

Objectives

At the end of this lecture, the student should be able to understand the following:

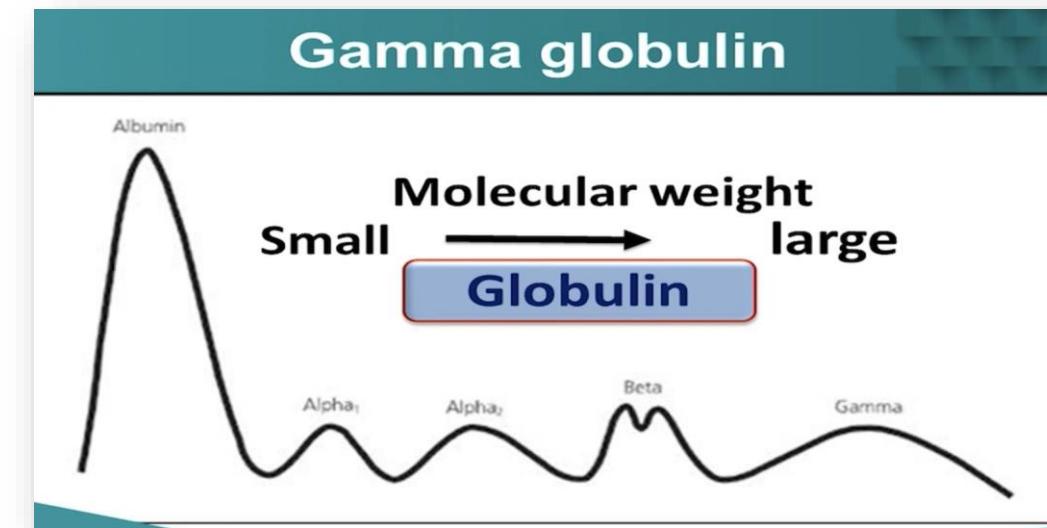
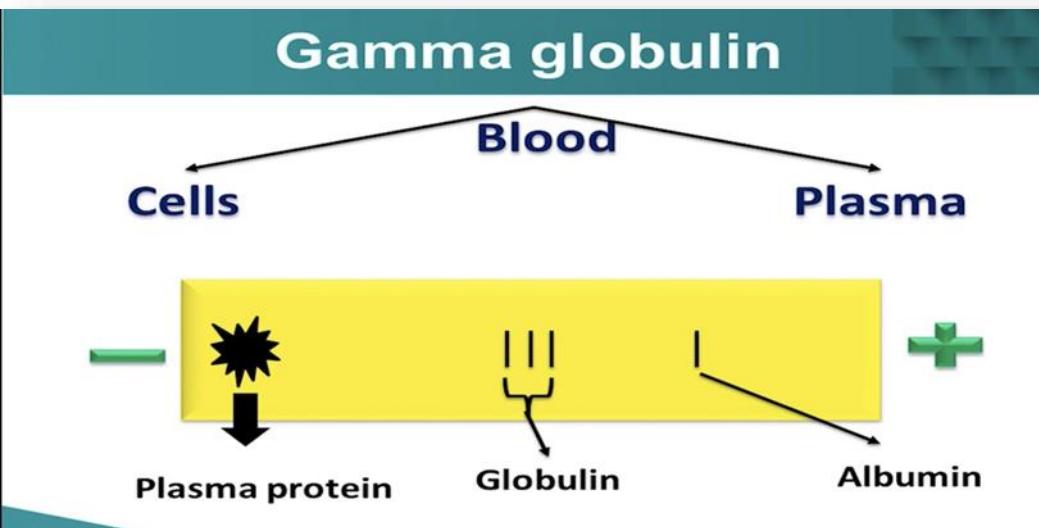
1- Definition of immunoglobulins or Abs

2- Steps of Abs production

3- Structure of Igs

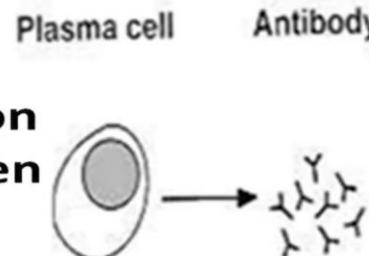
4- Functions of Igs

Why antibodies also called immunoglobulins?

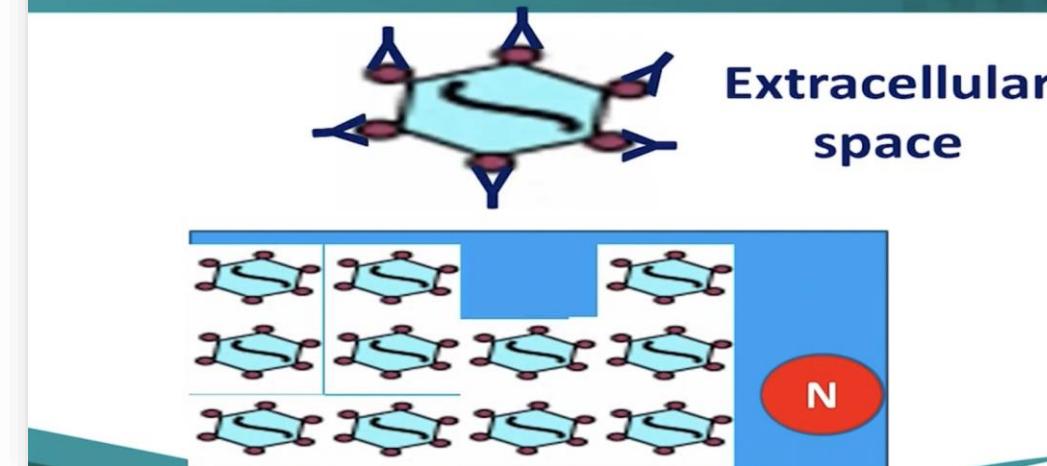


I- Definition of immunoglobulin

- Glycoprotein molecules
- plasma cells
- response to antigen
- contribute in destruction of extracellular pathogen
- present in the serum & tissue fluids.



Role of B cell in intracellular pathogen

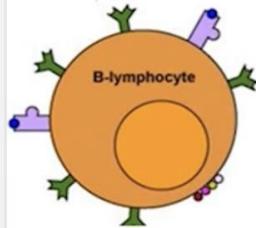


What is the difference between Ig's and Abs?

I- Definition of Antibody

Immunoglobulins

Antibodies



OR

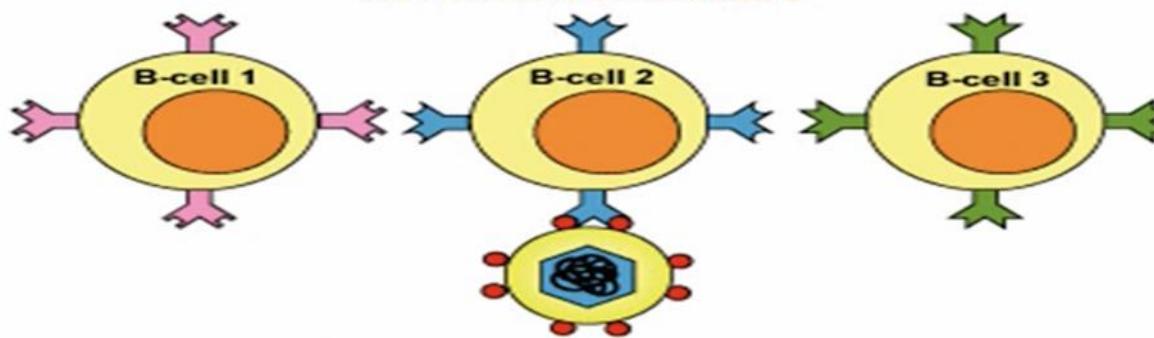


Steps of Abs production

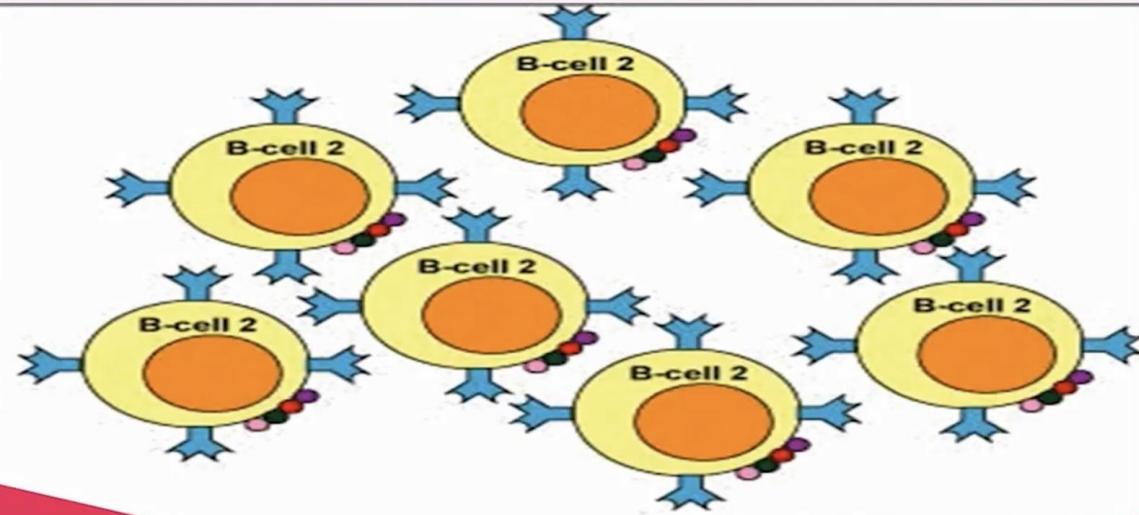
We must understand some general concepts on B cells

Clonal selection theory

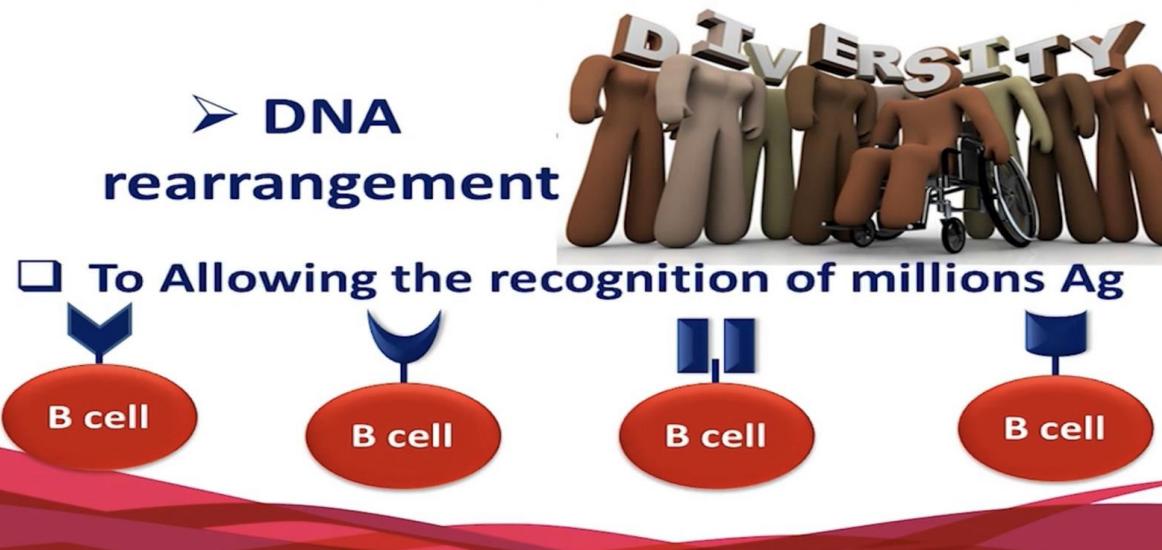
ACTIVATION OF B-CELL 2



Clonal selection theory



Antibodies production



□ B cell development

➤ Heavy chain (VDJ)

