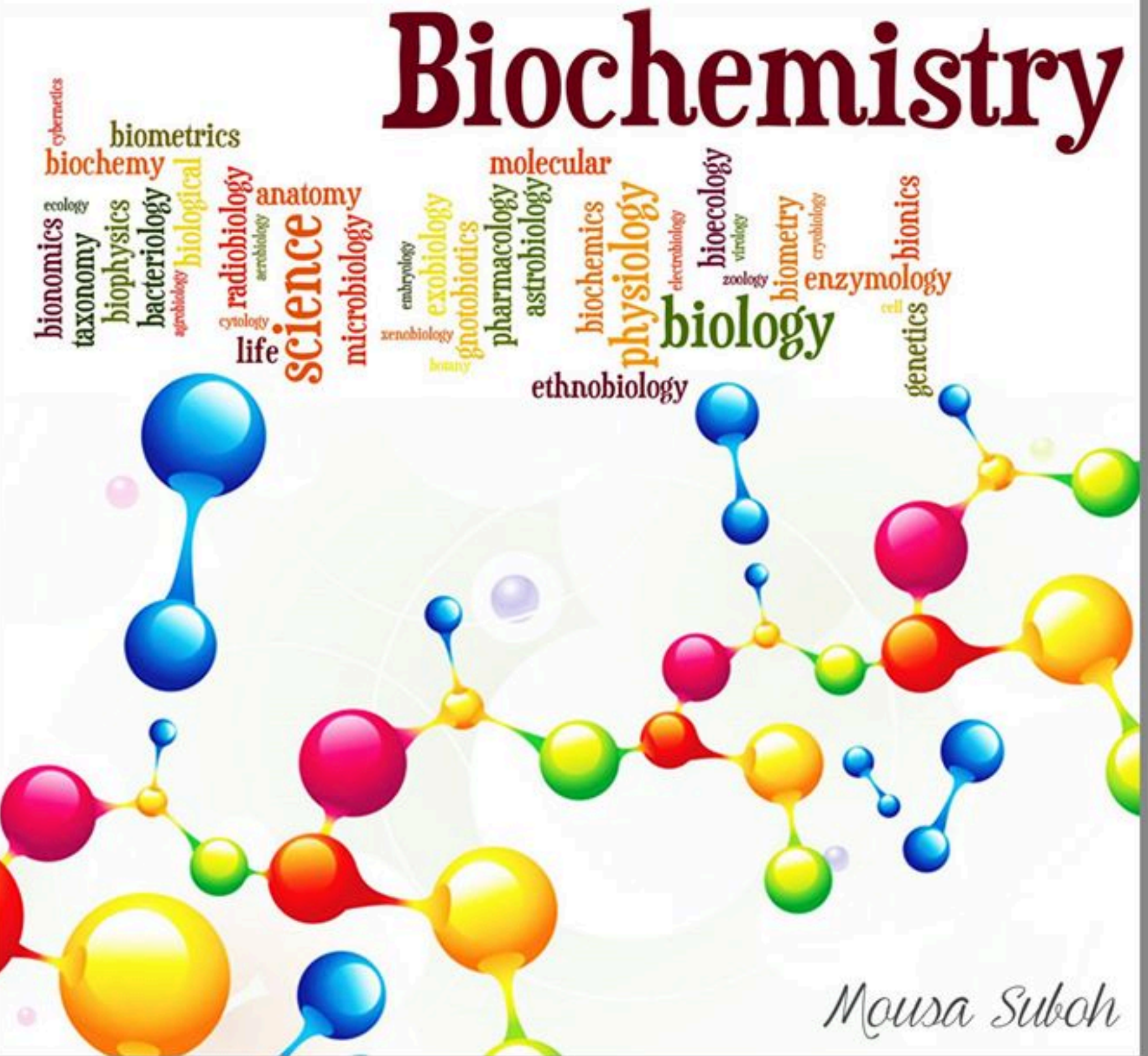
Slide : 2-Immunoglobulins

Dr. Name : Dr. Nafeth Abu Tarboush

Sections : .....

■ Slide ☐ Sheet

Medical Committee
The University of Jordan



Nafith Abu Tarboush
DDS, MSc, PhD
natarboush@ju.edu.jo
www.facebook.com/natarboush

Immunoglobulins

Defense lines (specific vs. non-specific)

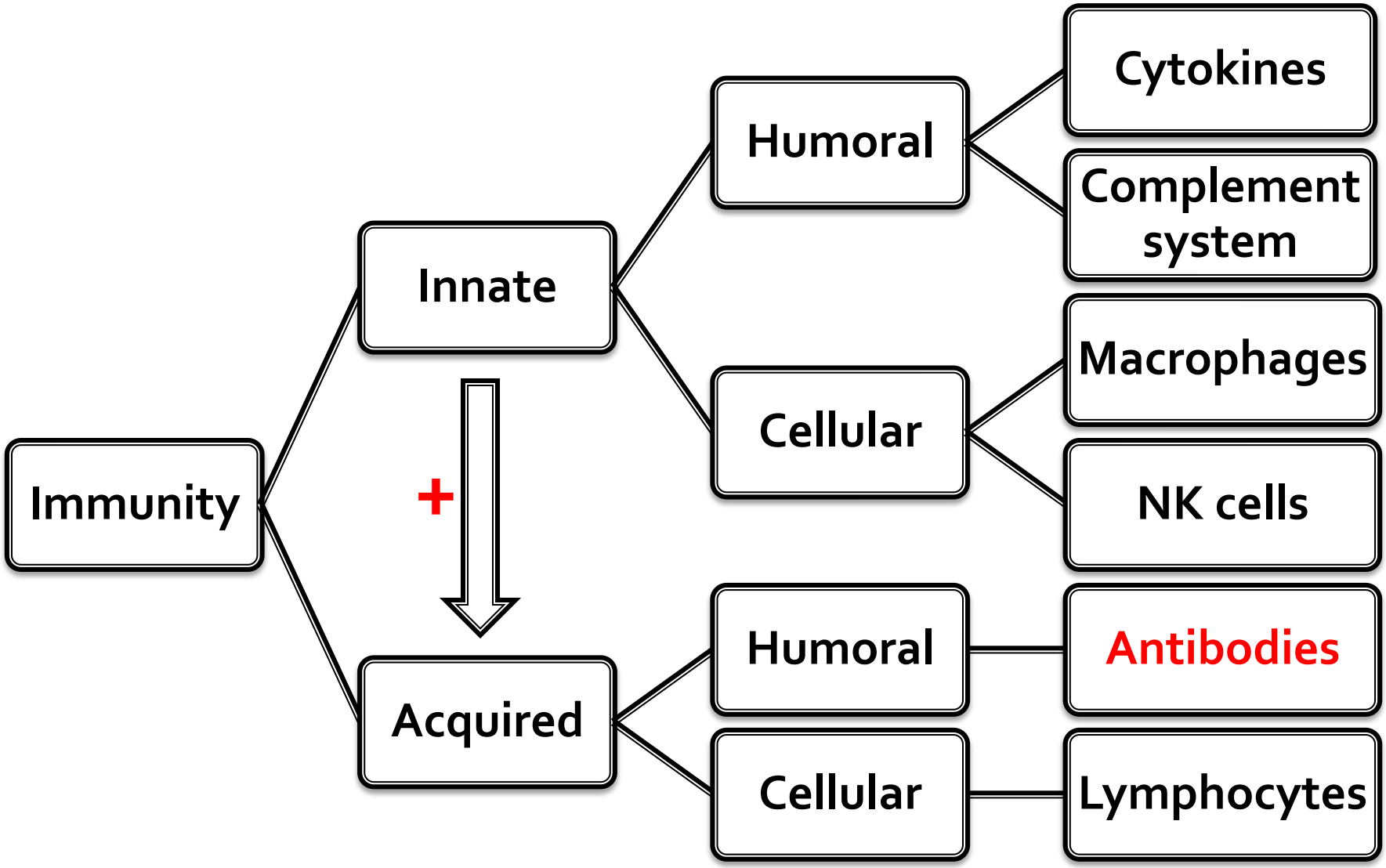
- **The immune system plays a major role in the body's defense mechanisms**

Non-specific (innate)		Specific (acquired)
➢ First line	➢ Second line	➢ Third line
✓Barriers ✓ physical: skin, hair, mucous membranes ✓chemical: sweat, tears, saliva, stomach acid, urine	✓Phagocytic WBCs ✓Antimicrobial proteins ✓Inflammatory response	✓Lymphocytes ✓**Antibodies**

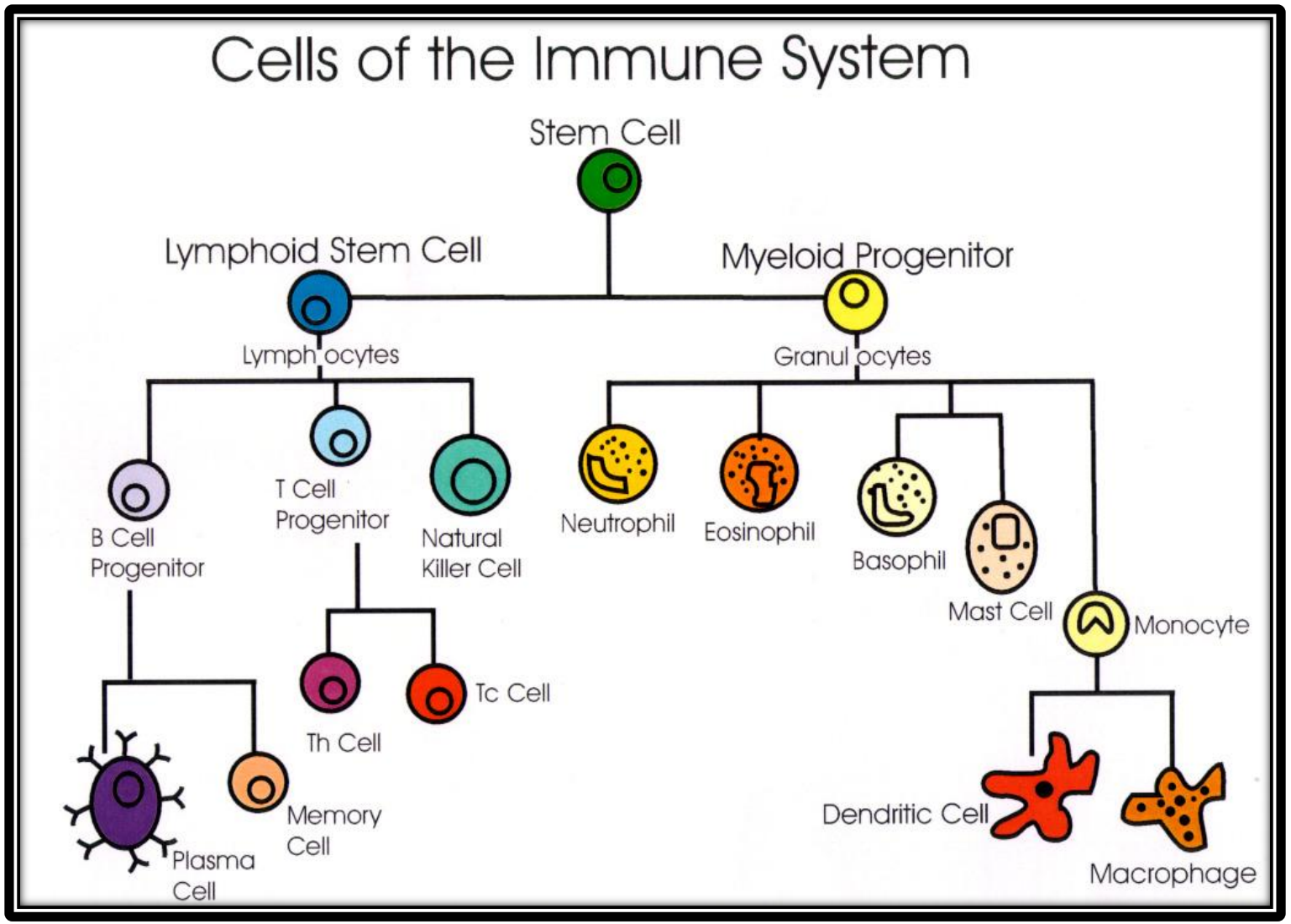
Innate vs. Acquired Immunity

- Innate:
  - Natural or native immunity
  - Cellular & biochemical defense mechanisms (non-specific)
  - Non-adaptive upon repeated infections
  - Only recognize microbial agents
- Acquired:
  - Develops as a response to infection & adapts to the infection
  - Increase in magnitude & defensive capabilities with each successive exposure to a particular microbe
  - High specificity & memory for distinct molecules
  - Recognize & react to microbial & non-microbial substances

