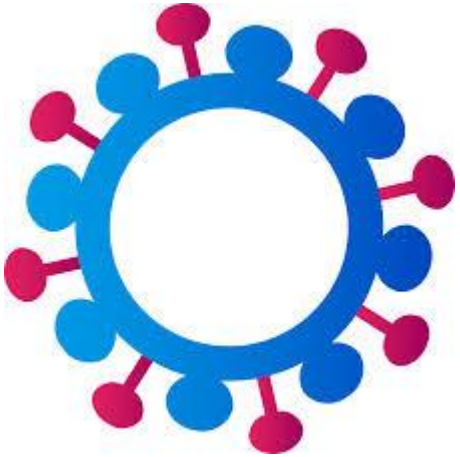
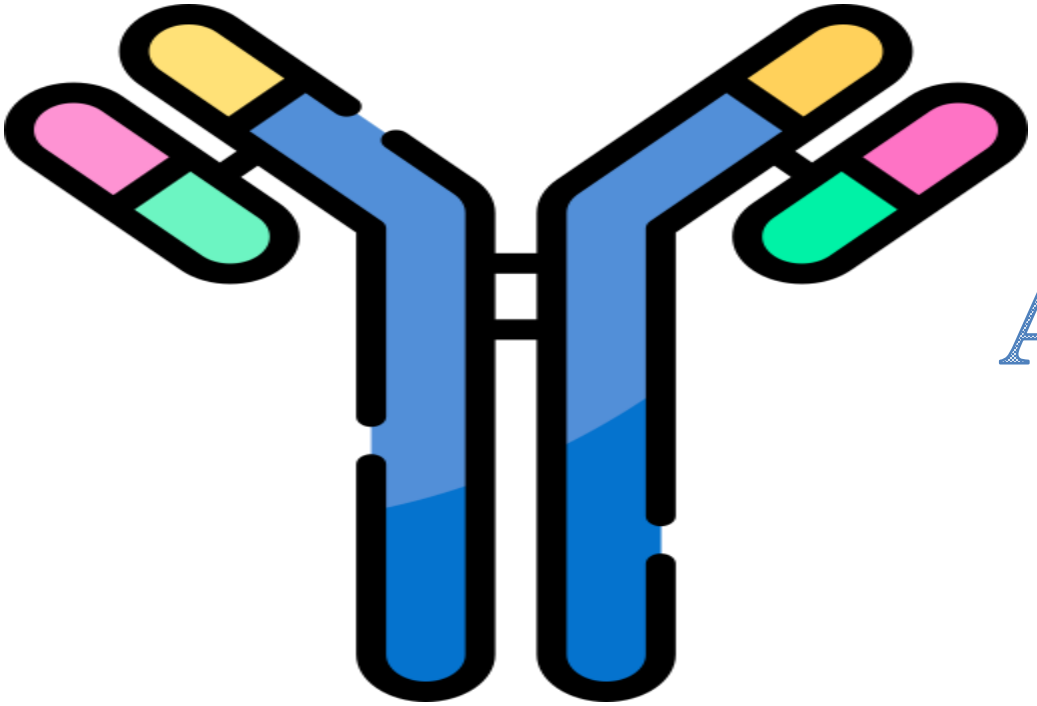


Antigens

and



Antibodies

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Learning outcomes

- By the end of this lecture the students will be able to :

- Define antigens and epitopes
- Classify functional types of antigens according to immunogenicity.
- Clarify factors affecting immunogenicity
- Recognize T-cell independent Ag and T-cell dependent Ag.
- Define immunoglobulins and describe their production and distribution.
- Define the structure of antibodies and forms
- Compare different immunoglobulin classes
- Explain monoclonal antibodies production
- Identify the applications of monoclonal antibodies.

Lecture Outline

- Antigens
- Immunoglobulins
- Monoclonal antibodies

Case scenario, Clinical Correlate, Practice points

- A 10-month-old boy is brought to the paediatrician by his parents because of fever, cough, and difficulty breathing. A profile of the patient's immunoglobulin isotypes shows low IgA, low IgG, and **markedly elevated IgM** levels. The number of T and B lymphocytes is normal.

What is the etiology of the increased level of IgM in this patient?

Antigens

Antigens

- **Antigen:**

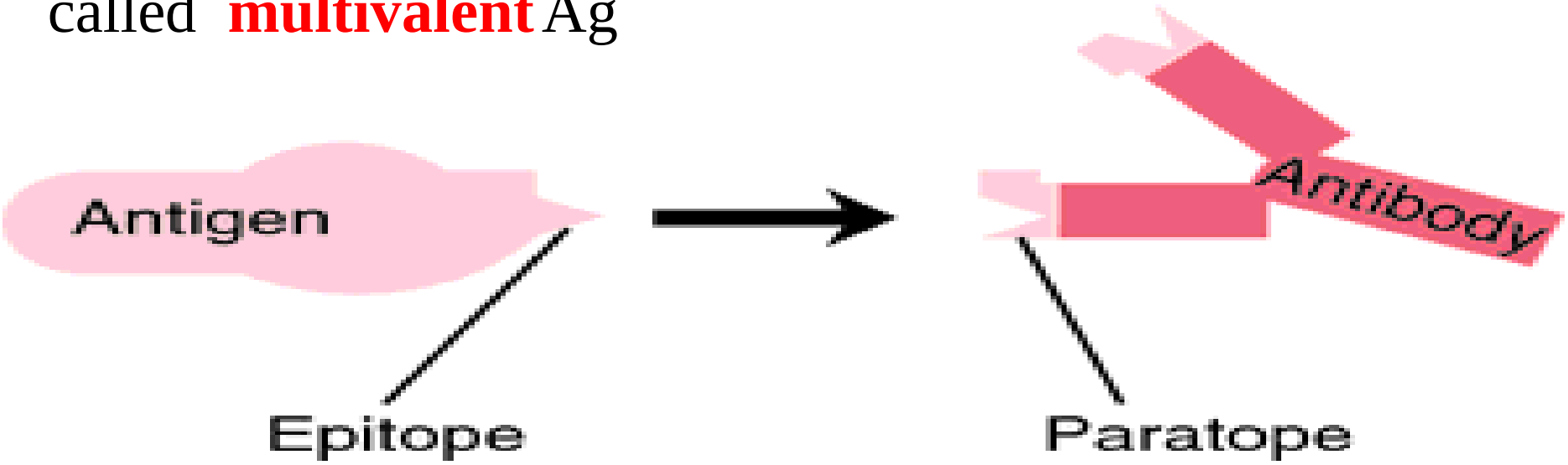
  Substance that is recognized by immune system which may be:

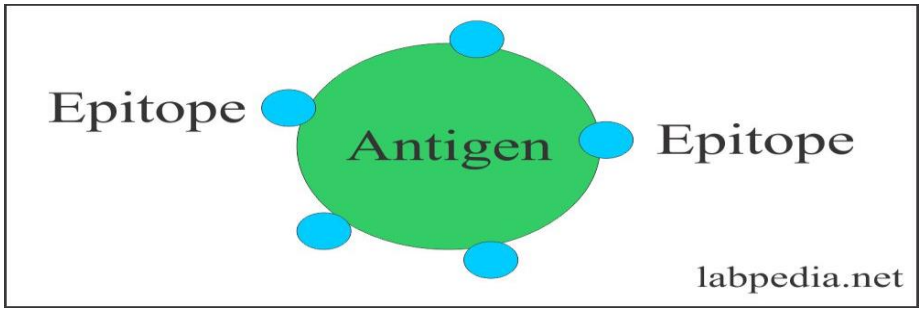
  - Simple or complex
  - Carbohydrate, lipid, protein, glycoprotein, nucleic acid, phospholipids
  - **B** cell recognize **any** biological Ag
  - T cell recognize **peptide** Ag presented on MHC

Epitopes (antigenic determinants):

Smallest part on Ag which bind with B cell receptors (BCR) & T cell receptors (TCR).

