Overview Of The Immune System

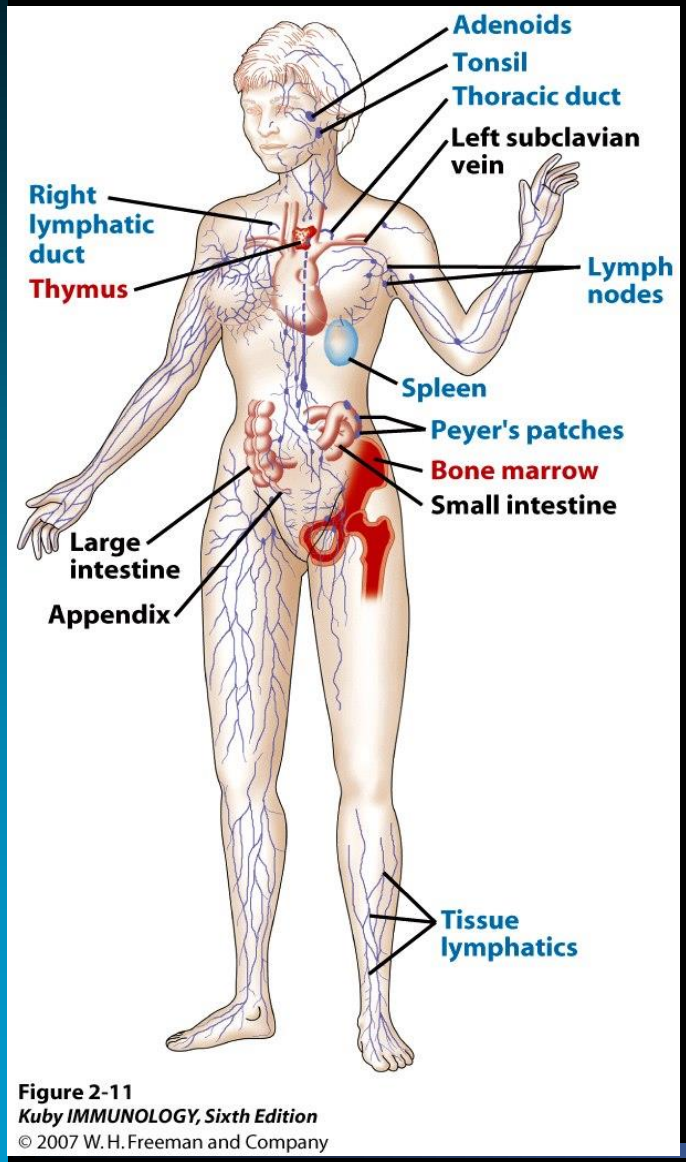

Figure 2-11
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Dr.Manjunath N.S

Immune System

[a] Defence System

[b] Extremely adaptable

[c] Generates a variety of cells and molecules

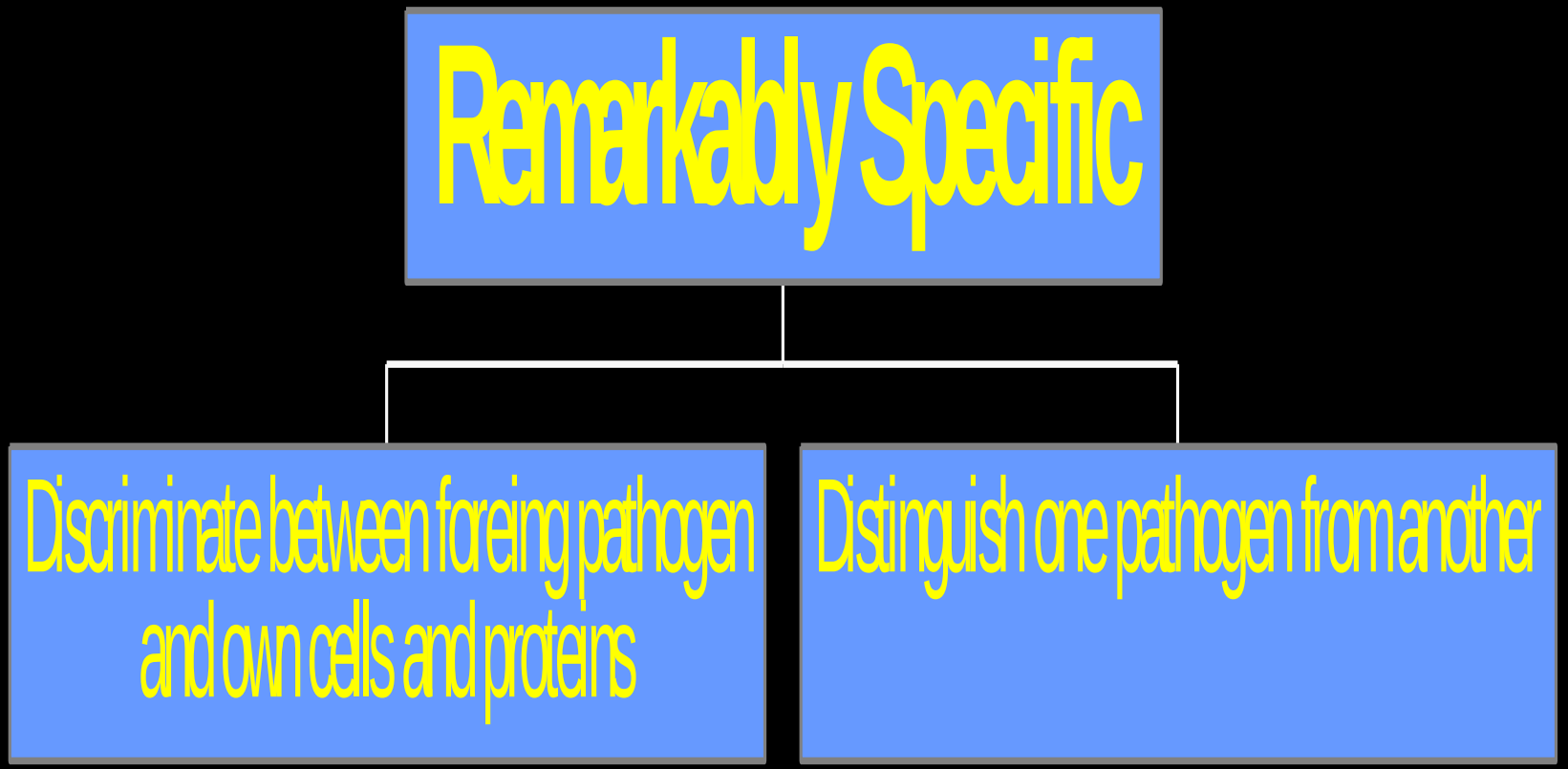
Immune Response

Two interrelated activities

[1] **R**ecognition

[2] **R**esponse

Recognition

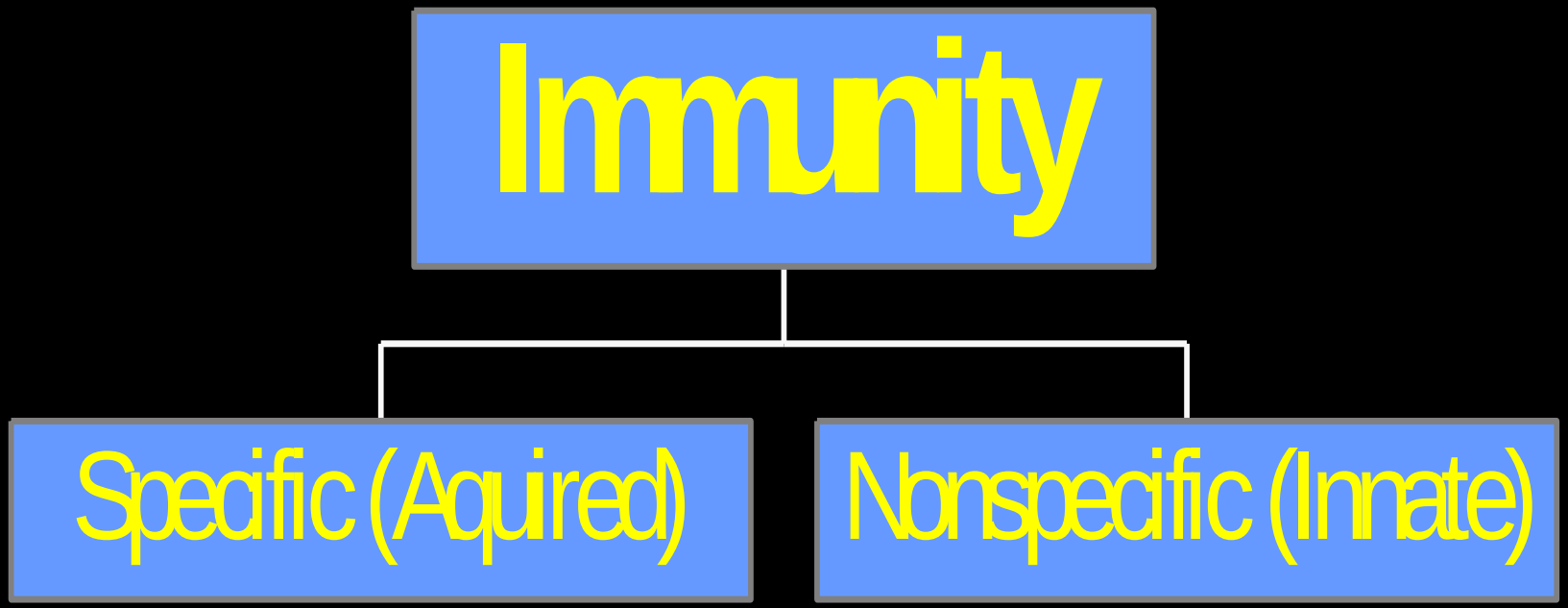


Response

- Also known as effector function
- Eliminate or neutralize foreign organisms
- Later exposure to same foreign organism ⇒ memory response ⇒ heightened immune reactivity.

Immunity

"State of protection from infectious diseases"



Innate (Nonspecific) Immunity

"Basic resistance to disease that a species possesses"

1.Anatomic Barriers

- **Skin**

Sebaceous Glands ⇒ secrete sebum ⇒ low pH (3-5)
⇒ Inhibitory to growth of most microorganisms

- **Mucous membranes**

Respiratory/ Gastrointestinal/ Urogenital tracts

Secrete mucus ⇒ traps microorganisms and expels them by movement of cilia

2.Physiologic / Endocytic Barriers

Temperature - Fever - Inhibition of Microbes

pH- Acidity of stomach

Soluble factors -

Gastric juice acidic ⇒ organisms can't survive.

Newborns ⇒ less acidic gastric juice ⇒ more susceptible to infections.

Soluble factors

Complement = serum proteins that are non active.

when pathogen enters $\Rightarrow$ activated $\Rightarrow$ membrane damaging reactions $\Rightarrow$ clear infections

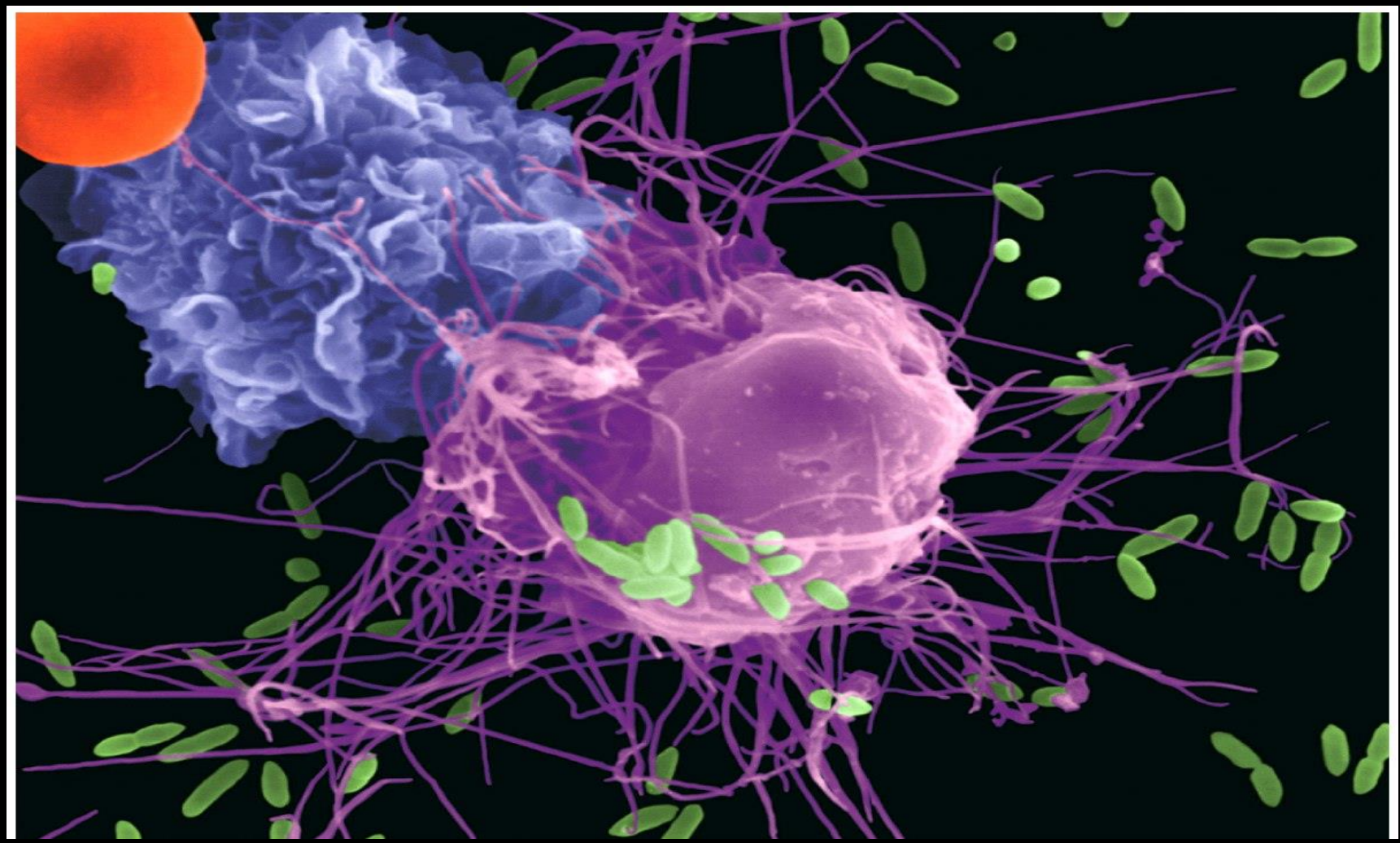
3.Endocytic and Phagocytic Barriers

Endocytosis "Macromolecules in the internalized by cells"

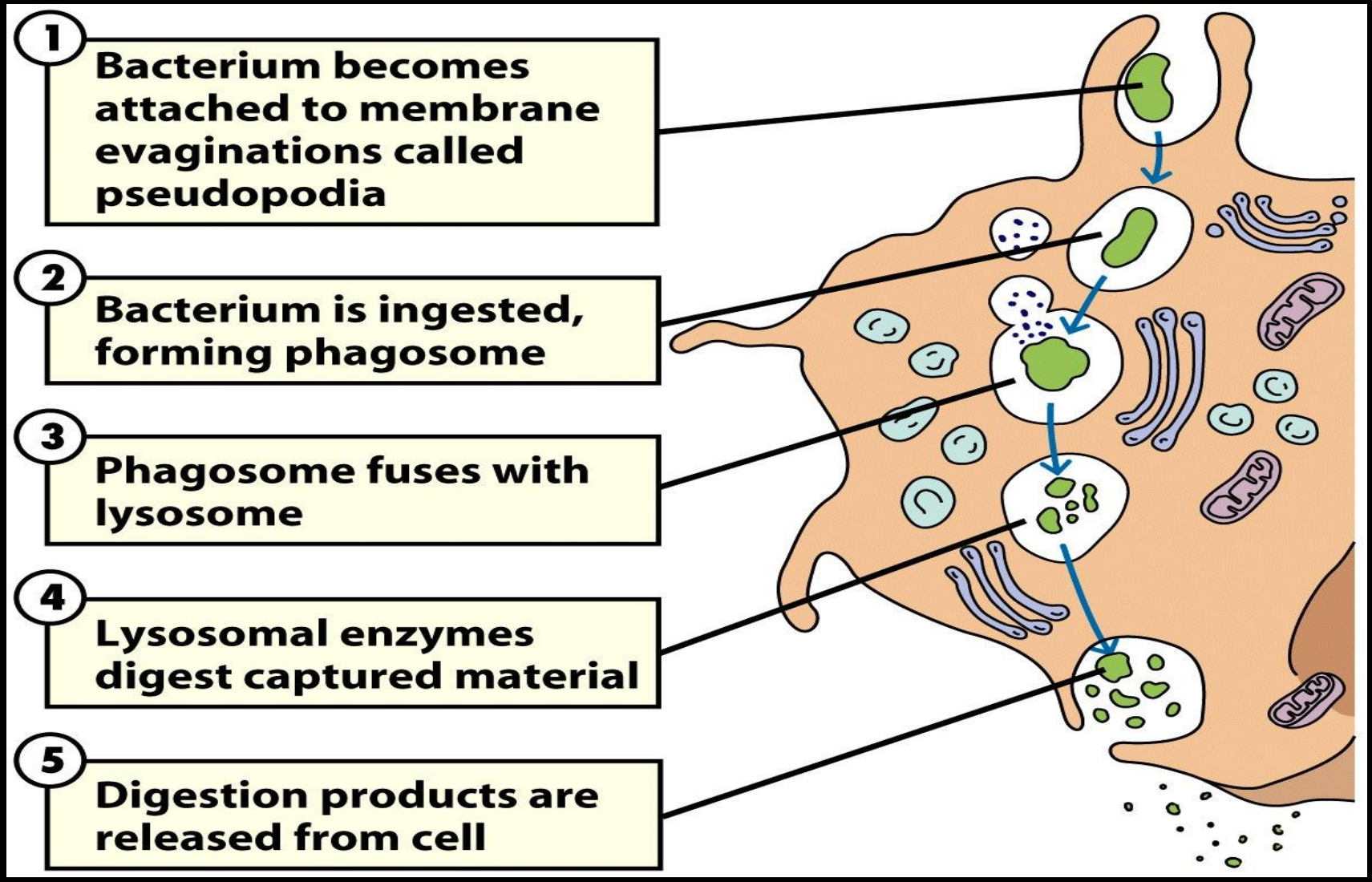
Phagocytosis - "More specialised and involves plasma membranes expanding around macromolecules

Specialized phagocytic cells include:
Monocytes,
Macrophages
Neutrophils.

(a) Electron micrograph of macrophage (center, pink) attacking *Escherichia coli* (green)



(b) Steps involved in Phagocytic activity



4. Inflammatory Response

Tissue damage & Infections – Influx of Phagocytic cells into affected area (leakage of vascular fluid).

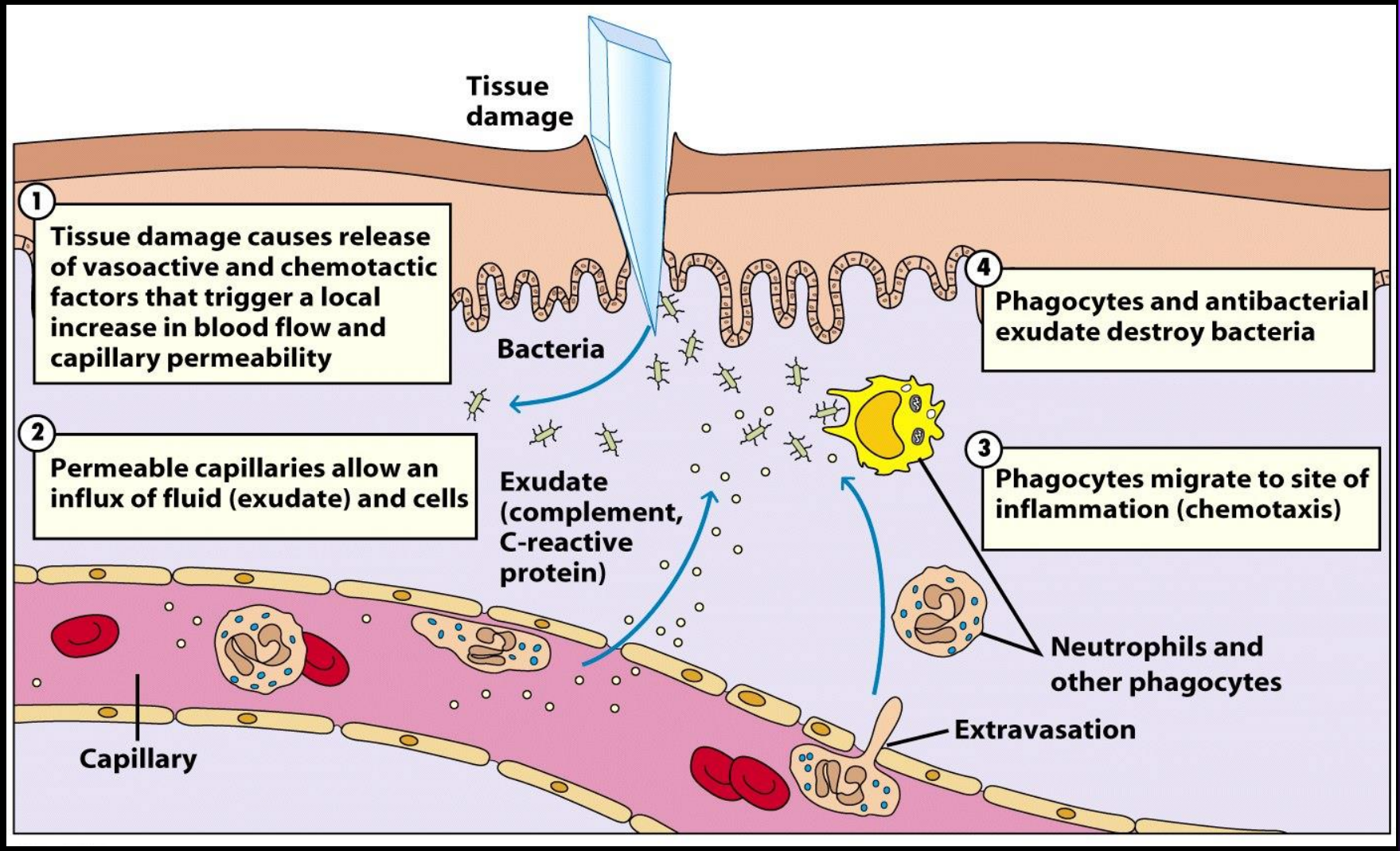
Signs:

Redness	(Ruber)
Swelling	(Tumor)
Heat	(Colar)
Pain	(Dolor)

Three major events:

(1) Vadodilation -Blood capillaries -Redness,Temperature
(2) Increased capillary permeability – Edema,margination
(3) Influx of phagocytic cells (chemotaxis, Margination, Extavasation)

Major events in the inflammatory response - Recruitment of macrophages and antimicrobial agents from the bloodstream



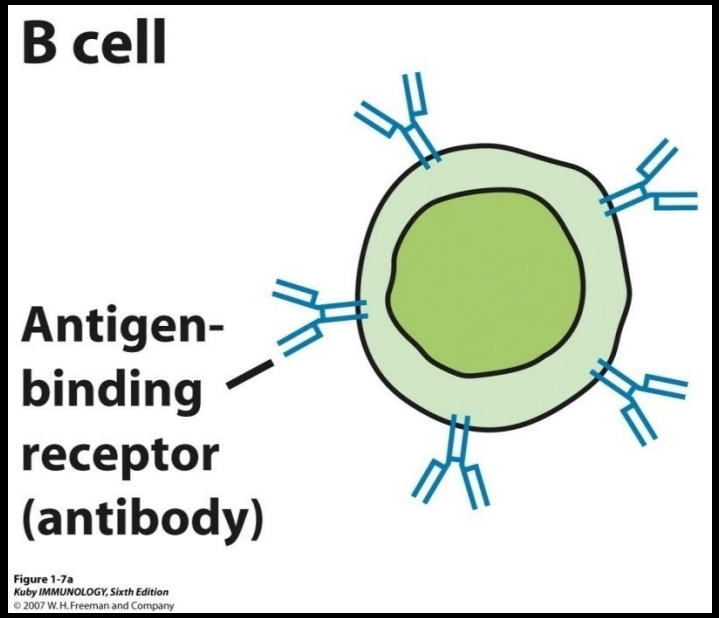
Adaptive (Specific) Immunity

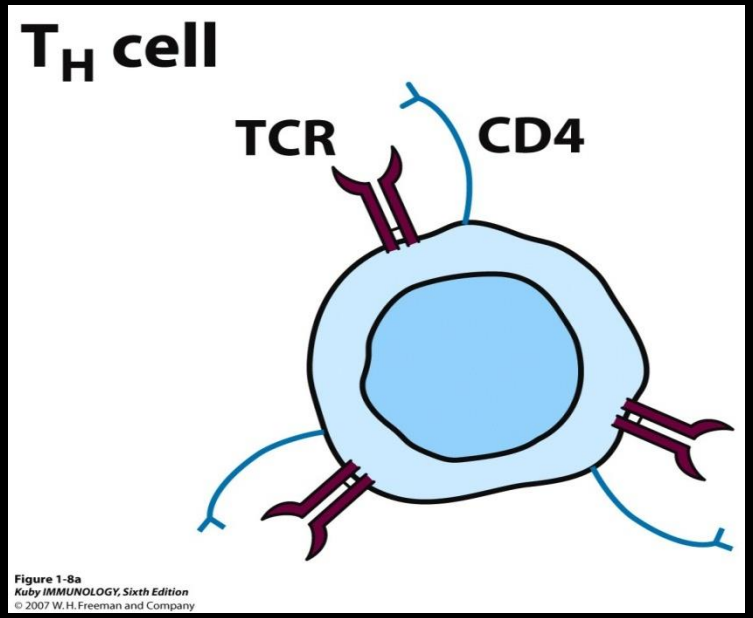
"Reflecting the presence of a specific and functional immune system"

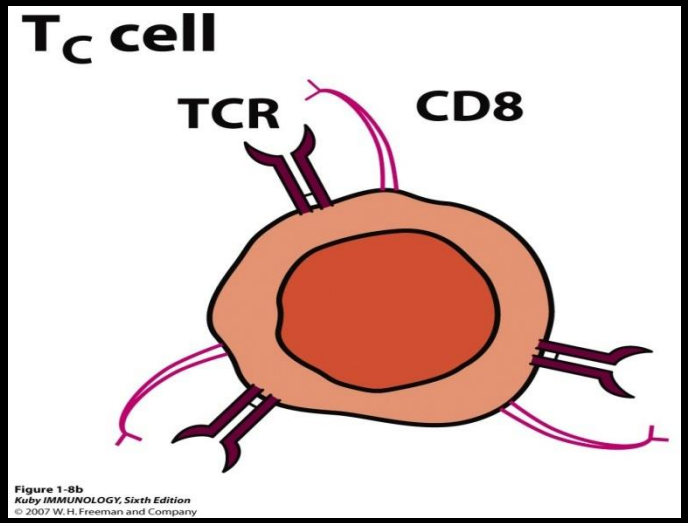
Properties of Adaptive immunity:

- Specificity
- Diversity
- Immunologgic Memory
- Self/ Nonself recognition

B-Cells, T-Cells, Antigen presenting Cells







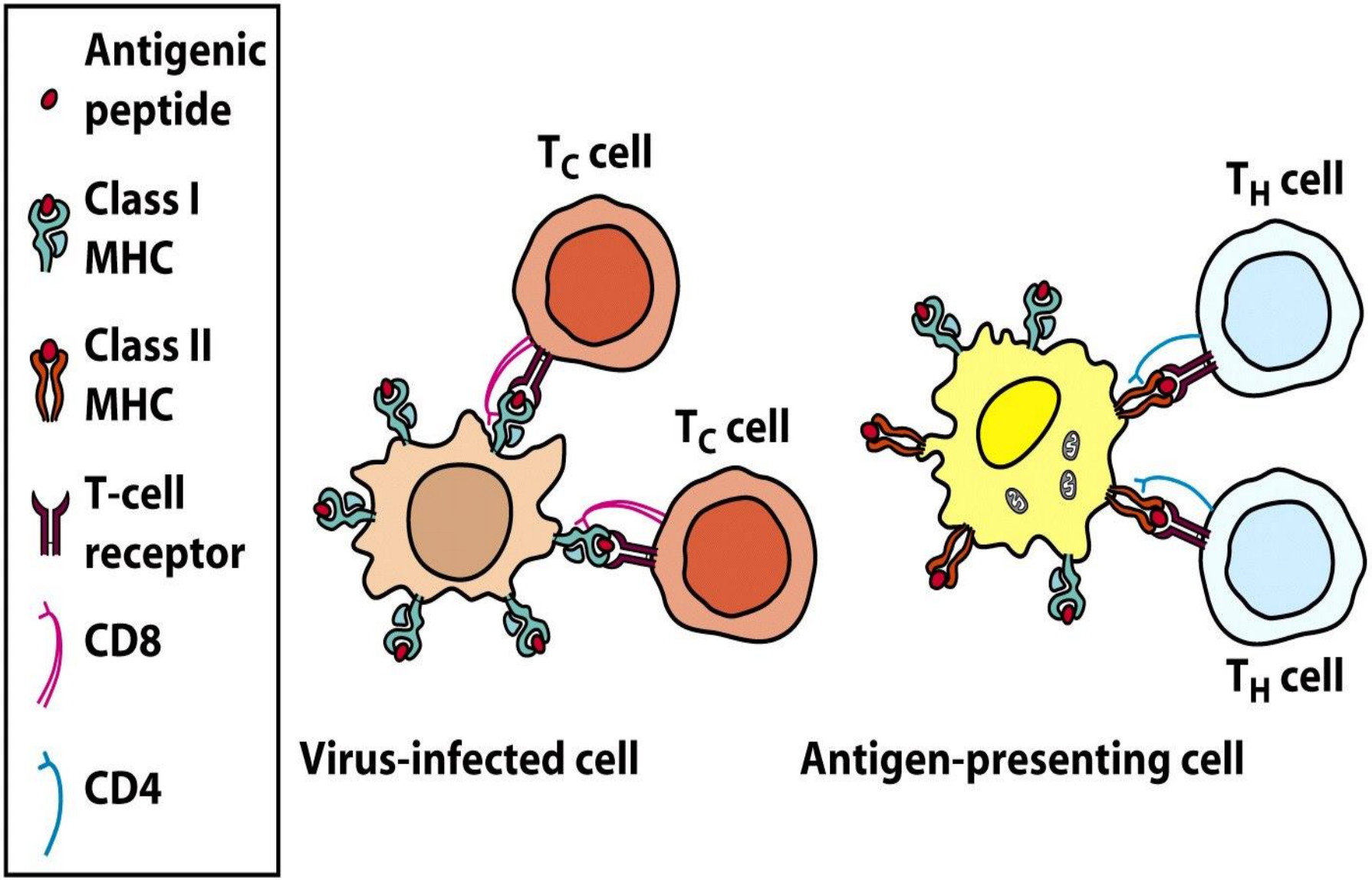


Figure 1-11
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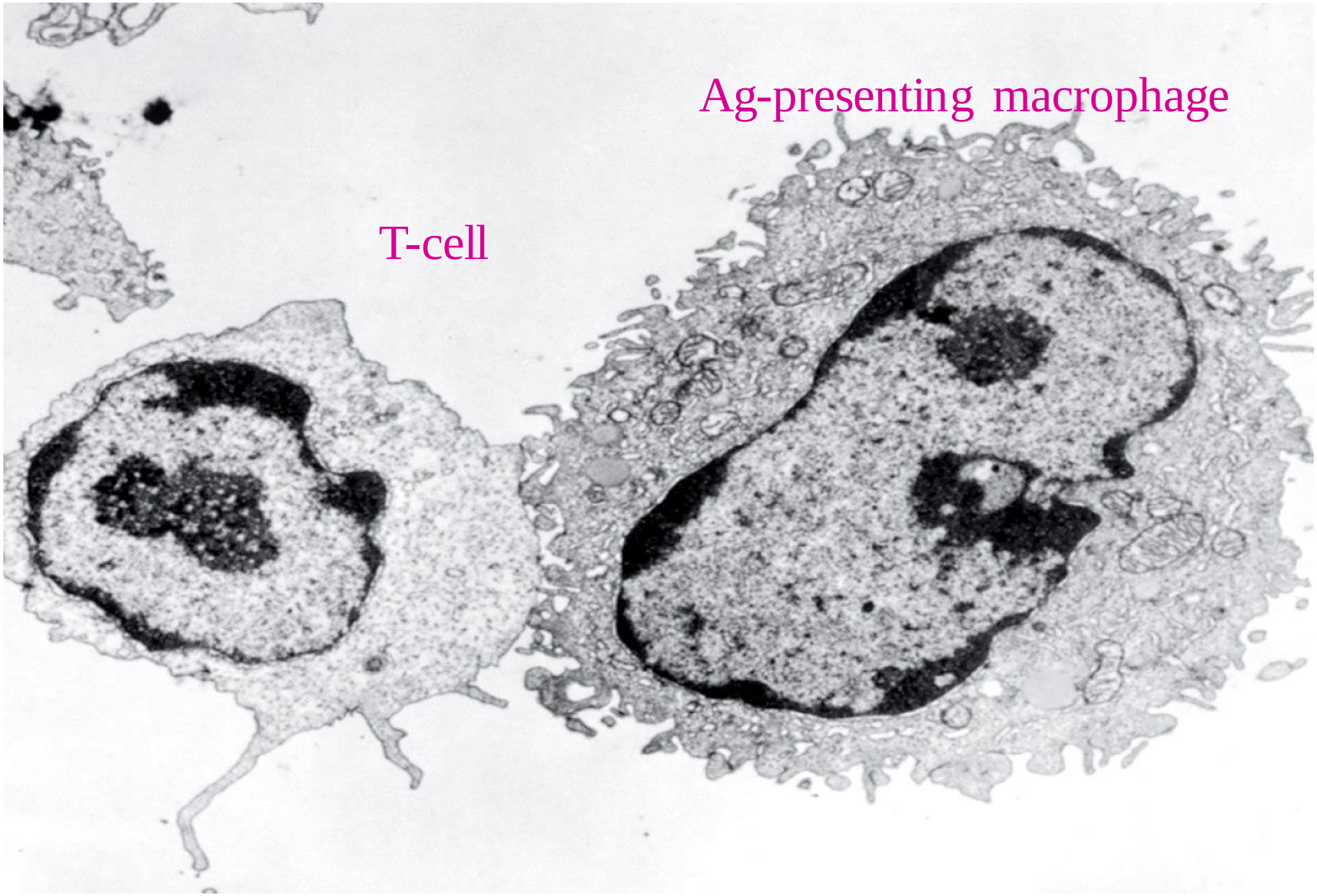

Figure 1-9
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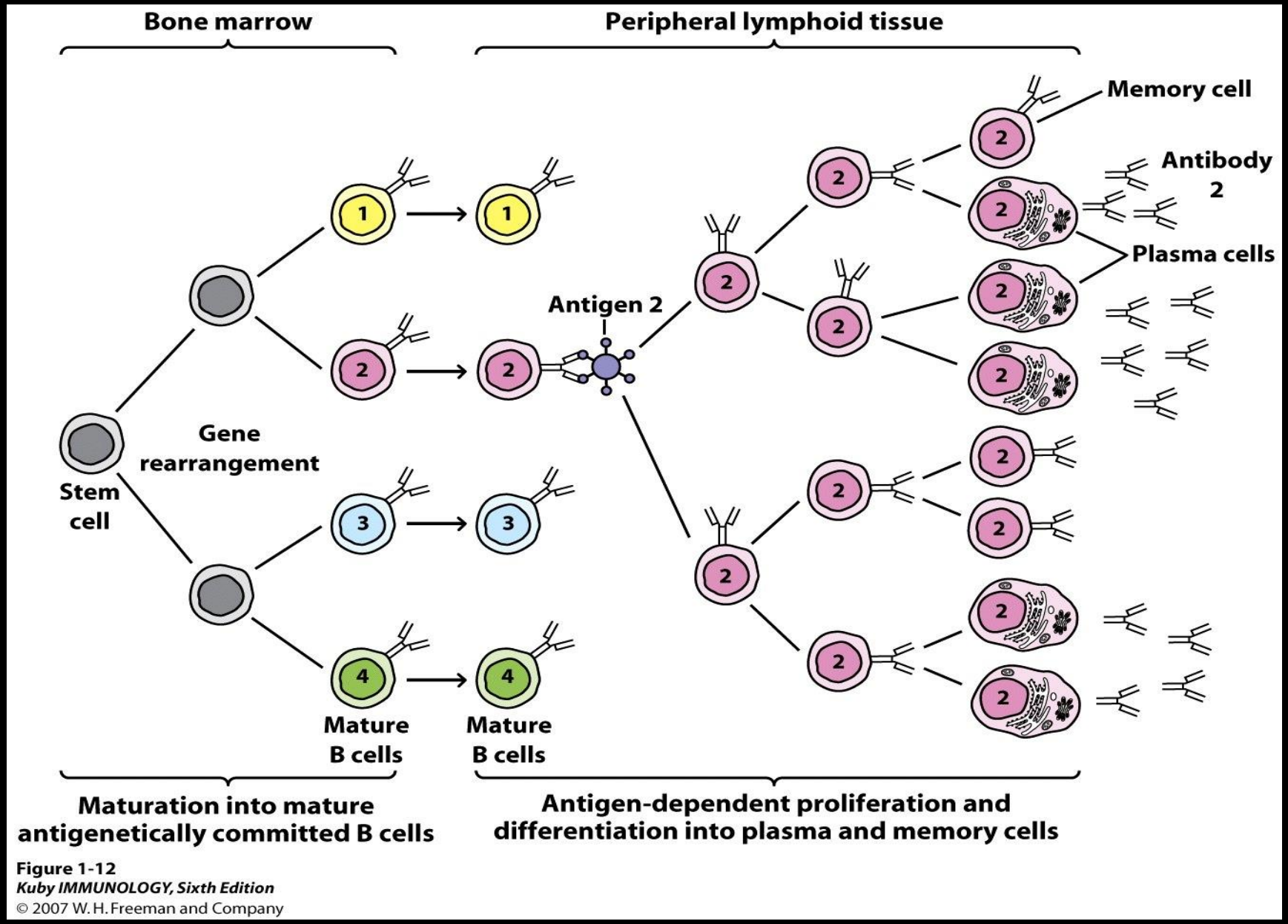

Figure 1-12
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Overview of Humoral and Cell mediated Immunity

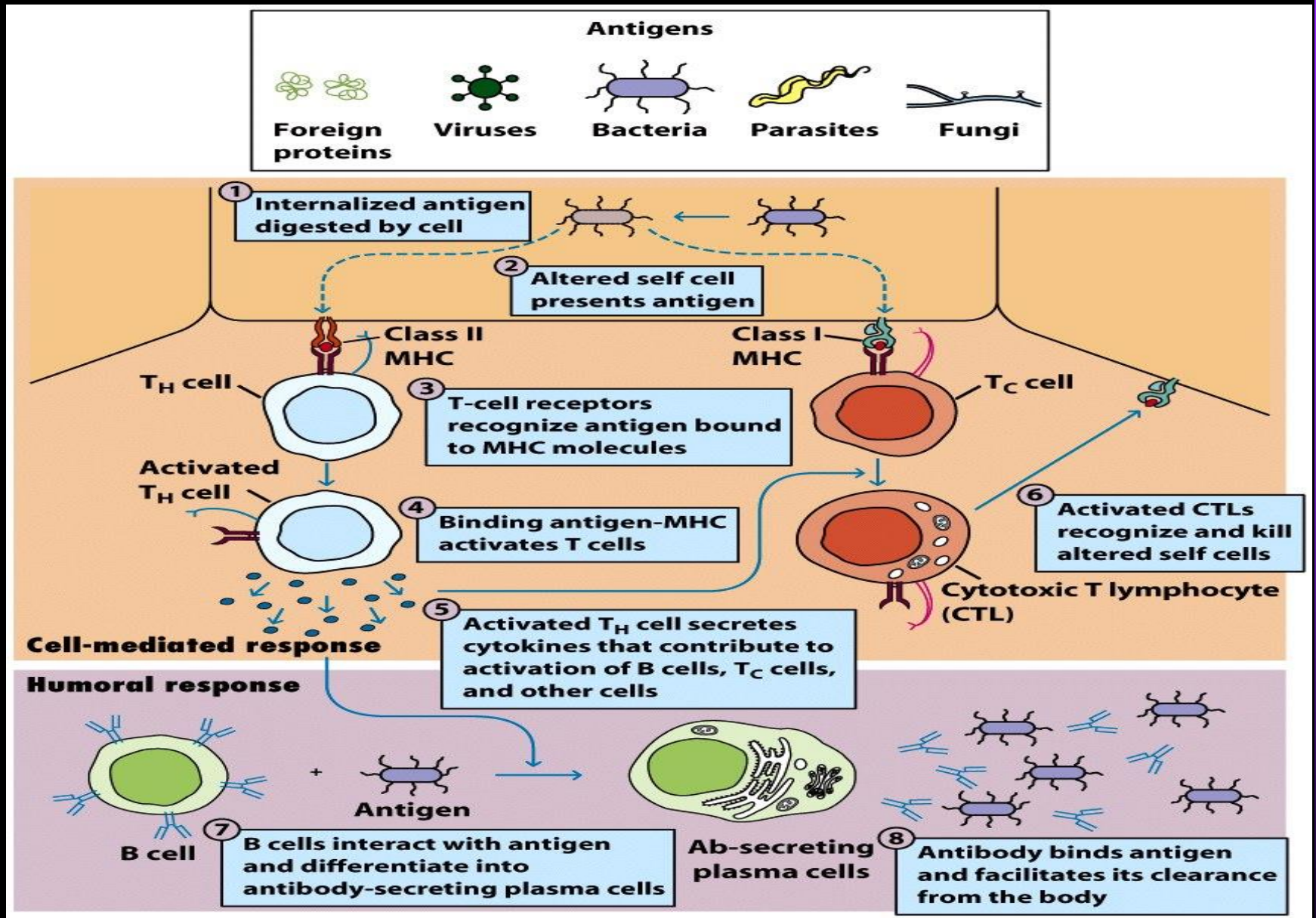

Figure 1-10
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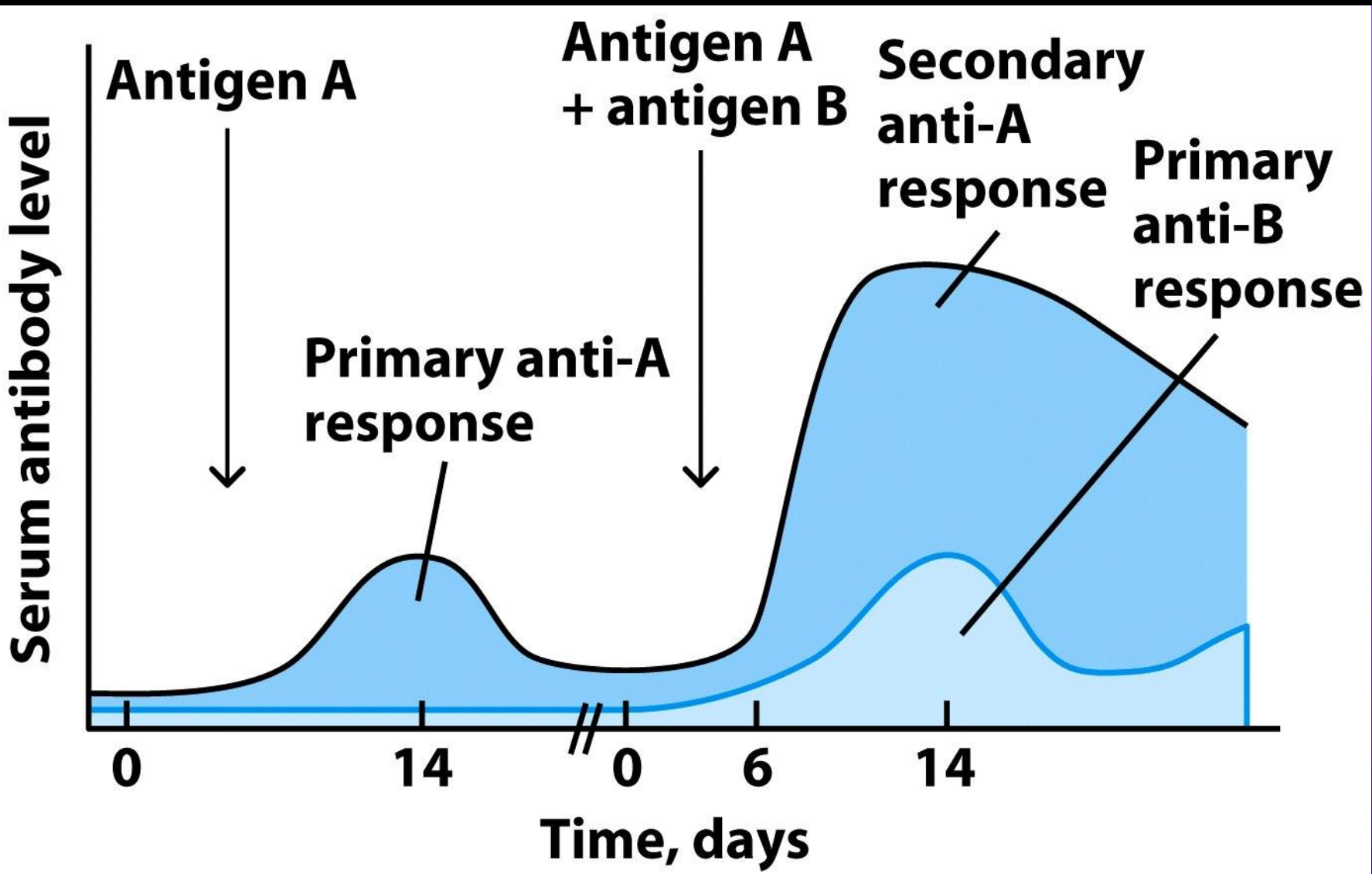

Figure 1-13
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Chapter 2

Cells and Organs of the Immune System

IMMUNOLOGY

Kindt • Goldsby • Osborne

Kuby IMMUNOLOGY

Sixth Edition

Chapter 2:

Cells and Organs
of the Immune System

Hematopoiesis

- All blood cells arise from Hematopoietic Stem Cells (HSC)
  - Study of these cells is difficult
    - Scarce
    - Difficult to grow in vitro

Hematopoiesis

- Early in hematopoiesis, stem cell differentiates to either
  - Lymphoid progenitor cell
  - Myeloid progenitor cell

    - Progenitor cells have lost ability for self renewal and are committed to particular cell lineage

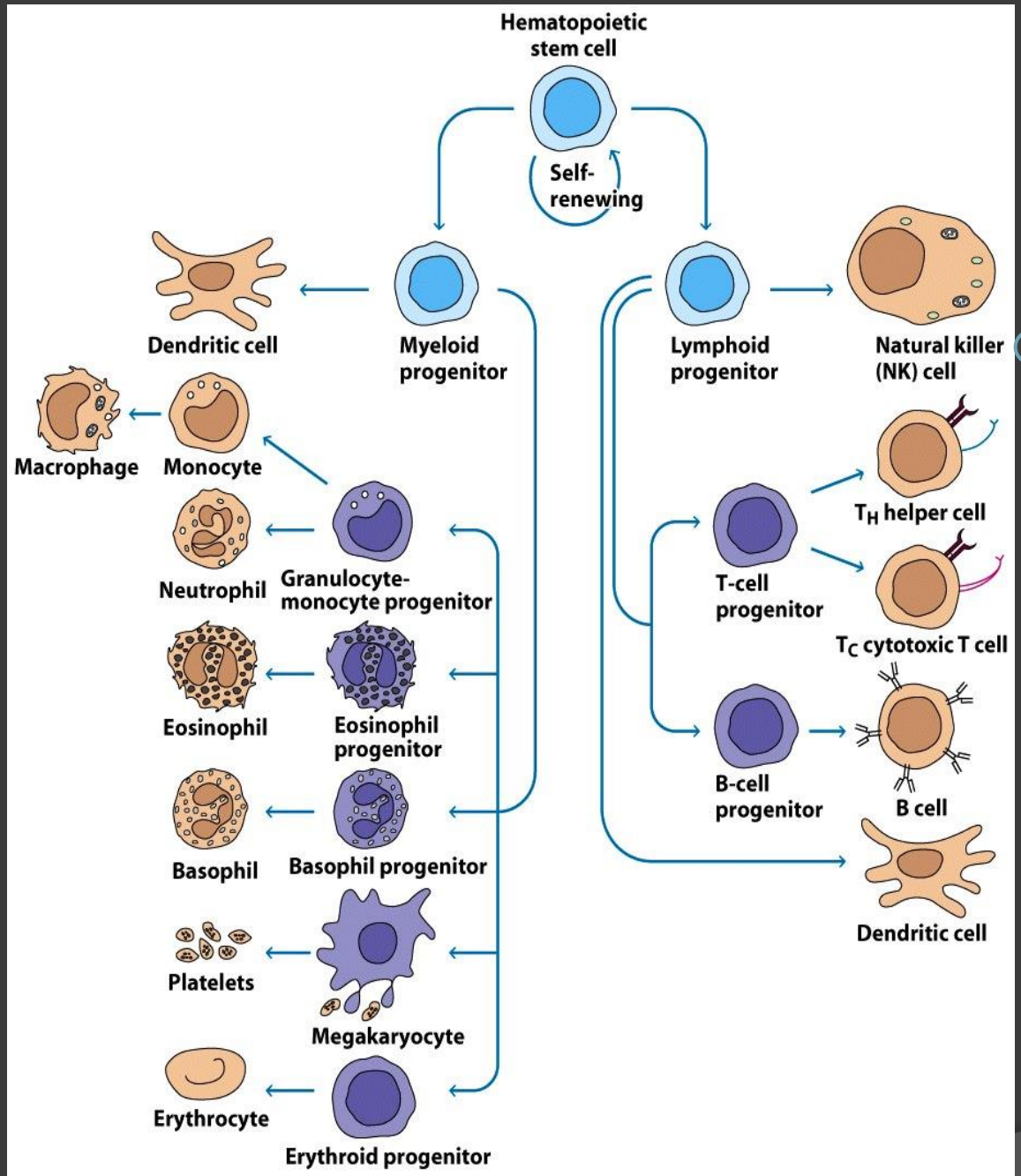

Figure 2-2
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- Organized hierarchy
  - Most of proliferation takes place in differentiated precursors (that are NOT self-renewing) rather than hematopoietic stem cell
  - Lowers chance of cancer

Hematopoiesis

- Regulated at gene level
  - Transcription factors play important roles in hematopoiesis
  - Studies using “knockout” mice
    - Gene inactivated, if RBC or a particular WBC fails to develop, it is concluded that protein was involved in development of that cell

TABLE 2-1 Some transcription factors essential for hematopoietic lineages

Factor	Dependent lineage
GATA-1	Erythroid
GATA-2	Erythroid, myeloid, lymphoid
PU.1	Erythroid (maturational stages), myeloid (later stages), lymphoid
Bmi-1	All hematopoietic lineages
Ikaros	Lymphoid
Oct-2	B lymphoid (differentiation of B cells into plasma cells)

Table 2-1
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Hematopoietic Homeostasis

- Erythrocyte
  - Average life span: 120 days
  - Phagocytosed by macrophages in spleen
- WBC - LEUKOCYTES
  - Life spans from 1 day to 20-30 years
- Apoptosis – programmed cell death

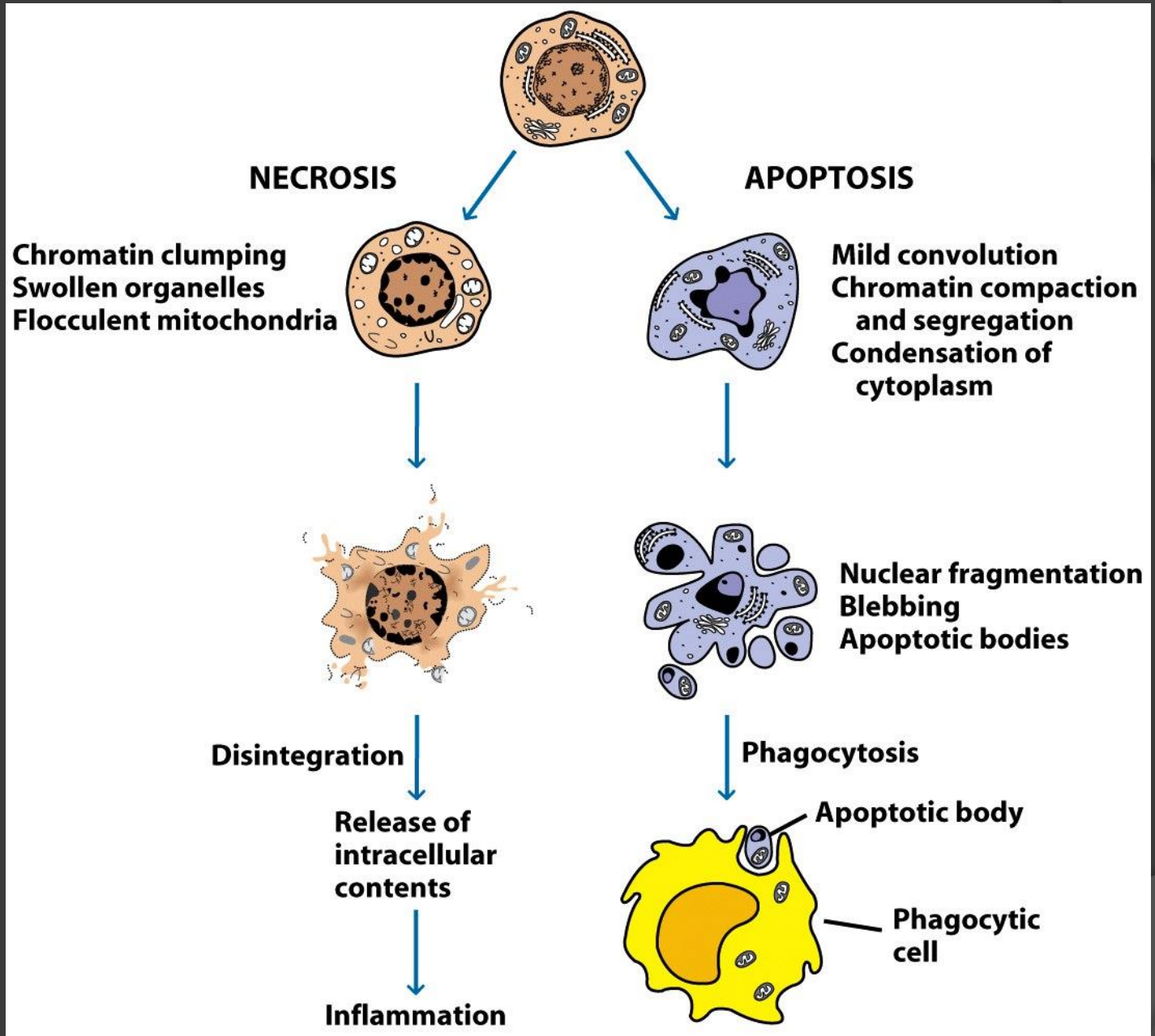

Figure 2-3
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TABLE 2-2 Genes that regulate apoptosis

Gene	Function	Role in apoptosis
bcl-2	Prevents apoptosis	Inhibits
bax	Opposes *bcl-2*	Promotes
bcl-X_L (bcl-Long)	Prevents apoptosis	Inhibits
bcl-X_S (bcl-Short)	Opposes *bcl-X_L*	Promotes
caspase (several different ones)	Protease	Promotes
Fas	Induces apoptosis	Initiates

Table 2-2
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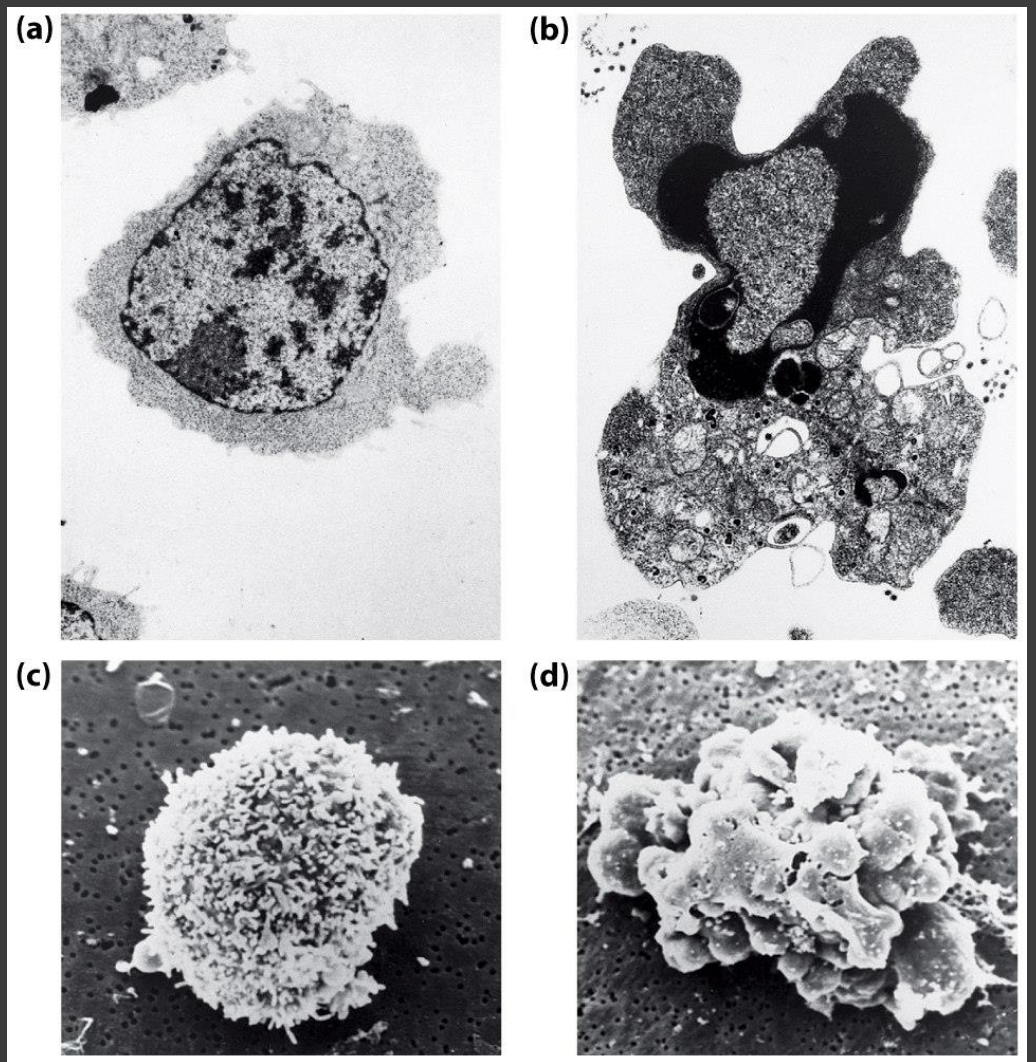

Figure 2-4
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Normal WBC

WBC going through apoptosis

Cells of the Immune System

- **Lymphocytes**
  - 20-40% of WBC , 90% of Lymph
  - 3 populations
    - B cells
    - T cells
    - Natural Killer Cells

TABLE 2-4 Normal adult blood cell counts

Cell type	Cells/mm^3	Total leukocytes (%)
Red blood cells	5.0×10^6	
Platelets	2.5×10^5	
Leukocytes	7.3×10^3	
Neutrophil	$3.7–5.1 \times 10^3$	50–70
Lymphocyte	$1.5–3.0 \times 10^3$	20–40
Monocyte	$1–4.4 \times 10^2$	1–6
Eosinophil	$1–2.2 \times 10^2$	1–3
Basophil	$<1.3 \times 10^2$	<1

Table 2-4
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Lymphocytes

- **B cells and T cells**
  - Adaptive immunity
  - Small lymphocytes
  - Those that have not interacted with antigen are called naïve
  - Interaction with antigen – proliferation into effector cells (i.e. plasma cells) and memory cells

Lymphocytes

- B and T cells

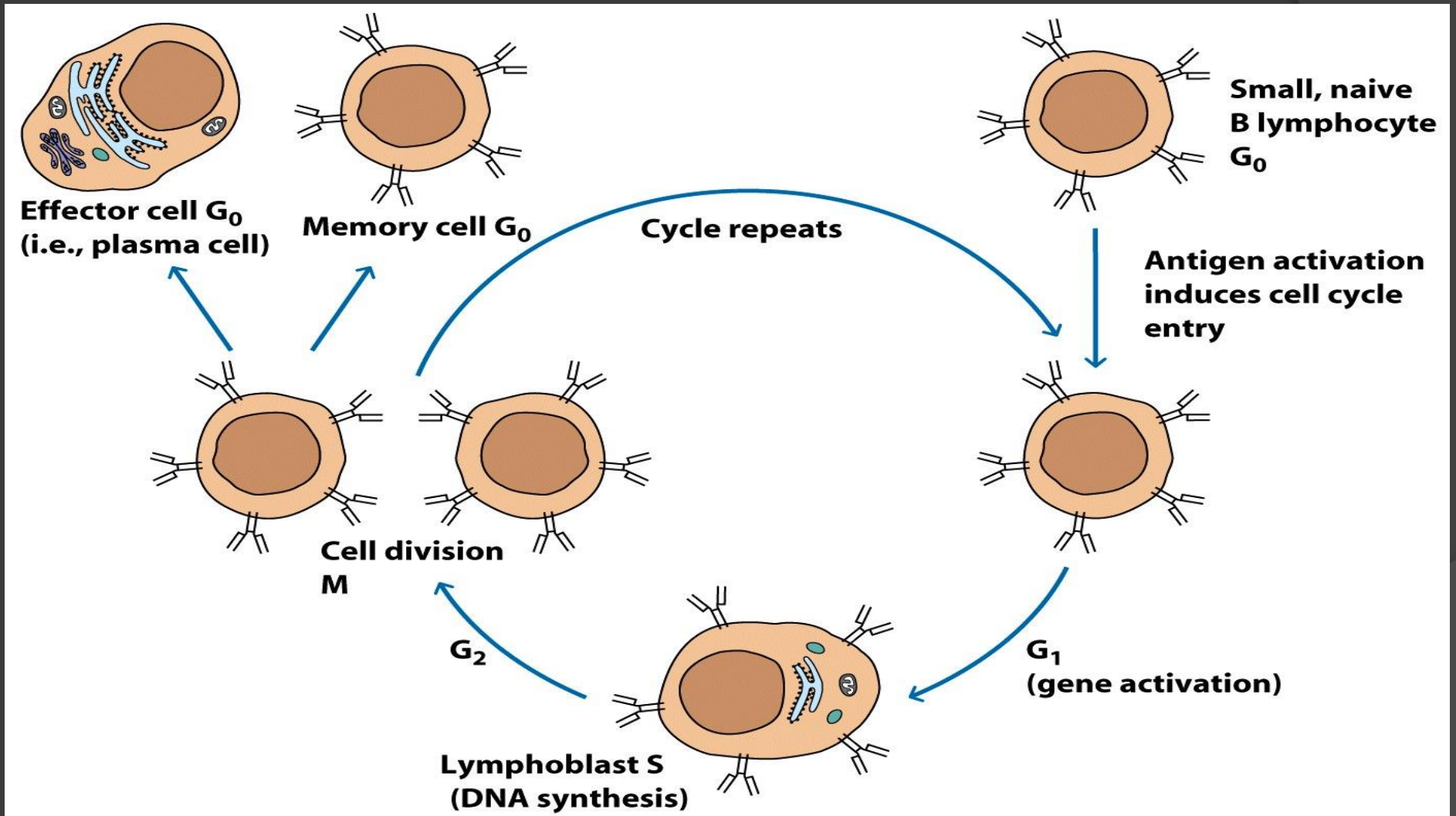

Figure 2-6a
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Lymphocytes

- **B Lymphocytes (B cells)**
  - **Site of maturation**
    - Bursa of fabricus in birds
    - Bone marrow in mammals
  - Display membrane-bound immunoglobulin (antibody)
  - **Once antigen is encountered:**
    - **Differentiation**
      - **Plasma cells – antibody can be secreted, die within 1-2 weeks**
      - **Memory B cells – same membrane-bound antibody as parent B cell, longer life span**

Lymphocytes

- T Lymphocytes (T cells)
  - Site of maturation
    - Thymus
  - T cell receptor
    - Only recognize antigen that is bound to cell membrane proteins called major histocompatibility complex (MHC)
    - Once antigen in encountered with MHC:
      - Differentiation
        - Effector T cells
        - Memory T cells
  - 2 subpopulations
    - T helper (T_H)
    - T cytotoxic (T_C)
    - And now T regulatory (T_{reg})

Lymphocytes

- T helper cells
  - CD4 glycoprotein
  - “help” activation of B cells, T_C cells, macrophages in immune response

Lymphocytes

- T cytotoxic cells
  - CD8 glycoprotein
  - Recognition of MHC-antigen complex initiates differentiation into effector cell called cytotoxic T lymphocyte
    - Eliminates infected cells or cancerous cells

Lymphocytes

- T regulatory cells
  - CD4 and CD25 glycoproteins
  - Help suppress the immune system

Lymphocytes

- Natural Killer Cells
  - Innate immune response
  - Large, granular
  - Recognize tumor or virus-infected cells
  - CD16 – which can recognize a region of antibody that has attached to cell infected by virus

TABLE 2-5 Common CD markers used to distinguish functional lymphocyte subpopulations

CD designation*	Function	B cell	T cell		NK cell
			T_H	T_C	
CD2	Adhesion molecule; signal transduction	−	+	+	+
CD3	Signal transduction element of T-cell receptor	−	+	+	−
CD4	Adhesion molecule that binds to class II MHC molecules; signal transduction	−	+ (usually)	− (usually)	−
CD5	Unknown (subset)	−	−	+	+
CD8	Adhesion molecule that binds to class I MHC molecules; signal transduction	−	− (usually)	+ (usually)	+ (variable)
CD16 (FcγRIII)	Low-affinity receptor for Fc region of IgG	−	−	−	+
CD21 (CR2)	Receptor for complement (C3d) and Epstein-Barr virus	+	−	−	−
CD28	Receptor for costimulatory B7 molecule on antigen-presenting cells	−	+	+	−
CD32 (FcγRII)	Receptor for Fc region of IgG	+	−	−	−
CD35 (CR1)	Receptor for complement (C3b)	+	−	−	−
CD40	Signal transduction	+	−	−	−
CD45	Signal transduction	+	+	+	+
CD56	Adhesion molecule	−	−	−	+

*Synonyms are shown in parentheses.

