Imunoglobulins

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BCHEM 471

Learning objective

Define immunoglobulins/antibody

Know the structure of antibody

Different fragment following enzymatic activities

Various classes of antibody

Functions of the classes

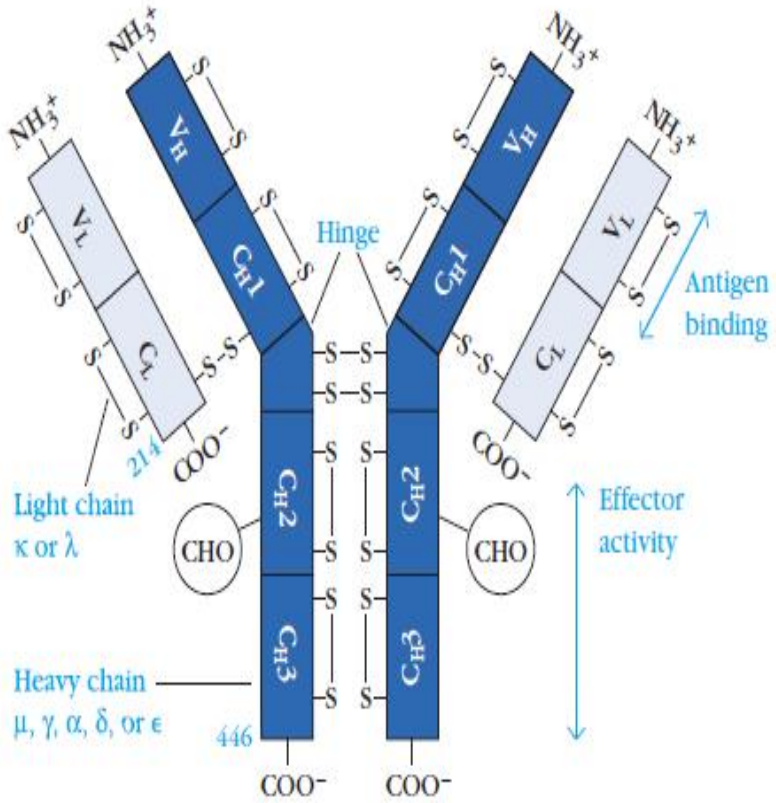
- Immunoglobulins of all classes have a fundamental four-chain structure, consisting of two identical light (L) and two identical heavy (H) chains.
- Through disulfide bonds, each light chain is linked to a heavy chain, and the two heavy chains are linked to each other.
- Immunoglobulins are expressed in two forms: a membrane-bound antibody present on the surface of B cells and a secreted antibody produced by plasma cells.

