NETAJI SUBHAS OPEN UNIVERSITY

STUDY MATERIAL

POST GRADUATE
ZOOLOGY

Paper - 6

Group : B

Immunology and
Microbiology

PREFACE

In the curricular structure introduced by this University for students of Post-Graduate Degree Programme, the opportunity to pursue Post-Graduate course in any subject introduced by this University is equally available to all learners. Instead of being guided by any presumption about ability level, it would perhaps stand to reason if receptivity of a learner is judged in the course of the learning process. That would be entirely in keeping with the objectives of open education which does not believe in artificial differentiation.

Keeping this in view, study materials of the Post-Graduate level in different subjects are being prepared on the basis of a well laid-out syllabus. The course structure combines the best elements in the approved syllabi of Central and State Universities in respective subjects. It has been so designed as to be upgradable with the addition of new information as well as results of fresh thinking and analysis.

The accepted methodology of distance education has been followed in the preparation of these study materials. Co-operation in every form of experienced scholars is indispensable for a work of this kind. We, therefore, owe an enormous debt of gratitude to everyone whose tireless efforts went into the writing, editing and devising of proper lay-out of the materials. Practically speaking, their role amounts to an involvement in 'invisible teaching'. For, whoever makes use of these study materials would virtually derive the benefit of learning under their collective care without each being seen by the other.

The more a learner would seriously pursue these study materials, the easier it will be for him or her to reach out to larger horizons of a subject. Care has also been taken to make the language lucid and presentation attractive so that they may be rated as quality self-learning materials. If anything remains still obscure or difficult to follow, arrangements are there to come to terms with them through the counselling sessions regularly available at the network of study centres set up by the University.

Needless to add, a great deal of these efforts is still experimental–in fact, pioneering in certain areas. Naturally, there is every possibility of some lapse or deficiency here and there. However, these do admit of rectification and further improvement in due course. On the whole, therefore, these study materials are expected to evoke wider appreciation the more they receive serious attention of all concerned.

Professor (Dr.) Subha Sankar Sarkar
Vice-Chancellor

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POST GRADUATE ZOOLOGY

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Writer & Editor
Prof. Amal Bhattacharya

Netaji Subhas Open University

PGZO–6
Quantitative Biology & Biotechnology, Immunology & Microbiology

Group - B
Immunology & Microbiology

Unit 1 ❐ Overview of Immune System, Components of Immunity, Innate and Adaptive Immunity

Structure

1.1 Overview of immune system

Immunity is the state of protection from infectious disease. The latin term 'immunis' meaning exempt is the main source of english word immunity. In proper sense this response is the defensive reactivity to a specific molecular configuration that develops following contact with it in vertebrate system. This special reactivity is the resistance to second infection or allergic response. The specific molecules released by the foreign substance are either pathogen or allergen. Immunity has both specific and non specific components. The non specific component, innate immunity is a set of disease resistant mechanism that are spontaneous and not specific to a particular pathogen. Phagocytic cells like macrophages play an important role in many aspects of innate immunity. In contrast, the specific components of adaptive immunity display high degree of specificity and the concept of memory for secondary response. The major cellular agents of adaptive immunity are lymphocytes and the antibodies and certain other molecules.

Historical concept : The belief of immunity in human population developed in course of time. Common human belief in society and scientist's observations equally contributed to the development of this discipline during early period. Jenner (1798) tried inoculation method in human body. Pasteur (1881) observed attenuation and gave vaccine idea (derived from latin word vacca meaning cow). In twentieth century scientists detected humoral immunity in blood serum, cellular immunity in body lymphocyte cell and phagocytosis by macrophage. Further biological procedures identified B and T lymphocyte, structure of antibody and major histocompatibility complex region of cell etc.

1.2 Components of immunity

There are two types of immunity e.g. innate and adaptive or acquired immunity. As because the adaptive immunity needs some time to work properly, innate immunity provides the first line of defence during critical period just at the beginning of infection at the primary level. The innate and adaptive immunity do not operate independently of each other, they function as a highly interactive and cooperative system, producing a total synchronized attack against the pathogen.

1.3 Theory of immunity

Erlich's selective theory (1900) and Horowitz's instructional theory were not considered valid. The clonal selection of Burnett (1950) only maintains the concept

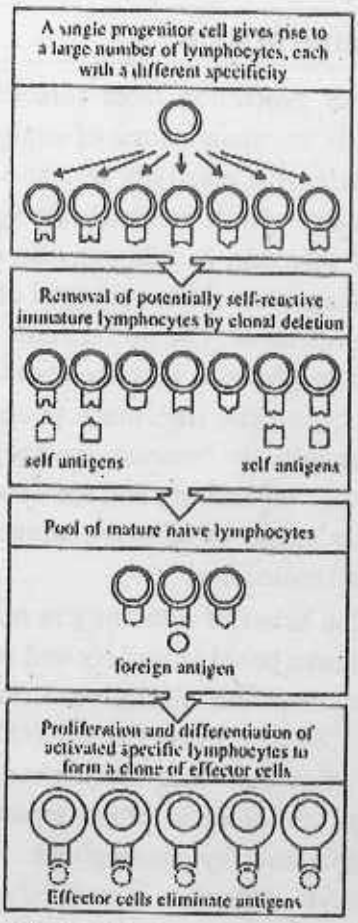

Fig. 1.1 Clonal selection theory

of acquired immunity. According to this theory an individual lymphocyte expresses unique membrance receptor which is specific for a dintinct antigen. This unique receptor of antigen binds to antibody or T lymphocytes and activates the lymphocyte cell to proliferate in a clone.

1.4 Features of innate and specific (adaptive) immunity

The following table will show the features of innate and specific (adaptive) immunity.

	Features	Innate	Specific
Characteristics	(1) Specificity for microbe	Relatively low	High
	(2) Diversity	Limited	Large
	(3) Specialization	Relatively stereotype	Highly specialized
	(4) Memory	No	Yes
Components	(1) Physical and chemical barriers	Skin mucosal epithalia, antimicrobal chemicals (e.g. defensins)	Cutaneous and mucosal immune sepheres; secreted antibodies
	(2) Blood proteins	Complement	Antibodies
	(3) Cells	Phagocytes (macrophages, neutrophils) natural killer cells	Lymphocytes

1.4.1 Innate (non-specific) immunity

Type	Mechanism
Anatomic barriers	
Skin	Mechanical barries retards entry of microbes. Acidic environment (pH 3-5) retards growth of microbes.
Mucous membranes	Normal flora compete with microbes for attachment sites and nutrients. Mucous entraps foreign microorganisms. Cilia propels microorgnisms out of the body.
Physiologic barriers	
Temperature	Normal body temperature inhibits growth of some pathogens. Fever response inhibits growth of some pathogens.

Type	Mechanism
Low pH	Acidity of stomach contents kills most of ingested microorganisms.
Chemical mediators	Lysozyme cleaves bacterial cell wall. Interferon induces antiviral state in uninfected cells. Complement lyses microorganisms or faciliates phagocytosis.
Phagocytic/endocytic barriers	Various cells internalize (endocytose) and breakdown foreign macromolecules. Specialised cells (blood monocytes, neutrophils, tissue macrophages) internalize (phagocytose), kill and digest whole microorganisms.
Inflammtory barriers	Tissue damage and infection induce leakage of vascular fluid, containing serum proteins with antimicrobial activity and influx of phagocytic cells into the affected area.

1.4.2 Adaptive Immunity : Adaptive immunity is capable of recognizing and selectively eliminating specific foreign microorganisms and molecules (antigens). Unlike innate immune responses, adaptive immune response reactions are specific antigenic challenges and display four attributes:

(1) Antigenic specificity
(2) Diversity
(3) Immunologic memory
(4) Self / non-self recognition

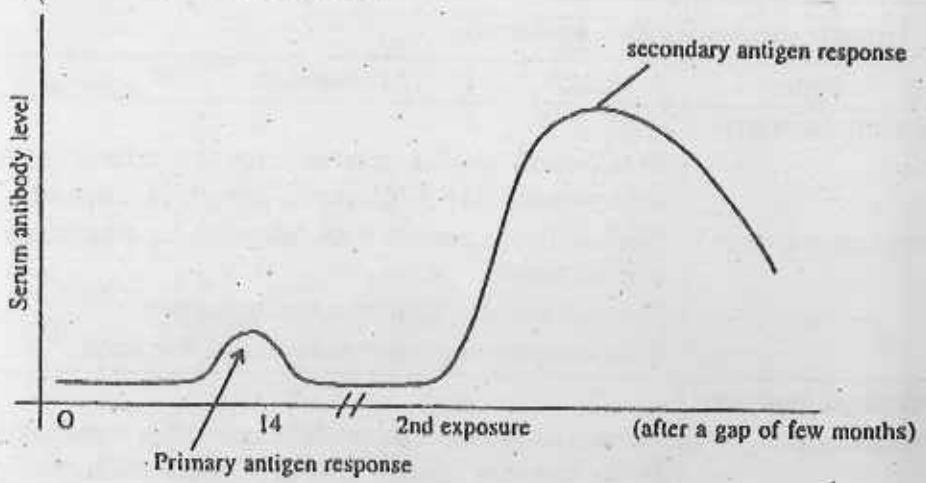

Fig. 1.2. Primary and secondary response serum level

An effective immune response involves two major groups of cells e.g. lymphocytes and antigen presenting cells (APC). The initial interaction of a naive lymphocyte with an antigen generates a primary response and the response is kept in memory so that in second contact of the host with the same antigen will induce a very rapid and hightened secondary response and the peak occurs in less time.

I. B Lymphocytes :

(1) They mature within bone marrow, however in bird it is processed by bursa fabricius. Bone marrow stem cell is the precursor of all blood cell. The mature B lymphocyte expresses a **unique antigen binding receptor** on its membrane.

(2) The B cell receptor is a membrane bound antibody molecule.

(3) On binding of antigen to a naive B cell, the cell divides rapidly; its progeny differentiate into **memory B cells and effector B Cells called plasma cells.**

(4) Memory B-cells continue to express same BCR as their parent naive B cell.

(5) Plasma cells produce antibody in a form that can be secreted.

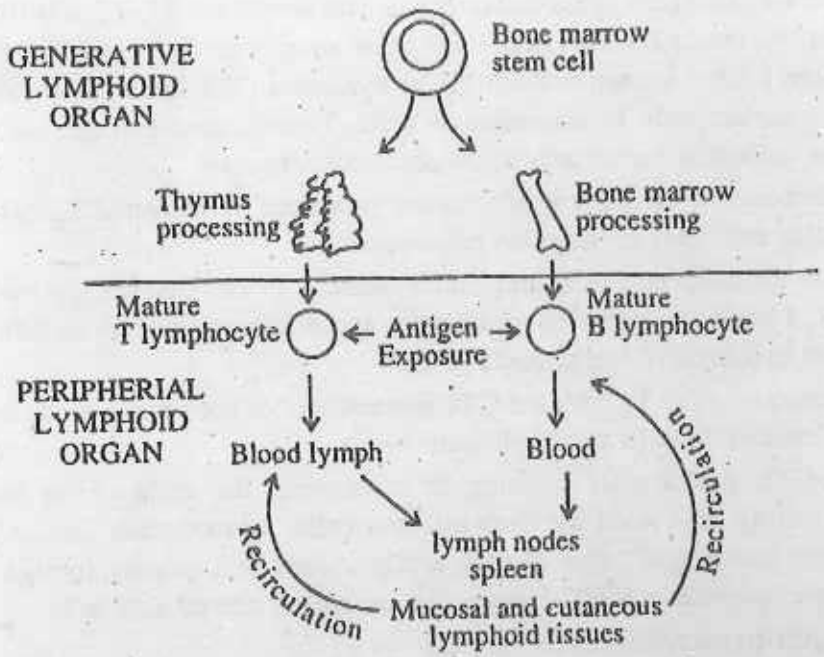

Fig. 1.3. ***Maturation of lymphocytes*** : Mature lymphocytes develop from bone marrow stem cell in generative (primary) lymphoid organ and immune response to foreign antigen develops in the peripheral lymphoid tissue.

II. T lymphosytes :

(1) T lymphocytes arise in bonemarrow but migrate to thymus for maturing.

(2) T cell expresses a unique antigen binding receptor (TCR) on its membrane. They can recognise only antigen that is bound to cell membrane proteins called major histocompatibility complex (MHC).

(3) There are two well defined subpopulations of T cells: T helper (T_H) and T-cytotoxic (Tc) cells. Although a third type of T cell called T supressor (Ts) has also been postulated.

(4) T helper and T cytotoxic cells can be distinguished from one another by the presence of either CD4 or CD8 membrane glycoproteins on their surface. CD stands for cluster of differentiation and refers to a molecule recognised by a cluster of monoclonal antibodies that can be used to identify the lineage or stage of differentiation of lymphocyte and thus to distinguish one class of lymphocytes from another.

(5) T cells displaying CD4 generally function as T_H cells, whereas those displaying CD8 function as Tc cells. Both of them perform intracellular killing mechanism of pathogen.

(6) After a T_H cell recognises and interacts with an antigen-MHC class II molecule complex, the cell is activated–it becomes an effector cell that secretes various growth factors known collectively as cytokines. The secreted cytokines play an important role in activating B cells, Tc cell, macrophages and various other cells that participate in the immune response.

(7) Differences in the pattern of cytokine produced by activated T_H cells result in different types of immune responses.

(8) Under the influence of T_H-derived cytokine, a Tc cell that recognizes antigen-MHC I molecule complex proliferates and differentiates into an effector cell celled cytotoxic T lymphocyte (CTL).

(9) In contrast to the T_H cell, the CTL generally does not secrete many cytokines and instead exhibits cytotoxic activity.

(10) The CTL has a vital function in monitoring the cells of the body and eliminating any, such as virus-infected cells, tumour cells and cells of a foreign tissue graft, that display antigen, cells that display foreign antigen complexed with a MHC I molecule are called altered self cells.

1.4.3 Antigen-presenting cells (APC)

(1) Certain specialized cells, which includes macrophage, B lymphocytes and dendritic cells are distinguished by 2 properties : i) they express class II

MHC molecules on their membranes and ii) they are able to deliver a co-stimulatory signal that is necessary for T_H cell activation.

(2) APCs first internalize antigen either by phagocytosis or by endocytosis and then display a part of that antigen bound to a class II MHC molecule on their membrane.

1.4.4 Interaction of lymphyocytes and APC

The APC (mainly macrophage) takes up the antigen and present it to T or B cell. T cells can act independant of B Cell. B cells require co-stimulation from T cell. T cells are activated on stimulation by APC and antigen attached to MHC. Complement activation process of the serum protein also form an effector arm of the humoral response.

1.5 Selective questions

(1) Define immunity. Explain the differences between innate and acquired immunity.

(2) Comment on primary and secondary immune response.

(3) Mention the maturation process of lymphocytes.

(4) State the role of antigen presenting cell (APC), B lymphocyte and T lymphocyte.

1.6 Selected readings

(1) Coico, R., Sunshine, G, and Benjamini, E. 2003. **Immunology** 5th Edn. Wiley-Liss, New Jersy.

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Unit 2 ❐ Cells and Organs of the Immune System

Structure

2.1 Introduction

Our immune system in body is controlled by white blood cells. The lymphocyte performs the role of acquired immune function and possess the major attributes of acquired immunological principles.

Embryonic Stem Cell is the precursor of all different types of cells that form the basis of all organ formations. Haematopoietic Stem Cells (HSC) generate blood cells (WBC and RBC). The process begins in embryonic sac during 1st week of development. In the 3rd month of pregnancy the HSC migrate from the yolksac to the foetal liver and then to the spleen. Later on after 6 months bone marrow is the precursor for differentiation (Fig. 2.1).

HSC give rise to i) lymphoid progenitor cell–B and T lymphocytes, natural killer cell. ii) Myeloid proganitor cell–RBC and WBC cells. The WBC are neutrophil, eosinophil, basophil, monocyte, mast cell and platelets (Fig. 2.2) The lymphon is a collective term for the primary and secondary lymphoid organs and their interconnecting blood vassels and lymphatics (Fig. 2.3). The blood cells enter circulation after differentiating in bone marrow. Various growth factors are involved during differentiation. These are : i) A group of acidic glycoprotein for colony stimulating factor (CSF) which are all cytokine material.The CSF's are Multilineage CSF, Interleukin-3, Macrophage colony stimulating factor (M-CSF), granulocyte CSF (G-CSF); granulocyte-macrophage CSF (GM-CSF). Another important haematopoietic cytokine detected by the method is a glycoprotein called erythropoietin (EPO). The expression of a progenitor cell to a particular differentiation pathway is controlled by a specific cytokine. It has been shown that regulation of haematopoiesis is controlled by certain gene like GATA-2.The steadystate regulation of haematopoiesis is controlled by factors like i) control of the level and types of cytokine produced by bonemarrow stromal cells, ii) release of cytokins by activated T cells and macrophage cells iii) regulation of cytokine production by stem cells

and progenitor cells iv) Apoptosis process of regulated cell death.

