



# Immunoglobulins

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**Immunoglobulin (Ig) are the critical ingredients at every stage of a humoral acquired immune response. When expressed on the surfaces of resting B lymphocytes, they serve as receptors that can detect and distinguish among the vast array of potential antigens present in an environment.**

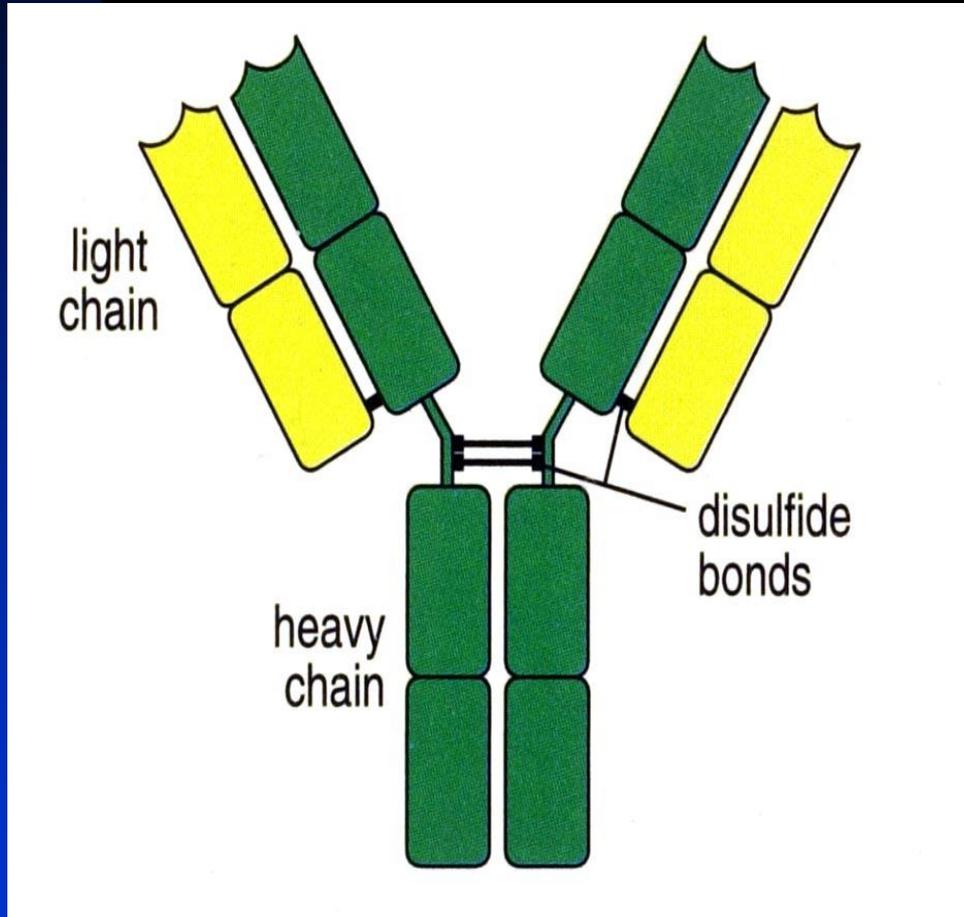


The **Ig** that secreted as a result then **function as** antibodies, traveling through the tissue fluids to seek out and bind to the specific **antigens** that **triggered** their production.



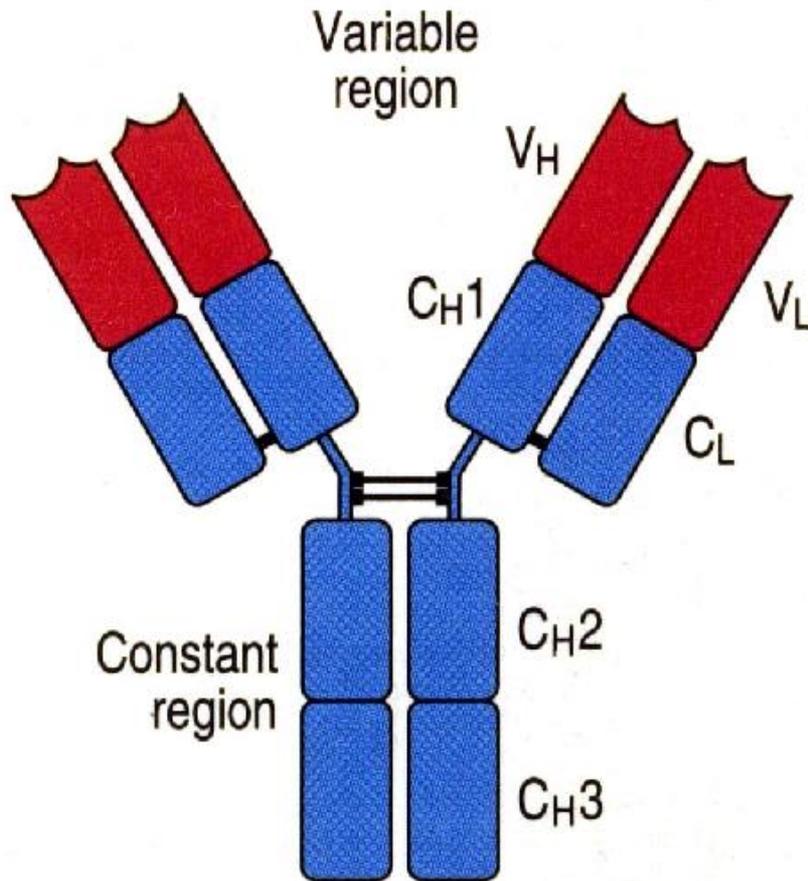
Figure 3-1a Immunobiology, 7ed. (© Garland Science 2008)

# Antibody structure



- Two heavy chains
- Two light chains
- \* H chain---50KD
- \* L chain---25KD

# Antibody structure?



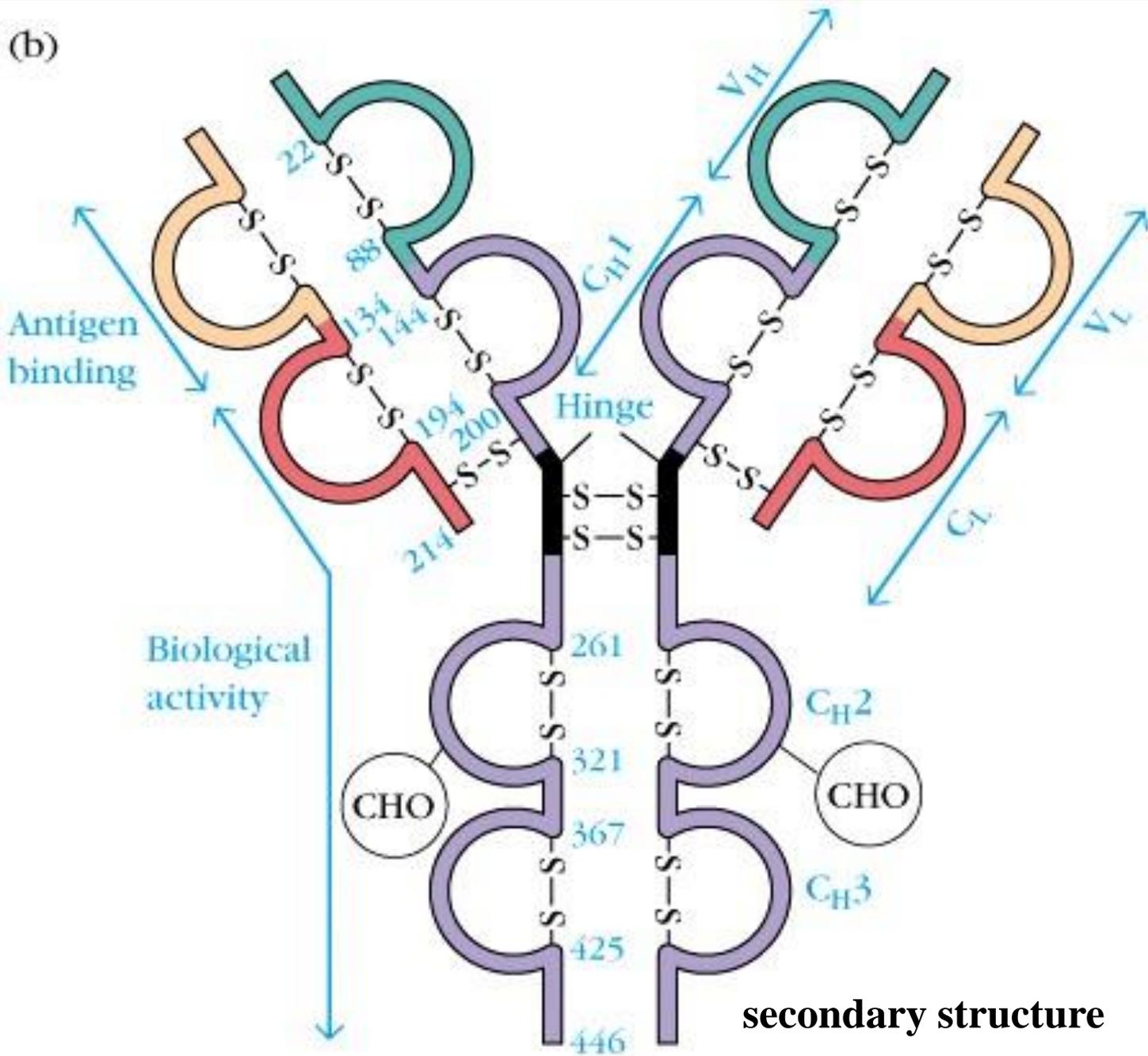
## V region (domain)

Regions vary greatly in amino acid sequence in different Abs, involved in Ag recognition.

**C region (domain)** relatively constant in sequence, engaging the effector functions.



(b)



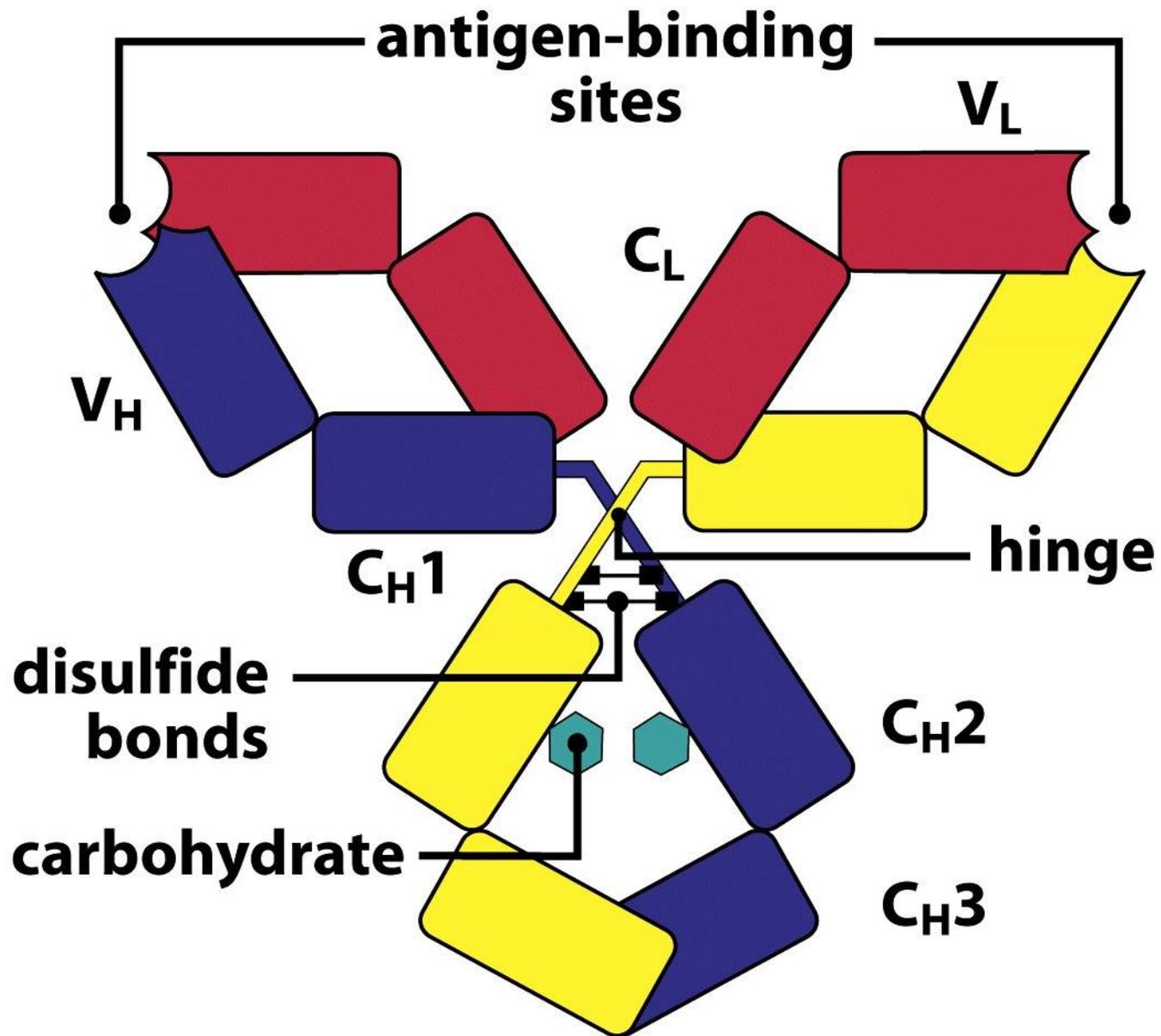
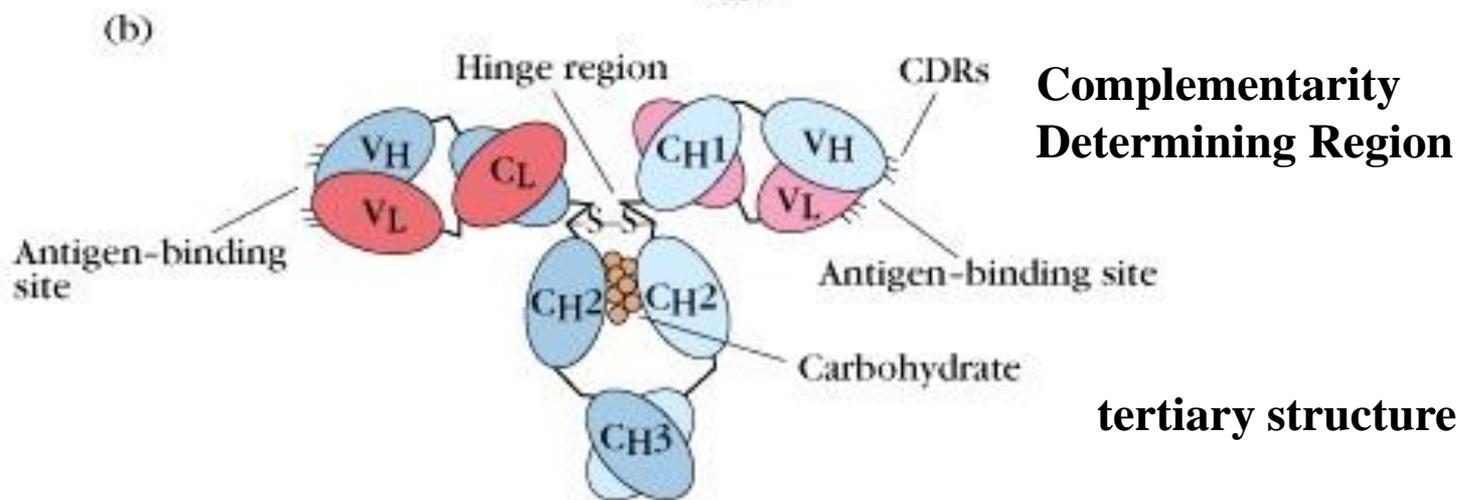
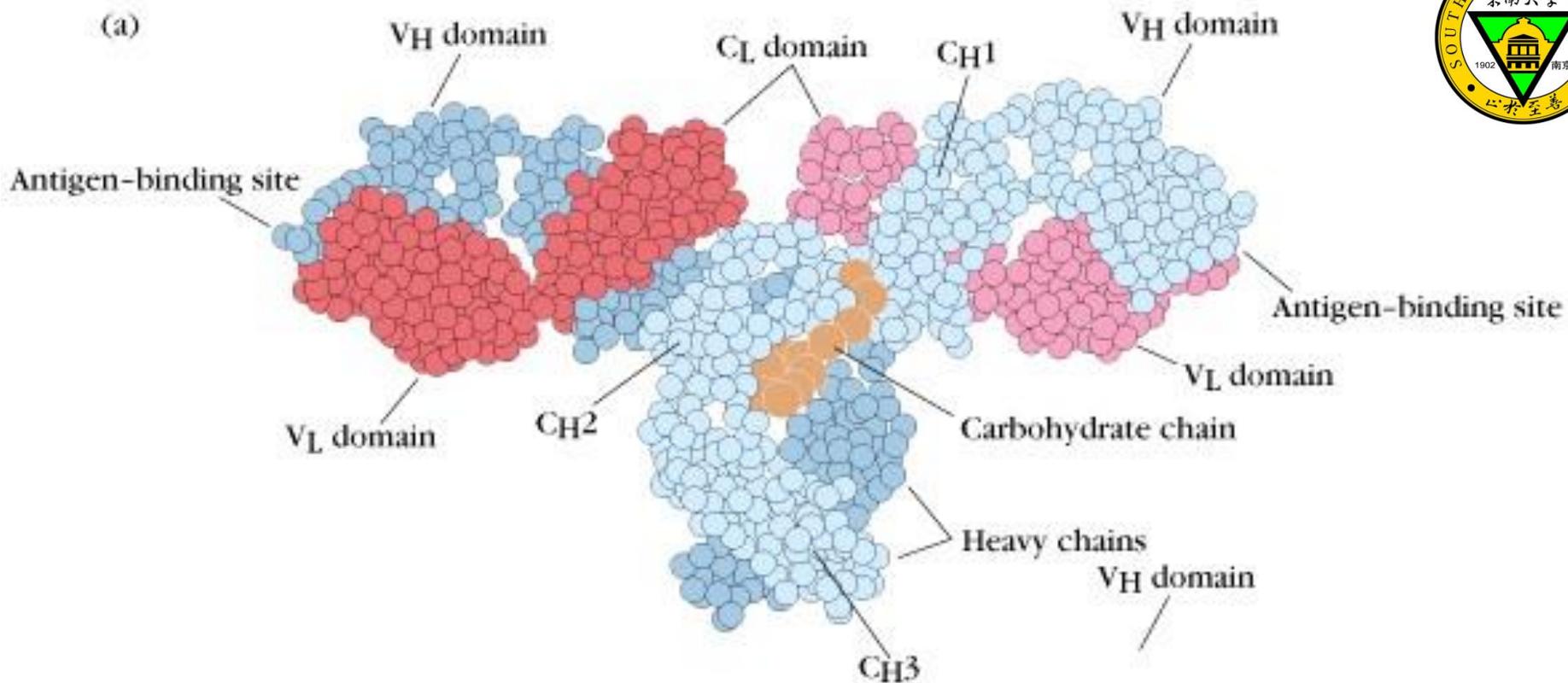
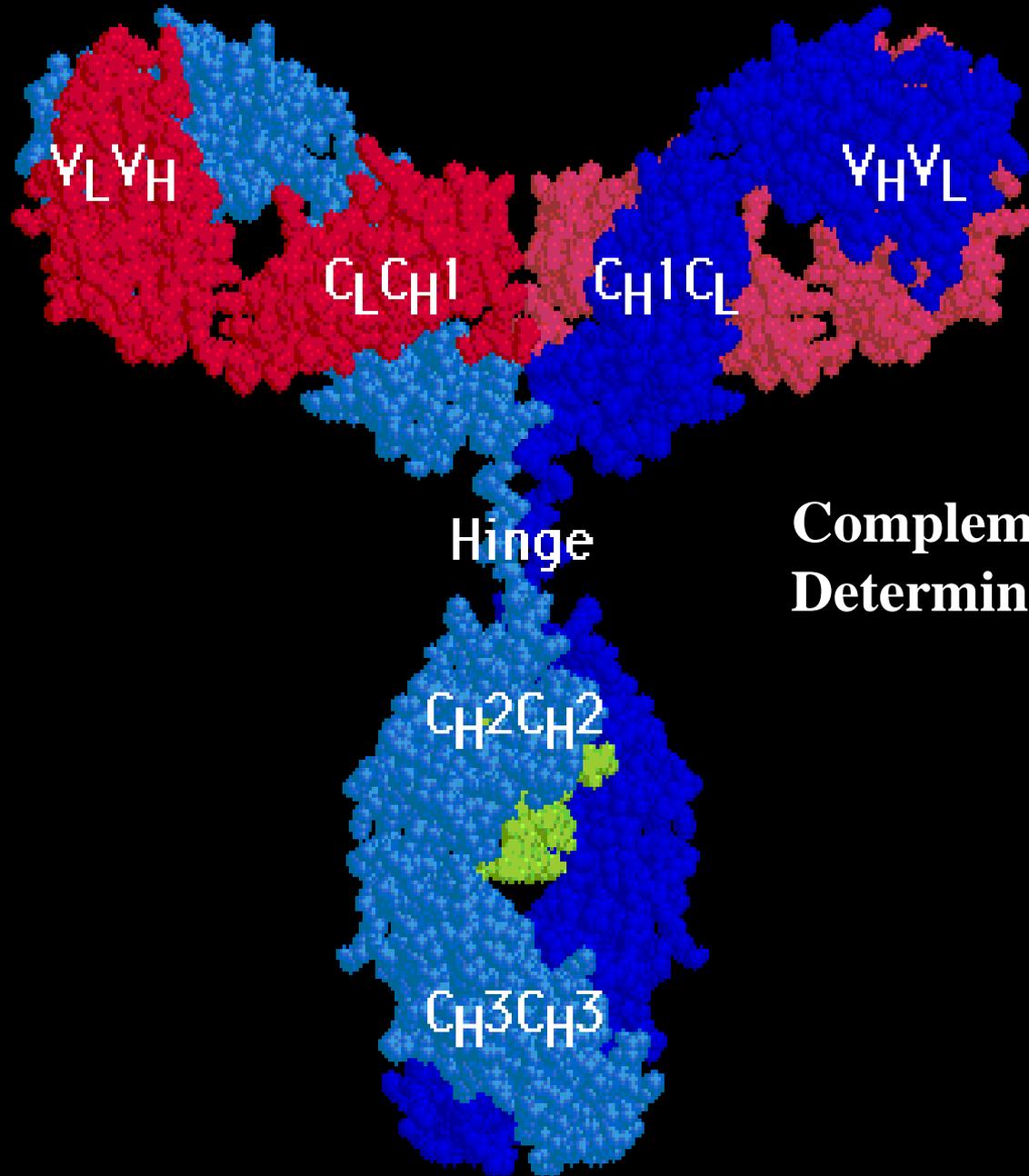


