

Humoral immunity

Antibodies structure & function

Humoral Immunity

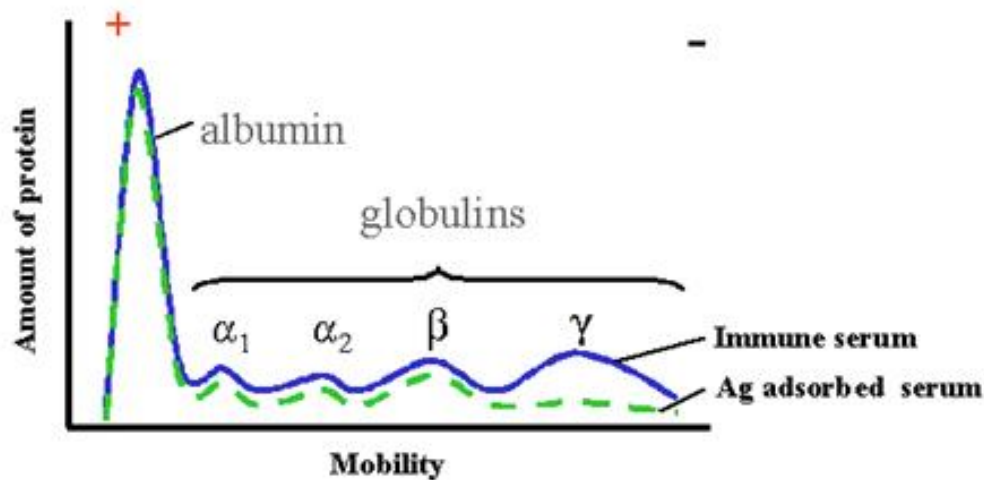
- Also called **Antibody mediated immunity**
- Humoral immune response constitutes **3rd line of defense**
- Humoral immune response results in production of special proteins called **antibodies** or **immunoglobulins**
- Antibodies are produced by B cells
- Antibody secreting B cell is called **plasma cells**
- Antibodies can be found in secretions or on the cell surface

Immunoglobulins

- **Immunoglobulin** - Glycoprotein molecules that are produced by plasma cells in response to an immunogen and functions as antibody

Discovery

- Human serum electrophoresis
- **Adsorbed serum** experiments revealed antibodies belong to gamma peak of globulins
- Gamma globulin shot



Immunoglobulins

- Lets study general function first
- Structure of antibodies
- Structure function relationship

General functions of Abs

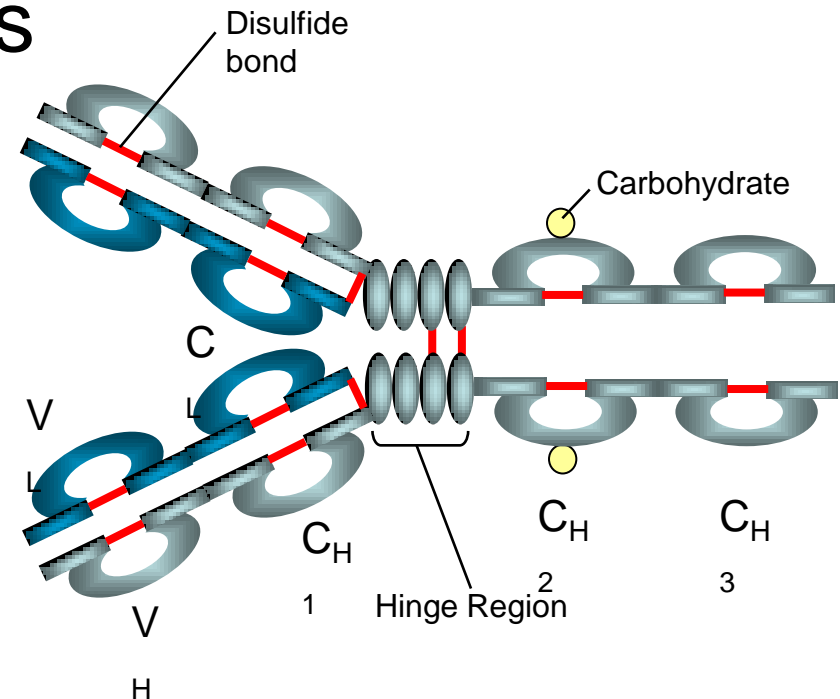
- **Antigen binding**
 - Just by binding to antigen can offer protection
 - Bacteria or virus needs to attach to host cell to infect. If Abs block it, no infection
- **Valance**
 - Each Ab molecule will bind 2 epitopes

General functions of Abs

- **Effector functions**
 - Complement fixation
 - Fixes complement after binding to antigen
 - Binding to various cells
 - Many cells like macrophages, neutrophils and **mast cells**... have receptors for antibodies
 - **Receptor mediated endocytosis**