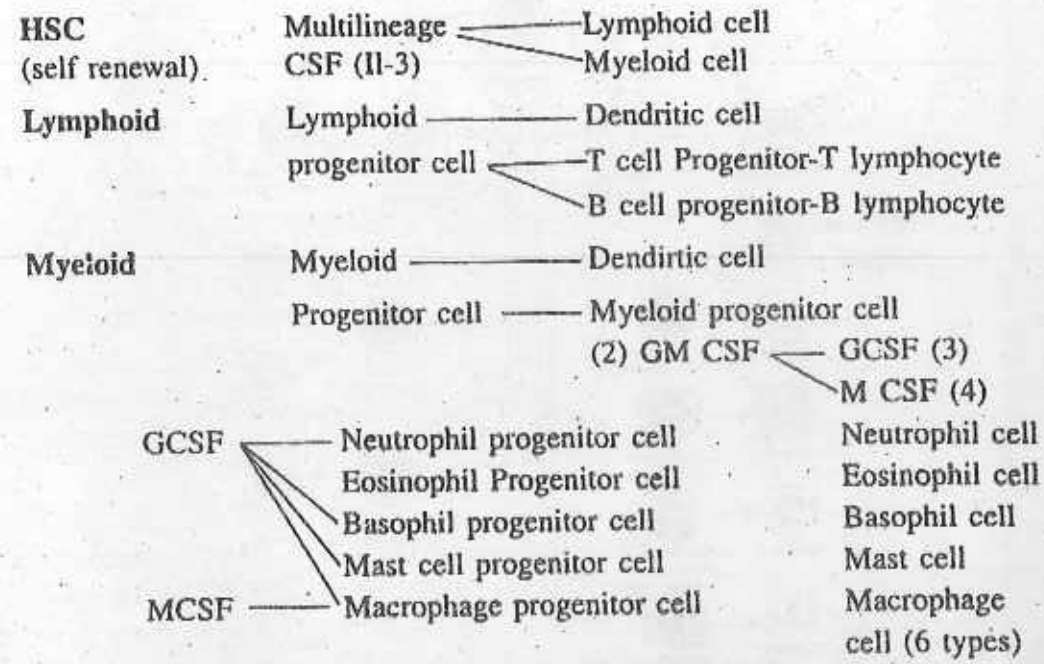

Chart : showing the process of immune cell formation

Abbreviations :

HSC	-	Haematopoietic stem cell
CSF	-	Colony stimulating factor
GMCSF	-	Granulocyte monocyte colony stimulating factor
GCSF	-	Granulocyte colony stimulating factor
M CSF	-	Monocyte colony stimulating factor
1-4	-	`Nos mark the colony stimulating factors

2.2 Cells of the immune system

Lymphoid cell : The lymphocytes constitute 20-40% of the WBC population. They are B and T lymphocytes and Natural Killer Cell. Natural Killer (Null) Cells have no surface markers like B and T lymphocytes. B and T lymphocytes (Naive stage) are 8 to 10 μm in diameter with large nucleus having dense heterochromatin. The cells have thin layer of cytoplasm with mitochondrion, ribosome and lysosome. B lymphocyte's receptor is a membrane bound antibody molecule. It is processed by bonemarrow or bursa in bird. In presence of antigen the naive B lymphocyte differentiates into memory B cell, effector B cell (Plasma cell). The mature B cell, has some markers on its surface like i) B 220 (a form of CD 45) ii) CRI (CD 35)

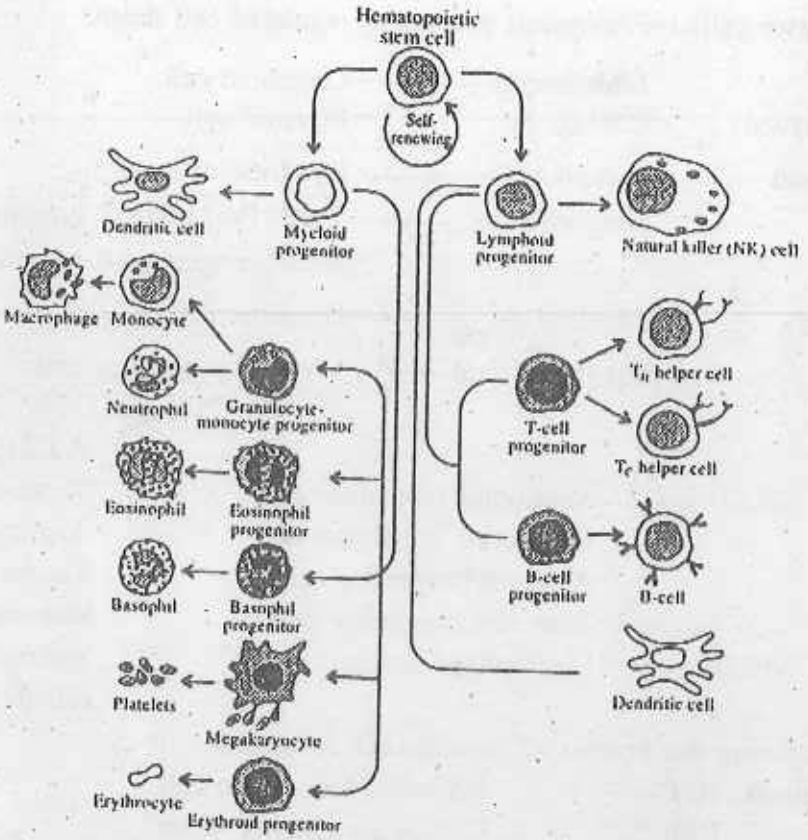

Fig. 2.1 Haematopoiesis—an outline of the formation of myeloid and lymphoid cells

and CR II (CD 21) receptors for complement factors iii) FcR II (CD 32) receptor for IgG (during ADCC) iv) B7-1 (CD 80) and B7-2 (CD 86) for interacting with CD 28 and CTLA-4 on the surface of T cell v) CD 40 for interaction with T helper cell.

B cells are activated by T helper cytokine factors and are differentiated to plasma cell and memory cell line. All clonal progeny from a given B cell secrete antibody molecule with same specificity. Plasma cells are teminally differentiated cell.

T lymphocytes are processed in thymus. they have membrane receptor for antign like B cell but they do not recognise a free antigen. It recognises antigen only when it is bound to a self molecule encoded by genes. So the antigen must be displayed together with MHC molecule on the surface of antigen presenting cell, virus infected cell, cancer cell and grafts. T lymphocytes are of two types mainly as T helper and T cytotoxic cell with receptor molecule CD4 and CD8 respectively.

Null cells are lymphocytes in peripheral blood which do not express the membrane molecule and receptors. They have no specificity like B and T lymphocyte and no memory cells. Most members of the population are large, granular lymphocyte and are natural killer (NK) cells. They constitute about 5% to 10% of the lymphocytes in human peripheral blood. They act against tumour cell and virally infected cell by releasing IFN-γ. NK cell kills virally infected cell along with T cytotoxic cell in a process called antibody-dependent cell mediated cytotoxicity where it is attached to antibody molecule.

Myeloid GM Cell : Monocyte (or macrophage in tissue)–It is a mononuclear cell in blood circulation and in tissue it becomes enlarged (5 to 10 times) and called macrophages, with phagocytic and killing mechanism. A number of antimicrobial and cytotoxic substances are produced by activated macrophage and can destroy phagocytosed microganisms. In an oxygen dependent killing mechanism activated phagocytes produce a number of reactive oxygen intermediate and reactive nitrogen intermediates that have potent microbial activity. Activated macrophage release lysozyme, Tumour necrosis factor and various hydrolytic enzymes which are oxygen independent killing mechanism. Macrophages in tissues are called alveolar cell (in lung), Histiocytes (in connective tissue). Kupffer cells (in liver), Mesengial cell (in kidney), Microglial cell (in Brain) and Osteoclasts (in bone).

Granulocyte cells are neutrophils, eosinophil, basophil and mast cells.

Neutrophil : Neutrophils are with multilobed nucleus and granulated cytoplasm. It stains both in acid and basic dyes. They are chemotactic and phagocytic. Movement of circulating neutrophils into tissue is called extravasation. Few chemotactic factors are accumulted at infiltration site during movement of neutrophil. These chemotactic factors are complement factors, blood clotting chemicals, cytokines released by activated T helper and macrophage cells. Neutrophils are phagocytic. The lytic enzymes and antimicrobial substances are contained in primary and secondary granules. Like macrophase they have also oxygendependent and independent pathways to generate antimicrobial substances.

Eosinophils : Eosinophils are with bilobed nucleus and stain in acid dye eosin. They act against parasite infection. They are also phagocytic in nature sometimes.

Basophils : Basophils have lobed nucleus and bind basic dyes. They express high affinity for $F_C\varepsilon$ receptor and can be triggered by antigen-binding to IgE and thereby mediate immediate hypersensitivity reaction to antigen. This character is also shared by mast cell. Basophils enter tissue only when they are recruited into inflammatory site. They have structural and functional similarities to mast cells.

Mast cells : They are like basophil and act against allergy. They are found

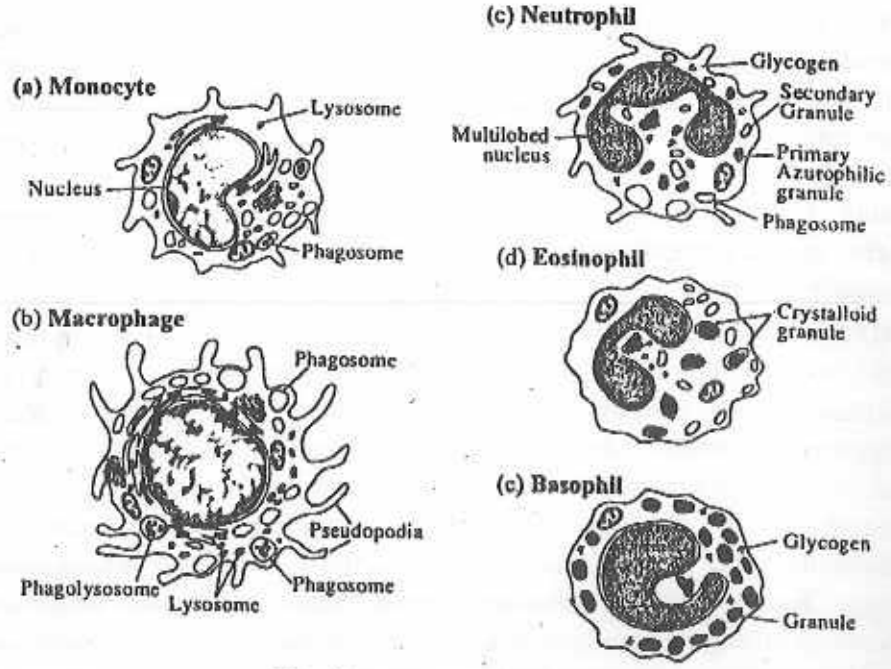

Fig. 2.2. Diagram of granulocyte cells

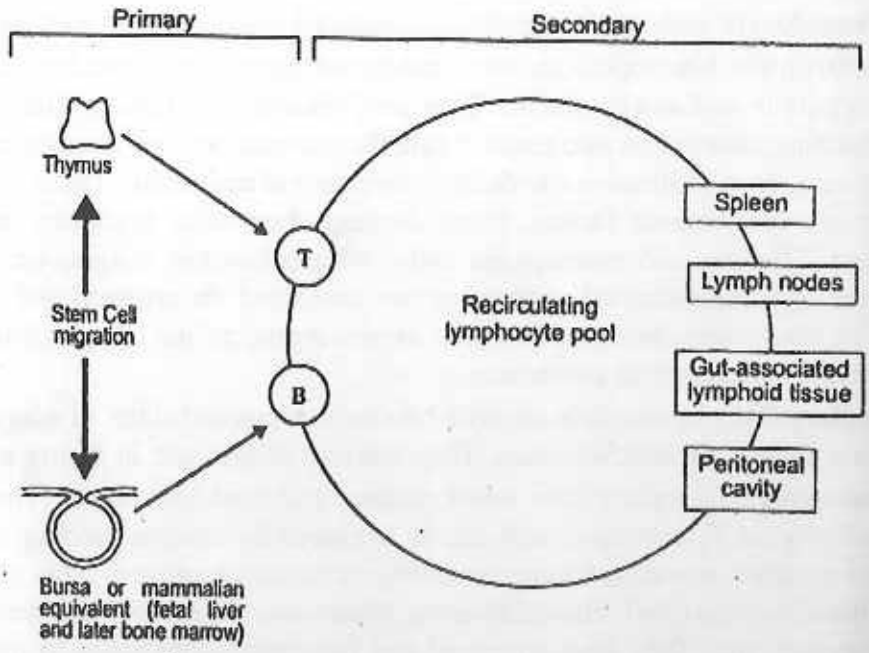

Fig. 2.3. The Lymphoid system and its relation with organs involved

throughout the body, predominantly located near blood vessels, nerves, lymphoid organs and beneath epithelium. They may be round, oval or spindle like with round nuclear. The cytoplasm contains membrane bound granules and lipid bodies. Mast cells found in the mucosa of gastrointestinal tract have chondroitin sulphate as their major granule proteoglycan where histamine production is minor. Whereas mast cells found in lungs and serosa of body tissue contain heparin as their major granule proteoglycan and histamine is produced in large quantity.

Dendritic cell process and present antigen to T_H cell. They are classified by their location as Langerhans cell in epidermis (skin) and mucous membrane, interstitial dendritic cell in heart, lung, liver, kidney, GI tract; intendigitating dendritic cell present in T cell areas of secondary lymphoid tissue or thymic medulla and follicular dendritic cells in cell rich region.

2.3 Organs of the immune system

Mature lymphocytes develop from bonemarrow stem cell in the generative (primary) lymphoid organ like Bone marrow, Bursa of fabricius and Thymus. Immune responses to foreign antigens occur in the peripheral lymphoid organs like lymph nodes, spleen, mucosal and cutaneous lymphoid tissues (secondary and tertiary lymphoid organs). In both T and B lymphocytes development, a selection process eliminates immature lymphocytes that react with self antigen, in addition, thymocytes that do not recognise self MHC molecules are also eliminated.

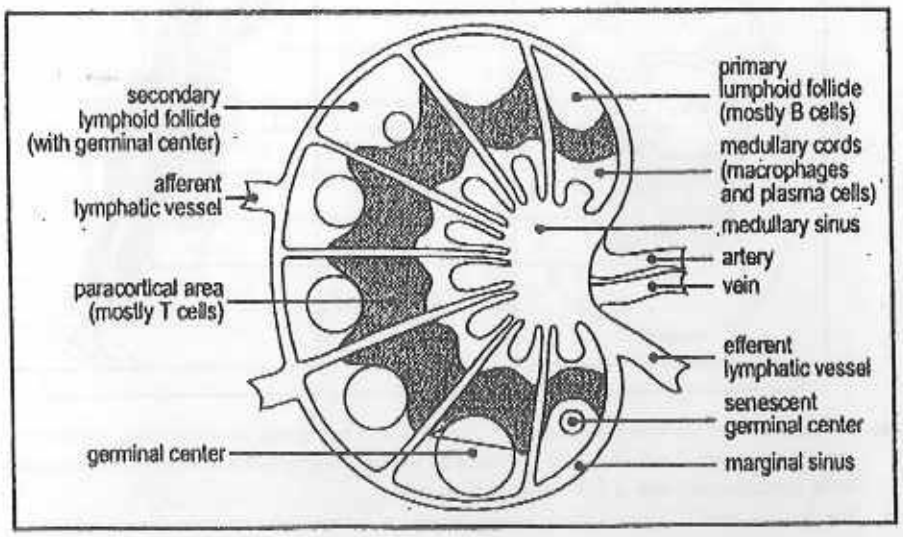

Fig. 2.4a. Organization of lymphnode. The node consists of an outermost cortex and an inner medulla.

In secondary lymphoid organs lymphocytes interact with antigen and undergo clonal proliferation and differentiation into effector cells. Lymph nodes trap antigen from regional tissue space and spleen collects antigens from blood. Lymph node and spleen are encapsulated organ (Fig. 2.4a, b). Less organized lymphoid tissues are in

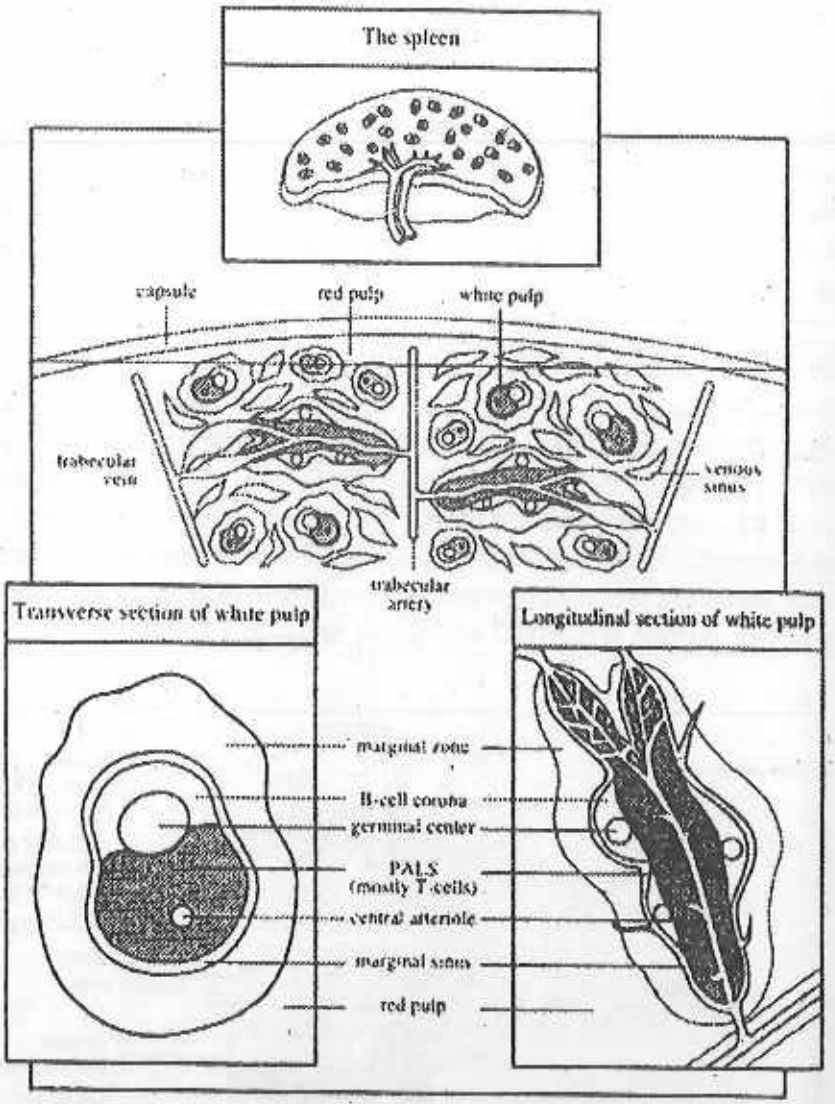

Fig. 2.4b Organization of spleen (top diagram) The spleen is composed of white pulp, rich in lymphoid cells, and red pulp, which contains many sinuses as well as large quantities of erythrocytes and macrophage, some lymphocytes and a few other cells types.
The bottom two diagrams show enlargements of a transverse section (lower left) and longitudinal section (lower right) of white pulp.

mucous membrane and are encapsulated. These include loose clusters of lymphoid follicles in the intestinal lamina propria and Peyer's patches found on the walls of the intestine. Cutaneous associated lymphoid tissue constitute the tertiary lymphoid organ (Fig. 2.5a, b and 2.6)

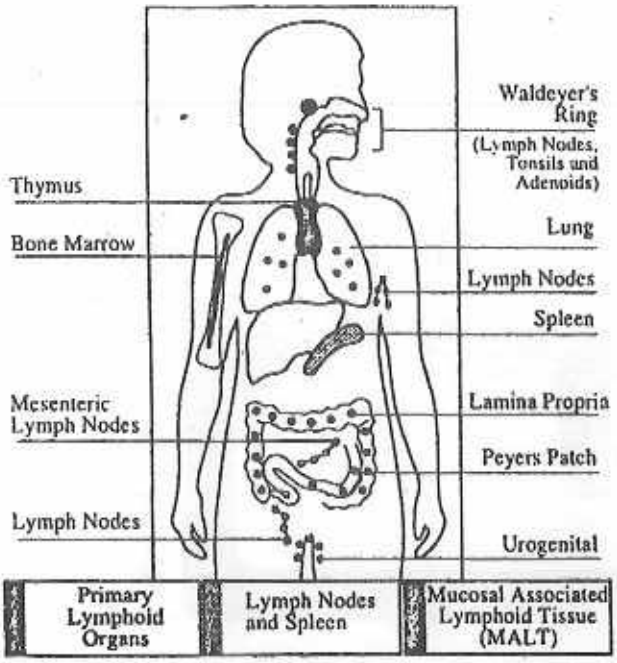

Fig. 2.5a The distribution of major lymphoid organs and tissues throughout the body.

