells and cytotoxic T cells can recognize and target cancer cells, and induce apoptosis through the action of perforins and granzymes. In addition, activated cytotoxic T cells can bind to cell-surface proteins on abnormal cells and induce apoptosis by a second killing mechanism called the CD95 (Fas) cytotoxic pathway.

Despite these mechanisms for removing cancerous cells from the body, cancer remains a common cause of death. Unfortunately, malignant tumors tend to actively suppress the immune response in various ways. In some cancers, the immune cells themselves are cancerous. In leukemia, lymphocytes that would normally facilitate the immune

response become abnormal. In other cancers, the cancerous cells can become resistant to induction of apoptosis. This may occur through the expression of membrane proteins that shut off cytotoxic T cells or that induce regulatory T cells that can shut down immune responses.

The mechanisms by which cancer cells alter immune responses are still not yet fully understood, and this is a very active area of research. As scientists' understanding of adaptive immunity improves, cancer therapies that harness the body's immune defenses may someday be more successful in treating and eliminating cancer.

Cancer Vaccines

There are two types of cancer vaccines: preventive and therapeutic. Preventive vaccines are used to prevent cancer from occurring, whereas therapeutic vaccines are used to treat patients with cancer. Most preventive cancer vaccines target viral infections that are known to lead to cancer. These include vaccines against human papillomavirus (HPV) and hepatitis B, which help prevent cervical and liver cancer, respectively.

Most therapeutic cancer vaccines are in the experimental stage. They exploit tumor-specific antigens to stimulate the immune system to selectively attack cancer cells. Specifically, they aim to enhance TH1 function and interaction with cytotoxic T cells, which, in turn, results in more effective attack on abnormal tumor cells. In some cases, researchers have used genetic engineering to develop antitumor vaccines in an approach similar to that used for DNA vaccines (see Micro Connections: DNA vaccines). The vaccine contains a recombinant plasmid with genes for tumor antigens; theoretically, the tumor gene would not induce new cancer because it is not functional, but it could trick the immune system into targeting the tumor gene product as a foreign invader.

The first FDA-approved therapeutic cancer vaccine was sipuleucel-T (Provenge), approved in 2010 to treat certain cases of prostate cancer. This unconventional vaccine is custom designed using the patient's own cells. APCs are removed from the patient and cultured with a tumor-specific molecule; the cells are then returned to the patient. This approach appears to enhance the patient's immune response against the cancer cells. Another therapeutic cancer vaccine (talimogene laherparepvec, also called T-VEC or Imlygic) was approved by the FDA in 2015 for treatment of melanoma, a form of skin cancer. This vaccine contains a virus that is injected into tumors, where it infects and

lyses the tumor cells. The virus also induces a response in lesions or tumors besides those into which the vaccine is injected, indicating that it is stimulating a more general (as opposed to local) antitumor immune response in the patient.

Using Viruses to Cure Cancer

Viruses typically destroy the cells they infect—a fact responsible for any number of human diseases. But the cell-killing powers of viruses may yet prove to be the cure for some types of cancer, which is generally treated by attempting to rid the body of cancerous cells. Several clinical trials are studying the effects of viruses targeted at cancer cells. Reolysin, a drug currently in testing phases, uses reoviruses (respiratory enteric orphan viruses) that can infect and kill cells that have an activated Ras-signaling pathway, a common mutation in cancerous cells. Viruses such as rubeola (the measles virus) can also be genetically engineered to aggressively attack tumor cells. These modified viruses not only bind more specifically to receptors overexpressed on cancer cells, they also carry genes driven by promoters that are only turned on within cancer cells. Herpes virus and others have also been modified in this way.

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6. Effector Mechanisms: Mucosal immunity, Peyer's patches, gut barriers, oral immunization, Oral tolerance, Cytotoxic response, ADCC, NK cells, CTL, Th, T regulation, Immunoregulation, anergy, tolerance, anti idiotype, Mechanisms of antiviral innate immune response.

Objective: In this unit we will discuss about different mechanisms of innate immunity. Different kinds of cells which are involved in immune respose modulators are also discussed.

Organization of Immune System:

The immune system consists of a network of diverse organs and tissue which vary structurally as well as functionally from each other (Fig. 2). These organs remain spreaded throughout the body. Basically, immune system is a complex network of lymphoid organs, tissues and cells.

The immune system consists of several organs distributed throughout the body (Fig. 1).

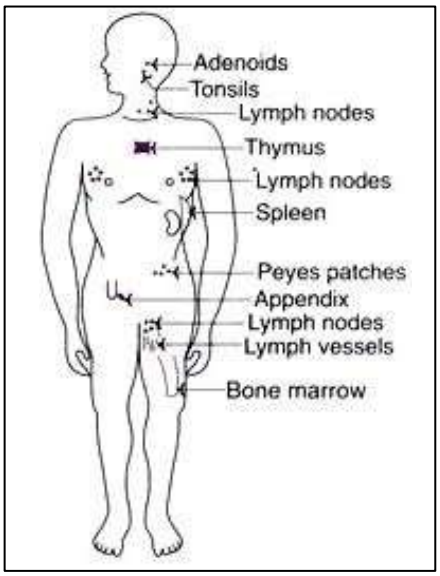

Fig.: A diagrammatic representation of human lymphatic system.

The Organs of the Immune System:

The immune system is made of the primary lymphoid and the secondary lymphoid organs. The primary lymphoid includes the bone marrow and the thymus, while the others such as the spleen, Peyer's patches of small intestine and the lymph nodes are included in the second category.

These lymphoid organs are categorized as primary and secondary.

1. Primary lymphoid organs:

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

(i) Bone marrow:

Bone marrow is the main lymphoid organ where all blood cells including lymphocytes

are formed. Maturation of B-lymphocytes occurs here.

(ii) Thymus:

Thymus is the site of T lymphocyte maturation. Thymus is situated near the heart. Thymus is quite large in size at the time of birth but keeps reducing with age. As stated earlier, T-lymphocytes and B-lymphocytes are responsible for cellular and humoral immune response respectively.

2. Secondary lymphoid organs:

After maturation, B lymphocytes and T lymphocytes migrate via blood vascular and lymphatic system to the secondary lymphoid organs where they undergo proliferation and differentiation. The acquired immune response to antigens usually develops in these organs and become effector cells.

In the secondary lymphoid tissues, the lymphocytes do not remain, and move from one lymphoid organ to another through blood and lymph. The secondary lymphoid organs are lymph nodes, spleen, tonsils, Peyer's patches of the small intestine and mucosal associated lymphoid tissues (MALT).

(i) Lymph nodes:

These are small solid structures found at intervals along the lymphatic system. They are composed of lymphoid tissue and act as filters for the lymph, preventing foreign particles from entering the bloodstream. Lymph nodes also produce lymphocytes and plasma cells.

(ii) Spleen:

It is a bean shaped organ which is the largest single mass of lymphoid tissue in the body. In foetus the spleen produces all types of blood cells but in adult it only produces lymphocytes. Macrophages of spleen are phagocytic.

(iii) Tonsils:

Usually there are six tonsils. They act as filters to protect body from bacteria and aid in the formation of white blood cells.

(iv) Peyer's patches:

These are clusters of lymph nodules found in small intestine, especially along the ileum. They produce lymphocytes.

(v) Mucosal-Associated Lymphoid Tissues (MALT):

MALT is significant aggregations of lymphoid tissues which are seen in relation to the mucosa of the major tracts like respiratory, alimentary canal and urinogenital tracts. It constitutes about 50 percent of the lymphoid tissue in human body.

They do not serve as filters of lymph. Larger aggregations extend into the submucosa. However, they are centres of lymphocyte production. Apart from B-lymphocytes and T-lymphocytes, phagocytic macrophages and dendritic cells are present.

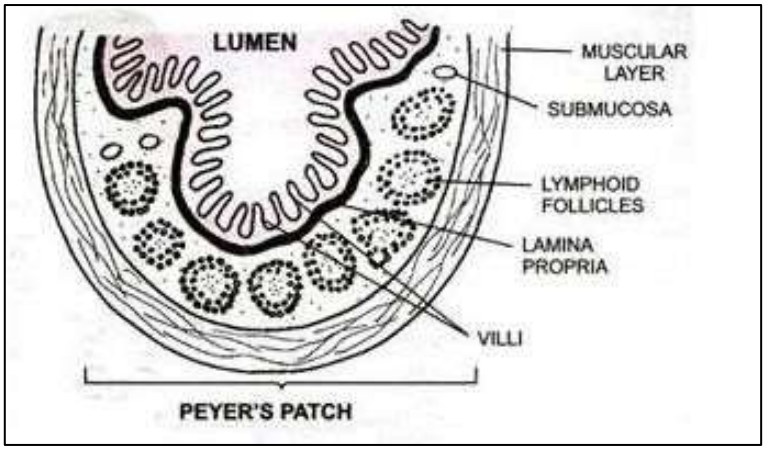

Fig.: Diagram showing Peyer's patch in the submucosa of small intestine.

Cells of the Immune System:

Leukocytes (white blood cells) are immune system cells involved in defending the body against infectious disease and foreign materials. Five different types of leukocytes exist, all produced and derived from a multipotent cell in the bone marrow known as a hematopoietic stem cell. The innate leukocytes include the phagocytes, mast cells, eosinophils, basophils, and natural killer cells. These cells identify and eliminate pathogens and are important mediators in the activation of the adaptive immune system.

Neutrophils and macrophages are phagocytes that travel throughout the body in pursuit of invading pathogens. Neutrophils are normally found in the bloodstream and are the most abundant type of phagocyte. During the acute phase of inflammation neutrophils migrate toward the site of inflammation and are usually the first cells to arrive at the scene of infection. Macrophages reside within tissues and produce a wide array of chemicals. They also act as scavengers, ridding the body of worn-out cells and other

debris, and as antigen-presenting cells that activate the adaptive immune system. Dendritic cells are phagocytes in tissues that are in contact with the external environment, and are located mainly in the skin, nose, lungs, stomach, and intestines. These cells serve as a link between the bodily tissues and the innate and adaptive immune systems, as they present antigen to T-cells, one of the key cell types of the adaptive immune system.

Mast cells reside in connective tissues and mucous membranes, and regulate the inflammatory response. They are most often associated with allergy and anaphylaxis.

Basophils and eosinophils are related to neutrophils. They secrete chemical mediators that are involved in defending against parasites, and play a role in allergic reactions, such as asthma.

Natural killer cells are leukocytes that attack and destroy tumor cells, or cells that have been infected by viruses. The cells of the adaptive immune system are special types of leukocytes, called lymphocytes. B cells and T cells are the major types of lymphocytes and are derived from hematopoietic stem cells in the bone marrow.

Here we will discuss seven important cells of immune system. The cells are: 1. Hematopoietic Stem Cell 2. Lymphocytes 3. Monocytes 4. Macrophages 5. Granulocytes 6. Dendritic Cells 7. Mast Cells.

Hematopoietic Stem Cell:

All blood cells arise from a type of cell called hematopoietic stem cell (HSC) (or stem cell). The stem cells are self-renewing, maintain their population by cell division, and differentiate into other cell types. This process of formation and development of blood cells (red and white blood cells) is called hematopoiesis.

It is remarkable that every functionally specialised, mature blood cell is derived from the same type of hematopoietic stem cell. In contrast to a unipotent cell, which differentiates into a single cell type, a hematopoietic stem cell is multi-potent or pluripotent as it is able to differentiate in various ways and thereby gives rise to various type of blood cells.

In humans, the formation and development of blood cells begins in the embryonic yolk sac during the first weeks of development. The hematopoietic stem cells differentiate into primitive erythroid cells that contain embryonic haemoglobin. In the third month of

gestation, hematopoietic stem cells migrate from the yolk sac to the foetal liver and then to the spleen.

Liver and spleen play major roles in hematopoiesis from the third to the seventh months of gestation. In later months, hematopoietic stem cells differentiate in the bone marrow and play major role in hematopoiesis, and by birth there is little or no hematopoiesis in the liver and spleen.

Multi-potent hematopoietic stem cell (or stem cell) in the bone marrow differentiates to form two lineages:

(1) **Common-lymphoid progenitor cell and**

(2) **Common myeloid progenitor cell**

The progenitor cells, unlike hematopoietic stem cell that is self-renewing, loss the capacity for self-renewal, and are committed to their specific cell linkage.

The common lymphoid progenitor cells give rise to B-lymphocytes (B-cells) that differentiate into antibody secreting plasma cells. T-lymphocytes (T-cells) that become activated T-cells. natural killer (NK) cells, and some dentritic cells.

The common myeloid progenitor cells give rise to erythroblasts that produce erythrocytes (red blood cells), megakaryoblasts that produce platelets (thrombocytes), myeloblasts that produce granulocytes (eosinophils, basophils, neutrophils), monoblasts that differentiate into monocytes which give rise to macrophages and dendritic cells, and an unknown precursor that produces mast cells.

