KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed University Established Under Section 3 of UGC Act 1956)
Coimbatore - 641021.
(For the candidates admitted from 2015 onwards)

DEPARTMENT OF BIOCHEMISTRY

SUBJECT : IMMUNOLOGY
SEMESTER : V
SUBJECT CODE : 15BCU503 **CLASS :** III B.Sc.(BC)

Semester V

15BCU503 **IMMUNOLOGY** **5H-5C**

Programme outcome: This course explains the basics and of immunology and components of immune system and its regulation in health and disease states. This course is intended to inculcate the basic understanding of immune system dysfunction viz., hyper-functioning and immunodeficiency states. Further, principle, working procedure, application, advantages and disadvantages of various immune-techniques used to assess the immune system are incorporated.

Programme learning outcome:

The students after completion of this course will have

- Clear understanding of immune system.
- The students can get clear understanding of immune components
- Basic understanding of immune system hyper-function and deficiency states
- Handling of immune-techniques

UNIT I

Basics of immunology : Introduction, Innate and acquired immunity, Cells of immune system. structure and function of T, B, NK cells, neutrophils, eosinophils and basophils. monocytes and macrophages ; Primary and secondary lymphoid organs. Humoral and cell mediated immunity.

UNIT II

Components of Immunity

Antigen: Definition, requirement for antigenecity, properties of antigen-specificity, cross reactivity, immunogenicity; epitopes, adjuvents, hapten.

Antibody-Definition, properties, classes, subclasses structure, specificity and distribution; self-antigens (MHC) - Class I, II, III molecules, role of MHC in antigen processing and presentation.

UNIT III
Hypersensitivity

Hypersensitivity- Type I, II, III & IV; Factors causing hypersensitivity; Mechanism, Pathogenesis, prevention and treatment**.**
Complement- definition, classical and alternate pathways, biological importance of complement system, complement deficiency diseases.

UNIT IV
Transplantation Immunology
Transplant-Mechanism of Allograft rejection; Auto immune diseases- Rheumatoid arthritis, myasthenia gravis, Graves's disease, Systemic lupus erythematosis.
Vaccination- passive and active. Preparation of live and attenuated vaccines, novel vaccines.

UNIT V
Immunotechniques

Antigen- antibody interaction-Precipitation reaction-immuno diffusion, immuno electrophoresis; Agglutination- blood grouping; Immuno techniques – Principle and application of RIA, ELISA, Fluorescent antibody techniques, immuno blotting, hybridoma technology - elementary concepts only.

TEXT BOOKS

Janis Kuby, 2013. Immunology,7th Edition. W.H. Freeman and Company, New York.

Pathak S., U.Palan, 2005 .Immunology essentials and fundamentals, capital publishing company, Banglore, 2nd edition.

Vaman Rao C. 2016. Immunolgy, Narosa publishing house, 2nd edition.

REFERENCES

Charles. A. Janeway and Jr. Paul Traverse, 2004. Immunobiology, Blackwell Scientific Publishers, Oxford

Ian R. Tizard, 2009. Immunology- An Introduction, 8th Edition.Saunders College Publishers, Sydney.

Ivan Riott and Janathar Brotoff, 2006. Immunology, 7th Edition Mosby Publishers, Sydney.

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LECTURE PLAN
DEPARTMENT OF BIOCHEMISTRY

STAFF NAME : Dr. M. SRIDHAR MUTHUSAMI
SUBJECT NAME : IMMUNOLOGY SUB.CODE: 15BCU503
SEMESTER: V CLASS : III B.Sc (BC)

S.No	Duration of period	Topics covered	Books referred	Page No	Web page referred
		UNIT-I			
1	1	Syllabi discussion			
2	1	Basics of Immunology-Introduction	T2	02-08	
3	1	Innate immunity	T1	06-11	
4	1	Acquired immunity	T1	1-31	
5	1	Cells of immune system-Structure and functions of Neutrophil, basophil and eosiniphil	T1	67-69	
6	1	Cells of immune system- Structure and functions of monocytes and macrophage	T1	64-65	
7	1	Cells of immune system- Structure and functions of T,B lymphocytes and NK cells	T1 T2	61-64 32-33	
8	1	Class test I			
9	1	Primary lymphoid organs	R2	110-115	

10	1	Secondary lymphoid organs	R2	116-122	
11	1	Humoral immunity	T2	203-208	
12	1	Cell mediated immunity	T2	251-258	
13	1	Revision of Unit I			
14	1	Possible questions discussion			
Total	**14**				
		Unit- II			
1	1	Components of Immunity-Antigen - Definiton, requirements for antigenicity	R2	34-41	
2	1	Properties of antigen –specificity, Cross reactivity, Immunogenicity	T1	87-90	
3	1	Epitopes	T1	92-98	
4	1	Adjuvants	T1	91-92	
5	1	Haptens	T1	100-101	
6	1	Class test			
7	1	Antibody-Definiton,properties	T1	107-122	
8	1	Classes, subclasses-Structure, specificity and distribution	T1	123-129	
9	1	Classes, subclasses-Structure, specificity and distribution (Contd..)	T1	123-129	
10	1	Self antigens(MHC)-introduction	R1	04.14	
11	1	Class I,II and III MHC molecules	R1		
12	1	Role of MHC in antigen processing and presentation	R1	4.14-4.19	
13	1	Revision of Unit II			

14	1	Possible questions discussion			
Total	**14**				
		Unit III			
1	1	Hypersensitivity-Introduction, types	T1	413-414	
2	1	Type I hypersensitivity-Factors, mechanism, pathogenesis, prevention and treatment	T1	415-430	
3	1	Type II hypersensitivity-Factors, mechanism, pathogenesis, prevention and treatment	T1	430-433	
4	1	Type III hypersensitivity-Factors, mechanism, pathogenesis, prevention and treatment	T1	434-436	
5	1	Type IV hypersensitivity-Factors, mechanism, pathogenesis, prevention and treatment	T1	436-437	
6	1	Class test			
7	1	Complement –Definition, activation pathwas	R2	234-235	
8	1	Classical pathway-Activation	R2	235-239	
9	1	Alternate pathway- Activation	R2	239-242	
10	1	Biological importance of complement system	R2	244-246	
11	1	Complement deficiency diseases	R2	246-247	
12	1	Revision of Unit II			
13	1	Possible questions discussion			
Total	**13**				
		Unit IV			
1	1	Transplantation immunology-Transplants	T1	555-556	

2	1	Mechanism of allograft rejection	T1	556-562	
3	1	Auto immune disease -Introduction	T1	485-486	
4	1	Rheumatoid arthritis and myasthenia gravis	T1	489-490	
5	1	Grave's disease, Systemic Lupus Erythematosis	T1	488-489	
6	1	Class test			
7	1	Vaccination-Passive	T1	444-445	
8	1	Vaccination- Active	T1	445-446	
9	1	Preparation of live vaccine	T1	446-447	
10	1	Preparation of attenuated vaccine	T1	448-449	
11	1	Novel vaccines	T1	451-456	
12	1	Revision of Unit II			
13	1	Possible questions discussion			
Total	**13**				
		Unit V			
1	1	Immuno techniques-Antigen antibody interactions-Introduction	T1	143-146	
2	1	Precipitation- Immuno diffusion	T1	148-152	
3	1	Precipitation- Immuno electrophoresis	T1	152-154	
4	1	Agglutination-Blood grouping	T1	154-156	
5	1	Class test			
6	1	Immuno techniques- RIA-Principle and applications	T1	156-157	
7	1	ELISA - Principle and applications	T1	157-158	

8	1	Fluorescent antibody technique- Principle and applications	T1	159-160	
9	1	Immuno blotting- Principle and applications	T1	158-159	
10	1	Hybridoma technology-Elementary concepts	T1	132-136	
11	1	Monoclonal antibody production and applications	T1	136-138	
12	1	Revision of Unit II			
13	1	Possible questions discussion			
Total	**13**				
		Previous year ESE question paper discussion			
1	1	Previous year question paper discussion			
2	1	Previous year question paper discussion			
3	1	Previous year question paper discussion			
4	1	Previous year question paper discussion			
5	1	Previous year question paper discussion			
Grand Total	**72**				

TEXT BOOKS

Text Books

T1- Janis Kuby, 2013. Immunology,6th Edition. W.H. Freeman and Company, New York.

T2-Pathak S., U.Palan, 2005 .Immunology essentials and fundamentals, capital publishing company, Banglore, 2nd edition.

T3-Vaman Rao C. 2006. Immunolgy, Narosa publishing house, 2nd edition.

Reference Books

R1- Ivan Riott and Janathar Brotoff, 2006. Immunology, 7th Edition Mosby Publishers, Sydney.

R2- Ian R. Tizard, 2009. Immunology- An Introduction, 8th Edition.Saunders College Publishers, Sydney.

R3- Charles. A. Janeway and Jr. Paul Traverse, 2004. Immunobiology, Blackwell Scientific Publishers, Oxford

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DEPARTMENT OF BIOCHEMISTRY

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SEMESTER : **V**
SUBJECT CODE : **15BCU503** **CLASS** : **III B.Sc.BC**

UNIT I - COURSE MATERIAL

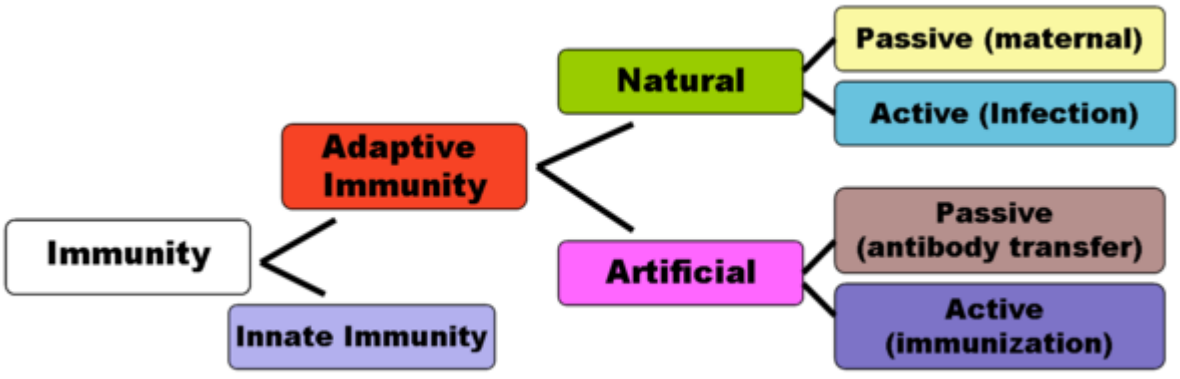
UNIT-I

Basics of immunology : Introduction, Innate and acquired immunity, Cells of immune system. structure and function of T, B, NK cells, neutrophils, eosinophils and basophils. monocytes and macrophages ; Primary and secondary lymphoid organs. Humoral and cell mediated immunity.

IMMUNITY

Immunity is a biological term that describes a state of having sufficient biological defenses to avoid infection, disease, or other unwanted biological invasion. In other words, it is nothing but the capability of the body to resist harmful microbes from entering the body. Immunity involves both specific and non-specific components.

There are two main types of immunity: They are innate immunity and adaptive or acquired immunity.



(I) **Innate immunity**, or nonspecific, immunity is the natural resistance with which a person is born. It provides resistance through several physical, chemical, and cellular approaches.

(i) Anatomic barrier:

Skin: Microbes first encounter the epithelial layers, physical barriers that line our skin and mucous membranes preventbthe entry of microbes

Sebum: Secretion of skin sebum, secretion of sebaseous glnad contains lactic and fratty acid , maintain a pH of 3-5 and inhibit the growth of microbes.

The conjunctivae , alimentary, respiratory and uorgenetal tracts are lined by mucous membrane and afford protection by entrapping foreign micro organism.

(ii)Physiological barrier:

The physiologic barrier that contribute to immunity include

Temperature- Some species body temperature prevent the growth of microbesEg: Chicken display innate immunity to anthrax because of its high temperature

pH : If ingested micro organism enter Into stomach , they can not survive with its low pH

Oxygen tension

Variable soluble factor : -Lysozyme a comnponnet of tears from eye is able to clear peptidoglyucan layer of bacterial cell wall

Interferon produced from the virus infected cell protects the near by cells from viral infection

Complement a circulating protein in serum activated thro immunologis response damage the membrane of micro organism

(iii) Endocytic and phagocytic barriers:

It is an important innate defence mechanism where extreacellular macromolecules are ingested via endocytosis and particulate material via phagocytosis and phagocytic activity is associated with the inflammatory response. The phagocytes express cell surface receptors that can bind and respond to common molecular patterns expressed on the surface of invading microbes. Through these approaches, innate immunity can prevent the colonization, entry, and spread of microbes.

(II) **Adaptive immunity** is often sub-divided into two major types depending on how the immunity was introduced. **Naturally acquired immunity** occurs through contact with a disease causing agent, when the contact was not deliberate, whereas **artificially acquired immunity** develops only through deliberate actions such as vaccination. Both naturally and artificially acquired immunity can be further subdivided depending on whether immunity is induced in the host or passively transferred from a immune host. **Passive immunity** is acquired through transfer of antibodies or activated T-cells from an immune host, and is short lived -- usually lasting only a few months

A further subdivision of adaptive immunity is characterized by the cells involved; **humoral immunity** is the aspect of immunity that is mediated by secreted antibodies, whereas the protection provided by **cell mediated immunity** involves T-lymphocytes alone. Humoral immunity is active when the organism generates its own antibodies, and passive when antibodies are transferred between individuals. Similarly, cell mediated immunity is active when the organisms' own T-cells are stimulated and passive when T cells come from another organism.

Passive immunity

Passive immunity is the transfer of active immunity in the form of readymade antibodies, from one individual to another. Passive immunity can occur naturally, when maternal antibodies are transferred to the fetus through the placenta, and can also be induced artificially, when high levels of human (or horse) antibodies specific for a pathogen or toxin are transferred to non-immune individuals. Passive immunization is used when there is a high risk of infection and insufficient time for the body to develop its own immune response, or to reduce the symptoms of ongoing or immunosuppressivediseases. Passive immunity provides immediate protection, but the body does not develop memory, therefore the patient is at risk of being infected by the same pathogen later.

Naturally acquired passive immunity

Maternal passive immunity is a type of naturally acquired passive immunity, and refers to antibody-mediated immunity conveyed to a fetus by its mother during pregnancy. Maternal antibodies (MatAb) are passed through the placenta to the fetus by an FcR receptor on placental cells. This occurs around the third month of gestation. IgG is the only antibody isotype that can pass through the placenta. Passive immunity is also provided through the transfer of IgA antibodies found in breast milk that are transferred to the gut of the infant, protecting against bacterial infections, until the newborn can synthesize its own antibodies.

Artificially acquired passive immunity

Artificially acquired passive immunity is a short-term immunization induced by the transfer of antibodies, which can be administered in several forms; as human or animal blood plasma, as pooled human immunoglobulin for intravenous (IVIG) or intramuscular (IG) use, and in the form of monoclonal antibodies (MAb). Passive transfer is used prophylactically in the case of immunodeficiency diseases, such as hypogammaglobulinemia. It is also used in the treatment of several types of acute infection, and to treat poisoning. Immunity derived from passive immunization lasts for only a short period of time, and there is also a potential risk for hypersensitivity reactions, and serum sickness, especially from gamma globulin of non-human origin.

Passive transfer of cell-mediated immunity

Passive or "adoptive transfer" of cell-mediated immunity, is conferred by the transfer of "sensitized" or activated T-cells from one individual into another. It is rarely used in humans because it requires histocompatible (matched) donors, which are often difficult to find. In unmatched donors this type of transfer carries severe risks of graft versus host disease. It has, however, been used to treat certain diseases including some types of cancer and immunodeficiency. This type of transfer differs from a bone marrow transplant, in which (undifferentiated) hematopoietic stem cells are transferred.

CELLS OF IMMUNE SYSTEM

Hematopoiesis

All blood cells arise from a type of cell called hematop[ietic stem cell(HSC). Stem cells that can differentiate into other cedll types. They are self renewing, they maintain thier population level by cell division. In human, hematopoiesis, the formation and development of red and white blood cells begins in the embryonic yolk sac during the first week of development.

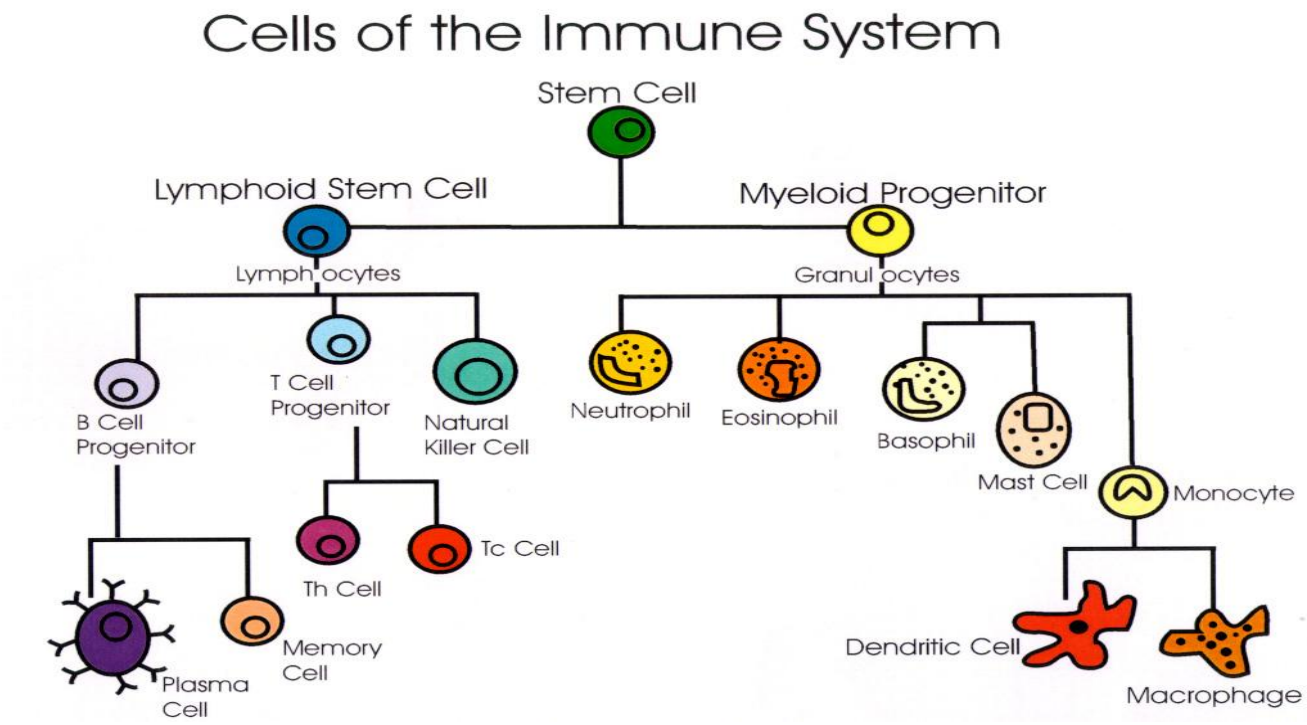


Fig: Cells of immune system

The Cells of the Immune System

Antigen-presenting cells	Cells which do not have antigen-specific receptors. Instead, they capture and process antigens, present them to T cell receptors. These cells include macrophages, dentritic cells and B cells.
B cells	Also known as *B cell lymphocytes*. B cells spend their entire early life in the bone marrow. Upon maturity, their job is to travel throughout the blood and lymph looking for antigens with which they can interlock. Once a B cell has identified an antigen, it starts replicating itself. These cloned cells mature into antibody-manufacturing *plasma cells*.
Basophils	Similar to mast cells, but distributed throughout the body. Like mast cells, basophils release histamine upon encountering certain antigens, thereby triggering an allergic reaction.
Cytotoxic T cells	Also called *cytotoxic T lymphocytes* or CTLs.
Dendritic cells	Mostly found in the skin and mucosal epithelium, where they are referred to as Langerhan's cells. Unlike macrophages, dendritic cells can also recognize viral particles as non-self. In addition, they can present antigens via **both** MHC I and MHC II, and can thus activate both CD8 and CD4 T cells, directly.
Granulocytes	Leukocytes (white blood cells) containing granules in the cytoplasm. Also known as a granular leukocyte. They seem to act as a first line of defense, as they rush toward an infected area and engulf the offending microbes. Granulocytes kill microbes by digesting them with killer enzymes contained in small units called lysosomes.
Helper T cells	These cells travel through the blood and lymph, looking for antigens (such as those captured by *antigen-presenting cells*). Upon locating an antigen, they notify other cells to assist in combating the invader. This is sometimes done through the use of cytokines (or specifically, lymphokines) which help destroy target cells and stimulate the production of

	healthy new tissue. Interferon is an example of such a cytokine.
Leukocytes	White blood cells. These are the cells which provide immunity, and they can be subdivided into three classes: lymphocytes, granulocytes and monocytes
Lymphocytes	Small white blood cells which are responsible for much of the work of the immune system. Lymphocytes can be divided into three classes: B cells, T cells and null cells.
Macrophages	Literally, "large eaters." These are large, long-lived phagocytes which capture foreign cells, digest them, and present protein fragments (peptides) from these cells and manifest them on their exterior. In this manner, they present the antigens to the T cells. Macrophages are strategically located in lymphoid tissues, connective tissues and body cavities, where they are likely to encounter antigens. They also act as effector cells in cell-mediated immunity.
Mast cells	Cells concentrated within the respiratory and gastrointestinal tracts, and within the deep layers of the skin. These cells release histamine upon encountering certain antigens, thereby triggering an allergic reaction.
Memory cells	Specialized B cells which grant the body the ability to manufacture more of a particular antibody as needed, in case a particular antigen is ever encountered again.
Monocytes	Large, agranular leukocytes with relatively small, eccentric, oval or kidney-shaped nuclei.
Plasma cells	Specialized B cells which churn out antibodies—more than two thousand per second. Most of these die after four to five days; however, a few survive to become *memory cells*.
T cells	Also known as *T cell lymphocytes.* Unlike B cells, these cells leave the marrow at an early age and travel to the thymus, where they mature. Here they are imprinted with critical information for recognizing "self" and "non-self" substances.

	Among the subclasses of T cells are *helper T cells* and *cytotoxic (or killer) T cells*.

Lymphoid Cells

Lymphocytes constitute 20%–40% of the body's white blood cells and 99% of the cells in the lymph (Table 2-4). There are approximately 10^{11} (range depending on body size and age: ~10^{10}–10^{12}) lymphocytes in the human body. These lym-phocytes continually circulate in the blood and lymph and are capable of migrating into the tissue spaces and lymphoid organs, thereby integrating the immune system to a high degree.

TABLE Normal adult blood-cell counts

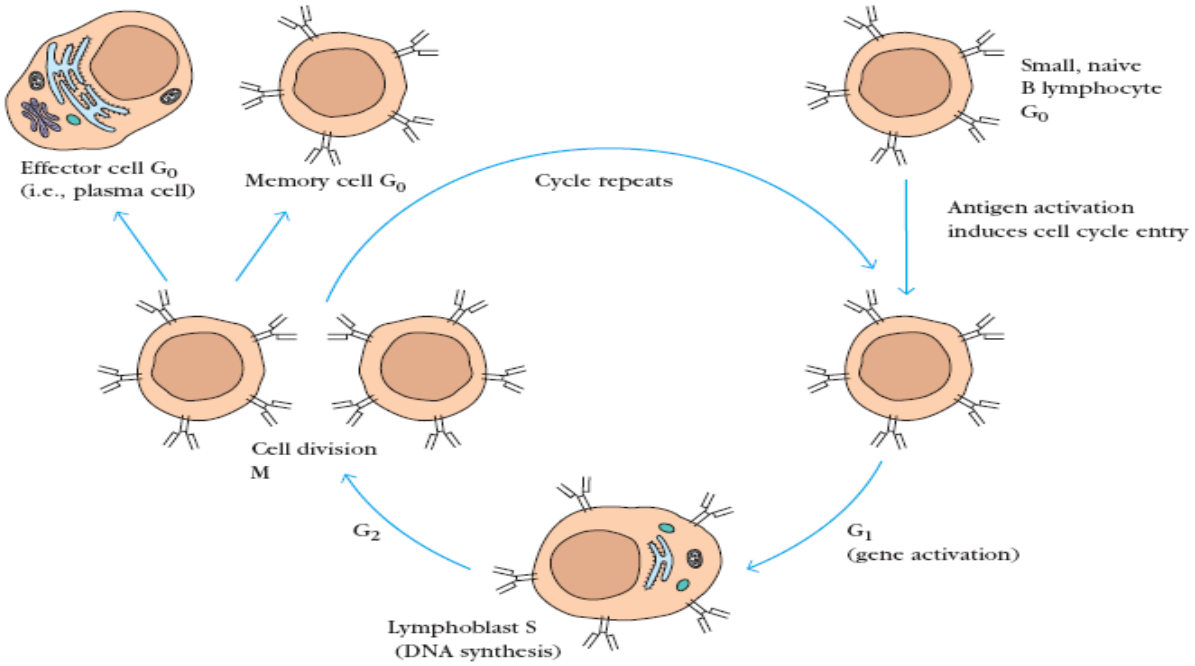
Cell type	Cells/mm^3	%
Red blood cells	5.0×10^6	
Platelets	2.5×10^5	
Leukocytes	7.3×10^3	
Neutrophil		50–70
Lymphocyte		20–40
Monocyte		1–6
Eosinophil		1–3
Basophil		<1

The lymphocytes can be broadly subdivided into three populations—B cells, T cells, and natural killer cells—on the basis of function and cell-membrane components. **Natural killer cells (NK cells)** are large, granular lymphocytes that do not express the set of surface markers typical of B or T cells. Resting B and T lymphocytes are small, motile, nonphago-cytic cells, which cannot be distinguished morphologically. B and T lymphocytes that have not interacted with antigen— referred to as **naive,** or unprimed—are resting cells in the G_0 phase of the cell cycle. Known as small lymphocytes, these cells are only about 6 m in diameter; their cytoplasm forms a barely discernible rim around the nucleus. Small lympho-cytes have densely packed chromatin, few mitochondria, and a poorly developed endoplasmic reticulum and Golgi

appa-ratus. The naive lymphocyte is generally thought to have a short life span. Interaction of small lymphocytes with anti-gen, in the presence of certain cytokines, induces these cells to enter the cell cycle by progressing from G_0 into G_1 and subsequently into S, G_2, and M . As they progress through the cell cycle, lymphocytes enlarge into 15 m-diameter blast cells, called **lymphoblasts;** these cells have a higher cytoplasm:nucleus ratio and more or-ganellar complexity than small lymphocytes .

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

Different lineages or maturational stages of lymphocytes can be distinguished by their expression of membrane mole-cules recognized by particular monoclonal antibodies (anti-bodies that are specific for a single epitope of an antigen; see Chapter 4 for a description of monoclonal antibodies). All of the monoclonal antibodies that react with a particular mem-brane molecule are grouped together as a **cluster of dif-ferentiation (CD).**



B LYMPHOCYTES

The B lymphocyte derived its letter designation from its site of maturation, in the *b*ursa of Fabricius in birds; the name turned out to be apt, for *b*one marrow is its major site of mat-uration in a number of mammalian species, including hu-mans and mice. Mature B cells are definitively distinguished from other lymphocytes by their synthesis and display of membrane-bound immunoglobulin (antibody) molecules,

which serve as receptors for antigen. Each of the approxi-mately 1.5 10^5 molecules of antibody on the membrane of a single B cell has an identical binding site for antigen. Among the other molecules expressed on the membrane of mature B cells are the following:

B220 (a form of CD45) is frequently used as a marker for B cells and their precursors. However, unlike antibody, it is not expressed uniquely by B-lineage cells.

Class II MHC molecules permit the B cell to function as an antigen-presenting cell (APC).

CR1 (CD35) and **CR2** (CD21) are receptors for certain complement products.

Fc RII (CD32) is a receptor for IgG, a type of antibody.

B7-1 (CD80) and **B7-2** (CD86) are molecules that interact with CD28 and CTLA-4, important regulatory

molecules on the surface of different types of T cells, including T_H cells.

CD40 is a molecule that interacts with CD40 ligand on the surface of helper T cells. In most cases this interaction is critical for the survival of antigen-stimulated B cells and for their development into antibody-secreting plasma cells or memory B cells.

Interaction between antigen and the membrane-bound anti-body on a mature naive B cell, as well as interactions with T cells and macrophages, selectively induces the activation and differentiation of B-cell clones of corresponding specificity. In this process, the B cell divides repeatedly and differentiates over a 4- to 5-day period, generating a population of plasma cells and memory cells. Plasma cells, which have lower levels of membrane-bound antibody than B cells, synthesize and secrete antibody. All clonal progeny from a given B cell se-crete antibody molecules with the same antigen-binding specificity. Plasma cells are terminally differentiated cells, and many die in 1 or 2 weeks.

T LYMPHOCYTES

T lymphocytes derive their name from their site of matura-tion in the *t* hymus. Like B lymphocytes, these cells have membrane receptors for antigen. Although the antigen-binding T-cell receptor is structurally distinct from im-munoglobulin, it does share some common structural features with the immunoglobulin molecule, most notably in the structure of its antigen-binding site. Unlike the mem-brane-bound antibody on B cells, though, the T-cell receptor (TCR) does not recognize free antigen. Instead the TCR rec-ognizes only antigen that is bound to particular classes of self-molecules. Most T cells recognize antigen only when it is bound to a self-molecule encoded by genes within the major histocompatibility complex (MHC). Thus, as explained in Chapter 1, a fundamental difference between the humoral and cell-mediated branches of the immune system is that the B cell is capable of binding soluble antigen, whereas the T cell is restricted to binding antigen displayed on self-cells. To be recognized by most T cells, this antigen must be displayed to-gether with MHC molecules on the surface of antigen-pre-senting cells or on virus-infected cells, cancer cells, and grafts. The T-cell system has developed to eliminate these al-tered self-cells, which pose a threat to the normal functioning of the body.

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

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

CD45, a signal-transduction molecule

T cells that express the membrane glycoprotein molecule CD4 are restricted to recognizing antigen bound to class II MHC molecules, whereas T cells expressing CD8, a dimeric membrane glycoprotein, are restricted to recognition of anti-gen bound to class I MHC molecules. Thus the expression of CD4 versus CD8 corresponds to the MHC restriction of the T cell. In general, expression of CD4 and of CD8 also defines two major functional subpopulations of T lymphocytes. CD4 T cells generally function as T helper (T_H) cells and are class-II restricted; CD8 T cells generally function as T cyto-toxic (T_C) cells and are class-I restricted. Thus the ratio of T_H to T_C cells in a sample can be approximated by assaying the number of CD4 and CD8 T cells. This ratio is approxi-mately 2:1 in normal human peripheral blood, but it may be significantly altered by immunodeficiency diseases, autoim-mune diseases, and other disorders.

The classification of CD4 class II–restricted cells as T_H cells and CD8 class I–restricted cells as T_C cells is not ab-solute. Some CD4 cells can act as killer cells. Also, some T_C cells have been shown to secrete a variety of cytokines and ex-ert an effect on other cells comparable to that exerted by T_H cells. The distinction between T_H and T_C cells, then, is not al-ways clear; there can be ambiguous functional activities. However, because these ambiguities are the exception and not the rule, the generalization of T helper (T_H) cells as being CD4 and class-II restricted and of T cytotoxic cells (T_C) as being CD8 and class-I restricted is assumed throughout this text, unless otherwise specified.

T_H cells are activated by recognition of an antigen–class II MHC complex on an antigen-presenting cell. After activa-tion, the T_H cell begins to divide and gives rise to a clone of effector cells, each specific for the same antigen–class II MHC complex. These T_H cells secrete various cytokines, which play a central role in the activation of B cells, T cells, and other cells that participate in the immune response. Changes in the pattern of cytokines produced by T_H cells can change the type of immune response that develops among other leukocytes. The **T_H1 response** produces a cytokine profile that supports inflammation and activates mainly cer-tain T cells and macrophages, whereas the **T_H2 response** ac-tivates mainly B cells and immune responses that depend upon antibodies. T_C cells are activated when they interact with an antigen–class I MHC complex on the surface of an altered self-cell (e.g., a virus-infected cell or a tumor cell) in the presence of appropriate cytokines. This activation, which results in proliferation, causes the T_C cell to differentiate into an effector cell called a **cytotoxic T lymphocyte (CTL).** In contrast to T_H cells, most CTLs secrete few cytokines. In-stead, CTLs acquire the ability to recognize and eliminate al-tered self-cells.

Another subpopulation of T lymphocytes—called **T sup-pressor (T_S) cells**—has been postulated. It is clear that some T cells help to suppress the humoral and the cell-mediated branches of the immune system, but the actual isolation and cloning of normal T_S cells is a matter of controversy and dis-pute among immunologists. For this reason, it is uncertain whether T_S cells do indeed constitute a separate functional subpopulation of T cells. Some immunologists believe that the suppression mediated by T cells observed in some sys-tems is simply the consequence of activities of T_H or T_C sub-populations whose end results are suppressive.

NATURAL KILLER CELLS

The natural killer cell was first described in 1976, when it was shown that the body contains a small population of large, granular lymphocytes that display cytotoxic activity against a wide range of tumor cells in the absence of any previous im-munization with the tumor. NK cells were subsequently shown to play an important role in host defense both against tumor cells and against cells infected with some, though not all, viruses. These cells, which constitute 5%–10% of lym-phocytes in human peripheral blood, do not express the membrane molecules and receptors that distinguish T- and B-cell lineages. Although NK cells do not have T-cell recep-tors or immunoglobulin incorporated in their plasma mem-branes, they can recognize potential target cells in two different ways. In some cases, an NK cell employs NK cell re-ceptors to distinguish abnormalities, notably a reduction in the display of class I MHC molecules and the unusual profile of surface antigens displayed by some tumor cells and cells infected by some viruses. Another way in which NK cells rec-ognize potential target cells depends upon the fact that some tumor cells and cells infected by certain viruses display anti-gens against which the immune system has made an anti-body response, so that antitumor or antiviral antibodies are bound to their surfaces. Because NK cells express CD16, a membrane receptor for the carboxyl-terminal end of the IgG molecule, called the Fc region, they can attach to these anti-bodies and subsequently destroy the targeted cells. This is an example of a process known as **antibody-dependent cell-mediated cytotoxicity (ADCC).**

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

Mononuclear Phagocytes

The mononuclear phagocytic system consists of **monocytes** circulating in the blood and **macrophages** in the tissues. During hematopoiesis in the bone marrow, granulocyte-monocyte progenitor cells differentiate into promonocytes, which leave the bone marrow and enter the blood, where they further differentiate into mature monocytes. Monocytes circulate in the bloodstream for about 8 h, during which they enlarge; they then migrate into the tissues and differentiate into specific tissue macrophages or, as discussed later, into dendritic cells.

Differentiation of a monocyte into a tissue macrophage involves a number of changes: The cell enlarges five- to ten-fold; its intracellular organelles increase in both number and complexity; and it acquires increased phagocytic ability, pro-duces higher levels of hydrolytic enzymes, and begins to se-crete a variety of soluble factors. Macrophages are dispersed throughout the body. Some take up residence in particular tissues, becoming fixed macrophages, whereas others remain motile and are called free, or wandering, macrophages. Free macrophages travel by amoeboid movement throughout

the tissues. Macrophage-like cells serve different functions in different tissues and are named according to their tissue location:

- **Alveolar macrophages** in the lung
- **Histiocytes** in connective tissues
- **Kupffer cells** in the liver
- **Mesangial cells** in the kidney
- **Microglial cells** in the brain
- **Osteoclasts** in bone

PHAGOCYTOSIS

Macrophages are capable of ingesting and digesting exoge-nous antigens, such as whole microorganisms and insoluble particles, and endogenous matter, such as injured or dead host cells, cellular debris, and activated clotting factors.

ANTIMICROBIAL AND CYTOTOXIC ACTIVITIES

A number of antimicrobial and cytotoxic substances pro-duced by activated macrophages can destroy phagocytosed microorganisms

i) OXYGEN - DEPENDENT KILLING MECHANISMS Activated phagocytes produce a number of **reactive oxygen intermedi-ates (ROIs)** and **reactive nitrogen intermediates that have** potent antimicrobial activity. During phagocytosis, a meta-bolic process known as the **respiratory burst** occurs in acti-vated macrophages. This process results in the activation of a membrane-bound oxidase that catalyzes the reduction of oxygen to superoxide anion, a reactive oxygen intermediate that is extremely toxic to ingested microorganisms.

ii) OXYGEN - INDEPENDENT KILLING MECHANISMS Activated macrophages also synthesize **lysozyme** and various hy-drolytic enzymes whose degradative activities do not require oxygen. In addition,

activated macrophages produce a group of antimicrobial and cytotoxic peptides, commonly known as **defensins.**

TABLE Mediators of antimicrobial and cytotoxic activity of macrophages and neutrophils

Oxygen-dependent killing	Oxygen-independent killing
Reactive oxygen intermediates	Defensins
$O^{\bullet}_2{}^{-}$ (superoxide anion)	Tumor necrosis factor α
$OH^{\bullet}$ (hydroxyl radicals)	(macrophage only)
H_2O_2 (hydrogen peroxide)	Lysozyme
ClO^{-} (hypochlorite anion)	Hydrolytic enzymes
Reactive nitrogen intermediates	
NO (nitric oxide)	
NO_2 (nitrogen dioxide)	
HNO_2 (nitrous acid)	
Others	
NH_2CL (monochloramine)	

Granulocytic Cells

The **granulocytes** are classified as neutrophils, eosinophils, or basophils on the basis of cellular morphology and cyto-plasmic staining characteristics. The **neurophil** has a multilobed nucleus and a granulated cytoplasm that stains with both acid and basic dyes; it is often called a polymorphonuclear leukocyte (PMN) for its multilobed nu-cleus. The **eosinophil** has a bilobed nucleus and a granulated cytoplasm that stains with the acid dye eosin red (hence its name). The **basophil** has a lobed nucleus and heavily granu-lated cytoplasm that stains with the basic dye methylene blue. Both neutrophils and eosinophils are phagocytic, whereas basophils are not. Neutrophils, which constitute 50%–70% of the circulating white blood cells, are much more numer-ous than eosinophils (1%–3%) or basophils (1%).

NEUTROPHILS

Neutrophils are produced by hematopoiesis in the bone marrow. They are released into the peripheral blood and circulate for 7–10 h before migrating into the tissues, where they have a life span of only a few days. In response to many types of infections, the bone marrow releases more than the usual number of neutrophils and these cells generally are the first to arrive at a site of inflammation. The resulting transient in-crease in the number of circulating neutrophils, called **leukocytosis,** is used medically as an indication of infection.

Neutrophils are normally found in the bloodstream and are the most abundant type of phagocyte, constituting 50% to 60% of the total circulating white blood cells.

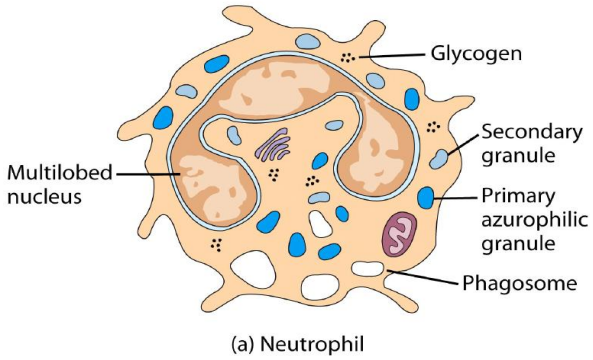


Fig: A neutrophil with a segmented nucleus (center and surrounded by erythrocytes), the intra-cellular granules are visible in the cytoplasm (Giemsa-stained high magnification)

Neutrophils have three strategies for directly attacking micro-organisms: phagocytosis (ingestion), release of soluble anti-microbials (including granule proteins), and generation of neutrophil extracellular traps (NETs). Neutrophils are professional phagocytes. they are ferocious eaters and rapidly engulf invaders coated with antibodies and complement, and damaged cells or cellular debris. The intra-cellular granules of the human neutrophil have long been recognized for their protein-destroying and bactericidal properties. Neutrophils can secrete products that stimulate monocytes and macrophages; these secretions increase phagocytosis and the formation of reactive oxygen compounds involved in intracellular killing. Neutrophils have two types of granules; primary (azurophilic) granules (found in young cells) and specific granules (which are found in more mature cells). Primary granules contain cationic proteins and defensins that are used to kill bacteria, proteolytic enzymes and cathepsin G to break down (bacterial) proteins, lysozyme to break down bacterial cell walls, and myeloperoxidase (used to generate toxic bacteria-killing substances). In addition, secretions from the primary granules of neutrophils stimulate the phagocytosis of IgG antibody-coated bacteria. The secondary granules contain compounds that are involved in the formation of toxic oxygen compounds, lysozyme, and lactoferrin (used to take essential iron from bacteria). Neutrophil extracellular traps (NETs) comprise a web of fibers composed of chromatin and serine proteases that trap and kill microbes

extracellularly. Trapping of bacteria is a particularly important role for NETs in sepsis, where NET are formed within blood vessels.

Movement of circulating neutrophils into tissues, called **extravasation,** takes several steps: the cell first adheres to the vascular endothelium, then penetrates the gap between adjacent endothelial cells lining the vessel wall, and finally penetrates the vascular basement membrane, moving out into the tissue spaces.

A number of substances generated in an inflam-matory reaction serve as **chemotactic factors** that promote accumulation of neutrophils at an inflammatory site. Among these chemotactic factors are some of the complement components,components of the blood-clotting system,and sev-eral cytokines secreted by activated T_H cells and macrophages.

Like macrophages, neutrophils are active phagocytic cells. Phagocytosis by neutrophils is similar to that described for macrophages, except that the lytic enzymes and bactericidal substances in neutrophils are contained within primary and secondary granules (see Figure 2-10a). The larger, denser pri-mary granules are a type of lysosome containing peroxidase, lysozyme, and various hydrolytic enzymes. The smaller sec-ondary granules contain collagenase, lactoferrin, and lyso-zyme. Both primary and secondary granules fuse with phagosomes, whose contents are then digested and elimi-nated much as they are in macrophages.

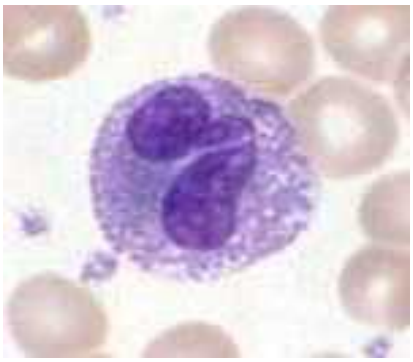
Neutrophils also employ both oxygen-dependent and oxygen-independent pathways to generate antimicrobial substances. Neutrophils are in fact much more likely than macrophages to kill ingested microorganisms. Neutrophils exhibit a larger respiratory burst than macrophages and con-sequently are able to generate more reactive oxygen interme-diates and reactive nitrogen intermediate. In addition, neutrophils express higher levels of defensins than macrophages do.

EOSINOPHILS

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

Eosinophils also have lobed nuclei (two to four lobes). The number of granules in an eosinophil can vary because they have a tendency to degranulate while in the blood stream. Eosinophils play a crucial part in the killing of parasites (e.g., enteric nematodes) because their granules contain a unique, toxic basic protein and cationic protein (e.g., cathepsin) receptors that bind to IgE are used to help with this task. These cells also have a limited ability to participate in phagocytosis, they are professional antigen-presenting cells, they regulate other immune cell functions (e.g., CD4+ T cell, dendritic cell, B cell, mast cell, neutrophil, and basophil functions),

they are involved in the destruction of tumor cells, and they promote the repair of damaged tissue. A polypeptide called interleukin-5 interacts with eosinophils and causes them to grow and differentiate; this polypeptide is produced by basophils.



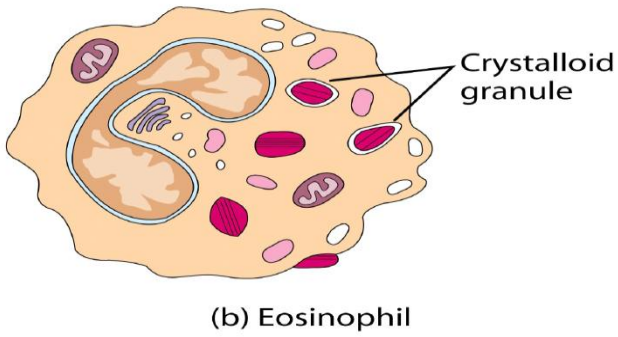
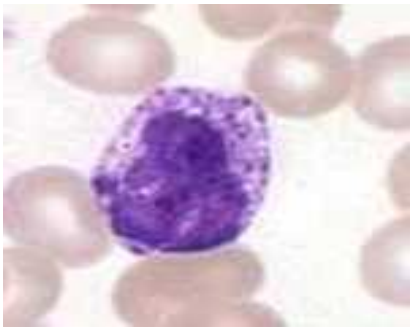


Fig: An eosinophil surrounded by erythrocytes

BASOPHILS

Basophils are nonphagocytic granulocytes that function by releasing pharmacologically active substances from their cy-toplasmic granules. These substances play a major role in cer-tain allergic responses.

Basophils are one of the least abundant cells in bone marrow and blood (occurring at less than two percent of all cells). Like neutrophils and eosinophils, they have lobed nuclei; however, they have only two lobes, and the chromatin filaments that connect them are not very visible. Basophils have receptors that can bind to IgE, IgG, complement, and histamine. The cytoplasm of basophils contains a varied amount of granules; these granules are usually numerous enough to partially conceal the nucleus. Granule contents of basophils are abundant with histamine, heparin, chondroitin sulfate, peroxidase, platelet-activating factor, and other substances.



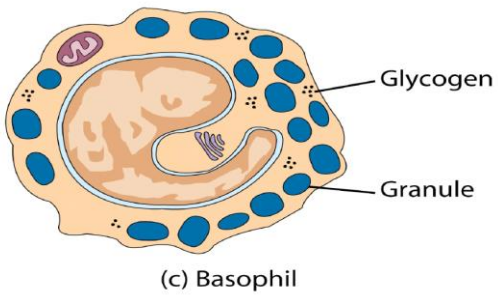


Fig:A basophil with lobed nuclei surrounded by erythrocytes

When an infection occurs, mature basophils will be released from the bone marrow and travel to the site of infection. When basophils are injured, they will release histamine, which contributes to the inflammatory response that helps fight invading organisms. Histamine causes dilation and increased permeability of capillaries close to the basophil. Injured basophils and other leukocytes will release another substance called prostaglandins that contributes to an increased blood flow to the site of infection. Both of these mechanisms allow blood-clotting elements to be delivered to the infected area (this begins the recovery process and blocks the travel of microbes to other parts of the body). Increased permeability of the inflamed tissue also allows for more phagocyte migration to the site of infection so that they can consume microbes.

MAST CELLS

Mast-cell precursors, which are formed in the bone marrow by hematopoiesis, are released into the blood as undifferenti-ated cells; they do not differentiate until they leave the blood and enter the tissues. Mast cells can be found in a wide vari-ety of tissues, including the skin, connective tissues of various organs, and mucosal epithelial tissue of the respiratory, geni-tourinary, and digestive tracts. Like circulating basophils, these cells have large numbers of cytoplasmic granules that contain histamine and other pharmacologically active sub-stances. Mast cells, together with blood basophils, play an im-portant role in the development of allergies.