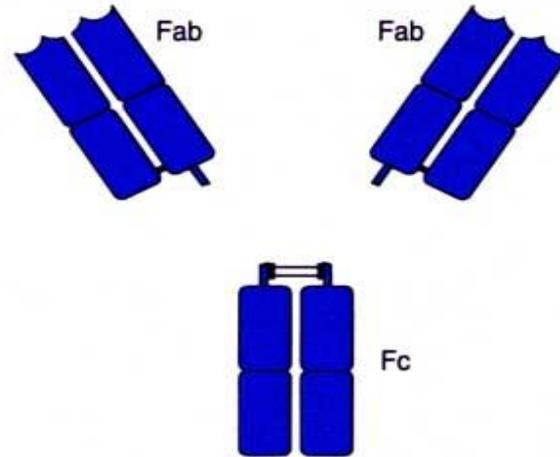
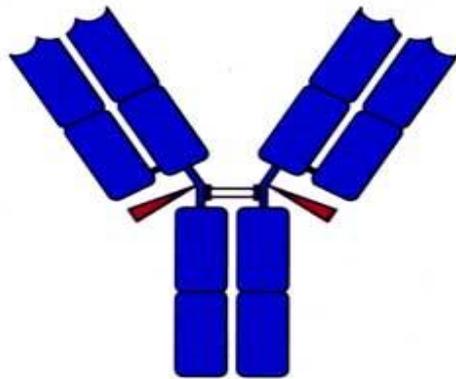
Figure 3-1b Immunobiology, 7ed. (© Garland Science 2008)



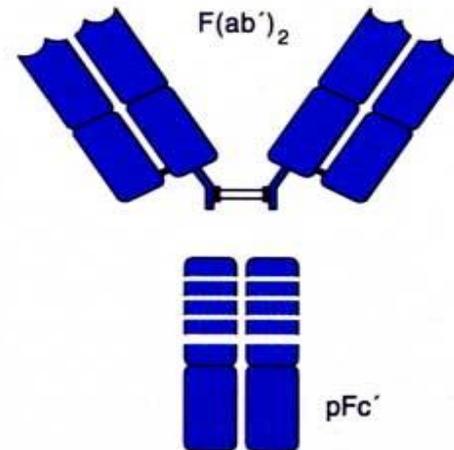
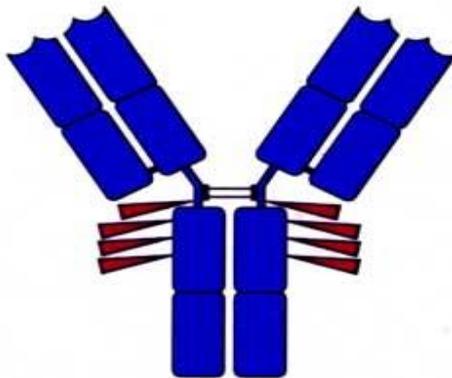


# Antibody structure

Proteolytic cleavage by papain



Proteolytic cleavage by pepsin



hydrolysis

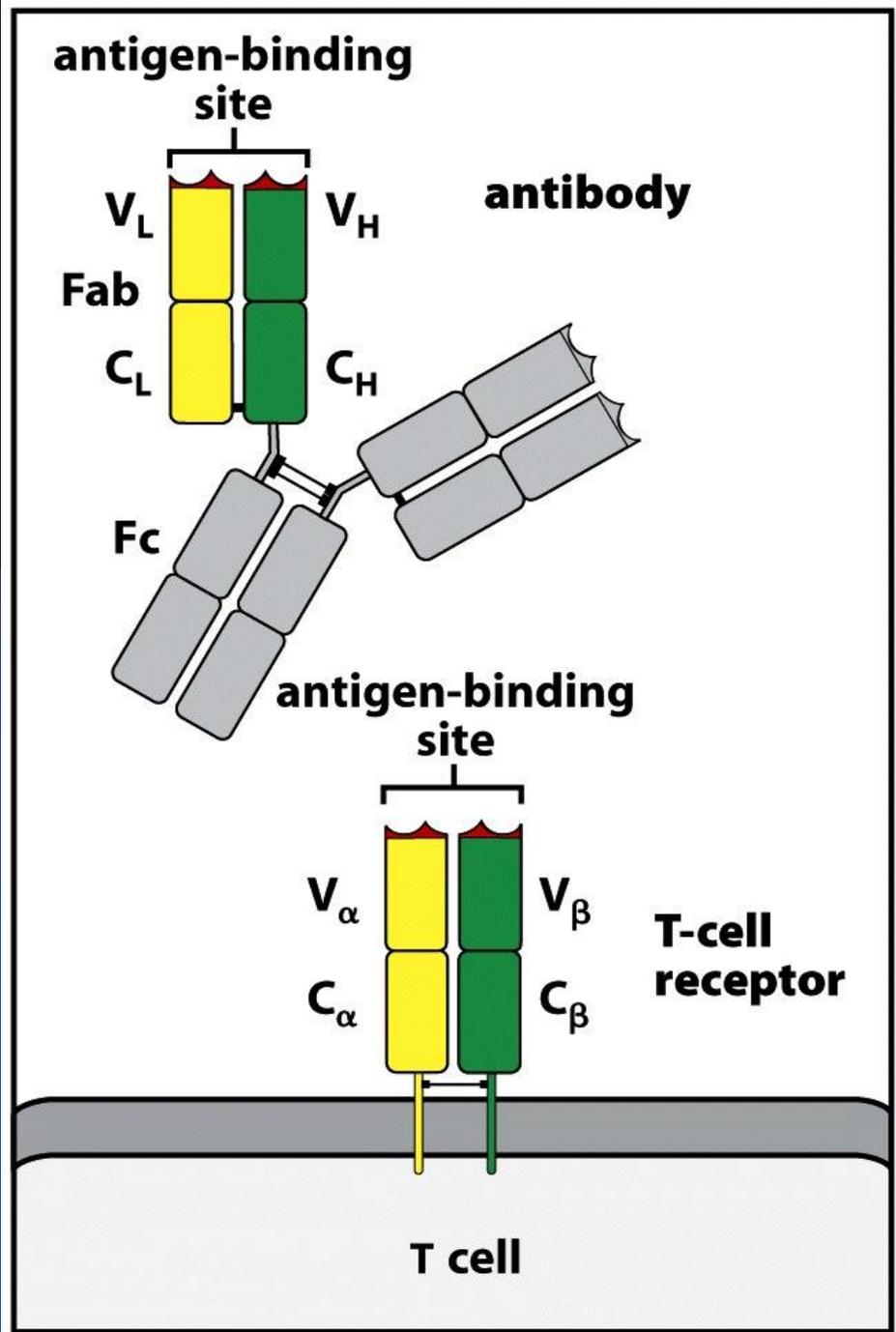


Figure 3-11 Immunobiology, 7ed. (© Garland Science 2008)

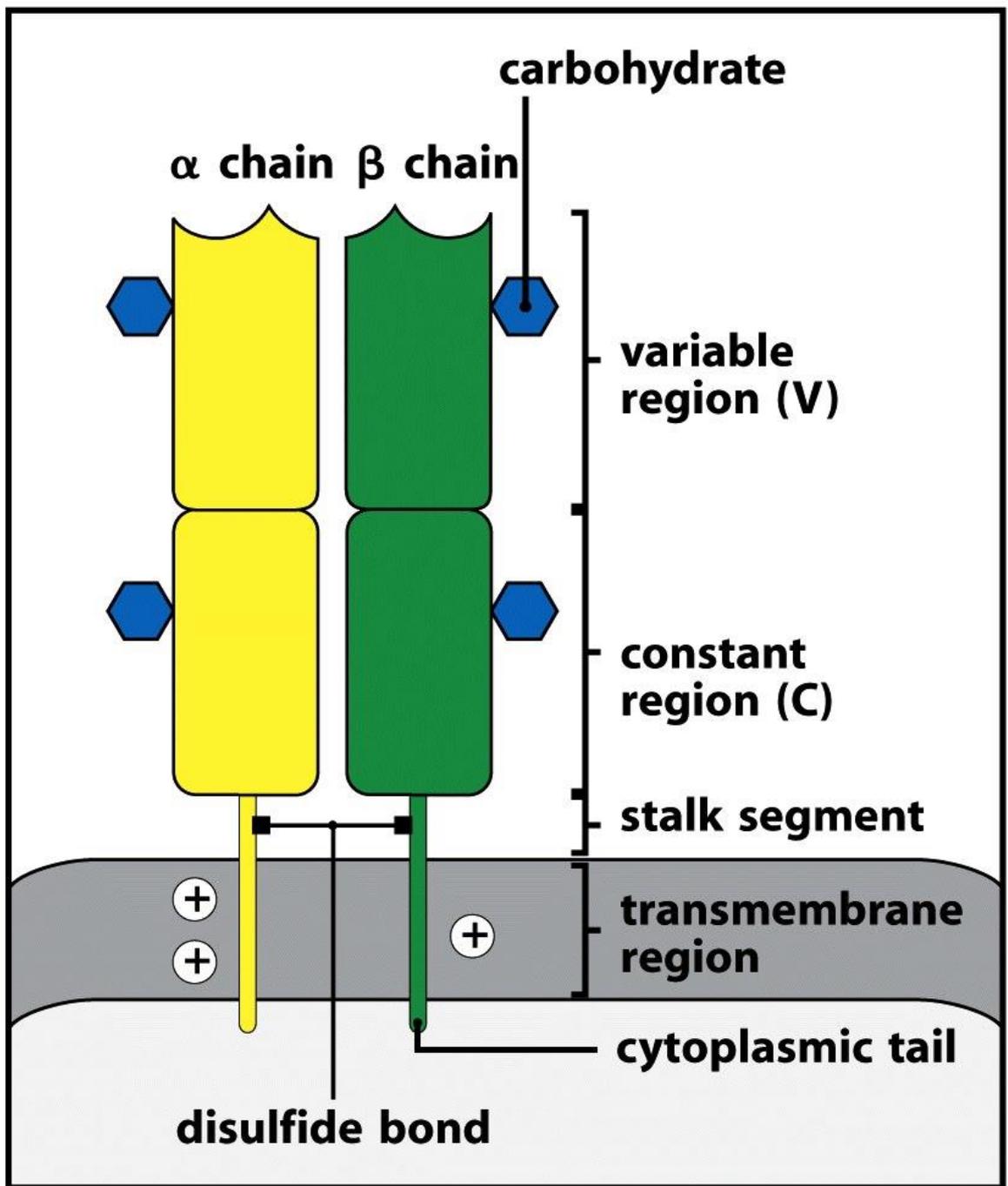
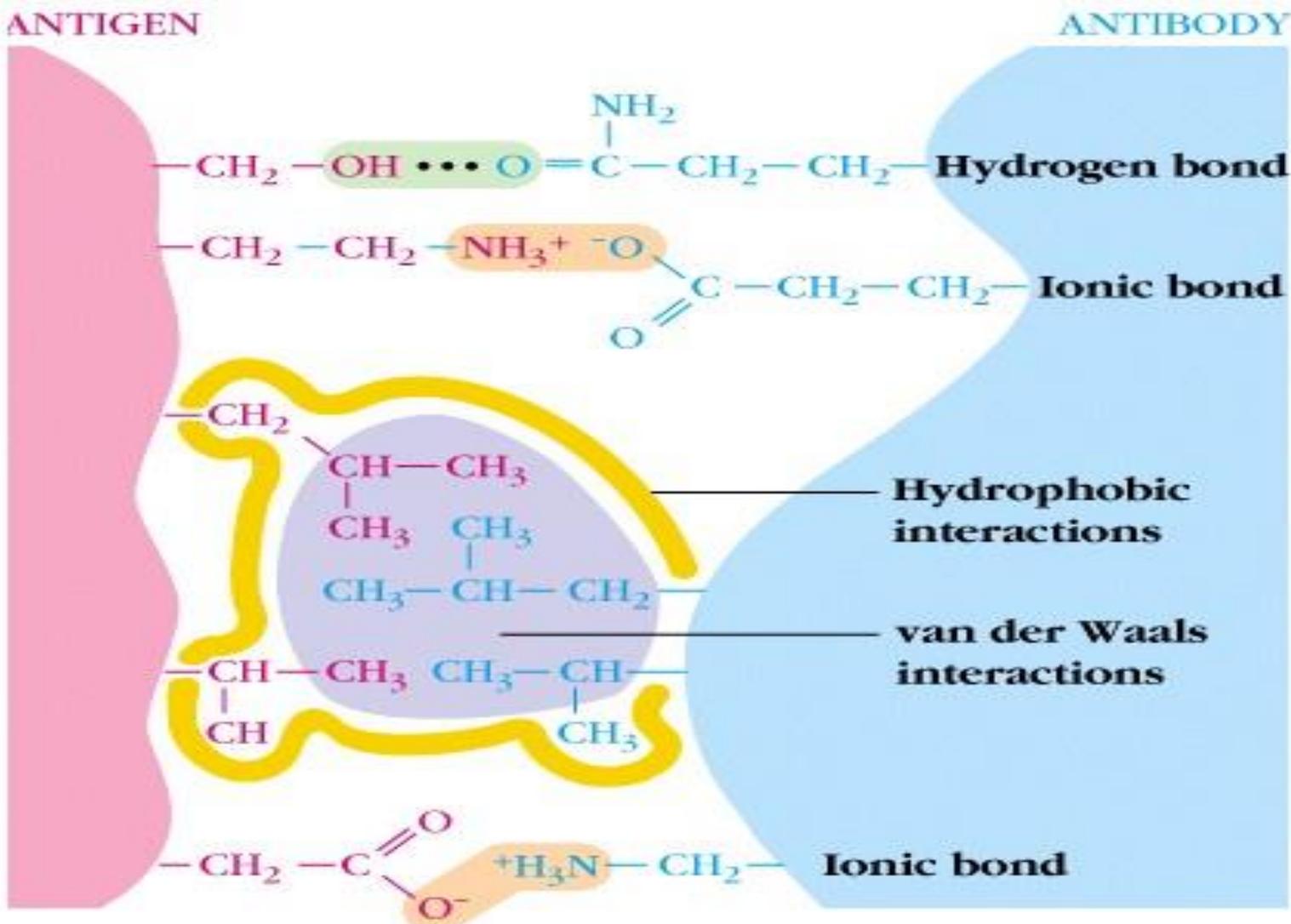


Figure 3-12 Immunobiology, 7ed. (© Garland Science 2008)

# Antigen Antibody Interactions



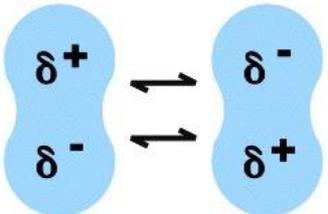
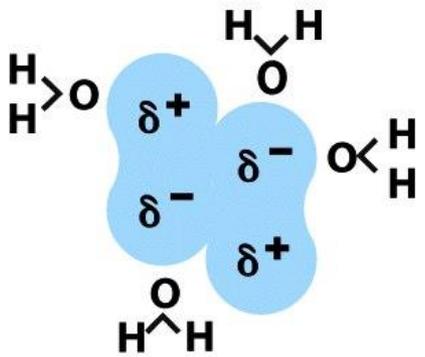
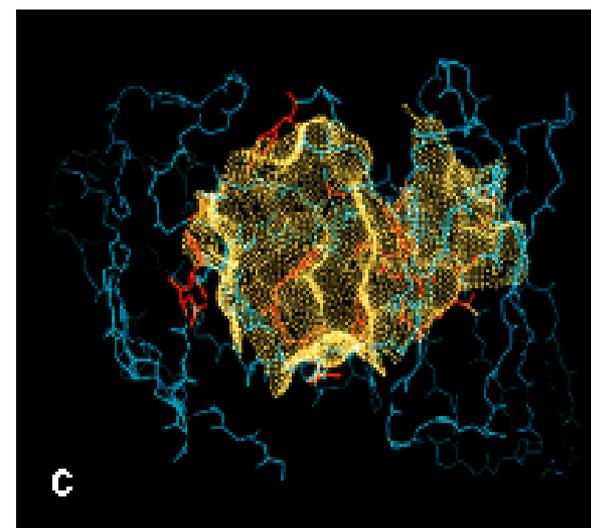
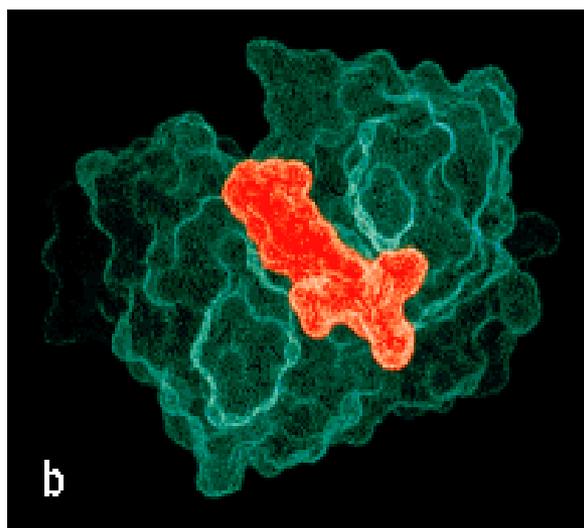
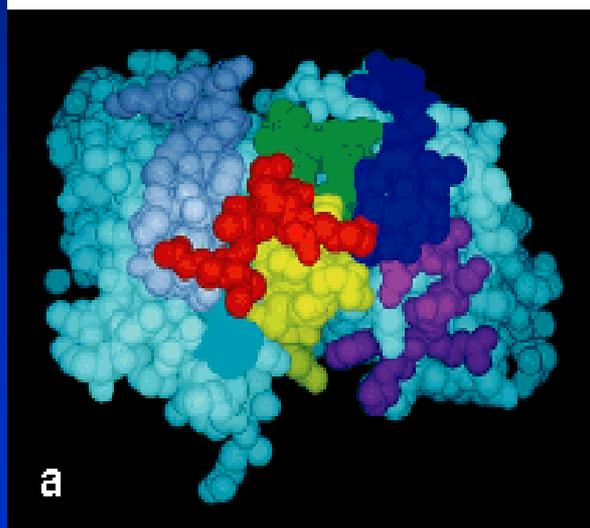
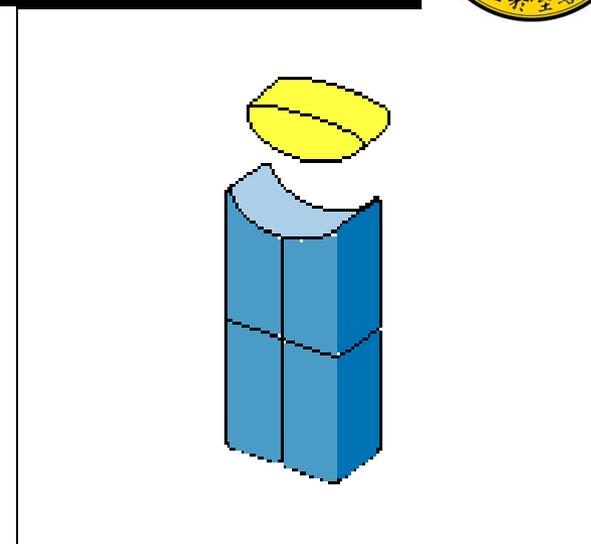
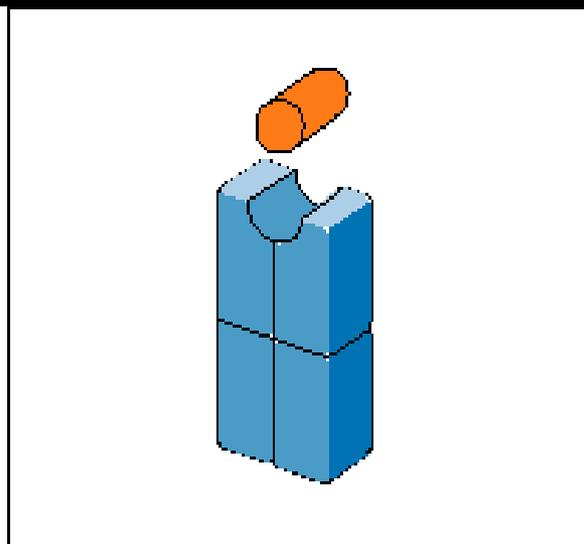
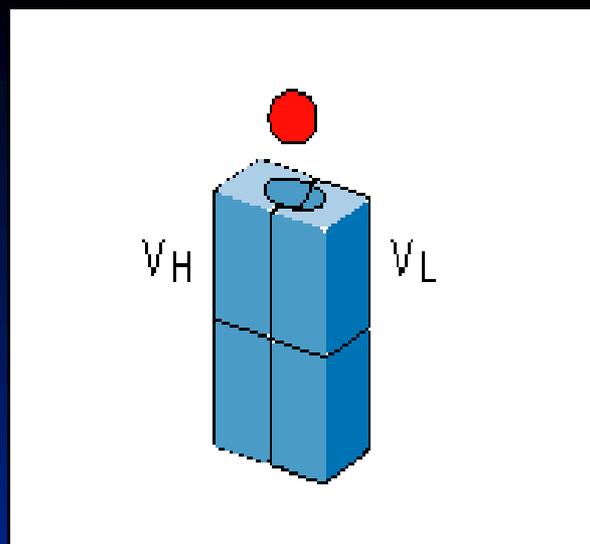
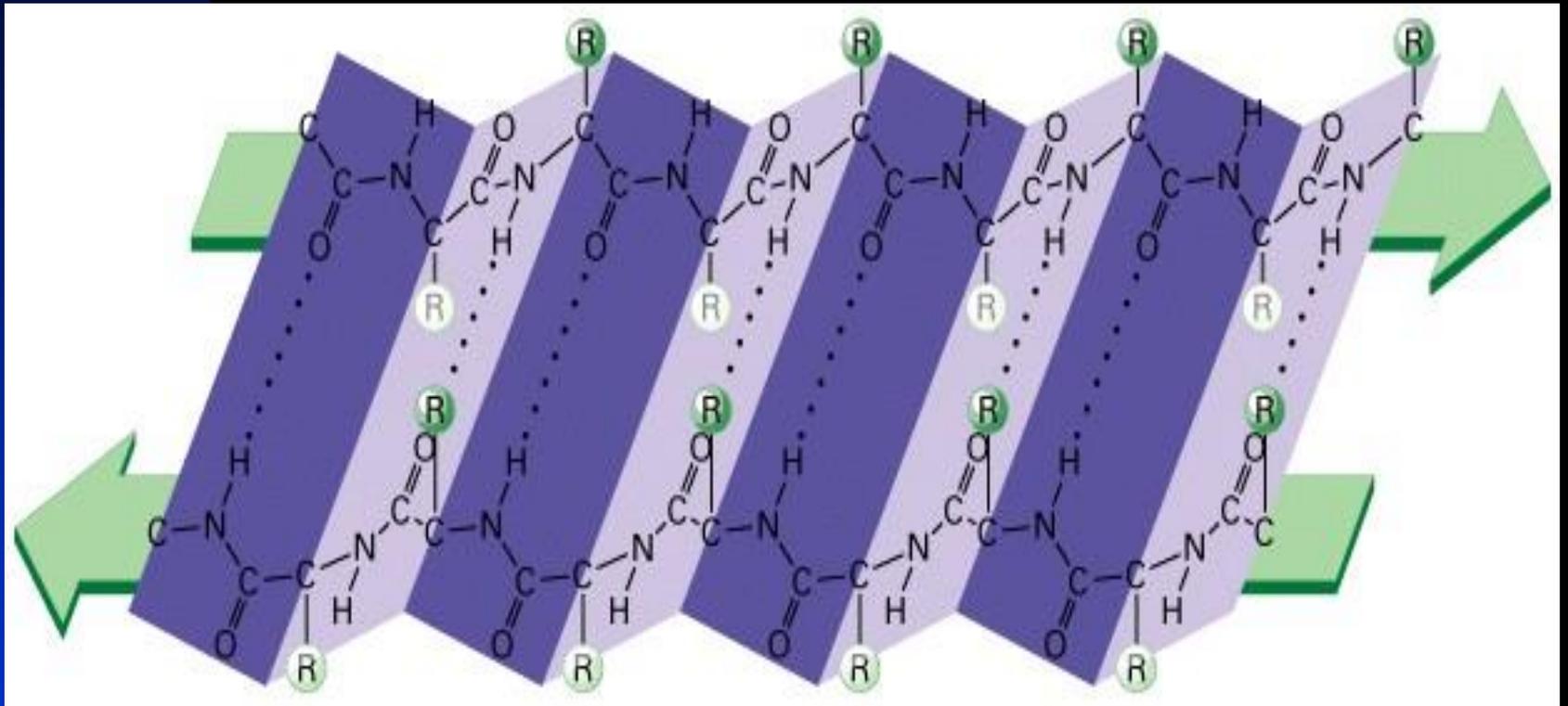
Noncovalent forces	Origin	
Electrostatic forces	Attraction between opposite charges	$-\overset{\oplus}{\text{N}}\text{H}_3 \quad \overset{\ominus}{\text{O}}\text{OC}-$
Hydrogen bonds	Hydrogen shared between electronegative atoms (N,O)	$\begin{array}{c} \diagdown \text{N} - \text{H} - - \text{O} = \text{C} \diagdown \\ \delta^- \quad \delta^+ \quad \delta^- \end{array}$
Van der Waals forces	Fluctuations in electron clouds around molecules oppositely polarize neighboring atoms	
Hydrophobic forces	Hydrophobic groups interact unfavorably with water and tend to pack together to exclude water molecules. The attraction also involves van der Waals forces	