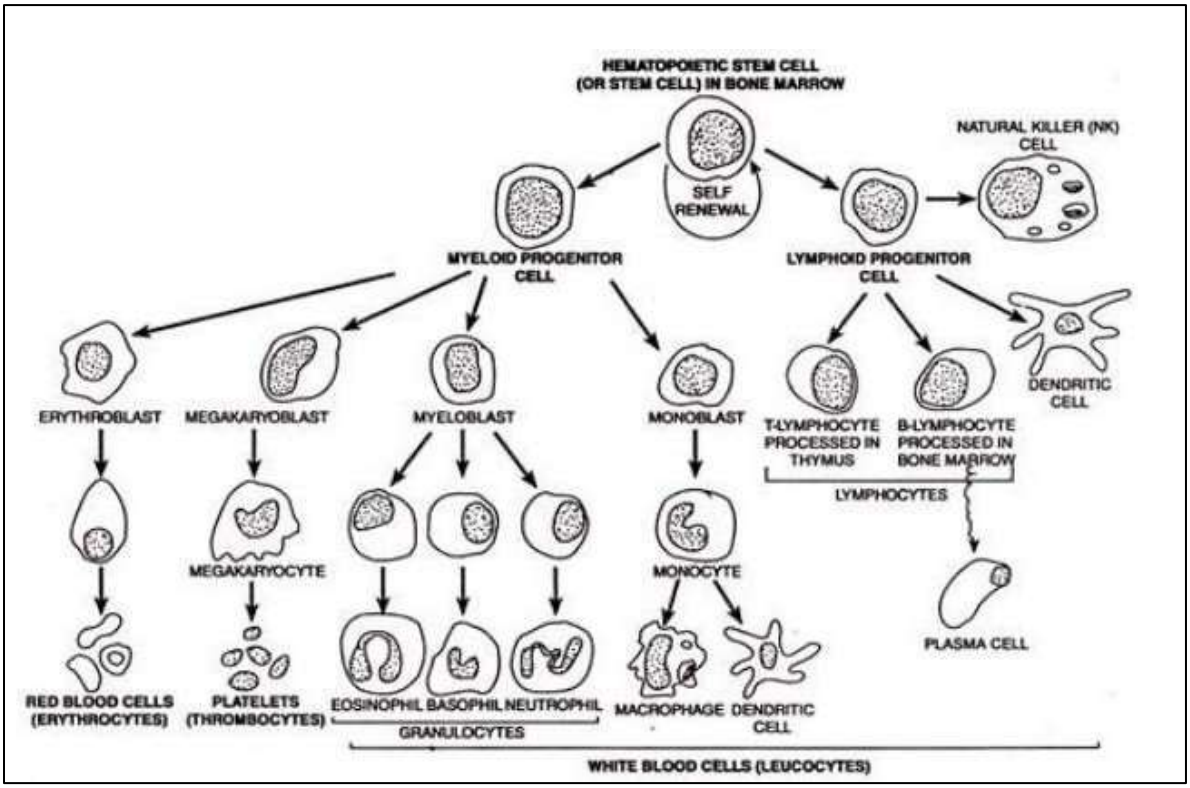

Fig.: Haematopoiesis.

However, B-lymphocytes (B-cells) T-lymphocytes (T-cells) and natural killer (NK) cells produced by lymphoid progenitor cell lineage and eosinophils, basophils, neutrophils, macrophages, and dendritic cells produced by myeloid progenitor cell lineage are collectively called white blood cells or leucocytes (Gk. leucos = white, kytos = cell). White blood cells or leucocytes are the cells that are responsible for nonspecific and specific immunity in the body.

Lymphocytes:

Lymphocytes (L. lympha = water, cyte = cell) are the most important effector cells of many cells involved in specific immune response. These cells are small, round and 5-15 µm in diameter. They are found in peripheral blood, lymph, lymphoid organs, and in many other tissues. Lymphocytes constitute 20% – 40% of the white blood cell (leucocyte) population in the body and 99% of the cells in the lymph.

They may be small (5-8 µm), medium (8-12 µm). and large (12-15 µm). The small lymphocytes are more numerous and may be short-lived with a life-span about two weeks or long-lived with a life-span of three years or more or even for life.

Short-lived lymphocytes act as effector cells in immune response, while long-lived ones

function as memory cells. Long-lived lymphocytes are mainly thymus derived. The formation and development of lymphocytes, i.e.. lymphopoiesis takes place in bone marrow, primary or central lymphoid organs, and secondary or peripheral lymphoid organs.

Lymphocytes are approximately 10^{11} in number in a human body; their number ranges from 10^{10} to 10^{12} depending on body size and age. Lymphocytes can be broadly subdivided into three populations: B-lymphocytes or B-cells, T-lymphocytes or T-cells, and null cells (natural killer cells or NK cells are included in this group).

1. B-Lymphocytes or B-Cells:

B-lymphocytes or B-cells derive their name from their site of maturation. They are so named since they were first detected in the bursa of Fabricius of birds and later from bone marrow of a number of mammalian species, including humans and mice. In birds, the multi-potent hematopoietic stem cells originating in the bone marrow migrate to the bursa of Fabricius and differentiate their into antibody synthesizing cells.

The bursa is a small pouch-like organ in the embryonic hind-gut of birds and is absent in mammals. In a number of mammalian species including humans and mice, the B-cells originate in the foetal lever and later migrate to the bone marrow which becomes the site for production of B-cells after embryonic life.

B-lymphocytes do not have the ability to synthesize antibody molecule during undifferentiated stage. During differentiation, each lymphocyte acquires the ability to synthesize antibody molecules when provoked by antigens.

2. T-Lymphocytes or T-Cells:

T-Lymphocytes or T-cells derive their name from their site of maturation in the thymus. They are major players in the cell-mediated immune response and also have an important role in B-cell activation. T-cells themselves do not secrete antibodies (immunoglobulin) like B-cells.

They are immunologically specific and are directly involved in cell-mediated immune responses, can carry a vast repertoire of immunologic memory, and can function in a variety of effector and regulatory way.

Monocytes:

Monocytes (G. monos = single; cyte = cell) are mononuclear phagocytic leucocytes

possessing an oval or kidney-shaped nucleus and granules in the cytoplasm that stain grey-blue (Figure).

Monocytes are produced in bone marrow. During hematopoiesis in bone marrow, granulocyte-monocyte progenitor cells differentiate into pro-monocytes, which-leave the bone marrow and enter the blood where they further differentiate into mature monocytes.

Mature monocytes circulate in the blood stream for about eight hours, enlarge in size, migrate into the tissues, and differentiate into specific tissue macrophages or into myeloid dendritic cells.

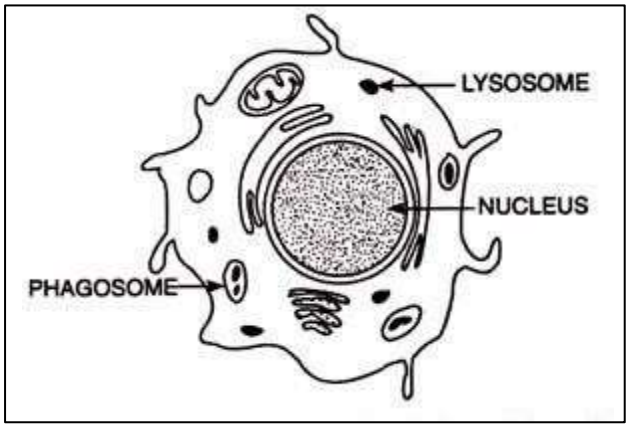

Fig.: Diagrammatic sketch of typical morphology of a monocyte.

Macrophages:

Macrophages (G. macros = large; phagein = to eat), as noted above, are differentiated from monocytes into the tissues of the body.

Differentiation of a monocyte into a tissue macrophage (Fig. 6) involves a number of changes:

(i) The monocyte enlarges five- to ten-fold,

(ii) Its intracellular organelles increase in both number (especially lysosomes and phagolysosomes) and complexity,

(iii) The cell acquires increased phagocytic ability,

(iv) Produces higher levels of hydrolytic enzymes,

(v) Begins to secrete a variety of soluble factors, and

(vi) Develops ruffles or microvilli on the surface of its plasma membrane.

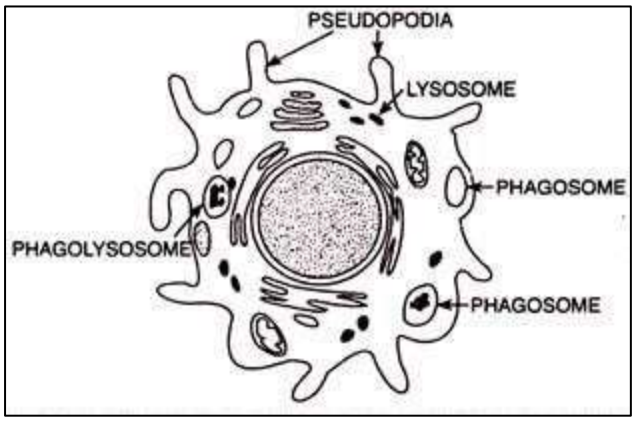

Fig.: Diagrammatic sketch of typical morphology of a macrophage which are 5 to 10 fold larger than monocytes and contain more organelles especially lysosomes and phagolysosomes.

Macrophages are transported throughout the body. Some macrophages reside in particular tissues and become fix macrophages. Others remain motile by amoeboid movement throughout the body and are called free or wondering macrophages.

Macrophages serve different functions i different tissues and are named according to their tissue location, e.g., histiocytes in connective tissues, osteoclasts in bone, microglial cells in the brain, alveolar macrophages in the lung, kupffer cells in the liver, and mesangial cells in the kidney.

Macrophages normally remain in a resting state and are activated for effective functioning. They are activated by a variety of stimuli such as interferon gamma (IFN-γ) secreted by activated T helper (T_H) cells, mediators of inflammatory response, components of bacterial cell walls, etc.

Activated macrophages secrete different types of cytotoxic proteins that help them eliminate large number of pathogens including vims-infected cells, malignant cells, and intracellular bacteria.

Activated macrophages also display class II MHC molecules that allow them to act more effectively as antigen-presenting cells (APCs). Thus, macrophages and T helper (T_H) cells facilitate each other's activation during the immune response. Macrophages are

highly phagocytic and they are capable of ingesting and digesting exogenous antigens (e.g., whole microorganisms and insoluble particles) and exogenous matter (e.g., injured or dead host cells, cellular debris, activated clotting factors).

Granulocytes:

Granulocytes (Fig. 6) are those white blood cells (leucocytes) which have irregular-shaped nuclei with two to five lobes and granulated cytoplasmic matrix.

Granules of cytoplasmic matrix contain reactive substances that kill microorganisms and enhance inflammation. Granulocytes are also called polymorphonuclear leucocytes (PMNs). Three types of granulocytes are recognised in the body and they are: basophils, eosinophils, and neutrophils.

1. Basophils:

Basophils (G. basis = base; philein = to love) possess bi-lobed irregular-shaped nucleus and cytoplasmic matrix granules that stain bluish-black with basic dyes (e.g., methylene blue). These granulocytes are non-phagocytic cell that function by releasing pharmacologically active substances (e.g., histamine, prostaglandins, serotonin, and leucotrienes) from their cytoplasmic granules upon appropriate stimulation.

Since these pharmacologically active substances influence the tone and diameter of blood vessels, they are collectively termed vasoactive mediators. Basophils possess high-affinity receptors for immunoglobulin-E (IgE) antibody and thereby become coated with these antibodies.

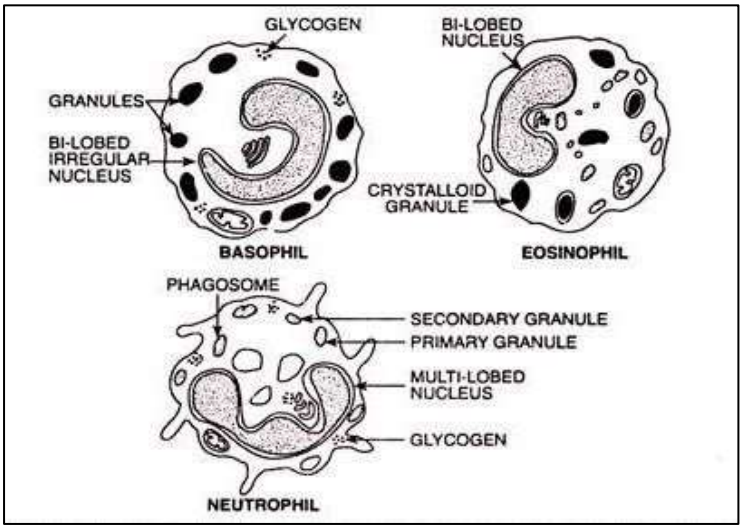

Fig.: Diagrammatic sketch showing the morphology of granulocytes

Once coated, antigens trigger the basophil cells to secrete vasoactive mediators which are inflammatory and play a major role in certain allergic responses (e.g., eczema, hay fever, and asthma). Basophils, however, comprise less than 1 % of white blood cells, are non-motile, and remain confined to the blood stream.

2. Eosinophils:

Eosinophils (G. eos = dawn; philein = to love) have a bi-lobed nucleus connected by a slender thread of chromatin and prominent acidophilic granules in cytoplasmic matrix. Eosinophils, like neutrophils, are motile cells that migrate from bloodstream into tissue spaces.

These granulocytes are considered to play a role in the defence against parasitic organisms (protozoans and helminth parasites) by phagocytosis.

They release mainly cationic proteins and reactive oxygen metabolites into the extracellular fluid. These substances damage the plasma membrane of the parasite. Eosinophils constitute only 3-5% of the white blood cells and their acidophilic granules stain red with acidic dyes.

3. Neutrophils:

Neutrophils (L. neuter – neither; philein = to love) possess a three- to five-lobed nucleus connected by slender threads of chromatin, and contain fine primary and secondary granules in cytoplasmic matrix. Neutrophils, like eosinophils, are motile cells that migrate from bloodstream into the tissue.

These granulocytes circulate in the bloodstream for 7 to 10 hours before their migration into the tissues where they enjoy a life span of only a few days. Approximately 60% of the circulating white blood cells (leucocytes) in human are the neutrophils. Like macrophages, the' primary function of neutrophils is phagocytosis of foreign or dead cells and pinnocytosis of pathological immune complexes.

Phagocytosis by neutrophils is similar to that operated by macrophages except that the lytic enzymes and bactericidal substances in neutrophils are contained within primary and secondary granules instead of lysosomes in macrophages. The primary granules are larger and denser and contain peroxidase, lysozyme, and various hydrolytic enzymes.

The secondary granules are smaller and contain collagenase, lactoferrin, and lysozyme. Both primary and secondary granules fuse with phagosome, whose contents are then

digested and the remains excreted much as they are in macrophages.