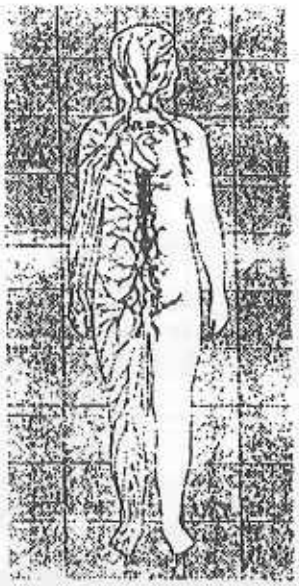

Fig. 2.5b The network of lymph nodes and lymphatics lymph nodes occur at junctions of the draining lymphatics. The lymph finally collects in the thoracic duct and hence returns to the blood stream via the left subclavian vein

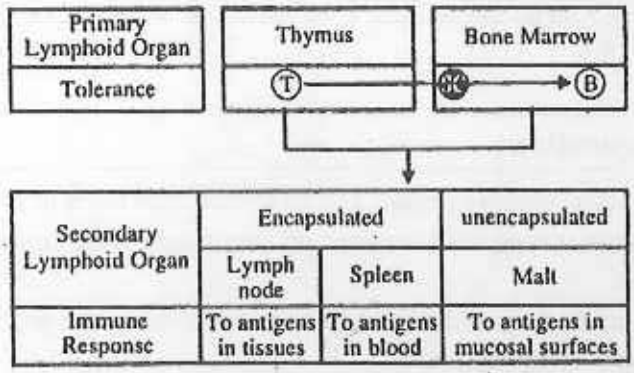

Fig 2.6. The functional organization of lymphoid tissue. T–Thymus; B–Bone marrow; SC–Stem cell; MALT–Mucous associated lymphoid tissue

Communication between these tissues and the rest of the body is maintained by a pool of recirculating lymphocytes which pass from the blood into the lymph node, spleen and other tissue and back to the blood by major lymphiatic channel as thoracic duct. (Fig. 2.7)

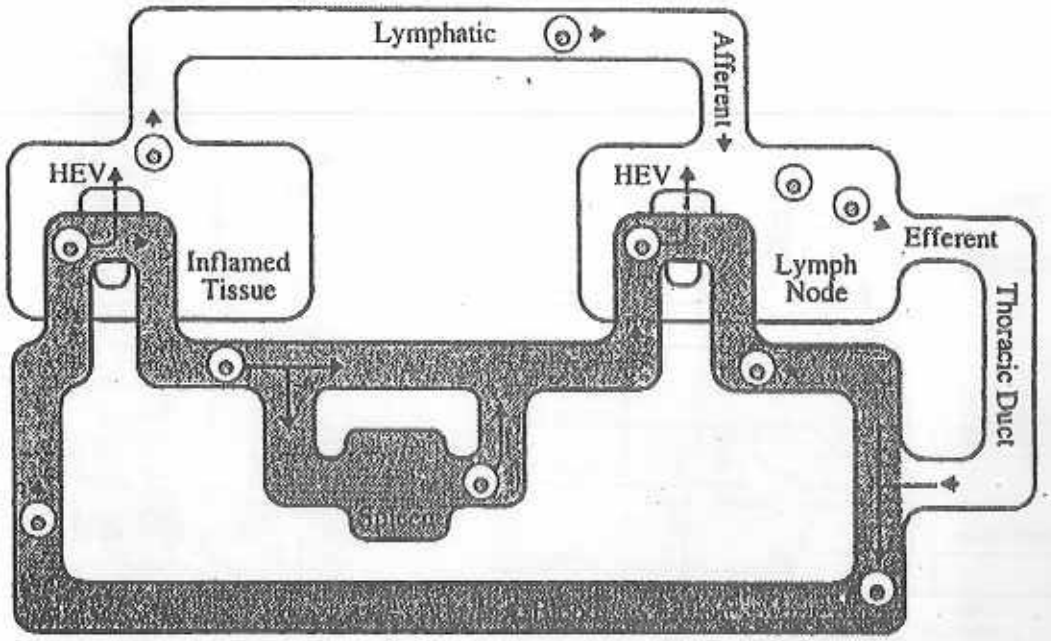

Fig. 2.7 The traffic of lymphocytes through out the body.
(HEV–Highwalled endothelium of the postcapillary venule)
Lymphocytes in blood circulation enter tissue and lymph nodes passing through HEV and leave via the draining lymphatics, the efficient lymphatics finally emerge from the node and enter thoracic duct which returns the lymphocytes to the blood stream. In the spleen lymphocytes enter the lymphoid area (while pulp) from the arterioles pass to the sinusoids of the erythroid area (red pulp) and leave by the splenic vein.

2.4 Selective questions

(1) List the primary lymphoid organs and state their functions in immune response.

(2) List the secondary lymphoid organs and summarize their functions in immune response.

(3) Give an outline diagram of Haematopoiesis and explain the process of formation of myeloid and lymphoid cells.

(4) Draw and describe different granulocyte cells.

2.5 Selected readings

(1) Janeway,C.A., Travers, P., Walport, M. and Capra J.D 1999. **Immuno Biology.** 4th edn. Current Biology Publications, New York

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Unit 3 ❐ Antigenicity and Immunogenicity, Immunogen properties, Adjuvant, Epitope, Hapten

Structure

3.1 Antigenicity and immunogenicity

Substances capable of evoking a specific immune response is generally called antigen. However we have to consider many points to determine if an antigen is also immunogen. **Immunogenicity** is the ability of an antigen to induce a humoral or cellular response. Whereas **Antigenicity** is the ability of the antigen simply to interact specifically with free antibody or with antigen-binding receptors on lymphocytes but fail to induce immune response B and T lymphocytes recognize small sites called antigenic determinats or epitopes on a complex immunogen. So this difference is mainly at functional level. The binding of antigen with B and T lymphocyte is highly specific; the immune components are capable of recognizing various physicochemical aspects of the component. There are several laws of physical chemistry controlling the phenamenon. These are Vender Waals forces, electrostatic interactions, hydrophobic interactions and hydrogen bonds function.

3.2 Immunogen properties

Factors that influence immunogenicity are mainly foreignness, molecular size, chemical composition of immunogen.

Foreignness : The immunogen must be a non-self molecule. The common experimental antigen bovine serum albumin (BSA) is not immunogenic when injected in cow but is strongly immunogenic when injected into rabbit. As a rule the more foreign the substance it becomes more immunogenic. In certain exceptional cases, as for example the body develops immune response against corneal tissue and sperm which are isolated from the immune system.

Molecular weight : The immunogen must have minimum molecular weight: small compounds with a molecular weight below 1000 Da (e.g. penicillin, progesterone, aspirin) are not immunogenic. Normally the chemicals of molecular weight above 6,000 Da are immunogenic (e.g. albumin, tetanus toxid).

Chemical complexity : Co-polymers of several amino acids are highly immunogenic. Protein structure of primary, secondary, tertiary and quaternary nature also have different nature during immunogenicity.

Degradability : The enzymatic degradation of protein antigen by antigen presenting cell is an important factor during binding with T cell. Carbohydrates are not processed and they cannot activate T cell although they can activate B cells.

3.3 Further requirements for immunogenicity

The genetic make up (genotype) of the organism play an important role in determining whether a given molecule will stimulate the immune response. So also in case of many parasitic infections the genotype of particular mice strain show either resistance or susceptibility during infection period.

The dosage and route of administration of antigen play a significant role in immunogenicity. Antigen administered via subcutaneously generally becomes very effective.

3.3.1 Adjuvant : Adjuvants (from latin adjuvare, to help) are substances that when mixed with an immunogen enhance the immunogenicity. They prolong antigen persistence, enhance co-stimulatory signal, induce granuloma formation, stimulate lymphocyte proliferation non specifically. Adjuvants contain microbial components (e.g. mycobacterial extracts). Other standard adjuvants are aluminium hydroxide and aluminium phosphate.

Table 3.1 Common adjuvants and their mode of action

Adjuvant	Composition	Mechanism of action
1) Alluminium hydroxide or aluminium phosphate (alum)	Aluminium hydroxide	Increases antigen uptake by APC and delayed release of antigen
2) Alum with a mycobacterial derived dipeptide	Aluminium Hydroxide gel with muramyl dipeptide	Enhanced antigen acceptance by APC, delayed release of antigen induction; Co-stimulation function of APC induced.

Adjuvant	Composition	Mechanism of action
3) Alum with *Bordetella pertusis*	Aluminium hydroxide gel with killed B pertusis	Increased uptake of antigen, delayed release of antigen and also costimulation function of APC induced.
4) Freund's complete adjuvant	oil in water with killed mycobacteria	Increased uptake of antigen by APC, delayed release of antigen. Co-stimulation function of APC induced.
5) Freund's incomplete adjuvant	Oil in water	Increased uptake of antigen by APC, delayed release of antigen.
6) Immune stimulatory complex	Open cage like structures with cholesterol and saponin.	Delivery of antigen to cytosol to induce cytotoxic T cell response

3.3.2 Epitopes

Lymphocytes recognize distinct sites on the macromolecule of antigen called epitopes or antigenic determinants. Epitopes are the active region of an immunogen that bind to lymphocytes. (Fig. 3.1).

Epitopes recognized by B and T cells

Protein antigens usually contain both sequential or non-sequential aminoacids.

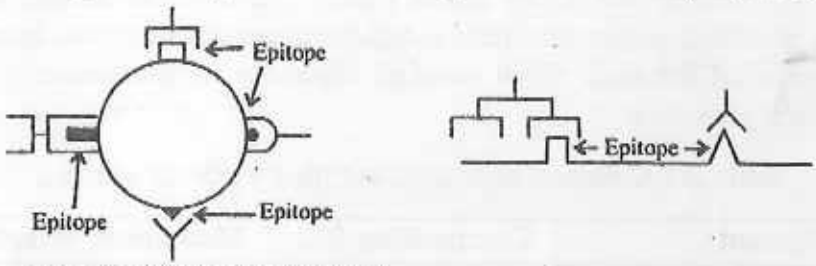


Fig. 3.1a. Different type of epitopes

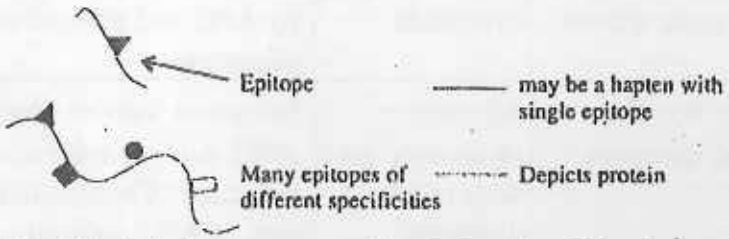


Fig. 3.1b. Antigenic structures containing single and multiple epitopes

Epitopes may be composed of sequential contiguous residues arranged in a sequence along the polypeptide chains or non-sequential residues from segments of the chain brought together by the folded confirmation of an antigen (Fig. 3.3). Normally the molecules at bends of α-helical regions are sequential (ex-sperm whale myoglobin) (Fig. 3.2). Spermwhale myoglobin also contains several non-sequential epitopes (or conformational types). Due to folding in tertiary protein structure this binding sites come close.

Table 3.2. Comparison of antigen recognition by T cells and B cells

Characteristics	B cells	T cells
1) Interaction with antigen	binary complex of immunoglobulin and antigen	Ternary complex T cell receptor, antigen and MHC molecule
2) Binding with soluble antigen	Yes	No
3) MHC Role	No	Yes
4) Chemical nature	Protein, Polysaccharide and Lipid	mostly protein
5) Property	Sequential or non sequential amino acid	Linear peptide by process-ed antigen and MHC molecule

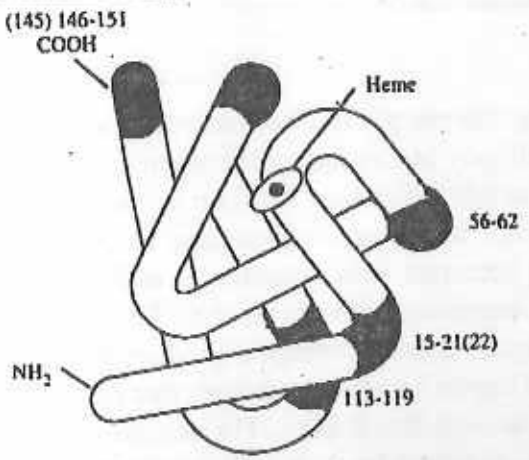


Fig. 3.2. Showing sperm whale myoglobin containing 5 linear B cell epitopes (black colour)

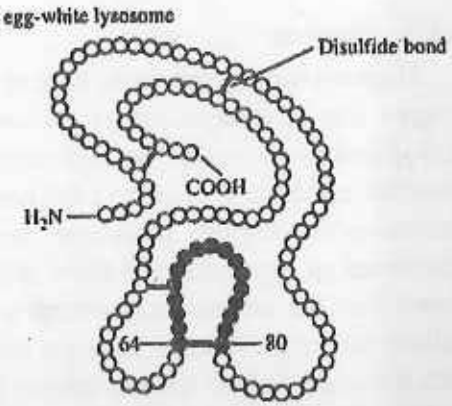


Fig. 3.3. Showing amino acid residues (circles) which forms a non-sequential epitope 'loop' (black colour) resulting from a disulfide bond between residues 64 and 80.

T cell epitope

Antigenic peptides recognized by T cells form trimolecular complexes with a T cell receptor and MHC molecule (Fig. 3.4).

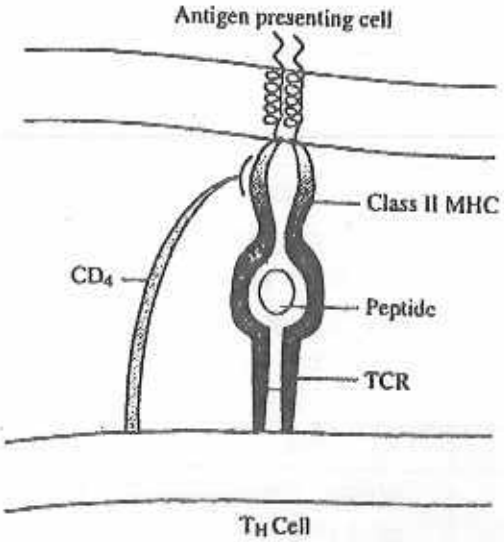
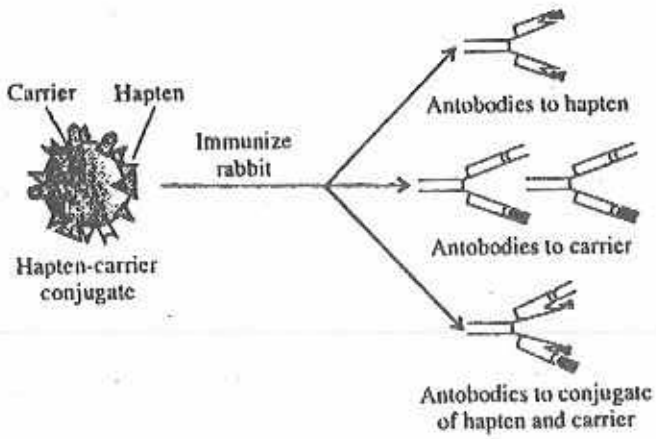


Fig. 3.4 Diagram of the ternary complex formed between a T cell receptor on a T_H cell, an antigen and a class II MHC mol.

Antigens that are recognized by T cells have two distinct interaction sites; an agrotope which interacts with a MHC molecule and an epitope which interacts with the T cell receptor

3.3.3 Hapten

Hapten (from greek word hapten meaning "no grasp") is a low molecular weight antigen that is recognised by preformed antibody but is not immunogenic unless conjugated to a 'carrier' or high molecular weight substance. The carrier molecule provides epitopes recognized by helper T cell. If carrier is conjugated to a non-immunogenic hapten molecule the latter becomes immunogenic. Landsteiner performed an experiment to show the role of hapten and carrier (fig. 3.5). The figure shows that the animal immunized with a hapten-carrier conjugate produced three distinct sets of antibodies. One set comprised hapten specific antibodies that reacted with the same hapten on any carrier as well as with free hapten. The second set of antibodies was specific for the carrier protein, as shown by their ability to bind both the hapten-modified and unmodified carrier protein. Lastly antibodies reacted only with specific conjugate of hapten and carrier used for immunization. This binding of haptens by antihapten antibodies is significant in defining the precision of antigen



Injection with	Antibodies formed
Hapten (DNP)	None
Protein carrier (BSA)	Anti-BSA
Hapten -carrier conjugate (DNP-BSA)	Anti-DNP (major) Anti-BSA (minor) Anti-DNP/BSA (minor)

Fig. 3.5. Landsteiner experiment to explain the role of hapten

binding by antibody molecules. Anti-hapten antibodies are important in medical science as they mediate allergic reactions (Type-1 Hypersensitivity reaction) to penicillin and other compounds which react with host protein to form a coupled hapten that can stimulate an antibody response.

Many biological substances like drugs, peptide hormones and steroid hormones can function as hapten. Conjugate of these haptens with large protein carrier can be used to produce hapten-specific antibodies. These antibodies are useful clinically for measuring the presence of various chemicals in our body.

A hapten-carrier conjugate with hapten (DNP-Dinitrophenol) chemically linked to a large protein carrier (BSA–Bovine serum albumin) is shown in Fig. 3.5. Immunization with DNP alone evokes no anti-DNP antibodies, but immunization with DNP-BSA combined elicits three predominant, indicating that in this reaction hapten is the immuno-deminant epitope is a hapten carrier conjugate.

3.4 Selective questions

(1) Define— Antigen, immunogen and show the difference between them.

(2) What is hapten and show its difference from carrier. Describe the Landsteiner's experiment on hapten-carrier conjugate.

(3) Define Adjuvaut. Discuss the role of different types of adjuvants.

(4) Explain the difference between sequential and conformational determinants with diagram.

3.5 Selected readings

(1) Abbas, A.K. and Lichtman, A.H. 2003. Cellular and Molecular Immunology, 5th edn. Elsevier Science, USA.

(2) Kuby J. 1999 Immunology 4th edn. Elsevier Publication, New York.

Unit 4 ❐ Complement System, MAC Mediated Lysis

Structure

4.1 Introduction

The complement system a component of the innate immune mechanism, comprises a group of more than 30 serum and cell surface proteins that interact with other immune system molecules in a highly regulated manner to provide many of the effector functions of humoral immunity and inflammation.

The name of the complement system derived from experiments performed by Charles Bordet shortly after the discovery of antibodies. He demonstrated if fresh serum containing an antibacterial antibody was added to the bacteria at a physiologic temperature (37^0C), the bacteria were lysed. If however the serum was heated to 56^0C or more, it lost its lytic property. As antibody is heat stable, so the heat labile factor that assists the reaction was named by Bordet as "complements" that enhances lytic function of antibodies.

