Biyani's Think Tank

Concept based notes

Immunology

B.Sc. Biotechnology Part-I

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Preface

I am glad to present this book, especially designed to serve the needs of the students. The book has been written keeping in mind the general weakness in understanding the fundamental concepts of the topics. The book is self-explanatory and adopts the "Teach Yourself" style. It is based on question-answer pattern. The language of book is quite easy and understandable based on scientific approach.

Any further improvement in the contents of the book by making corrections, omission and inclusion is keen to be achieved based on suggestions from the readers for which the author shall be obliged.

I acknowledge special thanks to Mr. Rajeev Biyani, *Chairman* & Dr. Sanjay Biyani, *Director* (*Acad.*) Biyani Group of Colleges, who are the backbones and main concept provider and also have been constant source of motivation throughout this Endeavour. They played an active role in coordinating the various stages of this Endeavour and spearheaded the publishing work.

I look forward to receiving valuable suggestions from professors of various educational institutions, other faculty members and students for improvement of the quality of the book. The reader may feel free to send in their comments and suggestions to the under mentioned address.

Author

IMMUNOLOGY

Q.1. What is immune system?

Ans. The immune system protect us from attack by microbes and worms. It uses specialized organs designed to filter out and respond to microbes entering the body tissues and a mobile force of molecules and cells in the blood-stream to respond rapidly to attack.

It has complex and sophisticated mechanism to regulate it. The system can fail, giving rise to tissue damage. Its comprised of much like the other body system e.g. respiratory and reproductive systems, in that its composed of a number of different cell types, tissue and organs.

Q.2. What is immunity?

Ans. All those physiological mechanism that endow the Living being with the capacity to recognize materials as foreign to itself and to neutralize, eliminate or metabolize them with or without injury to its own tissue. Its generally of 2 types :

(a) Adaptive (b) Innate (Natural)

Artificial Natural Artificial Natural

Innate immune system is the 'first live defense' this immunity is comprised of natural barriers like skin, sweat, effect of lysozymes. This is present at birth.

Phagocytes are important alls in the innate immunity system since they ingest and kill microbes.

Adaptive immune system this is the 'second line defence' this immunity comes into play when the antigens cross the first live defence system. This immunityr has far more specificity, and also remembers that a particular microbe has previously invaded the body. This lead to a more rapid expulsion of the microbe on its second and third time entry.

Interaction between Innate and Adaptive Immunity

The innate and adaptive immunity frequently work together. For example : Macrophages are phagocytic but produce important cytokines that help to induce the adaptive response.

Antibodies of Adaptive system

? ? Activated

Complement components of Innate immune system

Q.3. What are antigens?

Ans. Antigens are also known as "immunogens". Its defined as a molecule that provokes an immune response is c/as immunogen, and the other describes a molecule which reacts with the antibody produced or with the activated cellular constituents of CMI (Cell Mediated Immunity) is referred to as an antigen.

- Haptens are small well defined chemical groupings such as Dinitrophenyl (DNP) which are not immunogenic on their own but will react with performed antibodies.

- The part of the Ag molecule that makes contact with the paratope is called the **epitope.**

- As most Ag's are protein in nature they exist in a folded 3-D, tertiary structure. They may be a cluster of amino-acid sequences on the 3-D structure constituting a series of epitopes. Each of these epitope clusters is meant by an **antigenic determinant**.

Requirements for Immunogenicity

(1) It should be genetically foreign.

(2) Molecular Size

(3) Chemical Complexity

(4) Conformation

The Structure of Antigens

An antigen molecule may contain a no. of the same or different antigenic determinants to which individual abs or cell responses are made. The smallest unit (antigenic determinant) to which an antibody can be made is about 3 to 6 amino acids and about 5 to 6 sugar residues. All large molecules are multideterminant. Antibodies bind to conformational antigenic determinant Molecules which can stimulate an immune response (immunogens) should be distinguished from those which react with abs but cannot initiate an immune response (haptens or individual antigenic determinants).

Diagram

Antibody

Proteins with three antigenic determinants

Microbial surface

Fig : Antigenic determinants (epitope) recognized by abs.

