





## Micro L10: Introduction to immunology

### Immunity

Resistance, Protection, Non-susceptibility to invasion by foreign substances These substances may be:

- a. Infectious (Bacteria, viruses,...).
- b. Non-infectious (macromolecules; proteins, lipids)

### Immune and immunity

The term immune derived from the Latin word (immunitas) which means free burden or free from various civic duties or protected against diseases.

### Immune System

- Immune system is the system that protects the body from invasion by any invader i.e. like the **army**, which defends against enemy that invades any country.
- Any army includes **soldiers** and **weapons**.
- Soldiers of the immune system are the immunocompetent cells i.e. **leukocytes**,
- The weapons are called **secretory products** of immunocompetent cells like; [lysozymes, complement, cytokines and immunoglobulin molecules (antibodies)].
- The collection of these cells and their molecules is called immune system.
- The invader is called the **antigen**.

### Immune response

Collective and coordinated reactions of **immunocompetent cells** and their **secretory products** against the invader.

### Naive individual

Individual who has **not** previously encountered a microbe.

### Immune individual

Individual who is **exposed** to the antigens of a microbe, mount an active **response** to eradicate the infection and develops **resistance** to later infection by that microbe.

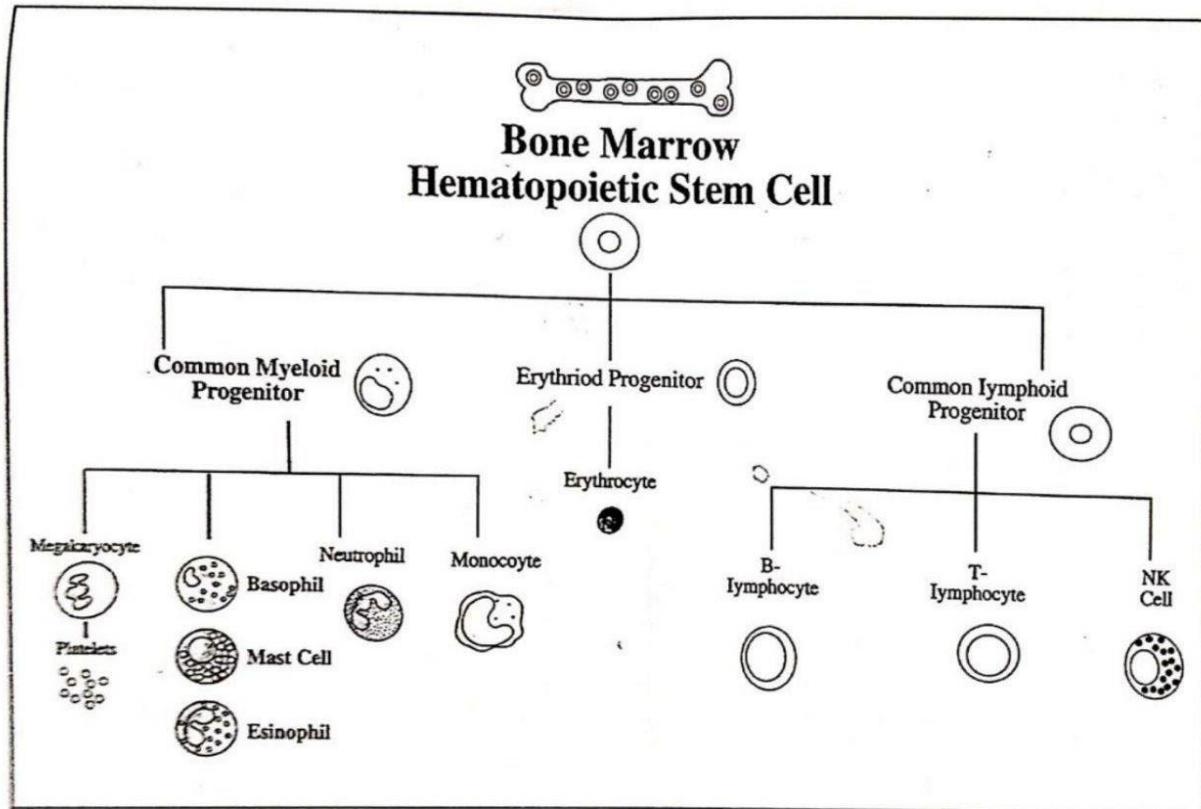


## Cells of Immune System:

They are called leukocytes; which are normally present as circulating cells in blood and lymph and as collections in lymphoid organs and scattered cells in all tissues.

**They are divided into:**

Lymphocytes	Mononuclear phagocytes	Granulocytes
<ul style="list-style-type: none"> <li>• B- cells</li> <li>• T- cells</li> <li>• Natural killer cells. (NK)</li> </ul>	<p>a) <b>Macrophages:</b> In the blood they are called monocytes and in tissues they are called histiocytes whose primary function is phagocytosis. They are considered as scavenger cells.</p> <p>b) <b>Dendritic cells.</b></p>	<p>Found in the blood, they contain abundant cytoplasmic granules.</p> <p>These leukocytes are often referred to as inflammatory cells e.g. neutrophils, eosinophils &amp; basophils.</p> <p>Basophils are found in blood, when present in tissues they are called mast cells.</p>





## COMPONENTS OF IMMUNE SYSTEM

Organs	Cells	Products
<b>Central (1ry)</b>  Where maturation of lymphocytes occur and expression of Ag receptor <ul style="list-style-type: none"> <li>▪ B.M</li> <li>▪ Thymus</li> </ul>	<b>Innate</b> <ul style="list-style-type: none"> <li>▪ Granulocytes</li> <li>▪ Mononuclear Phagocytes</li> <li>▪ Natural Killer cells</li> </ul> <b>Acquired</b> <ul style="list-style-type: none"> <li>▪ B (Ab forming cell)</li> <li>▪ T (and its subsets)</li> </ul>	<b>Innate</b> <ul style="list-style-type: none"> <li>▪ Cytokines (Monokines) Secreted by Macrophage               <ul style="list-style-type: none"> <li>- Interferon I</li> <li>- Interleukin 1</li> <li>- TNF alpha</li> </ul> </li> <li>▪ Lysozymes</li> <li>▪ C reactive Protein</li> <li>▪ Complement</li> <li>▪ Reactive oxygen radicals</li> </ul> <b>Acquired</b> <ul style="list-style-type: none"> <li>▪ Cytokines (Lymphokines) Secreted by T cells               <ul style="list-style-type: none"> <li>- Interferon II</li> <li>- Interleukin 2</li> <li>- TNF beta</li> </ul> </li> <li>▪ Antibodies Secreted by B cells</li> </ul>
<b>Peripheral (2ry)</b>  Where Lymphocytes Respond to foreign Ag <ul style="list-style-type: none"> <li>▪ Spleen</li> <li>▪ Lymph Node</li> <li>▪ Waldeyers ring</li> <li>▪ Diffuse Lymph tissue</li> </ul>		

### Functions of immune response

	Defense	Surveillance	Homeostasis
Normal	Resistance to infection by organisms	Recognition and destruction of abnormal cell types (mutants)	Removal of damage components.
Hyper Function	↑ Allergy		↑ Autoimmune ds
Hypo Function	Increase susceptibility To repeated infections (Immune deficiency disorder)	↓ Malignant diseases	



## Immunocompetent Cells

### 1) Cells of Non Specific Immunity

Members	Source
<p><b>A. Lymphocytes:</b> Natural killer (NK ) cells: They are of the following members: 1- Lymphokine Activated killer (LAK) cells. 2- Antibody dependent cell mediated cytotoxic cells (ADCC).</p> <p><b>B. Mononuclear phagocytes e.g.</b> 1- Macrophages. 2- Dendritic cells.</p> <p><b>C. Granulocytes:</b> 1- Neutrophils. 2- Basophils. 3- Eosinophils. 4- Mast cells.</p>	<p><b>A.</b> NK cells NOT undergo thymic maturation, they are derived from and mature in bone marrow.</p> <p><b>B.</b> Mononuclear Phagocytes are derived from common myeloid progenitor, obtained from hematopoietic stem cell which is found in the fetal liver and adult bone marrow</p> <p><b>C.</b> Granulocytes are derived from common myeloid progenitor obtained from bone marrow.</p>

### 2) Cells of Specific Immunity

Members	Source
<p><b>T &amp; B lymphocytes</b> T-cell subsets are:</p> <p><b>1) T- helper cells (TH, T4, CD4):</b> They include the following type of cells:</p> <ul style="list-style-type: none"> <li>a) T0: immature T cell.</li> <li>b) Th-1: mature T cell.</li> <li>c) Th-2: mature T cell.</li> <li>d) Th 4 &amp; CD25: (suppressor cells)</li> <li>e) Th17: (inflammatory cell)</li> </ul> <p><b>2) T- cytoxic cells (CTLs, T8, CD8).</b></p>	<ul style="list-style-type: none"> <li>- T &amp; B cells are derived from common lymphoid progenitor obtained from stem cell</li> <li>- Maturation of T- cell is completed in thymus.</li> <li>- Maturation of B - cell is completed in bone marrow.</li> </ul>

### 3) Antigen Presenting Cells (APCs)

#### 1) Macrophage MØ

#### 2) Dendritic cells (DC)

#### 3) B cells

- ❖ The common portals of entry for microbes, namely, the skin, gastrointestinal tract and respiratory tract, contain specialized cells located in the epithelium that capture protein antigens and transport them to peripheral lymphoid tissues.
- ❖ This function is understood for cell type called dendritic cells (DC) because of their long dendrite-like process. These cells display antigen to T lymphocytes.
- ❖ Macrophages (MØ) also are capable of displaying protein antigens to T cells.

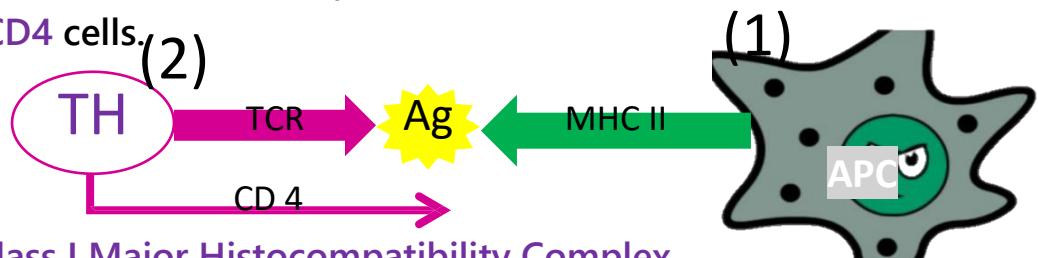


## Functions:

- 1) **Phagocytosis** of the antigens.
- 2) **Processing** of the antigens.
- 3) **Presentation of:**

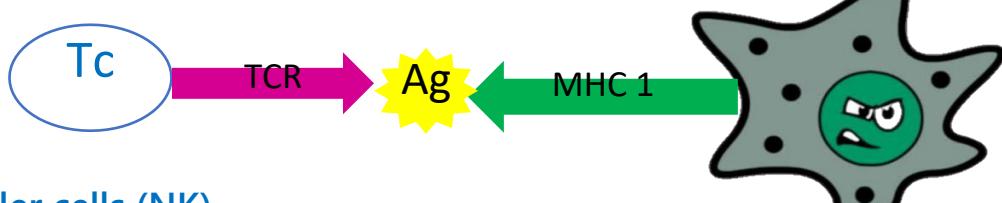
### A. Exogenous Ags by Class II Major Histocompatibility Complex (MHC):

- I. Protein antigen (Ag) taken from **extracellular** environment.
- II. Proteins are degraded by lysosomal proteases in endosome.
- III. The resulting peptides are presented by class **II** MHC molecules of APC to T cell receptor (TCR) of **CD4** cells.

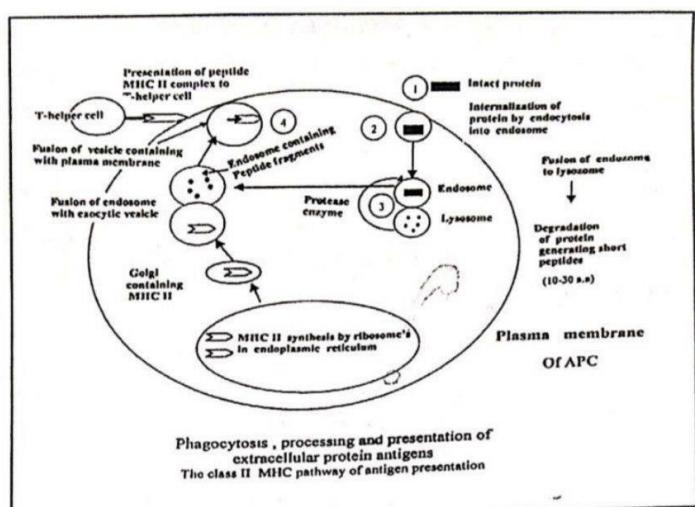
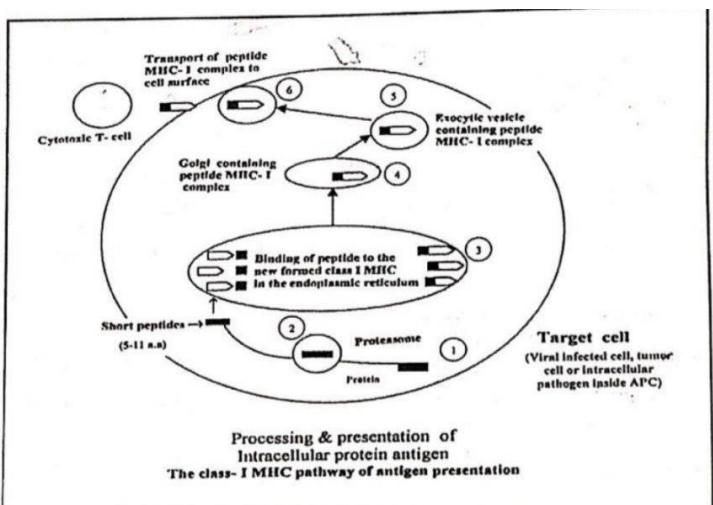


### B. Endogenous Ags by Class I Major Histocompatibility Complex (MHC):

- I. Cytosolic proteins e.g. **intracellular** microbes (viruses).
- II. Proteins are degraded into short peptides by proteasome.
- III. The resulting peptides are presented by class **I** MHC molecules of APC to T cell receptor (TCR) of **CD8** cells.

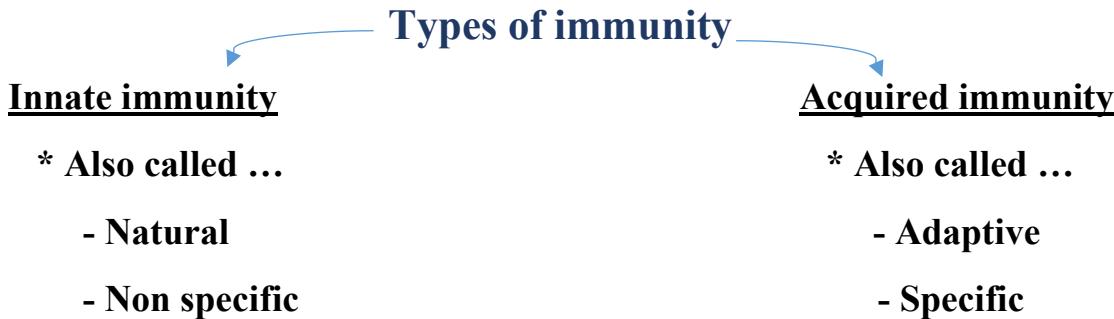


### 4) Activation of Natural Killer cells (NK).





## Micro L11 : Types of immunity



### Innate immunity

- Is considered as First Line of defense against the invading particles.
- It takes hours (0-12 hours).
- The two principal types of reactions of innate immunity are inflammation & antiviral defense.
- Innate immunity comprises a homologous clone of cells, , with no particular specialization in recognizing different types of foreign particles, so they are non-specific.

### Characters:

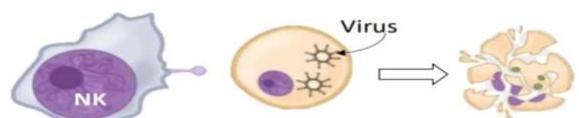
- Now they are considered specific,
- because they specifically target microbes and is a powerful early defense mechanism capable of controlling and even eradicating infection before adaptive immunity becomes active.
- They instruct adaptive immune system to respond to different microbes in effective ways.
- They recognize structures that are shared by various classes of microbes and are not present on host cells. These structures are often essential for the survival and infectivity of these microbes.
- Therefore, a microbe cannot evade innate immunity, simply because these structures are essential for their ability to live and ability to infect and colonize the host.
- Receptors on their surfaces are identical, Responds in the same way to repeat encounters with a microbe due to the absence of immunological memory.
- The cells of innate immunity are present in the germ line (chromosomes),  
So can recognize only less than 1000 microbial patterns (they are of limited specificities).
- Neutrophils ingest microbes in the circulation, and die after a few hours.



→ Monocytes in blood ingest microbes , -unlike neutrophils- they can survive for long periods , and in the tissues they differentiate into macrophages.

### Natural killer cells

- They comprise about 10% of the lymphocytes in the blood, and peripheral lymphoid organs.
- They contain abundant cytoplasmic granules.
- Their receptors recognize:
  - Cells infected with viruses and intracellular bacteria.
  - Tumor cells



### Adaptive (Acquired) immunity :

- Adaptive immunity is considered the second line of defense.
- It is made up of different cells (T & B lymphocytes).
- They require several days (1-7) after exposure to foreign particles before they become specifically operative.
- Their cells act specifically:
  - Specificity of this type of immunity is due the presence of different (heterogenous) clones of cells.
- Each one B or T cell can recognize only one type of antigen.
- The receptors for antigen recognition on T cell are called T- cell receptors (TCR) and the receptors on B- cell are called B-cell receptor (BCR).

#### Characters:

- 1- The ability to distinguish self from foreignness i.e. Antigen.
- 2- Specificity:
  - Immune system has the ability to recognize at least a billion different antigens.
  - This is due to the presence of different antigen receptors on their surfaces.
- 3- Diversity :
  - This is due to the presence of different receptors for antigens on lymphocyte.
  - Each clone of cell expresses an antigen receptor that is different from the receptors of all other clones.



- T-cell clones are called (T- cell repertoire) can recognize up to  $1 \times 10^{16-18}$  different antigenic determinants.
- B cell clones are called (B- cell repertoire) can recognize up to  $1 \times 10^{10-12}$  different antigenic determinants.

#### 4- Memory :

- The adaptive immune system mounts larger and more effective responses to repeated exposure to the same antigen.
- After the first challenge with a specific antigen, some of the activated B/T cells change to memory cells.
- If the same antigen is introduced for the second time this stimulate memory cells with higher affinity (faster and stronger response) to that antigen than in the first time.
- All normal immune responses decreased with time after antigenic stimulation.
- This is because the function of immune response is to eliminate antigens and thus elimination of the antigen stops the lymphocytes activation and these lymphocytes die or differentiated into memory cells.

#### 5- Self- elimination :

- All normal immune responses decreased with time after antigenic stimulation.
- This is because the function of immune response is to eliminate antigens and thus elimination of the antigen stops the lymphocytes activation and these lymphocytes die or differentiated into memory cells.

### Sources of Acquired immunity

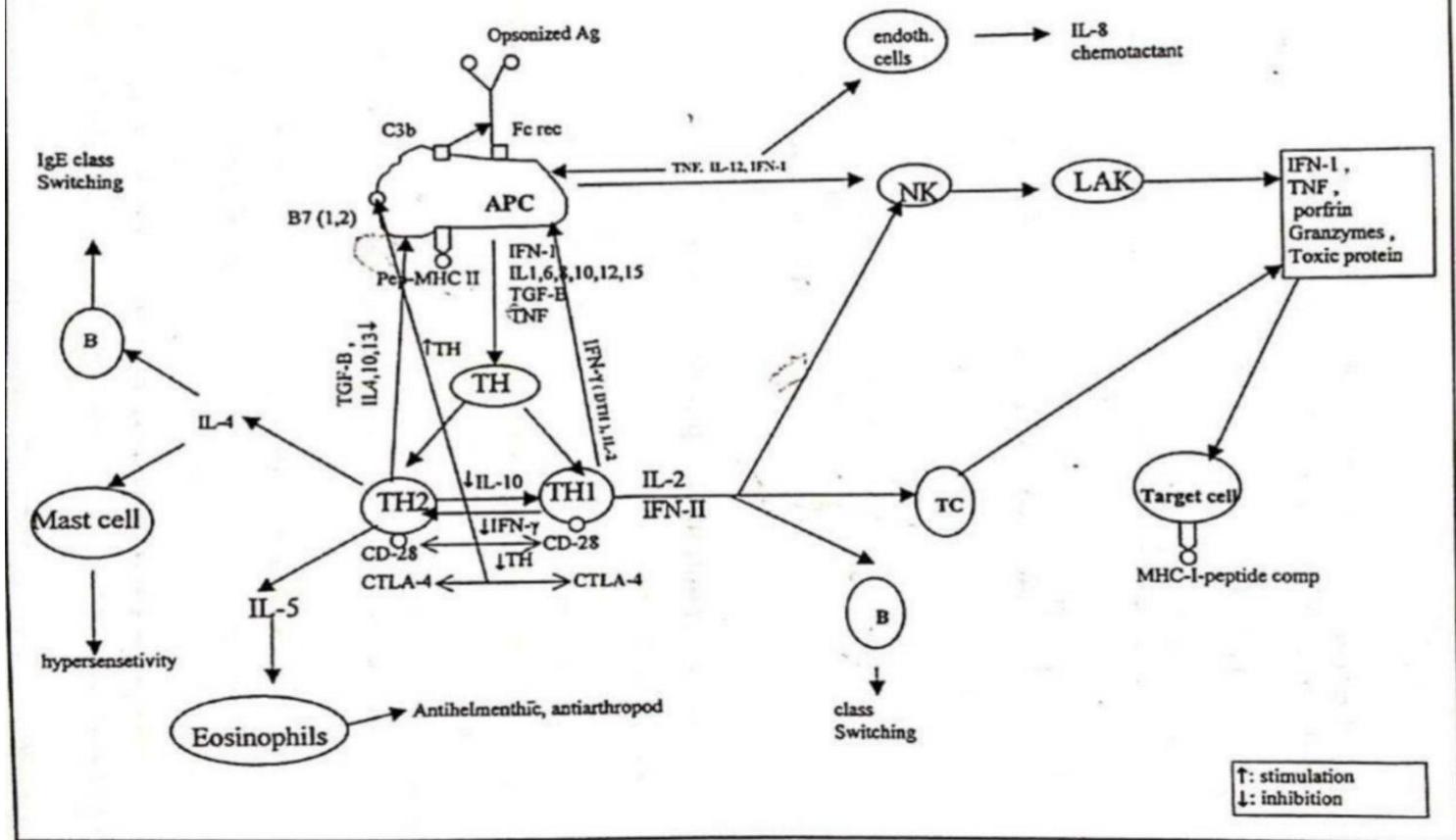
	Active	Passive
<b>Advantages</b>	<ul style="list-style-type: none"> <li>• Active humoral ( by antibodies )</li> <li>• Active cellular ( by T cells)</li> </ul>	<ul style="list-style-type: none"> <li>• Passive transfer of Antibodies</li> <li>• Passive transfer of previously activated T cell</li> </ul>
<b>Disadvantage</b>	<p><b>Slow onset of resistance</b>            - Need repeated contact with Ag            ( booster dose of vaccine )</p>	<p><b>Rapid protection.</b>  <b>Persist for short period</b>            - Hypersensitivity to foreign protein in serum</p>
<b>Type</b>	A) Natural active Recovery from infection	A) Natural passive: I- Abs from mother to fetus through placenta (IgG)



**B) Artificial active :**  
Vaccines

to protect him in first 6 months  
2- Maternal abs (IgA) during lactation  
**B) Artificial passive:**  
1 -Antiserum Antitoxic serum against tetanus.  
2-Administration of human immunoglobulin

Immune Regulatory Functions of Cytokines



**Q 1 Natural Passive Acquired immunity is obtained from ....**

- Infection.
- Vaccination.
- Mother Lactation.**
- Human Ig.

**Answer: C**

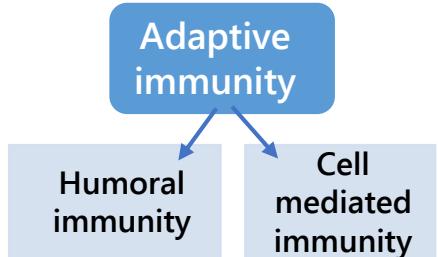


## Micro L12 : Types of Adaptive immunity

- Humoral and CMI are designed to provide defense against extracellular microbes and intracellular microbes respectively.

### Humoral immunity:

- ✓ Is mediated by **antibodies** secreted by **plasma cells** and present in plasma, lymph and body fluids (humors).
- ✓ Antibodies are synthesized exclusively by **B lymphocytes**.
- ✓ **This type of immunity can be transferred** from one person to another naïve person by plasma or serum.
- ✓ Antibodies neutralize and eliminate **extracellular microbes and their toxins** that are present in blood and lumens of mucosal organs such as respiratory tract & GIT, and stop these microbes from gaining access to and colonizing host cells.
- ✓ Antibodies **do not have access** to microbes that live and divide **inside** infected cells.



### Cell mediated immunity:

- ✓ It is mediated by cells called: “ **T- lymphocytes** ”.
- ✓ The term **cell- mediated immunity (CMI)** describes the localized reactions to organisms Usually (**intracellular pathogens**) mediated by **T-lymphocytes and phagocytes** .
- ✓ T-lymphocyte activates phagocytic cells (innate immunity) to destroy microbes that have been ingested by phagocytes.
- ✓ **This type of immunity could be transferred** to naive individuals by **T lymphocytes** from an immunized individual but **not** with plasma or serum.

### Function of CMI:

1. Immunity against intracellular bacteria like T.B., Leprosy, Salmonella, Brucella and Listeria.
2. Immunity against fungi.
3. Immunity against protozoa.
4. Immunity against viruses.
5. Immunity against tumor.
6. Graft rejections
7. Regulation of immune response.



## Types of T cells:

1) T- helper cells (CD4):	2) T- cytotoxic = TC (CD8)
<p>Help B cell to produce Abs and help phagocyte to destroy ingested microbe.</p> <p><b>Other types of T-helper cell (CD4):</b></p> <ul style="list-style-type: none"> <li>• T-regulatory (CD4 &amp; CD25) (Immune Suppressive cells)</li> <li>• Th-17 (inflammatory) secretes interleukin 17</li> </ul>	Kill Cells containing <b>Intra Cellular Microbes.</b>

## Components of Immune Response:

### I-Primary Immune Response:

- occurs due to the initial exposure to a foreign Ag
- This exposure runs in three phases:
  - **First phase** is called (**Recognition or Cognitive phase**).
  - **Second phase** is called (**activation phase**) during which lymphocytes proliferate and mature into plasma cells , and specifically reactive T cells.
  - **Third phase** is called (**effector phase** ) in which :

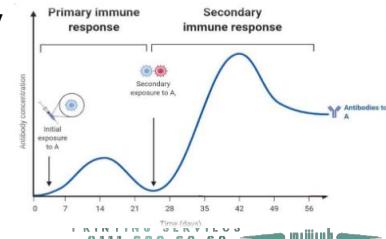
a. Plasma cells secrete antibodies (immunoglobulins) this is called humoral immunity.

Immunoglobulin produced is mainly **IgM**

b. Specifically reactive T cells secrete (lymphokines) this is called cell mediated immunity.

### II-Secondary Immune Response:

- On further contact with the same foreign substance (second exposure), increased resistance develops through the abundant production of specific antibodies or sensitized (activated) T- lymphocytes.
- The immunoglobulin response is shifted from **IgM** to **IgG** or any Other immunoglobulin this called (**Antibody Class Switch**).





## Questions:

**Q 1** Defense against extracellular microbes & their secreted toxins is mediated by ...

- a. Cell mediated immunity.
- b. Humoral immunity.
- c. NK.

**Q 2** Inhibitory signal for T cell activation is mediated by :

- a. CD28.
- b. CTLA-4.
- c. CD40L.
- d. CD40.



## Micro L13: Antigens

- Antigenic substance is any substance that could be recognized by a product of the immune system not necessarily induce an immune response.

### Forms of antigen:

#### 1. Complete antigen or immunogen:

- It is a substance that can induce specific immune response leading to production of antibodies or immune lymphocytes, and also reacts specifically with them.

#### 2. Incomplete antigen or Hapten:

- **Hapten** is an example of antigenic substances, but of low molecular weight, it can bind to antibody molecule, but not able to induce an immune response by itself; except if it is complexed to a carrier molecule e.g. (body protein molecule) causing immunologic response.
- The hapten can be defined as an exogenous determinant that is attached to a macromolecule.
- Examples of Haptens: Drugs (e.g. penicillin).

### Names of antigens:

1. Autoantigen: found within the same individual.
2. Isoantigen: found in genetically identical individuals e.g. identical twins.
3. Alloantigen: found in genetically dissimilar members of the same species.
4. Xenoantigens (heterophile): found in different species e.g. human and animal.

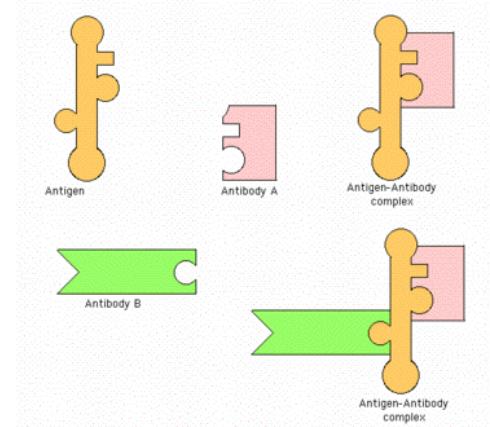
### Antigenic determinants (Epitopes):

#### Definition:

- They are sites either on or within the antigen against which the immune response is directed (valency = number of epitopes).

#### Characters:

- Epitopes determine the specificity of the antigen molecule.
- Some antigen are multivalent having different groups of epitopes, can react with different types of antibodies.





### According to T cell dependence Antigen may be:

- **T- cell dependent:** It depends on presence of T helper cells for activation of B-cells to produce antibodies (thymus dependent) e.g protein as bacterial enzymes.
- **T- cell independent:** This antigens directly stimulate B- cell to produce antibodies in the absence of T helper cells (thymus independent).e.g polysaccharide, lipid and non-protein.

