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MEDICAL IMMUNOLOGY

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BY

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Introduction to the Immune System

Immunology is the study of the ways in which the body defends itself from infectious agents and other foreign substances in its environment. The immune system protect us from pathogens. It has the ability to discriminate (differentiate) between the individual`s own cells and harmful invading organisms.

Immune system has two lines of defense:

a. Innate (non specific) immunity

b. Adaptive (specific) immunity

1.Innate immunity:

- **Characters**

1. 1st line of defense
2. Rapid defense
3. The same on re-exposure to Ag
4. No memory cell
5. Recognize and react against microbes only
6. Block entry of microbes and eliminate succeeded microbes which entered the host

Components:

1. **Barriers:**
   - **a. Physical barriers: protect against invasion of microbes eg epidermis & keratinocyte & epithelium of mucus membrane & cilia**
     b. **Mechanical barrier : longitudinal flow of air and fluid & movement of mucus by cilia**
c. **Chemical barriers:**

-Skin: α & β defensin & lysozyme & RNase & DNase

- **-Resp Tract: β defensin**
- **-GIT: α defensin & pepsin & lysozyme**
- **-HCL of stomach: kill ingested microbes**
- **-Tears in eye: lysozyme**

☞ d. **Biological barriers: commensal microbes or flora inhibit growth of pathogenic bacteria**

2. **Innate immune cells: phagocytes (Macrophage & neutrophil)& NK cells**

3. **Cytokines: TNF &IL1& IL12& IFNγ & chemokines**

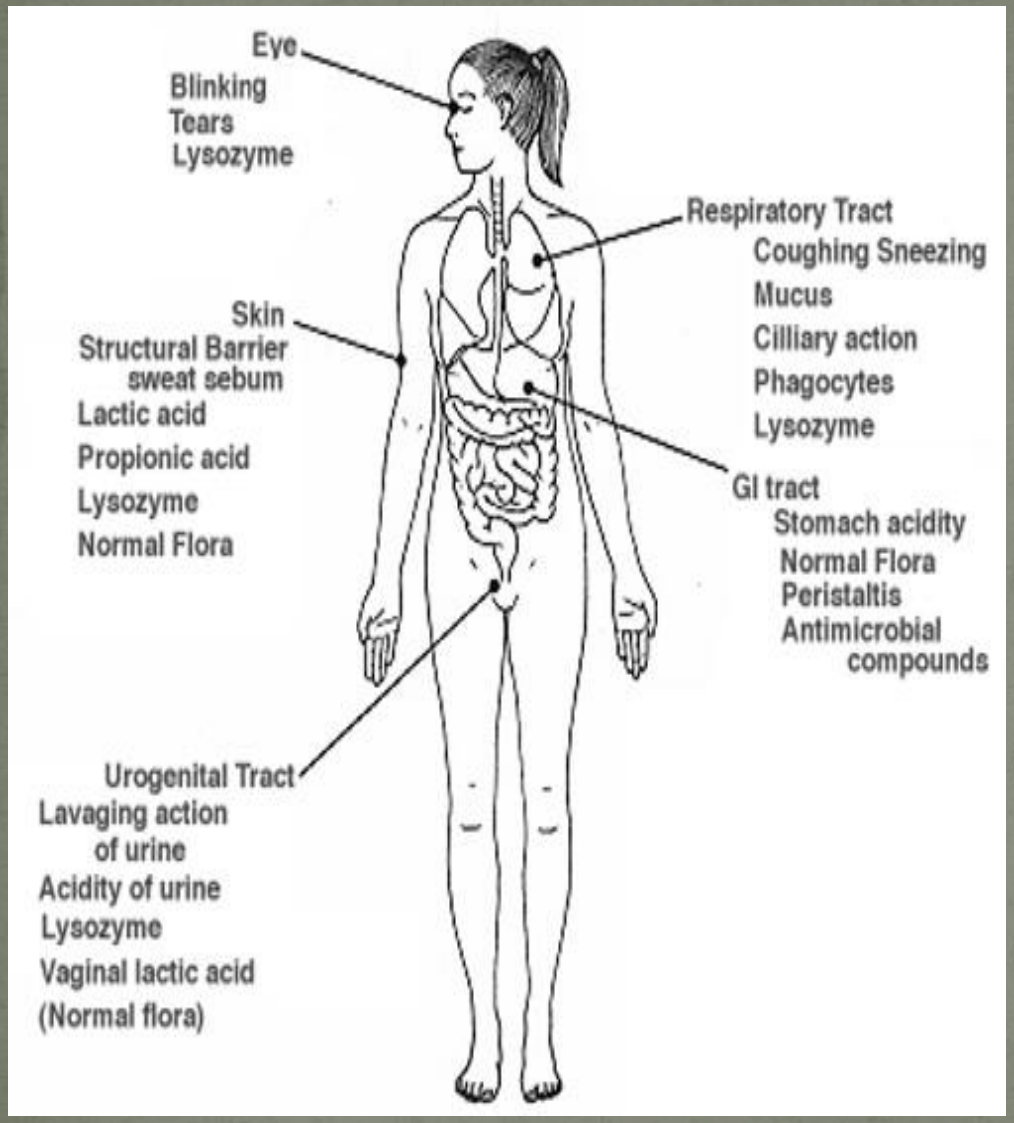
4. **Complement: Alternative pathway & lectin pathway**

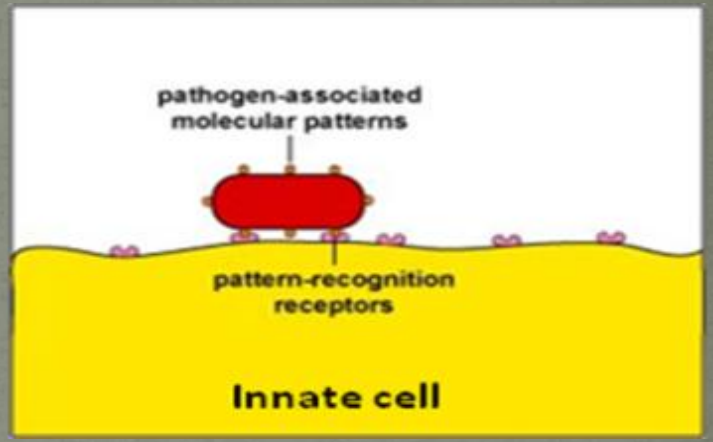
5. **Other plasma proteins (acute phase response):**

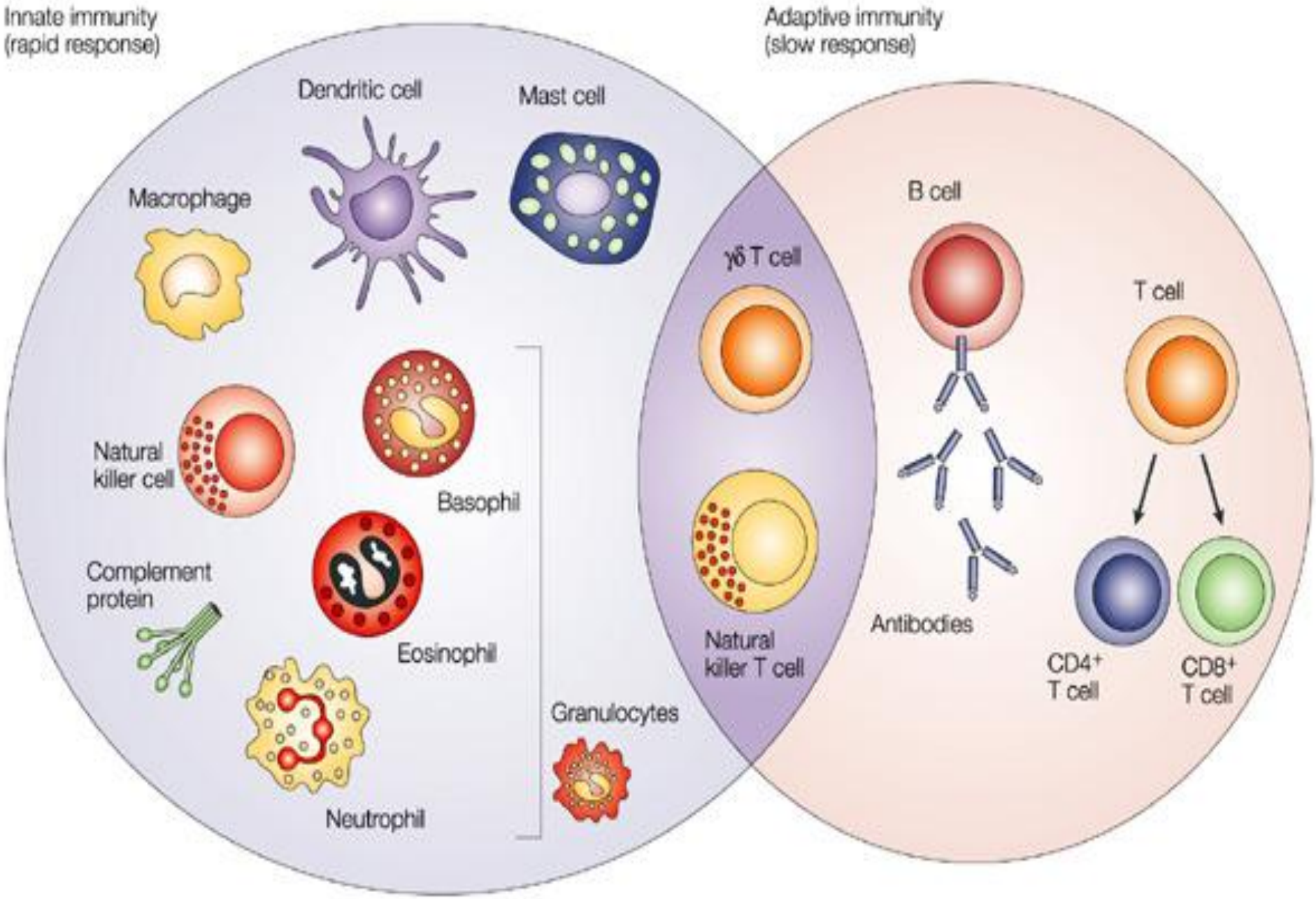
↑ **Mannose Binding Lectin : participate in lectin pathway of complement**

↑ **C Reactive Protein: coat microbes and help in phagocytosis**

NB: Recognition of microbes by the innate system: the receptors of innate cells (pathogen-recognition receptors) recognize structures called pathogen-associated molecular patterns (PAMPs) shared by different microbes


Eye
Blinking
Tears
Lysozyme
Respiratory Tract
Coughing Sneezing
Mucus
Cilliary action
Phagocytes
Lysozyme
Skin
Structural Barrier
sweat sebum
Lactic acid
Propionic acid
Lysozyme
Normal Flora
GI tract
Stomach acidity
Normal Flora
Peristaltis
Antimicrobial
compounds
Urogenital Tract
Lavaging action
of urine
Acidity of urine
Lysozyme
Vaginal lactic acid
(Normal flora)


pathogen-associated
molecular patterns
pattern-recognition
receptors
Innate cell


Innate immunity
(rapid response)
Adaptive immunity
(slow response)
Dendritic cell
Mast cell
Macrophage
γδ T cell
B cell
T cell
Natural
killer cell
Basophil
Complement
protein
Eosinophil
Natural
killer T cell
Antibodies
CD4+
T cell
CD8+
T cell
Granulocytes
Neutrophil

Adaptive immunity:

Characters

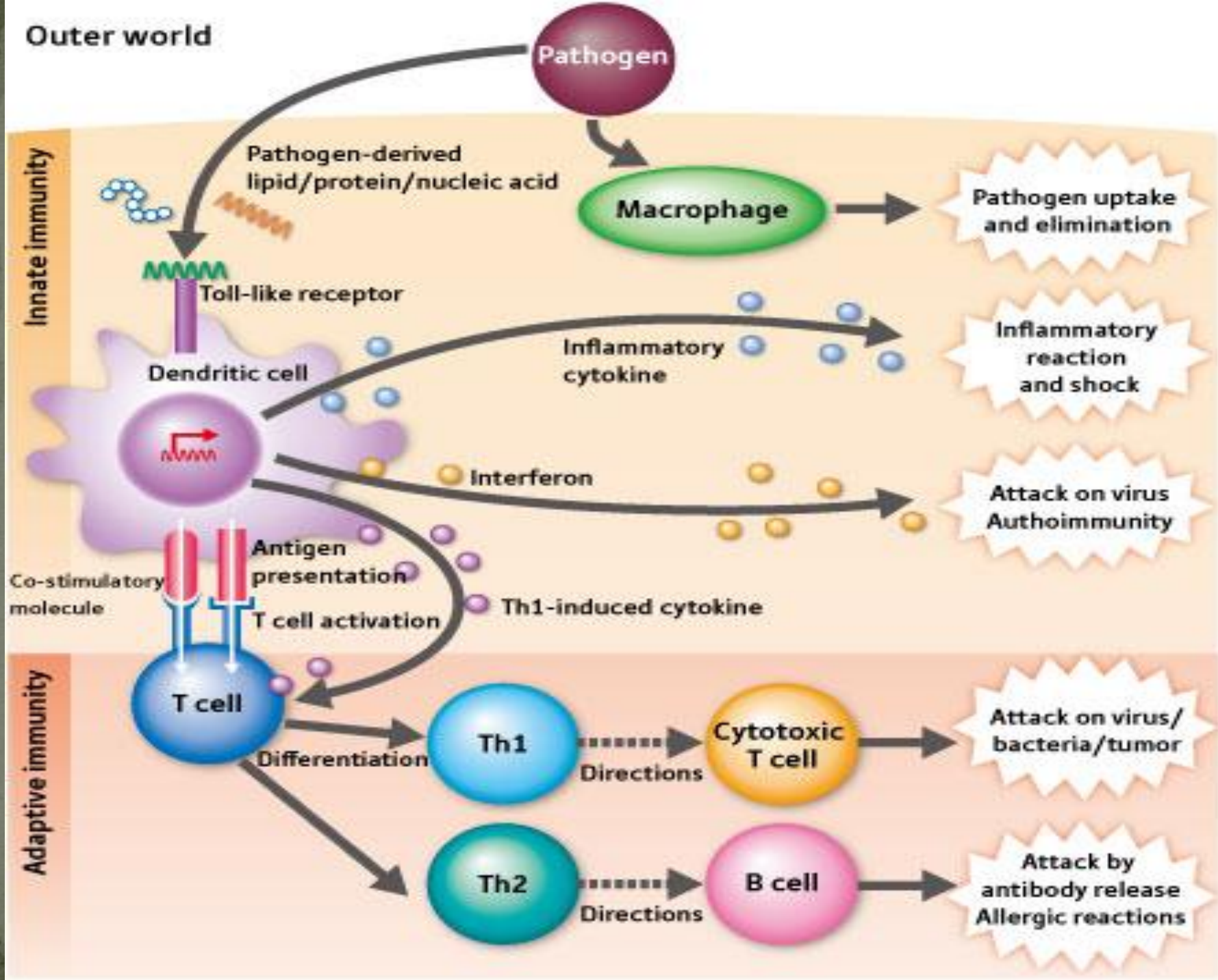
1. 2nd line of defense
2. Delayed as response to infection
3. Specific for microbes & Antigen (can differentiate Antigen)
4. Has memory cell which remember microbes and give strong immune response on re-exposure

components (sequential phases)

1. Ag recognition by lymphocyte through specific receptor to Ag
2. Activation of lymphocyte → proliferation → differentiation into memory cell & effector cell
3. Elimination of microbes
4. Decline & Termination of immune response
5. Long lived memory cell

Cells of adaptive immunity

1. **B lymphocyte : produce antibodies that neutralize and eliminate extracellular microbes and toxins(humoral immunity)**
2. **T lymphocyte: eradicate intracellular microbes (cell mediated immunity**


Outer world
Pathogen
Innate immunity
Pathogen-derived
lipid/protein/nucleic acid
Macrophage
Pathogen uptake
and elimination
Toll-like receptor
Dendritic cell
Inflammatory
cytokine
Inflammatory
reaction
and shock
Interferon
Attack on virus
Authoimmunity
Antigen
presentation
Co-stimulatory
molecule
T cell activation
Th1-induced cytokine
Adaptive immunity
T cell
Differentiation
Th1
Directions
Cytotoxic
T cell
Attack on virus/
bacteria/tumor
Th2
Directions
B cell
Attack by
antibody release
Allergic reactions

Cells of The Immune System

Hematopoitic stem cell in the bone marrow give

a. Lymphoid progenitor: give

- T lymphocyte
- B lymphocyte
- NK cell

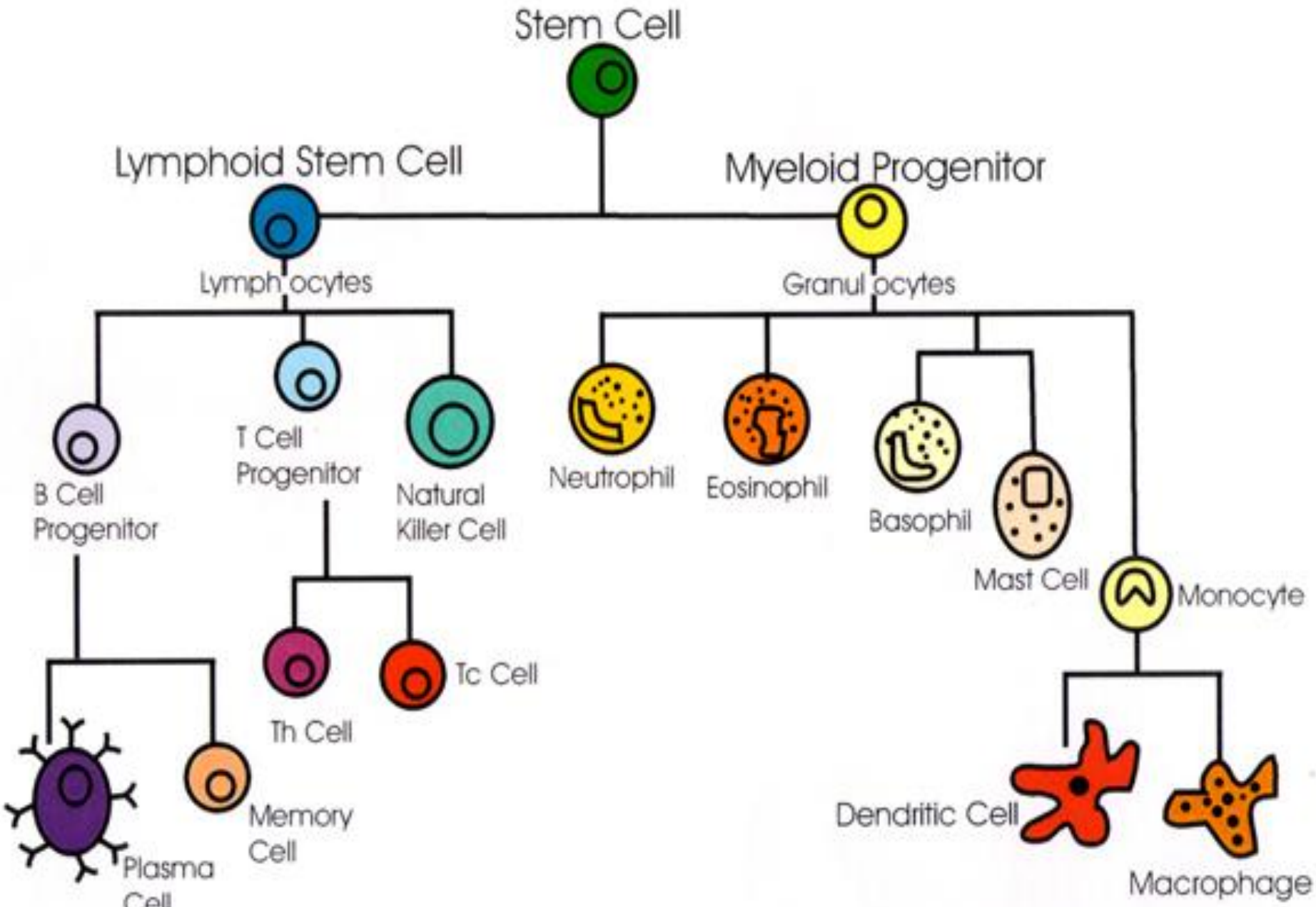
b. Myeloid progenitor: give

Leucocytes (neutrophils & eosinophils & basophils & mast cells & monocytes)

