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Immunoglobulins (1)

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

Innate vs. Acquired Immunity

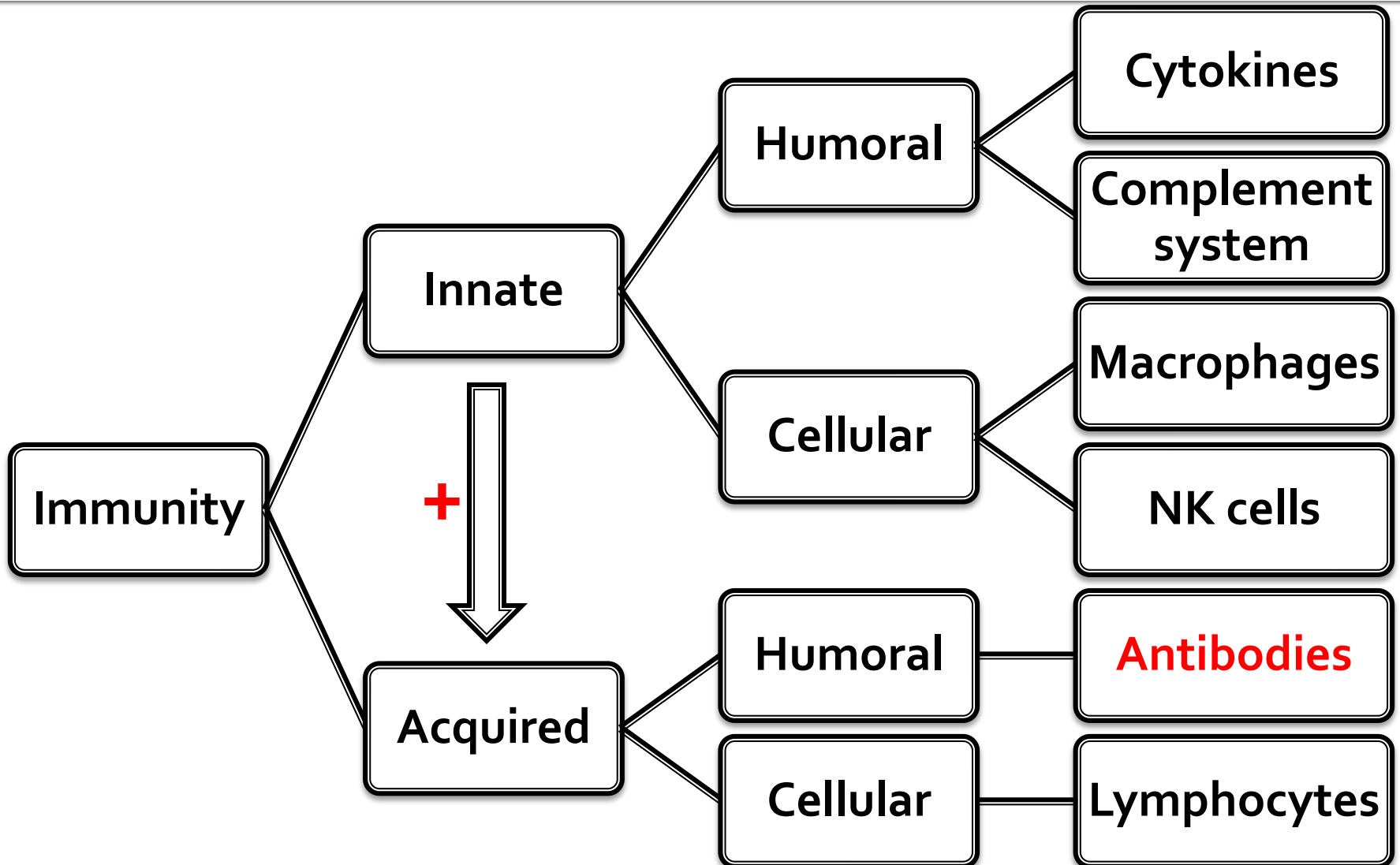
➤ Innate:

- Natural or native immunity
- Cellular and biochemical defense mechanisms (non-specific)
- Non-adaptive upon repeated infections
- Only recognize microbial agents

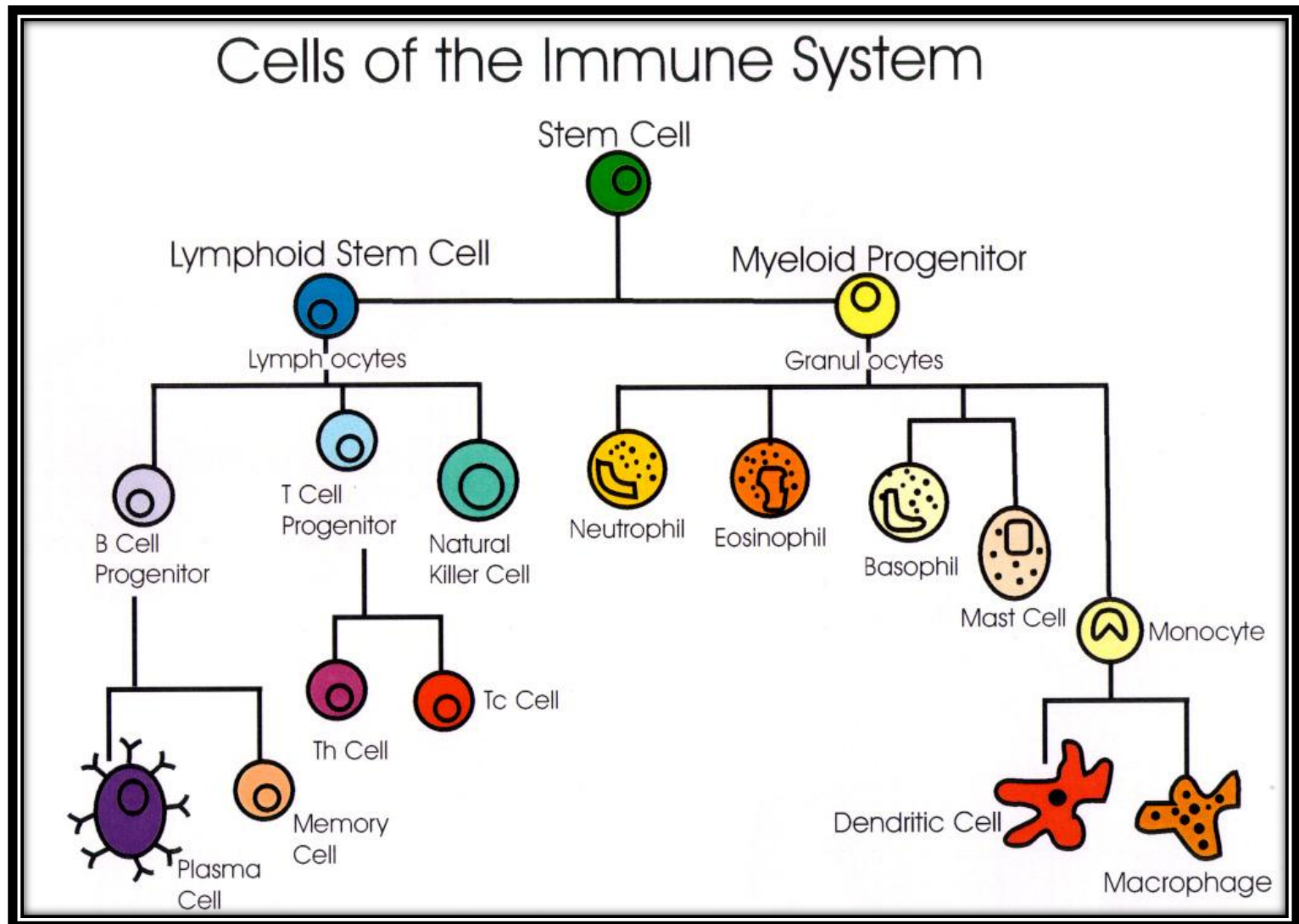
➤ Acquired:

- Develops as a response to infection & adapts to the infection
- Increase in magnitude and defensive capabilities with each successive exposure to a particular microbe
- Exquisite specificity & memory for distinct molecules
- Recognize and react to microbial and non-microbial substances

Innate vs. Acquired Immunity



Immune system cells



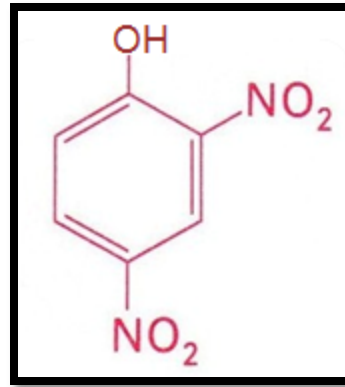
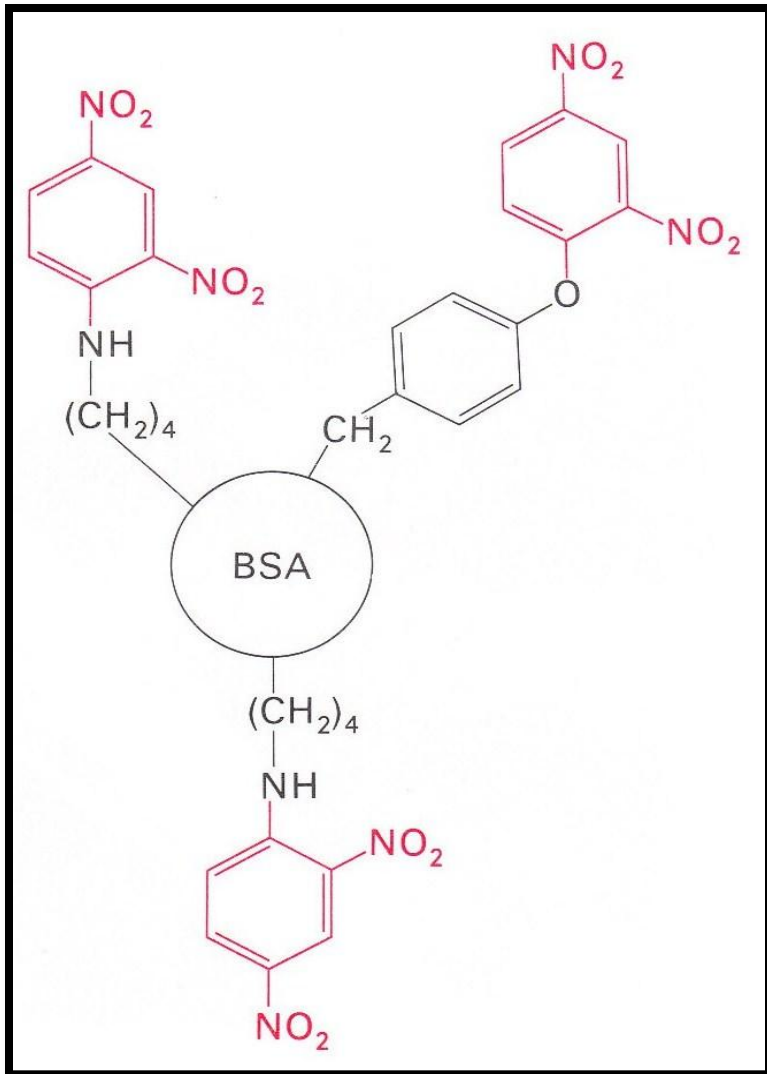
Acquired (specific) immunity

- Two major components:
 - 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
 - T lymphocytes (thymus, cell-mediated immunologic processes; graft rejection, hypersensitivity reactions, and defense against malignant cells and many viruses)
- Genetic deficiency is reported (recurrent infections)

