

# **Antigen and it's Types**

**Dr. Sandeep Sharma, BVSc & AH, MVSc and PhD  
Assistant Professor, PGIVER  
[drsharmask01@hotmail.com](mailto:drsharmask01@hotmail.com)  
Mob. 9414775879**

# Introduction

- ❖ The term **antigen** is Greek word means *anti-against & gen- to generate.*
- ❖ The **foreign materials, organisms, self and nonself substances** that elicit an immune response and react with the products (immunoglobulin receptor of B cells, or by the T-cell receptor when complexed with MHC) of that immune response are called **antigens**.



# Introduction

- ❖ Antigens include molecules such as proteins, nucleoproteins, polysaccharides, and some glycolipids.
- ❖ The ability of a substance to react with the specific antibodies or activated T cells that it induces is called *antigenicity*.



# Factors affecting antigenicity

- ❖ Foreignness
- ❖ Size (Molecular weight)
- ❖ Chemical complexity
- ❖ Solubility (biodegradability)
- ❖ Structural stability
- ❖ Genotype of the recipient animal
- ❖ Immunogen dosage and route of administration



# ANTIGENIC DETERMINANTS (EPITOPES)

- The antigen combining sites of antibodies or T cells are smaller than whole antigen and hence it is impossible for a whole antigen to bind entirely with antibodies or T cells.
- The antibodies or T cells bind with certain surface structures found on the antigen.
- These surface structures are referred as **antigenic determinants or epitopes**



- These epitopes have different shapes and even a simple microbe may have thousands of such epitopes.
- The immune response against all epitopes is not same.
- Certain **epitopes elicit large amount of antibody response**. Such epitopes are called as immuno-dominant epitopes.
- Over the surface of an antigen a particular epitope may be present more than once and
- If an antigen **contain only one type of epitopes it is called as monovalent** and if it contains more than one type of epitopes it is called as **polyvalent**.

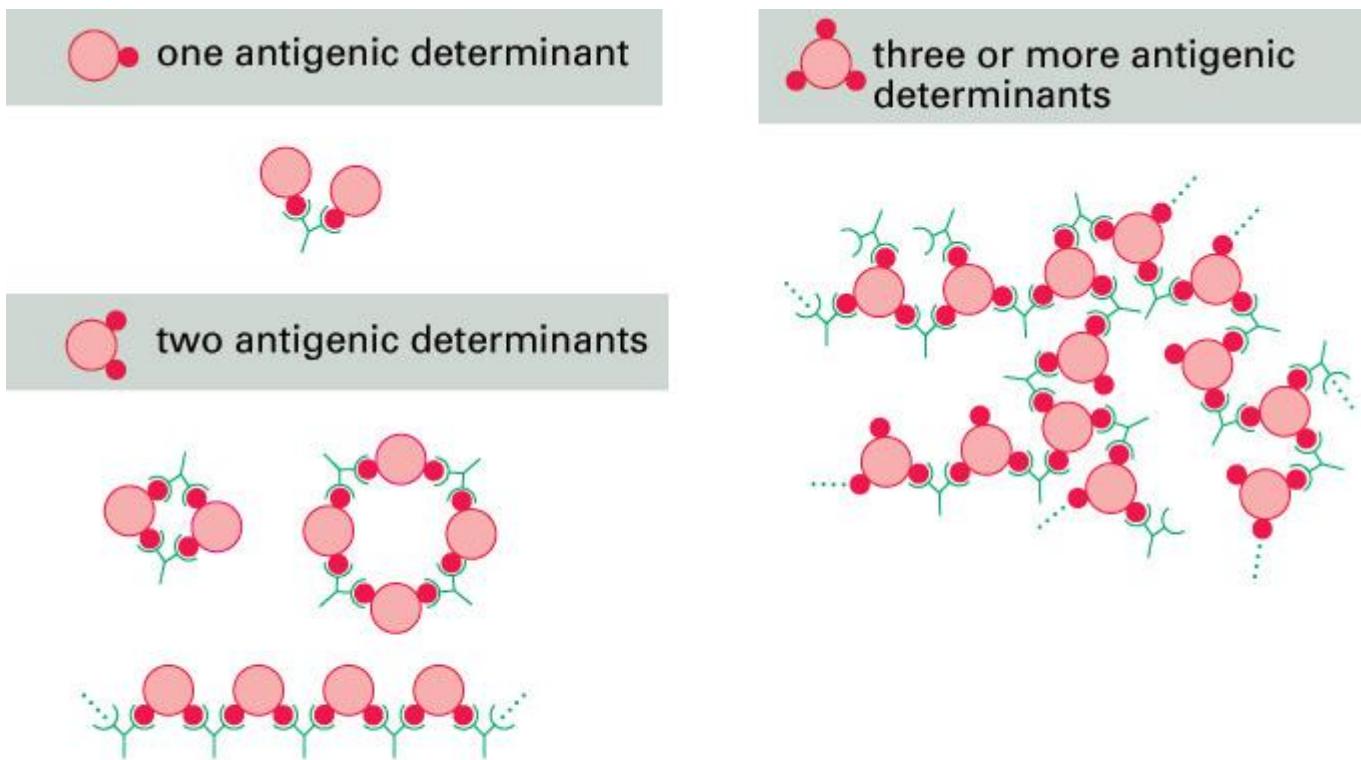


Figure 24–19. Molecular Biology of the Cell, 4th Edition.

- In certain antigens the epitopes are arranged spatially so that binding of an antibody to one may not interfere with binding of another antibody to different epitope. Such epitopes are referred as **non-overlapping epitopes**.
- Whereas in some antigen one epitope will interfere with binding of antibody to another epitope and such epitopes are called as **overlapping epitopes**.
- Some times binding of an antibody to an epitope will result in conformational changes that may in turn help in binding of another antibody to different epitope. This is called **allosteric effect**.

- The epitopes can be further classified into **linear epitopes**, **conformational epitopes** and **neo antigenic epitopes** depending upon the arrangement and post synthesis modifications of the building blocks.
- Multivalent antigens generally elicit a stronger immune response than do monovalent antigens.
- Antibody **affinity** known to be strength of binding between a single binding site of a molecule(antibody) and a ligand (of antigen) at a given antigen-binding site.
- The **avidity** of an antibody relates to its overall ability to bind antigen at all antigen-binding sites.

# TYPES OF ANTIGEN

## 1. Functional Classification

- Complete antigen:

Individually able to induce immune system. Produces a specific and observable immune response

Ex. Bacterial antigens *i.e.* **somatic antigen (O)**, **Capsular antigen (K)**, **flagella antigen (H)**

- Incomplete antigen:

Substances which individually not able to induce immune response by themselves but if joined with carrier (adjuvant) can provoke the immune system.

Ex. Haptens



# HAPTENS

- The concept of haptens emerged from the work of **Karl Landsteiner**, who pioneered to use synthetic haptens to study immunochemical phenomena.
- Origin from *Greek word Haptein meaning to Grasp or fasten*
- Haptens are small molecules (less than 1000 daltons) that cannot elicit immune response individually but they become immunogenic when coupled with larger molecules.
- These larger molecules that make the haptens immunogenic are called as **carrier molecules**.
- Haptens can react with pre formed antibodies. In other words haptens are antigenic but not immunogenic.



- Haptens are classified according to the number of antigenic determinants they have. If a hapten has **only epitope** it is classified as **simple hapten** and if it has got **two or more different epitopes** it is classified as **complex hapten**.
- Example:
  1. Penicillin ..... break in to penicilloyl..... when bind with serum protein such as albumin
  2. Urushiol protein from Ivy plant touches with any body protein such as skin protein

# CLASSIFICATION BASED ON ORIGIN OF ANTIGENS

## 1. Exogenous Ags:

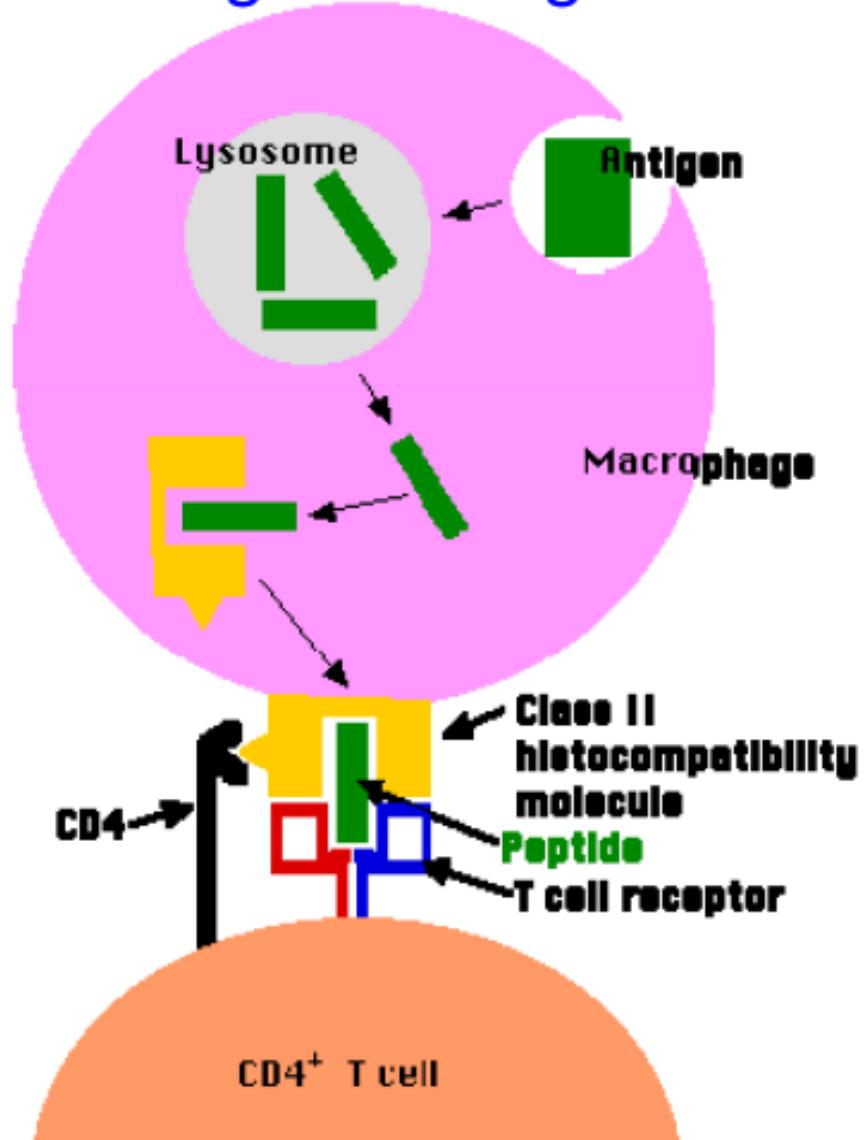
- Origin from outside of host
- Enter in the body by inhalation, ingestion or injection.
- Taken by the APCs and degraded into small peptides.
- APCs then present them to helper T cells by using **MHC type II** molecules.

## 2. Endogenous Ags:

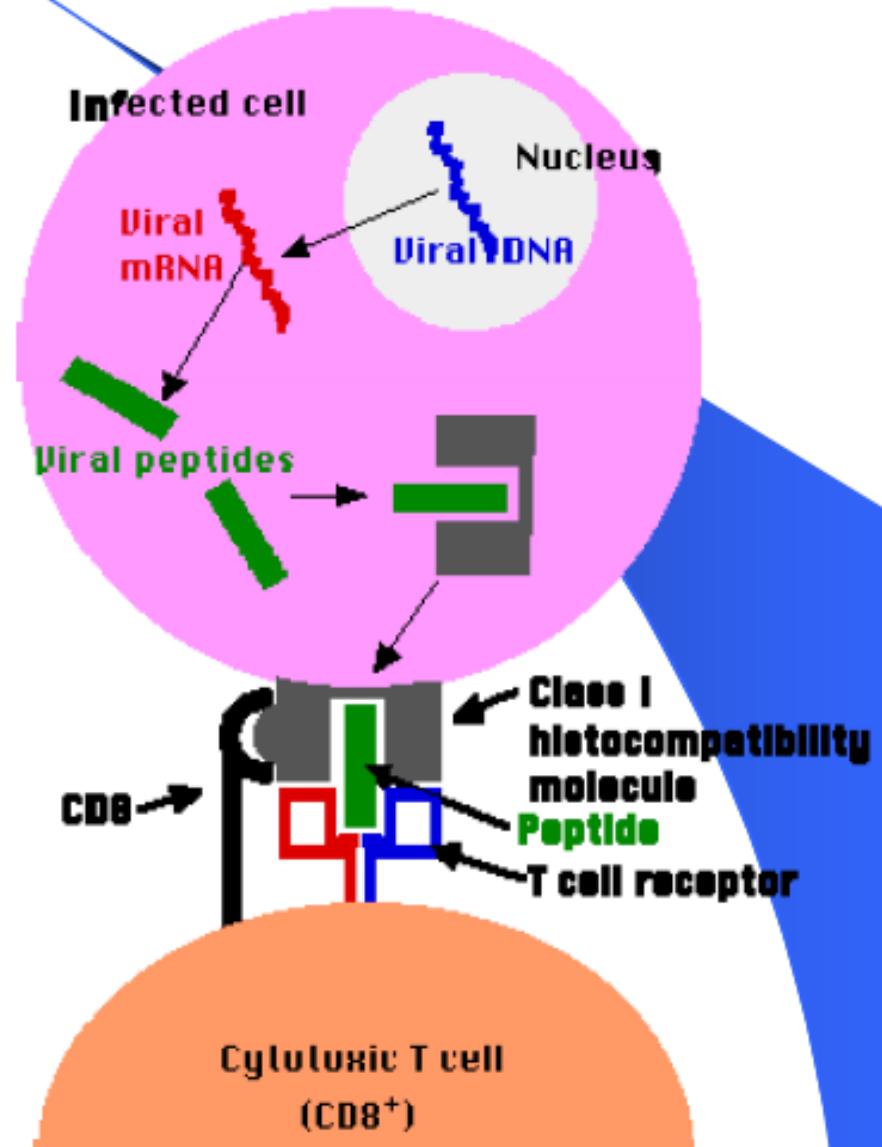
- Generated within the cell as a result of normal cell metabolism, or because of viral or intracellular pathogenic infection.
- The fragments are presented along with **MHC type I** molecules to cytotoxic T cells.



## Exogenous Ags



## Endogenous Ags



# BIOLOGICAL CLASSES (MECHANISMS) OF ANTIGEN

- Depending on the ability to induce antibody formation/ T cell activation, Antigens are classified as:
  1. T cell dependent (TD) Ags
  2. T cell independent (TI) Ags
- 1. T cell dependent (TD) Ags
  - Ags that require T cells to generate an immune response
  - Structurally complex e.g. RBCs, S. proteins
  - Immunogenic over a wide dose range and do not cause tolerance.
  - **Produce immunological memory**
  - Requires processing by APCs.
  - Rapidly metabolised

# BIOLOGICAL CLASSES (MECHANISMS) OF ANTIGEN

## 2. T cell independent (TI) Ags

- Directly stimulate Ab production by B cells, without the participation of T cells.
- Structurally simple, being composed of a limited number of repeating epitopes. e.g. Pneumococcal capsular polysaccharide, bacterial LPS, flagellar protein
- Immune response is dose dependent.
- Too little - non immunogenic
- Too much – tolerance
- Do not produce immunological memory.
- Do not require processing by APCs.
- Remain in the body for long periods

# BASED ON SOURCE OF AN ANTIGEN

1. **Microbial antigens/ Infectious:** The microbial antigens could be classified into bacterial, viral and other microbial antigens.

a. ***Bacterial antigens:***

- Bacteria possess different types of antigen mainly based on its morphological features. Some of the common bacterial antigens are **somatic antigen (O)**, **Capsular antigen (K)**, **flagella antigen (H)** and **fimbrial or pili antigen**.
- Exotoxins are highly immunogenic and stimulate the production of antibodies. **The antibodies against exotoxins are called antitoxins.**
- When these exotoxins are precipitated by mild protein denaturing agents such as formaldehyde, the exotoxin loses its pathogenicity but retains its immunogenicity. Such **precipitated toxins are called as toxoids.**

# BASED ON SOURCE OF AN ANTIGEN

## b. *Viral antigens:*

- The proteins of the outer coat (capsid) of virus are good antigens. These capsid proteins elicit good antibody response when they are present in the circulation.
- Types of virus proteins that are produced inside the cell are called **endogenous antigens**.

## c. *Other microbial antigens:*

- The other micro and macro parasites like fungi, protozoa and worms also are composed of different types of antigens composed of major building blocks like lipids, carbohydrates and proteins.
- They also elicit immune response in the body.



# BASED ON SOURCE OF AN ANTIGEN

2. **Non-microbial Antigens/ Non infectious:** The non-microbial antigens could be classified into cell surface antigens, auto (self) antigens, Non-self antigens and miscellaneous.

## a. **Cell surface antigens:**

- The surface of most of the cells is covered with different antigens. When these antigens are given to heterogenous host an immune response is mounted.
- Some of the important **cell surface antigens** are **blood group antigens**, and CD receptors of the leukocytes (The abbreviation CD stands for **cluster of differentiation**. There are more than 130 CD receptors.)



### b. Autoantigens:

- Belong to the host itself - not immunogenic.
- Hosts do not react to their own antigens by exhibiting a mechanism called immunological tolerance.
- Such substances are called as **autoantigens** and such immune response is called as **autoimmunity**.
- Some of the normal components of body against which immune response is elicited are basement membrane, myelin, mitochondrial proteins, nuclear proteins, hormones etc.
- Sometimes, the self-antigens are biologically altered (e.g. lens protein, tumor cells) and can become immunogenic.

# BASED ON SOURCE OF AN ANTIGEN

## c. Non-self or foreign antigens

- These are immunogenic
- Three different types based on their phylogenetic distance to host.
  - i. Xenoantigen – foreign Ag, from **different species** e.g. bacteria, viruses
  - ii. Alloantigen – **different individual from same species** e.g. blood group Ag
  - iii. Heterophile antigen – Common/ related Ags shared by different species e.g. M protein of *Streptococcus* spp. bears common antigen determinant with basement membrane of kidney. They share epitopes with each other.



**d. Miscellaneous antigens:**

- Non-microbial antigens like dust particle, certain type of food particle, pollen grains, venom etc. can also elicit immune response in certain individuals.
- These substances produce antibodies on entering the body.

Example

- Forssmann antigen
- Superantigens



# FORSSMANN ANTIGEN

- Forssman antigen was first described by J. Forssman in 1911 as a universal heterophile antigen.
- He immunized rabbits with homogenates of guinea pig organs and detected an antibody that hemolyzed sheep erythrocytes.
- Several groups proposed that Forssman antigen is not protein, but lipid
- It is a lipid carbohydrate complex present in all animals (except rat, rabbits, cattle and pig), plants and bacteria. Hence, anti-Forssmann antibody can be prepared in rabbits.



## **Diagnostic Applications of Heterophilic Antigen**

- **Weil-Felix test:**

Patients suffering from certain rickettsial diseases will produce antibodies that will react and agglutinate certain non-motile strains of Proteus (OXK, OX2, and OX19).

- **Paul-Bunnel test:**

Patients suffering from infectious mononucleosis due to Epstein-Barr virus (EBV) will produce antibodies that will react and agglutinate sheep erythrocytes.

- **Streptococcus MG agglutination test:**

Patients suffering from *Mycoplasma pneumoniae* develop heterophile antibodies to Streptococcus MG, which are titrated in a tube agglutination test.

# SUPERANTIGENS

- Superantigens are unconventional antigens which recognise immune receptors outside their usual recognition sites.
- These are unusual microbial toxins that can interact with a large number of different CD4+ T cells (up to 20% of T cells can be activated by a single type of superantigen).
- Most known superantigens are peptides of between 22 and 29kD that are resistant to proteases and heat inactivation and share common structural features.
- Superantigens can bind directly to MHC class II molecules and T-cell receptors on CD4+ T cells to cause T-cell activation without prior processing.



# SUPERANTIGENS

- Superantigen binding is predominantly controlled by the shape of the **TCR- $\beta$  variable region**, and superantigens typically bind to all T-cell receptors that derive from a single family of TCR- $\beta$  variable region gene segments (e.g., the **V $\beta$ 8**).

Example:

## Bacterial superantigen:

- ❖ Staphylococcal toxin- Toxic shock syndrome toxin-1(TSST-1);  
Exfoliative toxin; Enterotoxins
- ❖ Streptococcal toxin- Strptococcal pyrogenic exotoxin (SPE)-A and C
- ❖ Mycoplasma arthritidis mitogen-I

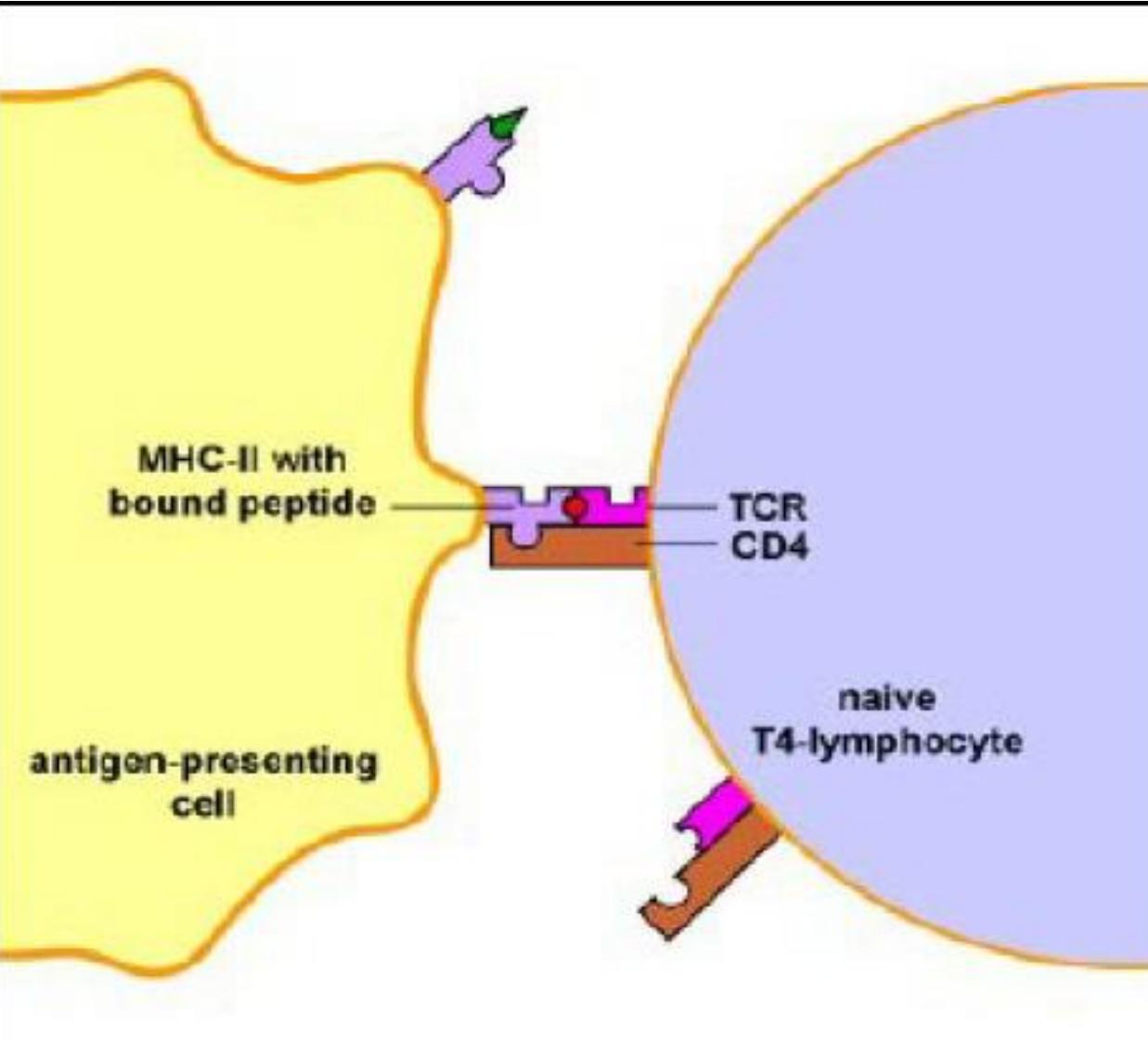


Fungal superantigen: *Malassezia furfur*

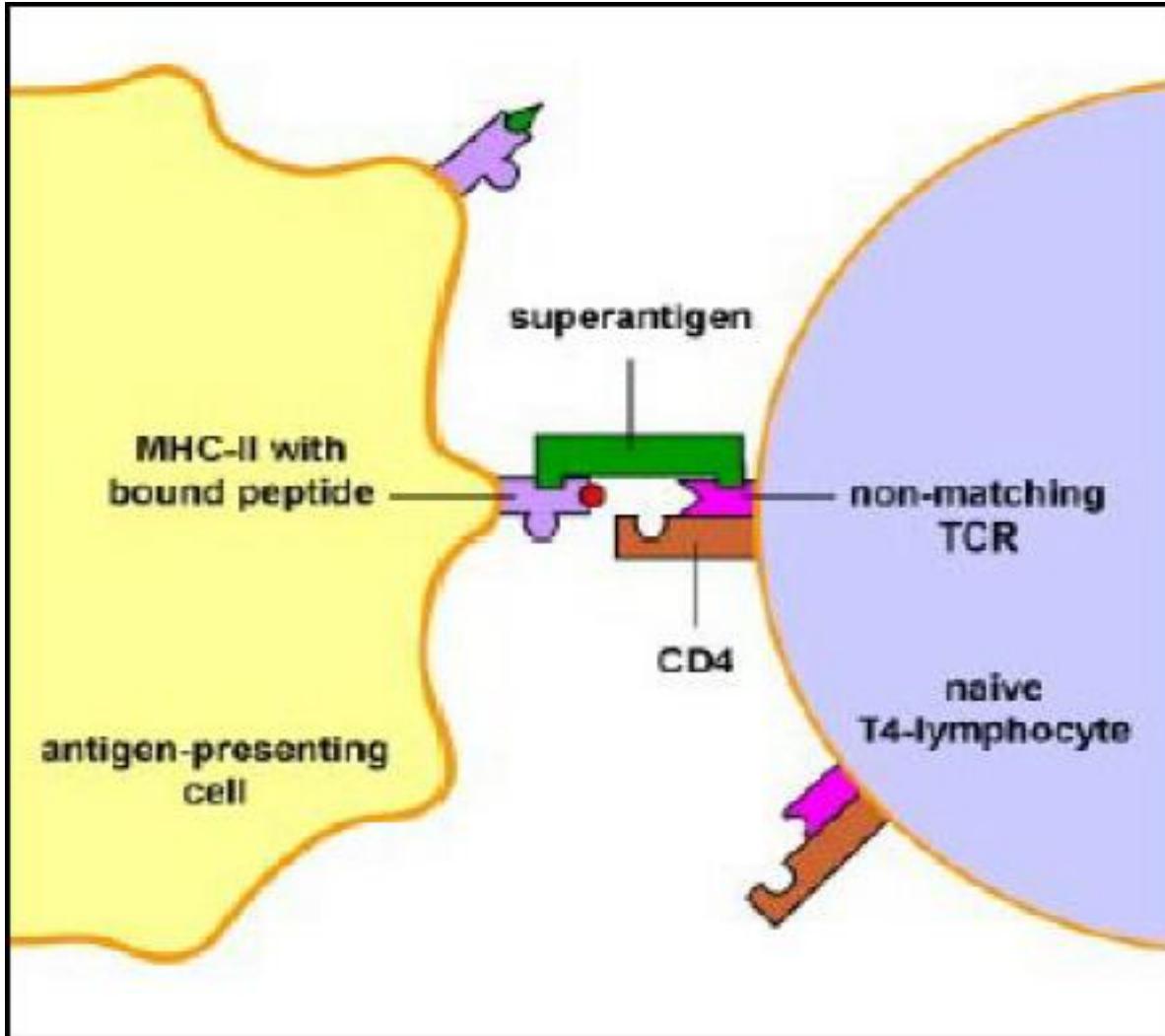
Viral superantigen:

- ❖ Epstein-Barr virus associated superantigen
- ❖ Cytomegalovirus associated superantigen
- ❖ Rabies nucleocapsid
- ❖ HIV encoded superantigen (nef- negative regulatory factor)





- ❖ Conventional antigens are only recognized by specific T4-lymphocytes having a specific TCR with a shape that corresponds to a peptide of that antigen processed and presented by an antigen presenting cell and bound to MHC-II molecules



- Super antigens bind directly to the outside of MHC-II molecules and the TCRs and activate many T4-lymphocytes.

A specific TCR is not required for activation.

# DISEASE ASSOCIATED WITH SUPERANTIGENS

- Toxic shock syndrome
- Food poisoning
- Scalded skin syndrome
- Rare conditions such as- Atopic dermatitis, Kawasaki syndrome, psoriasis, acute disseminated encephalomyelitis.



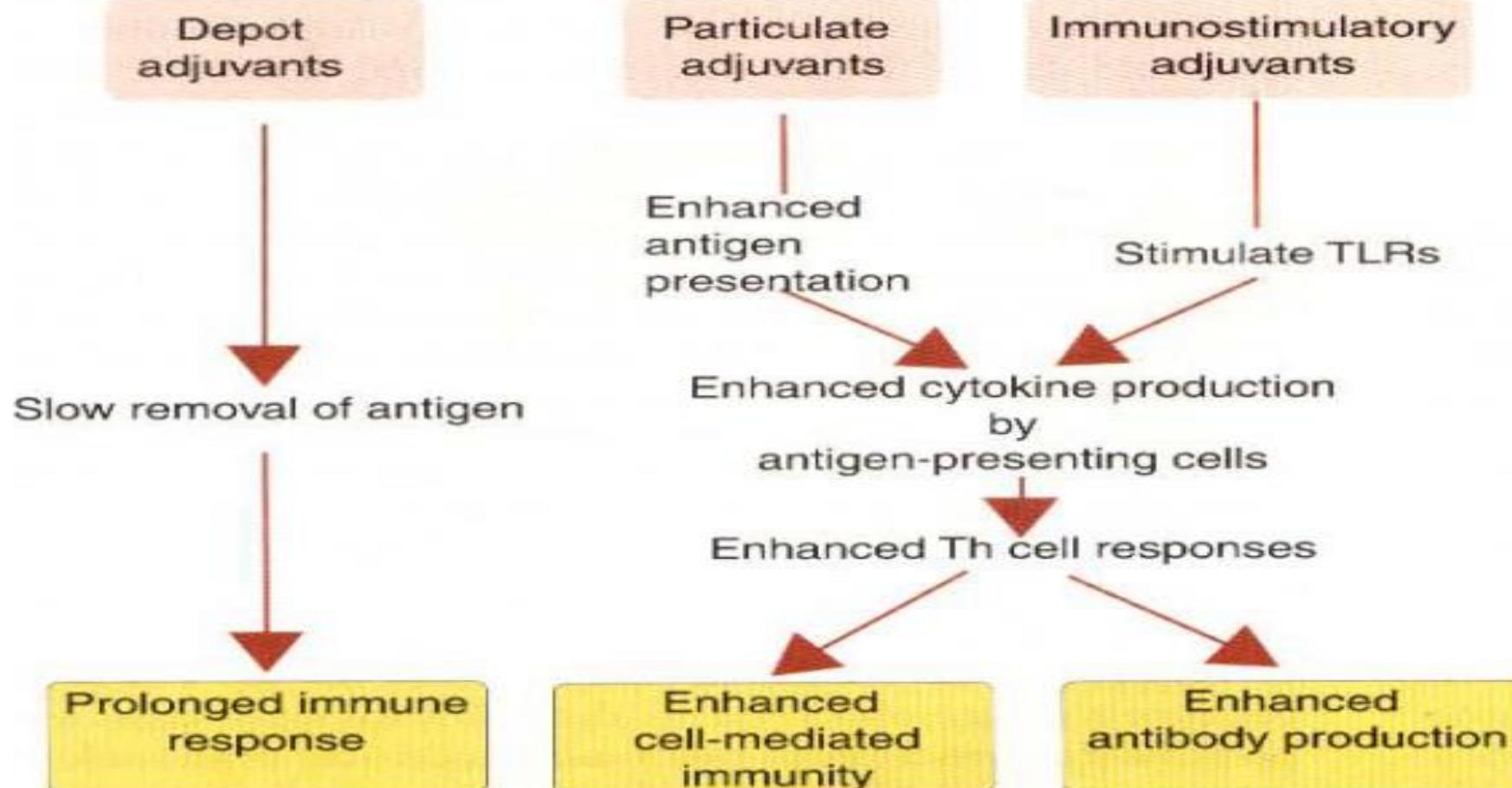
## CARRIER MOLECULES (ADJUVANTS)

- Adjuvants (origin from Latin *adjuvare*, to help) are substances that, when mixed with an antigen and injected with it, **enhance the immunogenicity of that antigen**.
- Adjuvants are often used to boost the immune response when an antigen has low immunogenicity or when only small amounts of an antigen are available.
- For example, the antibody response of mice to immunization with BSA can be increased fivefold or more if the BSA is administered with an adjuvant.



- Precisely how adjuvants augment the immune response is not entirely known, but they appear to exert one or more of the following effects.
  1. Antigen persistence is prolonged.
  2. Co-stimulatory signals are enhanced.
  3. Local inflammation is increased.
  4. The nonspecific proliferation of lymphocytes is stimulated.

# TYPES OF ADJUVANTS



# TYPES OF ADJUVANTS

Type	Adjuvant	Mode of Action
Depot adjuvants	Aluminum phosphate Aluminum hydroxide Alum Freund's incomplete adjuvant	Slow-release antigen depot Slow-release antigen depot
Immunostimulatory adjuvants	Anaerobic corynebacteria BCG Muramyl dipeptide <i>Bordetella pertussis</i> Lipopolysaccharide Saponin Lysolecithin Pluronic detergents Acemannan Glucans Dextran sulfate Liposomes ISCOMS Microparticles Freund's complete adjuvant	Macrophage stimulator Macrophage stimulator Macrophage stimulator Lymphocyte stimulator Macrophage stimulator Stimulates antigen processing Stimulates antigen processing Stimulates antigen processing Macrophage stimulator Macrophage stimulator Macrophage stimulator Stimulates antigen processing Stimulates antigen processing Stimulates antigen processing
Particulate adjuvants		
Mixed adjuvants		Water-in-oil emulsion plus <i>Mycobacterium</i>

## OTHER EXAMPLES

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



## FREUND'S ADJUVANT

- Water-in-oil adjuvants also prolong the persistence of antigen.
- A preparation known as **Freund's incomplete adjuvant** contains antigen in aqueous solution, mineral oil, and an emulsifying agent such as mannide monooleate, which disperses the oil into small droplets surrounding the antigen; the antigen is then released very slowly from the site of injection.
- This preparation is based on **Freund's complete adjuvant**, the first deliberately formulated highly effective adjuvant, developed by **Jules T. Freund** many years ago and containing heat-killed **Mycobacteria** as an additional ingredient.

- The immune response mediated by T- cytotoxic cells is called .....  
.....
- Write four intrinsic factors of antigen affecting immunogenicity
- Define the term active immunity
- Write a brief note on auto- immunity
- a. Define term immunity, b. Classification of immunity, c. briefly describe the mechanism of innate immunity
- ..... laboratory animal is considering as queen for production of hyper immune serum
- Which among the following is most antigenic  
1. Exotoxin                  2. Endotoxin                  3. viruses          4. none



- Heterophile antigen
- Adjuvants using in veterinary vaccines
- The smallest unit of an antigen molecule that is capable of binding with antibody or T- cell receptor is called .....
- Generation of immune response towards self- antigen lead to.....
- The antigen associated with bacterial flagella referred as
  - 1. K ag.    2. H ag.                      3. F ag              4. All
- Father of Immunology ???

- Define Immunogen and Haptens
- The antigenic determinant site of an antigen is called as.....
- Mode of action of adjuvants

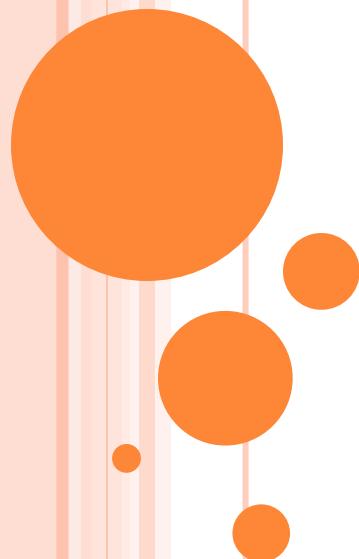


# **Thank You**

Dr. Sandeep Sharma, BVSc & AH, MVSc and PhD  
Assistant Professor, PGIVER  
[drsharmask01@hotmail.com](mailto:drsharmask01@hotmail.com)  
Mob. 9414775879



# CELLS OF IMMUNE SYSTEM



**Dr. Sandeep Sharma, BVSc & AH, MVSc and PhD  
Assistant Professor, PGIVER  
[drsharmask01@hotmail.com](mailto:drsharmask01@hotmail.com)  
Mob. 9414775879**

## INTRODUCTION

- The immune system is composed of different types of cells. These cells are generally found in the blood, lymph and local secretions.
- They are also scattered in various organs except the central nervous system.
- Few organs in the body are made up of a collection of these cells and such organs are called as **lymphoid organs**.
- The immune system is composed of two types of cells – lymphoid and non-lymphoid cells.



## INTRODUCTION

- The lymphoid cells are known as **lymphocytes** and there are three classes of lymphocytes – B-lymphocytes, T-lymphocytes and Natural Killer cells (NK cells).
- The **non-lymphoid** cells are also called as **accessory cells**. The important accessory cells of immune system are the mononuclear phagocytic cells (**Macrophage**) and polymorpho-nuclear cells (**PMNL**). The common PMNL are **Neutrophils** and **Eosinophils**.

# ORIGIN OF CELLS

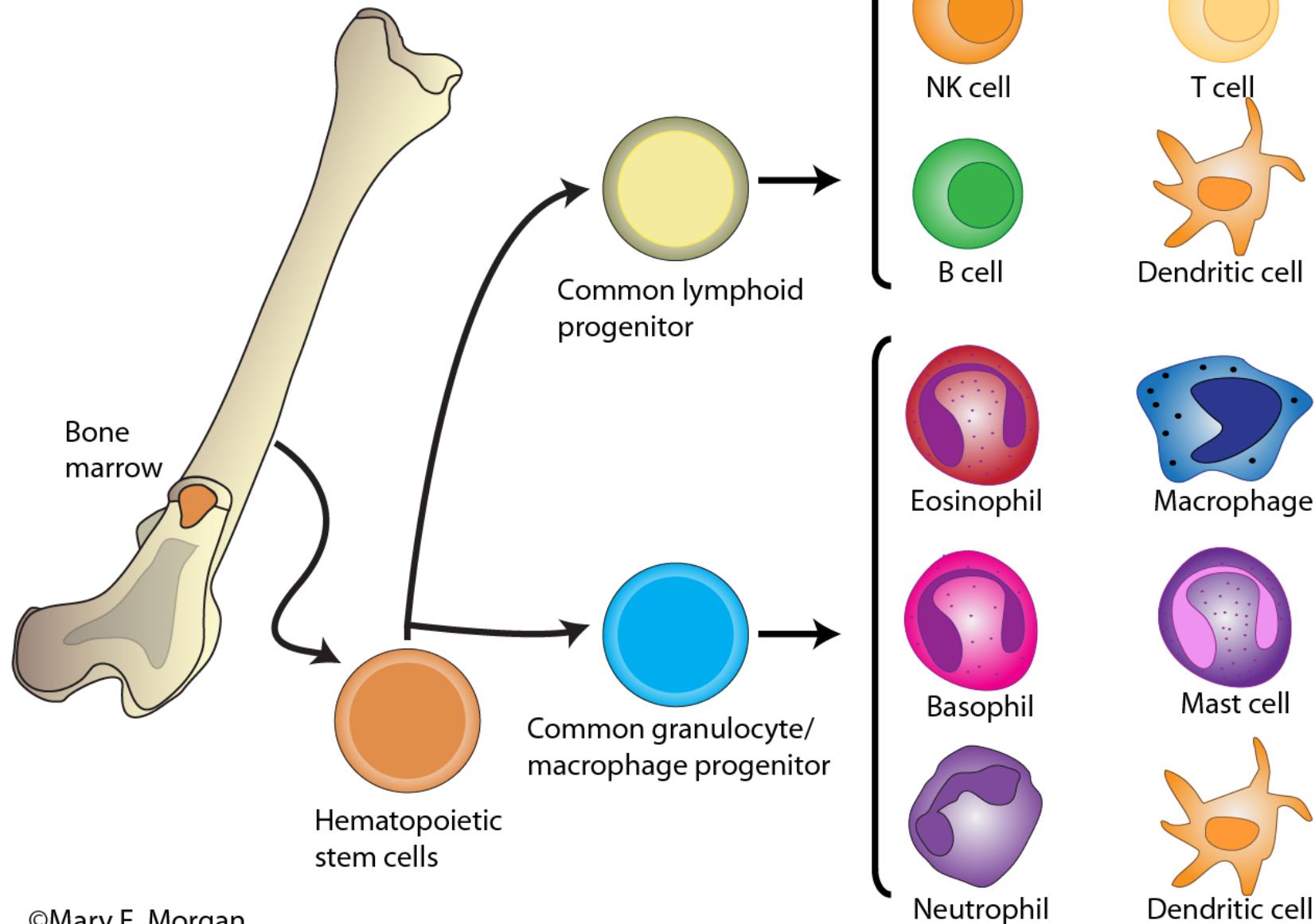
- All functionally specialized, mature blood cells (red blood cells, granulocytes, Macrophages, dendritic cells, lymphocytes) arise from a single cell type, **the hematopoietic stem cell (HSC)**.
- Early in the process, a multipotent stem cell differentiates along one of the two pathways, giving rise to either a common myeloid progenitor cell (CMP) or a common lymphoid progenitor cell (CLP).