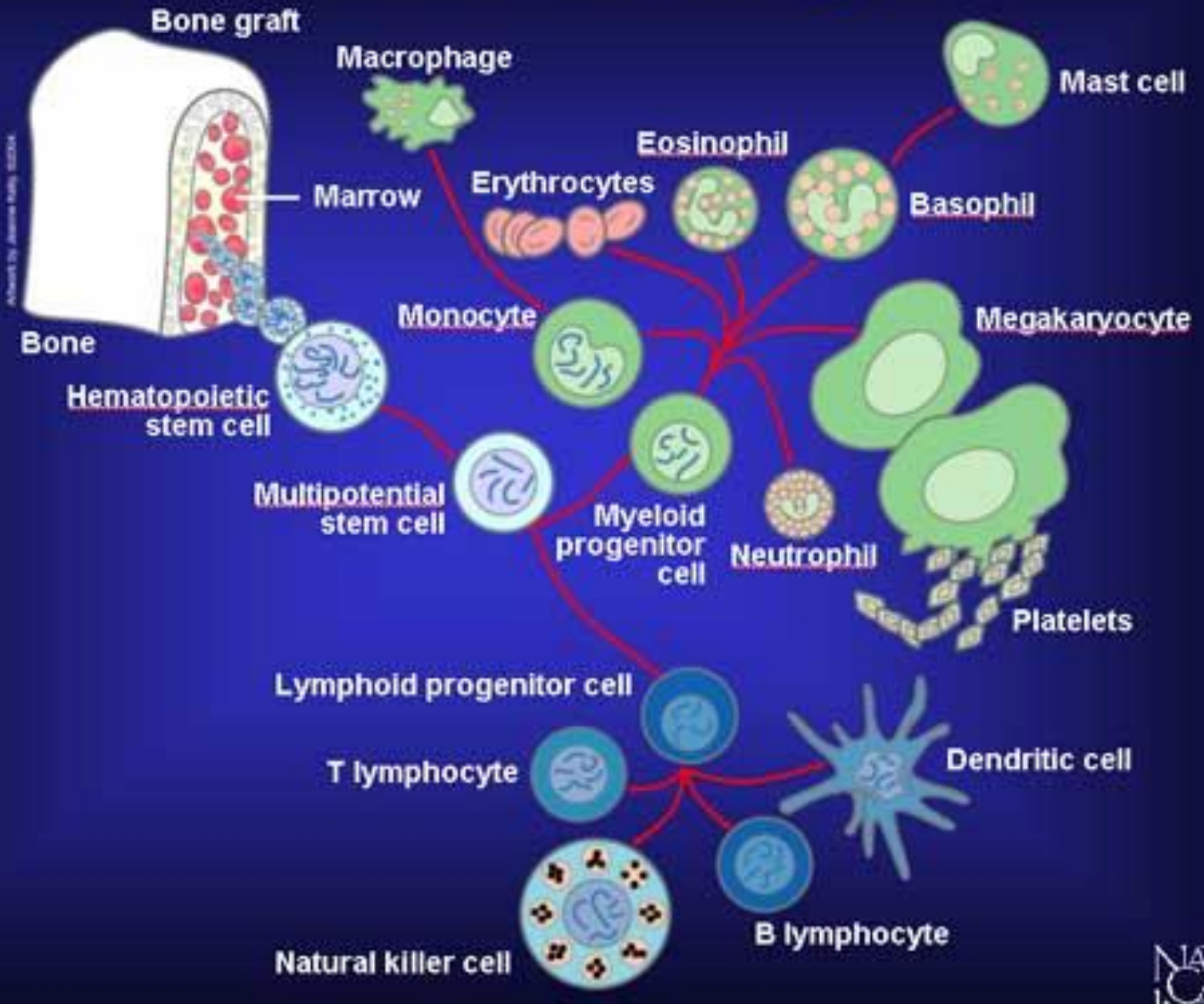
Erythrocyte

Platelets

Cells of the Immune System



Cells of the Immune System



Stem cells

Stem cells are undifferentiated (unspecialized) cells which possess

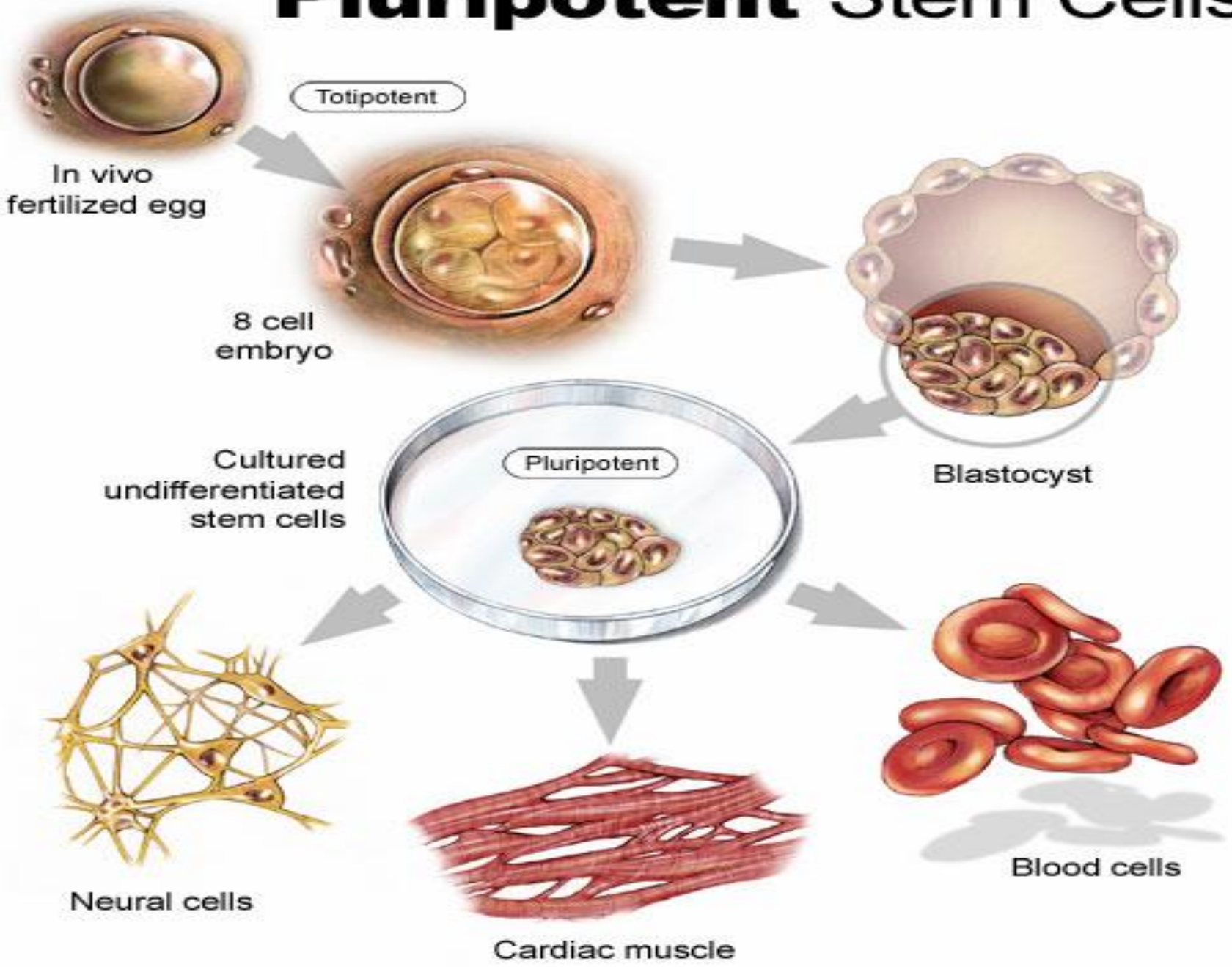
1. Self-renewal – and can be maintained in undifferentiated state.
2. Potency - the capacity to differentiate into specialized cell types e.g. muscle cell, a red blood cell, nerve cell or a brain cell.

Types of mammalian stem cells:

1. Embryonic stem cells (isolated from the inner cell mass of blastocysts)
2. Adult stem cells (umbilical cord blood and bone marrow).
    - **Medical importance:**
        - Has the potential to treatment of human diseases.

- Could be used in the generation of cells and tissues for transplantation.
- Bone marrow transplants that are used to treat leukemia.
- Possible to be introduced into damaged tissue in order to treat a disease or injury e.g. cancer, type1 diabetes mellitus , cardiac damage, Parkinson's disease, spinal cord injuries, and muscle damage.

Pluripotent Stem Cells



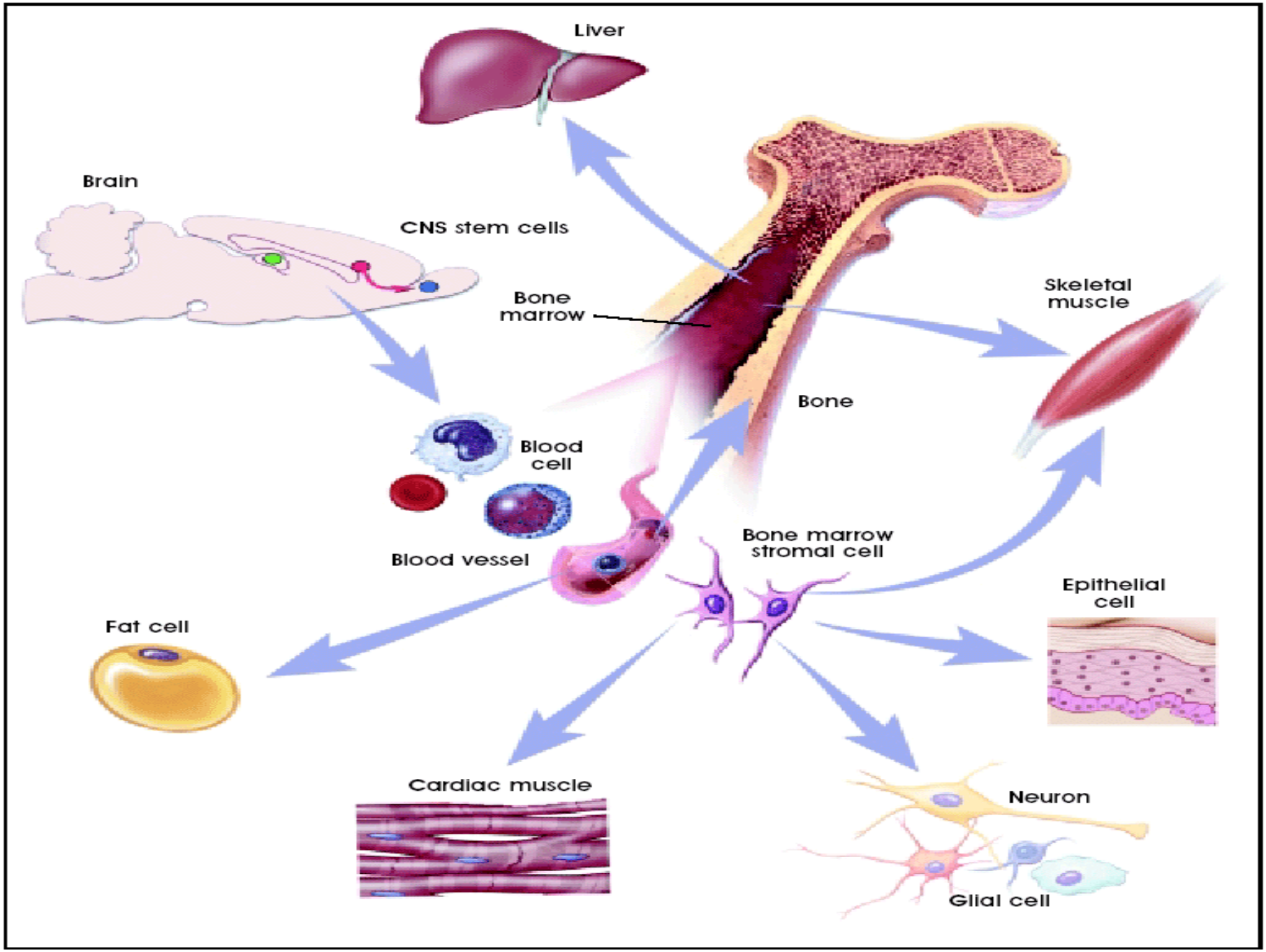
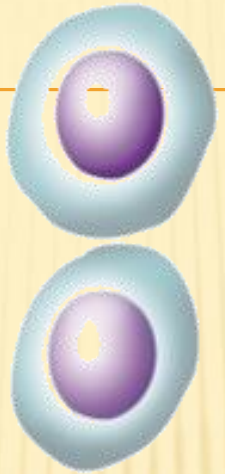


Figure 4.2. Preliminary Evidence of Plasticity Among Nonhuman Adult Stem Cells.

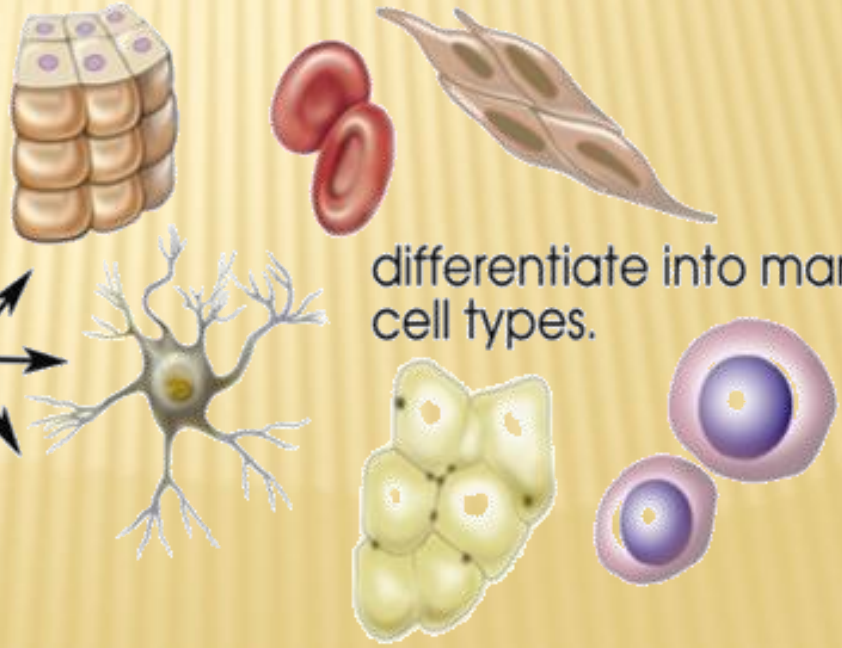
What is a stem cell?

A single cell that can

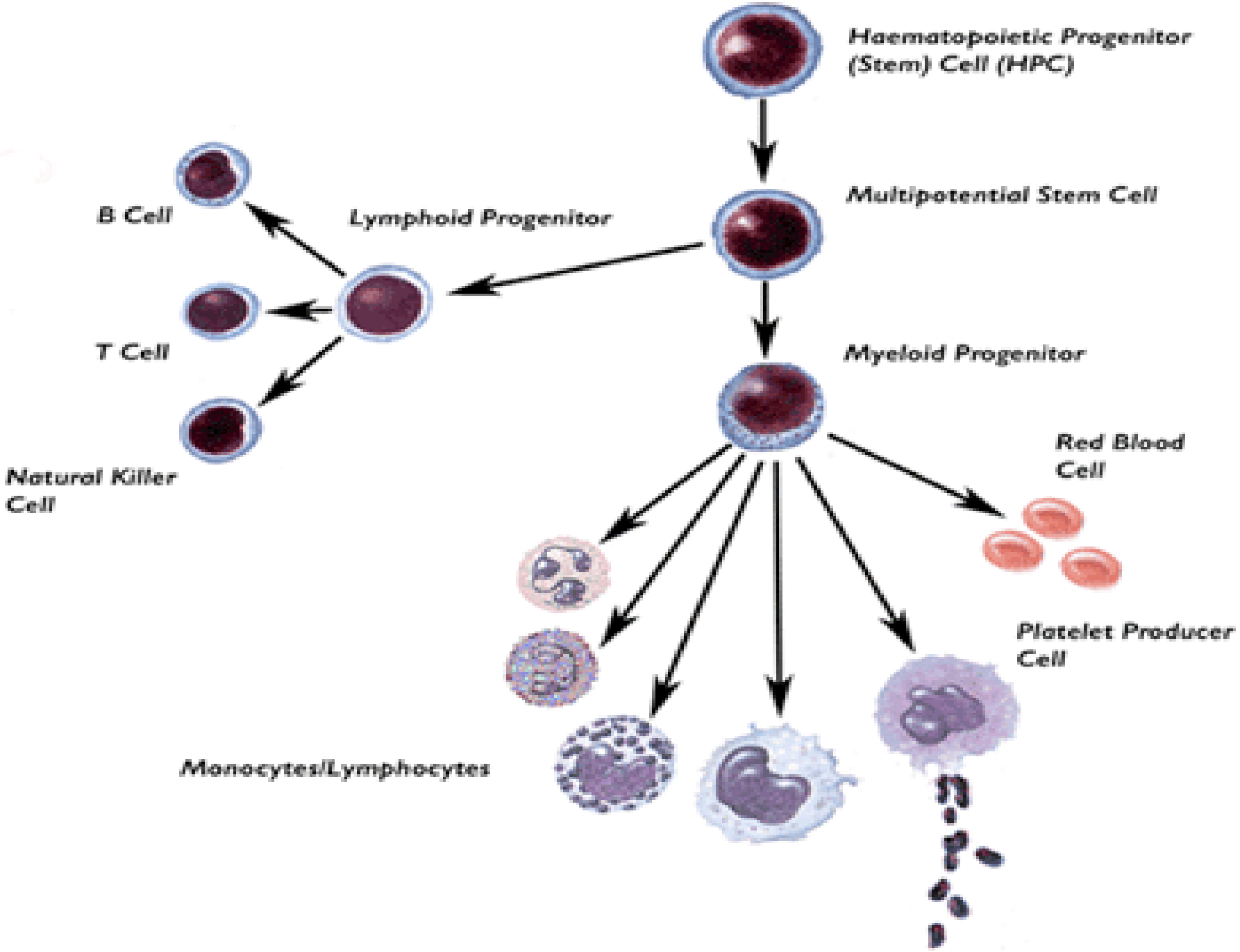
replicate itself, or...



differentiate into many cell types.



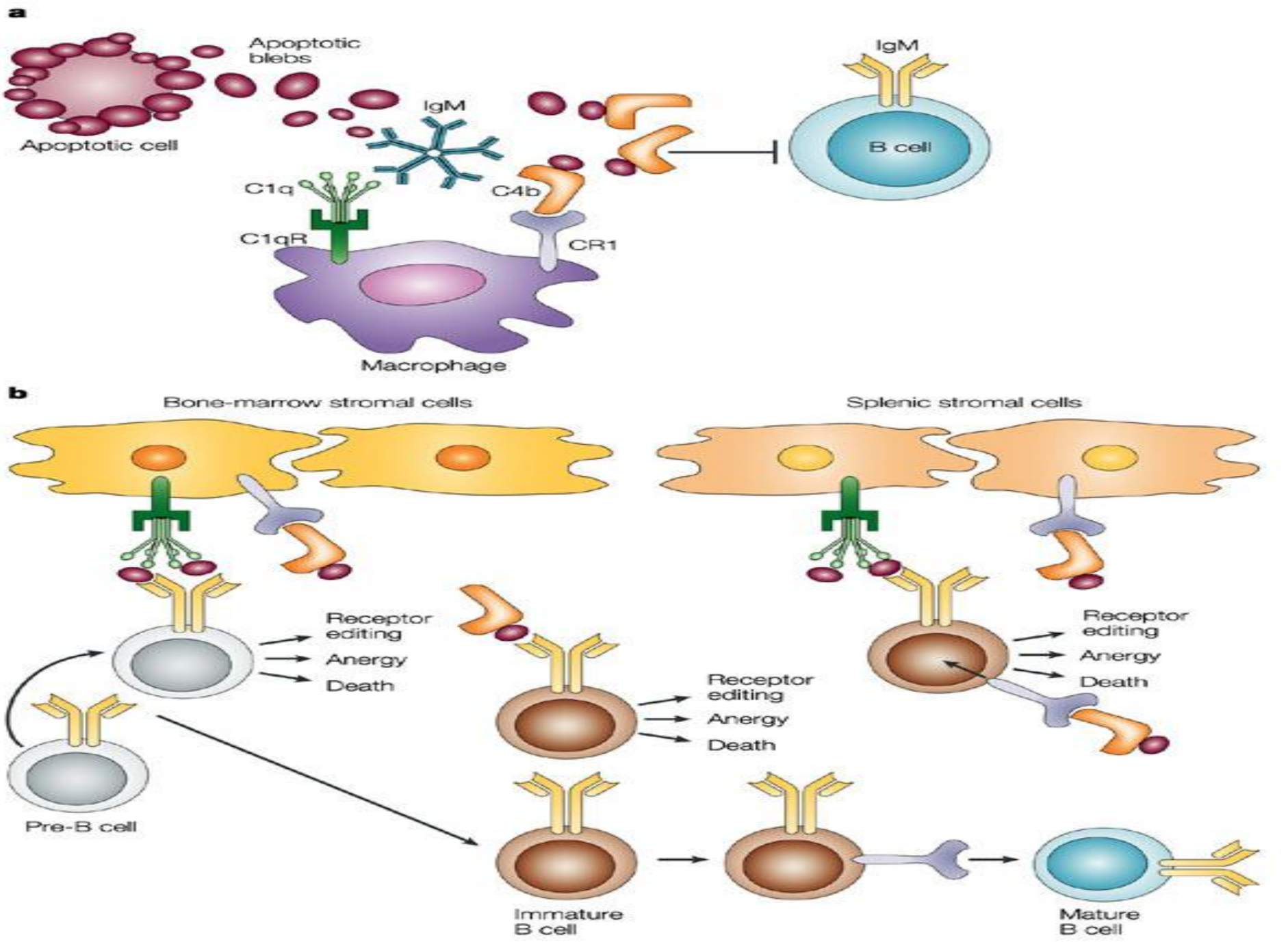
ge prepared by Catherine Twomey for the National Academies,
erstanding Stem Cells: An Overview of the Science and Issues
the National Academies, http://www.nationalacademies.org/stemcells.


