وزارة التعليم العالي والبحث العلمي
الجامعة التقنية الجنوبية
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قسم تقنيات المختبرات الطبية

الحقيبة التدريسية لمادة

المناعة / نظري
Immunology / theoretical
المرحلة الثانية

تدريسي المادة
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الفصل الدراسي الاول

جدول الخطة الدراسية لمادة المناعة

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Aims of subject

General objective:-

At the end of studying year the study able to collect of principle of immunology & serology with immunologic tests, performance of the tests, accuracy recognition of part of

immune system at operation, resist to disease.

Student able to know about the lab. Materials and how to deal with specimens.

Specific objective:-

1- Deal with lab. Specimens of immunology

2- Perform serologic tests – reporting & and reading, micro titration & macro.

3- Perform the serologic test.

4- Preparation of some Ages & Abs.

5- Detection of some febrile diseases by immunologic assays.

6- Detection about AID.

7- Detection about bacterial disease.

8- Diagnosis of parasitic disease..

11- Solution of immune tests preparations e.g. Normal saline & buffer solution.

Target group :

Second stage / medical laboratory techniques

Activities Used:

1- Interactive classroom activities

2- Brainstorming questions

3- Group activities (if required)

4- Homework

5- Online homework (classroom)

6- Quick written exam .

Lecture: 1

Immunology

Immunology is the study of the immune system, including its responses to microbial pathogens and damaged tissues and its role in disease.

Immune system & Immune Response

The collection of cells, tissues, and molecules that mediate resistance to infections is called the **immune system,** and the coordinated reaction of these cells and molecules to infectious microbes comprises an **immune response.**

Immunity:

Immunity is defined as resistance to disease, specifically infectious disease.

Immunity is classified in to two major groups:

- Innate Immunity.
- Specific immunity (Acquired Immunity).

Innate Immunity

Innate Immunity

Innate immunity, also called natural immunity, is always present in healthy individuals, function to block the entry of microbes and to rapidly eliminate microbes that do succeed in entering host tissues.

It has the following characteristics in common:

1. Are present intrinsically with or without previous stimulation.
2. Have limited specificity for shared microbe and cellular structures (pathogen associated molecular patterns [PAMPs] and damage-associated molecular patterns [DAMPs]).
3. It involves several defensive barriers including Anatomical, Humoral, and Cellular barriers:

Anatomical barriers, Humoral Barriers and *Cellular Barriers*

Anatomical barriers:

1. Mechanical (physical) Factors
2. Chemical Factors
3. Biological Factors

A. Anatomical barriers

1. Mechanical (physical) Factors:

- ***Skin*** is example of the first line of defense against infection.
- ***Coughing and sneezing*** mechanically eject pathogens from respiratory tract.
- ***Tears and urine flow*** also mechanically expels pathogens,
- ***mucus membrane*** secreted by the respiratory and gastrointestinal tract serves to trap microorganisms.

2.Chemical factors:

- ***Low pH*** (3-5) of skin due to fatty acids in sebum which is fungi and bacterial static,
- ***β- defensin* in the** respiratory tract act as antimicrobial.
- **Enzymes** like ***lysozyme and phospholipase*** in saliva, tears and breast milk are also antibacterial.
- In the stomach, ***low pH (1.2-3.0) of gastric juice and proteases*** serve as powerful chemical defenses against ingested pathogens.

3. Biological Factors:

Within the genitourinary and gastrointestinal tracts, ***normal flora*** is found

A person's normal flora is formed when non-pathogenic bacteria colonize epithelial surfaces. Normal flora protects the host by:

* Competing with pathogenic bacteria for nutrients and attachment sites
* Production of antibacterial substances.

The use of antibiotics can disrupt the normal flora, making pathogenic organisms more likely to cause disease

B. Humoral Barriers (against penetrating pathogens):

1. Complement System:

It is the major humoral non-specific defense mechanism. Once activated complement can lead to increase vascular permeability, recruitment of phagocytic cells, and lysis and opsonization of bacteria.

2. Coagulation System:

For example

1. ***beta-lysine***, a protein produced by platelet during coagulation can lyse many Gr+ bacteria act as antimicrobial.
2. ***Interferons***: Are protein that can limit virus replication in cells.
3. ***Lysozyme***: breaks down the cell wall of bacteria.
4. ***Interleukin-1***, IL-4, IL-6 and TNF: induce fever and production of acute phase proteins, some of which are antimicrobial because they can opsonize bacteria.

C. Cellular Barrier to infection:

1. **Neutrophil**: polymorphonuclear cells (PMNs) attack invading organisms and kill them intracellularly.
2. **Macrophages**: Tissue macrophages, intracellular killing of microorganisms.
3. **Natural Killer** (NK) cells: Kill virus infected and tumor cells.
4. **Eosinophils**: Have proteins in granules that are effective in killing certain parasites.
5. **Mast cells, platelets (thrombocytes), and endothelial cells**.

Lecture 2:

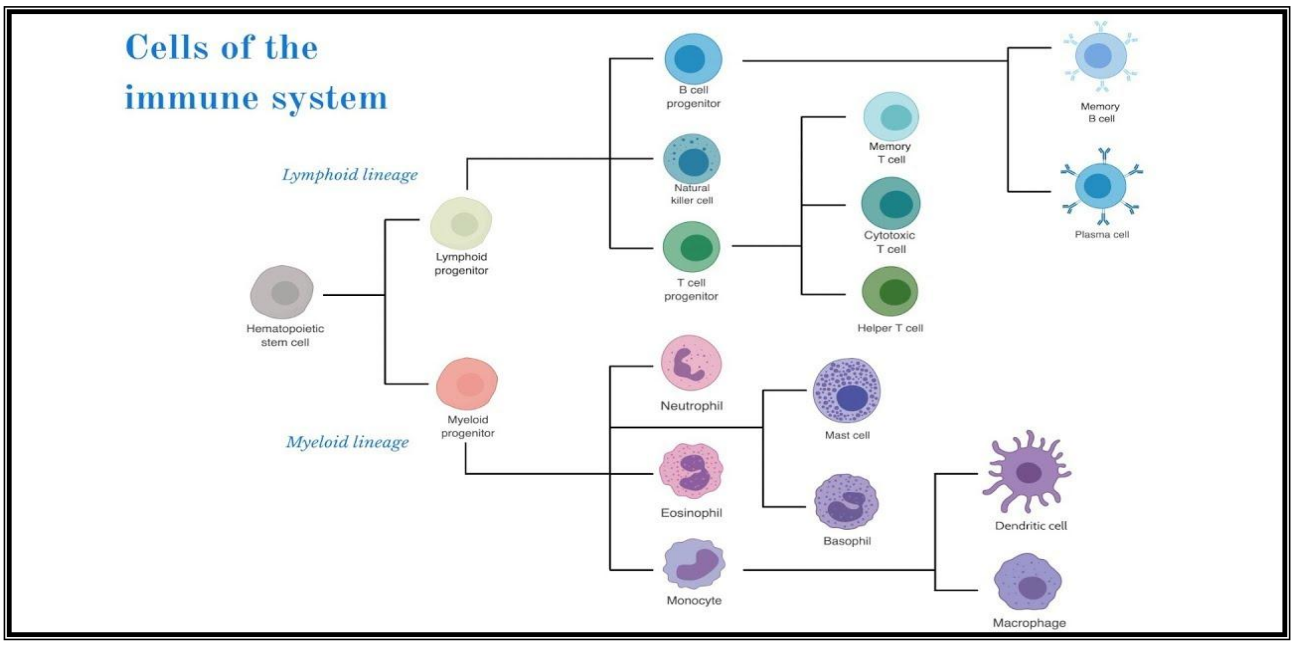
Cells and organs of the immune system

Immune system:

Immune system is the collection of organs, cells and molecules which are responsible for defending us against infection.

Stem cells in the bone marrow (B.M) is the origin of all blood cells.

Cells of the Immune System:



Cells of the Innate Immune System:

Innate immune system consists of many cells and molecules that represent the 2nd line of immunity.

Cells	Molecules
Neutrophil	
Macrophage	C3b
NK cell	INF

Neutrophil:

- Originates from myeloid stem cell in the B.M.
- Constitutes 65% of circulating white blood cells (WBC_s)
- Its nucleus is segmented, with variable number of segment (2-5 lobes). Thus, it is named polymorphonuclear (PMN) leukocyte.
- Its cytoplasm is granular, these granules are stained pale pink (neutral) by using Wright stain.
- These cytoplasmic granules are the lysosomes which contain many degradative enzymes, such as:

1. Myeloperoxidase (green)
2. Lysosomes
3. Lactoferrin.

} these enzymes kill the pathogen (antigen).

4. Proteinase, nuclease, and lipase

Neutrophil has the following receptors on its surface:

- TLR (toll-like receptor): used for binding to antigen
- FcR (Fc receptor): used, for binding to Fc fragment of IgG and IgM (antibodies).
- C3bR (C3b receptor): used for binding to C3b complement component.

<u>The function of neutrophil</u> in innate immunity, it acts as phagocyte (engulf and kill pathogen {antigen} by phagocytosis.

The number of neutrophils in blood is increased (neutrophilia) in acute pyogenic bacterial infection.

Macrophage:

- Originates from myeloid stem cell as monocyte. Monocyte stay to blood for three days and then go to tissue to become macrophage.
- Monocyte constitutes 5-7 % Of circulating (WBC_s).

- Monocyte-macrophage has single large kidney-shaped nucleus (mono-one).
- Its cytoplasm has fine granules (so it appears a granular) and these cytoplasmic granules are the lysosomes which contain many degradative enzymes.

Macrophage has the following receptors on its surface:

1. TLR (toll-like receptor): used for binding to antigen
2. FcR (Fc receptor): used for binding to Fc fragment of IgG and IgM (antibody).
3. C3bR (C3b receptor): used for binding to C3b complement component.

The function of macrophage in innate immunity:

Phagocytosis: it acts as phagocyte: engulf and kill pathogen [antigen}.

Cytokine production: when the macrophage engulf antigen, it produces several cytokines, such as:

1. IL-1 (lnterleukin-1): which induce inflammation
2. TNF (tumor necrosis factor): which has an inflammatory function
3. IL-8: Attracts neutrophil, NK cell to the site of infection
4. NO (nitric oxide): kill extra-cellular pathogen

The number of monocytes in blood is increased (monocytosis) in chronic granulomatous bacterial infection and in viral infection.

Lecture: 3.

Inflammatory response and phagocytosis (Humoral Barriers)

Inflammation

Inflammation is the recruitment of circulating blood leukocytes (e.g., phagocytes and lymphocytes) and various plasma proteins (e.g., complement, antibodies, fibrinogen) to sites of infection, where they function to destroy the microbes and repair damaged tissue.

Some of the many causes of inflammation include:

1. Infection and microbes,
2. Immune reactions between antigen and antibody.
3. Trauma, burns, physical or chemical agent and tissue necrosis.
4. Other causes of inflammation include: temperature extremes, oxygen

The duration of inflammation lasts from a few minutes to a few years. Depending on:

- ✓ the extent of the injury
- ✓ the type of injury
- ✓ the vascularity of the tissue

Signs of Inflammation

- ✓ Erythema
- ✓ Heat (fever)
- ✓ Edema
- ✓ Pain
- ✓ Loss of Function

These are signs of inflammation and it caused by:

1. Vasodilation: increase in the diameter of blood vessels (capillary) which is responsible for redness (erythema) and increase temperature (heat).
2. Increase permeability in the walls of blood vessels leading to escape of fluid and cells into space & surrounding tissues (swelling and edema).

Fever (pyrexia): this occurs due to bacterial products such as endotoxins. The functions of fever are:

1. Inhibit multiplication of bacteria.
2. Interfere with nutrition of bacteria.

3. Increase stimulates immune reaction like phagocytosis and specific immune response.

Functions of Inflammation

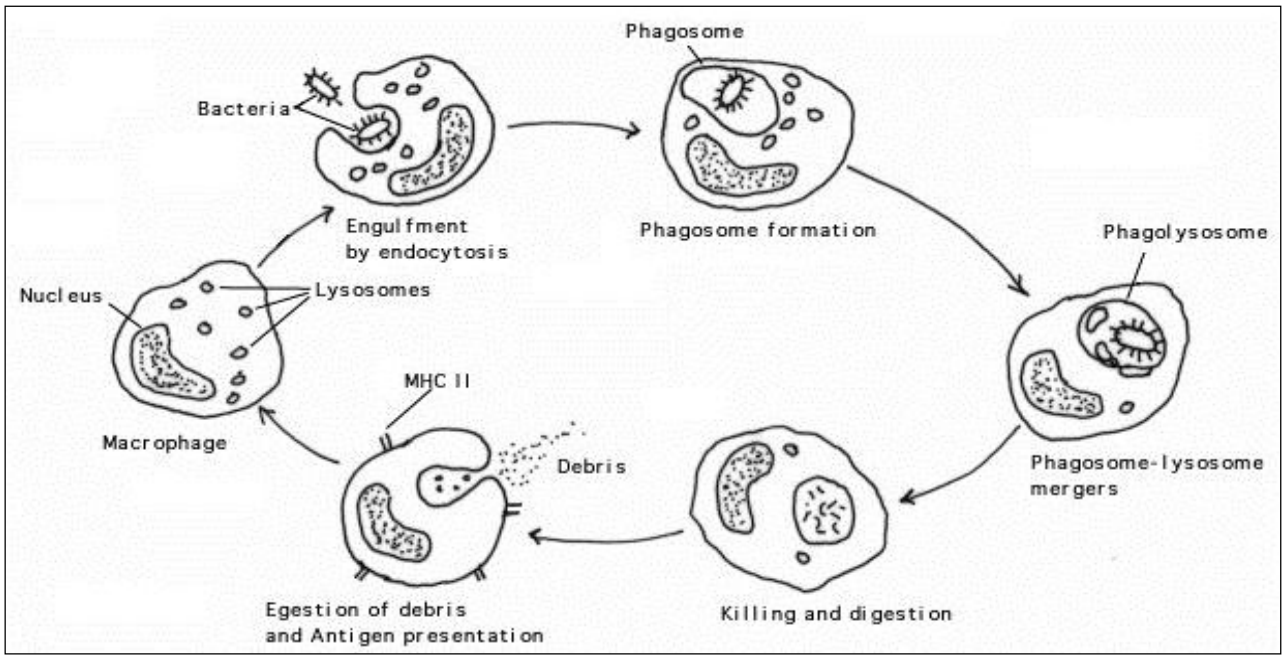
- ✓ Inactivate injurious agent
- ✓ Break down and remove dead tissue
- ✓ Initiate healing of tissue

Phagocytosis:

The primary method used by the body to remove free microorganisms in the blood and tissue fluids , by engulfing, ingestion particles and destroying them enzymatically (lysozyme, myeloperoxidase, Protease) by phagocytic cells like macrophages.