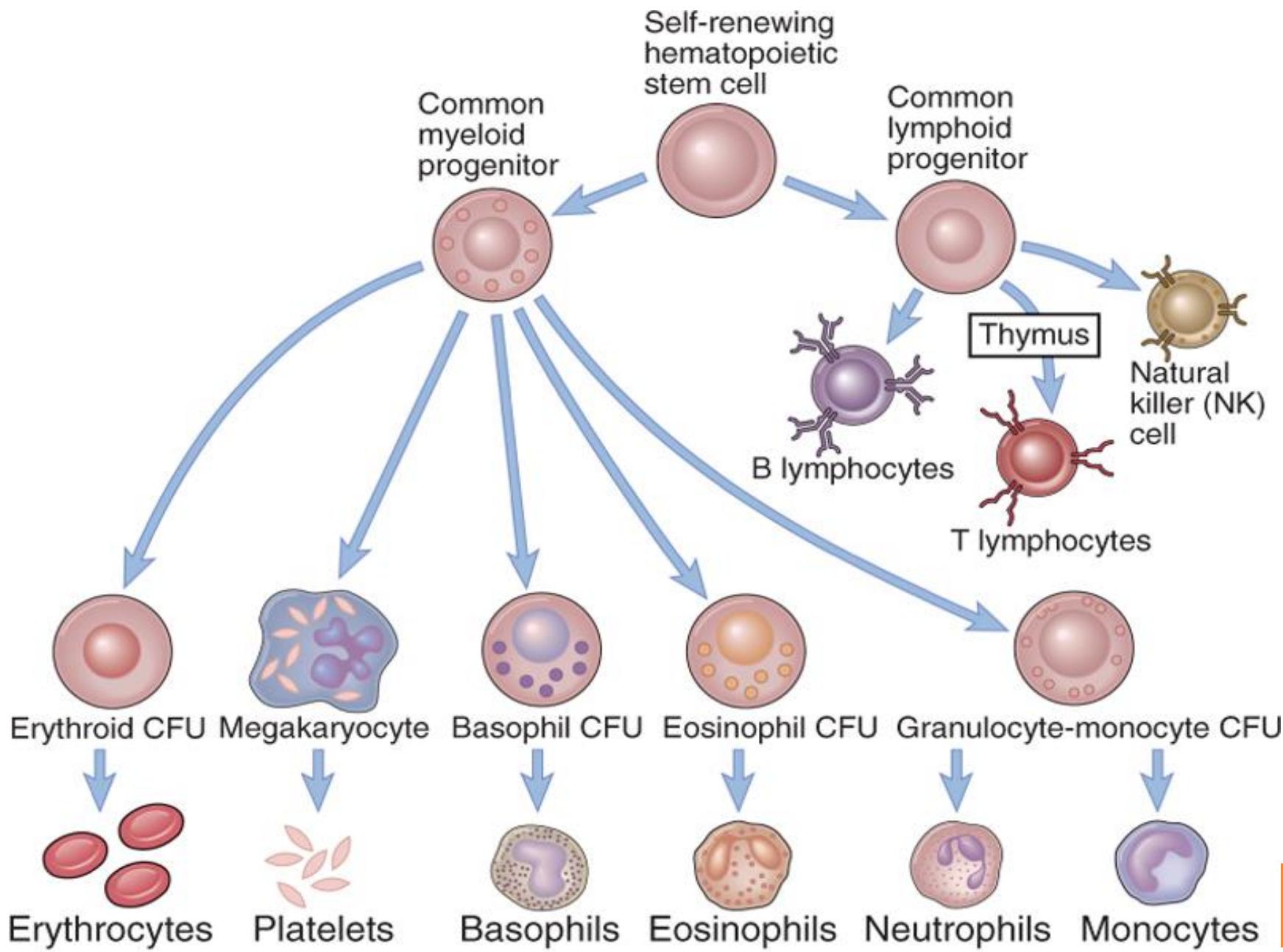
**1. Common myeloid-erythroid progenitor cells (CMP)**-which gives rise to all red blood cells (the erythroid lineage), granulocytes, monocytes, and macrophages (the myeloid lineage)

**2. Common lymphoid progenitor cells (CLP)**-which gives rise to B lymphocytes, T lymphocytes, and NK cells.



# Immune Cell Generation





## A. LYMPHOCYTES

- The lymphocytes are small, round cells found in blood and lymphoid organs such as thymus, lymph nodes and spleen.
- The diameter of each lymphocyte range from 7-15 $\mu$ m. They have a large and round nucleus that takes the haematoxylin intensely.
- They possess a thin rim of cytoplasm containing mitochondria, free ribosomes and a small Golgi apparatus.

- The surface of few lymphocytes are covered by small projections whereas, the surface is smooth for some lymphocytes.

## Classes of lymphocytes:-

- Though lymphocytes are morphologically same, there are distinct subsets that differ functionally and in their protein products.
- The three important classes are **B-lymphocytes, T-lymphocytes and NK cells.**



- ❑ The B-lymphocytes (B cells) are called thus, since they were first shown to mature in an organ in birds called bursa of Fabricius.
- ❑ However, in mammals they **mature in bone marrow**. B cells are the cells in immune system capable of producing antibodies.
- ❑ The T-lymphocytes (T cells) **arise from bone marrow and matures in thymus**.



- The T cells are divided into distinct functional groups called **helper T cells and cytotoxic T cells.**
- The main role of T cells is to regulate immune response against all protein antigens and to produce specific immunity against intracellular antigens.
- Besides these two sub classes of T cells there is also one more sub class called **T suppressor cells** that suppress the immune response.

- ❑ The origin and role of T suppressor cells are still controversial. The surface membrane proteins of T cells mainly identify the sub classes.
- ❑ The surface membrane proteins are also called as receptors or markers of lymphocytes. The receptors of lymphocytes are identified by CD nomenclature. The T helper cells are identified by presence of CD4 receptor (old name T4 receptor) and cytotoxic T cells are identified by presence of CD8 receptor.



- The third class of lymphocytes NK cells lack surface markers.
- These cells are also referred as **null cells**.
- They have a large cytoplasm filled with cytoplasmic granules.
- They lyse the virus infected and tumour cells without any antigenic stimulation.



- ❑ From the lymphoid stem cell pre B and Pre T cell arise. The pre B cells develop into B cells and pre T cells develop into T cells.
- ❑ Upon antigens stimulation, the B cells differentiate into memory B cells and plasma cells. Whereas the T cells differentiates into cytotoxic T cells, helper T cells and memory T cells.



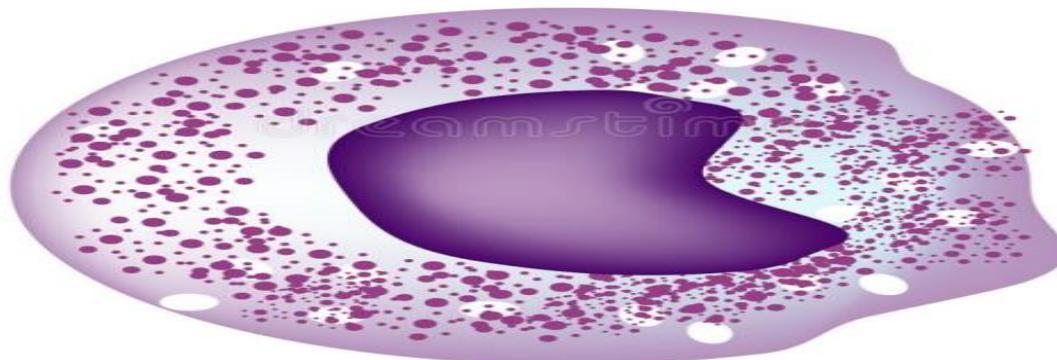
# Identifying features of T and B cells

S.No	Property	B cells	T cells
1.	Site of development	Bone marrow, bursa, Peyer's patches,Lymph node cortex.	Thymus
2.	Distribution	Splenic Follicles	Spleen periarticular sheath
3.	Circulate	No	Yes
4.	Antigen receptors	BCR	TCR
5.	Important surface antigens	Immunoglobulins	CD2, CD3, CD4, CD8
6.	Antigens recognised	Free foreign proteins	Processed foreign proteins on MHC
7.	Tolerance induction	Difficult	Easy
8.	Progeny cells	Plasma cells, memory cells	Helper T cell, cytotoxic cells
9.	Secreted proteins	Immunoglobulins	Cytokines

## B. MONONUCLEAR PHAGOCYTIC SYSTEM – MACROPHAGES

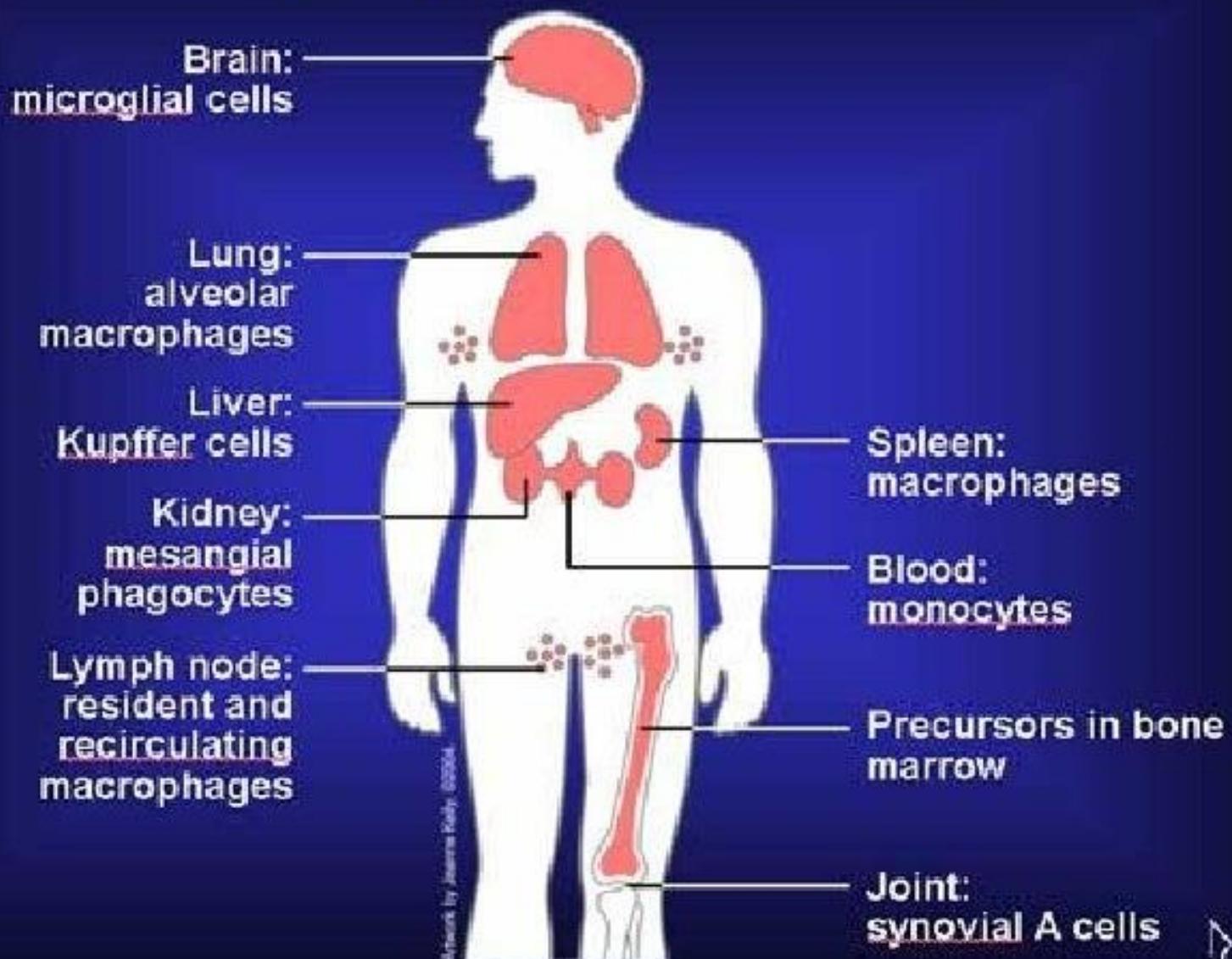
- ❑ One of the four important cells that constitute the immune system is the macrophage. The other three being B cells, T cells and NK cells.

### **Macrophage**



- The phagocytic cells can be divided into two distinct groups based on the morphology of nucleus into mononuclear (macrophages and their precursor monocytes) and polymorphonuclear (neutrophils and eosinophils) cells.
- Of the two groups, the macrophages are considered as powerful phagocytic cells and are referred as **big eaters** or **garbage collectors**.

# Phagocytes in the Body



**3. Development of macrophages:** Macrophages develop from a myelomonocytic stem cell through monoblast, promonocyte and monocyte till they reach structurally developed final stage called macrophage.

- ❑ The development of monocyte from promonocyte is stimulated by few factors called **colony-stimulating factors**.
- ❑ Before becoming macrophages they remain in the blood stream as monocyte for a period of time.



## 4. Characteristics of macrophages:

Macrophages have important characteristics that determine its role in the immune response. The characteristics are as follows.

- They are mononuclear cells.
- They show peroxidase and esterase activity.
- They bear specific receptors for antibody and complement.
- They have strong phagocytic properties



The characteristics are as follows.

- They are stimulated into active state by stimulation.
- They have varied secretory property.
- They can adhere to glass or plastic surface whereas T cells and B cells cannot adhere.



**Surface markers of macrophages:** The macrophages are characterized by presence of Fc receptors like (CD16, CD32, CD64), complement receptors (C11b, CD35), interleukin receptor (CD25), transport receptor (CD71) and Class I and II MHC molecules. The most important CD marker of macrophages is CD68, which is otherwise called as **macrosialin**.



## Functions of macrophages:

**1. Phagocytosis:** The primary function of macrophage is phagocytosis.

- They are potent phagocytic cells and can kill any foreign substance like microbe, carbon particles etc.
- They are also capable of doing repeated phagocytosis, which is not common among other phagocytic cells.
- The actual mechanism of macrophage-mediated phagocytosis is by **receptor-mediated endocytosis** followed by lysosomal enzyme degradation.



## 2. Antigen presentation:

- Macrophages break the antigen into different epitopes and present them to helper T cells via MHC II molecules on their surface.
- Helper T cells will accept epitopes only when presented by MHC II on macrophages.
- This step is essential for stimulating specific immune response. Hence, they are known as antigen presenting cells (APC).



### **3. Secretory property:**

They are strong secretory cells that secrete over 100 important substances. These substances play important role in lymphocyte differentiation, effector capability etc.

### **4. Activation:**

Certain agents stimulate macrophages and convert them from accessory cells to effector cells.

This process is called activation of macrophages and the macrophages are referred as activated macrophages.

- In normal conditions, the macrophages remain in circulation as monocytes or resting macrophages.
- When they reach the site of inflammatory response they possess enhanced phagocytic property with expression of Fc and complement receptors.
- At this stage the macrophages are referred as **inflammatory macrophages**. These inflammatory macrophages are further stimulated by certain substances like interferon into **activated macrophages**.



- ❑ The activated macrophages are bigger in size, increased mobility, and increased expression of Fc and complement receptors, increased lysosomal activity, increased MHC II expression and increased ability to kill bacteria.
- ❑ In certain chronic infections or in case of foreign substances remaining in body for a long time, a large number of macrophages accumulate at the site around the material and resemble epithelial cells.

□ These accumulated cells are called as **epitheloid cells**. These cells may fuse to form multinucleated giant cells. Giant cell formation is seen when the particle to be ingested too big for a single cell.

**5. Regulation of immune response:** The exact regulatory mechanisms are not clear. But macrophages through monokines control T cell proliferation.



**6. Wound healing:** Apart from phagocytosis, macrophages also help in wound healing. Macrophages secrete proteases that help in breakdown of connective tissue and regulate the production of collagenase by fibroblasts and help in formation of blood vessels. Thus it helps in wound healing.



- Granulocytes are group of blood leukocytes that are characterized by presence of number of cytoplasmic granule.
- Granulocytes are composed of three distinct populations of cells called **neutrophils, eosinophils and basophils**.
- This classification is based on the ability of the cytoplasmic granules to take up stains and the cell's function.



- The granules of neutrophils do not take any stain, eosinophils take acidic dye like eosin and basophils take basic dye haematoxylin.
- These blood leukocytes are called **inflammatory cells** since they play an important role in inflammatory processes, innate immunity and in removal of dead tissues.



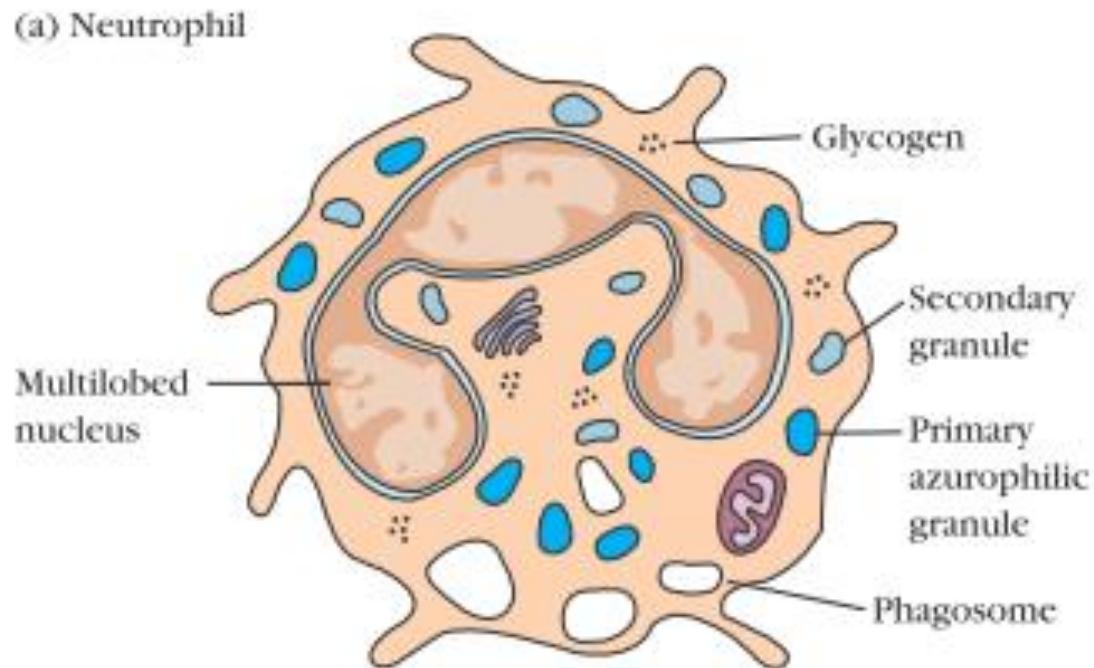
- ❑ Besides these functions, they also have effector functions in specific immune response.
- ❑ The granulocytes neutrophils and eosinophils constitute the polymorphonuclear phagocytic system.
- ❑ They are called so since the nucleus is multi-lobulated.
- ❑ The granulocytes originate from myeloid stem cells, which in turn originate from pluripotent stem cells.

# 1. NEUTROPHILS

- ❑ This is the major cell type of granulocytes. They are formed in bone marrow and arrive at blood stream within 12 hours. They have a very short life span of just few days.
- ❑ The percentage of neutrophils in blood circulation among animals varies widely.
- ❑ It is 60-70% in carnivores, 20-30% in ruminants and 50% in horses. Their presence in circulation indicates a mild infection.

## Morphology:

- They are round cells with a diameter of  $12\mu\text{m}$ .
- The cytoplasm is granular with a central sausage or segmented nucleus.



## Morphology:

- The cytoplasmic granules are of two types primary and secondary granules.
- The primary granules are rich in bactericidal enzymes such as myeloperoxidase and lysozyme etc. and secondary granules contain lysozyme, collagenase and iron binding protein lactoferrin.



- The cytoplasm also contains a small Golgi apparatus, few mitochondrion and very few ribosomes or rough endoplasmic reticulum. The neutrophils are characterized by inability of the cells to divide and perform protein synthesis
- **Surface receptors:** The neutrophils have cell adhesion molecules (CD11a/CD18, CD11c/CD18), complement receptors (CD35, CD11b/CD18) and Fc receptor (CD32) on their surface



- The cell adhesion molecules on neutrophils are called integrins. There are three types of integrins. Integrins are mixture of two receptors CD11 and CD18.

**Functions:** The main function of neutrophils is **phagocytosis**. They also have effector function in specific immune response.

Neutrophils can phagocytose only bacteria. Unlike macrophage they cannot perform repeated phagocytosis.



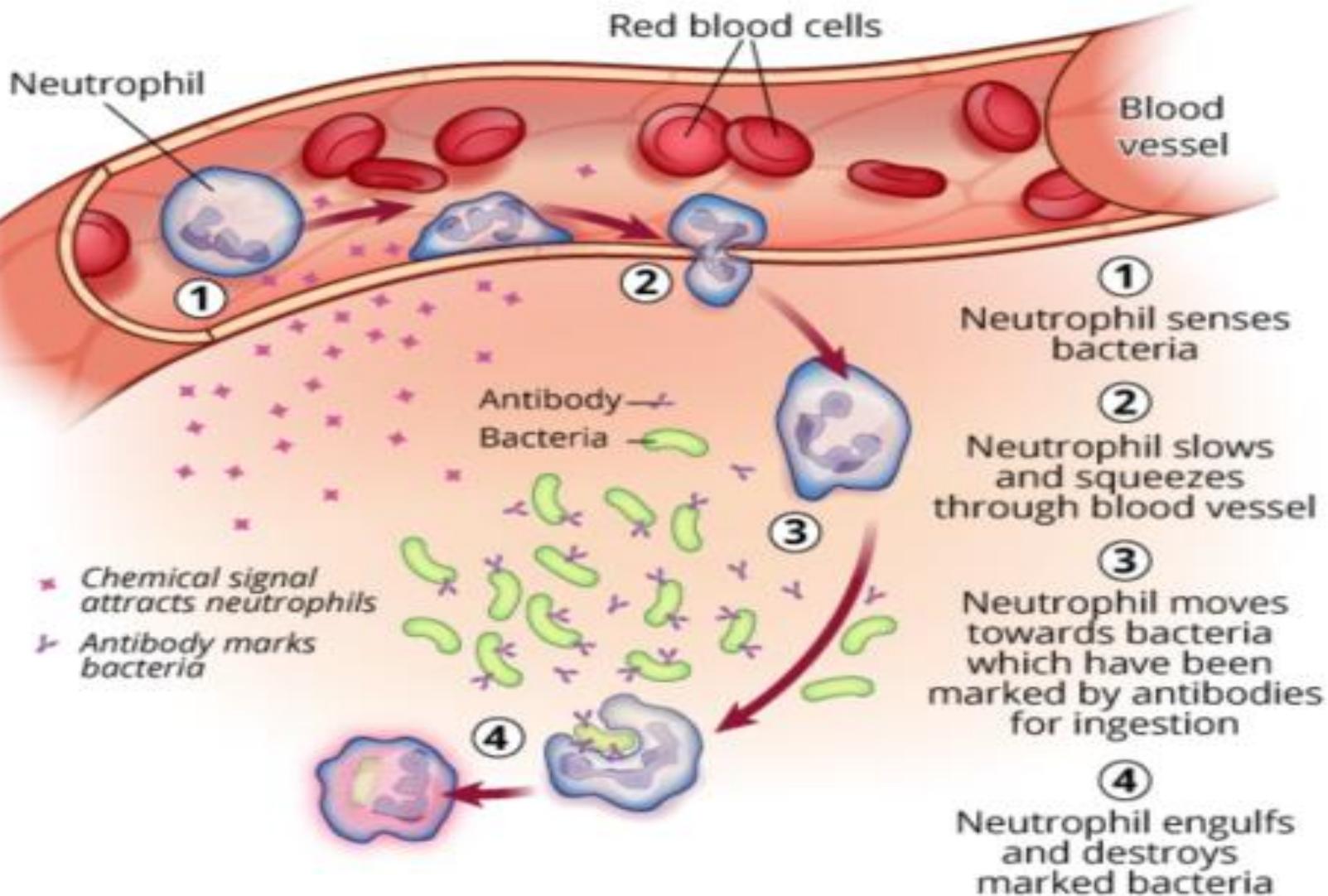
- ❑ A neutrophil can do only one phagocytosis.

At the end of phagocytosis neutrophils are also killed.

- ❑ However, they secrete certain substances that in turn attract macrophages to the site.
- ❑ Neutrophils also have receptors for Fc portion of antibodies (CD32) and for complement protein C3b (CD35) on their surface.



## Neutrophils in the Immune System



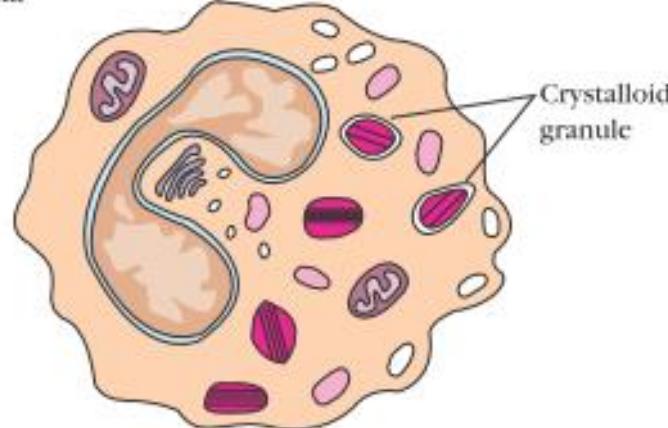
- Hence, an antigen neutralised by an antibody or antigen covered with C3b can be taken by neutrophils.
- Thus the antigen antibody complex formed is removed from this system. This process is called **opsonisation** and antibodies and C3b are referred as **opsonin**.



## 2. EOSINOPHILS

- This is the second major type of granulocytes.
- It is called so since the granules in the cytoplasm take eosin dye and appears pink.
- They are formed in the bone marrow and then they reach spleen where they mature.

(b) Eosinophil



- ❑ After maturation, they leave for blood stream where they remain for a shorter time (half life 30 minutes). Subsequently they migrate in to tissues where they remain relatively for a longer period (half life 12 days)
- ❑ In contrast to neutrophils, the percentage of eosinophils is only 2% for dogs and 10% for cattle.
- ❑ They are bigger than neutrophils and contain a bi-lobed nucleus.



- The cytoplasm consists of two types of granules - small primary granule and crystalloid granules.
- The small primary granules contain **arylsulfatase, eosinophil peroxidase and acid phosphatase.**
- The crystalloid granules contain four major proteins – major basic protein (MBP), eosinophilic cationic protein (ECP), eosinophil peroxidase (EPO) and eosinophil derived neurotoxin (EDN).

- ❑ In a crystalloid granule, the MBP is arranged as core and remaining proteins are arranged as matrix proteins.
- ❑ The cell membrane is characterized by the presence of large amount of lysophospholipase.

### Surface receptors:

- ❑ Eosinophils possess receptors for Fc portion of antibodies (CD16 and CD32) and complement proteins(CD35 and CD23).
- ❑ They also possess receptors for IgE.

## A condition that affects eosinophilia

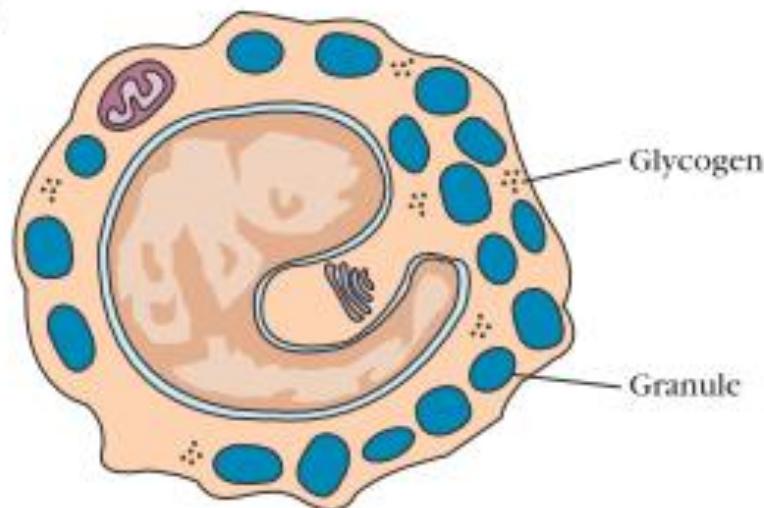
- **Eosinopenia:-** Ex:- Cushing's syndrome and sepsis are common causes of Eosinopenia.
- **Eosinophilia:-** Alcohol intoxication, allergies, gastrointestinal disorder, leukemia, overproduction of cortisol, and parasitic infection are causes of eosinophilia condition.



### 3. BASOPHILS

- They are less important type of granulocytes.
- They are called so since the granules in the cytoplasm take basophilic dyes like haematoxylin.

(c) Basophil



- ❑ They constitute only 0.5% of the blood cells.
- ❑ They are not normally found in extravascular tissues.
- ❑ They migrate into the tissue upon stimulation by the lymphocytes.
- ❑ The granules are rich in vasoactive amines like histamine and serotonin.
- ❑ They also possess surface receptors for IgE.
- ❑ Hence, inside the tissue, they stimulate inflammatory response along with mast cells and eosinophils.

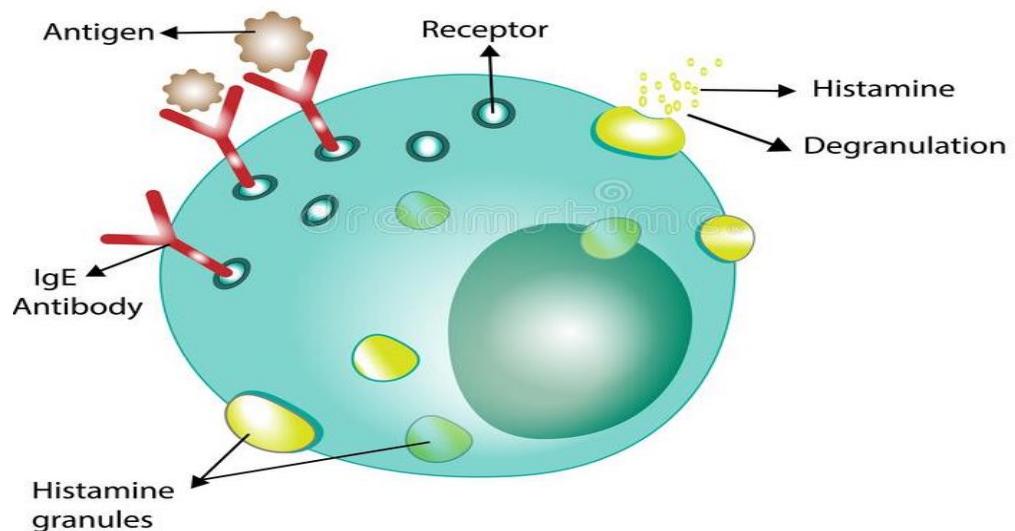


## 4. MAST CELLS

- Mast cells are formed in the bone marrow.
- They are released from the bone marrow into the blood as undifferentiated cells, and when they enter the tissues they then mature.
- Mast cells can be found in a wide variety of tissues, including the skin, connective tissues of various organs, and mucosal epithelial tissue of the respiratory, genitourinary, and digestive tracts.



- Like circulating basophils, these cells have large numbers of cytoplasmic granules that contain histamine and other pharmacologically active substances.
- Mast cells, together with blood basophils, play an important role in the development of allergies.



## 5. DENDRITIC CELLS

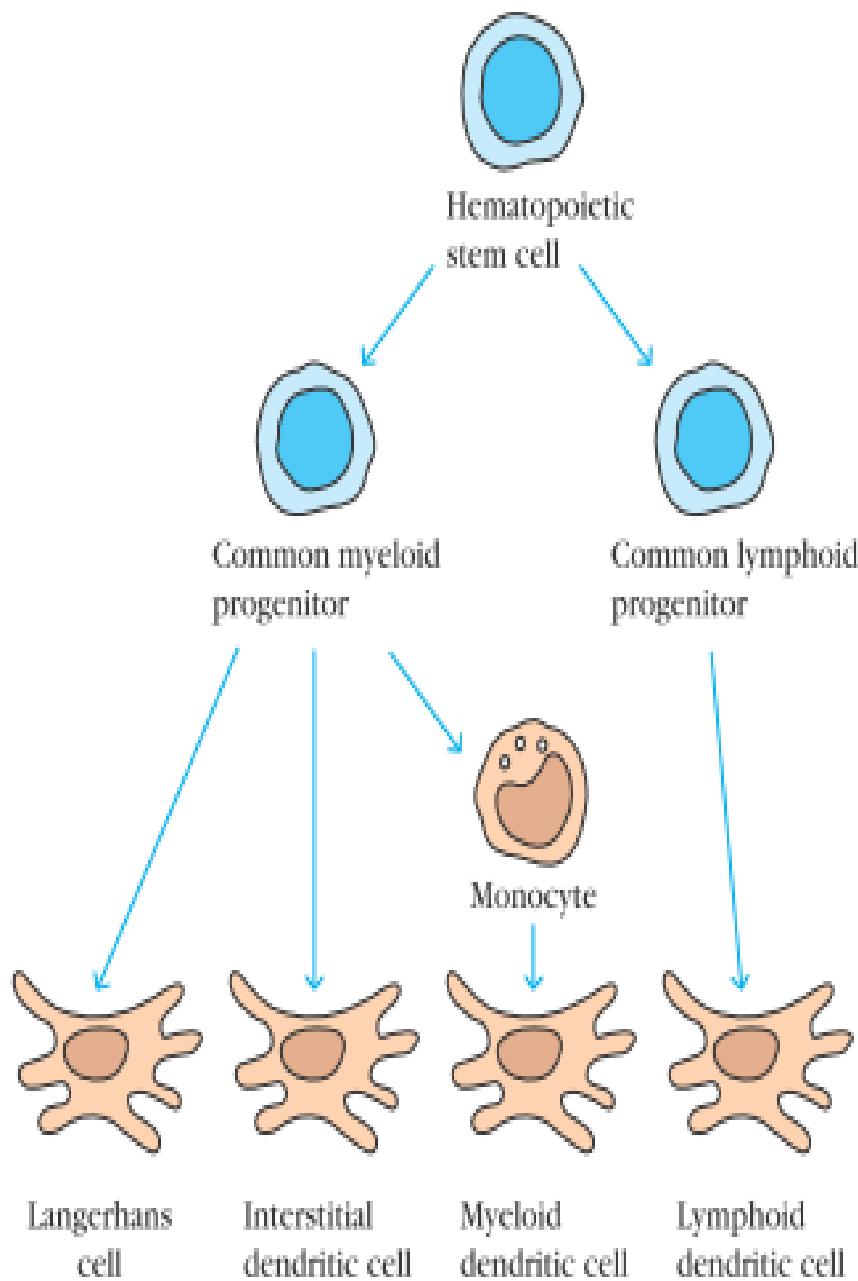
- They are accessory cells and play important roles in the induction of immune responses.
- Dendritic cells are population of specialised mononuclear cells that are positioned at the possible places of antigen entry into the body.
- They are poorly phagocytic cells. However, their main role is to present the antigen to lymphocytes for its effective removal from the system.



**Morphology:** The cells are characterized by lobulated nuclei, clear cytoplasm and filamentous cytoplasmic processes called **dendrites**.

- The cytoplasm is filled with a special granule (cytoplasmic organelle) called **Birbeck granules**.
- There are many different types of dendritic cells with different properties and functions.





- Dendritic cells arise from both the myeloid and lymphoid lineages.
- The myeloid pathway that gives rise to the monocyte/macrophage cell type also gives rise to dendritic cells.
- Some dendritic cells also arise from the lymphoid lineage.

- There are many types of dendritic cells, although most mature dendritic cells have the same major function, the presentation of antigen to T<sub>H</sub> cells.
- Four types of dendritic cells are known: Langerhans cells, interstitial dendritic cells, myeloid cells, and lymphoid dendritic cells.
- Each arises from hematopoietic stem cells via different pathways and in different locations.
- However, they all constitutively express high levels of both class II MHC



- Immature or precursor forms of each of these types of dendritic cells acquire antigen by phagocytosis or endocytosis; the antigen is processed, and mature dendritic cells present it to  $T_H$  cells.
- Following microbial invasion or during inflammation, mature and immature forms of Langerhans cells and interstitial dendritic cells migrate into draining lymph nodes, where they make the critical presentation of antigen to  $T_H$  cells that is required for the initiation of responses by those key cells.