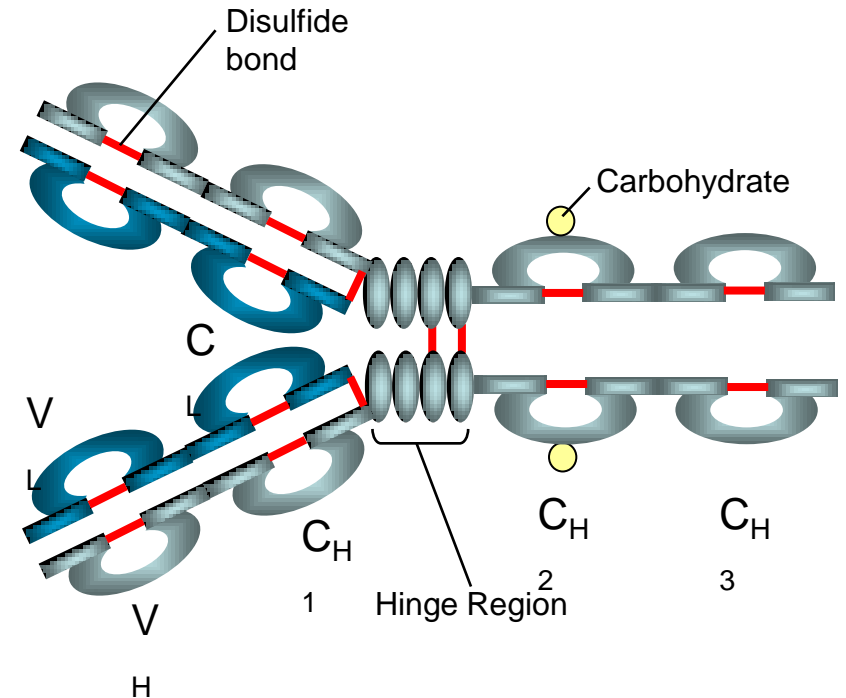
Structure of Abs

- Major problem to study structure is the heterogeneous nature
- Myeloma proteins gave homogenous immunoglobulins
- Heavy & light chains
- Disulfide bonds
 - Inter chain
 - Intra chain



Structure of Abs

- Variable & Constant regions
 - Part of molecule is highly variable rest is fairly constant
 - V_L & C_L
 - V_H & C_H
- Hinge regions
 - Gives flexibility



