Antibody structure and Humoral Immunity

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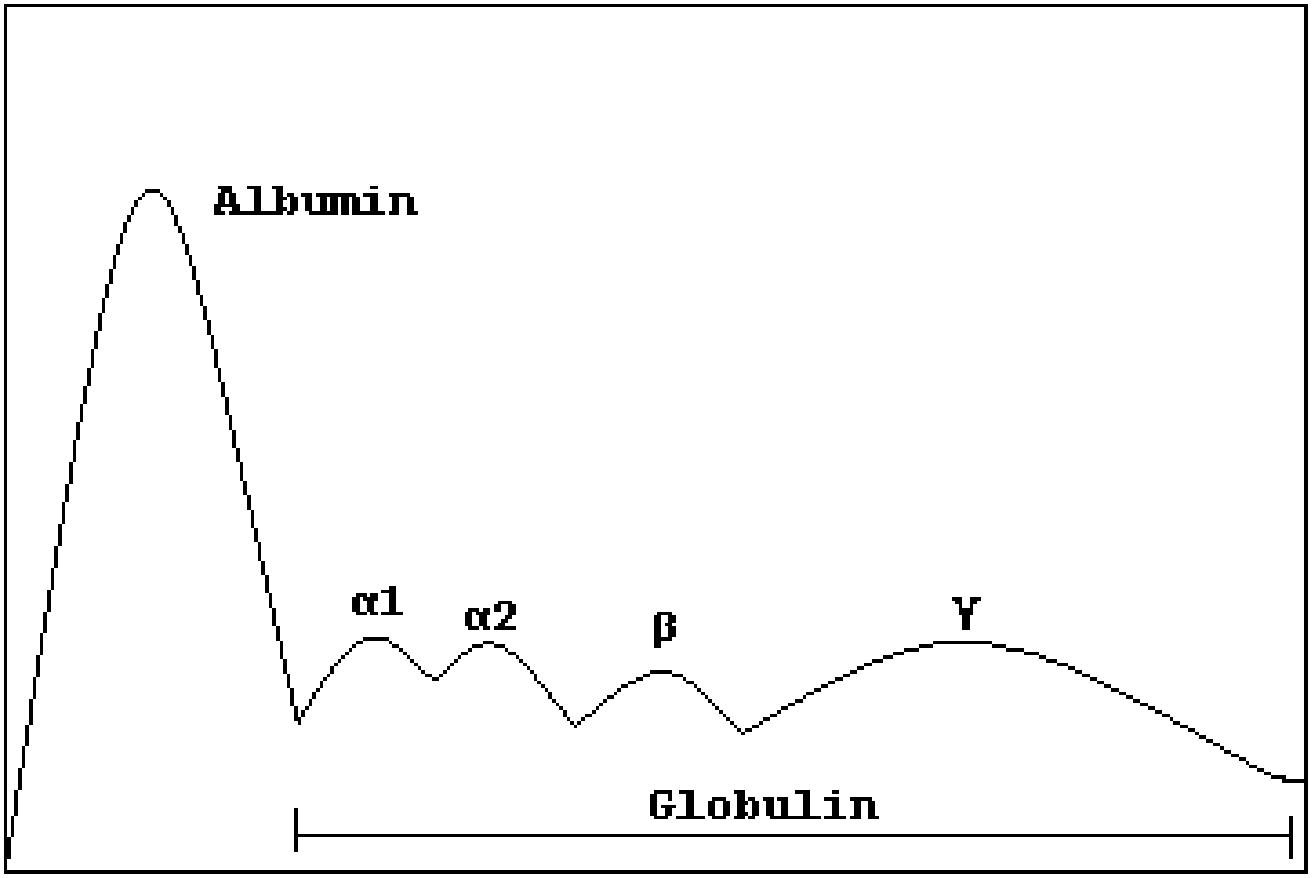
- When red blood clot, the remaining fluid called serum which include antibody and serology is any study include serum and antibody detection
- 3g of antibody produced daily and most of them is IGA in GIT and RT secretions
- Whereas, In serum, the most distributed antibody is IGG

