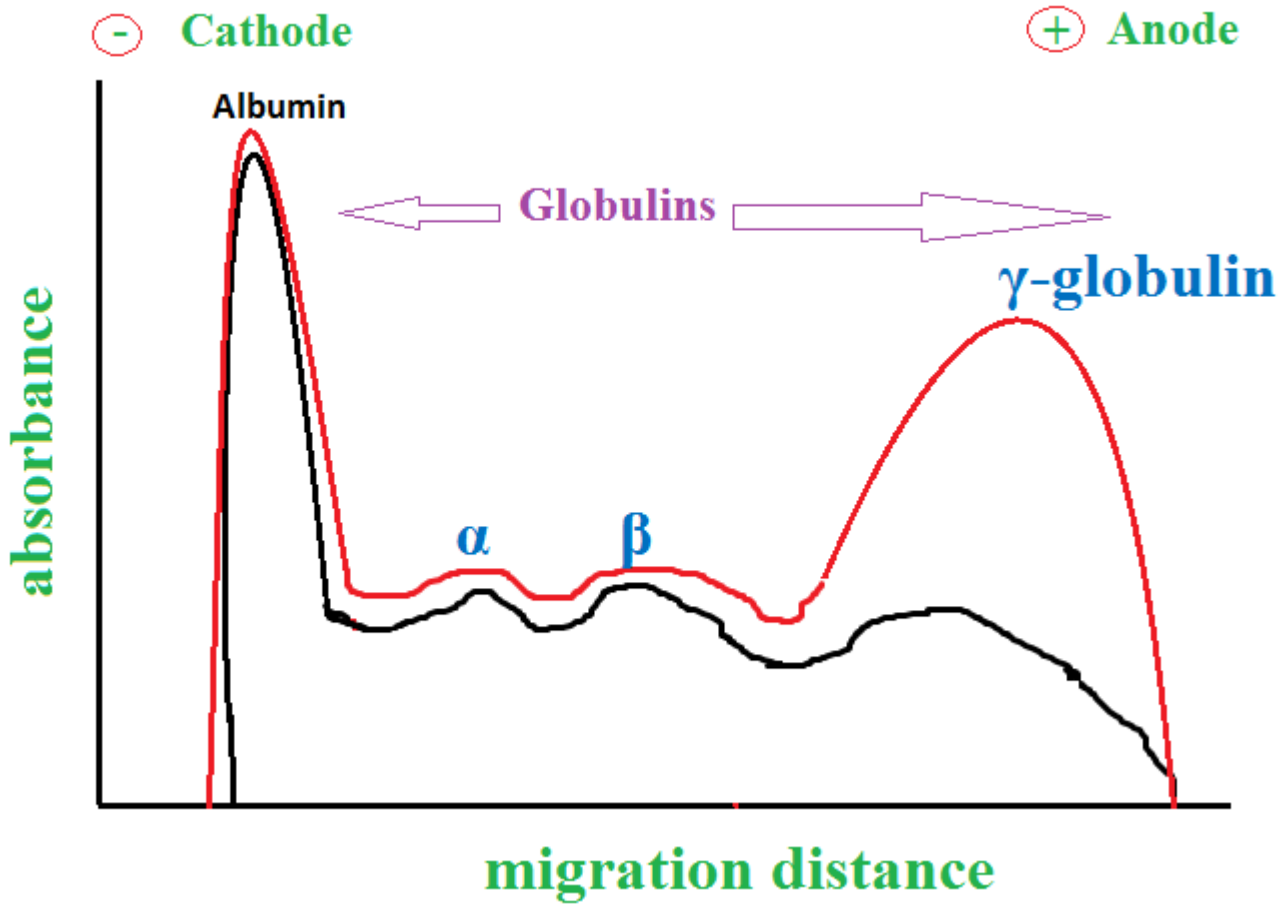
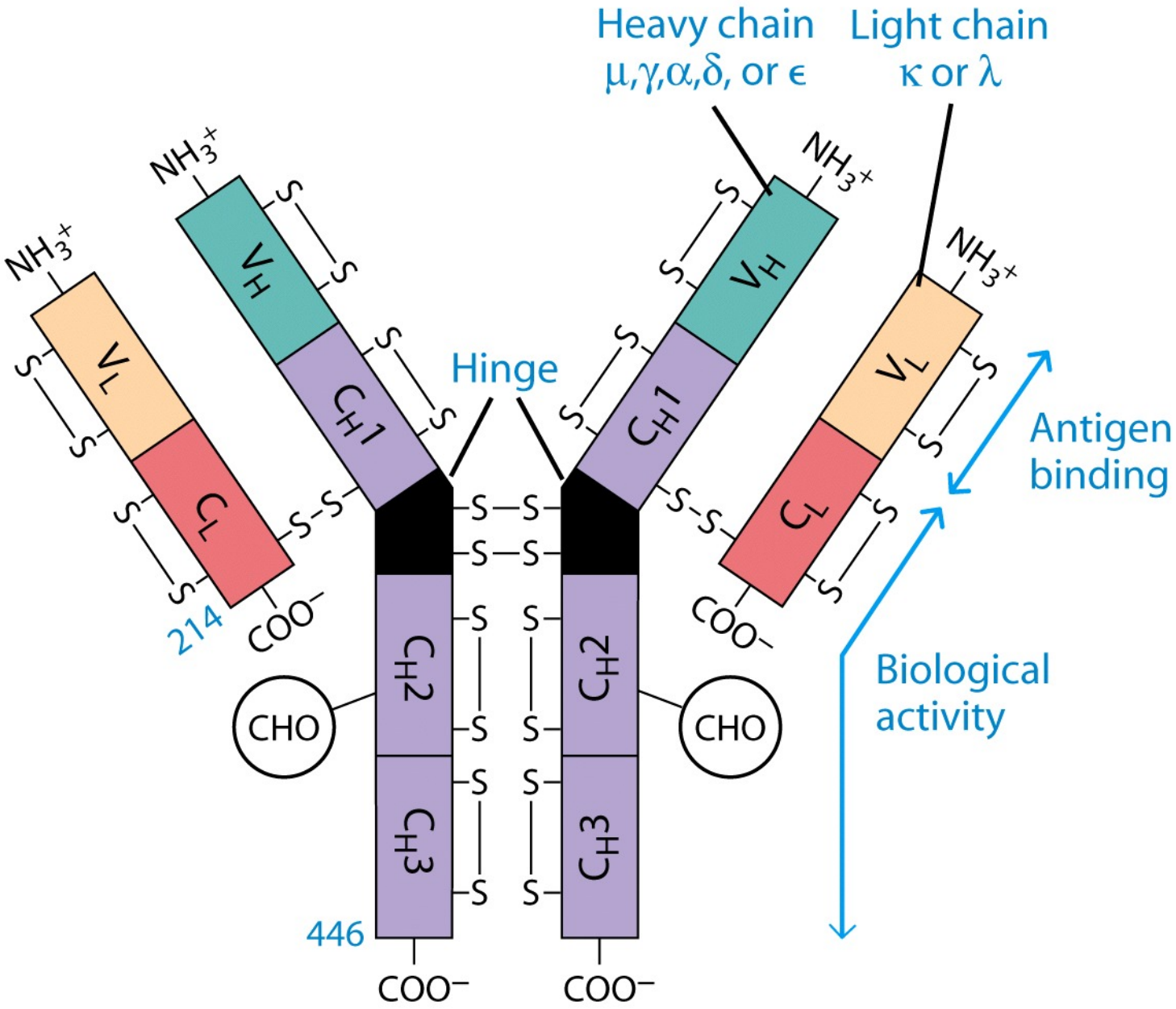
Antibodies: Structure and Function

1939, A. Tiselius and E. A. Kabat:

Rabbits were immunized with ovalbumin → immunized rabbits' serum → (i) one aliquot reveals four peaks on electrophoresis: **albumin and α, β, and γ-globulins** (ii) another aliquot treated with ovalbumin(Ag) and centrifuged. Supernatant gives peaks for : albumin, and α and β-globulins as for (i), but significant drop of γ-globulin peak. **Hence, γ-globulin fraction contained serum antibodies → immunoglobulin**




Heavy chain
μ,γ,α,δ, or ε
Light chain
κ or λ
NH3+
VH
CH1
VL
CL
Hinge
S—S
S-S
214
COO−
CHO
CH2
CH3
446
Antigen
binding
Biological
activity

Antibodies Are Made Up Of:

2 Light Chains (identical) ~25 KDa

2 Heavy Chains (identical) ~50 KDa

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**

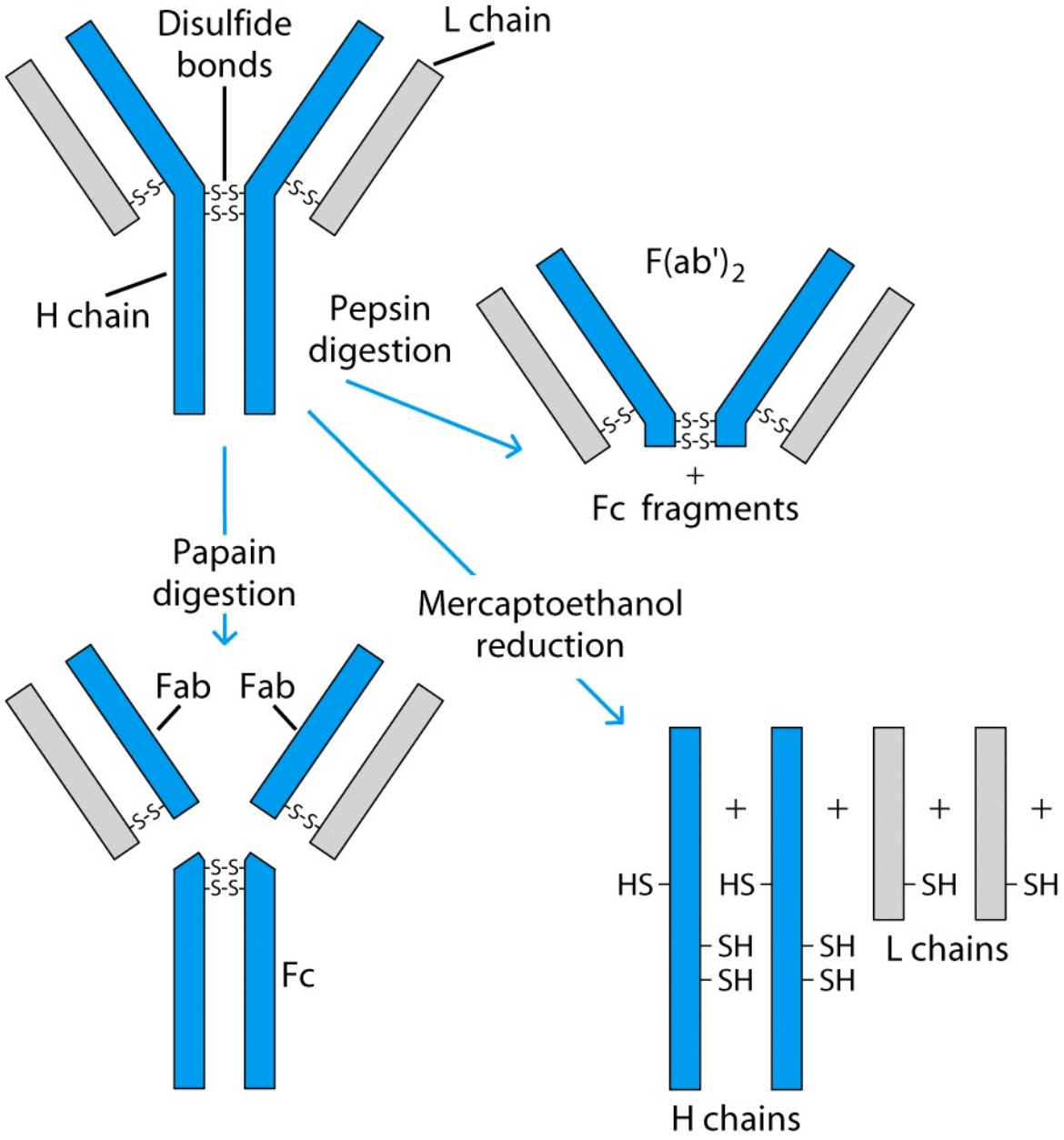

Disulfide bonds
L chain
H chain
Pepsin digestion
F(ab')2
+
Fc fragments
Papain digestion
Mercaptoethanol reduction
Fab
Fab
Fc
HS
HS
SH
SH
SH
SH
SH
SH
L chains
H chains

TABLE 4-3 Chain composition of the five immunoglobulin classes in humans

Class*	Heavy chain	Subclasses	Light chain	Molecular formula
IgG	γ	$\gamma 1, \gamma 2, \gamma 3, \gamma 4$	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	$\alpha 1, \alpha 2$	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	$\delta_2\kappa_2$ $\delta_2\lambda_2$

*See Figure 4-1 for general structures of five antibody classes.

Table 4-3
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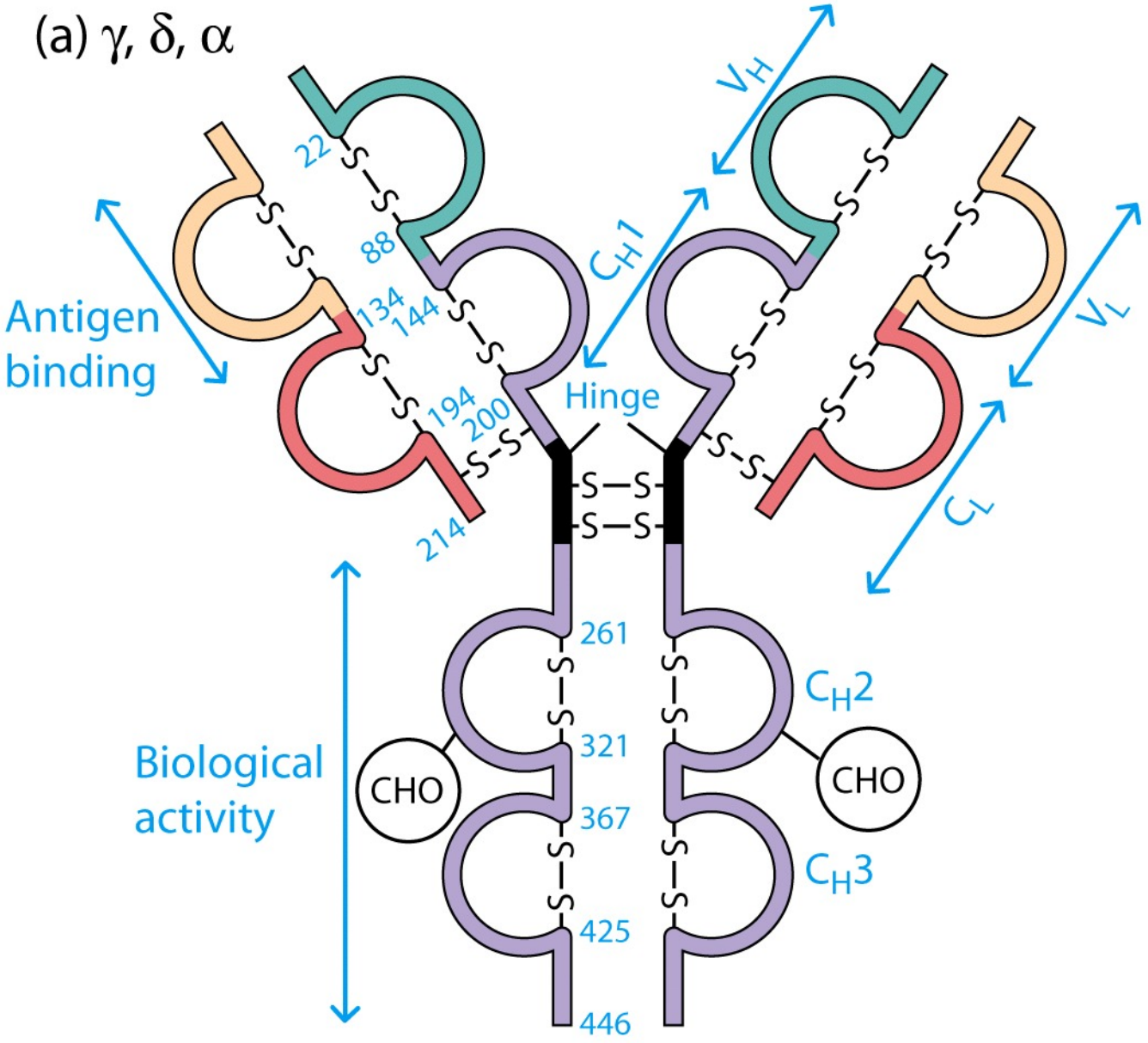
(a) γ, δ, α
V_H
C_H1
V_L
C_L
Antigen binding
Hinge
22
88
134
144
194
200
214
261
321
367
425
446
C_H2
C_H3
CHO
CHO
Biological activity