### Adjuvants:

- These are substances that when mixed with an immunogen it will enhance the immune response against the immunogen.

### Examples of adjuvants:

- Freund's adjuvant (composed of killed T.B., mineral oil and detergent) used for animal immunization.
- Alum-precipitated toxoid (adjuvants are added to vaccines to enhance the antibody response).

### Function of adjuvant:

- Their biological effects are multiple:
  - 1) They increase the efficiency of macrophage in processing the antigen.
  - 2) They can acts as depots and prolong the period of exposure to the antigen.
  - 3) They may amplify the proliferation of lymphocytes by enhancing the release and the action of lymphokines.
  - 4) They increase surface area of the antigen.

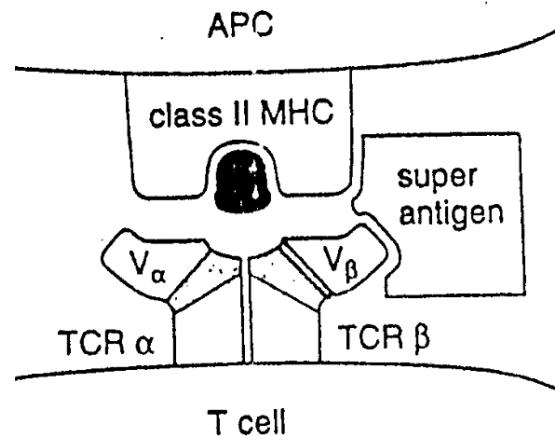
### Requirements of immunogenicity:

- Foreignness: Antigen must be foreign to the host.
- Chemical complexity:
- The strongest immunogens are proteins.
- The more complex the structure of proteins, the more potent is immunogenicity.
- Molecular size: Usually the larger molecular weight, (not less than 10.000).
- Degradability: susceptible to partial enzymatic degradation that occurs during antigen processing by presenting cells such as macrophages.



## Super antigens:

- These comprise a large group of bacterial toxins and retroviral proteins that can stimulate T-lymphocyte proliferation without regard for antigenic specificity of the T-cell.
- They have the ability to bind both class II MHC molecules and the TCR  $\beta$ -chain, acting as clamp between the two, without being presented by macrophages providing a signal for T-cell activation and release large amount of cytokines.
- Superantigens interact with MHC molecule outside the peptide – binding groove.
- The massive T cell activation and release of large amount of cytokines.



## Examples of superantigens:

- Toxic shock syndrome toxin.
- Group A streptococcal pyrogenic toxin A.
- Diphtheria and tetanus toxins.
- Staphylococcal enterotoxins.
- Retroviral proteins.

	Antigen	Superantigens
Processing in APCs	Occurs	Doesn't occur
Presentation by MHC	Essential	Doesn't occur
Binding to MHC	Into the peptide-binding groove	Into the peptide-binding groove
TCR bind by	Variable regions of both $\alpha$ and $\beta$ chains	Variable region of $\beta$ chain only
Interaction with and activation of T cells	Specific Few T cells only	Non-specific Many T cells
Cytokines released	The required levels	Very high harmful levels
Development of memory	Occurs	Doesn't occur
Outcome	Acquired immunity (beneficial to host)	Anergy (harmful to host)
Example	All ordinary antigens	<ul style="list-style-type: none"> <li>• Toxic shock syndrome toxin.</li> <li>• Staphylococcal enterotoxins.</li> </ul>



## Heterophil antigens & cross reactivity

- Heterophile antigens: They are groups of related but not identical antigens occurring in a wide range of unrelated molecules in different species.
- If one of these antigens is introduced into the body it will stimulate production of antibody
- which reacts not only with that antigen but also reacts with related antigens.

### Examples of heterophil antigens and practical applications of cross reaction:

- In vivo: In acute rheumatic fever, antibodies to *Streptococcus pyogenes* cross react with heart tissue leading to rheumatic carditis and valve destruction.
- In vitro: Several laboratory tests are based on detection of antibodies in patient's sera:
  - 1) **Paul Bunnell test:**
    - It is used in diagnosis of glandular fever caused by EB virus.
    - Serum contains heterophil antibodies which agglutinates not only the virus but also agglutinates sheep RBCs.
  - 2) **Weil Felix reaction:**
    - It is used in diagnosis of typhus fever (caused by rickettsia).
    - Serum contains heterophil antibody not only agglutinate rickettsia but also agglutinate proteus ox strains due to some antigenic sharing.
  - 3) **Cold agglutinins:**
    - It is used in diagnosis of mycoplasma pneumoniae.
    - Serum of patient with mycoplasma pneumoniae contains antibody that agglutinate human group O red cells in cold (at 4°C).
  - 4) **The regain antibody:**
    - It is used in diagnosis of syphilis as VDRL or RPR.
    - Serum of patient with syphilis contains antibody which reacts with cardiolipin antigen (alcoholic extract of beef heart muscle).



## MCQ:

1. A hapten:
  - a. Is usually a high molecular weight substance.
  - b. Acts as an antigen if coupled to protein molecule.
  - c. Is capable of inducing immune response alone.
  - d. Determines specificity of an antigen.
2. Superantigen:
  - a. Is processed inside antigen presenting cell.
  - b. Leads to development of memory T cell.
  - c. Stimulates an acquired immune response.
  - d. Leads to release of huge amount of non beneficial cytokines.



## Micro L 14: Immunoglobulins

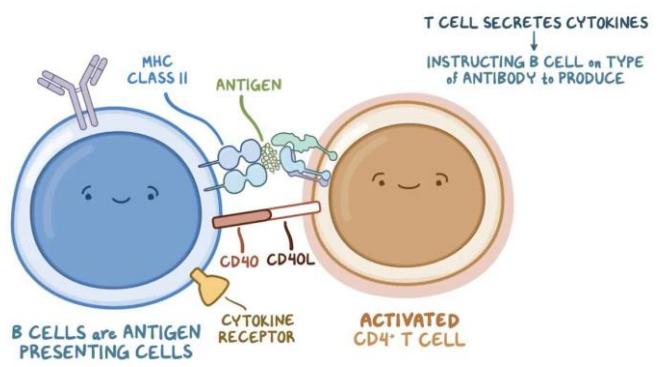
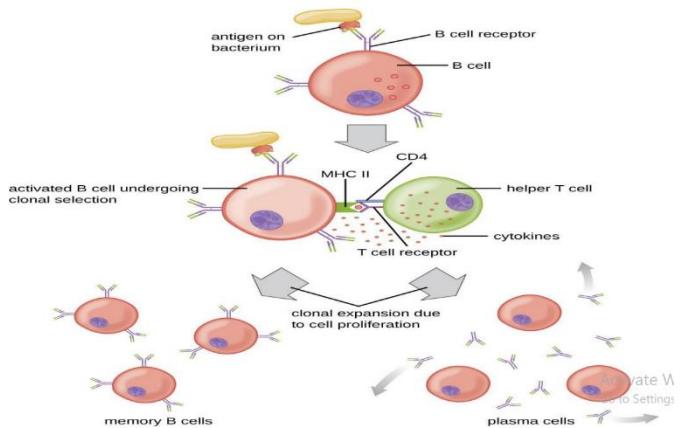
### The immunoglobulin or antibodies

- They are a group of **glycoprotein** present in the serum and tissue fluids of all mammals.
- There are several features essential for their participation in the immune response.
  - ✓ Specificity
  - ✓ Secondary biologic activities

### Origin and distribution of antibodies (Abs)

- Antibodies are synthesized in human lymphoid B - cells in bone marrow, spleen, and lymph node.
- Immunoglobulins are expressed as:
  - ✓ Secretory antibodies: are produced by plasma cells, the terminally differentiated B- cells that serve as antibody factories, Immunoglobulin binds specifically to the antigen, which induced its formation
  - ✓ The membrane –bound antibody is present on the surface of B- cells where it serves as the antigen specific receptor.
- When B lymphocytes are stimulated by antigens and with the help of Th cells and cytokines.
- They proliferate and differentiate to plasma cells which secrete the antibodies.

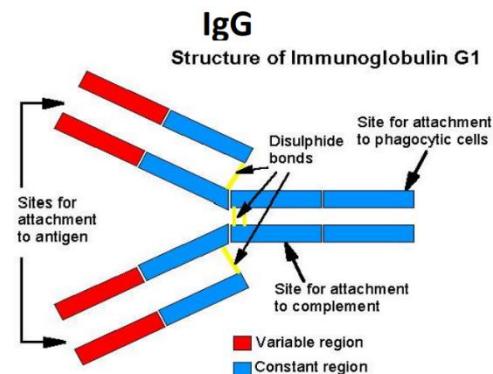
### B cell activation





### Structure of immunoglobulins or antibodies:

- The basic immunoglobulin unite (**monomer**) consists of **4 polypeptide chains**.
  - ✓ Two identical heavy chains (H)
  - ✓ Two identical light chains (L)
- Held together by disulphide bonds.
- Each chain containing variable region (V) and constant region (C )
- Both light and heavy polypeptide chains consist of subunits or domains.

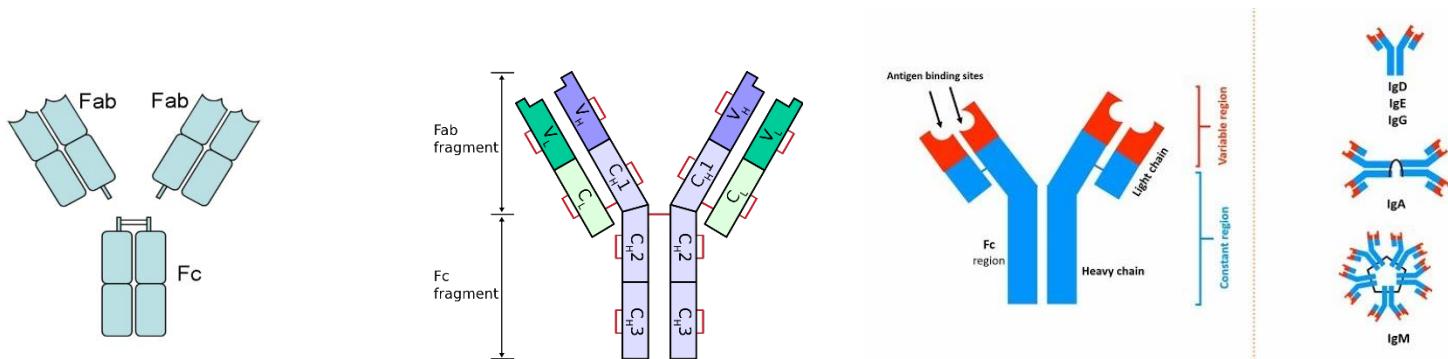


Light chains	Heavy chains
<ul style="list-style-type: none"> <li>There are two antigenic types called:           <ul style="list-style-type: none"> <li>a) kappa (k)</li> <li>b) Lambda (<math>\lambda</math>)</li> </ul> </li> <li><b>N.B</b> Only <b>one</b> type of light chain is found in any individual molecule. i.e the light chains are either both K or both <math>\lambda</math>.</li> <li>Light chains are made up of one V domain (VL)and one C domain (CL)</li> </ul>	<ul style="list-style-type: none"> <li>Five classes ( isotypes) are found according to the differences in the amino acid sequence of their heavy chain C regions</li> <li>Heavy chains are designated by Greek alphabet corresponding to the isotype of antibody           <ul style="list-style-type: none"> <li>Heavy chain <math>\gamma</math> (Gamma) <math>\rightarrow</math> IgG</li> <li>Heavy chain <math>\alpha</math> (alpha) <math>\rightarrow</math> IgA</li> <li>Heavy chain <math>\mu</math> ( Mu) <math>\rightarrow</math> IgM</li> <li>Heavy chain <math>\delta</math> (delta) <math>\rightarrow</math> IgD</li> <li>Heavy chain <math>\epsilon</math> (epsilon) <math>\rightarrow</math> IgE</li> </ul> </li> <li>Heavy chains is made up of one variable domain (VH)and 3 or 4 constant domains (CH1, CH2, CH3 &amp; CH4 )</li> </ul>



## Regions of immunoglobulin molecule

- Regions of Ig molecule are named according to the properties of proteolytic fragments
- Papin, a proteolytic enzyme, splits the antibody molecule into three fragments
  - Two identical pieces called **Fab fragments**
  - The third piece called **Fc fragment (fragment-crystalline)**



## Hypervariable regions:

- The variable regions of both L and H chains have 3 extremely variable (hyper variable) amino acid sequences that form the antigen – binding site (or complementarity determining regions (CDRs) to the antigenic determinant or epitope.

## Hinge region

- In the immunoglobulin (with possible exception of IgM and IgE).
- The hinge region is composed of a short segment of amino acids and **is found between the CH1 and CH2 regions of the heavy chains.**
- It permits flexibility between the two Fab areas of the γ –shaped antibody molecule to accommodate different antigens .

The biological functions of the Fc fragment are:

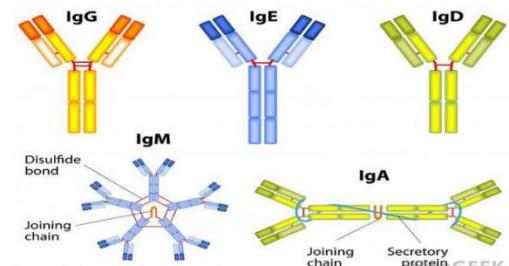
- Opsonization (IgG).
- Complement fixation (IgM and IgG).
- Transplacental transfer (IgG).
- Binding to mast cells (IgE).
- Mucosal immunity (IgA).



### The differences between heavy and light chains

Heavy chain(H)	Light chain(L)
They are structurally different for each of the five classes: $\alpha$ , $\gamma$ , $\mu$ , $\delta$ & $\epsilon$ for IgA, IgG, IgM, IgD and IgE respectively	<ul style="list-style-type: none"> <li>They are of two polypeptide chains Kappa (<math>\kappa</math>) and Lambda (<math>\lambda</math>) chains.</li> <li>An individual Ig molecule bears either two <math>\kappa</math> or two <math>\lambda</math>, never one of each.</li> </ul>
Have a molecular weight and a number of amino acids approximately twice that of light chain.	Have a molecular weight and a number of amino acids half that of heavy chain.
Have one variable domain (VH) followed by three or four constant domains. CH1, CH2, CH3, and CH4 according to the type of Ig class.	Each light chain consists of two domains: one variable ((VL) and one constant (CL))
A hinge region located between CH1 and CH2 in certain isotypes confers the flexibility of the antibody molecule.	The light chain does not mediate or influence the effector functions of antibodies.

	IgE	IgD	IgM	IgA	IgG
Subclass	1	1	1	1,2	1,2,3,4
H chain	$\epsilon$	$\delta$	$\mu$	$\alpha$ (1 or 2)	$\gamma$ (1,2,3 or 4)
L chain	$\kappa$ or $\lambda$				
Heavy chain V Domains	1	1	1	1	1
Heavy chain C Domains	4	3	4	3	3
Secretory piece	-	-	-	+	-
Serum concentration (mg/dl)	0.02	Trace	150	300	1200
Half-life in days	2-3	2-3	5	5.5	21
Functions					
Complement fixation	-	-	+++	-	++
Opsonization	-	-	-	+	++++
Mast cell sensitization	+++	-	-	-	-
Placental transfer	-	-	-	-	+



### Immunoglobulin classes and subclasses



## Functions of antibody isotypes

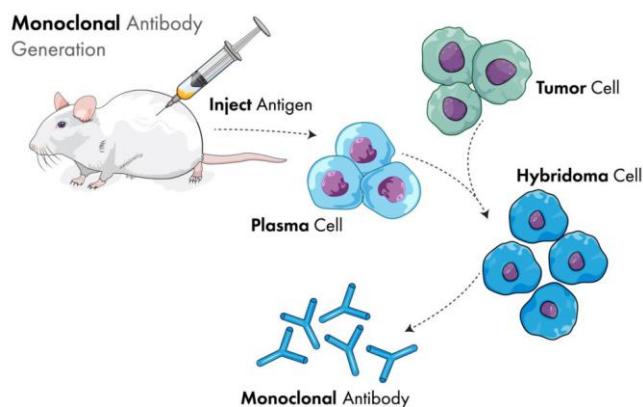
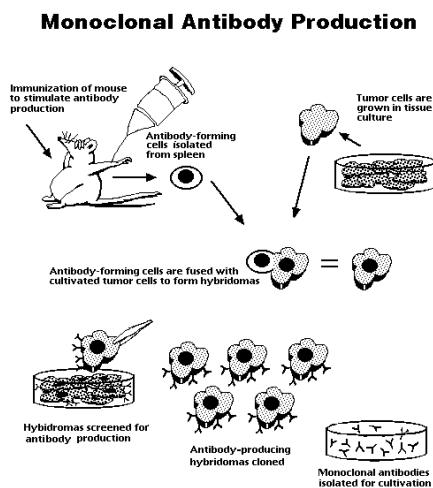
1. Neutralization of microbes and microbial toxins
2. Opsonization and phagocytosis (IgG)
3. Antibody dependent cell mediated cytotoxicity (ADCC), (IgG & IgE)
4. Activation of the complement by( IgG & IgM)
5. Mucosal immunity (IgA)
6. Neonatal immunity (IgG)neonates are protected from infection by maternal antibodies(IgG) transported across the placenta to the fetal circulation.

## Heavy chain class(isotype) switching (switch from one Ig isotype to another)

After activation of B lymphocytes , the antigen specific clone of B cells proliferate and differentiate into progeny that secrete antibodies, some of the progeny secrete IgM, and the other progeny of the same B cells produce antibodies of different isotypes to mediate different functions and combat different types of microbes.

## Monoclonal antibodies

- A monoclonal antibody is a highly specific antibody that is specific to a **single epitope** produced by **B cells** derived from single clone .
- It is artificially produced by fusing a specific antibody producing B –cell with a myeloma cell (i.e. malignant plasma cell)
- This fusion generates a new cell called "**hybridoma**" that acquire the property of immortality from the myeloma cell and the single antibody specificity from the B –cell , so that it can produce **unlimited quantities of highly specific monoclonal Abs .**





## Clinical application of the monoclonal antibodies:

### a) Diagnostic uses:

1. Diagnosis of infectious diseases (known monoclonal Ab+ unknown organism)
2. Assay of hormones .
3. Identification of cell
4. Surface markers, e.g. T – cell subsets .
5. Tissue (HLA) typing .
6. Detection of tumor antigens or tumor markers .

### b) Therapeutic uses:

1. **Antitumor therapy** by using monoclonal Abs to tumor specific antigens either alone or after coupling to cytotoxic drugs (magic bullet) .
2. **Immunosuppressive therapy**, e.g. using monoclonal antibodies to particular T – cell subsets, e.g. anti –CD3 to prevent graft rejection .
3. **Anti-RhD** to prevent Rh –incompatibility.
4. **Passive immunotherapy** in treatment of viral infections .
5. **neutralize drug toxicity** .e.g, yticixot silatigid.

## Monoclonal antibody used now in therapy:

- Anti- TNF for treatment of rheumatoid arthritis.
- Anti CD20 for treatment of B cell leukaemia.
- Anti CD3 for immunosuppression and prevention of graft rejection.

## Immunoglobulin functions:

- IgA ---- → Mucosal or local immunity
- IgD ---- → Naïve B cell antigen receptor (BCR)
- IgE ---- → Defense against parasites + immediate hypersensitivity )
- IgG ---- → Opsonization +complement activation +ADCC + secondary immune response
- IgM---- → primary immune response + complement activation.+ ---- → Naïve B cell receptor (BCR) +



## MCQ:

Q1: The domain unit of an immunoglobulin :

- a) only include the variable regions
- b) only recognizes the epitope
- c) only fixes complement
- d) Present in both heavy and light chains
- e) is polysaccharides

Q2: Injection into rabbits of a preparation of pooled human IgG could stimulate production of:

- a) Anti-  $\gamma$  heavy-chain antibody
- b) Anti-  $\kappa$  chain antibody.
- c) Anti  $\lambda$  chain antibody.
- d) Anti-Fc antibody.
- e) All of the above.



## Micro L15&16 : complement system & cytokines and lysozymes

### **\*Objectives:**

- Identify the characters of complement components.
- Define the role of complement in immune response.
- Differentiate between complement pathways.
- List the biological functions of complement.
- Describe the general features of cytokines.
- Identify the functional categories of cytokines.
- Understand the role of cytokines in immune response.
- Recognize the different types of cytokines.
- Enumerate the therapeutic uses of cytokines.

**\*History:** Research in complement started in 1890s when Jules Bordet at the Institute Pasteur of Paris. conducted experiment using sheep antiserum.

He named those substances as Alexins. He showed that sheep antiserum to the bacterium Vibrio cholera caused lysis of the bacteria and that heating the antiserum destroyed its bacteriolytic activity. Paul Ehrlich in Berlin independently carried out similar experiments and coined the term complement, defining it as "the activity of blood serum that completes the action of antibody." In ensuing years, researchers discovered that the action of complement was the result of interactions of a large and complex group of proteins.



### **\*Overview:**

- The complement system is part of the innate immune system.
- It is named “complement system” because it was first identified as a heat- labile component of Serum that “complemented” antibodies in the killing of bacteria.
- It is now known that it consists of over than 30 proteins.
- Many of its proteins are zymogens i.e. ( proenzymes ) requiring proteolytic cleavage in order to Gain enzymatic activity .
- Complement is activated sequentially in a cascading manner i.e. (each protein activating the Protein that directly follows it in the sequence).
- Complement proteins first appear in the fetus during the second month of pregnancy.
- The concentration of most complement components at birth about 50% of the adult levels.
- Nine major components are designated by C, followed by numeral from [1to 9] i.e. ( C 1 C4 C2 C3 C5 C678).

### **\*Site of origin of complement:**

- The complement system proteins are synthesized mainly by liver hepatocytes, although Significant amount are produced by blood monocytes, tissue macrophages and epithelial cells Of GI tract.
- Components are designated by numerals (C1-C9), by letter symbols.

### **Complement pathway:**

#### **Pathways of complement activation:**

- There are three major pathways of complement activation.
- Two initiated by microbes in the absence of antibody, called alternative and lectin Pathways, and the third is initiated by certain types of antibodies attached to Antigens called the classical pathway.



- Pathways for activation require multiple steps categorized as :

1-Recognition

2-Enzyme activation

3-Biological activity

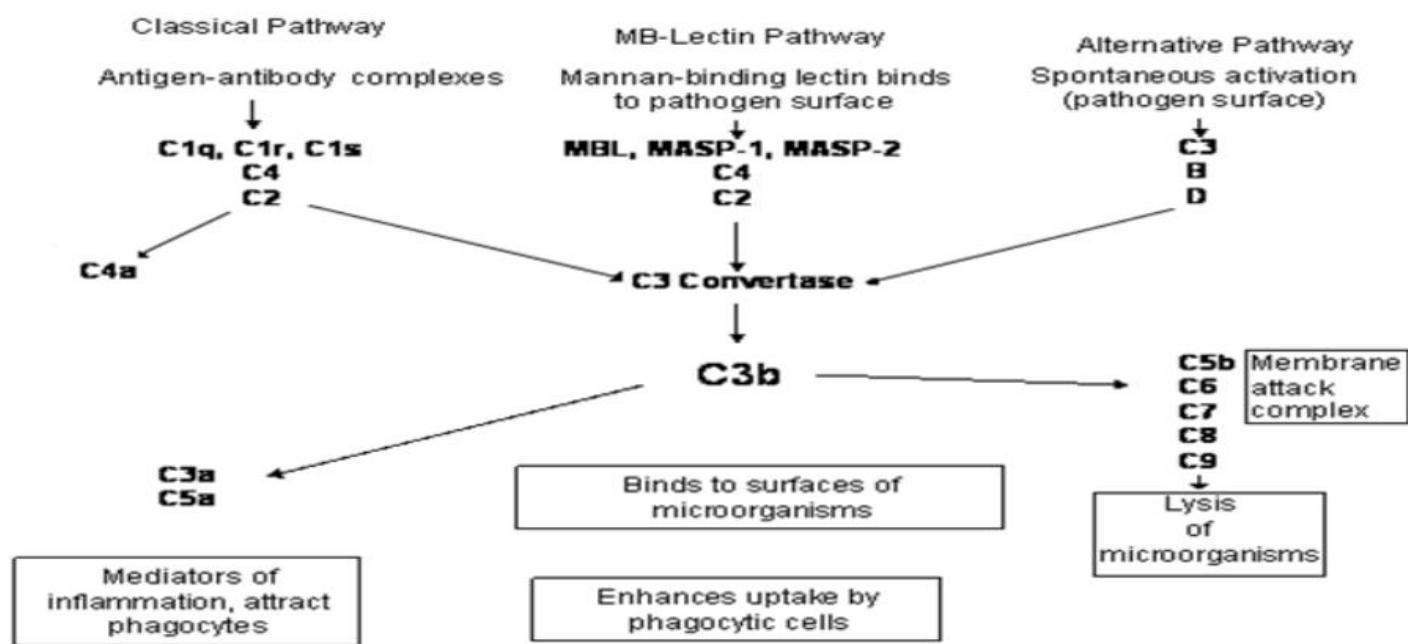
- Unique proteins for 1St, 2ND steps & common proteins for the last one.

Classical pathway	Alternative pathway
<p><b>1-C1</b> activated by Ag-Ab (i.e) immune complex. -Antibody molecules is either IgG (IgG1 &amp; IgG3) or IgM.</p> <p><b>2</b>-Needs calcium ions for C1 activation. This is followed by Activation of C4 and C2 followed by C3 ,C5, C6789</p> <p><b>3</b>-Serves as a major effector mechanism for the humoral immunity (i.e.) second line of defense.</p> <p><b>4</b>-It provides specific immunity, because it needs Ab for its activation.</p> <p><b>5</b>-It requires the interaction of all nine major complement components C1 C9</p> <p><b>6</b>-Its activation needs 5-7 days .This time is needed for Ab production.</p>	<p><b>1.</b> The activating agents are: a-Some intact cells e.g. : certain bacteria and fungi b-Endotoxin (lipopolysaccharides of Gram-ve bacteria). c- Zymozan (from yeast cell walls). d-Aggregated IgA or IgD classes.</p> <p><b>2.</b> Needs magnesium ions for C3 activation. The subsequent steps are the same as the classical pathway i.e. C5, 6, 7, 8 &amp; 9.</p> <p><b>3.</b> Regarded as a primitive defense system and as the first line of defense against invading organism.</p> <p><b>4.</b> It provides non- specific immunity because it does not need Ab for its activation.</p> <p><b>5.</b> It requires the interaction of component from C3 to C9.</p> <p><b>6.</b> It triggers the same antimicrobial actions as the classical pathway without the delay required for antibody production</p>



### \*Lectin pathway:

- The lectin pathway is activated by the binding of mannose-binding lectin (MBL) to Mannose residues on carbohydrates on the surface of microorganisms
- MBL is structurally similar to C1 of the classical pathway, and serves to activate C4 and C2.
- The subsequent steps are the same as the classical pathway i.e. (C3, C5, and C 6, 7, 8 &9).



### \*Biological function of complement:

1. **Cytotoxicity**: Is the final stage of the complement cascade, in which the Membrane attack complex (MAC) [C5b6789] inserted into cell wall lipid bilayers, Causing osmotic lysis of the target cells (bacteria or tumor cell) leading to their Death.
2. **Opsonization and immune adherence (C3b)**: C3b complement component

Facilitate phagocytosis of microbe through opsonization, because they have C3b Receptor on macrophages & neutrophil, so C3b enhance the immune adherence of Antigen to phagocytic cells, followed by its phagocytosis.



3. Inflammatory function ( anaphylatoxins ) Ca [4,2,3,5] : C4a, C2a, C3a, and C5a are involved in the release of histamine from mast cells and basophils during Acute anaphylaxis causing inflammatory reactions, so they are called Anaphylatoxins.

4. Chemotaxis (C5a): C5a component attracts the circulating phagocytes to The site of infection (mediate chemotaxis).

### \*Regulation of complement system:

Complement activation is associated with potent biological functions that, if left unchecked, Would exhaust the complement system and cause significant damage to the host.

Regulation is Achieved by: The natural instability and short active life of some of the activated components.

Serum inhibitors, e.g. Cl inhibitor and Factor I Membrane inhibitors,

Deficiency of these inhibitors can result in certain diseases e.g. congenital absence of Cl inhibitor Results in familial hereditary angioneurotic edema.

### \*Deficiency in complement component:

1) Deficiency of C3 results in profound susceptibility to infections and is usually fatal in Early life.

2) Deficiency of C2 and C4 does not cause severe immune deficiencies.

3) Deficiency of C2 and C4 are associated with an increased incidence of immune complex Diseases resembling systemic lupus erythematosus, perhaps because the classical pathway Functions to eliminate immune complexes from the circulation.

4) Deficiency in C9 and leads to incomplete MAC that result in increased susceptibility to Neisseria infections.

### \*Quiz: The membrane attack complex in the complement pathway consists of:

a-C3b3b Bb.                  B-C5b,6,7,8,9.

C-Colicins.                  D-OH.

E-Properdin.



## \*Cytokines:

### \*General features of cytokines:

- They are proteins of small – molecular weight, produced by the immune cells (cyto-) during the activation and effector phases of natural and acquired immunity. They communicate and influence the function (kines) of other cells through specific surface receptors.
- Most cytokines act in either paracrine manner (i.e., on nearby cells) or an autocrine manner (i.e., on the producing cell itself) or an endocrine manner (i.e. on distant cell).
- They have immunoregulatory functions. The ability of one cytokine to enhance OR suppress the production of others may provide important positive and negative regulatory mechanisms for immune and inflammatory responses.
- The cytokines include lymphokines (secreted by lymphocytes), monokines (secreted by Macrophages), interleukins (IL), interferons, tumor necrosis factor (TNF) and chemokines.

### A cytokine may act in 3 ways

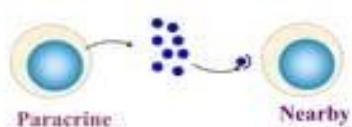
#### Autocrine

Cytokine binds to receptor on cell that secreted it.