4.2 Function of complement system

i) Certain activated complement components mediate **cytolysis** by polymerizing on cell surfaces to form pores by disrupting the integrity of the phospholipid bilayer in the membranes of these cells. Hence the microbes can be killed by **osmoticlysis.**

ii) **Opsonization** of foreign organisms or particles occurs by binding of complement proteins to their surfaces. These complement proteins are called **opsonins.** Phagocytic leukocytes express specific receptors for these opsonins. In this way, opsonins promote phagocytosis of particles or organisms.

iii) **Activation of inflammation** occurs in response to the generation of certain proteolytic fragments of complement proteins. These complement-derived peptides act on several targets. They activate mast cells, causing reactions that resemble immediate hypersensitivity; in extreme cases, this reaction can mimic

anaphylaxis and these complement fragments are sometimes called **anaphylotoxins.** Other targets of complement derived peptides include vascular endothelium, smooth muscle, and inflammatory leucocytes.

iv) The complement system promotes **solubilization and phagocytic clearance of immune complexes,** thereby minimizing the damage caused when immune complexes that are formed in the circulation deposit in tissues or vessel walls.

v) The complement system plays a significant role in **promoting humoral immune responses** by aiding in antigen presentation to B cells in germinal centres and by lowering the threshold of sensitivity of B cellls activation by antigens. These functions are mediated by receptors for complement fragments expressed on follicular dendritic cells and on B-lymphocytes. (Fig 4.1)

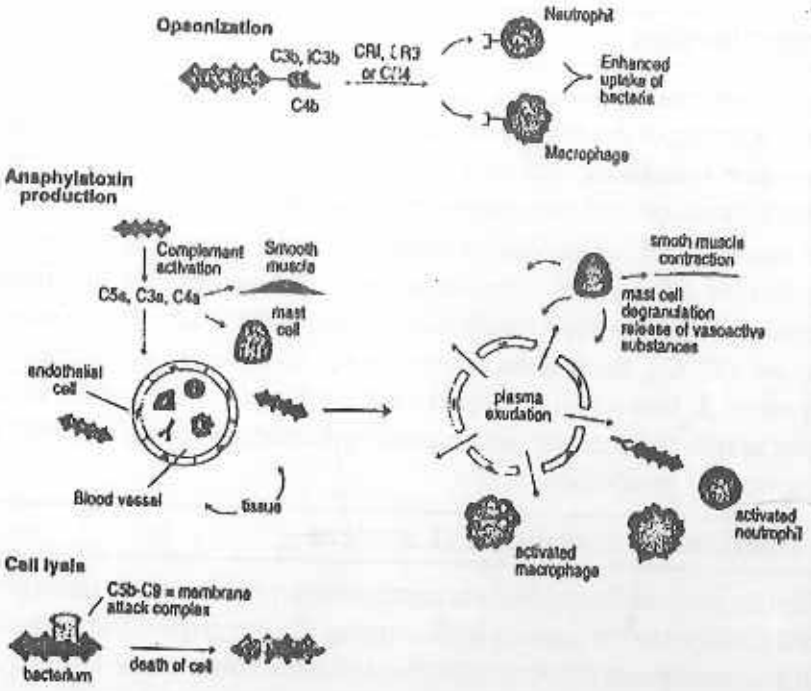


Fig. 4.1. Major functions of complement (1) : production of opsonins and anaphylatoxins, and cell lysis.

4.3 Properties of complement system

(1) The complement systems amplifies the response to microbes by means of an enzymatic cascade.

Proteolytic cascades allow for tremendous amplification, since each individual

enzyme molecule activated at one step can generate multiple activated enzymes or activated fragments, at the next step.

(2) Two converging pathways of complement activation co-exist, each one initiated by a specific set of stimuli, but both pathways share homologous molecules with similar functions. The pathways are :

a) alternative pathway, (Table 4.2 and Table 4.4)

b) classical pathway (Table 4.1 and 4.4)

Both pathways converge to a common final pathway that generates an assembly of proteins with cytolytic activity called the **membrane attack complex.** (Table 4.3).

(3) Although the components of the proteolytic cascades of the complement systems are present as soluble serum proteins, they are inactive or have only a low level of spontaneous activation in the circulation. Two mechanisms ensure that **initiation of complement cascades occurs only at certain sites and stay localized where it will be most useful.**

First, some complement components are only activated by binding to certain types of molecules that are present on the surfaces of infectious organisms but not on normal host cells.

Second, the requirement for immune complex for classical pathway initiation focuses complement activation to sites where specific antibodies bind to foreign antigens.

(4) The various biologic functions of the complement system are mediated in two general ways, First, complexes of complement proteins become directly bound to, and influence the fate of, microbes or immune complexes. Secondly, soluble fragments of complement proteins are generated during activation. These fragments diffuse from the sites where they are generated, bind to specific receptors or other nearby cells and thereby activate effector functions of those cells.

(5) The complement system is highly regulated by several soluble and cell membrane associated proteins that inhibit complement activation at multiple steps (Table 4.5). These regulatory mechanisms have two main functions. Firstly they limit or stop complement activation after the system has appropriately performed its functions. Secondly they prevent abnormal or constitutive complement activation in the absence of microbes and antibodies. Thus these regulatory mechanisms in effect enable the complement system to distinguish self from non-self and thereby prevent damage to normal tissues.

Classical Pathway :

Table 4.1 : Classical complement pathway : Proteins that participate in formation of C5 convertase

Component	Active protein/ split product	Immunologic function
C1 (6A+6B+ 6C+2r+2s)	C1q	Binds to Fc region of IgM/IgG
	C1r	serine protease : enzymatically activates C1S.
	C1s	Serine protease : enzymatically activates C4 & C2.
C4 ($\alpha + \beta + \gamma$)	C4a	Peptide mediator of inflammation (anaphylatoxin)
	C4b	Binds C2 forming complex that is cleaved by C1s to yield C4b2a.
C2 (α)	C2a	serine protease : C4b2a acts as C3 converstase
	C2b	? (unknown function)
C3 ($\alpha + \beta$)	C3a	Peptide mediator of inflammation (anaphylatoxin)
	C3b	Binds to C4b2a to form C4b2a3b (C5 convertase); major opsonin.

Alternative Pathway :

Table 4.2 : Alternative complement pathway : Proteins that participate in formation of C5 converstase

Component	Active protein / Split product	Immunologic function
C3 ($\alpha + \beta$)	C3a	Peptide mediator of inflammation (anaphylatoxin)
	C3b	Binds factor B, forming complex that is cleaved by factor D to yeield C3bBb
Factor B (α)	Ba	? (unknown function)
	Bb	serine potease : (C3bBb acts as C3 covertase, which generatee (C3B Bb 3b) (C5 convertase).
Factor D (α)	D	Serine protease : cleaves factor B that is bound to C3b to form C3 convertase.
	C3	
Properdin (α_4)		Binds to and stabilizes C3bBb (C3 convertase).

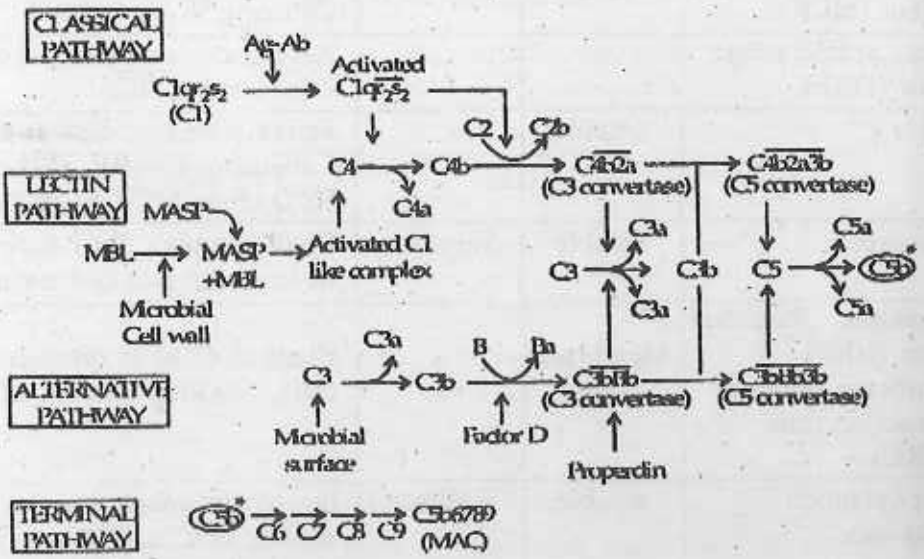
Terminal Complement Pathway :

Table 4.3 : Terminal complement pathway: Proteins involved in the formation of the Membrane Attack Complex (MAC)

Component	Active protein / Split product	Immunologic function
C5 ($\alpha + \beta$)	C5a	Peptide mediator of chemotaxis and inflammation (anaphylatoxin)
	C5b	Binds C6 to initiate formation of MAC
C6 (α)	C6	Bind to C5b to form C5b6; binds C7
C7	C7 (α)	After an amphophilic transition of C5b67, the resulting complex inserts into the lipid bilayer.
C8 ($\alpha + \beta + \gamma$)	C8	C5b678 binds multiple C9 molecules, initiating their polymerization
C9	C9	polymerizes to complete formation of MAC pore.

Combined Pathway :

Table 4.4 : Combined Pathway



In all the tables shown in text the complexes with eazymatic activity are designated by a bar over the number or symbol like C $\overline{4b2a}$, $\overline{C3bBb}$.

4.4 Regulation of complement system

Table. 4.5 Proteins that regulate complement system

Protein	Type	Pathway affected	Immunologic function
C1 inhibitor (C1 inh)	soluble	classical	Serine protease inhibitor ; Causes $C1r_2s_2$ to dissociate from C1q
C4b binding Protein (C4b BP)	,,	classical & lectin	Blocks formation of C3 convertase by binding C4b ; cofactor for cleavage of C4b by factor I
Factor H	,,	Alternative	Blocks formation of C3 convertase by binding C3b; co-factor for cleavage of C3b by factor I
Complement receptor (CRI) type I membrane co-factor protein (MCP)	Membrane-bound	all	Block formation of C3 convertase by binding C4b or C3b; co-factor for cleavage of C4b or C3b by factor I
Decay accelerating factor (DAF)	,,	,,	Accelerates dissociation of $\overline{C4b2a}$ and $\overline{C3bBb}$
Factor I	soluble	,,	Serine protease; cleaves C4b & C3b using C4bBP, CRI, factor H, DAF or MCF as co-factor
S-protein	soluble	Terminal	Binds soluble C5b67 & prevents its insertion into cell membrane
Homologus Restriction factor (HRF) Membrane Inhibitor of reactive lysis (MIRL)	Membrane bound	,,	Binds to C5b678 on autologous cells, blocking binding of C9
Anaphylatoxin inactivator	soluble	Effector	Inactivates anaphylatoxin activity of C3a, C4a and C5a by carboxy peptidase N removal of C-terminal Arginine

4.5 MCA mediate lysis

The terminal pathway of complement activation polymerize to form pores in the membrane of the pathogen (Table 4.3). The membrane attack complex start with action of C5 convertase on C5. C5 is cleaved to form C5b and C5a. Subsequently C5b the initiator of the complex binds to C6 and the C5b, 6 complex then binds to C7. The hydrophobic domain of C7 acts on lipid bilayer of the pathogen.C567 is bound with C8 and C9. The complex protein of all these components induces polymerization into the annular or ringstructure on the membrane and is called membrane-attack complex. The process leads to complete lysis of the pathogen membrane (fig. 4.2). The final step in formation of the complex is the terminal molecule C9 binding and polymerization. C9 is a perforin like chemical and can move into the pore created in membrane by hydrophilic and amphiphilic property. The free flow of MAC chemical through the pore destroys the osmotic balance of the cell and damage it completely.

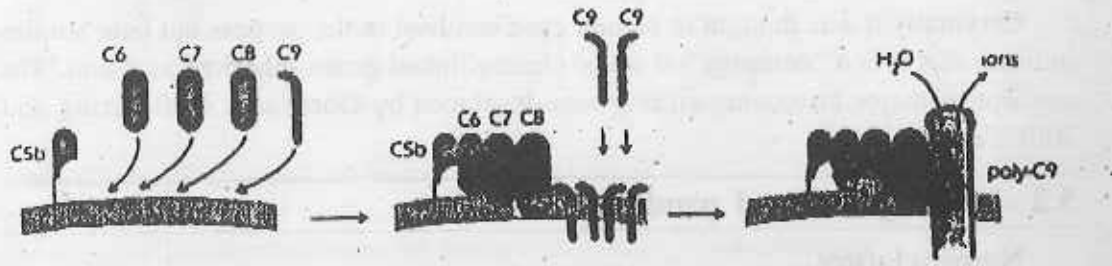


Fig. 4.2. Formation of the membrane attack complex. Late stage complement components C5b-C9 bind sequentially to form a complex on the cell surface. Multiple C9 components bind to this complex and polymerize to form poly-C9, creating a channel that disrupts the cell membrane.

4.6. Selective questions

(1) Mention the functions of the complement system.
(2) With a schematic diagram show the relation between classical and alternative complement pathways. Mention the role of different factors (components) of the complement.
(3) Describe the membrane–attack complex process involving three major pathways.

4.7 Selected readings

(1) Abbas, A.K. and Lichtman, A.H. 2003 **Cellular and Molecular Immunology,** 5th edn. Elsevier Science, USA.
(2) Janeway, C.A., Travers, P., Walport, M and Capra J.D. 1999 **Immuno Biology** 4th edn. current Biology Publication, New York.

Unit 5 ❐ Structure of MHC (elemenatary idea)

Structure

5.1 Introduction

The term major histocompatibility complex is arrived at from research oriented for acceptance or rejection of tissues-literally **histocompatibility**-transplanted between different members of the same species.

Originally it was thought to be one gene involved in the process but later studies indicate that it is a "complex"- a set of closely linked genes inherited as a unit. The concept of major histocompatibility was developed by Gorer and Snell during mid 20th Century.

5.2 MHC genes and products

Nomenclature

Human chorosome 6 contain human MHC, known as HLA (human leucocyte antigen). Names of other species for example are BOLA for bovine system, SLA for swine. The name of mouse MHC is H-2 locus located on chromosome 17.

Two major sets of MHC genes, known as **MHC class I** and **MHC class II** and their cell-surface expressed products are involved in T cell responses. The three independent genes that code for human class I MHC molecule are called HLA-A, HLA-B and HLA-C. The MHC class II molecules are obtained from 3 sets of genes --HLA-DP, HLA-DQ and HLA-DR.

Each MHC Class II subregion contains an A & B gene that code for a chain, α or β respectively of a two chain MHC class II molecule. Thus HLA-DPA gene codes for DPα of the DP molecule and HLA -DPB gene codes for the other chain DP-β of the HLA-DP molecule.

Mouse MHC Class I molecules are coded by K, D & L gene H2 has 2 MHC class II regions-rather than 3 in humans known as I-A, I-E that code for I-A α β and I-E α β molecules respectively.

Pattern of MHC molecules expression in different cells

MHC Class I molecules are expressed at varying levels on almost every nucleated cell in the body. MHC II molecules have a more limited distribution than class I molecule. They are present on Antigen presenting cell (APC).

Expression of MHC-I & MHC II molecules are coordinate, in that all molecules of each class can be expressed on the cell surface at the same time. They are however under distinct control.

5.3 Structure of MHC molecules

The simplified structure of MHC I and MHC II are shown in Fig. 5

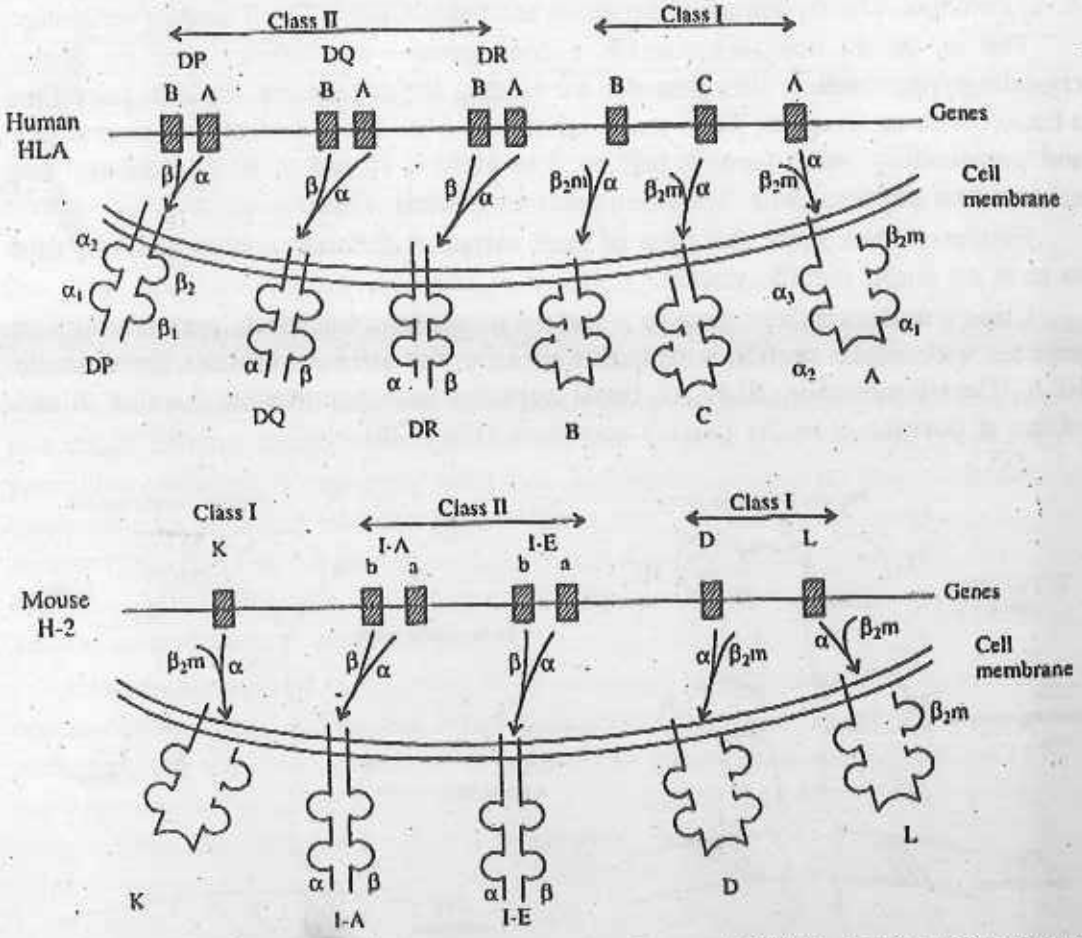


Fig.5.1 a and b Simplified depiction of the human (A) and mouse (B) MHC, showing regions & genes coding for polymorphic MHC Class I and Class II molecules. β_2m = β_2 microglobulin, encoded outside MHC.

5.3.1 Structure of MHC Class I molecules

Each Class I-gene codes for a transmembrane glycoprotein of approximate molecular weight 43 K Da, which is referred to as α, or heavy chain. It has 3 extra cellular domains α_1, α_2, α_3. It is expressed at the surface of a cell in covalent association with a small invariant polypeptide called β_2 microglobulin (β_2 m; molecular weight 12 K Da). β_2 m is coded by another chromosome and has a domain analogous to a single Ig domain.

At the cell surface, MHC I & β_2 m appear as a 4-domain molecule with α_3 & β_2m justaposed closest to the membrane.

The sequence differences between different Class-I molecules is restricted to α_1 & α_2 domains. The α_3 domain is invariant and binds CD8, a T-cell surface molecule.

The α_1 & α_2 domains contain a deep groove or cleft as seen by X-ray crystallographic studies. This groove in the binding site of peptides. the cleft resembles a basket with an irregular floor, made up of amino acid in a β plated sheet structure and surrounding walls form α helices. The cleft is closed at both ends and can accomodate peptides with 8-9 aminoacids in a linear way.

Further studies show that floor of each variant is different and thus can be said to have an allele specific pocket.

Class I molecules can bind to a variety of peptides but binds preferentially to peptides with certain **motifs.** Such motifs are known as **anchor residues.** For example HLA Class-I molecule HLA-A2 binds peptides with peptides at position 2 and valine at position 9 in the peptide sequence. (Fig 5.2b).