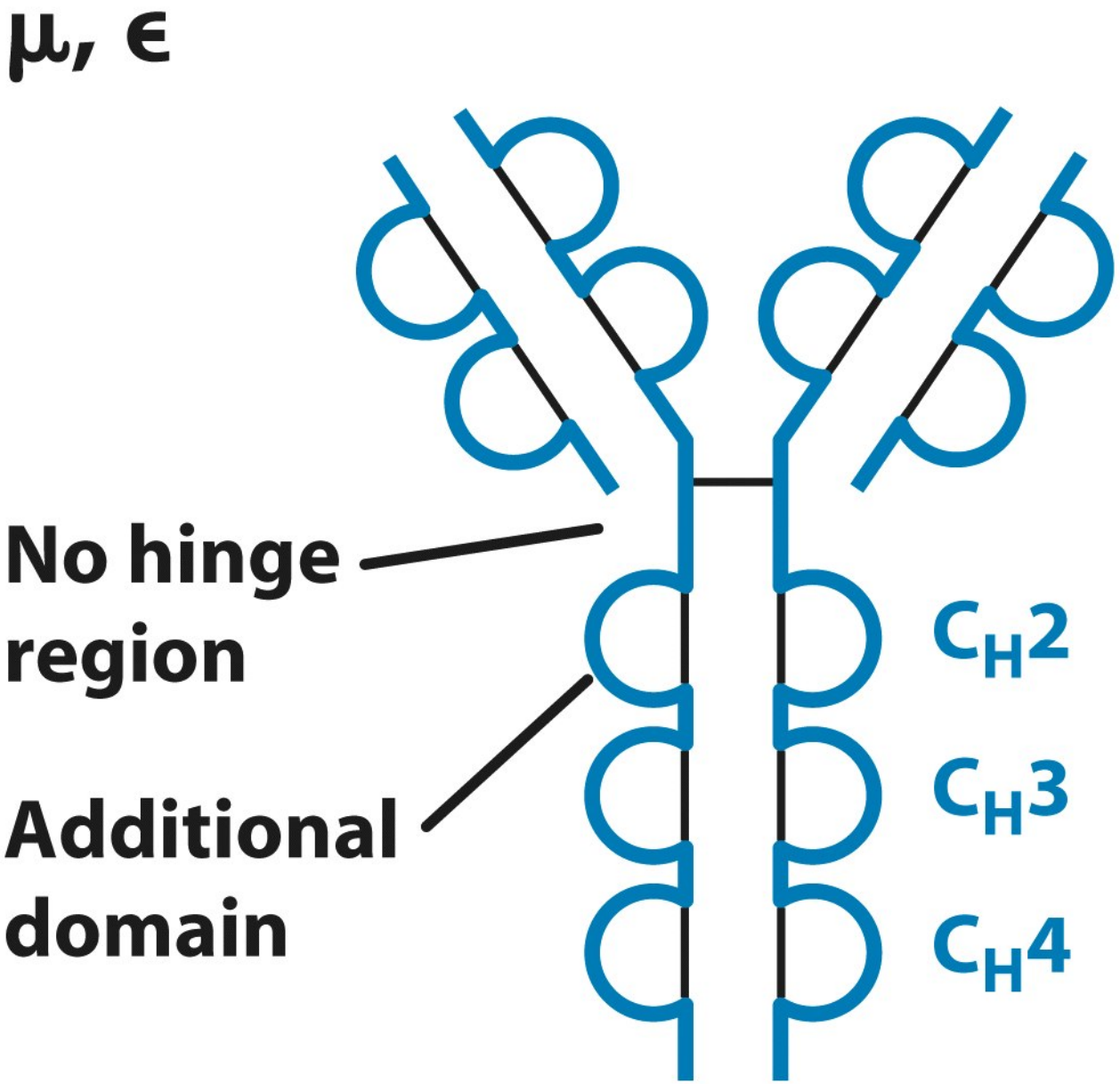

Figure 4-10b
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Secondary

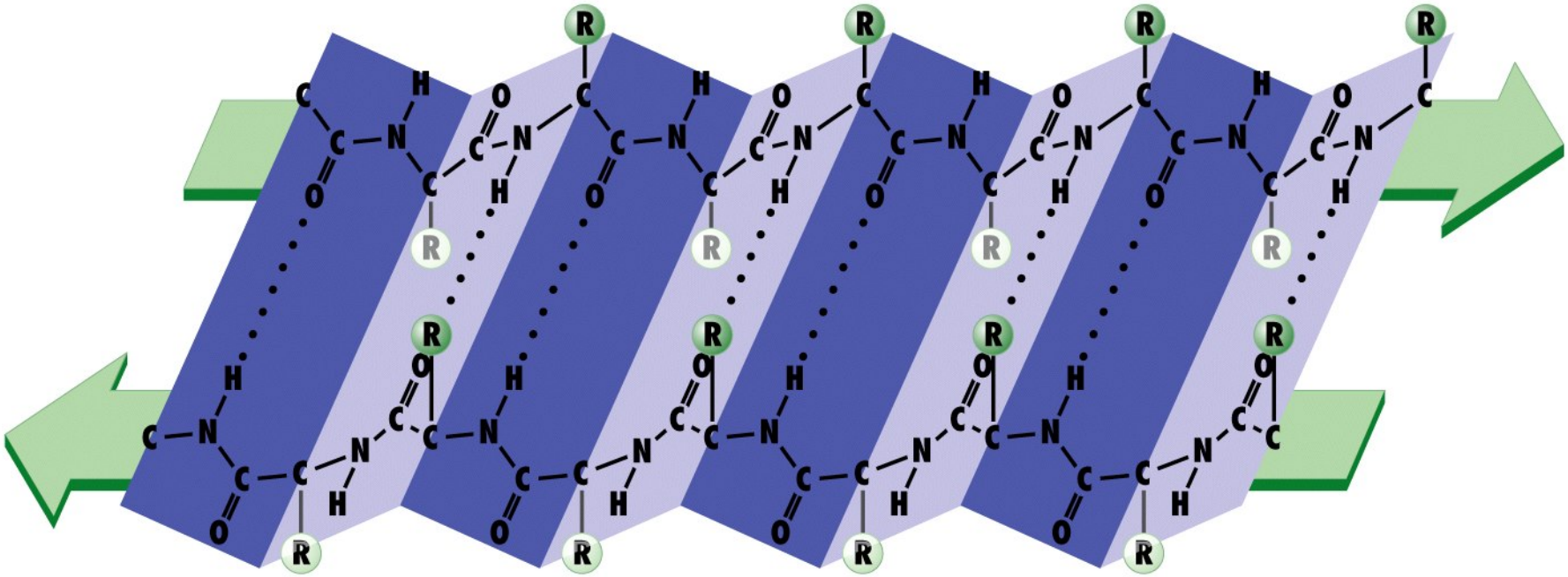

Figure 4-8
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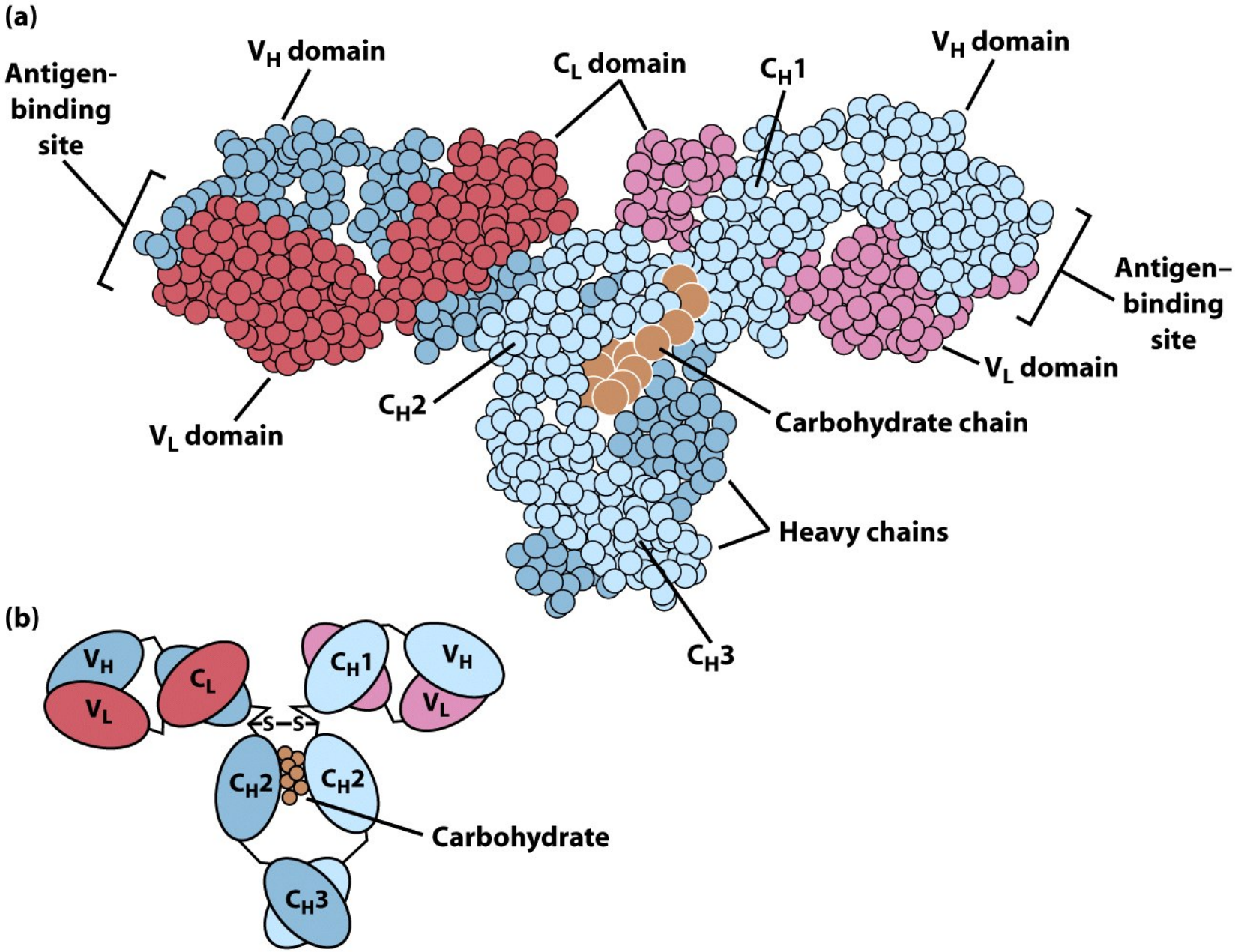

Figure 4-9
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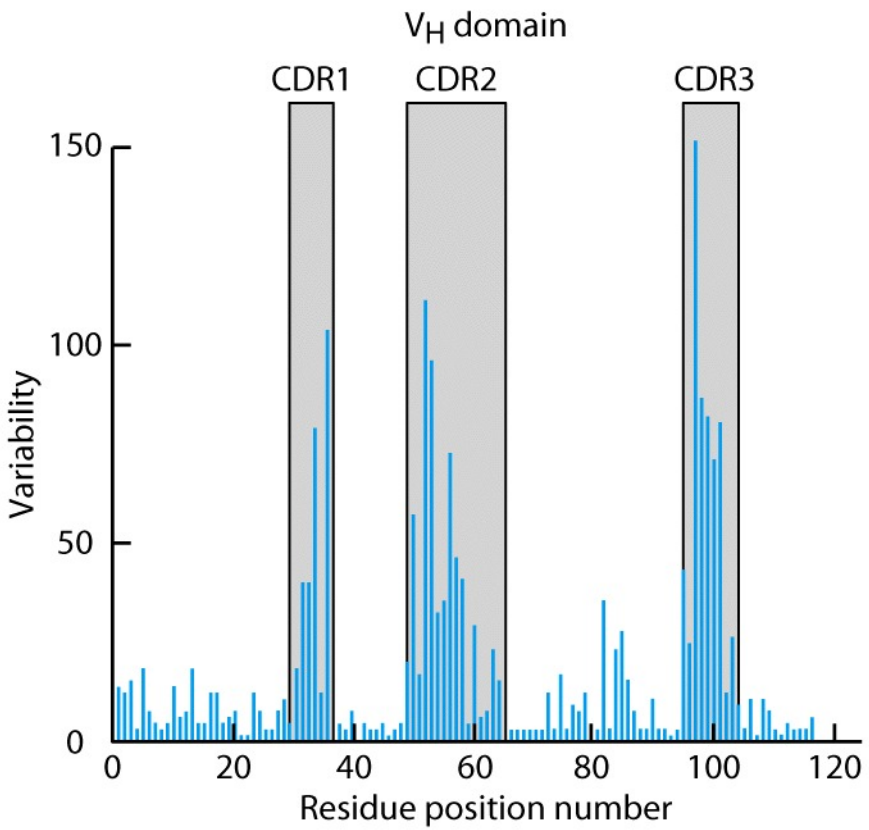
CDR (Complementarity Determining Regions) Are What Bind Ag



