Biyani's Think Tank

Concept based notes

Immunology

B.SC(BioTech)

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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 is a remarkable versatile defense system that has evolved to protect animals from invading pathogenic microorganisms and cancer. It is able to generate an enormous variety of cells and molecules capable of specifically recognizing and eliminating an apparently limitless variety of foreign invaders. These cells and molecules act together in a dynamic network whose complexity rivals that of the nervous system.

Q.2. What is immunity?

Ans. Immunity—the state of protection from infectious disease —has both a less specific and more specific component. The immunity is of two types:

a) The less specific component, **innate immunity,** provides the first line of defense against infection. Most components of innate immunity are present before the onset of infection and constitute a set of disease-resistance mechanisms that are not specific to a particular pathogen. Phagocytic cells, such as macrophages and neutrophils, barriers such as skin, and a variety of antimicrobial compounds synthesized by the host all play important roles in innate immunity.

b) The more specific component, **adaptive immunity,** comes into play when there is an antigenic challenge to the organism. Adaptive immunity responds to the challenge with a high degree of specificity as well as the remarkable property of "memory." Typically, there is an adaptive immune response against an antigen within five or six days after the initial exposure to that antigen. Exposure to the same antigen some time in the future results in a memory response: the immune response to the second

challenge occurs more quickly than the first, is stronger, and is often more effective in neutralizing and clearing the pathogen. The major agents of adaptive immunity are lymphocytes and the antibodies and other molecules they produce.

Q.3. What are antigens?

Ans. Substances that can be recognized by the immunoglobulin receptor of B cells, or by the T cell receptor when complexed with MHC, are called **antigens**. **Antigenicity** is the ability to combine specifically with the final products of the immune response responses i.e., antibodies and/or cell-surface receptors.

When an antigen induces a specific immune response, then, it is more appropriately called an **immunogen. Immunogenicity** is the ability to induce a humoral and/or cell mediated immune response.

The particular macromolecules of an infectious agent, generally either proteins or polysaccharides act as an immunogens. Proteins are the most potent immunogens, with polysaccharides ranking second. In contrast, lipids and nucleic acids of an infectious agent generally do not serve as immunogens unless they are complexed with proteins or polysaccharides. Proteins or polysaccharides acts as important immunogens in humoral immunity. For cell-mediated immunity, only proteins and some lipids and glycolipids serve as immunogens.

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

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

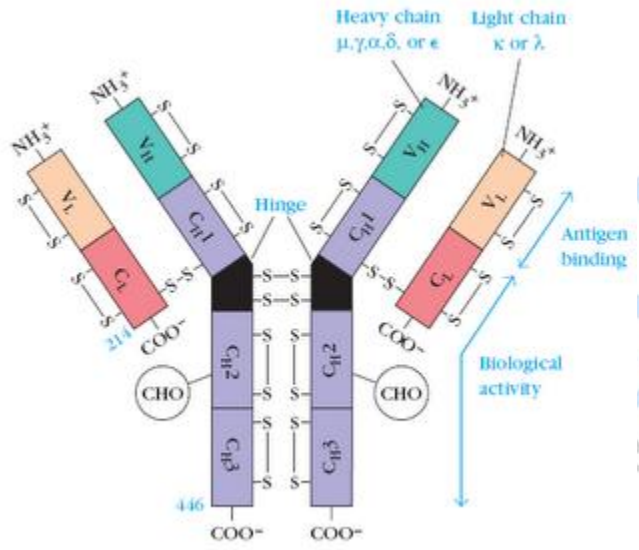
Structure of antibodies

Antibody molecules have a common structure of four peptide chains. This structure consists of two identical **light (L) chains,** polypeptides of about 25,000 molecular weight, and two identical **heavy (H) chains,** larger polypeptides of molecular weight 50,000 or more. Like the antibody molecules they constitute, H and L chains are also called immunoglobulins. Each light chain is bound to a heavy chain by a disulfide bond, and by such noncovalent interactions as salt linkages, hydrogen bonds, and hydrophobic bonds, to form a heterodimer (H-L). Similar noncovalent interactions and disulfide bridges link the two identical heavy and light (H-L) chain combinations to each other to form the basic four-chain $(H\text{-}L)_2$ antibody structure, a dimer of dimers. The exact number and precise positions of these interchain disulfide bonds differ among antibody classes and subclasses.

The first 110 or so amino acids of the amino-terminal region of a light or heavy chain varies greatly among antibodies of different specificity. These segments of highly variable sequence are called *V regions:* V_L in light chains and V_H in heavy. All of the differences in specificity displayed by different antibodies can be traced to differences in the amino acid sequences of V regions. In fact, most of the differences among antibodies fall within areas of the V regions called *complementarity- determining regions (CDRs),* and it is these CDRs, on both light and heavy chains, that constitute the antigen binding site of the antibody molecule. The regions of relatively constant sequence beyond the variable regions have been dubbed C regions, C_L on the light chain and C_H on the heavy chain. Antibodies are glycoproteins; with few exceptions, the sites of attachment for carbohydrates are restricted to the constant region.

The **constant (C) region** of light chain had two basic amino acid sequences. This led to the recognition that there were two light chain types, **kappa (κ)** and **lambda (λ).** In humans, 60% of the light chains are kappa and 40% are lambda, whereas in mice, 95% of the light chains are kappa and only 5% are lambda. A single antibody molecule contains only one light chain type, either κ or λ never both.

The constant region of the heavy chain revealed five basic sequence patterns, corresponding to five different constant (C) regions (μ, γ, α, δ and ε). Each of these five different heavy chains is called an **isotype.** The length of the constant regions is approximately 330 amino acids for δ, γ and α and 440 amino acids for μ and ε. The heavy chains of a given antibody molecule determine the class of that antibody: IgM(μ), IgG(γ),
IgA(α), IgD(δ), or IgE(ε). Each class can have either κ or λ light chains.



Q.5. Explain in detail different types of antibodies with their function.

Ans. There are five different types of antibodies. Each class is distinguished by unique amino acid sequences in the heavy-chain constant region that confer class-specific structural and functional properties.

Ig G : IgG, the most abundant class in serum, constitutes about 80% of the total serum immunoglobulin. The IgG molecule consists of two γ heavy chains and two κ or two λ light chains. There are four human IgG subclasses, distinguished by differences in γ -chain sequence and numbered according to their decreasing average serum concentrations: IgG1, IgG2, IgG3, and IgG4. The structural characteristics that distinguish these subclasses from one another are the size of the hinge region and the number and position of the interchain disulfide bonds

between the heavy chains. The subtle amino acid differences between subclasses of IgG affect the biological activity of the molecule:

- IgG1, IgG3, and IgG4 readily cross the placenta and play an important role in protecting the developing fetus.
- IgG3 is the most effective complement activator, followed by IgG1; IgG2 is less efficient, and IgG4 is not able to activate complement at all.
- IgG1 and IgG3 bind with high affinity to Fc receptors on phagocytic cells and thus mediate opsonization. IgG4 has an intermediate affinity for Fc receptors, and IgG2 has an extremely low affinity.

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.

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.

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.6. Explain the functions of cells and organs of the immune system.

Ans. The reticuloendothelial system mainly comprise of phagocytic cells whose function is to engulf microbes, immune complex from blood and tissues and participate in inflammation. This way they contribute to non-specific immunity. These cells also participate in specific immunity by way of antigen presentation and cytokine secretions. The deficiency of phagocytic system can lead to disorders such as Chronic Granulomatous Disease. The major phagocytic cells are:

- Polymorphonuclear leucocytes (PMNLs), also called neutrophils, microphages
- Blood and tissue monocytes.

They both are derived form the bone marrow during hematopoiesis.

Neutrophils have short life span. They circulate in the blood for 6-7 hours, then migrate through the endothelial cell junctions and reside in tissue spaces where they live only for few days and do not multiply. Neutrophils are the most abundant of the leukocytes, normally accounting for 54-75% of the WBCs. An adult typically has 3,000-7,500 neutrophils/mm^3 of blood but the number may increase two- to three-fold during active infections. Adult body usually produces 1011 neutrophils per day. Some neutrophils may remain attached to endothelial lining of large veins and can be mobilised during inflammation. The nucleus of a neutrophil is segmented into 3-5 connected lobes, hence the name polymorphonuclear leukocyte. They are called neutrophils because their granules stain poorly with the mixture of dyes used in staining leukocytes. Because of the granules, they are considered as one of the granulocytes. There are two types of granules, the specific granules and azurophilic granules. Specific granules are present in abundance and contain proteolytic enzymes such as lysozyme, collagenase and elastase. They stain neither with acidic nor basic dyes. The azurophilic granules are actually lysosomes.

Monocytes have rounded or kidney-shaped nuclei with finely granular cytoplasm, measure 12-15 μm and have half-life of 3 days in circulation. Monocytes

normally make up 2-8% of the WBCs (100-500/mm3 of blood). Once monocytes leave circulation and enter tissue, they are called **macrophages**. There are two types of macrophages, one that wander in the tissue spaces and the other that are fixed to vascular endothelium of liver, spleen, lymph node and other tissue. Tissue macrophages survive for months and can multiply. Macrophages present in different organs have been given different names. They are Histiocytes (in tissue), Kupffer cells (in liver), Alveolar macrophages (in lungs), Peritoneal macrophages (in peritoneum), Microglial cells (in brain), Mesangial cells (in kidneys) and Osteoclasts (in bone). Some macrophages develop abundant cytoplasm and are called epitheloid cells. Macrophages can fuse to form multi-nucleated giant cells. Some mononuclear cells differentiate into dendritic cells. Functions of macrophage include killing of microbes, infected cells, tumor cells, secretion of immunomodulatory cytokines, antigen processing and presentation to T cells. Macrophages respond to infections as quickly as neutrophils but persist much longer; hence they are dominant effector cells in the later stage of infection.

Microbial killing by phagocytes:

Phagocytosis involves two steps namely attachment and ingestion. Following attachment of the organism, invagination of the phagocyte results in the formation of a phagosome. Some capsulated bacteria don't attach to the phagocyte, but they can still be phagocytosed if they are coated with opsonins such as IgG and complement component (C3b). The engulfed bacteria are held inside a vacuole called phagosome. The formation of phagosome triggers respiratory bursts and fusion of lysosome with phagosome to form phagolysosome. The phagocytes appear to kill engulfed bacteria by two pathways, oxygen independent pathway and oxygen dependent pathway. The microbicidal mechanisms of the respiratory burst are termed oxygen dependent and phagolysosome formations are termed oxygen independent. Oxygen dependent mechanism involves catalytic conversion of molecular oxygen to oxyhalide free radicals, which are highly reactive oxidizing agents. The phagocyte oxidase present in the plasma membrane and phagolysosome reduce oxygen into reactive oxygen intermediates such as

superoxide radicals. Superoxide is converted to H_2O_2, which is used by enzyme myeloperoxidase to convert unreactive halide ions to reactive hypohalous acids that are toxic to bacteria. Oxygen independent mechanism involves release of lysosomal contents into phagolysosomes. The content of lysosome includes lactoferrin, cathepsin G, lysozyme and defensins etc. In addition to the phagocyte oxidase system, macrophages have free-radical generating system, namely inducible nitric oxide synthase. This cytosolic enzyme is absent in resting macrophages but can be induced in response to bacterial lipopolysaccharides and IFN-γ. This enzyme catalyses the conversion of arginine to citrulline, and in the process releases nitric oxide gas. Nitric oxide may then combine with H2O2 or superoxide to form highly reactive peroxynitrite radicals that kill the microbes.

Dendritic cells:

These cells are derived from myeloid progenitor in the bone marrow and are morphologically identified by spiny membranous projection on their surfaces. Immature dendritic cells are located in epithelia of skin, gastrointestinal tract and respiratory tract and are called langerhan cells. They express low levels of MHC proteins on their surface

and their main function is to capture and transport protein antigen to the draining lymph node. During their migration to the lymph node, dendritic cells mature into excellent antigen presenting cells (APC). Mature dendritic cells reside in the T cell area (paracortex) of the lymph node. Here, they are referred as interdigitating dendritic cells. These cells are distinct from the dendritic cells that occur in the germinal centers of lymphoid follicles (follicular dendritic cells) in lymph node, spleen and MALT. The follicular dendritic cells are not derived from the bone marrow and their role is to present antigen-antibody complex and complement products to B cell.

Lymphoid system:

Lymphoid organs are stationed throughout the body and are concerned with the growth, development and deployment of lymphocytes. These structurally and

functionally diverse lymphoid organs and tissues are interconnected by the blood vessels and lymphatic vessels through which lymphocytes circulate. The organs involved in specific as well as non-specific immunity are classified as primary (central) lymphoid organs and secondary (peripheral) lymphoid organs. The blood and lymphatic vessels that carry lymphocytes to and from the other structures can also be considered lymphoid organs. Recently, it has become accepted that the liver is also a hematopoietic organ, giving rise to all leukocyte lineages.

PRIMARY LYMPHOID ORGANS:

Also called central lymphoid organs, these are responsible for synthesis and maturation of immunocompetant cells. These include the bone marrow and the thymus.

BONE MARROW:

All the cells of the immune system are initially derived from the bone marrow through a process called hematopoiesis. During foetal development hematopoiesis occurs initially in yolk sac and para-aortic mesenchyme and later in the liver and spleen. This function is taken over gradually by the bone marrow. During hematopoiesis, bone marrow-derived stem cells differentiate into either mature cells or into precursors of cells that migrate out of the bone marrow to continue their maturation in thymus. The bone marrow produces B cells, natural killer cells, granulocytes and immature thymocytes, in addition to red blood cells and platelets. It is both a primary and secondary lymphoid organ. The proliferation and maturation of precursor cells in the bone marrow are stimulated by cytokines, many of which are called colony stimulating factors (CSFs). The bone marrow also contains antibody secreting plasma cells, which have migrated from the peripheral lymphoid tissue.