Structure function relationship

- Fragments of immunoglobulin

- *Papain digestion*

- Gives 2 fragments

- Fab

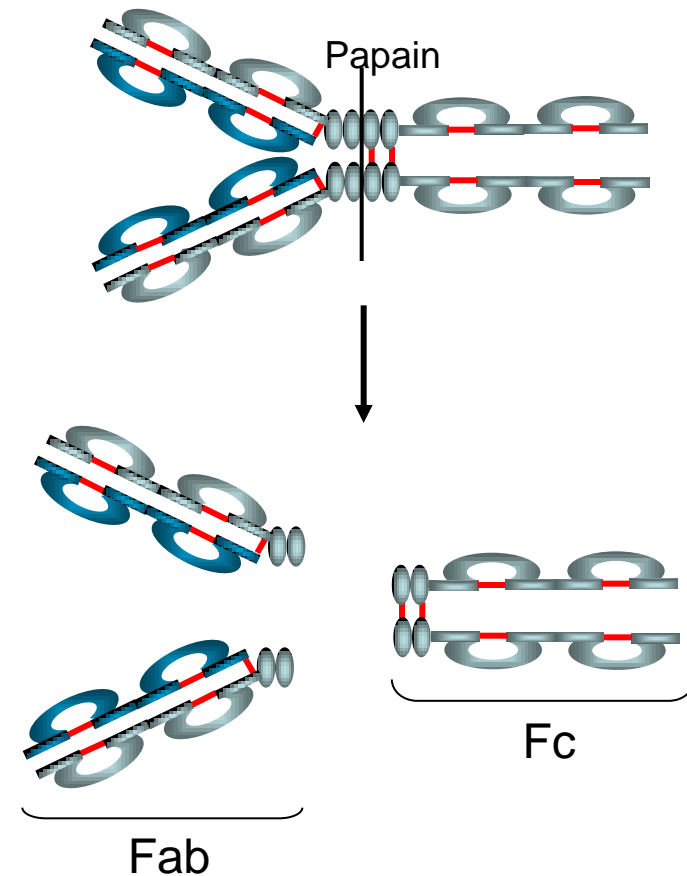
- Ag binding

- Specificity determined
By V_H and V_L regions

- Fc

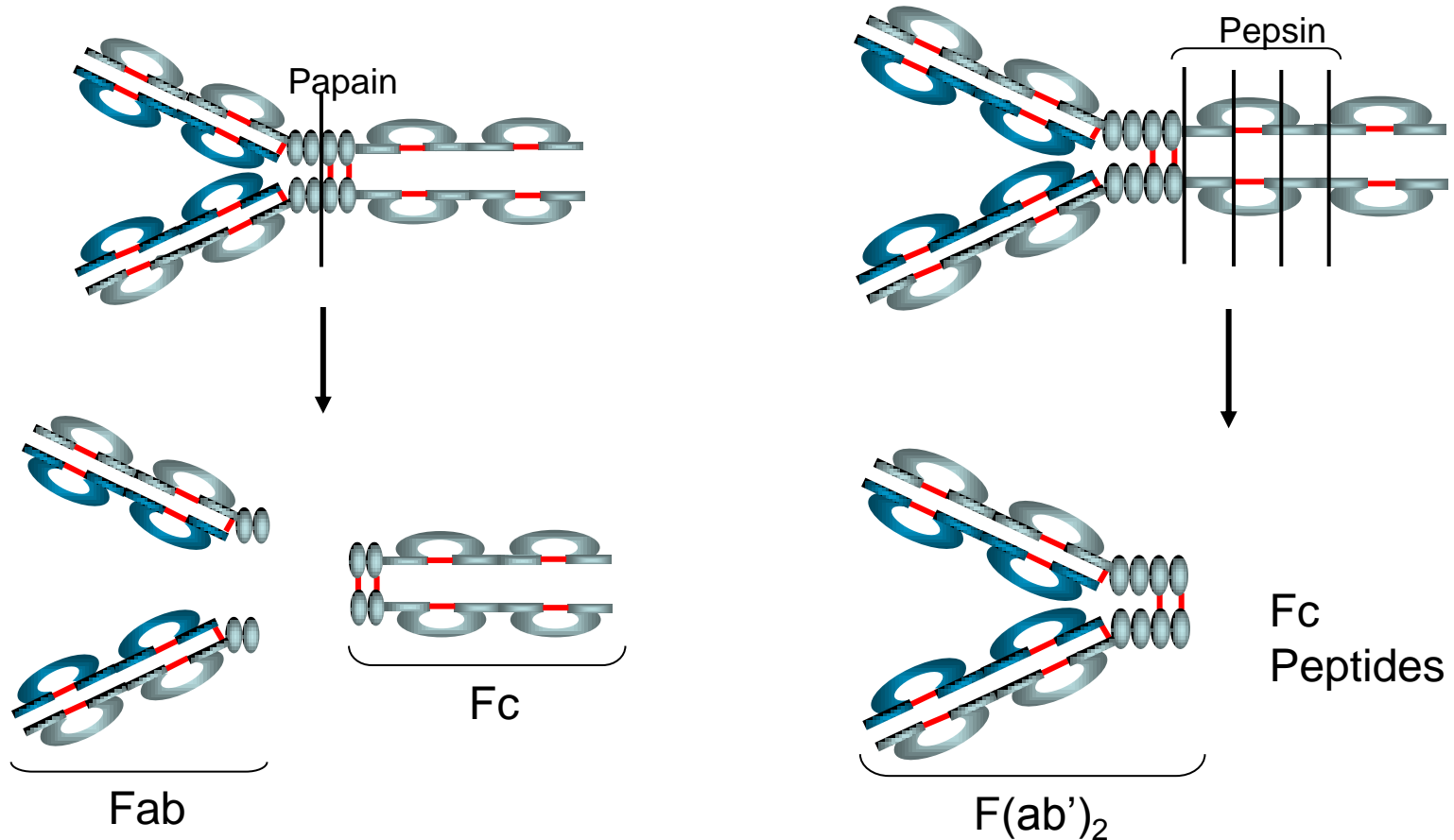
- Crystallizable fragment

- Biological activity

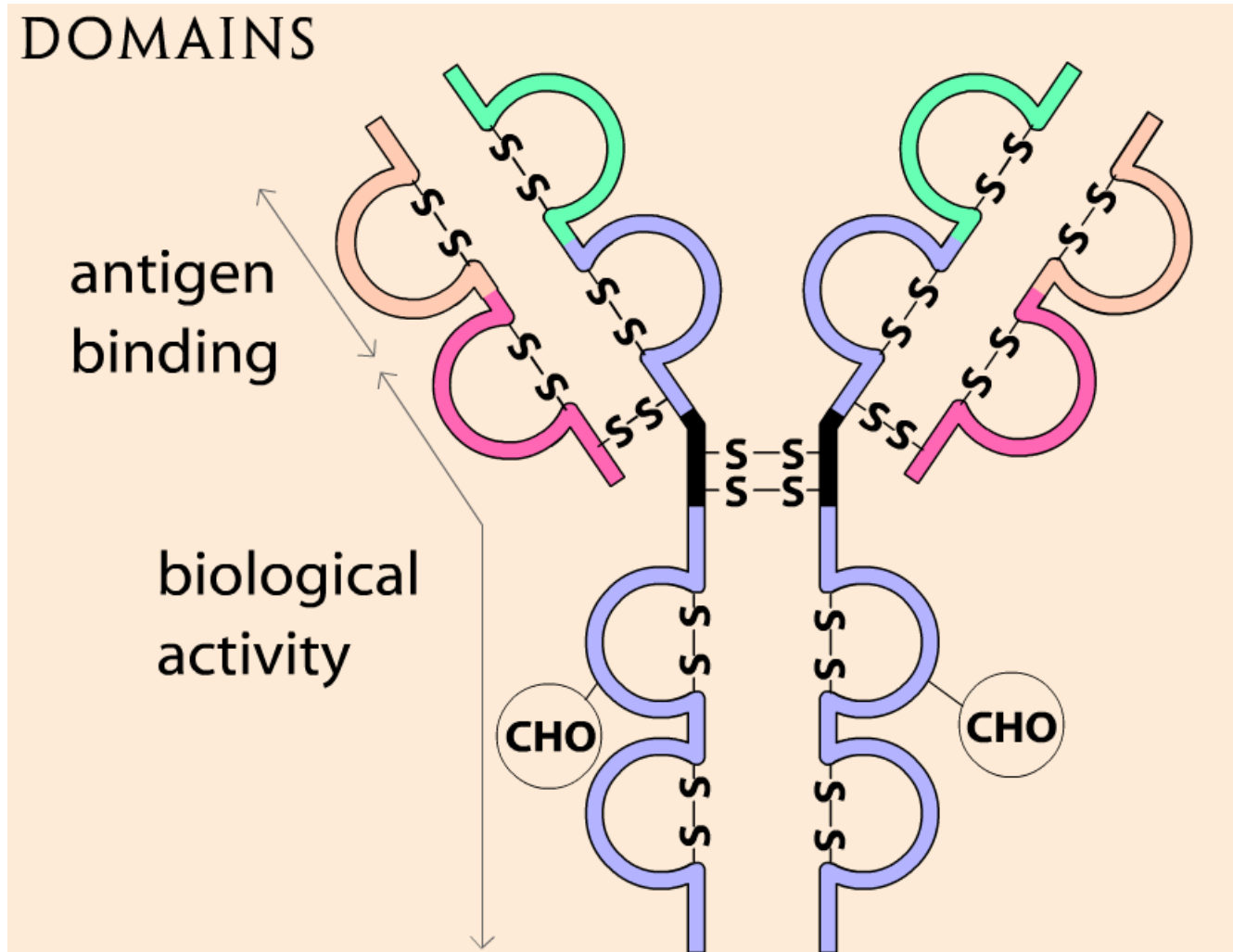


Structure function relationship

- Fragments of immunoglobulin
 - *Pepsin digestion*



Domains of Ab.



Classes of immunoglobulins

- Based on the amino acid sequence of constant region of heavy (C_H) chain 5 **classes** of Ig
- IgG – gamma (γ) - IgG1, IgG2, IgG3, IgG4