Mast cell (also known as *mastocyte* and *labrocyte)* is a resident cell of several types of tissues and contains many granules rich in histamine and heparin. Although best known for their role in allergy and anaphylaxis, mast cells play an important protective role as well, being intimately involved in wound healing and defense against pathogens.

Two types of mast cells are recognized, those from connective tissue and a distinct set of mucosal mast cells. The activities of the latter are dependent on T-cells.

Mast cells are present in most tissues characteristically surrounding blood vessels and nerves, and are especially prominent near the boundaries between the outside world and the internal milieu, such as the skin, mucosa of the lungs and digestive tract, as well as in the mouth, conjunctiva and nose.

Mast cells play a key role in the inflammatory process. When activated, a mast cell rapidly releases its characteristic *granules* and various hormonal mediators into the interstitium. Mast cells can be stimulated to degranulate by direct injury (e.g. physical or chemical [such as opioids, alcohols, and certain antibiotics such as polymyxins]), cross-linking of Immunoglobulin E (IgE) receptors, or by activated complement proteins.

In allergic reactions, mast cells remain inactive until an allergen binds to IgE already in association with the cell. Other membrane activation events can either prime mast cells for subsequent degranulation or can act in synergy with FceRI signal transduction. Allergens are generally proteins or polysaccharides. The allergen binds to the antigen-binding sites, which are situated on the variable regions of the IgE molecules bound to the mast cell surface. It appears

that binding of two or more IgE molecules (cross-linking) is required to activate the mast cell. The clustering of the intracellular domains of the cell-bound Fc receptors, which are associated with the cross-linked IgE molecules, causes a complex sequence of reactions inside the mast cell that lead to its activation. Although this reaction is most well understood in terms of allergy, it appears to have evolved as a defense system against intestinal worm infestations (tapeworms, etc.)

The molecules thus released into the extracellular environment include

- preformed mediators (from the granules):
  - serine proteases, such as tryptase
  - histamine (2-5 pg/cell)
  - serotonin
  - proteoglycans, mainly heparin (active as anticoagulant)
- newly formed lipid mediators (eicosanoids):
  - thromboxane
  - prostaglandin D2
  - leukotriene C4
  - platelet-activating factor
- cytokines
  - Eosinophil chemotactic factor

Histamine dilates post capillary venules, activates the endothelium, and increases blood vessel permeability. This leads to local edema (swelling), warmth, redness, and the attraction of other inflammatory cells to the site of release. It also irritates nerve endings (leading to itching or pain). Cutaneous signs of histamine release are the "flare and wheal"-reaction. The bump and redness immediately following a mosquito bite are a good example of this reaction, which occurs seconds after challenge of the mast cell by an allergen

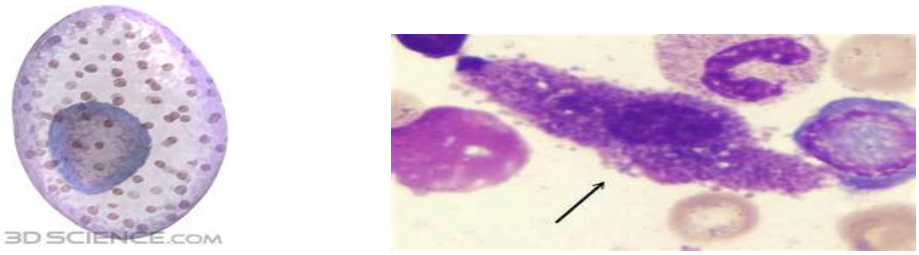


Fig: Mast cell

Fig: Mast cell activation and release of allergic activators

DENDRITIC CELLS

Dendritic cells (DCs) are immune cells forming part of the mammalian immune system. Their main function is to process antigen material and present it on the surface to other cells of the immune system. That is, dendritic cells function as antigen-presenting cells. They act as messengers between the innate and adaptive immunity.

Dendritic cells are present in tissues in contact with the external environment, such as the skin (where there is a specialized dendritic cell type called Langerhans cells) and the inner lining of the nose, lungs, stomach and intestines. They can also be found in an immature state in the blood. Once activated, they migrate to the lymph nodes where they interact with T cells and B cells to initiate and shape the adaptive immune response. At certain development stages they grow branched projections, the *dendrites* that give the cell its name

Natural Killer Cells

Natural killer cells, often referred to as NK cells, are similar to the killer T cell subset (CD8+ T cells). They function as effector cells that directly kill certain tumors such as melanomas, lymphomas and viral-infected cells, most notably herpes and cytomegalovirus-infected cells. Unlike conventional T cells that recognize peptide antigen presented by major histocompatibility complex (MHC) molecules, NKT cells recognize glycolipid antigen presented by a molecule called CD1d. Once activated, these cells can perform functions ascribed to both T_h and T_c cells (i.e., cytokine production and release of cytolytic/cell killing molecules). They are also able to recognize and eliminate some tumor cells and cells infected with herpes viruses.

Mechanism:

NK cells are cytotoxic; small granules in their cytoplasm contain proteins such as perforin and proteases known as granzymes. Upon release in close proximity to a cell slated for killing, perforin forms pores in the cell membrane of the target cell, creating an aqueous channel through which the granzymes and associated molecules can enter, inducing either apoptosis or osmotic cell lysis. The distinction between apoptosis and cell lysis is important in immunology: lysing a virus-infected cell would only release the virions, whereas apoptosis leads to destruction of the virus inside.

NK cells are activated in response to interferons or macrophage-derived cytokines. They serve to contain viral infections while the adaptive immune response is generating antigen-specific cytotoxic T cells that can clear the infection. Patients deficient in NK cells prove to be highly susceptible to early phases of herpes virus infection.

In order for NK cells to defend the body against viruses and other pathogens, they require mechanisms that enable the determination of whether a cell is infected or not. The exact mechanisms remain the subject of current investigation, but recognition of an "altered self" state is thought to be involved. To control their cytotoxic activity, NK cells possess two types of surface receptors: *activating receptors* and *inhibitory receptors*.

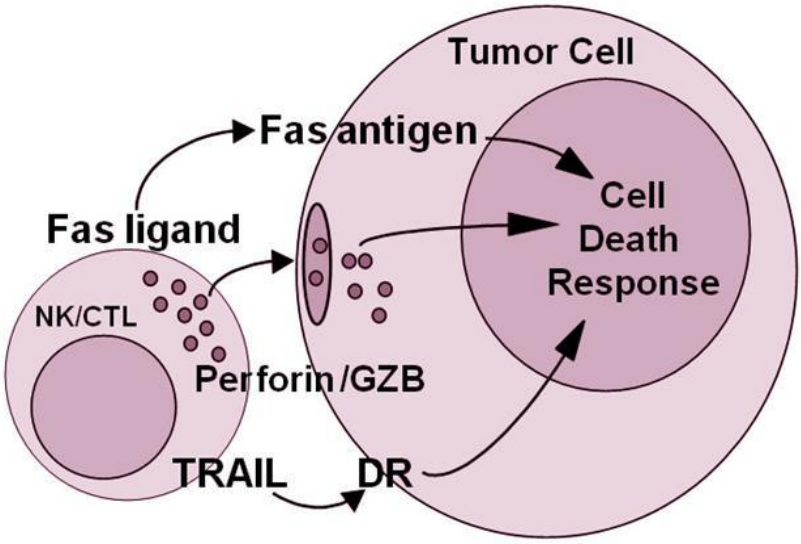


Fig: Diagram indicating the activities of NK cells.

ORGANS OF THE IMMUNE SYSTEM

A number of morphologically and functionally diverse or-gans and tissues have various functions in the development of immune responses. These can be distinguished by func-tion as the primary and secondary lymphoid organs (Figure). The thymus and bone marrow are the primary (or central) lymphoid organs, where maturation of lymphocytes takes place. The lymph nodes, spleen, and various mucosal-associated lymphoid tissues (MALT) such as gut-associated lymphoid tissue (GALT) are the secondary (or peripheral) lymphoid organs, which trap antigen and provide sites for mature lymphocytes to interact with that antigen. In addi-tion, tertiary lymphoid tissues, which normally contain fewer lymphoid cells than secondary lymphoid organs, can import lymphoid cells during an inflammatory response. Most prominent of these are cutaneous-associated lymphoid tissues.

PRIMARY LYMPHOID ORGANS:

Immature lymphocytes generated in hematopoiesis mature and become committed to a particular antigenic specificity within the primary lymphoid organs. Only after a lympho cyte has matured within a primary lymphoid organ is the cell immunocompetent (capable of mounting an immune re-sponse). T cells arise in the thymus, and in many mammals—humans and mice for example—B cells origi-nate in bone marrow.

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

(i) BONE MARROW:

In humans and mice, bone marrow is the site of B-cell origin and development. Arising from lymphoid progenitors, im-mature B cells proliferate and differentiate within the bone marrow, and stromal cells within the bone marrow interact directly with the B cells and secrete various cytokines that are required for development. Like thymic selection during T-cell maturation, a selection process within the bone marrow eliminates B cells with self-reactive antibody receptors.

Bone marrow is not the site of B-cell development in all species. In birds, a lymphoid organ called the bursa of Fabricius, In cattle and sheep, the primary lymphoid tis-sue hosting the maturation, proliferation, and diversification of B cells early in gestation is the fetal spleen. Later in gesta-tion, this function is assumed by a patch of tissue embedded in the wall of the intestine called the ileal Peyer's patch The rabbit, too, uses gut-associated tissues such as the appendix as primary lym-phoid tissue

(ii) 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.

The thymus is the site of T-cell development and maturation. It is a flat, bilobed organ situated above the heart. Each lobe is surrounded by a capsule and is divided into lobules, which are separated from each other by strands of connective tissue called trabeculae. Each lobule is organized into two compart-ments: the outer compartment, or *cortex,* is densely packed with immature T cells, called thymocytes, whereas the inner compartment, or *medulla,* is sparsely populated with thymo-cytes.

Both the cortex and medulla of the thymus are criss-crossed by a three-dimensional stromal-cell network com-posed of epithelial cells, dendritic cells, and macrophages, which make up the framework of the organ and contribute to the growth and maturation of thymocytes. Many of these stromal cells interact physically with the developing thymo-cytes (Figure). Some thymic epithelial cells in the outer cortex, called nurse cells, have long membrane extensions that surround as many as 50 thymocytes, forming large mul-ticellular complexes. Other cortical

epithelial cells have long interconnecting cytoplasmic extensions that form a network and have been shown to interact with numerous thymocytes as they traverse the cortex.

The function of the thymus is to generate and select a repertoire of T cells that will protect the body from infection. As thymocytes develop, an enormous diversity of T-cell re-ceptors is generated by a random process that produces some T cells with receptors capable of recognizing antigen-MHC complexes. However, most of the T-cell recep-tors produced by this random process are incapable of recog-nizing antigen-MHC complexes and a small portion react with combinations of self antigen-MHC complexes. Using mechanisms that are discussed in Chapter 10, the thymus in-duces the death of those T cells that cannot recognize anti-gen-MHC complexes and those that react with self-antigen– MHC and pose a danger of causing autoimmune disease. More than 95% of all thymocytes die by apoptosis in the thy-mus without ever reaching maturity.

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.

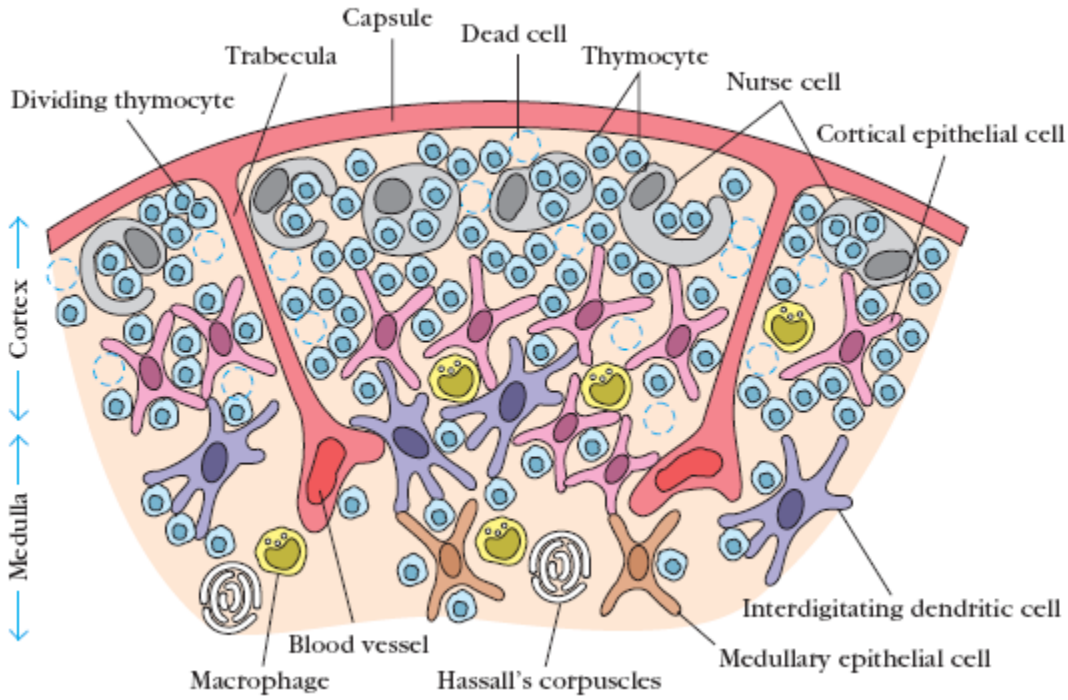


Fig: Diagrammatic cross section of a portion of the thymus, showing several lobules separated by connective tissue strands (trabeculae). The densely populated outer cortex is thought to con-tain many immature thymocytes (blue), which undergo rapid prolif-eration coupled with an enormous rate of cell death. Also present in the outer cortex are thymic nurse cells (gray), which are specialized epithelial cells with long membrane extensions that surround as many as 50

thymocytes. The medulla is sparsely populated and is thought to contain thymocytes that are more mature. During their stay within the thymus, thymocytes interact with various stromal cells, including cortical epithelial cells (light red), medullary epithelial cells (tan), interdigitating dendritic cells (purple), and macrophages (yellow). These cells produce thymic hormones and express high lev-els of class I and class II MHC molecules. Hassalls corpuscles, found in the medulla, contain concentric layers of degenerating ep-ithelial cells.

Lymphatic System

As blood circulates under pressure, its fluid component (plasma) seeps through the thin wall of the capillaries into the surrounding tissue. Much of this fluid, called interstitial fluid, returns to the blood through the capillary membranes. The remainder of the interstitial fluid, now called lymph, flows from the spaces in connective tissue into a network of tiny open lymphatic capillaries and then into a series of pro gressively larger collecting vessels called lymphatic vessels (Figure).

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

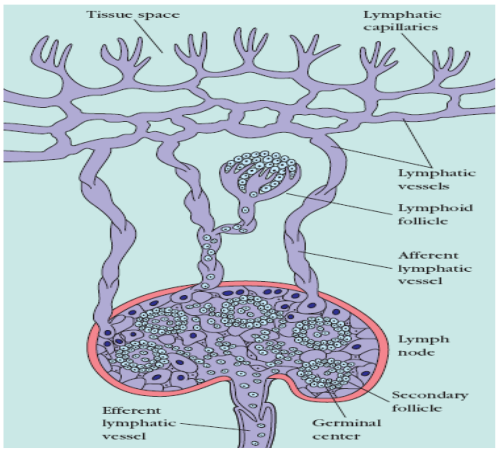


Fig: Lymphatic vessels. Small lymphatic capillaries open-ing into the tissue spaces pick up interstitial tissue fluid and carry it into progressively larger lymphatic vessels, which carry the fluid, now called lymph, into regional lymph nodes. As lymph leaves the nodes, it is carried

through larger efferent lymphatic vessels, which eventu-ally drain into the circulatory system at the thoracic duct or right lymph duct

PERIPHERAL LYMPHOID ORGANS (SECONDARY):

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).

(i) LYMPH NODE:

Morphologically, a lymph node can be divided into three roughly concentric regions: the cortex, the paracortex, and the medulla, each of which supports a distinct microenviron-ment (Figure 2-18). The outermost layer, the cortex, contains lymphocytes (mostly B cells), macro-phages, and follicular dendritic cells arranged in primary follicles. After antigenic challenge, the primary follicles enlarge into secondary folli-cles, each containing a germinal center. In children with B-cell deficiencies, the cortex lacks primary follicles and germinal centers. Beneath the cortex is the paracortex, which is popu-lated largely by T lymphocytes and also contains interdigitat-ing dendritic cells thought to have migrated from tissues to the node. These interdigitating dendritic cells express high levels of class II MHC molecules, which are necessary for pre-senting antigen to T_H cells. Lymph nodes taken from neona-tally thymectomized mice have unusually few cells in the paracortical region; the paracortex is therefore sometimes re-ferred to as a thymus-dependent area in contrast to the cor-tex, which is a thymus-independent area. The innermost layer of a lymph node, the medulla, is more sparsely popu-lated with lymphoid-lineage cells; of those present, many are plasma cells actively secreting antibody molecules.

As antigen is carried into a regional node by the lymph, it is trapped, processed, and presented together with class II MHC molecules by interdigitating dendritic cells in the para-cortex, resulting in the activation of T_H cells. The initial acti-vation of B cells is also thought to take place within the T-cell-rich paracortex. Once activated, T_H and B cells form small foci consisting largely of proliferating B cells at the edges of the paracortex. Some B cells within the foci differen-tiate into plasma cells secreting IgM and IgG. These foci reach maximum size within 4–6 days of antigen challenge. Within 4–7 days of antigen challenge, a few B cells and T_H cells migrate to the primary follicles of the cortex. It is not known what causes this migration. Within a primary follicle, cellular interactions between follicular dendritic cells, B cells, and T_H cells take place, leading to development of a sec-ondary follicle with a central germinal center. Some

of the plasma cells generated in the germinal center move to the medullary areas of the lymph node, and many migrate to bone marrow.

Afferent lymphatic vessels pierce the capsule of a lymph node at numerous sites and empty lymph into the subcapsu-lar sinus (Figure b). Lymph coming from the tissues percolates slowly inward through the cortex, paracortex, and medulla, allowing phagocytic cells and dendritic cells to trap any bacteria or particulate material (e.g., antigen-antibody complexes) carried by the lymph. After infection or the introduction of other antigens into the body, the lymph leav-ing a node through its single efferent lymphatic vessel is en-riched with antibodies newly secreted by medullary plasma cells and also has a fiftyfold higher concentration of lympho-cytes than the afferent lymph.

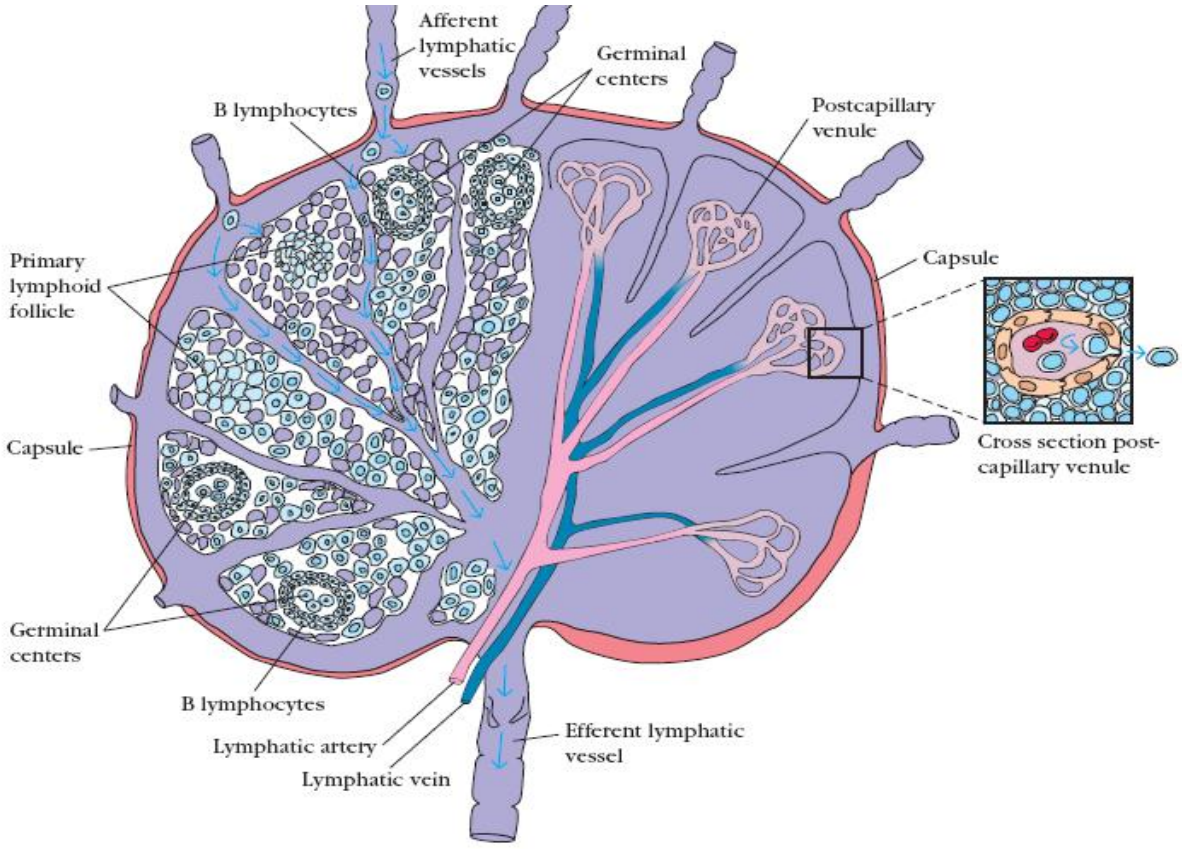


Fig: Structure of a lymph node.Depicts the arrangement of reticulum and lymphocytes within the various regions of a lymph node. Macrophages and dendritic cells, which trap antigen, are present in the cortex and paracortex. T_H cells are concentrated in the paracortex; B cells are located primarily in the cortex, within follicles and germinal centers. The medulla is populated largely by antibody-producing plasma cells. Lymphocytes circu-lating in the lymph are carried

into the node by afferent lymphatic vessels; they either enter the reticular matrix of the node or pass through it and leave by the efferent lymphatic vessel. The right side of (b) depicts the lymphatic artery and vein and the postcapillary venules. Lymphocytes in the circulation can pass into the node from the postcapillary venules by a process called extravasation

SPLEEN

The spleen plays a major role in mounting immune responses to antigens in the blood stream. It is a large, ovoid secondary lymphoid organ situated high in the left abdomi-nal cavity and weighing about 150 grams. It is the largest single lymphoid organ in the body.While lymph nodes are specialized for trapping antigen from local tissues, the spleen specializes in filtering blood and trapping blood-borne antigens; thus, it can re-spond to systemic infections. Unlike the lymph nodes, the spleen is not supplied by lymphatic vessels. Instead, blood-borne antigens and lymphocytes are carried into the spleen through the splenic artery. Experiments with radioactively labeled lymphocytes show that more recirculating lympho-cytes pass daily through the spleen than through all the lymph nodes combined.

PALS. These follicles are rich in B cells and some of them con-tain germinal centers. The marginal zone, located peripheral to the PALS, is populated by lymphocytes and macrophages.

Blood-borne antigens and lymphocytes enter the spleen through the splenic artery, which empties into the marginal zone. In the marginal zone, antigen is trapped by interdigi-tating dendritic cells, which carry it to the PALS. Lympho-cytes in the blood also enter sinuses in the marginal zone and migrate to the PALS.

The initial activation of B and T cells takes place in the T-cell-rich PALS. Here interdigitating dendritic cells capture antigen and present it combined with class II MHC mole-cules to T_H cells. Once activated, these T_H cells can then acti-vate B cells. The activated B cells, together with some T_H cells, then migrate to primary follicles in the marginal zone. Upon antigenic challenge, these primary follicles develop into char-acteristic secondary follicles containing germinal centers (like those in the lymph nodes), where rapidly dividing B cells (centroblasts) and plasma cells are surrounded by dense clusters of concentrically arranged lymphocytes.

In children, splenectomy often leads to an increased incidence of bacterial sepsis caused primarily by *Streptococcus pneumoniae, Neisse-ria meningitidis,* and *Haemophilus influenzae.* Splenectomy in adults has less adverse effects, although it leads to some in-crease in blood-borne bacterial infections (bacteremia).

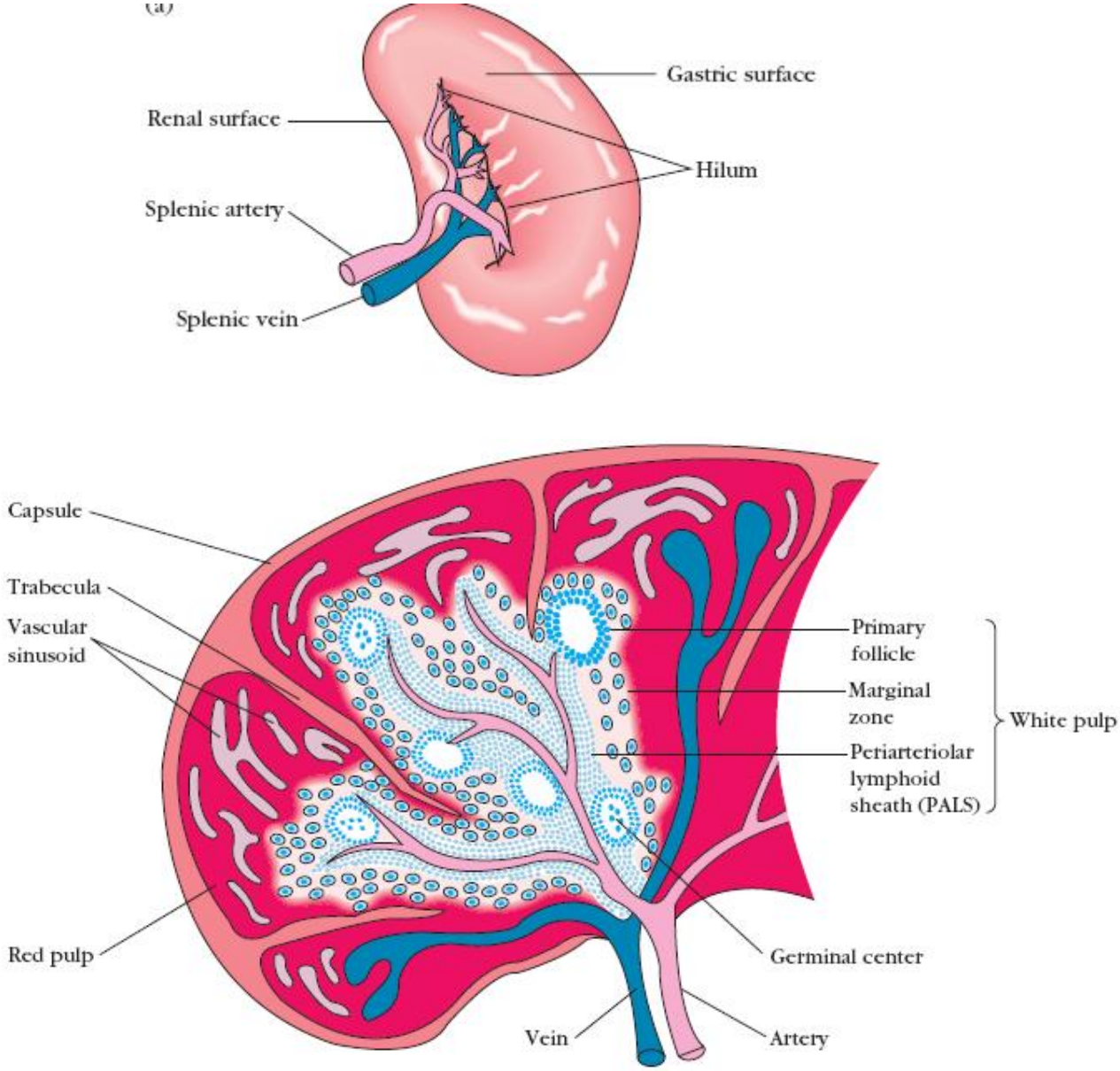


Fig: (a) The spleen, which is about 5 inches long in adults, is the largest secondary lymphoid or-gan. It is specialized for trapping blood-borne antigens**. (b) Diagram-matic cross section of the spleen**. The splenic artery pierces the capsule and divides into progressively smaller arterioles, ending in vascular sinusoids that drain back into the splenic vein. The erythrocyte-filled red pulp surrounds the sinusoids. The white pulp forms a sleeve, the periarteriolar lymphoid sheath (PALS), around the arteri-oles; this sheath contains numerous T cells. Closely associated with the PALS is the marginal zone, an area rich in B cells that contains lymphoid follicles that can develop into secondary follicles contain-ing germinal centers

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 endocytosise, 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.

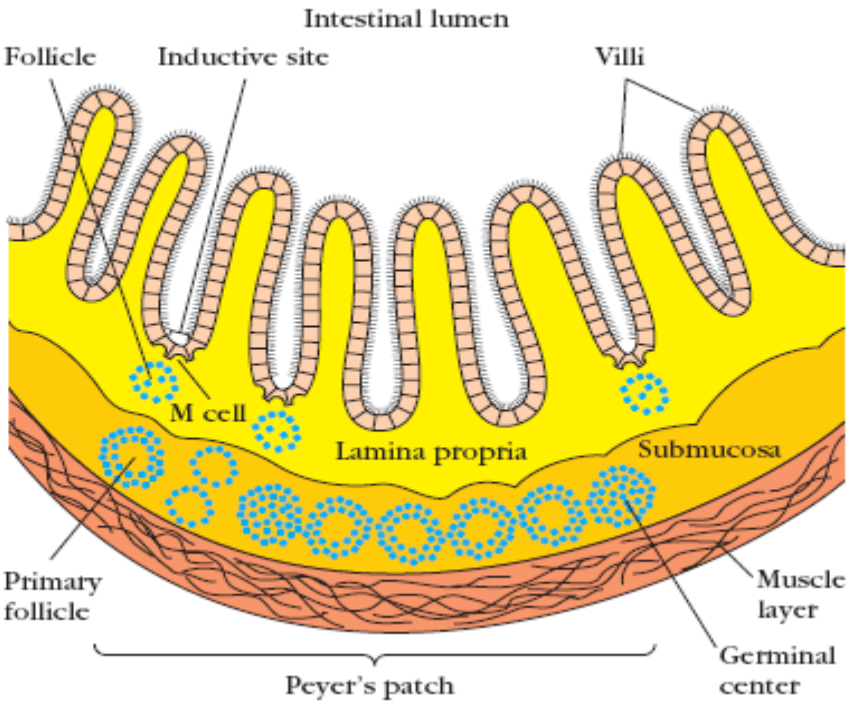


Fig: Cross-sectional diagram of the mucous membrane lining the intestine showing a nodule of lymphoid follicles that con-stitutes a Peyer's patch in the submucosa. The intestinal lamina propria contains loose clusters of lymphoid cells and diffuse follicles.

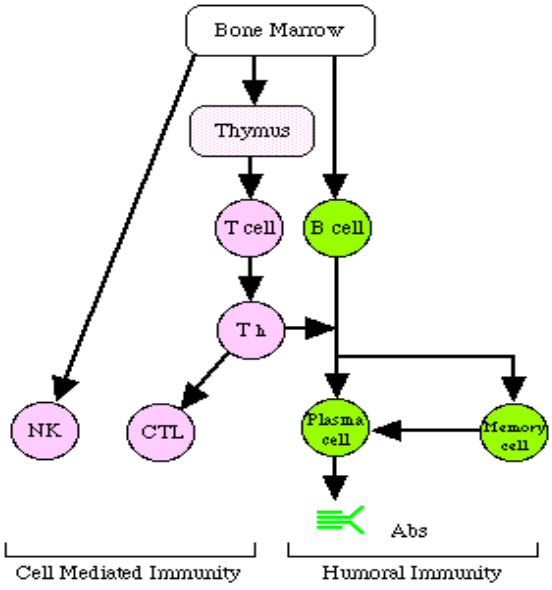
CUTANEOUS-ASSOCIATED LYMPHOID TISSUE(CALT)

The skin is an important anatomic barrier to the external environment, and its large surface area makes this tissue important in nonspecific (innate) defenses. The epidermal (outer) layer of the skin is composed largely of specialized epithelial cells called keratinocytes. These cells secrete a number of cytokines that may function to induce a local inflammatory reaction. In addition, keratinocytes can be induced to express class II MHC molecules and may function as antigen-presenting cells. Scattered among the epithelial-cell matrix of the epidermis are Langerhans cells, a type of dendritic cell, which internalize antigen by phagocytosis or endocytosis. The Langerhans cells then migrate from the epidermis to regional lymph nodes, where they differentiate into interdigitating dendritic cells. These cells express high levels of class II MHC molecules and function as potent activators of naive TH cells.

IMMUNE RESPONSE

The immune response is how the body recognizes and defends itself against bacteria, viruses, and substances that appear foreign and harmful. There ar two types of immune response

1. Cell mediated immune response
2. Humoral mediated immune response



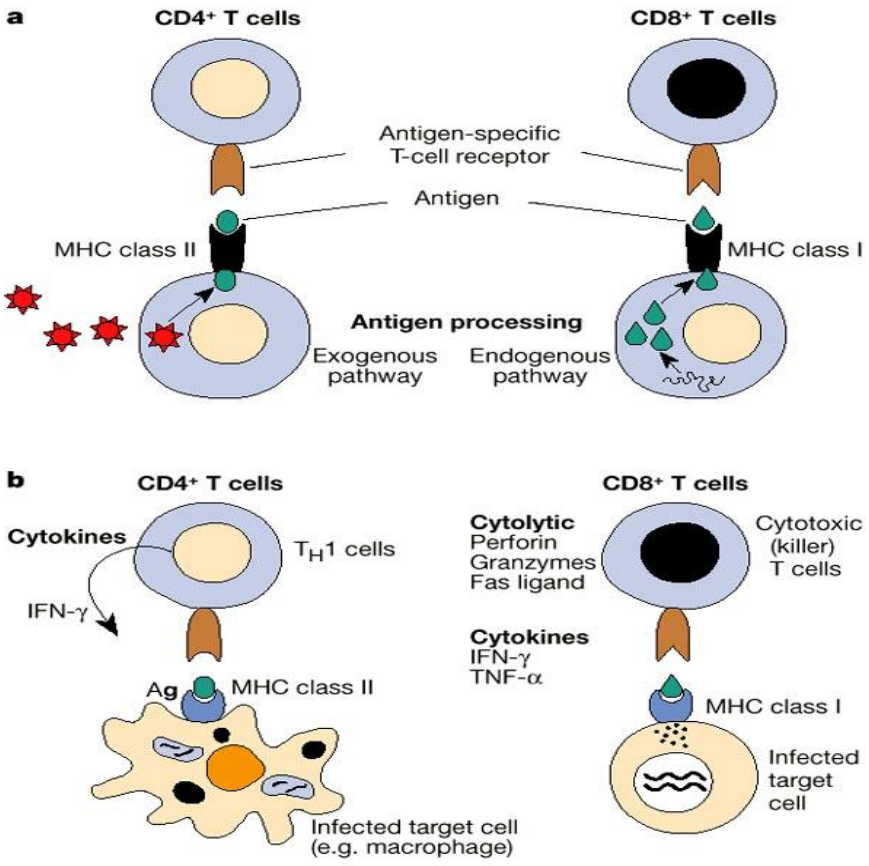
1. CELL MEDIATED IMMUNITY

Cell-mediated immunity

> These $CD4^+$ cells bind to antigen presented by antigen-presenting cells (APCs) like phagocytic macrophages and dendritic cells. The T cells then release lymphokines that attract other cells to the area. The result is inflammation: the accumulation of cells and molecules that attempt to wall off and destroy the antigenic material (the rash following exposure to poison ivy is an example).

CM is adaptive immune response against intracellular microbes is mediated by T cells and can be transferred from immunized to naïve individuals to T cells and not by antibodies.

There are two types (main) cell mediated immunity. In one, which is exemplified by DTH reactions, CD4+ Th1 Cells, as well as CD8 Th2 cells, recognize antigens of microbes that have been ingested by phagocyte and activate the phagocytes to kill the microbes. In the second type of CM, CD8+ CTLs kill any nucleated cell that contains foreign antigens (microbial /tumor antigens) in cytosol.



Cell mediated immune response consists of several steps;

- naive cell recognition of cell- Associated antigens in peripheral lymphoid organs,
- clonal expansion of T cells and their differentiation into effector cells.
- Migration of affecter cell to the site of infection or antigen challenge and elimination of microbes or antigen.

CD4 helper T lymphocytes may differentiate into specialized effector cells Th1 cells that secrete IFN-g which favors phagocyte mediated immunity, or into Th2 cells that secrete Il-1and Il4 and Il5, which favor IgE and eosinophil/ mast cell mediated immune reaction. The differentiation of naïve CD4+Tcells into Th1 and Th2 population is controlled by cytokines produced by the T cells themselves. CD8+ T cells differentiate in to effector CTLs acquiring the capacity to kill targets, under the influence of co stimulators and help from CD4+T c ells.

The migration of T cells to sites of infection is mediated by chemokines and the binding of adhesion molecules to their ligands on activated endothelium.

The activation of macrophages by TH1 cells is mediated by IFN-g and CD40L-CD40 interactions. Activated macrophages kill phogocytosed microbes, stimulate inflammation, and repair damaged tissues. If the infection is not fully resolved activated macrophages cause tissue damage and fibrosis.

CD8+CTLs kill cells that express peptides derived from cytosolic antigens, e.g. viral antigens that are presented in association with class I MHC molecules. CTL – mediated killing is mediated mainly by granule exocytosis, which release granzymes and perforin. Perforin facilitates granzymes entry into the cytoplasm of target cells and granzymes initiate several pathways of apoptosis. CTLs also express FasL, which engages Fas on target cell membranes and trigger apoptosis of target cells.

Examples of Cell-Mediated Immunity

Delayed-Type Hypersensitivity (DTH)

A tiny amount of protein, extracted from the bacteria, is injected into the skin. If the subject is currently infected, or has ever been infected, with the bacteria, a positive test results. In 24 hours or so, a hard, red nodule develops at the site of the injection. This nodule is densely packed with lymphocytes and macrophages

DTH is a cell-mediated response (in fact, anti-tuberculin antibodies are rarely found in tuberculin-positive people). The T cells responsible for DTH are members of the $CD4^+$ subset.

Contact Sensitivity

Many people develop rashes on their skin following contact with certain chemicals. Nickel, certain dyes, and the active ingredient of the poison ivy plant are common examples. The response takes some 24 hours to occur, and like DTH, is triggered by $CD4^+$ T cells.

Killing intracellular parasites

Some human pathogens avoid exposure to antibodies by taking up residence within cells. These include all viruses (discussed in the next section), and some bacteria

Anti-Viral Immunity

Any cell in the body is a potential target for one kind of virus or another. However, all cells express class I histocompatibility molecules at their surface. These can display antigenic fragments of viral components. $CD8^+$ T cells that can bind to these epitopes can then destroy the cell

Graft Rejection

Grafts of a kidney, heart, lung, liver, etc. from one human to another always (unless donated by an identical twin) are seen by the recipient's immune system as antigenic and elicit an immune response. If unchecked, this response will eventually lead to destruction of the graft. Both $CD4^+$ and $CD8^+$ T cells participate in graft rejection. They are responding to differences between donor and host of their class II and class I histocompatibility molecules (respectively).

2. HUMORAL IMMUNITY

Humoral immunity is mediated by secreted antibodies and its physiological function is defense against extracellular microbes (including viruses) and microbial exotoxins. Humoral immunity can be transferred to other individuals by the transfer of serum (antibodies). Defect in humoral immunity leads to enhanced infections by bacteria and fungi. Antibodies also participate in autoimmune disorders and hypersensitivity.

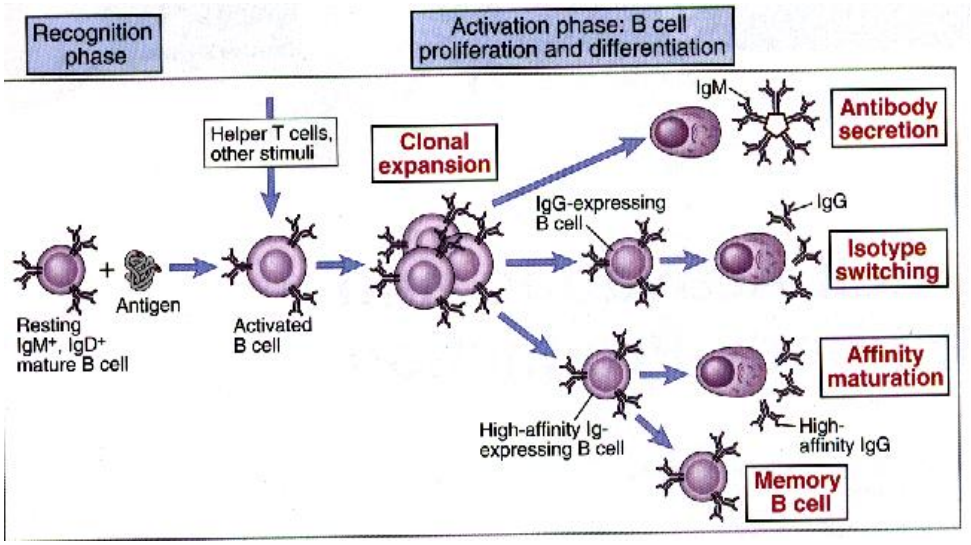
When an antigen with multiple epitopes gains entry into the body, different clones of B cells recognize and produce antibodies against different epitopes, thus the natural response is said to by polyclonal. However, by using hybridoma technology it is possible to develop a clone of B cells directed against a single epitope, and produce monoclonal antibodies.

Antibodies are produced by plasma cells in the secondary lymphoid organs, but antibodies can perform their effector functions at any site in the body. Once the antibodies enter the circulation or mucosa, they can easily reach sites of infection. Circulating antibodies can recognize antigen present in blood or can pass through the endothelium into tissue spaces and render their effector functions.

The first exposure to a microbe or an antigen, either by infection or by vaccination, leads to the activation of naive B lymphocytes. These B cells differentiate into antibody producing plasma cells and memory cells. Some of the antibody producing cells migrate to the bone marrow and live in this site for several years, where they continue to produce antibodies even when antigen has been eliminated. It is estimated that over half the IgG found in serum of normal individuals is derived from these long-lived antibody producing cells, which were induced by exposure to various antigens throughout the life of the individual. When the same antigen enters the body again, the circulating antibodies provide immediate protection against infection. At the same

time, memory cells too are activated by the antigen and the resulting secondary response provides high level of protection.

Antibody production by activated B cells is the core the humoral response: antibody effects, such as complement activation by IgM and certain IgGs, opsonization via F(c)Rs and pathogen/toxin neutralization by high-affinity IgG and IgA; and processes related to B cell activation, such as T_H2 activation and cytokine production, germinal center formation, isotype switching, affinity maturation and memory cell production. The F(c) region of IgG binds to F(c) receptors, playing a critical role (along with receptors for complement byproducts) in clearing extracellular bacteria. Intracellular bacteria are cleared by cell-mediated immunity.