THYMUS:

The thymus is a gland located in the anterior mediastinum just above the heart, which reaches its greatest size just prior to birth, then atrophies with age. This

lymphoepithelial organ develops from ectoderm derived from the third branchial cleft and endoderm of the third branchial pouch. Immature lymphocytes begin to accumulate in the thymus of human embryos at about 90-100 days after fertilization. Initially most of these immature lymphocytes have come from the yolk sac and fetal liver rather than the bone marrow. Cells from the bone marrow, later migrate to the thymus as precursors and develop into mature peripheral T cells. Once the immature lymphocytes have passed the blood-thymus barrier they are called thymocytes. Mature T cells migrate from the thymus to secondary lymphoid organs such as lymph node, Peyer's patches and spleen. Ultimately the thymus becomes an encapsulated and consists of many lobes, each divided into an outer cortical region and an inner medulla. The cortex contains mostly immature thymocytes, some of which mature and migrate to the medulla, where they learn to discriminate between self and non-self during foetal development and for a short time after birth. T cells leave the medulla to enter the peripheral blood circulation, through which they are transported to the secondary lymphoid organs. About 98% of all T cells die in the thymus. The greatest rate of T cell production occurs before puberty. After puberty, the thymus shrinks and the production of new T cells in the adult thymus drops away. Children with no development of thymus suffer from DiGeorge syndrome that is characterized by deficiency in T cell development but normal numbers of B cells.

PERIPHERAL LYMPHOID ORGANS

While primary lymphoid organs are concerned with production and maturation of lymphoid cells, the secondary or peripheral lymphoid organs are sites where the lymphocytes localise, recognise foreign antigen and mount response against it. These include the lymph nodes, spleen, tonsils, adenoids, appendix, and clumps of lymphoid tissue in the small intestine known as Peyer's patches. They trap and concentrate foreign substances, and they are the main sites of production of antibodies. Some lymphoid organs are capsulated such as lymph node and spleen while others are non-capsulated, which include mostly mucosa-associated lymphoid tissue (MALT).

LYMPH NODE:

Clusters of lymph nodes are strategically placed in the neck, axillae, groin, mediastinum and abdominal cavity, where they filter antigens from the interstitial tissue fluid and the lymph during its passage from the periphery to the thoracic duct. The key lymph nodes are the axillary lymph nodes, the inguinal lymph nodes, the mesenteric lymph nodes and the cervical lymph nodes. Lymph nodes that protect the skin are termed somatic nodes, while deep lymph nodes protecting the respiratory, digestive and genitourinary tracts are termed visceral nodes. Each lymph node is surrounded by a fibrous capsule that is pierced by numerous afferent lymphatics that drain lymph into marginal sinus. The lymph flows through the medullary sinus and leaves through efferent lymphatics. Each lymph node is divided into an outer cortex, inner medulla and intervening paracortical region. The cortex is also referred as B cell area, which mainly consists of B cells. The cortex is a high traffic zone where recirculating Tand B lymphocytes enter from the blood. Aggregates of cells called follicles are present in the cortex, which in turn may have central areas called germinal centers. Follicles without germinal centers are called primary follicles and those with germinal centers are called secondary follicles. Primary follicles are rich in mature but resting B cells. Germinal centers develop in response to antigenic stimulation and consist of follicular dendritic cells and reactive B cells. The medulla contains a mixture of B cells, T cells, plasma cells and macrophages. The medulla consists of medullary cords that lead to the medullary sinus. The cords are populated by plasma cells and macrophages. Between these two zones, lie the paracotex (T cell area) that contains T lymphocytes, dendritic cells and mononuclear phagocytes. Most of the T cells (70%) located there are CD4+ helper cells.

SPLEEN:

Situated in the left upper quadrant of the abdomen and weighing about 150 grams, spleen is the largest single lymphoid organ in the body. It has a dense fibrous capsule with muscular trabeculae extending inward to subdivide the spleen into

lobules. It filters blood and is the major organ in which antibodies are synthesized and released into circulation. In addition to capturing foreign antigens from the blood that passes through the spleen, migratory macrophages and dendritic cells also bring antigens to the spleen via the bloodstream. Persons lacking spleen (eg. splenectomy) are highly susceptible to infections with capsulated bacteria such as pneumococci and meningococci. Spleen is the major site for phagocytosis of antibody coated bacteria and destruction of aged RBCs. It is supplied by splenic artery, which pierces the capsule at hilum and divides into smaller branches that are surrounded by fibrous trabeculae. The spleen is composed of two types of tissue, the red pulp and the white pulp. The red pulp contains vascular sinusoids, large number of erythrocytes, resident macrophages, dendritic cells, granulocytes, few plasma cells and lymphocytes. It is the site where aged platelets and erythrocytes are destroyed. The white pulp contains the lymphoid tissue clustered around small arterioles and is known as a periarteriolar lymphoid sheath (PALS). PALS contain mainly T lymphocytes, about 75% of which are CD4+ helper T cells. Attached to this are lymphoid follicles, some of which contain germinal centers. Follicles and germinal center predominantly contain B cells. The PALS and follicles are surrounded by rim of lymphocytes and macrophages, called marginal zone. Marginal zone is composed of macrophages, B cells, and CD4+ helper T cells. The arterioles end in vascular sinusoids in the red pulp, which in turn end in venules that drain into splenic vein. Antigens and lymphocytes enter the spleen through vascular sinusoids. Activation of B cells occurs at the juncton between follicle and PALS. Activated B cells then migrate to the germinal centers or into the red pulp.

MUCOSA ASSOCIATED LYMPHOID TISSUE (MALT):

Approximately >50% of lymphoid tissue in the body is found associated with the mucosal system. MALT is composed of gut-associated lymphoid tissues (GALT) lining the intestinal tract, bronchus-associated lymphoid tissue (BALT) lining the respiratory tract, and lymphoid tissue lining the genitourinary tract. The respiratory, alimentary and genitourinary tracts are guarded by subepithelial

accumulations of lymphoid tissue that are not covered by connective tissue capsule. They may occur as diffuse collections of lymphocytes, plasma cells and phagocytes throughout the lung and lamina propria of intestine or as clearly organised tissue with well-formed lymphoid follicles. The well-formed follicles include the tonsils (lingual, palatine and pharyngeal), Peyer's patches in the intestine and appendix. The major function of these organs is to provide local immunity by way of sIgA (also IgE) production. Diffuse accumulations of lymphoid tissue are seen in the lamina propria of the intestinal wall. The intestinal epithelium overlying the Peyer's patches is specialized to allow the transport of antigens into the lymphoid tissue. This function is carried out by cuboidal absorptive epithelial cells termed "M" cells, so called because they have numerous microfolds on their luminal surface. M cells endocytose, transport and present antigens to subepithelial lymphoid cells. Majority of intra-epithelial lymphocytes are T cells, and most often CD8+ lymphocytes. The intestinal lamina propria contains $CD4^+$ lymphocytes, large number of B cells, plasma cells, macrophages, dendritic cells, eosinophils and mast cells. Peyer's patches contain both B cells and $CD4^+$ T cells.

LYMPHOCYTES:

Lymphocytes are stem cells derived cells that mature either in the bone marrow or thymus. Lymphocytes comprise 20-40% (1000 - 4000 cells/µl) of all leukocytes. The lymphocytes are distributed to blood, lymph and lymphoid organs. Typically, lymphocyte is small, round, cell with diameter of 5-10µm, spherical nucleus, densely compacted nuclear chromatin and scanty cytoplasm. Though the cytoplasm contains mitochondria and ribosomes, other organelles are not detectable. Such mature but resting lymphocytes are known as naïve cells. They are mitotically inactive but when stimulated can undergo cell division. Naïve lymphocytes have a short life span and die in few days after leaving bone marrow or thymus unless they are stimulated. Once the lymphocyte is activated (stimulated), they become large (10-12µm), have more cytoplasm and more organelles. Activated lymphocytes may undergo several successive rounds of cell

division over a period of several days. Some of the progeny cells revert to the resting stage and become memory cells, but can survive for several years in the absence of any antigenic stimulus. There are three major types of lymphocyte, B lymphocyte, T lymphocyte and NK cells. Different lymphocytes are identified by certain protein markers on their surface called "cluster of differentiation" or "CD" system. One marker that all leukocytes have in common is CD45. The presence of the markers can be detected using specific monoclonal antibodies.

B LYMPHOCYTE:

Also called B-cells, they are so called because in birds they were found to mature in bursa of fabricius. Humans don't have an anatomical equivalent to bursa, but the development and maturation of these cells occur in bone marrow. In mammals, the early stages of B cell maturation occur in the fetal liver and bone marrow. B cell development begins in the fetal liver and continues in the bone marrow throughout life. The stages in B cell development in the bone marrow are:

Stem cell > pro-B cell > pre-B cell > small pre-B cell > immature B cell > mature B cell.

Distribution:

They account for 5-15% of lymphocytes (250 cells/μl) in circulation and 80-90% in bone marrow, 20-30% in lymph node and 50-60% in spleen.

Surface markers:

The most important surface marker on the surface of mature B cell is the surface immunoglobulin. The surface immunoglobulins are of IgM and IgD type. A B cell will have approximately 109 immunoglubulins of single specificity on its surface. Markers/Receptors on B cells are Surface Immunoglobulin (IgM and IgD), CD40, B7, ICAM-1, LFA-1, MHC II, CD32 (Ig Fc receptor), CD35 (Receptor for complement component) and additional markers that distinguish B cells such as CD19, CD20, CD21 and CD22.

Demonstration of B cells:

EAC (Erythrocyte Amboceptor Complement) Rosettes: When sheep RBCs coated with antibody and treated with complement and B cells, a rosette is formed due to the presence of complement receptor on B cells. B cells can be demonstrated by immunofluorescence with fluorescent-labelled monoclonal antibodies against surface markers such as surface immunoglobulin. On stimulation by pokeweed mitogen, they undergo blast transformation.

Functions of B-cells:

Direct antigen recognition and Antigen presentation B cells may differentiate into plasma cells (which secrete large amounts of antibodies) or into memory B cells. Memory cells can survive 20 years or more.

Plasma cells:

These are the effector cells of the B-cell lineage and are specialised in secreting immunoglobulins. When activated B cells divide, some of its progeny become memory cells and the reminder become immunoglobulin-secreting plasma cells. Plasma cells are oval or egg shaped, have eccentrically placed nuclei, have abundant cytoplasm containing dense rough endoplasmic reticulum (the site of antibody production), perinuclear Golgi body (where immunoglobulins are converted to final form and packaged). Unlike B cells, immunoglobulins are not present on the surface of plasma cells. They have a short life span of few days to few weeks.

T LYMPHOCYTE:

Ontogeny:

The name "T-cell" is an abbreviation of "thymus dependent lymphocyte". T lymphocytes arise in the bone marrow as T-cell precursors, then migrate to and mature in the thymus. After entry into the thymus T-cell precursors are also referred to as "thymocytes". In the thymus there are rearrangements at gene segments coding for the variable part of the TCR (T Cell Receptor) resulting in

generation of diversity. T Cell Receptors are then expressed on the surface, which is followed by expression of either CD8 or CD4 surface molecules. Those cells expressing receptors that can interact with self MHC molecules are positively selected while those cells that express receptors that recognize peptides derived from self protein in association with self MHC are negatively selected. Such cells undergo clonal deletion or anergy.

Distribution:

T cell accounts for 70-80% (1500 cells/µl) lymphocytes in peripheral blood, 5-10% in bone marrow, 70-80% in lymph node and 30-40% in spleen.

Surface markers:

The most important surface receptor is TCR. TCR are polypeptides that belong to the immunoglobulin superfamily. There are two kinds of TCR, one composed of a α-β heterodimer (TCR2) and the other composed of a γ-δ heterodimer (TCR1). An individual T cell can express either α-β or γ-δ as its receptor but never both. 95% of T cells express the α-β heterodimer. The other markers/receptors present on the surface are IL-2R, IL-1R, CD2, CD3, CD4/CD8, CD28, ICAM-1 and LFA-1. Nearly all the mature T lymphocytes express both CD2 and CD3 on their surface. CD3, which is always found closely associated with TCR, is necessary for signal transduction following antigen recognition by the TCR.

Subsets of T Cells:

There are two major types of T cells, Helper (CD4) and Cytotoxic/Suppressor (CD8) T cells. CD4 cells account for 45% (900/µl) of lymphocytes while CD8 cells account for 30% (600/µl).

Helper T cells (TH) secrete cytokines that promote the proliferation and differentiation of cytotoxic T cells, B cells and macrophages and activation of inflammatory leukocytes. TH cells are identified by the presence of the CD4 marker. They recognize antigen when presented along with Class II MHC

molecules. TH cells are further subdivided into the TH1 and TH2 subsets on the basis of the kinds of cytokines they produce. TH1 cells produce interleukin-2 (IL-2), interferon-gamma (IFNγ), and tumour necrosis factor-beta (TNF-β) while TH_2 cells produce IL-4, IL-5, IL-6, IL-10 and TGF-β.

Cytotoxic T cells (T_C) lyse cells with foreign antigens, e.g. tumour cells, virus-infected cells, and foreign tissue grafts. TC cells are identified by the presence of the CD8 marker. They recognize antigen presented when presented along with Class I MHC molecules. The suppressor T cells have a role in down regulation of immune response.

