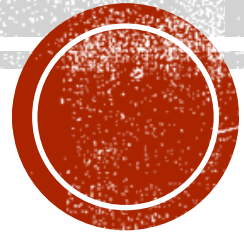
IMMUNOGLOBULINS



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 ✓ The inflammatory response	Lymphocytes Antibodies

ACQUIRED (SPECIFIC) IMMUNITY

- Two major components:
  - T lymphocytes (thymus, cell-mediated immunologic processes; graft rejection, hypersensitivity reactions, & defense against malignant cells and many viruses)
  - B lymphocytes (bone marrow, synthesis of circulating, humoral antibodies; Igs)
    - plasma cells: specialized B cells that synthesize and secrete immunoglobulins into the plasma in response to exposure to antigens
- Genetic deficiency is reported (recurrent infections)

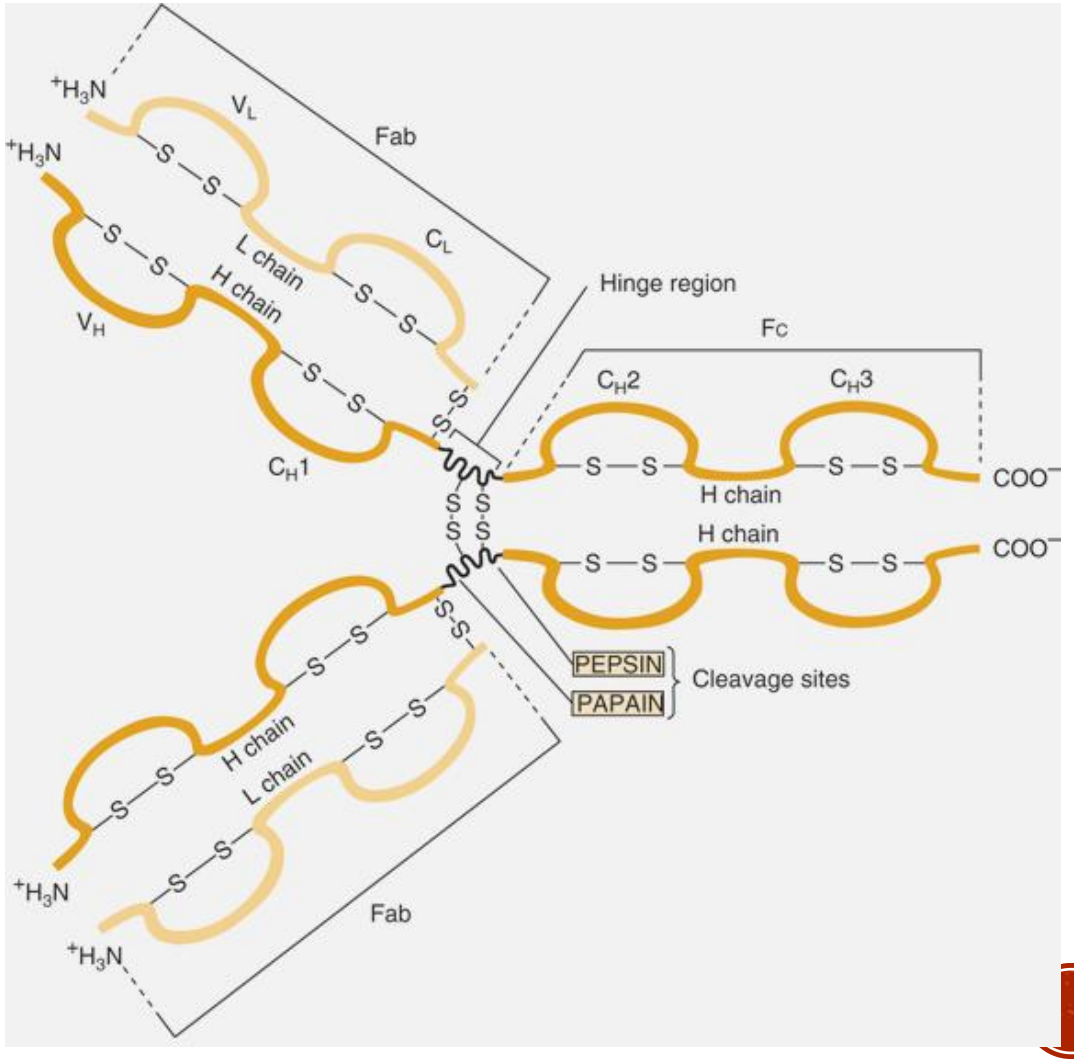
IMMUNOGLOBULINS & ANTIGENS

- Antibodies: glyco-proteins 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

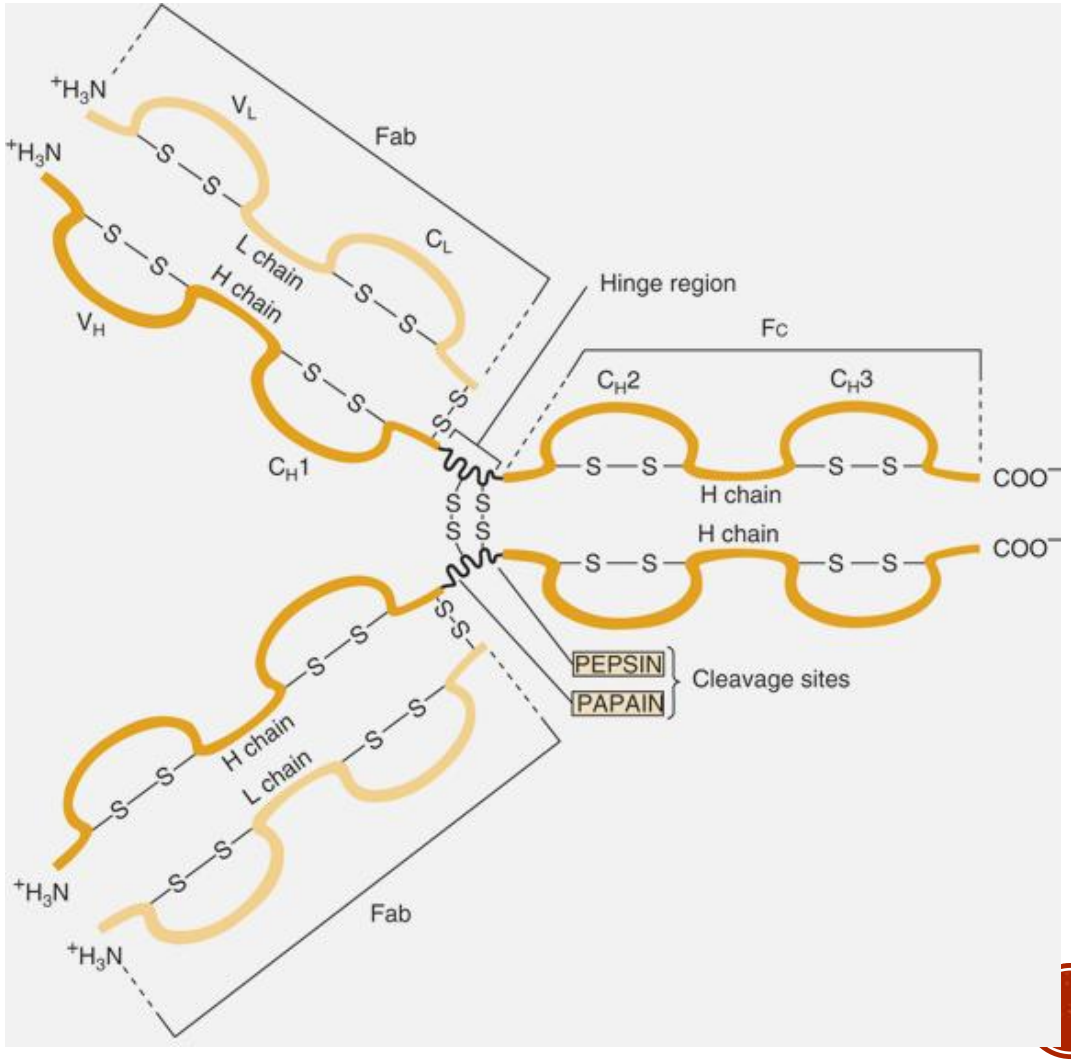
IMMUNOGLOBULINS - STRUCTURE

- 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)



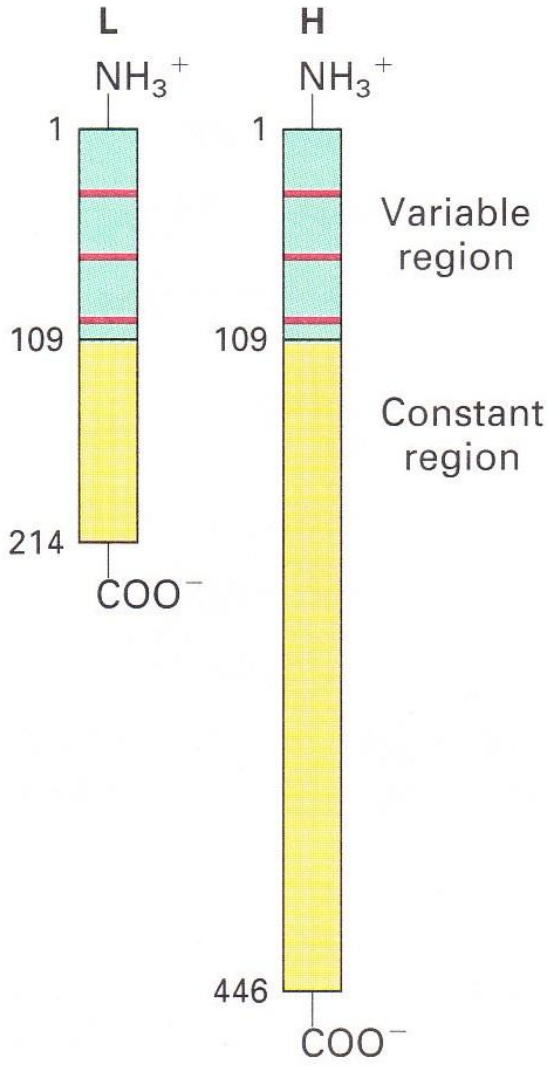
IMMUNOGLOBULINS - STRUCTURE

- Antigen binds V_H & V_L domains
- Papain: 2 antigen-binding fragments (Fab) and one crystallizable fragment (Fc)
- Pepsin: one $(Fab)_2$ fragment and one crystallizable fragment (Fc)
- Hinge region: C_H1 & C_H2 domains; flexibility & independent movement
- Fc & hinge regions differ in different classes of antibodies