Innate vs. Acquired Immunity



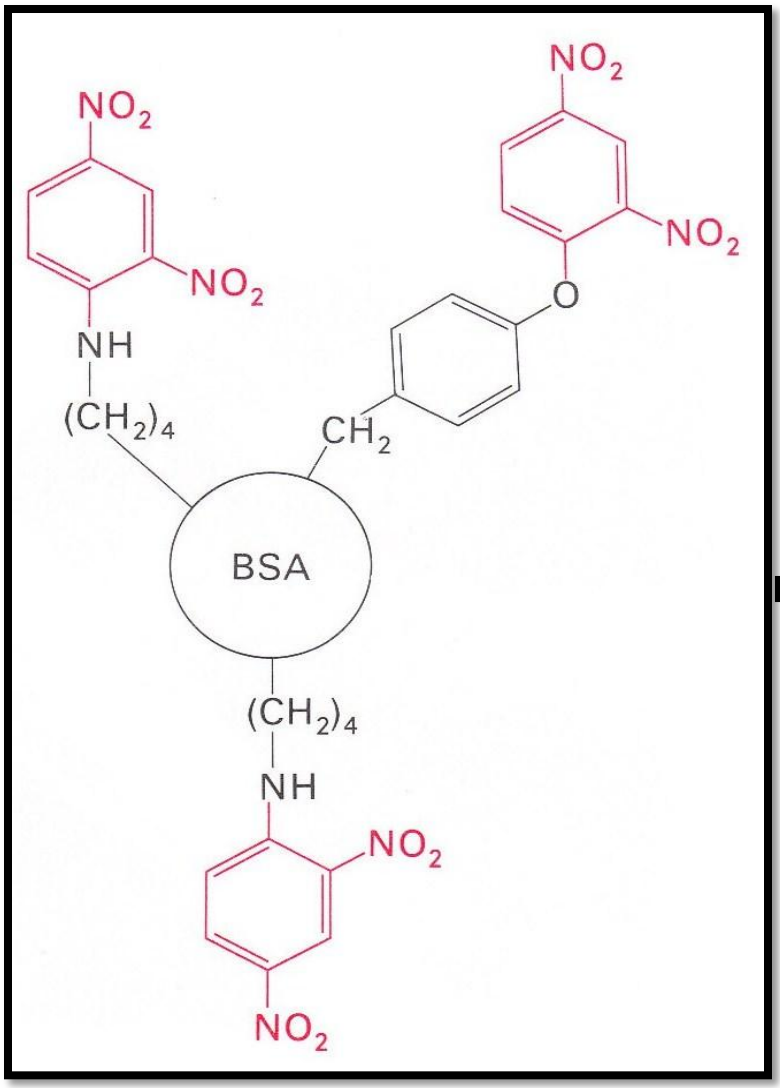
Immune system cells

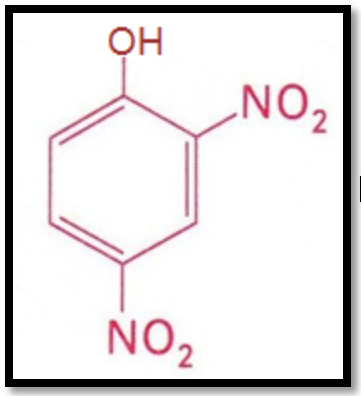


Immunoglobulins & antigens

- Antibodies: "glycoproteins" synthesized by plasma cells & able to bind foreign molecules even if not encountered before
  - ✓High specificity & high affinity
  - ✓Huge number of different kinds (~10^8)
  - ✓Synthesis is stimulated by having an immunogen
  - ✓Induces the "effector functions": Inactivation, degradation, lysis
- Antigen: Foreign molecules to which Igs bind
  - ✓Can elicit antibody formation (immounogen)
  - ✓Macromolecule; Protein, polysaccharide, nucleic acid
  - ✓Epitope (Antigenic determinant): each epitope is recognized by a different antibody
  - ✓Hapten: small molecule, antigen if attached to a macromolecule

Hapten-immunogenic response



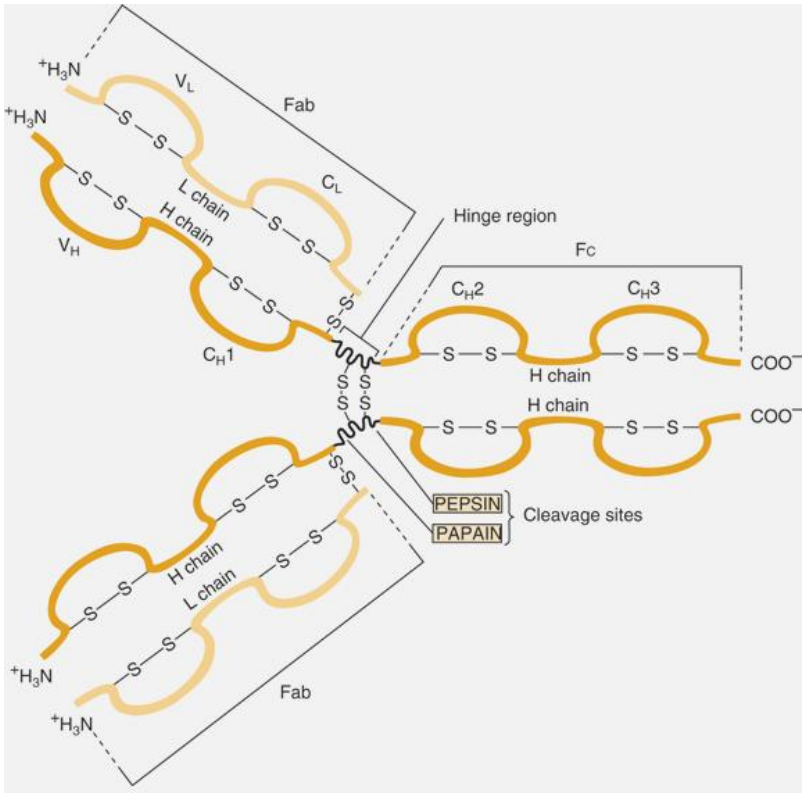


No response

Response

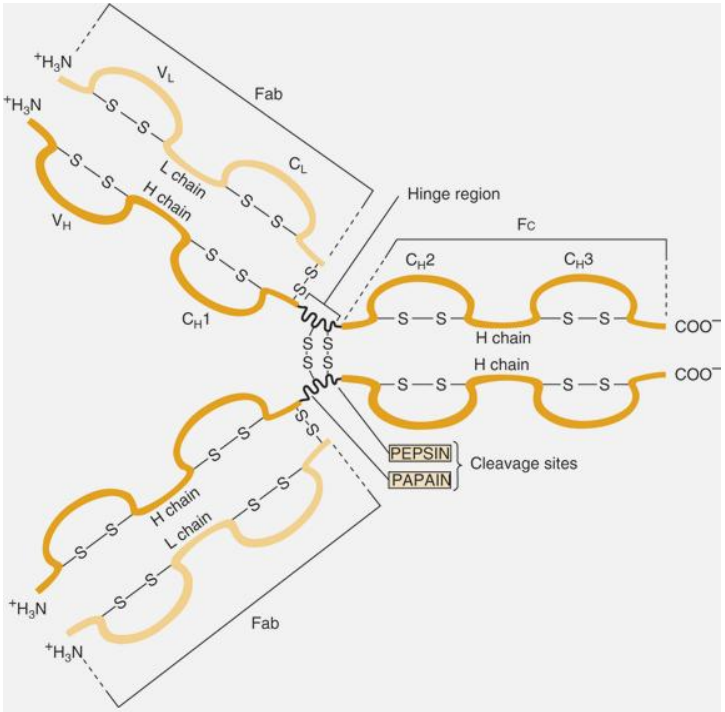
Immunoglobulins - structure

- All contain a minimum of 2 identical light chains (25 kDa) & 2 identical heavy chains (50 kDa)
- Held together by disulfide bonds
- Y-shaped: binding of antigen at both tips
- Each chain has specific domains
- L chain: amino half (V_L), carboxylic half (C_L)
- H chain: ¼ amino (V_H), ¾ carboxylic (C_H1, C_H2, C_H3)



Immunoglobulins - structure

- Antigen binds V_H & V_L domains
- Hinge region:
  - C_H1 & C_H2
  - Flexibileity
  - Independent movement
- F_c & hinge regions differ in the different classes of antibodies
- Papain: 2 antigen-binding fragments (F_{ab}) & one crystallizable fragment (F_c)
- Pepsin: one $(F_{ab})_2$ fragment & one crystallizable fragment (F_c)

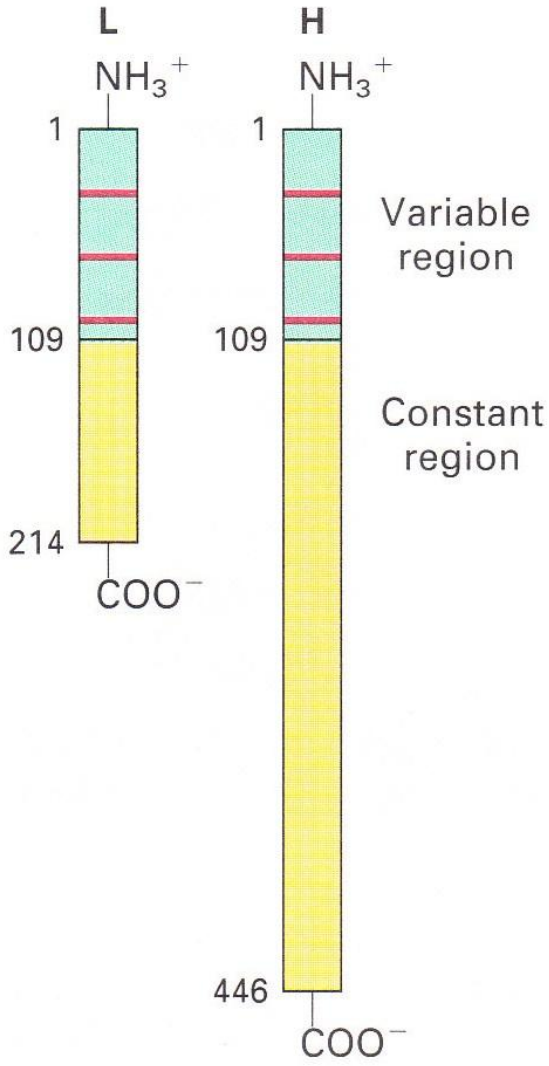


Immunoglobulins - structure

- 2 L chains 25 kDa 214 AA
- 2 H chains 50 kDa 446 AA

- Light chain:
  - ✓ 1- 110 variable, 111 – 214 similar
- Heavy chain:
  - ✓ 1- 113 variable, 114 – 446 similar

- 3 stretches (7-12 amino acids) "hypervariable"



