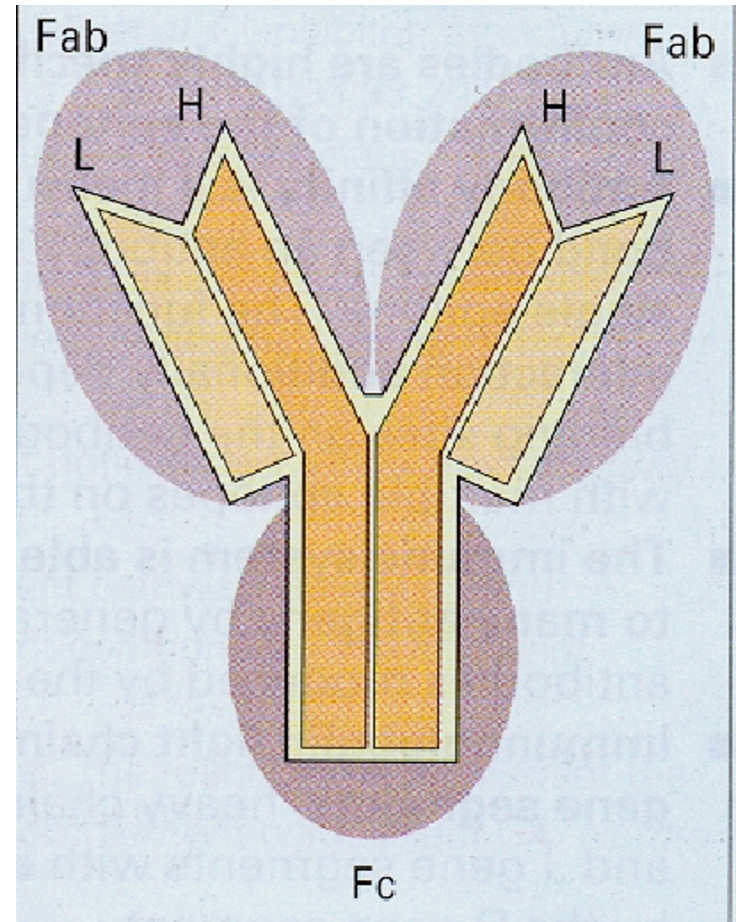


Lecture 14

31 Aug 2023



IMMUNOGLOBULINS



Immunoglobulins

- ▶ Humoral basis of immunity – 19th century.

Introduction of an Ag into an animal



Abs appeared in the serum & body fluids

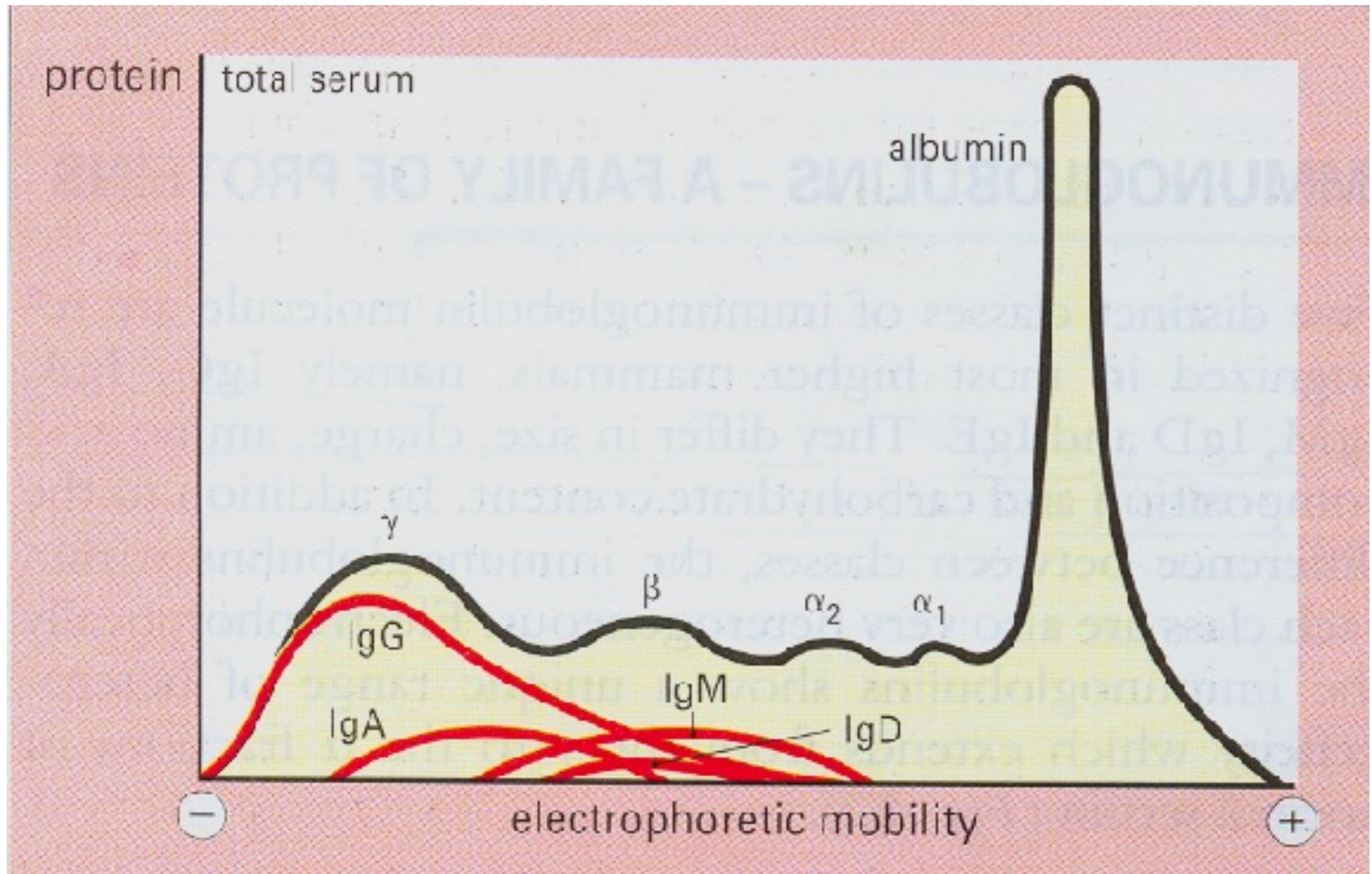


Immune sera

- ▶ Abs react with Ag in a specific & observable manner.

Distribution of Major Human Ig

- ▶ Electrophoresis of human serum separated serum proteins into 2 major components :
 1. Soluble Albumins
 2. Insoluble Globulins - α , β & γ
- ▶ Ab activity - γ globulin fraction of serum proteins.
- ▶ 1964 – ‘Immunoglobulin’ by WHO.