Demonstration of T cells:

- T cells can be demonstrated by immunofluorescence using fluorescent-labelled monoclonal antibodies against TCR or other surface markers.
- E-Rosette/ SRBC rosette: T cells bind to sheep RBCs at 37^oC forming rosettes.
- They undergo blast transformation on treatment with mitogens such as phytohemagglutinin (PHA) or Concanavalin A.

Functions of Helper T-cells (T_H):

Promotes differentiation of B-cells and cytotoxic T-cells

Activates macrophages

Functions of Cytotoxic/Suppressor T-cells (CTL):

Kills cells expressing appropriate antigen

Downregulates the activities of other cells

NK CELLS (LARGE GRANULAR LYMPHOCYTES):

Also called Large Granular Lymphocytes (LGLs), these are large lymphocytes containing azurophilic granules in the cytoplasm. NK cells derive form bone marrow but don't require thymus for development. NK cells are so called because they kill variety of target cells (such as tumour cells, virus-infected cells, transplanted cells) without the participation of MHC molecules. They can kill target cell without a need

for activation unlike cytotoxic T lymphocytes. Hence they mediate a form of natural (innate) immunity.

Distribution:

They account for 10-15% of blood lymphocytes. They are rare in lymph nodes and don't circulate through lymph.

Surface markers:

NK cells lack any surface immunoglobulins, TCR or CD4 makers; instead they have CD16 (Immunoglobulin Fc receptor) and CD56. Approximately 50% of human NK cells express only one form of CD8. Other receptors include IL-2R, CD2, ICAM-1 and LFA-1.

Functions:

NK cells are activated by recognition of antibody-coated cells, virus infected cell, cell infected with intracellular bacteria and cells lacking MHC I proteins. Activation of NK cell results in cytolysis of target and cytokine secretion but no clonal expansion. Interestingly, NK cells are inhibited on contact with MHC I proteins. NK cells can kill antibody-coated target cells, which is mediated through Fc receptor present on its surface. This is called antibody-dependent cell cytotoxicity (ADCC). NK cells also participate in Graft vs Host reaction in recipient of bone marrow transplants. NK cells can be activated by IL-2 so that their cytotoxic capacity is enhanced. Such cells are called Lymphokine Activated Killer cells (LAK) and have been used clinically to treat tumours. LAK cells have enhanced cytolytic activity and are effective against wide range of tumour cells. Activated NK cells produce cytokines such as IFN-γ, TNFα, GM-CSF and CSF-1 all of which are immunomodulators.

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) Pliotropy : 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 include contact dermatitis and graft rejection.

Q.9. Explain Transplantation and its mechanism.

Ans. Transplantation refers to the action of transferring cells, tissues or organs from one site to another.

- The transferred or implanted tissue is called as graft or transplant.

- An individual from graft is taken is called as donor and from whom its taken is called as recipient.

Types of Transplantation :

(a) Autograft : Self tissue transfer e.g. skin.
(b) Isograft : Between genetically identicaller species.
(c) Allograft : Transplantation between genetically different species.
(d) Xenograft : Between different species.
After transplantation two conditions occur

(i) Graft Acceptance
(ii) Graft Rejection : This is classified in two types :
(a) Host versus Graft Rejection

(b) Graft versus Host Reaction.

Before transplantation tissue typing is done to minimize the graft rejection. For tissue typing microcytotoxicity test is done.

Q.10. What is autoimmunity. Name the various diseases associated with it.

Ans. It is a condition in which structural or functional damage is produced by the action of Ab against own cells.

- Autoimmunity is the protection against self cells and injury to self cells so called auto allergy.
- Cell or tissues undergo antigenic alteration due to physical, chemical or biological influences. These are :

(a) Physical : Irradiation, photo-sensitivity.

(b) Chemical : Combines with cells and result in neo or altered antigen.

(c) Biological : Infectious agents like viruses etc.

(d) Mutations : Altered Ags may rise due to mutations in cells.

Based on the involvement and nature of lesions autoimmune disease are classified as :

(I) Haemolytic Autoimmune Diseases

(a) Autoimmune haemolytic anemia

- Auto antibodies are generated against own erythrocytes.

(b) Autoimmune thrombocytopenia

- Auto antibodies are directed against platelets.

(c) Autoimmune Leucopenia

- Auto antibodies are generated against leukocytes.

(II) Localized (Organ Specific) Autoimmune Disease

(a) Autoimmune disease of thyroid Gland

(i) Hashimatos Disease

- Enlargement of thyroid gland.

(ii) Grave's Disease

- Patients possess Ab to thyroglobulin.

(b) Addison's Disease

- It occurs due to Ab's formation against cells of Zona glomerulosa.

(c) Pernicious Anaemia

- Ab acts against parental cells of gastric mucosa.

- Ab's against intrinsic factor and prevents adsorption of vit B12.

(d) Good Pasteur's Syndrome

- Auto antibodies are generated specific for certain basement membrane antigens.

(e) Myasthenia gravis

(f) Insulin dependent diabetes mellitus.

(III) Systemic (Non-specific) Autoimmune Disease

(a) Systemic Lupus Erythematous (SLE)

- Antibodies are generated against vast array of tissue antigens.

- Interaction of these autoantibodies with their specific Ag produces various symptoms.

(b) Rheumatoid Arthritis

- Auto antibodies are called as rheumatoid factors, which react with Fe of Ig G, resulting in deposition of Ig M and Ig G complexes in joint.

Q.11. What do you mean by immuno deficiency diseases?

A**ns.** **Immunodeficiency** (or **immune deficiency**) is a state in which the immune system's ability to fight infectious disease is compromised or entirely absent. Immunodeficiency may also decrease cancer immunosurveillance. Most cases of immunodeficiency are acquired ("secondary") but some people are born with

defects in their immune system, or primary immunodeficiency. Transplant patients take medications to suppress their immune system as an anti-rejection measure, as do some patients suffering from an over-active immune system. A person who has an immunodeficiency of any kind is said to be **immunocompromised**. An immunocompromised person may be particularly vulnerable to opportunistic infections, in addition to normal infections that could affect everyone.

Types

By affected component

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

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

Primary or secondary

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

Primary immunodeficiency (PID)

A number of rare diseases feature a heightened susceptibility to infections from childhood onward. Primary Immunodeficiency is also known as congenital immunodeficiencies. Many of these disorders are hereditary and are autosomal recessive or X-linked. There are over 80 recognised primary immunodeficiency syndromes; they are generally grouped by the part of the immune system that is malfunctioning, such as lymphocytes or granulocytes. The treatment of primary immunodeficiencies depends on the nature of the defect, and may involve antibody infusions, long-term antibiotics and (in some cases) stem cell transplantation.

Secondary immunodeficiencies

Secondary immunodeficiencies, also known as acquired immunodeficiencies, can result from various immunosuppresive agents, for example, malnutrition, aging and particular medications (e.g. chemotherapy, disease-modifying antirheumatic drugs, immunosuppressive drugs after organ transplants, glucocorticoids). For medications, the term immunosuppression generally refers to both beneficial and potential adverse effects of decreasing the function of the immune system, while the term *immunodeficiency*generally refers solely to the adverse effect of increased risk for infection. Many specific diseases directly or indirectly cause immunosuppression. This includes many types of cancer, particularly those of the bone marrow and blood cells (leukemia, lymphoma, multiple myeloma), and certain chronic infections. Immunodeficiency is also the hallmark of acquired immunodeficiency syndrome (AIDS), caused by thehuman immunodeficiency virus (HIV). HIV directly infects a small number of T helper cells, and also impairs other immune system responses indirectly.

Q.12. What is tumor and explain tumor immunology.

Ans. When there is normal growth control mechanism becomes defective, it leads to abnormal growth and proliferation leads to the formation of tumor or neoplasm.

Tumor is of 2 types :

(a) Benign : A tumor cell which is not capable of indefinite growth and does not invade the surrounding healthy tissue.

(b) Malignant : Tumor cell which is capable of continuous growth and becomes progressively invasive, these tumors also exhibit metastasis.

Types of Cancer :

(a) Carcinoma

(b) Leukanua

(c) Lymphoma

(d) Sarcoma

- Tumor appearance depends on the tumor antigen which appear due to mainly 3 reasons :

(a) Mutation

(b) Gene Activation

(c) Clonal Amplification

Various categories of tumor antigens are :

(a) Normal Cellular Gene Products

(i) Oncofetal Antigens

e.g. – Melanoma Associated Ag's, Carcinoembryonic antigen, á-fetoprotein

(b) Mutant Cellular Gene Products

(c) Tumor Antigens Concoded by Oncogenes.

- Some tumors are caused by viruses e.g. : Epstein Bars Vines.

- The effactor mechanism which is functional are :

(a) By NK Cell

(b) Cytotoxic T-Lymphocytes

(c) Cytokines

Q.13. Explain the immune responses to various infectious diseases.

A**ns.** Majorally four types of pathogen which causes infection are:

(a) Viruses

(b) Bacteria

(c) Protoza

(d) Helminths

(**A) Immune response to viral infection is**

Response	**Effactor Molecules**	**Activity**
Humoral	Ig A, Ig M, Ig G	- Blocks the binding of virus to the host cell. - Activate Complement System.
Cell Mediated Immunity	Interferons, NK cells and macrophages	Have direct anti-viral activity active effactor CTL they kill the virus infected host cells.

(B) Immune responses to Bacterial Infection

Infection Process	**Host Defence**	**Bacterial Evasion Mech.**
(i) Attachment to host cells	Blockage of attachment by IgA	Secretion of proteases
(ii) Proliferation		
(a) Phagocytosis		
(b) Complement Lysis	Production of surface structure like polysacc capsules.	
(iii) Invasion of host tissue	Ab mediated agglutn reaction.	Secretion of elastase mech.
(iv) Toxins which damage the host cell	Neutralization	Secretion of hylauronilase.

(c) Immune response to Protozoan Disease

- In general humoral ab's are effective against it but once the protozoans has infected the host cell than CMI (Cell Mediated Immunity) is necessary.

(D) Immune response to helminths disease

- A low level of immunity is developed as helminthes normally do not multiply within the cell.

Q.14. What are Ag-Ab reactions?

Ans. NATURE OF ANTIGEN-ANTIBODY REACTIONS

Lock and Key Concept

The combining site of an antibody is located in the Fab portion of the molecule and is constructed from the hypervariable regions of the heavy and light chains. X-Ray crystallography studies of antigen-antibody interactions show that the antigenic determinant nestles in a cleft formed by the combining site of the antibody. Thus, our concept of antigen-antibody reactions is one of a key (*i.e.* the antigen) which fits into a lock (*i.e.* the antibody).

Non-covalent Bonds

The bonds that hold the antigen to the antibody combining site are all non-covalent in nature. These include hydrogen bonds, electrostatic bonds, Vander Waals forces and hydrophobic bonds. Multiple bonding between the antigen and the antibody ensures that the antigen will be bound tightly to the antibody.

Reversibility

Since antigen-antibody reactions occur via non-covalent bonds, they are by their nature reversible.

AFFINITY AND AVIDITY

Affinity

Antibody affinity is the strength of the reaction between a single antigenic determinant and a single combining site on the antibody. It is the sum of the attractive and repulsive forces operating between the antigenic determinant and the combining site of the antibody. Most antibodies have a high affinity for their antigens.

Avidity

Avidity is a measure of the overall strength of binding of an antigen with many antigenic determinants and multivalent antibodies. Avidity is influenced by both the valence of the antibody and the valence of the antigen. Avidity is more than the sum of the individual affinities. Affinity refers to the strength of binding between a single antigenic determinant and an individual antibody combining site whereas avidity refers to the overall strength of binding between multivalent antigens and antibodies.

SPECIFICITY AND CROSS REACTIVITY

Specificity

Specificity refers to the ability of an individual antibody combining site to react with only one antigenic determinant or the ability of a population of antibody molecules to react with only one antigen. In general, there is a high degree of specificity in antigen-antibody reactions. Antibodies can distinguish differences in:

- The primary structure of an antigen
- Isomeric forms of an antigen
- Secondary and tertiary structure of an antigen

Cross reactivity

Cross reactivity refers to the ability of an individual antibody combining site to react with more than one antigenic determinant or the ability of a population of antibody molecules to react with more than one antigen. Cross reactions arise because the cross reacting antigen shares an epitope in common with the immunizing antigen or because it has an epitope which is structurally similar to one on the immunizing antigen (multispecificity).

TESTS FOR ANTIGEN-ANTIBODY REACTIONS

Factors affecting measurement of antigen-antibody reactions

The only way that one knows that an antigen-antibody reaction has occurred is to have some means of directly or indirectly detecting the complexes formed between the antigen and antibody. The ease with which one can detect antigen-antibody reactions will depend on a number of factors.

Affinity

The higher the affinity of the antibody for the antigen, the more stable will be the interaction. Thus, the ease with which one can detect the interaction is enhanced.

Avidity

Reactions between multivalent antigens and multivalent antibodies are more stable and thus easier to detect.

Antigen to antibody ratio

The ratio between the antigen and antibody influences the detection of antigen-antibody complexes because the size of the complexes formed is related to the concentration of the antigen and antibody.

Physical form of the antigen

The physical form of the antigen influences how one detects its reaction with an antibody. If the antigen is a particulate, one generally looks for agglutination of the antigen by the antibody. If the antigen is soluble one generally looks for the precipitation of the antigen after the production of large insoluble antigen-antibody complexes.

Agglutination Tests

Agglutination/Hemagglutination

When the antigen is particulate, the reaction of an antibody with the antigen can be detected by agglutination (clumping) of the antigen. The general term agglutinin is used to describe antibodies that agglutinate particulate antigens. When the antigen is an erythrocyte the term hemagglutination is used. All antibodies can theoretically agglutinate particulate antigens but IgM, due to its high valence, is particularly good agglutinin and one sometimes infers that an antibody may be of the IgM class if it is a good agglutinating antibody.