Stages of Phagocytosis:

1. Recognition and attachment of microbes by phagocytes.
2. Ingestion of microbes and other materials by phagocytes.
3. Enzymatic destruction of microbes and other materials by fusion of lysosome with phagosome creates phagolysosome,
4. Waste material is expelled or assimilated.



Types of phagocytic cells :

1. **Polymorphonuclear cells (PMNs):** predominate in acute pyogenic infection, short-lived, circulating, granulated contains lysozyme and myeloperoxidase (degradative enzymes) and produce toxic substance (NO) which accelerate destruction of infectious microorganisms.

2. **Macrophages / Monocytes:**

Monocytes are less abundant in the blood than neutrophils, numbering 500 to 1000 per μL. They also ingest microbes in the blood and in tissues. During inflammatory reactions, monocytes enter extravascular tissues and differentiate into cells called **macrophages**, which, unlike neutrophils, survive in these sites for long periods. Macrophages present in tissues in different forms **such as:**

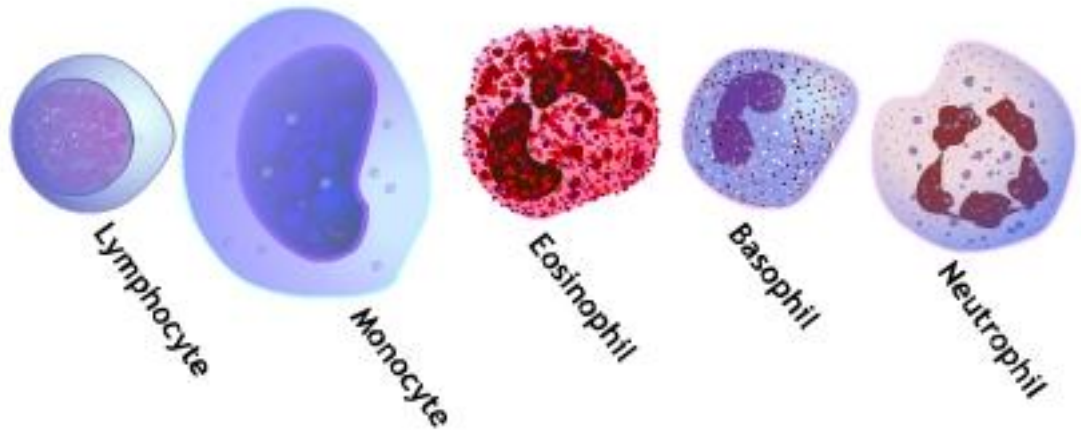
Alveolar macrophages in lungs.
Kupffer cells in Liver.
Splenic macrophages in white pulp of spleen.
Microglial cells in brain.
Mesangial cells in kidneys.
Osteoclasts in bones.
Langerhans cells in skin and mucous membrane

These cells long-lived also migrate, engulf, and kill the bacteria multiply within the cells, do not possesses myeloperoxidase, secrete plasminogen enzyme.

3. **Eosinophil and Basophil**:

Eosinophil: a bone marrow–derived granulocyte that is abundant in the inflammatory infiltrates of immediate hypersensitivity and contributes to many of the pathologic processes in allergic diseases. Eosinophils are important in defense against extracellular parasites, including helminths.

Basophil: a type of bone marrow–derived circulating granulocyte with structural and functional similarities to mast cells that has granules containing many of the same inflammatory mediators as mast cells and expresses a high-affinity Fc receptor for IgE. Basophils that are recruited into tissue sites where antigen is present may contribute to immediate hypersensitivity reactions. release inflammatory mediators.



Lecture: 4

Adaptive (acquired or specific immunity) immunity

Adaptive immunity, also called specific immunity or acquired immunity, requires expansion and differentiation of lymphocytes in response to microbes before it can provide effective defense; that is, it adapts to the presence of microbial invaders.

Whereas the mechanisms of innate immunity recognize structures shared by classes of microbes, the cells of adaptive immunity (lymphocytes) express receptors that specifically recognize a much wider variety of molecules produced by microbes as well as noninfectious substances.

Characteristics of Acquired Immunity:

1. Specificity Ensures that distinct antigens elicit specific responses.
2. Diversity: Enables immune system to respond to a large variety of antigens
3. Memory: Leads to enhanced responses to repeated exposures to the same antigens
4. Clonal expansion: Increases number of antigen-specific lymphocytes from a small number of naive lymphocytes
5. Specialization: Generates responses that are optimal for defense against different types of microbes
6. Contraction and homeostasis: Allow immune system to respond to newly encountered antigens.
7. Non-reactivity to self: Prevents injury to the host during responses to foreign antigens

adaptive immunity can be subdivided into two major types depending on how immunity was introduced.

- ✓ Naturally acquired immunity occurs through contact with a disease-causing agent.
- ✓ Artificially acquired immunity develops for example by vaccination.

Both naturally and artificially acquired immunity can be further subdivided into **active** or **passive**, depending on whether immunity is induced in the host or passively transferred from immune host. and each of these types can in turn be naturally or artificially acquired.

Passive immunity

Passive immunity is protection by products produced by an animal or human and transferred to another human, usually by injection. Passive immunity often

provides effective protection, but this protection wanes (disappears) with time, usually within a few weeks or months. They are divided into two:

I. Naturally acquired passive immunity: Refers to antibodies transferred from mother to fetus across the placenta and to the newborn in colostrum and breast milk during the first few months of life.

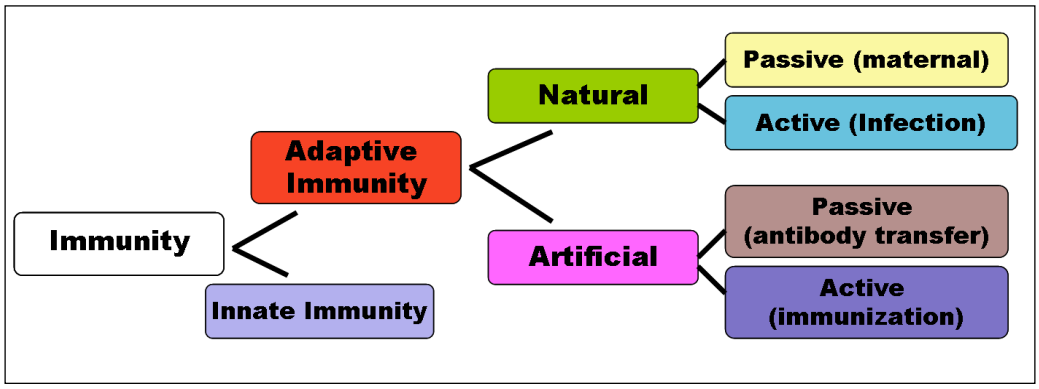
II. Artificially acquired passive immunity: Is introduction of antibodies that are formed by an animal or a human to an individual to prevent or treat infection.

Active immunity

Active immunity is protection that is produced by the person's own immune system. This type of immunity usually lasts for many years, often during a lifetime.

I. Naturally acquired active immunity: Is immunity that comes from infections encountered in daily life.

II. Artificially acquired active immunity: It is stimulated by the use of vaccines to artificially establish a state of immunity.



Comparison between Active and Passive Acquired Immunity:

	ACTIVE	PASSIVE
1. SOURCE	Self	other human or lower animal
2. EFFECTIVENESS	High	moderate to low
3.METHOD	infection or vaccination or immunization	through placenta or injection γ- globulin
4.TIME TO DEVELOP	7-10 days	immediately
5. DURATION	long time (may be years)	short time (few days or several weeks)
6. EASY OF REACTIVITY	easy by booster dose	dangerous (may cause anaphylaxis)

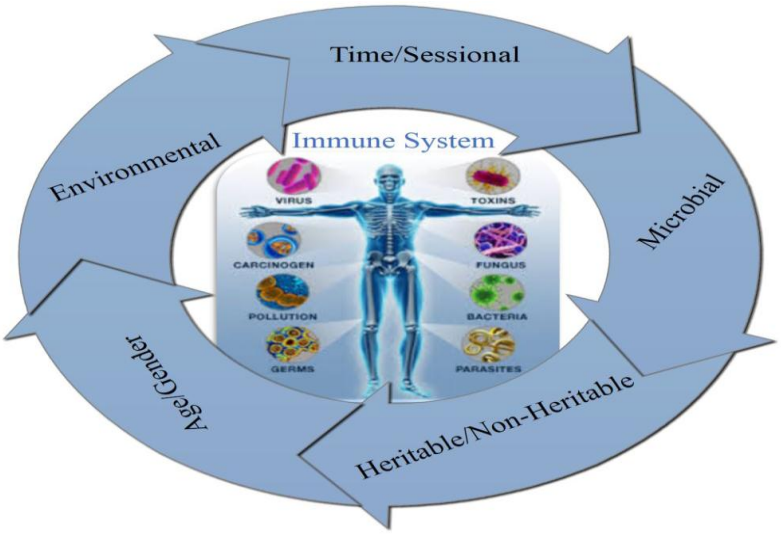
Differences between innate & acquired immunity:

Innate Immunity	Acquired immunity
1. Nonspecific to invader	Specific
2. It presented from birth	developed later in life
3. Genetically determination	Non
4. Consist of physical chemical barriers & cells	antibody & lymphocytes
5. No memory cells	memory cells

Factors affecting the Immune System

Many factors appear to be responsible for differences in the immunity of individuals

1. **Age**: play vital role in immune system regulation, i.e., young children and aged people is more vulnerable to infections than other age groups.
2. **Time**: during an immune response seems to be an ongoing moving target within a specific individual.
3. **Gender:** Comparatively women are more prone to various immune mediated abnormalities identified by > 80% patients with immune system disorders.
4. **Genetic Factors:** In many patients host genetics are deemed to be the most influential cause of infection.
5. **Microbial exposure :** The development of the immune system also depends on the encounter of microorganisms' types and their threshold.
6. **Other Environmental Factors**



Lecture: 5

Vaccines

A vaccine is a biological preparation that provides active acquired immunity to a particular infectious disease. Vaccines are dead or inactivated organisms or purified products derived from them.

Vaccination is the process of stimulating protective adaptive immune responses against microbes by exposure to nonpathogenic forms or components of the microbes.

There are several types of vaccines in use.

They are:

- ✓ Live attenuated vaccines (Bacillus Calmette-Guérin & Polio, influenza, rabies)
- ✓ Subunit (antigen) vaccines (Tetanus toxoid, diphtheria toxoid).
- ✓ Conjugate vaccines (*Haemophilus influenzae*, pneumococcus).
- ✓ Synthetic vaccines Hepatitis (recombinant proteins).
- ✓ Viral vectors (Clinical trials of HIV antigens in canarypox vector).
- ✓ DNA vaccines (Clinical trials ongoing for several infections).

Properties of ideal vaccine:

1. Provide long lasting immunity.
2. Should induce both humoral and cellular immunity.
3. Should not induce autoimmunity or hypersensitivity.
4. Should be inexpensive to produce, easy to store and administer.
5. Vaccines must also be perceived to be safe.

Vaccination (Immunization): is a way to trigger the immune response by giving small doses of an antigen which activate the immune system memory (activated B cells and sensitized T cells). *Immunization may be passive or active, natural or artificial:*

Active Immunization:

Achieved by natural infection with a microorganism or artificially by administration of a vaccine. This type of immunization will give long term protection by formation of memory cells and reactive B & T cells.

Passive Immunization:

Performed by transferred antibodies to recipient, either naturally by transfer of maternal Abs across placenta to the fetus, or by injecting recipient with performed Abs.

Passive immunization is routinely administrated to individuals exposed to tetanus, botulism…… etc, & and it can provide immediate protection to travelers and health-care workers.

Classification of Vaccines:

1. **Killed (Whole Pathogen) Vaccines.**

   These are preparations of the normal (wild type) infectious, pathogenic microorganisms that have been rendered nonpathogenic, usually by treatment with using heat, formaldehyde or gamma irradiation so that they cannot replicate at all. Such killed vaccines vary greatly in their efficacy. Example : Cholreae, Rabies, HAV vaccine … etc.

2. **Live Attenuated Vaccine.**

   These vaccines are composed of live, attenuated microorganisms (viruses) that cause a limited infection in their hosts sufficient to induce an immune response, but insufficient to cause disease. Ex: Bacillus Calmette Guerin (BCG).