Haematopoietic Progenitor (Stem) Cell (HPC)
Multipotential Stem Cell
Lymphoid Progenitor
B Cell
T Cell
Natural Killer Cell
Myeloid Progenitor
Red Blood Cell
Platelet Producer Cell
Monocytes/Lymphocytes

Lymphocytes

- Lymphocytes are the only cells with specific receptors for antigens and are
- the key mediators of adaptive immunity.
- They can be distinguished by surface proteins identified by monoclonal
- antibodies..... the standard nomenclature fot these proteins is the"CD"
- (cluster of differentiation) and a number ; for example CD1, CD2, CD3,
- ….etc.
- **Lymphocytes include:**
  - B lymphocytes: mediators of humoral immunity
- T lymphocytes: mediators of cell-mediated immunity.
- Natural killer cells: cells of innate immunity

	B lymphocyte	T lymphocyte
arise from	Bone marrow	
mature in	Bone marrow	Thymus
Name	Bone marrow lymphocytes	Thymus derived lymphocytes
% of total blood lymphocyte	10 – 15 %	Majority
Steps in maturation	Stem cell → lymphoid progenitor → pre B cell → immature B cell → mature/naïve B cell → leave bone marrow to meet antigen in the 2nd lymphoid organs	Stem cell → lymphoid progenitor → immature T cell → leave bone marrow to thymus gland → maturation & selection → mature/naïve T cells T helper (CD4) T cytotoxic (CD8) → leave thymus to meet antigen in the 2nd lymphoid organs
phenotypic markers	1. CD 19 & CD 21 2. Fc receptor 3. class II MHC molecule	1. CD 3 2. CD 4 or CD 8 3. T cell receptor (TCR)
Function	Antibody production (humoral immunity)	Cell mediated immunity
Antigen recognized	Protein , polysaccharide, lipid, nucleic acid and small chemicals (free & soluble)	Protein only - CD4 cell recognize → peptide + MHC II molecule - CD8 cell recognize → peptide + MHC I molecule
Antigen recognition receptor	**B cell receptor (BCR)**: membrane Immunoglobulin (Ig M & Ig D)	**TCR**: 2 types α/β TCR & γ/δ TCR **α/β TCR:** common type → 2 poly peptide chain α & β
Stimulation by Ag	B cell proliferation → differentiation into → memory cell & plasma cell which produce antibodies to eliminate Ag	**TCR complex**: - Ag presented on MHC, bind with variable domain of α & β of **TCR** - **CD3 & zeta protein** (signal transduction) → activate T cell
Signaling molecules	2 polypeptide chains → Ig α & β transmit signal inside B cell → B cell proliferation & differentiation into plasma cell	TCR & CD3 & zeta protein
Types	1. naïve B cell 2. plasma cell 3. memory cell	**1.** **T helper (CD4)** → produce cytokines which help other cells eg **2.** **Th1** help B cell to produce antibodies **3.** **Th2** help macrophage to destroy ingested microbes **4.** **T cytotoxic (CD8)** Also called cytolytic as lyse virus infected cell & kill tumor cells & graft rejection **1.** **T regulatory (Treg)** Suppress the immune response


a
Apoptotic blebs
IgM
IgM
Apoptotic cell
B cell
C1q
C4b
C1qR
CR1
Macrophage
b
Bone-marrow stromal cells
Splenic stromal cells
Receptor editing
Anergy
Death
Receptor editing
Anergy
Death
Receptor editing
Anergy
Death
Pre-B cell
Immature B cell
Mature B cell

- Are large lymphocytes with numerous cytoplasmic granules

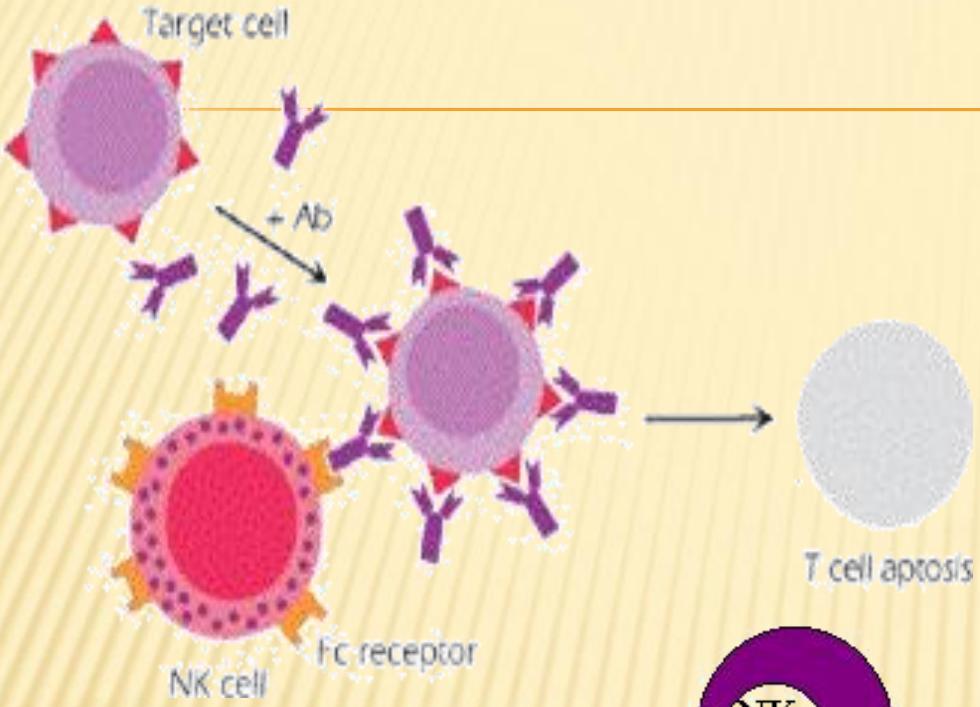
- NK cells comprise about 10% of blood lymphocytes.
- NK cells receptors :
  - Killer activation receptors (KARs) , initiate killing of target cells after recognition and binding stress molecules on its surface
  - Killer inhibitory receptors (KIRs) , inhibit killing of target cells after binding to MHC I molecules on its surface

- **Function of NK cells:**

  Activated by IL-12 →

  - Killing tumor cells.
  - Killing virus-infected cells.
- Produce IFN-γ which activate macrophages.

Antibody-dependent cellular cytotoxicity (ADCC



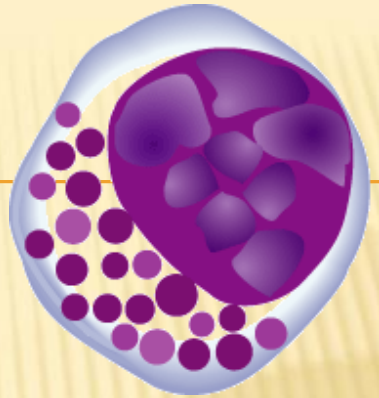
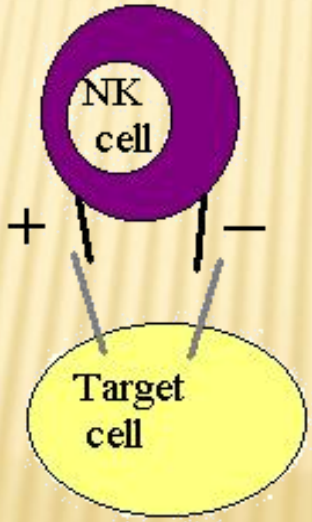
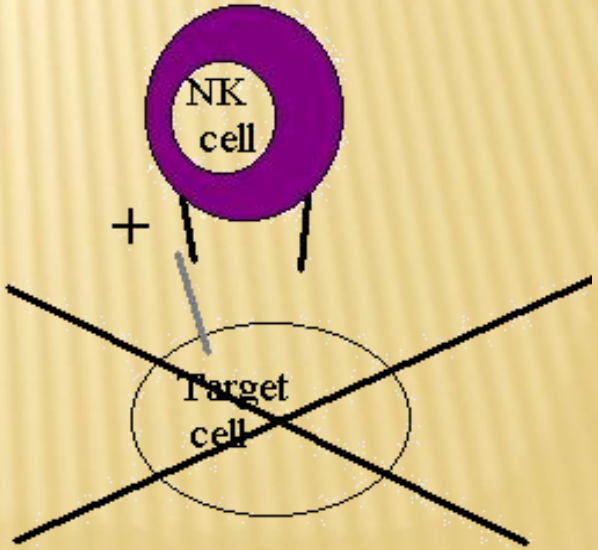


Fig.6 Nk cells



Both signals: Cell is not killed

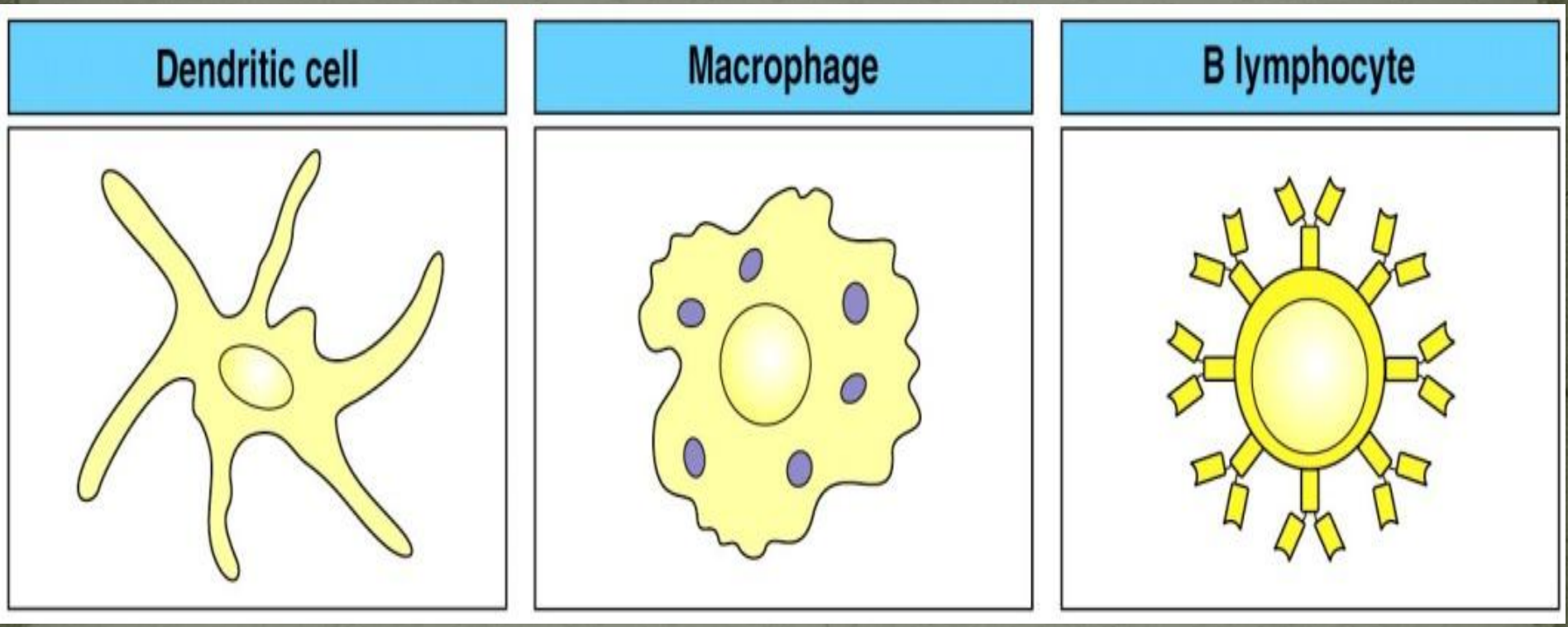


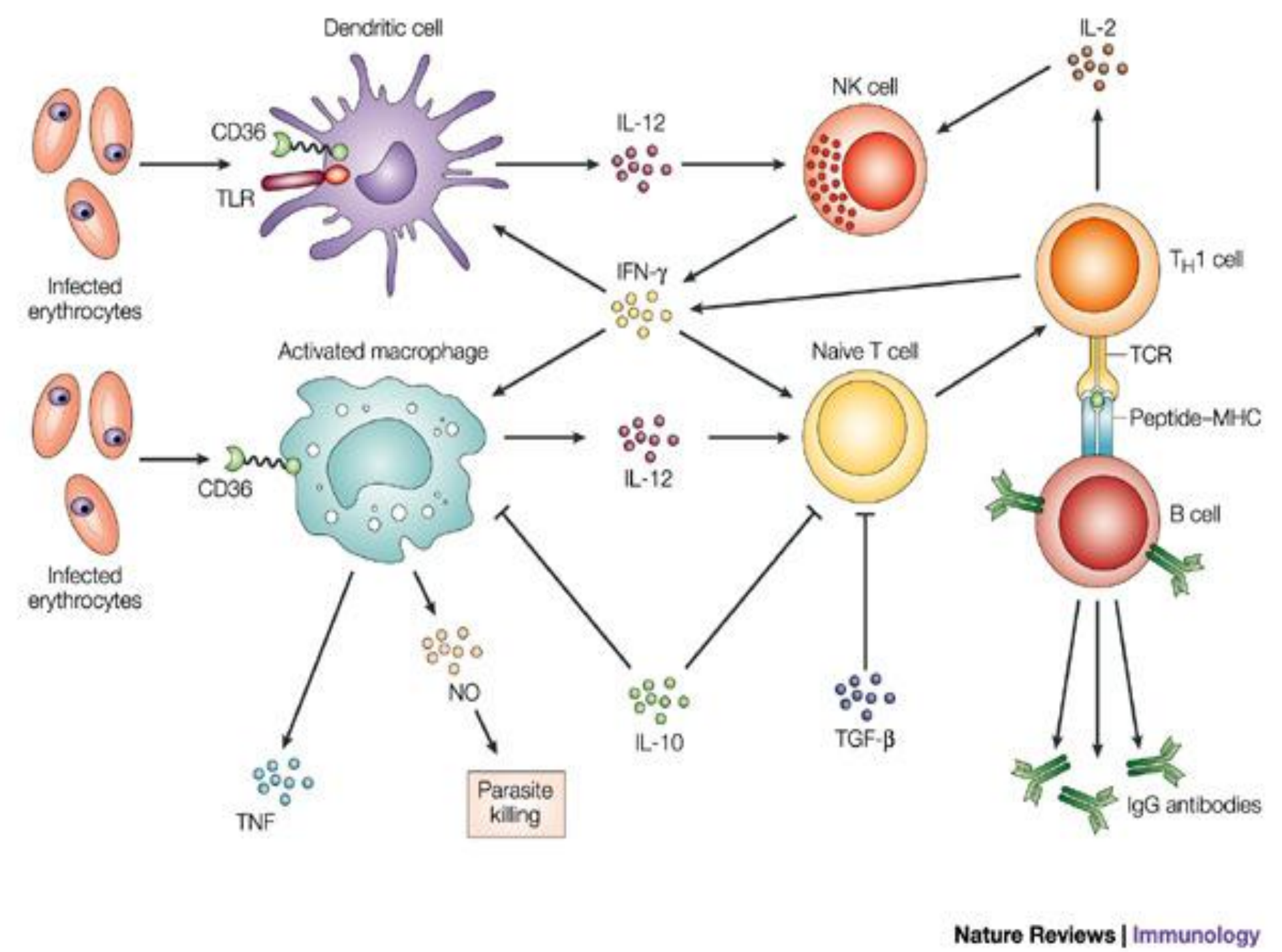
One Signal: Cell is killed

Antigen Presenting Cells (APCs)

- ✓ **These include:**
  - a Dendritic cells
  - b Macrophages
  - c B lymphocytes
- ✓ **Occurance: in the epithelium of the skin, gastrointestinal tract, respiratory tract (the common portal of entry of microbes).**
- ✓ **Functions:**
  - a Capture and transport antigens to the peripheral lymphoid tissues
  - b process antigens

Present the peptides derived from these antigens to T lymphocytes


Dendritic cell
Macrophage
B lymphocyte


Dendritic cell
IL-2
NK cell
CD36
TLR
IL-12
Infected erythrocytes
IFN-γ
T_H1 cell
Activated macrophage
Naive T cell
TCR
Peptide–MHC
CD36
IL-12
B cell
Infected erythrocytes
NO
IL-10
TGF-β
TNF
Parasite killing
IgG antibodies

- ✓ They are **rich in class II MHC molecules**

✓ **Pathways of antigen processing & presentation:**

a. Class II MHC pathway:

i. Protein antigen taken from extracellular environment.

i. Proteins are degraded by lysosomal proteases.

The resulting peptides are presented to CD4+ cells with class II MHC molecules.

b. Class I MHC pathway:

i. Cytosolic proteins e.g. intracellular microbes.

i. Proteins are degraded by a structure called *proteosome.*

i. The resulting peptides are presented to CD8+ cells with class I MHC molecules.

Phagocytes

Definition: Cells which can recognize & ingest & kill microbes &foreign bodies

Types:

	Neutrophil	Macrophage
% in blood	Most **numerous** WBCs ↑ in acute infection	**Few**
Size	**Small**	**Large** & mononuclear
Life	**Few hours**	**Days**

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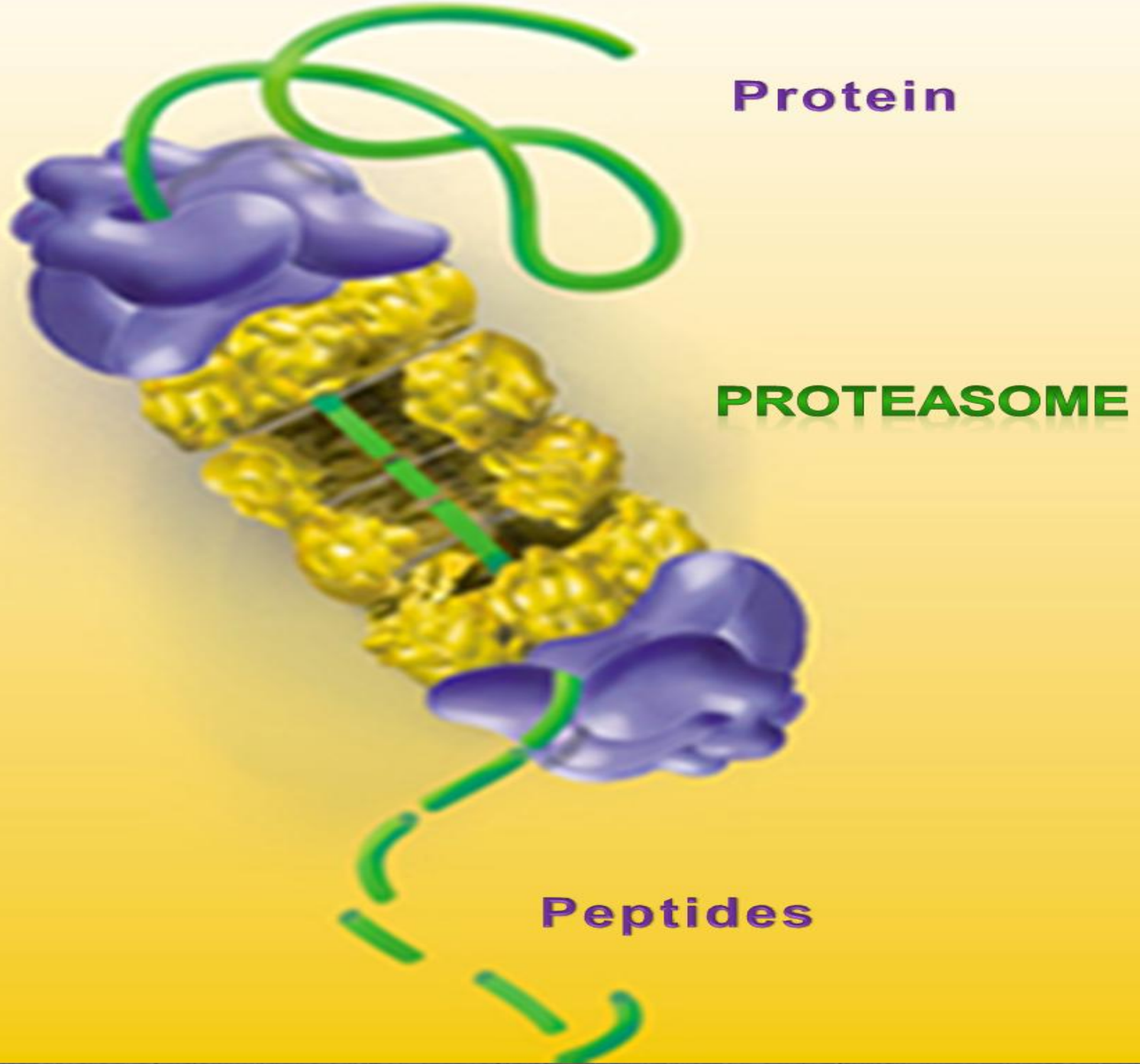
Protein
PROTEASOME
Peptides

Phagocytes

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Stages of phagocytosis:

1 Delivary of phagocytic cell to site if infection:

✓ **Diapedisis: histamine → stimulates phagocytes to migrate through wall of blood vessel to enter tissue**

✓ **Chemotaxis : chemotactic factors as chemokines & complement (C3a & C4a & C5a) attract phagocytes towards microbes in tissue**

2 Recognition of microbes:

- Phagocytes can recognize and bind microbes through receptor on its outer surface eg mannose receptor & Toll-Like receptor

3 dhAerence to target (opsonization)

✓ Microbe coated with IgG or complement (C3b)

✓ IgG or C3b bind with their receptors on phagocytic cells

✓ So they bring microbe near phagocytic cell →easy , helped phagocytosis.