Table 2-5
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Other Leukocytes

- Mononuclear phagocytes
  - Monocytes circulate in blood and then migrate into tissue and differentiate into specific macrophage
  - Macrophages
    - Intestinal macrophages in gut
    - Alveolar macrophages in lung
    - Histiocytes in connective tissue
    - Kupffer cells in the liver
    - Mesangial cells in the kidney
    - Microglial cells in the brain
    - Osteoclasts in bone

    - Activated macrophages are more effective than resting ones

Monocyte

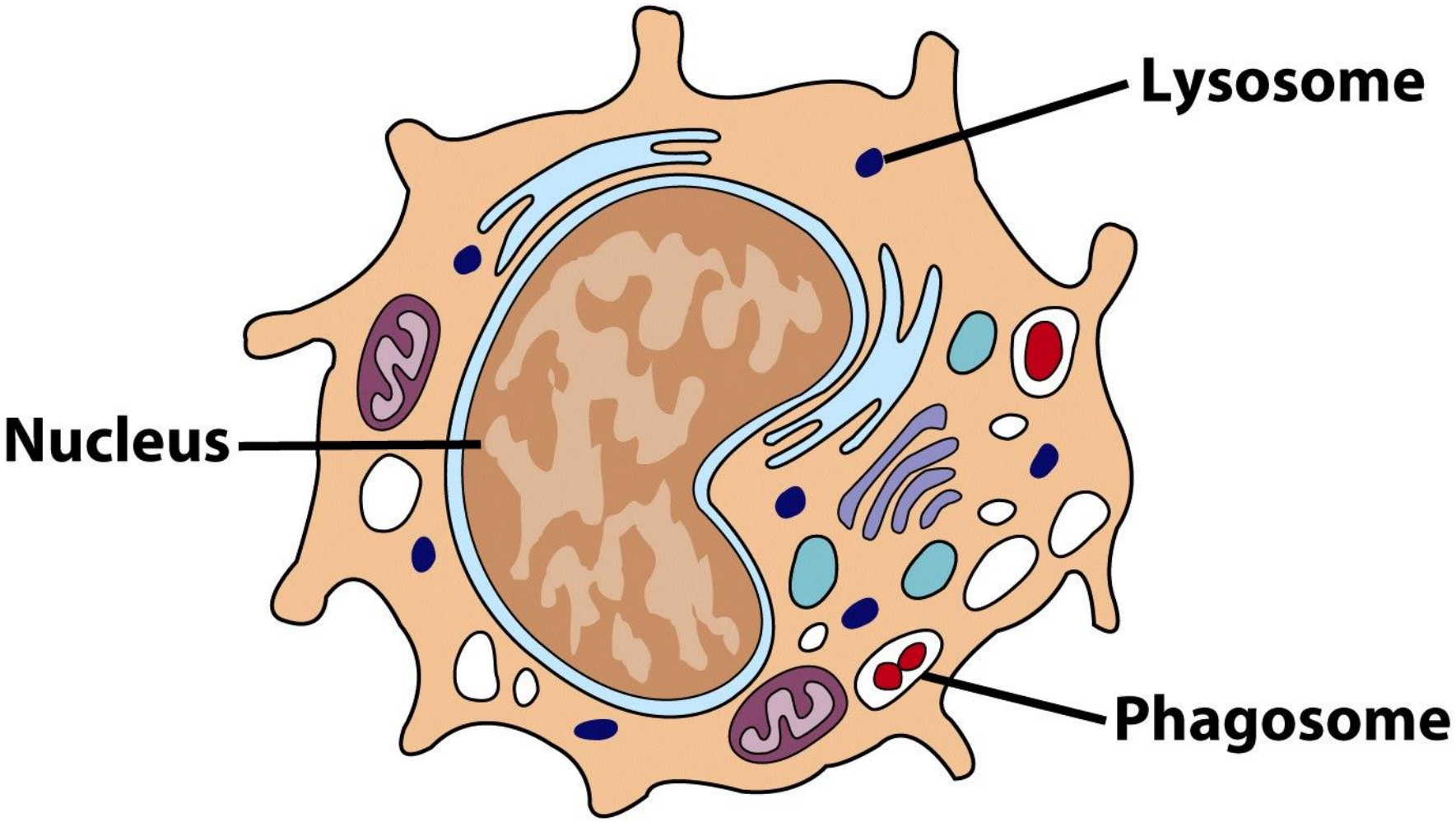

Figure 2-7a
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Macrophage

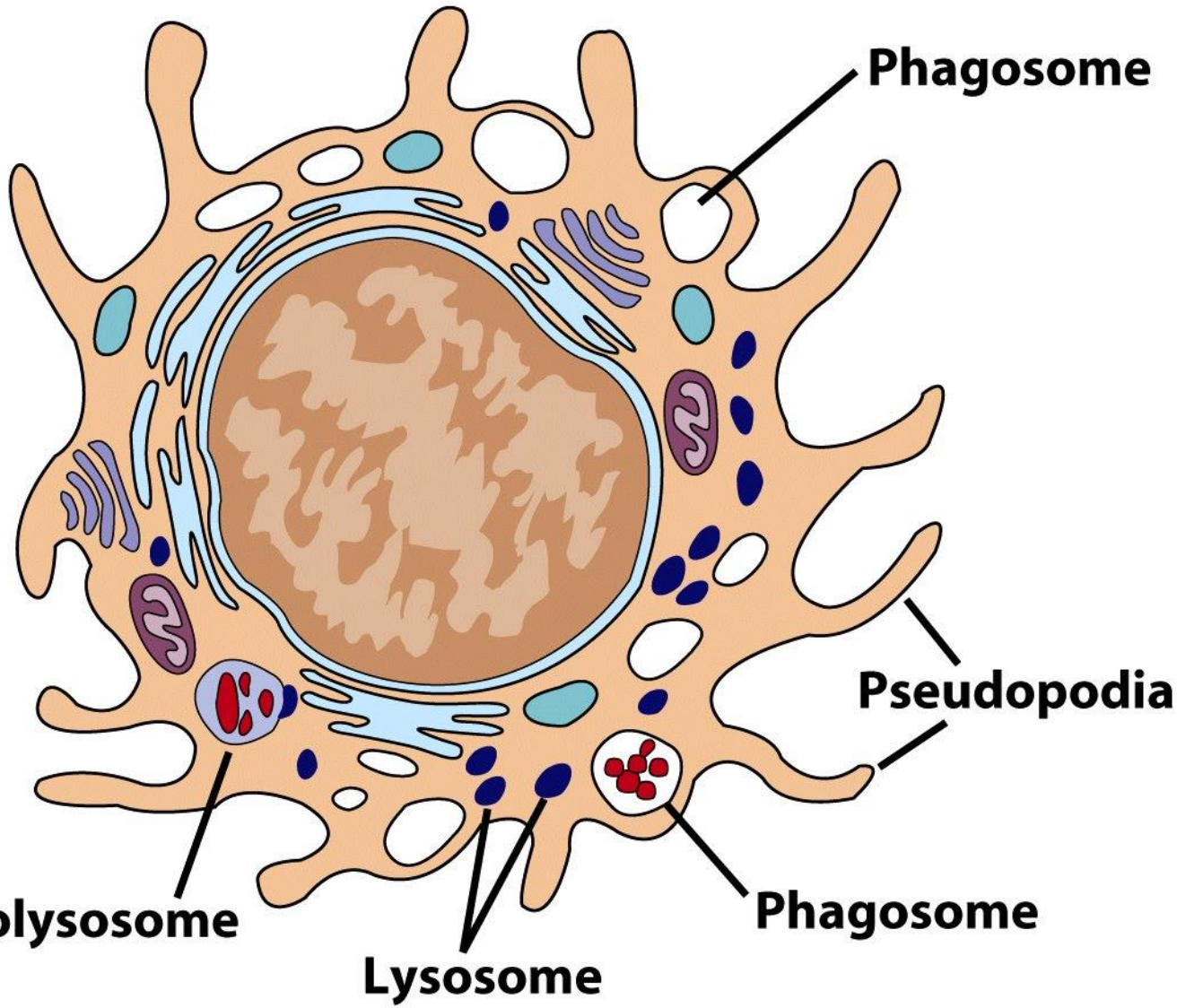

Figure 2-7b
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Other Leukocytes

- Mononuclear phagocytes
  - Complex antigens are phagocytized, the resulting phagosome fuses with a lysosome
  - The digested antigen is then eliminated through exocytosis
    - Some of it is presented on membrane on MHC
  - Phagocytosis is enhanced when antibody is attached to the antigen
    - Antibody acts as opsonin: molecule that binds to both antigen and phagocyte

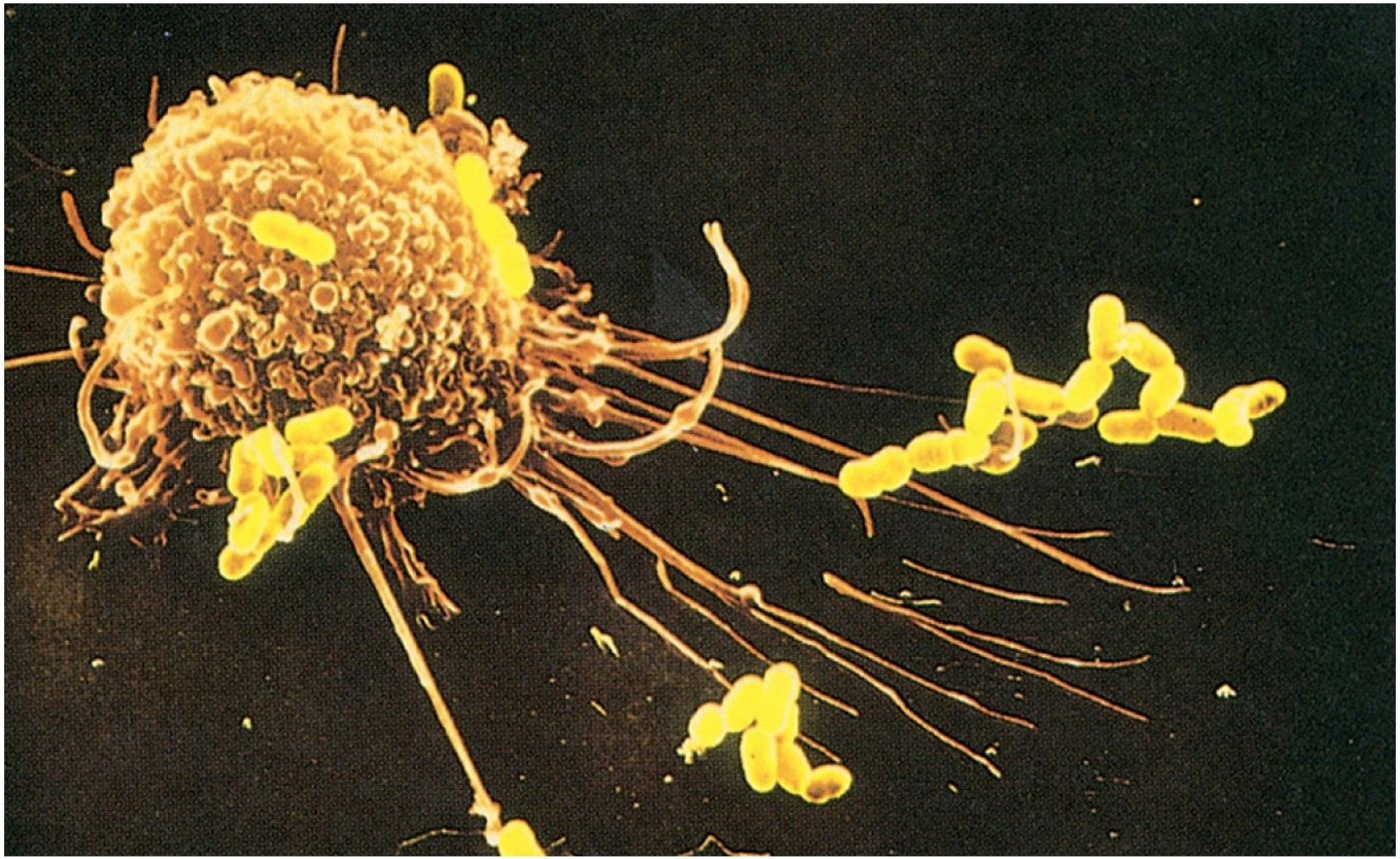

Figure 2-8a
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Macrophage and bacteria

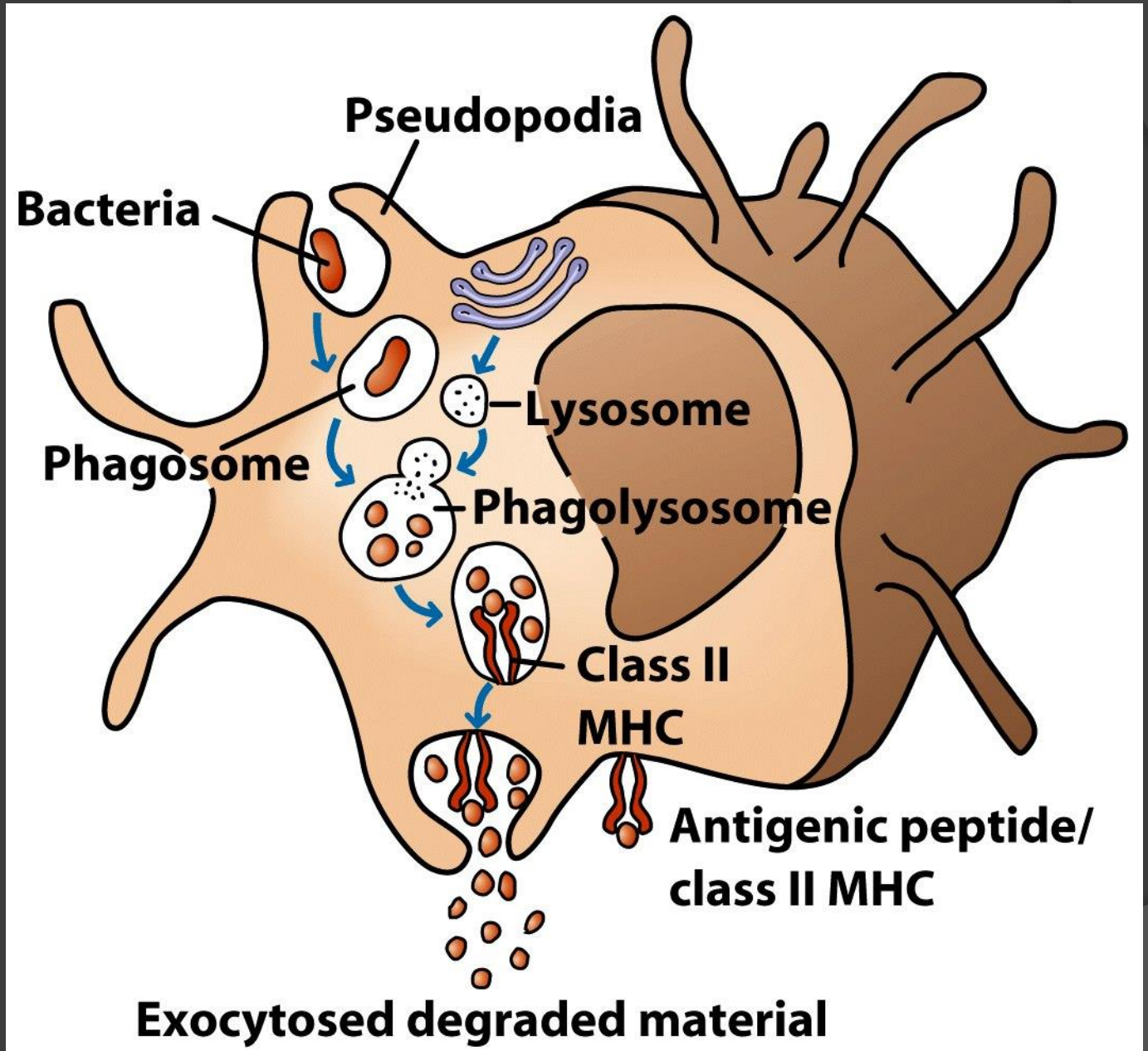

Figure 2-8b
Kuby IMMUNOLOGY, Sixth Edition

Other Leukocytes

- Granulocytes
  - Neutrophils
  - Eosinophils
  - Basophils

Other Leukocytes

- Granulocytes – Neutrophils
  - Multi-lobed nucleus, light granules
  - 1st to arrive at site of inflammation
  - High #'s is 1st indication of infection
  - Phagocytize
  - Generate antimicrobial agents

Neutrophil

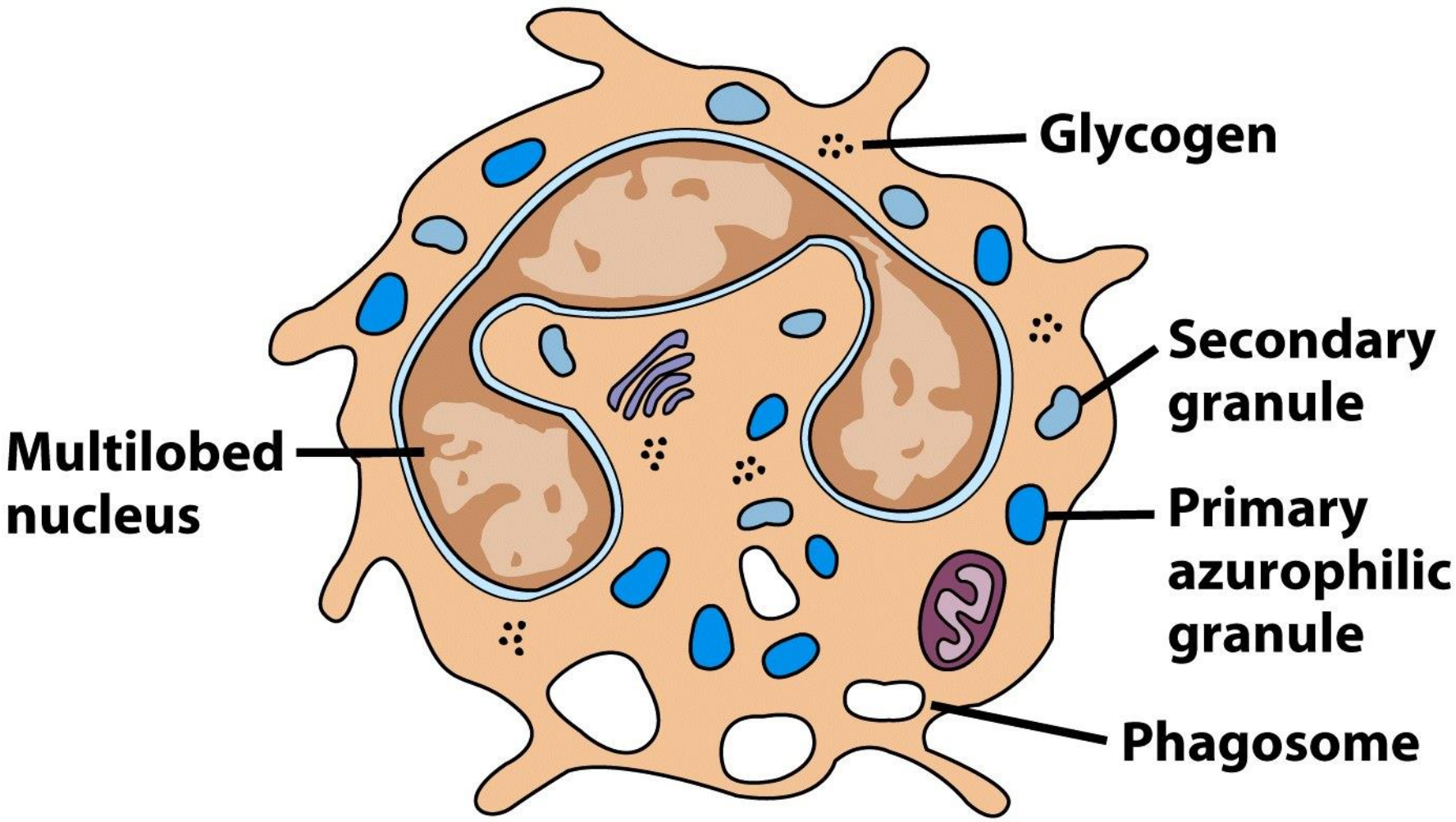

Figure 2-9a
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Other Leukocytes

- Granulocytes – Eosinophils
  - Phagocytize
  - Play a role in parasitic organisms

Eosinophil

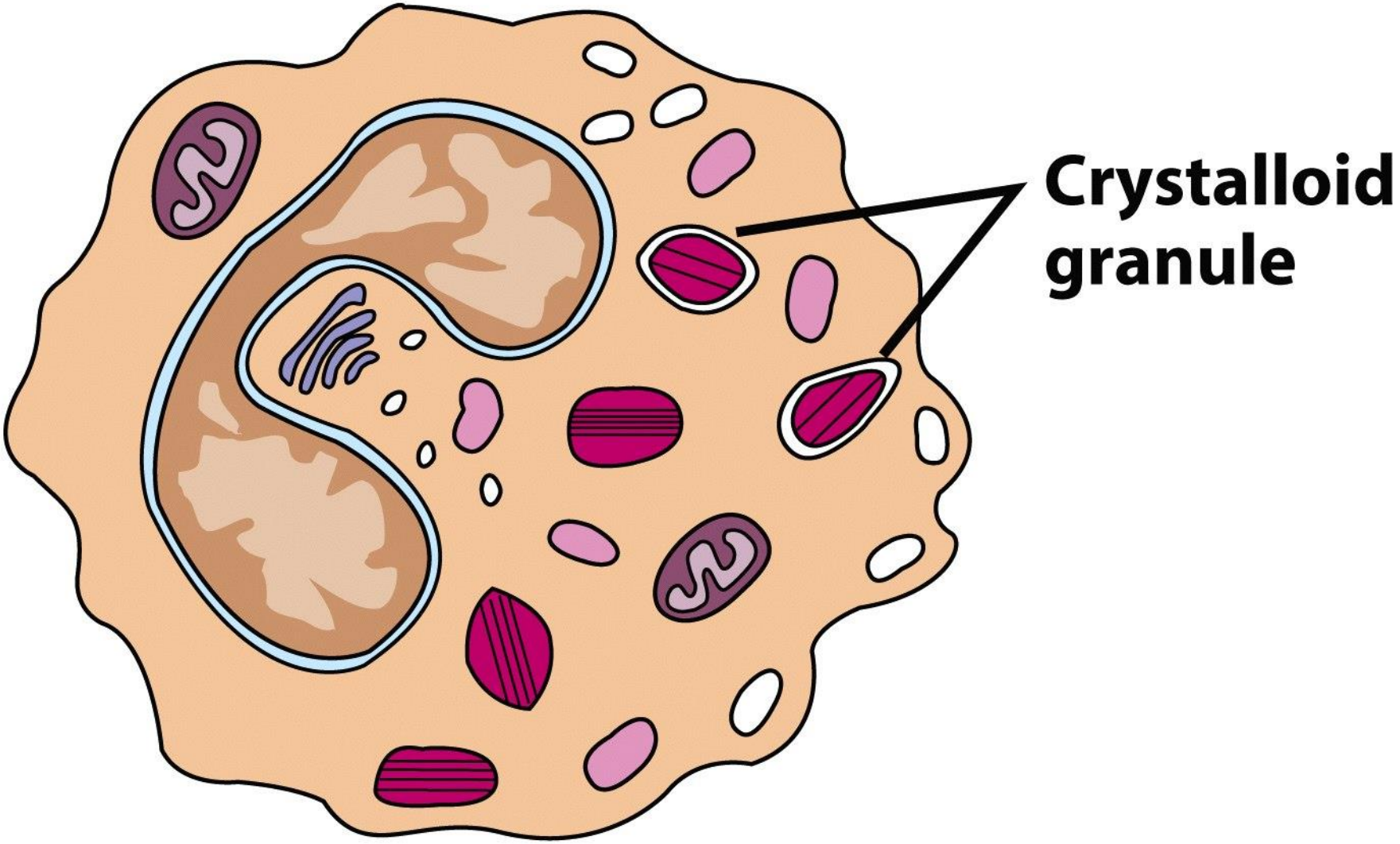

Figure 2-9b
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Other Leukocytes

- Granulocytes – Basophils
  - Nonphagocytic
  - Play a role in allergic reactions

Basophil

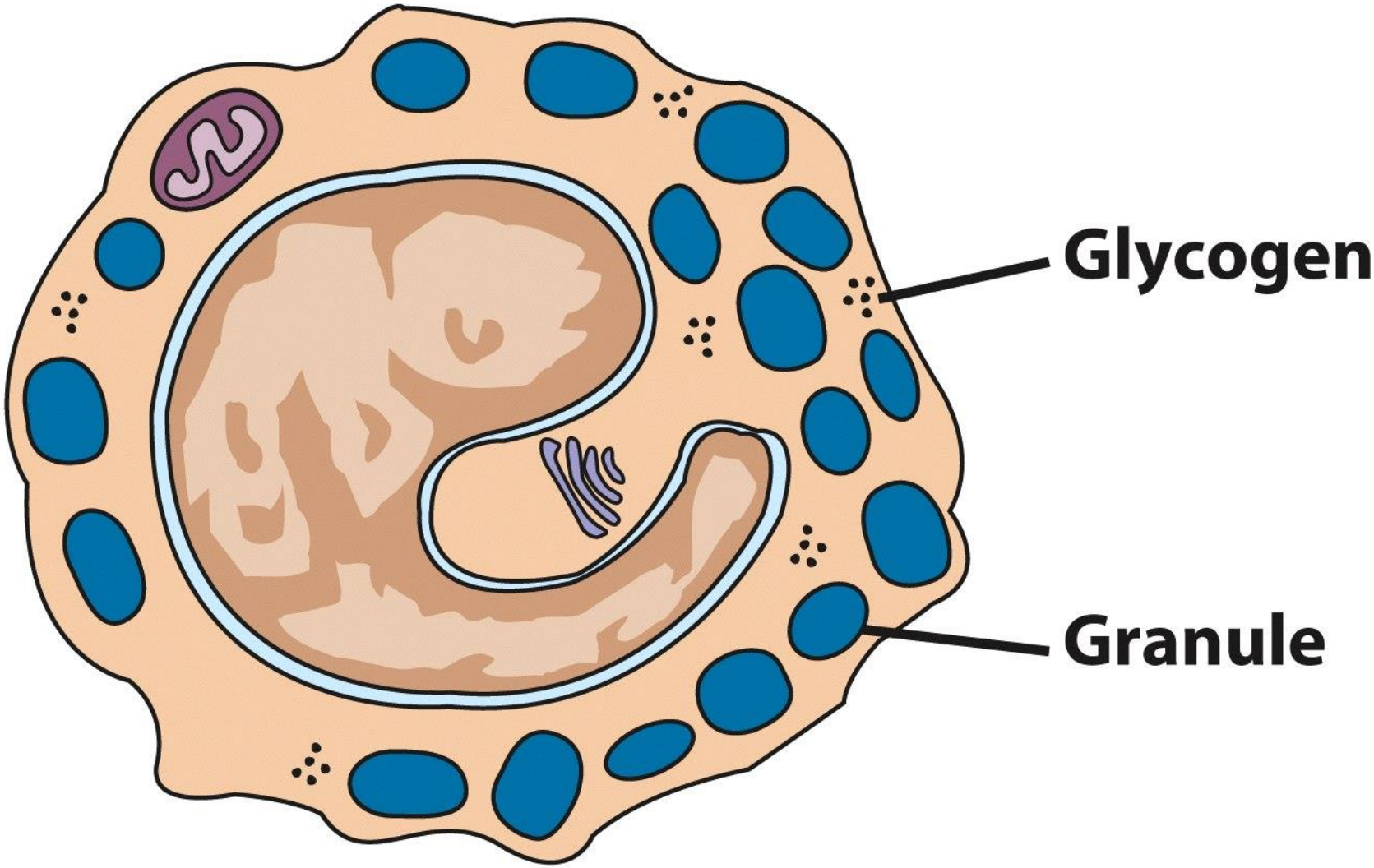

Figure 2-9c
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Other Leukocytes

- Mast cells
  - Play important role in development of allergies

Other Leukocytes

- Dendritic cells
  - Long membranous extensions, look like dendrites on nerve cells
  - Antigen presentation
  - 4 major groups:
    - Langerhans DC
    - Interstitial DC
    - Monocyte-derived DC
    - Plasmacytoid-derived DC
- Follicular dendritic cells
  - Involved with B cell maturation

Organs of the Immune System

- Primary
  - Thymus and bone marrow
  - Place of maturation of lymphocytes
- Secondary
  - Lymph nodes, spleen, mucosa-associated lymphoid tissues such as gut-associated lymphoid tissues
  - Mature lymphocytes interact with antigen

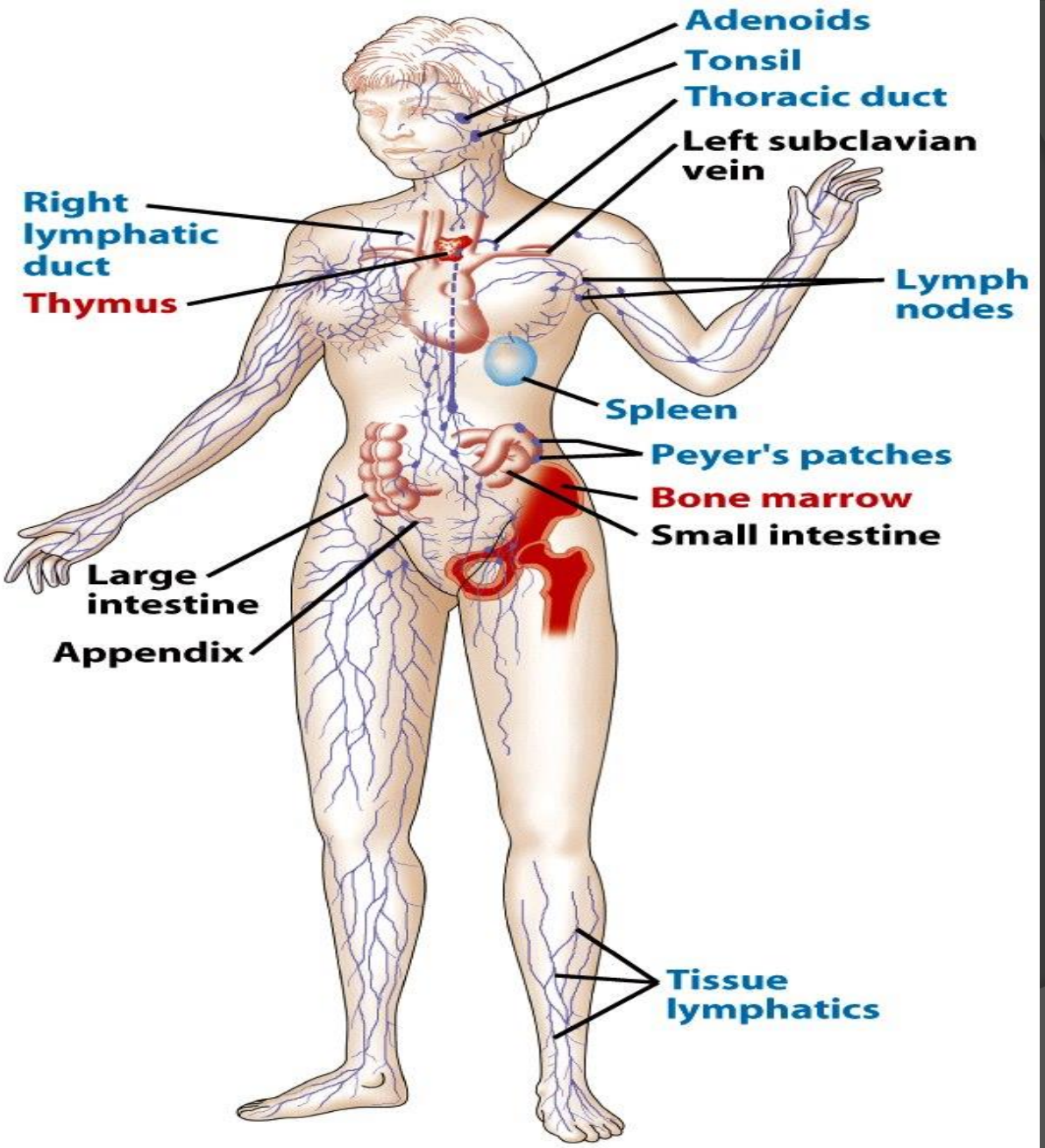

Figure 2-11
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Primary Lymphoid Organs

- Bone marrow
  - Lymphocytes arise there, T cells go to thymus to mature
  - B cells mature here
  - 90% of plasma IgG and IgA comes from B cells in the bone marrow

Primary Lymphoid Organs

- Thymus
  - T cell development and maturation
  - Bilobed organ above heart
    - Surrounded by capsule and divided into lobules
    - Outer part of lobule is cortex, inner is medulla
    - Network of epithelial cells, dendritic cells, and macrophages
  - Thymus will induce death of those T cells that can't:
    - Recognize self-MHC molecules
    - Those that interact with MHC molecules too strongly (could produce autoimmune disorder)
  - Function decreases with age

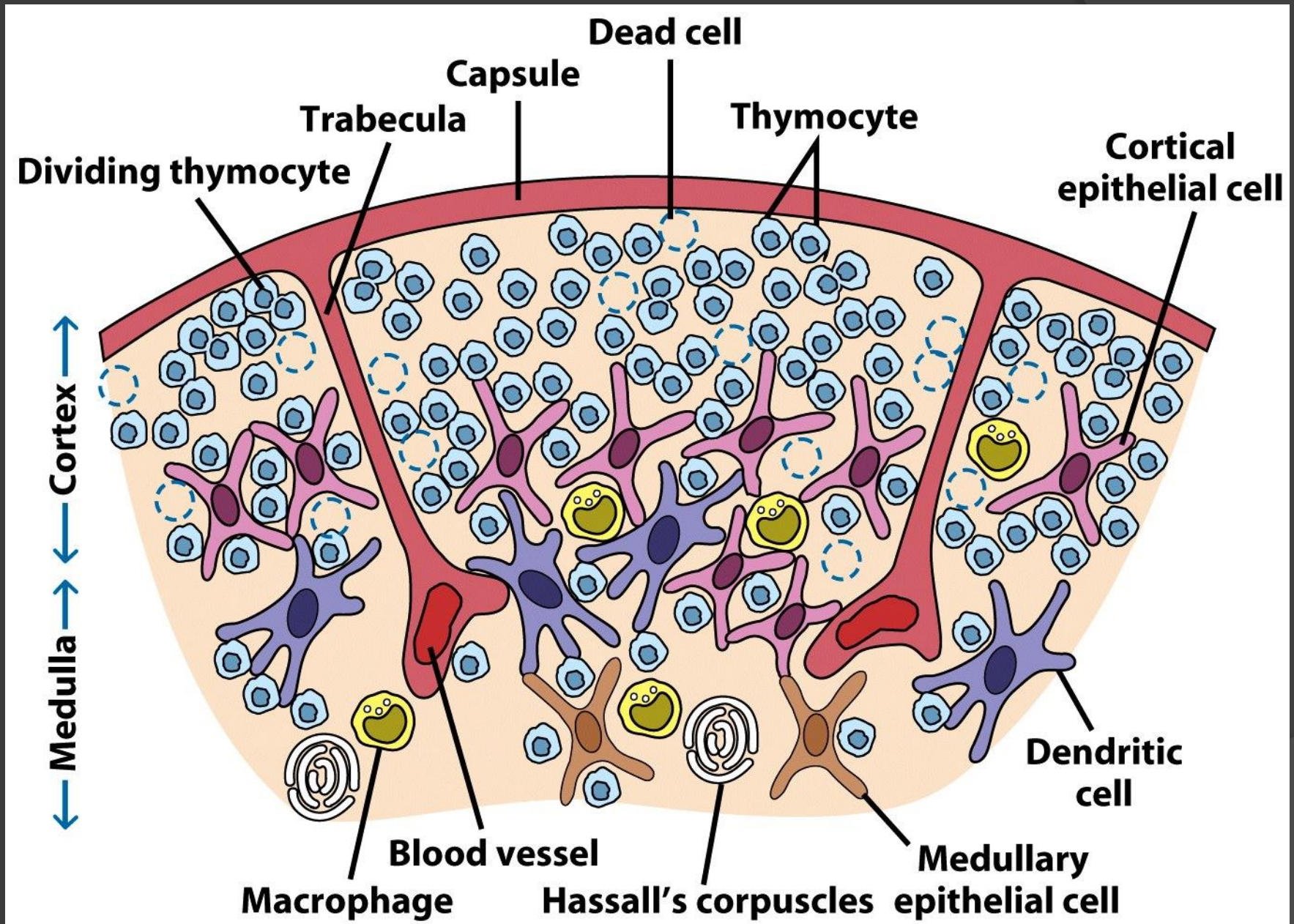

Figure 2-12
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Lymphatic System

- Interstitial fluid (the portion that doesn't enter venous system) is returned to circulatory system by lymphatic vessels
- Largest lymphatic vessel – thoracic duct
  - Enters left subclavian vein
  - Lymph from right arm and right side of head enters through right lymphatic duct, drains into right subclavian
- Antigen is carried by lymph to lymph nodes

Secondary Lymphoid Organs

- Primary follicle
  - Unactivated lymphoid follicle
- Secondary follicle
  - Follicle that is activated by antigen
  - Ring of B cells that surround germinal center
    - Proliferating B cells and T helper cells

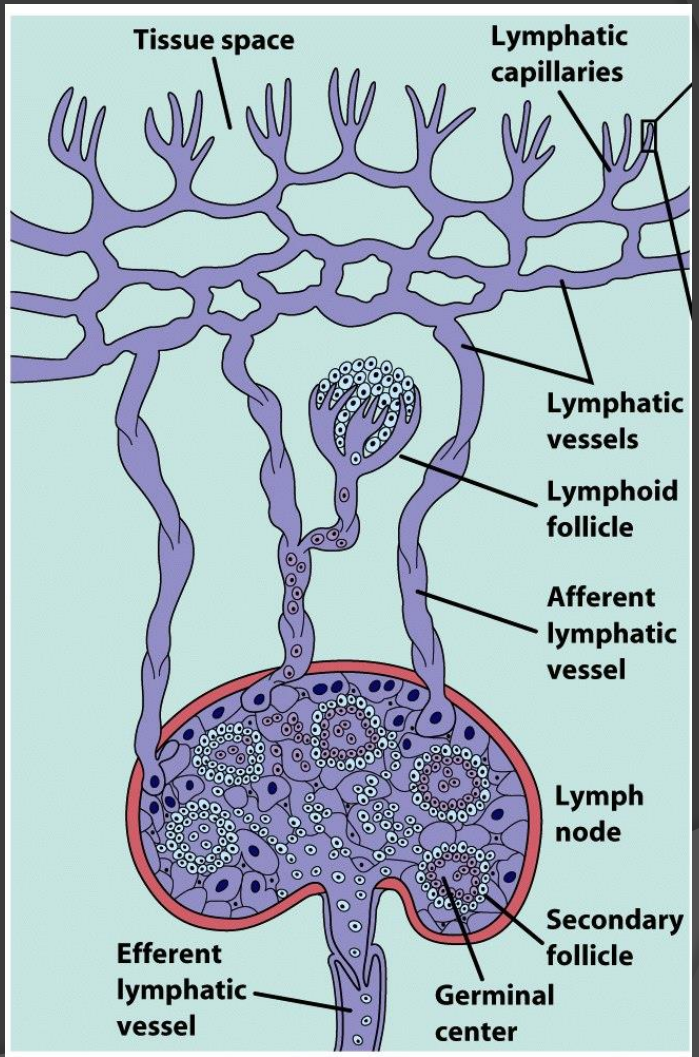

Figure 2-14
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Secondary Lymphoid Organs

- Lymph Nodes
  - Encapsulated
  - 3 regions:
    - Cortex
      - B cells, macrophages, dendritic cells
      - Primary follicles
    - Paracortex
      - T cells, dendritic cells
    - Medulla
      - Plasma cells secreting antibody

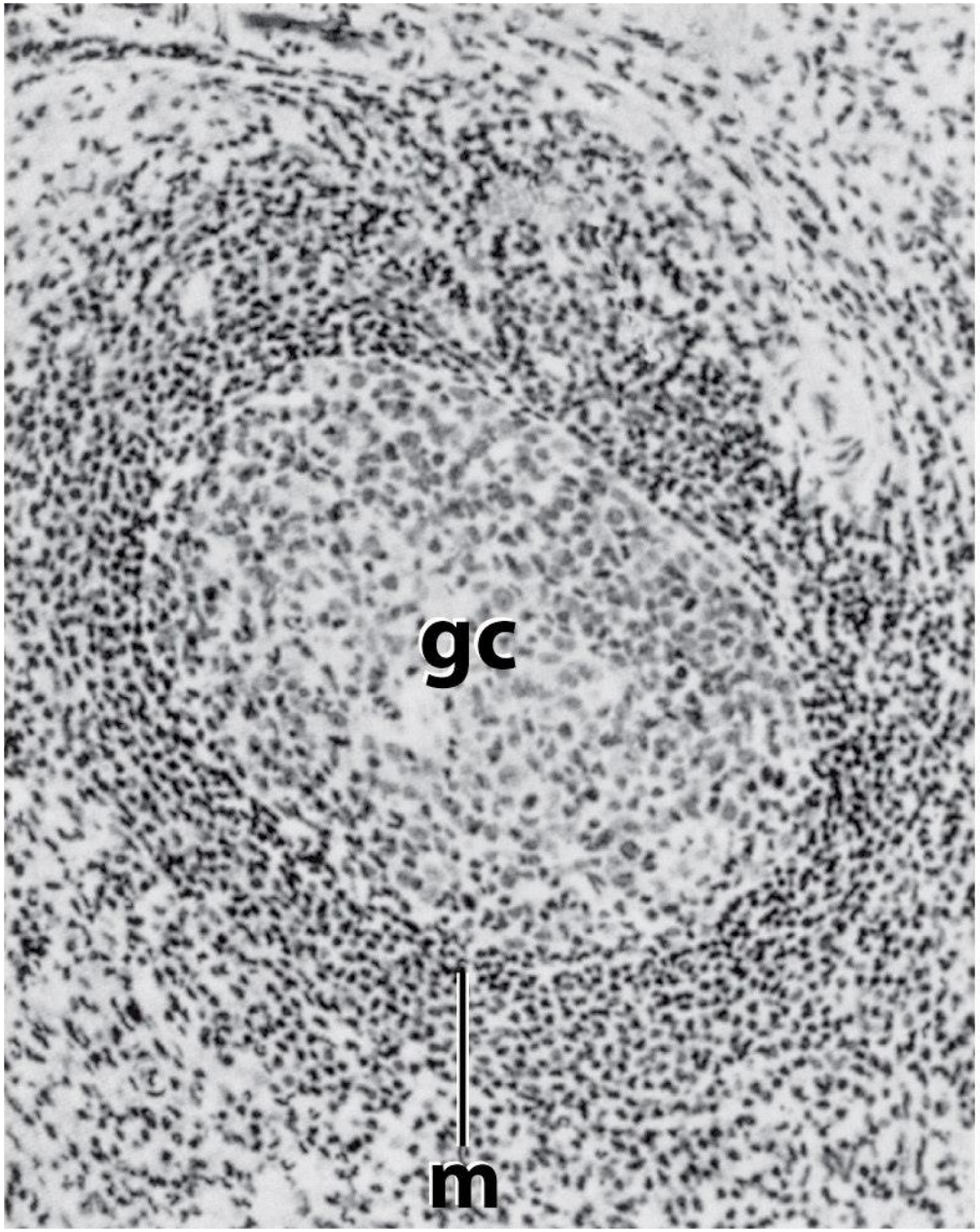

Figure 2-15
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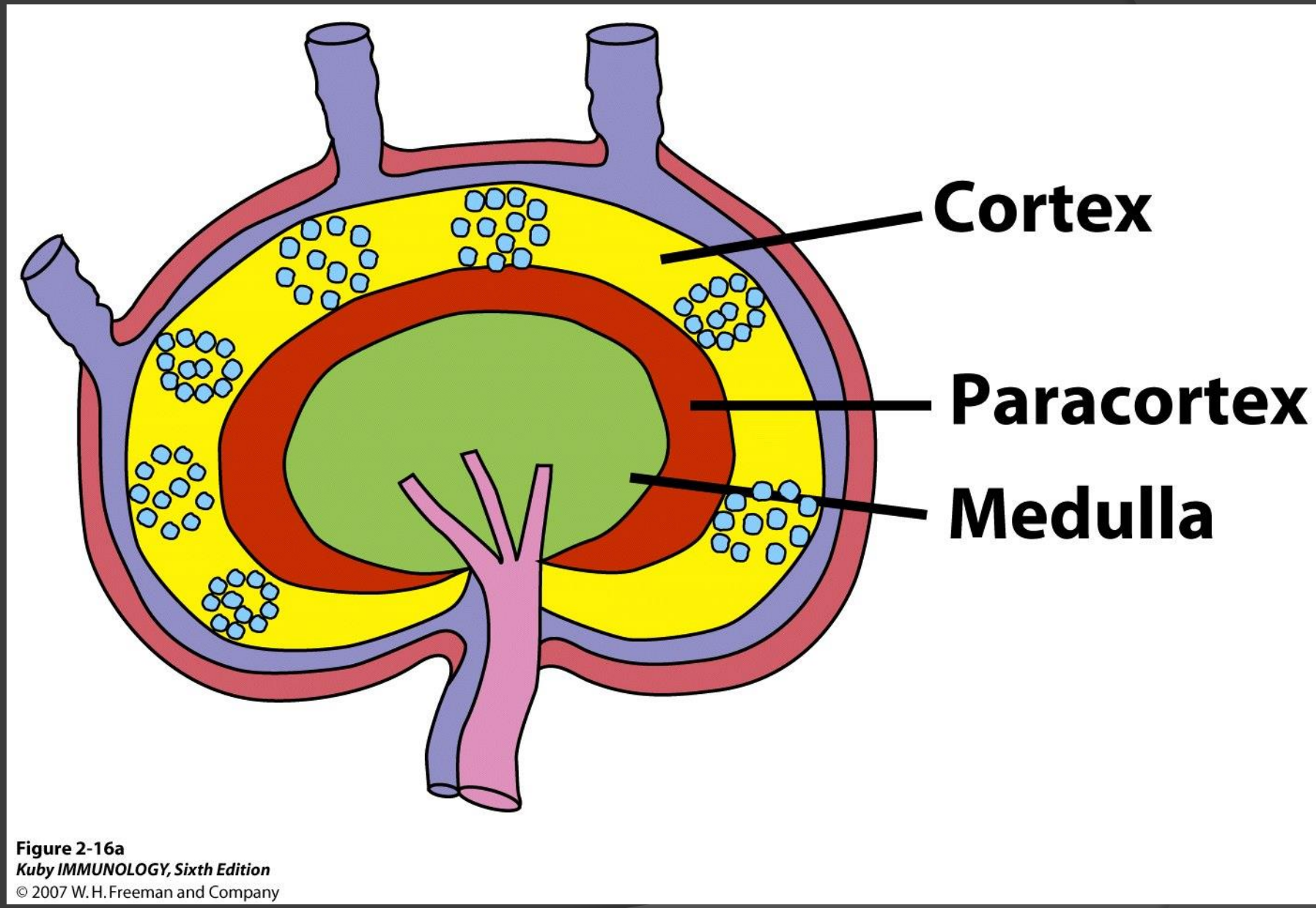

Figure 2-16a
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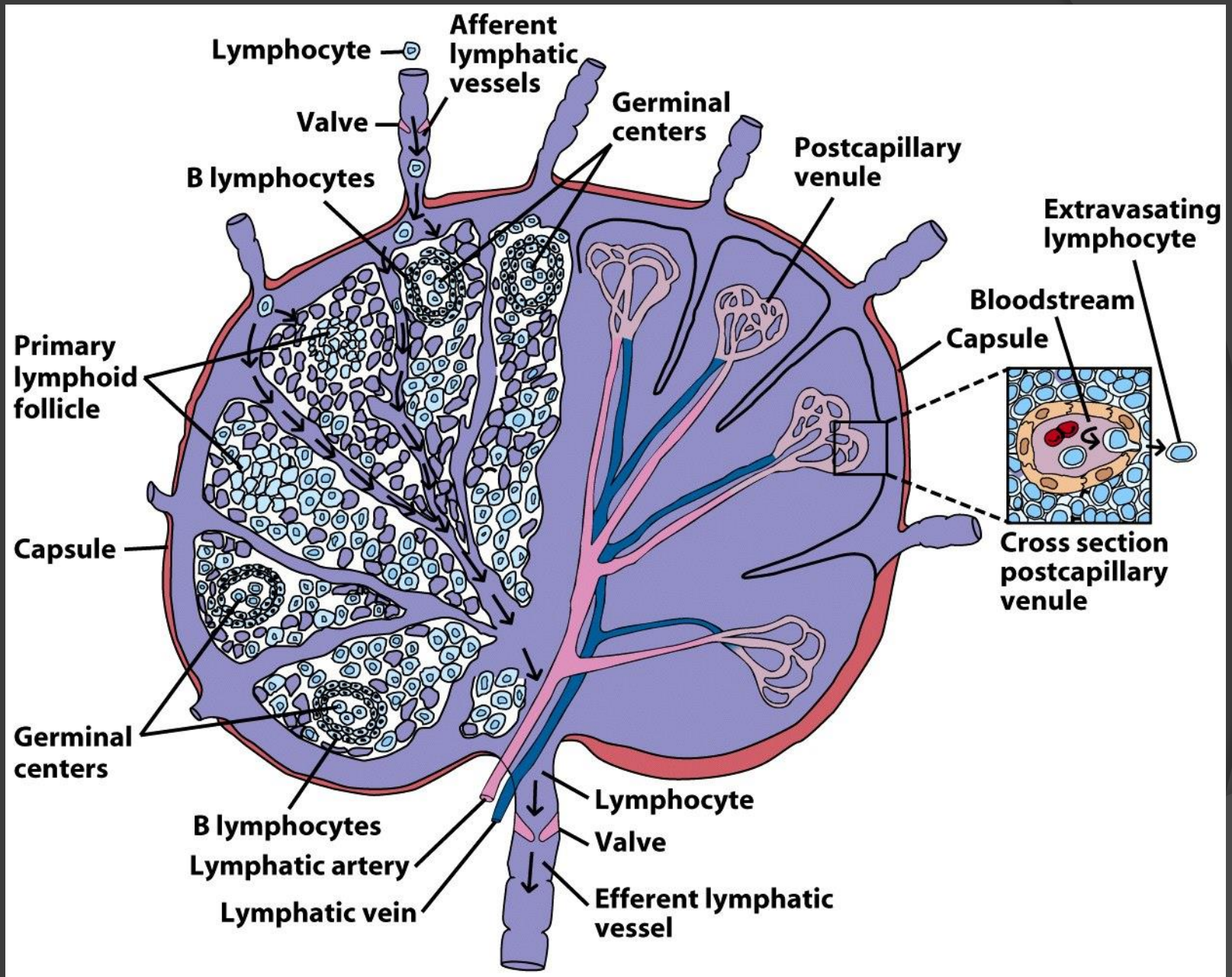

Figure 2-16b
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Secondary Lymphoid Organs

- Spleen
  - Filters blood, traps blood-bourne antigens
    - Important in systemic infections
  - Blood enters through splenic artery
  - Encapsulated
  - Structure:
    - Projections from capsule form trabeculae
    - Compartments:
      - Red pulp
        - Macrophages, red blood cells
      - White pulp
        - Surrounds branches of splenic artery
        - Forms PALS (periarteriolar lymphoid sheath)
        - Primary follicles rich in B cells

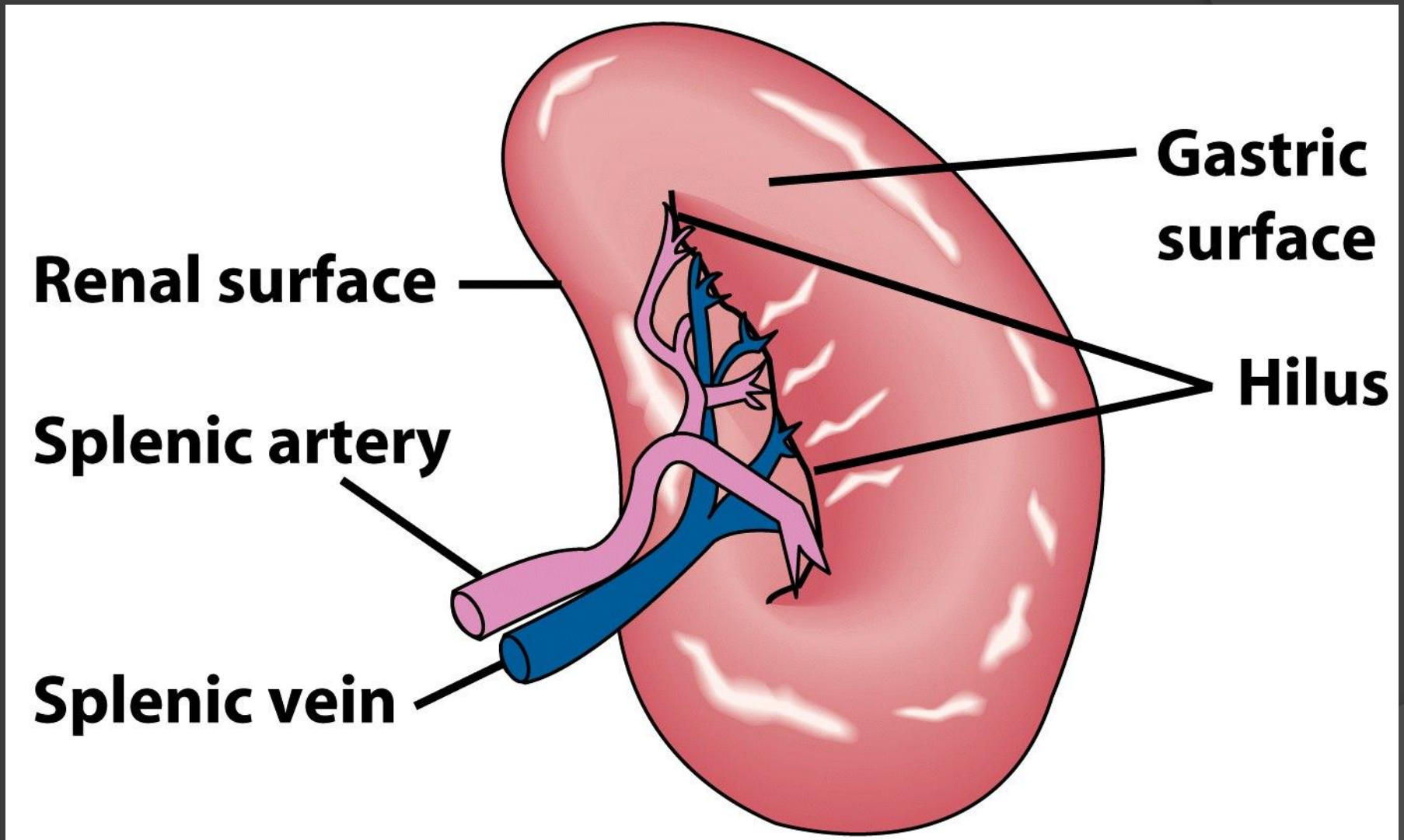

Figure 2-17a
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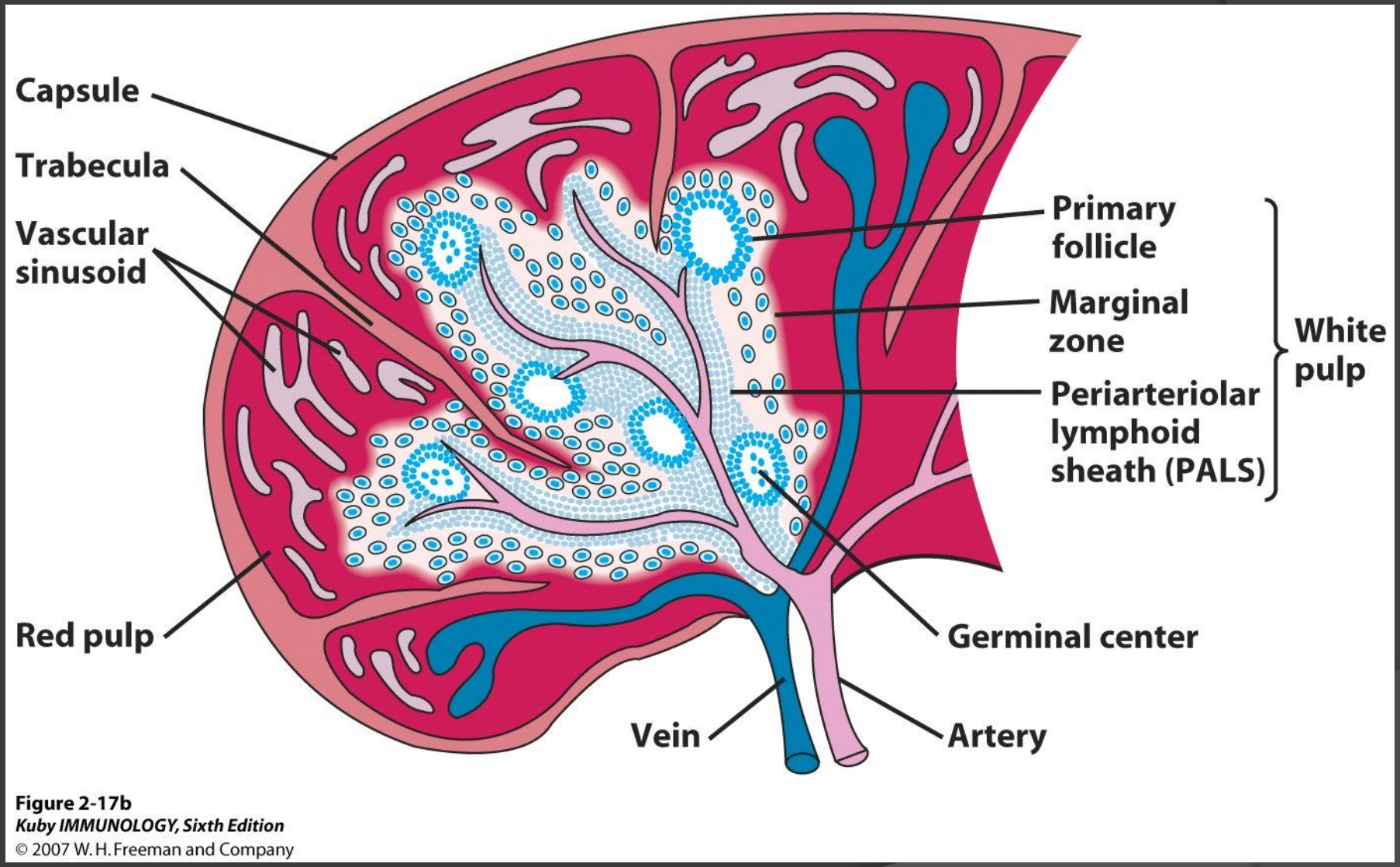

Figure 2-17b
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Secondary Lymphoid Organs

- Mucosa-Associated Lymphoid Tissue
  - MALT
  - Organized areas along digestive, respiratory, and urogenital tracts
    - Very well organized areas in intestine are referred to as Peyer's patches
    - Includes tonsils and appendix

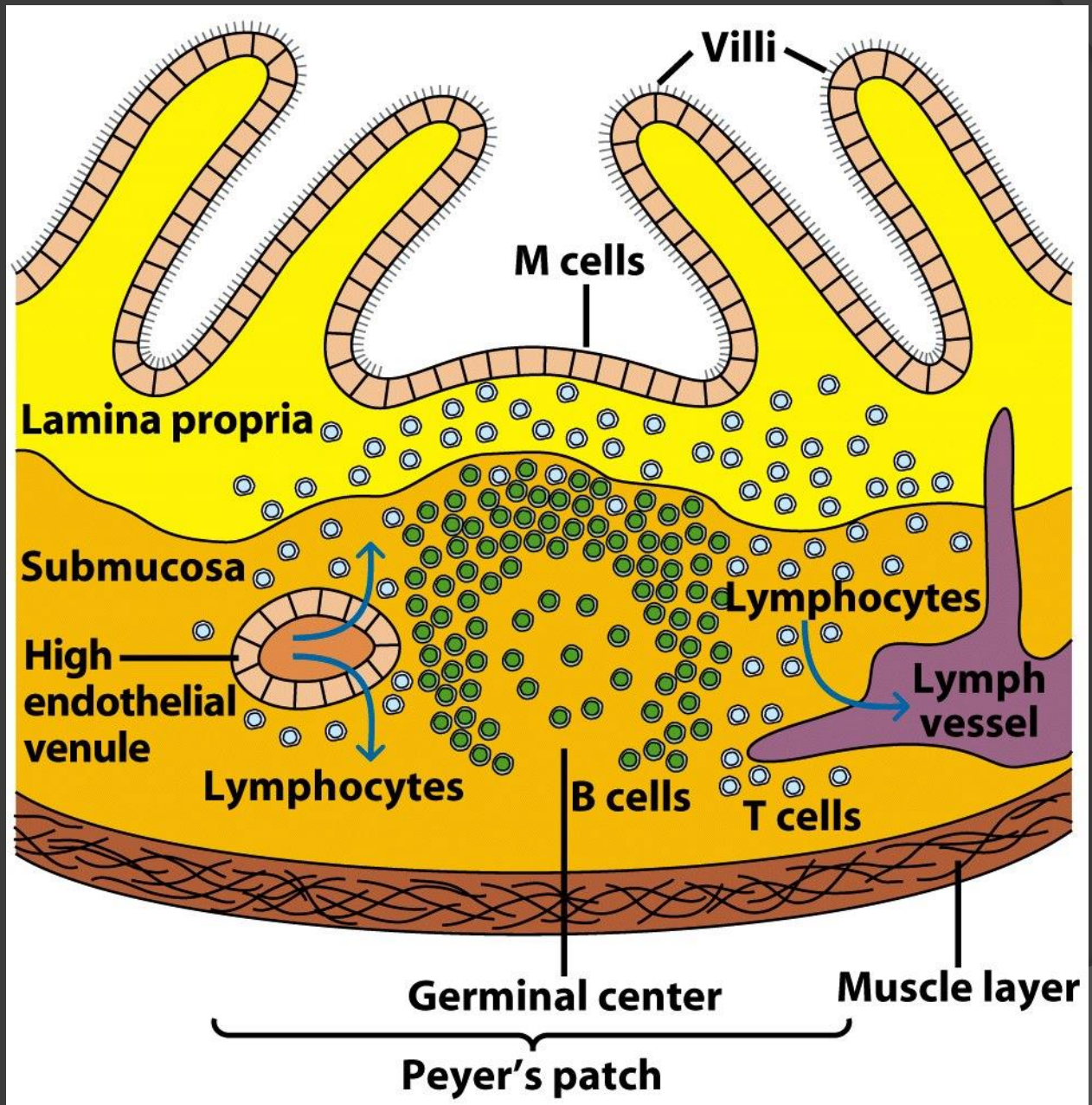

Figure 2-18
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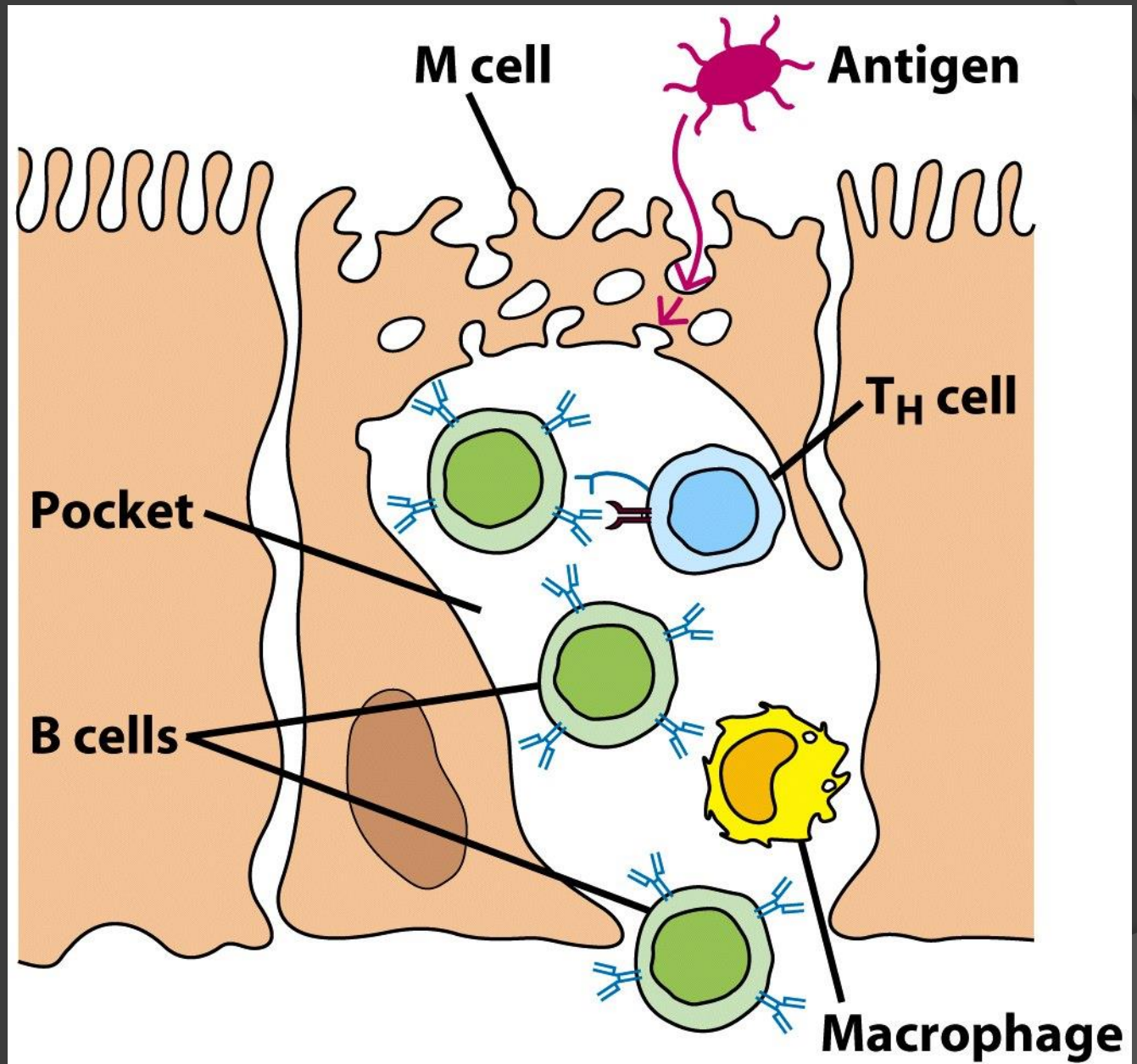