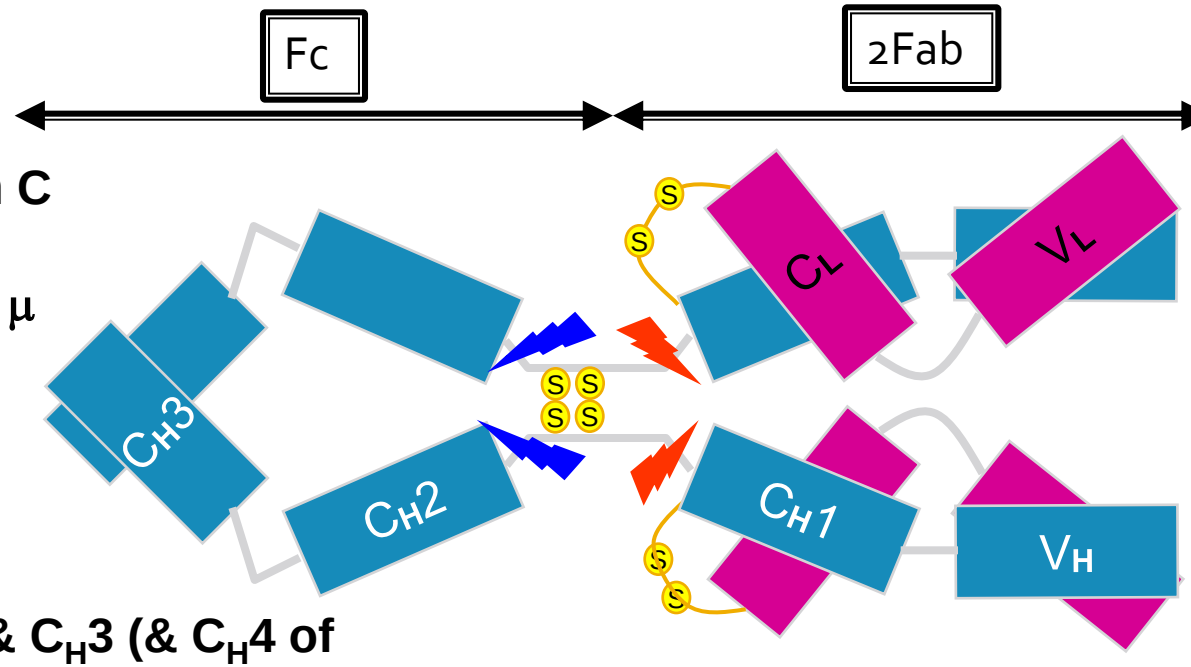
Immunoglobulin - Interactions

- With antigen (infinite):
  - ✓ Electrostatic, Hydrogen, Van der Waal's, Hydrophobic
    - The $(F_{ab})2$ fragment CAN:
      - ✓ Detect & bind the antigen
      - ✓ Block the active sites of toxins
      - ✓ Block interactions between host & pathogen

- With other cells and molecules through the Fc portion (finite)
    - The $(F_{ab})2$ fragment <u>CANNOT</u> activate:
      - ✓ Inflammatory functions associated with cells
      - ✓ Inflammatory functions of complement proteins
      - ✓ Intracellular cell signaling molecules

Domain Structural variation of Immunoglobulins – constant region

Domains are folded, compact, protease resistant structures



Heavy chain **C** domains **α, δ, ε, γ, or μ**

Light chain **C** domains **κ or λ**

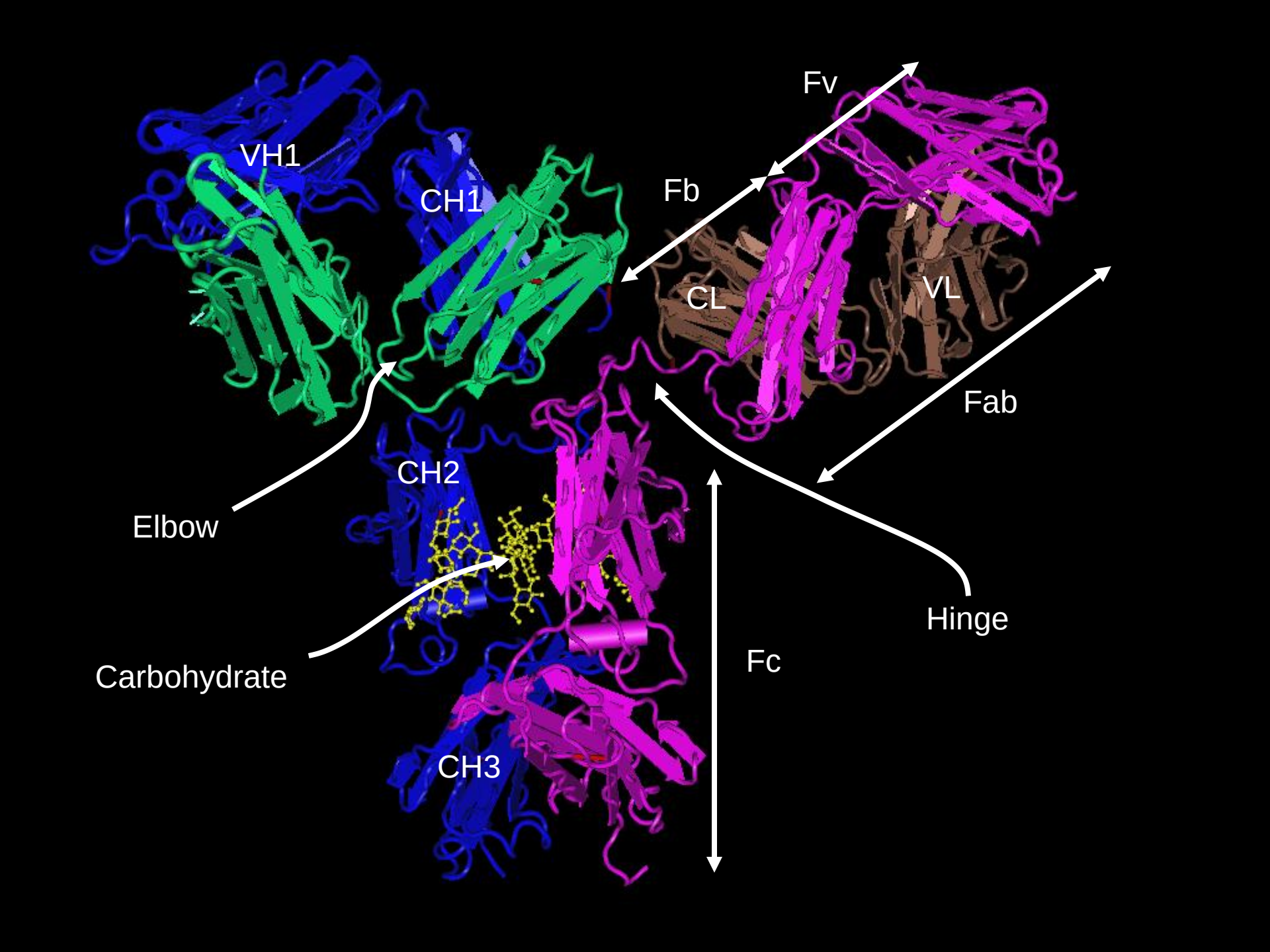
Never a mixture

Mainly, **C_H2 & C_H3 (& C_H4 of IgM & IgE)**, are responsible for the class-specific functions of different Igs

The L chains and H chains are synthesized seperately

Pepsin cleavage sites - 1 x $(Fab)_2$ & 1 x Fc

Papain cleavage sites - 2 x Fab 1 x Fc


Fv
VH1
CH1
Fb
CL
VL
Fab
CH2
Elbow
Hinge
Carbohydrate
Fc
CH3

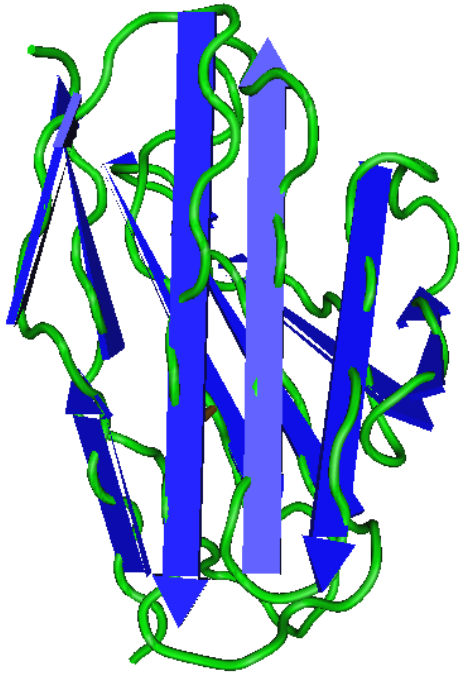
The Immunoglobulin Fold

The characteristic structural motif of all Ig domains

A barrel

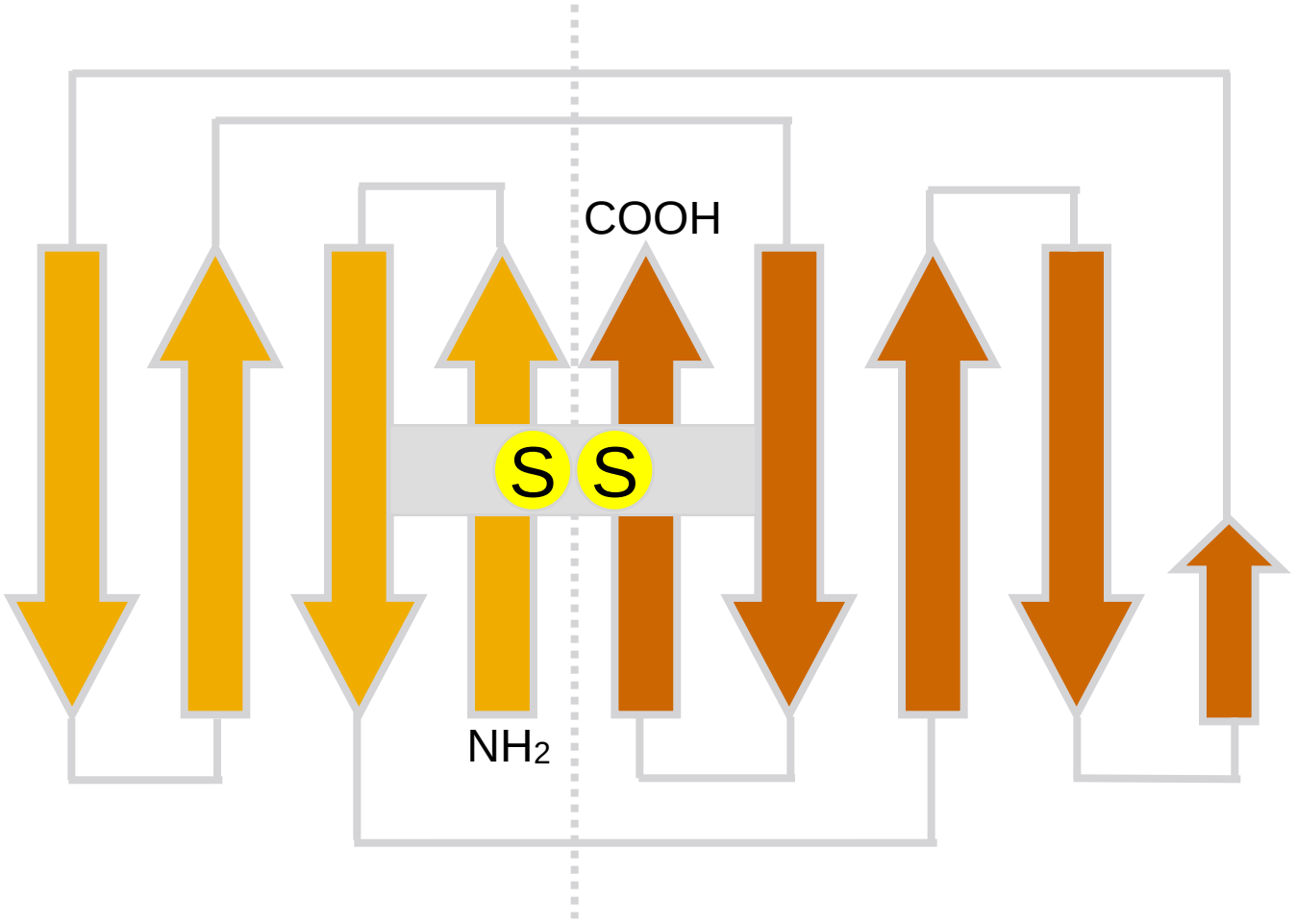
Barrel under construction

A β barrel of 7 (C_L) or 8 (V_L) polypeptide strands connected by loops and arranged to enclose a hydrophobic interior