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) hyper-variable



IMMUNOGLOBULIN - INTERACTIONS

- With antigen (infinite):
  - Electrostatic, Hydrogen, Van der Waal's, Hydrophobic
  - The (Fab)2 fragment CAN:
    - Detect, bind & precipitate the antigen
    - Block the active sites of toxins
    - Block interactions between host and pathogen

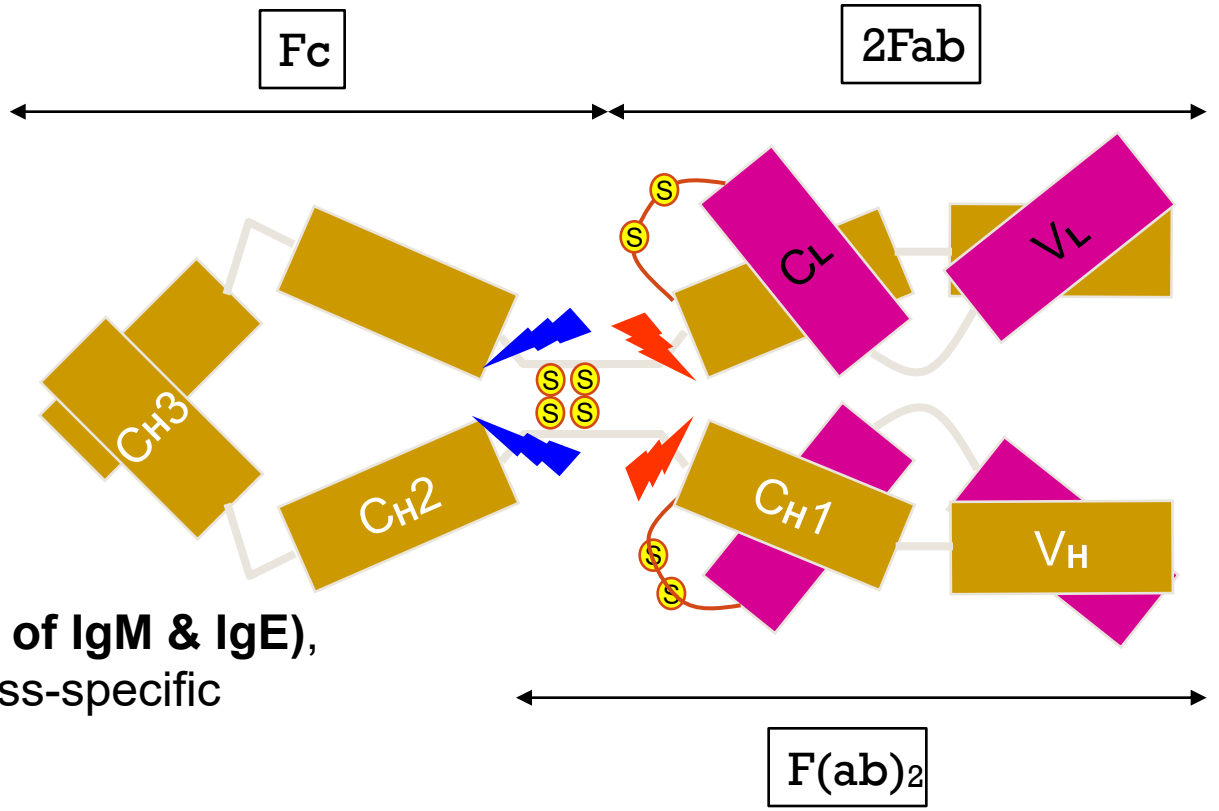
- With other cells and molecules through the Fc portion (finite)
  - The (Fab)2 fragment CANNOT activate:
    - Inflammatory functions associated with cells
    - Inflammatory functions of complement proteins
    - Intracellular cell signaling molecules

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

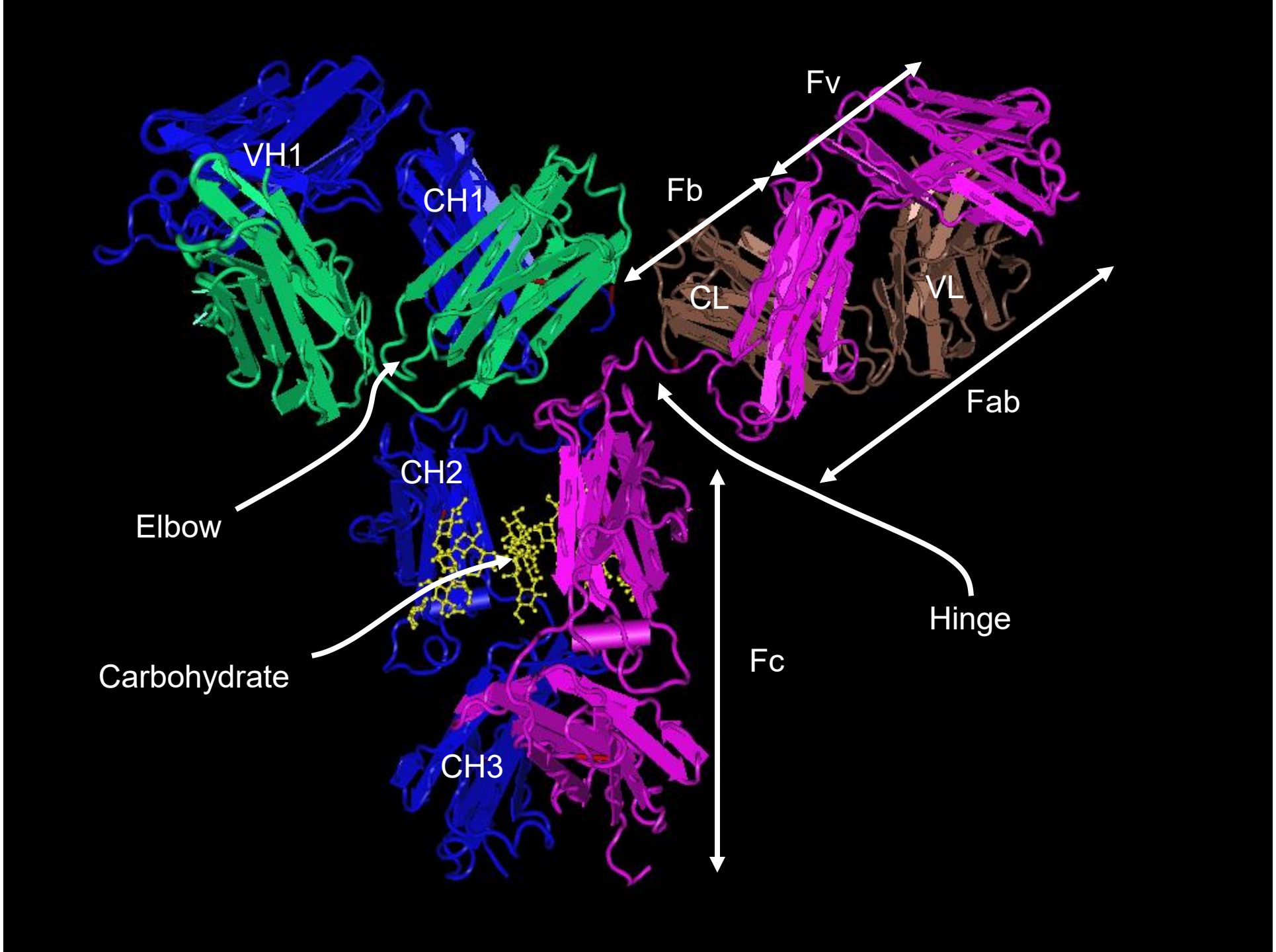


Mainly, **C_H2 & C_H3 (& C_H4 of IgM & IgE)**, are responsible for the class-specific functions of different Igs