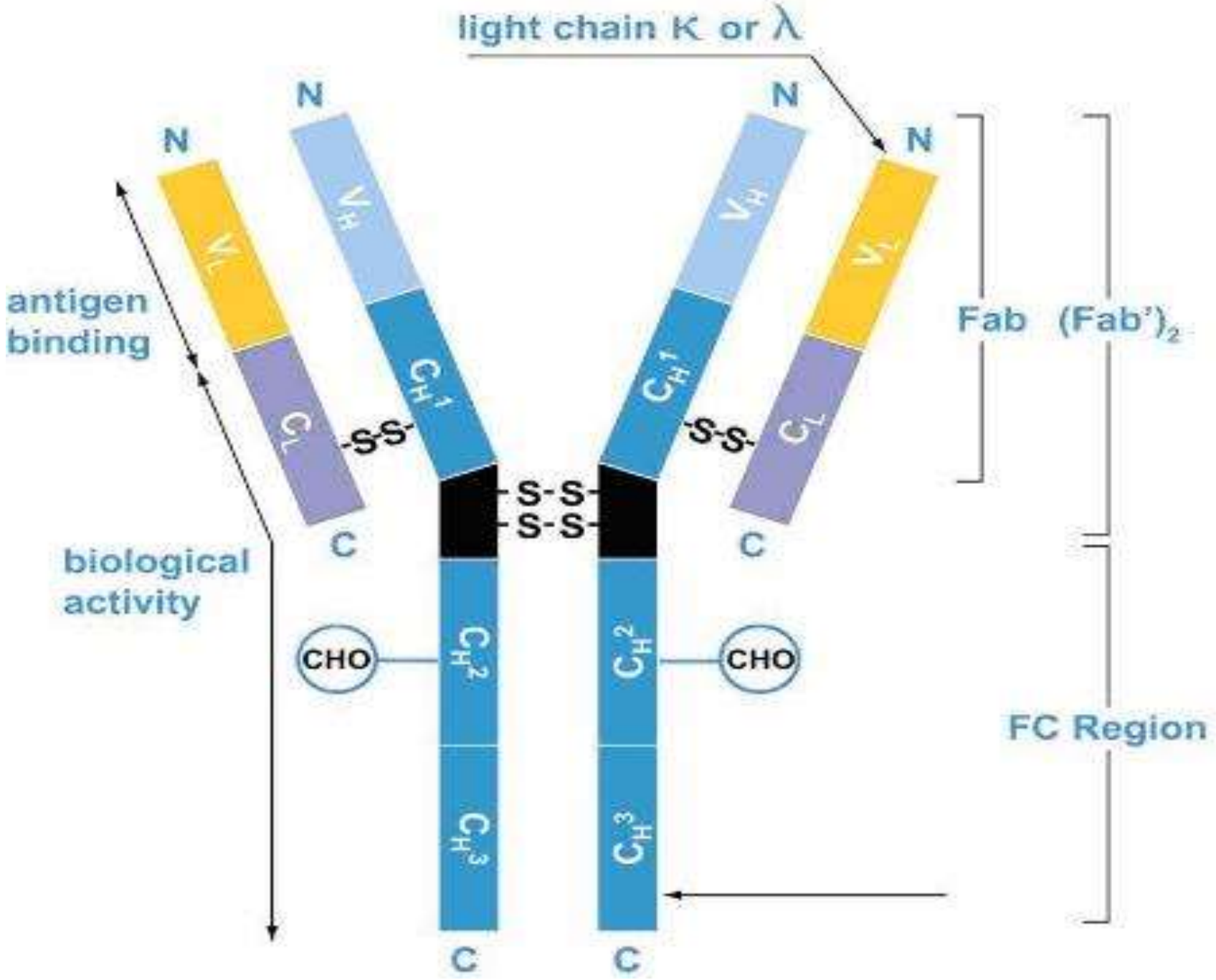
Antibody structure

- it is difficult to use normal human blood to study antibody structure as it has variable types or clones of antibodies (different variable regions) so that hypridoma technique that can produce one type of antibody was used
- This technique is; stimulation B cells with certain antigen to be antibody producing cells then fuse these cells with cancerous cell plastocytoma (the fused complex called hypridoma) by this we make these B cells to proliferate and continuously producing one type of antibodies called monoclonal antibodies against that antigen


Albumin
α1
α2
β
γ
Globulin

General structure

- 4 polypeptide chains, 2 identical heavy chains and 2 identical light chains combined by di-sulphide bonds
- Heavy chain constitute of one variable and 3-4 constant domains depending on the class of immunoglobulin
- The constant domains of the heavy chain are called depending on class of antibody (Cϒ for IGG, Cδ for IGD, Cε for IGE, Cμ for IGM and Cα for IGA), little difference in structure
- Constant L chains (2 classes)**- one or the other not both** Lambda **(λ) [40% in humans] and Kappa (κ) [60% in humans]**


light chain κ or λ
N
N
N
N
antigen binding
biological activity
V_L
V_H
V_H
V_L
C_L
C_{H1}
C_{H1}
C_L
-S-S-
-S-S-
S-S
S-S
C
C
CHO
CHO
C_{H2}
C_{H2}
C_{H3}
C_{H3}
C
C
Fab
$(Fab')_2$
FC Region

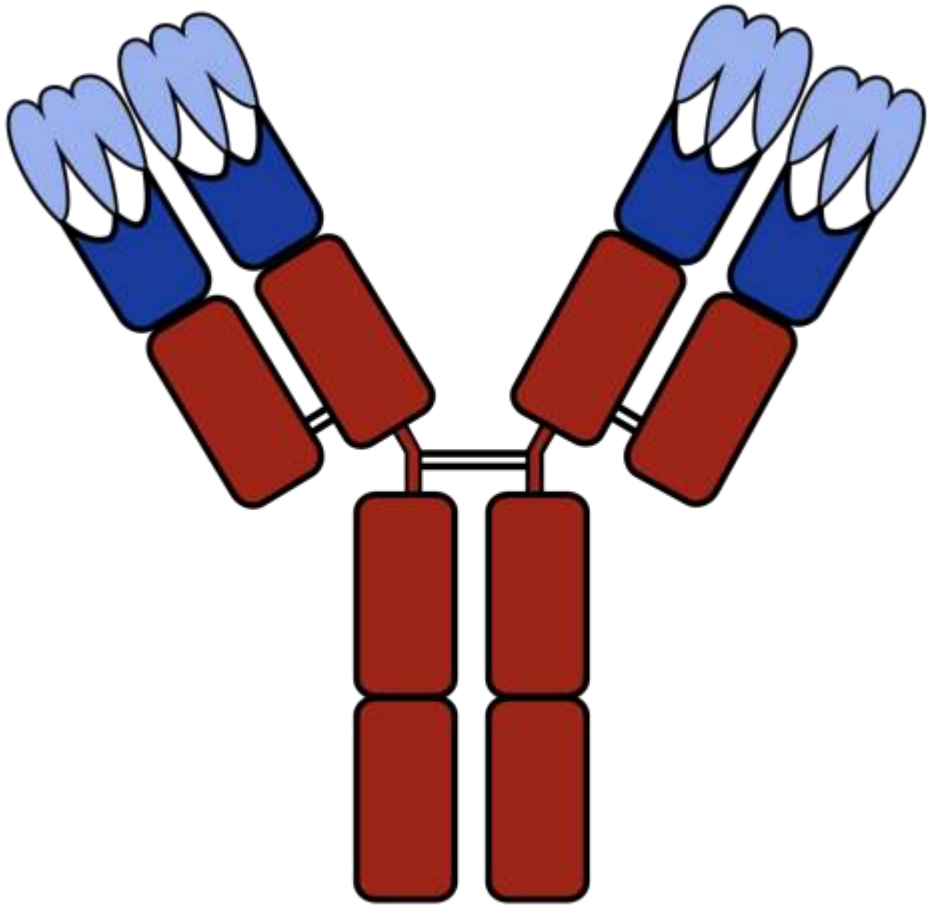
- The N-terminal part; 2 identical antigen binding sites. Each is formed by one variable domain of light and one of heavy domains

- The carboxy terminal consist only constant of heavy chain (Fc part).
- Heavy chain constant part determine
  - the type of antibody
  - and do the functional effect of Ab;
  - bind to Fc receptors on innate cells