Diagram

Hapten

Carrier Protein

Antigen as a carrier-hapten complex

B Cell

MHC Class II

Peptide derived from carrier protein presented in MHC Class II

Diagram

Fig : Response to hapten by B Cells require carrier protein which permits help from T Cells.

Q.4. What are antibodies and explain their structure.

Ans. Antibodies often termed 'immunoglobulins' are glycoproteins that bindantigens with high specificity and affinity. There are 5 chemically and physically distinct classes of ab's :

(a) Ig G

(b) Ig A

(c) Ig M

(d) Ig D

(e) Ig E

Basic Structure of Antibodies

Antibodies have a basic unit of four polypeptide chains – two identical pairs of **Light (L)** chains and **heavy (H)** chains bound together by covalent disulfide bridges as well as by non-covalent interactions. These molecules can be proteolytically cleaved to yield two Fab fragments (antigen binding part of the molecules) and an Fc fragment (the part of the molecule responsible for effector functions e.g. complement activation). Both H- and L- chains are divided into V and C regions – the V regions containing the antigen binding site and the C-region determining the fate of the antigen.

Diagram

Fig : Immunoglobulin Structure

(1) Ig G : It is the most abundant class of immunoglobulins present in serum. It also exist in polymerized form. Ig G is equally distributed in intra and extra vascular compartments. Ig G is the only maternal antibody that is normally transported across the placenta and provides maternal passive immunity.

(2) Ig M : It is 5-10% of total immunoglobulin serum and is the first antibody to appear after the infection. This Ig M is pentameric in structure and consists of five 'Y' shaped monomers which are joined together by Fc linked protein which is called as Joining Chain (J-chain). This J-Chain is the disulfide bond which is present between the carboxyl terminal. In this structure the 5 monomers are arranged with Fc in the centre and 10 Ag binding sites is in periphery of the molecules because of its more efficient than any other isotype.

(3) Ig A : This is usually a monomer but later on diners or trimers are seen which contain J-chain. Being secretory it has role in secretory function they prevent the attachment and Lyse the virus and bacteria attached to the epithelial surface. Ig A are found in many body secretion like Saliva, mucous etc.

(4) IG E : This antibody is present in serum only in nanograms. Its level is lately elevated during allergic conditions like Asthma, rashes etc. Its is chiefly produced in lining of respiratory and intestinal tract.

Ig D : It constitutes about 0.2% of total serum. This do not activate complement system and cannot pass placenta. Ig D present together with Ig M on lymphocyte surface. Together with Ig M are present on Ag receptor which control lymphocyte activation and suppression. The hinge regions of Ig d is fully extened and is fully extended and is protected by carbohydrate because of it, its more susceptible to Ag's, because of this extended hinge region it has short life span.

Q.5. Explain the functions of cells and organs of the immune system.

Ans. All blood cells arise from type of cells called as **Haematopoietic Stem Cells**. Haematopoesis means formation and development of blood cells. Haematopoisis starts in the embryonic yolk sac. This process starts in first week. At this time yolk stem cells differentiate into primitive erytheroid cells (these cells contain haemoglobin), now these cells are called as haematopoletic Stem cell, these cells migrate from the yolk sac to liver and from here goes to Spleen. Liver and Spleen plays a major role for 7 months and later on this process occurs in bone marrow.

Combryonic yolk sac

(In 1st weet)

?

Yolk stem cells differentiation occurs to

?

Primitive Erythroid Cells

(contain Haemoglobin)

Also called as Haematopoitic Stem Cell

?

Cell migrate from yolk sac to

?

Liver and spleen (7 months, development)

?

Bone marrow

CELLS OF IMMUNE SYSTEM

They are phagocytic in nature and kills the micro-organisms. It is of 2 types :

(1) Microphages also called as Polymorphonuclear Granulocytes

(2) Macrophages/Monocytes

(I) Microphages : On the basis of lobes and irregularly shaped nucleus and staining properties of cytoplasmic granules with acidic or basic dyes are classified as :

(A) ***Neutrophils*** **:** They can take both the stain that is acidic and basic. These are the first cells to arrive at the site of inflammation. These uses oxygen dependent and oxygen independent to generate anti-microbial substance. They are much more active than macrophage to kill the ingested organisms.