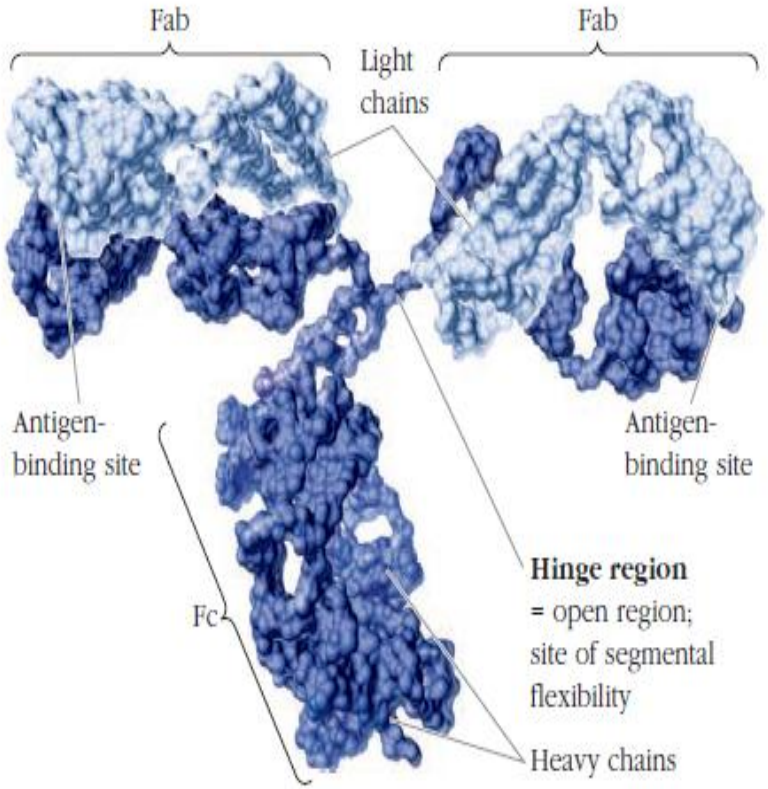
Antibody Structure

- 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

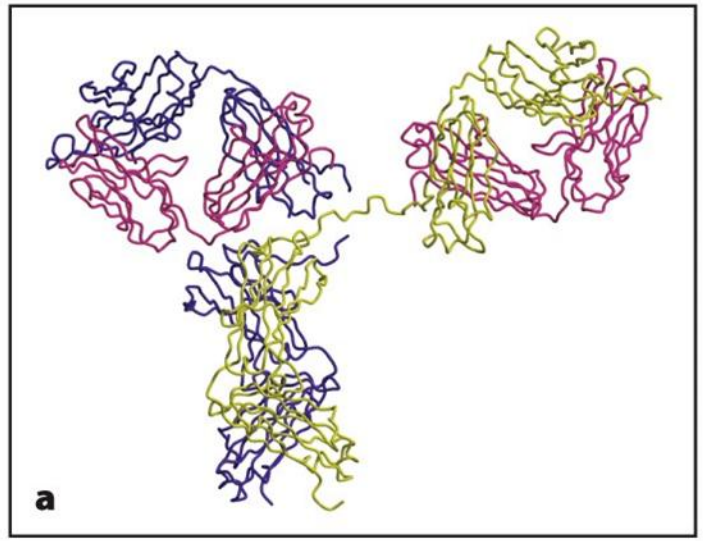
Antibody Structure

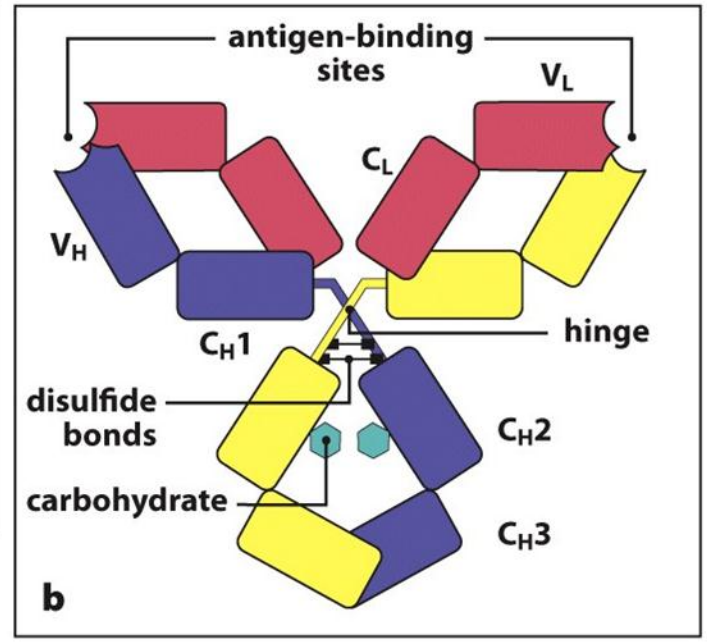
- Repeating Domains of ~110 a/a
  - Intrachain disulfide bonds within each domain
- Heavy chains
  - 1 V_H and either 3 or 4 C_H (C_H1, C_H2, C_H3, C_H4)
- Light chains
  - 1 V_L and 1 C_L
- Hinge Region
  - Rich in proline residues (flexible)
  - Hinge found in IgG, IgA and IgD
  - Proline residues 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

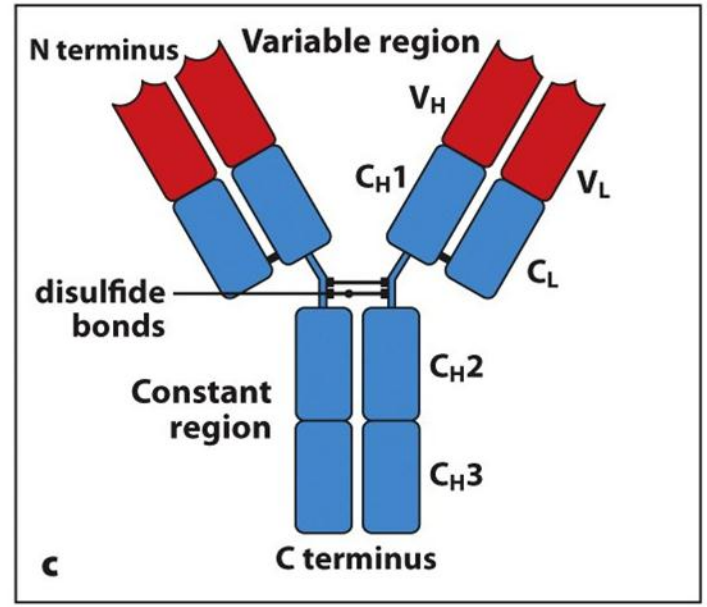




An antibody molecule comprises three equal-sized globular portions joined by a flexible stretch of polypeptide chain known as the **hinge region**


a


antigen-binding sites
V_L
C_L
V_H
hinge
C_H1
disulfide bonds
C_H2
carbohydrate
C_H3
b


N terminus
Variable region
V_H
C_H1
V_L
C_L
disulfide bonds