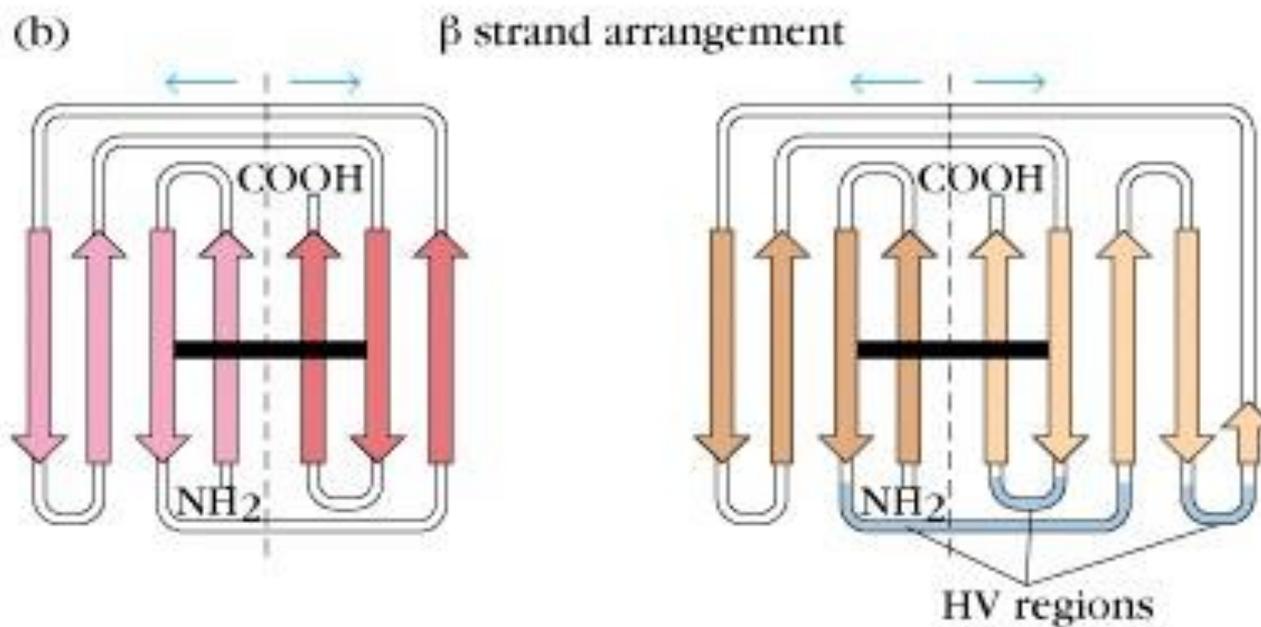
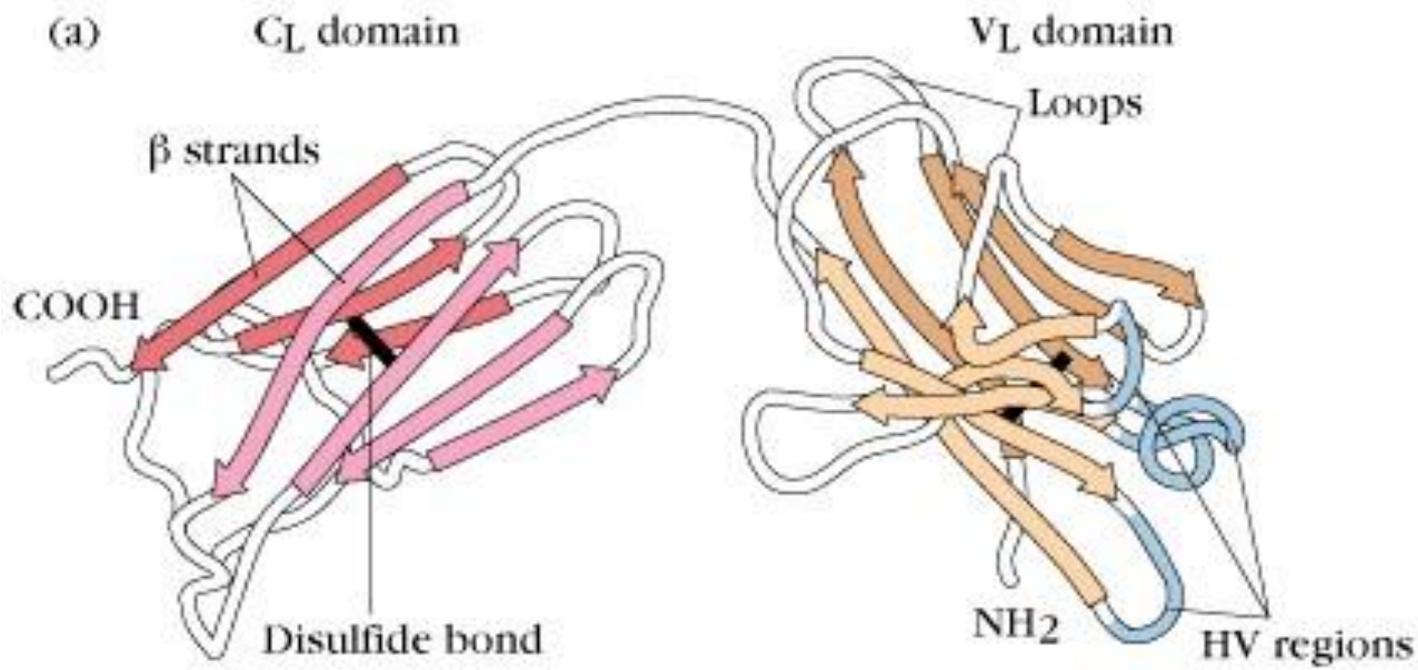
Figure 3-9 Immunobiology, 7ed. (© Garland Science 2008)

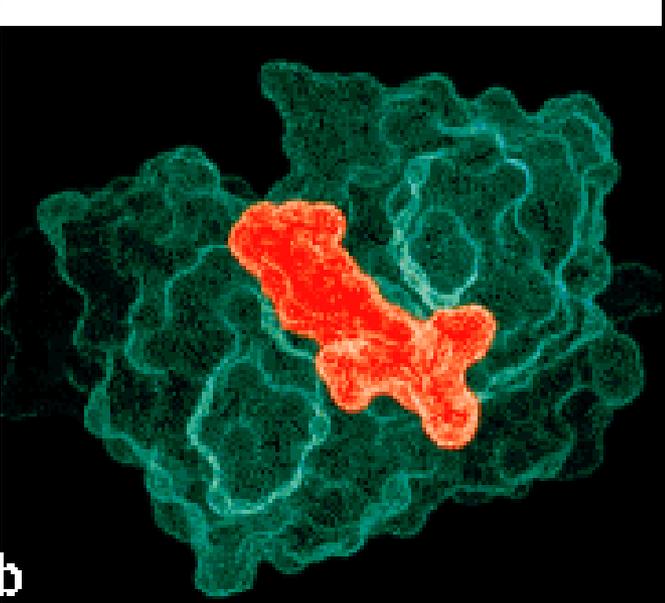
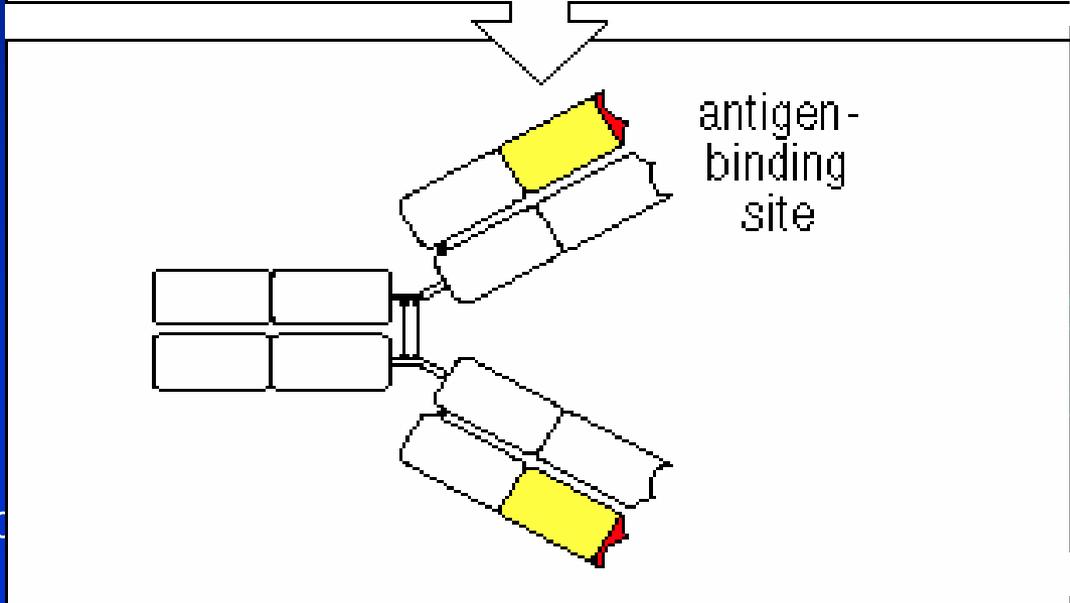
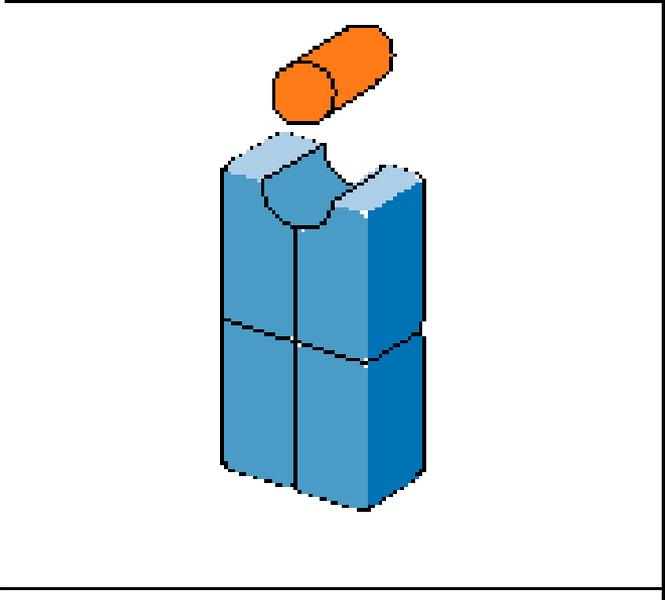
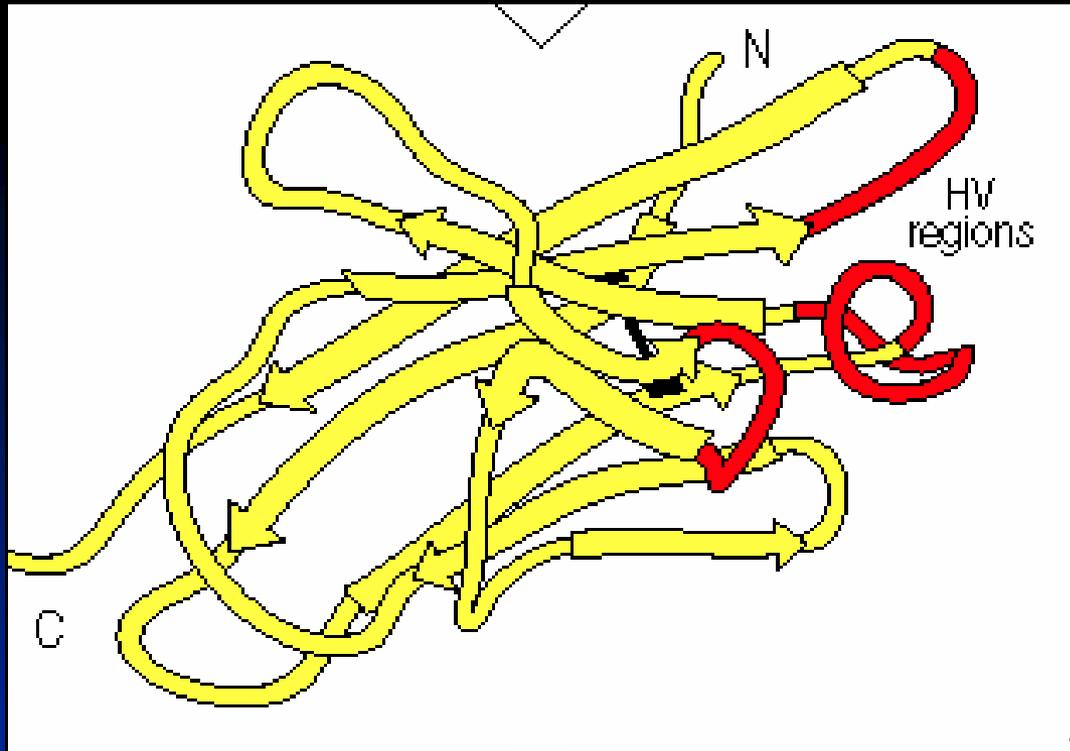


# The Sheet

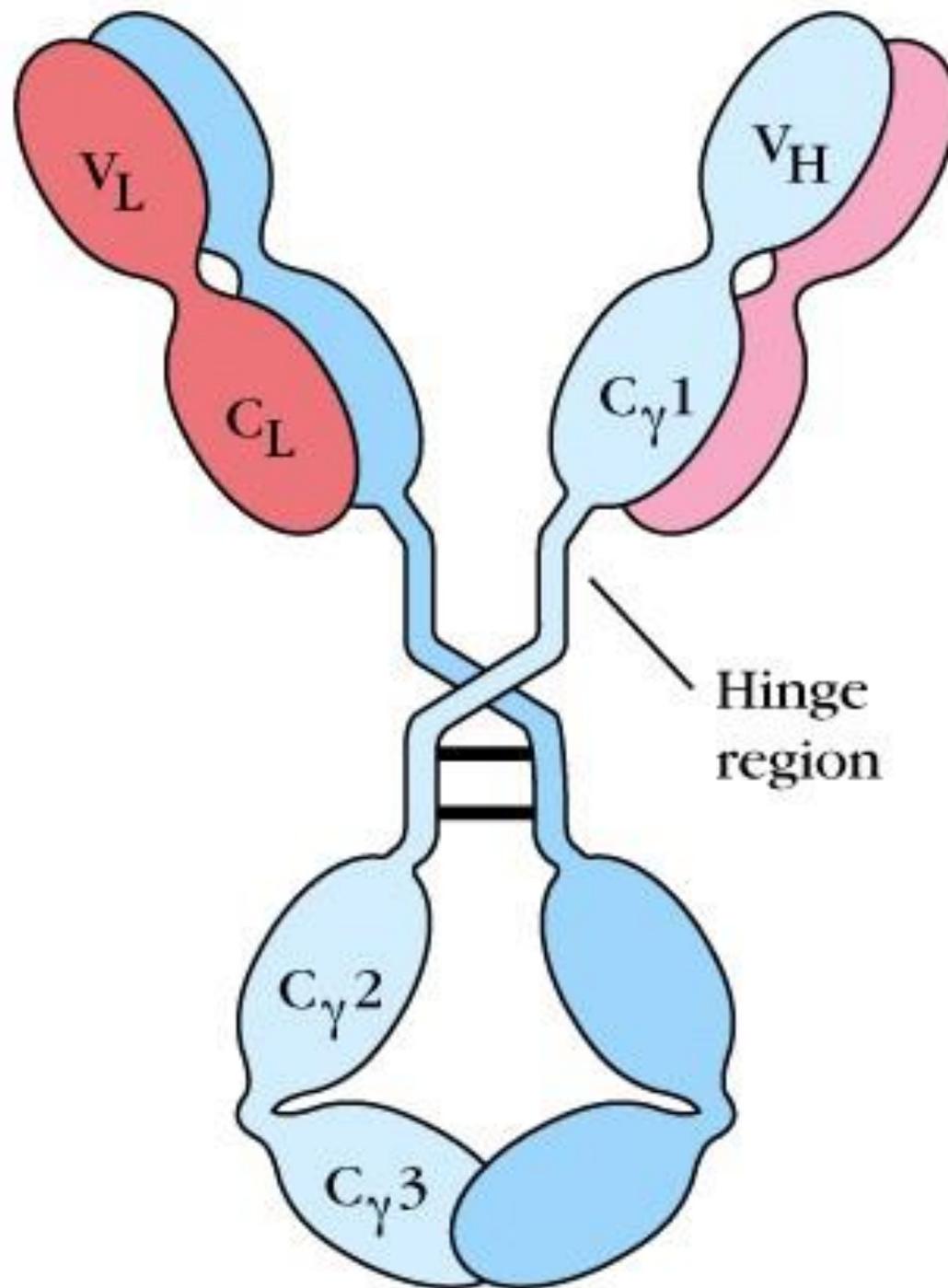


## Anti-parallel $\beta$ Sheet

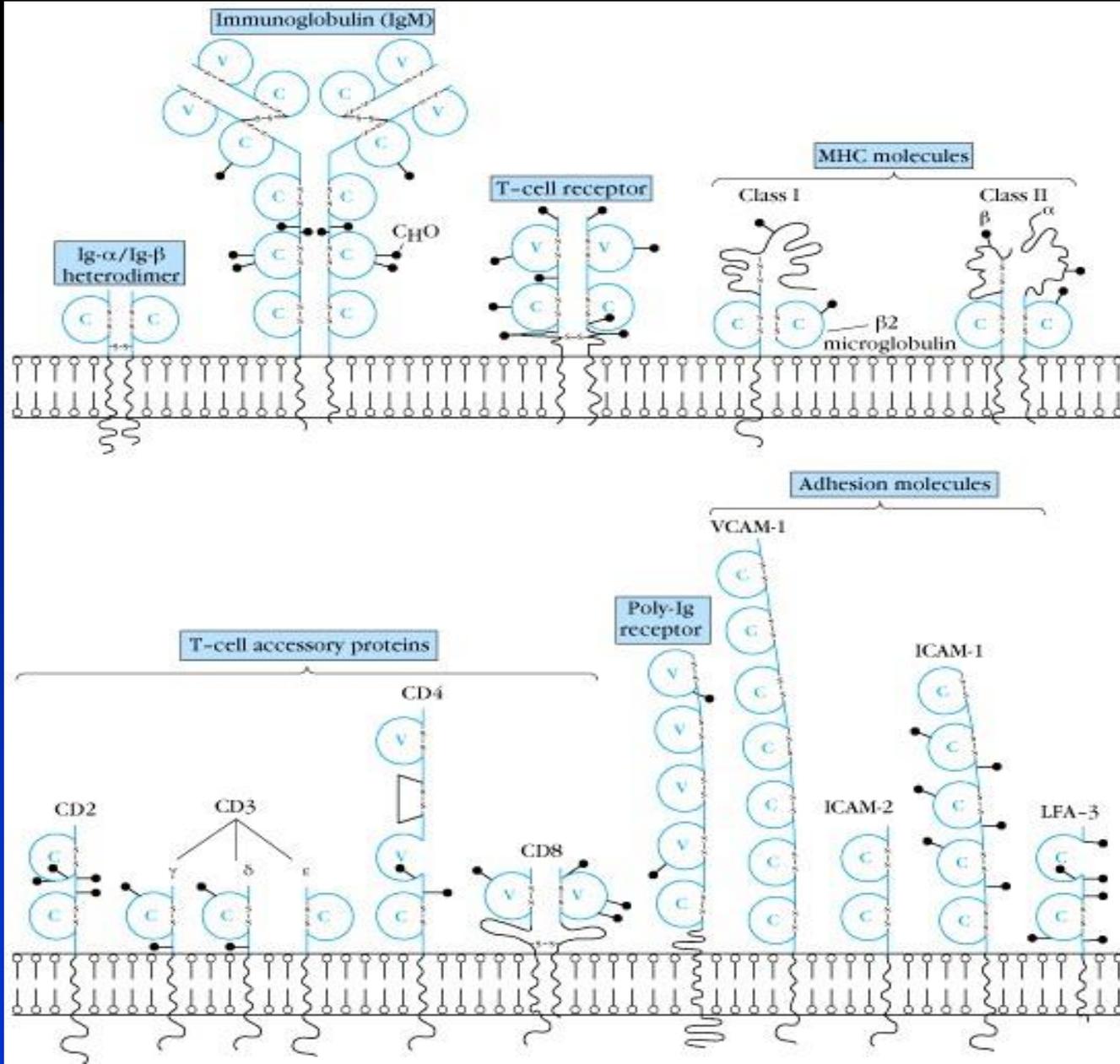


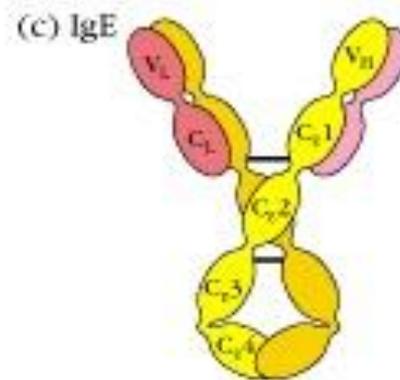
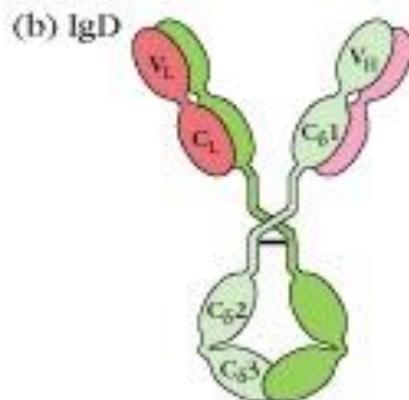
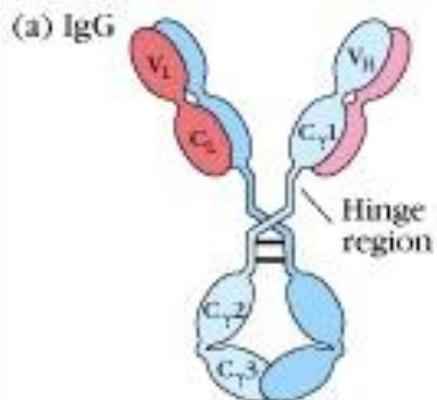


(a) IgG



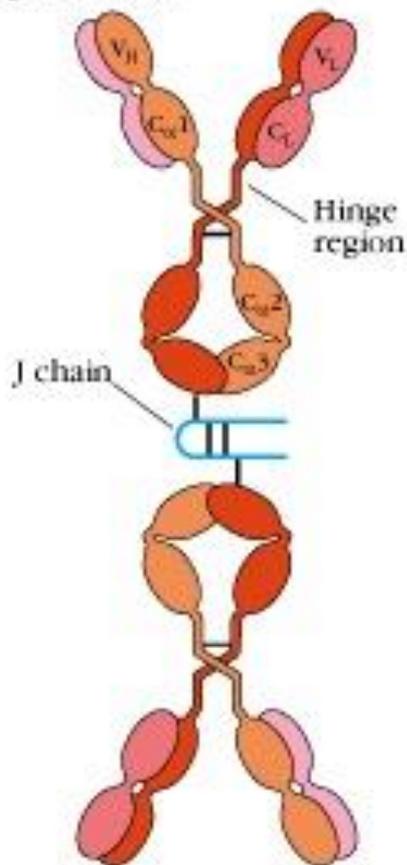
# Immunoglobulin Supergene Family.