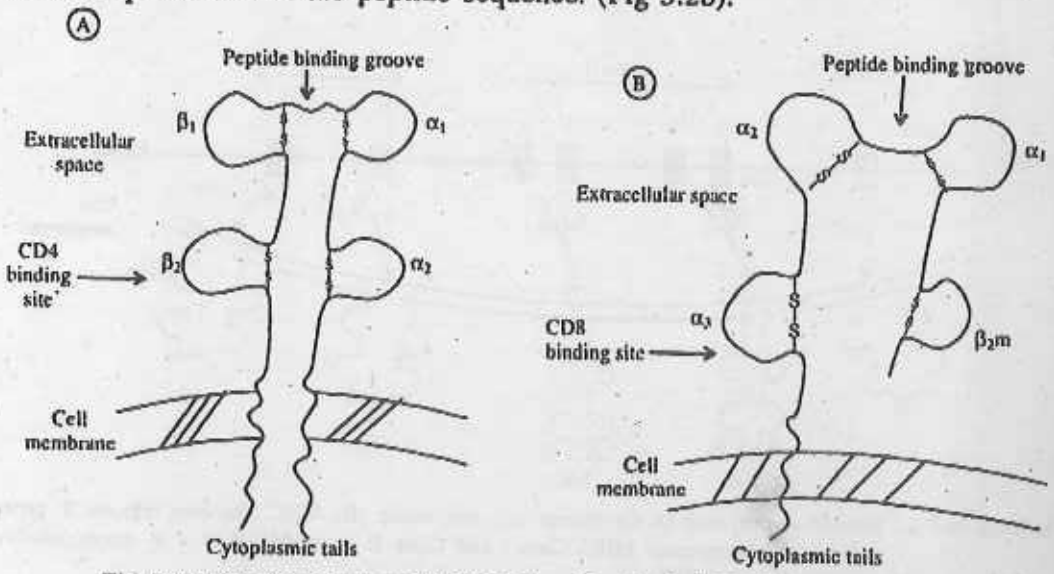


Fig. 5.2A & B. Depictions of (A) MHC Class II molecule (B) MHC Class-I molecule.

5.3.2 Structure of MHC class II molecule

MHC Clas-II α & β genes code for chains of approximate molecular weight 35,000 Da respectively. Each chain is a transmembrane glycoprotein molecule with cytoplasmic tails and extracellular Ig-like domains, the domains referred to as α_1, α_2 and $\beta_1$and β_2. It is made up of variable or polymorphic regions α_1 and β_2 and invariant or nonpolymorphic regions α_2 and β_2. The T-cell molecule CD4 binds to the invariant region.

The peptide binding groove of MHC II molecule in formed by interaction between domains of different chains, the α_1 and β_1 domain. The floor consists of 8 β-pleated sheets with each α_1 and β_1 contributing 4 each. The groove is open at both ends, allowing larger peptides to bind. The MHC Class-I binds peptide varying in length from 12 to approximately 17 aminoacids in a linear array.

Peptide binding to MHC Class II also exhibit motifs. Because the length of peptides are variable the motif is generally seen in the central region of the peptide, the region that fits inside the MHC class-II binding groove. (Fig. 5.2A).

5.4 Function of MHC molecules

MHC Class I : (1) Associated with antigenic peptides of infecting pathogens produced within the host cell.

(2) Vesicular transport of MHC-I peptide complex to the infected cells membrane.

(3) Interaction with TCR of Tc cells; co-induction by CD8 molecule.

(4) Activation of Tc cell and destruction of infected cell.

MHC-Class II : (1) Vescicular transport towards endolysome.

(2) Fragmentation of foreign peptides by lysomal enzymes present in endolysome; peptides derives from phagocytosed pathogen.

(3) Association of MHC-Class II with foreign peptide.

(4) vescicular transport to cell membrane and expression/ presentation of foreign peptide MHC II complex.

(5) Association with TCR of T_H cells; co-induction of T_H cells with CD4 molucules.

(6) Secretion of cytokines by TH cells, recruitment of macrophages, NK cells & Tc cells to infection site and formation of their effector population.

5.5 Selective questions

(1) Draw and describe class I MHC and class II MHC molecule.
(2) Write notes on :
   (a) Features of class I MHC and class II MHC
   (b) Function of two types of MHC molecules.
   (c) Pattern of MHC molecule expression in different cells.

5.6 Selected readings

Kuby, J. 1999 Immunology 4th edn. Elsevier publication, New york.

Unit 6 ❐ Structural Diversity of Immunoglobulin

Structure

6.1 Introduction

Immunoglobulins function as antibodies, the antigen binding proteins that are present on the B cell membrane and also are secreted by plasma cell. The class of an immunoglobulin molecule is determined by its heavy chain (isotype). They are classified as IgM, IgG, IgD, IgE and IgA. The antigenic specificity of each B cell is determined by the membrane bound antigen binding receptor of antibody expressed by the cell. The antibody on a B lymphoblast can recognize epitopes on macromolecules with incredible precision. Protein antigens that differ by only a single aminoacid often can be distinguished from each other.

The amazing feature of the vertebrate immune system is its ability to respond to an apparently unlimited number of foreign antigens. It has been estimated at the present situation that an individual can build up B (and T) cells with different antigenic specificities upto range of 10^{15} to 10^{18} times. The genome of the individual contain only less number of genes e.g 30,000 to 40,000 approximately. This diversity is generated due to participation of multiple germ like gene segments generating antibody along with some special arrangements and procedures. It may also be noted here that this maturation process and also incase of T lymphocyte it is accompanied with another selection process by which self reactive antibodies and T cells are all eliminated. This process develops **tolerance** or nonresponsiveness towards body cell antigen.

6.2 Points of diversity

Multiple gene segments code for antibody structure. Light chains are composed of Kappa (κ) and Lamda (λ) chain and heavy chains are determined by H chains. The structure of the constant region determines whether κ or λ arrangement will be taken in light chain. The κ or λ light chains and the heavy chains are encoded by separate multigene families situated on different chromosome. The arrangement of genes in Mouse are in 6, 16, 12 and in Human are 2, 22 and 14 respectively for K,

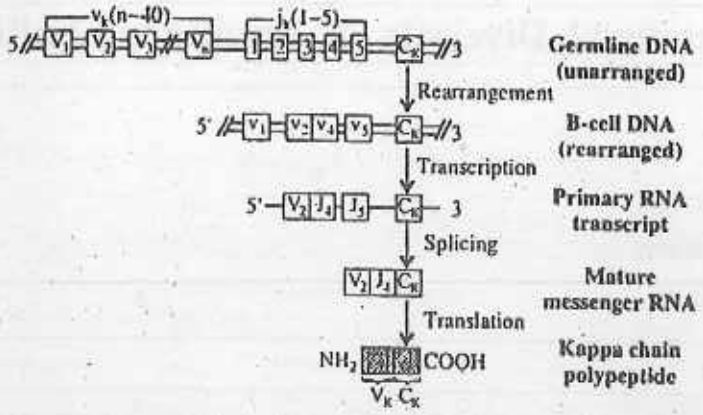


Fig. 6.1. The genetic events leading to the synthesis of a kappa light chain.

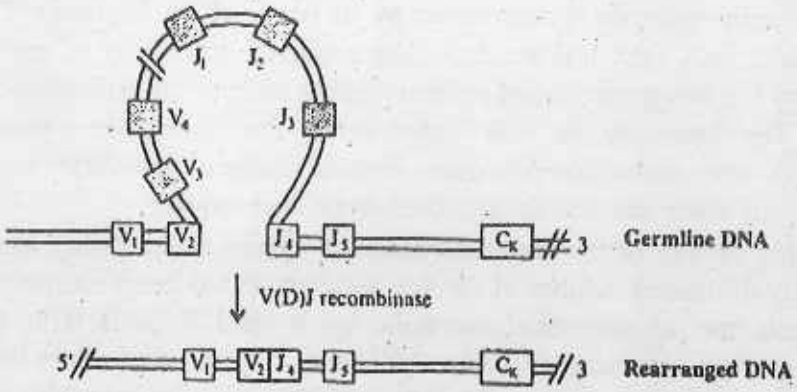


Fig. 6.2. Rearrangement of DNA coding for a kappa light chain

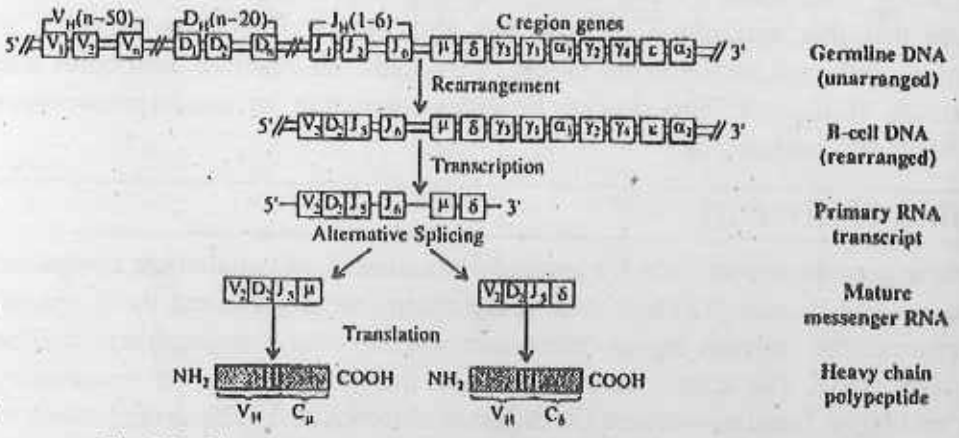


Fig. 6.3. The genetic events leading to the synthesis of a human heavy chain

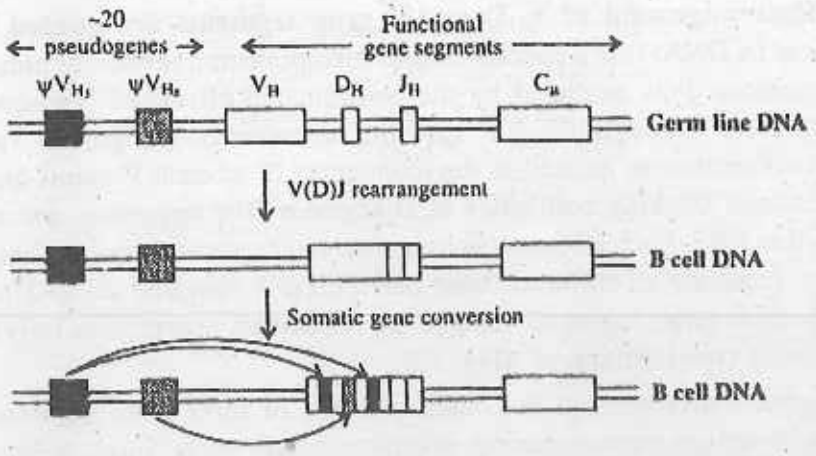


Fig. 6.4. Somatic gene conversion generates diversity in Ig genes of several species. The figure illustrates the phenomenon in the chicken Ig heavy-chain locus; short sequences of DNA from one or more pseudogenes (3 and 8 in the figure) are copied into the rearranged B-cell VDJ unit.

λ and H chains. In germ line DNA there are multigene families--gene segments (exons) which are separated by noncoding introns (silent). The segment arrangement of light chains are VJC segment and in heavy chain VDJ for variable region and C for constant region (Fig. 6.1, 6.2, 6.3, 6.4). The process of anitbody structure formation is same as in any other peptide chain formation during protein synthesis like; germline DNA–B cell DNA–Primary transcript–Transcription–primary transcript splicing–m RNA formation–Translation–protein (Peptide) chain formation according to leader sequence.

The rearrangement of Ig gene and its subsequent diversified structure is achieved by several means. (1) Compete V regions are generated by the somatic recombination of separate gene segments as shown in diagram. (2) V region genes are present in multiple copies. According to Janeway *et al* (2005) the segments for light and heavy chains are as in Table 6.1.

Number of functional gene segments in human immunoglobulin

Table. 6.1 A glimpse of different segment

Segment	Light chain		Heavy chain
	K	λ	H
Variable (V)	40	30	65
Diversity (D)	0	0	27
Joining (J)	5	4	6

(3) **Rearrangement of V, D and J gene segments are guided by flanking sequences in DNA.** It is a special kind of recombination involving non-homologous gene segments. It is mediated by the co-ordinated effects of lymphocyte specific recombinases. The lymphocyte specific recombinas recognizes specific DNA recognition sequences located in the intervening 3′ of each V exons and 5′ of each J segment and flanking both sides of D segment. The sequences are one turn RSS and two turn RSS. Each RSS contains a conserved heptamer sequence and a conserved nonamer sequence of different base pair (12bp 1 turn and 23bp-2 turn). During rearrangement gene segments flanked by a one-turn spacer join only to segments flanked by a two turn spacer. (Fig. 6.5).

The gene rearrangement that combines two or three gene segments to form a complete V-region exon generates diversity in two ways. First, there are multiple different copies of each type of gene segment, and different combinations of gene segments can be used in different rearrangement procedures. This is called **combinatorial diversity.** Secondly **Junctional diversity** is introduced at the joints between the different gene segments as a result of addition and subtraction of nucleotides by the recombination process. A third source of diversity is also combinatorial, arising from the many possible different combinations of heavy and light chain V-regions that pair to form that antigen binding site in the immunoglobulin molecule. The two means of generating combinatorial diversity alone could give rise to more than 10^6 different antibody molecules. Those two combinatorial diversity along with junctional diversity can lead to total receptors of 10^{11} different types that form the repertoire of receptors of B cell. Finally somatic hypermutation introduces point mutation into the rearranged V-region genes of activated B cells creating further diversity.

The steps can be elaborated in more detail as mentioned below.

The ultimate antibody diversity is generated by four main process three of which are consequences of the process of recombination and the last one is a mutational process.

(1) **Inherited gene segments are used in different combinations–** The V,D,J gene segments have multiple copies for combination. For human K light chain there are approximately 40 functional V_k gene segments and 5 J_k gene segments and so there is scope for 200 different V_k regions. For λ light chain there are approximately 30 functional V_λ gene segments and 4 J_λ gene segment, yielding 120 possible V_λ regions. So in all, 320 different light chains can be made. Similarly for heavy chains in human there are 65 functional V_H gene segments, approximately 27 D_H gene segments and 6 J_H gene segments, and thus around 11,000 different possible V_H

region (65×27×6 is round 11,000). The ability to create different combinations of a small number of gene segments is known as **combinatorial diversity**. The gene segments which do not take part in encoding protein are called Pseudogenes.

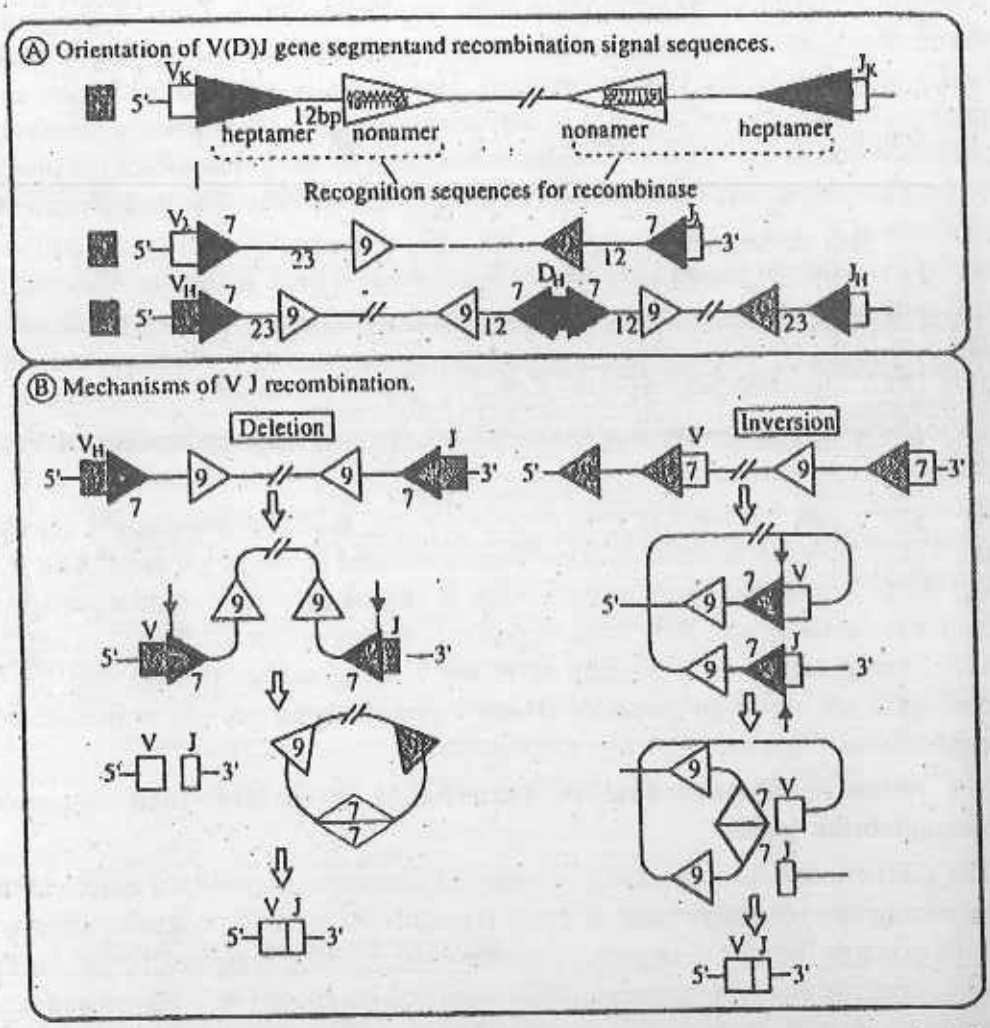


Fig. 6.5. V(D)J recombination. The DNA sequences and mechanisms involved in recombination in the Ig gene loci are depicted. The same sequences and mechanisms apply to recombinations in the TCR loci.
A. Conserved heptamer (7 bp) and nonamer (9 bp) sequence, separated by 12 or 23-bp spacers are located adjacent to V and J exons (for κ and λ loci) or V, D, and J exons (n the H chain locus). The V(D)J recombination recognizes these recombination signal sequenses and brings the exons together.
B. Recombination of V and J exons may occur by deletion of intervening DNA and ligation of the V and J segments (*left panel*) or, if the V gene is in the opposite orientation, by inversion of the DNA followed by ligation of adjacent gene segments (*right panel*). Arrows indicate the sites where germline sequences are cleaved and later rejoined.

(2) Variable addition and subtraction of nucleotides at the junctions between the gene segments encoding the V region contributes to diversity in the third hypervariable region (CDR3)

The third region of hypervariable light and heavy chain falls at the junction between the V gene segment and the J gene segment and in the heavy chain is partially encoded by the D gene segment. The diversity of the third hypervariable segment. The diversity of the third hypervariable region is significantly increased by the addition and deletion of nucleotides at two steps in the formation of the junctions between gene segments. This is known as **junctional diversity**. The added nucleotides are known as P- nucleotides and N-nucleotides. (Fig. 6.6). P-nucleotides sequences make up palindromic sequences added to the end of the gene segments, N-nucleotides are so called because they are non-template-encoded. They are added by an enzyme called terminal deoxy-nucleotidyl transferase (TdT) to single stranded ends of the coding DNA after hairpin, cleavage (see Fig. 6.6).