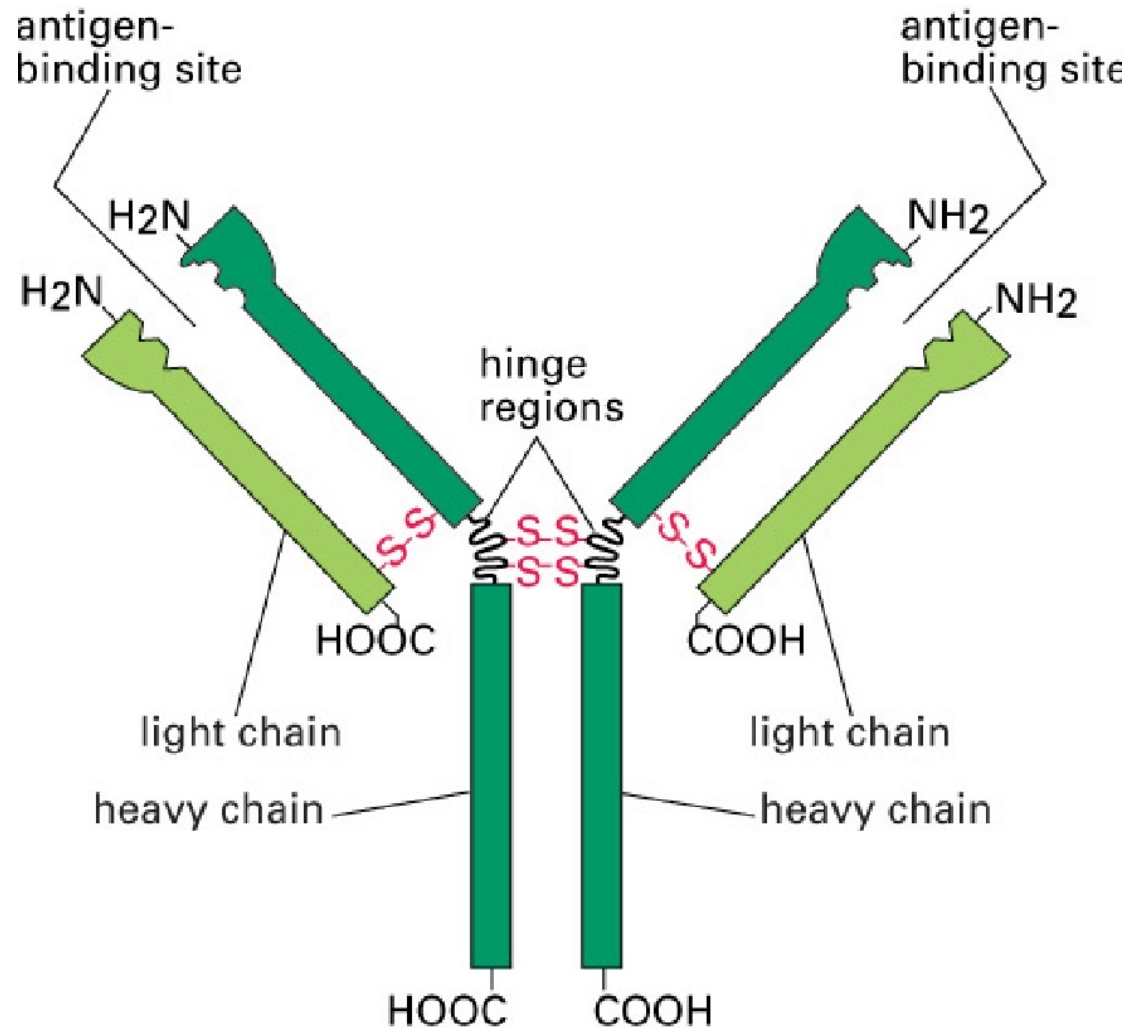


-
- ▶ Igs are synthesised mainly by the plasma cells & to some extent by the lymphocytes.
 - ▶ Constitutes 20-25% of total serum proteins.
 - ▶ 5 classes of Igs – IgG, IgA, IgM, IgE & IgD.
(**GAMED**: To recall)

Structure of an Ig

- ▶ Glycoproteins.
- ▶ 2 pairs of polypeptide chains – 2 light (L) chains & 2 heavy (H) chains.
- ▶ “L” chain – smaller chain
 - low molecular wt (25,000 Da)
- ▶ “H” chain – larger chain
 - high molecular wt (50,000 Da)

Structure of an Ig



Structure

- ▶ L chain attached to H chain by disulphide & non-covalent bonds.
- ▶ L chains : 2 forms – kappa (κ) & lambda (λ)
- ▶ Each molecule of Ig can have either κ or λ , but never both.
- ▶ H chains : structurally & antigenically distinct for each class.