If Ag contain multiple and identical epitopes , it is called **multivalent** Ag





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

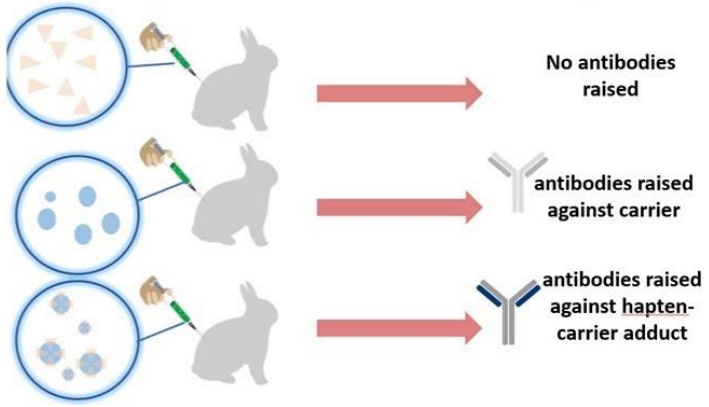
A. Immunogens:

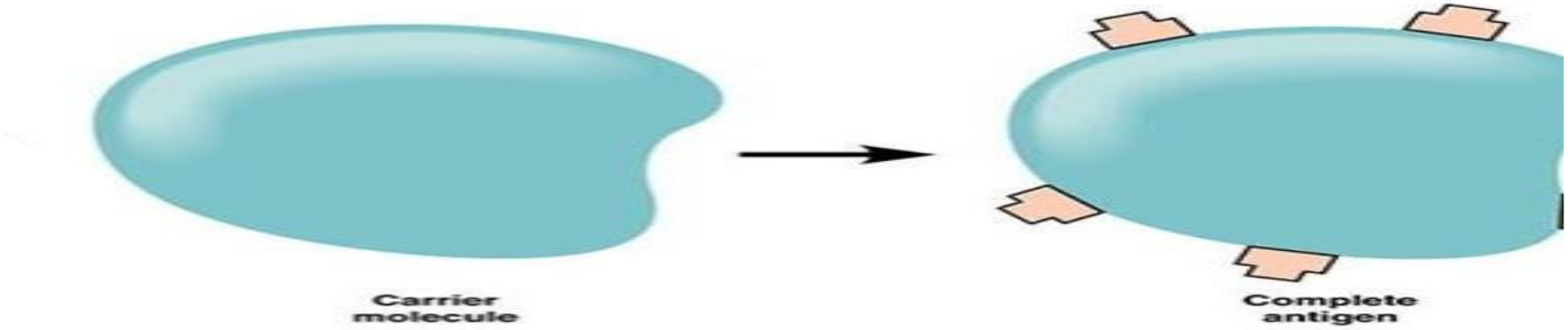
- **Contain epitopes that induce a specific immune response and are the targets of that response**
- **(Notice that not all antigens are immunogens)**

B. Hapten

- A small antigen that can elicit an immune response **only when attached to a larger carrier** such as a **protein**.
- → immune response against epitopes of hapten & carrier.

Haptens are minute molecules that elicit an imm
response only when attached to a large carrier





c. Tolerogens

Substances that induce immunological tolerance in an organism.

Immunological tolerance refers to a state in which the immune system does not cause an immune response against specific antigens, even though it is capable of recognizing them.

Self Ag normally not stimulate immune system

Factors that influence immunogenicity:

Size: **proteins > 10 KDs are more immunogenic**

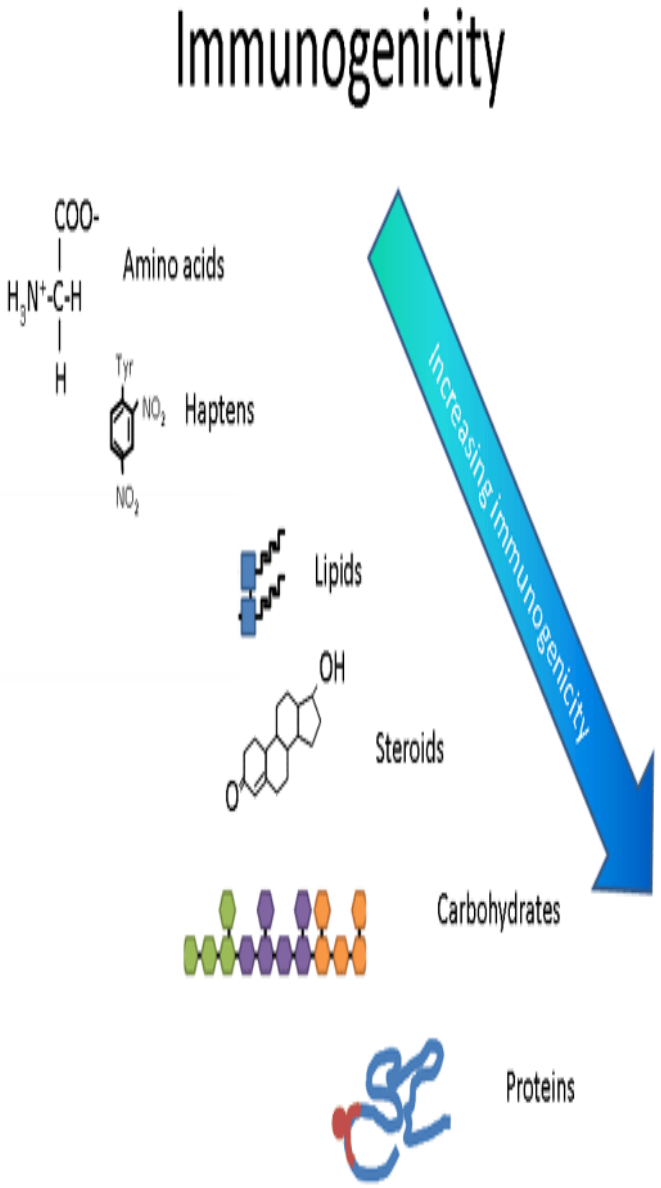
complex proteins : with numerous, diverse epitopes are more to induce an immune response than are simple peptides that contain only one or few epitopes.

Conformation and accessibility:

Epitopes must be"seen by" and be accessibile to the immune system.

