BP 605 T. Pharmaceutical Biotechnology (Theory)

Structure of Immunoglobulins

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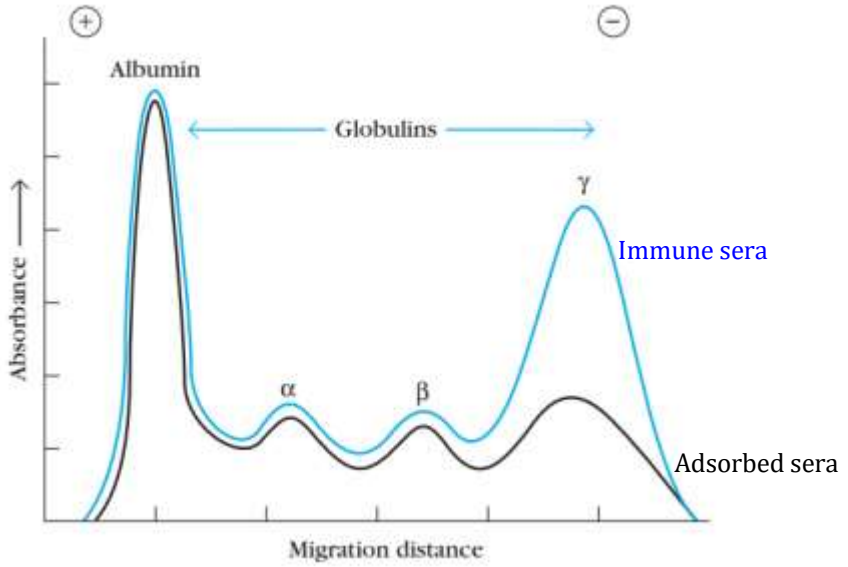
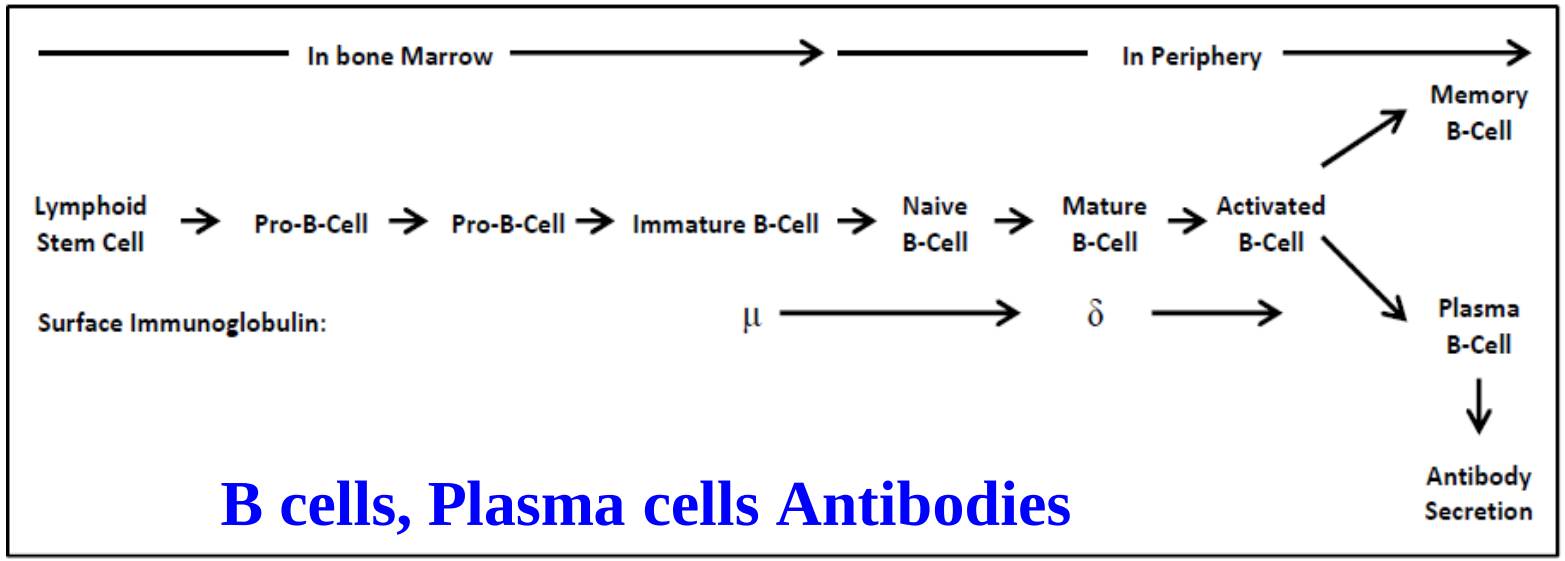
Overview

- Definition and Introduction
- Immunoglobulins Structure
- Immunoglobulins Function