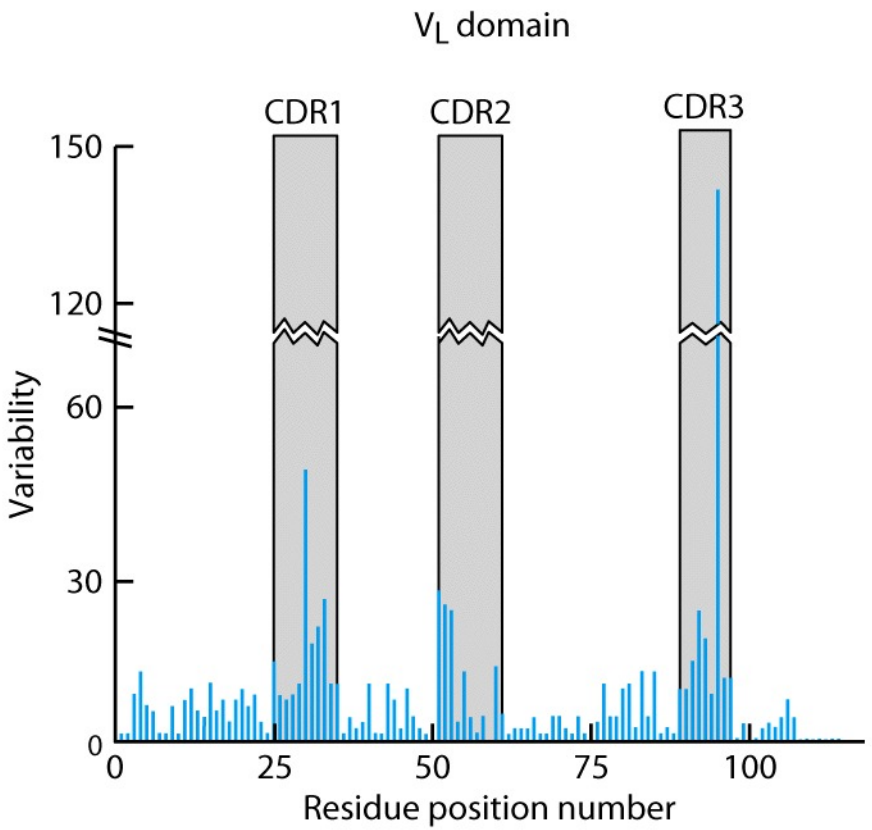


TABLE 4-4 Properties and biological activities* of classes and subclasses of human serum immunoglobulins

	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.

Table 4-4
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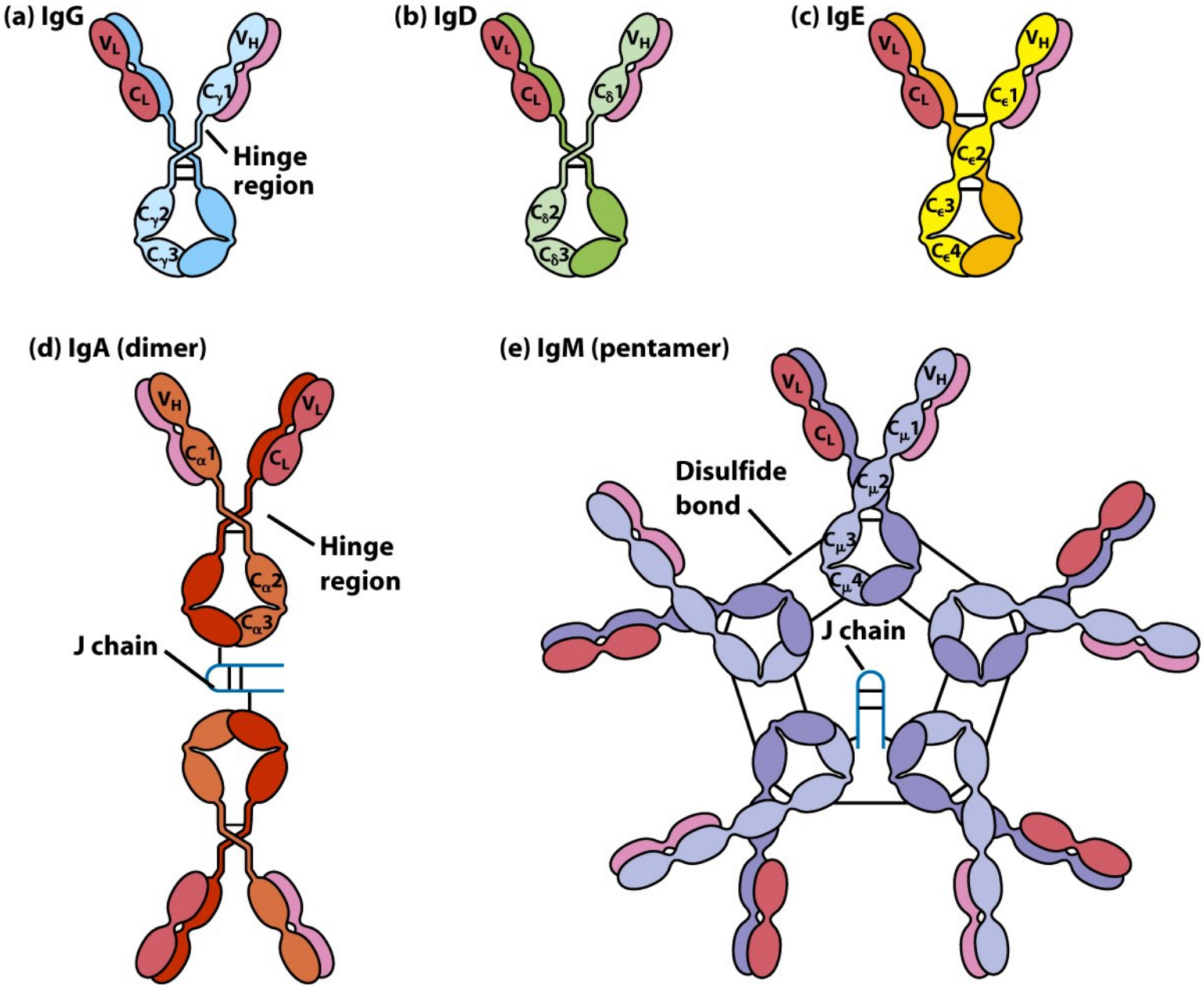

Figure 4-17
Kuby IMMUNOLOGY, Sixth Edition

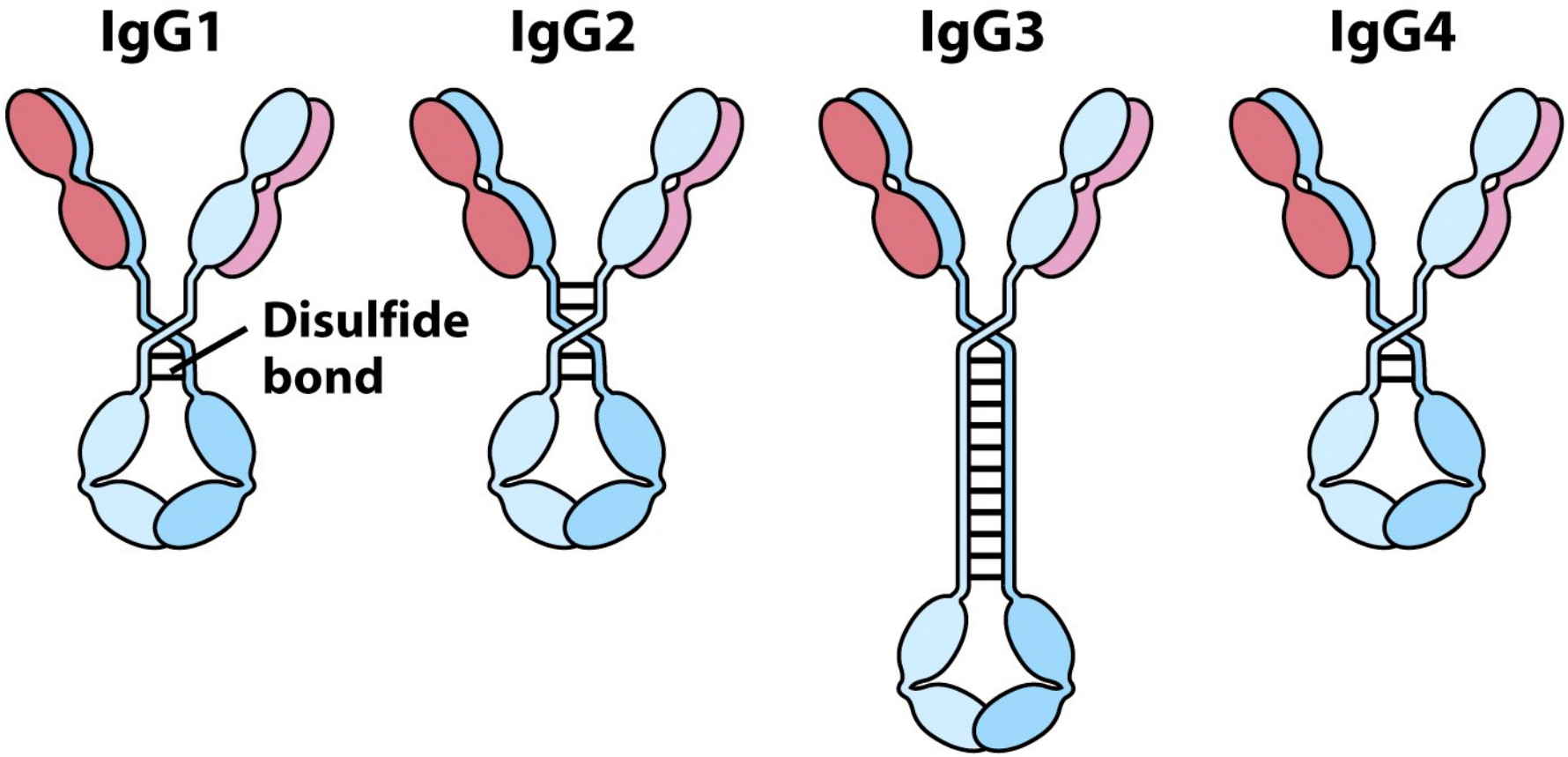

Figure 4-18
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IgG

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

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

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

- Predominant class in secretions
  - J chain and secretory component helps with transport across intestinal wall
  - J chain makes IgA more resistant to acids and enzymes found in digestive tract
  - IgA and macrophages restrict commensal bacteria that occasionally enter the tissues from the intestines
    - Better for IgA to interact than IgG – this is because the Fc portion of IgG has high affinity for receptors of immune cells and would constantly trigger inflammatory responses

