Humoral Immunity
(Nature, Mechanisms and Kinetics)

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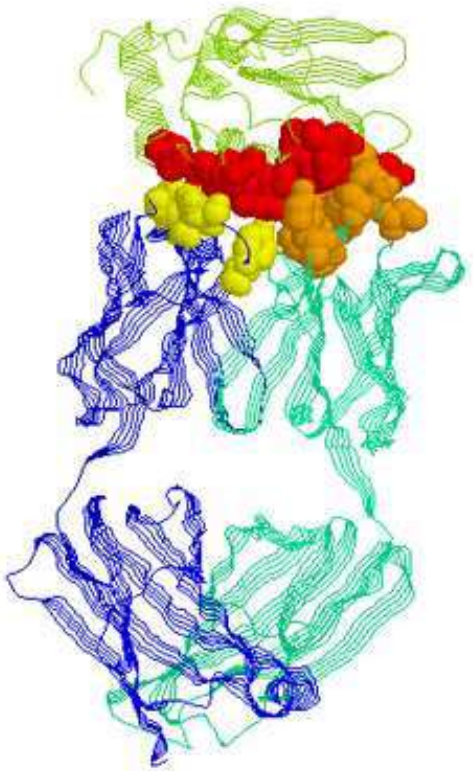
Nature of Ag/Ab Reactions

- **Lock and Key Concept**
- **Non-covalent Bonds**
  - Hydrogen bonds
  - Electrostatic bonds
  - Van der Waal forces
  - Hydrophobic bonds
  - **Require very close fit**
- **Multiple Bonds**
- **Reversible**
- **Specificity**

http://www.med.sc.edu:85/chime2/lyso-abfr.htm



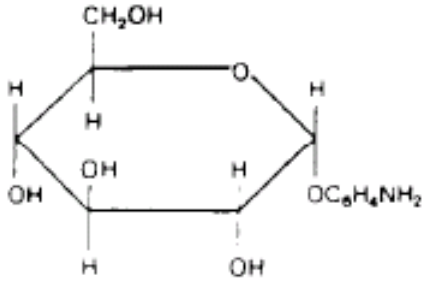
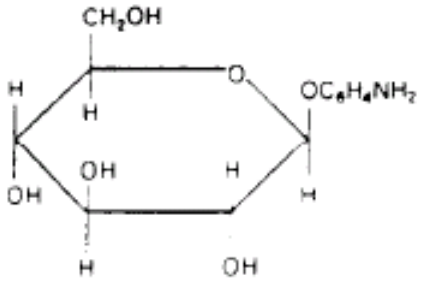
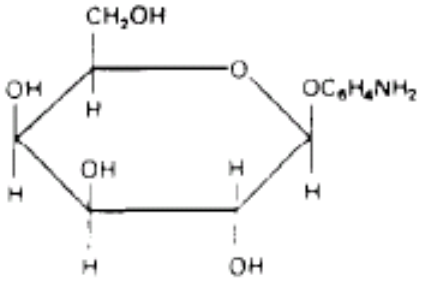
Source: Li, Y., Li, H., Smith-Gill, S. J., Mariuzza, R. A., Biochemistry 39, 6296, 2000

Specificity

- The ability of an individual antibody combining site to react with only one antigenic determinant.

- The ability of a population of antibody molecules (polyclonal antiserum) to distinguish minor structural differences between the original antigen or hapten and similar, yet structurally different antigens or haptens.

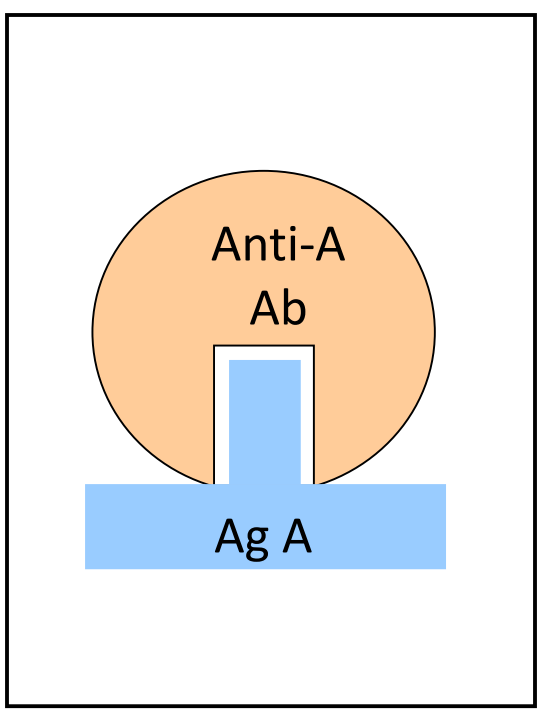
Table 3–2. Reactions of antisera with isomeric glucoside protein conjugates.*
(+ represents precipitation)

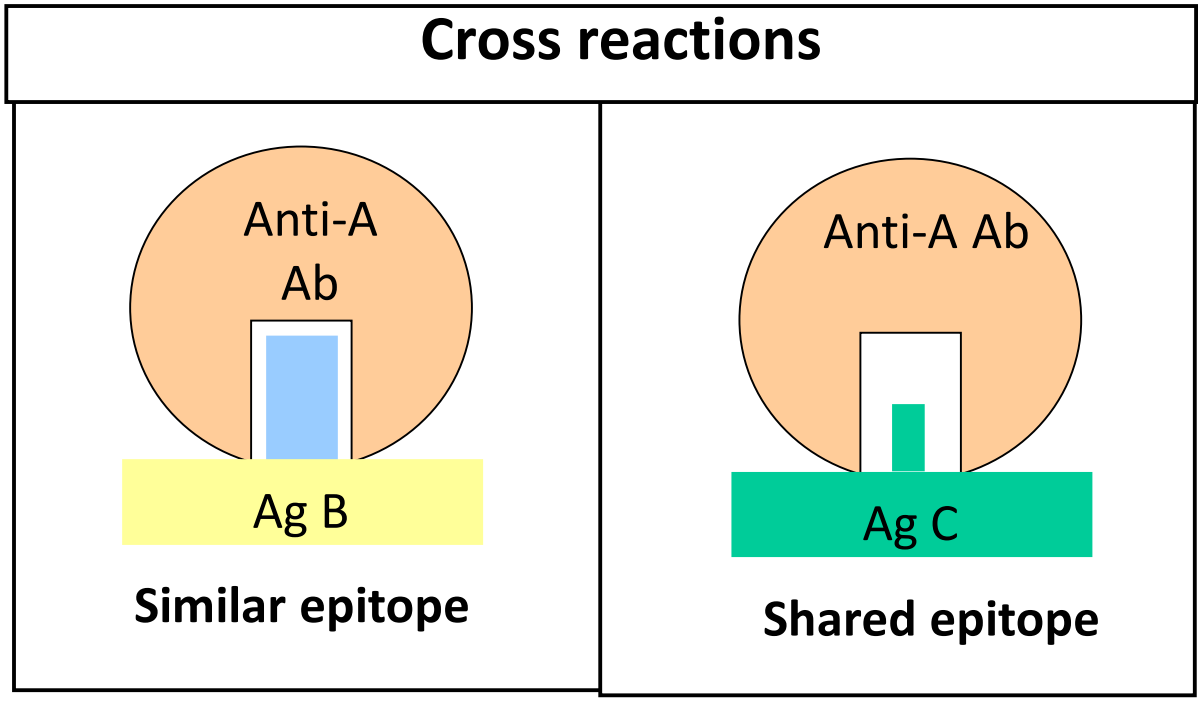
	p-Aminophenol α-glucoside	*p*-Aminophenol β-glucoside	*p*-Aminophenol β-galactoside
			
Antisera against:			
α-Glucoside	+++	++	0
β-Glucoside	++	+++	0
β-Galactoside	0	0	+++

*Reproduced, with permission, from Humphrey JH, White RG: *Immunology for Students of Medicine.* Davis, 1970.

Cross Reactivity

- The ability of an individual Ab combining site to react with more than one antigenic determinant.
- The ability of a population of Ab molecules to react with more than one Ag.
- The two may share one or more identical epitopes or one or more structurally similar, yet different epitopes





AFFINITY, AVIDITY AND VALENCY

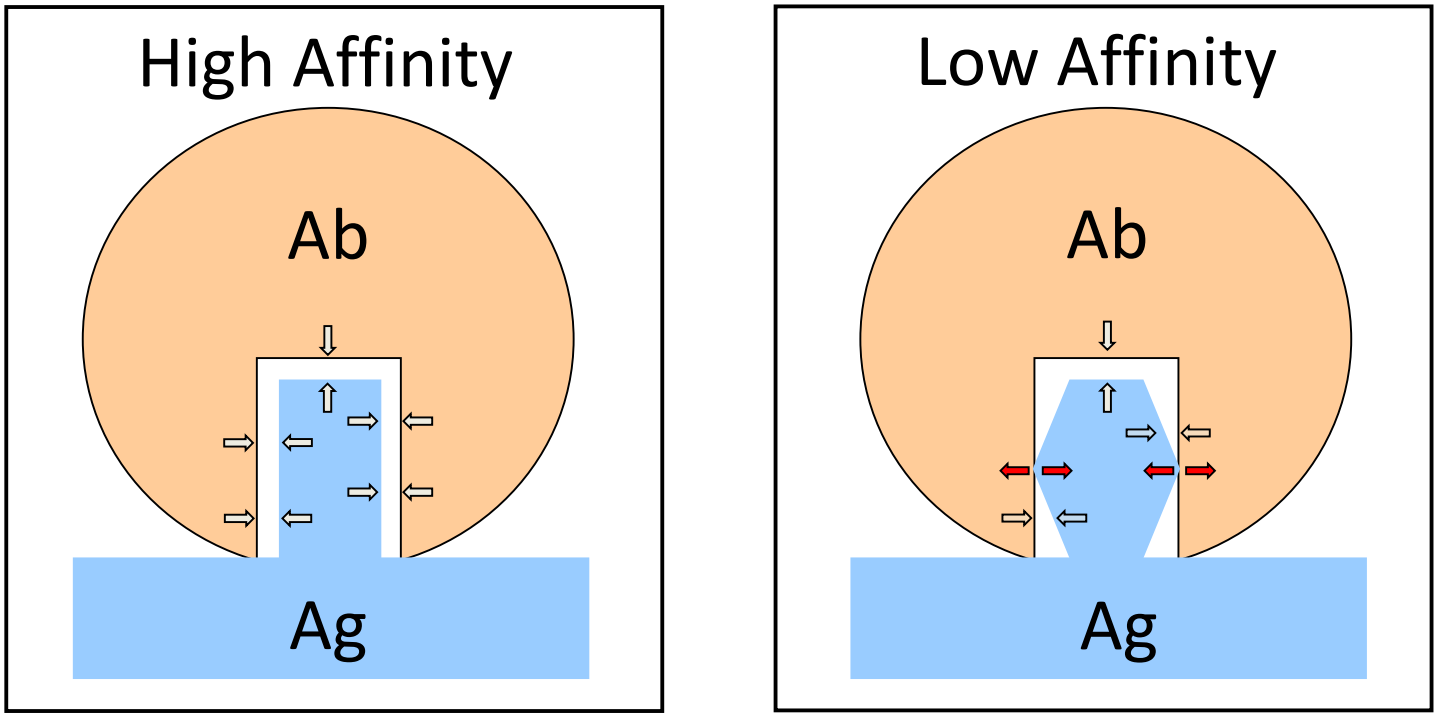
- **Affinity** refers to strength of binding of single epitope on an antigen to its paratope in an antibody molecule. It refers to the intrinsic reaction between paratope and epitope

