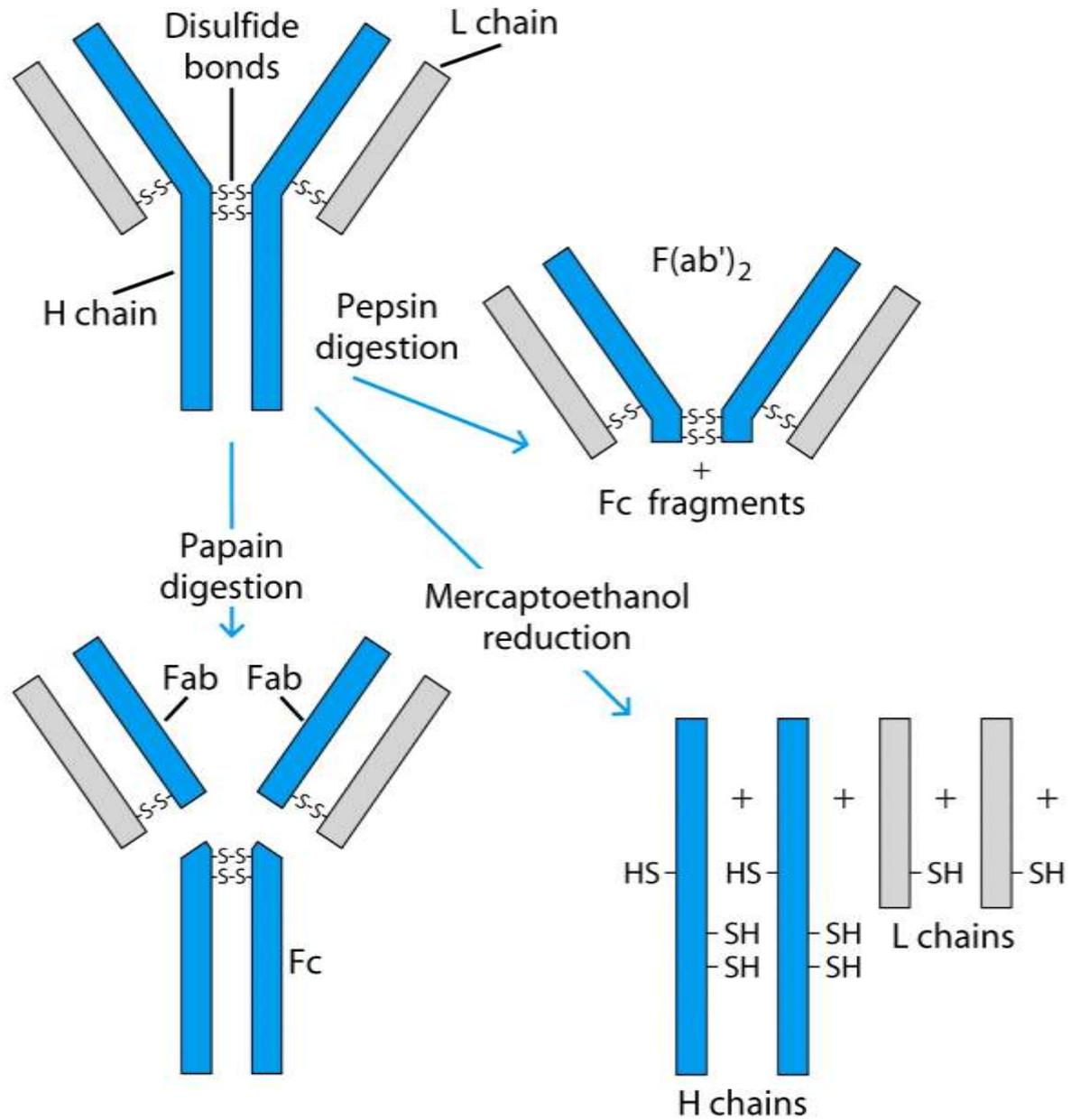


# Antibodies: Structure And Function



# Antibody Structure

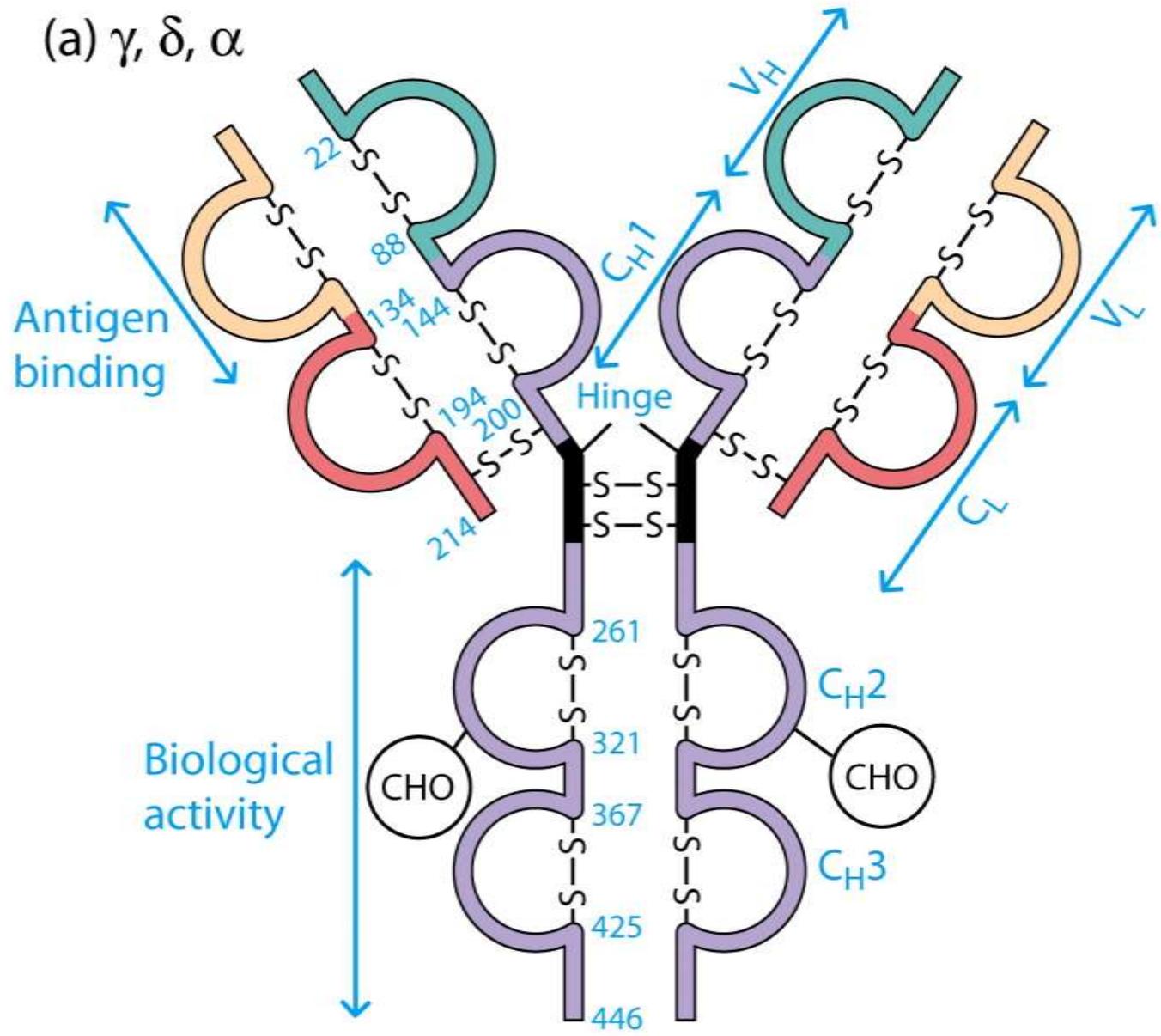
- Antibodies Are Made Up Of:
  - 2 Light Chains (identical) ~25 KDa
  - 2 Heavy Chains (identical) ~50 KDa
- Each Light Chain Bound To Heavy Chain By Disulfide (H-L)
- Heavy Chain Bound to Heavy Chain (H-H)
- First 100 a/a Of Amino Terminal Vary of Both H and L Chain Are Variable
- Referred To As  $V_L$ ,  $V_H$ ,  $C_H$  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<sub>H</sub> and either 3 or 4 C<sub>H</sub> (C<sub>H</sub>1, C<sub>H</sub>2, C<sub>H</sub>3, C<sub>H</sub>4)
- Light chains
  - 1 V<sub>L</sub> and 1 C<sub>L</sub>
- 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<sub>H</sub>4 Domain