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 ($\sim 10^8$)
 - ✓ 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 (immunogen)
 - ✓ 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

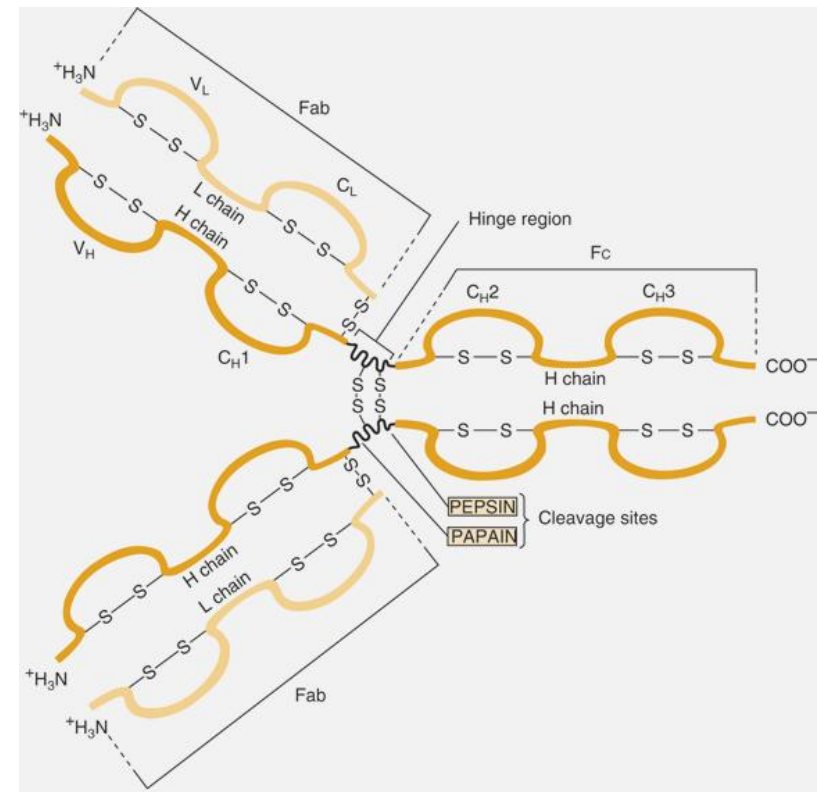


⇒ No response

⇒ Response

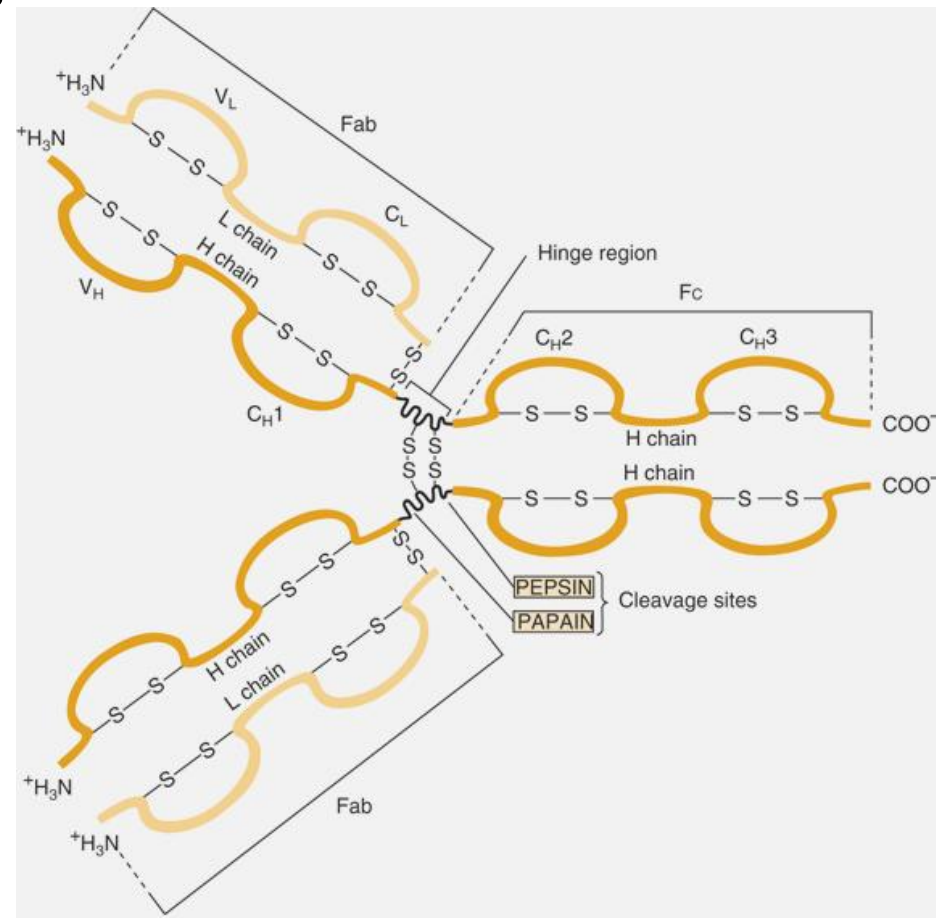
Immunoglobulins - structure

- 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: $\frac{1}{4}$ amino (V_H), $\frac{3}{4}$ carboxylic (C_{H1} , C_{H2} , C_{H3})



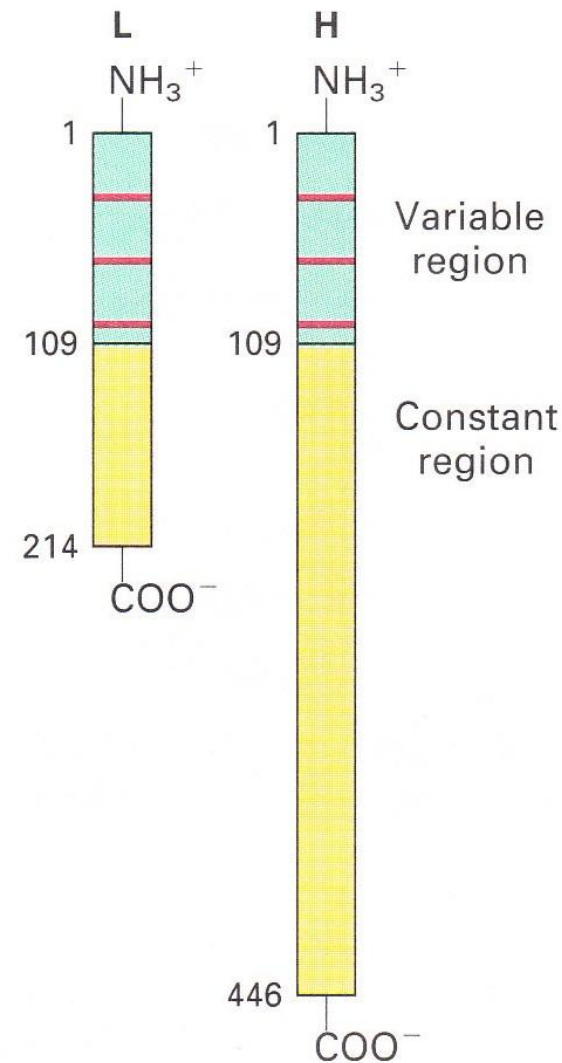
Immunoglobulins - structure

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



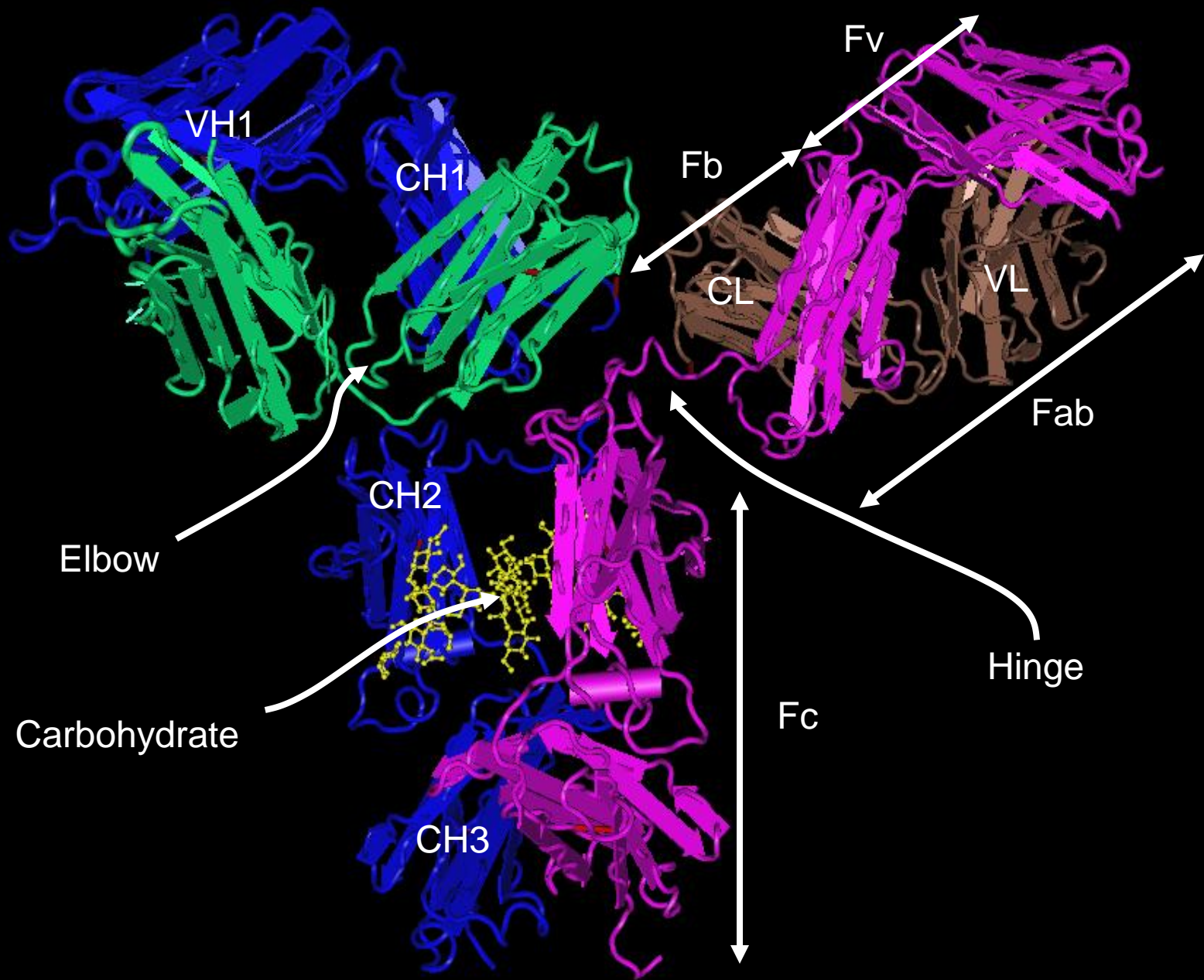
Immunoglobulins - structure

- 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 (Fab)₂ fragment CAN:
 - ✓ Detect & bind 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 signalling molecules



The Immunoglobulin Fold

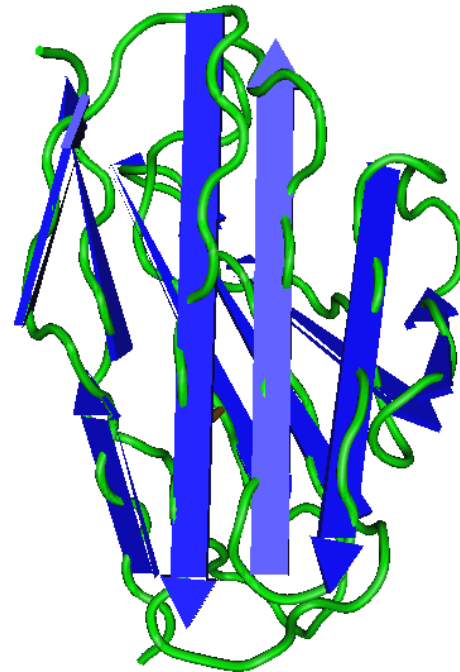
The characteristic structural motif of all Ig domains