- **Avidity** refers to total binding strength of an antibody molecule to an antigen. Thus avidity (strength of binding) is influenced by both affinity (Ka of single binding site) x Valence of interaction (number of interacting binding sites). It refers to the functional enhancement of affinity due to multivalency of antibody

- **Valency of antibody** refers to the number of antigenic determinants that an individual **antibody** molecule can bind. The **valency** of all **antibodies** is at least two and in some instances more. Thus IgG has valency of 2 (bivalent) and IgM has of 10 (decavalent)

Affinity

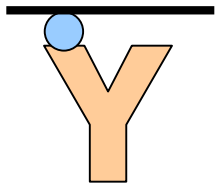
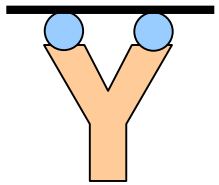
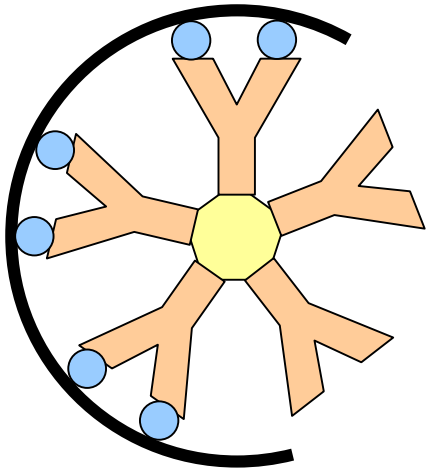
Strength of the reaction between a single antigenic determinant (epitope) and a single Ab combining site (paratope)



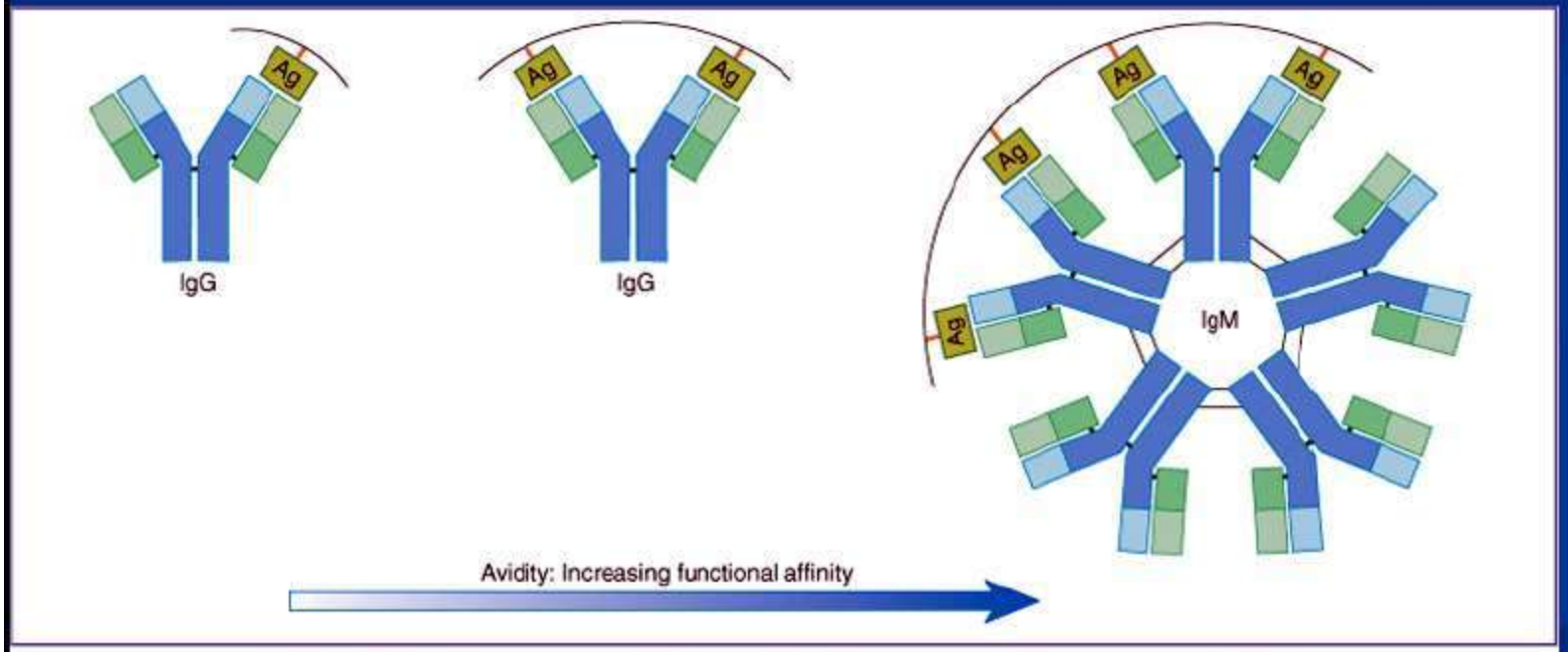
Affinity = ∑ attractive and repulsive forces

Avidity

The overall strength of binding between an Ag (with multiple determinants) and multivalent Abs

			
K_{eq} =	10^4	10^6	10^{10}
	Avidity	Avidity	Avidity

Affinity vs. Avidity



Avidity-Refers to the functional enhancement of affinity due to multivalency of Ab.

MECHANISMS OF ACTION OF ANTIBODIES *IN VIVO*

- **Neutralization:** Antibody “neutralizes” toxins, binds to attachment molecules
- **Preventing Bacterial Adherence**
- **Opsonization:** Antibody binds to pathogen surface molecules and promotes phagocytosis
- **Complement activation:** occurs on antibody bound to pathogens
- **Antibody dependent cell cytotoxicity**

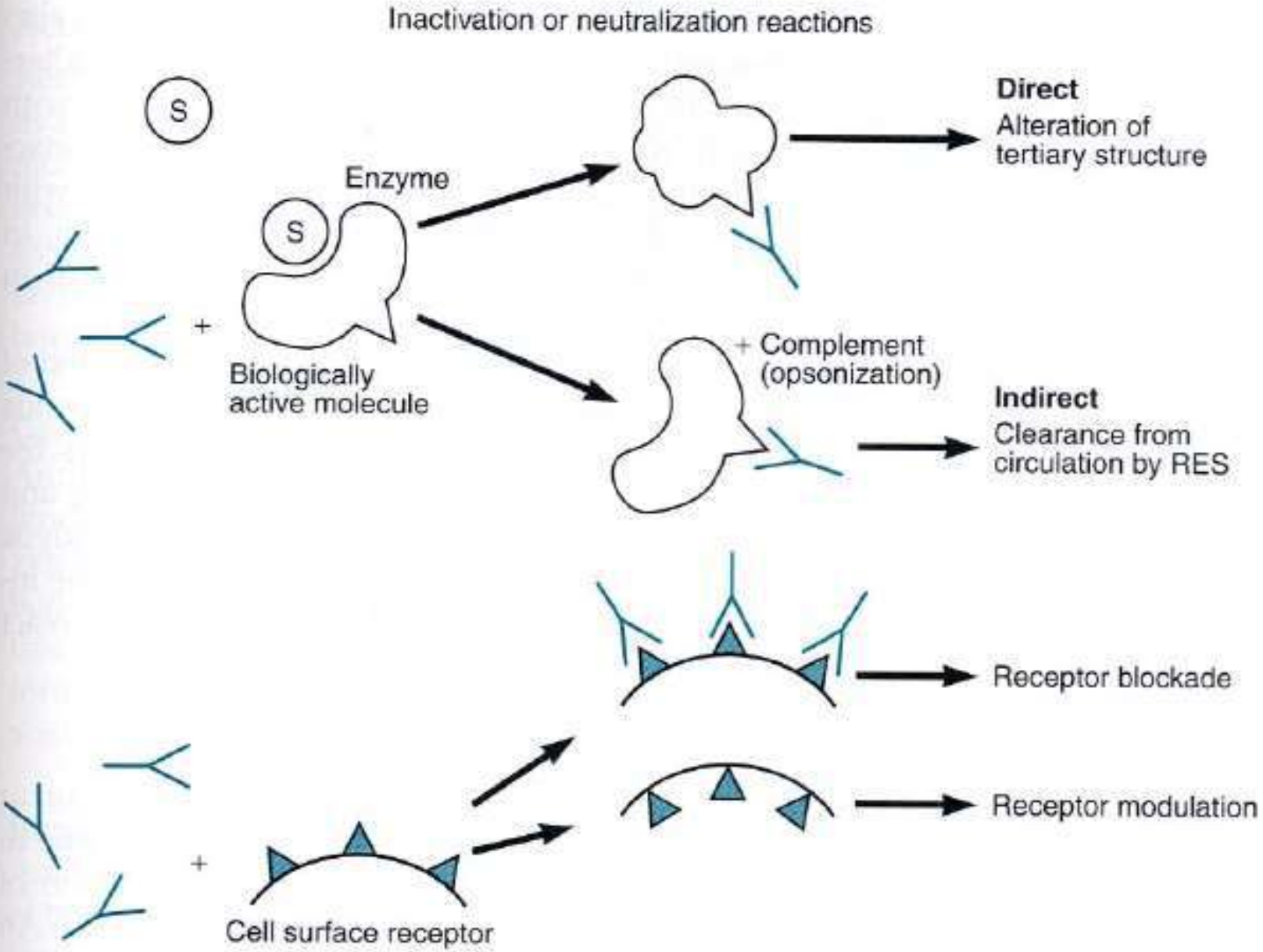
Neutralization

Definition:

Binding of an antibody to an epitope resulting in inactivation, neutralization or abnormal activation.

Mechanisms:

- Ab binding to protein can inhibit binding to substrate or alter its conformation resulting in inactivation
- Ab binding to a pathogen can block receptors, alter structure or opsonize
- Autoimmune Abs against a hormone or a neurotransmitter receptor can either block or activate the receptor


Inactivation or neutralization reactions
S
Enzyme
S
+
Biologically
active molecule
Direct
Alteration of
tertiary structure
+ Complement
(opsonization)
Indirect
Clearance from
circulation by RES
Receptor blockade
Receptor modulation
+
Cell surface receptor

Neutralization

Binding of an antibody to an epitope resulting in inactivation, neutralization or abnormal activation.