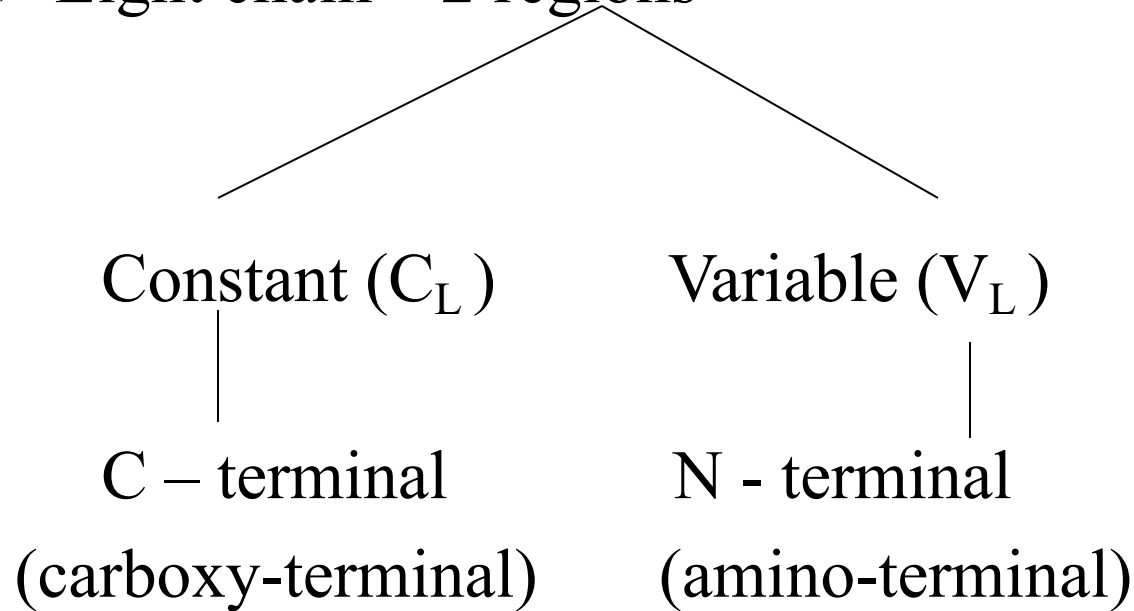
H chain

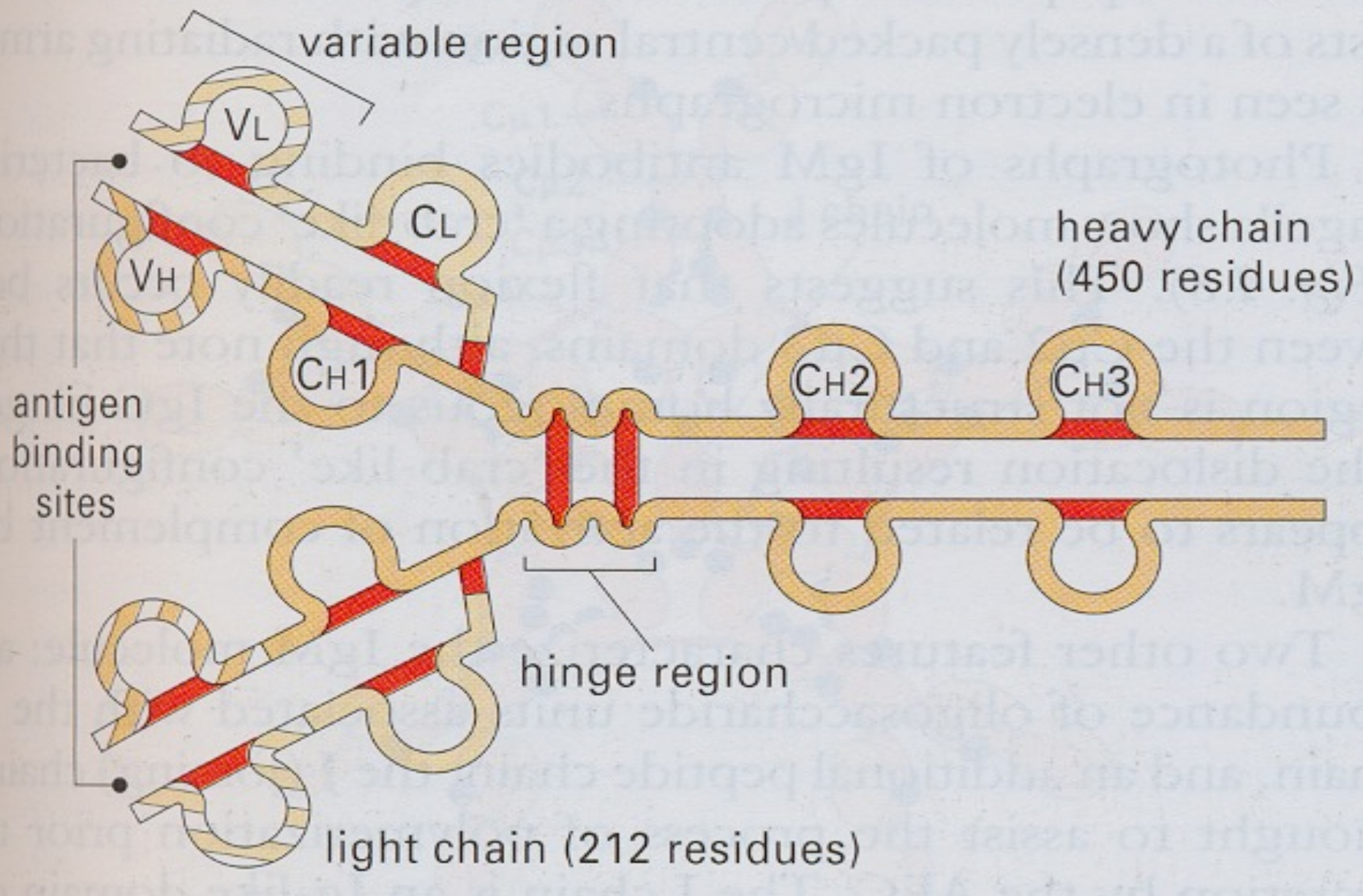
- H chain designated by Greek letter.

Ig class	H chain
Ig G	γ
Ig A	α
Ig M	μ
Ig E	ϵ
Ig D	δ

Structure

- ▶ Light chain – 2 regions



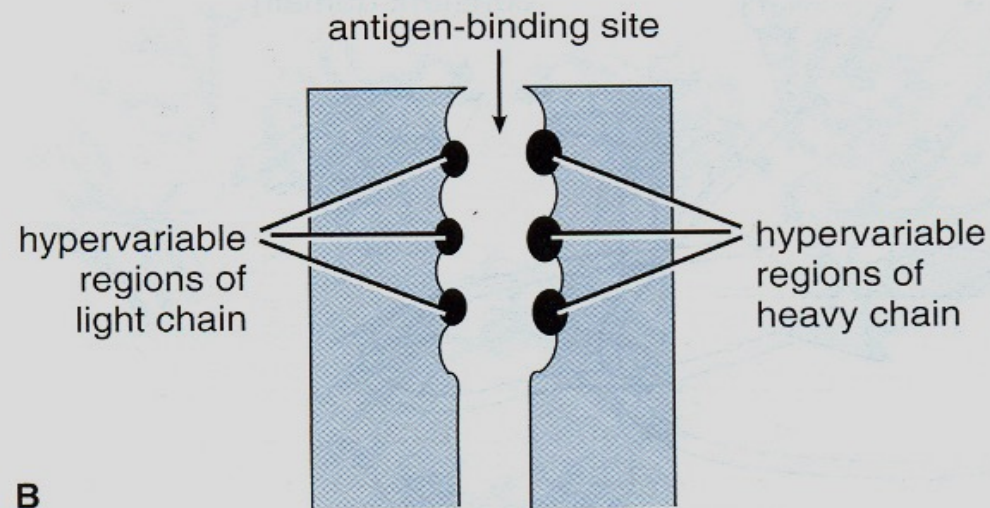
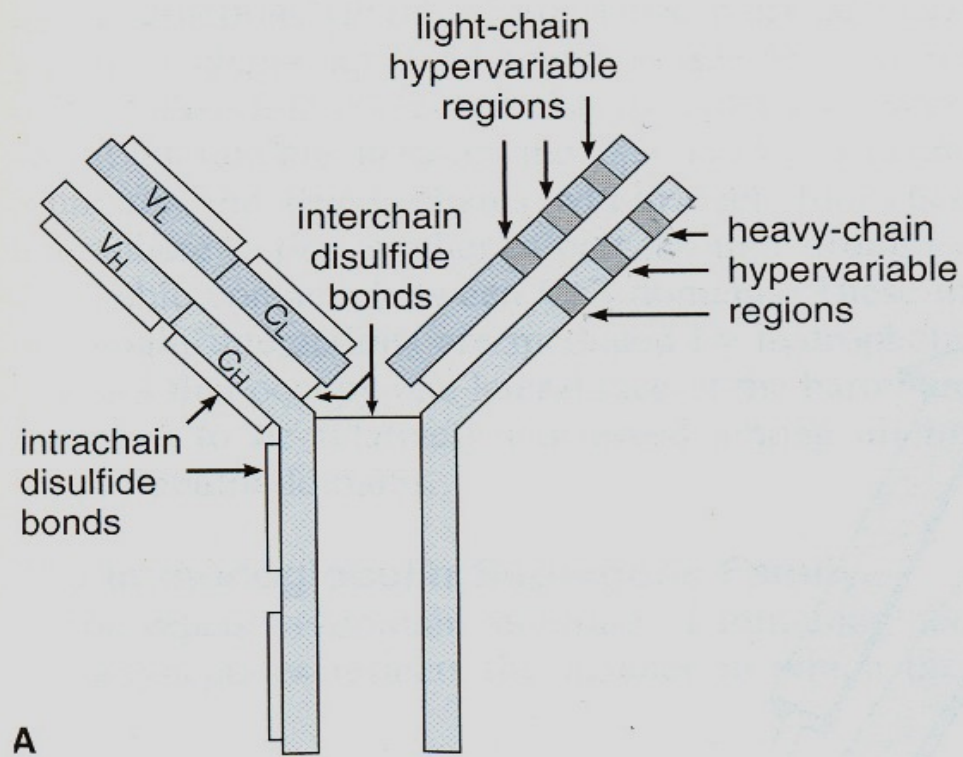


Structure

- ▶ H chain also divided into V_H & C_H regions; the C_H region is further divided into C_H1 , C_H2 & C_H3 .
- ▶ Regions also called as DOMAINS :
 - globular in shape
 - stabilized by intrachain disulphide bonds
- ▶ Ag binding sites are located in the variable domains.