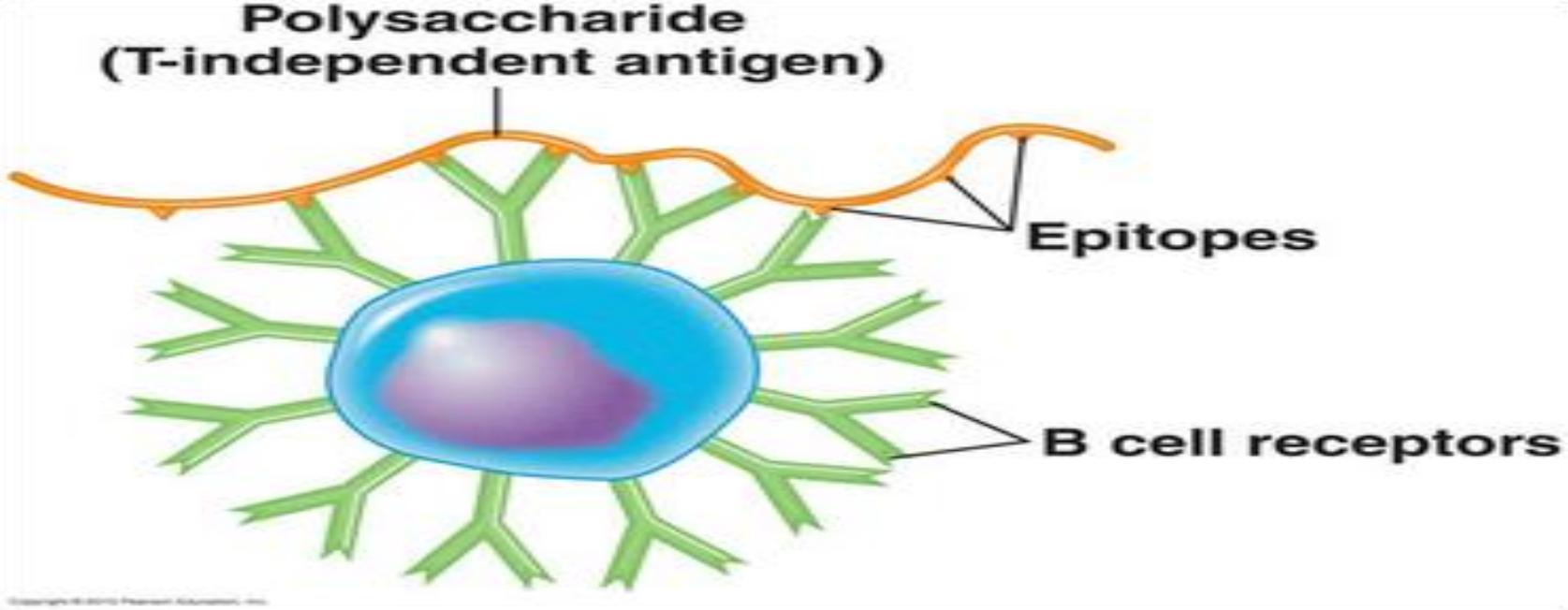
Chemical properties:

- **A protein is good immunogen.**
- **Many charbohydrates, steroids, and lipids are poor immunogens.**
- **Amino acids and haptens are, not immunogenic.**