Property	Primary Response	Secondary Response
Responding B Cell :	Naïve B cell	Memory B cell
Lag Period:	4-7 days	1-3 days
Length of Response:	7-10 days	3-5 days
Magnitude:	Varies based on antigen	100-1000x greater than 1° response
Isotype Produced:	Initially IgM, then IgG	Mostly IgG
Antigens:	Thymus-dependent and -independent	Thys-dependent
Antibody Affinity:	Lower	Higher

	QUESTION	A	B	C	D	Answer
UNIT- I	A hematopoietic stem is a	Progeniter	Pluripotent	Stem cell	Mast cell	Pluripotent
2	Lymphoid and myeloid stem cells differentiate into	Progeniter cells	White blood cells	Red blood cells	Lymphoid cells	Progeniter cells
3	In adult bone marrow, the hematopoietic cells grow and mature on a meshwork of	Stem cells	Lymphoid	Stromal cells	Myeloid cells	Stromal cells
4	The colony stimulating factor is a	Acidic glycoproteins	Basic glycoproteins	Neutral glycoproteins	glycoprotein	Acidic glycoproteins
5	Multilineage CSF is also known as	IL-2	IL-3	Il-4	IL-7	IL-3
6	Erytropoietin is a	Protein	Glycans	Glycoproteins	Cytokines	Glycoproteins
7	Cells undergoing programmed cell death is reffered to as	**Apoptosis**	Degeneration	Denaturation	Cell cycle	Apoptosis
8	Apoptosis differs markly from	Necrosis	Cell cycle	Degeneration	Denaturation	Degeneration
9	A normal adult has about 5litres of blood with about……lymphocytes/mm^3	3000	2000	4000	2500	2000
10	Activated lymphocytes have been found to express lower levels of	Bcl-X^2	Bcl-.3	Bcl-2	Bcl-4	Bcl-2
11	Lymphocytes constitute …….. of WBC	20-40%	40-60%	30-50%	40-50%	20-40%
12	T,B& Null cells are	phagocytic cells	Non-phagocytic cells	Mast cells	WBC	Non-phagocytic cells
13	CD^{34+} are	Potent	Stem cells	Pluripotent stem cells	Mast cells	Pluripotent stem cells
14	Lymphocytes enlarge into 15mm diameter blast cells known as	lymph cells	lymphoid cells	lymphocytes	lymphoblasts	lymphoblasts
15	CD is a	Cluster of differentiation	Cluster of designation	Colony of differentiation	Colony of designation	Cluster of differentiation
16	B^{220} is also a	CD45	CD20	CD30	CD50	CD45
17	Absence of NK cells results in disorder	Cushion syndrome	Chediak-Higashi syndrome	Necrosis	X-linked syndrome	Chediak-Higashi syndrome
18	Alveolar macrophages is found in	Kidney	Brain	Lung	Tissue	Lung
19	Kupffer cells is found in	Brain	Liver	Lung	Kidney	Liver
20	Macrophages present in kidney is known as	Mesangial cells	Microglial cells	Kupffer cells	Histocytes	Mesangial cells
21	Microglial cells found in	Kidney	Lung	Tissue	Brain	Brain
22	The neutrophil has a…….. nucleus	Bilobed	Lobed	Multilobed	Polylobed	Multilobed
23	The eosinophil has a…….. nucleus	Bilobed	Lobed	Multilobed	Polylobed	Lobed
24	Neutrophils are produced in	Bone marrow	Tissue	Liver	Blood	Bone marrow
25	Movement of circulating neutrophils into tissues called	Vasation	Vascular endothelium	Extravasation	Endothelial cells	Extravasation
26	Chemotactic factors promote accumulation of	Basophils	Eosinophils	Neutrophils	Mast cells	Neutrophils
27	…… cells found in epidermis and mucous membranes.	Interstitial dendritic	Inter digitating	Circulating	Langerhans	Langerhans
28	The pre-TCR consists of protein	CD2	CD4	CD3	CD5	CD3
29	The pre-TCR consists of	Alpha chain	Beta chain	Gamma chain	Alpha-Beta cells	Beta chain
30	Thy-1 is a	Protein	Membrane	Membrane protein	Membrane molecules	Membrane protein
31	TH-Cell activation is initiated by	TCR-CD2	TCR-CD4	TCR-CD5	TCR-CD3	TCR-CD3
32	Exogenous super antigens are soluble proteins secreted by	Bacteria	Fungus	Algae	Chlorophyll	Bacteria
33	Exogenous super antigens are soluble	Cells	Tissues	Membranes	Proteins	Proteins
34	Gram positive bacteria secretes	Endotoxins	Toxins	Exotoxins	Mesotoxins	Exotoxins
35	Endogenous super antigens are	Cell protein	Cell-membrane protein	Membrane protein	Protein	Cell-membrane protein
36	The generation of mature B cells first occurs in	Cell	Tissue	Embryo	Bone marrow	Embryo
37	B-cells response to antigens	TA	TB	TC	TD	TD
38	Antigens that activate B-cells in the absence of TH cells are known as	TI	TG	TH	TK	TI
39	Polyclonal B-cell activatirs are known as	Mesogens	Mitogens	Mycogens	Minogens	Mitogens
40	Signals that drives B cell from G_0 into G_1 is	Progression	Pre-Progression	Competence	Pre-competence	Competence
41	Signal that drives the B-Cell from G_1 into S is	Competence	Pre-competence	Progression	Pre-progression	Progression
42	T1 antigens are in nature of	monovalent	Divalent	Trivalent	Multivalent	Multivalent
43	Initial activation of bothB&T cells are thought to take place in	Cortex	Paracortex	Precortex	Procortex	Paracortex
44	The proliferating activated B cells known as	Centromeres	Centrocytes	Centroblasts	Centeromeres	Centroblasts
45	TH cells failsto express CD40 results in	X-Linked hyper Ig-M syndrome	X-Linked hyper Ig-K syndrome	X-Linked hyper Ig-L syndrome	X-Linked hyper Ig-I syndrome	X-Linked hyper Ig-M syndrome
46	NK cells play an important role in	Mesocells	Mast cell	Tumour cells	TH cells	Tumour cells
47	A signal transduction molecule is	CD 43	CD 45	CD 46	CD 47	CD 45
48	The adhesion molecule is a	CD 52	CD 53	CD 55	CD 56	CD 56
49	B & T lymphocytes recognize discrete sites on the antigen called	Epitopes	Endotopes	Exotopes	Isotopes	Epitopes
50	The main component of the blood clot	Platelets	Fibrin	Fibrous	Fibroblast	Fibrin
51	The B cell receptor is a membrane bound …… molecule	Antigen	Antibody	Epitope	Endotope	Antibody
52	Memory B cells & effector B cells are called as	Mast cells	Mesocells	Plasma cells	Membrane cells	Plasma cells
53	Receptor mediated endocytosis is also known as	Phagocytosis	Endocytosis	Exocytosis	Pinocytosis	Pinocytosis
54	The engorged capillaries are responsible for tissue rednessis known as	edema	erythema	extravasation	Chemotaxis	extravasation
55	An accumulation of fluid results in tissue swelling leads to	edema	erythema	extravasation	Chemotaxis	edema
56	Which hydrolytic enzyme found in mucous secretions	Lytizyme	Pre- Lytizyme	Lysozyme	Pre-Lysozyme	Lysozyme
57	……..comprises a group of proteins produced by virus- in fected cells.	Interferon	Interleukins	Cytokinins	Cytooxic.	Interferon
58	The adhesion molecule that binds to class I MHC molecule is	CD5	CD 6	CD7	CD 8.	CD 8.
59	Monocytes is found in	Blood	bone marrow	tissue	Cells.	Blood
60	Macrophages is found in	Blood	Bone marrow	tissues	Cells.	tissues

KARPAGAM ACADEMY OF HIGHER EDUCATION
(Deemed University Established Under Section 3 of UGC Act 1956)
Coimbatore - 641021.
(For the candidates admitted from 2015 onwards)
DEPARTMENT OF BIOCHEMISTRY

SUBJECT : **IMMUNOLOGY**
SEMESTER : **V**
SUBJECT CODE : **15BCU503** **CLASS** : **III B.Sc.BC**

UNIT I - COURSE MATERIAL

UNIT II
Components of Immunity

Antigen: Definition, requirement for antigenecity, properties of antigen-specificity, cross reactivity, immunogenicity; epitopes, adjuvents, hapten. *Antibody-Definition, properties, classes, subclasses structure, specificity and distribution; self-antigens (MHC) - Class I, II, III molecules, role of MHC in antigen processing and presentation.*

Definition of Antigen:

An antigen is any substance that causes immune system to produce antibodies against it. An antigen may be a foreign substance from the environment such as chemicals, bacteria, viruses, or pollen. An antigen may also be formed within the body, as with bacterial toxins or tissue cells.

Properties of Antigen:

- Antigen, foreign substance that, when introduced into the body, is capable of stimulating an immune response, specifically activating lymphocytes
- Virtually any large foreign molecule can act as an antigen, including those contained in bacteria, viruses, protozoa, helminths, foods, snake venoms, egg white, serum components, red blood cells, and other cells and tissues of various species including humans.
- An antigen that induces an immune response stimulates the lymphocytes to produce antibody
- On the surface of the antigens are regions, called antigenic determinants(epitope), that fit and bind to receptor molecules of complementary structure on the surface of the lymphocytes
- The binding of the lymphocytes' receptors to the antigens' surface molecules stimulates the lymphocytes to multiply and to initiate an immune response by the production of antibody, activation of cytotoxic cells, or both .

- The amount of antibody formed in response to stimulation depends on the kind and amount of antigen involved, the route of entry to the body, and individual characteristics of the host

Factors influencing antigenecity:
Molecular size: Large molecules are better antigan thansmall moleculesEg: hemocyanin- a large protein is a potent antigen
Structural stability: To recognise a molecule or part of a molecule as foreign, the cells of immune system must recognise its specific shape. Consequently, highly flexible molecules that have fixed shape are poor antigens.Eg: Gelatin-poor antigen
Degradability: The cells of immune system recognise small molecular fragments and soluble antigens. If a moleculecannot be broken up or solubilised, then it cannot acts as an antigen.Eg-Stainless steel pin
Foreignness: The cells whose function is ti respond to antigen are selected in such a way that they do not usually respond to normal body componenets. They will respond, however, to foreign molecules differ even in minor respects from those usually found within the body. This property is **immunogenecity** and this depends on the degree of foreingnness.

Specificity and Cross-Reactivity

Specificity measures the degree to which the immune system differentiates between different antigens. **Cross-reactivity** measures the extent to which different antigens appear similar to the immune system. The molecular determinants of specificity and cross-reactivity define the nature of antigenic variation and the selective processes that shape the distribution of variants in populations.

Immunogenicity
Immunogenicity is the ability of a particular substance, such as an antigen or epitope, to provoke an immune response in the body of a human or animal. In other words, immunogenicity is the ability to induce a humoral and/or cell mediated immune response.
The ability of an antigen to elicit immune responses is called immunogenicity, which can be humoral and/or cell-mediatedimmuneresponses.
Differentiation has to be made between wanted and unwanted immunogenicity.

- Wanted immunogenicity is typically related with vaccines, where the injection of an antigen (the vaccine) has to lead to an immune response against the pathogen (the virus, bacterium or substance).
- Unwanted immunogenicity is when the organism mounts an immune response against an antigen which is undesired. Unwanted immunogenicity is strongly linked with therapeutic proteins. A fraction of the patient treated with those drugs mount anti-drug-antibodies, which leads to inactivation of the drug and in rare cases to adverse effects .

Immunogenicity Versus Antigenicity

Immunogenicity and antigenicity are related but distinct immunologic properties that sometimes are confused. **Immunogenicity** is the ability to induce a humoral and/or cell-mediated immune response:

B cells + antigen → effector B cells + memory B cells
↓
(plasma cells)

T cells + antigen → effector T cells + memory T cells
↓
(e.g., CTLs, T_Hs)

Although a substance that induces a specific immune re-sponse is usually called an antigen, it is more appropriately called an **immunogen.**

Antigenicity is the ability to combine specifically with the final products of the above responses (i.e., antibodies and/or cell-surface receptors). Although all molecules that have the property of immunogenicity also have the property of antigenicity, the reverse is not true. Some small molecules, called *haptens*, are antigenic but incapable, by themselves, of inducing a specific immune response. In other words, they lack immunogenicity

Immunogenic potency of antigens

Proteins are significantly more immunogenic than polysaccharides. T cell response is required to drive immunogenicity.

Since lipids and nucleicacids arenon-immunogenic haptens,theyrequire conjugation with an epitope such as a protein or polysaccharide before they can evoke an immunologic response.

- Proteins or polysaccharides are used for studies of humoral immune response.
- Only proteins can serve as immunogens for cell-mediated immunity.

Immunogenicity is influenced by multiple characteristics of an antigen:

- Phylogenetic distance
- Molecular size
- Epitope density
- Chemical composition and heterogeneity
- Protein structure, aa-polymers, Glu-Lys, Tyr, Phe
- Degradability ability to be processed & presented to T cells
- D-amino acids

The Biological System is also contributes to Immunogenicity. They are

- Genotype of the recipient animal
- Immunogen dosage and route of administration

EPITOPE

An **epitope**, also known as **antigenic determinant**, is the part of an antigen that is recognized by the immune system, specifically by antibodies, B cells, or T cells. The part of an antibody that recognizes the epitope is called a paratope.

B CELL EPITOPE -Features

- The ability to function as a B-cell epitope is determined by the nature of the antigen-binding site on the antibody molecules displayed by B cells.
- The B-cell epitopes on native proteins generally are composed of hydrophilic amino acids on the protein **surface** that are topographically accessible to membrane-bound or free antibody.
- B-cell epitopes can contain sequential or nonsequential amino acids. In general antigenic determinants are small and are limited to approximately 4-8 residues
- B-cell epitopes tend to be located in flexible regions of an immunogen and display site mobility.
- Complex proteins contain multiple overlapping B-cell epi-topes, some of which are immunodominant.

T CELL EPITOPE

Composition

- Antigenic determinants recognized by T cells are created by the primary sequence of amino acids in proteins
- Free peptides are not recognized by T cells, rather antigen processing is required to generate peptides that interact specifically with MHC molecules. and it is the complex of MHC molecules + peptide that is recognized by T cells
- Antigenic peptides recognized by T cells form trimolecular complexes with a T-cell receptor and an MHC molecule
- Epitopes recognized by T cells are often **internal.**
- T cells do not recognize polysaccharide or nucleic acid antigens
- In general antigenic determinants are small and are limited to approximately 8-15 amino acids

Adjuvants

Adjuvants (from Latin *adjuvare*, to help) are substances that, when mixed with an antigen and injected with it, enhance the immunogenicity of that antigen, without having any specific antigenic effect in itself.. Adjuvants are often used to boost the immune response when an antigen has low immunogenicity or when only small amounts of an antigen are available. For example, the antibody response of mice to im-munization with BSA can be increased fivefold or more if the BSA is administered with an adjuvant. Precisely how adju-vants augment the

immune response is not entirely known, but they appear to exert one or more of the following effects :

- Antigen persistence is prolonged.
- Co-stimulatory signals are enhanced.
- Local inflammation is increased.
- The nonspecific proliferation of lymphocytes is stimulated.

Adjuvants in immunology are often used to modify or augment the effects of a vaccine by stimulating the immune system to respond to the vaccine more vigorously, and thus providing increased immunity to a particular disease. Adjuvants accomplish this task by mimicking specific sets of evolutionarily conserved molecules, so called PAMPs, which include liposomes, lipopolysaccharide (LPS), molecular cages for antigen, components of bacterial cell walls, and endocytosed nucleic acids such as double-stranded RNA (dsRNA), single-stranded DNA (ssDNA), and unmethylated CpG dinucleotide-containing DNA. Because immune systems have evolved to recognize these specific antigenic moieties, the presence of an adjuvant in conjunction with the vaccine can greatly increase the innate immune response to the antigen by augmenting the activities of dendritic cells (DCs), lymphocytes, and macrophages by mimicking a natural infection. Furthermore, because adjuvants are attenuated beyond any function of virulence, they pose little or no independent threat to a host organism.

Inorganic adjuvants

Aluminium salts

There are many adjuvants, some of which are inorganic (such as alum), that also carry the potential to augment immunogenicity. Two common salts include aluminium phosphate and aluminium hydroxide. These are the most common adjuvants in human vaccines.

Aluminum potassium sulfate (alum) prolongs the persis-tence of antigen. When an antigen is mixed with alum, the salt precipitates the antigen. Injection of this alum precipitate results in a slower release of antigen from the injection site, so that the effective time of exposure to the antigen increases from a few days without adjuvant to several weeks with the adjuvant. The alum precipitate also increases the size of the antigen, thus increasing the likelihood of phagocytosis.

Organic adjuvants

While Aluminium salts are popularly used in human vaccines, the organic compound Squalene is also used. However, organic adjuvants are more commonly used in animal vaccines.

Oil-based

Oil-based adjuvants are commonly used in some veterinary vaccines.

Water-in-oil adjuvants- also prolong the persistence of antigen. A preparation known as **Freund's incomplete ad-juvant** contains antigen in aqueous solution, mineral oil, and an emulsifying agent such as mannide monooleate, which disperses the oil into small droplets surrounding the antigen; the antigen is then released very slowly from the site of injection. This preparation is based on **Freund's complete adjuvant,** the first deliberately formulated highly effective adjuvant, developed by Jules Freund many years ago and containing heat-killed

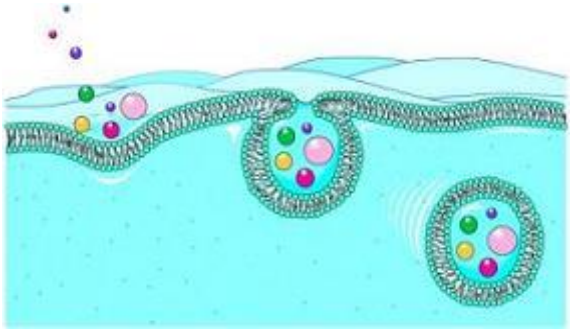
Mycobacteria as an additional ingredient. Muramyl dipeptide, a component of the mycobacterial cell wall, activates macrophages, making Freund's complete adjuvant far more potent than the in-complete form. Activated macrophages are more phago-cytic than unactivated macrophages and express higher levels of class II MHC molecules and the membrane mole-cules of the B7 family. The increased expression of class II MHC increases the ability of the antigen-presenting cell to present antigen to T_H cells. B7 molecules on the antigen-presenting cell bind to CD28, a cell-surface protein on T_H cells, triggering co-stimulation, an enhancement of the T-cell immune response. Thus, antigen presentation and the requisite co-stimulatory signal usually are increased in the presence of adjuvant.

Alum and Freund's adjuvants also stimulate a local, chronic inflammatory response that attracts both phagocytes and lymphocytes. This infiltration of cells at the site of the adjuvant injection often results in formation of a dense, macrophage-rich mass of cells called a **granuloma.** Because the macrophages in a granuloma are activated, this mecha-nism also enhances the activation of T_H cells.

Other adjuvants (e.g., synthetic polyribonucleotides and bacterial lipopolysaccharides) stimulate the nonspecific pro-liferation of lymphocytes and thus increase the likelihood of antigen-induced clonal selection of lymphocytes

Virosomes

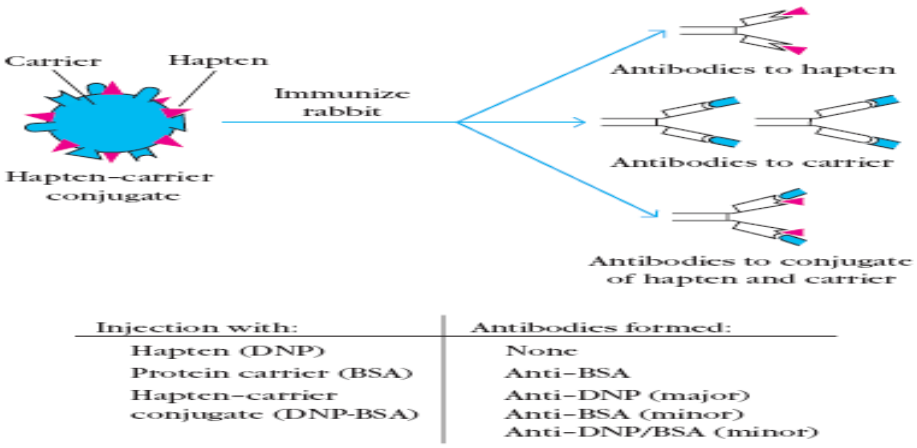
Another market-approved adjuvant and carrier system are virosomes. During the last two decades, a variety of technologies have been investigated to improve the widely-used adjuvants based on aluminium salts. These salts are unfavorable, since they develop their effect by inducing local inflammation, which is also the basis for the extended side-effect pattern of this adjuvant. In contrast, the adjuvant capabilities of virosomes are independent of any inflammatory reaction. Virosomes contain a membrane-bound hemagglutinin and neuraminidase derived from the influenza virus, and serve to amplify fusogenic activity and therefore facilitate the uptake into antigen presenting cells (APC) and induce a natural antigen-processing pathway. The delivery of the antigen by virosomes to the immune system in a way that mimics a natural path may be a reason why virosome-based vaccines stand out due to their excellent safety profile



Today, adjuvants play an important role in the efficacy of vaccines. Stimulating the correct immune response is a must when selecting an adjuvant to use for a new vaccine. Since one adjuvant alone is rarely optimal for all antigens, it is critical to have a selection of different types of adjuvants for evaluation with one antigen.

HAPTEN

- A **hapten** is a small molecule that can elicit an immune response only when attached to a large carrier such as a protein; the carrier may be one that also does not elicit an immune response by itself. (In general, only large molecules, infectious agents, or insoluble foreign matter can elicit an immune response in the body.) Once the body has generated antibodies to a hapten-carrier adduct, the small-molecule hapten may also be able to bind to the antibody, but it will usually not initiate an immune response; usually only the hapten-carrier adduct can do this. Sometimes the small-molecule hapten can even block immune response to the hapten-carrier adduct by preventing the adduct from binding to the antibody, a process called *hapten inhibition*.
- **Haptens** are low-molecular weight molecules which contain an antigenic determinant but which are not itself antigenic unless complexed with an immunogenic carrier. Classical haptens include di- and trinitrophenol (DNP & TNP), dimethylaminonaphthalene sulfonate (dansyl), and a numer of toxins, including urushiol, which is the toxin found in poison ivy.
- A substance that is capable of reacting with a specific antibody but cannot induce the formation of antibodies unless bound to a carrier protein or other molecule. Also called *incomplete antigen*, *partial antigen*.
- The first haptens used were aniline and its carboxyl derivatives (o-, m-, and p-aminobenzoic acid). A well-known example of a hapten is urushiol, which is the toxin found in poison ivy. When absorbed through the skin from a poison ivy plant, urushiol undergoes oxidation in the skin cells to generate the actual hapten, a reactive molecule called a quinone, which then reacts with skin proteins to form hapten adducts. Usually, the first exposure causes only sensitization, in which there is a proliferation of effector T-cells. After a second exposure later, the proliferated T cells can become activated, generating an immune reaction, producing the typical blisters of poison ivy exposure.Some haptens can induce autoimmune disease. An example is hydralazine, a blood pressure-lowering drug that occasionally can produce drug-induced lupus erythematosus in certain individuals. This also appears to be the mechanism by which the anaesthetic gas halothane can cause a life-threatening hepatitis, as well as the mechanism by which penicillin-class drugs cause autoimmunehemolytic anemia.Other haptens that are commonly used in molecular biology applications include fluorescein, biotin, digoxigenin, and dinitrophenol.
- Penicillin bound to a protein can result in Ab production to the penicillin (results in an allergy to penicillin



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

Fig: A hapten-carrier conjugate contains multiple copies of the hapten—a small nonimmunogenic organic compound such as dinitrophenol (DNP)—chemically linked to a large protein carrier such as bovine serum albumin (BSA). Immunization with DNP alone elicits no anti-DNP antibodies, but immunization with DNP-BSA elicits three types of antibodies. Of these, anti-DNP antibody is predominant, indicating that in this case the hapten is the immuno-dominant epitope in a hapten-carrier conjugate, as it often is in such conjugates.

Many biologically important substances, including drugs, peptide hormones, and steroid hormones, can function as haptens.

ANTIBODY

Antibodies are glycoprotein belonging to the immunoglobulin superfamily; the terms antibody and immunoglobulin are often used interchangeably. They present on the B-cell membrane and secreted by plasma cells. Membrane-bound antibody confers antigenic specificity on B cells; antigen-specific prolifer-ation of B-cell clones is elicted by the interaction of membrane antibody with antigen. Secreted antibodies cir-culate in the blood, where they serve as the effectors of hu-moral immunity by searching out and neutralizing antigens or marking them for elimination. All antibodies share struc-tural features, bind to antigen, and participate in a limited number of effector functions.

The antibodies produced in response to a particular anti-gen are heterogeneous. Most antigens are complex and contain many different antigenic determinants, and the immune system usually responds by producing antibodies to several epitopes on the antigen. This response requires the recruit-ment of several clones of B cells. Their outputs are mono-clonal antibodies, each of which specifically binds a single antigenic determinant. Together, these monoclonal antibod-ies make up the polyclonal and heterogeneous serum anti-body response to an immunizing antigen.

Immunoglobulins generally assume one of two roles: immunoglobulins may act as i) plasma membrane bound antigen receptors on the surface of a B-cell or ii) as antibodies free in cellular fluids functioning to intercept and eliminate antigenic determinants.

ANTIBODY-Structure

- Antibodies are typically made of basic structural units—each with two large heavy chains and two small light chains.

Structure

- Antibodies are heavy (~150 kDa) globular plasma proteins
- They have sugar chains added to some of their amino acid residues, so antibodies are glycoproteins.
- Immunoglobulins are composed of four polypeptide chains: two "light" chains (lambda or kappa), and two "heavy" chains (alpha, delta, gamma, epsilon or mu).
- The type of heavy chain determines the immunoglobulin isotype (IgA, IgD, IgG, IgE, IgM, respectively).
- Light chains are composed of 220 amino acid residues while heavy chains are composed of 440-550 amino acids. Each chain has "constant" and "variable" regions as shown in the figure.
- Variable regions(V region) are contained within the amino (NH_2) terminal end of the polypeptide chain (amino acids 1-110). When comparing one antibody to another, these amino acid sequences are quite distinct.
- Constant regions (C region), comprising amino acids 111-220 (or 440-550), are rather uniform, in comparison, from one antibody to another, within the same isotype.
- "Hypervariable" regions, or "Complementarity Determining Regions" (CDRs) are found within the variable regions of both the heavy and light chains. These regions serve to recognize and bind specifically to antigen.
- The four polypeptide chains are held together by covalent disulfide (-S-S-) bonds

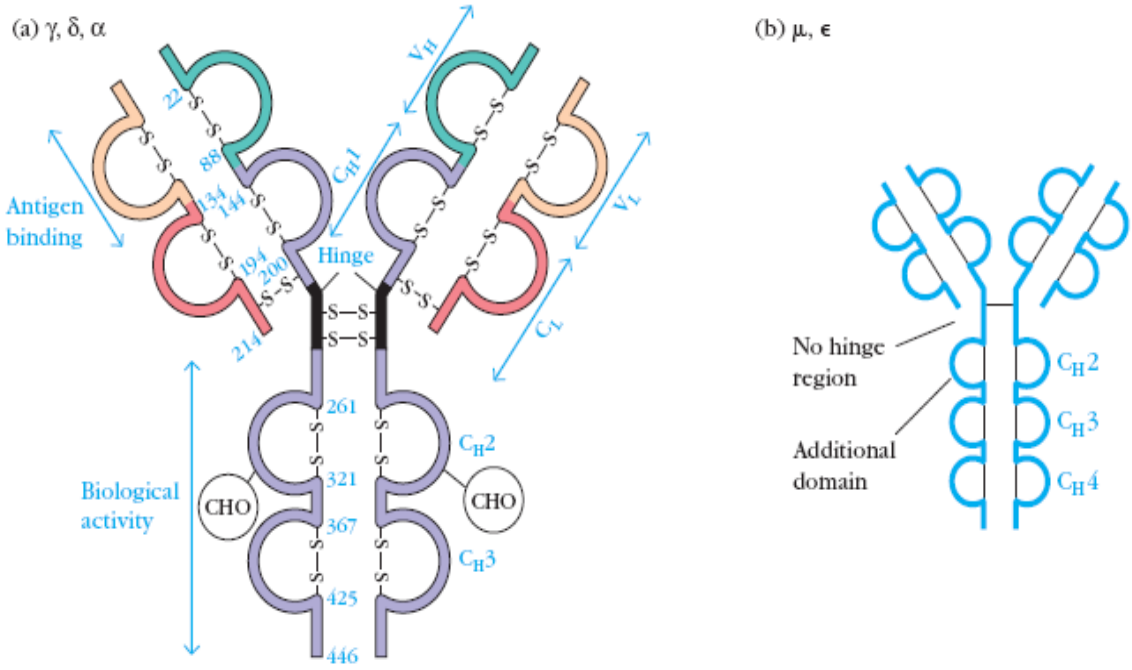


Fig:Heavy and light chains are folded into domains, each containing about 110 amino acid residues and an intrachain disulfide bond that forms a loop of 60 amino acids. The amino-terminal domains, corresponding to the V regions, bind to antigen effector functions are mediated by the other domains. (b) The and heavy chains contain an additional domain that replaces the hinge region.

Immunoglobulin domains

The Ig monomer is a "Y"-shaped molecule that consists of four polypeptide chains; two identical *heavy chains* and two identical *light chains* connected by disulfide bonds. Each chain is composed of structural domains called immunoglobulin domains. These domains contain about 70-110 amino acids and are classified into different categories (for example, variable or IgV, and constant or IgC) according to their size and function. They have a characteristic immunoglobulin fold in which two beta sheets create a "sandwich" shape, held together by interactions between conserved cysteines and other charged amino acids.

Heavy chain

There are five types of mammalian Ig heavy chain denoted by the Greek letters: α, δ, ε, γ, and μ. The type of heavy chain present defines the *class* of antibody; these chains are found in IgA, IgD, IgE, IgG, and IgM antibodies, respectively. Distinct heavy chains differ in size and

composition; α and γ contain approximately 450 amino acids, while μ and ε have approximately 550 amino acids.

1. Fab region

2. Fc region

3. Heavy chain (blue) with one variable (V_H) domain followed by a constant domain (C_H1), a hinge region, and two more constant (C_H2 and C_H3) domains.

4. Light chain (green) with one variable (V_L) and one constant (C_L) domain

5. Antigen binding site (paratope)

6. Hinge regions.

Each heavy chain has two regions, the *constant region* and the *variable region*. The constant region is identical in all antibodies of the same isotype, but differs in antibodies of different isotypes. Heavy chains γ, α and δ have a constant region composed of *three* tandem (in a line) Ig domains, and a hinge region for added flexibility; heavy chains μ and ε have a constant region composed of *four* immunoglobulin domains. The variable region of the heavy chain differs in antibodies produced by different B cells, but is the same for all antibodies produced by a single B cell or B cell clone. The variable region of each heavy chain is approximately 110 amino acids long and is composed of a single Ig domain.

Light chain

In mammals there are two types of immunoglobulin light chain, which are called lambda (λ) and kappa (κ). A light chain has two successive domains: one constant domain and one variable domain. The approximate length of a light chain is 211 to 217 amino acids. Each antibody contains two light chains that are always identical; only one type of light chain, κ or λ, is present per antibody in mammals. Other types of light chains, such as the iota (ι) chain, are found in lower vertebrates like sharks (Chondrichthyes) and bony fishes (Teleostei).

Immunoglobulin Classes and Subclasses

- Immunglobulin molecules are divided into distinct classes and subclasses in terms of the differences in amino acid sequence of constant region of heavy chain, i.e.γ,α,μ,δ,and εchains
- Immunoglobulin Classes of Mammals
  - IgG - Gamma (γ) heavy chains
  - IgM - Mu (μ) heavy chains
  - IgA - Alpha (α) heavy chains
  - IgD - Delta (δ) heavy chains
  - IgE - Epsilon (ε) heavy chains

- IgA is divided into two subclasses, IgA1 and IgA2(sheep)
- Mammalian antibodies can be divided into five classes: IgG, IgM, IgA, IgD and IgE, based on the number of Y units and the type of heavy chain
- The light chains of any antibody can be classified as either a kappa or Antibody Structure lambda type based on small polypeptide structural differences
- The heavy chain determines the subclass of each antibody
- The subclasses of antibodies differ in the number of disulfide bonds and the length of the hinge region
- The most commonly used antibody in immunochemical procedures is of the IgG class because they are the major immunoglobulin (Ig) released in serum

IgG Immunoglobulins

- IgG, the most abundant class in serum, constitutes about 80% of the total serum immunoglobulin. The IgG molecule consists of two heavy chains and two or two light chain.s There are four human IgG subclasses, dis-tinguished by differences in -chain sequence and numbered according to their decreasing average serum concentrations: IgG1, IgG2, IgG3, and IgG4 .

- 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.

Properties of IgG:

- Molecular weight: 150,000
- H-chain type (MW): gamma (53,000)
- Serum concentration: 10 to 16mg/mL
- Percent of total immunoglobulin: 80%
- Glycosylation (by weight): 3%
- Distribution: intra- and extravascular
- Function: secondary response

IgM Immunoglobulins

Serum IgM exists as a pentamer in mammals, predominates in primary immune responses to most antigens, is the most efficient complement fixing immunoglobulin and comprises

approximately 10% of normal human serum Ig content. IgM is also expressed on the plasma mem- brane of the B lymphocytes as a monomer. It is the B cell antigen receptor and the H chains each contain an additional hydrophobic domain for anchoring in the membrane. Monomers of serum IgM are bound together by disulfide bonds and a joining (J) chain.

IgM is se-creted by plasma cells as a pentamer in which five monomer units are held together. The five monomer subunits are arranged with their Fc regions in the center of the pentamer and the ten antigen-binding sites on the periphery of the molecule. Each pentamer contains an additional Fc-linked polypeptide called the **J (joining) chain,** which is disulfide-bonded to the carboxyl-terminal cysteine residue of two of the ten chains.

IgM is the first immunoglobulin class produced in a primary response to an antigen, and it is also the first im-munoglobulin to be synthesized by the neonate. Because of its pentameric structure with 10 antigen-binding sites, serum IgM has a higher valency than the other isotypes. An IgM molecule can bind 10 small hapten molecules.

Because of its large size, IgM does not diffuse well and therefore is found in very low concentrations in the intercel-lular tissue fluids. The presence of the J chain allows IgM to bind to receptors on secretory cells, which transport it across epithelial linings to enter the external secretions that bathe mucosal surfaces.

Properties of IgM:

- Molecular weight: 900,000
- H-chain type (MW): mu (65,000)
- Serum concentration: 0.5 to 2mg/mL
- Percent of total immunoglobulin: 10%
- Glycosylation (by weight): 12%
- Distribution: mostly intravascular
- Function: primary response

IgA Immunoglobulins

Although IgA constitutes only 10%–15% of the total im-munoglobulin in serum, it is the predominant im-munoglobulin class in external secretions such as breast milk, saliva, tears, and mucus of the bronchial, genitouri-nary, and digestive tracts. In serum, IgA exists primarily as a monomer, but polymeric forms (dimers, trimers, and some tetramers) are sometimes seen, all containing a J-chain polypeptide. The IgA of external secre-tions, called **secretory IgA,** consists of a dimer or tetramer, a J-chain polypeptide, and a polypeptide chain called **secretory component**

Properties of IgA:

- Molecular weight: 320,000 (secretory)
- H-chain type (MW): alpha (55,000)

- Serum concentration: 1 to 4mg/mL
- Percent of total immunoglobulin: 15%
- Glycosylation (by weight): 10%
- Distribution: intravascular and secretions
- Function: protect mucus membranes.IgA-secreting plasma cells are concentrated along mucous membrane surfaces. Breast milk contains secretory IgA and many other molecules that help protect the newborn against infection during the first month of life

IgD and IgE Immunoglobulins

IgD and IgE are found in serum in much smaller quantities than other Igs. Membrane IgD is a receptor for antigen found mostly on mature B-lymphocytes. IgE primarily defends against parasitic invasion and is responsible for allergic reactions.

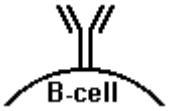
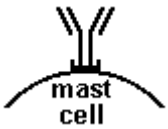
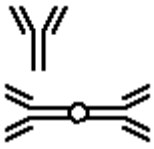
Properties of IgD:

- Molecular weight: 180,000
- H-chain type (MW): delta (70,000)
- Serum concentration: 0 to 0.4mg/mL
- Percent of total immunoglobulin: 0.2%
- Glycosylation (by weight): 13%
- Distribution: lymphocyte surface
- Function: unknown
- IgD was first discovered when a patient developed a multiple myeloma whose myeloma protein failed to react with anti-isotype antisera against the then-known isotypes: IgA, IgM, and IgG. When rabbits were immunized with this myeloma protein, the resulting antisera were used to identify the same class of antibody at low levels in normal human serum. The new class, called IgD, has a serum concentration of 30 g/ml and constitutes about 0.2% of the total immunoglobulin in serum. IgD, together with IgM, is the major membrane-bound immunoglobulin expressed by mature B cells, and its role in the physiology of B cells is under investigation. No biological effector function has been identified for IgD.

Properties of IgE:

- Molecular weight: 200,000
- H-chain type (MW): epsilon (73,000)
- Serum concentration: 10 to 400ng/mL
- Percent of total immunoglobulin: 0.002%
- Glycosylation (by weight): 12%
- Distribution: basophils and mast cells in salive and nasal secretions
- Function: protect against parasites
- IgE binds to Fc receptors on the membranes of blood ba-sophils and tissue mast cells. Cross-linkage of receptor-bound IgE molecules by antigen (allergen) induces basophils

and mast cells to translocate their granules to the plasma membrane and release their contents to the extracellular en-vironment, a process known as degranulation. As a result, a variety of pharmacologically active mediators are released and give rise to allergic manifestations

Isotype	Structure	Placental transfer	Binds mast cell surfaces	Binds phagocytic cell surfaces	Activates complement	Additional features
IgM		-	-	-	+	First Ab in development and response.
IgD		-	-	-	-	B-cell receptor.
IgG		+	-	+	+	Involved in opsonization and ADCC. Four subclasses; IgG1, IgG2, IgG3, IgG4.
IgE		-	+	-	-	Involved in allergic responses.
IgA		-	-	-	-	Two subclasses; IgA1, IgA2. Also found as dimer (sIgA) in secretions.

MHC- SELF-ANTIGENS

The **major histocompatibility complex** (**MHC** is a large genomic region found in most vertebrates that encodes MHC molecules. MHC molecules play an important role in the immune system and autoimmunity.

The MHC proteins act as "signposts" that serve to alert the immune system if foreign material is present inside a cell. They achieve this by displaying fragmented pieces or *antigens* on the host cell's surface. These antigens may be *self* or *nonself*. The constitutive presentation of MHC peptide on cell surfaces allows for pathogen surveillance by immune cells, usually a T cell or natural killer (NK) cell.

Every mammalian species studied to date possess a tightly linked cluster of genes, the **major histocompatibility complex (MHC),** whose products play roles in intercellular recognition and in dis-crimination between self and nonself. The MHC partici-pates in the development of both humoral and cell-mediated immune responses. While antibodies may react with antigens alone, most T cells recognize antigen only when it is combined with an MHC molecule.

The MHC Encodes Three Major Classes of Molecules

The MHC gene family is divided into three subgroups - class I, class II, and class III.

The major histocompatibility complex is a collection of genes arrayed within a long continuous stretch of DNA on chromosome 6 in humans and on chromosome 17 in mice. The MHC is referred to as the **HLA complex** in humans and as the **H-2 complex** in mice. Although the arrangement of genes is somewhat different, in both cases the MHC genes are organized into regions encoding three classes of molecules (Figure 7-1):

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

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

Class III MHC genes encode, in addition to other products, various secreted proteins that have immune functions, including components of the complement system and molecules involved in inflammation.

The class I and class II MHC molecules have common structural features and both have roles in antigen processing. By contrast, the class III MHC region, which is flanked by the class I and II regions, encodes molecules that are critical to immune function but have little in common with class I or II molecules. Class III products include the complement com-ponents C4, C2, BF (see Chapter 13), and inflammatory cy-tokines, including tumor necrosis factor (TNF) and heat-shock proteins

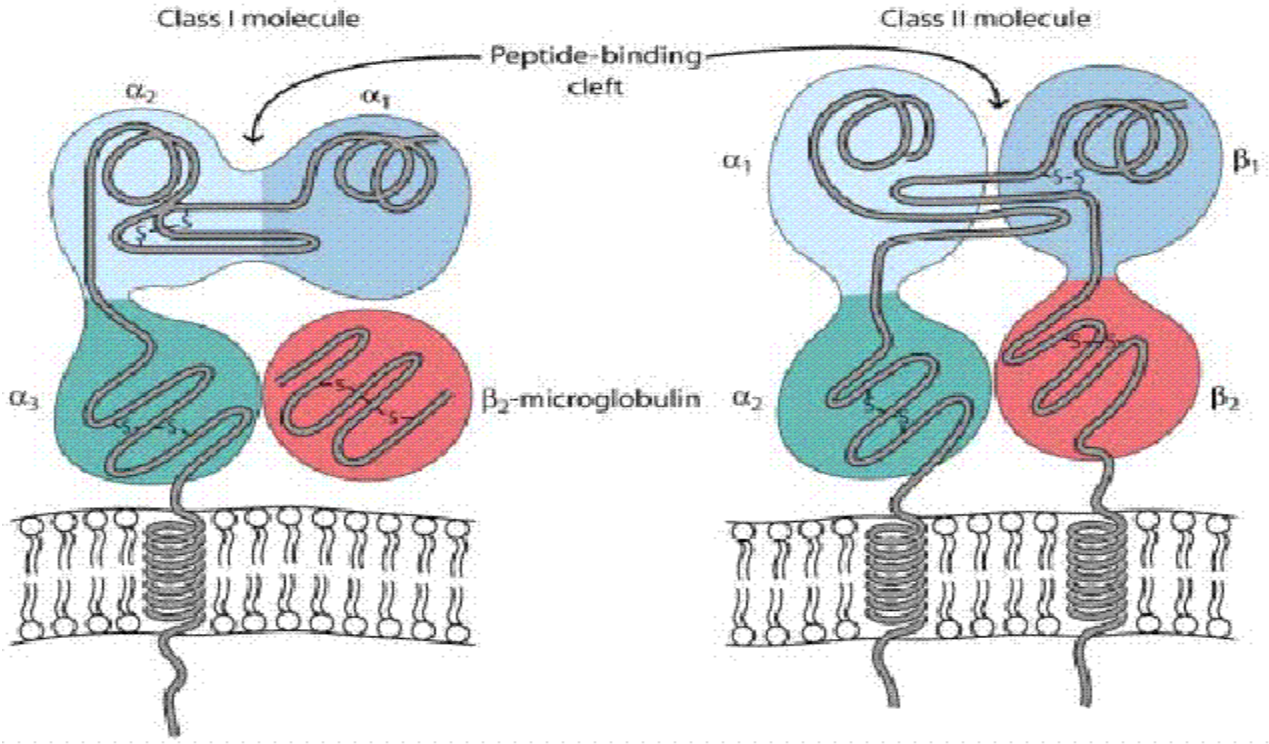


Fig: Schematic diagrams of a class I and a class II MHC molecule showing the external domains, transmembrane segment, and cytoplasmic tail. The peptide-binding cleft is formed by the mem-brane-distal domains in both class I and class II molecules. The membrane-proximal domains possess the basic immunoglobulinfold structure; thus, class I and class II MHC molecules are classified as members of the immunoglobulin superfamily

Role of MHC inantigen presentation and processing

Class I MHC molecules are found **on all nucleated cells** of the body. Thus, all nucleated cells can present antigen to CD8(+) T-cells.MHC proteins are essential to **antigen processing** and **antigen presentation**.

Class II proteins present pieces of **exogenous antigens**. These are antigens that are **ingested (or phagocytosed) by "professional" antigen presenting cells (APC's).** Processed antigen fragments are then presented to CD4(+) T-cells.
This is diagramed in figure:

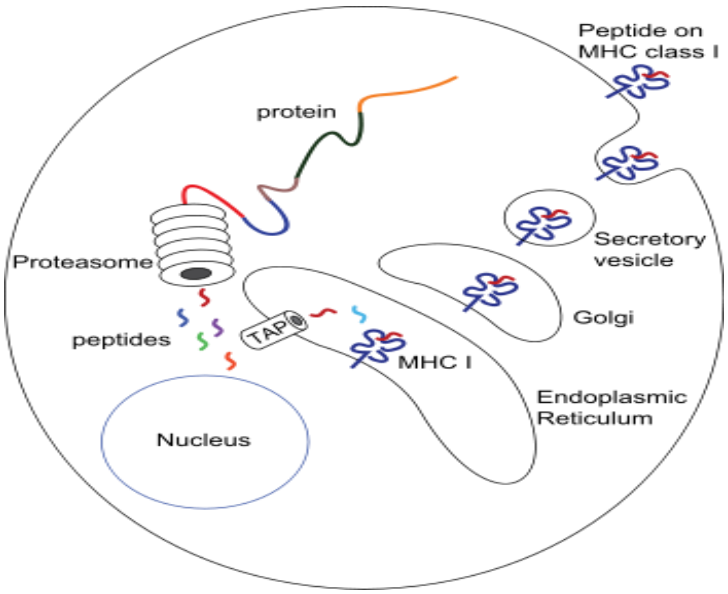
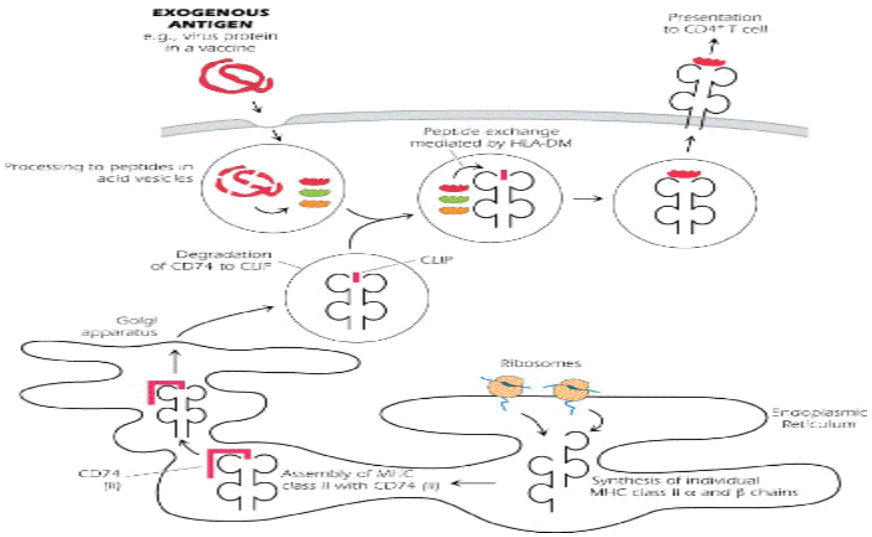
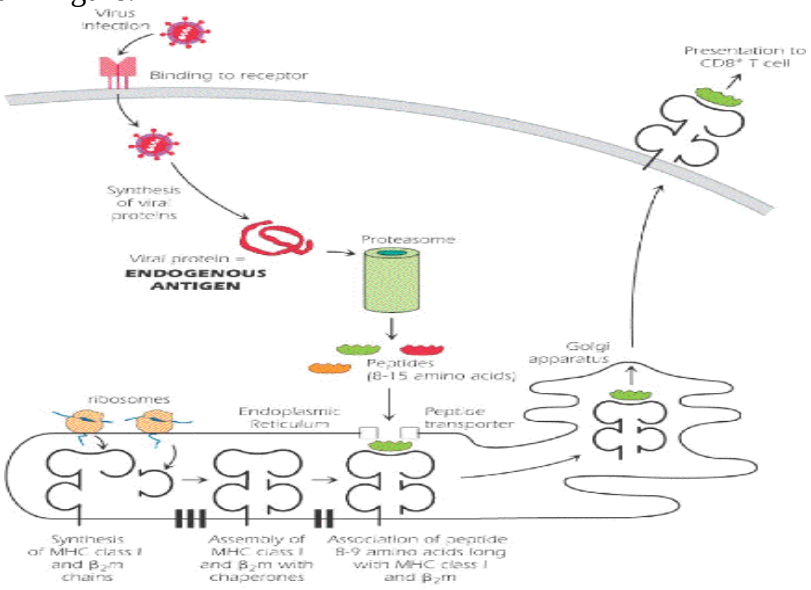


Fig:Peptide processing for peptides associated to MHC-I molecules:

MHC molecules can display only **peptides**. For this reason, as T lymphocytes can recognize an antigen only if it is displayed by an MHC molecule, they only can react to antigens of proteic origin (coming from microbes) and not to other types of chemical compounds (neither lipids nor nucleic acids nor sugars). Each MHC molecule can display **only one peptide** each time, because the cleft in the molecule has space only to load one peptide. However, one given MHC molecule has a **broad specificity**, because it can display many different peptides (although not all).



Class I proteins present pieces of **endogenous antigens**. These are antigens that are made by the cell itself - **antigens that come from within the antigen presenting cell**. These are usually thought of as cells that are infected with virus or some other intracellular parasite. Such processed antigen fragments are then presented to CD8(+) T-cells.
This is diagramed in figure:



Self antigen and MHC

Adaptive immunity includes both a humoral response produced by antibodies, and a cell-mediated response produced by T cells that have the ability to destroy other cells. The cell-mediated adaptive immune response is regulated by the major histocompatibility complex (MHC), so named because it is responsible for graft rejection, or tissue compatibility. Individuals identical for this region can exchange grafts more successfully than those with different MHC combinations, which is not an easy task

In humans, the MHC genes encode the human leukocyte antigens (HLAs) on the cell surface. Proteins inside the cell are broken down into short fragments that can be displayed as peptide antigens by MHC molecules on the surface of the cell. MHC molecules display both 'self' peptides derived from their own proteins, and foreign peptides derived from invading pathogens. The immune system is constantly monitoring the surfaces of cells, and the MHC-presented peptides help immune cells to discriminate between normal antigens on the surface of all cells, and those that are foreign and potentially dangerous. The immune system also monitors the amount of MHC-presented antigens, which helps them to target and destroy cancerous cells that often display increased amounts of self-antigens. Defects in certain MHC genes lead to autoimmune disorders in which the body fails to recognize self-antigens, such as occurs in

diseases like multiple sclerosis, inflammatory bowel disease, and in some forms of arthritis and diabetes.