Figure 2-19a
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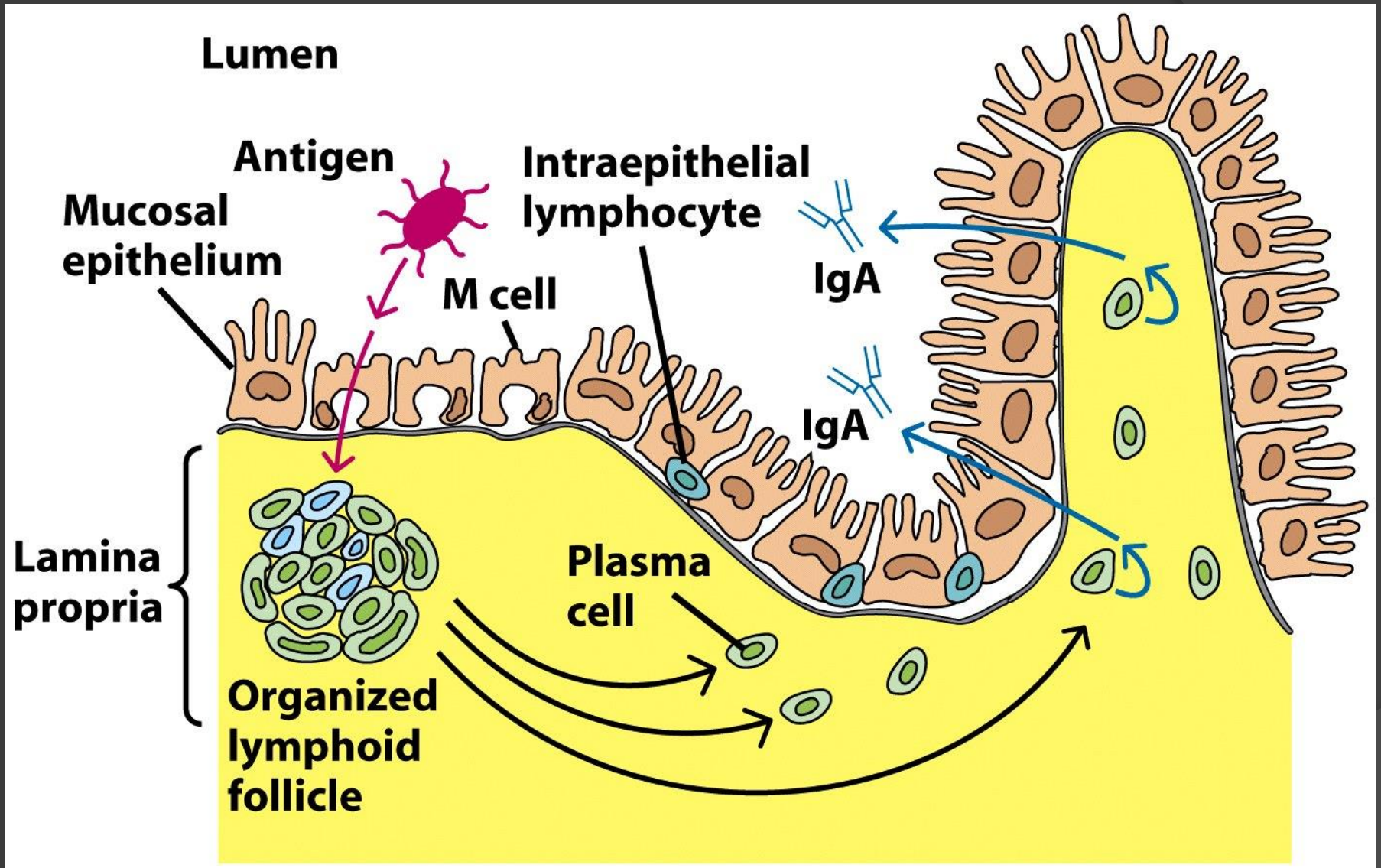

Figure 2-19b
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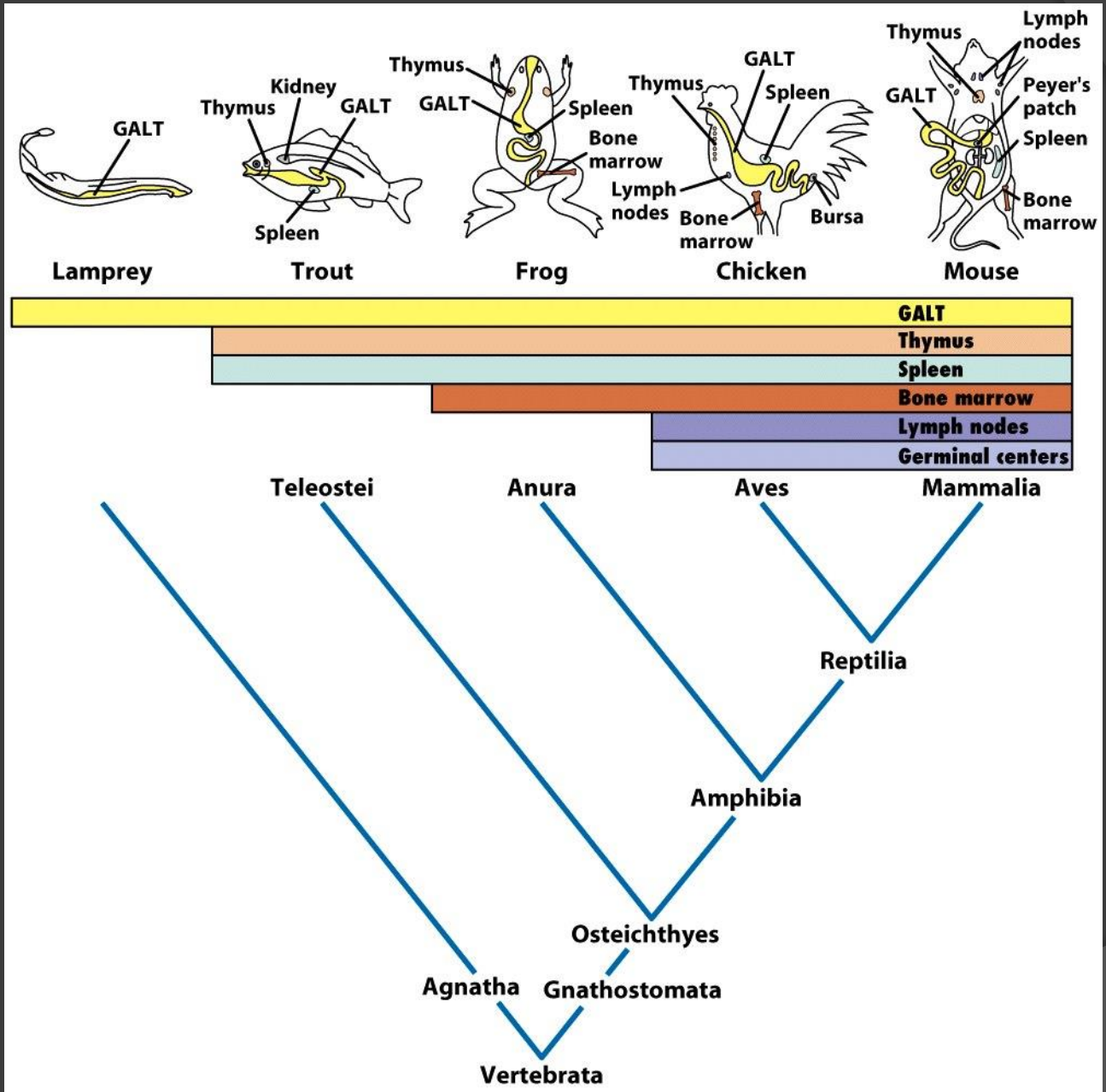

Figure 2-20
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Chapter 4

Antigens and Antibodies

Dr. Capers

IMMUNOLOGY

Kindt • Goldsby • Osborne

Kuby IMMUNOLOGY

Sixth Edition

Chapter 4

Antigens and Antibodies

- Hallmark molecules of adaptive immunity
  - Antibody and T-cell receptor
  - Innate immunity recognizes patterns, whereas antibodies and T cell receptors have high degree of specificity

- Antibodies and T cell receptors
  - Recognize epitopes
    - Immunologically active regions of immunogen that bind to antigen-specific antibodies or T-cell receptors

Antibodies (Abs)

- Epitope binding proteins
  - Membrane bound on B cells OR
  - Secreted in blood
    - Humoral immunity
- Share structural features, bind to antigen, and participate in number of effector functions
- Known collectively as Immunoglobulins (Igs)

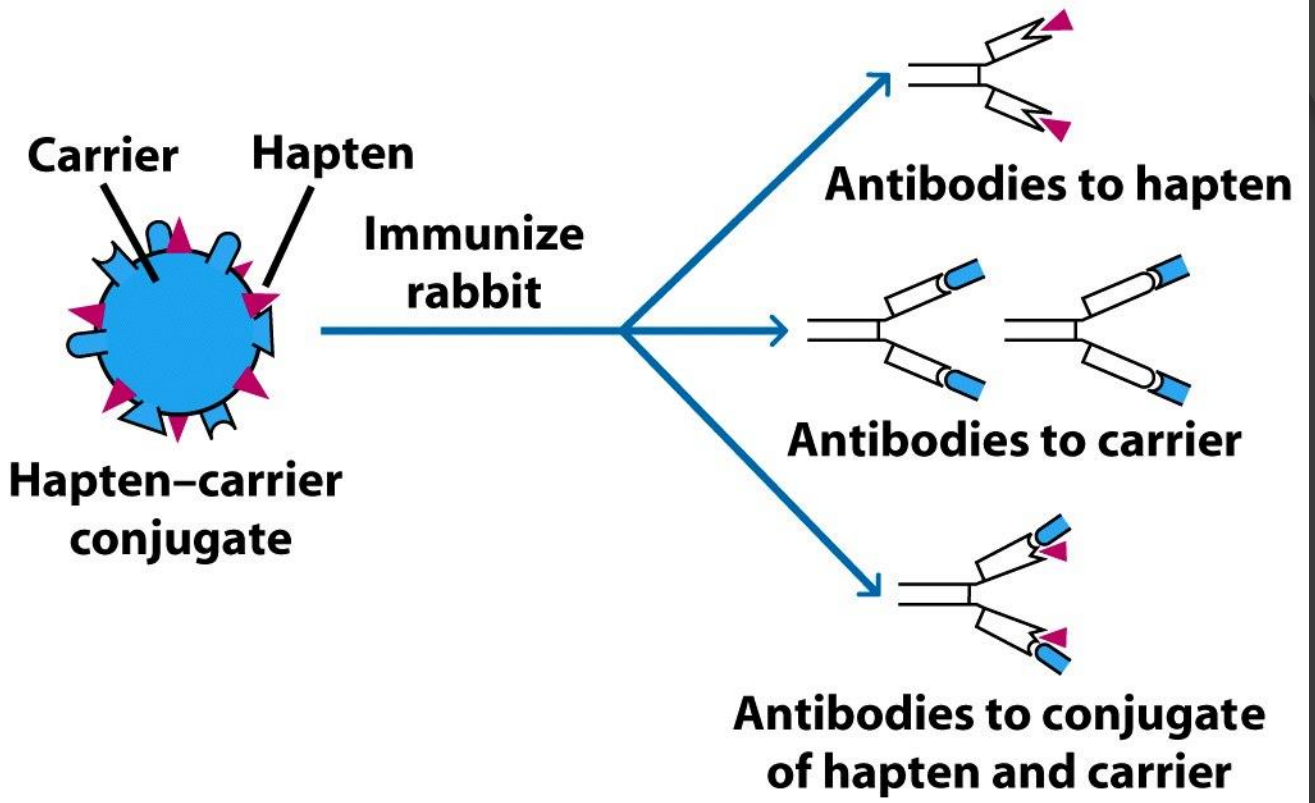
T cell Receptor

- T Cell Receptor
  - Expressed on surface of T cells
  - Recognize processed antigen complexed with MHC molecules

- Immunogenicity
  - Ability to induce humoral and/or cell-mediated immune response
  - Immunogen is substance that induces response
- Antigenicity
  - Ability to combine specifically with Abs or T-cell receptor/MHC
  - Not all antigens are immunogenic
    - Haptens

Haptens

- Hapten – too small, lack immunogenicity
  - If hapten is coupled to carrier protein, immune response can be induced
  - Hapten-carrier conjugate
    - Produces 3 types of antigenic determinants
      - Antibodies to hapten
      - Antibodies to carrier
      - Antibodies to hapten-carrier conjugate



Injection with:	Antibodies formed:
Hapten (DNP)	None
Protein carrier (BSA)	Anti–BSA
Hapten–carrier conjugate (DNP-BSA)	Anti–DNP (major) Anti–BSA (minor) Anti–DNP/BSA (minor)

Figure 4-1
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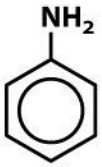
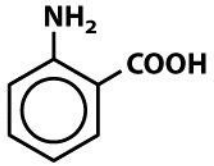
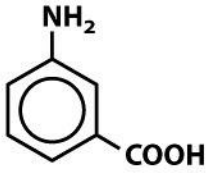
TABLE 4-1 Reactivity of antisera with various haptens

	REACTIVITY WITH			
				
Antiserum against	**Aminobenzene (aniline)**	***o*-Aminobenzoic acid**	***m*-Aminobenzoic acid**	***p*-Aminobenzoic acid**
Aminobenzene	+	0	0	0
***o*-Aminobenzoic acid**	0	+	0	0
***m*-Aminobenzoic acid**	0	0	+	0
***p*-Aminobenzoic acid**	0	0	0	+

KEY: 0 = no reactivity; + = strong reactivity

SOURCE: Based on K. Landsteiner, 1962, *The Specificity of Serologic Reactions,* Dover Press. Modified by J. Klein, 1982, *Immunology: The Science of Self-Nonself Discrimination,* Wiley.

Table 4-1
Kuby IMMUNOLOGY, Sixth Edition

Properties of Immunogen contribute to Immunogenicity

- 4 Properties
  - Foreignness
  - Molecular size
  - Chemical composition and complexity
  - Ability to be processed and presented on MHC

- Foreignness
  - Lymphocytes that do not bind to self antigens are allowed to further develop
    - Therefore they will later only recognized nonself antigens
  - For example:
    - Bovine serum albumin (BSA) is not immunogenic when injected into cow but is when injected into chicken
    - Some macromolecules are highly conserved throughout evolution and display little immunogenicity
      - Cytochrome c, collagen

- Molecular Size
  - Active (good) immunogens
    - > 100,000 Daltons
  - Poor immunogens
    - < 5,000-10,000 Daltons

- Chemical Composition
  - Polymers composed of multiple copies of same amino acid or sugar tend to be poor immunogens
  - Lipids are haptens and need to be congugated with carrier to produce antibodies
    - Important for assays for detection of some steroids, vitamins

- Susceptibility to antigen processing
  - Large, insoluble macromolecules are more likely to be phagocytized for processing

The biological system contributes to immunogenicity

- Host Genetic make-up
- Manner in which material is presented
- Use of agents (adjuvants) to enhance immunogenicity

- Genotype of recipient animal
  - Genes of MHC
  - Genes in coding for specific antibodies

- Material presentation – immunogen dosage and route of administration
  - Too low or high of dosage can induce tolerance
  - Single dose is often not enough – booster is needed
  - Route
    - Intravenous (iv)
    - Intradermal (id)
    - Subcutaneous (sc)
    - Intramuscular (im)
    - Intraperitoneal (ip)
      - Antigen administered iv would travel to spleen; administered sc would travel to lymph nodes

- Adjuvants
  - Enhance immunogenicity
  - Not exactly sure how they work but are recognized by Toll-like receptors
  - Water-in-oil adjuvants
    - Freund's incomplete adjuvant – antigen in aqueous solution, mineral oil, and emulsifying agent
      - Antigen is then released very slowly from injection site
      - Based on Freund's complete adjuvant - also contained heat –killed *Mycobacteria*

Epitopes

- Antigenic determinants recognized by B cells and T cells
  - B cell epitopes tend to be on the outside of the antigen
    - For example, the hydrophilic amino acids on a protein's surface
  - T cell epitopes from proteins derived from enzymatic digestion of peptide and then association with MHC

- B cell epitopes have characteristic properties
  - Located on surface of immunogen – accessible to antibody
  - When talking about proteins, the epitopes can be sequential or nonsequential (referring to amino acid sequence) depending on protein folding

TABLE 4-2 Comparison of antigen recognition by T cells and B cells

Characteristic	B cells	T cells
Interaction with antigen	Involves binary complex of membrane Ig and Ag	Involves ternary complex of T-cell receptor, Ag, and MHC molecule
Binding of soluble antigen	Yes	No
Involvement of MHC molecules	None required	Required to display processed antigen
Chemical nature of antigens	Protein, polysaccharide, lipid	Mostly proteins, but some lipids and glycolipids presented on MHC-like molecules
Epitope properties	Accessible, hydrophilic, mobile peptides containing sequential or nonsequential amino acids	Internal linear peptides produced by processing of antigen and bound to MHC molecules

Table 4-2
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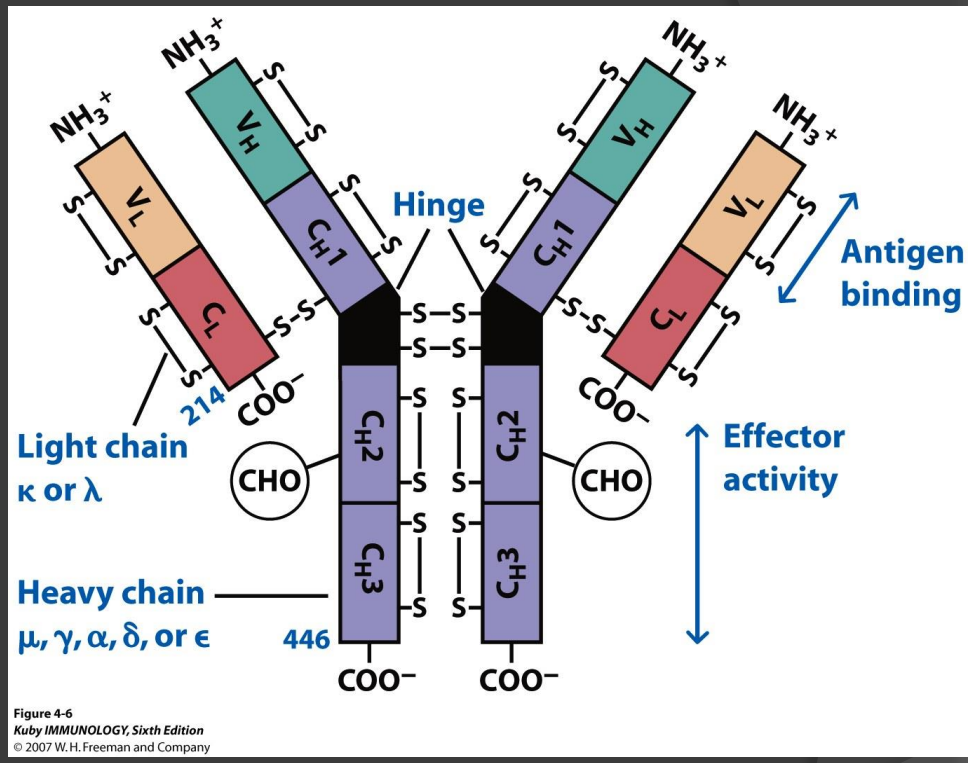
Basic Structure of Antibodies

- Known since late 19th century that antibodies are in serum
  - Serum is fluid phase that remains after plasma is allowed to clot
  - Antibodies are also found in other secretions

- Antibodies are heterodimers
  - 2 light chains
    - ~ 22, 000 daltons each
  - 2 heavy chains
    - ~ 55,000 daltons each
  - First 110 aa of amino-terminal end of heavy and light chain vary depending on antibody specificity



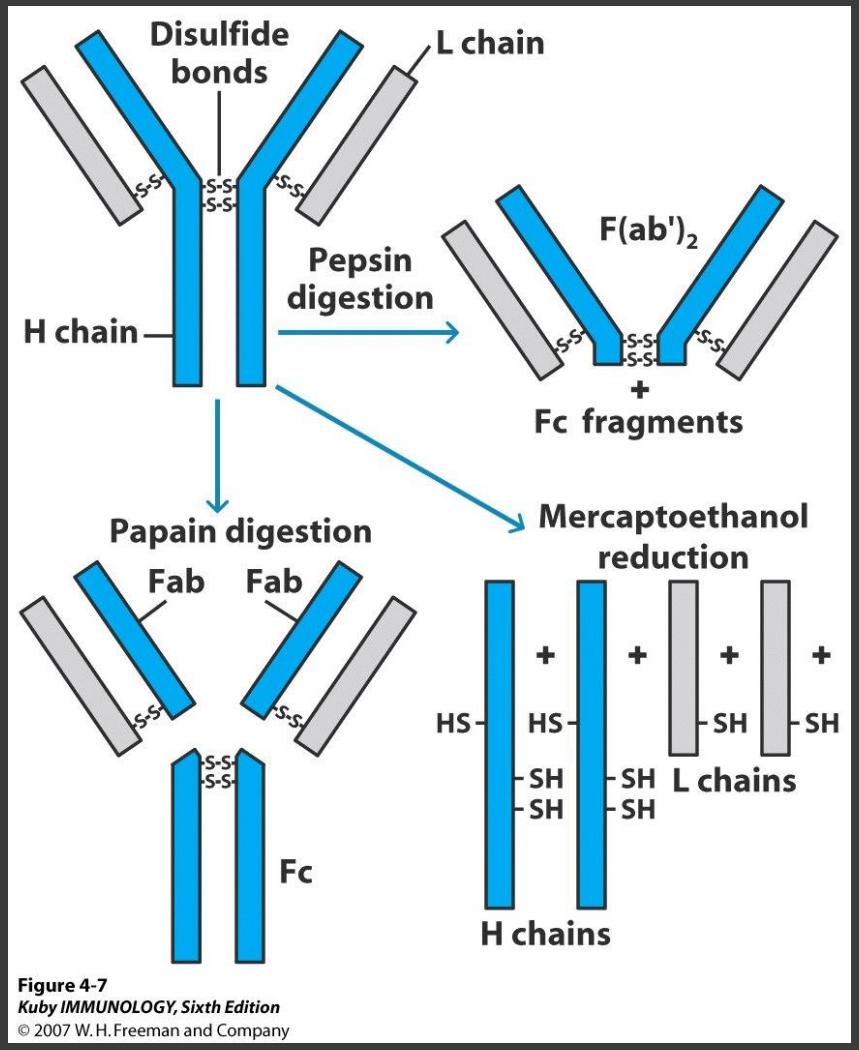


Figure 4-7
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- Different digestion procedures reveal different fragments
- F(ab')^2 still shows antigen binding capability

Light Chains

- When aa sequences of light chains from several individuals were sequenced, pattern emerged:
  - Amino-terminal end (110 aa) varied
  - Other part remained constant
    - Were found to be either kappa (κ) OR
    - Lambda (λ)
      - In mice and humans, different lambda subtypes have been found

Heavy Chains

- Amino-terminal end also shows variability
- 5 different heavy chain constant regions (isotypes)
  - IgM – μ
  - IgG – γ
  - IgA – α
  - IgD – δ
  - IgE – ε

  Some subisotypes have been discovered in some species

  Each antibody has 2 identical heavy chains, 2 identical light chains

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

Class*	Heavy chain	Subclasses	Light chain	Molecular formula
IgG	γ	$\gamma1, \gamma2, \gamma3, \gamma4$	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	$\alpha1, \alpha2$	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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Overall structure of immunoglobulin

- Primary – sequence of amino acids
- Secondary – folding into series of β pleated sheets
- Tertiary – compact globular domains
- Quarternary – adjacent light and heavy chains interact

Secondary

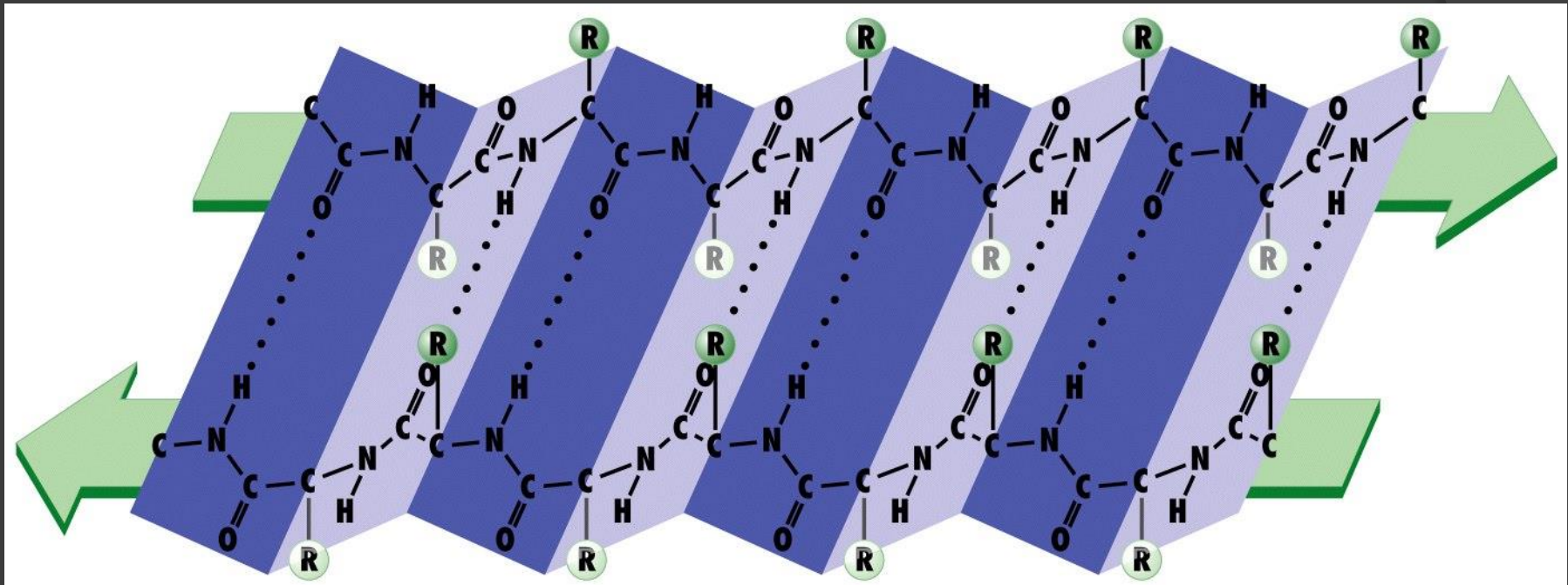

Figure 4-8
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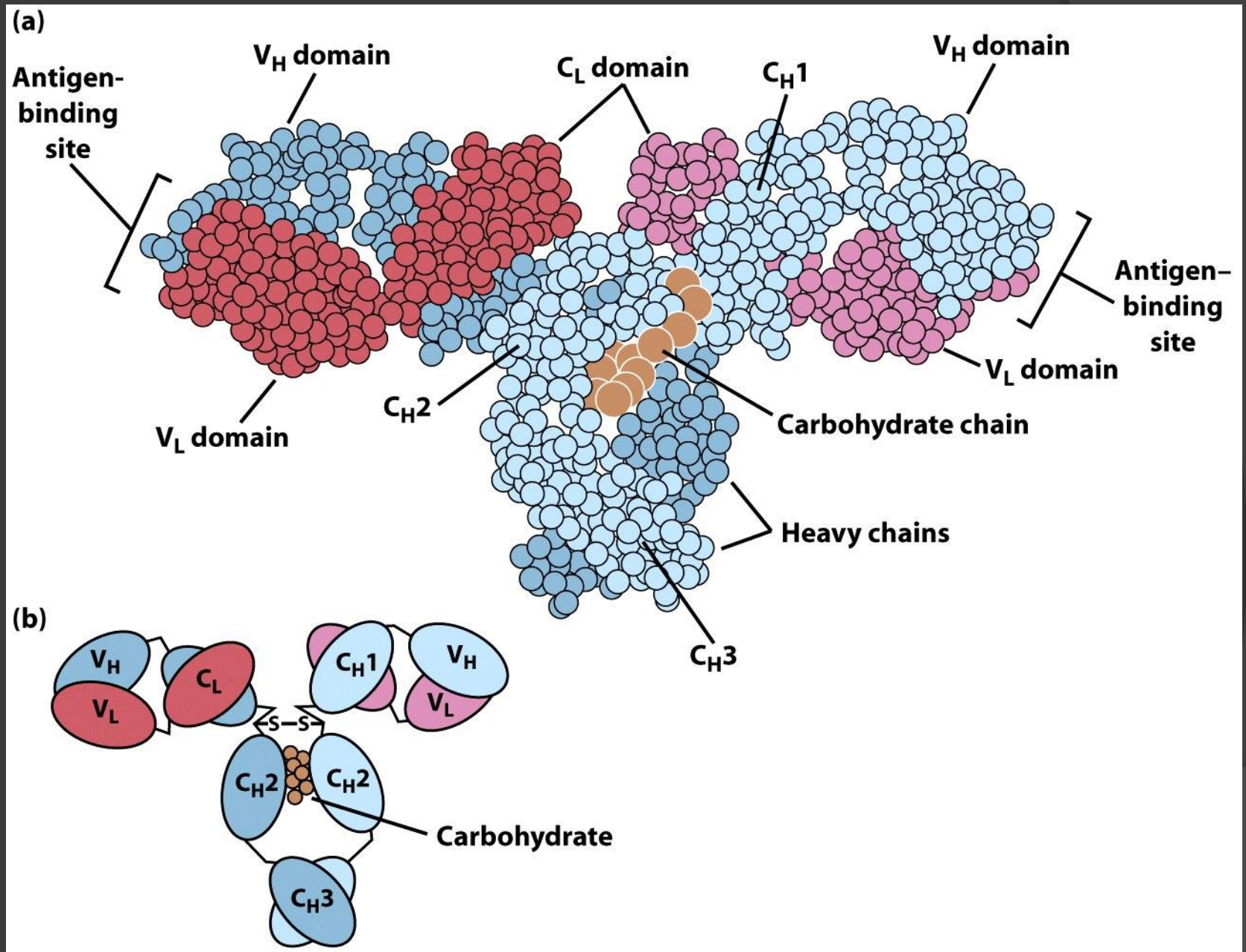

Figure 4-9
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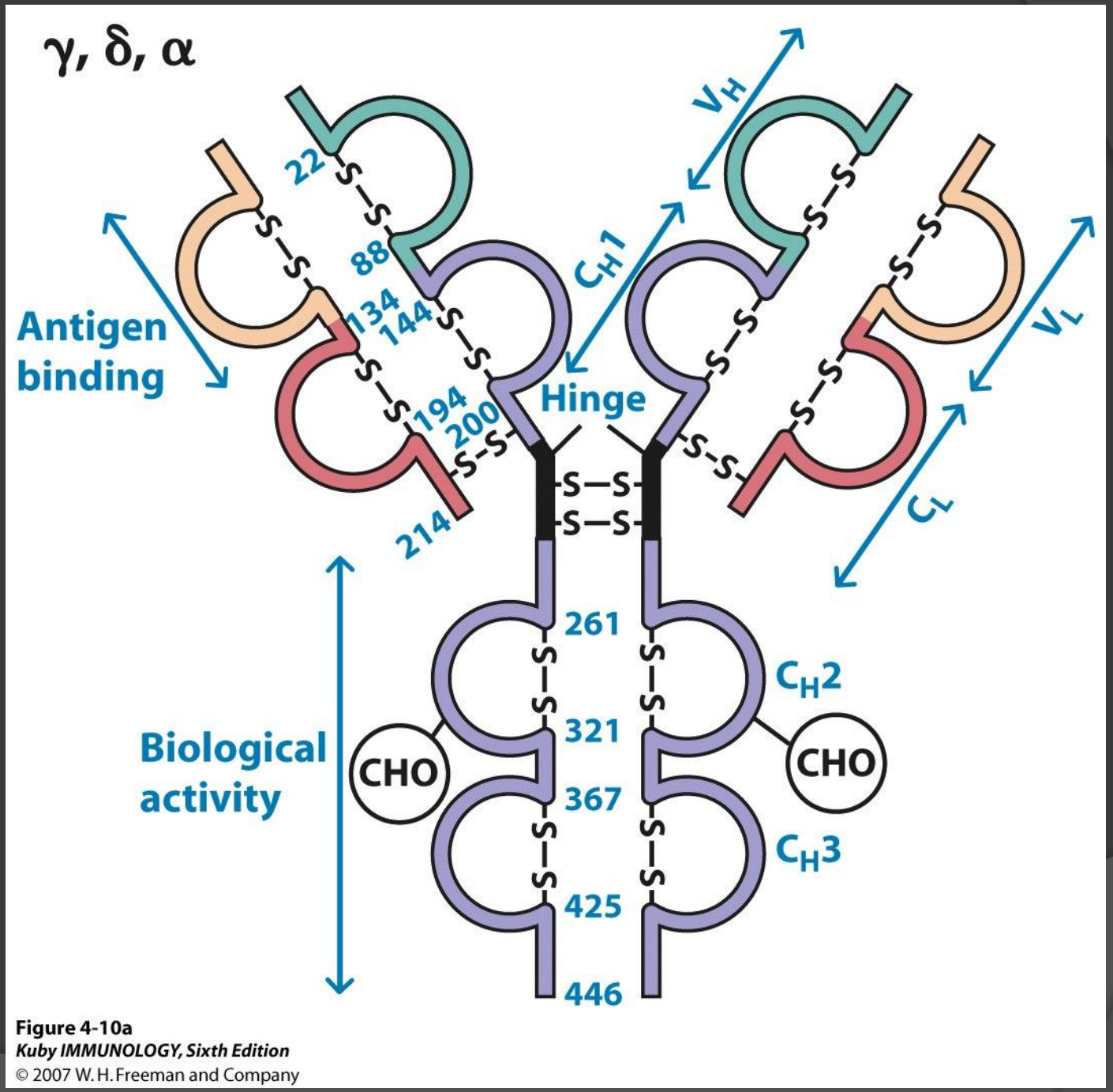

Figure 4-10a
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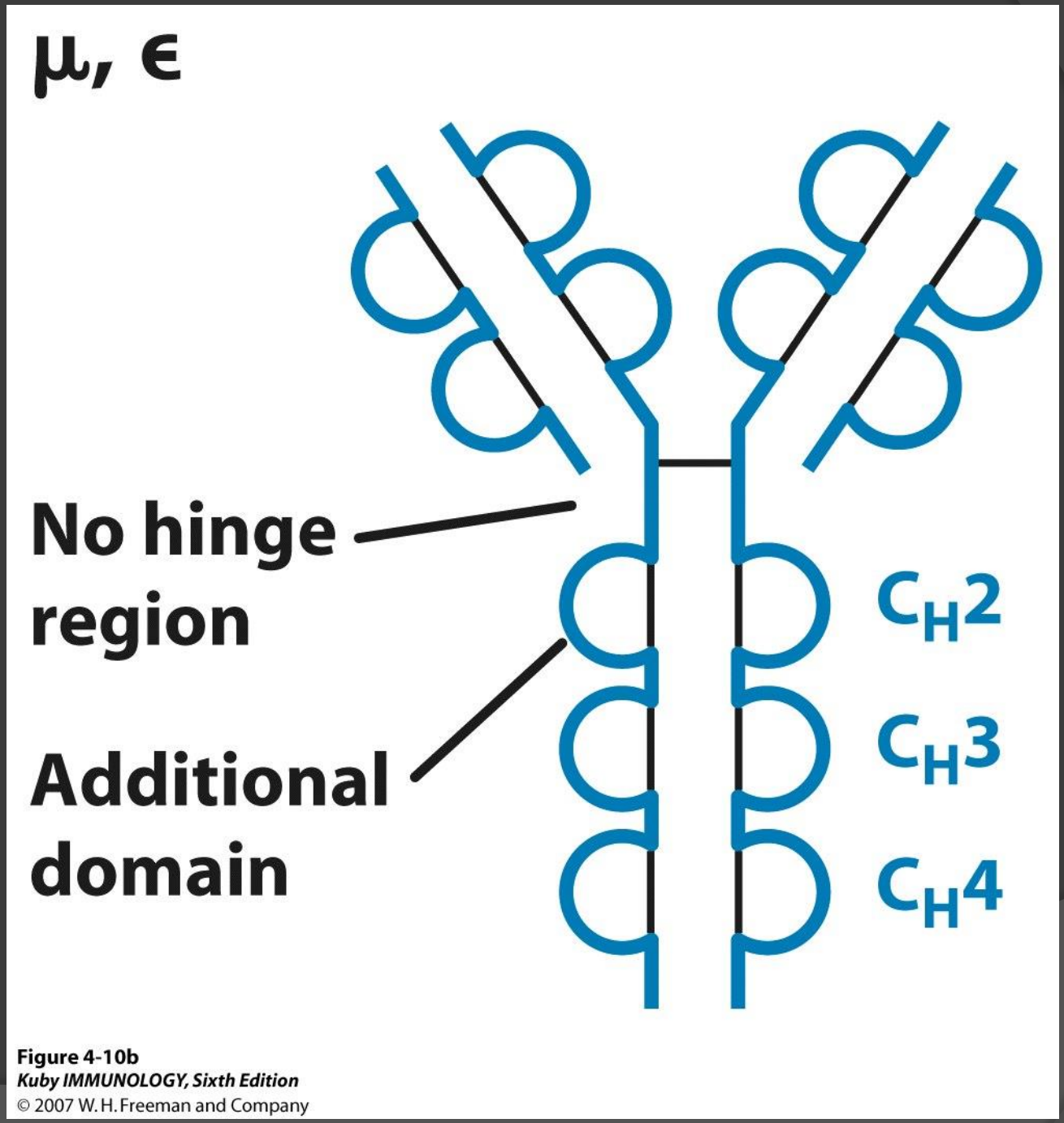

Figure 4-10b
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- Hypervariable regions = complimentarity-determining regions (CDRs)
  - Complimentary to epitopes that they will bind

- Ab-antigen interaction
  - Smaller antigens will fit in pockets in the variable regions of Abs
  - Larger antigens will interact with flatter regions of the variable region
- 15-22 amino acid residues on antibody will interact with residues on antigen

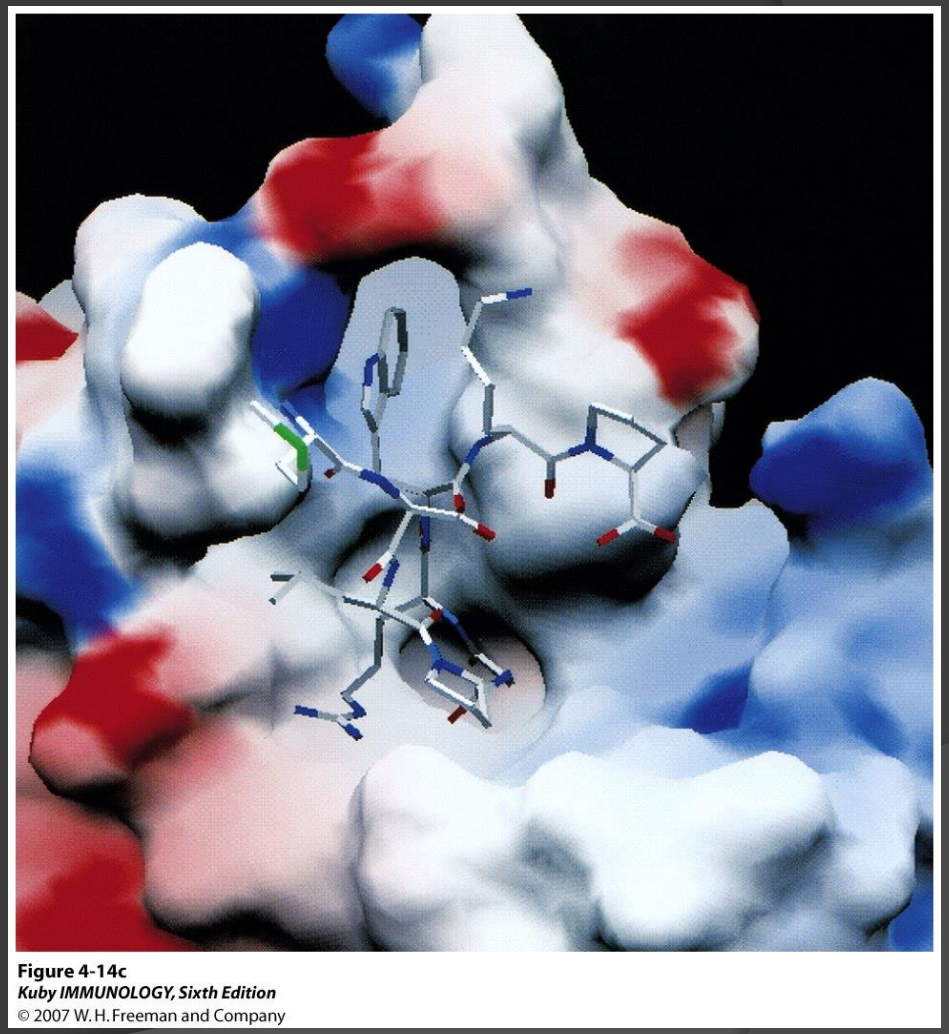

Figure 4-14c
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- Hinge Region
  - γ (gamma), δ (delta), and α (alpha) heavy chains have extended peptide sequence
    - Rich in proline and cysteine
    - Gives flexibility
- Immunoglobulins can be secreted or membrane-bound
    - Membrane-bound differ in the carboxyl-terminal end:
      - Extracellular “spacer” of 26 aa
      - Hydrophobic transmembrane sequence
      - Cytoplasmic tail

Antibody-mediated Effector Functions

- In addition to binding antigen, Abs can:
  - Promote phagocytosis (opsonization)
  - Activate complement
  - Antibody dependent cell mediated cytotoxicity (ADCC)
    - Natural killer cells have receptor for F_c portion of antibody
  - Some can cross epithelial layers to be excreted through mucous or across placenta

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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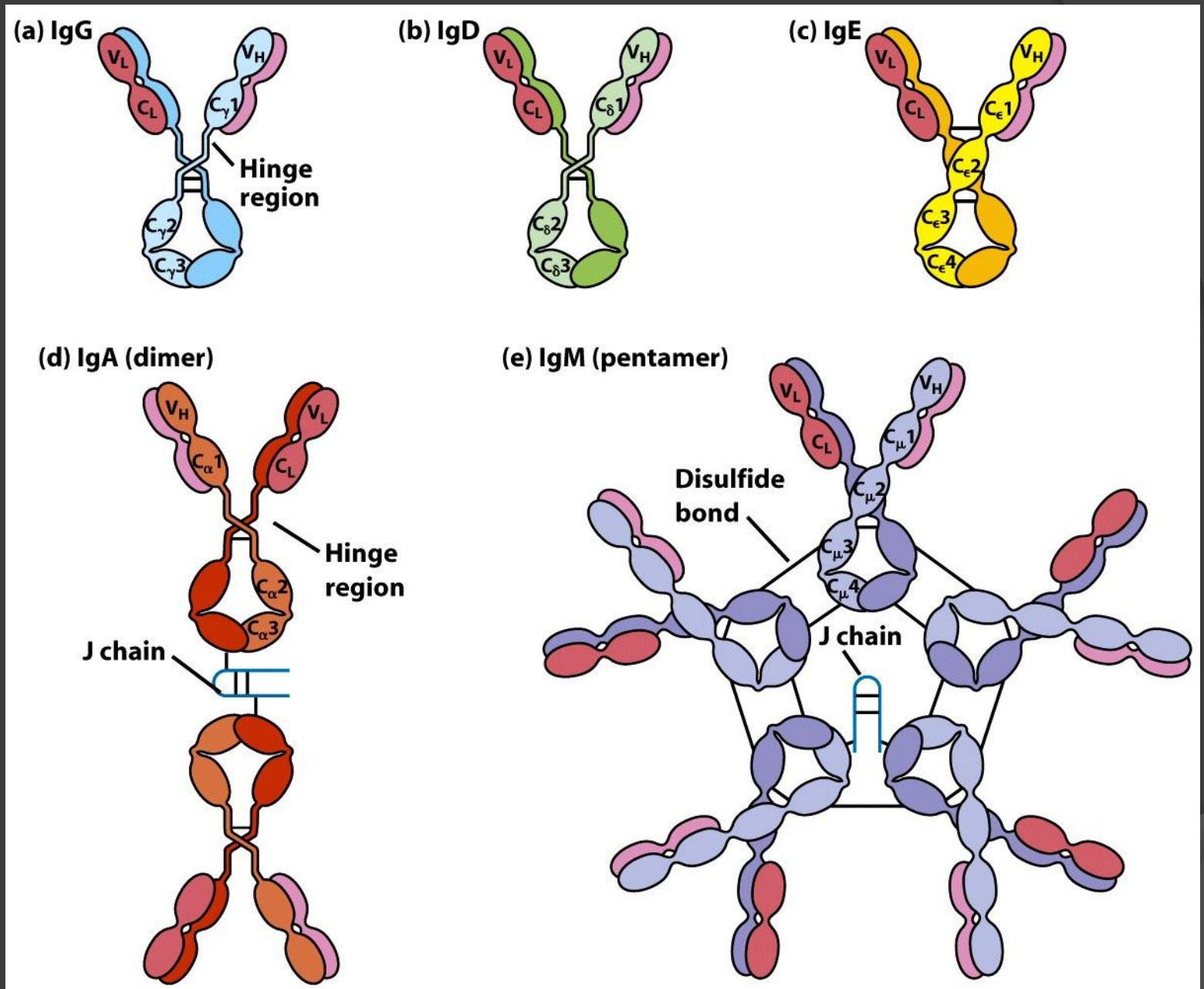

Figure 4-17
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- Monomeric IgM expressed on B cells
- Secreted is pentameric
- 1st class produced in primary response
- Activates complement
- Very good at agglutination

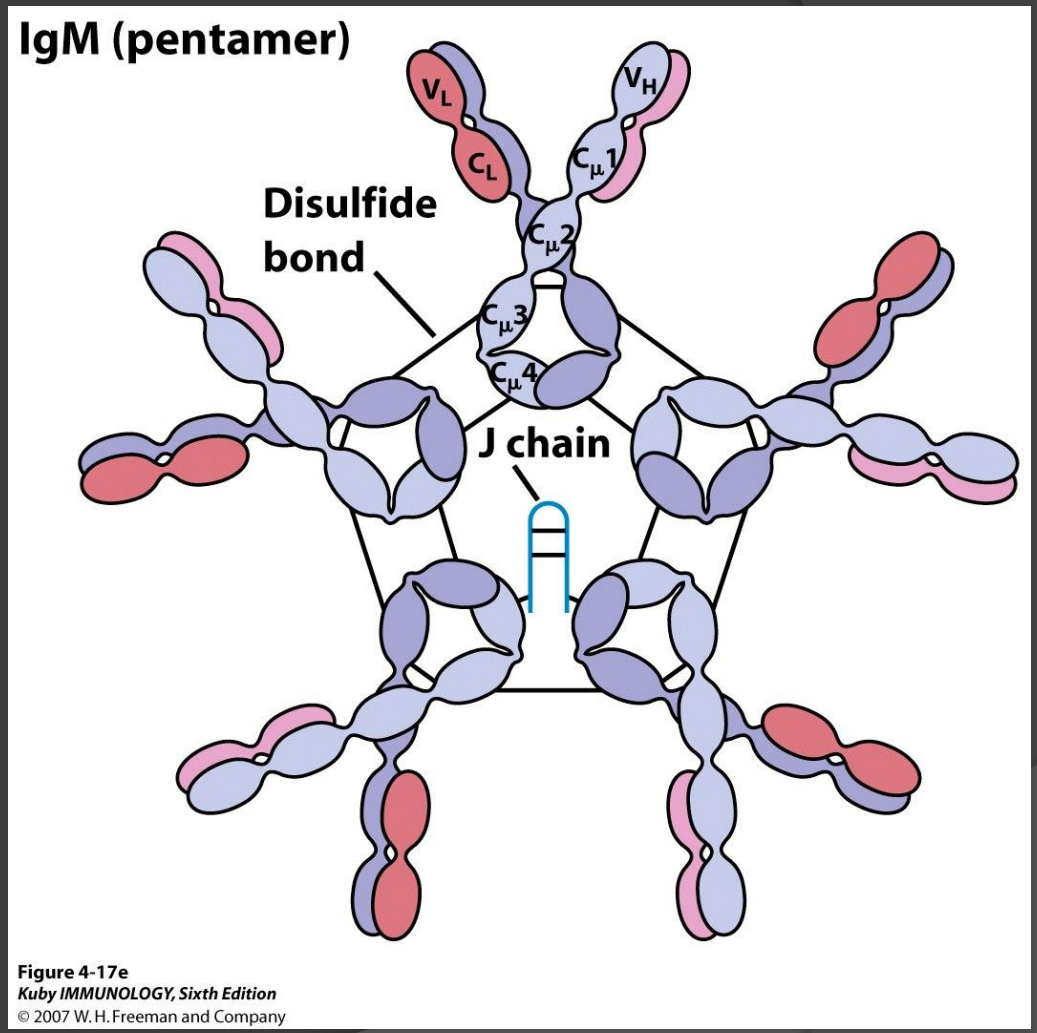

Figure 4-17e
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- Membrane bound on B cells

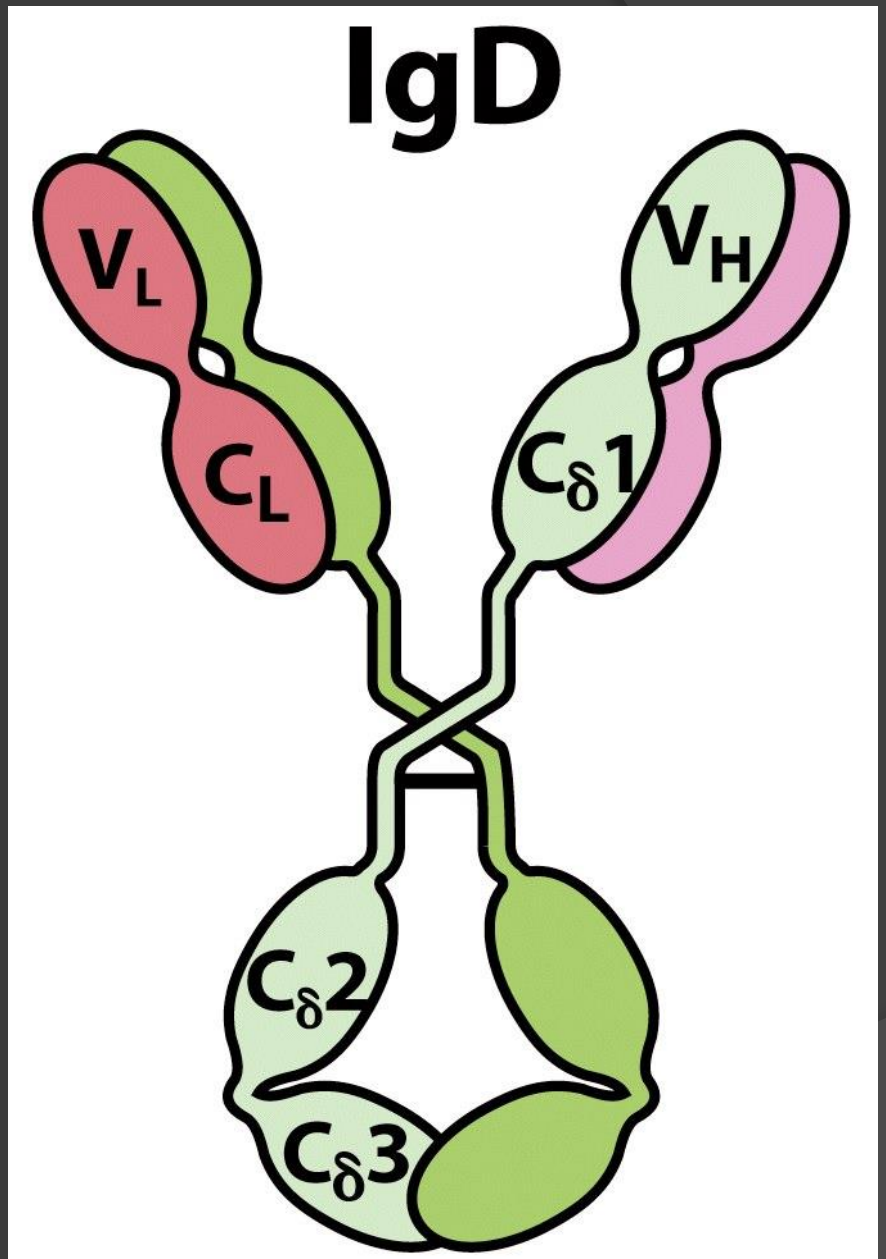

Figure 4-17b
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- Most abundant
- 4 human subclasses
- Crosses placenta
- Involved in complement

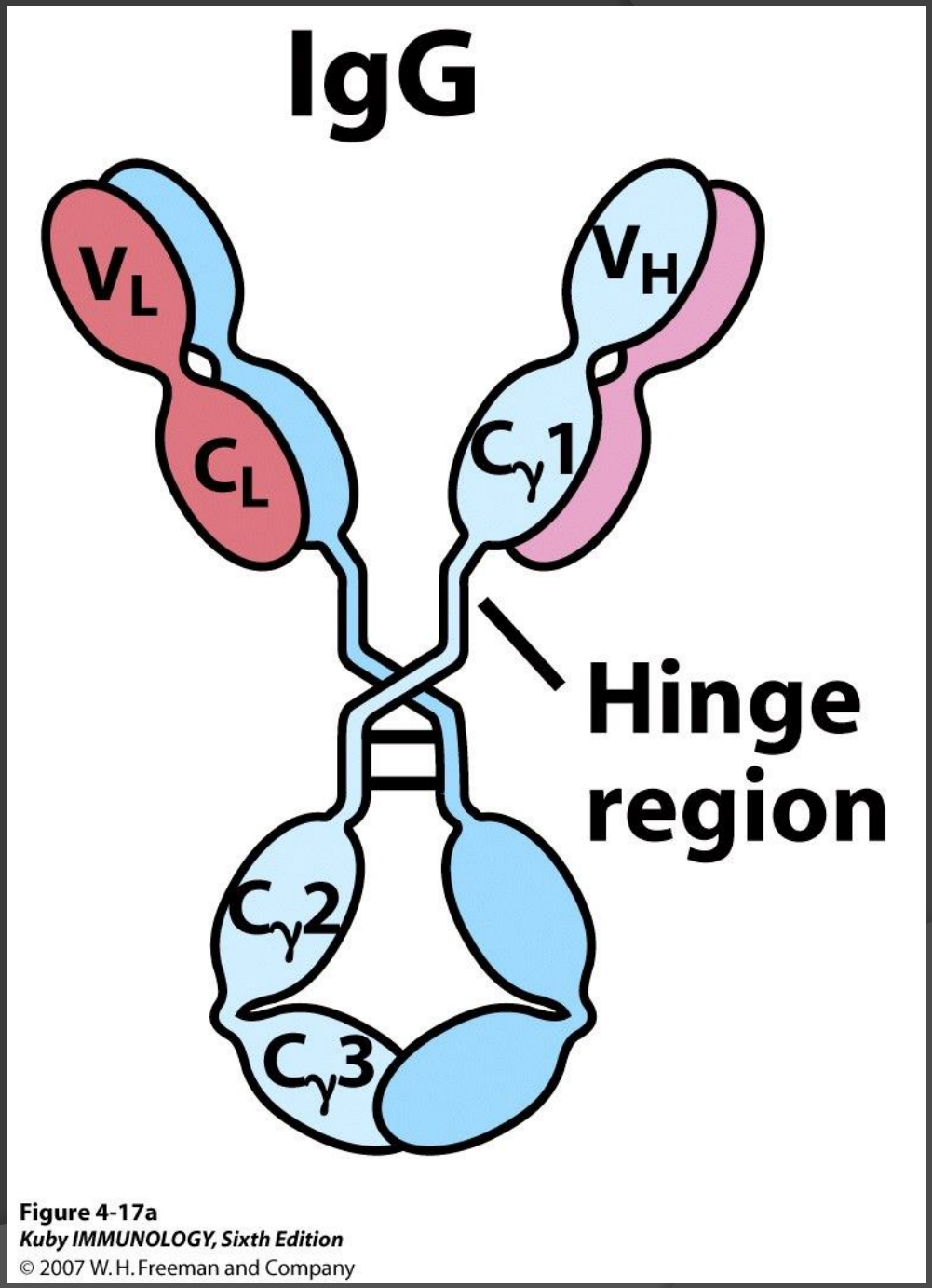

Figure 4-17a
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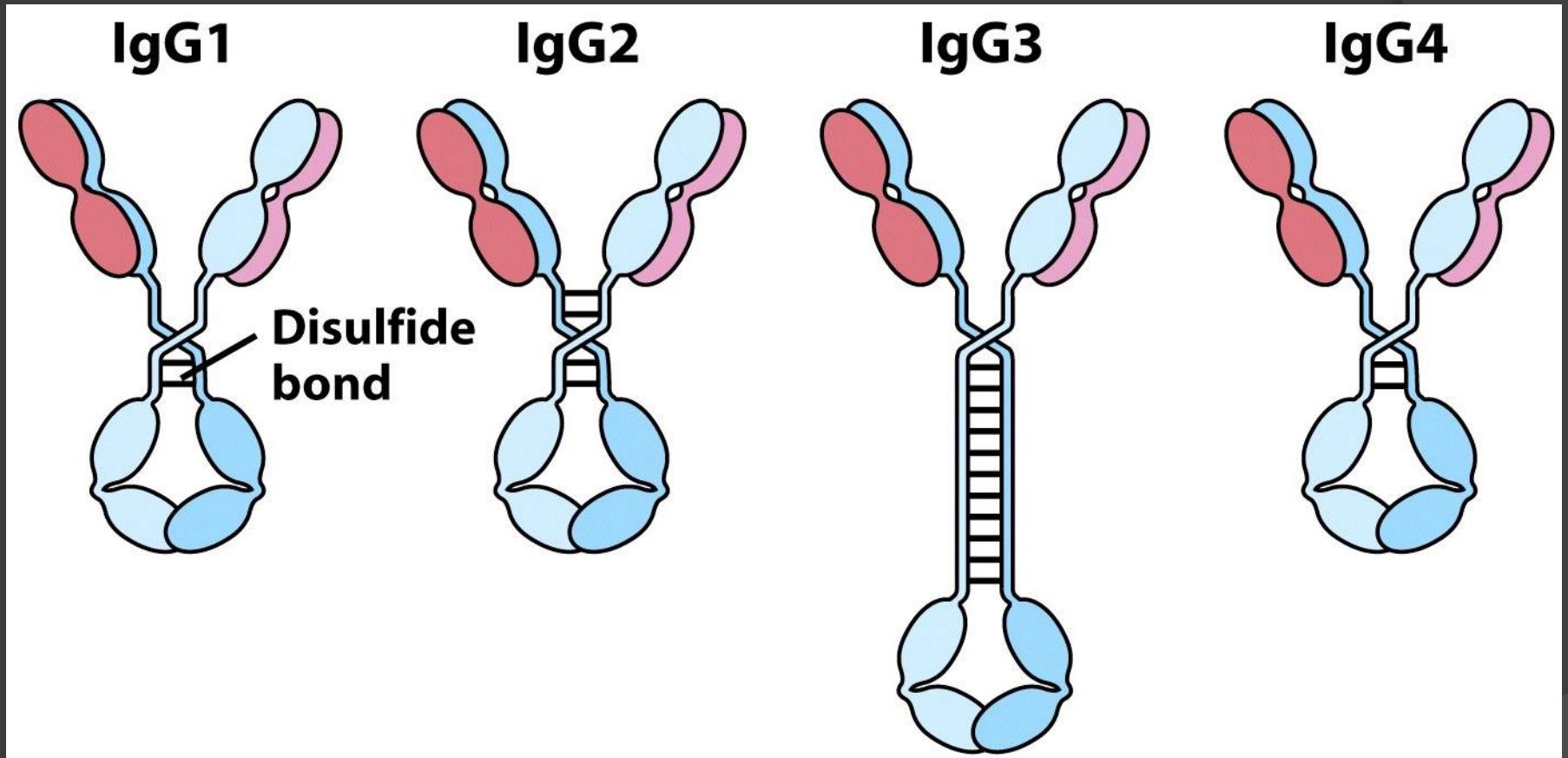

Figure 4-18
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- Involved in allergic reactions
- Involvement in parasitic infections