Qualitative agglutination test

Agglutination tests can be used in a qualitative manner to assay for the presence of an antigen or an antibody. The antibody is mixed with the particulate antigen and a positive test is indicated by the agglutination of the particulate antigen. For example, a patient's red blood cells can be mixed with antibody to a blood group antigen to determine a person's blood type. In a second example, a patient's serum is mixed with red blood cells of a known blood type to assay for the presence of antibodies to that blood type in the patient's serum.

Quantitative agglutination test

Agglutination tests can also be used to measure the level of antibodies to particulate antigens. In this test, serial dilutions are made of a sample to be tested for antibody and then a fixed number of red blood cells or bacteria or other such particulate antigen is added. Then the maximum dilution that gives agglutination is determined.

The maximum dilution that gives visible agglutination is called the titer. The results are reported as the reciprocal of the maximal dilution that gives visible agglutination.

Prozone effect - Occasionally, it is observed that when the concentration of antibody is high (i.e. lower dilutions), there is no agglutination and then, as the sample is diluted, agglutination occurs. The lack of agglutination at high concentrations of antibodies is called the prozone effect. Lack of agglutination in the prozone is due to antibody excess resulting in very small complexes that do not clump to form visible agglutination.

Applications of agglutination tests

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

ii. To assess bacterial infections

e.g. A rise in titer of an antibody to a particular bacterium indicates an infection with that bacterial type. N.B. a fourfold rise in titer is generally taken as a significant rise in antibody titer.

Passive hemagglutination

The agglutination test only works with particulate antigens. However, it is possible to coat erythrocytes with a soluble antigen (e.g. viral antigen, a polysaccharide or a hapten) and use the coated red blood cells in an agglutination test for antibody to the soluble antigen. This is called passive hemagglutination. The test is performed just like the agglutination test. Applications include detection of antibodies to soluble antigens and detection of antibodies to viral antigens.

Hemagglutination Inhibition

The agglutination test can be modified to be used for the measurement of soluble antigens. This test is called hemagglutination inhibition. It is called hemagglutination inhibition because one measures the ability of soluble antigen to inhibit the agglutination of antigen-coated red blood cells by antibodies. In this test, a fixed amount of antibodies to the antigen in question is mixed with a fixed amount of red blood cells coated with the antigen (see passive hemagglutination above). Also

included in the mixture are different amounts of the sample to be analyzed for the presence of the antigen. If the sample contains the antigen, the soluble antigen will compete with the antigen coated on the red blood cells for binding to the antibodies, thereby inhibiting the agglutination of the red blood cells. By serially diluting the sample, you can quantitate the amount of antigen in your unknown sample by its titer. This test is generally used to quantitate soluble antigens and is subject to the same practical considerations as the agglutination test.

Precipitation tests

Radial Immunodiffusion (Mancini)

In radial immunodiffusion antibody is incorporated into the agar gel as it is poured and different dilutions of the antigen are placed in holes punched into the agar. As the antigen diffuses into the gel, it reacts with the antibody and when the equivalence point is reached a ring of precipitation is formed.

The diameter of the ring is proportional to the log of the concentration of antigen since the amount of antibody is constant. Thus, by running different concentrations of a standard antigen one can generate a standard cure from which one can quantitate the amount of an antigen in an unknown sample. Thus, this is a quantitative test. If more than one ring appears in the test, more than one antigen/antibody reaction has occurred. This could be due to a mixture of antigens or antibodies. This test is commonly used in the clinical laboratory for the determination of immunoglobulin levels in patient samples.

Immunoelectrophoresis

In immunoelectrophoresis, a complex mixture of antigens is placed in a well punched out of an agar gel and the antigens are electrophoresed so that the antigen is separated according to their charge. After electrophoresis, a trough is cut in the gel and antibodies are added. As the antibodies diffuse into the agar, precipitin lines are produced in the equivalence zone when an antigen/antibody reaction.

This tests is used for the qualitative analysis of complex mixtures of antigens, although a crude measure of quantity (thickness of the line) can be obtained. This test is commonly used for the analysis of components in a patient' serum. Serum is placed in the well and antibody to whole serum in the trough. By comparisons to normal serum, one can determine whether there are deficiencies on one or more serum components or whether there is an overabundance of some serum component (thickness of the line). This test can also be used to evaluate purity of isolated serum proteins.

Radioimmunoassay (RIA)/Enzyme Linked Immunosorbent Assay (ELISA)

Radioimmunoassays (RIA) are assays that are based on the measurement of radioactivity associated with immune complexes. In any particular test, the label may be on either the antigen or the antibody. Enzyme Linked Immunosorbent Assays (ELISA) are those that are based on the measurement of an enzymatic reaction associated with immune complexes. In any particular assay, the enzyme may be linked to either the antigen or the antibody.

Competitive RIA/ELISA for Ag Detection

By using known amounts of a standard unlabeled antigen, one can generate a standard curve relating radioactivity (cpm) (Enzyme) bound versus amount of antigen. From this standard curve, one can determine the amount of an antigen in an unknown sample.

The key to the assay is the separation of the immune complexes from the remainder of the components. This has been accomplished in many different ways and serves as the basis for the names given to the assay:

Precipitation with ammonium sulphate

Ammonium sulphate (33 - 50% final concentration) will precipitate immunoglobulins but not many antigens. Thus, this can be used to separate the immune complexes from free antigen. This has been called the Farr Technique

Anti-immunoglobulin antibody

The addition of a second antibody directed against the first antibody can result in the precipitation of the immune complexes and thus the separation of the complexes from free antigen.

Immobilization of the Antibody

The antibody can be immobilized onto the surface of a plastic bead or coated onto the surface of a plastic plate and thus the immune complexes can easily be separated from the other components by simply washing the beads or plate. This is the most common method used today and is referred to as Solid phase RIA or ELISA. In the clinical laboratory, competitive RIA and ELISA are commonly used to quantitate serum proteins, hormones, drugs metabolites.

Non-competitive RIA/ELISA for Ag or Ab

Non-competitive RIA and ELISAs are also used for the measurement of antigens and antibodies. The bead is coated with the antigen and is used for the detection of antibody in the unknown sample. The amount of labeled second antibody bound is related to the amount of antibody in the unknown sample. This assay is commonly employed for the measurement of antibodies of the IgE class directed against particular allergens by using a known allergen as antigen and anti-IgE antibodies as the labeled reagent. It is called the RAST test (radioallergosorbent test). The bead is coated with antibody and is used to measure an unknown antigen. The amount of labeled second antibody that binds is proportional to the amount of antigen that bound to the first antibody.

Tests for Cell Associated Antigens

Immunofluorescence

Immunofluorescence is a technique whereby an antibody labeled with a fluorescent molecule (fluorescein or rhodamine or one of many other fluorescent dyes) is used to detect the presence of an antigen in or on a cell or tissue by the fluorescence emitted by the bound antibody.

Direct Immunofluorescence

In direct immunofluorescence, the antibody specific to the antigen is directly tagged with the fluorochrome

Indirect Immunofluorescence

In indirect immunofluorescence, the antibody specific for the antigen is unlabeled and a second anti-immunoglobulin antibody directed toward the first antibody is tagged with the fluorochrome. Indirect fluorescence is more sensitive than direct immunofluorescence since there is amplification of the signal.

Flow Cytometry

Flow cytometry is commonly used in the clinical laboratory to identify and enumerate cells bearing a particular antigen. Cells in suspension are labeled with a fluorescent tag by either direct or indirect immunofluorescence. The cells are then analyzed on the flow cytometer.

In a flow cytometer, the cells exit a flow cell and are illuminated with a laser beam. The amount of laser light that is scattered off the cells as they passes through the laser can be measured, which gives information concerning the size of the cells. In addition, the laser can excite the fluorochrome on the cells and the fluorescent light emitted by the cells can be measured by one or more detectors.

The type of data that is obtained from the flow cytometer is represented in a one parameter histogram, increasing amount of fluorescence (*e.g.* green fluorescence) is

plotted on the x axis and the number of cells exhibiting that amount of fluorescence is plotted on the y axis. The fraction of cells that are fluorescent can be determined by integrating the area under the curve. In a two parameter histogram, the x axis is one parameter (*e.g.* red fluorescence) and the y axis is the second parameter (*e.g.* green fluorescence). The number of cells is indicated by the contour and the intensity of the color.

Complement Fixation

Antigen/antibody complexes can also be measured by their ability to fix complement because an antigen/antibody complex will "consume" complement if it is present, whereas free antigens or antibodies do not. Tests for antigen/antibody complexes that rely on the consumption of complement are termed complement fixation tests and are used to quantitate antigen/antibody reactions. This test will only work with complement fixing antibodies (IgG and IgM are best).

Antigen is mixed with the test serum to be assayed for antibody and antigen/antibody complexes are allowed to form. A control tube in which no antigen is added is also prepared. If no antigen/antibody complexes are present in the tube, none of the complement will be fixed. However, if antigen/antibody complexes are present, they will fix complement and thereby reduce the amount of complement in the tube. After allowing complement fixation by any antigen/antibody complexes, a standard amount of red blood cells, which have been pre-coated with anti-erythrocyte antibodies is added. The amount of antibody-coated red blood cells is predetermined to be just enough to completely use up all the complement initially added, if it were still there. If all the complement was still present (i.e. no antigen/antibody complexes formed between the antigen and antibody in question), all the red cells will be lysed. If antigen/antibody complexes are formed between the antigen and antibody in question, some of the complement will be consumed and, thus, when the antibody-coated red cells are added not all of them will lyse. By simply measuring the amount of red cell lysis by measuring the release of hemoglobin into the medium, one can indirectly quantitate antigen/antibody complexes in the tube. Complement fixation tests are

most commonly used to assay for antibody in a test sample but they can be modified to measure antigen.

Q 15. What is complement system?

Ans. The complement system helps or "complements" the ability of antibodies and phagocytic cells to clear pathogens from an organism. It is part of the immune system called the innate immune system that is not adaptable and does not change over the course of an individual's lifetime. However, it can be recruited and brought into action by the adaptive immune system.

The complement system consists of a number of small proteins found in the blood, generally synthesized by the liver, and normally circulating as inactive precursors (pro-proteins). When stimulated by one of several triggers, proteases in the system cleave specific proteins to release cytokines and initiate an amplifying cascade of further cleavages. The end-result of this activation cascade is massive amplification of the response and activation of the cell-killing membrane attack complex. Over 25 proteins and protein fragments make up the complement system, including serum proteins, serosal proteins, and cell membrane receptors. They account for about 5% of the globulin fraction of blood serum.

Three biochemical pathways activate the complement system: the classical complement pathway, the alternative complement pathway, and the mannose-binding lectin pathway.

Functions of the Complement

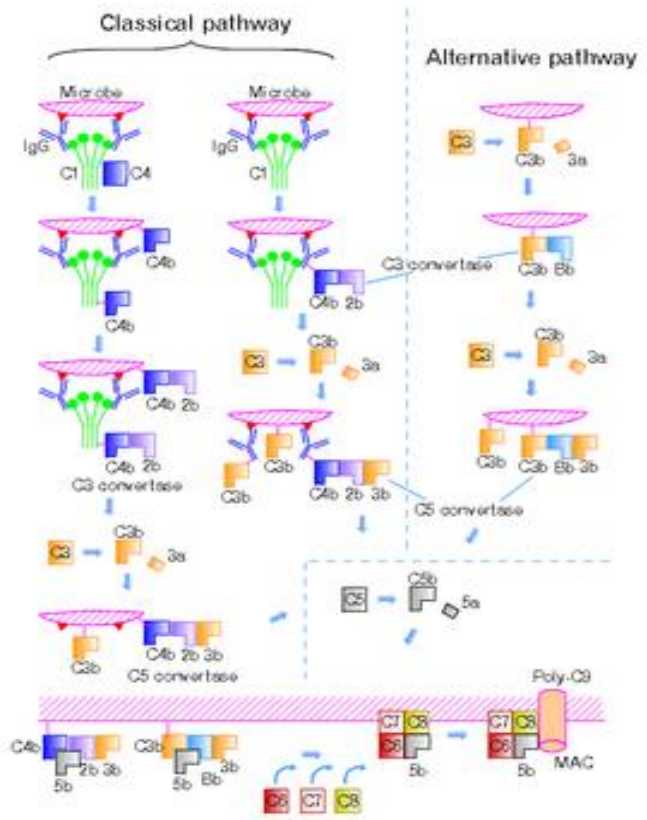
The following are the basic functions of the complement

1. **Opsonization** - enhancing phagocytosis of antigens
2. **Chemotaxis** - attracting macrophages and neutrophils
3. **Cell Lysis** - rupturing membranes of foreign cells
4. **Clumping of antigen-bearing agents**

Overview

The proteins and glycoproteins that constitute the complement system are synthesized by the liver hepatocytes. But significant amounts are also produced by tissue macrophages, blood monocytes, and epithelial cells of the genitourinal tract and gastrointestinal tract. The three pathways of activation all generate homologous variants of the protease C3-convertase. The classical complement pathway typically requires antigen:antibody complexes for activation (specific immune response), whereas the alternative and mannose-binding lectin pathways can be activated by C3 hydrolysis or antigens without the presence of antibodies (non-specific immune response). In all three pathways, C3-convertase cleaves and activates component C3, creating C3a and C3b, and causing a cascade of further cleavage and activation events. C3b binds to the surface of pathogens, leading to greater internalization by phagocytic cells by opsonization. C5a is an important chemotactic protein, helping recruit inflammatory cells. C3a is the precursor of an important cytokine (adipokine) named ASP and is usually rapidly cleaved by carboxypeptidase B. Both C3a and C5a have anaphylatoxin activity, directly triggering degranulation of mast cells as well as increasing vascular permeability and smooth muscle contraction. C5b initiates the membrane attack pathway, which results in the **membrane attack complex** (MAC), consisting of C5b, C6, C7, C8, and polymeric C9. MAC is the cytolytic endproduct of the complement cascade; it forms a transmembrane channel, which causes osmotic lysis of the target cell. Kupffer cells and other macrophage cell types help clear complement-coated pathogens. As part of the innate immune system, elements of the complement cascade can be found in species earlier than vertebrates; most recently in the protostome horseshoe crab species, putting the origins of the system back further than was previously thought.