UNIT II						
1	Which one has the ability to induce a humoral immune response	Antigenicity	immunogencity	T cells	B cells.	immunogencity
2	Substances that induce a specific immune response is known as	Antigen	Antibody	haptens	Cytokines.	haptens
3	Which one has the ability to combine specifically to the cell surface receptors.	haptens	Cytokines	Antigenicity	immunogenicity.	haptens
4	Haptens are	large molecules	small molecule	very large molecules	very small molecules.	small molecule
5	In cell mediated immunity which one serves as immunogens.	antigen	Antibody	protein	haptens.	protein
6	Analogue of dihydrofolic acid is _____	xanthine	uric acid	aminopterin	arginine	aminopterin
7	Which one of the following acts as fusogen	hypoxanthine	PRPP	polyethylene glycol	glycerol	polyethylene glycol
8	Which are the substances serves to enhance the immunogenicity.	proteins	cytokines	haptens	Adjuvants	Adjuvants
9	The formation of macrophage- trich mass of cells called as	Granuloma	granules	endo granules	exogranules.	Granuloma
10	---------- are the immunologically active regions of an immunogen	exotopes	epitopes	endotopes	exotopes.	epitopes
11	Sperm whale myoglobin contains abundance of ---------- regions.	µ - helical	b - helical	g- helical	helical	µ - helical
12	Complex proteins contains multiple overlapping ---------- epitopes.	T cell	B cell	NK cells	T_H cells.	B cell
13	Haptens are	Immunogenic	immune	antigens	antigenic	antigenic
14	Chemical coupling of a hapten to a large protein called as	Carrier	hapten – carrier	antigen – carrier	antibody carrier.	Carrier
15	Which one is capable of inducing cell division in a high percentage of T and B cells	mesogens	mitogens	endogens	exogens.	mitogens
16	polyclonal activators are also known as	exogens	endogens	mesogens	mitogens.	mitogens.
17	Mitogens are sugar-binding protein called as	lectins	endolectin	exolectin	mesolectin.	lectins
18	Cancerous plasma cell is also called as	Myeloma cell	plasma cell	cancer cell	grauloma cell	grauloma cell
19	The light chains in urine of myleoma patients	Jonce protiens	Bence Jones proteins	proteins	myleoma proteins.	Bence Jones proteins
20	The clones of maliginant plasma cells that develop are called	Plasma	cytoma	plasmacytomas	Myeloma	plasmacytomas
21	Which is a transmembrane protein complex	B-cell receptor	T cell	T cell receptor	B cell.	B-cell receptor
22	The g- chain allotypes are referred to as	GI markers	Gl markers	Gn markers	Gm markers.	Gm markers.
23	Which Immunogloubulin is found abundance in serum	Ig K	IgG	IgM	IgG1.	IgG
24	5% - 10% of the total serum immunoglobulin was	IgG	IgG1	Ig K	IgM	IgM
25	Monomeric IgM has a molecular weight of	180,000	188,000	181,000	190,000	180,000
26	Antigenic cells are readily aggluitaned by	IgG	IgA	Ig K	IgM	IgM
27	The biological role of -------- is found in the development of allergic symptoms	IgG	IgA	IgE	IgM	IgE
28	Second largest class of immunogluoblins present in human serum	IgG	IgA	IgE	IgM	IgA
29	The two monomeric IgA molecules are cross linked by short polypeptide chain called	J chain	B chain	A chain	K chain.	J chain
30	Immunogluoblins, which are isotypically as well as allotypically similar are refferd to as	IgE	IgM	Idiotypes	endotypes.	Idiotypes
31	Who proposed the basic structure of immunoglobulin	Rodney proter	Koular & milstien	Perlman	Dreyer	Rodney proter
32	When an immunogloublin does not reactswith an antigen, it is known as	Antigen	Antibody	Antigen – antibody	Immunoglobulins.	Immunoglobulins.
33	When the immunogloublin contains kappa chain, it is known as	L type	K type	KL type	K_1 type.	K type
34	When the immunogloublin contains lambda chain, it is known as	L type	K type	KL type	K_1 type	L type
35	g- chain in the immunogloublin is	Ig A	Ig M	I gG	Ig D	I gG
36	a- chain in the immunogloublin is	Ig A	Ig M	I gG	Ig D	Ig A
37	m- chain in the immunogloublin is	Ig A	Ig M	I gG	Ig D	Ig M
38	d - chain in the immunogloublin is	Ig A	Ig M	I gG	Ig D	Ig D
39	Epsilon chain in the immunogloublin is	Ig A	Ig M	I gE	Ig D	I gE
40	Antigen binding fragments is also known as	Fab	Fc	VL	VHL	Fab
41	Crystalline fragment is also known as	Fab	Fc	VL	VHL	Fc
42	Amino acid sequence and the shape of the combining site together constitute the	epitope	endotope	paratope	exotope	paratope
43	Paratope is complementary to the specific shape of the ---------- in the antigen	epitope	endotope	paratope	exotope.	epitope
44	---------- is the only immunogloublin that crosses the human placenta.	Ig A	Ig M	I gG	Ig D	I gG
45	IgG neutralizes	Bacteira	Viruses	fungi	algae.	Viruses
46	Which one is the largest immunoglobulin	Ig A	Ig M	I gG	Ig D	Ig M
47	Which immunogloublin is limited to the blood serum	Ig A	Ig M	I gG	Ig D	Ig D
48	Which one is heat stable immunogloublins.	Ig E	Ig M	I gG	Ig G	Ig E
49	Which immunogloublin has a special attraction to mast cells	Ig A	Ig E	I gG	Ig D	Ig E
50	In IgE carbohydrate content found to be	12 %	13%	14 %	15%	12 %
51	---------- is a process of clumping of particulate antigens.	Precipitation	agglutination	covalent binding	Cross – linking	agglutination
52	The first injection of the antigen producing primary immune response is called as	Primary dose	secondary dose	tertiary dose	Quantery dose.	Primary dose
53	The injection of the same antigen for the second time is called as	Primary dose	Booster dose	tertiary dose	Quantery dose.	Booster dose
54	The amount of antibodies produced by the immune response.	Antigen titre	antibody titre	antigen- antibody titre	immuno titre	Antigen titre
55	Aminopterin blocks which DNA synthetic pathway	Salwage pathway	Denovo pathway	Both	cAMP Pathway	Denovo pathway
56	Catalytic antibodies are called as	enzymes	abzymes	ribozymes	ribulozyme	
57	Among the following which one is not involved in antibody diversity	junctional flexibility	somatic hyper mutation	P addition	negative selection	negative selection
58	In heavy chain gene rearrangement which genes are involved	V genes	D genes	J genes	all	all
59	Among the following which plays important role in antibody diversity	class switching	flipflop movement	Transition	transversion	class switching
60	In human heavy chain genes are located at _____ chromosome	2	7	14	21	14

KARPAGAM ACADEMY OF HIGHER EDUCATION
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Coimbatore - 641021.
(For the candidates admitted from 2015 onwards)
DEPARTMENT OF BIOCHEMISTRY

SUBJECT : **IMMUNOLOGY**
SEMESTER : **V**
SUBJECT CODE : **15BCU503** **CLASS** : **III B.Sc.BC**

UNIT III - COURSE MATERIAL

UNIT III- *Hypersensitivity*
Hypersensitivity- Type I, II, III & IV; Factors causing hypersensitivity; Mechanism, Pathogenesis, prevention and treatment**.**
Complement- definition, classical and alternate pathways, biological importance of complement system, complement deficiency diseases.

HYPERSENSITIVITY : DEFINITION AND TYPES

Hypersensitivity refers to excessive, undesirable (damaging, discomfort-producing and sometimes fatal) reactions produced by the normal immune system. Hypersensitivity reactions require a pre-sensitized (immune) state of the host. Hypersensitivity reactions can be divided into four types: type I, type II, type III and type IV, based on the mechanisms involved and time taken for the reaction. Frequently, a particular clinical condition (disease) may involve more than one type of reaction.

HYPERSENSITIVE REACTIONS

TYPE I HYPERSENSITIVITY

Type I hypersensitivity is also known as immediate or anaphylactic hypersensitivity. The reaction may involve skin (urticaria and eczema), eyes (conjunctivitis), nasopharynx (rhinorrhea, rhinitis), bronchopulmonary tissues (asthma) and gastrointestinal tract (gastroenteritis). The reaction may cause a range of symptoms from minor inconvenience to death. The reaction usually takes 15 - 30 minutes from the time of exposure to the antigen, although sometimes it may have a delayed onset (10 - 12 hours).

Immediate hypersensitivity is mediated by IgE. The primary cellular component in this hypersensitivity is the mast cell or basophil. The reaction is amplified and/or modified by

platelets, neutrophils and eosinophils. A biopsy of the reaction site demonstrates mainly mast cells and eosinophils.

The mechanism of reaction involves preferential production of IgE, in response to certain antigens (often called allergens). The precise mechanism as to why some individuals are more prone to type-I hypersensitivity is not clear. However, it has been shown that such individuals preferentially produce more of TH2 cells that secrete IL-4, IL-5 and IL-13 which in turn favor IgE class switch. IgE has very high affinity for its receptor (Fcε; CD23) on mast cells and basophils.

A subsequent exposure to the same allergen cross links the cell-bound IgE and triggers the release of various pharmacologically active substances (figure 1). Cross-linking of IgE Fc-receptor is important in mast cell triggering. Mast cell degranulation is preceded by increased Ca++ influx, which is a crucial process; ionophores which increase cytoplasmic Ca^{++} also promote degranulation, whereas, agents which deplete cytoplasmic Ca^{++} suppress degranulation.

The agents released from mast cells and their effects are listed in Table 1. Mast cells may be triggered by other stimuli such as exercise, emotional stress, chemicals (*e.g.*, photographic developing medium, calcium ionophores, codeine, *etc.*), anaphylotoxins (*e.g.*, C4a, C3a, C5a, *etc.*). These reactions, mediated by agents without IgE-allergen interaction, are not hypersensitivity reactions, although they produce the same symptoms

Table 1. Pharmacologic Mediators of Immediate Hypersensitivity

MEDIATOR	
Preformed mediators in granules	
histamine	bronchoconstriction, mucus secretion, vasodilatation, vascular permeability
tryptase	Proteolysis
kininogenase	kinins and vasodilatation, vascular permeability, edema
ECF-A (tetrapeptides)	attract eosinophil and neutrophils

Newly formed mediators

leukotriene B_4	basophil attractant
leukotriene C_4, D_4	same as histamine but 1000x more potent
prostaglandins D_2	edema and pain
PAF	platelet aggregation and heparin release: microthrombi

The reaction is amplified by PAF (platelet activation factor) which causes platelet aggregation and release of histamine, heparin and vasoactive amines. Eosinophil chemotactic factor of anaphylaxis (ECF-A) and neutrophil chemotactic factors attract eosinophils and neutrophils, respectively, which release various hydrolytic enzymes that cause necrosis. Eosinophils may also control the local reaction by releasing arylsulphatase, histaminase, phospholipase-D and prostaglandin-E, although this role of eosinophils is now in question.

Diagnostic tests for immediate hypersensitivity include skin (prick and intradermal) tests (fig. 1A), measurement of total IgE and specific IgE antibodies against the suspected allergens. Total IgE and specific IgE antibodies are measured by a modification of enzyme immunoassay (ELISA).

Symptomatic treatment is achieved with anti-histamines which block histamine receptors. Chromolyn sodium inhibits mast cell degranulation, probably, by inhibiting Ca^{++} influx. Late onset allergic symptoms, particularly bronchoconstriction which is mediated by leukotrienes, are treated with leukotriene receptor blockers (Singulair, Accolate) or inhibitors of the cyclooxygenase pathway (Zileutoin). Symptomatic, although short term, relief from bronchoconstriction is provided by bronchodilators (inhalants) such as isoproterenol derivatives (Terbutaline, Albuterol). Thophylline elevates cAMP by inhibiting cAMP-phosphodiesterase and inhibits intracellular Ca^{++} release is also used to relieve bronchopulmonary symptoms. Hyposensitization (immunotherapy or desensitization) is another treatment modality which is successful in a number of allergies, particularly to insect venoms and, to some extent, pollens. Suppressor T cells that specifically inhibit IgE antibodies may play a role.

The use of IgG antibodies against the Fc portions of IgE that binds to mast cells has been approved for treatment of certain allergies, as it can block mast cell sensitization.

EG-Asthma, atopic dermatitis, Food allegy

TYPE II HYPERSENSITIVITY

Type II hypersensitivity is also known as cytotoxic hypersensitivity and may affect a variety of organs and tissues. The antigens are normally endogenous, although exogenous chemicals (haptens) which can attach to cell membranes can also lead to type II hypersensitivity. Drug-induced hemolytic anemia, granulocytopenia and thrombocytopenia are such examples. The reaction time is minutes to hours.

Type II hypersensitivity is primarily mediated by antibodies of the IgM or IgG classes and complement (Figure). Type II hypersensitive reactions involve antibody-mediated destruction of cells. Antibody can activate the complement system, creating pores in the membrane of a foreign cell (see Figure) , or it can mediate cell destruction by antibody-dependent cell-mediated cytotoxicity (ADCC). In this pro-cess, cytotoxic cells with Fc receptors bind to the Fc region of antibodies on target cells and promote killing of the cells (see Figure). Antibody bound to a foreign cell also can serve as an opsonin, enabling phagocytic cells with Fc or C3b re-ceptors to bind and phagocytose the antibody-coated cell

Eg: **i)Blood transfusion reaction**

The clinical manifestations of transfusion reactions result from massive intravascular hemolysis of the transfused red blood cells by antibody plus complement.

ii) Hemolytic Disease of the Newborn

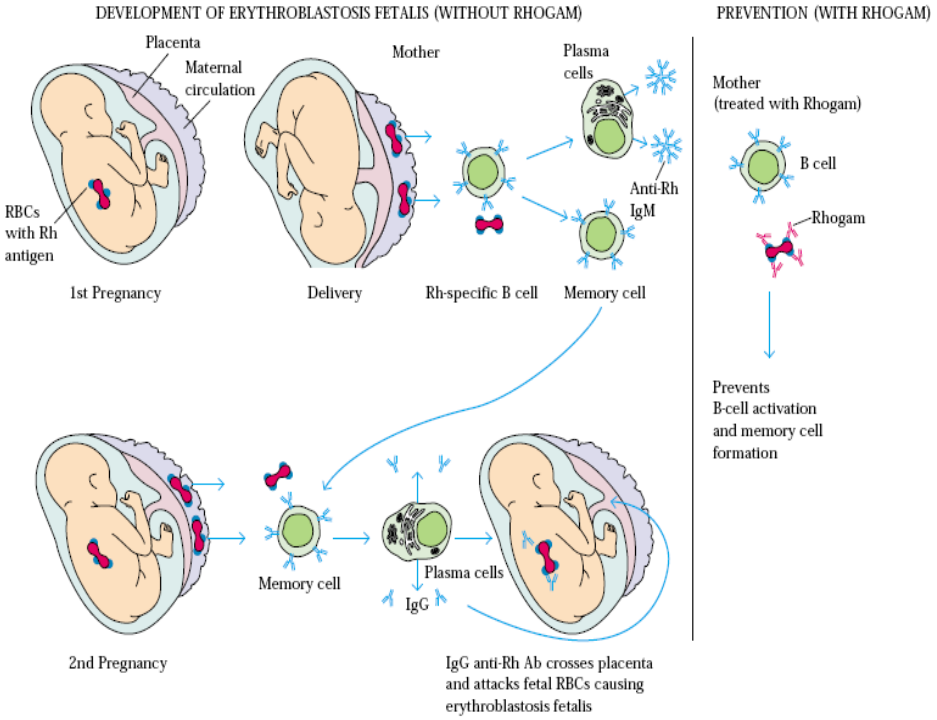


Figure: Type II hypersensitivity mechanisms

Hemolytic disease of the newborn develops when maternal IgG antibodies specific for fetal blood-group antigens cross the placenta and destroy fetal red blood cells. The conse-quences of such transfer can be minor, serious, or lethal. Severe hemolytic disease of the newborn, called **erythroblas-tosis fetalis,** most commonly develops when an Rh^+ fetus ex-presses an **Rh antigen** on its blood cells that the Rh^- mother does not express.

Diagnostic tests include detection of circulating antibody against the tissues involved and the presence of antibody and complement in the lesion (biopsy) by immunofluorescence. The staining pattern is normally smooth and linear, such as that seen in Goodpasture's nephritis (renal and lung basement membrane) and pemphigus (skin intercellular protein, desmosome).

Treatment involves anti-inflammatory and immunosuppressive agents.

TYPE III HYPERSENSITIVITY

Type III hypersensitivity is also known as immune complex hypersensitivity. The reaction may be general (*e.g.*, serum sickness) or may involve individual organs including skin (*e.g.*, systemic lupus erythematosus, Arthus reaction), kidneys (*e.g.*, lupus nephritis), lungs (*e.g.*, aspergillosis), blood vessels (*e.g.*, polyarteritis), joints (*e.g.*, rheumatoid arthritis) or other organs. This reaction may be the pathogenic mechanism of diseases caused by many microorganisms.

The reaction may take 3 - 10 hours after exposure to the antigen (as in Arthus reaction). It is mediated by soluble immune complexes. They are mostly of the IgG class, although IgM may also be involved. The antigen may be exogenous (chronic bacterial, viral or parasitic infections), or endogenous (non-organ specific autoimmunity: *e.g.*, systemic lupus erythematosus, SLE). The antigen is soluble and not attached to the organ involved. Primary components are soluble immune complexes and complement (C3a, 4a and 5a). The damage is caused by platelets and neutrophils (Figure3).

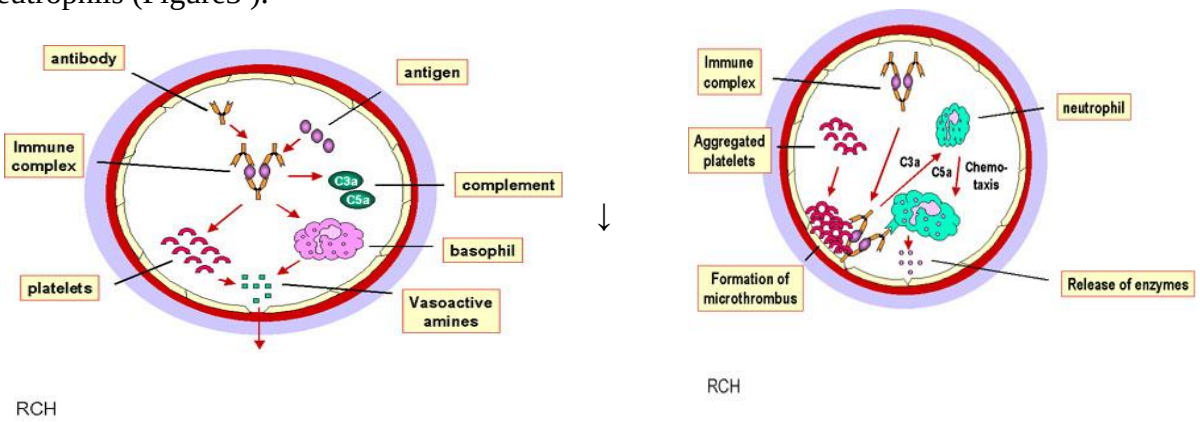


Figure 3: Mechanism of damage in type-III hypersensitivity

The lesion contains primarily neutrophils and deposits of immune complexes and complement. Macrophages infiltrating in later stages may be involved in the healing process.

The affinity of antibody and size of immune complexes are important in production of disease and determining the tissue involved.

Mechanism:

The reaction of antibody with antigen generates immune complexes. Generally this complexing of antigen with anti-body facilitates the clearance of antigen by phagocytic cells. In some cases, however, large amounts of immune complexes can lead to tissue-damaging type III hypersensitive reactions. The magnitude of the reaction depends on the quantity of immune complexes as well as their distribution within the body. When the complexes are deposited in tissue very near the site of antigen entry, a localized reaction develops. When the complexes are formed in the blood, a reaction can de-velop wherever the complexes are deposited. In particular, complex deposition is frequently observed on blood-vessel walls, in the synovial membrane of joints, on the glomerular basement membrane of the kidney, and on the choroid plexus of the brain. The deposition of these complexes initiates a reaction that results in the recruitment of neutrophils to the site. The tissue there is injured as a consequence of granular release from the neutrophil.

Type III hypersensitive reactions develop when immune complexes activate the complement system's array of immune effector molecules (see Figure). The C3a, C4a, and C5a complement split products are anaphylatoxins that cause localized mast-cell de-granulation and consequent increase in local vascular per-meability. C3a, C5a, and C5b67 are also chemotactic factors for neutrophils, which can accumulate in large numbers at the site of immune-complex deposition. Larger immune com-plexes are deposited on the basement membrane of blood-vessel walls or kidney glomeruli, whereas smaller complexes may pass through the basement membrane and be deposited in the subepithelium. The type of lesion that results depends on the site of deposition of the complexes.

Much of the tissue damage in type III reactions stems from release of lytic enzymes by neutrophils as they attempt to phagocytose immune complexes. The C3b complement component acts as an opsonin, coating immune complexes. A neutrophil binds to a C3b-coated immune complex by means of the type I complement receptor, which is specific for C3b. Because the complex is deposited on the basement-membrane surface, phagocytosis is impeded, so that lytic enzymes are released during the unsuccessful attempts of the neutrophil to ingest the adhering immune complex. Further activation of the membrane-attack mechanism of the com-plement system can also contribute to the destruction of tis-sue. In addition, the activation of complement can induce aggregation of platelets, and the resulting release of clotting factors can lead to formation of microthrombi.

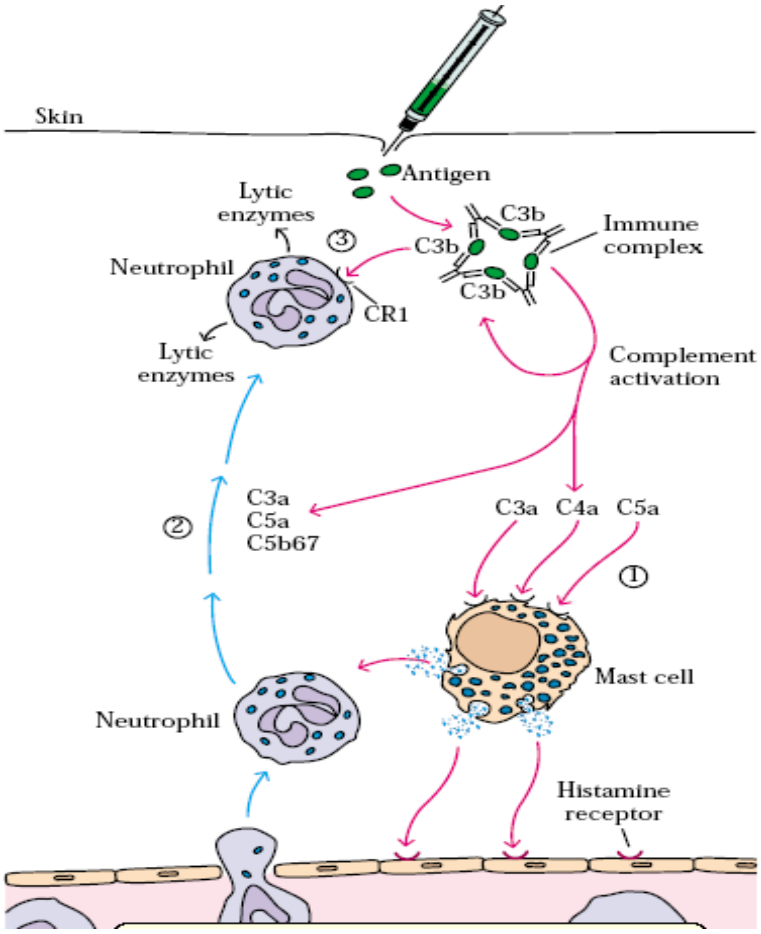


Fig:Development of a localized Arthus reaction (type III hypersensitive reaction).

Diagnosis involves examination of tissue biopsies for deposits of immunoglobulin and complement by immunofluorescence microscopy. The immunofluorescent staining in type III hypersensitivity is granular (as opposed to linear in type II such as seen in Goodpasture's syndrome). The presence of immune complexes in serum and depletion in the level of complement are also diagnostic. Polyethylene glycol-mediated turbidity (nephelometry) are also utilized to detect immune complexes.

Treatment includes anti-inflammatory agents.

TYPE IV HYPERSENSITIVITY

Type IV hypersensitivity is also known as cell mediated or delayed type hypersensitivity. The classical example of this hypersensitivity is tuberculin (Montoux) reaction (figure 5) which peaks

48 hours after the injection of antigen (PPD or old tuberculin). The lesion is characterized by induration and erythema.

Type IV hypersensitivity is involved in the pathogenesis of many autoimmune and infectious diseases (tuberculosis, leprosy, blastomycosis, histoplasmosis, toxoplasmosis, leishmaniasis, *etc.*) and granulomas due to infections and foreign antigens. Another form of delayed hypersensitivity is contact dermatitis (poison ivy , chemicals, heavy metals, *etc.*) in which the lesions are more papular. Type IV hypersensitivity can be classified into three categories depending on the time of onset and clinical and histological presentation.

Table 3 - Delayed hypersensitivity reactions

Type	Reaction time	Clinical appearance	Histology	Antigen and site
contact	48-72 hr	eczema	lymphocytes, followed by macrophages; edema of epidermis	Epidermal (organic chemicals, poison ivy, heavy metals, *etc.*)
tuberculin	48-72 hr	local induration	lymphocytes, monocytes, macrophages	intradermal (tuberculin, lepromin, *etc.*)
granuloma	21-28 days	hardening	macrophages, epitheloid and giant cells, fibrosis	Persistent antigen or foreign body presence (tuberculosis, leprosy, *etc.*)

Mechanisms of damage in delayed hypersensitivity include T lymphocytes and monocytes and/or macrophages. Cytotoxic T cells (Tc) cause direct damage whereas helper T (TH1) cells secrete cytokines which activate cytotoxic T cells and recruit and activate monocytes and macrophages, which cause the bulk of the damage. The delayed hypersensitivity lesions mainly contain monocytes and a few T cells.

Major lymphokines involved in delayed hypersensitivity reaction include monocyte chemotactic factor, interleukin-2, interferon-gamma, TNF alpha/beta, *etc.*

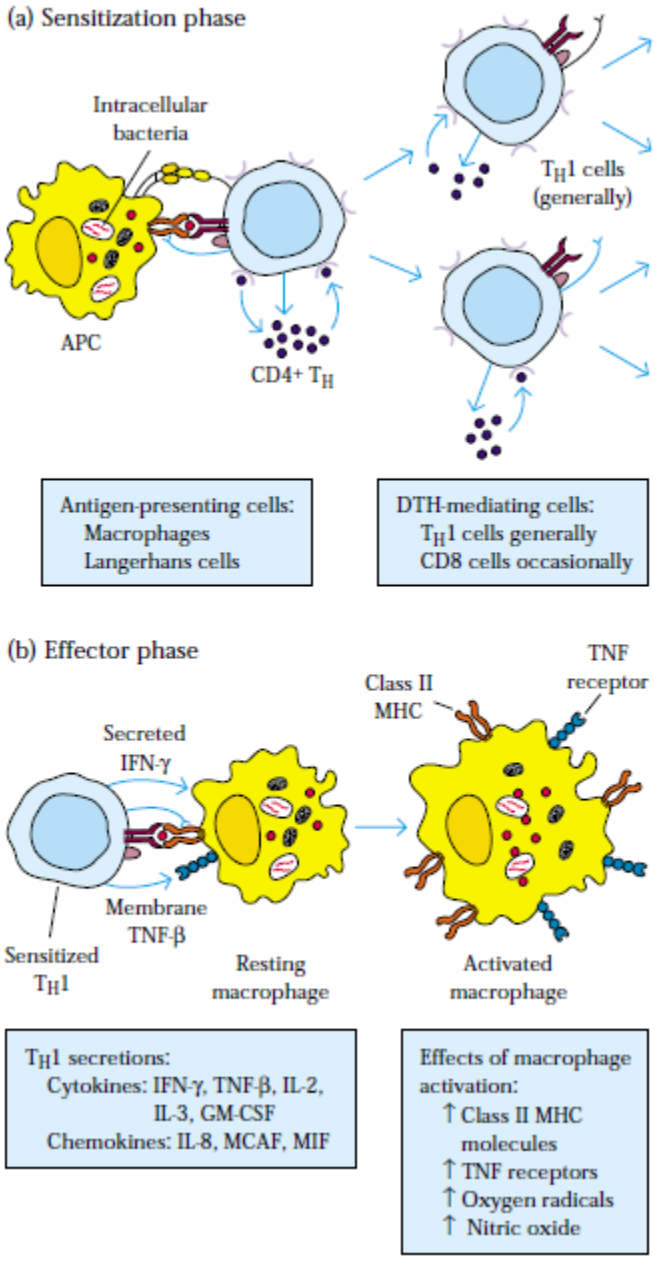
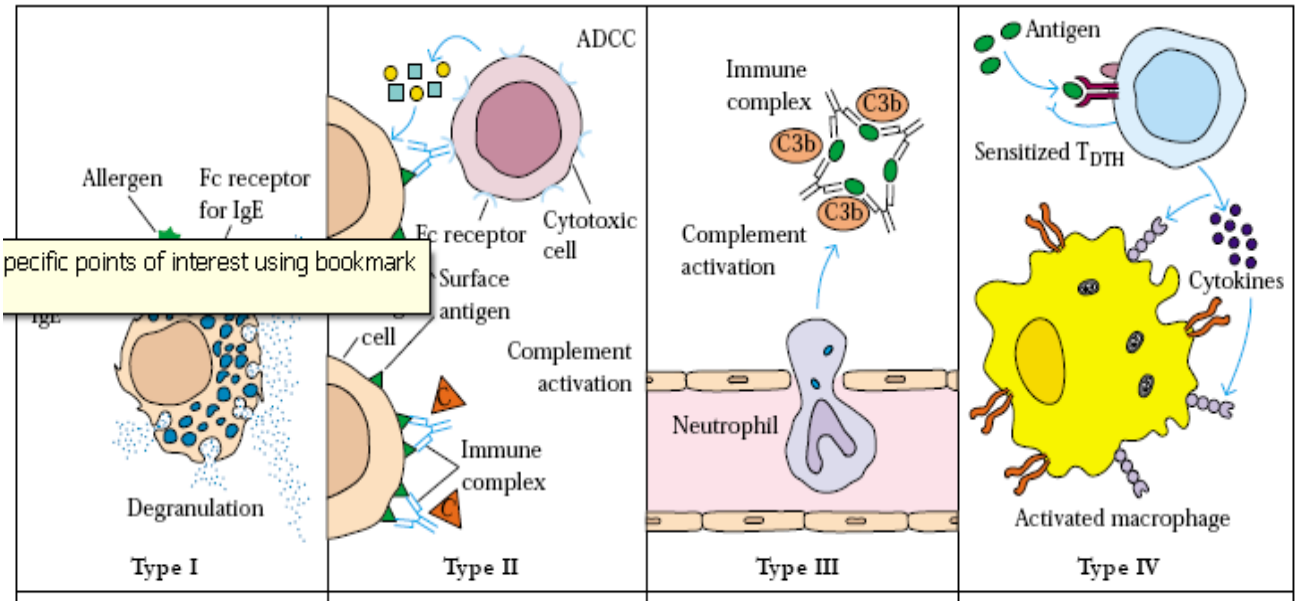


Figure 4. Mechanisms of damage in delayed hypersensitivity

Diagnostic tests *in vivo* include delayed cutaneous reaction (*e.g.* Montoux test (figure 5)) and patch test (for contact dermatitis). In vitro tests for delayed hypersensitivity include mitogenic response, lympho-cytotoxicity and IL-2 production.

Corticosteroids and other immunosuppressive agents are used in **treatment**



Type I	Type II	Type III	Type IV
IgE-Mediated Hypersensitivity	IgG-Mediated Cytotoxic Hypersensitivity	Immune Complex-Mediated Hypersensitivity	Cell-Mediated Hypersensitivity
Ag induces crosslinking of IgE bound to mast cells and basophils with release of vasoactive mediators	Ab directed against cell surface antigens meditates cell destruction via complement activation or ADCC	Ag-Ab complexes deposited in various tissues induce complement activation and an ensuing inflammatory response mediated by massive infiltration of neutrophils	Sensitized T_H1 cells release cytokines that activate macrophages or T_C cells which mediate direct cellular damage
Typical manifestations include systemic anaphylaxis and localized anaphylaxis such as hay fever, asthma, hives, food allergies, and eczema	Typical manifestations include blood transfusion reactions, erythroblastosis fetalis, and autoimmune hemolytic anemia	Typical manifestations include localized Arthus reaction and generalized reactions such as serum sickness, necrotizing vasculitis, glomerulnephritis, rheumatoid arthritis, and systemic lupus erythematosus	Typical manifestations include contact dermatitis, tubercular lesions and graft rejection

THE COMPLEMENT SYSTEM

Complement (C) was used to refer to a heat-labile serum component that was able to lyse bacteria (activity is destroyed (inactivated) by heating serum at 56 degrees C for 30 minutes). However, complement is now known to contribute to host defenses in other ways as well. Complement can opsonize bacteria for enhanced phagocytosis; it can recruit and activate various cells including polymorphonuclear cells (PMNs) and macrophages; it can participate in regulation of antibody responses and it can aid in the clearance of immune complexes and apoptotic cells. Complement can also have detrimental effects for the host; it contributes to inflammation and tissue damage and it can trigger anaphylaxis.

Complement comprises over 20 different serum proteins (see Table 1) that are produced by a variety of cells including, hepatocytes, macrophages and gut epithelial cells. Some complement proteins bind to immunoglobulins or to membrane components of cells. Others are proenzymes that, when activated, cleave one or more other complement proteins. Upon cleavage some of the complement proteins yield fragments that activate cells, increase vascular permeability or opsonize bacteria.

The following are the basic functions of the complement

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

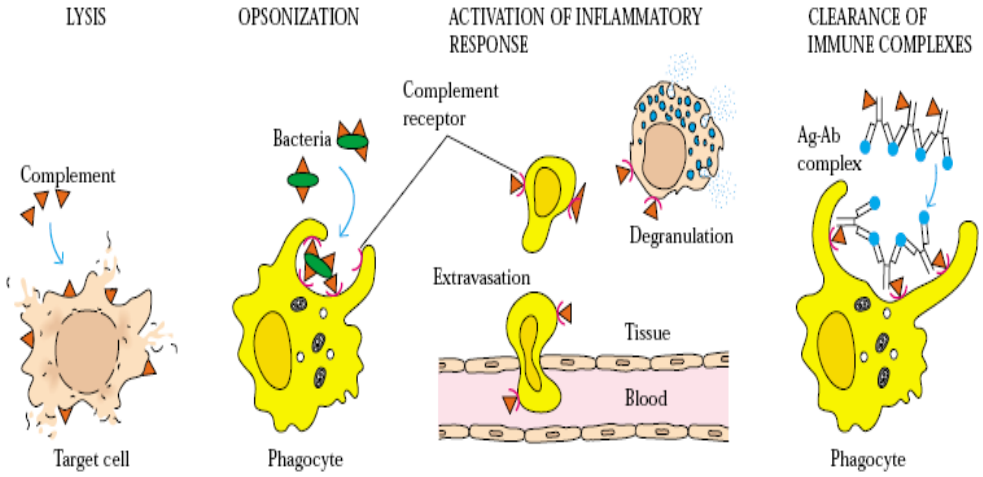


Fig: multiple activities of the complement system.

Features of the complement system

- The complement system consists of some 30 proteins circulating in blood plasma.
- Most of these are inactive until
  - they are cleaved by a protease which, in turn,
  - converts them into a protease.
- Thus many components of the system serve as the substrate of a prior component and then as an enzyme to activate a subsequent component.

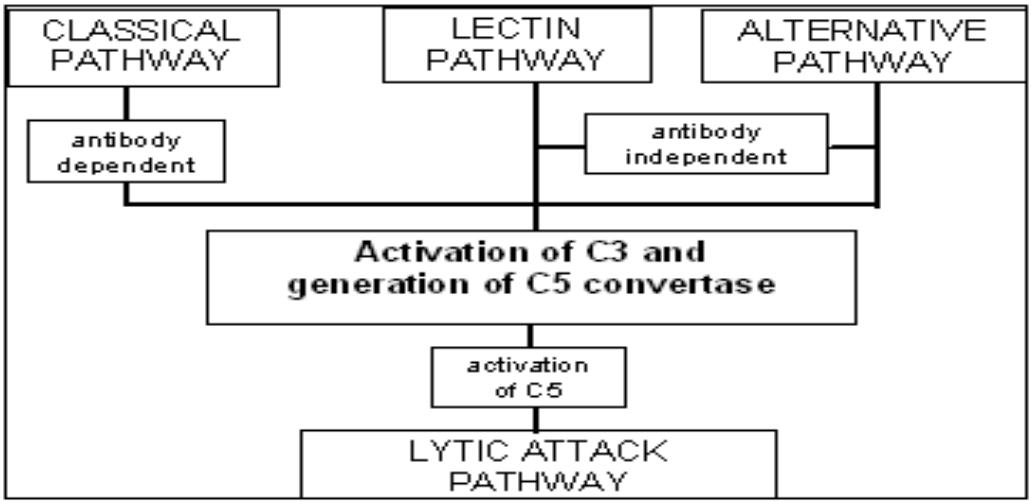
  This pattern of sequential activation produces an expanding cascade of activity (reminiscent of the operation of the blood clotting system.

  Complement proteins are activated by the antibodies IgM and IgG, which are located on the membrane of B cells. When these antibodies recognize and bind to antigens, a

conformational change of the IgM or IgG occurs. This means that its structure is slightly altered. This alteration results in the binding site for the first complement protein, Cl, to be exposed. Once Cl binds to the Ag-Ab complex, more complements proteins can also bind. Once complement proteins 1-6 bind to the Ag-Ab complex, this forms what is called the MAC complex. MAC stands for membrane attacking complex. This complex, as it implies, perforates, or punches holes in the target cell that is expressing the antigen. This allows surrounding water to flow into the cell and electrolytes to flow out of the cell resulting in cells death and therefore antigen death.

PATHAYS OF COMPLEMENT ACTIVATION

Complement activation can be divided into four pathways (figure 1): the classical pathway, the lectin pathway, the alternative pathway and the membrane attack (or lytic) pathway. Both classical and alternative pathways lead to the activation of C5 convertase and result in the production of C5b which is essential for the activation of the membrane attack pathway.



i) The Classical Pathway

The binding of antibody to its antigen often triggers the complement system through the so-called classical pathway. It can occur in solution or — as shown here — when the antibodies have bound to antigens on a cell surface.

The proteins of the classical pathway 1

C1 exists in blood serum as a molecular complex containing:

- 6 molecules of C1q
- 2 molecules of C1r

- 2 molecules of C1s

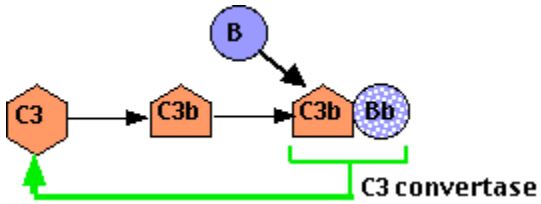
The constant regions of mu chains (IgM) and some gamma chains (IgG) contain a binding site for C1q. (A single molecule of IgM is enough to initiate the pathway. IgG is far less efficient, requiring many molecules to do so.)

- Binding of C1q activates **C1s** and **C1r**.
- Activated C1s (a serine protease) cleaves two serum proteins:
    - **C4** is cleaved into a large fragment
        - **C4b**, which binds covalently to sugar residues on cell-surface glycoproteins, and a smaller, inactive, fragment of
        - C4a which diffuses away.
    - **C2** is cleaved into
        - **C2b**, which binds noncovalently to a site on **C4b**, leaving a smaller, inactive, fragment of
        - C2a which diffuses away.
    - The complex of **C4b•2b** is called "**C3 convertase**" because it catalyzes the cleavage of **C3**. (C4b•2b is also a serine protease.)

C3

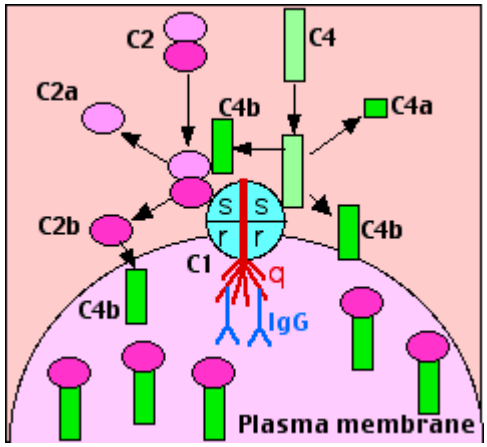
C3 is the most abundant protein of the complement system (~1.3 mg/ml). Because of its abundance and its **ability to activate itself** (by a mechanism described below), it greatly magnifies the response.

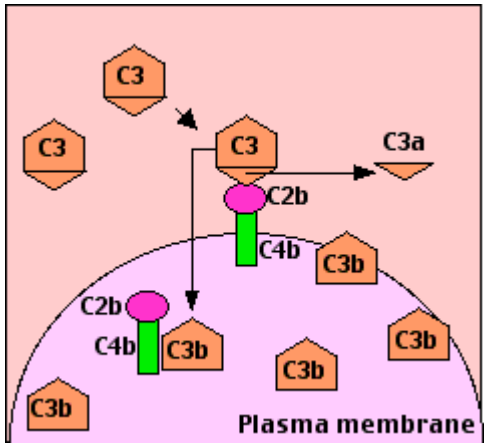
- **C4b•2b** cuts **C3** into two major fragments:
    - **C3b**, which binds covalently to glycoproteins scattered across the cell surface. Macrophages and neutrophils have receptors for **C3b** and can bind the C3b-coated cell or particle preparatory to phagocytosis. This effect qualifies C3b as an **opsonin**.
    - **C3a** This small fragment is released into the surrounding fluids. It can bind to receptors on basophils and mast cells triggering them to release their vasoactive contents (e.g., histamine). Because of the role of these materials in anaphylaxis, C3a is called an **anaphylatoxin**.
- Some of the C3b binds to molecules of C5 creating an allosteric change that exposes them to cleavage by C4b•2b (which is thus a "C3/C5 convertase".)



C5

Cleavage of C5 by the C3/C5 convertase initiates the assembly of a set of complement proteins that make up the membrane attack complex. (The membrane attack complex can also be formed by another C5 convertase produce by the "alternative pathway" [Link].)





The Membrane Attack Complex

Cleavage of C5 by the C3/C5 convertase, produces:

- **C5a**, which is released into the fluid surroundings where it
  - is a potent anaphylatoxin (like C3a)
  - is a chemotactic attractant for neutrophils
- **C5b**, which serves as the anchor for the assembly of a single molecule each of
  - C6;
  - C7, and
  - C8.
- The resulting complex **C5b•6•7•8** guides the polymerization of as many as 18 molecules of **C9** into a tube inserted into the lipid bilayer of the plasma membrane. This tube forms a channel allowing the passage of ions and small molecules. Water enters the cell by osmosis and the cell lyses.

The electron micrograph (courtesy of Drs. J. H. Humphrey and R. Dourmashkin) shows holes punched through the cell wall of the Gram-negative bacterium **Shigella dysenteriae** by the

terminal components of the complement system. (Some of the holes are larger than expected for C9 channels and probably were enlarged later by the action of lysozyme.)

LECTIN PATHWAY

The lectin pathway is very similar to the classical pathway. It is initiated by the binding of mannose-binding lectin (MBL) to bacterial surfaces with mannose-containing polysaccharides (mannans). Binding of MBL to a pathogen results in the association of two serine proteases, MASP-1 and MASP-2 (MBL-associated serine proteases). MASP-1 and MASP-2 are similar to C1r and C1s, respectively and MBL is similar to C1q. Formation of the MBL/MASP-1/MASP-2 tri-molecular complex results in the activation of the MASPs and subsequent cleavage of C4 into C4a and C4b. The C4b fragment binds to the membrane and the C4a fragment is released into the microenvironment. Activated MASPs also cleave C2 into C2a and C2b. C2a binds to the membrane in association with C4b and C2b is released into the microenvironment. The resulting C4bC2a complex is a C3 convertase, which cleaves C3 into C3a and C3b. C3b binds to the membrane in association with C4b and C2a and C3a is released into the microenvironment. The resulting C4bC2aC3b is a C5 convertase. The generation of C5 convertase is the end of the lectin pathway.

The biological activities and the regulatory proteins of the lectin pathway are the same as those of the classical pathway.

ALTERNATIVE PATHWAY

The **alternative pathway** of the complement system is an innate component of the immune system's natural defense against infections.

The alternative pathway is one of three complement pathways that opsonize and kill pathogens. The pathway is triggered when the C3b protein directly binds the microbe.

The alternative pathway begins with the activation of C3 and requires Factors B and D and Mg^{++} cation, all present in normal serum.

It is initiated by the spontaneous hydrolysis of C3, which is abundant in the plasma in the blood. "Tickover" occurs through the spontaneous cleavage of the thioester bond in C3 to form $C3(H_2O)$.

This change in shape allows the binding of plasma protein Factor B, which allows Factor D to cleave Factor B into Ba and Bb.

Bb remains part of the C3(H_2O) to form C3(H_2O)Bb. This complex is also known as a fluid-phase C3 convertase. This convertase, although only produced in small amounts, can cleave multiple C3 proteins into C3a and C3b.

The alternative pathway C3-convertase consists of the activated B and D factors, forming an unstable compound that can become stable after binding properdin, a serum protein.

After the creation of C3 convertase, the complement system follows the same path regardless of the means of activation (alternative, classical, or lectin). Binding of another C3b-fragment to the C3-convertase of the alternative pathway creates a C5-convertase analoguous to the lectin or classical pathway.

The C5-convertase of the alternative pathway consists of C3bBbC3b also referred to as $C3b_2Bb$ (instead of C4b2a3b in the other pathways).

MEMBRANE ATTACK (LYTIC) PATHWAY

C5 convertase from the classical (C4b2a3b), lectin (C4b2a3b) or alternative (C3bBb3b) pathway cleaves C5 into C5a and C5b. C5a remains in the fluid phase and the C5b rapidly associates with C6 and C7 and inserts into the membrane. Subsequently C8 binds, followed by several molecules of C9. The C9 molecules form a pore in the membrane through which the cellular contents leak and lysis occurs. Lysis is not an enzymatic process; it is thought to be due to physical damage to the membrane. The complex consisting of C5bC6C7C8C9 is referred to as the membrane attack complex (MAC).

C5a generated in the lytic pathway has several potent biological activities. It is the most potent anaphylotoxin. In addition, it is a chemotactic factor for neutrophils and stimulates the respiratory burst in them and it stimulates inflammatory cytokine production by macrophages. Its activities are controlled by inactivation by carboxypeptidase B (C3-INA).