Neutrophils, like macrophages, also use oxygen-dependent and oxygen-independent pathways to generate antimicrobial substances and defensins to kill ingested microorganisms. Neutrophils generate more reactive oxygen intermediates and reactive nitrogen intermediates and express higher levels of defensins than macrophages do.

Dendritic Cells:

Dendritic cells constitute only 0.2% of WBCs (leucocytes) in the blood and are present in even smaller numbers in skin and mucous membranes of the nose, lungs, and intestines. They derive their name due to long membrane extensions resembling the dendrites of nerve cells.

Dendritic cells arise from hematopoietic stem cells in the bone marrow via different pathways and in different locations (Fig. 8); they descend through both the myeloid and lymphoid lineages. Stem cell-originated dendritic cells are of four types: Langerhans cells, interstitial dendritic cells, myeloid dendritic cells, and lymphoid dendritic cells.

Despite differences, all the stem cell-originated mature dendritic cells perform the same major function of presenting antigen to T helper (T_H) cells by expressing high levels of both class II MHC molecules and members of the co-stimulatory B-7 family, and thereby play an important accessory role in the specific immune response.

This pattern of functioning makes dendritic cells more potent antigen-presenting cells (APCs) than macrophages and B-lymphocytes, both of which need to be activated before they can function as antigen-presenting cells (APCs).

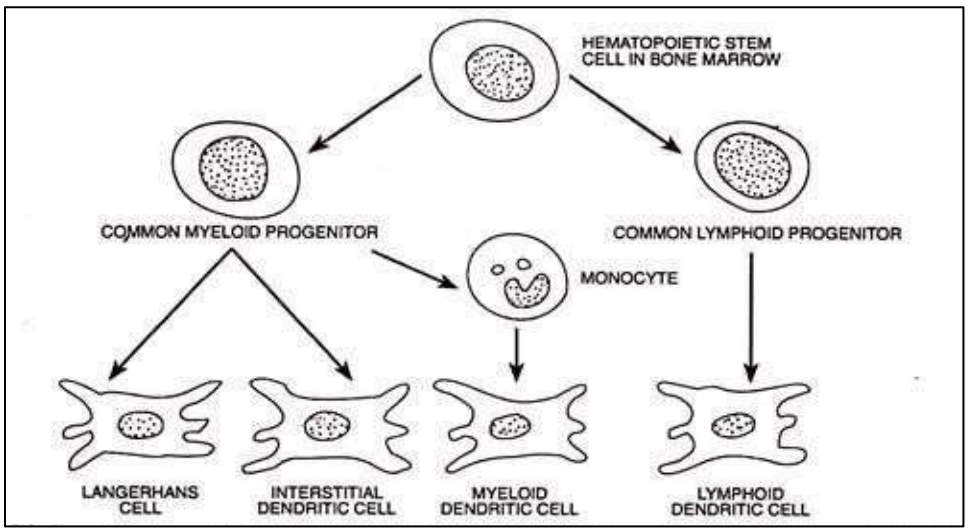

Fig.: Different types of dendritic cells originated by haematopoietic stem cell in bone marrow. Dendritic cells arise from both the myeloid and lymphoid lineages.

In addition to dendritic cells originated in bone marrow, there are another type of dendritic cells, the follicular dendritic cells, that do not arise in bone marrow and perform their function in a different ways as they do not express class II MHC molecules and do not act as antigen-presenting cells (APCs).

Follicular dendritic cells express high levels of membrane receptors for antibody; which allows the binding of antibody complexes. The interaction of B-lymphocytes with this bound antigen can have important effects on B-lymphocyte responses.

Mast Cells:

Mast cell precursors originate in the bone marrow and are released into the blood as undifferentiated cells. Mast cells are not differentiated from their precursors until the latter leave the blood and enter the tissues. Mast cells are found in a variety of tissues including the skin, connective tissues of various organs, and mucosal epithelial tissue of the respiratory, genitourinary, and digestive tracts.

These cells, like basophils, possess large numbers of granules in cytoplasmic matrix. The granules in cytoplasm contain histamine and other pharmacologically active substances that contribute to the inflammatory response. Mast cells, together with basophils, play an important role in the development of allergies and hypersensitivities.

Innate Immunity:

Innate immunity is non-specific, and represents the inherent capability of the organism to offer resistance against diseases. It consists of defensive barriers.

First line of defense:

The skin is the largest organ in the human body, constituting about 15% of the adult body weight. The skin provides mechanical barrier to prevent the entry of microorganisms and viruses. The acidic (pH 3-5) environment on the skin surface inhibits the growth of certain microorganisms. Further, the sweat contains an enzyme lysozyme that can destroy bacterial cell wall.

Second line of defense:

Despite the physical barriers, the microorganisms do enter the body. The body defends itself and eliminates the invading organisms by non-specific mechanisms such as sneezing and secretions of the mucus. In addition, the body also tries to kill the pathogens by phagocytosis (involving macrophages and complement system). The inflammatory response and fever response of the body also form a part of innate immunity.

(i) Physical Barrier:

Skin is the first line of mucous coating on defence. It prevents the entry of the pathogens of the body. Mucous coating on the epithelium lining the respiratory, gastrointestinal and urogenital tracts also help in trapping microbes.

(ii) Physiological Barrier:

Acid in the stomach, saliva in the mouth, tears from the eyes, etc., prevent the entry of microbes.

(iii) Cellular Barrier:

Special types of cells in our body, which kill the disease causing agents. Example are WBCs, Lymphocytes, Polymorpho Nuclear Leukocytes (PMNL—neutrophils, monocytes, macrophages, etc.

(iv) Cytokine Barrier:

Cells which are virus-infected, release types of protein called interferon's. Interferons protect the uninfected cells from further infection.

Physical (or Mechanical) Barriers:

Physical (or mechanical) barriers of the host in cooperation with chemical barriers (secretions) act as the first line of defence against pathogenic microorganisms and foreign materials. These barriers include skin, mucous membranes, respiratory system, gastrointestinal tract, genitourinary tract, eye, bacteriocins, and beta-lysin and other polypeptides.

Skin, mucous membranes, respiratory system, gastrointestinal tract, genitourinary tract, and eyes are the barriers that provide both physical and chemical defence (e.g., gastric juices, lysozyme, lactoferrin, glycoproteins, urea etc.) in cooperation. In addition, bacteriocins and beta-lysin and other polypeptides are the defensive chemicals against microorganisms.

1. Skin:

Intact skin is a very effective physical or mechanical barrier to block the entry of microbial pathogens into the body. With few exceptions the microorganisms fail to penetrate the skin because its outer layer consists of thick, closely packed cells called keratinocytes that produce keratins.

Keratins are scleroproteins comprising the main components of hair, nails, and outer skin cells. These scleroproteins are not easily degradable enzymatically by microorganisms. They resist the entry of microbe-containing water and thus function as physical barrier to microorganisms.

In addition to direct prevention of penetration, continuous shedding of the outer epithelial cells of skin removes many of those microbial pathogens that manage to adhere on the surface of the skin.

2. Mucous membranes:

Mucous membranes of various body systems such as respiratory, gastrointestinal, genitourinary, and eye prevent invasion by microorganisms with the help of their intact stratified squamous epithelium and mucous secretions, which form a protective covering that resists penetration and traps many microorganisms.

3. Respiratory system:

An average person inhales about 10,000 microorganisms per day usually at the rate of eight microorganisms per minute. These microorganisms are deposited on the moist,

sticky mucosal surfaces of the respiratory tract. The mucociliary blanket of the respiratory epithelium traps the microorganism less than 10 μm in diameter and transports them by ciliary action away from the lungs.

Microorganisms larger than 10 μm normally are trapped by hairs and cilia lining the nasal cavity which beat towards the pharynx so that the mucus with its trapped microorganisms is moved towards the mouth and expelled. Coughing and sneezing also help removal of microorganisms from the respiratory tract.

They make clear the respiratory system of microorganisms by expelling air forcefully from the lungs through the mouth and nose, respectively. Salivation also washes microorganisms from the mouth and nasopharyngeal areas into the stomach.

4. Gastrointestinal system:

Microorganisms may manage to reach the stomach. Many of them are destroyed by the gastric juice of the stomach. The gastric juice is a mixture of hydrochloric acid, proteolytic enzymes, and mucus, and is very acidic with a pH 2 to 3. This juice is normally sufficient to kill most microorganisms and destroy their toxins.

Furthermore, the normal microbial population of the large intestine is extremely significant in not allowing the establishment of pathogenic microorganisms in it.

For convenience, many commensalistic microorganisms in the intestinal tract secrete metabolic products (e.g., fatty acids) that prevent "unwanted" microorganisms from becoming established in the tract. In small intestine, however, the microbial pathogens are often killed by various pancreatic enzymes, bile, and enzymes in intestinal secretions.

5. Genitourinary system:

Kidneys, ureters, and urinary bladder are sterile under normal conditions. Kidney medulla is so hypertonic that it allows only few microorganisms to survive.

Urine destroys some microorganisms due to its low pH and the presence of urea and other metabolic end-products like uric acid, hippuric acid, mucin, fatty acids, enzymes, etc. The lower urinary tract is flushed with urine eliminating potential microbial pathogens. The acidic environment (pH 3 to 5) of vagina also confers defence as it is unfavourable to most microorganisms to establish.

6. Eye:

The conjunctiva of eye lines the interior surface of each eyelid and the exposed surface of the eyeball. It is a specialised mucus-secreting epithelial membrane and is kept moist by continuous flushing action of tears secreted by the lacrimal glands. Tears contain lysozyme and lactoferrin and thus act as physical as well as chemical barriers.

7. Bacteriocins:

The surfaces of skin and mucous membranes are inhabited by normal microbial flora. Of this, many bacteria synthesize and release toxic proteins (e.g., colicin, staphylococcin) under the direction of their plasmids. These toxic proteins are called bacteriocins, which kill other related species thus provide an adaptive advantage against other bacteria.

Inflammation (Inflammatory Response):

Inflammation (L. inflammatio = to set on fire) is an innate (nonspecific) defence response of the body to pathogenic infection or tissue injury and helps localizing the infection or injury in its local area. Many of the classic features of inflammation were described as early as 1600 BC in Egyptian papyrus writings.

In the first century AD, the Roman physician Celsus described the four cardinal signs of inflammation as redness (rubor), swelling (tumor), heat (color) and pain (dolor). In the second century AD, another physician, Galen added a fifth sign: altered function (functio laesa).

Types of immunity:

Broadly there are two types of immunity.

1. **Innate or natural immunity**
2. **Acquired immunity**

<u>Innate or Natural immunity:</u>

Innate immunity is antigen-nonspecific defense mechanisms that a host uses immediately or within several hours after exposure to almost any microbe. This is the immunity one is born with and is the initial response by the body to eliminate microbes and prevent infection. Innate immunity can be divided into immediate innate immunity and early induced innate immunity.

Immediate innate immunity begins 0 - 4 hours after exposure to an infectious agent and involves the action of soluble preformed antimicrobial molecules that circulate in the blood, our found in extracellular tissue fluids, and are secreted by epithelial cells. These include: antimicrobial enzymes and peptides; complement system proteins; and anatomical barriers to infection, mechanical removal of microbes, and bacterial antagonism by normal flora bacteria. These preformed innate defense molecules will be discussed in greater detail later in this unit.

Early induced innate immunity begins 4 - 96 hours after exposure to an infectious agent and involves the recruitment of defense cells as a result of pathogen-associated molecular patterns or PAMPS binding to pattern-recognition receptors or PRRs. These recruited defense cells include: phagocytic cells: leukocytes such as neutrophils, eosinophils, and monocytes; tissue phagocytic cells in the tissue such as macrophages; cells that release inflammatory mediators: inflammatory cells in the tissue such as macrophages and mast cells; leukocytes such as basophils and eosinophils; and natural killer cells (NK cells).

Examples of innate immunity include anatomical barriers, mechanical removal, bacterial antagonism, antigen-nonspecific defense chemicals, the complement pathways, phagocytosis, inflammation, fever, and the acute-phase response. In this current unit we will look at each of these in greater detail.

- Immunity with which an individual is born is called innate or natural immunity.
- Innate immunity is provided by various components such as Skin, mucus membrane, Phagocytic cells etc
- Innate immunity acts as first line of defense to particular microorganisms.

Mechanism of innate immunity:

1. **Anatomical barrier**
2. **Physicochemical barrier**
3. **Phagocytic barrier or Phagocytosis**
4. **Inflammatory barrier or Inflammation**

Anatomical barriers:

Skin and mucus membrane are the examples of anatomical barriers that provides immunity.

Skin and mucus membrane:

- Skin consists of two distinct layer; a thin outer layer called epidermis and thick inner layer called dermis.
- Epidermis consists of mostly dead cell filled with keratin. Dermis is composed of connective tissue, hair follicle, sebaceous gland and sweat gland.
- Skin provides first line of defense by preventing entry of microorganisms. However skin may be penetrated by injury or insects.
- Below skin, the mucus membrane prevents the entry of microorganism to the body. And also it secrets mucus that entraps microorganisms.

Anatomical barriers provide immunity by following ways.

- At first skin and mucus membrane prevent entry of microorganism into host body by mechanical separation. For example, Skin surrounds the host body from external and mucus membrane surrounds the body tracts.
- They also have mechanism to kill the pathogen before entry to body. For example; lysozyme, acidic pH, sebum, high salt concentration in sweat are antimicrobial agents found in skin and mucus membrane.
- Skin and mucus membrane provides first line of defense against microorganism as they are first component to encounter with microorganism.

Physico-chemical barriers:

- Physicochemical barrier includes physiological barrier and chemical barrier.
- Physiological conditions of body such as normal body temperature, normal body pH etc provides immunity.
- Some species are resistant to certain disease simply because of their higher body temperature. For example, mammals are susceptible to anthrax but birds are resistant to anthrax. It is because *Bacillus anthracis* are killed by higher body temperature of birds (39°C).