The L chains and H chains are synthesized seperately

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

Papain cleavage sites - 2 x $(Fab)_1$ & 1 x Fc


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

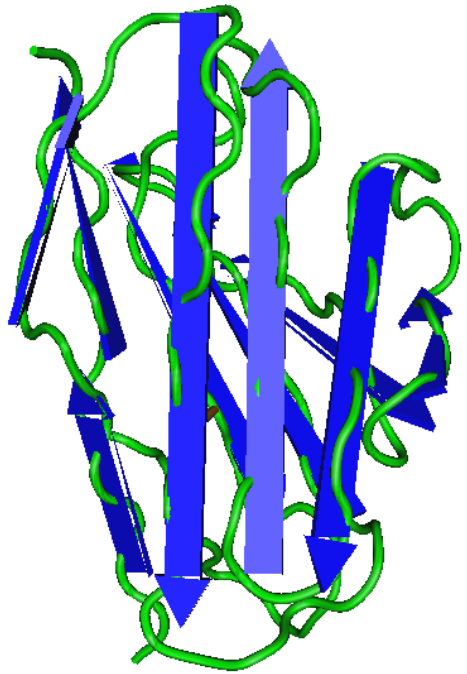
THE IMMUNOGLOBULIN FOLD

The characteristic structural motif of all Ig domains

A barrel

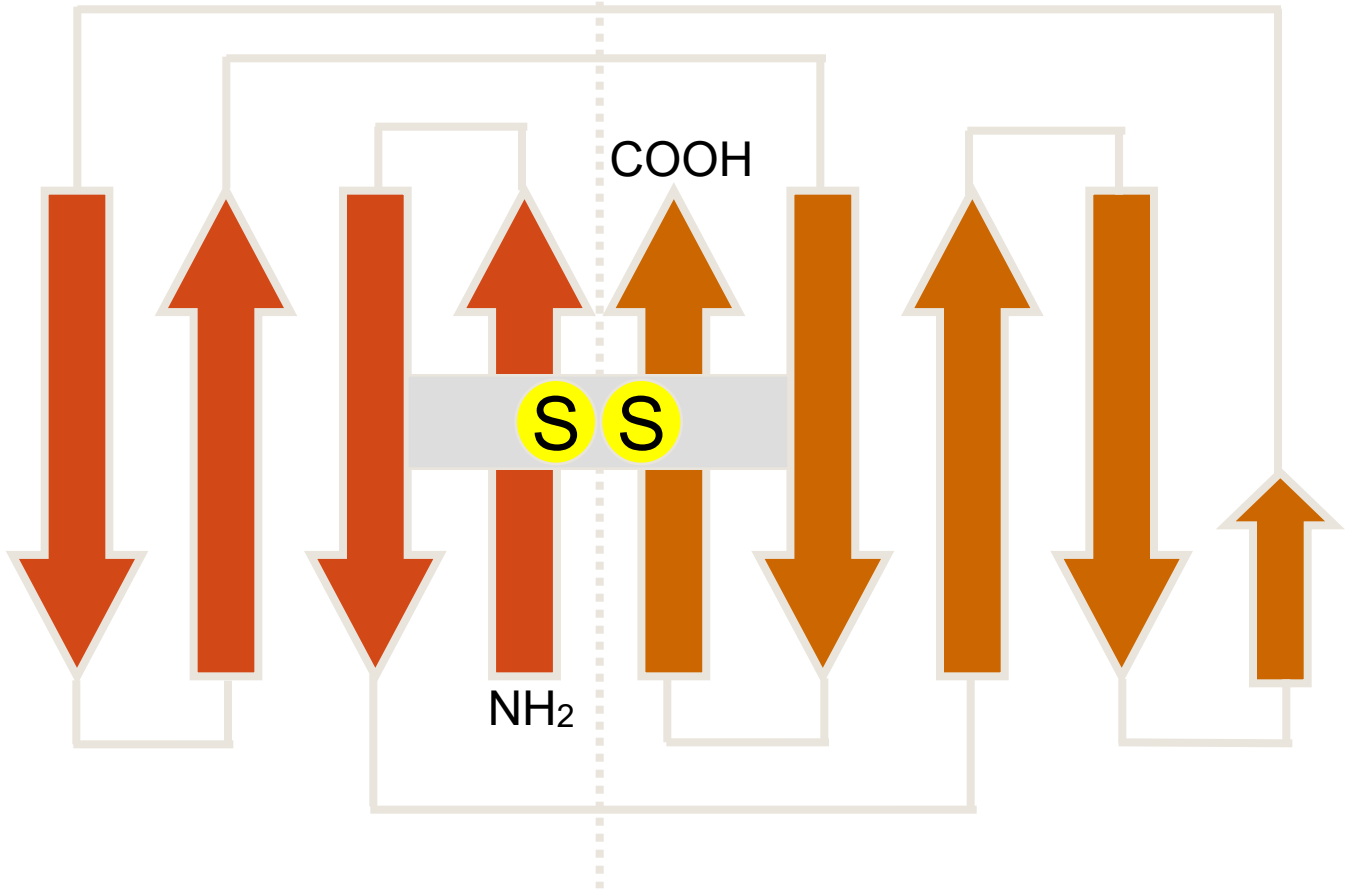
Barrel under construction

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

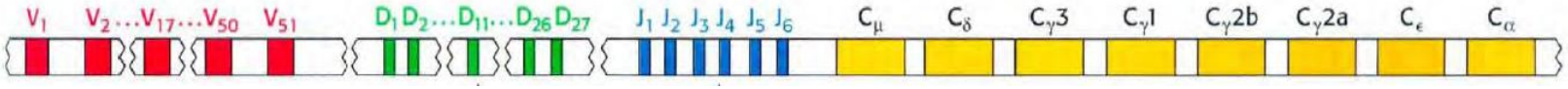
GENES INVOLVED & DIVERSITY

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

- Immune system can generate > 10^8 antibodies
- Human genome contains ~ 25,000 genes !

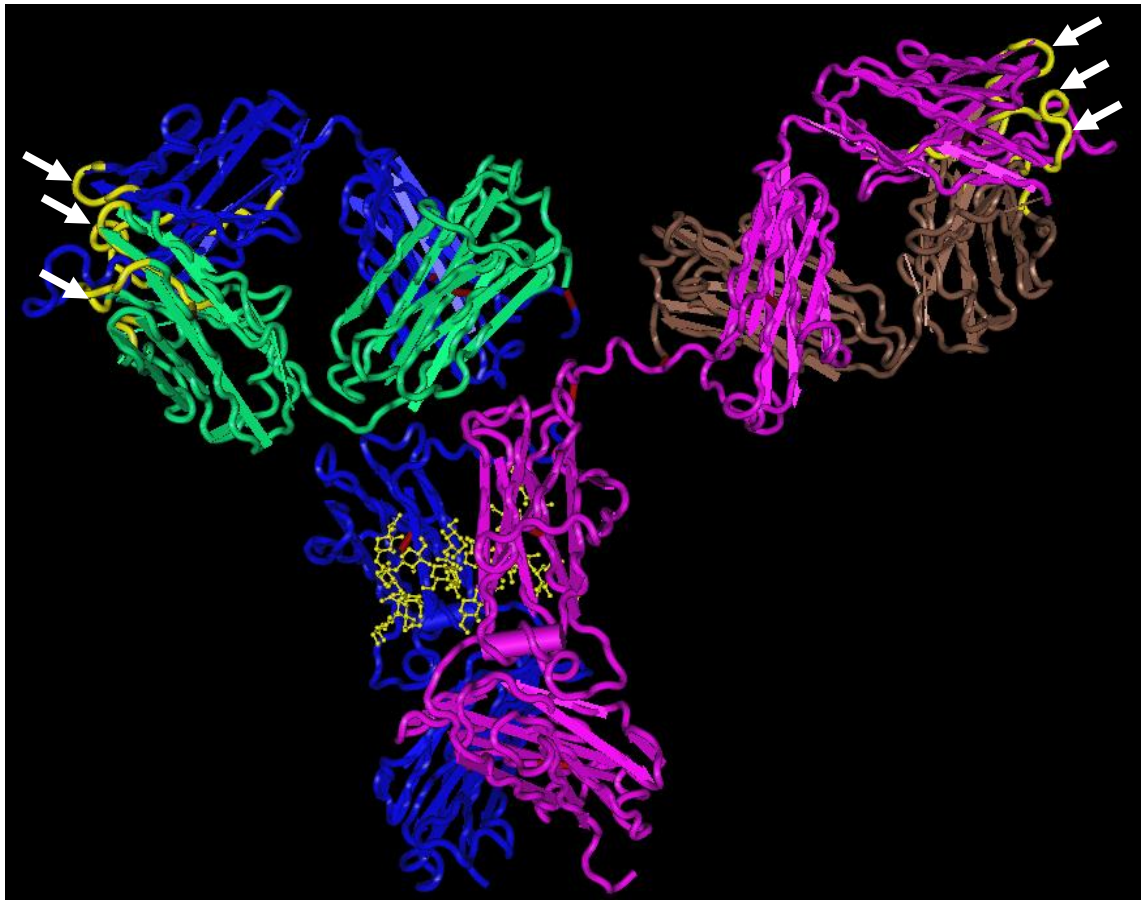
- Light chain is a product of at least 3 genes:
  - Variable (V_L) gene
  - Joining region (J) gene
  - Constant region (C_L) gene

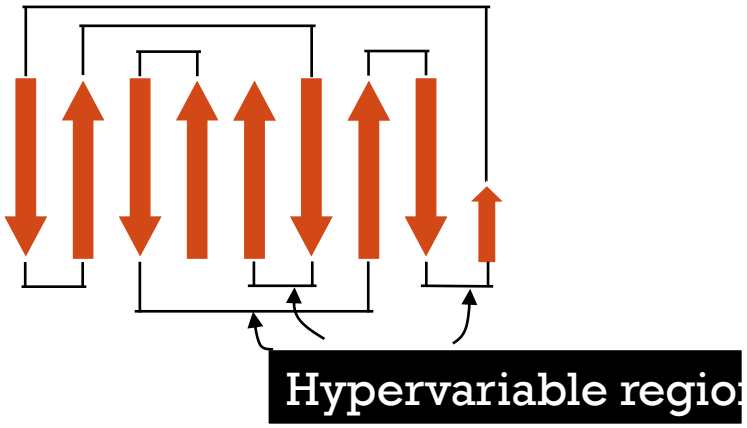
- Heavy chain is a product of at least 4 genes :
  - Variable region (V_H) gene
  - Diversity region (D) gene
  - Joining region (J) gene
  - Constant region (C_H) gene



VARIABLE REGIONS

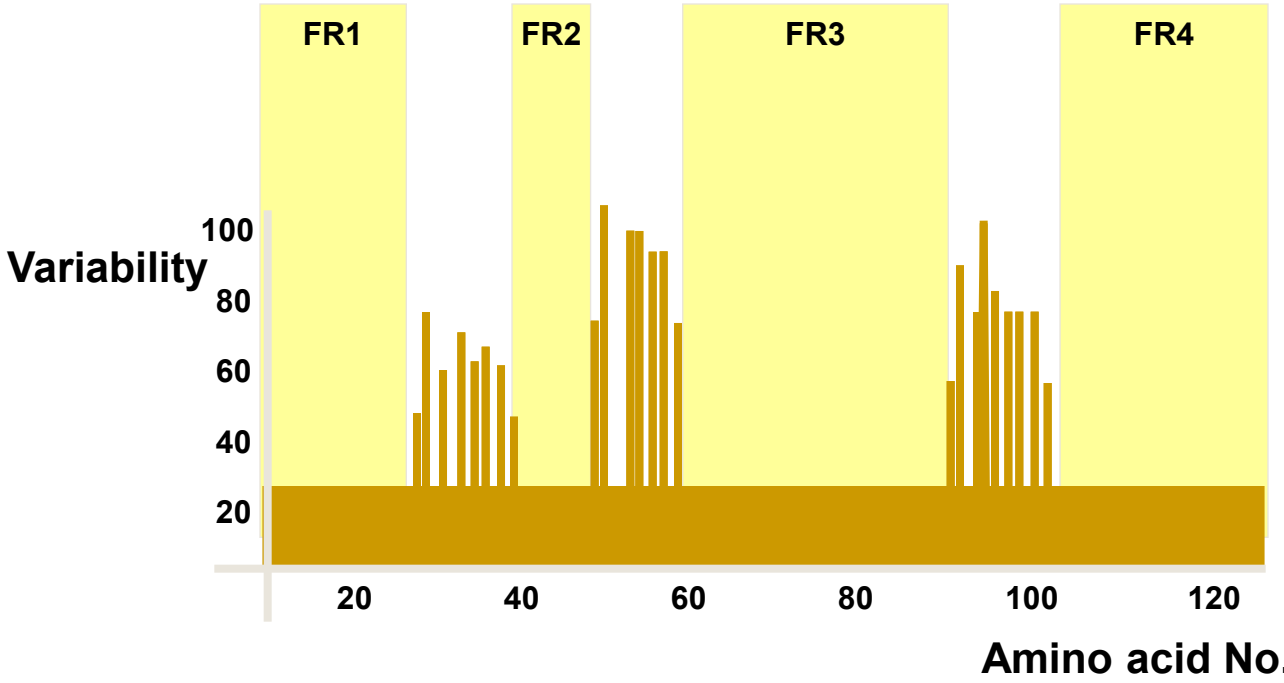
- No two variable regions in different humans are identical
- Relatively invariable regions and other hypervariable regions
- L chains have 3 hypervariable regions (in V_L) and H chains have four (in V_H)
- These hypervariable regions comprise the antigen-binding site
- Dictate the amazing specificity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