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

A barrel

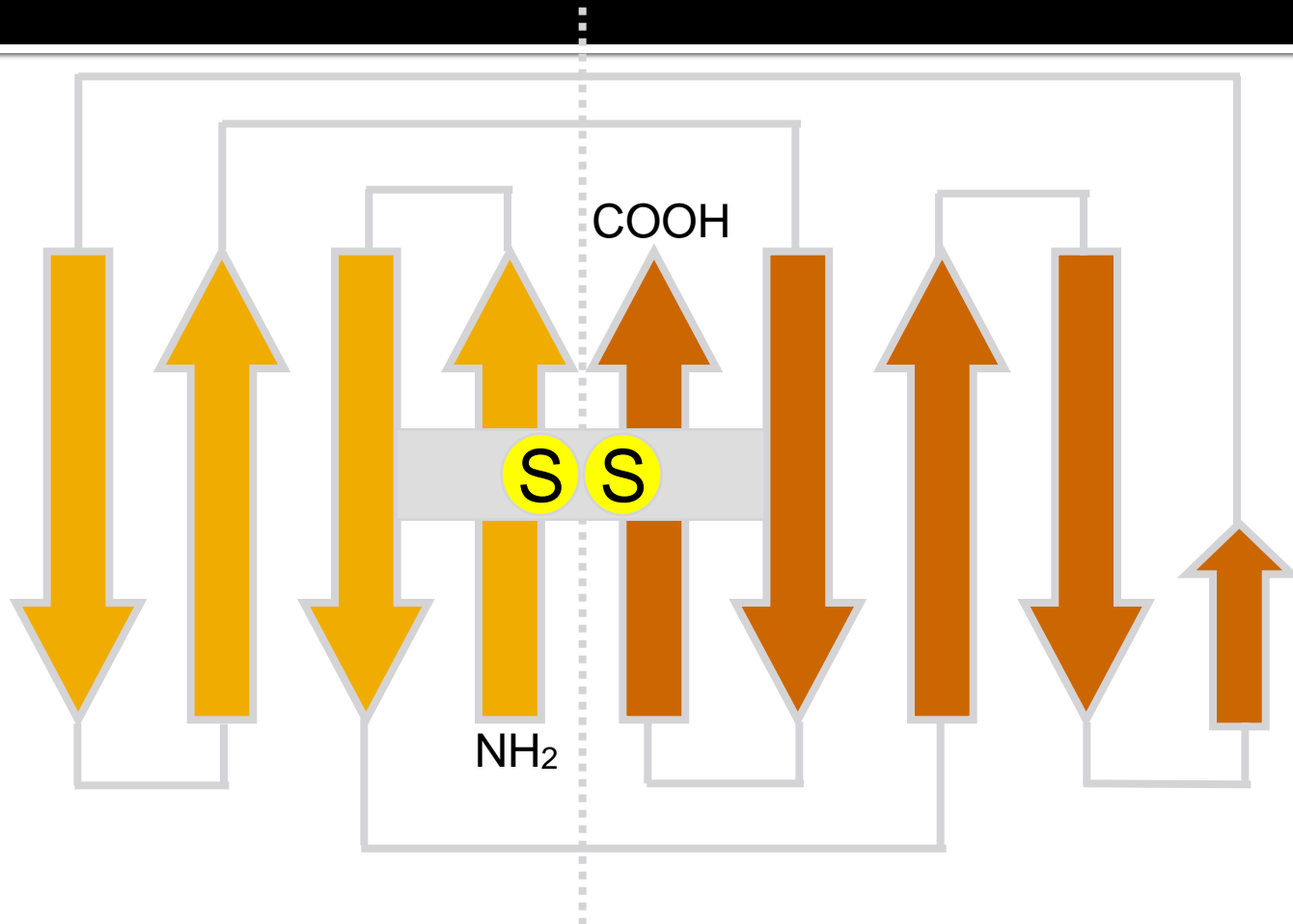


Barrel under construction



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

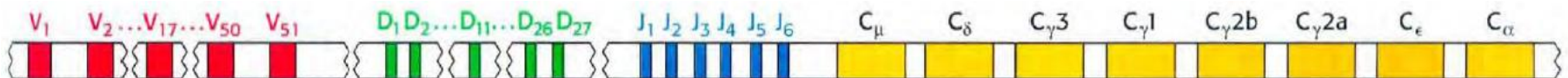
➤ Light chain is a product of at least 3 genes:

- Variable (V_L) gene
- Joining region (J) gene
- Constant region (C_L) gene



➤ Heavy chain is a product of at least 4 genes :

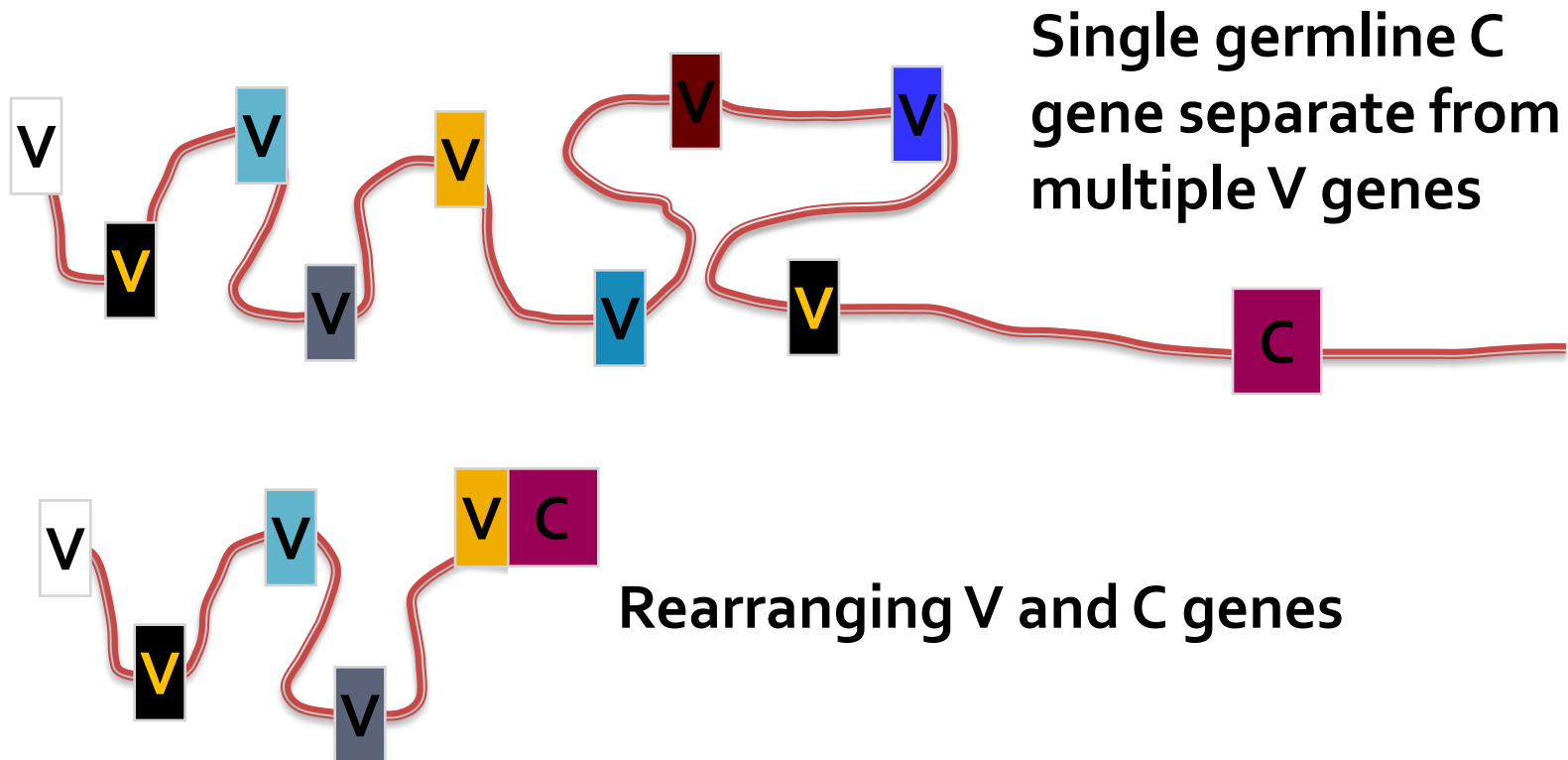
- Variable region (V_H) gene
- Diversity region (D) gene
- Joining region (J) gene
- Constant region (C_H) gene



Combinatorial diversity: How does diversity occur?

Dreyer - Bennett hypothesis

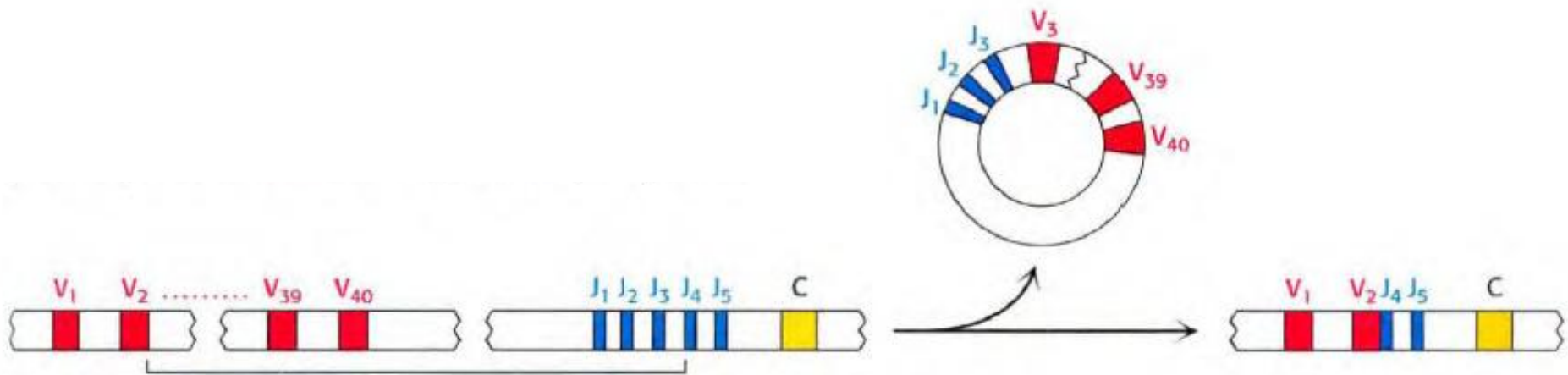
- Immune system can generate $> 10^8$ antibodies
- Human genome contains ~ 40,000 genes !



How does diversity occur?