(a)  $\gamma, \delta, \alpha$



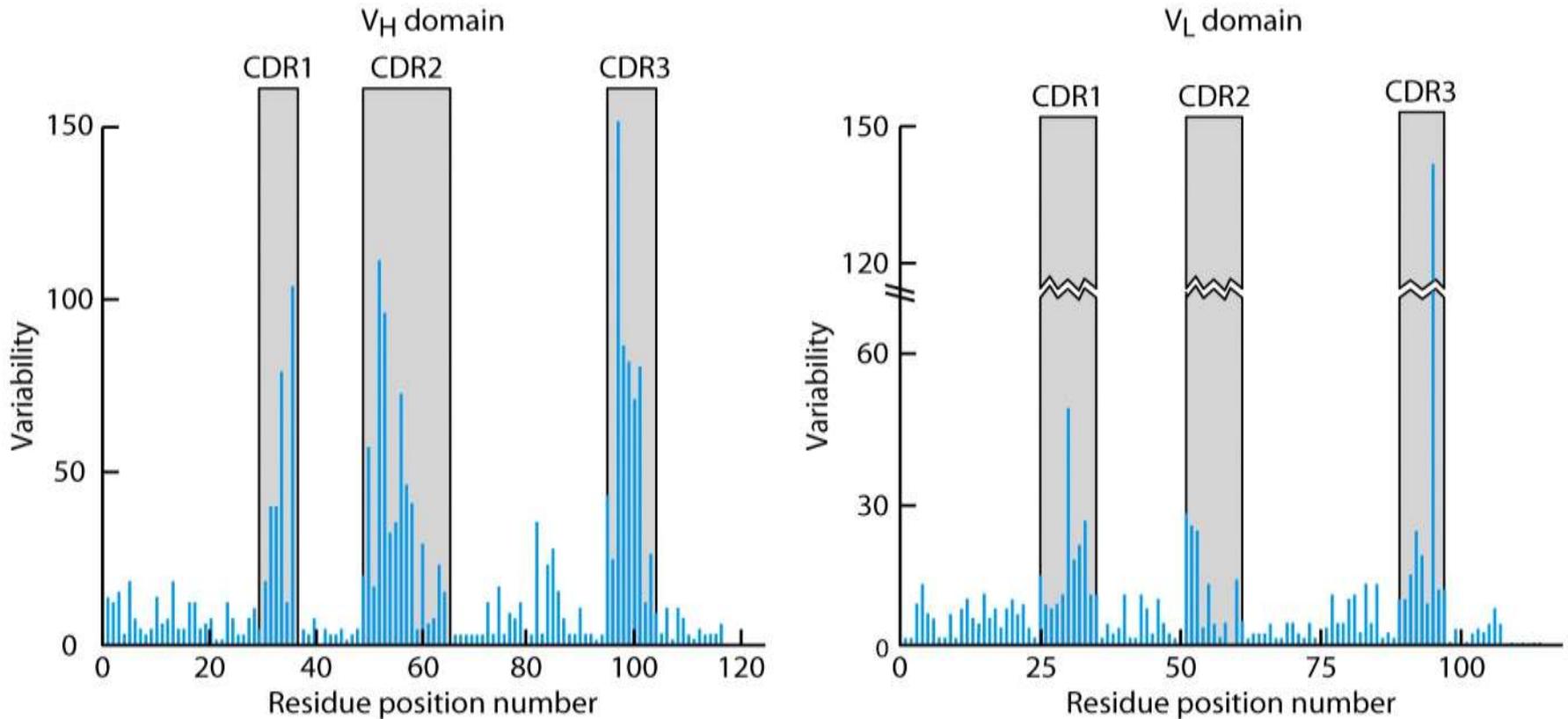
# Enzymatic Digestion Of Antibodies

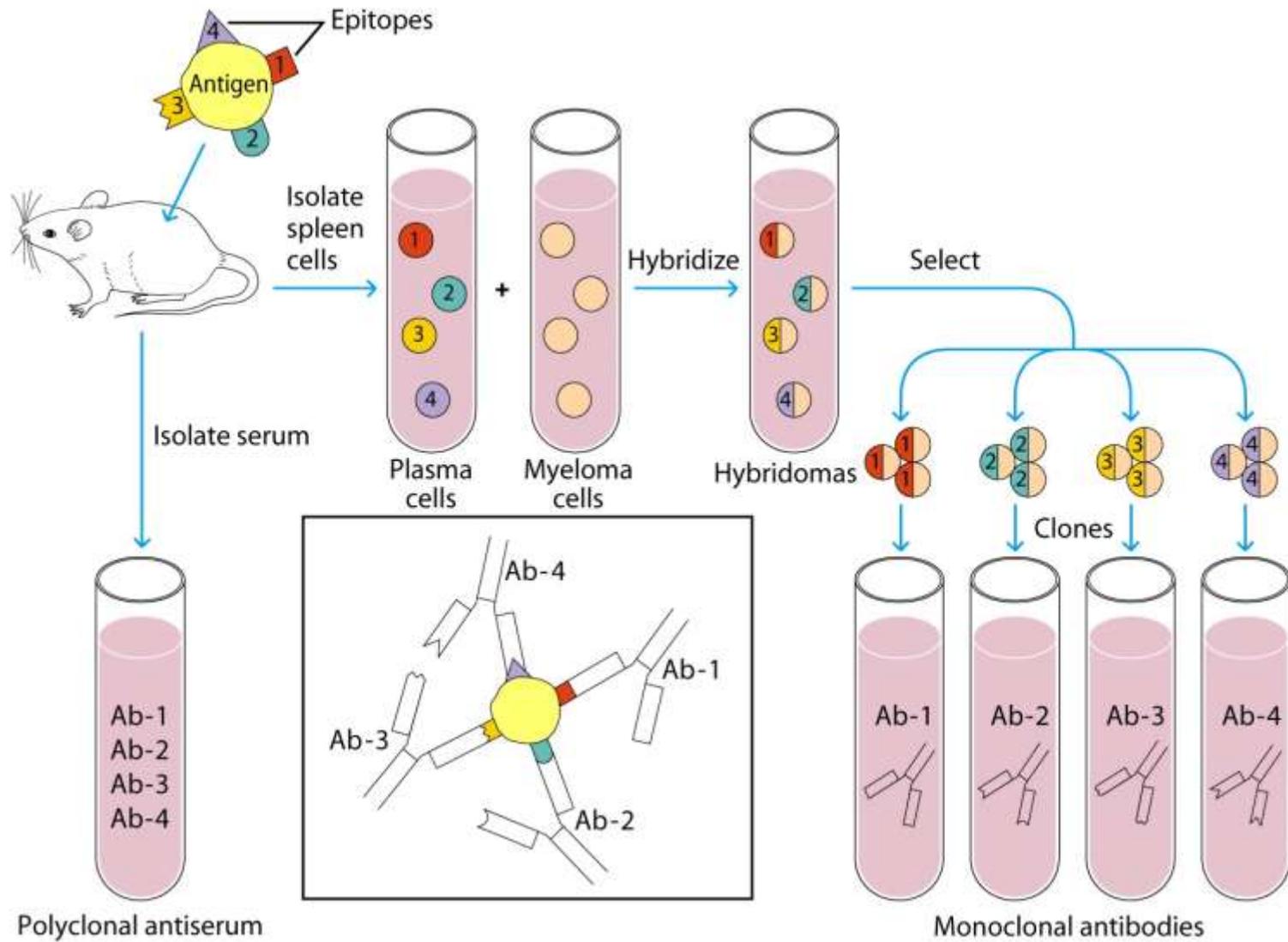
- Digestion With Papain Yields
  - 3 Fragments
  - 2 identical Fab and 1 Fc
  - Fab Because Fragment That is Antigen Binding
  - Fc Because Found To Crystallize In Cold Storage
- Pepsin Digestion
  - $F(ab')_2$
  - No Fc Recovery, Digested Entirely
- Mercaptoethanol Reduction (Eliminates Disulfide Bonds) 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  $\kappa$  or  $\lambda$  light chains
    - Minor Differences Led To Sub-classes For IgA and IgG
      - IgA1, IgGA2 and IgG1, IgG2, IgG3, IgG4