- Another type of dendritic cell, the **follicular dendritic cell** does not arise in bone marrow and has a different function from the antigen-presenting dendritic cells described above.
- Follicular dendritic cells do not express class II MHC molecules and therefore do not function as antigen presenting cells for  $T_H$ -cell activation.
- These dendritic cells were named for their exclusive location in organized structures of the lymph node called lymph follicles

# **Thank You**

**Dr. Sandeep Sharma, BVSc & AH, MVSc and PhD  
Assistant Professor, PGIVER  
drsharmask01@hotmail.com  
Mob. 9414775879**

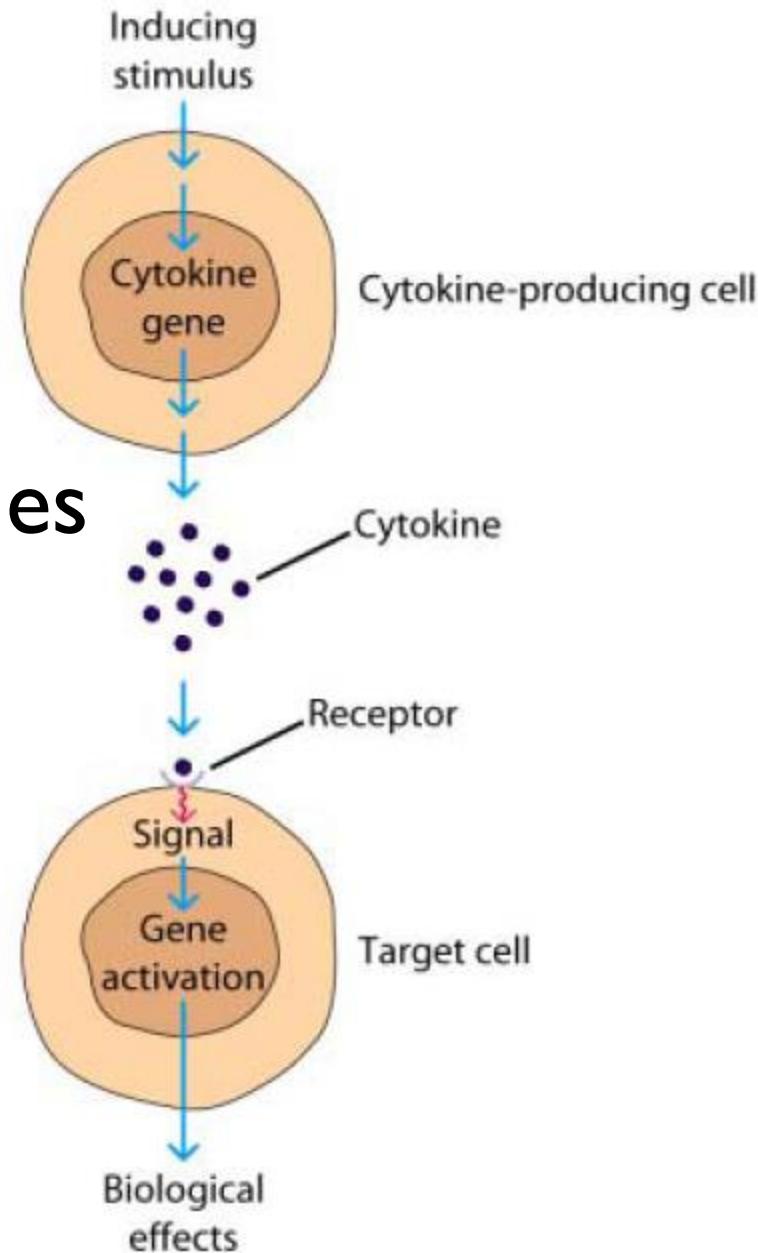




# Cytokines

# Contents

- Definition of cytokines
- Classification of cytokines
- Structure of cytokines
- Functions of cytokines



# History

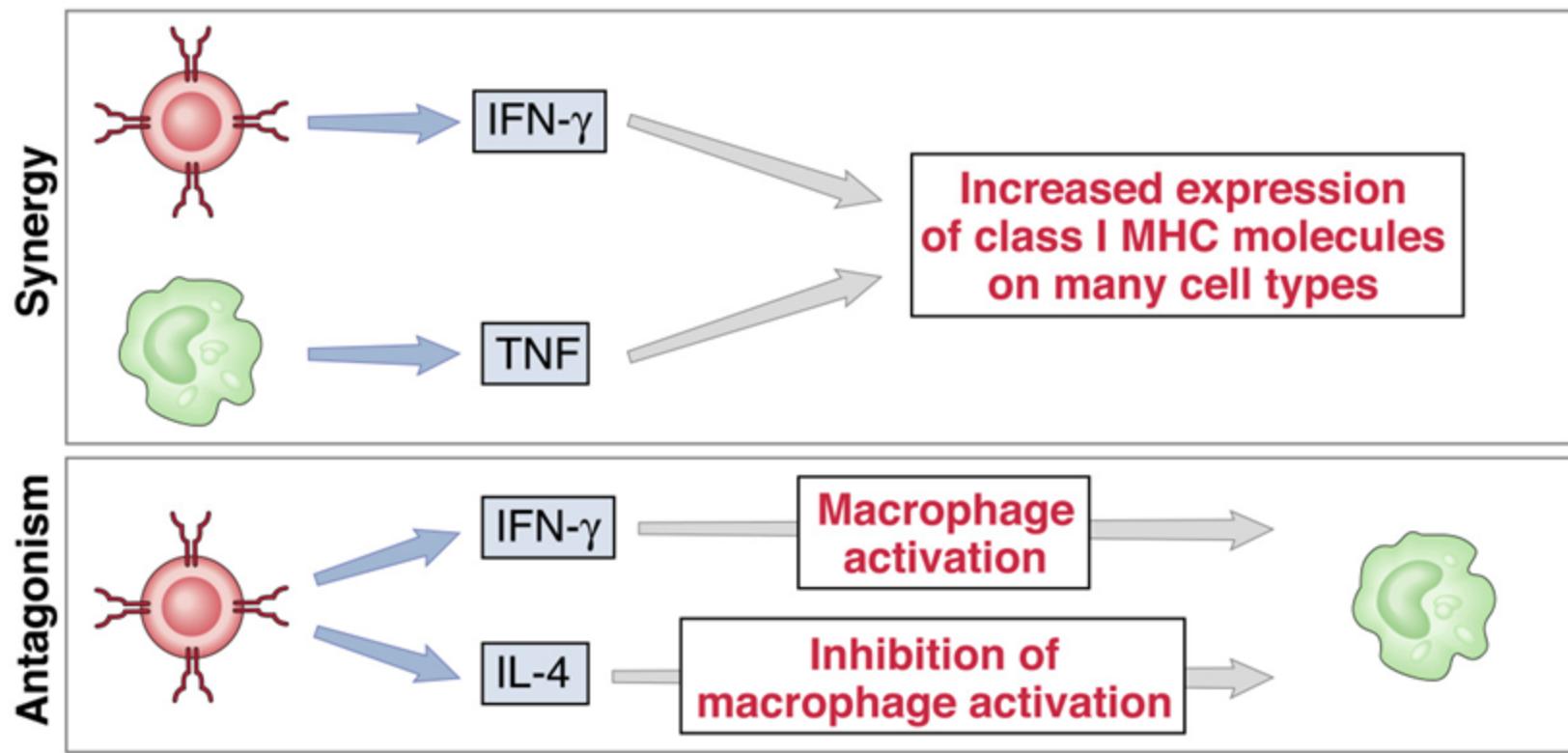
- The term cytokine was proposed by Cohen *et al* in 1974 to replace lymphokine, a term coined in the late 1960's to denote lymphocyte-derived soluble proteins that possess immunological effects.

- Cytokines (Greek *cyto-*, cell; and *-kinos*, movement) are small cell-signaling proteins, peptides, or glycoproteins molecules that are secreted by numerous types of cells especially by monocytes and lymphocytes.
- Low molecular weight (20- 30 KDa)
- Cytokines are chemical messengers; they are extremely potent and act at very low concentrations ( $10^{-10}$ - $10^{-12}$  M).
- They are very specific, and act through specific receptors of the target cell membrane.

# Properties of cytokines

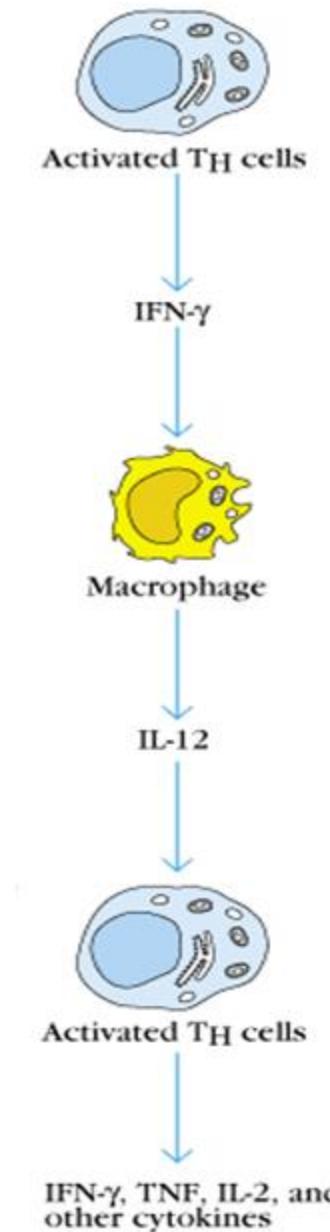
- Synergy
- Antagonism
- Pleiotropy
- Redundancy and
- Cascade Induction

# Synergy and Antagonism



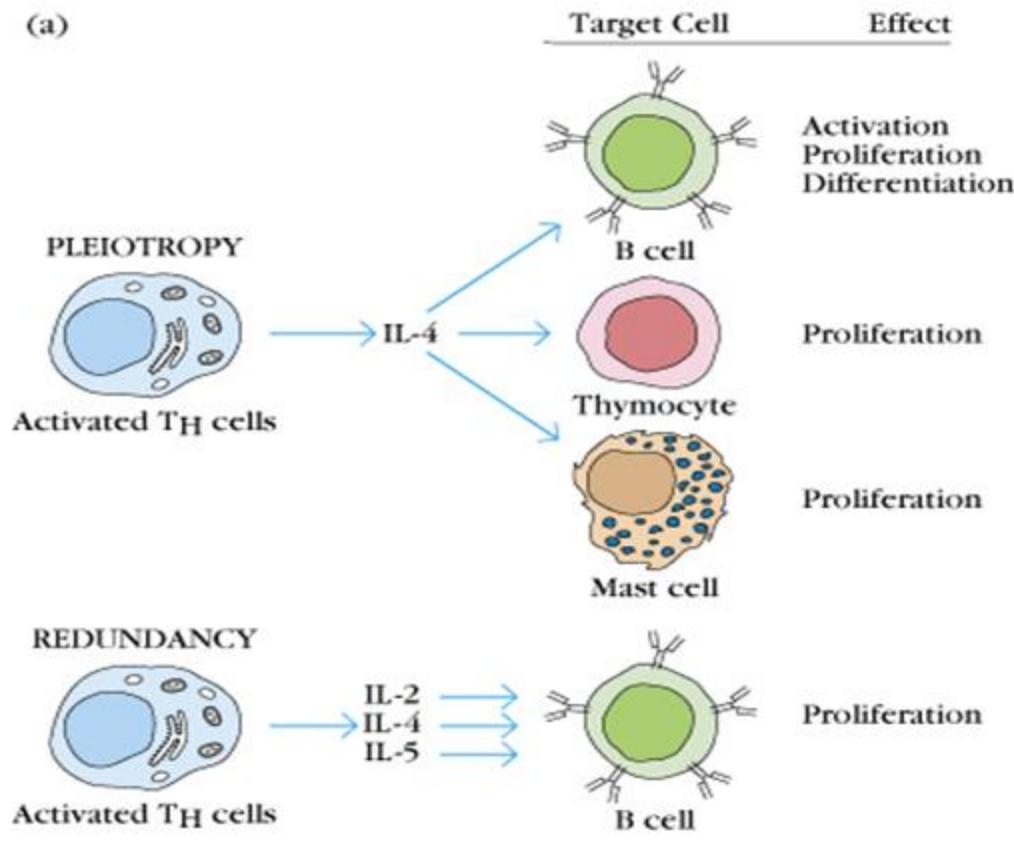
From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 11-2b

(b) CASCADE INDUCTION



- Pleiotropy
- Redundancy and
- Cascade Induction

(a)



# Classification of cytokines

- General classification of cytokines
- Cytokines classified into various families
- Classification based on secretory cells
- Classification based on mode of action
- Classification based on cytokine receptors

# General classification of cytokines

**Table 1: Important classes of Cytokines**

## 1. Growth Factors:

- a. Haemopoietic Growth Factors
  - Granulocyte – Colony Stimulating Factor (G-CSF)
  - Granulocyte Macrophage – Colony Stimulating Factor (GM-CSF)
  - Erythropoietin (EPO)
  - Thrombopoietin
  - Stem Cell Factor or c-kit ligand
- b. Epidermal Growth Factor
- c. Platelet Derived Growth Factor
- d. Transforming Growth Factor  $\beta$
- e. Fibroblast Growth Factor
- f. Insulin like Growth Factor
- g. Nerve Growth Factor

## 2. Interleukins

IL – 1 to IL – 18

## 3. Interferons

IFN –  $\alpha$   
IFN –  $\beta$   
IFN –  $\gamma$

## 4. Miscellaneous

Tumour Necrosis Factor (TNF), etc.

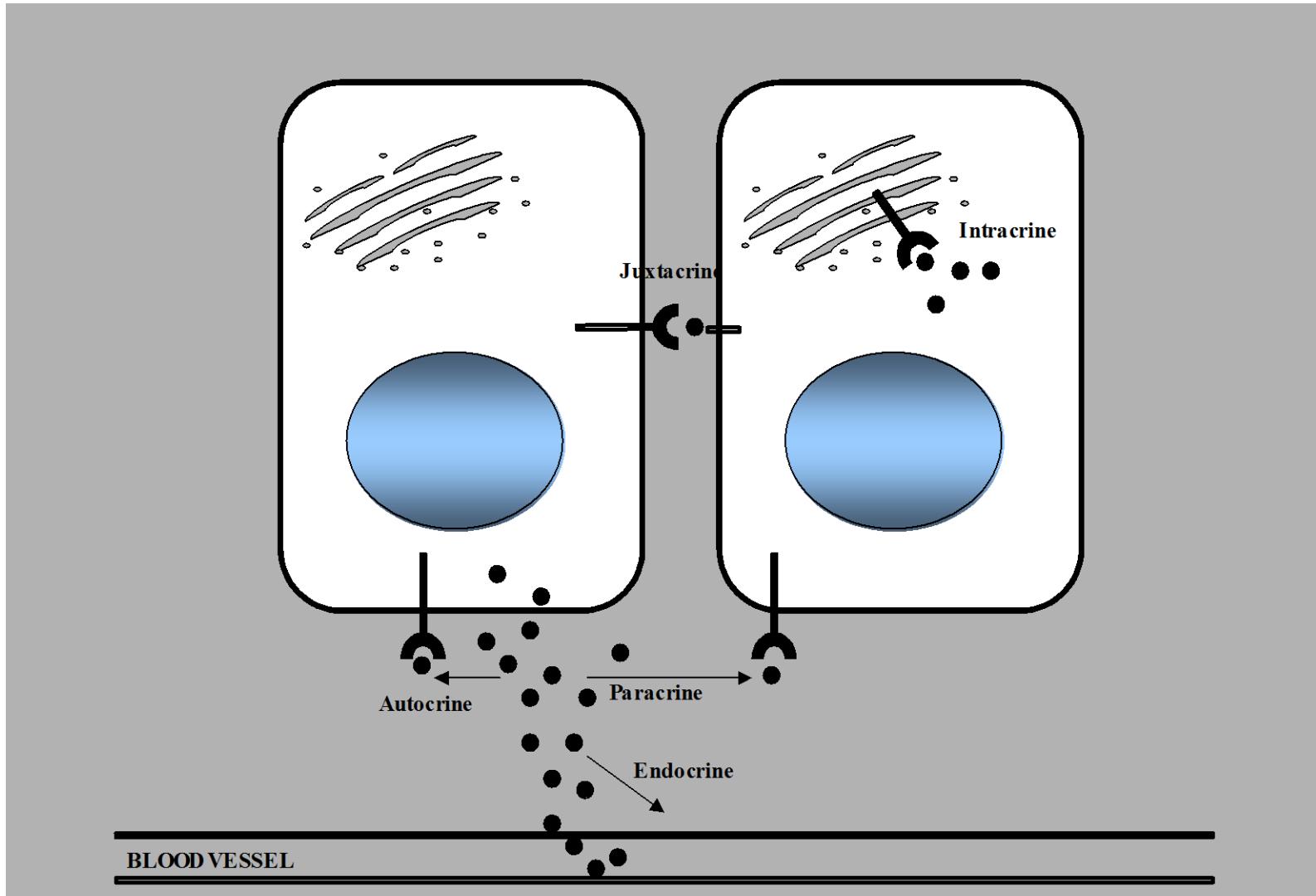
# Cytokines classified into various families

- Hematopoietin family
- Interferon family
- Chemokine family
- Tumor necrosis factor (TNF) family

# Classification based on secretory cells

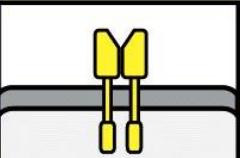
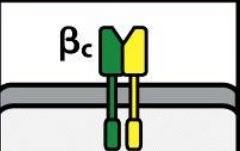
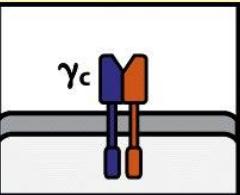
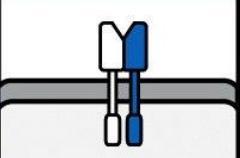
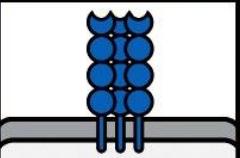
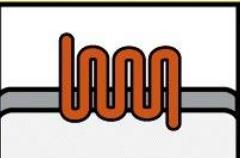
- **Lymphokines:** secreted by lymphocytes
- **Monokines:** secreted by monocytes and macrophages
- **Interleukins:** secreted by some leukocytes and act upon other leukocytes
- **Interferons:** natural killer (NK) and natural killer T (NKT) cells
- **Tumor necrosis factors:** macrophages, CD4+ lymphocytes and NK cells
- **Chemokines:** endothelial cells, myeloblasts, erythroblasts, and megakaryoblasts

# Classification based on mode of action



Bafico & Aaronson, Cancer Med, 2002

# Classification based on cytokine receptors

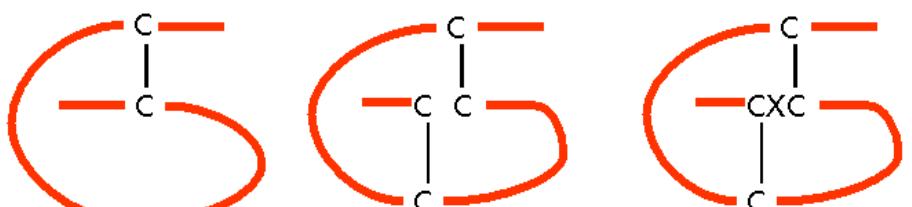
<b>Homodimeric receptors</b>		Receptors for erythropoietin and growth hormone
<b>Heterodimeric receptors with a common chain</b>		Receptors for IL-3, IL-5, GM-CSF, share a common chain, CD131 or $\beta_c$ (common $\beta$ chain)
		Receptors for IL-2, IL-4, IL-7, IL-9 and IL-15, share a common chain, CD132 or $\gamma_c$ (common $\gamma$ chain). IL-2 receptor also has a third chain, a high-affinity subunit IL-2R $\alpha$ (CD25)
<b>Heterodimeric receptors (no common chain)</b>		Receptors for IL-13, IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$ , IL-10
<b>TNF-receptor family</b>		Tumor necrosis factor (TNF) receptors I and II CD40, Fas (Apo1, CD95), CD30, CD27, nerve growth factor receptor
<b>Chemokine - receptor family</b>		CCR1–10, CXCR1–5, XCR1, CX3CR1

# Chemokines

Produced by many cell types and binds to glycosaminoglycans (GAGs)

- induce directed chemotaxis of leukocytes
- regulate leukocyte migration in development, homeostasis and activation
- several groups with many members based on location of cystein residues near NH<sub>2</sub>-terminus
- C, CC, CXC and CX<sub>3</sub>C

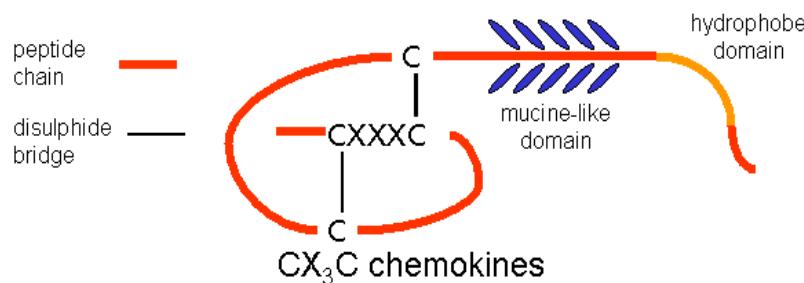
Structure of chemokine classes



C chemokines

CC chemokines

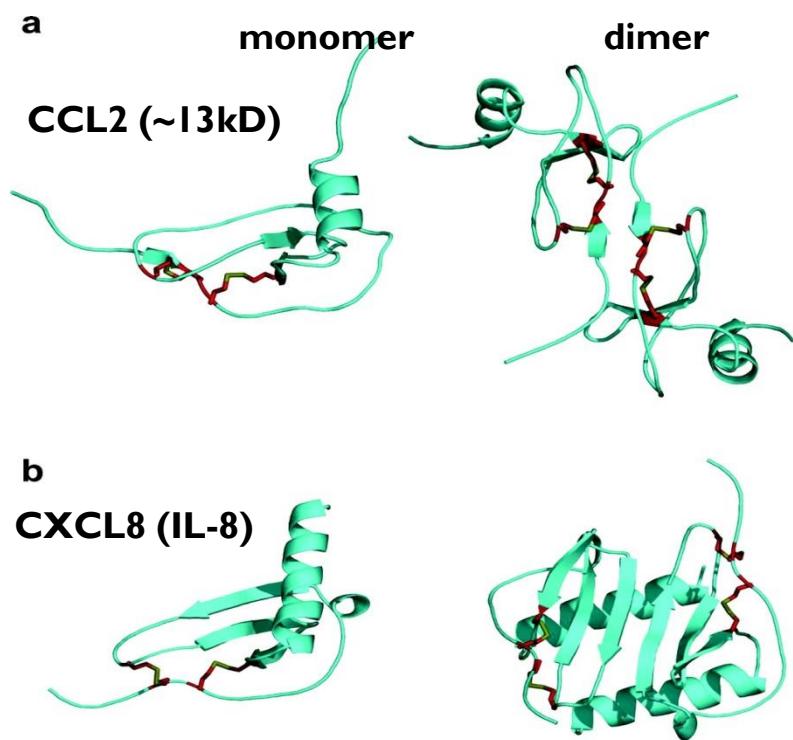
CXC chemokines



CX<sub>3</sub>C chemokines

© Kohidai, L.

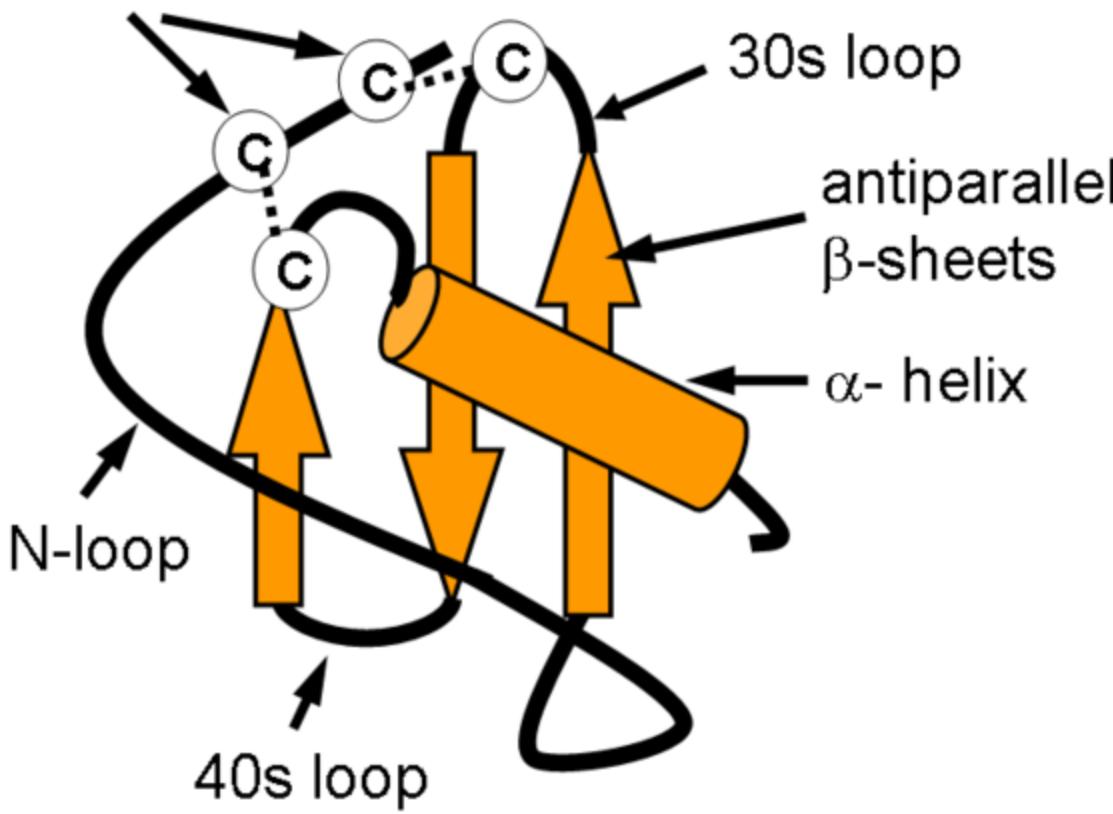
- Form dimers in solution, but interact with receptors as monomers



Allen SJ, et al. 2007.  
Annu. Rev. Immunol. 25:787–820

# Three dimensional structure of chemokines

disulphide bridges of Cys-Cys



© Kohidai, L.

Typical Greek key structure that is stabilized by disulfide bonds between conserved cysteine amino acid residues.

# Interferons

## Type-I Interferon (IFN-I)

- produced by many cell types: leukocytes, endothelia, fibroblasts, etc
- Induced by viral double stranded RNA: recognized by TLR3
- Induced by bacterial cell wall components by TLR4
- IFN- $\alpha$ : 14 subtypes
  - drug for hepatitis B and C virus and cancer (melanoma & leukemia)
- IFN- $\beta$ : single gene product
  - drug for multiple Sclerosis – effective at an early stage
- induces production of IL-15
- plasmacytoid dendritic cells can make large amounts

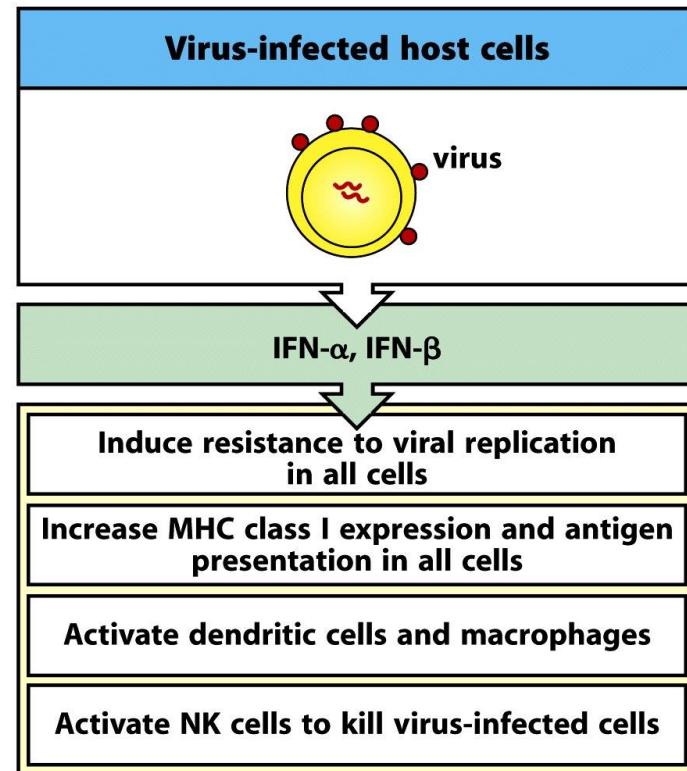
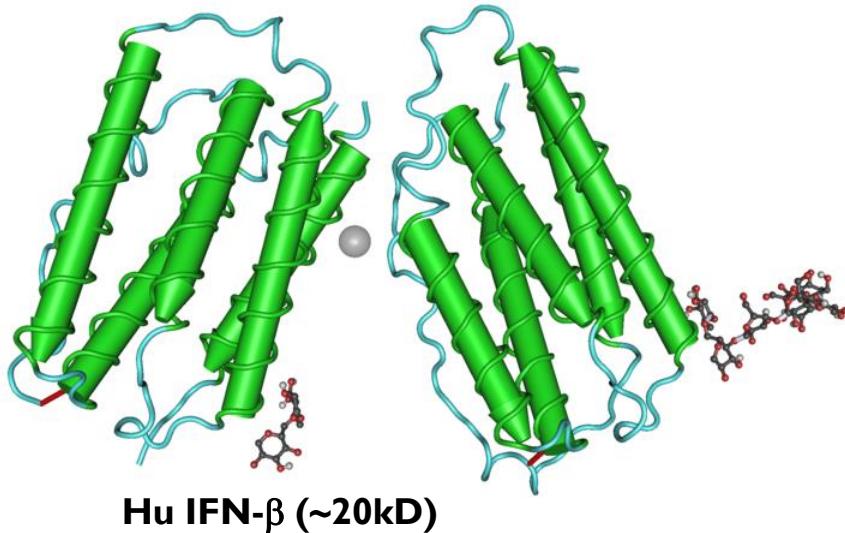
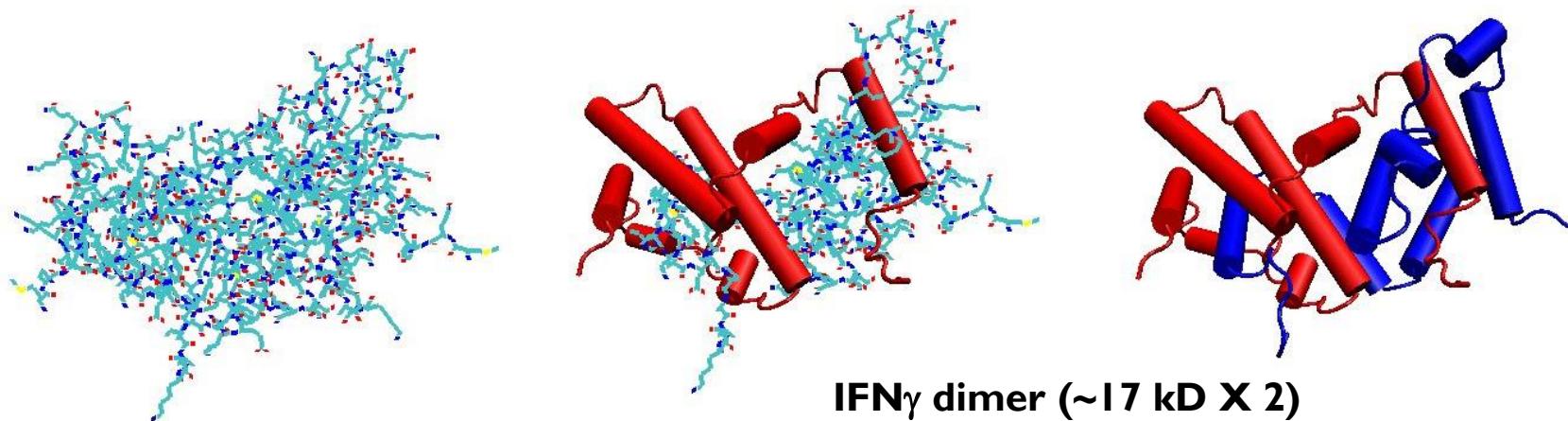


Figure 2-54 Immunobiology, 7ed. (© Garland Science 2008)

# Gamma Interferons

Type-II Interferon (IFN-II, IFN- $\gamma$ ):

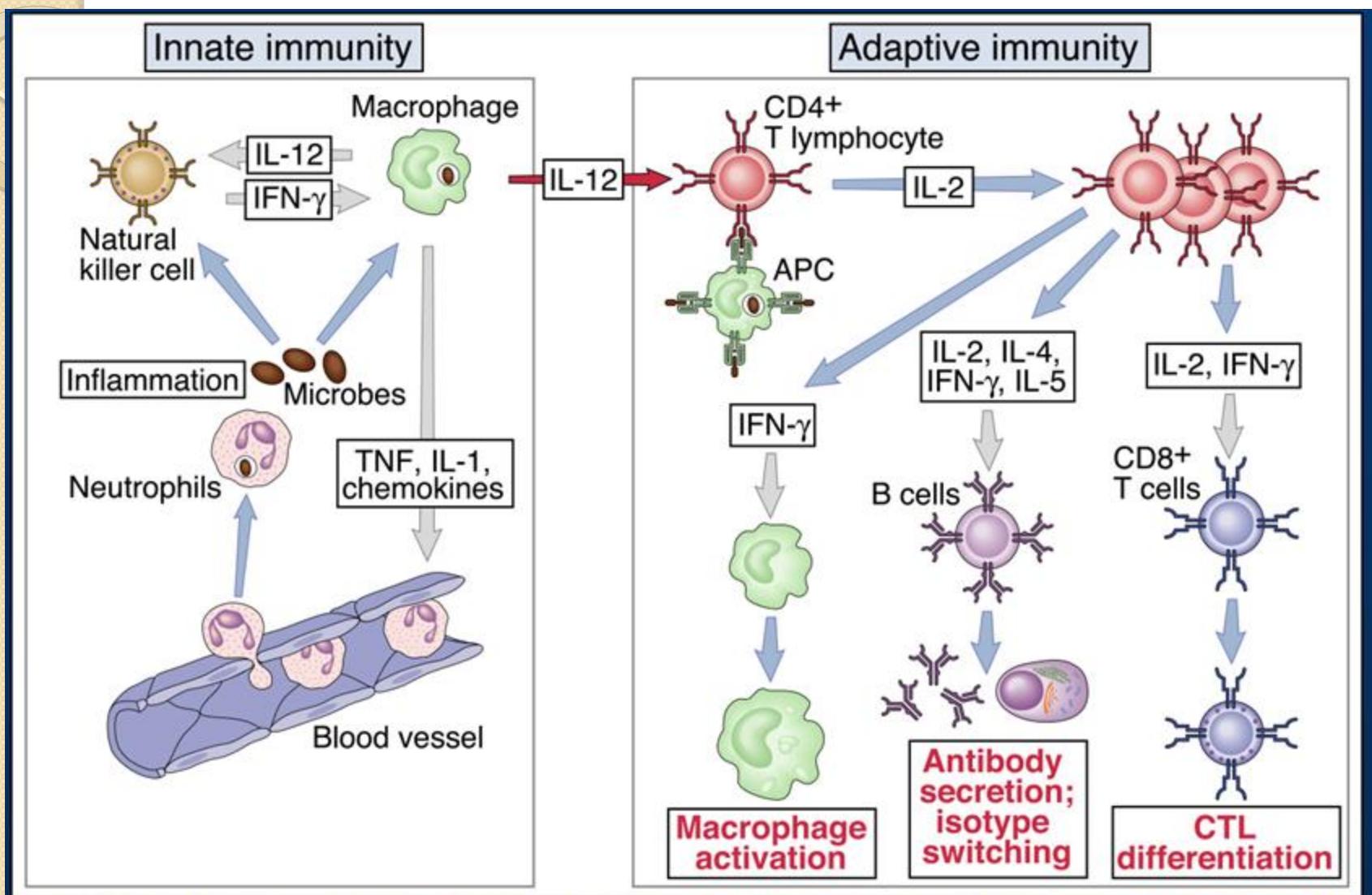
- Produced by NK, NKT, activated T cells and DC
- Increased Ag presentation and expression of MHC by Antigen presenting cells
- Promote leukocyte migration
- Induce NK cell activity
- Induces Th1 cell production and activity
- Suppresses Th2 cell production and activity



# Tumor necrosis factor (TNF)

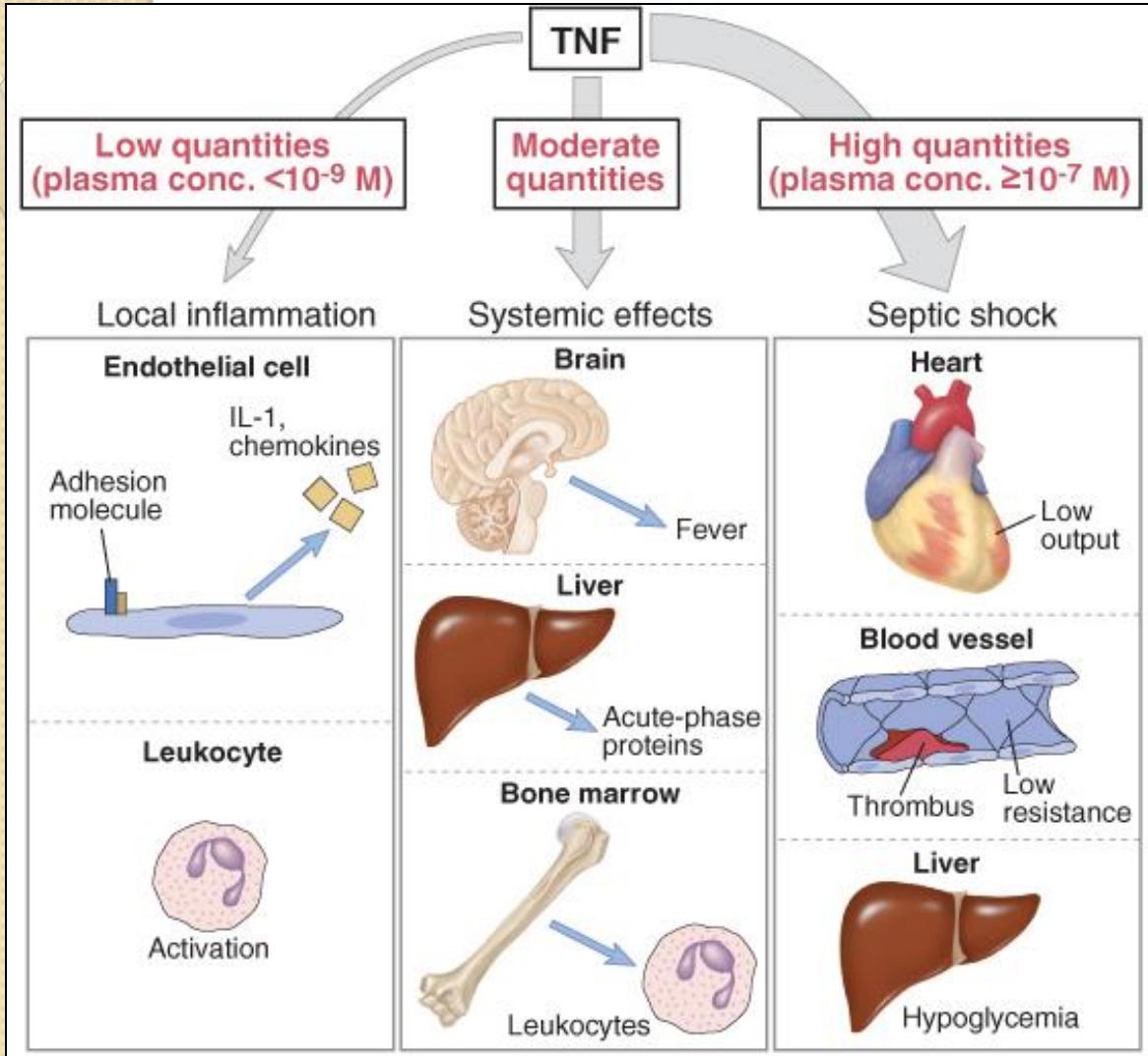
- It is produced by macrophages, lymphocytes, fibroblasts and keratinocytes.
- TNF- $\alpha$ , is the best-known member of this class.
- TNF- $\alpha$  is a monocyte-derived cytokine that has been implicated in tumor regression, septic shock, and cachexia.
- Other one is Lymphotoxin-alpha, formerly known as TNF- $\beta$ , is a cytokine that is inhibited by interleukin 10.

# Functions of cytokines



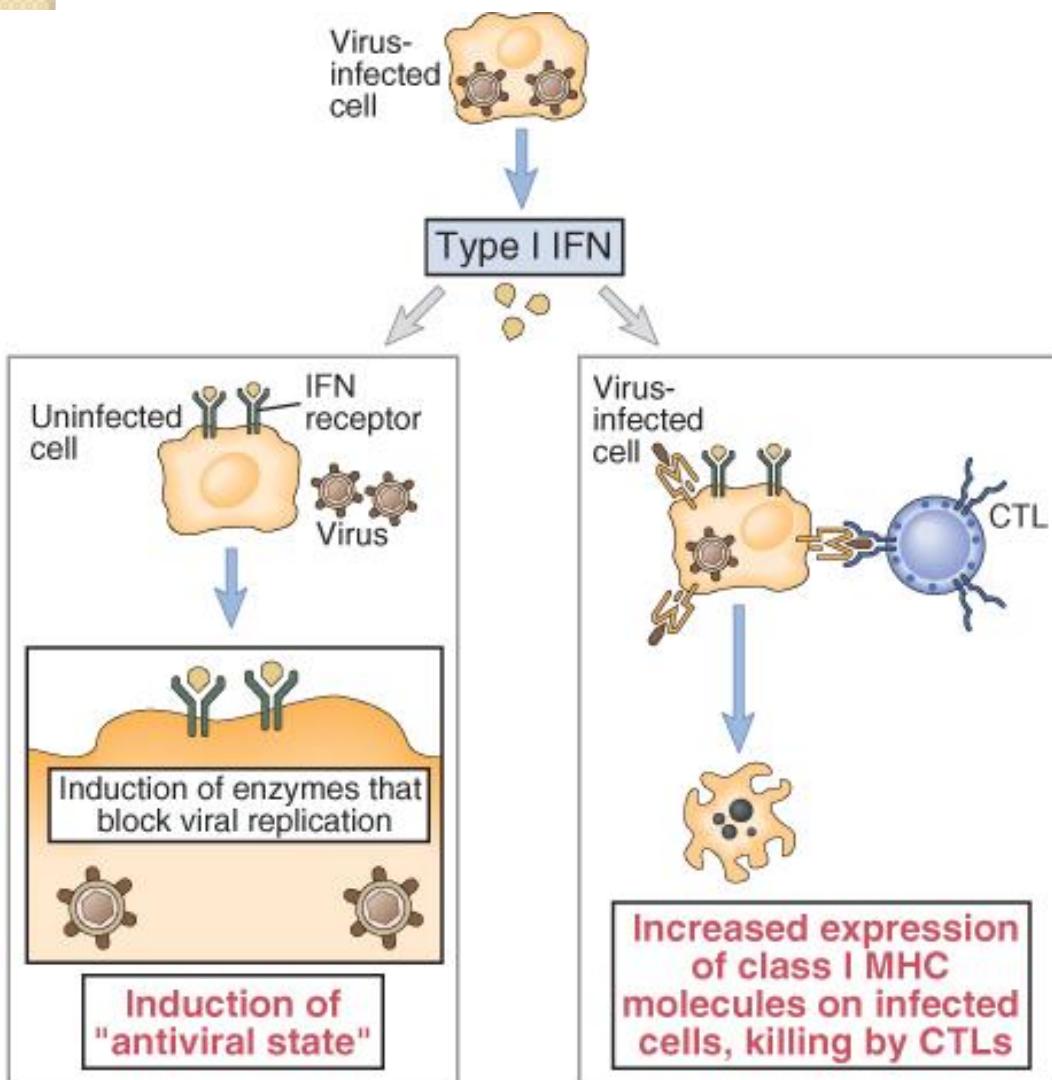
From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 11-1

# Functions of Tumor Necrosis Factor (TNF)



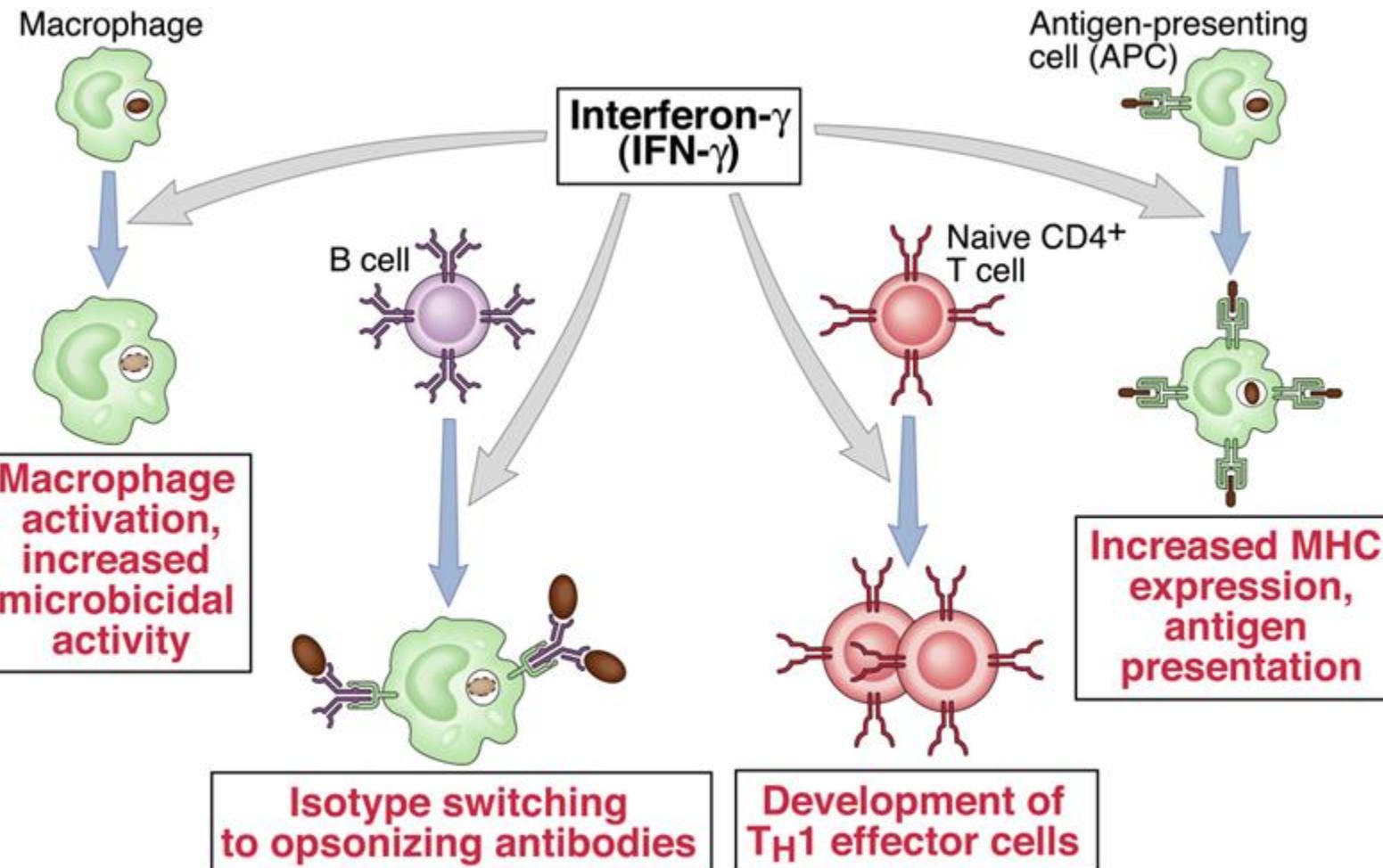
- The release of TNF- $\alpha$  by macrophages induces local protective effects, but TNF- $\alpha$  can have damaging effects when released systemically (septic shock).