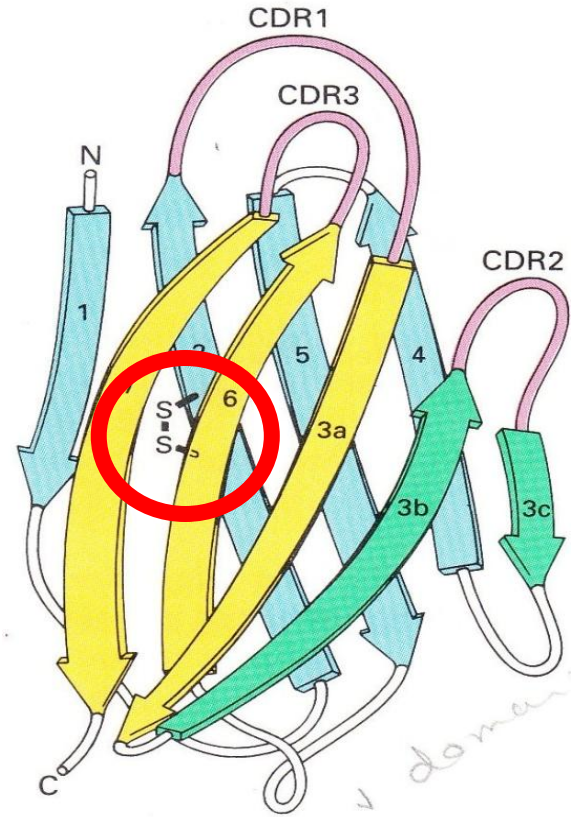




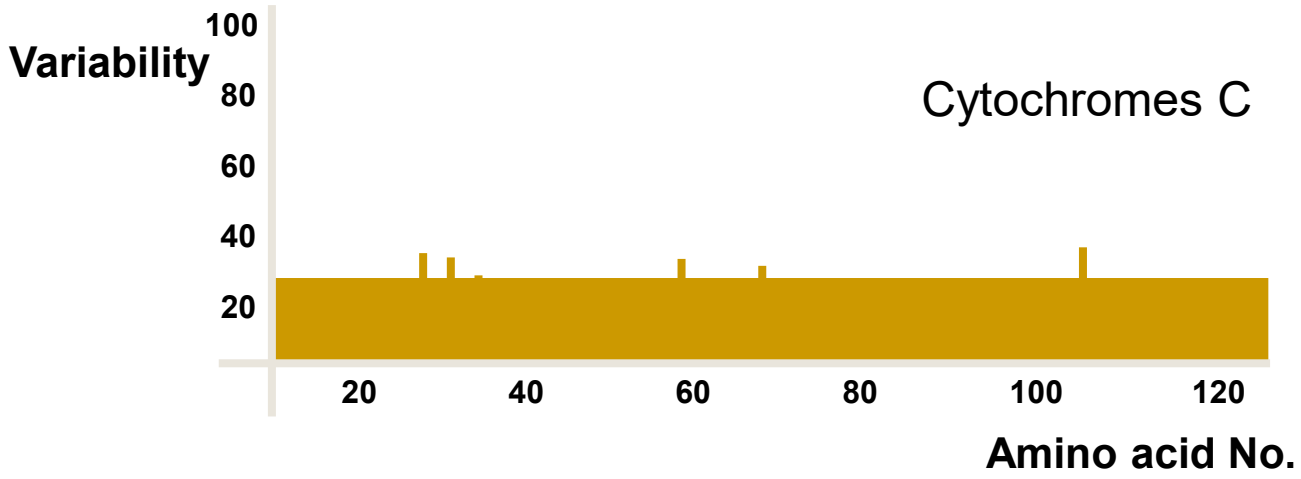
HYPERVARIABLE REGIONS
COMPLEMENTARITY-DETERMINING REGIONS (CDRS)

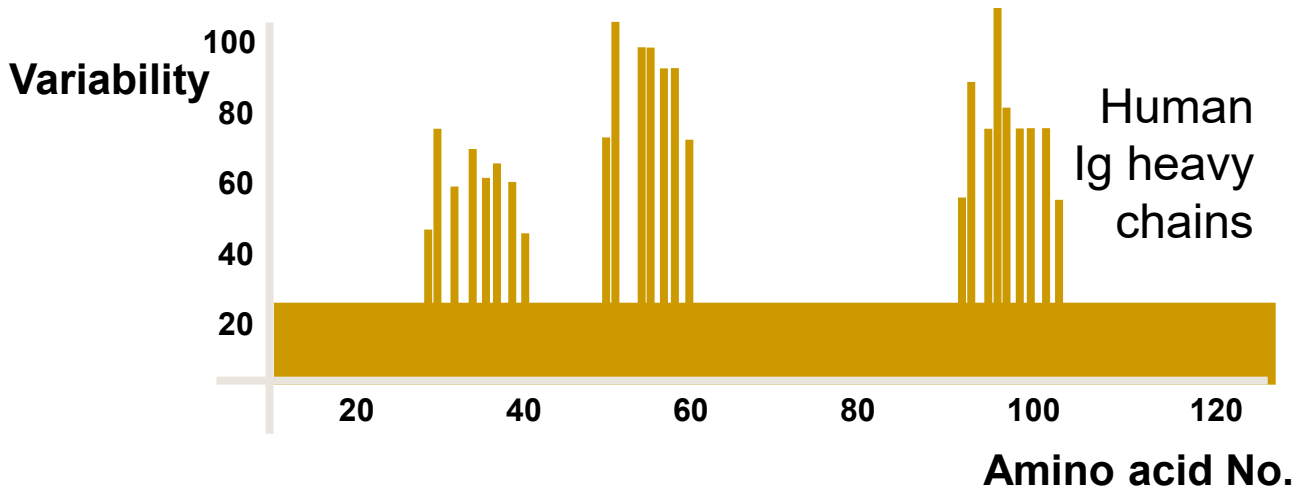
- About 7-12 amino acids in each one that contribute to the antigen-binding site
- CDRs are located on small loops of the variable domains
- Framework regions: the surrounding polypeptide regions among the hypervariable regions





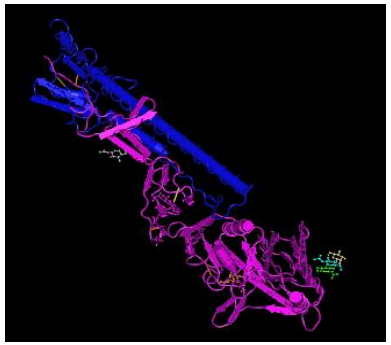
VARIABILITY IN OTHER PROTEINS



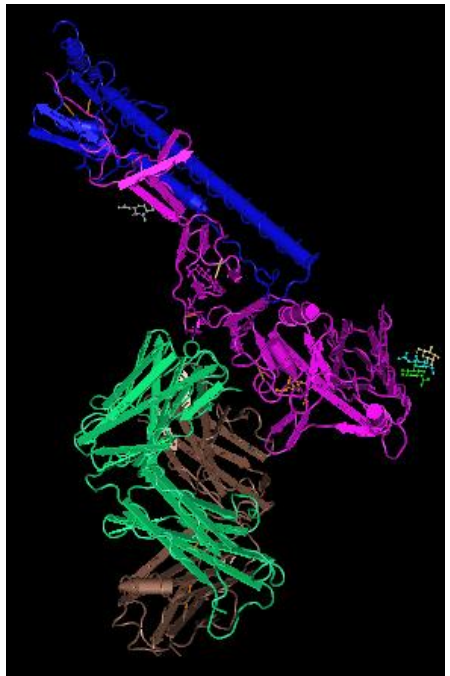


CDRs interaction with antigens

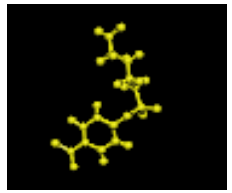
- Antigen-antibody interactions is based on mutual complementarity between surfaces
- Large antigens: interact with all of the CDRs of an antibody
- Small antigens: interact with only one or a few CDRs that form a pocket or groove in the antibody molecule