C_H2
Constant region
C_H3
C terminus
c

- The N-terminal domains of both heavy and light chains are the variable (V) regions and contain the hypervariable regions, also called ***complementarity determining regions*** (CDRs), which make up the combining site of the antibody and vary according to the specificity of the antibody.
- The constant (C) region domains of L and H chains are similar within each of the L and H chain isotypes, respectively

- There are two major class of light chains: **(kappa)** or **(lambda) chains**

- There are five major classes of heavy chains. **These are IgG, IgM, IgD, IgA and IgE**

Papsin digestion

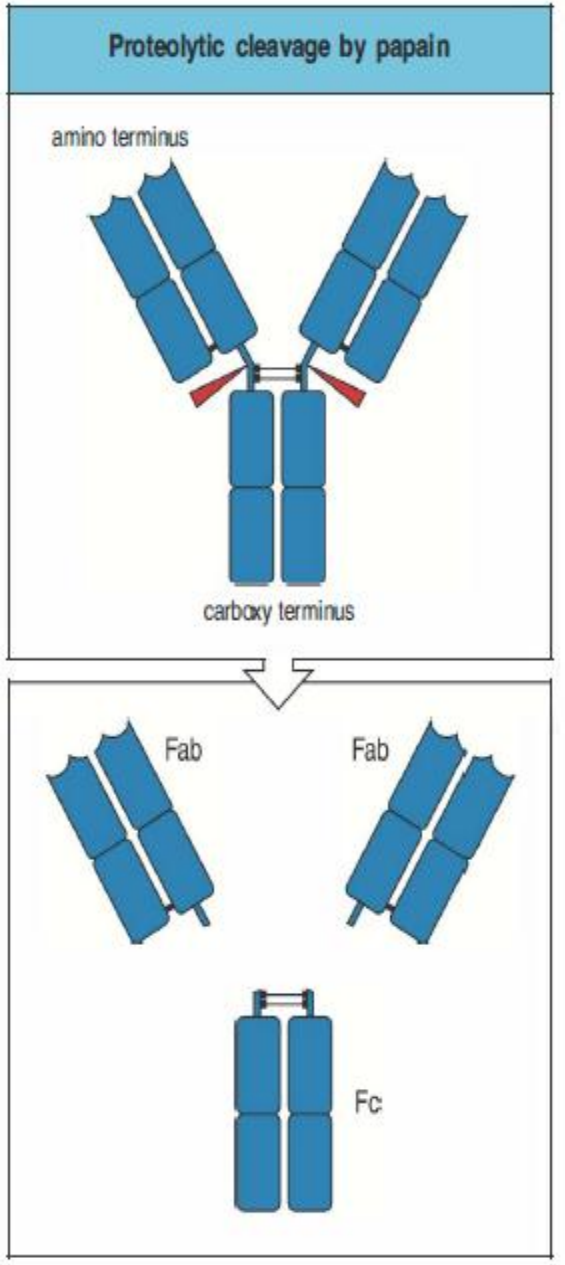
Papain cuts the antibody molecule on the amino-terminal side of the disulfide bonds that link the two heavy chains, releasing the two arms of the antibody molecule as two identical fragments that contain the antigen-binding activity

