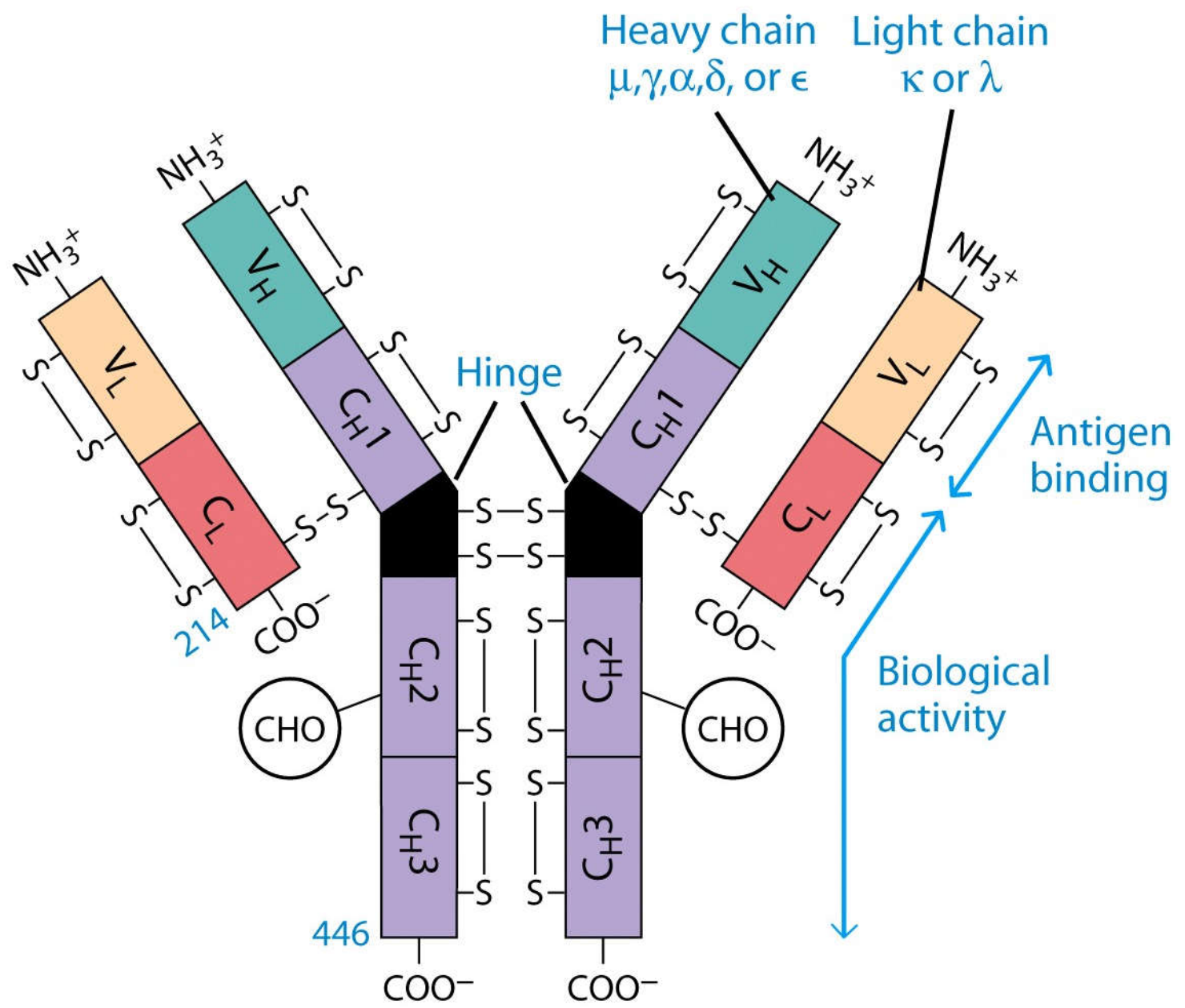
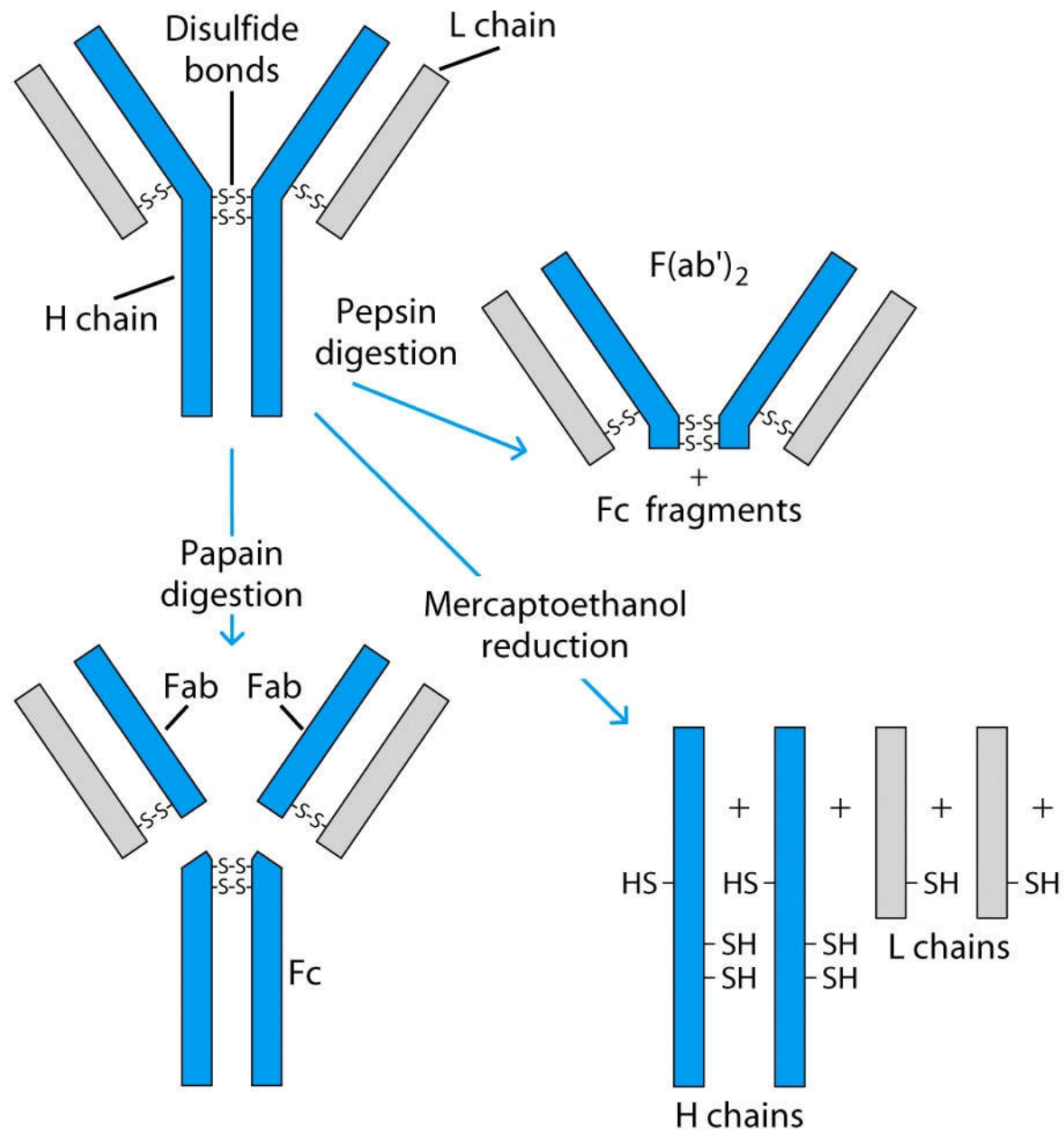