Antigen binding site

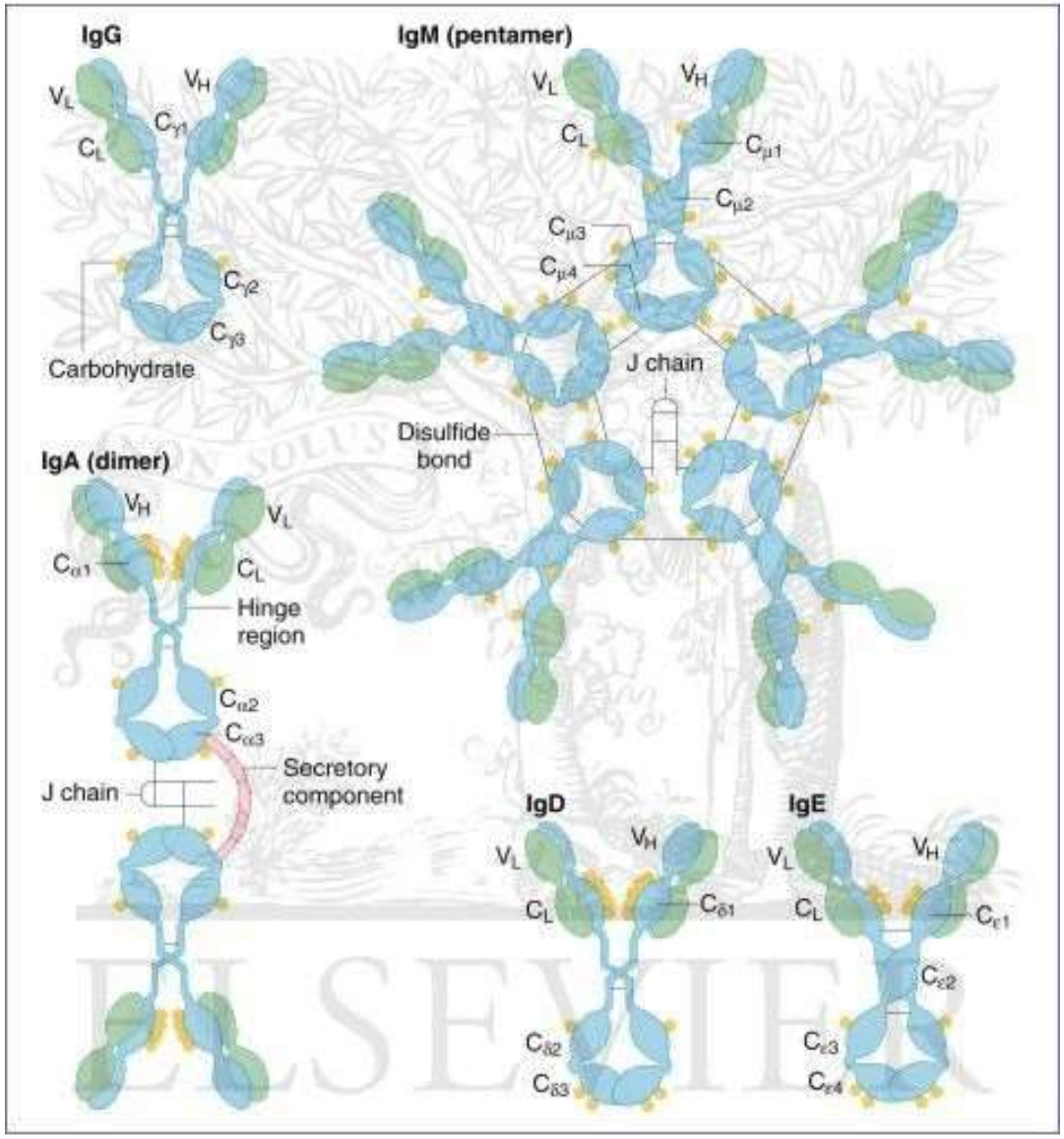
- Much of the variation between Igs located in 3 hot spots of hypervariable regions (HVR1, 2, 3) or called or complementary determining regions CDR1, 2, 3 found in variable region. Because they determine the complementary fit of antibody to antigen, and contribute to different antigenic specificities
- antigen combining sites consist of 3 HVR from light and 3HVR from heavy chains
- The most variable is on heavy chain on antibody and BCR and on beta chain on TCR
- Each HVR is about 10 amino acid residues in length.
- Intervening sequences between the CDRs have restricted variability and show little difference in amino acid sequence between chains. These invariant segments make up the **framework residues**

Hypervariable regions

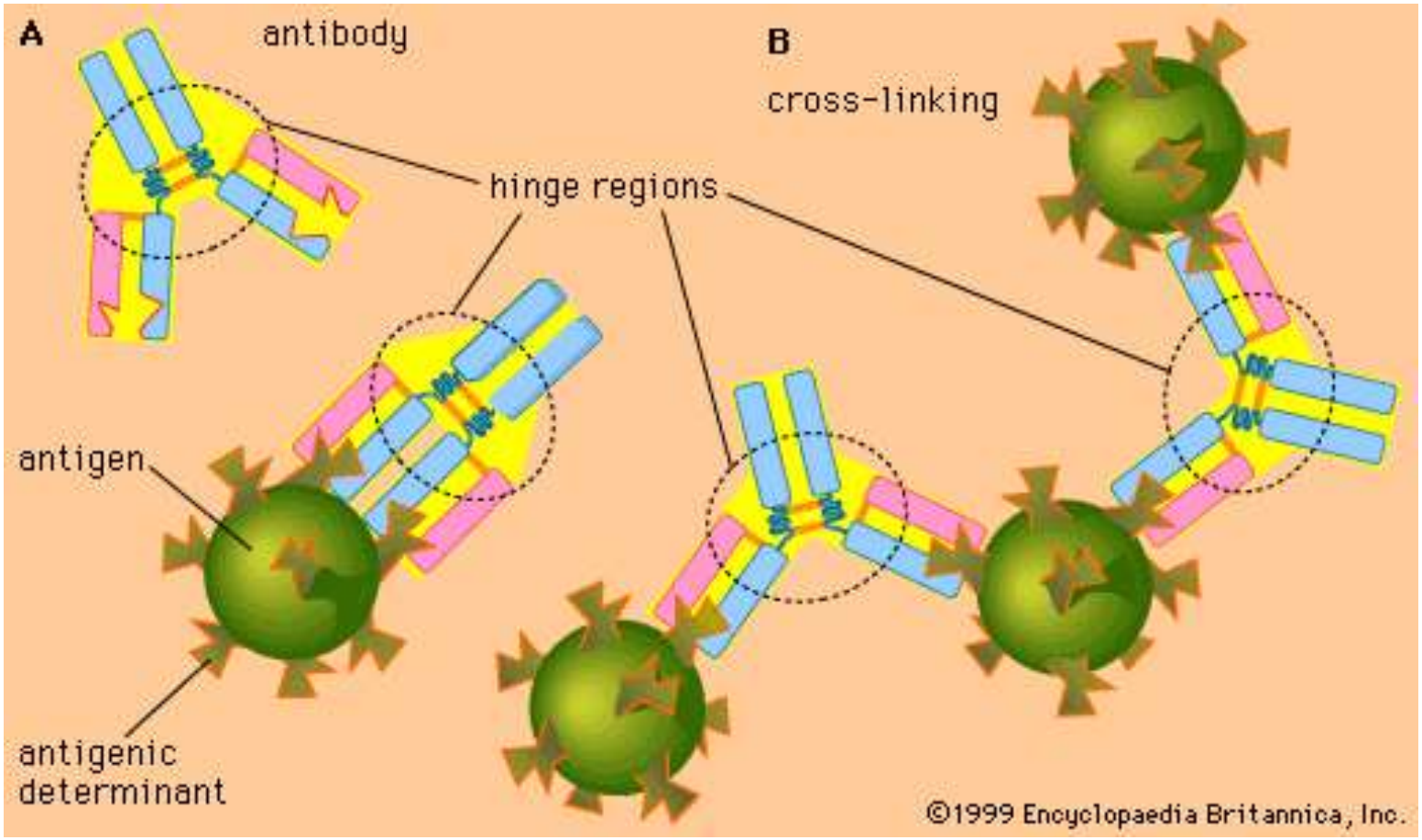


Further additions on the structure

- Antibodies also demonstrate **segmental flexibility,** which means that the two Fab portions can move relative to one another on antigen binding. The angle varies from 60 to 180 degrees. This flexible region where the arms meet the stem of the Y is called the **hinge region** and is located between the CH1 and CH2 domains. Only IgG, IgA, and IgD antibody molecules have hinge regions
- IgM and IgA also have a polypeptide called the **joining (J) chain,** which is disulfide- linked to the tail of the antibody and stabilizes the multimeric structure.
- **Secretory part in IGA**



- How many molecules can a single antibody molecule bind (*i.e* how many combining sites does it have, called valency (in IGM they are 10 sites whereas in IGA are 4 and 2 in IGG, IGE and IGD)
- What is the strength of binding of the epitope to single combining site on the antibody molecule called affinity whereas the combining strength of all combining sites to the epitopes on surface of same antigen called avidity