Classical pathway



The classical and alternative complement pathways.

The classical pathway is triggered by activation of the C1-complex. The C1-complex is composed of 1 molecule of C1q, 2 molecules of C1r and 2 molecules of C1s, or $C1qr^2s^2$. This occurs when C1q binds to IgM or IgG complexed with antigens. A single IgM can initiate the pathway, while multiple IgGs are needed. This also occurs when C1q binds directly to the surface of the pathogen. Such binding leads to conformational changes in the C1q molecule, which leads to the activation of two C1r molecules. C1r is a serine protease. They then cleave C1s (another serine protease). The $C1r^2s^2$ component now splits C4 and then C2, producing C4a, C4b, C2a, and C2b. C4b and C2a bind to form the classical pathway C3-convertase (C4b2a complex), which promotes cleavage of C3 into C3a and C3b; C3b later joins with C4b2a (the C3 convertase) to make C5

convertase (C4b2a3b complex). The inhibition of C1r and C1s is controlled by C1-inhibitor.

C3-convertase can be inhibited by Decay accelerating factor (DAF), which is bound to erythrocyte plasma membranes via a GPI anchor.

Paroxysmal nocturnal hemoglobinuria is caused by complement breakdown of RBCs due to an inability to make GPI. Thus the RBCs are not protected by GPI anchored proteins such as DAF.

Alternative pathway

The alternative pathway is continuously activated at a low level, analogous to a car engine at idle, as a result of spontaneous C3 hydrolysis due to the breakdown of the internal thioester bond(C3 is mildly unstable in aqueous environment). The alternative pathway does not rely on pathogen-binding antibodies like the other pathways.[1] C3b that is generated from C3 by a C3 convertase enzyme complex in the fluid phase is rapidly inactivated by factor H and factor I, as is the C3b-like C3 that is the product of spontaneous cleavage of the internal thioester. In contrast, when the internal thioester of C3 reacts with a hydroxyl or amino group of a molecule on the surface of a cell or pathogen, the C3b that is now covalently bound to the surface is protected from factor H-mediated inactivation. The surface-bound C3b may now bind factor B to form C3bB. This complex in the presence of factor D will be cleaved into Ba and Bb. Bb will remain associated with C3b to form C3bBb, which is the alternative pathway C3 convertase. The C3bBb complex is stabilized by binding oligomers of factor P. The stabilized C3 convertase, C3bBbP, then acts enzymatically to cleave much more C3, some of which becomes covalently attached to the same surface as C3b. This newly-bound C3b recruits more B,D and P activity and greatly amplifies the complement activation. When complement is activated on a cell surface, the activation is limited by endogenous complement regulatory proteins, which include CD35, CD46, CD55 and CD59, depending on the cell. Pathogens, in general, don't have complement regulatory proteins (there are many exceptions, which reflect

adaptation of microbial pathogens to vertebrate immune defenses). Thus, the alternative complement pathway is able to distinguish self from non-self on the basis of the surface expression of complement regulatory proteins. Host cells don't accumulate cell surface C3b (and the proteolytic fragment of C3b called iC3b) because this is prevented by the complement regulatory proteins, while foreign cells, pathogens and abnormal surfaces may be heavily decorated with C3b and iC3b. Accordingly, the alternative complement pathway is one element of innate immunity.

Once the alternative C3 convertase enzyme is formed on a pathogen or cell surface, it may bind covalently another C3b, to form C3bBbC3bP, the C5 convertase. This enzyme then cleaves C5 to C5a, a potent anaphylatoxin, and C5b. The C5b then recruits and assembles C6, C7, C8 and multiple C9 molecules to assemble the membrane attack complex. This creates a hole or pore in the membrane that can kill or damage the pathogen or cell.

Lectin pathway

The lectin pathway is homologous to the classical pathway, but with the opsonin, mannose-binding lectin (MBL), and ficolins, instead of C1q. This pathway is activated by binding mannose-binding lectin to mannose residues on the pathogen surface, which activates the MBL-associated serine proteases, MASP-1, and MASP-2 (very similar to C1r and C1s, respectively), which can then split C4 into C4a and C4b and C2 into C2a and C2b. C4b and C2a then bind together to form C3-convertase, as in the classical pathway. Ficolins are homologous to MBL and function via MASP in a similar way. In invertebrates without an adaptive immune system, ficolins are expanded and their binding specificities diversified to compensate for the lack of pathogen-specific recognition molecules.

Q.16. Explain in detail the structure of major histocompatibility complex.

Ans. **Major histocompatibility complex** (**MHC**) is a cell surface molecule encoded by a large gene family in all vertebrates. MHC molecules mediate interactions of leukocytes, also called white blood cells (WBCs), which are immune cells,

with other leukocytes or body cells. MHC determines compatibility of donors for organ transplant as well as one's susceptibility to an autoimmune disease via crossreacting immunization. In humans, MHC is also called human leukocyte antigen (HLA).

Protein molecules—either of the host's own phenotype or of other biologic entities—are continually synthesized and degraded in a cell. Occurring on the cell surface, each MHC molecule displays a molecular fraction, called epitope, of a protein, somewhat like a hot dog (epitope) within a bun (MHC). The presented antigen can be either *self* or*nonself*. On the cell membrane, the MHC population in its entirety is like a meter indicating the balance of proteins within the cell.

The MHC gene family is divided into three subgroups—class I, class II, and class III. Diversity of antigen presentation, mediated by MHC classes I and II, is attained in multiple ways: (1) the MHC's genetic encoding is polygenic, (2) MHC genes are highly polymorphic and have many variants, (3) several MHC genes are expressed from both inherited alleles.

MHC genes

MHC gene families are found in all vertebrates, though they vary widely, chickens having among the smallest known MHC regions (19 genes). In humans the MHC region occurs on chromosome 6, between the flanking genetic markers MOG and COL11A2, and contains 140 genes spanning 3.6 mega base pairs (3.6 Mb or 3 600 000 bits). About half have known immune functions.

The same markers in the gray short-tailed opossum (*Monodelphis domestica*), a marsupial, span 3.95 Mb yielding 114 genes, 87 shared with humans. Marsupial MHC genotypic variation lies between eutherian mammals and birds—taken as minimal MHC encoding—but is closer in organization to that of nonmammals, and MHC class I genes of marsupials have amplified within the class II region, yielding a unique class I/II region.

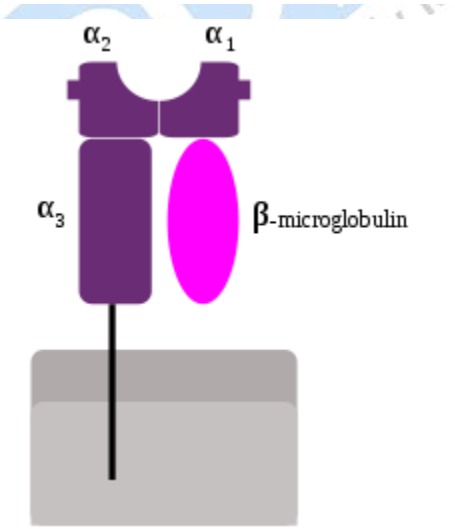
Class III function very differently from class I and class II, but its locus occurs between the other two classes—on chromosome 6 in humans—and are frequently discussed together.

MHC proteins

MHC proteins have immunoglobulin-like structure.

Class I

MHC I occurs as an α chain composed of three domains—α1, α2, α3. The α1 rests upon a unit of the non-MHC molecule β2 microglobulin (encoded on human chromosome 15). The α3 subunit is transmembrane, anchoring the MHC class I molecule to the cell membrane. The peptide being presented is held by the floor of the peptide-binding groove, in the central region of the α1/α2 heterodimer (a molecule composed of two nonidentical subunits). The genetically encoded and expressed sequence of amino acids, the sequence of *residues*, of the peptide-binding groove's floor determines which particular peptide residues it binds.



MHC class I protein molecule

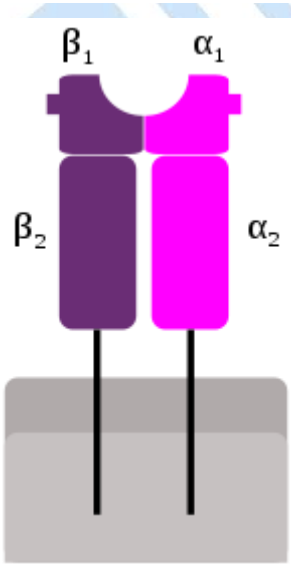
Classical MHC molecules present epitopes to the TCRs of CD8+ T lymphocytes. **Nonclassical molecules** (MHC class IB) exhibit limited polymorphism, expression patterns, and presented antigens; this group is subdivided into a group encoded within MHC loci (e.g., HLA-E, -F, -G) as well

as those not (e.g., stress ligands such as ULBPs, Rae1, H60); the antigen/ligand for many of these molecules remain unknown, but they can interact with both CD8+ T cells, NKT cells, and NK cells.

Class II

MHC class two is formed of two chains, α and β, each having two domains—α1 and α2 and β1 and β2—each chain having a transmembrane domain, α2 and β2, respectively, anchoring the MHC class II molecule to the cell membrane. The peptide-binding groove is formed of the heterodimer of α1 and β1.

MHC class II molecules in humans have five to six isotypes. **Classic molecules** present peptides to CD4+ lymphocytes. **Nonclassic molecules**, accessories, with intracellular functions, are not exposed on cell membranes, but in internal membranes in lysosomes, normally loading the antigenic peptides onto classic MHC class II molecules.



MHC class II protein molecule

Class III

Class III molecules have physiologic roles unlike classes I and class II, but are encoded between them in the short arm of human chromosome 6. Class III

molecules include several secreted proteins with immune functions: components of the complement system (such as C2, C4, and B factor), cytokines (such as TNF-α, LTA, LTB), and heat shock proteins (hsp).

Multiple Choice Questions

1 Which of the following does **not** protect body surfaces:

b) Skin
c) Mucus
d) Gastric acid
e) Salivary amylase
f) Gut microflora.

2 The mononuclear phagocyte system does not include:

a) Monocytes
b) Kupffer cells
c) Kidney mesangial cells
d) Lymph node medullary macrophages
e) Endothelial cells

3 A polymorphonuclear neutrophil (PMN):

a) Is a bone marrow stem cell.
b) Is closely similar to a mast cell.
c) Contains microbicidal cytoplasmic granules.
d) Is not a professional phagocytic cell.
e) Has granules which stain with eosin.

4 Which of the following is not produced following activation of the NADPH oxidase microbicidal pathway

a) O_2^-
b) O_2
c) H_2O_2
d) NO
e) OH

5 Neutrophil defensins are

a) Anti-toxins.
b) Oxygen-dependent
c) Enzymes
d) Glycolipids
e) Peptide antibiotics

6 Complement component C3 is cleaved by:

b) C3b
c) C3bBb
d) Factor B
e) Factor D
f) Factor H

7 The membrane attack complex consists of:

a) OH
b) Colicins
c) C3b3b,Bb
d) C5b,6,7,8,9
e) Properdin

8 C3b:

a) Is chemotactic.
b) Is an anaphylatoxin.
c) Opsonizes bacteria.
d) Directly injures bacteria.
e) Is the inactive form of C3.

9 Acute inflammation characteristically involves:

a) Constriction of arterioles.
b) Capillary endothelial cell enlargement.
c) Influx of macrophages.
d) Influx of mast cells.
e) Influx of neutrophils.

10 All of the following are true with respect to IgM antibodies EXCEPT which one

a) they fix complement

b) they occur on the surface of lymphocytes

c) they predominate in the primary response to antigen

d) they are glycoproteins

e) they mediate allergic reaction

11 One principal function of complement is to

a) inactivate perforins

b) mediate the release of histamine

c) Bind antibodies attached to cell surfaces and to lyse these cells

d) phagocytize antigens

e) cross link allergens

12 One principal function of the Class I and Class II major histocompatibility complex proteins is to

a) transduce the signal to the T-cell interior following antigen binding

b) mediate immunoglobulin class switching

c) present antigen for recognition by the T-cell antigen receptor

d) stimulate production of interleukins

e) bind complement

13 The major role of the complement system is to work in conjunction with

a) antibodies to lyse cells via the C8 and C9 components

b) the major histocompatibility complex for cell recognition

c) antibodies to opsonize cells

d) the T-cell receptor for production of lymphokines

e) antibodies to lyse cells via the perforin molecules

14 T-cell antigen receptors are distinguished from antibodies by which of the following

a) T-Cell receptors are glycosylated

b) T-cell receptors must interact with antigen uniquely presented by other cells but not with free antigen

c) T-Cell receptors bind various cytokines

d) T-Cell receptors bind complement to lyse cells

e) T-cell receptors are mediators of allergic reactions

15 T-cell receptors or antibodies react with antigens

a) because both are made by lymphocytes

b) because of complementary of molecular fit of both with antigen

c) because both 'have light chain and heavy chain polypeptides

d) cause histamine release

e) facilitate perforin release

16 All of the following are true of antigen EXCEPT which one of the following?

a) They contain epitopes.

b) They will react with antibodies.

c) They contain antigenic determinants.

d) They can elicit an immune response.

e) They contain paratopes.