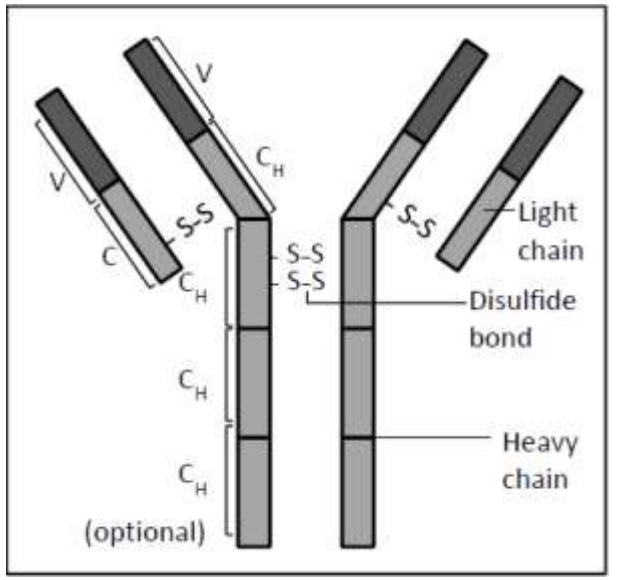
Immunoglobulins

- ✓ **Definition:** Immunoglobulins are glycoprotein molecules **belonging to γ-globulins class of plasma proteins** produced in response to a non-self or an altered self immunogen and act as antibodies in humoral adaptive immune response.
- ✓ Immunoglobulins are produced in vertebrates by plasma cells, which are the terminally differentiated B lymphocytes



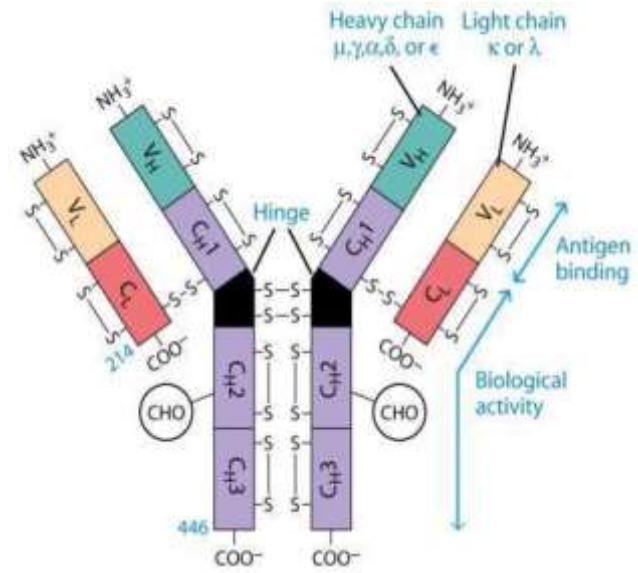


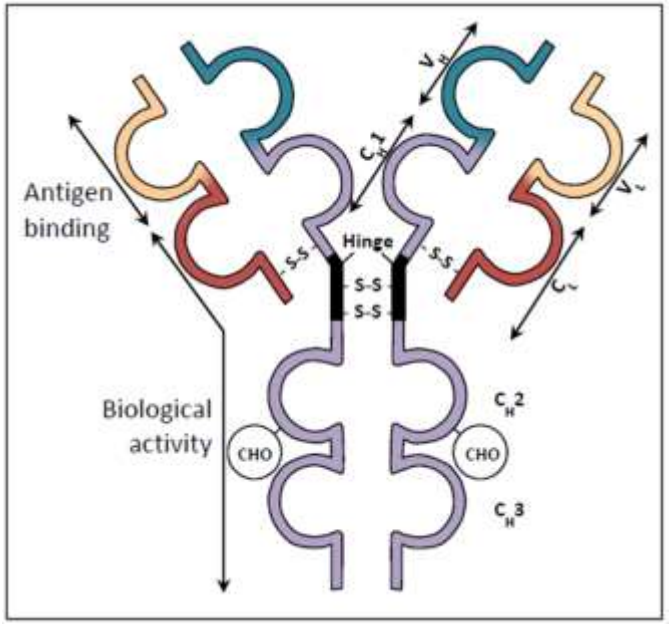
B cells, Plasma cells Antibodies



Basic Immunoglobulin Structure

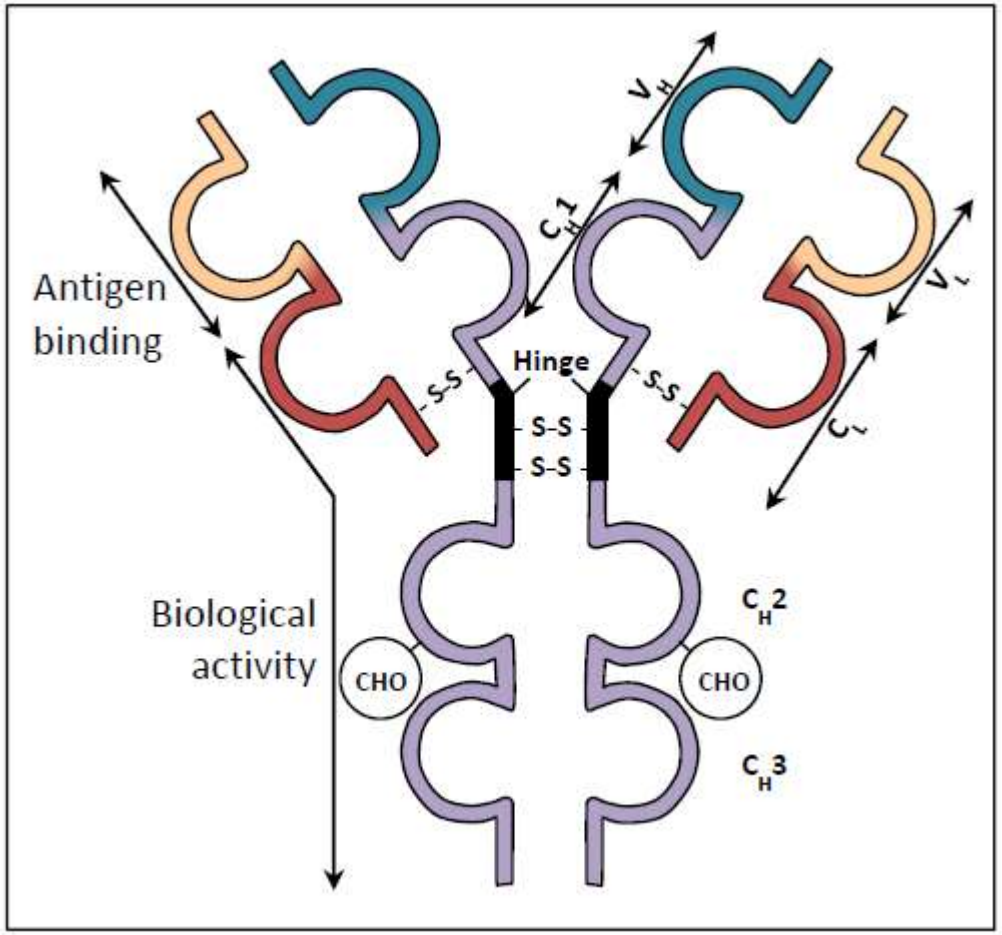
- ✓ γ-globulin
- ✓ glycoprotein
- ✓ heterodimer
- ✓ 'Y' shaped molecule
- ✓ coded by immunoglobulin supergene family
- ✓ Secreted antibodies are the major effector molecules of humoral immunity