Single V_L domain

The Immunoglobulin Fold



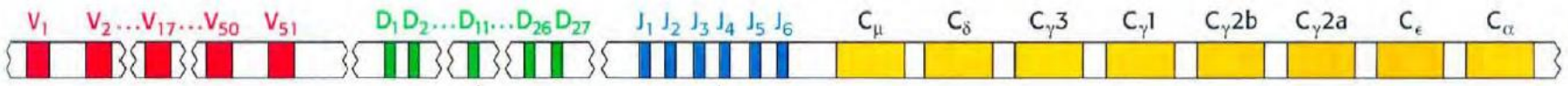
Unfolded V_L region showing 8 antiparallel β-pleated sheets connected by loops

Genes involved

The "one gene, one protein" concept is not valid

- **Light chain is a product of at least 3 genes:**
  - **Variable (V_L) gene**
  - **Joining region (J) gene**
  - **Constant region (C_L) gene**

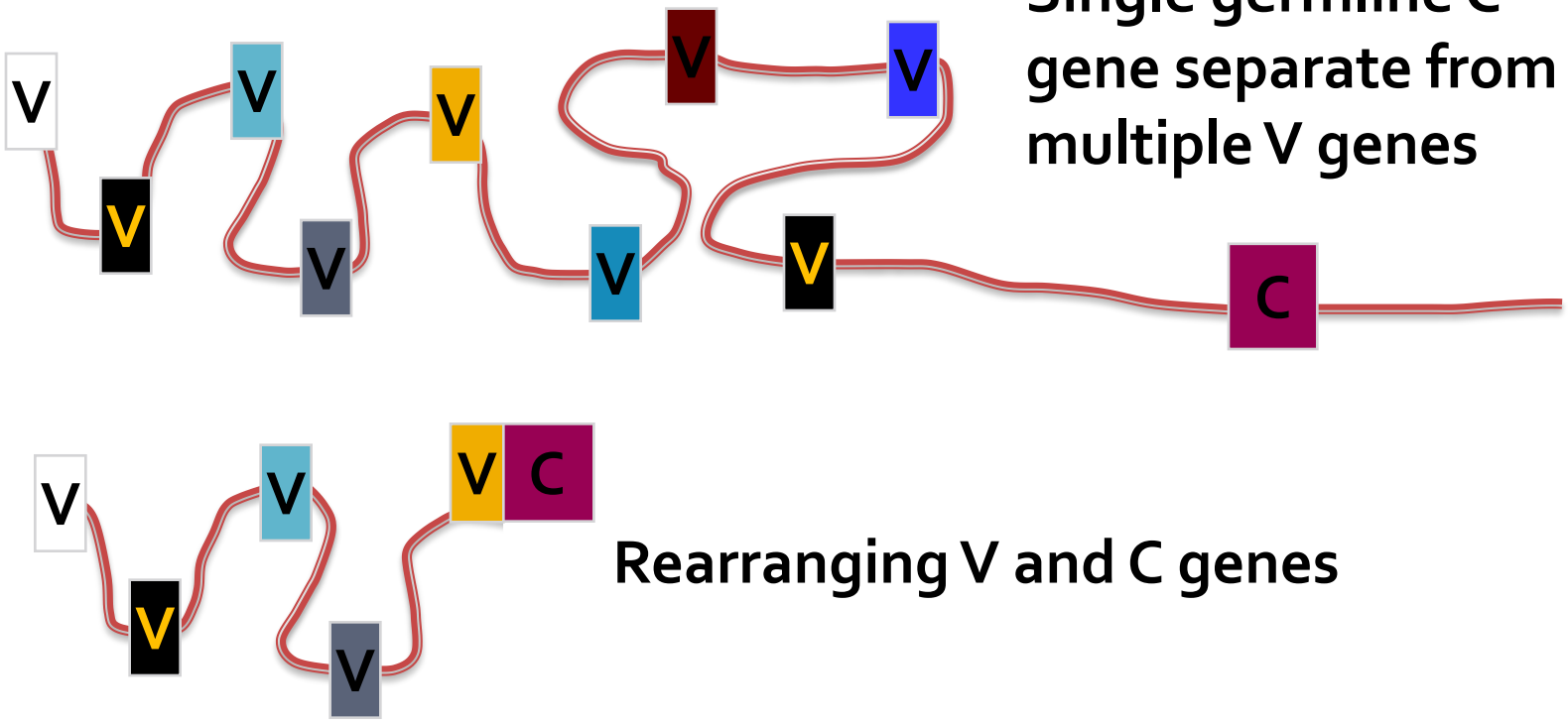
- **Heavy chain is a product of at least 4 genes :**
  - **Variable region (V_H) gene**
  - **Diversity region (D) gene**
  - **Joining region (J) gene**
  - **Constant region (C_H) gene**



Combinatorial diversity: How does diversity occur?

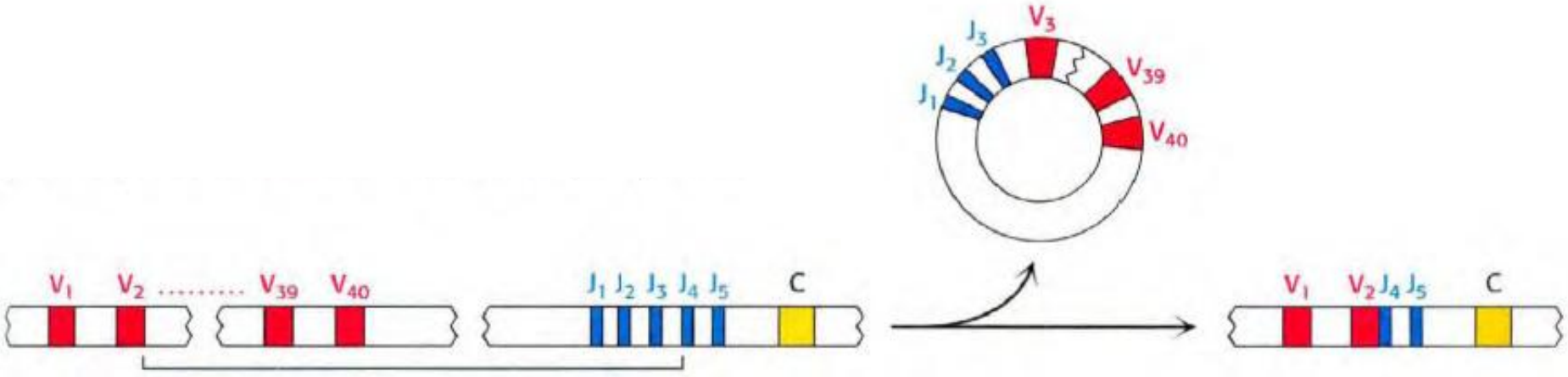
Dreyer - Bennett hypothesis

- Immune system can generate > 10^8 antibodies
- Human genome contains ~ 40,000 genes !

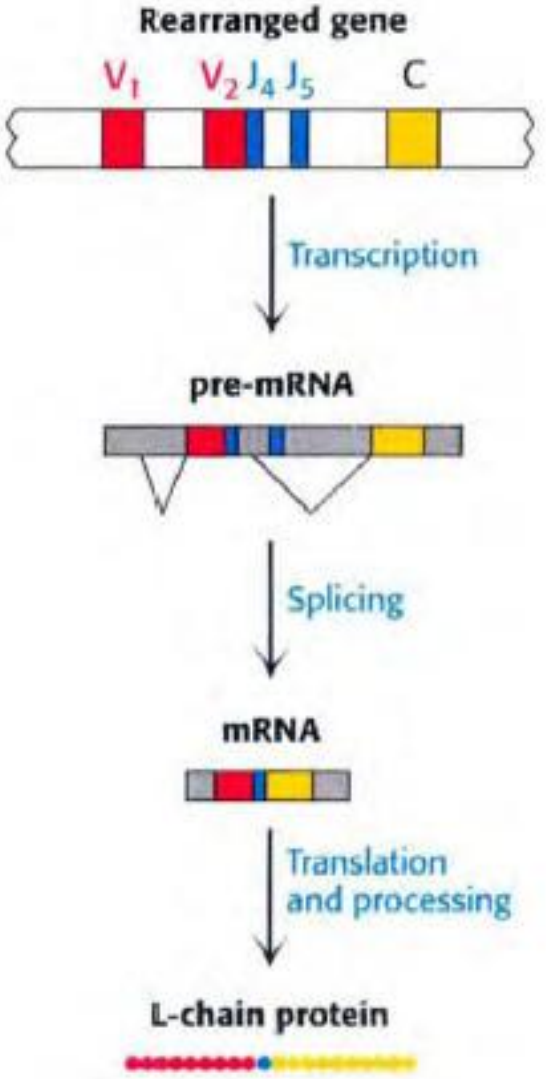


How does diversity occur? Rearrangement & splicing (L chain)

- V genes encode the first 97 amino acids
- J genes encode the last 13 amino acids
- Possible combinations (kappa, κ) = 40* 5 = 200
- Possible combinations (lambda, λ) = 30 * 4 = 120

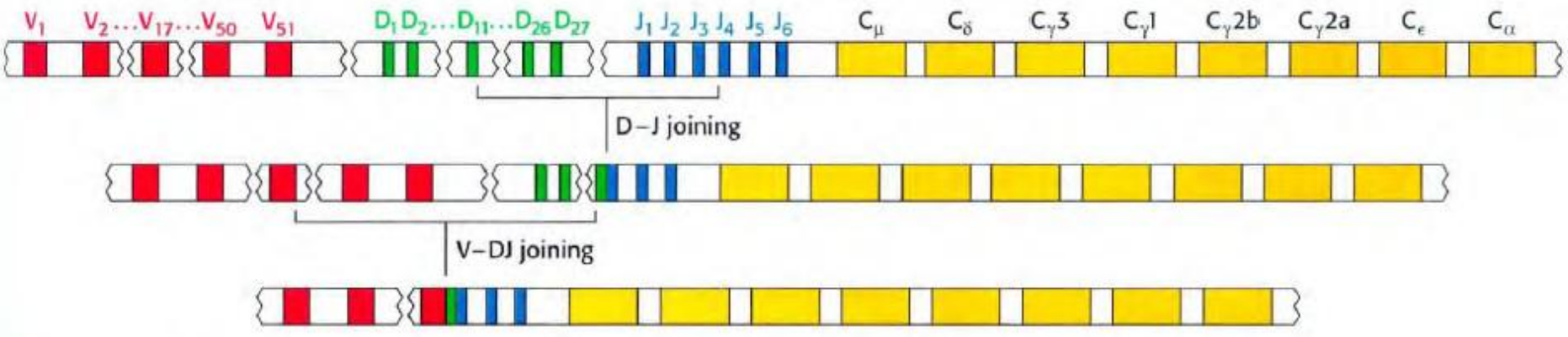


How does diversity occur? Rearrangement & splicing



How does diversity occur? Rearrangement & splicing (H chain)