Eosinophile **:**These are the motile phagocytic cells they can migrate from the blood to the tissue spaces and these play an role in defence against parisitic organism. The secretion of eosionophil damages the membrane of parasite.

Basophiles : These are granular leucocyte their granules are stained with basic dyes. They arise from the bone marrow and enter blood some of these basophils leaves the blood and enter the solid tissue and in solid tissue converts into mast cells. Basophiles and mast cells have granules and they contain heparin, leucotinens, histamines, these chemicals promote inflammatory reaction. They also contain chemotactic factors which attracts the cosinophils at the place of inflammation.

(II) Macrophages : These are derived from bone marrow cells which are called as pro-monocytes. These pro-monocytes differentiate into blood monocyte which finally settles in tissue as macrophage where they constitute the mononuclor phagocytic system. These are present throughout the connective tissue and along the basement membrane of small blood vessels. These macrophages are particularly concentrated lungs, liver, spleen lymph nodes and these places acts as filter to prevent the micro-organisms to enter by any route.

The changes occur when monocyte converts to macrophages are the cell enlarges 5-10 times their intracellular content increases in number and complexity. The macrophages acquire increased phagocytic ability as compared to monocytes and macrophages produces large amount of hydrolytic enzymes and variety of soluble factors.

According to distribution, they are named as :

Lungs ? Alveolar

Connective Tissue ? Histiocytes

Brain ? Mesongial

Bone tissue ? Osteoblast

Liver ? Kupffer

In resting stage they are activated by no. of stimuli and these stimuli comes from the immune response.

Null Cells ? These are small groups of lymphocyte which are of Iytotoxic type. These cells have receptors on their surface for Fc portion of Ig G, they kill the sensitized cell which are sensitized with the Ig G and lysis the complex they are known as killer cells. These cells are responsible for antibody dependent cytotoxicity. The sub-population of null cells is called as natural killer cells.

Lymphocytes

They are the major types of WBC/Leucocytes. They are of 2 types :

(A) B - Lymphocytes

(B) T- Lymphocytes

B- Lymphocytes carry humoral immune response whereas T cells carryinfected cells.

These lymphocytes originate from stem cells in bone marrow in foetus. Initially all lymphocytes are alike but later on they differentiate into B and T Lymphocytes.

Lymphocytes that migrate from bone marrow to thymus (present near heart) develop in T-Lymphocytes or T-cell (T-stands for thymus). Another type of lymphocytes remain in bone marrow and continues their maturation in bone marrow and develops into B-cells. After maturation, B & T cells are most conc in lymph nodes, spleen and lymphatic organ.

T-Cells – Specialized to operate against cells which bears intracellular micro-organism or pathogens. T-cells recognize antigens only when they are present on the surface of cells. T-cells have receptors on their surface called as T-cell receptor, with the help of these receptors T-cell recognize Ag along with surface markers.

Its of 3 types :

(a) T Helper Cells (b) Cytotoxic T-cells (c) Suppressor T-cell

T Helper Cells : After recognizing antigen, T_H secretes Interleukin and other cytokines activate T_H cells, B-cells and Tc cells. T_H metabolizes both the arms of immune response that is cell mediated and humoral arms.

Diagram

T-Cell Receptor

T- Cell

T_H cell activated and give rise to clones of T_H cells

Attack on infected cell and generate cell mediated imunity

T_H secretes IH-1 and other cytokines

B- Cell shows humoral immunity. Secrete Ab in plasma

Class II MHC molecule along with antigen

Macrophage (Antigen Presenting Cell)

Cytotoxic T-Cells : Have wide range of specificity clones express large number of different surface receptors but each lymphocyte is programmed for Type I receptors in presence of surface markers secretes cytokines, these attack on infected cells and cancer cells and secrete a protein called as perforin. Teytotoxic require help from T Helper cells for their function.

Suppressor T-Cells : As the name indicates their function is to suppress the immune response or regulate the activity of T Helper cells.

Immune response is regulated by mutually opposing influence of helper and repressor cells.

Helper cells constitute about 65%.

Suppressor cells constitute about 35% of circulating T-cells.