- Can cross-link large antigens
    - Exists as dimer

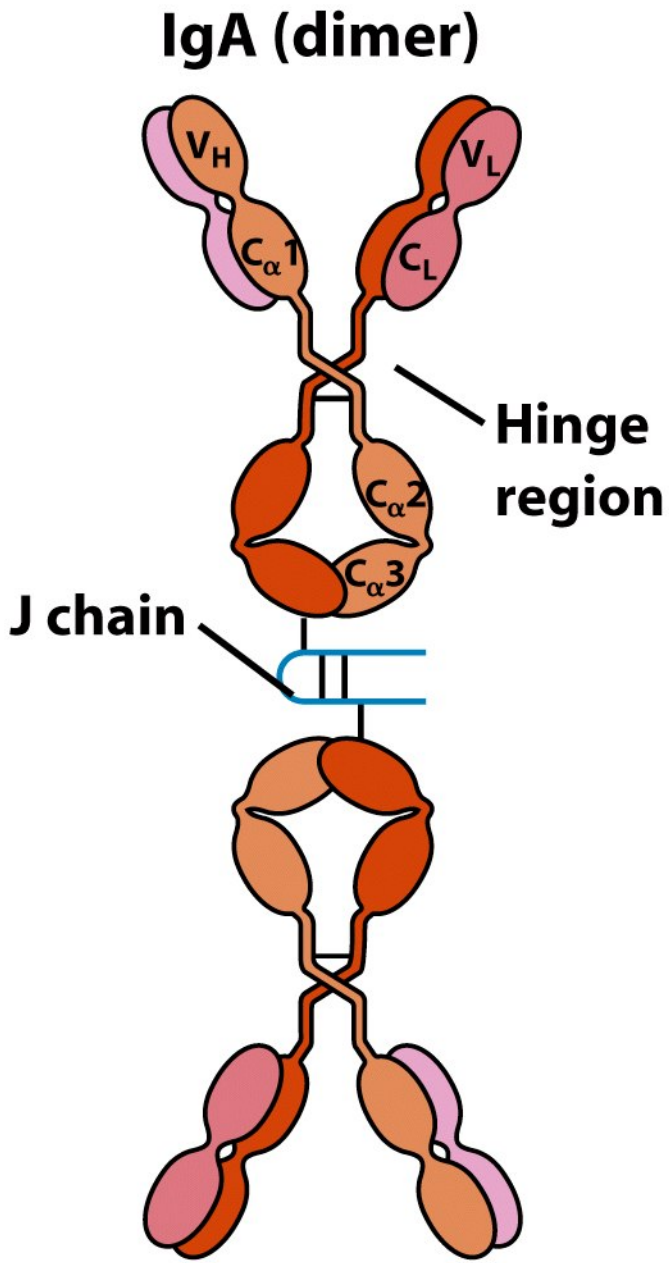

Figure 4-17d
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Structure of secretory IgA

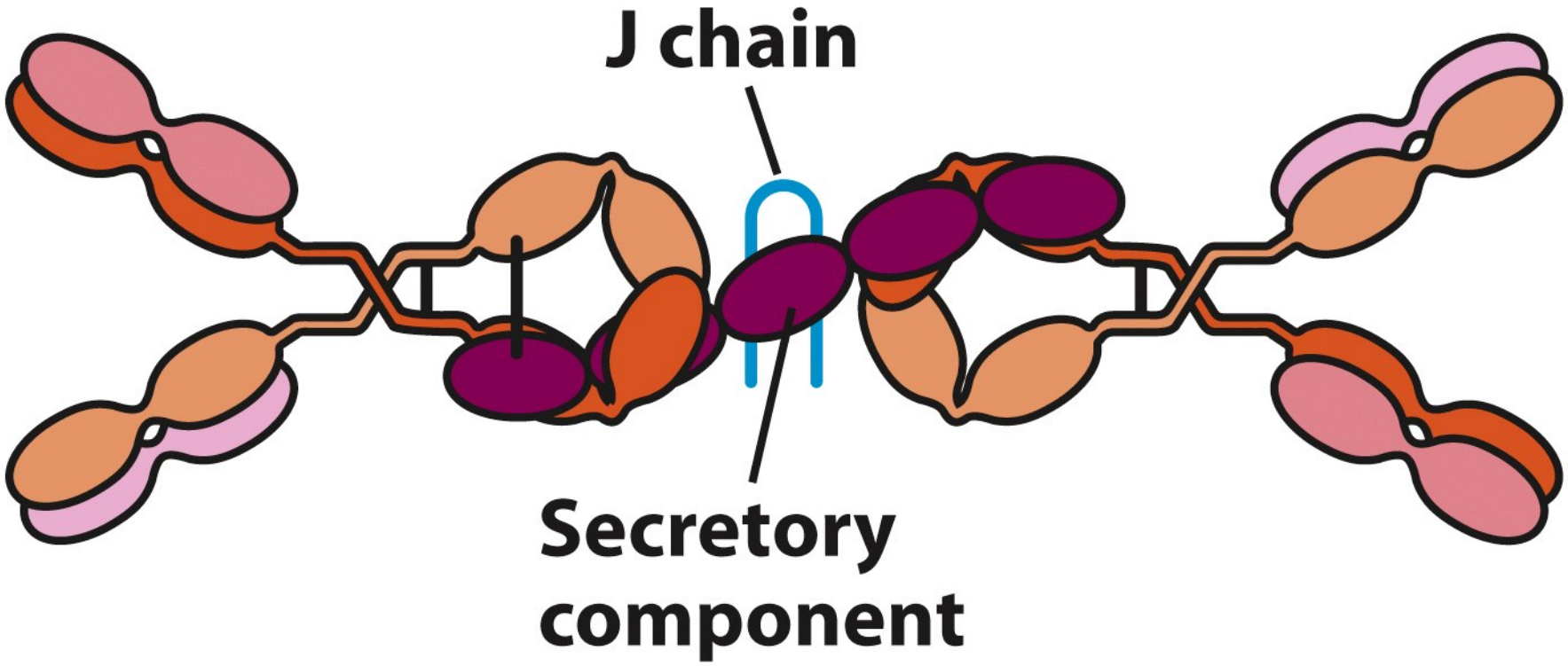

Figure 4-19a
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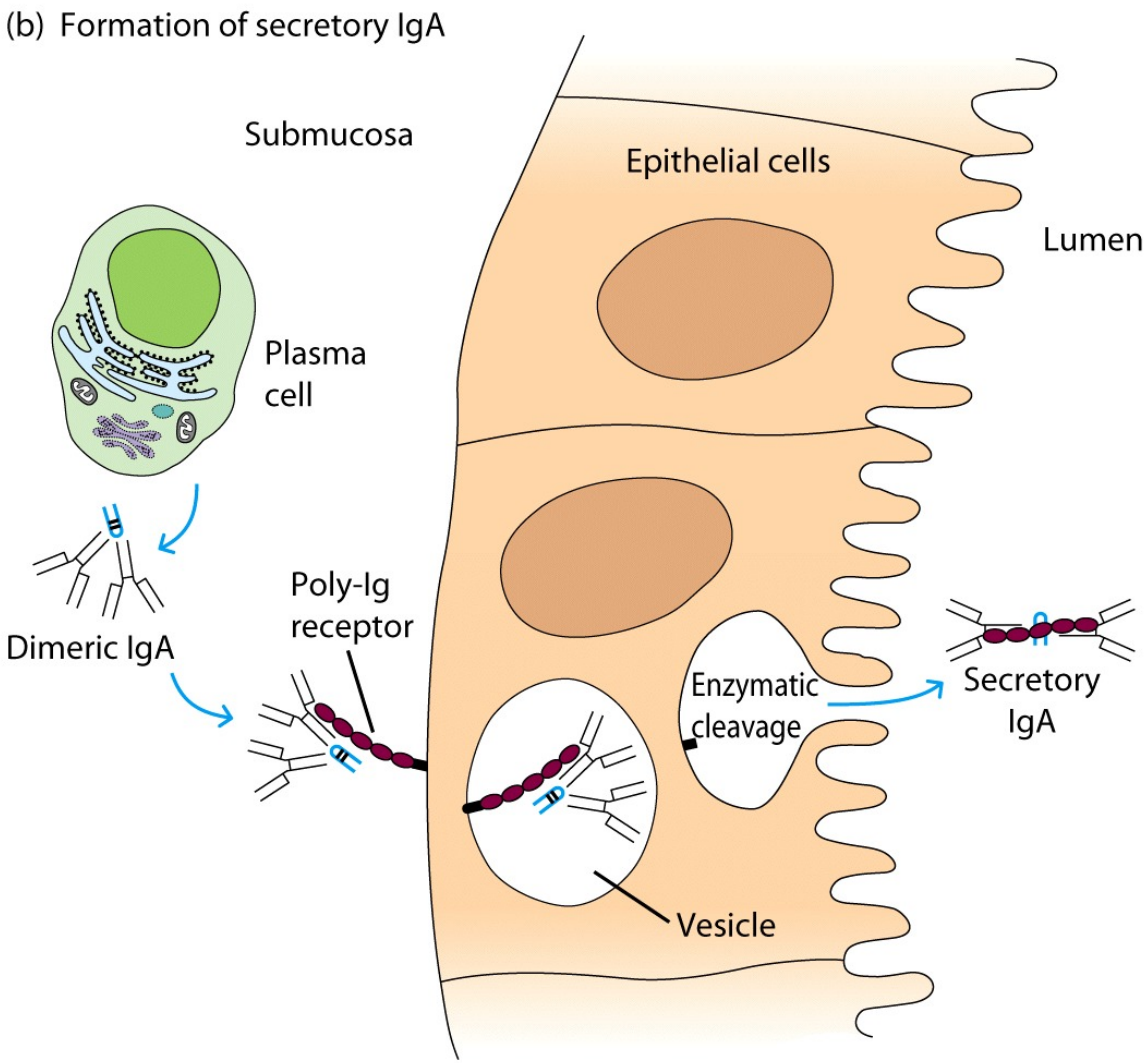
IgA Antibody Transport Across Cell (Transcytosis)