17 All of the following are true with respect to IgE molecules, EXCEPT which one?

a) They are the principal immunoglobulin class involved in allergic reactions

b) They are involved in mediating anti-parasitic immune responses

c) They will cross the placenta and fix complement

d) They can effect the release of histamine and other chemical mediators

e) They are the least abundant immunoglobulin in the serum

18 Which of the following immunoglobulins is present normally in plasma at the highest concentration?

a) IgG

b) IgM

c) IgA

d) IgD

e) IgE

19 All of the following are true about antibodies, EXCEPT which one?

a) They fix complement.

b) They occur on the surface of B-lymphocyte

c) They predominate the primary immune response to antigen.

d) They are glycoproteins.

e) They are molecule with a single, defined amino acid sequence.

20 The major immunoglobulin family to which a particular immunoglobulin belongs can be determined by sequential analysis of the 110 amino acids beginning from the

a) Amino terminus of the light chain

b) Carboxy terminus of the light chain

c) Amino terminus of the heavy chain

d) Carboxy terminus of the heavy chain

e) None of the above

21 The immunoglobulin Joining chain (J-chain) is

a) only produced by T-Cells

b) only produced by neutrophils

c) associated with only multimeric forms of lgM and IgA

d) associated with IgE for histamine release

e) only produced by mast cells

22 All of the following are true EXCEPT

a) An epitope is a small portion of a macromolecule

b) the variable region domains contain the antigen recognition site

c) an antigenic determinant is a paratope

d) The class of an immunoglobulin is determined by its heavy chain

e) An IgG antibody is bivalent

23 Which immunoglobulin is the principal one found in secretions such as milk?

a) IgG

b) IgM

c) IgA

d) IgD

e) IgE

24. The immunoglobulin class which is the least abundant in the normal adult is

a) IgG

b) IgA

c) IgM

d) IgD

e) IgE.

25. Class switching of immunoglobulins occurs

a) Usually with booster immunizations, going from IgM to IgG

b) binds complement

c) causes the histamine release

d) mediates immunoglobulin class switching

e) results in the glycosylation of immunoglobulins

26. When a B-cell undergoes immunoglobulin class switching

a) the variable region of the light chain changes, but its constant region remains the same

b) the variable region of the light chain remains the same, but its constant region changes

c) the variable region of the heavy chain remains the same but its constant region changes

d) the variable region of the heavy chain changes but its constant region remains the same

e) both the variable and constant regions change

27. The class of an immunoglobulin

a) is determined by Class I and Class II major histocompatibility complex proteins

b) is determined by the carbohydrate attached to the light chain is

c) determined by the antigen

d) is determined by the heavy chain type

e) Is determined by the J-chain

28. The class of an immunoglobulin is determined by

a) the variable region

b) the J-chain

c) the heavy chain

d) the carbohydrate

e) the T3 polypeptide complex

29. Light chains are

a) specific for each class of antibody

b) not specific for each class of antibody

c) reactive with antigen

d) have only a constant region

e) are composed only of carbohydrate

30. Each of the following is a characteristic of antibodies, EXCEPT which one?

a) they are proteins with variable and constant regions

b) they contain carbohydrates

c) they are only secreted by T-cells

d) they can combine very specifically with antigen

e) they are structurally organized in globular domains

31. Cytokines are produced by cells of the immune system in response to various physiological stimuli

a) modulate cell function through subsequent cell differentiation or cell proliferation

b) facilitate cell lysis

c) cause glycosylation of Immunoglobins

d) cause histamine release

e) facilitate perforin release

32. Which of the following uniquely distinguishes the T-cell receptor (TCR) from an antibody?

a) The TCR can bind an antigen fragment only in a trimolecular complex with either the class I or class II surface proteins of the major histocompatibility complex

b) The TCR can function as a cell surface antigen receptor

c) The TCR is composed of two different types of polypeptide chains

d) The TCR is capable of participating in a cytotoxic reaction.

e) The TCR polypeptides is composed of domains - an amino terminal variable portion at determines the binding specificity and a constant portion that determines the class of the polypeptide chain.

33. Which of the following is NOT true of interleukins?

a) They are cytokines which can be produced by various cells of the immune system.

b) They are hormones which allow one cell to communicate with another cell.

c) They are in need of receptors on the target cell in order to mediate their effects.

d) They are able bind antigen with a high level of specificity.

e) They are able to modulate various aspects of the B-cell arm of immune system.

34. Which of the following is NOT true of the ability of the T-cell receptor (TCR) to specifically recognize antigen?

a) The antigen must be "processed" first by an accessory cell of immune system in order for it to bind to the TCR.

b) The recognition of the antigen by the TCR can mediate helper, suppressor or cytotoxic function.

c) The recognition of antigen by the TCR can result in cytokine secretion and/or an increase in cell proliferation within the immune system.

d) The antigen is recognized by the T3-TCR complex only when it is associated with a protein of the major histocompatibility complex

e) Only the alpha chain of the TCR is necessary for antigen

35 Which category of hypersensitivity BEST describes hemolytic disease of the newborn caused by Rh incompatibility?

a) atopic or anaphylactic

b) cytotoxic

c) immune complex

d) delayed

36 The principal difference between cytotoxic (type II) and immune complex (type III) hypersensitivity is

a) the class (isotype) of antibody.

b) the site where antigen-antibody complexes are formed.

c) the participation of complement.

d) the participation of T cells.

37 A child stung by a bee experiences respiratory distress within minutes and lapses into unconsciousness. This reaction is probably mediated by

a) IgE antibody.

b) IgG antibody.

c) sensitized T cells.

d) complement.

e) IgM antibody.

38 Which of the following is not characteristic of an innate immune response?

a) Inflammation

b) increase in blood levels of specific antibodies

c) increase in phagocytic cells at the site of infection

d) activation of complement

39 Of the following cell types, which ones function in both innate and acquired immune responses and as "professional" antigen presenting cells?

a) Dendritic cells

b) CTLs

c) TH lymphocytes

d) B cells

40 Professional antigen presenting cells have _____, whereas most other cells have _____.

a) MHC I; MHC I and II

b) MHC II; MHC I

c) MHC I and II; MHC II

d) MHC I; MHC II

Glossary

acquired immune response: Immunity mediated by lymphocytes and characterized by antigen-specificity and memory.

acute phase proteins: Serum proteins, mostly produced in the liver, which rapidly change in concentration (some increase, some decrease) during the initiation of an inflammatory response.

addressin: Cell adhesion molecule present on the luminal surface of blood and lymph vessel endothelium, and recognized by homing molecules which direct leukocytes to tissues with the appropriate 'address'.

adjuvant: Any substance which nonspecifically enhances the immune response to antigen.

affinity (intrinsic affinity): The strength of binding (affinity constant) between a receptor (e.g. one antigen-binding site on an antibody) and a ligand (e.g. epitope on an antigen).

affinity chromatography: The use of immobilized antibody (or antigen) to select specific antigen (or antibody) from a mixture. The purified ligand is then released by disrupting for antibody–antigen interaction, for example by changing the pH.

allele: Variants of a polymorphic gene at a given genetic locus.

allelic exclusion: The phenomenon whereby, following successful rearrangement of one allele of an antigen receptor gene, rearrangement of the other parental allele is suppressed.

allergen: An antigen which causes allergy.

allergy: IgE-mediated hypersensitivity, e.g. asthma, eczema, hayfever and food allergy.

allogeneic: Refers to the genetic differences between individuals of the same species.

allograft: Tissue or organ graft between allogeneic individuals.

allotype: An allelic variant of an antigen which, because it is not present in all individuals, may be immunogenic in members of the same species which have a different version of the allele.

alternative pathway (of complement activation): Activation pathway involving complement components C3, Factor B, Factor D and Properdin which, in the presence of a stabilizing activator surface such as microbial polysaccharide, generates the alternative pathway C3 convertase C 3bBb.

anaphylatoxin: A substance (e.g. C3a, C4a or C5a) capable of directly triggering mast cell degranulation.

anaphylaxis: An often fatal hypersensitivity reaction, triggered by IgE or anaphylatoxin-mediated mast cell degranulation, leading to anaphylactic shock due to vasodilatation and smooth muscle contraction.

antibody-dependent cellular cytotoxicity (ADCC): A cytotoxic reaction in which an antibody-coated target cell is directly killed by an Fc receptor-bearing leukocyte, e.g. NK cell, macrophage or neutrophil.

antigen: Any molecule capable of being recognized by an antibody or T-cell receptor.

antigen-presenting cell (APC): A term most commonly used when referring to cells that present processed antigenic peptide and MHC class II molecules to the T-cell receptor on CD4 + T-cells, e.g. dendritic cells, macrophages, B-cells. Note, however, that most types of cell are able to present antigenic peptides with MHC class I to CD8 + T-cells, e.g. as occurs with virally infected cells.

antigenic determinant: A cluster of epitopes (*see* epitope).

apoptosis: A form of programmed cell death, characterized by endonuclease digestion of DNA.

atopic allergy: IgE-mediated hypersensitivity, i.e. asthma, eczema, hayfever and food allergy.

autologous: From the same individual.

avidity (functional affinity): The binding strength between two molecules (e.g. antibody and antigen) taking into account the valency of the interaction. Thus the avidity will always be equal to or greater than the intrinsic affinity (*see* affinity).

β_2-microglobulin: A 12 kDa protein, not itself encoded within the MHC, but forming part of the structure of MHC class I-encoded molecules.

basophil: A type of granulocyte found in the blood and resembling the tissue mast cell.

BCG (bacille Calmette–Guérin): Attenuated *Mycobacterium tuberculosis* used both as a specific vaccine for tuberculosis and as an adjuvant.

bispecific antibody: An artificially produced hybrid antibody in which each of the two antigen-binding arms is specific for a different antigenic epitope. Such antibodies, which can be produced either by chemical cross-linkage or by recombinant DNA techniques, can be used to link together two different antigens or cells, e.g. a cytotoxic T-cell and a tumor cell.

bursa of Fabricius: A primary lymphoid organ in avian species, located at the cloacal-hind gut junction; it is the site of B-cell maturation.

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capping: An active process whereby cross-linking of cell surface molecules (e.g. by antibody) leads to aggregation and subsequent migration of the molecules to one pole of the cell.

caspases: A family of cysteine proteases involved in generating apoptosis.

carrier: Any molecule which when conjugated to a non-immunogenic molecule (e.g. a hapten) makes the latter immunogenic by providing epitopes for helper T-cells which the hapten lacks.

CD antigen: Cluster of differentiation designation assigned to leukocyte cell surface molecules which are identified by a given group of monoclonal antibodies.

CD3: A trimeric complex of γ, δ and ε chains which together with a ζζ homodimer or ζη heterodimer acts as a signal transducing unit for the T-cell receptor.

CD4: Cell surface glycoprotein, usually on helper T-cells, that recognizes MHC class II molecules on antigen-presenting cells.

CD8: Cell surface glycoprotein, usually on cytotoxic T-cells, that recognizes MHC class I molecules on target cells.

cell-mediated immunity (CMI): Refers to T-cell mediated immune responses.

chemokines: A family of structurally-related cytokines which selectively induce chemotaxis and activation of leukocytes. They also play important roles in lymphoid

organ development, cell compartmentalization within lymphoid tissues, Th1/Th2 development, angiogenesis and wound healing.

chemotaxis: Movement of cells up a concentration gradient of chemotactic factors.

chimeric: Composite of genetically distinct individuals, e.g. following an allogeneic bone marrow graft.

class switching: The process by which a B-cell changes the class but not specificity of a given antibody it produces, e.g. switching from an IgM to an IgG antibody.

class switch recombination: The recombination of immunoglobulin heavy chain constant region gene segments, e.g. switching from Cu and Cδ to Cγ1 to convert an IgM (and IgD) antibody into an IgG1 antibody.

classical pathway (of complement activation)**:** Activation pathway involving complement components C1, C2 and C4 which, following fixation of C1q, e.g. by antigen–antibody complexes, produces the classical pathway C3 convertase C 4 b 2a.

clonal deletion: A process by which contact with antigen (e.g. self antigen) at an early stage of lymphocyte differentiation leads to cell death by apoptosis.

clonal selection: The selection and activation by antigen of a lymphocyte bearing a complementary receptor, which then proliferates to form an expanded clone.

clone: Identical cells derived from a single progenitor.

colony stimulating factors (CSF): Factors that permit the proliferation and differentiation of hematopoietic cells.

combinatorial diversity: That component of antibody and T-cell receptor (TCR) diversity that is generated by the recombination of variable (V), diversity (D, for immunoglobulin heavy chains, and for TCR β and δ chains) and joining (J) gene segments.

complement: A group of serum proteins, some of which act in an enzymatic cascade, producing effector molecules involved in inflammation (C3a, C5a), phagocytosis (C3b), and cell lysis (C5b–9).

complementarity determining regions (CDR): The hypervariable amino acid sequences within antibody and T-cell receptor variable regions which interact with complementary amino acids on the antigen or peptide–MHC complex.

ConA (concanavalin A): A T-cell mitogen.

congenic: Animals which only differ at a single genetic locus.

conjugate: Covalently linked complex of two or more molecules (e.g. fluorescein conjugated to antibody).

convergent evolution: Independent evolution of similarity between molecules or between species.

Coombs' test: Diagnostic test using anti-immuno globulin to agglutinate antibody-coated erythrocytes.

cortex: Outer (peripheral) layer of an organ.

