Applied Immunology

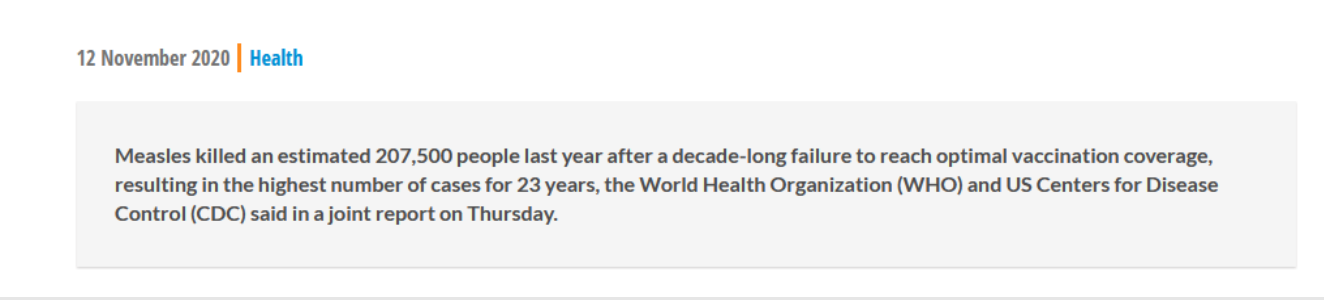
Normal immune homeostasis.

Applied Immunology Lecture-Practice 2025 on Wednesdays from 10:00 to 13:30

week	date	course type	title	participants
1.	05.Feb	lecture	Overview of the immune system: components, structure, functions. Immunological recognition.	Németh P
			Types of immune responses (elimination or tolerance).	Németh P
			Normal immune homeostasis and its potential disturbances.	Boldizsár F
			Introduction to hypersensitive reactions.	Boldizsár F
2.	12.Feb	lecture	Allergy (IgE-mediated hypersensitivity).	Berki T
			Delayed type hypersensitivity.	Boldizsár F
			Primary immunodeficiencies.	Balogh P
			Secondary (acquired) immunodeficiencies, HIV, AIDS	Engelmann P
3.	19.Feb	practice	Ficoll, Flow cytometry, blood smear/cytoprep, cell counting/staining	Chayenne, Dávid Ernszt
4.	26.Feb	practice	ELISA from immunized mouse blood (?)	Katalin Böröcz, Dávid Szinger
5.	05.Mar	lecture	From the humoral immune response to artificial antibody production (Hybridoma technique)	Németh P
			From the humoral immune response to artificial antibody production (Hybridoma technique)	Németh P
			Applications of monoclonal antibodies (medical diagnostics)	Berki T
			Monoclonal antibodies for therapy	Berki T
6.	12.Mar	lecture	Immunomodulation	Boldizsar F
			Immunomodulation	Boldizsar F
			Non-immune functions of lymphocytes-tissue regeneration. Ectopic and artificial lymphoid tissues.	Balogh P
			CAR T cells	Balogh P
7.	19.Mar	practice	Hybridoma from OVA immunized mice spleen (next day: checking the selection, changing medium), counting, cloning	Péter B., Móni, Dia, Dávid Szinger
8.	26.Mar	practice	ELISA - hybridoma practice	Katalin Böröcz, Dávid Szinger
9.	02.Apr	practice	ELISA - hybridoma practice	Katalin Böröcz, Dávid Szinger
10.	09.Apr	lecture	Physiological and pathological mucosal immune responses-cells, processes and potential targets for restoration.	Balogh P
			Physiological- and pathological autoimmunity	Németh P
			Physiological- and pathological autoimmunity	Németh P
			Complex animal models of autoimmunity (RA)	Boldizsár F
11.	16.Apr	lecture	Complex animal models of autoimmunity (SLE, Hemolytic anemia)	Boldizsár F
			Complex animal models of innate immunity	Engelmann P
			Immune response against pathogens	Berki T
			Immune response against pathogens	Berki T
12.	30.Apr	practice	Antibody purification; Tissue section (cryostat)	Péter B., Móni, Dia;Chayenne
13.	07.May	lecture	Interactions between the microbiom and the immune system	Olasz K
			Interactions between the microbiom and the immune system	Olasz K
			Immune response against tumors	Németh P
			Immunological aspects of organ transplantation	Németh P
14.	14.May	practice	Immunodiagnostic laboratory.	Katalin Böröcz, Dávid Szinger, Berki T

Introducing the subject 6.

- **What is the significance of immunology?**
  - The immune system is involved one way or another in almost all human pathological conditions.
  - Many of the laboratory diagnostics are based on immunological methods. (see later)
  - More and more diseases get treatable by manipulating the immune system. (see later)
  - Autoimmune diseases affect 7-8% of the population, they are chronic and generally incurable, yet many can be treated effectively. (see later)
  - The number of immunocompromised patients has increased recently. (therapeutic immunosuppression and HIV, see later)
  - COVID-19/SARS-COV2 pandemy
  - Laypeople also seem to have strong opinions regarding immunology. → Media tends to mix medical facts with quackery and pseudoscience.


12 November 2020 | Health

Measles killed an estimated 207,500 people last year after a decade-long failure to reach optimal vaccination coverage, resulting in the highest number of cases for 23 years, the World Health Organization (WHO) and US Centers for Disease Control (CDC) said in a joint report on Thursday.

Main tasks of the immune system

Preserving the integrity of an organism

Defense against **external pathogens** (e.g. viruses, bacteria, parasites)

Elimination of one's **pathologically altered cells** (e.g. virally infected cells, cancer cells)

Altered or foreign structures must be **recognized** and **distinguished** from the organism's own healthy cells.

IMMUNE RESPONSE (either an aggressive response or immunological tolerance)

Components of the immune system

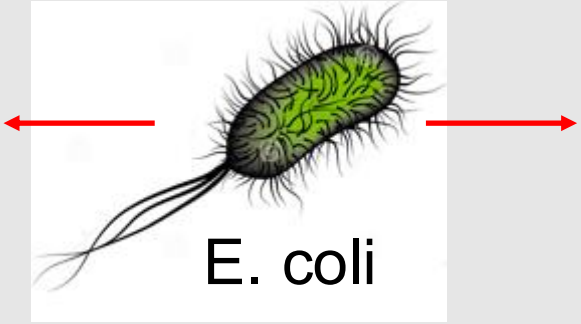
- The components of immune system can be classified into subsystems (See the lectures for more details):
  - **Innate immunity** (e.g. granulocytes, macrophages, NK cells, complement system)
  - **Natural immunity** (e.g. B1 B cells, γδ T cells)
  - **Adaptive immunity** (e.g. αβ T cells, B2 cells, antibodies)
- The distinction above is artificial, in the organisms these work hand in hand.
- You will mostly learn about the adaptive immunity throughout the semester.

Innate vs adaptive immunity

	Innate	Adaptive
Recognition	**Pattern-based (not antigen-specific)**	**Antigen-specific**
Kinetics	**Quick (minutes, hours)**	**Slow (days, weeks)**
Amplification of response	**Linear**	**Exponential**
Immunological memory	**No**	**Yes**

Antigen: Substance recognized by T and B cell receptors (TCR and BCR) which induces either an immune response or immunological tolerance.
Difference between pattern-recognition and antigen-recognition:

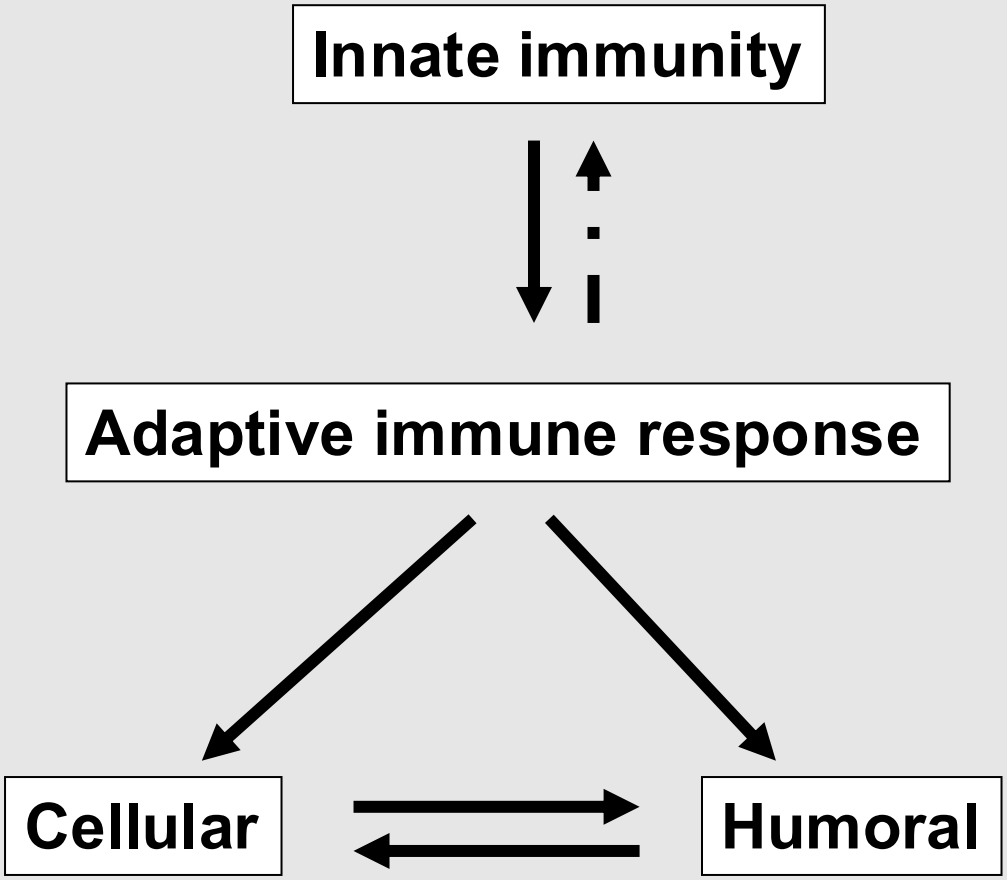
Innate: „The cell surface is full of carbohydrates usually found on bacteria - it must be some kind of bacteria."