IgE

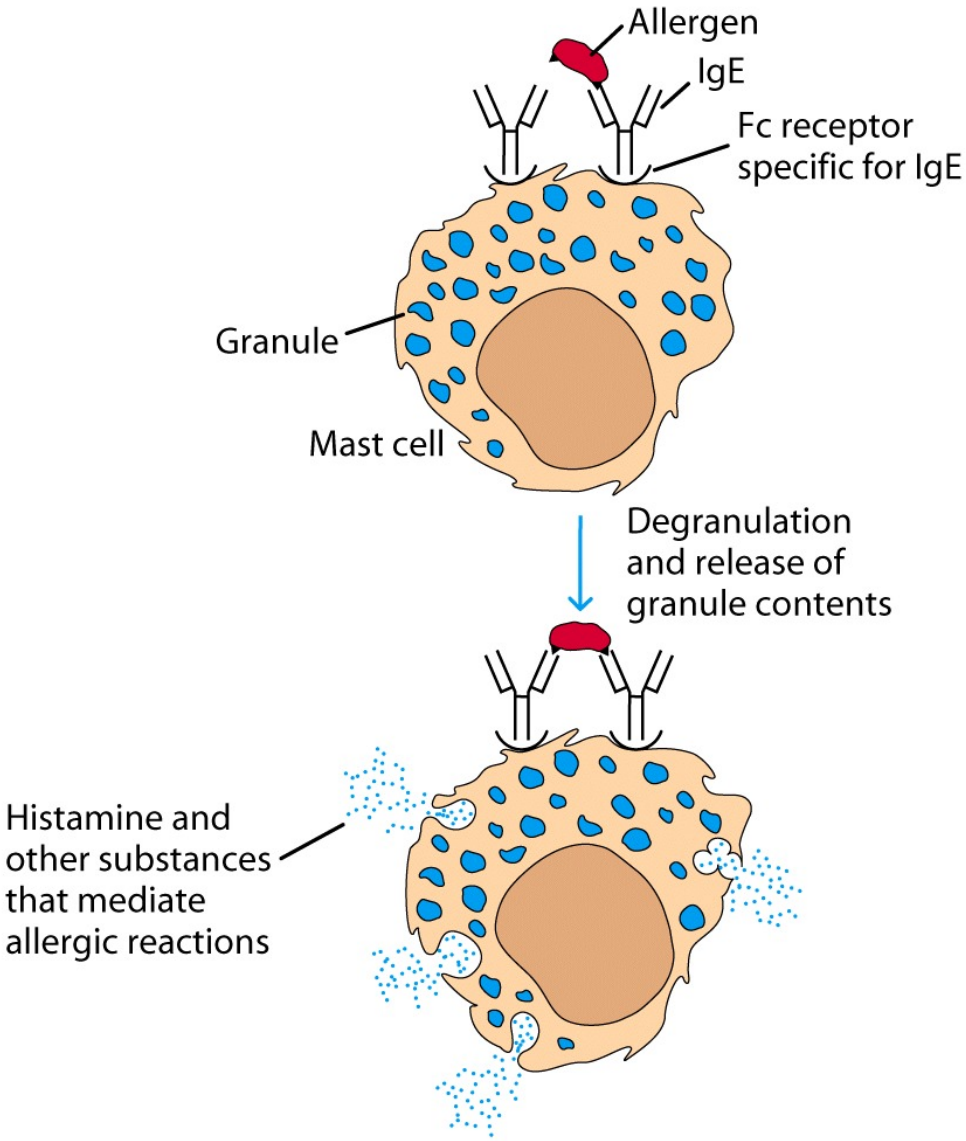
Very low serum concentration, 0.3μg/mL

Participate in immediate hypersensitivities reations. Ex. Asthma, anaphylaxis, hives.

Binds Mast Cells and Blood Basophils thru FcεR

Binding causes degranulation (Histamine Release)

Cross-Linkage of Bound IgE Antibody With Allergen Causes



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

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

Allotype

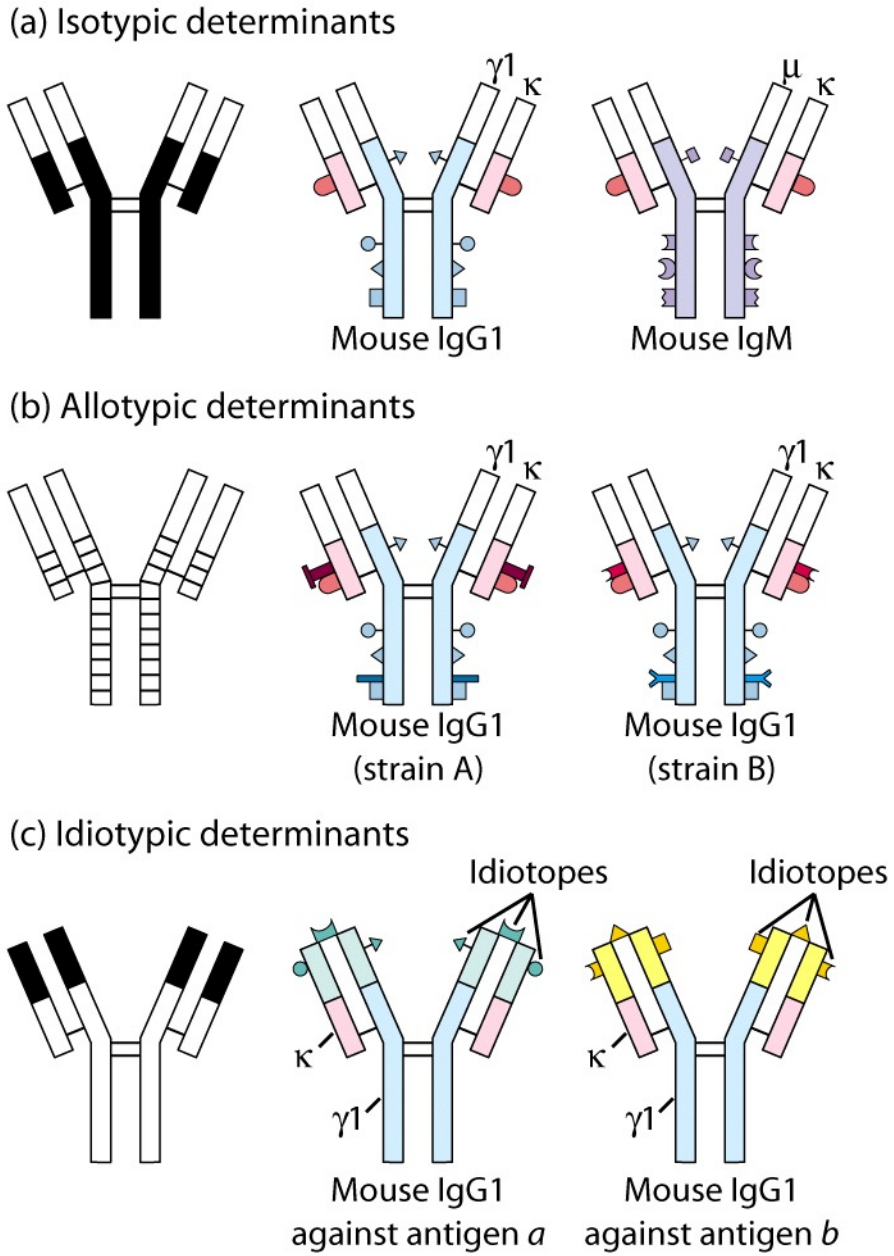
- Even though same isotypes within one species small differences (1-4 a/a) arise in different individuals (form of polymorphism)
- If injected with such Ab you generate anti-allotype Ab
  - Ex. During pregnancy
  - Blood transfusion

Idiotype

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
γ1
κ
μ
κ
Mouse IgG1
Mouse IgM
(b) Allotypic determinants
γ1
κ
γ1
κ
Mouse IgG1
(strain A)
Mouse IgG1
(strain B)
(c) Idiotypic determinants
Idiotopes
Idiotopes
κ
γ1
κ
γ1
Mouse IgG1
against antigen a
Mouse IgG1
against antigen b