A
antibody
B
cross-linking
hinge regions
antigen
antigenic
determinant
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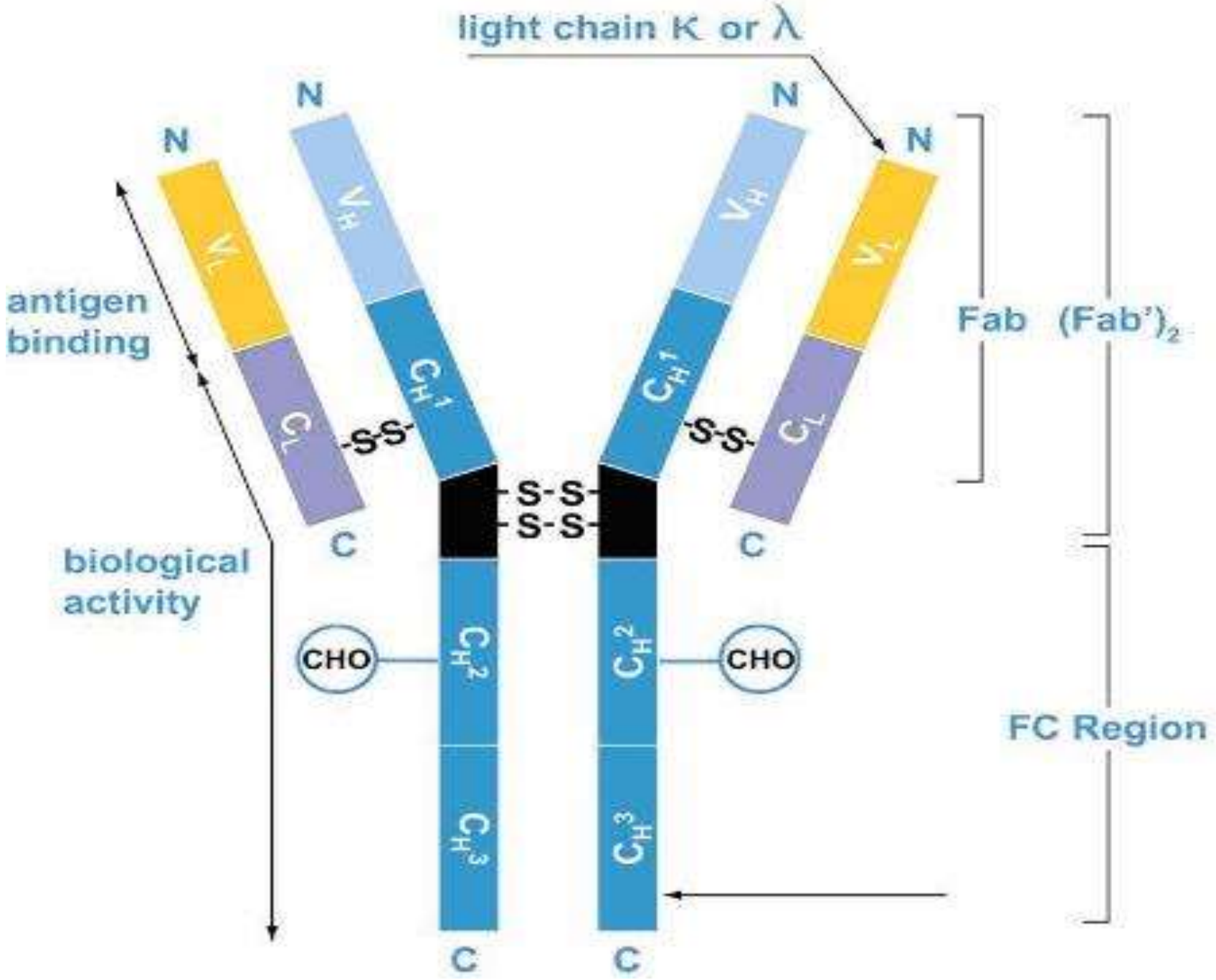
Generation of antibody fragments

- Papain enzyme digest the antibody in the n terminal side of the disulphide bonds result in 2 fab and one Fc
- Pepsin in the c-terminal side of the bonds and result in $f(ab)_2$ and smaller fc fragments (pfc)


light chain κ or λ
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antigen binding
biological activity
V_L
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C_L
-S-S-
-S-S-
S-S
S-S
C
C
CHO
CHO
C_{H2}
C_{H2}
C_{H3}
C_{H3}
C
C
Fab
$(Fab')_2$
FC Region

Classes and subclasses

- Abs can be classified as isotypes or allotypes or as idiotypes. Her we will use the first system
- 5 classes or isotypes; IGG, IGM, IGA, IGE and IGD
- IGG into 4 subclasses, IGG1, 2, 3, 4. IGA into IGA1, IGA2 while no subclasses in IGE, IGM and IGD. all of these classes and subclasses found in every person
- Antibody isotypes differ in their chemical (charge, size, and solubility) and function

Antibody classifications

- **Allotypes**, in some races structures of constant regions are nearly identical except change in 1 amino acid may occur kappa constant chain and gamma constant chain (KM and GM **allotypes respectively) the types of allotypes depend on races.**
- **Idiotypic determinants The structure formed by the CDR is known as the idiotope***. They are unique to immunoglobulins of particular antigenic specificity. These determinants classify antibodies into* **idiotypes.**

	Immunoglobulin								
	IgG1	IgG2	IgG3	IgG4	IgM	IgA1	IgA2	IgD	IgE
Heavy chain	γ_1	γ_2	γ_3	γ_4	μ	α_1	α_2	δ	ε
Molecular weight (kDa)	146	146	165	146	970	160	160	184	188
Serum level (mean adult mg ml^{-1})	9	3	1	0.5	1.5	3.0	0.5	0.03	5×10^{-5}
Half-life in serum (days)	21	20	7	21	10	6	6	3	2
Classical pathway of complement activation	++	+	+++	−	+++	−	−	−	−
Alternative pathway of complement activation	−	−	−	−	−	+	−	−	−
Placental transfer	+++	+	++	−/+	−	−	−	−	−
Binding to macrophages and other phagocytes	+	−	+	−/+	−	+	+	−	+
High-affinity binding to mast cells and basophils	−	−	−	−	−	−	−	−	+++
Reactivity with staphylococcal Protein A	+	+	−/+	+	−	−	−	−	−

IGM

- IgM, primary response to polysaccharide and protein antigens, is largest antibody,it is pentamer that makes up about 8%
- The H chain have 1 v and 4 c chains
- The five monomeric IgM molecules are arranged radially, the Fab fragments pointing outward and the Fc fragments pointing to the center of the circle
- IgM is the first antibody to appear during an immune response and the first formed by a developing fetus.
- Because of its many antigen-binding sites, IgM can quickly clump antigen (agglutinate)
- IgM acts as one of the main receptors on the surface of mature B cells, along with IgD. When IgM is a surface receptor, it is in its monomeric form.
- the CH1 and CH3 domains are the parts of the m chain where the J chain binds. The CH2 domain of the m chain is equivalent to the hinge regions
- The membrane form of IgM is made up of additional transmembrane segment
- Function; -complement activation
  - Indirect opsonization for phagocytosis
  - Antigen clumping and precipitation
  - In IGA deficiency IGM can appear in secretions linked to secretory piece
  - Complement activation