Some of the C5b67 complex formed can dissociate from the membrane and enter the fluid phase. If this were to occur it could then bind to other nearby cells and lead to their lysis. The damage to bystander cells is prevented by Protein S (vitronectin). Protein S binds to soluble C5b67 and prevents its binding to other cells

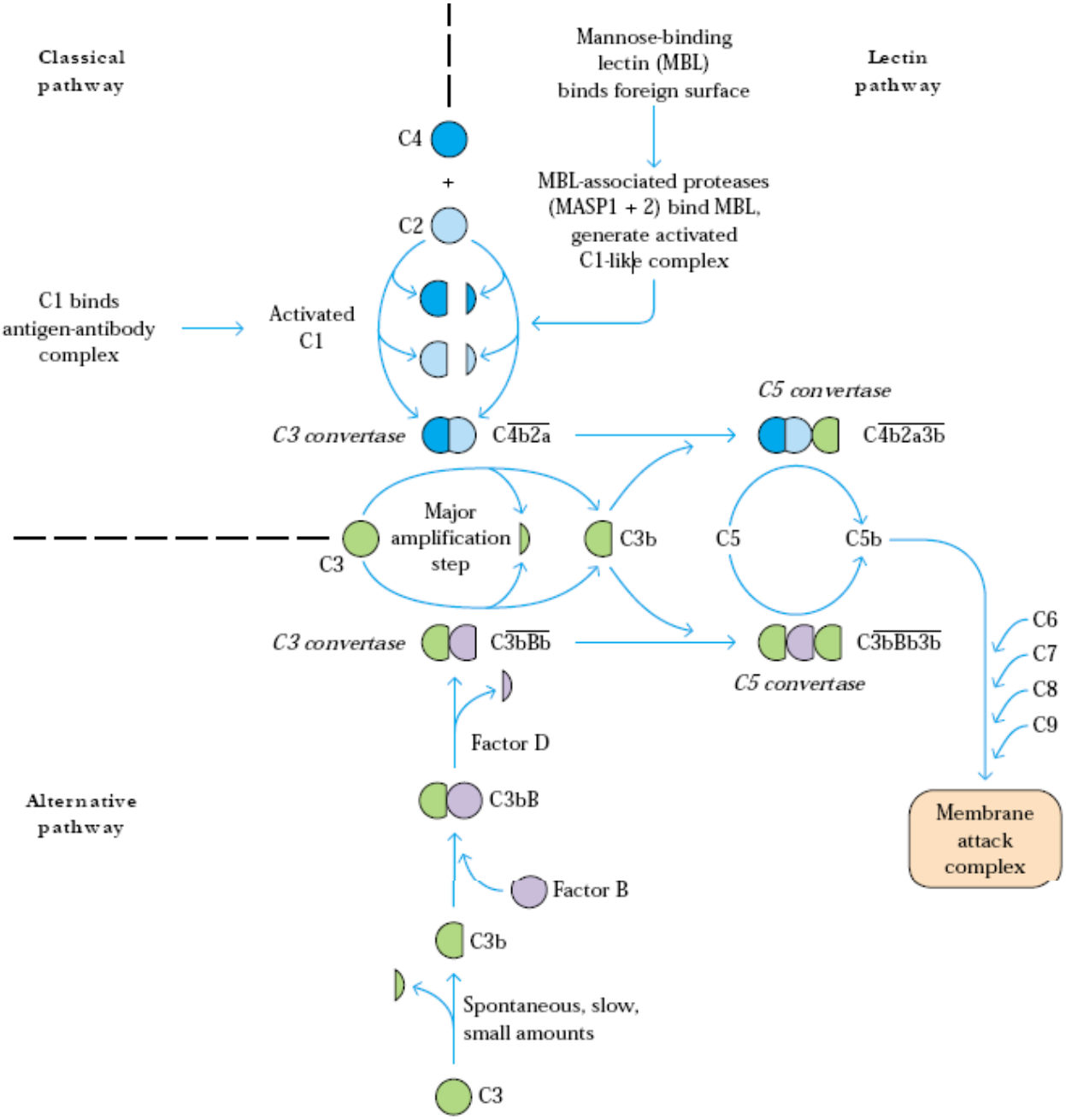


FIG: Overview of the complement activation pathways. The classical pathway is initiated when C1 binds to antigen-antibody complexes. The alternative pathway is initiated by binding of spontaneously generated C3b to activating surfaces such as microbial cell walls. The lectin pathway is initiated by binding of the serum protein MBL to the surface of a pathogen. All three pathways generate C3 and C5 convertases and bound C5b, which is converted into a mem brane-attack complex (MAC) by a common sequence of terminal reactions. Hydrolysis of C3 is the major amplification step in all path-ways, generating large amounts of C3b, which forms part of C5

con-vertase. C3b also can diffuse away from the activating surface and bind to immune complexes or foreign cell surfaces, where it func-tions as an opsonin.

Biological Consequences of Complement Activation

Activation of complement results in the production of several biologically active molecules which contribute to resistance, anaphylaxis and inflammation.

Complement serves as an important mediator of the humoral response by amplifying the response and converting it into an effective defense mechanism to destroy invading microorganisms. The MAC mediates cell lysis, while other complement components or split products participate in the inflammatory response, opsonization of antigen, viral neutralization, and clearance of immune complexes

The Membrane-Attack Complex Can Lyse a Broad Spectrum of Cells

The membrane-attack complex formed by complement acti-vation can lyse gram-negative bacteria, parasites, viruses, erythrocytes, and nucleated cells.

Cleavage Products of Complement Components Mediate Inflammation

various peptides generated during formation of the MAC play a decisive role in the de-velopment of an effective inflammatory response . The smaller fragments resulting from complement cleavage, C3a, C4a, and C5a, called **anaphylatoxins,** bind to receptors on mast cells and blood basophils and induce de-granulation, with release of histamine and other pharmaco-logically active mediators. The anaphylatoxins also induce smooth-muscle contraction and increased vascular perme-ability. Activation of the complement system thus results in influxes of fluid that carries antibody and phagocytic cells to the site of antigen entry.

C3a, C5a, and C5b67 can each induce monocytes and neutrophils to adhere to vascular endothelial cells, ex-travasate through the endothelial lining of the capillary, and migrate toward the site of complement activation in the tis-sues. C5a is most potent in mediating these processes, effec-tive in picomolar quantities.

C3b and C4b Binding Facilitates Opsonization

C3b is the major **opsonin** of the complement system, al-though C4b and iC3b also have opsonizing activity. The am-plification that occurs with C3 activation results in a coating of C3b on immune complexes and particulate antigens. Phagocytic cells, as well as some other cells, express comple-ment receptors (CR1, CR3, and CR4) that bind C3b, C4b, or iC3b (see Table 13-4). Antigen coated with C3b binds to cells bearing CR1. If the cell is a phagocyte (e.g., a neutrophil, monocyte, or macrophage), phagocytosis will be enhanced .

The Complement System Also Neutralizes Viral Infectivity

For most viruses, the binding of serum antibody to the re-peating subunits of the viral structural proteins creates par-ticulate immune complexes ideally suited for complement activation by the classical pathway. Some viruses (e.g., retro-viruses, Epstein-Barr virus, Newcastle disease virus, and rubella virus) can activate the alternative, lectin, or even the classical pathway in the absence of antibody.

The complement system mediates viral neutralization by a number of mechanisms. Some degree of neutralization is achieved through the formation of larger viral aggregates, simply because these aggregates reduce the net number of in-fectious viral particles.

The Complement System Clears Immune Complexes from Circulation

The importance of the complement system in clearing im-mune complexes is seen in patients with the autoimmune dis-ease systemic lupus erythematosus (SLE). These individuals produce large quantities of immune complexes and suffer tis-sue damage as a result of complement-mediated lysis and the induction of type II or type III hypersensitivity (see Chapter 16). Although complement plays a significant role in the devel-opment of tissue damage in SLE, the paradoxical finding is that deficiencies in C1, C2, C4, and CR1 predispose an indi-vidual to SLE; indeed, 90% of individuals who completely lack C4 develop SLE.

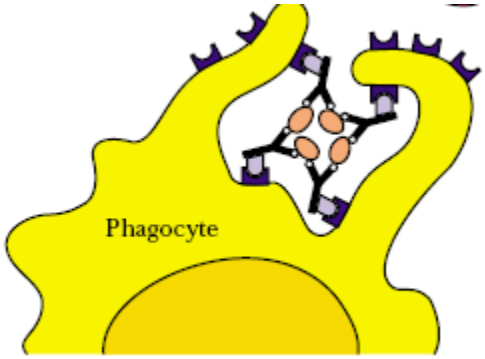


Fig: Clearance of circulating immune complexes by reaction with receptors for complement products on erythrocytes and removal of these complexes by receptors on macrophages in the liver and spleen.

Disorders of the Complement System

With so many proteins involved, it is not surprising that inherited deficiencies of one or another are sometimes encountered in humans. Four examples:

Homozygous deficiencies in any of the early components of the classical pathway (C1q, C1r, C1s, C4, and C2) exhibit similar symptoms, notably a marked increase in immune-complex diseases such as systemic lupus erythe-matosus, glomerulonephritis, and vasculitis.Individuals with such complement deficien-cies may suffer from recurrent infections by pyogenic (pus-forming) bacteria such as streptococci and staphylococci. These organisms are gram-positive and therefore resistant to the lytic effects of the MAC.

Deficiencies in factor D and properdin—early components of the alternative pathway—appear to be associated with *Neisseria* infections but not with immune-complex disease.

Patients with C3 deficiencies have the most severe clinical manifestations, reflecting the central role of C3 in activation of C5 and formation of the MAC.

- . **C9**. Another curiosity: most people who cannot make C9 have no more of a problem with bacterial infections than those who can. Laboratory studies suggest that the **C5b•6•7•8** complex by itself is able to lyze bacteria although not as efficiently as C9.
- **C1INH**. A deficiency of C1INH produces **hereditary angioedema**. Patients are at risk of occasional explosive triggering of the complement system. The massive release of anaphylatoxins (C3a, C5a) may cause dangerous swelling (edema) of the airways, as well as of the skin and intestine.
- **Paroxysmal nocturnal hemoglobinuria** (PNH) is a rare, acquired, life-threatening disease of the blood characterized by destruction of red blood cells by the complement system, a part of the body's innate immune system. This destructive process occurs due to the presence of defective surface proteins on the red blood cell, which normally function to inhibit such immune reactions. Since the complement cascade attacks the red blood cells within the blood vessels of the circulatory system, the red blood cell destruction (hemolysis) is considered an intravascular hemolytic anemia.

UNIT III

1	---------- Reactions are responsible for tackling invasion of foreign antigens.	Antigen	Immune	Antibody	Antigen- antibody	Immune
2	The visible feature of hypersensitive states is the inflammation developed at the site of	Antigen	Antibody	Antigen- antibody	Foreign antigens.	Antigen- antibody
3	Type I is acute in nature and mediated by	IgM	IgA	Ig D	IgE	IgE
4	Which type of hypersensitivity resulting mainly from blood transfusion methods.	Type I	Type II	Type III	TypeIV	Type II
5	Which hypersentivity involves formationof complexes btwn Ab &Ag lodged capillaries	Type I	Type II	Type III	TypeIV	Type III
6	Which type hypersentivity involves in delayed type of reaction mediated by lymphocytes.	Type I	Type II	Type III	TypeIV	TypeIV
7	-------- release causes peripheral vasodilation	Histamine	anaphylaxis	Edema	erytherma.	Histamine
8	Histamine is a	amine	bio amine	di amine	tri amine.	bio amine
9	In anaphlyaxis ,antibodies are only the indirect cause of the	Influx	lnfection	lnflammation	Vasodilation	lnflammation
10	The antibodies are collectively called	reagins	pre-reagins	exo-reagins	endo-reagins	reagins
11	Influx of Ca_2+ ions activate phospholipase	A_1	A_2	A_3	A_4	A_2
12	The prostagladin pathway is initiated by the enzyme	oxygenase	non-cyclooxygenase	cyclo oxygenase	endooxygenase	cyclo oxygenase
13	Prostagladin biosynthesis may be blocked by	aspirin	salicylate	salicylic acid	streptomycin	aspirin
14	The PGE_1 also enhances immunogenecity of	liver cells	spleen cells	mast cells	lymph cells	mast cells
15	Antigens which give rise to allergic reactions are called	allergic	allergic response	allergens	allergen response	allergens
16	ABO blood group antigens are	Lipids	Glycolipids	Oligosaccharides	galactose	Glycolipids
17	The T cell subsets operate by releasing chemical substances called	lymphocytes	lymphokines	lymphoma	pre-lymphocytes	lymphokines
18	C_3 has molecular weight of	190KD	192KD	194KD	195KD	195KD
19	Activation of first complement component is triggered by	Ag complex	Antibody complex	Ag-Ab complex	Cross-linking	Ag-Ab complex
20	The…… region has key role in activating the complement	Fc	fab	C_1	C_3	Fc
21	The complement system can activate phagocytes , a phenomenon called as	phagocytosis	opsonisation	Immune response	endotoxins	opsonisation
22	Classical pathway is a ……dependant	Ag	Ag-Ab	Ab	C_3	Ab
23	……is the most potent antibody to activate classical pathway	IgG	IgM	IgG_1	IgG_3	IgM
24	The alternative pathway starts at	C_3	C3a	C3b	C5a	C_3
25	SLE disease is caused by the deficiency of	C1	C1p	C1 q	C1 r	C1 q
26	Meningococcal infections is caused by the deficiency of	C5	C6	C7	C9	C6
27	Gococcal infections is caused by the deficiency of	C_5	C_6	C_7	C_9	C_5
28	---------- has proved to be chemo tactic	C_5	C_{5a}	C_6	C_9	C_{5a}
29	Bacteria are coated with ---------- molecules.	Antigen	divalent	Antibody	Antigen- antibody	Antibody
30	Which is the agent that enhances phagocytosis by immune adherence are	C3	C3a	C3a1	C3b	C3b
31	Histocompability antigen found on the surface of	mast cell	nucleated cell	spleen cell	stem cell	nucleated cell
32	Histocompability antigen are highly	Monomer	Dimer	Polymorphic	multimorphic	Polymorphic
33	All MHC genes are	silent	Activate	intermediate	suppress.	silent
34	Which region in HLA complex contains several different genes	D	R	DR	HLA-D	DR
35	HLA-D antigens are	hetero dimer	dimer	homodimer	hexodimer.	hetero dimer
36	The MHC genes coding for …….. antigens in the mouse	H	H-1	H-2	H-3	H-2
37	HLA genes are	dominant	recessive	co-recessive	co-dominant	co-dominant
38	In MHC class II Ag, which one is found on surface of B cells,macrophages &active cells	Ag	Ab	Ag-Ab	B cell Ag	Ag
39	The human MHC region has …….coding sequence	1	2	3	4	3
40	In mouse MHC region has	H-2K	H-2D	H-K&H-D	H2K-H-2D	H2K-H-2D
41	HLA locus has about ……different alleles.	23	24	25	26	25
42	In mouse H-2 region has more than ……different alleles.	210	220	230	200	200
43	Class I antigens are	glycans	glycoproteins	proteins	peptides	glycoproteins
44	In MHC classII antigen α chain has its molecular weight	30,000	29,000	31,000	32,000	32,000
45	In MHC classII antigen ß chain has its molecular weight	30,000	29,000	31,000	32,000	29,000
46	MHC classI antigens cause cell mediated lysis of……infected cell.	bacteria	fungi	virus	algae	virus
47	MHC classII antigens play an important role in	graft	autograft	isograft	allograft	graft
48	Blood transfusion,is a good example of transplantation of	liver	tissue	heart	kidney	tissue
49	grafting of one part to another in same individual is	xeno graft	autograft	isograft	allograft	isograft
50	Trasplantation of two individuals from identical twins are called as	xeno graft	autograft	isograft	allograft	isograft
51	Transplantation of tissue between two animals of same species	autograft	isograft	allograft	xenograft	allograft
52	Grafts between two individuals of different species is	xeno graft	autograft	isograft	allograft	xeno graft
53	Humoral Ab play an important role in……rejection	hyper acute	acute	graft	delayed	graft
54	Syngeneic are also known as	Isograft	allograft	autograft	xenogaft	Isograft
55	Acute rejection occurs usually in about	7 days	8 days	9 days	10 days	10 days
56	Antigen from donor kidney is processed by a	macrophage	monocyte	mast cell	lymphocytes	macrophage
57	Exposure to radiation causes depletion of	macrophage	monocyte	mast cell	lymphocytes	lymphocytes
58	Bone-marrow grafting leads to	leukemia	hypertension	Wilson's disease	Renal failure	leukemia
59	Graft rejection is controlled by	cytotoxic	cytokines	lymphoma	lymphocytes	cytotoxic
60	According to Sir Peter Medawar,graft rejection is mainly due to…….reactions	Ag-Ab	immune	cytotoxic	Ab reaction	cytotoxic

KARPAGAM ACADEMY OF HIGHER EDUCATION
(Deemed University Established Under Section 3 of UGC Act 1956)
Coimbatore - 641021.
(For the candidates admitted from 2015 onwards)
DEPARTMENT OF BIOCHEMISTRY

SUBJECT : IMMUNOLOGY
SEMESTER : V
SUBJECT CODE : 15BCU503 CLASS : III B.Sc.BC

UNIT IV - COURSE MATERIAL

UNIT IV- *Transplantation Immunology*
Transplant-Mechanism of Allograft rejection; Auto immune diseases- Rheumatoid arthritis, myasthenia gravis, Graves's disease, Systemic lupus erythematosis. Vaccination- passive and active. Preparation of live and attenuated vaccines, novel vaccines.

Transplantation

Types of graft (figure 1)

- **Xenograft**
  Grafts between members of different species (also known as heterologous, xenogeneic or heterografts)
- **Allograft**
  Grafts between two members of the same species (also known as allogeneic or homograft)
- **Isograft**
  Grafts between members of the same species with identical genetic makeup (identical twins or inbred animals)

Haplotype
A group of genes on a single chromosome

PRINCIPLES OF TRANSPLANTATION

An immunocompetent host recognizes the foreign antigens on grafted tissues (or cells) and mounts an immune response which results in rejection. On the other hand, if an immunocompromised host is grafted with foreign immunocompetent lymphoid cells, the immunoreactive T-cells in the graft recognize the foreign antigens on the host tissue, leading to damage of the host tissue.

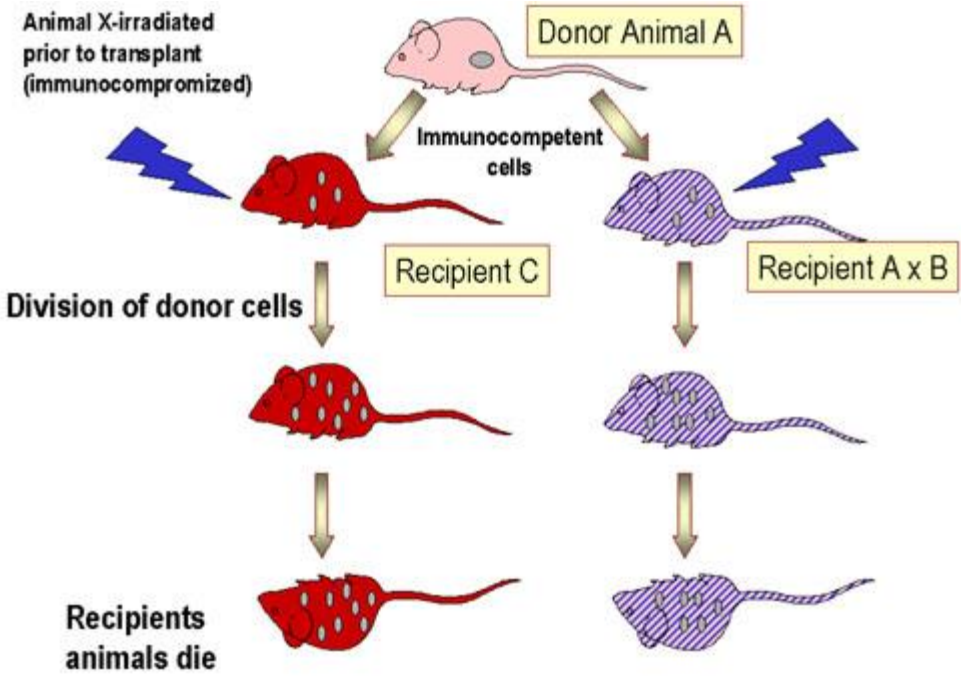
Host-versus-graft-reaction

he duration of graft survival follows the order, xeno- < allo- < iso- = auto- graft. The time of rejection also depends on the antigenic disparity between the donors and recipient. MHC antigens are the major contributors in rejection, but the minor histocompatibility antigens also play a role. Rejection due to disparity in several minor histocompatibility antigens may be as quick or quicker than rejection mediated by an MHC antigen. As in other immune responses, there is immunological memory and secondary response in graft rejection. Thus, once a graft is rejected by a recipient, a second graft from the same donor, or a donor with the same histocompatibility antigens, will be rejected in a much shorter time.

Graft-versus-host (GVH) Reaction

Histocompatible lymphoid cells, when injected into an immunocompromised host, are readily accepted. However, the immunocompetent T lymphocytes among the grafted cells recognize the alloantigens and, in response, they proliferate and progressively cause damage to the host tissues and cells. This condition is known as graft-versus-host (GVH) disease (figure 3) and is often fatal.

Common manifestations (figure 4) of GVH reaction are diarrhea, erythema, weight loss, malaise, fever, joint pains, etc. and ultimately death.



Transplant rejection occurs when a transplanted organ or tissue is not accepted by the body of the transplant recipient. This is explained by the concept that the immune system of the recipient

attacks the transplanted organ or tissue. This is expected to happen, because the immune system's purpose is to distinguish foreign material within the body and attempt to destroy it, just as it attempts to destroy infecting organisms such as bacteria and viruses. When possible, transplant rejection can be reduced through serotyping to determine the most appropriate donor-recipient match and through the use of immunosuppressant drugs.

ALLOGRAFT REJECTION

The clinical significance of the MHC is realized in organ transplantation. Cells and tissues are routinely transplanted as a treatment for a number of diseases. However, reaction of the host against allo-antigens of the graft (HVG) results in its rejection and is the major obstacle in organ transplantation. The rejection time of a graft may vary with the antigenic nature of the graft and the immune status of the host and is determined by the immune mechanisms involved (Figure 8 and Table 1).

Hyper-acute rejection

This occurs in instances when the recipient has preformed high titer antibodies. A graft may show signs of rejection within minutes to hours due to immediate reaction of antibodies and complement.

Accelerated (2nd set; secondary) rejection

Transplantation of a second graft, which shares a significant number of antigenic determinants with the first one, results in a rapid (2 - 5 days) rejection. It is due to presence of T-lymphocytes sensitized during the first graft rejection. Accelerated rejection is mediated by immediate production of lymphokines, activation of monocytes and macrophages, and induction of cytotoxic lymphocytes.

Acute (1st set; primary) rejection

The normal reaction that follows the first grafting of a foreign transplant takes 1 - 3 weeks. This is known as acute rejection and is mediated by T lymphocytes sensitized to class I and class II antigens of the allograft, elicitation of lymphokines and activation of monocytes and macrophages.

Chronic rejection

Some grafts may survive for months or even years, but suddenly exhibit symptoms of rejection. This is referred to as chronic rejection, the mechanism of which is not entirely clear. The hypotheses are that this may be due infection, causes which led to failure of the first organ, loss of tolerance induced by the graft, etc.

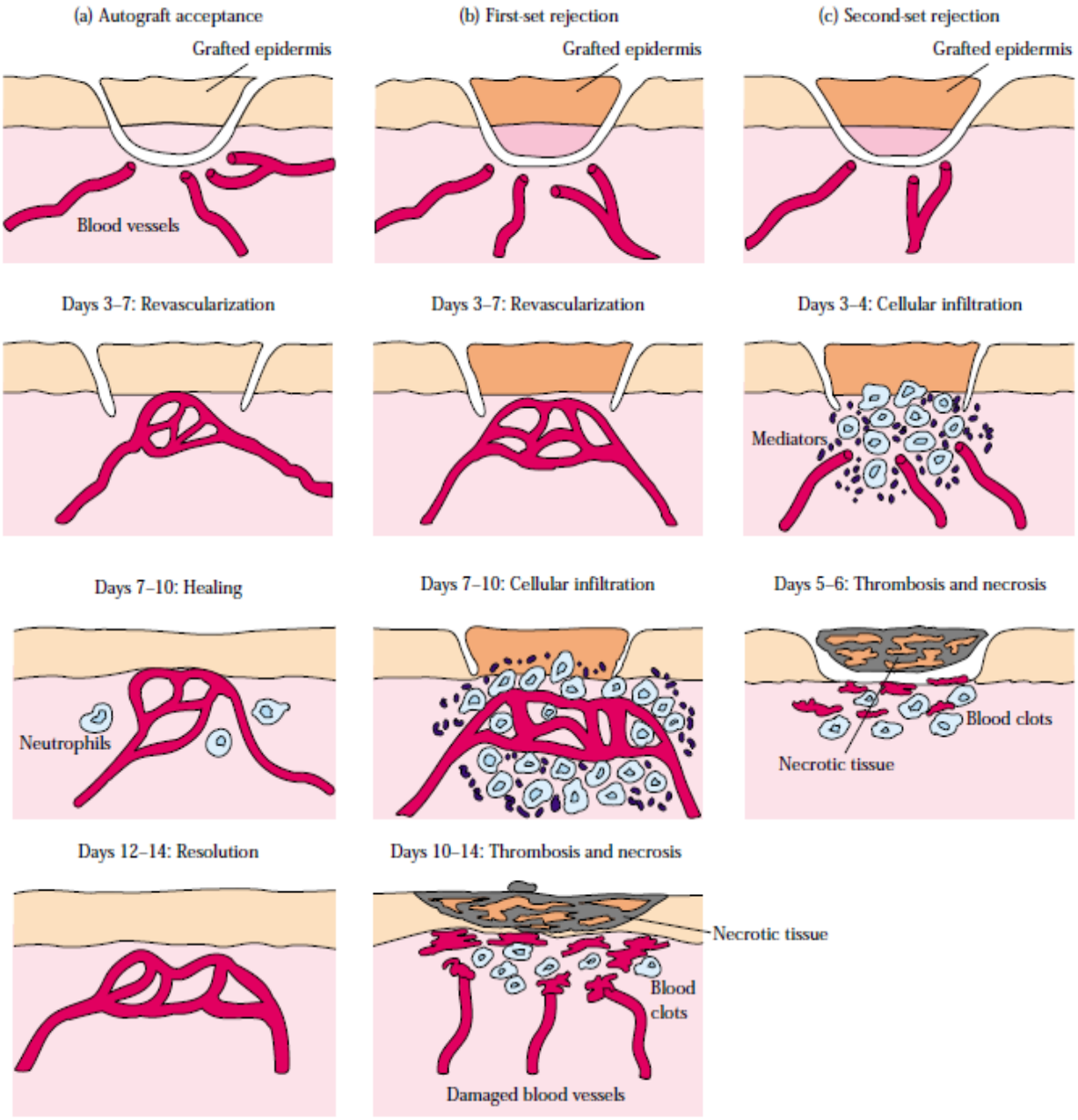


Fig: Schematic diagram of graft rejection and acceptance

REJECTION MECHANISMS

Graft rejection is caused principally by a cell-mediated im-mune response to alloantigens (primarily, MHC molecules) expressed on cells of the graft. Both delayed-type hypersensi-tive and cell-mediated cytotoxicity reactions have been im-plicated. The process of graft rejection can be divided into two stages: (1) a sensitization phase, in which antigen-reactive lymphocytes

of the recipient proliferate in response to allo-antigens on the graft, and (2) an effector stage, in which im-mune destruction of the graft takes place.

i) Sensitization Stage

During the sensitization phase, $CD4^+$ and $CD8^+$ T cells rec-ognize alloantigens expressed on cells of the foreign graft and proliferate in response. Both major and minor histo-compatibility alloantigens can be recognized. In general, the response to minor histocompatibility antigens is weak, al-though the combined response to several minor differences can sometimes be quite vigorous. The response to major histo-compatibility antigens involves recognition of both the donor MHC molecule and an associated peptide ligand in the cleft of the MHC molecule. The peptides present in the groove of allogeneic class I MHC molecules are derived from proteins synthesized within the allogeneic cell. The peptides present in the groove of allogeneic class II MHC molecules are gener-ally proteins taken up and processed through the endocytic pathway of the allogeneic antigen-presenting cell.

A host T_H cell becomes activated when it interacts with an antigen-presenting cell (APC) that both expresses an appro-priate antigenic ligand–MHC molecule complex and pro-vides the requisite co-stimulatory signal. Depending on the tissue, different populations of cells within a graft may func-tion as APCs. Because dendritic cells are found in most tis-sues and because they constitutively express high levels of class II MHC molecules, dendritic cells generally serve as the major APC in grafts. APCs of host origin can also migrate into a graft and endocytose the foreign alloantigens (both major and minor histocompatibility molecules) and present them as processed peptides together with self-MHC mole-cules.

In some organ and tissue grafts (e.g., grafts of kidney, thy-mus, and pancreatic islets), a population of donor APCs called *passenger leukocytes* has been shown to migrate from the graft to the regional lymph nodes. These passenger leuko-cytes are dendritic cells, which express high levels of class II MHC molecules (together with normal levels of class I MHC molecules) and are widespread in mammalian tissues, with the chief exception of the brain. Because passenger leuko-cytes express the allogeneic MHC antigens of the donor graft, they are recognized as foreign and therefore can stimulate immune activation of T lymphocytes in the lymph node. In some experimental situations, the passenger cells have been shown to induce tolerance to their surface antigens by dele-tion of thymic T-cell populations with receptors specific for them. Consistent with the notion that exposure to donor cells can induce tolerance are data showing that blood tran-fusions from the donor prior to transplantation can aid ac-ceptance of the graft.

Passenger leukocytes are not the only cells involved in im-mune stimulation. For example, they do not seem to play any role in skin grafts. Other cell types that have been implicated in alloantigen presentation to the immune system include

Langerhans cells and endothelial cells lining the blood ves-sels. Both of these cell types express class I and class II MHC antigens.

Recognition of the alloantigens expressed on the cells of a graft induces vigorous T-cell proliferation in the host. This proliferation can be demonstrated in vitro in a mixed-lymphocyte reaction (see Figure 21-4c). Both dendritic cells and vascular endothelial cells from an allogeneic graft induce host T-cell proliferation. The major proliferating cell is the $CD4^+$ T cell, which recognizes class II alloantigens directly or alloantigen peptides presented by host antigen-presenting cells. This amplified population of activated T_H cells is thought to play a central role in inducing the various effector mechanisms of allograft rejection.

ii)Effector Stage

A variety of effector mechanisms participate in allograft re-jection (Figure 21-6). The most common are cell-mediated reactions involving delayed-type hypersensitivity and CTL-mediated cytotoxicity; less common mechanisms are antibody-plus-complement lysis and destruction by antibody-dependent cell-mediated cytotoxicity (ADCC). The hallmark of graft rejection involving cell-mediated reactions is an influx of T cells and macrophages into the graft. Histologically, the in-filtration in many cases resembles that seen during a delayed-type hypersensitive response, in which cytokines produced by T_{DTH} cells promote macrophage infiltration (see Figure 14-15). Recognition of foreign class I alloantigens on the graft by host $CD8^+$ cells can lead to CTL-mediated killing (see Figure 14-4). In some cases, $CD4^+$ T cells that function as class II MHC–restricted cytotoxic cells mediate graft rejection.

In each of these effector mechanisms, cytokines secreted by T_H cells play a central role (see Figure 21-6). For example, IL-2, IFN- , and TNF- have each been shown to be impor-tant mediators of graft rejection. IL-2 promotes T-cell pro-liferation and generally is necessary for the generation of effector CTLs (see Figure 14-1). IFN- is central to the devel-opment of a DTH response, promoting the influx of macro-phages into the graft and their subsequent activation into more destructive cells. TNF- has been shown to have a di-rect cytotoxic effect on the cells of a graft. A number of cyto-kines promote graft rejection by inducing expression of class I or class II MHC molecules on graft cells. The interferons (, , and), TNF- , and TNF- all increase class I MHC ex-pression, and IFN- increases class II MHC expression as well. During a rejection episode, the levels of these cytokines increase, inducing a variety of cell types within the graft to express class I or class II MHC molecules. In rat cardiac allo-grafts, for example, dendritic cells are initially the only cells that express class II MHC molecules. However, as an allograft reaction

begins, localized production of IFN- in the graft induces vascular endothelial cells and myocytes to express class II MHC molecules as well, making these cells targets for CTL attack.

Rejection is an adaptive immune response via cellular immunity (mediated by killer T cells inducing apoptosis of target cells) as well as humoral immunity (mediated by activated B cells secreting antibody molecules), though the action is joined by components of innate immune response (phagocytes and soluble immune proteins). Different types of transplanted tissues tend to favor different balances of rejection mechanisms.

Humoral immunity in rejection

Developed through an earlier primary exposure that primed adaptive immunity—which matured before the transplant occurring as secondary exposure—a transplant recipient can bear specific antibody crossreacting with donor tissue. This is typical after earlier mismatching among A/B/O blood types. Then components of innate immunity—soluble immune proteins called complement and innate immune cells called phagocytes—inflame and destroy the transplanted tissue.

An antibody molecule, secreted by an activated B cell, then called plasma cell, is a soluble immunoglobulin (Ig) whose constituent unit is configured like the letter *Y*: the two arms are the Fab regions and the single stalk is the Fc region. Each Fab tip is the paratope, which ligates (binds) a cognate (matching) molecular sequence as well as its 3D shape (its conformation), altogether called epitope, within a specific antigen.

When the paratope of Ig class *gamma* (IgG) ligates its epitope, IgG's Fc region conformationally shifts and can host a complement protein, initiating the complement cascade that terminates by punching a hole in a cell membrane. With so many holes punched fluid rushes into the cell and ruptures it. Molecular motifs of necrotic cell debris are recognized as damage associated molecular patterns (DAMPs) when they ligate Toll-like receptors (TLRs) on membranes of innate immune cells, which phagocytes are thereby activated to secrete proinflammatory cytokines recruiting more phagocytes to traffic to the area by sensing the concentration gradient of the secreted cytokines (chemotaxis). IgG's Fc region also enables opsonization by a phagocyte—such as neutrophils in blood and macrophages in tissues—which attains improved uptake of cell debris and tissue by seizing the IgG molecule's Fc stalk.

Cellular immunity in rejection

Transplanted organs are often acquired from a cadaver—usually a host who had succumbed to trauma—and the tissues had already sustained ischemia or inflammation. Dendritic cells (DCs) of the donor tissue migrate to the recipient's peripheral lymphoid tissue—lymphoid follicles and lymph nodes—and present the donor's *self peptides* to the recipient's naive helper T cells. Primed toward these allogeneic HLA peptides, the helper T cells effect immunomemory at either 1) the donor's self peptides, 2) the allogeneic HLA molecules, or 3) both.

The primed helper T cells establish alloreactive killer T cells whose CD8 receptors dock to the transplanted tissue's MHC class I molecules presenting self peptides, whereupon the T cell receptors (TCRs) of the killer T cells recognize their epitope—self peptide now coupled within MHC class I molecules—and transduce signals into the target cell prompting its programmed cell death by apoptosis.

When the CD4 receptors of helper T cells dock to their hosts, MHC class II molecules, expressed by select cells, their own TCRs—the paratope—might recognize their matching epitope being presented, and thereupon approximate the secretion of cytokines that had prevailed during their priming event, an aggressively proinflammatory balance of cytokines.

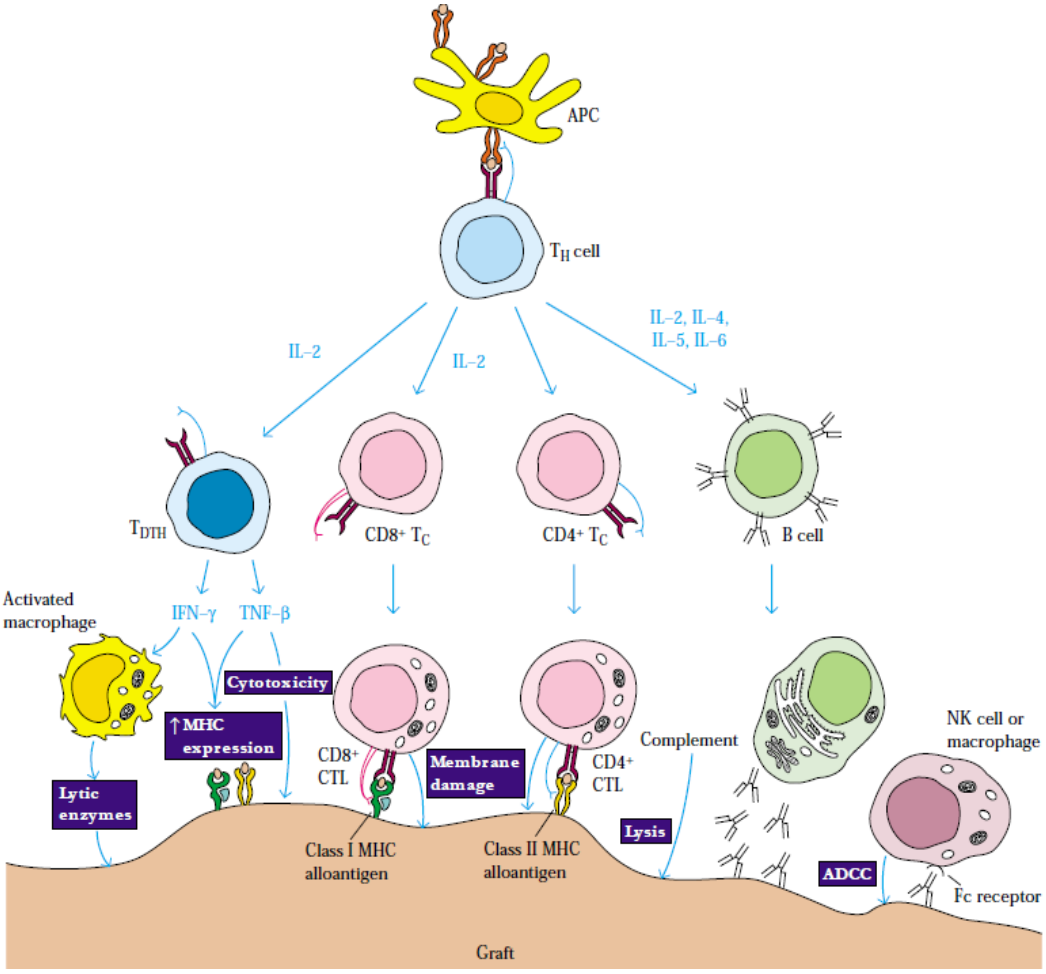


Fig:Effector mechanism involved in rejection

Rejection detection

The laboratory pathologist generally seeks three main histological signs: (1) infiltrating T cells, perhaps accompanied by infiltrating eosinophils, plasma cells, and neutrophils, particularly in

telltale ratios, (2) structural compromise of tissue anatomy, varying by tissue type transplanted, and (3) injury to blood vessels.

Rejection treatment

Hyperacute rejection manifests severely and within minutes, and so treatment is immediate: removal of the tissue. **Chronic rejection** is generally considered irreversible and poorly amenable to treatment—only retransplant generally indicated if feasible—though inhaled cyclosporine is being investigated to delay or prevent chronic rejection of lung transplants. **Acute rejection** is treated with one or multiple of a few strategies like immuno supressive therapy, antibobody based treatments and blood transfer

Rejection mechanisms

Rejection is an adaptive immune response and is mediated through both T cell mediated and humoral immune (antibodies) mechanisms. The number of mismatched alleles determines the speed and magnitude of the rejection response. Different mechanisms tend to act against different grafts.

Organ/tissue	Mechanism
Blood	Antibodies (isohaemagglutinins)
Kidney	Antibodies, CMI
Heart	Antibodies, CMI
Skin	CMI
Bonemarrow	CMI
Cornea	Usually accepted unless vascularised, CMI

CMI=Cell mediated immunity

Treatment of rejection

Chronic transplant rejection is irreversible and cannot be treated effectively. Treatments with inhaled ciclosporin are being investigated as a means to delay or prevent chronic rejection of the lungs. At present the only definitive treatment is re-transplantation, if patients can be re-allocated and if donors are available.

Acute transplant rejection can be treated using chemotherapeutic drugs designed to suppress the immune system (see list below). Acute rejection is normally treated initially with a short course of high-dose corticosteroids, which is usually sufficient. If this is not enough, the course can be repeated or a *triple therapy* regimen can be used, consisting of a corticosteroid plus a calcineurin inhibitor and an anti-proliferative agent. Antibodies against specific components of the immune system can be added to this regimen, especially for high-risk patients. mTOR inhibitors can be used in selected patients, where calcineurin inhibitors or steroids are contraindicated. Acute rejection refractory to these treatments may require blood transfusions to remove antibodies against the transplant.

If a bone marrow transplant can be performed, the transplant recipient's immune system can be replaced with the donor's immune system, thus enabling the recipient's body to accept the new organ without risk of rejection. This requires that the bone marrow, which produces the immune cells, be from the same person as the organ donation (or an identical twin or a clone). There is a risk of graft versus host disease (GVHD) in which the lymphoid cells co-injected with the bone marrow transplant recognize the host tissues as foreign and attack and destroy them accordingly.

Immunosuppressive drugs used to treat transplant rejection

- **Calcineurin inhibitors**
  - Ciclosporin
  - Tacrolimus
- **mTOR inhibitors**
  - Sirolimus
  - Everolimus
- **Anti-proliferatives**
  - Azathioprine
  - Mycophenolic acid
- **Corticosteroids**
  - Prednisolone
  - Hydrocortisone
- **Antibodies**
  - Monoclonal anti-IL-2Rα receptor antibodies
    - Basiliximab
    - Daclizumab
  - Polyclonal anti-T-cell antibodies
    - Anti-thymocyte globulin (ATG)
    - Anti-lymphocyte globulin (ALG)
  - Monoclonal anti-CD20 antibodies
    - Rituximab

The monoclonal anti-T cell antibody OKT3 was formerly used in the prevention of rejection, and is occasionally used in treatment of severe acute rejection, but has fallen out of common use due to the severe cytokine release syndrome and late post-transplant lymphoproliferative disorder,

which are both commonly associated with use of OKT3; in the United Kingdom it is available on a named-patient use basis only.

Current diagnosis of organ rejection following transplantation relies on tissue biopsy, which is not ideal due to sampling limitations and risks associated with the invasive procedure. Cellular MRI of in vivo labeled immune cells offers a noninvasive approach to detect and monitor graft rejection after solid organ transplantation. Clinical application of a reliable and noninvasive technique to detect the early signs of graft rejection will improve not only the therapeutic treatment of transplant patients but also improve their quality of life. (Magnetic Resonance in Medicine (2011)

Auto immune diseases- Rheumatoid arthritis, myasthenia gravis.

Autoimmune diseases

Autoimmune diseases arise from an overactive immune response of the body against substances and tissues normally present in the body. In other words, the body actually attacks its own cells. The immune system mistakes some part of the body as a pathogen and attacks it. This may be restricted to certain organs (e.g. in chagas disease) or involve a particular tissue in different places (e.g. Goodpasture's disease which may affect the basement membrane in both the lung and the kidney).

The treatment of autoimmune diseases is typically with immunosuppression—medication which decreases the immune response.

The cause of autoimmune diseases is unknown, but it appears that there is an inherited predisposition to develop autoimmune disease in many cases. In a few types of autoimmune disease (such as rheumatic fever), a bacteria or virus triggers an immune response, and the antibodies or T-cells attack normal cells because they have some part of their structure that resembles a part of the structure of the infecting microorganism.

EFFECTOR MECHANISMS IN AUTOIMMUNE DISEASES

Both antibodies and effector T cells can be involved in the damage in autoimmune diseases.

GENERAL CLASSIFICATION

Autoimmune diseases are generally classified on the basis of the organ or tissue involved. These diseases may fall in an organ-specific category in which the immune response is directed against antigen(s) associated with the target organ being damaged or a non-organ-specific category in which the antibody is directed against an antigen not associated with the target organ. The antigen involved in most autoimmune diseases is evident from the name of the disease .

Autoimmune disorders fall into two general types: those that damage many organs (systemic autoimmune diseases) and those where only a single organ or tissue is directly damaged by the autoimmune process (localized). However, the distinctions become blurred as the effect of localized autoimmune disorders frequently extends beyond the targeted tissues, indirectly affecting other body organs and systems. Some of the most common types of autoimmune disorders include:

Systemic Autoimmune Diseases

- Rheumatoid arthritis (RA) and Juvenile RA (JRA) (joints; less commonly lung, skin)
- Lupus [Systemic Lupus Erythematosus] (skin, joints, kidneys, heart, brain, red blood cells, other)
- Scleroderma (skin, intestine, less commonly lung)
- Sjögren's syndrome (salivary glands, tear glands, joints)
- Goodpasture's syndrome (lungs, kidneys)
- Wegener's granulomatosis (blood vessels, sinuses, lungs, kidneys)
- Polymyalgia Rheumatica (large muscle groups)
- Guillain-Barre syndrome (nervous system)

Localized Autoimmune Diseases

- Type 1 Diabetes Mellitus (pancreas islets)
- Hashimoto's thyroiditis, Graves' disease (thyroid)
- Celiac disease, Crohn's disease, Ulcerative colitis (GI tract)
- Multiple sclerosis (There is still some debate as to whetherMS is an autoimmune disease.)
- Addison's disease (adrenal)
- Primary biliary cirrhosis, Sclerosing cholangitis, Autoimmune hepatitis (liver)
- Temporal Arteritis / Giant Cell Arteritis (arteries of the head and neck)

ETIOLOGY OF AUTOIMMUNITY DISEASE

The exact etiology of autoimmune diseases is not known. However, various theories have been offered. These include sequestered antigen, escape of auto-reactive clones, loss of suppressor cells, cross reactive antigens including exogenous antigens (pathogens) and altered self antigens (chemical and viral infections).

Sequestered antigen

Lymphoid cells may not be exposed to some self antigens during their differentiation, because they may be late-developing antigens or may be confined to specialized organs (*e.g.*, testes,

brain, eye, *etc*.). A release of antigens from these organs resulting from accidental traumatic injury or surgery can result in the stimulation of an immune response and initiation of an autoimmune disease.

Escape of auto-reactive clones

The negative selection in the thymus may not be fully functional to eliminate self reactive cells. Not all self antigens may be represented in the thymus or certain antigens may not be properly processed and presented.

Lack of regulatory T cells

There are fewer regulatory T-cells in many autoimmune diseases

Cross reactive antigens

Antigens on certain pathogens may have determinants which cross react with self antigens and an immune response against these determinants may lead to effector cell or antibodies against tissue antigens. Post streptococcal nephritis and carditis, anticardiolipin antibodies during syphilis and association between *Klebsiella*and ankylosing spondylitis are examples of such cross reactivity.