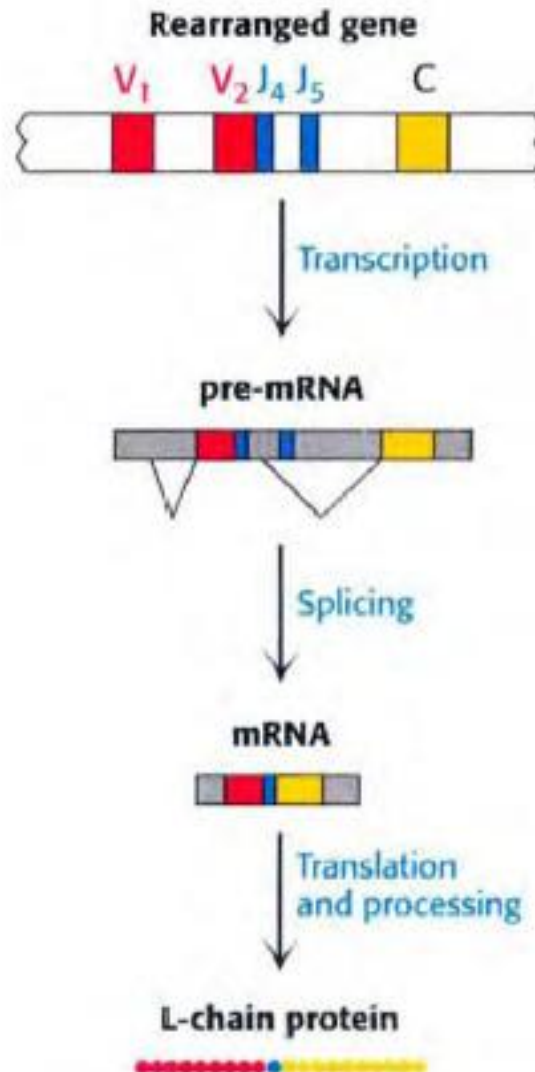
Rearrangement & splicing (L chain)

- 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?

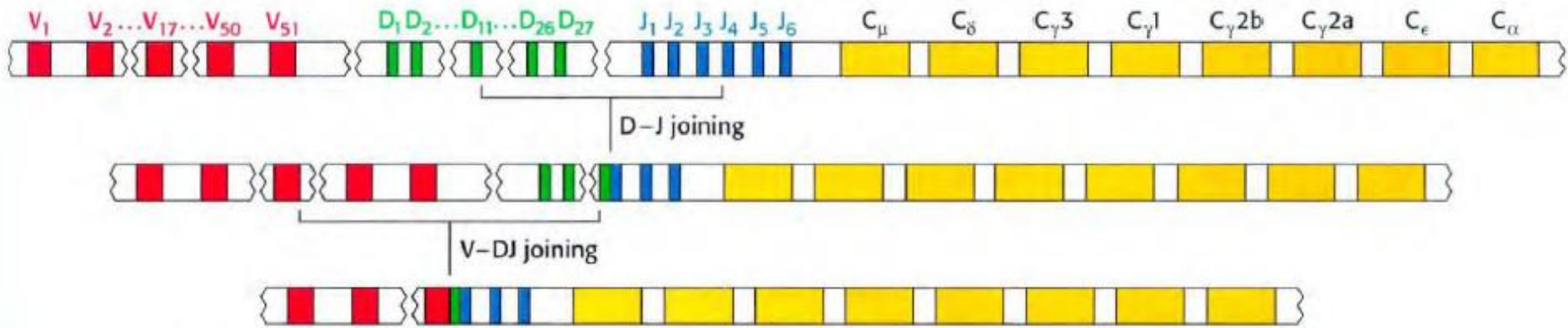
Rearrangement & splicing



How does diversity occur?

Rearrangement & splicing (H chain)

➤ Possible combinations = $51 * 27 * 6 = 8262$



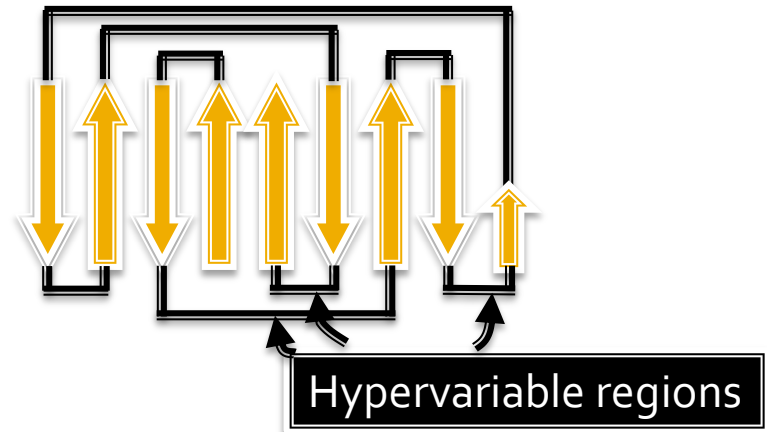
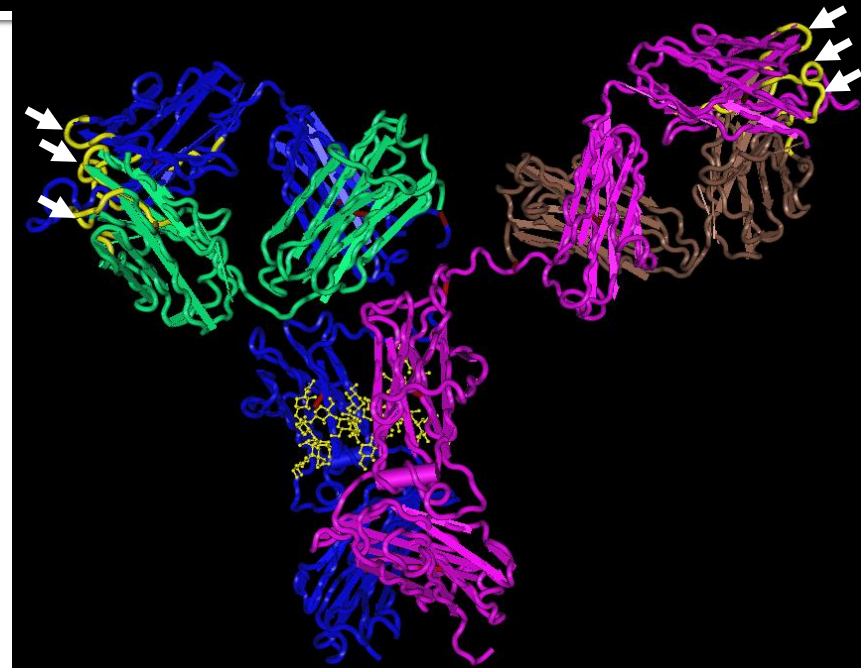
➤ All possible combinations (L&H) = $(200 + 120) * 8262 =$

$$2.6 * 10^6$$

➤ Somatic mutations increases the diversity

Variable Regions

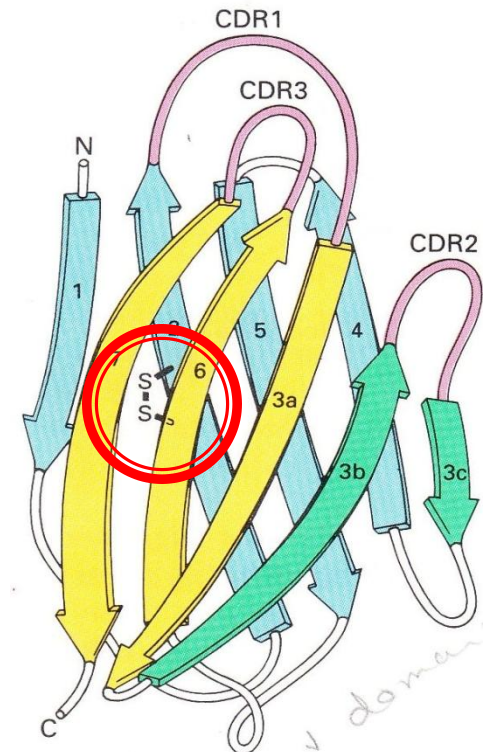
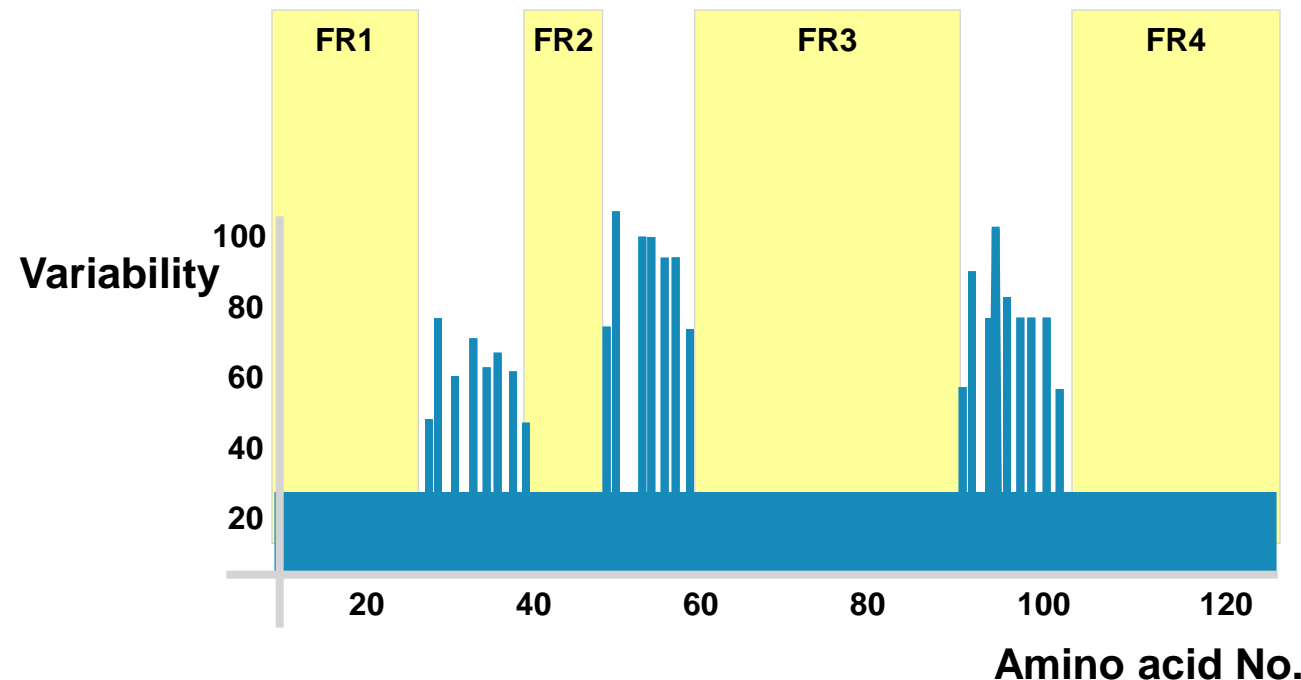
- No two variable regions in different humans are identical
- Relatively invariable regions and other hypervariable regions
- L chains have 3 hypervariable regions (in V_L) and H chains have four (in V_H)
- These hypervariable regions comprise the antigen-binding site
- Dictate the amazing specificity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