- Similarly, body pH also provides immunity. For example acidity of stomach kills most of the ingested bacteria and provides immunity. In infants stomach is less acidic. This is the reason why infants suffer more from gastrointestinal disturbance than adults.
- **Chemical barriers** include various antimicrobial chemicals found in body fluids. For examples, Lysozyme found in tear and mucus kills many Gram +ve bacteria.
- Interferon found in blood and lymph kills viruses. Other antimicrobial chemicals found in body fluids include complement protein, collectins, etc.

Phagocytosis or Phagocytic barrier of immune system (neutrophil macrophage functions):

- Phagocytosis is an important defense mechanism of host to provide immunity. Most of the bacteria that enter into host are killed by phagocytic cells such as Neutrophils, monocytes and macrophages.
- Phagocytosis is an example of endocytosis.
- There are two types of endocytosis; phagocytosis and pinocytosis.

Steps of phagocytosis

1. At first phagocyte approaches to the site of infection
2. Phagocyte extends pseudopodia around bacterial cell.
3. Pseudopodia gradually increase in size and finally fused so that bacteria are engulfed in the form of phagosome or food vacuole.
4. The phagosome and lysosome come nearer to each other and fuse to form phago- lysosome.
5. Inside phago-lysosome ingested bacteria is killed by hydrolytic and digestive enzyme of lysosome.
6. Required materials released from digested bacteria are absorbed into surrounding cytoplasm and undigested residues are excreted out by exocytosis.

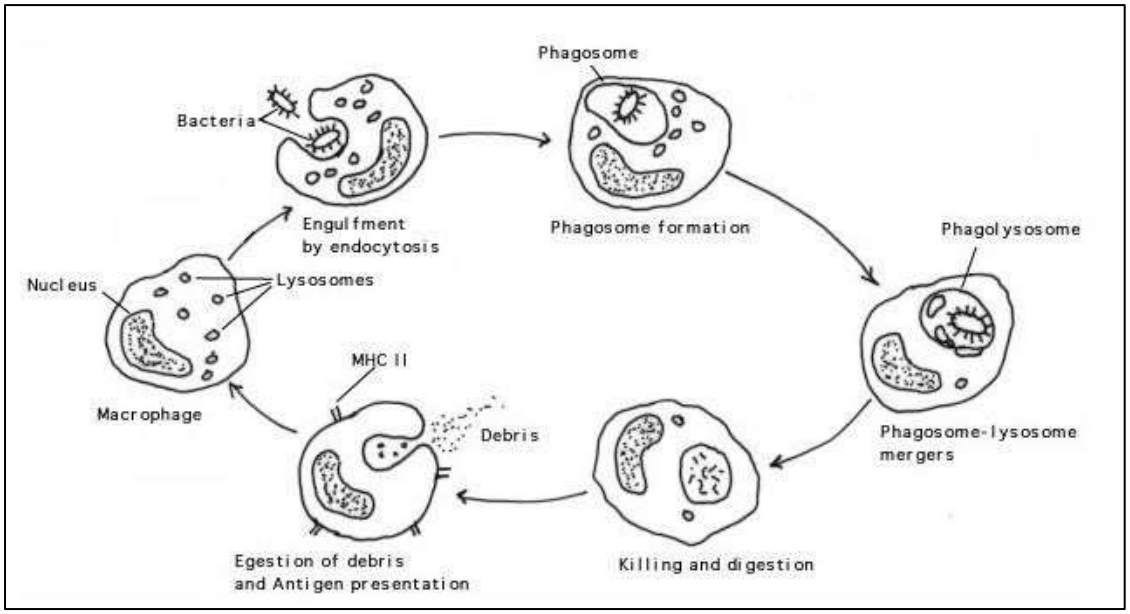

Fig.: Steps in phagocytosis.

Killing Mechanism of phagocytosis:

Killing of ingested bacteria during phagocytosis occur by two different mechanism

1. Oxygen dependent mechanism
2. Oxygen independent mechanism

1. Oxygen dependent mechanism:

- During phagocytosis, phagocytic cell increases uptake of O2. At the same time rateof pentose phosphate pathway increases to generate more NADPH.
- NADPH reduces molecular O2 to produce various toxic metabolic products such as Hydroxyl free radical, H2O2 and superoxide ions that kill ingested bacteria.
- This process is also known as respiratory burst.
- It is the major mechanism of killing of ingested bacteria during phagocytosis.

2. Oxygen independent mechanism:

- In this mechanism, ingested bacteria are killed by hydrolytic and digestive enzymes of lysozyme.

Inflammation, a response triggered by damage to living tissues. The inflammatory response is a defense mechanism that evolved in higher organisms to protect them from infection and injury. Its purpose is to localize and eliminate the injurious agent and to remove damaged tissue components so that the body can begin to heal. The response consists of changes in blood flow, an increase in permeability of blood vessels, and the migration of fluid, proteins, and white blood cells (leukocytes) from the circulation to the site of tissue damage. An inflammatory response that lasts only a few days is called acute inflammation, while a response of longer duration is referred to as chronic inflammation.

Example of acute inflammation is; the response to tissue damage or **Example of** chronic inflammation is; Arthritis, cancer etc.

Main aim of inflammation is to prevent spread of injected microorganism or toxin from site of injection and kill them on spot by phagocytosis.

Characteristics of inflammation:

1. Rubor: redness
2. Tumor: swelling
3. Calor: heat
4. Dolor: pain
5. Functio laesa: loss of function

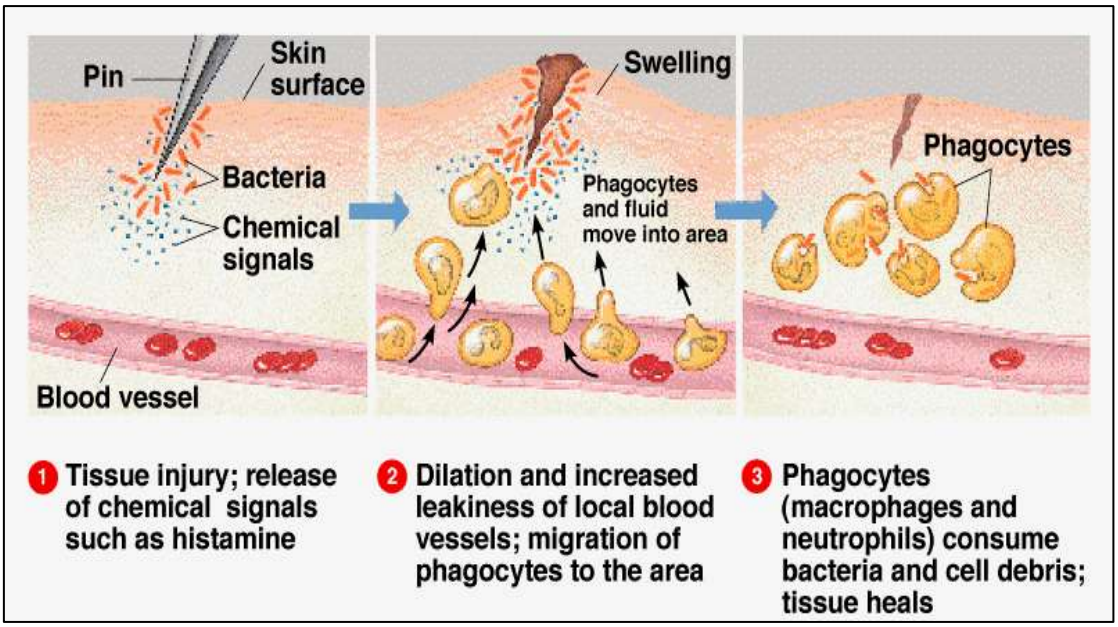

Fig.: Inflammation process.

Steps of inflammatory response:

Step I: Tissue damage and Release of histamine:

- Tissue damage caused by toxin, microorganism or mechanical injury release histamine.

Step II: Vasodilation:

- Histamine acts on surrounding blood capillaries and causes vasodilation.
- When vasodilation occurs, speed of blood flow decreases so that Neutrophils get chance to settle at the site of infection.

Step III: Increased permeability:

- At the same time histamine increases the permeability of blood capillaries leading to leakage of fluid from blood capillaries. This results in accumulation of fluid causing edema.

Step IV: Extravasation:

- Within few hours, Neutrophil migrates to the site of tissue damage by the process of chemotaxis and passes through capillaries wall and enter into tissue space by the process called extravasation.
- Extravasation completes in 4 steps:
- **Rolling:** neutrophils attach loosely to endothelium by low affinity

interaction between glycoprotein-mucin of Neutrophil.

- **Activation of chemotactic stimus:** chemokines are secreted and Neutrophil are attracted.
- **Arrest and adhesion:** ICAMS and integrin stabilize adhesion of neutophil and endothelium
- **Transendothelial migration:** Neutrophil enter through endothelium layer.

Step V: Phagocytosis:

- Neutrophil kills the injected microorganism or toxins by phagocytosis and release molecular mediators that contributes to inflammatory response. At the same time activates effectors cells.

Step VI: Inflammatory response:

- As inflammatory response develops, various cytokines and other inflammatory mediators act on endothelium of local blood vessels, including increased expression of cell adhesion molecules (CAMs). The epithelium is then said to be inflamed.
- Neutrophils are the first cell types to bind to inflamed endothelium and extravasate into tissue.

Natural Killer (NK) cells:

Natural killer (NK) cells are effector lymphocytes of the innate immune system that control several types of tumors and microbial infections by limiting their spread and subsequent tissue damage

The natural killer cell was first described in 1976, when it was shown that the body contains a small population of large, granular lymphocytes that display cytotoxic activity against a wide range of tumour cells in the absence of any previous immunization with the tumor. NK cells were subsequently shown to play an important role in host defense both against tumor cells and against cells infected with some, though not all, viruses. These cells, which constitute 5%–10% of lymphocytes in human peripheral blood, do not express the membrane molecules and receptors that distinguish T- and B-cell lineages. 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 tumour cells and cells infected by certain viruses display antigens against which the immune system has made an 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 defence against tumours. 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 tumour cells.

There has been growing recognition of a cell type, the NK1-T cell, that has some of the characteristics of both T cells and NK cells. Like T cells, NK1-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 CD16 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 the cytokines needed to support antibody production by B cells as well as inflammation and 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 responses are still developing.

7. Immune Regulation Mechanisms: Brief account on immuno-induction, immunosuppression, immuno-tolerance, immuno-potentiation.

Objective: In this unit we will discuss about immune regulation mechanisms such as immuno-induction, immuno suppression, immuno-tolerance, immuno-potentiation.

Immuno-induction:

The induction of immune responses requires critical interaction between innate parts of the immune system, which respond rapidly and in a relatively nonspecific manner, and other specific parts, which recognize particular epitopes on an antigen. A critical element in this interaction is the role played by dendritic cells (DCs), which represent "professional antigen-presenting cells." DCs endocytose and process antigen to peptide presented on the cell surface in association with major histocompatibility complex (MHC) molecules. This presentation results in interaction with and stimulation of helper T (Th) lymphocytes, which recognize peptide in association with either MHC class II or cytotoxic T (Tc) lymphocytes, which recognize peptide in association with MHC class I. Stimulation of Th lymphocytes produces the growth and differentiation factors (cytokines) essential for the B lymphocytes that have responded to a more intact form of the antigen and that differentiate into antibody-producing cells. The precise interaction between the cells depends on cognate ligand-receptor recognition between the B and Th lymphocytes. DCs also play a direct role with the stimulation of the B lymphocytes. It appears that DC can deliver antigen to the B lymphocytes in a more intact form than the processed form essential for stimulating T lymphocytes, and can release cytokines that assist the differentiation of the B lymphocytes into antibody-producing cells. This close relationship among the three cell types and the cytokines that are produced ensures the precise control and regulation necessary for immune response development.

Activation of Innate Immune Defenses:

Some local tissue damage occurs after immunization or infection and results in the release of exogenous immune response mediators referred to as "danger signals". These mediators interact with histiocytes (MΦs and neutrophils), DCs, and mast cells at the

site of injury or vaccine deposition, and result in the production of endogenous mediators that promote immune defense system recruitment through the local inflammatory immune reaction. An important element in this process is the local increased endothelium permeabilization, concomitant with upregulation of integrin and other adhesion molecule expression on the endothelium. Chemokines released from the endothelium recruit histiocytes and other leukocytes from the blood to adhere to the endothelium with modulated adhesion molecule expression. The increased binding at the endothelium permits extravasation of the recruited leukocytes between the junctions of the endothelium into the site of injury. This recruitment of leukocytes is due to chemotaxis, and is a key element in orchestrating selective recruitment of leukocytes to inflammatory sites.

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Neutrophils and monocytes, including DCs, typically dominate migratory leukocytes in innate defense responses. The mononuclear phagocytes (monocytes and MΦs) and neutrophils are central to the acute inflammatory reaction of innate effector immune defenses. They are important immune defense agents because they are actively phagocytic and have a wide distribution throughout the body tissues and organs. By comparison, the DCs, which are recruited to the site of antigen presence, are primarily

involved in antigen processing and presentation to antigen-specific lymphocytes. For this activity to occur, the DCs must migrate from the site of antigen uptake to the lymphoid organs and tissues to interact with the antigen-specific lymphocytes. Thus, DC uptake of antigen results in the transport of that antigen to the lymphoid organs and tissues wherein specific responses develop.