DIAGNOSIS

Diagnosis of autoimmune diseases is based on symptoms and detection of antibodies (and/or very early T cells) reactive against antigens of tissues and cells involved. Antibodies against cell/tissue associated antigens are detected by immunofluorescence. Antibodies against soluble antigens are normally detected ELISA or radioimmunoassay (see table above). In some cases, a biological /biochemical assay may be used (*e.g.*, Graves diseases, pernicious anemia). Autoimmune disorders are diagnosed, evaluated, and monitored through a combination of autoantibody blood tests, blood tests to measure inflammation and organ function, clinical presentation, and through non-laboratory examinations such as X-rays.

TREATMENT

There is currently no cure for autoimmune disorders, although in rare cases they may disappear on their own. The goals of treatment of autoimmune disorders are to reduce symptoms and control the autoimmune response while maintaining the body's ability to fight infections. Treatments vary widely and depend on the specific disease and symptoms: Anti-inflammatory (corticosteroid) and immunosuppressive drug therapy (such as cyclophosphamide, azathioprine, cyclosporine) is the present method of treating autoimmune diseases. Extensive research is being carried out to develop innovative treatments which include: anti-TNF alpha therapy against arthritis, feeding antigen orally to trigger tolerance, anti-idiotype antibodies, antigen peptides, anti-IL2 receptor antibodies, anti-CD4 antibodies, anti-TCR antibodies, etc.

MYASTHENIA GRAVIS

Myasthenia gravis is a chronic autoimmune disorder that affects the voluntary muscles of the body. It is characterized by weakness or rapid muscle fatigue. The condition is supposed to occur when there is a dysfunction between the muscles and the nerves that control the same. A defect in the transmission of nerve impulses to the muscles gives rise to the symptoms of the condition.

Myasthenia gravis is usually not inherited but it develops with age. It often affects women after the age of 40 and men after 60. Muscle weakness is a common symptom of many other

conditions and hence a diagnosis for myasthenia gravis is often delayed and learnt about quite late.

Pathophysiology:
In MG, the autoantibodies **most commonly act against the nicotinic acetylcholine receptor (nAChR),** the receptor in the motor end plate for the neurotransmitteracetylcholine that stimulates muscular contractions. Some forms of the antibody impair the ability of acetylcholine to bind to receptors. Others lead to the destruction of receptors, either by complement fixation or by inducing the muscle cell to eliminate the receptors through endocytosis.

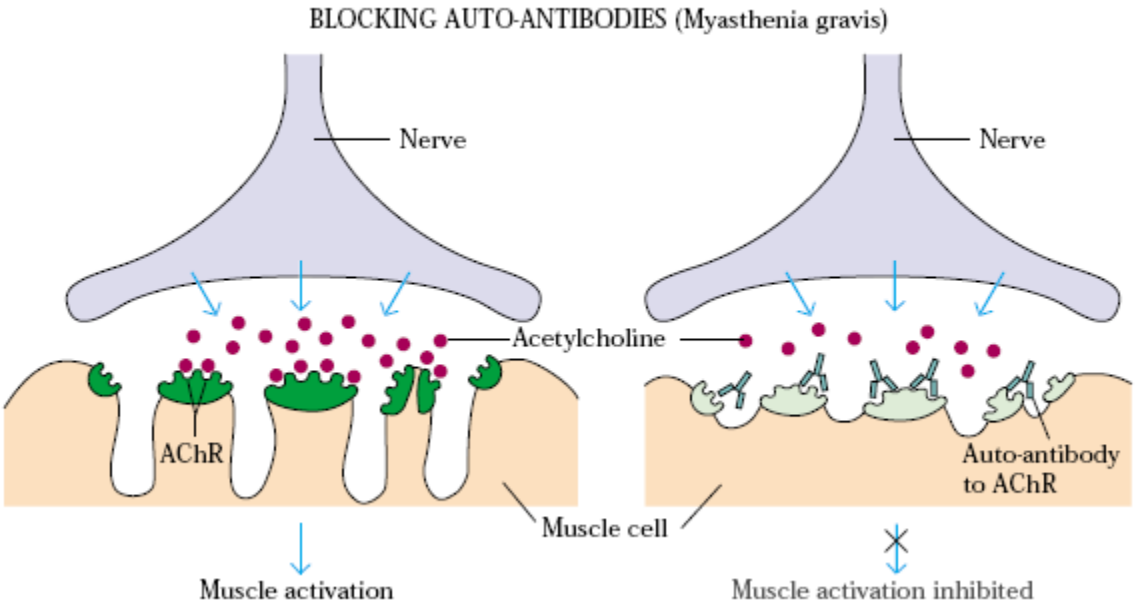
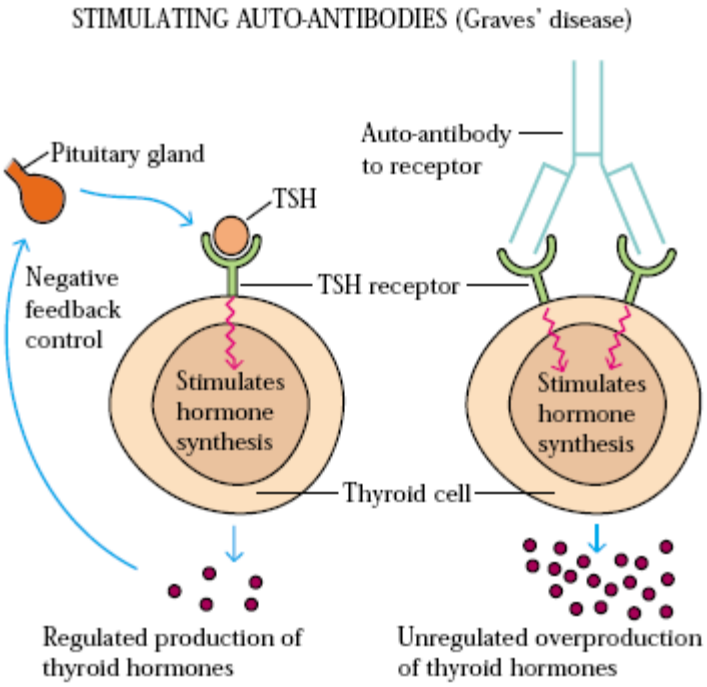


Fig:In MG , binding of auto antibodies to acetyl choline receptor blockksthe normal binding of acetyl choline and subsequent muscle activation. In addition the antiAchR auto antibodies activates complement which damage the muscle end plate

GRAVES' DISEASE

The production of thyroid hormones is carefully regulated by thyroid-stimulating hormone (TSH), which is produced by the pituitary gland. Binding of TSH to a receptor on thyroid cells activates adenylate cyclase and stimulates the synthesis of two thyroid hormones, thyroxine and triiodothyronine. A patient with **Graves' disease** produces auto-antibodies that bind the receptor for TSH and mimic the normal action of TSH, activating adenylate cyclase and resulting in produc-tion of the thyroid hormones. Unlike TSH, however, the auto-antibodies are not regulated, and consequently they over-stimulate the thyroid. For this reason these auto-antibodies are called long-acting thyroid-stimulating (LATS) antibod-ies (Figure).



In Graves' disease, binding of auto-antibodies to the receptor for thyroid-stimulating hormone (TSH) induces unregu-lated activation of the thyroid, leading to overproduction of the thy-roid hormones (purple dots).

Common signs and symptoms of Graves' disease include:

Graves' disease is an immune system disorder that results in the overproduction of thyroid hormones (hyperthyroidism). Although a number of disorders may result in hyperthyroidism, Graves' disease is a common cause.Because thyroid hormones affect a number of different body systems, signs and symptoms associated with Graves' disease can be wide ranging and significantly influence your overall well-being. Although Graves' disease may affect anyone, it's more common among women and before the age of 40.

- Anxiety and irritability
- A fine tremor of your hands or fingers
- Heat sensitivity and an increase in perspiration or warm, moist skin
- Weight loss, despite normal eating habits

- Enlargement of your thyroid gland (goiter)
- Change in menstrual cycles
- Erectile dysfunction or reduced libido
- Frequent bowel movements
- Bulging eyes (Graves' ophthalmopathy)
- Thick, red skin usually on the shins or tops of the feet (Graves' dermopathy)
- Rapid or irregular heartbeat (palpitations)

RHEUMATOID ARTHRITIS

Rheumatoid arthritis is a chronic inflammatory disorder that typically affects the small joints in hands and feet. Unlike the wear-and-tear damage of osteoarthritis, rheumatoid arthritis affects the lining of joints, causing a painful swelling that can eventually result in bone erosion and joint deformity.

An autoimmune disorder, rheumatoid arthritis occurs when immune system mistakenly attacks own body's tissues. In addition to causing joint problems, rheumatoid arthritis sometimes can affect other organs of the body — such as the skin, eyes, lungs and blood vessels.

Although rheumatoid arthritis can occur at any age, it usually begins after age 40. The disorder is much more common in women than in men. Treatment focuses on controlling symptoms and preventing joint damage.

Rheumatoid arthritis signs and symptoms may vary in severity and may even come and go. Periods of increased disease activity, called flares, alternate with periods of relative remission — when the swelling and pain fade or disappear. Over time, rheumatoid arthritis can cause joints to deform and shift out of place.

Etiology

Rheumatoid arthritis is a common autoimmune disorder, most often affecting women from 40 to 60 years old. The major symptom is chronic inflammation of the joints, although the hematologic, cardiovascular, and respiratory systems are also frequently affected. Many individuals with rheumatoid arthritis produce a group of auto-antibodies called **rheumatoid factors** that are reactive with determi-nants in the Fc region of IgG. The classic rheumatoid factor is an IgM antibody with that reactivity. Such auto-antibodies bind to normal circulating IgG, forming IgM-IgG complexes that are deposited in the joints. These immune complexes can

activate the complement cascade, resulting in a type III hypersensitive reaction, which leads to chronic inflammation of the joints.

Systemic Autoimmune Diseases(SLE)

In systemic autoimmune diseases, the response is directed toward a broad range of target antigens and involves a number of organs and tissues. These diseases reflect a general defect in immune regulation that results in hyperactive T cells and B cells. Tissue damage is widespread, both from cell-mediated immune responses and from direct cellular dam-age caused by auto-antibodies or by accumulation of immune complexes

Systemic Lupus Erythematosus AttacksMany Tissues

One of the best examples of a systemic autoimmune disease is **systemic lupus erythematosus (SLE),** which typically appears in women between 20 and 40 years of age; the ratio of female to male patients is 10:1. SLE is characterized by fever, weak-ness, arthritis, skin rashes, pleurisy, and kidney dysfunction (Figure 20-6). Lupus is more frequent in African-American and Hispanic women than in Caucasians, although it is not known why this is so. Affected individuals may produce auto-antibodies to a vast array of tissue antigens, such as DNA, his-tones, RBCs, platelets, leukocytes, and clotting factors; inter-action of these auto-antibodies with their specific antigens produces various symptoms. Auto-antibody specific for RBCs and platelets, for example, can lead to complement-mediated lysis, resulting in hemolytic anemia and thrombocytopenia, respectively. When immune complexes of auto-antibodies with various nuclear antigens are deposited along the walls of small blood vessels, a type III hypersensitive reaction devel-ops. The complexes activate the complement system and generate membrane-attack complexes and complement split products that damage the wall of the blood vessel, resulting in vasculitis and glomerulonephritis.

Excessive complement activation in patients with severe SLE produces elevated serum levels of the complement split products C3a and C5a, which may be three to four times higher than normal. C5a induces increased expression of the type 3 complement receptor (CR3) on neutrophils, facilitating neutrophil aggregation and attachment to the vascular endothelium. As neutrophils attach to small blood vessels, the number of circulating neutrophils declines (neutropenia) and various occlusions of the small blood vessels develop (vasculitis). These occlusions can lead to widespread tissue damage.

Laboratory diagnosis of SLE focuses on the characteristic antinuclear antibodies, which are directed against double-stranded or single-stranded DNA, nucleoprotein, histones, and nucleolar

RNA. Indirect immunofluorescent staining with serum from SLE patients produces various characteris-tic nucleus-staining patterns.

VACCINES

A **vaccine** is a biological preparation that improves immunity to a particular disease. A vaccine typically contains an agent that resembles a disease-causing microorganism, and is often made from weakened or killed forms of the microbe or its toxins. The agent stimulates the body's immune system to recognize the agent as foreign, destroy it, and "remember" it, so that the immune system can more easily recognize and destroy any of these microorganisms that it later encounters.

Vaccines can be prophylactic (e.g. to prevent or ameliorate the effects of a future infection by any natural or "wild" pathogen), or therapeutic (e.g. vaccines against cancer are also being investigated; see cancer vaccine

The immune system recognizes vaccine agents as foreign, destroys them, and "remembers" them. When the virulent version of an agent comes along the body recognizes the protein coat on the virus, and thus is prepared to respond, by (1) neutralizing the target agent before it can enter cells, and (2) by recognizing and destroying infected cells before that agent can multiply to vast numbers.

When two or more vaccines are mixed together in the same formulation, the two vaccines can interfere. This most frequently occurs with live attenuated vaccines, where one of the vaccine components is more robust than the others and suppresses the growth and immune response to the other components. This phenomenon was first noted in the trivalent Sabin polio vaccine, where the amount of serotype 2 virus in the vaccine had to be reduced to stop it from interfering with the "take" of the serotype 1 and 2 viruses in the vaccine.[11] This phenomenon has also been found to be a problem with the dengue vaccines currently being researched, where the DEN-3 serotype was found to predominate and suppress the response to DEN-1, -2 and -4 serotypes.[12]

Vaccines have contributed to the eradication of smallpox, one of the most contagious and deadly diseases known to man. Other diseases such as rubella, polio, measles, mumps, chickenpox, andtyphoid are nowhere near as common as they were a hundred years ago. As long as the vast majority of people are vaccinated, it is much more difficult for an outbreak of disease to occur, let alone spread. This effect is called herd immunity. Polio, which is transmitted only between humans, is targeted by an extensive eradication campaign that has seen endemic polio restricted to only parts of four countries (Afghanistan, India, Nigeria and Pakistan).[1] The difficulty of reaching all children as well as cultural misunderstandings, however, have caused the anticipated eradication date to be missed several times.

Immunization, or **immunisation**, is the process by which an individual's immune system becomes fortified against an agent (known as the immunogen).

When this system is exposed to molecules that are foreign to the body (*non-self*), it will orchestrate an immune response, but it can also develop the ability to quickly respond to a subsequent encounter (through immunological memory). This is a function of the adaptive immune system. Therefore, by exposing an animal to an immunogen in a controlled way, its body can learn to protect itself: this is called active immunization.

The most important elements of the immune system that are improved by immunization are the B cells (and the antibodies they produce) and T cells.Memory B cell and memory T cells are responsible for a swift response to a second encounter with a foreign molecule. Passive immunization is when these elements are introduced directly into the body, instead of when the body itself has to make these elements.

Immunization be done through various techniques, most commonly vaccination. Vaccines against microorganisms that cause diseases can prepare the body's immune system, thus helping to fight or prevent an infection. The fact that mutations can cause cancer cells to produce proteins or other molecules that are unknown to the body forms the theoretical basis for therapeutic cancer vaccines. Other molecules can be used for immunization as well, for example in experimental vaccines against nicotine (NicVAX) or the hormone ghrelin (in experiments to create an obesity vaccine).

Passive and active immunization

Immunization can be achieved in an active or passive fashion: vaccination is an active form of immunization.

Active immunization

Active immunization entails the introduction of a foreign molecule into the body, which causes the body itself to generate immunity against the target. This immunity comes from the T cells and the B cells with their antibodies.

Active immunization can occur naturally when a person comes in contact with, for example, a microbe. If the person has not yet come into contact with themicrobe and has no pre-made antibodies for defense (like in passive immunization), the person becomes immunized. The immune system will eventually create antibodies and other defenses against the microbe. The next time, the immune response against this microbe can be very efficient; this is the case in many of the childhood infections that a person only contracts once, but then is immune.

Artificial active immunization is where the microbe, or parts of it, are injected into the person before they are able to take it in naturally. If whole microbes are used, they are pre-treated, Attenuated vaccine.

Passive immunization

Main article: Passive immunity

Passive immunization is where pre-synthesized elements of the immune system are transferred to a person so that the body does not need to produce these elements itself. Currently, antibodies can be used for passive immunization. This method of immunization begins to work very quickly, but it is short lasting, because the antibodies are naturally broken down, and if there are no B cells to produce more antibodies, they will disappear.

Passive immunization occurs physiologically, when antibodies are transferred from mother to fetus during pregnancy, to protect the fetus before and shortly after birth.

Artificial passive immunization is normally administered by injection and is used if there has been a recent outbreak of a particular disease or as an emergency treatment for toxicity (for

example, fortetanus). The antibodies can be produced in animals ("serum therapy") although there is a high chance of anaphylactic shock because of immunity against animal serum itself. Thus, humanized antibodies produced *in vitro* by cell culture are used instead if available.

Vaccines

Types of Vaccines

Scientists take many approaches to designing vaccines against a microbe. These choices are typically based on fundamental information about the microbe, such as how it infects cells and how the immune system responds to it, as well as practical considerations, such as regions of the world where the vaccine would be used. The following are some of the options that researchers might pursue:

* Live, attenuated vaccines
* Inactivated vaccines
* Subunit vaccines
* Toxoid vaccines
* Conjugate vaccines
* DNA vaccines
* Recombinant vector vaccines

Live, Attenuated Vaccines

Live, attenuated vaccines contain a version of the living microbe that has been weakened in the lab so it can't cause disease. Because a live, attenuated vaccine is the closest thing to a natural infection, these vaccines are good "teachers" of the immune system: They elicit strong cellular and antibody responses and often confer lifelong immunity with only one or two doses.

Despite the advantages of live, attenuated vaccines, there are some downsides. It is the nature of living things to change, or mutate, and the organisms used in live, attenuated vaccines are no different. The remote possibility exists that an attenuated microbe in the vaccine could revert to a virulent form and cause disease. Also, not everyone can safely receive live, attenuated vaccines. For their own protection, people who have damaged or weakened immune systems— because they've undergone chemotherapy or have HIV, for example—cannot be given live vaccines.

Another limitation is that live, attenuated vaccines usually need to be refrigerated to stay potent. If the vaccine needs to be shipped overseas and stored by health care workers in developing countries that lack widespread refrigeration, a live vaccine may not be the best choice.

Live, attenuated vaccines are relatively easy to create for certain viruses. Vaccines against measles, mumps, and chickenpox, for example, are made by this method. Viruses are simple microbes containing a small number of genes, and scientists can therefore more readily control their characteristics. Viruses often are attenuated through a method of growing generations of them in cells in which they do not reproduce very well. This hostile environment takes the fight

out of viruses: As they evolve to adapt to the new environment, they become weaker with respect to their natural host, human beings.

Live, attenuated vaccines are more difficult to create for bacteria. Bacteria have thousands of genes and thus are much harder to control. Scientists working on a live vaccine for a bacterium, however, might be able to use recombinant DNA technology to remove several key genes. This approach has been used to create a vaccine against the bacterium that causes cholera, *Vibrio cholerae,* although the live cholera vaccine has not been licensed in the United States.

Inactivated Vaccines

Scientists produce inactivated vaccines by killing the disease-causing microbe with chemicals, heat, or radiation. Such vaccines are more stable and safer than live vaccines: The dead microbes can't mutate back to their disease-causing state. Inactivated vaccines usually don't require refrigeration, and they can be easily stored and transported in a freeze-dried form, which makes them accessible to people in developing countries.

Most inactivated vaccines, however, stimulate a weaker immune system response than do live vaccines. So it would likely take several additional doses, or booster shots, to maintain a person's immunity. This could be a drawback in areas where people don't have regular access to health care and can't get booster shots on time.

Subunit Vaccines

Instead of the entire microbe, subunit vaccines include only the antigens that best stimulate the immune system. In some cases, these vaccines use epitopes—the very specific parts of the antigen that antibodies or T cells recognize and bind to. Because subunit vaccines contain only the essential antigens and not all the other molecules that make up the microbe, the chances of adverse reactions to the vaccine are lower.

Subunit vaccines can contain anywhere from 1 to 20 or more antigens. Of course, identifying which antigens best stimulate the immune system is a tricky, time-consuming process. Once scientists do that, however, they can make subunit vaccines in one of two ways:

* They can grow the microbe in the laboratory and then use chemicals to break it apart and gather the important antigens.
* They can manufacture the antigen molecules from the microbe using recombinant DNA technology. Vaccines produced this way are called "recombinant subunit vaccines."

A recombinant subunit vaccine has been made for the hepatitis B virus. Scientists inserted hepatitis B genes that code for important antigens into common baker's yeast. The yeast then produced the antigens, which the scientists collected and purified for use in the vaccine. Research is continuing on a recombinant subunit vaccine against hepatitis C virus.

Toxoid Vaccines

For bacteria that secrete toxins, or harmful chemicals, a toxoid vaccine might be the answer. These vaccines are used when a bacterial toxin is the main cause of illness. Scientists have found that they can inactivate toxins by treating them with formalin, a solution of formaldehyde and sterilized water. Such "detoxified" toxins, called toxoids, are safe for use in vaccines.

When the immune system receives a vaccine containing a harmless toxoid, it learns how to fight off the natural toxin. The immune system produces antibodies that lock onto and block the toxin. Vaccines against diphtheria and tetanus are examples of toxoid vaccines.

Conjugate Vaccines

If a bacterium possesses an outer coating of sugar molecules called polysaccharides, as many harmful bacteria do, researchers may try making a conjugate vaccine for it. Polysaccharide coatings disguise a bacterium's antigens so that the immature immune systems of infants and younger children can't recognize or respond to them. Conjugate vaccines, a special type of subunit vaccine, get around this problem.

When making a conjugate vaccine, scientists link antigens or toxoids from a microbe that an infant's immune system can recognize to the polysaccharides. The linkage helps the immature immune system react to polysaccharide coatings and defend against the disease-causing bacterium.

The vaccine that protects against *Haemophilus influenzae* type B (Hib) is a conjugate vaccine.

DNA Vaccines

Once the genes from a microbe have been analyzed, scientists could attempt to create a DNA vaccine against it.

Still in the experimental stages, these vaccines show great promise, and several types are being tested in humans. DNA vaccines take immunization to a new technological level. These vaccines dispense with both the whole organism and its parts and get right down to the essentials: the microbe's genetic material. In particular, DNA vaccines use the genes that code for those all-important antigens.

Researchers have found that when the genes for a microbe's antigens are introduced into the body, some cells will take up that DNA. The DNA then instructs those cells to make the antigen molecules. The cells secrete the antigens and display them on their surfaces. In other words, the body's own cells become vaccine-making factories, creating the antigens necessary to stimulate the immune system.

A DNA vaccine against a microbe would evoke a strong antibody response to the free-floating antigen secreted by cells, and the vaccine also would stimulate a strong cellular response against the microbial antigens displayed on cell surfaces. The DNA vaccine couldn't cause the disease because it wouldn't contain the microbe, just copies of a few of its genes. In addition, DNA vaccines are relatively easy and inexpensive to design and produce.

So-called naked DNA vaccines consist of DNA that is administered directly into the body. These vaccines can be administered with a needle and syringe or with a needle-less device that uses high-pressure gas to shoot microscopic gold particles coated with DNA directly into cells. Sometimes, the DNA is mixed with molecules that facilitate its uptake by the body's cells. Naked DNA vaccines being tested in humans include those against the viruses that cause influenza and herpes.

Recombinant Vector Vaccines

Recombinant vector vaccines are experimental vaccines similar to DNA vaccines, but they use an attenuated virus or bacterium to introduce microbial DNA to cells of the body. "Vector" refers to the virus or bacterium used as the carrier.

In nature, viruses latch on to cells and inject their genetic material into them. In the lab, scientists have taken advantage of this process. They have figured out how to take the roomy genomes of certain harmless or attenuated viruses and insert portions of the genetic material from other microbes into them. The carrier viruses then ferry that microbial DNA to cells. Recombinant vector vaccines closely mimic a natural infection and therefore do a good job of stimulating the immune system.

Attenuated bacteria also can be used as vectors. In this case, the inserted genetic material causes the bacteria to display the antigens of other microbes on its surface. In effect, the harmless bacterium mimics a harmful microbe, provoking an immune response.

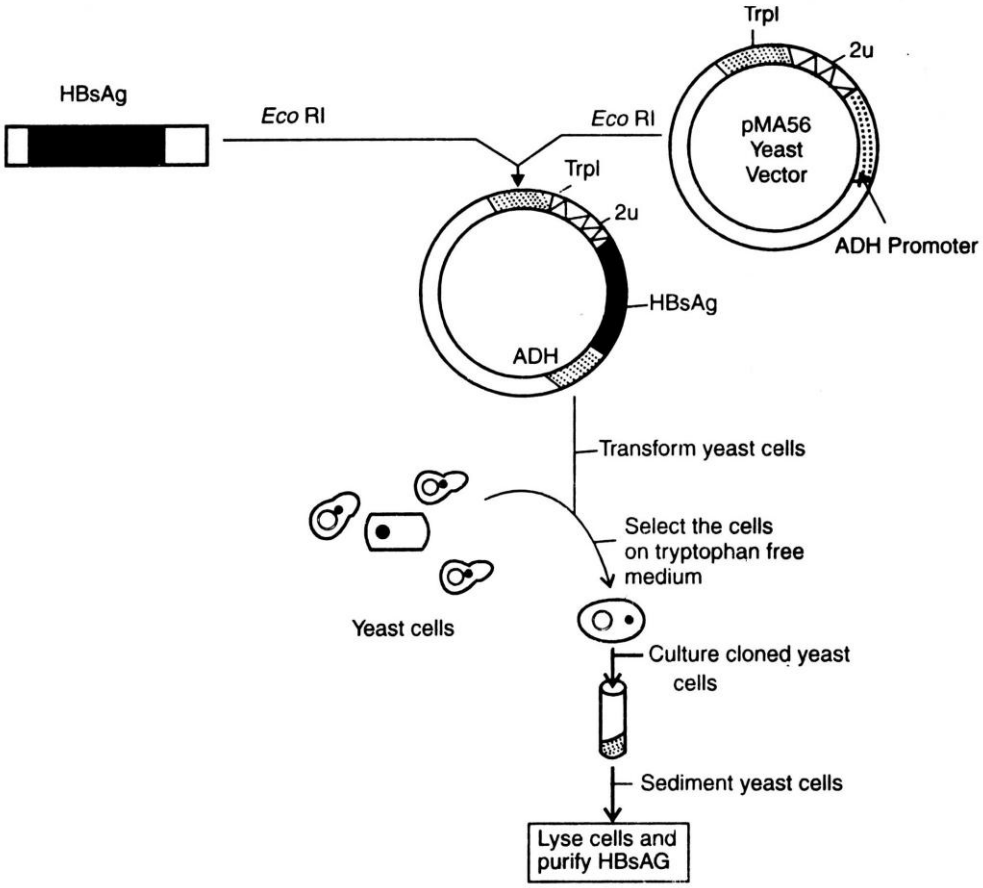
Researchers are working on both bacterial and viral-based recombinant vector vaccines for HIV, rabies, and measle

Hepatitis B vaccine

It is a vaccine developed for the prevention of hepatitis B virus infection. The vaccine contains one of the viral envelope proteins, hepatitis B surface antigen (HBsAg). It is produced by yeast cells, into which the genetic code for HBsAg has been inserted.

RECOMBIVAX HB Hepatitis B Vaccine (Recombinant) is a sterile suspension of non-infectious subunit viral vaccine derived from HBsAg produced in yeastcells. A portion of the hepatitis B virus gene, coding for HBsAg, is cloned into yeast, and the vaccine for hepatitis B is produced

from cultures of this recombinant yeast strain according to methods developed in the Merck Research Laboratories.



The antigen is harvested and purified from fermentation cultures of a recombinant strain of the yeast *Saccharomyces cerevisiae* containing the gene for the adw subtype of HBsAg. The fermentation process involves growth of *Saccharomyces cerevisiae* on a complex fermentation medium which consists of an extract of yeast, soy peptone, dextrose, amino acids and mineral salts. The HBsAg protein is released from the yeast cells by cell disruption and purified by a series of physical and chemical methods. The purified protein is treated in phosphate buffer with formaldehyde and then coprecipitated with alum (potassium aluminum sulfate) to form bulk vaccine adjuvanted with amorphous aluminum hydroxyphosphate sulfate. Each dose contains less than 1% yeast protein. The vaccine produced by the Merck method has been shown to be

comparable to the plasma-derived vaccine in terms of animal potency (mouse, monkey, and chimpanzee) and protective efficacy (chimpanzee and human).

The vaccine against hepatitis B, prepared from recombinant yeast cultures, is free of association with human blood or blood products.

A course of two to three (2–3) vaccine injections is given, the second injection at least one month after the first dose and the third injection being administered six months after the first dose. The first and second dose offer complete protection. The final injection (second or third depending on number of vaccines being administered) is to prolong protection against the hepatitis B virus. Afterward an immune system antibody to HBsAg is established in the bloodstream. The antibody is known as *anti-HBs*. This antibody and immune system memory then provide immunity to hepatitis B infection. The first vaccine became available in 1981.

RECOMBIVAX HB Hepatitis B Vaccine (Recombinant) is supplied in three formulations.
Pediatric/Adolescent Formulation (Without Preservative), 10 mcg/mL: each 0.5 mL dose contains 5 mcg of hepatitis B surface antigen.

Adult Formulation (Without Preservative), 10 mcg/mL: each 1 mL dose contains 10 mcg of hepatitis B surface antigen.

Dialysis Formulation (Without Preservative), 40 mcg/mL: each 1 mL dose contains 40 mcg of hepatitis B surface antigen.
All formulations contain approximately 0.5 mg of aluminum (provided as amorphous aluminum hydroxyphosphate sulfate, previously referred to as aluminum hydroxide) per mL of vaccine. In each formulation, hepatitis B surface antigen is adsorbed onto approximately 0.5 mg of aluminum (provided as amorphous aluminum hydroxyphosphate sulfate) per mL of vaccine. The vaccine contains < 15 mcg/Ml residual formaldehyde.

UNIT IV

1	---------- Reactions are responsible for tackling invasion of foreign antigens.	Antigen	Immune	Antibody	Antigen- antibody	Immune
2	The visible feature of hypersensitive states is the inflammation developed at the site of	Antigen	Antibody	Antigen- antibody	Foreign antigens.	Antigen- antibody
3	Type I is acute in nature and mediated by	IgM	IgA	Ig D	IgE	IgE
4	Which type of hypersensitivity resulting mainly from blood transfusion methods.	Type I	Type II	Type III	TypeIV	Type II
5	Which hypersentivity involves formationof complexes btwn Ab &Ag lodged capillaries	Type I	Type II	Type III	TypeIV	Type III
6	Which type hypersentivity involves in delayed type of reaction mediated by lymphocytes.	Type I	Type II	Type III	TypeIV	TypeIV
7	Maternal antibodies present in colostrums provide…….immunity to infant	passive	active	cellular	humoral	passive
8	……..immunization used to provide immediate protection toindividuals exposed to infectious organisms	humoral	active	cellular	Passive	Passive
9	Attenuated strain of Mycobacterium bovis	BCG	Polio	DPT	Hepatitis	BCG
10	The major disadvantage of attenuated vaccine	diversion	inversion	reversion	deletion	inversion
11	One limitation of polysaccharide vaccines is their inability to activate	B cells	Tc cells	NK cells	T_H cells	T_H cells
12	The first recombinant antigen vaccine approved for human use was	Polio	DPT	Hepatitis B	BCG	Hepatitis B
13	A DNA vaccine only induces a response to a single	cytokines	epitopes	haptens	paratopes	epitopes
14	Activated T_H 1 cells produces large number of	Cytokines	epitopes	haptens	paratopes	Cytokines
15	Main components of cell-mediated antiviral defence CD8+ in	T cells	Tc cells	B cell	NK cell	Tc cells
16	Interferon µ and b induce an antiviral protein called	DAI	antibody	cytokine	lnterleukines	DAI
17	The causative agent of common cold	Hepatitis virus	rhinovirus	Epstein-barr virus	herpus virus	rhinovirus
18	The immune system goes against self components of the body reffered	Humoral immunity	Innate immunity	Autoimmunity	cell mediated immunity	Autoimmunity
19	Adrenal hyperplasia and progressive malfunctioning of the adrenal cortex	Addison's disease	Multiple sclerosis	Agranulocytosis	sjogren's syndrome	Addison's disease
20	…….is an autoimmune disease that affects the CNS and causes neurological disability	Addison's disease	Multiple sclerosis	Agranulocytosis	sjogren's syndrome	Multiple sclerosis
21	The genetic basis of most autoimmune disease is	bigenic	isogenic	Agenic	polygenic	polygenic
22	…… play a major role in natural & innate immunity	Neutrophils	phagocytes	macrophages	lymphocytes	phagocytes
23	Reduction in neutrophil counts	neutropenia	aneutropenia	Agranulocytosis	jogren's syndrome	neutropenia
24	Complete absence of neutrophils	Neutropenia	agranulocytosis	agranulopenia	aneutropenia	agranulocytosis
25	Agranulopenia causes decrease in the production of	CSF	M- CSF	G- CSF	C-CSF	G- CSF
26	A autoimmune disease called --------- leads to complete absence of neutrophils.	sjogren's syndrome	Graves disease	Addison' s disease	Digeorge syndrome	sjogren's syndrome
27	Which is an indirect test used for the deletion of serum antibody to HIV	RIA	ELISA	DIBA	ELI SPOT	ELISA
28	------- is an example of a cell mediated immune defect.	Digeorge syndrome	sjogren's syndrome	Graves disease	Addison' s disease	Digeorge syndrome
29	Failure to express MHC molecule is	Digeorge syndrome	sjogren's syndrome	bare lymphocyte syndrome	Graves disease	bare lymphocyte syndrome
30	Most commonly observed clinical feature with HIV infection is the reduction number of	CD^{4+} T cells	CD^{4+} B cells	CD^{4+}	CD^{8+} T cells	CD^{4+} T cells
31	The disease conditions in which immune function is defective	Immune response	Antigencity	Immunodeficiency	Immunogenicity	Immunodeficiency
32	Another name for the gel- diffusion technique	single diffusion	Double diffusion	Diffusion	Immuno diffusion	Immuno diffusion
33	The disease characterized by the absence of g- globulins in the blood	X- linked Agamma gloubulinemia	Y- linked Agamma gloubulinemia	X- linked Beta gloubulinemia	Y- linked Beta gloubulinemia	X- linked Agamma gloubulinemia
34	Reduction in blood platelets leads to	Wiskott –Aldrich syndrome	Digeorge syndrome	sjogren's syndrome	Graves disease	Wiskott –Aldrich syndrome
35	---------- are antigens expressed on tumor cells but not normal cells.	Tumor specific antibody	Tumor specific antigen	Tumor cells	NK cells.	Tumor specific antigen
36	Solid malignant tumors of lymphoid tissues are called	Lymphocytes	Lymphokines	lymphomas	leukocytes	lymphomas
37	The cytokines produced by activated macrophages that kill tumors but not normal cells a	Malignant tumors	necrosis	Tumor cells	Tumor necrosis factor	Tumor necrosis factor
38	Solid malignant tumors of bone marrow & bloodborn of lymphoctes or other are called	leukemia	Lymphokines	lymphomas	leukocytes	leukemia
39	Cancers derived from epithelial cells are	Sarcoma	Lymphoma	Carcinoma	Myleoma	Carcinoma
40	Malignant tumors of mesenchymal tissues arising from cells like fibroblasts, and fat cells	Sarcoma	Lymphoma	Carcinoma	Myleoma	Sarcoma
41	The antigens do not stimulate immunologic response in the host are	Tumor associated antibody	Tumor associated antigen	Antigen	Antibody	Tumor associated antigen
42	--------- Provide effective anti- tumor immunity invivo	Cytotoxic T-lymphocytes	T- lymphocytes	lymphocytes	cytokines.	Cytotoxic T-lymphocytes
43	Mononuclear cells are found in human solid tumors are	lymphocytes..	Tumor infiltrating lymphocytes	Cytotoxic T-lymphocytes	T- lymphocytes	Tumor infiltrating lymphocytes
44	---------- plays an important role in anti- tumor response.	CD 4 helper T cells	CD^{4+} B cells	CD^{4+}	CD^{8+} T cells	CD 4 helper T cells
45	---------- are known to secrete TMF & IFN g on activation by tumor antigens	CD cells	CD 8 cells	CD 4 cells	T cells	CD 4 cells
46	The tumoricidal activity of NK cells is enhanced by	T- lymphocytes	lymphocytes	Cytotoxic T-lymphocytes	Cytokines	Cytokines
47	Tumorcells transfected genes encodingsygernic ---produce effectivecell cancer vaccine	MHC	MHC class I	MHC class II	both of MHC class	MHC class II
48	---------- on carcinogen or radiation induced rodent tumors	Tumor specifc transplantation Ag	Tumor specifc transplantation Ab	Transplantation Antigen	Thymus dependent Ag.	Tumor specifc transplantation Ag
49	Grave's disease, autoantibodies are produced against	Thyroid stimulating hormone receptor	T cell receptor	B cell receptor	T & B cell receptor	Thyroid stimulating hormone receptor
50	---------- is characterized by the abnormal weakness of voluntary muscles.	Wiskott –Aldrich syndrome	Digeorge syndrome	sjogren's syndrome	Myasthenia Graves disease	Myasthenia Graves disease
51	--------characterized by adrenal hyperplasia & progressive malfunctioning adrenal water	Addison's disease	Digeorge syndrome	sjogren's syndrome	Myasthenia Graves disease	Addison's disease
52	--------- is autoimmune disease that affects the CNS and causes neurological disability	Sclerosis	single sclerosis	Multiple sclerosis	poly sclerosis	Multiple sclerosis
53	A class rheumatoid factor	Ig M	Ig G	Ig D	Ig A	Ig M
54	In innate immune system ---- play important role in inhibiting tumor growth & metastasis	T cells	B cells	NK cells	T_H cells	NK cells
55	------produced resultinfection rolein rhythmicity of sleep & immunity regulator molecules	T- lymphocytes	lymphocytes	Cytotoxic T-lymphocytes	cytokines	cytokines
56	------- capableof internalizing antigensby phagocytosis(or)endocytosis process cytoplasm	Antigen presenting cell	Antibody presenting cell	Antigen	antibody.	Antigen presenting cell
57	The non-cellular part of blood	Tissues	serum	blood	platelets.	serum
58	The state of protection from infections disease	Immunity	Immune response	T cell	B cell	Immunity
59	A group of proteins produced by virus infected cells	Interluekins	Interluekins-2	Interferon	leukocytes	Interferon
60	A group of serum proteins that circulate in an inactive proenzyme state.	non- complement	complement	alternate	classical	complement

KARPAGAM ACADEMY OF HIGHER EDUCATION
(Deemed University Established Under Section 3 of UGC Act 1956)
Coimbatore - 641021.
(For the candidates admitted from 2015 onwards)
DEPARTMENT OF BIOCHEMISTRY

SUBJECT : IMMUNOLOGY
SEMESTER : V
SUBJECT CODE : 15BCU503 CLASS : III B.Sc.BC

UNIT V- *Immunotechniques*

Antigen- antibody interaction-Precipitation reaction-immuno diffusion, immuno electrophoresis; Agglutination- blood grouping; Immuno techniques – Principle and application of RIA, ELISA, Fluorescent antibody techniques, immuno blotting, hybridoma technology - elementary concepts only.

ANTIGEN-ANTIBODY REACTIONS

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 as illustrated in Figure 1. 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 includehydrogen bonds, electrostatic bonds, Van der 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.

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

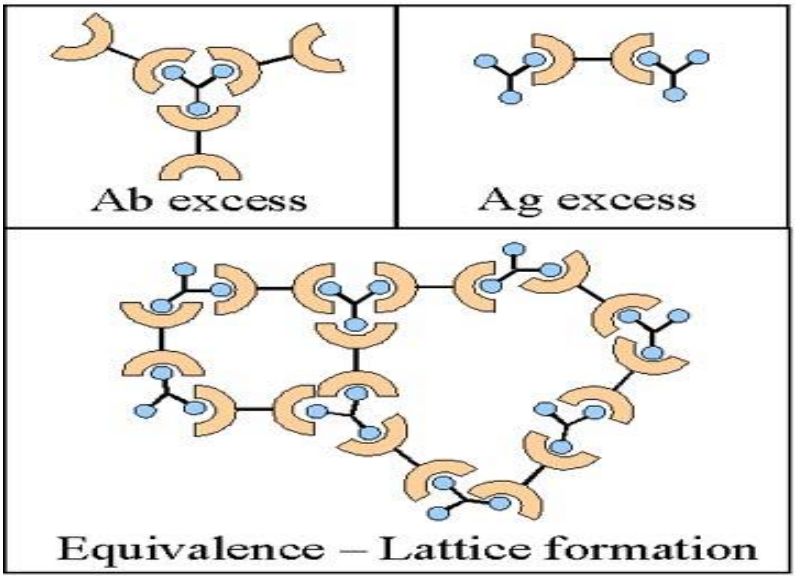
The higher the affinity of the antibody for the antigen, the more stable will be the interaction. Thus, the ease with which one can detect the interaction is enhanced.

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. This is depicted in Figure .



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.

IMMUNO TECHNIQUES

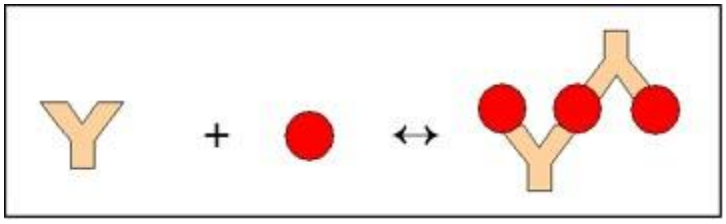
Agglutination Tests

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. (Figure).

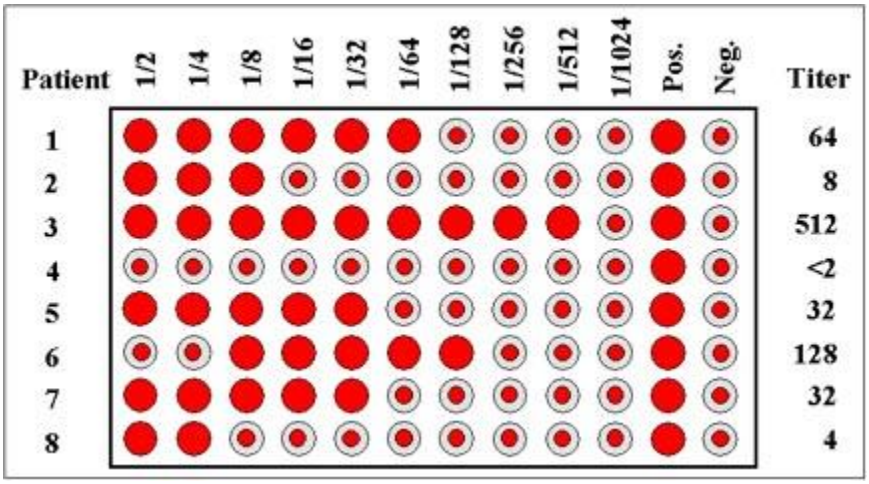


For example, a patient's red blood cells can be mixed with antibody to a blood group antigen to determine a person's blood type. In a second example, a patient's serum is mixed with red blood cells of a known blood type to assay for the presence of antibodies to that blood type in the patient's serum.

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.

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.

Agglutination reactions (Figure 6-7) are routinely performed to type red blood cells (RBCs). In typing for the ABO antigens, RBCs are mixed on a slide with antisera to the A or B blood-group antigens. If the antigen is present on the cells, they agglutinate, forming a visible clump on the slide. Determination of which antigens are present on donor and recipient RBCs is the basis for matching blood types for transfusions.

Figure: Blood grouping

Hemagglutination Is Used in Blood Typing

Agglutination reactions (Figure 6-7) are routinely performed to type red blood cells (RBCs). In typing for the ABO antigens, RBCs are mixed on a slide with antisera to the A or B blood-group antigens. If the antigen is present on the cells, they agglutinate, forming a visible clump on the slide. Determination of which antigens are present on donor and recipient RBCs is the basis for matching blood types for transfusions.

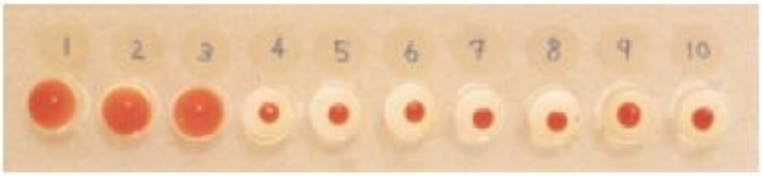


FIGURE Demonstration of hemagglutination using antibodies against sheep red blood cells (SRBCs). The control tube (10) contains only SRBCs, which settle into a solid "button." The experimental tubes 1–9 contain a constant number of SRBCs plus serial two-fold dilutions of anti-SRBC serum. The spread pattern in the experimental series indicates positive hemagglutination through tube

ii)Bacterial Agglutination Is Used To Diagnose Infection

ii. To assess bacterial and viral 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.

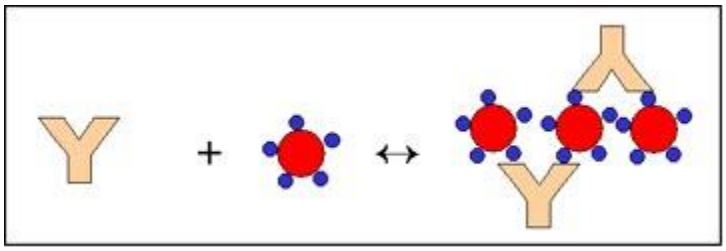
A bacterial infection often elicits the production of serum antibodies specific for surface antigens on the bacterial cells. The presence of such antibodies can be detected by bacterial agglutination reactions. Serum from a patient thought to be infected with a given bacterium is serially diluted in an array of tubes to which the bacteria is added. The last tube showing visible agglutination will reflect the serum antibody **titer** of the patient. The agglutinin titer is defined as the reciprocal of the greatest serum dilution that elicits a positive agglutina-tion reaction. For example, if serial twofold dilutions of serum are prepared and if the dilution of 1/640 shows agglu-tination but the dilution of 1/1280 does not, then the agglu-tination titer of the patient's serum is 640. In some cases serum can be diluted up to 1/50,000 and still show agglutina-tion of bacteria.

The agglutinin titer of an antiserum can be used to diag-nose a bacterial infection. Patients with typhoid fever, for ex-ample, show a significant rise in the agglutination titer to *Salmonella typhi*. Agglutination reactions also provide a way to type bacteria. For instance, different species of the bac-terium *Salmonella* can be distinguished by agglutination re-actions with a panel of typing antisera.

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 (Figure). This

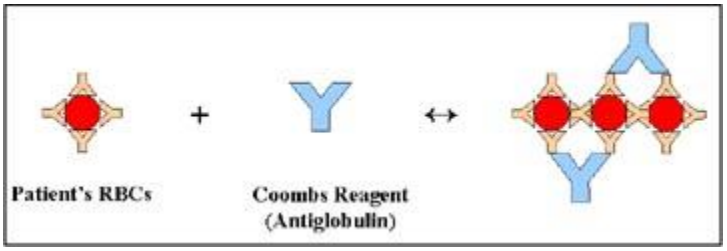
is called passive hemagglutination. The test is performed just like the agglutination test. Applications include detection of antibodies to soluble antigens and detection of antibodies to viral antigens.