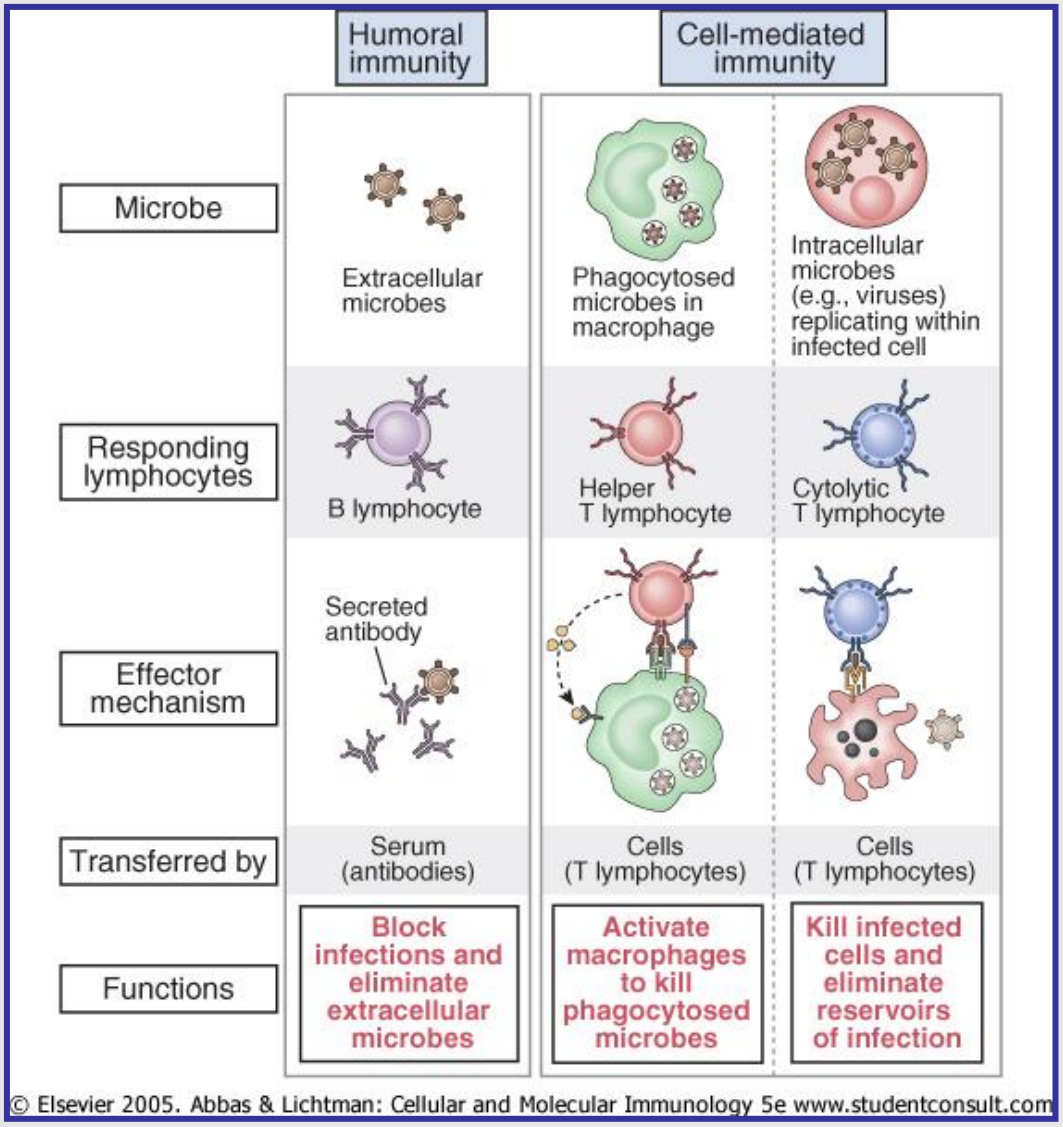
Adaptive: „This is the 45-60 amino acid segment of *E. coli* flagellin."

Organs of the immune system

- The immune system is organized into a **network** of cells and organs. (the entire body must be protected from pathogens)
- Lymphoid organs:
  - Primary (production of immune cells)
    - **Bone marrow, thymus**, embryonic liver (+bursa of Fabricius in birds [nomenclature: „B" lymphocytes originating from the bursa and „T" cells from the thymus[1.]])
  - Secondary (site of antigen recognition, immune response)
    - **Lymph nodes, spleen, MALT** (mucosa-associated lymphoid tissue), **SALT** (skin-associated lymphatic tissue)
  - Tertiary (pathological, usually due to chronic inflammation)
    - E.g. ectopic (=at an abnormal site) lymphoid follicles


Innate immunity
Adaptive immune response
Cellular
Humoral

The type of pathogens determine the type of immune response



Main steps of the adaptive immune response

Recognition
Molecular- and cellular cooperation
Activation
Differentiation and clonal expansion
Effector functions
Memory fromation
Suppression