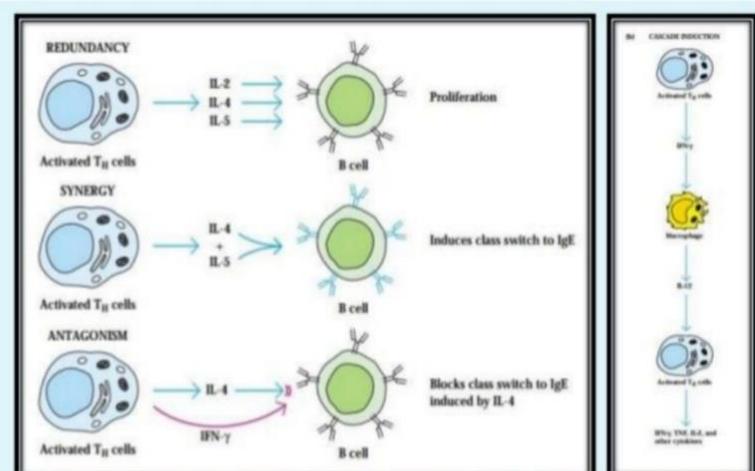
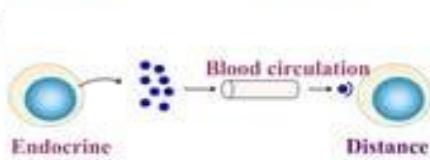
#### Paracrine

Cytokine binds to receptors on nearby cells.



#### Endocrine

Cytokine binds cells in distant parts of the body



- Two or more cytokines that mediate similar functions are said to be redundant.
- Cytokine synergism occurs when the combined effect of two cytokines on cellular activity.
- Antagonism, that is, the effects of one cytokine inhibit or offset the effects of another cytokine.



### **\*Functional Categories of Cytokines:**

- **Mediators and regulators of innate immunity:** These are produced by **activated macrophages and NK cells** in response to microbial infections. They act mainly on endothelial cells and leucocytes to **stimulate the early inflammatory reactions to microbes**. They include; IL-1, IL-6, IL-10, IL-12, IL-15, IL-18, TNF-a, chemokines, and type I interferon (IFN-a and IFN-B).
- **Mediators and regulators of acquired (adaptive) immunity:** These are **produced mainly by T lymphocytes** in response to specific recognition of foreign antigens. They include IL-2, IL-4, IL-5, IL-13, IFN-γ, transforming growth factor-β (TGF-β) and lymphotoxin.
- **Stimulators of hematopoiesis:** These are produced by bone marrow stromal cells, leucocytes, and other cells. They **stimulate the growth and differentiation of immature leucocytes**. These include; stem cell factor, IL-7, IL-3 and granulocyte monocyte colony stimulating factor (GM- CSF).

### **\*Therapeutic uses of cytokines:**

1-IL-2 and LAK cells are used in immunotherapy of tumors.

2-GM-CSF is of value in treatment of leucopenia after chemotherapy, radiation therapy or AIDS-associated leucopenia.

3-Interferons have been proven to be useful in:

a-Treatment of patients with chronic active hepatitis B and C infections.

b-Interferon treatment may be helpful in certain severe viral infections (rabies, hemorrhagic fever, herpes encephalitis).

C- Topical interferon in the eye may suppress herpetic keratitis.

### **\*Therapeutic uses of cytokines:**

1-In adult T cell leukemia, antibodies to the IL-2R α induced therapeutic responses in one third of the patients.

2-Anti-TNF antibodies may be very effective in treating patients with septic shock due to infections with gram negative bacteria.



3-Anti-cytokine antibodies and soluble cytokine receptors may be used in management of autoimmune diseases and transplant rejection, e.g.:

- Anti-TNF is approved for use in rheumatoid arthritis.
- Anti-IL-2R (anti-Tac) therapy to reduce graft rejection.
- Anti-IL-4 is under trial for treatment of allergies

### **\*Chemokines:**

Group of cytokines that can be attract either macrophages or neutrophils to the site of Infection. It is produced by various cells in infected area as (endothelial cells, NK and activated T cells). Its function is:

- 1-Chemoattractant activity for neutrophils, lymphocytes and fibroblasts.
- 2-Mediator of acute and chronic inflammatory reactions.
- 3-Promote lymphocyte adhesion, migration, activation and differentiation e.g. IL8.

### **\*Quiz:**

The major purpose of lymphokines is to:

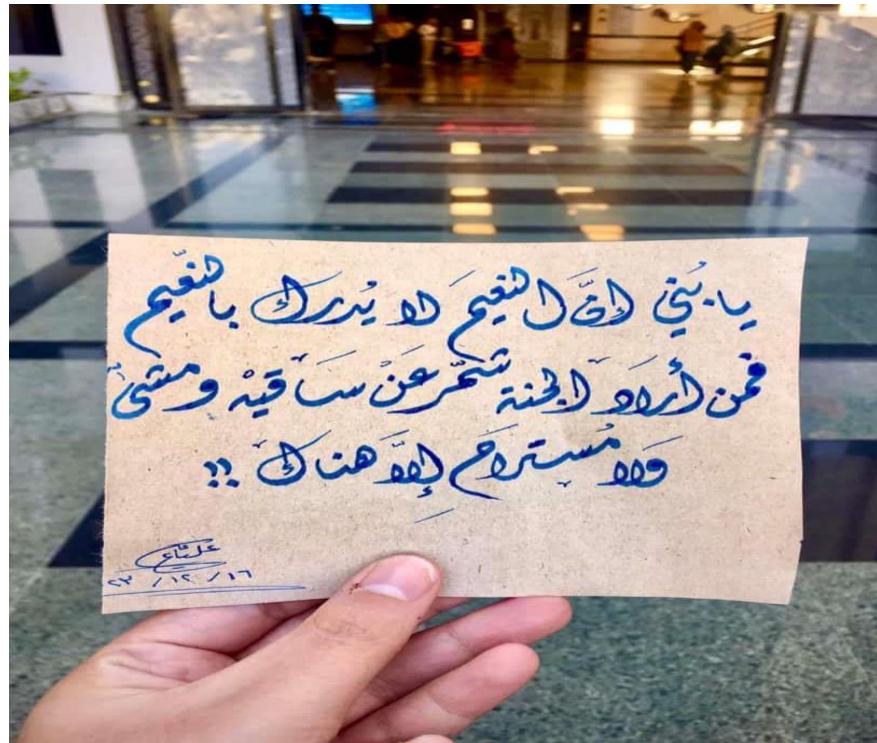
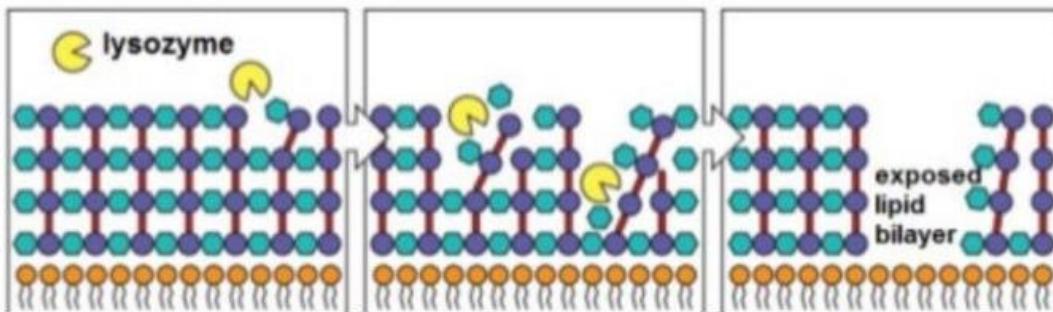
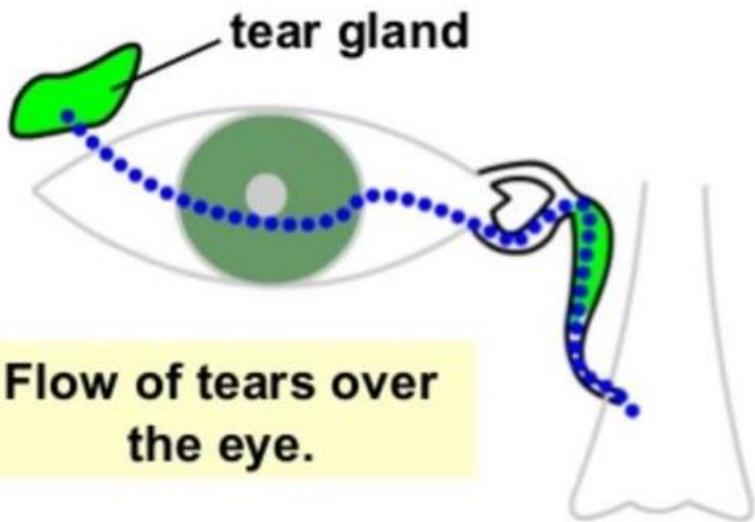
- A)Bind to class I major histocompatibility molecules for cytotoxic function.
- B)Specifically recognize antigens or their fragments.
- C)Stimulate the production of complement.
- ~~D)Help control and regulate the cells of the Immune system.~~

### **\*Lysosomes:**

- Lysozymes are one of the secretory products of cells of the immune system.
- These are saccharolytic enzymes, present in tears, nasal secretions, on the skin, and in lower Concentrations in serum.
- They lyse certain bacteria, chiefly Gram – positive cocci.
- They inhibit the transpeptidation Reaction in the cell wall of Gram – positive bacteria. They also potentiate the action of Complement on Gram – negative bacteria.



**Tear fluid contains lysozyme : destroys bacterial cell walls**





## L17:Surface markers

### Immunoglobulin superfamily

- The cell surface & soluble proteins that mediate Recognition, binding and adhesion functions in **Immune system** are derived from a common Precursor genes.
- The genes encoding all these molecule Ig gene superfamily, and the protein Molecules encoded by these genes are referred To as members of the Ig superfamily
- Ig superfamily is a large diverse family Of membrane proteins shared the Presence of one or more of the **Ig Domain**.
- They are formed of polypeptide chains, Each has
  - Extracytoplasmic,
  - A transmembrane and
  - Intracytoplasmic regions.
- These members are not share similar Functions.

### Members of the immunoglobulin superfamily:

- Adhesion molecules.
- Major Histocompatibility Complex (MHC).
- Cell receptors (T-cell receptor & B-cell Receptor )

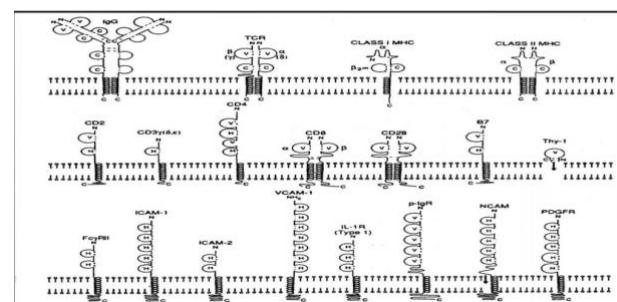
### I) Adhesion molecules

- Cell adhesion molecules (CAMs ) are cell Surface proteins involved in the **binding of Cells**, usually leukocytes to each other , to **Endothelial cells** , or to extra-cellular Matrix.
- They also regulate the **movement of Lymphocytes** out of blood vessels to Inflammatory site and **retention** of T cell in Tissues.

### Examples of adhesion molecules

- Leukocyte function associated antigen ( LFA-1,2 & 3).
- Intracellular adhesion molecules (ICAM-1&2).
- Clusters of differentiation (CDs).

### Some members of IGSF





## Cluster of differentiation or Cluster determination (CDs) (cell surface marker)

- CD molecules are
  - Marker on immunocompetent cells
  - Formed of a polypeptide chain, which has an External domain, a trans-membrane segment and Cytoplasmic extension.
  - Function primarily as adhesion molecules, although They may serve signaling functions.

### Maturation of T cells

- Maturation of T cells begin in the cortex of the thymus

With the appearance of CD2 , followed by the Appearance of CD3 with T-cell receptor ( TCR ) and with The concomitant expression of CD4 and CD8. (CD4+CD8+ )

- In the medulla of the thymus , a loss of some CDs Occurs to produce two populations of T- cells.
  - ✓ One carrying CD2,CD3 ,CD4 &TCR, (CD4+ CD8- ) and Represent T- helper cell
  - ✓ The other carrying CD2 ,CD3 , CD8 &T CR , (CD4- CD8+) Called T- cytotoxic cell and are called T =cytotoxic .

## II)Major Histocompatibility Complex (MHC).Human Leukocyte Antigen (HLA)

### Major Histocompatibility Complex (MHC)

\_MHC is antigens expressed on the surface of the Nucleated cells e.g leukocytes.

\_They are glycoprotein in nature, have an external Domain, a trans-membrane segment and a Cytoplasmic extension.

\_They are encoded by a set of genes called the major Histocompatibility gene complex that is located on The chromosome No 6

\_They are found in three classes ( I , II & III)

\_Class I and class II belong to Ig superfamily.

### Gene of the Major Histocompatibility Complex (MHC) locus

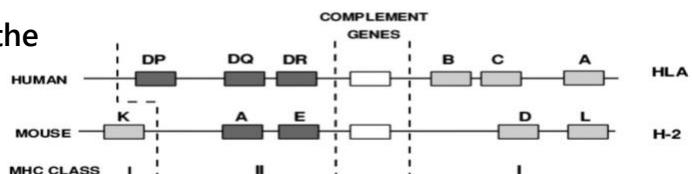
- MHC is a cluster of genes important in immune Recognition and cooperation between cells of the Immune system.



- MHC contains about 40 genes, grouped into 3 Regions.

- MHC class I genes contains 3 sets of genes A, B and C. ( HLA-A, HLA-B & HLA-C )
- MHC class II region which contains 3 sets of genes DP, DQ
- and DR ( HLA- DP, HLA- DQ & HLA- DR )
- MHC class III region between region B and D.

schematic maps show the human and mouse Genes of (MHC) locus



- These genes determine the antigenic specificity of the MHC surface molecules.

## Allele

- One of different forms of a gene present at a Particular chromosomal locus
- An individual who is heterozygous at a locus has Two different alleles, each on a different member

Of a pair of chromosomes , one inherited from the Mother and one from the father

- If there are many different alleles for a particular Gene in a population , the gene or locus is said to

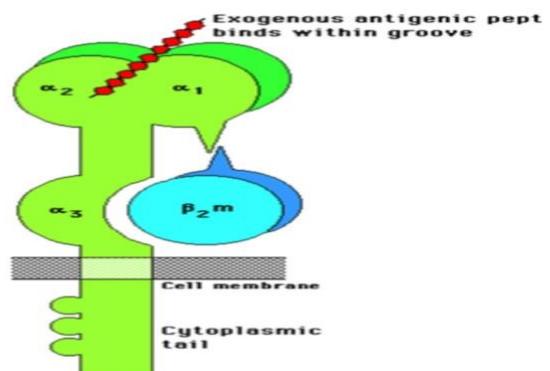
Be polymorphic. The major histocompatibility Locus is extremely polymorphic.

## MHC haplotype:

- MHC haplotype is the total set of MHC Alleles present on the same chromosome.
- Two haplotypes, one is inherited from each Parent,
- Each allele is given a numerical designation. For instance, an HLA haplotype of an Individual could be HLA-A2, HLA-B5,HLA-DR3 , and so on .

## Class I MHC antigens:

- They include HLA-A, HLA-B & HLA-C
- MHC class I molecules consist of a single Polypeptide chain ( $\alpha$  chain ) associated with a Molecule known as  $\beta 2$  micro-globulin.
- The  $\alpha$  chain is formed of 3 domains ( $\alpha_1$ ,  $\alpha_2$  and  $\alpha_3$  ).
- $\alpha_3$  molecule is the site of binding non covalently to
  - A  $\beta 2$  micro-globulin
  - Is the ligand to CD8 .





- Amino acid arrangement on eht senimretd niahc  $\alpha$

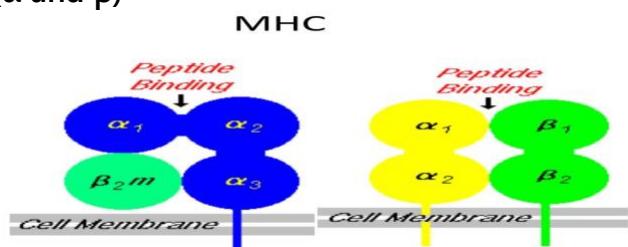
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- They are found on all nucleated cells.

## Class II MHC antigens :

- They are products of human MHC class II . They are Three cell surface molecules HLA-DP, HLA-DQ and HLADR, each comprising an  $\alpha$  and  $\beta$  chain
- MHC class II molecules consist of 2 polypeptide chains ( $\alpha$  and  $\beta$ )
- The  $\alpha$  chain is formed of 2 domains ( $\alpha_1$  and  $\alpha_2$  ).
- $\beta$  chain is formed of 2 domains ( $\beta_1$  and  $\beta_2$ ).

B2 domain Is the ligand to CD4 molecule.



- These antigens are glycoproteins, found Mainly on antigen presenting cells (dendritic Cells, macrophages & B lymphocytes).

- MHC class II molecule consists of  $\alpha$  and  $\beta$ Polypeptide chain.
- It occupies region D and include HLA–DP, HLA – DQ and HLA –DR.
- Helper T –cells recognize foreign antigen on Surface of APC only if they are associated With class II MHC molecules having the same MHC (MHC restriction).

## Class III MHC antigen:

- The region between the class I and Class II loci , contains genes for :
- Tumor necrosis factor  $\alpha$  (TNF $\alpha$ )   • Tumor necrosis factor  $\beta$  (TNF  $\beta$  )
- Some complement component like
  - \_ C2 and C4.
  - \_ Factor B (FB)

## Functions of MHC:

- 1.Presentation of processed protein antigen in APCs or tumor or Viral infected cells to T helper cell or to T cytotoxic cell
- .2Immune recognition and graft rejection of incompatible Transplant, this is done by class I MHC.
- 3.Association with certain diseases, especially autoimmune Diseases (e.g HLA – B8 with myasthenia gravis.)



## Comparison of the properties and function of MHC class I and class II

Class I MHC	Class II MHC
<b>Expressed on the surface of all nucleated cells</b> including APC as HLA-A, HLA-B and HLA-C.	<b>Express on the APCs ( B cells, dendritic cells, macrophage &amp; endothelial cells)</b> as DP,DQ & DR
<b>Formed of one polypeptide chain (<math>\alpha</math>) attached non covalently to a <math>\beta 2</math>-microglobulin chain which is encoded by chromosome number 15.</b>	<b>Formed of two polypeptide chains <math>\alpha</math> &amp; <math>\beta</math></b>
Is associated with the expression of <b>intracellular protein antigen</b> (cytosolic proteins) on virus infected cells or any transformed cells e.g.tumor cell	is associated with the presentation of <b>extracellular protein antigen</b> (endocytosed proteins ) on APC.
<b>Is recognized by cell carrying CD8 molecule and having the same and identical class I MHC</b>	<b>Is recognized by cell carrying CD4 molecule and having the same MHC II</b>

### Tests of MHC Antigens

- Serological detection of MHC antigens is done By microcytotoxicity.
  - This is done by using lymphocytes from donor and Recipient which carry MHC class I and class II Antigens and tested against antibodies to these Molecules.
- Mixed lymphocyte reaction
- Genotyping is done by PCR

### III) Cell Receptors

#### B cell receptor:

- B-cell receptors are structurally homologues to Immunoglobulins molecules, except they are **bound to The B cell surface and has an intracytoplasmic tails**.
- B cell receptors on the resting B cell are of IgM and IgD Type. They have short cytoplasmic tails, these tails are too Small to transduce signals to the cell interior, so signaling Is actually transduce by two other molecules (Ig-  $\alpha$  and Ig- $\beta$ ) forming a B cell receptor complex.



## T cell receptor (TCR)

- The TCR for antigen consists of **two polypeptide chains α And β**, linked by disulfide bonds. , having variable and Constant regions.
- They are found on CD4 and CD8 T cell .
- In human less than 5 % of T cells express other form of TCR polypeptide chains called ( $\gamma$  &  $\delta$ ) .
- $\gamma\delta$  TCR are abundant in epithelia
- The  $\alpha/\beta$  chains of TCR provide T cell the ability to **recognize Peptide antigen bound to MHC molecule**.
- Activation of T cells is dependent on up to **five other transmembrane Proteins** that are non-covalently associated with  $\alpha/\beta$  chains.
  - Three proteins are called CD3 molecule **designated ( $\gamma, \delta, \epsilon$ )** .
  - The remainder two proteins are of ( $\zeta, \text{Zeta}$ ) type. These proteins form the

### Functional TCR complex.

- T cell specificities are dependent on TCR  $\alpha/\beta$  chains , TCR are differ Among T cell clones.
- The cytoplasmic domain of TCR is of short size, so cannot transduce Signals to the cell interior, while that of the **CD3 and  $\zeta\zeta$  molecules are Of long size, therefore can transduce signals to the cell interior**.



**Q1:The HLA complex:**

- A) Induces complement activation
- B) Controls T cell recognition and elicits allograft reaction
- C) Is involved in the adherence to endothelial cell surface
- D) Is involved in platelet aggregation
- E) Consists of HLA -A and HLA -B gene clusters only.

**Q2:MHC class I molecules are normally present on :**

- A) B cells only
- B) Antigen – presenting cells only
- C) Most nucleated cells
- D) T cell only
- E) Erythrocytes and platelets only.

**Q3 • One principal function of class I and class II major Histocompatibility complex proteins is to**

- Bind complement
- Transduce the signal to the T –cell interior following Antigen binding
- Mediate immunoglobulin class switching
- Present antigen for recognition by the T –cell antigen Receptor

**Q 4 :The T-cell receptor**

1. Is composed of four polypeptide chains
2. Is secreted into the plasma by the T-cell
3. Is the recognition element of the humoral arm of The immune system
4. Recognizes antigen fragments via the alpha and Beta chains
5. Fix complement.

**Q5 :All of the following statements are properties of B – Cell receptor EXCEPT**

1. Recognize antigen or antigen fragments
2. Recognize antigen presented on MHC class-I
3. Are composed of multiple polypeptide chains folded into Discrete domain units
4. They contain variable and constant region domains
5. They are associated with accessory proteins necessary for Signal transduction after antigen recognition

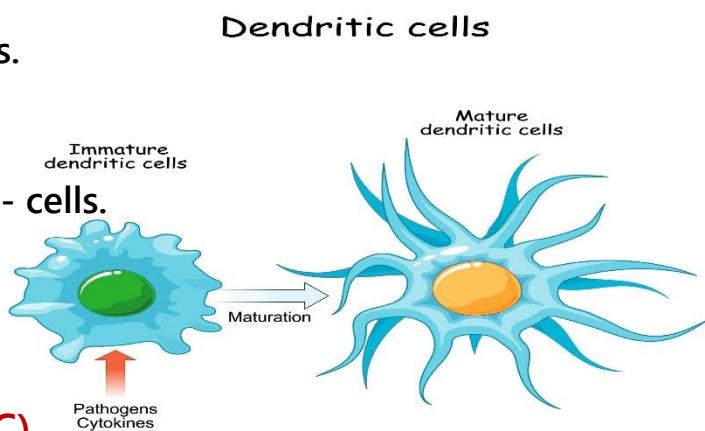


## Micro L19

## Antigen presentation and lymphocyte activation

## Stages Of CMI

1. **Phagocytosis** of the antigen by phagocytic cells.
2. **Processing** i.e. (grinding) of the antigen.
3. **Presentation** of the processed antigen to the T- cells.
4. **Activation** of T-helper cells.
5. **Activation** of cytotoxic T- cells.



## Professional Antigen- Presenting Cells (APC)

- Also known as accessory cells, these cells are capable of capturing raw antigens and process them to be presented to T lymphocytes.
- Dendritic cells, macrophages, and B cells are the principal APCs.

## Functions of APCs:

\_Internalizing of antigens, either by phagocytosis (e.g. macrophages, Dendritic cells) or by receptor-mediated endocytosis (B cells)

\_Antigen processing and presentation to T cells.

\_Activation of Lymphocytes by secreting some cytokines e.g. interleukin -12, tumor necrosis factor (TNF), interferon -1(IFN-1).

\_Dendritic cells are the most important and efficient APCs.

\_They are so-called because they have cytoplasmic projections called dendrites.

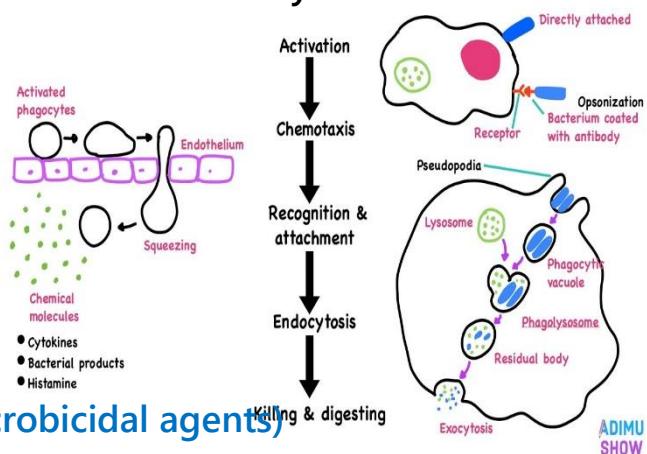
\_Dendritic cells are found in tissue that has contact with the outside environment, such as lung mucosa, epithelial cells of the skin, and the linings of the nose and the gastrointestinal tract



## Phagocytosis

### Stages of Phagocytosis

1. **Chemotaxis:** Phagocytes are chemically attracted to site of infection. These chemical signals are :
  - a) Cytokine called (IL8) ,
  - b) A component of complement called (C5a)
2. **Adherence:** Phagocyte plasma membrane attaches to surface of pathogen by receptors on macrophages e.g. Toll – like receptors (TLRs)
3. **Ingestion:** Plasma membrane of phagocytes extends projections (pseudopods) which engulf the microbe. Microbe is enclosed in a sac called phagosome.
4. **Digestion(processing):** Inside the cell, phagosome fuses with lysosome to form a phagolysosome.



### Killing (two microbicidal routes)

- a- **Oxygen dependent system (powerful microbicidal agents)**

Oxygen converted to superoxide anion, hydrogen peroxide, activated oxygen and hydroxyl radicals.

- b- **Oxygen-independent system (anaerobic conditions)**

Digestion and killing by lysozyme, lactoferrin, low pH, and hydrolytic and proteolytic enzymes

### Antigen processing and presentation

-Antigen processing is the degradation of a foreign protein antigen into small antigenic peptide fragments which become bound MHC molecules within the cell interior.



-These peptide – MHC complexes are then transported to the cell membrane and presented at the cell surface to be recognized by a T cell.

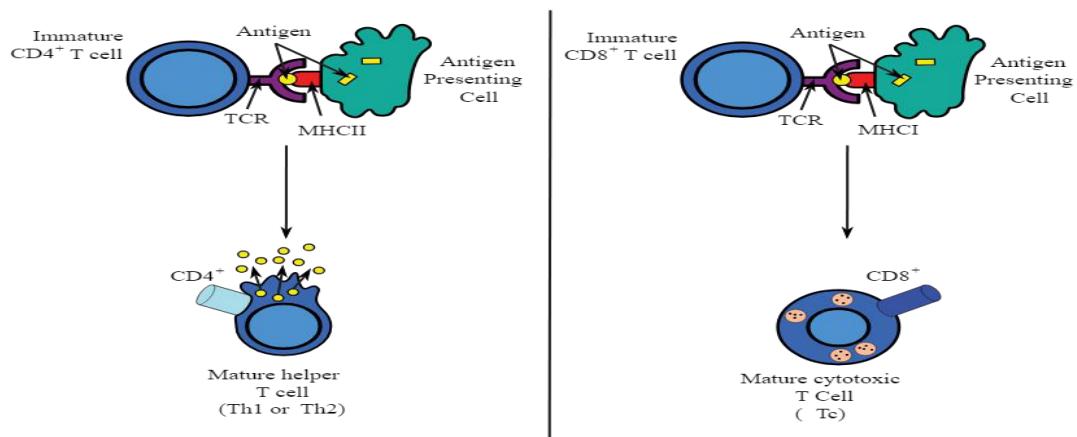
\_There are 2 pathways of processing **protein** antigen to be presented to T lymphocytes in the form of peptides

#### a. Class II MHC pathway: (Exogenous antigens)

- I. Antigen taken from extracellular environment
- II. degraded by lysosomal proteases.
- III. The resulting peptides are presented to CD4+ T cells with class II MHC molecules.

#### b. Class I MHC pathway: .(Endogenous antigens)

- I. cytosolic proteins e.g. intracellular microbes e.g. viruses, intracellular bacteria
- II. degraded by proteosome.
- III. the resulting peptides are presented to CD8+ T cells with class I MHC molecules.



Peptides antigens generated in the cytosolic compartment (intracellular infection, e.g. virus) bind to    MHC molecules for presentation to    T cells. Peptide antigens generated in vesicles (extracellular infection, e.g. bacteria) bind to    MHC molecules for presentation to    T cells.

Class I; CD4+; Class II; CD8+

Class II; CD4+; Class I; CD8+

Class I; CD8+; Class II; CD4+

Class II; CD8+; Class I; CD4+



## Activation of T- cells

### 1) First signal for activation (antigen induce signal)

-Binding of peptide Ag and MHC molecule (MHC complex) on APC to TCR of (CD4 or CD8) molecule (according to the type of T-cell).

After binding :signals are transduced by other associated protein complex called (CD3 & ζ ζ).

This complex is usually not sufficient for full T-cell activation

### 2) Second signal (Costimulatory signals)

#### a) Accessory molecules

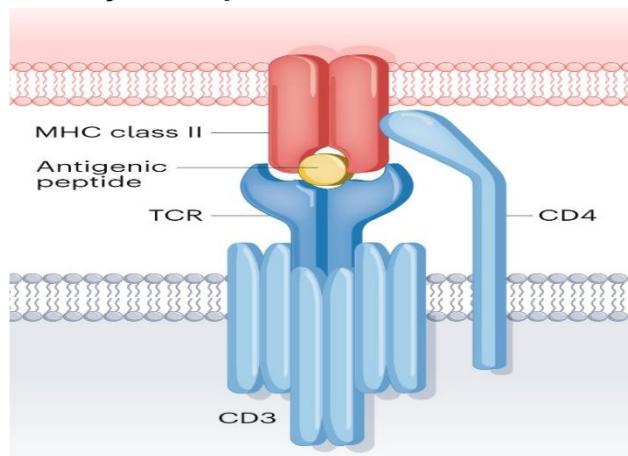
1- on APCs called [B7(I&2)] which bind to specific receptor on T-cell called [CD28].