C-reactive protein: An acute phase protein which is able to bind to the surface of microorganisms where it functions as a stimulator of the classical pathway of complement activation, and as an opsonin for phagocytosis.

cyclophosphamide: Cytotoxic drug used as an immunosuppressive.

cyclosporine A: A T-cell specific immunosuppressive drug used to prevent graft rejection.

cytokines: Low molecular weight proteins that stimulate or inhibit the differentiation, proliferation or function of immune cells.

cytophilic: Binds to cells.

cytotoxic: Kills cells.

cytotoxic T lymphocyte (CTL, Tc): T-cells (usually CD8 +) which kill target cells following recognition of foreign peptide–MHC molecules on the target cell membrane.

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danger-associated molecular pattern (DAMP): A structure or molecule produced by necrotic cells and which provides danger signals to activate the immune response following tissue damage.

defensins: A family of small basic antimicrobial peptides, produced by both animals and plants.

delayed-type hypersensitivity (DTH): A hypersensitivity reaction occurring within 48–72 hours and mediated by cytokine release from sensitized T-cells.

dendritic cell: Refers to an interdigitating dendritic cell which is MHC class II-positive, Fc receptor-negative, and presents processed antigens to T-cells in the T-cell areas of secondary lymphoid tissues. (NB a different cell type to follicular dendritic cells).

differentiation antigen: A cell surface molecule expressed at a particular stage of development or on cells of a given lineage.

DiGeorge syndrome: Immunodeficiency caused by a congenital failure in thymic development resulting in a lack of mature functional T-cells.

diversity (*D*) gene segments: Found in the immunoglobulin heavy chain gene and T-cell receptor β and δ gene loci between the *V*and *J*gene segments. Encode part of the third hypervariable region in these antigen receptor chains.

domain: a structural element of a polypeptide.

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edema: Swelling caused by accumulation of fluid in the tissues.

effector cells: Cells which carry out an immune function, e.g. cytokine release, cytotoxicity.

ELISA (*e*nzyme-*l*inked *i*mmuno*s*orbent *a*ssay)**:** Assay for detection or quantitation of an antibody or antigen using a ligand (e.g. an anti-immunoglobulin) conjugated to an enzyme which changes the color of a substrate.

endocytosis: Cellular ingestion of macromolecules by invagination of plasma membrane to produce an intracellular vesicle which encloses the ingested material.

endogenous: From within.

endosomes: Intracellular smooth surfaced vesicles in which endocytosed material passes on its way to the lysosomes.

endotoxin: Pathogenic cell wall-associated lipopolysaccharides of Gram-negative bacteria.

eosinophil: A class of granulocyte, the granules of which contain toxic cationic proteins.

epitope: That part of an antigen recognized by an antigen receptor (*see* antigenic determinant).

Epstein–Barr virus (EBV): The virus responsible for infectious mononucleosis and Burkitt's lymphoma. Can be used to immortalize human B-cells *in vitro*.

equivalence: The ratio of antibody to antigen at which immunoprecipitation of the reactants is virtually complete.

erythema: The redness produced during inflammation due to erythrocytes entering tissue spaces.

erythropoiesis: Erythrocyte production.

exotoxin: Pathogenic protein secreted by bacteria.

exudate: The extravascular fluid (containing proteins and cellular debris) which accumulates during inflammation.

Fab: Monovalent antigen-binding fragment obtained following papain digestion of immunoglobulin. Consists of an intact light chain and the N-terminal V H and C H1 domains of the heavy chain.

F(ab´)$_2$: Bivalent antigen-binding fragment obtained following pepsin digestion of immunoglobulin. Consists of both light chains and the N-terminal part of both heavy chains linked by disulfide bonds.

Fas: A member of the TNF receptor gene family. Engagement of Fas (CD95) on the surface of the cell by the Fas ligand (CD178) present on cytotoxic cells, can trigger apoptosis in the Fas-bearing target cell.

Fc: Crystallizable, non-antigen-binding fragment of an immunoglobulin molecule obtained following papain digestion. Consists of the C-terminal portion of both heavy chains which is responsible for binding to Fc receptors and C1q.

Fc receptors: Cell surface receptors which bind the Fc portion of particular immunoglobulin classes.

fibroblast: Connective tissue cell which produces collagen and plays an important part in wound healing.

fluorescein isothiocyanate (FITC): Green fluorescent dye used to ‘tag’ antibodies for use in immunofluorescence.

fluorescent antibody: An antibody conjugated to a fluorescent dye such as FITC.

follicular dendritic cell: MHC class II-negative Fc receptor-positive dendritic cells which bear immune complexes on their surface and are involved in the generation of antibody-secreting cells and maintenance of B-cell memory in germinal centres. (N.B. a different cell type to interdigitating dendritic cells).

follicular helper T-cell: Subset of helper T-cells that direct B-cell development, class switch recombination and survival within germinal centers.

framework regions: The relatively conserved amino acid sequences which flank the hypervariable regions in immunoglobulin and T-cell receptor variable regions and maintain a common overall structure for all V-region domains.

Freund's adjuvant: Complete Freund's adjuvant is an emulsion of aqueous antigen in mineral oil that contains heat-killed *Mycobacteria*. Incomplete Freund's adjuvant lacks the *Mycobacteria*.

Fv: The variable region fragment of an antibody heavy or light chain.

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gammaglobulin: The serum proteins, mostly immunoglobulins, which have the greatest mobility towards the cathode during electrophoresis.

germ line: The arrangement of the genetic material as transmitted through the gametes.

germinal center: Discrete areas within secondary lymphoid tissues where B-cell maturation and memory development occur.

giant cell: Large multinucleate cell derived from fused macrophages and often present in granulomas.

graft versus host (g.v.h.) reaction: Reaction occurring when T lymphocytes present in a graft recognize and attack host cells.

granulocyte: Myeloid cells containing cytoplasmic granules (i.e. neutrophils, eosinophils and basophils).

granuloma: A tissue nodule containing proliferating lymphocytes, fibroblasts, and giant cells and epithelioid cells (both derived from activated macrophages), which forms due to inflammation in response to chronic infection or persistence of antigen in the tissues.

granzymes: Serine esterases present in the granules of cytotoxic T lymphocytes and NK cells. They induce apoptosis in the target cell which they enter through perforin channels inserted into the target cell membrane by the cytotoxic lymphocyte.

gut-associated lymphoid tissue (GALT): Includes Peyer's patches, appendix and solitary lymphoid nodules in the submucosa.

H-2: The mouse major histocompatibility complex (MHC).

haplotype: The set of allelic variants present at a given genetic region.

hapten: A low molecular weight molecule that is recognized by preformed antibody but is not itself immunogenic unless conjugated to a 'carrier' molecule which provides epitopes recognized by helper T-cells.

helper T lymphocyte (Th): A subclass of T-cells which provide help (in the form of cytokines and/or cognate interactions) necessary for the expression of effector function by other cells in the immune system.

hemagglutinin: Any molecule which agglutinates erythrocytes.

hematopoiesis: The production of erythrocytes, leukocytes and platelets.

hematopoietic stem cells: Self-renewing stem cells that are capable of giving rise to all of the formed elements of the blood (i.e. leukocytes, erythrocytes and platelets).

heterozygous: Possessing different alleles at a given locus on the two homologous chromosomes.

high endothelial venule (HEV): Capillary venule composed of specialized endothelial cells allowing migration of lymphocytes into lymphoid organs.

hinge region: Amino acids between the Fab and Fc regions of immunoglobulin which permit flexibility of the molecule.

histamine: Vasoactive amine present in basophil and mast cell granules which, following degranulation, causes increased vascular permeability and smooth muscle contraction.

HLA (*h*uman *l*eukocyte *a*ntigen)**:** The human major histocompatibility complex (MHC).

homing receptors: Cell surface molecules that direct leukocytes to specific locations in the body.

homozygous: Possessing the same allele at a given locus on the two homologous chromosomes.

humanized antibody: A genetically engineered monoclonal antibody of non-human origin in which all but the antigen-binding CDR sequences have been replaced with sequences derived from human antibodies. This procedure is carried out to minimize the immunogenicity of therapeutic monoclonal antibodies.

humoral: Pertaining to extracellular fluid such as plasma and lymph. The term humoral immunity is used to denote antibody-mediated immune responses.

hybridoma: Hybrid cell line obtained by fusing a lymphoid tumor cell with a lymphocyte which then has both the immortality of the tumor cell and the effector function (e.g. monoclonal antibody secretion) of the lymphocyte.

hypersensitivity: Excessive immune response which leads to undesirable consequences, e.g. tissue or organ damage.

hypervariable regions: Those amino acid sequences within the immunoglobulin and T-cell receptor variable regions which show the greatest variability and contribute most to the antigen or peptide–MHC binding site.

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idiotope: An epitope made up of amino acids within the variable region of an antibody or T-cell receptor which reacts with an anti-idiotope.

idiotype: The complete set of idiotopes in the variable region of an antibody or T-cell receptor which react with an anti-idiotypic serum.

idiotype network: A regulatory network based on interactions of idiotypes and anti-idiotypes present on antibodies and T-cell receptors.

immune complex: Complex of antibody bound to antigen which may also contain complement components.

immunoadsorption: Method for removal of antibody or antigen by allowing it to bind to solid phase antigen or antibody.

immunofluorescence: Technique for detection of cell- or tissue-associated antigens by the use of a fluorescently tagged ligand (e.g. an anti-immunoglobulin conjugated to fluorescein isothiocyanate).

immunogen: Any substance which elicits an immune response. Whilst all immunogens are antigens, not all antigens are immunogens (*see* hapten).

immunoglobulin superfamily: Large family of proteins characterized by possession of 'immunoglobulin-type' domains of approximately 110 amino acids folded into two β-pleated sheets. Members include immunoglobulins, T-cell receptors and MHC molecules.

immunological synapse: A contact point between the T-cell and antigen-presenting cell which is generated by reorganization and clustering of cell surface molecules in lipid rafts. The synapse facilitates interactions between TCR and MHC and between adhesion molecules, thereby potentiating the TCR-mediated activation signal.

immunotoxin: A biochemical conjugate, or recombinant fusion protein, consisting of an immune targeting molecule such as an antibody or antibody fragment together with a cytotoxic molecule.

inflammation: The tissue response to trauma, characterized by increased blood flow and entry of leukocytes into the tissues, resulting in swelling, redness, elevated temperature and pain.

innate immunity: Immunity which is not intrinsically affected by prior contact with antigen, i.e. all aspects of immunity not directly mediated by lymphocytes.

integrins: A family of heterodimeric cell adhesion molecules.

interdigitating dendritic cell: MHC class II-positive, Fc receptor-negative, antigen-presenting dendritic cell found in T-cell areas of lymph nodes and spleen. (NB a different cell type to follicular dendritic cells).

interferons (IFN): IFNα and IFNβ (type I interferons) can be induced in most cell types, whereas IFNγ (type II interferon) is produced by T lymphocytes. All three types induce an anti-viral state in cells and IFNγ additionally acts in the regulation of immune responses.

interleukins (IL): Designation for some of the cytokines secreted by leukocytes.

internal image: An epitope on an anti-idiotype which binds in a way that structurally and functionally mimics the antigen.

invariant chain: A polypeptide which binds MHC class II molecules in the endoplasmic reticulum, directs them to the late endosomal compartment and prevents premature association with self peptides.

isotype: An antibody constant region structure present in all normal individuals, i.e. antibody class or subclass.

ITAM: *I*mmunoreceptor *T*yrosine-based *A*ctivation *M*otifs are amino acid consensus sequences recognized by src-family tyrosine kinases. These motifs are found in the cytoplasmic domains of several signaling molecules including the signal transduction units of lymphocyte antigen receptors and of Fc receptors.

ITIM: *I*mmunoreceptor *T*yrosine-based *I*nhibitory *M*otifs present in the cytoplasmic domains of certain cell surface molecules, e.g. Fcγ RIIB, inhibitory NK cell receptors, and which mediate inhibitory signals.

J chain: A molecule which forms part of the structure of pentameric IgM and dimeric IgA.

joining (*J*) gene segments: Found in the immunoglobulin and T-cell receptor gene loci and, upon gene rearrangement, encode part of the third hypervariable region of the antigen receptors.

junctional diversity: Diversity of the splice junctions in the recombined variable (V), diversity (D, for immunoglobulin heavy chains, and for TCR β and δ chains) and joining (J) gene segments of antibody and T-cell receptor (TCR) genes.

K (killer) cell:: A generic term for any leukocytes which mediates antibody-dependent cellular cytotoxicity (ADCC)

kinins: A family of polypeptides released during inflammatory responses and which increase vascular permeability and smooth muscle contraction.

Kuppfer cells: Fixed tissue macrophages lining the blood sinuses in the liver.

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lamina propria: The connective tissue underlying the epithelium at mucosal sites.

Langerhans' cell: Fc receptor and MHC class II-positive antigen-presenting dendritic cell found in the skin.

large granular lymphocyte (LGL): Large lymphocytes which contain cytoplasmic granules and function as natural killer (NK) and killer (K) cells. Activated $CD8^+$ cytotoxic T lymphocytes (Tc) also assume an LGL morphology.

lectins: A family of proteins which bind specific sugars on glycoproteins and glycolipids. Some plant lectins are mitogenic (e.g. PHA, ConA).

leukocyte: White blood cells, which include neutrophils, basophils, eosinophils, lymphocytes and monocytes.

leukotrienes: Metabolic products of arachidonic acid which promote inflammatory processes (e.g. chemotaxis, increased vascular permeability) and are produced by a variety of cell types including mast cells, basophils and macrophages.

ligand: General term for a molecule recognized by a binding structure such as a receptor.

lipopolysaccharide (LPS): Endotoxin derived from Gram-negative bacterial cell walls which has inflammatory and mitogenic actions.

lymph: The tissue fluid which drains into and through the lymphatic system.

lymphadenopathy: Enlarged lymph nodes.

lymphotoxin (also called TNFβ): A T-cell derived cytokine which is cytotoxic for certain tumor cells and also has immunoregulatory functions.

lysosomes: Cytoplasmic granules containing hydrolytic enzymes involved in the digestion of phagocytosed material.

lysozyme: Anti-bacterial enzyme present in phagocytic cell granules, tears and saliva, which digests peptidoglycans in bacterial cell walls.