Coomb's Test (Antiglobulin Test)

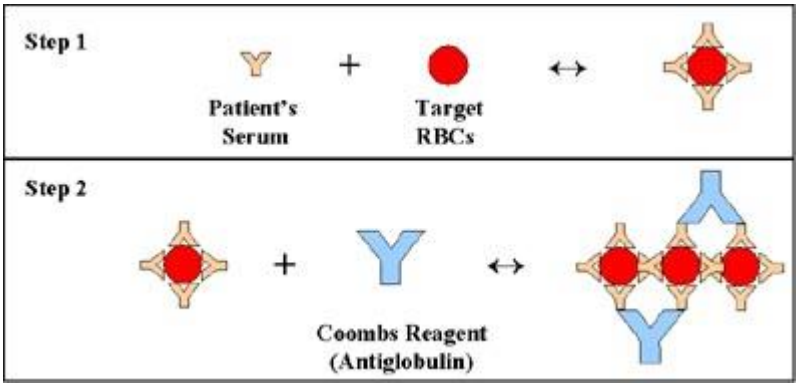
Direct Coomb's Test

When antibodies bind to erythrocytes, they do not always result in agglutination. This can result from the antigen/antibody ratio being in antigen excess or antibody excess or in some cases electrical charges on the red blood cells preventing the effective cross linking of the cells. These antibodies that bind to but do not cause agglutination of red blood cells are sometimes referred to as incomplete antibodies. In no way is this meant to indicate that the antibodies are different in their structure, although this was once thought to be the case. Rather, it is a functional definition only. In order to detect the presence of non-agglutinating antibodies on red blood cells, one simply adds a second antibody directed against the immunoglobulin (antibody) coating the red cells. This anti-immunoglobulin can now cross link the red blood cells and result in agglutination. This test is illustrated in Figure and is known as the Direct Coomb's test.



Indirect Coomb's Test

If it is necessary to know whether a serum sample has antibodies directed against a particular red blood cell and you want to be sure that you also detect potential non- agglutinating antibodies in the sample, an Indirect Coomb's test is performed (Figure). This test is done by incubating the red blood cells with the serum sample, washing out any unbound antibodies and then adding a second anti-immunoglobulin reagent to cross link the cells.



Applications

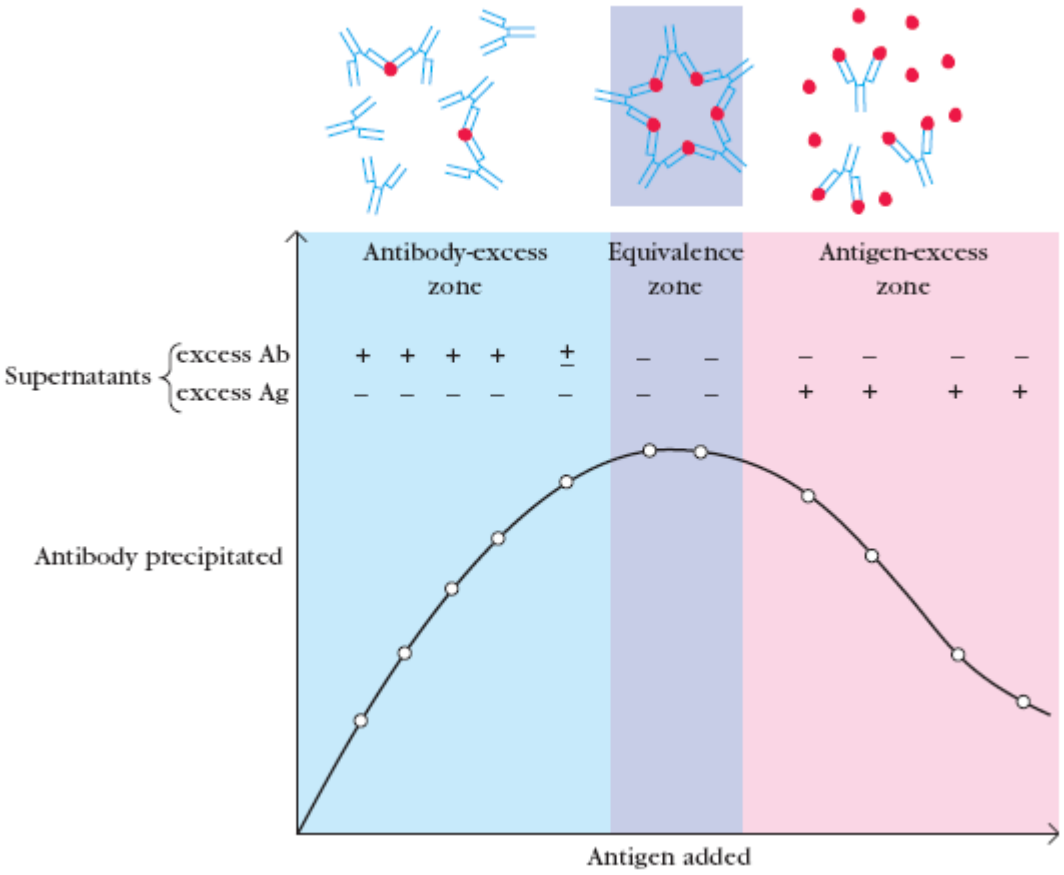
These include detection of anti-rhesus factor (Rh) antibodies. Antibodies to the Rh factor generally do not agglutinate red blood cells. Thus, red cells from Rh^+ children born to Rh^- mothers, who have anti-Rh antibodies, may be coated with these antibodies. To check for this, a direct Coombs test is performed. To see if the mother has anti-Rh antibodies in her serum an Indirect Coombs test is performed.

Precipitation Reactions

The interaction of soluble antigens with IgG or IgM antibodies leads to precipitation reactions. Antibodies that aggregate soluble antigens are called **precipitins.**
Precipitation reactions depend on the formation of lattices and occur best when antigen and antibody are present in optimal proportions. Excesses of either component decrease lattice formation and subsequent precipitation.Formation of an Ag-Ab lattice depends on the valency of both the antibody and antigen:The antibody must be bivalent; a precipitate will not form with monovalent Fab fragments.The antigen must be either bivalent or polyvalent;

Precipitation Reactions in Fluids Yield a Precipitin Curve

A quantitative precipitation reaction can be performed by placing a constant amount of antibody in a series of tubes and adding increasing amounts of antigen to the tubes. At one time this method was used to measure the amount of antigen or antibody present in a sample of interest. After the precipitate forms, each tube is centrifuged to pellet the pre-cipitate, the supernatant is poured off, and the amount of precipitate is measured. Plotting the amount of precipitate against increasing antigen concentrations yields a precipitin curve. As in Figure, excess of either antibody or antigen interferes with maximal precipitation, which occurs in the so-called **equivalence zone,** within which the ratio of antibody to antigen is optimal. As a large multimolecular lattice is formed at equivalence, the complex increases in size and precipitates out of solution. As shown in Figure, under conditions of *antibody excess* or *antigen excess*, extensive lattices do not form and precipitation is inhibited.



Precipitation Reactions in Gels Yield Visible Precipitin Lines

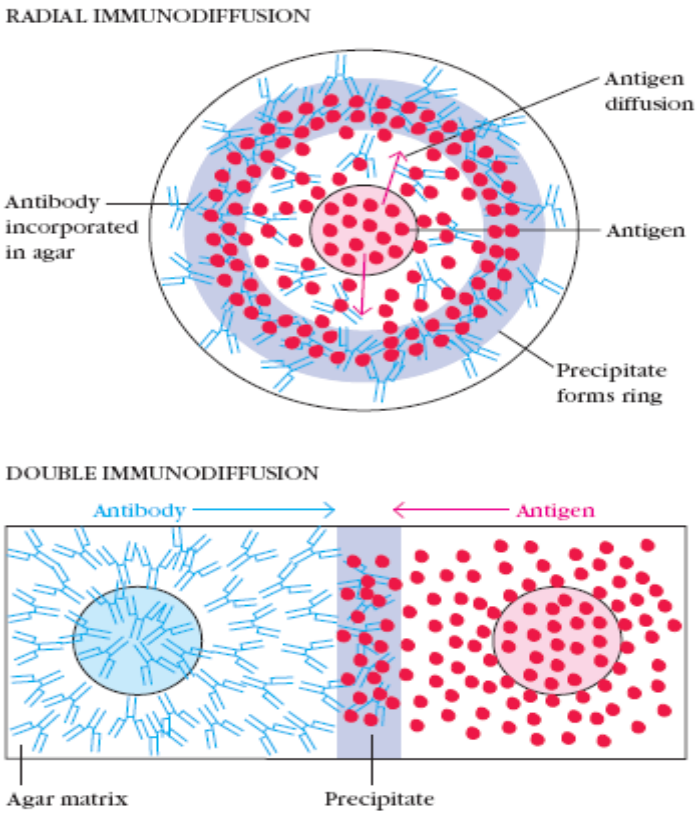
Immune precipitates can form not only in solution but also in an agar matrix. When antigen and antibody diffuse toward one another in agar, or when antibody is incorporated into the agar and antigen diffuses into the antibody-containing matrix, a visible line of precipitation will form. As in a precipitation re-action in fluid, visible precipitation occurs in the region of equivalence, whereas no visible precipitate forms in regions of antibody or antigen excess. Two types of *immunodiffusion reactions* can be used to determine relative concentrations of an-tibodies or antigens, to compare antigens, or to determine the relative purity of an antigen preparation. They are **radial im-munodiffusion** (the Mancini method) and **double immun-odiffusion** (the Ouchterlony method); both are carried out in a semisolid medium such as agar.

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 as illustrated in Figure 13.

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.



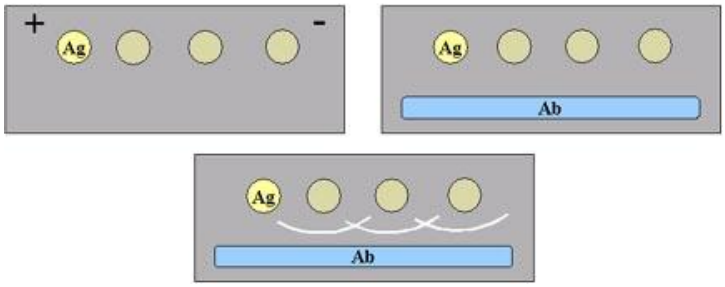
In the Ouchterlony method (Double diffusion), both antigen and antibody diffuse radially from wells toward each other, thereby establishing a concentration gradient. As equiv-alence is reached, a visible line of precipitation, a precipitin line, forms.

IMMUNOELECTROPHORESIS COMBINES ELECTROPHORESIS AND DOUBLE IMMUNODIFFUSION

In **immunoelectrophoresis,** the antigen mixture is first electrophoresed to separate its components by charge. Troughs are then cut into the agar gel parallel to the direction of the electric field, and antiserum is added to the troughs. Antibody and antigen then diffuse toward each other and produce lines of precipitation where they meet in appropriate proportions (Figure). Immunoelectrophoresis is used in clinical laboratories to detect the presence or absence of proteins in the serum. A sample of serum is electrophoresed, and the individual serum components are identified with antisera specific for a given protein or im-munoglobulin class (Figure).

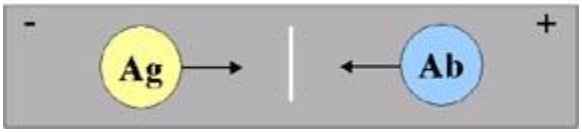
This technique is useful in determining whether a patient produces abnormally low amounts of one or more isotypes, characteristic of certain immunodeficiency diseases. It can also show whether a pa-tient overproduces some serum protein, such as albumin, immunoglobulin, or transferrin. The immunoelectropho-retic pattern of serum from patients with multiple myeloma, for example, shows a heavy distorted arc caused by the large amount of myeloma protein, which is monoclonal Ig and therefore uniformly charged (Figure 6-6b). Because immu-noelectrophoresis is a strictly *qualitative* technique.

A related *quantitative* technique, **rocket electrophore-sis,** does permit measurement of antigen levels. In rocket electrophoresis**,** a negatively charged antigen is elec-trophoresed in a gel containing antibody. The precipitate formed between antigen and antibody has the shape of a rocket, the height of which is proportional to the concen-tration of antigen in the well. One limitation of rocket electrophoresis is the need for the antigen to be negatively charged for electrophoretic movement within the agar matrix. Some proteins, immunoglobulins for example, are not sufficiently charged to be quantitatively analyzed by rocket electrophoresis; nor is it possible to measure the amounts of several antigens in a mixture at the same time.



Countercurrent electrophoresis

In this test the antigen and antibody are placed in wells punched out of an agar gel and the antigen and antibody are electrophoresed into each other where they form a precipitation line as illustrated in Figure 15. This test only works if conditions can be found where the antigen and antibody have opposite charges. This test is primarily qualitative, although from the thickness of the band you can get some measure of quantity. Its major advantage is its speed.



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.

Fluorescent molecules absorb light of one wavelength (excitation) and emit light of another wavelength (emission). If antibody molecules are tagged with a fluorescent dye, or **fluorochrome,** immune complexes containing these fluorescently labeled antibodies (FA) can be detected by colored light emission when excited by light of the appropriate wave-length. Antibody molecules bound to antigens in cells or tissue sections can similarly be visualized. The emitted light can be viewed with a fluorescence microscope, which is equipped with a UV light source. In this technique, known as **immunofluorescence,** fluorescent compounds such as fluorescein and rhodamine are in common use, but other highly fluorescent substances are also routinely used, such as phycoerythrin, an intensely colored and highly fluorescent pigment obtained from algae. These molecules can be conjugated to the Fc region of an antibody molecule without affecting the specificity of the antibody. Each of the fluo-rochromes below absorbs light at one wavelength and emits light at a longer wavelength:

Fluorescein, an organic dye that is the most widely used label for immunofluorescence procedures, absorbs blue light (490 nm) and emits an intense yellow-green fluorescence (517 nm).

Rhodamine, another organic dye, absorbs in the yellow-green range (515 nm) and emits a deep red fluorescence (546 nm). Because it emits fluorescence at a longer wavelength than fluorescein, it can be used in two-color immunofluorescence assays. An antibody specific to one determinant is labeled with fluorescein, and an antibody recognizing a different antigen is

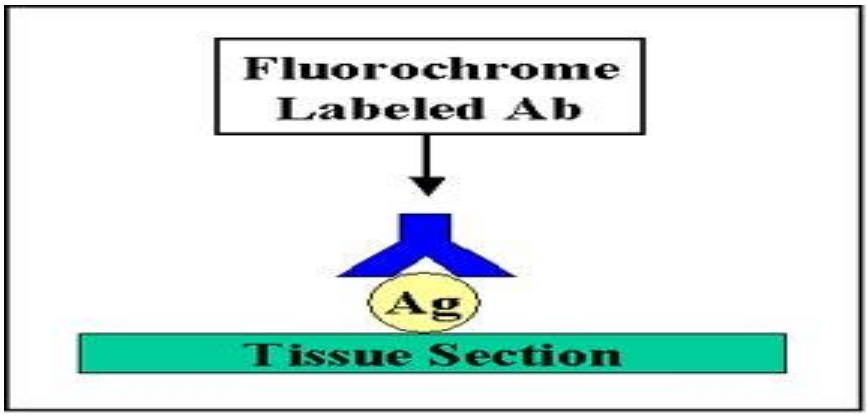
labeled with rhodamine. The location of the fluorescein-tagged antibody will be visible by its yellow-green color, easy to distinguish from the red color emitted where the rhodamine-tagged antibody has bound. By conjugating fluorescein to one antibody and rhodamine to another antibody, one can, for example, visualize simultaneously two different cell-membrane antigens on the same cell.

Phycoerythrin is an efficient absorber of light (~30-fold greater than fluorescein) and a brilliant emitter of red fluorescence, stimulating its wide use as a label for immunofluorescence.

Fluorescent-antibody staining of cell membrane mole-cules or tissue sections can be direct or indirect (Figure).

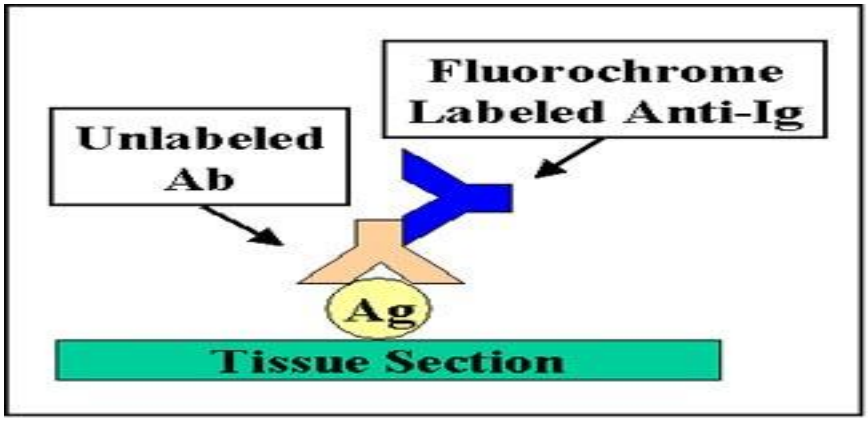
Direct Immunofluorescence

In direct immunofluorescence, the antibody specific to the antigen is directly tagged with the fluorochrome (Figure). The antibody recognizes the target molecule and binds to it, and the fluorophore it carries can be detected via microscopy. This technique has several advantages over the secondary (or indirect) protocol below because of the direct conjugation of the antibody to the fluorophore. This reduces the number of steps in the staining procedure making the process faster and can reduce background signal by avoiding some issues with antibody cross-reactivity or non-specificity



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 (Figure 21). Indirect fluorescence is more sensitive than direct immunofluorescence since there is amplification of the signal.



Indirect immunofluorescence staining has two advantages over direct staining. First, it avoid the loss of antibody that usually occurs du-ing the conjugation reaction. Second, indirect methods increase the sensitivity of staining

Applications

- Immunofluorescence has been applied to identify a number of subpopulations of lymphocytes, notably the CD4 and CD8 T-cell subpopulations.
- The technique is also suit-able for identifying bacterial species, detecting Ag-Ab com-plexes in autoimmune disease,
- detecting complement components in tissues,
- and localizing hormones and other cellular products stained in situ.
- Indeed, a major application of the fluorescent-antibody technique is the localization of antigens in tissue sections or in subcellular compartments. Because it can be used to map the actual location of target antigens, fluorescence microscopy is a powerful tool for relat-ing the molecular architecture of tissues and organs to their overall gross anatomy.
- Immunofluorescence can be used on tissue sections, cultured cell lines, or individual cells, and may be used to analyse the distribution of proteins, glycans, and small biological and non-biological molecules

Flow Cytometry and Fluorescence

The fluorescent antibody techniques described are extremely valuable qualitative tools, but they do not give quantitative data. This shortcoming was remedied by development of the flow cytometer, which was designed to automate the **analysis and separation of cells stained with fluorescent antibody**.

The flow cytometer uses a laser beam and light detector to count single intact cells in suspension (Figure 6). Every time a cell passes the laser beam, light is deflected from the

detector, and this interruption of the laser signal is recorded. Those cells having a fluorescently tagged antibody bound to their cell surface antigens are excited by the laser and emit light that is recorded by a second detector system located at a right angle to the laser beam. The simplest form of the instrument counts each cell as it passes the laser beam and records the level of fluorescence the cell emits; an attached computer generates plots of the number of cells as the ordinate and their fluorescence inten-sity as the abscissa. More sophisticated versions of the in-strument are capable of sorting populations of cells into different containers according to their fluorescence profile. Use of the instrument to determine which and how many members of a cell population bind fluorescently labeled antibodies is called analysis; use of the instrument to place cells having different patterns of reactivity into different containers is called cell sorting.

Applications

The flow cytometer has multiple applications to clinical and research problems.

- Flow cytometry now occupies a key position in immunology and cell biology, and it has become an indispensable clinical tool as well. In many medical centers, the flow cytometer is one of the essential tools for the detection and classification of leukemias. It deter-mine the kind and number of white blood cells in blood samples. It would be possible to determine the percentage of T cells in the total white blood cell population. Then, using the cell-sorting capabilities of the flow cytometer, it would be possible to isolate the T-cell fraction of the leukocyte population The choice of treatment for leukemia depends heavily on the cell types involved, making precise identification of the neoplastic cells an essential part of clinical practice.
- Likewise, the rapid measurement of T-cell subpopulations, an important prognostic indicator in AIDS, is routinely done by flow-cytometric analysis. In this procedure, labeled monoclonal antibodies against the major T-cell subtypes bearing the CD4 and CD8 antigens are used to determine their ratios in the patient's blood. When the number of CD4 T cells falls below a certain level, the patient is at high risk for oppor-tunistic infections.

- The size of cells is also derived from analysis of the light-scattering properties of members of the cell population under examination.

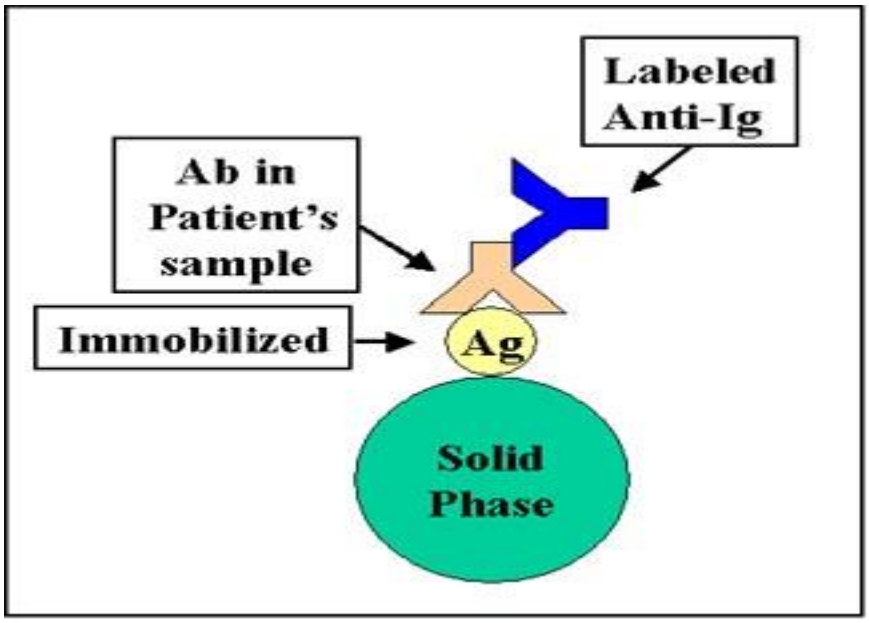
Radioimmunoassay

One of the most sensitive techniques for detecting antigen or antibody is **radioimmunoassay (RIA).** The technique was first developed in 1960 by two endocrinologists, S. A. Berson and Rosalyn Yalow, In 1977, some years after Berson's death, the significance of the technique was acknowledged by the award of a Nobel Prize to Yalow.

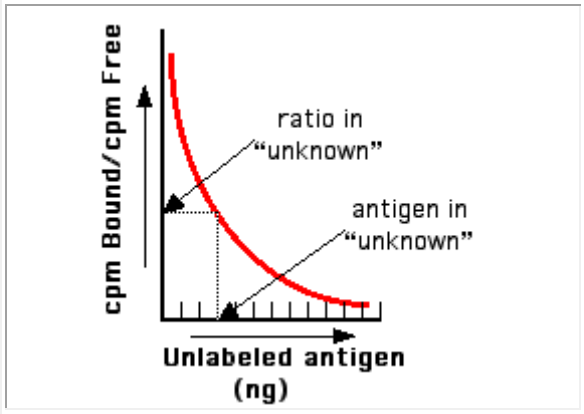
The principle of RIA involves competitive binding of ra-diolabeled antigen and unlabeled antigen to a high-affinity antibody.

Competitive RIA for Ag Detection

To perform a radioimmunoassay, a known quantity of an antigen is made radioactive, frequently by labeling it with gamma-radioactive isotopes of iodine attached to tyrosine. This radiolabeled antigen is then mixed with a known amount of antibody for that antigen, and as a result, the two chemically bind to one another. Then, a sample of serum from a patient containing an unknown quantity of that same antigen is added. This causes the unlabeled (or "cold") antigen from the serum to compete with the radiolabeled antigen ("hot") for antibody binding sites. As the concentration of "cold" antigen is increased, more of it binds to the antibody, displacing the radiolabeled variant, and reducing the ratio of antibody-bound radiolabeled antigen to free radiolabeled antigen. The bound antigens are then separated from the unbound ones, and the radioactivity of the free antigen remaining in the supernatant is measured using a gamma counter. Using known standards, a binding curve can then be generated which allows the amount of antigen in the patient's serum to be derived.



To determine the amount of labeled antigen bound, the Ag-Ab complex is precipitated to separate it from free antigen (antigen not bound to Ab), and the radioactivity in the precipitate is measured. A standard curve can be generated using unlabeled antigen samples of known concentration (in place of the test sample), and from this plot the amount of antigen in the test mixture may be precisely determined.



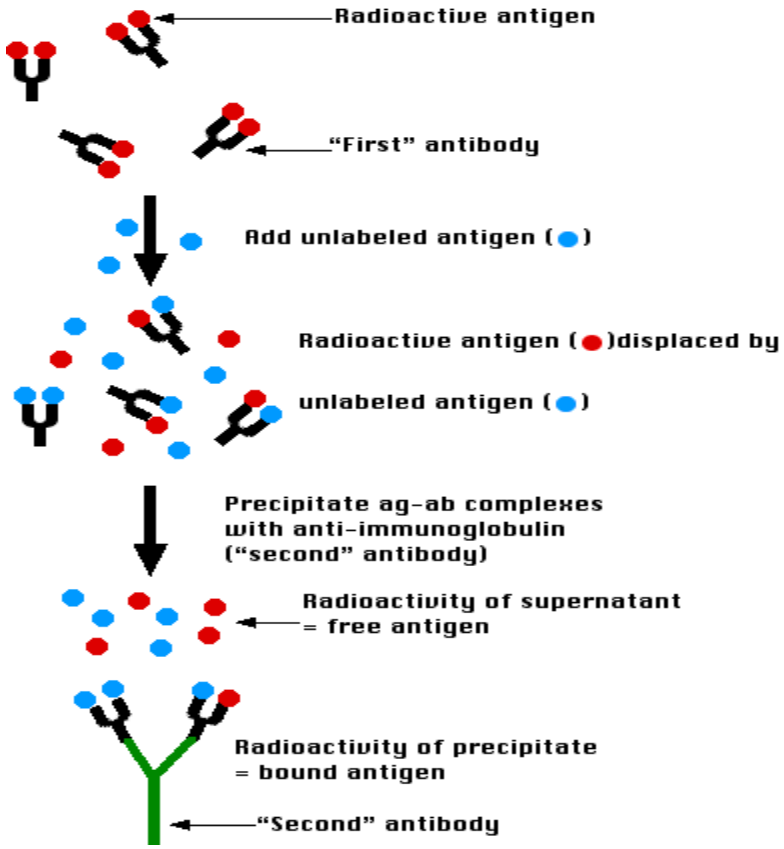
Several methods have been developed for separating the bound antigen from the free antigen in RIA. One method involves precipitating the Ag-Ab complex with a secondary anti-isotype antiserum. For example, if the Ag-Ab complex contains rabbit IgG antibody, then goat anti-rabbit IgG will bind to the rabbit IgG and precipitate the complex. Another method makes use of the fact that protein A of *Staphylococcus aureus* has high affinity for IgG. If the Ag-Ab complex contains an IgG antibody, the complex can be precipitated by mixing with formalin-killed *S. aureus.* After removal of the complex by either of these methods, the amount of free la-beled antigen remaining in the supernatant can be measured in a radiation counter; subtracting this value from the total amount of labeled antigen added yields the amount of la-beled antigen bound.

Various solid-phase RIAs have been developed that make it easier to separate the Ag-Ab complex from the unbound antigen. Alternatively, the antibody can be immobilized on polystyrene or polyvinylchloride wells and the amount of free labeled antigen in the supernatant can be determined in a radiation counter. In another ap-proach, the antibody is immobilized on the walls of mi-crotiter wells and the amount of bound antigen determined. Because the procedure requires only small amounts of sam-ple and can be conducted in small 96-well microtiter plates (slightly larger than a 3 5 card), this procedure is well suited for determining the concentration of a particular antigen in large numbers of samples.

Appliations:

- A microtiter RIA has been widely used to screen for the presence of the hepatitis B virus. RIA screening of donor blood has sharply reduced the incidence of hepatitis B infections in recipients of blood transfusions.
- for measuring hormones, serum proteins, drugs, and vitamins at concentrations of 0.001 *micrograms* per milliliter or less.
- The technique of radioimmunoassay has revolutionized research and clinical practice in many areas, e.g.,
- Blood banking

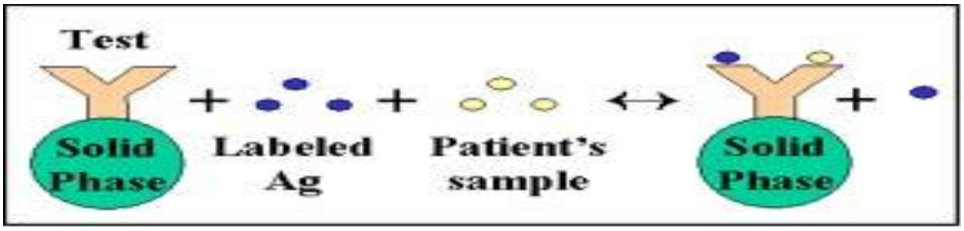
- Diagnosis of allergies

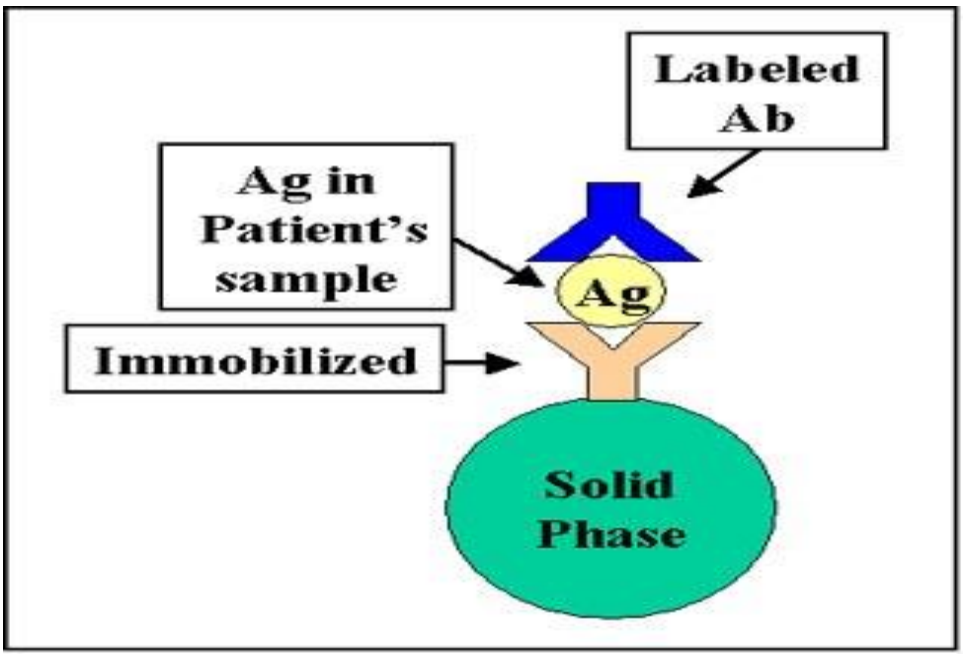


Fig; RIA

Non-competitive RIA for Ag or Ab

Non-competitive RIA and ELISAs are also used for the measurement of antigens and antibodies. In Figure, 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). In Figure, 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.





ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

The principle of Enzyme Linked Immunosorbent Assays (ELISA) is 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.

Performing an ELISA involves at least one antibody with specificity for a particular antigen. The sample with an unknown amount of antigen is immobilized on a solid support (usually a polystyrene microtiter plate) either non-specifically (via adsorption to the surface) or specifically (via capture by another antibody specific to the same antigen, in a "sandwich" ELISA). After the antigen is immobilized, the detection antibody is added, forming a complex with the antigen. The detection antibody can be covalently linked to an enzyme, or can itself be detected by a secondary antibody that is linked to an enzyme through bioconjugation. Between each step, the plate is typically washed with a mild detergent solution to remove any proteins or antibodies that are not specifically bound. After the final wash step, the plate is developed by adding an enzymatic substrate to produce a visible signal, which indicates the quantity of antigen in the sample

INDIRECT ELISA

Antibody can be detected or quantitatively determined with an indirect ELISA (Figure a). Serum or some other sam-ple containing primary antibody (Ab_1) is added to an anti-gen-coated microtiter well and allowed to react with the antigen attached to the well. After any free Ab_1 is washed away, the presence of antibody bound to the antigen is de-tected by adding an enzyme-conjugated secondary anti-iso-type antibody (Ab_2), which binds to the primary antibody. Any free Ab_2 then is washed away, and a substrate for the en-zyme is added. The amount of colored reaction product that forms is measured by specialized spectrophotometric plate readers, which can measure the absorbance of all of the wells of a 96-well plate in seconds.

Indirect ELISA is the method of choice to detect the pres-ence of serum antibodies against human immunodeficiency virus (HIV), the causative agent of AIDS. In this assay, re-combinant envelope and core proteins of HIV are adsorbed as solid-phase antigens to microtiter wells. Individuals in-fected with HIV will produce serum antibodies to epitopes on these viral proteins. Generally, serum antibodies to HIV can be detected by indirect ELISA within 6 weeks of infection

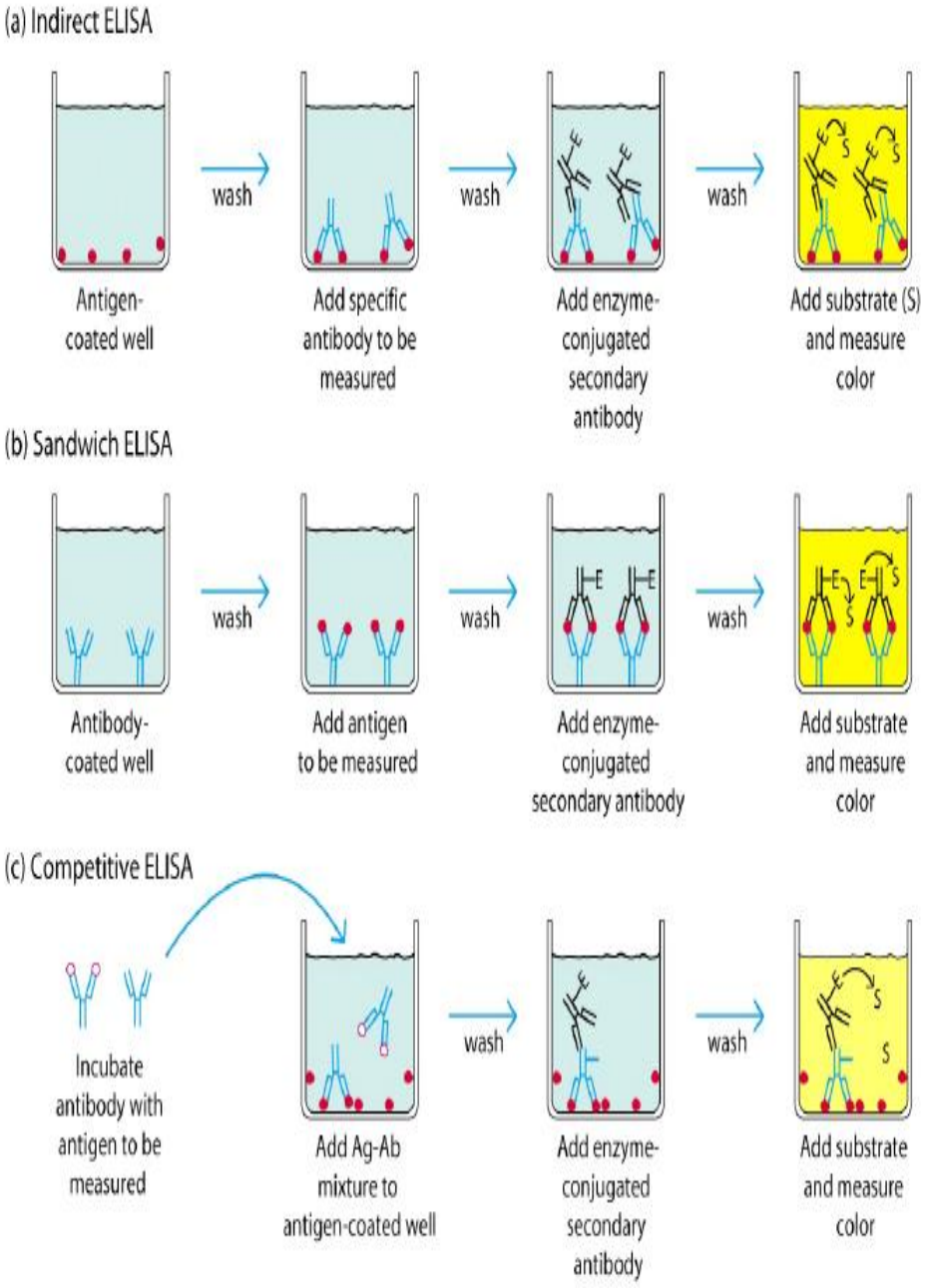
SANDWICH ELISA

Antigen can be detected or measured by a sandwich ELISA (Figure b). In this technique, the antibody (rather than the antigen) is immobilized on a microtiter well. A sample containing antigen is added and allowed to react with the immobilized antibody. After the well is washed, a second en-zyme-linked antibody specific for a different epitope on the antigen is added and allowed to react with the bound anti-gen. After any free second antibody is removed by washing, substrate is added, and the colored reaction product is measured.

COMPETITIVE ELISA

Another variation for measuring amounts of antigen is com-petitive ELISA (Figure c). In this technique, antibody is first incubated in solution with a sample containing antigen. The antigen-antibody mixture is then added to an antigen-coated microtiter well. The more antigen present in the sam-ple, the less free antibody will be available to bind to the antigen-coated well. Addition of an enzyme-conjugated sec-ondary antibody (Ab_2) specific for the isotype of the primary antibody can be used to determine the amount of primary antibody bound to the well as in an indirect ELISA. In the competitive assay, however, the higher the concentration of antigen in the original sample, the lower the absorbance

Immunologic tests: Types of ELISA's…



The steps of "indirect" ELISA follows the mechanism below:-

A buffered solution of the antigen to be tested for is added to each well of a microtiter plate, where it is given time to adhere to the plastic through charge interactions.

A solution of non-reacting protein, such as bovine serum albumin or casein, is added to block any plastic surface in the well that remains uncoated by the antigen.

Next the primary antibody is added, which binds specifically to the test antigen that is coating the well. This primary antibody could also be in the serum of a donor to be tested for reactivity towards the antigen.

Afterwards, a secondary antibody is added, which will bind the primary antibody. This secondary antibody often has an enzyme attached to it, which has a negligible effect on the binding properties of the antibody.

A substrate for this enzyme is then added. Often, this substrate changes color upon reaction with the enzyme. The color change shows that secondary antibody has bound to primary antibody, which strongly implies that the donor has had an immune reaction to the test antigen. This can be helpful in a clinical setting, and in R&D.

The higher the concentration of the primary antibody that was present in the serum, the stronger the color change. Often a spectrometer is used to give quantitative values for color strength.

The enzyme acts as an amplifier; even if only few enzyme-linked antibodies remain bound, the enzyme molecules will produce many signal molecules. A major disadvantage of the indirect ELISA is that the method of antigen immobilization is non-specific; when serum is used as the source of test antigen, all proteins in the sample may stick to the microtiter plate well, so small concentrations of analyte in serum must compete with other serum proteins when binding to the well surface. The sandwich or direct ELISA provides a solution to this problem, by using a "capture" antibody specific for the test antigen to pull it out of the serum's molecular mixture.

In quantitative ELISA, the optical density (OD) of the sample is compared to a standard curve, which is typically a serial dilution of a known-concentration solution of the target molecule.

IMMUNO BLOTTING

Identification of a specific protein in a complex mixture of proteins can be accomplished by a technique known as **Western blotting,** named for its similarity to Southern blotting, which detects DNA fragments, and Northern blotting, which detects mRNAs. In Western blotting, a protein mixture is electrophoretically separated on an **SDS-polyacrylamide gel (SDS-PAGE),** a slab gel infused with sodium dodecyl sulfate (SDS), a dissociating agent (Figure 6-12). The protein bands are transferred to a nylon membrane by electrophoresis and the individual protein

bands are identified by flooding the nitrocellulose membrane with radiolabeled or enzyme-linked polyclonal or monoclonal antibody specific for the protein of interest. The Ag-Ab complexes that form on the band containing the protein recognized by the antibody can be visualized in a variety of ways. If the protein of interest was bound by a radioactive antibody, its position on the blot can be determined by exposing the membrane to a sheet of x-ray film, a procedure called autoradiography. However, the most generally used detection procedures employ enzyme-linked antibodies against the protein. After binding of the enzyme-antibody conjugate, addition of a chromogenic substrate that produces a highly colored and insoluble product causes the appearance of a colored band at the site of the target antigen. The site of the protein of interest can be determined with much higher sensitivity if a chemiluminescent compound along with suitable enhancing agents is used to produce light at the antigen site.

Western blotting can also identify a specific antibody in a mixture. In this case, known antigens of well-defined molec-ular weight are separated by SDS-PAGE and blotted onto ni-trocellulose. The separated bands of known antigens are then probed with the sample suspected of containing antibody specific for one or more of these antigens. Reaction of an an-tibody with a band is detected by using either radiolabeled or enzyme-linked secondary antibody that is specific for the species of the antibodies in the test sample. The most widely used application of this procedure is in confirmatory testing for HIV, where Western blotting is used to determine whether the patient has antibodies that react with one or more viral proteins

MONOCLONAL ANTIBODIES (MAB)

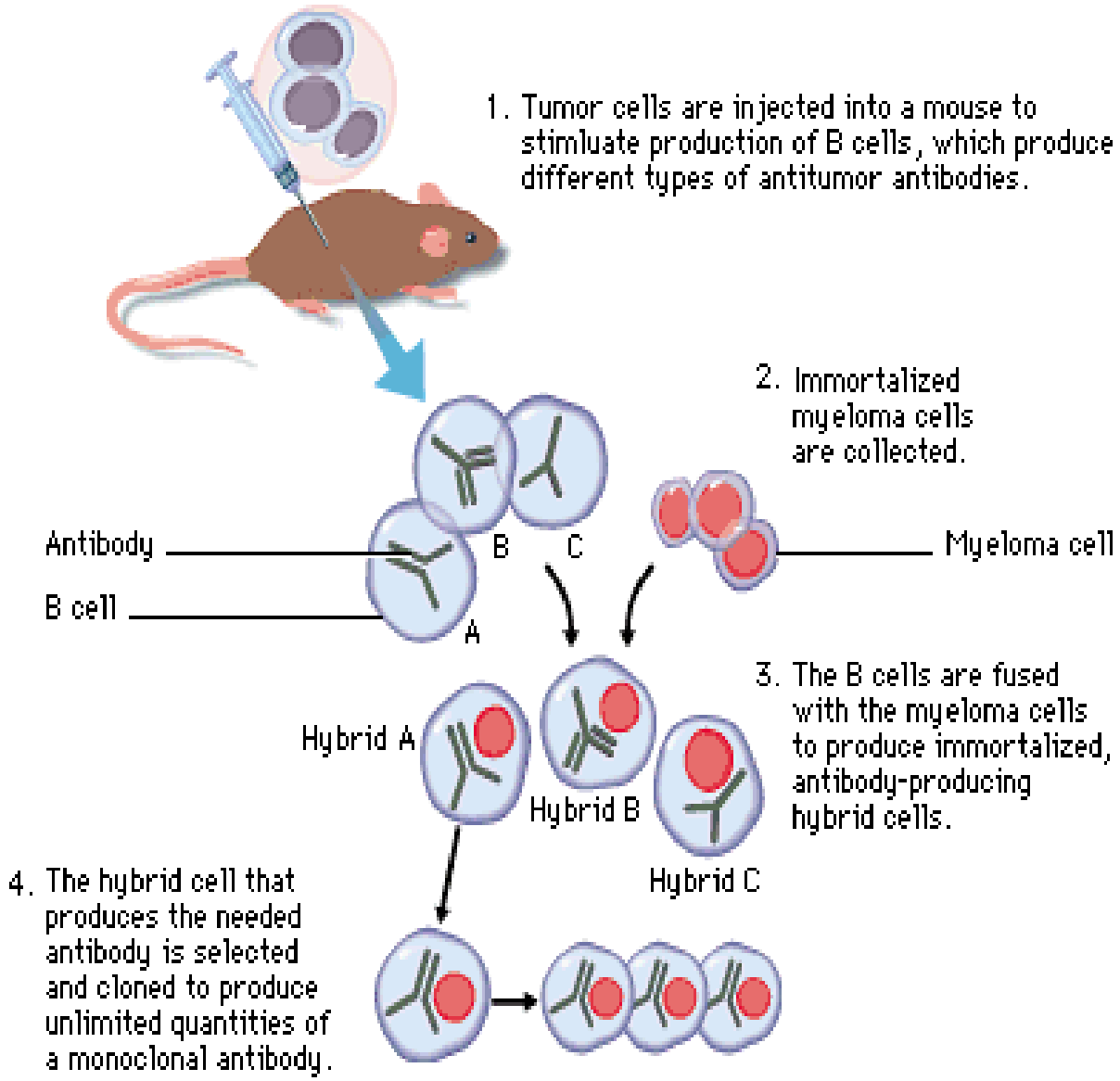
Production:

- fusion of cancerous tumor cells + B-lymphocytes = hybridomas
    - use tumor cells because, by being cancerous, they divide without limit
    - use B-lymphocytes which are a clone producing the antibody desired
    - thus, the hybridomas produce specific antibodies in large quantities indefinitely
- Monoclonal antibodies are typically made by fusing myeloma cells with the spleen cells from a mouse that has been immunized with the desired antigen. However, recent advances have allowed the use of rabbit B-cells. Polyethylene glycol is used to fuse adjacent plasma membranes, but the success rate is low so a selective medium (HAT-Hypoxanthine, Amiopterin and Thymidine)) in which only fused cells can grow is used.
- The selective culture medium is called HAT medium because it contains hypoxanthine, aminopterin, and thymidine. This medium is selective for fused (hybridoma) cells. Unfused myeloma cells cannot grow because they lack HGPRT, and thus cannot replicate their DNA. Unfused spleen cells cannot grow indefinitely because of their limited life span. Only fused hybrid cells, referred to as hybridomas, are able to grow indefinitely in

the media because the spleen cell partner supplies HGPRT and the myeloma partner has traits that make it immortal (as it is a cancer cell).