- Possible combinations = 51 * 27 * 6 = 8262



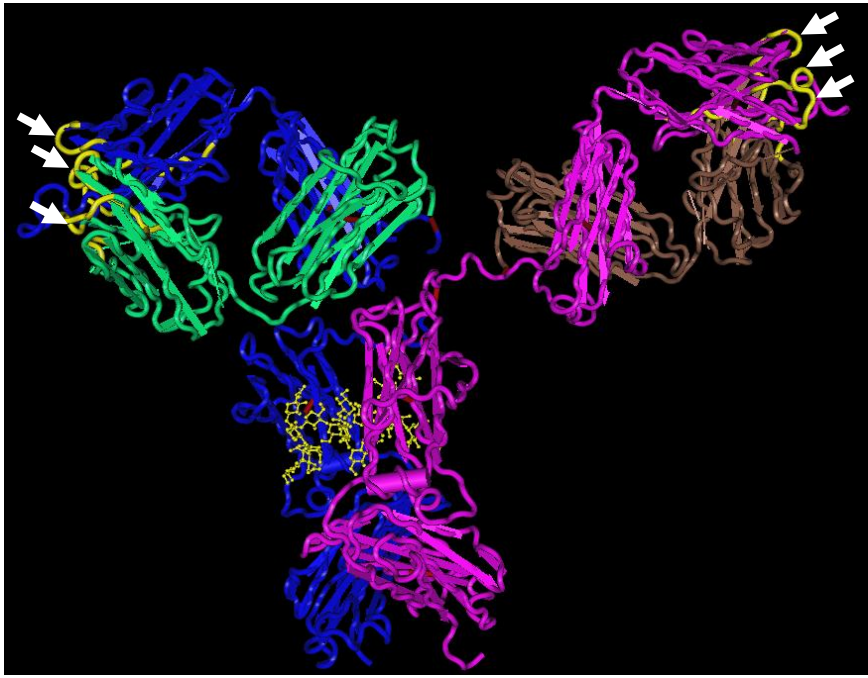
- All possible combinations (L&H) = (200 + 120) * 8262 =

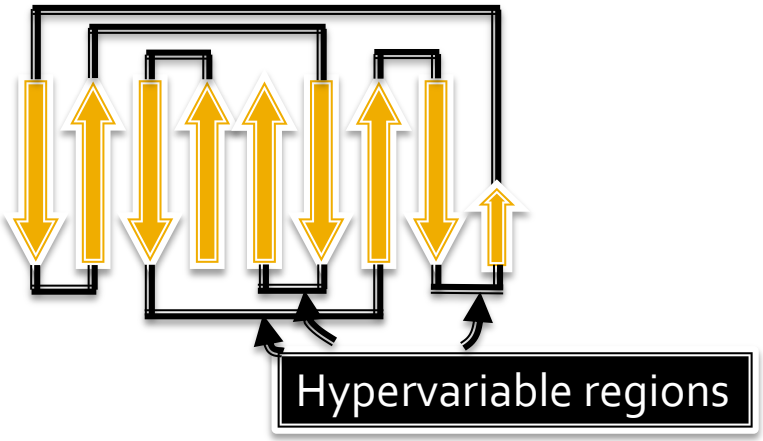
$$2.6 * 10^6$$

- Somatic mutations increases the diversity

Variable Regions

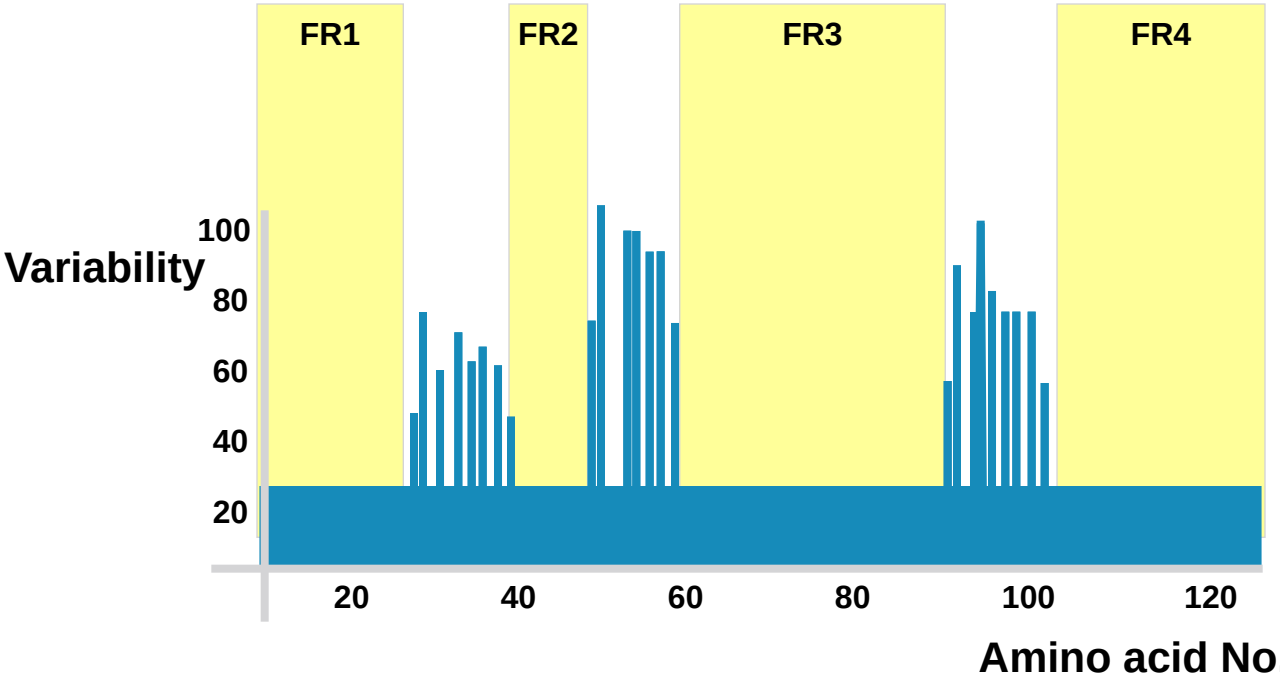
- No two variable regions in different humans are identical
- Relatively invariable regions & other hypervariable regions
- L chains have 3 hypervariable regions (in V_L) & H chains have 4 (in V_H)
- These hypervariable regions comprise the antigen-binding site
- Dictate the amazing specificity of antibodies

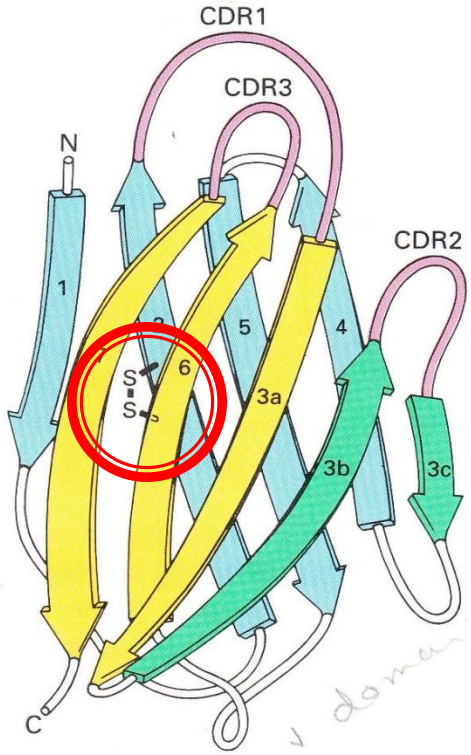




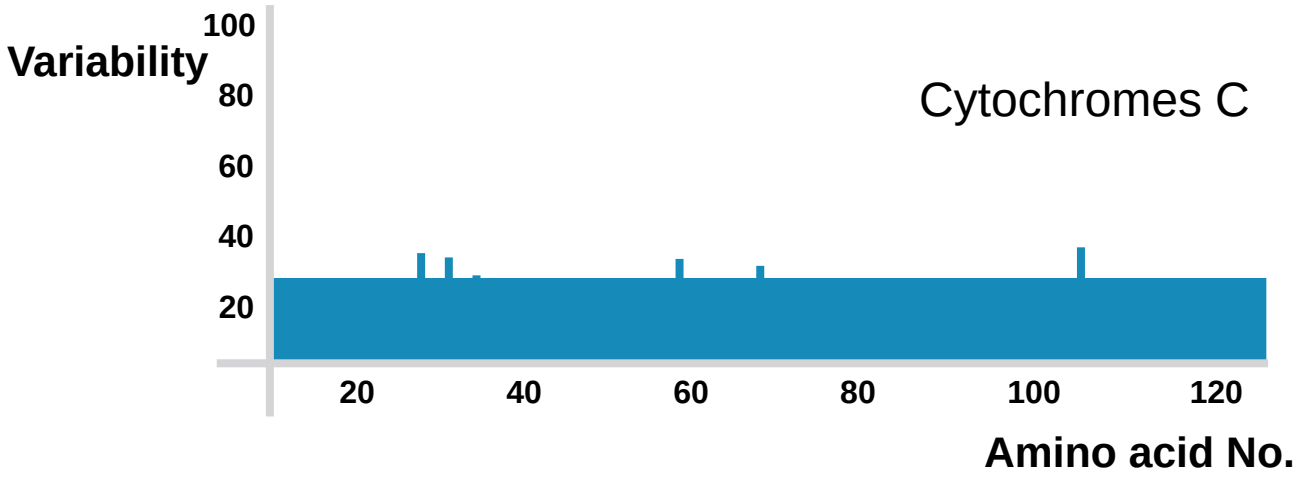
Hypervariable regions
Complementarity-determining regions (CDRs)

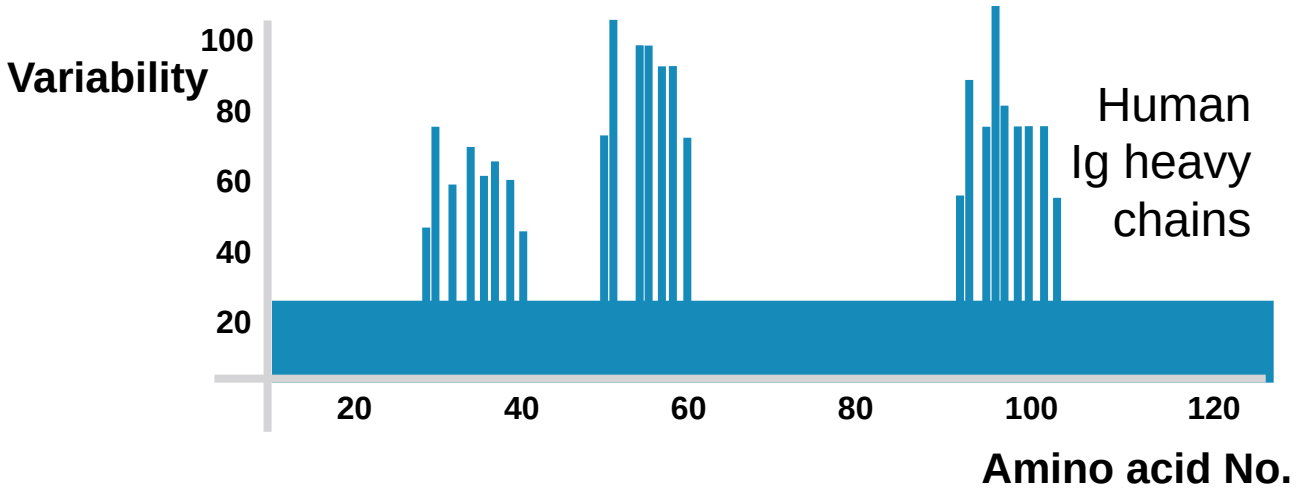
- About 7-12 amino acids in each one that contribute to the antigen-binding site
- CDRs are located on small loops of the variable domains
- Framework regions: the surrounding polypeptide regions among the hypervariable regions