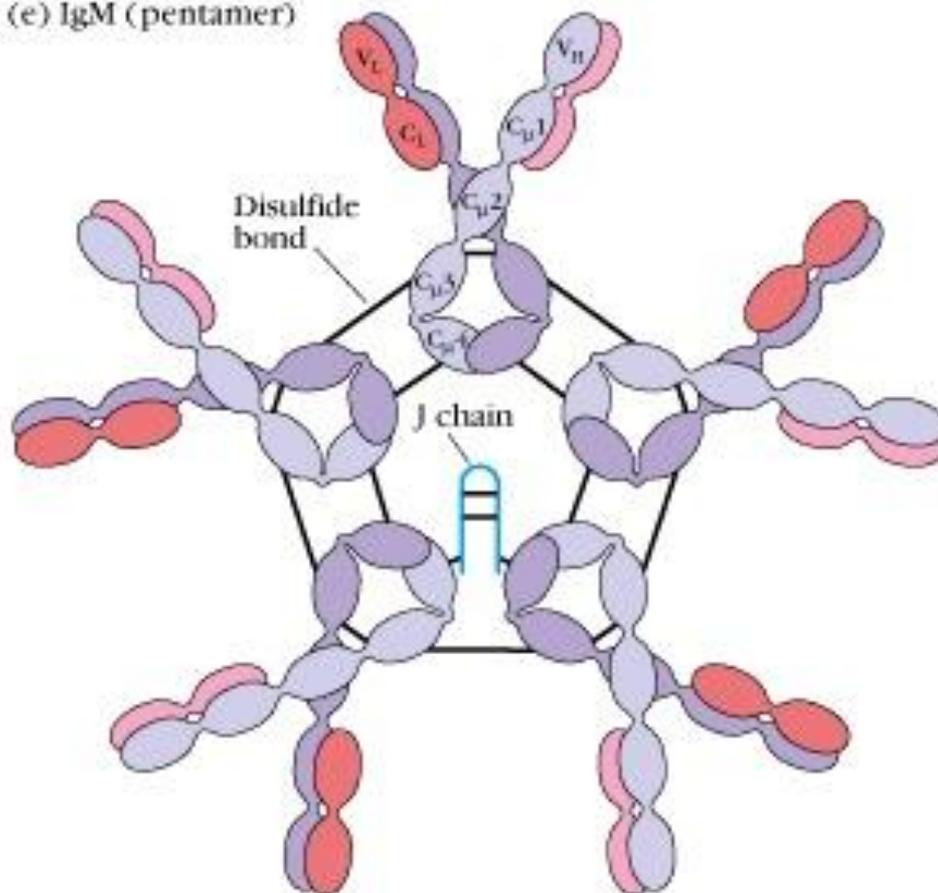


*monomer*

(d) IgA (dimer)

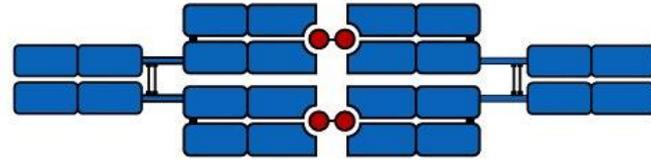


(e) IgM (pentamer)

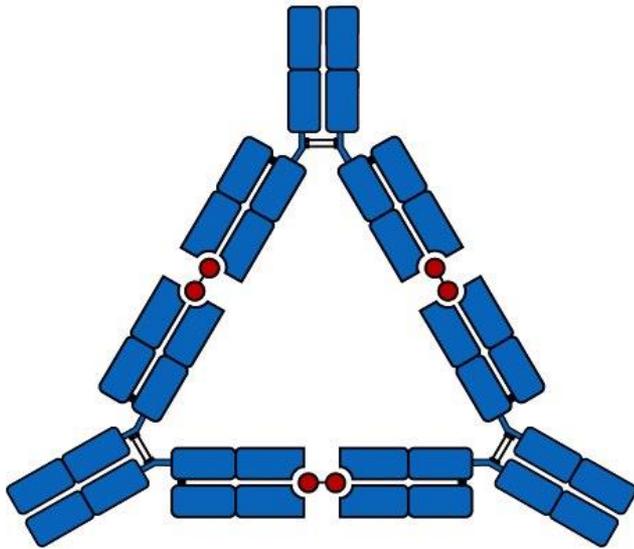




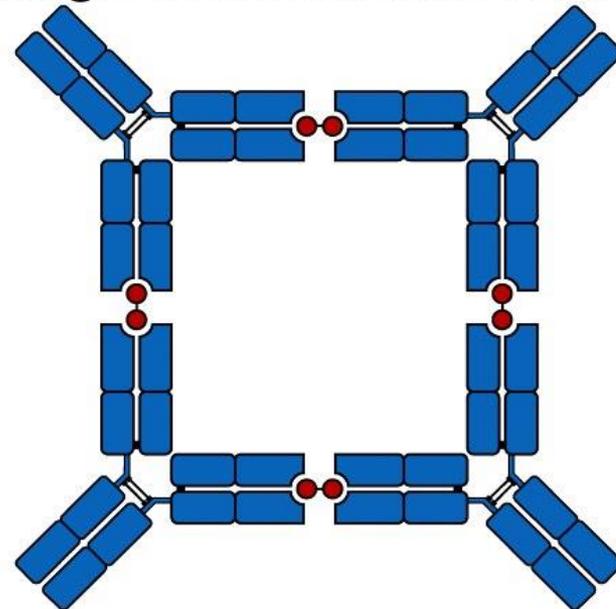
Angle between arms is  $0^\circ$



Angle between arms is  $60^\circ$



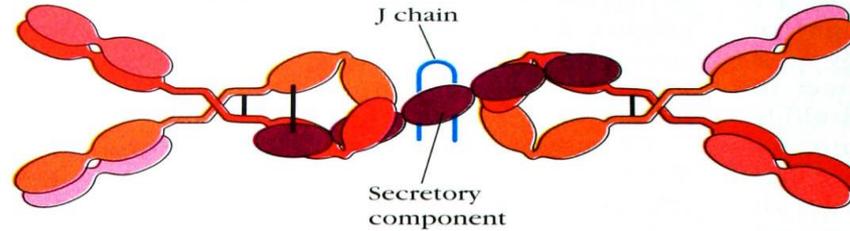
Angle between arms is  $90^\circ$



# Generation of Secretory Immunoglobulins

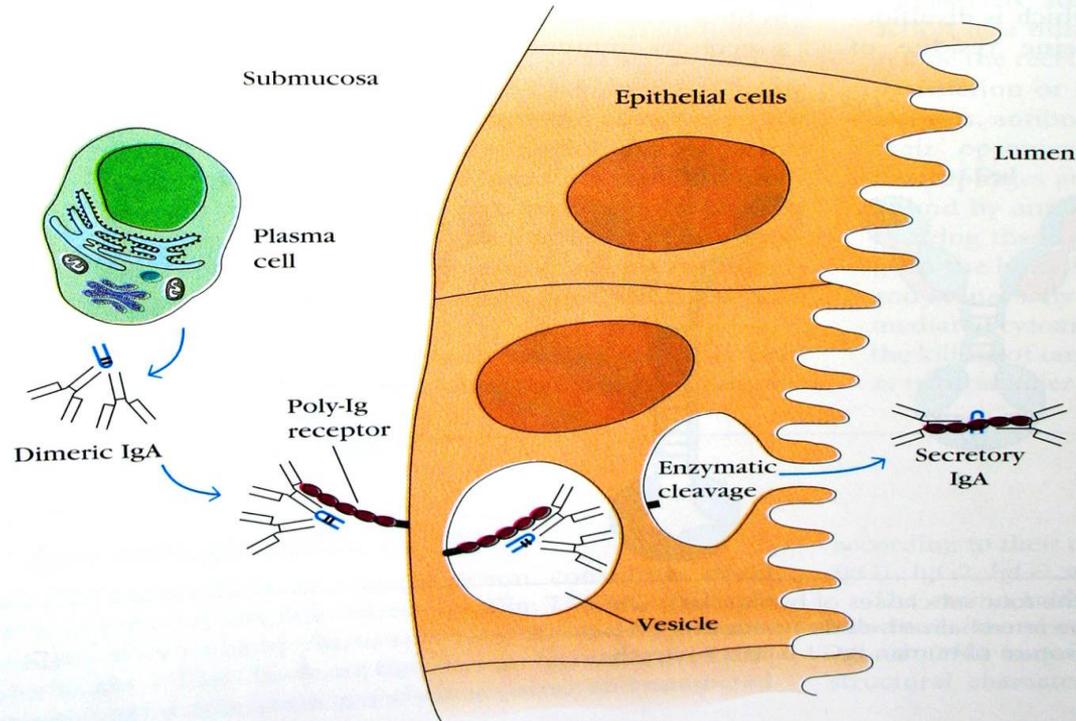
IgA

(a) Structure of secretory IgA



dimer

(b) Formation of secretory IgA





**TABLE 4-3 IMMUNE BENEFITS OF BREAST MILK**

Antibodies of secretory IgA class	Bind to microbes in baby's digestive tract and thereby prevent their attachment to the walls of the gut and their subsequent passage into the body's tissues.
B <sub>12</sub> binding protein	Reduces amount of vitamin B <sub>12</sub> , which bacteria need in order to grow.
Bifidus factor	Promotes growth of <i>Lactobacillus bifidus</i> , a harmless bacterium, in baby's gut. Growth of such nonpathogenic bacteria helps to crowd out dangerous varieties.
Fatty acids	Disrupt membranes surrounding certain viruses and destroy them.
Fibronectin	Increases antimicrobial activity of macrophages; helps to repair tissues that have been damaged by immune reactions in baby's gut.
Hormones and growth factors	Stimulate baby's digestive tract to mature more quickly. Once the initially "leaky" membranes lining the gut mature, infants become less vulnerable to microorganisms.
Interferon (IFN- $\gamma$ )	Enhances antimicrobial activity of immune cells.
Lactoferrin	Binds to iron, a mineral many bacteria need to survive. By reducing the available amount of iron, lactoferrin thwarts growth of pathogenic bacteria.
Lysozyme	Kills bacteria by disrupting their cell walls.
Mucins	Adhere to bacteria and viruses, thus keeping such microorganisms from attaching to mucosal surfaces.
Oligosaccharides	Bind to microorganisms and bar them from attaching to mucosal surfaces.

SOURCE: Adapted from J Newman, 1995, How breast milk protects newborns, *Sci. Am.* 273(6): 76.

**TABLE 4-2 PROPERTIES AND BIOLOGICAL ACTIVITIES\*  
OF CLASSES AND SUBCLASSES OF HUMAN SERUM IMMUNOGLOBULINS**



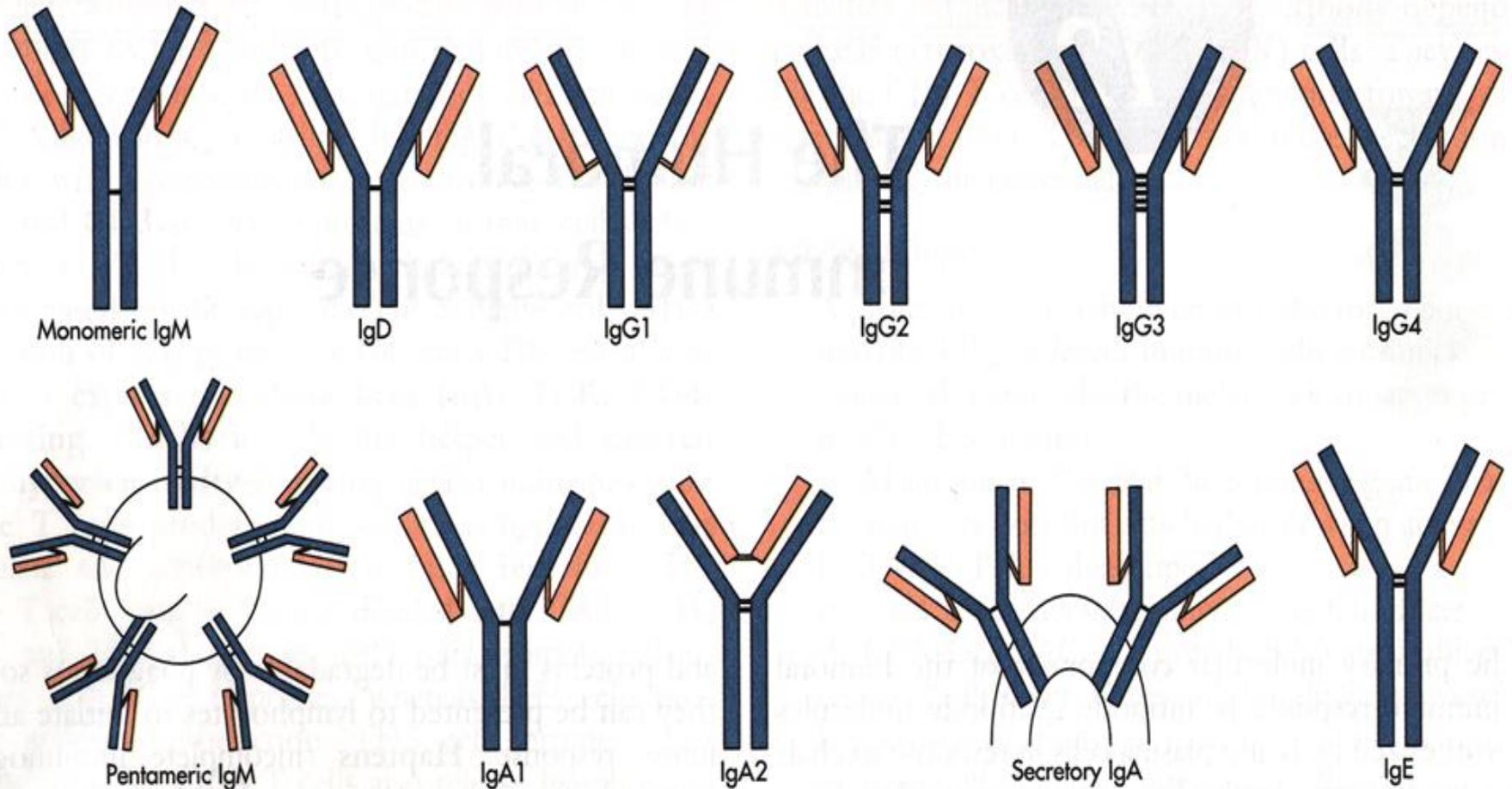
Property/Activity	IgG1	IgG2	IgG3	IgG4	IgA1	IgA2	IgM <sup>†</sup>	IgE	IgD
Molecular weight <sup>‡</sup>	150,000	150,000	150,000	150,000	150,000– 600,000	150,000– 600,000	900,000	190,000	150,000
Heavy-chain component	γ1	γ2	γ3	γ4	α1	α2	μ	ε	δ
Normal serum level (mg/ml)	9	3	1	0.5	3.0	0.5	1.5	0.0003	0.03
In 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.

<sup>†</sup>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.

<sup>‡</sup>IgM is the first isotype produced by the neonate and during a primary immune response.

# Immunoglobulin Subtypes



**FIGURE 12-1** Comparative structures of the immunoglobulin classes and subclasses in humans. IgA and IgM are held together in multimers by the J chain. IgA can acquire the secretory component for the traversal of epithelial cells.



# Functions of Antibody

**Neutralize toxins**

**Bind to bacterial surfaces and aid in opsonization**

**Bind to bacterial surfaces and aid in complement killing**

**Inactivate viral particles**

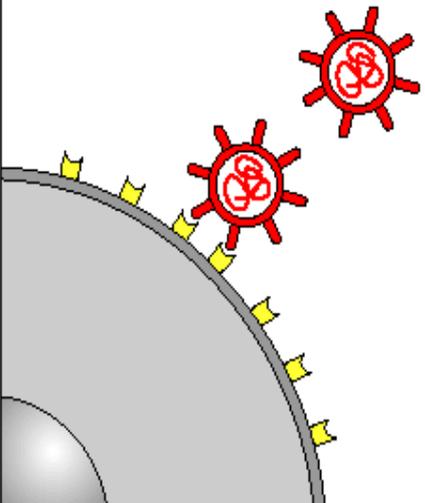
**Provide immunity to the unborn**

**Provide immunity to the newborn**

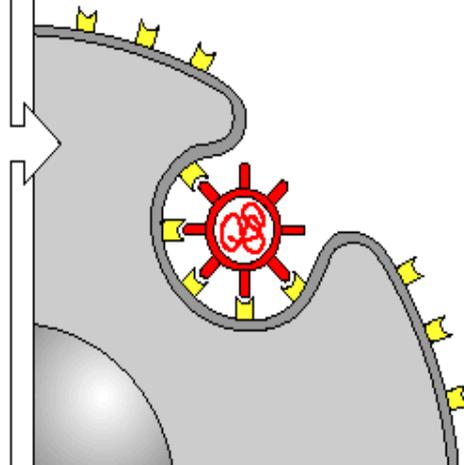
# Functions of Antibody

- Neutralization
- Opsonization
- Complement Activation

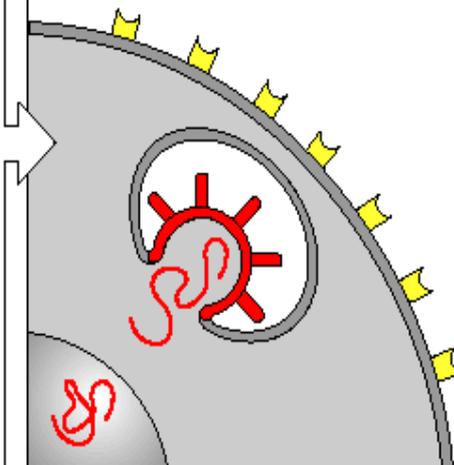
Viruses bind to receptor on cell surface



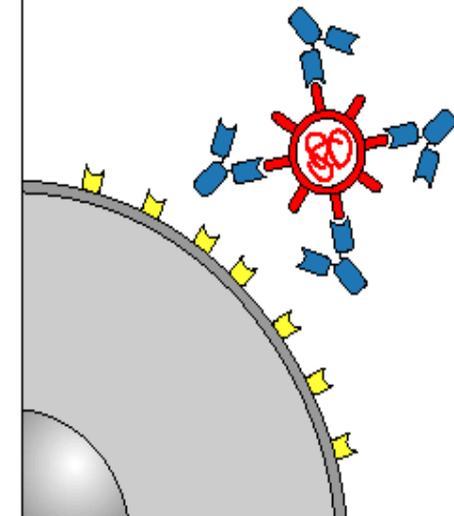
Receptor-mediated endocytosis of virus



Acidification of endosome after endocytosis triggers fusion of virus with cell and entry of viral DNA