- IgA – alpha (α) - IgA1, IgA2
- IgM – Mu (μ)
- IgD – Delta (δ)
- IgE – Epsilon (ϵ)

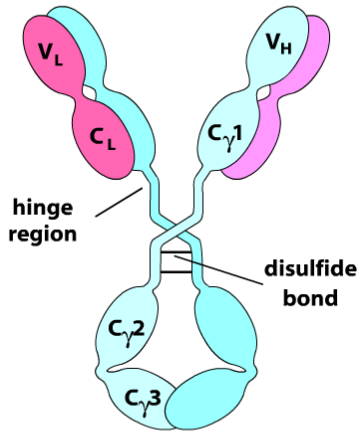
Classes of immunoglobulins

- Based on light chain amino acid sequence two **types** light chains
 - Kappa (κ)
 - Lambda (λ)
 - λ has 4 sub types
 - $\lambda 1$
 - $\lambda 2$
 - $\lambda 3$
 - $\lambda 4$

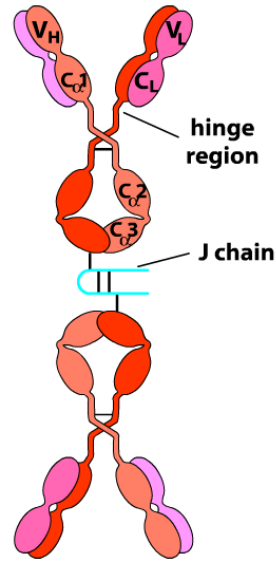
Classes of immunoglobulins

- **Nomenclature**
- Names are given in such a way that it tells what type of chains are there in Ig
 - IgM (kappa)
 - IgA1 (lambda 2)
 - IgG
- Combination of these heavy and light chains gives heterogeneity to immunoglobulins