Papain cleaves the immunoglobulin molecule into three:

- two Fab fragments
- one Fc fragment

Fab fragments still capable of binding
to antigen (Fab-*fragment antigen binding*)
because they contain the V (variable) regions

Fc fragment (fragment crystallizable)



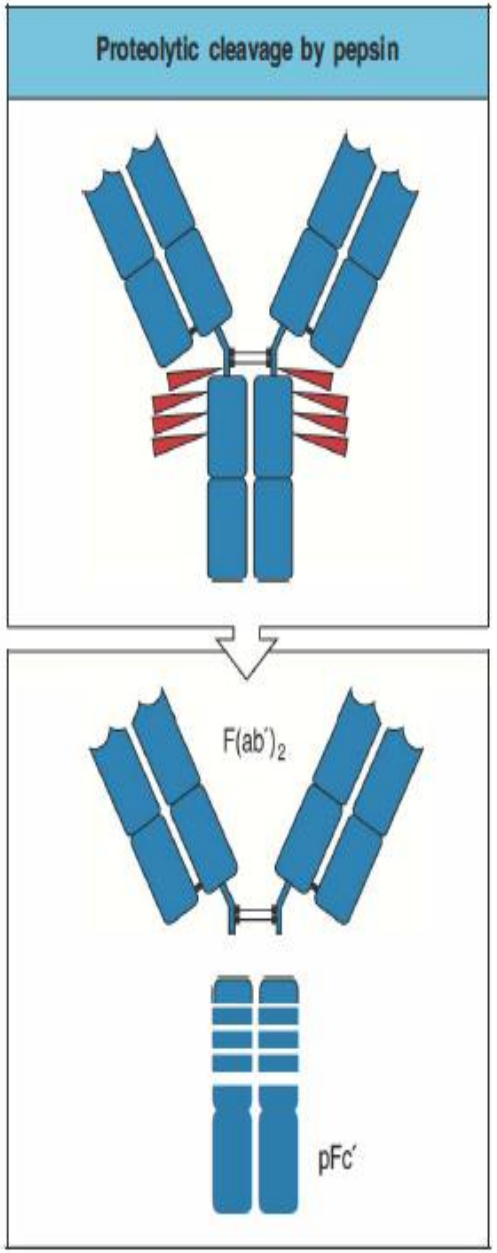
Pepsin Digestion

Pepsin, cuts on the carboxy-terminal side of the disulfide bonds

Pepsin cleaves immunoglobulin to yield

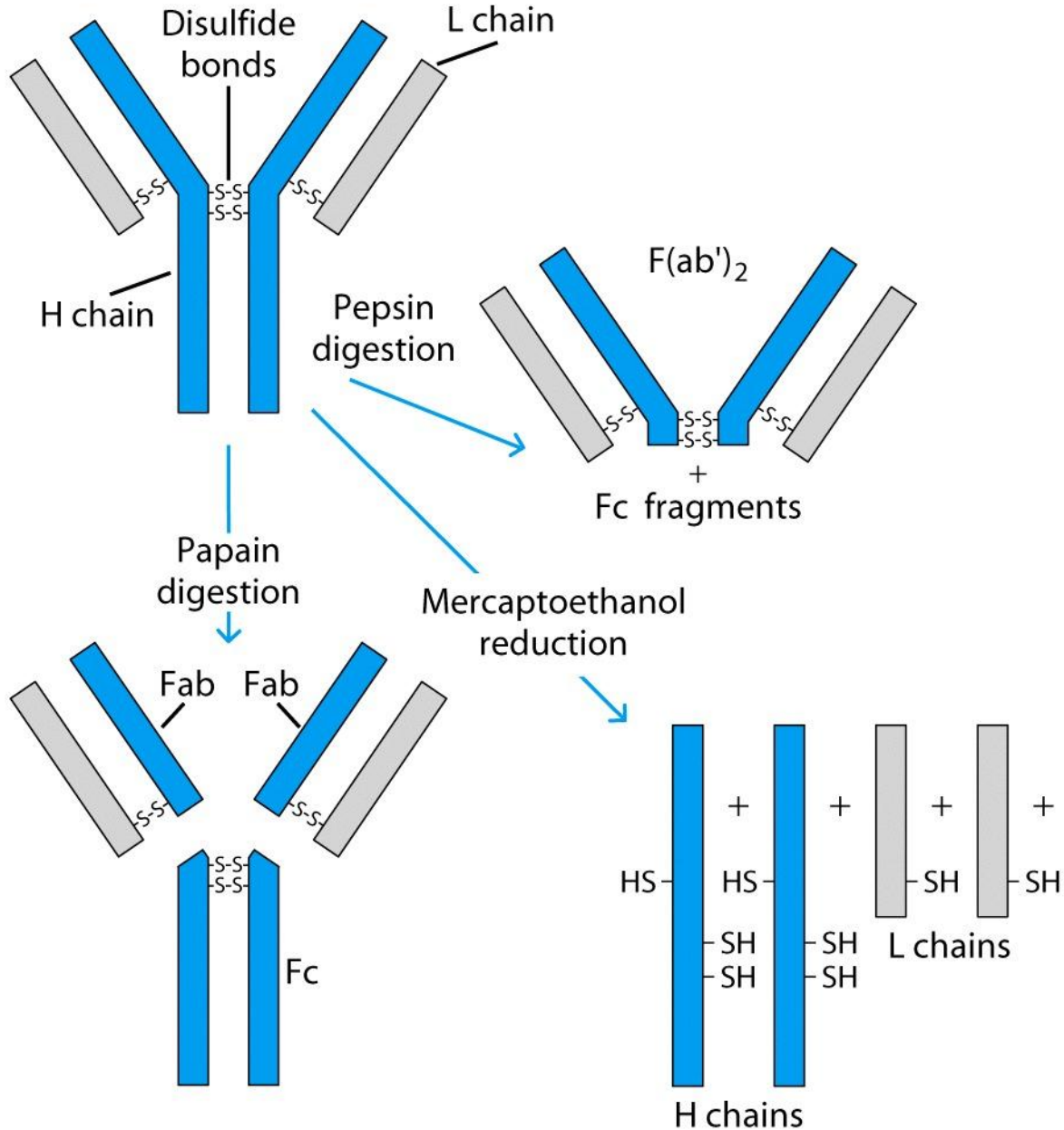
- one F(ab}2 fragment and
- pFc' fragment.