✓ **Opsonins: antibodies (IgG) or C3b which are capable of of enhancing phagocytosis.**

4 Ingestion of target

- Cell membrane of phagocytes invaginate to enclose microbe → microbe become in cytoplasms surrounded by cell membrane (vacuole) phagosome

1 Phagolysosome formation

Phagosome fuses with lysosome forming phagolysosome to kill microbe

2 Intracellular killing

a) <u>O2 dependent system (Respiratory bursts):</u>

- O2 free radicals as H2O2 , O2 & OH .
- Toxic nitrogen oxide.

b) O2 independent agents

- **Lysosomal granules: basic ptn → damage permeability barrier in bacteria & fungi & virus**
- Lactoferrin → **chelating iron ptn → ↓ iron → ↓ bacterial growth**
- Lysosomal enzymes → **as lysozyme & nuclease & phospholiase**

7. Digestion by macrophage

Microbes now are digested into small antigen peptides which presented on MHC to T helper cell.

5. Phagolysosome formation

Phagosome fuses with lysosome forming phagolysosome to kill microbe

6. Intracellular killing

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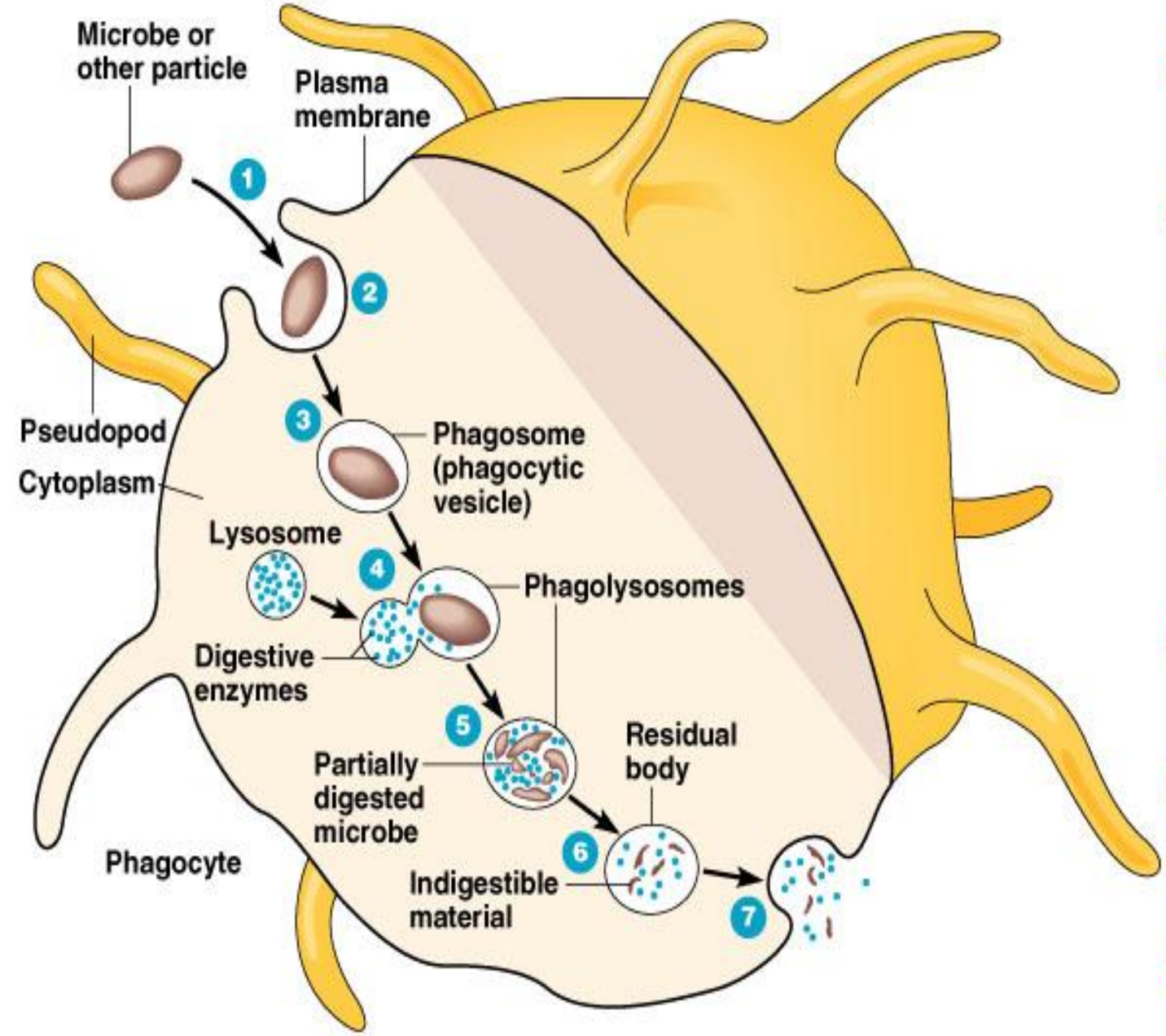
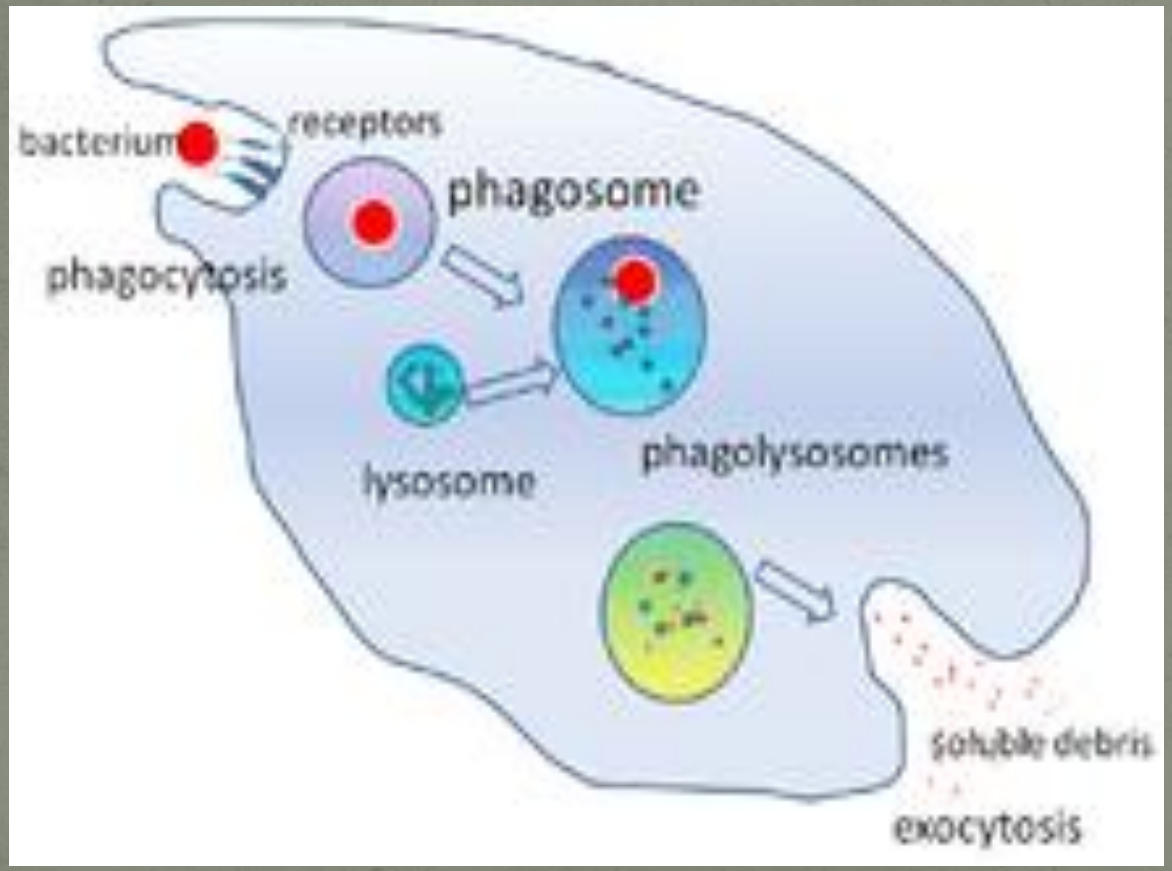


1. Chemotaxis and adherence of microbe to phagocyte.
2. Ingestion of microbe by phagocyte.
3. Formation of a phagosome.
4. Fusion of the phagosome with a lysosome to form a phagolysosome.
5. Digestion of ingested microbe by enzymes.
6. Formation of residual body containing indigestible material.
7. Discharge of waste materials.

(a) Phases of phagocytosis

Fig.9 steps of phagocytosis



Bacterial defense against phagocytosis:

Grow at sites which phagocyte can't reach

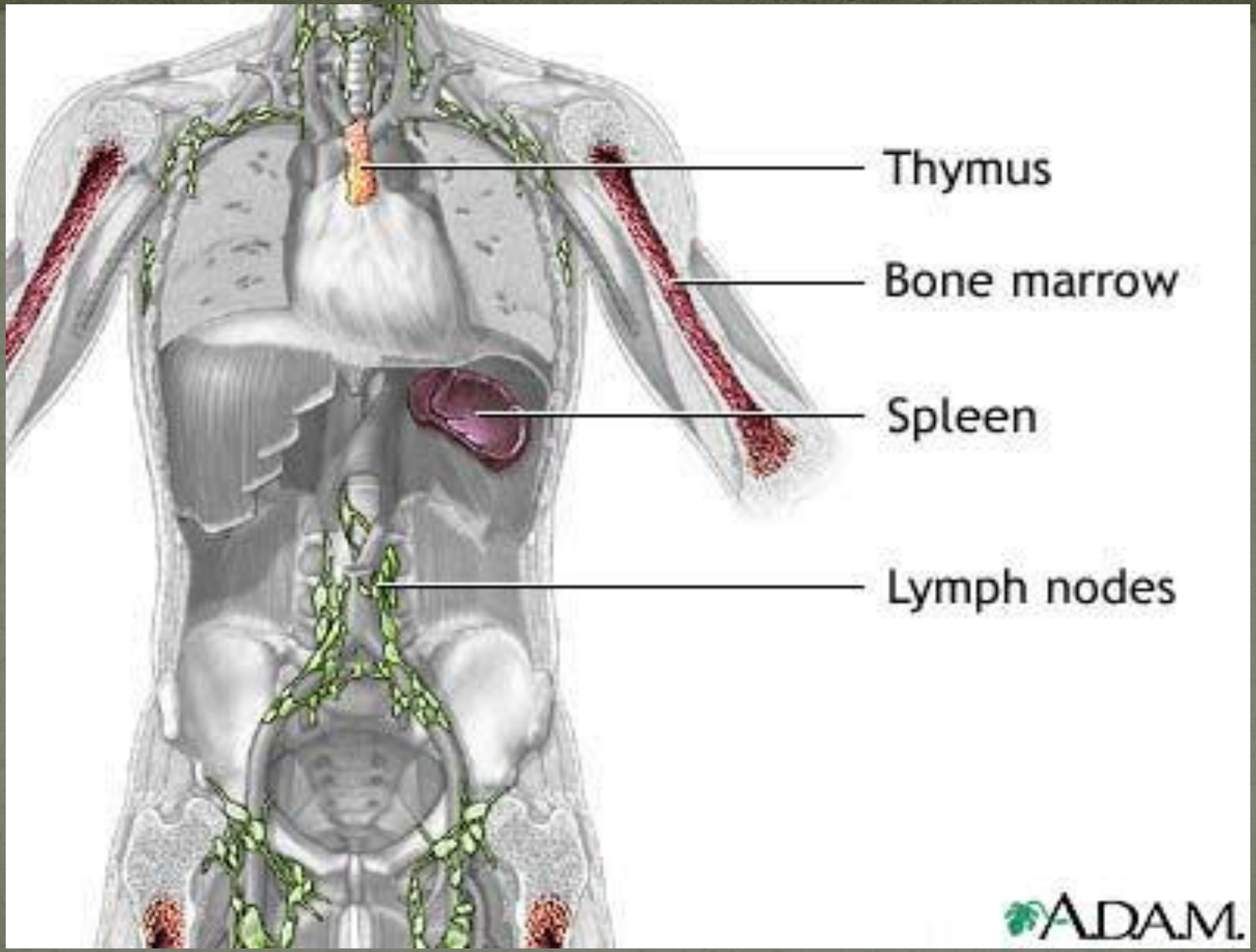
Capsule prevent phagocytosis

Can kill phagocyte either before or after phagocytosis

Can survive inside macrophage as intracytoplasmic pathogens

Lymphoid tissue & organs

1ry lymphoid organ		2nd lymphoid organ
Bone marrow	Thymus gland	
- B cell maturation - Blood cell generation ie hematopioesis	- **T cell maturation** - **Educate T cell how** to differentiate between self Antigen (MHC peptide) & non self Antigen - **T cell selection :** **Positive selection:** only T cell which can bind with MHC is allowed to grow **Negative selection:** only T cell which bind efficiently with MHC (auto-reactive T cell)is eliminated by apoptosis as it is a dangerous cell	- **Lymph node** - **Spleen** - **Tonsils** - **Payer's patches** Sites for contact between lymphocyte & Antigen to initiate immune response


Thymus
Bone marrow
Spleen
Lymph nodes
A.D.A.M.

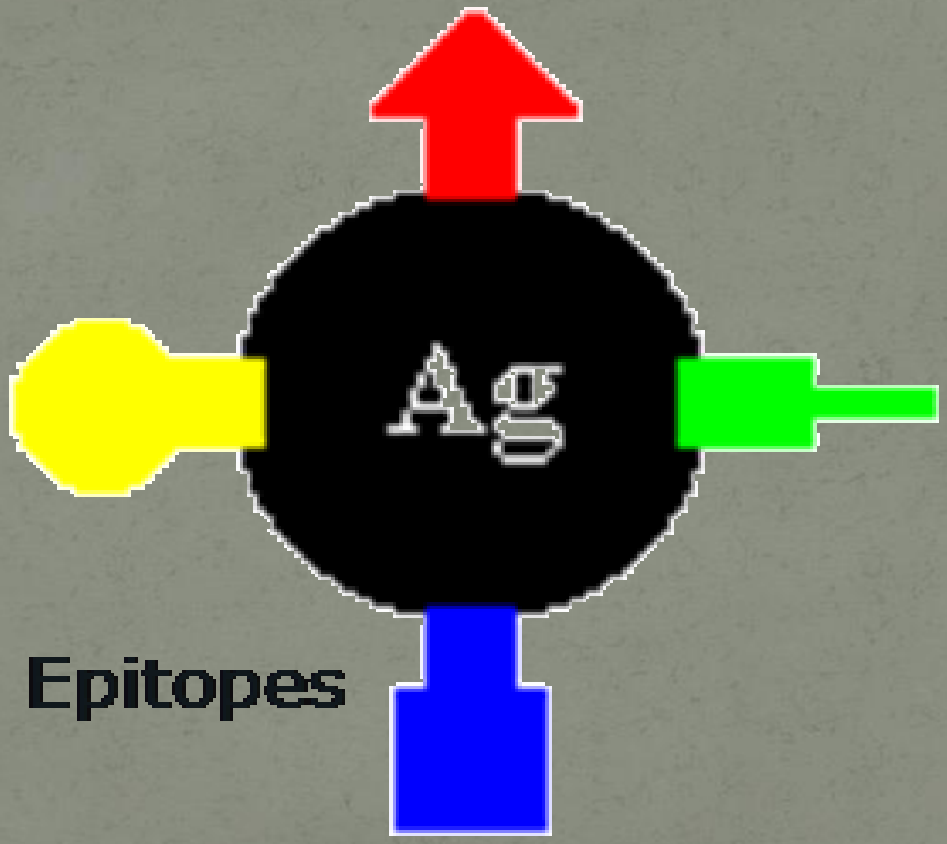
Antigens (Ags)

Antigen: Substance recognized by immune system which may be

- Simple or complex
- Carbohydrate, lipid, protein, nucleic acid, phospholipids
- B cell recognize any biological Ag
- T cell recognize peptide Ag presented on MHC

Epitopes (antigenic determinants):

- Smallest part on Ag which bind with BCR & T cell receptors
- determines the specificity of Ag
- if Ag contain multiple epitopes , it is called multivalent Ag


Ag
Epitopes

Depending on the nature of immune responses they trigger, antigens/epitopes are divided into 3 fuctional types:

a. Immunogens

- ✓ Large Ag with epitopes capable of binding with immune receptor & inducing immune response.

✓ Always a macromolecule (protein, polysaccharide).

✓ Notice that not all antigens are immunogens

b. Haptens

- ✓ A small Ag with epitopes capable of binding with immune receptor & without inducing immune response

✓ BUT can **produce immune response only when conjugated with large carrier molecule (as a protein) → immune response against epitopes of hapten & carrier.**

c. Tolerogens

- ✓ Self Ag (MHC) normally not stimulate immune system

✓ Lack of immune response against self Ag called self tolerance

Factors that influence immunogenicity:

1. **Size: proteins > 10 KDs are more immunogenic.**
2. **Complexity: complex proteins with numerous, diverse epitopes are more to induce an immune response than are simple peptides that contain only one or few epitopes.**
3. **Conformation and accessibility: epitopes must be "seen by" and be accessibile to the immune system.**
4. **Chemical properties:**
   - A protein is good immunogens.
- Many charbohydrates, steroids, and lipids are poor immunogens.

Amino acids and haptens are, by themselves, not immunogenic

Types of Antigens

1. **T-cell independent antigens** (TI): activate B cells without help from T cell ; e.g. polysaccharides (Pneumococcal polysaccharide, LPS)
2. **T-cell dependent antigens: Requires T cell help for B cell activation; e.g. proteins (microbial proteins & non-self or altered-self proteins).**

Superantigens (SAGs)

Proteins produced by pathogens.

Not processed by antigen presenting cells.

Intact protein binds to variable region of β chain on TCR of T cells and to MHC class II on antigen presenting cells

They induce massive T cell activation

Large numbers of activated T cells release large amount of cytokines leading to systemic toxicity and skin syndrome

diseases.

Examples

Staphylococcal enterotoxins

Staphylococcal toxic shock syndrome toxin-1 (TSST-1)

SAGs and diseases:

Staphylococcal toxic shock syndrome .

Staphylococcal scalded skin syndrome.