B Cells : Are of lymphocyte whose differentiation occur in bone marrow in man and bursa of fabricus in birds. Each B-Lymph is programmed to make only one antibody. This antibody is placed on the surface B-cell where antibody acts as a receptor. Each B-cell have order of 10^5 identical Ab molecules on its surface. Now when the antigen enters into the blood and circulates in blood. It will bind only to the B-cells which have receptor suitable for that antigen. So once, the reaction occurs between Ag- B cells the lymphocyte receives triggering signal and then develops into plasma cells. These plasma cells produces more antibody of same types.

ORGANS OF THE IMMUNE SYSTEM

There are number of organs and tissue involved in development of immune response. These organs are morphologically and functionally distinct. According to their function they are classified as :

(A) Primary Lymphoid Organs Or Central Lymphoid Organ

(B) Secondary Lymphoid Organs Or Peripheral Lymphoid Organ

Primary Lymphoid Organs : major site for lymphocyte maturation, lymphocytes are formed from lymphoid stem cells. In mammals later on these lymphocytes mature in bone marrow.

Various primary lymphoid organs are :

Thymus : Its made of 2 lobes each lobe is further divide into lobules by connective tissue called as trabecula. In connective tissue, thymusdivide in 2 parts :

(a) Outer Cortex

(b) Inner Medulla (thymocytes are arranged)

Cortex part is densely packed with immature T cells, where as medulla contain more mature cells. This shows a differentiation gradient from cortex to medulla. Both in cortex and medulla stromal cells network is present. This network is formed by cortical epithelial cells, interdigitating membrane. In Cortex nurse cells are also present. Nurse cells have numerous membrane extensions and these extension surrounds as many as 50 Lymphocytes and provide nourishment. T-Lymphocytes become mature and functional only after a period of residue in thymus.

Bursa of Fabricus : Its an avian lymphoid organ which is present in the gut. This organ provide primary site for T-cell maturation. Its a pouch like structure which is connected to return near the cloacal opening like thymus this organ also undergo involution on the onset of maturity. This is a site of Lymphocyte called as proliferation and differentiate.

SECONDARY LYMPHOID ORGANS

Lymph Nodes : These are the most highly organized secondary lymphoid organs these are small kidney shaped organs which are distributed throughout the body.

Diagram

Afferent Vessel

B-Lymphocytes

Post capillary venule

Effleuent lymphatic vessel

The Lymph nodes are divided into :

(i) Cortex

(ii) Paracortex

(iii) Medulla

Lymph nodes have filtered lymph coming from the tissue and macrophages are found in the lymph. They remove phago cytically all the antigen which has entered into the tissue. In lymph nodes T cells and B-cells are distinct.

Cortex contain B-cells in dense lymphoid conicals whereas T-cells are present in paracortex regions. Lymphocyte enters the node through affrent vessels and leaves through efferent vessels. B-cells on exposure to Ag converts into plasma cells.

Diagram

Spleen

It is a large, oboid secondary lymphoid organ. It is situated in left at dominal cavity. This organ is specialized to trap localized Ag from regional tissue spacts, adopted to filtering blood and trapping blood borne antigens. Thus they can respond to systemic infection.

It is surrounded by a capsule and separated by trabeculae into compartment (red pulp and white pulp).

Red pulp consist of a network of sinusoids populated macrophages and numerous RBC : It is the site where the old K infective RBC's destroyed and removed. Macrophages are present in red pulp.

White pulp surrounds the arteries, forming a periarteriolar lymphoid sheath (PALS) populated mainly by T-Lymphocytes.

Q.6. What are Ag-Ab reactions?

Ans. They serve for several purposes like diagnose infections, identification of infectious and non-infectious agents. Ag-Ab reactions occur in three stages :

(1) Primary Stage

This is an initial interaction between Ag and Ab without any visible effect. This reaction may occur even at low temperature. The combination between Ag and Ab molecules being affected by the weaker intermolecular forces such as Wander Vaal's force.

(2) Secondary Stage

This reactions leads to demonstrable events such as precipitation, agglutination lysis of cells, killing of living antigens, neutralization oftoxins, fixing of complement in mobilization of micro-organisms and enhancement of phagocytosis.