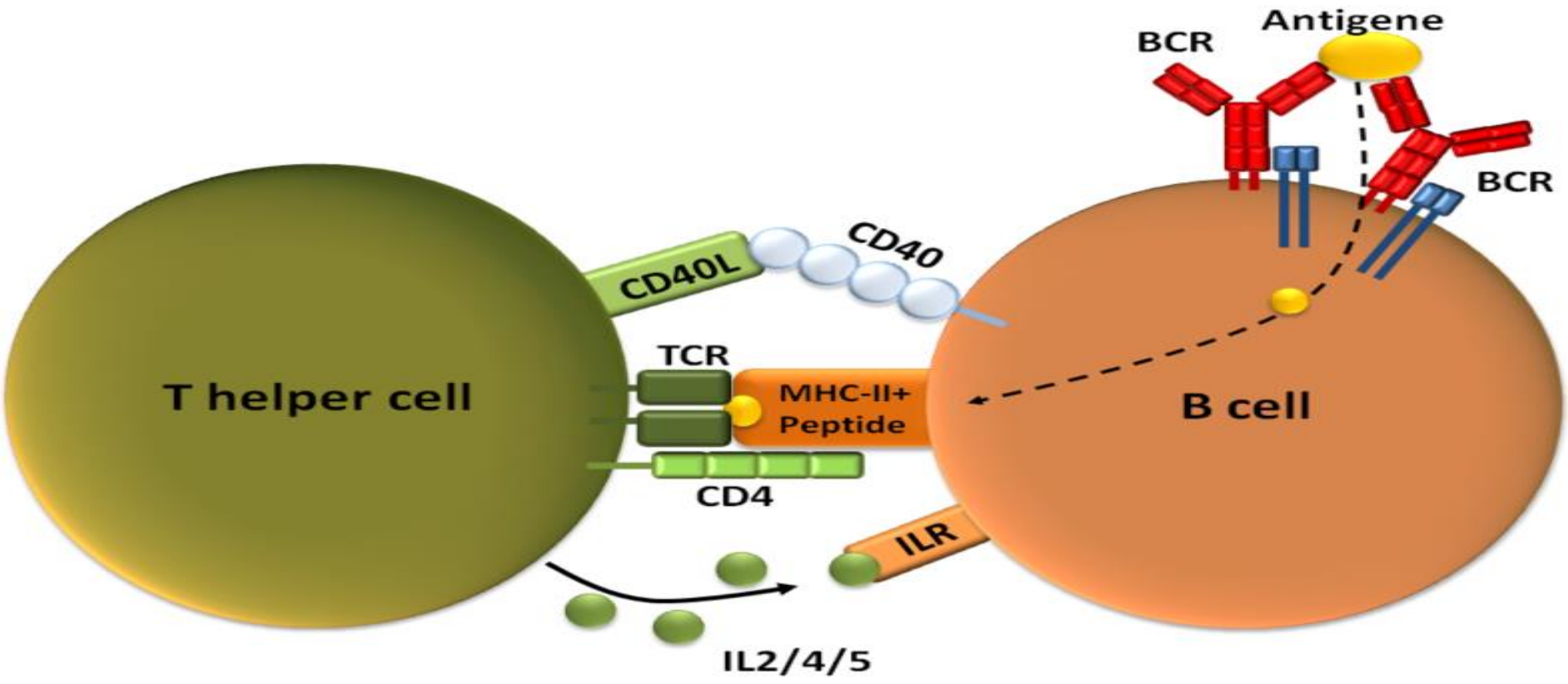


Types of Antigens

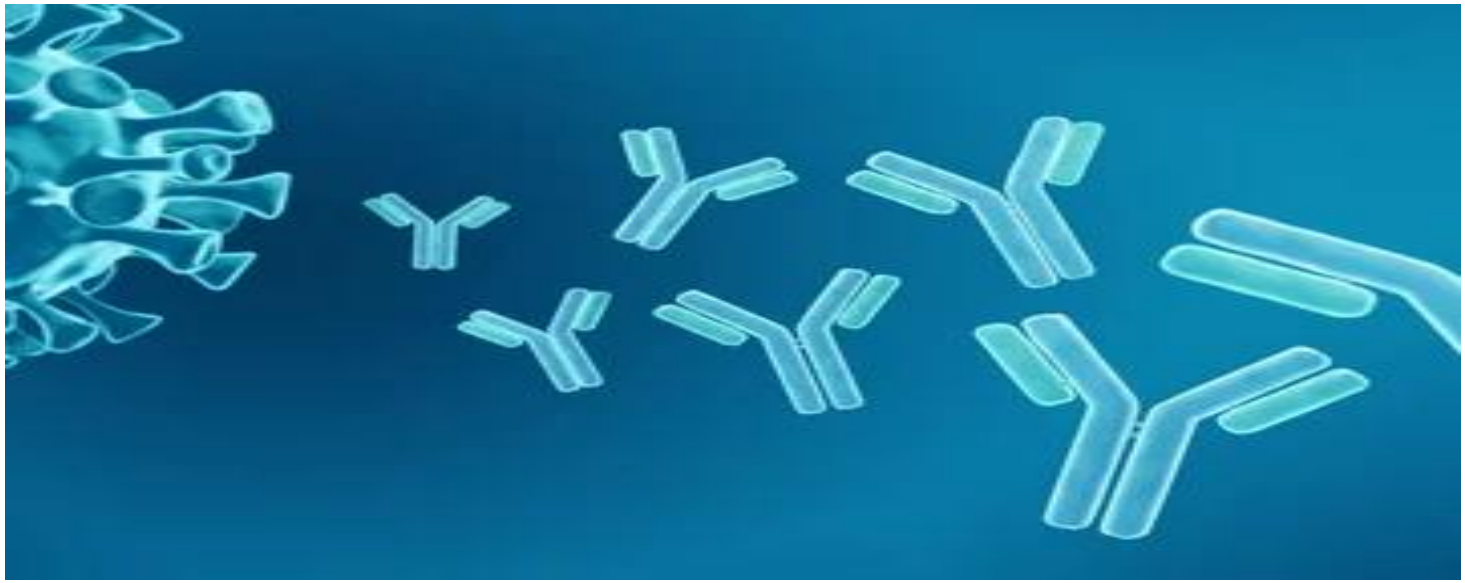
1. **T-cell independent Ag (TI): activate B cells without help from T-cell ; e.g. polysaccharides (Pneumococcal polysaccharide, LPS)**



2. T-cell dependent Ag:Requires T-cell help for B cell activation; e.g. proteins (microbial proteins & non-self or altered-self proteins).



Immunoglobulins (Igs) (antibodies)

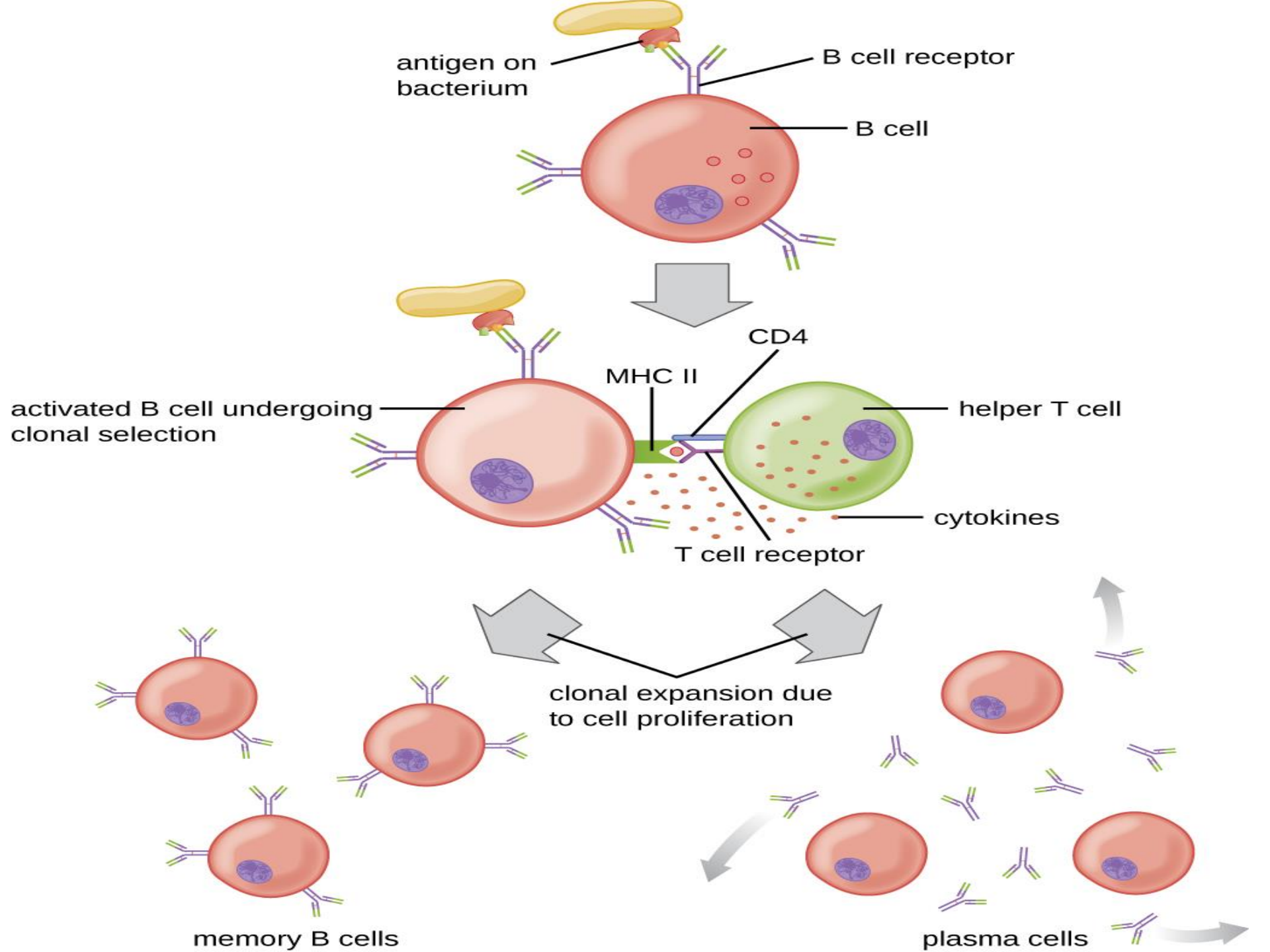


Def:

Immunoglobulins are glycoprotein which mediate humoral immunity.