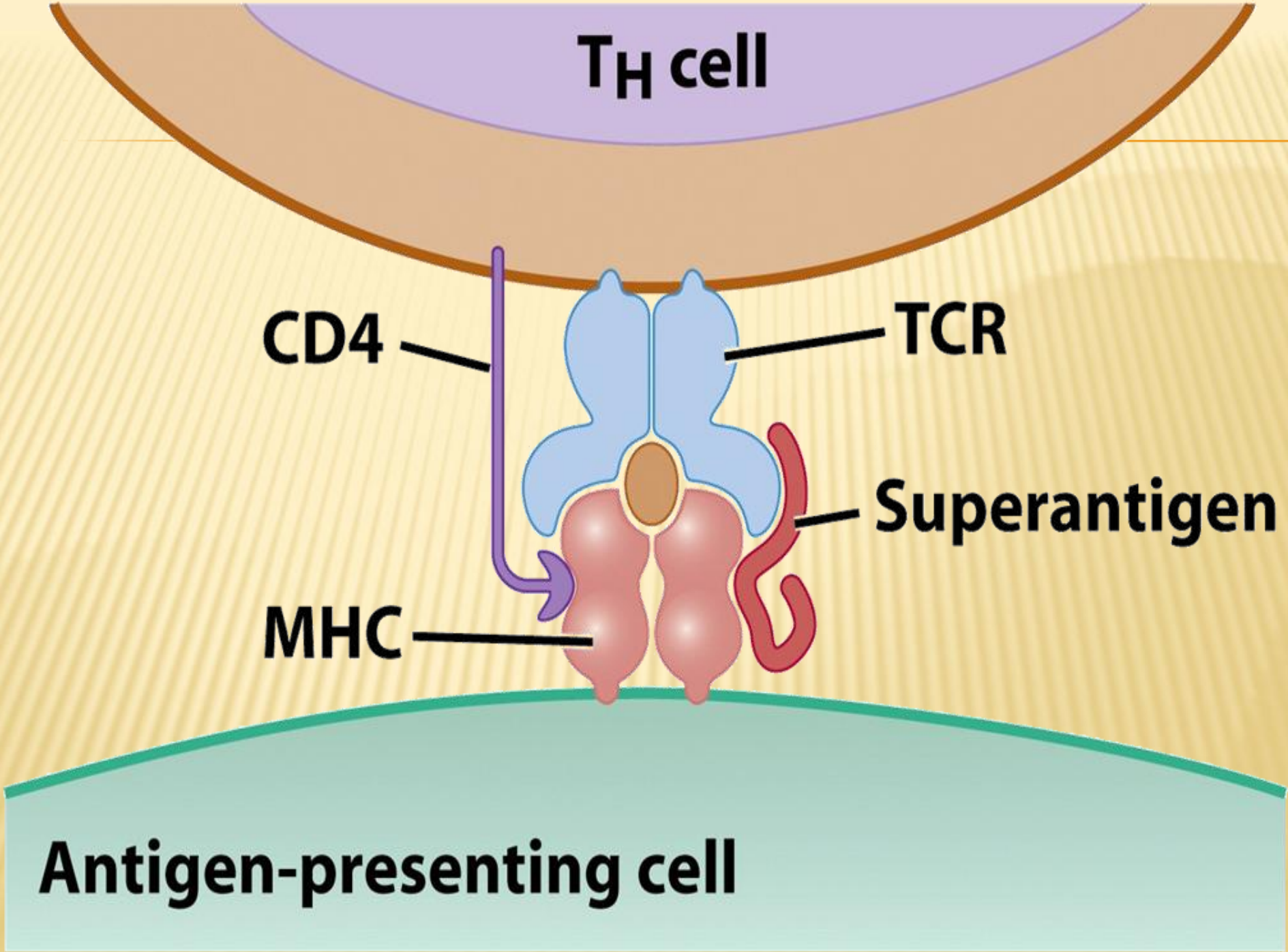
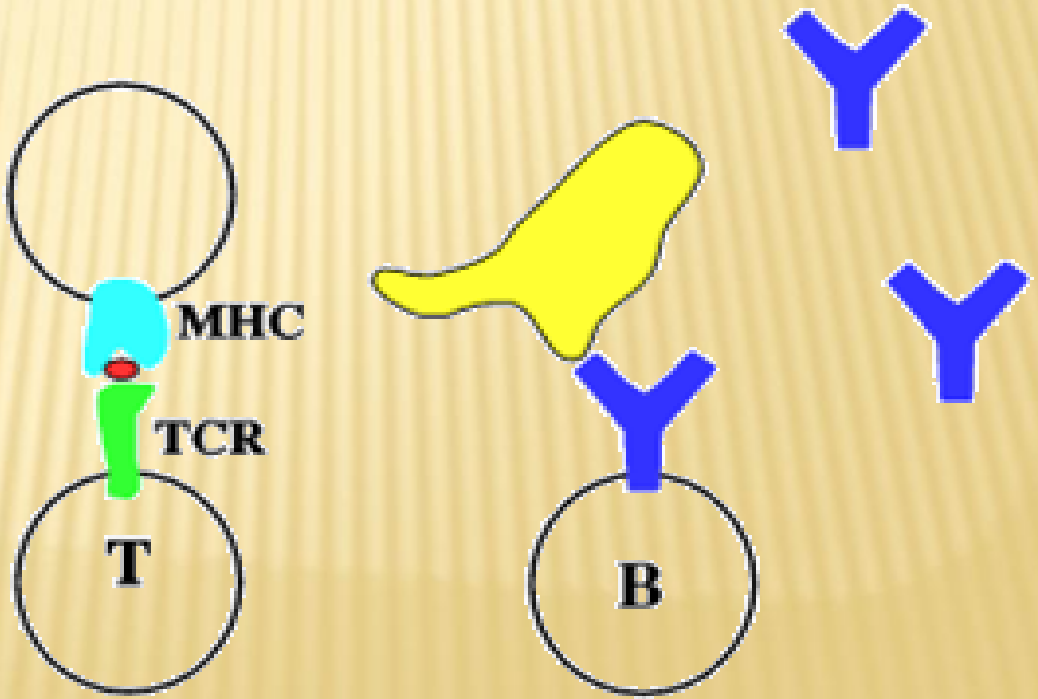

T_H cell
CD4
TCR
Superantigen
MHC
Antigen-presenting cell

FIG. 1 SAGS
ANTIGEN BINDING MOLECULES OF THE IMMUNE SYSTEM

- Immunoglobulins (Igs):
- T cell receptors (TCR):
- MHC molecules.



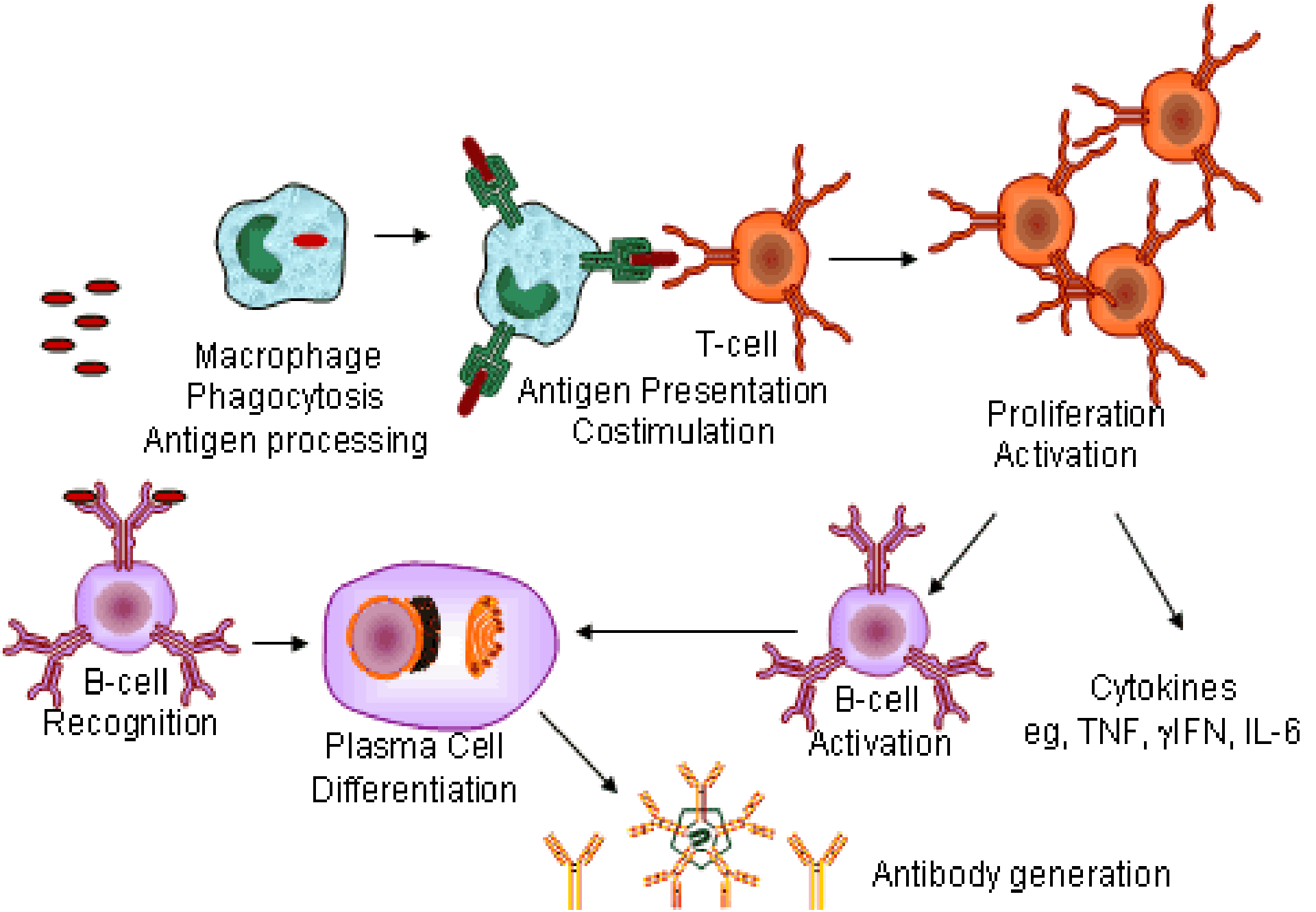
Immunoglobulins (Igs) (antibodies)

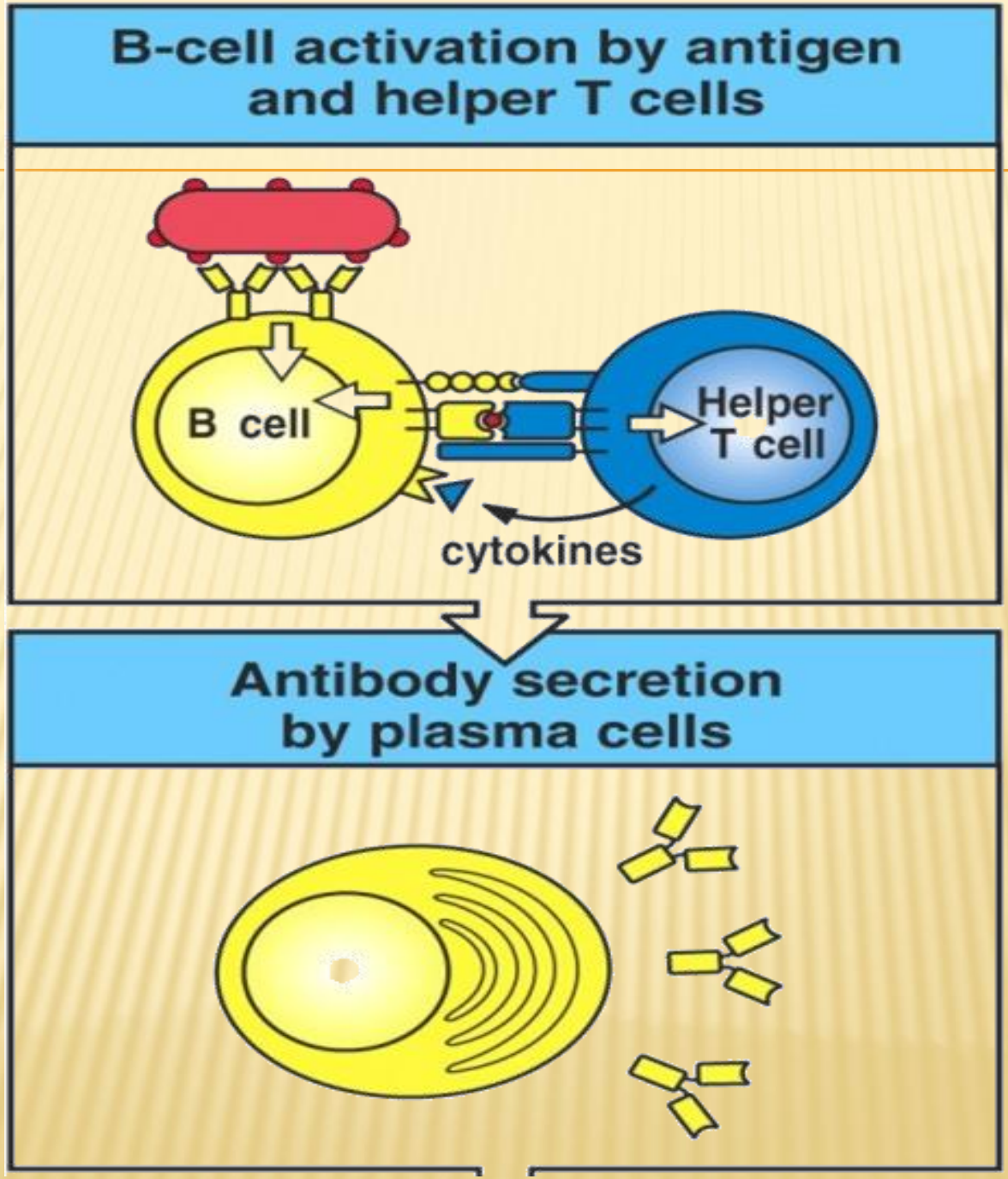
Immunoglobulins (Igs) (antibodies)

Def: Immunoglobulins are glycoproteins which mediate humoral or antibody mediated immunity

Production & distribution of antibodies

- In lymph node → antigenic stimulation of B cells with help of T helper cytokines → B cell proliferate → differentiate into plasma cell which secrete antibodies → enter circulation → site of infection
- Also mature B cell in Bone Marrow express membrane bound antibodies (BCR)
- So antibodies are produced in lymphoid tissue & bone marrow


Macrophage
Phagocytosis
Antigen processing
T-cell
Antigen Presentation
Costimulation
Proliferation
Activation
B-cell
Recognition
Plasma Cell
Differentiation
B-cell
Activation
Cytokines
eg, TNF, γIFN, IL-6
Antibody generation

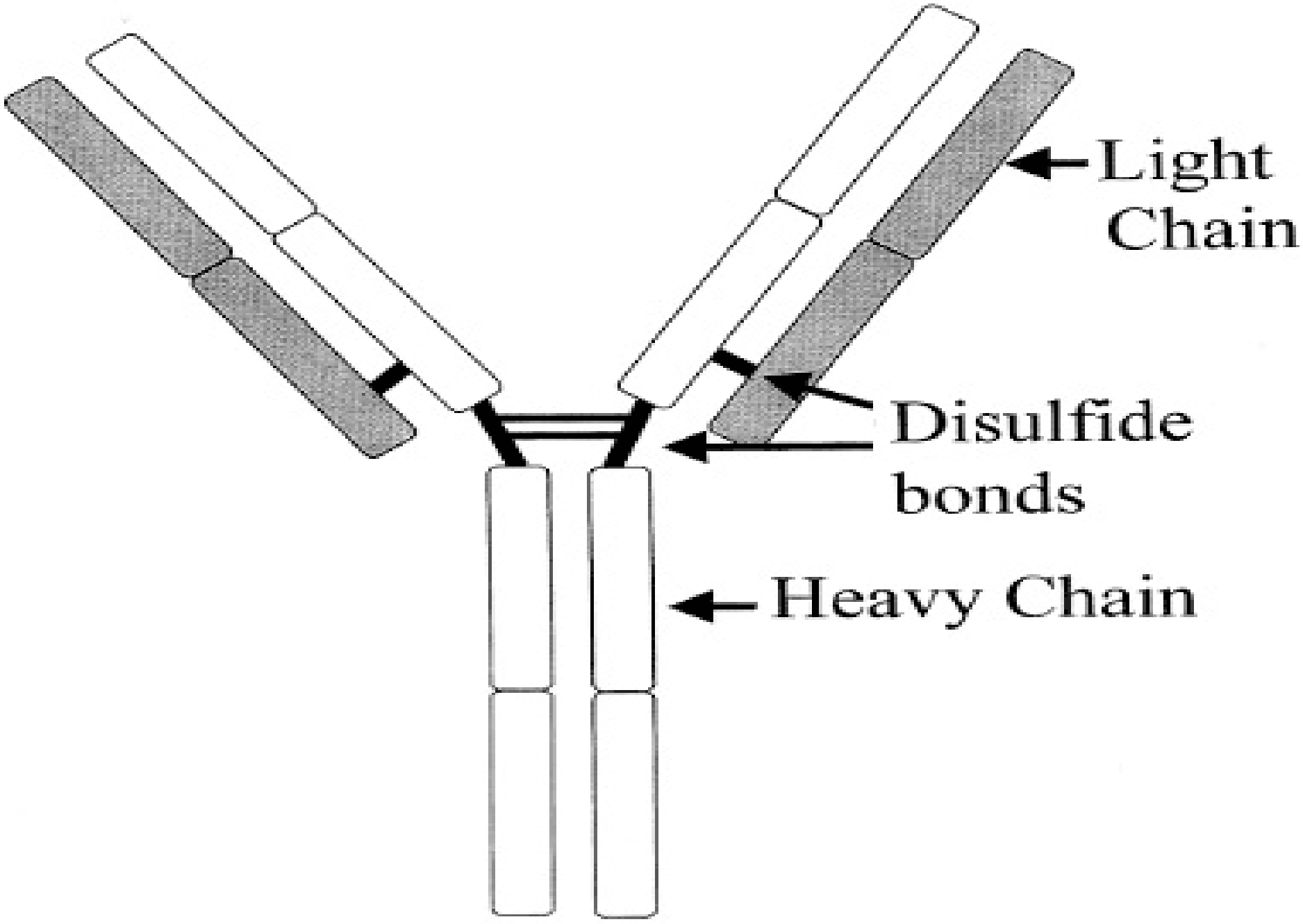

B-cell activation by antigen and helper T cells
B cell
Helper T cell
cytokines
Antibody secretion by plasma cells

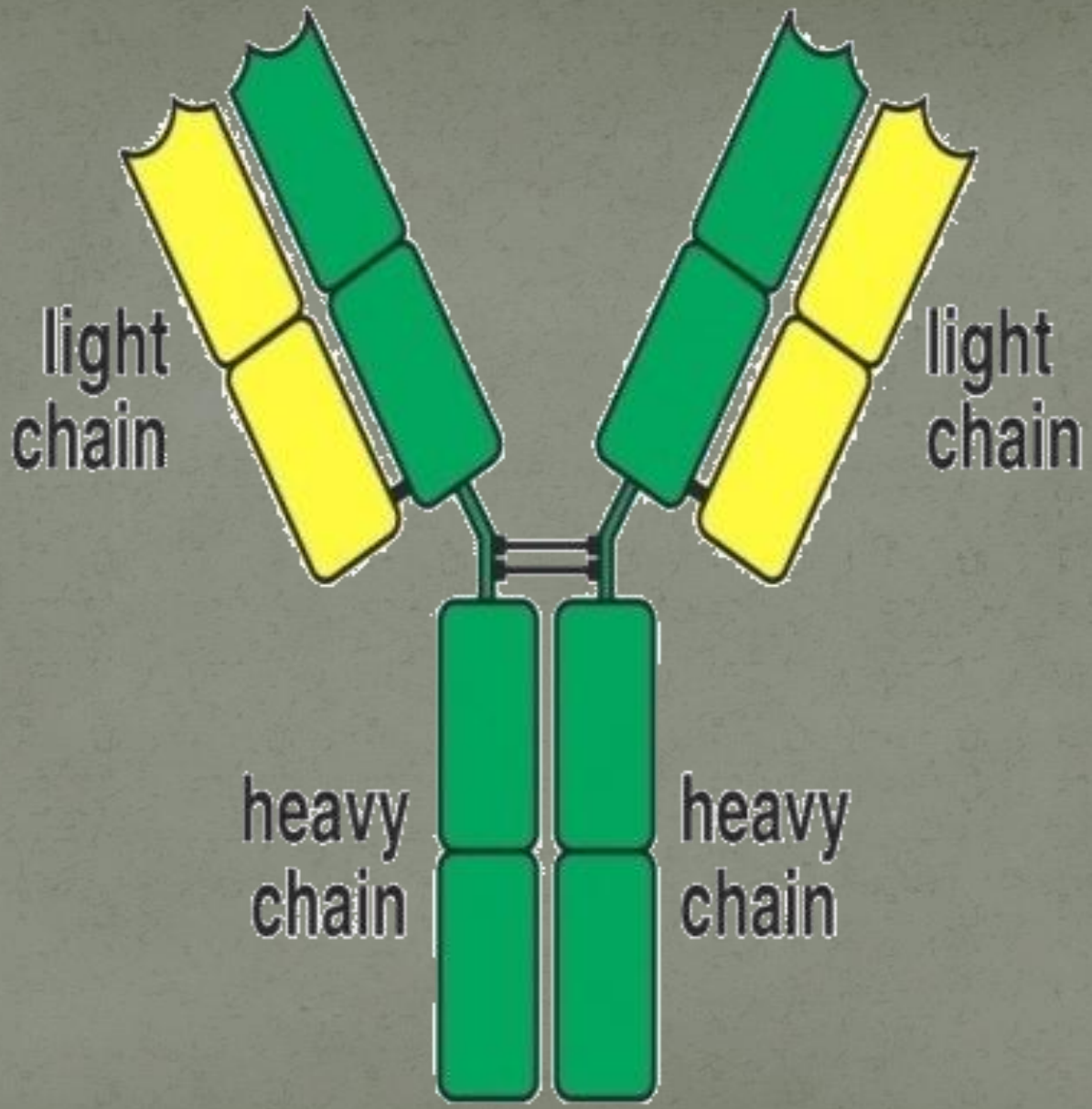
Forms of Antibodies

Membrane bound Ig	Secreted Ig
Expressed on B cell surface (IgM & IgD) as BCR for Ag If bind with Ag → initiate B cell response	- in plasma & mucosa & interstitial fluids of tissues

Structure of antibodies

- Y shaped molecules of 4 polypeptide chains
- 2 identical heavy chain → each chain → 1 variable domain (VH) & 3 or 4 constant domains (CH)
- 2 identical light chain → each chain → 1 variable domain (VL) & 1 constant domains (CL)
- Each variable domain (VL or VH) contains 3 hypervariable regions called complementary determining repeats (CDR)
- Disulfide bond connect heavy chain with light chain & heavy chain with heavy chain


Light Chain
Disulfide bonds
Heavy Chain


light chain
light chain
heavy chain
heavy chain

R egions of antibody according to proteolytic fragments of Ig

Fab = fragment Ag binding	Fc = fragment crystalline	Hinge region
Contain whole light chain + VH + CH1 2 in number Part for Ag recognition and binding	Tend to crystallize in solution One in number Contain remaining of both heavy chains C domain Give effector & biological function of antibody	- Flexible region lies between Fab & Fc to give mobility to both Fab to accommodate different Ag

C terminal end of heavy chain may be anchored in plasma membrane of B cell (IgM & IgD) → act as BCR

Ig classes (isotypes)

A. Immunoglobulin classes

Immunoglobulins →divided into five different classes → according to the difference in structure in constant domains of heavy chain

1. Gamma heavy chains → IgG
2. Alpha heavy chains → IgA
3. Mu heavy chains → IgM
4. Epsilon heavy chains → IgE
5. Delta heavy chains → IgD

Different classes and subclasses of antibodies perform different effector functions (table 1).

There are two types of light chains, called κ (kappa) and λ (lambda). An antibody has either two κ light chains or two λ light chains.