Antibodies: Structure And Function



Antibody Structure

- Antibodies Are Made Up Of:
 - 2 Light Chains (identical) ~25 KDa
 - 2 Heavy Chains (identical) ~50 KDa
- Each Light Chain Bound To Heavy Chain By Disulfide (H-L)
- Heavy Chain Bound to Heavy Chain (H-H)
- First 100 a/a Of Amino Terminal Vary of Both H and L Chain Are Variable
- Referred To As V_L , V_H , C_H And C_L
- CDR (Complementarity Determining Regions) Are What Bind Ag
- Remaining Regions Are Very Similar Within Same Class



Antibody Structure

- Repeating Domains of ~ 110 a/a
 - Intrachain 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 proline residues (flexible)
 - Hinge found in IgG, IgA and IgD
 - Proline residues are target for proteolytic digestion (papain and pepsin)
 - Rich in cysteine residues (disulfide bonds)
 - IgM and IgE lack hinge region
 - They instead have extra C_H4 Domain

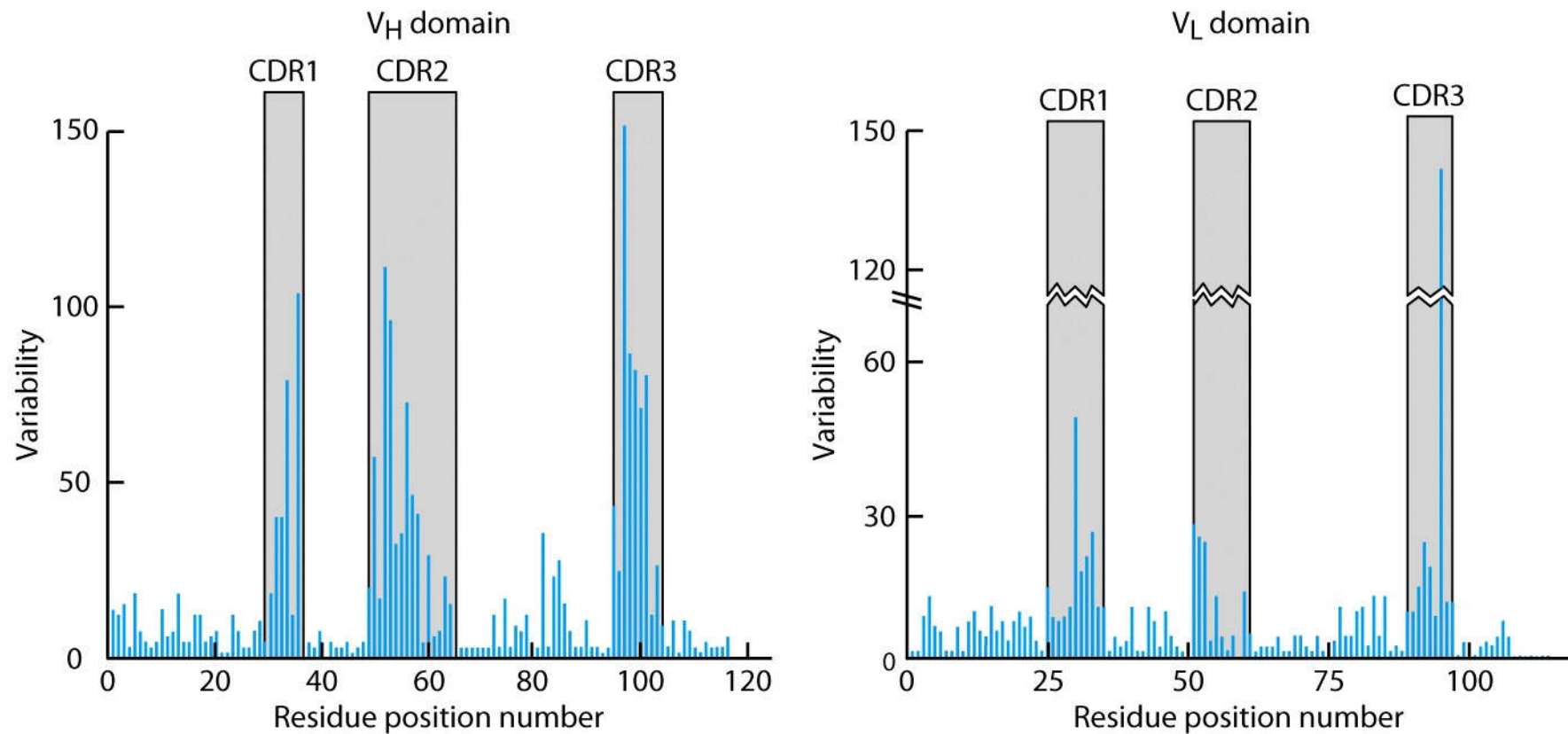
Enzymatic Digestion Of Antibodies

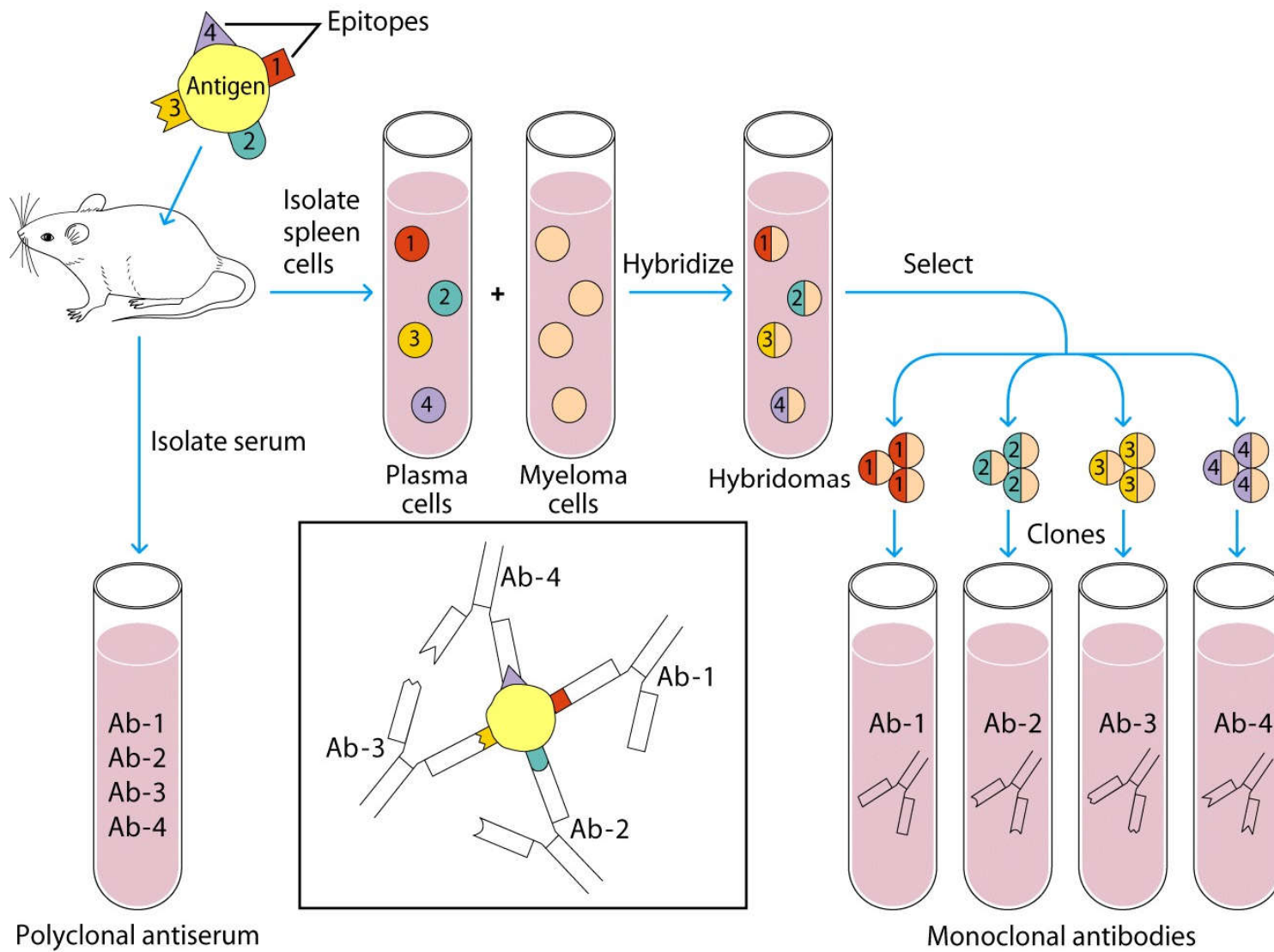
- Digestion With Papain Yields
 - 3 Fragments
 - 2 identical Fab and 1 Fc
 - Fab Because Fragment That is Antigen Binding
 - Fc Because Found To Crystallize In Cold Storage
- Pepsin Digestion
 - $F(ab')_2$
 - No Fc Recovery, Digested Entirely
- Mercaptoethanol Reduction (Eliminates Disulfide Bonds) And Alkylation Showed