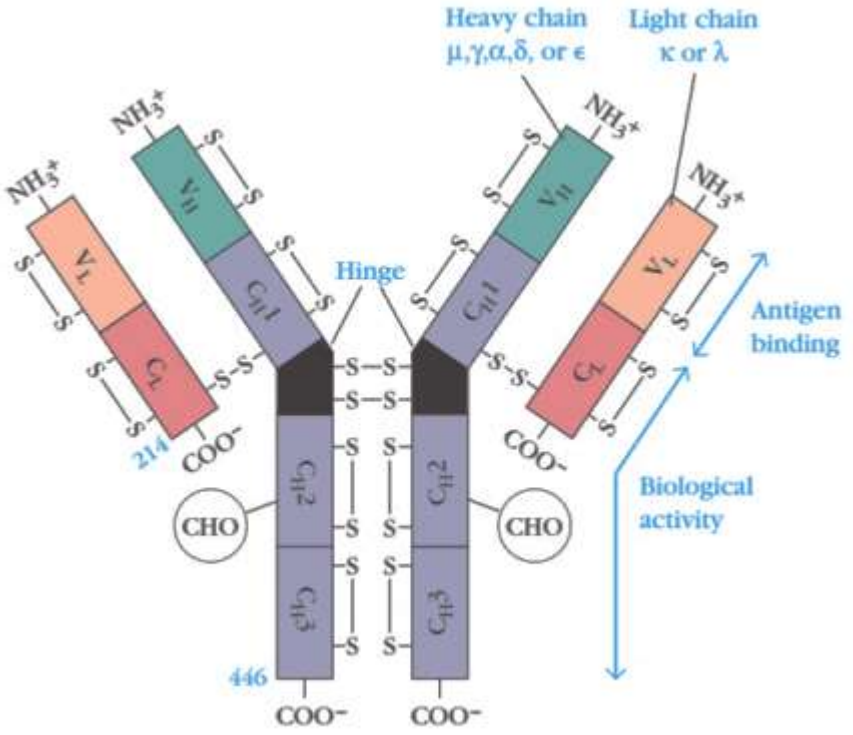
Immunoglobulin Structure – a monomer (H2L2)

- ✓ 2 Heavy & 2 Light chains
- ✓ Disulfide bonds
  - Inter-chain
  - Intra-chain
- ✓ Variable & Constant regions in each chain
  - V_L & C_L
  - V_H & C_H
- ✓ Forms globular loop like structure called as domains
- ✓ Hinge Region: proline-rich (The μ and ε heavy chains, which lack a hinge region, contain an additional domain in the middle of the molecule).