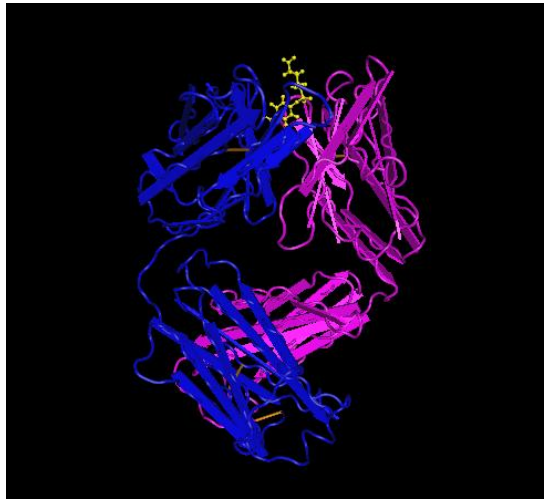


Protein: Influenza haemagglutinin

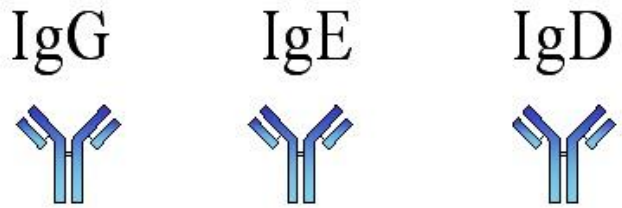


Hapten: 5-(para-nitrophenyl phosphonate)-pentanoic acid

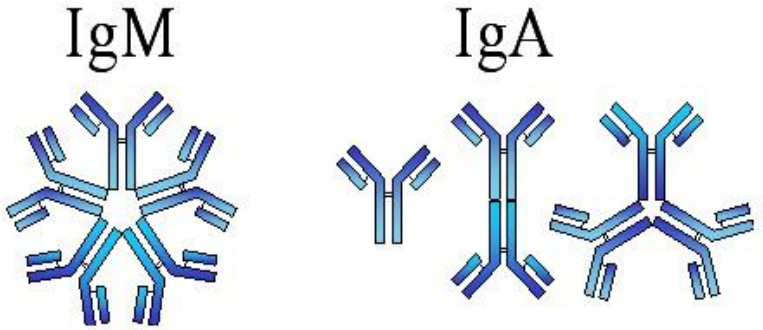




IMMUNOGLOBULIN CLASSES - OVERVIEW

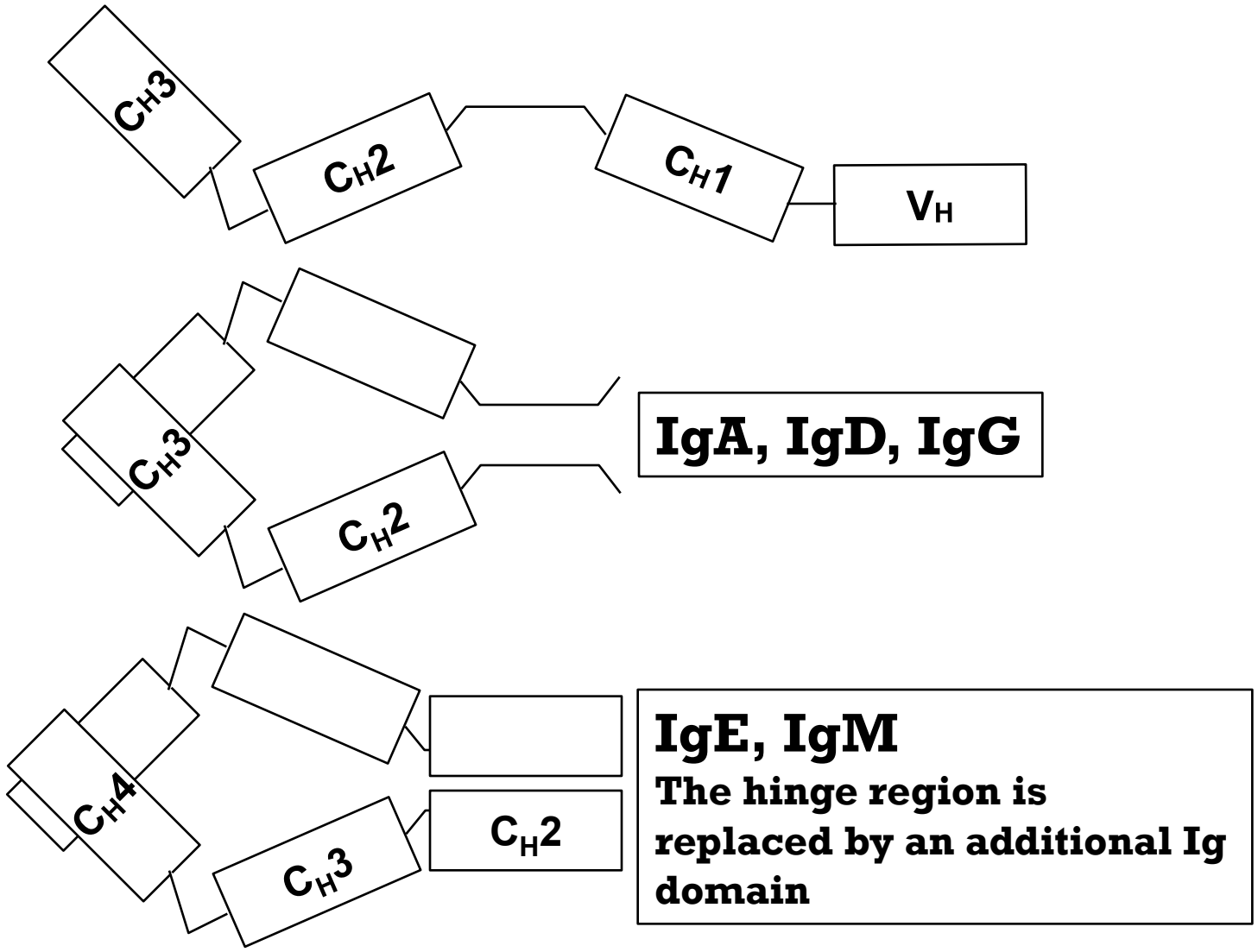


- Igs are classified based on the nature of their heavy chain



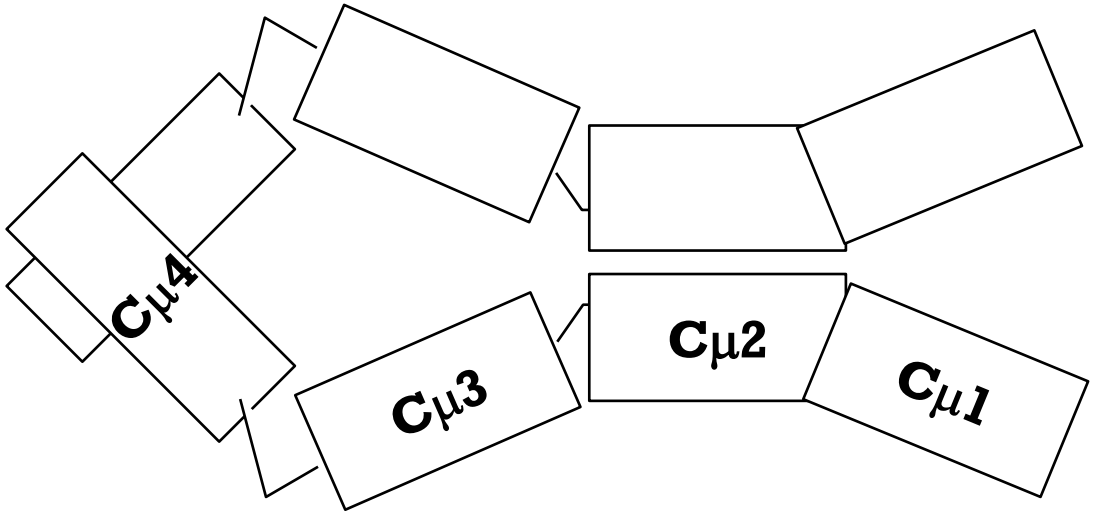
Class	Heavy chain	Chains structure	% in serum	$T_{1/2}$ (days)	Comp. fixation	Placental crossing
IgM	μ	Mono-, penta-, & hexa	5-10	5-10	++++	No
IgG	γ	Monomer	80	23	++	Yes
IgA	α	Mono-, di-, or tri	10-15	6	-	No
IgD	δ	Monomer	0.2-1	3	-	No
IgE	ε	Monomer	0.002	2	-	No

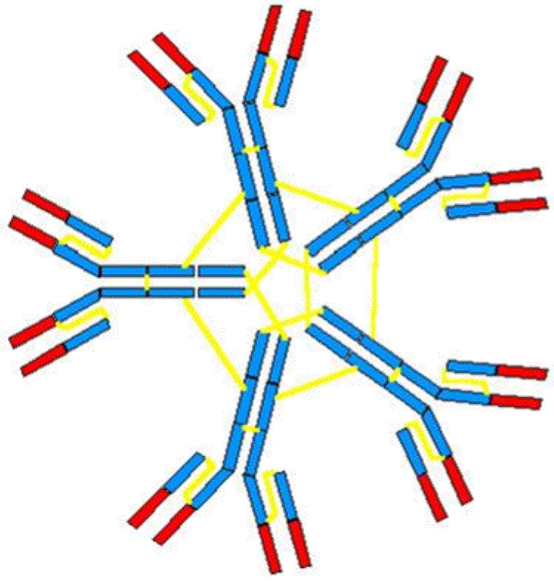
DOMAINS IN DIFFERENT CLASSES (H-CHAIN)