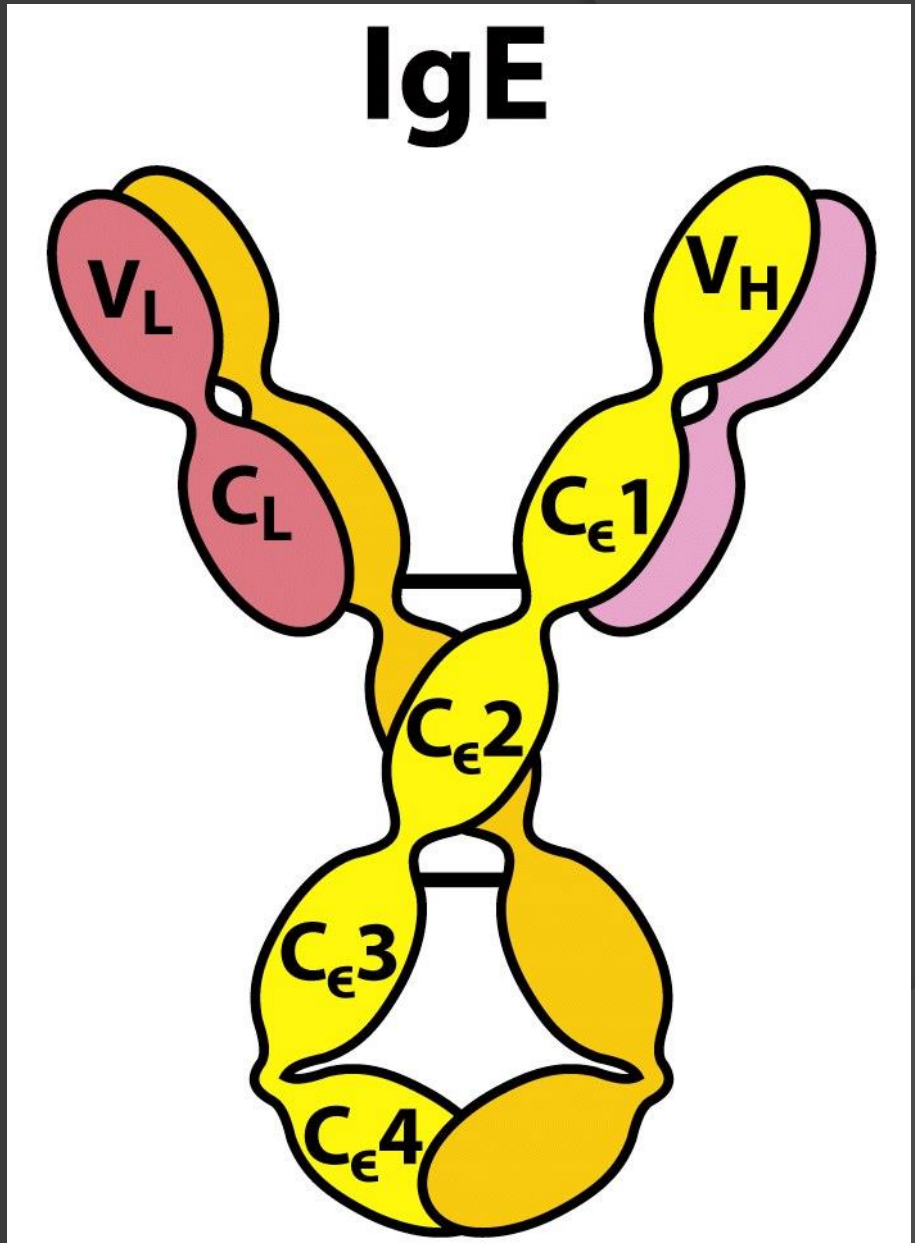

Figure 4-17c
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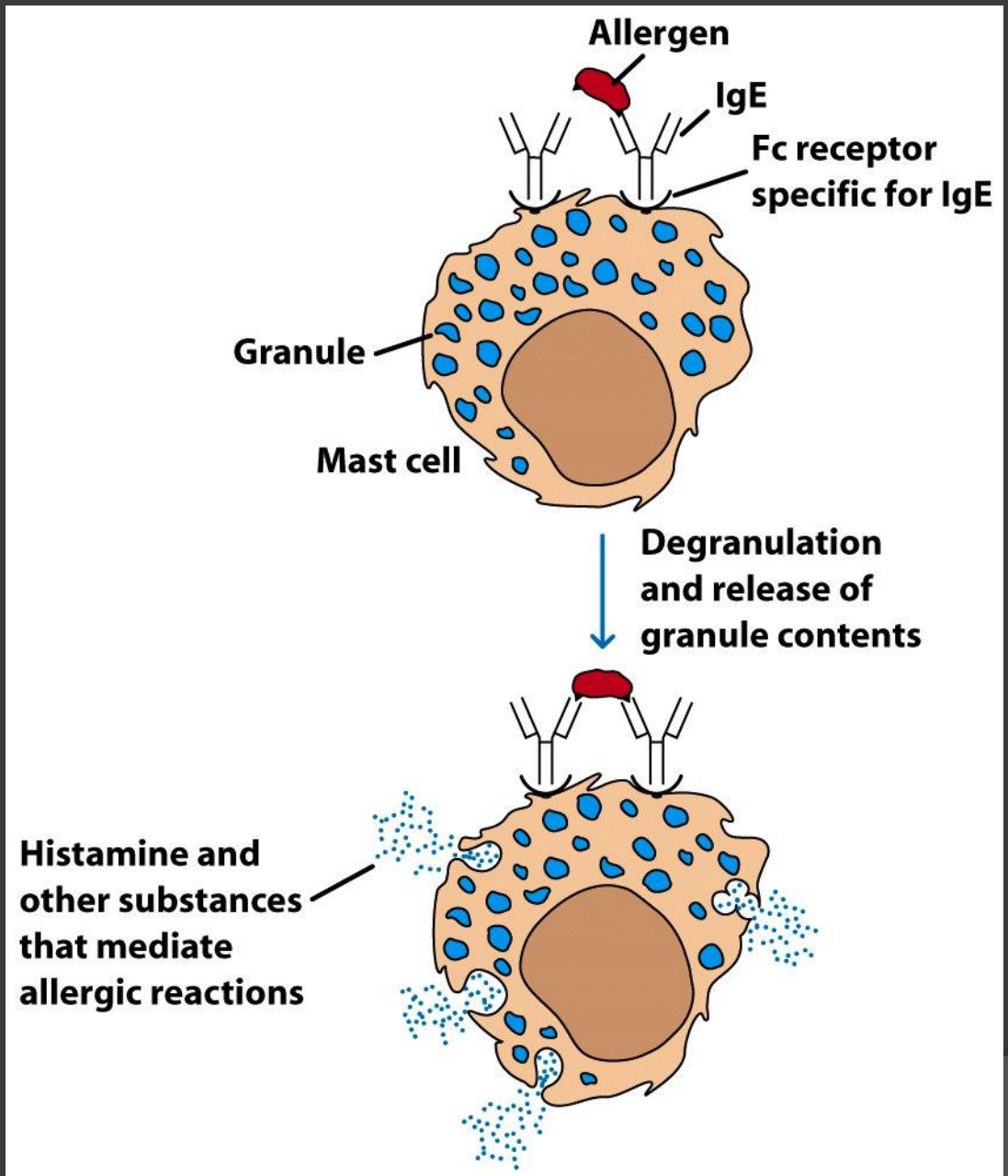

Figure 4-20
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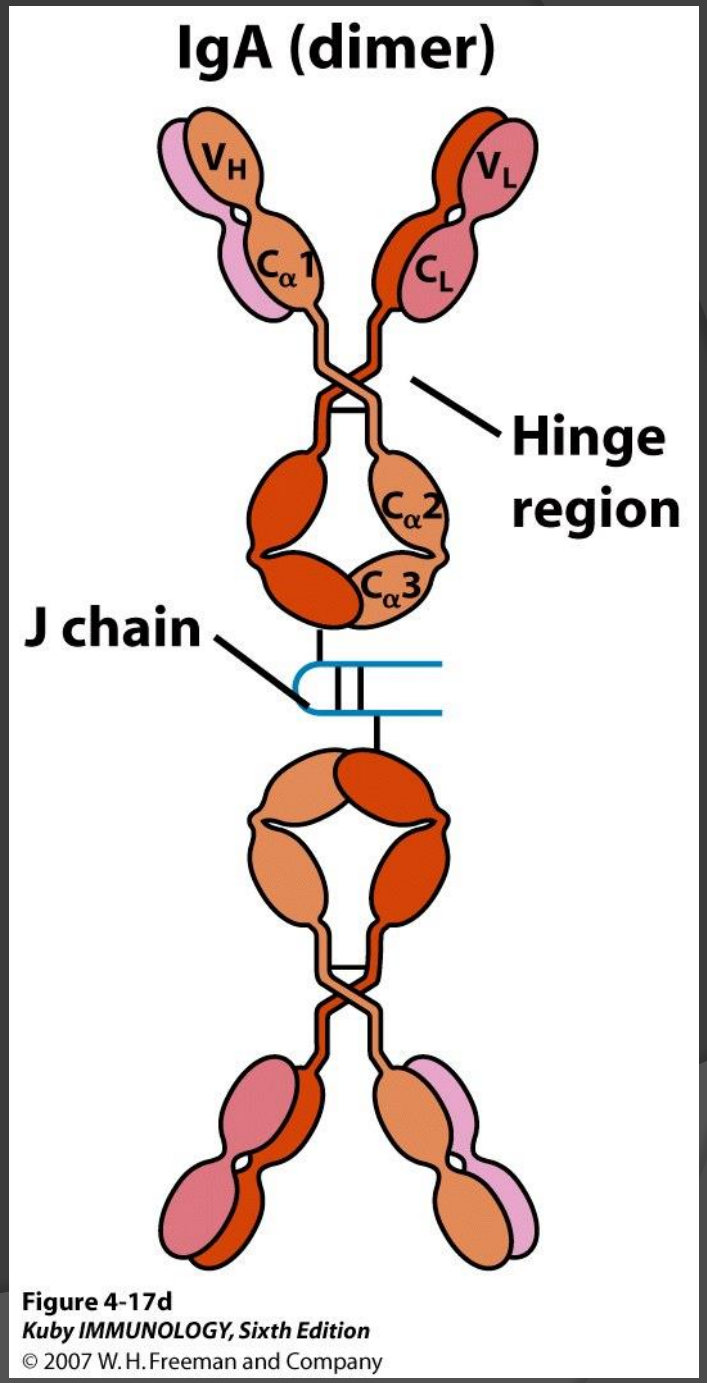
- Predominant class in secretions
  - Exists as dimer
- Can cross-link large antigens



Structure of secretory IgA

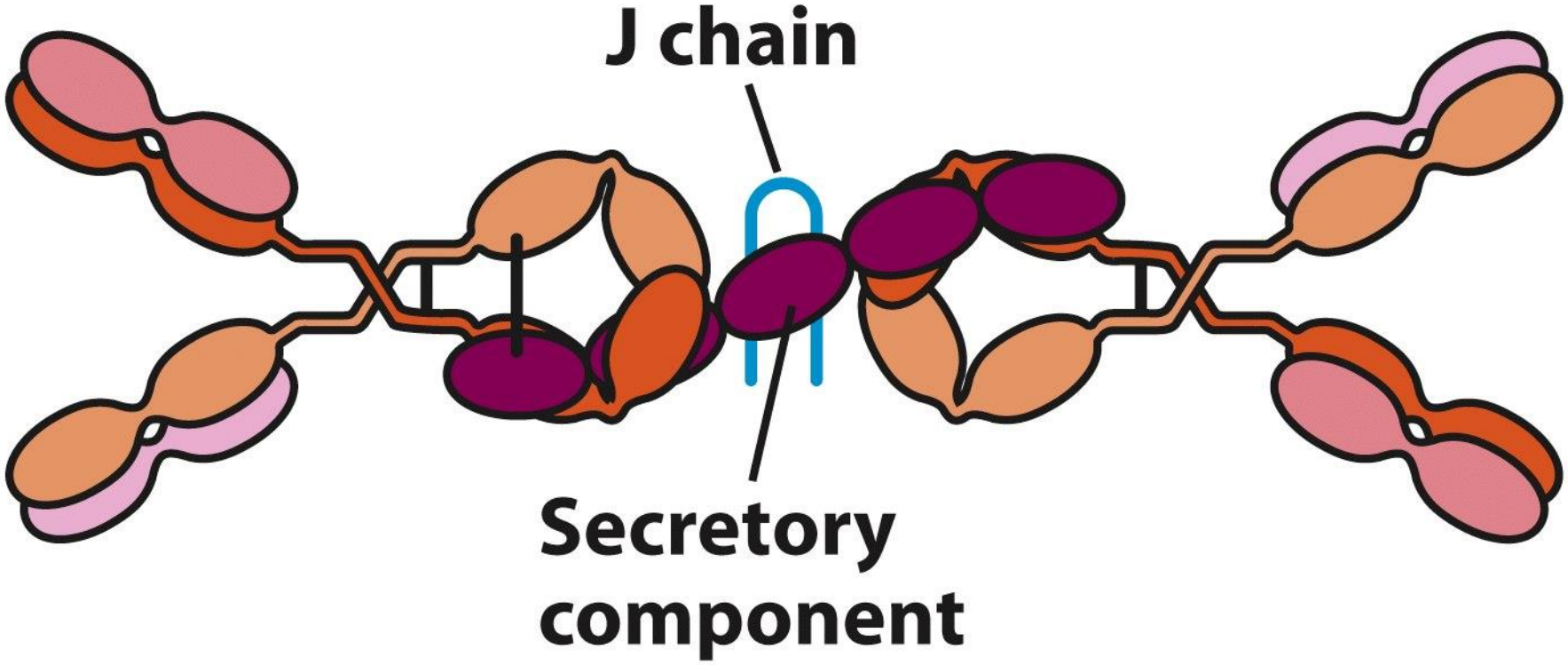


Figure 4-19a
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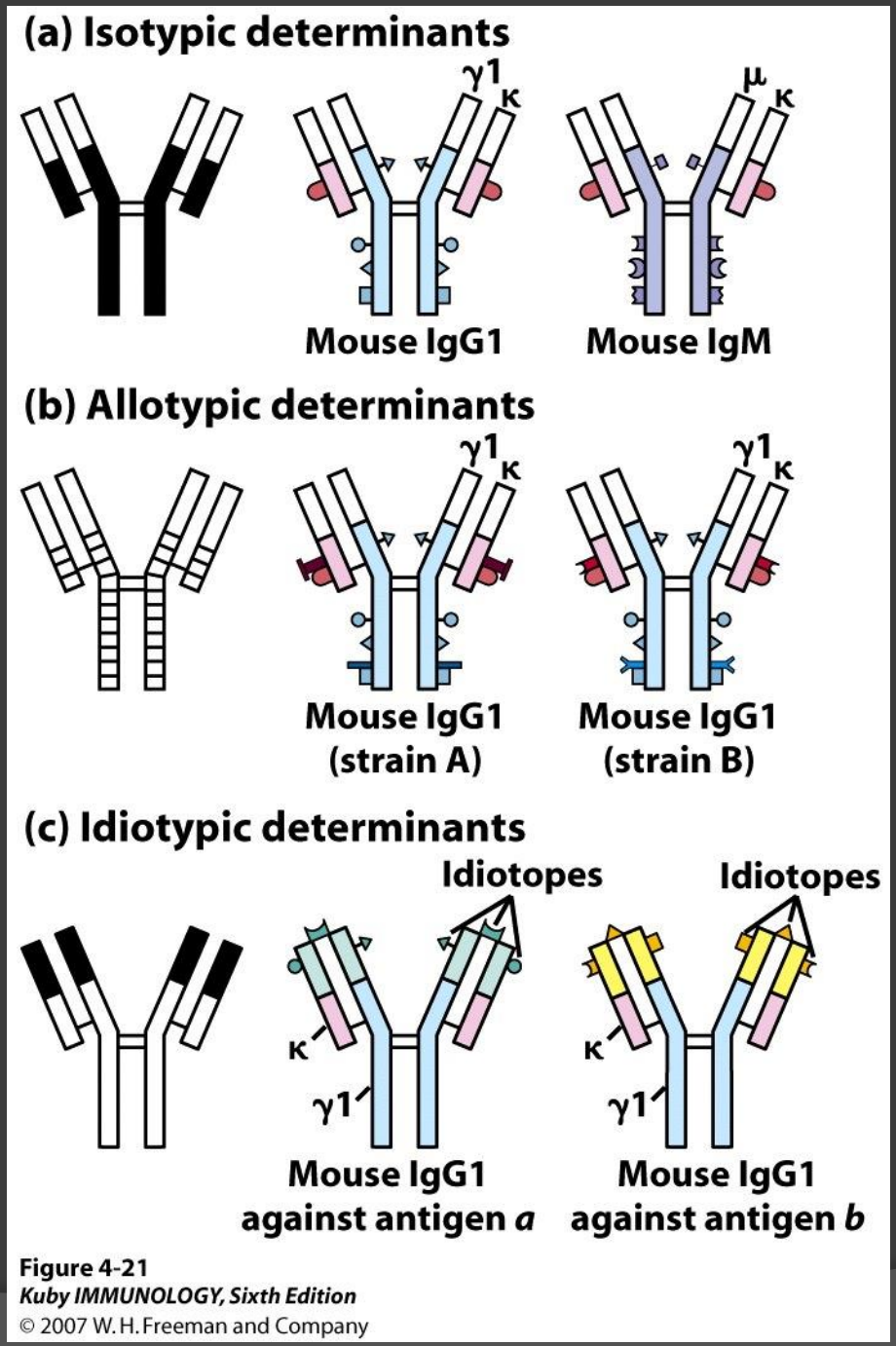

Figure 4-21
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- Immunoglobulins when injected into another species can be immunogenic
  - Isotypic – differences in constant region from one species to another
  - Allotypic – differences (alleles) that occur in some individuals
  - Idiotypic – differences in variable regions; will differ even on Abs of same isotype

Immunoglobulin Superfamily

- Similar structures
- Examples:
  - Antibodies
  - T-cell receptors
  - Class I and II MHC molecules
  - Part of B cell receptor
  - Most members of immunoglobulin superfamily cannot bind antigen

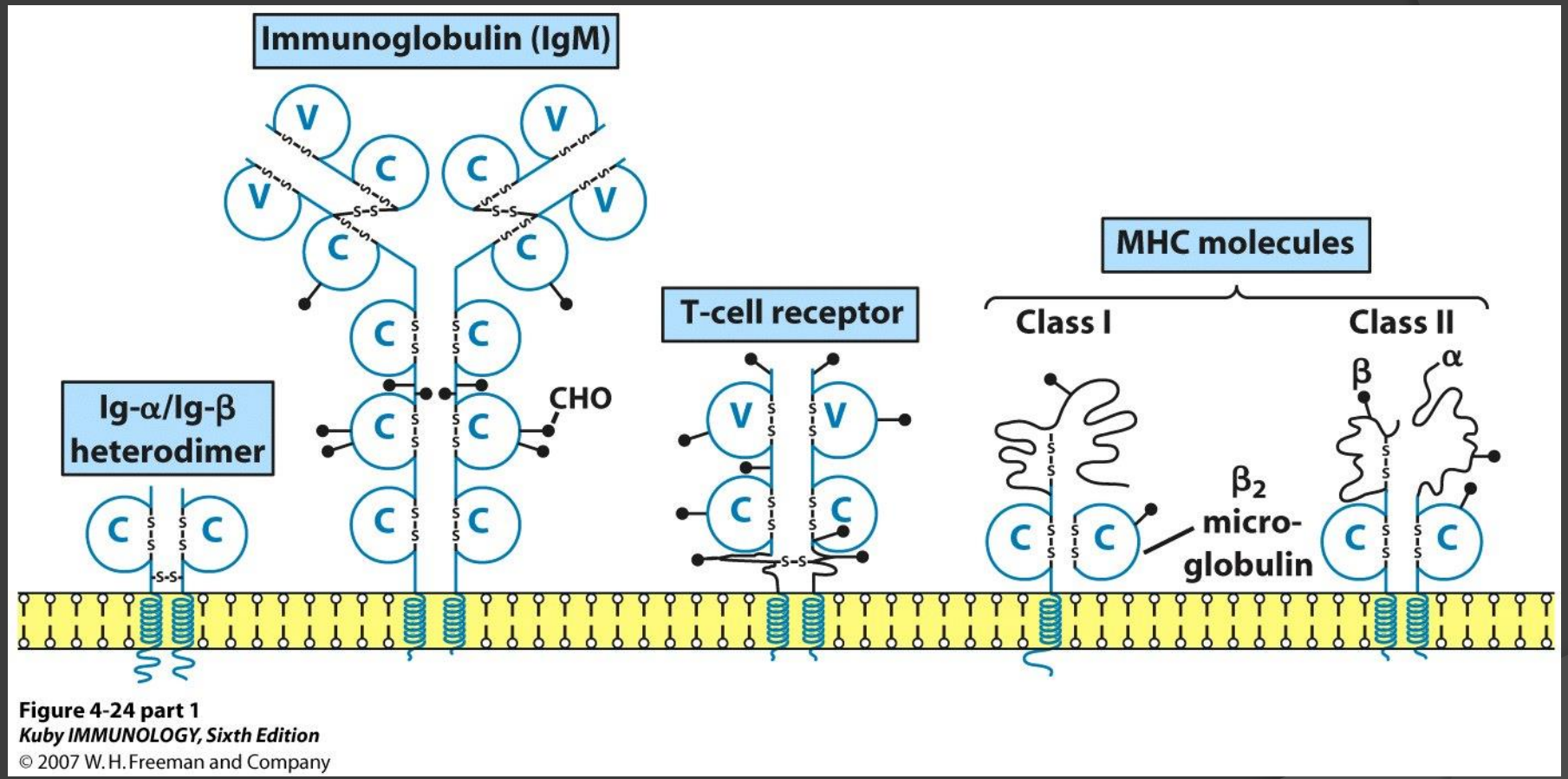

Figure 4-24 part 1
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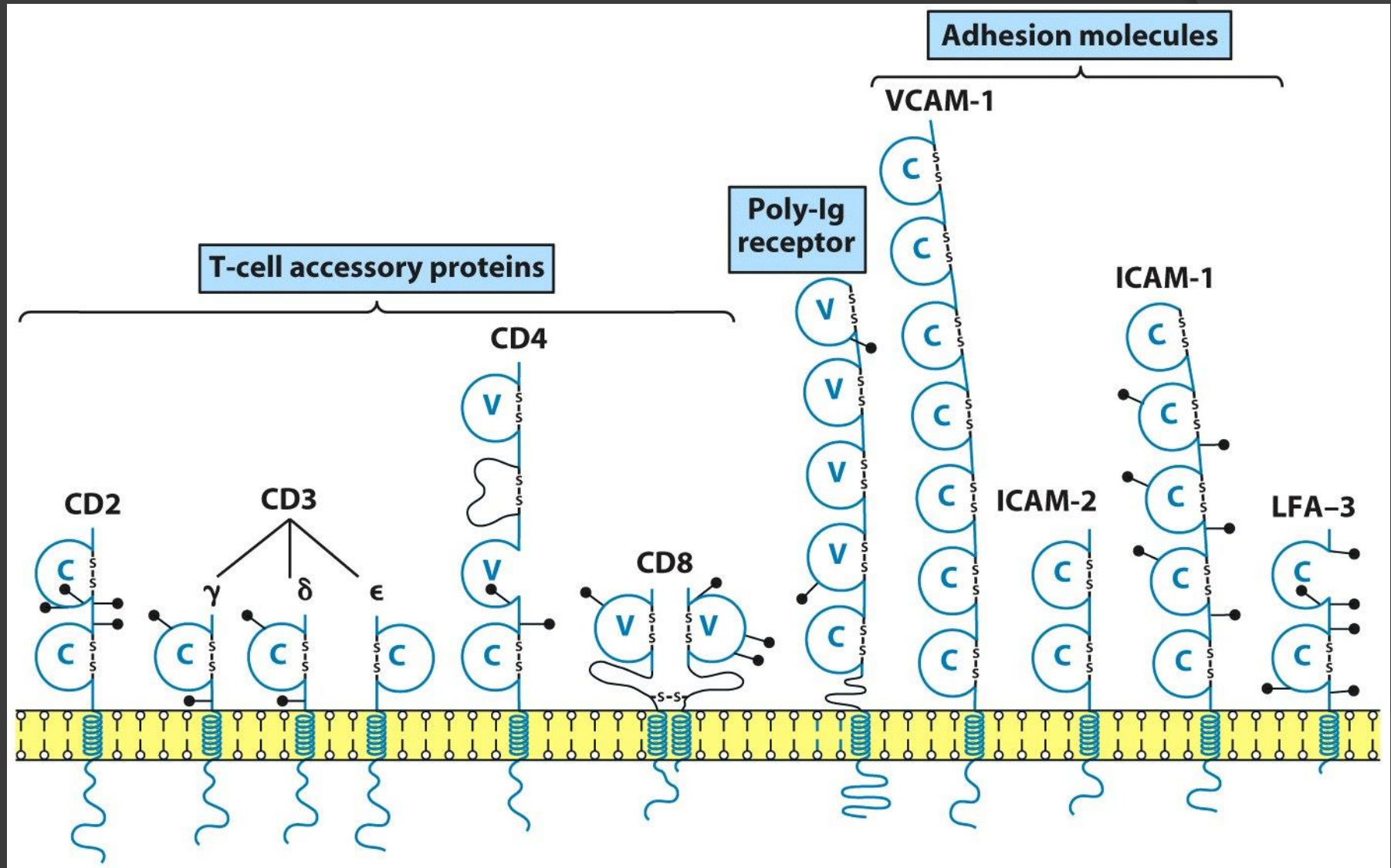

Figure 4-24 part 2
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Monoclonal Antibodies

- Most antigens offer multiple epitopes
- However, a single B cell will only produce antibody specific to single epitope
- Antibodies found in serum are from many different B cells
  - Polyclonal antibodies
  - However, for diagnostic uses, monoclonal antibodies are needed

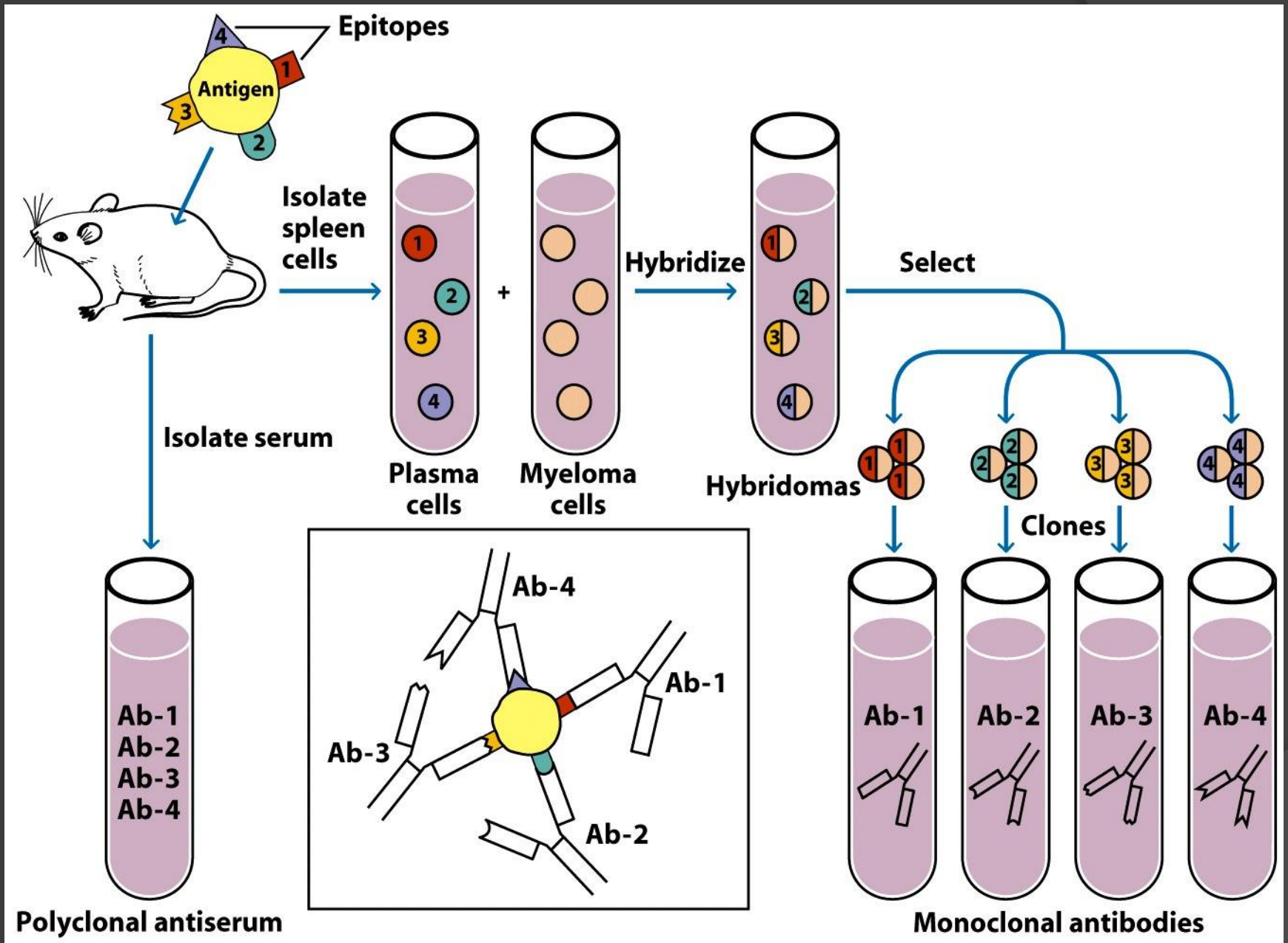

Figure 4-25
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Chapter 6

Antigen-Antibody Interactions

Dr. Capers

IMMUNOLOGY

Kindt • Goldsby • Osborne

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Chapter 6

Antigen-Antibody Interactions: Principles and Applications

- Antigen-Antibody (Ag-Ab) Interaction
  - Similar to enzyme-substrate interaction
    - However, does not lead to irreversible chemical alteration in either the antibody or antigen

- Noncovalent Interactions
  - Hydrogen bonds
  - Ionic bonds
  - Hydrophobic reactions
  - Van der Waals
    - All weak so need combination of all of them to make strong interaction

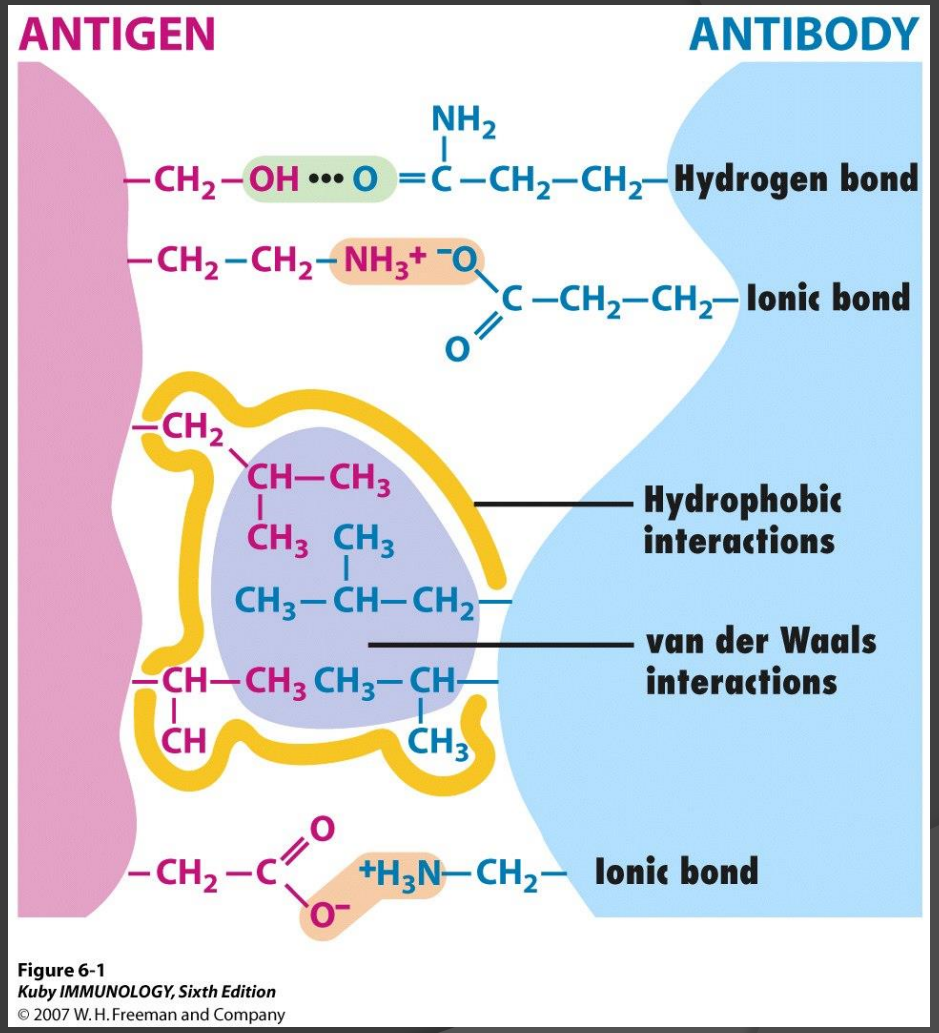

Figure 6-1
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Cross-reactivity

- Mechanisms of tolerance prevent formation of Abs against one's own blood group antigens
  - However, exposure to microbial antigens on intestinal bacteria induce formation of Abs, these antigens share similarity to blood group antigens

TABLE 6-2 ABO blood types

Blood type	Antigens on RBCs	Serum antibodies
A	A	Anti-B
B	B	Anti-A
AB	A and B	Neither
O	Neither	Anti-A and anti-B

Table 6-2
Kuby IMMUNOLOGY, Sixth Edition

- Immunoassays – measuring Ag-Ab interactions
  - Vital roles:
    - Diagnosing disease
    - Monitoring level of humoral response
    - Identifying molecules of biological or medical interest

TABLE 6-3 Sensitivity of various immunoassays

Assay	Sensitivity* (μg antibody/ml)
Precipitation reaction in fluids	20–200
Precipitation reactions in gels	
Mancini radial immunodiffusion	10–50
Ouchterlony double immunodiffusion	20–200
Immunoelectrophoresis	20–200
Rocket electrophoresis	2
Agglutination reactions	
Direct	0.3
Passive agglutination	0.006–0.06
Agglutination inhibition	0.006–0.06
Radioimmunoassay (RIA)	0.0006–0.006
Enzyme-linked immunosorbent assay (ELISA)	~0.0001–0.01
ELISA using chemiluminescence	~0.00001–0.01†
Immunofluorescence	1.0
Flow cytometry	0.006–0.06

*The sensitivity depends on the affinity of the antibody used for the assay as well as the epitope density and distribution on the antigen.
†Note that the sensitivity of chemiluminescence-based ELISA assays can be made to match that of RIA.

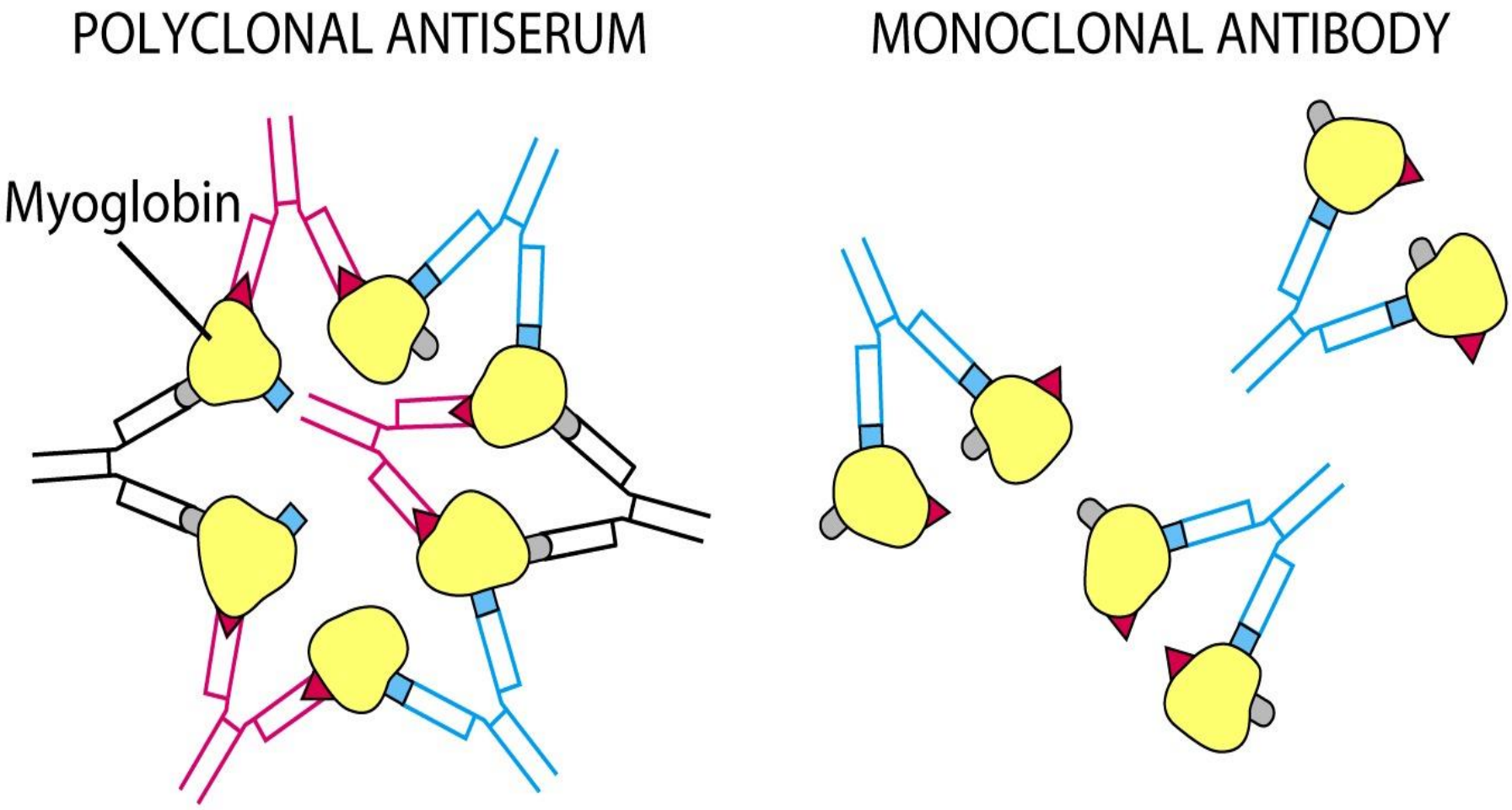
SOURCE: Updated and adapted from N. R. Rose et al., eds., 1997, *Manual of Clinical Laboratory Immunology,* 5th ed., American Society for Microbiology, Washington, DC.

Table 6-3
Kuby IMMUNOLOGY, Sixth Edition

Precipitation Reactions

- Ag-Ab interactions can form visible precipitate
  - Examples:
    - Radial immunodiffusion
    - Double immunodiffusion
    - immunoelectrophoresis

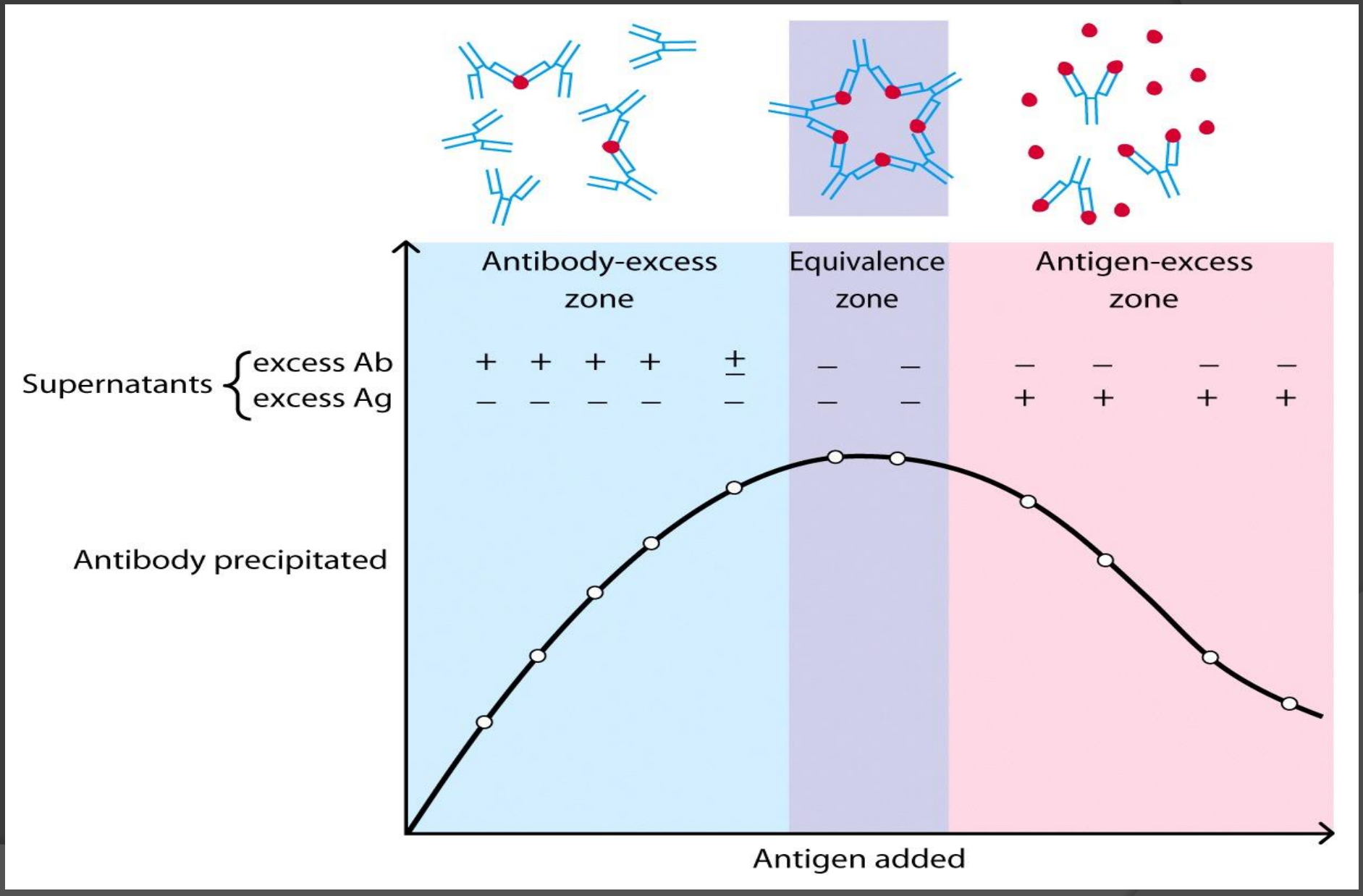
Precipitation reactions

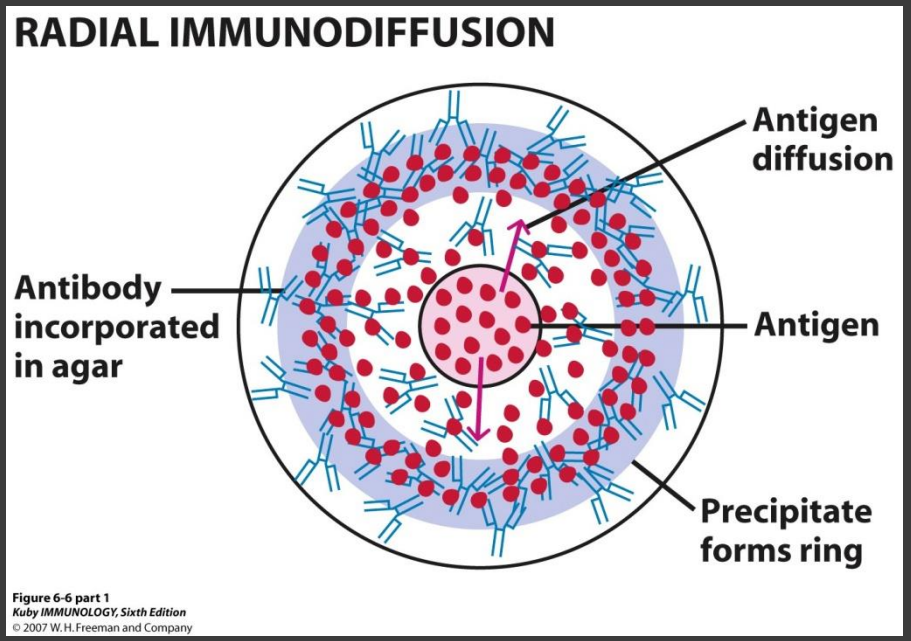


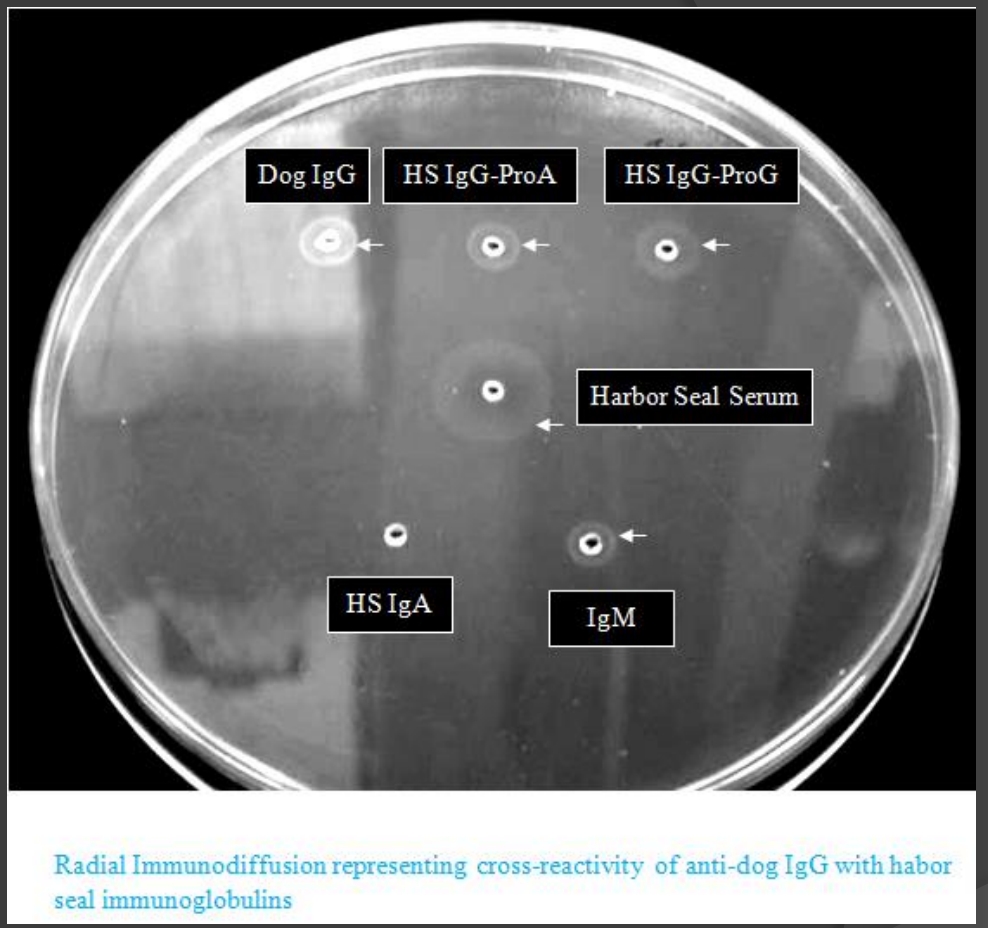
(Lattices or large aggregates)

no precipitate is formed if an Ag contains only a single copy of each epitope

Precipitation curve







Radial Immunodiffusion representing cross-reactivity of anti-dog IgG with habor seal immunoglobulins

In this example, Anti-dog IgG is Mixed in agar so only what is Placed in wells (Ag) diffuses out

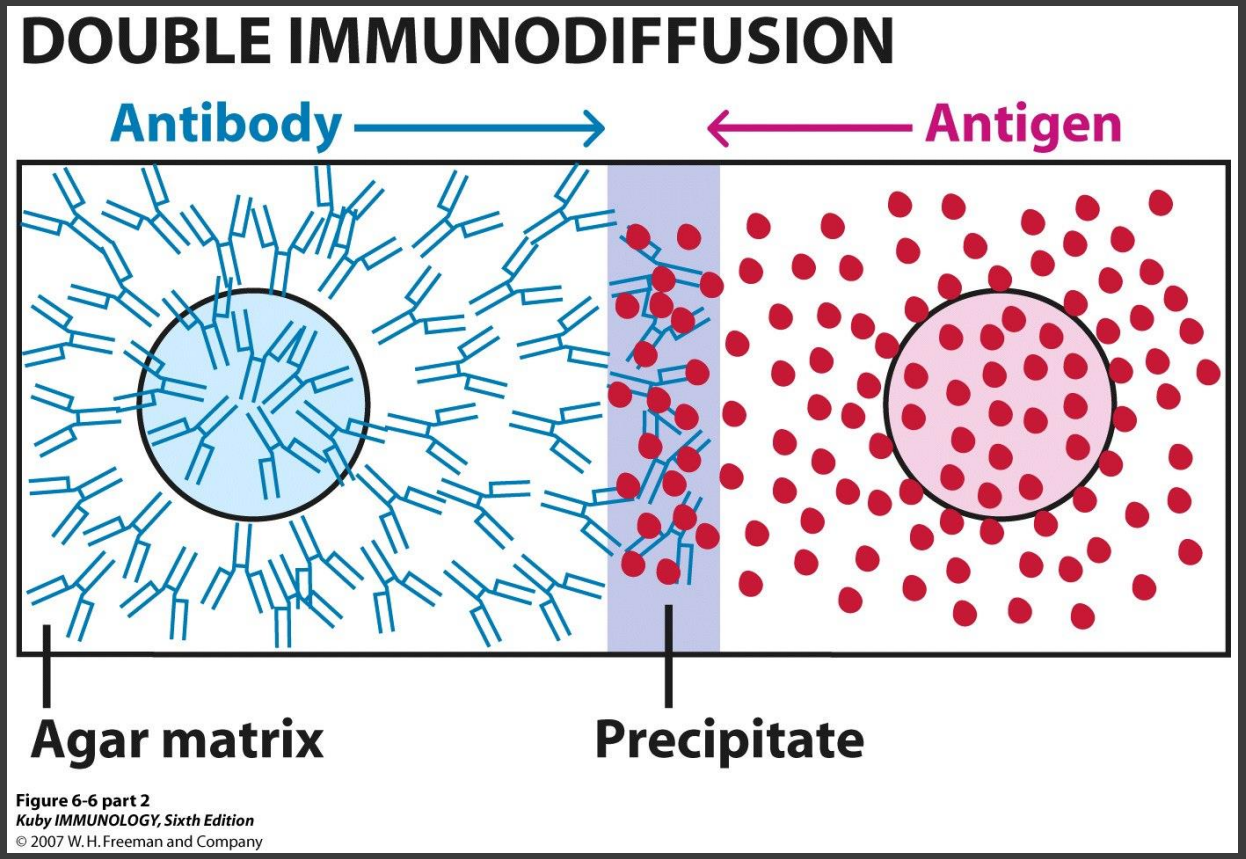


Figure 6-6 part 2
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In this example, both antibody and antigen diffuse Out of wells

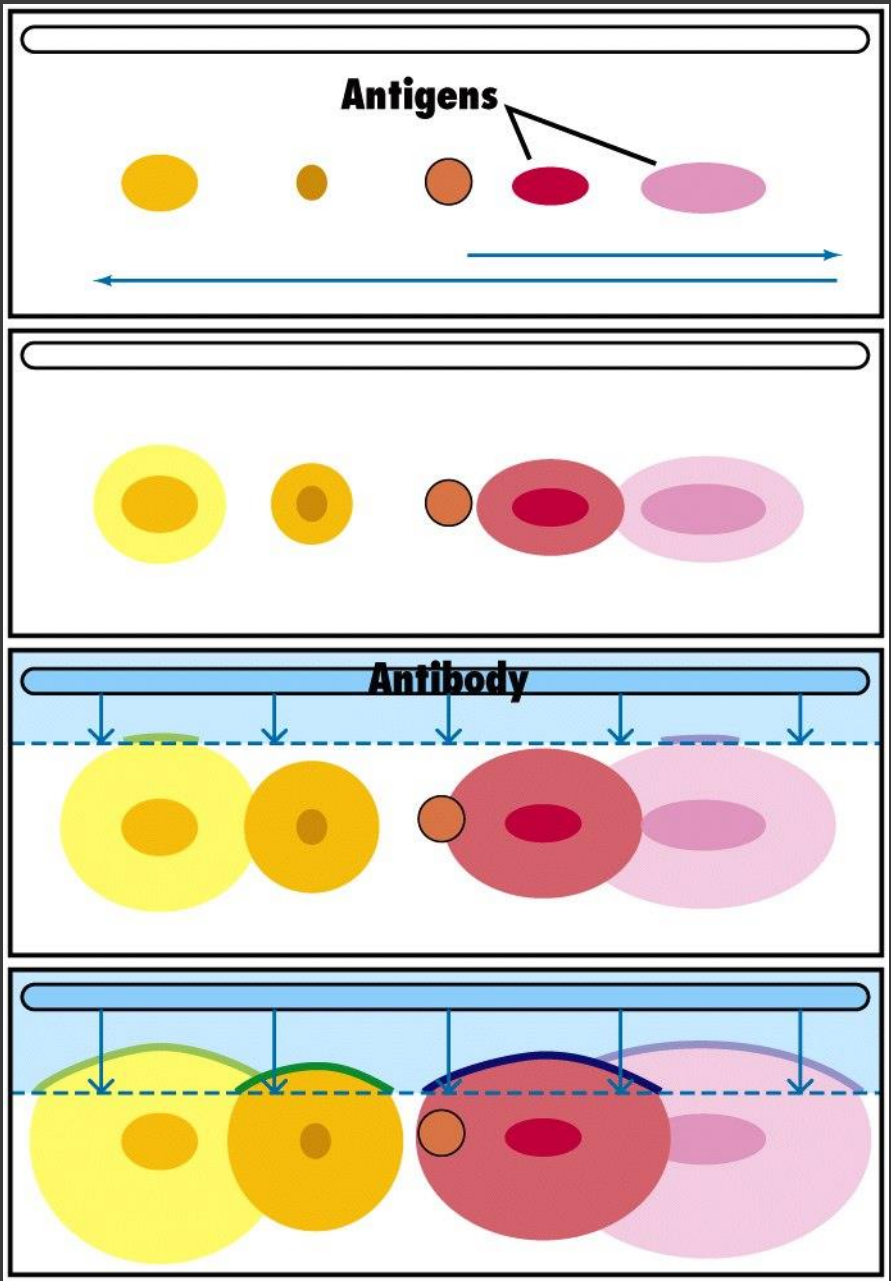

Figure 6-7
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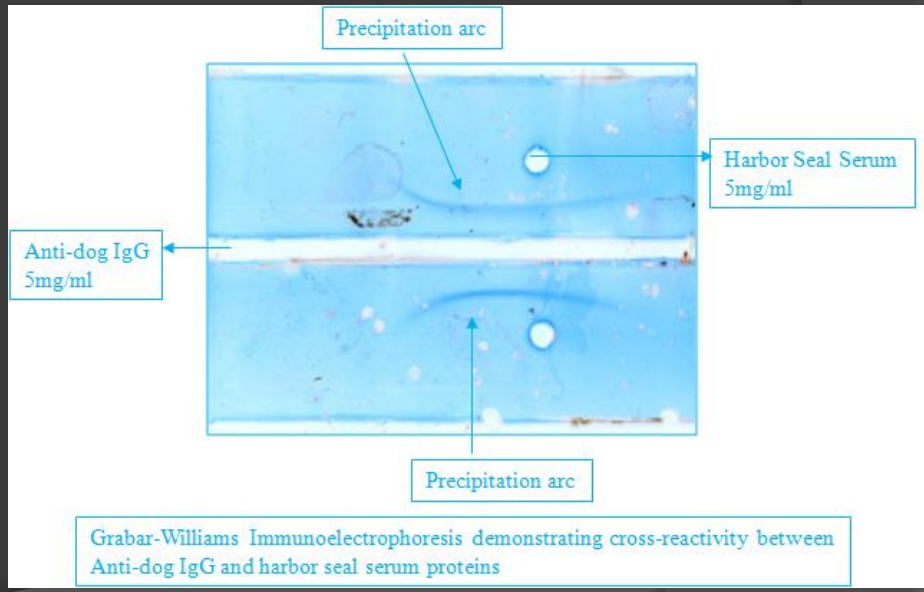
Immunoelectrophoresis – Antigen is 1st put into wells, charge is applied to separate components of antigen mixture, then troughs are cut and antibody is allowed to diffuse through gel



Grabar-Williams Immunoelectrophoresis demonstrating cross-reactivity between Anti-dog IgG and harbor seal serum proteins

Agglutination Reactions

- Visible clumping – agglutination
  - Examples:
    - Hemagglutination
    - Bacterial Agglutination
- Important in all tests for Antibody to be in right concentation
  - Too much antibody will cause univalent binding, need multivalent for preciptitate or agglutination to occur

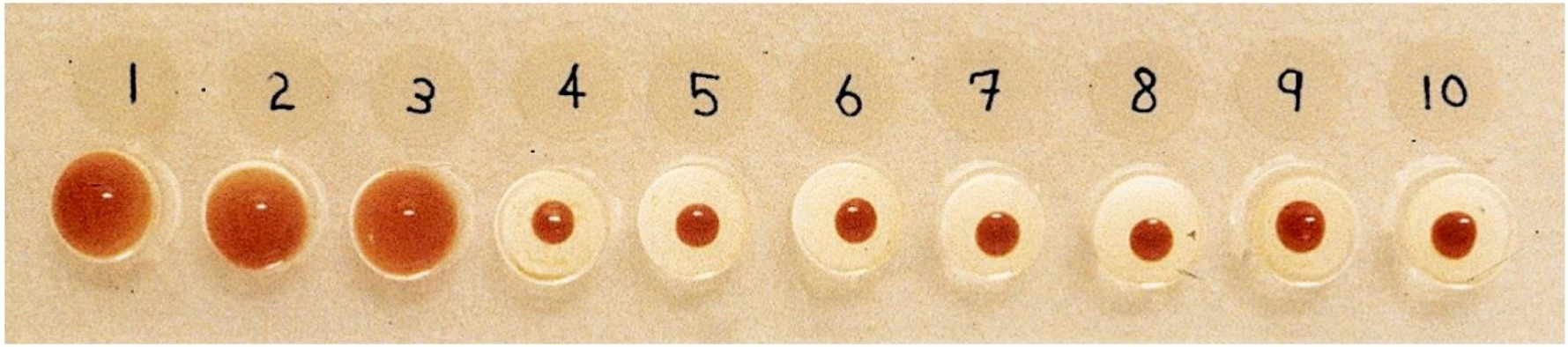


Figure 6-8
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Bacterial agglutination

- Diagnosis of infection
- Typhoid
- *Salmonella typhi*
- Titer
- Dilution of antiserum – 1/640 ; 1/1280
- The last tube of visible agglutination

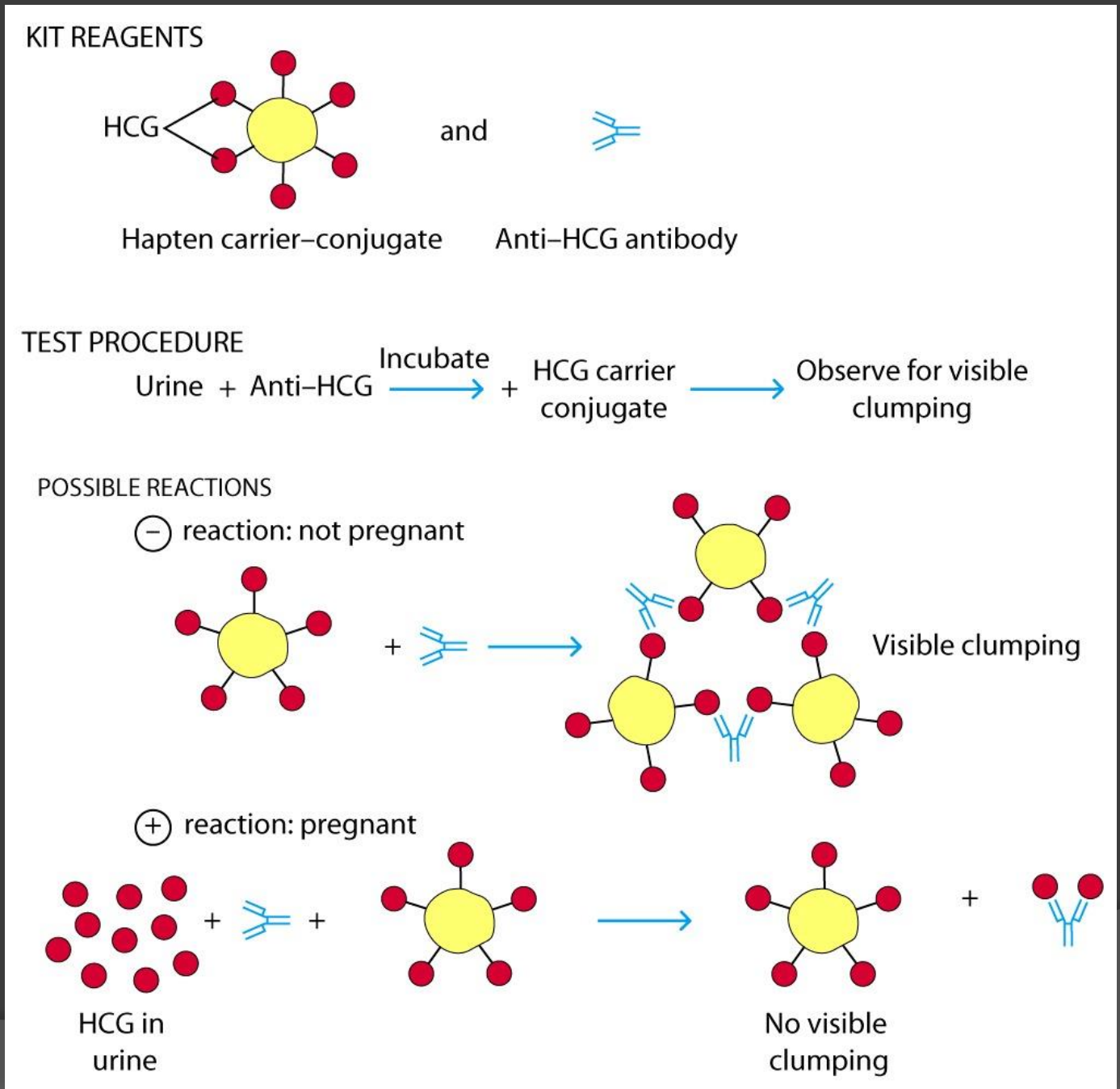
Passive Agglutination

- Useful with soluble antigens
- Preparation of Ag- coated RBC's treated with Tannic acid & NaCl (Adsorption)
- Serial dilution of serum containing Ab's in micro titer plate wells
- Ag coated RBC's are then added
- Agglutination is assessed
- Alternative to RBC's – Synthetic Latex beads – consistency, uniformity & stability

Agglutination inhibition

- Home pregnancy test
- Illicit drug adicts
- Cocaine
- Heroin

- problems – similarity in chemical structures of legal & Illicit drugs



-The original home pregnancy test kit employed hapten inhibition (agglutination inhibition) to determine the presence or absence of human chorionic gonadotropin (HCG) >>> The kits currently on the market use ELISA-based assays.
-Also used to determine the use of illegal drugs, & immunity (Ab) to virus (rubella).

ELISA

- Enzyme-Linked Immunosorbent Assay
  - Examples:
    - Indirect ELISA
    - Sandwich ELISA
    - Competitive ELISA

Indirect ELISA

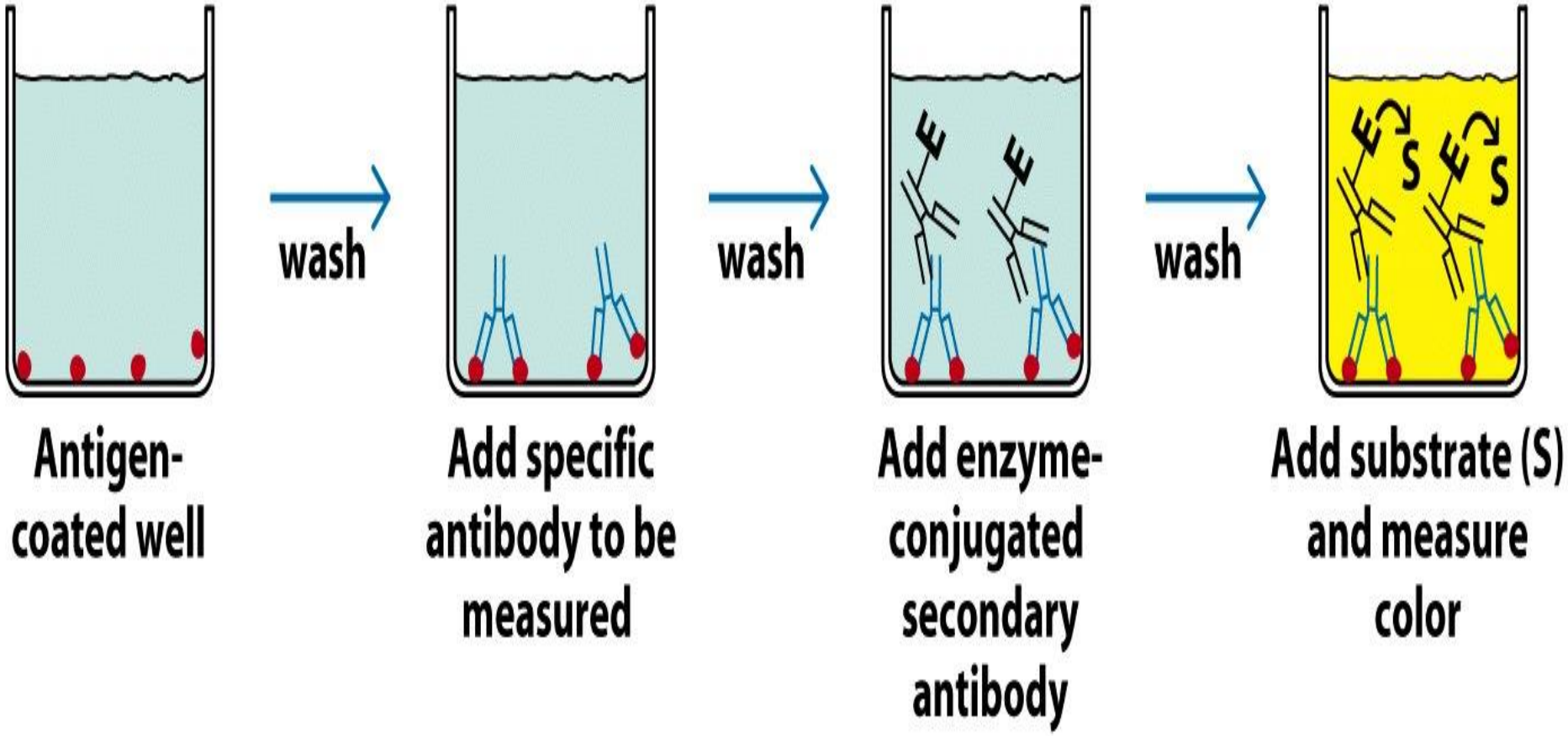


Figure 6-10a
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Sandwich ELISA

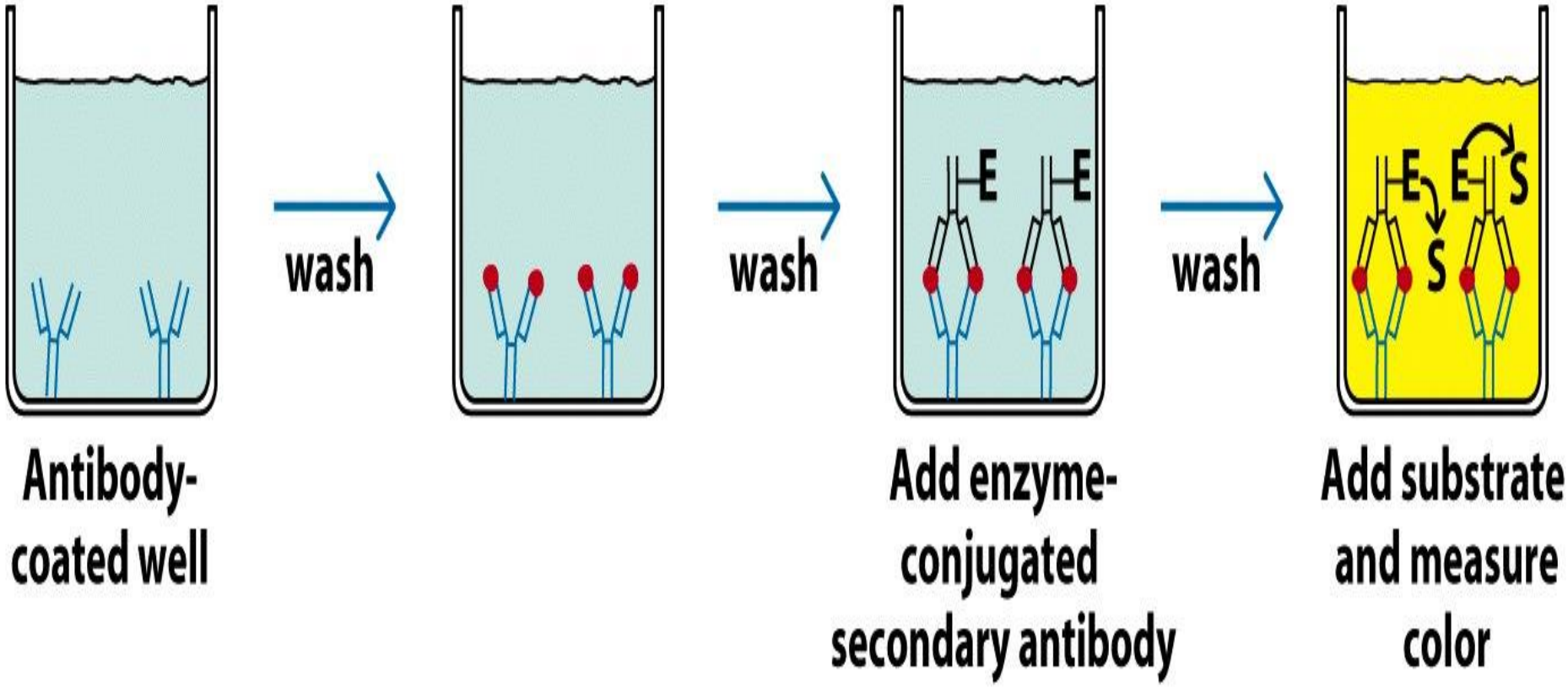

Figure 6-10b
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1. Antisera that is cross-reactive with harbor seal immunoglobulin is coated onto a plate and purified harbor seal immunoglobulin is added.