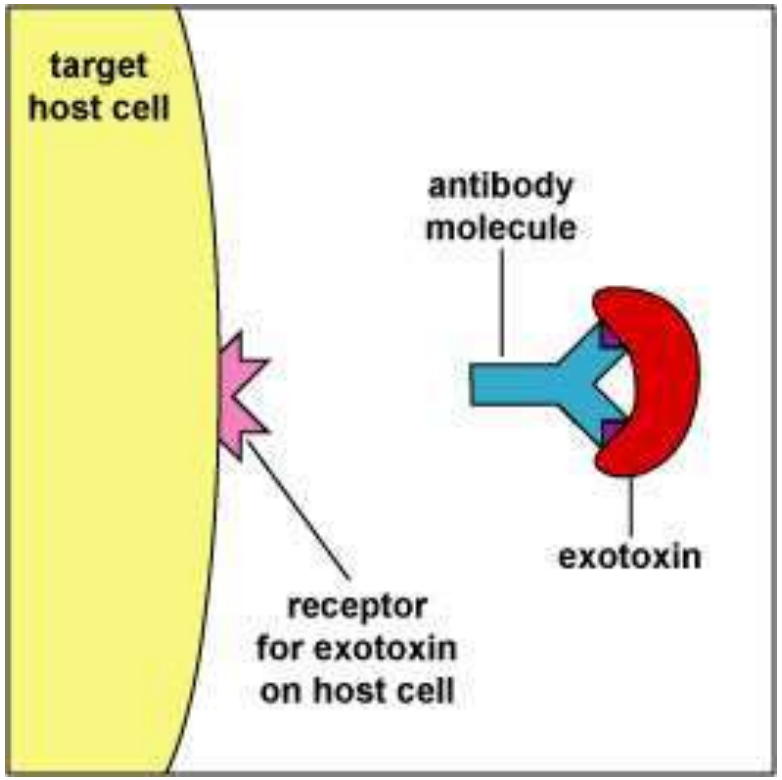
Neutralization of Toxin

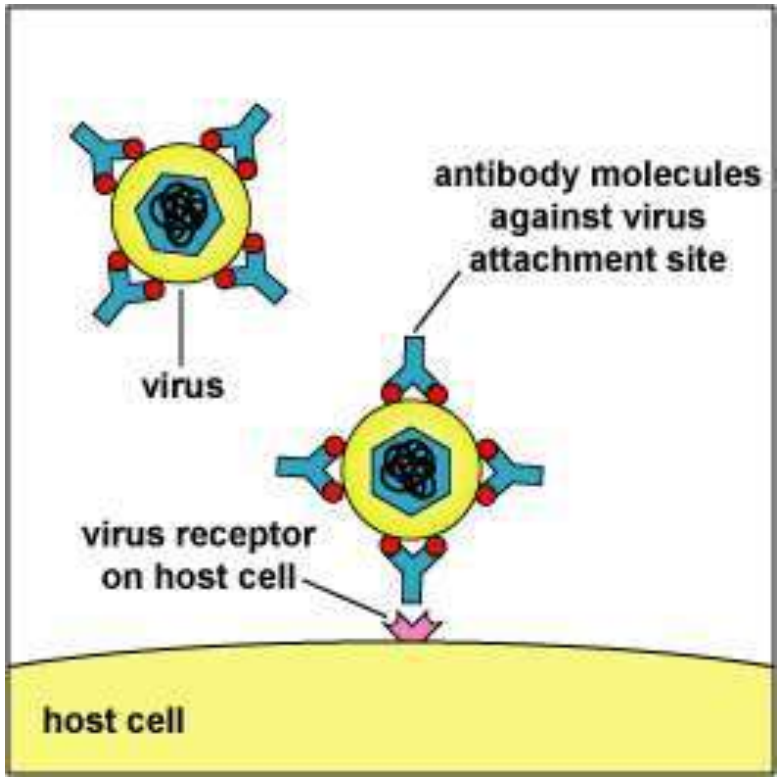
- An exotoxin must first bind to receptors on a susceptible host cell.
- Antitoxin antibodies are made against microbial exotoxins. The Fab portion binds to the exotoxin molecules before they can interact with host target cells and thus neutralizes the toxin.
- IgG neutralizes toxins in tissues while IgA neutralizes toxins at mucosal surfaces within the body.

Neutralization of Virus

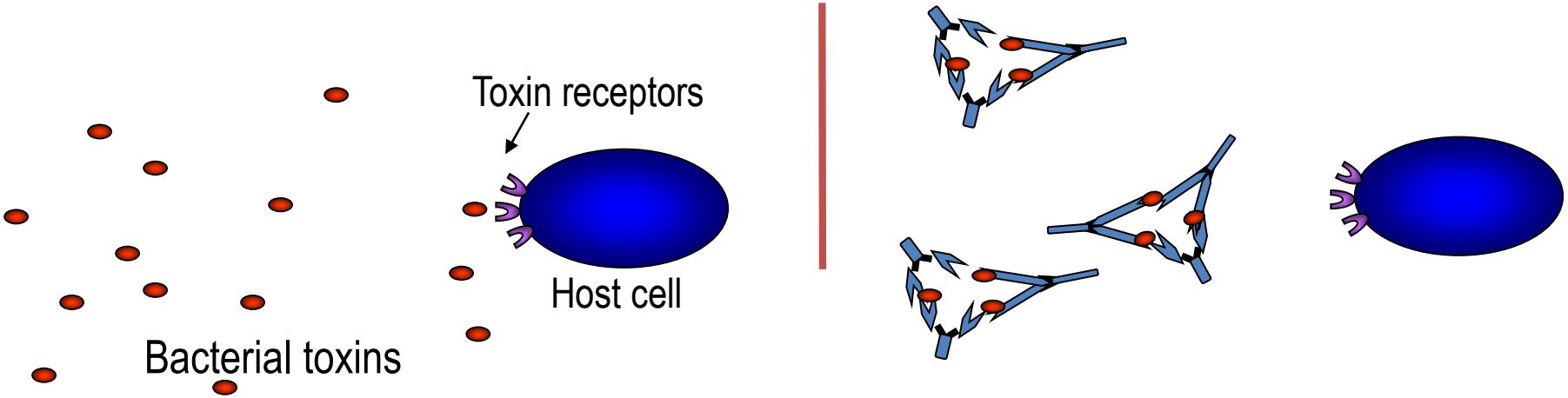
- In order for viruses to infect a cell and replicate, they must first adsorb to receptors on the host cell's plasma membrane.
- Antibodies made against viral capsids or envelope glycoproteins binds to and masks the viral attachment molecules.
- This prevents viral adsorption to host cells..

Neutralization

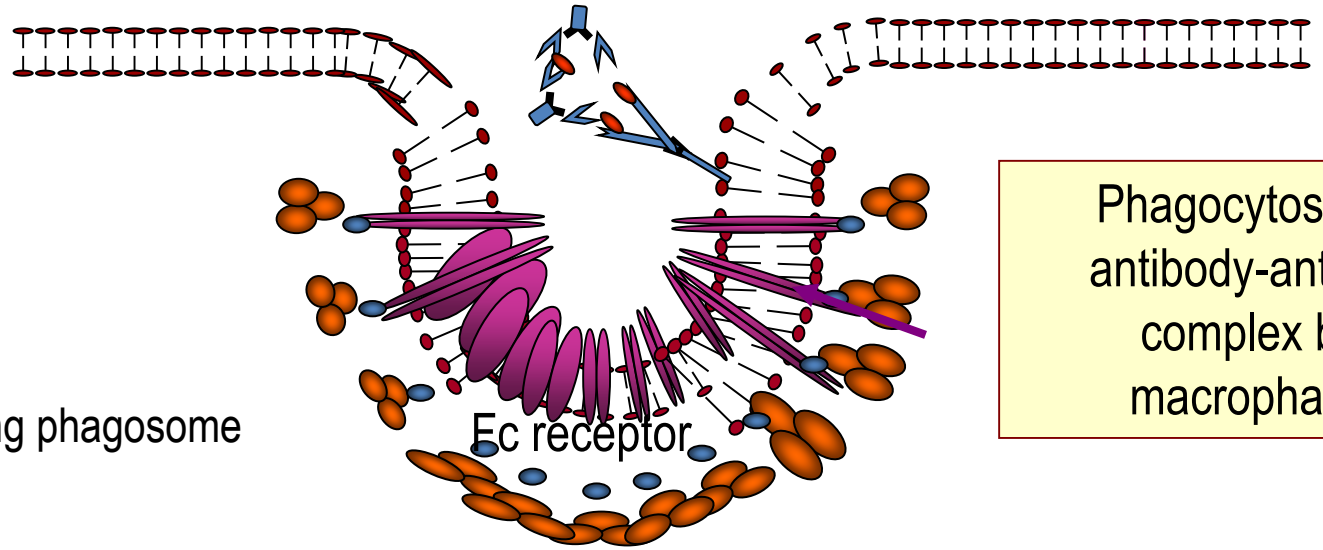




Neutralization



Neutralization by antibody



Phagocytosis of antibody-antigen complex by macrophage

Preventing Bacterial Adherence

- Bacteria resist physical removal by means of pili, cell wall adhesin proteins, and/or biofilm-producing capsules.
- Antibodies are made against pili, capsules, and cell wall adhesins.
- The binding of the Fab portion of the antibody to the adhesive tip of the pili, the cell wall adhesins, or the capsular molecules prevents the bacteria from adhering to and colonizing host cells.
- IgG blocks adherence of bacteria in tissues while IgA blocks adherence of bacteria at mucosal surfaces within the body.

Inactivation or Neutralization Reactions: Medical Aspects (Examples)

- Protective
  - Toxin inactivation (e.g. *C. diphtheriae* and *C. tetani* toxins)
  - Virus neutralization (polio, influenza, measles, mumps, rubella)
- Immunopathologic
  - Myasthenia gravis: autoimmune Ab to ACh receptors
  - Graves disease: Ab to TSH receptor activate thyroid cells (hyperthyroidism)
  - Pernicious anemia: Ab against intrinsic factor blocks binding of vitamin B12 in gut, B12 deficiency, anemia

Cytotoxic mechanisms

Definition:

Antibody binding to cell surfaces resulting in opsonization, complement activation, or antibody dependent cell cytotoxicity.

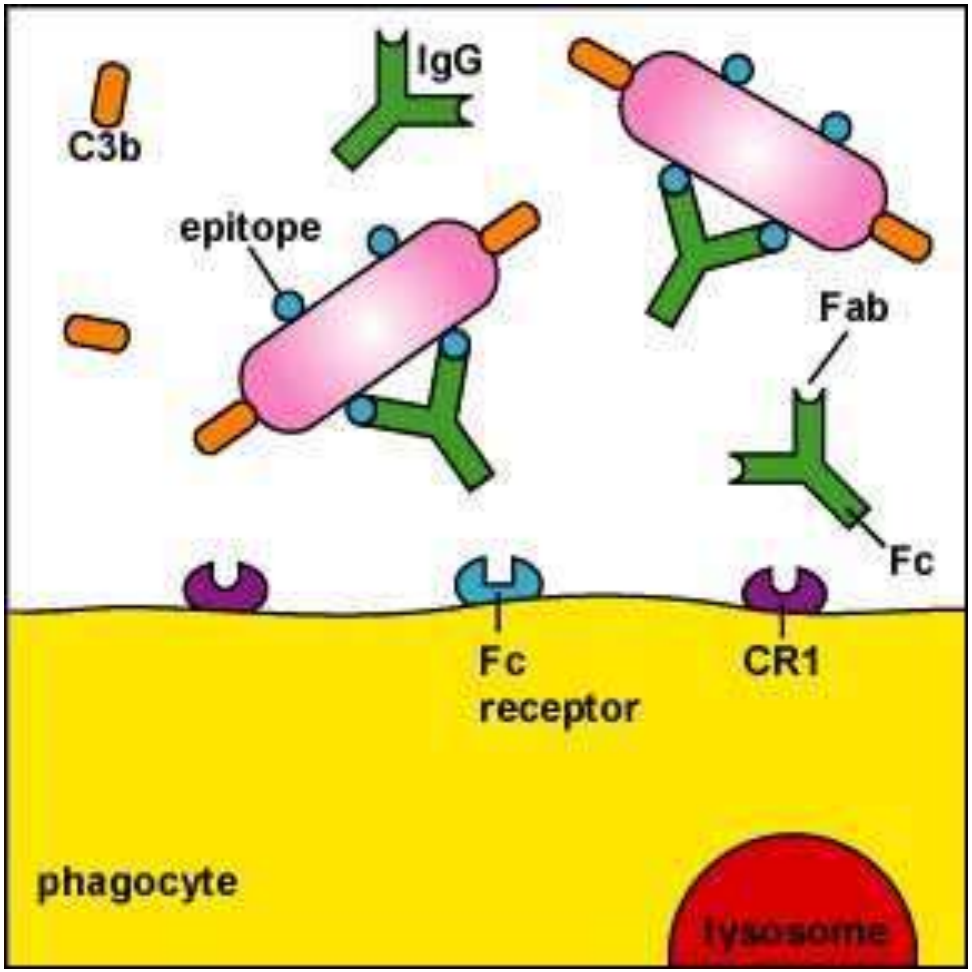
Mechanisms:

- Opsonization by IgG or complement components (C3b) resulting in enhanced phagocytosis.
- Activation of classical complement pathway resulting in formation of MAC
- ADCC via macrophages, NK or NKT cells
- IgE mediated binding of eosinophils to helminths (PMD)

Opsonization

- **Opsonization, or enhanced attachment**, refers to the antibody molecules, the complement proteins (C3b and C4b), and other opsonins attaching antigens to phagocytes and resulting in a much more efficient phagocytosis.
- The process starts with antibodies of the isotype IgG, IgA, or IgM being made against a surface antigen of the organism or cell to be phagocytosed.
- The Fab portions of the antibody react with epitopes of the antigen.
- The Fc portion of IgG (but not IgM) can then bind to receptors on neutrophils and macrophages thus sticking the antigen to the phagocyte.
- The Fc portion of secretory IgA can also bind to macrophages and neutrophils for opsonization.