For Monoclonal Antibody Production

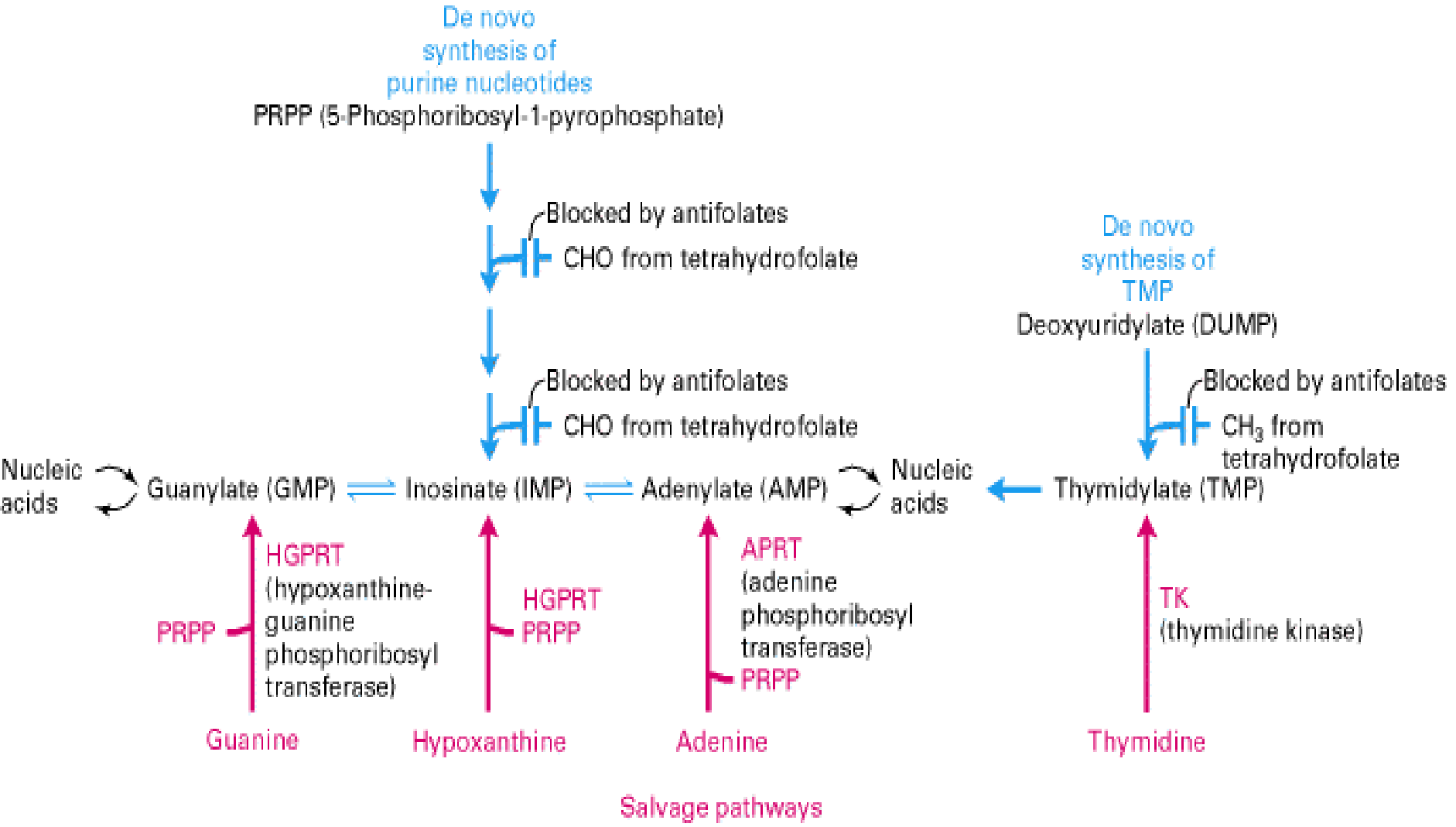
- Animal Is Immunized With Antigen
- Spleen Cells Are Isolated

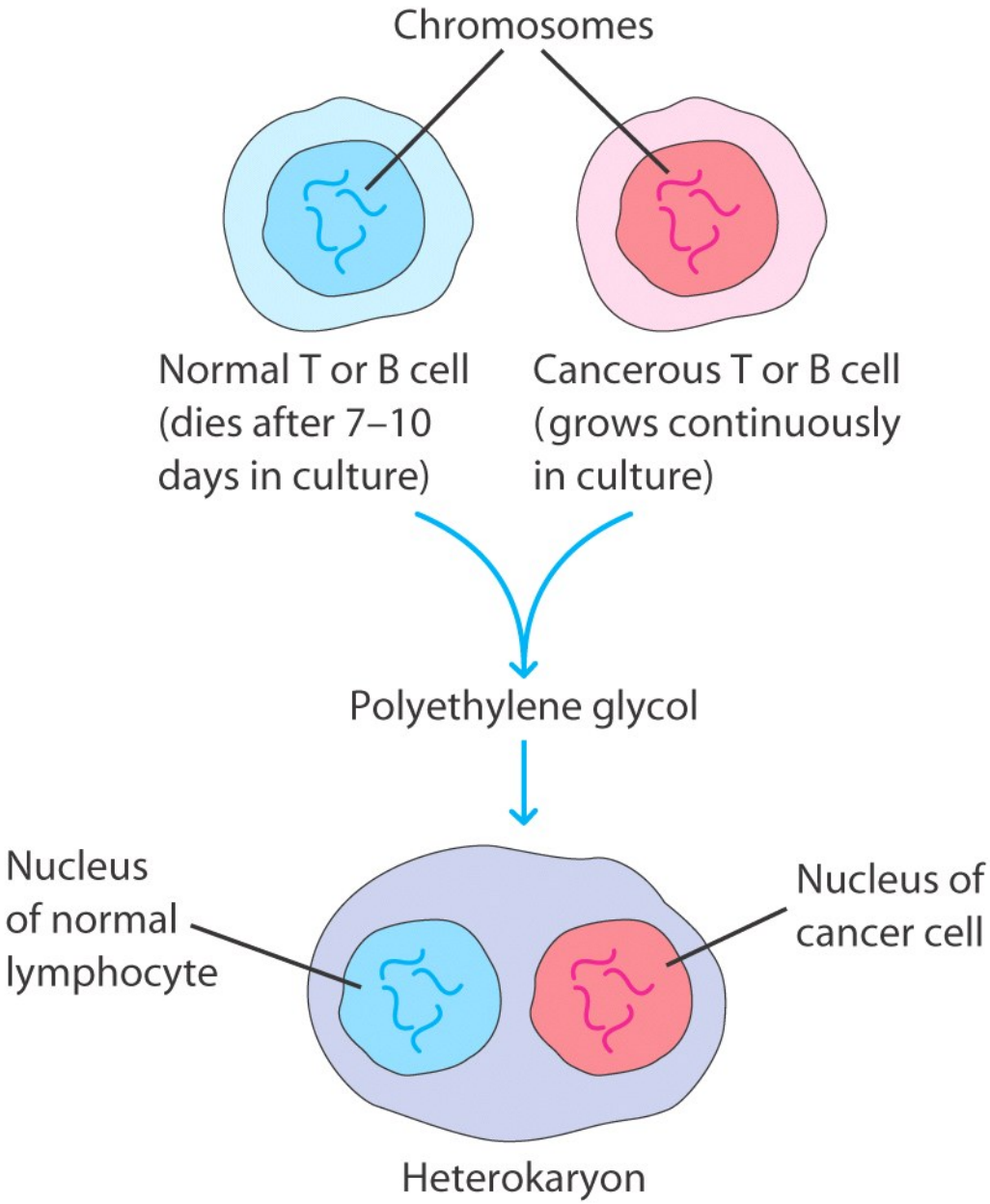
Intraperitoneal Immunization

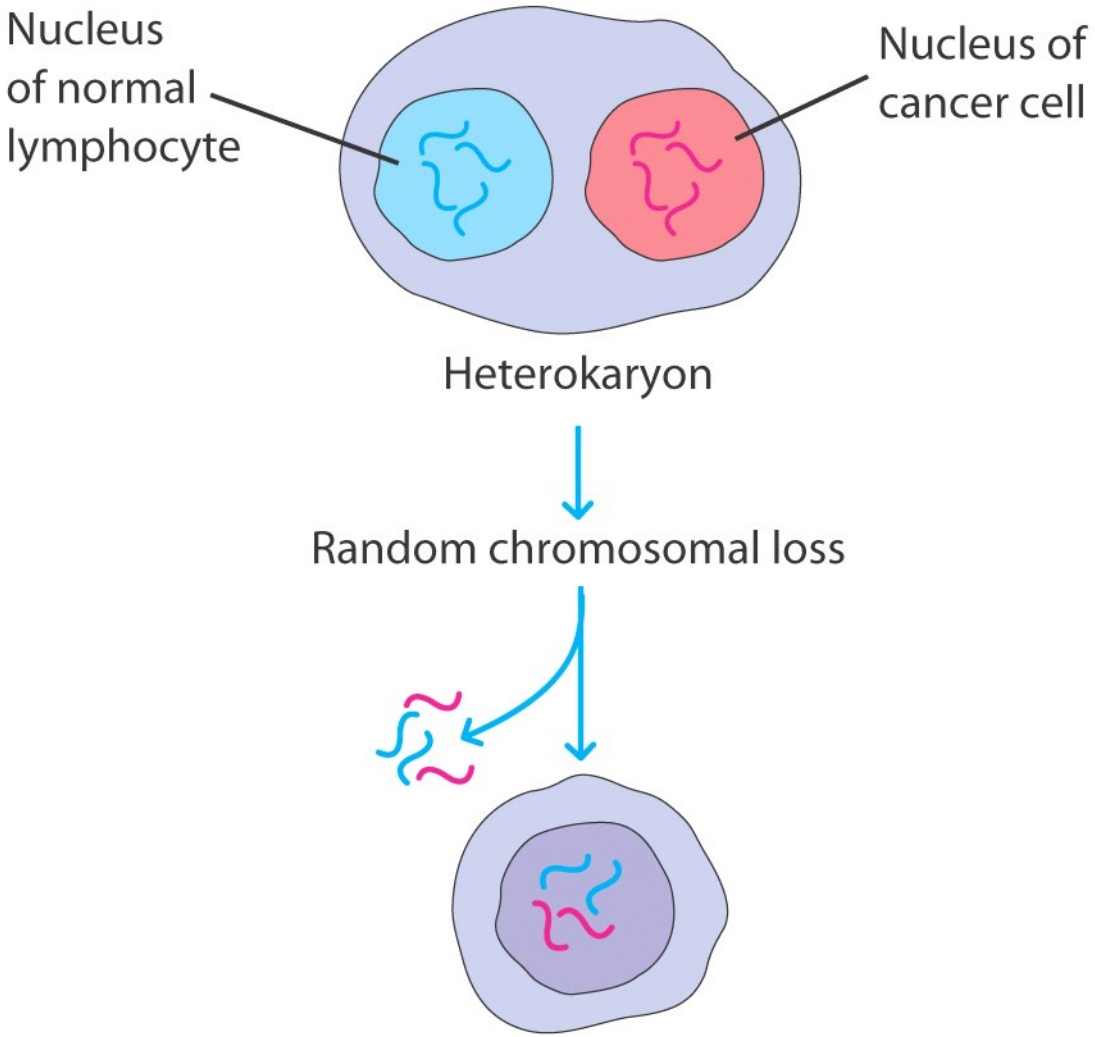
- Balb/c Mouse
- Day 0: 50 μg of Ag In Complete Freund's Adjuvant
- Day 14: 25 μg of Ag In Incomplete Freund's Adjuvant
- Day 28: 25 μg of Ag In D-PBSA
- Bleed Animal Test Reactivity To Antigen
  - Serum Is Diluted 1:30
- Final Boost of 10 μg Antigen i.v or i.p 3 days before fusion