# CDR Are Hypevariable



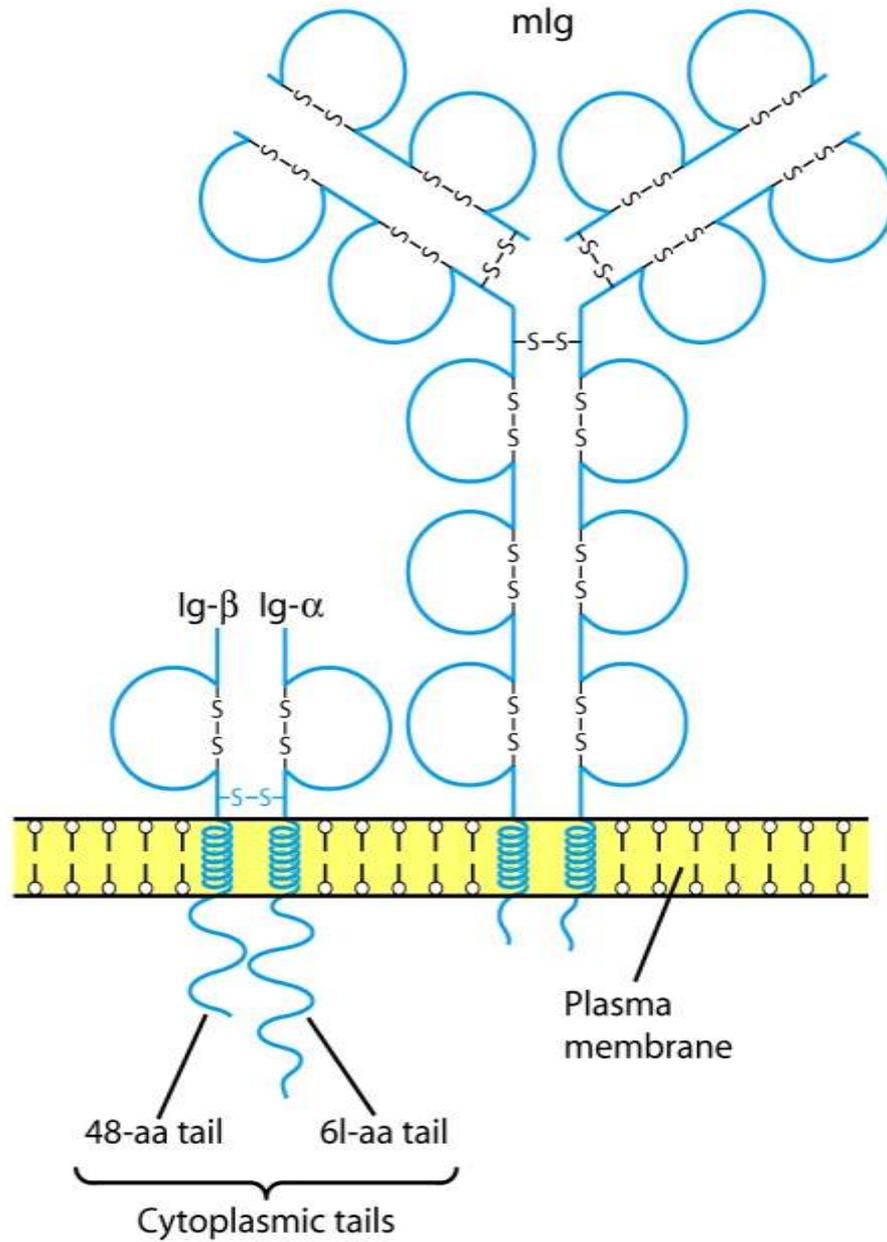


# Monoclonal Antibodies

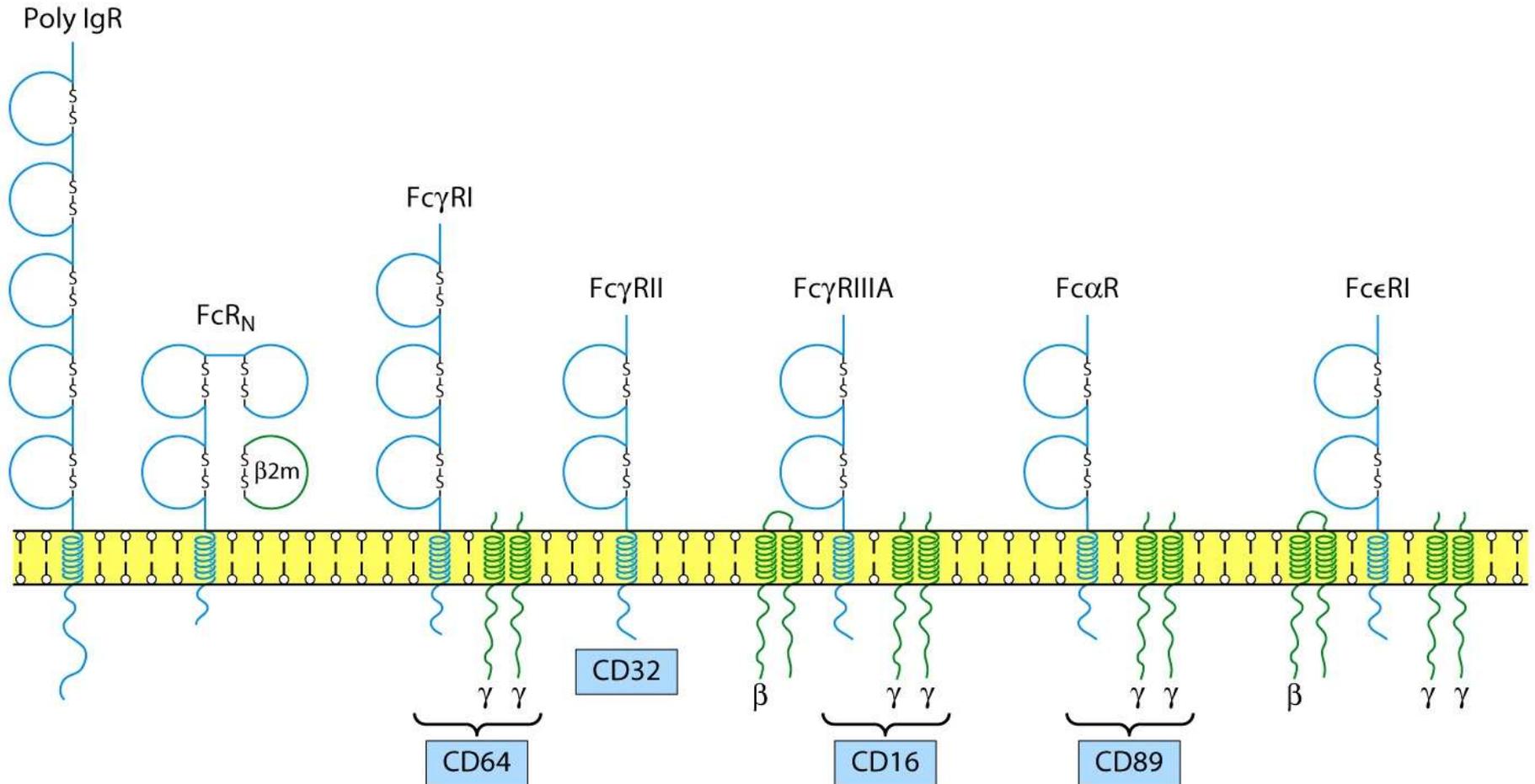
- Immunize Animal With Antigen
- Multiple Clones Are Generated, Good For In Vivo
- For Clinical Diagnosis, Research, One Clone That Reacts To Single Epitope Is Preferred
- Solution By Kohler and Milstein
  - Fuse A Myeloma Cell (Cancerous) With A Normal Plasma Cells
  - Resulting Clones Can Be Cultured 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- $\alpha$ /Ig- $\beta$   
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<sub>N</sub>
  - 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