2-on APCs called CD40 molecule bind to CD40 Ligand (CD40L) on T cell.

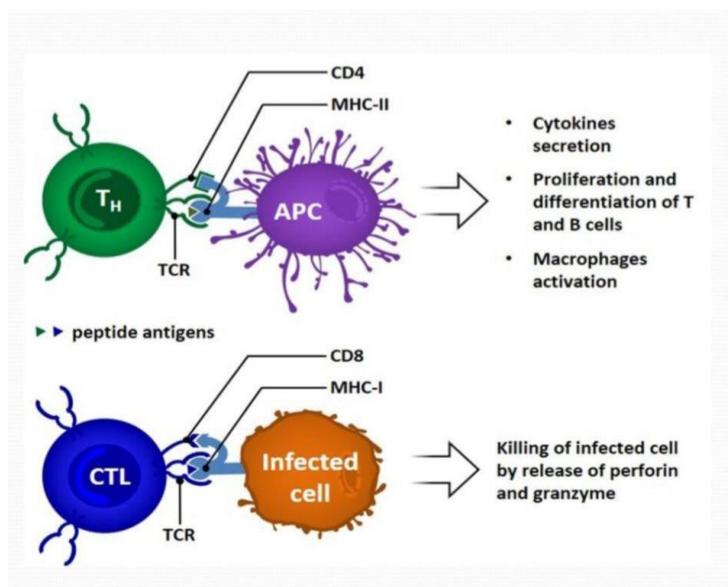
#### b) Role of cytokines

\_ Monokines of APC e.g. interleukin (IL) 1, 6, 8, 10, 12 & 15 assist in T-cell activation.

\_ Lymphokines: interferon (IFN) γ & IL-2 secreted by T-helper-1 cell activates APC



## Effector Functions of T cells





## Inhibitory signal

In contrast to CD28 molecule:

molecule (cytotoxic T lymphocyte Ag - 4) on T cells binds to B-7 and transmits inhibitory signals that inhibit T-cell activation.

- الزهرة لا تفكر بمنافسة الزهرة المجاورة لها!  
هي فقط تزهـر.

@GHOZYDES

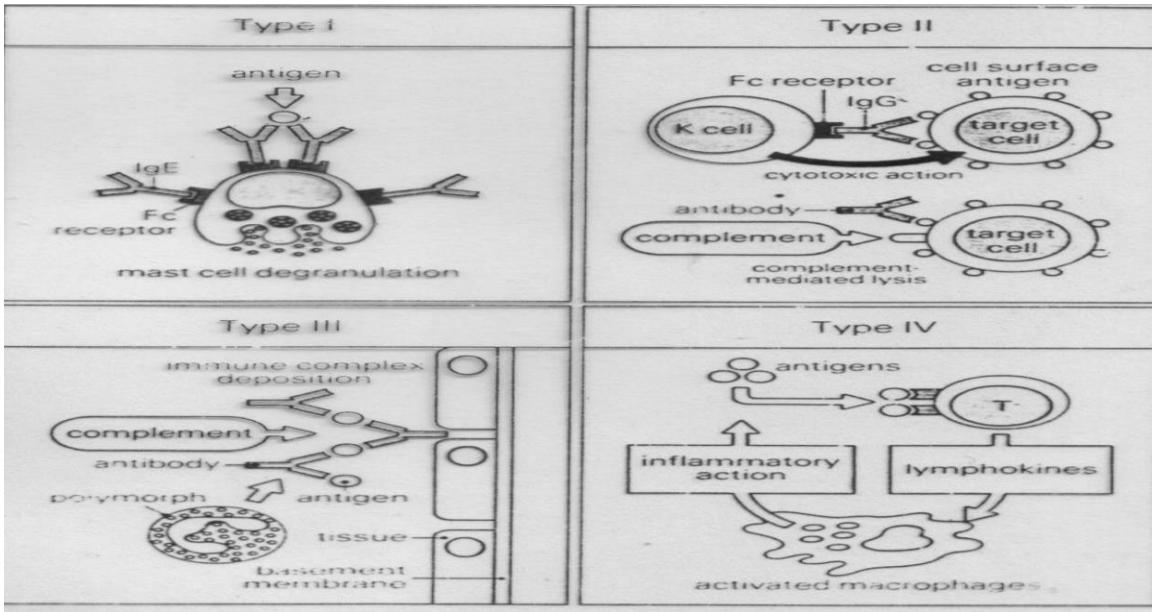
كُنْ أَنْتَ الْأَسْنَاءِ الْجَمِيلَ لِكُلِّ  
قَوَاعِدِ الْقُبُحِ الَّتِي حَوَّلَكَ.



الْعُسْرُ مَهْمَا قَسِي فَالْيُسْرَ يَتَّبِعُهُ، وَعَدَ مِنَ اللَّهِ وَهَذَا الْوَعْدُ يَكْفِينَا.



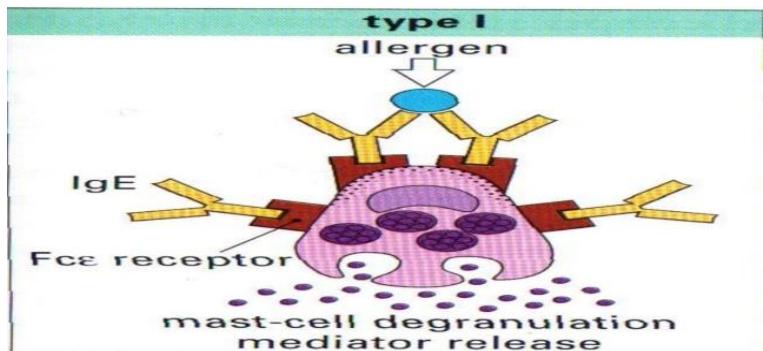
## Micro L20: Hypersensitivity



### Type I hypersensitivity

- (Anaphylactic) Antibody is IgE (reagin or homocytotropic) on mast cells

#### Mechanism:



#### Mediators:

- 1▪ Histamine.
- 2▪ Heparin.
- 3▪ Serotonin.
- 4▪ Leukotriens (Lt C4 , Lt D4 & Lt E4).

#### Predisposing factors:

- Immunoglobulin deficiency such as SIgA.
- Immunoregulation deficiency such as lack of T suppressor cells.

#### Examples:

- |                       |                 |                     |              |
|-----------------------|-----------------|---------------------|--------------|
| ▪ Anaphylactic shock. | ▪ Atopy.        | ▪ Infantile eczema. | ▪ Hay fever. |
| ▪ Asthma.             | ▪ Food allergy. | ▪ Urticaria.        |              |



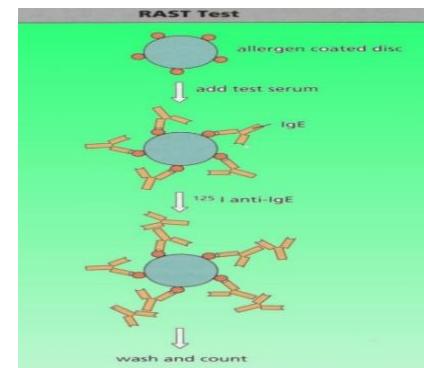
## Diagnostic Aspects of Allergy:

- History .
- Physical Examination.
- Laboratory Diagnosis of allergy disease :-
  - A. Test for organ function.
  - B. Tissue diagnosis.
  - C. Diagnostic tests that assess the patient's environment (Dust sampling).
  - D. Skin test.
  - E. In vitro tests.
  - F. Provocative tests.



Skin testing for allergens

Immunoblotting



RAST

Radio Allergo Sorbent Technique

## In Vitro Tests:

- IgE measurement total & specific (RAST & immunoblotting).
- Eosinophil count.

## Treatment:

- 1) Allergen avoidance.
- 2) Drug treatment.
- 3) Immunotherapy of allergy (desensitization).
- 4) Leukotriene modifiers (montelukast).
- 5) Other Immunological approaches for the treatment of allergy.

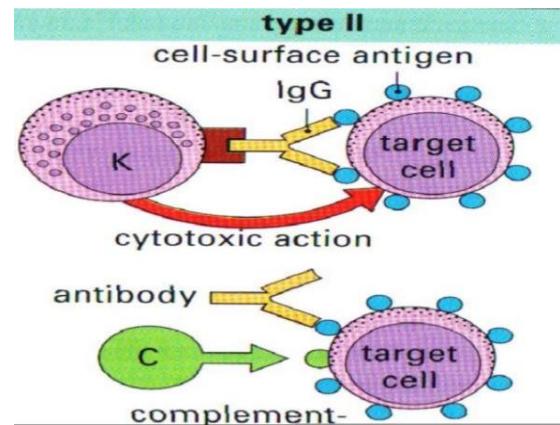


## Type II (Cytotoxic) hypersensitivity

Antibody (or killer cell) + Target cell (antigen) + Complement

### Examples:

- ABO & Rh incompatibility.
- Some autoimmune disorders.
- Drug induced hemolytic anemia & thrombocytopenia.



### Drug induced hypersensitivity

Could be in the form of :

1. Type I.
2. Type II.
3. Type IV.

## Type III hypersensitivity (Immune complex mediated)

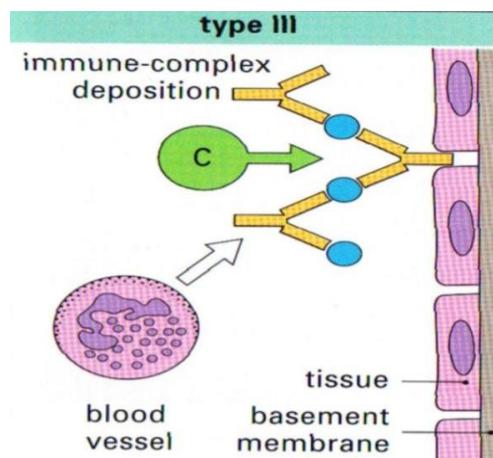
Excess Ag Ab + C → precipitation & Damage

### Mechanism of IC diseases:

Due to failure of clearance of soluble IC from circulation.

### Examples:

- Serum sickness (general).
- Arthus reaction (local).
- Farmer's lung.
- IC diseases like T.B., leprosy, SLE, rheumatoid and neoplasm.

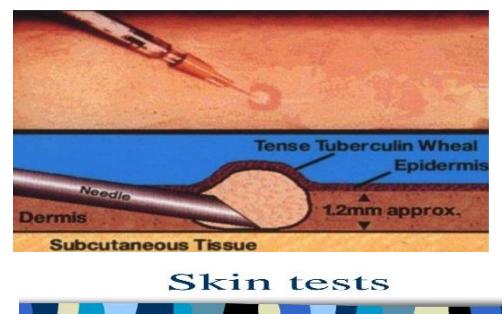


## Type IV(Delayed type) hypersensitivity

Depends on Cell mediated immunity.

### Examples:

- Immunity to infection (T.B.).
- Intradermal testing.
- Contact dermatitis.



## Type V (stimulatory) hypersensitivity

Ab react with a key surface component such as hormone receptor → switching ON the cell to be activated

- Example: Gravis disease.



## L 21: Tolerance and autoimmune diseases

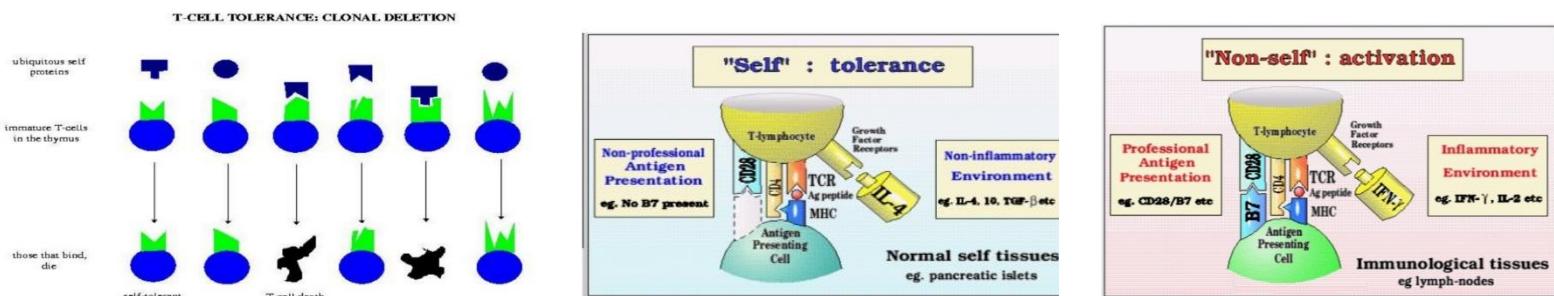
**Autoimmunity:** Failure of tolerance to self antigens.

**Tolerance:** A state of unresponsiveness of the immune system to particular antigen.

-When this occurs to self antigen it is known as self tolerance

### Mechanisms of self tolerance:

1. Clonal deletion (central, at embryonic life).
2. Clonal anergy (peripheral Functional inactivation of T-lymphocytes).



### Etiology of autoimmune diseases

- ✓ It occurs due to defects in self recognition.
- ✓ It can be mediated by humoral or cellular mechanisms.
- ✓ Mechanisms by which self tolerance broken, predisposing factors, and mechanisms of tissue damage all together explain the stat of autoimmunity.

### Mechanisms by which self tolerance broken:

#### 1-Antigens:

- ✓ Release of sequestered antigens (eye lens and sperms).
- ✓ Cross reactivity between self antigen and foreign antigen, molecular mimicry, (rheumatic fever).
- ✓ Altered form of antigen under effect of drug or chemical.

2-lymphocyte activation: Due to B- cell activators like bacterial LPS or EB virus.

3-Failure of T-Suppressor regulatory function.

### Predisposing factors for autoimmune diseases:

#### 1-Genetic factors:

- ✓ Correlation between Rheumatoid arthritis & HLA-DR4.
- ✓ Correlation between Hashimoto thyroiditis & HLA-DR5.

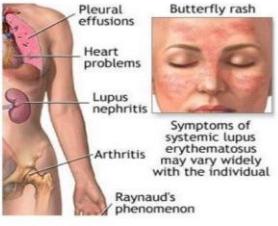
2-Hormonal factors: Rheumatoid arthritis is more in female and improves with pregnancy or with androgen therapy.     3-Infection or damage of tissues.



**Mechanisms of tissue damage:** The mechanisms of tissue damage in autoimmune diseases are the same that operate in hypersensitivity type I, II, III& IV reactions.

- ✓ Type II (cytotoxic reaction) Autoimmune haemolytic anaemia.
- ✓ Type III (immune complex) Rheumatoid arthritis and SLE.
- ✓ Type IV (T- cell mediated diseases) Ulcerative colitis

**Autoimmune diseases:** Autoimmune diseases can be classified into organ specific and organ non specific diseases.

Organ specific autoimmune diseases	Organ non specific autoimmune diseases:
<p>Directed to particular tissue such as:</p> <ol style="list-style-type: none"> <li>1. Auto antibodies to thyroid gland in Hashimoto's thyroiditis.</li> <li>2. Antibodies to acetyl choline in Myasthenia gravis.</li> <li>3. Antibodies to pancreatic Islet cells in insulin dependent diabetes.</li> </ol> <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">  <p>Rough, pitted cartilage and bone</p> <p>Rheumatoid arthritis</p> </div> <div style="text-align: center;">  <p>Pleural effusions</p> <p>Heart problems</p> <p>Lupus nephritis</p> <p>Arthritis</p> <p>Raynaud's phenomenon</p> <p>Butterfly rash</p> <p>Symptoms of systemic lupus erythematosus may vary widely with the individual</p> <p>Systemic lupus</p> </div> </div>	<p>-Antibodies are directed to wide spread antigens leading to disseminated diseases as anti-DNA in SLE &amp; Rheumatoid factor in rheumatoid arthritis</p>

## Laboratory diagnosis of autoimmune diseases:

- 1-There is elevation in serum immunoglobulins.
- 2-Detection of auto-antibodies in serum (ANA, ASMA, Rheum. Factor, ANCA,...)
- 3-Detection of specific antibodies to particular antigen as anti thymoglobulin
- 4-Decrease in serum complement.
- 5-Detection of immune complexes in serum or tissue sections.
- 6-Elevation in ESR and acute phase proteins (CRP) indicating disease activity

## Management of autoimmune diseases.

- 1-Anti-inflammatory drugs.
- 2-Immune suppressive & cytotoxic drugs.
- 3-Plasmapheresis (plasma exchange therapy).





## Micro L22: Tumor immunology

### Functions of immune response (I.R)

#### 1) Defense

- ❖ Resistance against infection
- ❖ If hyperfunction ( $\uparrow$ )  $\rightarrow$  Allergy
- ❖ If hypofunction ( $\downarrow$ )  $\rightarrow$  more susceptibility to repeated infections and Immune deficiency disorders

#### 2) Homestasis

- ❖ Removal of damage self-components
- ❖ If increased ( $\uparrow$ )  $\rightarrow$  exaggerated  $\rightarrow$  autoimmune diseases

#### 3) Surveillance

- ❖ Detection and destruction of abnormal cell mutants which constantly arise within body
- ❖ If the function deviated  $\rightarrow$  Malignancy.

### Tumor Antigens

- |                                 |                                   |
|---------------------------------|-----------------------------------|
| ❖ Tumor specific antigens (TSA) | ❖ Tumor associated antigens (TAA) |
|---------------------------------|-----------------------------------|

Tumor specific antigens (TSA)	Tumor Associated Antigens (TAA)
<ul style="list-style-type: none"> <li>❖ May be antigenic.</li> <li>❖ May induce immune response.</li> <li>❖ On the tumor cells and metastases.</li> <li>❖ Target for immune cells.</li> </ul>	<ul style="list-style-type: none"> <li>❖ Not antigenic ,not induce immune response.</li> <li>❖ Present in the blood.</li> <li>❖ Not target for immune cells.</li> <li>❖ Increase or decrease by original tumor.</li> <li>❖ Some of the present with normal conditions (e.g AFP increase with pregnancy, CEA increases with heavy smokers)</li> <li>❖ Used for diagnosis and follow up to tumors (tumor markers).</li> </ul>

### Tumor associated antigens (Tumor markers)

#### 1) Oncofetal antigens

Antigen	Tumor	Antigen	Tumor
AFP	liver carcinoma	CA125	Cancer breast
CEA	Gut tumors	CA15.3	Ovarian tumor
CA19.9	Pancreatic tumor		





## 2) Antigens of Viral origin

- ❖ EBV → Burkitt lymphoma & Nasopharyngeal carcinomas.
- ❖ Hepatocellular carcinoma → HCV & HBV.
- ❖ Osteogenic sarcomas.
- ❖ Soft tissue sarcoma.
- ❖ Malignant melanoma.
- ❖ Neuroblastoma.

## 3) Hormonal

Hormone	Tumor
HCG	Choriocarcinoma (Vesicular mole)

## 4) Enzymes

Enzyme	Tumor	Enzyme	Tumor
Alkaline phosphatase	Bone tumor	Acid phosphatase	Prostatic tumors

## 5) Others

Substance	Tumor	Substance	Tumor	Substance	Tumor
Ferritin	Leukemia	Casein	Breast	PSA	Prostatic carcinoma

## Tumor escapes

- ❖ Antigenic modulation → masking ,shedding & endocytosis.
- ❖ Antigenic Masking by glycocalyx.
- ❖ Tumor products like prostaglandin inhibits NK & k cells.
- ❖ Sneaking through due to failures of immuno-surveillance.
- ❖ Down expressions of class I MHC in tumors.
- ❖ No expression of class II MHC on most tumor cells.
- ❖ Tolerance low dose early in tumor later high dose tolerance.
- ❖ Mutation or deletion of genes encoding tumor Ag lead to non-expression of tumor Ags

## Tumor therapy

- ❖ Surgical
- ❖ Irradiation
- ❖ Chemotherapy
- ❖ Immunotherapy



## Immunotherapy (specific and non-specific)

### 1) Specific immunotherapy

- ❖ Modifications to tumor antigens by conjugation with happen, adjuvants and infection with virus (transfection) increase antigenicity.
- ❖ Monoclonal Abs to tumor Ages conjugated with radioactive substance, cytotoxic drugs, diphtheria toxin, ricin or tumoricidal substances.
- ❖ Infusions of tumor infiltrating lymphocytes back to original patient after activation with lymphokines (Lak cells).
- ❖ Infusions of ab carry AntiCD3 & AntiCD16 Conjugated with monoclonal abs to tumor antigens (Bifunctional ab technology)

### 2) Nonspecific immunotherapy

- ❖ BCG locating malignant melanomas & systemic in acute leukemia.
- ❖ Muramyl dipeptide (mimics the action of mycobacteria).
- ❖ Corynebacterium parvum.
- ❖ Levamisole (ketrex).
- ❖ Echinacosides.
- ❖ Transfer factor (lysate of lymphocytes from cancer patients).
- ❖ Plasmapharesis decrease immune suppression.
- ❖ Interferon A → local in cervical carcinoma & systemic in osteogenic sarcomas

## Test yourself

### 1) What are functions of immune response?

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### 2) The use of BCG vaccine in cancer immunotherapy is an example of

- A. Adoptive immunotherapy.
- B. Non – specific immuno-stimulation.
- C. Specific passive immunotherapy (immunotoxins).
- D. Specific active immunotherapy



## Micro L23: Transplantation immunology

### Graft types:

- A. Autograft: (auto= self graft) the same individual.
- B. Synegraft: (isograft) genetically identical.
- C. Allograft: (homograft) between allogenic individuals of the same species.
- D. Xenograft: (heterograft)between different species.

### Phases of graft rejection:

- Hyper acute rejection: minutes to hours.
- Acute rejection.
- Frist set 11 to 17 days.
- Second set rejection 5 to 10 days.
- Chronic rejection.

### How to avoid graft rejection:

1. Proper choice of donors (ABO matching).
2. Tissue typing =histocompatibility typing=HLA typing.
3. Postoperative immune suppression therapy.

### Immunosuppression:

#### 1) General immunosupression:

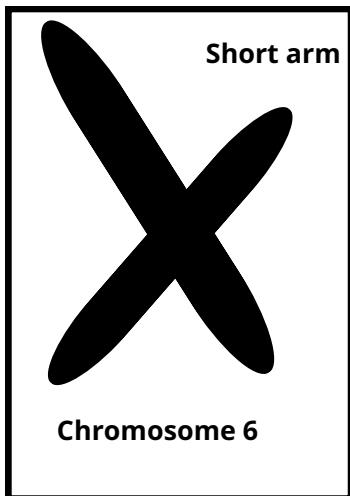
- Cyclosporine.
- Corticosteroids.
- Azathioprine.
- OKT3.

#### 2) Antigen-specific immunosuppression.

**Q1:** List types of graft?

**Q2:** The immunosuppressive drugs include all of the following except: -

- a) Cyclosporine.
- b) Corticosteroids.
- c) Azathioprine.
- d) Prostaglandins.



MHC COMPLEX GENE

يحمل الكود (الشفرة)

هي الـ بتطبع  
MHC Ags

Major MHC

Minor

- cummulative effect

I II

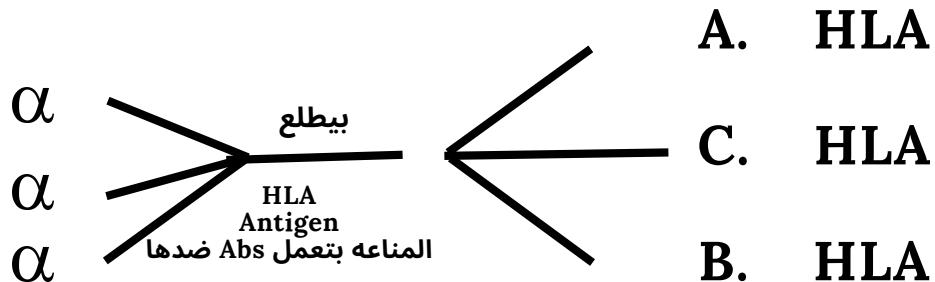
- Blood vessels  
(all nucleated cells)

- B\_lymphocyte
- Macrophage
- Monocyte
- Some epithelial cells

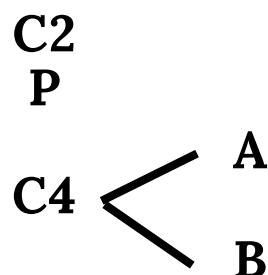
نفس Ags ال على Organ هي نفسها ال على  
Human leukocyte cells

عشان كدا سميت HL Ag او HLA Ags

## Class I gene

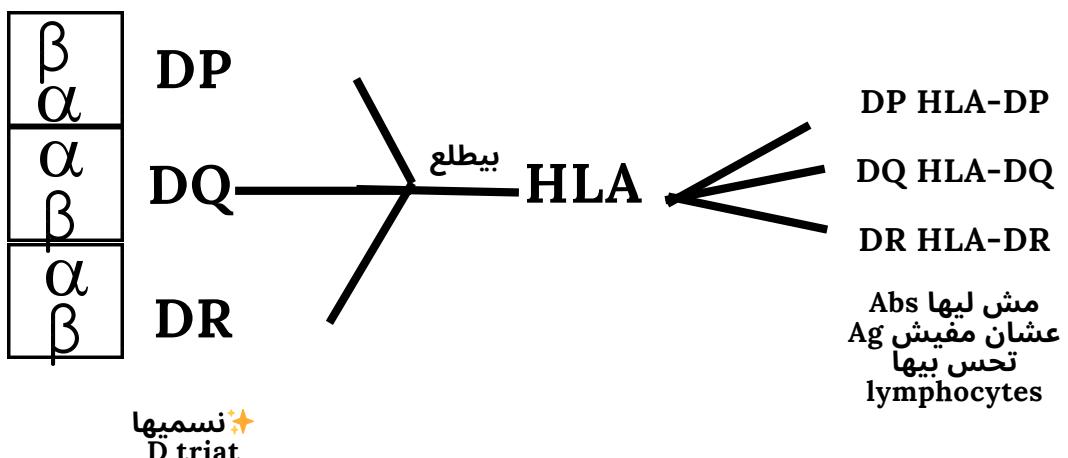


## Class III gene Complements genes



## Class II gene

خاصية منش  
صرير ليس  
Abs له



# Microcyto toxicity test

## اختبار التوافق

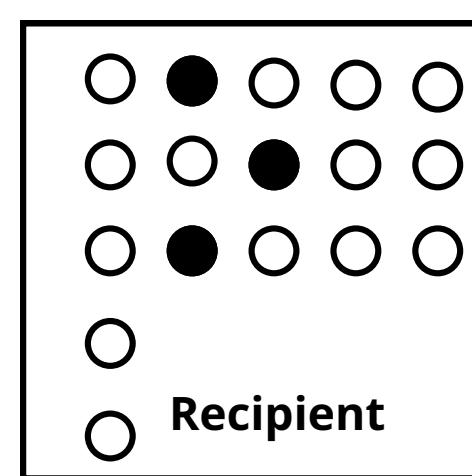
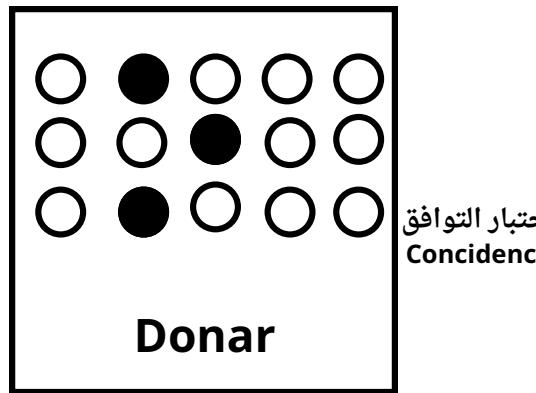
Class I → HLA  
 A  
 B  
 C

هنا هنشوف التشابه الجيني Anti genic similarity between donar and recipient

كل واحد لوحدة **المعطى** donar  
**المستقبل** recipient > نحضر lymphocytes من كل واحد

★ اعمل لكل واحد منها لطاقة جينية (اعرف فيها آل Ags آل بيحملها)  
 Antigenic composition of both donar and recipient is determine

لو فيه Ag في  
 recipient موجودة  
 ومش موجودة في  
 donar مش مهم  
 ولكن العكس لا



- Antiserum for most important Ags
- Lymphocytes separated from both donar and recipient

Lymphocytes  
 HLA Ag عليها + Anti HLA - anti serum (Abs)  
 Complement + للخلية لو موجودة → Death

☆ Trypanblue أو Eosin في الحالة دي ناخد الصبغه Cyto toxicity

☆ Micro cyto toxicity wells صغيرة علشان كدا بنسميهem بحفظهم في

☆ Donar في recipient ونحسب عدد Ags في

Recipient donar concidence بين (التوافق) نشوف هنا

Mixed lymphocytes culture (MLR)

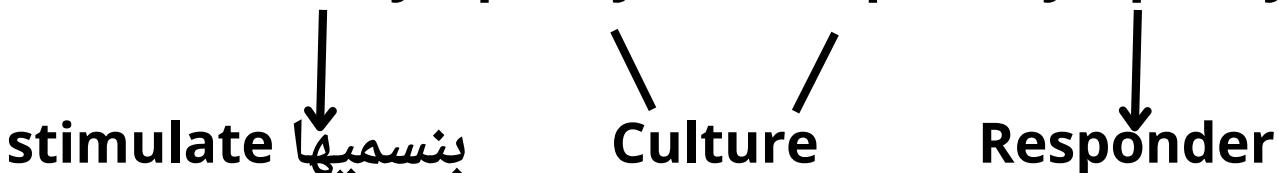
اختبار التضاد عشان CLASS II

Antigen هي هنا خاصية مش ✨

- Measured Ags that are the most critical to graft success
- Reflect disparities in the major ages

دا اختبار التضاد (Antagonism)

Donar lymphocyte + recipient lymphocyte



Ag forigen غريب لو عليها  $\xrightarrow{\text{هيحصل}}$  Proliferation - mitosis - transformation

لو مش غريبيه مش  
هيحصل حاجه



## Micro L2.4: Immune deficiency diseases

### ❑ Definition:

An abnormality of the immune system that renders a person susceptible to diseases normally prevented by a normal functioning immune system.