2. Harbor seal immunoglobulin, if present, attaches to the antisera.

3. Biotinylated antisera is added.

4. eAAP is added.

5. pNP is added and a color change appears if there was harbor seal immunoglobulin present (color intensity, absorbance, increases as amount of immunoglobuin increases).

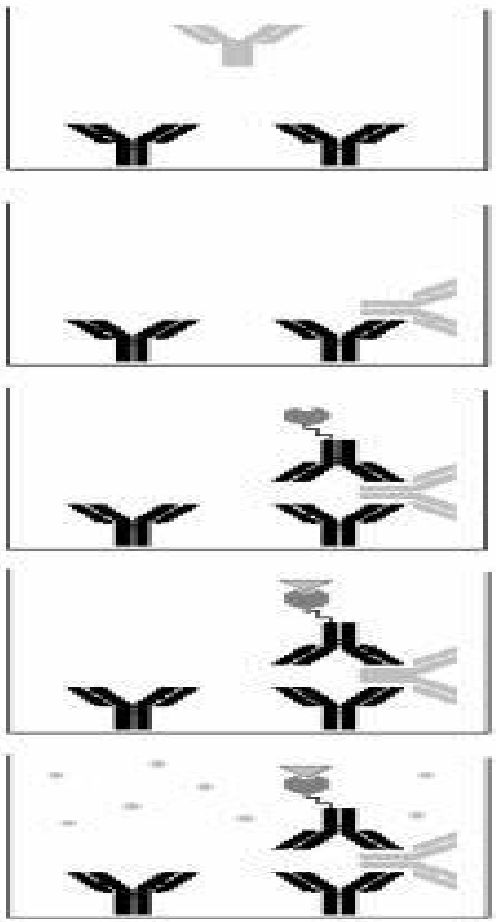

Figure 1. Overview of Sandwich ELISA coating with antisera.

Competitive ELISA

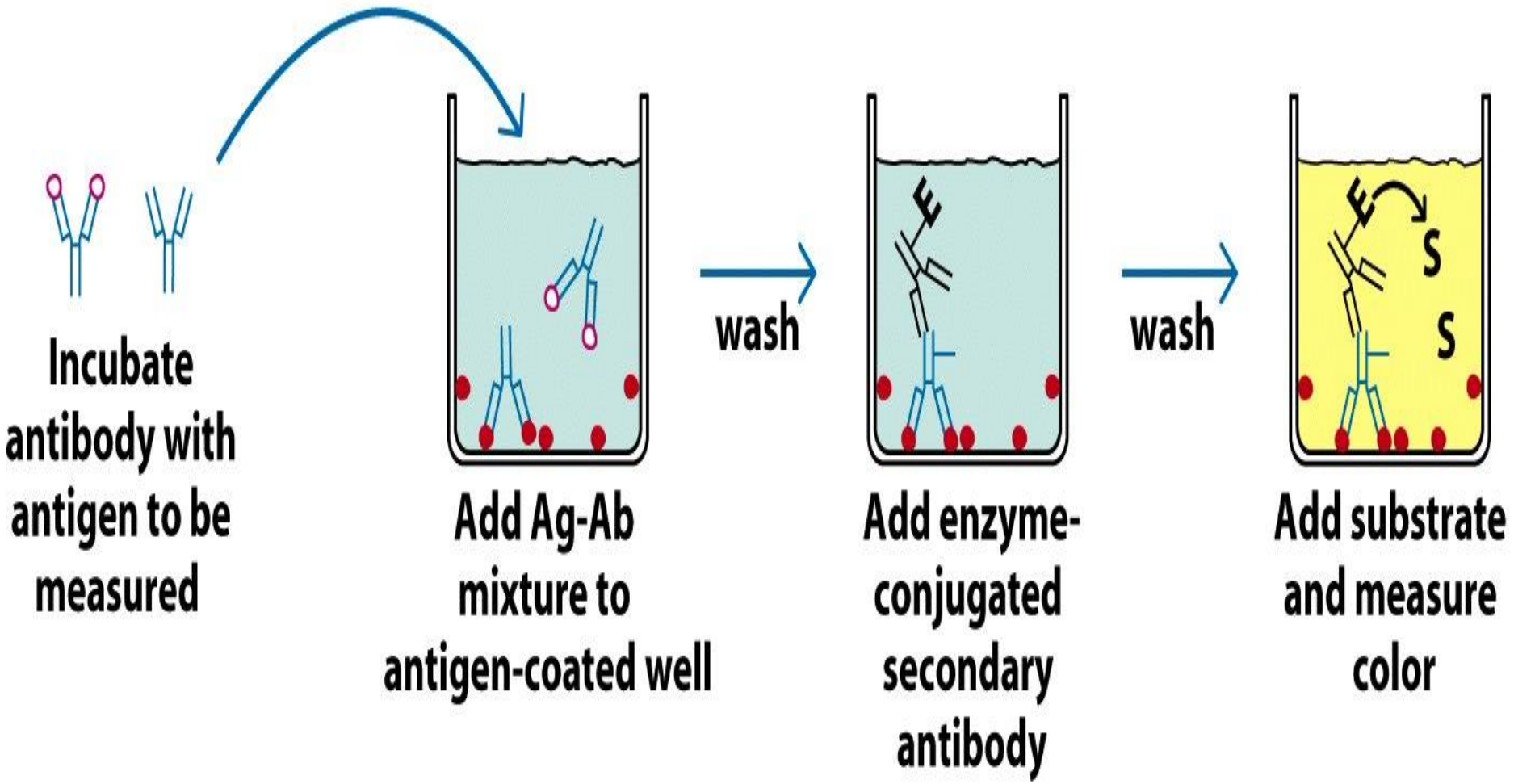

Figure 6-10c
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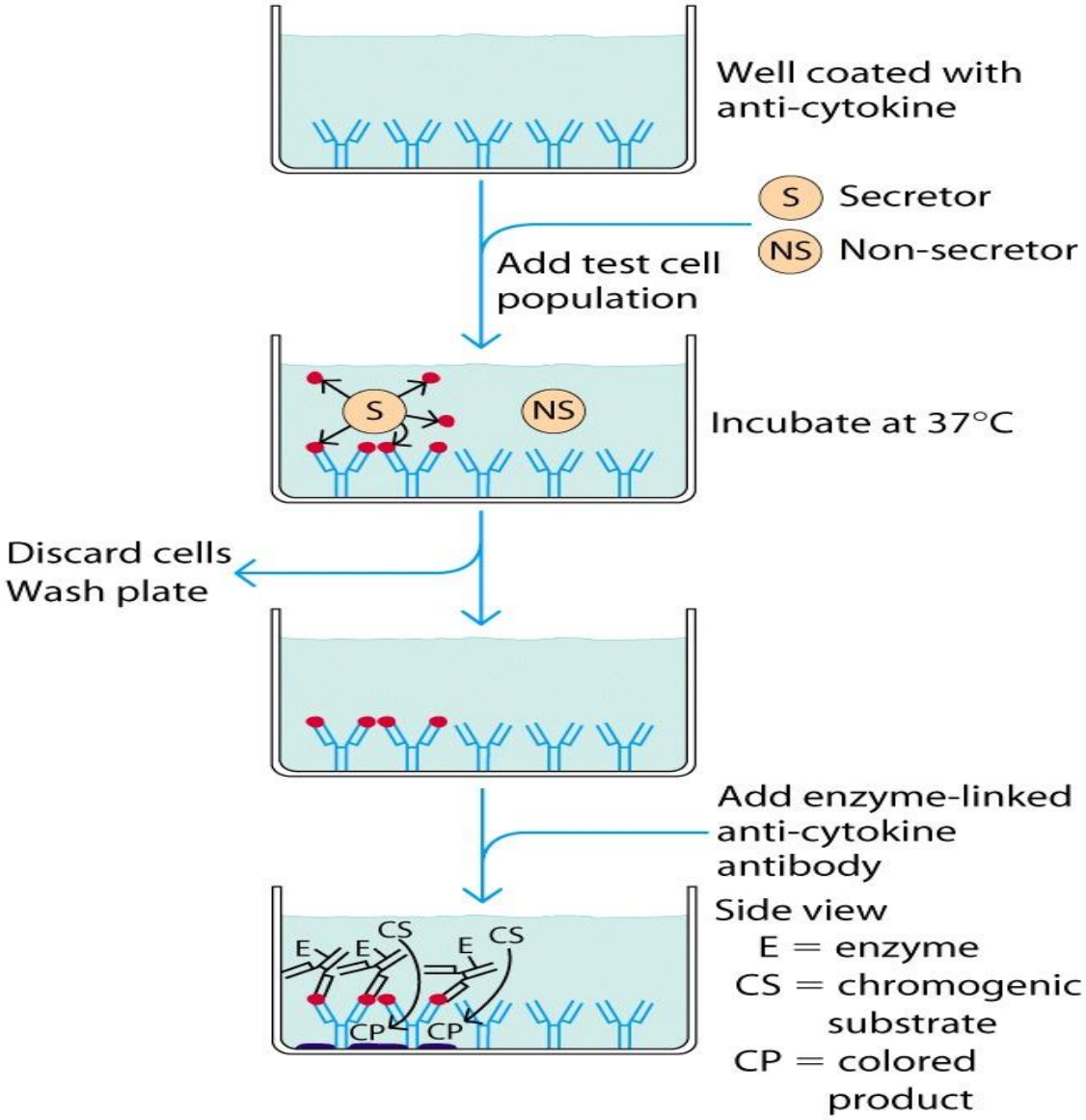
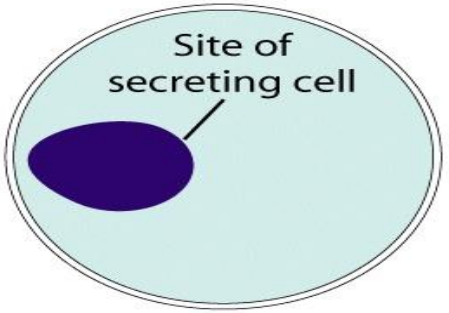
ELISSPOT ASSAY





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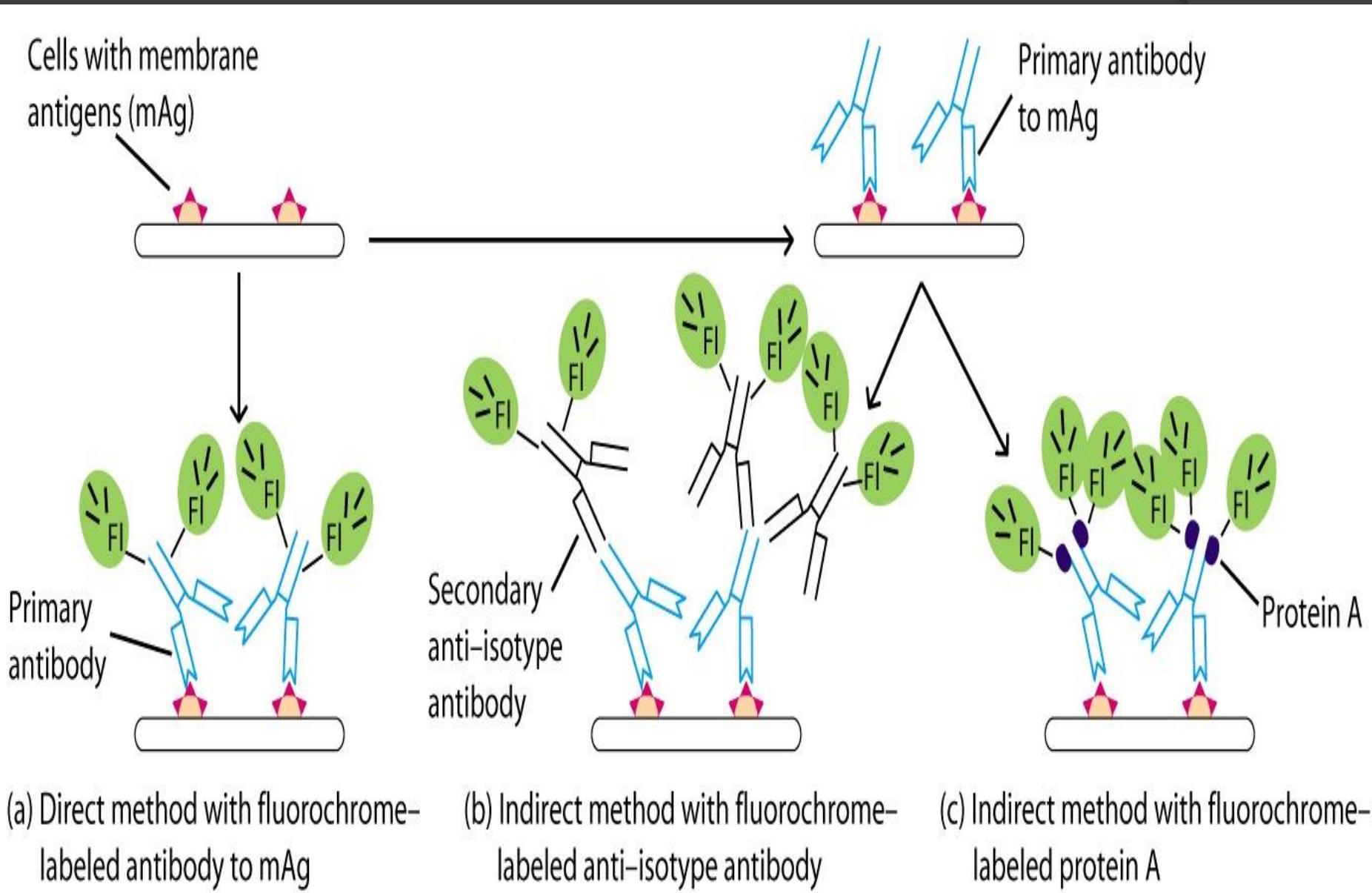

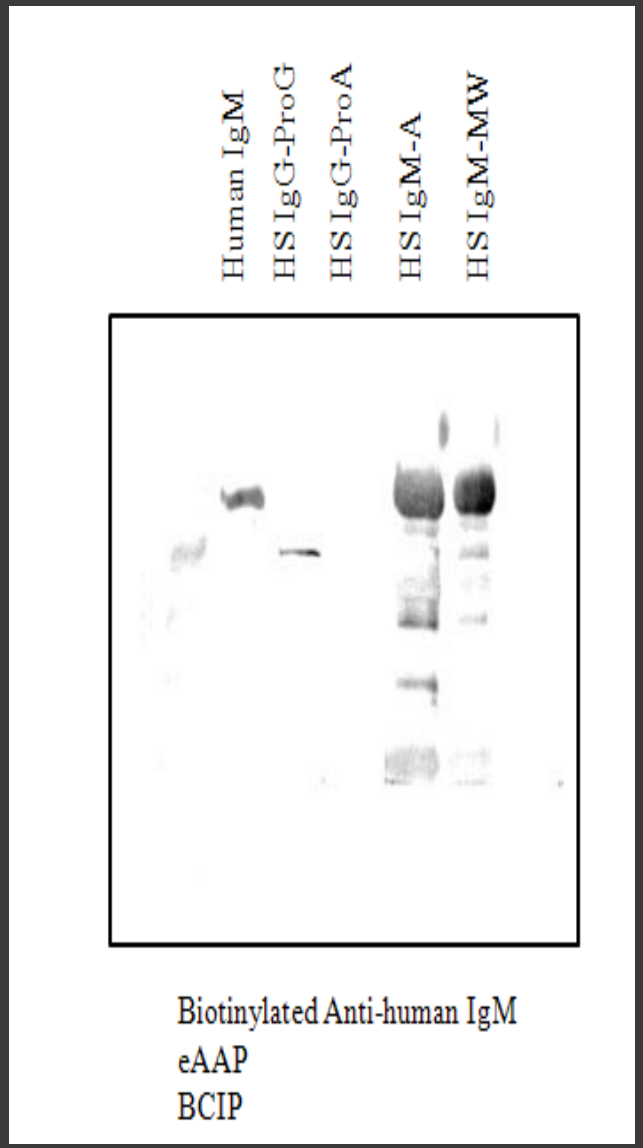

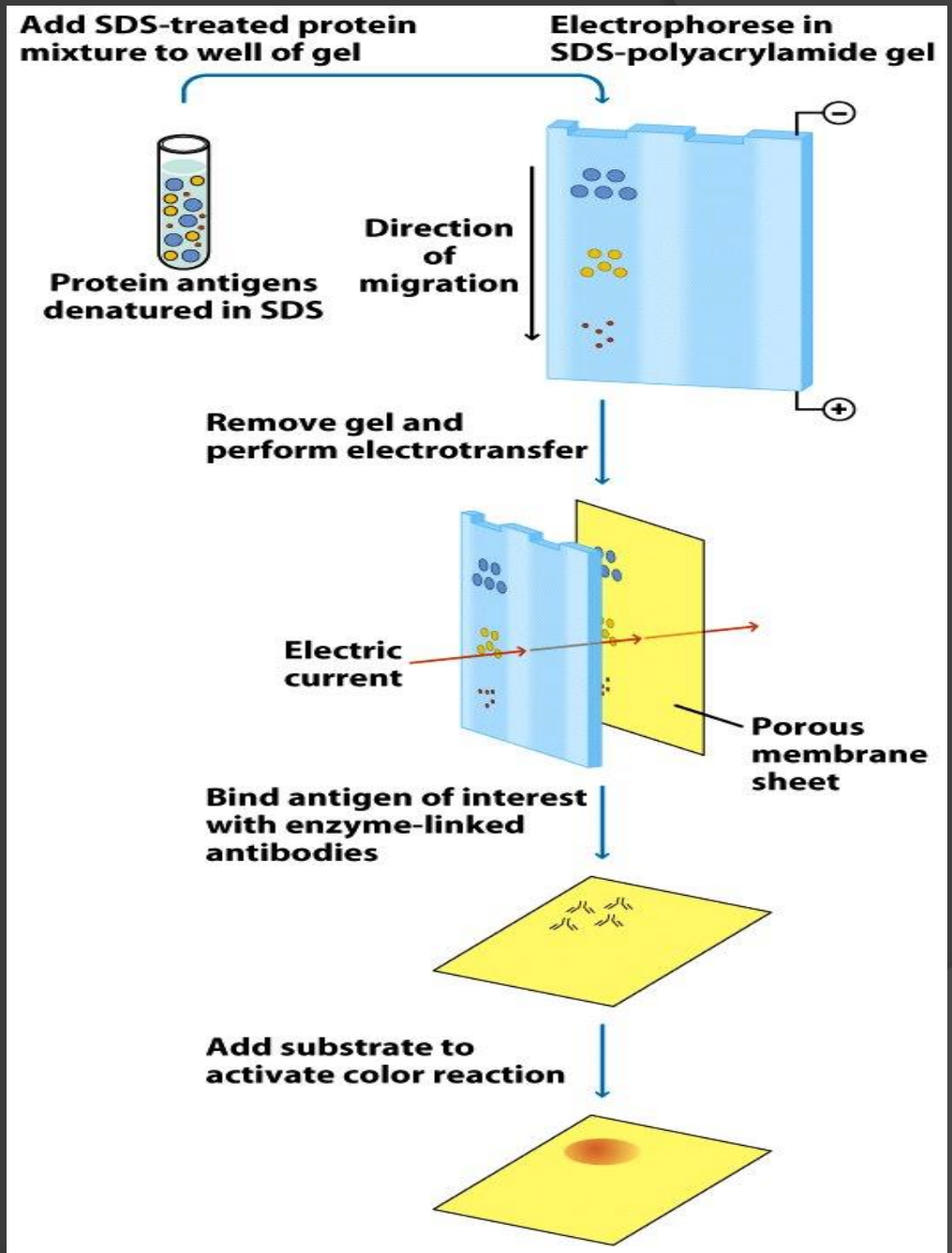

Figure 6-12
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Immunoprecipitation

- Using microscopic magnetic beads
  - Below is cell with magnetic beads attached

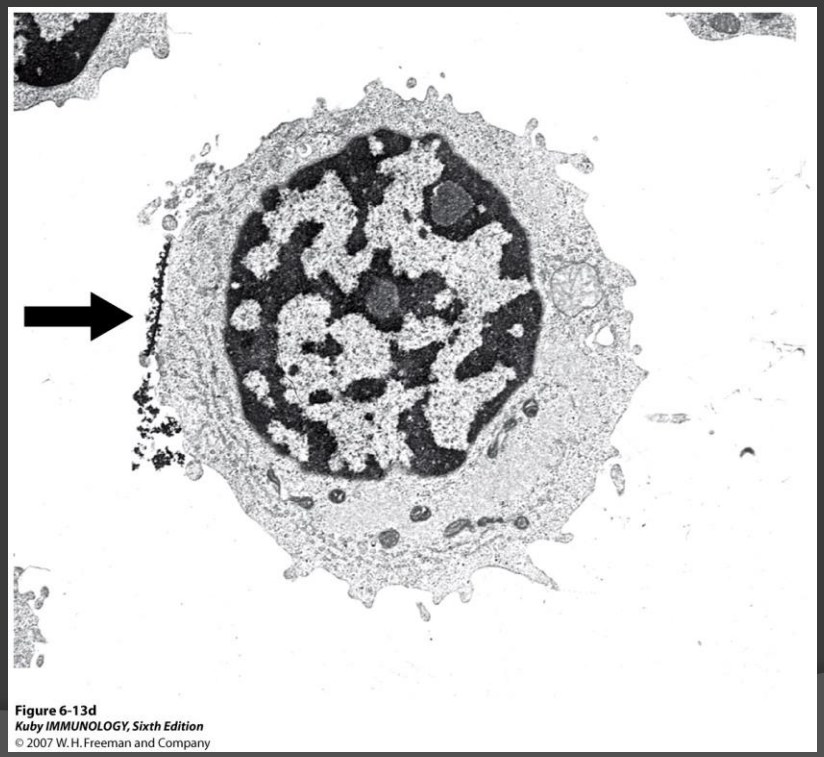

Figure 6-13d
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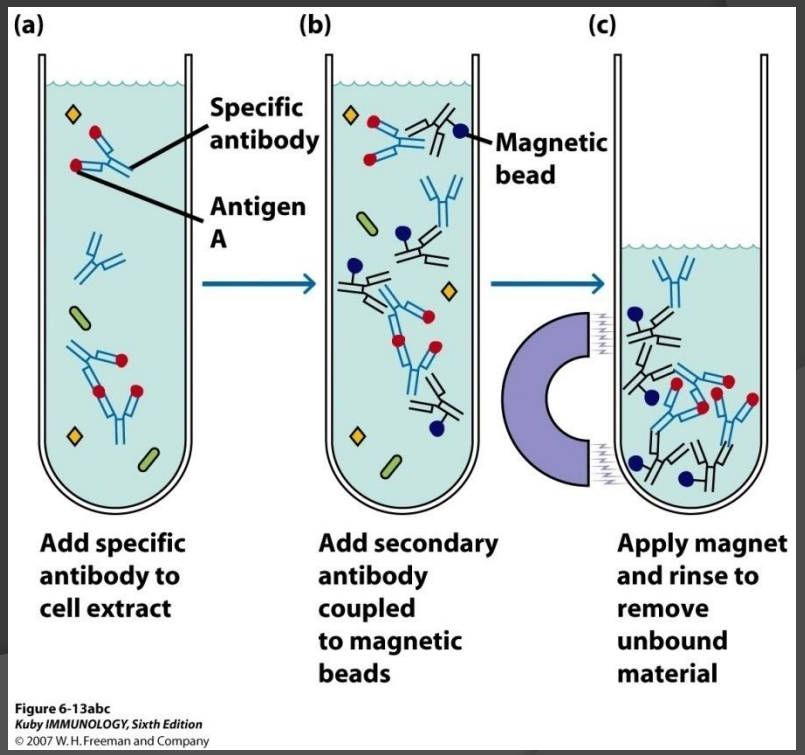

Figure 6-13abc
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Immunofluorescence

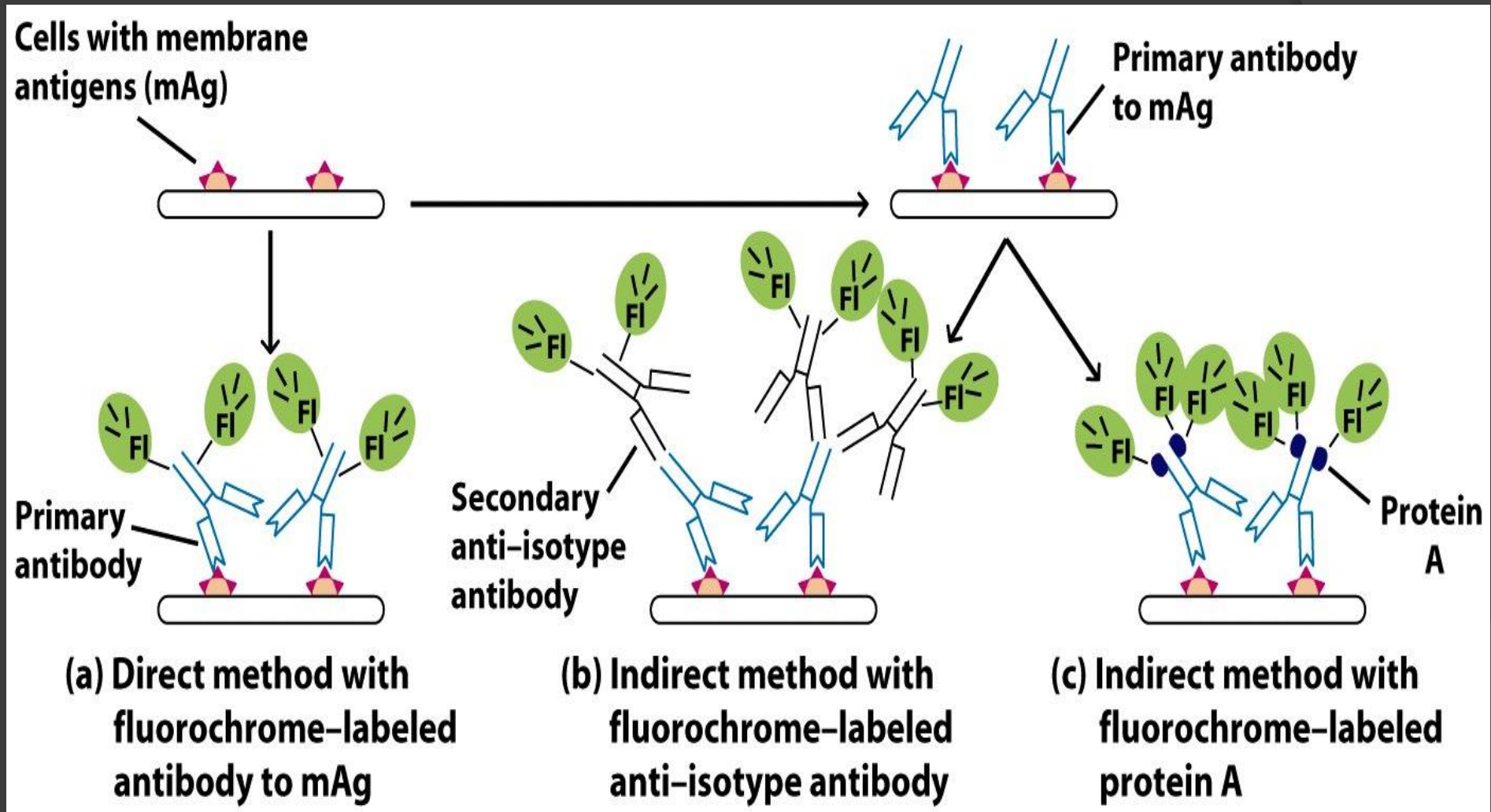

Figure 6-14abc
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Flow Cytometry

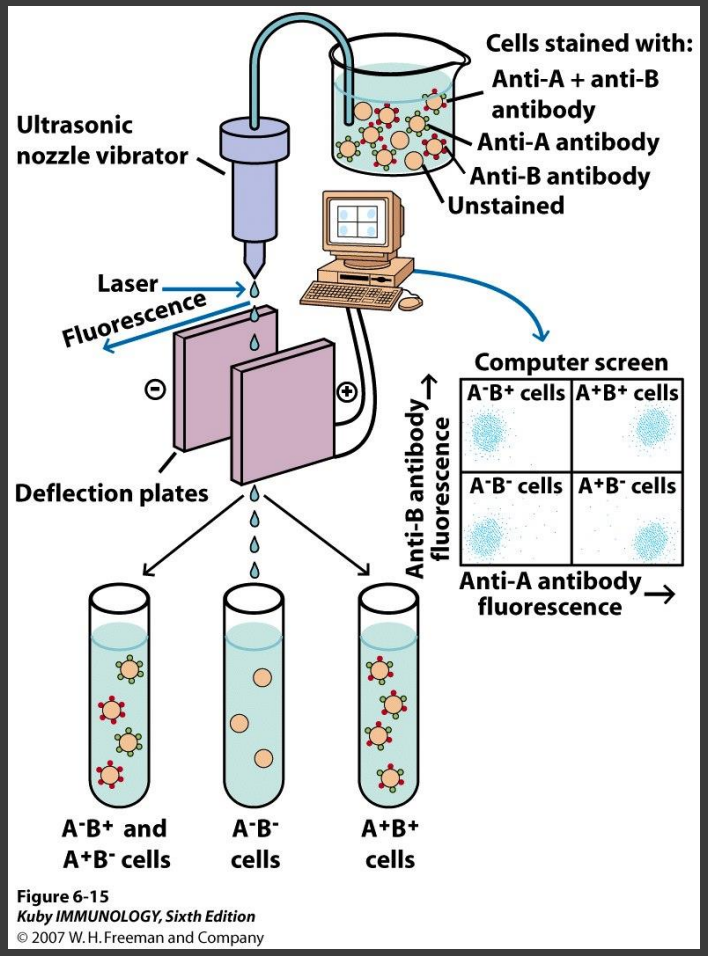

Figure 6-15
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- Can provide quantitative data

Chapter 16

Tolerance and Autoimmunity and Transplants

Dr. Capers

IMMUNOLOGY

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Chapter 16

Tolerance and Autoimmunity

“Horror Autotoxicus”

- Failure of host’s humoral and cellular immune systems to distinguish self from non-self
  - Autoimmunity
  - Can result in tissue and organ damage, can be fatal

TABLE 16-1 Some autoimmune diseases in humans

Disease	Self antigen	Immune response
	ORGAN-SPECIFIC AUTOIMMUNE DISEASES	
Addison's disease	Adrenal cells	Auto-antibodies
Autoimmune hemolytic anemia	RBC membrane proteins	Auto-antibodies
Goodpasture's syndrome	Renal and lung basement membranes	Auto-antibodies
Graves' disease	Thyroid-stimulating hormone receptor	Auto-antibody (stimulating)
Hashimoto's thyroiditis	Thyroid proteins and cells	T_H1 cells, auto-antibodies
Idiopathic thrombocyopenia purpura	Platelet membrane proteins	Auto-antibodies
Insulin-dependent diabetes mellitus	Pancreatic beta cells	T_H1 cells, auto-antibodies
Myasthenia gravis	Acetylcholine receptors	Auto-antibody (blocking)
Myocardial infarction	Heart	Auto-antibodies
Pernicious anemia	Gastric parietal cells; intrinsic factor	Auto-antibody
Poststreptococcal glomerulonephritis	Kidney	Antigen-antibody complexes
Spontaneous infertility	Sperm	Auto-antibodies
	SYSTEMIC AUTOIMMUNE DISEASES	
Ankylosing spondylitis	Vertebrae	Immune complexes
Multiple sclerosis	Brain or white matter	T_H1 cells and T_C cells, auto-antibodies
Rheumatoid arthritis	Connective tissue, IgG	Auto-antibodies, immune complexes
Scleroderma	Nuclei, heart, lungs, gastrointestinal tract, kidney	Auto-antibodies
Sjögren's syndrome	Salivary gland, liver, kidney, thyroid	Auto-antibodies
Systemic lupus erythematosus (SLE)	DNA, nuclear protein, RBC and platelet membranes	Auto-antibodies, immune complexes

Table 16-1
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Tolerance

- # of mechanisms are in place to protect individual from self-reactive lymphocytes
  - Central tolerance – deleting T or B clones before maturity if they have receptors that recognize self-antigens with great affinity
  - Peripheral tolerance – kills lymphocytes in secondary lymphoid tissue
    - Also, life span of lymphocytes regulated by apoptosis

Central tolerance

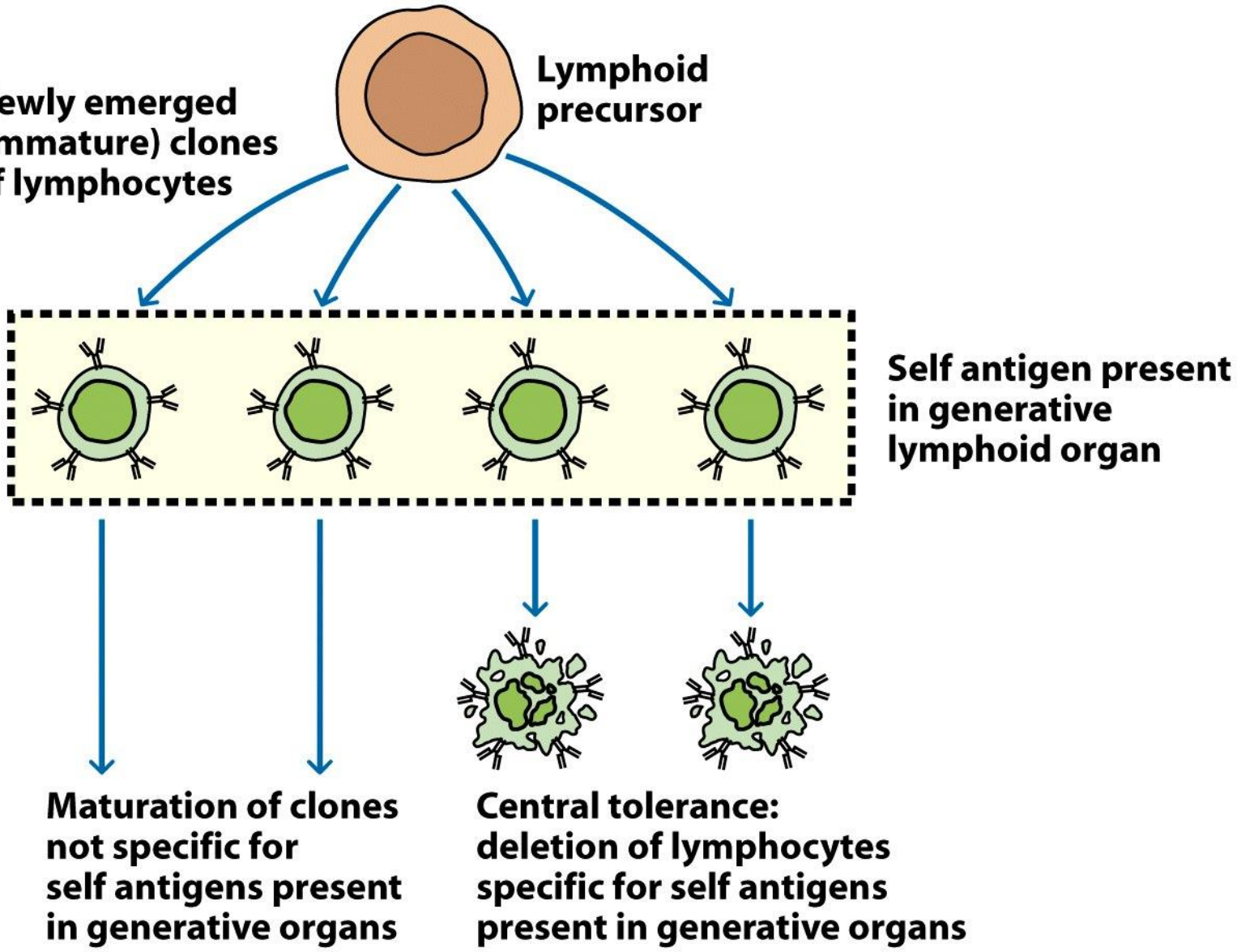

Figure 16-1a
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Peripheral tolerance

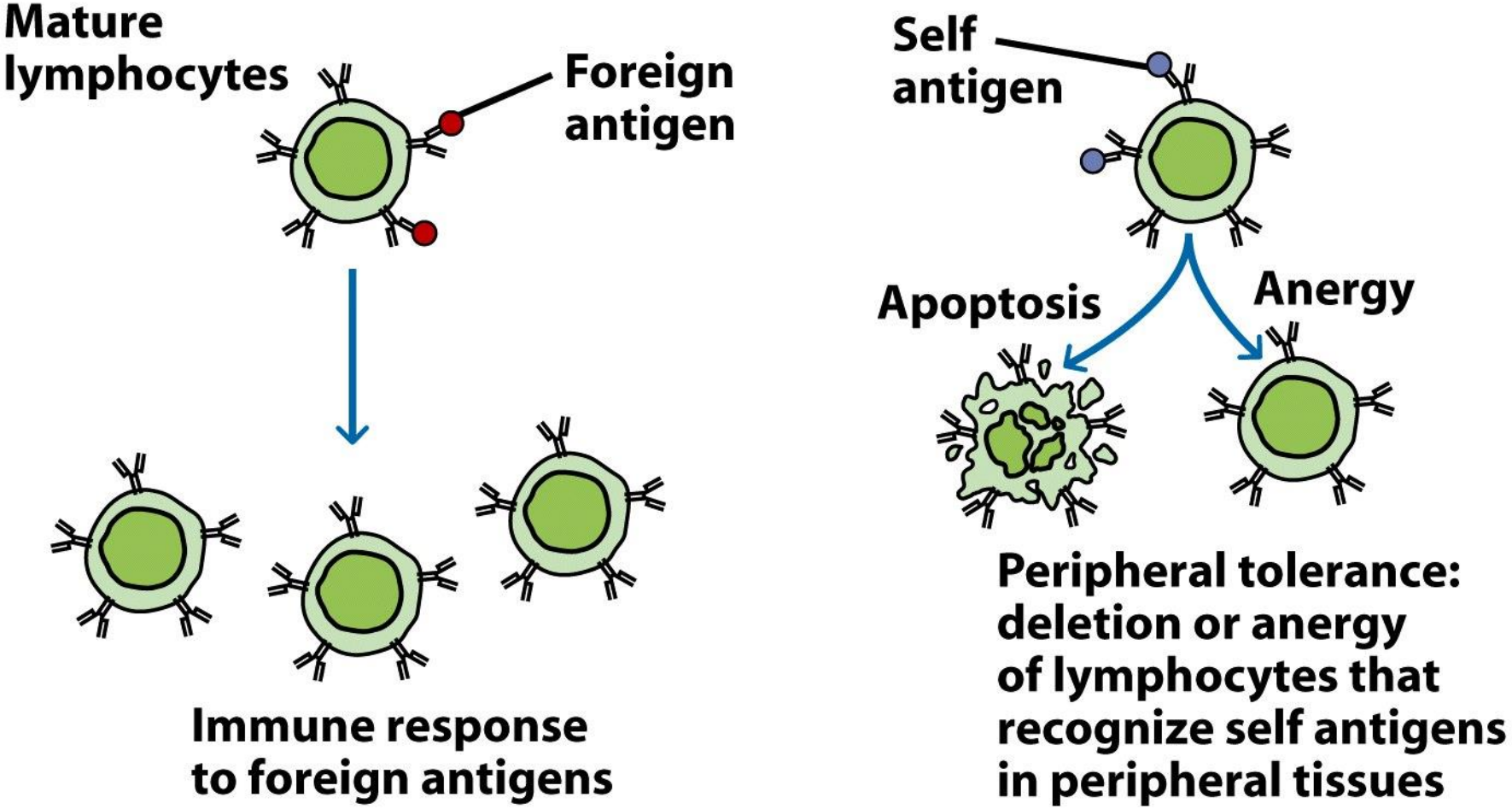

Figure 16-1b
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- Some antigens can produce tolerance
  - Termed tolerogens rather than immunogens
    - High dosages of antigen
    - Persistance of antigen in host
    - IV or oral introduction
    - Absence of adjuvants
    - Low levels of costimulators
      - CD28 will bind to B7 and provide activating signals; however, it was discovered that another receptor, CTLA-4 will bind to B7 and inhibit

- Anergy
  - Unresponsiveness to antigenic stimulus

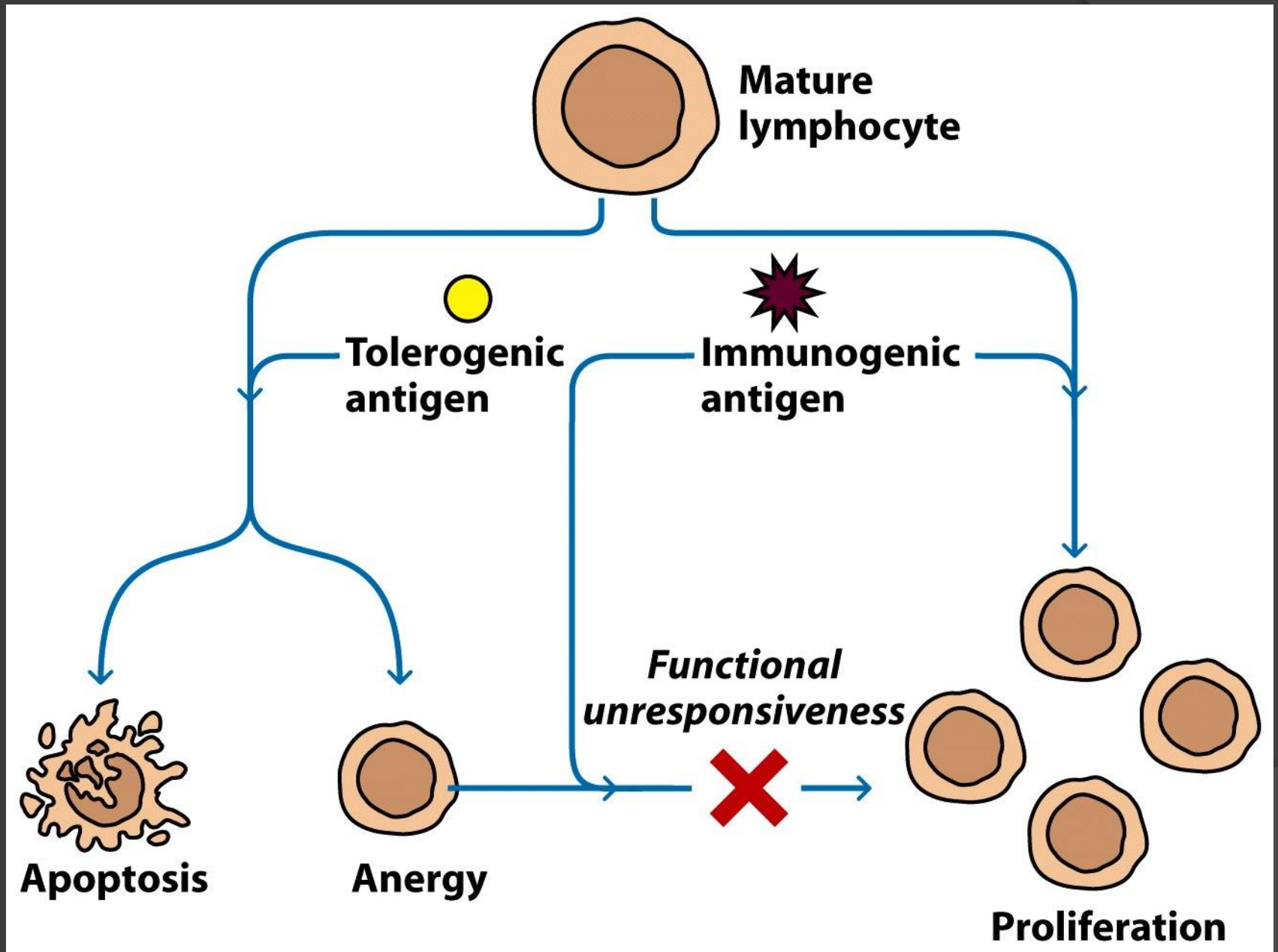

Figure 16-2
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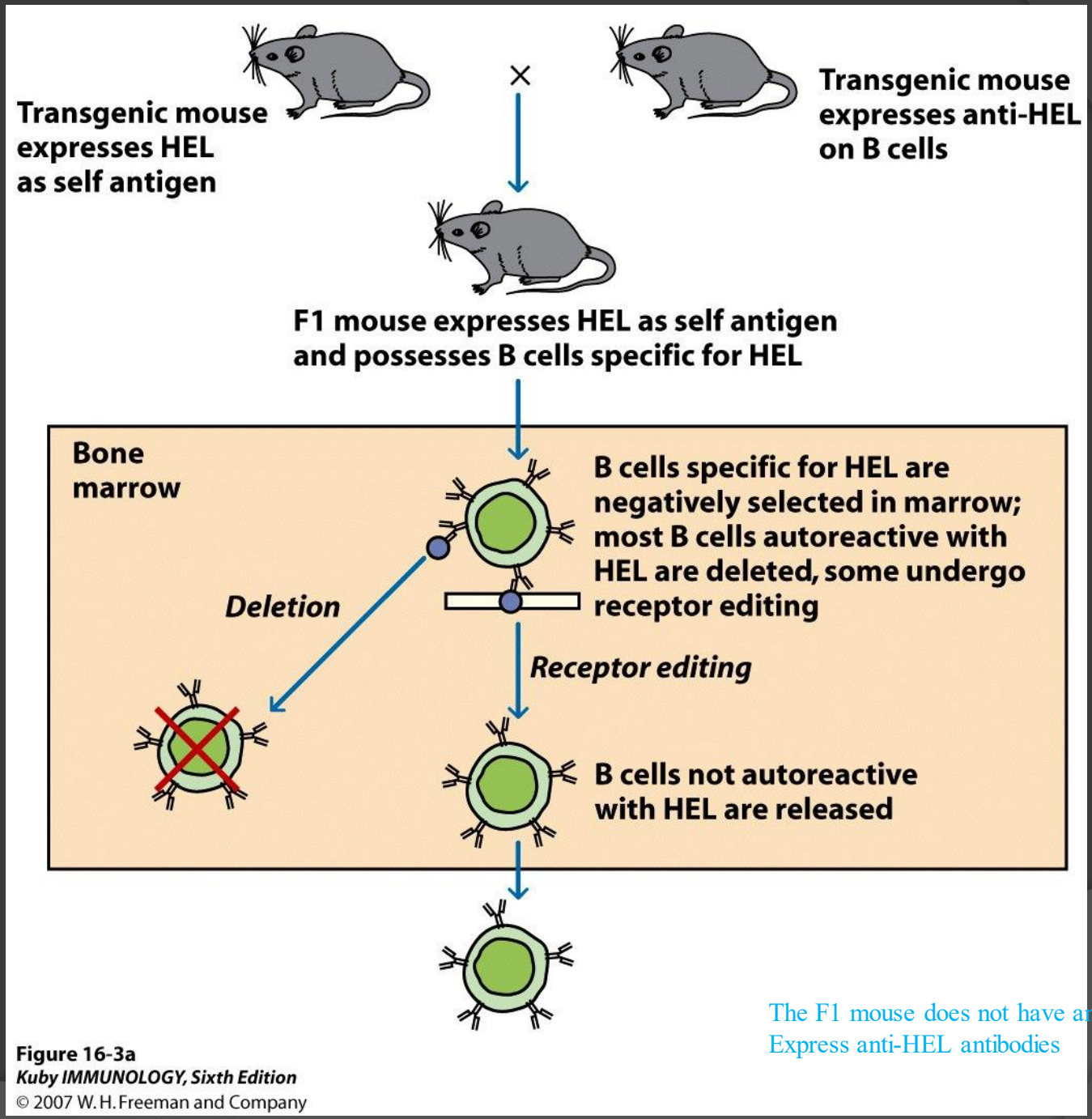

The F1 mouse does not have any B cells that Express anti-HEL antibodies

Figure 16-3a
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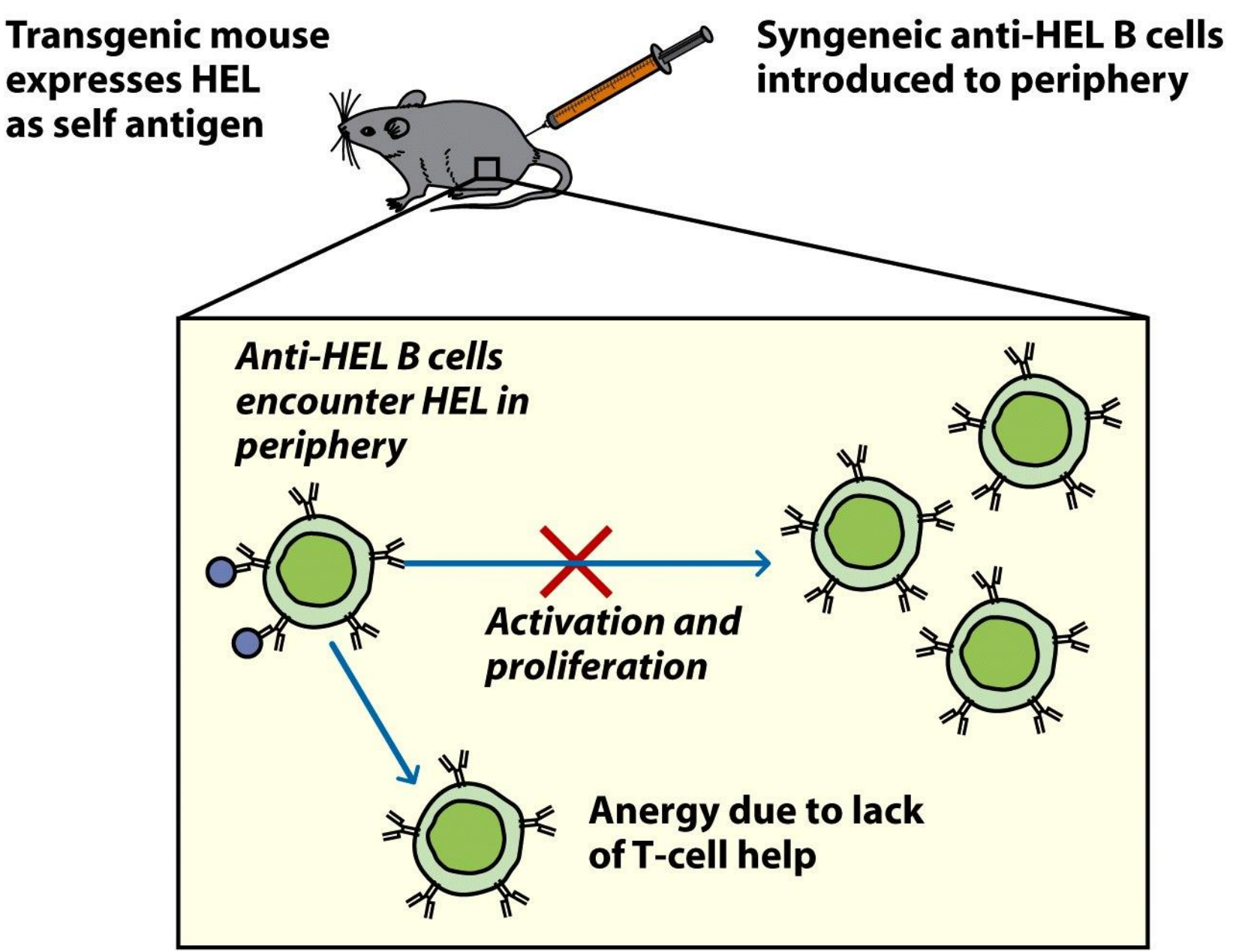

Figure 16-3b
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- Peripheral Tolerance
  - May be induced by T_{reg} cells
    - Unique group of CD4+ T cells
    - Recognize self-antigens on immune system cells and seem to be able to suppress immune system
    - Induce cell death in some immune cells

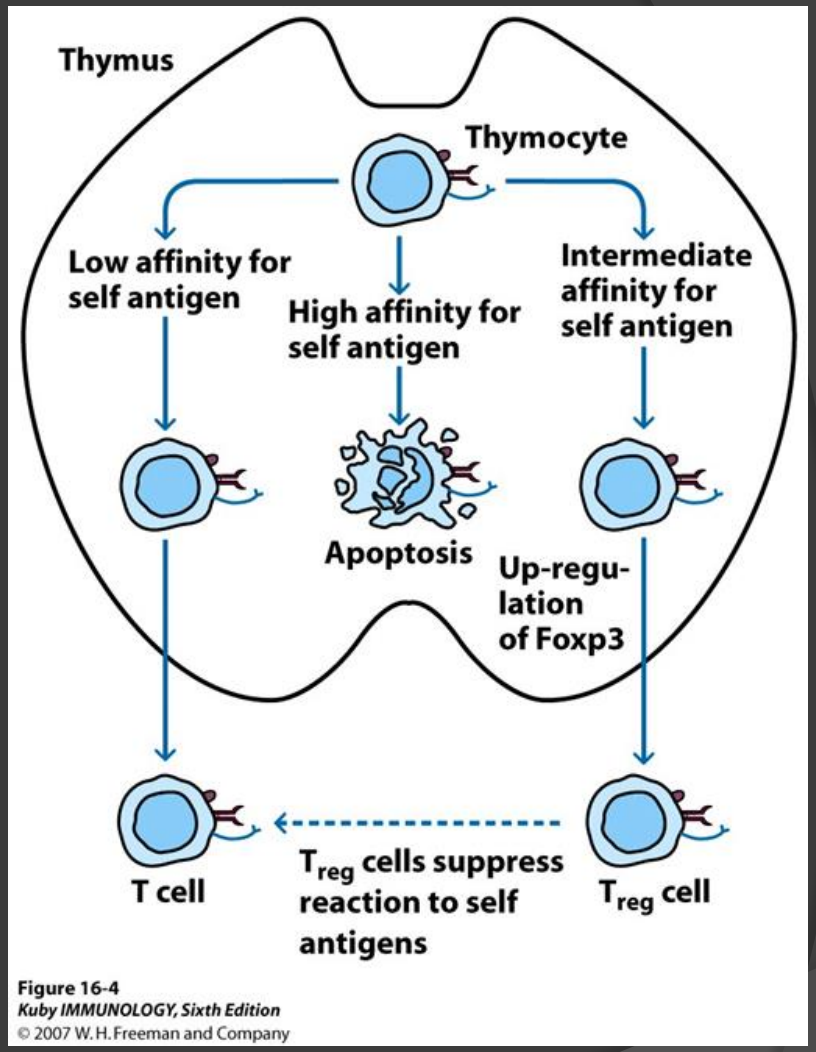

Figure 16-4
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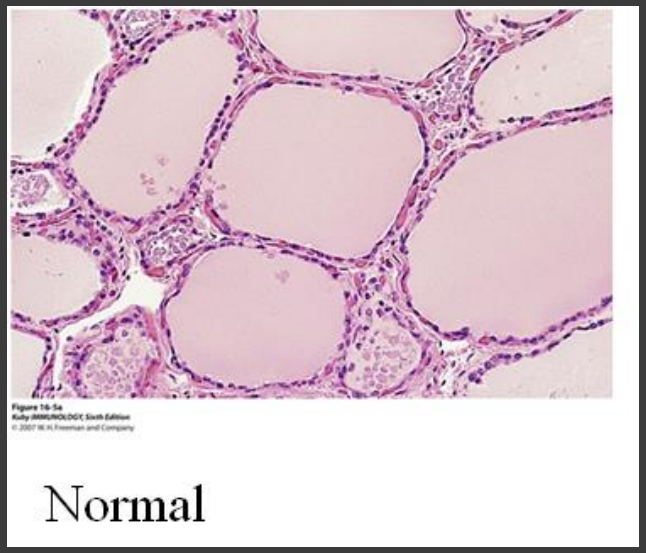
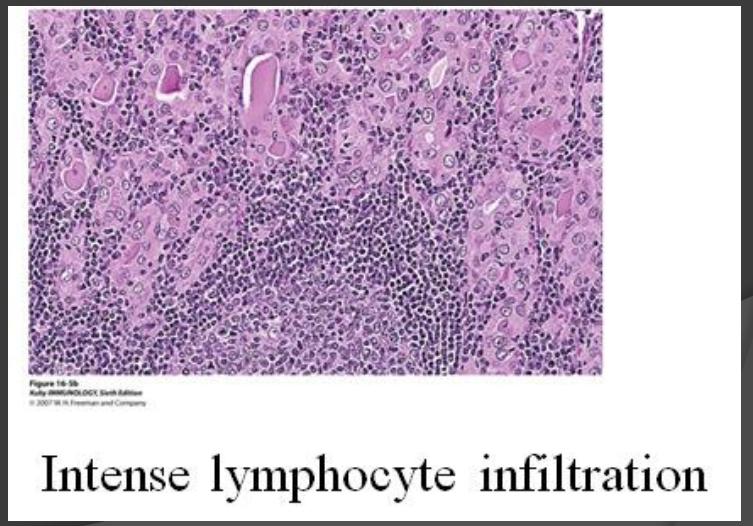
Organ-specific autoimmune diseases

- Target antigen specific to organ or gland
- Cellular lysis and chronic inflammation that can damage organ

- Hashimoto's Thyroiditis
  - Mainly middle-aged women
  - Target is thyroid antigens
  - Goiter can form
  - Hypothyroidism - decrease



Normal



Intense lymphocyte infiltration

- Autoimmune anemias
  - Pernicious anemia
    - Ab against membrane bound intestinal protein that uptakes B_{12} - needed for hematopoiesis
  - Hemolytic anemia
    - Abs to red-blood cell antigens
  - Drug-induced anemia

- Goodpasture's syndrome
  - Abs against basement membranes in glomeruli and aveoli
  - Leads to kidney damage and pulmonary hemmorhage

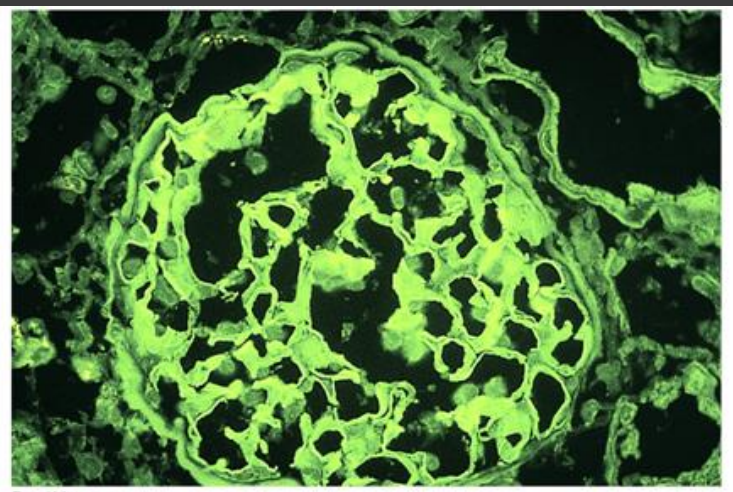


Figure 16-6
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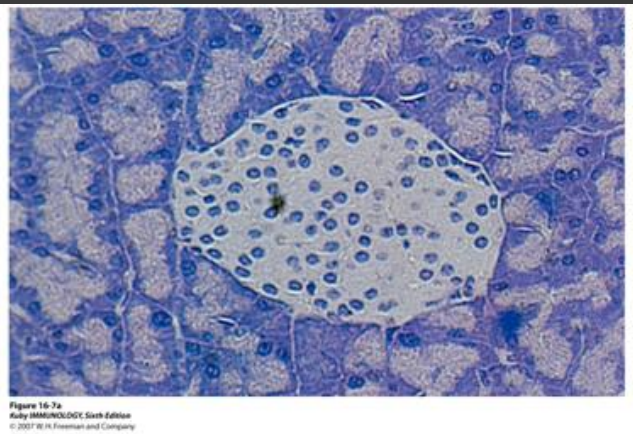
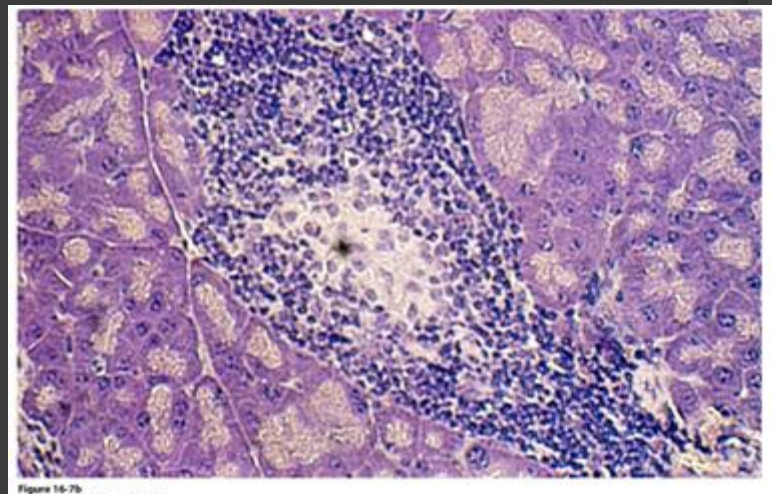
Glomerulus of kidney – fluorescent labeled anti-IgG reveals a large amount of IgG (autoantibodies) attached to glomerulus

- Insulin-Dependent Diabetes Mellitus
  - Abs against beta cells that produce insulin
  - Insulin is needed by cells to uptake glucose needed for cellular respiration



Normal islet with beta cells in pancreas



Islet that is infiltrated by lymphocytes

- In some autoimmune diseases, antibodies act as agonists
  - Bind inappropriately to receptors, resulting in overproduction
    - For example, up-regulating a hormonal response without the presence of that hormone
    - Grave's Disease – auto-Ab binds to receptor for thyroid stimulating hormone resulting in over-stimulation of thyroid
    - Myasthenia gravis
      - Auto-Abs bind acetylcholine receptors on motor end plate of muscles – progressively weakened skeletal muscles

STIMULATING AUTO-ANTIBODIES (Graves' disease)

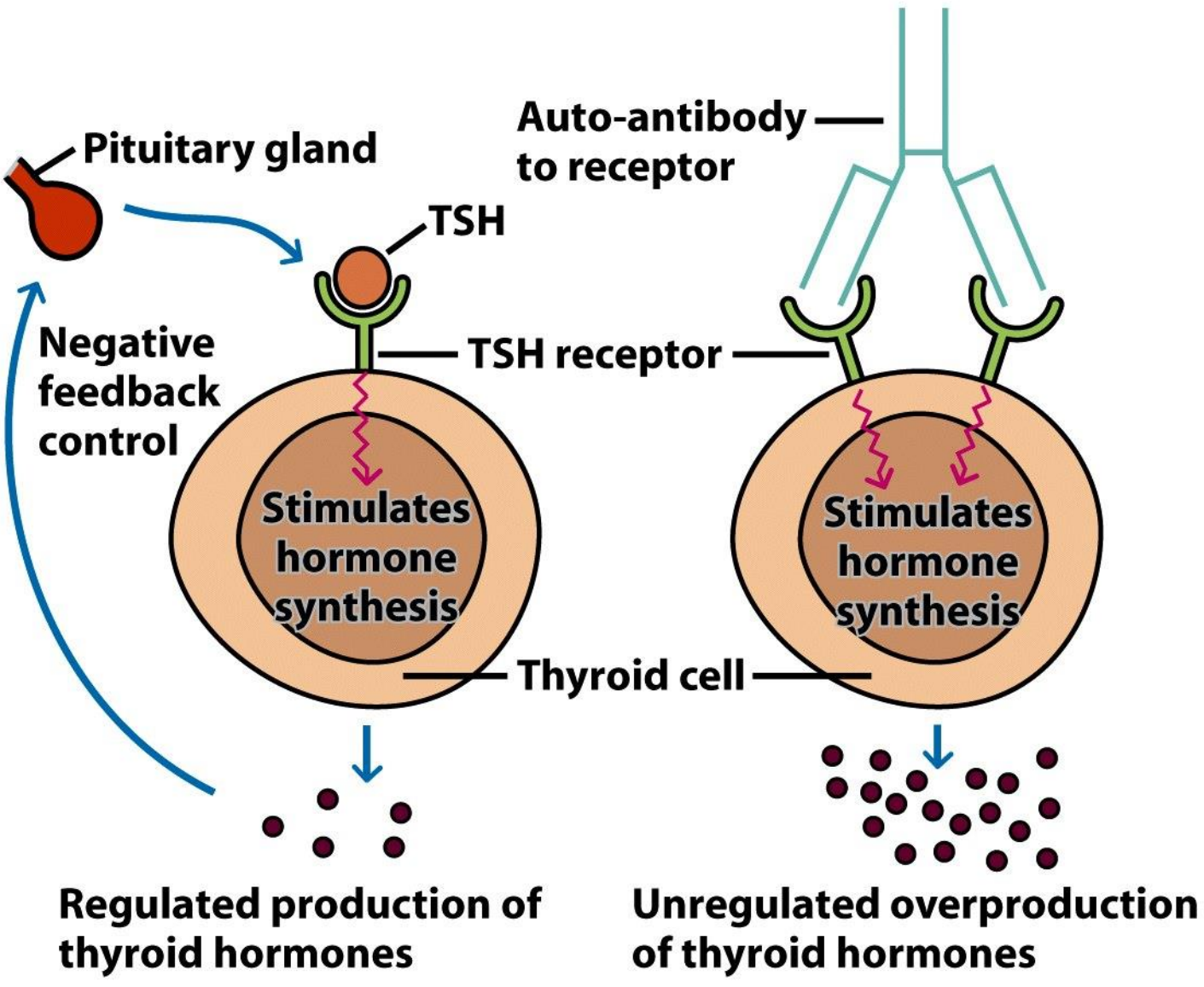


Figure 16-8
Kuby IMMUNOLOGY, Sixth Edition

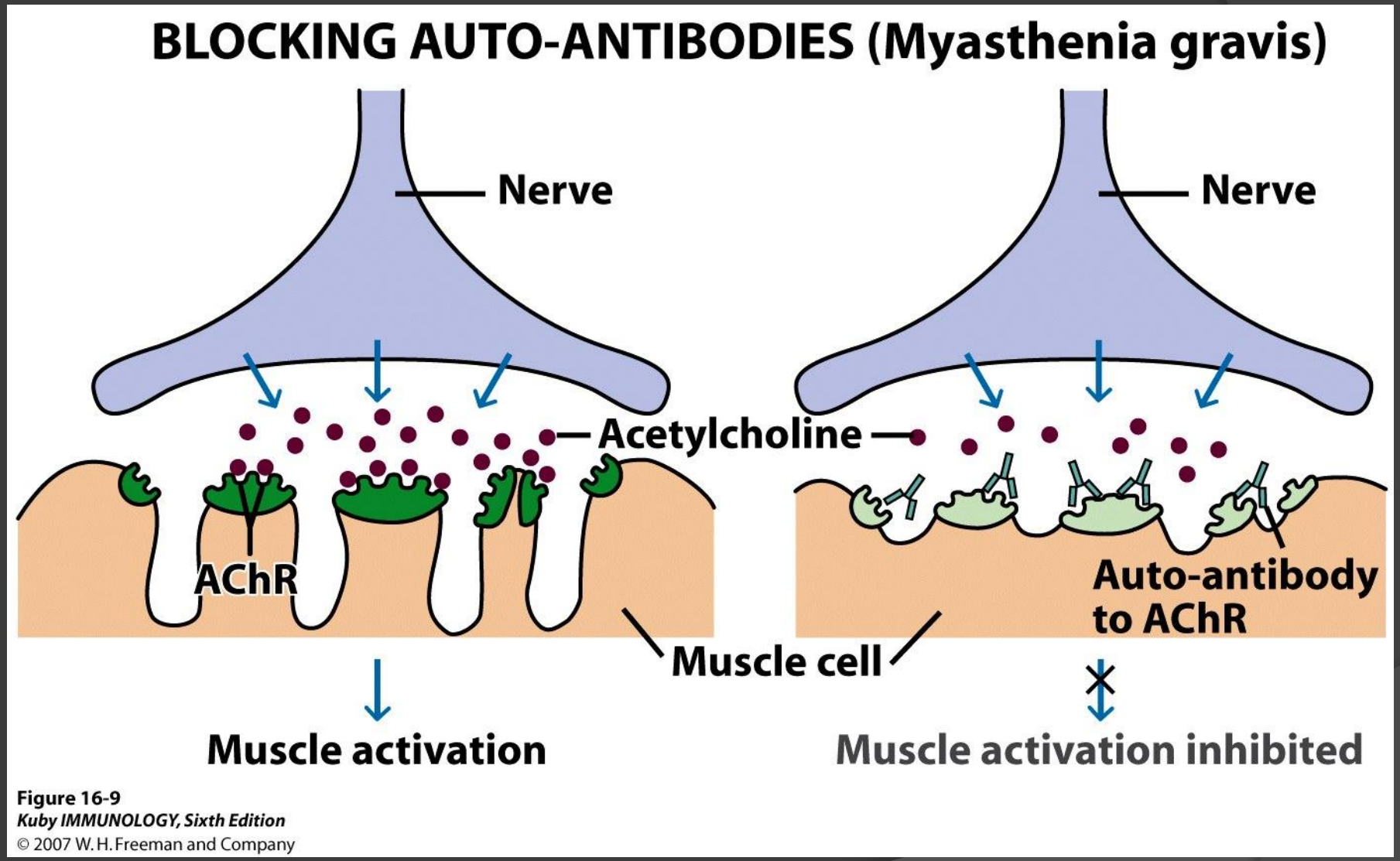


Figure 16-9
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Systemic Autoimmune Diseases

- Response is directed toward wide range of target antigens

- Systemic Lupus Erythematosus
  - Typically middle-aged women
  - Fever, weakness, arthritis, skin rash, kidney problems
  - Produce auto-Abs to DNA, histones, platelets, leukocytes, clotting factors
  - Excessive complement activation

- Multiple sclerosis
  - Numbness, paralysis, vision loss
  - Inflammatory lesions in myelin sheath caused by T cells
  - Epidemiology
    - Frequent in African American and Hispanic women
    - More common in Northern Hemisphere, more common north of 37th parallel
    - Environmental components as well as genetic components

- Rheumatoid Arthritis
  - Chronic inflammation of joints
  - Produce auto-Abs that bind Fc portion of IgG circulating in blood that creates immune complexes

Animal Models

- Autoimmunity develops spontaneously in some lab animals and can be induced with manipulation
  - Rabbits injected with acetylcholine receptors from eels
    - Soon developed muscular weakness as seen with Myasthenia gravis

TABLE 16-2 Experimental animal models of autoimmune diseases

Animal model	Possible human disease counterpart	Inducing antigen	Disease transferred by T cells
SPONTANEOUS AUTOIMMUNE DISEASES			
Nonobese diabetic (NOD) mouse	Insulin-dependent diabetes mellitus (IDDM)	Unknown	Yes
(NZB × NZW) F_1 mouse	Systemic lupus erythematosus (SLE)	Unknown	Yes
Obese-strain chicken	Hashimoto's thyroiditis	Thyroglobulin	Yes
EXPERIMENTALLY INDUCED AUTOIMMUNE DISEASES*			
Experimental autoimmune myasthenia gravis (EAMG)	Myasthenia gravis	Acetylcholine receptor	Yes
Experimental autoimmune encephalomyelitis (EAE)	Multiple sclerosis (MS)	Myelin basic protein (MBP); proteolipid protein (PLP)	Yes
Autoimmune arthritis (AA)	Rheumatoid arthritis	*M. tuberculosis* (proteoglycans)	Yes
Experimental autoimmune thyroiditis (EAT)	Hashimoto's thyroiditis	Thyroglobulin	Yes

*These diseases can be induced by injecting appropriate animals with the indicated antigen in complete Freund's adjuvant. Except for autoimmune arthritis, the antigens used correspond to the self antigens associated with the human disease counterpart. Rheumatoid arthritis involves reaction to proteoglycans, which are self antigens associated with connective tissue.