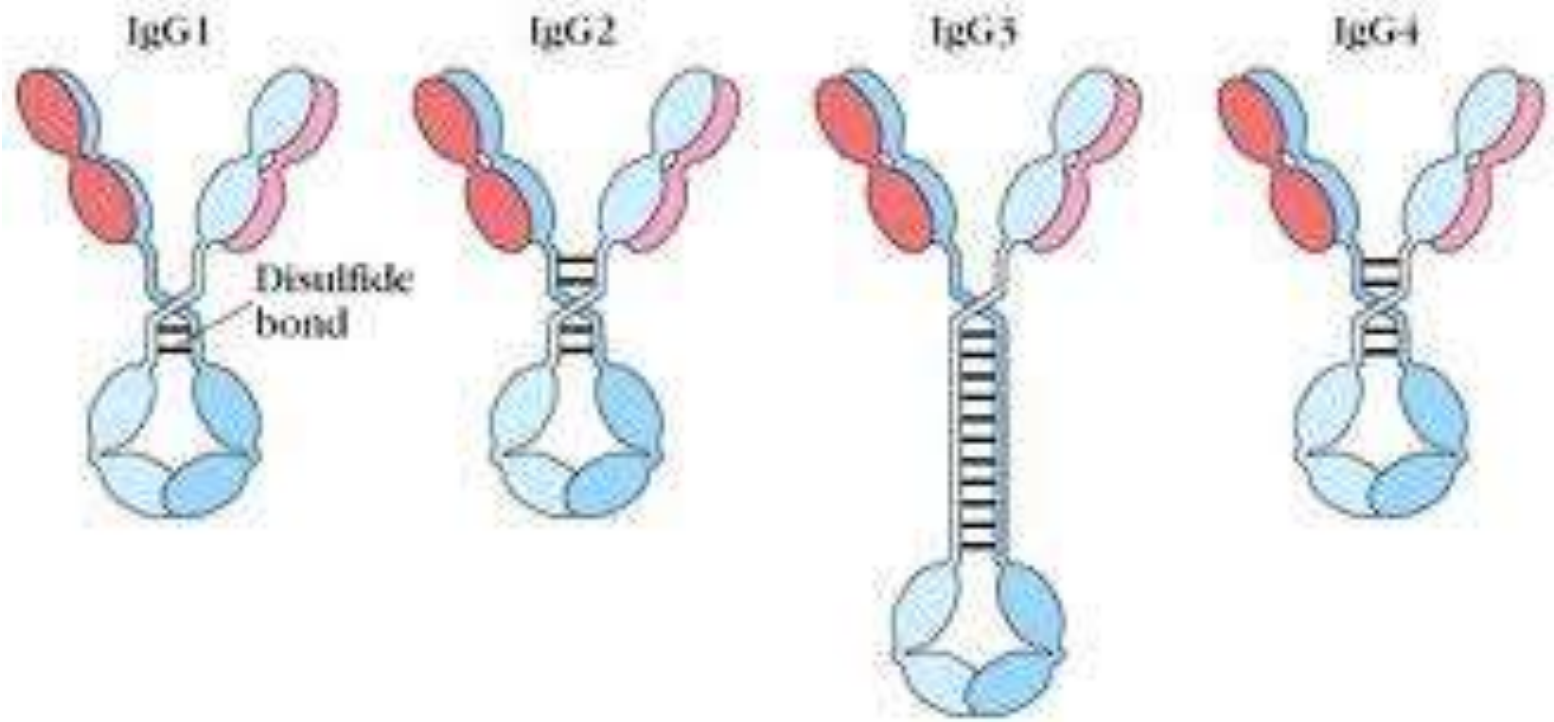
Natural antibodies

- T cell independent antigens also contribute to the generation of natural antibodies, mainly IGM, Most natural antibodies are low-affinity anti-carbohydrate antibodies, postulated to be produced by peritoneal B-1 cells stimulated by bacteria that colonize the gastrointestinal tract and by marginal zone B cells in the spleen.

IGG

- IgG, induced by protein antigens, constitutes about 80% (12.5 mg/ml) of the antibody in serum.
- Human IgG consists of four subclasses, which are numbered in order of their serum concentrations (IgG1, IgG2, IgG3, and IgG4). The four subclasses have 90 to 95% identity with each other.
- The Heavy chain is made up of four domains, one in the V portion and three in the C portion of the chain.
- The chief distinguishing characteristic among the four IgG subclasses is the pattern of interchain linkages in the hinge region.
- Produced particularly in secondary immune response
- It's presence indicate previous exposure and the higher the titer the higher the protection is.
- It activate the classical complement pathway via Cγ2 domain
- IGG and IGG3 interact with the 3 Fc receptors expressed on various cells.
- Function;
  - FcγR1 and 2 and 3 on phagocytes help in phagocytosis, low affinity FcγR3A on NK help in extracellular killing,(ADCC) , FcγR2B for B cell inhibition
  - Complement activation
  - Opsonization
  - IGG cross the placenta to give babies their immunity
  - Do neutralization of toxins

- A higher than normal IgG antibody level can suggest an IgG monoclonal gammopathy, such as *multiple myeloma* — a cancer of the blood and bone marrow
- A lower than normal IgG antibody level may suggest some types of leukemia or nephrotic syndrome, which often results in kidney damage.


IgG1
IgG2
IgG3
IgG4
Disulfide bond

Name	Percent	Crosses placenta easily	Complement activator	Binds to Fc receptor on phagocytic cells
IgG1	66%	yes (1.47)†	second-highest	high affinity
IgG2	23%	no (0.8)†	third-highest	extremely low affinity
IgG3	7%	yes (1.17)†	highest	high affinity
IgG4	4%	yes (1.15)†	no	intermediate affinity

†: Quota cord/maternity concentrations blood. Based on data from a Japanese study on 228 mothers.

IGA

- Human IgA constitutes only 13% (2.1 mg/ml) of the antibody in human serum, but it is the predominant class of antibody in extravascular secretions. The IgA present in secretions (tears, saliva, nasal secretions, bronchial and digestive tract mucus, and mammary gland secretions) is **secretory IgA.**
- The *J chain* is synthesized by plasma cells and attaches to IgA (or IgM) either before or at the time of secretion. The J chain attaches to the carboxyl-terminal of either the a or the m chain.
- IGA may be monomeric in serum or dimeric in secretions
- The alpha chain is made up of one V domain and three C domains. IgA1 is the most prevalent form in serum, but IgA2 is slightly more prevalent in secretions.
- Another difference between IgA subclasses is the size of their hinge regions.
- Increase in secondary immune response to antigen gaining access via mucosa.
- Function; -Bind neutrophils through FCαR and mediate phagocytosis
  - although most of its protection result from direct neutralization of toxins in gut and RT
  - Do agglutination of antigens gaining access via mucosa
  - They secreted in breast milk help in infant immunity
  - Complement activation