Sequencing Of Heavy Chains

- Sequencing Of Several Immunoglobulins Revealed
 - 100-110 Amino Terminus, Highly Variable (V)
 - Five Basic Sequence Patterns
 - $\alpha, \gamma, \delta, \epsilon, \mu$
 - IgA, IgG, IgD, IgE and IgM
 - The Above Classes Are Called Isotype
 - Each class can have either κ or λ light chains
 - Minor Differences Led To Sub-classes For IgA and IgG
 - IgA1, IgGA2 and IgG1, IgG2, IgG3, IgG4

CDR Are Hypervariable



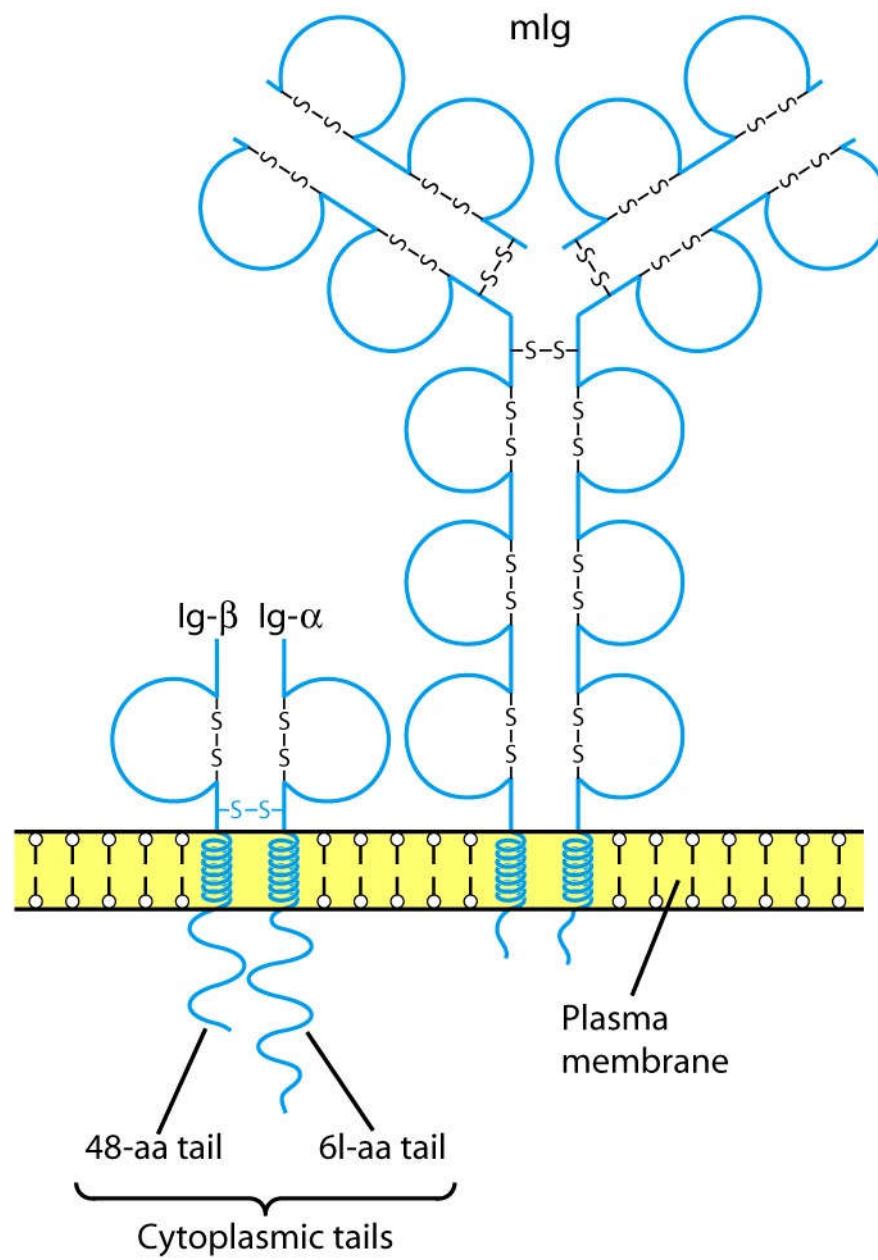


Monoclonal Antibodies

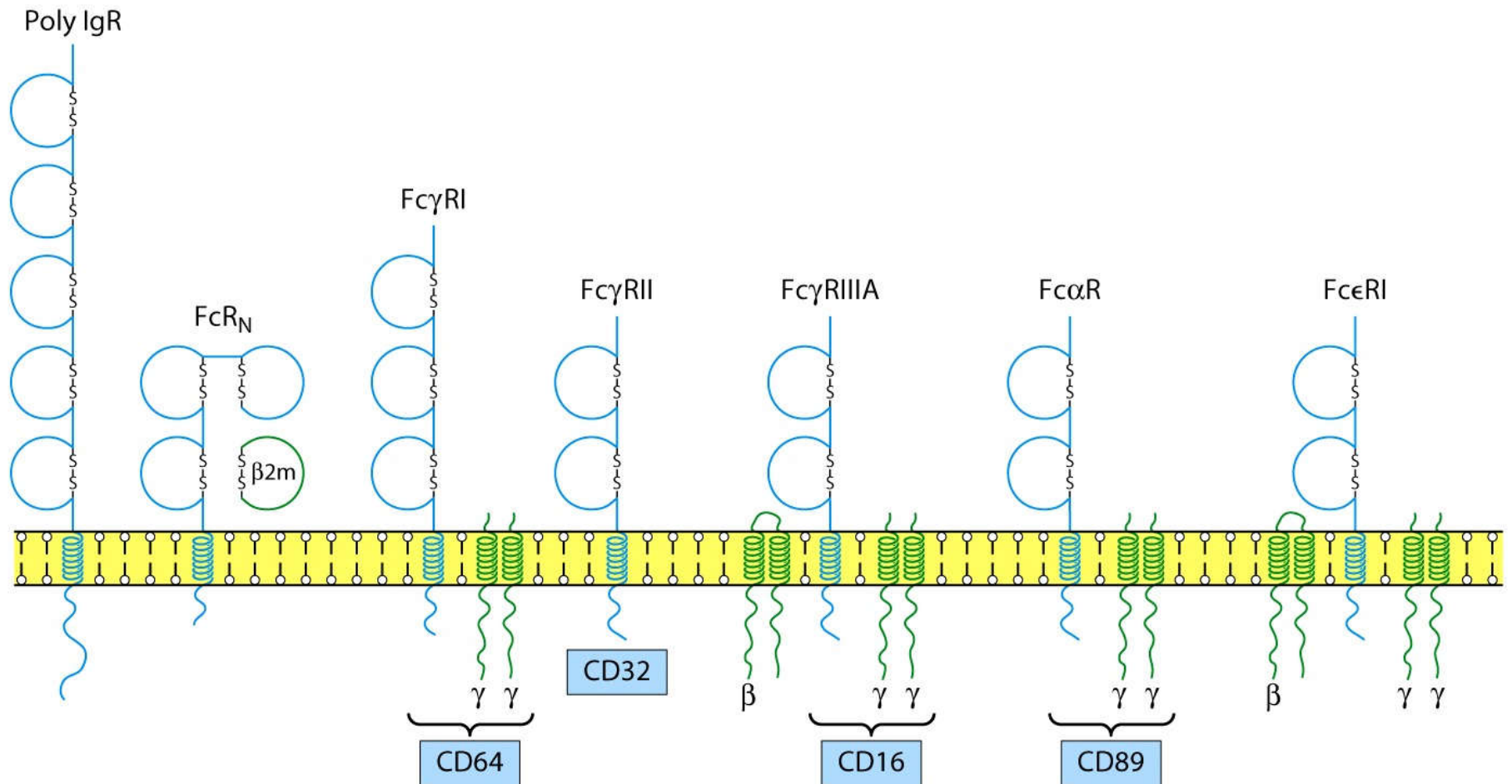
- Immunize Animal With Antigen
- Multiple Clones Are Generated, Good For In Vivo
- For Clinical Diagnosis, Research, One Clone That Reacts To Single Epitope Is Preferred
- Solution By Kohler and Milstein
 - Fuse A Myeloma Cell (Cancerous) With A Normal Plasma Cells
 - Resulting Clones Can Be Cultured Indefinitely
 - Produces An Antibody Recognizing One Epitope