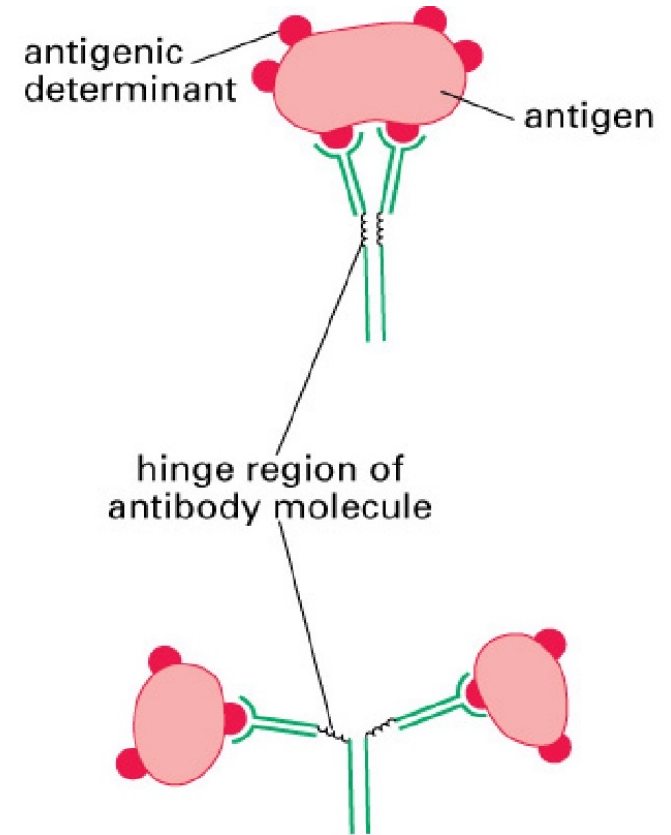
Hypervariable regions

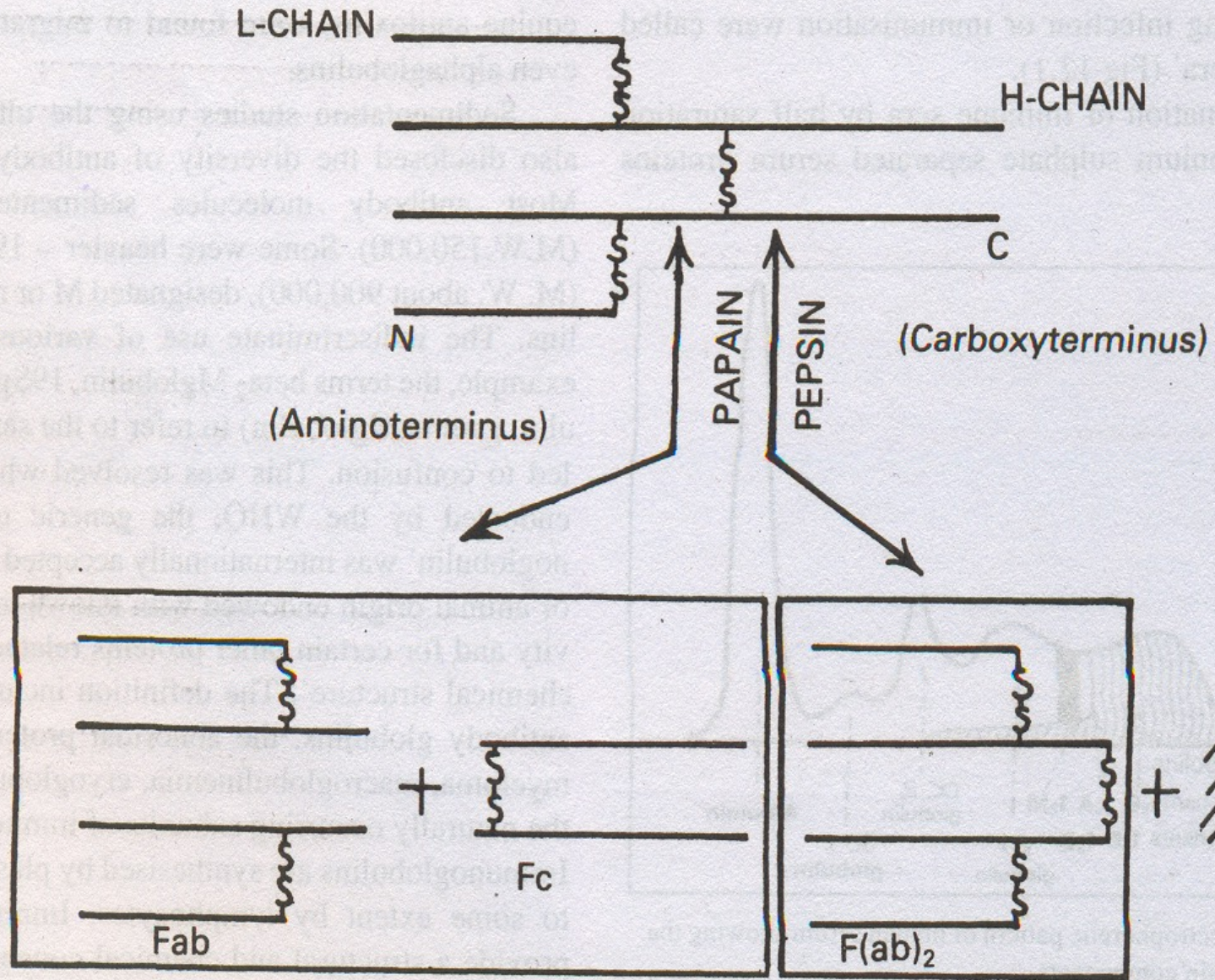
- ▶ Amino acid sequence in the variable region of L & H chains are not uniformly variable.
- ▶ Consists of some highly variable (hypervariable) & some relatively invariable zones.
- ▶ Highly variable zones actually make contact with the epitope on an Ag and are called as **Complementarity Determining Regions (CDRs)**
- ▶ 3 CDRs – each made up of 9 -12 amino acids. CDR3 is the longest & most variable of the three.



Structure

- ▶ ‘Hinge’ region – segment of H chain between C_H1 & C_H2 .
- ▶ Flexibility to Ab
- ▶ Susceptible to enzymes & chemicals.
- ▶ Studies involving the cleavage of Ig molecule by pepsin & papain have led to a detailed picture of Ig structure.





Fragments of Ig

- ▶ Fab – Ag binding.