# Functions of interferons



- Antiviral effects of Type I Interferons (IFN-a/b)

# Functions of interferon- $\gamma$



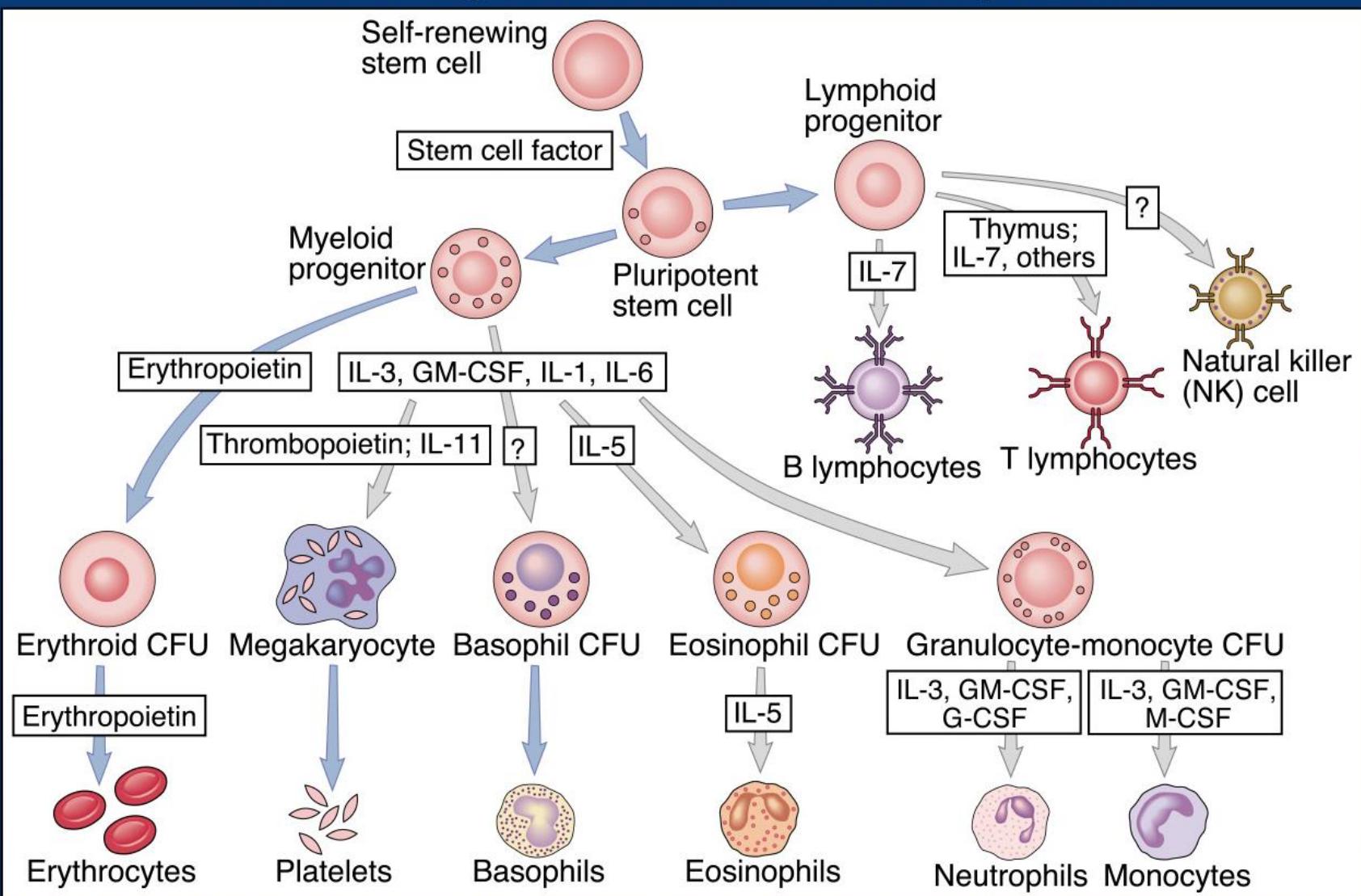
From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 11-14

### Role of cytokines in regulating Ig isotype expression

Cytokines	IgM	IgG3	IgG1	IgG2b	IgG2a	IgE	IgA
IL-4	Inhibits	Inhibits	Induces		Inhibits	Induces	
IL-5							Augments production
IFN- $\gamma$	Inhibits	Induces	Inhibits		Induces	Inhibits	
TGF- $\beta$	Inhibits	Inhibits		Induces			Induces

Fig 9.7 © 2001 Garland Science

# Roles of cytokines in hematopoiesis



# Cytokines required for organization of lymphoid tissues

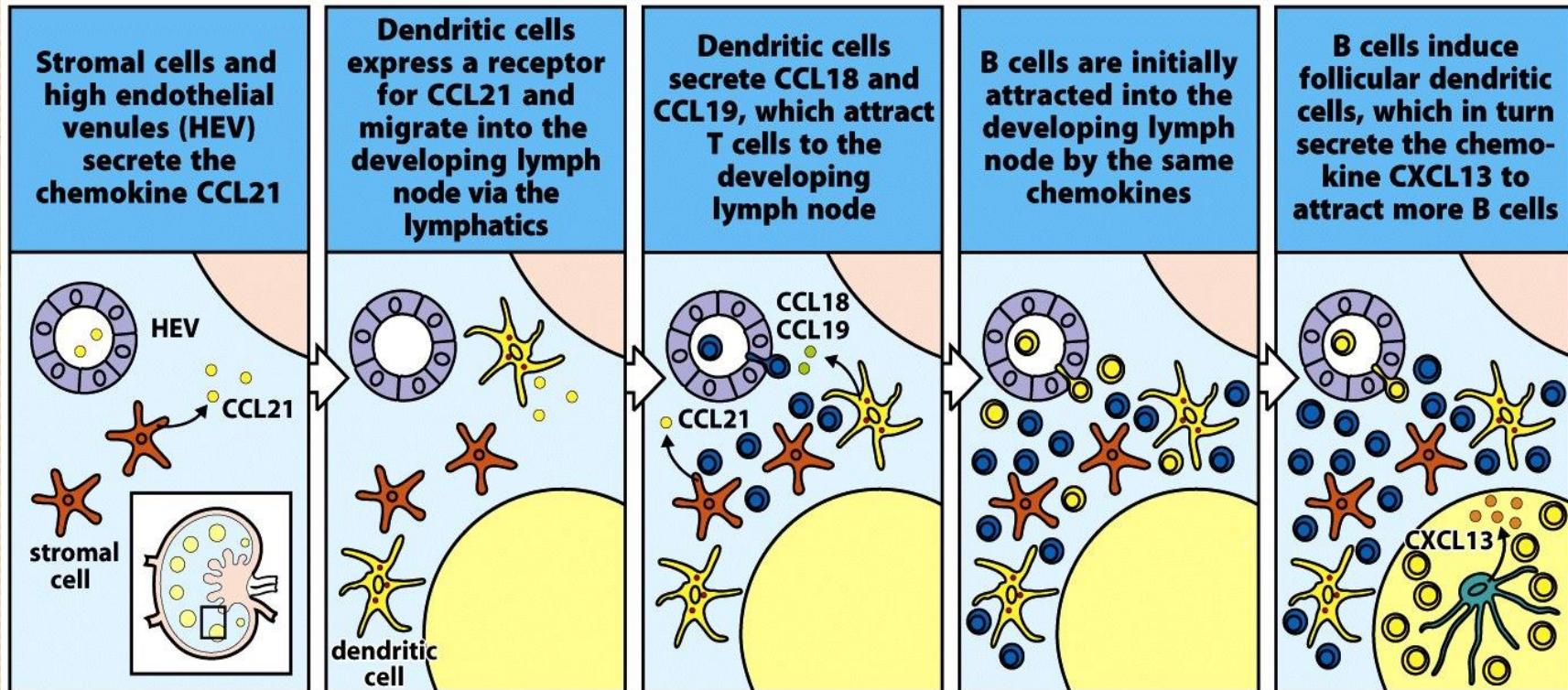


Figure 7-38 Immunobiology, 7ed. (© Garland Science 2008)

## CCL21, CCL19

- produced by stromal cells, HEV and interdigititating DC
- recruits T cells and DC into LN and T cell areas (mutual reinforcement)
- recruits B cells into LN (B cells pass through T cell area to follicle)

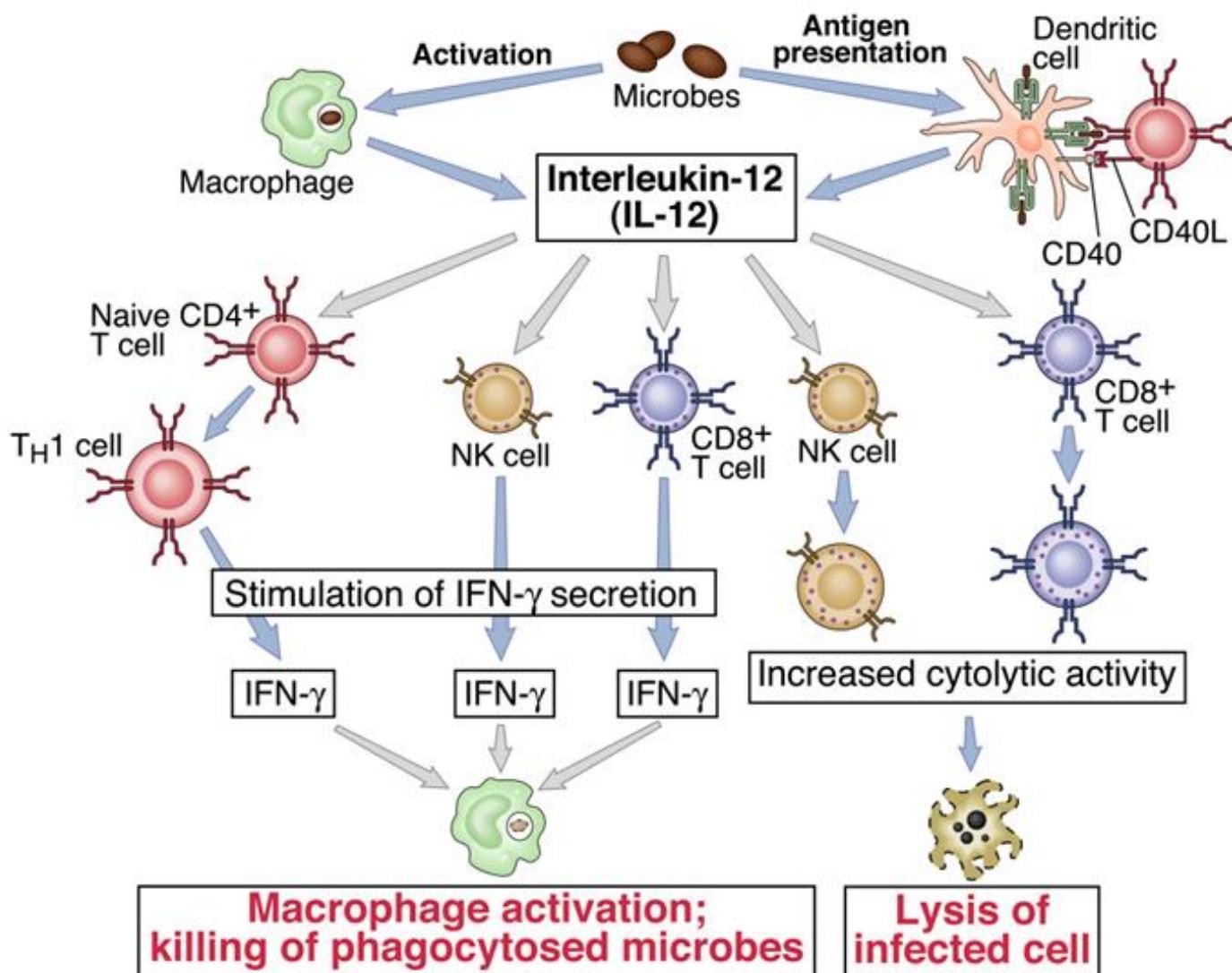
## CXCL13

- produced by follicular dendritic cells
- recruits B and activated T cells ( $T_{FH}$ ) into B cell follicles
- B cells in turn produce Lymphotoxin
- promotes development of follicular DC

## CCL18

- produced by APC, including DC
- recruits B and T cells into LN

# Functions of interleukin - 12



# Cytokines have many other biological functions

TABLE 12-1 Functional groups of selected cytokines<sup>1</sup>

Cytokine*	Secreted by**	Targets and effects
SOME CYTOKINES OF INNATE IMMUNITY		
Interleukin 1 (IL-1)	Monocytes, macrophages, endothelial cells, epithelial cells	Vasculature (inflammation); hypothalamus (fever); liver (induction of acute phase proteins)
Tumor necrosis factor- $\alpha$ (TNF- $\alpha$ )	Macrophages	Vasculature (inflammation); liver (induction of acute phase proteins); loss of muscle, body fat (cachexia); induction of death in many cell types; neutrophil activation
Interleukin 12 (IL-12)	Macrophages, dendritic cells	NK cells; influences adaptive immunity (promotes T <sub>H</sub> 1 subset)
Interleukin 6 (IL-6)	Macrophages, endothelial cells	Liver (induces acute phase proteins); influences adaptive immunity (proliferation and antibody secretion of B cell lineage)
Interferon $\alpha$ (IFN- $\alpha$ ) (this is a family of molecules)	Macrophages	Induces an antiviral state in most nucleated cells; increases MHC class I expression; activates NK cells
Interferon $\beta$ (IFN- $\beta$ )	Fibroblasts	Induces an antiviral state in most nucleated cells; increases MHC class I expression; activates NK cells
SOME CYTOKINES OF ADAPTIVE IMMUNITY		
Interleukin 2 (IL-2)	T cells	T-cell proliferation; can promote AIID; NK cell activation and proliferation; B-cell proliferation
Interleukin 4 (IL-4)	T <sub>H</sub> 2 cells; mast cells	Promotes T <sub>H</sub> 2 differentiation; isotype switch to IgE
Interleukin 5 (IL-5)	T <sub>H</sub> 2 cells	Eosinophil activation and generation
Interleukin 25 (IL-25)	Unknown	Induces secretion of T <sub>H</sub> 2 cytokine profile
Transforming growth factor $\beta$ (TGF- $\beta$ )	T cells, macrophages, other cell types	Inhibits T-cell proliferation and effector functions; inhibits B-cell proliferation; promotes isotype switch to IgE; inhibits macrophages
Interferon $\gamma$ (IFN- $\gamma$ )	T <sub>H</sub> 1 cells; CD8 $^{+}$ cells; NK cells	Activates macrophages; increases expression MHC class I and class II molecules; increases antigen presentation

\*Many cytokines play roles in more than one functional category.

\*\*Only the major cell types providing cytokines for the indicated activity are listed; other cell types may also have the capacity to synthesize the given cytokine.

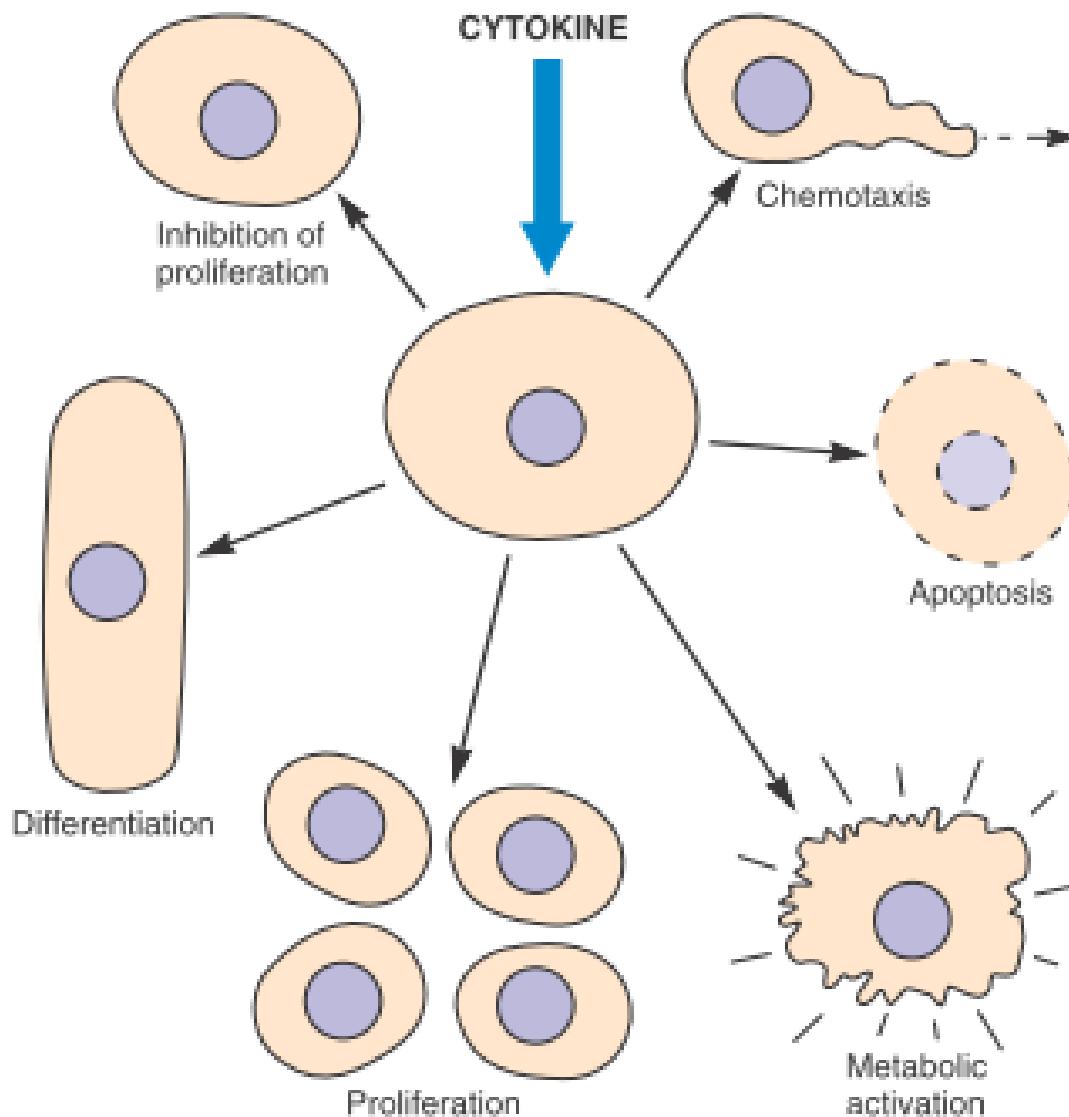
\*\*Also note that activated cells generally secrete greater amounts of cytokine than unactivated cells.

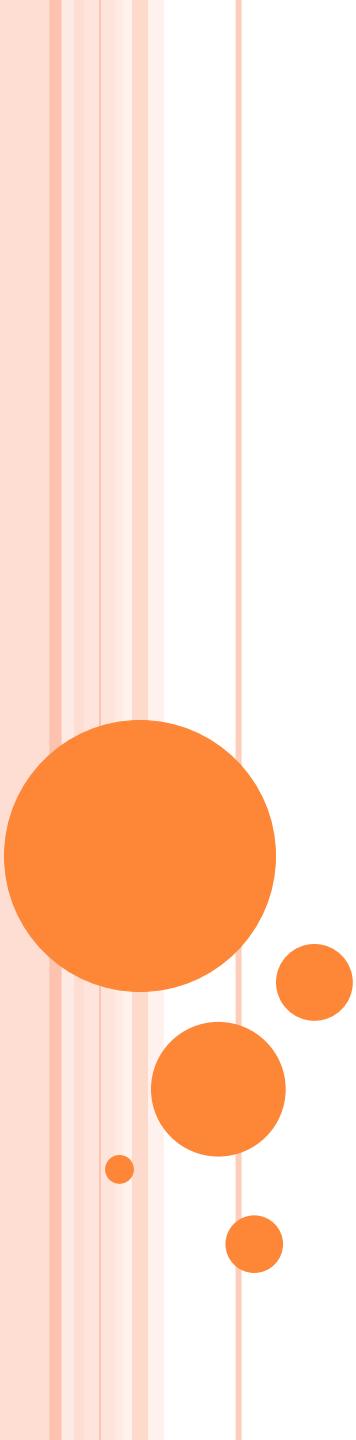
# Commercially available cytokines

Factor	Clinical uses	Dose	Side effects
(i) Recombinant -human Erythropoietin (Epoget; Procrit)	<ul style="list-style-type: none"> <li>Anaemia of chronic renal disease (in predialysis, dialysis dependent and chronic anaemia patients)</li> <li>Treatment of anaemia in cancer patients on chemotherapy.</li> <li>Anaemia in adult cancer patients with platinum chemotherapy</li> <li>Autologous predonation blood collection</li> <li>Anaemia in HIV infection to permit use of AZT</li> <li>MDS</li> <li>Post autologous peripheral blood stem cell transplantation</li> </ul>	(i) 50 – 100 u/kg thrice weekly (ii) In MDS 150 – 300 ug per kilogram per day	Hypertension; Seizures; Iron Deficiency; Thrombosis
(ii) Recombinant human Dysuria; granulocyte colony- stimulating factor (G- CSF) (Filgastrim; grade children; Neupogen)	<ul style="list-style-type: none"> <li>Chemo induced Neutropenia</li> <li>Optimization of chemotherapy</li> <li>Escalation of chemotherapy</li> <li>Peripheral progenitor cell mobilization</li> <li>Mobilization of donor's stem cells</li> <li>Congenital Neutropenia</li> </ul>	(i) 5 ug/kg.s.c/d until ANC $\geq$ 1.0 X10 <sup>9</sup> /l (ii) For mobilization of peripheral blood stem cells: 4.0 ug/kg, S.C/day with apheresis collection after 5 <sup>th</sup> and 6 <sup>th</sup> daily dose	Musculoskeletal pains; Liver enzymes elevation; Transient Hypotension; Allergic type reactions; Splenomegaly; Sweet's Syndrome; Low fever; Osteopenia in Capillary leak syndrome
(iii) Recombinant – human macrophage colony- pain;Chills; stimulating factor (GM- CSF) (Sargramostim;Leucomex) leak;Thrombotic Hypotension;Conjuctivitis;	<ul style="list-style-type: none"> <li>Acceleration of myeloid recovery in patients with Lymphoma who are undergoing autologous bone marrow transplantation</li> </ul>	250ug/s.c/day Or 5 ug/kg/d/s.c	Fever; Nausea; Fatigue; Headache; Bone Myalgias; Diarrhoea; Anorexia;  Arthralgias; Skin rashes; Facial flushing; Capillary events;

v) Interferon - $\alpha$	<ul style="list-style-type: none"> <li>• Chronic Myeloid Leukaemia</li> <li>• Chronic Hepatitis C</li> <li>• Hairy Cell Leukaemia</li> <li>• Kaposi Sarcoma</li> <li>• Cutaneous T cell Lymphoma</li> <li>• Low grade NHL</li> <li>• Multiple Myeloma</li> </ul>	3 millions U thrice weekly S.C • Chronic Hepatitis B	Fever; Shivering; Myalgias; Flu – like features; Lethargy; Malaise; Anorexia; Alopacia; Thrombocytopenia; Hyperthyroidism.
Pegylated IFN (Peg- Intron)		0.5 – 2.0 ug/kg body weight Once weekly; S.C	
(v) Thrombopoietin	<ul style="list-style-type: none"> <li>• To treat thrombocytopenia due to myelosuppressive therapy</li> <li>• To increase platelet yield in plateletpheresis donors</li> <li>• Advanced renal cell carcinoma</li> </ul>	1 – 3 ug/kg body weight	
(vi) Interleukin 2 (Proleukin)			
(vii) Recombinant Interleukin -11 (Neumega)	<ul style="list-style-type: none"> <li>• Treatment of severe thrombocytopenia due to myelosuppressive therapy in patients with non-myeloid malignancies</li> </ul>		
(viii) IL- 3 reaction;fever;	<ul style="list-style-type: none"> <li>• Chemotherapy induced myelosuppression</li> </ul>	,5,10 or 15ug/kg s.c or continuous i.v infusion	Injection site headache;rash;flu- like symptoms; facial flushing.

# In conclusion cytokines works for





# **HYBRIDOMA AND MONOCLONAL ANTIBODIES**

**Dr. Sandeep Kumar Sharma, BVSc & AH, MVSc and PhD  
Assistant Professor, PGIVER  
[drsharmask01@hotmail.com](mailto:drsharmask01@hotmail.com)  
Mob. 9414775879**

- Hybridoma technology includes formation of hybrid cell lines (hybridomas) by fusing a specific antibody-producing B cell with a myeloma (cancer cell) cell.
- Can grow in tissue culture (*in-vitro*) and have a ability to produce specific antibodies known as monoclonal antibodies (single specificity)
- The term hybridoma was coined by **Leonard Herzenberg**.
- The production of monoclonal antibodies was invented by **Cesar Milstein and Georges J. F. Köhler** in 1975.
- They shared the Nobel Prize of 1984 for Medicine and Physiology with Niels Kaj Jerne.



# **ANTIBODIES**

## **POLYCLONAL**

**Each identifying a different epitope**

**Derived from different B Lymphocytes cell lines**

**Batch to Batch variation affecting Ab reactivity & titre**

**NOT powerful tools for clinical diagnostic tests**

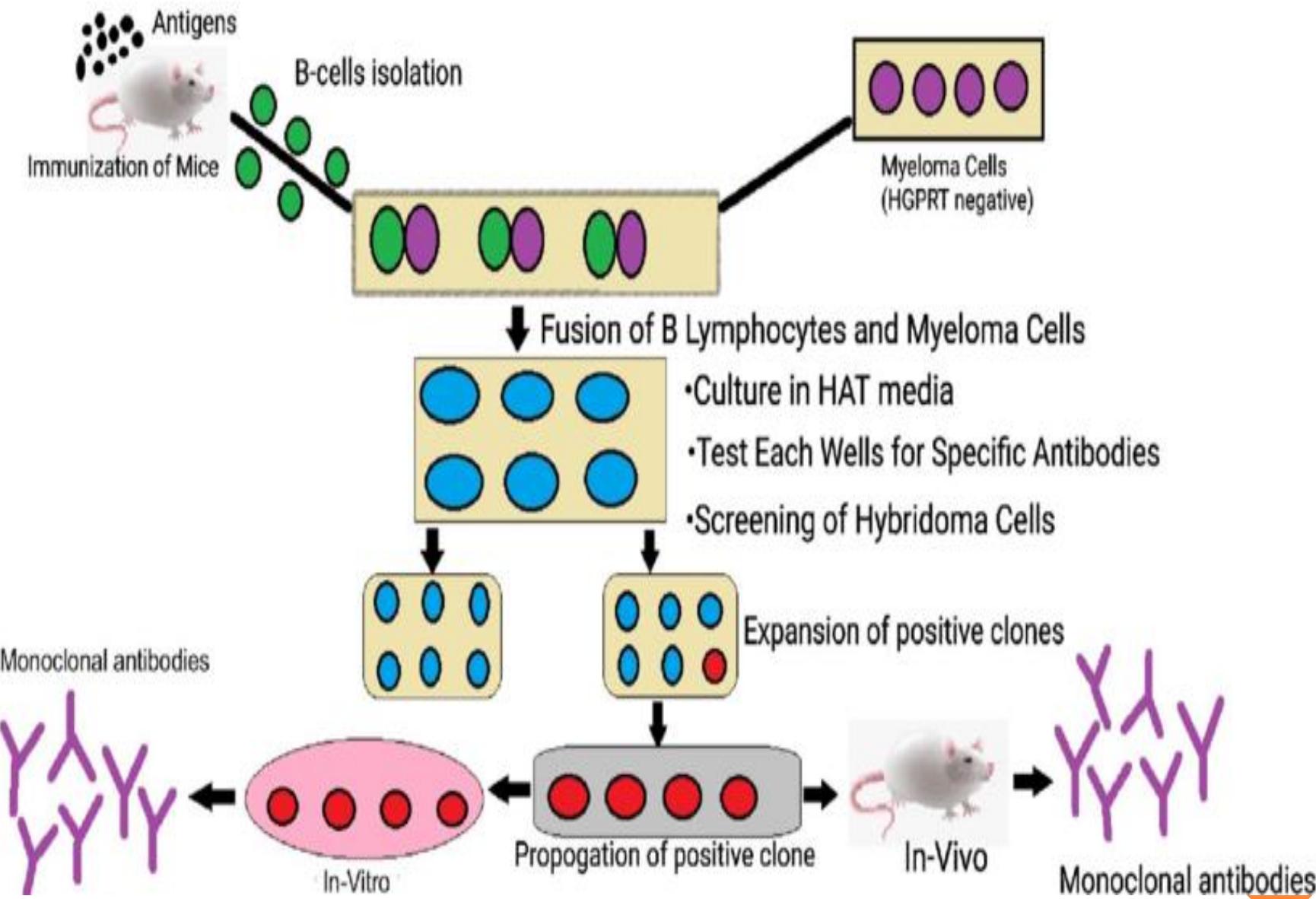
## **MONOCLONAL**

**They bind to the same epitope**

**Derived from a single B cell clone**

**mAb offer Reproducible, Predictable & Potentially inexhaustible supply of Ab with exquisite specificity**

**Enable the development of secure immunoassay systems.**



# Preparation of monoclonal antibodies using hybridoma

## ❖ *Immunization / Specific Antigen Inoculation*

- The first step involves injecting the laboratory animals like rabbits or mice with a selected antigen against which the antibodies are raised through a series of injections over a period of several weeks to stimulate B cell differentiation into plasma B cells and memory B cells.



# Preparation of monoclonal antibodies using hybridoma

## ❖ *Isolation of B lymphocytes*

- Following sacrifice, the spleen is removed in aseptic conditions to **isolate the activated B-cells.**
- This procedure is performed using density gradient centrifugation.
- The presence of antibodies in the serum is identified using methods like ELISA.



# Preparation of monoclonal antibodies using hybridoma

## ❖ *Preparation of Myeloma Cell Lines*

- Few weeks before the cell fusion, metastatic tumor cells are incubated in **8-azaguanine** to get non-functional hypoxanthine-guanine phospho ribosyl transferase (HGPRT) genes in the myeloma cells.
- Non-functional HGPRT can stop the assembly of nucleotides from the salvage pathway and makes the metastatic tumor cells sensitive to HAT media.



# Preparation of monoclonal antibodies using hybridoma

## ❖ *Cell fusion*

- Cell fusion is the process in which the activated B lymphocytes are fused with HAT-sensitive myeloma cells.
- This step is performed by centrifugation of freshly obtained activated B-cells with HAT-sensitive myeloma cells in a fusion-promoting media.
- Polyethylene glycol(PEG) is used in this procedure.



# Preparation of monoclonal antibodies using hybridoma

## ❖ *Hybridoma Selection*

- In the PEG-containing media, cells are fused to form hybridoma cells but even the most efficient fusion method will allow the formation of **only about 1 to 2% of fused hybridoma cells.**
- Furthermore, about 1 in 100 cells will be viable hybrid cells. Therefore, there are a number of un fused cells within the media.
- This step allows the selection of the fused cells from all the un fused cells.

# Preparation of monoclonal antibodies using hybridoma

## ❖ *Hybridoma Selection*

- This is achieved by incubating the cell mixture followed by culturing for 10–14 days in HAT media (a selection media).
- HAT medium contains Hypoxanthine Aminopterin Thymidine.
- Aminopterin present in HAT media blocks the power of cells to synthesize nucleotides by the **de novo synthesis pathway**.
- Hypoxanthine and deoxy thymidine allow cells with functional hypoxanthine-guanine phospho ribosyl transferase (HGPRT) genes to survive through **salvage pathways**.



# Preparation of monoclonal antibodies using hybridoma

## ❖ *Hybridoma Selection*

- Due to a limited life span, un fused B cells perish within a few days.
- Un fused malignant neoplastic cells die as a result of the lack of the hypoxanthine-guanine phospho ribosyl transferase (HGPRT) gene.
- The presence of aminopterin blocks their ability to synthesize nucleotides through the de novo pathway.
- Therefore, the remaining viable cells left in the media are the hybrid cells; these hybrid cells have the ability to grow and divide on HAT media.

# **Preparation of monoclonal antibodies using hybridoma**

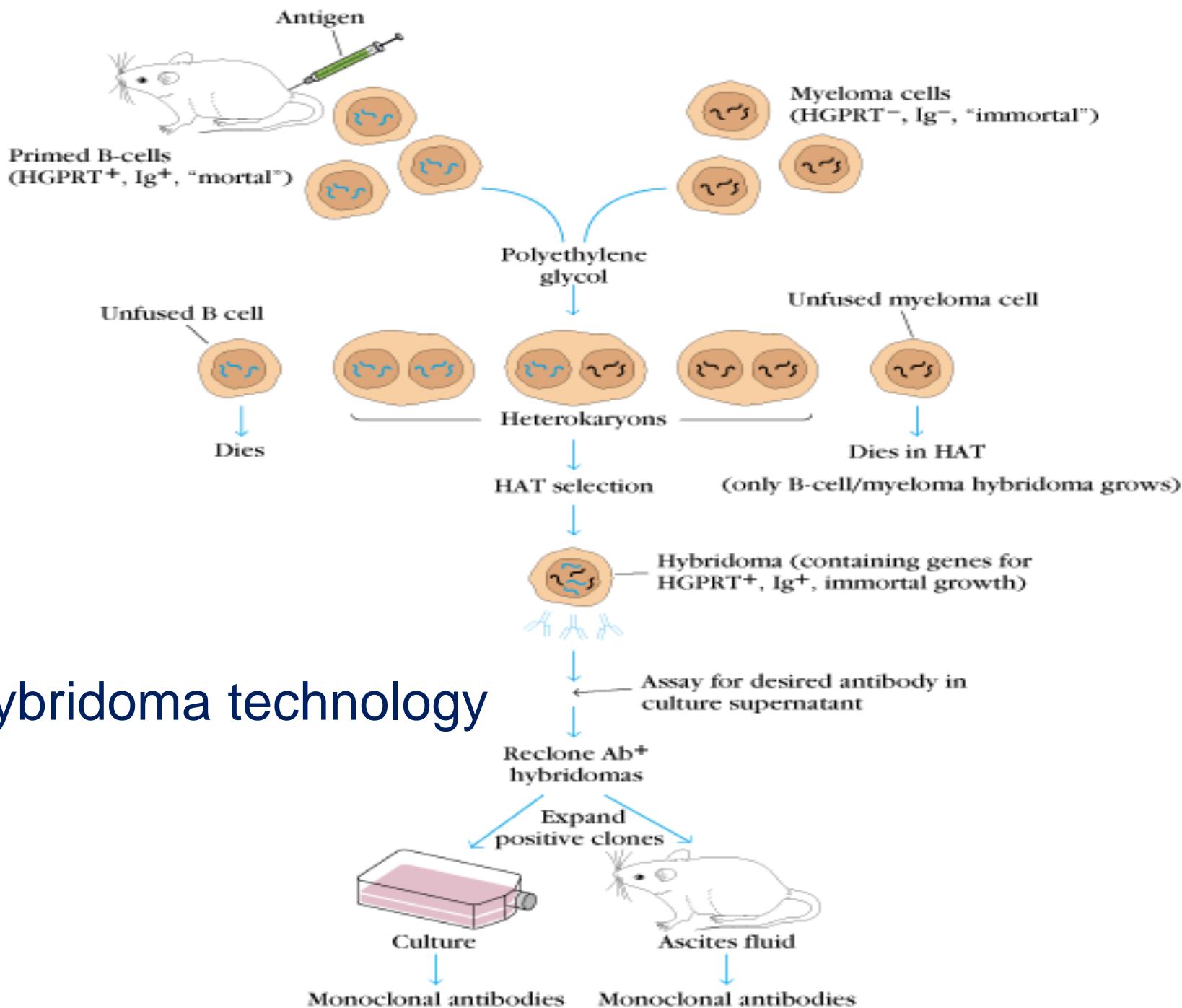
## ❖ *Screening of Hybridoma Cells*

- HAT-selection hybridoma cells are transferred to ELISA plates, where each well houses a single hybridoma cell.
- This is achieved using the limiting dilution method.

## ❖ *Cloning and propagation of hybridoma cell*

- Hybridomas producing desired antibodies are selected and are then transferred into large culture vessels or flasks
- The hybridoma cell lines are cultured using in vivo or in vitro methods.





# Two different pathways to synthesis nucleotide in mammalian cells

## DE NOVO PATHWAY

Phosphoribosyl  
pyrophosphate  
+  
Uridylate



## SALVAGE PATHWAY

Thymidine

TK<sup>+</sup>

(thymidine kinase)



Hypoxanthine

HGPRT<sup>+</sup>

(hypoxanthine guanine phosphoribosyl transferase)

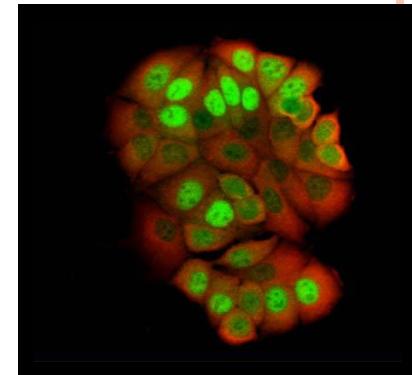
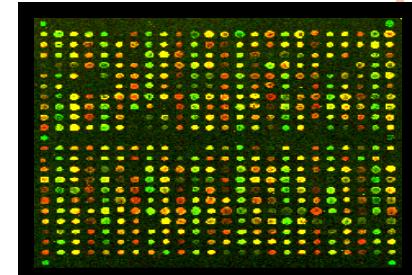


**Myeloma cells used in hybridoma technology are double mutants, they lack the HGPRTase and lose the ability to produce Ig**

- Myeloma cells are immortal but lack the HGPRT (Hypoxanthine Guanine Phospho Ribosyl Transferase) gene and
- Aminopterin in the HAT medium causes myeloma cells death, as they cannot produce nucleotides by the de novo or salvage medium blocks the pathway that allows for nucleotide synthesis.
- B- cells have short life span but can produce antibody

# APPLICATIONS OF MONOCLONAL ANTIBODIES

1. Diagnostic Applications.  
Biosensors & Microarrays.
2. Therapeutic Applications.  
Transplant rejection (Muronomab-CD3).  
Cardiovascular disease (Abciximab).  
Cancer (Rituximab).  
Infectious Diseases (Palivizumab).  
Inflammatory disease (Infliximab).
3. Clinical Applications.  
Purification of drugs, Imaging the target.
4. Future Applications.  
Fight against Bioterrorism.