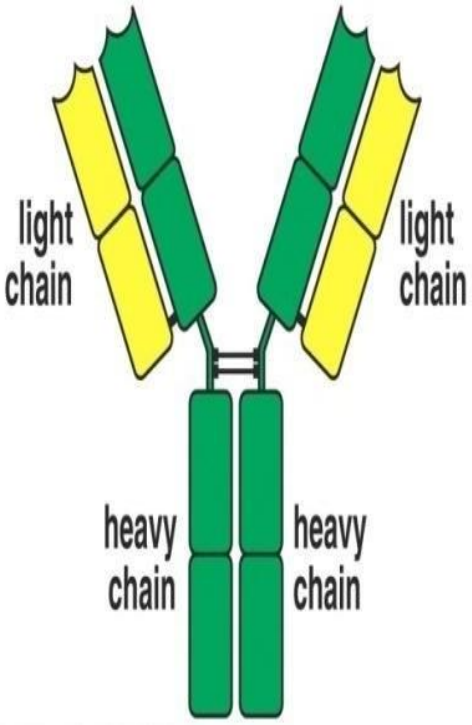
Production & distribution of antibodies

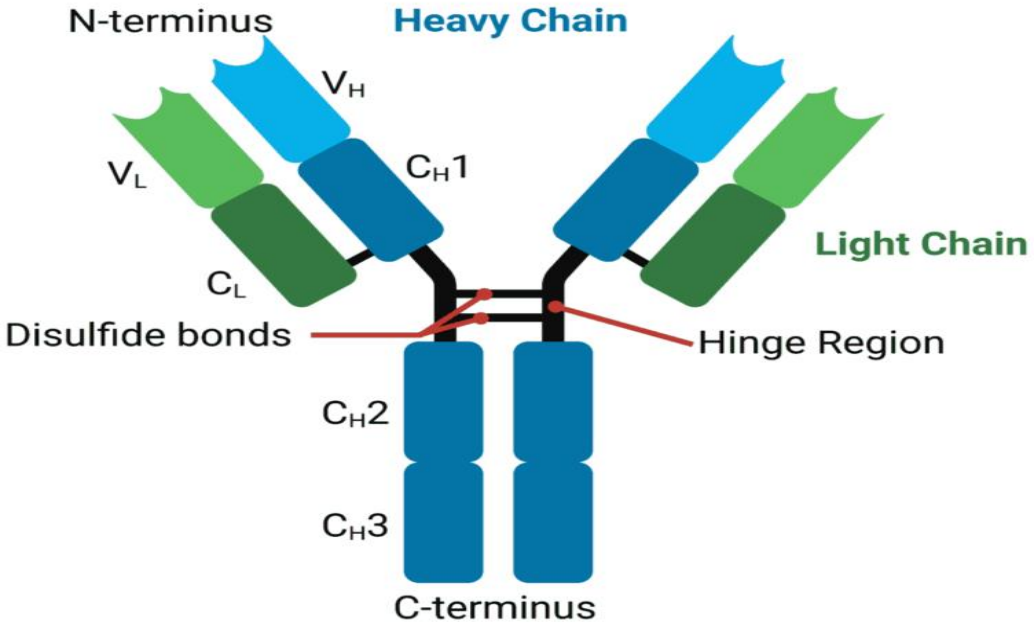
- In lymph node → Ag stimulation of B cells (may be with help of T helper cytokines) → B cell proliferate → differentiate into plasma cell which secrete antibodies → enter circulation → site of infection
- Also, mature B-cell in Bone Marrow express membrane bound antibodies (BCR).
- So, antibodies are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


antigen on bacterium
B cell receptor
B cell
CD4
MHC II
activated B cell undergoing clonal selection
helper T cell
cytokines
T cell receptor
clonal expansion due to cell proliferation
memory B cells
plasma cells

The structure of antibodies:

- **Y shaped molecules of 4 polypeptide chains**
- **2 identical heavy chain (1 variable domain (VH) & 3 constant domains (CH)**
- **2 identical light chain (1 variable domain (VL) & 1 constant domains (CL)**

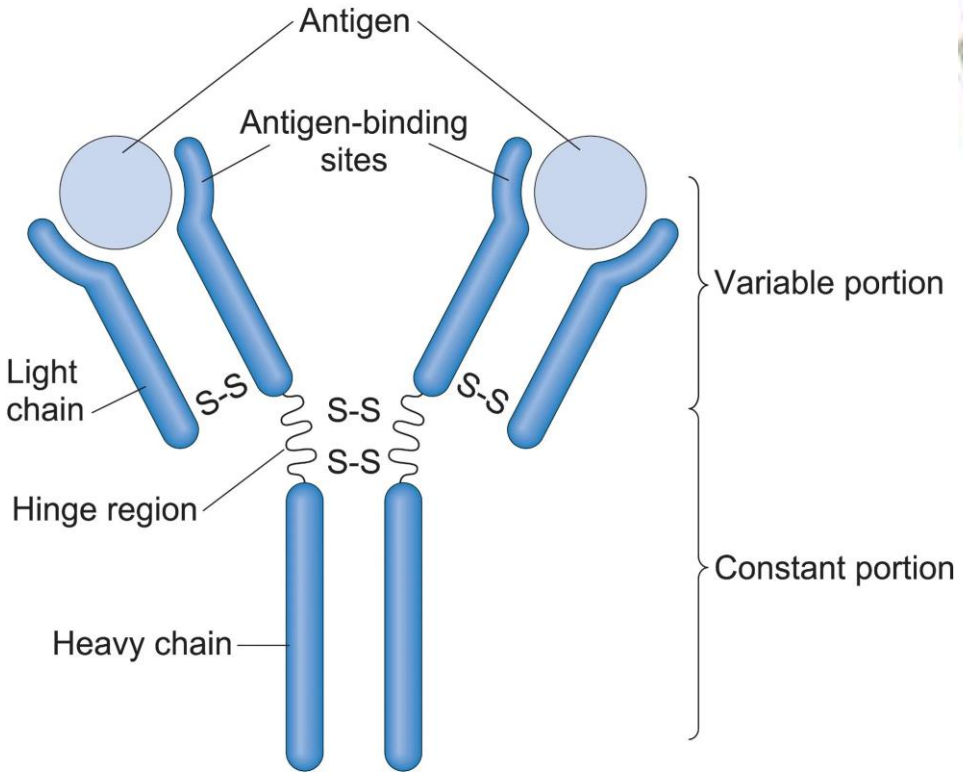


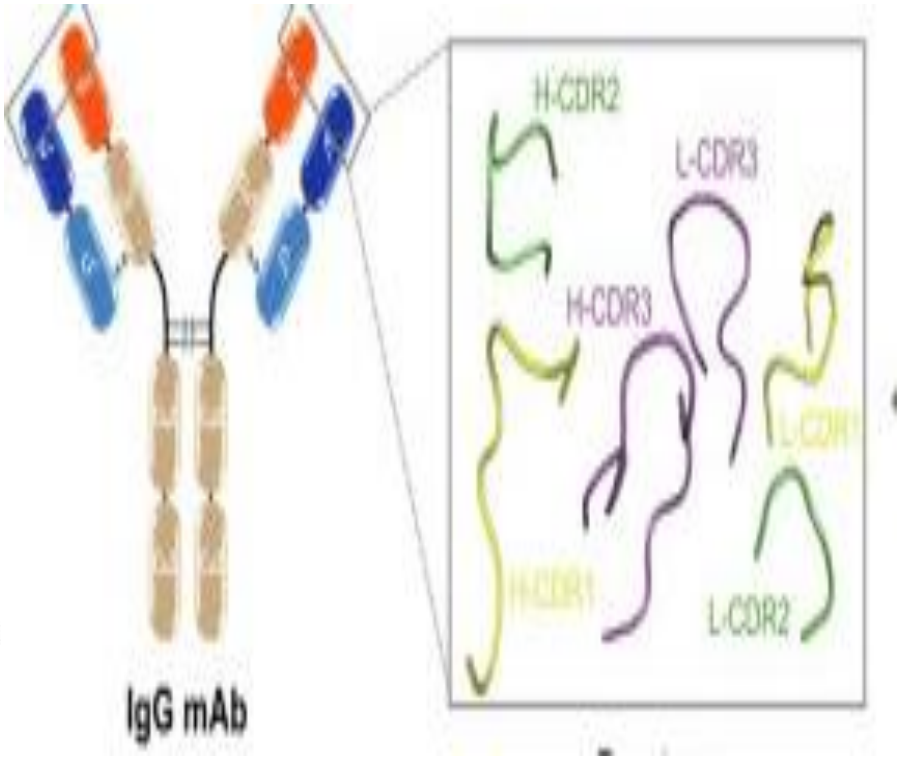


light chains

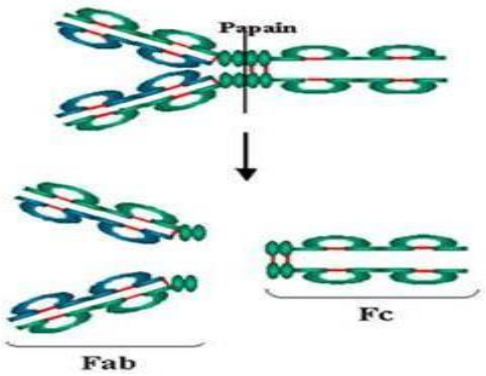
There are two types of light chains, called κ (kappa) and λ (lambda). An antibody has either two κ or two λ light chains but never one of each.