Live Attenuated *VS* Killed Vaccines

Feature	Live	Killed
Dose	Low	High
No. of dose	Single	Multiple
Need of adjuvant	No	Yes
Duration of Immunity	Many years	Less
Antibody response	IgG	IgG, IgA
CMI (cell mediated immunity)	Good	Poor
Reversion of Virulence	Possible (non safe)	Not possible

3. **Synthetic Vaccines:**

   These include Conjugate Vaccines, recombinant vaccines DNA vaccines and subunit vaccines

The success of vaccination in eradicating infectious disease is dependent on several properties of the microbes.

Vaccines are most effective if the infectious agent does not establish latency, does not undergo antigenic variation, does not interfere with the host immune response.

It is difficult to effectively vaccinate against microbes such as HIV, which establishes latent infection and is highly variable. Vaccines are also most effective against infections that are limited to human hosts and do not have animal reservoirs.

Factors playing a role in immunization:

1- Choice of vaccine type eg: live attenuated is best but disadvantage is possibility of reversion of its pathogenicity.

2- Route is very important eg: Oral or intranasal is more effective in many viral respiratory or gastrointestinal diseases.

3- Time of primary and secondary dose.

4- Adjuvant, the best one is combination of alum with oil gives prolong immune response.

Childhood Immunization Schedule in Iraq

	Types of Vaccines	Age of Vaccination	Number of Doses
1	BCG(Bacillus Calmette Guerin)	Within 72 Hours after birth	Single dose Zero dose First dose
2	DTP (Diphtheria, Tetanus, Pertussis) Oral poliomyelitis Hepatitis B	At 12 months of age	First dose First dose Second dose
3	DTP (Diphtheria, Tetanus, Pertussis) Oral poliomyelitis Hepatitis B	At 4 months of age	Second dose
4	DTP (Diphtheria, Tetanus, Pertussis) Oral poliomyelitis Hepatitis B	At 6 months of age	Third dose
5	Measles	at 9 months of age	Single dose
6	Mixed measles (Measles, Mumps, Rubella	At 15 months of age	First dose
7	DTP (Diphtheria, Tetanus, Pertussis) Oral poliomyelitis	At 18 months of age	First booster dose
8	DTP (Diphtheria, Tetanus, Pertussis) Oral poliomyelitis	From 6-7 years of age	Second booster dose
9	Mixed measles (Measles, Mumps, Rubella	At 6 years	Second dose

Note : BCG vaccine must be repeated if there is no scare appeared after 2 months after vaccination

Lecture: 6

The Structure of The Immune System

Lymphoid Tissue: Specialized connective tissues and organs where the lymphocytes form the major cellular components.

The immune system consists of primary organs (central lymphoid organ) and secondary organs (Peripheral lymphoid organ).

The Primary Lymphoid Organ

The **primary lymphoid organs** are primary lymphoid tissues involved in the production and early selection of lymphocytes and consisting of:

1- **The Thymus:**

Thymus is bi-lobed organ situated above the heart, each lobe divided into outer part Cortex and inner part Medulla. Thymic function decline with ages, most active during the neonatal and pre-adolescent periods, the growth continue until reaches its maximum size at puberty and then undergo atrophy with aging and replaced by adipose tissue.

Cortex contain immature T-cells, highly dividing, highly dying thymocyte, about 95 % of T-cell will be die, 1-5 % will remain and migrate down to the medulla. There are cortical epithelial cells which also helps the thymocyte in their proliferation and maturation by secreting thymic hormones (Thymopoietin, Thymoline and Thymosine).

Medulla contain mature, less dividing, less dying thymocytes. That is mean thymus deals with proliferation and maturation of T-Lymphocytes and the letter (T) is related to it.

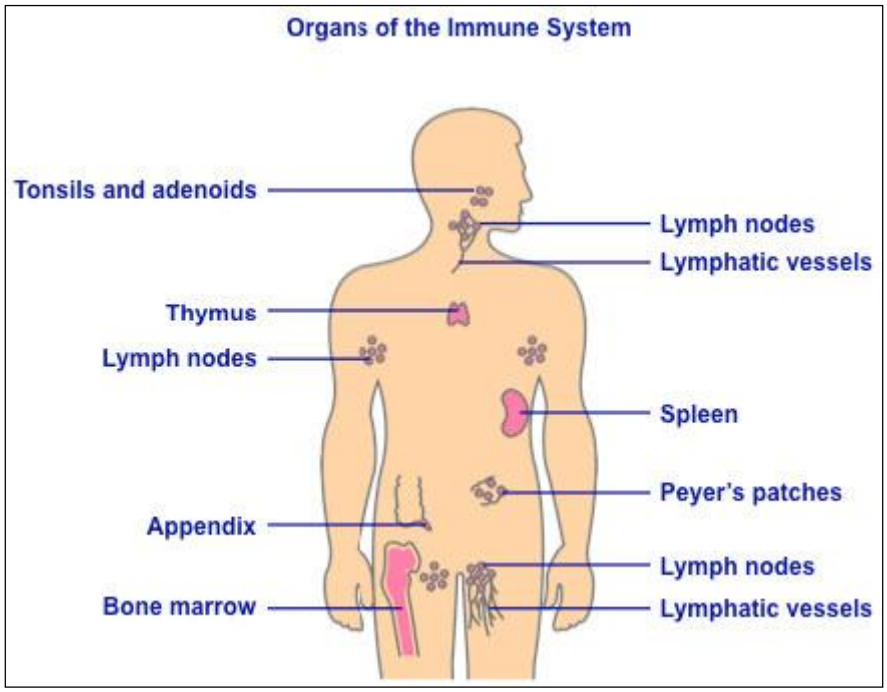
2- **Bone Marrow:**

Is the flexible tissue found in the interior of bones, it is the site of B-cell origin and development in human and mice.

Secondary lymphoid organs : plays a major role in mounting immune response to antigen,

1- Encapsulated organs : include spleen, lymph nodes, appendix, tonsil, skin (Langerhans cells) and liver. These organs act as filters or traps for foreign antigens, and that plays a major roles in mounting immune response to Ags in the blood stream which is come into contact with macrophages and lymphocytes (B & T cells).

2- Un-capsulated organs : called mucosa associated lymphoid tissue (MALT), is found in various body sites which includes Payer's patches (aggregates of cells) in small intestine, and numerous lymphoid follicles in intestine, respiratory tract and genitourinary tract as well.



Primary & Secondary Lymphoid Organs

Features	Primary Lymphoid organ	Secondary Lymphoid Organ
Component	Bone marrow, fetal liver, thymus	Spleen, lymph nodes, and mucosa-associated lymphoid tissue (MALT) including tonsils, adenoids, respiratory, genitourinary, and gastrointestinal tracts
Proliferation and differentiation	Antigen-independent	Antigen-dependent
Product	Immunocompetent cells (B cells and T cells)	Effector cells (antibody-secreting plasma cells for humoral immune response and T helper and T cytotoxic cells for cell-mediated immune response)
Event	Development and maturation of B Cells and T cells	Induction of immune response: encounter of antigens and antigen-presenting cells (APC) with mature B and T cells, generation of effector cells, and memory cells

Cells of Immune system:

1- Lymphocytes : produced in the primary lymphoid organs then migrates by the circulation to the secondary lymphoid organs, is a type of white blood cells. The three major types of lymphocytes are T-cell, B-cell and natural killer (NK cells). B-cells and T-cells (bone marrow cells) are major cellular components of the adaptive immune response.

a- T- Lymphocytes: classified according to their function into:

I- **Cells with $CD4^+$ marker** are called **helper T-cells**, become activated by antigen presenting macrophages and then help in generating T-

cytotoxic cells and cooperating with B-cells in production of antibodies.

II- **Cells with $CD8^+$ marker** are **cytotoxic T-cells** (Tc cells), cytotoxic against tumor cells and host cells infected with intracellular pathogens on the basis of MHC presenting foreign proteins.

III- **T-suppressor / regulator cells** which suppress T and B-cell responses. T-regulatory are subpopulation of T-helper cells, serve as regulator of T-cells responses by secreting inhibitory cytokines that decrease the activity of both T & B-cells.

b- **B-lymphocytes:** develop from stem cells in the bone marrow, these cells produce antibodies with specificity for antigen after determination into plasma cells and make it easier for immune cells to destroy the antigens.

2- Natural Killer (NK) cells: are population of lymphoid cells characterized by large granulated cytoplasm, they don't have T or B-cell receptors, they attack and destroy tumor cells or cells that have been infected by viruses nonspecifically (they don't need to recognize foreign antigens presented on the target cell). NK cells distinguish tumors and infected cells from normal and uninfected cells by recognizing changes on the surface molecule called MHC class I. they named natural killer because they don't require prior activation in order to kill cells which are missing MHC class I.

3- Antigen presenting cells (APC): are found primarily in the skin, lymph nodes, spleen and thymus such as macrophages, dendritic cells and activated B-cells. Main function of APCs is present antigen to antigen sensitive lymphocytes.

Cluster of Differentiation (CD):

Unique cell surface molecules, molecules given number designation. The CD describes the cluster of determinants, the number describes the order in which it was discovered, as of March 2010 the list of determinants was to CD 350.

CD specific markers have been useful for determining the function of proteins. Hence, for example CD4 antigens are expressed by T-helper cells while CD8 antigens are expressed by cytotoxic and suppressor T-cells.

Major histocompatibility complex (MHC):

Refers to cluster of gens responsible for immune response. MHC also called human leukocyte antigen in human (HLA). The importance of MHC proteins is that they allow T-cells to distinguish self from non-self. In every cells of our bodies, antigens are constantly broken up and presented to passing T-cells. Without this presentation, other

aspects of the immune response cannot occur. MHC determines compatibility of donors for organ transplants as well.

MHC class I molecules: found in all nucleated cell surfaces presents antigens to cytotoxic T-cells ($CD8^{+)}$.

MHC class II molecules: found only on B-cells, macrophages and other cells that presents antigens to T-helper cells, that is participate in Ag presentation to T-helper cells ($CD4^{4}$).

Features	**B-Cell**	**T-Cell**
Origin	Bone marrow	Bone marrow
Site of maturation	Bone marrow	Thymus
Antigen receptor	B cell receptor (BCR)	T cell receptor (TCR)
Target of binding	Soluble antigens	Biomolecular complex displayed at the surface of APC
Branch of immune response	Antibody-mediated immune response	Cell-mediated and antibody-mediated immune response

Lecture: 7

Complement System

The complement system is a collection of circulating and membrane-associated proteins (C1 – C9) that are important in defense against microbes. Many complement proteins are proteolytic enzymes, and complement activation involves the sequential activation of these enzymes.

Properties of complement:

1. Inactivated by heating at 56°c for 30 minutes.
2. Activated by microbes.
3. Cause destruction (lysis) of target cell.

Complement Activation:

Three major steps appeared in complement activation :

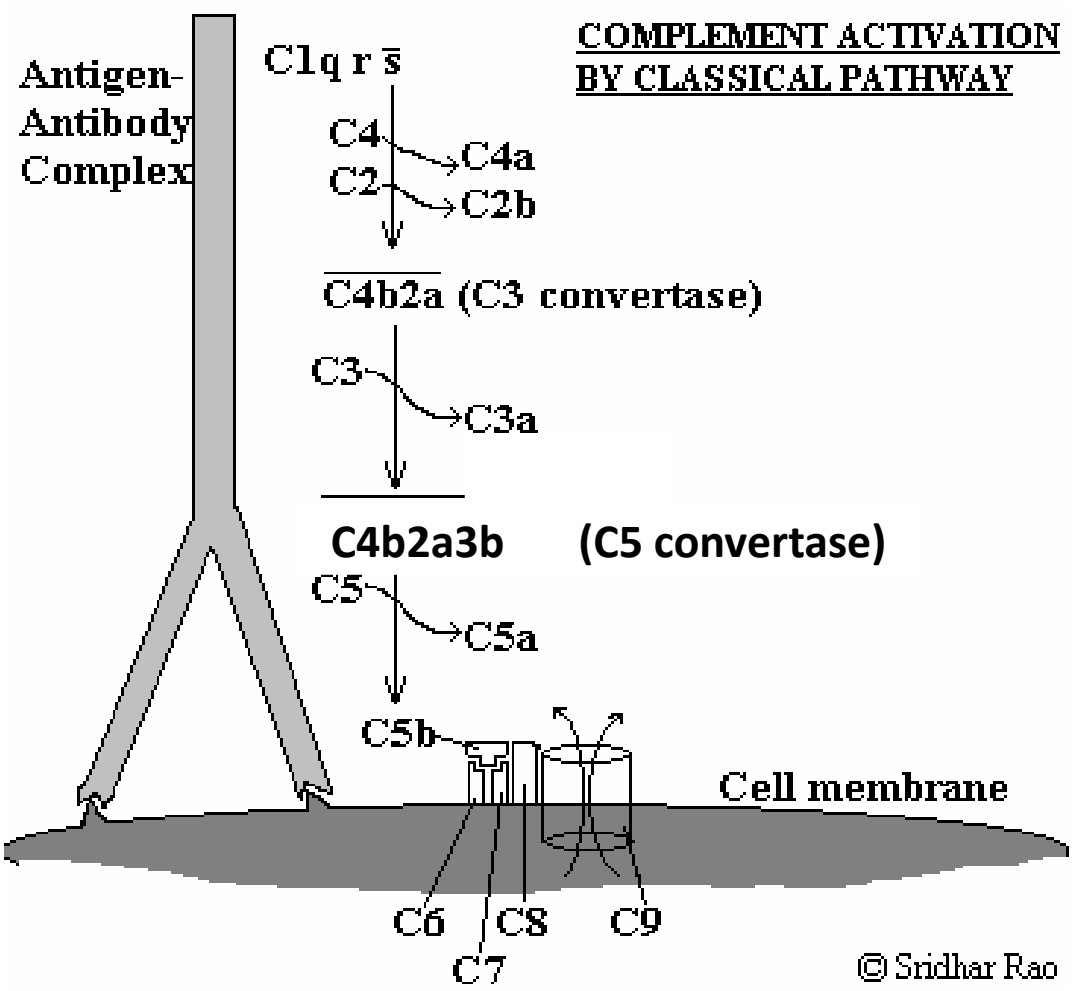
1. Recognition.
2. Enzyme activation.
3. Cell membrane change.