Normal immune homeostasis

HUMORAL IMMUNE RESPONSE

Phases of the Humoral Immune Response

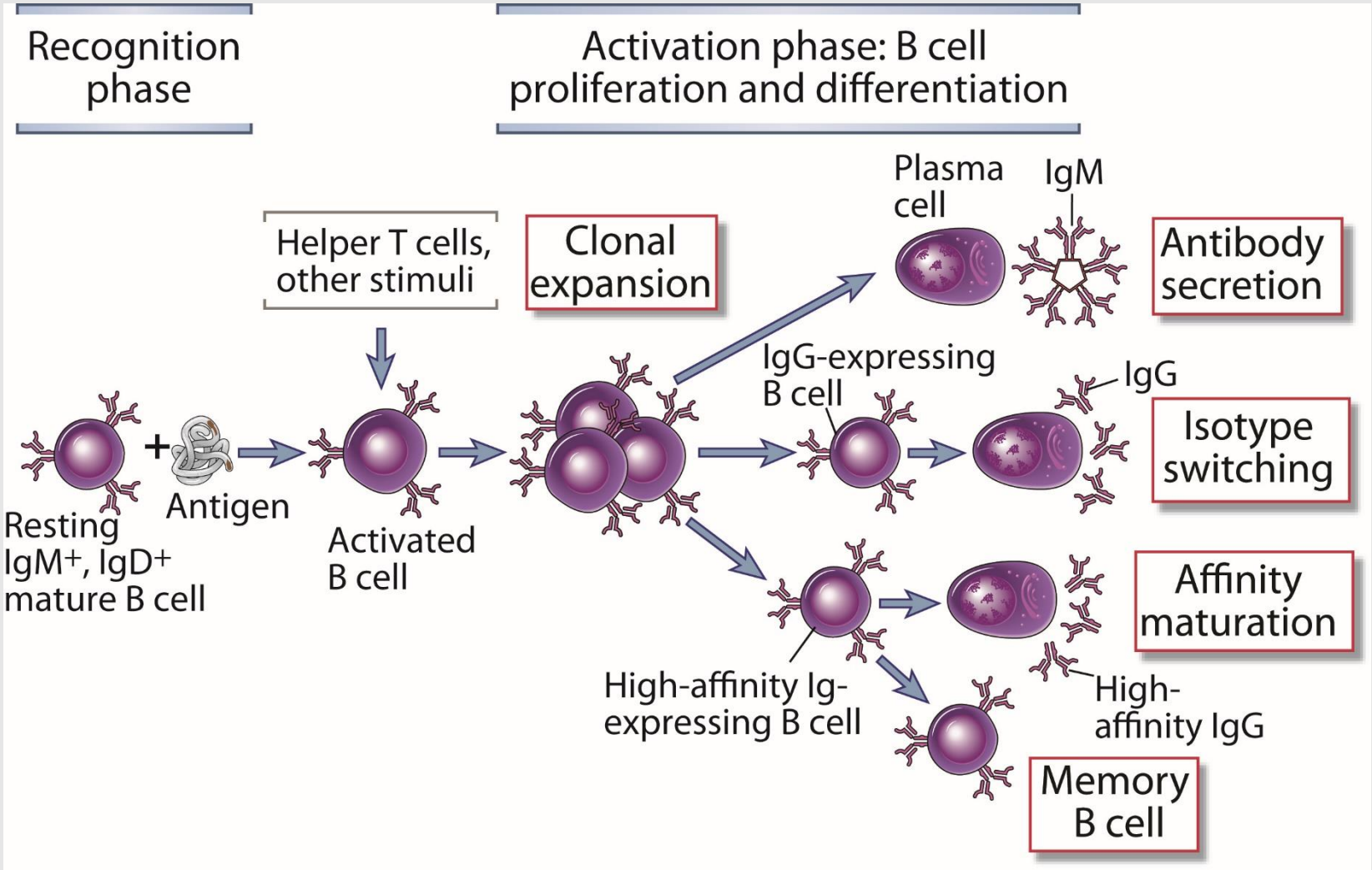


Fig. 11-1

Antibody production

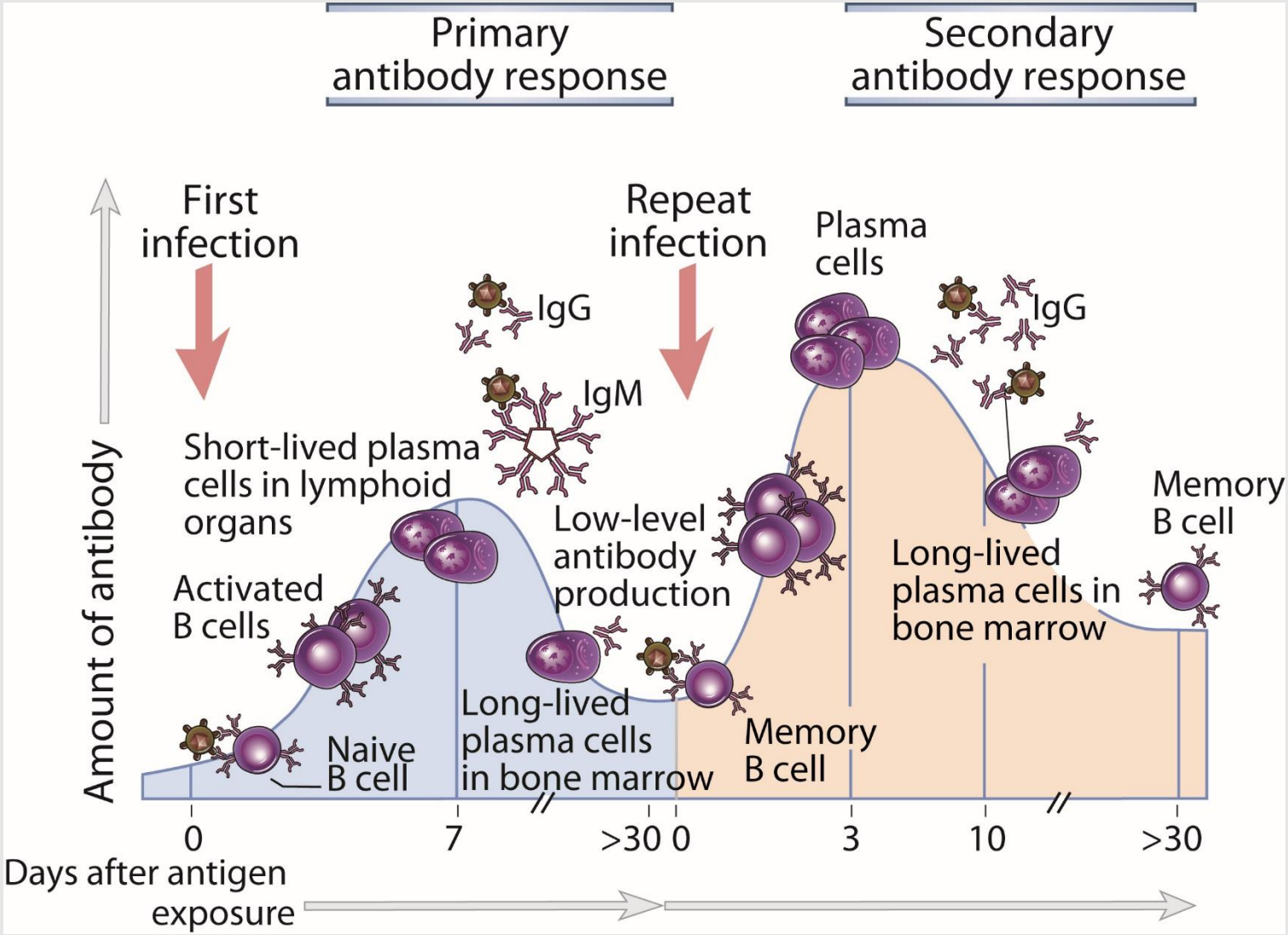
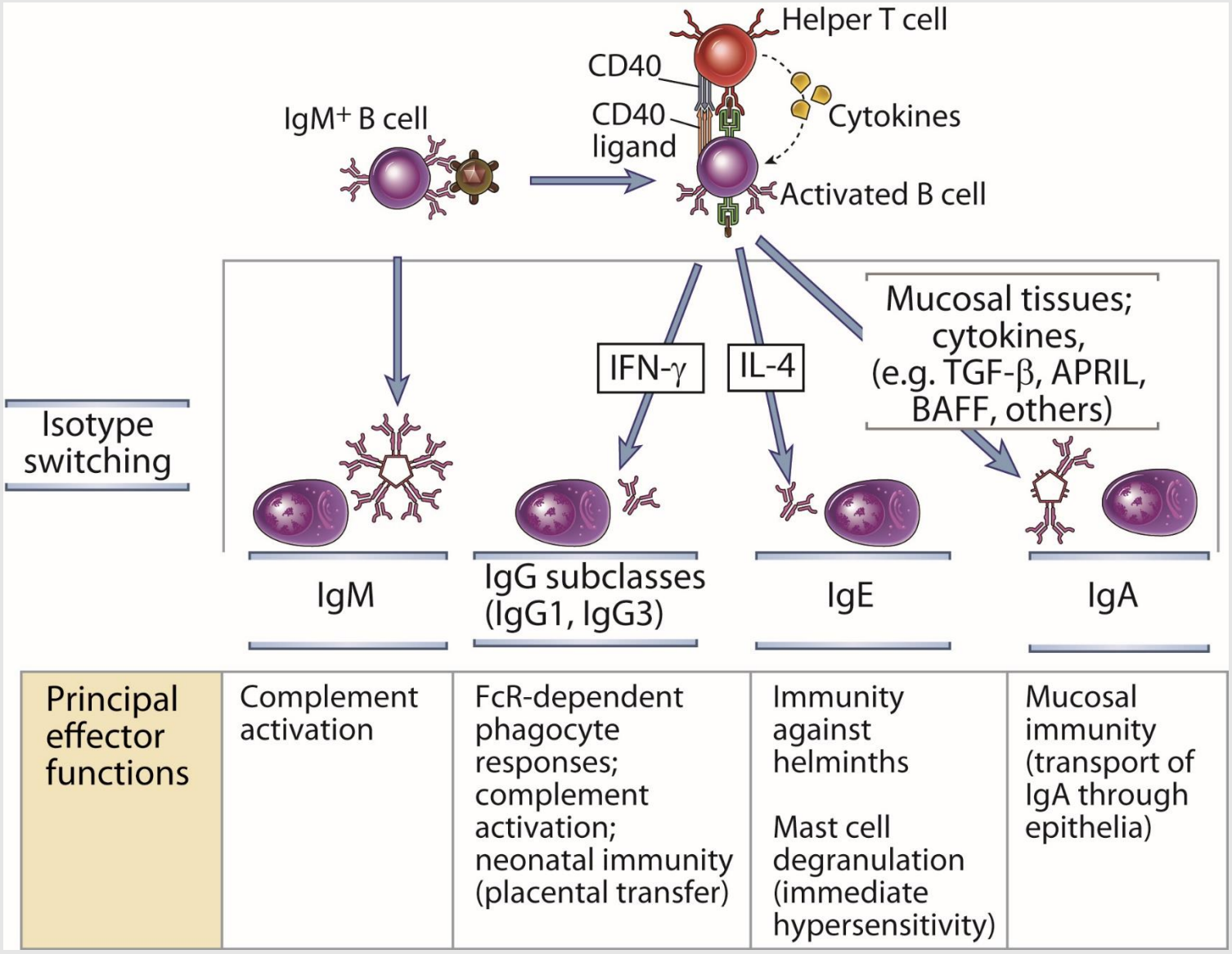


Fig. 11-2

Ig heavy chain isotype switching → development of functional diversity



Principal effector functions	Complement activation	FcR-dependent phagocyte responses; complement activation; neonatal immunity (placental transfer)	Immunity against helminths Mast cell degranulation (immediate hypersensitivity)	Mucosal immunity (transport of IgA through epithelia)

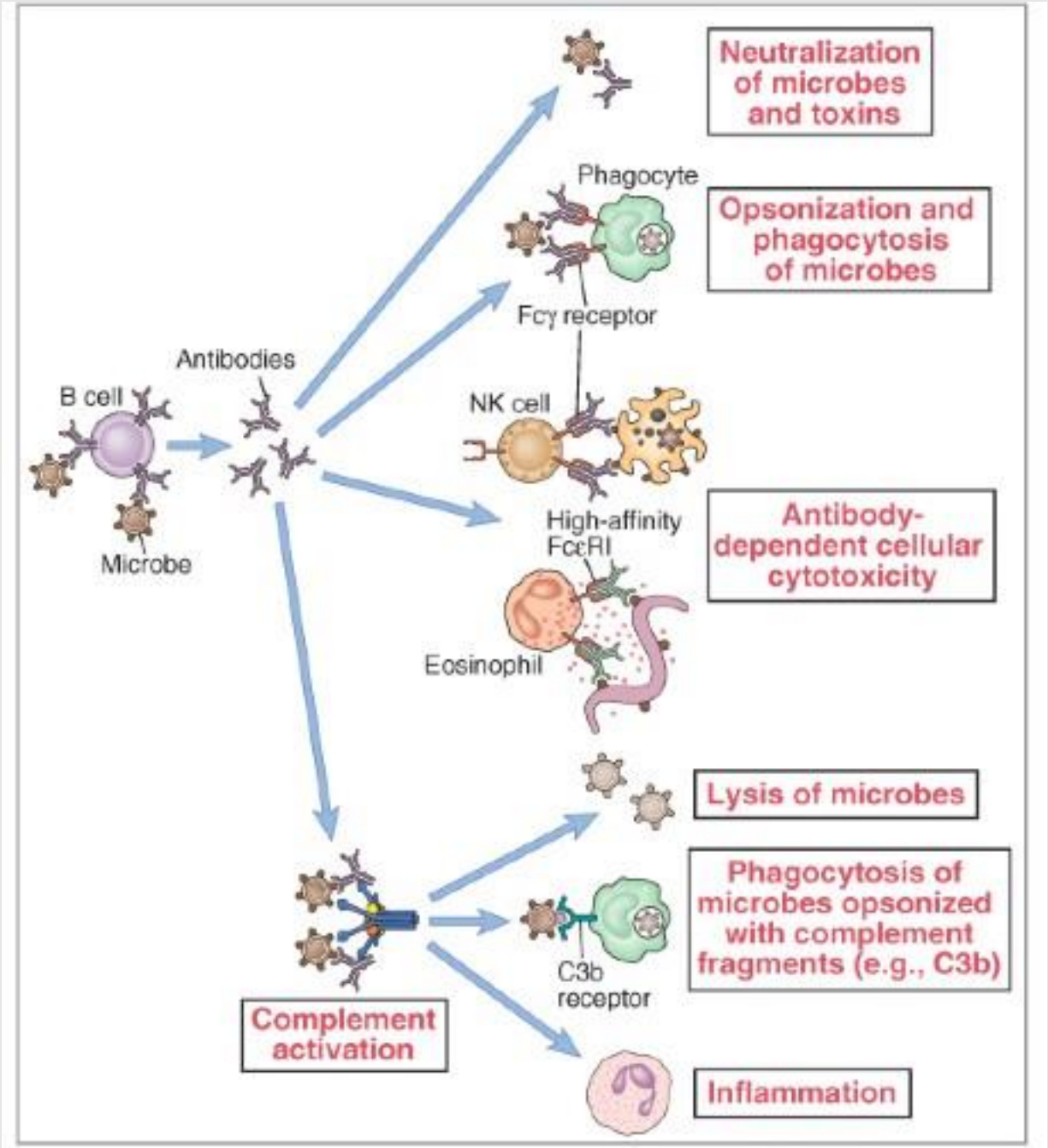
Immungolobulin effector functions

I. Neutralization of the antigen

II. Complement activation

III. Immunocomplex binding to Fc receptor and enhancing phagocytosis (opsonization)

IV. Antibody dependent cell-mediated cytotoxicity (ADCC)



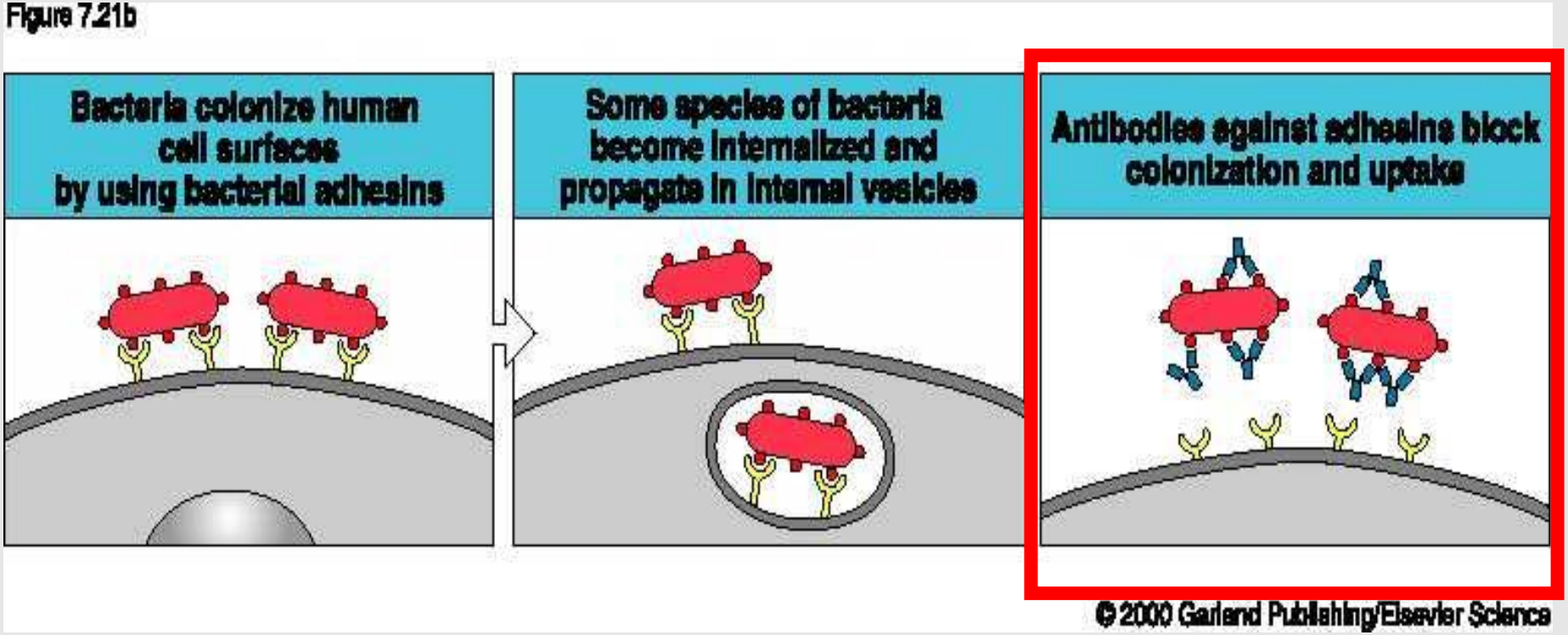
Immunoglobulins of various isotypes have different functions