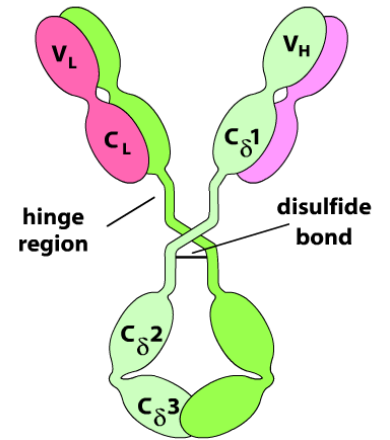
Properties of antibodies



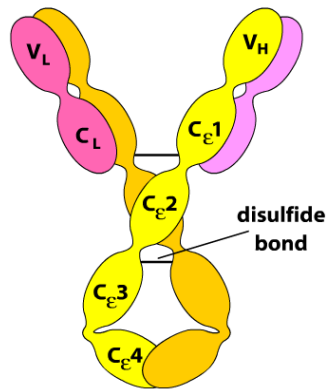
IgG



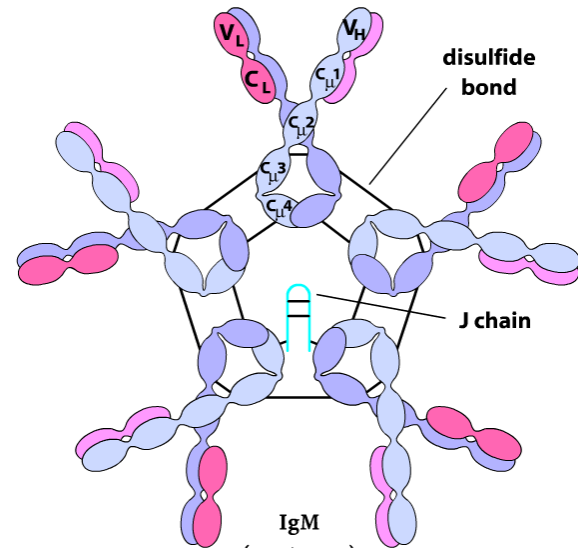
IgA (dimer)



IgD



IgE



IgM
(pentamer)

Properties of antibodies

Ig Class	Heavy chain	subclasses	Light Chain	Molecular formula
IgG	γ	$\gamma 1$ $\gamma 2$ $\gamma 3$ $\gamma 4$	κ or λ	$\gamma_2\kappa_2$ $\gamma_2\lambda_2$
IgA	α	$\alpha 1$ $\alpha 2$	κ or λ	$(\alpha_2\kappa_2)_{1, 2, 3, \text{ or } 4}$ $(\alpha_2\lambda_2)_{1, 2, 3, \text{ or } 4}$
IgM	μ	None	κ or λ	$(\mu_2\kappa_2)_{1 \text{ or } 5}$ $(\mu_2\lambda_2)_{1 \text{ or } 5}$
IgE	ϵ	None	κ or λ	$\epsilon_2\kappa_2$ $\epsilon_2\lambda_2$
IgD	δ	None	κ or λ	$\delta_2\kappa_2$ $\delta_2\lambda_2$

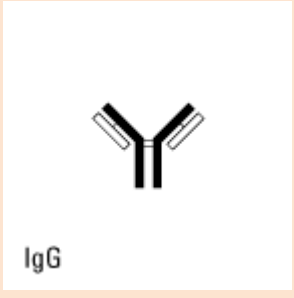

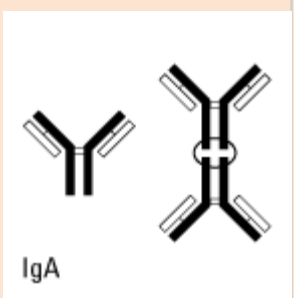
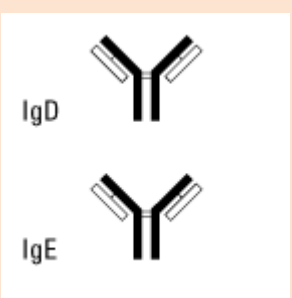

Properties of Antibodies

Ig Class	subclasses	Heavy-chain	Molecular weight	Polymer Unit
IgG	IgG1	$\gamma 1$	150,000	Monomer
	IgG2	$\gamma 2$		
	IgG3	$\gamma 3$		
	IgG4	$\gamma 4$		
IgA	IgA1	$\alpha 1$	150,000- 600,000	Monomer
	IgA2	$\alpha 2$		Dimer Trimer Tetramer
IgM		μ	900,000	Monomer (membrane) Pentamer (secreted)
IgE		ϵ	190,000	Monomer
IgD		δ	150,000	Monomer

Properties of Antibodies

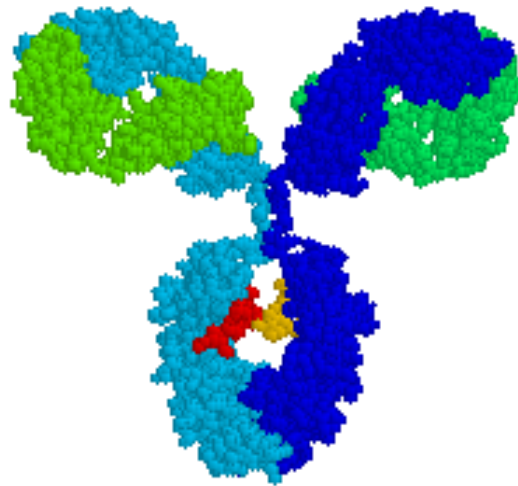
Ig Class, subclass	Serum levels (mg/ml)	Half-Life (days)	Biological Activity
IgG1	9.0	23	A. Activates classical complement pathway. (IgG1+, IgG2+/-, IgG3++, IgG4 -)
IgG2	3.0	23	B. Crosses placenta. (IgG1++, IgG2+/-,
IgG3	1.0	8	IgG 3+, IgG4 -)
IgG4	0.5	23	C. Binds to Fc receptors of phagocytes. (IgG1++, IgG2+/-, IgG3++, IgG4 +)
IgA1	3.0	6	Mucosal transport. (1 and 2 ++)
IgA2	0.5	6	
IgM	1.5	5	A. Activates classical complement pathway. (+++) B. Present on membrane of mature B cells. (+) C. Mucosal transport. (+) D. May bind to Fc receptors of phagocytes. (?) E. First isotype produced by neonate and during a primary response.
IgE	0.0003	2.5	Induces mast cell degranulation. (+)
IgD	0.03	3	Present on membrane of mature B cells. (+)