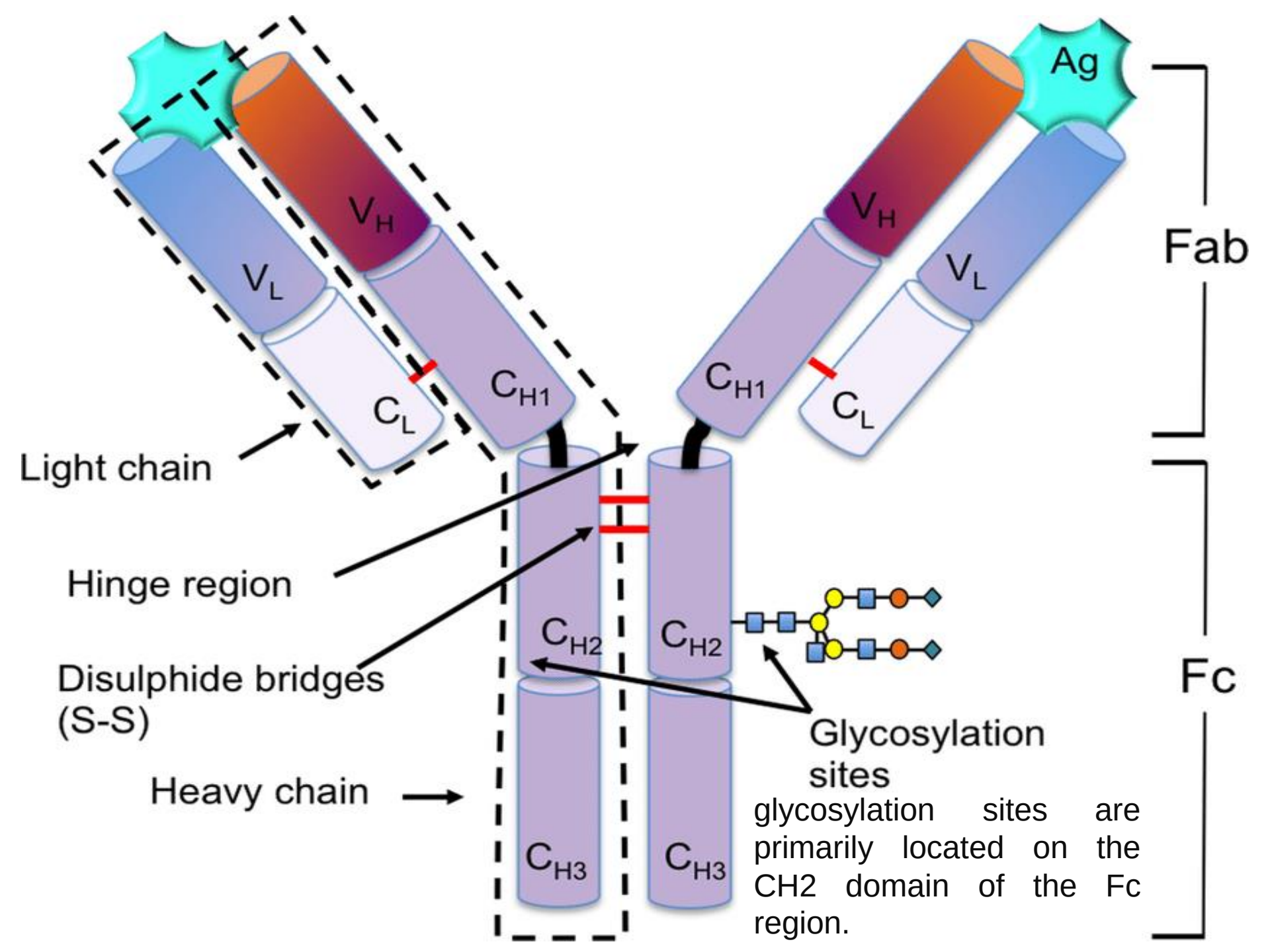
- Each variable domain (VL or VH) contains **3 hypervariable regions** called complementary determining repeats (**CDR**)
- **Disulfide** bond connect heavy chain with light chain & heavy chain with heavy chain





Regions of Ab according to proteolytic fragments:

Fab = fragment Ag binding	Fc= fragment crystalline	Hinge region
Contain whole light+VH+ CH1 2 in number  	Tend to crystallize in solution 1 in number Contain remaining of both heavy chains C domain. effector & biological function.	- Flexible region lies between Fab &Fc to give mobility to both Fab to accommodate different Ag



glycosylation sites are primarily located on the CH2 domain of the Fc region.