F(ab}2 is written with a prime because it contains a few more amino acids than Fab, including the cysteines that form the disulfide bonds



Functions of Fc Fragments

- The Fc portion encodes the effector functions of the immunoglobulin.
- These functions are generally inflammatory reactions that include
- fixation of complement
- activation of complement
- Binding to Fc receptors on the surface of other cells


Disulfide bonds
L chain
H chain
Pepsin digestion
F(ab')2
+
Fc fragments
Papain digestion
Mercaptoethanol reduction
Fab
Fab
Fc
HS
HS
SH
SH
SH
SH
SH
SH
L chains
H chains

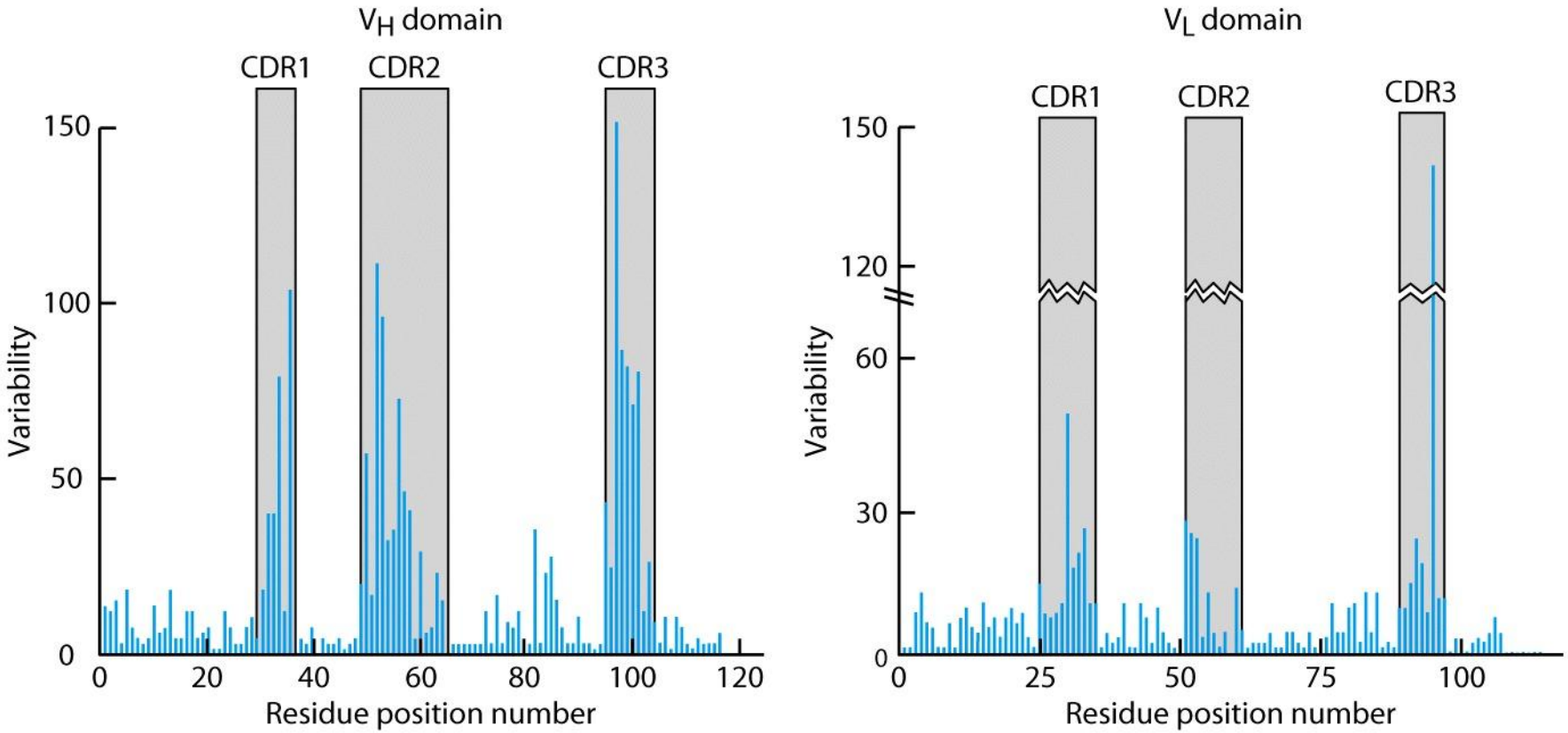
Enzymatic Digestion Of Antibodies

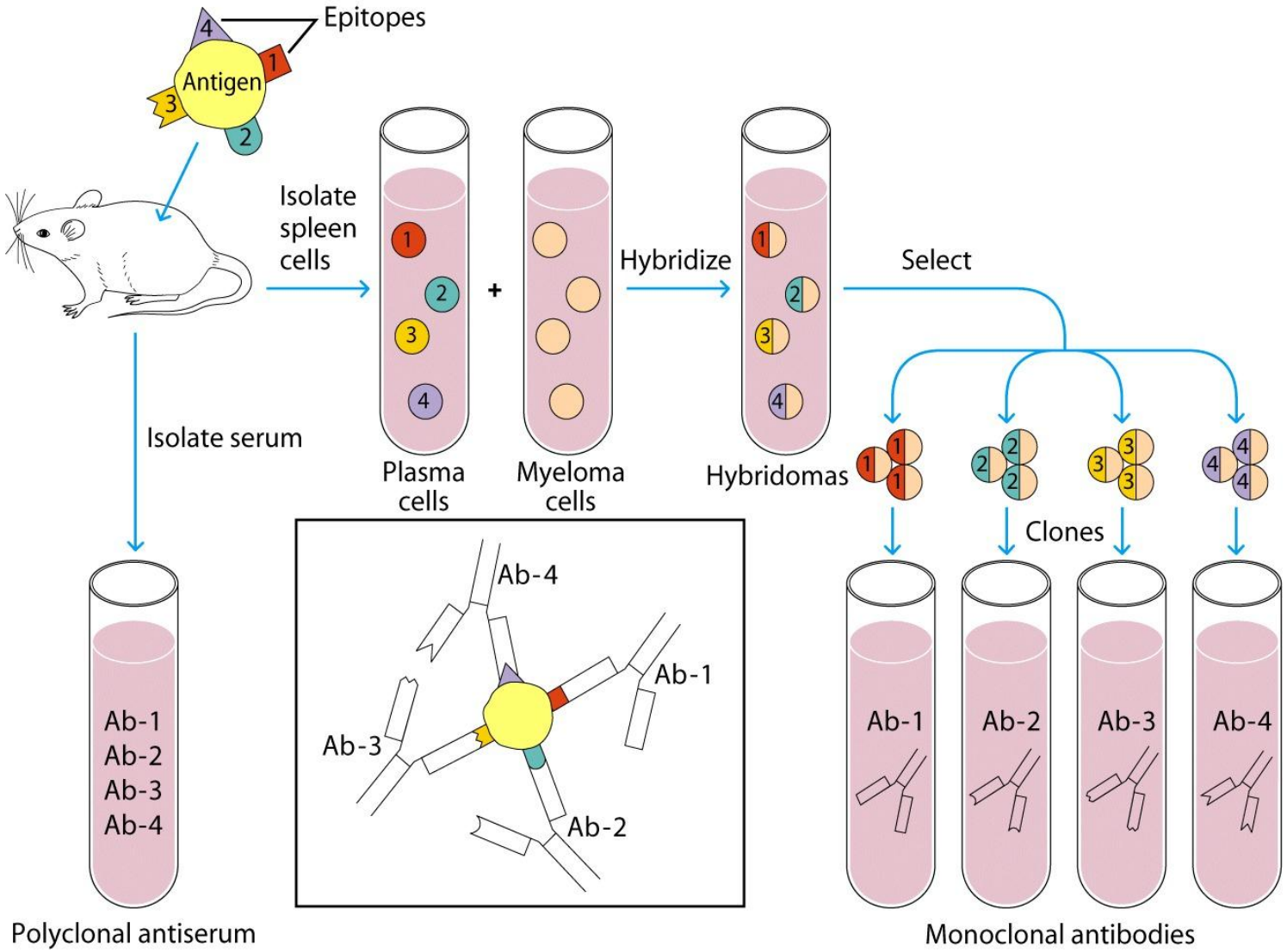
- Digestion With Papain Yields
  - 3 Fragments
  - 2 identical Fab and 1 Fc
  - Fab Because Fragment That is Antigen Binding
  - Fc Because Found To Crystallize In Cold Storage
- Pepsin Digestion
  - F(ab`)2
  - No Fc Recovery, Digested Entirely
- Mercaptoethanol Reduction (Eliminates Disulfide Bonds)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

Sequencing Of Heavy Chains

- Sequencing Of Several Immunoglobulins Revealed
  - 100-110 Amino Terminus, Highly Variable (V)
  - Five Basic Sequence Patterns
  - α,γ, δ, ε, μ
  - 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 Hypevariable