Functional activity	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Neutralization	+	–	++	++	++	++	++	–
Opsonization	+	–	+++	*	++	+	+	–
Sensitization for killing by NK cells	–	–	++	–	++	–	–	–
Sensitization of mast cells	–	–	+	–	+	–	–	+++
Activates complement system	+++	–	++	+	+++	–	+	–

Figure 9-19 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)

NEUTRALIZATION

Neutralization: the antibody can inhibit the binding of bacteria to the host cells



Secretory IgA inhibits binding to mucous membranes

Opsonization by IgG → enhanced phagocytosis
IgG & IgM →complement activation → lysis

Antibody-mediated agglutination → inhibits entrance into the host tissues

Neutralization of bacterial toxins

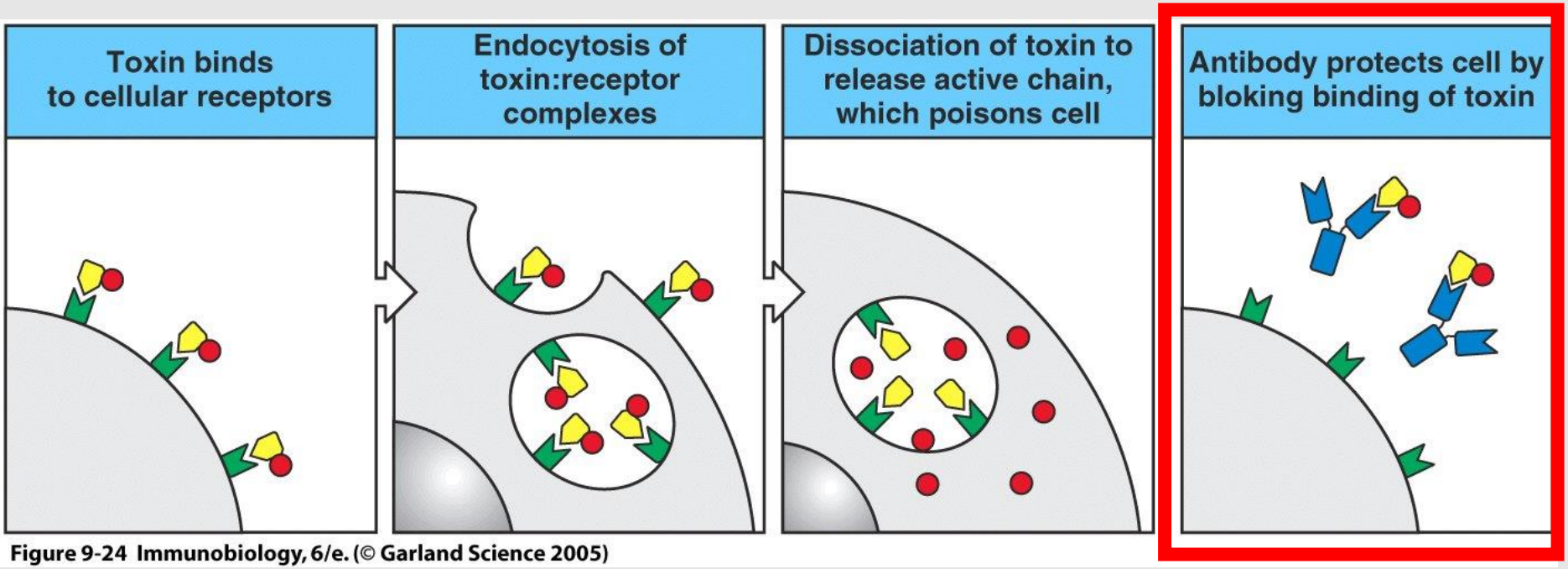


Figure 9-24 Immunobiology, 6/e. (© Garland Science 2005)

Diphtheria,Tetanus exotoxin → Toxoid (inactivated exotoxin) for vaccination

Diseases caused by bacterial toxins

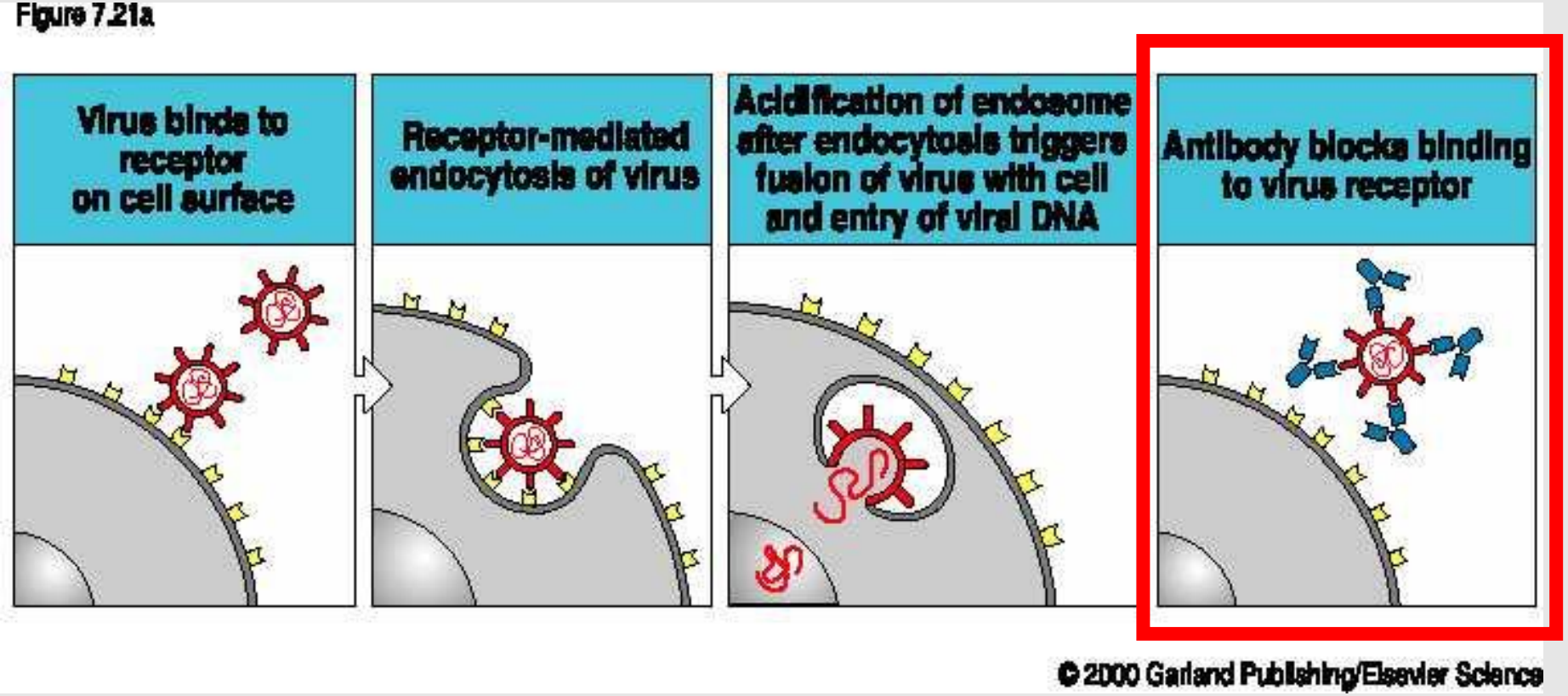
Disease	Organism	Toxin	Effects *in vivo*
Tetanus	*Clostridium tetani*	Tetanus toxin	Blocks inhibitory neuron action, leading to chronic muscle contraction
Diphtheria	*Corynebacterium diphtheriae*	Diphtheria toxin	Inhibits protein synthesis, leading to epithelial cell damage and myocarditis
Gas gangrene	*Clostridium perfringens*	Clostridial toxin	Phospholipase activation, leading to cell death
Cholera	*Vibrio cholerae*	Cholera toxin	Activates adenylate cyclase, elevates cAMP in cells, leading to changes in intestinal epithelial cells that cause loss of water and electrolytes
Anthrax	*Bacillus anthracis*	Anthrax toxic complex	Increases vascular permeability, leading to edema, hemorrhage,and circulatory collapse
Botulism	*Clostridium botulinum*	Botulinum toxin	Blocks release of acetylcholine, leading to paralysis
Whooping cough	*Bordetella pertussis*	Pertussis toxin	ADP-ribosylation of G proteins, leading to lymphoproliferation
		Tracheal cytotoxin	Inhibits cilia and causes epithelial cell loss
Scarlet fever	*Streptococcus pyogenes*	Erythrogenic toxin	Vasodilation, leading to scarlet fever rash
		Leukocidin Streptolysins	Kill phagocytes, allowing bacterial survival
Food poisoning	*Staphylococcus aureus*	Staphylococcal enterotoxin	Acts on intestinal neurons to induce vomiting. Also a potent T-cell mitogen (SE superantigen)
Toxic-shock syndrome	*Staphylococcus aureus*	Toxic-shock syndrome toxin	Causes hypotension and skin loss. Also a potent T-cell mitogen (TSST-1 superantigen)

Figure 9-23 Immunobiology, 6/e. (© Garland Science 2005)