- This mixture of cells is then diluted and clones are grown from single parent cells on microtitre wells. The antibodies secreted by the different clones are then assayed for their ability to bind to the antigen (with a test such as ELISA or Antigen Microarray Assay) or immuno-dot blot. The most productive and stable clone is then selected for future use.
- The hybridomas can be grown indefinitely in a suitable cell culture medium.They can also be injected into mice (in the peritoneal cavity, surrounding the gut). There, they produce tumors secreting an antibody-rich fluid called ascites fluid.

Hybridoma cell production



MAB Applications

i) Diagnostic tests

Monoclonal antibodies are proving to be very useful as diagnostic, imaging, and therapeutic reagents in clinical medi cine. Tthe monoclonal antibody diagnostic reagents now available are

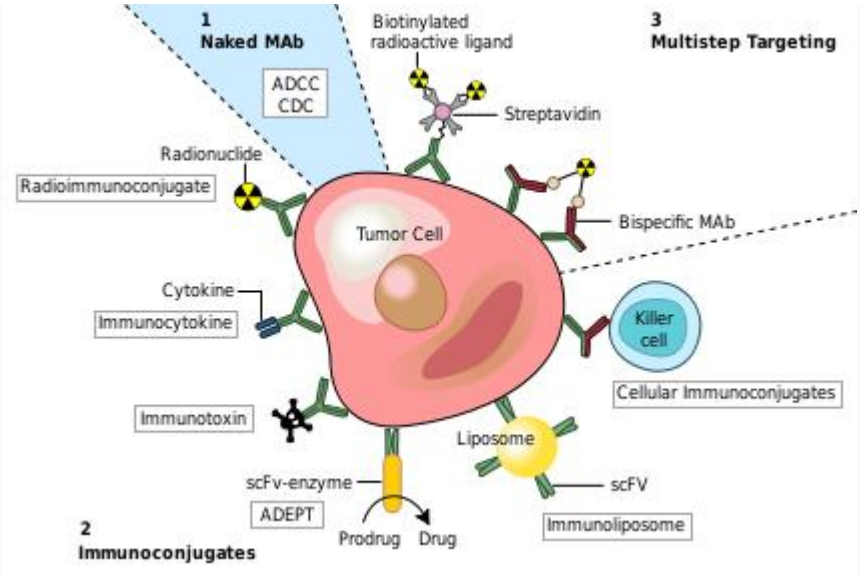
- for detecting pregnancy,
- diagnosing numerous pathogenic mi-croorganisms(HIV infection),
- measuring the blood levels of various drugs,
- matching histocompatibility antigens, and
- **detecting antigens shed by certain tumors:**
  Radiolabeled monoclonal antibodies can also be used in vivo for detecting or locating tumor antigens, permitting ear-lier diagnosis of some primary or metastatic tumors in patients. For example, monoclonal antibody to breast-cancer cells is labeled with iodine-131 and introduced into the blood to detect the spread of a tumor to regional lymph nodes. This monoclonal imaging technique can reveal breast-cancer metastases that would be undetected by other, less sensitive scanning techniques.

ii) Therapeutic treatment

- monoclonal antibodies can be produced which selectively locate and adhere to cancer cells
- anti-cancer drugs can be attached to the monoclonal antibodies
  - so that they deliver their effects directly to the targeted cancer cells

Cancer treatment

One possible treatment for cancer involves monoclonal antibodies that bind only to cancer cell-specific antigens and induce an immunological response against the target cancer cell. Such mAb could also be modified for delivery of a toxin, radioisotope, cytokine or other active conjugate; it is also possible to design bispecific antibodies that can bind with their Fab regions both to target antigen and to a conjugate or effector cell. In fact, every intact antibody can bind to cell receptors or other proteins with its Fc region.



Monoclonal antibodies for cancer. ADEPT, antibody directed enzyme prodrug therapy; ADCC, antibody dependent cell-mediated cytotoxicity; CDC, complement dependent cytotoxicity; MAb, monoclonal antibody; scFv, single-chain Fv fragment.[20]

The illustration below shows all these possibilities:

MAbs approved by the FDA include

- Bevacizumab
- Cetuximab
- Panitumumab
- Traztuzumab

Immunotoxins composed of tumor-specific monoclonal antibodies coupled to lethal toxins are potentially valuable therapeutic reagents. The toxins used in preparing immuno-toxins include ricin, *Shigella* toxin, and diphtheria toxin, all of which inhibit protein synthesis. These toxins are so potent that a single molecule has been shown to kill a cell. Each of these toxins consists of two types of functionally distinct polypeptide components, an inhibitory (toxin) chain and one or more binding chains, which interact with receptors on cell surfaces; without the binding polypeptide(s) the toxin cannot get into cells and therefore is harmless. An immuno-toxin is prepared by replacing the binding polypeptide(s) with a monoclonal antibody that is specific for a particular tumor cell (Figure). In theory, the attached mono-clonal antibody will deliver the toxin chain specifically to tu-mor cells, where it will cause death by inhibiting protein synthesis (Figure) . The initial clinical responses to such immunotoxins in patients with

leukemia, lymphoma, and some other types of cancer have shown promise, and re-search to develop and demonstrate their safety and effective-ness is underway.

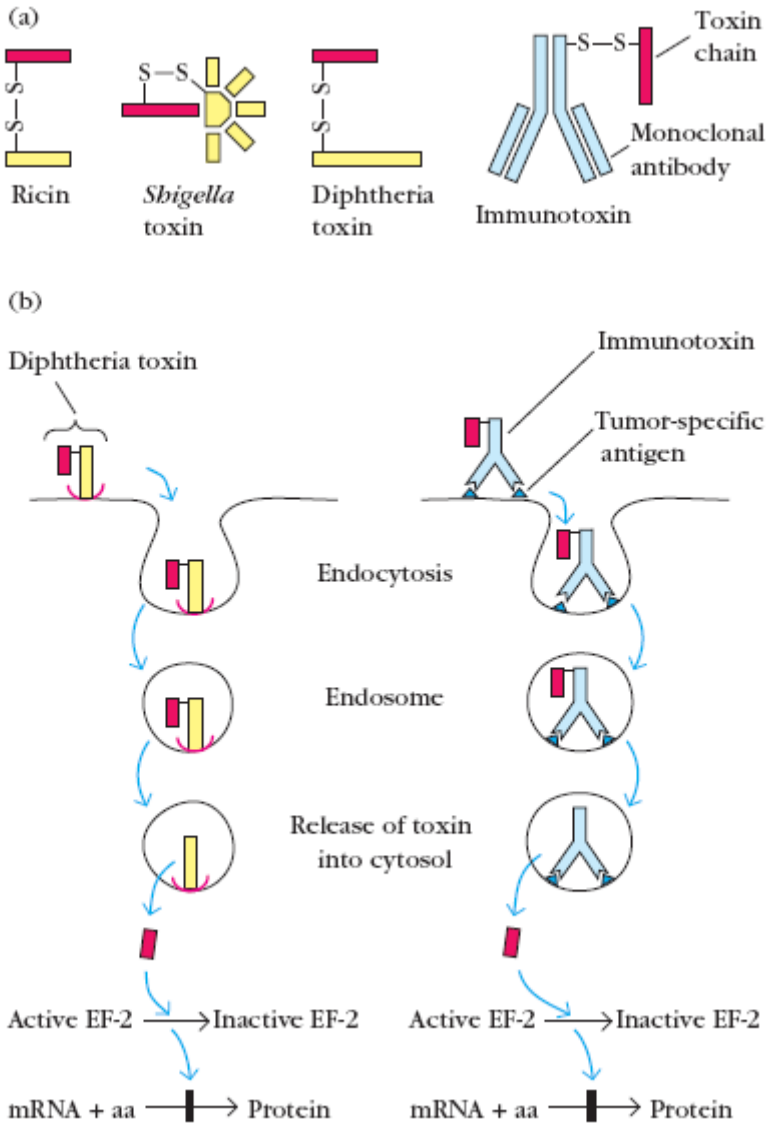


Fig:Toxins used to prepare immunotoxins include ricin, *Shigella* toxin, and diphtheria toxin. Each toxin contains an in-hibitory toxin chain (red) and a binding component (yellow). To make an immunotoxin, the binding component of the toxin is replaced with a monoclonal antibody (blue). (b) Diphtheria toxin binds to a cell-membrane receptor (*left*) and a diphtheria-immunotoxin binds to a tumor-associated antigen (*right*). In either case, the toxin is in-ternalized in an endosome. The toxin chain is then released into the cytoplasm, where it inhibits protein synthesis by catalyzing the inac-tivation of elongation factor 2 (EF-2).

Autoimmune diseases

Monoclonal antibodies used for autoimmune diseases include infliximab and adalimumab, which are effective in rheumatoid arthritis, Crohn's disease and ulcerative Colitis by their ability to bind to and inhibit TNF-α. Basiliximab and daclizumab inhibit IL-2 on activated T cells and thereby help prevent acute rejection of kidney transplants. Omalizumab inhibits human immunoglobulin E (IgE) and isused in severe allergic asthma

Abzymes Are Monoclonal Antibodies That Catalyze Reactions

The catalytic activity of these antibodies was highly specific; that is, they hydrolyzed only esters whose transition-state structure closely resembled the transition state analogue used as a hapten in the immu-nizing conjugate. These catalytic antibodies have been called **abzymes** in reference to their dual role as antibody and enzyme.

A central goal of catalytic antibody research is the deriva-tion of a battery of abzymes that cut peptide bonds at specific amino acid residues, much as restriction enzymes cut DNA at specific sites. Such abzymes would be invaluable tools in the structural and functional analysis of proteins. Addition-ally, it may be possible to generate abzymes with the ability to dissolve blood clots or to cleave viral glycoproteins at specific sites, thus blocking viral infectivity.

UNIT- V

1	Precipitins are antibodies that insolublise	Ag	Ab	Ag-Ab	antigenic determinants	Ag
2	Antibodies are …….molecules	Mono	Di	unique	poly	unique
3	An ……that produces a precipitate when mixed with antigen solution	Ag	Ab	Ag-Ab	Antiserum	Antiserum
4	T he quantitative precipitin test,it was developed by	Kendall	Heidelberg	Heidelberg &Kendall	Rodney porter	Heidelberg &Kendall
5	The VDRL test is used to diagnosis	AIDS	Gonorrhea	Genital Herpus	Syphilis	Syphilis
6	The binding of complement to Ag-Ab complex is called	antigen binding	complement fixation	antibody binding	non-complement fixation	complement fixation
7	ELISA technique was first introduced by	Kohlar &Milstein	Engral&Perlma	Dreyer&Burnet	Rodney porter	Engral&Perlma
8	Set of antigens on the surface of all nucleated cells of human bidy	Transplantation ag	human leucocyte ag	Thymus dependent ag	thymus independent ag	human leucocyte ag
9	Hybridoma technology was developed by	Kohlar &Milstein	Engral&Perlman	Dreyer&Burnet	Rodney porter	Kohlar &Milstein
10	A method of measuring Ag or Ab using radiolabelled Ag	ELISA	DIBA	RIA	Immunodiffusion	RIA
11	Well-felix reaction is used to test	typhus	malaria	cholera	small pox	typhus
12	Which test is used for the detection of anti Rh Ab	Widal test	Coomb's test	Rh blood typing	Well-felix reaction	Coomb's test
13	Test used for the diagnosis of typhoid fever	Widal test	Coomb's test	Rh blood typing	Well-felix reaction	Widal test
14	Anti-streptomycin-O test is used to detect	rheumatoid fever	malaria	typhus	cholera	cholera
15	Corticosteroids &cyclosporin are used in prevention of	cancer	graft rejection	typhus	cholera	graft rejection
16	Which are involved in cytotoxicity and destruction of tumor cells	Macrophage	T cells	Phagocytes	NK cells	NK cells
17	……..are used for the treatment of autoimmune disease	Corticosteroids&cyclosporin	chloramphenicol	Quinone	Sulphonamides	Corticosteroids&cyclosporin
18	…….is used for the treatment of lepromatous leprosy	µ interferon	b interferon	¶ interferon	g interferon	g interferon
19	Burkitti lymphoma is caused by	Hepatitis-B virus	Epstein-Barr virus	Herpes virus	Retro virus	Epstein-Barr virus
20	Test which is used to detect both Ag & Ab using labeled anti- human Ab	Solid phase RIA	Single Radial immunodiffusion	Radial immunodiffusion	Single linear immunodiffusion	Solid phase RIA
21	Gastric auto-Ag reacting with auto Ab present in serum of patient suffering from	Pernicious anaemia	Haemolytic anaemia	rheumatoid fever	malaria	Pernicious anaemia
22	Which test is used to detect autoantibodies and antibodies to tissue & cellular antigen	Immunoassay	Complement fixation	Immunofluorescence	Immunoelectrophoresis	Immunofluorescence
23	The complement fixation test detects	Antigen	Antibody	lymphocytes	Rh factor	Antibody
24	Identification of specific protein in complex mixture of proteins can be accomplished by	Northern blotting	Western blotting	Southern blotting	Southern hybridisation	Western blotting
25	The technique used for the isolation of the antigen of interest for further analysis	Immunoprecipitation	Immunodiffusion	Immunofluorescence	Complement fixation	Immunoprecipitation
26	……is the most widely used organic dye for the immunofluorescence procedure	crystal violet	Azo dyes	bromophenol blue	Fluorescein	Fluorescein
27	……is the injection of Ag into body to produce immunity and protect against disease	Innate immunity	Vaccination	Complement fixation	Passive immunity	Vaccination
28	Vaccines prepared from the toxins and chemicals of microbes	Cellular	sub-cellular	Attenuated	Homologous	sub-cellular
29	Vaccines prepared from the polysaccharide or protein units of bacteria	Cellular	sub-cellular	Toxoid	sub unit	sub unit
30	The immunity transferred from the mother to the child	Natural passive immunization	Active immunization	cell mediated	Humoral	Natural passive immunization
31	Substance,which stimulatespecific immune response when introduced intobody	Antibody	Antiserum	Immunogen	Allergen	Allergen
32	The immunization of an individual with antigens from within its own species	Alloimmunization	Active immunization	cell mediated	Autoimmunization	Alloimmunization
33	The strength of binding between antigen and antibody is termed	Fixation	Avidity	Atopy	Eczema	Avidity
34	Having the ability to step cell growth	Cytostatic	cytotoxic	cytophilic	cytokines	cytokines
35	Having the ability to kill cells	Cytostatic	cytotoxic	cytophilic	cytokines	cytophilic
36	A large, primitive looking, cell capable of division & differentiation	B cell	T cell	NK cell	Blast cell	B cell
37	The substance, which increase immunity response to an antigen	allergen	alloantigens	adjuvants	aggluitnogens	adjuvants
38	Erythema causes inflammation in redness of ----------	Skin	lung	tissues	liver	Skin
39	Second antibody molecules in the sandwich technique are simply	gloublins	antigens	anti- gloublins	antibody - gloublins	anti- gloublins
40	Ab against immunogloublin,by injecting immunogloubulin intoanimal of other	Antiserum	antitoxins	antibodies	anti- gloublins	anti- gloublins
41	A local inflammatory reaction due to a type III hypersensitive reaction	Asthma	Arthus reaction	haemolysis	oesnophilia	Arthus reaction
42	An animal that contains cells from two or more genetically different individual	Chimera	genera	non-genera	non-chimera	Chimera
43	Cell surface molecule classified accordingtointernationally accepted differentiation	haptens	epitopes	CD molecule	Interferon	CD molecule
44	Which of the following is a quantitative precipitation technique	Gel diffusion	diffusion	immunoelectrophoresis	radial immunodiffusion	radial immunodiffusion
45	Which of these antibody assays is primary binding test	Fluorescent antibody	immunoelectrophoresis	Agglutination	Complement fixation	Fluorescent antibody
46	Serum IgM levels may be measured by means of	RID	direct agglutination	passive agglutination	Active agglutination	passive agglutination
47	The gel diffusion technique is	1^0 binding test	2^0 binding test	3^0 binding test	4^0 binding test	2^0 binding test
48	The most sensitive immunological test in terms of the amount of Ab detectable is	RIA	RID	ELISA	gel-precipitation test	RIA
49	Incomplete antibodies are antibodies that	lack a Fc region	lack a Fab region	cannot bind Ag	cannot agglutinate Ag	cannot agglutinate Ag
50	The ligand for the small protein avidin is	Ig	biotin	Fluorescein	Ferrilin	Ferrilin
51	Which of these is secondary binding test	ELISA	RIA	Immunoelectrophoresis	mouse protection test	Immunoelectrophoresis
52	Immune complex precipitates are formed in	Ag excess	Ab excess	The zone of equivalence of Ag&Ab	Absence of electrolyte	The zone of equivalence of Ag&Ab
53	Antiglobulins are	Incomplete Ag	Abs against Ig	Agglutinating Ab	None of these	Abs against Ig
54	The major forces linking Ag&Ab are	hydrogen bonds	Covalent bonds	Ionic bonds	Hydrophobic bonds	Hydrophobic bonds
55	The Ag –combining site of an antibody molecule determines its	Isotype	Idiotype	Allotype	Hypertype	Hypertype
56	One major vaccine component that causes allergic reaction	Egg Ag	Viral Ag	Bacterial Ag	Endotoxin	Viral Ag
57	Deficiency of which minerals is most likely to lead to an immunodeficiency	Calcium	Zinc	Lead	Iron	Zinc
58	The drug that is mainly used to treat AIDS patients is	Azidiothymidine	Tetracyclin	Imuran	Cortisone	Azidiothymidine
59	Which of the following blood group Ags is not determined by carbohydrate epitopes	A	Rhesus	O	B	Rhesus
60	The complement present in higher concentration in the blood is	C_4	C_1	C_3	C_5	C_3

Reg. No.....

[15BCU503]

KARPAGAM UNIVERSITY
Karpagam Academy of Higher Education
(Established Under Section 3 of UGC Act 1956)
COIMBATORE – 641 021
(For the candidates admitted from 2015 onwards)

B.Sc., DEGREE EXAMINATION, NOVEMBER 2017
Fifth Semester

BIOCHEMISTRY

IMMUNOLOGY

Time: 3 hours Maximum : 60 marks

PART – A (20 x 1 = 20 Marks) (30 Minutes)
(Question Nos. 1 to 20 Online Examinations)

PART B (5 x 8 = 40 Marks) (2 ½ Hours)
Answer ALL the Questions

21. a. Give a brief account on acquired immunity.
Or
b. Explain the structure and functions of lymph node.

22. a. Define – Antigenecity, epitope, adjuvant, hapten.
Or
b. Explain the structure of immunoglobulin.

23. a. Discuss on anaphylactic reaction.
Or
b.Explain the classical pathway of complement activation.

24. a. Give a detailed note on Myasthenia gravis and Rheumatoid arthritis.
Or
b. Discuss about live and attenuated vaccine.

25. a. Write a detailed note on precipitation reaction.
Or
b. Explain about ELISA.

Reg. No.....

[12BCU502]

KARPAGAM UNIVERSITY

(Under Section 3 of UGC Act 1956)
COIMBATORE – 641 021
(For the candidates admitted from 2012 onwards)

B.Sc. DEGREE EXAMINATION, NOVEMBER 2014

Fifth Semester

BIOCHEMISTRY

INTRODUCTORY IMMUNOLOGY

Time: 3 hours Maximum : 100 marks

PART – A (15 x 2 = 30 Marks)
Answer ALL the Questions

1. Enumerate the secondary lymphoid organs and their functions
2. Brief about the cells of immune system
3. Narrate the role of thymus, bone marrow in immune system
4. List the properties of antigen
5. What is hapten? Give the importance of it
6. What is MHC? Give its role in immunity
7. Define the term hypersensitivity? Narrate its classification
8. What is the mechanism behind the type III hypersensitivity?
9. Enumerate the pathogenesis of type IV hypersensitivity
10. Brief the role of MHC in rejection of transplant
11. Give an illustration on myaethenia gravis
12. Illustrate the pathology behind the auto immunity with an example
13. Give the principle of immuno electrophoresis
14. Add note on the principle of fluorescent antibody technique
15. Give the principle of RIA

PART B (5 X 14= 70 Marks)
Answer ALL the Questions

16. a) Discuss in detail about the classes and subclasses of antibodies with neat diagram

Or

b) Explain the following :
i) Mechanism of cell mediated immunity ii) Adjuvants

17. a) Explain the following
i) Mechanism of Type II hypersensitive reaction
ii) Classical pathway of complement activation

Or

b) Discuss in detail about the causes, mechanism, pathogenesis and treatment of Type I hypersensitive reaction

18. a) Explain the mechanism of graft rejection and also add note on role of immuno suppressors in transplantation

Or

b) Explain the following : i) Rheumatoid arthritis ii) AIDS

19. a) Explain in detail about the principle and application and types of ELISA

Or

b) Explain the following : i) Recombinant vaccines ii) Immuno diffusion

20. **Compulsory : -**

i. Why spleenectomy persons are susceptible to bacterial infection

ii. Why might the body requires two phagocytic cell system-the myeloid and mononuclear phagocytic system (Macrophage and monocytes). What advantage were there in having two system.

Reg. No. : ----------

[15BCU503]

KARPAGAM ACADEMY OF HIGHER EDUCATION
(Deemed to be University *established Under Section 3 of UGC Act 1956*)
DEPARTMENT OF BIOCHEMISTRY
III B.Sc., BIOCHEMISTRY - Fifth Semester
15BCU503- IMMUNOLOGY
MODEL EXAMINATION – September 2017

Date: **Maximum Marks :60**
Time: 3 hrs

SECTION – A (20 X 1 = 20 marks)
Answer ALL the questions

1. Memory B cells and Plasma B cells are called as
   a. Mast cells b. Mesocells
   c. **Effector cells** d. Membrane cells

2. Macrophages present in kidney is known as
   a. **Mesangial cells** b. Microglial cells
   c. Kupffer cells d. Histiocytes

3. Plasma cells have
   a. **Have a highly developed rough endoplasmic reticulum**
   b. Are derived from T-cells
   c. Develop into B-cells
   d. Secrete large amounts of gamma interferon

4. An accumulation of fluid results in tissue swelling leads to
   a. **edema** b. erythema
   c. extravasation d. Chemotaxis

5. Among the following which is not an antigen presenting cell
   a. Macrophages b. Dentritic cell
   c. **T cell** d. B cell

6. Which of the following statements does not apply to IgG?:
   a. **Appears early in the primary immune response.**
   b. Neutralizes bacterial toxins.
   c. Can fix complement.
   d. Crosses the human placenta.

7. MHC class II antigens play an important role in
   a. Inflammation b. **Antigen presentation**
   c. Blood clotting d. Extravation

8. Which Immunogloubulin is found abundance in serum
   a. Ig E b. **IgG**
   c. IgM d. IgD.

9. Type I hypersensitivity is acute in nature and mediated by
   a. IgM b. IgA
   c. Ig D d. **IgE**

10. Which type of hypersensitivity resulting mainly from blood transfusion methods.
   a. Type I b. **Type II**
   c. Type III d. Type IV

11. Which is the agent that enhances phagocytosis by immune adherence are
   a. C3 b. C3a
   c. C2a d. **C3b**

12. Classical pathway is a ……dependant
   a. Antigen b. **Ag-Ab**
   c. Antibody d. C3

13. Acute rejection occurs usually in about
   a**. 7 -10 days** b. 10-15 days
   c. 1-3 days d. 15-18 days

14. Trasplantation of two individuals from identical twins are called as
   a. xeno graft c. autograft
   b. **isograft** d. allograft

15. Maternal antibodies present in colostrums provide……..immunity to infant
   a. **passive** b. active
   c. cellular d. humoral

16. The first vaccine was developed by
   a. Louis Pasteur c. **Edward Jenner**
   c. Carl Landsteiner d. Joseph Miester

17. ELISA technique was first introduced by
   a. Kohlar &Milstein b**. Engral&Perlma**
   c. Dreyer&Burnet d. Rodney porter

18. Hybridoma technology was developed by
   a. **Kohlar &Milstein** b. Engral&Perlma
   c. Dreyer&Burnet d. Rodney porter

19. Which test is used for the detection of anti Rh Antibodies
   a. Widal test b. **Coomb's test**
   c. Rh blood typing d. Well-felix reaction

20. Test used for the diagnosis of typhoid fever
   a. **Widal test** b. Coomb's test
   c. Rh blood typing d. Well-felix reaction

SECTION – B (5 X 8 = 40 marks)
Answer ALL the questions

21. a) **Describe the cells of immune system**

The response to pathogens is orchestrated by the complex interactions and activities of the large number of diverse cell types involved in the immune response. The innate immune response is the first line of defense and occurs soon after pathogen exposure. It is carried out by phagocytic cells such as neutrophils and macrophages, cytotoxic natural killer (NK) cells, and granulocytes. The subsequent adaptive immune response includes antigen-specific defense mechanisms and may take days to develop. Cell types with critical roles in adaptive immunity are antigen-presenting cells including macrophages and dendritic cells. Antigen-dependent stimulation of various cell types including T cell subsets, B cells, and macrophages all play critical roles in host defense.

* B Cells
* Dendritic Cells
* Granulocytes
* Innate Lymphoid Cells (ILCs)
* Megakaryocytes
* Monocytes/Macrophages
* Myeloid-derived Suppressor Cells (MDSC)
* Natural Killer (NK) Cells
* Platelets
* Red Blood Cells (RBCs)
* T Cells
* Thymocytes

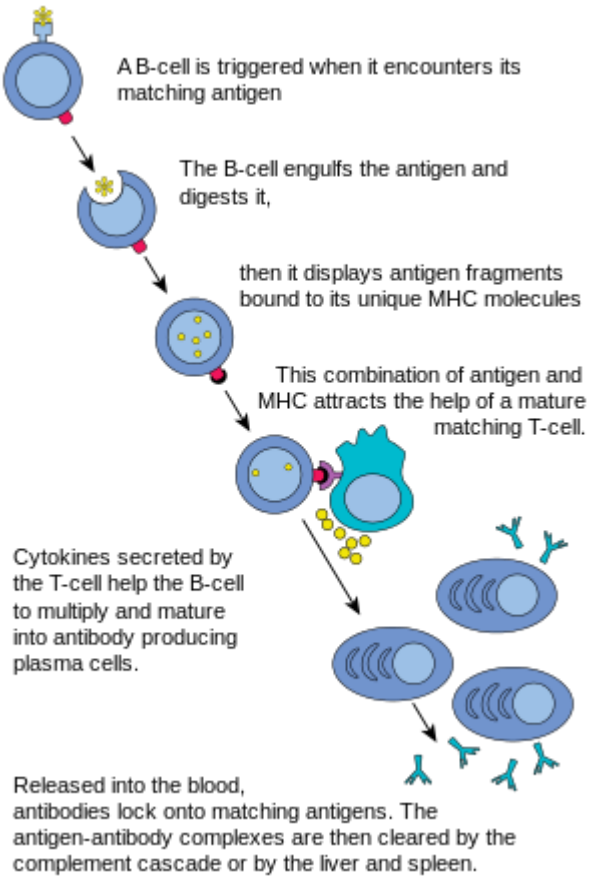
OR

b) **Explain about Humoral and cell mediated immunity**

Humoral immunity or humoural immunity is the aspect of immunity that is mediated by macromolecules found in extracellular fluids such as secreted antibodies, complement proteins, and certain antimicrobial peptides. Humoral immunity is so named because it involves substances found in the humors, or body fluids. It contrasts with cell-mediated immunity. Its aspects involving antibodies are often called antibody-mediated immunity.

The study of the molecular and cellular components that form the immune system, including their function and interaction, is the central science of immunology. The immune system is divided into a more primitive innate immune system, and acquired or adaptive immune system of vertebrates, each of which contains humoral and cellular components.

Humoral immunity refers to antibody production and the accessory processes that accompany it, including: Th2 activation and cytokine production, germinal center formation and isotype switching, affinity maturation and memory cell generation. It also refers to the effector functions of antibodies, which include pathogen and toxin neutralization, classical complement activation, and opsonin promotion of phagocytosis and pathogen elimination



Cell-mediated immunity is an immune response that does not involve antibodies, but rather involves the activation of phagocytes, antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to an antigen.

Historically, the immune system was separated into two branches: humoral immunity, for which the protective function of immunization could be found in the humor (cell-free bodily fluid or serum) and cellular immunity, for which the protective function of immunization was associated with cells. CD4 cells or helper T cells provide protection against different pathogens. Naive T cells, mature T cells that have yet to encounter an antigen, are converted into activated effector T cells after encountering antigen-presenting cells (APCs). These APCs, such as macrophages, dendritic cells, and B cells in some circumstances, load antigenic peptides onto the MHC of the cell, in turn presenting the peptide to receptors on T cells. The most important of these APCs are highly specialized dendritic cells; conceivably operating solely to ingest and present antigens.

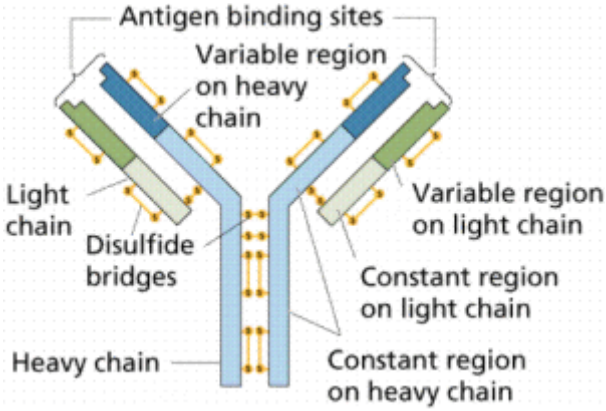
Activated Effector T cells can be placed into three functioning classes, detecting peptide antigens originating from various types of pathogen: The first class being Cytotoxic T cells, which kill infected target cells by apoptosis without using cytokines, the second class being TH1 cells, which primarily function to activate macrophages, and the third class being TH2 cells, which primarily function to stimulate B cells into producing antibodies.

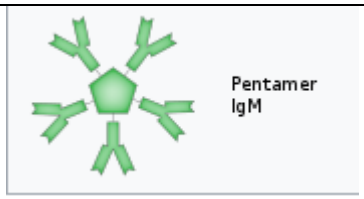
The innate immune system and the adaptive immune system each comprise both humoral and cell-mediated components.

Cellular immunity protects the body by:

T-cell mediated immunity or T-cell immunity : activating antigen-specific cytotoxic T cells that are able to induce apoptosis in body cells displaying epitopes of foreign antigen on their surface, such as virus-infected cells, cells with intracellular bacteria, and cancer cells displaying tumor antigens; activating macrophages and natural killer cells, enabling them to destroy pathogens; and stimulating cells to secrete a variety of cytokines that influence the function of other cells involved in adaptive immune responses and innate immune responses.[citation needed]
Cell-mediated immunity is directed primarily at microbes that survive in phagocytes and microbes that infect non-phagocytic cells. It is most effective in removing virus-infected cells, but also participates in defending against fungi, protozoans, cancers, and intracellular bacteria. It also plays a major role in transplant rejection.

22. a) **Discuss in detail about the classes and subclasses of antibodies with neat diagram**



Name	Type	Description	Antibody
IgA	**2**	**Found in mucosal areas, such as the gut, respiratory tract and urogenital tract, and prevents colonization by pathogens. Also found in saliva, tears, and breast milk.**	
IgD	**1**	**Functions mainly as an antigen receptor on B cells that have not been exposed to antigens. It has been shown to activate basophils and mast cells to produce antimicrobial factor**	
IgE	**1**	**Binds to allergens and triggers histamine release from mast cells and basophils, and is involved in allergy. Also protects against parasitic worms**	
IgG	**4**	**In its four forms, provides the majority of antibody-based immunity against invading pathogens. The only antibody capable of crossing the placenta to give passive immunity to the fetus.**	
IgM	**1**	**Expressed on the surface of B cells (monomer) and in a secreted form (pentamer) with very high avidity. Eliminates pathogens in the early stages of B cell-mediated (humoral) immunity before there is sufficient IgG**	

OR

b) Explain the following

i). **Role of MHC in antigen processing and presentation**

ii). **Adjuvants**

Role of MHC in antigen processing and presentation

MHC class I molecules are one of two primary classes of major histocompatibility complex (MHC) molecules (the other being MHC class II) and are found on the cell surface of all nucleated cells in the bodies of jawed vertebrates. They also occur on platelets, but not on red blood cells. Their function is to display peptide fragments of non-self proteins from within the cell to cytotoxic T cells; this will trigger an immediate response from the immune system against a particular non-self antigen displayed with the help of an MHC class I protein. Because MHC class I molecules present peptides derived from cytosolic proteins, the pathway of MHC class I presentation is often called cytosolic or endogenous pathway.

MHC class II molecules are a class of major histocompatibility complex (MHC) molecules normally found only on antigen-presenting cells such as dendritic cells, mononuclear phagocytes, some endothelial cells, thymic epithelial cells, and B cells. These cells are important in initiating immune responses.

The antigens presented by class II peptides are derived from extracellular proteins (not cytosolic as in MHC class I).

Loading of a MHC class II molecule occurs by phagocytosis; extracellular proteins are endocytosed, digested in lysosomes, and the resulting epitopic peptide fragments are loaded onto MHC class II molecules prior to their migration to the cell surface.

In humans, the MHC class II protein complex is encoded by the human leukocyte antigen gene complex (HLA). HLAs corresponding to MHC class II are HLA-DP, HLA-DM, HLA-DOA, HLA-DOB, HLA-DQ, and HLA-DR.

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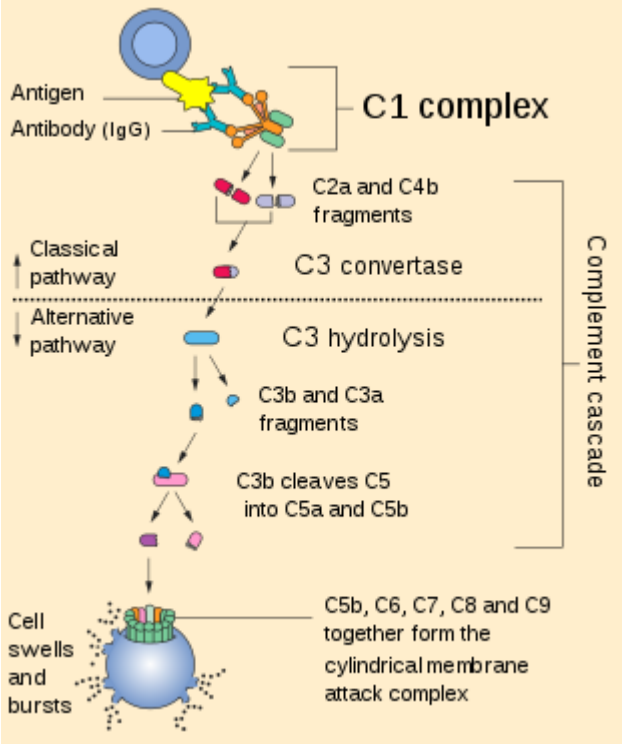
Adjuvants
An adjuvant is a pharmacological or immunological agent that modifies the effect of other agents. Adjuvants may be added to a vaccine to modify the immune response by boosting it such as to give a higher amount of antibodies and a longer-lasting protection, thus minimizing the amount of injected foreign material.

Examples
Alum. Alum, the most commonly used vaccine adjuvant, consists of aluminum salts that are not soluble in water.
Virosomes.
Cytokines.

23. a) Explain classical and alternative pathways for complement activation



OR

b) Discuss in detail about the causes, mechanism, pathogenesis and treatment of Type I hypersensitive reaction

In type 1 hypersensitivity, B-cells are stimulated (by CD4+TH2 cells) to produce IgE antibodies specific to an antigen. The difference between a normal infectious immune response and a type 1 hypersensitivity response is that in type 1 hypersensitivity, the antibody is IgE instead of IgA, IgG, or IgM. During sensitisation, the IgE antibodies bind to Fcε receptors on the surface of tissue mast cells and blood basophils.

Mast cells and basophils coated by IgE antibodies are "sensitised". Later exposure to the same allergen cross-links the bound IgE on sensitised cells, resulting in degranulation and the secretion of pharmacologically active mediators such as histamine, leukotriene (LTC4 and LTD4), and prostaglandin that act on the surrounding tissues. The principal effects of these products are vasodilation and smooth-muscle contraction

Type 1 hypersensitivity can be further classified into immediate and late-phase reactions. The immediate hypersensitivity reaction occurs minutes after exposure and includes release of vasoactive amines and lipid mediators, whereas the late-phase reaction occurs 2–4 hours after exposure and includes the release of cytokines

Treatment and prognosis
Epinephrine, antihistamines, and corticosteroids.

If the entire body is involved, then anaphylaxis can take place, which is an acute, systemic reaction that can prove fatal.

24. a) Explain the mechanism of graft rejection and also add note on role of immuno suppressors in transplantation

Transplant rejection occurs when transplanted tissue is rejected by the recipient's immune system, which destroys the transplanted tissue. Transplant rejection can be lessened by determining the molecular similitude between donor and recipient and by use of immunosuppressant drugs after transplant.

Rejection is an adaptive immune response via cellular immunity (mediated by killer T cells inducing apoptosis of target cells) as well as humoral immunity (mediated by activated B cells secreting antibody molecules), though the action is joined by components of innate immune response (phagocytes and soluble immune proteins). Different types of transplanted tissues tend to favor different balances of rejection mechanisms.

Immunosuppressive therapy
A short course of high-dose corticosteroids can be applied, and repeated. Triple therapy adds a calcineurin inhibitor and an anti-proliferative agent. Where calcineurin inhibitors or steroids are contraindicated, mTOR inhibitors are used.

Immunosuppressive drugs:

* Corticosteroids
* Prednisolone
* Hydrocortisone
* Calcineurin inhibitors
* Cyclosporin
* Tacrolimus
* Anti-proliferatives
* Azathioprine
* Mycophenolic acid
* mTOR inhibitors
* Sirolimus
* Everolimus

OR

b) **Explain the following**
(i) Rheumatoid arthritis (ii) Paroxysmal nocturnal hemoglobinuria

Rheumatoid arthritis is a chronic inflammatory disorder that can affect more than just your joints. In some people, the condition also can damage a wide variety of body systems, including the skin, eyes, lungs, heart and blood vessels.

An autoimmune disorder, rheumatoid arthritis occurs when your immune system mistakenly attacks our own body's tissues. Unlike the wear-and-tear damage of osteoarthritis, rheumatoid arthritis affects the lining of your joints, causing a painful swelling that can eventually result in bone erosion and joint deformity. The inflammation associated with rheumatoid arthritis is what can damage other parts of the body as well. While new types of medications have improved treatment options dramatically, severe rheumatoid arthritis can still cause physical disabilities.

Complications

Rheumatoid arthritis increases your risk of developing:

Osteoporosis. Rheumatoid arthritis itself, along with some medications used for treating rheumatoid arthritis, can increase your risk of osteoporosis — a condition that weakens your bones and makes them more prone to fracture.

Rheumatoid nodules. These firm bumps of tissue most commonly form around pressure points, such as the elbows. However, these nodules can form anywhere in the body, including the lungs.

Dry eyes and mouth. People who have rheumatoid arthritis are much more likely to experience Sjogren's syndrome, a disorder that decreases the amount of moisture in your eyes and mouth. Infections. The disease itself and many of the medications used to combat rheumatoid arthritis can impair the immune system, leading to increased infections.

Carpal tunnel syndrome. If rheumatoid arthritis affects your wrists, the inflammation can compress the nerve that serves most of your hand and fingers.

Heart problems. Rheumatoid arthritis can increase your risk of hardened and blocked arteries, as well as inflammation of the sac that encloses your heart.

Lung disease. People with rheumatoid arthritis have an increased risk of inflammation and scarring of the lung tissues, which can lead to progressive shortness of breath.

Lymphoma. Rheumatoid arthritis increases the risk of lymphoma, a group of blood cancers that develop in the lymph system.

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired, life-threatening disease of the blood. The disease is characterized by destruction of red blood cells (hemolytic anemia), blood clots (thrombosis), and impaired bone marrow function (not making enough of the three blood components). PNH affects 1-1.5 persons per million of the population and is primarily a disease of younger adults. The median age of diagnosis is 35-40 years of age, with occasional cases diagnosed in childhood or adolescence. PNH is closely related to aplastic anemia. In fact, up to 30% of newly diagnosed cases of PNH evolve from aplastic anemia. Similarly, the risk of developing PNH after treatment for aplastic anemia with immunosuppressive therapy (anti-thymocyte globulin and cyclosporine) is approximately 20-30%. The median survival after diagnosis is 10 years; however, some patients can survive for decades with only minor symptoms.

PNH occurs when mutations of a gene called PIG-A occur in a bone marrow stem cell. Stem cells give rise to all the mature blood elements including red blood cells , which carry oxygen to our tissues; white blood cells , which fight infection; and platelets, which are involved in forming blood clots. In PNH, the affected stem cell passes the PIG-A mutation to all cells derived from the abnormal stem cell. Cells harboring PIG-A mutations are deficient in a class of proteins called GPI-anchored proteins. Certain GPI-anchored proteins protect red blood cells from destruction, some are involved in blood clotting, and others are involved in fighting infection. The majority of PNH-related issues, including destruction of red blood cells (hemolytic anemia), blood clots (thrombosis), and infection, result from a deficiency of these proteins.

25. a) Explain in detail about the principle, instrumentation and applications of immunodiffusion techniques.

Radial immunodiffusion (RID) or Mancini method, Mancini immunodiffusion or single radial immunodiffusion assay, is an immunodiffusion technique used in immunology to determine the quantity or concentration of an antigen in a sample.

Preparation

A solution containing antibody is added to a heated medium such as agar or agarose dissolved in buffered normal saline. The molten medium is then poured onto a microscope slide or into an open container, such as a Petri dish, and allowed to cool and form a gel. A solution containing the antigen is then placed in a well that is punched into the gel.The slide or container is then covered or closed to prevent evaporation.

The antigen diffuses radially into the medium, forming a circle of precipitin that marks the boundary between the antibody and the antigen. The diameter of the circle increases with time as the antigen diffuses into the medium, reacts with the antibody, and forms insoluble precipitin complexes. The antigen is quantitated by measuring the diameter of the precipitin circle and comparing it with the diameters of precipitin circles formed by known quantities or concentrations of the antigen.

Antigen-antibody complexes are small and soluble when in antigen excess. Therefore, precipitation near the center of the circle is usually less dense than it is near the circle's outer edge, where antigen is less concentrated

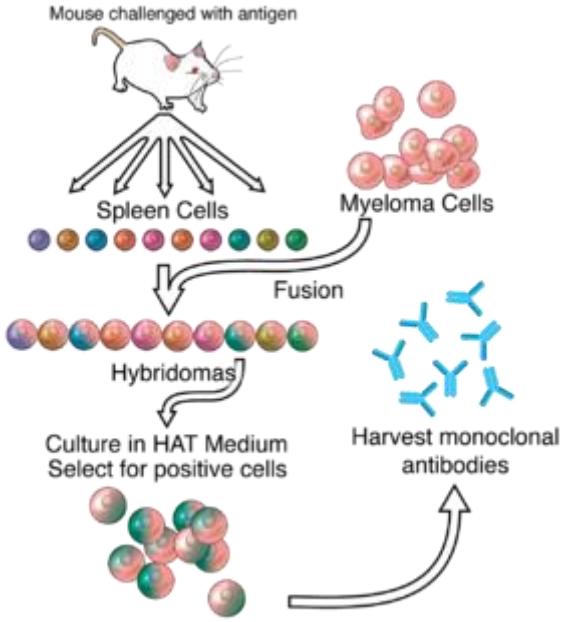
Expansion of the circle reaches an end point and stops when free antigen is depleted and when antigen and antibody reach equivalence. However, the clarity and density of the circle's outer edge may continue to increase after the circle stops expanding.

OR

b) Explain the following

i) Hybridoma technology
ii) RIA

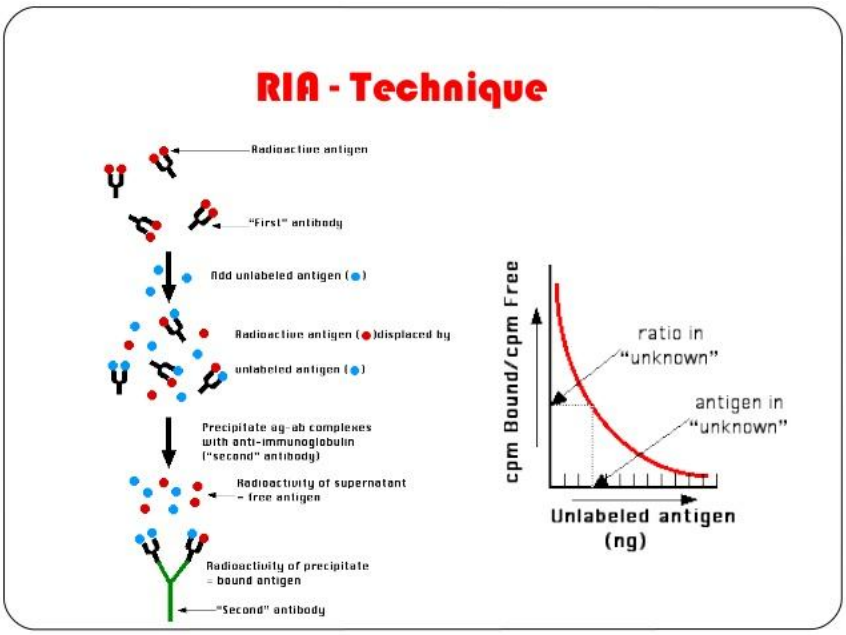
Hybridoma Technology



Hybridoma technology is a method for producing large numbers of identical antibodies (also called monoclonal antibodies). This process starts by injecting a mouse (or other mammal) with an antigen that provokes an immune response. A type of white blood cell, the B cell that produces antibodies that bind to the antigen are then harvested from the mouse. These isolated B cells are in turn fused with immortal B cell cancer cells, a myeloma, to produce a hybrid cell line called a hybridoma, which has both the antibody-producing ability of the B-cell and the exaggerated longevity and reproductivity of the myeloma. The hybridomas can be grown in culture, each culture starting with one viable hybridoma cell, producing cultures each of which consists of genetically identical hybridomas which produce one antibody per culture (monoclonal) rather than mixtures of different antibodies (polyclonal). The myeloma cell line that is used in this process is selected for its ability to grow in tissue culture and for an absence of antibody synthesis. In contrast to polyclonal antibodies, which are mixtures of many different antibody molecules, the monoclonal antibodies produced by each hybridoma line are all chemically identical.

RIA

Radioimmunoassay (RIA) is a very sensitive in vitro assay technique used to measure concentrations of antigens (for example, hormone levels in blood) by use of antibodies. As such, it can be seen as the inverse of a radiobinding assay, which quantifies an antibody by use of corresponding antigens.


RIA - Technique
Radioactive antigen
"First" antibody
Add unlabeled antigen ( )
Radioactive antigen ( )displaced by
unlabeled antigen ( )
Precipitate ag-ab complexes
with anti-immunoglobulin
("second" antibody)
Radioactivity of supernatant
= free antigen
Radioactivity of precipitate
= bound antigen
"Second" antibody
cpm Bound/cpm Free
ratio in
"unknown"
antigen in
"unknown"
Unlabeled antigen
(ng)

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