Antibody blocks binding to virus receptor and can also block fusion event



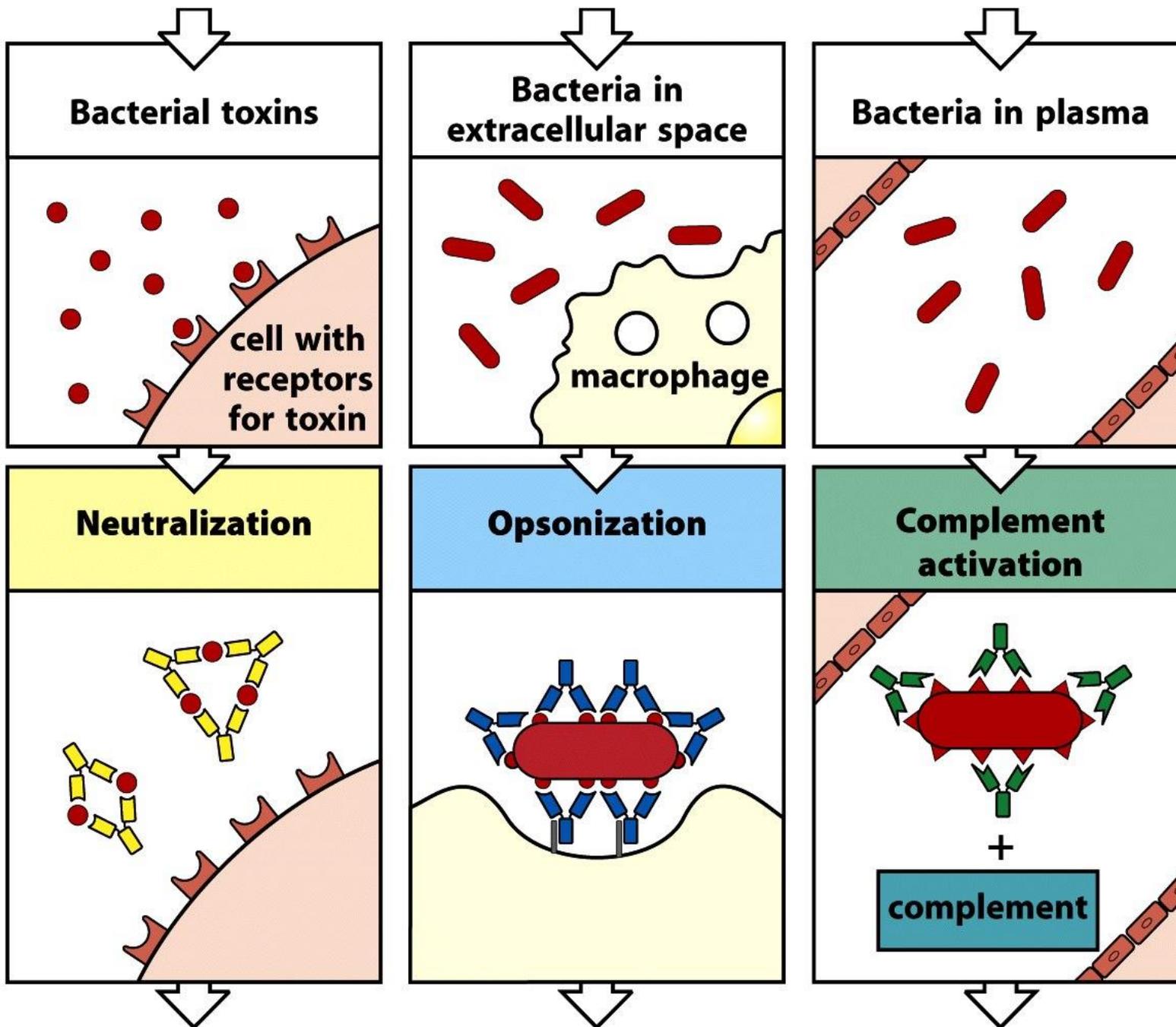


Figure 1-26 part 2 of 3 Immunobiology, 7ed. (© Garland Science 2008)

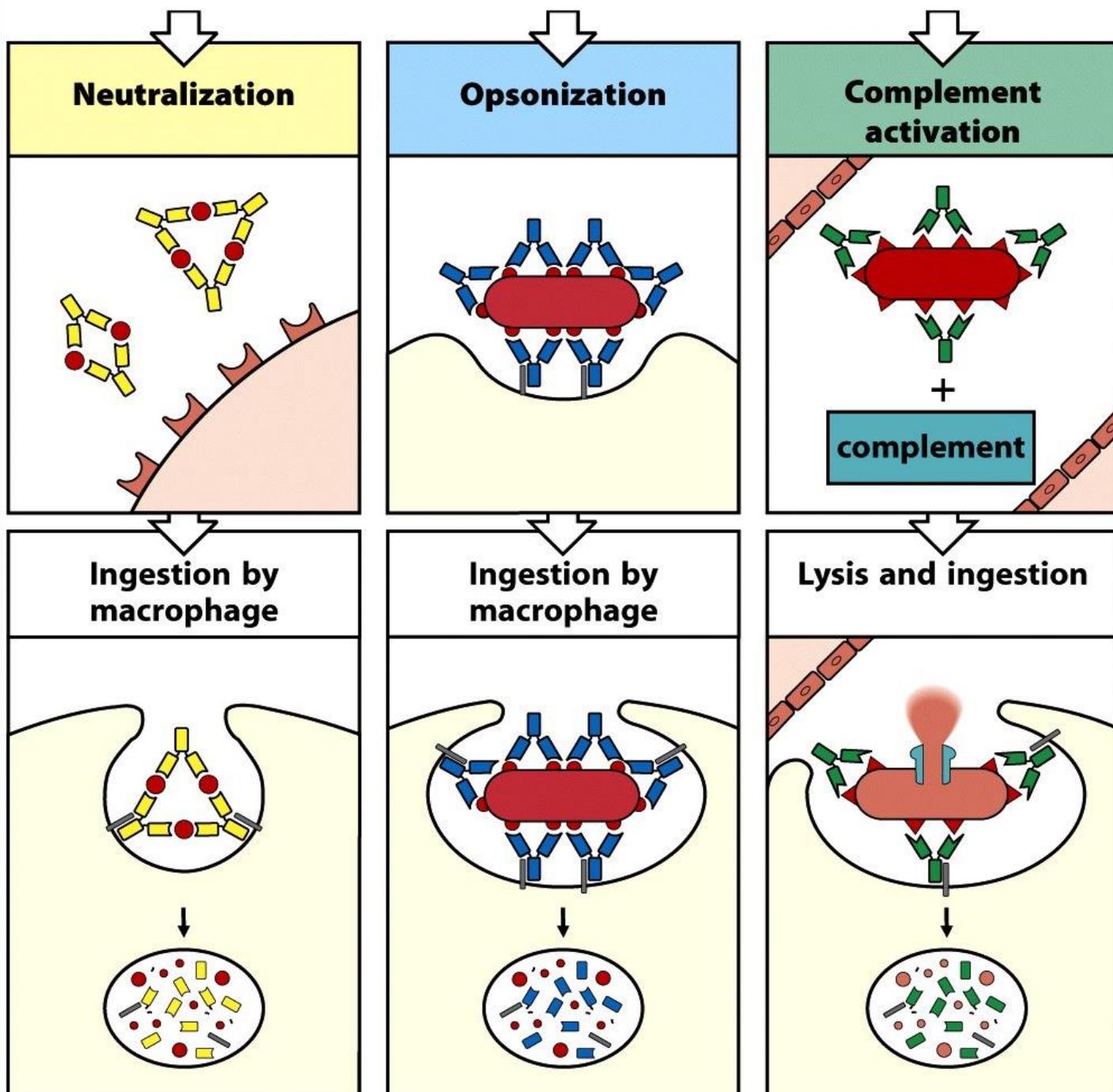
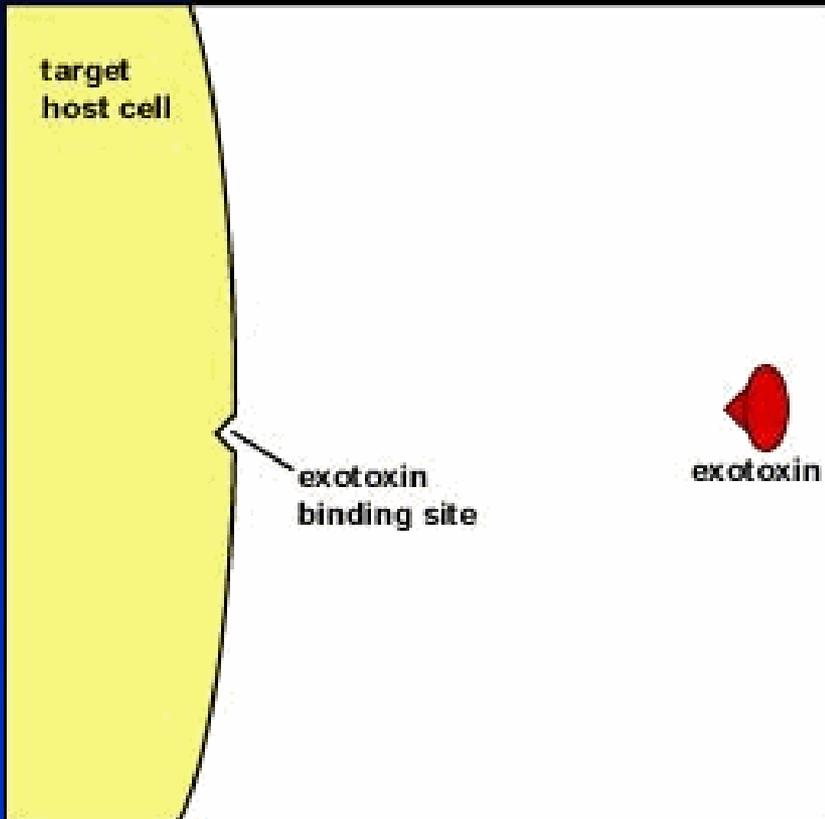
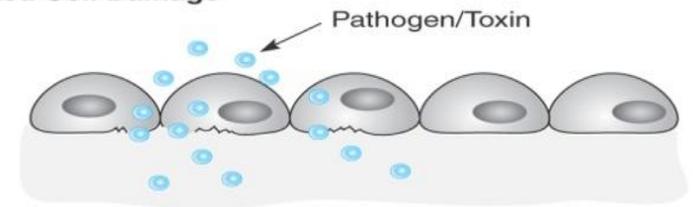


Figure 1-26 part 3 of 3 Immunobiology, 7ed. (© Garland Science 2008)

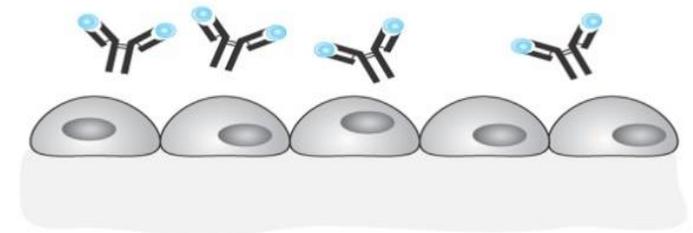
# Neutralization toxicant



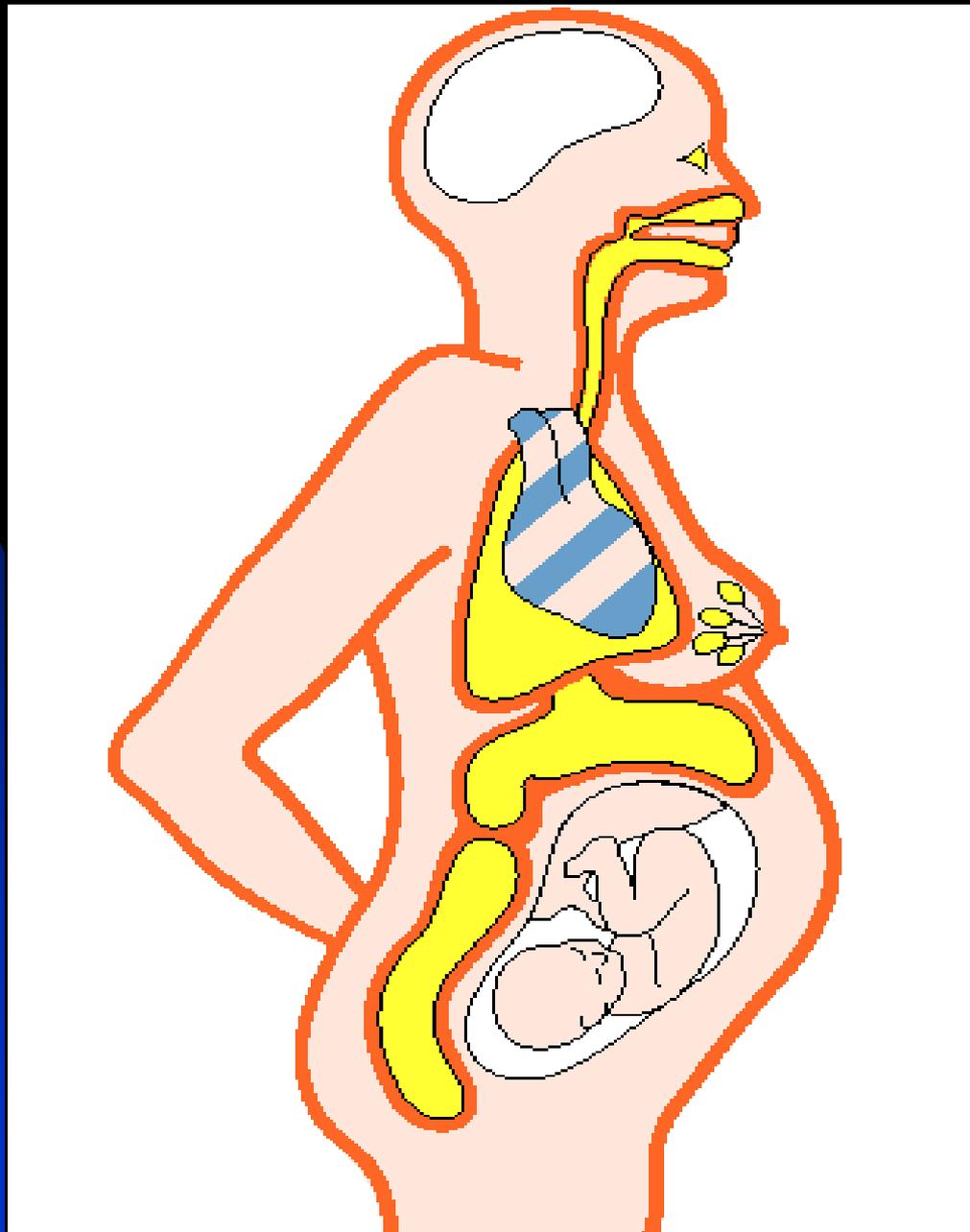
## A. Infected Cell Damage



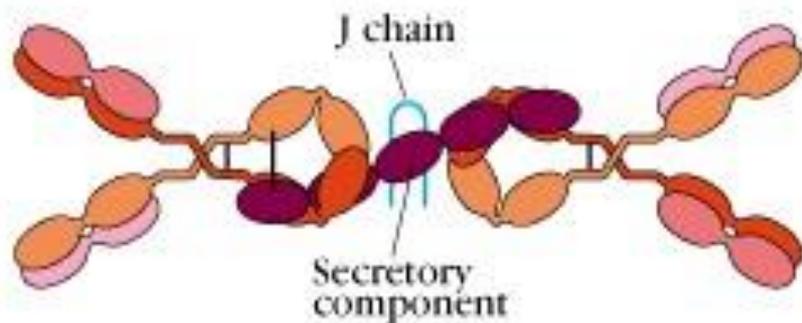
## B. Protected Cells



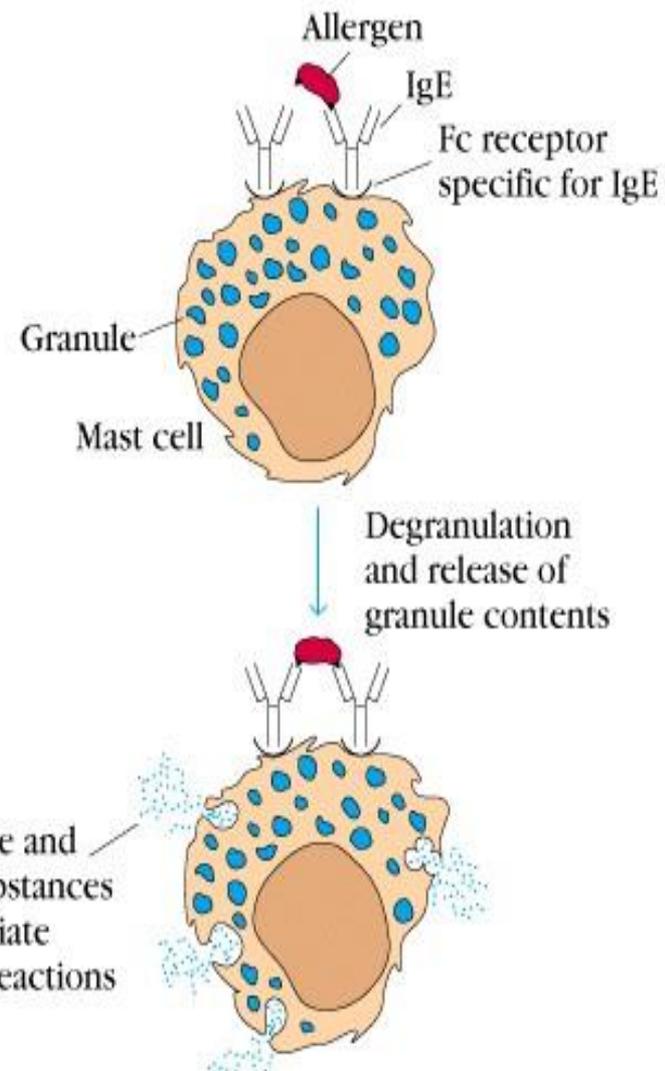
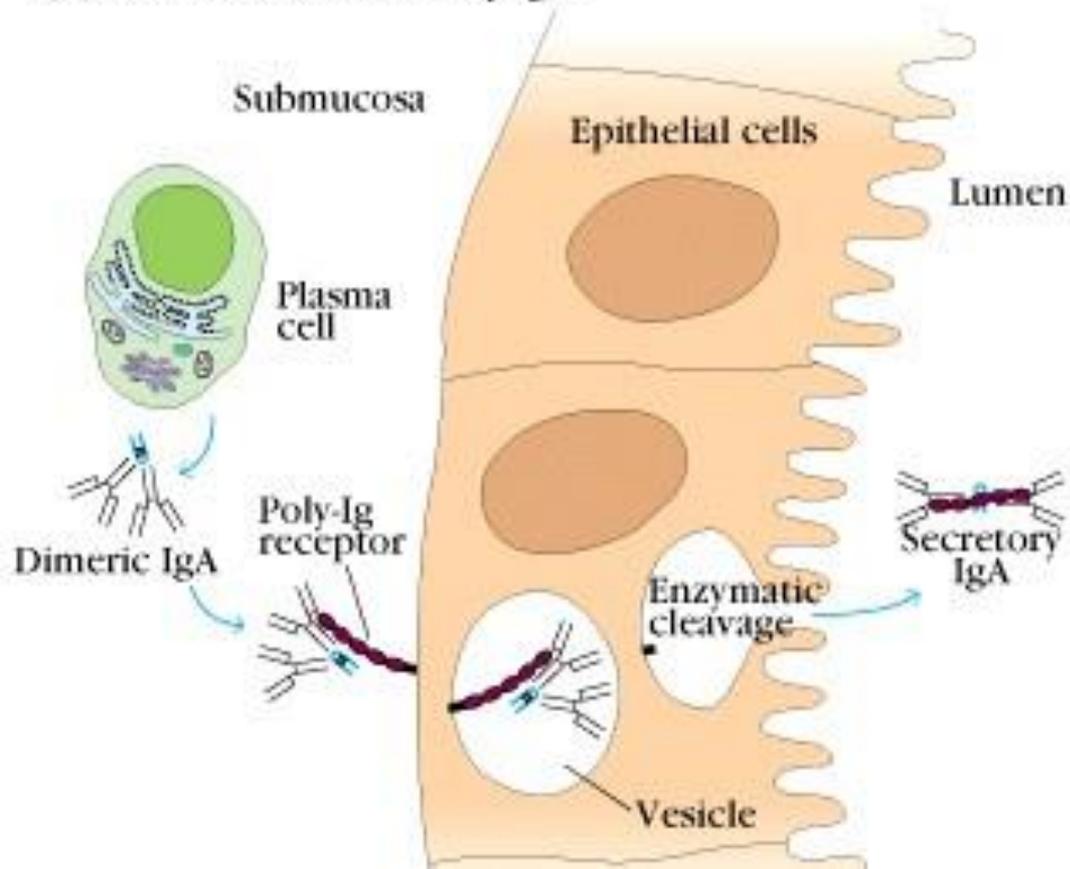
Copyright 2008 Elsevier, Inc.



(a) Structure of secretory IgA



(b) Formation of secretory IgA





# Immunoglobulin Isotypes

- **Isotypes** are the **antigenic features** of a class of immunoglobulin H (heavy chain).
- Example for  $\mu$  chain is isotypical different from  $\gamma$  chain.
- The genes for  $\gamma 1, \gamma 2, \gamma 3, \gamma 4, \mu, \alpha 1, \alpha 2, \delta, \epsilon, \kappa$  chains are present in the human genome, and are therefore isotypes.



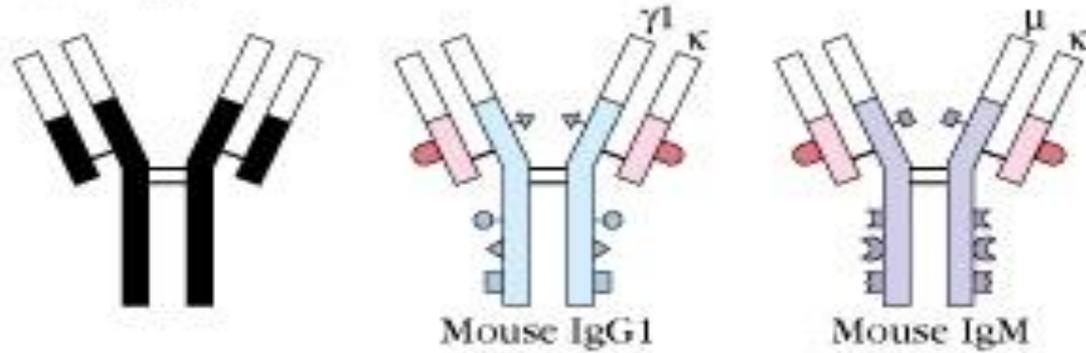
- **IgM Big, First, Complement**
- **IgD Membrane receptor**
- **IgG Secondary response, prototype**
- **IgA Secretions**
- **IgE Allergy (**Wheeze**)**

Isotype

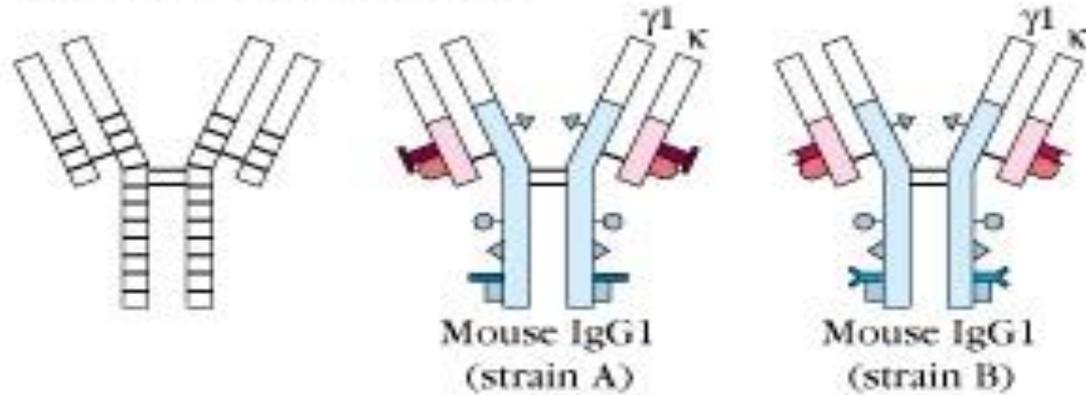
Allotype

Idiotype

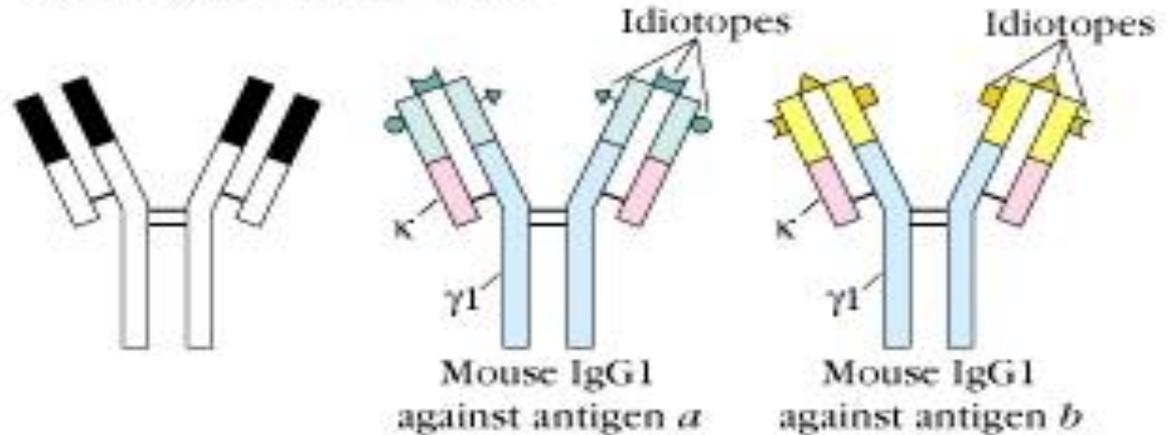
(a) Isotypic determinants



(b) Allotypic determinants



(c) Idiotypic determinants





## ■ Allotype

- Allotypes are additional features of immunoglobulines that **vary among individuals** within a species and are under genetic control.
- Allotypes can be shared by some members of a species.