Fc fragment

- ▶ Composed of carboxy terminal of H chain.
- ▶ Determines the biological properties of Ig molecule.
- ▶ Receptors for Fc portion expressed by mononuclear cells

neutrophils

NK cells

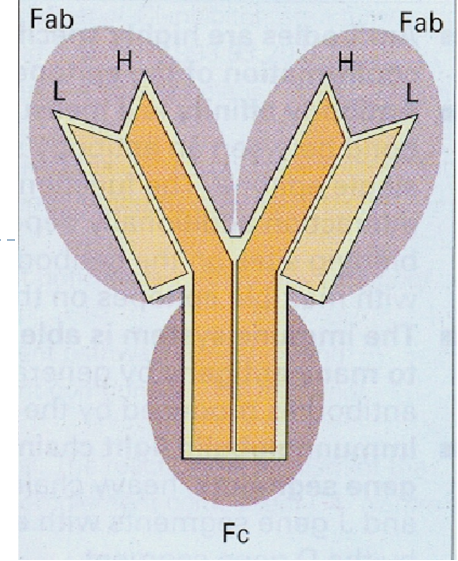
eosinophils &
mast cells

phagocytosis

tumour cell killing

mast cell degranulation

Ig G



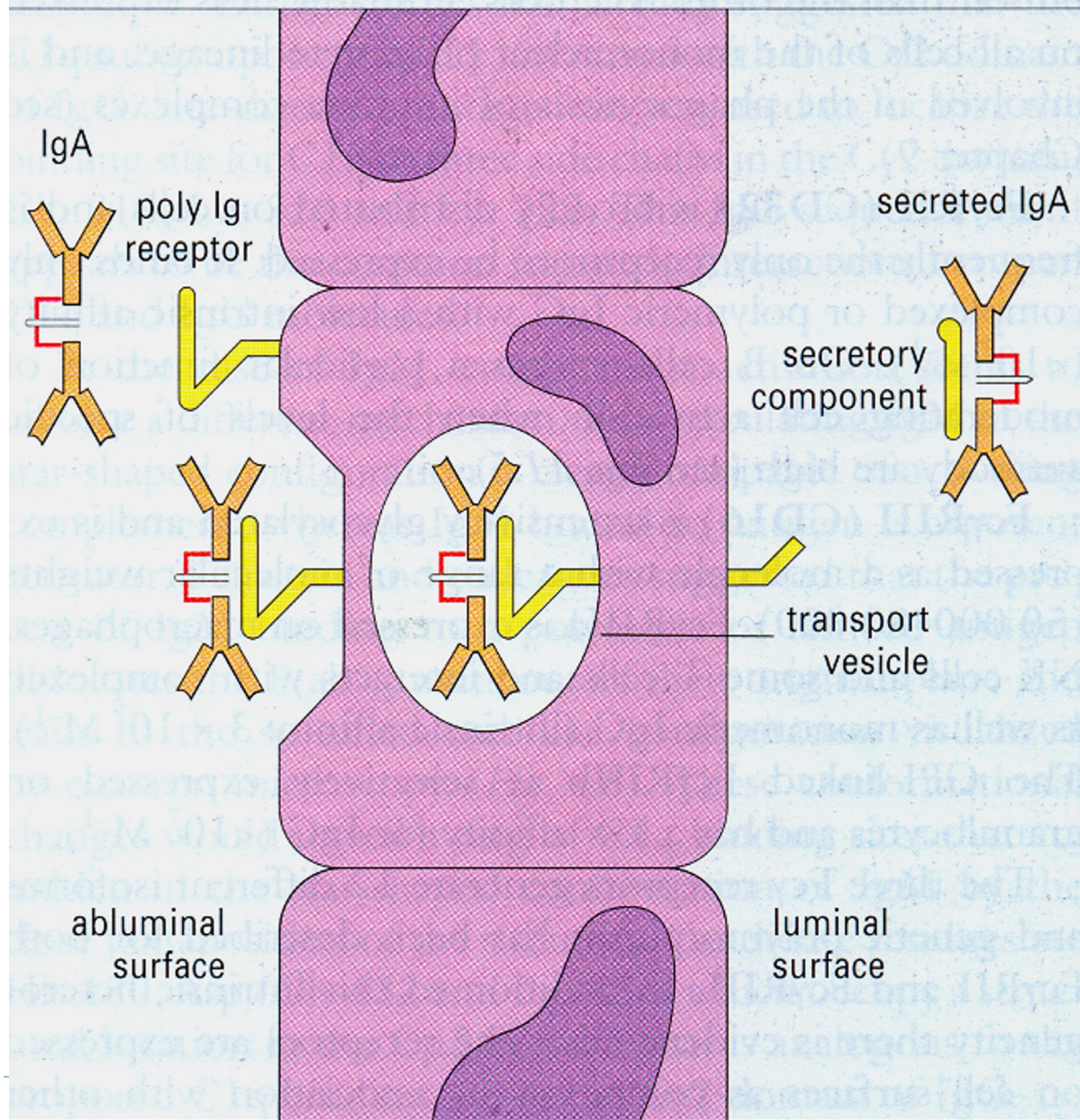
- ▶ Major serum Ig
- ▶ Constitutes 75% of total Igs.
- ▶ 4 subclasses found in humans – IgG1, IgG2, IgG3 & IgG4, each having a distinct type of gamma chain.
- ▶ Major Ab of secondary response, found both in serum & body fluids.
- ▶ Only maternal Ig to be transported across placenta – natural passive immunity in newborn.
- ▶ Participates in complement fixation, precipitation & neutralisation of viruses & toxins.

Ig A

- ▶ 2nd most abundant, constitutes 10-13 %.
- ▶ Major Ig in the **colostrum, saliva, tears & other body fluids**.
- ▶ Occur in 2 forms : IgA1 & IgA2.
- ▶ Secretory IgA is always in **dimeric form** – composed of 2 basic chain units, a **J chain** & the **secretory** component.
- ▶ Secretory component helps to transport the dimer from the submucosa to the mucosal cell surface.
- ▶ Secretory component protects IgA from proteolytic digestion and denaturation.

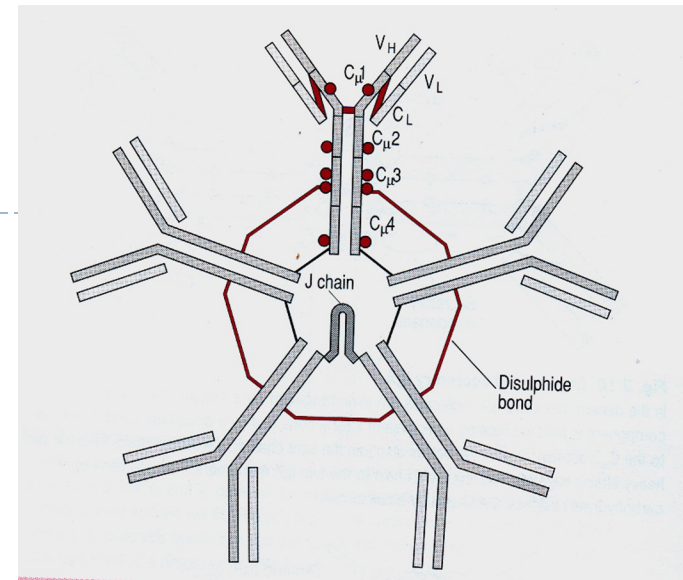


Transport of IgA across the mucosal epithelium



Ig M

- ▶ 5-8 % of serum Igs.
- ▶ Short lived Abs.
- ▶ **Pentameric** structure.
- ▶ Predominant Ab in primary immune response.
- ▶ Earliest Ab to be synthesized by the fetus.
- ▶ Confined to the intravascular pool due to its large size.
- ▶ **Not transported across placenta.**
- ▶ Presence of IgM in newborn indicates intra uterine infection.
- ▶ Useful in the diagnosis of congenital infections like syphilis, rubella, HIV, toxoplasmosis etc.