(3) Tertiary Stage

Some Ag-Ab reactions occur in-vivo, which leads to neutralization or destruction of injurious Ag's or tissue damage. They generate one of the branch of immune response to do so such as humoral or all mediated immune response.

General properties of Ag-Ab reactions :

(1) The reaction is specific.

(2) Some-times cross-reaction may occur due to antigenic similarity.

(3) Entire molecules reacts not only the fragments.

(4) There is no denaturation of the Ag or Ab during the reaction.

(5) Combination occur at the surfaces.

(6) Combination is firm but reversible. The firmness of the union is influenced by the affinity and avidity.

Affinity : Intensity attraction between Ag and Ab molecules.

Avidity : Strength of the bond after the formation of Ag-Ab complex.

(7) Both Ag's and Ab's participate in the formation of agglutinates or precipitates.

(8) Ag and Ab can combine in varying proportions.

(9) Ag's are multivalent whereas Ab's are bivalent.

Measurement of Ag and Ab

Sensitivity : It's the ability of the test to detect even very minute quantity of Ag and Ab.

Specificity : It's the ability of the test to detect reactions between homologus Ag's Ab's only.

In-vitro Ag- Ab Reactions

PRECIPITATION REACTIONS

- Soluble Ag combines with its Ab in the presence of electrolytes at suitable temp and pH.

- The complex formation is seen as insoluble precipitate called **precipitation.**

- Instead of sedimenting, the precipitate remains suspended as floccules called flocculation.

- Precipitation can take place in liquid media or in gels such as agar, agarose or poly acrylamide.

- Ag and Ab involved in the precipitation reaction called as precipitinogen and precipitin respectively.

- The amount of precipitate formed is greatly influenced by relative proportions of Ag's and Ab.

- To the same amount of anti serum in different tubes, if increasing quantities of Ag's are added, the precipitation occurs abundantly in one of the middle tubes, in which Ag and Ab are in optimal quantity.

- The Preceding tubes in which the Ab is in excess and later tubes in which the Ag is excess, the precipitation will be weak or even absent.

- Three phases occur:

(a) Pro-zone (zone of Ab excess)

(b) A peak (zone of equivalence)

(c) Post-zone (zone of Ag excess)

- This is called as **Zone Phenomenon.**

Lattice hypothesis

(Mechanism of Precipitation)

- Marrack (1934) proposed the lattice hypothesis to explain the mechanism.

Diagram *

Lattice Formation

- Multivalent antigen combined with bivalent antibody in varying proportion depending on the Ag-Ab ratio in the mixture.

- Precipitation occurs when a large lattice is formed consisting of alternating Ag and Ab molecules. This is possible only in the zone of equivalence.

- In the zone of antigen or antibody molecules excess, the lattice does not enlarge as the valencies of Ab and Ag does not equal respectively.

Application of Precipitation Reaction

This reaction can be carried out either is qualitatively or as quantitatively.

(A) Precipitation Reactions in Liquid.

(I) Ring Test – Simplest type of test.

- Layering Ag solution over a column of antiserum in a narrow tube, precipitate forms at the junction of two liquids.

Diagram

(II) Immunodiffusion (precipitation in gel)

- Precipitation occurs easily in gel rather than in a Liquid medium and this reaction is visible in a distinct band.

- Different immunodiffusion test :

(a) Single diffusion in one dimension (Oudin Procedure)

- Ab is incorporated in agarose gel in a tube and Ag solution is layered over it.

- Ag diffuses downwards through agar gel and forms a line of precipitation that appears to move downwards.

- Precipitation is formed at the advancing front of the Ag.

- Conc. of Ag increases due to diffusion and no. of bands indicate the no. of different Ag's present.

(b) Double diffusion in one dimension (Okakley Fulthrope Procedure)

- Ab is incorporated in gel, above this is placed a coloumn of plain agar. The Ag is layered on top.

- Ag and Ab move towards each other through the coloumn of plain agar, and form a band of precipitate.

(c) Single Diffusion in two dimension (Radial Immuno diffusion)

- Antiserum is incorporated in agar gel placed on flat surface.