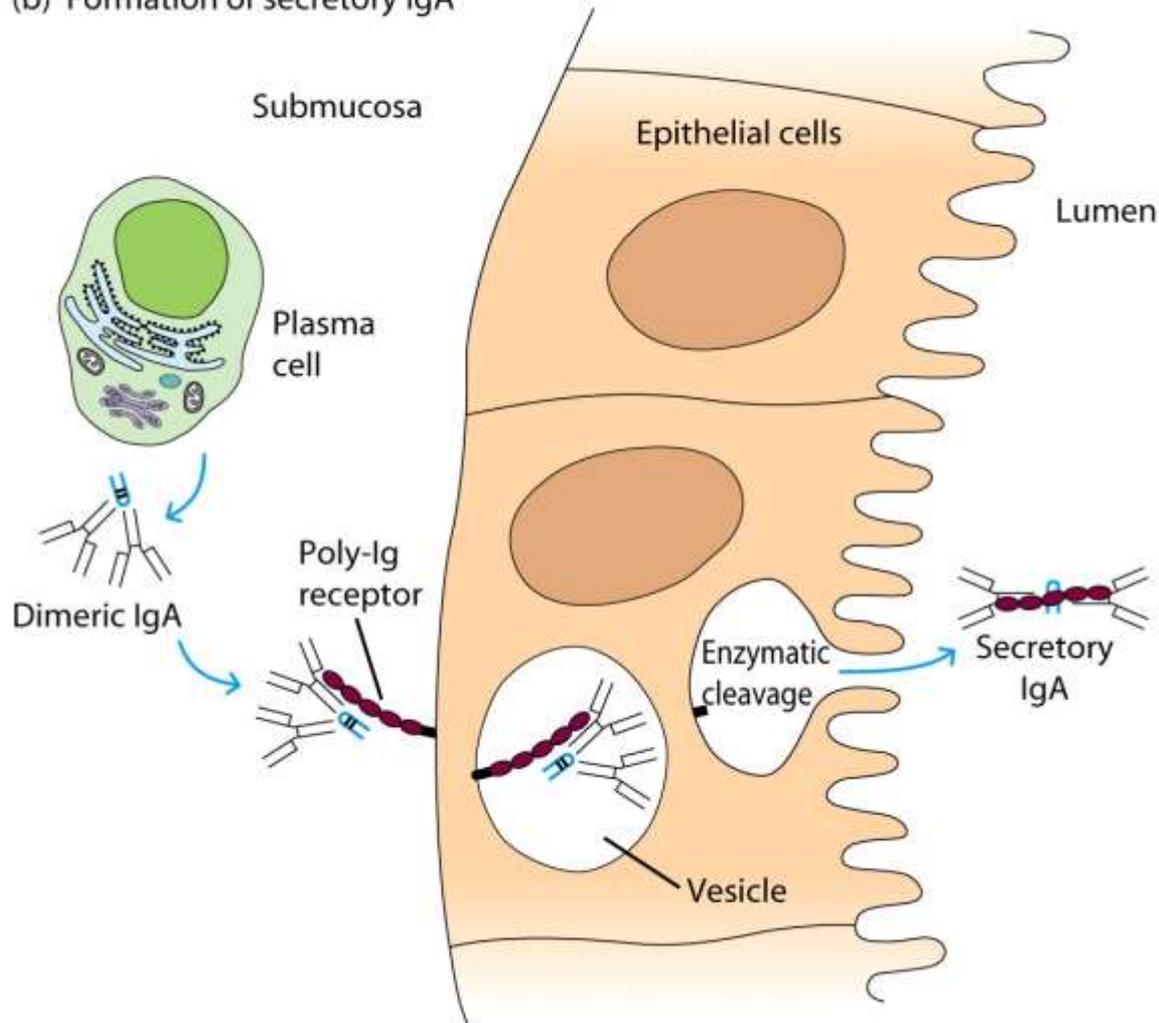
- IgM
  - 5-10% of serum immunoglobulin
  - 1.5mg/mL
  - mIgM (also IgD) expressed on B-cells as BCR
  - Pentameric version is secreted
  - First Ig of primary immune response
  - High valence Ig (10 theoretical), 5 empirical
  - More efficient than IgG in complement activation