## ■ **Idiotype**

- The term **idiotype** **refers to the unique V-region** amino acid sequences of the homogeneous immunoglobulin molecules produced by a single B cell clone.
- Thus, there are as many **idiotypes** as there are B cell clones (perhaps about  **$10^8$**  in an adult). It is now known that anti-idiotype Ab specificity recognize sequences in the hypervariable regions of the target Ab that **induced it.**



An anti-idiotypic immune response (Ab or T cell mediated) expresses its own idiotypic which in turn can be recognized as foreign and an **anti-idiotypic immune response** made against this idiotypic.

**Jerne** described a **Network Theory** which proposes that a series of **idiotypic-anti-idiotypic** reactions are partially responsible for regulation of the immune response.



**How does the immune system  
make millions or billions of  
different antibody molecules?**

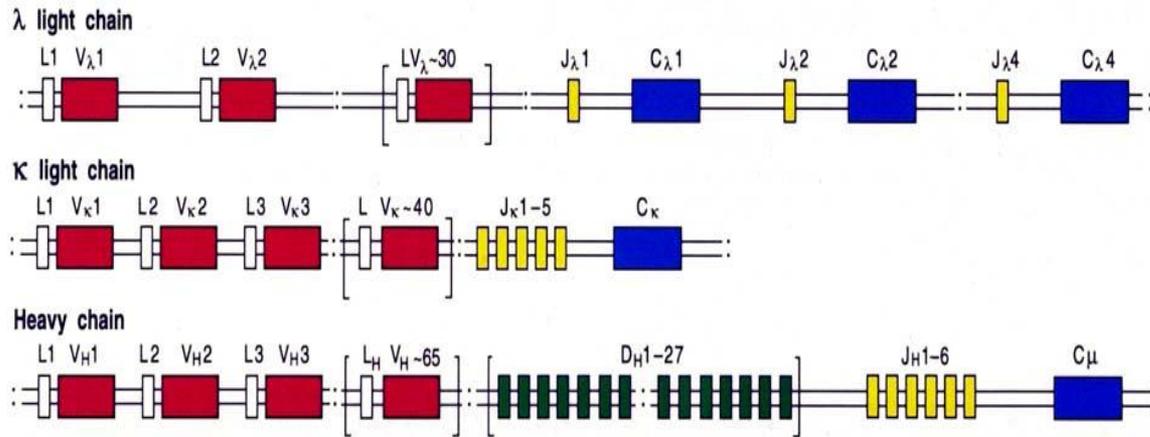


# Mechanisms for Generation of Diversity

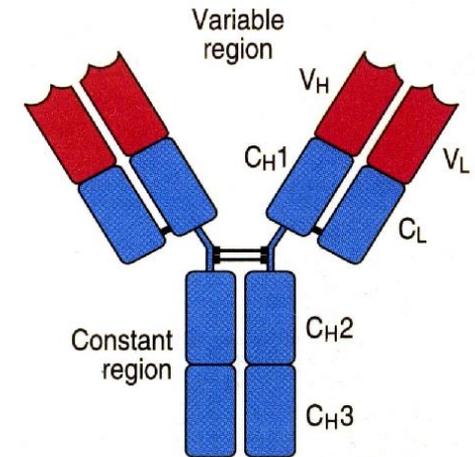
- **Random assortment of light and heavy chains.**
- **Multiple V region gene segments.**
- **Somatic rearrangement.**
- **Faulty joining.**
- **Somatic mutation of rearranged genes.**

# How to determine Ig gene structure?

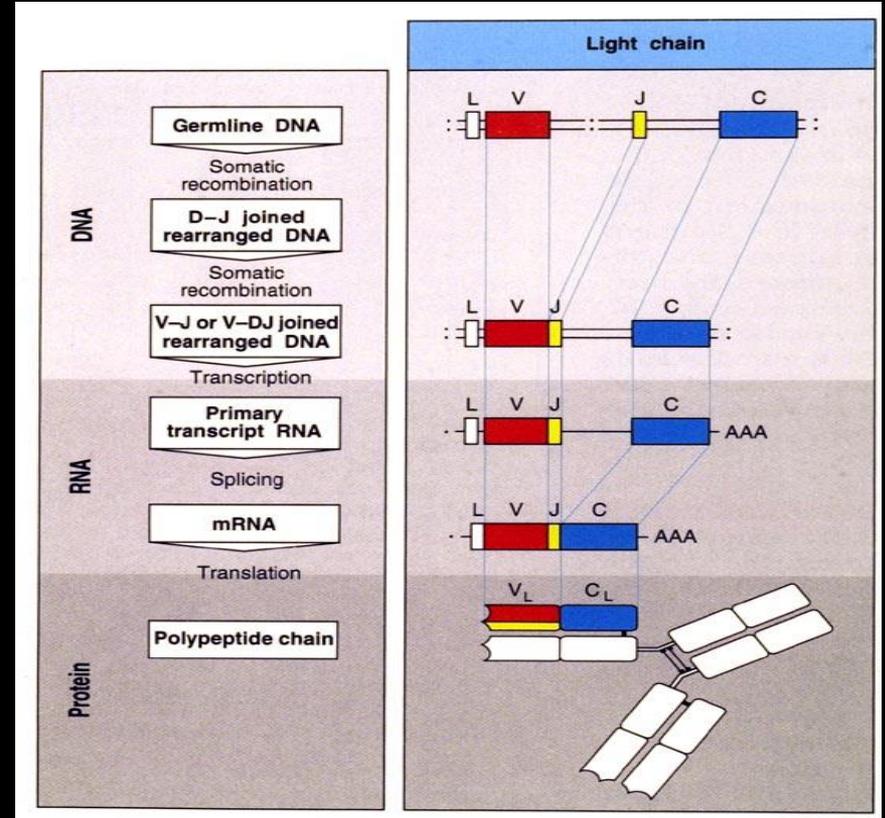
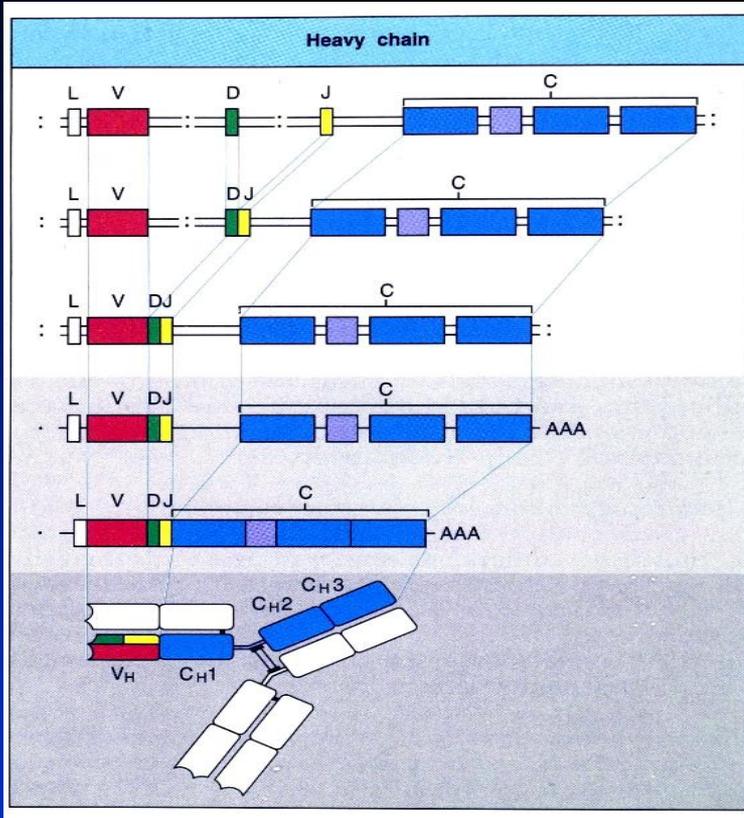
## Genetic structure (DNA)



## Ab (peptide)

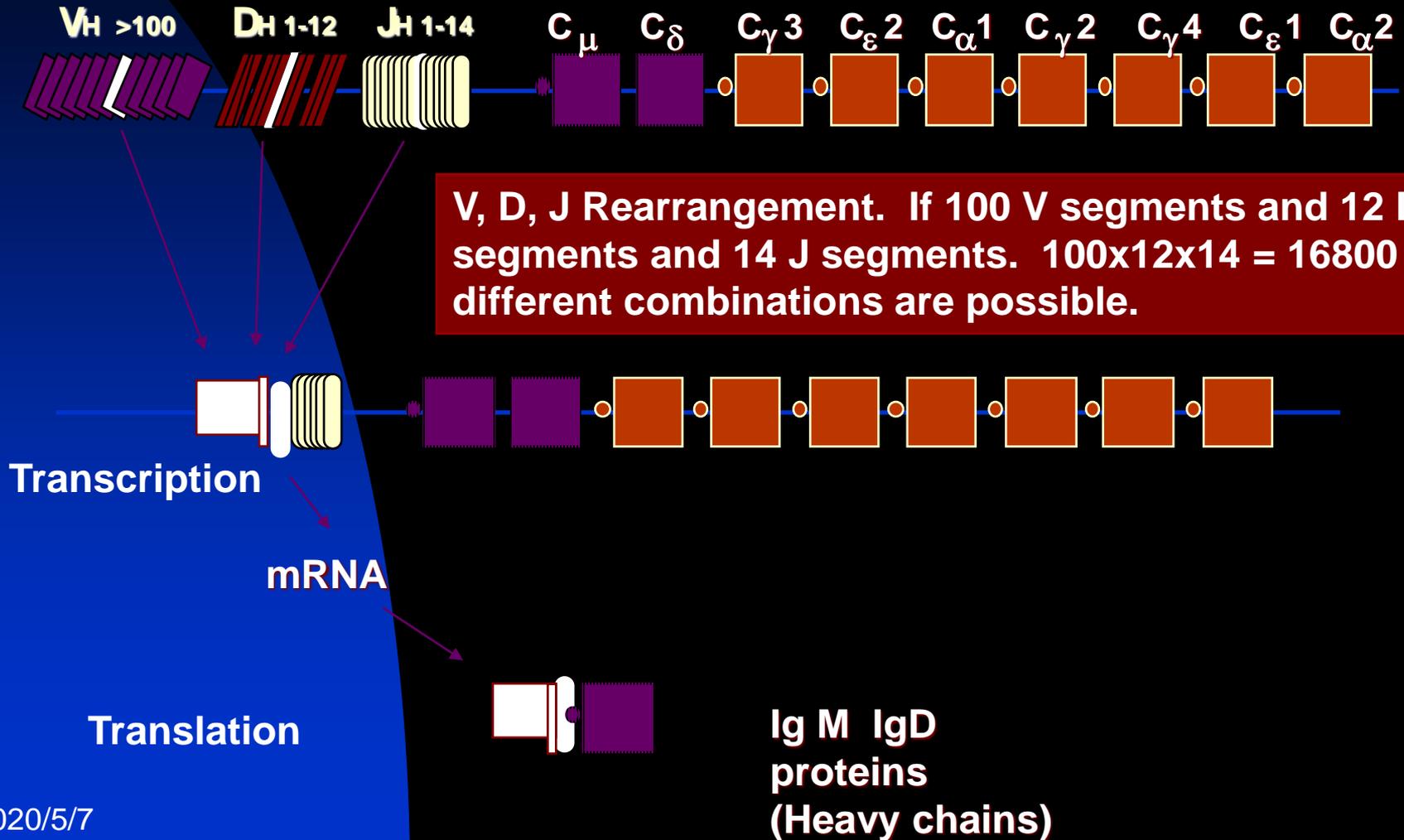


# How are Ig genes rearranged?



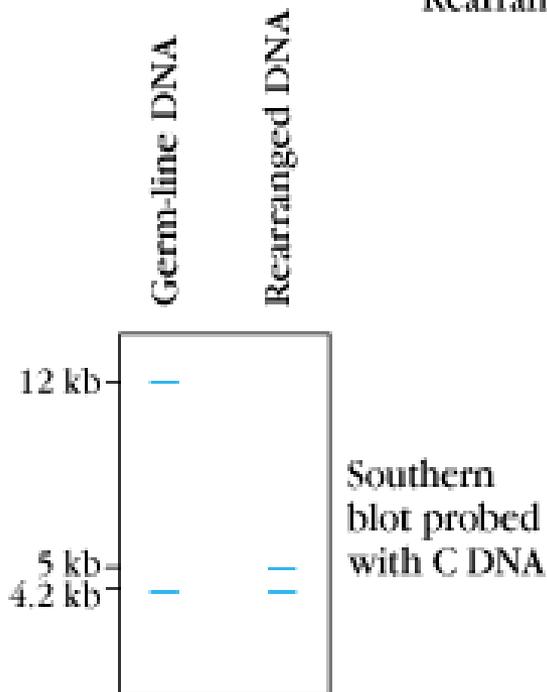
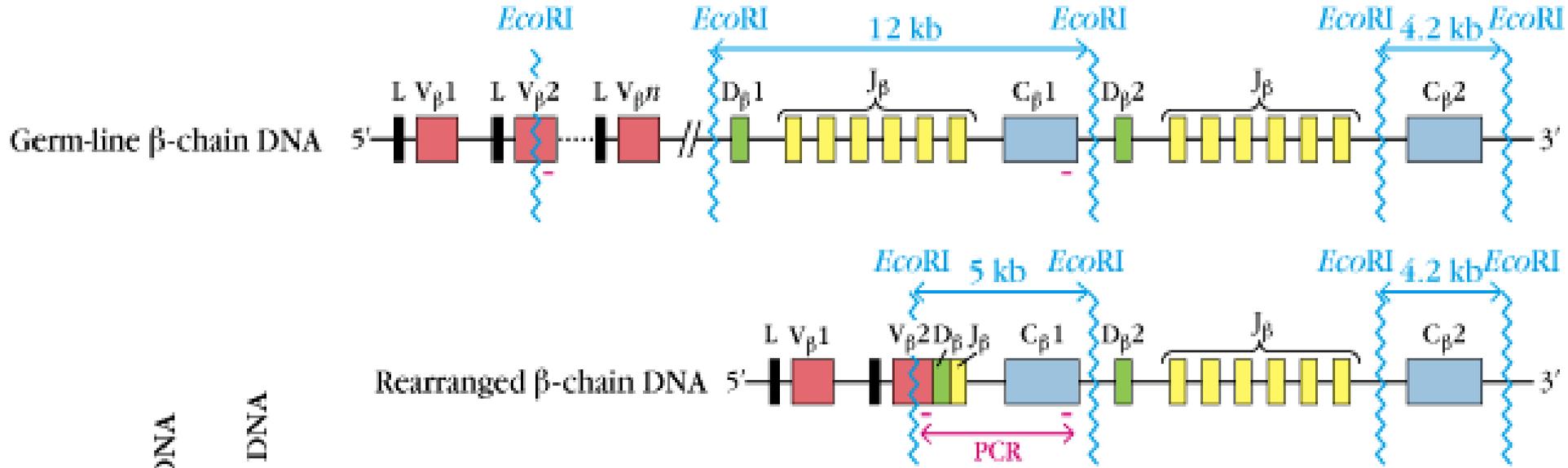
**\*\*H chain genes are rearranged first, followed by L chain genes**

# Somatic Rearrangement of Ig genes occurs during B cell differentiation





# TCR genes construct and rearrangement



## Southern blot analysis

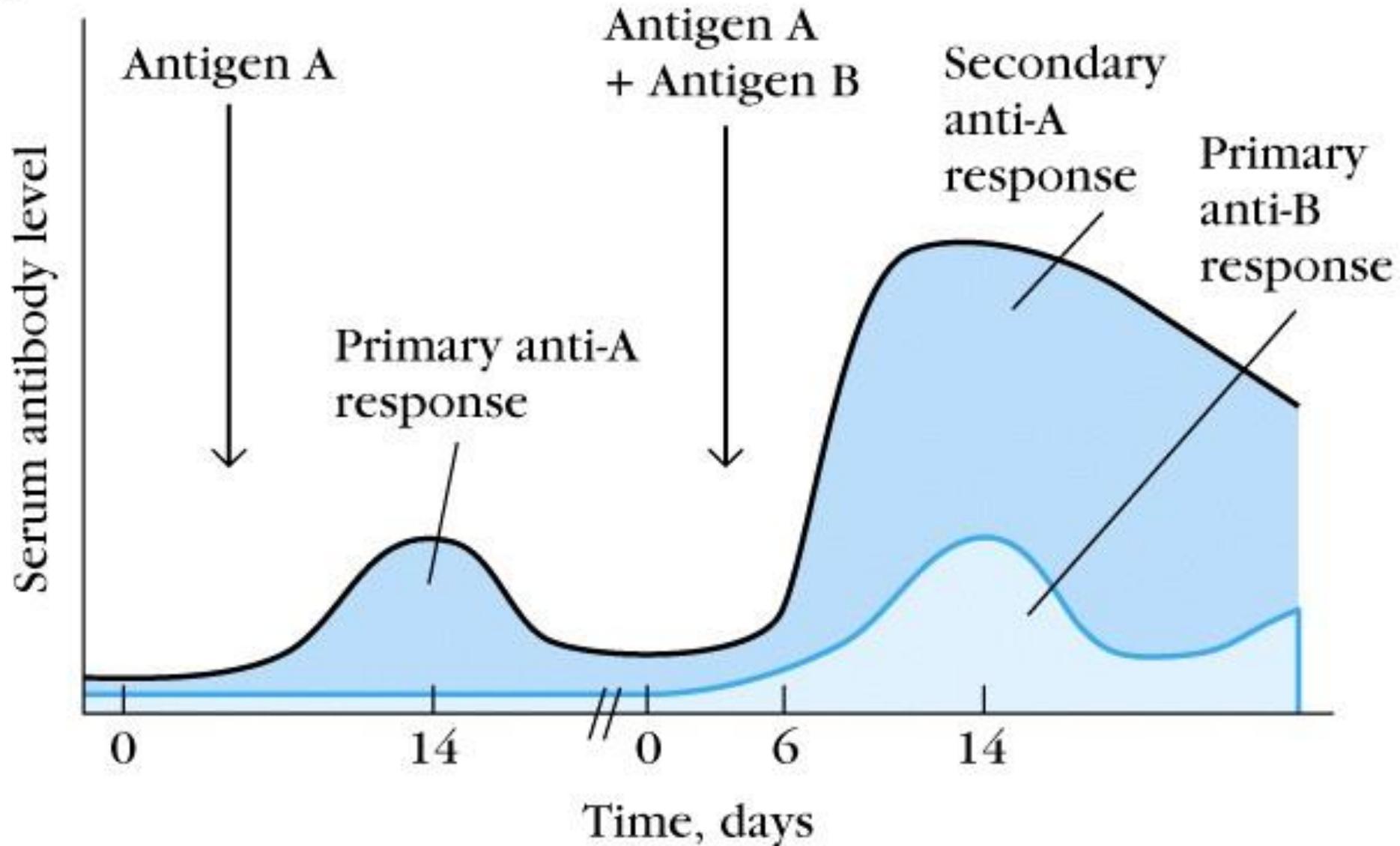
*Eco*RI digesting PCR products

TCR— $\alpha$  and  $\beta$  genes use a strategy of recombination similar to that of Ig genes.

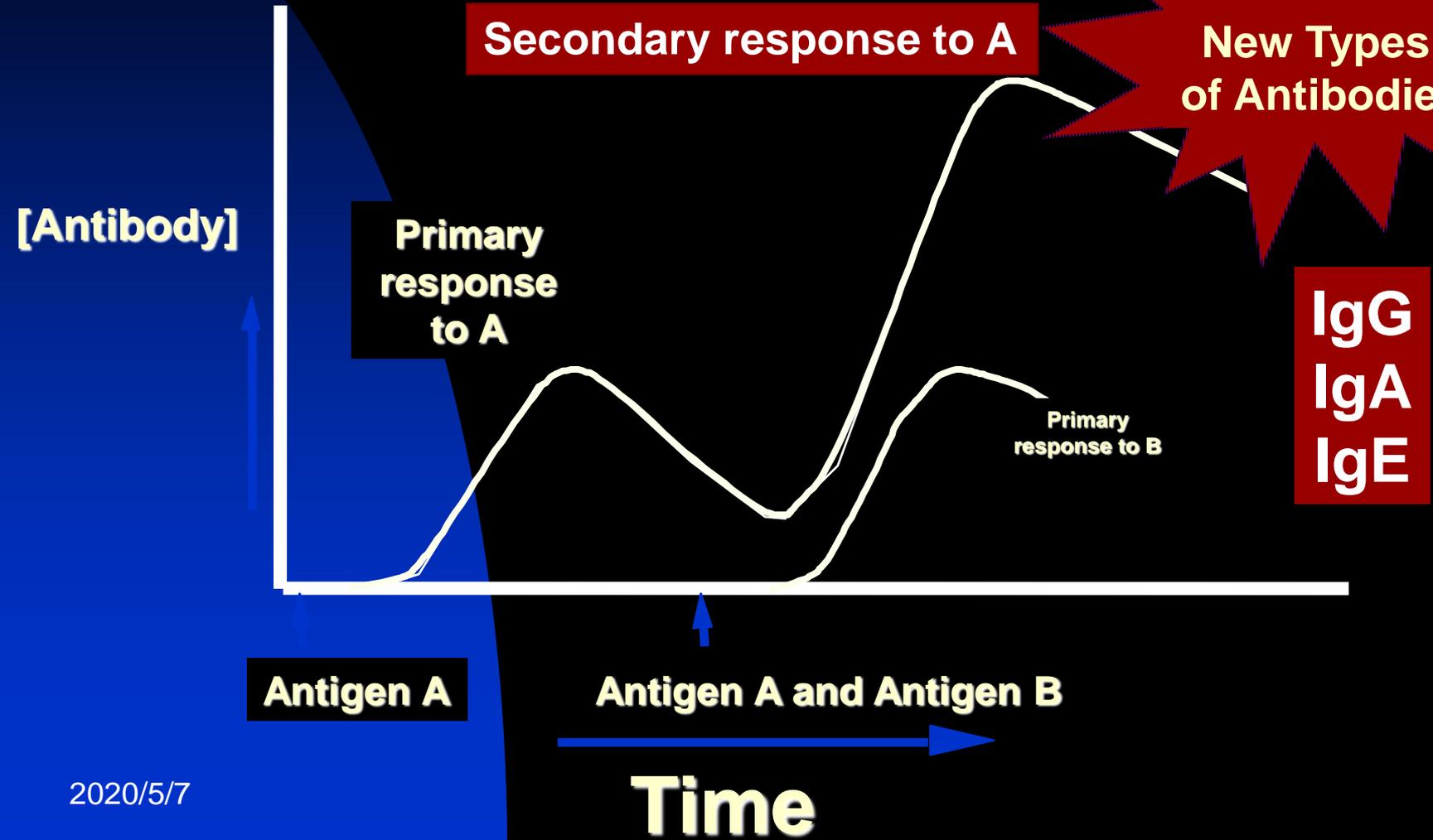
# Primary and Secondary Responses. Specific Adaptive or Acquired Immunity.