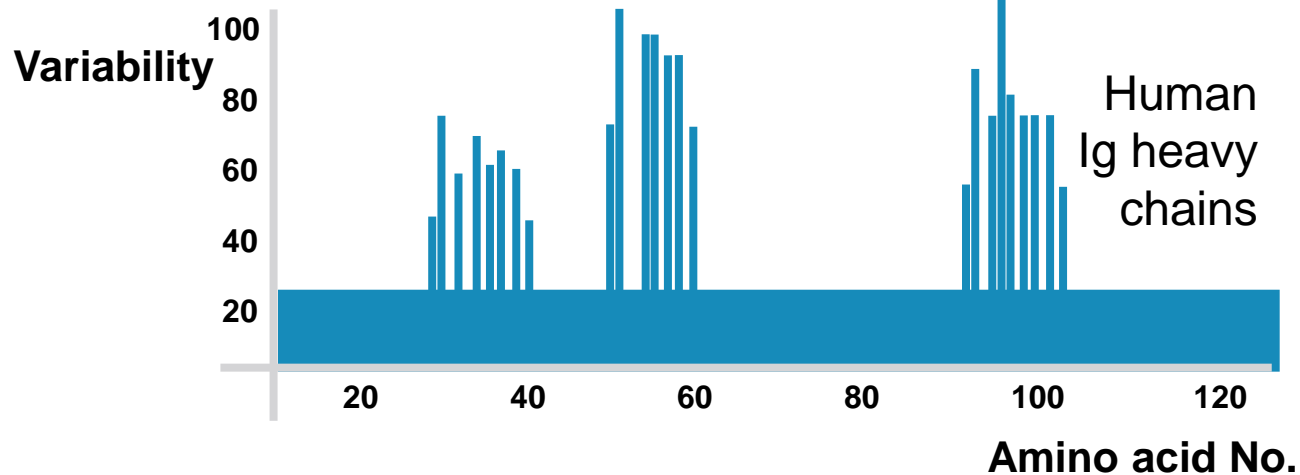
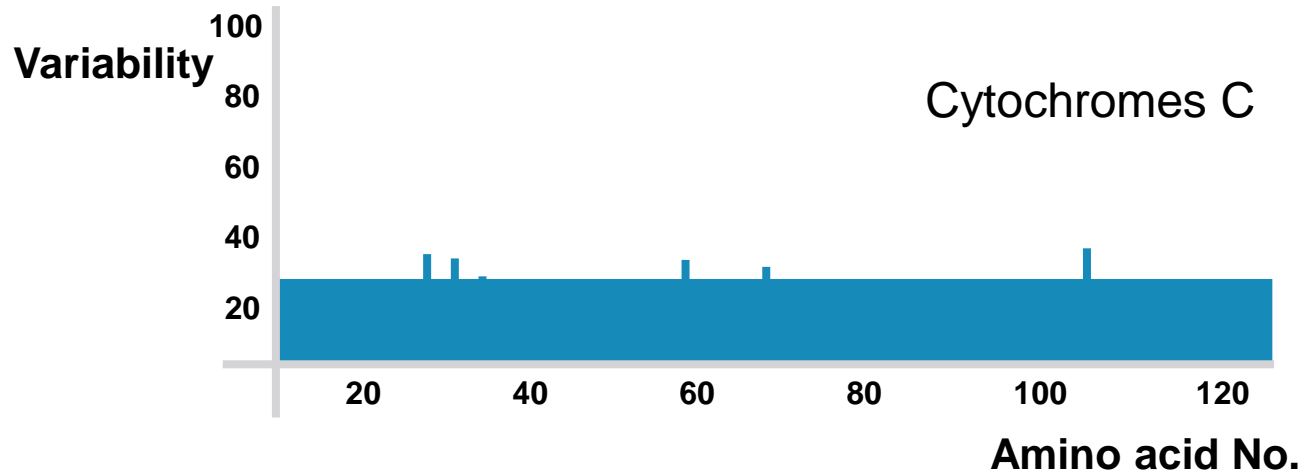
Hypervariable regions

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

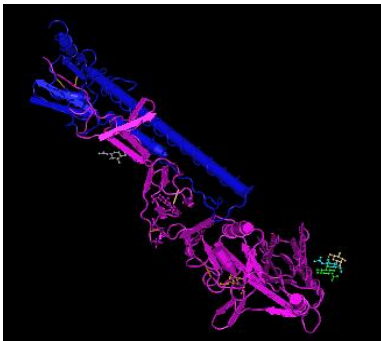


Variability in other proteins

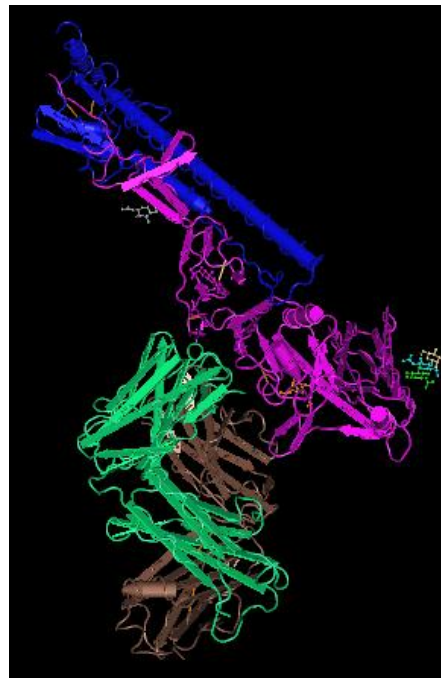


CDRs interaction with antigens

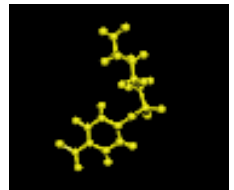
- Antigen-antibody interactions is based on mutual complementarity between surfaces
- Large antigens: interact with all of the CDRs of an antibody
- Small antigens: interact with only one or a few CDRs that form a pocket or groove in the antibody molecule



Protein:
Influenza
haemagglutinin



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



Immunoglobulin classes - overview

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

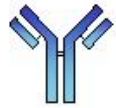
IgG



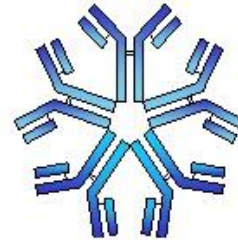
IgE



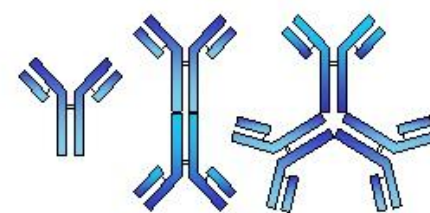
IgD



IgM

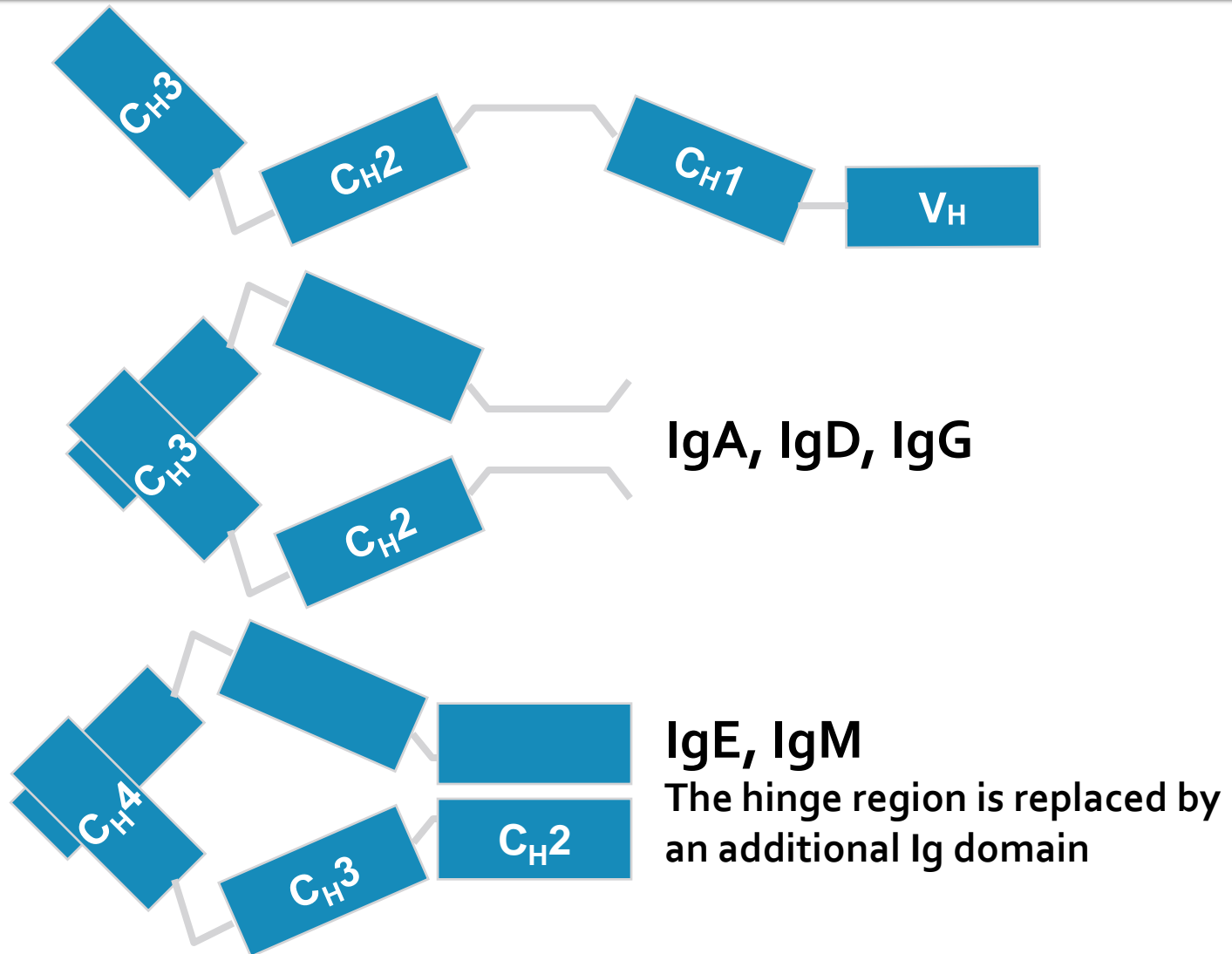


IgA



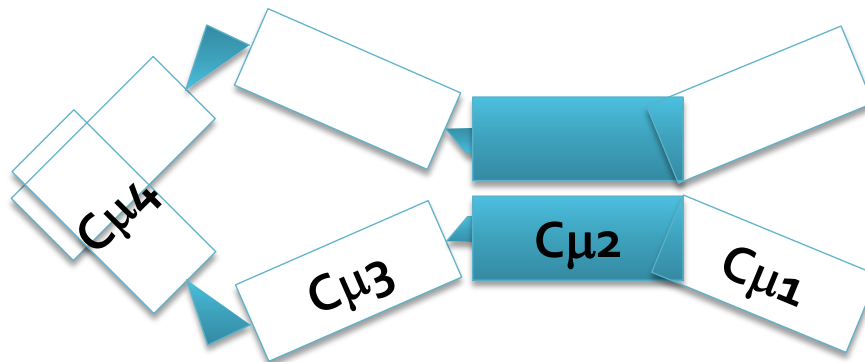
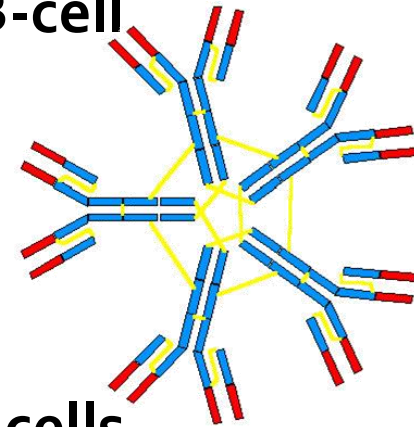
Class	Heavy chain	Chains structure	% in serum	T _{1/2} (days)	Comp. fixation	Placental crossing
IgM	μ	Mono-, penta-, & hexa	5-10	5-10	++++	No
IgG	γ	Monomer	80	23	++	Yes
IgA	α	Mono-, di-, or tri	10-15	6	-	No
IgD	δ	Monomer	0.2-1	3	-	No
IgE	ε	Monomer	0.002	2	-	No

Domains in different classes (H-chain)