Interaction of the Innate and Specific Parts of the Immune System:

DCs at the local inflammatory site include dermal DCs and Langerhans cells. In addition, DCs referred to as "immature DCs" migrate from the blood into the inflammatory site. Together, they play an important role in linking the innate with the adaptive immune system. DCs at the mucosal surfaces, including the mucosal-associated lymphoid tissue ("MALT") and tonsils, are particularly important in terms of how they interact with foreign material. The latter can be transported across or through the epithelial barrier at these sites, and passively taken up from the epithelial cells by DCs. In addition, there is evidence that local DCs can open the tight junctions between epithelial cells at mucosal surface barriers in a way that retains the integrity of these junctions. This capability permits the DCs to send a cellular protrusion into the lumen at the mucosal surfaces, "capture" the foreign material, and transport it into lymphoid tissue for presentation. Once in the lymphoid tissues or organs, the DCs enter follicles to deliver antigen to the B lymphocytes and to present processed antigen in association with MHC molecules to T lymphocytes. As a result of this process, specific immune responses are induced.

Innate-specific Interactions: Antigen Processing/Presentation:

Although monocytes and MΦs had been considered as APCs of the immune system, the principle APCs are the DCs, which are found in all lymphoid tissues, the blood, lymphatic circulation, and nonlymphoid tissues. The main function of DCs is antigen presentation, in contrast to other cells that can function as APCs (e.g., as MΦs and B cells). DCs are actually a heterogeneous population of cells, as exemplified by recent studies in swine. Nevertheless, one consistent element is the "maturation" of DCs during antigen processing/presentation. DCs are compartmentalized into immature DCs (iDCs 1) and mature DCs (mDCs 1). Langerhans cells and dermal DCs, which are good examples of iDC, seek out antigenic material for endocytosis that leads to processing and presentation. Once iDCs endocytose and commence the processing of antigenic cargo,

they begin to mature. Inflammatory “danger” signals, including proinflammatory cytokines and factors released during tissue damage (e.g., uric acid) promote this maturation. Microbial exogenous signals can also induce maturation through the PAMPs described above. It is important to consider these elements during the formulation of vaccines.

DC Presentation of Antigen to Th Lymphocytes:

DCs can internalize antigen, or cells such as tumor, virus-infected, and apoptotic cells. Internalization is achieved either by constitutive macropinocytosis of intracellular fluid (“environmental sampling”) or binding to one of the numerous receptors possessed by DCs. When iDCs endocytose antigen, the uptake can use one of the different forms of endocytosis—clathrin-dependent endocytosis, macropinocytosis, pinocytosis, or phagocytosis—depending on the size and nature of the antigen or cell to be internalized. The internalized material is degraded into peptide fragments within the endosomal system for processing that involves MHC class II molecules, or is transferred to the cytosol for processing that involves MHC class I molecules. The MHC class II-dependent presentation of antigen is essential for stimulating the Th lymphocytes.

Antigenic peptide-containing endosomal structures fuse with specialized structures that contain MHC class II molecules, the so-called MHC class II-rich part (MIIC 1). In human iDCs, newly synthesized MHC class II molecules carrying the invariant chain (Ii 1) first appear on the cell surface, from where they are internalized and targeted to the endosomal part through signaling via the cytoplasmic tail of the Ii. The Ii is cleaved by cathepsin S, leaving what is referred to as the class II-associated Ii peptide (CLIP 1). In the MIIC, the antigenic peptides are catalytically exchanged for the CLIP, which allows interaction of the antigenic peptide with the peptide binding cleft of the MHC class II molecules that is capable of “recognizing” the peptide amino acid sequence. These peptide-loaded MHC class II molecules are then transferred to the cell surface, where they are stably expressed—an important characteristic of mDC. MHC class II molecules that lack peptide loading are not stably expressed on the cell surface but are continually recycled between the surface and the MIIC or are degraded by lysosomes.

The process described above has been observed with human DCs. However, with murine iDCs, newly synthesized MHC class II molecules can be retained in the lysosomal

part and do not reach the cell surface until they are loaded with antigenic peptide. Furthermore, not all MHC class II alleles behave identically. With mice at least, the cellular localization and peptide loading characteristics of MHC class II molecules depend on their haplotype. In addition, with both human and murine DCs, the MHC haplotypes determine the amino acid sequence of the peptide to be recognized. The requirement for MHC-peptide interaction introduces a limitation in the number of processed peptides that can be presented by APCs, due to the MHC haplotypes. Antigen presentation depends on the alleles in the loci encoding the MHC class I or II binding site of the APCs. Individuals show a variation in their peptide recognition patterns through the possession of different alleles, due to differences in the MHC molecule "recognition" of the peptides)—referred to as MHC restriction. Polymorphism in the MHC class II loci leads to species-specific and individual-specific repertoires for the efficient recognition of antigenic peptides by $CD4^+$ T lymphocytes.

Despite the differences described above, efficient antigen processing and presentation will result in the stable expression of peptide-loaded MHC class II molecules on the mDC surface. This stability of expression is critical because it facilitates the presentation of the molecules to the TCR on Th ($CD4^+$) lymphocytes.

DC Presentation of Antigen to Cytotoxic T (Tc 1) Lymphocytes:

MHC class I molecules also bind intracellular peptides and transport them to the cell surface, for presentation to Tc lymphocytes. This process is critically important in defending against intracellular pathogens such as viruses and certain bacteria. The Tc lymphocyte responses are also essential in the immune defense against tumors due to their role in recognizing antigens presented in the context of the host's own MHC class I—the so-called "altered self." For the initiation of such responses, pathogen or tumor cell antigens must be processed by APCs for presentation on their surface in the context of the MHC class I molecules Again, it is the DCs that are the most potent at performing this function of antigen presentation. As with antigen presentation in the context of MHC class II to Th lymphocytes, the additional involvement of costimulatory receptors is required to promote the interaction between the DC and the Tc lymphocytes. The DCs can express MHC class I molecules that carry the peptide antigen in question at sufficiently high levels, along with the required costimulatory molecules, to stimulate naive Tc lymphocytes.

For stimulation of Tc lymphocytes by DCs, the antigen must be processed in the DC cytoplasm. Although this requirement may appear similar to that involving MHC class II, the similarity ends there. The site of processing differs for MHC class II- and MHC class I-dependent presentation. For MHC class I presentation, many of the antigens processed into this pathway are referred to as endogenous, because the origin of the peptides interacting with the MHC Class I molecules are derived from the host cell or intracellular pathogens. In the majority of cases, these antigenic peptides are generated by the proteolytic cleavage of misfolded proteins, collectively termed "defective ribosomal products" (DRiPs 1). Following DC activation, DRiPs accumulate in cytosolic aggregates termed "dendritic cell aggresome-like inducible structures" ("DALIS"). Ubiquitination of these complexes generates substrates for the cytosolic proteasome, which cleaves them into small peptides for transportation into the lumen of the endoplasmic reticulum (ER 1) by the transporter associated with antigen presentation ("TAP"). The transported peptides are further degraded into eight to nine amino acid peptides by the ER-aminopeptidase I. The ER chaperones calnexin, calreticulin, and tapasin load the peptides onto MHC class I chains in the "MHC class I loading complex" to form the mature MHC class I molecules. The latter can then dissociate from the "loading complex" for transportation onto the surface of the cell, where they are stably expressed on the plasma membrane.

Although the description above explains the presentation of endogenous antigen such as that coming from intracellular pathogens, it does not explain how DCs present antigens from tumor cells. It is clear that DCs can process and present both endogenous (generated within the DCs) and exogenous (endocytosed by the DC) antigens in the context of MHC class II. Similarly, MHC class I processing can utilize either endogenous or exogenous sources of antigen. The latter explains how DCs can present antigen from tumor cells, pathogen-infected cells, and even apoptotic cells to Tc lymphocytes. This presentation in the context of MHC class I is referred to as "cross-priming" or "cross-presentation". Indeed, cross-presentation has now been demonstrated to occur with DCs endocytosing virus antigens, particulate antigens, and proteins such as albumins, tumor cells, and apoptotic cells both in vitro and in vivo.

Depending on the nature of the antigen or cell to be endocytosed, internalization by the DC uses one of several forms of endocytosis. In this context, it relates to uptake by DC

for processing into the MHC class II presentation pathway. The difference is in the cytosolic targeting. With MHC class II processing, the target is the endosomal/lysosmal processing pathway that leads into the MIIC. For MHC class I processing to be activated, the endocytosed material must escape the lysosomal degradation by translocation into the cytosol. From here, the same process as for endogenous antigen processing occurs: ubiquination, proteosome degradation, and transport into the

Although DCs are important for cross-presentation, evidence has shown that other cells such as macrophages can also perform this function. Nevertheless, DCs are the most potent effectors of cross-presentation despite the fact that not all DCs perform this function equally. The DC family is a collection of subsets, and evidence has shown that the murine $CD8\alpha^+ CD205^+$ DC subset is the major subset involved in cross-presentation. Although this work must still be expanded into other species, it is clear that approaches to vaccine targeting of a particular aspect of immune defenses should include consideration of the subsets of leukocytes likely to be involved and therefore to be efficient for the immunization process.

DC Interaction with B Lymphocytes:

DCs form clusters with B lymphocytes in vivo and can be involved in their activation through antigen delivery. This interaction with B lymphocytes is related to "delivery" and is not a MHC-dependent "presentation" of antigen. It has also been reported that this DC-B cell interaction can result in stimulation of B cell differentiation into antibody-producing cells without the need for T cell help. The antigen is unprocessed and has no MHC involvement. In addition, DCs play an important role in the regulation of humoral immunity through accessory molecule-dependent regulation of B cell survival and proliferation. Examples include cognate interactions such as the CD40-dependent signaling, as well as secreted molecules such as the TNF-family ligands BAFF and APRIL. The presentation of unprocessed antigen during DC-B cell interaction is not a consistent event, and it apears to be related to the antigen load delivered by the DC. The T cell-independent response is more readily identified under conditions of high antigen load. It is important to understand this factor when developing methods for the delivery of vaccines, and considering the antigen payload therein.

Stimulation of Specific Immune Responses:

T Lymphocytes:

The $CD4^+$ Th lymphocytes are essential for efficient execution of the majority of specific immune responses, including antigen-activated B lymphocyte production of antibody and antigen-specific cytotoxic T lymphocytes (Tc) effector function. An important consideration is that not all Th lymphocytes recognize the same peptide; only distinctive Th lymphocyte clones recognize particular peptide sequences. This specificity is due to the antigen processing by the APCs, which results in several peptides that are capable of stimulating the Th lymphocytes for which they are specific. These peptides carry what are referred to as T cell epitopes, which in general are continuous or sequential epitopes. Most show haplotype restriction, meaning that they will not be recognized by T lymphocytes from all individuals. Once stimulated, Th lymphocytes provide their "help" (hence the name T helper) via both cognate interactions and the release of soluble signaling mediators. The latter, cytokines, are responsible for driving antigen-stimulated B and Tc lymphocytes into becoming antibody-producing and effector cells, respectively. Of course, such cytokine signaling requires appropriate cognate interactions.

B Lymphocytes:

Activation of B lymphocytes requires continued stimulation of both the membrane immunoglobulin-like receptors on the B lymphocytes by the antigen and the surface cytokine receptors by factors produced by the Th lymphocytes. In addition, a physical interaction between the B and Th lymphocytes is required. For example, CD40 and its ligand play a key role in permitting selective differentiation. Similar to the T lymphocyte responses, those of B lymphocytes are also restricted to particular clones that can recognize one of the epitopes on the antigen, referred to as the B cell epitopes. APCs are not required to process and present antigen to stimulate B lymphocytes, but it appears that they play a role for the "delivery" of antigen into the B lymphocyte areas of the lymphoid follicles. This antigen must be "delivered" relatively intact because the epitopes recognized by B lymphocytes are most often discontinuous—they rely on the tertiary and quarternary (conformational) structure of the antigenic determinants on the antigen. Once a B lymphocyte interacts with the epitope for which it is specific, via the BCR, the cell is induced into capping its BCR in preparation for division and

differentiation. At this point, the interaction with the Th lymphocytes, and in particular the involvement of the T cell cytokines, becomes critical. Without this interaction, the B lymphocyte reverts back to its resting stage, as before interaction with the antigen. In the presence of the correct cognate interaction with the Th lymphocytes and the involvement of the T cell cytokines, the B lymphocyte begins its differentiation pathway into the antibody-producing plasma cell. It is for this reason that a number of the T cell cytokines involved were originally referred to as B cell differentiation factors.

Innate-specific Interactions: Effector Immune Defenses:

Interaction with the innate immune parts continues after induction of the specific immune defenses. At this point, the immune response is in a phase termed "the effector phase." This term refers to the processes directly involved in effecting protection against the danger in question—pathogen, toxin, or other foreign substance considered by the immune system recognition processes as presenting a danger to the host. Interaction between the innate and specific immune parts can be seen with both antibody-based and cytotoxic effector immune defenses.

When antibody specific for the antigen in question binds to that antigen, the resultant immune complexes must be removed from the host. When the antigen is a pathogen, it it not possible to guarantee that the pathogen will not continue to infect its target cells. Furthermore, immune complexes themselves can cause immunopathological disorders. MΦs are important innate defenses that interact with antibody-based effector defenses, and remove and degrade immune complexes. Without the presence of the antibody, the MΦs can phagocytose and destroyed the pathogen, although this action may be delayed and may even allow survival and transport of the pathogen in question. Furthermore, many viruses and certain bacteria actually target MΦs and other cells of the innate immune system for infection and replication.

The cytotoxic immune defenses also utilize both specific (Tc lymphocytes) and innate (NK plus lymphokine-activated killer cells) processes. As mentioned above, the Tc lymphocytes are activated by MHC Class I-dependent recognition. NK cells employ MHC-independent recognition, which permits the immune defenses to identify, for example, virus-infected cells in which viral antigenic peptides are not presented on MHC molecules (see below). These NK cells will recognize what are referred to as "NK-specific, triggering surface molecules"—examples are NKp46, NKp30, and NKp44, but

other triggering receptors are known. Another subset of T lymphocytes—the γδ T cells—can recognize antigen without requiring MHC and therefore antigen processing. This knowledge has led to the proposal that such reactions of γδ T cells may fulfill more of an innate than a specific defensive role. The innate and specific immune parts also interact in the regulation of cytotoxic defenses. NK cells produce IFN-γ and TGF-β, potent regulators of MΦs and lymphocytes, while MΦs and DCs can produce IL-12, which is capable of inducing NK cell and T lymphocyte production of the IFN-γ involved in growth regulation of these latter cells.