B-Cell Receptor

- BCR Is An Antibody On Surface Of Cell
mIg
- Very Short Cytoplasmic Tail, Cannot
Transduce Signal
- Heterodimeric Molecule Ig- α /Ig- β
Transduces (long cytoplasmic tail)



Fc Receptors (FcR)



Fc Receptors (FcR) Functions

- To Transport Abs Across Membranes
 - Secretion of IgA Across Epithelium into lumen
 - Transport of maternal Abs Across Placenta (IgG)
- Many Cell Types Use FcR
 - Ex. Mast Cells, Macrophages, Neutrophils, B, T, NK
- Opsonization, ADCC
- Poly IgR
 - Transport of IgA across epithelium
- FcR_N
 - Transport of maternal IgG to fetus

Antibody Classes And Biological Activities

- IgG
 - Most abundant immunoglobulin 80% of serum Ig
 - ~10mg/mL
 - IgG1,2,3,4 (decreasing serum concentration)
 - IgG1, IgG3 and IgG4 cross placenta
 - IgG3 Most effective complement activator
 - IgG1 and IgG3 High affinity for FcR on phagocytic cells, good for opsonization

Antibody Classes And Biological Activities

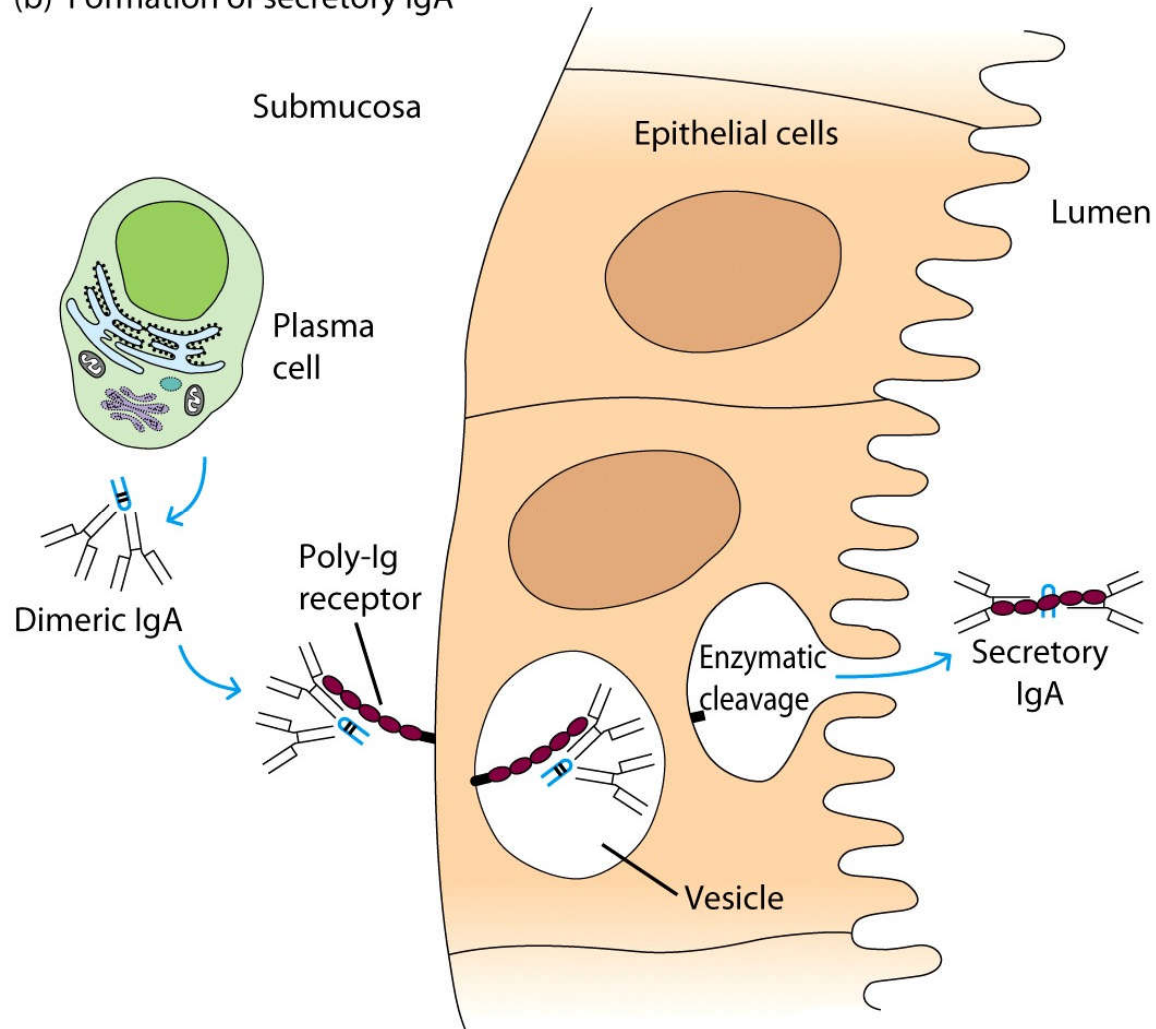
- IgM
 - 5-10% of serum immunoglobulin
 - 1.5mg/mL
 - mIgM (also IgD) expressed on B-cells as BCR
 - Pentameric version is secreted
 - First Ig of primary immune response
 - High valence Ig (10 theoretical), 5 empirical
 - More efficient than IgG in complement activation