# 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

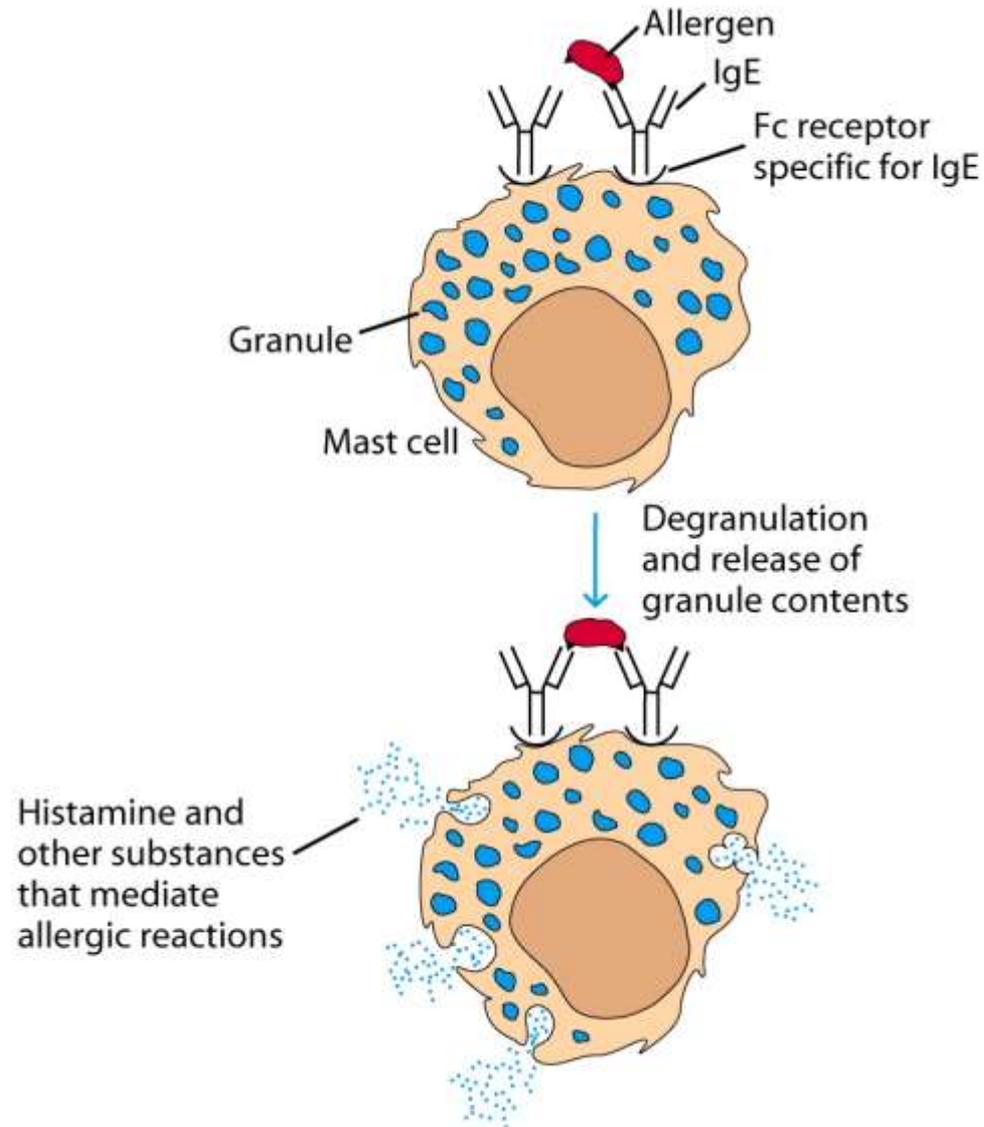
- IgE
  - Very low serum concentration,  $0.3\mu\text{g/mL}$
  - Participate in immediate hypersensitivities reactions. Ex. Asthma, anaphylaxis, hives
- Binds Mast Cells and Blood Basophils thru  $\text{Fc}\epsilon\text{R}$
- Binding causes degranulation (Histamine Release)