Virus neutralization

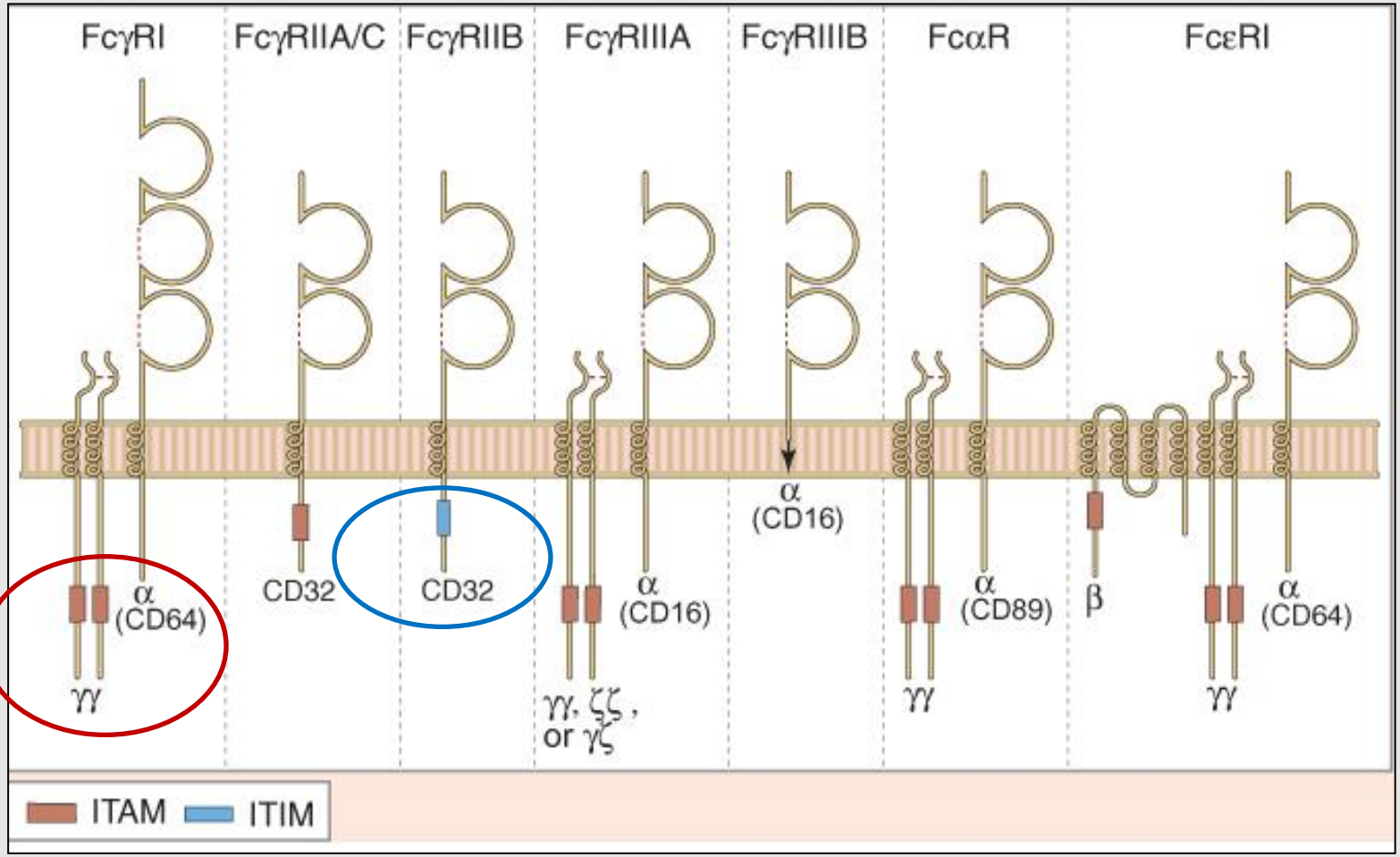
Antibody inhibits the binding of the virus to the host cell and the infection:

-Influenza virus binds to syalic acid residues of cell membrane glycoproteinns
-Rhinovirus bind to ICAM-1
-Epstein-Barr virus binds to CR2



Fc-RECEPTOR BINDING

Activatory and inhibitory role of FcγReceptors



Fc receptors (FcR)

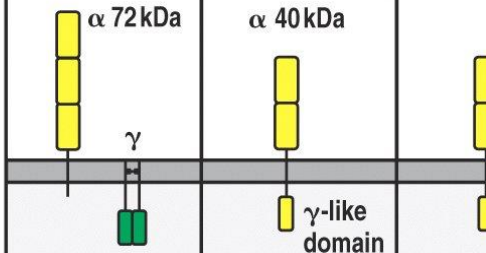
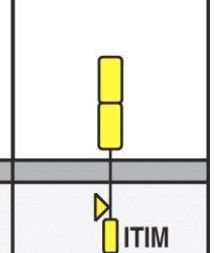
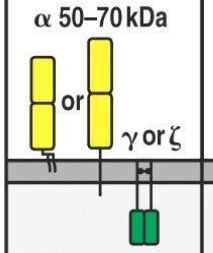
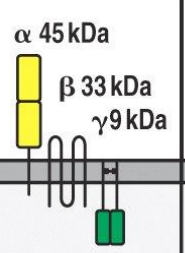
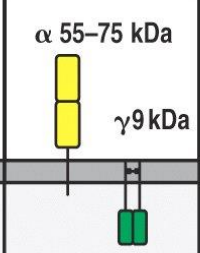
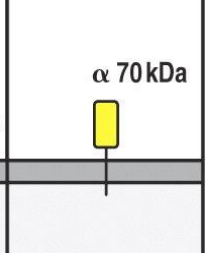
Receptor	FcγRI (CD64)	FcγRII-A (CD32)	FcγRII-B2 (CD32)	FcγRII-B1 (CD32)	FcγRIII (CD16)	FcεRI	FcαRI (CD89)	Fcα/μR
Structure								
Binding Order of affinity	IgG1 10^8 M^{-1} 1) IgG1=IgG3 2) IgG4 3) IgG2	IgG1 2×10^6 M^{-1} 1) IgG1 2) IgG3=IgG2* 3) IgG4	IgG1 2×10^6 M^{-1} 1) IgG1=IgG3 2) IgG4 3) IgG2	IgG1 2×10^6 M^{-1} 1) IgG1=IgG3 2) IgG4 3) IgG2	IgG1 5×10^5 M^{-1} IgG1=IgG3	IgE 10^{10} M^{-1}	IgA1, IgA2 10^7 M^{-1} IgA1=IgA2	IgA, IgM 3×10^9 M^{-1} 1) IgM 2) IgA
Cell type	Macrophages Neutrophils† Eosinophils† Dendritic cells	Macrophages Neutrophils Eosinophils Platelets Langerhans' cells	Macrophages Neutrophils Eosinophils	B cells Mast cells	NK cells Eosinophils Macrophages Neutrophils Mast cells	Mast cells Eosinophils† Basophils	Macrophages Neutrophils Eosinophils‡	Macrophages B cells
Effect of ligation	Uptake Stimulation Activation of respiratory burst Induction of killing	Uptake Granule release (eosinophils)	Uptake Inhibition of stimulation	No uptake Inhibition of stimulation	Induction of killing (NK cells)	Secretion of granules	Uptake Induction of killing	Uptake

Figure 9-30 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)

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Opsonization and Phagocytosis by Antibodies