Cytotoxic immune defenses are important for destroying modified host cells such as tumor cells, as well as host cells infected with intracellular pathogens such as viruses. Tc lymphocytes and NK cells are major players in this context, recognizing antigenic peptides on an infected cell surface. Both of these groups of cytotoxic cells, as well as the cytotoxic γδ T lymphocytes, will employ what are referred to as granule-associated cytotoxic proteins (GACPs 1) to lyse their targets—the tumor or virus-infected host cell. Examples of these GACPs are the T cell intracellular antigen-1, perforin, and granzyme B. In addition, there is evidence that Fas-Fas ligand interactions relating to induction of apoptosis may be implicated in the cytotoxic immune defenses. When considering cytotoxic immune defenses, it is important to note that MΦs also play a role. MΦs can recognize antibody and complement components interacting with viral proteins expressed on the infected cell surface.

Taking all of these effector cytotoxic immune defenses together, it becomes evident that they are involved in destruction of infected cells only when the host cell surface is modified. Certain intracellular pathogens can evade detection, by not expressing antigenic proteins or peptides on the cell surface, or otherwise not modifying the cell surface, to avoid detection by the immune defenses. Nevertheless, the immune defenses can still recognize such infected cells if the infection results in cell death (apoptosis)—this is a capacity found with MΦs and other phagocytic cells of the immune system.

Immunological Memory:

When the threat posed by an antigen is overcome (i.e., when no more free antigen is stimulating the lymphocytes), the immune system enters the phase of memory development. The important signal is the replacement of antigen by antigen/antibody complexes due to the progression of the immune system into antibody production. The

low-affinity Fc receptor type II for immunoglobulin G (FcγRII 1) and Fc receptor type III for immunoglobulin G (FcγRIII 1) on phagocytes readily bind antibody that is complexed with antigen, rather than free antibody. Through such interactions, the cytoplasmic portion of the FcγRII and FcγRIII is modified, resulting in enhanced phagocytosis. This event is important in effector immune defense involving antibody and phagocytes.

An additional consequence of antibody/antigen complex formation is the interaction with B lymphocytes, in which case the low-affinity immunoglobulin receptor FcγRIIB is involved. With B lymphocytes specific for the antigen, the antibody in the complexes interacts with the FcγRIIB while the antigen in the complex interacts with the BCR. This cross-linking of the two receptors induces a different signaling within the B lymphocyte from that induced by antigen alone. The B cell differentiation switches from antibody-producing plasma cell to memory B lymphocyte development. Modifications of the surface interactions between B and Th lymphocytes and the cytokines involved are also implicated in the switch from antibody production to immunological memory development. In addition, the Th lymphocyte activity moves into the development of memory Th lymphocytes. Moreover, memory development concomitantly increases the number of antigen-reactive B and T lymphocytes, as well as the affinity of the BCR for the antigenic determinants for which they are specific. These modifications reflect the somatic mutation and selection involved with the increasing avidity of antibody and B lymphocyte receptor upon repeated exposure to the same or related antigens.

Cytokines:

In addition to the cellular components, cytokines are important for the development of immune responses involved in the regulation of those responses. The cytokines that are produced depend on the cells involved. For example, DCs can secrete IL-1, IL-6, IL-10, IL-12, TNFα, and Type I interferons; T lymphocytes produce IL-2, IL-4, IL-5, IL-10, TNFβ, and IFNγ. Th lymphocyte cytokines can be described as “Th1” or “Th2” cytokines, due to the discovery in mice that individual Th lymphocyte clones secrete a particular pattern of cytokines. Th1 cytokines include IL-2, IFNγ, and TNFβ. Th2 cytokines include IL-4, IL-5, IL-6, IL-10, and IL-13. Although such discrimination relates to the characteristics of an immune response, there is not the same subset relationship to Th1 and Th2

lymphocytes in humans that is seen in mice. The situation is further complicated by the fact that certain Th2 cytokines (e.g., IL-6 and IL-10) are also produced by monocytes and DCs.

Cytokines do not act in isolation. Rather, their effects are due to interactions with other cytokines. The result is a control over the development and characteristics of the immune response. It appears that this control is often designed to meet the threat imposed, such as responding when there is a danger to the host but regulating the response when the threat of danger has been controlled. Such measures ensure that the host does not respond against substances that should be tolerated (e.g., food), or continues responding in the absence of a threat. Such events can occur when the control procedures break down, leading to intolerance and even food allergy, or in the case of immunopathological disorders, when responses occur in the absence of a threat. Certain cytokines have been found to have particularly profound roles in development and control of immune responses. For example, interaction of Th lymphocytes with antigen-presenting DCs that are still in an immature state (iDCs) results in IL-10 production, which enhances regulatory T lymphocyte (Treg 1) activity and anergy of T cell responsiveness. If the interaction involves mDC rather than iDC with Th lymphocytes, the latter produce IL-2 and IFN-γ, which promotes Th lymphocyte expansion. Importantly, when these Treg lymphocytes become involved, a shift occurs in cytokine expression from IFN-γ to IL-10, along with the downregulation of Th cell responses.

When dealing with immunization, one encounters the use of the term “Th1 cytokine response”. This term denotes that the immune activity involves cytokines that are characteristic of those produced by Th1 cells in mice. However, the term can be misleading with respect to vaccine efficacy. A Th1 cytokine profile is rarely induced in isolation from a Th2 cytokine profile. Certainly the cytokine profile may be dominated more by the Th1 cytokines than by the Th2 cytokines, but both groups are likely to be present. This characteristic is important for the regulation of the immune response, which relies on the balance between these groups.

Designing Vaccines and Immunization Strategy with the Aid of Immunological Profiles:

Using Immunological Memory:

In immunological memory, as mentioned above, the recall immune response in an immune host is more rapid and avid upon subsequent encounter with the antigen for which it is specific. In other words, the efficacy of immune defense is increased. It is this increased efficacy that is sought through any immunization, including vaccination—the aim of immunization in general is to induce a desired immune response, whereas vaccination is somewhat more targeted in preparing the host for defense against the pathogen or toxin in question. Clearly one must initiate the immune response process with a naive host (i.e., begin with the primary immune response). With this method, the efficient targeting of DCs is critical (see below) due to their unique role in antigen presentation with primary immune response development. A second immunization will increase the efficiency of the induced immune defenses to respond against the antigen in question. Although other APCs, including B lymphocytes, can be involved at this point, the most efficient APC and therefore immunization target remains the DCs for stimulation of all parts of specific immune defense. The value of the secondary or booster immunizations arises from the fact that the populations of responding lymphocytes have been expanded, and their affinity for recognized epitopes has increased. In other words, both the rate and efficacy of antigen recognition and immune response are increased. With booster immunizations, these pools of high-affinity lymphocytes are further elaborated both in size and in the strength of epitope recognition. This elaboration leads to an increase in the efficiency with which the immune defenses can respond to subsequent encounter with the antigen, and indeed defend the host against that antigen when the goal is efficacious vaccination.

Promoting Appropriate Cytokine Profiles:

The efficiency with which immune defenses can be stimulated by immunization (both effector immune defense and memory development) relies on the efficacy of the antigen or vaccine being employed. The result depends on how much is actually processed by the APCs and how much reaches the lymphoid follicles and germinal centers wherein the specific lymphocytes will be stimulated. Increasing the likelihood that the antigen or vaccine will be efficacious at inducing immune responses relies on appropriate targeting of the immune system (see below), as well as the application of adjuvants. Although adjuvants can assist targeting, the focus in this area has tended to be toward

augmenting immune response development. In this latter context, certain cytokines can be applied as adjuvants. It should be remembered, however, that cytokines are growth factors and can be a double-edged sword—both stimulating immune responsiveness when that response is required and downregulating the responses when the threat to the host has been "neutralized." Application of cytokines as adjuvants can be advantageous, certainly when initiating immune responses, but such cytokine application should be considered carefully. It is essential to ensure that the correct form of interaction with the immune system is being effected.

An alternative to cytokines as adjuvants is to use biologically defined adjuvants that induce a known cytokine profile. In this area, lipoproteins, lipopeptides, CpG-ODN motifs, and antigenic structures such as Baypamun are particularly potent, and have had some success. It is important to characterize and have a detailed understanding of the cytokine profiles induced by particular adjuvants. For example, application of aluminium hydroxide as an adjuvant can promote primarily a Th2-like response. However, the type of response induced actually depends on the antigen in question, and the influence of whether a primary or secondary immune response is being activated.

It is also important to understand adjuvant-induced cytokine profiles because of the reason for their application—to enhance induction of efficacious immune defenses. It is important to exercise care when applying anything with immunomodulatory capacity, because the immune responses will be modified depending on the cytokine profiles induced. Furthermore, immune defenses against particular pathogens will benefit from a profile dominated by Th1- or Th2-like cytokines depending on the pathogen (e.g., see reviews by authors. Adjuvants can also influence in an immunological targeting sense, because particular DC subsets are involved in the regulation of Th1 and Th2 cytokine responses. Clearly it is important to understand the characteristics of an efficacious defense against the pathogen or antigen in question. Only with this knowledge can one determine the appropriate components to be employed in an immunization or vaccination.

Immunological Target for Vaccination:

Induction of long-lasting protective immunity is the primary aim in vaccination against infectious diseases or toxins. As mentioned above, the pivotal cell in the complex

immune network is the DC. This distinct family of white blood cells belongs to the first line of immune defense against pathogen attack. Due to their migration throughout the body (including the subepithelial areas at mucosal surfaces) as they seek out anything presenting a danger for the host, they capture invading pathogens or the administered vaccine. The DCs can carry this material into the lymphoid follicles and germinal centers for delivery to B lymphocytes and presentation of the processed antigen to T lymphocytes. By this method, protective immune responses—the desired end-product of vaccination—are stimulated. Because of the critical nature of the DC activity for the efficiency of the immune response induction, the design must take into account both the lymphocytes' recognition of antigen specificity within the vaccine and the need for efficient DC activation. As described above, when DCs capture pathogen or vaccine antigen, they are in an 'immature' state. For efficient delivery and processing of that antigen, the DCs must respond to what is described as a danger signal, which is responsible for inducing their maturation. Therefore, vaccines must target DCs as well as induce their maturation. Only with both processes can one be assured that the DCs will efficiently stimulate lymphocytes into an active immune defense response. Without DC maturation, the induced response will be ineffective or may even result in a state of tolerance.

Numerous methods for targeting DC have been reported, including ligands for receptors on the DC surface. Of particular interest are the TLRs, which can be used not only to target the DC but also for interaction, which activates the DC and often results in maturation. A good example of these activities is seen with adjuvants containing lipoprotein or lipopeptide moieties. These adjuvants target TLR2 and/or TLR4, and stimulate DC maturation. It is not always necessary for the receptors to be on the cell surface. For example, although TLR9 is internal, it is the receptor for CpG-ODN. Actually, CpG-ODNs do not react with all DCs, but only with the subset known as plasmacytoid DCs, or natural interferon-producing cells (NIPCs 1). This result is a clear contrast between the murine and human immune systems: CpG-ODNs stimulate all DCs in murine cells, but only the NIPCs in human. In addition, with porcine DCs, only NIPCs respond to CpG-ODNs, making this animal model more appropriate for adjuvant studies using CpG-ODNs.

CpG-ODN stimulation of NIPCs is particularly important due to the high levels of IFN-α along with TNF-α that are induced. IFN-α and TNF-α are important stimulators of DC

maturation and NK cell activation, and they enhance humoral immunity. This is one reason for the success of CpG-ODN application as adjuvants; however, the reasoning is more complex because only type A CpG-ODNs stimulates NIPCs. Another type, type B, is reported to stimulate B lymphocytes directly, whereas type C can stimulate both NIPC and B lymphocytes. The direct stimulation of B cells by type B or C CpG-ODN not only induces a polyclonal activation with proliferation but also increases production of IL-6 and chemokines as well as increasing antibody secretion. It is evident that these potent effects of CpG also have the potential to cause damage such as autoimmune diseases.

Immunosuppression:

This is a reduction of the activation or efficacy of the immune system. Some portions of the immune system itself have immunosuppressive effects on other parts of the immune system, and immunosuppression may occur as an adverse reaction to treatment of other conditions.

In general, deliberately induced immunosuppression is performed to prevent the body from rejecting an organ transplant. Additionally, it is used for treating graft-versus-host disease after a bone marrow transplant, or for the treatment of auto-immune diseases such as systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome, or Crohn's disease. This is typically done using medications, but may involve surgery (splenectomy), plasmapheresis, or radiation. A person who is undergoing immunosuppression, or whose immune system is weak for some other reasons (such as chemotherapy or HIV), is said to be immunocompromised

Deliberately induced:

Administration of immunosuppressive medications or immunosuppressants is the main method for deliberately inducing immunosuppression; in optimal circumstances, immunosuppressive drugs primarily target hyperactive components of the immune system. People in remission from cancer who require immunosuppression are not more likely to experience a recurrence. Throughout its history, radiation therapy has been used to decrease the strength of the immune system. Dr. Joseph Murray of Brigham and Women's Hospital was given the Nobel Prize in Physiology or Medicine in 1990 for work on immunosuppression.

Immunosuppressive drugs have the potential to cause immunodeficiency, which can increase susceptibility to opportunistic infection and decrease cancer immunosurveillance. Immunosuppressants may be prescribed when a normal immune response is undesirable, such as in autoimmune diseases.