# HUMAN MONOCLONAL ANTIBODIES

- Production of human monoclonal antibody
  - There are numbers of technical difficulties
    - The lack of human myeloma cells to exhibit immortal growth, be susceptible to HAT selection, to not secrete antibody, and support antibody production in the hybridoma made with them
    - Human B cell sometimes have immortality
    - That is the difficulty of readily obtaining antigen-activated B cells
  - To culture human B cells in vitro to produce human monoclonal antibody
    - Transplant human cells with immune response into SCID mice (lack a functional immune system)

# Thank You

Dr. Sandeep Kumar Sharma, BVSc & AH, MVSc and PhD  
Assistant Professor, PGIVER  
[drsharmask01@hotmail.com](mailto:drsharmask01@hotmail.com)  
Mob. 9414775879



# **MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)**



**Dr. Sandeep Kumar Sharma, BVSc & AH, MVSc and PhD**  
**Assistant Professor, PGIVER**  
**[drsharmask01@hotmail.com](mailto:drsharmask01@hotmail.com)**  
**Mob. 9414775879**

# HISTORY

- In the mid 1930s, the concept of histocompatibility antigens originated from the work of Peter Gorer, who observed that the rejection of foreign tissue is the result of an immune response to cell-surface molecules.
- During the 1940s, Medawar and his colleagues demonstrated that tissue graft rejection in rabbits was indeed due to an immune response attacking the foreign tissue graft.



# HISTORY

- In the 1940s and 1950s by Gorer and George Snell established that antigens encoded by the genes in the group designated II took part in the rejection of transplanted tumors and other tissue.
- Snell called the genes controlling tissue rejection ‘histocompatibility (H) genes’, In 1980 Snell was awarded the Nobel prize for this work

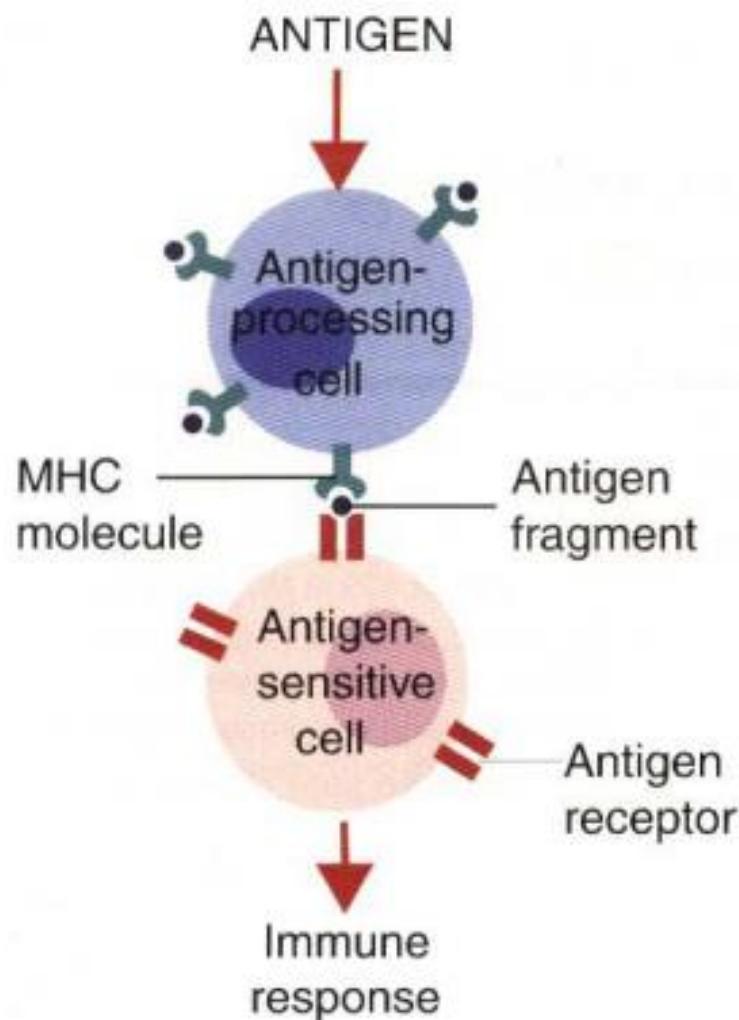


# HISTOCOMPATIBILITY MOLECULES

- Antigen presentation:
  - In order to trigger acquired immunity, antigens phagocytosed by cells are degraded.
  - The degraded antigens are presented by these cells on plasma membrane for recognition by T-cells.
- But for this antigen presentation, these antigen fragments are bound to antigen-presenting receptors called **Histocompatibility molecules**.
- Antigens can trigger immune response only when they are bound to these histocompatibility molecules.

- Histocompatibility molecules are glycoproteins encoded by genes belonging to a gene cluster known as **Major Histocompatibility Complex (MHC)**.

- Therefore these receptors are also called **MHC Molecules**.
- This gene complex can be very large.
- for example, the human MHC is about 04mb in size, which is about the same size as the total genome of the bacterium *E. coli*



# MAKE UP OF MHC GENE CLUSTER

- Three classes of gene loci are found:

**Loci I :** Code for MHC molecules found on **all nucleated cells.**

subclasses Ia, Ib, Ic and Id.

Ia is **highly polymorphic.**

Present antigen to **cytotoxic T cells.**

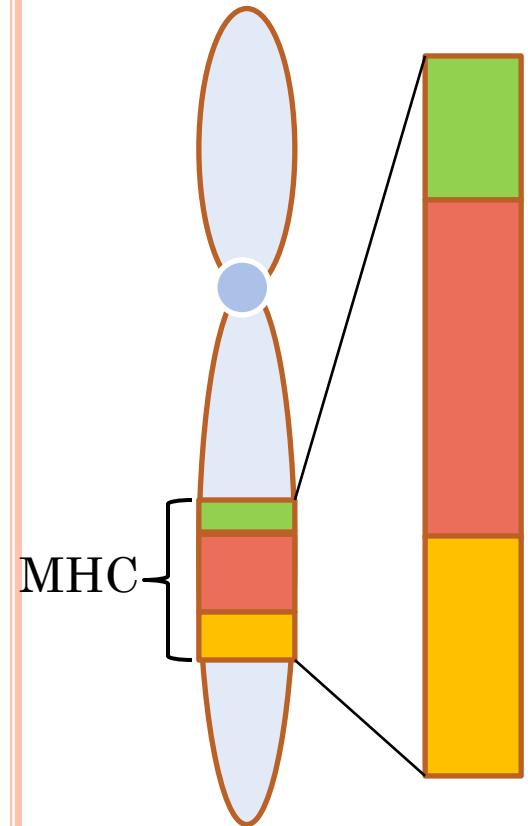
T cell mediated toxicity.

**Loci II :** Code for MHC found on **antigen presenting cells.**

Present antigen to **helper T cells.**

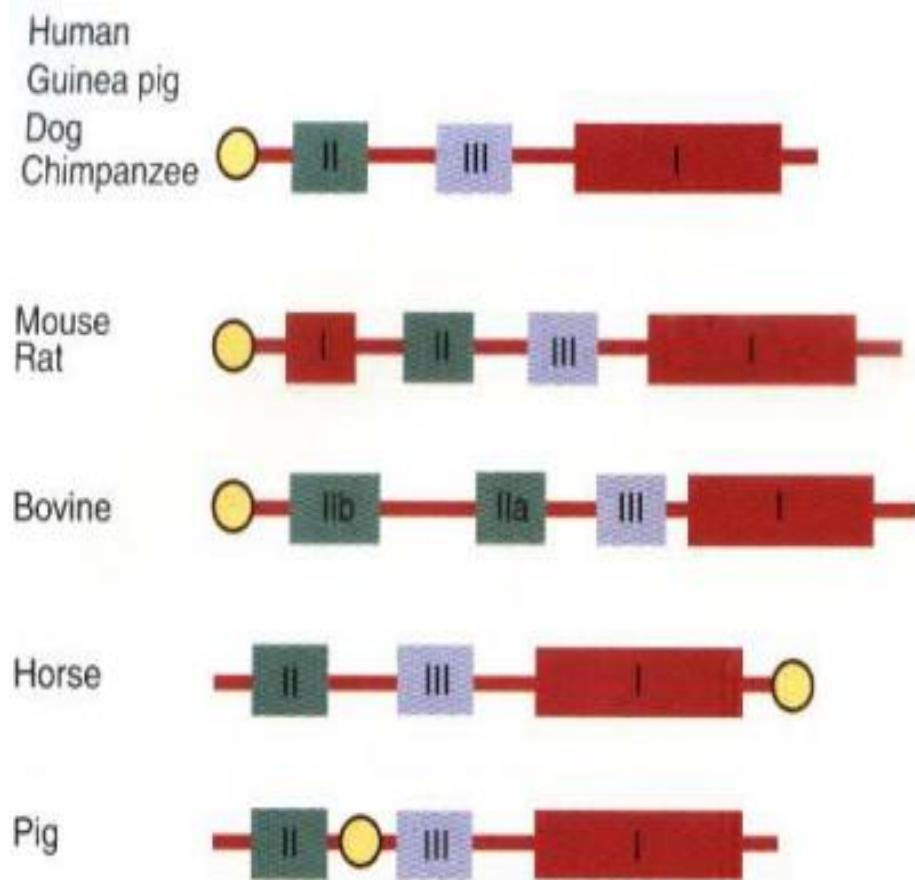
T cell mediated stimulation/help.

**Loci III :** Code for proteins with **diverse functions.** Ex. Some complement proteins C2, C4, Factor B, Cytokine TNF- $\alpha$ , some heat shock proteins etc.



- Each MHC contains all three classes of loci, although their name, number and arrangement vary with different species for example
- Human- HLA (6), Dog- DLA (12), Horse- ELA (20), Cattle- BoLA(23), Pig-SLA (7)

& The complete set of alleles found within an animal's MHC is called its MHC Haplotype.



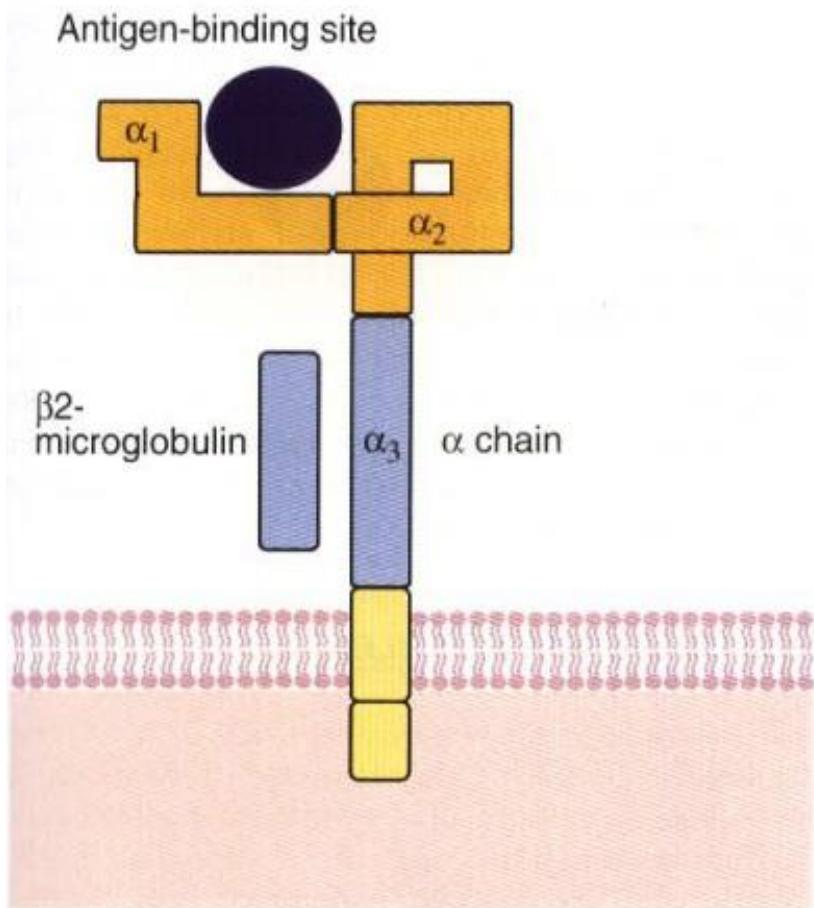
# STRUCTURE OF CLASS I MHC MOLECULE

- Class I MHC are **Glycoproteins** expressed on all nucleated cells, for example, in pigs detected on lymphocytes, platelets, granulocyte, hepatocyte, kidney cells and sperms.
- Usually not found on mammalian red cells, gametes, neurons or tropholast cells
- Structure: Made up of two glycoprotein chains
  - One 45 kD  **$\alpha$  chain**, and
  - Second small 12 kD  **$\beta_2$  microglobulin ( $\beta_2$  M)** chain.
- $\alpha$  chain consists of 5 domains.
- $\alpha_1$  and  $\alpha_2$  domains form the antigen binding site.
- $\beta$  2 chain stabilize the structure.

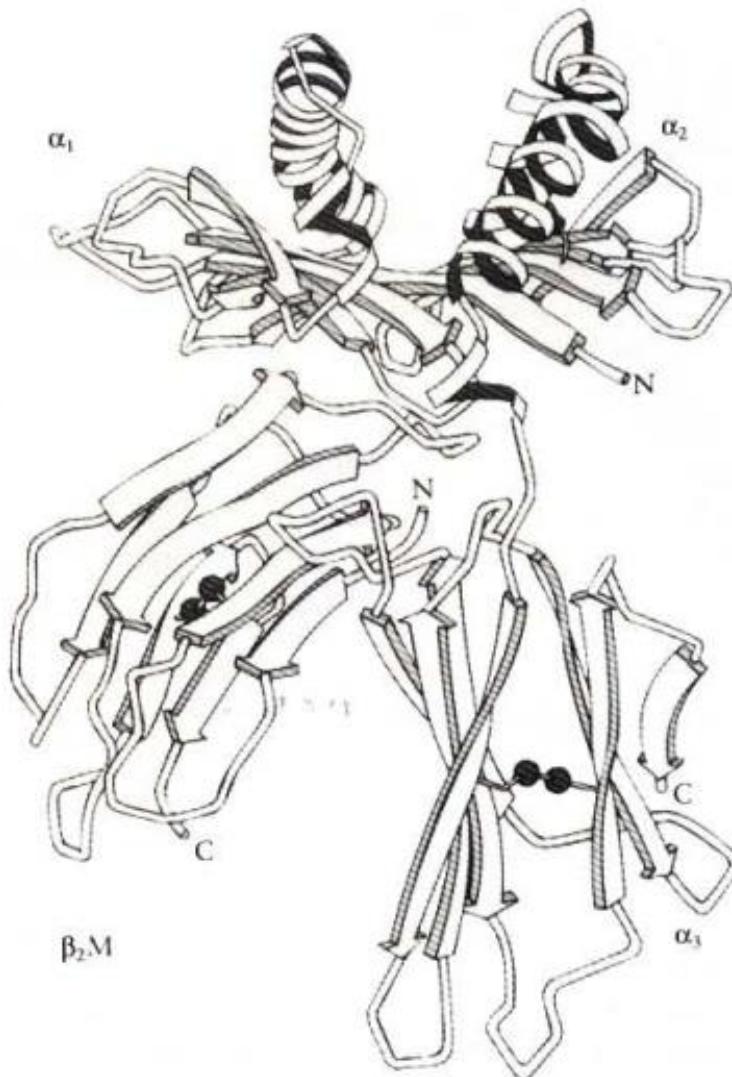


- The alpha chain is attached to the cell where as the beta chain is not attached to the cell and is bound non-covalently with the alpha chain.
- There are four discrete domains in a MHC I molecules
  - i. External polypeptide binding domain
  - ii. Immunoglobulin like domain
  - iii. Transmembrane domain
  - iv. Cytoplasmic domain
- The external polypeptide-binding domain is made up of 180 amino acids with two homologous segments called as alpha 1 and alpha 2 segments.
- This two segments form a cleft like structure with approximate dimension of ( $25\text{\AA} \times 10\text{\AA} \times 11\text{\AA}$ ).

- This cleft can take a polypeptide of 9-11 amino acid (epitope) and presents to T cells.
- The immunoglobulin like domain is made up of 90 amino acids and have disulphide bonds bound a loop.
- The amino acids in this region are highly conserved and no variations are found between different MHC I molecules.
- This domain is also named as alpha3 domain and is responsible for binding with CD8 receptor.
- The remaining transmembrane domain and cytoplasmic domain help in anchoring the MHC I molecules to cell surface.

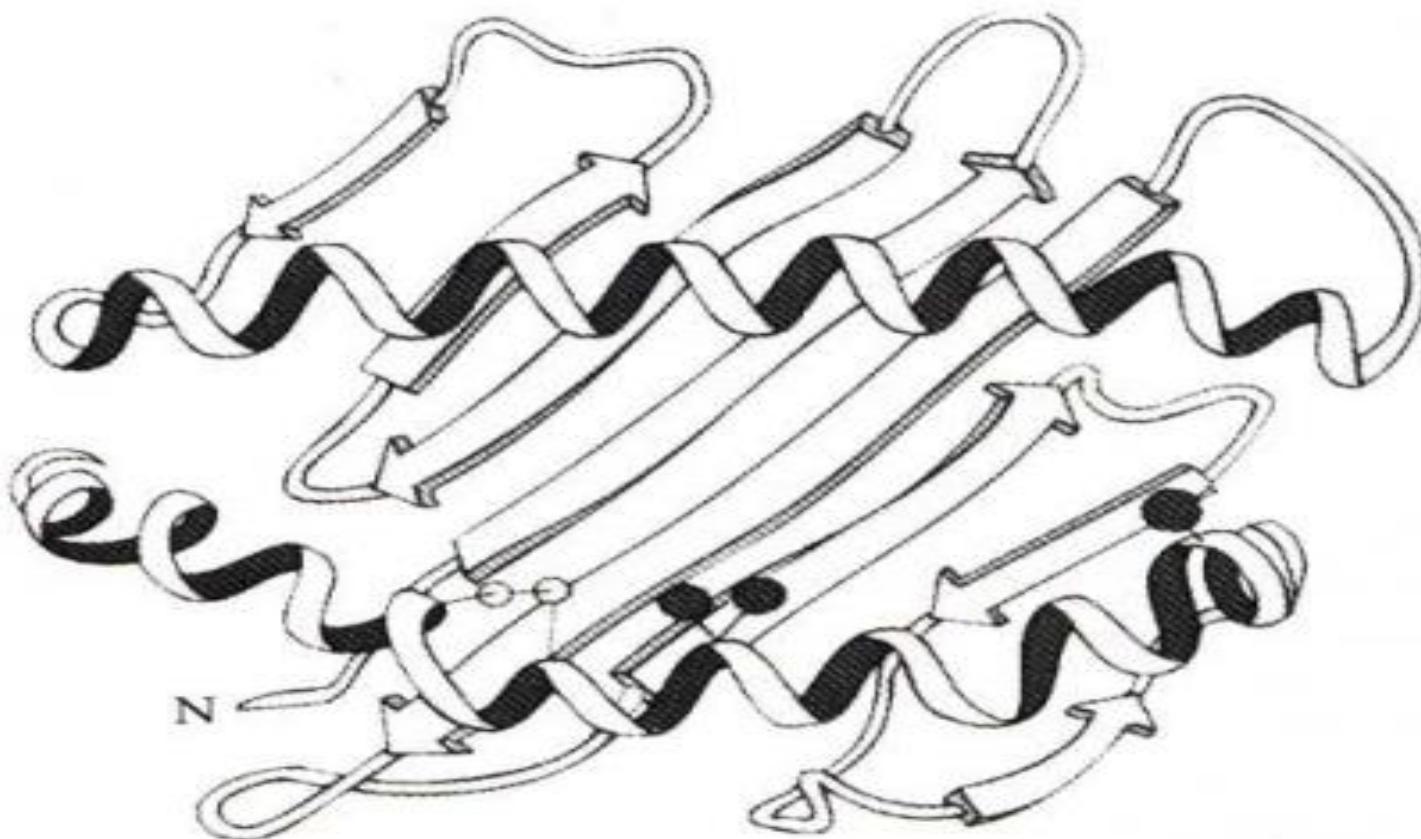


**Figure 7-3.** Diagram showing the structure of a class Ia MHC molecule on a cell membrane. Its antigen-binding site is formed by the folding of its  $\alpha_1$  and  $\alpha_2$  domains.



**Figure 7-5.** Schematic three-dimensional view of the complete structure of HLA-A2 derived by x-ray crystallography. The antigen-binding groove at the top is formed by the  $\alpha_1$  and  $\alpha_2$  domains, whereas the  $\alpha_3$  domain binds to the cell membrane. The  $\beta$  chain ( $\beta 2$ -microglobulin) has no direct role in antigen binding. (From *Nature* 320:506, 1987. Macmillan Magazines Ltd.)

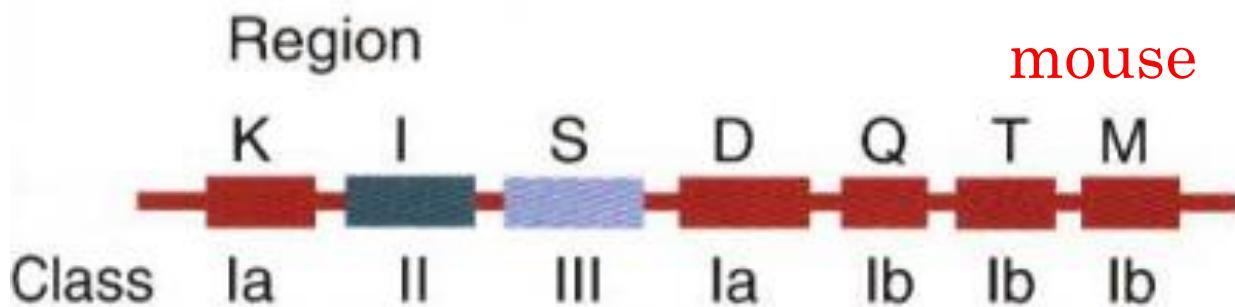
# Structure of Class I MHC molecule



**Figure 7-6.** A view (from above) of the antigen-binding groove on a MHC class I molecule. The floor of the groove is formed by an extensive  $\beta$ -pleated sheet. The walls of the groove are formed by two parallel  $\alpha$  helices. This structure is formed by the folding of the  $\alpha_1$  and  $\alpha_2$  domain of the  $\alpha$  chain. (From *Nature* 320:506, 1987. Macmillan Magazines Ltd.)

# GENE ARRANGEMENT

- The total number of class I loci varies greatly between mammals.
- Rats have more than 60, mice 30, human 20, cattle 13 to 15 and pig have 11 but not all these loci as functional and code for cell surface protein.
- For example mice only two or three class Ia gene are expressed.
- In humans the functional loci are called *A*, *B*, *C* and in mice they called *K* and *D* (some strains, *L*)



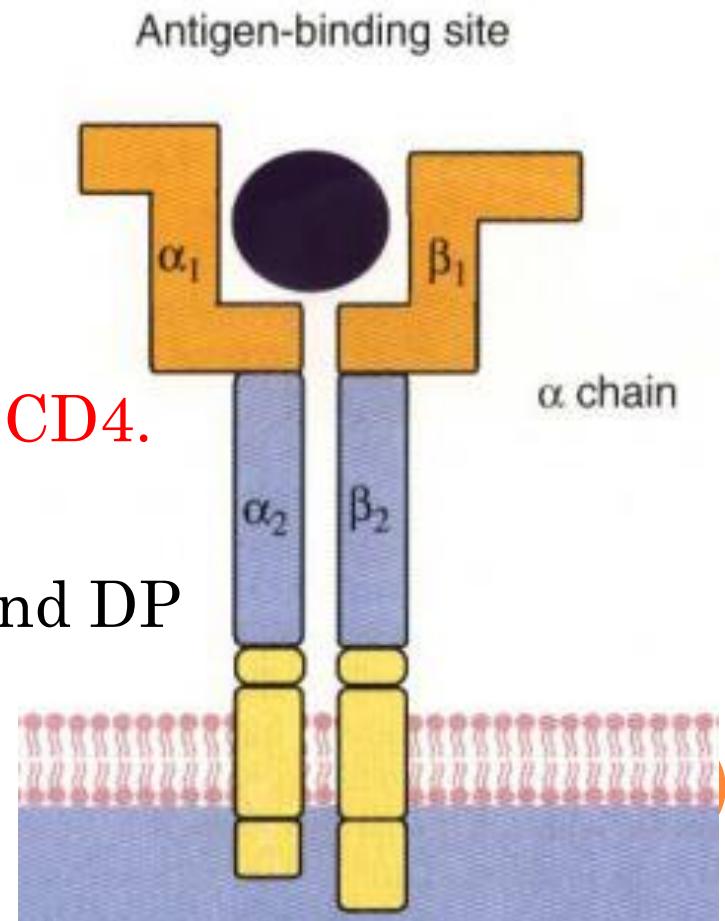
# Polymorphism in Class I MHC molecule

- Some of the class Ia gene loci code have a large number of alleles, these allelic differences cause variations in the amino acid sequence of the  $\alpha 1$  and  $\alpha 2$ . This variation is called polymorphism.
- These variable regions are restricted to three to four discrete regions within the  $\alpha 1$  and  $\alpha 2$  domains while other domains of MHC class Ia molecules are highly conserved and not show evident sequence variation.
- This nucleotide sequence variability in MHC alleles result of
  - Point mutation,
  - Reciprocal recombination and
  - Gene conversion



# STRUCTURE OF CLASS II MHC MOLECULE

- Class II MHC molecule encode glycoproteins expressed primarily on antigen-presenting cells (macrophages, dendritic cells and B cells) where they present processed antigenic peptides to T<sub>H</sub> cells.
- Two protein chains –  $\alpha$  and  $\beta$ .
- $\alpha$  chain = 31 to 34 kDa
- $\beta$  chain = 25 to 29 kDa
- Third protein chain = Ii or  $\gamma$
- The  $\beta_2$  is the binding site for the CD4.
- The  $\alpha$  chains are the HLA - DR
- The  $\beta$  chains are the HLA - DQ and DP

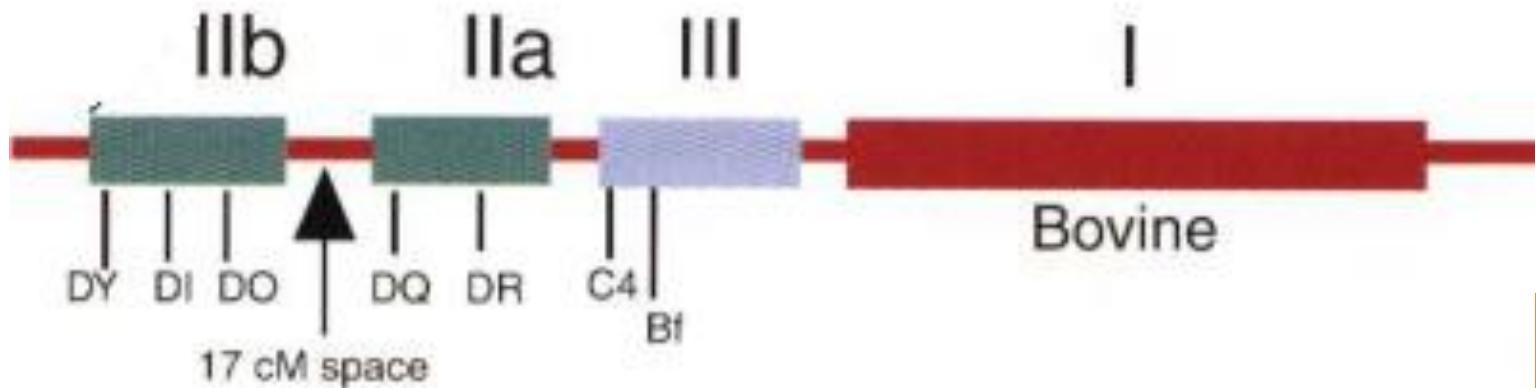


- The alpha chain is slightly larger than the beta chain. As in MHC I molecule the MHC II molecules also contain four discrete domains
  - i. External polypeptide binding domain
  - ii. Immunoglobulin like domain
  - iii. Transmembrane domain
  - iv. Cytoplasmic domain
- The **external polypeptide binding** site of the MHC II molecule is made up 90 amino acids and is formed by interaction of part of alpha and beta chains (alpha 1 and beta 1).
- The **immunoglobulin like domain** is made up of 90 amino acids and have disulphide bonds bound a loop. This domain is formed by part of alpha and beta chains (alpha 2 and beta 2).

- The amino acids in this region are highly conserved and no variations are found between different MHC II molecules and are responsible for binding with CD4 receptor.
- The remaining transmembrane domain and cytoplasmic domain help in anchoring the MHC II molecules to cell surface.

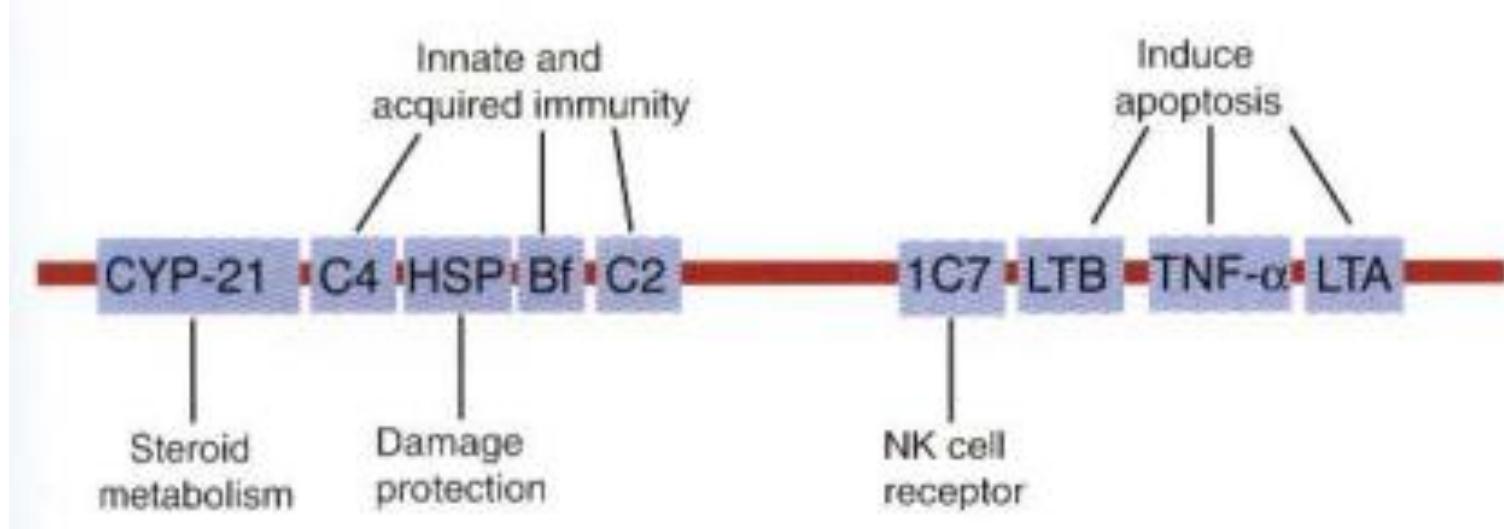
## GENE ARRANGEMENT

- Within the MHC class II region there are six loci arranged in order DP, DOA, DM, DOB, DQ and DR
- Not all loci contain genes for both chain and some contain many pseudogenes. These pseudogenes may be used to generate additional class II polymorphisms
- While nonpolymorphic loci such as DM and DO, whose function is to regulate the loading of antigen fragments into the groove



# MHC CLASS III MOLECULES

- They code for proteins with many different functions Such as four genes for complement components: two for C4 and one each for factor B and C2
- Code for the enzyme 21-hydroxylase involved in steroid synthesis, for cytochrome P450, for tumor necrosis factor  $\alpha$ , for several lymphotoxins, for some NK cell receptors and for several heat-shock proteins (HSP)



& In mice, MHC is called H2 complex. Present on chromosome 17.

Mouse H-2 complex

Complex	H-2						
MHC class	I	II		III		I	
Region	K	IA	IE	S		D	
Gene products	H-2K	IA αβ	IE αβ	C' proteins	TNF-α TNF-β	H-2D	H-2L

- In humans, it is called Human Leukocyte Antigen (HLA) Complex, present on chromosome 6.

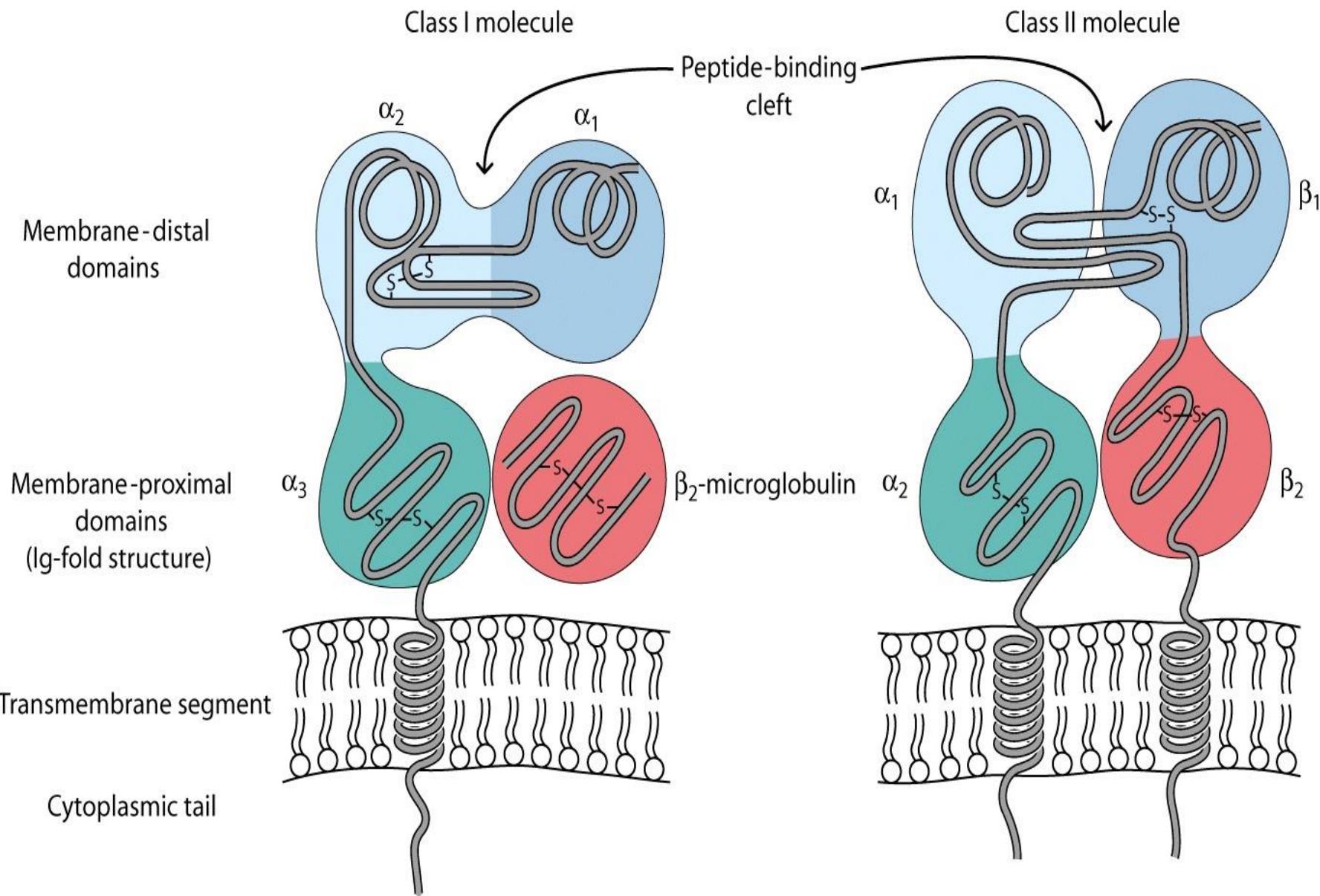
Human HLA complex

Complex	HLA							
MHC class	II			III		I		
Region	DP	DQ	DR	C4, C2, BF		B	C	A
Gene products	DP αβ	DQ αβ	DR αβ	C' proteins	TNF-α TNF-β	HLA-B	HLA-C	HLA-A

# Comparison of MHC class I & II molecule

Feature	Class I MHC	Class II MHC
<b>Polypeptide chains</b>	$\alpha$ (44-47kD) $\beta_2$ - Microglobin (12kD)	$\alpha$ (32-34kD) $\beta$ (29 -32kD)
<b>Locations of polymorphic residues/ Peptide binding domain</b>	$\alpha 1$ and $\alpha 2$ domains	$\alpha 1$ and $\beta 1$ domains
<b>Binding site for T cell coreceptor</b>	$\alpha 3$ regions binds CD8	$\beta 2$ regions binds CD4
<b>Nature of peptide binding cleft</b>	Closed at one ends	Open at both ends
<b>General size of bound peptides</b>	8-10 amino acids	13-18 amino acids
<b>Peptide motifs involved in binding to MHC molecules</b>	Anchor residues at both ends of peptide; generally hydrophobic carboxyl-terminal anchor	Anchor residues distributed along the length of the peptide
<b>Nature of bound peptide</b>	Extended structure in which both ends interact with MHC cleft but middle arches up away from MHC molecule	Extended structure that is held at a constant elevation above the floor of MHC cleft
<b>Nomenclature</b>		
<b>Human</b>	HLA- A, B, C	HLA- DR, DQ, DP
<b>Mouse</b>	H- K, D, L	I- A, E

# Comparison of MHC class I & II molecule



# Mechanism of MHC molecules-I (Processing of Endogenous Antigens)

- Endogenous antigens are type of antigens that are generated inside the cells.
- Some of the common examples of endogenous antigens are **virus proteins and cancer proteins**.
- The antigen processing is taken care by all nucleated cells.
- In other words all nucleated cells act APC for endogenous antigens. The MHC molecules involved in presentation is MHC Class I molecules.

- The peptides (epitopes) that bind to MHC I molecules are proteolytically generated inside the cytoplasm.
- The major mechanism for the generation of peptides from endogenous antigens is **proteolysis** is special complexes inside the cell called proteasomes.
- These proteasomes are large multiprotein complex with a broad range of proteolytic activity.
- Two different types of proteasomes are found inside the cell. This classification is based on the size.



- One proteosome with 700 kD size appears as cylinder composed of stacked array of four inner and four outer rings with each ring composed of seven distinct subunits. The subunits of inner rings are the catalytic sites for proteolysis.
- The second type of proteosome is of 1500 kD in size and it is composed of 700 kD structure along with additional subunits that regulate proteolytic activity.
- The endogenous antigens that are found inside the cell are taken to this proteosomes by a special polypeptide called ubiquitin.

- Several ubiquitins are found attached to a single endogenous antigen just like string of pearls. This process is called as polyubiquitination.
- The endogenous antigens are broken into small polypeptides of 5-10 amino acids inside the proteosome complexes. This breaking resembles the act of meat grinding in meat grinder.
- The small polypeptides thus formed are modified structurally suitable for fixing into the cleft of MHC I molecule. Now the small peptides are ready for binding with MHC I molecules for presentation to cytotoxic T cells.

- The two chains of MHC I molecules are synthesised separately at the rough ER and the move to smooth ER where they are assembled into a dimer structure. This structure formation is facilitated and stabilized by calnexin, BiP and TAP proteins.
- Then the MHC I molecules move to proteosomes, bind with small peptides and move through Golgi to the surface of cell by exocytic vesicles. At the surface of the cells they are recognized by cytotoxic cells.