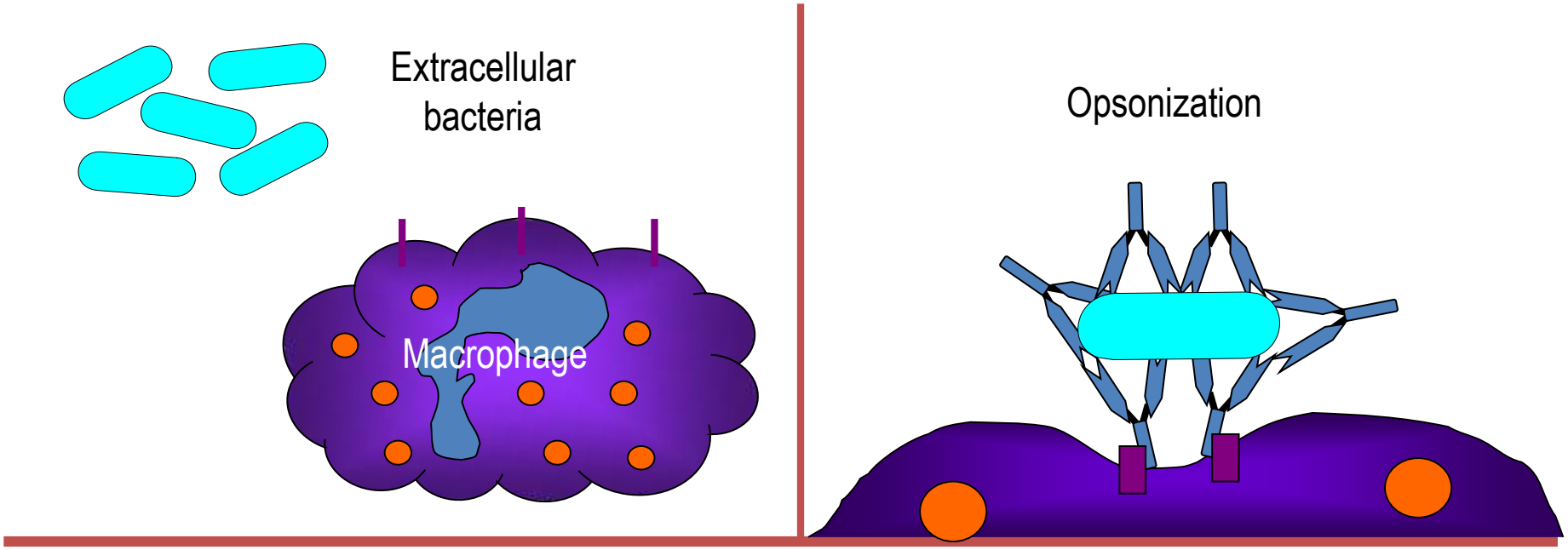
Opsonization



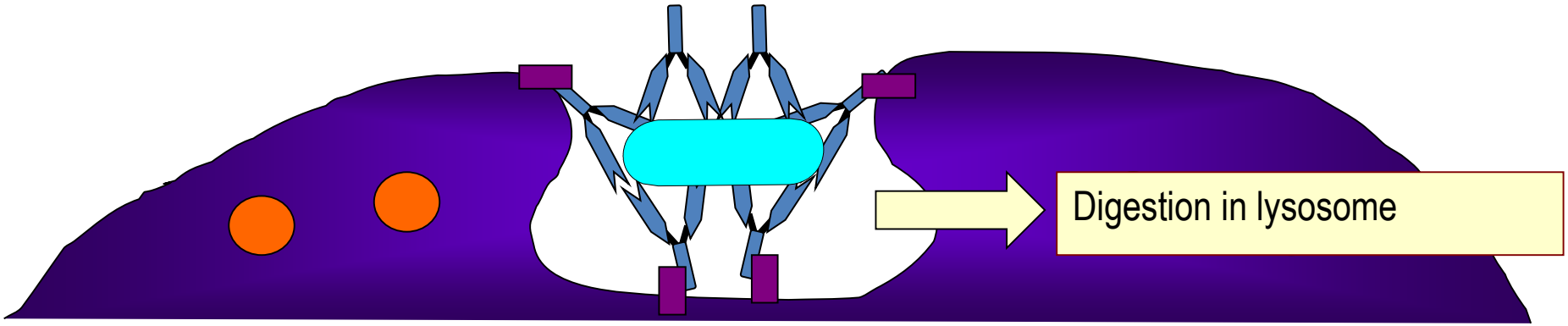
Opsonization

- Alternately, IgG, IgA, and IgM can activate the complement pathways and C3b or C4b, thus produced, can act as an opsonin and attach the antigen to phagocytes.
- One portion of the C3b binds to proteins and polysaccharides on microbial surfaces; another portion attaches to CR1 receptors on phagocytes, B-lymphocytes, and dendritic cells for enhanced phagocytosis.
- Opsonization is especially important against microorganisms with anti-phagocytic structures such as capsules since opsonizing antibodies made against the capsule are able to stick capsules to phagocytes