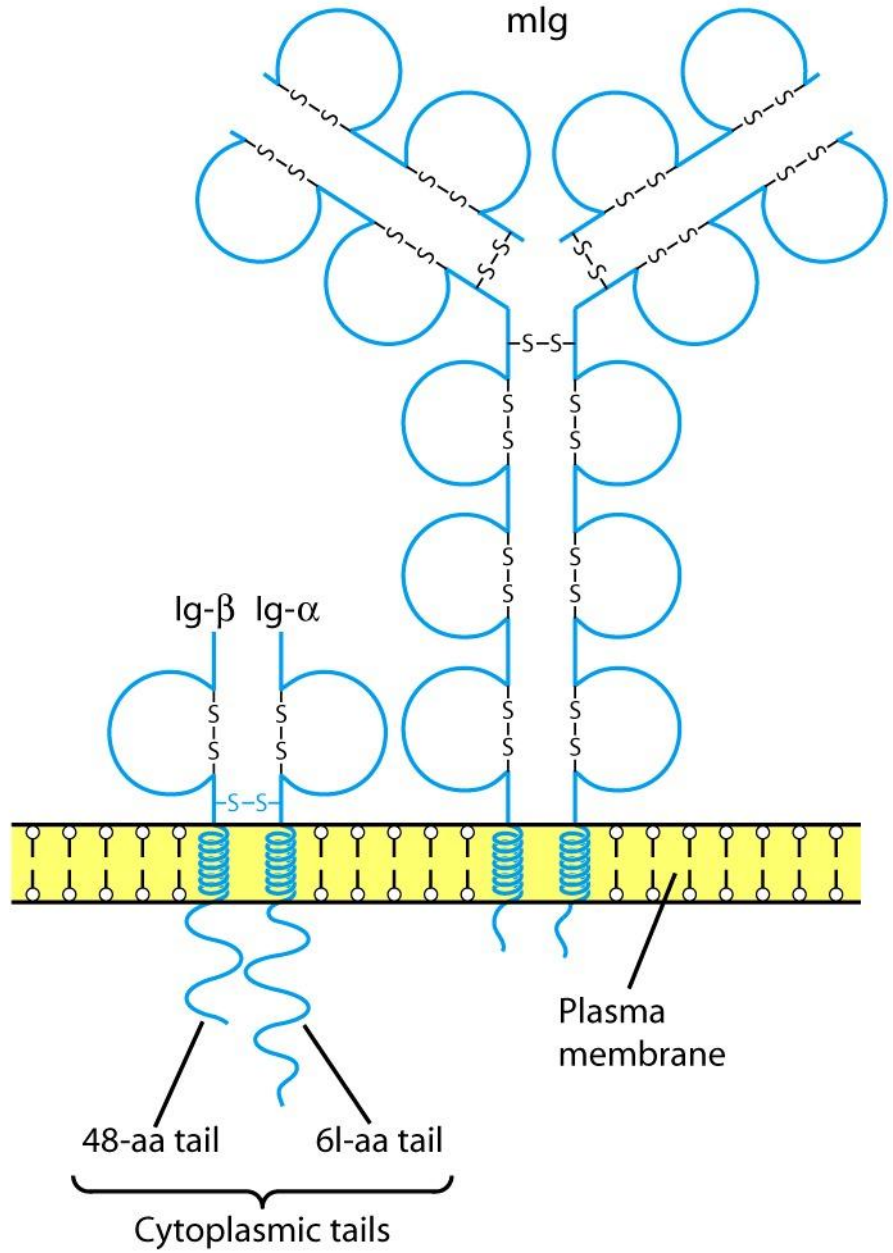

Epitopes
Antigen
Isolate spleen cells
Plasma cells
Myeloma cells
Hybridize
Hybridomas
Select
Clones
Isolate serum
Ab-1
Ab-2
Ab-3
Ab-4
Polyclonal antiserum
Monoclonal antibodies

Monoclonal Antibodies

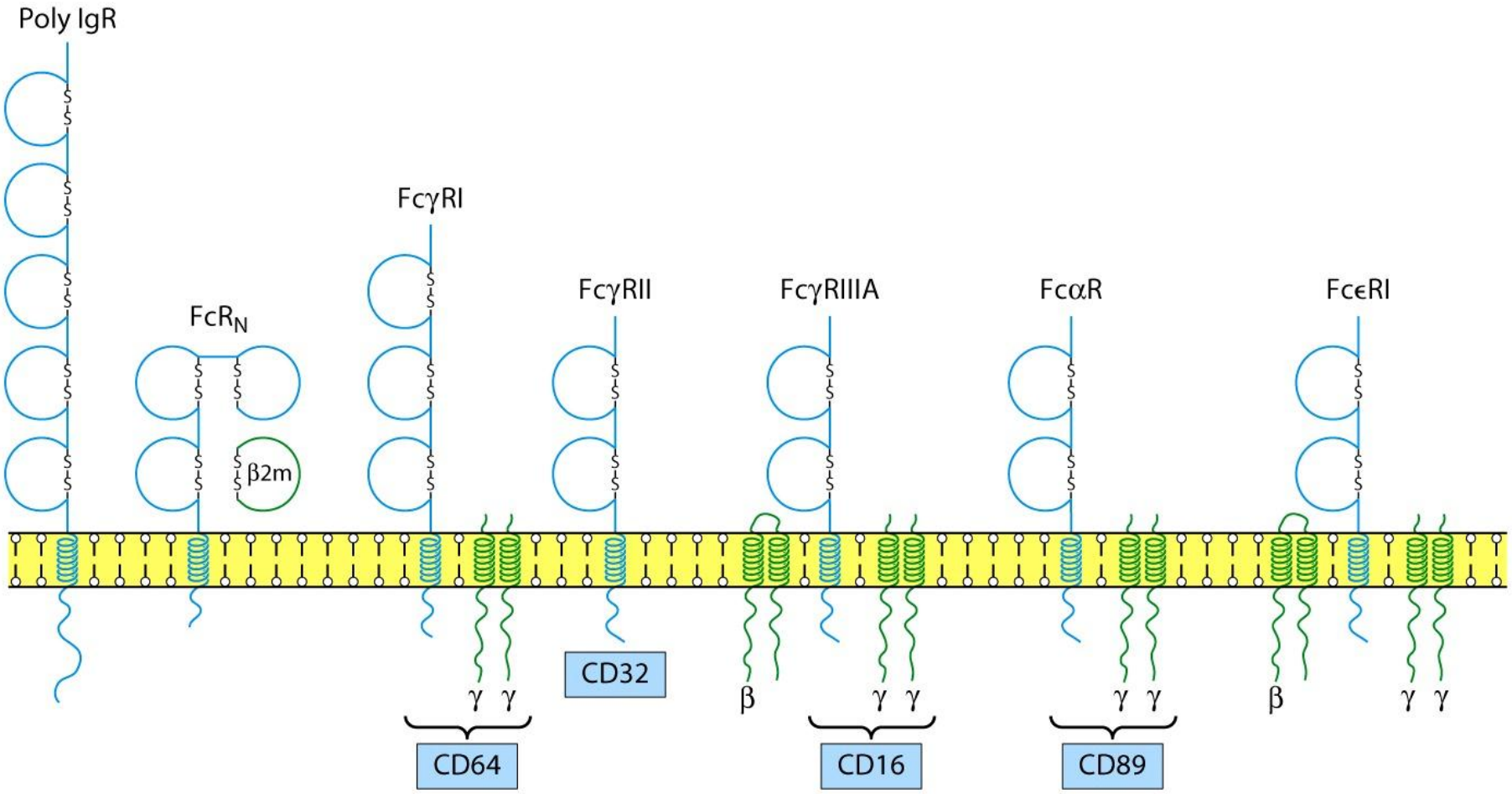
- Immunize Animal With Antigen
- Multiple Clones Are Generated, Good For In Vivo
- For Clinical Diagnosis, Research, One Clone That Reacts To Single Epitope Is Preferred
- Solution By Kohler and Milstein
  - Fuse A Myeloma Cell (Cancerous) With A Normal Plasma Cells
  - Resulting Clones Can Be Cultured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


mIg
Ig-β
Ig-α
Plasma membrane
48-aa tail
61-aa tail
Cytoplasmic tails

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 immunoglobin 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