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macrophage: Large phagocytic cell, derived from the blood monocyte, which also functions as an antigen-presenting cell and can mediate ADCC.

mannose binding protein (mannose binding lectin): A member of the collectin family of calcium-dependent lectins, and an acute phase protein. It functions as a stimulator of the classical pathway of complement activation, and as an opsonin for phagocytosis by binding to mannose, a sugar residue usually found in an exposed form only on the surface of microorganisms.

marginal zone: The outer area of the splenic periarteriolar lymphoid sheath (PALS) which is rich in B cells, particularly those responding to thymus-independent antigens.

margination: Leukocyte adhesion to the endothelium of blood vessels in the early phase of an acute inflammatory reaction.

mast cell: A tissue cell with abundant granules which resembles the blood basophil. Both these cell types bear high affinity Fc receptors for IgE, which when crosslinked by IgE and antigen cause degranulation and the release of a number of mediators including histamine and leukotrienes.

medulla: Inner (central) region of an organ.

megakaryocyte: A bone marrow precursor of platelets.

membrane attack complex (MAC)**:** Complex of complement components C5b –C9 which inserts as a pore into the membrane of target cells leading to cell lysis or apoptosis.

memory (immunological): A characteristic of the acquired immune response of lymphocytes whereby a second encounter with a given antigen produces a secondary immune response; faster, greater and longer lasting than the primary immune response.

memory cells: Clonally expanded T- and B-cells produced during a primary immune response and which are 'primed' to mediate a secondary immune response to the original antigen.

MHC (*m*ajor *h*istocompatibility *c*omplex)**:** A genetic region encoding molecules involved in antigen presentation to T-cells. Class I MHC molecules are present on virtually all nucleated cells and are encoded mainly by the H-2K, D and L loci in mice and by HLA-A, B and C in man, whilst class II MHC molecules are expressed on antigen-presenting cells (primarily dendritic cells, macrophages and B-cells) and are encoded by H-2A and E in mice and HLA-DR, DQ and DP in man. Allelic differences are associated with the most intense graft rejection within a species.

MHC restriction: The necessity that T-cells recognize processed antigen only when presented by MHC molecules of the original haplotype associated with T-cell priming.

minor histocompatibility antigens: Non-MHC-encoded cell surface processed peptides which, in association with MHC-encoded molecules, contribute to graft rejection, albeit not usually as severe as that due to MHC mismatch.

mitogen: A substance which nonspecifically induces lymphocyte proliferation.

mixed lymphocyte reaction (MLR): A T-cell proliferative response induced by cells expressing allogeneic MHC.

monoclonal antibody: Homogeneous antibody derived from a single B-cell clone and therefore all bearing identical antigen-binding sites and isotype.

monocyte: Mononuclear phagocyte found in blood and which is the precursor of the tissue macrophage.

mononuclear phagocyte system: A system comprising blood monocytes and tissue macrophages.

mucosa-associated lymphoid tissue (MALT): Lymphoid tissue present in the surface mucosa of the respiratory, gastrointestinal and genitourinary tracts.

multiple myeloma: Plasma cell malignancy resulting in high levels of monoclonal immunoglobulin in serum and of free light chains (Bence–Jones protein) in urine.

myeloma protein: Monoclonal antibody secreted by myeloma cells.

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naive lymphocyte: A mature T- or B-cell which has not yet been activated by encounter with antigen.

negative selection: Deletion by apoptosis in the thymus of T-cells which recognize self peptides presented by self MHC molecules, thus preventing the development of autoimmune T-cells. Negative selection of developing B-cells also occurs if they encounter high levels of self antigen in the bone marrow.

neutrophil: The major circulating phagocytic polymorphonuclear granulocyte. Enters tissues early in an inflammatory response and is also able to mediate antibody-dependent cellular cytotoxicity (ADCC).

NK (natural killer) cell: Large granular lymphocyte which does not rearrange nor express either immunoglobulin or T-cell receptor genes but is able to recognize and destroy certain tumor and virally infected cells in an MHC and antibody-independent manner.

nude mouse: Mouse which is T-cell deficient due to a homozygous gene defect (*nu*/*nu*) resulting in the absence of a thymus (and also lack of body hair).

N-nucleotides: Nontemplated nucleotides added to the junctions between antibody (and T-cell receptor) variable (V), diversity (D) and joining (J) gene segments during gene rearrangement.

Nod-like receptor: A family of cytoplasmic pattern recognition receptors involved in sensing the presence of pathogens.

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oncofetal antigen: Antigen whose expression is normally restricted to the fetus but which may be expressed during malignancy in adults.

opsonin: Substance, e.g. antibody or C3b, which enhances phagocytosis by promoting adhesion of the antigen to the phagocyte.

opsonization: Coating of antigen with opsonin to enhance phagocytosis.

PAF (platelet activating factor): An alkyl phospholipid released by a variety of cell types including mast cells and basophils, which has immunoregulatory effects on

lymphocytes and monocytes/macrophages as well as causing platelet aggregation and degranulation.

paracortex: The part of an organ (e.g. lymph node) which lies between the cortex and the medulla.

pathogen-associated molecular pattern (PAMP): Molecules such as lipopolysaccharide, peptidoglycan, lipoteichoic acids and mannans, which are widely expressed by microbial pathogens as repetitive motifs but are not present on host tissues. They are therefore utilized by the pattern recognition receptors (PRRs) of the immune system to distinguish pathogens from self antigens.

pattern recognition receptor (PRR): Receptors found on many different cell types in the immune system which enable them to recognize pathogen-associated molecular patterns (PAMPs). Amongst the large number of different PRRs are the mannose receptor (CD206), macrophage scavenger receptor (CD204) and the Toll-like receptors.

perforin: Molecule produced by cytotoxic T-cells and NK cells which, like complement component C9, polymerizes to form a pore in the membrane of the target cell leading to cell death.

periarteriolar lymphoid sheath (PALS): The lymphoid tissue which forms the white pulp of the spleen.

peripheral tolerance: Specific immunological tolerance occurring outside of the primary lymphoid organs.

Peyer's patches: Part of the gut associated lymphoid tissue (GALT) and found as distinct lymphoid nodules mainly in the small intestine.

PHA (phytohemagglutinin): A plant lectin which acts as a T-cell mitogen.

phagocyte: Cells, including monocytes/macrophages and neutrophils, which are specialized for the engulfment of cellular and particulate matter.

phagolysosome: Intracellular vacuole where killing and digestion of phagocytosed material occurs following the fusion of a phagosome with a lysosome.

phagosome: Intracellular vacuole produced following invagination of the cell membrane around phagocytosed material.

plaque forming cell (PFC): Antibody-secreting plasma cell detected *in vitro* by its ability to produce a 'plaque' of lysed antigen-sensitized erythrocytes in the presence of complement.

plasma cell: Terminally differentiated B lymphocyte which actively secretes large amounts of antibody.

pluripotent stem cell: A cell which has the potential to differentiate into many different cell types.

pokeweed mitogen (PWM): A plant lectin which is a T-cell dependent B-cell mitogen.

polyclonal: Many different clones, or the product of many different clones, e.g. polyclonal antiserum.

poly-Ig receptor: A receptor molecule which specifically binds J-chain containing polymeric Ig, i.e. dimeric secretory IgA and pentameric IgM, and transports it across mucosal epithelium.

polymorphic: Highly variable in structure or sequence.

positive selection: The selection of those developing T-cells in the thymus which are able to recognize self MHC molecules. This occurs by preventing apoptosis in these cells.

precipitin: Precipitate of antibody and multivalent antigen due to the formation of high molecular weight complexes.

primary immune response: The relatively weak immune response which occurs upon the first encounter of naive lymphocytes with a given antigen.

primary lymphoid organs: The sites at which immunocompetent lymphocytes develop, i.e. bone marrow and thymus in mammals.

prime: The process of giving an initial sensitization to antigen.

prostaglandins: Acidic lipids derived from arachidonic acid which are able to increase vascular permeability, mediate fever, and can both stimulate and inhibit immunological responses.

proteasome: Cytoplasmic proteolytic enzyme complex involved in antigen processing for association with MHC.

protein A: *Staphylococcus aureus* cell wall protein which binds to the Fc region of IgG.

protein tyrosine kinases: Enzymes which are able to phosphorylate proteins on tyrosines, and often act in a cascade-like fashion in the signal transduction systems of cells.

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radioimmunoconjugate: A biochemical conjugate consisting of an immune targeting molecule such as an antibody or antibody fragment together with a cytotoxic radionuclide.

regulatory idiotope: An antibody or T-cell receptor idiotope capable of regulating immune responses via interaction with lymphocytes bearing complementary idiotopes (anti-idiotopes).

regulatory T-cell: T-cells, mostly CD4 +, which suppress the functional activity of lymphocytes and dendritic cells.

respiratory burst: The increased oxidative metabolism which occurs in phagocytic cells following activation.

reticuloendothelial system (RES): A rather old term for the network of phagocytes and endothelial cells throughout the body.

rheumatoid factor: IgM, IgG and IgA autoantibodies to the Fc region of IgG.

rosette: Particles or cells bound to the surface of a lymphocyte (e.g. sheep erythrocytes around a human T-cell).

scavenger receptors: Cell surface receptors, for example on phagocytic cells, that recognize cells or molecules that require clearance from the body.

SCID (*s*evere *c*ombined *i*mmuno*d*eficiency)**:** Immunodeficiency affecting both T and B lymphocytes.

secondary immune response: The qualitatively and quantitatively improved immune response which occurs upon the second encounter of primed lymphocytes with a given antigen.

secretory component: Proteolytic cleavage product of the poly-Ig receptor which remains associated with dimeric IgA in sero-mucus secretions.

secretory IgA: Dimeric IgA found in sero-mucus secretions.

somatic hypermutation: The enhanced occurence of point mutations in the recombined immunoglobulin variable region V[D]J genes which occurs following antigenic stimulation and acts as a mechanism for increasing antibody diversity and affinity.

stem cell: Multipotential cell from which differentiated cells derive.

stochastic: A process involving at least some element of randomness.

superantigen: An antigen which reacts with all the T-cells belonging to a particular T-cell receptor V region family, and which therefore stimulates (or deletes) a much larger number of cells than does conventional antigen.

surface plasmon resonance: A technique based upon changes in the angle of reflected light which occur upon ligand binding to an immobilized target molecule on a biosensor chip. This permits the observation of protein–protein interactions (such as antibody binding to an antigen) in 'real-time', i.e. by continuous monitoring of the association and dissociation of the reversible reaction.

switch sequences: Highly conserved repetitive sequences which mediate class switching in the immunoglobulin heavy chain gene locus.

syngeneic: Genetically identical, e.g. a fully inbred strain of mice.

systemic: Throughout the body.

TAP: The *T*ransporters associated with *A*ntigen *P*rocessing (TAP-1 and TAP-2) are molecules which carry antigenic peptides from the cytoplasm into the lumen of the endoplasmic reticulum for incorporation into MHC class I molecules.

T-cell receptor (TCR): The heterodimeric antigen receptor of the T lymphocyte exists in two alternative forms, consisting of α and β chains, or γ and δ chains. The αβ TCR recognizes peptide fragments of protein antigens presented by MHC molecules on cell surfaces. The function of the γδ TCR is less clearly defined but it can recognize native proteins on the cell surface.

T-dependent antigen: An antigen which requires helper T-cells in order to elicit an antibody response.

T-independent antigen: An antigen which is able to elicit an antibody response in the absence of T-cells.

thymocyte: Developing T-cell in the thymus.

titer: Measure of the relative 'strength' (a combination of amount and avidity) of an antibody or antiserum, usually given as the highest dilution which is still operationally detectable in, for example, an ELISA.

tolerance: Specific immunological unresponsiveness.

tolerogen: An antigen used to induce tolerance. Often depends more on the circumstances of administration (e.g. route and concentration) than on any inherent property of the molecule.

Toll-like receptors (TLRs): A family of pattern recognition receptors involved in the detection of structures associated with pathogens or damaged host tissues.

toxoid: Chemically or physically modified toxin that is no longer harmful but retains immunogenicity.

tumor antigens: Antigens whose expression is associated with tumor cells.

tumor necrosis factor (TNF, also called TNFα): Together with the related cytokine lymphotoxin (TNFβ), was originally named for its cytotoxic effect on certain tumor cells, but also has important inflammatory and immunoregulatory functions.

variable (*V*) gene segments: Genes that rearrange together with *D* (diversity) and *J*(joining) gene segments in order to encode the variable region amino acid sequences of immunoglobulins and T-cell receptors.

vascular addressins: Cell adhesion molecules present on the luminal surface of blood and lymph vessel endothelium recognized by homing molecules which direct leukocytes to tissues with the appropriate 'address'.

vasoactive amines: Substances including histamine and 5-hydroxytryptamine which increase vascular permeability and smooth muscle contraction.

xenogeneic: Genetic differences between species.

xenograft: A tissue or organ graft between individuals of different species.

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