Opsonization



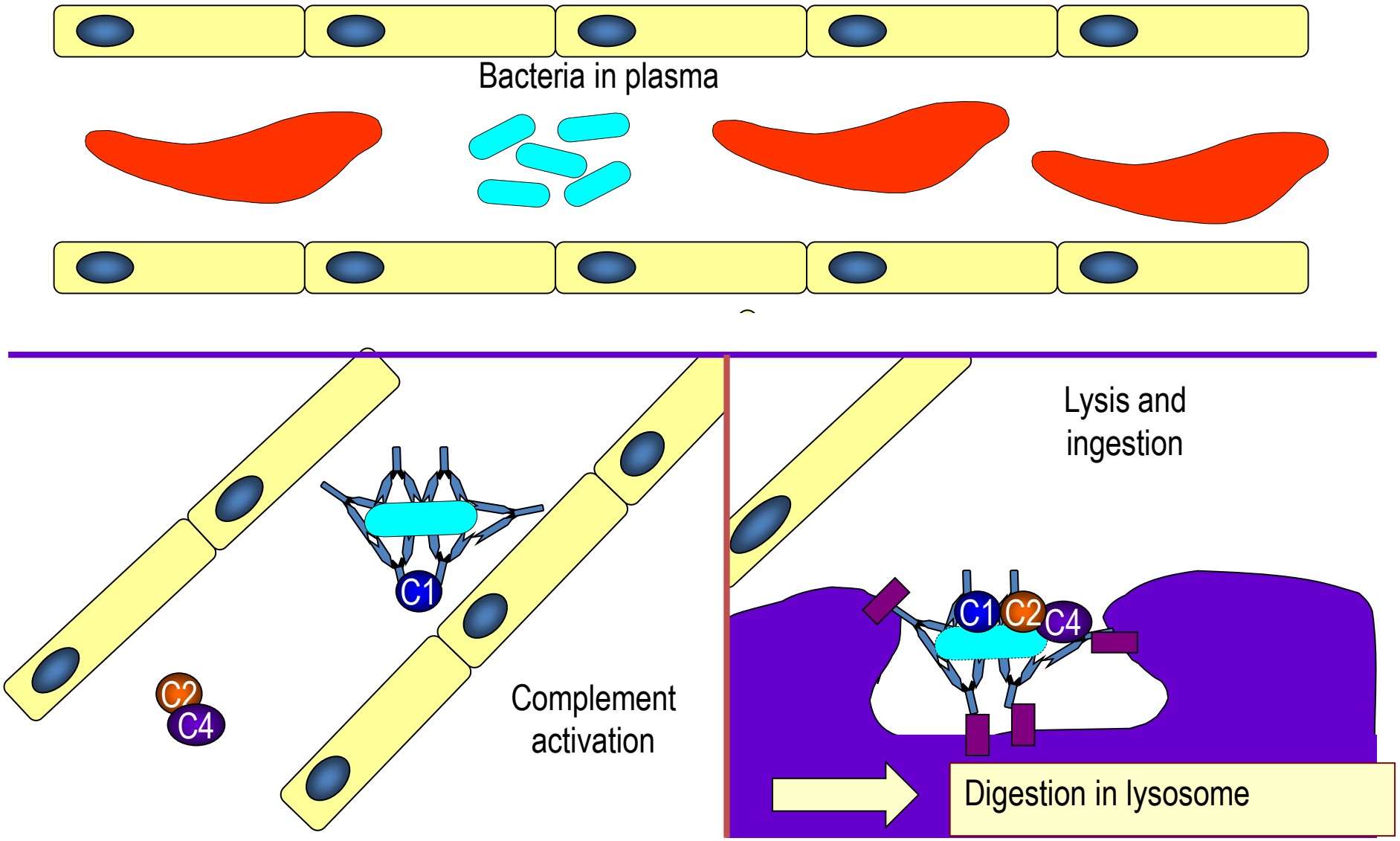
Ingestion by macrophage



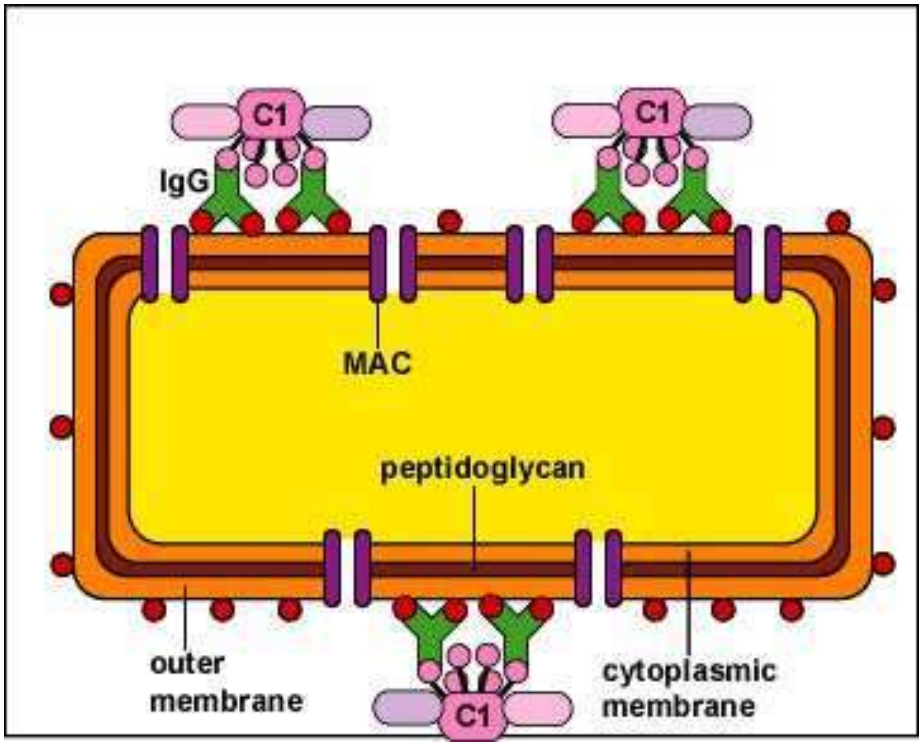
Complement Activation

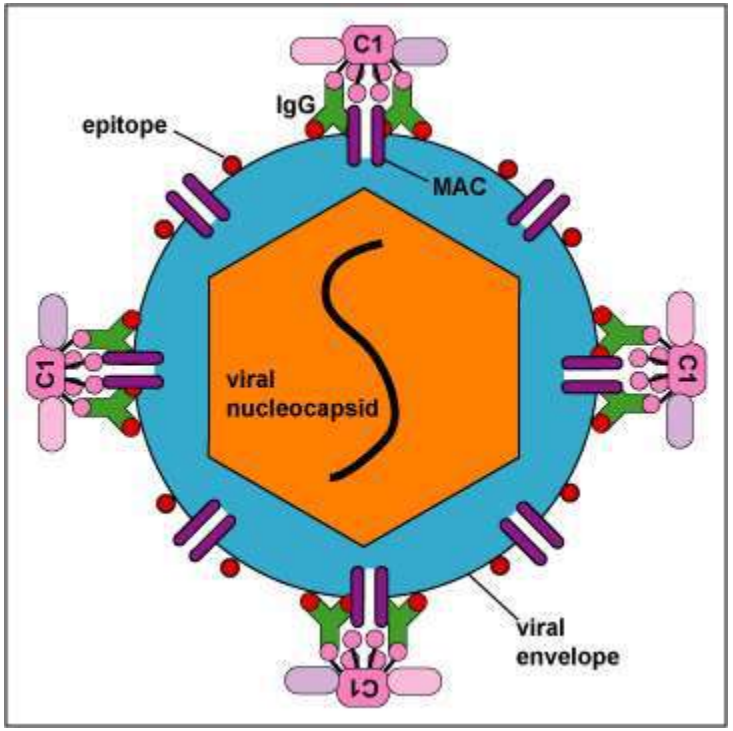
- The process starts with the antibody isotypes IgG or IgM being made against epitopes on heterologous membranes (e.g. bacterial cell wall).
- The Fab portion of IgG or IgM interacts with the epitopes on the membrane.
- The Fc portion of the antibody then activates the classical complement pathway to form $C5b6789_n$ (the membrane attack complex or MAC), which then punch holes in the heterologous membrane and results in the cytolysis.
- In the case of bacteria, MAC can punch holes in the outer membrane and possibly the cytoplasmic membrane of the Gram-negative cell wall causing lysis.
- In enveloped viruses, the MAC can damage the viral envelope

Complement Activation



Complement Activation

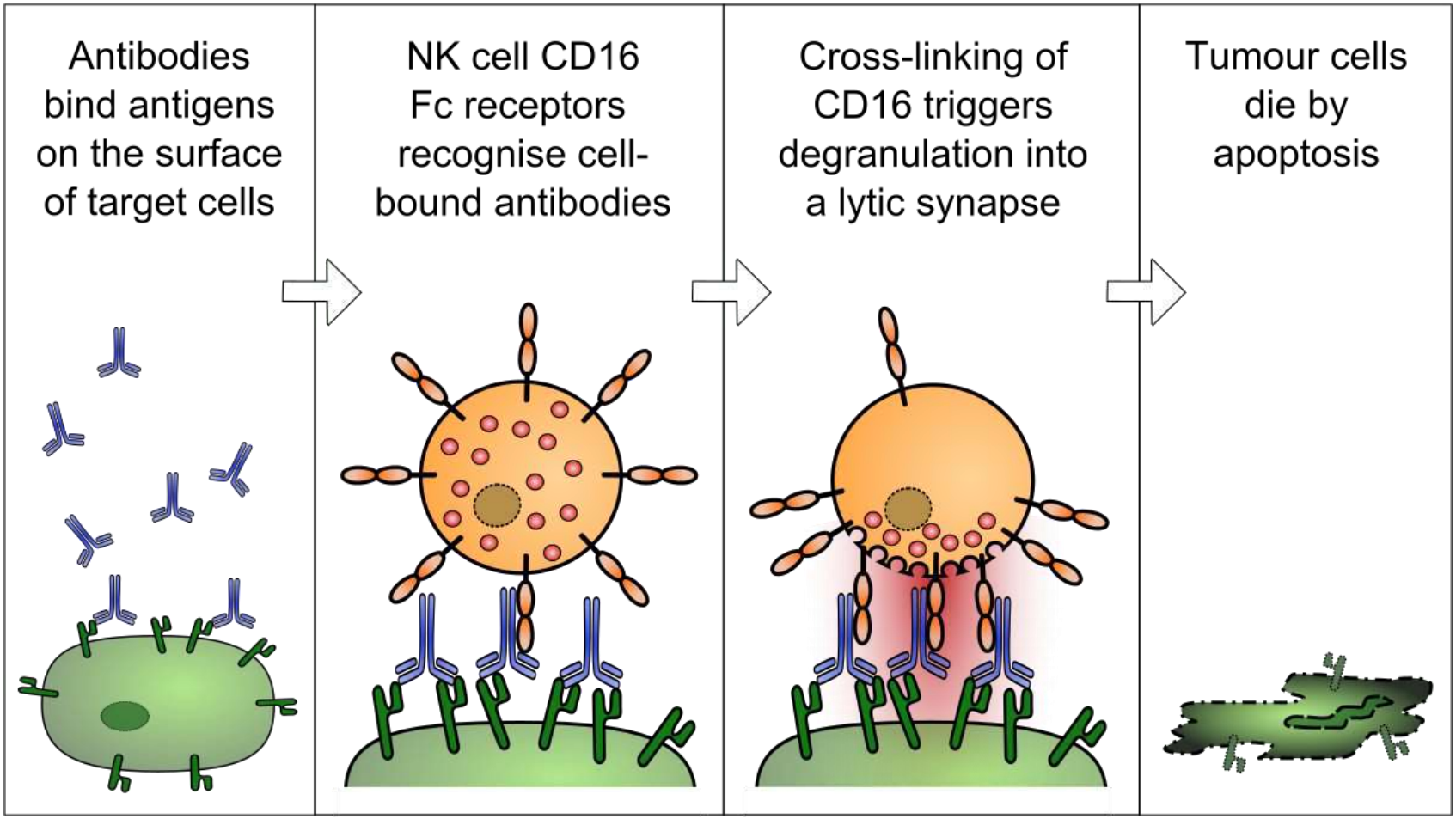




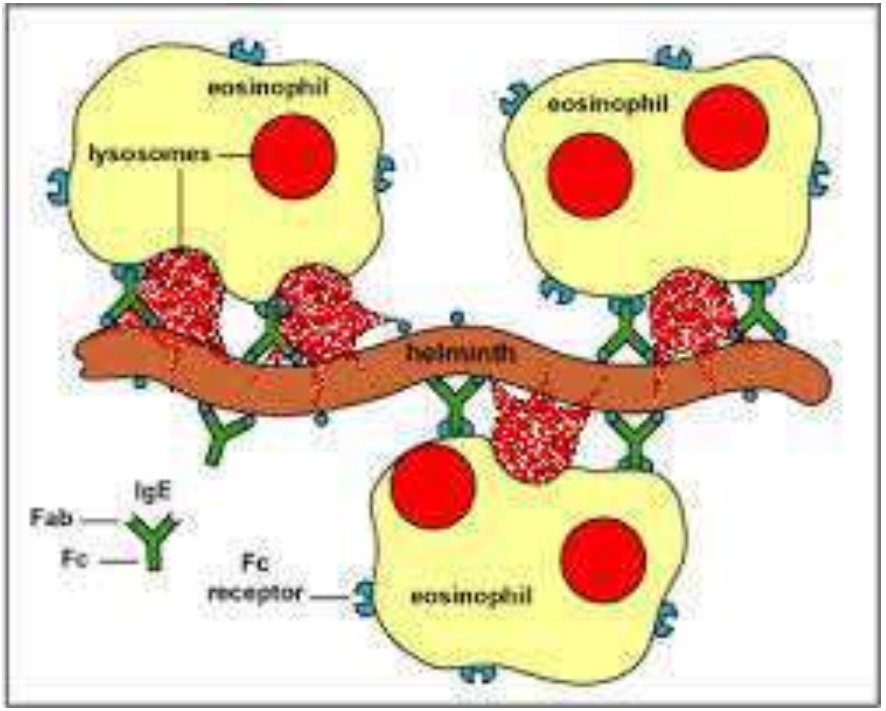
Antibody dependent cell cytotoxicity (ADCC)

- **ADCC is a mechanism where effector cells (NK or NKT cells) secrete cytotoxic molecules and lyse antibody-coated target cells.**
- ADCC depends on the bifunctional structure of IgG molecules.
- The fragment antigen-binding (Fab) of the Ab molecule bind to a specific viral or TAA associated on the surface tumor or the target cell.
- The fragment Fc of Ab bind with FcγRIII (CD16) present on surface of NK cell.
- On engagement of both ADCC is initiated since this creates a bridge from the tumor/target cell to the effector NK cell.
- The recognition of target cells is then combined to a lytic attack on the target cell mounted by effector cells.
- ADCC does not depend on the immune complement system in which targets are also lysed but no other cell is required.