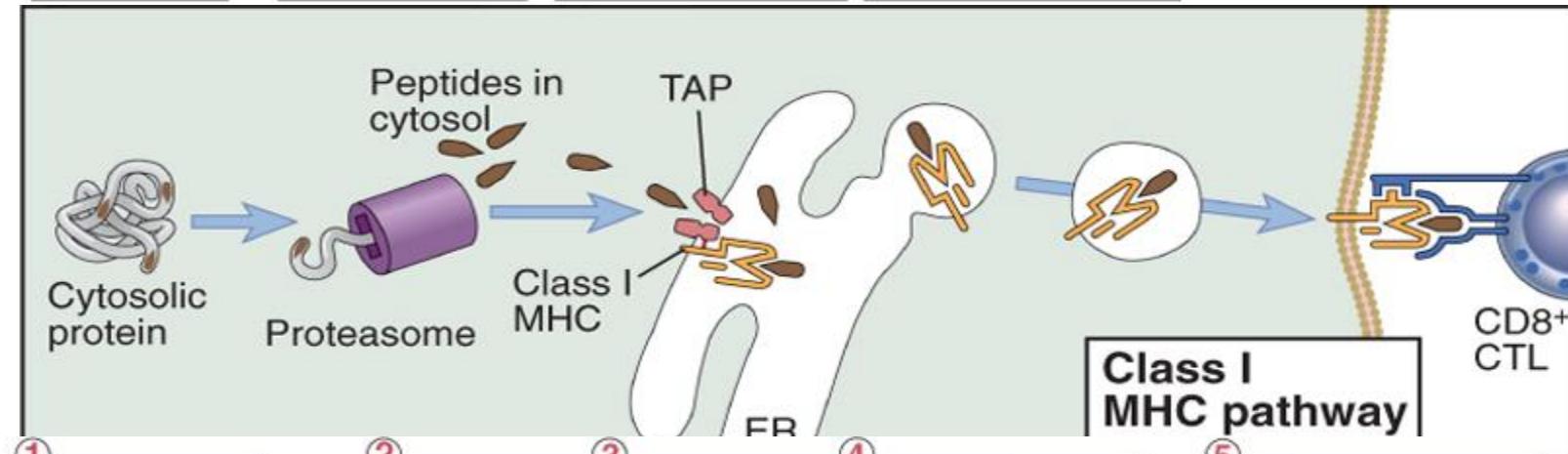


Antigen uptake

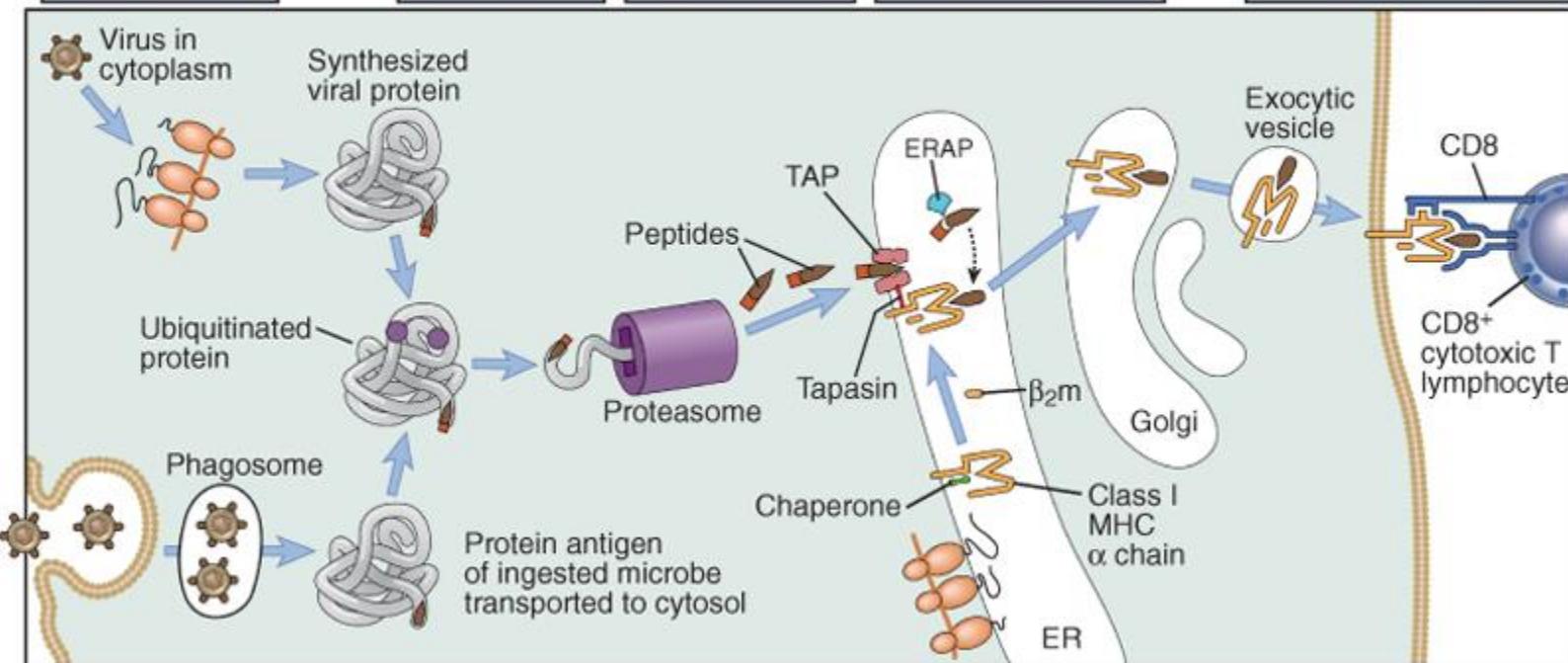
Antigen processing

MHC biosynthesis

Peptide-MHC association



- ① Production of proteins in the cytosol
- ② Proteolytic degradation of proteins
- ③ Transport of peptides from cytosol to ER
- ④ Assembly of peptide-class I complexes in ER
- ⑤ Surface expression of peptide-class I complexes



# Mechanism of MHC molecules-II (Processing of Exogenous Antigens)

- Internalization of antigen: For antigen processing to take place first the antigens should enter into APC. This usually takes place by phagocytosis, pinocytosis or endocytosis.
- Macrophages on their surface exhibit receptors for Fc portion of antibody. Hence opsonised bacteria can easily be taken into the cell. Besides this macrophages also exhibit receptors that bind with mannose residues of the polysaccharide on bacterial cell wall.
- The internalized antigen becomes localized inside the APC in a membrane bound vesicular structure called endosome.



- The proteolytic processing of proteins takes place either in endosomes or in lysosomes. Both endosomes and lysosomes have an acidic pH that is pre requisite for antigen processing.
- Proteolytic processing requires proteases enzymes and some of the common protease enzymes found in endosomes and lysosomes are cathepsin and leupeptin.
- These enzymes break the peptide antigen into small peptides of 10-30 amino acids long that are capable of binding with clefts of MHC II molecules.



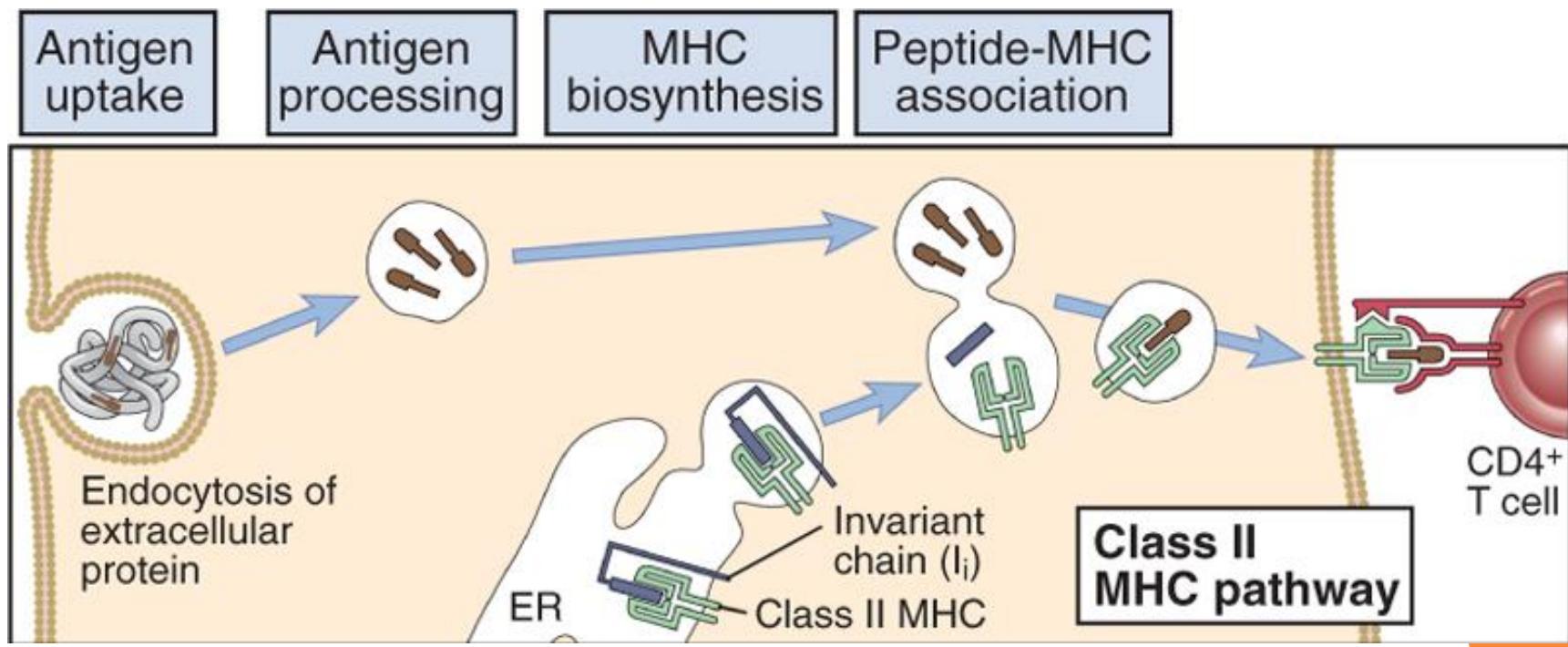
- The alpha and beta chains of MHC II molecules are synthesized and associate with each other in endoplasmic reticulum.
- Certain proteins like calnexin that are, help in proper assembly and transport of the MHC molecule.
- Another protein called invariant chain prevents the nascent or any other unfolded peptide in ER from binding to MHC II molecules.
- This invariant chain also directs the MHC II molecule to endosomes or lysosomes where internalised proteins are broken into small peptides. This transport occurs in vesicle like structure. It is called MHC class II compartment or MIIC in macrophages and Class II vesicle or CIIV in B cells.



- Inside the MIIC or CIIC the invariant chain is removed.
- Removal of invariant chain is essential for processed peptides of endogenous antigens to bind with cleft.
- This removal is facilitated by proteases. Initially these enzymes leave a small fragment of invariant chain called Class II associated invariant chain polypeptide (CLIP) in the cleft.
- But this is also removed later. Only after removal of CLIP, the MHC II molecule is ready for binding with peptides of exogenous antigens.
- Each MHC II molecule takes a peptide of 10-30 amino acids length.

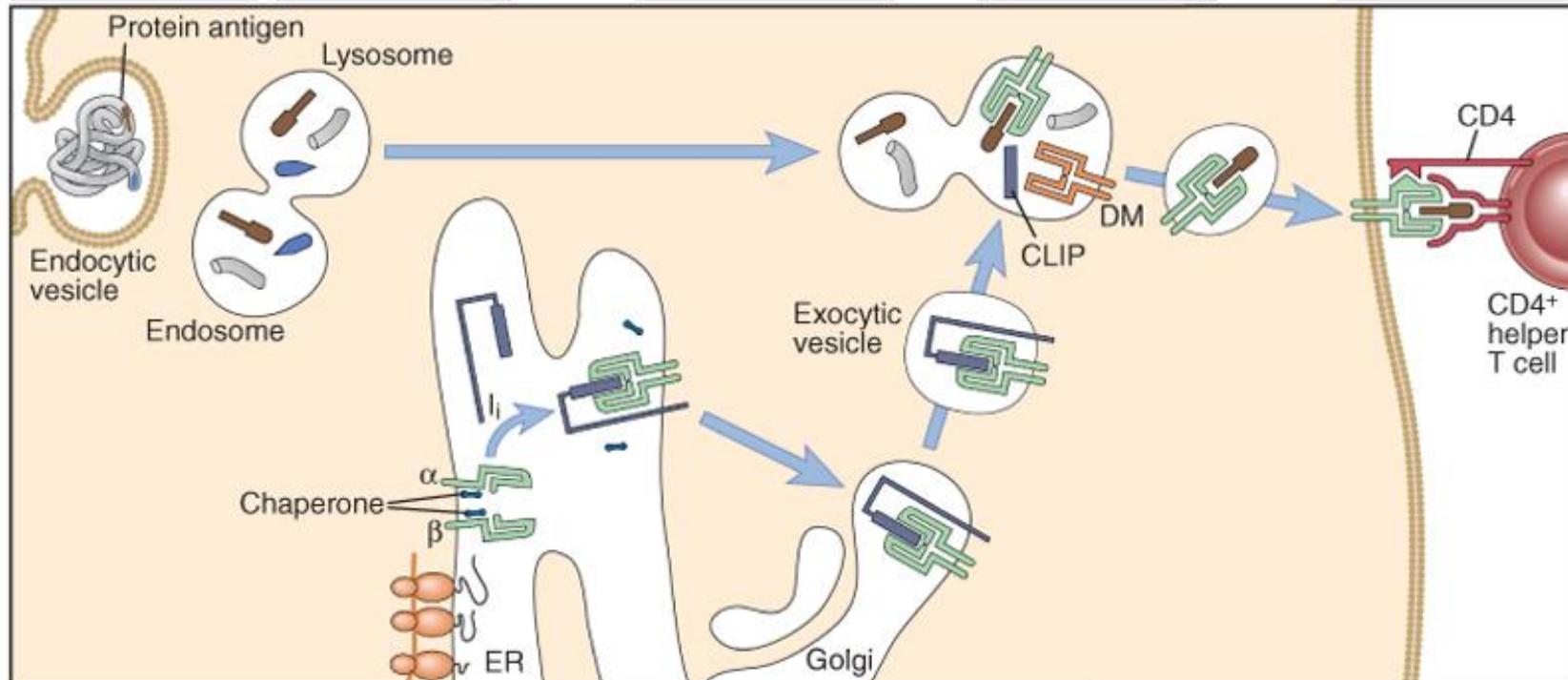


- Once, a MHC II molecules bind with peptides they are taken in a vesicle that fuses with plasma membrane and thus the peptides are displayed to T helper cell for binding.



# Mechanism of MHC molecules-II (Processing of Exogenous Antigens)

- 1 Uptake of extracellular proteins into vesicular compartments of APC
- 2 Processing of internalized proteins in endosomal/lysosomal vesicles
- 3 Biosynthesis and transport of class II MHC molecules to endosomes
- 4 Association of processed peptides with class II MHC molecules in vesicles
- 5 Expression of peptide-MHC complexes on cell surface



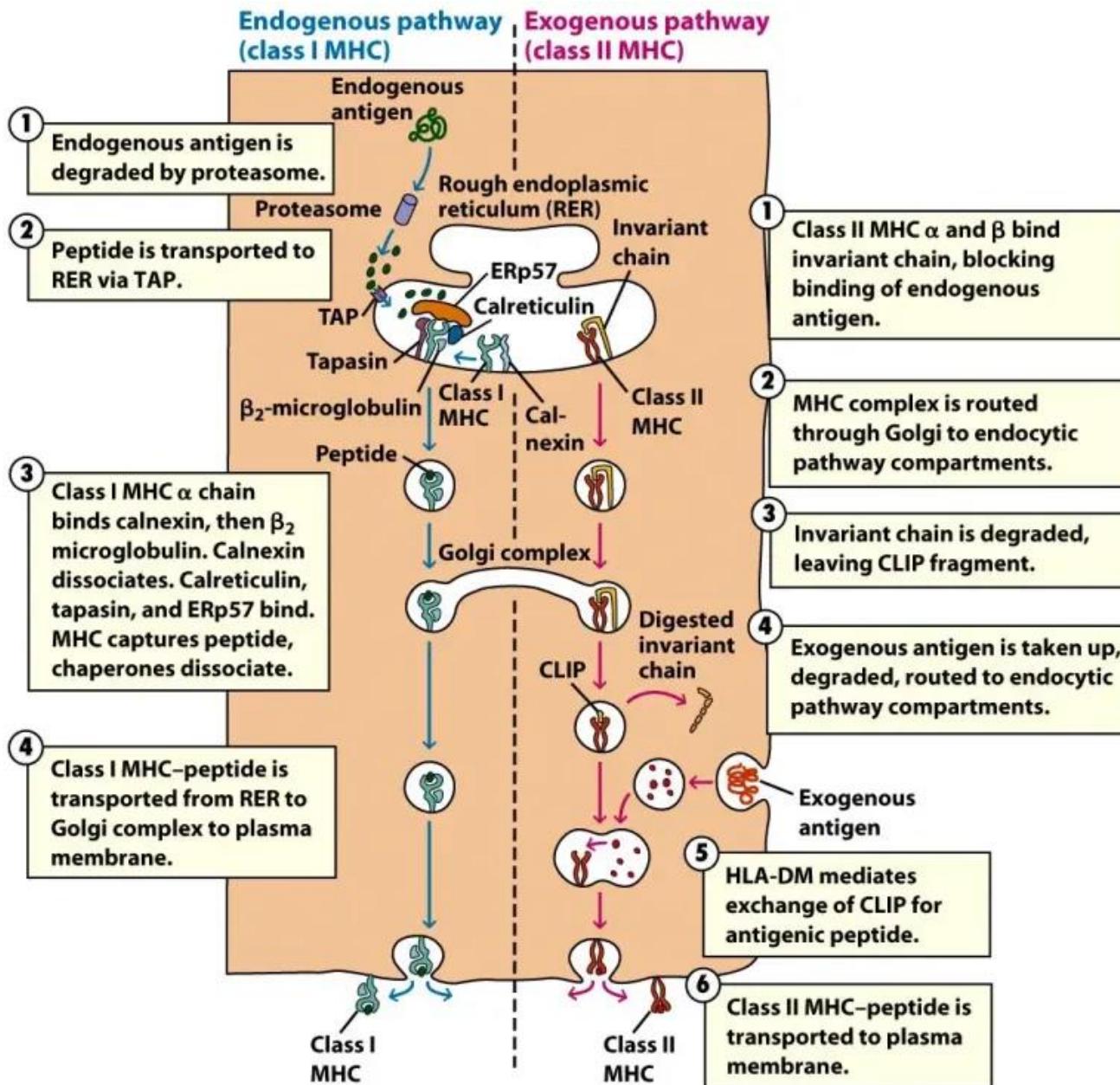


Figure 8-23

Kuby IMMUNOLOGY, Sixth Edition

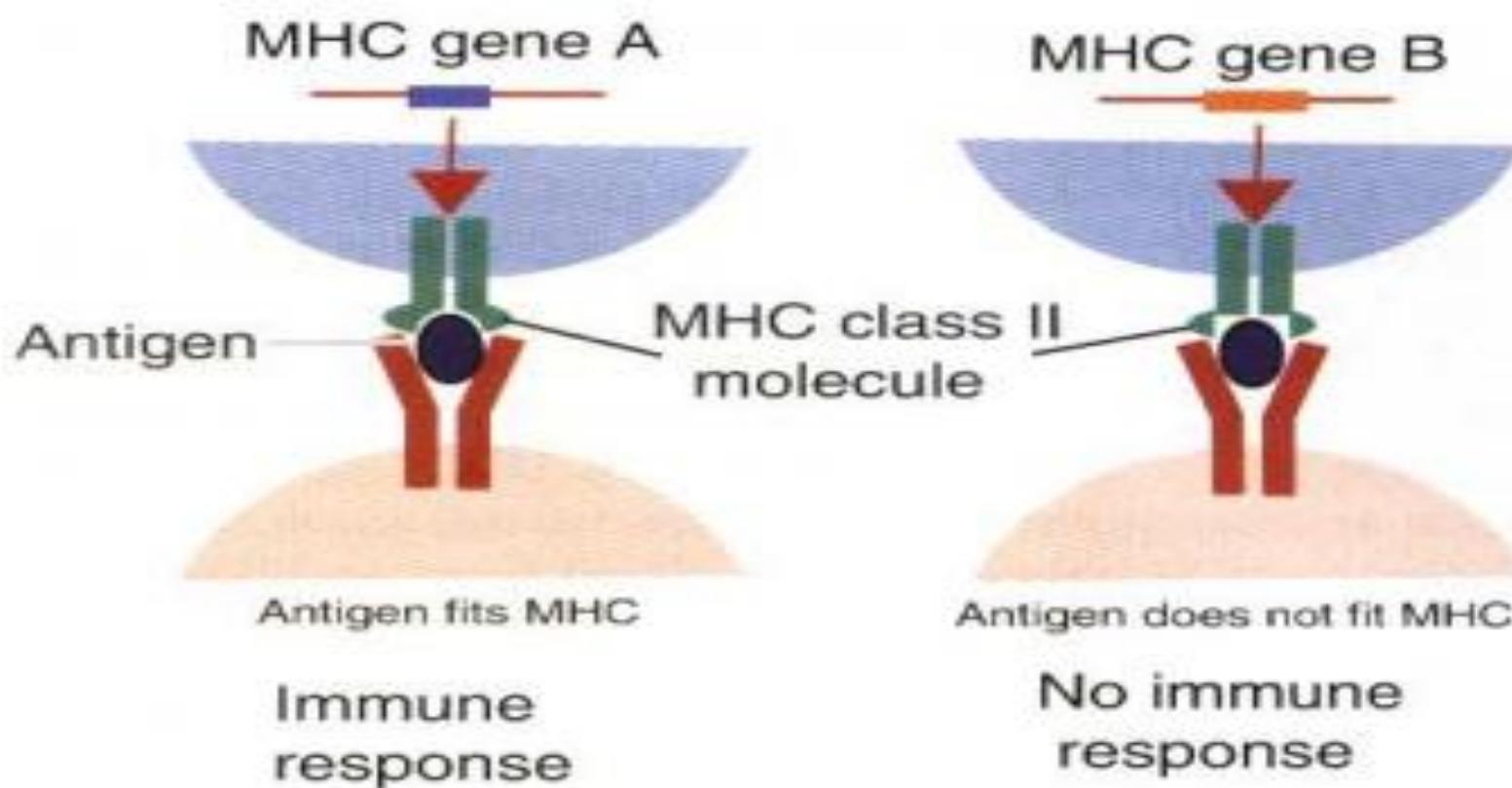
© 2007 W.H. Freeman and Company

# FUNCTION OF MHC MOLECULES

- **Specific tissue markers**:- they reflect the specific and unique genetic make-up of an individual thus serve as specific tissue markers, may be identified by in vitro tissue typing.
- **Recognition of foreign antigen**:- MHC molecules can bind specifically to the altered target cells and present the MHC- antigenic protein (peptide) complex to T cells (CD8+/ CD4+) for recognition and destruction. This type of antigen recognition in the context of MHC molecule is referred to as **MHC-restricted antigen recognition**



- Dr. Peter Doherty and his colleague, Rolf Zinkernagel were first to show the phenomena of MHC restriction



**Figure 7-16.** MHC molecules regulate the immune response. Only molecules that can bind in the groove of a MHC molecule will trigger an immune response. This is called MHC restriction. Thus the MHC genes that code for these molecules also regulate immune responsiveness.

# Function of MHC molecules

## MHC and body odors:-

- The class I region of mice, cattle, and pigs contains at least four genes coding for pheromone olfactory receptors.
- As the result, MHC haplotype affects the recognition of individual odors in an allelic –specific fashion and thus influences the mating preferences of mammals



# Function of MHC molecules

## Graft rejection:-

- MHC molecules act themselves as antigens and can provoke immune response in the recipient—thus transplant rejection.

1) Both TH and TC are activated

- TC cells destroy graft cells by direct contact

TH cells secrete cytokines that attract and activate macrophages, NK cells and polymorphs leading to cellular infiltration and destruction of graft



2) B cells recognize foreign antigens on the graft and produce antibodies which bind to graft cells and

- ❖ Activate complement causing cell lysis
- ❖ Enhance phagocytosis, i.e. opsonization

3) Immune complex deposition on the vessel walls induce platelets aggregation and microthrombi leading to ischemia and necrosis of graft



# GRAFT VERSUS HOST (GVH) REACTION

- \* An immunologically competent graft is transplanted into an immunologically suppressed recipient (host)
- \* The grafted cells survive and react against the host cells i.e instead of reaction of host against the graft, the reverse occurs
- \* GVH reaction is characterized by fever, pancytopenia, weight loss, rash , diarrhea, hepatosplenomegaly and death

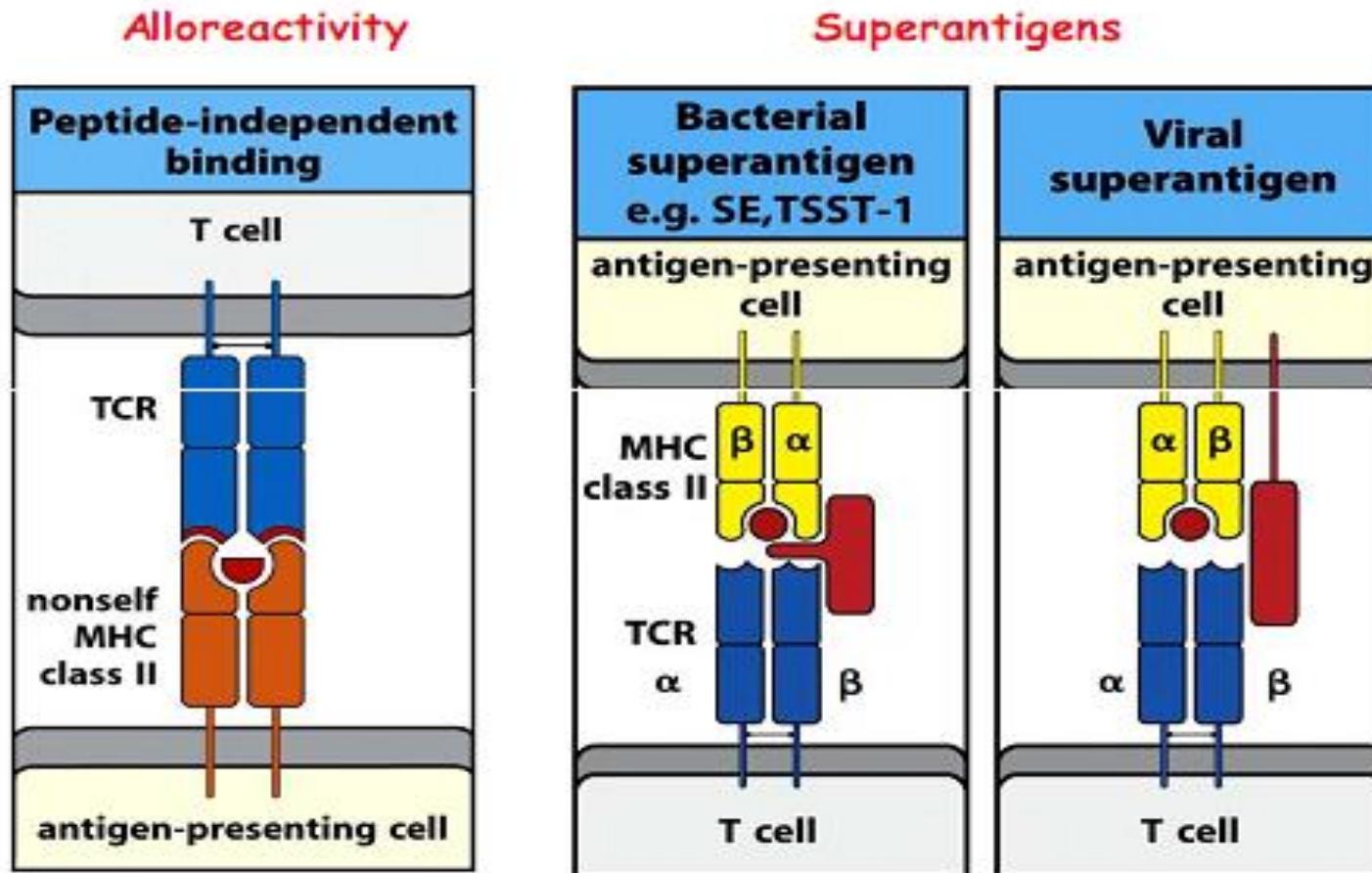


# DISEASES ASSOCIATED WITH MHC MOLECULE

Disease	HLA type
Ankylosing spondylitis	B27
Goodpasture's syndrome	DR2
Insulin-dependent diabetes mellitus	DQ2
Multiple sclerosis	DR2
Pemphigus vulgaris	DR4
Rheumatoid arthritis	DR4
Systemic lupus erythematosus	DR3

**Alloantigens**:- non-self MHC molecules. 1-10% of all T lymphocyte recognizes allogenic MHC- transplant rejection.

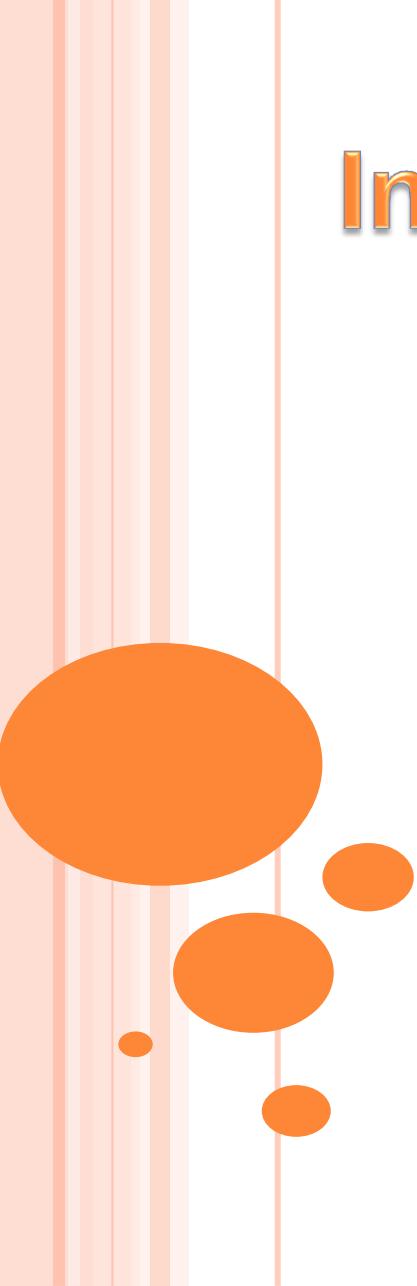
**Superantigens**:- bacterial or viral proteins/lipid/sugars that associate extracellularly with the MHC – II (HLA-DQ) and are recognized by 5- 10% of all T lymphocyte- endotoxic shock.



# Thank You

Dr. Sandeep Kumar Sharma, BVSc & AH, MVSc and PhD  
Assistant Professor, PGIVER  
[drsharmask01@hotmail.com](mailto:drsharmask01@hotmail.com)  
Mob. 9414775879





# **Introduction: Immunology and Types of Immunity**

**Dr. Sandeep Kumar Sharma, BVSc & AH, MVSc and PhD  
Assistant Professor, PGIVER  
[drsharmask01@hotmail.com](mailto:drsharmask01@hotmail.com)  
Mob. 9414775879**

# Introduction

- ❖ **Immunology** is defined as study about structure and functioning of immune system.
- ❖ It also deals with the study of host responses to the introduction of foreign substances into the tissues and the methods by which the body tries to eliminate them and protect itself from further invasion by them.



# Introduction

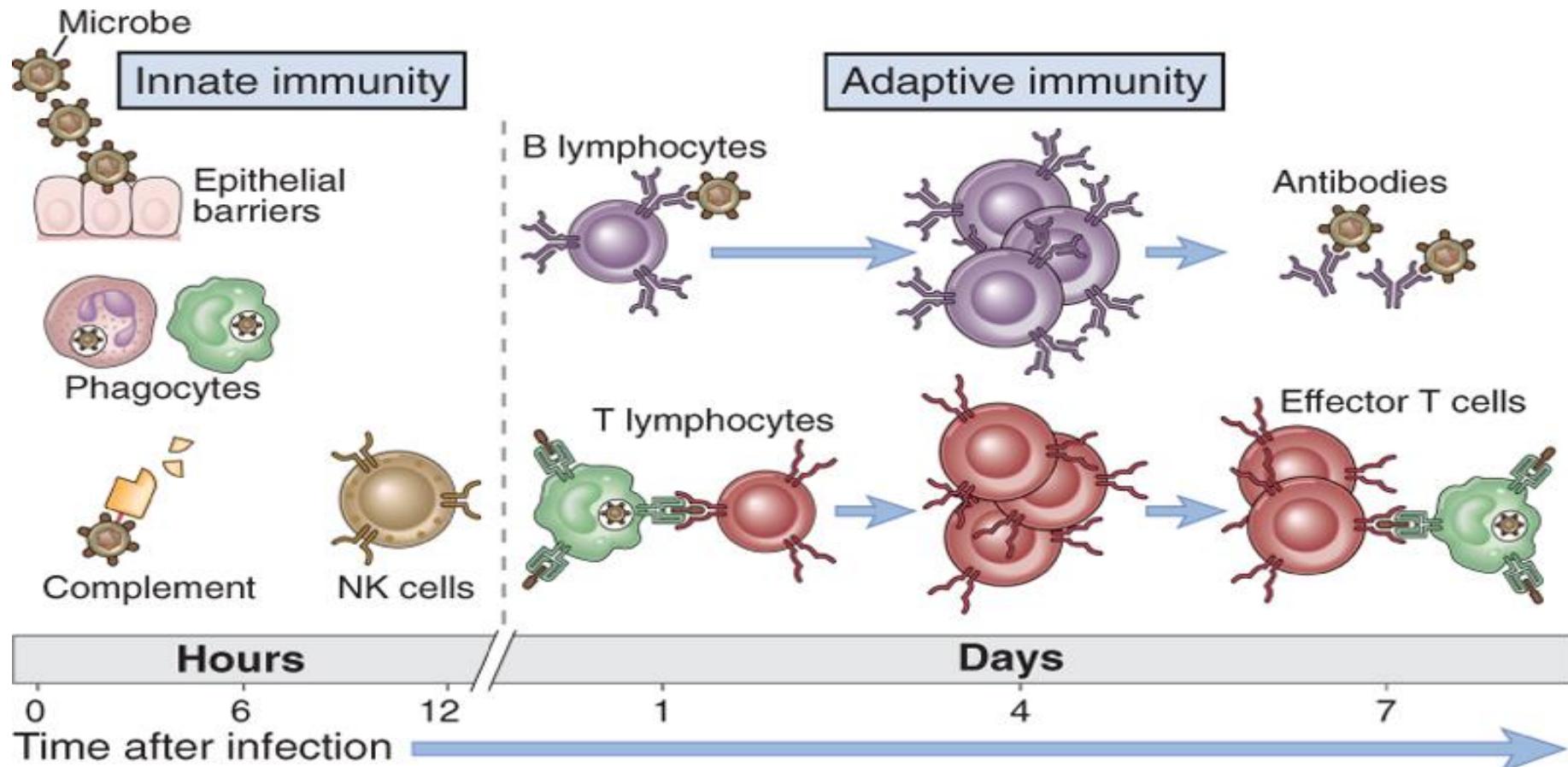
- ❖ The term immunity is derived from the *Latin word immunis / immunitas*, meaning “exempt,” is the source of the English word immunity, meaning the state of protection from infectious and non infections disease.
- ❖ The cells and molecules responsible for immunity constitute the **immune system** and their collective and coordinated response to the introduction of foreign substances is defined **as immune response**.



# Types of Immunity

- ❖ The term immunity that otherwise means protection or to protect comprise of two different mechanisms innate (natural or native, Nonspecific immunity, nonspecific resistance) immunity and adaptive (acquired or specific) immunity.
- ❖ Both specific and innate immunities act closely in removal of antigens





© Elsevier. Abbas et al: Cellular and Molecular Immunology 6e - [www.studentconsult.com](http://www.studentconsult.com)

Figure: Innate and adaptive immunity

# Innate Immunity

- ❖ Innate immunity (also called natural or native immunity) consists of cellular and biochemical defense mechanisms that are in place even before infection and poised to respond rapidly to infections.
- ❖ These mechanisms react only to microbes and not to non infectious substances, and they respond in essentially the same way to repeated infections



# Innate Immunity

- ❖ The principal components of innate immunity are
  1. Physical and chemical barriers, such as epithelia and antimicrobial substances produced at epithelial surfaces
  2. Phagocytic cells (neutrophils, macrophages) and NK (natural killer) cells
  3. Blood proteins, including members of the complement system and other mediators of inflammation
  4. Proteins called cytokines that regulate and coordinate many of the activities of the cells of innate immunity.

# Innate Immunity

## 1. Physical barriers

Physical barrier	Major activity
Skin	Prevents penetration of pathogens
Body fluids	Prevents entry of pathogens
Tears	Contain lysozymes that prevent the entry of pathogens
Ciliated epithelium	Acts as a filter against invaded pathogen
Mucus	Prevents entry of pathogen
Acidic pH of stomach	Kills organisms



# Innate Immunity

## 2. Phagocytosis

- ❖ Phagocytosis involves the engulfment and destruction of pathogens and particulate matter by specialised cells that fall in two categories Polymorphonuclear (Neutrophils and Eosinophils) and Mononuclear (Macrophages and Monocytes).
- ❖ These phagocytes are also called first line of defence.



# Innate Immunity

## 3. Inflammation

- ❖ The term inflammation that means ‘setting on fire’ is the **third component of innate immunity** and is initiated when phagocytosis fails to control the infection.
- ❖ Inflammation collectively involves a sequence of vascular events, which serve as a defence mechanism.
- ❖ The events are clotting mechanism activation, increased blood flow, increased capillary permeability and increased influx of phagocytic cells.



# Adaptive (Specific) Immunity

- ❖ Immune responses that are stimulated by exposure to infectious agents and increase in magnitude and defensive capabilities with each successive exposure to a particular microbe.
- ❖ Because this form of immunity develops as a response to infection and adapts to the infection, it is called **adaptive immunity**.
- ❖ it has an extraordinary capacity to distinguish among different, even closely related, microbes and molecules, and for this reason it is also called **specific immunity**.
- ❖ It is also sometimes called **acquired immunity**



# Adaptive (Specific) Immunity

- ❖ The specific (adaptive) immune system of vertebrates has three major functions:
  1. To recognize anything that is foreign to the body (“nonself ”)
  2. To respond to this foreign material; and
  3. To remember the foreign invader.



# **Adaptive (Specific) Immunity**

## ❖ Cardinal Features of Adaptive Immune Responses:

<b>Feature</b>	<b>Functional significance</b>
1. Specificity	Ensures that distinct antigens elicit specific responses
2. Diversity	Enables immune system to respond to a large variety of antigens
3. Memory	Leads to enhanced responses to repeated exposures to the same antigens



# **Adaptive (Specific) Immunity**

## ❖ Cardinal Features of Adaptive Immune Responses:

<b>Feature</b>	<b>Functional significance</b>
4. Specialization	Generates responses that are optimal for defense against different types of microbes
5. Self-limitation	Allows immune system to respond to newly encountered antigens
6. Non reactivity to self	Prevents injury to the host during responses to foreign antigens



## Cardinal Features of Adaptive Immune Responses:

- ❖ ***Specificity and diversity:***
- ❖ ***Specificity:*** Immune responses are specific for distinct antigens and, in fact, for different portions of a single complex protein, polysaccharide, or other macromolecule.
- ❖ The parts of such antigens that are specifically recognized by individual lymphocytes are called determinants or epitopes.



## Cardinal Features of Adaptive Immune Responses:

- ❖ ***Specificity and diversity:***
- ❖ ***Diversity:*** The total number of antigenic specificities of the lymphocytes in an individual, called the **lymphocyte repertoire**, is extremely large.
- ❖ It is estimated that the immune system of an individual can discriminate  $10^7$  to  $10^9$  distinct antigenic determinants. This property of the lymphocyte repertoire is called **diversity**.



## Cardinal Features of Adaptive Immune Responses:

- ❖ **Memory:** Exposure of the immune system to a foreign antigen enhances its ability to respond again to that antigen.
- ❖ Responses to second and subsequent exposures to the same antigen, called secondary immune responses, are usually more rapid, larger, and often qualitatively different from the first, or primary, immune response.
- ❖ Immunologic memory occurs partly because each exposure to an antigen expands the clone of lymphocytes specific for that antigen.

## Cardinal Features of Adaptive Immune Responses:

- ❖ **Specialization:** the immune system responds in distinct and special ways to different microbes, maximizing the efficiency of antimicrobial defense mechanisms.
- ❖ Thus, humoral immunity and cell-mediated immunity are elicited by different classes of microbes or by the same microbe at different stages of infection (extracellular and intracellular),  
and each type of immune response  
protects the host against that class of  
microbe.

## Cardinal Features of Adaptive Immune Responses:

- ❖ ***Self-limitation***: All normal immune responses wane with time after antigen stimulation, thus returning the immune system to its resting basal state, a process called **homeostasis**.
- ❖ Homeostasis is maintained largely because immune responses are triggered by antigens and function to eliminate antigens, thus eliminating the essential stimulus for lymphocyte survival and activation.