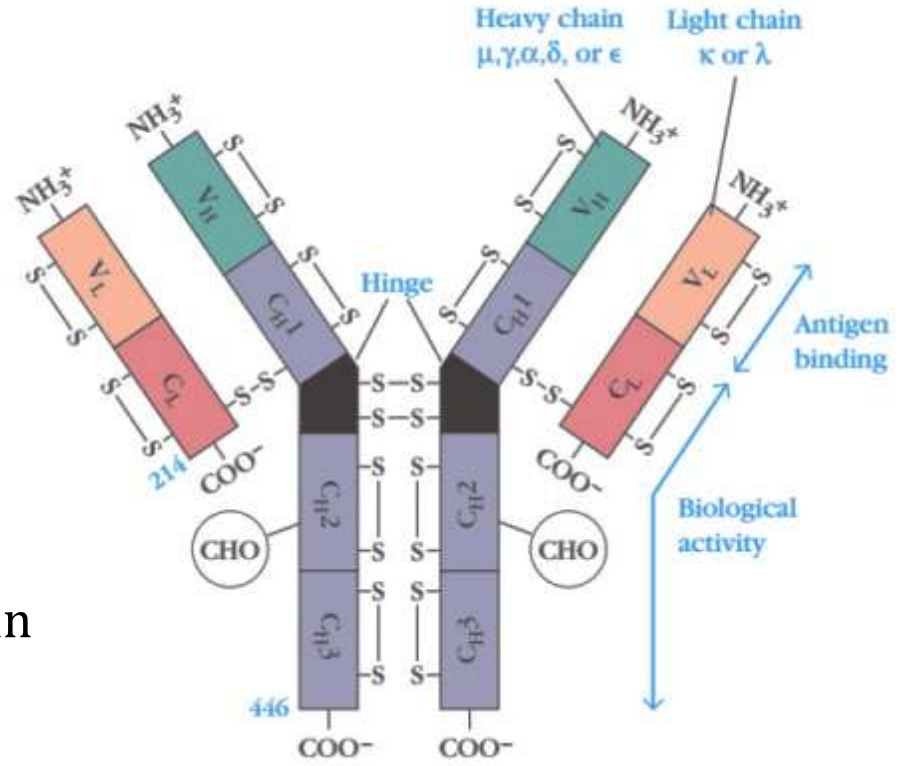
Basic Immunoglobulin Structure

- ✓ A monomer (H2L2) of an immunoglobulin molecule is made up of:
  - 2 Light Chains (identical) ~25 KDa
  - 2 Heavy Chains (identical) ~50 KDa
- ✓ Each light chain bound to heavy chain by disulfide bonds (H-L)
- ✓ Each heavy chain bound to heavy chain by disulfide bonds (H-H)
- ✓ The ¼ portion of each H chain and ½ of each L chain towards amino terminal are more variable (110 aa each - V_H and V_L) in amino acid composition as compared to the remaining portion towards carboxyl terminal (C_H and C_L) in each monomer, which has nearly constant composition in each domain of a given isotype.
- ✓ CDR (Complementarity Determining Regions) are actual areas where antigen binds and are present within variable region.



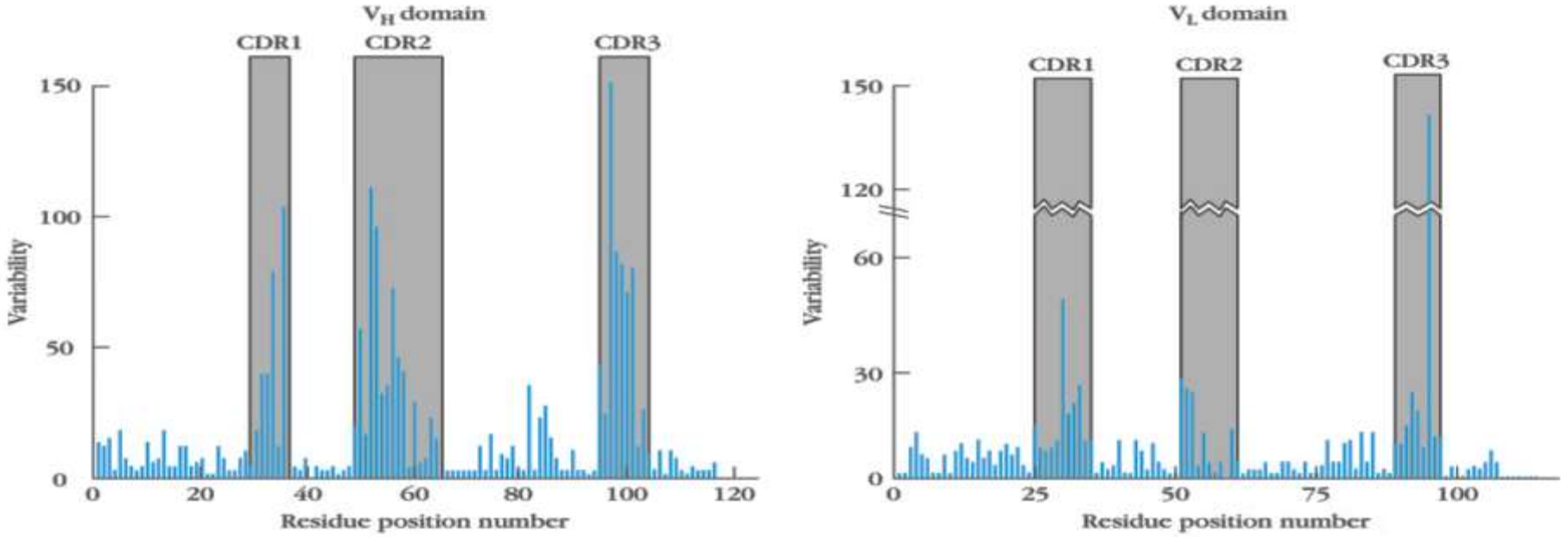
Basic Immunoglobulin Structure

- ✓ **Repeating Domains of ~110 a/a**
  - Intra-chain disulfide bonds within each domain
- ✓ **Heavy chains**
  - 1 V_H and either 3 or 4 C_H (C_H1, C_H2, C_H3, C_H4)
- ✓ **Light chains**
  - 1 V_L and 1 C_L
- ✓ **Hinge Region**
  - Rich in cysteine residues (disulfide bonds)
  - Rich in proline residues (flexible)
  - Proline residues are target for proteolytic digestion (papain and pepsin)
  - Hinge found in IgG, IgA and IgD
  - IgM and IgE lack hinge region
  - They instead have extra C_H4 Domain
- ✓ **Oligosaccharides**