### ❑ Classification of Immunodeficiency.

#### 1- Primary or Congenital:

- Inherited (due to Mutation in genes controlling immune cells).
- Susceptible to recurrent, severe infection; starting in children.
- Cannot recover without treatment.
- >125 immunodeficiency disorders.

#### 2- Secondary:

- As a consequence of other diseases or environmental factors (e.g.: Infection, Malignancy, Aging, Starvation, Medication, Drugs, protein calorie malnutrition)
- Acquired Immunodeficiency Syndrome (Human Immunodeficiency Virus).

### ❑ Primary Immunodeficiency

A- Specific Immunity disorders	B- Non Specific Immunity disorders
1- B-cell defect.      2- T-cell defect. 3- Combined B and C cell defects.	1- Phagocytic cell defect. 2- Complement deficiency.

### ❑ Defects in Humoral Mediated Immunity

- Caused by the improper production of one or all of the immunoglobins (antibodies)
- Results in an increased of infections from Staphylococcus, Streptococcus, Haemophilus and Pseudomonas.
- Humoral Immunodeficiencies **include**:
  - a. Bruton's X-Linked Agammaglobulinemia.
  - b. Common Variable Immunodeficiency.
  - c. Selective Immunoglobin A Deficiency.

### ❑ X-linked agammaglobulinemia (XLA) [Bruton agammaglobulinemia]

- It is an inherited immunodeficiency disease caused by mutations in the gene coding for Bruton tyrosine kinase (BTK).
- BTK is critical to the maturation of pre-B cells to differentiating mature B cells.



## ❑ Defects in Humoral Mediated Immunity

- Caused by **defects in T lymphocyte development** (both CD4+ helper cells and CD8+ cytotoxic killer cells)
- Symptoms are more severe than with humeral immunodeficiencies.
- **Children rarely survive beyond infancy or childhood.**
- Cell Mediated Immunodeficiency disorders include:
  - a. DiGeorge Syndrome
  - b. X-Linked Immunodeficiency with Hyper-IgM.

### DiGeorge syndrome:

- It is a disorder caused by a **defect in chromosome 22**.
- Medical problems commonly associated with DiGeorge syndrome **include**:
  - ✓ Heart defects.
  - ✓ Poor immune system function.
  - ✓ A cleft palate.
  - ✓ complications related to low levels of calcium in the blood
  - ✓ Delayed development with behavioral and emotional problems.

**Symptoms**

Depending on the severity...

- Weakness or tiring easily
- Failure to thrive
- Failure to gain weight
- Poor muscle tone
- Shortness of breath
- Frequent infections
- Difficulty feeding
- Delayed speech development
- Learning delays or difficulties
- A gap in the roof of the mouth (cleft palate) and other issues
- Certain facial features, such as low-set ears, wide-set eyes or a narrow groove in the upper lip
- Bluish skin due to poor circulation of oxygen-rich blood
- Twitching or spasms around the mouth, hands, arms or throat
- Delayed development, such as delays in rolling over, sitting up or other infant milestones



## ❑ Combined T-cell and B-cell immunodeficiencies

### Examples:

- Severe Combined Immunodeficiency (SCID).
- Ataxia Telangiectasia.
- Wiskott Aldrich syndrome

### DiGeorge syndrome:

- Commonly known as "**bubble boy**" disease, is a defect in the specialized white blood cells (B- and T-lymphocytes) that defend us from infection by viruses, bacteria and fungi.
- SCID patients are **susceptible to recurrent infections** such as pneumonia, meningitis and chicken pox and can die before the first year of life.
- Though invasive, new treatments such as **bone marrow and stem-cell transplantation** save as many as 80% of SCID patients.



## ❑ Complement Deficiency Disorders

- Examples:
- C1 inhibitors deficiency ----- Angioneurotic edema.
  - C3 deficiency ----- Recurrent infections.
  - C1,C4,C2 & C3 deficiency ----- ICS diseases.
  - C5,6,7,8,9 deficiency ----- Recurrent Neisseria infections.

## ❑ Phagocytic cell Disorders

- Recurrent pyogenic skin infections.

Examples:

- Defect In **chemotaxis and ingestion**-----Chediak-Higashi syndrome.
- Defect in **intracellular killing**-----Glucose-6- phosphatase deficiency.
- Chronic granulomatous disease.

### Chédiak-Higashi syndrome (CHS)

- It is a rare childhood **autosomal recessive** disorder that affects multiple systems of the body.
- Patients with CHS **shows hypopigmentation** of the skin, eyes and hair.
- Prolonged bleeding time recurrent infections and peripheral neuropathy.

## Assessment of immune competence:

### A-Assessment of T-cell disorders:

1- Enumeration of T-cells by monoclonal antibodies.

2- Testing the function of T-cells:

➤ Skin test:

- To detect delayed hypersensitivity to a variety of antigens to which the patient is supposed to be exposed during his life, e.g. tuberculin (PPD) or Candida.
- These skin tests are useful **to assess T-effector cell function**.
- Negative skin tests indicate T-cell deficiency.

➤ Test for lymphocyte proliferation using mitogens:

phytohemagglutinin (PHA), concanavalin A (con-A) or specific antigens such as PPD or tuberculin.

### B- Assessment of B cell disorders:

1- Enumeration of B-cells by monoclonal antibodies.

2- Testing the function of B-cells:

- Quantitation of total serum immunoglobulins **by single radial immunodiffusion.**
- Stimulation of production of specific antibodies to killed vaccines or toxoids.
- Cell proliferation using B-cell mitogens e.g. polysaccharides.



## C- Assessment of phagocytic cell disorders:

- **Assessment of chemotaxis** by testing the ability of phagocytic cells to migrate through membranes towards a chemotactic substance.
- **Assessment of the ability of phagocytes** to engulf and kill bacteria or yeast.
- **Assessment of the intracellular killing (oxidative burst):**  
by testing the ability of phagocytes to deoxidize (reduced) colorless nitroblue tetrazolium (NBT) dye to blue colour.

## D- Assessment of complement disorders:

- 1-Estimation of total hemolytic activity of complement.
- 2-Estimation of individual complement components by RIA, ELISA or immunodiffusion.

**Choose the correct answer**

### A-The thymus gland fails to develop in which of the following disorders:

- a) AIDS.
- b) Congenital agammaglobulinemia.
- c) DiGeorge syndrome.
- d) Severe combined immune deficiency.

### B-Skin test used to assess function of:

- a) B cells.
- b) T- cells.
- c) Complement.
- d) Phagocytes.



## Micro Tut 4: Immuno-prophylaxis

### Case

- Ten days after having a wound on his right foot, a 20-year-old boy came to the emergency room with generalized muscle spasms. Upon arrival, he developed a progressive spasm in the jaw muscles that developed into lock jaw, and sustained generalized muscle spasms. He was diagnosed as tetanus and management plan was immediately started in the form of tetanus immune globulin (TIG), ventilatory support, high-calorie nutritional support, diazepam, wound debridement, penicillin G and a booster dose of DTaP vaccine.

- 1) What is the role of TIG in management of this patient ?
- 2) What is the type of DTaP vaccine and what disease does it protect against?

### Immunization

- ❖ Providing specific protection against most common and dangerous pathogens.

### Specific immunity can be either

- 1) Passive (from mother, antiserum)
  - 2) Active (infection, vaccine)
- ❖ SO, both may be natural (mother, infection ) or artificial (antiserum, vaccine)

### **Passive immunization (Receiving preformed antibodies)**

#### Artificial passive immunity

- ❖ Administration of preformed antibodies derived from seropositive humans / animals or produced by genetic engineering (monoclonal Ab) .
- ❖ Human immunoglobulin is preferable to animal immunoglobulin because there is little risk of a hypersensitivity reaction (serum sickness).

#### Natural passive immunity

- ❖ Newborns receive natural passive immunity from maternal immunoglobulin (placenta /mother's milk)

#### Passive immunization may be used

- ❖ To prevent disease after a known exposure (e.g., needlestick injury with blood that is contaminated with HBV).
- ❖ To protect immunodeficient individuals.
- ❖ To block the action of bacterial toxins or venoms and prevent the diseases they cause.



## Types

### **1) Tetanus antitoxin**

- ❖ It is used in the treatment of tetanus and in its prevention along with tetanus toxoid (passive– active immunity) .
- ❖ Made in humans

### **2) Diphtheria antitoxin**

- ❖ It is used in the treatment of diphtheria.
- ❖ Prepared in horses.

### **3) Immune globulins against viral infections**

- ❖ HAV,HBV,VZV, CMV, RSV, Rabies, Measles

## **Passive - Active Immunity**

- ❖ Providing both immediate (but short term) protection in the form of immunoglobulins (passive immunization) and long-term protection in the form of vaccine (active immunization)

## Examples

- 1) Prevention of tetanus in an unimmunized person who has sustained a contaminated wound. Both tetanus antitoxin and tetanus toxoid should be given.
- 2) Used with rabies and hepatitis B as a post-exposure prophylaxis
  - ❖ They should be given at different sites so that the antibodies in the antitoxin do not neutralize the toxoid.

## **Active immunization**

- ❖ When an immune response is stimulated by immunogen through:
- 1) Exposure to an infection Natural active immunity
  - 2) In vaccines Artificial active immunity
- ❖ On subsequent exposure → secondary immune response is activated that is faster and more effective, or antibody is present to block the spread or virulence of the agent

## **MCQ: Which of the following confer(s) passive immunity:**

- A. Hepatitis B vaccine
- B. MMR vaccine
- C. Hepatitis B immunoglobulin
- D. Infection with measles virus
- E. Transfer of maternal antibodies to the fetus
- F. across the placenta





## Vaccine

- ❖ It is a biological preparation that provides active acquired specific immunity to a particular disease.
- ❖ The term vaccine is derived from vaccinia virus, which is a less virulent member of the poxvirus family that is used to immunize people against smallpox.

### The protective immunity conferred by a vaccine may be

- ❖ Lifelong (MMR)
- ❖ May last as little as six months (cholera) → booster doses are required

### Types of vaccines

#### 1) Inactivated (killed) vaccine

- ❖ Organisms can be inactivated by heat, chemical or UV irradiation
- ❖ Use a killed virus to trigger an immune response.

#### 1. Advantages

- ❖ Safe for pregnant woman, immunocompromised.
- ❖ It is heat stable (easier to store & transport).
- ❖ Use of the whole organism stimulates immunity to antigens in their natural conformation

#### 2. Disadvantages

- ❖ Larger doses must be used.
- ❖ Immunity is not usually lifelong (short lasting and need boosting).
- ❖ Do not stimulate cytotoxic T cell response (only humoral is induced) in contrast to live attenuated vaccines.
- ❖ Less effective in stimulating local immunity (not elicit a local IgA response).
- ❖ Given by injection (costly to administer).

#### 3. Types

##### A. Killed Bacterial Vaccines

- ❖ killed cholerae vaccine.
- ❖ Killed pertussis vaccine (Discontinued owing to safety concerns replaced by safe acellular vaccine).

##### B. Killed Viral Vaccines

- ❖ Salk vaccine (poliomyelitis).
- ❖ Hepatitis A vaccine.



## 2) Live Attenuated Vaccines

- ❖ The pathogen within the vaccine has a limited ability to cause disease (e.g., avirulent or attenuated microbes).
- ❖ Use a weakened virus to trigger the immune response.

### 1. Attenuation

- ❖ Genetic engineering (No reversion to virulence).
- ❖ Repeated cultivation under abnormal conditions then selection of mutants (which are non-pathogenic).
- ❖ Related virus from a different species that has a common antigen with the human virus (e.g. bovine rotavirus).

### 2. Advantages

- ❖ Humoral & cellular immune responses are developed.
- ❖ Immunity is generally long-lived.
- ❖ Can mimic the normal immune response to the infecting agent if administered in the same way.
- ❖ Can be administered orally, which is less expensive than giving injections.

### 3. Disadvantages

- ❖ Reversion to virulence → The vaccine may revert to a virulent viral form.
- ❖ Hypersensitivity.
- ❖ Not safe for immunosuppressed people or pregnant women.
- ❖ The viability of the vaccine must be maintained ( storage and transport concerns).

### 4. Types

#### A. Live Attenuated Bacterial Vaccines

- ❖ BCG vaccine (for tuberculosis): A live attenuated strain of *Mycobacterium bovis* called bacillus Calmette-Guérin (BCG)
- ❖ Oral, live attenuated cholera vaccine.

#### B. Live , Attenuated viral Vaccines

- ❖ Live , attenuated polio (sabin, oral).
- ❖ Cold adapted influenza vaccine(nasal spray).





## 5. Live Vs Inactivated vaccines

Property	Live	Inactivated
Route of administration	Natural " or injection	Injection
Dose of antigen	Low	High
Number of doses, amount	Single, low	Multiple, high
Need for adjuvant	No	Yes
Duration of immunity	Long term	Short term
Antibody response	IgG, IgA	IgM, IgG
Cell-mediated immune response	Good	Poor
Potential lability	Yes	More stable
Reversion to virulence	Rarely	None

### 3) Subunit vaccine

- ❖ Contains only bacterial or viral components (isolated by biochemical means or prepared by genetic engineering( recombinant technology ) i.e. purified antigens rather than the whole organisms
- ❖ Use only a portion of a virus to teach the immune system to recognize the whole virus

#### 1. Purified Protein Vaccines

##### A. Pertussis acellular vaccine

- ❖ The principal antigen in the acellular vaccine is inactivated pertussis toxin (pertussis toxoid) + other bacterial proteins. Protect against whooping cough.
- ❖ Usually given in combination with diphtheria and tetanus toxoids (DTaP vaccine).

##### B. HBV vaccine

- ❖ Recombinant subunit vaccine containing HBsAg

#### 2. Capsular Polysaccharide Vaccines

##### A. Streptococcus pneumoniae

- ❖ Contains the capsular polysaccharide of the 23 most prevalent

#### 3. Advantages

- ❖ Not infectious, so they can safely be given to immunosuppressed people. Less likely to induce unfavorable immune reactions that may cause side effects (e.g. hypersensitivity).

#### 4. Disadvantages

- ❖ The antigens may not retain their native conformation, so that antibodies produced against the subunit may not recognize the same protein on the pathogen surface
- ❖ Isolated protein does not stimulate the immune system like a whole organism vaccine.
- ❖ **Polysaccharide vaccines (T-independent antigens):** are not effective in infants and young children (under 18–24 months), Induce only short-term immunity

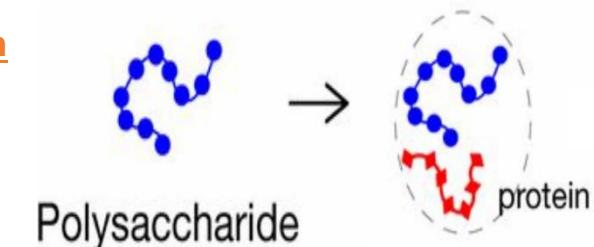


#### 4) Conjugate vaccines

- ❖ Developed to pathogens with **polysaccharide capsules**, by linking the polysaccharides to protein carrier converting them into T-dependent antigens (i.e. class switching, memory)

##### 1. T-dependent antigen → T-independent antigen

- ❖ Improved immune and memory response.
- ❖ Longer lasting protection.
- ❖ The protection of infants and toddlers



##### 2. Types

###### A. *Neisseria meningitidis*

- ❖ Polysaccharide vaccines conjugated to either diphtheria toxoid or tetanus toxoid

###### B. Pneumococcal conjugate vaccine

- ❖ Capsular polysaccharide coupled to diphtheria toxoid

###### C. *Haemophilus influenzae*

- ❖ Type b polysaccharide conjugated to diphtheria toxoid or other carrier protein.

#### 5) Toxoid vaccines

- ❖ A toxoid is an inactivated toxin that has lost its ability to cause disease but has retained its immunogenicity

##### 1. *Corynebacterium diphtheriae* vaccine

- ❖ Contains diphtheriae toxoid (formaldehyde-treated exotoxin) for protection against diphtheriae

##### 2. *Clostridium tetani* vaccine

- ❖ Contains tetanus toxoid (formaldehyde-treated exotoxin) protect against tetanus

#### 6) Recombinant vaccines

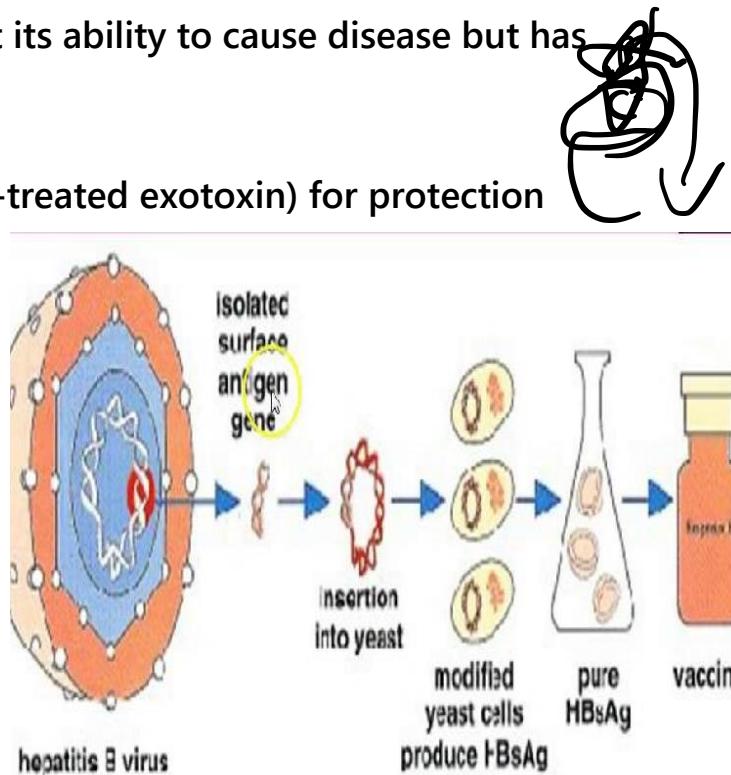
- ❖ Prepared by recombinant DNA technology

##### 1. Recombinant antigen vaccine

- ❖ Antigens are synthesized by inserting the coding genes into *E. coli*, yeast cell or other cells e.g. HBV

###### A. Hepatitis B virus (HBV) vaccine

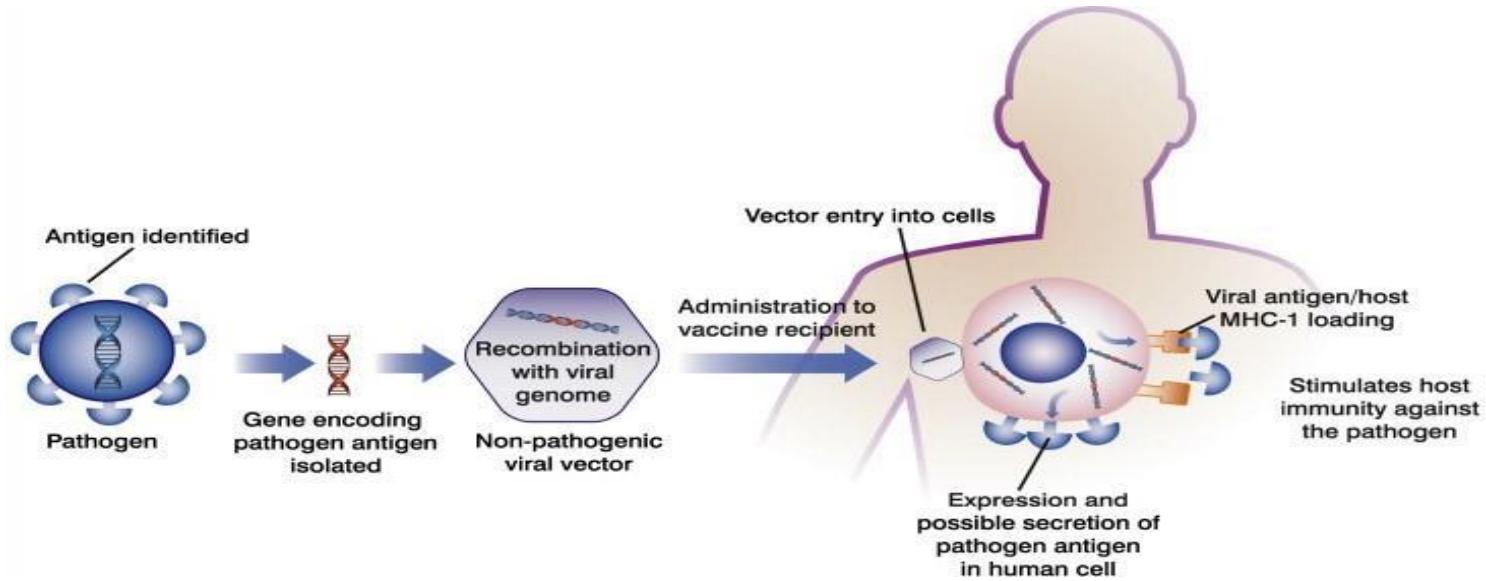
- ❖ Hepatitis B surface antigen is produced from a gene transfected into yeast cells and purified for injection as a subunit vaccine. This is much safer than using attenuated HBV, which could cause lethal hepatitis or liver cancer if it reverted to its virulent phenotype.





## 2. Viral vector vaccine

- The genes coding for the antigen (protein) is inserted into genome of an avirulent vector (e.g. adenovirus) e.g. Covid- 19 vaccine Janssen (Johnson& Johnson)



## 7) Nucleic acid vaccines

- New promising vaccines for combating infectious diseases and cancer.
- Nucleic acid-based vaccines: DNA (as plasmids) and RNA [as mRNA].
- Use virus DNA or RNA to enable human cells to manufacture portions of a virus to trigger the immune response.

### 1. DNA vaccines

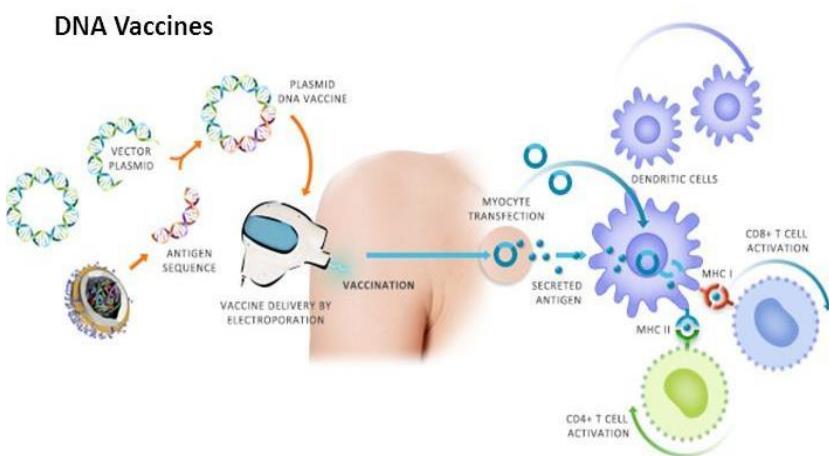
- More stable than mRNA vaccines and
- Can be stored at room temperature
- Easier to transport and distribute.
- Can be produced more quickly and cheaply than mRNA vaccines.

### 2. Mechanism of Action:

#### 1. DNA vaccines

- Contain plasmids that encodes the target antigen gene
- Plasmid is injected into a muscle cell → enters the cell's nucleus → transcribed into mRNA → translated into the antigen → displayed on the cell surface & or secreted.
- This induce an immune response (production of antibodies and immune cells)
- Example: COVID-19 DNA vaccine: Inovio

DNA Vaccines

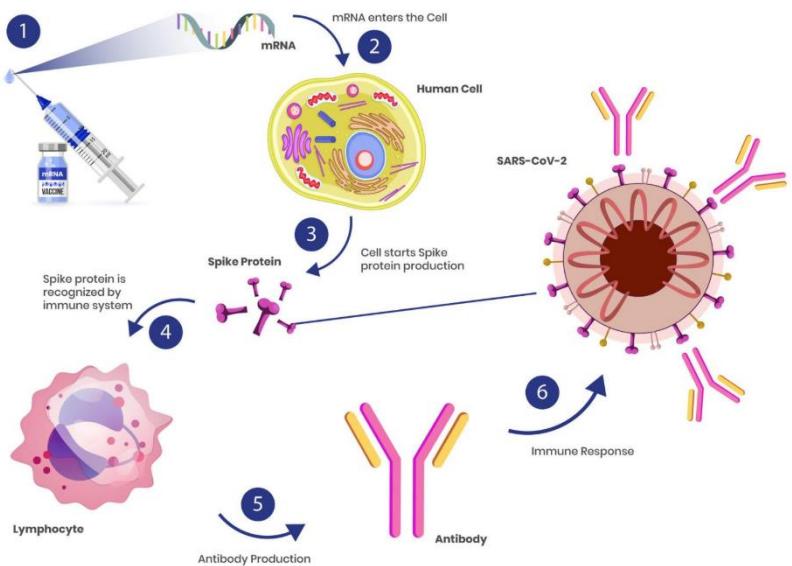




## 2. RNA vaccines

- ❖ Contain messenger RNA (mRNA) that encodes an antigen → injected into a muscle cell → translated into the antigen → displayed on the cell surface & or secreted.
- ❖ This induce an immune response (production of antibodies and immune cells)
- ❖ Example: Pfizer COVID-19 vaccine: mRNA that codes for the virus spike protein

### How does mRNA vaccine work



## ADJUVANTS

- ❖ Adjuvants are substances added to vaccines to enhance the immune response to the antigen
- ❖ Example: Aluminum salts : in HBV, Polio , DTP.

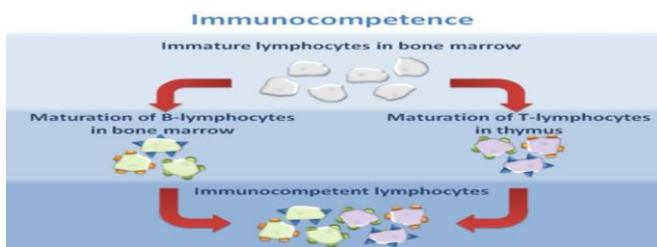
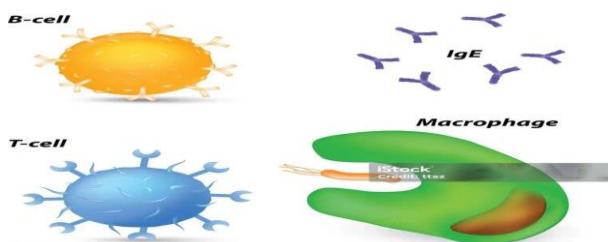
## Remember

- ❖ Passive immunity is immunity acquired by the transfer of preformed antibodies (immune globulins).
- ❖ Passive-active immunity consists of administering both immune globulins and a vaccine.
- ❖ Active immunity is most often elicited by vaccines containing killed , purified protein/ polysaccharide subunits, conjugated , toxoid or live, attenuated (weakened) pathogen.
- ❖ In general, live attenuated vaccines are preferable to killed vaccines.
- ❖ Recombinant DNA technology is promising for production of new vaccines.
- ❖ Nucleic acid vaccines are new, and many are under development.