(a)



# Primary and Secondary Immune Response to Antigen





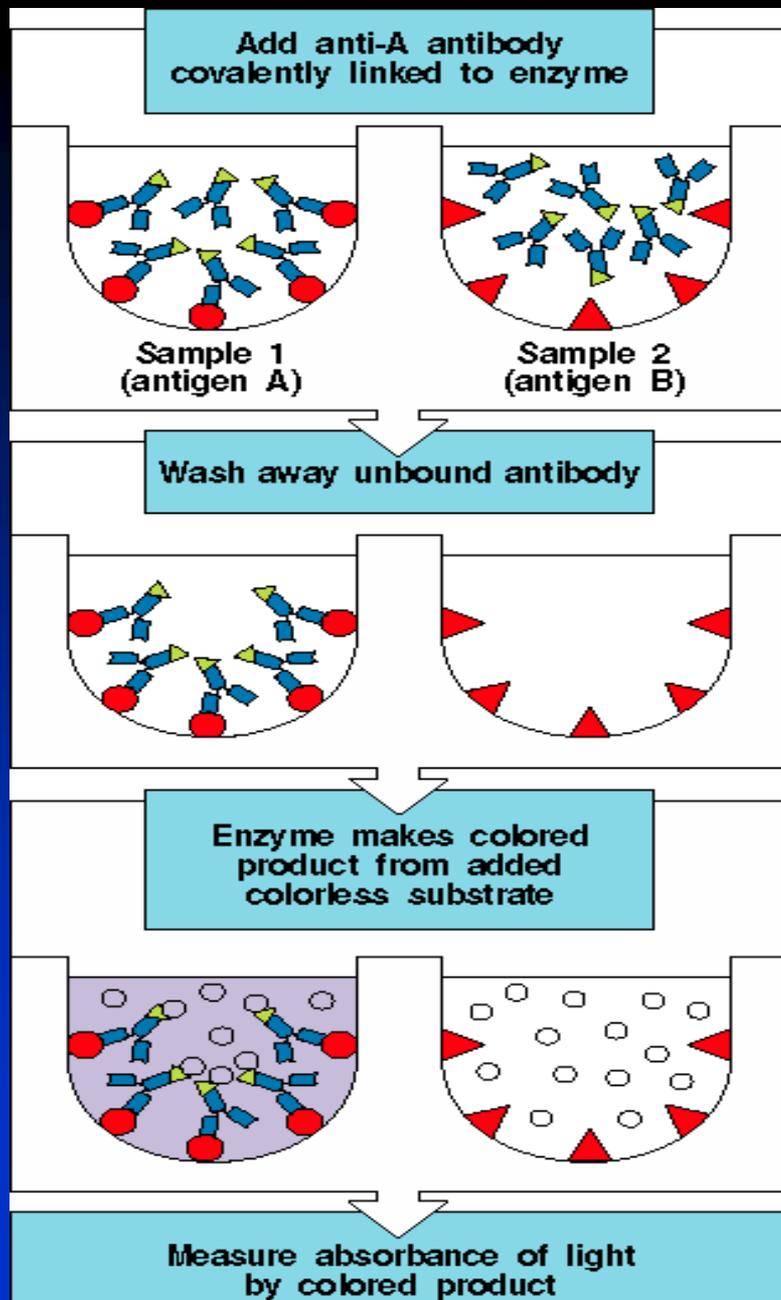
# Antibody Use in Medicine

- **Detecting assays**
- **Drugs, chemicals, hormones, pregnancy tests, HIV tests, etc**
- **Enzyme linked immunosorbent assay (ELISA)**
- **Blood typing**
- **Microbial identification**

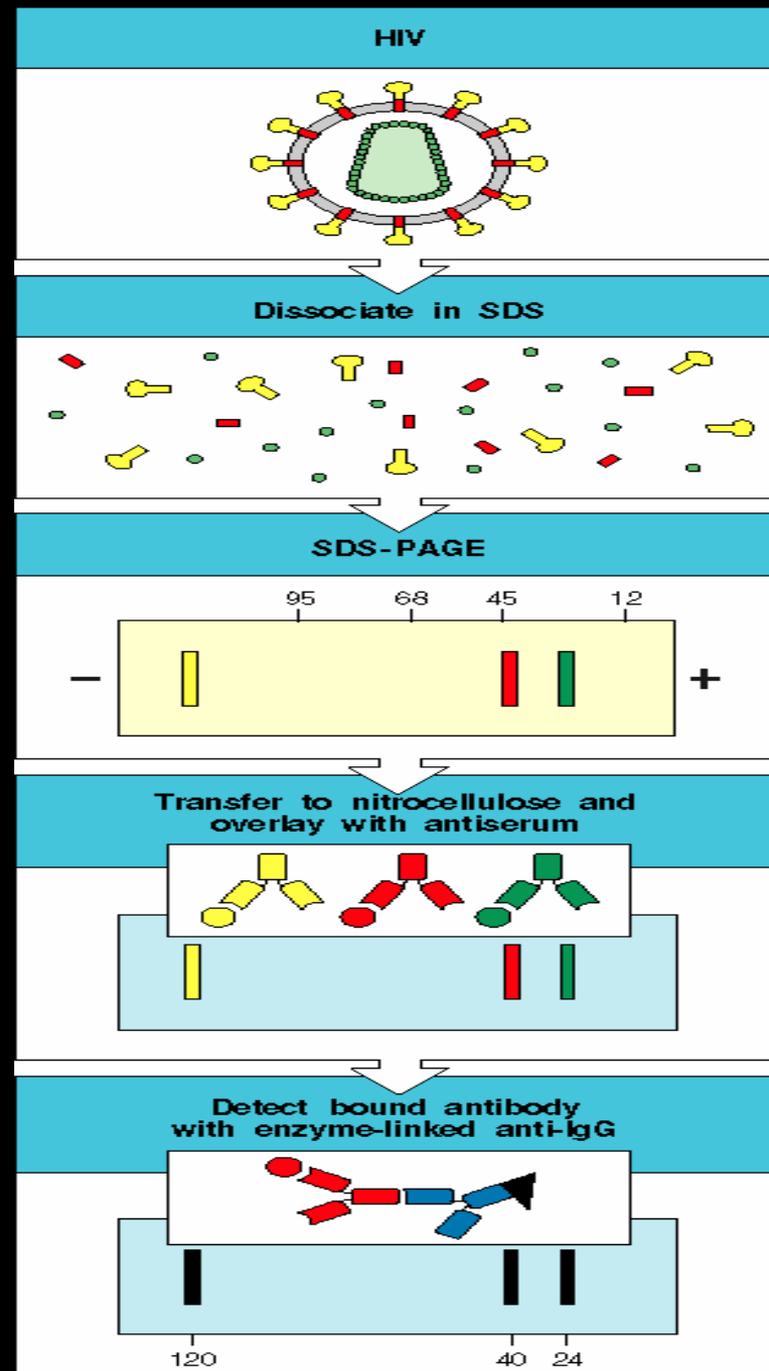


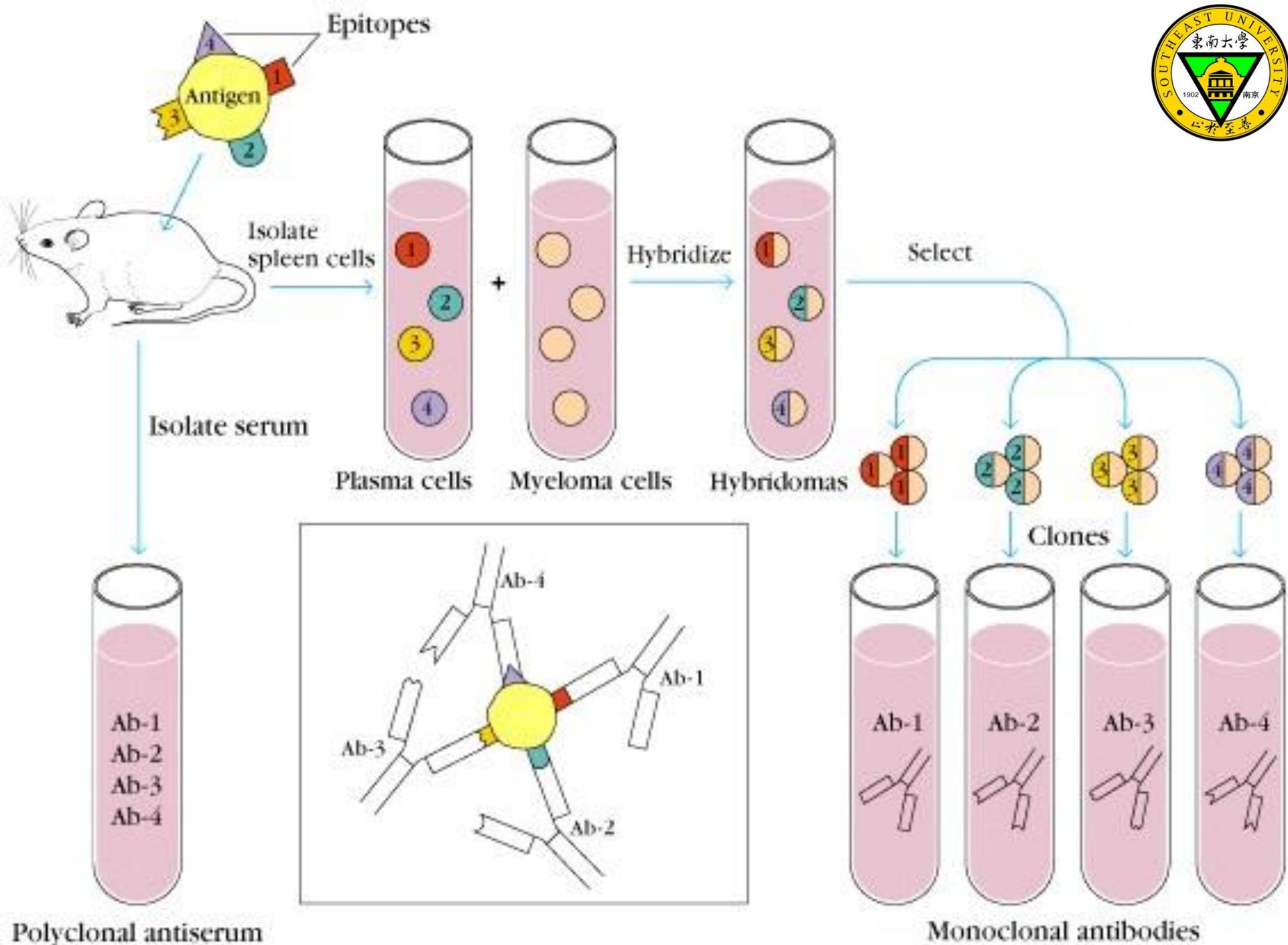
- **Passive Antibody Therapy.**
- e.g. Gamma globulins
- **Imaging**
- **Immunotoxins. Cancer therapy**
- **Neutralize inflammatory cytokines. e.g. antibody to TNF**
- **Catalytic antibodies.**
- **Etc.**

# ELISA



# Western Blot





## Polyclonal antibody

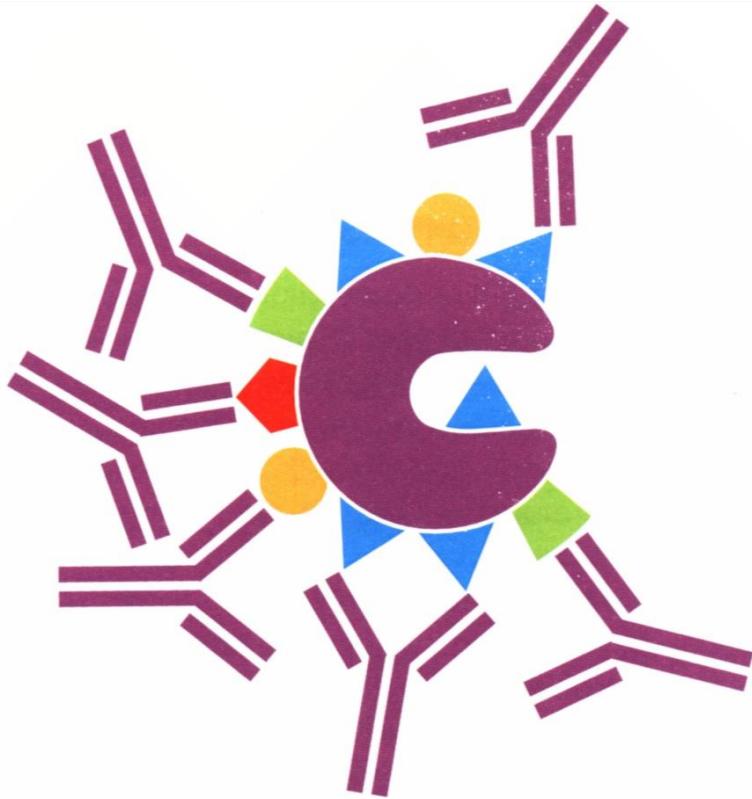


Figure 4: Schematic diagram of polyclonal antibodies binding to various epitopes on an antigen.

## Monoclonal antibody (McAb)

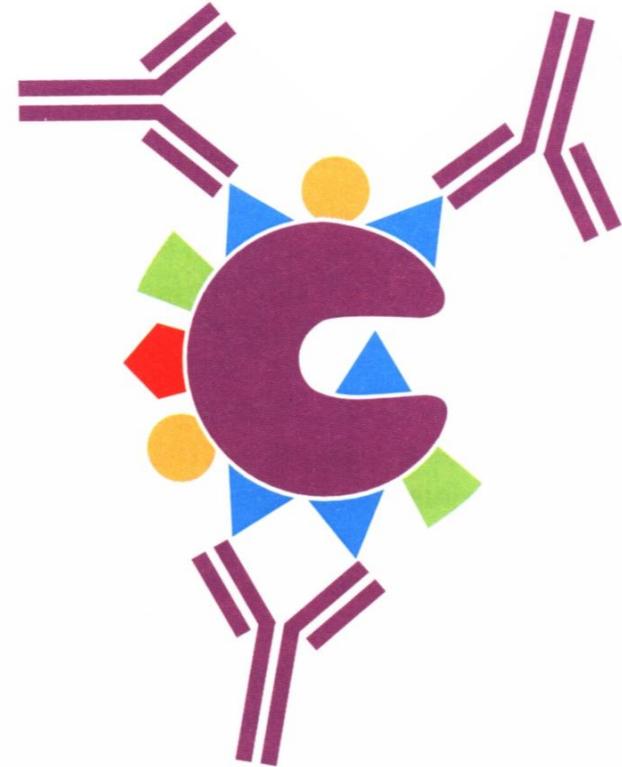
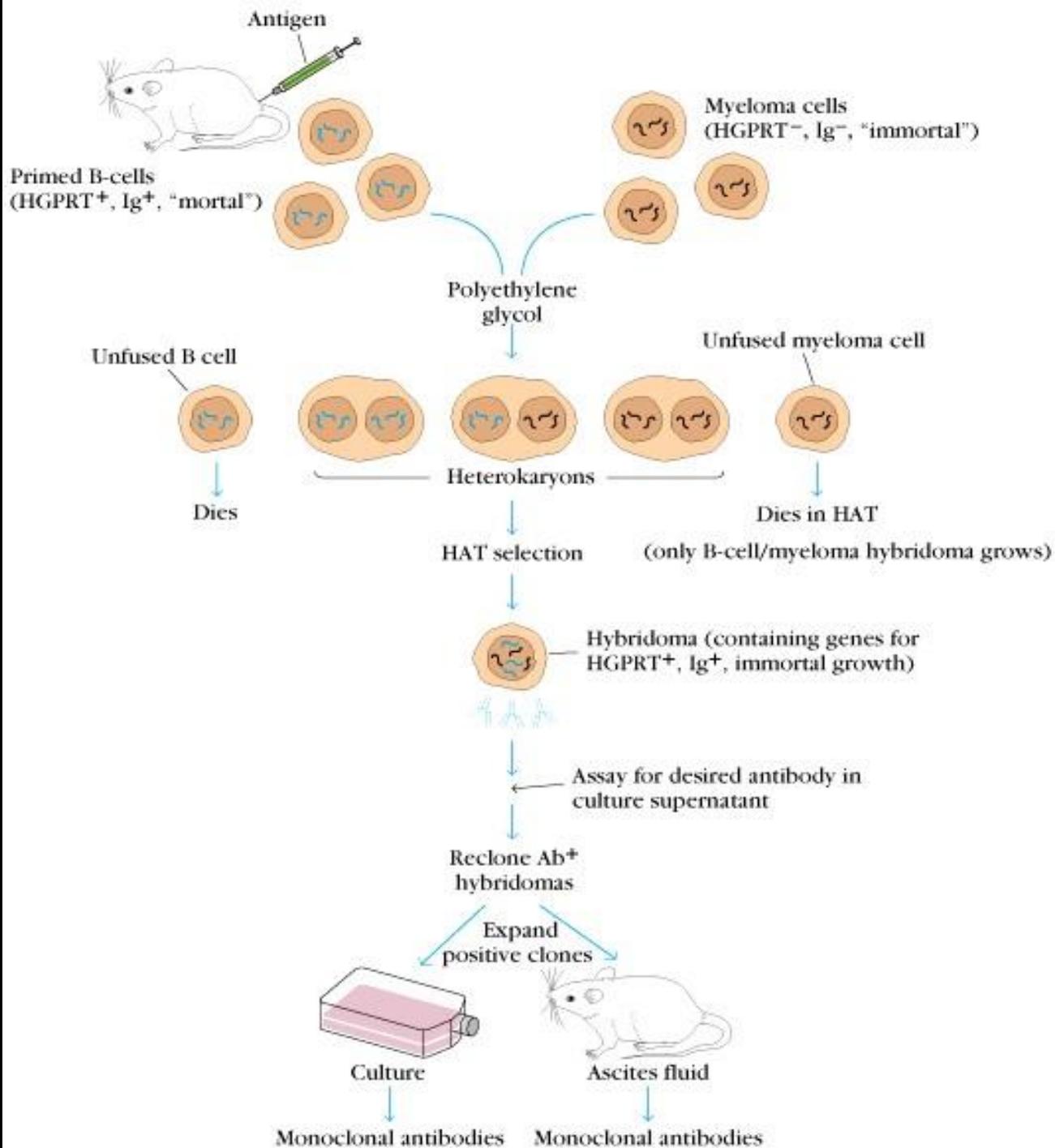
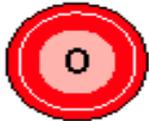
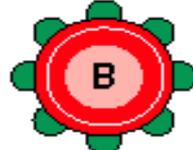


Figure 5: A given clone of monoclonal antibodies reacts with a specific epitope on an antigen.