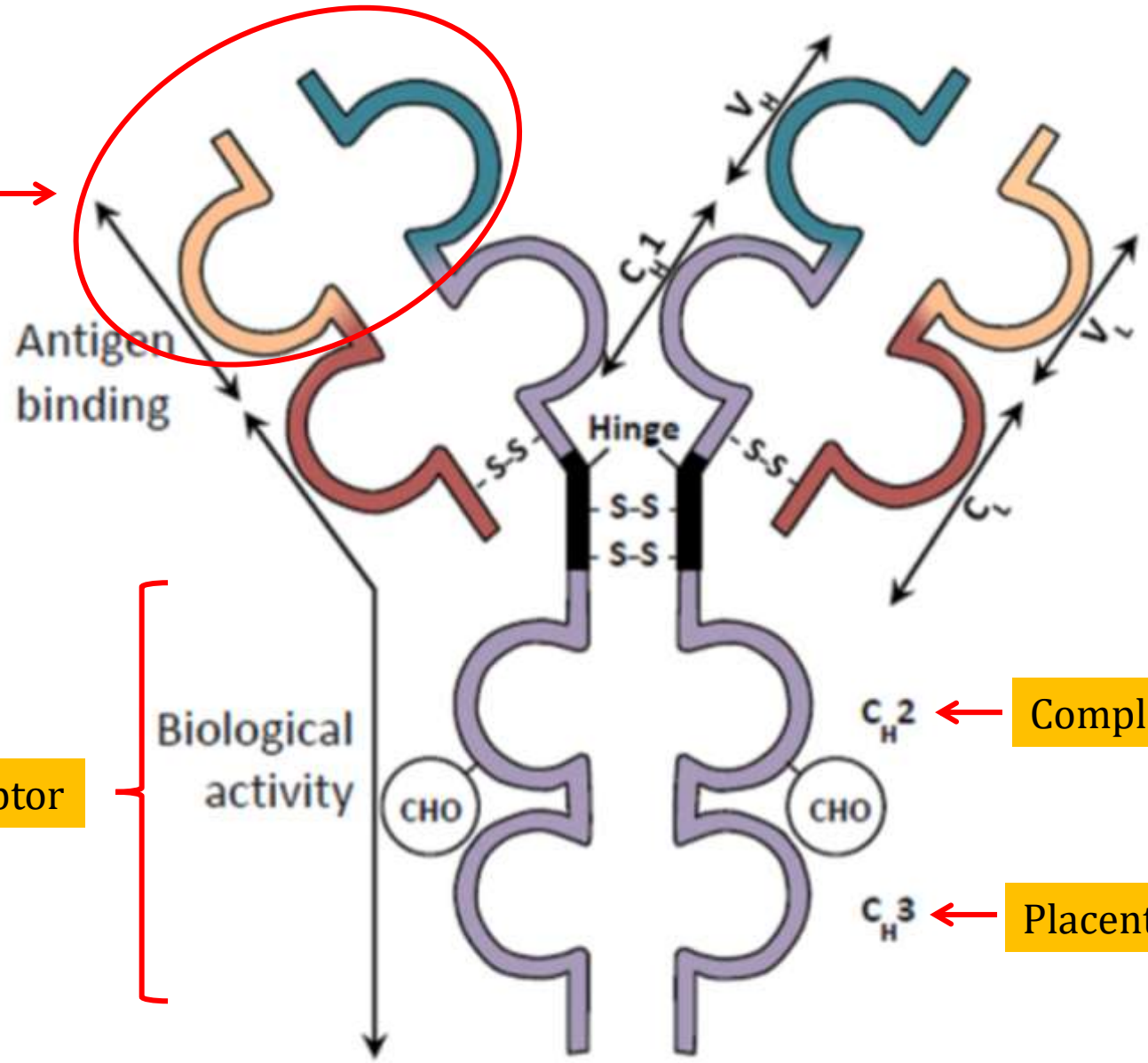


Structure of the Variable Region

- ✓ Hypervariable (HVR) or Complementarity determining regions (CDR) – hot spots within variable region of both H and L chains which exhibit more variation in aa composition than other regions
- ✓ HVRs form paratope – the epitope binding region on antibody
- ✓ Framework regions (FR)