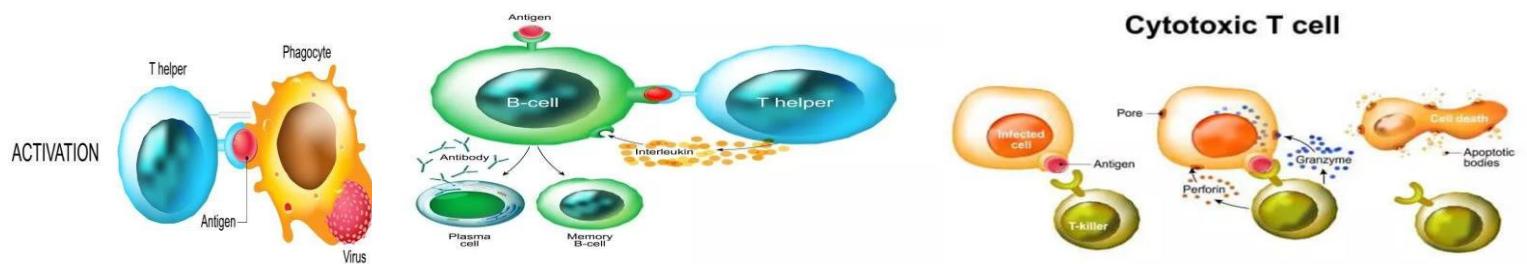
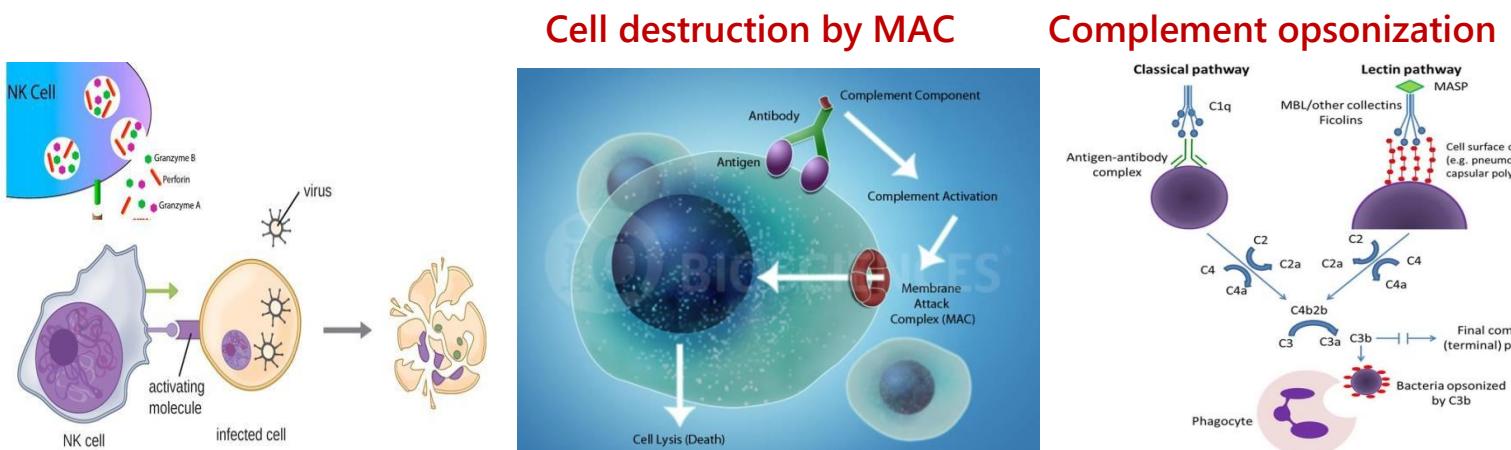
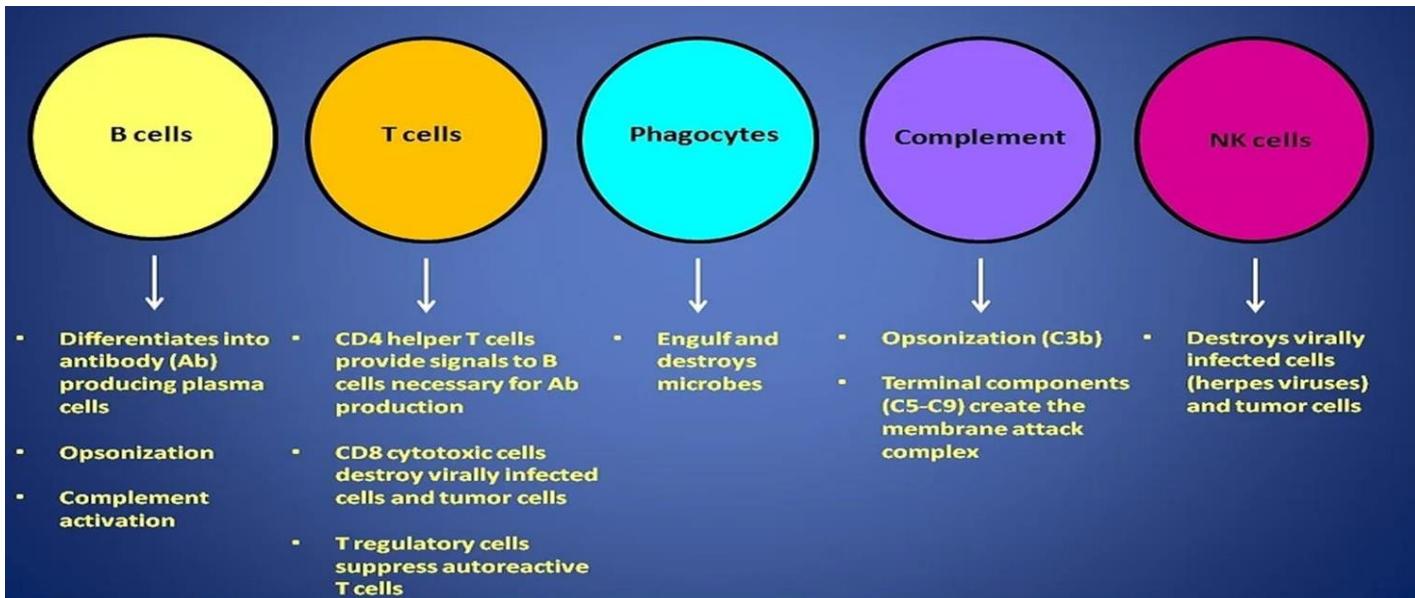
## Micro TUT 5 : Assessment of immunocompetence

- The main function of the immune system is to protect the body against foreign pathogens.
- **Immunocompetence** is the ability of the body to resist pathogens through production of a normal immune response following exposure to an antigen.
- So, being immunocompetent means that the immune system is working properly and the body is capable of mounting an appropriate immune response, when necessary.
- Immunocompetence is the opposite of immunodeficiency.
- Immunodeficient (immuno-incompetence or immuno- compromised) individual has an immune system that not working as it should be.
  - Several factors can affect the immune system's performance, such as:
- ✓ Nutrition.
- ✓ Sleep.
- ✓ Stress.
- ✓ Life history events (e.g. migration, reproduction).
- ✓ Some medications e.g. Corticosteroids.
- ✓ Chronic diseases.
- ✓ Smoking.
- ✓ Alcohol, etc.....
- ✓ Immunologic competence, begins to develop during embryonic life. It is incomplete at the time of birth but becomes fully established soon after birth.
- ✓ Immunocompetence requires the combination of the innate (e.g. phagocytes & complement) and adaptive (B & T cells) immune mechanisms.

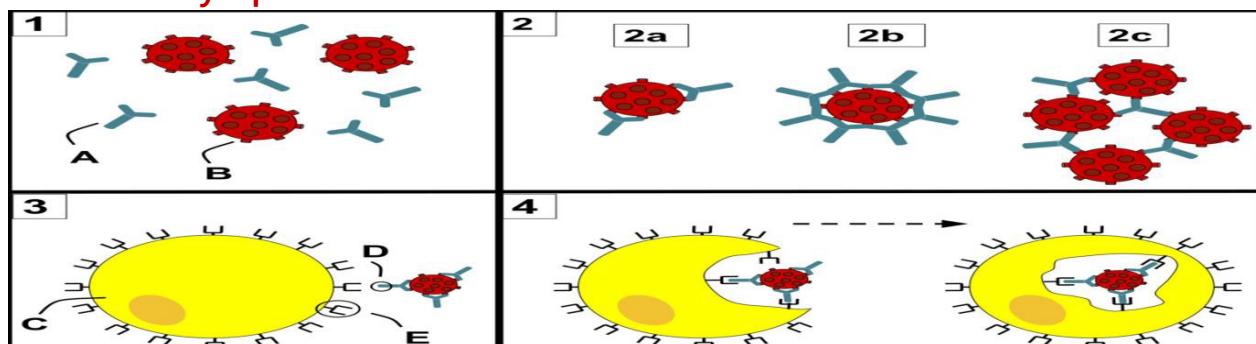




## Function of immune components



## Antibody opsonization





## Evaluation

	B cell	T cell	Phagocyte	Complement	NK cell
QUANTITATIVE	IgG, IgM, IgA	Absolute Lymphocyte Count (ALC)	Absolute Neutrophil Count (ANC)	Individual Complement Components	NK cell numbers
FUNCTIONAL	B cell numbers (CD19, CD20)	T cell numbers (CD3, CD4, CD8)	Mitogen proliferation	DHR Assay	CH50
	Antibody titers Tetanus, Diphtheria, Hib, Pneumococcus				NK cell Functional Assay

**N.B.** The dihydrorhodamine (DHR) test has been performed to assess neutrophil superoxide production. Dihydrorhodamine is a fluorescent dye oxidized by neutrophil superoxides.

### Assessment of immune competence: A- Assessment of T-cell disorders:

1 Enumeration of T-cells by monoclonal antibodies (Flow cytometry).

2 Testing the function of T-cells:

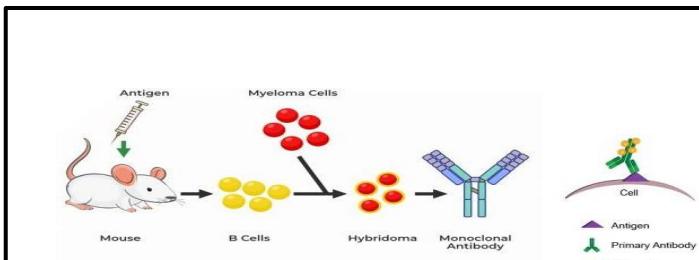
#### a) Skin test:

- To detect delayed hypersensitivity to a variety of antigens to which the patient is supposed to be exposed during his life, e.g. tuberculin (PPD) or Candida.
- These skin tests are useful to assess T-effector cell function.
- Negative skin tests indicate T-cell deficiency.

#### b) Test for lymphocyte proliferation using mitogens:

- Phytohemagglutinin (PHA), concanavalin A (con-A) or specific antigens such as PPD or tuberculin

1- A monoclonal antibody is a type of antibody able to operate against only one type of antigen

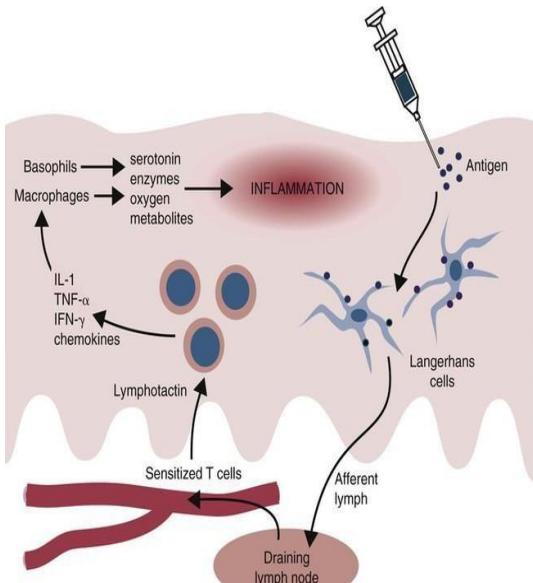
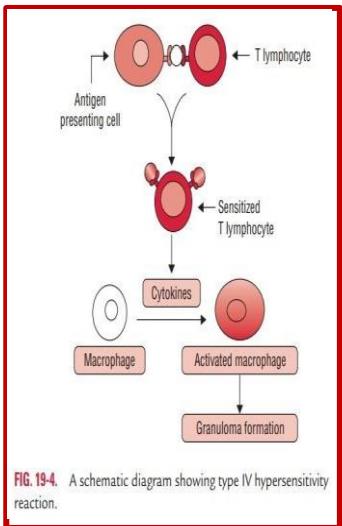




## 2- a) Skin test:

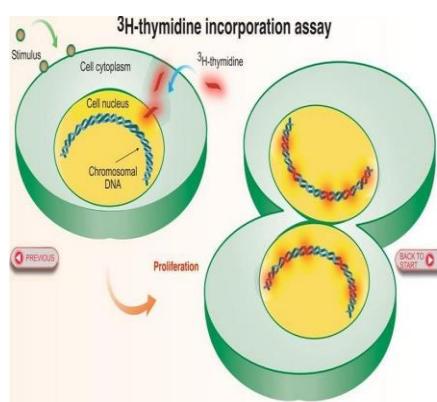
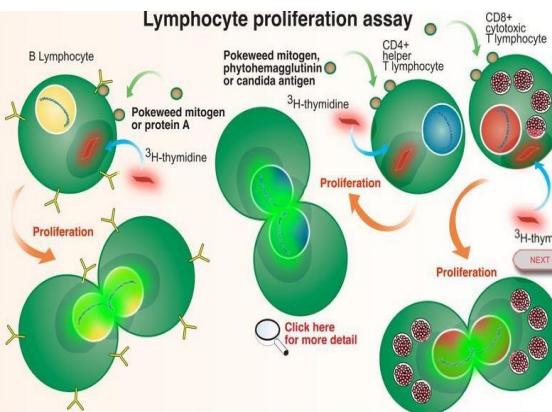
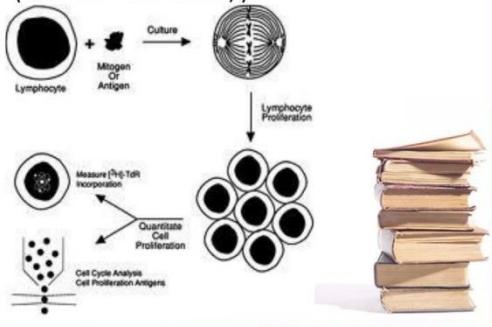
### What is delayed-type hypersensitivity?

Delayed-type hypersensitivity is a type of immune response involving T cell activation after antigen exposure. It causes redness, swelling and tissue damage at the site, appearing at least 12-24 hours after exposure and up to 48-72 hours after exposure.



## 2- b) Lymphocyte proliferation using mitogens

### Lymphocyte proliferation (stimulation assay)



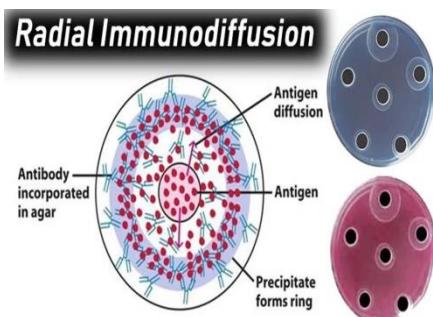
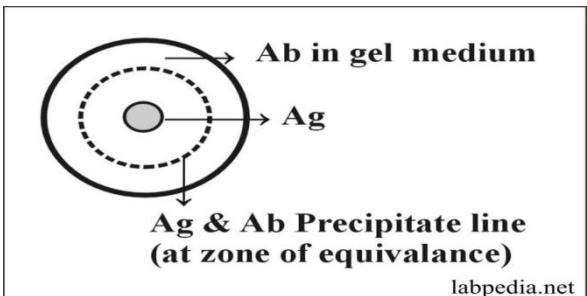
## B- Assessment of B cell disorders:

Enumeration of B-cells by monoclonal antibodies.

Testing the function of B-cells:

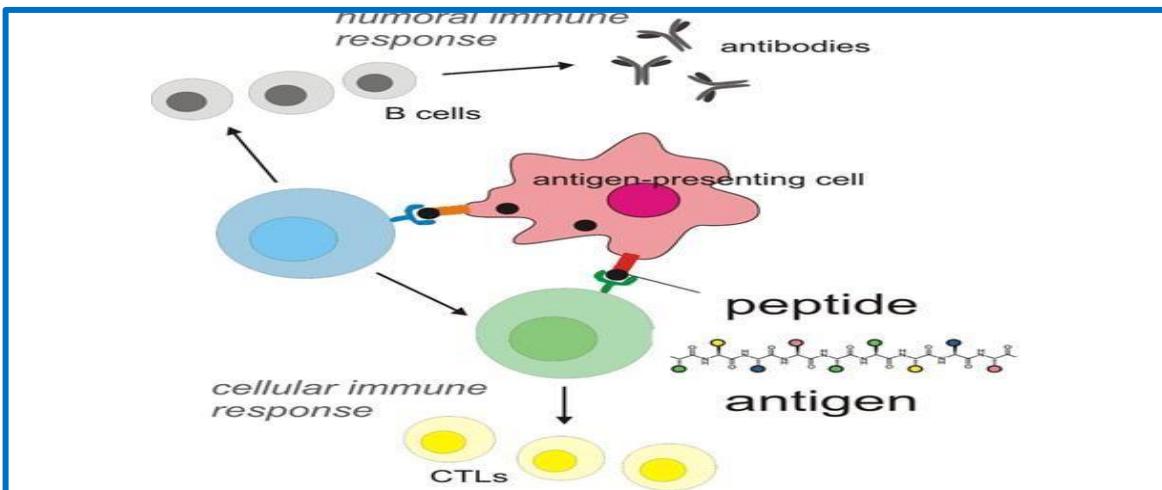
- Quantitation of total serum immunoglobulins by single radial immunodiffusion.
- Stimulation of production of specific antibodies to killed vaccines or toxoids.
- Cell proliferation using B-cell mitogens e.g. polysaccharides.

## 2- a) Quantitation of total serum immunoglobulins by single radial immunodiffusion.





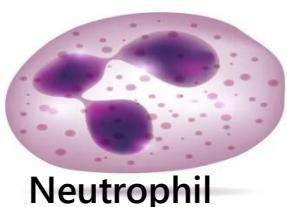
## 2- b) Stimulation of production of specific antibodies.



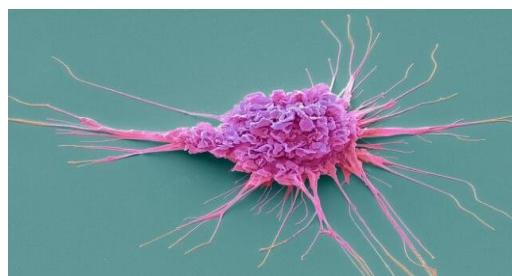
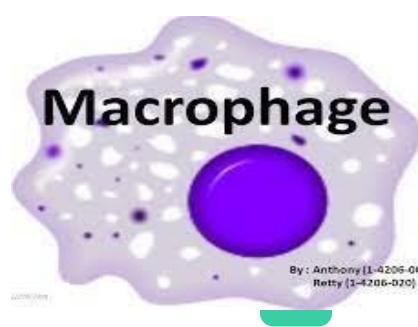
### C- Assessment of phagocytic cell disorders:



- **Assessment of chemotaxis** by testing the ability of phagocytic cells to migrate through membranes towards a chemotactic substance.
- Assessment of the ability of phagocytes (neutrophils, dendritic cells and macrophages) to *engulf* and *destroy* bacteria or yeast.
- **Assessment of the oxidative burst** by testing the ability of phagocytes to deoxidize (reduce) the yellow colored nitroblue tetrazolium (NBT) dye to blue color.
- Phagocytosis (means to eat) is a process refers to the engulfing of microorganisms, cells or foreign particles by cells.
- It is the first line of defense mechanism of the body against microorganisms provided by white blood cells.
- Cells performing phagocytosis are called phagocytes which mainly are:
  - Neutrophils [polymorphnuclear leukocytes (PMNs)]; found in blood.
  - Macrophages; resident in tissues.
  - Dendritic cells.



Neutrophil

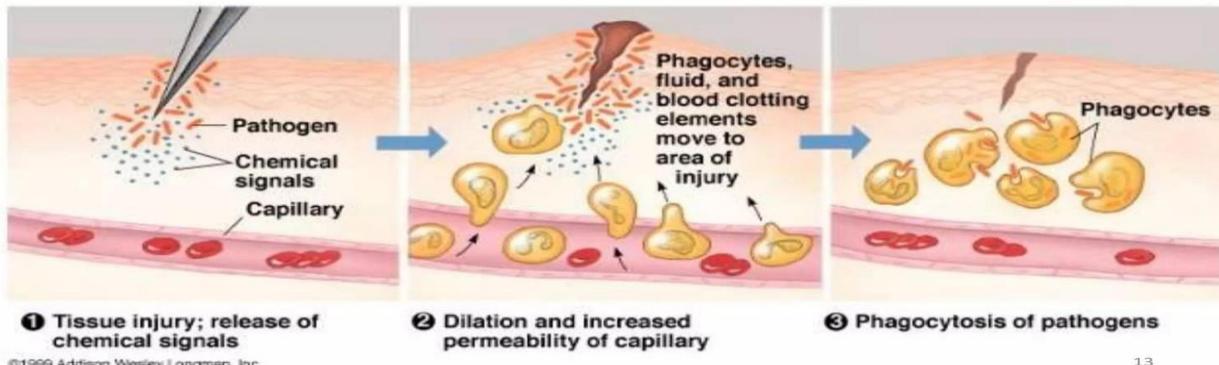


Dendritic cell



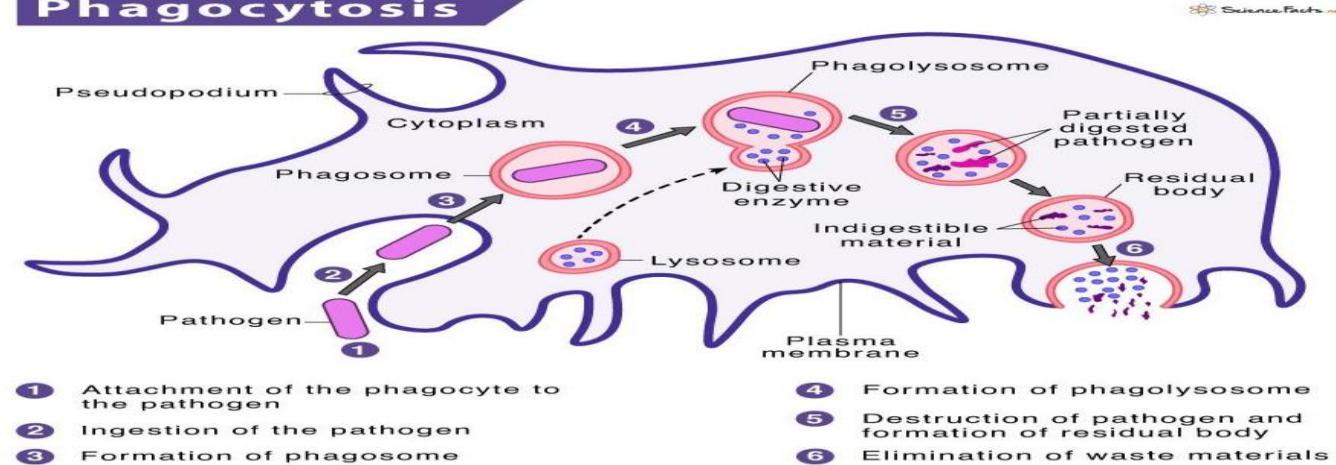
- **Chemotaxis** is the process of attraction and migration of phagocytes toward micro-organisms by the effect of chemical substances (chemo-attractants) released by the infecting organism or the inflammatory cells at the site of infection.

### PHAGOCYTES ARE ATTRACTED TO SITE OF INFECTION BY CHEMOTAXIS



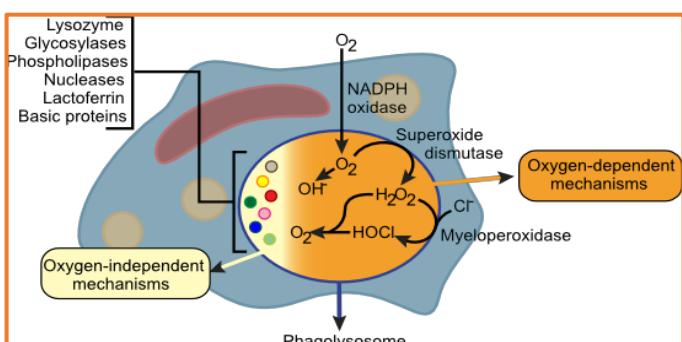
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### Phagocytosis

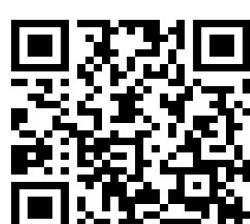


- Pathogen killing can occur in one of two ways:

- The oxygen dependent pathway (oxidative burst) involves: reactive oxygen species (ROS) such as (superoxide radicals & hydrogen peroxide).
- The oxygen independent pathway involves: lysosomal enzymes (such as lysozyme), tumor necrosis factor (TNF) and hydrolytic enzymes.

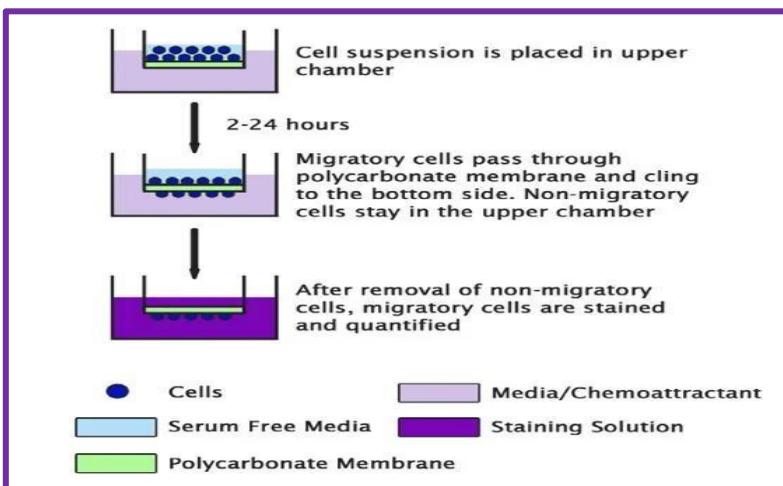
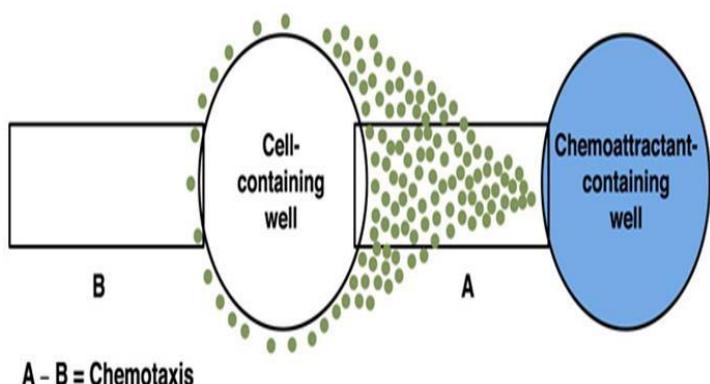


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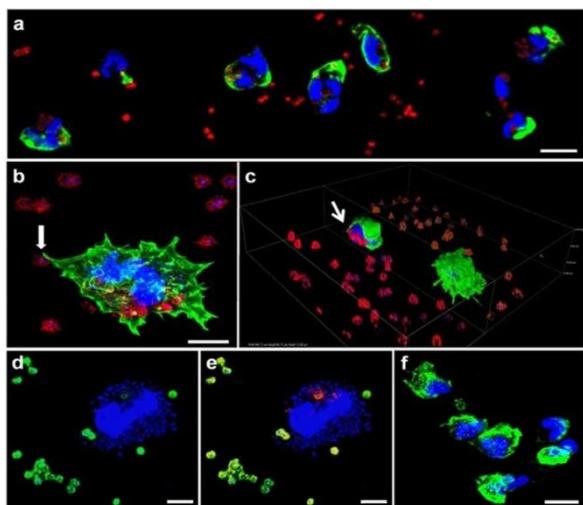
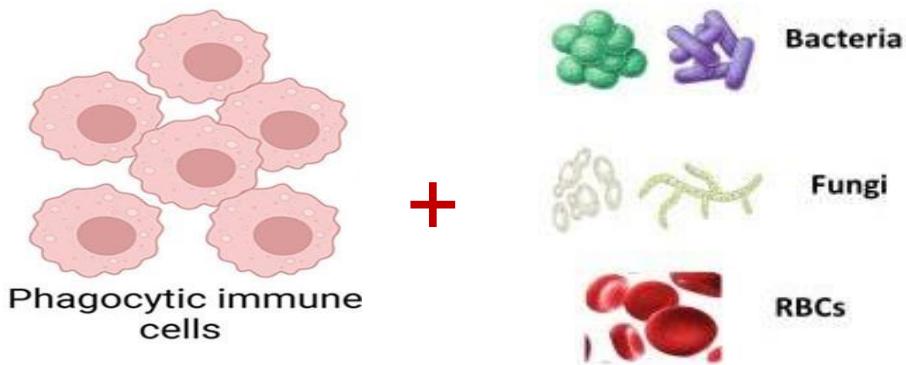




## Assessment of Chemotaxis



## Assessment of phagocytosis engulfment

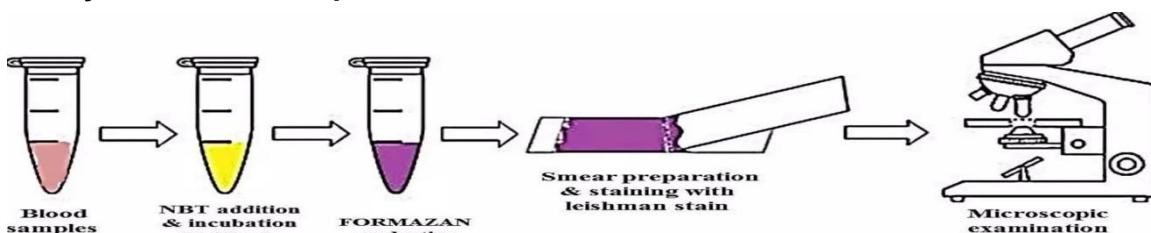


## Assessment of the oxidative burst

- The oxygen dependent pathway (oxidative burst) of pathogen killing by phagocytes is assessed by the ability of phagocytes to reduce the yellow color nitroblue tetrazolium (NBT) into blue color formazan.

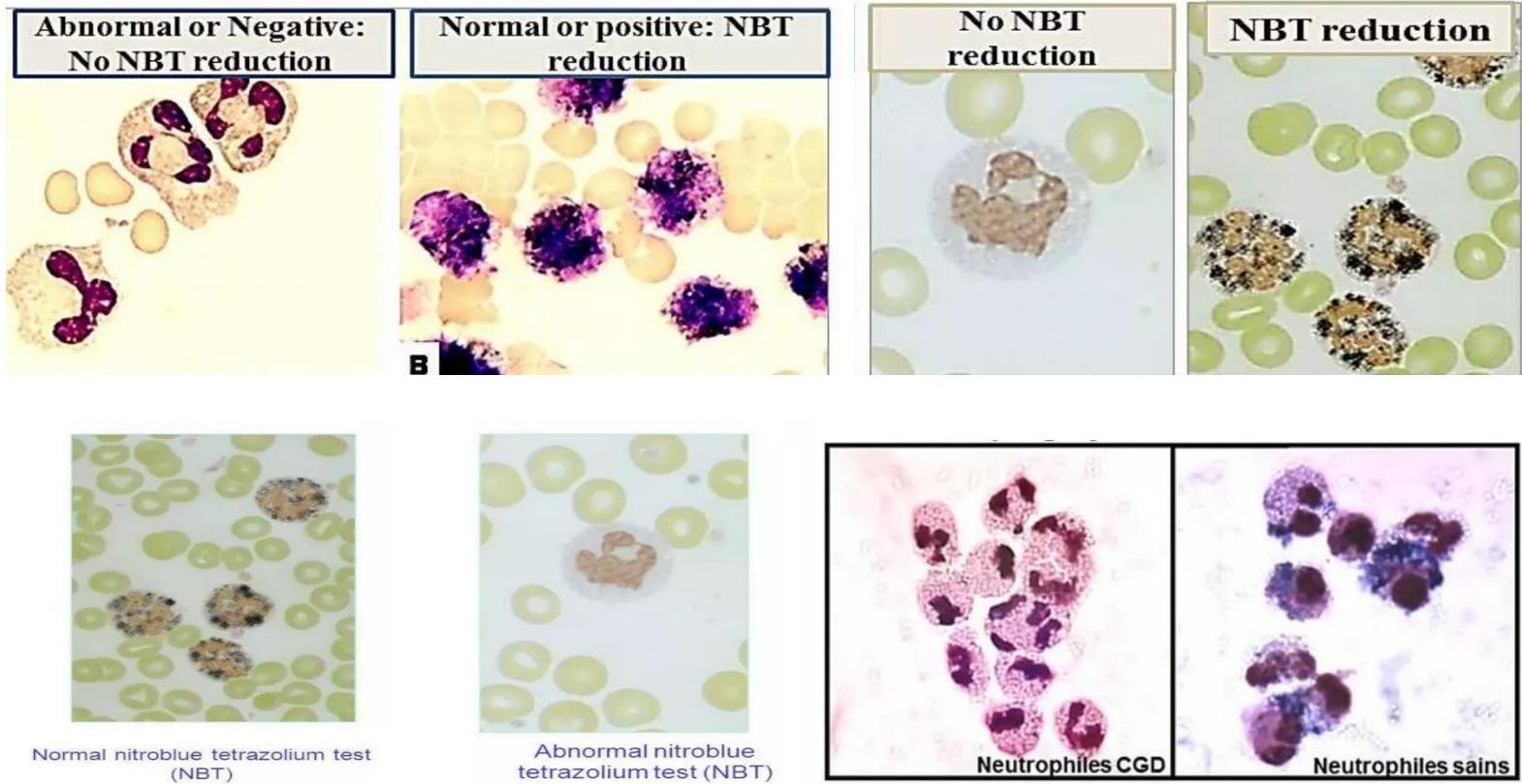


- In this test NBT is added to the blood sample leads to formation of insoluble complex which is engulfed and reduced by phagocytes that can be examined by the microscope.