(3) Specialized enzymes are required for somatic recombination of V gene segments

Two genes that stimulate Ig gene recombination, called recombination activating genes 1 & 2 (RAG-1 & RAG-2) have been identified in the pre B cells. The RAG-1 and RAG-2 proteins form a dimer that is responsible for lymphocyte-specific recombinase activity and they have important properties like (i) They are cell-type specific, being acting only in cells of B and T lymphocytes lineage and (ii) The recombinase are active in imnature B and T lymphocytes but not in mature cells. (Fig.6.6).

(4) Somatic hypermutation introduces diversity into expressed immunoglobulin genes

The mechanisms for generating diversity as discussed in previous points all take place during the rearrangement of gene segments in the initial development of B cells in primary lymphoid organs. However in secondary lymphoid organ there is an additional mechanism that generates diversity throughout the V region and which operates on B cells in secondary lymphoid organs after functional antibody genes have been assembled. This process is known as **somatic hypermutation** and it introduces point mutations into the V region of the rearranged heavy and light chain genes at a very high rate giving rise to mutant immunoglobulin molecules on the surface of the B cell. The antibody diversity in adult individual is mainly derived from somatic hypernutations acquired during life time. This system of heritable and acquired components of diversity are present in mammal. Whereas Birds do not use somatic recombination or hypermutation to maintain diversity, but create their

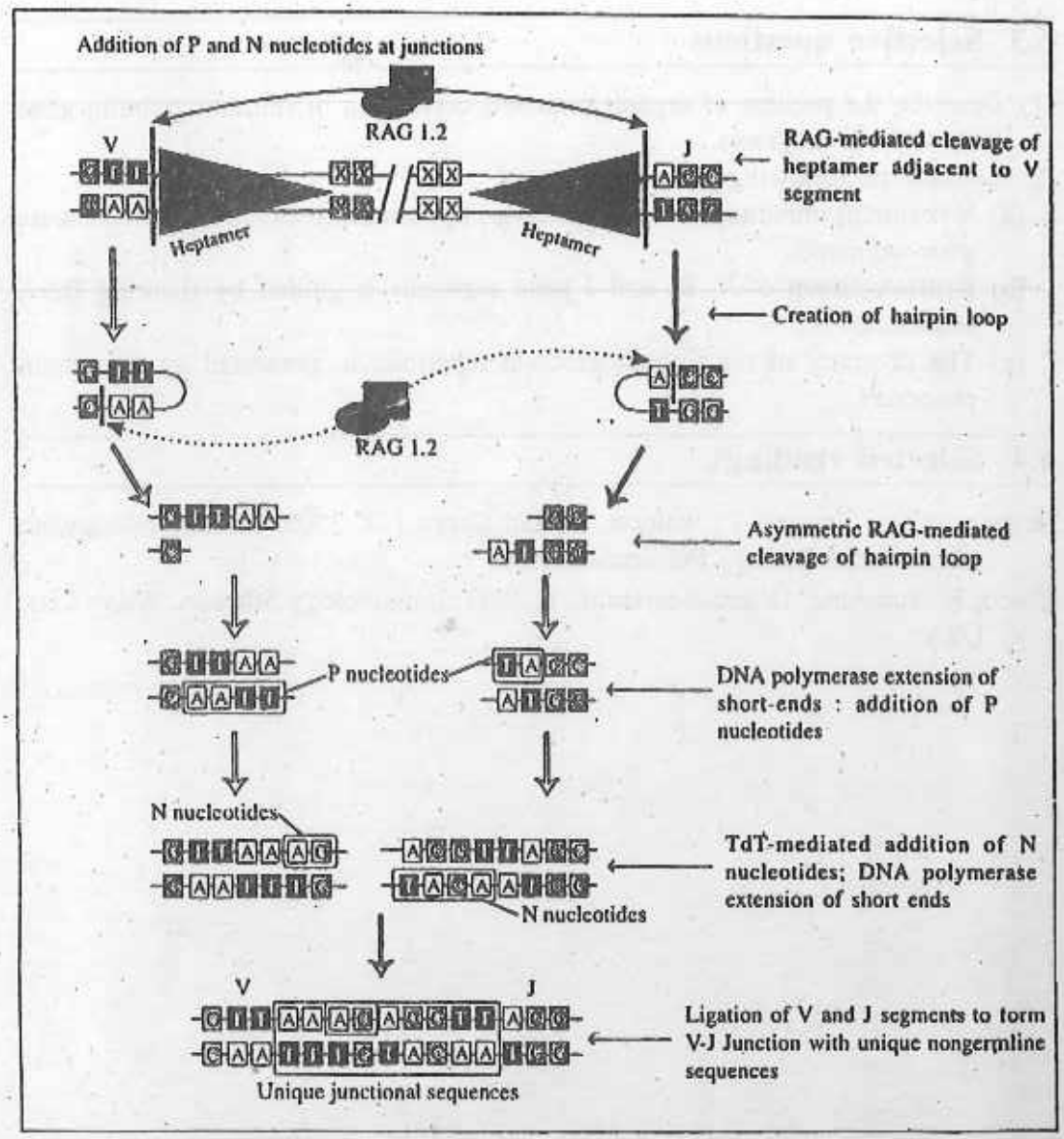


Fig. 6.6. Nucleotides (P sequences) may be added to broken DNA ends to repair asymmetric breaks. Other nucleotides (N regions) may be added to the sites of VD, VJ, or DJ junctions by the action of the enzyme terminal deoxyribonucleotidyl transferase (TdT), generating new sequences that are not present in the germline.

antibody pool gene conversion from germline pseudogenes. So at the end it can be presumed that the combination of all these sources of diversity creates a vast resource of antibody specificity from a limited number of genes.

6.3 Selective questions

(1) Describe the process of organization and expression of immunoglobulin gene with suitable diagrams.

(2) Explain the following points :

(a) V region of immunoglobulin is generated by somatic recombination of separate gene segments.

(b) Rearrangement of V, D, and J gene segments is guided by flanking DNA sequences.

(c) The diversity of the immunoglobulin repertoire is generated by four main processes.

6.4 Selected readings

Janeway, C.A., Travers, P., walport, M and Capra J.D. 2005. Immunobiology,6th edn..Current Biology Publication, N.Y.

Coico, R. Sunshine, G and Benjamini, E 2003. Immunology 5th edn. Wiley Liss. USA

Unit 7 ❐ Hypersensitivity

Structure

7.1 Introduction

Immune response to antigen or allergen with hightened or inappropriate nature upon reexposure is called Hypersensitivity. The reactions are broadly considered as of four types.

7.2 Type I hypersensitive reaction (IgE-mediated hypersensitivity)

It is induced by certain type of antigens, referred to as allergens. The term allergen refers specifically to non-parasitic antigens capable of stimulating type I responses in allergic individuals.

The IgE class of antibody binds with high affinity to Fc receptor on the surface of tissue mast cells and blood basophils and are said to be sensitized. A later exposure to the same allergen crosslinks the membrane-bound IgE on sensitized mast cells & basophils, causing degranulation of these cell. The pharmacologically active mediators released from the granules act on the surrounding tissues. The principal effects are-vasodilation and smooth muscle contraction. It may be either systemic or localized, depending on the extent of mediator release.

7.2.1 Common allergens of this type of reaction.

1) **Protein**
   Foreign serum
   vaccines
2) **Plant pollens**
   Rye grass
   Ragwood
   Timothy grass
   Birch trees
3) **Drugs**
   Penicillin
   Sulfonamides
   Local anesthetics
   Salicylates
4) **Food**
   Nuts
   Sea foods
   Eggs
   Pea, Beans & Milk
5) **Insect products**
   Bee venom
   Wasp venom
   Ant Venom
   Cockroach calyx
   Dust mites
6) Mold spores
7) Animal hair & dander

7.2.2 Components of Type I reactions

(1) Allergens (2) Reaginic Antibody (IgE) (3) Mast Cells & Basophils

The reaginic activity of IgE depends on its ability to bind to a receptor specific for the Fc region of the ε chain e.g. FcεRI and Fcε R II.

Mast cells and Basophils express FcεRI, the high affinity IgE receptor. This helps to bind IgE despite its low concentration (1×10^{-7}/M).

7.2.3 Structure of FcεRI (Fig. 7.1a)

(1) FcεRI contains 4 polypeptide chains : an 'α' & a 'β' chain and 2 identical disulphide-linked 'γ' chain.

(2) The external region of the 'α' chain contains 290 aa domains that exhibit homology with Ig domain. The domains interact with C_H3/C_H3 & C_H4/C_H4.

(3) The β chain spans the plasma membrane four times and is thought to link the α, chain to the γ homodimer.

(4) The 2 γ chain are **disulfide linked** & extend a considerable distance into the cytoplasm.

(5) Each γ chain has a conserved sequence in its cytosolic domain known as an **immunoreceptor hyrosine-based activation motif** (ITAM).

(6) Allergen mediated crosslinkage of the bound IgE results in aggregation of the FcεRI receptor & rapid tyrosine phosphorylation, which initiates the process of mast-cell degranulation.

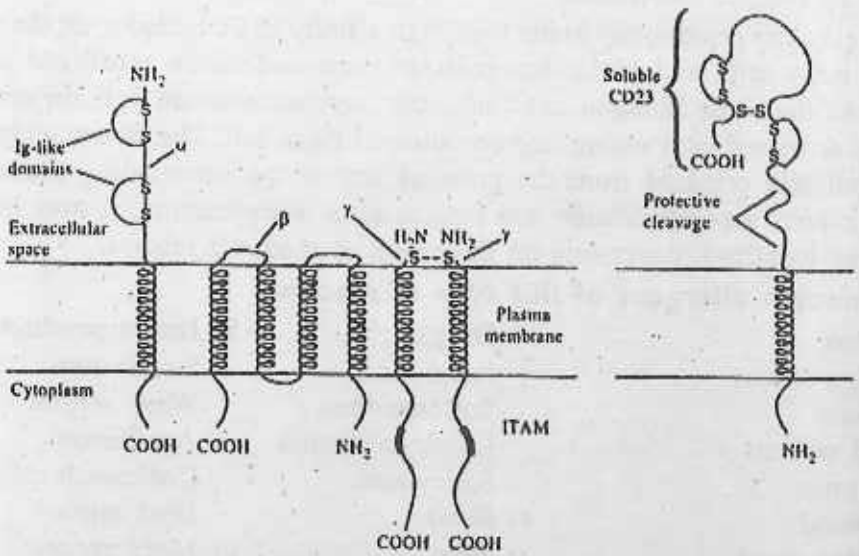


Fig. 7.1. Schematic diagrams of the high affinity FcεRI and low affinity FcεRII receptors that bind the Fc region of IgE. Fig. 7.1(a) shows active ITAM motif 7.1(b) shows NH_2 terminal towards cell internal space and COOH terminal towards extracellular space.

7.2.4 Structure of Fc ε RII/CD 23) (Fig. 7.1b)

(1) It has a lower affinity for IgE & specific for C_H3/C_H3 domain of the IgE

(2) Consists of a single polypeptide with a large extracellular domain, a single transmembrane domain & a short cytoplasmic tail.

(3) An important character is that the c-terminus of the polypeptide is extracellular & the N-terminus is cytosolic.

(4) A soluble form of $F_C\varepsilon RII$ (CD 23) may be generaed by autoproteolysis of the membrane receptor.

(5) IG-E crosslinking Fc ε RII has been shown to activate B-cells, alveolar macrophages & eosinophils; the soluble form has been shown to enhance Ig-E production by B cells.

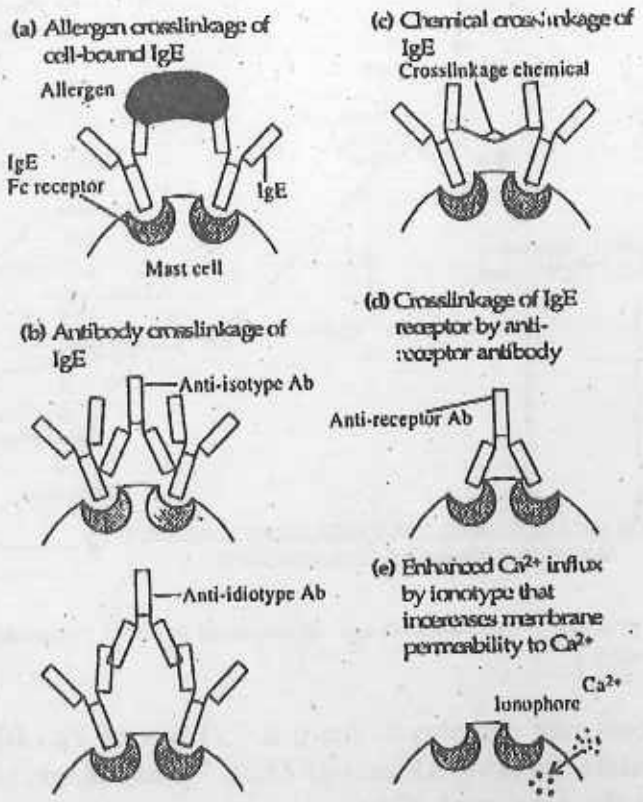


Fig. 7.2. Schematic diagrams of the mechanisms that can trigger degranulation of mast cells. Note that mechanims (b) and (c) not require allergen; mechanisms (d) and (e) require neither allergen *nor* IgE and mechanism (e) does not even require receptor crosslinkage.

7.2.5 Mechanism of Ig-E mediated degranulation

(1) **Receptor crosslinkage :** IgE mediated degranulation begins when an allergen crosslinks Ig-E that is bound to the Fc receptor on the surface of a mast cell or basophil. (fig. 7.2) Crosslinking can be mediated by (a) polyvalent allergen; (b) anti-isolypic or antiidiotypic antibody (c) Crosslinking Chemicals, (d) Antirecptor antibody, (e) influx of Ca^{+2} ions.

Note : The importance of crosslinkage is indicated by the inability of monovalent allergens, which cannot crosslink the fixed Ig-E, to trigger degranulation.

(2) **Intracellular events :** The events are shown in Chart 7.1

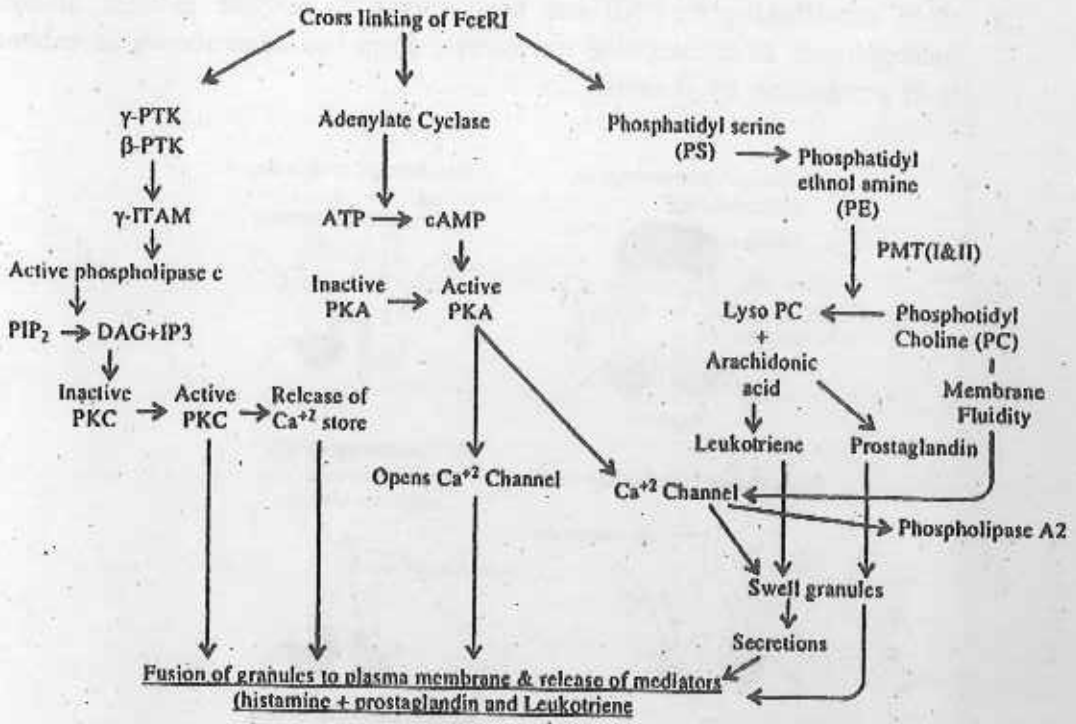


Chart 7.1 Fusion of granules to plasma membrane & release of mediaors (histamine + prostaglandin and Leukotriene)

(i) The cytoplasmic domains of the β & γ chains of **Fc ε RI are associated with protein tyrosine kinases (PTKs), Crosslinkage** of FcεRI receptor **activates** the associated PTKs.

(ii) **This results in phosphorylation of tyrosines with the ITAMs of the γ as well as phospholipase C.**

(iii) Phospholipase C converts phosphatidylinositol 4-5 bisphosphate (PIP_2) into diacylglycerol (DAG)& inositol triphosphate (IP_3)

(iv) DAG activate protein kinase C (PKC); IP_3 mobilizes intra cellular Ca^{+2} stores; PKC & Ca^{+2} is necessary for microtubulor assembly & fusion of granules with plasma membrane.

(v) Crosslinkage of FcεRI activates an enzyme that converts Phosphatidyl serine (PS) to Phosphatidyl ethanolanine (PE). PE is methylated to Phosphatidyl chohine (PC) by enzymes Phospholipid methyl transferase I (PMTI) & PMTII. Accumulation of PC cause an increase in membrane fluidly & faciliated the formation Ca^{+2} channels.

(vi) Influx of Ca^{+2} activates Phospholipale A2; this promotes breakdown of PC to lysophosphatidyl choline (lysoPC) & arachidonic acid.

(vii) Arachidonic acid is converted into potent mediators; the leukotrienes & prostaglandin D_2.

(viii) Crosslinking also activates adenylate cyclase, leading to increase in c-AMP.

(ix) c-AMP dependant protein kinases are thought to phosphorylate the granule memrane proteins, changing its pemeability to water & Ca^{+2}.

(x) The consequent swelling facilitates fusion with the plasma membrane & release of mediators.

7.2.6 Mediators of Type-I reactions

The clinical manifestations of type I hypersensitive reactions are related to the biological effects of the mediators released during mast cell or basophil degranulation.

The mediators can be classified as either primary or secondary. The primary mediators are produced before degranulation and stored in the granules. The secondary mediators either are synthesized after target cell activation or are released by the breakdown of membrane phospholipids during the degranulation process.

Leukotrienes and Prostaglandins :

They are not formed until the mast cell degranulates and enzymaic breakdown of phospholipids in the plasma membrane takes place. Their effects are more pronounced and longer lasting than that of histamine.

Leukotrienes mediate broncho constriction, increased vascular permeability and mucous production.

Prostaglandin D2 causes bronchoconstriction.

Cytokines :

Human mast cells secrete IL-4, IL-5, IL-6 and TNF-α. These cytokines alter the

local environment, eventually leading to recruitment of inflammatory cells such as neutrophils and eosinophils. IL-4 increases IgE production by B cells. IL-5 is important for recruitment of eosinophils. TNF-Y contributes to shock in systemic anaphylaxis.

Histamine : Formed by decarboxylation of amino acid histidine; major component of mast cell granules; about 10% of granule weight.

There are 3 types of histamine receptors H_1, H_2 and H_3; they have different tissue distribution and function.