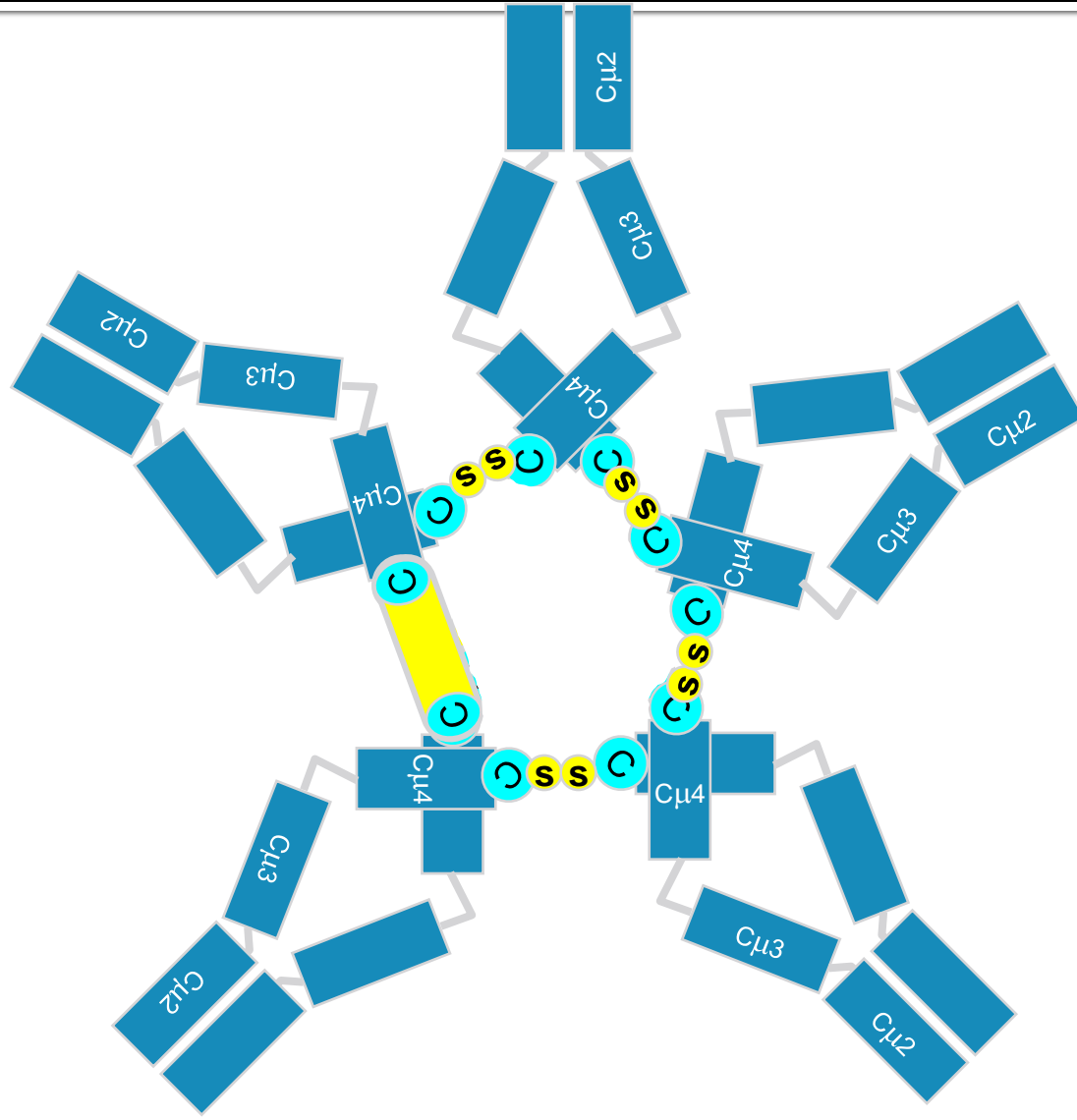


IgM Class

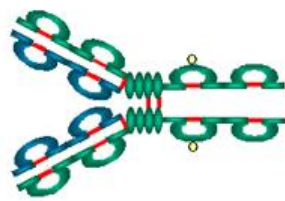
- Location: Mainly intravascular (blood & lymph), B-cell surface (monomer)
- Known Functions:
 - ✓ primary immune response (1st produced)
 - ✓ Primary role in antigen agglutination (ex. ABO)
- IgM only exists as a monomer on the surface of B cells
- Monomeric IgM has a very low affinity for antigen
- A J-chain is involved in the process of multimerization
- C μ 4 mediates multimerisation (C μ 3 may also be involved)



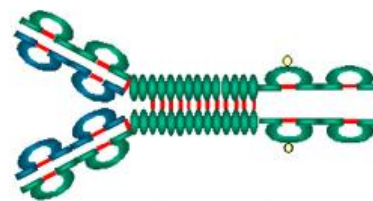
The process of IgM Multimerisation



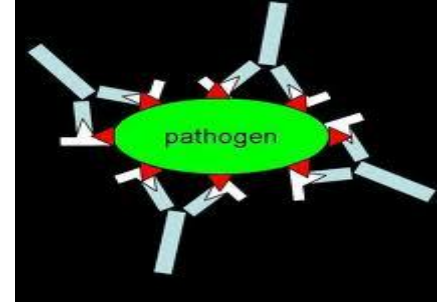
IgG Class



IgG1, IgG2 and IgG4



IgG3



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

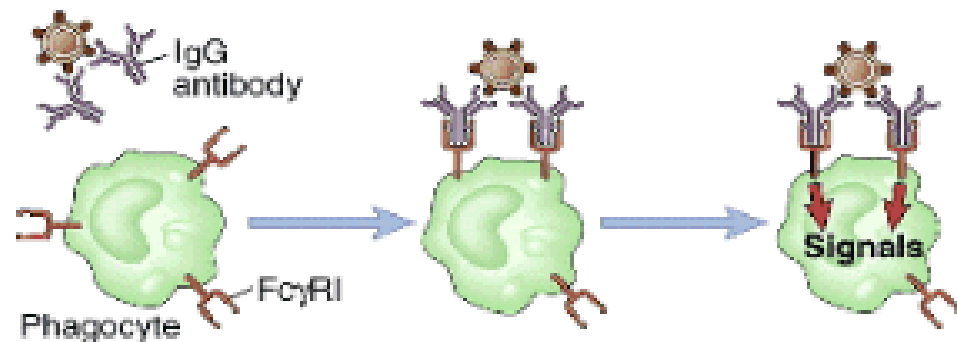
➤ Known Functions

- ✓ The predominant antibody produced in the 2^o 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)

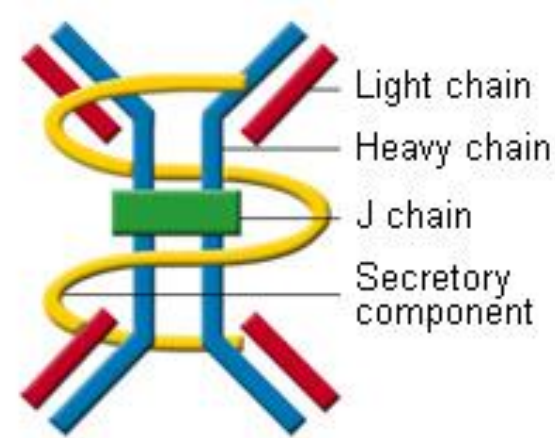
Opsonization
of microbe
by IgG

Binding of
opsonized microbes
to phagocyte
Fc receptors (FcγRI)

Fc receptor
signals
activate
phagocyte



IgA class

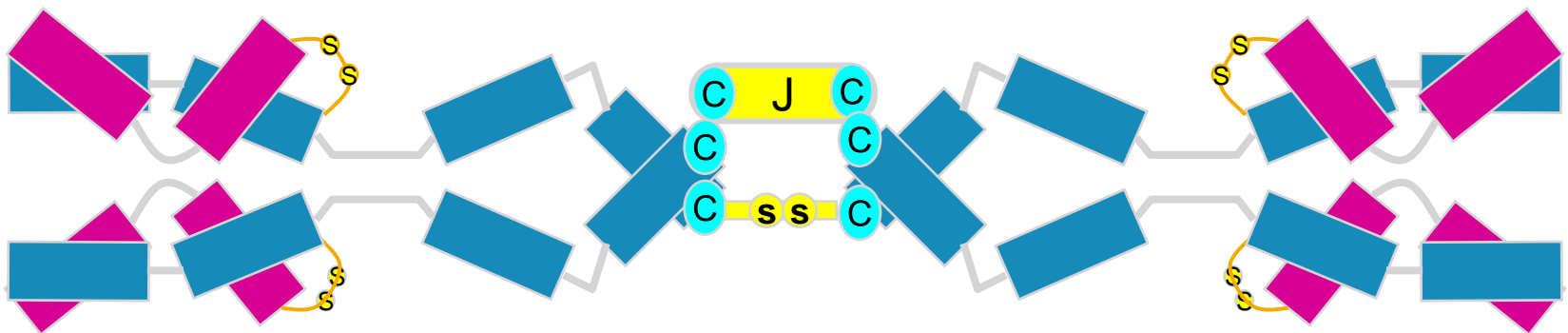


➤ Structure & location:

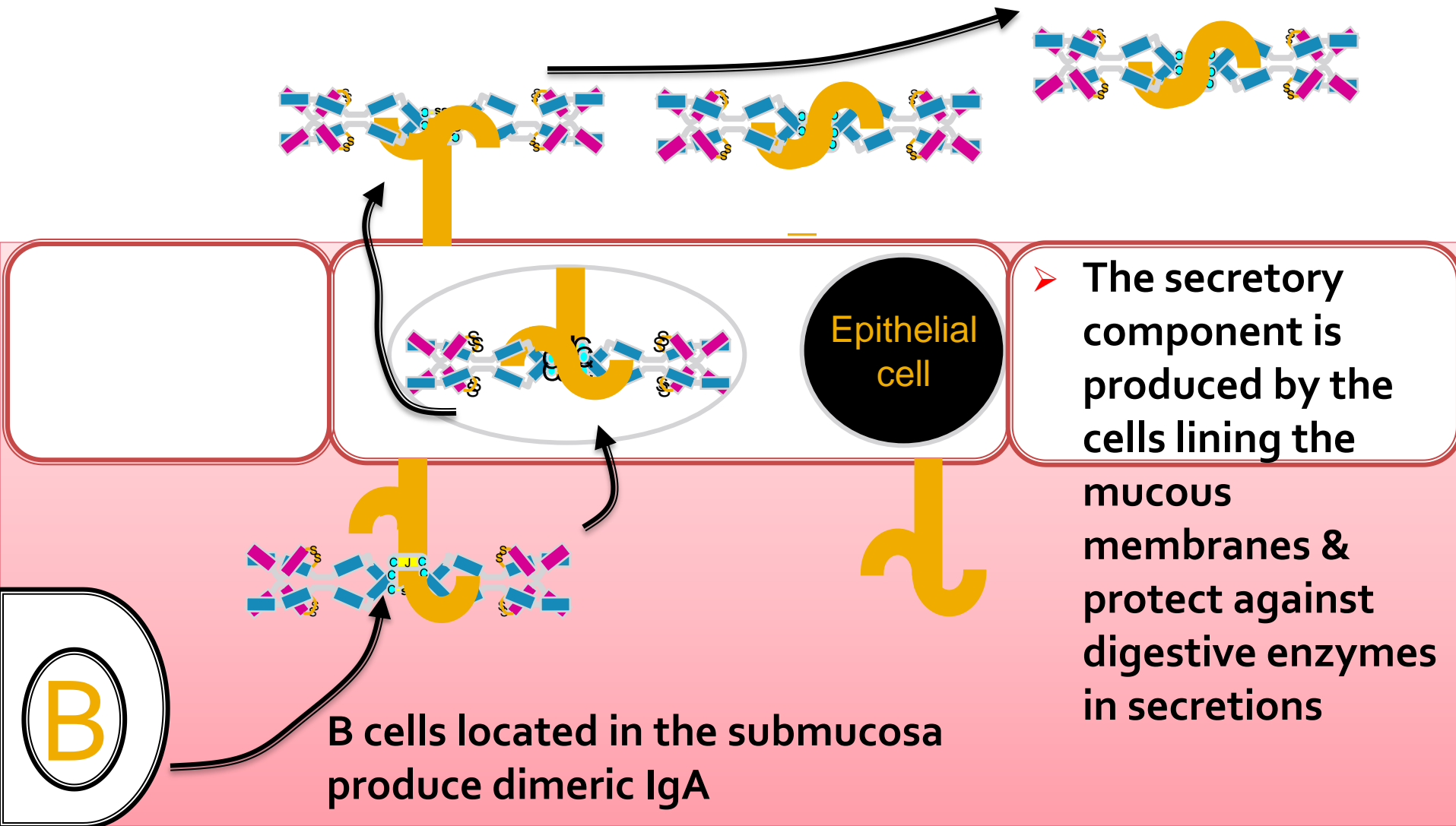
- ✓ Plasma → monomer, dimer, or trimer
- ✓ Secretions (tears, saliva, intestines, milk, bronchial secretion, urine)
→ dimer attached to “secretory component”