IGD

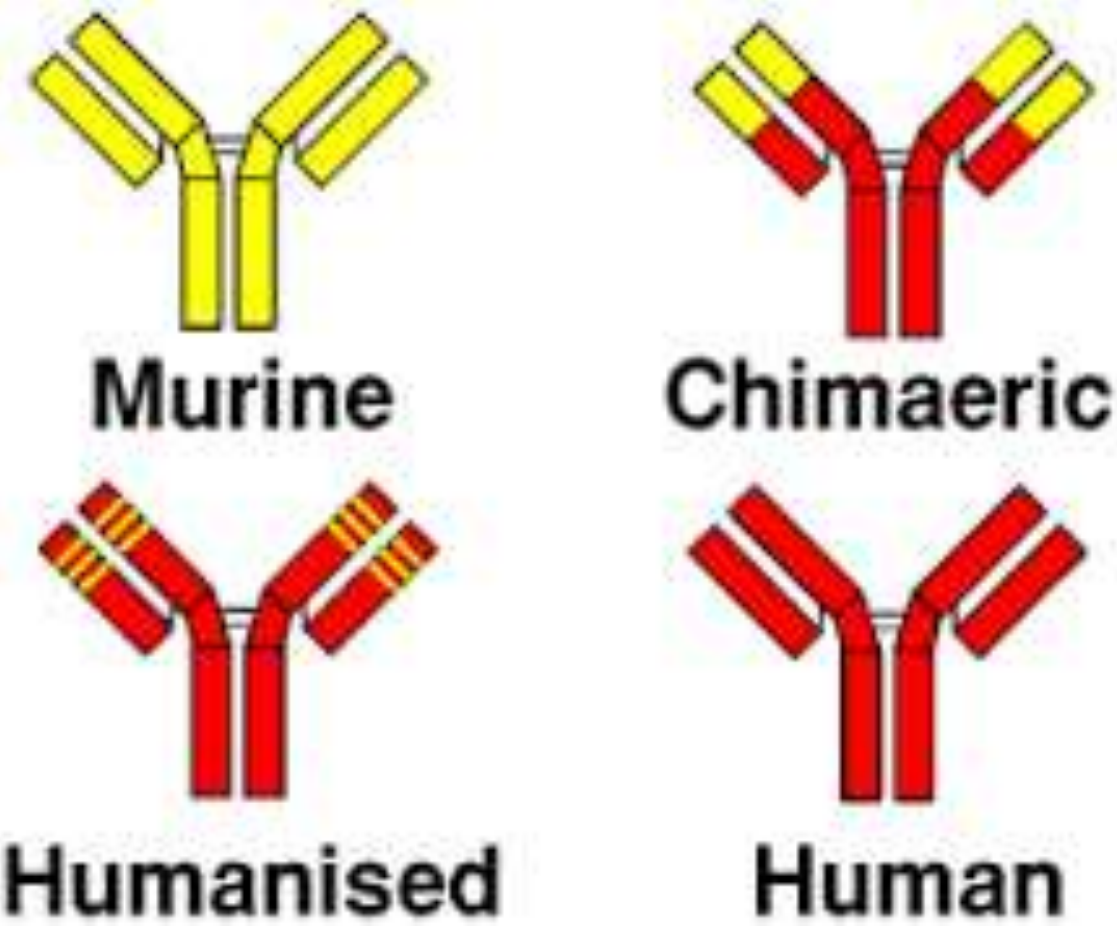
- IgD constitutes less than 1% of the antibody in human serum.
- IgD is an antibody whose function remains unknown, even though it is one of the main receptors on mature B cells and may regulate cell activation.
- The d-chain C region is divided into three domains
- The hinge region of IgD longer than any other antibody class

IGE

- Human IgE makes up less than 0.003% of the antibody in serum.
- IgE binds through its high affinity FcεR1 part to mast cells or basophils. On later exposure to the same antigen, mast cells and basophils bind antigen with IgE and trigger allergic reactions.
- IgE protects against parasites by binding low affinity Fc εR1 on eosinophils and then releasing mediators (ADCC).
- FC εR2 on B cells unknown function
- Like the m chain, the e chain contains four C-region domains.

Monoclonal antibodies

- Are pure antibodies with single antigen specificity produced from one B cell clone by hypridoma technique
- Uses;
  - Diagnostic uses
    - Identification and separation of microbe antigens; identification of autoimmune disease, level of vaccination, diagnose immune complex disease, diagnose pregnancy
  - Therapeutic uses;
    - antitumor therapy alone or with cytotoxic agents (magic bullet),
    - Immunosuppressive; anti-CD3 (T cell) in graft rejection
    - Neutralize drug toxicity; digitalis
    - Anti RH in RH incompatibility (hemolytic disease of newborn)
    - Passive immunotherapy to protect against vericella zoster and CMV


Murine
Chimaeric
Humanised
Human

FC receptors

FcR	Affinity for immunoglobulin	Cell distribution	Function
FcγRI (CD64)	High ($K_d \sim 10^{-9}$ M); binds IgG1 and IgG3, can bind monomeric IgG	Macrophages, neutrophils; also eosinophils	Phagocytosis; activation of phagocytes
FcγRIIA (CD32)	Low ($K_d > 10^{-7}$ M)	Macrophages, neutrophils; eosinophils, platelets	Phagocytosis; cell activation (inefficient)
FcγRIIB (CD32)	Low ($K_d > 10^{-7}$ M)	B lymphocytes	Feedback inhibition of B cells
FcγRIIIA (CD16)	Low ($K_d > 10^{-6}$ M)	NK cells	Antibody-dependent cell-mediated cytotoxicity
FcγRIIIB (CD16)	Low ($K_d > 10^{-6}$ M); GPI-linked protein	Neutrophils, other cells	Phagocytosis (inefficient)
FcεRI	High ($K_d > 10^{-10}$ M); binds monomeric IgE	Mast cells, basophils, eosinophils	Cell activation (degranulation)
FcεRII (CD23)	Low ($K_d > 10^{-7}$ M)	B lymphocytes, eosinophils, Langerhans cells	Unknown
FcαR (CD89)	Low ($K_d > 10^{-6}$ M)	Neutrophils, eosinophils, monocytes	Cell activation?

Fc receptors and antibody functions

- Immunoglobulin Fc receptors (FcRs) are expressed on all hematopoietic cells
- Binding of antigen-antibody to FcR activates effector cells, leading to

  1- Opsonization of microbe (coating to make it obvious) using IGG, IGA or IGM. Then phagocytosis
  - 2 types
  - Direct opsonization by IGG
  - Indirect opsonization by IGM + complement