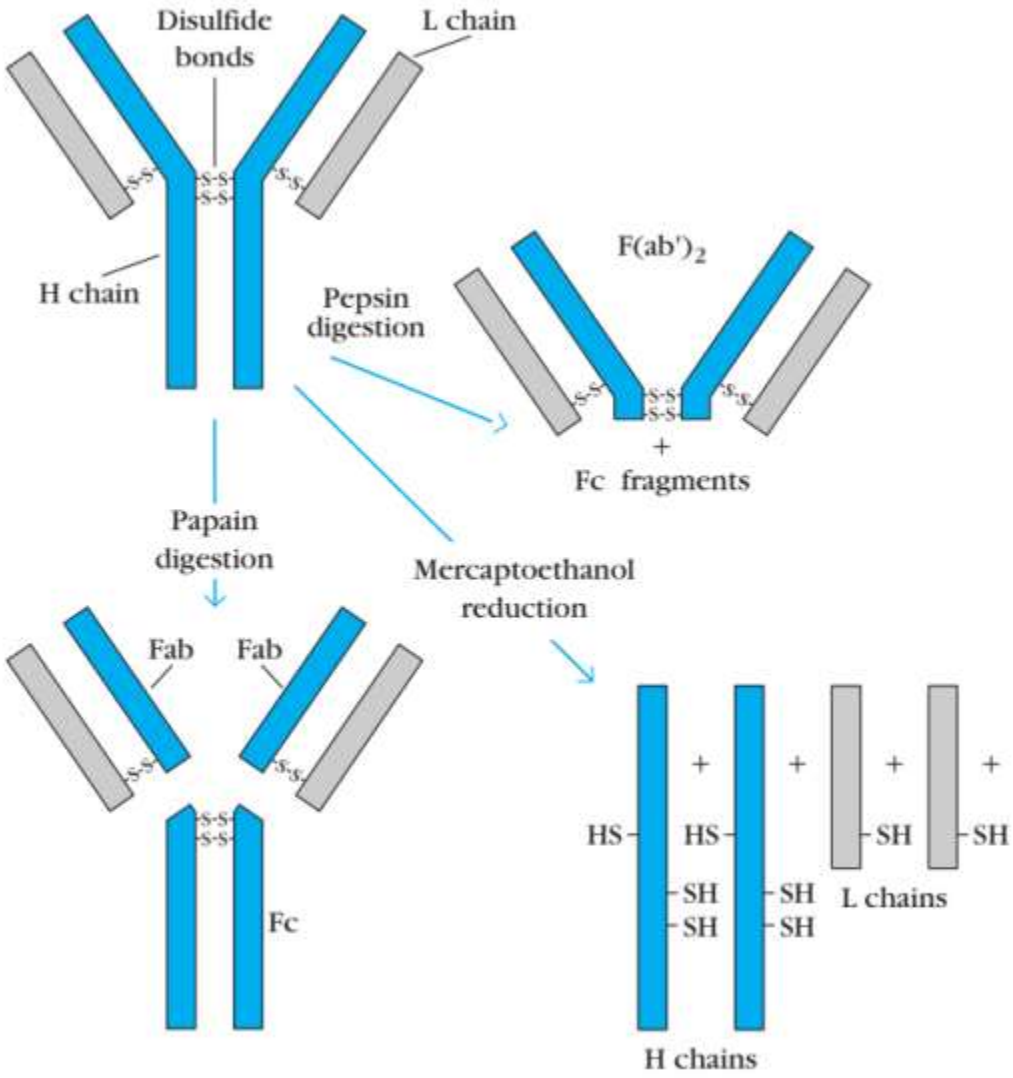


Immunoglobulin Fragments: Structure/Function Relationships



Enzymatic digestion of antibodies

- ✓ Digestion with Papain yields
  - – 3 fragments
  - – 2 identical Fab (each monovalent) and 1 Fc
  - – Fab - fragment that is antigen binding
    - (Specificity determined by V_H and V_L)
  - – Fc - crystallize in cold storage
    - – Effector functions
- ✓ Digestion with Pepsin yields
  - – F(ab`)$_2$ (divalent)
  - – No Fc recovery; digested entirely
- ✓ Mercapto-ethanol reduction eliminates disulfide bonds



Immunoglobulin Classes

✓ Sequencing of heavy chains of several immunoglobulins in human beings and mice revealed:

- A highly variable (V) region of 100-110 amino acids at amino terminus of each H chain
- Five basic amino acid sequence patterns in remaining constant (C) region of H chains which differ between H chains of each pattern, but not in all H chains of a given pattern

– α,γ, δ, ε, μ types of heavy chains

– IgA, IgG, IgD, IgE and IgM classes of immunoglobulins

– The above classes are called isotype named on basis of type of heavy chain

– κ or λ light chains; each class can have either of these

– Minor differences led to sub-classes

TABLE 4-1 Chain composition of the five immunoglobulin classes in humans

Class	Heavy chain	Subclasses	Light chain	Molecular formula
IgG	γ	γ1, γ2, γ3, γ4	κ or λ	$\gamma_2\kappa_2$ $\gamma_2\lambda_2$
IgM	μ	None	κ or λ	$(\mu_2\kappa_2)_n$ $(\mu_2\lambda_2)_n$ $n = 1$ or 5
IgA	α	α1, α2	κ or λ	$(\alpha_2\kappa_2)_n$ $(\alpha_2\lambda_2)_n$ $n = 1, 2, 3$, or 4
IgE	ϵ	None	κ or λ	$\epsilon_2\kappa_2$ $\epsilon_2\lambda_2$
IgD	δ	None	κ or λ	$\delta_2\kappa_2$ $\delta_2\lambda_2$