Ig E

- ▶ Present in very low levels in serum.
 - ▶ Found on the surface of mast cells & basophils which have specific receptors for the Fc portion of IgE.
 - ▶ Chiefly produced in the linings of respiratory & intestinal tracts.
 - ▶ Responsible for anaphylactic type of hypersensitivity.
 - ▶ Defense against parasitic infections.
-



Ig D

- ▶ Resembles Ig G structurally.
- ▶ Occurs along with Ig M on the surface of B cells
- ▶ Very susceptible to proteolytic attack.



TABLE 4-2 Properties and biological activities* of classes and subclasses of human serum immunoglobulins

Property/Activity	IgG1	IgG2	IgG3	IgG4	IgA1	IgA2	IgM [‡]	IgE	IgD
Molecular weight [†]	150,000	150,000	150,000	150,000	150,000–600,000	150,000–600,000	900,000	190,000	150,000
Heavy-chain component	γ1	γ2	γ3	γ4	α1	α2	μ	ε	δ
Normal serum level (mg/ml)	9	3	1	0.5	3.0	0.5	1.5	0.0003	0.03
In vivo serum half life (days)	23	23	8	23	6	6	5	2.5	3
Activates classical complement pathway	+	+/-	++	—	—	—	+++	—	—
Crosses placenta	+	+/-	+	+	—	—	—	—	—
Present on membrane of mature B cells	—	—	—	—	—	—	+	—	+
Binds to Fc receptors of phagocytes	++	+/-	++	+	—	—	?	—	—
Mucosal transport	—	—	—	—	++	++	+	—	—
Induces mast-cell degranulation	—	—	—	—	—	—	—	+	—

*Activity levels indicated as follows: ++ = high; + = moderate; +/- = minimal; — = none; ? = questionable.

[†]IgG, IgE, and IgD always exist as monomers; IgA can exist as a monomer, dimer, trimer, or tetramer. Membrane-bound IgM is a monomer, but secreted IgM in serum is a pentamer.

[‡]IgM is the first isotype produced by the neonate and during a primary immune response.

Lecture 15

4 Sept 2023



Antibody diversity

- ▶ An individual produces a large number of Abs to cope with the vast number of different Ags.
- ▶ This Ab diversity is due to the Ig genes.
- ▶ Genes coding for the variable & constant portions of the chains are separate
- ▶ One or only few genes code for C region whereas many genes code for the V region.



To date, seven means of antibody diversification have been identified in mice and humans:

- Multiple germ-line gene segments
- Combinatorial V-(D)-J joining
- Junctional flexibility
- P-region nucleotide addition (P-addition)
- N-region nucleotide addition (N-addition)
- Somatic hypermutation
- Combinatorial association of light and heavy chains



Table 3.2 Diversity in immunoglobulin (Ig) and T-cell receptor (TCR) genes. TCR has fewer germ line V gene segments but a much greater junctional diversity.

Mechanism	Ig		TCR		TCR	
	H	κ	α	β	γ	δ
V gene segments	100–250	100	75	75	8	4
Diversity in V gene segments	$H \times \kappa$ 10 000–25 000		$\alpha \times \beta$ 5625		$\gamma \times \delta$ 32	
D gene segments	10	0	0	2	0	2*
D read in cell frames	rarely	—	—	often	—	often
J gene segments	6	5	75	12	5	3
N-region additions	V–D D–J	—	V–J	V–D D–J	V–J	V–D D–D D–J
Potential using all mechanisms†	10^{11}		10^{16}		10^{18}	

* In all δ chains more than one D gene segment can be included.

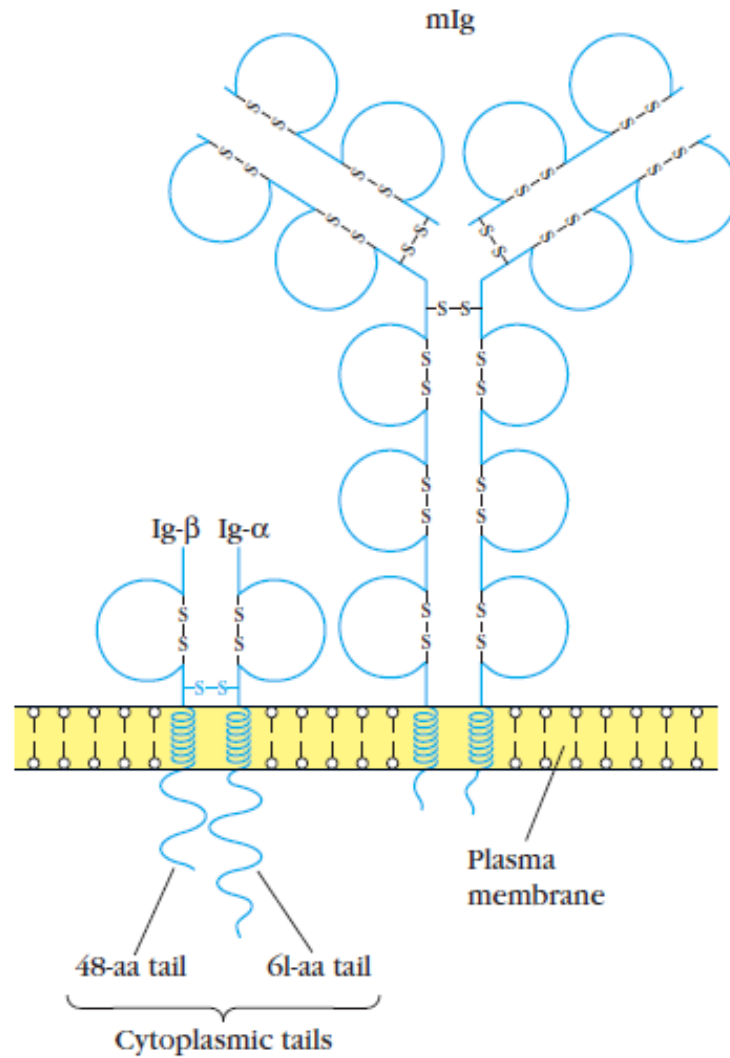
† Somatic mutations not included.