There are 3 major pathways for complement activation the classical, alternate pathway and lectin pathway.

The Classical path way

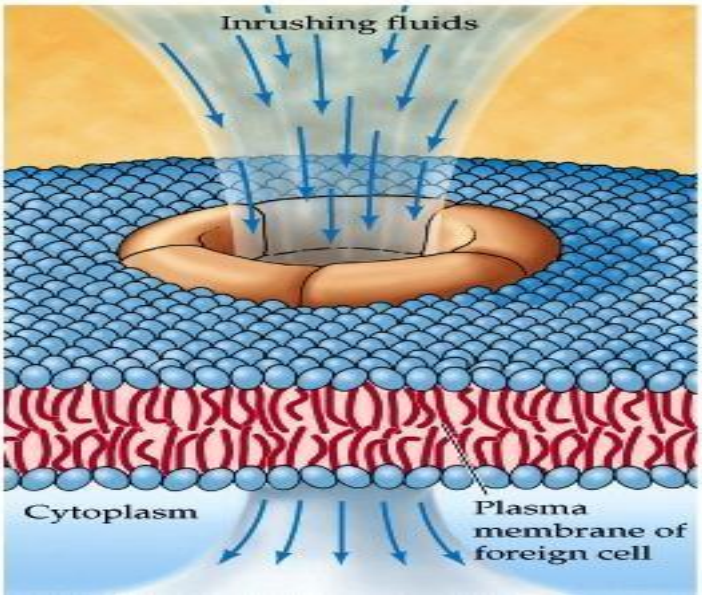
- ✓ The classical pathway is triggered by immune complexes (Ag + Ab IgG or IgM) in the presence of complement components C1, C4, C2, C3, Ca^{+2} and Mg^{+2} cations.
- ✓ While IgG1, IgG2 and IgG3 (most effective) can activate complement, IgG4 is not able to activate at all.

The classical pathway is illustrated in the following diagram:



Biological activity of the classical pathway products

Component	Biological activity
C3a	Anaphylatoxin: can activate basophils and mast cells to degranulate resulting in increased vascular permeability and contraction of smooth muscle cells which may lead to anaphylaxis.
C3b	Opsonin: promote phagocytosis by binding to complement receptors. Activation of phagocytic cells.



Alternative Pathway

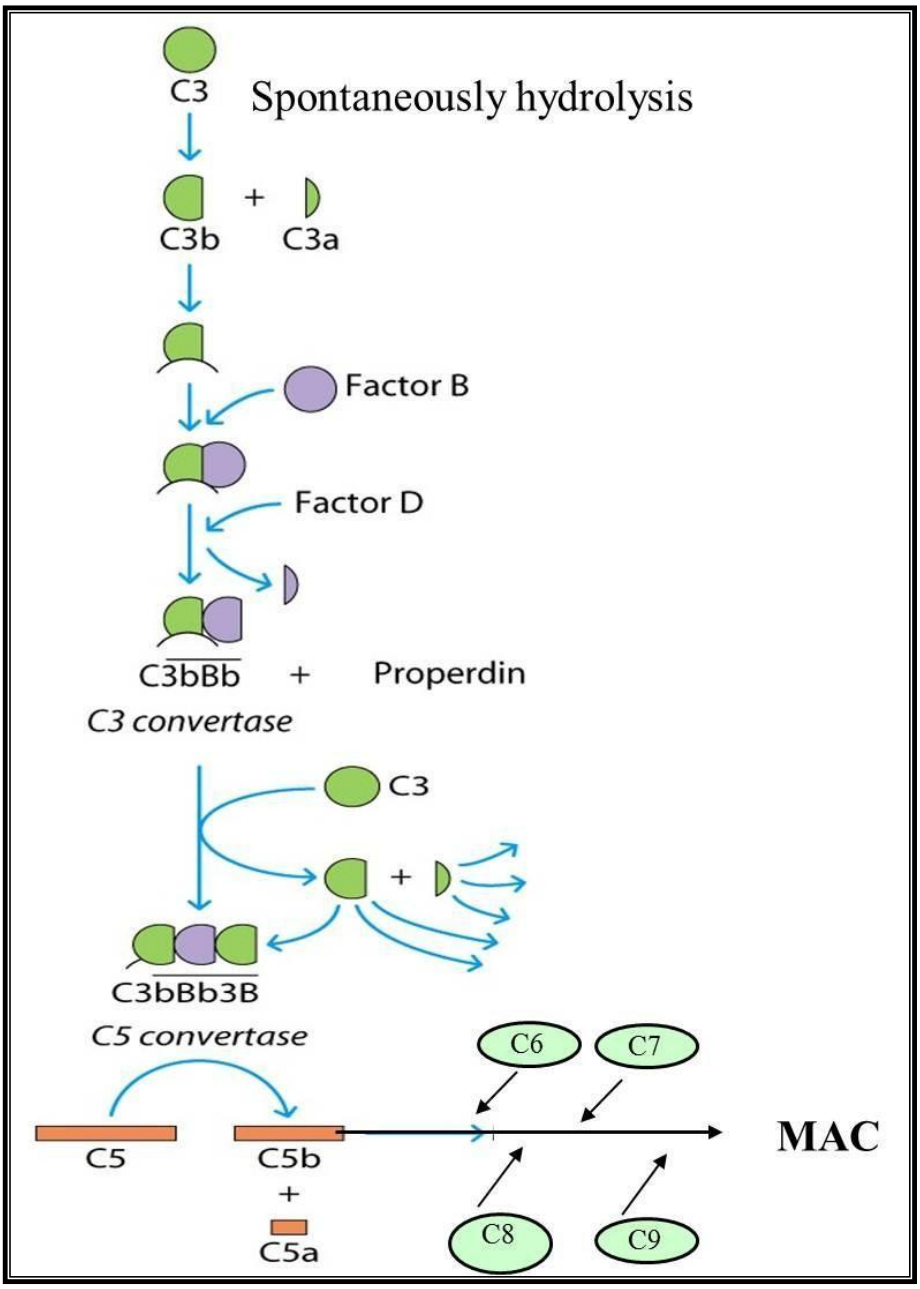
- ✓ Alternate pathway is a non-antibody-initiated pathway.
- ✓ This pathway does not require C1, C2 and C4.
- ✓ Activated by bacterial cell wall (lipopolysaccharide), fungi, viruses envelop, parasites (Trypanosoma) and some other proteins (eg: proteases).
- ✓ It begins with the spontaneous activation of C3 in serum and requires Factors B and D and Mg^{+2}, all present in normal serum.

It activated as the following:

1. Factor B: binds to the c3b (in the Ag_c3b complex) to form C3bB complex.
2. Factor D: cleaves the factor B into Ba and Bb. The Bb binds to c3b to form c3bBb complex (C3 convertase).
3. Factor P: (properdin) binds to Bb to form c3bBbp complex convert c3 from inactive form to active form (by cleaving the c3 into c3a and c3b).
4. Additional c3b will bind to the complex to form c3bBbpc3b complex. (c3bBbpc3b) acts as c5 convertase and convert c5 from inactive form to active form by cleaving the c5 into c5a and c5b.
5. C5b insert into the cell membrane of the Ag.
6. C6 C7 C8 C9 will be inserted subsequently to the cell membrane of Ag to form C5b,6,7,8,9 complex. (C5b,6,7,8,9 is called membrane attack complex or MAC)

7. MAC leads to formation of pore in the cell membrane cell lysis (cytolysis)

Note: the "b" fragments complement participate in the activation pathway. whereas, the (a) fragments are split off and have other activities (i.e. act as anaphylatoxins or chemotaxins).



The lectin pathway:

Lectin: are proteins that binds to mannose residues (glycoproteins or carbohydrate on the surface of pathogens).

- The lectin pathway is homologous to the classical pathway, but with the opsonin, mannose-binding lectin (MBL), instead of C1q.
- This pathway is activated by binding of MBL to mannose residues on the pathogen surface, which activates the MBL-associated serine proteases, MASP-1, and MASP-2 (very similar to C1r and C1s, respectively), which can then split C4 into C4a and C4b and C2 into C2a and C2b.
- C4b and C2a then bind together to form the classical C3-convertase, as in the classical pathway.
- Represent the second pathway depending on bacterial, fungal, viruses and parasites carbohydrates in activation.
- It is the more important pathway in early infection especially in babies between (6-18) months (in the period between decreases antibodies passively transferred from mother and full development of their adaptive immune system).

The Role of Activated Complement in Immune Response

1- Viral neutralization.

2- Cytotoxic function (lysis).

3- Opsonization.

4- Immune complexes (IC) clearance.

5- Inflammatory function.

Lecture :8

The Antigen

An antigen is defined as an organism, a molecule, or part of a molecule that is recognized by the immune system. **or**

Any substance (usually foreign) that binds specifically to an antibody or a T-cell receptor; often is used as a synonym for **immunogen**.

An Immunogens:

Immunogen is a specific type of antigen which is capable of inducing an immune response and binds to the products of the immune response,

Note: *the antigen is able to combine with the products of the immune response once they are made but it may not have the ability to induce it.*

Note: all immunogens are antigens, but some antigens (e.g., **haptens**) are not immunogens.

The foreign substances that induce an immune response possess two properties:

1. **Immunogenicity**: is the ability of a substance (immunogen) to induce a specific immune response, resulting in the formation of antibodies or cell mediated immune response.

2. **Antigenicity:** is the property of a substance (antigen) that causes it to react specifically with the final products of the immune response (secreted antibodies and/or surface receptors on T-cells)

Hapten:

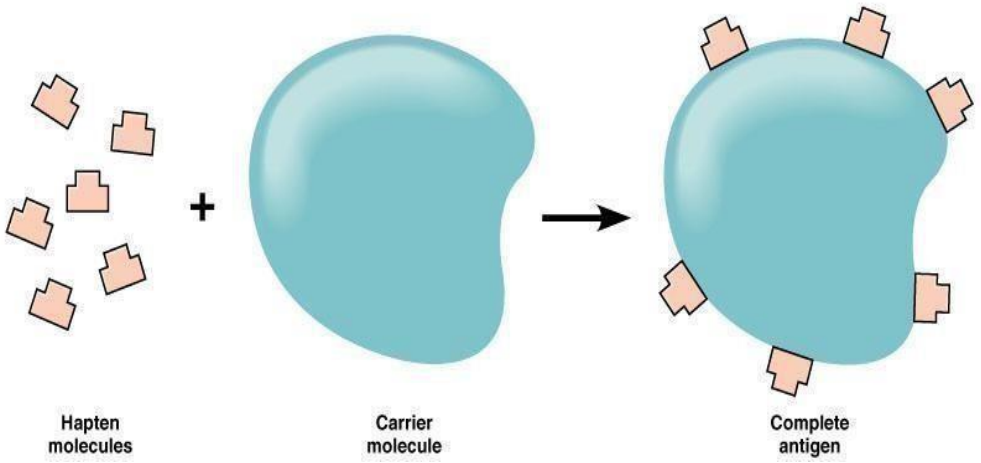
Hapten is a molecule or substance with low molecular weight (non-immunogenic) that cannot induce an immune response on its own such as antibiotics, analgesics, penicillin and other low- molecular weight compounds

However, if a hapten is combined with larger macromolecules (usually proteins) which serve as carriers then a response can be induced.

Hapten-carrier conjugate A covalent combination of a small molecule (**hapten**) with a large carrier molecule or structure.

Carrier An immunogenic molecule containing antigenic determinants recognized by T cells including albumins, globulins, or synthetic polypeptides.

Conjugation of a carrier to a non-immunogenic **hapten** renders the hapten immunogenic. Ex: Serum Protein such as Albumin or Globulin**.**



An adjuvant: Factors that are added to a vaccine mixture to enhance the immune response to antigen by activating innate immune cells. Dead mycobacterium were among the original adjuvants, but more refined preparation include alum, cytokines, and/or lipids are used.