Binding of histamine to H1 induces contraction of smooth muscles of intestine & branches; increased permeability of venules; increased mucous secretion by goblet cells.

Interaction with H2 receptors increases vasopermeability and dilation and stimulate exocrine glands.

Remark : Histamine binding to H2 receptros on mast cell and basophil supresses degranulation; thus has a negative feedback on mediator release.

7.2.7 Consequences

(A) Systemic Anaphylaxis :

It is a shock like and often fatal state whose onset occurs within minute of type-I hypersensitive reactions.

On injection of antigen to a sensitized guineapig, its respiration is laboured, blood pressure drops. As smooth muscle of GI tract and bladder contracts, the animal defaecates and urinates. Finally bronchial constriction results in death by asphyxiation within 5 minutes of injection.

All these events stem from systemic vasodilation and smooth muscle contraction brought on by mediators released.

Epinephrine counteracts the effect of mediators by relaxing the smooth muscles and reducing vascular permeability.

Localized Anaphylaxis (Atopy)

In it, the reaction is limited to a specific target tissue, often involving epithetial surface at the site of allergen entry. The tendency to manifest localized anaphylactic reaction is inherited and is called **Atopy.**

Allergic Rhinitis

This results from the reaction of airborne allergens with sensitized mast cells in the conjunctival and nasal mucosa to induce the release of pharmalogically active mediator from mast cells. The mediator caused localized vasodiltion and increased capillary permeability.

Symptoms : watery exudation of the conjunctival, nasal mucosa, as well as sneezing & coughing.

Asthma

It is triggred by degranulation of mast cells with release of mediators, but instead of occuring in nasal mucosa, the reaction develop in **lower respiratory tract.**

The resulting contraction of the bronchial smooth muscles leads to bronchoconstriction. Airway edema, *mucous* secretion, and inflammation contribute to the bronchial constriction and to airway obstruction.

The asthmatic response can be divided into early and late responses. Early response occurs within minutes of allergen exposure & primarily involve histamine, leukotrienes & prostaglandins. The effect lead to brochoconstriction, vasodilation and buildup of mucous.The late response occurs hours later and involves IL-4, IL-5, Il-16 and TNF-α, eosinophil chemoactie factor (ECF), and platelet acivating factor (PAF). The overall effects are endothelial cell adhesion, and recruitment of eosinophils and neurophil in bronchial tissue.

Food allergies

Allergens crosslinking IgE on mast cells along the upper or lower GI tract can induce localized smooth muscle contraction and vasodilation.

Mast cell degranulation along the gut increase permeability of mucus membranes, so that allergens can enter blood stream. Various symptoms can develop depending on site of deposit of allergen. Symptoms include, asthma, **wheel and flare** reaction.

Atopic dermatitis : (Allergic eczema)

In allergic individuals, serum IgE levels are often elevated. The individuals develop skin eruptions that are erythromatous and filled with pus. The allergic individual shows an elevated TH_2 cells response and increased number of eosinophils.

7.3 Type II Antibody mediated cytoloxic hypersensitivity

(1) Antibody can mediat cell destruction by activating complement system to create pores in the membrane of foreign cells.

(2) Antibody can also mediate destruction by ADCC (antibody dependent cell-mediated cytotoxicity). In this process, cytotoxic cells with Fc receptors bind to the Fc region of antibodies on target cells and promote killing of cells.

(3) Antibody bound to a foreign cell can also serve as an opsonin, enabling phagocytic cells with Fc or C3b receptors to find and phagocytose the

antibody coated cell.

Examples : i) Transfusion reactions

ii) Hemolytic disease of newborn (erythroblastosis foetalis)

iii) Drug induced haemolytic anemia

7.4 Type III immune complex mediated hypersensitivity

They develop when immune complexes activate the complement systems array of immune effector molecules. C3a, C4a, C5a complement split products are anaphyltoxins and cause localized mast cell degranulation and consequent increase in local vascular permeability. They also function as chemotactic factors of nontrophils.

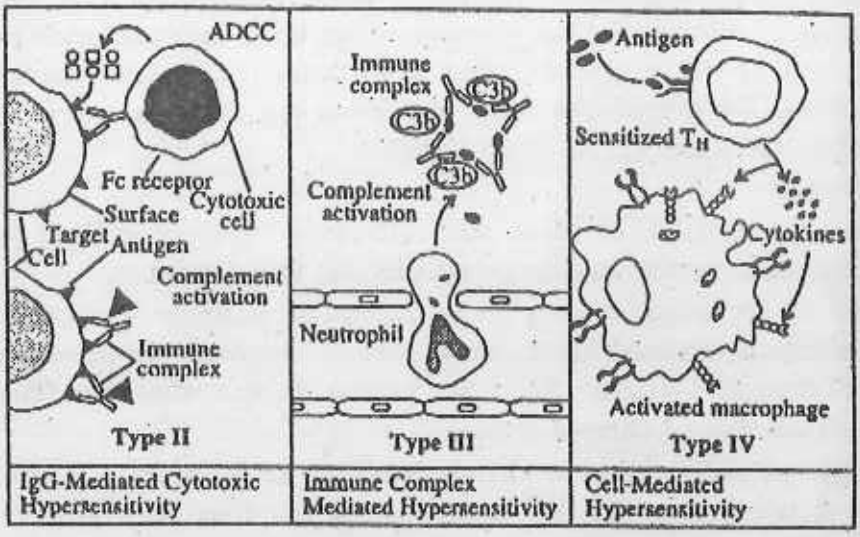


Fig. 7.3. Outlines drawings three types of Hypersensitive reactions (Type II, III and IV)

The lytic enzymes released from neutrophils as they attempt to clear the immune complex destroys localized host tissue.

Activation of complement induce agregation of platelets, resulting in release of clotting factors and lead to formation of microthrombi.e.g. Insect bite, meningitis, malaria, rheumatoid arthritis,

7.5 Type IV (DTH) : delayed type hypersensitivity (DTH)

Activation of DTH of T lymphocytes causes release of cytokine like IL-2, IFN-γ macrophage inhibiting factor (MIF) and TNF-β.

The overall effect is to draw macrophage in the area of action and activate them. Activated macrophage release enzymes and local tissue destruction results. Time lapse for reaction is typically 48-72 hours and hence named DTH e.g. *Mycrobacterium leprae* infection; *Mycobacterium tuberculosis* infecion

7.6 Selective questions

1. Mention the IgE-mediated common allergic reactions. State the syndrome, common allergeus involved and response.
2. Allergic responses can be divided into immediate and latephase responses. Explain all different types of allergic responses involved in both the cases.
3. What is delayed type hypersensitive reaction. Explain with proper diagram.
4. What are the Type II and Type III hypersensitive reaction. Explain both the reactions.

7.7 Selected Readings

Kuby, J. 1999 Immumology, Elsevier Publication, New York

Unit 8 ❐ Elementary Concept of Invertebrate Immunity

Structure

8.1 Introduction

The defence mechanism and its evolution is connected with the stepwise modification of unicellular organism to assending phyla of the invertebrate kingdom. In invertebrate phyla different types of cells, certain organs and body fluids are primarily involved in non-specific (innate) type of immune response. The major difference of this non-specific immunity from adaptive immunity is the i) absence of lymphoid organ and tissue ii) absence of immunoglobin molecule and no memory or genetic basis for synthesis of defence organs. However on the otherhand they are capable of distinguishing between self and non-self. Invertebrates possess an extremely effective physicochemical barriers as their first line of defence. Coelenterates, annelids, mollusks and tunicates has thick layer of mucus that surrounds their body, entraps and kills potential pathogens.Tough tests in coelentenates, molluscs, echinoderms and arthropods form barrier to invasion. The invaders after overcoming barriers are exposed to a range of interacting cellular and partly humoral defence reactions like :

i) Blood clotting / co-agulation and wound healing
ii) Phagocytosis
iii) Encapsulation responses
iv) Natural and inducible antimicrobial factors.

These reactions consider non-self factor and have receptor molecules role in blood cell surface. Recent observations on the evolution of immune systems supports the idea of a well developed defence mechanism in coelomate groups. Toll-like receptors is considered as a method of pathogen recognition system. The attached diagram (fig 8.3) shows the relation of *Drosphila* and mammalian Toll signalling

pathway. Toll receptor in ***Drosophila*** acts in host defense mechanism. This type of receptor has also been found in mouse.

8.2 Cellular component of immunity in invertebrates (Fig. 8.1)

Porifera

Cells-like choanocytes, pinacocytes and archeocytes are all able to internalize various materials. The archeocytes represent the major cells type in defense system

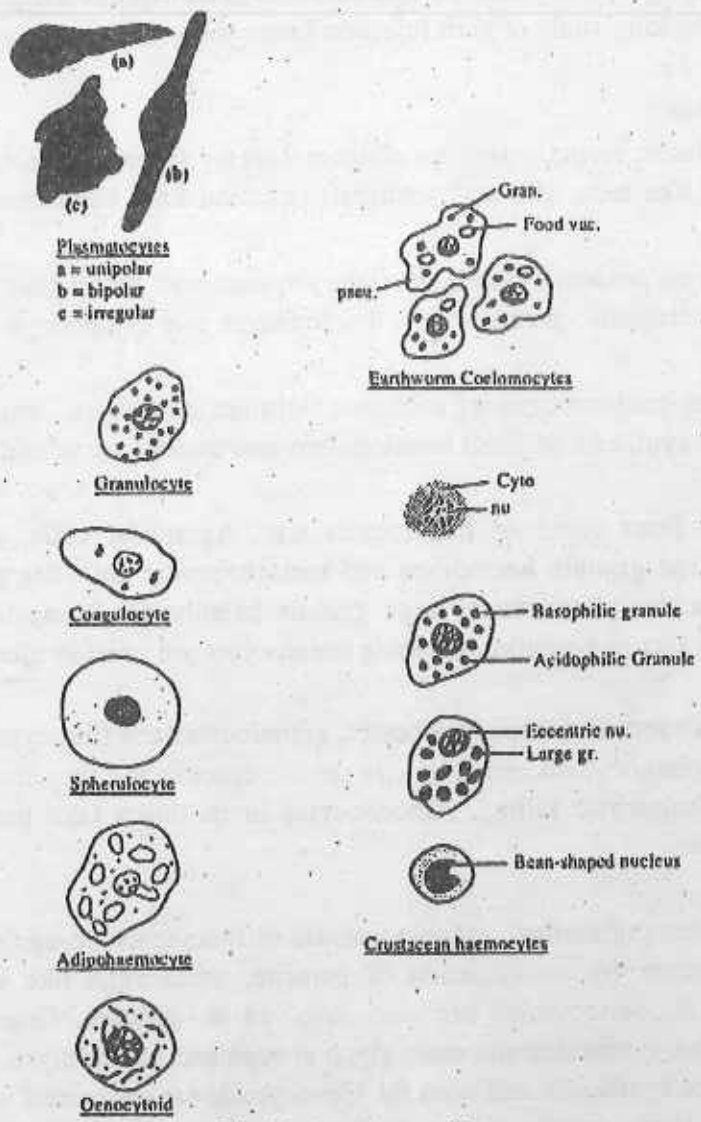


Fig. 8.1. Invertebrate cells

in sponges. The sponges also exhibit some degree of lectin formation, wound healing, histocompatibility and cytotoxic reaction. Phagocytosis is the major defense reaction and the archeoegtes are the effector cells assisted by pinococytes, coelencytes and spherulous cells.

Coelenterate

Phagocytosis, wound repair, allograft rejection has been shown by coelenterates. The regeneration power in colenterate is assumed to be most powerful type in whole invertebrate kingdom. study of graft rejection factor shows allogeneic and xenogenieic recognition power.

Platyhelminthes

These triblasitc animals perform phagocytosis by amoeboid neoblast. Common graft rejection like auto, allo and xenograft rejection have been observed.

Nemertea

Ribbonworms possess a closed circulatory system with blood cells like basophilic granulocytes, nerophilic granulocytes, macrophages and lymphocyte like cells.

Annelida

Earthworms-coelomocytes of coelomic fluid act in defense, while haemocytes are involved in synthesis of fresh haemoglobin and catabolism of old haemoglobin.

Arthropoda

Crustacea- Four types of haemocytes e.g., Agranular cells, small granule haemocytes, large granule haemocyte and metachromatic cells are present. Small granule haemocytes phagocytise, large granule haemocytes encapsulate and their 76KDa protein acts as opsonin. Agranulr haemocytes are used in clotting of blood.

Molluses

In molluscs haemocytes are halinocytes, granulocytes and fibrocytes. Halinocytes are engaged in phagocytosis, granulocytes secrete opsonin and they secrete lysosonal enzymes for phagocytic killing. Amoebocytes in molluscs take part in humoral defence reaction.

Insects

Plasmatocytes (agranular), granulocytes do phagocytosis, co-agulocytes release chemotactic factors for co-agulation of parasite, other cells like spherulocytes, adipohaemocytes, oenocytoids are also involved in defence. Granulocytes and spherulocytes can synthesize and store glyco protein and mucopolysacharides while adipohaemocytes synthesize and store fat. Oenocytoids are concerned with darkening of cuticle after larval moults and co-agulocytes help in blood coagulation.

8.3 Major functional components of the invertebrate immune mechanism

i) Opsonin in phagocytosis

It is a process in which foreign particles or micorbes get coated by certain blood borne substances known as opsonin. In invertebrale haemaglutinin acts as opsonin. (Fig. 8.2)

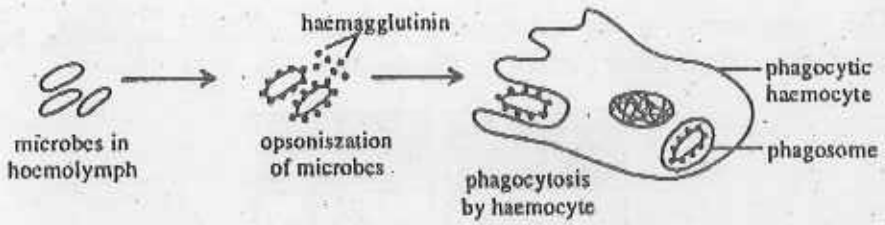


Fig. 8.2. A model of opsonization in invertebrates

ii) Nodule formation in mollusca

It is a kind of defence reaction of certain gastropods and bivalve against invading parasite. It is a process of formation of a fibrous capsule around large sized parasites and leading to death by asphyxiation. The process is much similar but not identical to another process called encapsulation as observed in insects and crustaeans. In encapsulation process blood cells or haemocytes form capsule whereas in nodule the capsule is formed by fibres of fibroblast cells.

iii) Cytotoxic reaction in invertebrates

Neither thymocytes or T-lymphocytes are present in invertebrate. However graft-implantation experiments indicate that some haemocytes of invertebrates have cytotoxic effect over foreign or non-self cells. This observation has been made in annelida and mollusca. Allograft rejection has been observed in starfish (echinodermata).

iv) Phagocytosis by insect haemocytes

Phgocytosis occurs in three steps-a) recognition of pathogen by receptor moleculs b) ingestion of pathogen and c) final disposal.

Opsonization in haemocytic phagocytosis enhance the recognition and phagocytosis process.

v) Encapsulation in insects and in crustacea

Granulocytes and coagulocytes act in the process of capsule fomation while

plasmatocytes play the important role in producing a capsule in insect. Granulocytes, the largegranule haematocytes of decapod crustaceans can encapsulate metazoan parasites and fungal hyphae.

vi) **Components of a complement system of vertebrate in echinoderm**

The alternative pathwy of complement with factors-like factor B, factor D are present in echinoderm.

A lectin pathway of complement activation is present in higher invertebrates like urochordates.

vii) The components of the mammation Toll-like receptor signalling pathway has similar functional pattern in ***Drosophila*** (Fig. 8.3).

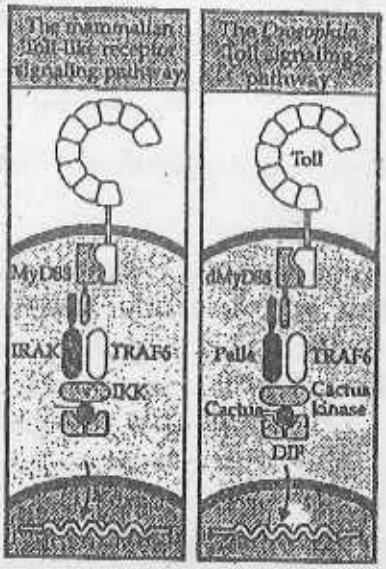


Fig. 8.3. The components of the mammalian toll-like receptors signalling pathway has similar functional pattern as in *Drosoptila,* different abbreviation stand for chemicals in pathways like My D88 or dMy D88 is an adaptor protein, IRAK, TRAFE, IKB, IKK and NFKB are components of signalling pathway.

The mid step in both signalling pathway occurs via the interaction of death domains, between MyD88 and IRAK is mammal and between d MyD88 and Pelk in *Drosophila.* Both IRAK and Pelle are serive xinase. Simlarly the end point reactions involving TRAF6, IKB, IKK in mammal and cactus and cactus kinase are homologous in nature (Adapted from Janeway, *et al,* 2005).

8.4 Naturally occuring humoral type defense process in invertebrate

There is no immunoglobulin in invertebrate, but the fluid in body possess a range of humoral defence factors. Agglutinins, lysozyme, lysin, non-lysozyme bacteriocidins, lysosomal enzymes and immobilization factor are few examples to

name. Components parallel to vertebrate complement system has also been reported. Phagocytes bear c3b-like receptors and can enhance phagocytosis. In insects many antibacterial proteins can be induced within few hours of antigen injection. One such factor, a ceropin, called P4 or haemolin has been obseved to have homology with certain immunoglobulin. Cytokine like factors are found in protozoan pheromore Er-1, similar to IL-2 in functional aspect. Similarly molecules like IFN-γ, IFN-β and TNF have also been isolated and characterised from annelids, echinoderms and tunicates.

8.5 Selective questions

1. Draw and describe the role of different cells in invertebrates involved in defence function.
2. Compare the toll like receptors of *Drosophila* with mammalian system.

8.6 Selected readings

Sima, P and Vetvicka, V. 1993. Evolution of immune reactions. Critical Review in immunology CRC Pross 13(2) : 83-114.

Janeway C., Travers. P, Walpart, M. and shlomchik, M.J. 2005 Immunobiology 6th edn., Churchill livingstone, London, 823 pp.

Unit 9 ❐ Epidemiology of Microbe Related Diseases

Structure

9.1 Introduction

Epidemiology is difined as the overall situation of the particular disease and related informations like nature, distribuion, cause transfer procedure, preventive, measure and management of the disease. It is also known as the "natural history of disease" or can be called "the human face of ecology".

Surveillance, a term closely linked with the study of epidemiology has three main elements—

1) Systemic collection of pertinent data

2) The orderly consolidation and evaluation of the data

3) The prompt dissemination of the findings, especially to those who can take appropriate action.

9.2 Factors of eipdemiology

The infection process is actually a dynamic state involving three main factors : the microorganism, the host and the environment.

The micro-organisms

The concept of **Virulence** is an important point to understand the epidemiological consequence of microbe. It is difined as the degree of pathogenicity of an infectious agent indicated by fatality rates and / or its ability to invade and damage the tissues of the host. The degree of virulence depends on **invasiveness**, the capacity of organism to spread widely through the body, and **toxigenicity** ,the toxin producing property of the organism.