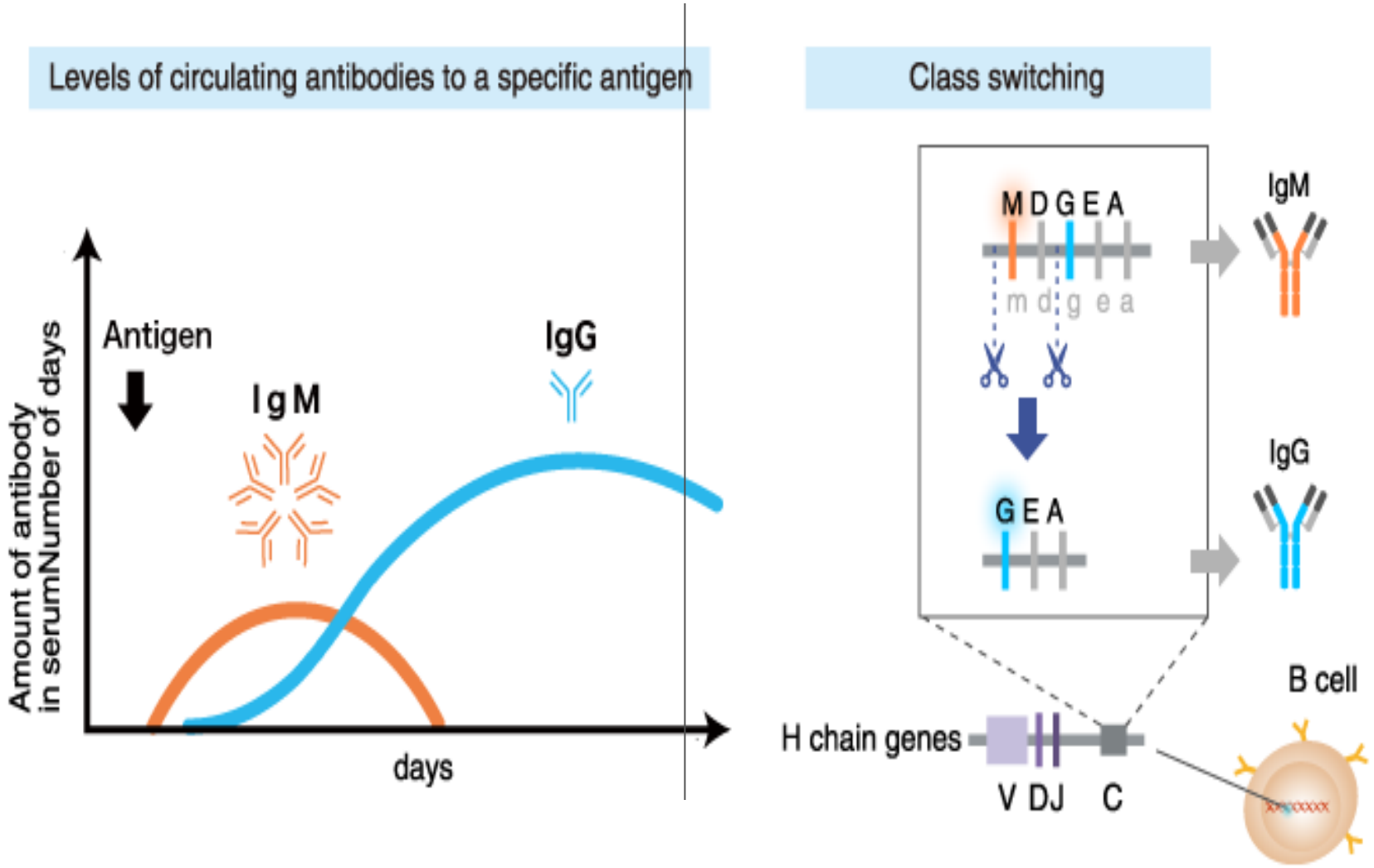
Ig classes (isotypes)

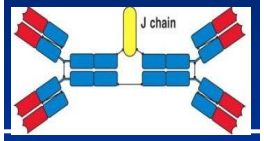
A. Immunoglobulin classes

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


Levels of circulating antibodies to a specific antigen
Amount of antibody in serumNumber of days
Antigen
IgM
IgG
days
Class switching
M D G E A
m d g e a
G E A
IgM
IgG
B cell
H chain genes
V DJ
C

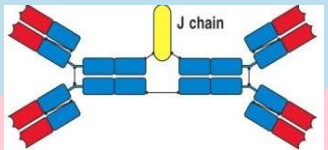
	Subtypes	H chain	Serum mg/ml	Secreted form	functions
IgA	IgA1, 2	α1 or α2	3.5 	Monomer dimer, tri	Mucosal immunity
IgD	-	δ	traces 0.02	monomer	-B cell receptor
IgE	-	ε	0.05	monomer	-Parasite -Allergy
IgG	IgG 1-4	γ (1,2,3,4)	13.5	monomer	-Opsoniz. -Comp. -ADCC
IgM	-	μ	1.5	pentamer	-B cell rec. -Comp.

5 Types of Antibodies

Antibodies or immunoglobulins (Ig) are Y-shaped proteins that recognize unique markers (antigens) on pathogens.

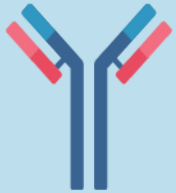
IgA

Secreted into mucous, saliva, tears, colostrum. Tags pathogens for destruction.



IgD

B-cell receptor. Stimulates release of IgM.

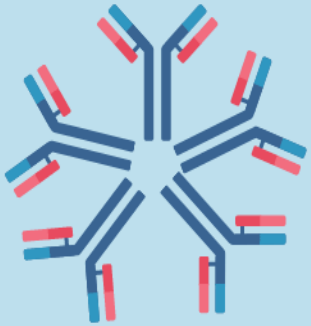


IgE

Binds to mast cells and basophils. Allergy and antiparasitic activity.

IgG

Binds to phagocytes. Main blood antibody for secondary responses. Crosses placenta.

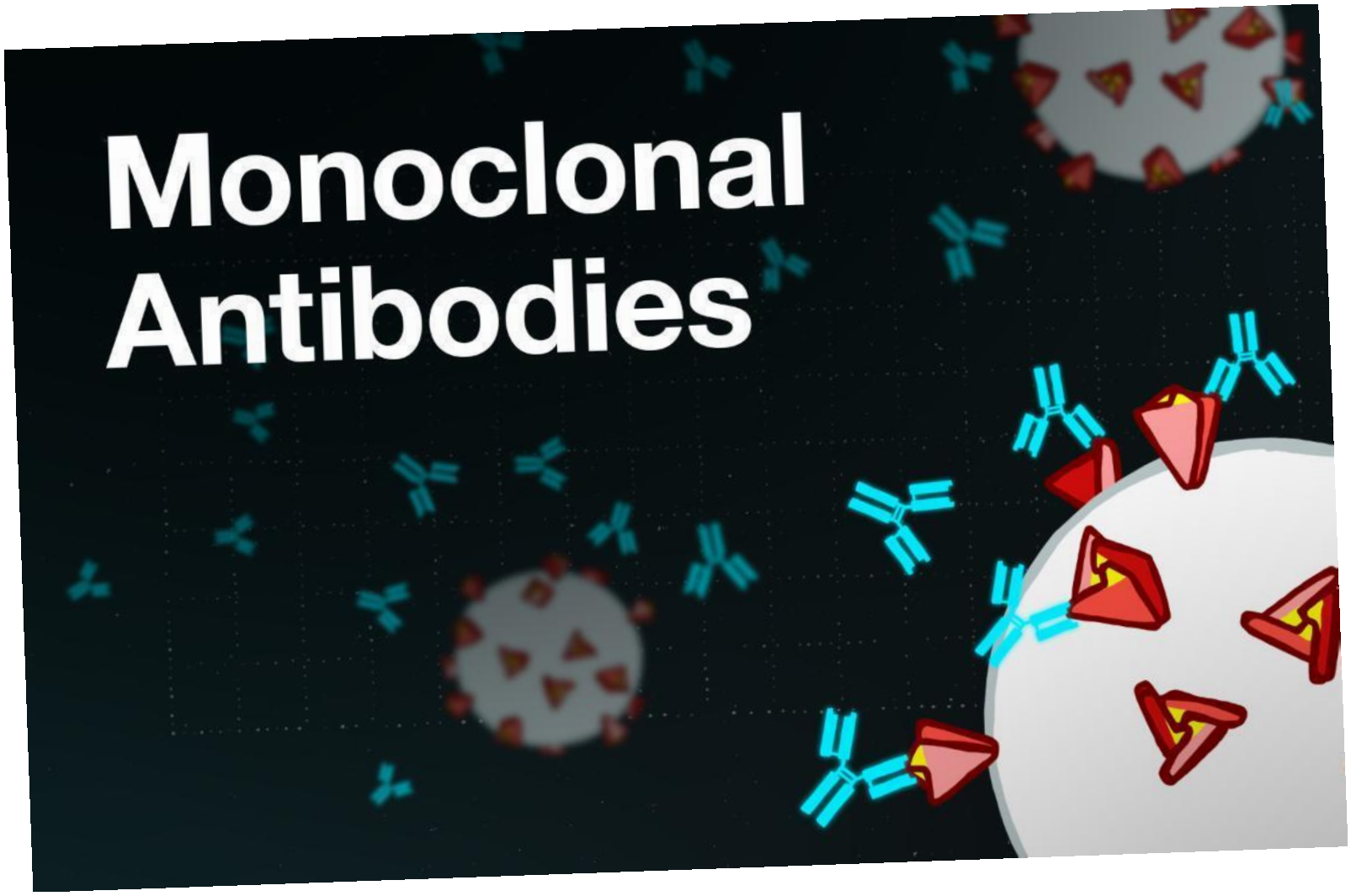


IgM

Fixes complement. Main antibody of primary responses. B-cell receptor. Immune system memory.