Antibody Classes And Biological Activities

- IgA
 - 10-15% of serum IgG
 - Predominant Ig in secretions
 - Milk, saliva, tears, mucus
 - 5-15 g of IgA released in secretions!!!!
 - Serum mainly monomeric, polymers possible not common though
 - Secretions, as dimer or tetramer+J-chain polypeptide+secretory component (Poly IgR)

IgA Antibody Transport Across Cell (Transcytosis)

(b) Formation of secretory IgA



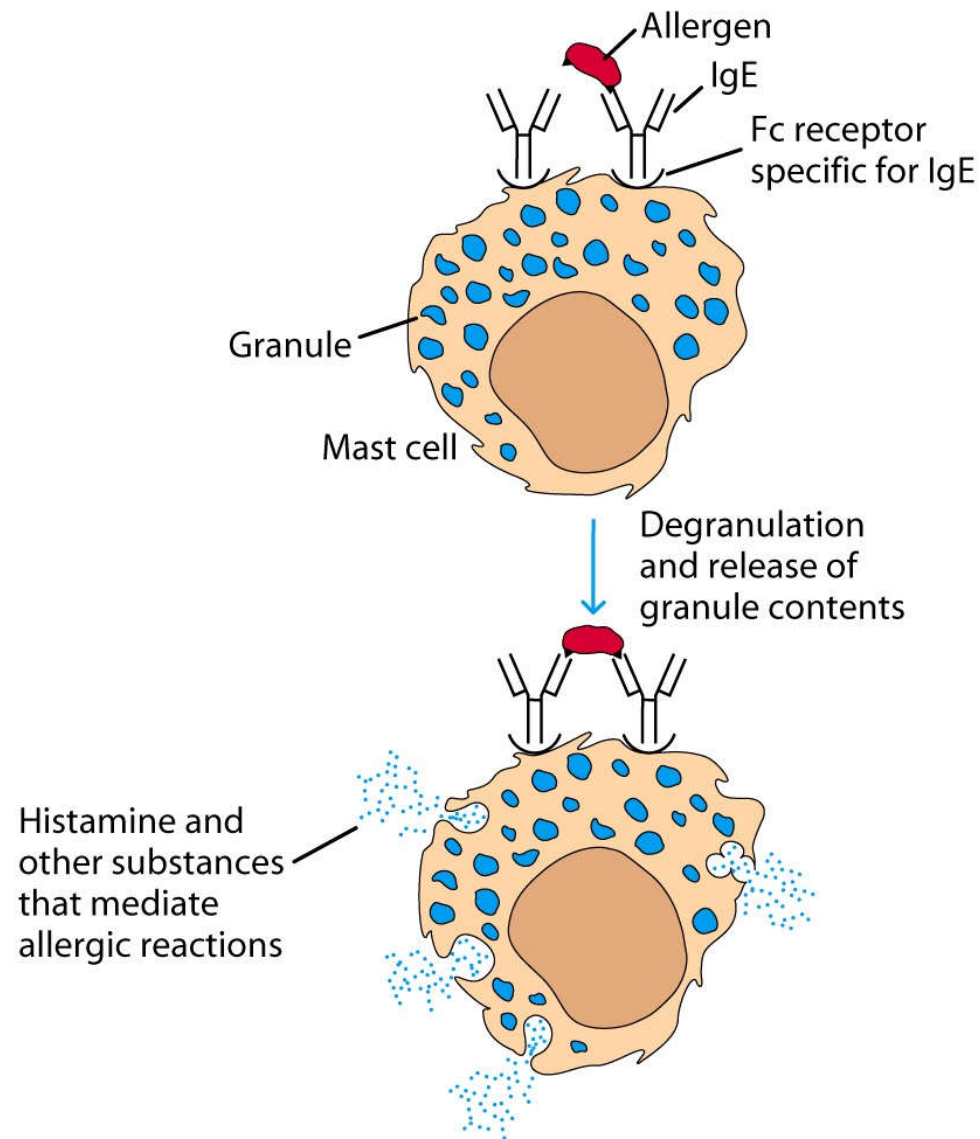
Antibody Classes And Biological Activities

- IgE
 - Very low serum concentration, $0.3\mu\text{g/mL}$
 - Participate in immediate hypersensitivities reactions. Ex. Asthma, anaphylaxis, hives
- Binds Mast Cells and Blood Basophils thru $\text{Fc}\epsilon\text{R}$
- Binding causes degranulation (Histamine Release)