Generation of diversity

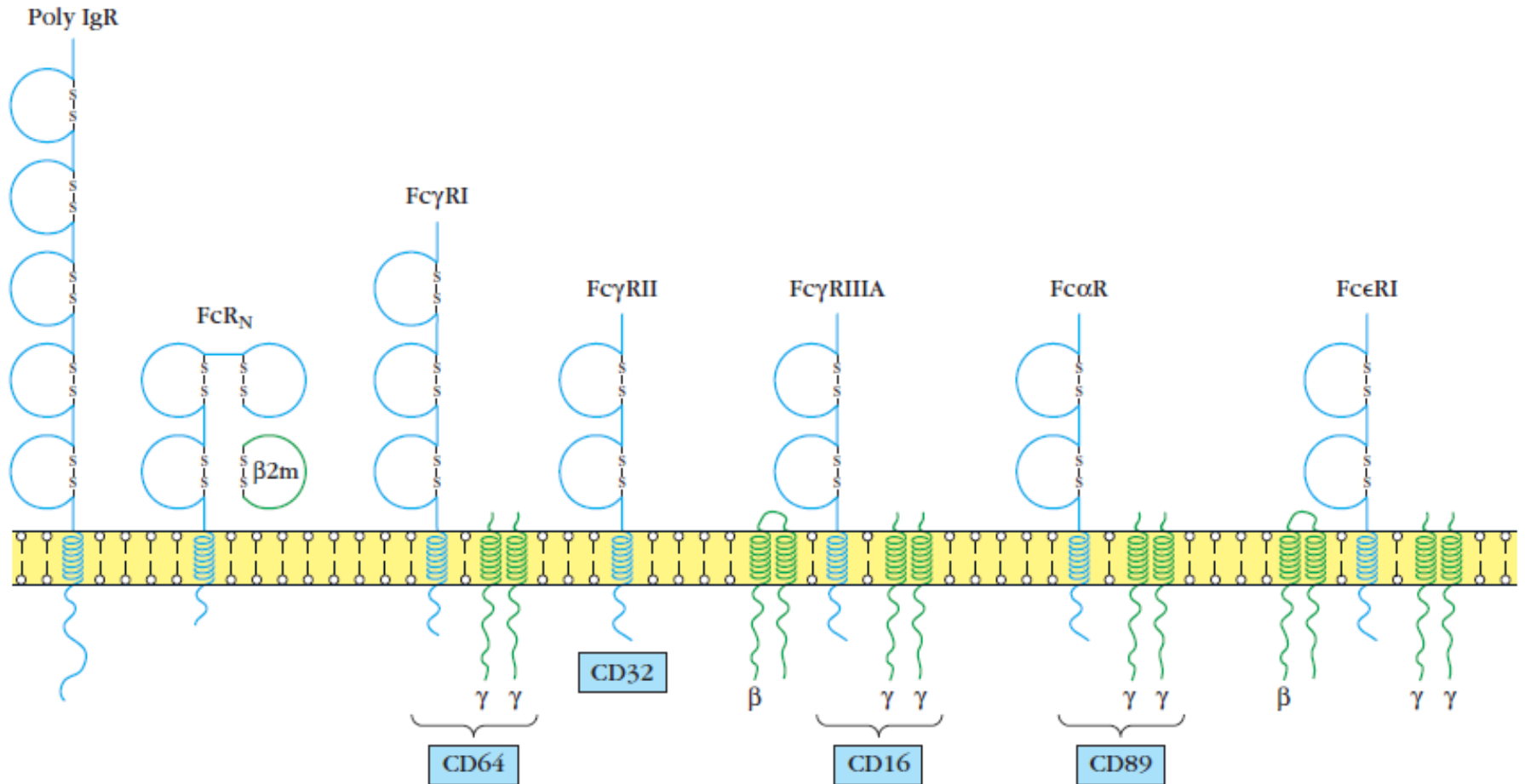
- ▶ Multiple V- region genes.
- ▶ V-J recombination in light chain & V-D-J recombination in heavy chain.
- ▶ Junctional diversity
 1. Nucleotide addition – extra nucleotides may get inserted between V_H & D, and between D & J_H segments