Adjuvants function in one or more of the following ways;

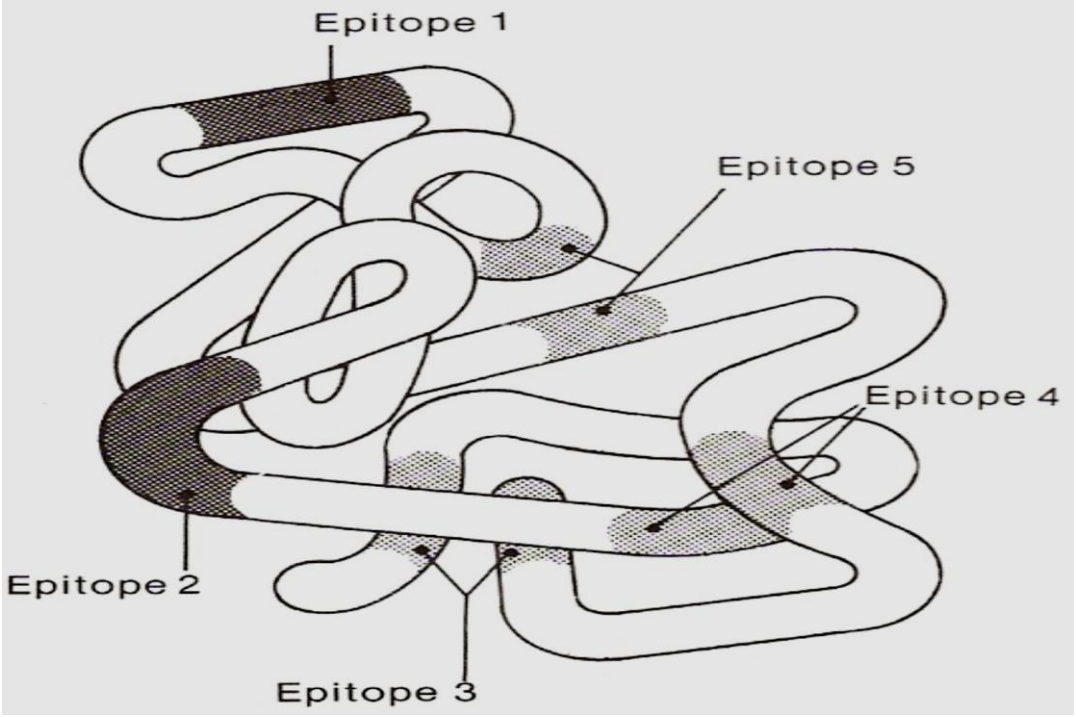
1- By prolonging retention of the immunogen,

2- By increasing the effective size of the immunogen,

3- By stimulating the local influx of macrophages and/or other immune cells to the injection site and promoting their subsequent activities.

The Epitope (antigenic determinant or antigenic specificity):

- Epitopes (also called **determinant groups or antigenic determinants**) are the sites either on or within the antigen with which antibodies react.
- Antibodies are specific for epitopes.
- A particular antigen molecule may have many different epitopes or determinant, each of which can be a target for antibody binding.
- The epitopes on an antigen can be linear or conformational



Types of Antigens:

1. **Heterophil Antigens:** are identical antigens found in the cells of different species.

2. **Graft Antigen:** divided in three types:

- **Autoantigens:** These are the antigens belonging to host itself.

- **Alloantigens or Isoantigens:** are antigens found in different members of the same species (the red blood cell antigens A and B are examples).

- **Xenoantigen:** An antigen found in more than one species. Certain proteins of brain, kidney, thyroglobulin and lens protein of one species share specificity with that of another species

3. **Bacterial Antigen:**

4. **Super antigens (SAgs)** : are a class of antigens that cause non-specific activation of T-cells at the T cells receptor (TCR) resulting in polyclonal T cell activation and massive secretion of cytokine (e.g. IL-2, IL-1, IL-6, TNFα), then resulting in toxic shock syndrome. SAgs are produced by some pathogenic viruses and bacteria most likely as a defense mechanism against the immune system**.** SAgs including retroviral protein and bacterial toxins (staphylococcal enterotoxins, toxic shock syndrome toxin**.**

Factors Influencing Immunogenicity:

1. **Chemical complexity:**

   - Proteins are usually very good immunogens.
   - Pure polysaccharides and lipopolysaccharides are good immunogens.
   - Nucleic acids are usually poorly immunogenic.
   - Lipids are non-immunogenic, although they may be haptens.

2. **Foreignness**

   - An antigen must be foreign to the host with which it makes contact to serve as an immunogen.
   - The degree of immunogenicity is dependent upon the degree of foreignness.
   - The greater the phylogenetic difference between species, the more foreign something becomes with high immunogenicity.

3. **Molecular weight**

- High molecular weight increase immunogenicity that induces immune response.
- The best immunogens are in the range more than 10000 Dalton (Da.) and the most active immunogen is with 100000 Da., while the small molecules with 5-10,000 Dalton (Da.) are generally poor immunogens.

4. **Degradability**

- The molecules with the ability to biodegrade are the best immunogens to be presented by MHC molecules to activate T-cells (Ag processing by Ag- presenting cells (APC).
- Macromolecules that cannot be degraded and presented with MHC molecules are poor immunogens. Example such as polystyrene

5. **Rout of immunization**

The rout of antigen administration plays an important role in immunogenicity. According to high immunogenicity the routs divided as following:

- Intravenous (iv): into a vein (non-favorite route because it is minimizing the immune response).
- Intradermal (id): into the skin.
- Subcutaneous (sc): beneath the skin (best route).

- Intramuscular (im): into a muscle (the best route because it is prolonging the period of immune response).
- Intraperitoneal (ip): into the peritoneal cavity (best route)

6. **Stability:** Ag with high stability acts as immunogen due to its ability to activate the immune response while the opposite is not immunogens because they are haven't a rigid structure enough to be stably bound by antibodies such as lipids and gelatin.
7. **Antigen dose:**
    - Too high or low dose of Ag will fail to activate enough immune response and cause immunologic unresponsiveness state (Tolerance).
    - Appropriate dose of Ag cause optimum antigenicity.

Lecture: 9

Immunoglobulins (Antibodies)

Antibody: Antibodies are glycoproteins, which are sensitized, and secreted by plasma cells in response to specific antigenic stimulation and it forms about 20% of plasma protein.

Functions of Antibodies:

1. Neutralizing viruses and toxins or toxoids, which neutralize the antigen.
2. Agglutinin: immobilize motile bacteria and aggregate cells forming clumps.
3. Precipitins: which form complexes with soluble antigens forming precipitates.
4. Lysine: antibodies together with complement lyse the antigenic cells.
5. Opsonins: antibodies combine with surface components of microbs and other cells so that they are more readily phagocytized.
6. Activation & fixation of complement: IgM and most IgG subclasses can activate complement system resulting to cell lysis.

Basic Structure of Immunoglobulins

Immunoglobulin is a y shaped molecule consists of four amino acid chains:

- Two heavy (H) chains (50 000 Daltons)
- Two light (L) chains (25000 Daltons).

The antibody molecule could be anatomically divided into two fragments:

- **Fab** fragments (for Ag binding).
- **FC** fragment (for Ag elimination).

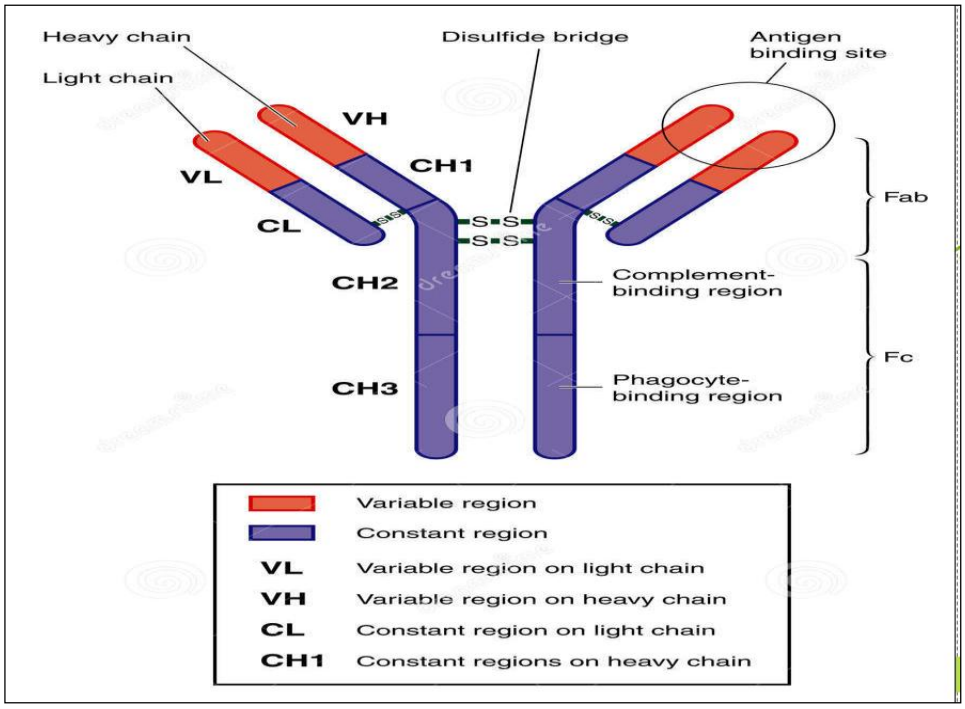
Each Chains contain two regions:

- Variable (V) region: contain variable amino acid sequence.
- Constant (C) region: contain constant amino acid sequence

Each chain has many domains:

- Each heavy chain has four domains (CH1, CH2, CH3, and VH).
- Each light chain has two domains (CL and VL)

The constant region of the light chain is determining the mechanisms to destroy antigens. This region of light chain is either Kappa (K) or Lambda (λ) with ratio 2:1.



Light Chain:

1. **Variable region**: contains the following structures:
    - **Hot spots (hyper variable area):** these are three areas in the variable region which characterized by high variability in amino acid sequencing to form the Ag binding site.
    - V_L domain.
2. Constant region: contains the C_L domain

Note: There are two types of the light chain: **Kappa and Lambda**

Heavy Chain:

1. **Variable region: contain the following structure**
    - Hot spots (hyper variable area): these are three areas in the variable region which characterized by high variability in amino acid sequencing to form Ag binding site.
    - VH domain.
2. Constant region: contain the following structure:
    - CH1 domain.
    - CH2 domain: it is the site of binding to complements.
    - CH3 domain: it is the site of binding to phagocyte and NK cell.

Note: Antibodies are divided into 5 classes (isotypes) based on their constant region structure (amino acid sequences) of heavy chain and immune function:

1. **Immunoglobulin Gamma (γ) IgG.**
2. **Immunoglobulin Alpha (α) IgA.**
3. **Immunoglobulin Mu (μ) IgM.**
4. **Immunoglobulin Delta (δ) IgD.**
5. **Immunoglobulin Epsilon (ε) IgE.**

In addition, there are subtypes of IgA (IgA1, IgA2), while IgG have 4 subtypes (IgG1-IgG4).

Hinge Region:

This is the region at which the arms of the antibody molecule form a Y shape. It is called the hinge region because there is some flexibility in the molecule at this point.

Note: in case if IgM and lgE there is additional domain in the heavy chain (CH4 domain)

Areas:

1-Switch area → located between V_L and C_{H1} domains.

It is the site of class switching (transformation from lgM to lgG or lgA or lgE)

2.Hinge area → located between $C_{H1}$and C_{H2} domains.

It is the site of opening of Immunoglobulin (transformation from inactive to active lg)

3.Paratope light → a cleft formed by the three hot spots of

Chain and adjacent three hot spots chain. It is the site of Ag binding.

Properties of Human Immunoglobulins

Properties	IgG	IgA	IgM	IgD	IgE
Structure	Monomer	Monomer, Dimer	Pentamer	Monomer	Monomer
Number of binding sites	2	2,4	10	2	2
Biological function	Long term immunity	Secretory Ab on mammary mammalian	Produced on end response to Ag	Receptor on B-cell	Ab of allergy and worm infections
Molecular weight x1000 Da	150	150, 60	900	150	190
Complement fixation	+	0	++++	0	0
Serum concentration approximate (mg/d/)	1000	200	120	3	0.05
Serum half-life (days)	23	6	5	3	2
Placental transfer	+	0	0	0	0
Binding to Fc receptor on	Phagocyte	Phagocyte	B-cell	B-cell	Mast cell and basophil

Lecture: 10

Antigen - Antibody Interactions

Principle of Antigen Antibody Interactions

Known antigen suspension or antiserum is used to detect and measure unknown antibody or microbial antigen.

The reaction between antigen and either antibody can be divided into 3 levels:

1. Primary antigen – antibody reaction.
2. Secondary antigen – antibody reaction.
3. Tertiary antigen – antibody reaction.

Primary antigen – antibody reaction.

The initial binding between Ag-Ab & and is rarely visible, the assay of this reaction includes several techniques:

- ✓ Ammonium sulfate precipitation.
- ✓ Radio Immuno assay (RIA).
- ✓ Fluorescent Immuno assay (FIA).
- ✓ Enzyme Immuno assay (EIA) or Enzyme Linked Immuno Assay (ELISA).

Secondary antigen – antibody reaction.

This reaction include:

- ✓ Agglutination.
- ✓ Precipitation.
- ✓ Complement fixation.
- ✓ Cytolysis.

Agglutination Reactions

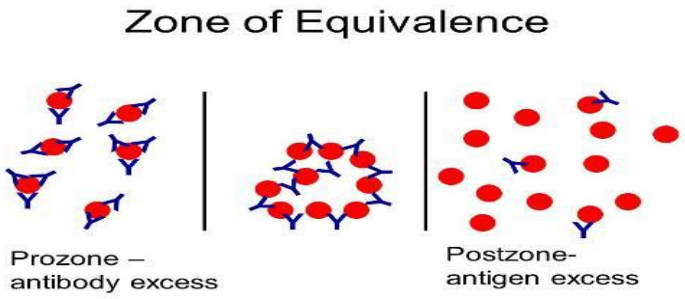
Agglutination is the visible clumping together of bacteria, cells, or particles, by an antigen combining with its specific antibody.

The Titer

The highest dilution of serum that still causes agglutination, but beyond which no agglutination occurs, is termed the ***titer***.

The tubes with high concentrations of serum, where agglutination does not occur, represent a ***prozone***.