Steroids were the first class of immunosuppressant drugs identified, though side-effects of early compounds limited their use. The more specific azathioprine was identified in 1960, but it was the discovery of ciclosporin in 1980 (together with azathioprine) that allowed significant expansion of transplantation to less well-matched donor-recipient pairs as well as broad application to lung transplantation, pancreas transplantation, and heart transplantation. After an organ transplantation, the body will nearly always reject the new organ(s) due to differences in human leukocyte antigen between the donor and recipient. As a result, the immune system detects the new tissue as "foreign", and attempts to remove it by attacking it with white blood cells, resulting in the death of the donated tissue. Immunosuppressants are administered in order to help prevent rejection; however, the body becomes more vulnerable to infections and malignancy during the course of such treatment

Non-deliberate immunosuppression:

Non-deliberate immunosuppression can occur in, for example, ataxia–telangiectasia, complement deficiencies, many types of cancer, and certain chronic infections such as human immunodeficiency virus (HIV). The unwanted effect in non-deliberate immunosuppression is immunodeficiency that results in increased susceptibility to pathogens, such as bacteria and viruses.

Immunodeficiency is also a potential adverse effect of many immunosuppressant drugs, in this sense; the scope of the term *immunosuppression* in general includes both beneficial and potential adverse effects of decreasing the function of the immune system.

B cell deficiency and T cell deficiency are immune impairment that individuals are born with or are acquired, which in turn can lead to immunodeficiency problems. Nezelof syndrome is an example of an immunodeficiency of T-cells

Immunodeficiency, also known as **immunocompromisation**, is a state in which the immune system's ability to fight infectious diseases and cancer is compromised or

entirely absent. Most cases are acquired ("secondary") due to extrinsic factors that affect the patient's immune system. Examples of these extrinsic factors include HIV infection and environmental factors, such as nutrition. Immunocompromisation may also be due to genetic diseases/flaws such as SCID.

In clinical settings, immunosuppression by some drugs, such as steroids, can either be an adverse effect or the intended purpose of the treatment. Examples of such use is in organ transplant surgery as an anti-rejection measure and in patients with an overactive immune system, as in autoimmune diseases. Some people are born with intrinsic defects in their immune system, or primary immunodeficiency.

A person who has an immunodeficiency of any kind is said to be **immunocompromised**. An immunocompromised individual may particularly be vulnerable to opportunistic infections, in addition to normal infections that could affect anyone. It also decreases cancer immunosurveillance, in which the immune system scans the body's cells and kills neoplastic ones. They are also more susceptible to infectious diseases owing to the reduced protection afforded by vaccines.

Types:

By affected component:

- → Humoral immune deficiency (including B cell deficiency or dysfunction), with signs or symptoms depending on the cause, but generally include signs of hypogammaglobulinemia (decrease of one or more types of antibodies) with presentations including repeated mild respiratory infections, and/or agammaglobulinemia (lack of all or most antibody production) which results in frequent severe infections and is often fatal.
- → T cell deficiency, often causes secondary disorders such as acquired immune deficiency syndrome (AIDS).
- → *Granulocyte deficiency*, including decreased numbers of granulocytes (called as granulocytopenia or, if absent, agranulocytosis) such as of neutrophil granulocytes (termed neutropenia). Granulocyte deficiencies also include decreased function of individual granulocytes, such as in chronic granulomatous disease.
- → Asplenia, where there is no function of the spleen.

→ Complement deficiency is where the function of the complement system is deficient.

In reality, immunodeficiency often affects multiple components, with notable examples including severe combined immunodeficiency (which is primary) and acquired immune deficiency syndrome (which is secondary).

Primary or secondary:

The distinction between primary versus secondary immunodeficiencies is based on, respectively, whether the cause originates in the immune system itself or is, in turn, due to insufficiency of a supporting component of it or an external decreasing factor of it.

Primary immunodeficiency:

A number of rare diseases feature a heightened susceptibility to infections from childhood onward. Primary Immunodeficiency is also known as congenital immunodeficiencies. Many of these disorders are hereditary and are autosomal recessive or X-linked. There are over 95 recognized primary immunodeficiency syndromes; they are generally grouped by the part of the immune system that is malfunctioning, such as lymphocytes or granulocytes.

The treatment of primary immunodeficiencies depends on the nature of the defect, and may involve antibody infusions, long-term antibiotics and (in some cases) stem cell transplantation. The characteristics of lacking and/or impaired antibody functions can be related to illnesses such as X-Linked Agammaglobulinemia and Common Variable Immune Deficiency.

Secondary immunodeficiencies:

Secondary immunodeficiencies, also known as acquired immunodeficiencies, can result from various immunosuppressive agents, for example, malnutrition, aging, particular medications (e.g., chemotherapy, disease-modifying antirheumatic drugs, immunosuppressive drugs after organ transplants, glucocorticoids) and environmental toxins like mercury and other heavy metals, pesticides and petrochemicals like styrene, dichlorobenzene, xylene, and ethylphenol. For medications, the term *immunosuppression* generally refers to both beneficial and potential adverse effects of decreasing the function of the immune system, while the term *immunodeficiency* generally refers solely to the adverse effect of increased risk for infection.

Many specific diseases directly or indirectly cause immunosuppression. This includes many types of cancer, particularly those of the bone marrow and blood cells (leukemia, lymphoma, multiple myeloma), and certain chronic infections. Immunodeficiency is also the hallmark of acquired immunodeficiency syndrome (AIDS), caused by the human immunodeficiency virus (HIV). HIV directly infects a small number of T helper cells, and also impairs other immune system responses indirectly.

Various hormonal and metabolic disorders can also result in immune deficiency including anemia, hypothyroidism and hyperglycemia. Smoking, alcoholism and drug abuse also depress immune response. Heavy schedules of training and competition in athletes increases their risk of immune deficiencies.

Causes:

The cause of immunodeficiency varies depending on the nature of the disorder. The cause can be either genetic or acquired by malnutrition and poor sanitary conditions. Only for some genetic causes, the exact genes are known.

Diagnosis:

Patients with immune deficiencies can present with variable clinical phenotypes. This often translates into a significant delay in their diagnosis, and resultant patient morbidity. A structured approach on when to suspect an immunodeficiency and the initial investigations pathway is given in the publication by Grammatikos et al.

Treatment:

Available treatment falls into two modalities: treating infections and boosting the immune system.

Prevention of Pneumocystis pneumonia using trimethoprim/sulfamethoxazole is useful in those who are immunocompromised. In the early 1950s Immunoglobulin(Ig) was used by doctors to treat patients with primary immunodeficiency through intramuscular injection. Ig replacement therapy are infusions that can be either subcutaneous or intravenously administered, resulting in higher Ig levels for about three to four weeks, although this varies with each patient.

Prognosis:

Prognosis depends greatly on the nature and severity of the condition. Some deficiencies cause early mortality (before age one), others with or even without treatment are lifelong conditions that cause little mortality or morbidity. Newer stem cell transplant technologies may lead to gene based treatments of debilitating and fatal genetic immune deficiencies. Prognosis of acquired immune deficiencies depends on avoiding or treating the causative agent or condition (like AIDS).

What is Tolerance in Immunology?

Immune tolerance, or immunological tolerance, is the process by which immune cells are made unresponsive to self-antigens to prevent damage to healthy tissues. It prevents an immune response to antigens produced by the body itself or recognized from a prior encounter. Tolerance is built by the body's ability to determine self vs non-self cells.

Regulatory T Cells for Immune Tolerance

Immune cell functionality—via essential antigen-recognizing abilities—is integral to peripheral immune tolerance. One subtype of T cells called regulatory T cells, or Treg cells, are indispensable to this process.

Treg cells are created during the immune system's normal selection process in the thymus. They maintain immune tolerance by distinguishing self-antigens from foreign antigens and finding overactive effector cells that evade other mechanisms of negative selection. Treg cells will also suppress or halt an inflammatory reaction once it becomes detrimental to the body.

There are two types of immune tolerance: self-tolerance and induced tolerance and both play an integral role in defending the body from harmful inflammation.

Self-Tolerance:

The immune system can identify and not react against self-produced antigens, which are known as self-tolerance. Autoimmune disease may occur if this ability is lost and the body begins to attack its cells.

Throughout an individual's life, there is an ongoing active process of immune tolerance to self. Autoreactive lymphocytes are eliminated during development, and regulatory T cells help to keep them in check during circulation.

- Learn More about Sample Preparation for Rare Cell Isolation (Tregs) using Microbubbles
- Download the “Quick and Gentle Isolation of Unique Cell Populations” App Note
- Shop for the Human CD4+ T Cell Isolation Kit

Possible Causes for a Lack of Self-Tolerance

The loss of self-tolerance is the primary driver of autoimmune diseases. Self-tolerance and autoimmunity can have numerous causes.

The mechanism that controls tolerance involves deleting overactive and autoreactive cells during negative selection processes in the thymus before naive T cells mature. This process screens naive T cells for reactivity to self-elements. Naive T cells that show naive cell activation to self-antigens during development are negatively selected and apoptosis is induced.

This mechanism can be manipulated based on the concentrations of negative selection control and cell surface receptors, such as **Fas** and **FasLigand**, which regulate cellular apoptosis. T cell suppression is mediated by cytokines, and some foreign particles and pathogens can modify the abilities, structure, and number of cytokines circulating.

Risks of Losing Self-Tolerance

A decrease in self-tolerance can cause various autoimmune diseases. The immune system’s ability to distinguish pathogens from self-antigens is critical to the functionality of its infection protections. When the immune system fails to tolerate self-cells, the body will go into an autoimmune state, attacking self-tissues, leading to illness and, in some cases, death.

Induced Tolerance:

Induced tolerance occurs when the immune system actively avoids responding to an external antigen. Previous encounters with that antigen induce this immunological tolerance. An example of induced tolerance is a deliberate manipulation of the immune system to avoid the rejection of transplanted organs or to provide protection from allergic reactions.

Practical Applications of Inducing Tolerance:

Current practices in transplantation medicine immune tolerance mechanisms to good advantage by manipulating the immune system to tolerate transplanted organs and

tissues through immunosuppressive drugs. Inflammation and immune reactions to transplanted cells are common complications of major organ transplants. By exploiting the body's natural self-tolerance processes, a transplant patient is better protected against rejection of the transplanted cellular material.

In addition to transplant medicine, induction of immune tolerance is done regularly in the treatment of allergies. Antigen immunotherapy introduces very low doses of the antigen (below the activation threshold of the immune cells) either by injection or sublingually to retrain the immune system to a tolerant state.

Central Tolerance vs. Peripheral Tolerance: What's the Difference?

Immune tolerance mechanisms are separated into two categories: central tolerance and peripheral tolerance. These mechanisms occur at different stages of the lymphocyte life cycle, and a deficiency in either category can result in serious consequences to the body.

Central Tolerance

Central tolerance mechanisms occur during lymphocyte development, either in the thymus for T cells or in the bone marrow for B cells. Through this process, immune cells with T cell receptors (TCRs) or B cell receptors (BCRs) that can recognize and bind to self-antigens are eliminated—or, for some T cells, differentiated into Tregs. By preventing the maturation of autoreactive lymphocytes, central tolerance helps the immune system discriminate between self-antigens and foreign materials.

Elimination of self-reactive lymphocytes can occur by one of several immune tolerance mechanisms:

- ✓ **Deletion:** Cell death is induced in autoreactive immune cells.
- ✓ **Anergy:** Autoreactive immune cells receive signals that cause their antigen simulation to cease, leaving them functionally incapable of differentiating into effector cells.
- ✓ **Ignorance:** Autoreactive immune cells that either do not encounter their self-antigen or bind to their self-antigen so weakly that they are "ignorant" of their reaction, do not differentiate into effector cells.

Some autoreactive lymphocytes, however, are not eliminated or differentiated into Tregs during development. For these cells—or for ignorant lymphocytes that encounter their self-antigen later—there are additional immune tolerance mechanisms in place.

Examples of Central Tolerance

Central tolerance processes take place during immune cell development, modifying the number of naive lymphocytes circulating the periphery. Central tolerance— referring to the location of these processes—occurs in the central lymphoid organs, the bone marrow, and the thymus. When autoreactive T cells are deleted for binding to self-antigens in the thymus, central tolerance properly enacts immune cell regulation.

Peripheral Tolerance

After mature lymphocytes are released into the lymph nodes or other tissues, **peripheral tolerance** mechanisms occur to prevent autoreactive immune cells from causing damage in the periphery. Tregs are one mechanism of peripheral tolerance that induces suppression or anergy of autoreactive cells that have escaped other mechanisms of central tolerance, preventing the immune system from overreacting to self- or other non-harmful antigens.

Examples of Peripheral Tolerance

The regulation of the inflammatory response in the peripheral lymphoid organs occurs during regular immune cell circulation. These organs include secondary lymphoid organs such as the mucosal membranes and lymph nodes. Peripheral tolerance enacts the second stage of immune tolerance, safeguarding the body from harmful inflammation when autoreactive cells escape central tolerance selection and reach the periphery.

Anergy is a type of peripheral tolerance that occurs when a cell is activated without the necessary co-stimulation and histocompatibility complex. Although anergic cells remain alive and reversible, they are unable to respond to antigenic activation. Another crucial aspect of peripheral tolerance is the involvement of Treg cells, cells differentiated by exposure to specific cytokines, which help regulate overactive T cells.

Immuno-potentiation:

Immuno-potentiation is the effort to enhance the body's natural immune response. This is mediated by adjuvants and vaccination.

Adjuvants (from Latin adjuvare, to help) are substances that, when mixed with an antigen and injected with it, enhance the immunogenicity of that antigen. Adjuvants are

often used to boost the immune response when an antigen has low immunogenicity or when only small amounts of an antigen are available, limiting the immunizing dosage.
As for example, the antibody response in mice following immunization with BSA can be increased five fold or more if the BSA is administered with an adjuvant.