Properties of Antibodies

	IgG	IgM	IgA	IgD	IgE
Mol. Wt.	150,000	900,000	320,000	180,000	200,000
H chain type	gamma	mu	alpha	Delta	Epsilon
Serum conc. mg/ml	10 - 16	0.5 - 2	1 - 4	0 - 0.4	10 - 400 ng/ml
Distribution	Intra and extra vascular	Mostly intra vascular	Secretions and intra vascular	Surface of lymphocytes	Mast cells, basophils, nasal secretions
Function	Major role in secondary response	Primary response	Protect mucous membranes	Probably antigen presentation	Against parasites and allergies
	 <p>IgG</p>	 <p>IgM</p>	 <p>IgA</p>	 <p>IgD</p>  <p>IgE</p>	

Next class

Functions of Antibodies.....

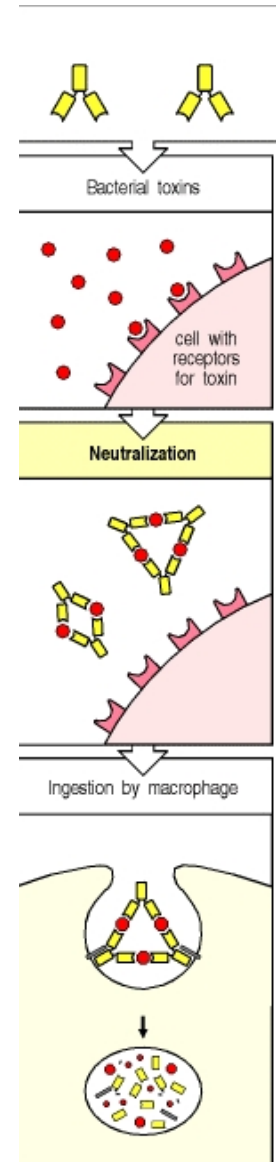


Functions of Ab

- Mainly 3 functions
 - Neutralization
 - Opsonization
 - Complement fixation

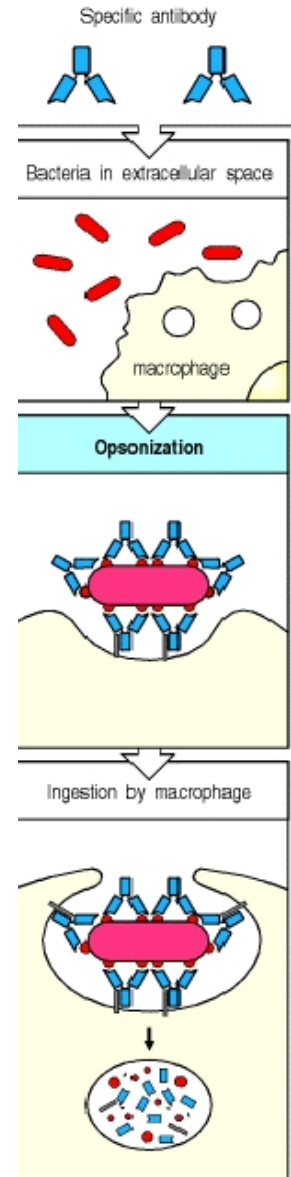
Nutralization

- **Nutralization**
 - Antibody participating in nutralization reaction is called **nutralizing antibody**
 - By binding to the antigen effects the biological function of the antigen
 - Forms complex with antigen which in turn are phagocytosed



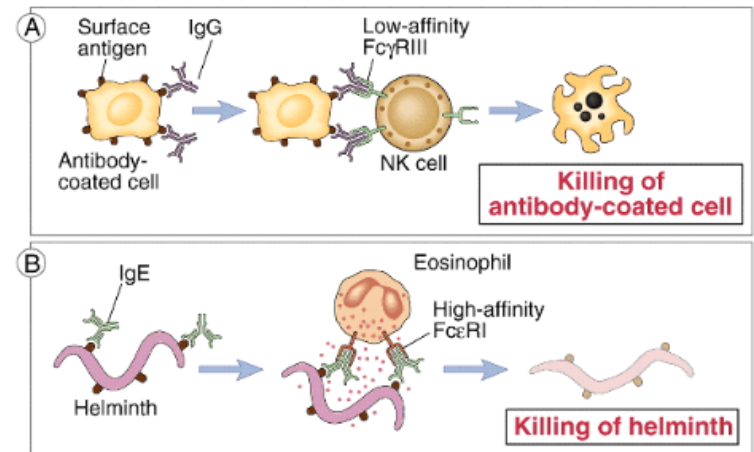
Opsonization

- **Opsonization**
 - Antibody participating in Opsonization reaction is called **Opsonin**
 - By binding to the antigen effects the biological function of the antigen
 - Forms complex with antigen which in turn are phagocytosed
 - Opsonin acts as mark / stamp to phagocytosis