Heavy chain class (isotype) switching: is the switch from one Ig isotype to another. After activation of B lymphocytes, the antigen-specific clone of B cells proliferate and differentiate into progeny that secrete antibodies; some of the progeny secrete IgM, and other progeny of the same B cells produce antibodies of different isotypes to mediate different functions and combat different types of microbes.

Table 1
Immunoglobulins classes, subclasses and their functions

Isotype	Subtypes	H chain	Serum conc. mg/ml	Secreted form	Functions
IgA	IgA1 IgA2	α1 α2	3.5	Monomer, dimer, trimer	Mucosal (local) immunity
IgD	None	δ	Trace	None	Naïve B cell antigen receptor BCR
IgE	None	ε	0.05	Monomer	1.Defense against helminthic parasites 2.Immediate hypersensitivity
IgG	IgG1 IgG2 IgG3 IgG4	γ1 γ2 γ3 γ4	13.5	Monomer	1.Opsonization, 2.Complement activation 3.ADCC 4. 2ry immune response
IgM	None	μ	1.5	Pentamer	1. Naïve BCR 2. 1ry immune response 3. Complement activation

Monoclonal Antibodies

Definition: identical monospecific antibodies that are produced by one type of **immune cell** that are all **clones** of a single parent cell.

In contrast, antibodies obtained from the blood of an immunized host are called polyclonal antibodies.

Production: Hybridoma technology

Steps:

1. A mouse is immunized with the antigen of interest
2. B cells are isolated from the spleen of the animal
3. B cells (Ab-producing cell) are then fused with myeloma cells (malignant cell) in vitro by using a fusion agent as poly-ethylene glycol, a virus.

The cell fusion form an immortalized antibody-producing cell “hybridoma”.

5. Hybrids (fused cells) are selected for growth in special culture media. The B cells that fuse with another B cell or do not fuse at all die because they do not have the capacity to divide indefinitely. Only hybridomas between B cells and myeloma cells survive.
6. Hybridomas, secrete a large amount of mAbs.

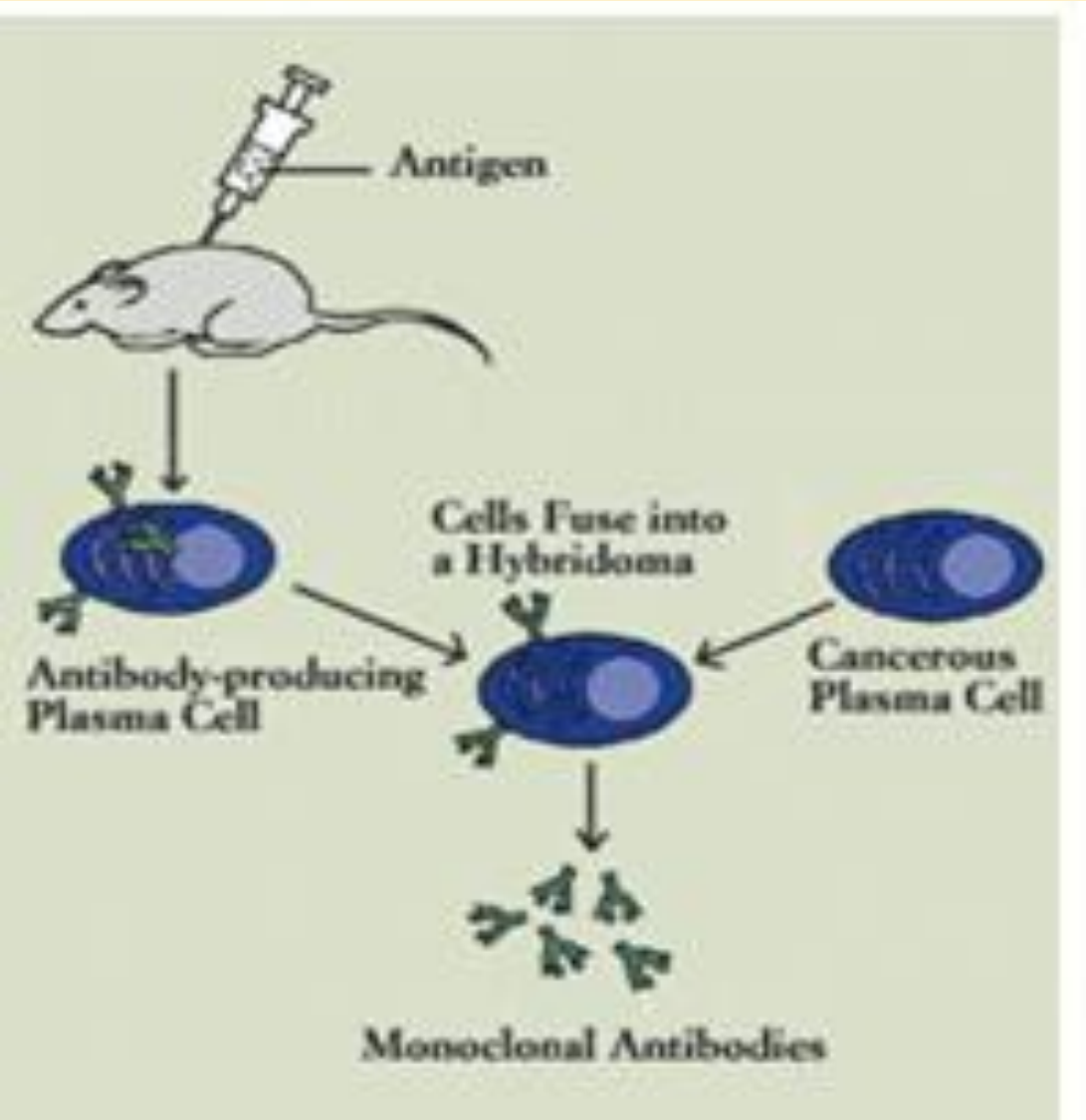


Fig.13 Hybridoma

Applications

1. To define clusters of differentiation (CD markers) on lymphocytes
2. Diagnosis of many viral, bacterial and fungal infections by using their specific monoclonal antibodies.
3. Diagnosis and treatment of cancer
4. Treatment of autoimmune diseases as rheumatoid arthritis
5. Prevention of graft rejection

Humoral (Antibody-Mediated) Immunity

- ✓ Humoral immunity is mediated by secreted antibodies

Its physiologic function is defense against extracellular microbes and microbial toxins

Functions of antibody isotypes

1. **Neutralization of microbes and microbial toxins:**
   a. Antibodies blocks and prevent binding of microbe to cells i.e. prevent infection of cells
   b. Antibodies inhibit the spread of microbes from an infected cell to an adjacent cell.

Antibodies block binding of toxin to cellular receptors, and thus inhibit pathologic effects of the toxin.

2. **Opsonization and phagocytosis:**
   - Antibodies of IgG isotype opsonize (coat) microbes and promote their phagocytosis by binding to Fc receptors on phagocytic cells

3. **Antibody-dependent cell-mediated cytotoxicity (ADCC):**
   a. IgG antibodies bind to infected cells are recognized by Fc receptors on NK cells. → activation of NK cells → killing of antibody-coated cells.
   b. IgE antibodies bind to helminthic parasites, and are recognized by Fc receptors on eosinophils → activation of eosinophils →release their granule contents, → killing of parasites.

Activation of the complement by IgG and IgM

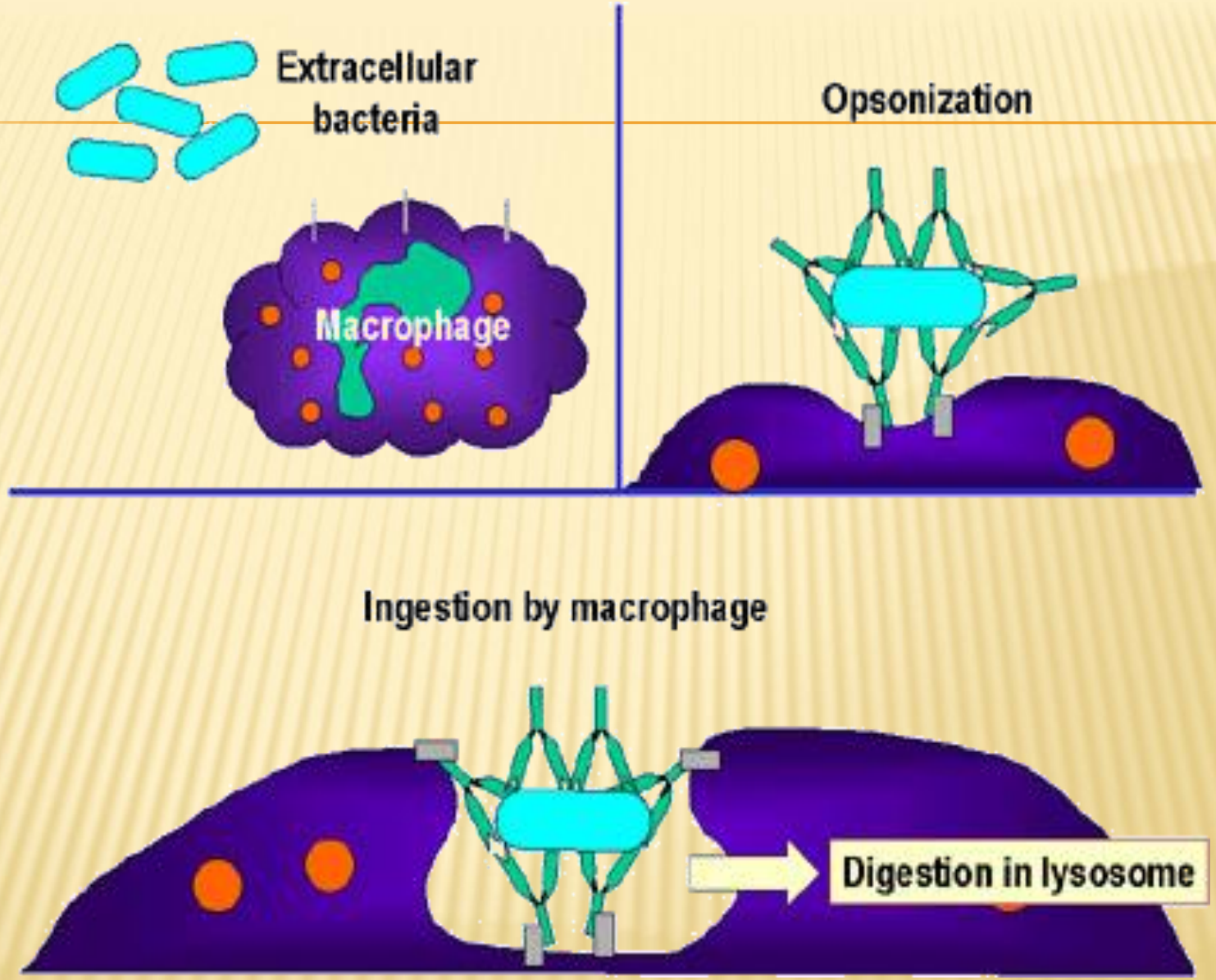


Fig.14 Opsonization

1. **Functions of antibodies at special sites:**
   a. **Mucosal immunity: IgA is the major class produced by the mucosa-associated lymphoid tissues (MALT) in the GIT and RT and transported to the lumens of organs. In mucosal secretions, IgA binds to microbes and toxins present in the lumen and neutralize them by blocking their entry into the host.**
   b. **Neonatal immunity: neonates are protected from infection by maternal antibodies (IgG) transported across the placenta into the fetal circulation and by antibodies in ingested milk transported across the gut epithelium of newborns.**

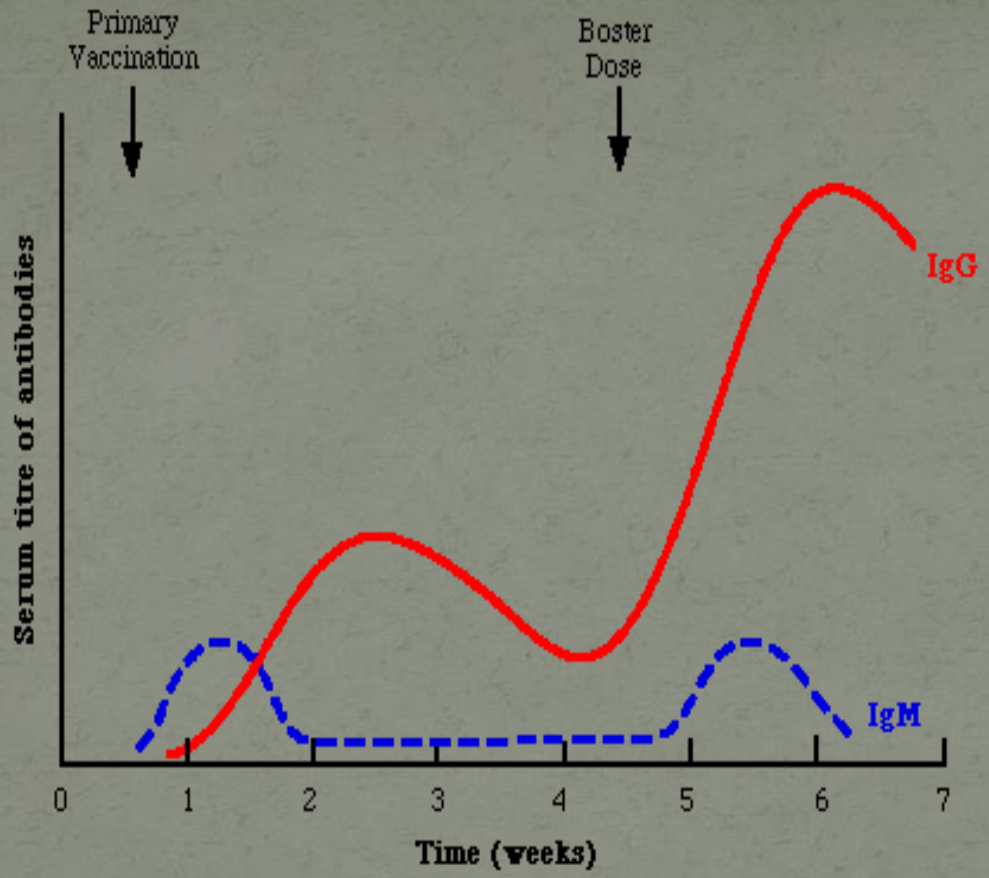
Primary and Secondary Immune Response

- **The *primary response***

When we are exposed to an antigen for the first time, there is a lag of several days (10 days) before specific antibody becomes detectable. This antibody is IgM. After a short time, the antibody level declines.

- **The *secondary response***

If at a later date we are re-exposed to the same antigen, there is more rapid appearance of antibody, and in greater amount. It is of IgG class and remains detectable for months or years.


Primary Vaccination
Boster Dose
Serum titre of antibodies
IgG
IgM
0
1
2
3
4
5
6
7
Time (weeks)

If at the same time that we are re-exposed to an antigen, we are exposed to a different antigen for the first time, the properties of the specific response to this antigen are those of the primary response:

Primary Response	**Secondary Response**
• **Slow in Onset** • **Low in Magnitude** • **Short Lived** • **IgM**	• **Rapid in Onset** • **High in Magnitude** • **Long Lived** • **IgG (Or IgA, or IgE)**

This phenomenon is possible because the immune system possesses specific immunologic memory for antigens.
During the primary response, some B lymphocytes, become memory cells which are long lived. Thus we can see that the secondary response requires the phenomen known as class switching (IgM to IgG).

Major Histocompatibility Complex (MHC)

- **Def.: group of genes on short arm of chromosome 6 which produce MHC molecules present on cell surfaces and responsible for display of protein Ag to T cell**
- **Also called human leucocytic Ag = HLA**

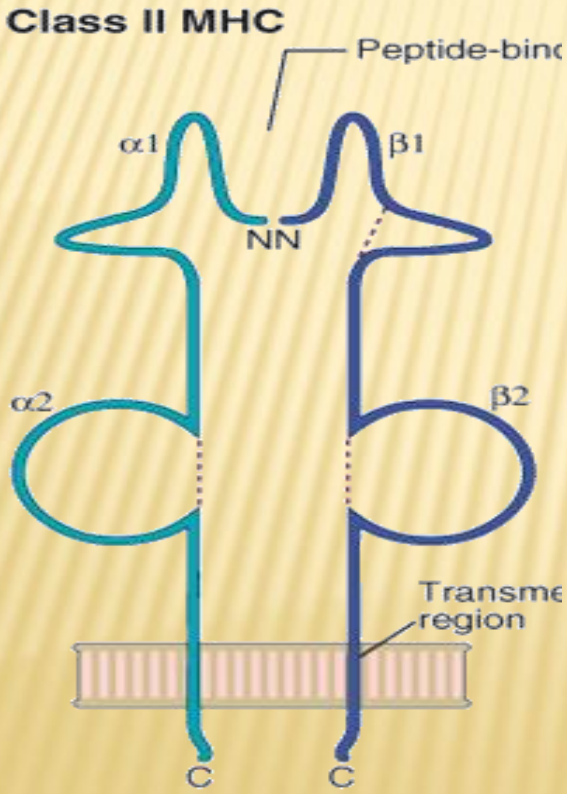
Classification of genes

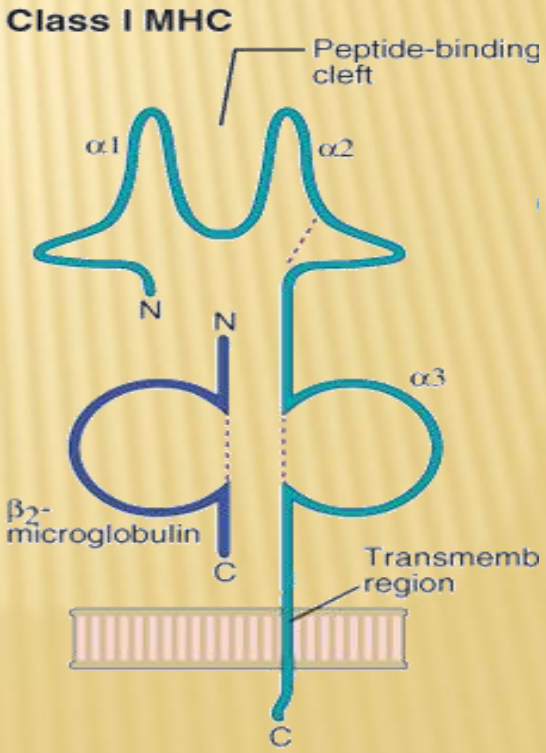
Class I MHC genes → HLA-A & HLA-B & HLA-C → role in Ag presentation to Tc

Class II MHC genes → HLA-D region (HLA-DR & HLA-DP & HLA-DQ) → role in Ag presentation to Th

Class III MHC genes → lies between class I & II & not produce MHC but produce some complement components & TNF-α.

	Class I molecules	Class II molecules
Structure	• 2 poly peptide chain • α chain → (α1 & α2 & α3 domains) encoded by MHC I genes • ß2-microglobulin → not encoded by MHC genes	• 2 poly peptide chain (encoded by MHC II) • α chain → (α1 & α2) • β chain → (β1 & β2)
Groove of Ag presentation	between α1 & α2 domain	( ) β 1 & α1 domain
Distribution	Any nucleated cell in body	Ag P C (macrophage & dendritic & B cells)
Present Ag to	Tc (CD8)	Th (CD4)





Complement System

A system of circulating and membrane-associated proteins that function in both the innate and adaptive branches of the immune system.

Component nomenclature

- Components C1 – C9 , factor B, D, and P.
- Fragments of complement components: each component can be cleaved into 2 fragments indicated by a lowercase letter (e.g. C5a, C5b , C4a, C4b…).
- A horizontal bar above a component or complex indicate enzymatic activity e.g. C4b2b = C3 convertase enzyme.

Complement activation

1. In *innate immunity, complement can be activated in two ways: via the alternative pathway or via the mannan-binding lectin pathway.*
- In *adaptive immune system: Complement can also be activated via the classical pathway*

The classical pathway

Activated by antigen-antibody (IgG, IgM) complex.

C1(C1q,r,s) complement component bind Ag-Ab complex, lead to activation of C1 .

C1 act as an enzyme and cleave both C2 and C4.