Table 16-2
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- Animal models have implicated CD4+ T cells to be primary mediator of some autoimmune responses
  - Treatment with anti-CD4 antibodies can help

- Some studies have shown association between expressing particular MHC allele and susceptibility to autoimmunity
  - Individuals that express HLA-B27 have 90 times greater chance of having ankylosing spondylitis (spine inflammation)
    - Interestingly, most of those are male even though women are more likely to suffer from autoimmune disease

- Proposed mechanisms for induction of autoimmunity
  - Release of sequestered antigens
    - Blood-brain barrier, sperm released into tissues during vasectomy
  - Molecular mimicry
  - Inappropriate expression of Class II MHC
    - Non-antigen presenting cells will for some reason express Class II MHC
      - Can be caused by viral infection
    - This allows them to present self antigen to T helper cells – leads to inappropriate reaction

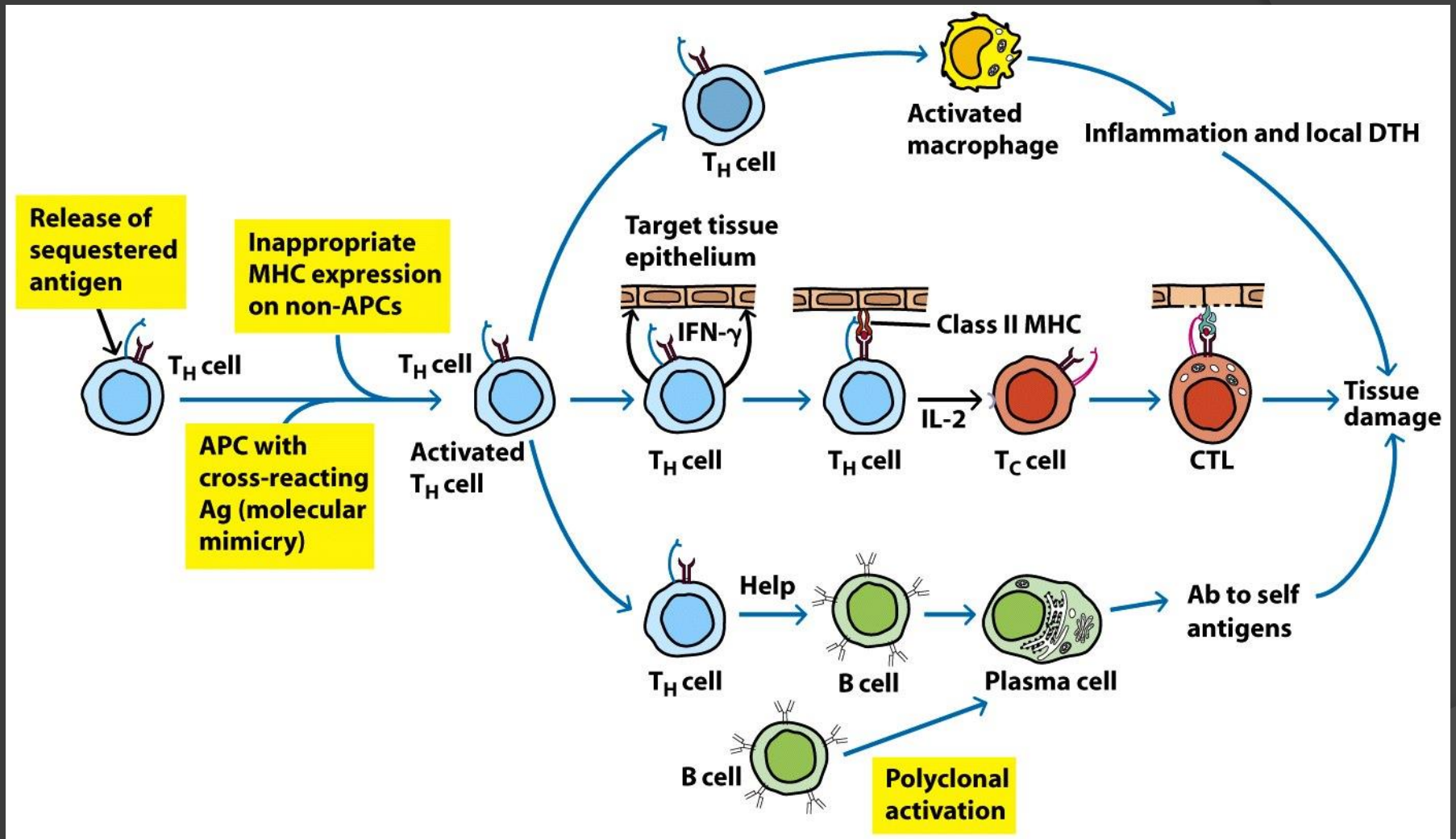

Figure 16-12
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TABLE 16-3 Molecular mimicry between proteins of infectious organisms and human host proteins

Protein*	Sequence†
Human cytomegalovirus IE2	79 P D P L G R P D E D
HLA-DR molecule	60 V T E L G R P D A E
Poliovirus VP2	70 S T T K E S R G T T
Acetylcholine receptor	176 T V I K E S R G T K
Papilloma virus E2	76 S L H L E S L K D S
Insulin receptor	66 V Y G L E S L K D L
Rabies virus glycoprotein	147 T K E S L V I I S
Insulin receptor	764 N K E S L V I S E
Klebsiella pneumoniae nitrogenase	186 S R Q T D R E D E
HLA-B27 molecule	70 K A Q T D R E D L
Adenovirus 12 E1B	384 L R R G M F R P S Q C N
α-Gliadin	206 L G Q G S F R P S Q Q N
Human immunodeficiency virus p24	160 G V E T T T P S
Human IgG constant region	466 G V E T T T P S
Measles virus P3	13 L E C I R A L K
Corticotropin	18 L E C I R A C K
Measles virus P3	31 E I S D N L G Q E
Myelin basic protein	61 E I S F K L G Q E

*In each pair, the human protein is listed second. The proteins in each pair have been shown to exhibit immunologic cross-reactivity.

†Amino acids are indicated by a single-letter code. Identical residues are shown in blue. Numbers indicate amino acid position in the intact protein.

SOURCE: Adapted from M. B. A. Oldstone, 1987, *Cell* **50**:819.

Table 16-3
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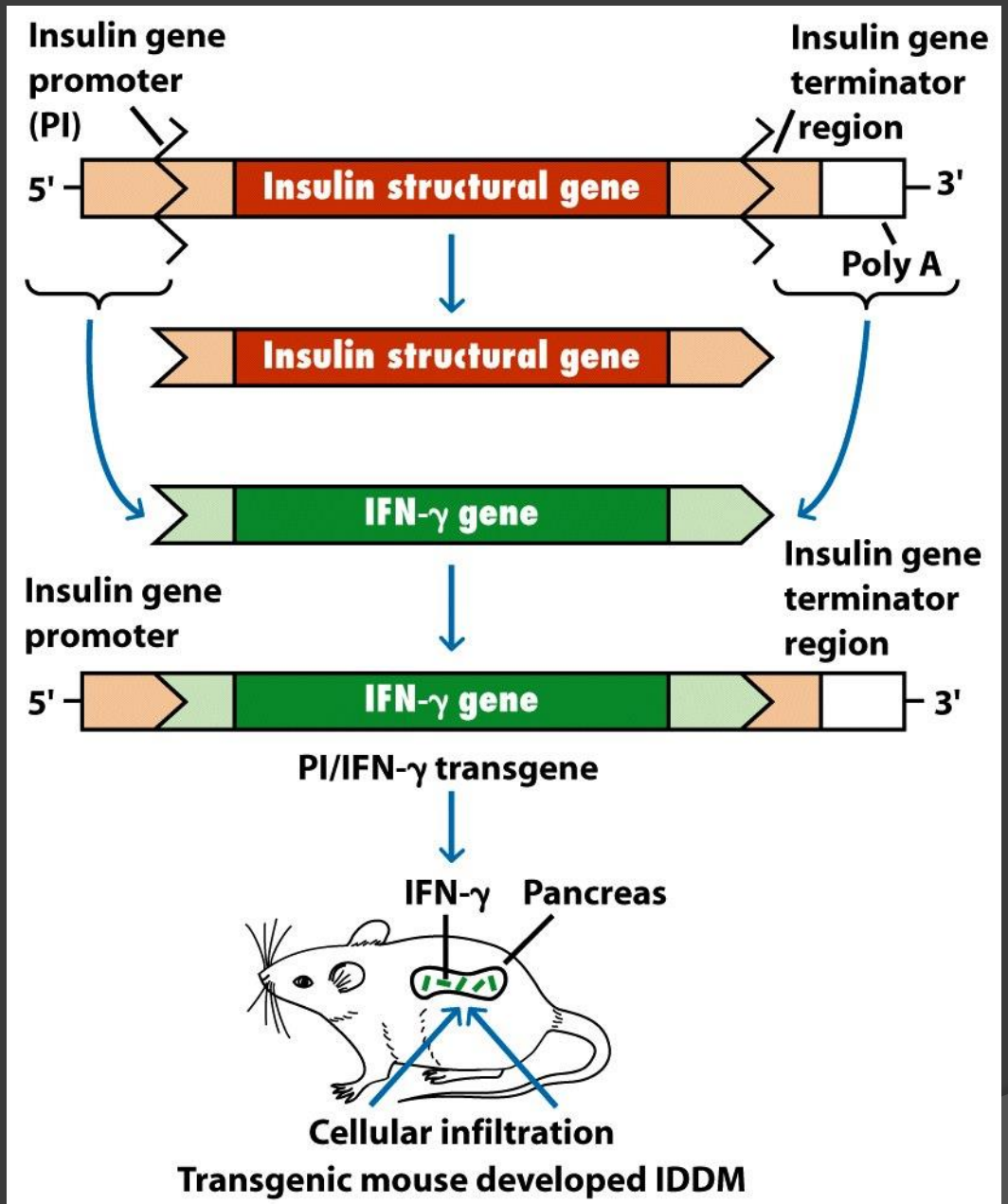

Figure 16-13a
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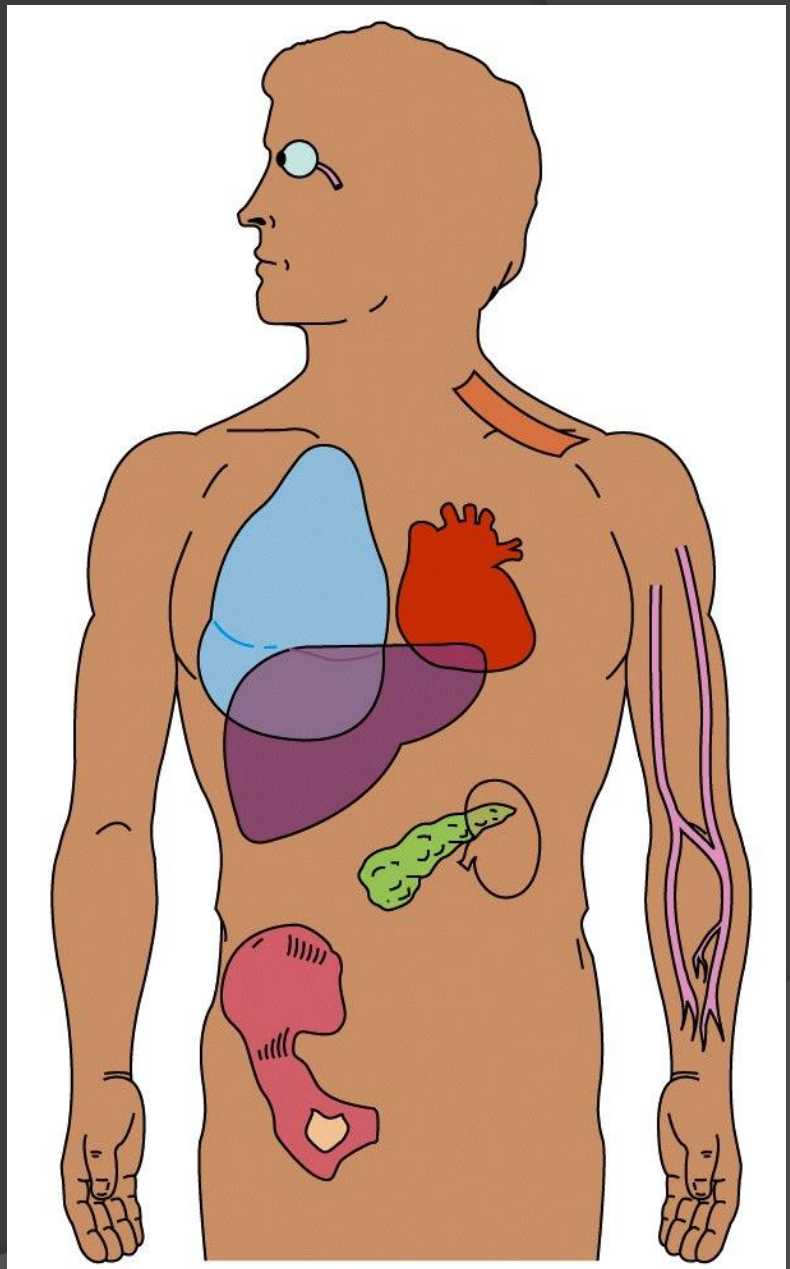
Treatment

- Immunosuppressive drugs
- Removal of thymus (for example, with myasthenia gravis)
- Plasmapheresis – removing plasma and then returning RBCs (removes extra immune complexes)
- Treating the inflammation
- Antigen given orally can induce tolerance

- Transplantation
  - Transfer of cells, tissues, or organs
- 1st human kidney transplant
  - 1935
  - Patient died to mistake in blood typing



Chapter 17 Opener
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- Immunosuppressive Agents
  - Delay or prevent rejection
  - Majority of these have overall immunosuppressive effect
  - New methods being developed
    - Inducing specific tolerance to graft without suppressing other immune responses

Different types of Transplants

- Autograft
  - Self tissue transferred from one part of body to another
- Isograft
  - Tissue transferred between genetically identical individuals
- Allograft
  - Tissue transferred between genetically different members of same species
    - Most of our transplants
- Xenograft
  - Tissue transferred between different species

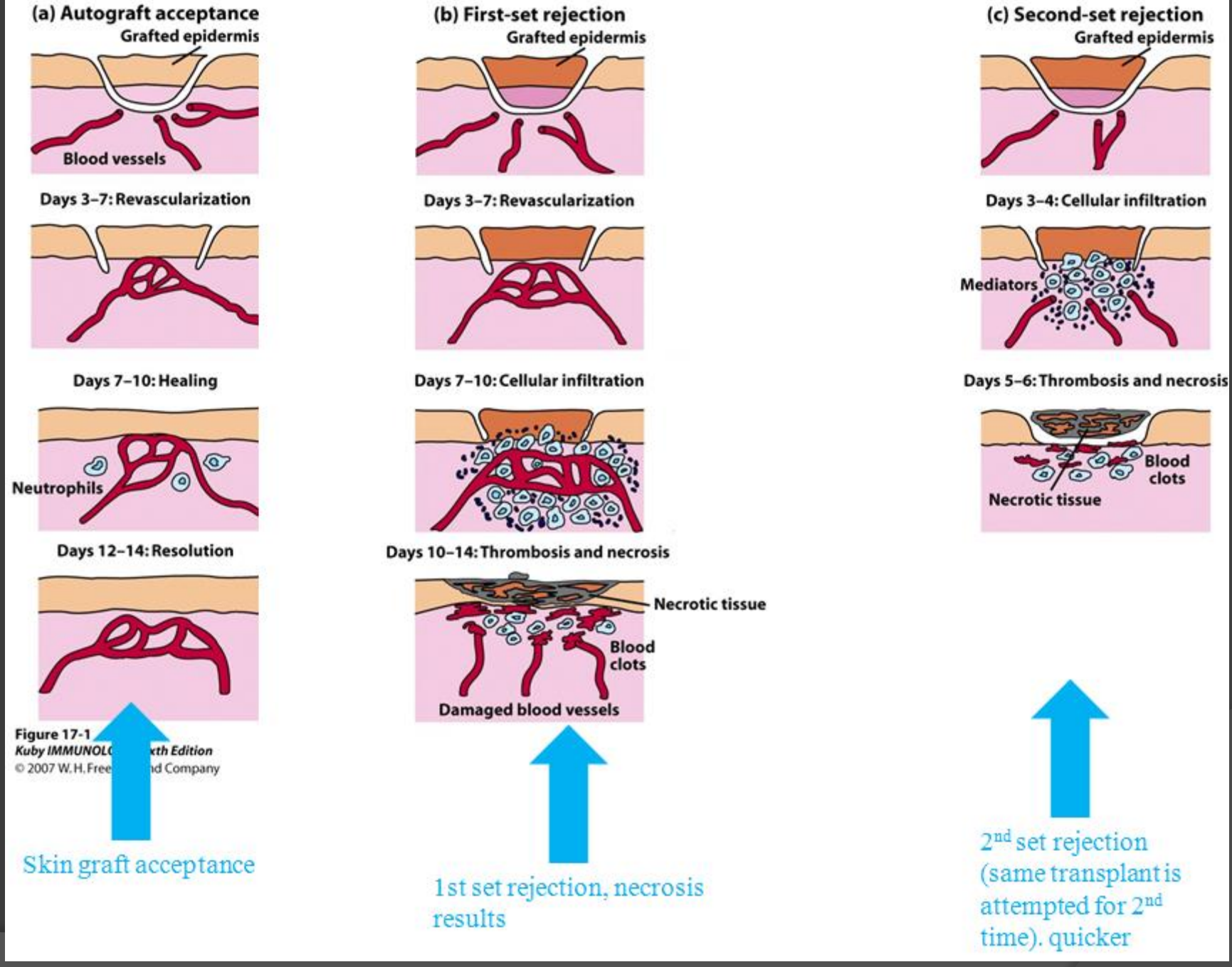

Figure 17-1
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Skin graft acceptance

1st set rejection, necrosis results

2nd set rejection (same transplant is attempted for 2nd time). quicker

- T cells play key role in allograft rejection
  - Both CD4+ and CD8+ populations present

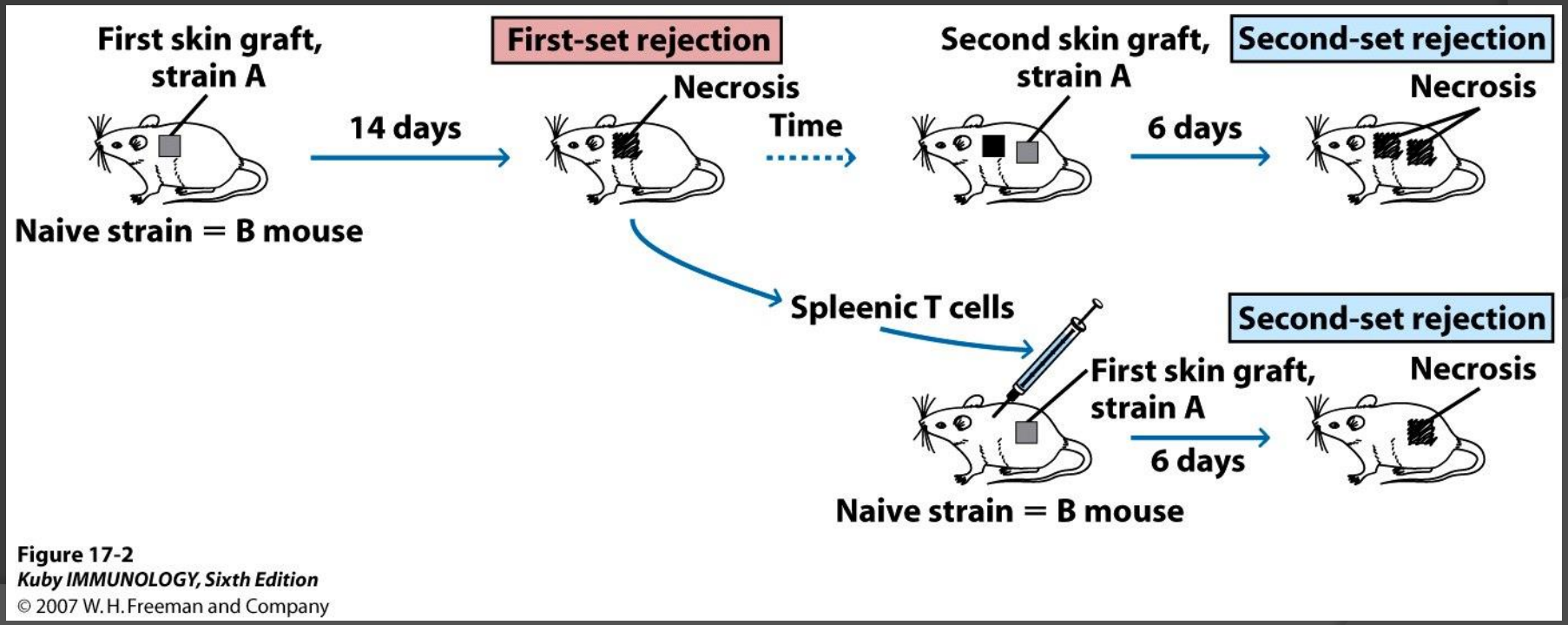

Figure 17-2
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- Tissues that are antigenically similar – *histocompatible*
- Loci most responsible for the most vigorous allograft rejection are within MHC complex
  - Test donors to get matching haplotype
    - Mismatches with Class II are more likely to lead to rejection than mismatches with Class I
  - Also test for blood type

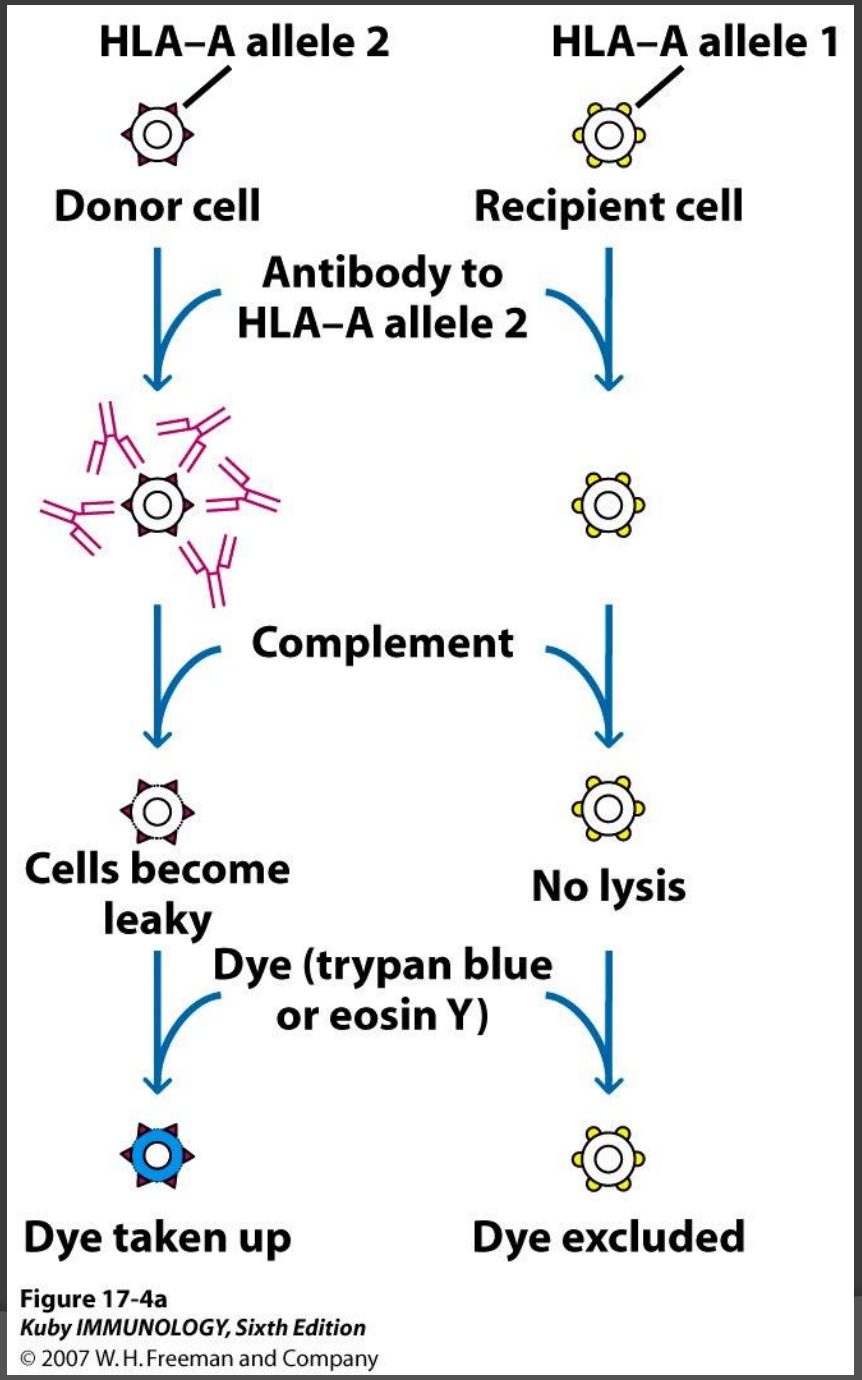

Figure 17-4a
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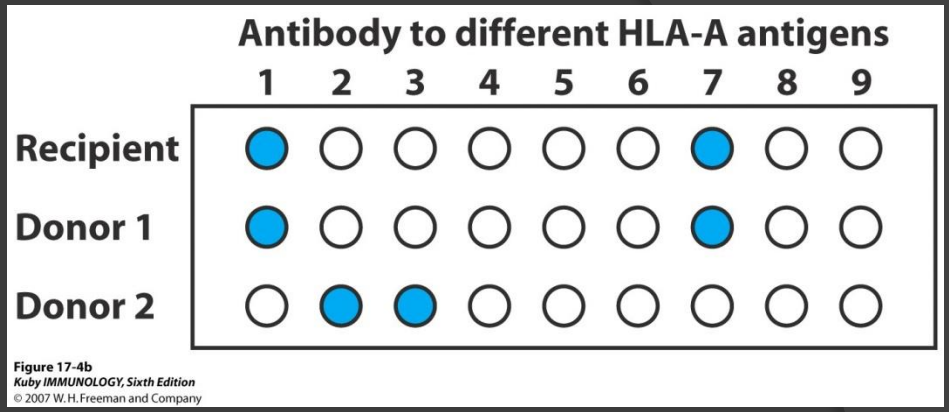

Figure 17-4b
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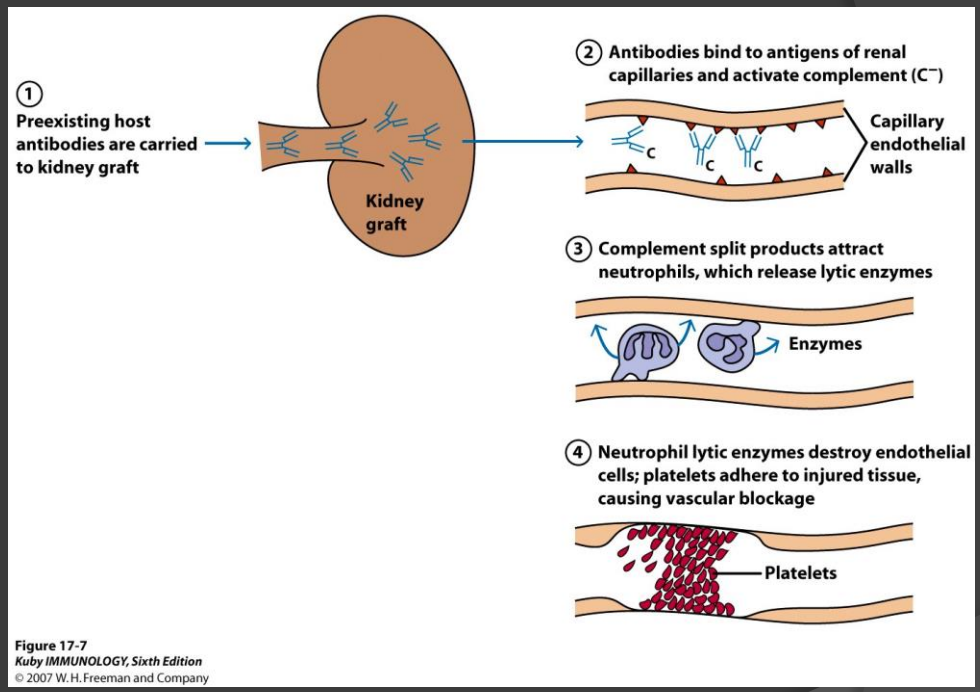
- Microcytoxicity assay for MHC haplotypes
- If antigen is present on cell, complement will lyse it, and it will uptake dye (blue)
- Donor 1 has antigens in common with recepient

Clinical Manifestations of Graft Rejections

- Hyperacute
  - Within hours
- Acute
  - Within weeks
- Chronic
  - Months to years

Clinical Manifestations of Graft Rejection

- Hyperacute
  - Pre-existing recipient antibodies
  - Graft never become vascularized



Immunosuppressive Therapy

- Mitotic inhibitors
  - i.e. Azathioprine
  - Help lower T cell proliferation
- Methotrexate
  - Folic acid antagonist – blocks purine synthesis
- Corticosteroids
  - Reduces inflammation
- X-irradiation of recipient before grafting
- Antibodies specific for immune cells to keep them at lower numbers

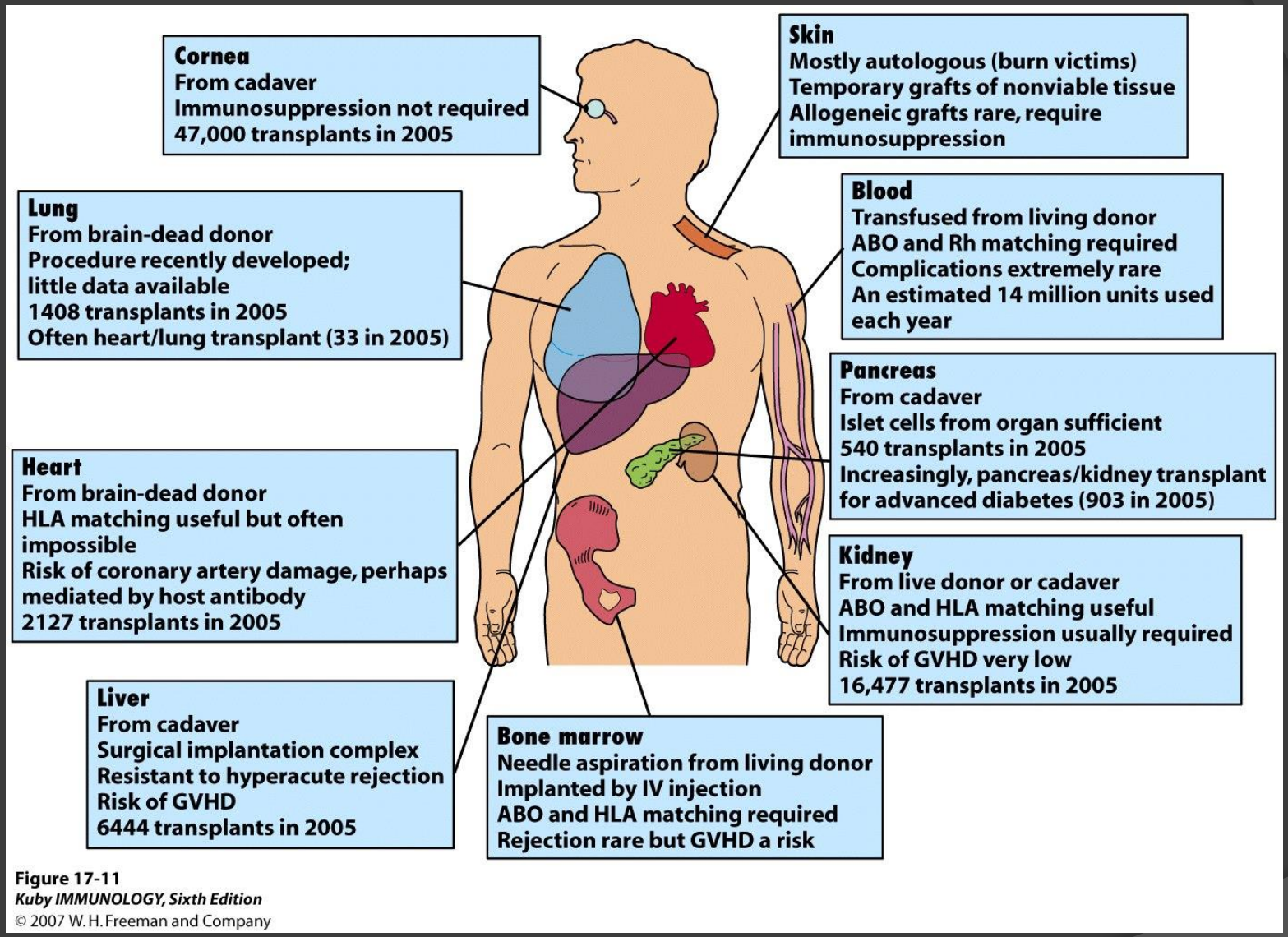


Figure 17-11
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GVHD – Graft versus Host Disease (donor T cells start reacting with host

- Xenotransplantation
  - Shortage of human donors
  - Obstacles with immune system
  - Closely related species have more success
    - However, taking risk of creating new viruses by recombination in graft

Chapter 11

B-Cell Generation, Activation, and Differentiation

Dr. Capers

IMMUNOLOGY

Kindt • Goldsby • Osborne

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Sixth Edition

Chapter 11

B-Cell Generation, Activation, and Differentiation

Development of B cells

- In many vertebrates, including humans and mice, B cells generate in bone marrow
  - Antigen-independent phase
    - Ig-gene rearrangement to create antigen-specificity
- Immature B cell bearing IgM on membrane leaves bone marrow
  - Matures to express both IgM and IgD with single antigen specificity
  - NAÏVE B cells – have not encountered antigen
- Encounter antigen in secondary lymphoid tissue
  - Differentiate into plasma cells and memory cells
  - Class switching

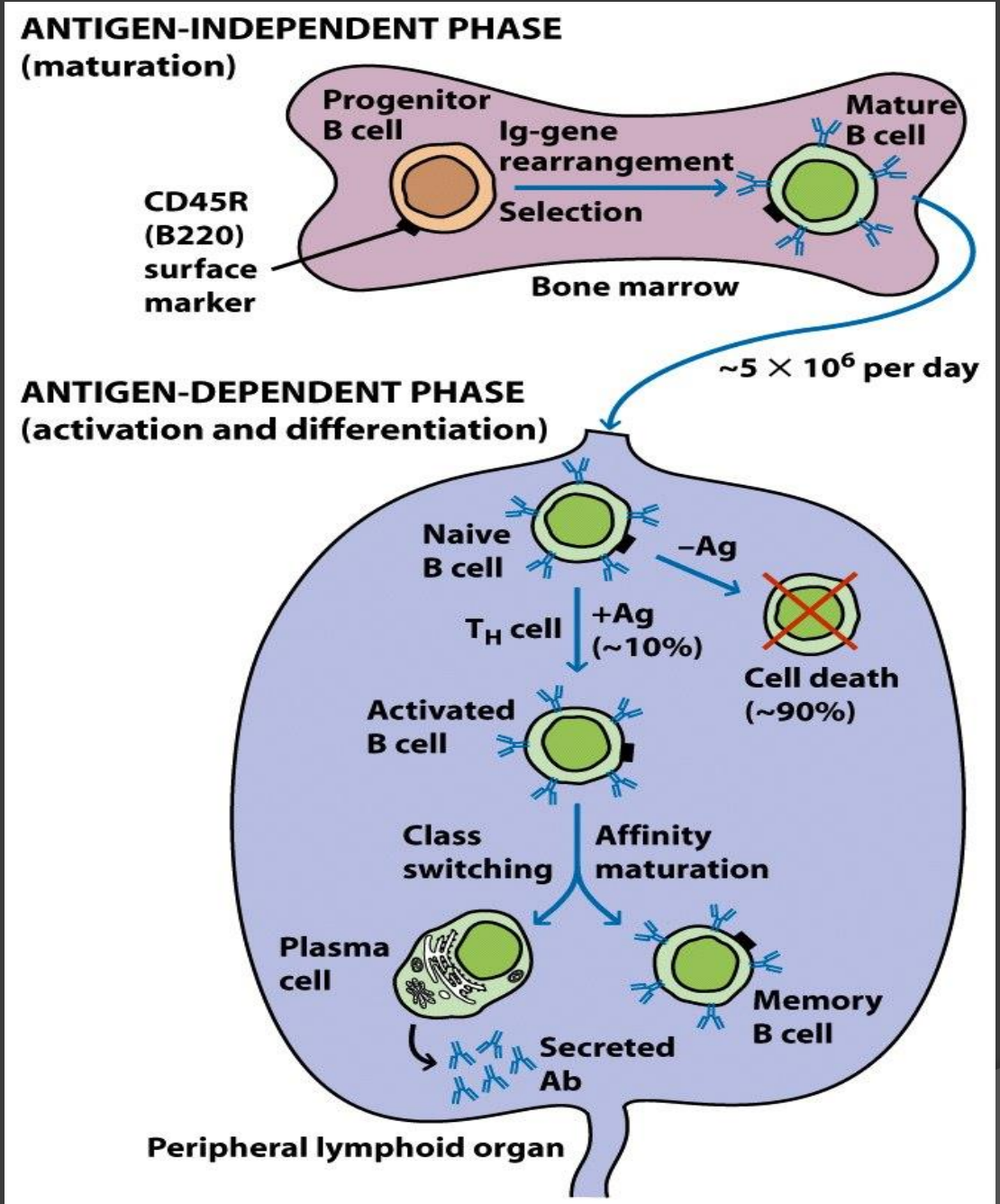

Figure 11-1
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- Bone marrow
  - Pro-B cell → precursor B cell
  - Stromal cell in bone marrow secrete IL-7 that help development into immature B cells

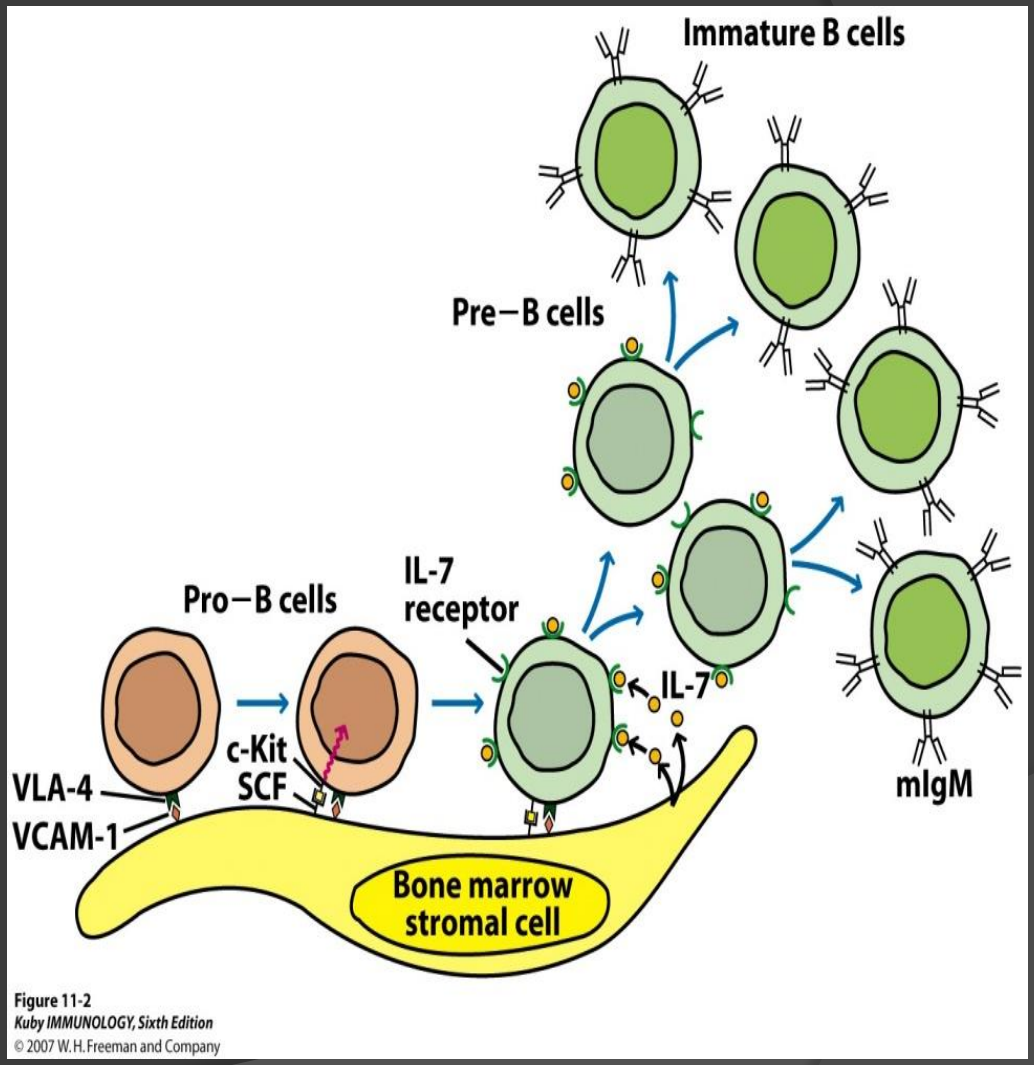

Figure 11-2
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- Pro-B Cell
  - Heavy chain rearrangement
- Pre-B cell
  - Light chain rearrangement
- Immature B cell
  - Is now committed to antigenic specificity and produces IgM
  - B cell not fully functional, must first express both IgM AND IgD on membrane

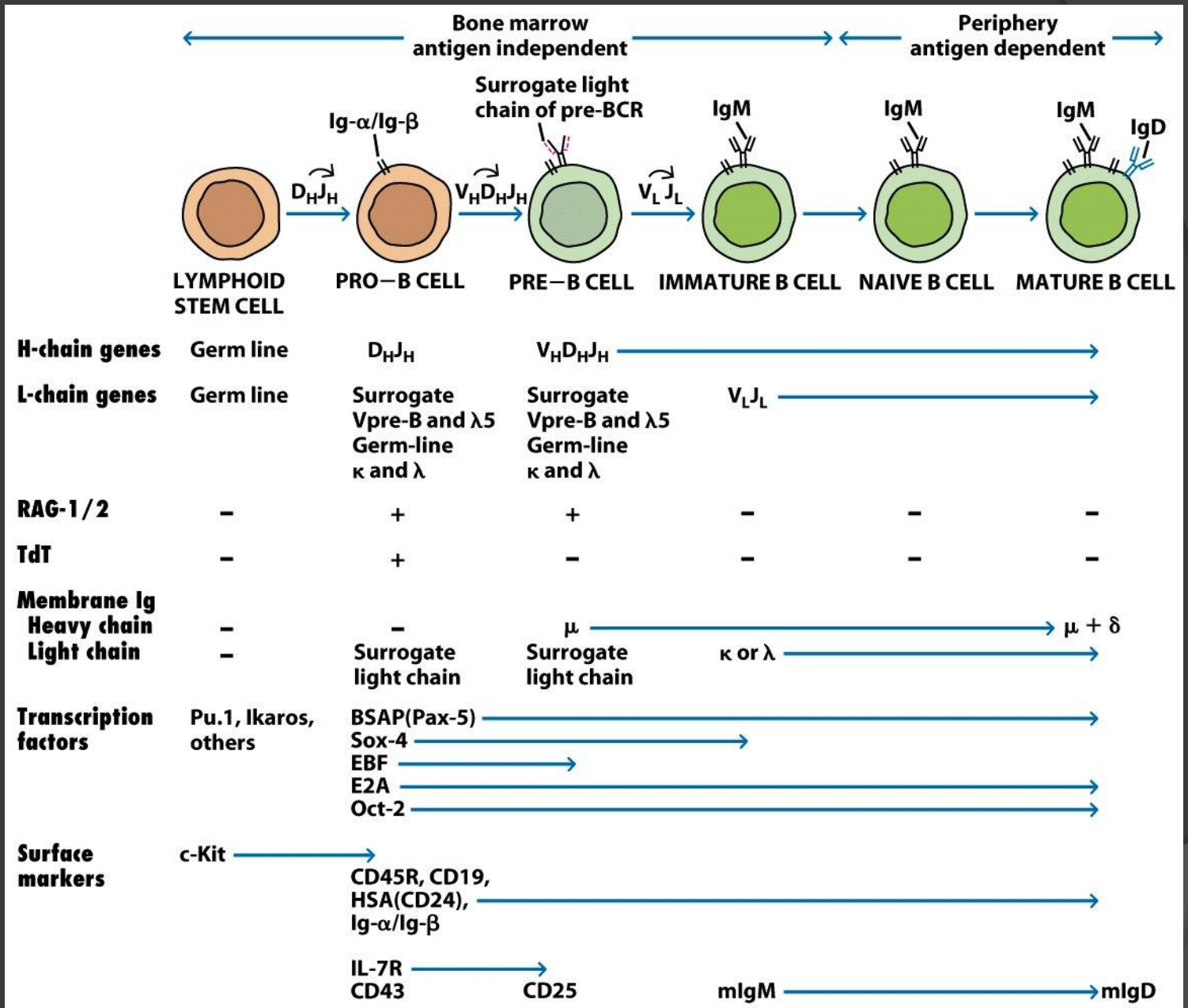

Figure 11-3
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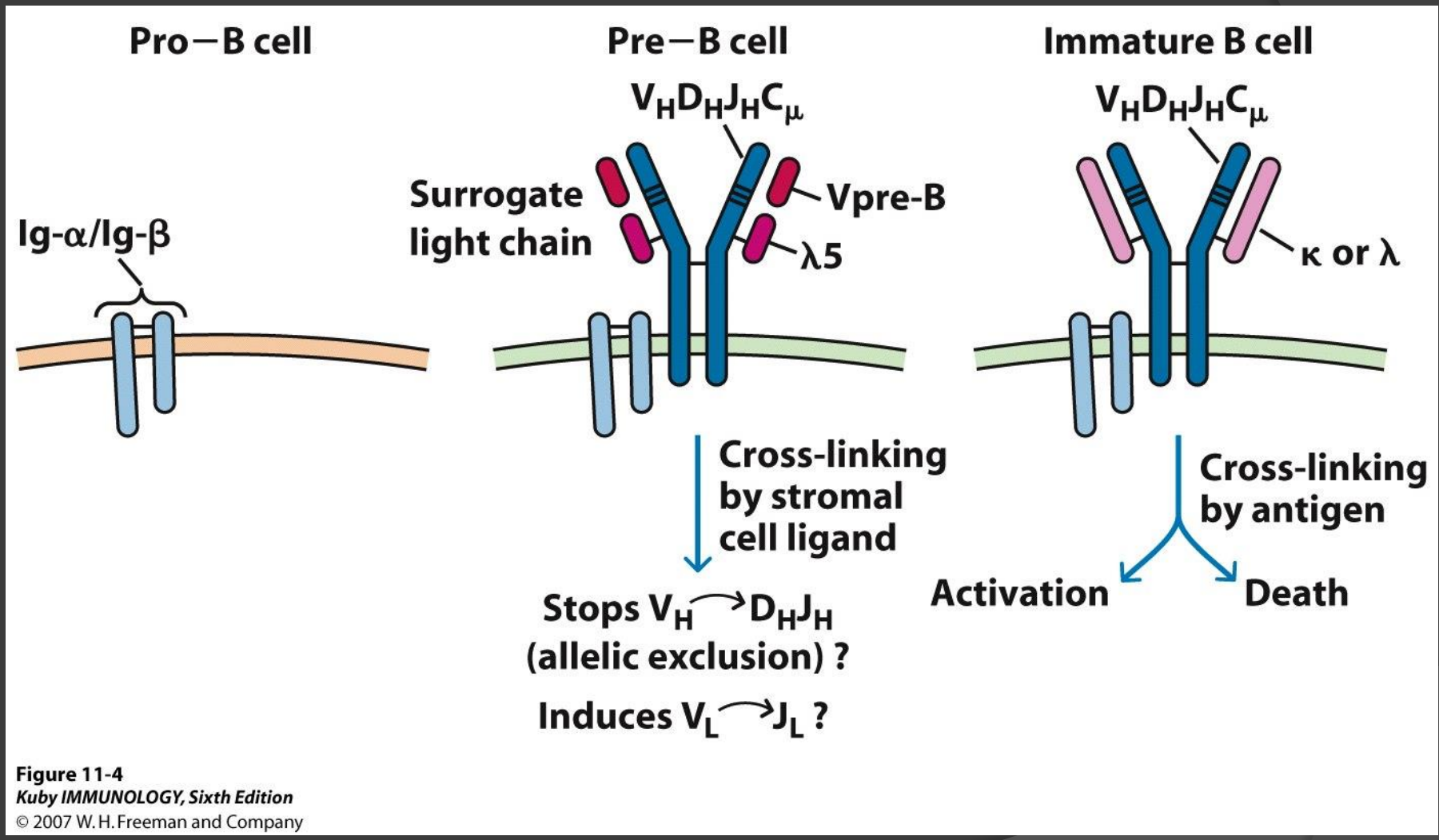

Figure 11-4
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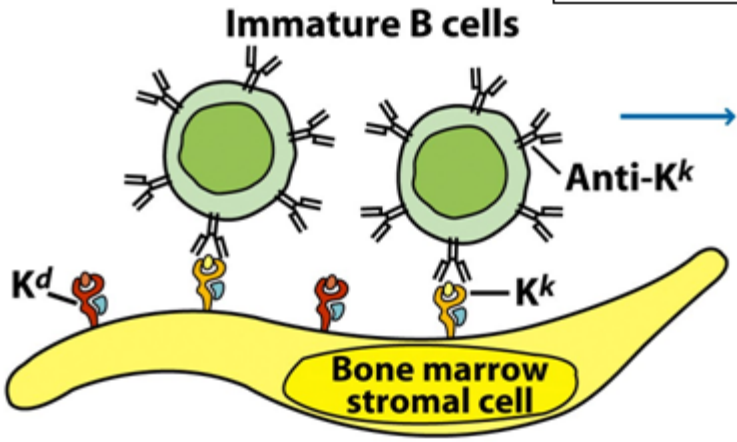
- In mice, 90% of B cells produced everyday die without ever leaving bone marrow
  - Negative selection due to cells that express auto-antibodies against self antigen in the marrow

H-2$^{d/k}$ transgenics

Scientists inserted transgene encoding an anti-MHC K^k



→ **No mature B cells express anti-K^k**

The anti-MHC K^k reacts with self MHC molecules Causing negative selection

Figure 11-6a
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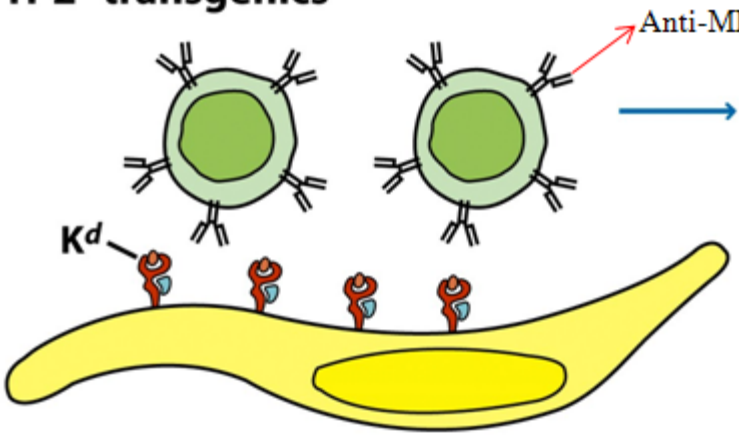
H-2^{d} transgenics



→ **25–50% of mature B cells express anti -K^k**

Figure 11-6b
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B cell Activation

- Thymus-dependent (TD) antigens
  - B cell required direct contact with T_H cell
- Thymus-independent antigens (TI)
  - These antigens activate B cells by different means
    - Type I (TI-1) – lipopolysaccharide
    - Type 2 (TI-2) – highly repititous molecules (bacterial flagella)

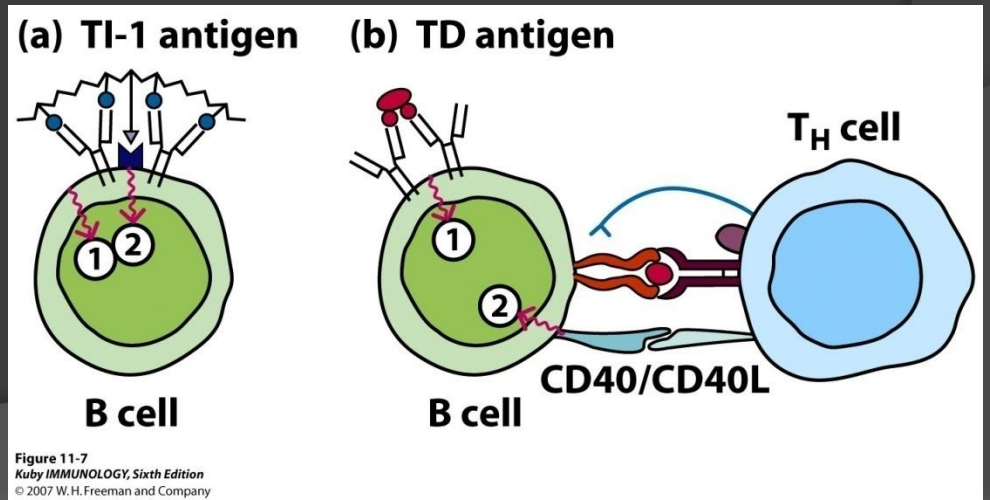

TABLE 11-2 Properties of thymus-dependent and thymus-independent antigens

Property	TD antigens	TI antigens	
		Type 1	Type 2
Chemical nature	Soluble protein	Bacterial cell- wall components (e.g., LPS)	Polymeric protein antigens; capsular polysaccharides
Humoral response			
Isotype switching	Yes	No	Limited
Affinity maturation	Yes	No	No
Immunologic memory	Yes	No	No
Polyclonal activation	No	Yes (high doses)	No

Table 11-2
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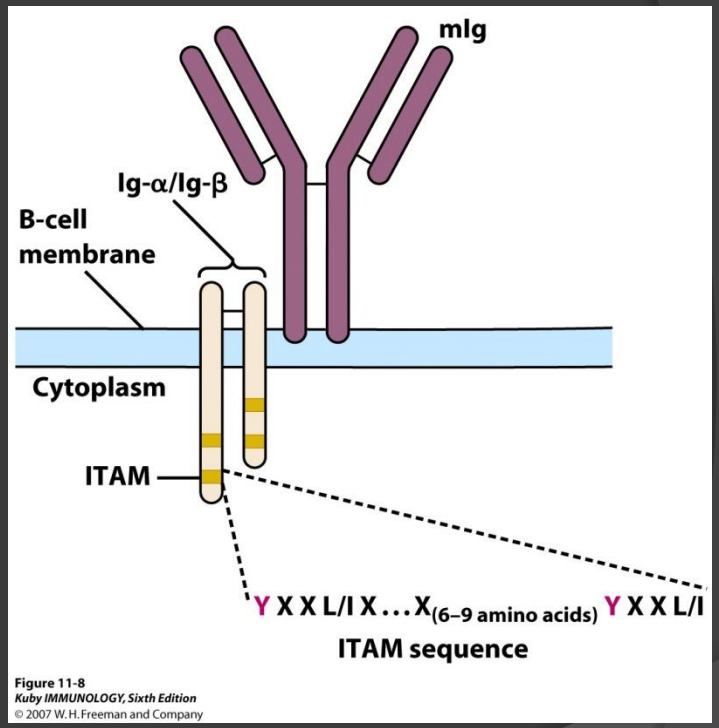
B cell Activation

- Membrane bound antibody have short cytoplasmic tails
  - Too short to generate signal by associating with tyrosine kinases and G proteins
- Membrane Ig must be associated with B-cell receptor
  - Ig-α/Ig-β