  2- and antibody-dependent cellular cytotoxicity (ADCC). By NK

Fc receptors and antibody functions

3- Mast cell degranulation in allergy

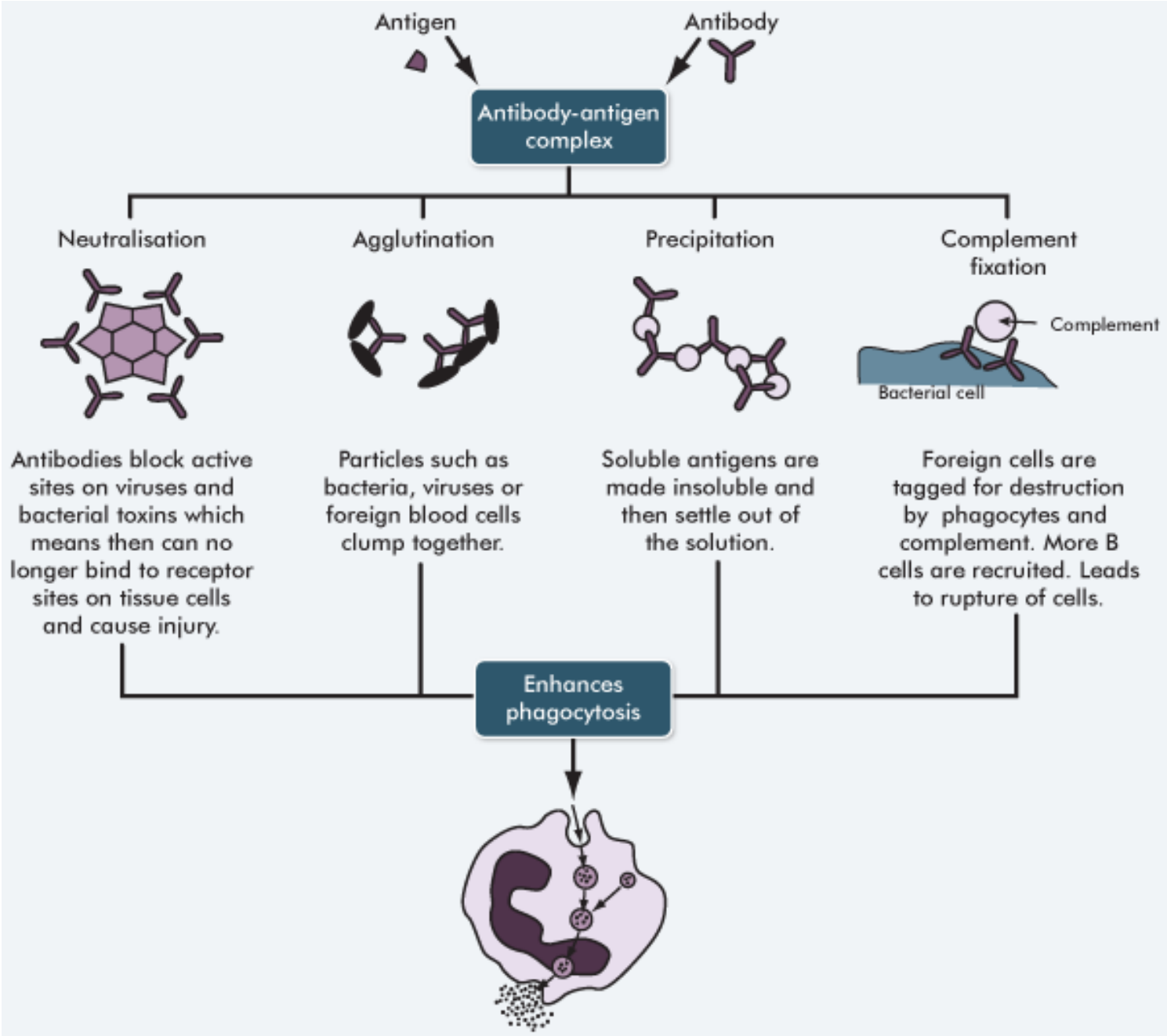
4- Extracellular killing by eosinophils (ADCC)

5- complement activation and cell lysis, IGG, IGM, IGA

6- Neutralization; Antibodies against microbes and microbial toxins block the binding sites of these toxins and viruses so un able to bind cellular receptors (IGG)

7- Precipitation and agglutination (IGM, IGA and IGG)

- To form immune complexes to be cleared from serum
- And to neutralize toxins and microbe to inactivate
- Enhance phagocytosis

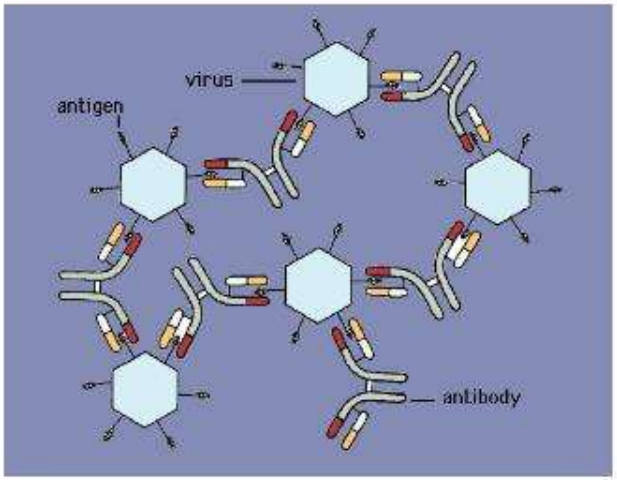

Antigen
Antibody
Antibody-antigen complex
Neutralisation
Agglutination
Precipitation
Complement fixation
Complement
Bacterial cell
Antibodies block active sites on viruses and bacterial toxins which means then can no longer bind to receptor sites on tissue cells and cause injury.
Particles such as bacteria, viruses or foreign blood cells clump together.
Soluble antigens are made insoluble and then settle out of the solution.
Foreign cells are tagged for destruction by phagocytes and complement. More B cells are recruited. Leads to rupture of cells.
Enhances phagocytosis

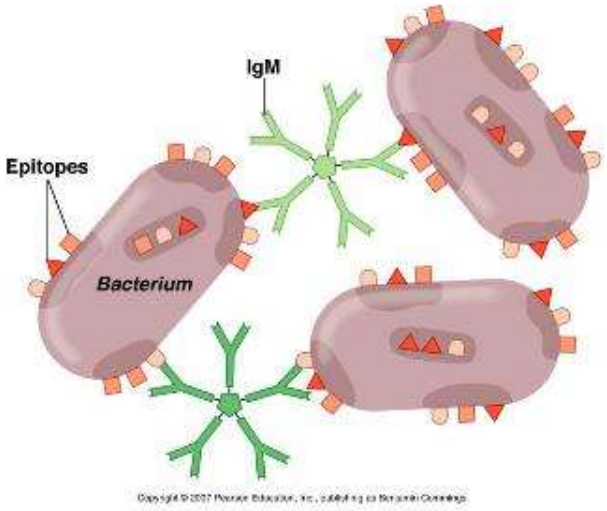
AG-AB binding in Serology

- Serology mean use serum (antibodies) to detect any infection or immune disease
- In Ag-Ab binding; Precipitation, Precipitation reactions are based on the interaction of antibodies and antigens. They are based on two soluble antigen and antibody that come together to make one insoluble product, the precipitate which appear as line between 2 solutions
- In Ag-Ab binding, Agglutination; Agglutination is the visible expression of the aggregation of antigens and antibodies. Agglutination reactions apply to cell bound antigens (on RBC or artificially fixed on particles) bind to antibody . The endpoint of the test is the observation of clumps resulting from that antigen-antibody complex formation.