V1 V2 V3 vn D1 D2 Dn J1 J2 Jn C
RAG1 & RAG2

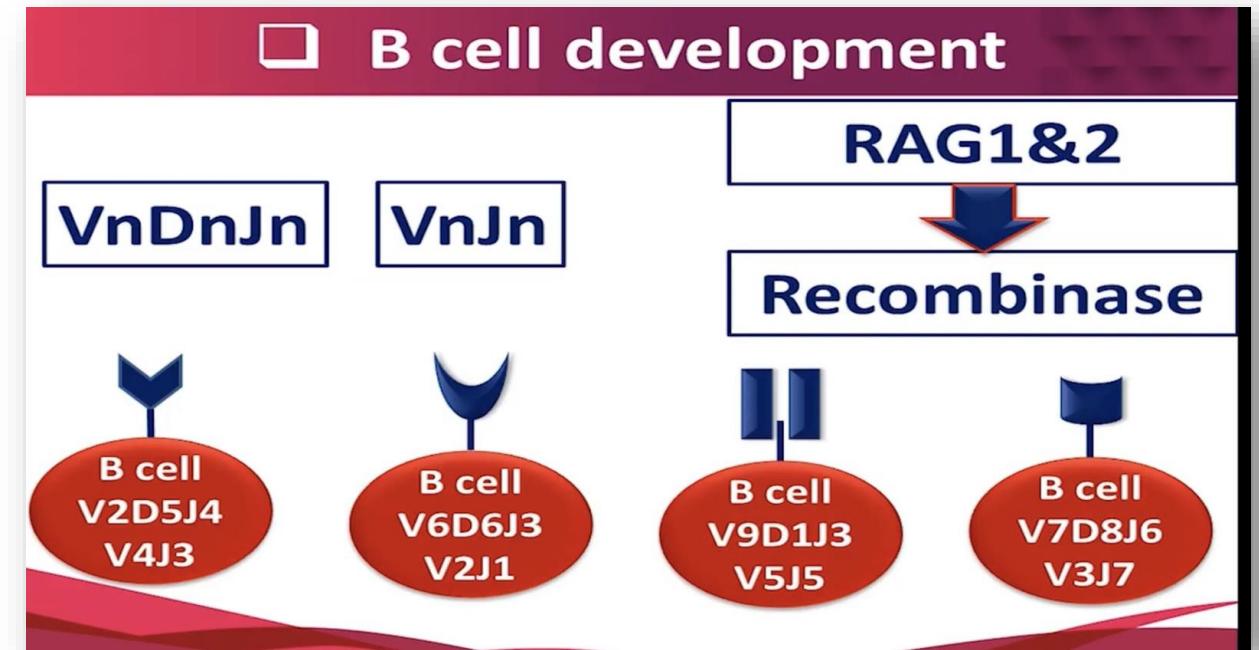
(Recombinant activating gene

Recombinase enzyme
➤ Light chain VJ

V1 V2 V3 vn J1 J2 Jn C



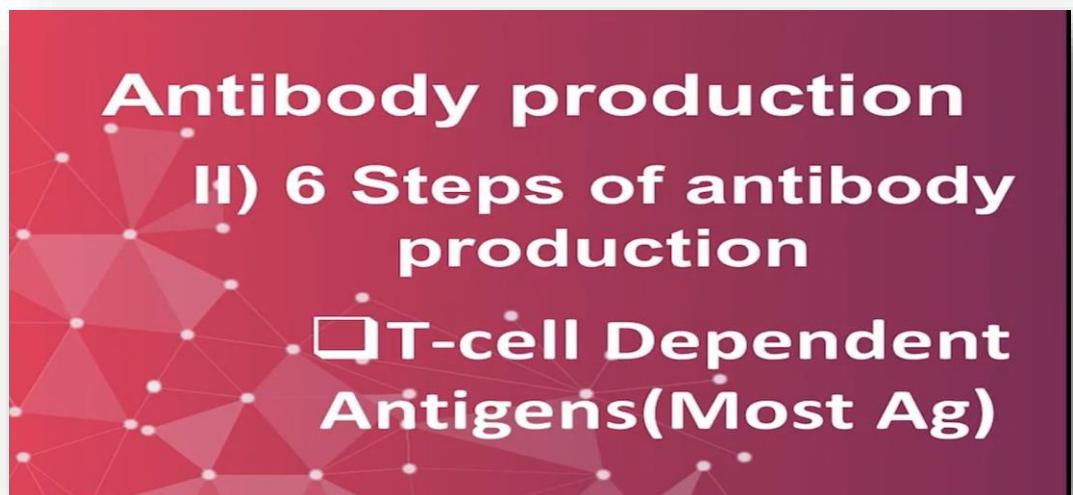
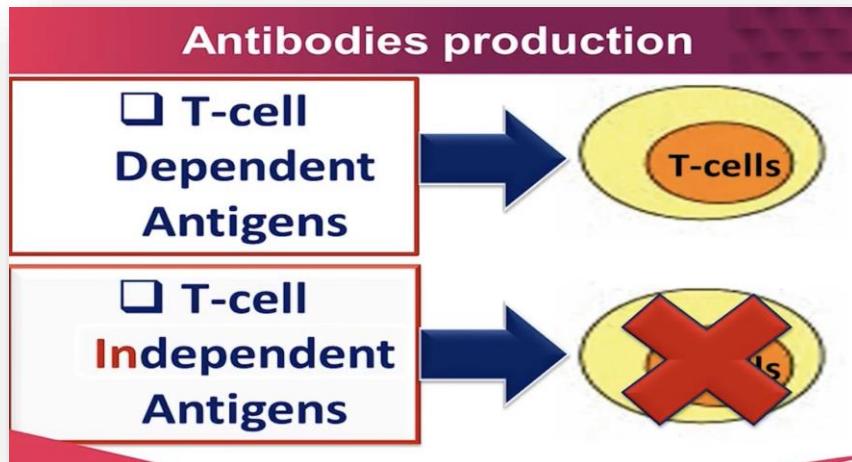
During B cell maturation in B.M, there are two genes called RAG1 and 2 which participate in DNA rearrangement in the genes responsible for making variable region of Ig (Both Heavy and light variable regions). By this mechanism millions of B cells will produce each with different BCR in order to be able to react with any possible antigens coming from invaders. The same mechanism happens with T cells to produce millions of different T cells with different TCRs.



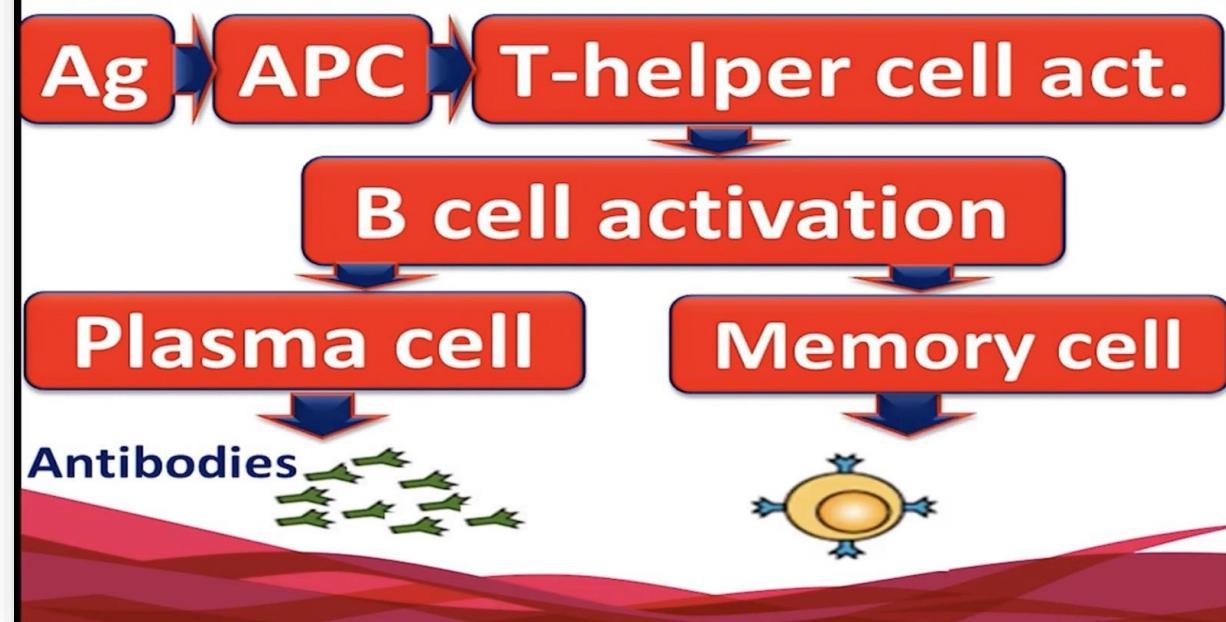
Imagine what will be happen if the **RAG 1 and 2** have been mutated

There will be no gene rearrangement for B and T cells and no diversity of B and T cells (lack of both T and B cells) which result **Severe Combined Immunodeficiency (SCID)**

Antibodies production

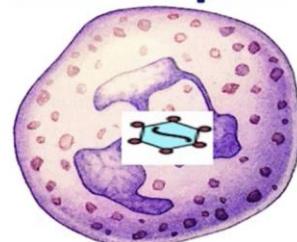


Antibodies production



1) Antigen entry

Neutrophil



Through out

Neutrophils engulf pathogen and breakdown then throw out into extracellular fluid

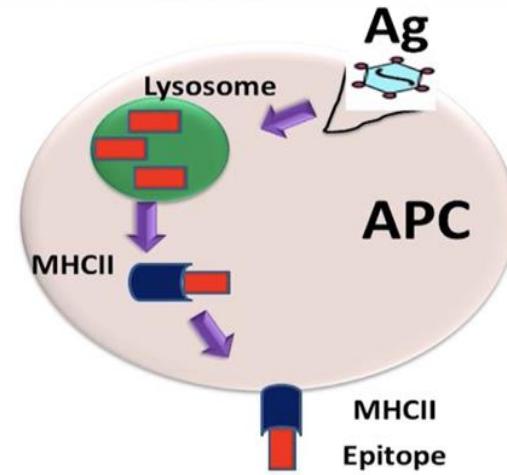
At the same time the same pathogen has been engulfed by MO and processed then presented by MHC class II

II) 6 steps of antibody production

- 1) Ag entry
- 2) Antigen presentation
- 3) Activation of T-helper cells
- 4) Activation of B cells
- 5) Production of antibodies
- 6) Memory cells

2) Antigen presentation

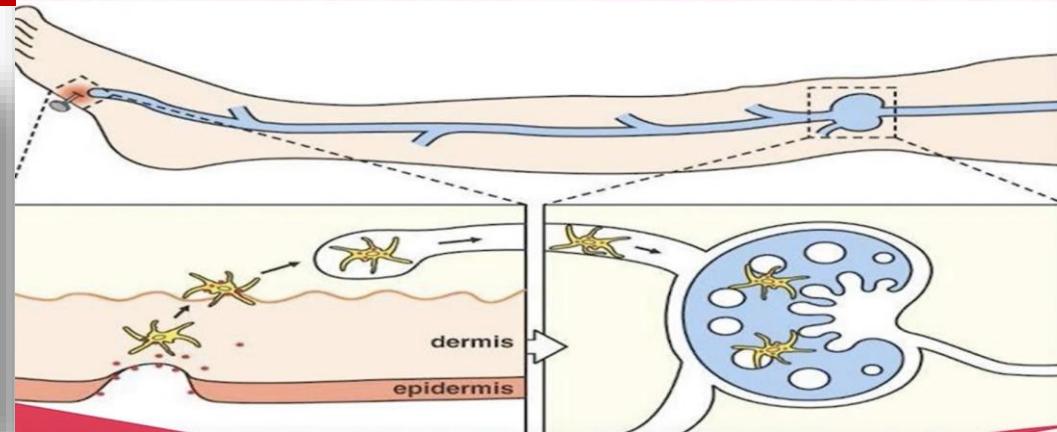
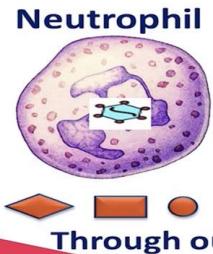
- MHC-II with epitope.
- IL-1