- Ag is added to the well cut surface of the gel.

- Ag diffuses radially from the well and forms ring shaped bands of precipitation (halos) around the well.

(d) Double diffusion in two dimensions (Ouchter long technique)

- Agar gel is poured on a slide and well are made using a template with gel bunch. The anti serum is placed in the central well and different Ag in the surrounding wells.

- If two adjacent Ag are identical, the lines of precipitate formed by them will diffuse.

- If unrelated, the lines will cross each other.

(III) Immuno-electrophoresis

- Resolving power of immunidiffusion was greatly influenced by immuno-electrophoresis.

- Performed on agar or agarose gel on slide with an Ag well and Ab is trough is cut on it.

- Test serum is placed in the Ag well and electrophorsed for an hour.

- Ab against is then placed in the trough and allowed to diffuse.

- The resulting precipiting lines can be stained for observation.

Fig : Various procedure of Precipitation Reaction

Diagram

(i) Oudin Procedure (ii) Okaley Fulthrope

(iii) Radial Immuno Diffusion (iv) Ouchterlony Double diffusion

(v) Immuno electrophores Ag

AGGLUTINATION REACTION

- Particulate Ag mixed with its homologus Ab in the presence of electrolytes at a suitable temp and pH.

- The particles are clumped on agglutinated.

- Agglutination is more sensitive than precipitation.

- Agglutination occurs when Ag's react with homologus antibody in equivalent proportions.

- Antigen and Antibody involved in agglutination reaction are called as agglutinogen and agglutin respectively.

- At higher conc, antibodies bind to antigen but do not induce agglutination are called as **incomplete antibodies.**

Applications of Agglutination Reactions

(I) Direct agglutination Test.

(a) Slide Agglutination – Antiserum is mixed with the uniform suspension of particulate antigen on a glass slide.

- It's a routine procedure for identification of many infectious diseases.

(b) Tube Agglutination : Its a Standard quantitative method for measurement of Ab's.

- A fixed volume of particulate antigen suspension is added to the equal volume of serial dilutions of an antiserum in test tubes.

- This test is done for typhoid, brucellosis.

(c) Antiglobulin Coomb's Test – Test was devised by Coombs, Mourant and Race for detection of anti RH- positive erythrocytes in saline.

- Rh ab's when added with incomplete Ab's, Ag-Ab reaction's does not takes place.

- Antiglobulin serum (complete Ab to unmunoglobulin) called Coomb's serum (Rabit serum against anti Rh Ab) is then added resulting in agglutination.

Diagram

Rh positive erythrocytes

Incomplete Antibody

Complete antibody to Immunoglobulins results in agglutination.

Antiglobulin serum is added.

Ab coat with Rh + ve erythrocyte.

COMPLEMENT FIXATION TEST (CFT)

- This test is adopted to determine the specific bacterial antibodies based on the presence of complement fixing antibodies present in serum.

It is done by 2 methods :

(I) DIRECT CFT

(a) Serum (sample) should be heated to 56^{o}C (inactivated) to destroy complement activity and to remove non-specific inhibitors of complement if present.

?

Complement obtained from guinea pig is added along with specific Ag.

?

SRBC and Rabbit antibody against SRBC (amboceptor) also used in this test as hemolytic indicator system.

?

Ag-Ab complement complex occurs and SRBC and anti-haemolysis in positive cases because of the presence of Ab against the specific Ag.

?

In negative cases the sample may be not contain specific Ab and the complement bind with anti SRBC and SRBC results in haemolysis indicated as red color formation.

Step – 1

Positive Patient's Serum

Diagram

Step – 2

Complement fixes antibodies, RBC's do not lyse.

Haemolytic indicator system

Diagram

Result

Diagram

Step I

Negative Patients Serum

Diagram

RADIO IMMUNO ASSAY (RIA)

- It is one of the most sensitive techniques for detecting Ag or Ab.

- In this method competitive binding of radio labeled antigen and unlabelled antigen to a high affinity antibody is used.

- Antigen is generally labeled with gamma-emitting isotope such as ^{125}I.

- The labeled antigen is mixed with antibody at a conc. that saturates the antigen binding sites of the antibody molecules.