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	−	−	−	−	−	−	−	+	−

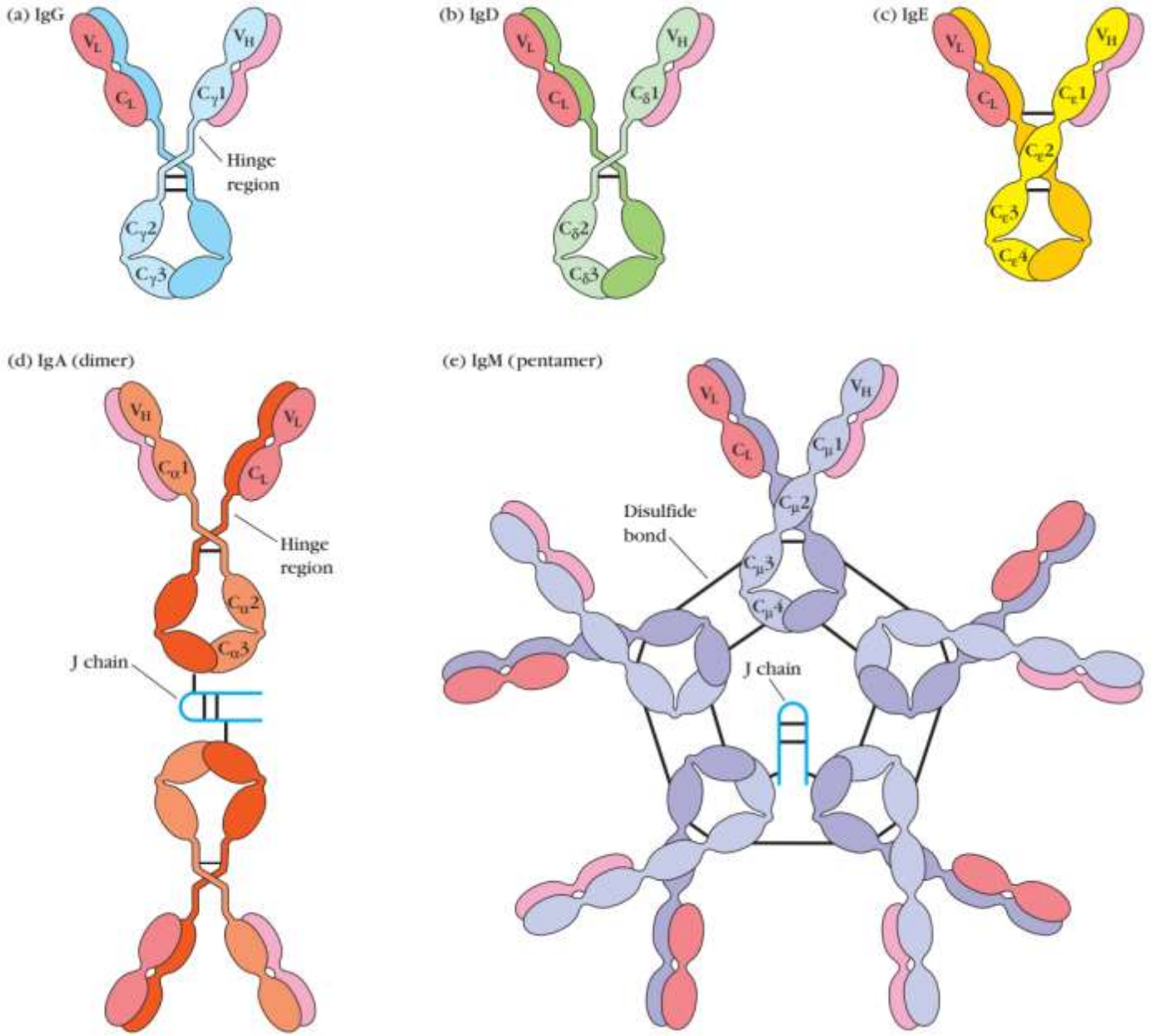
*Activity levels indicated as follows: ++ = high; + = moderate; +/− = minimal; − = none; ? = questionable.

†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.

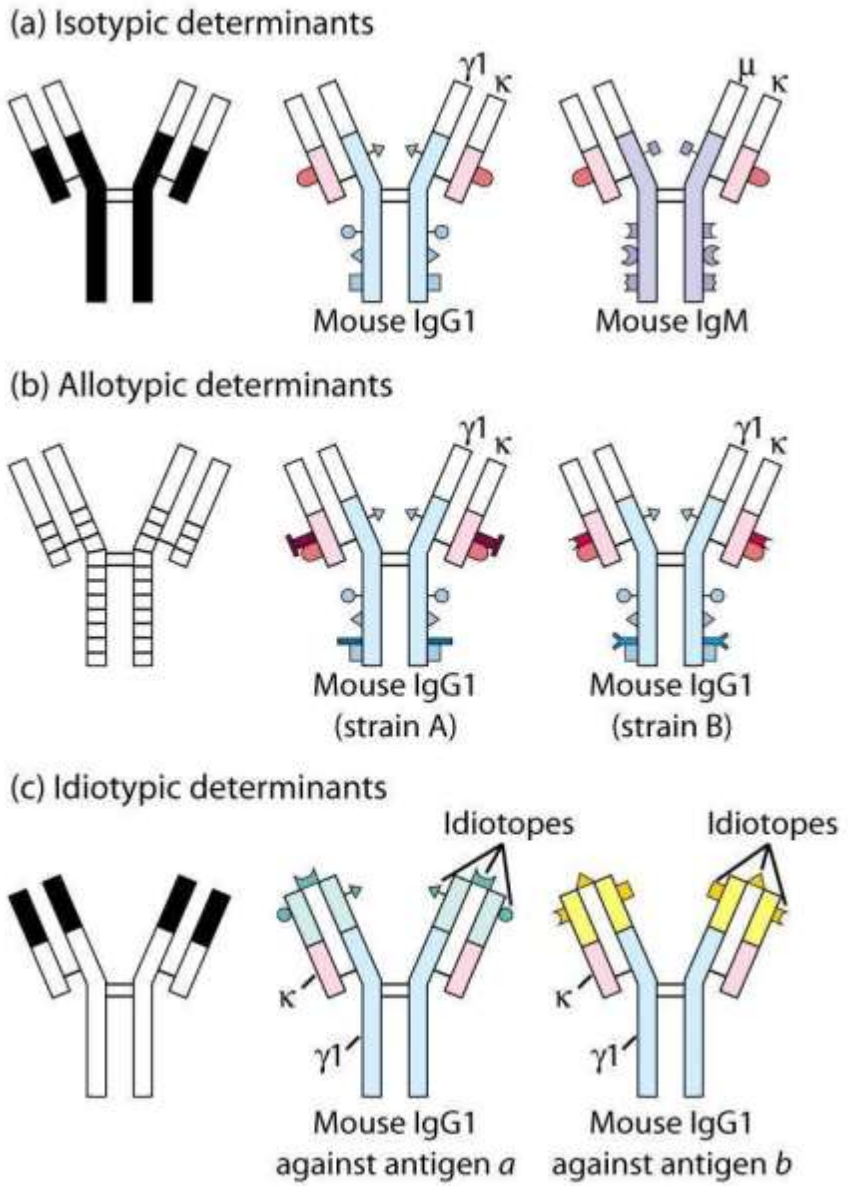
General structures of the five major classes of secreted antibody:

- ✓ The IgG, IgA, and IgD heavy chains (blue, orange, and green, respectively) contain four domains and a hinge region, whereas the IgM and IgE heavy chains (purple and yellow, respectively) contain five domains but no hinge region.
- ✓ The polymeric forms of IgM and IgA contain a polypeptide, called the J chain, that is linked by two disulfide bonds to the Fc region in two different monomers.
- ✓ Serum IgM is always a pentamer; most serum IgA exists as a monomer, although dimers, trimers, and even tetramers are sometimes present.



Antigenic determinants on immunoglobulins

- ✓ Immunoglobulins, being protein in nature, are themselves immunogenic for other individuals of same or different species, i.e. Igs also have epitopes,
- ✓ Antigenic Determinants on Abs are of three types:
  - Isotypic
  - Allotypic
  - Idiotypic



Isotypic determinants

- ✓ prefix ‘Iso’ means same in all members of the same species
- ✓ Antigenic determinants that characterize the classes and subclasses of heavy chains and types and subtypes of light chains in a species are called as isotypic determinants
- ✓ the isotypic determinants are present in the constant region of heavy and light chains
- ✓ the isotypic determinants between different species are not the same
- ✓ if you inject an Ab from one species in a different species then the injected antibodies are recognized as foreign, resulting in the induction of antibodies (anti-antibodies) - anti-isotype is generated
- ✓ if within same species, no anti-isotype produced

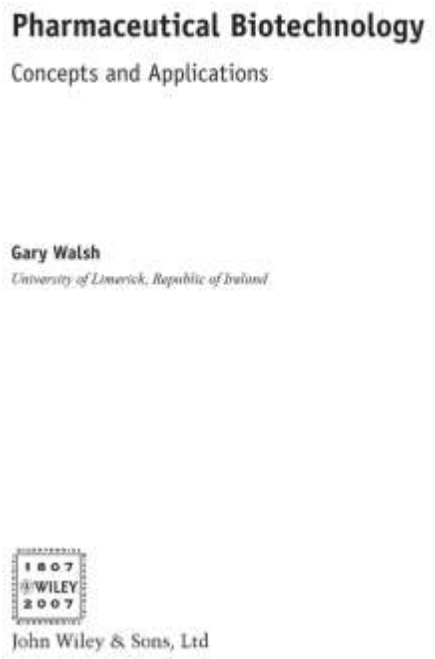
Allotypic determinants

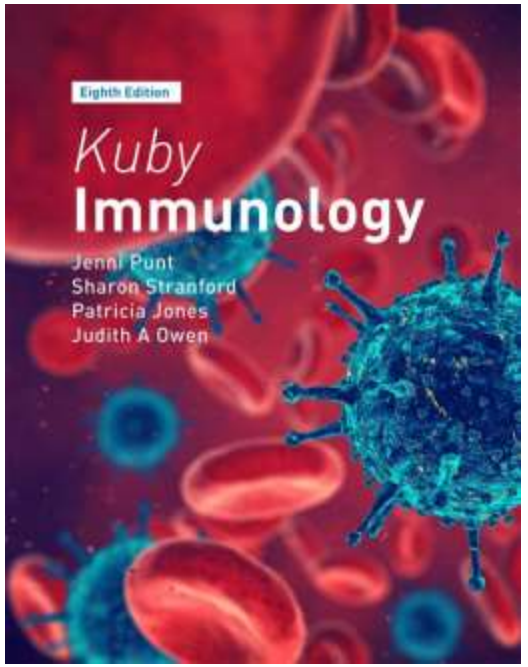
- ✓ the prefix ‘Allo’ means that different in individuals of the same species.
- ✓ even though same isotype, within one species small differences (1-4 a/a) arise in different individuals (due to polymorphism).
- ✓ antigenic determinants specified by allelic forms of the Ig genes are called as allotypic determinants.
- ✓ if an animal of one species is injected with such Ab from another animal of same species, the former will generate anti-allotype Abs, provided that the two animals differ in their allotypic determinants, e.g. A2m (1), A2m (2)
  - during pregnancy
  - blood transfusion

Idiotypic determinants

- ✓ Antigen-binding site in antibody molecule is formed by the hypervariable regions of the VH and VL chains. These HVRs also act as immunogen.
- ✓ The antigenic determinants of the VH and VL region, unique to an antibody molecule of a given specificity, are called idiotypic determinants or idiotopes.
- ✓ One antibody molecule has many idiotopes in the antigen-binding site or adjacent to it. The sum of the individual idiotopes in an antibody molecule is called the idiotype of the antibody (antigenic determinants created by the HVR = Idiotypes)
- ✓ The idiotopes are further designated alpha, beta, and gamma idiotopes.
  - **Alpha idiotope** lie outside the antigen-binding site of hyper-variable region.
  - **Beta idiotope** lie close to the antigen binding site of hyper- variable region.
  - **Gamma idiotope** is formed by the amino acids of the antigen binding site.
- ✓ If a monoclonal antibody against an idiotype is injected into a genetically identical recipient then anti-idiotypic antibodies are generated; no anti-isotypic and no anti-allotypic Abs will be generated

Acknowledgement





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