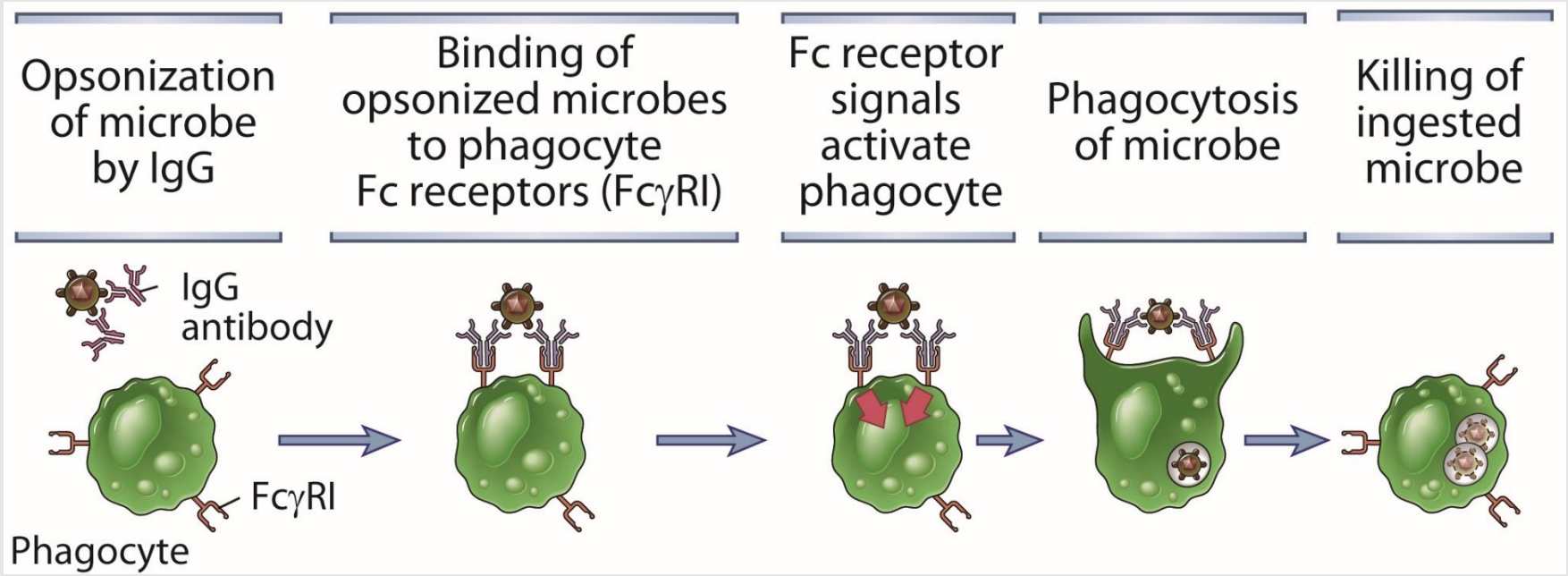


Fig. 12-4

Opsonization by antibody and complement C3b → FCr and CR mediated phagocytosis

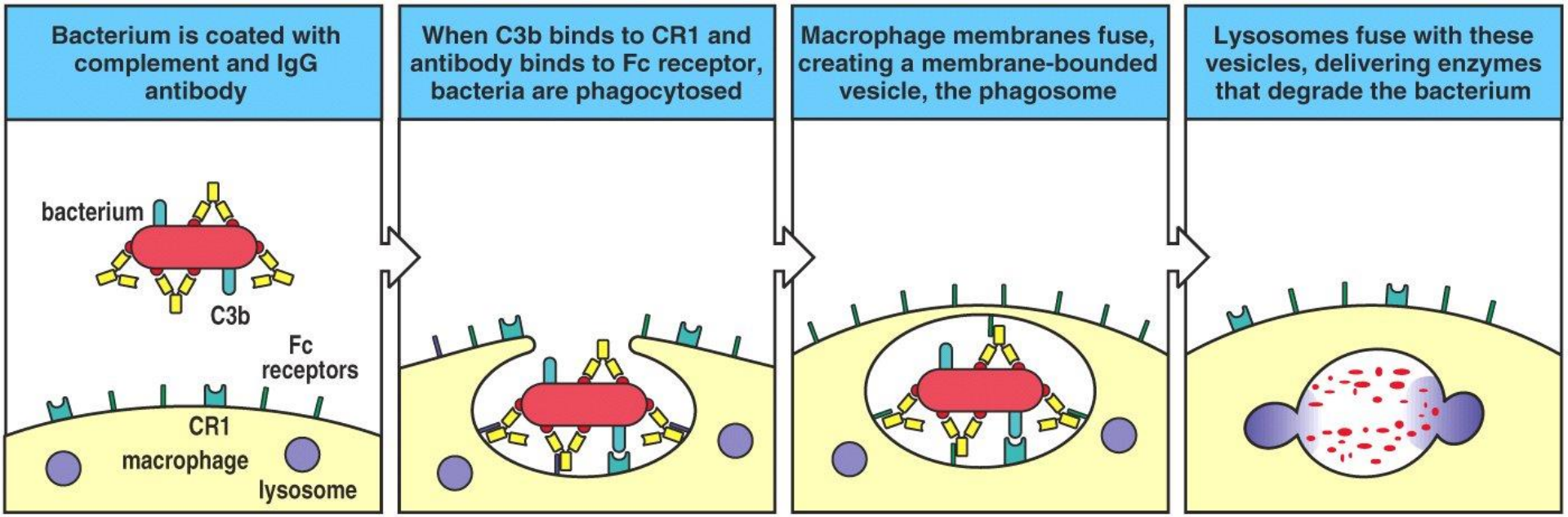
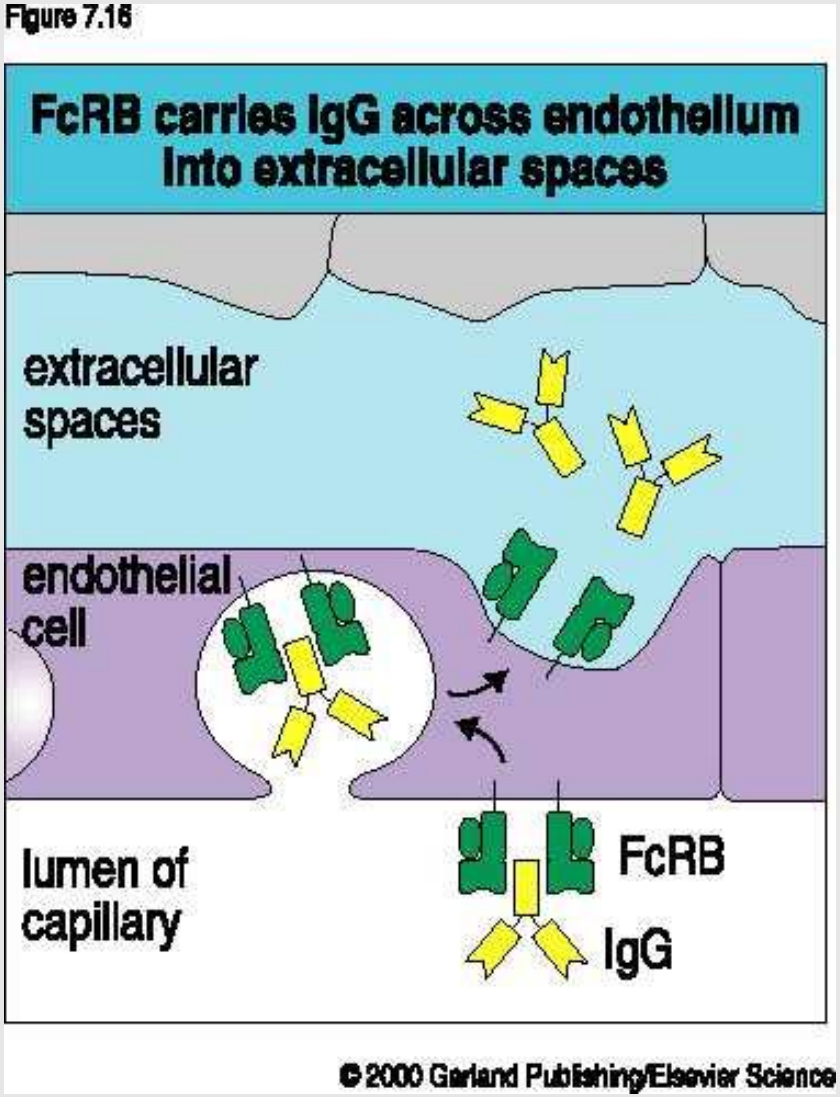


Figure 9-32 Immunobiology, 6/e. (© Garland Science 2005)