➤ Known Functions:

- Localized protection (respiratory, urinary tract and bowel infections)
- Provides immunity to infant's digestive tract & body (translocated)
- The process of dimerization

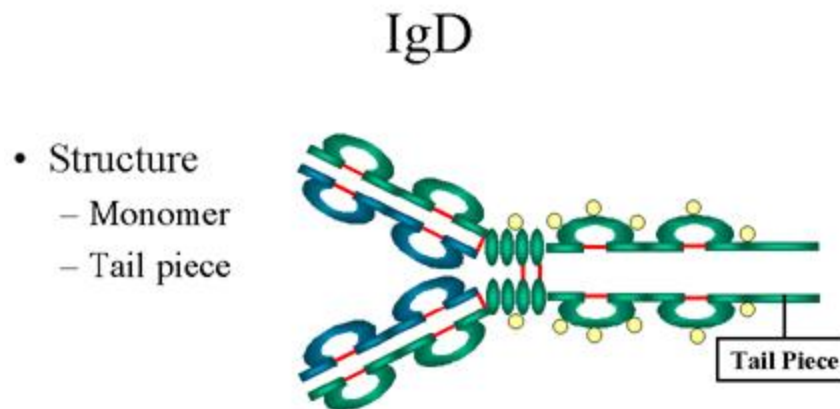


IgA & transcytosis



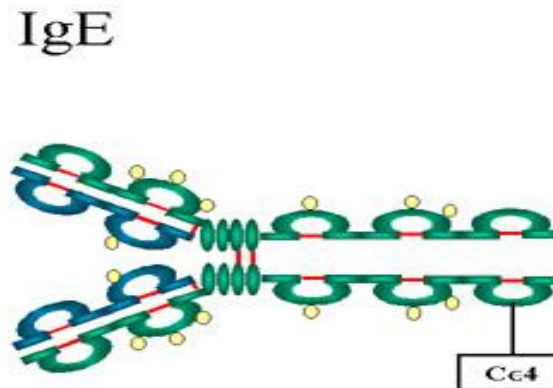
IgD class

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

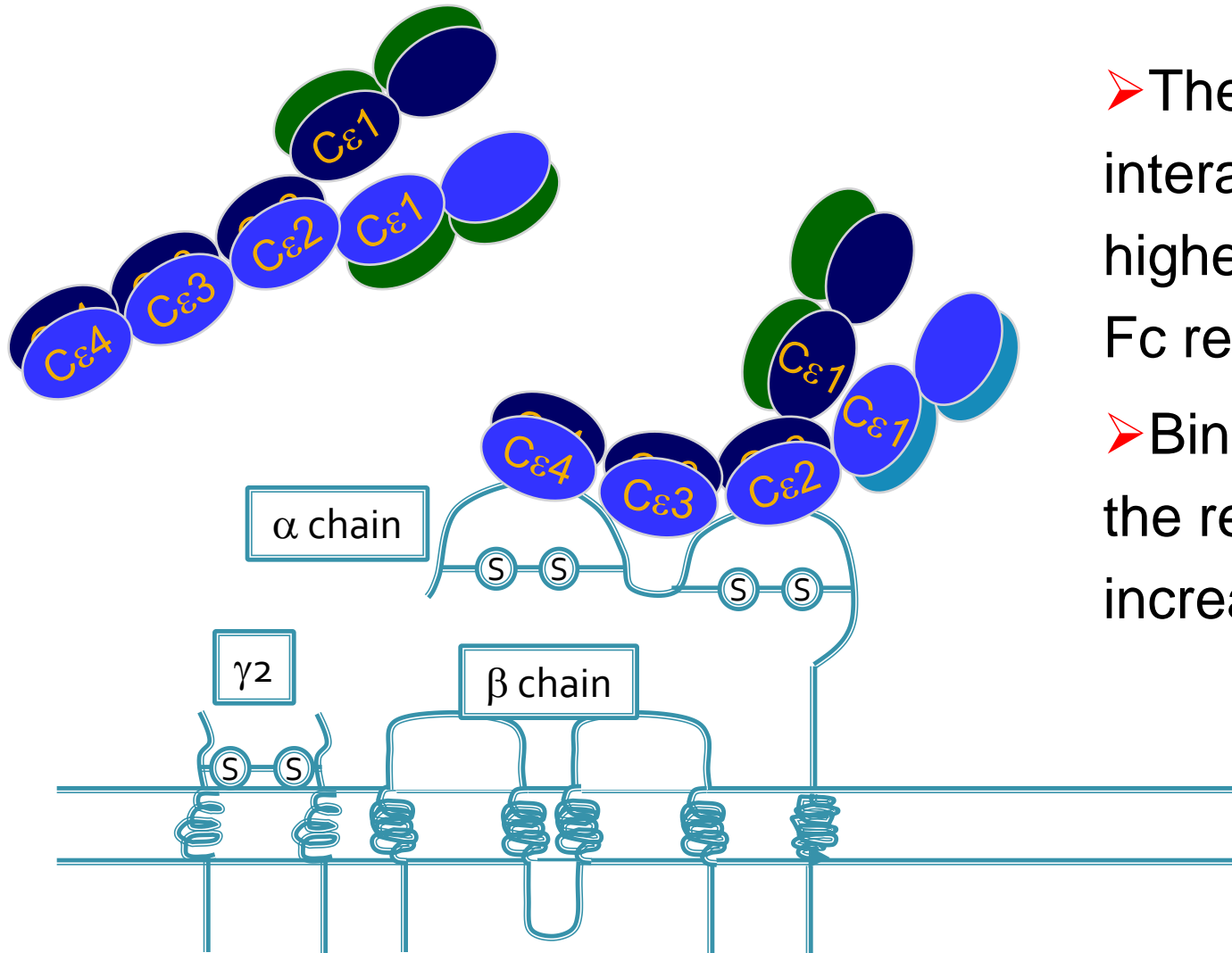


IgE class

- 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



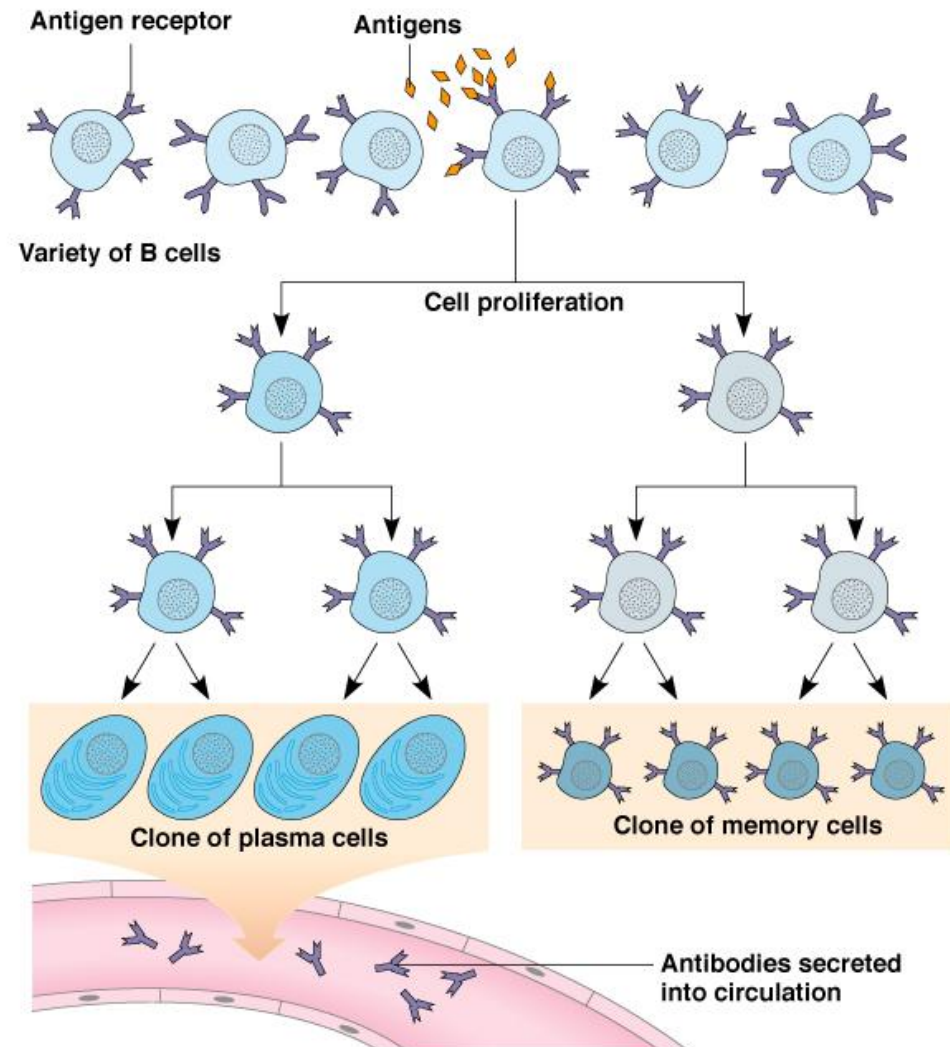
IgE-receptor affinity



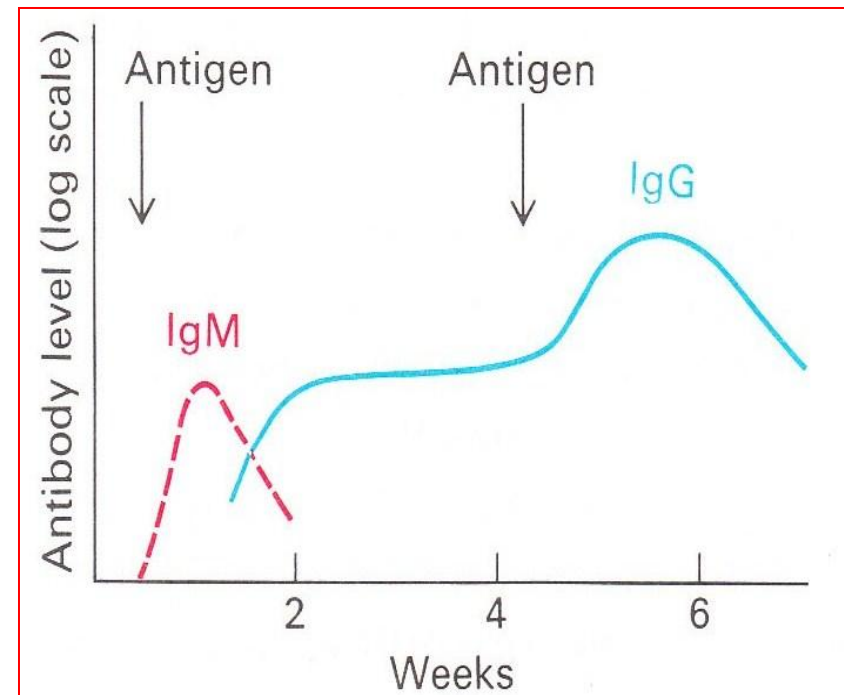
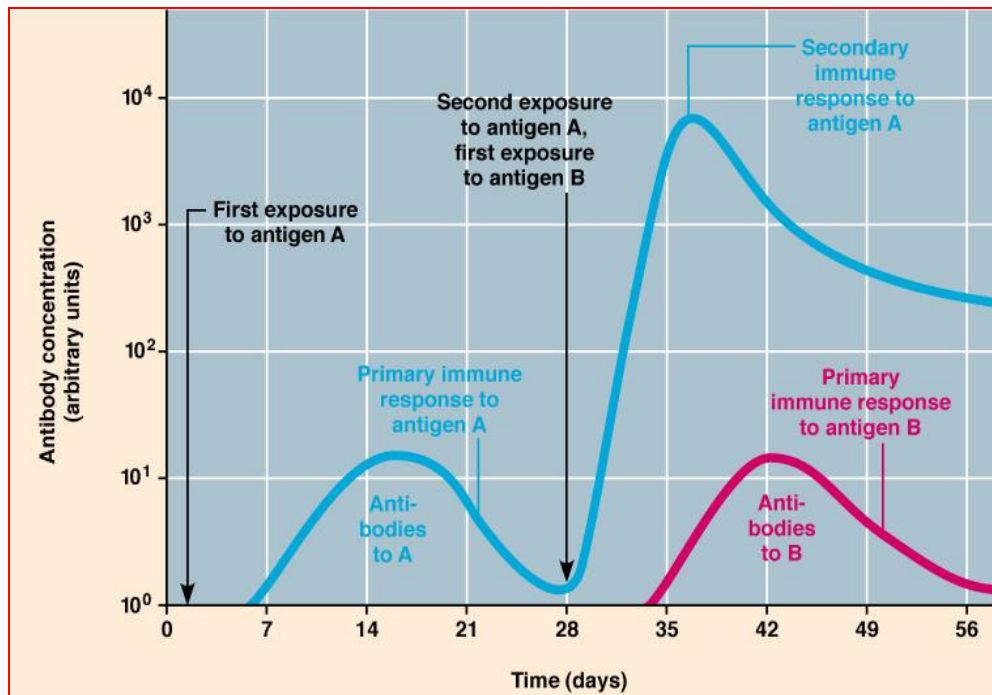
- The IgE–receptor interaction is the highest affinity of any Fc receptor
- Binding of IgE to the receptor increases the half life

How Do B Cells Produce Antibodies?

- B cells (bone marrow or liver) → maturation → migration to lymphoid organs (lymph node or spleen) → antigen → many clones of plasma cells → antibodies
- Each B cell produces antibodies that will recognize only one antigenic determinant

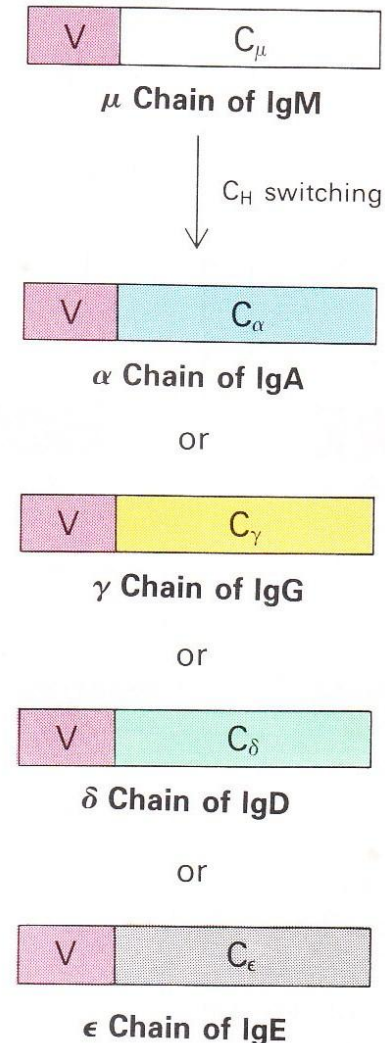