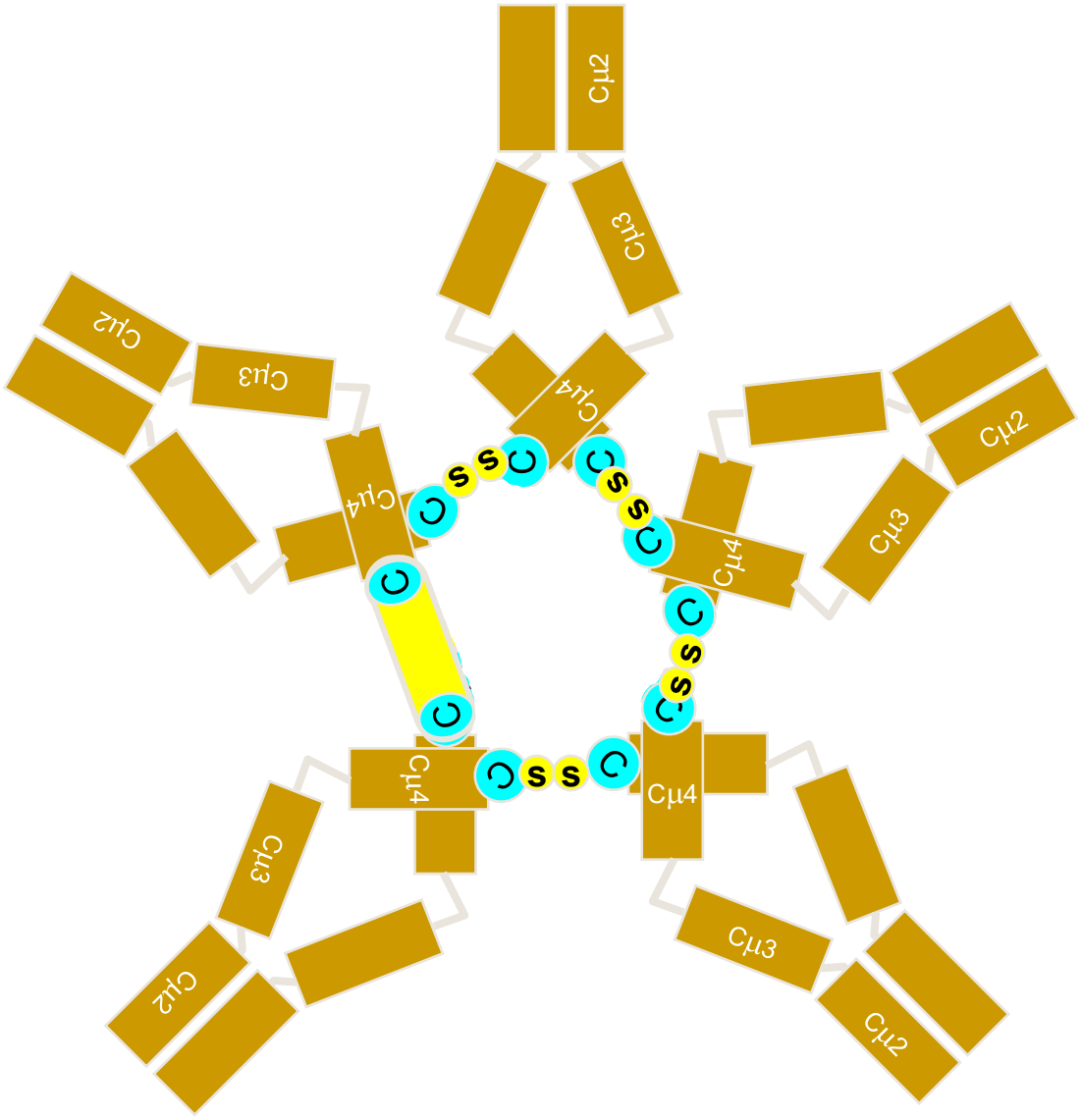
IgM CLASS

- Location: Mainly intravascular (blood & lymph), B-cell surface (monomer)
- Known Functions:
  - ✓Primary immune response (1st produced)
  - ✓Primary role in antigen agglutination (ex. ABO)
- 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)

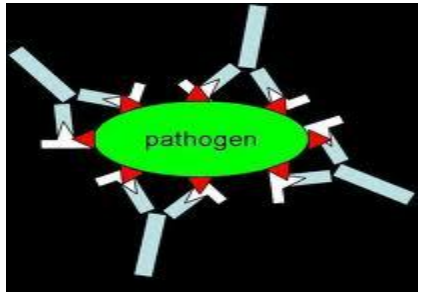




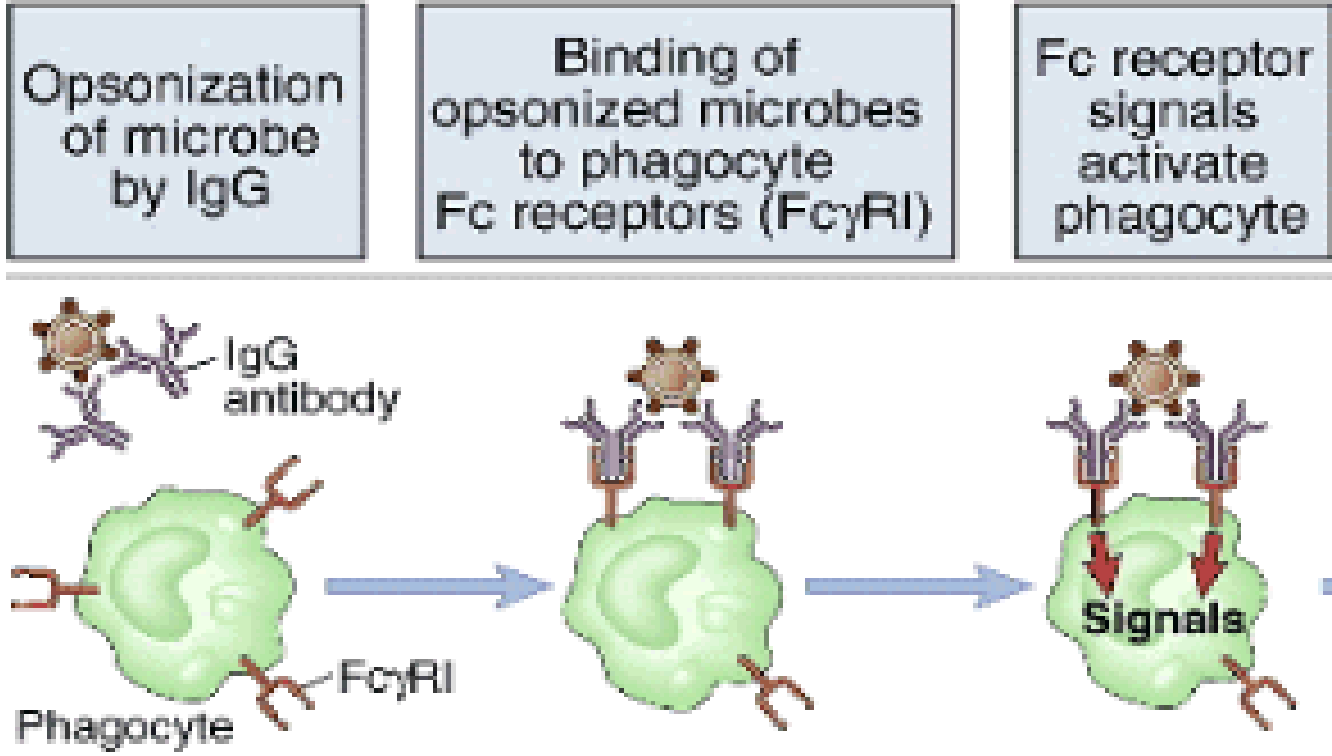
THE PROCESS OF IgM MULTIMERISATION

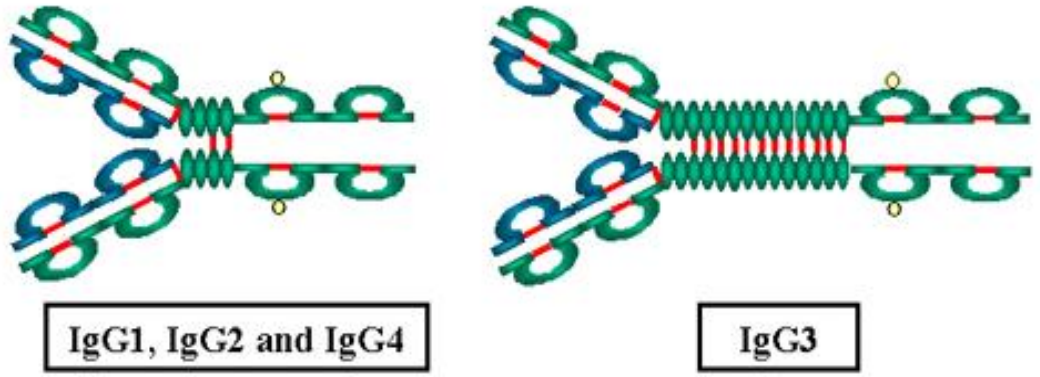


IgG CLASS

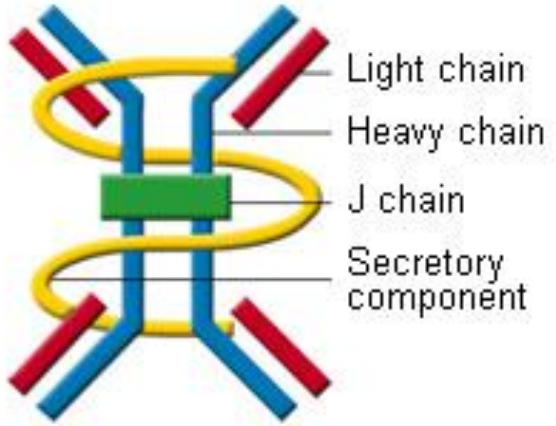


- Location: Blood, lymph, intestine
  - Produced in response to a wide variety of antigens, (ex. bacteria, viruses)
  - Known Functions
    - ✓ The predominant antibody produced in the 2^0 immune response
    - ✓ Provides the major line of defense for the fetus & during first few weeks of newborns
    - ✓ Coats organisms to enhance phagocytosis by neutrophils and macrophages (opsonization)