Both free and

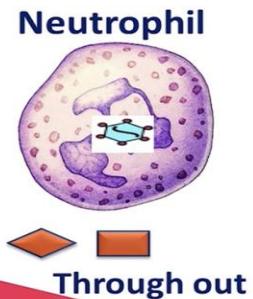
APC migrates into lymph nodes

1) Antigen entry

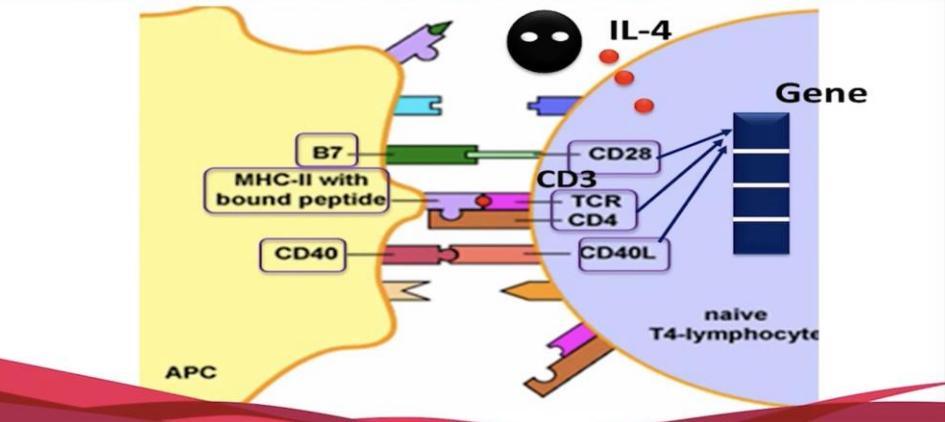


II) 6 Steps of antibody production

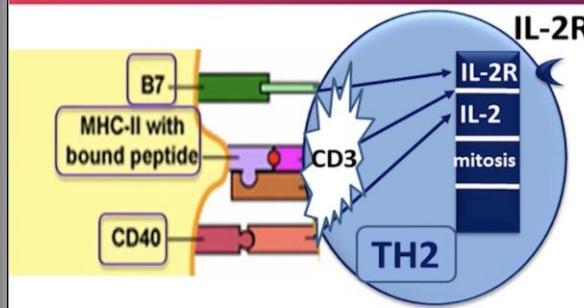
1) Antigen entry



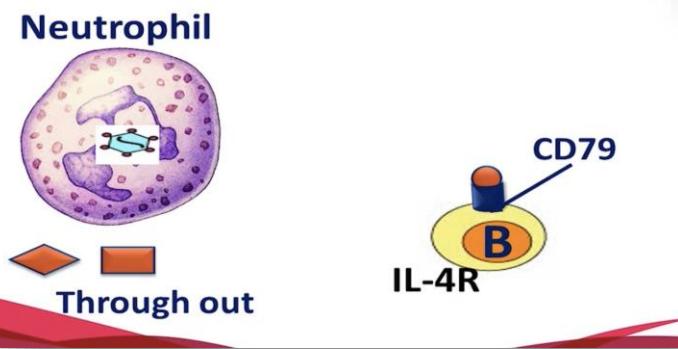
3) Activation of T helper cells



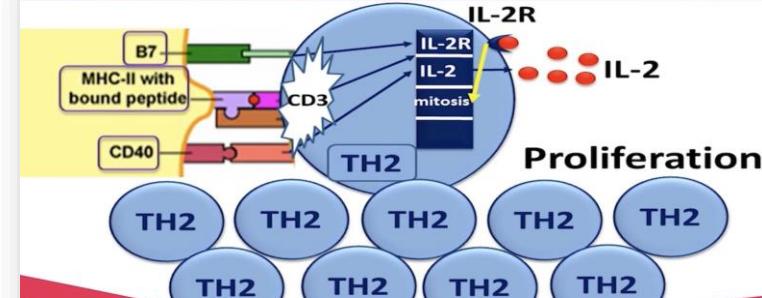
3) Activation of T helper cells



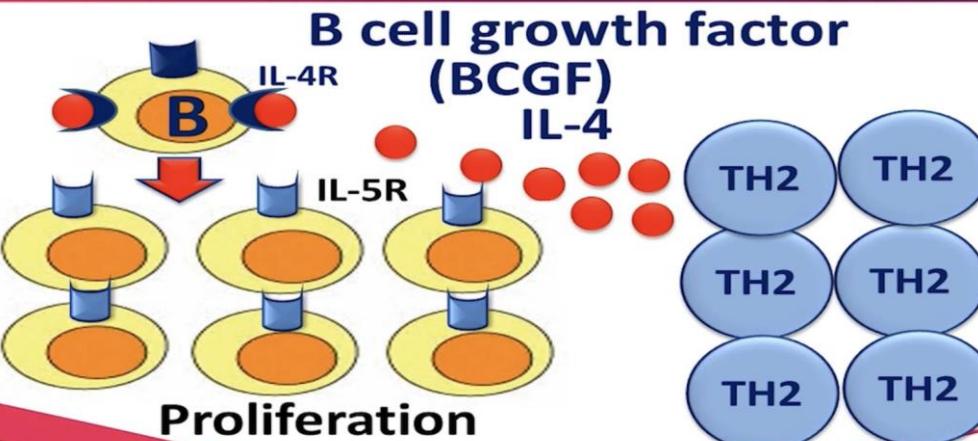
1) Antigen entry



3) Activation of T helper cells



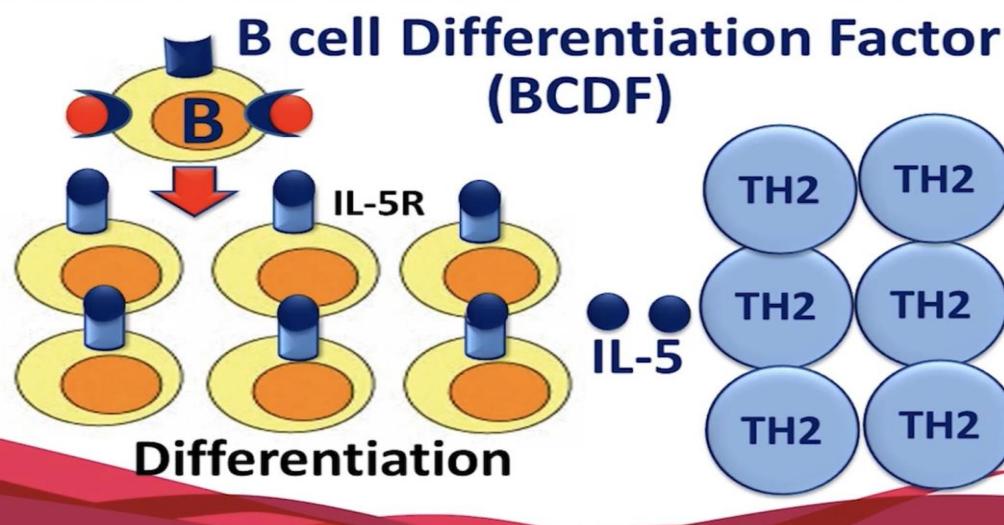
4) Activation of B cells



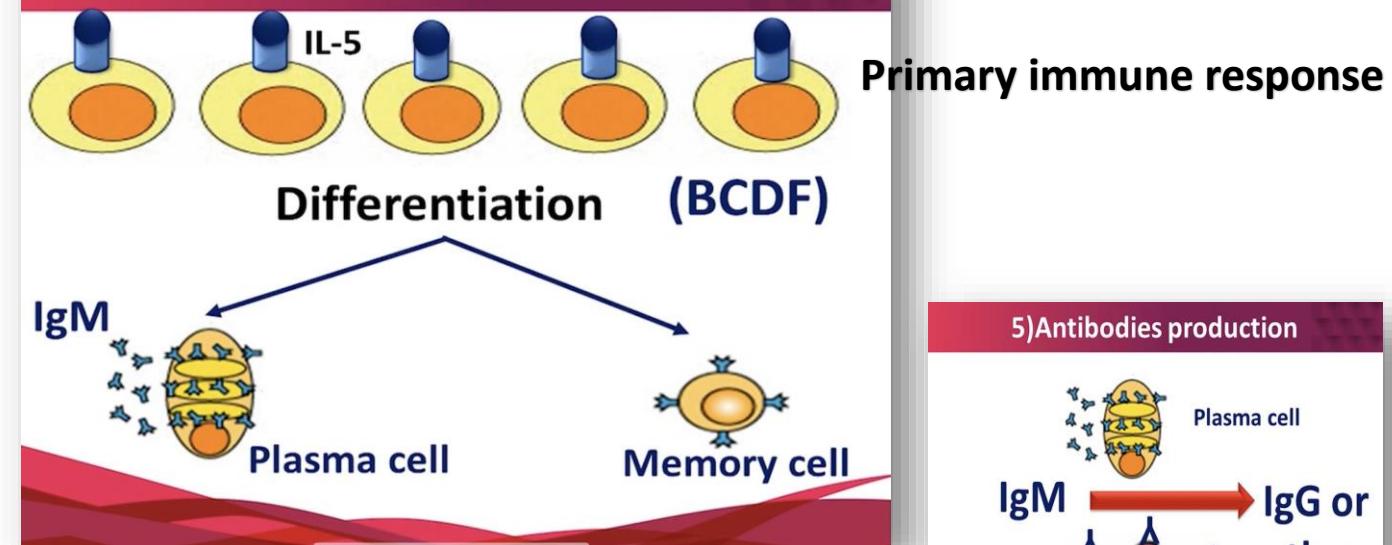
II) 6 Steps of antibody production

4) Activation of B cells

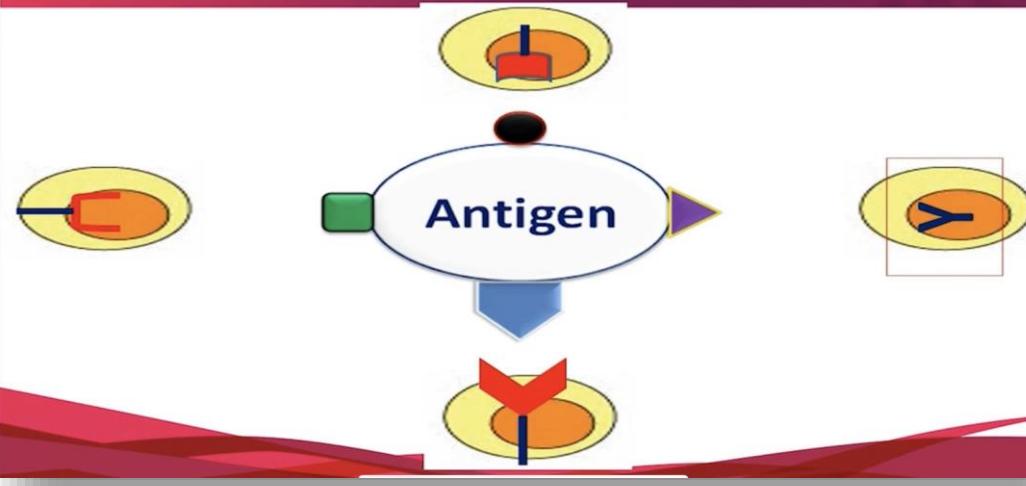
4) Activation of B cells



5) Antibodies production

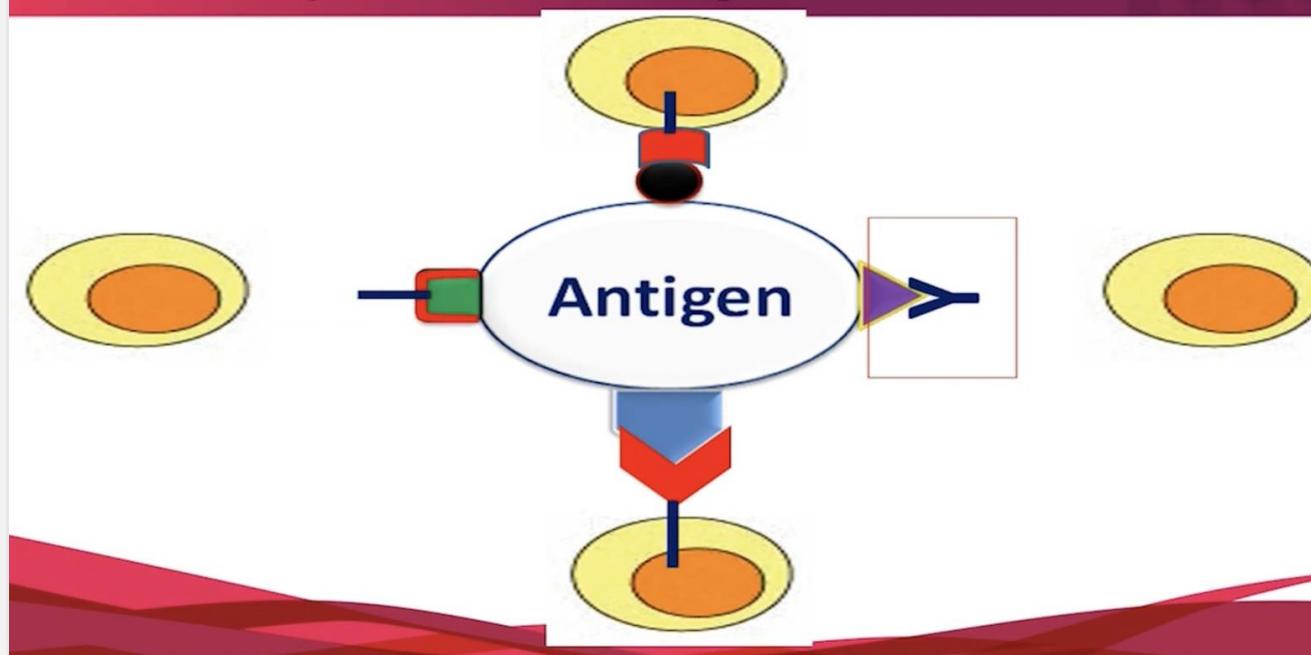


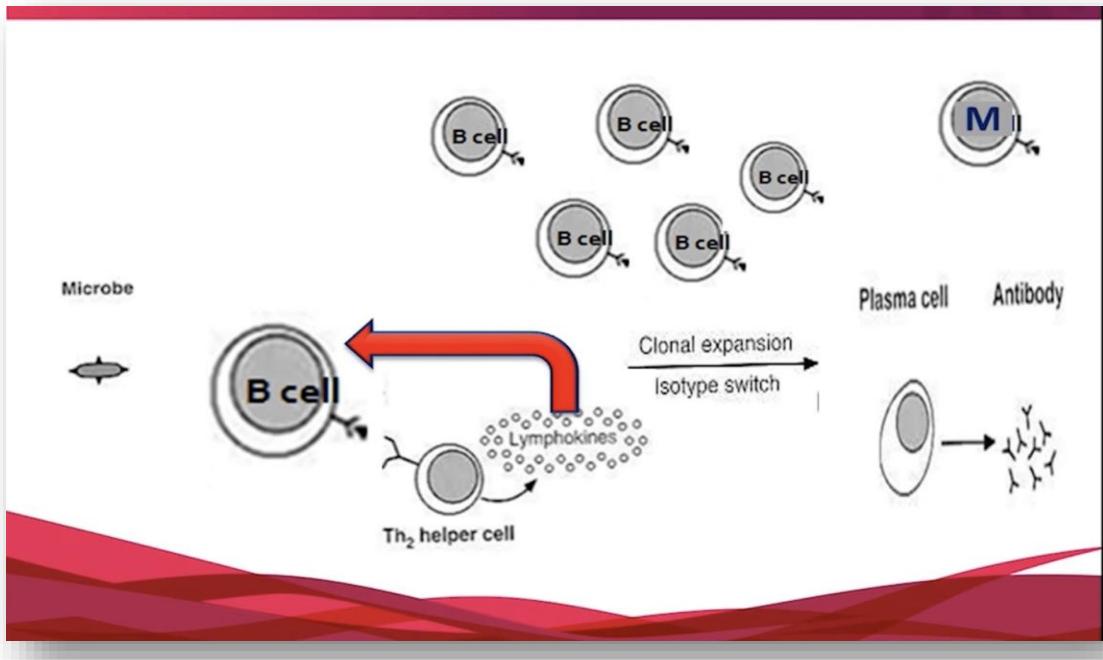
5)Antibodies production



This Antigen has 4 different epitopes and each one produce specific Abs (T dependent Ag) (polyclonal Abs) from different clones of B cells