- Then increasing amount of unlabelled antigen of unknown conc. is added.

- The antibody does not distinguish labeled from unlabelled antigen.

- So two kinds of antigen compete for available binding sites on the antibody.

- With increasing conc. of unlabelled antigen, more labeled antigen will be displaced from the binding sites.

ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

An enzyme conjugated to an antibody reacts with colourless substrate to generate a coloured reaction product.

?

Enzymes such as alkaline phosphatase, Horse reddish peroxidase have been employed.

?

These enzymes when mixed with suitable substrate generate a coloured reaction product.

?

This method is used for detection and quantitation of either Ag and Ab.

?

Types of ELISA are :

(a) Indirect ELISA

(b) Sandwich ELISA

(c) Competitive ELISA

Immuno Fluorescence

- Antibodies that are bound to cells or tissue sections can be visualized by tagging the antibody molecules with the fluorescent dye or fluorochrome.

- Dyes like Rhodamine and Fluroscein can be used.

- These dyes absorb light at one wavelength and emit light at a longer wavelength.

- Fluorescent-antibody staining of cell membrane molecular or tissue sections can be direct or indirect.

(a) Direct Staining : The specific Ab's Fc region is tagged.

(b) Indirect Staining : The specific antibody is unlabelled and is detected with an additional fluorochrome labeled reagent.

Western Blotting

- This technique is used for identification of specific protein in a complex mixture of protein.

- In this, a protein mixture is electrophoretically separated on SDS – PAGE.

- A Slab gel is incorporated with sodium-dodecyl sulfate which is dissociating agent of protein.

- Protein bands are transferred to nylon membn by electrophoresis.

- Then the individual protein bands are identified by flooding the nitro-cellulose membrane with enzyme conjugated Ab specific for protein interest.

- The Ag – Ab Complexes that form on the band containing the protein are recognized and identified by flooding the nitro-cellulose membn in substrate specific for enzyme, resulting in the generation of colour spot which indicates the presence of the protein interest.

THE COMPLEMENT SYSTEM

- Complement is defined as "the activity of blood serum that completes the action of antibody."

- The complement system carries out the following basic functions :

(a) Lysis of cells, bacteria and viruses.

(b) Opsonization, which promotes phagocytosis of particulate antigens.

(c) Activation of immune response such as inflammat^{n} and secretion of immunoregulatory molecules.

(d) Removal of Immune Complexes.

- Complement System needs to be activated before it takes effect, activation can occurs in one of the following ways :

(A) Classical Pathway

(B) Alternative Pathway

(C) Lectin Pathway

Classical Pathway Lectin Pathway

C1gr2S2 Mannose Binding

? Binds to Ag-A6 Complex Lectins (MBL)

Activated C1gr2S2 ? Binds to Microbial Surface

MBL – Bind to cell surface ? Mannose Associated Proteins (MASP)

MBL – MASP Complex Cleaves C4 – C4 – Cleaves C4

? C4a

O2 - C4b

C26 ?

C2a

Cleaves C3 – C3 – Cleaves C3

C462a

(C3 Convertase) C3a

C462a-3b C3b

C5 Convertase

? Cleaves C5 – Cleaves C5

C5 – C5a – C5b – Bind to cell surface (Bound)

Alternative Pathway

C3

C3a ? Spontaneous Cleavage

C3b

? Bind Microbial Surface

Bound C36

? Factor B

C36 B Complex

Ba ? Factor D

Cleaves B

C36 B6

(C3 Convertase)

?

C36B636

(C5 Convertase)

C6

C56 ? C566 ? C566789

Membⁿ Attack Complex

MAJOR HISTOCOMTABILITY COMPLEX (MHC)

- MHC is associated with intercellular recognition and with self/non self discrimination.

- They play a major role in development of humoral and cell mediated immune response.

- Transplanted tissue is accepted as Self (histo compatible) or rejected as foreign (histo incompatible).

- T_{cyto} cell requires the processed Ag available along with MHC I, whereas T_{Helper} Cell requires the processed Ag available along with MHC II. This MHC determines susceptibility to diseases and development of autoimmunity.