In the prozone, antibodies are present in excess. Agglutination may not occur at high ratio of antibody to antigen because every epitope on one particle may bind only to a single antibody molecule, preventing cross-linking between different particles.
Instead, absence of precipitation with excess antigen is described as the **postzone** phenomenon



Agglutination reaction may be Direct or Indirect:

Direct agglutination:

This reaction usually involves IgM antibodies that cross-link epitopes on cells or particles.

Indirect agglutination:

The sensitivity of the agglutination test may be enhanced by the addition of an anti-immunoglobulin reagent (rabbit antihuman immunoglobulin) in the so-called indirect or passive agglutination technique. Addition of these second-step antibodies is used to increase binding over a greater span and to increase valence by virtue of their ability to bind to the primary antibody

Latex particles: these are polystyrene particles that can be coated with either known antigen or specific antibody.

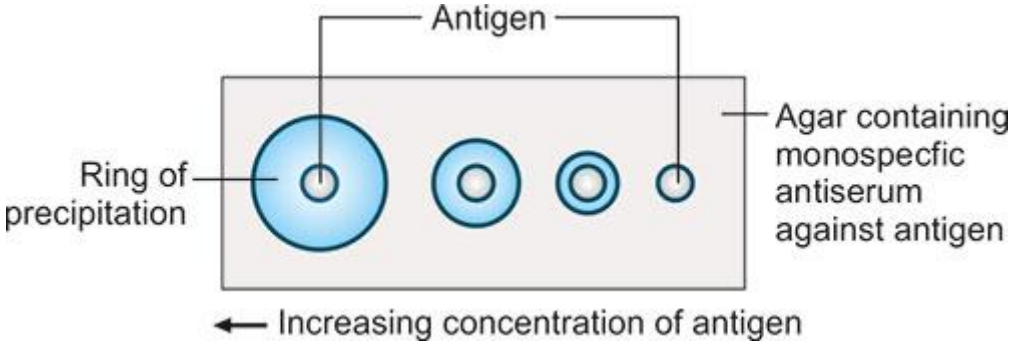
Precipitation Reaction

Combination of soluble Ag with specific Ab to give a complex from soluble aggregates, many types of precipitation used for qualitative and quantitative test as the followings:

1. **Radial Immunodiffusion** (Mancini technique)**:**

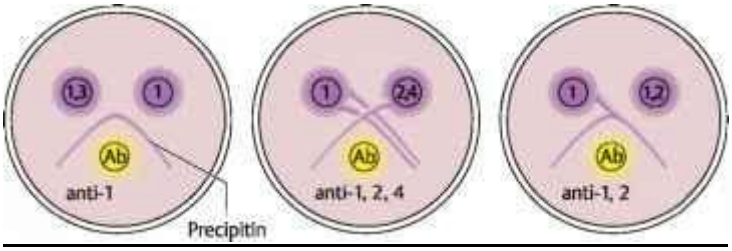
   This test is based on the diffusion of soluble antigen within an agar gel that contains a uniform concentration of antibody. Antibody containing molten agar is poured onto a glass slide or plastic dish. When the agar cools and solidifies, wells are cut into the gel matrix, and soluble antigen is placed into the well.

Antigen diffuses radially from the well, forming a precipitin ring at equivalence. The diameter of the ring is directly proportional to the amount of antigen loaded into the well. (https://www.youtube.com/watch?v=Bn-w6P_9TUA).



2. **Double-diffusion (or the Ouchterlony technique):**

This test is based on the diffusion of both antigen (loaded in one well) and antibody (loaded in another well) through an agar gel. A precipitin line forms at equivalence. An advantage of this technique is that several antigens or antibodies can be compared to determine identity, partial identity, and nonidentity of antigens
and/or antibodies. (https://www.youtube.com/watch?v=Fnx5CkGRBEM).



Complement fixation Test (CFT):

Complement fixation is a classic method for demonstrating the presence of **antibody** in patient serum. The complement fixation test consists of two components. The first component is an indicator system that uses combination of sheep red blood cells, complement-fixing antibody such as immunoglobulin G produced against the sheep red blood cells and an exogenous source of complement usually guinea pig serum. When these elements are mixed in optimum conditions, the anti-sheep antibody binds on the surface of red blood cells. Complement subsequently binds to this antigen - antibody complex formed and will cause the red blood cells to lyse.

The second component is a known antigen and patient serum added to a suspension of sheep red blood cells in addition to complement. These two components of the complement fixation method are tested in sequence. Patient serum is first added to the known antigen, and complement is added to the solution. If the serum contains antibody to the antigen, the resulting antigen-antibody complexes will bind all of the complement. Sheep red blood cells and the anti-sheep antibody are then added. If complement has not been bound by an antigen-antibody complex formed from the patient serum and known antigens, it is available to bind to the indicator system of sheep cells and anti-sheep antibody. Lysis of the indicator sheep red blood cells signifies both a lack of antibody in patient serum and a negative complement fixation test. If the patient's serum does contain a complement-fixing antibody, a positive result will be indicated by the lack of red blood cell lysis.

Lecture : 11

Primary antigen – antibody reaction.

Immunoelectrophoresis (IEOP)

Immunoelectrophoresis is a general name for a number of biochemical methods for separation and characterization of proteins based on electrophoresis and reaction with antibodies. All variants of immunoelectrophoresis require antibodies reacting with the proteins to be separated or characterized.

Electrophoresis is used to increase the speed with which the antigen and antibody travel in the agar gel.

Principle of Counterimmunoelectrophoresis

In this test, specific antibody is placed in a well at the positive electrode (anode) end of the plate and the unknown antigen in a well at the negative electrode (cathode) end. An electric current is applied and the antibody and antigen move towards each other. Positive samples show a line of precipitation within 30-60 minutes.

Immunoelectrophoresis is used to detect extracellular antigens in cerebrospinal fluid.

Radio Immunoassay (RIA).

RIA is a competitive immunologic procedure for measuring very low concentrations of antigens (or antibodies) by using radioactively labeled antigens (or antibodies) as competitors. Radioactive isotopes such as 3H , 14C, 35S, 30P or 125I can be used for labeling. It is a highly sensitive method to detect low concentration of the unknown (unlabeled) antigen and is used to assay: Hormones, Drugs, Enzymes, Microbial antigens e.g. hepatitis B antigen. RIA can also be used for detection of antibody.

RIA technique utilizes three components:-

1. Patient antigen, the specific compound we wish to determine.
2. Labeled antigen, the same compound patient antigen which is attached a radioactive label.
3. Antibody, specific for the sample and labeled antigen.

There are two assay approaches in conventional RIA:

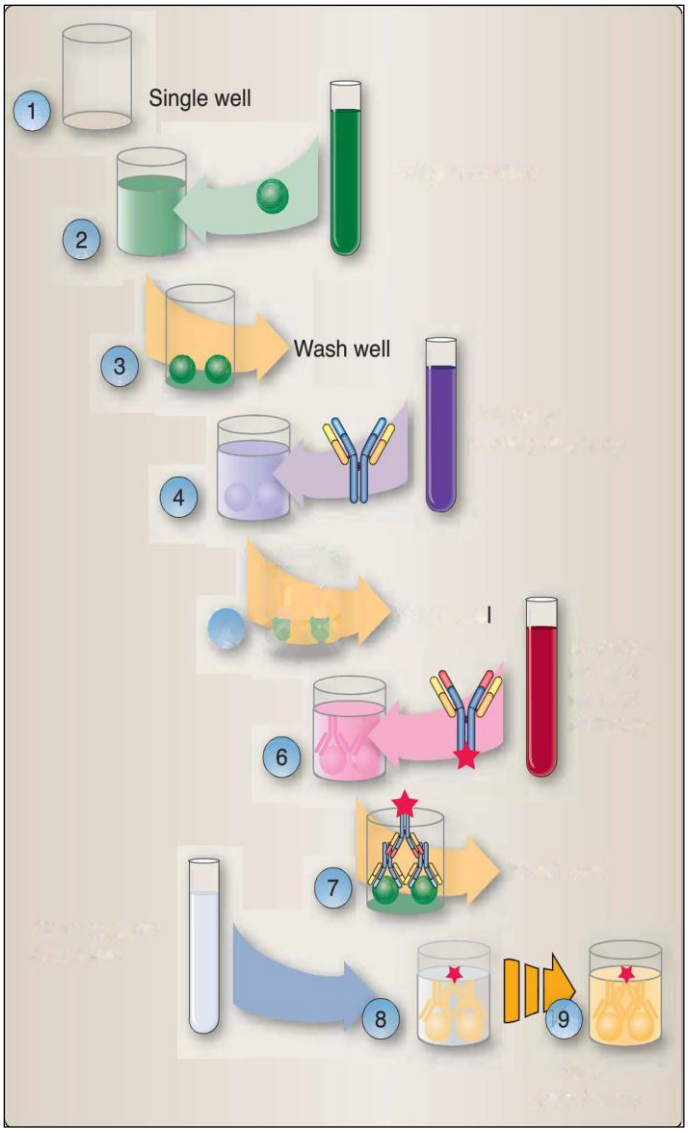
- ✓ Liquid phase Assay
- ✓ Solid phase Assay

Enzyme Linked Immunosorbent Assay ELISA

ELISA is a plate-based assay technique designed for detecting and quantifying peptides, proteins, antibodies and hormones.

Principle

In an ELISA, an antigen must be immobilized to a solid surface and then complexed with an antibody that is linked to an enzyme. Detection is accomplished by assessing the conjugated enzyme activity via incubation with a substrate to produce a measureable product. The most crucial element of the detection strategy is a highly specific antibody-antigen interaction.



ELISA Types

1. Direct ELISA

For direct detection, an antigen coated to a multi-well plate is detected by an antibody that has been directly conjugated to an enzyme.

2. Indirect ELISA

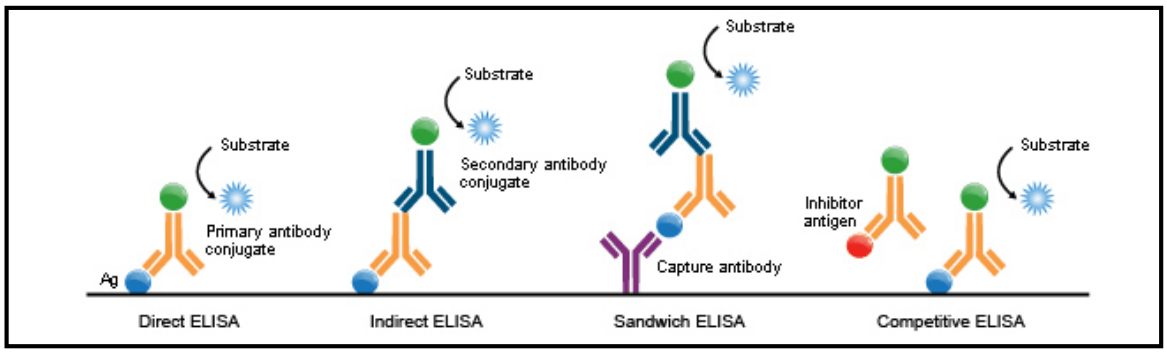
For indirect detection, the antigen coated to a multi-well plate is detected in two stages or layers. First an unlabeled primary antibody, which is specific for the antigen, is applied. Next, an enzyme-labeled secondary antibody is bound to the first antibody. The indirect assay, the most popular format for ELISA.

3.Sandwich ELISA:

Sandwich ELISAs typically require the use of matched antibody pairs, where each antibody is specific for a different, non-overlapping part (epitope) of the antigen molecule. A first antibody (known as capture antibody) is coated to the wells. The sample solution is then added to the well. A second antibody (known as detection antibody) follows this step in order to measure the concentration of the sample.

4. Competitive ELISA

The key event of competitive ELISA (also known as inhibition ELISA) is the process of competitive reaction between the sample antigen and antigen bound to the wells of a microtiter plate with the primary antibody. First, the primary antibody is incubated with the sample antigen and the resulting antibody–antigen complexes are added to wells that have been coated with the same antigen. After an incubation period, any unbound antibody is washed off. The more antigen in the sample, the more primary antibody will be bound to the sample antigen. Therefore, there will be a smaller amount of primary antibody available to bind to the antigen coated on the well, resulting in a signal reduction. The main advantage of this type of ELISA arises from its high sensitivity to compositional differences in complex antigen mixtures, even when the specific detecting antibody is present in relatively small amounts.



Membrane-Based Cassette Assays

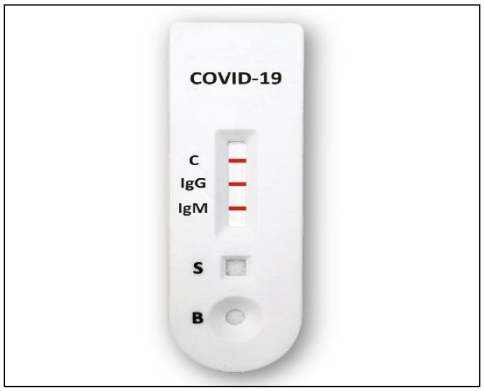
Membrane-based cassette assays are a relatively new type of enzyme immunoassay. They are rapid, easy to perform, and give reproducible results.

- ✓ Typically these are designed as single-use, disposable assays in a plastic cartridge.
- ✓ The membrane is usually nitrocellulose, which is able to easily immobilize proteins and nucleic acids.

- ✓ Either antigen or antibody can be coupled to the membrane, and the reaction is read by looking for the presence of a colored reaction product

Another type of rapid assay, called ***immunochromatography***, The analyte is applied at one end of the strip and migrates toward the distal end, where there is an absorbent pad to maintain a constant capillary flow rate. The labeling and detection zones are set between the two ends.