5)Antibodies production





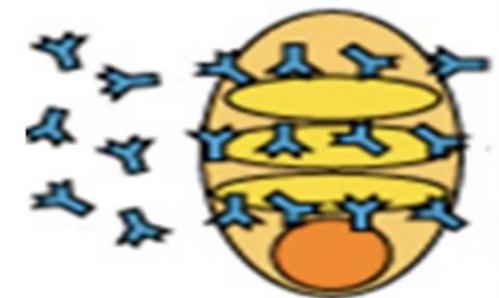
In this diagram, B cell acts as APC

Do you know that B cells comprise 10% only of lymphocytes so do you think this small percentage is enough to do their function? But if you look at the diagram below you will find that each activated B cell will produce thousands of plasma cells.

Of course **B cells** comprise 10% of Lymphocytes BUT

Plasma cell

Thousands of antibodies



Antibodies production

- T cell Independent antigen
(Certain Ag)

T cell Independent antigen

T cell independent Ag

Antibodies are produced in the absence
of T cells

(No T cells)


(Polysaccharides Ag)

T cell Independent antigen

1st) Ag-BCR

2nd) Ag-BCR
(Cross-linked)

Polysaccharides Ag

BCR

No T cell



No cytokines

IgM

Antibody production by T cell independent antigen (certain Ags)

Multivalent antigens have repeated units (multiple similar epitopes) like capsule of bacteria can cross link B cell receptors and activate B cells without help of T helper cells. These activated B cells directly converted into plasma cells which produce only IgM antibodies without class switching and memory cells. Such antigens are not sufficient for producing life long immunity, therefore can be converted into T dependent antigen like capsule of H.influenzae bind with a carrier protein so called **conjugate vaccine**.

		TD	TID
1	Type of Ag	Most (protein)	Few (Polysaccharide)
2	1ry signal	MHC-II-TCR.CD4	BCR-Ag
3	2ry signal	B7-CD28 CD40-CD40L	BCR-Ag (cross linking)
4	Onset of action	Slow	Rapid
5	First Ab	IgM	IgM
6	Class switching	Found	Absent
7	Memory cells	Found	Absent
8	Role of T helper cells	Found	Absent

Structure of antibodies

Ab consists of 2 heavy chains [4 domains(each domain has 110 a.a) and two light chains (2 domains) (either kappa or lambda) that are linked by a disulfide bond and give Y shape with 2 (Fabs) and 1 (Fc). Ag binds to the fab while Fc act as receptors for complement (CH2),NK cells (CH3= ADCC) and opsonization (CH3).

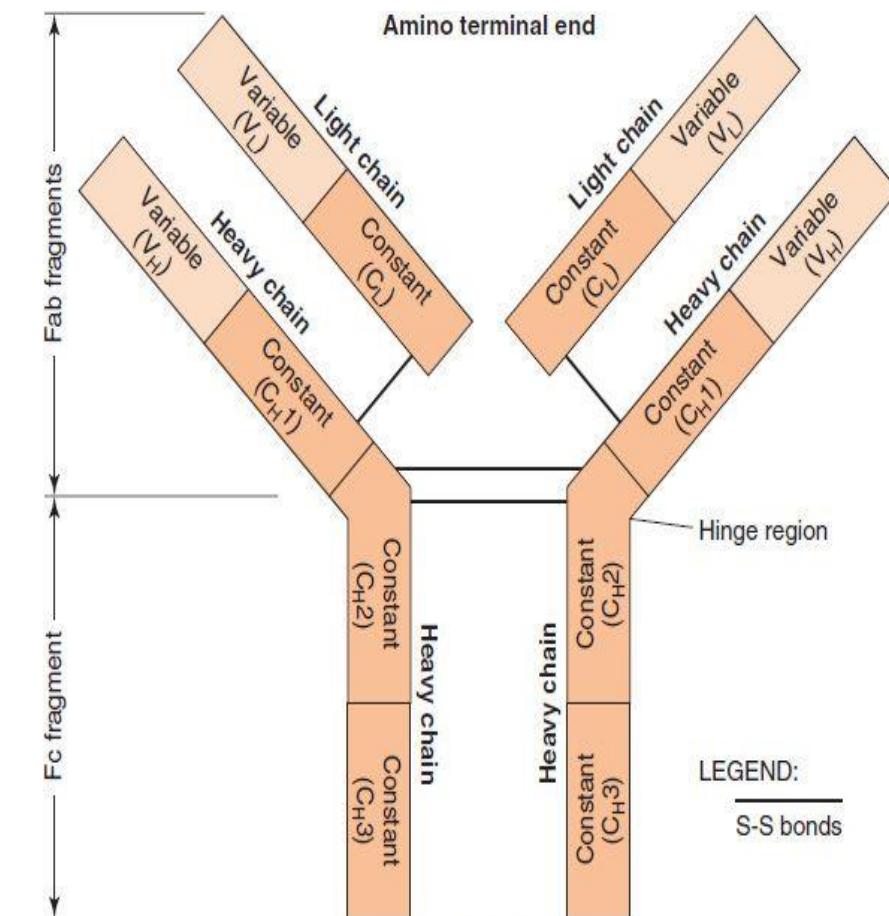
There are five different classes or Isotypes of Ig's according to the differences in their heavy chains named (MAJED) μ (IgM) , α (IgA), γ (IgG), ϵ (IgE) and δ (IgD).

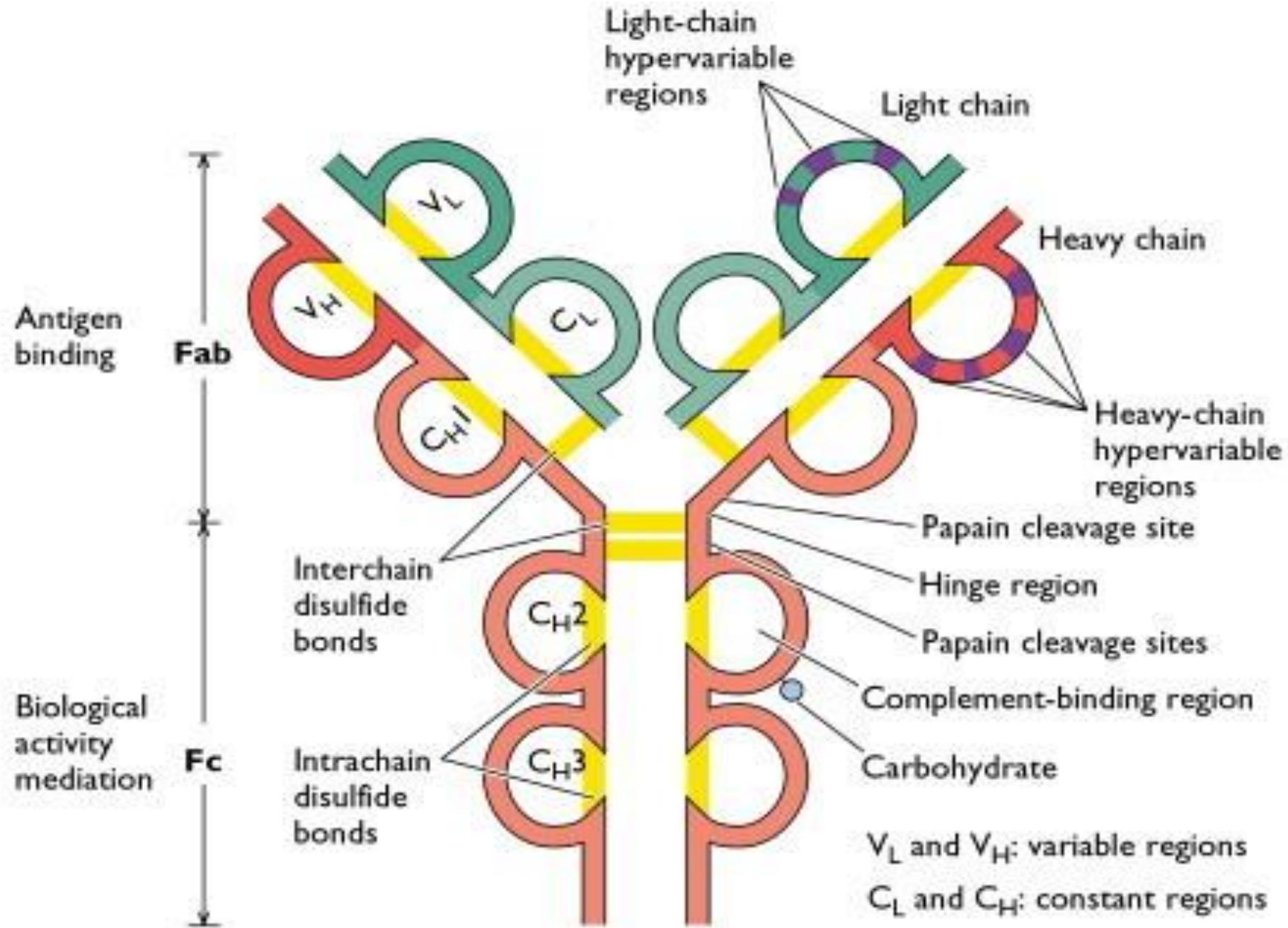
Fab region= VH +CH 1 and VL +CL1)

Antigen binding site is called paratope = VL + VH

Hypervariable regions or Complementary determining regions (CDRs) are 3 also known as idiotypes can produce anti idiotype antibodies

Type of heavy chain in each Ig class	
Immunoglobulin class	Heavy chain type
IgG	γ (gamma)
IgA	α (alpha)
IgM	μ (mu)
IgD	δ (delta)
IgE	ϵ (epsilon)





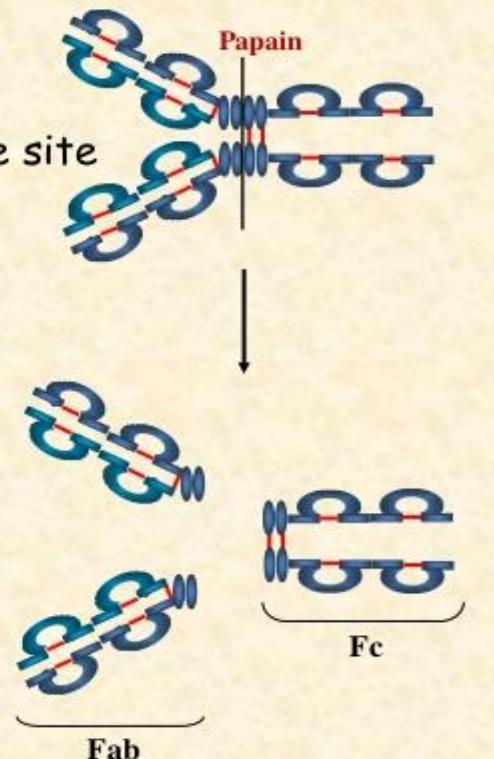
Enzymatic Digestion Of Antibodies

- Digestion With Papain Yields
 - 3 Fragments
 - 2 identical Fab and 1 Fc
 - Fab Because Fragment That is Antigen Binding
 - Fc Because Found To Crystallize In Cold Storage
- Pepsin Digestion
 - $F(ab')^2$
 - No Fc Recovery, Digested Entirely
- Mercaptoethanol Reduction (Eliminates Disulfide Bonds) inactivating IgM.