C4b + 2b , form the C3 convertase C4b2b which cleave C3

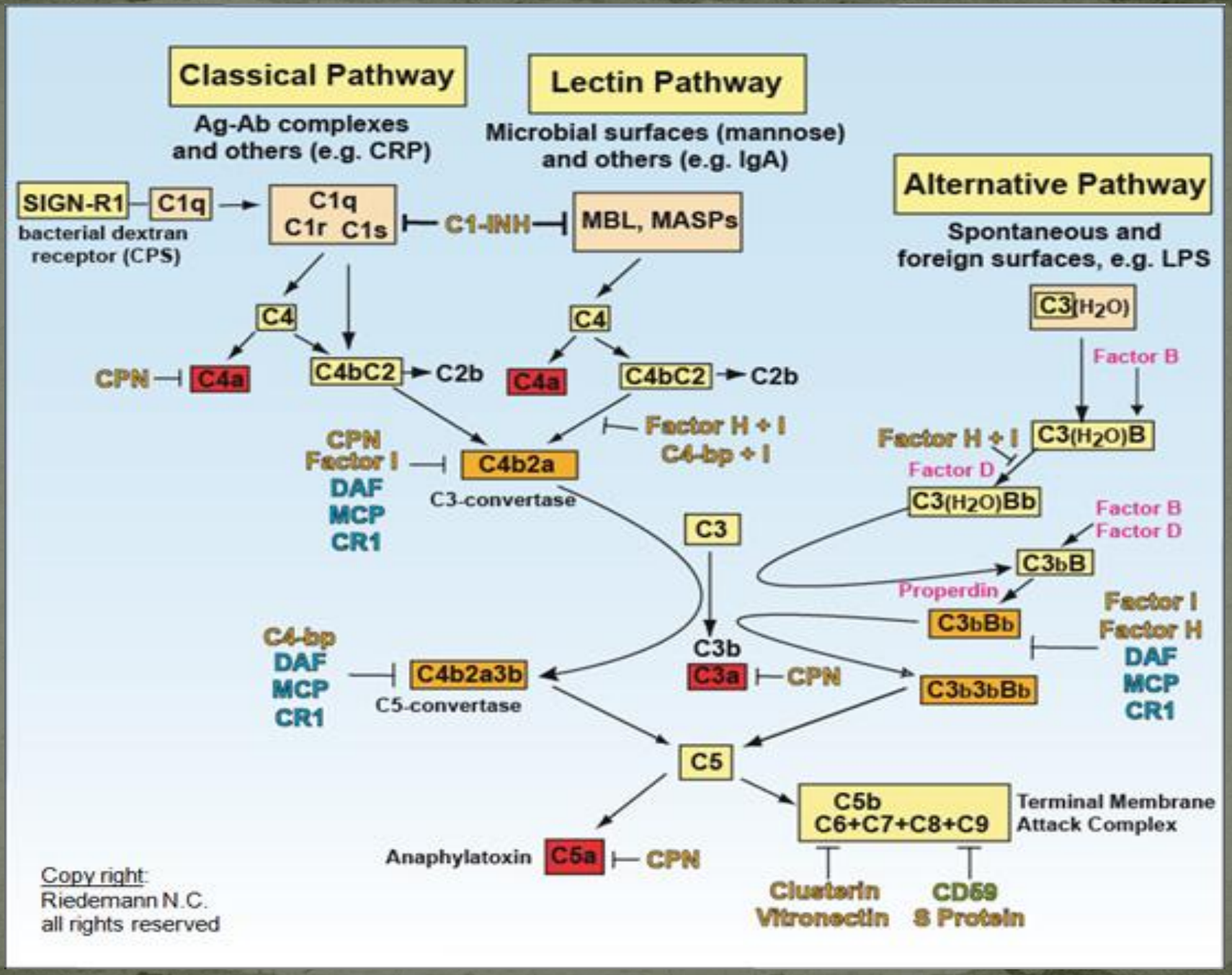
Binding of C3b to C4b2b lead to the formation of C4b2b3b which is the C5 convertase that cleave C5 to C5a & C5b which initiate the membrane attack complex.

The mannan-binding lectin (MBL) pathway

- MBL are serum proteins bind to specific charbohydrates
- This pathway is activated by binding of MBL to mannose residues of glycoproteins on certain microbes.
- Once MBL bind to mannose, it interact with 2 MBL-activated serine protease (MASP1 & MASP2).
- Activation of MASP lead to activation of C2, C4,and C3 in the same like the classical pathway.

The alternative pathway

- Initiated by cell-surface components of microbes that are recognized as foreign to the host as LPS
- Spontaneous breakdown of C3, the most abundant serum complement component
- C3b attaches to receptors on the surface of microbes
- C3b bind to factor B
- Factor B is cleaved by factor D to produce C3bBb, an unstable C3 convertase
- C3bBb binds properdin factor (factor P) to produce stabilized C3 convertase.
- Additional C3b fragments are added to form C5 convertase C3bBb3b.
- C5 convertase cleave C5 into C5a and C5b
- C5b inserts into the cell membrane of microbes , to begin the formation of membrane attack complex and cell lysis.

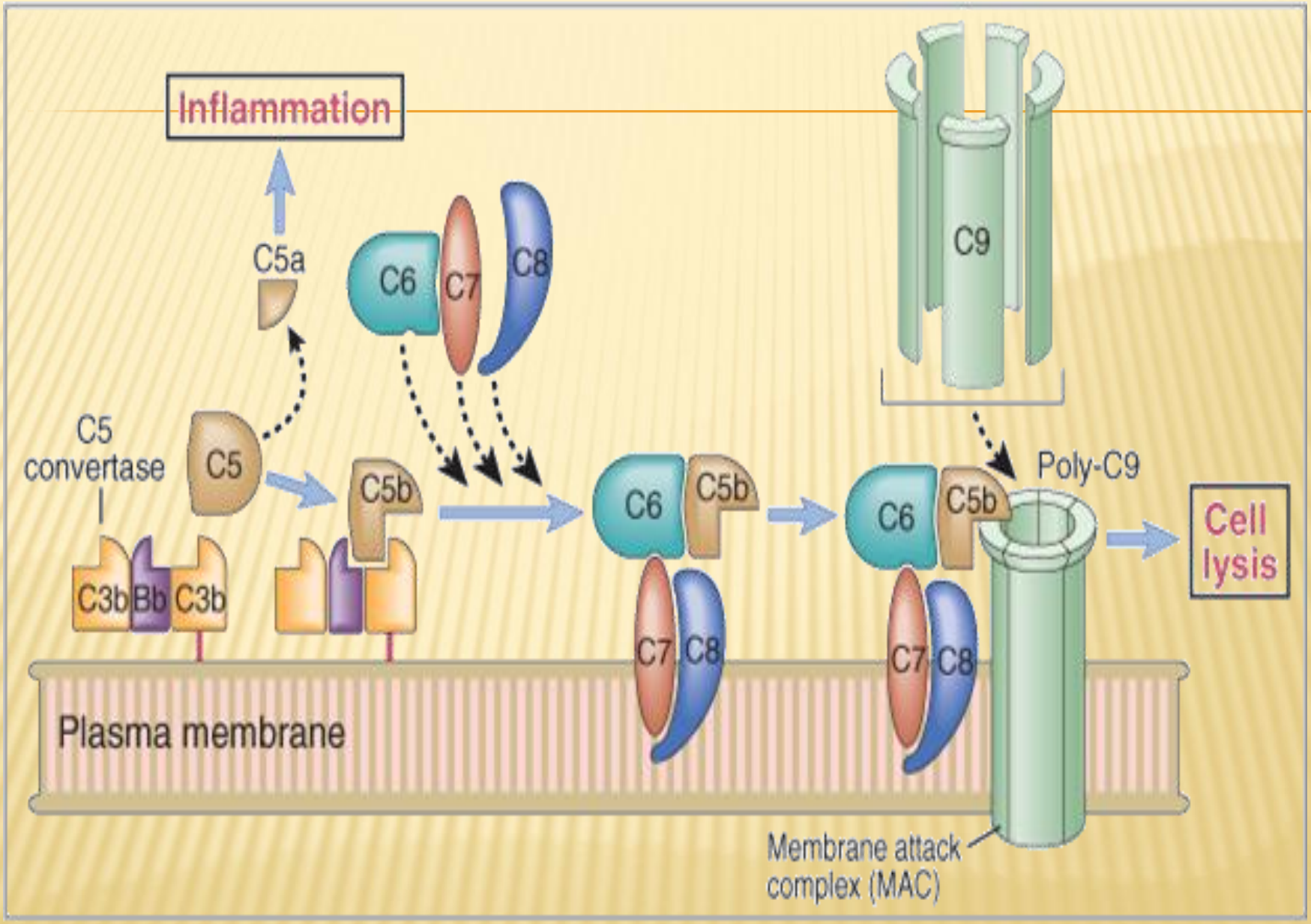


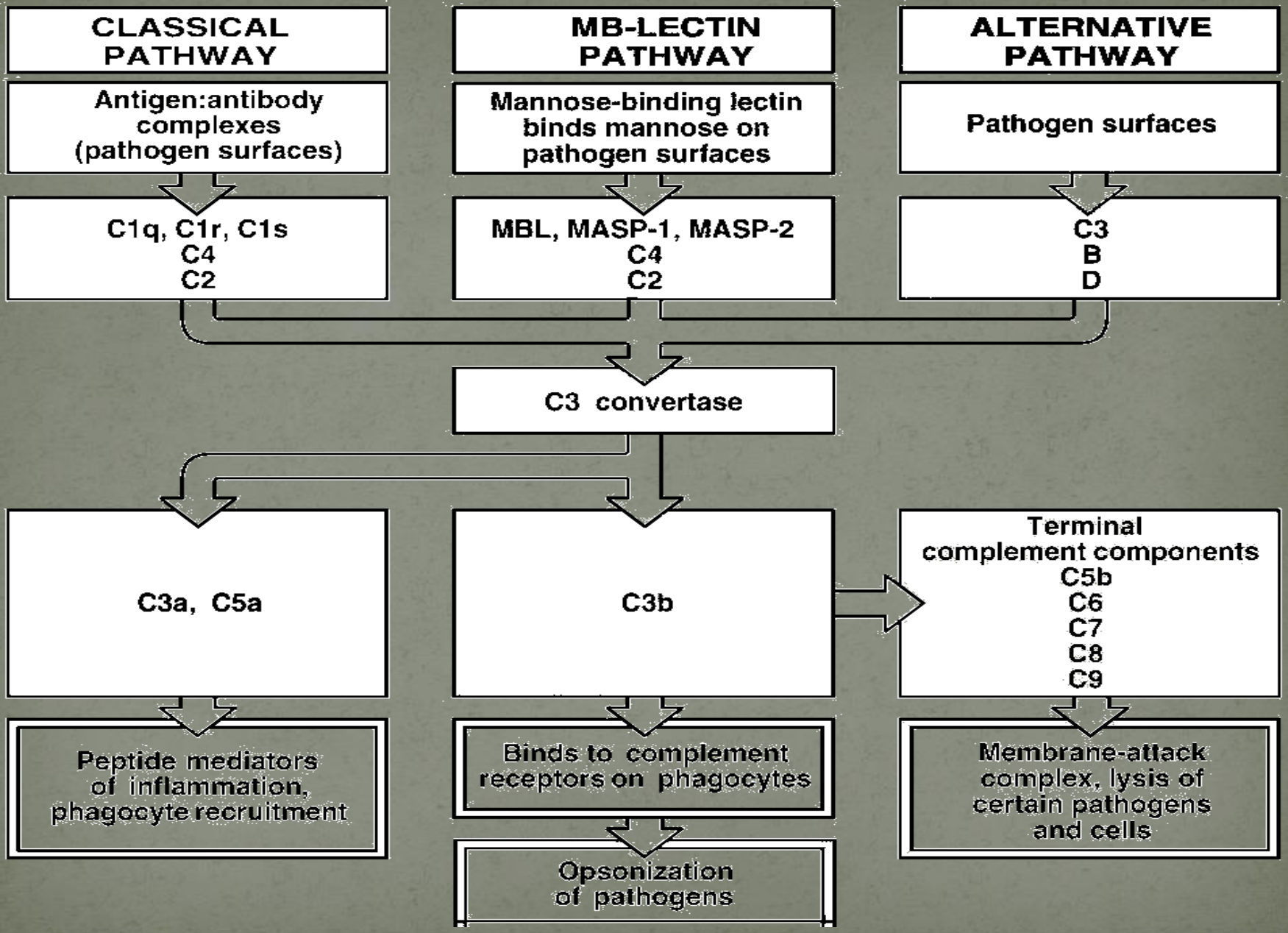
\ terminal or lytic pathway

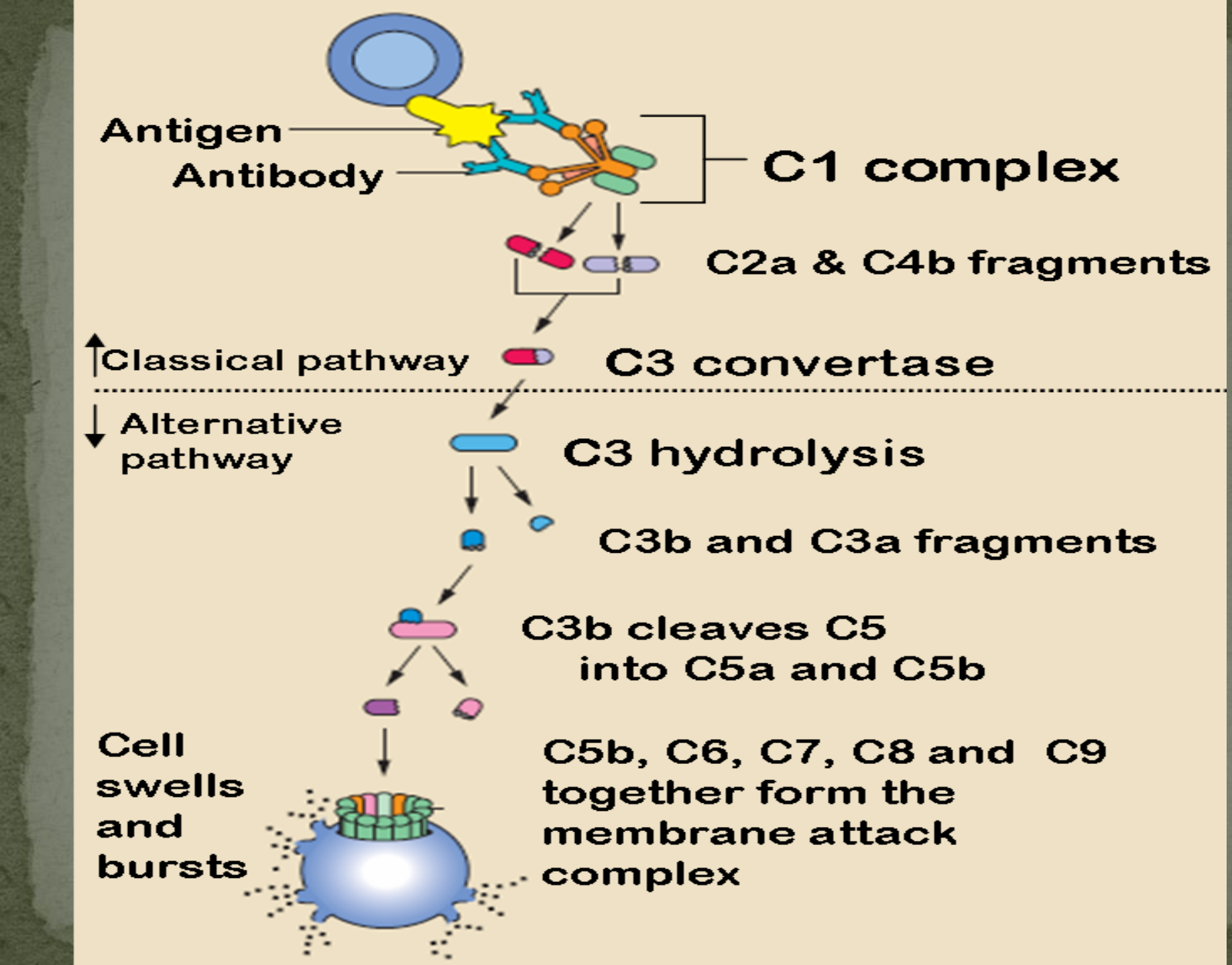
Can be entered from the classical, MBL, or classical pathway of complement activation. Attachment of C5b to the bacterial membrane initiate the formation of the membrane attack complex (MAC) and lysis of the cell.

- lysis.
- C5b attach to the cell membrane

- Addition of components C6, C7, C8 to form C5b678.
- Subsequent addition of multiple molecules of C9 (poly C9) to form pores in the cell membrane of microbe and lytic death of the cell.
- The MAC is C5b6789(n) .
- C9 is homologous to “perforin” found in Tc and NK cell granules


Inflammation
C5a
C6
C7
C8
C9
C5
convertase
C5
C5b
C3b
Bb
C3b
C6
C5b
C7
C8
C6
C5b
Poly-C9
C7
C8
Cell
lysis
Plasma membrane
Membrane attack
complex (MAC)


CLASSICAL PATHWAY
Antigen:antibody complexes (pathogen surfaces)
C1q, C1r, C1s
C4
C2
MB-LECTIN PATHWAY
Mannose-binding lectin binds mannose on pathogen surfaces
MBL, MASP-1, MASP-2
C4
C2
ALTERNATIVE PATHWAY
Pathogen surfaces
C3
B
D
C3 convertase
C3a, C5a
C3b
Terminal complement components
C5b
C6
C7
C8
C9
Peptide mediators of inflammation, phagocyte recruitment
Binds to complement receptors on phagocytes
Opsonization of pathogens
Membrane-attack complex, lysis of certain pathogens and cells


Antigen
Antibody
C1 complex
C2a & C4b fragments
Classical pathway
C3 convertase
Alternative pathway
C3 hydrolysis
C3b and C3a fragments
C3b cleaves C5 into C5a and C5b
Cell swells and bursts
C5b, C6, C7, C8 and C9 together form the membrane attack complex

Functions of the complement

- Opsonization and phagocytosis: C3b (or C4b) act as opsonins
- Complement-mediated lysis: the membrane attack complex MAC creates pores in cell membranes and induce osmotic lysis of the cells.
- Stimulation of inflammatory reactions: C5a, C3a, and C4a bind to receptors on neutrophils and stimulate inflammatory reactions that serve to eliminate microbes. Also, C5a, C3a and C4a are chemoattractants to neutrophils.
- Providing stimuli for B cell activation and the humoral immune response.

Cytokines

- **Def: proteins secreted by immune cells in response to microbes**
- **Role of cytokines:**
  1. Stimulate growth & differentiation of lymphocyte
  2. Activates immune cells to eliminate microbes & Ag
  3. Stimulate hematopoiesis
  4. Used in medicine as therapeutic agent

Nomenclature: according producing cell

Monokines from macrophage/monocyte

Lymphokines from lymphocyte

Interleukins from leucocytes & act on other leucocytes eg IL-1 & IL-2 & IL-3.....

Biologic response modifier : cytokine which used clinically to + or - immunity

- **General Properties:**

1. Not stored in granules
2. Action: either

Pleotropism: act on different cells giving different effects

Redundancy: multiple cytokines act on 1 cell giving same effect

3. Mode of action:

Autocrine : act on same cell that produce it

Paracrine: act on adjacent cell

Endocrine: act on distant cell at distant site

4. Bind with specific receptor on target cell
5. The effect on target cell → alter gene expression → new protein

Classification of cytokines

- **Mediators and regulators of innate immunity: produced mainly by**

  macrophages, and NK cells.

  - ✓ Tumor necrosis factor TNF-α ….. activation of neutrophils & inflammation.
- ✓ IL-1…….activation of neutrophils and inflammation
- ✓ IL-12…..activation of T & NK cells
- ✓ Interferon IFN-α and IFN-β…..antiviral action and increase expression of class I MHC in all cells.
  - Chemokines ……chemotaxis & migration of leukocytes into tissues

- **Mediators and regulators of adaptive immunity: produced by T lymphocytes.**
  - IL-2…….proliferation of T, NK, and B cells.
- IL-4…….B cell isotype switch to IgE and mast cell proliferation.
- IL-5…….B cell proliferation and eosinophils activation.
- IFN-γ…..macrophage activation and increase microbicidal functions.
  - **Stimulators of hematopoiesis:**
  - Graulocyte-monocytes colony stimulating factor GM-CSF.
- IL-3 and IL-7.

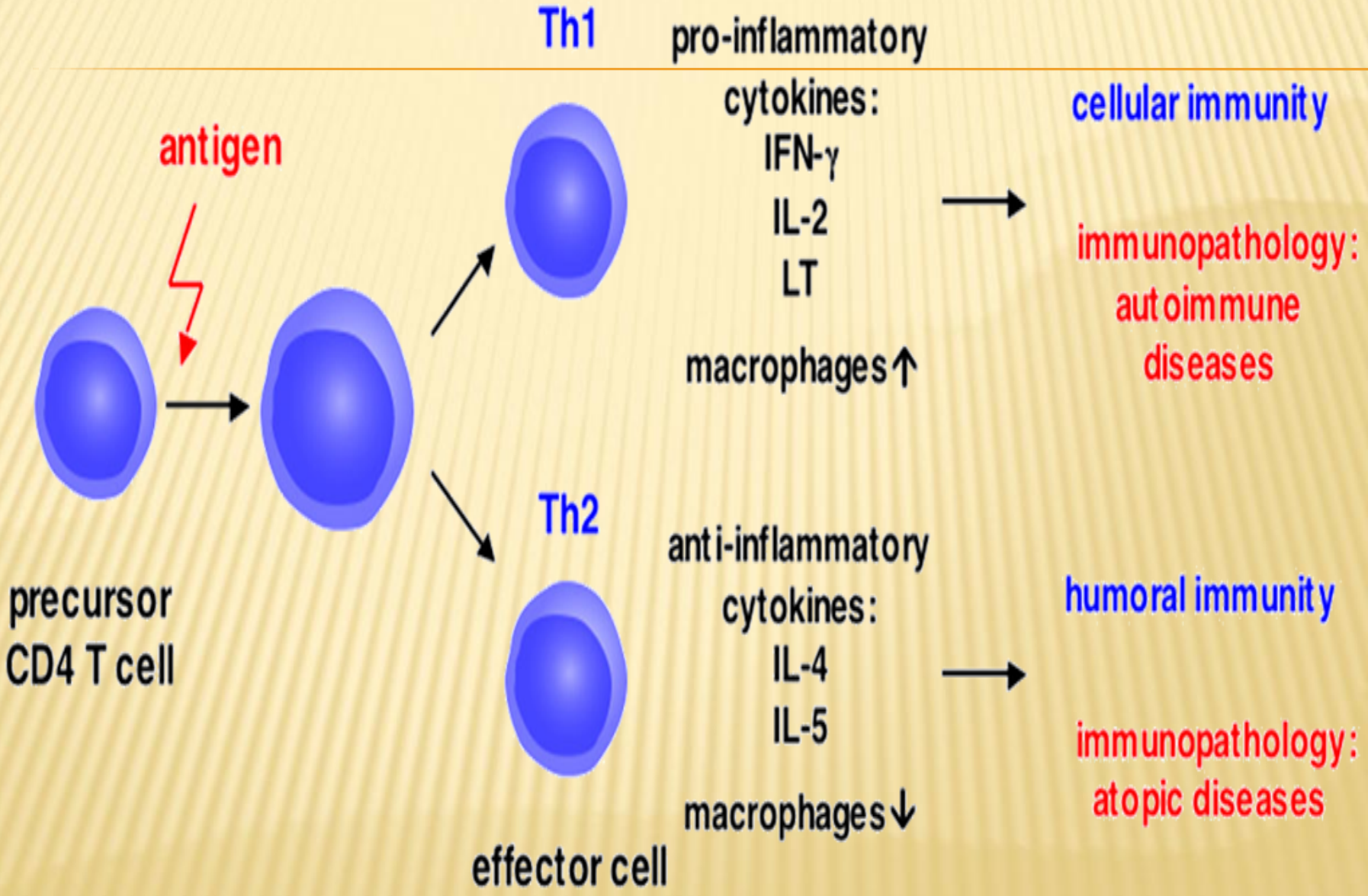
Cytokine Profiles of Th1 and Th2 Subsets

1-Th1 :

- ✓ Induced by IFN-γ and IL-12
- ✓ Produce : IFN-γ, IL-2, TNF, LT
- ✓ Function: cell-mediated immune response.