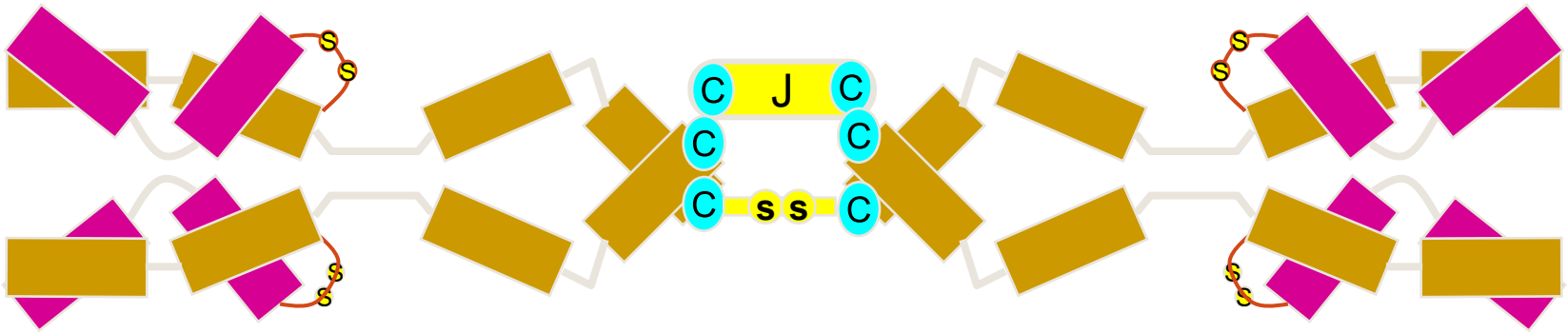




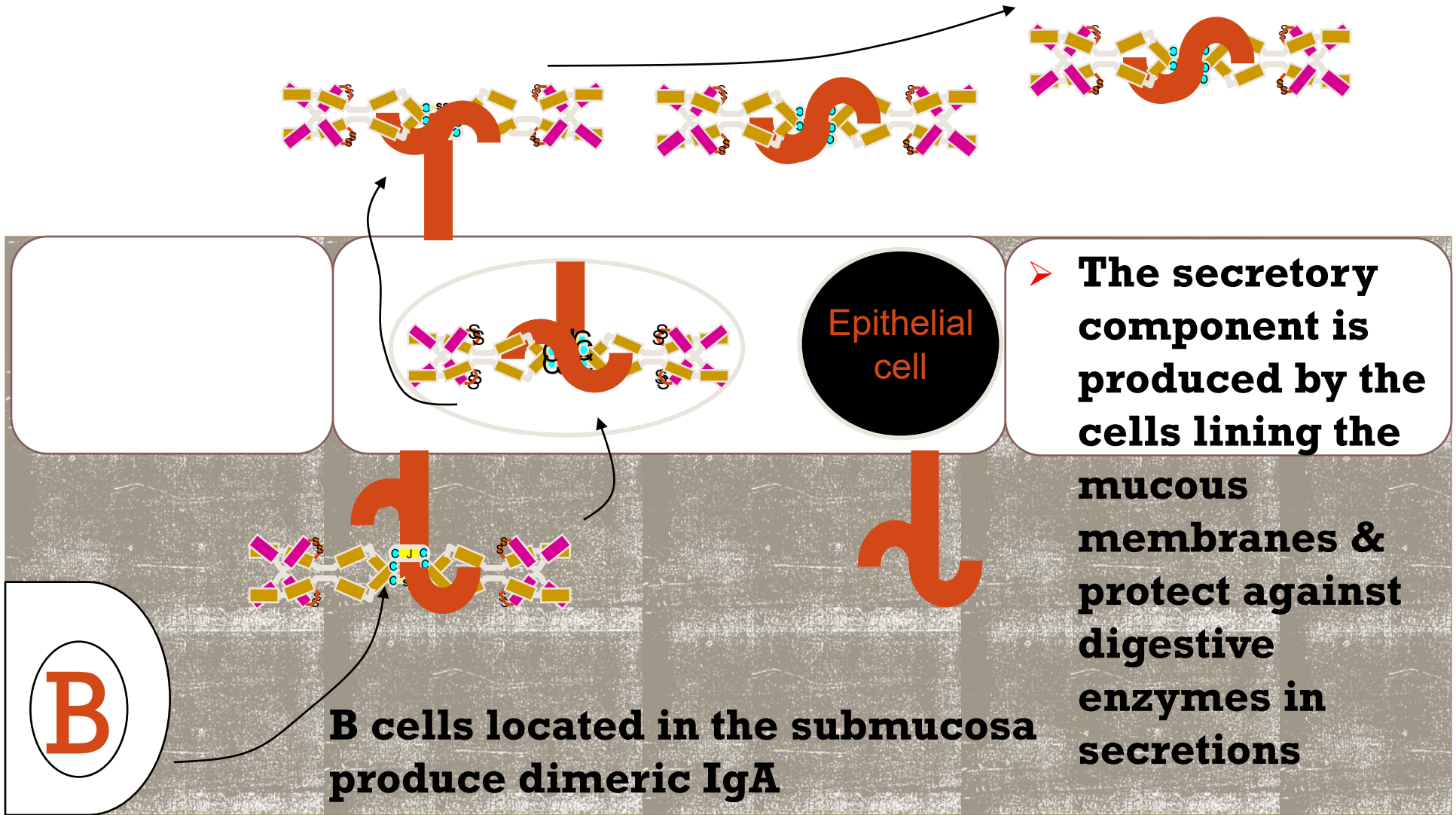
IgA CLASS



- 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 tract and 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**

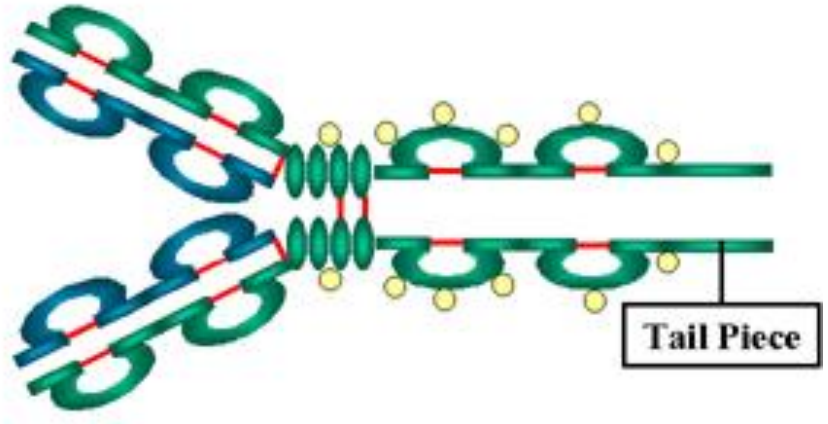
B cells located in the submucosa produce dimeric IgA