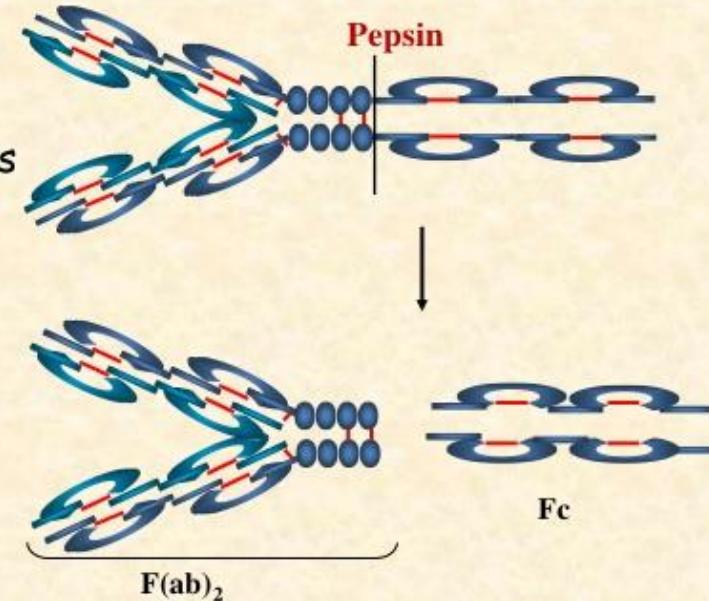
➤ Proteolytic cleavage of Ig :

➤ Papain enzyme split the Ig at the site Between CH1 and CH2 region (hinge region) into two fragments.

- Fab (fraction antibody)
- Ag binding
 - Specificity determined by V_H and V_L
- Fc (fraction crystallisable)
- complement binding



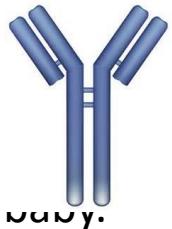
Another proteolytic enzyme, pepsin cleaves Ig at another site to yield $F(ab)_2$



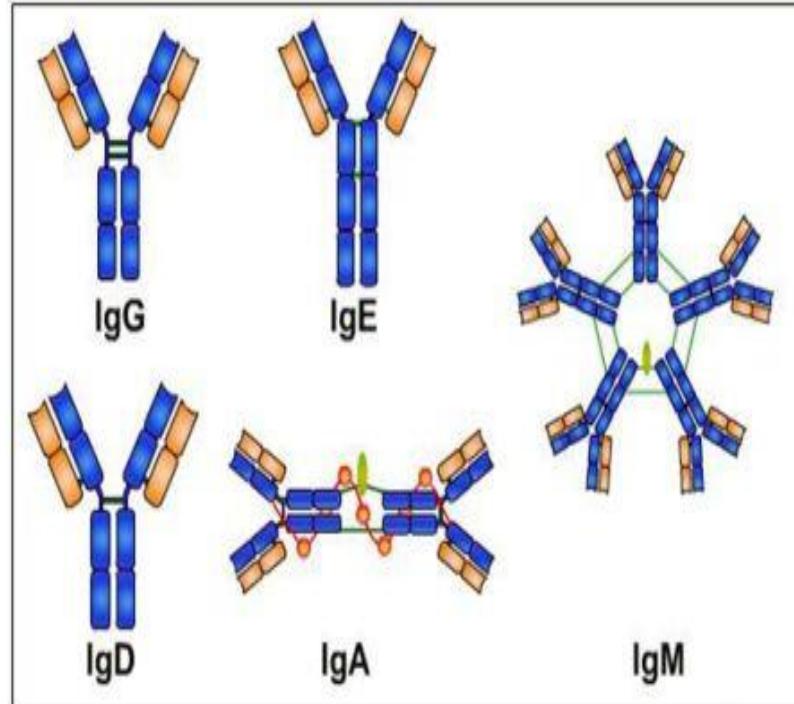
2-ME digest IgM which used for diagnosis of chronic Brucellosis (2-ME test)

IgG

Types of Ig's

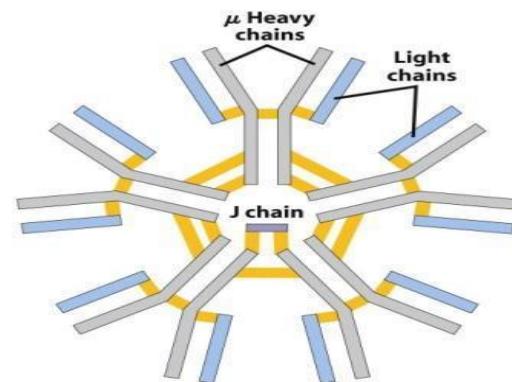


- 1- Monomer
- 2-Major Ig in the human serum (75%)
- 3- Across placenta and responsible for maternal immunity to the baby.
- 4- 4 subclasses, IgG1,2,3 and 4.
- 5- Responsible for secondary immune responses
- 6- Can persist in the body for years
- 7- Can be used as serological markers for past or chronic infections.
- 8- Neutralize toxins
- 9- It is a major opsonizing Ig in phagocytosis.
- 10- Activates complement except IgG4



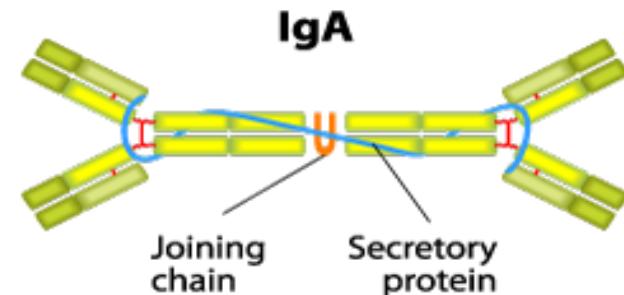
IgM

- 1- Its pentameric (the largest Ig with M.W of 900000 Daltons) consists of 5 basic units held by J-chain or monomeric as BCR.
- 2- It constitutes 10% of normal serum proteins.
- 3- It is the most efficient complement fixing Abs
- 4- Important in opsonization
- 5- Either free or bound with IgD on the surface of B cells as B cell receptors (BCR)
- 6- Cannot pass across placenta and if baby positive for this Ig it means infection.
- 7- Responsible for primary immune responses
- 8- Used as serological markers for recent or acute infection
- 9- Can persist for few months only.
- 10- Unable to do ADCC, opsonization and neutralization



IgA

- 1- Its dimeric
- 2- It constitutes 10-15% of normal serum proteins.
- 3- There are two subclasses, IgA1 (93%) and IgA2 (7%).
- 4- It is responsible for mucosal immunity with antiviral and bacterial activity.
- 5- Two forms, monomeric in serum and dimeric in body secretions.
- 6- Rich in breast milk giving maternal immunity to the breast feeding babies.



IgD

- 1- Its monomeric.
- 2- Found on the surface of B cells and acts as receptors for antigens.
- 3- Free form in blood with unknown function.

IgE

- 1- Its monomeric.
- 2- Responsible for allergic reaction and immunity to parasites

Roles of Antibodies

In Vivo

Inactivate their specific Ags (protection) or autodestructing in autoimmune diseases or not protective

In vitro

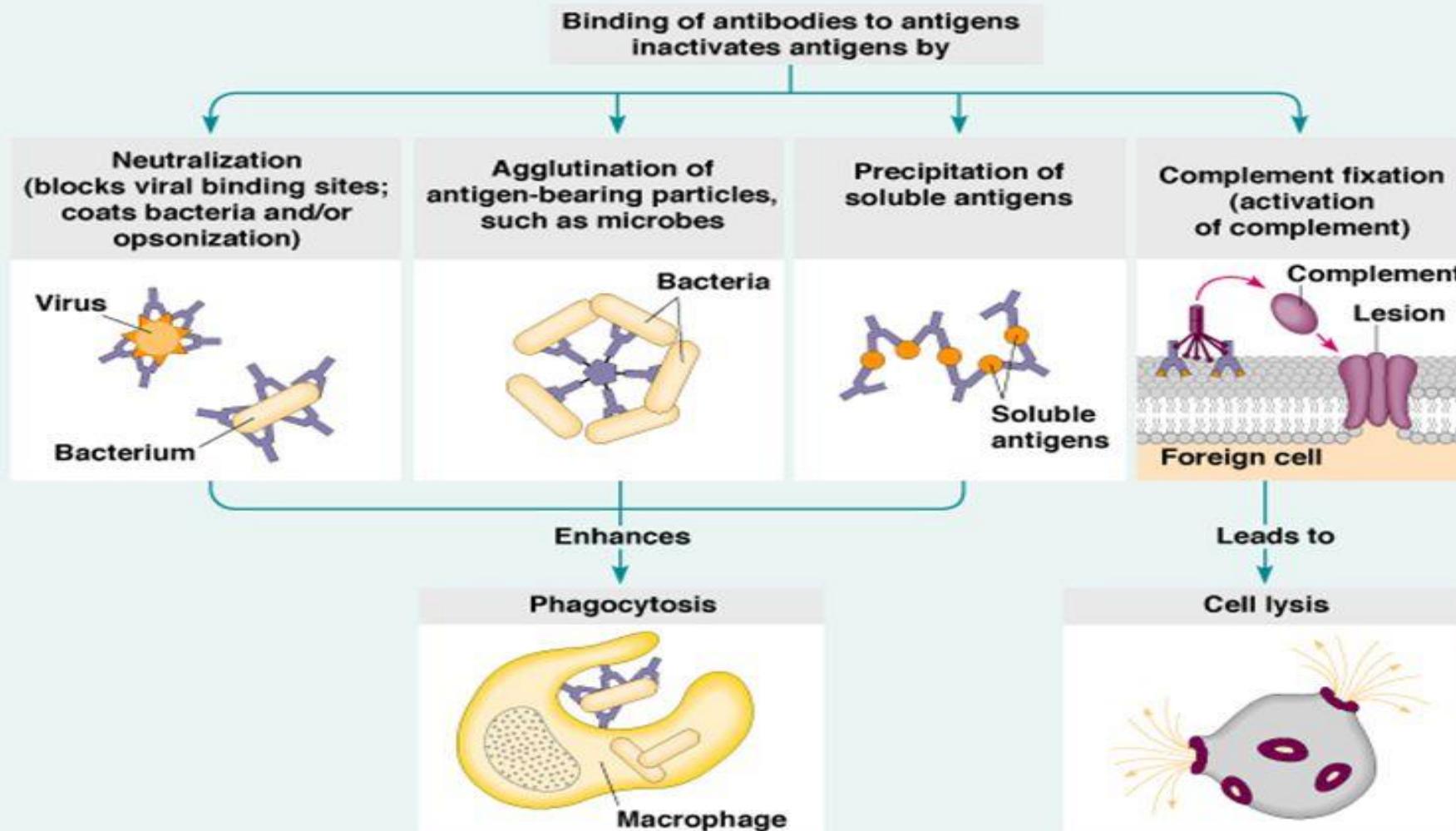
Used as serological markers for diagnosis of various infections

Functions of antibodies

The main functions of antibodies is to facilitate or contribute to the destruction of extracellular pathogens by different mechanism

- 1- Agglutination (IgM and IgG) of particulate Ag like bacteria or precipitate soluble Ag –Enhance phagocytosis
- 2- Neutralization of (Toxins, Viruses, Bacteria) (SIgA and IgG)-Enhance phagocytosis
- 3- Opsonization (IgG and IgM)
- 4- Complement fixation (IgM and IgG)
- 5- Natural passive immunity (Transplacental by IgG and breast milk (SIgA)
- 6- Killing by antibody dependent cell mediated cytotoxicity (ADCC) mainly by NK cells (IgG) also in case parasites by IgE which bring eosinophils (eosinophilia). The same mechanism which kill cancer cells upon administration of biological treatment using monoclonal antibodies like Herceptin in breast cancer.
- 7- Allergy or hypersensitivity type 1 in which IgE binds to the mast cells and after binding of allergens like pollen to the IgE and cause crosslinking that degranulate mast cells to release vasoactive amines such as histamine