A second variable is the **dosage** of the organism and this is clearly related to the virulence. A small number of organisms of high virulence is usually sufficient to cause disease in a susceptible person, whereas if the organism is of low verulence it often fails to cause disease.

Portal of entry is the third variable as many orgnisms have a predilection for a particulr tissue or organ. ***Salmonella typhi*** usually causes typhoid only when it enters the human body through the mouth in food or water.

The Host

Host reaction to a microorganism depends on the ability to resist infection. The individual may not possess sufficient resistance against a particular pathogen to prevent contraction of infection when exposed to the organism. Alternatively, the individual may possess specific protective antibodies or cellular immunity as a result of previous infection or immunization.

However, immunity is relative and may be overwhelmed by an excessive dose of the infectious agent or if the person is infected via an unusual portal of entry.

It may also be impaired by immunosupressive drug therapy, concurrent disease, or the aging process.

The three main factors of infection are shown in Fig. 9.1.

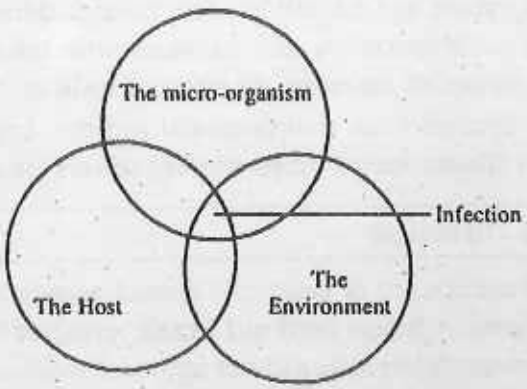


Fig. 9.1. The 3 main factors involved in the infectious process.

The Environment

The environment plays a major role in the occurrence, spread and control of infection. The virtual dissappearance of relapsing fever, plague and cholera, and rarity of indigenous typhoid fever and the relative infrequency of tuberculosis and bacillary dysentery from UK and USA are all indications of improvements which have taken place in environmental conditions. The decrease in overcrowding, and infestation together with the demand for cleaner water supplies, and better sanitation have been of paramount importance in producing these dramatic improvements.

9.3 The spread of infection

The infection spreads in an epidemiological pattern.

These are—

i) It spreads directly from one person to another. Clinical symptoms are easily detectatable and healthy carriers are not a feature. Ex.-Measles.

ii) The infection spreads through healthy carrier. Typhoid, paratyphoid , diptheria are examples where healthy individuals may harbour the bacilli responsile for such disease.

iii) Infections in which persons harbour the organism before the onset of clinical illness.

Streptococcus pneumonie may not cause any harm until an event such as skull fracture allows the transfer of the bacterian from middle ear to cerebrospinal space where it can potentially cause meningitis.

iv) Infection is derived from animal sources as for example leptospirosis, Q fever, anthrax, rabies and brucellosis are diseases derived from animals by zoonosis. These are spread by direct contact with the animal concerned or indirectly by means of ingestion of infected milk or bone products.

v) Infections are derived from environmental sources. Legionnaires disease is an example of illness spread from cooling towers or airconditioners.

9.4 Outbreaks of infection

It is defined as the occurrence of cases of a disease associated in time or locaion among a group of persons. A **house hold out break** involves two or more persons resident in the same private household and not apparently connected with any other case of outbreak, A general outbreak involves 2 or more persons who are not confined to one private household.

9.4.1 Patterns of outbreak

There are 3 main patterns of outbreak—

(i) **The explosive outbreak** : It is characterized by occurrence of a large proposition of cases in a relatively short period of time; there is a sharp rice and fall in the number of infected persons. It is also known as **common source** or **point source** outbreak. This pattern is often associated with food & water contamination (Fig. 9.2)

(ii) **Person to person spread** : Outbreaks caused by infections which are spread from person to person have a more protected course taking longer than explosive outbreaks to build up & to subside.

Diseases such as dysentery, hepatitis type A and gastro-enteritis which are usually spread by the faecal-oral route often follow the pattern of spread. (Fig. 9.3).

(iii) **Explosive outbreaks with subsequent person-to-person spread** : This pattern is often apparent when there is contamination of a common water or food source and the initial cases subsequently infect their contacts. Thus, the pattern of the outbreak in a combination of that process with an explosive outbreak, but followed by a slower decline. (Fig. 9.4)

9.4.2 Analysis of outbreak

The fundamental pieces of information which should be sought whenever an outbreak occurs are as follows :

(1) WHO gets infected?
(2) WHERE were those became infected?
(3) WHEN did the infection occur?
(4) WHAT was the common factor?
(5) HOW did those involved become infected?
(6) WHY did the infection occur?

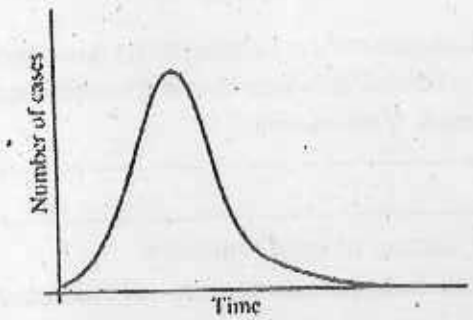


Fig. 9.2. Epidemic curve apparent when there is an explosive outbreak.

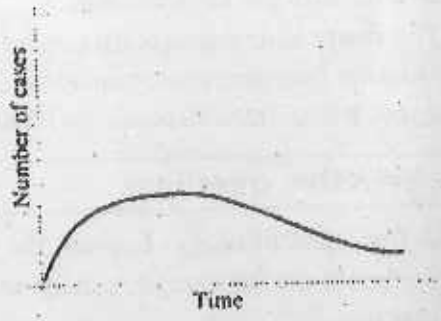


Fig. 9.3. Epidemic curve apparent when there is person-to-person spread of infection.

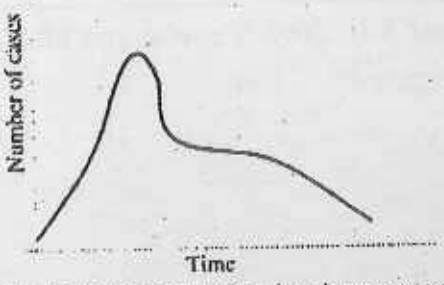


Fig. 9.4. Epidemic curve apparent when there is person-to person spread subsequent to a common source outbreak.

9.4.3 Investigation of outbreaks

In the investigation of outbreaks it is important to have a standardized approach to the various steps involved. Such an approach might have the following as a basis :

1. Verify the diagnosis.
2. Establish the existence of an outbreak.
3. Establish the extent of an outbreak.
4. Identify common charecteristics of experiences of the affected persons.
5. Investigate the source and vehicle of infection.
6. Analyse the findings.
7. Construct an hypothesis.

9.4.4 Control of outbreaks

The investigation of an outbreak should be carried out as swiftly as possible so that adequate control measures can be started without delay. Knowledge of the **source of infection** and **route of transmission** and the **persons at risk** should allow appropriate acion to be taken in order to achieve success.

The **source of infection** may be—(1) Human cases or carriers. (ii) Animal cases or carriers, (iii) the environment.

The **route of transmission** can be—(1) Direct or indirect contact, (ii) Air-borne transmission,(iii) percutaneous transmission (iv) Food & water-borne transmission, (v) Insect-borne transmission, (vi) Transplacental transmission.

9.5 Selective questions

1. Define epidemiology. Explain the role of factors of epidemiology.
2. Comment on the spread, pattern of outbreak and control measures of microbial infection.

9.6 Selected readings

Abbas, A.K. and Lichtman, A.H. 2003. Cellular and Molecular Immunology. 5th edn. Elsevier Science, USA.

Unit 10 ❐ Host Microbe Interaction–Immune Response to Protozoa, Bacteria and Virus

Structure

10.1 Introduction

The host microbe interaction is an integrated mechanism where a series of co-ordinated events must be overcome by pathogen to establish the infection in a susceptible host. The barriers to be overcome are—

i) Epithelial surface of skin, respriratory surface, gut.

ii) Mucous membrane of gut.

iii) lower pH value of stomach and upper intestine

iv) gastric enzymes.

v) antibacterial peptides.

On overcoming these physical barriers the pathogen face complement system and NK cells which are members of innate immunity along with its most important effector cell the phagocyte.

Even if this system is breached the pathogen has to deal with humoral and cell mediated immune system (acquired immunity) of the host. Potential outcomes of the interaction between a host and a microbe has been shown in a diagrammatic way below (Table 10.1)

10.2 Viral infections

Specific immune effector mechanism and nonspecific defence mechanism act in coordination to eliminate an infectiong virus. Virus, on the other hand, try to overcome one a more of these mechanisms & establish infection. The final outcome of host-virus, interaction depends on the effectivity of the hosts defensive mechanism and resistance offered by the virus. (Fig. 10.1 and 10.2)

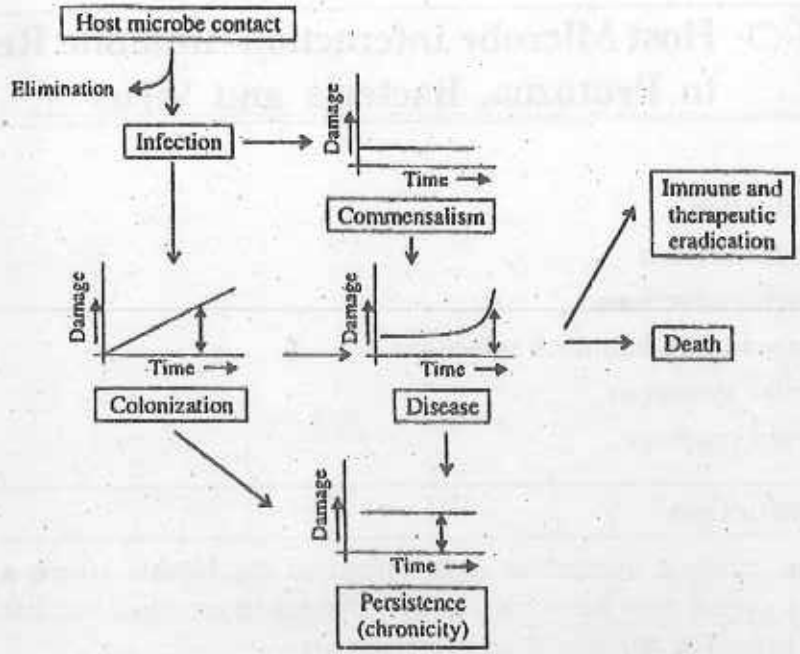


Table 10.1 Model of host microbe contact (Adapted from Casadevall and pirofski, 2000)

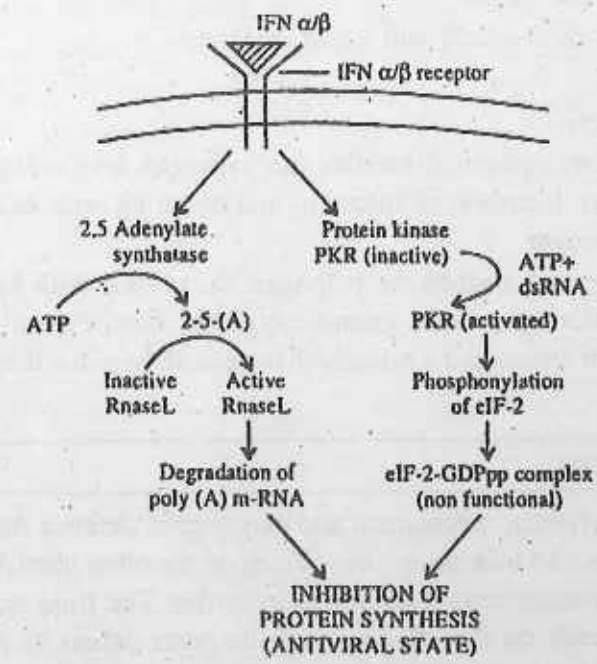


Fig.10.1 Induction of antiviral state by interferon α/β (IFNα/β)

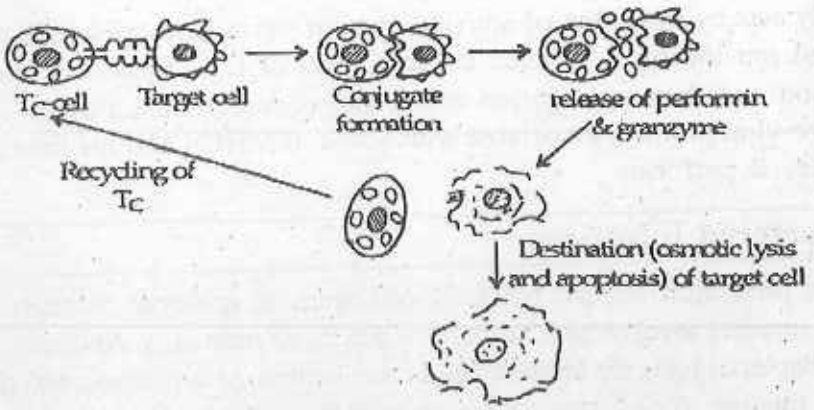


Fig. 10.2 Tc cell mediated killing of target cell.

Innate responses against viral infection is mediated by induction of type I interferous and by NK cells. Viral infection induce infected cells to produce and secrete IFN-α & IFN-β. On binding of IFN-α / IFN-β to their respective receptors on non-infected surrounding host cells, a state, better known as "antiviral state" occurs. The receptors of IFN-α / IFN-β transduce this signal to the cell interior via JAK/STAT signalling pathway. This in turn induces the expression of certain genes.

One of these genes code for an enzyme 2'-5' Adenylate synthetase, which in turn activates a ribonuclease, RNase L, that degrades viral DsRNA. Another function of IFN-α / IFN-β receptor is activation of a specific protein Kinase, PKR, which inactivates el F-2 a translation initiation factor. Viral protein synthesis is blocked and hence no new virus particles can be produced.

On binding of IFN-α/IFN-β to their specific receptor on NK cells, the NK cells are activated & they can effectively lyse & kill virally infected host cells.

Virus enter host cell by certain surface molecules which at as receptors for host cell surface molecules. Antibodies are particularly effective in protection against viral infection if they are localized at the site of entry of the virus into the body. Blocking of viral receptors renders them incapable of entering host cells and makes them easy target for phagocytic killing. The antibodies also acts as opsonins and help in viral clearance.

However, when infection has occured, the viral DNA is generally integrated in Host DNA and under such conditions, the antibodies become helpless. It is at this point that CD4+ TH1 cell & CD8+ Tc cells act in unison to alleviate viral infection. T_H1 cells secrete IL-2, IFNγ & TNF as defence against infection.

IFNγ acts by induction of antiviral state in surroundin cells cells by method as described previously. IL-2 helps in recruitment of CTL precesors into an effector population and their aggregation at the site of infection. CTLs or Tc cells then recognize viral proteins associated with host cell MHC-I and mediate its killing by granofmes & perforins.

10.3 Bacterial infections

Host protection against bacterial pathogens is achieved through a variety of mechanisms that involve both humoral and cellular immunity. Antibacterial defenses include bacterial lysis via antibody and complement, opsonization and phagocytosis, with elimination of phagocytosis bacteria by the liver, spleen and other components of the reticulo endothetial system.

Bacterial pathogens can be distinguished as intracellulor or extracellular pathogens. The defense mechanism of host vary along with the abode of the bacterial pathogen.

However antibodies to play an important role in negeting the toxins--endotoxin (bacterial cell wall component) & exotoxin (secretory product). By acting against exotoxins the anibodies act to reduce the pahtogenecity and damage caused by bacteria as most exotoxins interfere with normal physiological mechanisms of host cell. When targetted against endotoxins, the antibodies act as opsonins and help in their phagocytonis by NK cells and macrophages.

The complex peptidoglycan coat of gram positive bacteria contain teichoic and molecules which are highly immunogenic and form their major antigenic determinant. However their presence prevent lysis by complement activation. Gram negative bacteria on the other hand are susceptible to lysis by complement activation. The lipopolysacchride coat (LPS) act as immunogen as well as inducers to lectin binding pathway of complement activation.

Bacteria residing inside host cell, however escape the above described process of destruction/elimination. They can be eliminated by host, only if T_H & Tc acts in unision, i.e. by cellular immunity. MHC proteins along with viral anigens, activate T_H cells which release lytokines thereby recruiting phagocytic as well as Tc cells as effectors of clearance.

Phagocytic cells kill by injection, & then by acting upon endosomes with lysosomal enzymes. Tc cells utilize perform & granzyme pathway. The performin secreted by Tc cells attach to the membrane of the infected cell and form channel through which water can enter the injected cell and cause osmotic lysis. "Granzymes" a set of DNA degrading enzymes secreted by Tc cells also enter infected host cell

via peforin channel. They degrde the host cell DNA into fragments and thus induce regulated cell death.

10.4 Protozoan and helminth infection

As in the case of bacterial infection the protozoal infection may be divided into two blood groups–intracellular infection and extra cellular type.

In case of extracellular protozoans, the innate immunity mediated by complement system (Lectin and alternate pathway) and NK cells form the first line of defence. the specific antibodies against protozoan forms the humoral branch of immunity. They reader protection by activating classical complement pathway as well ad by acting as opsonius and phagocytic function by macrophage.

In case of intracellular protozoan infection, cellular immunity mediated by TH and Tc cells play an important role along with the immune reaction as in case of bacteria. The TH cells by secreting cytokines attract and activate Tc, NK cells & macrophages. Macrophages ingest protozoa infected with cells and destroy them. Tc cells interact with host MHC coupled with protozoan antigen and lyse infected cells by secretion of perforia and granzymes. Helminth parasites are large and they are blood parasite or tissue parasite in host. Some kind of immunity to reinfection is the general rule but existing worms are not always destroyed. This property of host system is a state called concomitant immunity.

Adult or even larval worms are too large to be destroyed by antibody with or without complement or by phagocytic cells. The normal effector mechanism is antibody-dependent cell mediated cytotoxicity (ADCC) in which the worms become coated with IgG or IgE antibody which binds eosinophils and other cells that actually destroy the parasite. As in chronic protozoa infections the immune responses are multifactorial and vary according to stage of development of the invading worm. Helminths are all capable of evading immune responses of the host and contribute much to the pathology of the infection in host's body.

Parasites (Protozoa and Helminth) can evade the immune response of the host by various methods. They become intracellular and avoid antibody like *Leishmania* spp., *Toxoplasma gondii* and *Trypanosoma cruzi*. Parasites like african trypanosoma (*T. brucei*) and malarial parasites (*Plasmodium*), nematode *Trichinella* show antigenic variation of their surface glycoprotein antigen. The non-specific way of evading the immune response is by interfering with the operation and control of its delicate balance. The mechanisms are directed towards damaging the normal functioning of host immune mechanism by immunodepression, polyclonal B cell activation etc.

10.5 Selective questions

1. Explain the hypothesis or potential outcomes of the interaction between a host and a microbe as proposed by Casadevall and Pirofski.
2. Elaborate the immunity to (a) virus (b) bacteria (c) protozoa and helminth parasites.
3. Explain briefly the mechanisms by which pathgens evade the immune response.

10.6 Selected readings

1. Abbas, A.K. and Lichtman, A.H. 2003. Cellular and Molecular Immunology. 5th edn. Elsevier Science, USA.
2. Casadevall, A and Pirofski, L 2000 Host pathogen miteractions; basic concepts of microbial commensalism, colonization, infection and disease. Infect Immun 68 : 6511.