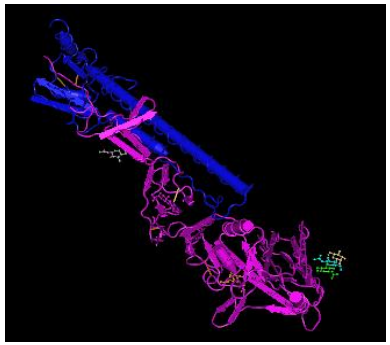
Variability in other proteins



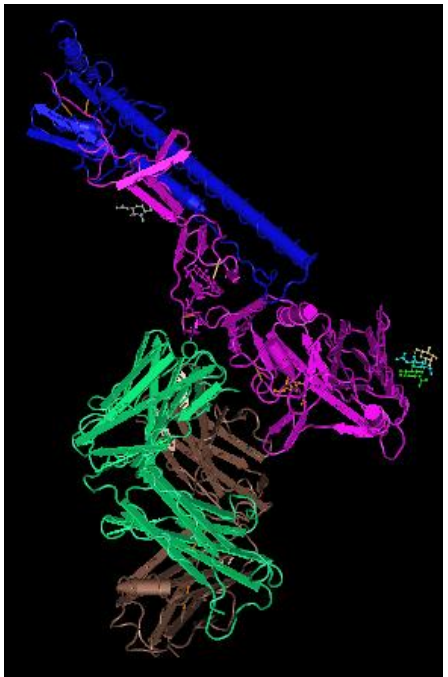


CDRs interaction with antigens

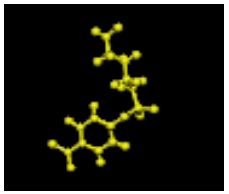
- **Antigen-antibody interactions is based on mutual complementarity between surfaces**
- **Large antigens: interact with all of the CDRs of an antibody**
- **Small antigens: interact with only one or a few CDRs that form a pocket or groove in the antibody molecule**



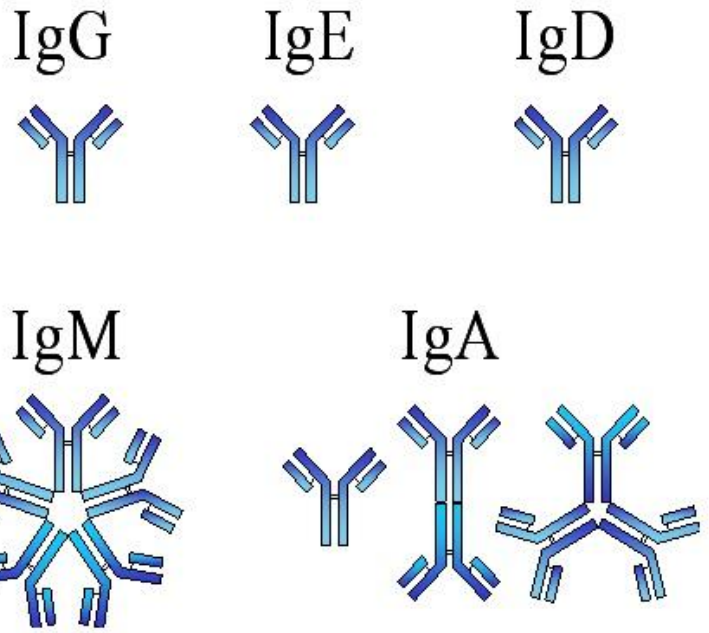
Protein:
Influenza haemagglutinin



Hapten: 5-(para-nitrophenyl phosphonate)-pentanoic acid



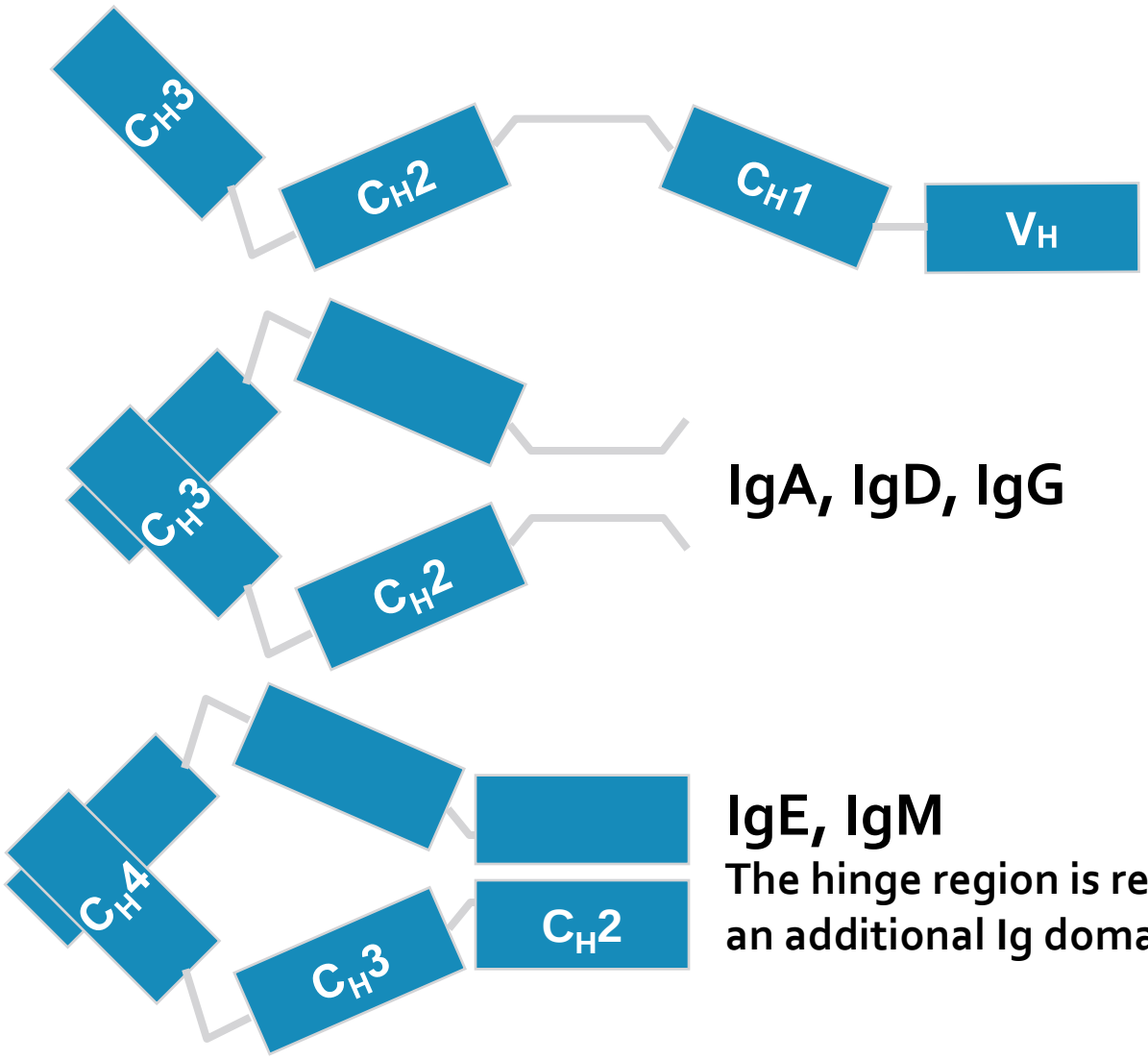
Immunoglobulin classes - overview



- Igs are classified based on the nature of their heavy chain

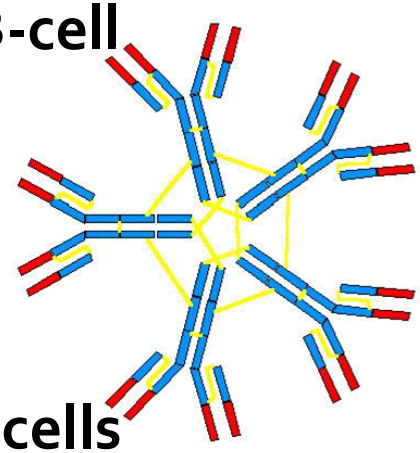
Class	Heavy chain	Chains structure	% in serum	$T_{1/2}$ (days)	Comp. fixation	Placental crossing
IgM	μ	Mono-, penta-, & hexa	5-10	5-10	++++	No
IgG	γ	Monomer	80	23	++	Yes
IgA	α	Mono-, di-, or tri	10-15	6	-	No
IgD	δ	Monomer	0.2-1	3	-	No
IgE	ε	Monomer	0.002	2	-	No

Domains in different classes (H-chain)

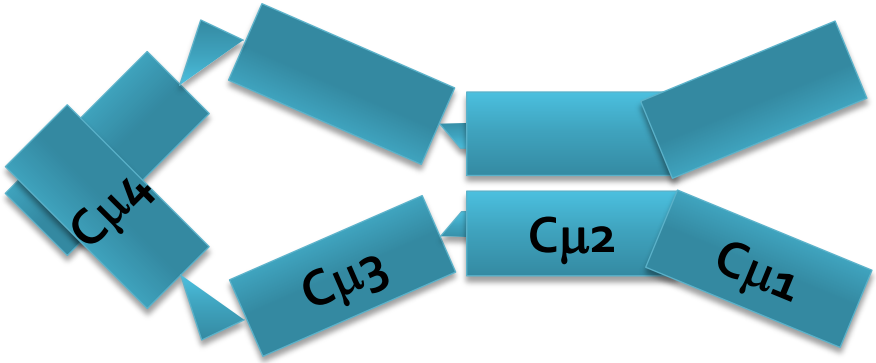


IgM Class

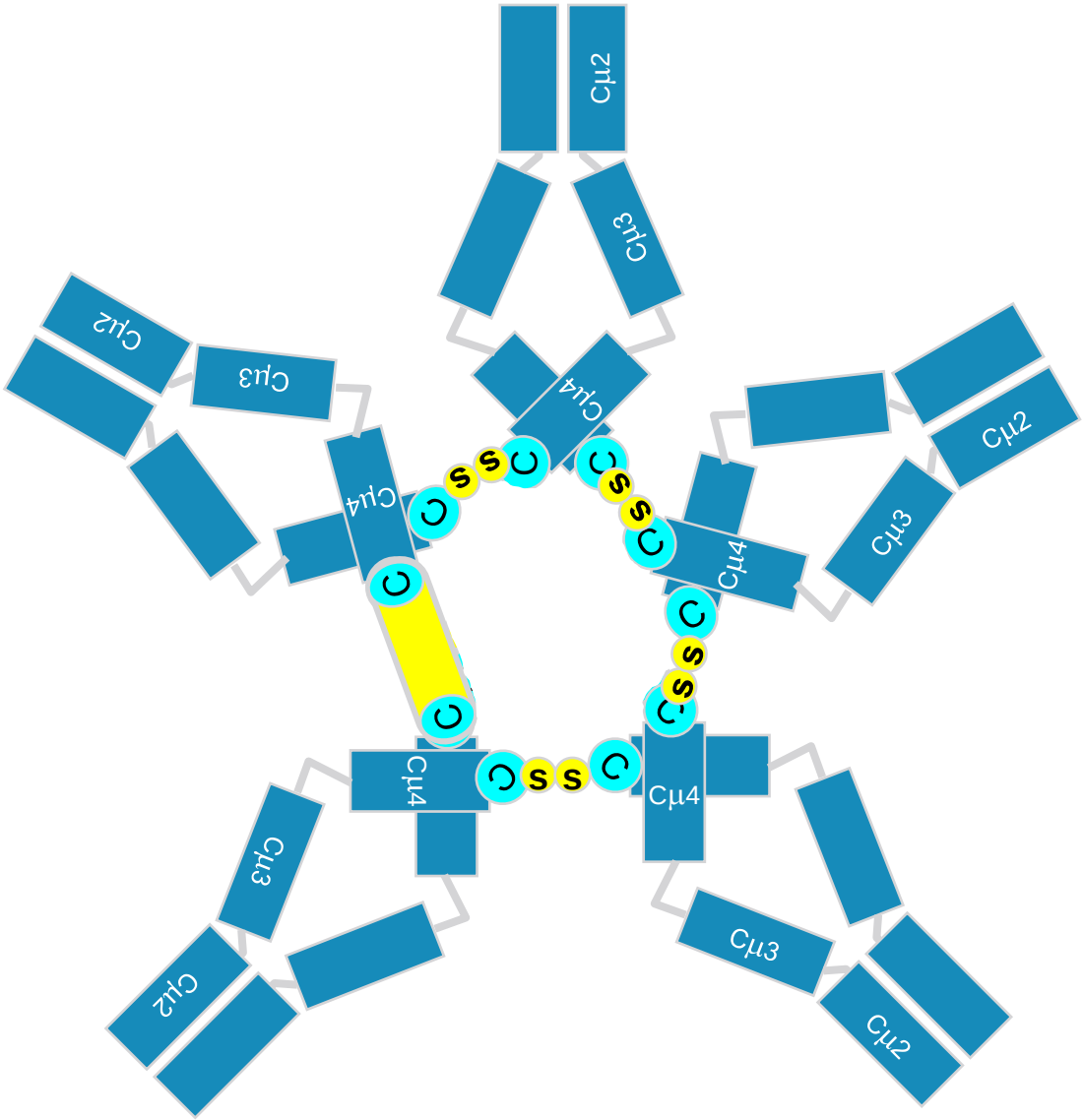
- Location: Mainly intravascular (blood & lymph), B-cell surface (monomer)
- Known Functions:
  - ✓ Primary immune response (1st produced)