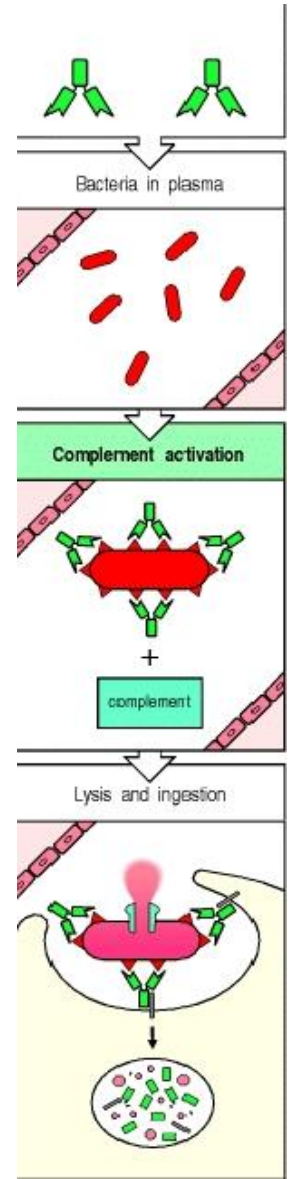
ADCC

- **Antibody dependent cellular cytotoxicity**
- Antibody coated cells are killed by released substances of effector cells like Neutrophils, Eosinophils, **Mast cells**.....
- Antibody coated cells are not phagocytosed



Complement fixation

- **Complement fixation**
 - Complement system helps (complements) the ability of antibody in clearing the antigen from the system
 - It is a system of many small proteins once activated or triggered leads to formation of **membrane attack complex**