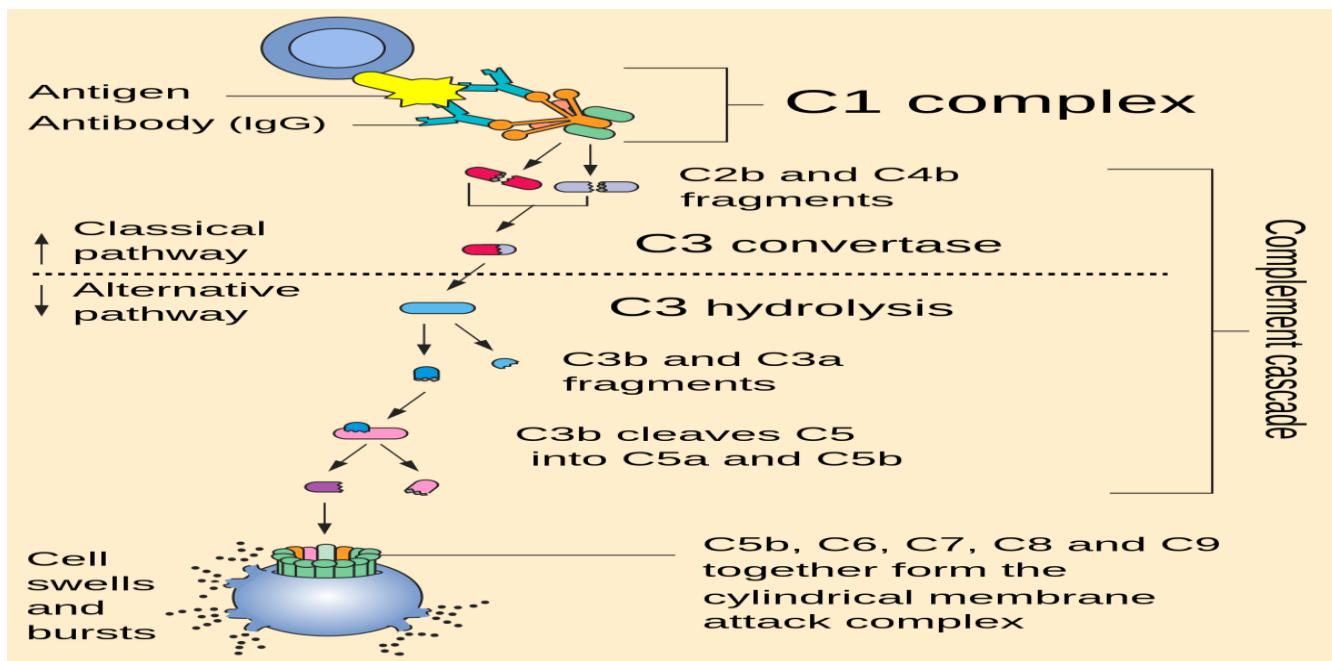


- Normal phagocytic cells show dark blue cytoplasmic granules of formazan.



### D- Assessment of complement disorders:

- Estimation of total hemolytic activity of complement.
- Estimation of individual complement components by immunodiffusion, ELISA or RIA.

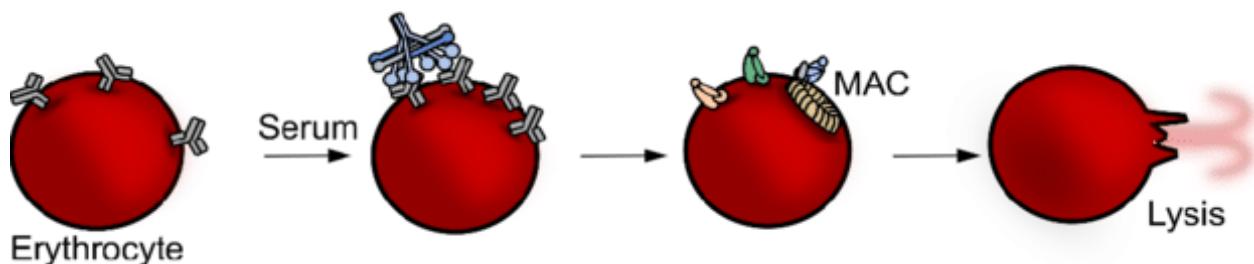




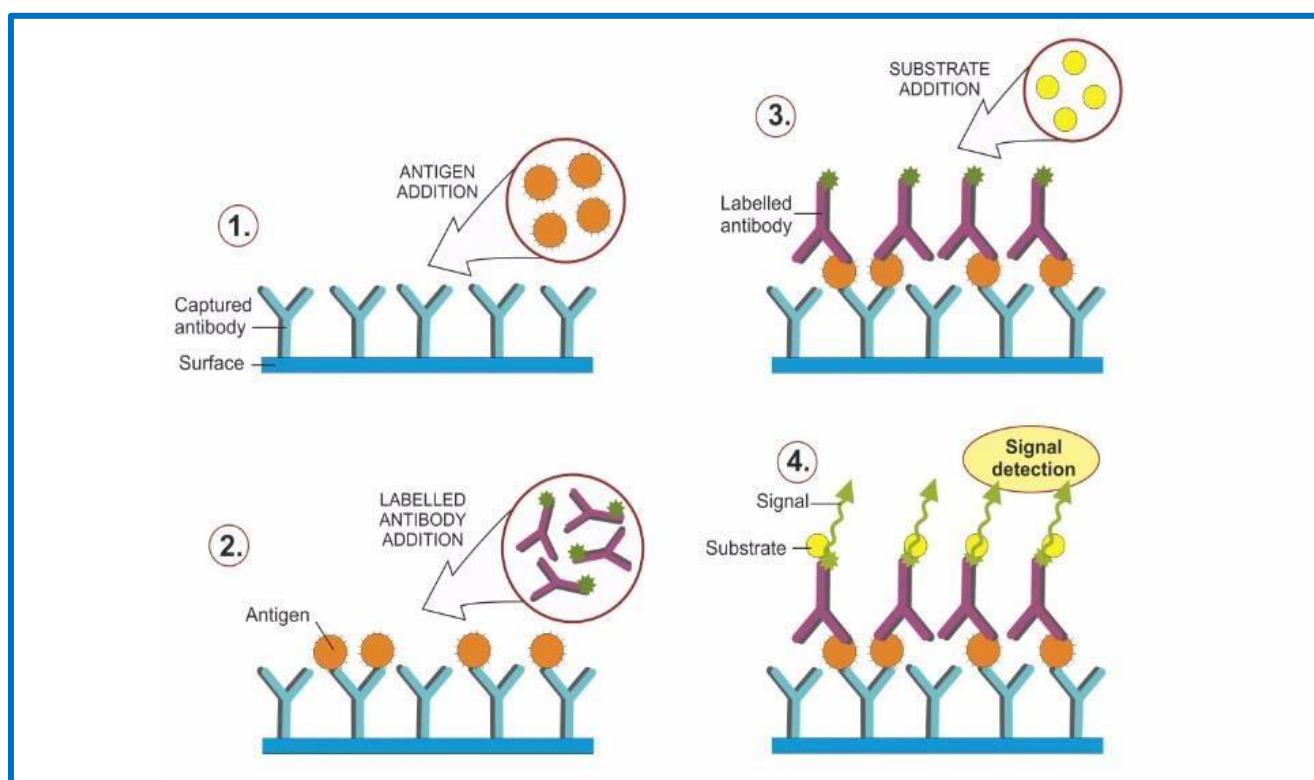
- Hemolytic assays have traditionally been used to assess the functional activity of the complement system. They provide insights into the integrity of the entire cascade reaction.
- These tests are particularly useful in the investigation of suspected complement deficiencies.

### Principle of the test:

- Serial dilutions of the sample to be analyzed are incubated with Ab-coated sheep RBCs (sensitized RBCs) in test tubes at a defined temp.
- Addition of the complement to the sensitized RBCs leads to RBCs hemolysis.
- The released Hb can be estimated by a photoelectric spectrophotometer.
- The hemolysis level is related to the total complement activity.



### Estimation of individual complement components by ELISA





## DISCUSSION

### CASE (1)

An 12 month old male presents to your clinic for evaluation of recurrent infections. He had no medical problems until 6 months of age. Since then, he has developed 6 ear infections as well as 1 episode of pneumonia requiring hospitalization and IV antibiotics. He has had some loose stools attributed to use of antibiotics.

- Based on the clinical history, what arm of the immune system would you suspect is impaired?
- What laboratory tests would you send?

### Case 1 (Answer)

- The history of recurrent sinopulmonary infections is concerning for B-cell or antibody defects. Enterovirus infections can cause chronic diarrhea. The lack of infections during the first several months of life can be explained by the presence of maternal antibodies in circulation that are transferred across the placenta.
- An quantitative screen of this arm of the immune system would include:
  - A total IgG, IgA, IgM levels as well as quantification of B-cells by flow cytometry (CD19 or CD20).
  - A functional evaluation of this arm of the immune system would include assessing specific antibody responses to tetanus, diphtheria, and pneumococcus vaccinations.

### CASE (2)

A 4 month old male infant is admitted for respiratory distress and severe hypoxia and is diagnosed with *Pneumocystis jiroveci* pneumonia. He had a normal weight at birth but has failed to gain weight with chronic loose stools. He has diffuse oral candidiasis refractory to nystatin therapy. His mother reports that her HIV test during pregnancy was negative.

- Based on the clinical history, what arm of the immune system would you suspect is impaired?



- b) What additional laboratory tests would you send for this patient?

### Case 2 (Answer)

- a) The history of *Pneumocystis jiroveci*, severe thrush and chronic diarrhea should raise concern for a defect in the T cell arm of the immune system.
- b) This patient should have lymphocyte flow cytometry to enumerate T cell, B cell, and NK cell populations.
- c) Lymphocyte culture with mitogens (PHA) should be performed to assess T cell function.
- e) Genetic testing.
- f) HIV PCR should also be a part of the laboratory evaluation.

### CASE (3)

A 6 year male presents with a *Staph aureus* skin abscess on the arm requiring drainage and IV antibiotics. The patient also has a history of pneumonia with *Nocardia* 1 year ago. An older brother also has a history of recurrent skin abscesses and *Serratia* osteomyelitis (two older sisters are presently healthy).

- a) Based on the clinical history, what arm of the immune system would you suspect is impaired?
- b) What is the most likely diagnosis for this patient?
- c) What screening laboratory test would you send?
- d) What confirmatory test would you send?

### Case 3 (Answer)

- a. The history of recurrent skin abscesses, pneumonia and osteomyelitis in these two male siblings should raise suspicion for defects in the phagocyte arm of the immune system.
- b. This history is concerning for Chronic Granulomatous Disease (specifically X-linked CGD given the presence of similar symptoms in the older brother). Patients have an increased susceptibility to catalase positive organisms - *Staph aureus*, *Nocardia*, *Serratia*, *Burkholderia cepacia*, and *Aspergillus* are the five most common organisms causing disease in CGD.
- c. This patient should have a screening test to assess neutrophil reactive oxygen intermediate production [this can be done by a Dihydrorhodamine (DHR) Assay or the older Nitroblue Tetrazolium (NBT) test].



- d. If the DHR assay or NBT test indicates poor reactive oxygen intermediate production, genetic testing should be performed for CGD [most likely X-linked CGD (CYBB gene) in this case]. If negative, testing for known autosomal recessive forms of CGD should be considered.





## Micro TUT6 : IMMUNITY TO MICROBES

- The principal physiologic function of the immune system is to protect the host against pathogenic microbes.



### \* General features of immunity against microbes:

1- Defense against microbes is mediated by both innate and adaptive immunity. Adaptive immunity enhances the protective mechanisms of innate immunity.

- Example: activation of macrophages by IFN- $\gamma$  produced by T helper cells.

2- The innate immune response to microbes plays an important role in determining the nature of the adaptive immune response.

- Production of IL-12 by macrophages, for example, leads to development of Th1 cells and consequently a good cell- mediated immune response.

3-The survival of pathogenic microbes in a host depends on the ability of these microbes to evade or resist the body's immune mechanisms.

4- During infections, tissue injury and disease may sometimes be caused by the host response to the microbe and its products rather than by the microbe itself.

### I. Immunity to Extracellular Bacteria

- Extracellular bacteria are capable of replicating outside host cells.

#### A- Innate Immunity:

- Phagocytosis by neutrophils, monocytes and tissue macrophages.

- Activation of complement by the alternative pathway:

- Complement can be activated by the peptidoglycan and lipopolysaccharide in the cell wall of bacteria.

- Complement activation leads to:

- production of opsonins,
- recruitment and activation of phagocytes and
- lysis of bacteria.



## B. Adaptive Immunity

### I. Humoral immune response :

- This is the main protective specific immune response against extracellular bacteria.

- Antibodies** perform several functions to eliminate extracellular bacteria:

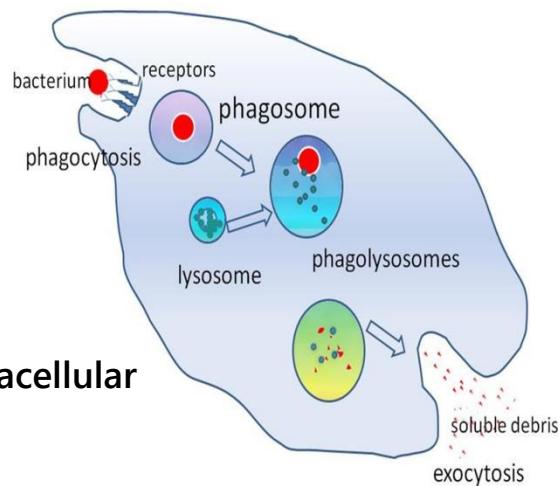
1) **Neutralization** of bacterial toxins, preventing their binding to target cells.

2) **Opsonization**: enhances phagocytosis

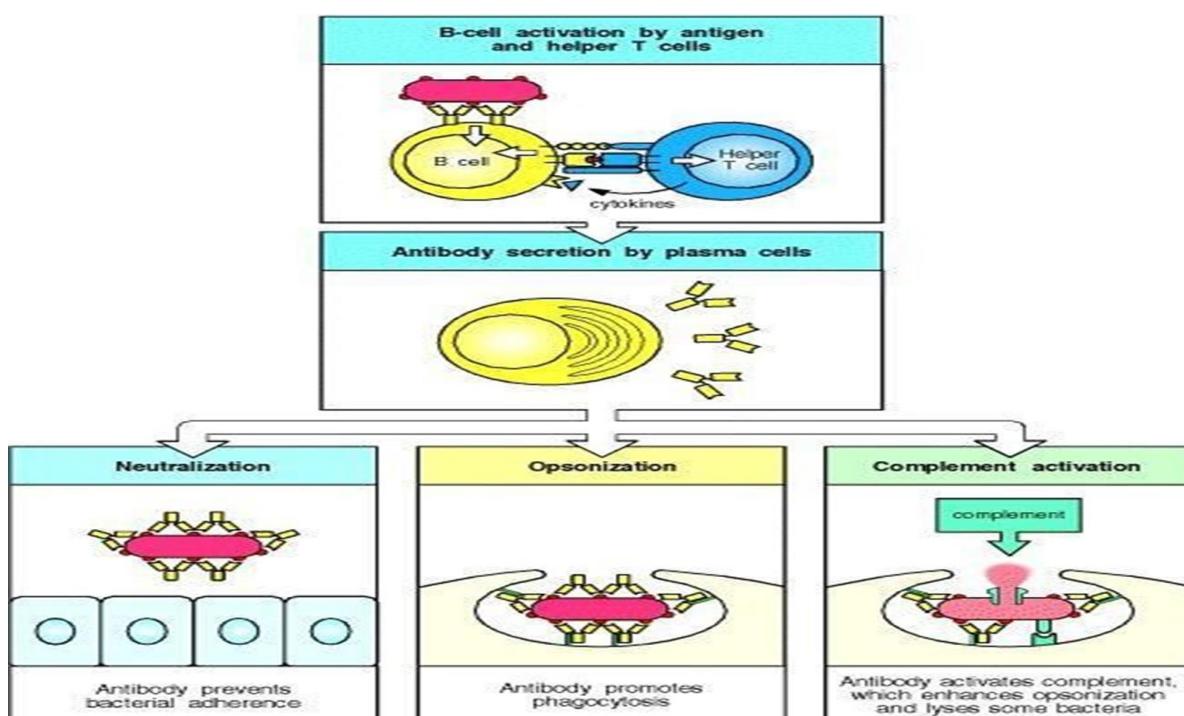
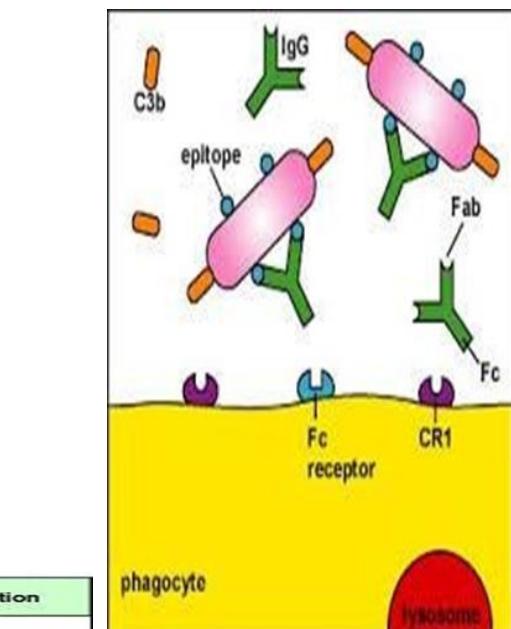
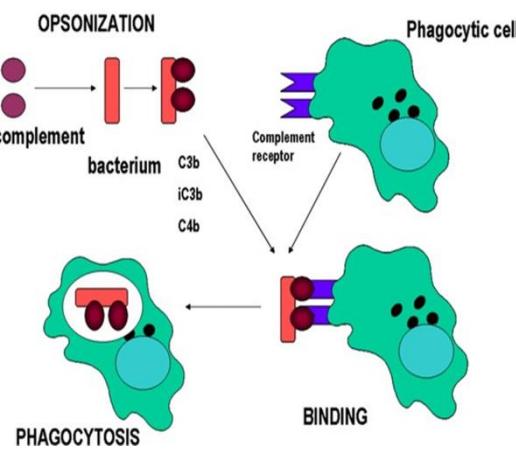
3) Activation of complement by the classical pathway, with all its consequences.

4) **Agglutination** of bacteria, preventing spreading and facilitating phagocytosis.

5) **Binding to pilin protein of the pili and inhibition of adhesion** of bacteria to host cells.



Opsonization and phagocytosis





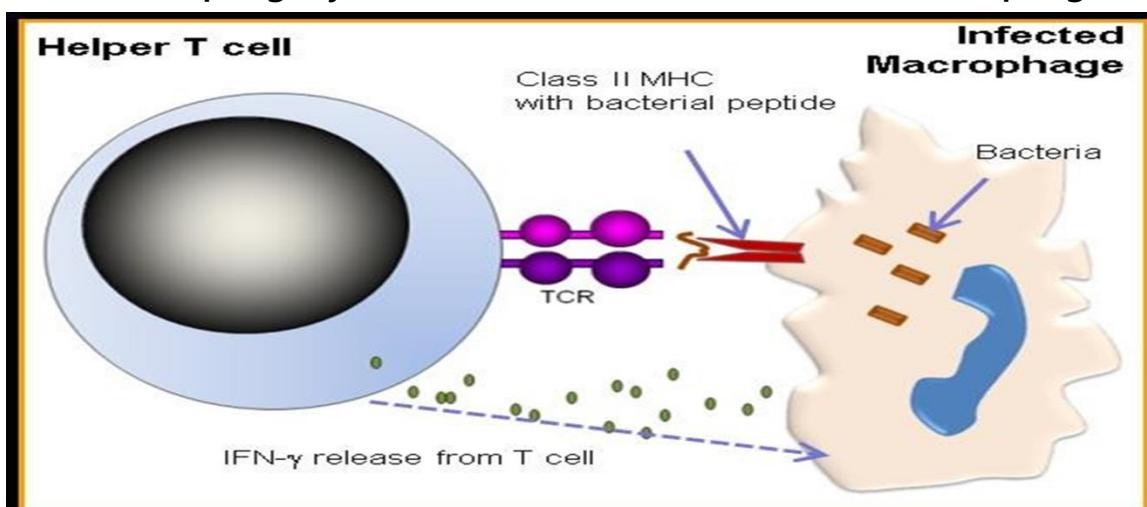
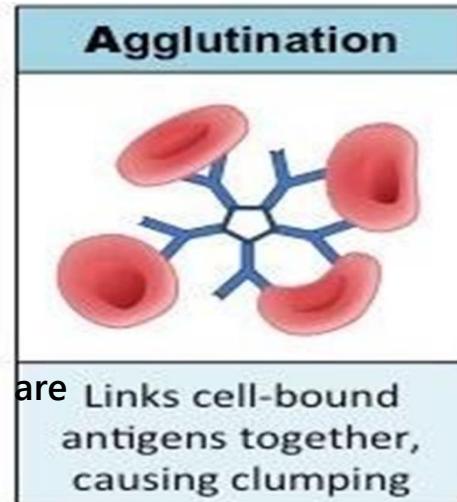
## II.T cell response

- Extracellular bacteria and their products are internalized by APCs and peptides from them are presented to T cells in association with MHC II molecules.

- So stimulate **T helper cells**.

- Their effector functions against extracellular bacteria mediated by the cytokines they secrete and include:

- Stimulation of antibody production.
- Induction of local inflammation.
- Enhancement of phagocytic and microbicidal activities of macrophages.



## II. Immunity to Intracellular Bacteria:

A characteristic of intracellular bacteria is their ability to **survive**, and even **replicate**, **within phagocytes**, e.g. *Mycobacterium tuberculosis*

### A- Innate Immunity:

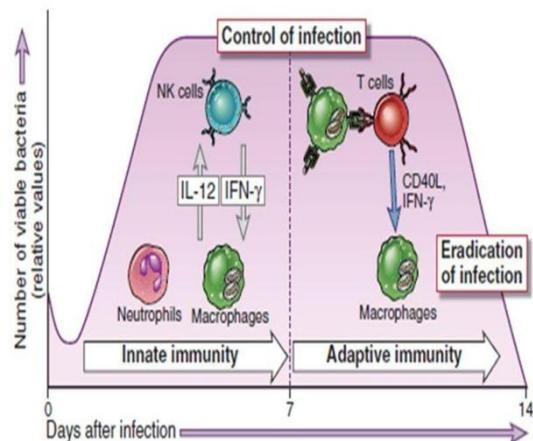
- Pathogenic intracellular bacteria are resistant to degradation within phagocytes.
- Thus, innate immunity is usually **ineffective** in controlling such infections
- **NK cells** provide early defense against these microbes before **adaptive** immunity develops. They produce IFN- $\gamma$  which activates macrophages to kill intracellular bacteria.



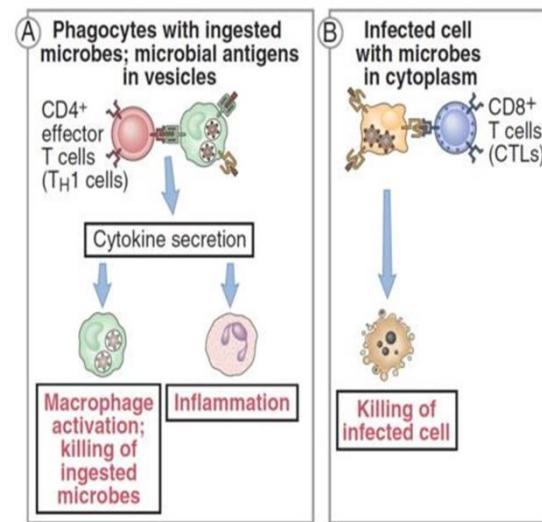
Innate and adaptive immunity to intracellular bacteria

**B- Adaptive immunity :**

- **Humoral immune response:-** (Has NO role; Since intracellular bacteria are inaccessible to circulating antibodies).
- **T cell response:-** Cell-mediated immunity (in the form of macrophage activation by Th1 cells) is the main protective immune response against intracellular bacteria.
- These bacteria induce the production of **IL-12** by macrophages and **IFN- $\gamma$**  by **NK cells**.
- Both cytokines promote the development of Th1 cells.
- **Th1 cells**, in turn, secrete IFN- $\gamma$  which :
  - activates the **macrophages** to kill the bacteria that they harbour.
  - activates **cytotoxic T lymphocytes (CTLs)** to kill the infected cell.



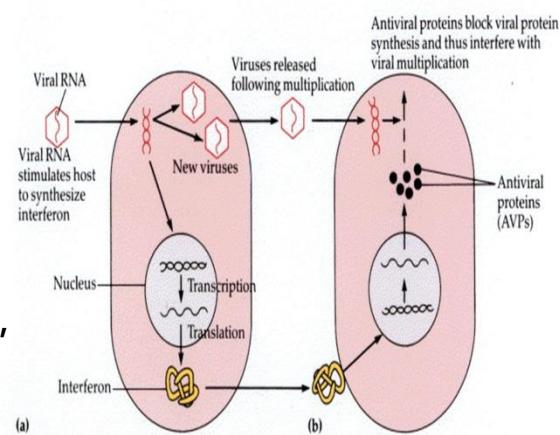
Cellular and Molecular Immunology, 7th ed., 2014 Elsevier

**III-Anti-viral immunity**

Antiviral action of interferon (Figure 16.15)

**A- Innate Immunity:****Interferon**

- Interferon **interferes with virus multiplication**; this is the property for which they are named.
- The antiviral action of interferon is primarily **paracrine**, in that a virally infected cell secretes IFN to protect neighboring cells not yet infected.
- Interferon provide the **first line** of defense against virus infections.





- **NK cells:** NK cells kill cells infected with a variety of viruses.

NK cells are important for immunity against viruses **early** in the course of infection, before adaptive immune responses have developed.

## B- Adaptive immunity :

### I. (Humoral immunity):

**1- Virus neutralization** is the most important mechanism: Antibodies can neutralize virus infectivity by preventing its attachment to receptor sites on susceptible cells.

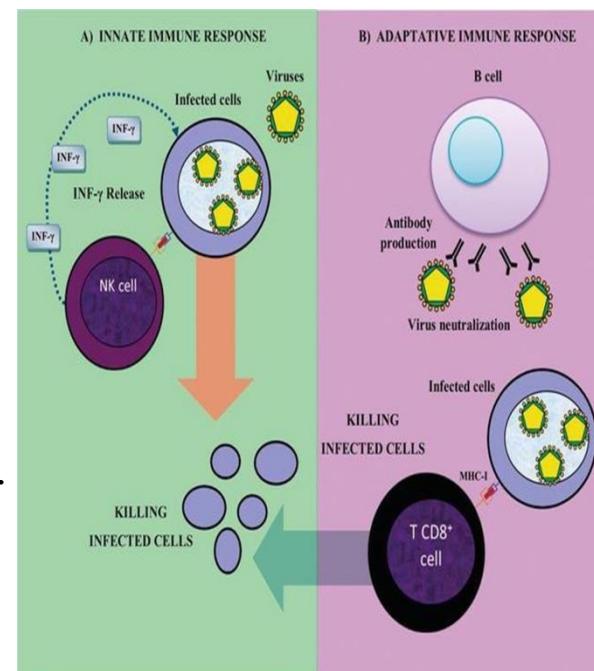
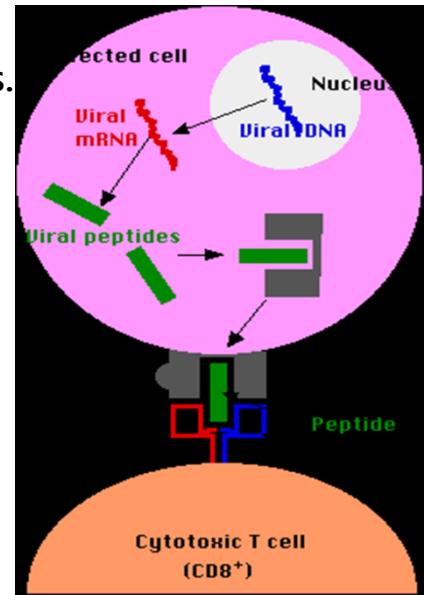
- (IgG and IgM) neutralize viruses which pass through the blood stream -causing viremia.
- (Secretory IgA) neutralizes virus infectivity at the mucous surfaces. Non-viremic infections e.g. influenza.

**2- Antibodies may also destroy free virus particles directly through:**

- a- Aggregation of virus and opsonization.
- b- Complement mediated lysis **of the virally infected cells**.

### II. Cell mediated immunity:

- Elimination of viruses that reside within cells is mediated by CTLs, which kill the infected cells.
- Most virus-specific CTLs are CD8+T cells that recognize cytosolic, usually endogenously synthesized, viral antigens in association with class I MHC molecules on any nucleated cell.





## IV. Immunity to Fungi

### A- Innate Immunity:

- The neutrophils are the most important cells of the innate immune system in combating fungi.
- Neutrophils liberate fungicidal substances and phagocytose fungi as well. People with neutropenia are extremely sensitive to fungal infections.
- Macrophages can also combat fungal infections.



### B- Specific Immunity:

Most pathogenic fungi behave like intracellular bacteria and specific immunity to them is quite similar.

- Humoral immune response: Antibodies are often produced against fungi, but do not appear to be useful in protection.
- T cell response: Cell mediated immunity is the major defense mechanism against fungal infections. It acts in the same way as for combating intracellular bacterial infections.
- Complete:**

Th1 cells are triggered by ..... and their effector cytokine is .....

- List the effector functions of Antibodies to eliminate extracellular bacteria:

1. .... 2. .... 3. .... 4. ....