Consequences of Antibody Binding



Ag-Ab interactions

- Nature of this reaction
- Measurement of this reaction

- Nature of this reaction

A- Like key and lock concept

B- Non covalent bond

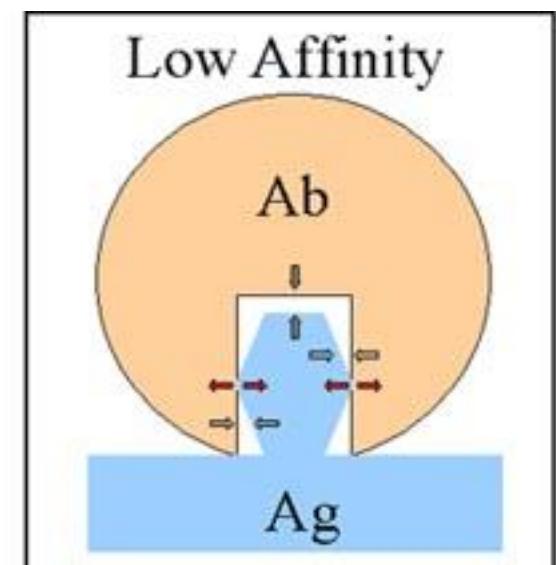
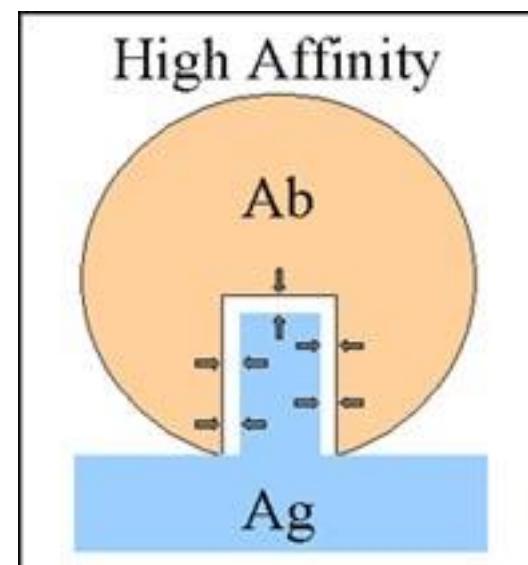
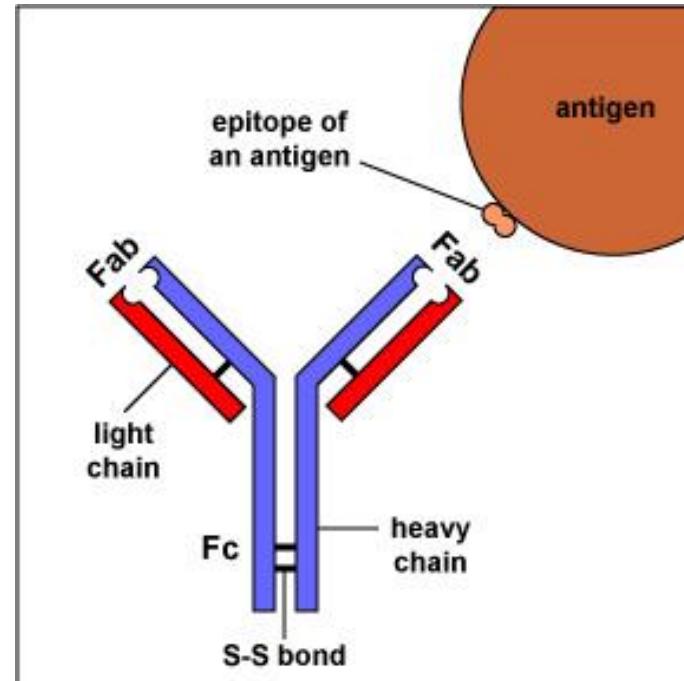
C- Irreversible reaction

- Measurement of this reaction

This reaction can be measured by affinity and avidity

A- Affinity

It is a strength of the reaction (attractive and repulsive forces) between a single antigenic determinant and a single combining site of the Ab.



B-Avidity

It is a measure of the all strength of binding of an Ag with many antigenic determinants and multivalent Ab.

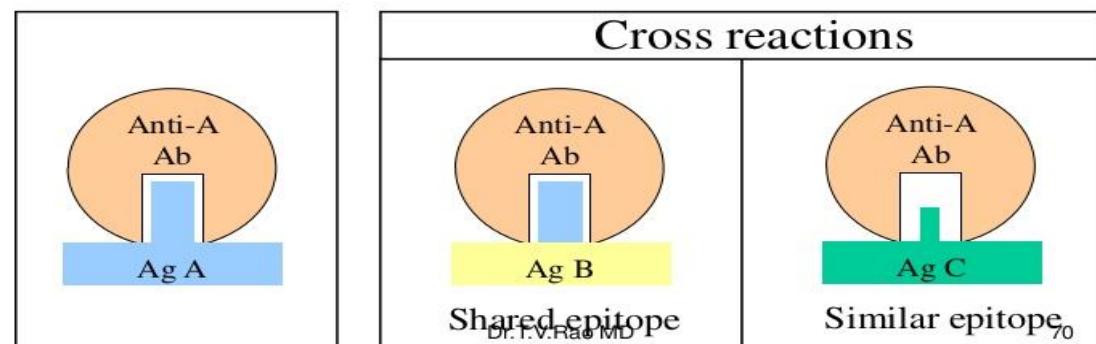
C-Specificity

It is the ability of Ab to combine with only one Ag

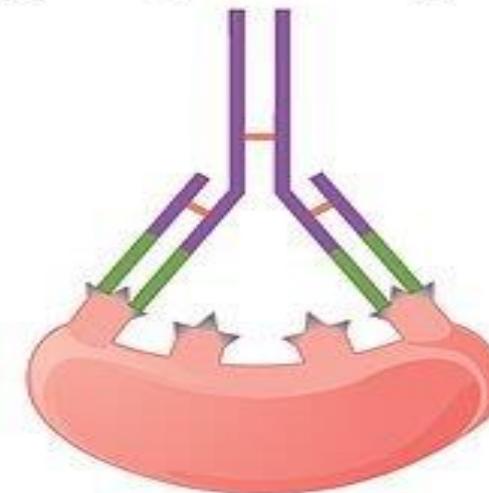
D-Cross reactivity ability of one antibody to bind with more than one Ag

Cross Reactivity

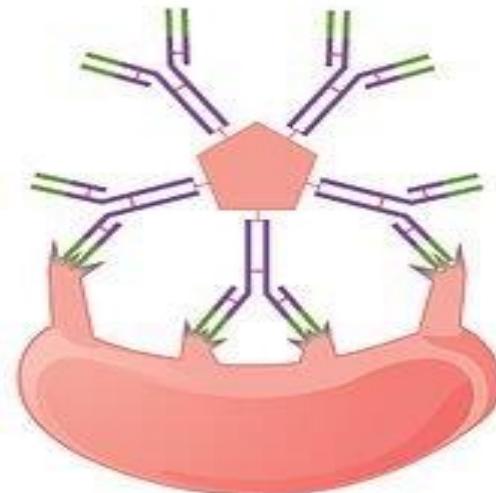
- The ability of an individual Ab combining site to react with more than one antigenic determinant.
- The ability of a population of Ab molecules to react with more than one Ag



(a) Affinity versus avidity



Affinity refers to the strength of a single antibody–antigen interaction. Each IgG antigen binding site typically has high affinity for its target.



Avidity refers to the strength of all interactions combined. IgM typically has low affinity antigen binding sites, but there are ten of them, so avidity is high.

Antibodies consists of

2 light chains and 1 heavy chain

1 light chain 2 heavy chains

1 light chain and 1 heavy chain

2 light chain and 2 heavy chain

The function of IL-2 in Aby production is:

Activation of B cell

Activation of APC

Converted the B cell into Plasma cell

Auto-activation of T-helper cell

The hypervariable region resides in the

Between 2 light chains

Between constant light & heavy chains

Between variable light & heavy chains

Between constant light chains

Antibodies are

Proteins

Glycoproteins

Carbohydrates

Nucleic acid

The sequences of production antibodies (TD) are

Macrophage-T-helper cell- B cell- Plasma cell - Antibodies

T-helper cell- B cell- Plasma cell - Antibodies

B cell- T-helper cell- Plasma cell - Antibodies

T-helper cell- Plasma cell- B cell - Antibodies

The sequences of production antibodies (TI) are

Polysaccharides Ag- T-helper cell- B cell –
Plasma cell- Antibodies

Polysaccharides Ag- T-helper cell- B cell –
Plasma cell- Antibodies

Polysaccharides Ag-B cell – Plasma cell-
Antibodies

Why antibody against ABO is only IgM?

IgM is the only antibody made to ABO blood group antigens on human erythrocytes due to the ABO are carbohydrate (Thymus independent)

1) IgG pass through Fc receptors in the placenta

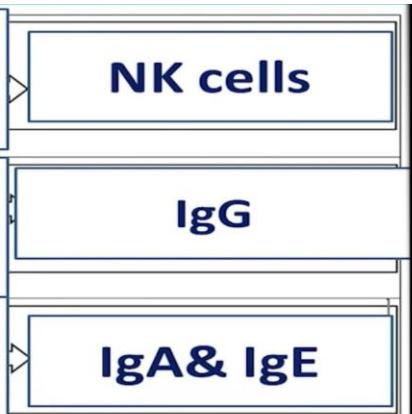
2) Passive protection to the newborn during few months of life

3) IgG have many activities (Neutralization, Opsonization, Complement activation & ADCC)

Scan the cell, if there is no MHC class I expression

Which antibody able to do ADCC, Opsonization, complement activation

Immunoglobulins cannot activation complement



IgM can not cross placenta, therefore, its presence in the newborn blood indicates intrauterine infection e.g. Syphilis, rubella, toxoplasma & cytomegalovirus

Property	IgG	IgM
Cross placenta	Yes	No
Opsonization	Yes	No
ADCC	Yes	No
Antigen receptor on B cell	No	Yes

Property	IgG	IgM
% in serum	75%	9%
M.W	Low	High
Structure	Monomer	Pentamer
H chain	Y	μ
J chain	No	Yes
Complement activation	+	++

Substance that can enhance the immunogenicity of antigen without altering their chemical nature

Adjuvants

T lymphocytes respond only when appropriate antigen presented in association with either self- MHC I or MHC II molecules

MHC restricted

Newborn suffered from fever after delivery, IgM for rubella appear.

Intrauterine infection

A molecule encoded by genes of the MHC that participates in antigen presentation to CD4 T-helper cells.

MHC-II

Antibody has receptor for Fc portion on mast cell and basophil

Substance that can enhance the immunogenicity of antigen without altering their chemical nature

T lymphocytes respond only when appropriate antigen presented in association with either self- MHC I or MHC II molecules

Newborn suffered from fever after delivery, IgM for rubella appear.

Enhancing of phagocytosis by coating the organism with antibody and or complement

Opsonization

After maturation of the cells in the primary lymphoid organs, its reside in

Secondary L.O

B cell growth factor (BCGF & BCDF)

IL-4 & IL-5

Immunoglobulin that does not cross placenta and its presence indicates recent infection

Macrophages, B cells and dendritic cells are take the organism, processing and present it in associated with MHCII

APC

When B cell activated, it differentiate into cell that produce antibodies and it called

Plasma cell

Cytokine that produce by T cell and lead to auto-activation and proliferation, it called

IL-2

Give reason

IgM can not do opsonization and ADCC by its Fc portion while it activates complement by its Fc portion



Scan the cell, if there is no MHC class I expression

NK cells

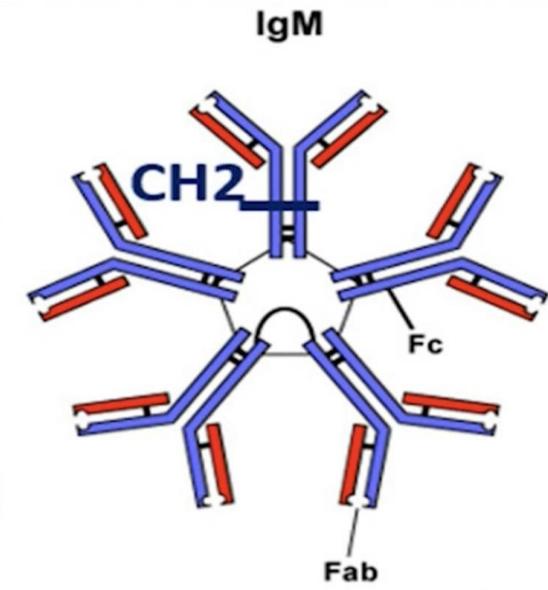
Which antibody able to do ADCC, Opsonization, complement activation

IgG

Immunoglobulins cannot activation complement

IgA & IgE

Opsonization occur through CH3 that joined with J chain & s-s bonds while Complement activation occur through CH2 that still free



II) Class switching

IgM

NO IgD

IgG

IgA

IgE

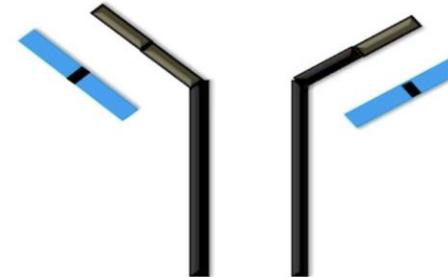
II) Class switching

➤ Change in the constant H chain

➤ No changes in:

- L chain
- V heavy chain

Why is change occur only in constant heavy chain?