Free immunoglobulins cannot bind to Fc receptor and enhance phagocytosis

Antigen bound antibody is capable of binding to FcR

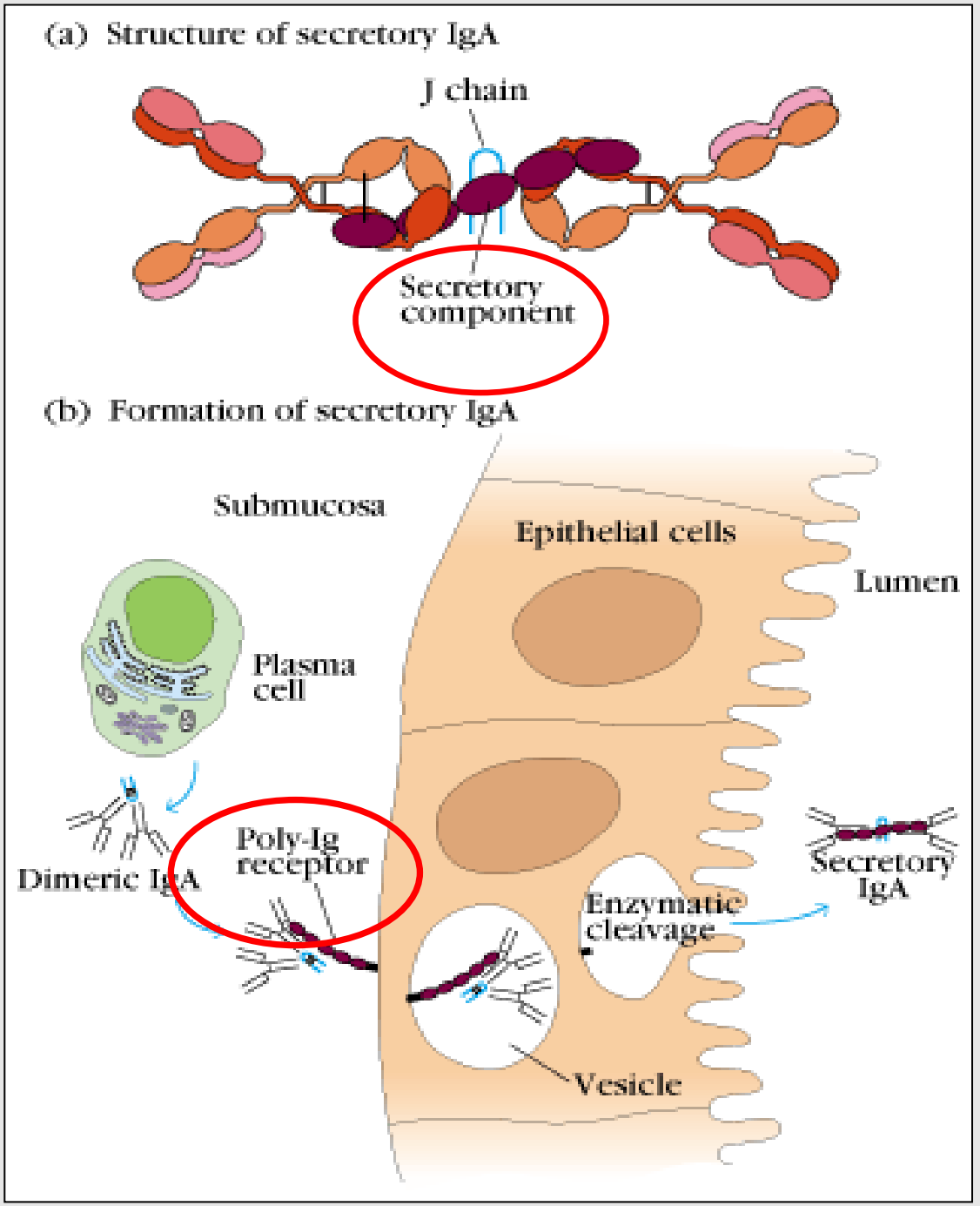
IgG transport from blood to tissues



Poly-Ig receptor

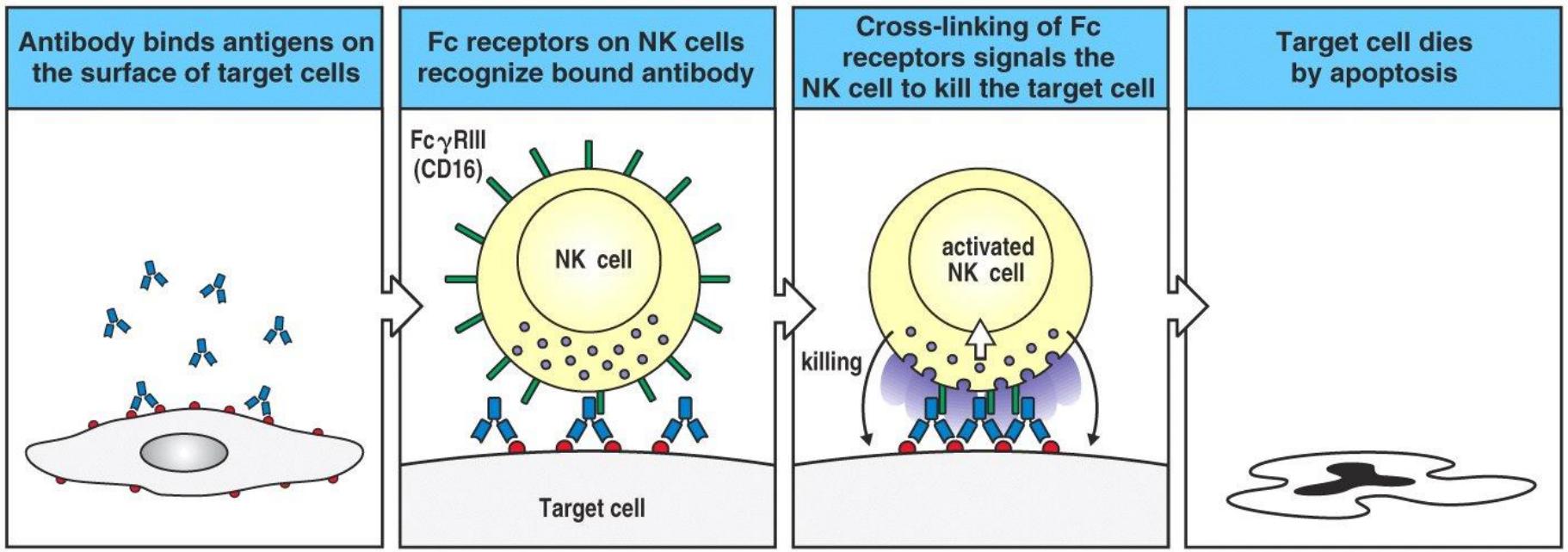
IgA/IgM transport

secretory component



ADCC = antibody dependent cellular cytotoxicity

ADCC



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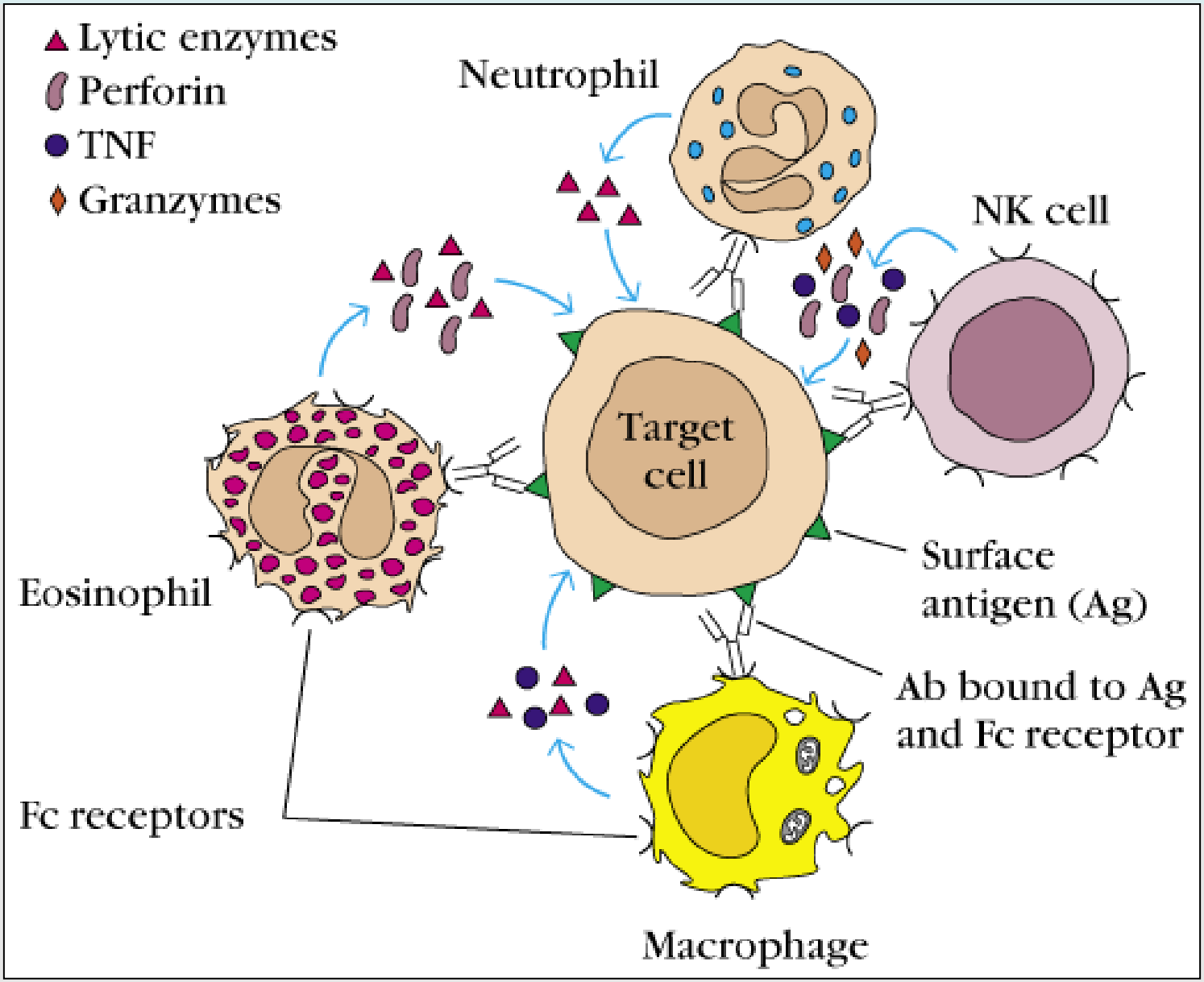
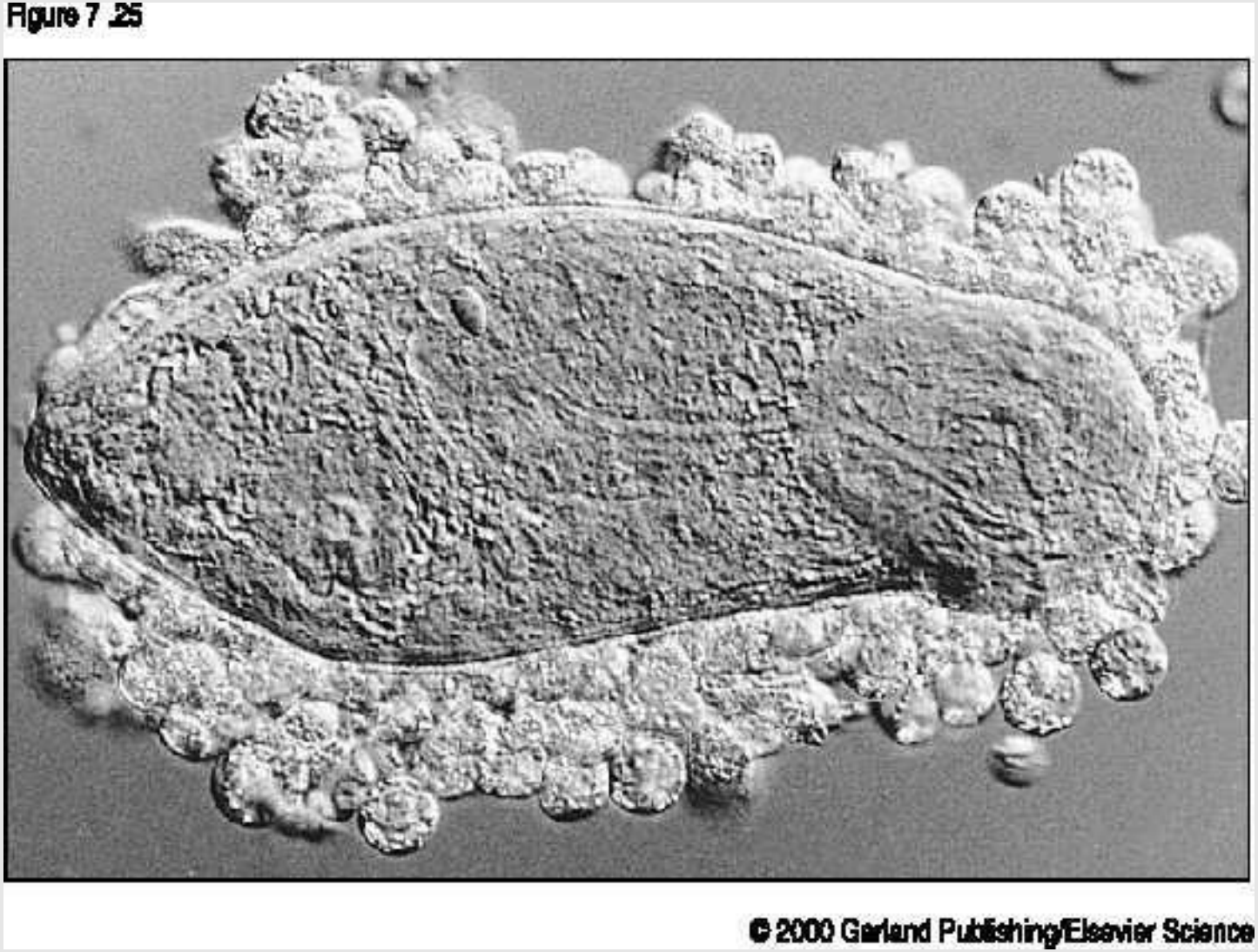
ADCC


Lytic enzymes
Perforin
TNF
Granzymes
Neutrophil
NK cell
Target cell
Eosinophil
Surface antigen (Ag)
Ab bound to Ag and Fc receptor
Fc receptors
Macrophage



Parasite covered by IgE > eosinophil activation > release of toxic granules

COMPLEMENT ACTIVATION

IgG & IgM antigen-antitbody complexes activate complement

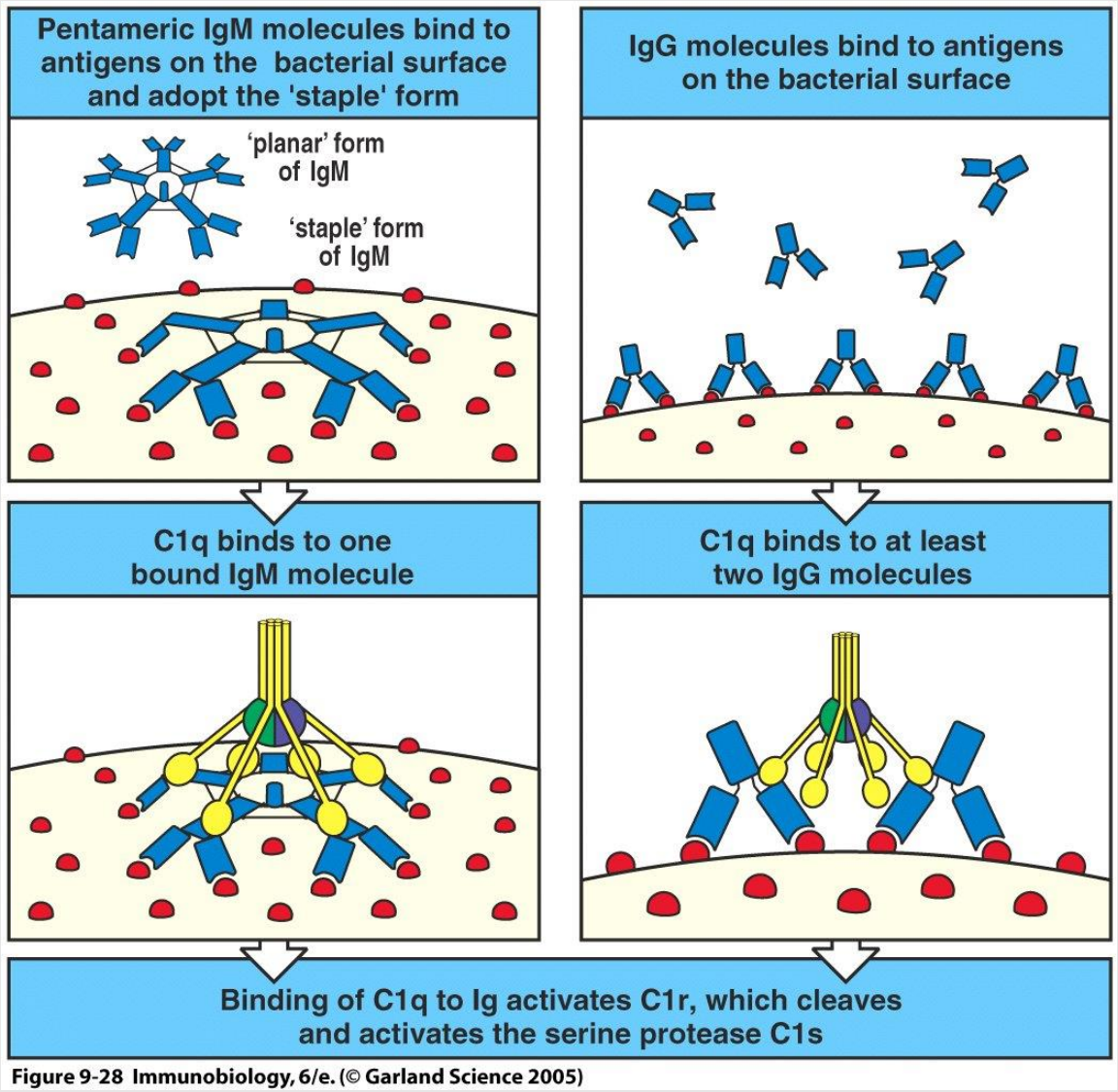


Figure 9-28 Immunobiology, 6/e. (© Garland Science 2005)