ITAM – immunoreceptor tyrosine-based activation motif

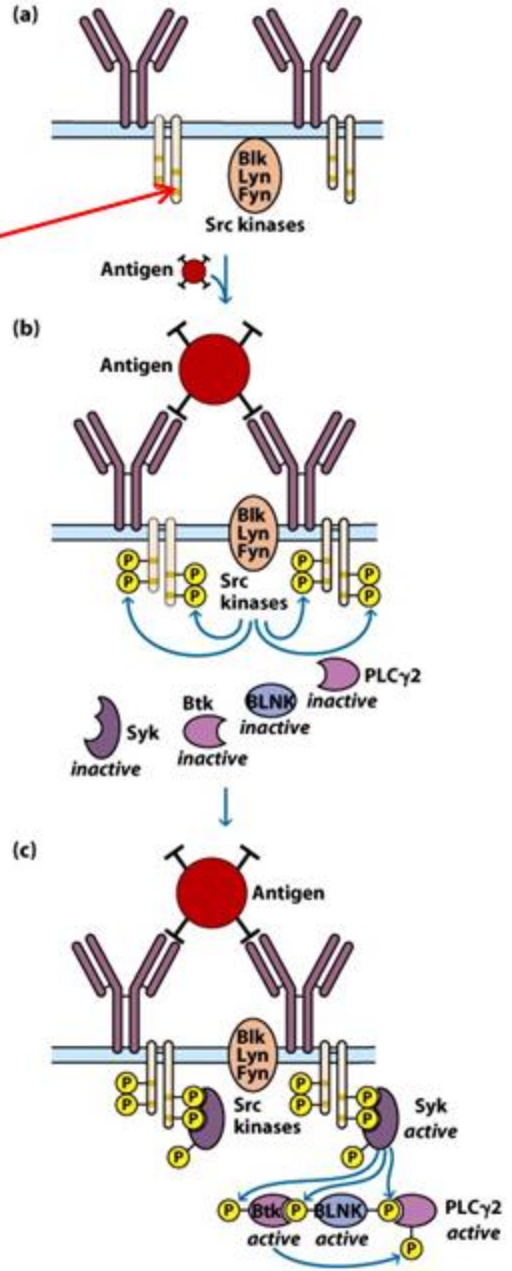


Figure 11-9
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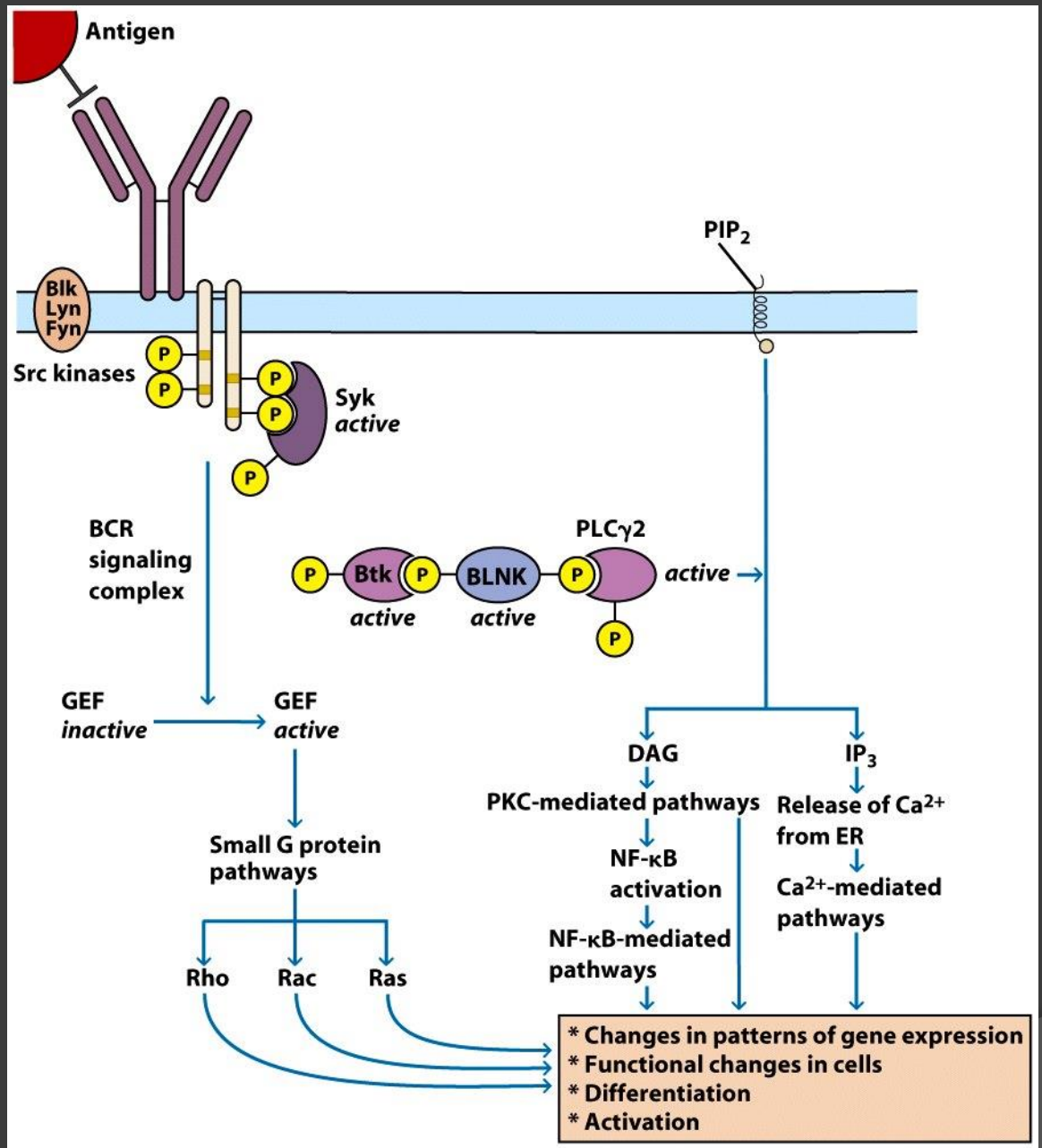
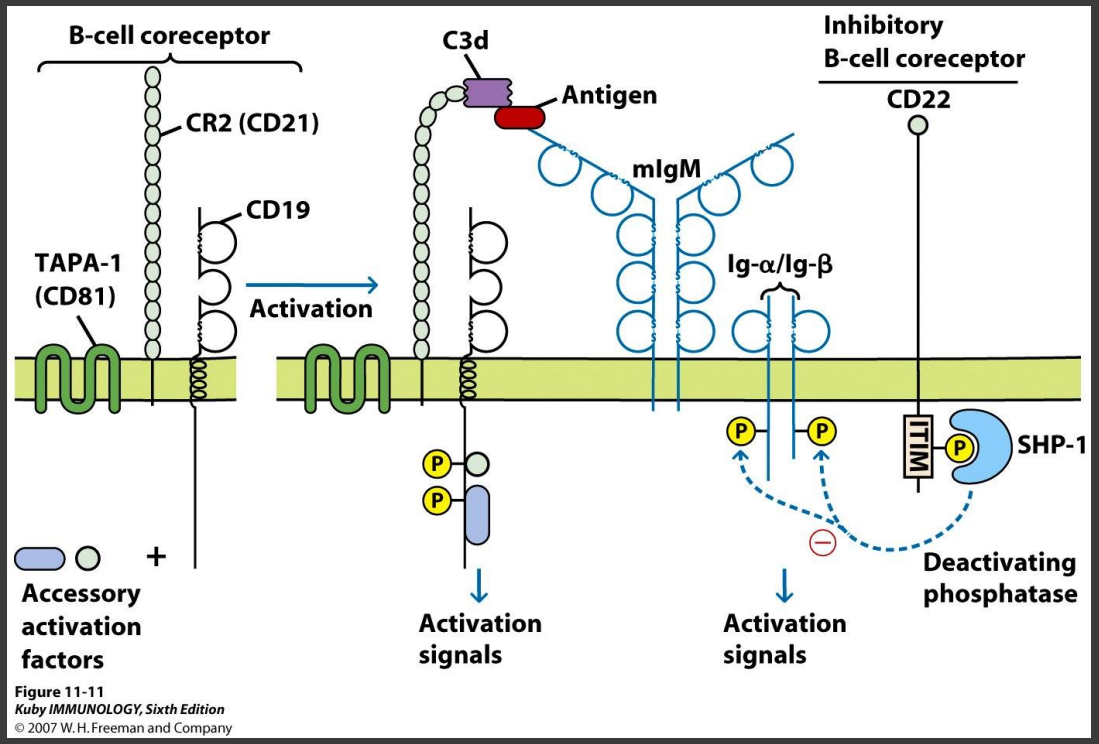


Figure 11-10
Kuby IMMUNOLOGY, Sixth Edition



- ITIM (immunoreceptor tyrosine inhibitory motif)
  - Associated with CD22
  - Functions to deactivate B cells – negative regulation
  - Important in preventing autoimmunity

- T_H cells play essential role in B cell repsonses

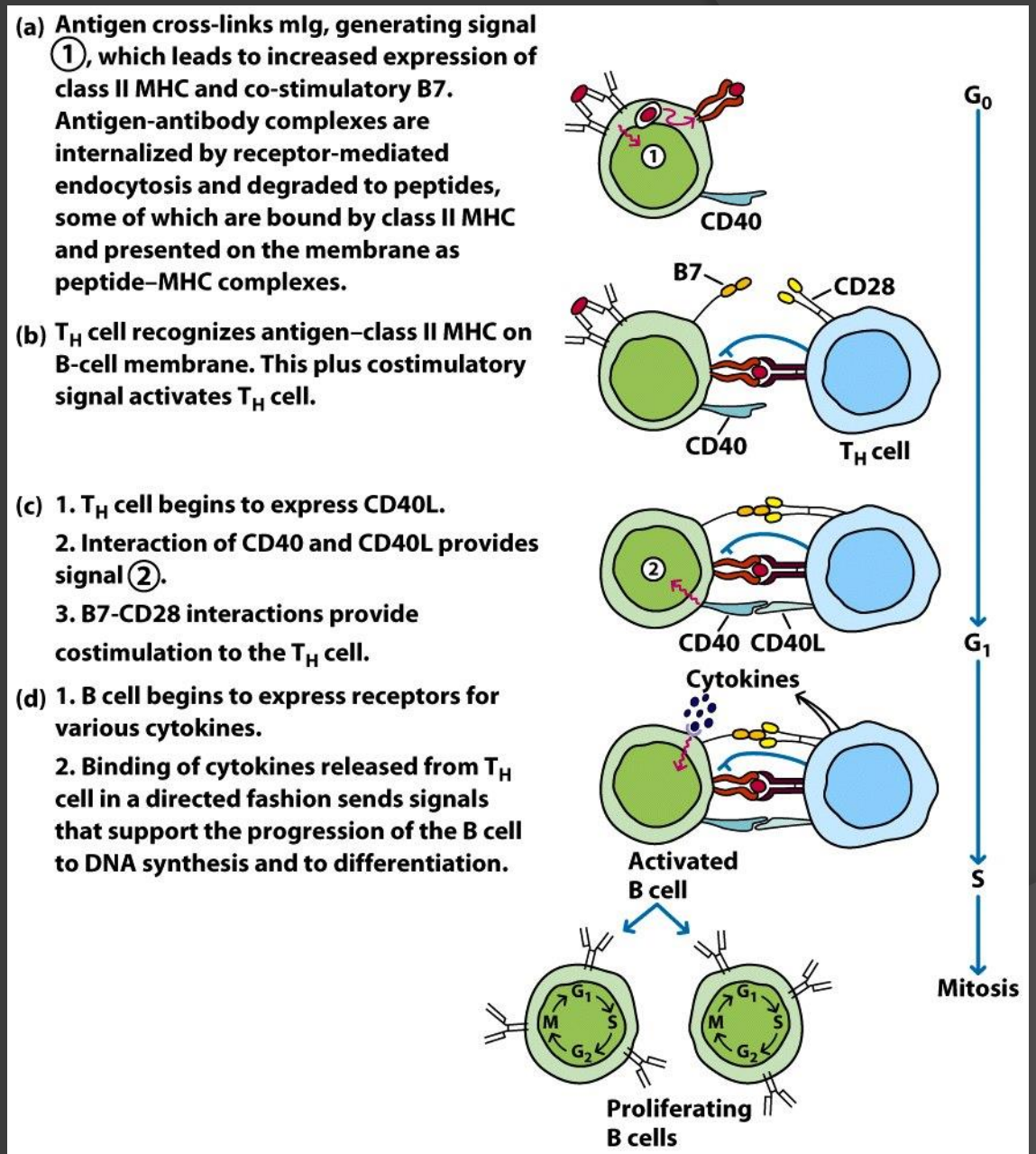
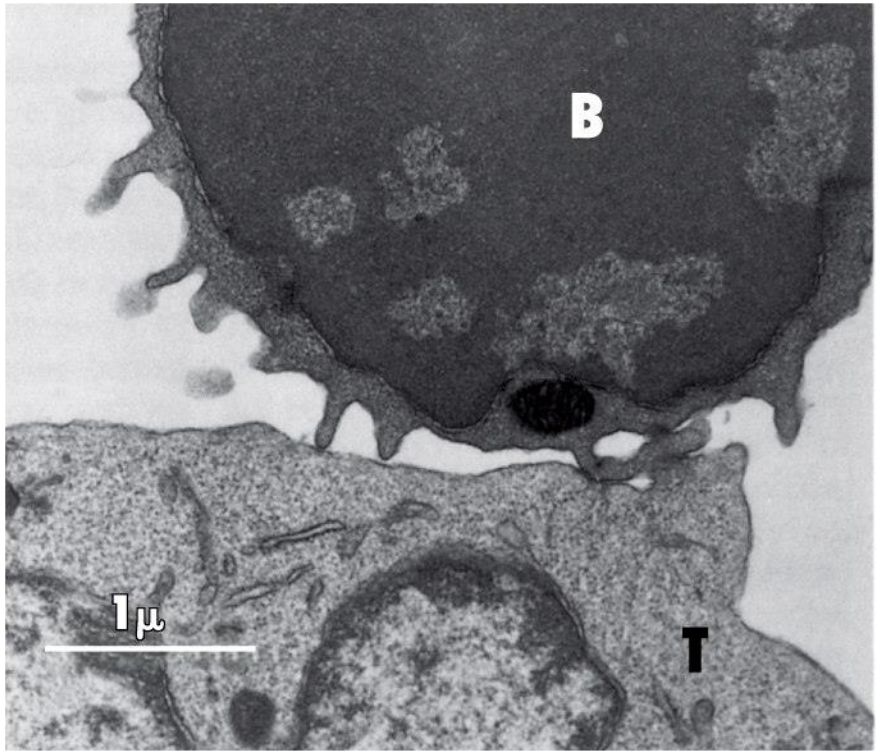


Figure 11-12
Kuby IMMUNOLOGY, Sixth Edition

- TEM of interaction between B cell and T cell



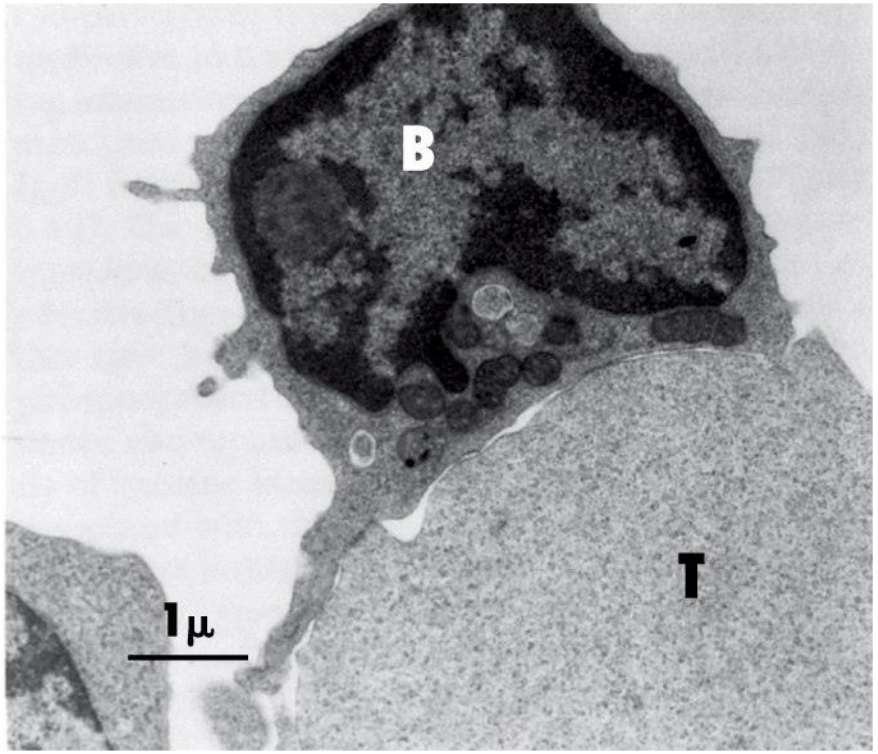


Figure 11-13
Kuby IMMUNOLOGY, Sixth Edition

Humoral Response – Primary vs Secondary

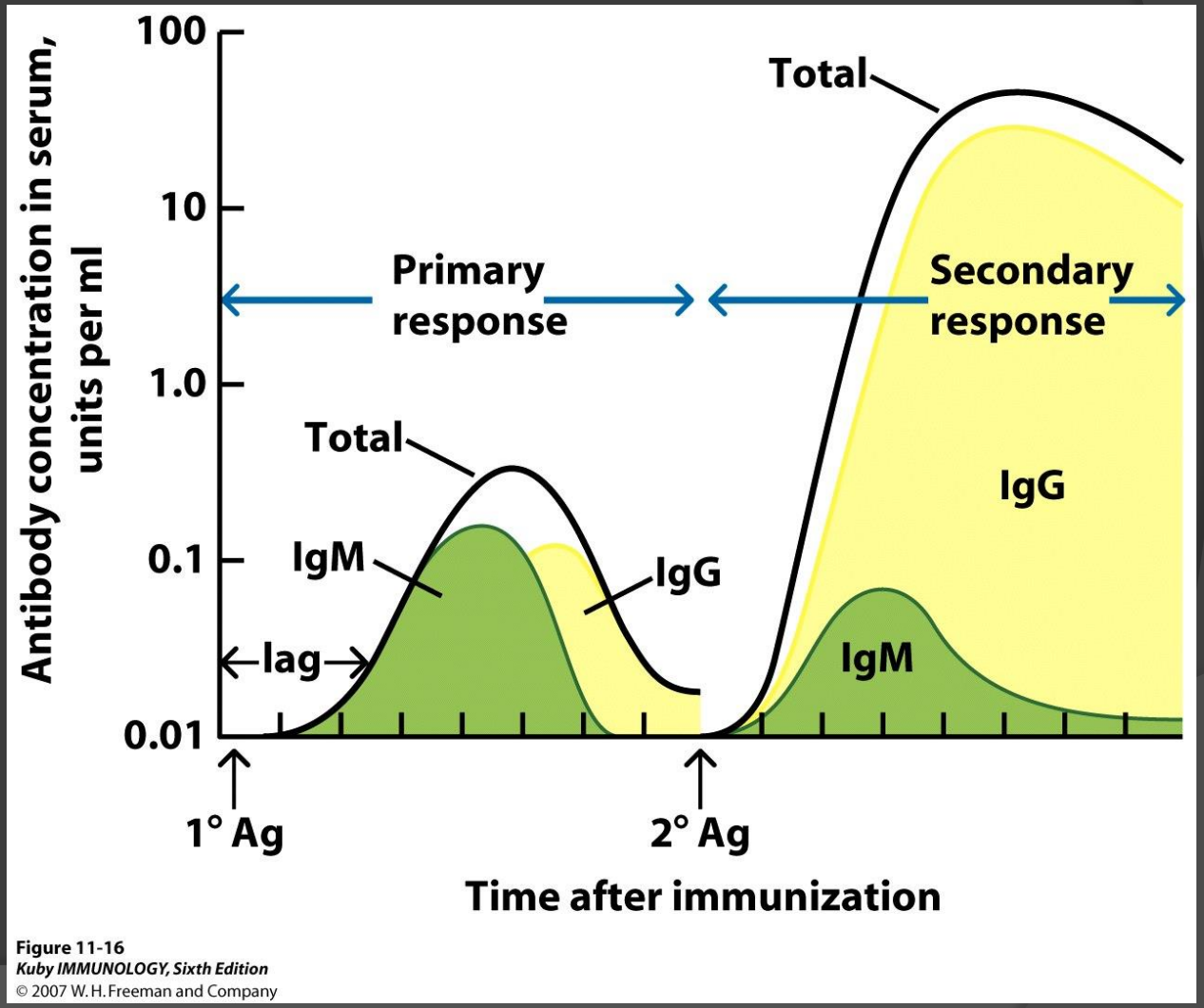


Figure 11-16
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TABLE 11-4 Comparison of primary and secondary antibody responses

Property	Primary response	Secondary response
Responding B cell	Naive B cell	Memory B cell
Lag period following antigen administration	Generally 4–7 days	Generally 1–3 days
Time of peak response	7–10 days	3–5 days
Magnitude of peak antibody response	Varies depending on antigen	Generally 100–1000 times higher than primary response
Isotype produced	IgM predominates early in the response	IgG predominates
Antigens	Thymus dependent and thymus independent	Thymus dependent
Antibody affinity	Lower	Higher

Table 11-4
Kuby IMMUNOLOGY, Sixth Edition

Hapten-carrier conjugates

- Hapten – low molecular weight molecule that won't itself induce a humoral response
  - Must be coupled to suitable carrier

TABLE 11-5 Common hapten-carrier conjugates used in immunologic research

Hapten-carrier abbreviation	Hapten	Carrier protein
DNP-BGG	Dinitrophenol	Bovine gamma globulin
TNP-BSA	Trinitrophenyl	Bovine serum albumin
NIP-KLH	5-Nitrophenyl acetic acid	Keyhole limpet hemocyanin
ARS-OVA	Azophenylarsonate	Ovalbumin
LAC-HGG	Phenyllactoside	Human gamma globulin

Table 11-5
Kuby IMMUNOLOGY, Sixth Edition

In vivo sites for induction of humoral responses

- Blood-bourne antigen is filtered by spleen
- Antigen from tissue spaces filtered by lymph nodes
  - Antigen either enters alone or with antigen-transporting cells
    - Langerhans cells
    - Dendritic cells
  - Encounters antigen-presenting cells
    - Dendritic cells
    - Macrophages
    - Follicular dendritic in follicles and germinal centers

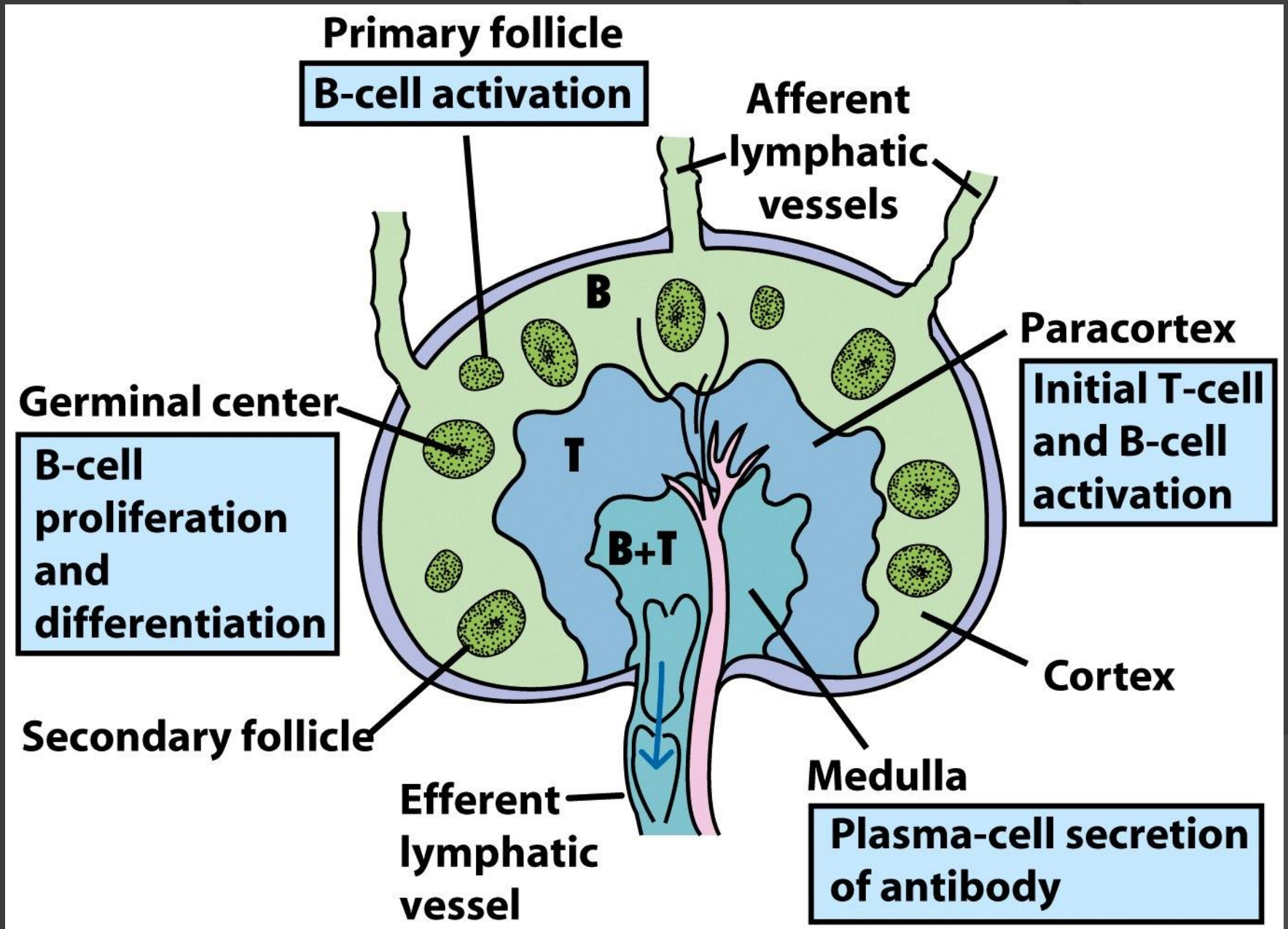
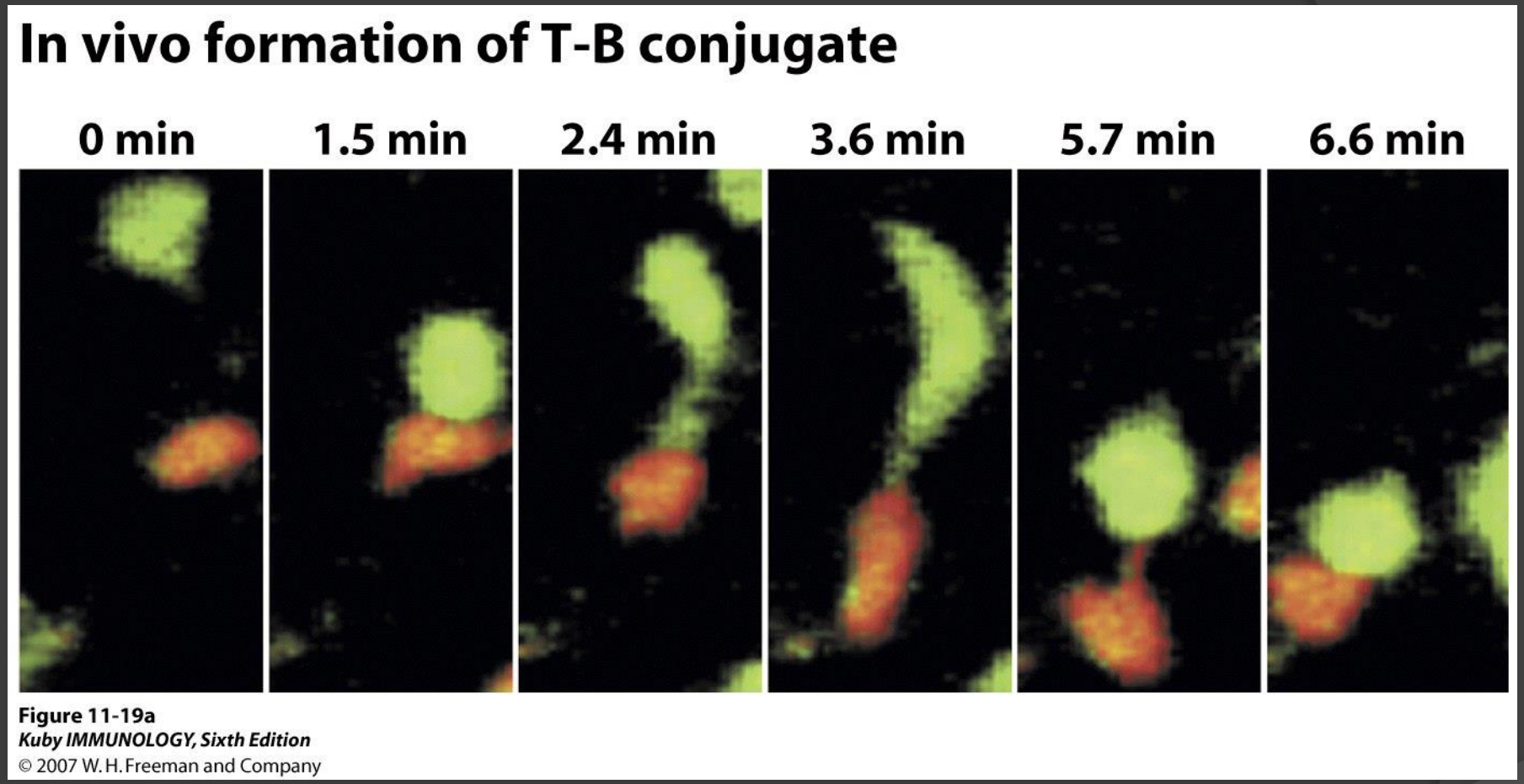


Figure 11-18
Kuby IMMUNOLOGY, Sixth Edition



T cells are green and B cells are red

- Germinal centers arise within 7-10 days after initial exposure to thymus-dependent antigen in lymph node
  - 3 events in germinal centers
    - Affinity maturation
      - Result of somatic hypermutation
    - Class switching
    - Formation of plasma and memory B cells

Cellular events in germinal centers

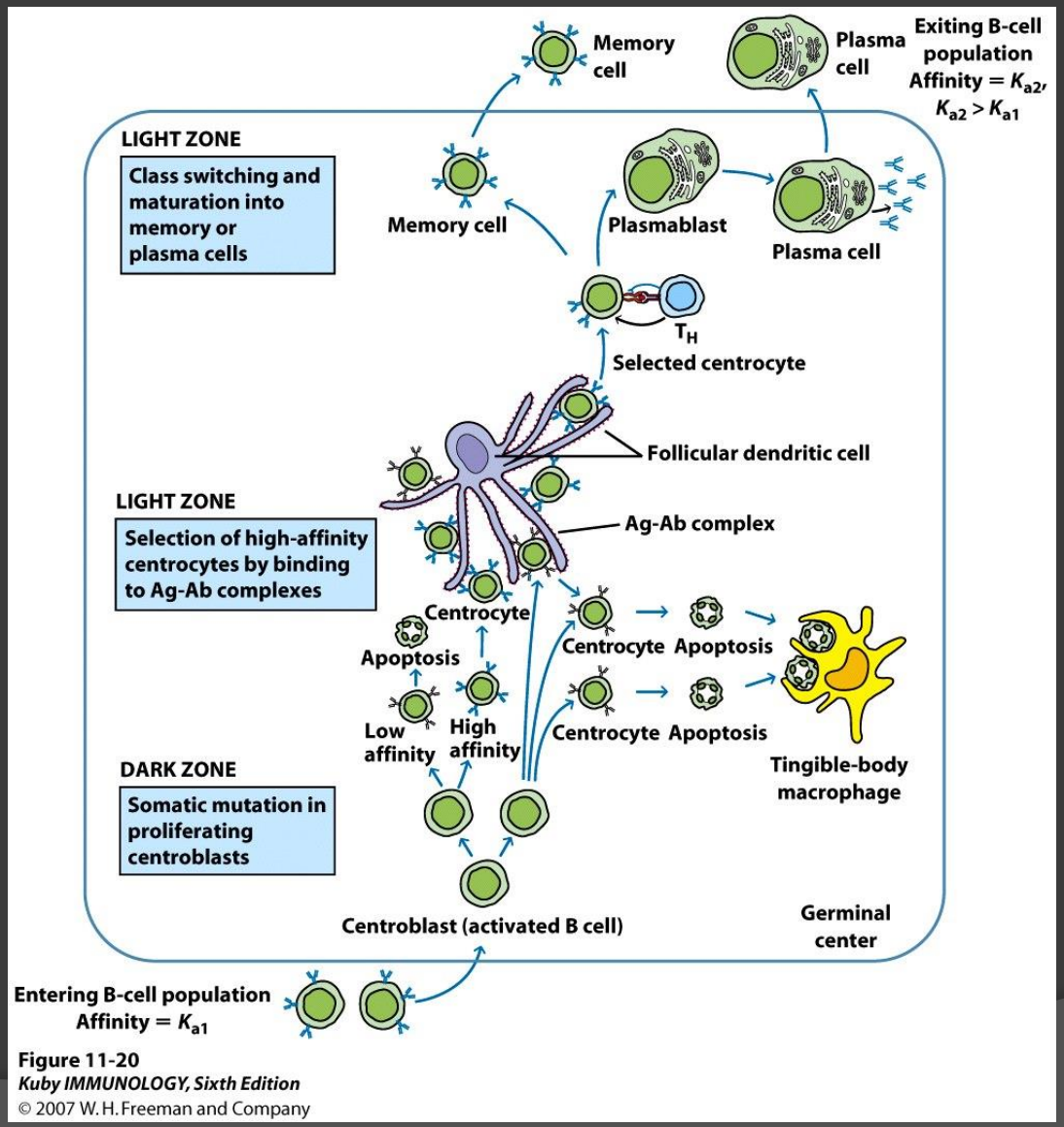


Figure 11-20
Kuby IMMUNOLOGY, Sixth Edition

Dendritic cell presents antigen to developing B cells to see which B cells are producing antibody with high-affinity for that antigen

Class Switching

- Dependent on cytokines to switch from IgM to other isotype
  - Thymus-dependent antigens
  - Interaction of CD40 on B cell and CD40L on T cell
  - X-linked hyper-M syndrome
    - T_H cells don't express CD40L, patients only produce IgM
      - No memory cell populations, no germinal centers

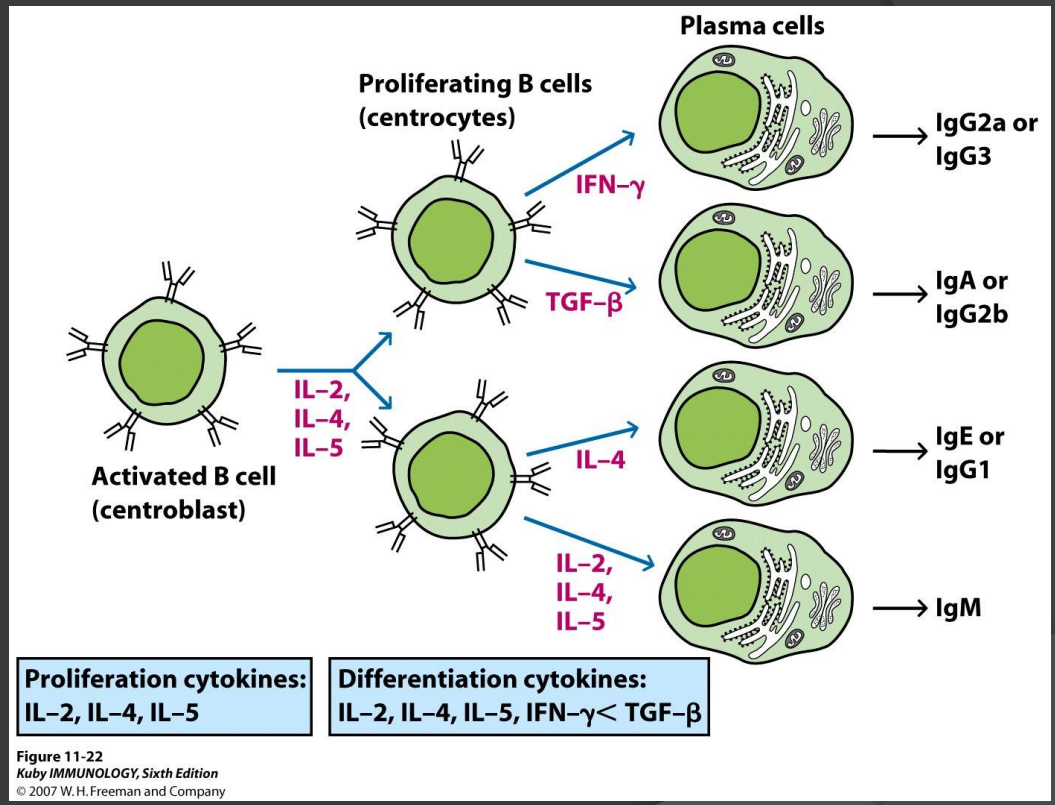


Figure 11-22
Kuby IMMUNOLOGY, Sixth Edition

Regulation

- Humoral and cell-mediated branches must be heavily regulated
- Cytokines play important role
- Antigenic competition
  - Previous encounter with antigen can render animal tolerant or may result in formation of memory cells
- Presence of antibody can suppress response to antigen
  - Some vaccines are given to babies after maternal IgG (that was transferred across placenta) has left system
  - Vaccination before this will prevent proper response and development of long-lasting memory cells

TABLE 11-7 Antigenic competition between sheep and horse RBCs

IMMUNIZING ANTIGEN		HEMOLYTIC PLAQUE ASSAY (DAY 8)	
Ag1 (day 0)	Ag2 (day 3)	Test Ag	PFC/10^{6}* spleen cells
None	HRBC	HRBC	205
SRBC	HRBC	HRBC	13
None	SRBC	SRBC	626
HRBC	SRBC	SRBC	78

*PFC = plaque-forming cells.

Table 11-7
Kuby IMMUNOLOGY, Sixth Edition

ABZYMES

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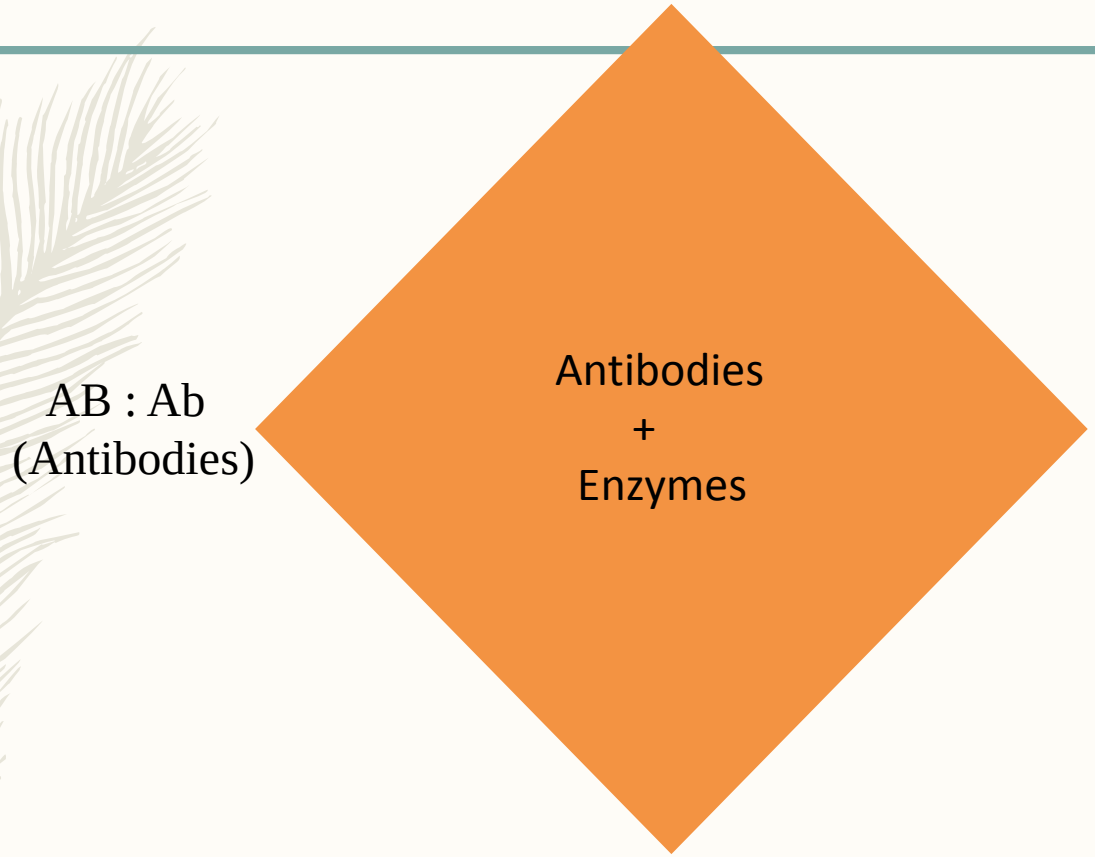
M. Tech IBT

ABZYMES



AB : Ab
(Antibodies)

ZYMES
(Enzymes)

Catalytic monoclonal antibodies

Introduction

- An abzyme (from antibody and enzyme), also called catmab (from catalytic monoclonal antibody), and most often called catalytic antibody, is a monoclonal antibody with catalytic activity.
- A single molecule of an antibody-enzyme, or abzyme, is capable of catalyzing the destruction of thousands of target molecules

- Abzymes are usually raised in lab animals immunized against synthetic haptans, but some natural abzymes can be found in normal humans (Intestinal peptide autoantibodies) and in patients with autoimmune diseases where they can bind to and hydrolyze DNA.

- One basic difference between antibodies and enzymes is that the former binds the complementary structure in its ground state while enzymes bind in high energy state

History

- The possibility of catalyzing a reaction by means of an antibody which binds the transition state was first suggested by William P. Jencks in 1969.
- In 1994, Peter G. Schultz and Richard A. Lerner received the prestigious Wolf Prize for developing catalytic antibodies.

- 1995-2011: Dr. Paul publishes first example of hydrolysis of HIV coat protein by an abzyme

- Antibodies and enzymes share the ability to bind with compounds with great specificity and high affinity. This property has been exploited in the development of antibodies with catalytic activity.

Principle

– The production of the abzymes is based on the following two principles:

1. Enzymes act by binding the transition state of a reactant better than the ground state.

2. Antibodies which bind to specific small molecules can be produced by coupling this small molecule to a protein carrier and using this protein for immunizing experimental animals.

- If the molecule is a transition state analog, then the antibodies that are produced to bind to this molecule will function as enzyme towards the substrate of this reaction.
- Abzymes are selected from monoclonal antibodies produced by immunizing mice with haptens that mimic the transition state of enzyme catalyzed reactions (Haptens are small molecules that elicit an immune response only when attached to a large carrier such as a protein).

Mechanism of Action

- Enzymes function by lowering the activation energy of the transition state of a chemical reaction, thereby enabling the formation of an otherwise less-favorable molecular intermediate between the reactant(s) and the product(s).

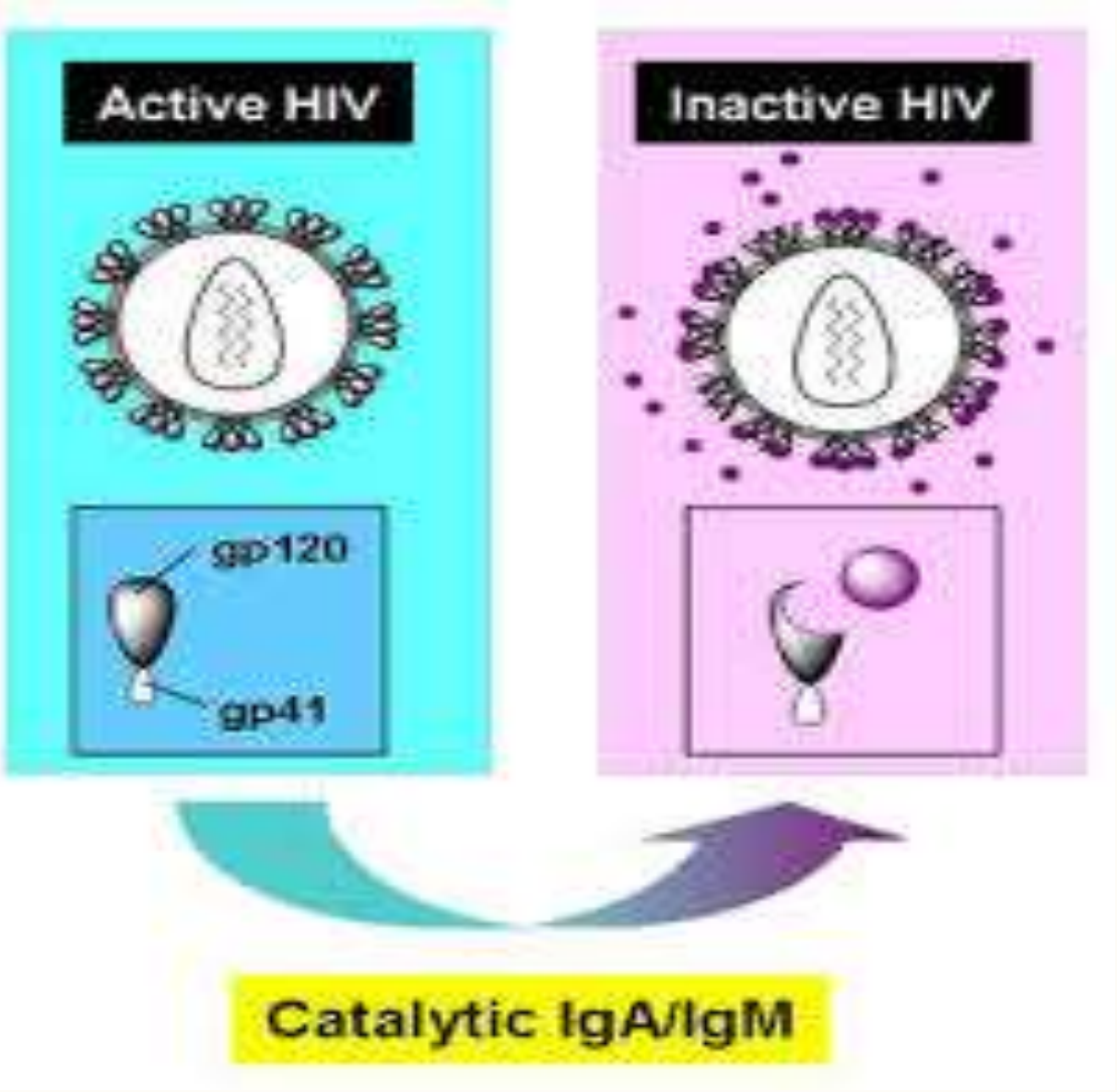
- If an antibody is developed to bind to a molecule that's structurally and electronically similar to the transition state of a given chemical reaction, the developed antibody will bind to, and stabilize, the transition state, just like a natural enzyme, lowering the activation energy of the reaction, and thus catalyzing the reaction.
- By raising an antibody to bind to a stable transition-state analog, a new and unique type of enzyme is produced.

Applications

- Treatment of cancer
- Abzymes in treatment of HIV
- Drug Detoxification
- Abzymes against weight gain
- Antibody directed enzyme prodrug therapy

CATALYTIC ANTIBODIES IN HIV TREATMENT

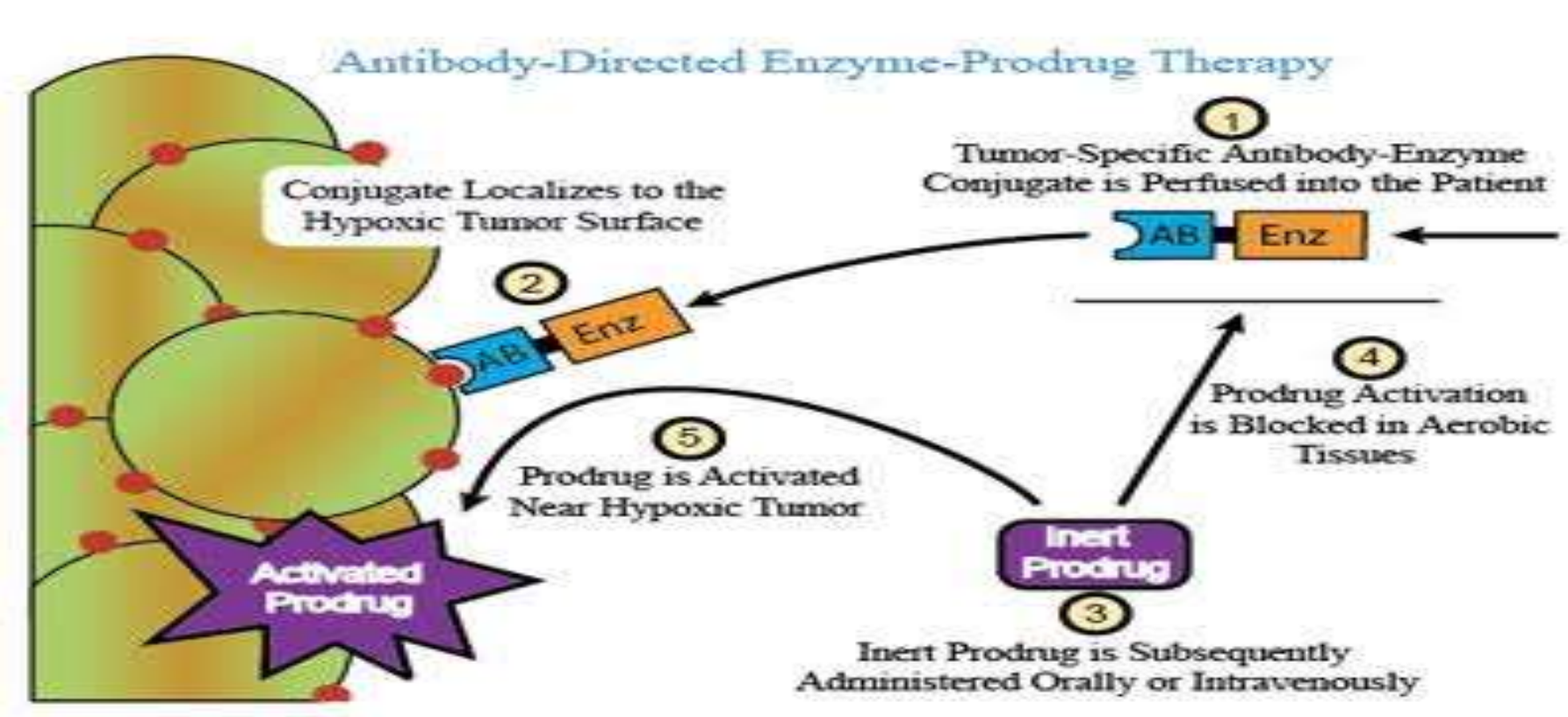
- CD4 binding site on surface HIV gp120 molecules, the mostly-unchanging binding site of the virus that reacts with host cell receptors.
- The abzyme does more than bind to the site, it catalytically destroys the site, rendering the virus inert, and then can attack other HIV viruses.
- A single abzyme molecule can destroy thousands of HIV viruses.


Active HIV
gp120
gp41
Inactive HIV
Catalytic IgA/IgM

ABZYMES IN DRUG DETOXIFICATION

- Cocaine taken by a person(in form of drug or stimulant) goes to brain via blood circulation(crossing the blood brain barrier) and may cause damage to brain.
- To overcome it **cocaine transition state** can be used as vaccines.
- When given to patient, antibodies will be generated against **cocaine transition state** which will detoxify cocaine if patient consumes it in future.

ANTIBODY DIRECTED ENZYME PRODRUG THERAPY



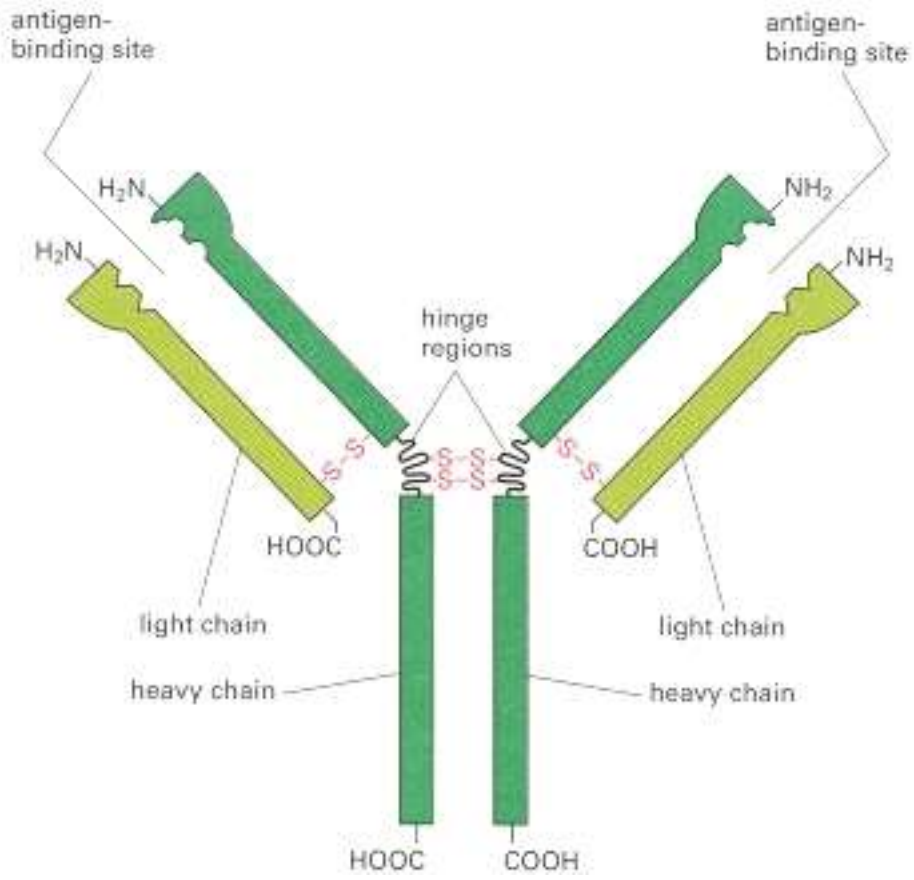
- In some cases unwanted protein- protein interaction can also be responsible for various problems or abnormalities in body.
- So to overcome it, catalytic antibodies can be used which binds to interacting domains of these proteins and prevent unwanted protein protein interactions.

ABZYMES AGAINST WEIGHT GAIN AND CONTROLLING OBESITY

Abzymes also plays important role in maintaining proper body weight through dedradation of ligands which binds to a specific receptor and show physiological effect.(eg- growth hormone receptors and LDL receptors).


Thank you

CLONAL SELECTION



Presented by-

Rahul Kumar Thaosen

M.Sc Life Science

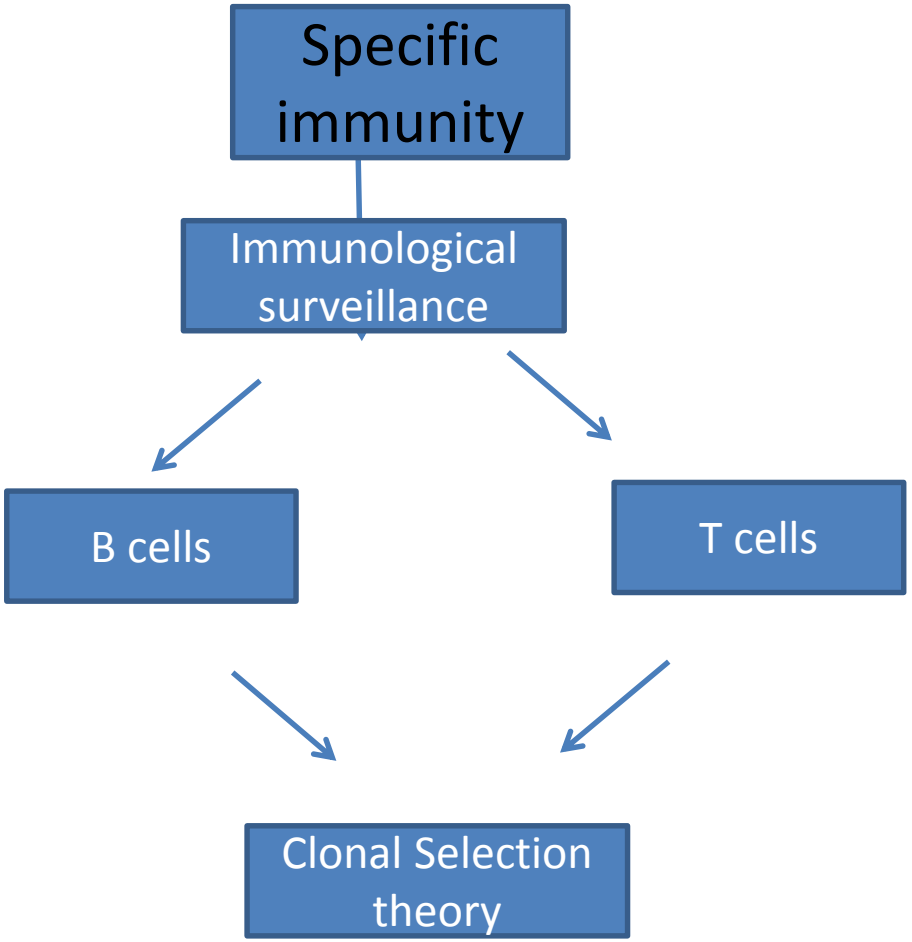
Sem III , BBAU

History

- In 1900, Paul Ehrlich proposed the so-called side chain theory of antibody production.

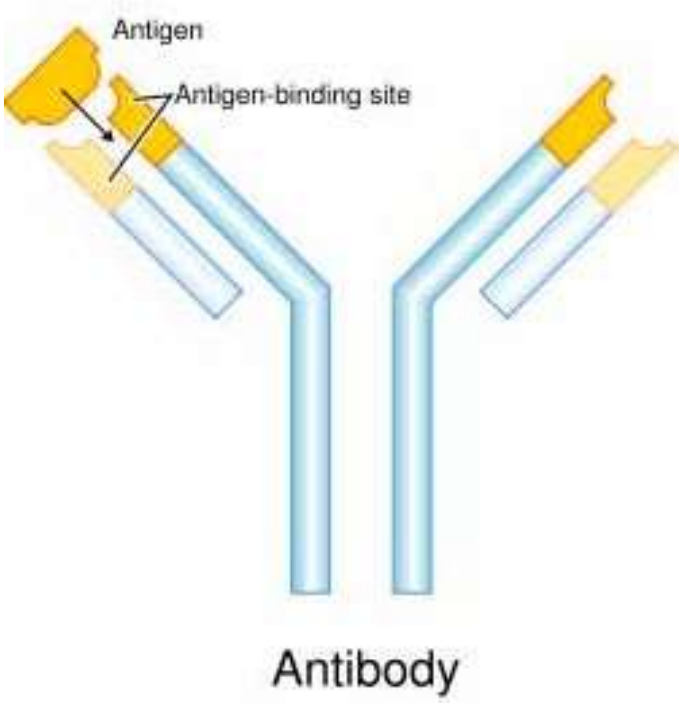
- In 1955, Danish immunologist Niels Jerne put forward a hypothesis that there is already a **vast array of soluble antibodies in the serum** prior to any infection. The entrance of an antigen into the body results in the selection **of only one type of antibody** to match it.

- In 1957, Frank Macfarlane Burnet published a paper titled ‘A modification of Jerne's theory of antibody production using the concept of clonal selection’ in a rather obscure *Australian Journal of Science*. In it Burnet expanded the ideas of Talmage and named it "**clonal selection theory**


Specific immunity
Immunological surveillance
B cells
T cells
Clonal Selection theory

B lymphocytes (b cells)

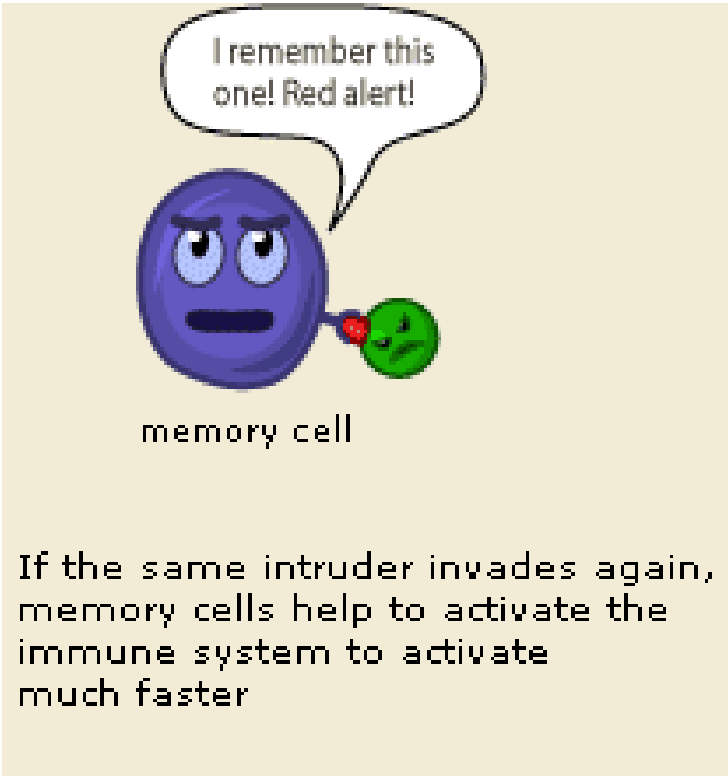
- Lymphocytes respond **specifically** to **antigens** on **foreign cells**, cells infected by pathogens and toxins released by pathogens.



- AN ANTIGEN IS A COMPLEX MOLECULE – RECOGNISABLE AS SELF OR FOREIGN/ NON-SELF
- ANTIGEN TRIGGERS PRODUCTION OF ANTIBODIES
- ANTIBODY IS A Y-SHAPED MOLECULE WITH A SPECIFIC RECEPTOR (BINDING SITE)

Memory cells

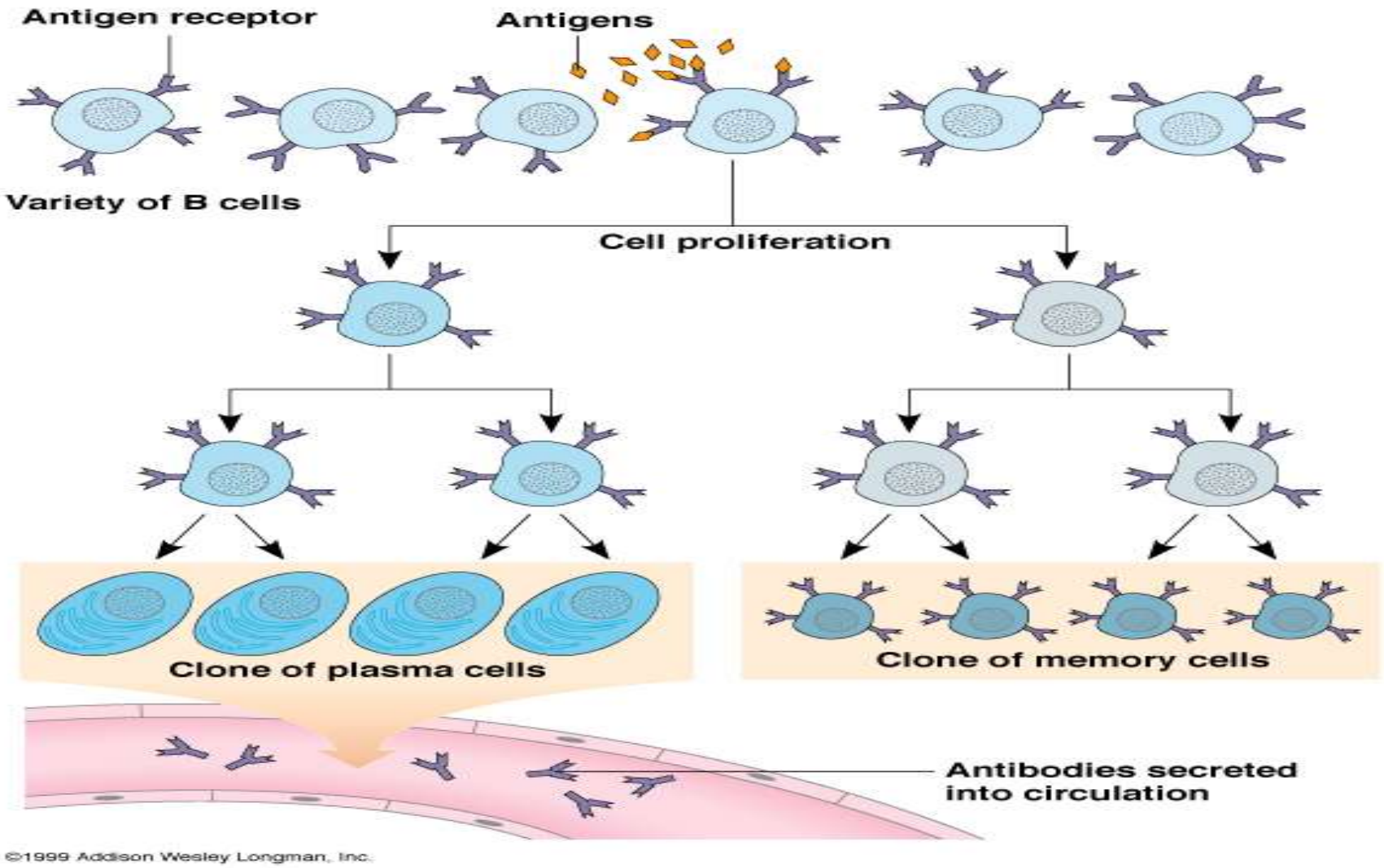
- Some **T and B lymphocytes** produced in response to antigens by clonal selection **survive long term** as **memory cells**.
- **A secondary exposure** to the same antigen rapidly gives rise to a new clone of lymphocytes producing **a rapid** and greater immunological **response**.



How Do B Cells Produce Antibodies?

- B cells develop from stem cells in the bone marrow of adults (liver of fetuses).
- After maturation B cells migrate to lymphoid organs (lymph node or spleen).
- **Clonal Selection**: When a B cell encounters an antigen it recognizes, it is stimulated and divides into many clones called plasma cells, which actively secrete antibodies.
- Each B cell produces antibodies that will recognize only one antigenic determinant.