II) Class switching

During the immune response, Plasma cells switch from producing IgM to produce IgG or IgA or IgE according to type of cytokines

H (VDJ)

S C μ | C δ

S C γ

S C ϵ

S C α

II) Class switching

H (VDJ)

S C μ | C δ

S C γ

S C ϵ

S C α

IgM/IgD

IgG

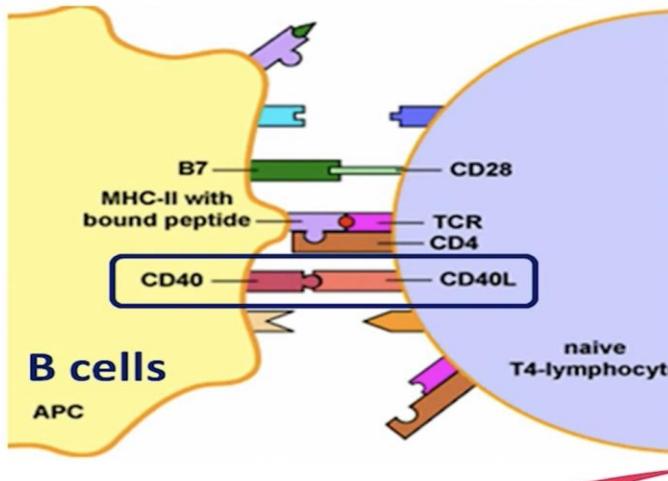
IgE

IgA

S = switch gene

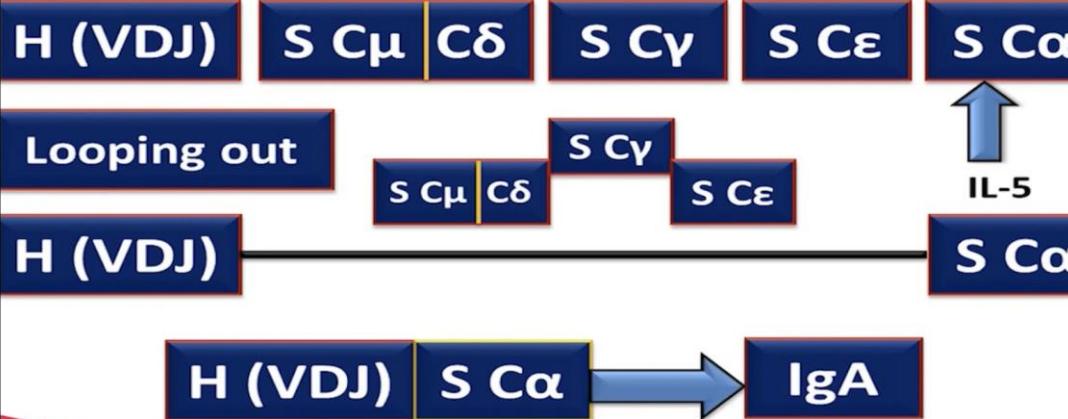
II) Class switching

It required for class switching
(CD40-CD40L)



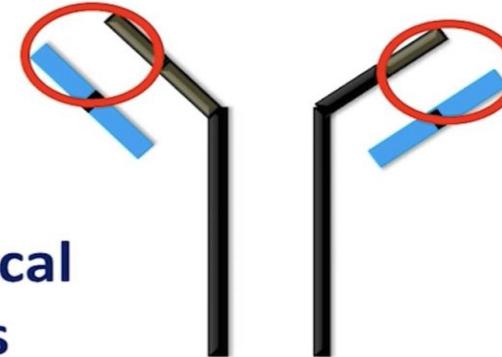
Cytokines responsible for class switching

II) Class switching



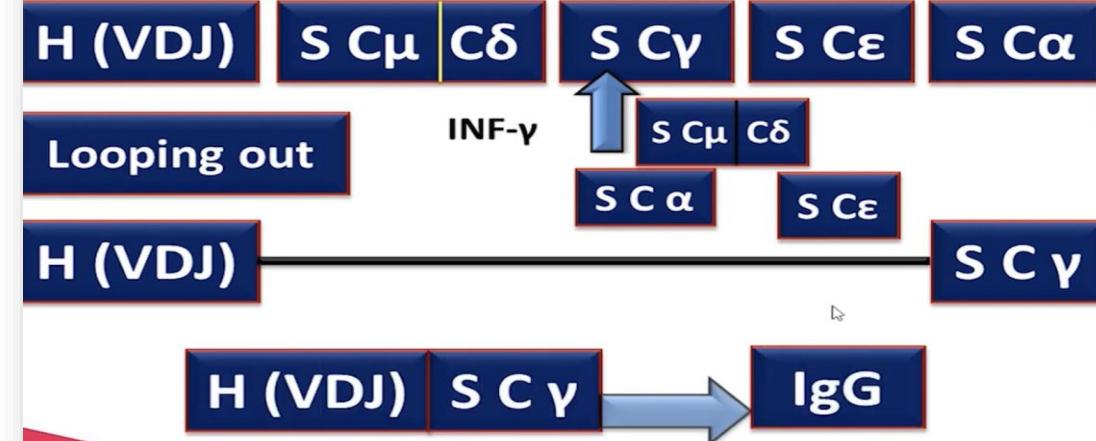
II) Class switching

No change in fab but change in Fc, IgM to IgG for the same antigen



- Result
 - Same specificity
 - Different biological characteristics

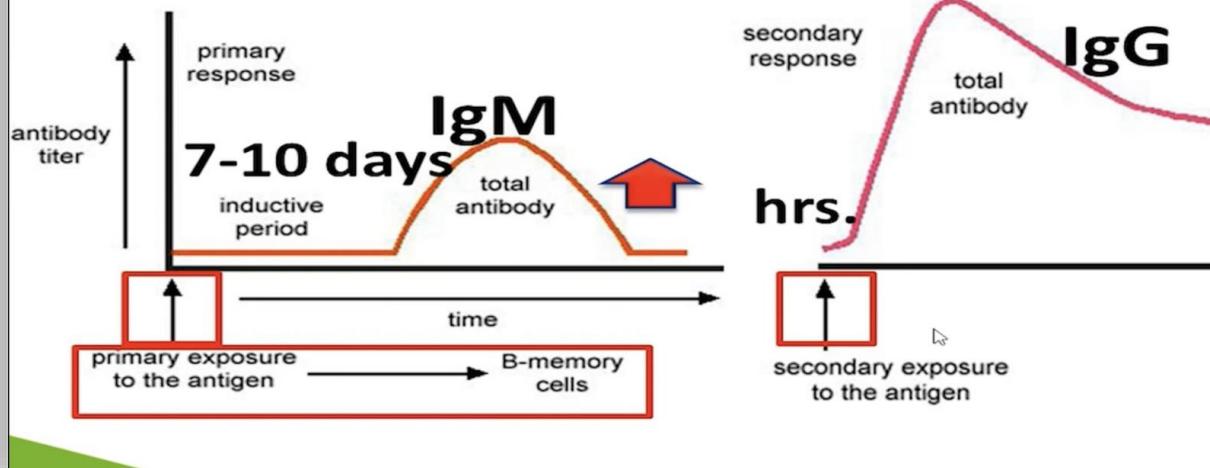
II) Class switching



Antibodies

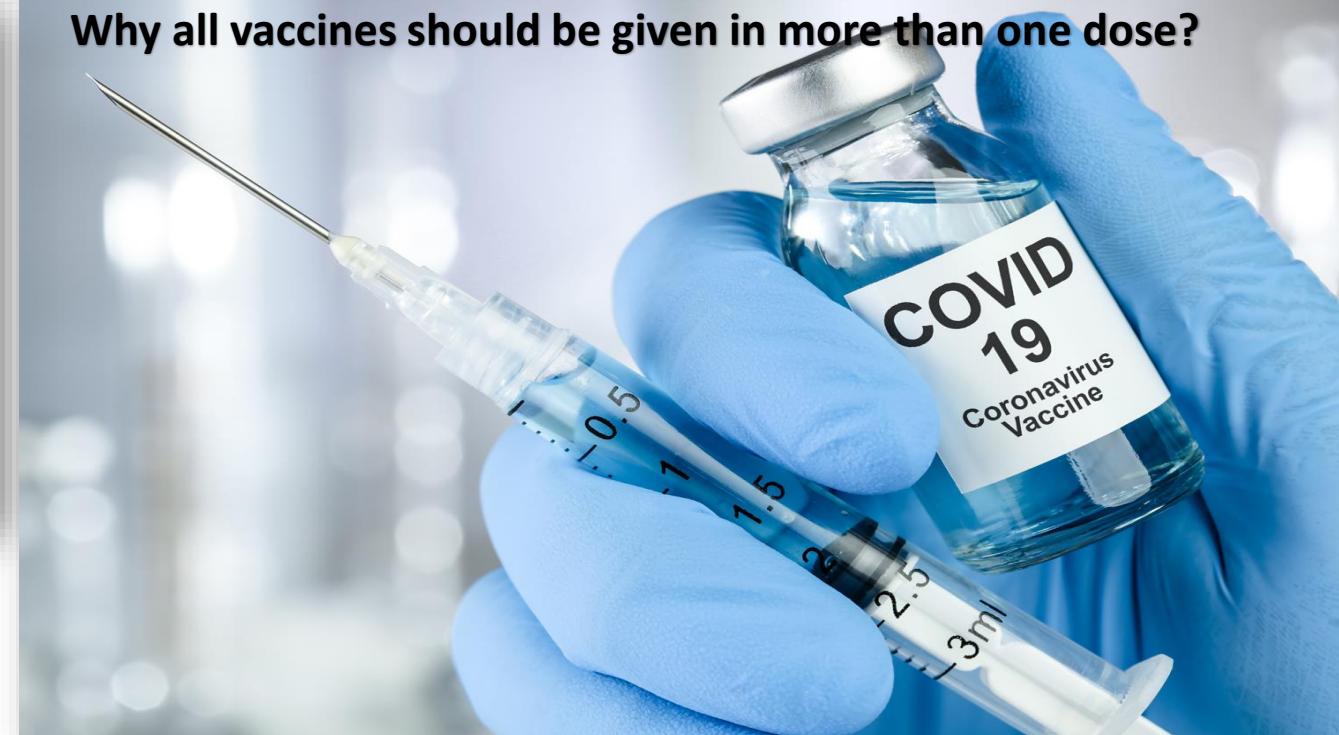
III) Primary & secondary antibodies

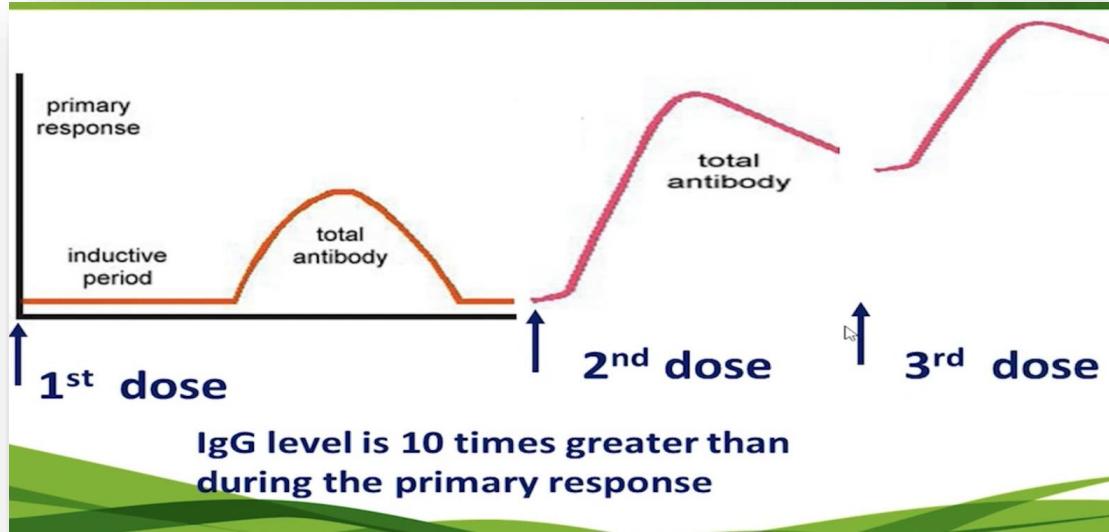
III) Primary & secondary antibodies



Items	Primary response	Secondary response
Occurs when	Ag is introduced for the first time	2 nd exposure to the same Ag
Induction period	Long (7-10 days)	Short (few hours)
Ig class	IgM	IgG
Antibody level	Low	high (10 times greater)
Items	Primary response	Secondary response
Memory cells	Absent	Present
Decline	Rapid	Slow

Why all vaccines should be given in more than one dose?