2- Th2:

- ✓ Induced by: IL-4
- ✓ Produce : IL-4, IL-5, IL-10, IL-13.
- ✓ Function : humoral immune response.

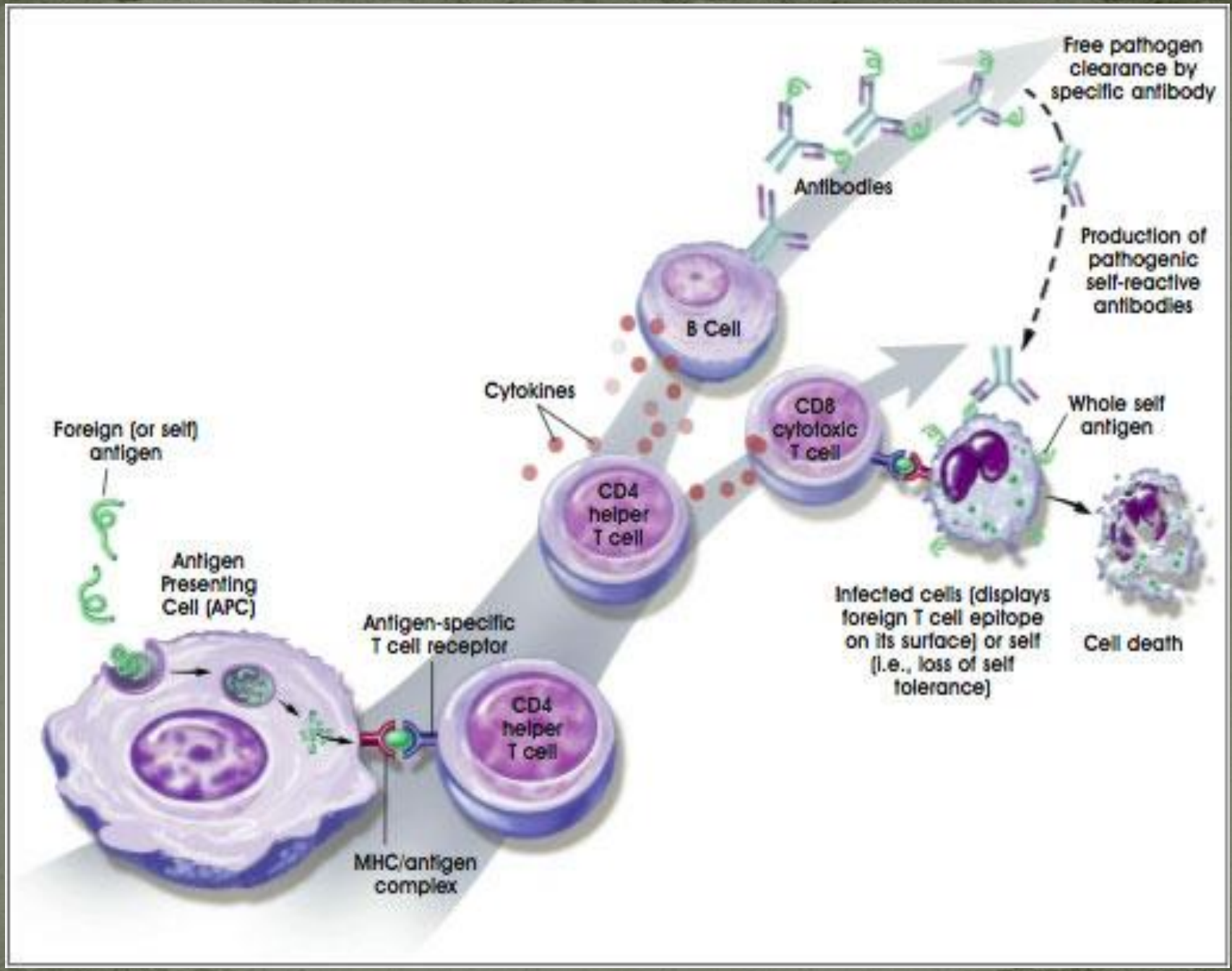

Th1
pro-inflammatory
cytokines:
IFN-γ
IL-2
LT
macrophages↑
cellular immunity
immunopathology:
autoimmune
diseases
antigen
precursor
CD4 T cell
Th2
anti-inflammatory
cytokines:
IL-4
IL-5
macrophages↓
humoral immunity
immunopathology:
atopic diseases
effector cell
Arthritis Research & Therapy

Cell-Mediated Immunity

- Eradicates infections by intracellular microbes.
- Consist of the activation of naïve T cells to proliferate and differentiate into effector cells ($CD4^+$ T helper cells and $CD8^+$ cytolytic cells; CTLs) and the elimination of the intracellular microbes.

Types of cell-mediated immunity

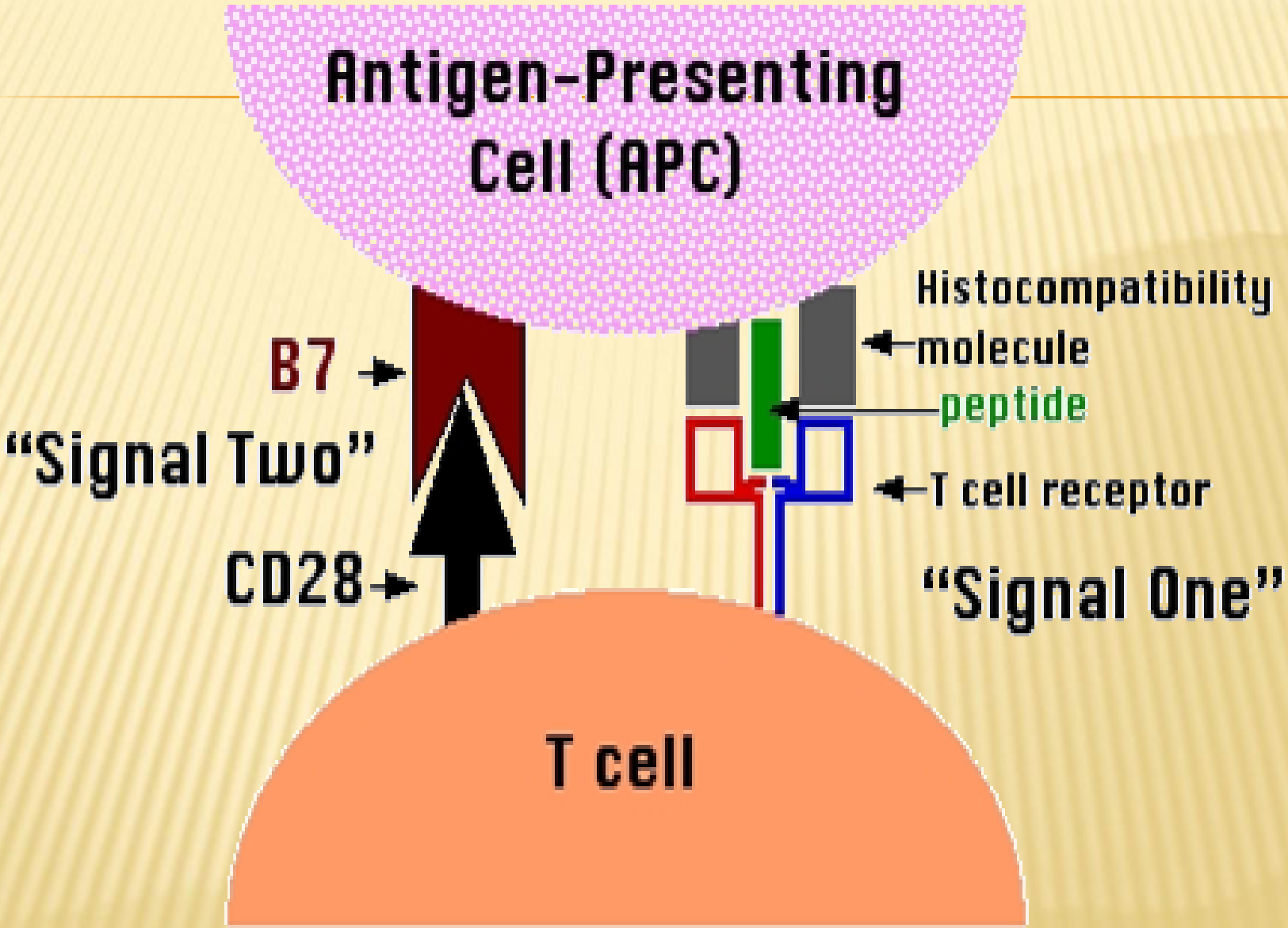
1. **$CD4^+$ T cells: activate macrophages to kill ingested microbes that are able to survive inside phagocytes. T_H1 activate macrophages by secretion of the macrophage-activating cytokine, IFN-γ.**
2. **$CD8^+$ T cells kill any cell containing microbes or microbial proteins in the cytoplasm (intracellular) by direct cell cytotoxicity, thus; eliminating the reservoir of infection.**


Free pathogen clearance by specific antibody
Antibodies
Production of pathogenic self-reactive antibodies
B Cell
Cytokines
CD8 cytotoxic T cell
Whole self antigen
Foreign (or self) antigen
CD4 helper T cell
Antigen Presenting Cell (APC)
Antigen-specific T cell receptor
Infected cells (displays foreign T cell epitope on its surface) or self (i.e., loss of self tolerance)
Cell death
CD4 helper T cell
MHC/antigen complex

Activation of T cells

Activated by two signals:

- **The 1st signal : peptide + MHC on the surface of APCs recognized by TCR-CD3**
- **The 2nd co-stimulatory signal: is the interaction of B7 molecule on APCs with CD28 on T cells .**
  - **In absence of 2nd signal, exposure of T cells to antigen lead to anergy (unresponsiveness**

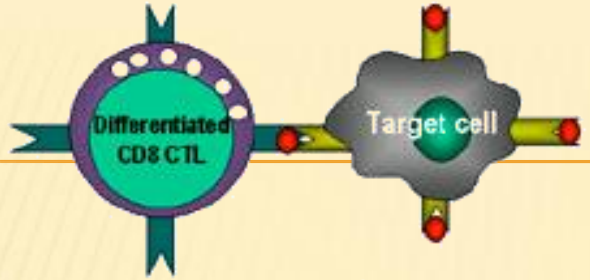

Antigen-Presenting Cell (APC)
B7
"Signal Two"
CD28
Histocompatibility molecule
peptide
T cell receptor
"Signal One"
T cell

Steps of killing of target cells by $CD8^+$ CTLs

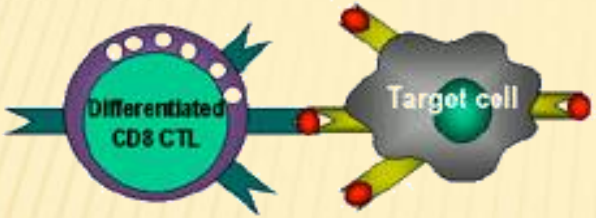
1. CTLs recognize class I MHC + peptides on the surface of "target" cell.
2. Formation of tight adhesions "conjugates" with these cells.
3. CTLs are activated by IL-2 & IFN-γ to release their granule contents toward the

target cell i.e. granule exocytosis.

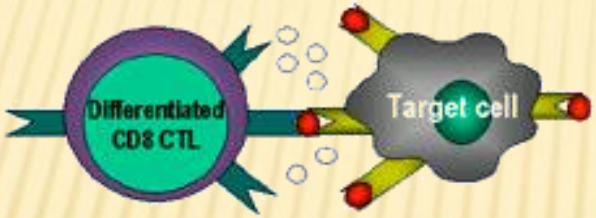
4. The granules contents include:
   a. Perforin, which form pores in the target cell membrane
   b. Granzymes, enter the target cells through these pores and induce apoptosis through the activation of caspases.
5. Detachment of CTL from target cells to kill other target cell.
6. Death of target cell by apoptosis.



1. CTL recognizes antigen on target cell



2. CTL is activated



3. A lethal hit is delivered by the CTL using agents such as perforin or granzyme B

4. The CTL detaches from the target cell

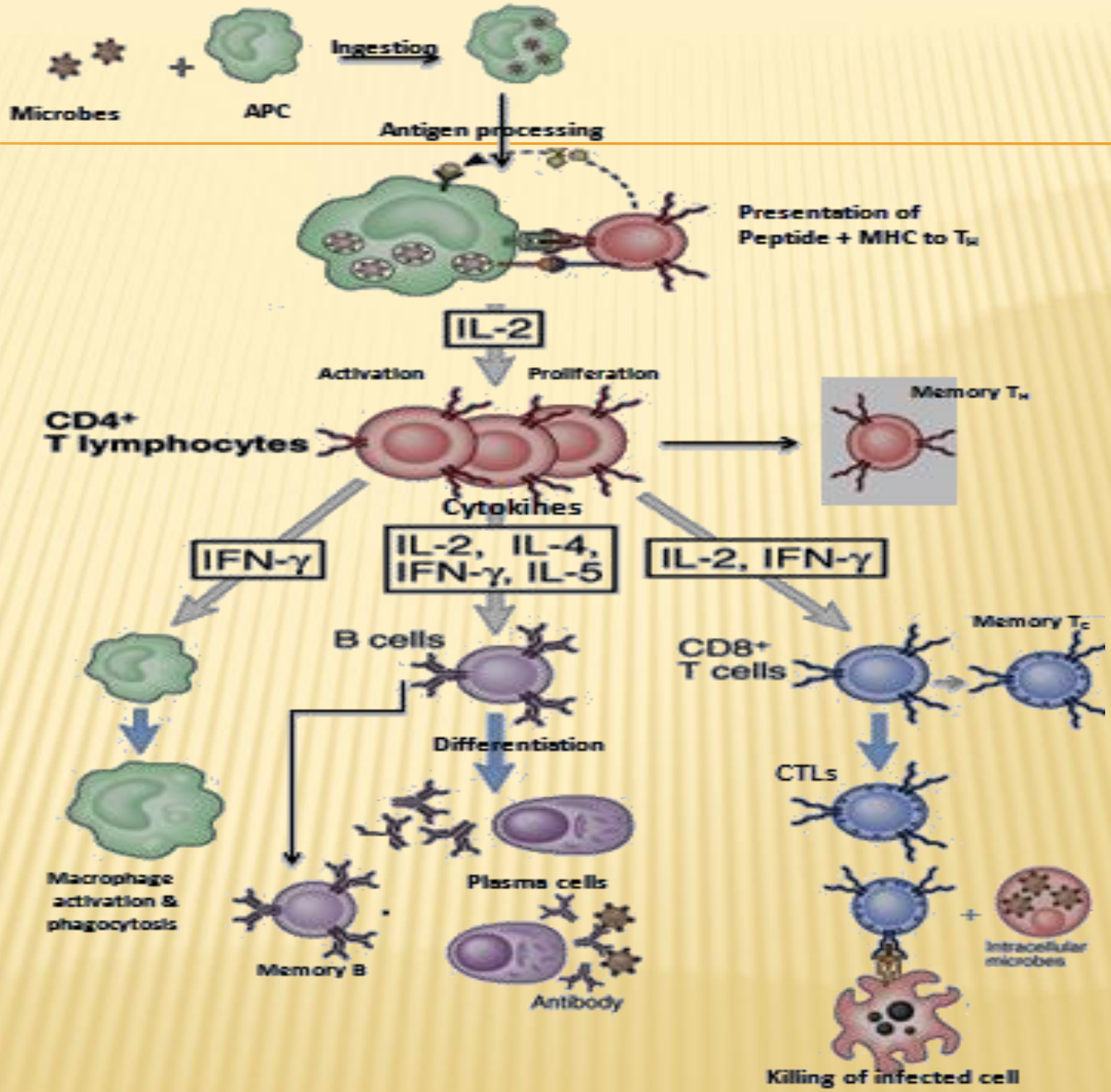
5. Target cell dies by apoptosis

Sequence of events of a prototypical immune response

Every immune response is a complex and highly regulated sequence of events involving several cell types that interact with each other either directly or through cytokines. (Fig.18):

- APCs capture a minute amount of the antigen by phagocytosis.
- Antigen processing: APCs process the antigen to very small fragments (peptides)
- Antigen presentation: the APCs present peptides plus class II MHC molecules to T helper cell.
- Binding of peptide-MHC complex to TCRs → activation of helper T lymphocytes
- Secretion of cytokines including IL-2 by the activated T_H cells that act on the producing T_H cells themselves leading to their proliferation and activation. IFN-γ activates macrophages & phagocytosis.
- Activation of B lymphocytes: need 2 signals. The first signal is binding of the antigen to BCR, and the second one is provided by helper factors (cytokines) secreted by T_H cells e.g.IL-2, IL-4, IL-5. The second signal is called *T cell help*.
- B cell proliferation and differentiation into either *memory* B lymphocytes or *plasma cells* which secrete antibodies.

Activation of cytotoxic T cells: T_c lymphocytes recognize antigen on the surface of target cell (e.g.virus-infected cell) which express class I MHC molecules. T_c cell activation


Microbes
APC
Ingestion
Antigen processing
Presentation of
Peptide + MHC to T_H
IL-2
Activation
Proliferation
CD4+
T lymphocytes
Memory T_H
Cytokines
IFN-γ
IL-2, IL-4,
IFN-γ, IL-5
IL-2, IFN-γ
B cells
CD8+
T cells
Memory T_C
Differentiation
CTLs
Macrophage
activation &
phagocytosis
Plasma cells
Memory B
Antibody
Intracellular
microbes
Killing of infected cell

Immunomodulation

Adjustment of the immune response to a desired level, as in immunopotentiation, or immunosuppression.

Immunopotentiation

Definition: enhancement of the immune response by increasing its rate or prolonging its duration by the administration of another substance (an adjuvant).

Adjuvant: an agent that stimulate the immune system and increase the response to a vaccine, without having any specific antigenic effect by itself :

Inorganic adjuvants like aluminium salts:

Organic adjuvants like squalene

Oil-based adjuvants like

- complete freund`s adjuvant
- Incomplete freund`s adjuvant

Virosomes: A virosome is a phospholipid bilayer vesicle containing hepatitis A and influenza antigens

Cytokines for example IL-12,

Mechanisms of action of adjuvants:

Prolong retention of the immunogen

Increase the size of immunogen and so promote phagocytosis and presentation by macrophages

Stimulate the influx of macrophages and other immune cells to the injection site

Increase local cytokine production

Immunosuppression

Def.: Suppression of immune system

Indications*:*

1- Hypersensitivity responses.

2- Autoimmune disease.

3- After transplantation to prevent rejection.

Induction:

1- Drugs.

2- Radiation**.**

3- Anticancer drugs

<u>Methods of immunosuppression:</u>

1. Cyclosporine & tacrolimus
2. Azathioprine & mycophenolate
3. Corticosteroids
4. Anti CD3 monoclonal antibodies
5. Anti-IL2 receptor antibody

Apoptosis

"Programmed Cell Death"

- Apoptosis is self suicide of cells when they are no longer needed or if they become damaged and cannot be repaired. e.g. modeling of tissues & organs during embryogenesis ; and shedding of uterine lining each month in females
- cells normally die and eliminated by phagocytosis
- In the immune system, apoptosis is important for elimination of unwanted and harmful lymphocytes during maturation and after activation at the end of an immune response to return the immune system back to its resting state after elimination of the antigen. Also apoptosis is essential in elimination of infected cells.
- Apoptosis is induced by the activation of proteolytic enzymes called *caspases i.e. cysteine proteases.*