Normal immune homeostasis

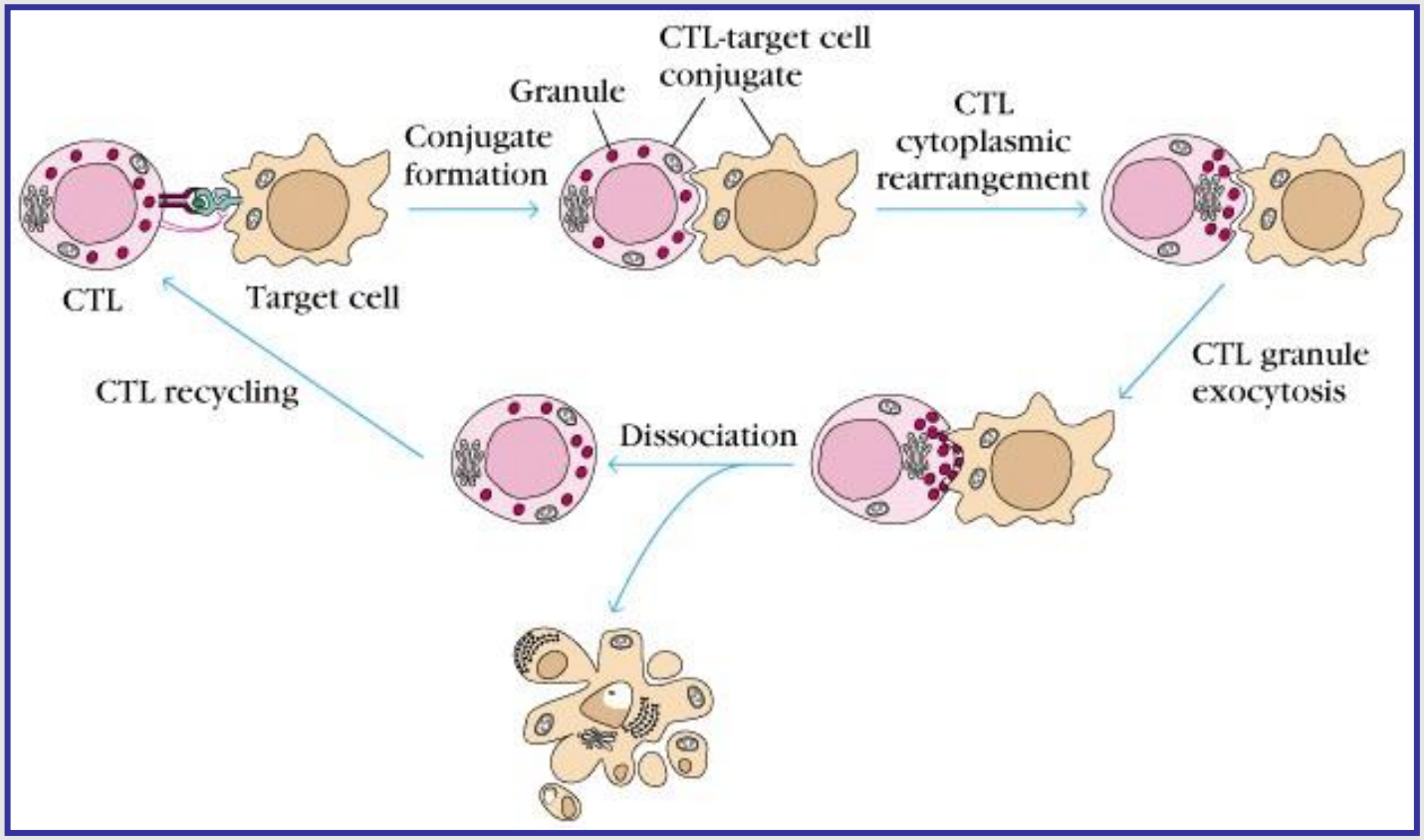
CELLULAR IMMUNE RESPONSE

Cell-mediated immuneresponse (CMI)

Cytotoxicity	***DTH***
Effector cells direct cytotoxic activity: **- CTL (CD8+ Tc),** **- γδ T cells** **- NK cells,** **- Macrphages**	**Effector cells cytokine production:** **- T_{DTH} cells = Th1 cells** **- Macrophages**
Target cell (cytosolic antigen): **- allogen cells (transplantation minor histocompatibility antigen)** **- malignant cells** **- virally infected cells** **- chemically modified cells**	**Antigen in phagolysosome:** **- intracellular bacterium, fungi, parasite, virus** **- contact antigens (small molecules (haptén) skin protein complexes)**

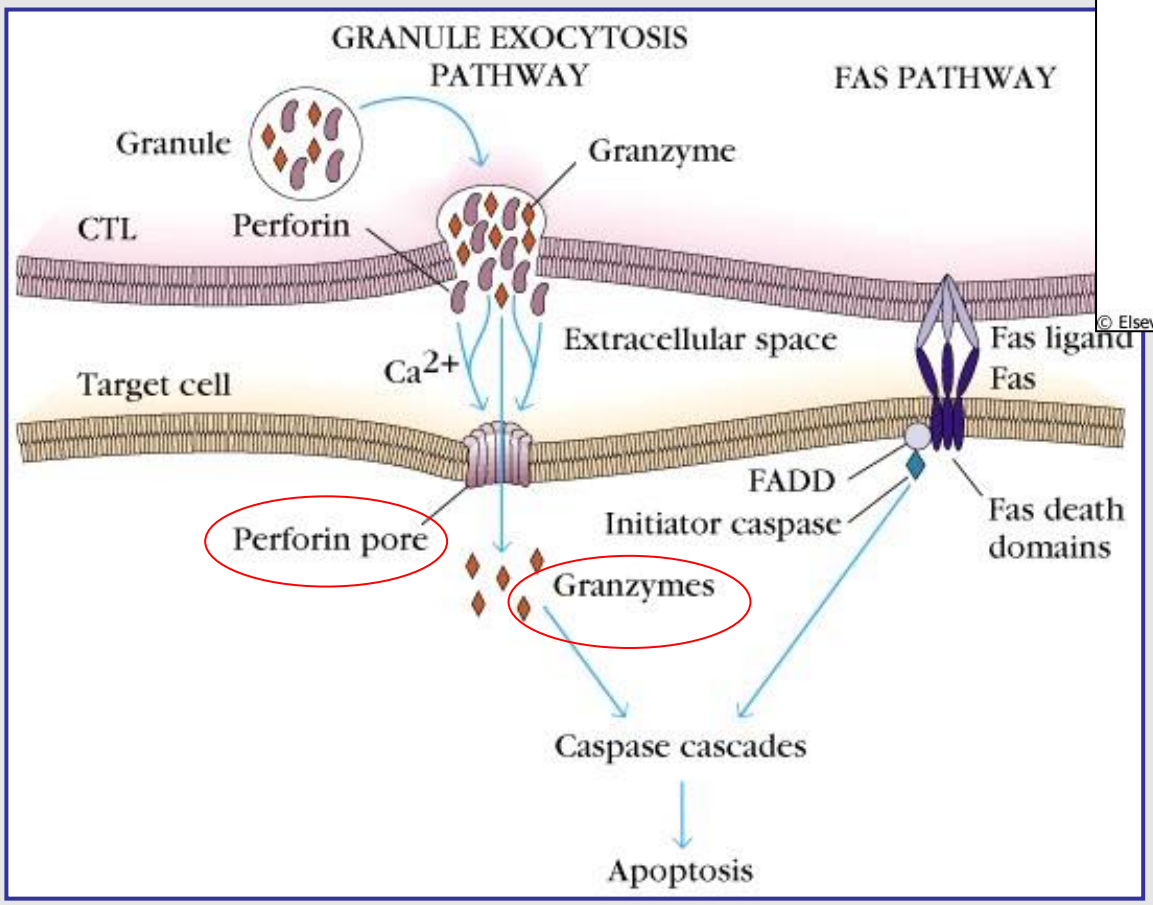
Cytotoxicity

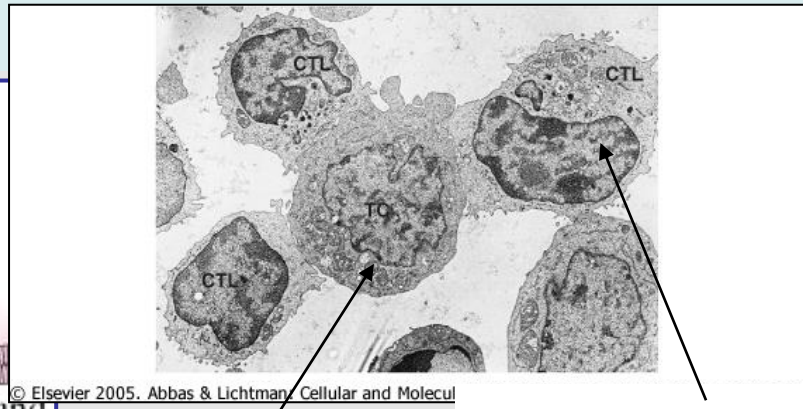
CTL-mediated target cell killing:



1. Antigen recognition 2. Conjugation 3. CTL cytoplasmic rearrangement
4. CTL degranulation 5. Target cell apoptózis 6. Dissociation

Mechanisms of CTL induced apoptosis:





Target cell

Cytotoxic T-cell

Soluble effector molecules: perforins and granzymes

Membrane-bound effector molecules: Fas/Fas ligand (FAS-L)