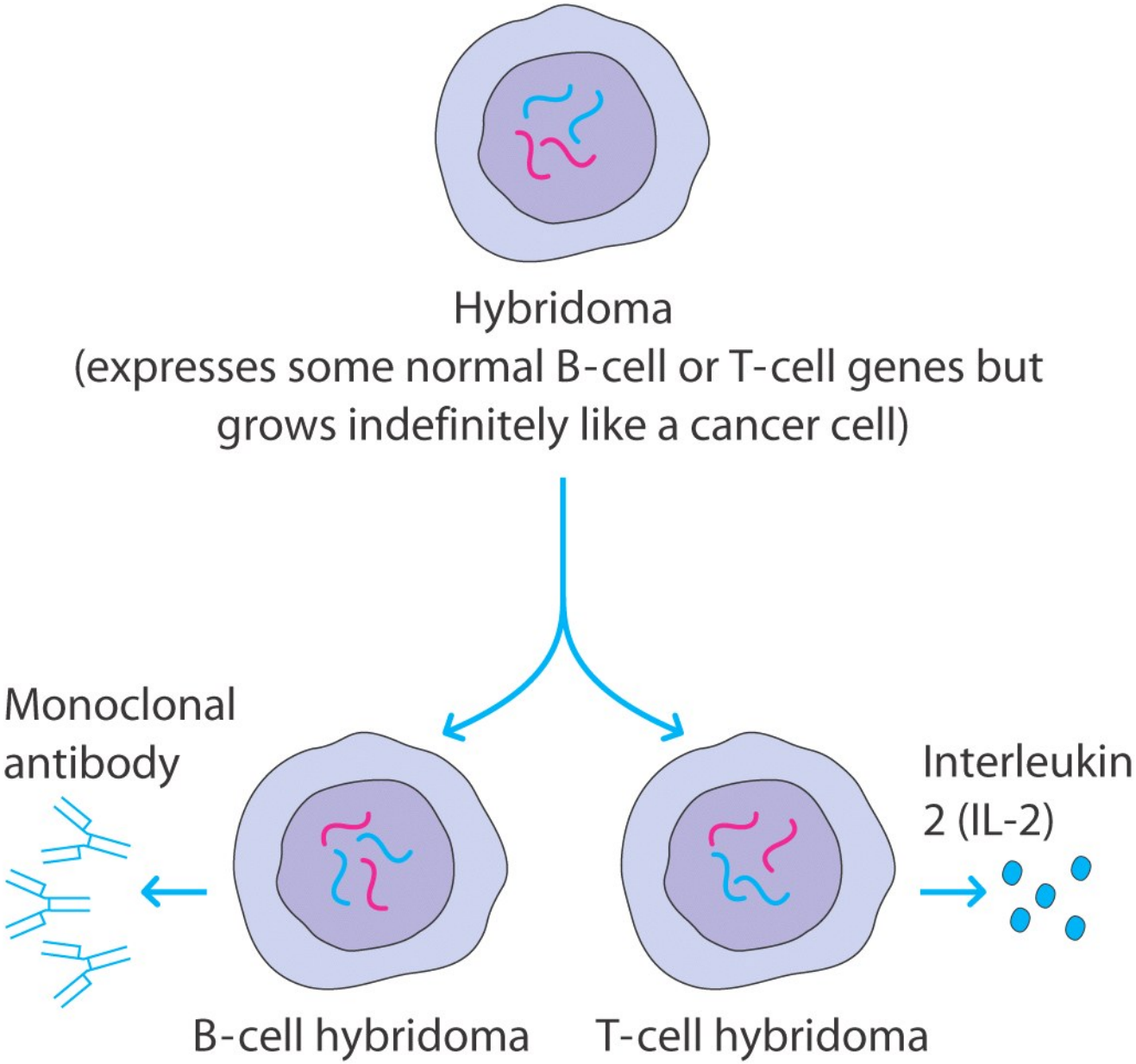
Aseptically Isolate Spleen Cells

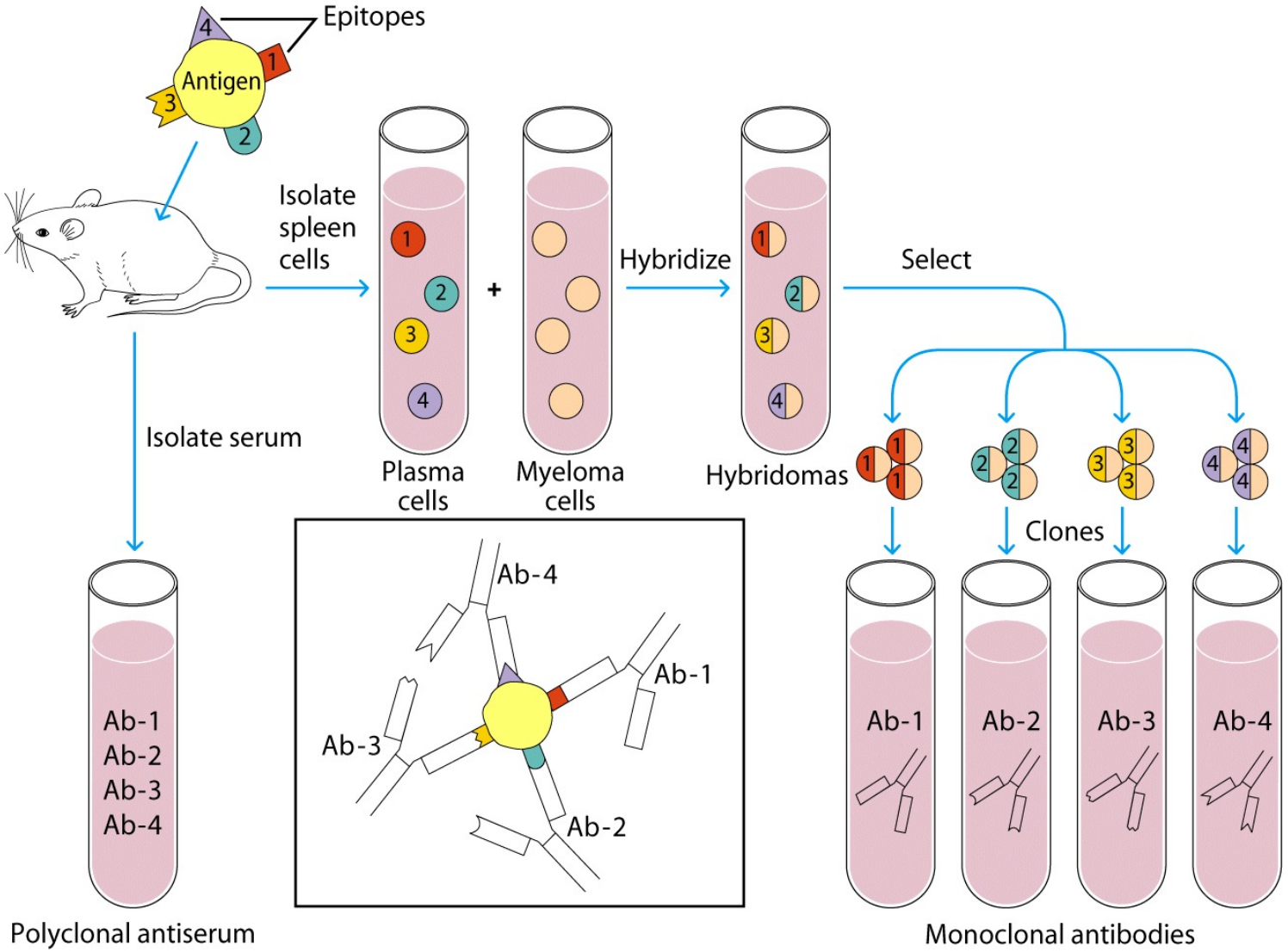
- This Cell Line Is Deficient In HGPRT (hypoxantine guanine phosphoribosyl transferase)
- Alternatively TK (thymidine kinase deficient)
- Cell Line Cannot Survive In Selection Medium
  - Aminopterin Inhibits "*De novo* Pathway", "Salvage Pathway" Is Not Possibe Due To HGPRT or TK Deficiency
- It Is Also Ig Deficient
  - It can not secret any immunoglobulins
- Aminopterin (folic acid antagonist) Blocks *De novo* Pathway
  - P3.653 cells die in the presence of aminopterin
  - They cannot utilize the "salvage pathway" because they are HGPRT deficient


De novo synthesis of purine nucleotides
PRPP (5-Phosphoribosyl-1-pyrophosphate)
Blocked by antifolates
CHO from tetrahydrofolate
Blocked by antifolates
CHO from tetrahydrofolate
De novo synthesis of TMP
Deoxyuridylate (DUMP)
Blocked by antifolates
CH_3 from tetrahydrofolate
Nucleic acids
Guanylate (GMP)
Inosinate (IMP)
Adenylate (AMP)
Nucleic acids
Thymidylate (TMP)
PRPP
HGPRT (hypoxanthine-guanine phosphoribosyl transferase)
HGPRT
PRPP
APRT (adenine phosphoribosyl transferase)
PRPP
TK (thymidine kinase)
Guanine
Hypoxanthine
Adenine
Thymidine
Salvage pathways


Chromosomes
Normal T or B cell (dies after 7–10 days in culture)
Cancerous T or B cell (grows continuously in culture)
Polyethylene glycol
Nucleus of normal lymphocyte
Nucleus of cancer cell
Heterokaryon


Nucleus of normal lymphocyte
Nucleus of cancer cell
Heterokaryon
Random chromosomal loss
Hybridoma
(expresses some normal B-cell or T-cell genes but grows indefinitely like a cancer cell)


Hybridoma
(expresses some normal B-cell or T-cell genes but
grows indefinitely like a cancer cell)
Monoclonal
antibody
Interleukin
2 (IL-2)
B-cell hybridoma
T-cell hybridoma


Epitopes
Antigen
4
1
3
2
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 Antibody Applications

Diagnostic Tests

Abs are capable to detect tiny amouns (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