ADCC

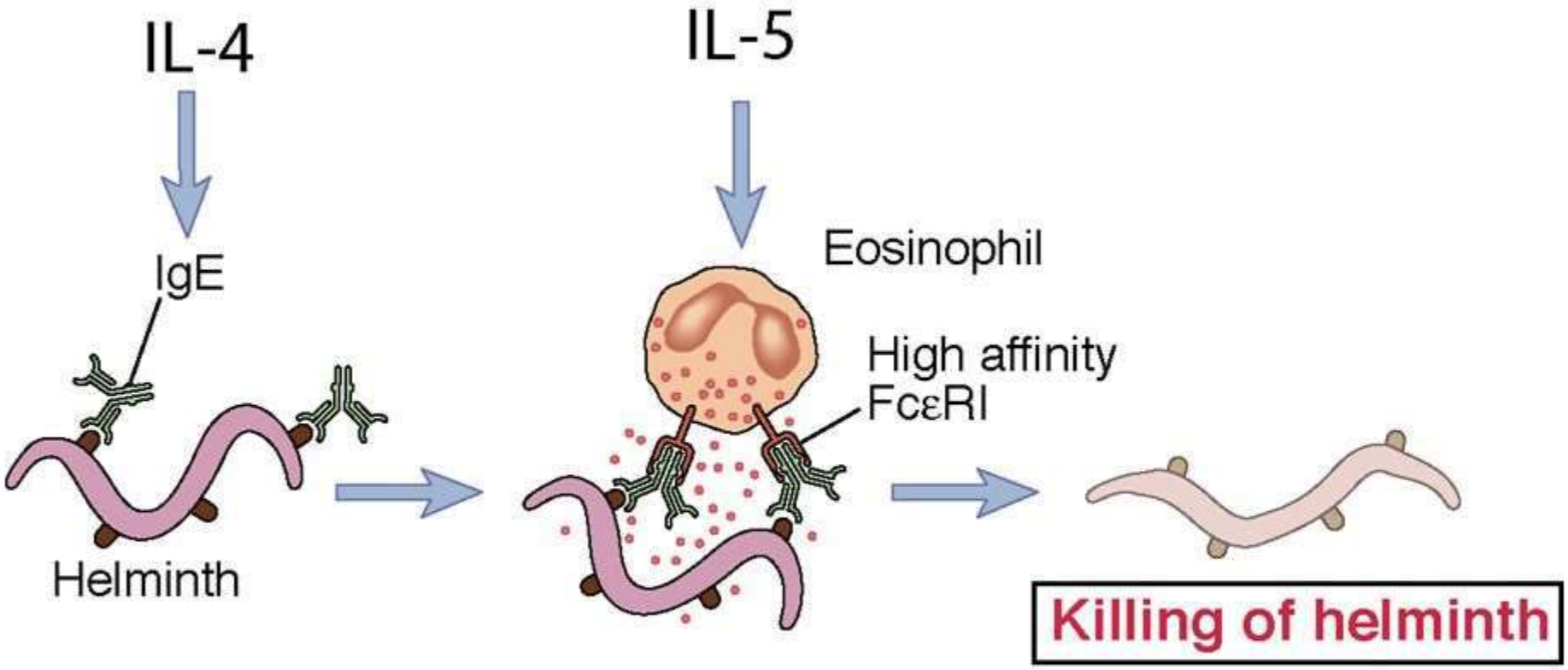

Antibodies bind antigens on the surface of target cells
NK cell CD16 Fc receptors recognise cell-bound antibodies
Cross-linking of CD16 triggers degranulation into a lytic synapse
Tumour cells die by apoptosis

IgE mediated Extracellular Destruction of Helminths



- IgE antibodies are attached to surface of eosinophils via FcεR.
- Cross linkikng of eptitopes present on surface of helminth by IgE, activates eosinophils.
- The activated eosiniphils will empty the contents of its lysosomes by a process called **piece meal degranulation** in order to kill the helminth parasites extracellularly.
- **Termed piece meal degranulation (PMD) because of a “piece by piece” release of secretory granule contents.**

The role of T cell-mediated responses in defense against helminths



Eosinophils are better at killing helminths than are other leukocytes; the T_H2 response and IgE provide a mechanism for bringing eosinophils to helminths and activating the cells.

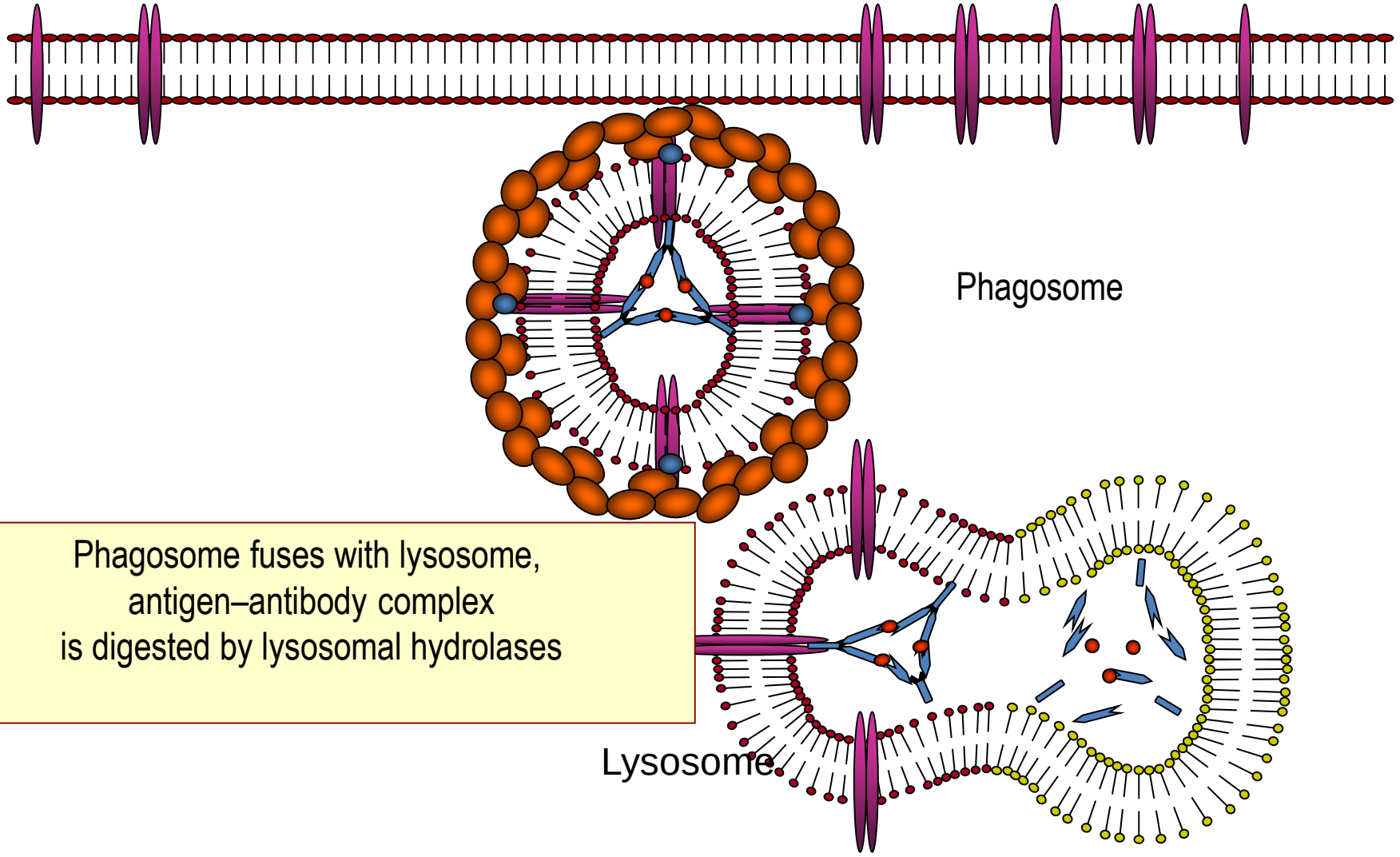
Cytotoxic Reactions: Medical Aspects (Examples)

- Protective
  - Ab and complement-mediated killing and opsonization of **pyogenic bacteria** (e.g. Staph and Strep)
  - Ab and complement-mediated killing and opsonization of protozoa, including *Plasmodium* and *Trypanosoma*
  - ADCC against virus-infected cells, tumor cells, protozoa, and helminths
- Immunopathologic
  - Transfusion reactions - lysis of transfused RBCs
  - Rh reactions - hemolytic disease of newborns
  - Hemolytic anemia- autoantibodies lyse, opsonize RBCs
  - Goodpasture's syndrome - anti-basement membrane Abs
  - Transplant rejection - recipient Abs cause hyperacute rejection

Common Fate of Pathogen or Toxin after Neutralization, Opsonization, or Complement Activation

- Fc or complement receptors on phagocytic cells bind pathogen/toxin complexed with antibody
- Endocytosed complex fuses with lysosomes containing acid hydrolases
- Complex digested by lysosomal hydrolases

Fate of Antibody-Toxin or Antibody-Pathogen Complexes

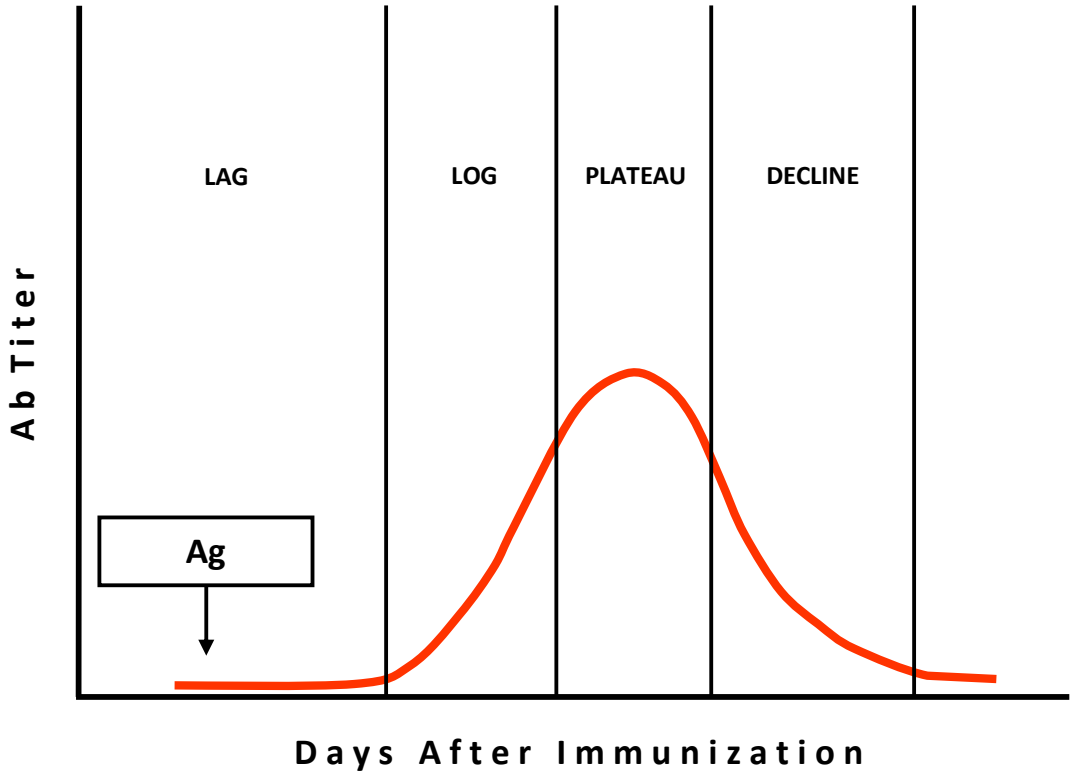


Phagosome fuses with lysosome,
antigen–antibody complex
is digested by lysosomal hydrolases