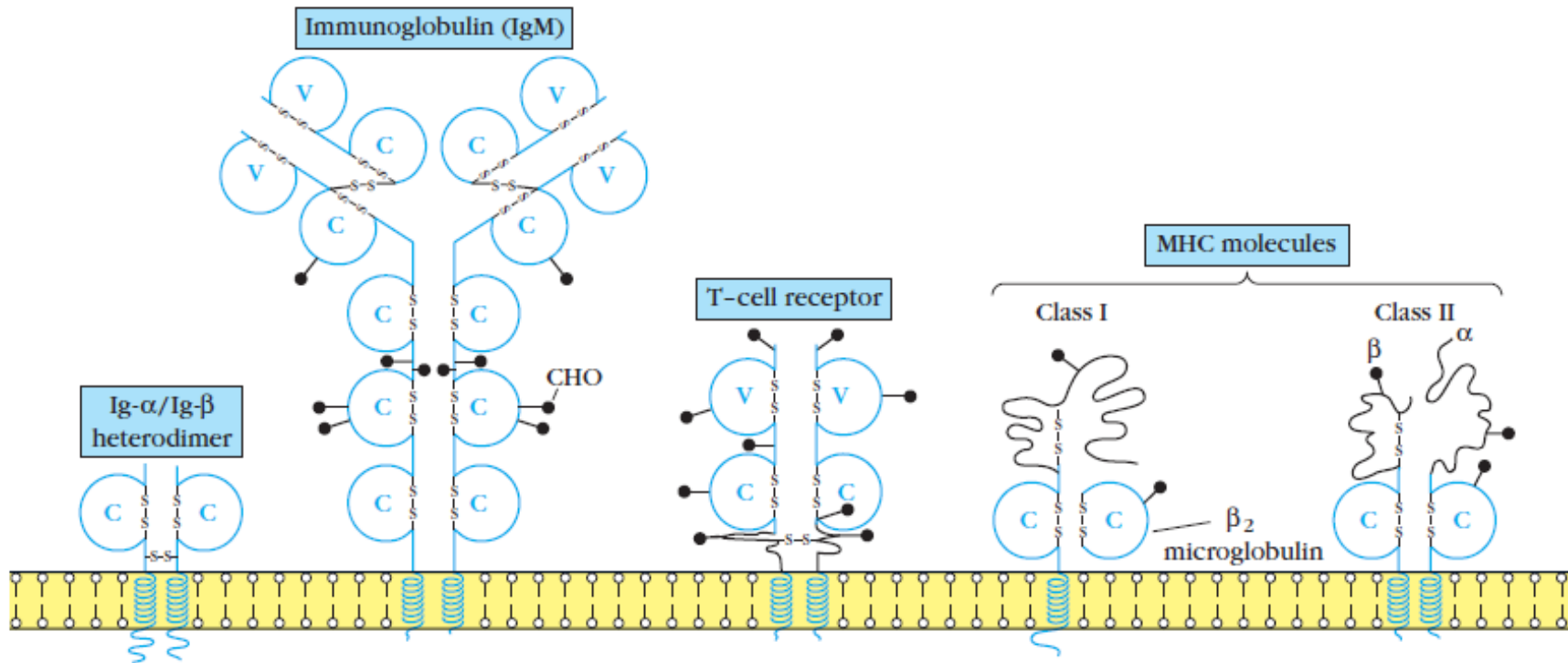
General structure of BCR



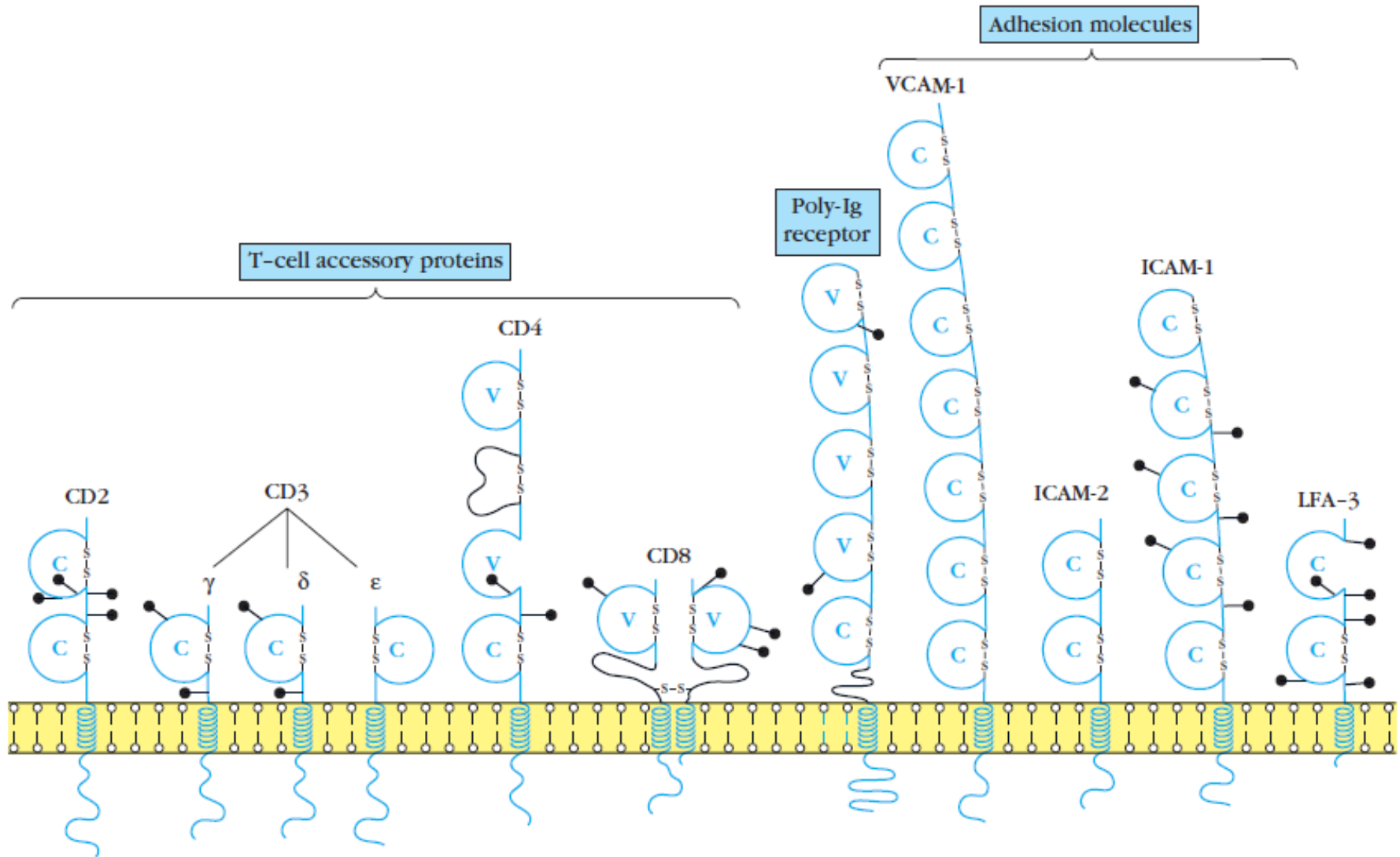
Structure of Fc-receptors membrane bound



Immunoglobulin superfamily membrane bound



Immunoglobulin superfamily membrane bound



Major Histocompatibility Complex



MHC

- ▶ Major Histocompatibility Complex
 - ▶ Cluster of genes found in all mammals
 - ▶ Its products play role in discriminating self/non-self
 - ▶ Participant in both humoral and cell-mediated immunity
- ▶ MHC act as antigen presenting structures
- ▶ In Human MHC is found on chromosome 6
 - ▶ Referred to as HLA complex (human leucocyte antigen)
- ▶ In Mice MHC is found on chromosome 17
 - ▶ Referred to as H-2 complex

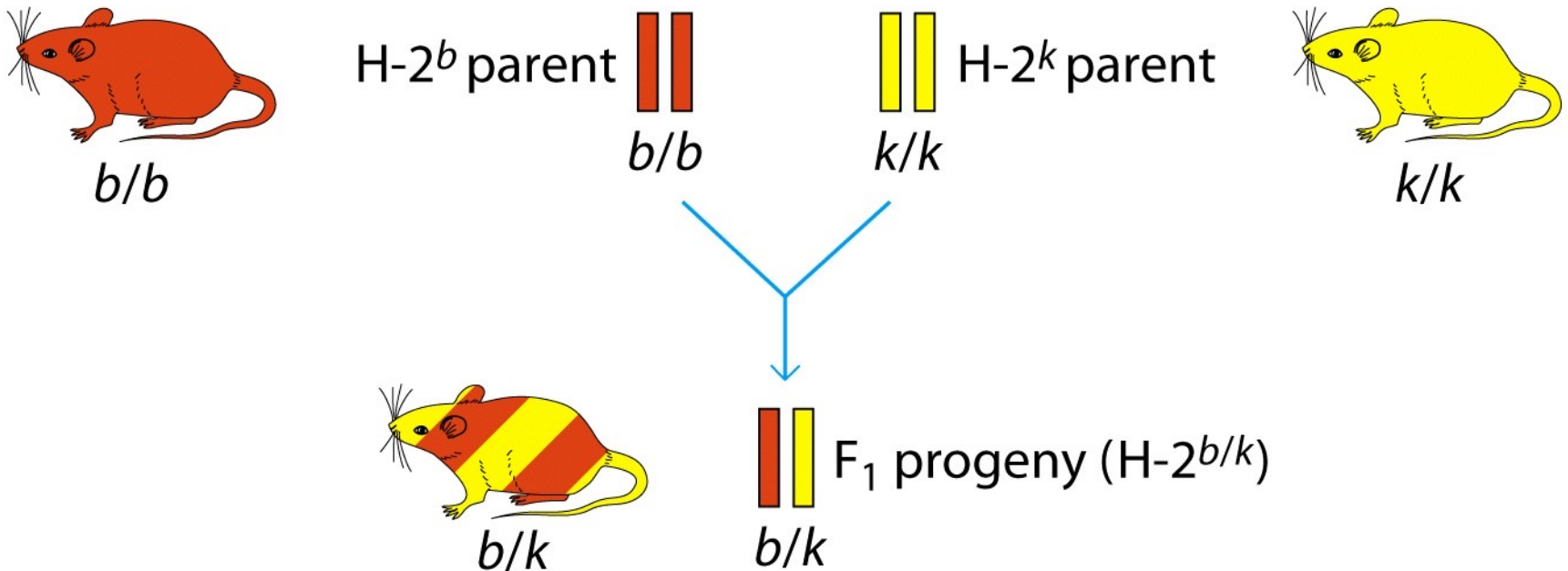
MHC

- ▶ Genes of MHC are organized in 3 Classes
 - ▶ Class I MHC genes
 - ▶ Glycoproteins expressed on all nucleated cells
 - ▶ Major function to present processed Ags to T_C (cytotoxic CD8 T cells)
 - ▶ Class II MHC genes
 - ▶ Glycoproteins expressed on MΦ, B-cells, DCs
 - ▶ Major function to present processed Ags to T_H (Helper CD4 T cells)
 - ▶ Class III MHC genes
 - ▶ Products that include secreted proteins that have immune functions. Ex. Complement system, inflammatory molecules

Skin graft acceptance is controlled by MHC

(a) Mating of inbred mouse strains with different MHC haplotypes

Homologous chromosomes with MHC loci



(b) Skin transplantation between inbred mouse strains with same or different MHC haplotypes

Parental recipient

Skin graft donor

Progeny recipient