Immunological Memory



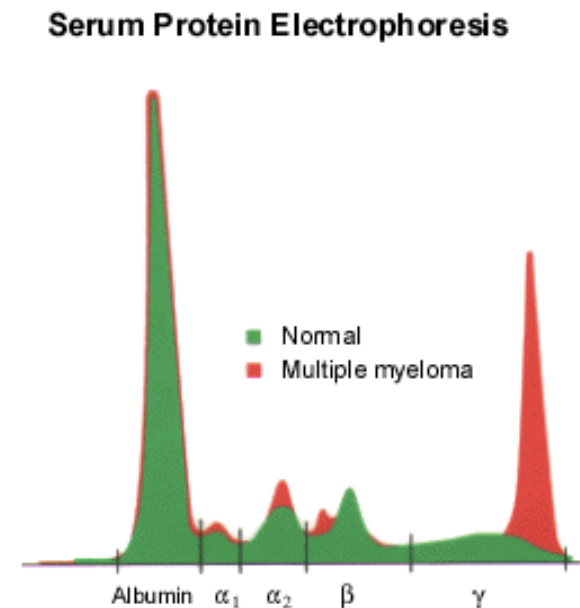
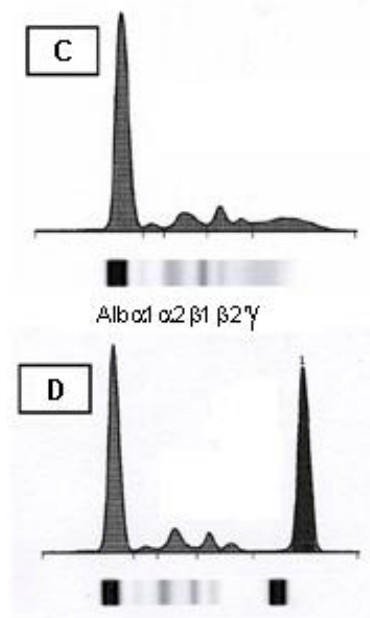
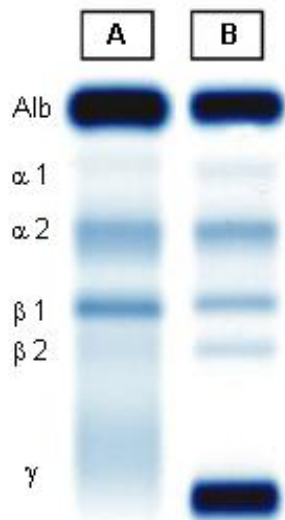
Class (Isotype) Switching

- 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



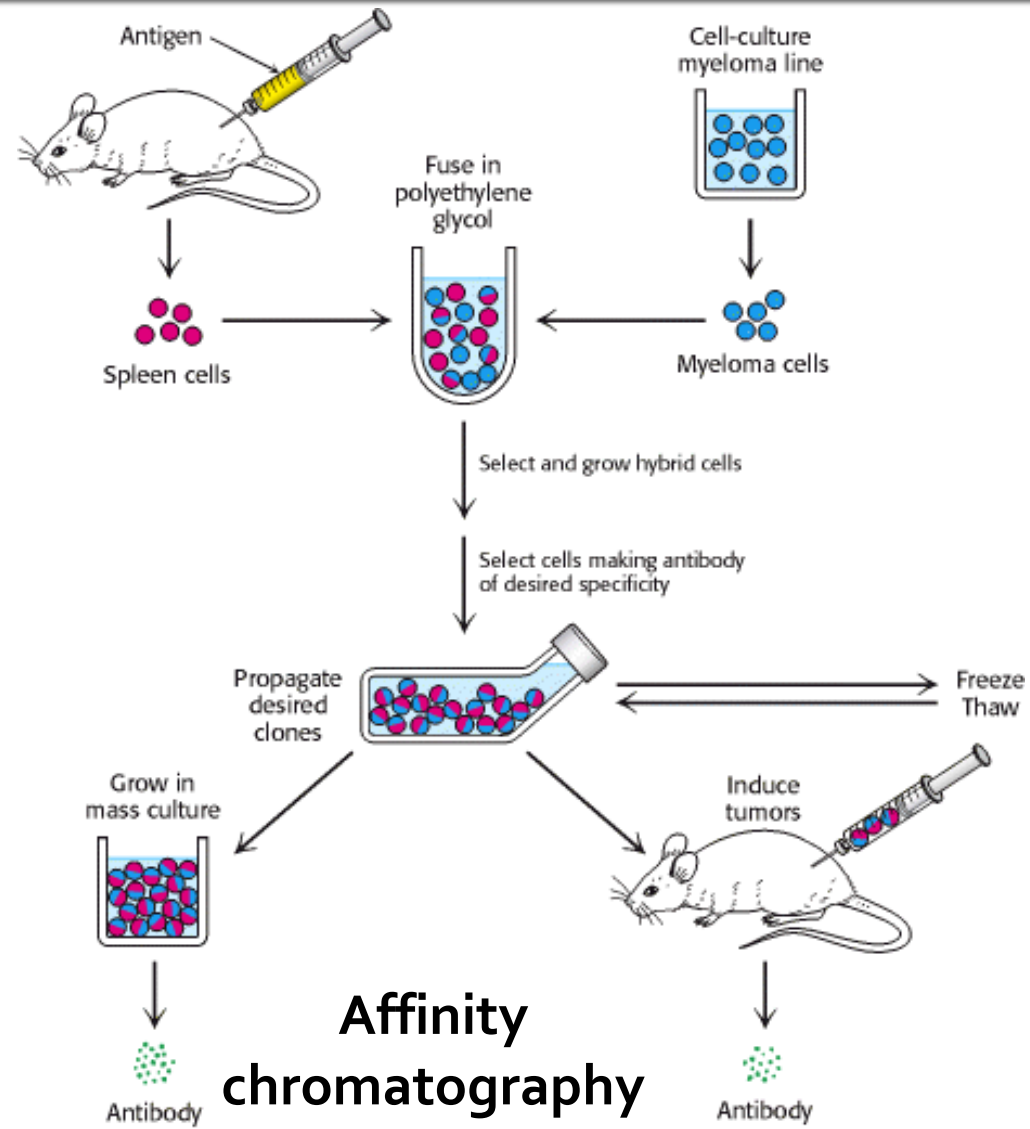
Diseases

- **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)**



Hybridomas & monoclonal antibodies

- Antigen injection → polyclonal antibodies (mixture of B cells)
- Polyclonal antibodies are not monospecific (different epitopes)
- How to make a specific monoclon?
- The technique provides long-term source of highly useful monoclonal antibodies



Benefits of monoclonal antibodies

- Can be used to measure the amounts of many individual proteins (eg, plasma proteins)
- Can determine the nature of infectious agents (eg, types of bacteria)
- Can be used to subclassify both normal (eg, lymphocytes) and tumor cells (eg, leukemic cells)
- Can be used to direct therapeutic agents to tumor cells
- Can be used to accelerate removal of drugs from the circulation when they reach toxic levels