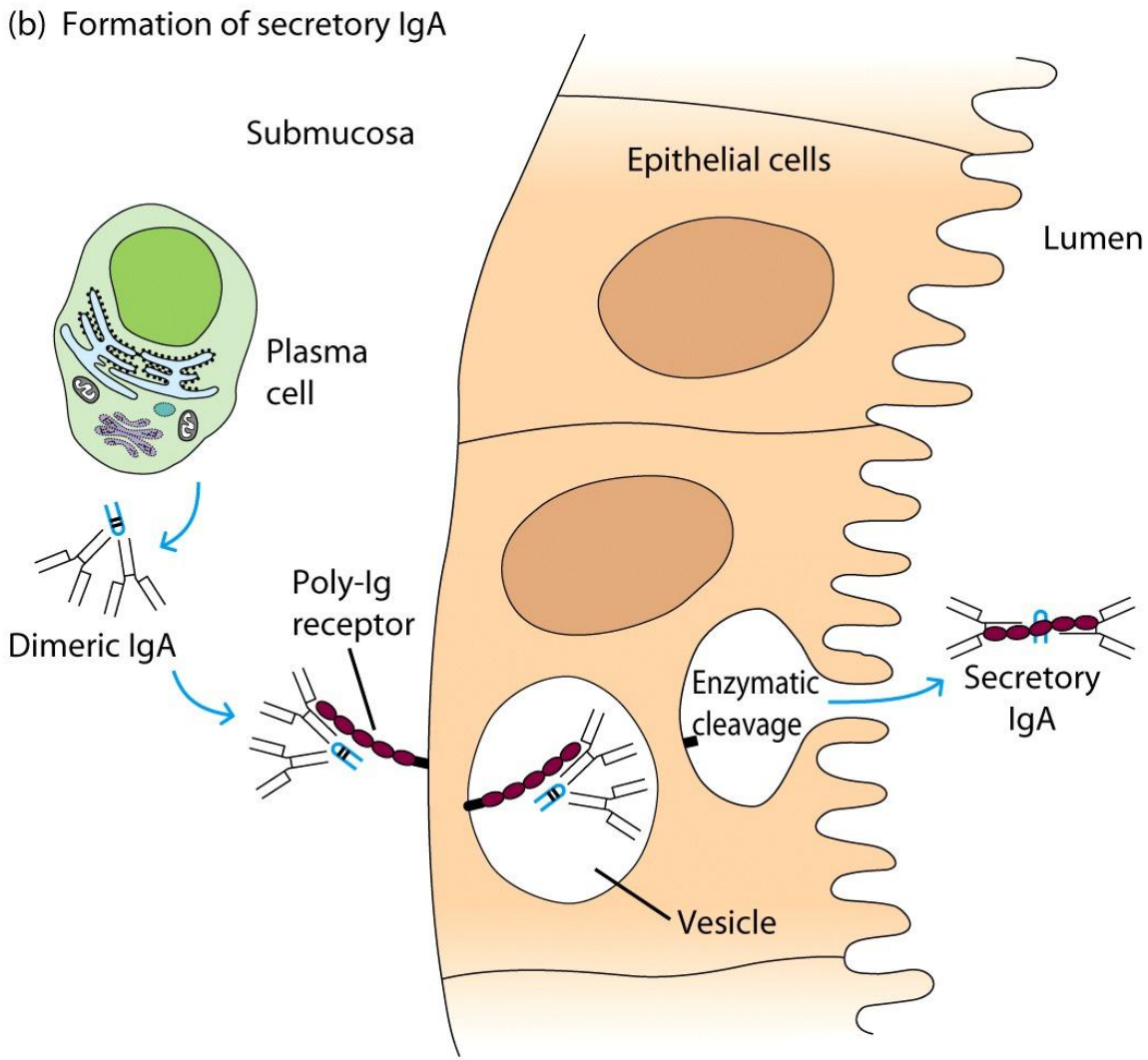
Antibody Classes And Biological Activities

- 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 polyptetide+secretory component (Poly IgR)

IgA Antibody Transport Across Cell (Transcytosis)



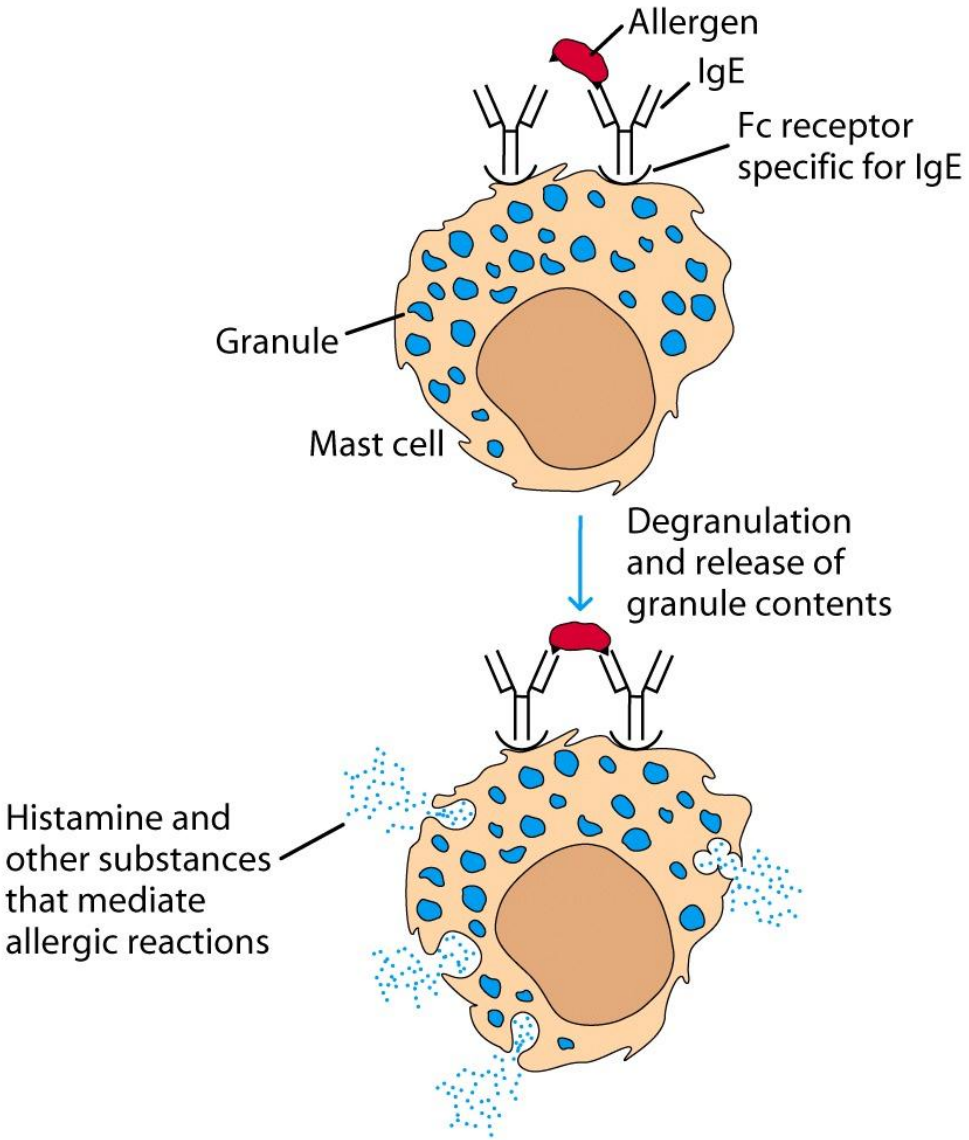
Antibody Classes And Biological Activities