Membrane attack complex

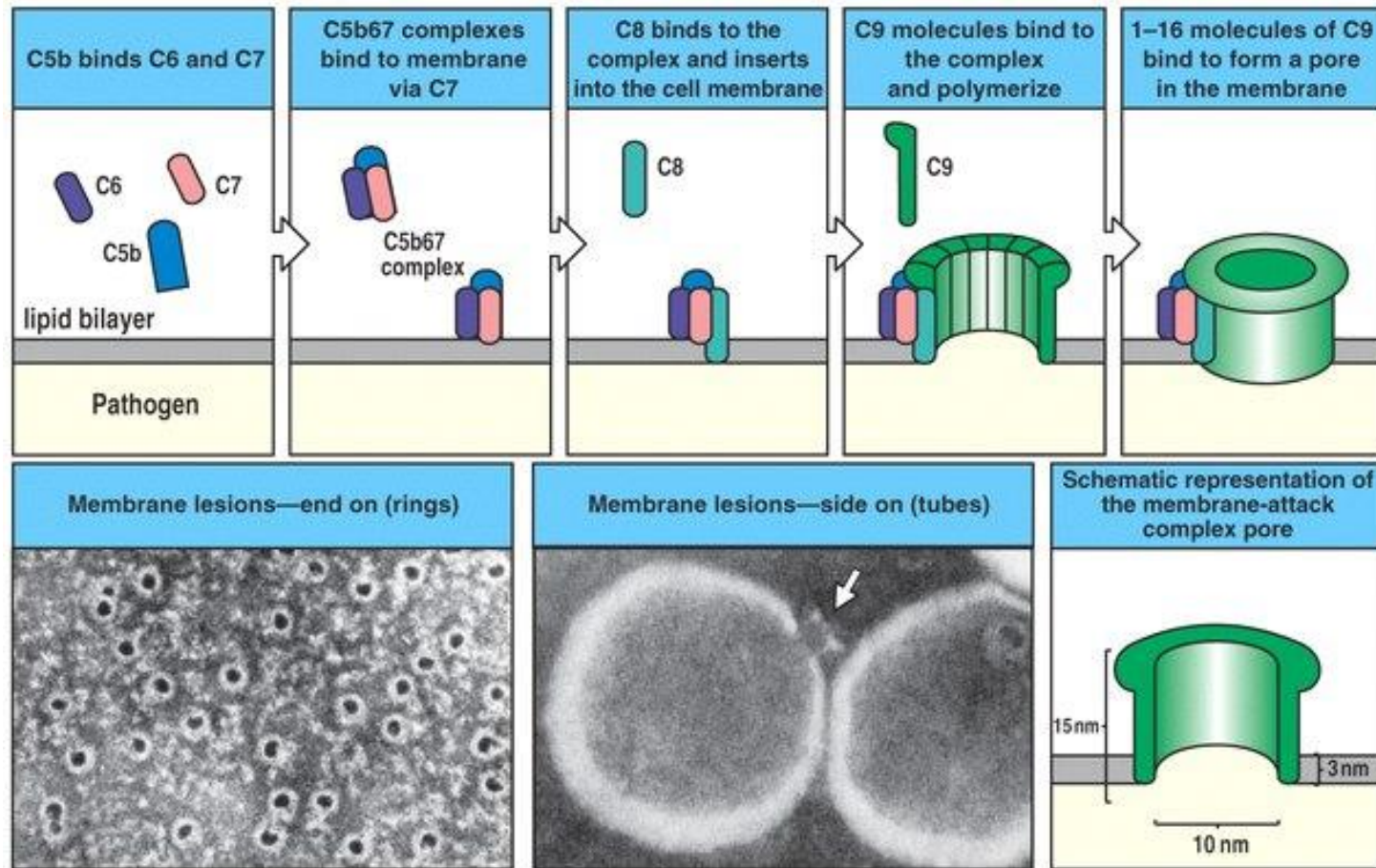
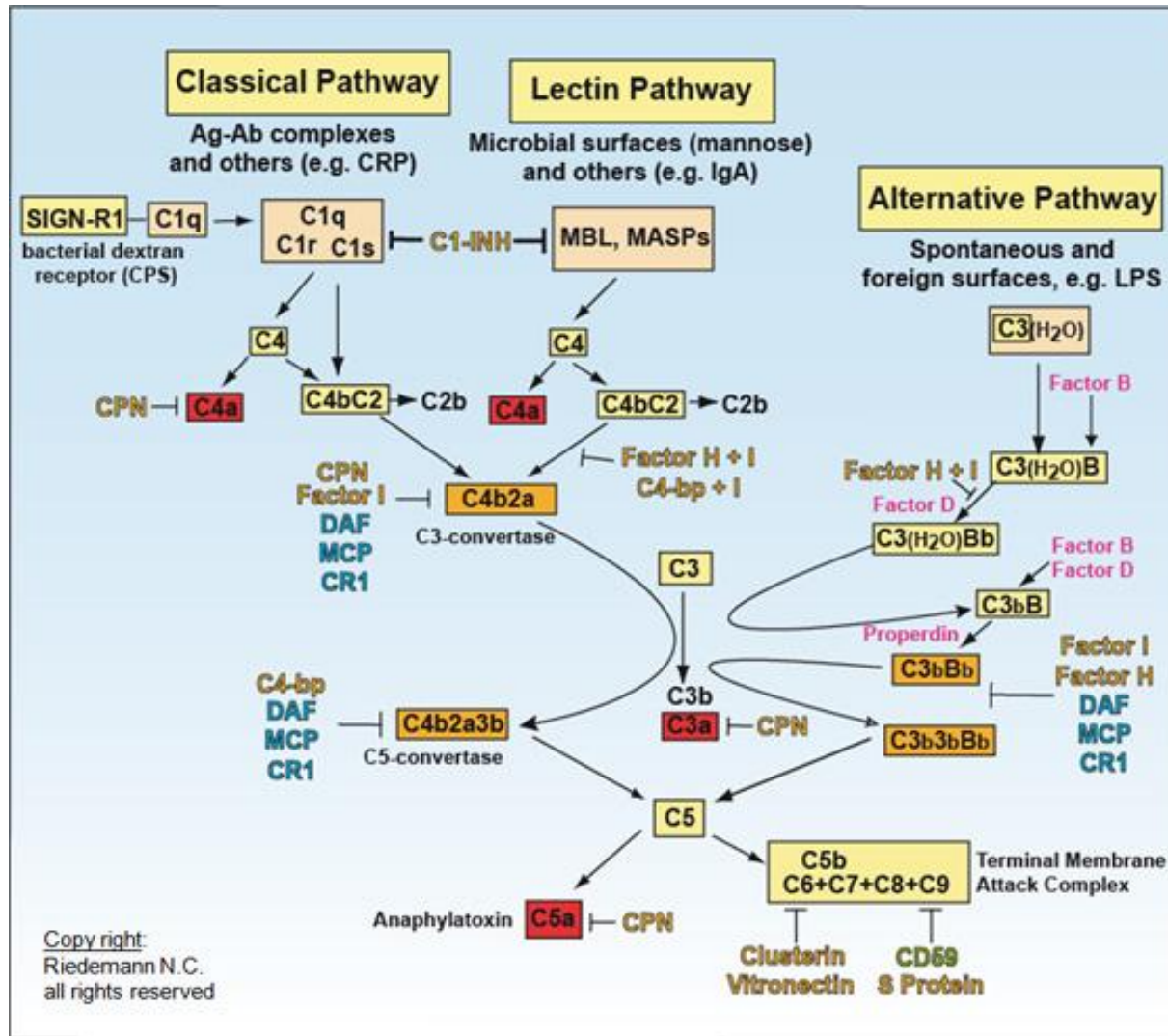


Figure 2-35 Immunobiology, 6/e. (© Garland Science 2005)

Complement pathways



Next class....

- Isotypes
- Allotypes
- Ideotypes
- Ig biosynthesis