# 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

## 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

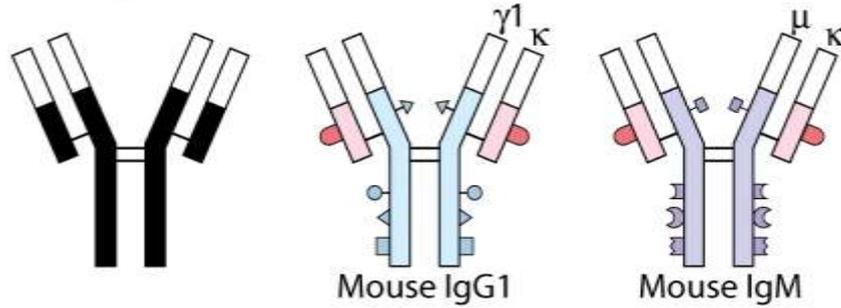
# Antibodies Act As Immunogens

- 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

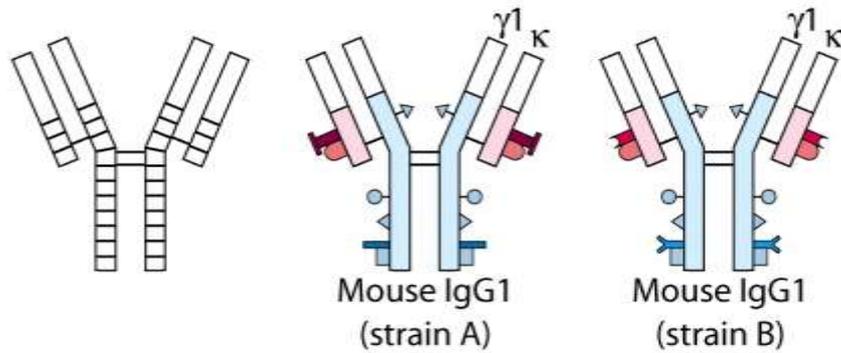
# Antibodies Act As Immunogens

- 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

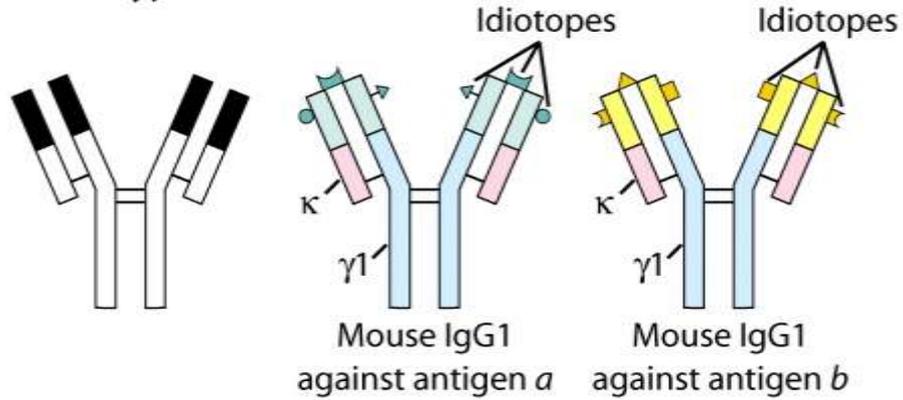
(a) Isotypic determinants



(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](http://www.elusys.com)