## Cardinal Features of Adaptive Immune Responses:

- ❖ ***Non reactivity to self***: One of the most remarkable properties of every normal individual's immune system is its ability to recognize, respond to, and eliminate many foreign (nonself) antigens while not reacting harmfully to that individual's own (self) antigenic substances.
- ❖ Immunologic unresponsiveness is also called **tolerance**.



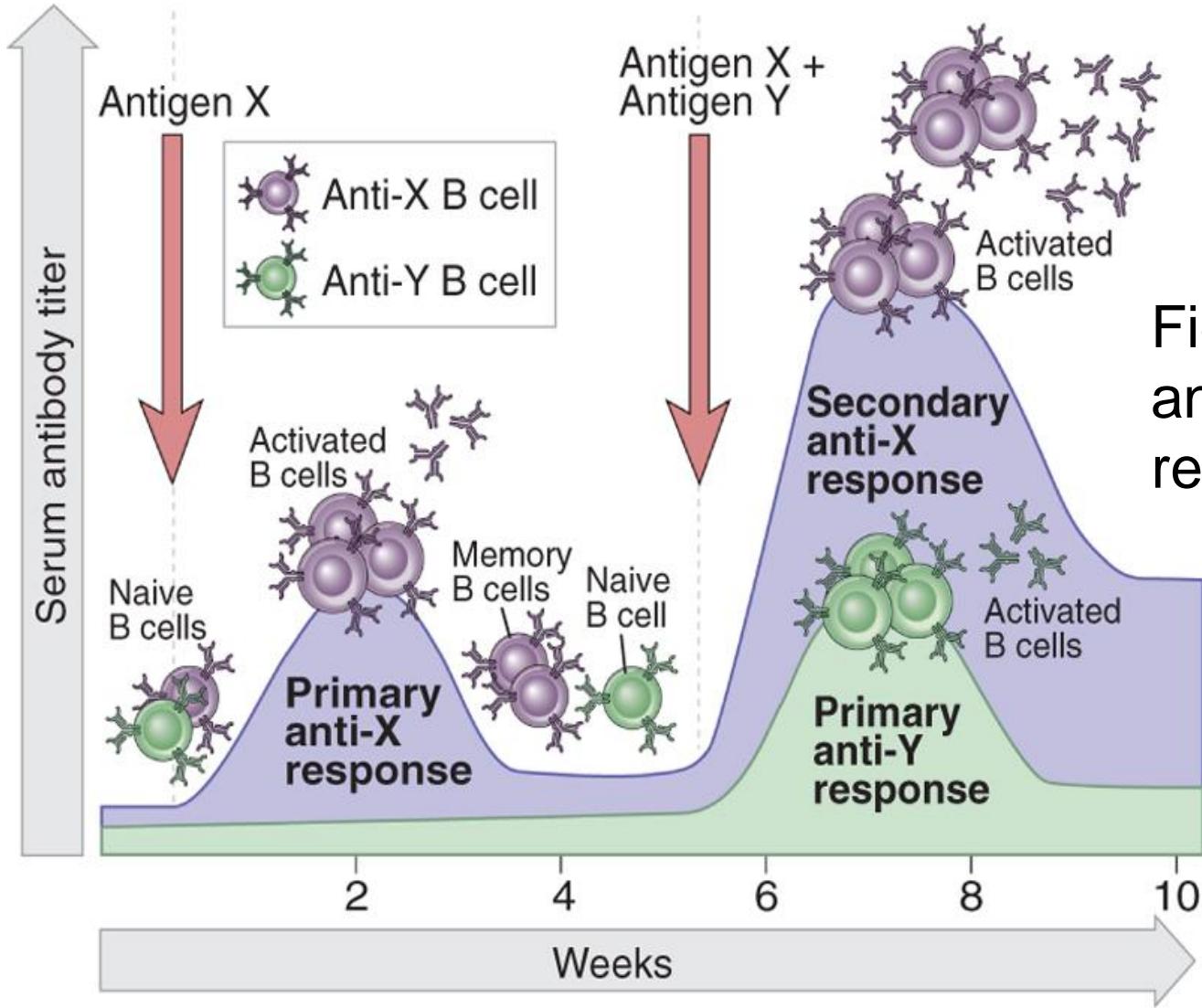


Figure: Specificity, memory, and self-limitation of immune responses.

## Cellular Components of the Adaptive Immune System:

- ❖ The principal cells of the immune system are **lymphocytes**, **antigen-presenting cells**, and **effector cells**.
- ❖ Lymphocytes:
  - **B lymphocytes** are the only cells capable of producing antibodies. They recognize **extracellular** (including cell surface) antigens and differentiate into antibody-secreting cells, thus functioning as the mediators of humoral immunity.



## Cellular Components of the Adaptive Immune System:

- ❖ Lymphocytes:

- **T lymphocytes** the cells of cell-mediated immunity, recognize the **antigens of intracellular** microbes and function to destroy these microbes or the infected cells.

- T lymphocytes have a restricted specificity for antigens thus T cells recognize and respond to cell surface-associated but not soluble antigens.

- **Helper T cells, cytolytic (cytotoxic), T lymphocytes** (CTLs) and **Regulatory T cells**.



## Cellular Components of the Adaptive Immune System:

- ❖ Lymphocytes:
- A third class of lymphocytes, **natural killer (NK) cells**, is involved in innate immunity against viruses and other **intracellular microbes**.

## Cellular Components of the Adaptive Immune System:

### ❖ Antigen-presenting cells (APCs):

- The initiation and development of adaptive immune responses require that antigens be **captured and displayed to specific lymphocytes**. The cells that serve this role are called **antigen-presenting cells (APCs)**.
- The most highly specialized APCs are **dendritic cells**.



## Cellular Components of the Adaptive Immune System:

### ❖ **Effector cells :**

- The activation of lymphocytes by antigen leads to the generation of numerous mechanisms that function to eliminate the antigen.
- Antigen elimination often requires the participation of cells called **effector cells**.
- Activated T lymphocytes, mononuclear phagocytes, and other leukocytes function as effector cells in different immune responses.



# Cellular Components of the Adaptive Immune System:

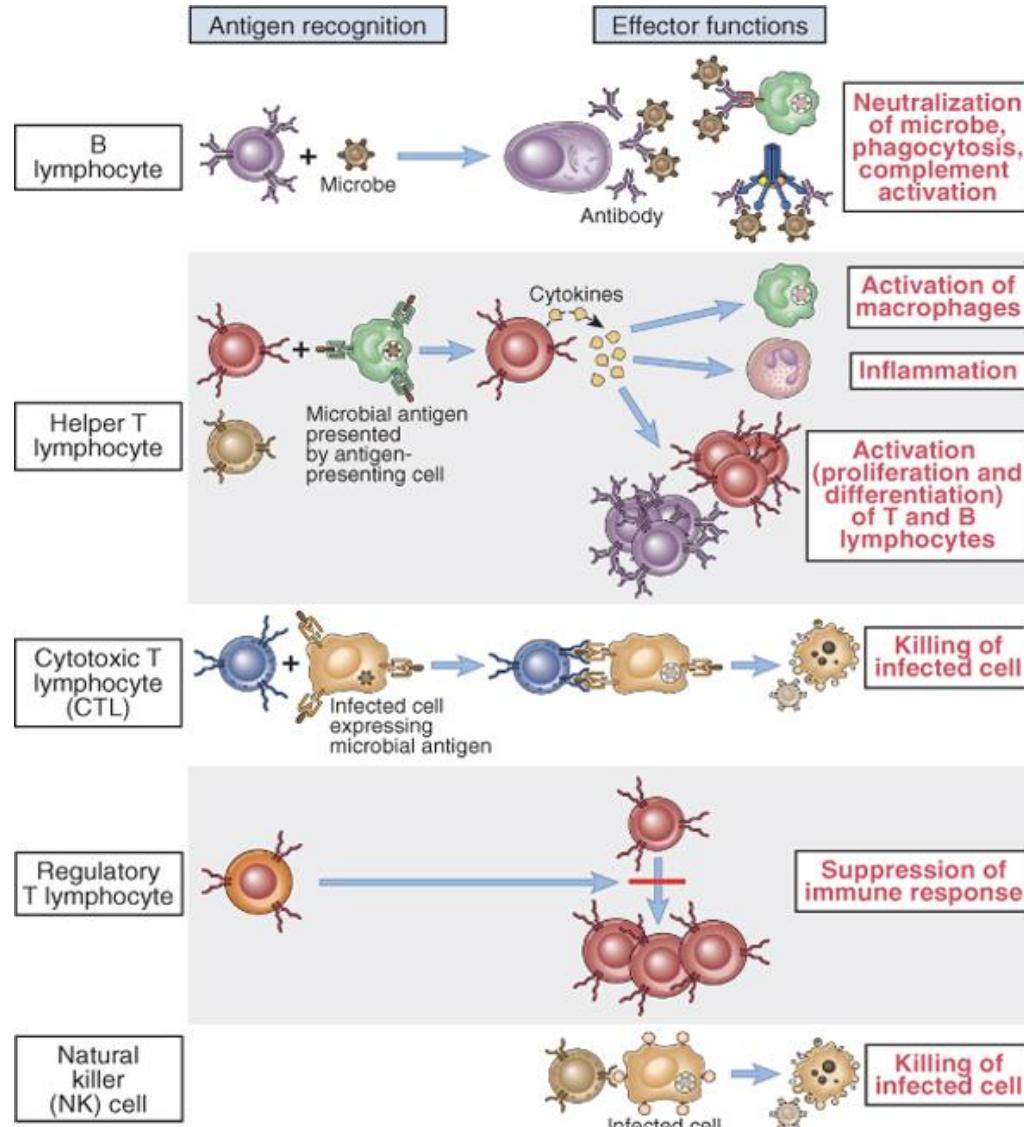


Figure: Classes of lymphocytes.

# Adaptive (Specific) Immunity

- ❖ The recognition response is **highly specific**.
- ❖ The immune system is able to distinguish one pathogen from another, to identify cancer cells, and to discriminate the body's own “self” proteins and cells as different from “nonself ” proteins, cells, tissues, and organs.
- ❖ After recognition of an invader, the specific immune system responds by amplifying and activating specific lymphocytes to attack it. **This is called an effector response.**



# Adaptive (Specific) Immunity

- ❖ A successful effector response either eliminates the foreign material or renders it harmless to the host.
- ❖ If the same invader is encountered at a later time, the immune system is prepared to mount a more intense and **rapid memory or anamnestic response** that eliminates the invader once again and protects the host from disease.
- ❖ This immunologic memory helps in mounting a faster and efficient removal when the same antigen enters subsequently.



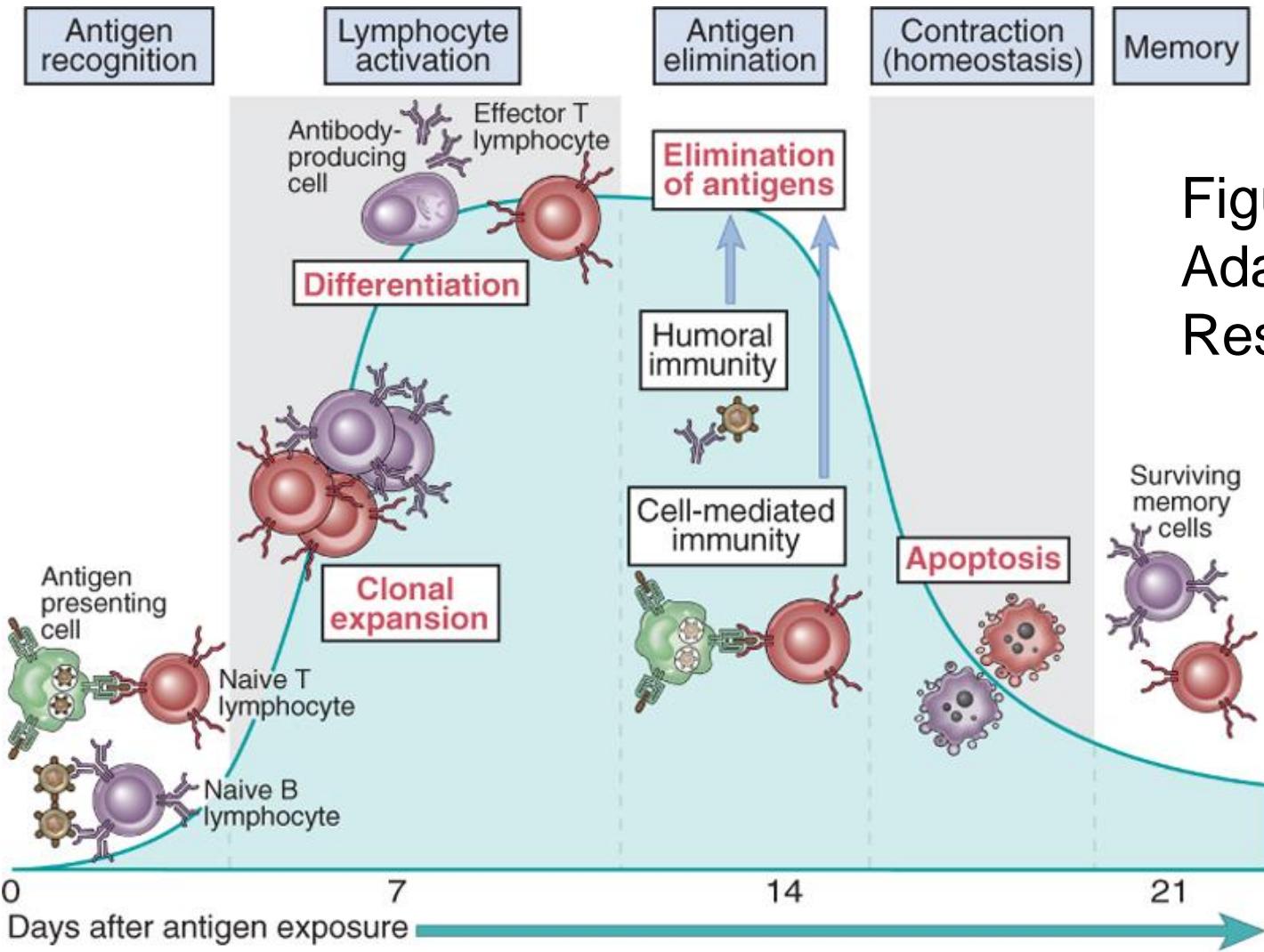
# Phases of Adaptive Immune Responses

❖ *Adaptive immune responses may be divided into distinct phases-*

- The recognition of antigen
- The activation of lymphocytes, and
- The effector phase of antigen elimination
- Followed by the return to homeostasis
- The maintenance of memory



# Figure: Phases of Adaptive Immune Responses



# Difference between Innate and Adaptive immunity

Characteristics	Innate immunity	Adaptive immunity
Specificity	For structures shared by groups of related microbes	For antigens of microbes and for non microbial antigens
Diversity	Limited; germline encoded	Very large; receptors are produced by somatic recombination of gene segments
Memory	None	Yes
Non reactivity to self	Yes	Yes

# Difference between Innate and Adaptive immunity

Characteristics	Innate immunity	Adaptive immunity
Physical and chemical barriers	Skin, mucosal epithelia; antimicrobial chemicals	Lymphocytes in epithelia; antibodies secreted at epithelial surfaces
Blood proteins	Complement	Antibodies
Cells	Phagocytes (macrophages, neutrophils), natural killer cells	Lymphocytes

# Types of Adaptive Immune Responses

- ❖ There are two types of adaptive immune responses, called humoral immunity and cell-mediated immunity, that are mediated by different components of the immune system and function to eliminate different types of microbes



## Humoral Immunity

- ❖ It is mediated by molecules in the blood and mucosal secretions, called **antibodies**, that are produced by cells called **B lymphocytes** (also called B cells).
- ❖ Antibodies recognize microbial antigens, neutralize the infectivity of the microbes, and target microbes for elimination by various effector mechanisms.



## Humoral Immunity

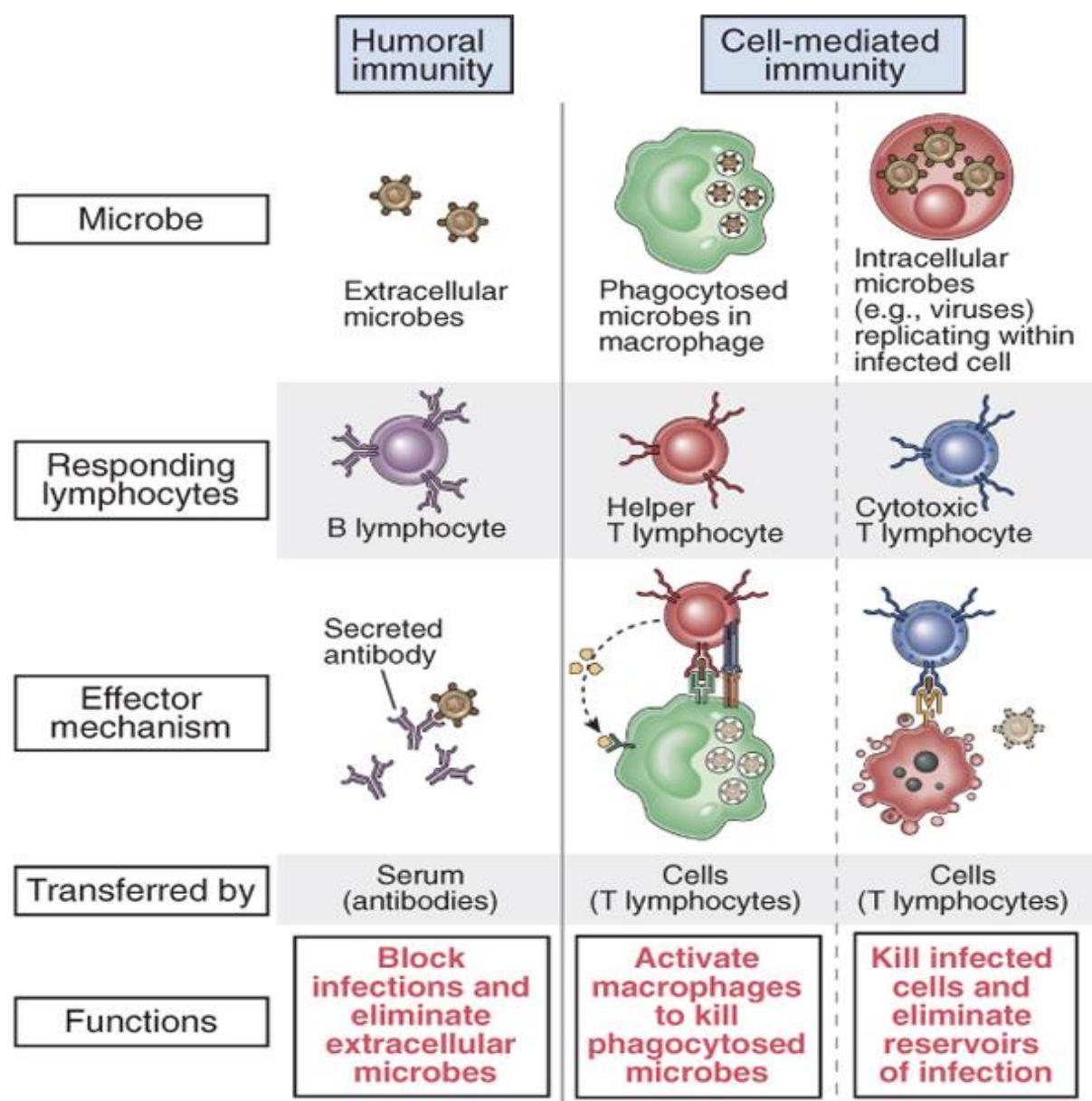
- ❖ Humoral immunity is the principal defense mechanism against **extracellular microbes** and their toxins because secreted antibodies can bind to these microbes and toxins and assist in their elimination.
- ❖ Antibodies themselves are specialized, and different types of antibodies may activate different effector mechanisms.



## Cell-mediated Immunity

- ❖ Cell-mediated immunity, also called cellular immunity, is mediated by **T lymphocytes** (also called T cells).
- ❖ Intracellular microbes, such as viruses and some bacteria, survive and proliferate inside phagocytes and other host cells, where they are inaccessible to circulating antibodies.
- ❖ Defense against such infections is a function of cell-mediated immunity, which promotes the destruction of microbes residing in phagocytes or the killing of infected cells to eliminate reservoirs of infection.





**Figure: Types of adaptive immunity**

# Differences between Humoral and Cell mediated immunity

Feature	Humoral immunity	Cell mediated immunity
Antigen	Extracellular antigens	Intracellular antigens
Responding lymphocytes	B lymphocytes	T lymphocytes
Effector mechanism	Antibody mediated elimination	Lysis of infected cell
Transferred by	Serum	T lymphocytes

## Active and Passive Immunity

- ❖ Humoral and cell mediated immunity can each be divided into active and passive immunity.
- ❖ Specific immune responses are stimulated when a host is exposed to and antigen.
- ❖ This immunity is called active immunity, which is acquired gradually, lasts longer and is highly protective.
- ❖ It also stimulates immunological memory.

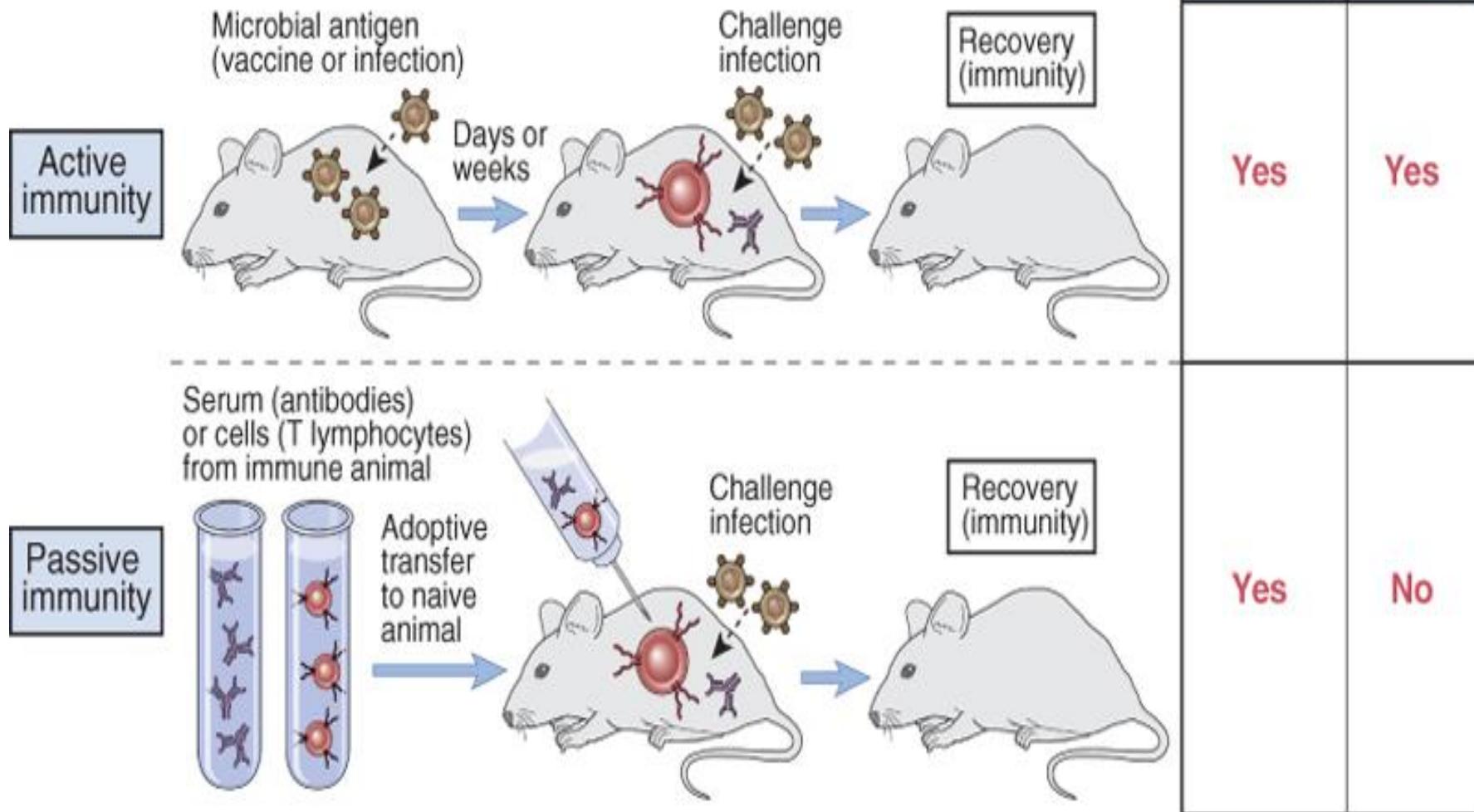


## Active and Passive Immunity

- ❖ Passive immunity is obtained either by **transfer of serum or T cell**.
- ❖ The recipient of such a transfer becomes immune to the particular antigen without ever having been exposed to or having responded to that antigen. Therefore, It is called **passive immunity**.
- ❖ The recipient of such transfer becomes immune immediately. However, the immunity is short lived with moderate protection.
- ❖ There is **no immunological memory** associated with passive immunity.



# Figure: Active and Passive Immunity



# Thank You

Dr. Sandeep Kumar Sharma, BVSc & AH, MVSc and PhD  
Assistant Professor, PGIVER  
[drsharmask01@hotmail.com](mailto:drsharmask01@hotmail.com)  
Mob. 9414775879



# Vaccines and Their Properties

**Dr. Sandeep Kumar Sharma,**

BVSc & AH, MVSc and PhD

**Assistant Professor, PGIVER**

**drsharmask01@hotmail.com**

**Mob. 9414775879**

# History

**1100s:** In Turkey, Africa, India, China, and Europe.

The **variolation** technique was developed, involving the inoculation of children and adults with dried scab material recovered from smallpox patients.

The terms *inoculation* and *variolation* were often used interchangeably.

**1721:** Variolation was introduced to Great Britain by English aristocrat **Lady Mary Wortley Montague**.

# History

**1798:** **Edward Jenner** published his work on the development of a vaccination that would protect against smallpox.

The word **Vaccine** is derived from the Latin **vacca**, meaning **cow**.

A virus that mainly affects cows (Cowpox) was used by **Edward Jenner** to protect the young boy (**James Phipps**) from smallpox by scratching liquid from cowpox sores into the boy's skin.

- *Louis Pasteur first used term vaccine*

# History

- **1879:** Louis Pasteur created the first live attenuated bacterial vaccine (chicken cholera)
- **1882:** Robert Koch identified the tubercle bacillus as the cause of tuberculosis, subsequently called Koch's bacillus.
- **1884:** The first live attenuated viral vaccine (rabies) was developed by Louis Pasteur, using dessicated brain tissue inactivated with formaldehyde.
- **1885:** Louis Pasteur first used rabies vaccine in humans.

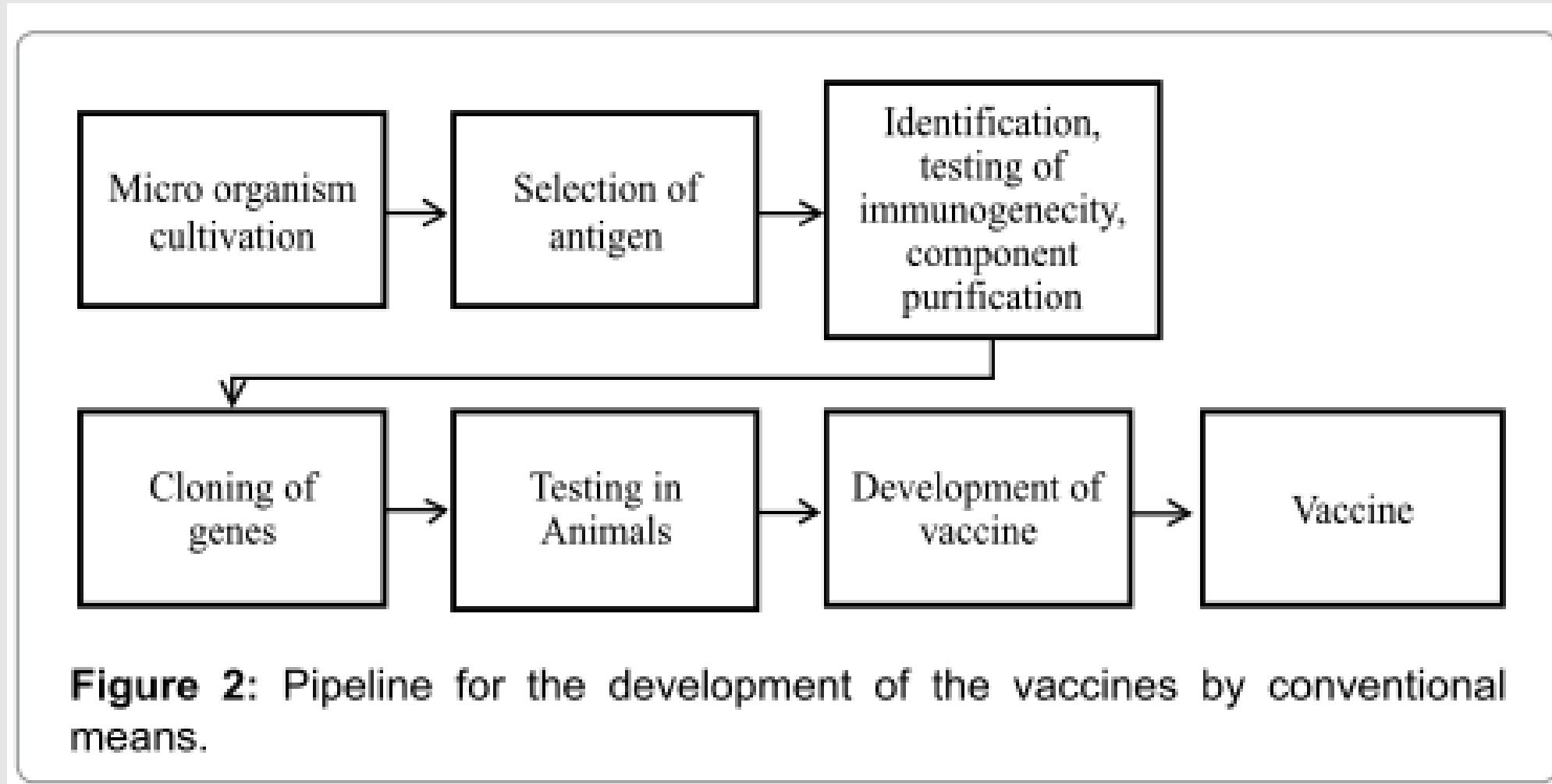
*Joseph Meister* (21 February 1876 – 24 June 1940)

# Terminology

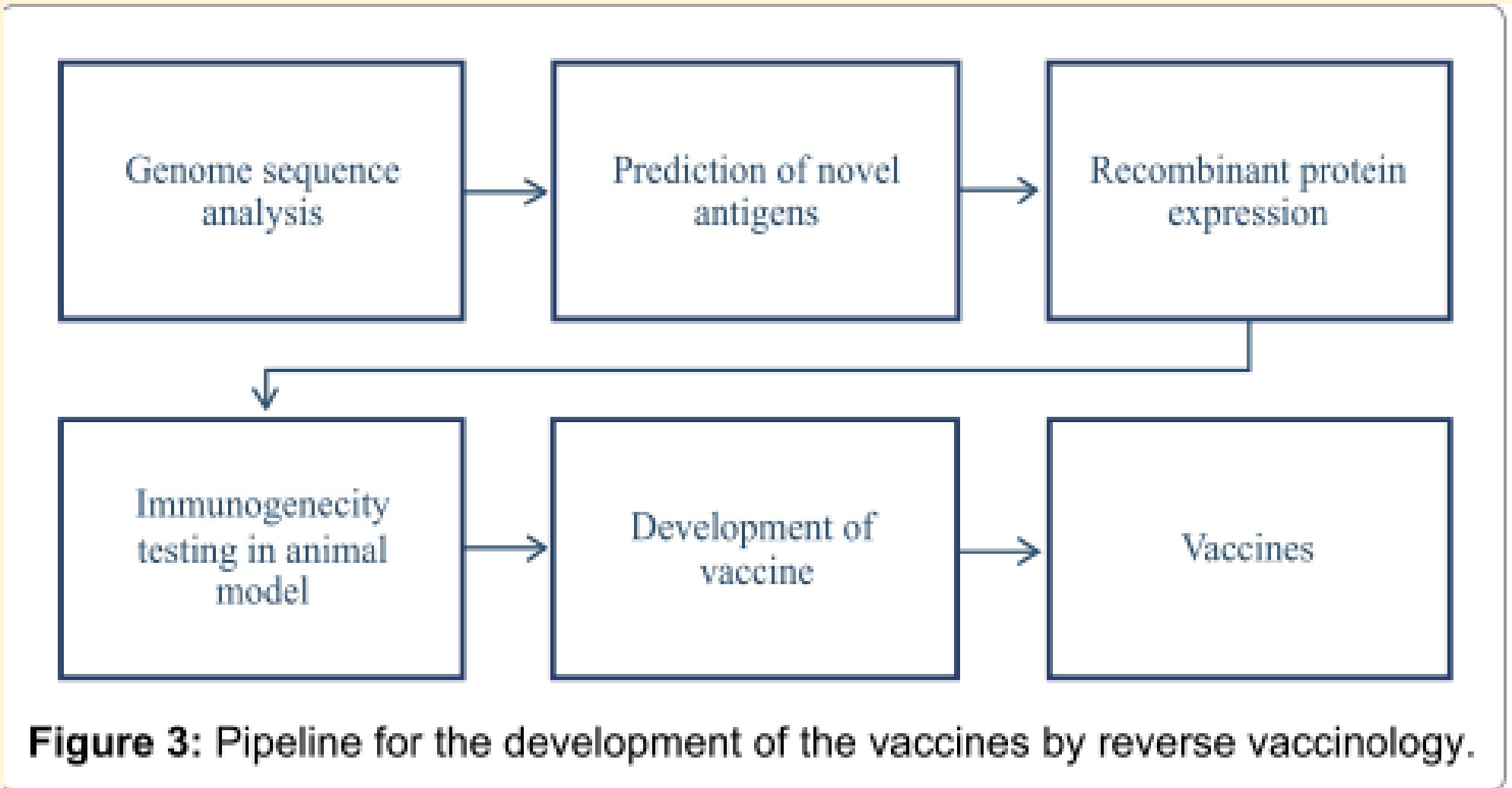
- A **vaccine** is a biological preparation that improves immunity to a particular disease. A vaccine typically contains an agent that resembles a disease-causing, and is often made from weakened or killed forms of the microbe, its toxins, one of its surface proteins or genetic material.
- **Immunogen**
  - ❖ **Immunogenicity**
- **Antigen(Ag)**
  - ❖ **Antigenicity**

## • *Vaccinology*

It is the science to study vaccine and it's properties for prevention and control of diseases.



# Reverse Vaccinology



# Properties of Ideal Vaccine

- Immunogenic
- Long lasting immunity
- Safe
- Stable in field conditions
- Combined
- Single dose
- Affordable (and accessible) to all

# Types of vaccines

- ***First generation vaccines***:- are whole-organism vaccines – either live and weakened, or killed forms.
- ***Second generation vaccines***:- subunit vaccines, consisting of defined protein antigens or protein components.
- ***Third generation vaccines***:- DNA vaccine or marker vaccine and synthetic peptide vaccine composed of DNA Sequence code for antigenic protein of pathogen.

# Live, Attenuated vaccines

- Contain a version of the living microbe that has been weakened in the lab so it can't cause disease.
- They elicit strong cellular and antibody responses
- **But** some time vaccine could revert to a virulent form and cause disease
- Need to be refrigerated to stay potent
- Difficult to create for bacteria because bacteria have thousands of genes and thus are much harder to control.

# Merits of live vaccine

- Fewer dose required
  - Adjuvants unnecessary
  - Less chance of hyper sensitivity
  - Induction of interferon
  - Relatively cheap
  - Smaller dose needed
  - Can be given by natural route
  - Stimulate both humoral and cell mediated immunity
  - Long lasting protection
- Ex:
- *Brucella abortus* strain 19 vaccine
  - BCG vaccine (*Bacillus of Calmette and Gaurin*) vaccine developed by growing *Mycobacterium bovis* in bile saturated for 13 years

# Killed/ inactivated vaccines

- Produce by killing the disease-causing microbe with chemicals, heat, or radiation.
- These are more stable and safer than live vaccines
- Usually don't require refrigeration, and they can be easily stored and transported in a freeze-dried form, which makes them accessible to people in developing countries.
- Here the organism is inactivated using various inactivating agents like **formaldehyde**, **ethylene oxide**, **ethylenimine**, **acetyl ethylenimine**
- Salk polio vaccine is inactivated with formaldehyde

# Merits of Killed /inactivated vaccine

- Stable on storage, unlikely to produce disease through residual virulence, do not replicate in recipient, unlikely to contain live contaminating organism
- Will not spread to others
- Safe in immunodeficient patients
- Easier to storage
- Lower development cost
- No risk of reversion

# Subunit vaccines

- Instead of the entire microbe, subunit vaccines include only the antigens that can best stimulate the immune system.
- Since vaccines contain only the essential antigens, these are very specific and sensitive.

These can prepared by one of two ways:

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

# Subunit vaccines

- Inactivated exotoxins, capsular polysaccharides, and recombinant microbial antigens
- Bacterial polysaccharide capsules are used as subunit vaccines
- The current vaccine for *Streptococcus pneumoniae*, which causes pneumococcal pneumonia, consists of 23 antigenically different capsular polysaccharides
- The vaccine for *Neisseria meningitidis*, a common cause of bacterial meningitis, also consists of purified capsular polysaccharides.
- Diphtheria and tetanus vaccines, for example, can be made by purifying the bacterial exotoxin and then inactivating the toxin with formaldehyde to form a **toxoid**.

# Toxoid vaccines

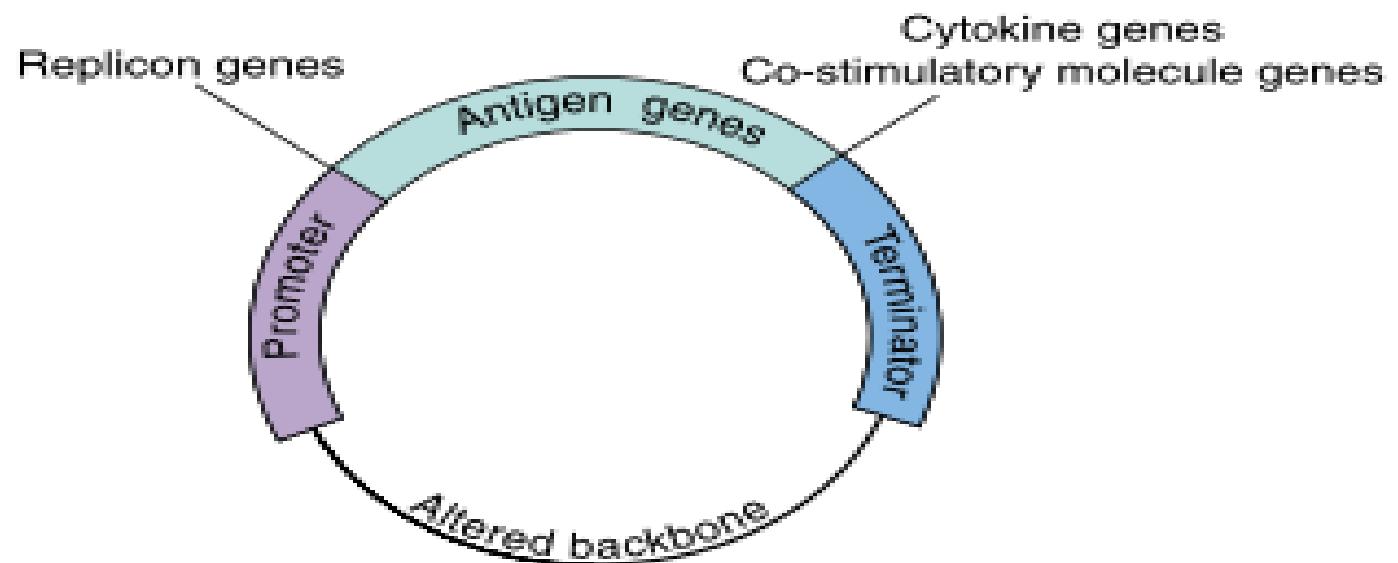
- Most of pathogenic bacteria secrete toxins, or harmful chemicals such harmful agents known as virulence factors, responsible for diseases.
- These can inactivate by treating with formalin, a solution of formaldehyde, heat treatment and sterilized water. Such “detoxified” toxins, called toxoids, are safe for use in vaccines.

# Conjugate vaccines

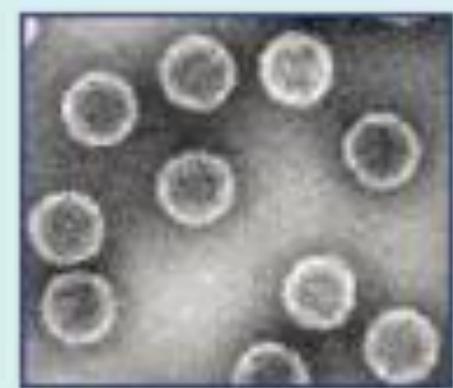
- Bacterium possesses an outer coating of sugar molecules called polysaccharides, known as capsule is less immunogenic but more virulent.
- Conjugated to protein (antigens/ toxoid) carriers or adjuvants to enhance immunogenicity and helps the immature immune system react to polysaccharide coatings and defend against the disease-causing bacterium.