Antibody Classes And Biological Activities

- IgD
 - Expressed on B-cell Surface
- IgM and IgD, Expressed on B-cell Surface
- We Do Not Know Any Other Biological Effector Activity
- Low serum concentrations, $\sim 30\mu\text{g/mL}$

Cross-Linkage of Bound IgE Antibody With Allergen Causes



Antibodies Act As Immunogens

- Antigenic Determinants on Abs Fall in 3 Categories
 - Isotypic
 - Allotypic
 - Idiotypic
- Isotypic
 - Constant Region Of Ab
 - If you inject Ab in a different species Anti-Isotype is generated
 - If within same species, No Anti-isotype

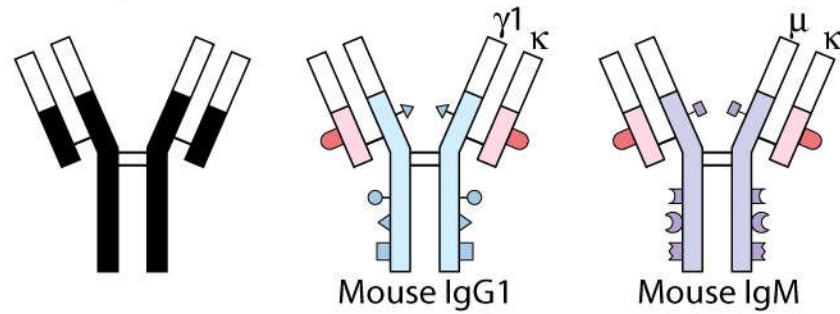
Antibodies Act As Immunogens

- Allotype
 - Even though same isotypes within one species small differences (1-4 a/a) arise in different individuals (form of polymorphism)
 - If injected with such Ab you generate anti-allotype Ab
 - Ex. During pregnancy
 - Blood transfusion

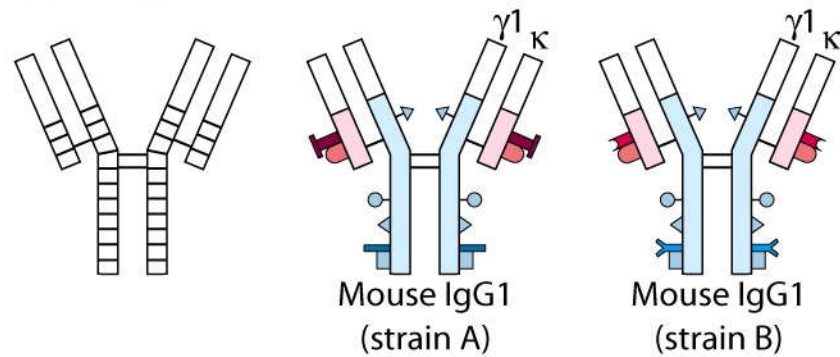
Antibodies Act As Immunogens

- Idiotypic
 - Unique V_H AND V_L binds antigen but can also behave as antigenic determinant
- If you inject a monoclonal antibody into a genetically identical recipient then anti-idiotypic antibodies are generated
- No anti-isotypic and no anti-allotypic Abs will be generated

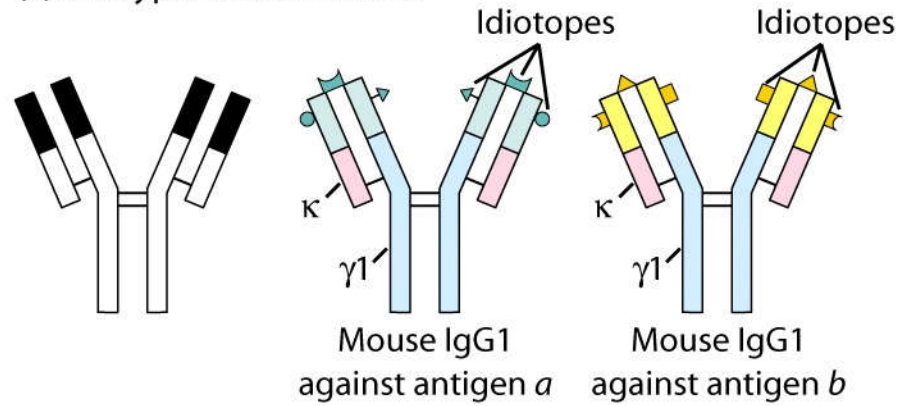
(a) Isotypic determinants



(b) Allotypic determinants



(c) Idiotypic determinants



Monoclonal Antibody Applications

- Diagnostic Tests
 - Abs are capable to detect tiny amounts (pg/mL) of molecules
 - Ex. Pregnancy hormones
- Diagnostic Imaging
 - mAbs that recognize tumor antigens are radiolabeled with iodine I-131
- Immunotoxins
 - mAbs conjugated with toxins
- mAbs To Clear Pathogens
 - www.elusys.com