KINETICS OF ANTIBODIES RESPONSE

Kinetics of the Ab Response to T-dependent Ag
Primary Immune Response

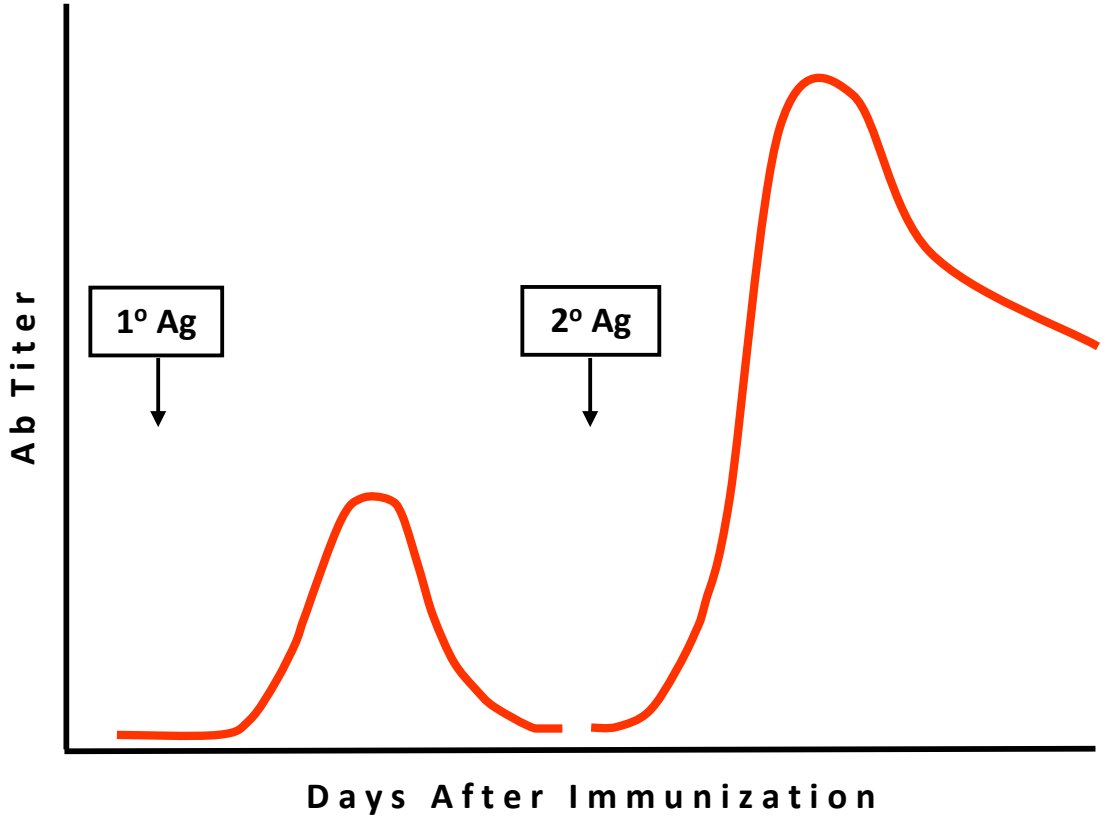
- Lag phase
- Log phase
- Plateau phase
- Decline phase



Kinetics of the Ab Response to T-dependent Ag
Secondary Immune Response

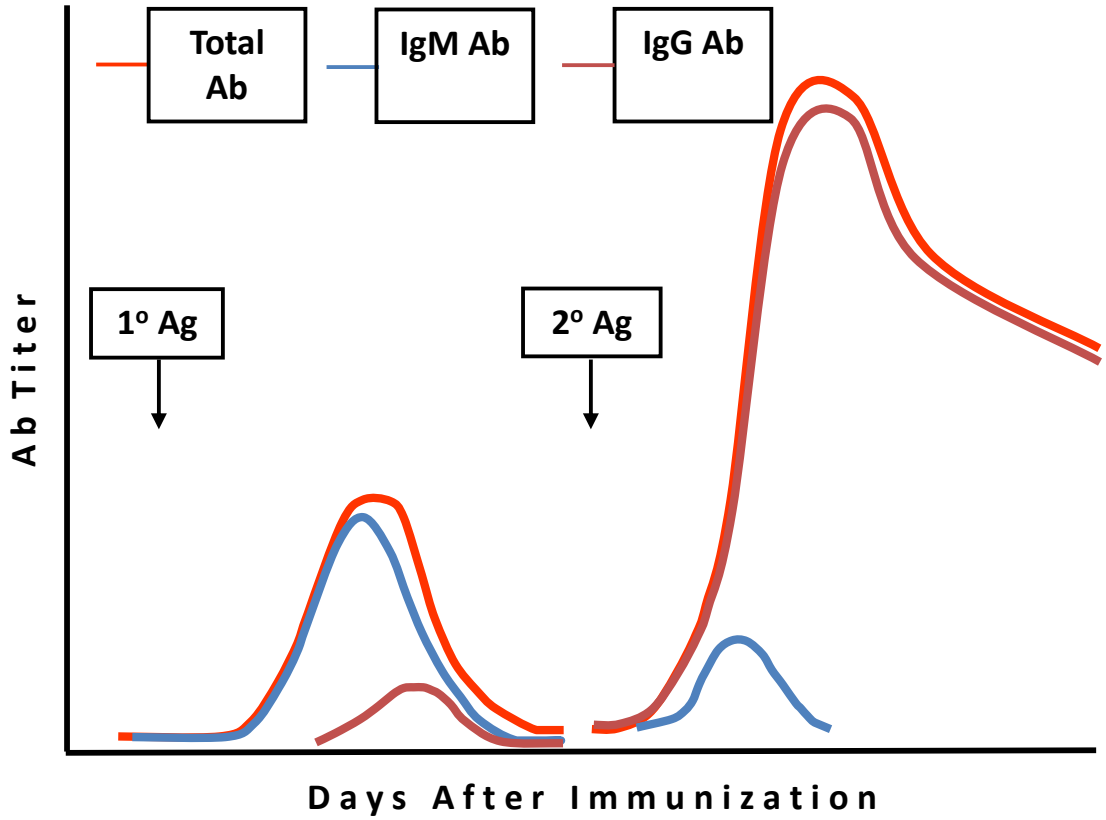
- Lag phase
- Log phase
- Plateau phase
- Decline phase

* Specificity



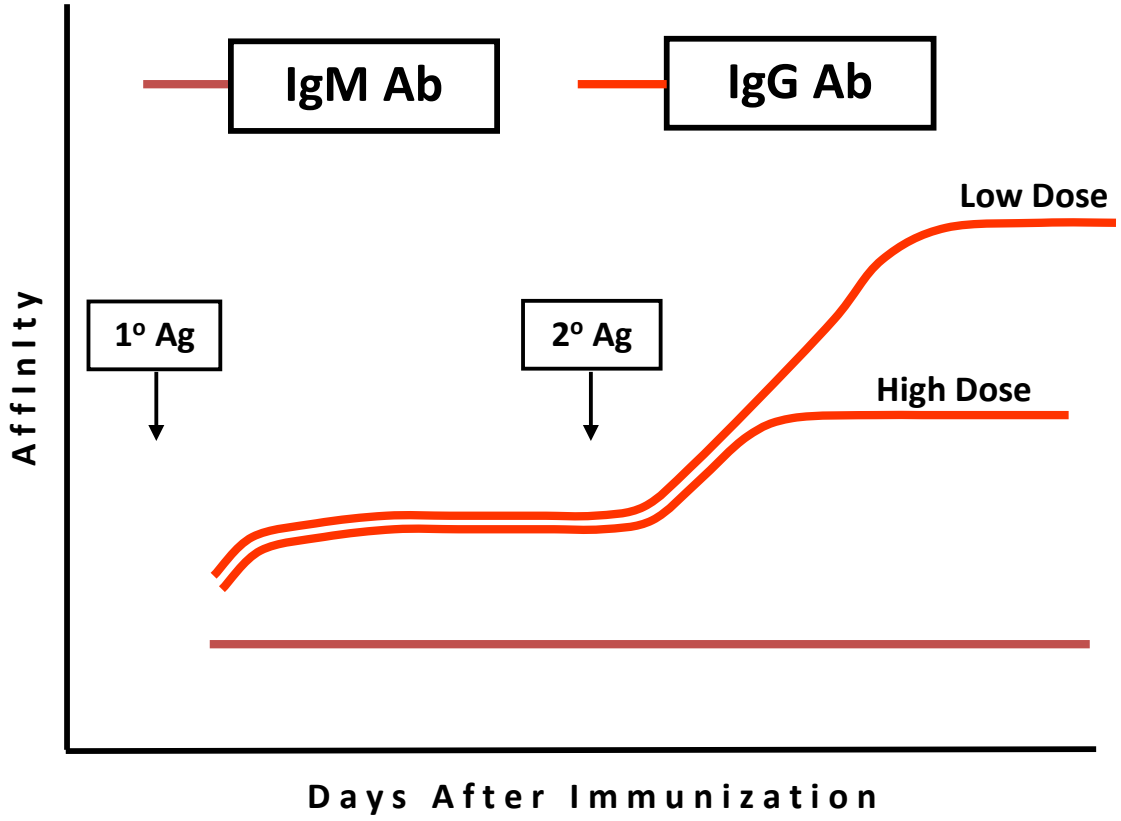
Qualitative Ab Changes during Primary and Secondary Immune Responses

- Class variation
  - 1º - IgM
  - 2º - IgG, IgA or IgE



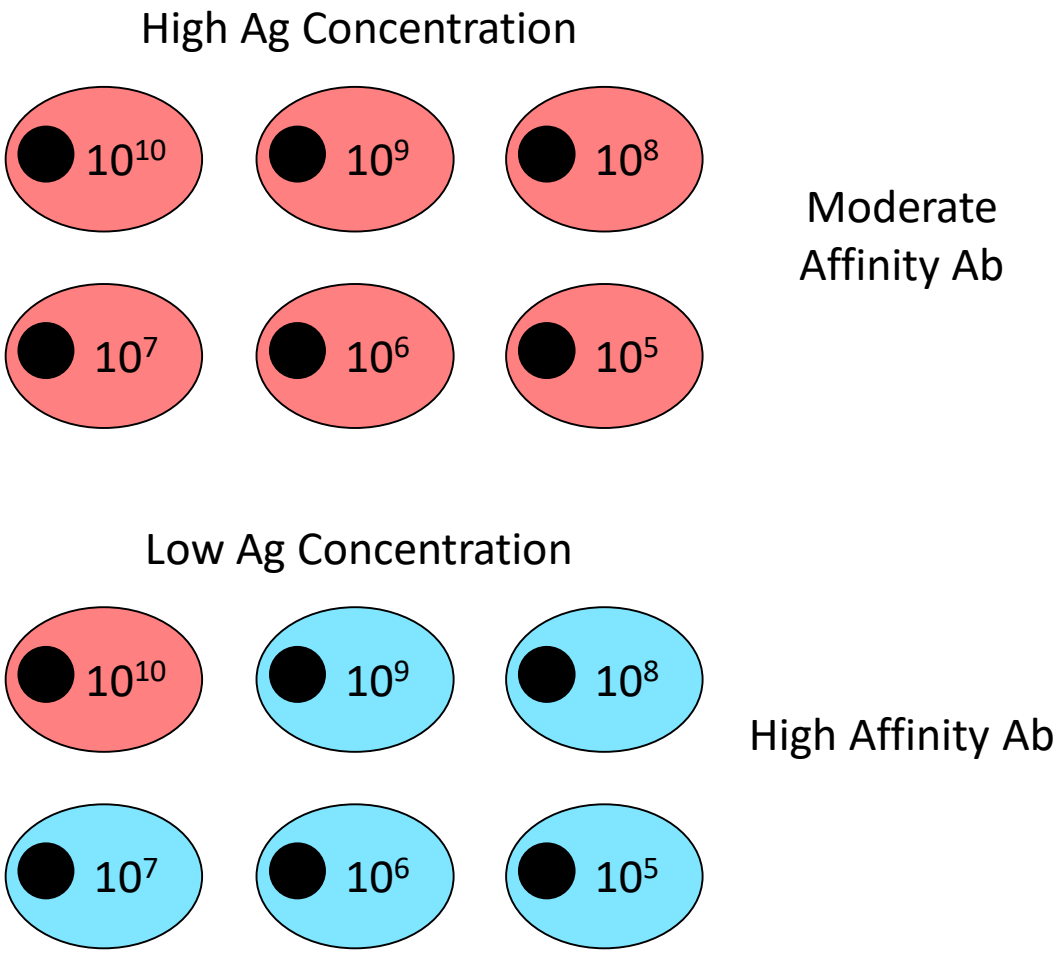
Qualitative Ab Changes during Primary and Secondary Immune Responses