As the sample is loaded, it reconstitutes the labeled antigen or antibody, and the two form a complex that migrates toward the detection zone. An antigen or antibody immobilized in the detection zone captures the immune complex and forms a colored line for a positive test. This type of test device has been used to identify microorganisms such as *Streptococcus pyogenes* and *Streptococcus agalactiae* and has been used to test for pregnancy, for troponin in a heart attack, and for hepatitis B surface antigen.



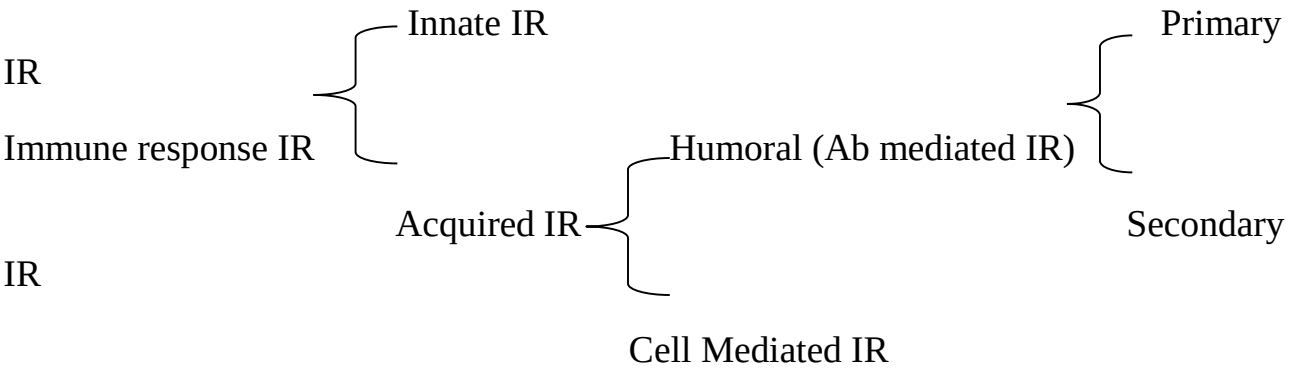
Lecture : 12

Immune Response

Immune response (IR): a protective response of the body's immune system to an antigen, especially a microorganism or that causes disease. The immune response is how your body recognizes and defends itself against bacteria, viruses and substances that appear foreign and harmful.

Mechanisms of IR:

1. Recognize, processed and presentation of antigen.
2. Cooperation of cells.
3. Destruction of specific Ag.



Humoral Immune Response :

For activation, B-cells needs the binding of antigen to specific surface Ig on B-cell leading to their proliferation and differentiation into effective plasma cells and memory cells. Plasma cells produce and secrets antibody specific for the antigen.

Functions:

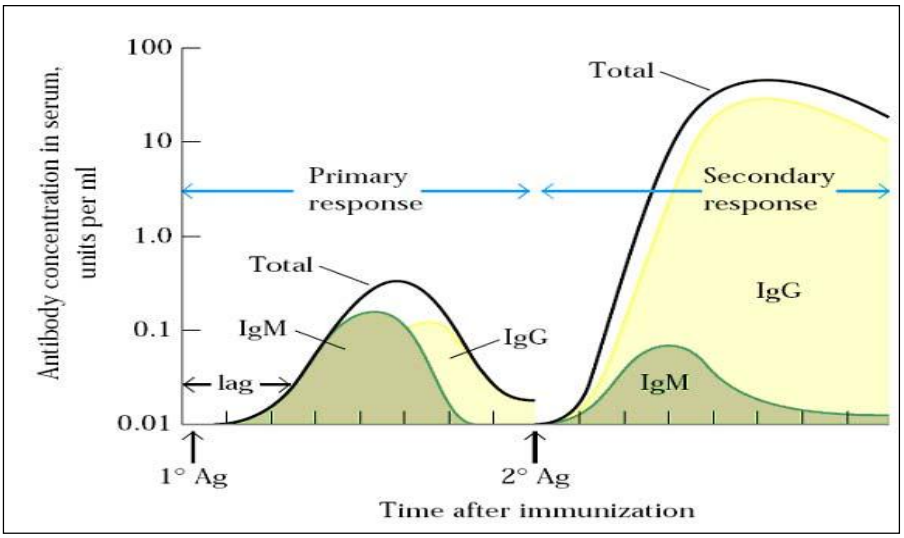
Humoral or Ab mediated immunity develops mainly against extracellular pathogens such as staphylococci, streptococci, encapsulated pathogens (eg: Pneumococcus, Neisseria) and against pathogens that secrets exotoxins (eg: Corynebacterium diphtheria). Antibodies neutralize exotoxins and some viruses(eg: rabies, Hepatitis A and B). IgM and IgG can activate the complement system which can opsonize pathogens leading to ingestion by phagocytic cells, or can cause lysis of the organisms.

Exposure to an Ag by an individual for the first time is called primary IR and re-exposure to the same antigen is called secondary IR which develops due to immunologic memory.

There are two types of humoral IR:

1. **Primary humoral IR: which characterized by :-**

- ✓ Latent phase (lag phase): in the first few days (7-10 days), is a period for T and B cells to bind Ag then activated and differentiated. Ab levels in the serum is zero (no Ab production).
- ✓ Exponential phase (log phase): increased of serum Ab level logarethmatically and reaches a peak.
- ✓ Steady phase (plateaus phase): production and degradation of Abs are balanced.
- ✓ Decline phase: the IR begins to shut down, and Abs concentration in the serum decreases rapidly due to combination with Ags.



2. **Secondary IR (anamnestic or memory IR):**

In the second exposure to same Ag, there is a rapid Ab response (the lag phase is only 3-5 days) this is related to memory cells produced after the first contact.

Comparison between Primary & Secondary IR:

Primary IR	Secondary IR
The lag phase is often longer, weeks or months	Very short (3-4days) due to memory presence cells
IgM predominant with low IgG	IgG predominant with low IgM
Antibody titer is low	Antibody titer is high
Cell (native B cell)	Memory B cell
Ab level decline rapidly	Ab level tend to remain high for longer

Cell-mediated immunity

The main defense is against intracellular pathogens like Mycobacterium, viruses, fungi and protozoa. It may be involved in tumor immunity. Immune response in this case is due to activation of T-cells leads to production of cell-mediated immunity by a direct attack on foreign cells.

Two different types of T-cells are generated, T-helper (CD^{+4}) and cytotoxic T-cells (CD^{+8}), their killing are specific.

Helper T-cells are killed the target cells specifically by producing lymphokines, while cytotoxic T-cells are responsible for direct usually of virally-infected cells.

Lecture 13.

Immunologic Tolerance and Autoimmunity

When Ag introduced into the body there will be an immune response (IR), but sometimes there is no IR or unresponsiveness (tolerance).

Immunologic Tolerance is defined as unresponsiveness to an antigen that is induced by previous exposure to that antigen.

- ✓ Antigens that induce tolerance are called **tolerogens**, or tolerogenic antigens, to distinguish them from immunogens, which generate immunity.
- ✓ Tolerance to self-antigens, also called **self-tolerance**, is a fundamental property of the normal immune system, and failure of self-tolerance results in immune reactions against self (autologous) antigens.
- ✓ Such reactions are called **autoimmunity**, and the diseases they cause are called **autoimmune diseases**.

General Characteristics of Tolerance:

- ✓ The mechanisms of tolerance eliminate and inactivate lymphocytes that express high-affinity receptors for self-antigens.
- ✓ Tolerance is antigen specific, resulting from the recognition of antigens by individual clones of lymphocytes.
- ✓ Self-tolerance may be induced in immature self-reactive lymphocytes in the generative lymphoid organs (**central tolerance**) or in mature lymphocytes in peripheral sites (**peripheral tolerance**).
- ✓ Tolerance can be existing in T and B cells or both of them. Tolerance also can be natural or induced.

Mechanism of Central Tolerance:

Normally, both B and T cells that bind self-epitopes at distinct early stages of development meet an apoptotic death, thus eliminating large numbers of potentially self-reactive cells before they enter the circulation. B cells express surface lgM as their BCRs. Epitope recognition by BCRs of developing B cells within the bone marrow triggers their apoptotic death , a process known as negative selection. Likewise, the binding of peptide-MHC complex (pMHC I or pMHC II) by TCRs of single positive ($CD4^{+4}$ or $CD8^{+}$) thymocytes causes them to undergo apoptotic death . This process removes many potentially autoreactive B and T cells before they enter the periphery.

Note:

A major caveat imposed on central tolerance is that not all self-epitopes are to be found in the primary lymphoid organs, especially those self-epitopes that arise after lymphogenesis, such as those that arise during puberty. Other means are needed to prevent the auto reactive cells among them from inflicting damage on the body.

Mechanism of Peripheral Tolerance

Several additional mechanisms, collectively called peripheral tolerance, control or eliminate autoreactive B and T cells after they exit the bone marrow or thymus.

1. **Anergy:** a state of non-responsiveness in lymphocytes after their receptors bind antigen (B cell) or pMHC (T cell).
2. **Suppression**: Tolerance to self-epitopes can also be induced by regulatory cells. The molecular bases for these regulatory actions are still unclear, but in most cases, the regulatory cells are T cells

Factors playing a role in inducing tolerance:

1. Time of introduction of toleragen: it means either in perinatal or adult period.
2. Nature (structure), high dose of toleragen, e.g: very simple molecule induce tolerance more rapidly than complex, and very high or very low doses may result in tolerance of an IR.
3. Persistence of toleragen in host, e.g: in cancer, there will be continuous shedding of antigens.

Autoimmune reaction:

Breakdown in self-tolerance lead to immune cells react with self-tissues antigens which result in tissue injury and leading to autoimmune diseases (AID).

Factors playing a role in induction of (AID):

1.Molecular Mimicry:

Is a process in which infection by particular microbes is associated with the subsequent development of specific autoimmune diseases.

Mechanism:

The antigenic molecules on some infectious agents are similar enough to some host self-molecules that B- and T-cell responses generated against the microbial antigens can result in damage to host cells bearing similar molecules.

Example:

The best understood example of this process is the cardiac damage resulting from rheumatic fever after infection by *Streptococcus pyagenes*. Strains of *S. pyogenes* express high levels of an antigen known as the M protein, a molecule that shares some structural similarities with molecules found on the valves and membranes of the heart. If the levels of IgM and IgG generated against the M protein during infection reach sufficient levels, there may be sufficient binding to host cells to induce damage and reduced cardiac function.

2.Epitope spreading

The epitope that initiates a response leading to autoimmunity might not be the epitope that is targeted by immune responses that develop later during the pathogenesis of the disease.

Mechanism

Initial responses against an infectious agent may result in damage that exposes self-epitopes in ways that subsequently trigger true autoimmune responses.

Example:

In some animal models of human multiple sclerosis, responses to particular viral epitopes regularly precede the development of response to specific epitopes associated with the myelin sheath that protects neuronal axons.

3.Loss of suppression

Suppressor cells of various types serve to maintain peripheral tolerance.

Mechanism:

Evidence suggests that the numbers of these suppressor cells decline with age, increasing the risk that previously suppressed autoreactive lymphocytes can become active. **Example:**

A pattern of increasing risk with increasing age is indeed seen in some autoimmune diseases, such as systemic lupus erythematosus (SLE).

4.Sequestered antigens

Mechanism

Some self-molecules are "sequestered" and are normally never exposed to the immune system for various reasons. As a result, if they do become exposed , as a result of injury for example, the immune system may view them as foreign and attack them.

Example

Among the best understood examples of sequestered antigens are those associated with spermatogonia and developing sperm within the lumen of testicular tubules. The tubules are sealed off early in embryonic development, prior to development of the immune system, by enclosure within a sheath of tightly joined Sertoli cells. Immune cells do not penetrate the barrier presented by the Sertoli cells and therefore are never exposed to self-molecules that are unique to the testicular tubule lumen. If these are exposed by injury (or by procedures such as surgery or vasectomy) , immune responses may occur against the self (but seemingly foreign) molecules. It is believed that some cases of male sterility are caused by this mechanism.

5.Neoantigens

Neoantigens are self-antigens that have been modified by some extrinsic factor (binding of a reactive chemical) so that they appear foreign to the immune system. Thus, they are not true autoantigens, and the reactions against them are not truly autoimmune. However, the effects of responses to neoantigens can be nearly identical to those against autoantigens

Lecture: 14.

Autoimmune diseases

Autoimmune diseases are conditions in which damage to organs or tissues results from the presence of autoantibody or autoreactive cells. Such diseases affect 5-7% of the population and are thought to be caused by the loss or breakdown of self-tolerance.

Autoimmune diseases are divided into:

1. Organ specific means injury occurs in one organ e.g.: gravies disease.
2. Non organ specific (Systemic): means injury occurs in tissues of many organs eg: Systemic lupus erythematosus

There is often a good bit of overlap between the two, because some diseases that start out as organspecific later affect other organs.

An example of autoimmune diseases:

1. Organ specific autoimmune diseases:

- ✓ **autoimmune hemolytic anemia**: autoantibodies to RBCs surface antigens, triggering complement – mediated lysis or Ab – mediated opsonization and phagocytosis of RBCs. Such anemia is drug induced like using penicillin or methyldopa which interact with RBCs and diagnosed by coombs test.
- ✓ **Insulin dependent diabetes mellitus (IDDM):** autoantibodies attack pancreas (islet – cell, enzymes) such as glutamine acid decarboxylase (GAD), insulin autoantibodies (IAA), insulinoma autoantibodies (IA2), these autoantibodies destroy islet – cells and resulting in increased levels of blood glucose.
- ✓ **Myasthenia gravis:** autoimmune disease mediated by blocking antibodies by producing autoantibodies that bind acetyl choline receptor (AChR) on the motor end plates of muscles and also inducing complement – mediated lysis of the cell, leading to weakened of skeletal muscles.