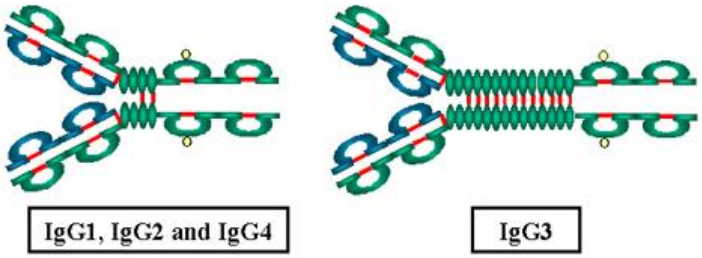
- IgM only exists as a monomer on the surface of B cells
- Monomeric IgM has a very low affinity for antigen
- A J-chain is involved in the process of multemerization
- Cμ4 mediates multimerization (Cμ3 may also be involved)

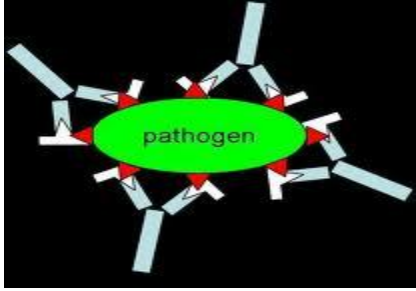


The process of IgM Multimerisation

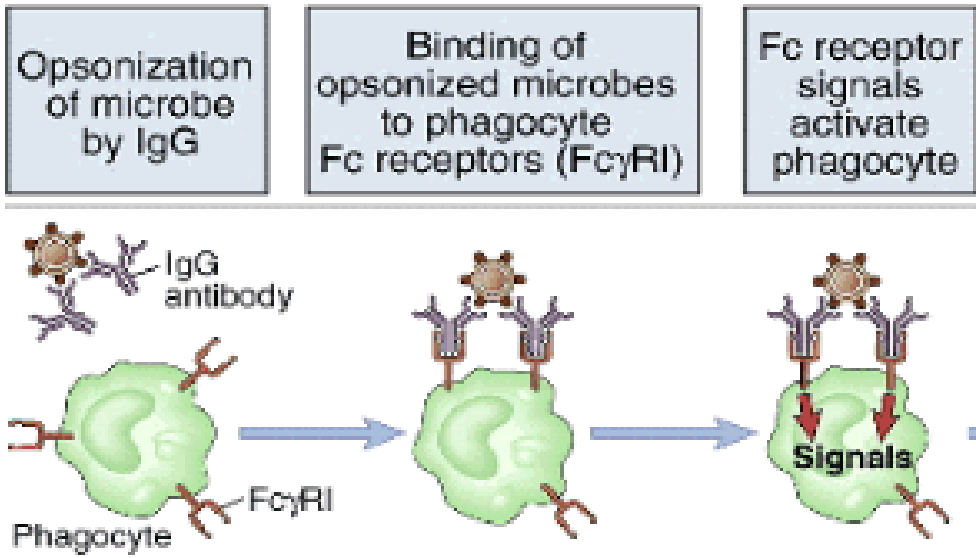


IgG Class



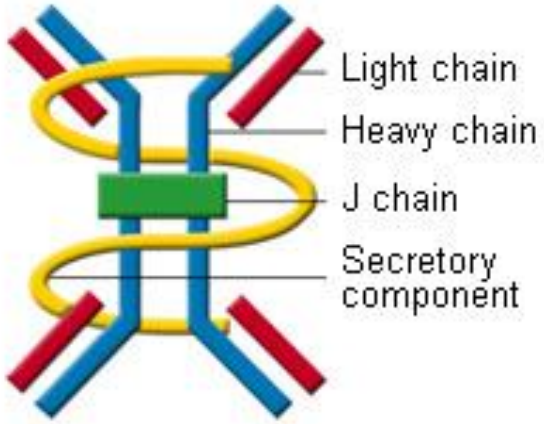


- Location: Blood, lymph, intestine
- Produced in response to a wide variety of antigens, (ex. bacteria, viruses)

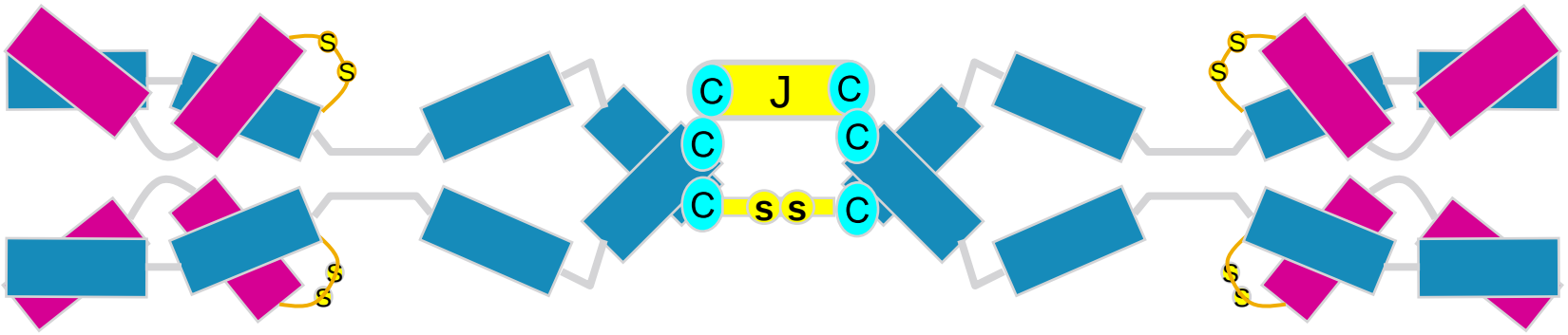


- Known Functions
  - ✓ The predominant antibody produced in the 2° immune response
  - ✓ Provides the major line of defense for the fetus & during first few weeks of newborns
  - ✓ Coats organisms to enhance phagocytosis by neutrophils & macrophages (opsonization)

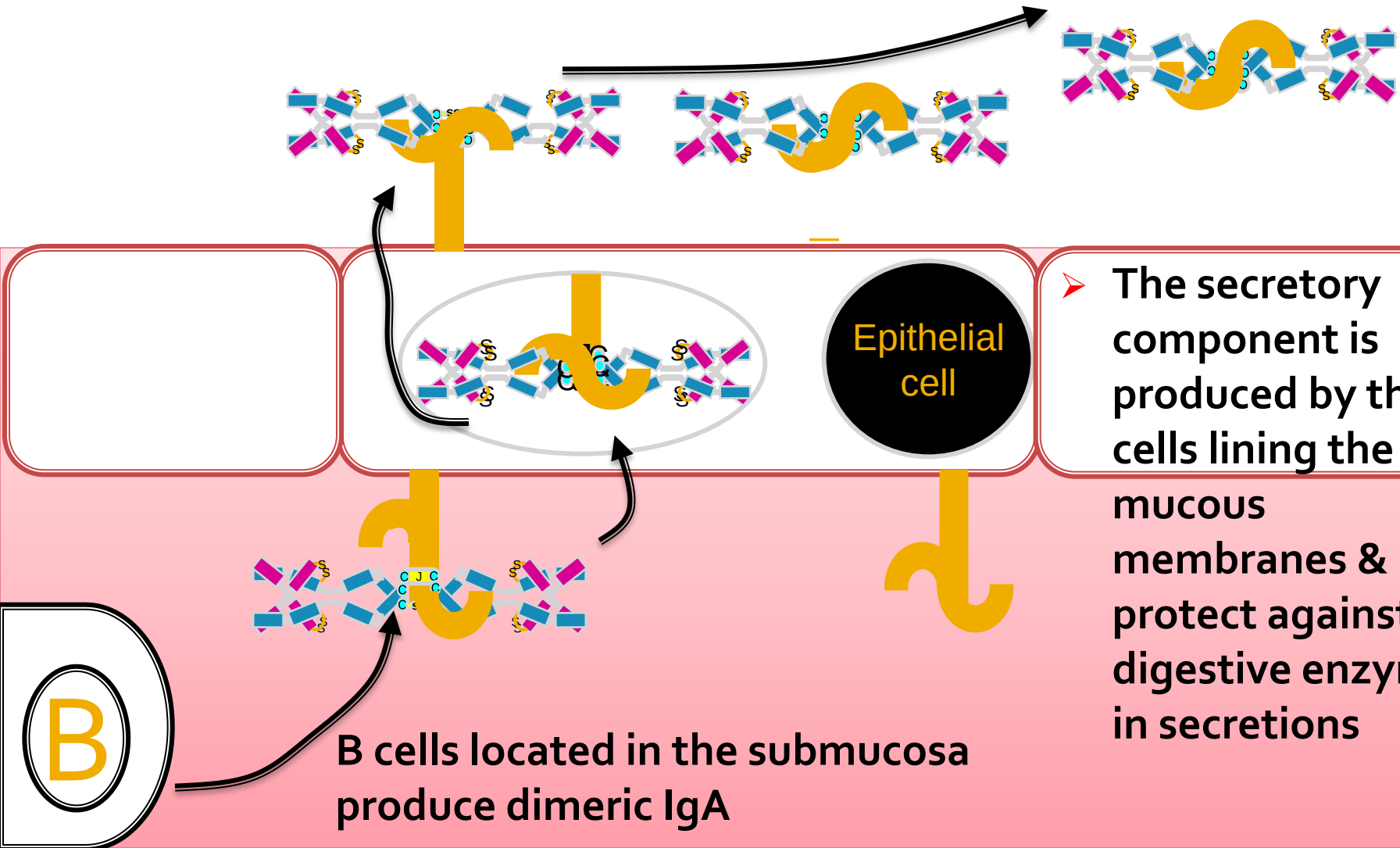
IgA class



- **Structure & location:**
  - ✓ **Plasma → monomer, dimer, or trimer**
  - ✓ **Secretions (tears, saliva, intestines, milk, bronchial secretion, urine) → dimer attached to "secretory component"**
- **Known Functions:**
  - **Localized protection (respiratory & urinary tracts & bowel infections)**
  - **Provides immunity to infant's digestive tract & body (translocated)**
- **The process of dimerization**



IgA & transcytosis



- The secretory component is produced by the cells lining the mucous membranes & protect against digestive enzymes in secretions