- Affinity
  - Affinity maturation



Qualitative Ab Changes during Primary and Secondary Immune Responses

- Affinity
  - Clonal selection
  - Somatic mutation



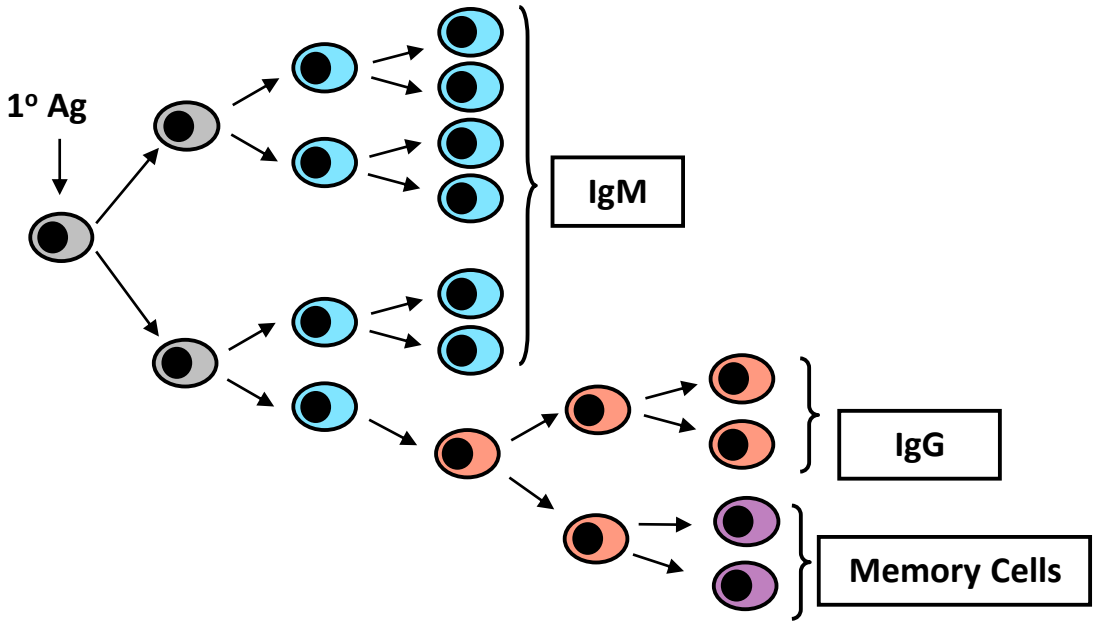
Qualitative Ab Changes during Primary and Secondary Immune Responses

- Avidity
- Cross reactivity

	Affinity of Ab for Ag	
	Early	Late
Immunizing Ag	10^6 +	10^9 ++
Cross reactive Ag	10^3 -	10^6 +

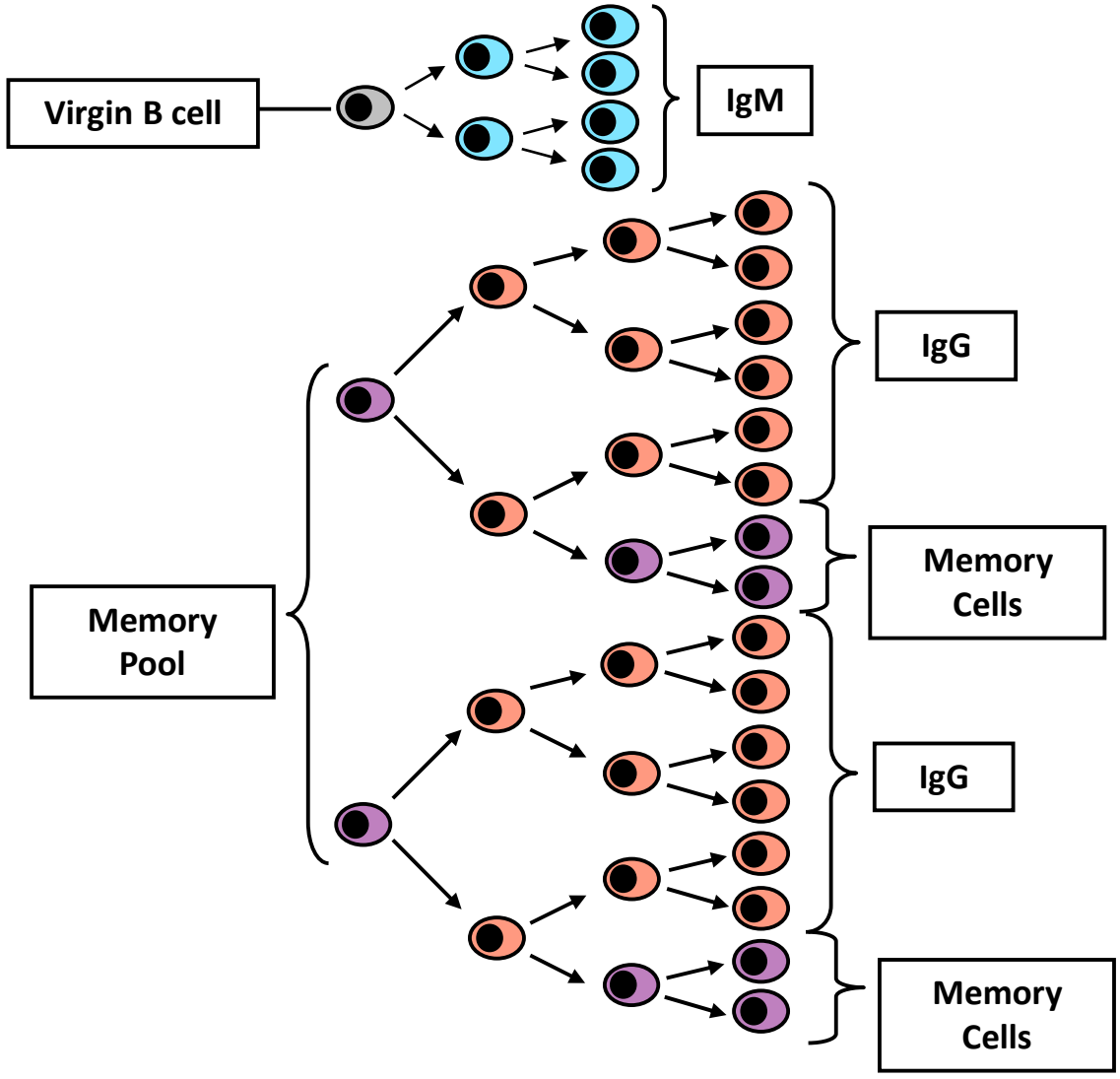
Cellular Events in 1º Response to T-dependent Ags

- Lag
  - Clonal selection
- Log
  - IgM
  - Class switching
- Stationary
- Decline
- Memory Cell Pool

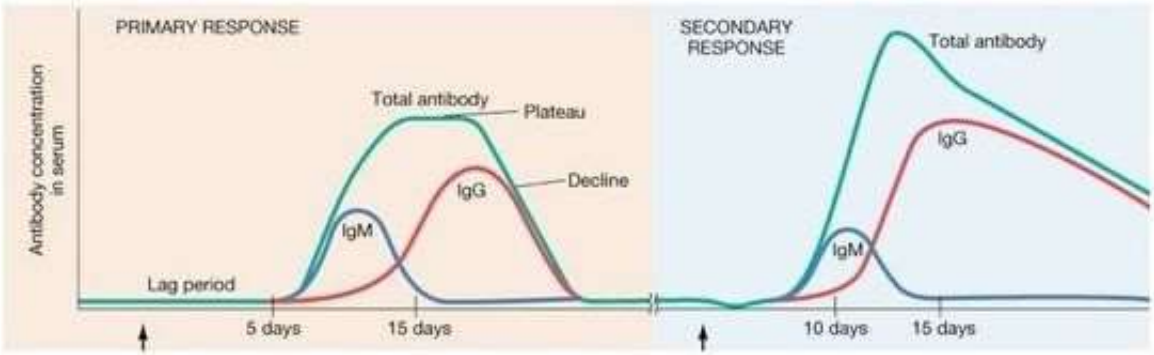


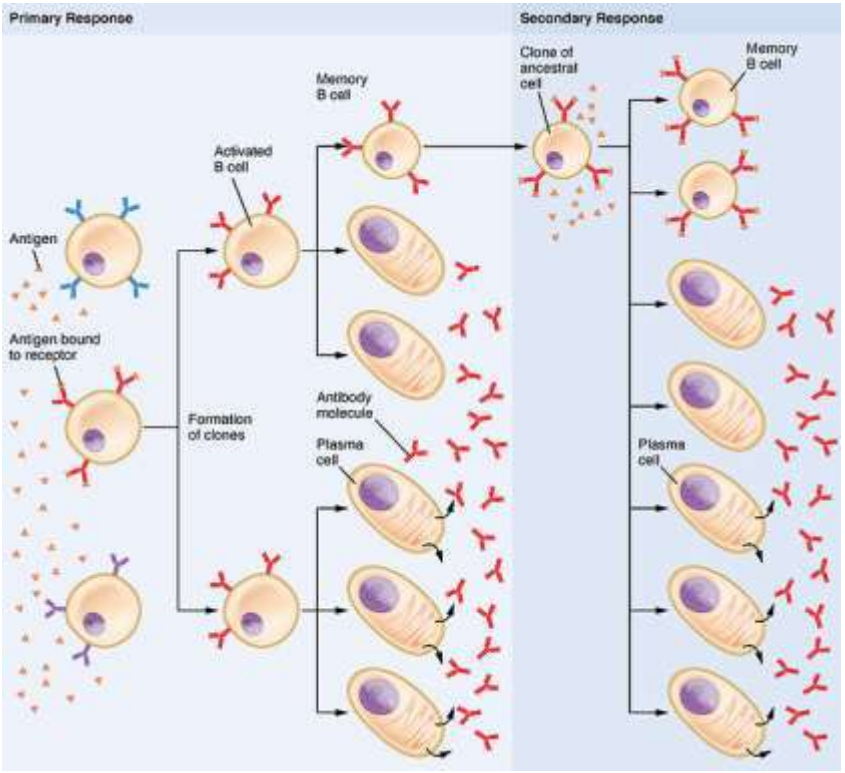
Cellular Events in 2º Response to T-dependent Ags

- Lag phase
  - Virgin cells
  - Memory cells
- Log phase
  - Pool size
  - IgG, IgA or IgE
- Stationary
- Decline
  - Sustained production



Primary *vs* secondary antibody responses





Difference Between Primary & Secondary Immune Response

Traits	Primary Imm. Res.	Secondary Imm. Res.
Exposure to antigen	First time	Second time or subsequent
Latent period (lag phase)	Long	Short
Rate of antibody production	Low	High
Peak antibody titer	Low	High
Persistence of antibody titer	Short	Long
Memory cells	No	Yes
Affinity of antibody	Low	High
Cross-reactive antibody	Low	High
Dose of antigen required	Large	Small
Predominant Ig class	IgM	IgG
Antigen Type	Both T dependent and T independent	Only T dependent
Responding cells	Naïve B or T cells	Memory B or T cells

Kinetics of Ab Response to T-independent Ags

- 4 Phases
- IgM antibody
- No secondary response