2. Non organic (systemic) autoimmune diseases:

Systemic lupus erythematosus (SLE): in this disease there is autoantibodies against DNA, nucleoproteins and other component of the nucleus. Symptoms vary between people and may be mild to severe. Common symptoms

include painful and swollen joints, fever, chest pain, hair loss, mouth ulcers, swollen lymph nodes, feeling tired, and a red rash which is most commonly on the face. Rates of disease vary between countries from 20 to 70 per 100,000. Women of childbearing age are affected about nine times more often than men. Diagnosis can be difficult and is based on a combination of symptoms and laboratory tests

Tests used to diagnose SLE may include:

1. Antinuclear antibody (ANA).
2. Anti-histone antibody
3. Anti-Deoxyribonucleoprotein (anti-DNP)
4. Extractable nuclear antibody (ENA)

Goodpasture syndrome:

Goodpasture syndrome is a group of acute diseases that affects the lungs and kidneys. The immune system mistakenly makes antibodies that attack the lungs and kidneys. This condition can quickly progress to an inflammation of the kidneys (glomerulonephritis) and kidney failure. It can be fatal if not quickly diagnosed and treated. Autoantibodies are formed against collagen in basement membrane of the kidneys and lungs. This disease primarily affects young men. Subsequent complement activation leads to direct cellular damage (glomerular and alveolar membrane) leading to kidney damage and lung hemorrhage. Death may occur in several months. Diagnosis by immunofluorescent – labeled anti – IgG.

Rheumatoid arthritis:

Sever chronic systemic inflammatory autoimmune disease of unknown etiology, characterized by inflammation of synovial membrane, affecting peripheral joints in a symmetric fashion, leads to cartilage destruction, bone erosion and joint deformities, extra – articular manifestations like vasculitis and subcutaneous nodules also can occur.

Symptoms are:

- ✓ Joint pain, tenderness, swelling or stiffness for six weeks or longer
- ✓ Morning stiffness for 30 minutes or longer
- ✓ More than one joint is affected
- ✓ Small joints (wrists, certain joints of the hands and feet) are affected
- ✓ The same joints on both sides of the body are affected.

Laboratory diagnosis :

Inflammation

Erythrocyte sedimentation rate (ESR, or "sed rate") and C-reactive protein (CRP) level are markers of inflammation. A high ESR or CRP is not specific to RA, but when combined with other clues, such as antibodies, helps make the RA diagnosis.

Immunologic Tests:

1. Rheumatoid factor (RF) is an antibody found in about 80% of people with RA during the course of their disease. Because RF can occur in other inflammatory diseases, it's not a sure sign of having RA.
2. Anti-cyclic citrullinated peptide (anti-CCP) – occurs primarily in patients with RA. That makes a positive anti-CCP test a stronger clue to RA. But anti-CCP antibodies are found in only 60-70% of people with RA and can exist even before symptoms start.
3. Other autoantibodies found include antikeratin antibody, antiperinuclear antibody, anti-filaggrin, and anti-Sa antibody.

Lecture: 15.

Immune Responses to Viruses

Immune response against viruses can be achieved by cytotoxic cells, interferons and antibodies.

Via cytotoxic cells

When a virus infects a person (host), it invades the cells of its host in order to survive and replicate. Once inside, the cells of the immune system cannot **'see'** the virus and therefore do not know that the host cell is infected. So, cells employ a system that allows them to show other cells what is inside them – they use or **MHC class I** to display pieces of protein from inside the cell upon the cell surface. If the cell is infected with a virus, these pieces of peptide will include fragments of proteins made by the virus to activate **cytotoxic T cell**

Cytotoxic T cells have specialized proteins on their surface that help them to recognize virally-infected cells. These proteins are called **T cell receptors** (**TCRs**) that can specifically recognize a particular antigenic peptide bound to an MHC molecule. If the T cell receptor detects a peptide from a virus, it warns its T cell of an infection. The T cell releases **cytotoxic factors** to kill the infected cell and, therefore, prevent survival of the invading virus

Some viruses stop MHC molecules from getting to the cell surface to display viral peptides. If this happens, the T cell doesn't know there's a virus inside the infected cell.

However, another immune cell specializes in killing cells that have a reduced number of MHC class I molecules on their surface – this cell is a **NK cell**. When the NK cell finds a cell displaying fewer than normal MHC molecules it releases toxic substances, which kill the virally-infected cell.

Mechanisms of cytotoxic IR against Virus

1. **Apoptosis :**

   Once inside the target cell, they initiate a process known as programmed cell death or **apoptosis,** causing the target cell to die.
2. Cytokines include **tumor necrosis factor** that transfer a signal from the T cell to the infected, or other neighboring cells, to enhance the killing mechanisms.

Cytotoxic factors (granules) of Cytotoxic cells are :

1. **Perforin**, a protein that can make pores in cell membranes; these pores allow entry of other factors into a target cell to facilitate destruction of the cell.
2. **Granulysin** is a substance released by cytotoxic T cells (CD8) and NK cell. It functions to create holes in the target cell membrane and destroy it. Granulysin is able to induce apoptosis in target cells and also has antimicrobial action.
3. Enzymes called **granzymes** are also stored in cytotoxic cells, and released from, the granules. Granzymes enter target cells through the holes made by perforin.

Via interferons

Virally infected cells produce and release small proteins called **interferons**, which

1. Prevent replication of viruses, by directly interfering with their ability to replicate within an infected cell.
2. They also act as signaling molecules that allow infected cells to warn nearby cells of a viral presence - this signal makes neighboring cells increase the numbers of MHC class I molecules upon their surfaces, so that T cells surveying the area can identify and eliminate the viral infection as described above.

Via antibodies

1. Firstly, the antibodies **neutralize** the virus, meaning that it is no longer capable of infecting the host cell.
2. Secondly, many antibodies can work together, causing virus particles to stick together in a process called **agglutination**. Agglutinated viruses make an easier target for immune cells than single viral particles.

3. The activation of phagocytes. A virus-bound antibody binds to receptors, called Fc receptors, on the surface of phagocytic cells and triggers **phagocytosis**, by which the cell engulfs and destroys the virus.
4. Finally, antibodies can also activate the complement system, which opsonizes and promotes phagocytosis of viruses. Complement can also damage the envelope (phospholipid bilayer) that is present on some types of virus.

IMMUNITY TO BACTERIA

Immunity to extracellular bacteria

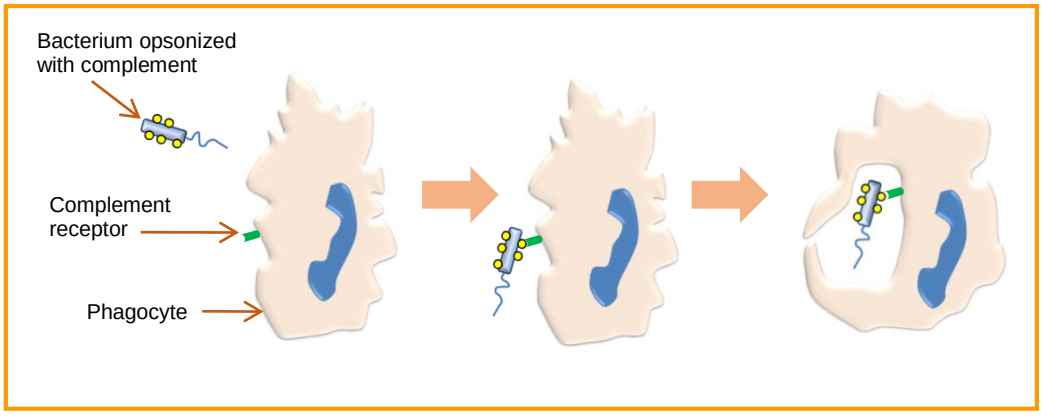
Extracellular bacteria are those that multiply and reside outside the host cell. These bacteria mainly affect the cells in two ways. They either attack by causing inflammation and tissue damage or by producing toxins.

Innate Immunity to Extracellular Bacteria

Innate immunity to extracellular bacteria essentially involves three processes.

1. **Stimulation of phagocytes:**

   Phagocytes takes the help of surface receptors and Fc receptors to identify extracellular bacteria and its opsonization with the help of antibodies, respectively. Most of these receptors are associated with promotion of phagocytic activity and microbicidal activity. After bacteria are ingested by phagocytosis, they are killed by various processes that occur inside the cell, and broken into small fragments by enzymes. Phagocytes present the fragments on their surface via **class II major histocompatibility** (**MHC class II**) **molecules**.



Circulating **helper T cells** recognize these bacterial fragments and begin to produce proteins called **cytokines**.

2. **Induction of inflammatory response:**

   Antigen presenting cells like dendritic cells in addition to phagocytes are stimulated by microbes and these cells secrete cytokines which are responsible for causing leukocyte infiltration at the site of inflammation.

3. **Activation of complement system:**

   Both gram positive and gram-negative bacteria stimulate alternative pathway of complement system and mannose expressing bacteria stimulate lectin pathway of complement system by binding to mannose binding lectin.

Adaptive Immunity to Extracellular Bacteria

The immunity that plays major role against extracellular bacteria is the humoral or antibody mediated immunity as it prevents the infection by neutralizing the toxins. Usually polysaccharide antigens are prototypic thymus-independent antigens and humoral immunity is the basic line of defense against polysaccharide-rich encapsulated bacteria.

Circulating helper T cells recognize bacterial fragments presented by antigen presenting cells and begin to produce cytokines. Two major groups of helper T cells are known as **Th1 and Th2** cells. These cell types differ in the types of cytokines they secrete. Th1 cells predominantly produce interferon-g (IFN-g), which promotes cell-mediated immune mechanisms. Th2 cells produce mostly interleukin-4 (IL-4), which promotes humoral immunity by activating B cells. B cells make antibodies that stick to extracellular bacteria and prevent their growth and survival.

The antibodies also defend the body by neutralization, opsonization, phagocytosis and stimulation of complement system. Bacterial specific CD4+

helper T cells also induces inflammation and phagocytic activity. Besides this, these antigens may cause some mutational disorders and also the affected individual may have reduced immune response towards microbial infections.

Immune Evasion by Extracellular Bacteria

1. Polysaccharide antigens or encapsulated bacteria are more lethal as compared to a strain devoid of capsule because they resist phagocytosis.
2. Capsulated bacteria inhibit alternate pathway of complement system due to the presence of sialic acid.
3. Genetic edition of surface antigens: E.g., surface antigen of some specific bacteria is contained in their pili. Pili contain a protein antigen called "pilin" and this pilin undergoes gene variation. Pili are the structures of bacteria responsible for bacterial adhesion to host cells.

Immunity to Intracellular Bacteria

Some intracellular bacteria like pathogenic or facultative are able to multiply within the phagocytes, so their elimination from the patients requires modified strategies.

Innate Immunity to Intracellular Bacteria

Phagocytes and natural killer cells provide innate immunity to the intracellular bacteria. However, some bacteria survive and multiply easily in the phagocytes, the phagocytes need to be stimulated by the secretions of these bacteria in order to clear the infection. The secretions from these bacteria are recognized by Toll-Like Receptors (TLRs) and cytoplasmic proteins of the NOD-like receptor (NLR) family so that they stimulate the phagocytes to degrade the invading bacteria. Although innate immunity provides protection from most of the bacteria but some intracellular bacteria like *Listeria monocytogenes* need cell mediated immunity in order to be eliminated from the body.

Adaptive Immunity to Intracellular Bacteria

Some bacteria engulfed during phagocytosis avoid the killing mechanisms of the phagocyte to survive inside cells. Macrophages are a common target for intracellular bacteria (e.g. Salmonella spp.) that live inside cell compartments. These bacteria cannot be detected by complement or antibody but, instead, are eliminated using a cell-mediated response. Infected macrophages present bacterial peptides on their cell surface using MHC class II molecules. This mechanism is called antigen presentation.

A helper T cell surveys MHC class II molecules with its T-cell receptor (TCR) to observe the peptides they hold. If a bacterial peptide is presented, the Th1 cell releases IFN-g. This cytokine stimulates killing mechanisms, (such as production of lysozyme) inside the infected macrophage to digest and destroy the invading bacterium. IFN-g also increases antigen presentation by cells, making the bacterium more visible to the immune system and more prone to attack.

Granulomatous inflammation acts as a marker for most of the infections due to intracellular bacteria, which occurs because of T-cell and macrophage stimulation. Macrophage stimulation that occurs as an antigenic response towards intracellular microbes is sometimes able to cause tissue damage. The response shown by different patients towards the intracellular microbes decides the development of the disease and its consequence.

One example of such type of response is shown by leprosy patients. Leprosy is a disorder caused by *Mycobacterium leprae* and it exists in two forms, the lepromatous and tuberculoid form. Lepromatous form is characterized by feeble cell-mediated immune response and high specific antibody titer while the tuberculoid form shows low specific antibody titer but very strong cell-mediated immune response. Although the reasons attributed to this type of response are still speculated and not yet verified, one of the factors that are given significance is regarding varied pattern of cytokine production and T-cell differentiation in patients.

Dodging of Immune System by Intracellular Bacteria

Intracellular bacteria tend to dodge the immune system in many ways comprising evading into the cytosol or preventing phagolysosome fusion and by overpowering the

reactive oxygen species by their microbicidal activity. These bacteria have the potential to cause chronic infections because they can survive the phagocyte mediated elimination and thrive for years in the body and may show reversion of the disease.