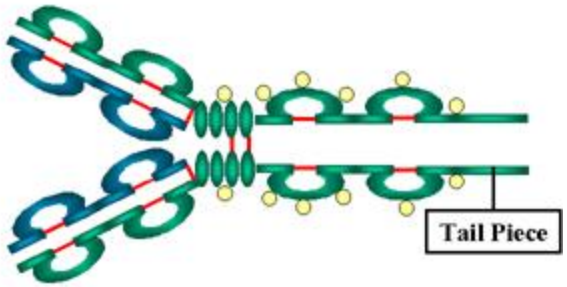
B cells located in the submucosa produce dimeric IgA

IgD class

- Location: B-cell surface (primarily), blood, & lymph
- Known Functions:
  - ✓ In serum: function is unknown
  - ✓ On B cell surface: initiate immune response

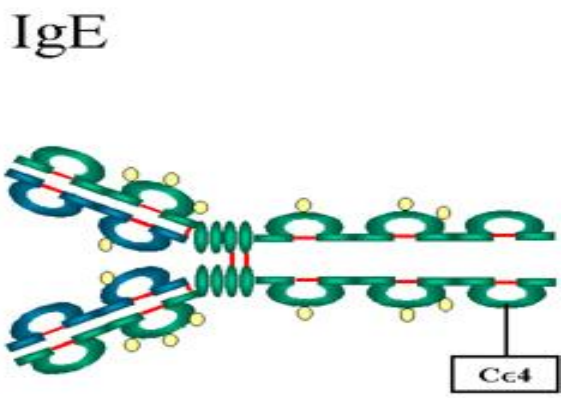
IgD

- Structure
  - Monomer
  - Tail piece

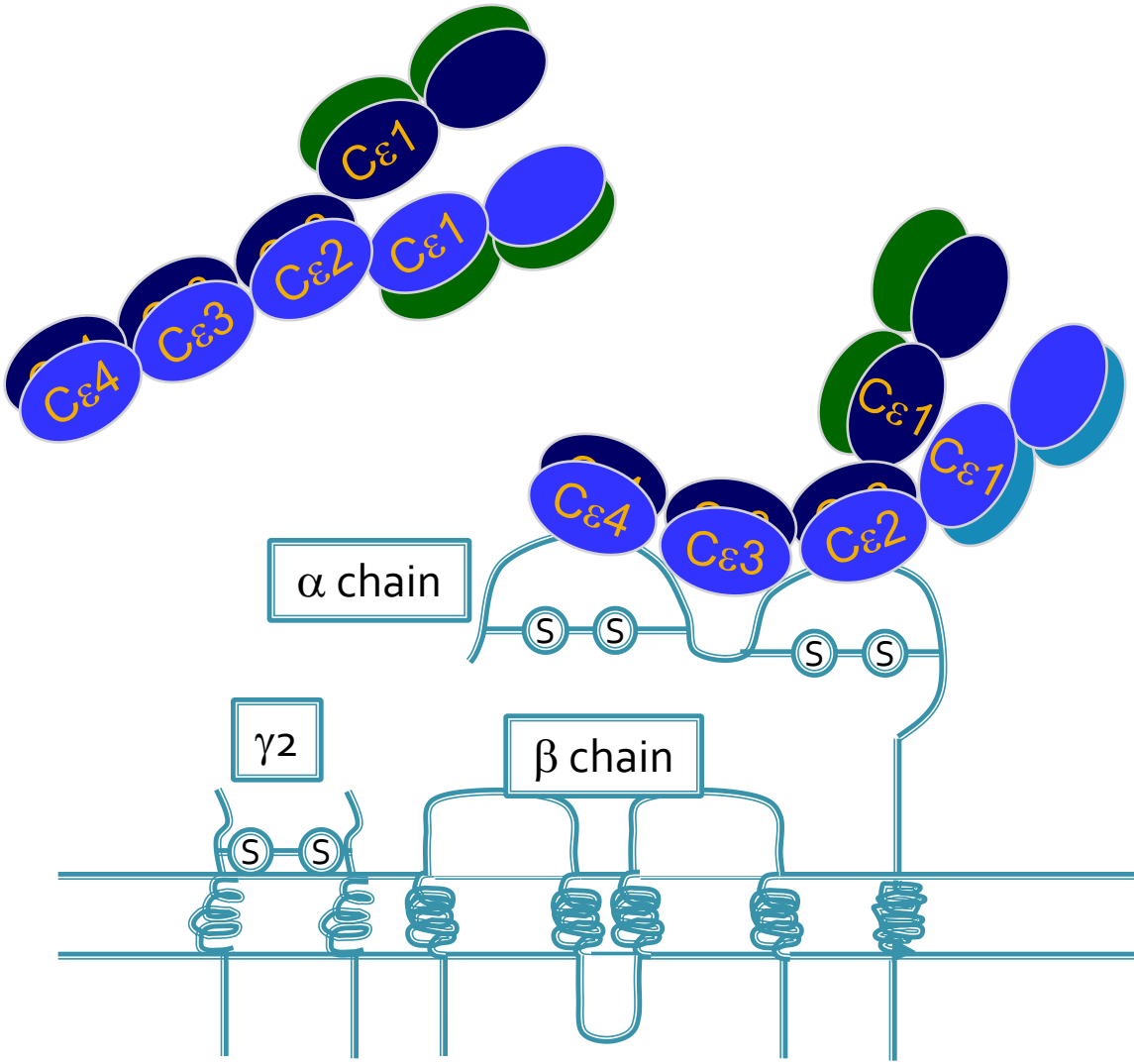


IgE class

- Location: Blood & bound to mast cells & basophils throughout body
- Known Functions:
  - Allergic reactions (histamines & heparin): increased vascular permeability, skin rashes, respiratory tract constriction (wheezing), & increased secretions from epithelium (watery eyes, runny nose)
  - Possibly lysis of worms

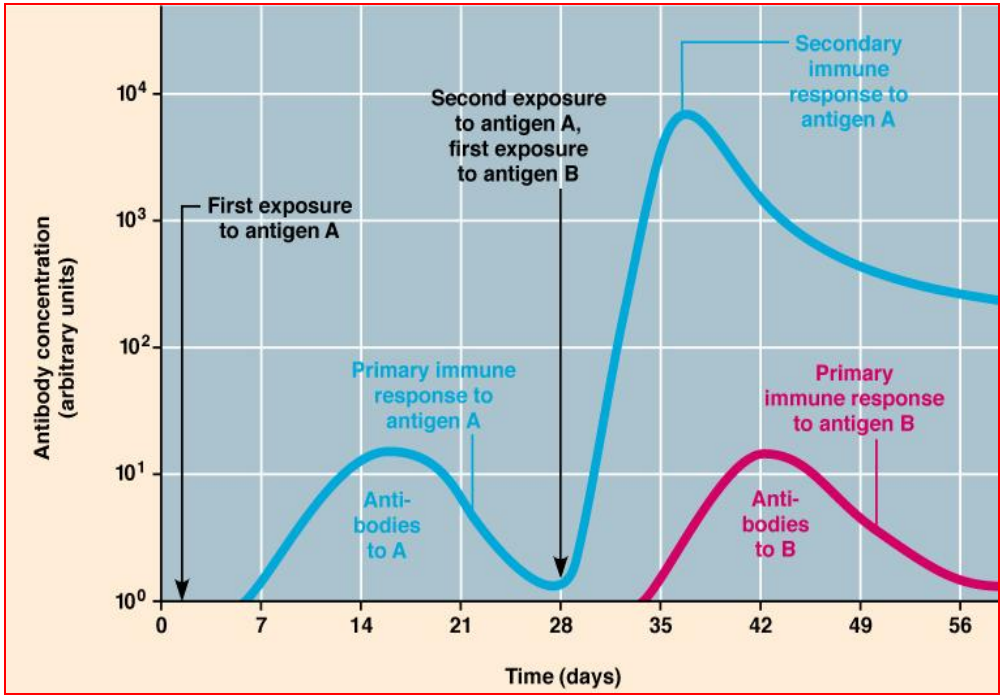


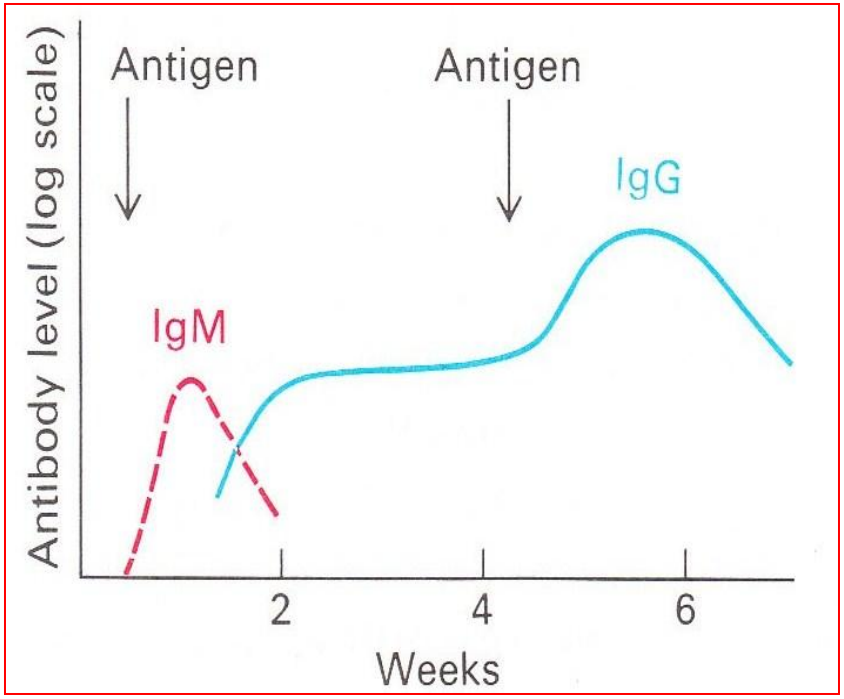
IgE-receptor affinity



- The IgE–receptor interaction is the highest affinity of any Fc receptor
- Binding of IgE to the receptor increases the half life

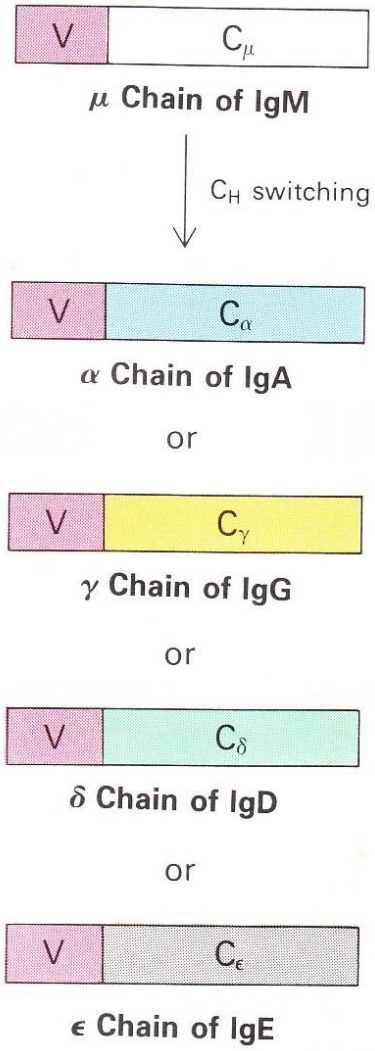
Immunological Memory





Class (Isotype) Switching

- Antibodies with identical specificity but of different classes
- Generated in a chronologic order in response to the antigen
- Gene rearrangement: movement of VDJ from a site near one C gene to a site near another C gene



Diseases

- Myelomas: increased production
- Multiple myeloma: a neoplastic condition, increase in one class, or a particular light chain (Bence Jones protein)
- Decreased production may be restricted to a single class or may involve underproduction of all classes (ex. agammaglobulinemia)