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, saliva, tears, colostrum & interstitial fluids of tissues.


Monoclonal
Antibodies

Monoclonal Antibodies

- **identical mono-specific antibodies that are produced by one type of immune cell that are all clones of a single parent cell.**

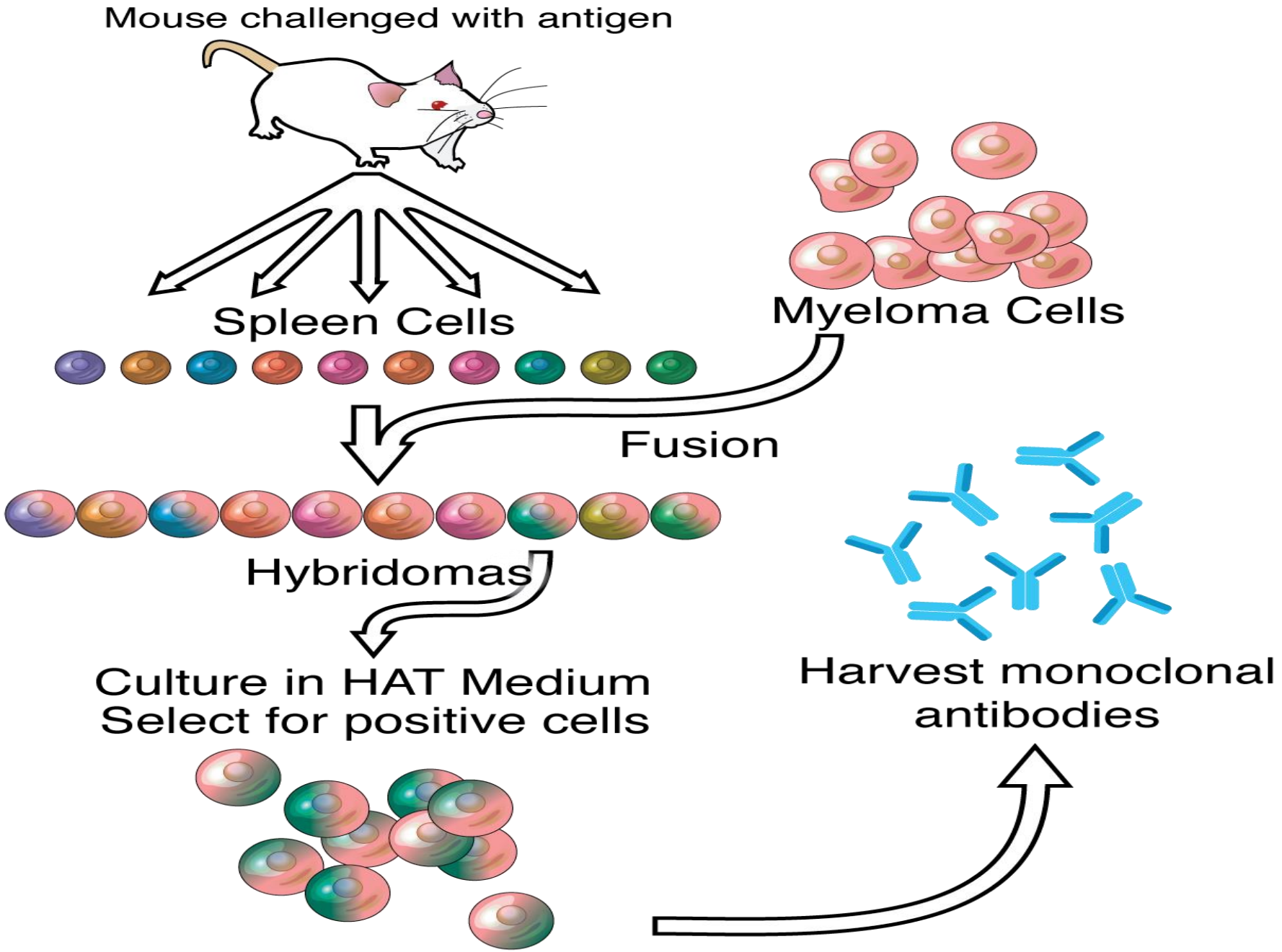
- **In contrast,** diverse group of antibodies that are produced by **different** B-lymphocytes, each recognizing a **different** epitope on an antigen **called polyclonal antibodies.**

Production:
Hybridoma technology

Steps:

- A mouse is immunized with the antigen .
- B cells are isolated from the spleen of the mouse.
- 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 antibody-producing cell "hybridoma".
- 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.
- Hybridomas, secrete a large amount of mAbs.


Mouse challenged with antigen
Spleen Cells
Myeloma Cells
Fusion
Hybridomas
Culture in HAT Medium
Select for positive cells
Harvest monoclonal
antibodies

Applications

1. Identification of phenotypic markers
   They have been used to define clusters of differentiation (CD markers) on lymphocytes.
2. Immunodiagnosis : the diagnosis of many infectious and systemic diseases relies on the detection of specific antigens or antibodies in the circulation or tissues by use of mAbs.

3. Tumor diagnosis: tumor-specific monoclonal antibodies are used for detection of tumors by imaging techniques.

4. Therapy: a number of mAbs are used therapeutically today:

- **e.g: Anti-CD3 for immunosuppression and prevention of graft rejection**

Case scenario, Clinical Correlate, Practice points

- A 10-month-old boy is brought to the pediatrician by his parents because of fever, cough, and difficulty breathing. A profile of the patient's immunoglobulin isotypes shows low IgA, low IgG, and markedly elevated IgM levels. The number of T and B lymphocytes is normal.

What is the etiology of the increased level of IgM in this patient?

- Which of the following immunoglobulins is present normally in plasma at the highest concentration?

- a. IgG
- b. IgM
- c. IgD
- d. IgE
- e. IgA

a. IgG

-A molecule that can be covalently linked to a non-immunogenic antigen to make it an immunogen is called a:

- a. Adjuvant.
- b. Carrier.
- c. Hapten.
- d. Mitogen.
- e. Superantigen.

c. Carrier.

- The Fab portion of IgG usually:

- a. Binds to an Fc receptor
- b. Crystalline
- d. involved in antigen recognition and binding.
- d. Mediates biological effector functions of Ab molecules(e.g. complement fixation)
- e. Activates mast cell

d. involved in antigen recognition and binding.

References or further readings

- Basic Immunology : functions and disorders of the immune system , fifth edition ; Abul K. Abbas, Andrew H. Lichtman and Shiv Pillai
- Immunology :7th edition ; David Male, Jonathan Brostoff, David Roth and Ivan Roitt

THANK YOU

Akbarian