## Micro CBL2: Cases on Type I hypersensitivity

### CASE 1

A 12-year-old boy is brought to the emergency department after being injected by penicillin. He initially complained of localized pain and swelling. Fifteen minutes later, he began to complain of shortness of breath. His parents observed, his chest was wheezing. He also said that he felt very weak and dizzy. His parents brought him immediately to the local emergency department

- Identify the possible diagnosis.
- Explain the mechanisms of this condition.
- Enumerate the lines of treatment of this condition.

### CASE 2

A 15-year-old girl presented with a prolonged wheezing attack which had suddenly come on 36h earlier. She had experienced several episodes of 'wheezy bronchitis' as a child and eczema as an infant. On examination, she was tired and unwell, with a rapid respiratory rate and tachycardia (140/min). Her sputum contained many eosinophils.

- What is the most likely diagnosis?
- What types of antibodies involved in this case?
- What is the treatment of this case?

### CASE 3

14-year-old girl complained that her nose was constantly running, causing a nasal blockage at night, exacerbating her sleep disturbance. Her eyes were red, itchy and often discharging clear tears. These symptoms triggered by tree pollens from March to mid-May. There was a strong family history of atopy. Her father had asthma and her mother had eczema.

- What is the most likely diagnosis?



## Definition of hypersensitivity:

- It is defined as an **exaggerated immune response** to a foreign agent resulting in **injury of the host**.

## Types

### ■ Antibody – mediated reactions:

1. Type I, atopic, or anaphylactic reactions.
2. Type II, cytolytic or cytotoxic reactions.
3. Type III, toxic – complex syndrome.

### ■ Delayed (cell – mediated) reactions:

4. Type IV.

## TYPE I- HYPERSENSITIVITY REACTION (IMMEDIATE HYPERSENSITIVITY) / IgE-mediated response

- It is immediate hypersensitivity reaction: occur within 2-30 mins.
- It is initiated by antigens reacting with cell bound IgE.
- It is manifested in many ways (dependent on the organ or tissue involved).
- It may range from Atopic allergy (e.g. food allergy or hay fever) to fatal Anaphylactic shock.

**Allergens**: are antigens that induces production of specific IgE.

The antigens included in this reaction are introduced to the body by:

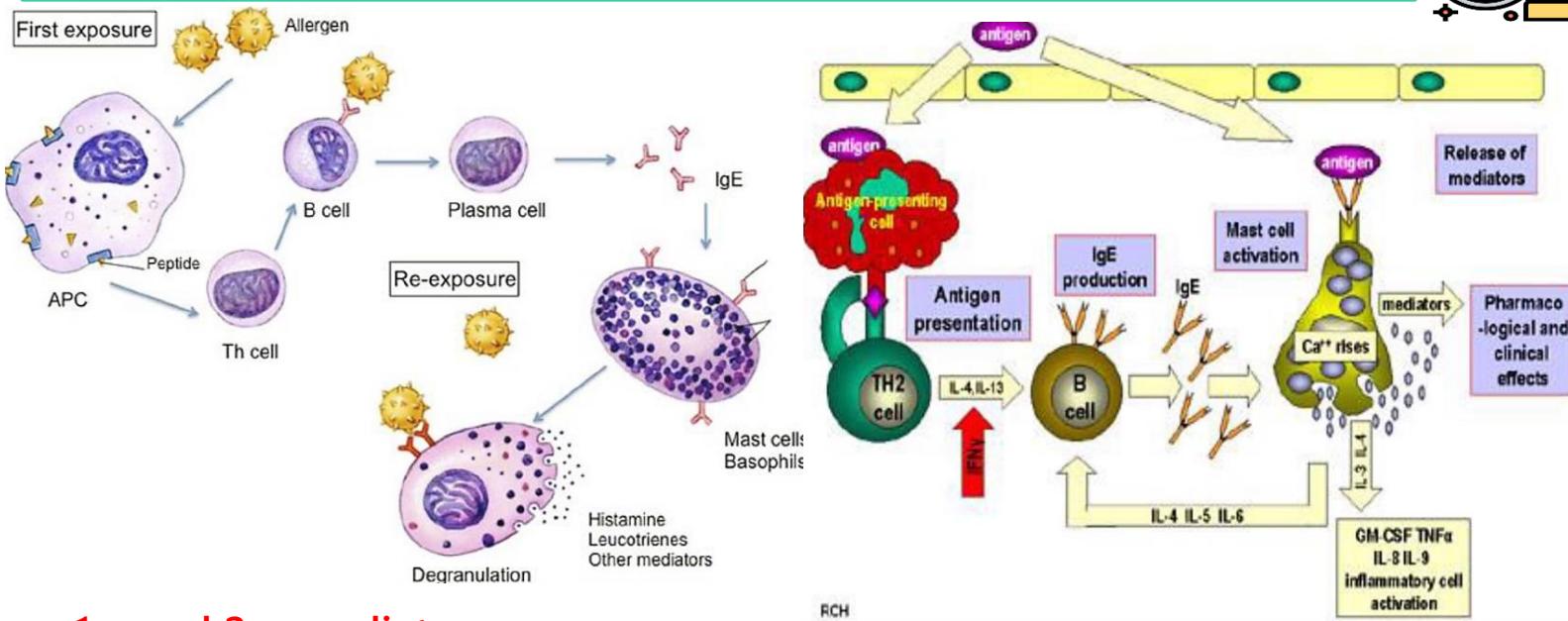
- **Injection** as drugs (penicillin's).
- **Inhalation** e.g. dust, fumes, pollens.
- **Skin contact**. e.g. wool, animal fur, nylon.
- **Ingestion** e.g. milk, wheat, egg, fish, strawberries, chocolate

**Reagin**: IgE antibody which is produced in response to a specific allergen

## Mechanism:

**First** exposure to certain antigens (allergens), stimulates production of IgE.

- ❖ IgE has very high affinity for its receptor on mast cells and basophils.



## 1ry and 2ry mediators

### Preformed mediators:- early manifestations:

- Histamine: Causes smooth muscle contraction and increased capillary permeability.
- Serotonin: vascular permeability, smooth muscle contraction.
- Chemo-attractants for Neutrophils and Eosinophils.

### Secondary mediators:- late manifestations:

- Leukotrienes: vascular permeability, smooth muscle contraction.
- Prostaglandins: vasodilatation, smooth muscle contraction, platelet activation.
- Th2 cytokines: IL-4, IL-5, IL-13. numerous effects including activation of vascular endothelium, eosinophils recruitment and activation.

## Examples of Type I Hypersensitivity

### Systemic (Anaphylactic shock):

It occurred in certain conditions after parenteral injection of a foreign antigen.

The anaphylactic shock is usually fatal.

### Localized (Atopy):-

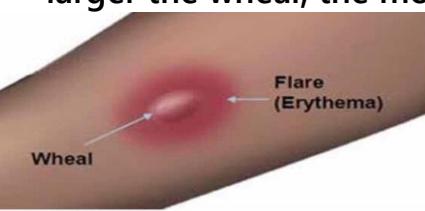
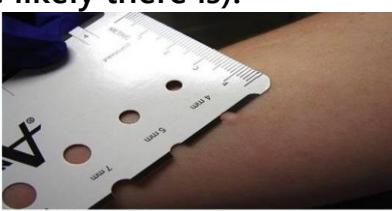
- The manifestations depend on the site of antigen antibody reaction.
- If it occurs in the bronchial tree, this will lead to **bronchial asthma**.
- If in the nose it will cause **rhinitis or hay fever**.
- If in the gastrointestinal tract it will cause **diarrhea and vomiting**.
- If it occurs in the skin it will result in **urticaria and skin rash**.
- If in the eye it will cause **conjunctivitis**.



## Diagnosis:

1. **Skin test:** These are done by intradermal injection of different groups of allergens.
2. Determination of **total serum IgE** level which is usually high in atopic individuals.
3. Determination of **specific IgE** levels to the different allergens
4. **Provocation test:** may be used, by challenging the patient with the allergen, in question, intranasal or otherwise.

## Procedure of skin prick test

Site	<ul style="list-style-type: none"> <li>▪ Usually on the <b>forearm</b> (In younger children on the thigh or sometimes on the back).</li> <li>▪ Placing a small amount of <b>allergen</b> on the forearm.</li> <li>▪ Followed by a gentle scratch using sterile <b>lancets</b> (These devices make small punctures in the skin).</li> <li>▪ Wait for <b>10-15 minutes</b>.</li> </ul> <div style="display: flex; justify-content: space-around; align-items: center;">    </div>
Results	<ul style="list-style-type: none"> <li>▪ If there is reaction (<b>a small, itchy spot</b>) which can indicate an allergy.</li> <li>▪ A <b>ruler</b> is used to measure the size of the resulting wheal (The presence of a reaction doesn't mean there is definitely an allergy to the substance but the larger the wheal, the more likely there is).</li> </ul> <div style="display: flex; justify-content: space-around; align-items: center;">   </div>
BUT this test contraindicated:	<ul style="list-style-type: none"> <li>❖ person had a <b>severe allergic reaction</b>. (as may be sensitive to certain substances that even the tiny amounts used in skin tests could trigger a life-threatening reaction (anaphylaxis)).</li> <li>❖ Take <b>medications</b> that could interfere with test results. These include antihistamines, many antidepressants and some heartburn medications.</li> <li>❖ Person with certain <b>skin conditions</b>. If severe eczema or psoriasis affects large areas of skin on arms and back.</li> </ul>



### Treatment:

1. Avoidance of specific allergen responsible for the condition.
2. **Desensitization:** weekly administration of the Ag to which the person is hypersensitive. This stimulates the production of IgG blocking antibodies in serum which can prevent subsequent Ag from reaching IgE on the mast cells, thus preventing the reaction.
3. Drugs are used for treatment e.g. corticosteroids & antihistamines

### Case for discussion

A 45-year male was taken to the local emergency department with anaphylactic shock. He was working on his house when he was attacked by bees. He was stung twice and subsequently experienced generalized body hives and decrease in systolic blood pressure to the 80s per the emergency medical system responders. The patient denied shortness of breath, nausea or vomiting or tongue swelling on admission. There was no prior exposure or allergic reaction.

- a. What is the most likely diagnosis?
- b. The type of antibodies is-----
- c. What the treatment of this case?



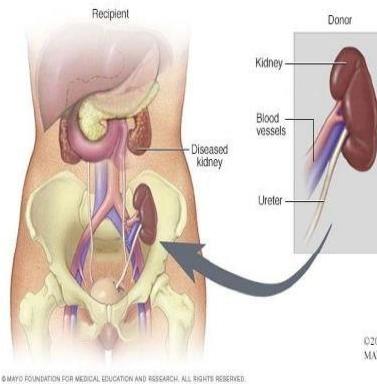
خليٰك واثق من نفسك وكمـل !



## CBL3 micro: Graft and graft rejection

### Case study:

A 55-year-old with a history of diabetic nephropathy and chronic kidney failure, who recently underwent a successful kidney transplant and is currently taking tacrolimus, presents with a 3-day fever. Physical examination reveals tenderness at the graft site. Lab results show a previous serum creatinine level of 1.1 mg/dL, a current temperature of 38.5°C (101.3°F), pulse rate of 88/minute, and blood pressure of 140/90 mmHg. The patient is referred for a biopsy of the transplanted kidney.



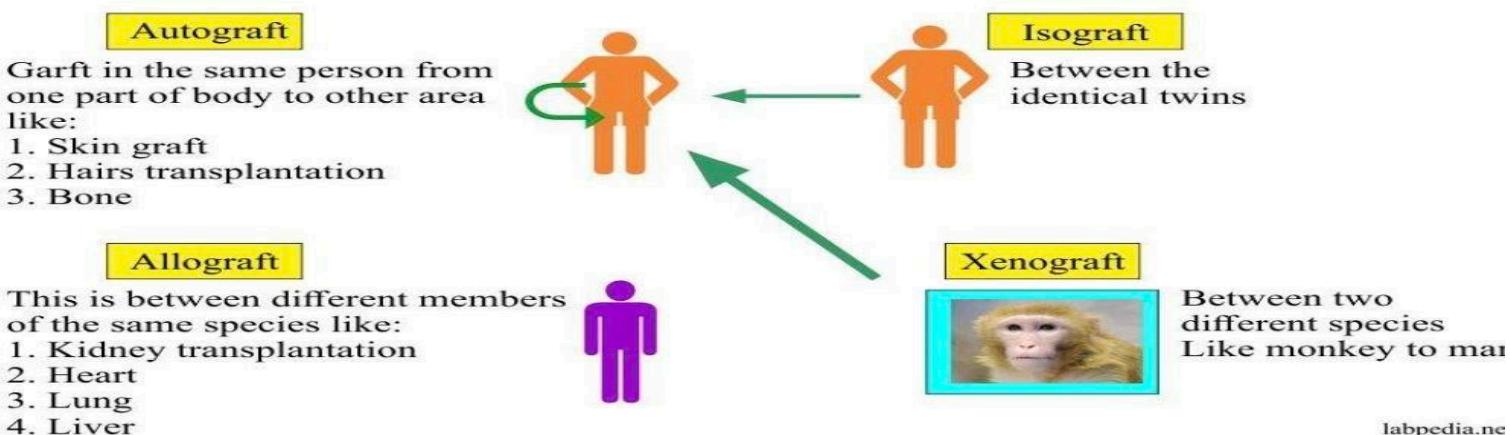
What is the most likely finding on histological examination of the biopsy?  
Can you figure it out?

Before answering remember .....

#### ➤ Facts

- The transplantation of tissues to replace diseased organs is now an important medical therapy.
- The grafting of organs or tissues from one individual to another is called **transplantation**.
- Tissue and organ grafts between **genetically distinct** individuals almost always elicit an adaptive immune response that causes **graft rejection**, the destruction of the grafted tissue by attacking lymphocytes • So immune responses are a major barrier to effective tissue transplantation.
- The **major antigens** that cause graft rejection are:
  - The blood group antigens (ABO) and these are genetically determined antigens, found on the surface of red cells as well as other tissues.
  - The HLA or MHC antigens

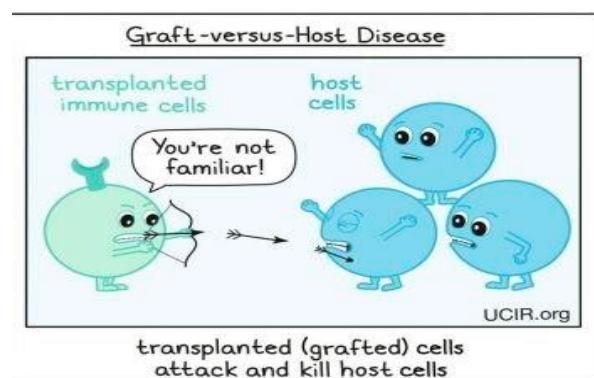
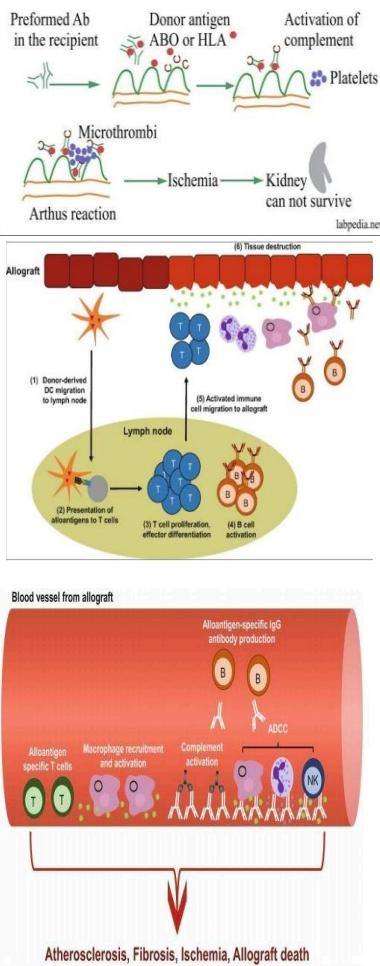
### Types of graft:





## Types of graft rejection:

- 1) **Hyper acute rejection** : This type of rejection occurs **immediately within minutes or hours** after transplantation is conducted . In these cases, **recipients already** have antibodies to donor antigens, which are **often blood group antigens** (blood group incompatibility). Hyperacute rejection can be overcome by transplanting organ to recipient **having the same blood type** as the blood type of the donor .
- 2) **Acute rejection** : Acute rejection may occurs **within days to weeks** of transplantation, due to inadequate immunosuppression therapy . it is caused by both cellular and humoral immune mechanisms . Allograft destruction results from the infiltration of activated immune cells, including T cells , monocytes/macrophages, neutrophils and antibody - producing B cells
- 3) **Chronic rejection** : It occurs after extended period (months or years) and is characterized by gradual loss of function of the graft . It may be due to a cellular immune response, antibody response or a combination of the two . T cells recognizing alloantigens can have direct cytotoxic effects on vessels as well as recruit macrophages and neutrophils . Antibodies produced by B cells promote vascular injury through complement activation(cause cell lysis) and ADCC (antibody dependent cellular cytotoxicity) . Chronic inflammation over the course of years leads to vascular smooth muscle cell proliferation, extracellular matrix deposition, fibrosis, atherosclerosis, organ ischemia and ultimately death of the allograft .
- 4) **Graft versus host rejection** : (The graft reacts against the body) . It is a major feature of bone marrow transplantation . 3 conditions should be fulfilled for this reaction to occur : 1. The host possesses antigen that the graft lacks . 2. The graft contains immunologically competent cells (lymphocytes ) . 3. The host is immunologically incompetent . It occurs when donor -derived T cells and B cells attack tissues in the recipient including the skin, liver, or intestine (presented by skin rash, hepatotoxicity and severe diarrhea.





## Advances in organ transplantation:

- Three major advances have made it possible to use organ transplantation routinely in the clinic:
  - The technical skill to carry out organ replacement surgery has been mastered by many people.
  - Networks of transplantation centers have been organized to ensure that the few healthy organs that are available are HLA typed and so matched with the most suitable recipient.
  - The use of powerful immunosuppressive drugs, as cyclosporin A and FK-506, known as tacrolimus, to inhibit T-cell activation that has increased graft survival rates dramatically.

## Steps towards prevention of graft rejection:

- Proper choice of donors
- Postoperative immunosuppressive therapy
- Antigen specific immunosuppression

### Proper choice of donor:

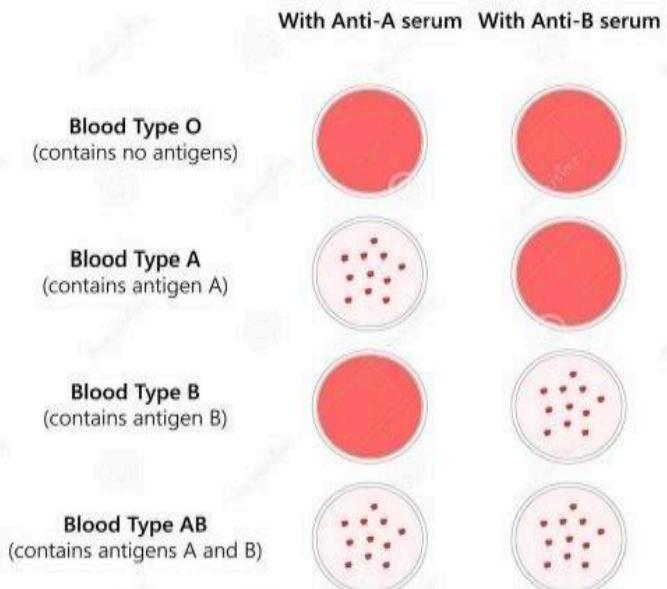
#### 1- ABO blood grouping:

#### The ABO antigens

	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies in plasma	Anti-B	Anti-A	None	Anti-A and Anti-B
Antigens in red blood cell	A antigen	B antigen	A and B antigens	None

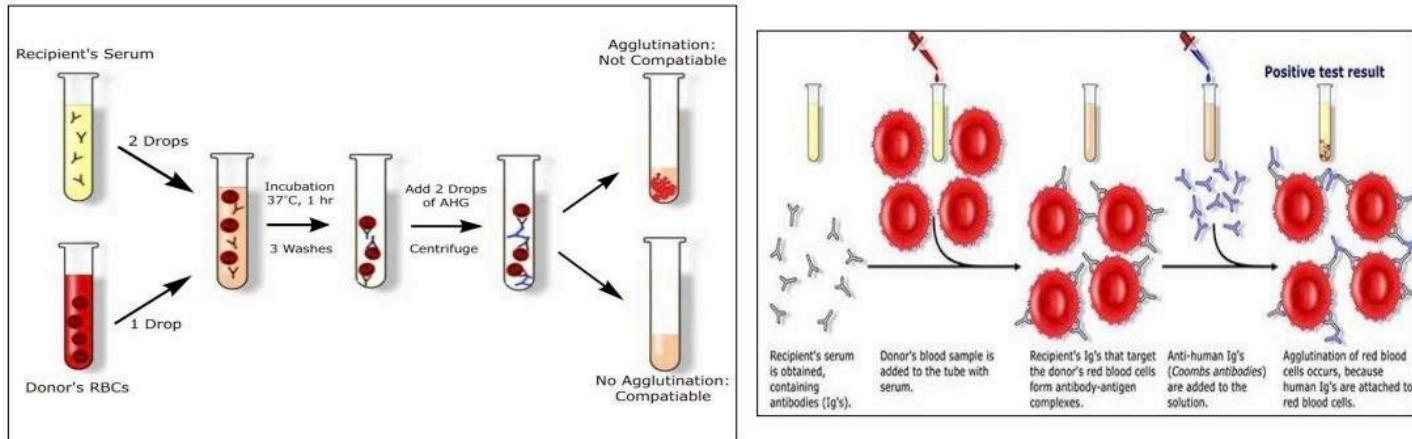
#### The test procedure

#### ABO Blood Group Test





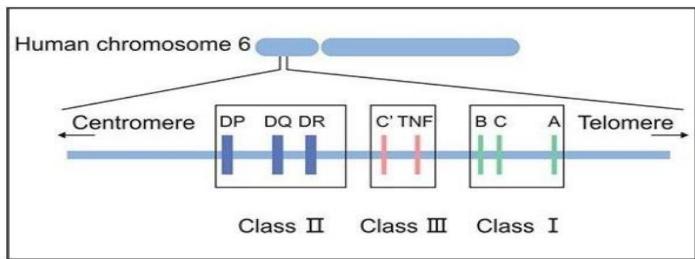
- 2- **Cross matching:** is performed prior to administration of blood or blood products (e.g. packed red blood cells). The purpose of the crossmatch is to test the recipient's serum for the presence of **preformed antibodies** against the donor's antigens.



- 3- **Tissue typing:** • Tissue typing ensures that an organ from a donor will be compatible with its recipient. The process starts with identifying the unique human leukocyte antigens (HLAs) for the organ donor and recipient, either from blood or tissue.

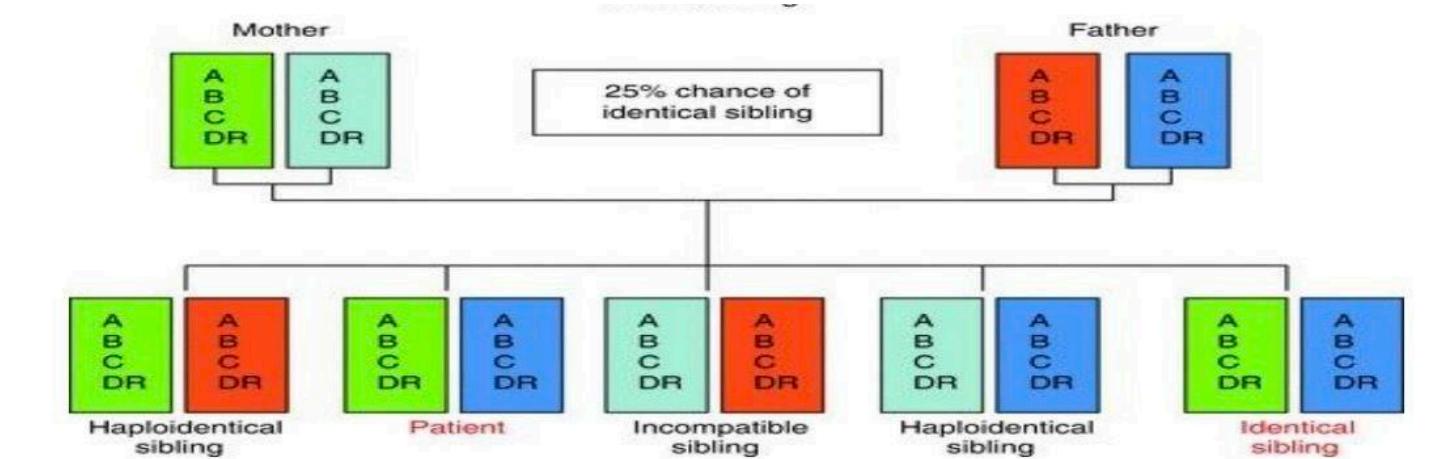
## What is HLA ?

These antigens are known as **human leucocyte antigens HLA** (or histocompatibility antigens) and are genetically determined by a set of genes called the **major histocompatibility gene complex (MHC)**.



## What is HLA genes?

- The MHC is the most polymorphic gene cluster in the human genome, having large numbers of alleles at several different loci.
- Transplantation within families significantly reduce alleles mismatch because the inheritance pattern of HLA genes





## Tissue typing: done by

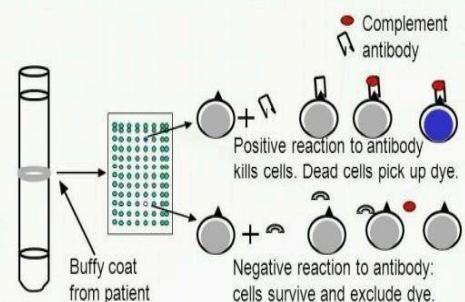
- Serologically
- Mixed lymphocyte reaction
- Molecular typing

### a - serological Tissue typing: (lymph cytotoxicity test):

Each of donor Lymphocytes and recipient Lymphocytes are reacted with the different antisera in the presence of complement . Lysis of lymphocytes indicates that they contain the antigen . Hence similarity of donor and recipient transplantation antigens is indicated by similarity in the specific antisera with which the 2 sets of cells react (or do not react) .

## Serological Typing

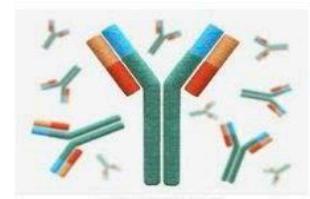
Lymphocytes are **HLA-typed** by crossmatching to panel reactive antibodies (**PRA**) using the complement-dependent cytotoxicity (**CDC**) test.



### Serologically lymphocytotoxicity tests

N.B.: The antisera (panel reactive AB) may be obtained from:

1. Women with multiple births
2. Multi -transfused patients
3. Transplant recipients.
4. Monoclonal antibodies prepared to the different HLA antigens.



### b - Mixed lymphocyte reaction :

MLR can be used Quantify the degree of MHC class 2 compatibility between potential Donor and Recipient.

#### PROCEDURE

Lymphocytes from the donor are irradiated or treated with Mitomycin C (to prevent cell division)

The irradiated Donor cells are added to the cell fro Recipient

If the Class II MHC antigens on the two cell population are same; then there is no reaction with donor and recipient, indicating that donor graft can be transplanted to the recipient

If the class II MHC Antigen on two cell population are different, the recipient cells rapidly.

Proliferation of Recipient T cell indicates T cell activation

Which can be measured by the uptake of [<sup>3</sup>H] Thymidine (Radioactive Nucleotides) into newly synthesized nuclear DNA

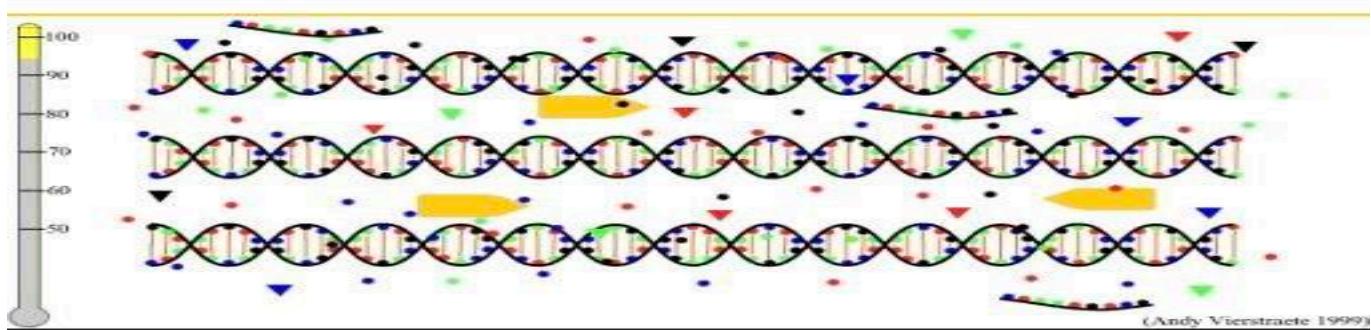
The amount of Radioactive is proportionate to the MHC class II differences between the Donor and Recipient Lymphocytes.



## c - Molecular typing:

- These methods are based on amplification of DNA segments on chromosome 6 carrying the genes for HLA class I or II by PCR .
- The amplified product may be further identified by DNA probes or by sequencing where the sequences are compared in donor and recipient .
- The higher the degree of matching between donor and recipient, the better the chance of survival of the graft . The best results (nearly 100 % ) are between identical twins .

### Molecular typing



**Now can you find the answer?**

**The answer.....**

1. This patient has fever, graft site tenderness, and abnormal kidney function test one month after kidney transplantation. This presentation is concerning for **acute graft rejection**.
2. Acute rejection develops due to recognition of the graft antigens by the immune system (**cellular and the humoral immune responses**).
3. Acute rejection develops from **one week to three months** after transplantation but still can be decreased immensely by using immunosuppressant drugs.
4. There is typically **decreased function** of the transplanted organ; as in our example, the patient develop anuria, metabolic abnormalities (e.g. hyperkalemia), and elevated serum creatinine, as seen in this patient.
5. In **biopsy**, acute graft rejection is characterized by mononuclear lymphocytic infiltrate in the interstitium of the graft tissue along with necrosis of the arterial walls (vasculitis).