- IgE
  - Very low serum concentration, 0.3μg/mL
  - Participate in immediate hypersensitivities reations. Ex. Asthma, anaphylaxis, hives
- Binds Mast Cells and Blood Basophils thru FcεR
- 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, ~30μg/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

Immunoglobulin Gene Rearrangement

- Virtually any substance can be the target of an antibody response, and the response to even a single epitope comprises many different antibody molecules each with a subtly different specificity for the epitope and a unique affinity, or binding strength

- The total number of antibody specificities available to an individual is known as the antibody repertoire or immunoglobulin repertoire, and in humans is at least 1011 and probably several orders of magnitude greater.

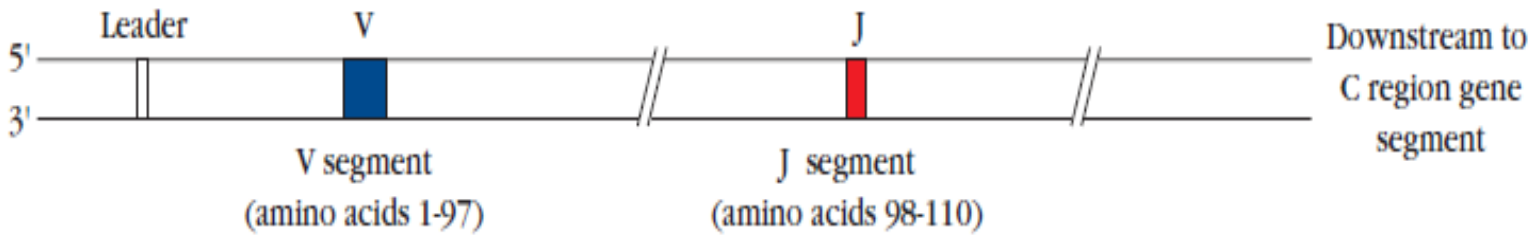
- The germ line theory suggested is a separate gene for each different immunoglobulin chain and that the antibody repertoire is largely inherited.
- In contrast, somatic diversification theories proposed that the observed repertoire is generated from a limited number of inherited V-region sequences that undergo alteration within B cells during the individual's lifetime
- Diversity is further enhanced by the process of somatic hypermutation in mature activated B cells. Thus the somatic diversification theory was essentially correct.

Segments of genomic DNA within the immunoglobulin genes are rearranged in cells of the B-lymphocyte lineage. This process of rearrangement is known as somatic recombination.

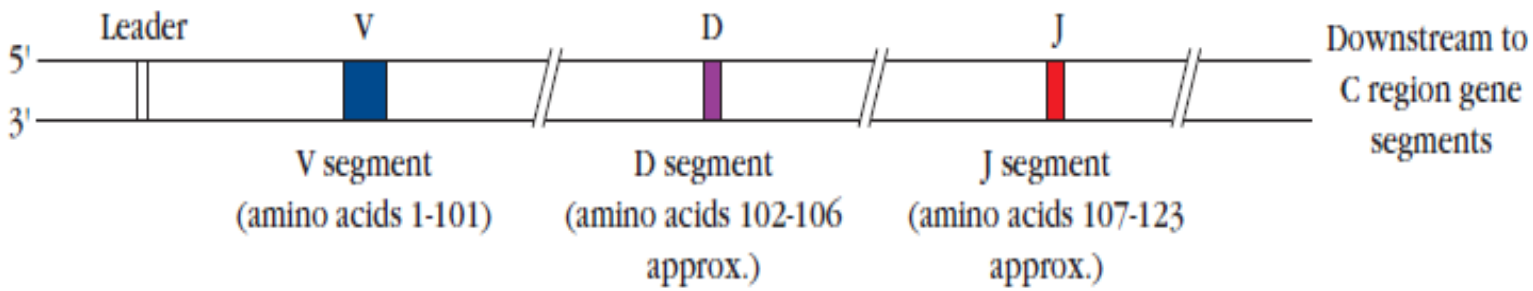
Two enzymes are primarily involved in immunoglobulin genes rearrangement .

These are recombinant activating gene 1 and 2 (RAG1/RAG2).

(a) Light chain V region gene segments in embryo (germline DNA)



(b) Heavy chain V region gene segments in embryo (germline DNA)



Five mechanisms accounts for antibody diversity

- Multiple gene segments exist at heavy (V, D, and J) and light-chain (V and J) loci.
- P nucleotide addition results when the DNA hairpin at the

coding joint is cleaved asymmetrically

- Exonuclease trimming sometimes occurs at the VDJ and VJ junctions, causing loss of nucleotides
- Non-templated N nucleotide addition in heavy chains
- Combinatorial diversity: The same heavy chain can combine
- with different light chains, and vice versa

Cytokines regulates Antibody classes

	Role of cytokines in regulating expression of antibody classes						
Cytokines	**IgM**	**IgG3**	**IgG1**	**IgG2b**	**IgG2a**	**IgE**	**IgA**
IL-4	Inhibits	Inhibits	Induces		Inhibits	Induces	
IL-5							Augments production
IFN-γ	Inhibits	Induces	Inhibits		Induces	Inhibits	
TGF-β	Inhibits	Inhibits		Induces			Induces

Figure 10.15 Janeway's Immunobiology, 8ed. (© Garland Science 2012)