IgD CLASS

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

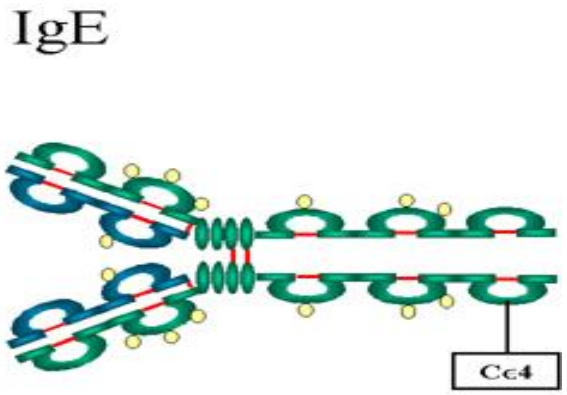
IgD

- Structure
  - Monomer
  - Tail piece

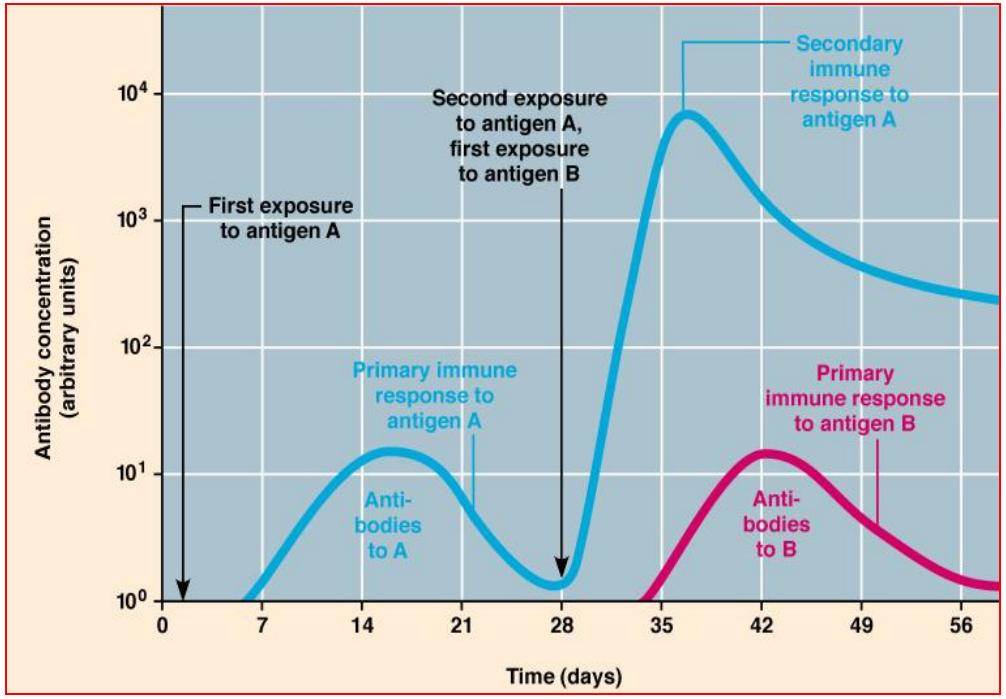


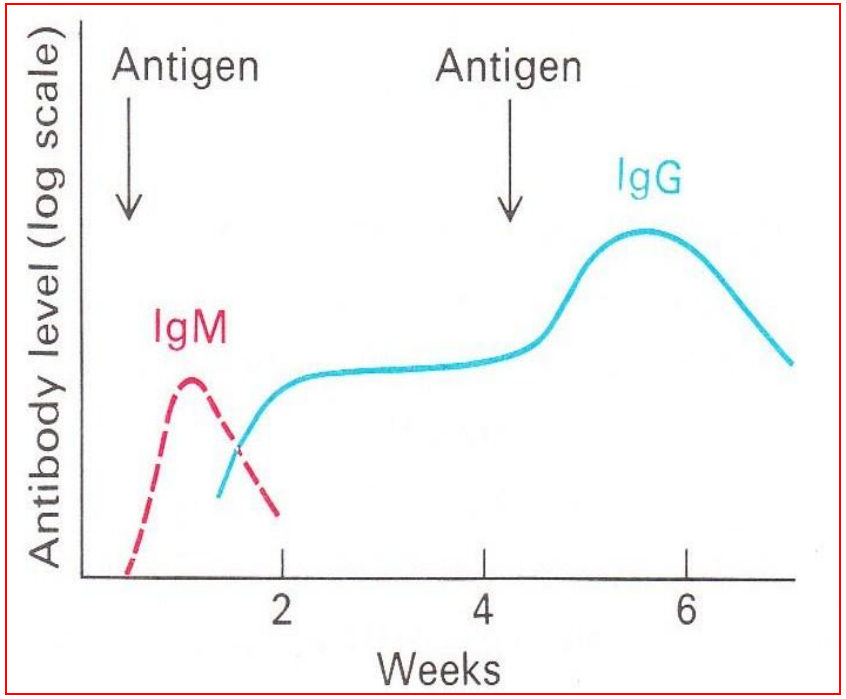
IgE CLASS

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



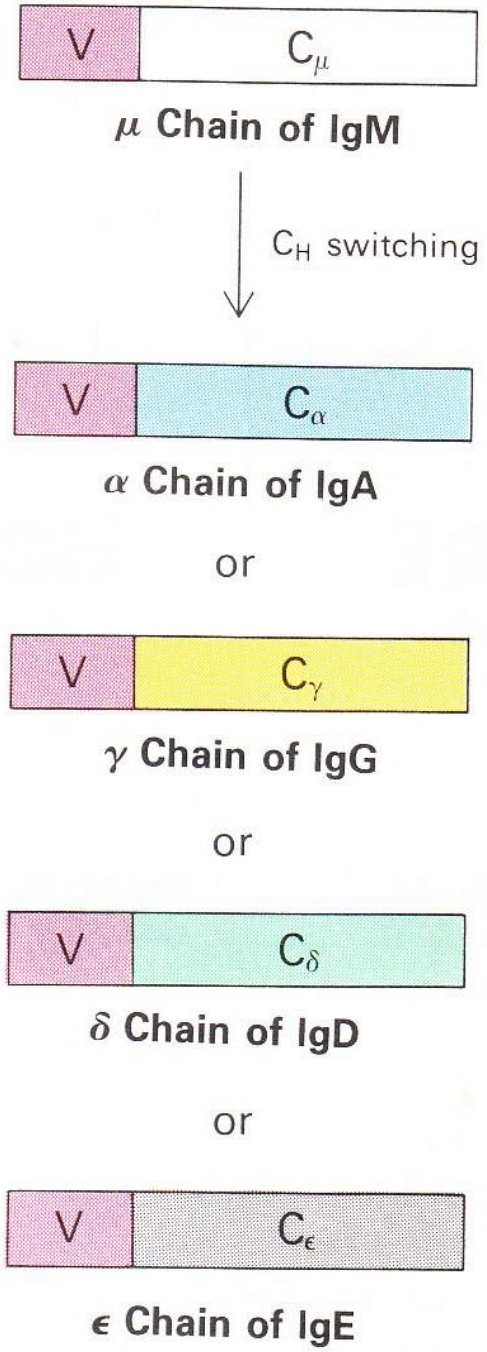
IMMUNOLOGICAL MEMORY





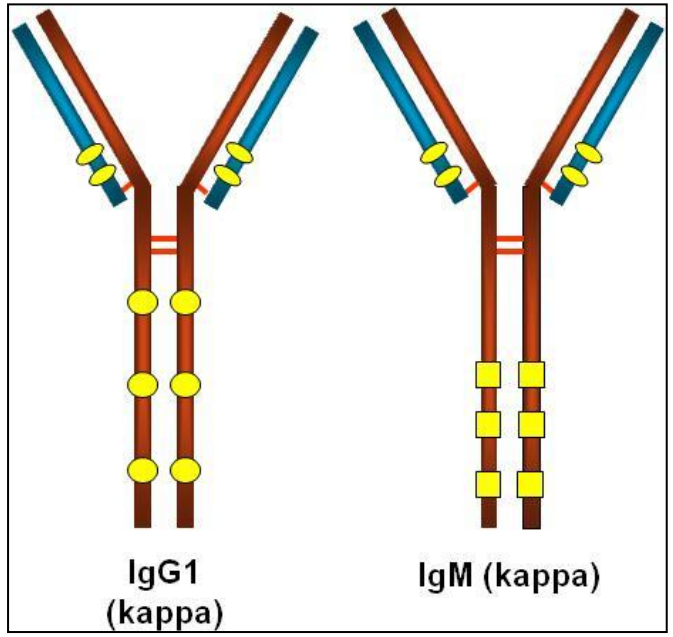
CLASS (ISOTYPE) SWITCHING

- 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

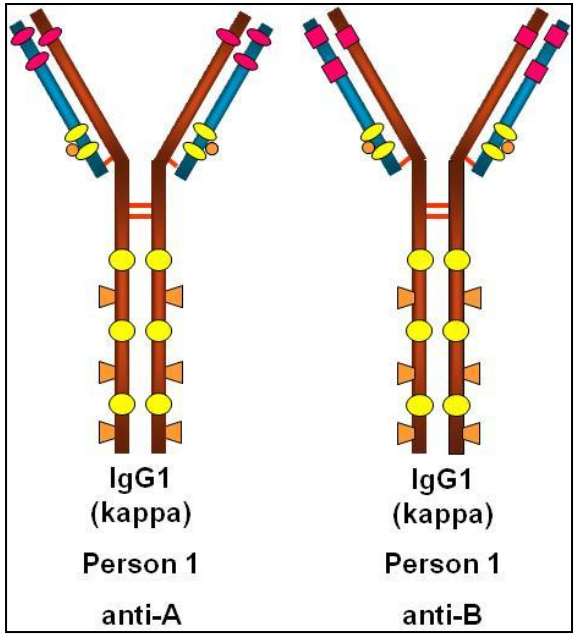


IDIOTYPE VS. ISOTYPES VS. ALLOTYPES

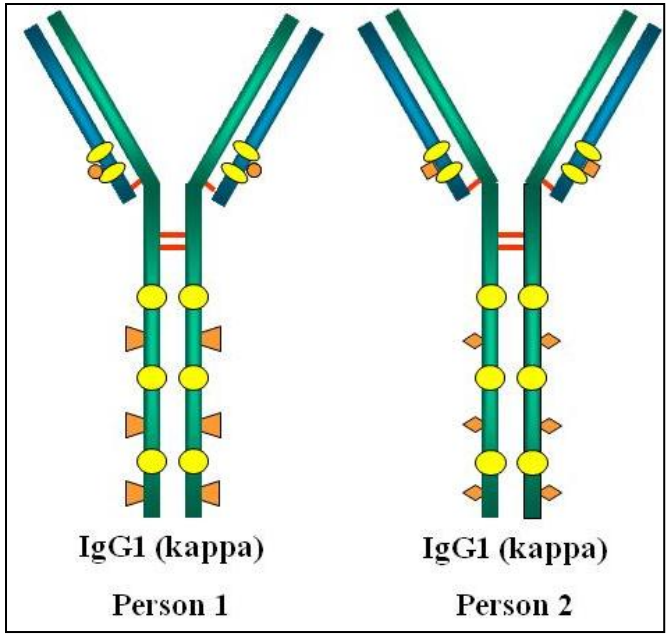
isotypes



idiotype

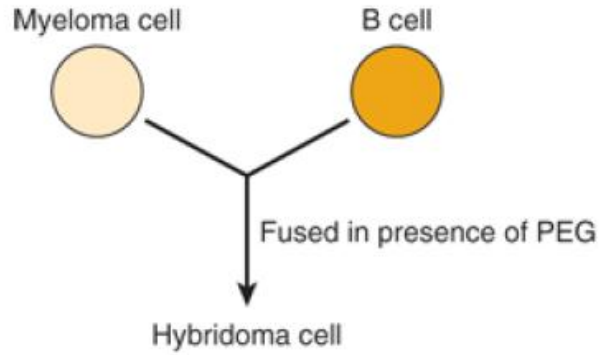


allotypes

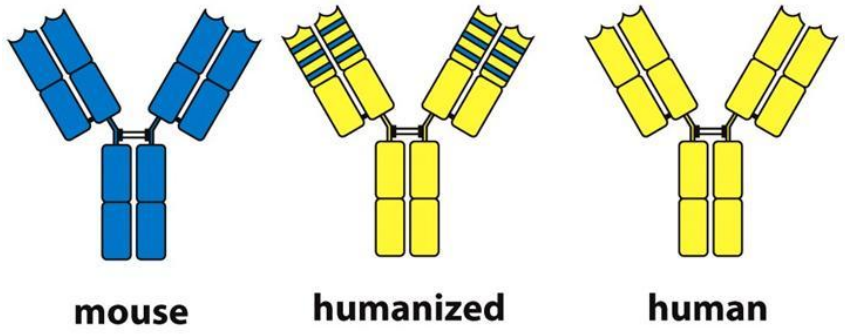


HYBRIDOMA AND MONOCLONAL ANTIBODIES

- When an antigen is injected into an animal, the resulting antibodies are polyclonal, meaning they are directed against a number of different epitopes on the antigen.
- In order to "create" an immortal B cell that produces a single antibody (monoclonal), a B cell hybridizes with a B cancer cell (myeloma).

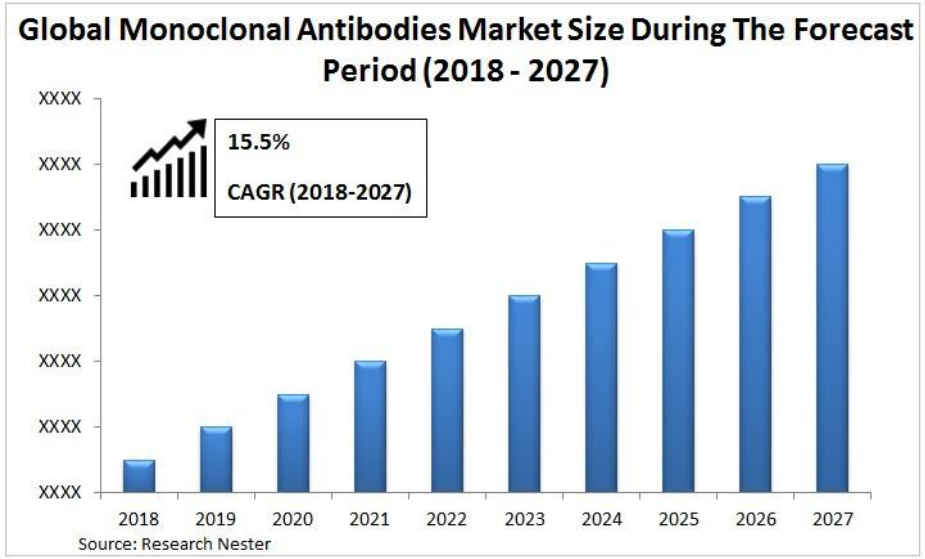


Monoclonal antibodies made in mice can be humanized by attaching the CDRs onto appropriate sites in a human immunoglobulin molecule.



BENEFITS OF MONOCLONAL ANTIBODIES

- Measure the amounts of many individual proteins and molecules (e.g. plasma proteins, steroid hormones).
- Determine the nature of infectious agents (e.g. types of bacteria).
- Used to direct therapeutic agents to tumor cells.
- Used to accelerate the removal of drugs from circulation when they reach toxic levels.



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)