AGGLUTINATION

- Abs can bind and cross-link cells or particles aggregate formation
- Entrap microbial invaders
- IgM & IgA are the most suitable *(IgG in sufficient amounts can agglutinate cells)*



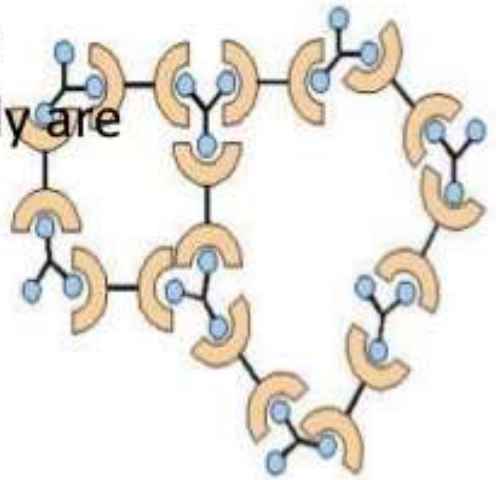


Precipitation

- **Principle**

  Soluble antigen combine with its soluble antibody and form a **lattice** that develops into a visible precipitate.

- One of the easiest of serological tests
- Occur best when antigen and antibody are present in **optimal proportions (Equivelance)**.



Equivalence – Lattice formation

- Fc receptors have been described for all classes of immunoglobulins:
  - FcγR and neonatal FcR (FcRn) for IgG,
  - FcεR for IgE,
  - FcαR for IgA,
  - FcδR for IgD,
  - and FcμR for IgM.
- Of these receptors, leucocyte FcγR and FcεR are characterized most extensively.
- Structurally, all known Fc receptors belong to the immunoglobulin superfamily, except for FcRn and FcεRII,

- Among them, FcγRI and FcεRI are high-affinity Fc receptors with dissociation constants ranging high.
- All other IgG receptors, such as FcγRII and FcγRIII, are low-affinity receptors with dissociation constants are low.

FcγR

- In addition to the affinity variations among the receptors, each Fcγ receptor displays distinct IgG subtype specificities; for example,
  - FcγRIII and FcγRI binds IgG_1 and IgG_3 better than IgG_2 and IgG_4.

FcγR

- FcγRII A, B,. These isoforms have similar extracellular domains and ligand specificities but differ in cytoplasmic tail structure, cell distribution, and functions
- FcγRI and FcγRII A and FcγRIII B, on phagocytes help in phagocytosis
- FcγRIIB deliver inhibitory signals to B lymphocytes and IVIG (intravenous immunoglobulin) may use this mechanism to treat B cell cancer

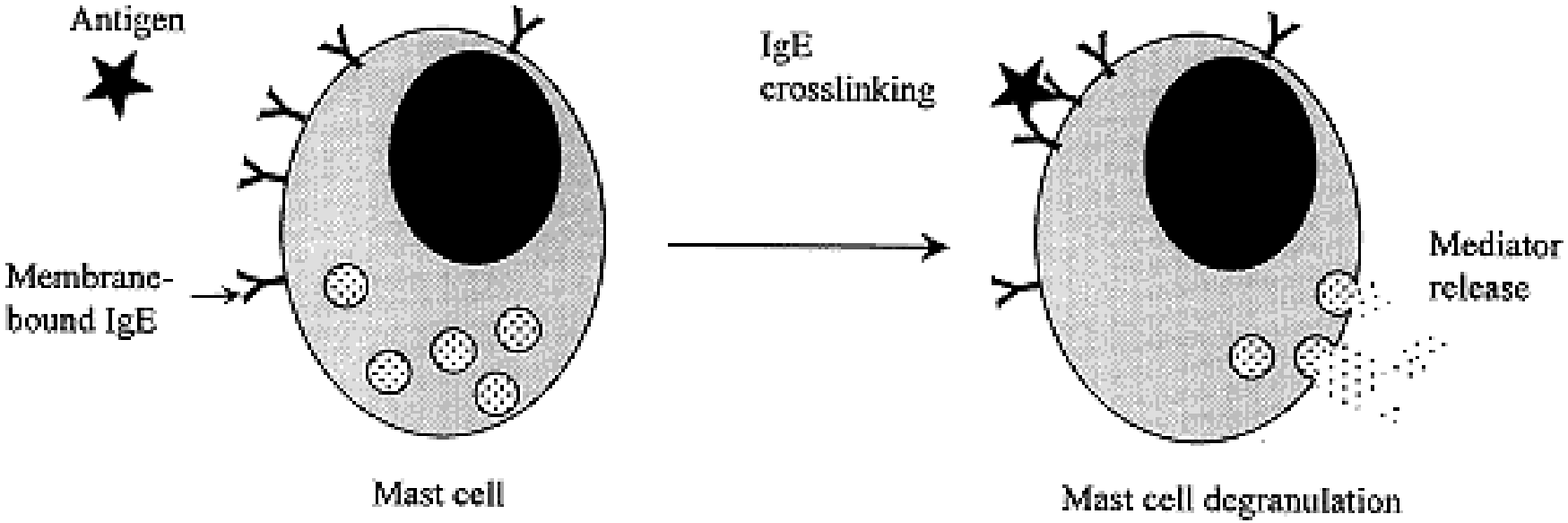
FcγR

- Natural killer (NK) cells and eosinophils bind to antibody-coated cells by Fc receptors and destroy these cells. This process is called antibody-dependent cell mediated cytotoxicity (ADCC).
- It was first described as a function of NK cells, which use their Fc receptor, low affinity FcγRIIIA (CD16), to bind to antibody-coated cells.
- After binding the receptors, NK secrete cytokines such as IFN-γ as well as to discharge the contents of their granules, which mediate the killing functions
- Giving treatment with CD20 antibody kill B cell–derived tumor cells by NK cells by ADCC

FcεR

- **FcεRI**
  - on eosinophils, function to mediate the killing and expulsion of some helminthic Parasites carrying IgE by ADCC. Killing molecules are secreted out side from eosinophils as major basic protein
  - Binding of FcεRI on Mast cell with an allergen mediate a rapid release of mediators (Histamine) that may induce bronchoconstriction and increased local motility, contributing to the formation of hypersensitivity reaction 1
- **FC εR2 on B cells unknown function**

Effecter phase



- Some data suggest the existence of FcRs for IgM and IgD on leukocytes have not been well defined.
- In addition, two epithelial cell FcRs have been well characterized:
  - FcRn (Neonatal Fc receptors) which mediates both IgG transport across the placenta and IgG uptake by neonatal intestinal epithelium, The FcRn is unique among Fc receptors in that it resembles a class I major histocompatibility complex (MHC)
  - and the pIgR (poly Ig receptor, also known as secretory component) which transports IgA into mucosal secretions.

Antibody Isotype	Isotype-Specific Effector Functions
IgG (also note: subclass differences)	Opsonization of antigens for phagocytosis by macrophages and neutrophils
	Activation of the classical pathway of complement
	Antibody-dependent cell-mediated cytotoxicity mediated by NK cells
	Neonatal immunity: transfer of maternal antibody across the placenta and gut
	Feedback inhibition of B cell activation
IgM	Activation of the classical pathway of complement
	Antigen receptor of naive B lymphocytes * good avidity bc they are pentamers
IgA	Mucosal immunity: secretion of IgA into the lumens of the gastrointestinal and respiratory tracts
	Activation of complement by the lectin pathway or by the alternative pathway
IgE	Mast cell degranulation (immediate hypersensitivity reactions)
IgD	Antigen receptor of naive B lymphocytes *