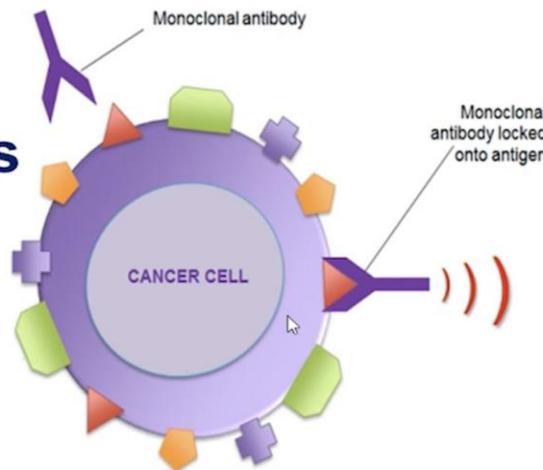


IV) Monoclonal antibodies

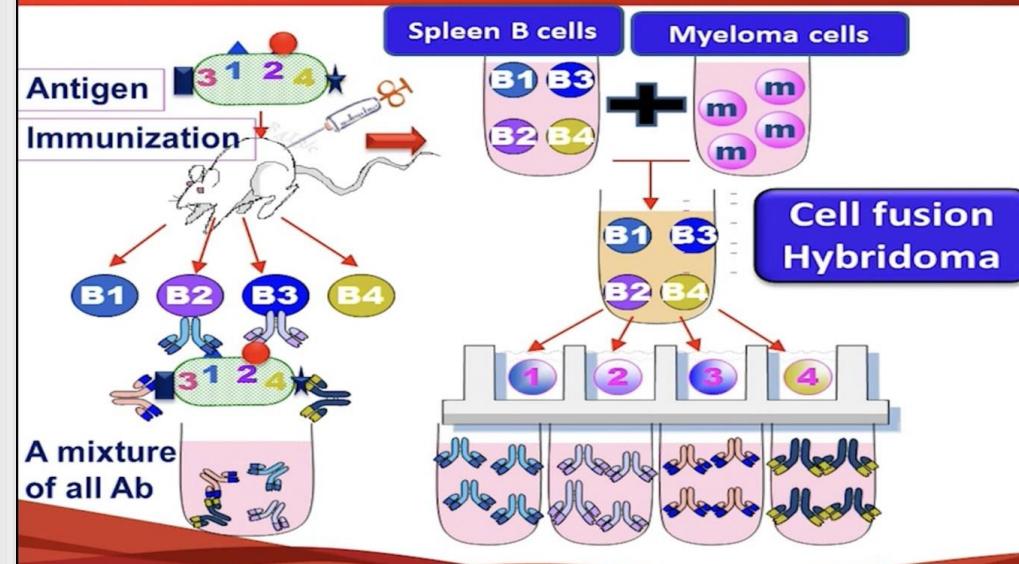
Monoclonal antibodies
(mab)

IV) Monoclonal antibodies

They are highly specific antibodies that produced against single epitope



IV) Monoclonal antibodies



IV) Uses of Monoclonal antibodies

A) Diagnostic



B) Therapeutic uses



A) Diagnostic uses

1) For identification antigens on cells & microorganisms



A) Diagnostic uses

CD4



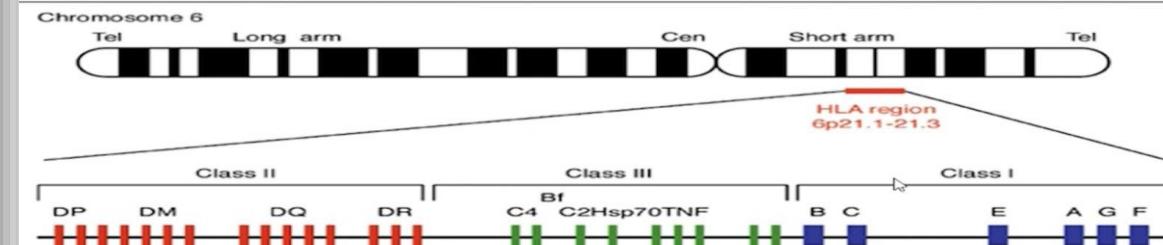
CD8



2) Determination of lymphocytes subset markers (CD)

A) Diagnostic uses

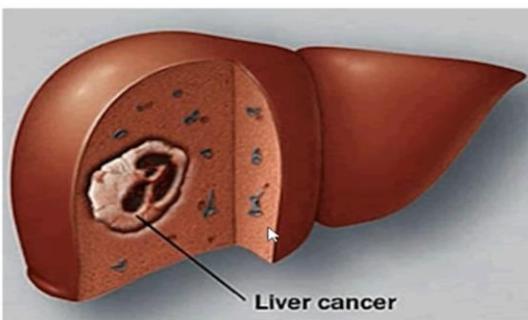
3) Detection of HLA antigen (tissue typing)



Gene map of the human leukocyte antigen (HLA) region
Reviews in Molecular Medicine ©2003 Cambridge University Press

A) Diagnostic uses

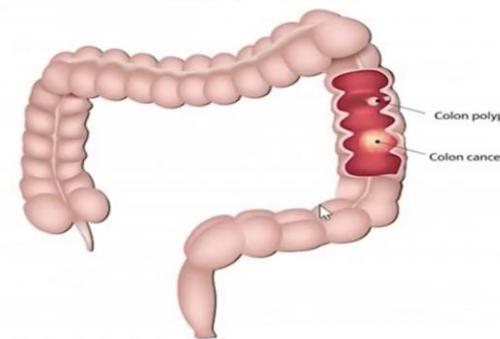
4) Detection of tumor antigen (Alpha feto-protein) AFP



A) Diagnostic uses

5) Detection of tumor antigen (Carcinogenic antigen) (CEA)

COLON CANCER AND POLYP



A) Diagnostic uses

6) Hormonal assay



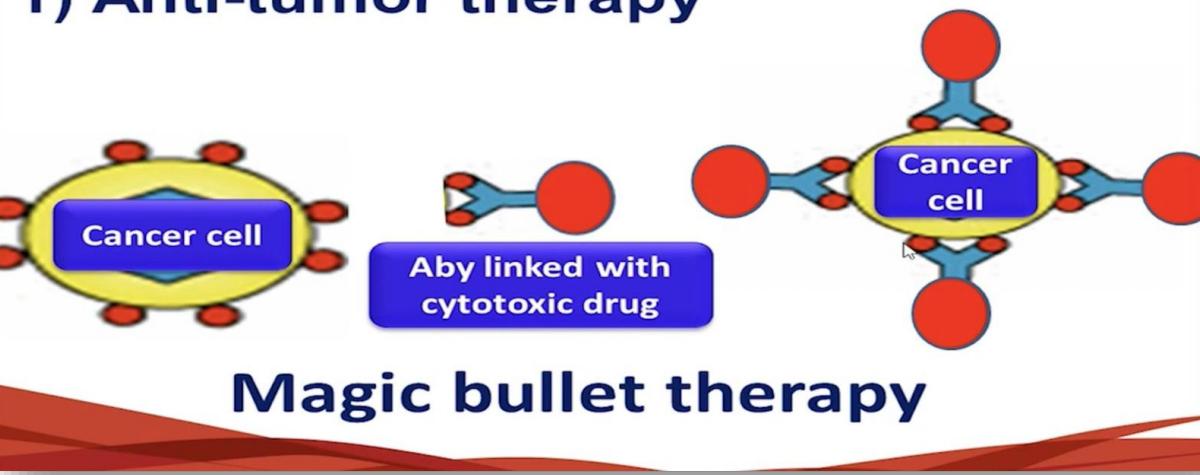
IV) Uses of Monoclonal antibodies

B) Therapeutic uses



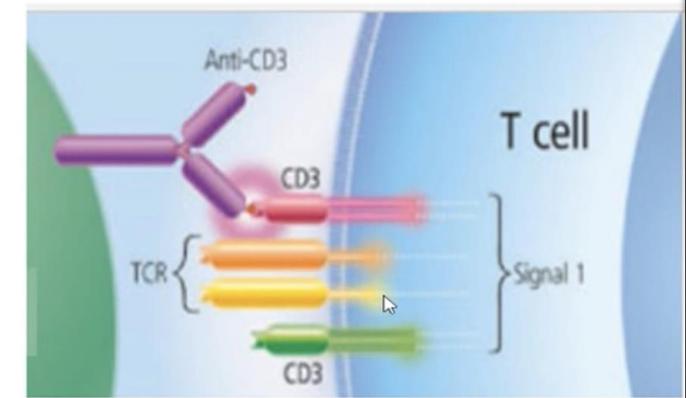
B) Therapeutic uses

1) Anti-tumor therapy



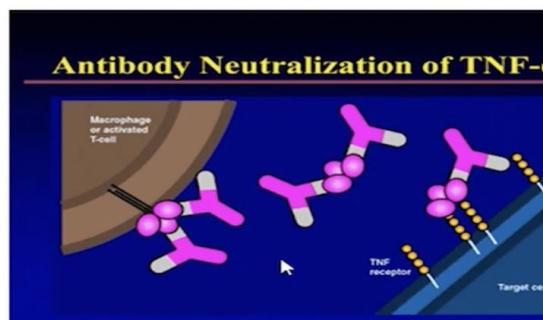
B) Therapeutic uses

2) Anti-CD3 to prevent graft rejection

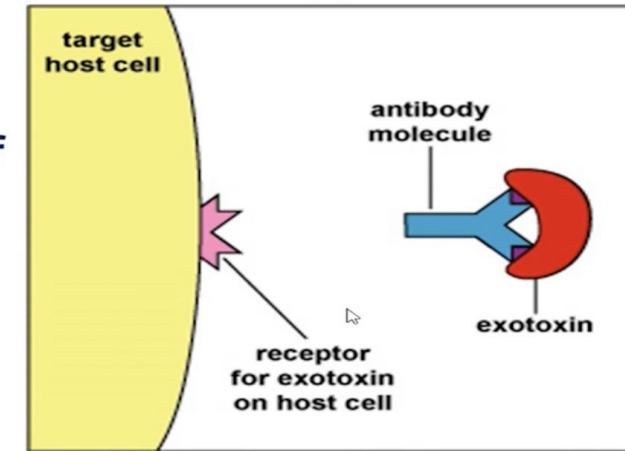


B) Therapeutic uses

3) Anti-TNF (a chimeric antibody) for treatment of rheumatoid arthritis



4) Neutralization of digitalis toxicity & anti-snake venom toxicity



B) Therapeutic uses

5) Anti-Rh (Anti-D) to prevent Rh-incompatibility



B) Therapeutic uses

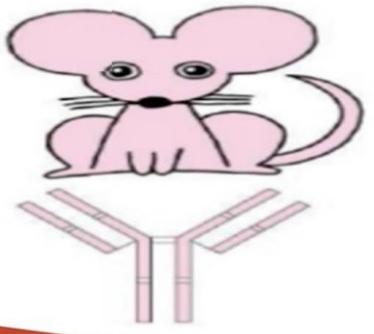
6) Passive immune therapy (VZV & CMV)



Types of Monoclonal antibodies

Types of monoclonal antibodies

Murine



Chimeric



Humanized

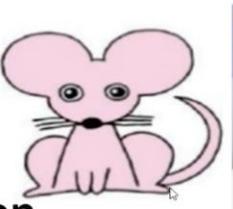


1) Murine

(Mouse)

suffix -omab

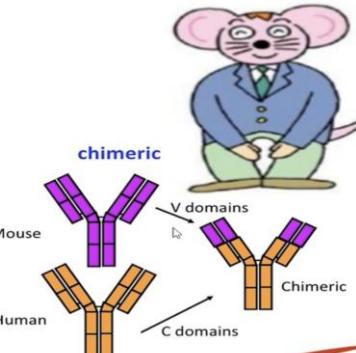
- Poor penetration
- Hypersensitivity



2) Chimeric

suffix - ximab

Antigen binding site
from mouse
+
constant region from
human



2) Chimeric

suffix - ximab

Rituximab
Anti- CD20 for
lymphoma



2) Chimeric

suffix - ximab

Infliximab
Anti-TNF α for treatment
arthritis



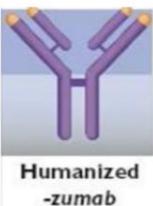
3) Humanized (95%)

(suffix -zumab)

Hypervariable
region from mouse

+

The remaining
from human



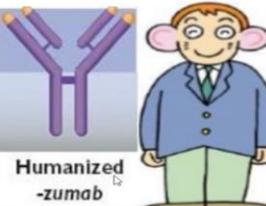
3) Humanized (95%)

(suffix -zumab)

Trastuzumab

(For breast cancer)

HER2 protein

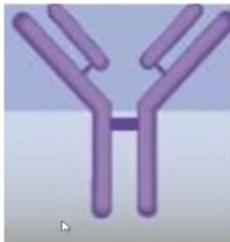


4) Human 100%

(suffix -umab)

Golimumab

Anti-TNF α



Human
-umab

Q1

humoral immune response against a pathogen leads to the production of

Polyclonal antibodies

Monoclonal antibodies

Activation of macrophages

Activation of cytotoxic T cells

Monoclonal antibodies are produced by

Lymphocytes

Myeloma cells

Plasma cells

Hybridomas

Q3

Hybridomas are made from combining

Monoclonal antibodies with myeloma cells

B cell with specific epitope

B cell with myeloma cell

monoclonal antigens with myeloma cells

Q4

The most vaccines are given in more than one dose because

Production of IgM

IgG is the main Aby produced

Aby Stay a long time

The level of Aby is high

Q5

Class switching occurring by change in:

Constant of light chain

Variable of light chain

Constant of heavy chain

Light and heavy chain

Q7

IgM is the only antibody made to ABO blood group antigens because

Thymus independent Ag

Thymus dependent Ag

Secondary immune response

Polysaccharides antigen

Q6

In isotype class switching

The specificity of the antibody is changed

The variable region genes are changed

The specificity of the antibody is not changed

Fc portion is not changed

Q8

SIgA not destroyed by digestive enzymes due to

J chain

Disulfide bond

Secretory piece

Dimeric form

Q9

The presence of IgM in the newborn blood indicates intrauterine infection

IgM cross the placenta

IgM can not cross the placenta

The fetus infected during pregnancy

It's the only Aby made by fetus

Q10

Why IgG is the only Ig can cross the placenta to the fetus?

Has receptor in the placenta

Provide passive immunity to the newborn

Major Aby of the 2nd immune response

Stay for a long period