Effect of Adjuvants:

Though augmentation of adjuvants in the immune response is not completely known but they exert several effects like**:**

(a) Help to prolong antigen persistence;

(b) Help to enhance co-stimulatory formation;

(c) Help to induce granuloma formation and

(d) Help to stimulate typhocyte proliferation non-specifically.

Examples of Adjuvants:

i. Aluminum-potassium sulfate (alum)

ii. Freund's incomplete adjuvant

iii. Freund's complete adjuvant

i. Aluminum-potassium sulfate (alum):

This alum acts to increase antigen persistence. When an antigen is mixed with alum, the salt precipitates the antigen. Injection of this alum precipitate, results in a slower release of antigen from the injection site, so that the effective time of exposure to the antigen increases from a few days (without adjuvant) to several weeks (with the adjuvants). The alum precipitate also increases the size of the antigen and thus increasing the likelihood of phagocytosis.

ii. Freund's incomplete adjuvant:

Freund's water in oil adjuvants prolong the persistence of antigens. It contains antigen in aqueous solution, mineral oil, and an emulsifying agent such as mannide monosoleate which disperses the oil into small droplets surrounding the antigen. The antigen is then released very slowly from the site of injection.

iii. Freund's complete adjuvant:

This contains heat-killed Mycobacteria in the water in oil emulsion. A muramul dipeptide component of the mycobacterial cell wall activates macrophages, making Freund's complete adjuvant more potent than the incomplete one.

The use of adjuvants in vaccines

The purpose of adding adjuvants into vaccines is to boost the immune system response and to allow for fewer doses or lesser quantities of the vaccine to be administered. Aluminum, one of the most commonly used adjuvants, was first discovered to have adjuvant properties back in 1926.

Since then numerous vaccines, such as hepatitis A, hepatitis B, diphtheria-tetanus, Haemophilus influenza type b, and pneumococcal vaccines have been developed with the use of aluminum adjuvants. Today, a number of different kinds of adjuvants have been discovered and successfully used to develop new vaccines. We discuss these below.

Scientists theorize that adjuvants may act through a number of mechanisms to have the impact of enhancing the immune system response. Studies have revealed that adjuvants are likely to influence mechanisms such as the induction of cytokines and chemokines, the formation of depot, the promotion of antigen transportation to drain the lymph nodes, and the enhancement of antigen uptake and presentation.

Research has revealed that adjuvants are likely generating immuno-competent environments at the location of the vaccine injection through the activation of an innate immune response. It is this innate response, the type that is activated, which governs how the quality of the adaptive immune responses is altered.

How do adjuvants work?

When adjuvants are added into a vaccine they work in four distinct ways to boost the immune response. The first of these pathways is the activation of antigen-presenting cells to signal to the immune system's T cells that foreign substances have infiltrated.

To do this adjuvants boost the activation of antigen-presenting cells, cells of the immune system that encompass foreign substances and break them up, presenting the resulting particles to the immune system's T cells. This activates the T cells, which has the impact of activating the antibody-producing B cells. The second way that adjuvants work is by activating T cells indirectly by discharging phagosomes that attach themselves to the T

cells. Following this binding, the T cells are induced to release cytokines that switch on the antibody-producing B cells.

The next process involves the targeting of antigens at specific locations. The location where an adjuvant is injected can induce immune system activity localized to that specific area. This activation incites T cells to travel through the bloodstream to that specific location. Finally, adjuvants can induce the slow release of an antigen. The depot effect refers to the process by which adjuvants can regulate the rate of antigen release into the bloodstream. To achieve this, the adjuvant is enclosed within a polymer along with an antigen. This has the impact of reducing the rate at which both the chemicals and antigens are released into the tissue and bloodstream.

Types of adjuvant:

Since the discovery of aluminum's function of an adjuvant back in 1926, many more substances have been recognized as adjuvants and used to create a variety of vaccines. To begin with, aluminum, as discussed, is a common type of adjuvant. These are often added into vaccines in the form of mineral salts. It is particularly competent at activating the Th2 immune response, which is characterized by the release of Interleukin 5 and is often associated with the removal of parasites.

However, it is not as effective at activating the Th1 response, which causes B cells to attach themselves to antigens to allow other immune cells to identify and kill whatever substance is clinging to the antibody. Oil emulsions are another type of widely used adjuvant. These mixtures of oil and water have proven their effectiveness at generating strong immune responses. Like aluminum, these substances are excellent at inducing the Th2 immune response. Also, they are good at creating a slow-release effect.

Microbial substances, such as sugars from the cell walls of microbes, can be used to induce intense immune reactions due to the body's natural response against microbes. Saponins are a group of chemical compounds that exist in abundance in numerous species of plants. These steroid molecules with attached sugar chains can also trigger an intense immune response at a low dose. Cytokines are a group of peptides that play a vital role in cell signaling. Interferons and interleukins are specific types of cytokines that are naturally released by cells in the immune system in order to generate mutual activations. Certain types of these cytokines can be used to evoke specific immune cell responses.

Finally, scientists have successfully established various synthetic adjuvants. Specifically, molecules have been designed that activate the immune cell's PRR and TLR receptors, having the impact of switching on genes that indicate the presence of an infection to neighboring cells.

Future directions:

Scientists will continue to investigate the mechanisms responsible for how adjuvants influence the immune response. Growth in the understanding of these processes will help to develop new and safe vaccines for a wider range of afflictions.

8. Let's sum up

- Major histocompatibility complex (MHC) is a collection of genes coding for glycoprotein molecules expressed on the surface of all nucleated cells.
- **Intracellular** peptides for MHC class I presentation are made by proteases and the proteasome in the cytosol, then transported into the endoplasmic reticulum via TAP (Transporter associated with Antigen Processing) to be further processed.
- **CD4** is present on T helper cells and only binds to antigen-MHC II complexes.
- **CD8** is present on cytotoxic T cells and only binds to antigen-MHC I complexes.
- All nucleated cells in the body have mechanisms for processing and presenting antigens in association with MHC molecules. This signals the immune system, indicating whether the cell is normal and healthy or infected with an intracellular pathogen. However, only macrophages, dendritic cells, and B cells have the ability to present antigens specifically for the purpose of activating T

cells; for this reason, these types of cells are sometimes referred to as antigen-presenting cells (APCs).

- The complement system consists of a series of heat-labile serum proteins that are activated in turn. The complements exist as soluble inactive precursors which once activated; a complement component may then act as an enzyme. Enzymatic chain reactions of this type are known as cascade reactions and usually require a "trigger" to initiate the reaction chain.

- On binding to antibody, one molecule of C1r is induced to cleave itself, becomes enzymatically active. Gradually it cleaves and activates the second C1r and both C1s molecules. The activated serine protease C1s binds, cleaves and activates the next two components of the classical pathway i.e. serine protease C4 and C2. Ultimately active CI component is called $C1qr_2s_2$.

- Formation of C3bBb accelerates the auto- catalyse of more C3 component and forms C3bBb3b as C5 convertase. Though structural basis of C3 and C5 convertase vary in these two pathways of complement system but their mode of action is alike.

- B-cells express on their surface intra-membrane immunoglobulin (Ig) molecules that function as B cell antigen receptors. Since all the receptors on a single B cell are identical, each B cell can bind only one antigen. This makes them much more efficient antigen-presenting cells than macrophages, which must ingest any foreign material that comes their way. Descendants of B-cells (plasma cells) produce antibodies.

- The chemical natures of several types of surface antigens are now known. Surface antigens have some biological importance and are involved in many functions. For examples, H-2 antigens may function during the mechanism of immunological surveillance.

- Vaccine-induced CD8 T cells directed to tumour-specific antigens are recognised as important components of protective and therapeutic immunity against tumours.

- The immune system is a complex system of interacting cells whose primary function is to identify foreign from self and eliminate it, usually referred to as “antigens”. The defense system of the body against this antigen usually involves the elicitation of both innate and adaptive branch of immunity.

- **Active Immunity** results when exposure to a disease organism triggers the immune system to produce antibodies to that disease. Active immunity can be acquired through natural immunity or vaccine-induced immunity.

- Acquired immunity concerns the production of protein molecules by B lymphocytes, called antibodies (or immunoglobulins), and of specific cells, including T-lymphocytes (also known as cell-mediated immunity) along with the innate immunity involving the cells like macrophages, complement mediated lysis, production of pro-inflammatory cytokines etc. to eliminate the antigen.

- Inflammation (L. inflammatio = to set on fire) is an innate (nonspecific) defence response of the body to pathogenic infection or tissue injury and helps localizing the infection or injury in its local area. Many of the classic features of inflammation were described as early as 1600 BC in Egyptian papyrus writings.

- **Immediate innate immunity** begins 0 - 4 hours after exposure to an infectious agent and involves the action of soluble preformed antimicrobial molecules that circulate in the blood, our found in extracellular tissue fluids, and are secreted by epithelial cells. These include: antimicrobial enzymes and peptides; complement system proteins; and anatomical barriers to infection, mechanical removal of microbes, and bacterial antagonism by normal flora bacteria.

- Immune tolerance mechanisms are separated into two categories: central tolerance and peripheral tolerance. These mechanisms occur at different stages of the lymphocyte life cycle, and a deficiency in either category can result in serious consequences to the body.

- After mature lymphocytes are released into the lymph nodes or other tissues, **peripheral tolerance** mechanisms occur to prevent autoreactive immune cells from causing damage in the periphery. Tregs are one mechanism of peripheral tolerance that induces suppression or anergy of autoreactive cells that have escaped other mechanisms of central tolerance, preventing the immune system from overreacting to self- or other non-harmful antigens.

9. Suggested Readings

1. Kindt T, Goldsby R, Osborne B, Kuby J, Kuby J. Kuby immunology. 2007. New York:W.H. Freeman.
2. Delves, Peter J.; Martin, Seamus J.; Burton, Dennis R.; Roitt, Ivan M. 2011. Roitt'sEssential Immunology. Hoboken, NJ: Wiley-Blackwell.
3. Murphy, K., Travers, P., Walport, M., & Janeway, C. 2008. Janeway's immunobiology.New York: Garland Science.
4. Abbas, A. K., Lichtman, A. H., & Pillai, S. 2010. Cellular and molecular immunology.Philadelphia: Saunders/Elsevier.

10. Assignments

1. Describe exogenous pathway of antigen presentation with suitable diagram.
2. Describe endogenous pathway of antigen presentation with suitable diagram.
3. Describe structure of class I and class II MHC molecule.
4. How antigens are presented with MHC-II?
5. How antigens are presented with MHC-I?
6. What are the functions of complement system?
7. Describe lectin pathway of complement system activation.

8. Describe alternative pathway of complement system activation.
9. Describe classical pathway of complement system activation.
10. Discuss the characteristics of an antigen.
11. Define epitope and paratope.
12. What are the characteristics of an epitope?
13. Discuss different types of surface antigens.
14. What is MHC Antigen ?
15. How ABO antigens are used to detect blood group?
16. Discuss polytope vaccines.
17. How gene regulation of MHC molecules happen?
18. Discuss disease associated with MHC molecules.
19. What is cytokines? Discuss characteristics of cytokines.
20. What do you mean by immunization?
21. What is vaccine and vaccination?
22. What do you mean by active immunization?
23. Describe the different types of vaccines administered in human.
24. What is toxoid? Give an example.
25. Explain the mechanism of action of a vaccine.
26. Elaborate the advantages of DNA vaccine.
27. What do you mean by antigen-antibody reaction?
28. Describe the nature of antigen antibody reaction.
29. What is affinity?
30. What do you mean by cross reactivity?
31. Write short notes on agglutination and precipitation reaction?
32. Describe sandwich ELISA?
33. Describe the process of Radioimmunoassay (RIA) with diagram.
34. What do you mean by Immunofluorescence? Which flurochromes are used in this teachnique?
35. Write down the full form of FISH and GISH.
36. What is FISH used for?
37. Write down the main steps of Genomic in situ hybridization with diagram.
38. What do you mean by Immunohistochemistry (IHC)?

39. List the primary lymphoid organs and summarize their functions in the immune response.
40. List the secondary lymphoid organs and summarize their functions in the immune response.
41. What are the two primary characteristics that distinguish hematopoietic stem cells and progenitor cells?
42. What are the two primary roles of the thymus?
43. What effect would removal of the bursa of Fabricius (bursectomy) have on chickens?
44. Highlight the difference between innate and adaptive immunity.
45. What do you mean by hematopoiesis?
46. Classify the immune cells on the basis of origin.
47. What is antibody dependent cell mediated cytotoxicity?
48. Classify macrophages on the basis of location.
49. What is GALT and MALT?
50. Define innate immunity?
51. State the differences between innate and adptive immunity.
52. Describe the mechanism of innate immunity?
53. Briefly describe the three major events in the inflammatory response.
54. Innate and adaptive immunity act in cooperative and interdependent ways to protect the host. Discuss the collaboration of these two forms of immunity.
55. What are the functions of NK cell?
56. Explain the role of macrophages in elimination of foreign particulates.
57. Define immune induction. How activation of innate immune system occurs?
58. How innate and adaptive immune system interacts with each other?
59. Discuss DC Presentation of Antigen to Cytotoxic T (Tc1) Lymphocytes.
60. How Dendritic cells interacts with B lymphocytes?
61. How immunosuppression occurs?
62. What is non deliberate immunosuppression?
63. Discuss deliberate immunosuppression.
64. Differentiate primary and secondary immunodeficiency.
65. What is immunotolerance?
66. Discuss Possible Causes for a Lack of Self-Tolerance.

67. What is induced tolerance and what are its applications?
68. Differentiate Central Tolerance and Peripheral Tolerance.
69. What is adjuvant? How it boost up immunity?
70. How adjuvants are used in preparation of vaccines?
71. Discuss different types of adjuvants.

All the materials are self writing and collected from e-book, journals and websites.