- Rejection of foreign tissue is the result of immune response to cell surface molecules called histointocompatible antigens.

- R.A. Goer and G.D. Snell proposed histoincompatibility antigens for the first time.

- MHC is a collection of genes within DNA on chromosome 6 in humans and 17 in mice.

Class I MHC molecules

- Encode glycoprotein expressed on the surface of all nucleated cells.

- They present peptide antigens of altered self cells for necessary activation of Tc cells.

- Class I MHC is encoded by ABC regions in humans.

Class II MHC Molecules

- Concode glycoprotein expressed on antigen presenting cells.

- They present processed antigenic peptide to T_H cells.

- These are encoded by DP, DQ and DR regions in humans.

Class III MHC genes

- Encode Secreted protein associated with the immune process including soluble serum proteins, components of the complement system and Humor necrosis factor.

- Class III MHC is encoded by soluble protein C11, C2 (Complement protein) and BF in human.

Q.7. What are cytokines? Explain their importance.

Ans. These are low molecular weight proteins, playing a major role in cell to cell communication and serve as a messenger of the immune system.

- The generic term for regulatory proteins secreted by a cell is cytokine.

- Cytokine secreted lymphocytes are called as Lymphokines.

They have 4 important aspects :

(a) Phiotropy : Cytokine has different biological effects on different target cells.

(b) Redundant : Two or more cytokine mediate similar function.

(c) Synergism : Combined effect of two cytokines.

(d) Antagonism : Inhibits the effect of other cytokines.

- They and their receptors exhibit very high affinity.

- Target cell is for a particular cytokine which is determined by the presence of specific membrane receptors.

Main Class of Cytokine :

(i) Interleukin : Regulate interaction between lymphocytes and other leukocytes.

(ii) Interferous : These are glycoproteins synthesized in response to viral infection.

(iii) Tumor Necrosis Factor : These are secreted cytokine, one is derived from macrophage and other from T-cell.

(iv) Chemokines : These are group of low molecular weight proteins that play important role in inflammatory reaction.

(v) Monokines : Are cytokines secreted by monocyter and macrophage.

General Structure of Cytokine Receptor

Receptors are various cytokines are structurally diverse and belongs to five families :

(a) Ig Super family receptor.

(b) Class I cytokine receptor family.

(c) Class II cytokine receptor family.

(d) TNF receptor family.

(e) Chemokine receptor family.

Q.8. What is hypersensitivity?

Ans. It is defined as the vigorous reaction of immune system leading to severe symptoms and even death in an individual.

The factors which causes hypersensitivity are various extrinsic or intrinsic factors such as :

(a) Drugs-penicillin, asperim.

(b) Airborne particles - pollen grains, grass.

(c) Food stuffs - Shell fish, nuts, eggs.

(d) Insect products – bee venom etc.

(e) Micro-organisms – bacteria, virus etc.

(f) Blood transfusion – mismatched blood.

- On the basis of time required for the manifestation of the reaction, it can be classified into :

(i) Immediate Type Hypersensitivity

(ii) Delayed Type Hypersensitivity

- A classification is made on the basis of different mechanism of pathogenesis :

(I) Type I Hypersensitivity

- Rxn takes place within 2-30 mins.

- Antigen induces cross-linking of IgE bound to mast cells and basophils.

- It include food allergy, eczema, hay fever.

(II) Type II Hypersensitivity

- It is Ab mediated cytotoxic hypersensitivity.

- It occurs within 5-8 hrs.

- In this, Ab is directed against cell surface antigens and cell destruction via complement activation.

- It include autoimmune haemolytic anaemia, erythroblastosis foetalis.

(III) Type III Hypersensitivity

- It is immune complex mediated hypersensitivity.

- It occurs within 2-8 hrs.

- In this Ag-Ab complex deposited in various tissues.

- It include Arthus reaction, serum sickness.

(IV) Type IV Hypersensitivity

- Also known as 'Delayed Type hypersensitivity'.
- It is cell mediated hypersensitivity.
- It occurs within 24-72 hrs.
- In this sensitized T_{DTH} cells release cytokines that activate macrophage.
- It includes contact dermatitis and graft rejection.