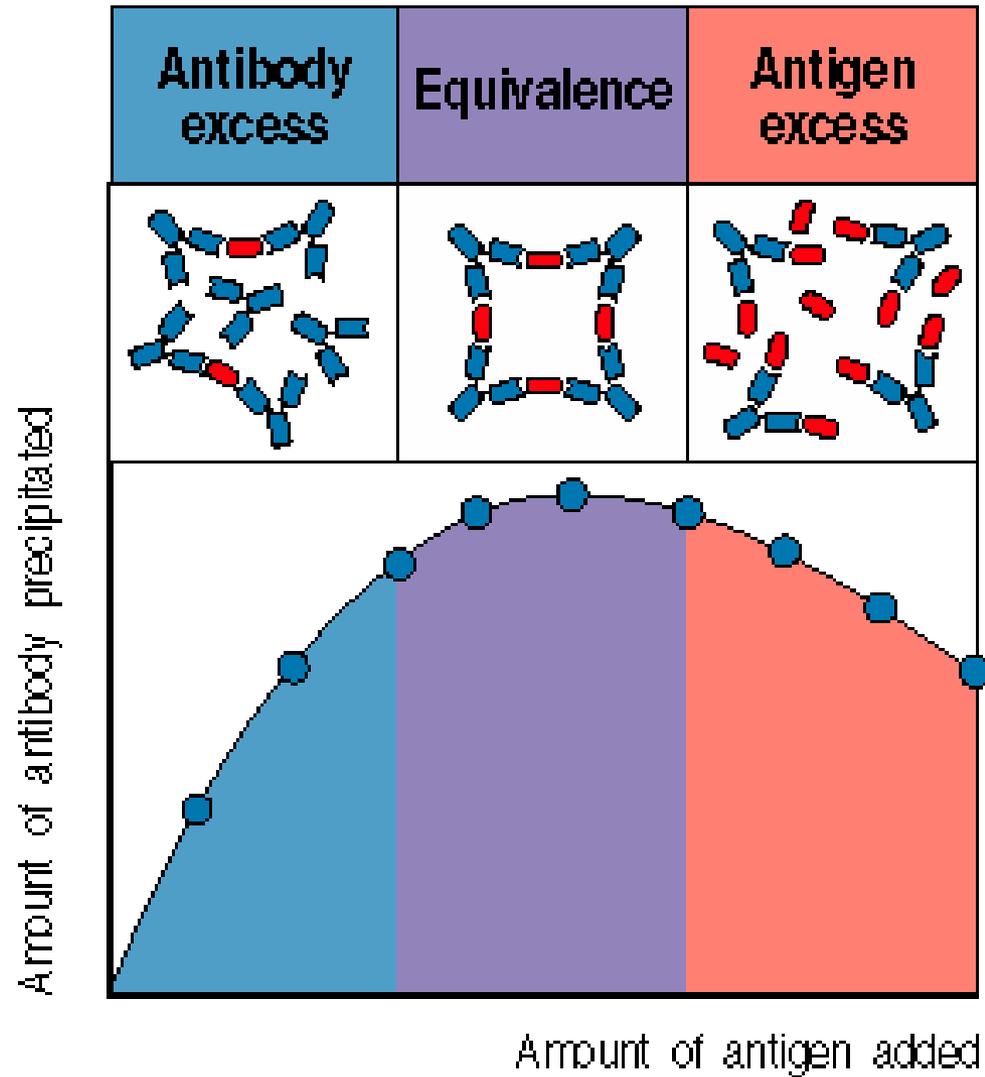
# Monoclonal Antibody



# Blood typing by agglutination

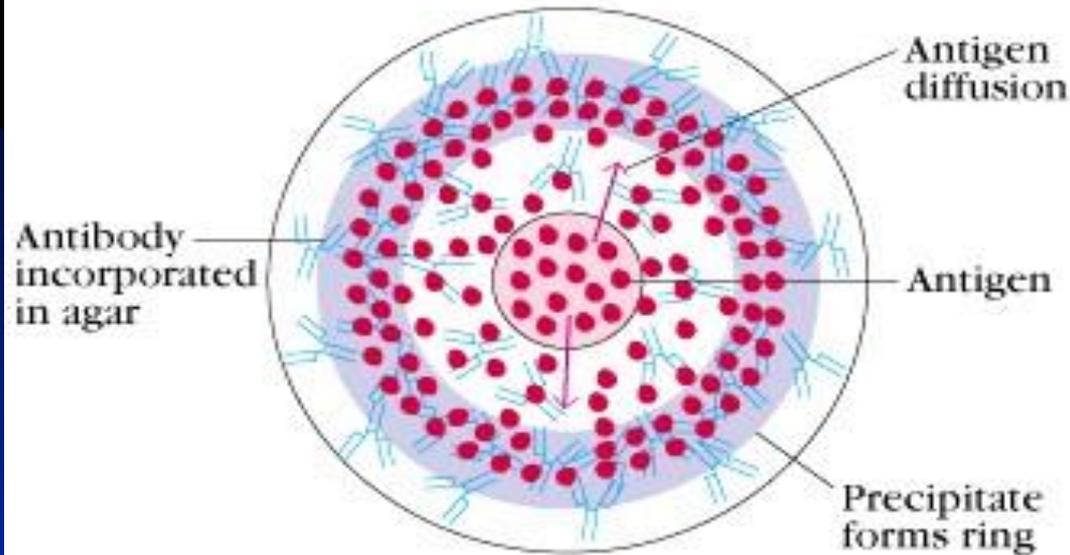
	Red blood cells from individuals of type			
				
	Express the carbohydrate structures			
Serum from individuals of type	R-GlcNAc-Gal   Fuc	R-GlcNAc-Gal-GalNAc   Fuc	R-GlcNAc-Gal-Gal   Fuc	R-GlcNAc-Gal-GalNAc   Fuc + R-GlcNAc-Gal-Gal   Fuc
 Anti-A and anti-B antibodies	no agglutination	agglutination	agglutination	agglutination
 Anti-B antibodies	no agglutination	no agglutination	agglutination	agglutination
 Anti-A antibodies	no agglutination	agglutination	no agglutination	agglutination
<b>AB</b> No antibodies to A or B	no agglutination	no agglutination	no agglutination	no agglutination

# Immunoprecipitation

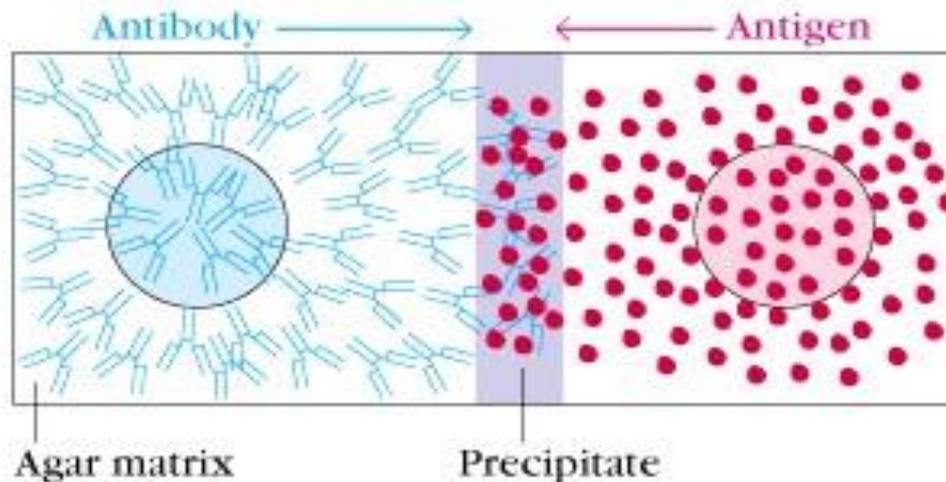


# Immunodiffusion

## RADIAL IMMUNODIFFUSION

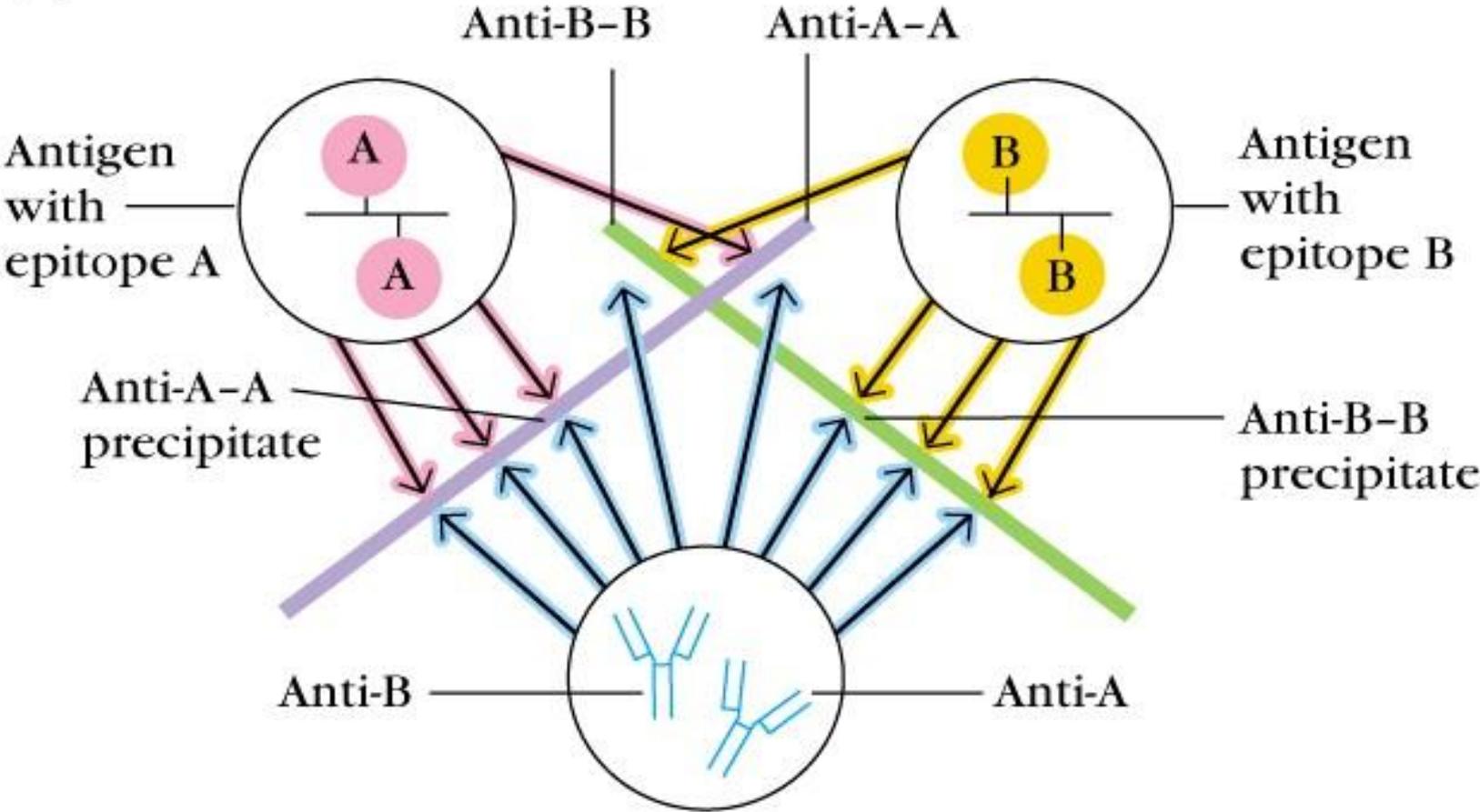


## DOUBLE IMMUNODIFFUSION

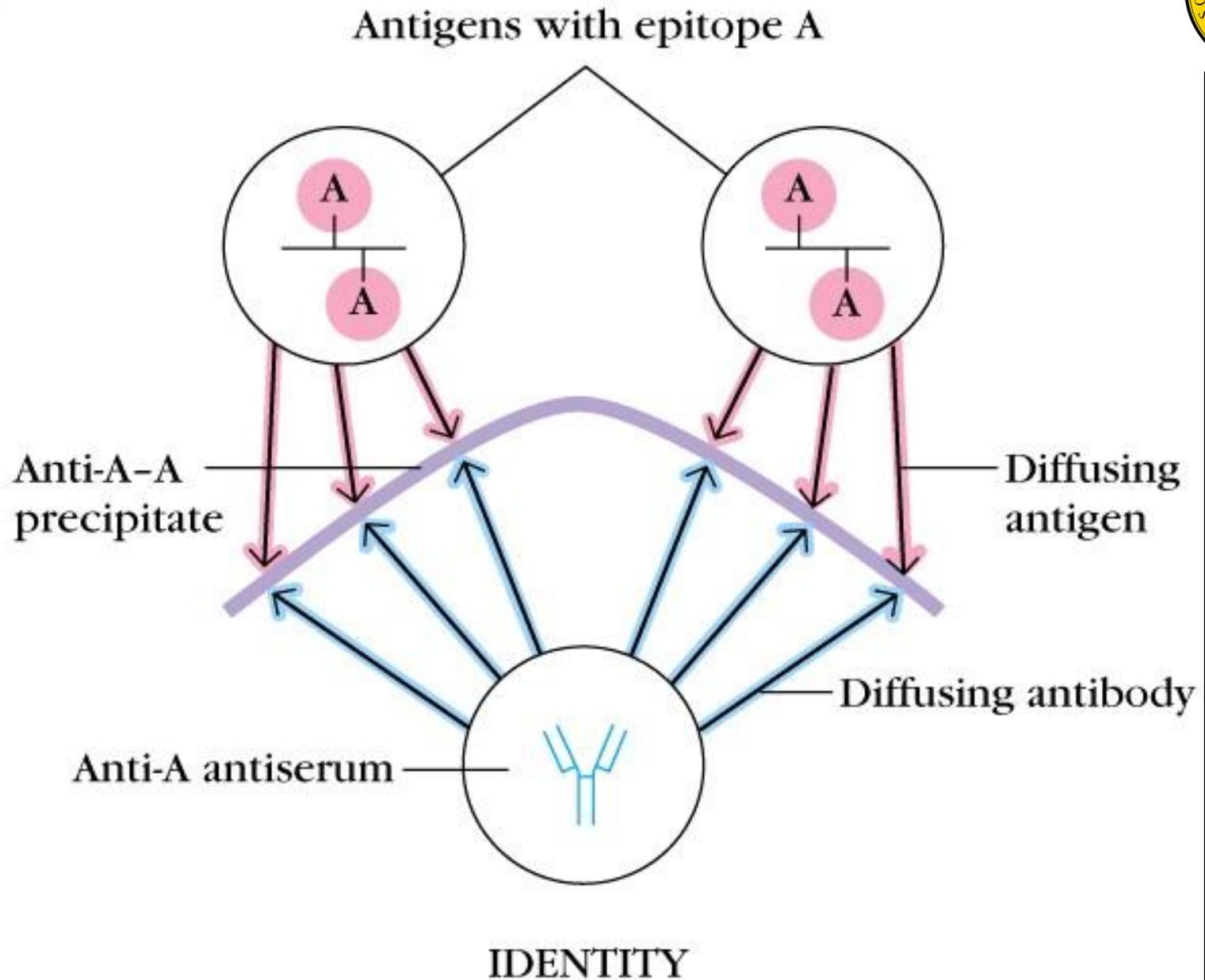


# Double Immunodiffusion

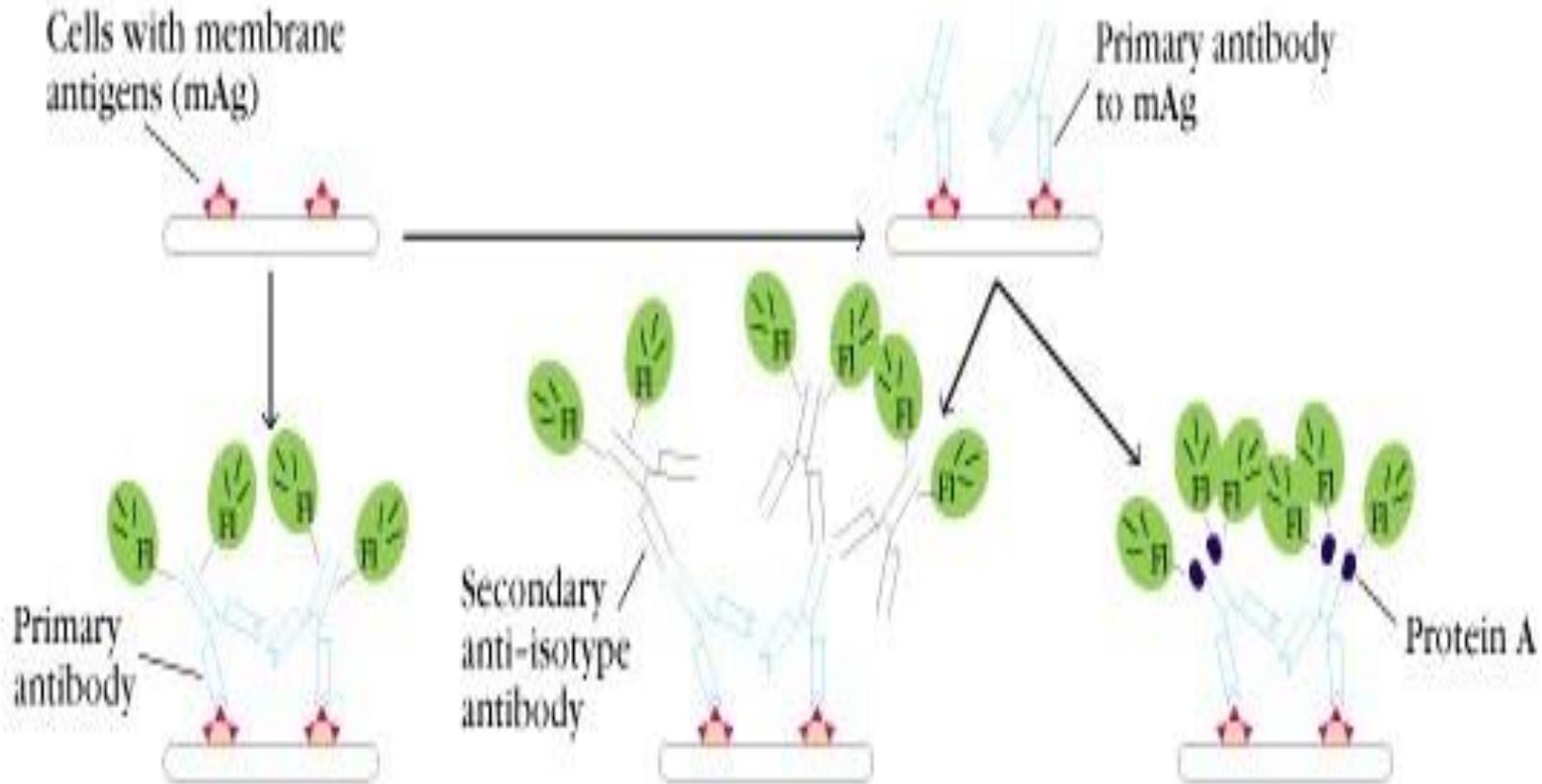
(b)



(a)



# Immunofluorescence

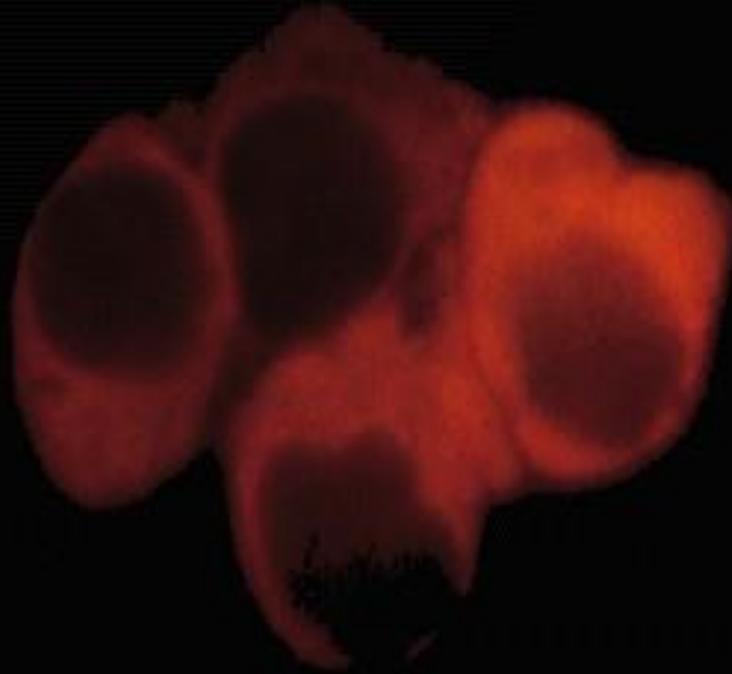


(a) Direct method with fluorochrome-labeled antibody to mAg

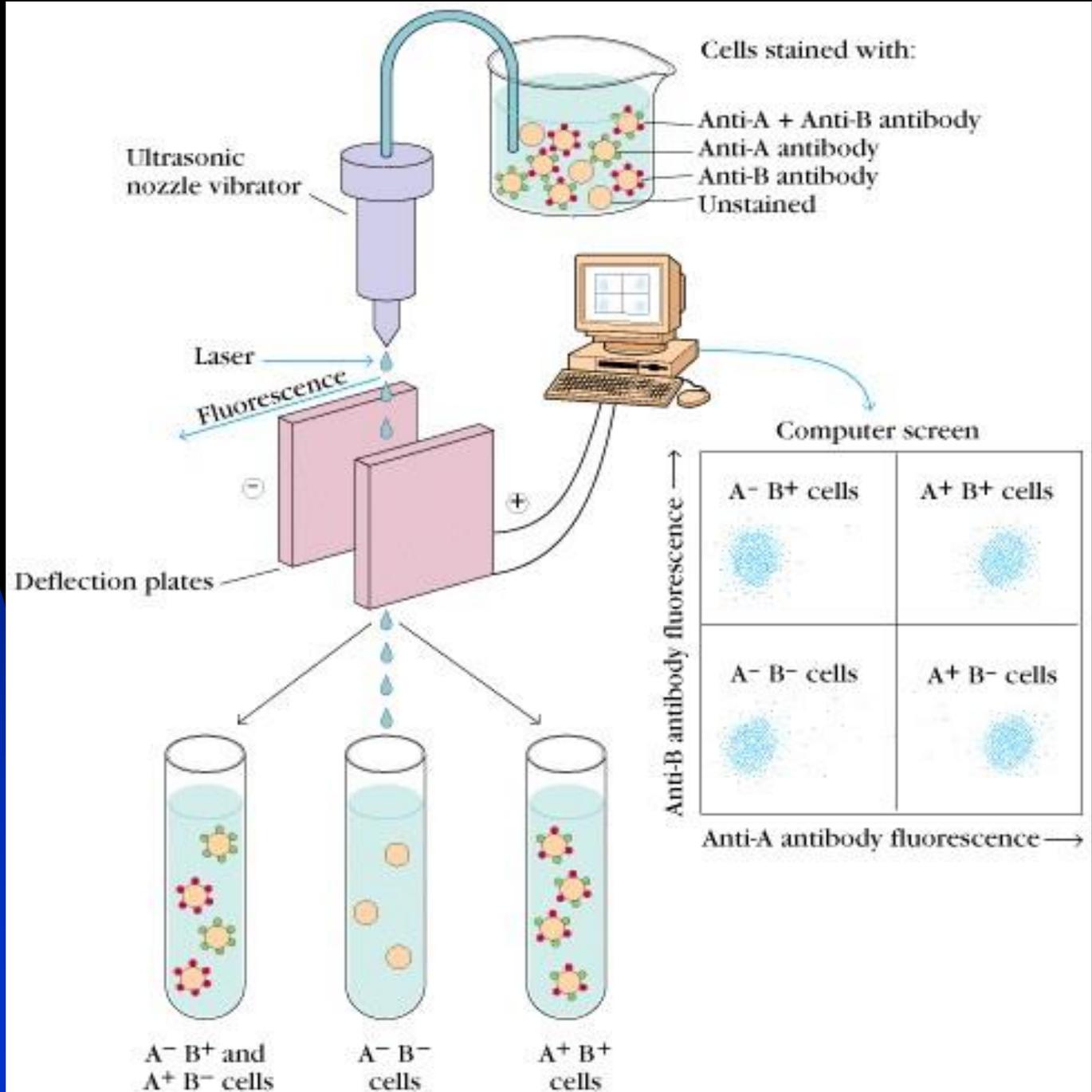
(b) Indirect method with fluorochrome-labeled anti-isotype antibody

(c) Indirect method with fluorochrome-labeled protein A

(d)



# FACS Or Flow Cytometry





# Humoral Immunity In Summary:

- **Antibody functions by binding antigen and by directing the activity of complement and phagocytes**
- **Antibody is a protein termed immunoglobulin**
- **There are several different Immunoglobulin classes or isotypes depending on the heavy chain expressed.**



- **Antibodies** can exist in millions or billions of different forms (antigen specificities) created via a process termed generation of diversity.
- **Antibody** production results from B cell proliferation of differentiation.
- **B cell proliferation and differentiation** usually requires helper T cells.



# ■ Extracurricular works

## ■ I. Please answer the concepts below:

- 1. Ig and Antibody(Ab)
- 2. Ig Isotype
- 3. Ig Allotype
- 4. Ig idotype
- 5. Monoclonal Ab and Polyclonal Ab
- 6. Neutralization
- 7. Opsonization
- 8. CDRs and  $V_H$
- 9. Fab and Fc
- 10. Noncovalent forces
- 11. Ig subtypes and sIgA



- **II. Please answer the questions below:**
- **1. Please demonstrate the basic structure of immunoglobulin.**
- **2. What are the functions of antibodies?**
- **3. What are the differences of primary and secondary response for antibody production?**
- **4. How to understand the passive Ab therapy?**
- **(hint: COVID-19 therapy)**