# DNA vaccines

- The DNA vaccines are simple rings of DNA containing a gene encoding an antigen, and a promoter/terminator to make the gene express in eukaryotic cells.
- They are a promising new approach for generating all types of desired immunity: **cytolytic T lymphocytes (CTL)**, **T helper cells** and **antibodies**.



## Principle of DNA vaccination



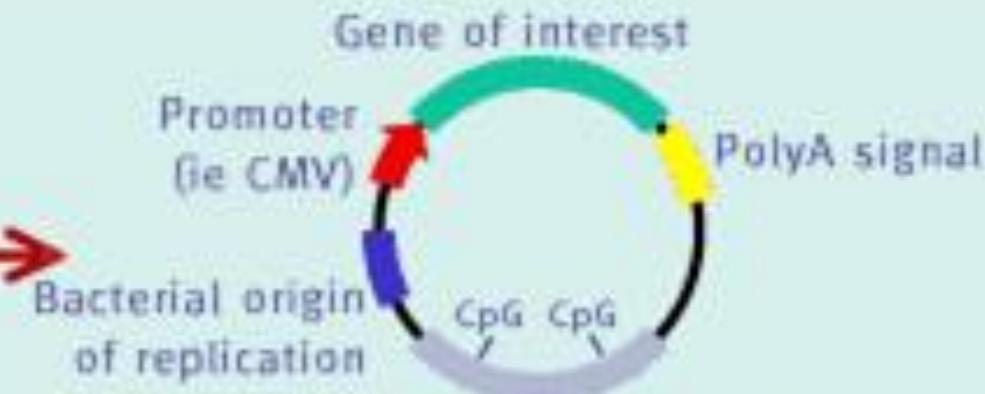
Pathogen



Injection



Isolation of gene  
for antigenic protein



Cloning into a vaccine plasmid



Purification of plasmid



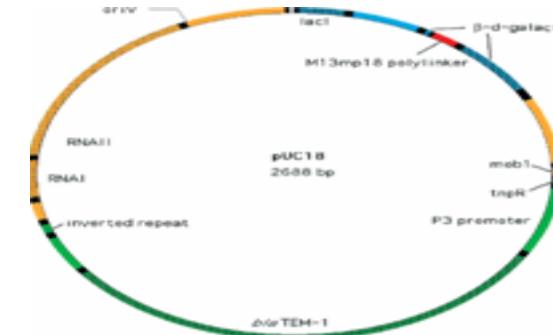
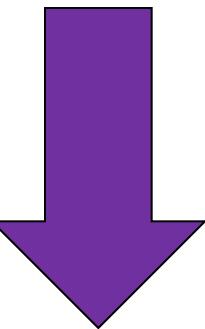
Production by bacteria

# *Preparation of DNA Vaccine*

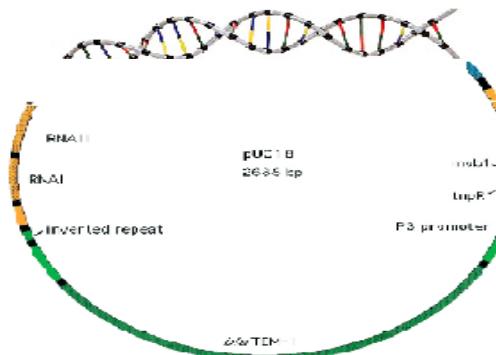


Antigen protein encoding gene

Recombinant DNA  
technology

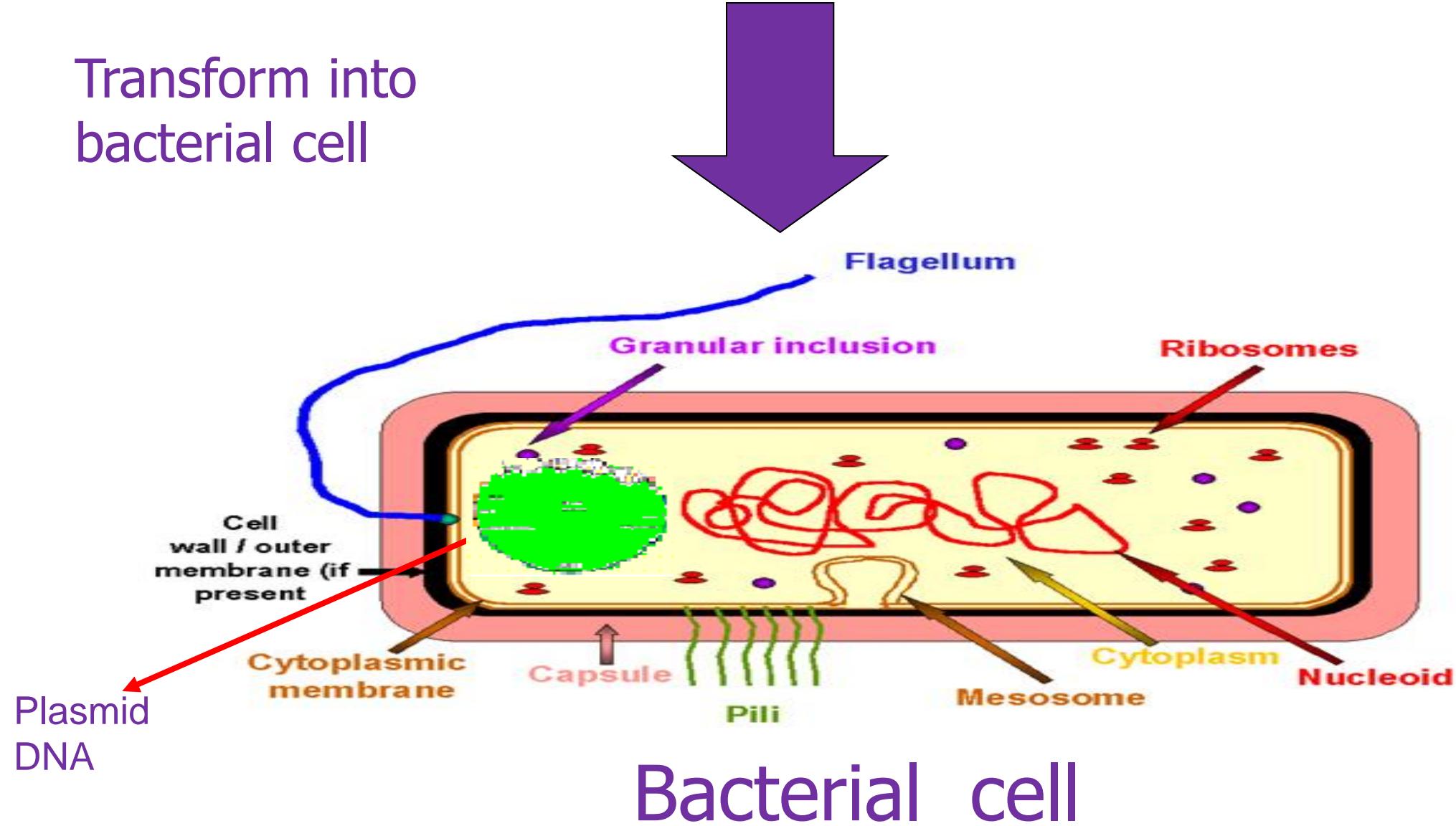


Expression plasmid

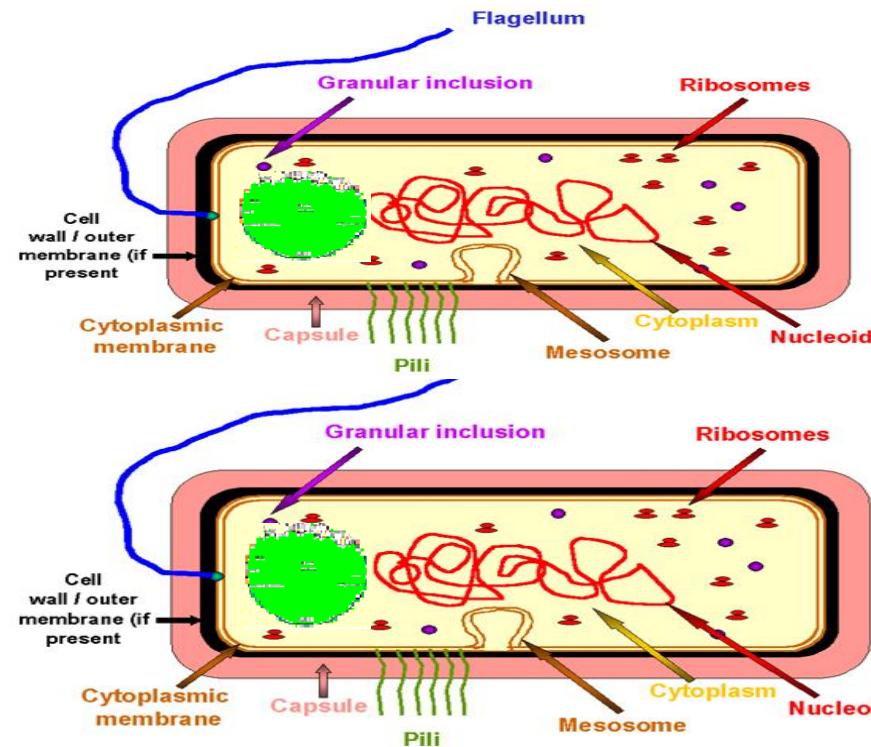
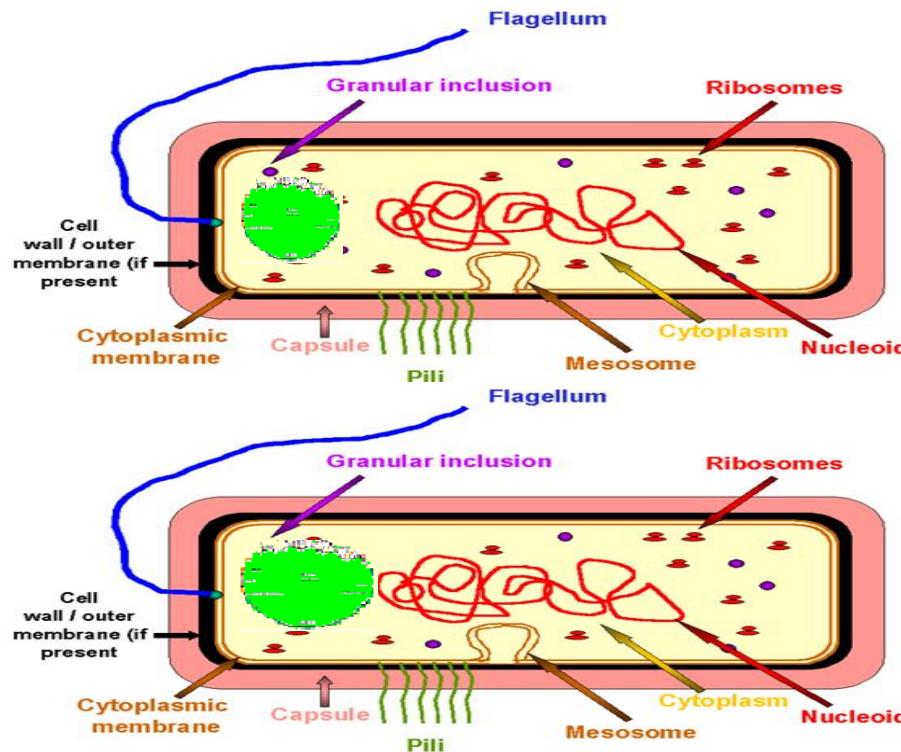
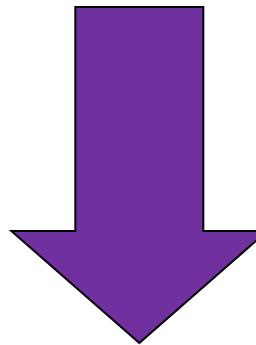


Plasmid with foreign gene

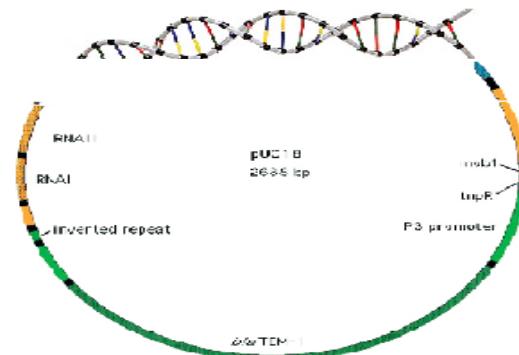
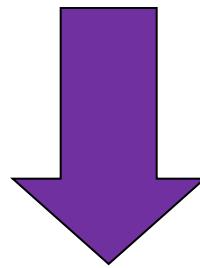
Transform into  
bacterial cell



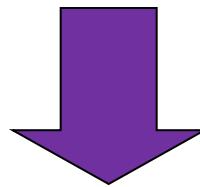
# Plasmid DNA get amplified



Plasmid DNA Purified



Ready to use



# Delivery methods

Method of Delivery	Formulation of DNA	Target Tissue	Amount of DNA
Parenteral	Injection (hypodermic needle)	Aqueous solution in saline	IM (skeletal), ID, IV, SC and intraperitoneal with variable success) Large amounts (approximately 100-200 µg)
	Gene Gun	DNA-coated gold beads	ED (abdominal skin), vaginal mucosa, surgically exposed muscle and other organs Small amounts (as little as 16 ng)
	Pneumatic (Jet) Injection	Aqueous solution	ED (abdominal skin) Very high (as much as 300 µg)
Topical application	Aqueous solution	Ocular, intravaginal	Small amounts (up to 100 µg)
Cytofectin-mediated	liposomes (cationic), microspheres, recombinant adenovirus vectors, attenuated Shigella vector, aerosolised cationic lipid formulations	IM, IV (to transfect tissues systemically), intraperitoneal, oral immunization to the intestinal mucosa; nasal/lung mucosal membranes variable	

Method of Delivery	Advantage	Disadvantage
Intramuscular or Intradermal injection	No special delivery mechanism Permanent or semi-permanent expression pDNA spreads rapidly throughout the body	Inefficient site for uptake due to morphology of muscle tissue Relatively large amounts of DNA used
Gene Gun	DNA bombarded directly into cells Small amounts DNA	Requires inert particles as carrier
Jet injection	No particles required DNA can be delivered to cells mm to cm below skin surface	Significant shearing of DNA after high-pressure expulsion 10-fold lower expression, and lower immune response Requires large amounts of DNA (up to 300 µg)
Liposome-mediated delivery	High levels of immune response Can increase transfection of intravenously delivered pDNA Intravenously delivered liposome-DNA complexes can potentially transfect all tissues Intranasally delivered liposome-DNA complexes can result in expression in distal mucosa as well as nasal mucosa and the generation of IgA antibodies	Toxicity Ineffectiveness in serum Risk of disease or immune reactions

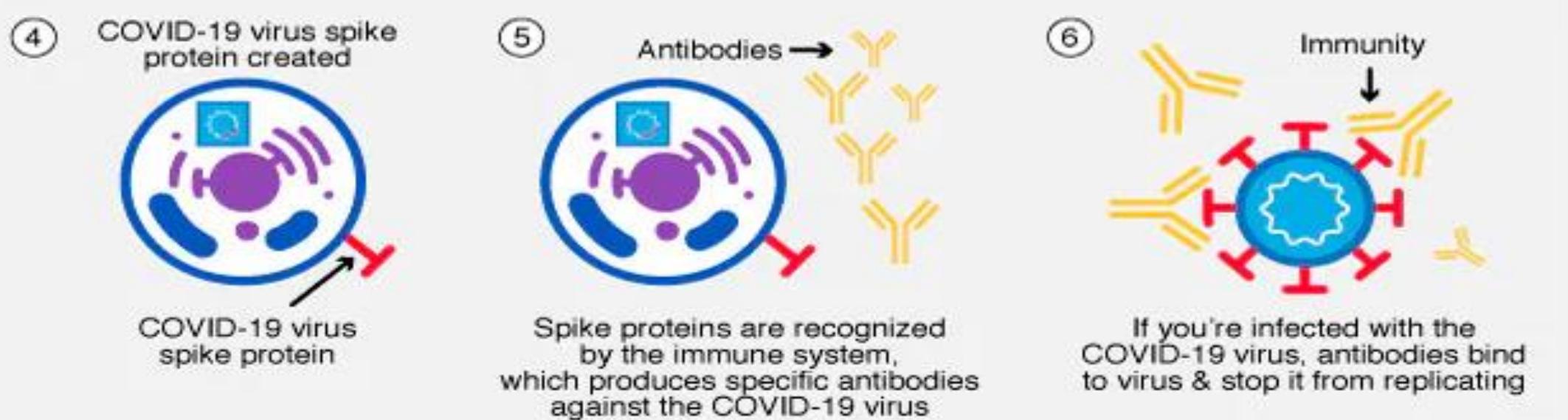
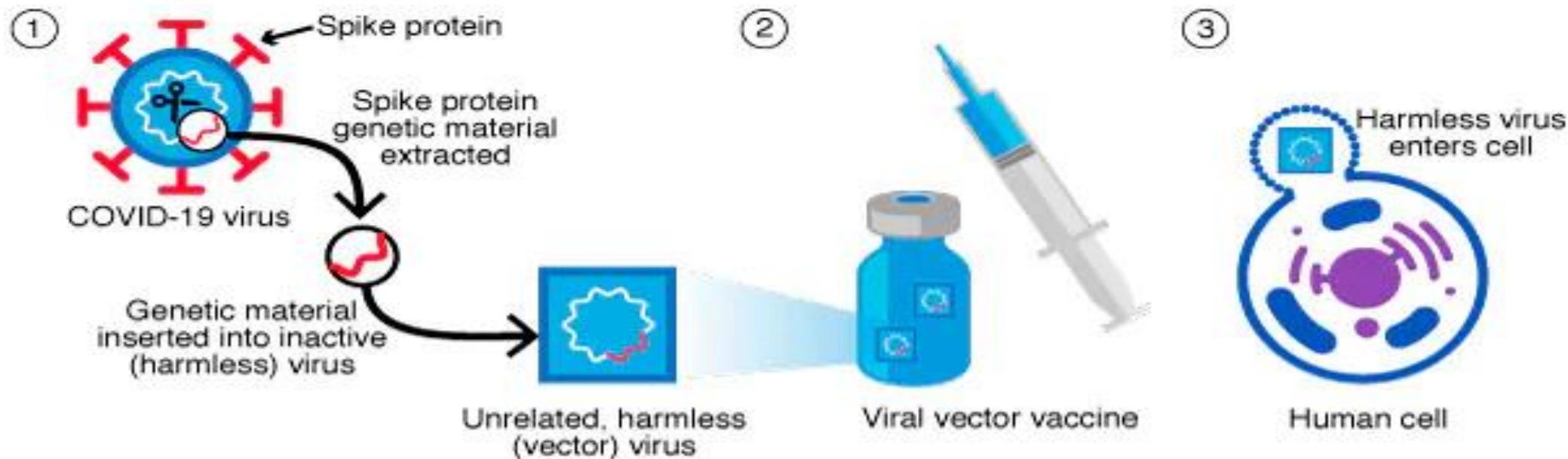
# Advantages and disadvantages of DNA vaccines

Advantages	Disadvantages
<ul style="list-style-type: none"><li>❖ Elicit both Humoral &amp; cell mediated immunity with no risk of infection</li><li>❖ Antigen presentation by both MHC class I and class II molecules</li><li>❖ Immune response focused only on antigen of interest</li><li>❖ Ease of development and production</li><li>❖ Refrigeration not required with stability of vaccine for storage and shipping, Cost-effectiveness</li><li>❖ Obviates need for peptide synthesis, expression and purification of recombinant proteins and the use of toxic adjuvants</li><li>❖ Long-term persistence of immunogen</li></ul>	<ul style="list-style-type: none"><li>• Limited to protein immunogens (not useful for non-protein based antigens such as bacterial polysaccharides)</li><li>• Risk of affecting genes controlling cell growth</li><li>• Possibility of inducing antibody production against DNA</li><li>• Possibility of tolerance to the antigen (protein) produced</li><li>• Potential for atypical processing of bacterial and parasite proteins</li><li>• Extended immuno stimulation leads to chronic inflammation</li></ul>

# Vector vaccines

- Vector vaccines are experimental vaccines similar to DNA vaccines, but they use an attenuated virus or bacterium to introduce microbial DNA to cells of the body.
- “Vector” refers to the virus or bacterium used as the carrier vector vaccines (DNA sequence coding for the foreign gene + harmless bacterium, plasmid & virus) closely mimic a natural infection and therefore do a good job of stimulating the immune system.

# Vector vaccines



# Marker vaccines

- Also known as “Gene Deleted / DIVA vaccines”
- Marker vaccines, being a form of the virus/bacterium without a particular gene coding for a non protective antigen so, do not produce a key antibody.
- it is a vaccine which allows for the differentiation between infected and vaccinated subjects so it has significant importance in diagnostic aspects.
- The first such vaccines were used to protect pigs against Aujeszky's disease (AD). The same principles were subsequently applied to the development of vaccines against infectious bovine rhinotracheitis (IBR).

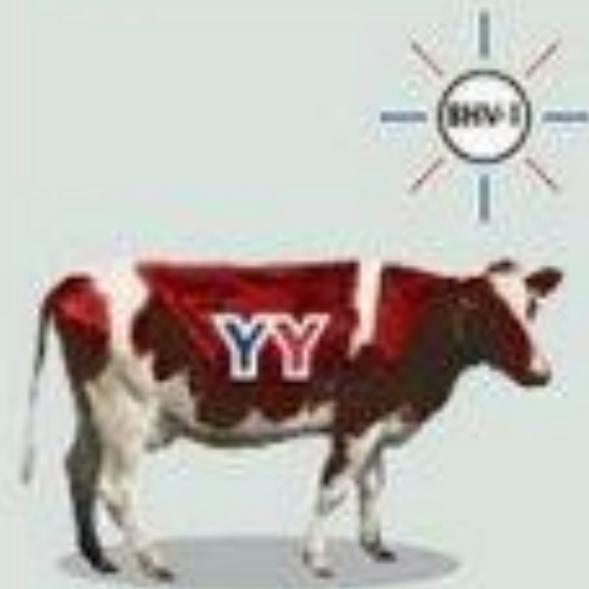
## Principle of marker vaccines

- Aujeszky's disease virus vaccines strain lack a specific glycoprotein gene (**gG**, **gE**, or **gC**).
- Infectious bovine rhinotracheitis (IBR) disease virus vaccines strain lack glycoprotein gene **gE** , **gB** (Envelope) and **tK** (Thymidine kinase, virulence).
- FMDV serotype O virus (vaccine strain O IND R2/1975) disease virus vaccines strain, lack a specific non-structural proteins **3A** and **3B**.

# Principle of marker vaccines

## Differential test for marker vaccinated and IBR positive cow

1. IBR infected cow



2. Traditional vaccine



3. Bovilis IBR marker



Test: Y=anti gB  
Y=anti gE

Test: Y=anti gB  
Y=anti gE

Test: Y=anti gB

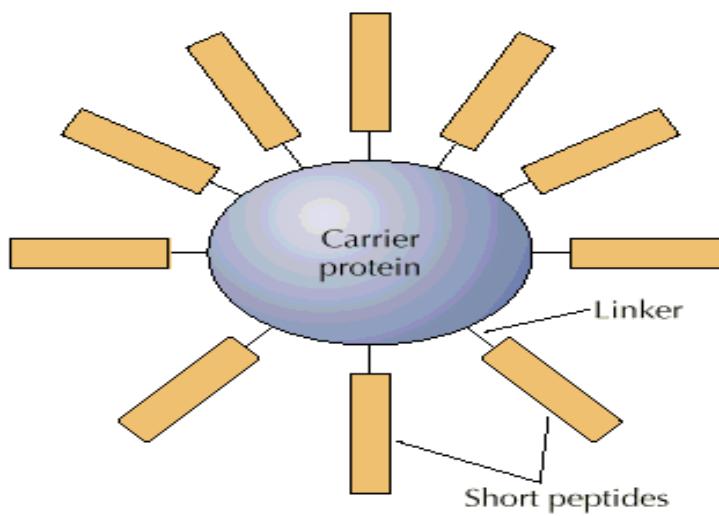
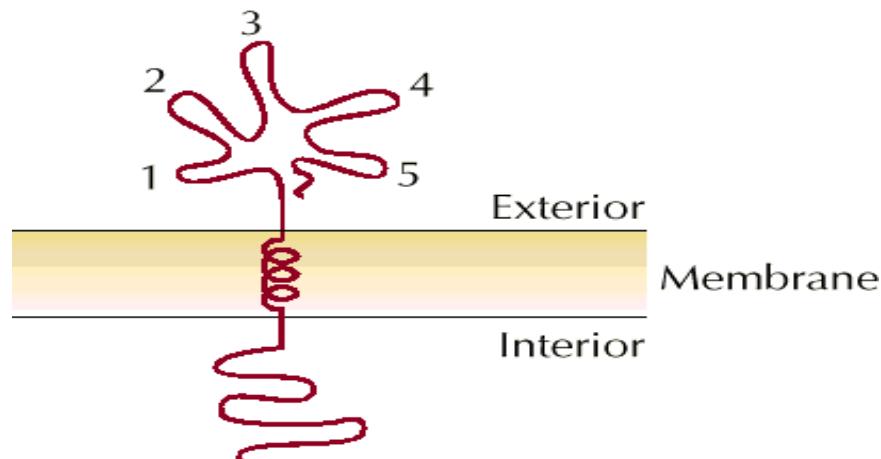
Y= Antibodies • gB and gE= Proteins on surface of virus

# Synthetic vaccine

Synthetic vaccines mainly consists of

- Synthetic peptides,
- Carbohydrates, or
- Antigens
- The development of synthetic vaccine based on the identification of immunogenic sites.
- Their antigens are precisely defined and free from unnecessary components which may be associated with side effects.

## Selection & delivery of vaccine peptides:



Use discrete portion (domain) of a surface protein as Vaccine

These domains are 'epitopes' (antigenic determinants) -> are recognized by antibodies

## CARRIER PROTEINS

Problem -> Small Peptides are often Digested  
-> no strong immune response

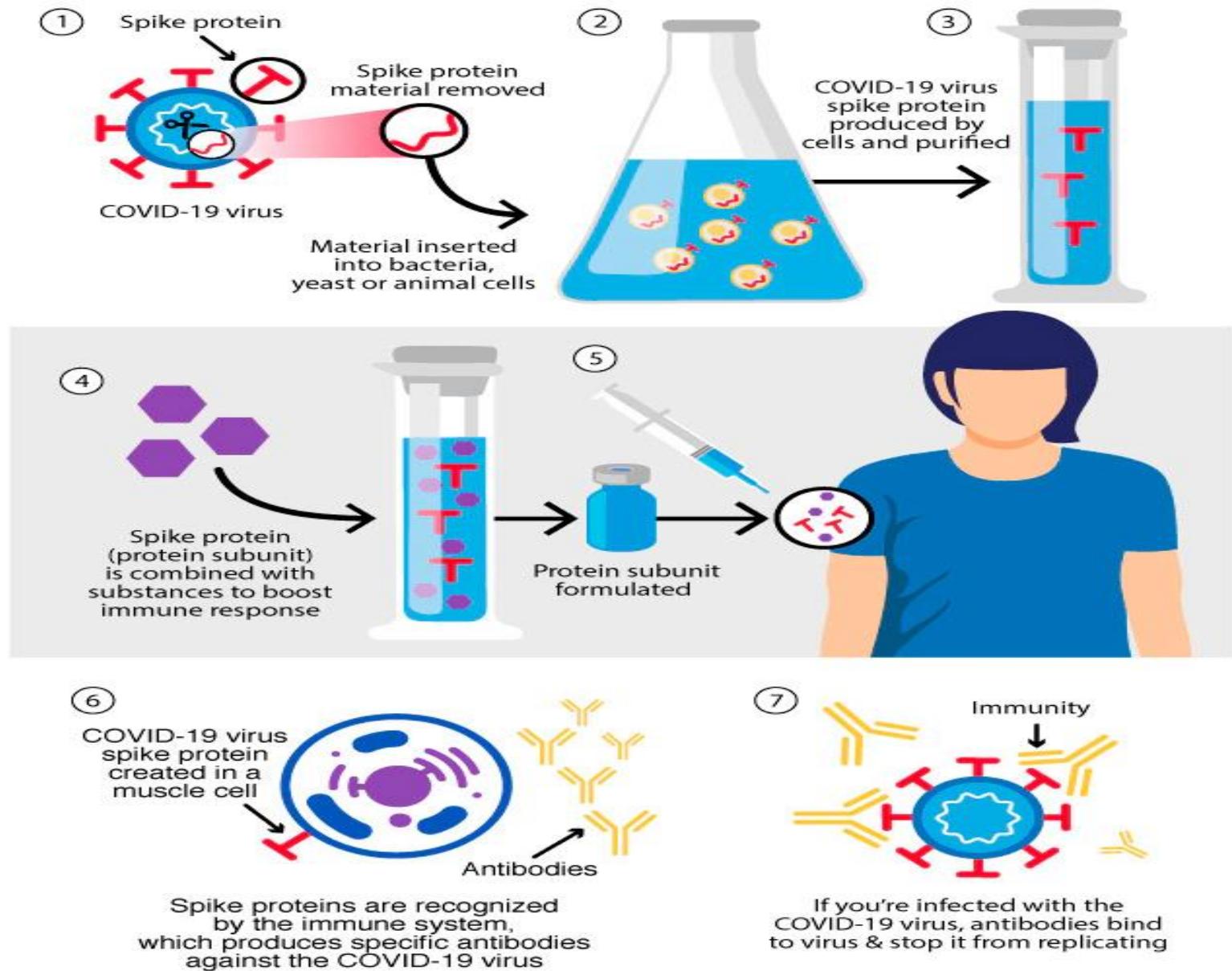
-> Carrier Proteins Make more Stable + stronger immune response

Make fusion protein of carrier + vaccine peptide  
-> inert carrier or highly immunogenic carrier (hepatitis B core protein)

# Advantages Synthetic Peptide Vaccines?

- Chemically defined product
- Stable indefinitely at ambient temperature
- No infectious agent present - hence no problems with innocuity
- No large-scale production plant required
- No downstream processing required
- Can be designed to stimulate appropriate immune responses
- Provide the opportunity to use delayed-release mechanisms

# Protein subunit vaccine



# Advances in veterinary vaccines

## **Non-replicating recombinant antigen(s)-vaccine:**

Feline Leukemia Vaccine, Killed Virus

Avian Influenza Vaccine, H5N3 Subtype, Killed Virus

Porcine Circovirus Vaccine, Type 1 -Type 2 Chimera, Killed Virus

Porcine Circovirus Vaccine, Type 2, Killed Baculovirus Vector

*Escherichia Coli* Bacterin-Toxoid

*Borrelia Burgdorferi* Bacterial Extract

Feline Leukemia Virus Antigen

## **Nucleic acid-mediated (not synthetic)-vaccine:**

West Nile Virus Vaccine, DNA

Canine Melanoma Vaccine, DNA

# Advances in veterinary vaccines

## **Live gene deleted:**

*Escherichia Coli* Vaccine, Live Culture

*Salmonella Dublin* Vaccine, Live Culture

Pseudorabies Vaccine, Modified Live Virus

*Salmonella Typhimurium* Vaccine, Live Culture

## **Live vectored:**

Marek's Disease Vaccine, Serotypes 1 & 3, Live Herpesvirus Chimera

Avian Influenza-Fowl Pox Vaccine, H5 Subtype, Live Fowl Pox Vector

Distemper Vaccine, Live Canarypox Vector

Equine Influenza Vaccine, Live Canarypox Vector

Canine Distemper-Adenovirus Type 2-Parvovirus Vaccine, Modified Live Virus, Canarypox Vector

Canine Distemper-Adenovirus Type 2-Parainfluenza-Parvovirus Vaccine, Modified Live Virus, Canarypox Vector

Rabies Vaccine, Live Canarypox Vector

West Nile Virus Vaccine, Live Canarypox Vector

Feline Leukemia Vaccine, Live Canarypox Vector

Rabies Vaccine, Live Vaccinia Vector

Newcastle Disease-Fowl Pox Vaccine, Live Fowl Pox Vector

Fowl Pox-*Mycoplasma Gallisepticum* Vaccine, Live Fowl Pox Vector

Fowl Pox-Laryngotracheitis Vaccine, Live Fowl Pox Vector

Bursal Disease-Marek's Disease Vaccine, Serotype 3, Live Marek's Disease Vector

Marek's Disease-Newcastle Disease Vaccine, Serotype 3, Live Marek's Disease Vector

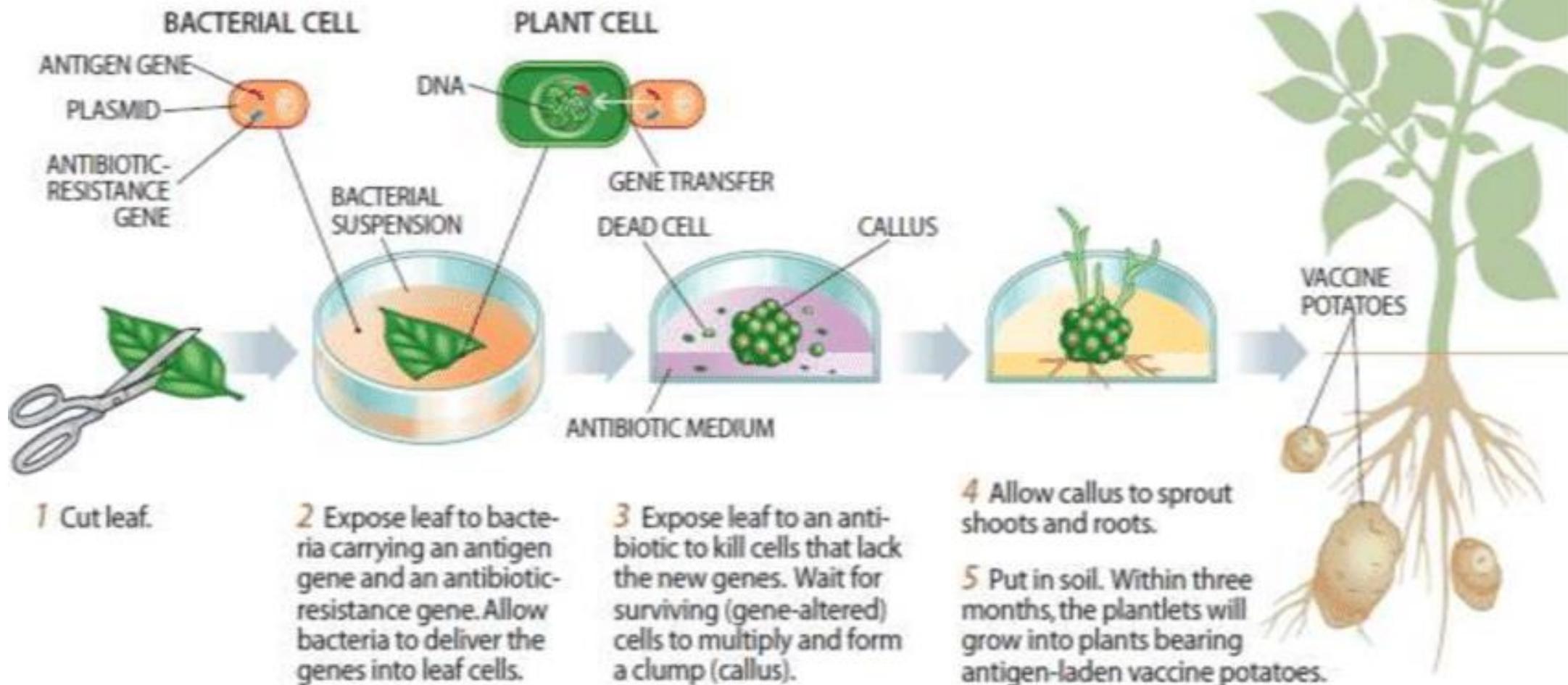
# Edible vaccine

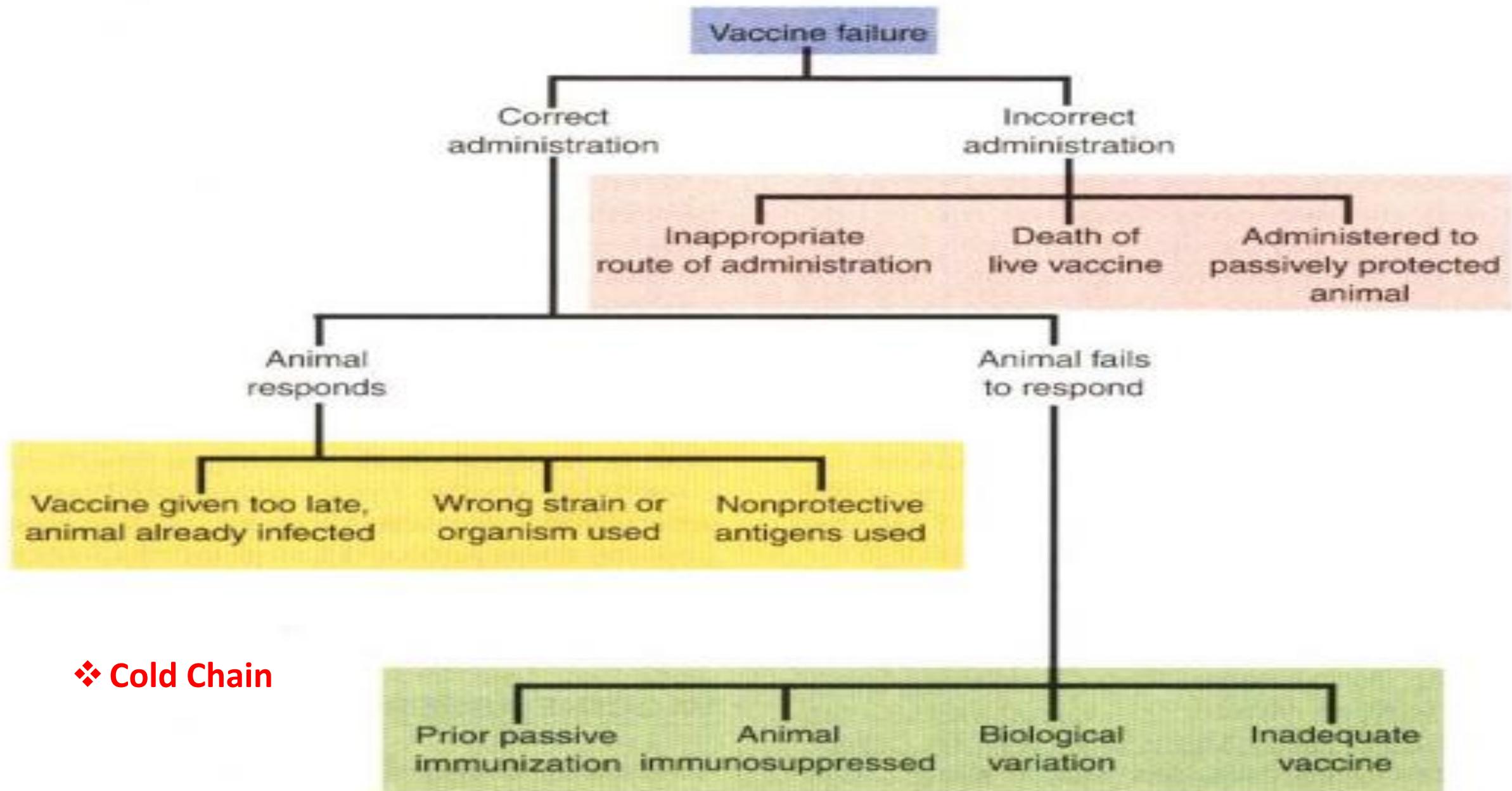
- Here edible plants are used as site of antigen production.
- Transgenic plants are developed expressing antigens derived from animal viruses / bacteria
- Here the antigenic gene carrying plasmid is introduced into the plant with the help of bacterium *Agrobacterium tumefaciens*.
- The first clinical trial conducted in 1997.
- Transgenic potatoes with a toxin of *E. coli* causing diarrhea.

# HOW TO MAKE AN EDIBLE VACCINE

One way of generating edible vaccines relies on the bacterium *Agrobacterium tumefaciens* to deliver into plant cells the genetic blueprints for viral or bacterial

"antigens"—proteins that elicit a targeted immune response in the recipient. The diagram illustrates the production of vaccine potatoes.





### ❖ Cold Chain

**Figure 22-1.** A classification of the causes of vaccine failure.

# Thank You

Dr. Sandeep Kumar Sharma, BVSc & AH, MVSc and PhD  
Assistant Professor, PGIVER  
[drsharmask01@hotmail.com](mailto:drsharmask01@hotmail.com)  
Mob. 9414775879