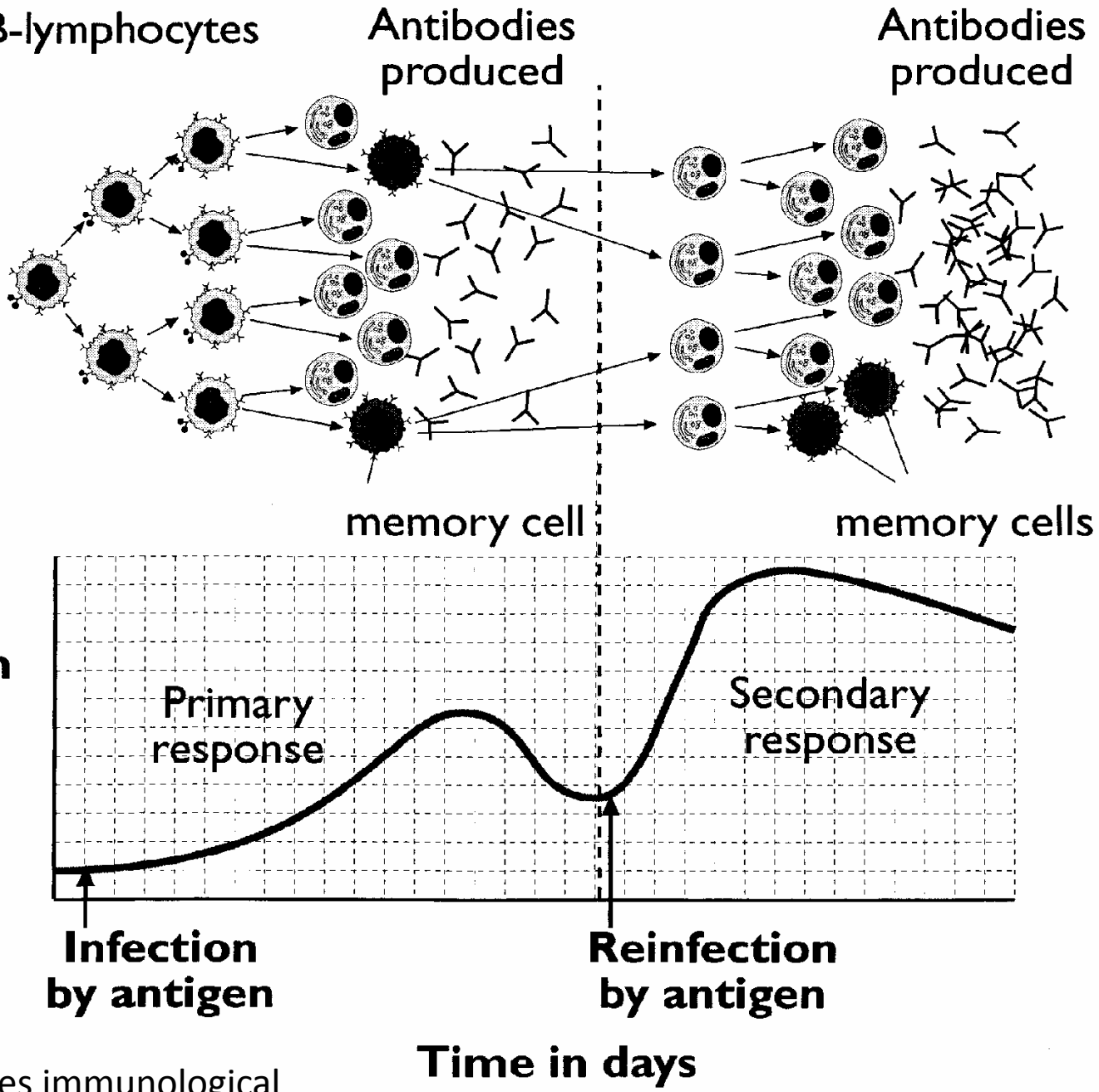
Clonal Selection of B Cells is Caused by Antigenic Stimulation



Types of Immune Response

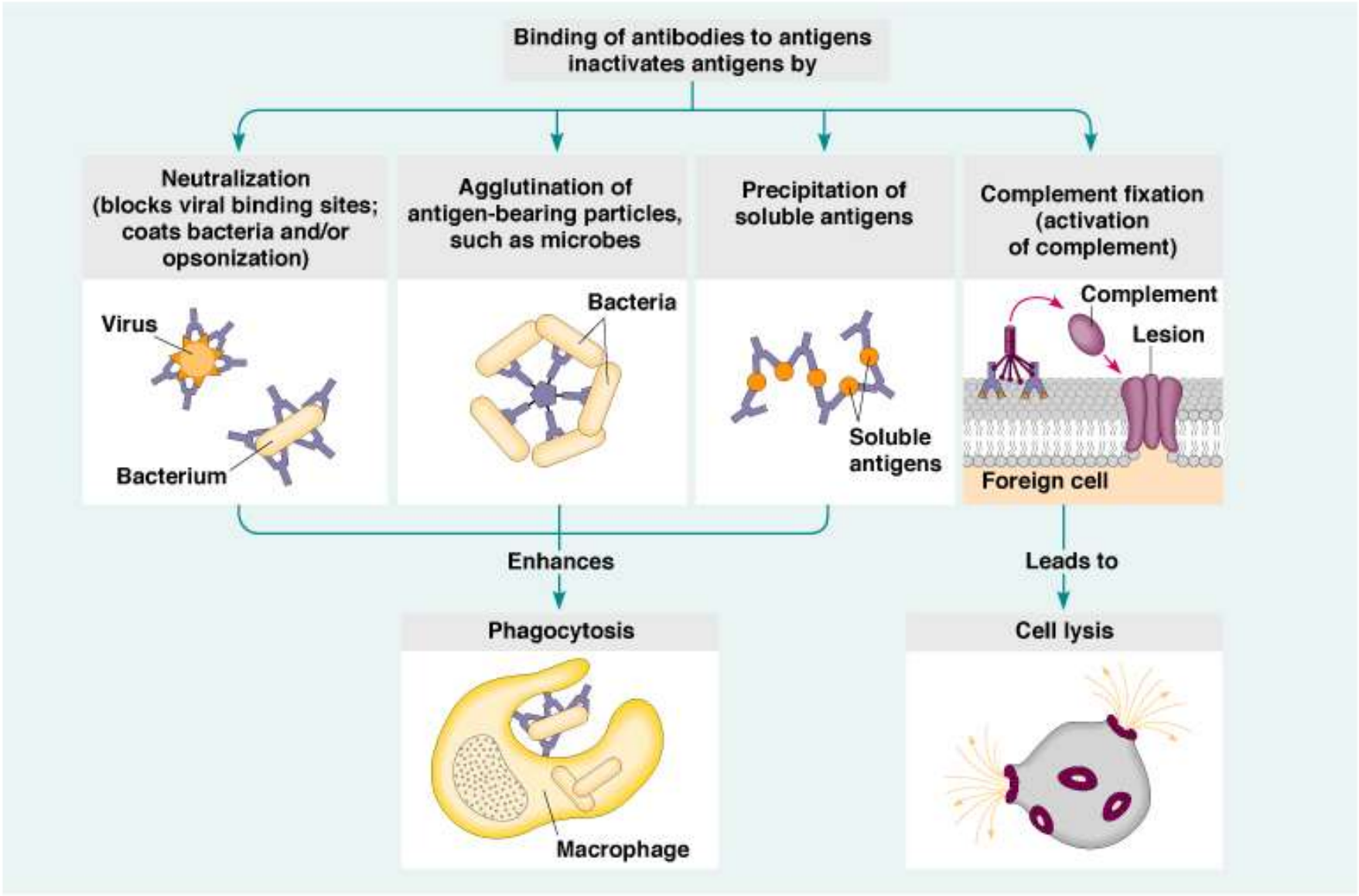
- Primary Immune Response
  - This is a response to an invader the First time the invader infects the body.
    - No measurable immune response for first few days.
    - Next 10 – 15 days antibody production grows steadily

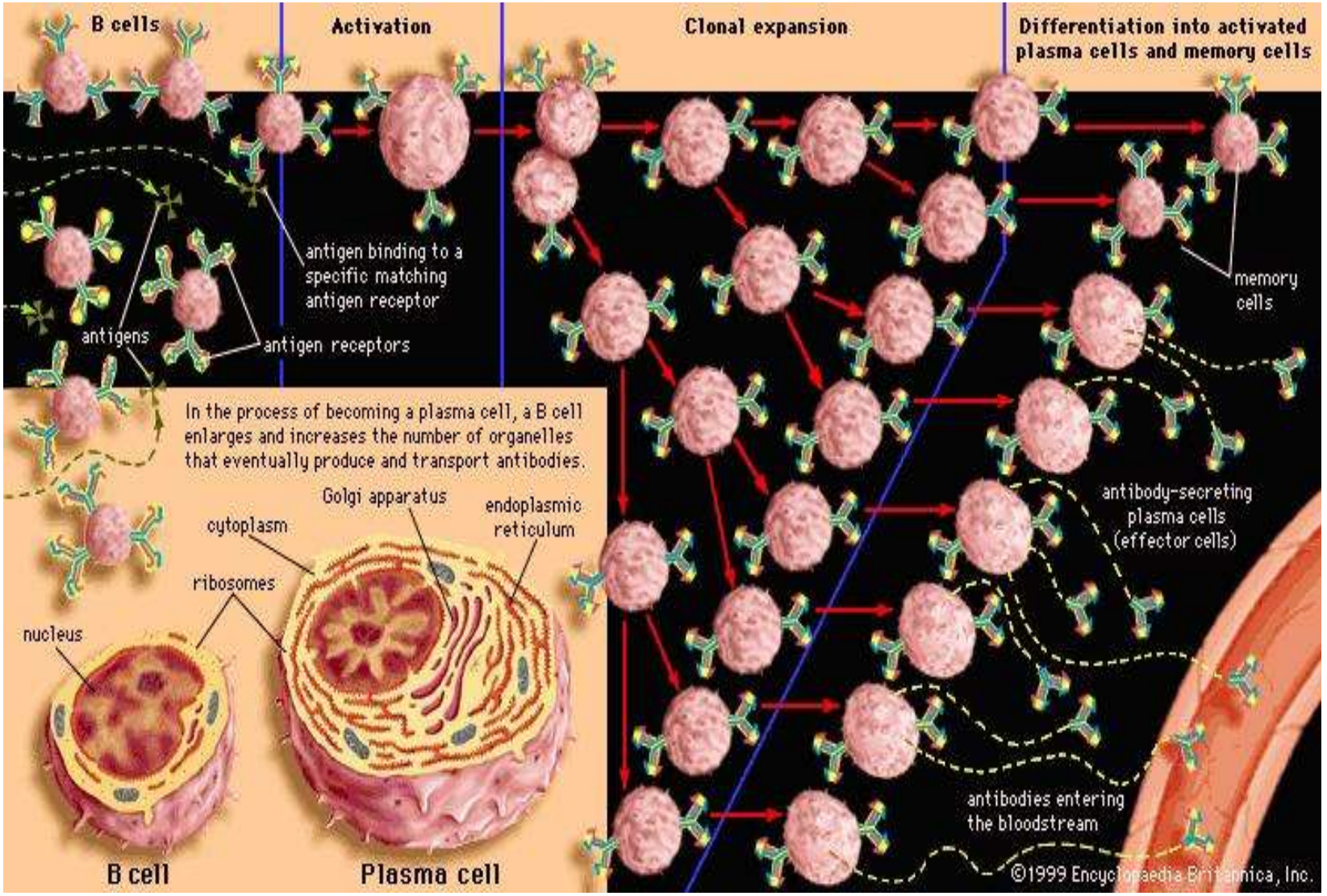
- Secondary Immune Response
  - A more rapid response to an invader the 2nd time it invades the body.
    - Antibody production increases dramatically and in a much shorter time period..



Primary – establishes immunological memory

Consequences of Antibody Binding




B cells
Activation
Clonal expansion
Differentiation into activated plasma cells and memory cells
antigen binding to a specific matching antigen receptor
antigens
antigen receptors
memory cells
In the process of becoming a plasma cell, a B cell enlarges and increases the number of organelles that eventually produce and transport antibodies.
Golgi apparatus
endoplasmic reticulum
cytoplasm
ribosomes
nucleus
antibody-secreting plasma cells (effector cells)
antibodies entering the bloodstream
B cell
Plasma cell
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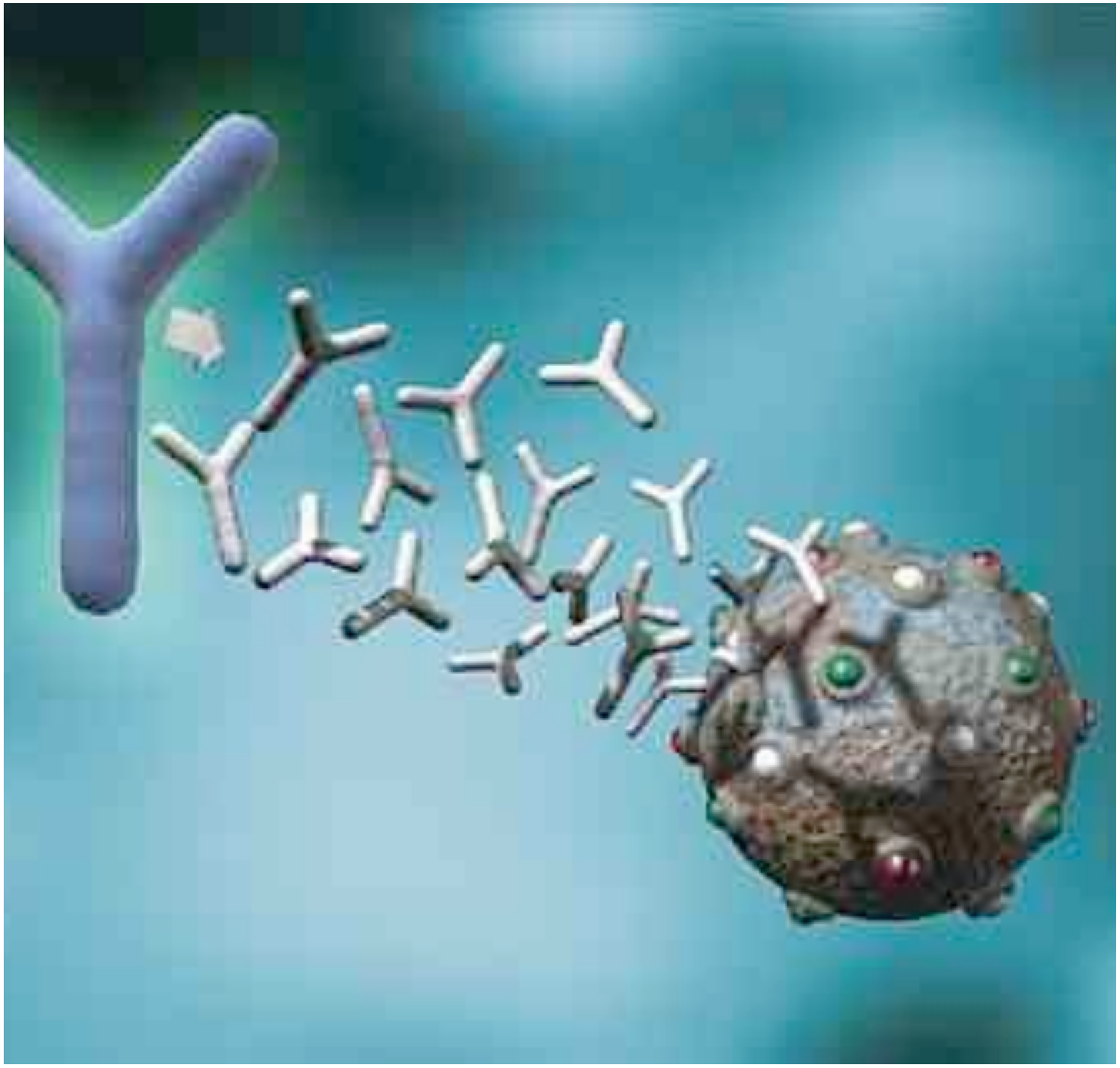
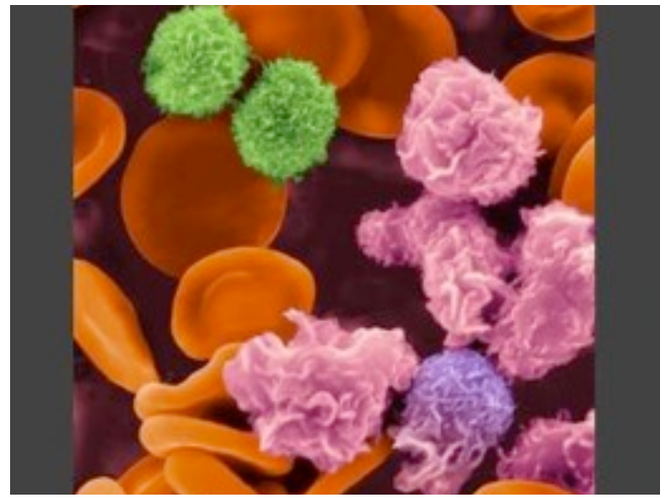
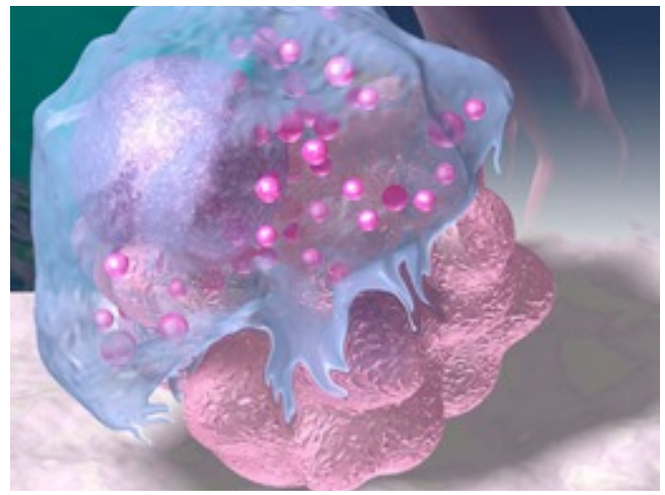
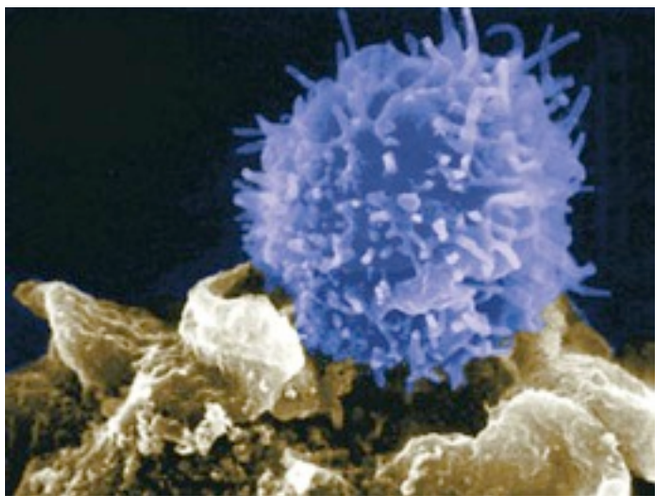
THANK YOU



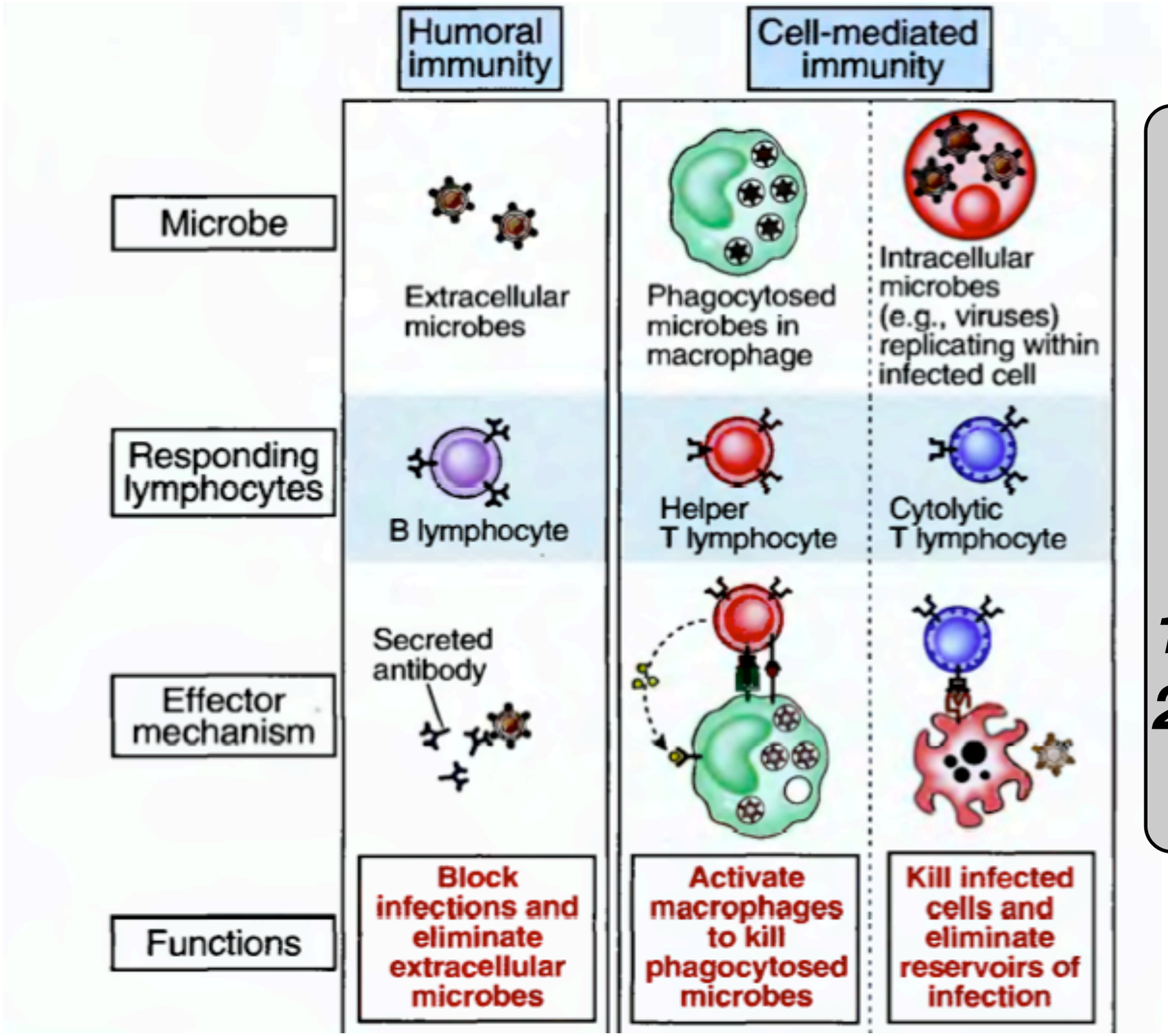






Biology 151 Lecture 4: Cell-mediated & Humoral Immunity

RECALL...



TWO ARMS OF ADAPTIVE IMMUNE RESPONSE:

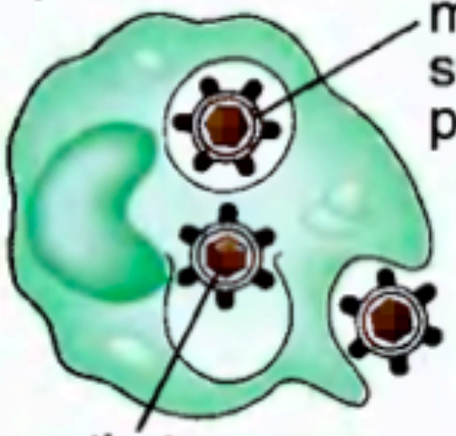
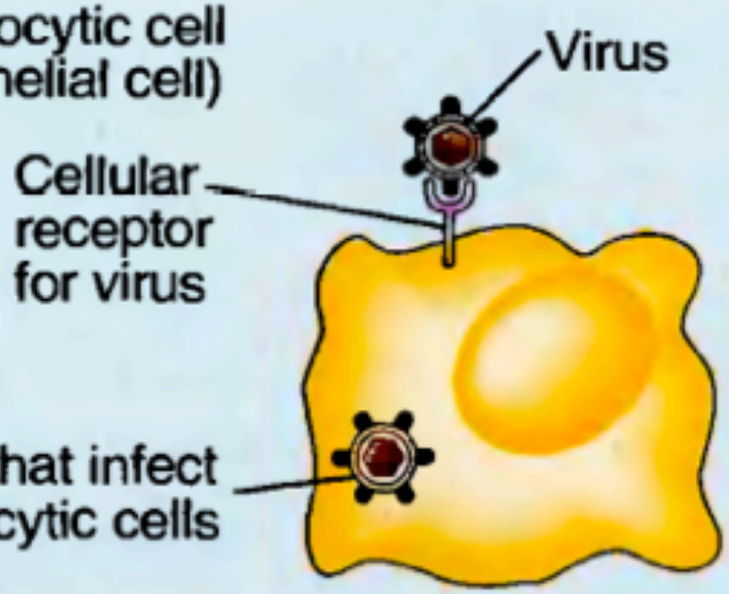
1. ***Humoral***
2. ***Cell-mediated***


CELL-MEDIATED
IMMUNITY

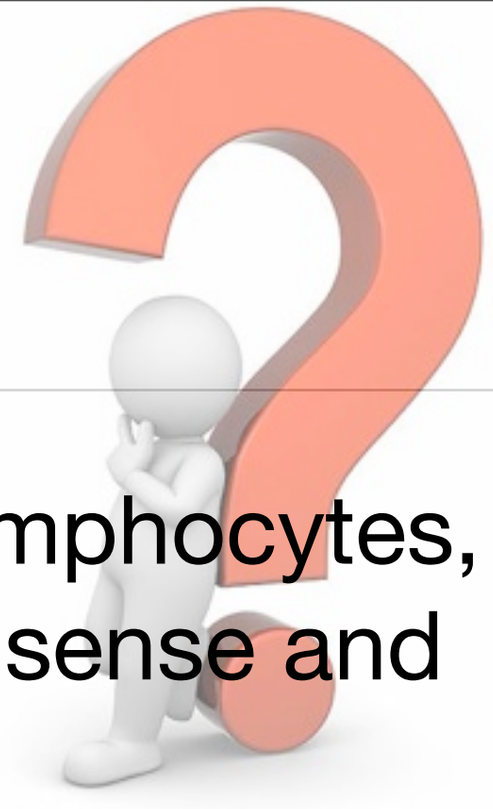
CELL-MEDIATED IMMUNITY

- combat infections by INTRACELLULAR microbes
- mediated by T-lymphocytes
- **TYPES OF INTRACELLULAR MICROBES:**
  - microbes ingested by phagocytes (early defense mechanism of innate that developed defense or evasion mechanisms) can enter cytoplasm and multiply
  - viruses that bind to host receptor and replicate in cytoplasm

TYPES OF INTRACELLULAR MICROBES

Intracellular microbes	Examples
(A) Phagocyte  	Intracellular bacteria: Mycobacteria *Listeria moriocytogenes* *Legionella pneumophila* Fungi: *Cryptococcus neoformans* Protozoa: *Leishmania* *Trypanosoma cruzi*
(B) Nonphagocytic cell (e.g. epithelial cell)  	Viruses: All Rickettsiae: All Protozoa: *Plasmodium falciparum* *Cryptosporidium parvum*

IMPORTANT POINTS

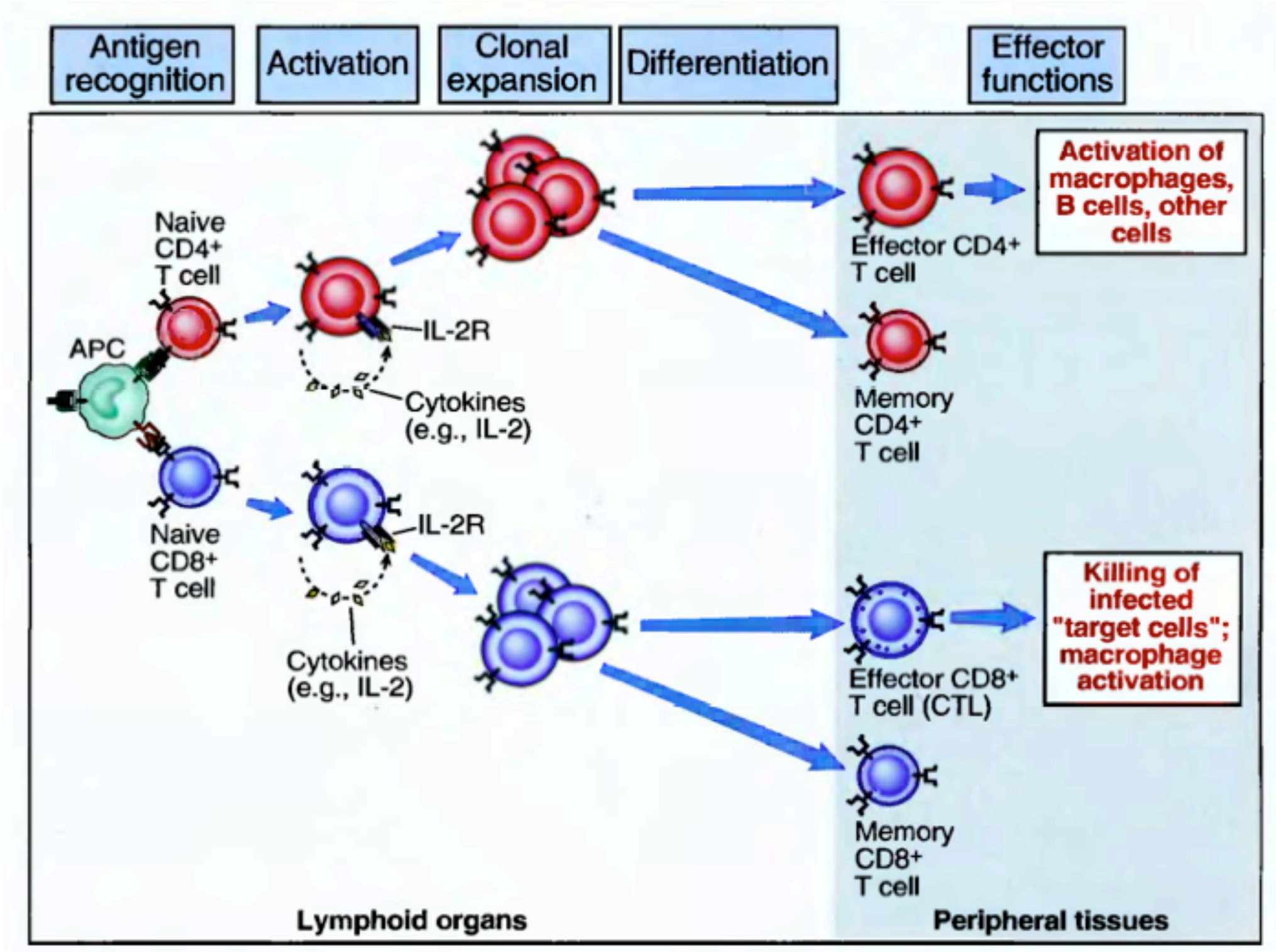


- What ***signals*** are needed to activate T-lymphocytes, and what ***cellular receptors*** are used to sense and respond to these signals?

- How are the few naive T-cells specific for any microbe ***converted*** into the large number of ***effector T-cells*** endowed with the ability to ***eliminate*** the microbe?

- What molecules are produced by T-lymphocytes that ***mediate their communications*** with other cells, such as macrophages and B-lymphocytes?

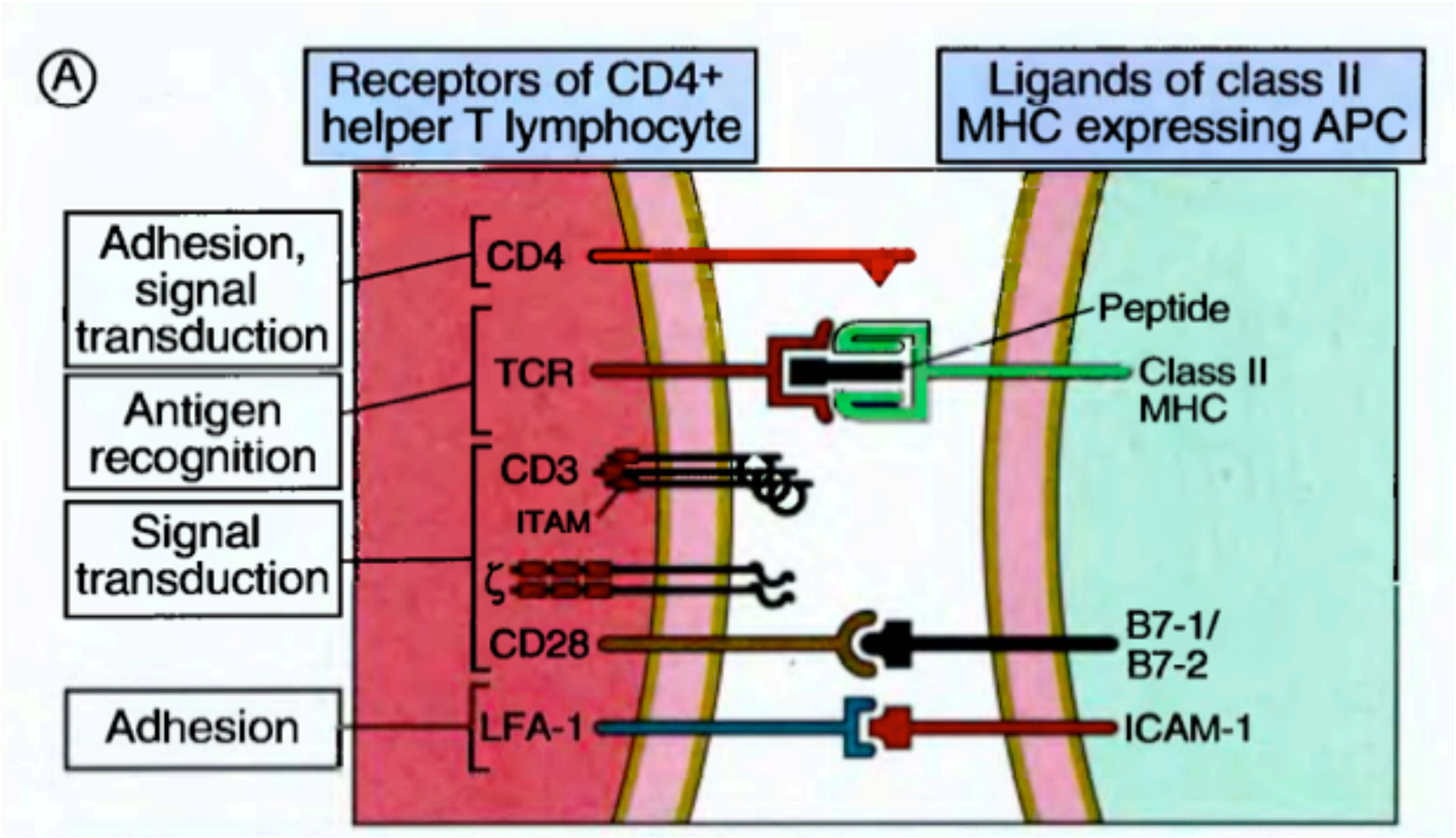
PHASES OF T-CELL RESPONSE



PHASES OF T-CELL RESPONSE

- The initiation of T cell responses requires multiple receptors on the T-cells recognizing ligands on APCs:
  - TCR recognizes MHC-associated peptide antigens
  - CD4 or CD8 coreceptors recognize the MHC molecules
  - Adhesion molecules strengthen the binding of T cells to APCs
  - Receptors for costimulators recognize second signals provided by the APCs

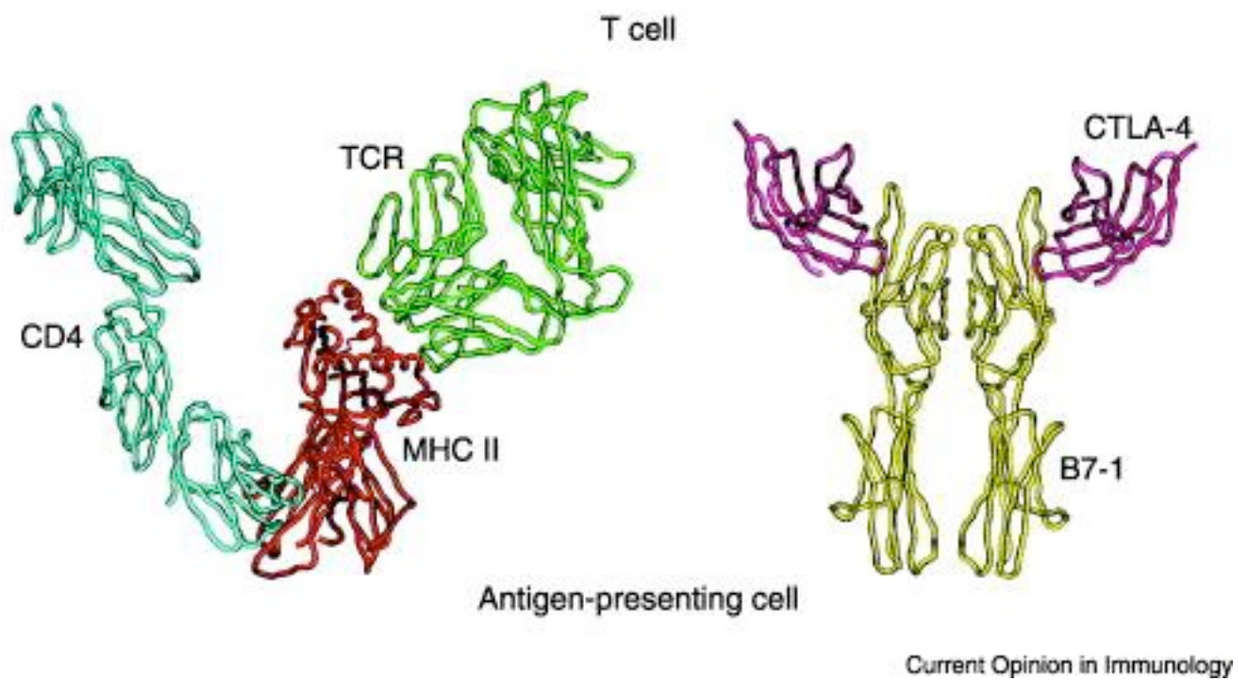
ANTIGEN RECOGNITION & STIMULATION (Receptor-Ligand Pairs in T-cell Activation)



PHASES OF T-CELL RESPONSE

- ***ACCESSORY MOLECULES***: molecules other than antigen receptors that are involved in T-cell responses to antigens
  - Invariant among all T cells
  - bind to different ligands
  - each of these interactions plays a distinct and and complementary role in the process of T-cell activation

- ***FUNCTION:***
  - Recognition
  - Signaling
  - Adhesion

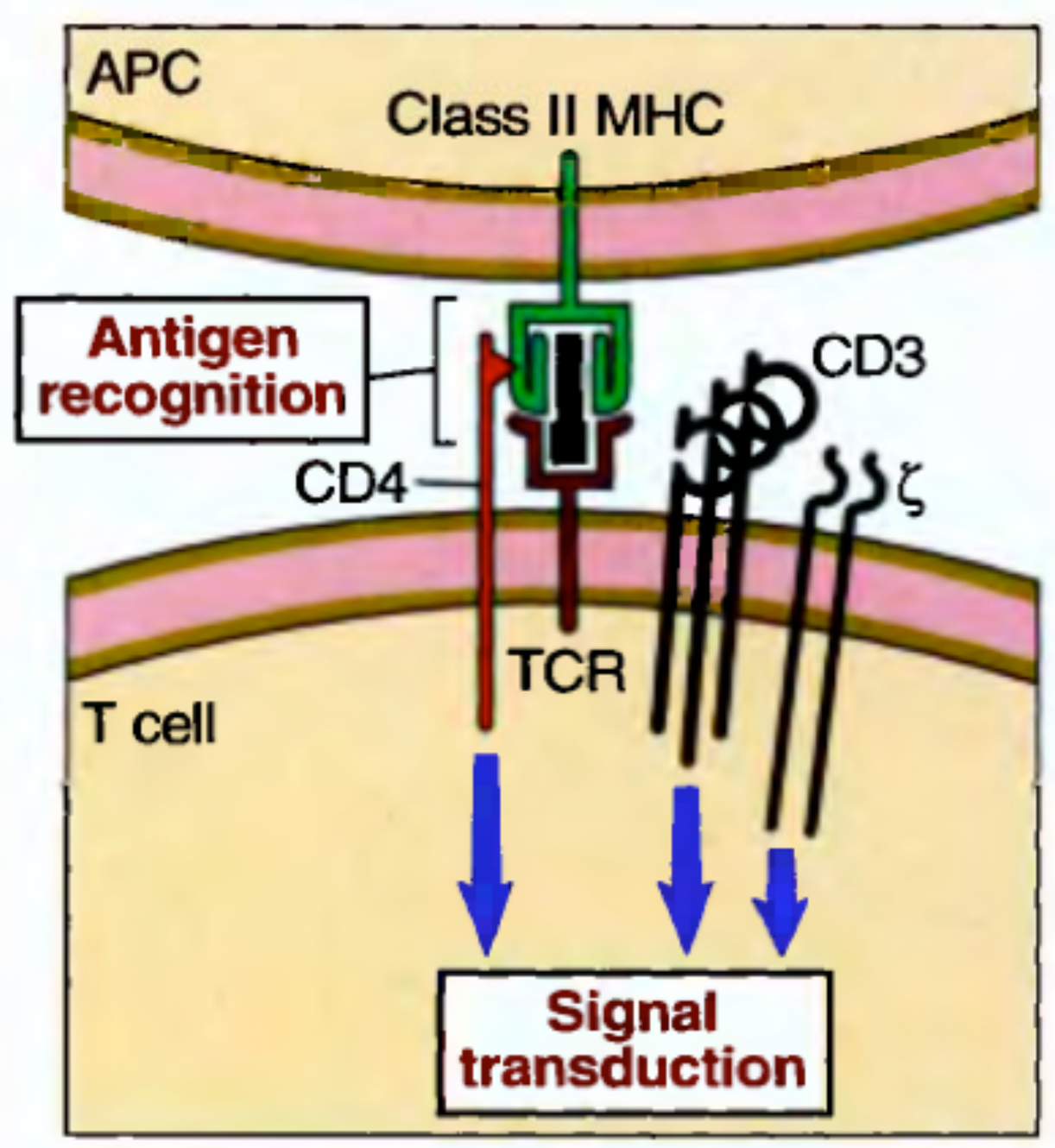


ANTIGEN RECOGNITION & STIMULATION (Receptor-Ligand Pairs in T-cell Activation)

Ⓑ T cell accessory molecule	Function	Ligand	
		Name	Expressed on
CD3	Signal transduction by TCR complex	None	
ζ	Signal transduction by TCR complex	None	
CD4	Adhesion and signal transduction	Class II MHC	Antigen-presenting cells
CD8	Adhesion and signal transduction	Class I MHC	Antigen-presenting cells, CTL target cells
CD28	Signal transduction (costimulation)	B7-1/B7-2	Antigen-presenting cells
CTLA-4	Signal transduction (negative regulation)	B7-1/B7-2	Antigen-presenting cells
LFA-1	Adhesion	ICAM-1	Antigen-presenting cells, endothelium
VLA-4	Adhesion	VCAM-1	Endothelium

RECOGNITION OF MHC-ASSOCIATED PEPTIDES

- **INITIATING SIGNAL FOR T-CELL ACTIVATION ANTIGEN RECOGNITION:**
  - T-cell receptor for antigen (the TCR) and the CD4 or CD8 coreceptor together recognize the complex of peptide antigens and MHC molecules on APCs
- **SIGNAL TRANSDUCTION** leading to T-cell activation:
  - biochemical signals are triggered by a set of proteins that are linked to the TCR to form TCR complex and by the CD4 and CD8 coreceptors (CD3, etc)



ROLE OF ADHESION MOLECULES IN T-CELL ACTIVATION

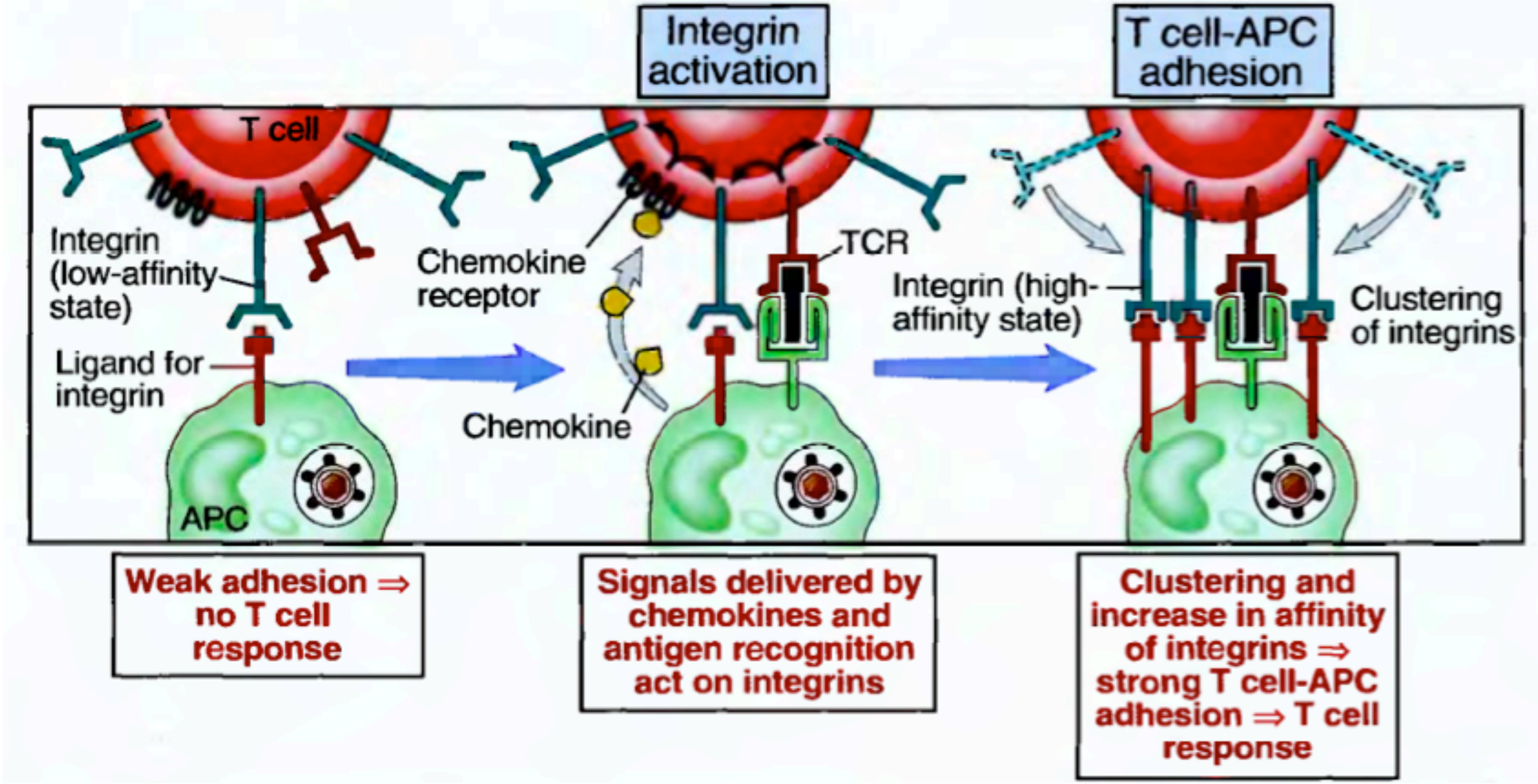
- Adhesion molecules on T-cells recognize their ligands on APCs and stabilize the binding of the T-cells to the APCs

- INTEGRINS (e.g. leukocyte function-associated antigen-1 or LFA-1; ligand is ICAM-1)

  - ***enhances*** T-cell responses to microbial antigens

  - ***directs*** migration of effector T-cells from circulation to sites of infection

REGULATION OF INTEGRIN AVIDITY



ROLE OF COSTIMULATION IN T-CELL ACTIVATION

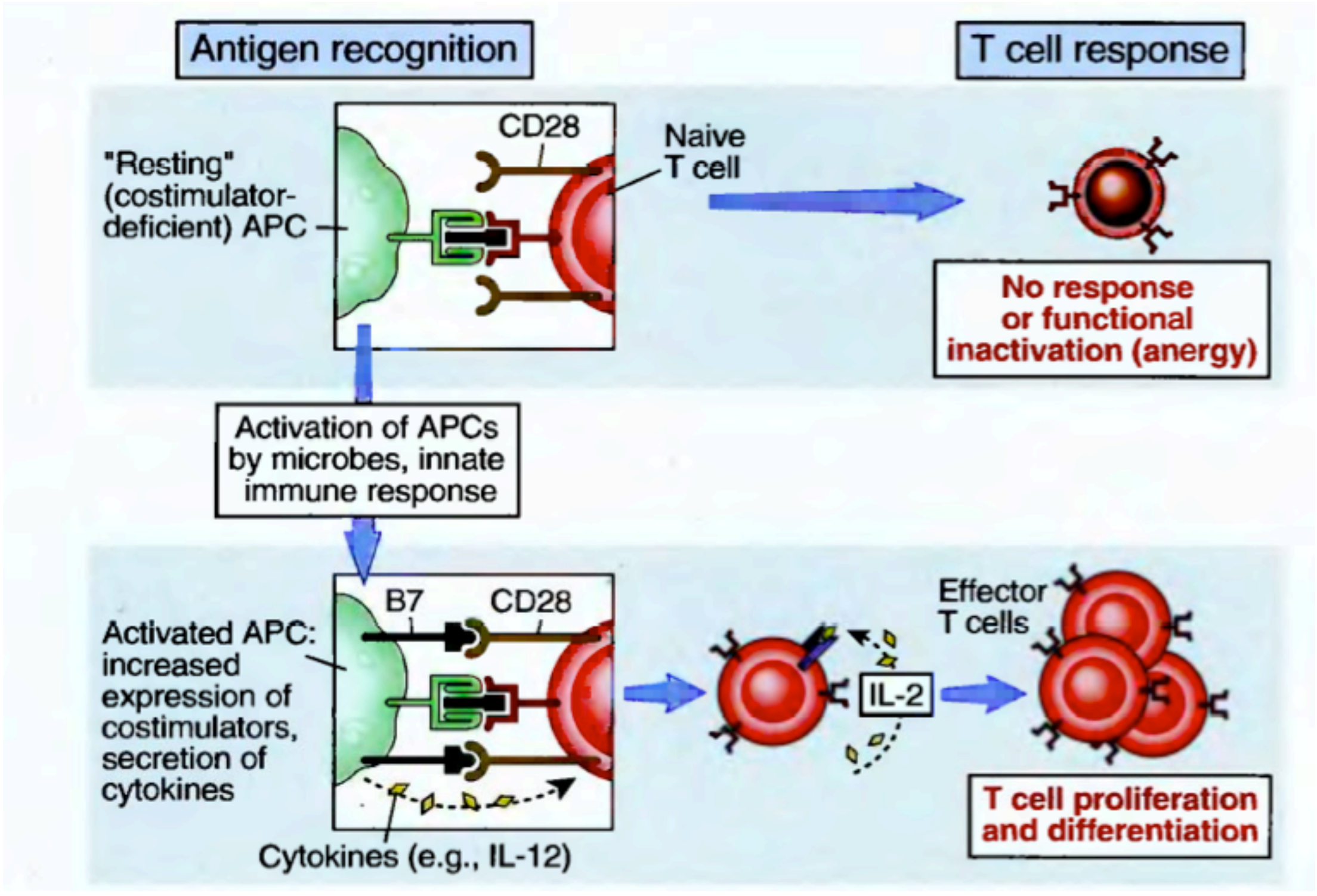
- The full activation of T-cells is dependent on the recognition of costimulators on APCs = “second signals” that provide ***stimuli*** to T-cells

- e.g. B7-1 (CD80) and B7-2 (CD86): expression is greatly increased when the APCs encounter microbes

    - B7 proteins are recognized by CD28 receptor which is expressed on virtually all cells

- costimulation ***ensures*** that naive T-lymphocytes are activated fully by microbial antigens

CO-STIMULATION IN T-CELL ACTIVATION



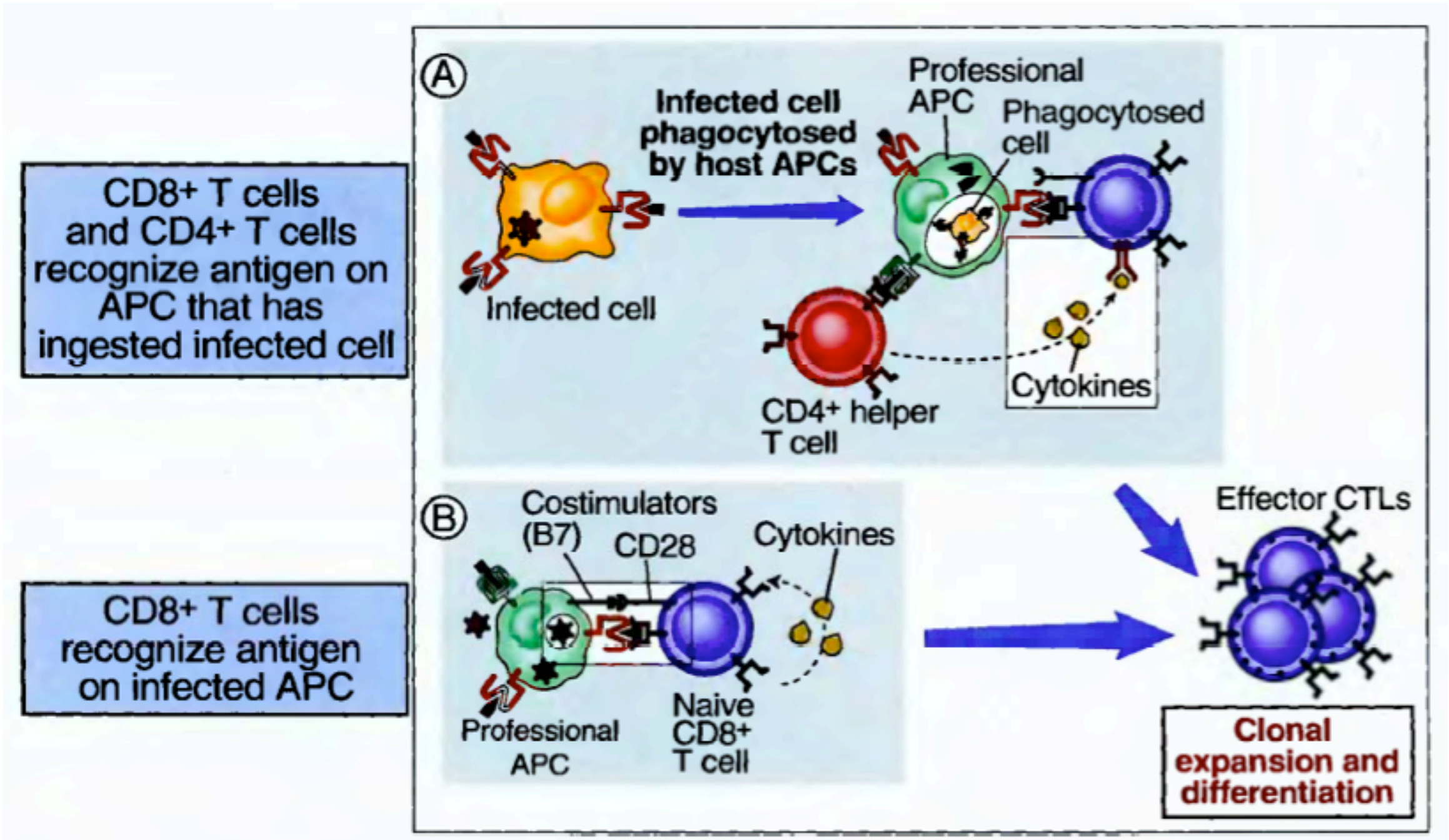
ACTIVATION OF CD8+ T-CELLS

- CD8+ T-cells recognize peptides that may be produced from cytoplasmic proteins, such as viral proteins, in any nucleated cell

- in SOME viral infections: requires the concomitant activation of CD4+ helper T-cells (co-presentation/cross-priming)

- ***<u>NOTE: HIV case on targeting CD4+ T-cells</u>***

ACTIVATION OF CD8+ T-CELLS



HOW T-LYMPHOCYTES RESPOND TO STIMULI

- many of the responses of T-cells are mediated by cytokines that are secreted by T-cells themselves

- in response to antigen and co-stimulators, T-lymphocytes (CD4+ T-cells), rapidly secrete several different cytokines that have diverse activities

CYTOKINES PRODUCED BY CD4+ T-CELL

Ⓐ General properties of cytokines

Property	Mechanism
Produced transiently in response to antigen	TCR signal and costimulation induce cytokine gene transcription
Usually acts on same cell that produces the cytokine (autocrine) or nearby cells (paracrine)	T cell activation induces expression of both cytokines and high-affinity receptors for cytokines
Pleiotropism: each cytokine has multiple biologic actions	Many different cell types may express receptors for a particular cytokine
Redundancy: multiple cytokines may share the same or similar biologic activities	Many cytokines use same conserved signaling pathways

CYTOKINES PRODUCED BY CD4+ T-CELL

Ⓑ Biologic actions of selected T cell cytokines

Cytokine	Principal action	Cellular source(s)
Interleukin-2 (IL-2)	T cell growth stimulation	$CD4^+$ and $CD8^+$ T cells
IL-4	B cell switching to IgE	$CD4^+$ T cells, mast cells
IL-5	Activation of eosinophils	$CD4^+$ T cells, mast cells
Interferon-γ (IFN-γ)	Activation of macrophages	$CD4^+$ and $CD8^+$ T cells, natural killer cells
TGF-β	Inhibition of T cell activation	$CD4^+$ T cells; many other cell types

INTERLEUKINS

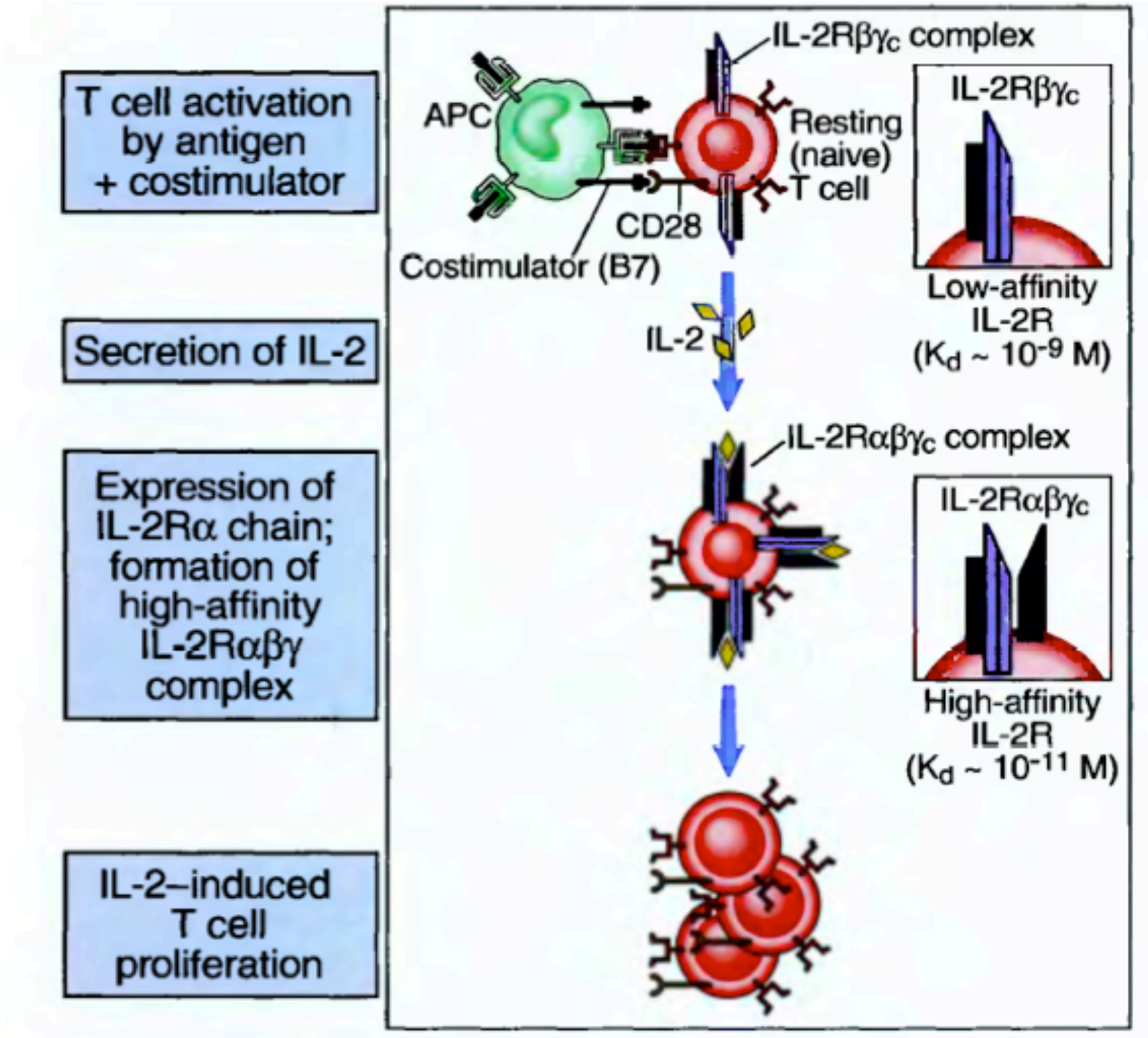
- proteins produced by leukocytes to act on leukocytes

- **IL-2:** first cytokine to be produced by CD4+ T-cells (within 1-2 hours after activation)

  - also called ***T-cell growth factor*** = principal action is to stimulate proliferation of T-cells

- ***NOTE:*** CD8+ T-lymphocytes that recognize antigen and costimulators do not appear to secrete large amounts of IL-2 ***BUT*** CD8+ activation may require help from CD4+ T-cells that are activated nearby to provide IL-2

IL-1 & IL-2 IN T-CELL PROLIFERATION



CLONAL EXPANSION

- within 1-2 days after activation, T-lymphocytes begin to proliferate, resulting in expansion of antigen-specific clones

- provides a large pool of antigen-specific lymphocytes from which effector cells can be generated to COMBAT infections

- ***FEATURES***:

    - not accompanied by increase in “bystander cells” that do not recognize that microbes

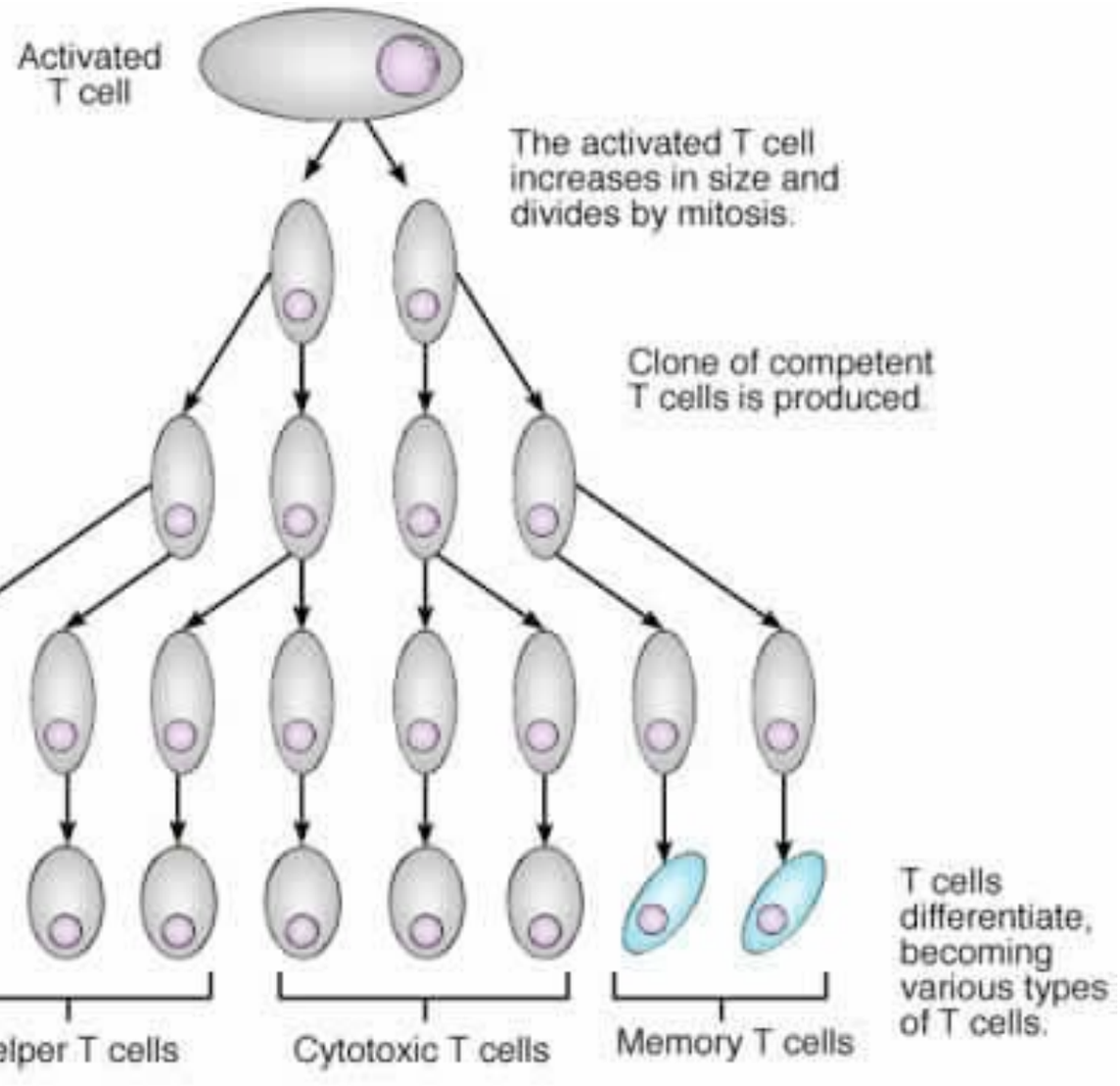
    - even in infections with complex microbes that contain many protein antigens, clones specific only for the immunodominant peptides of the microbe

CLONAL EXPANSION

- ***<u>NOTE: magnitude of expansion less in CD4+ than CD8+ T-cells due to difference in function</u>***

  - CD8+ CTLs = kill infected cells themselves, thus many are needed

  - CD4+ effector cells = activate other effector cells, small number may suffice



DIFFERENTIATION OF NAIVE T-CELLS INTO EFFECTOR CELLS

- result of changes in gene expression or cytolytic proteins
- appear within 3-4 days after exposure to microbes beginning with clonal expansion
- cells leave the peripheral lymphoid organs and migrate to the site of infection where they meet the antigens again (which stimulated their development)
- upon recognition, effector cells respond to eradicate the infection (thus each effector cell has a distinct pattern of differentiation

MOLECULES INVOLVED IN CD4+ EFFECTOR FUNCTIONS

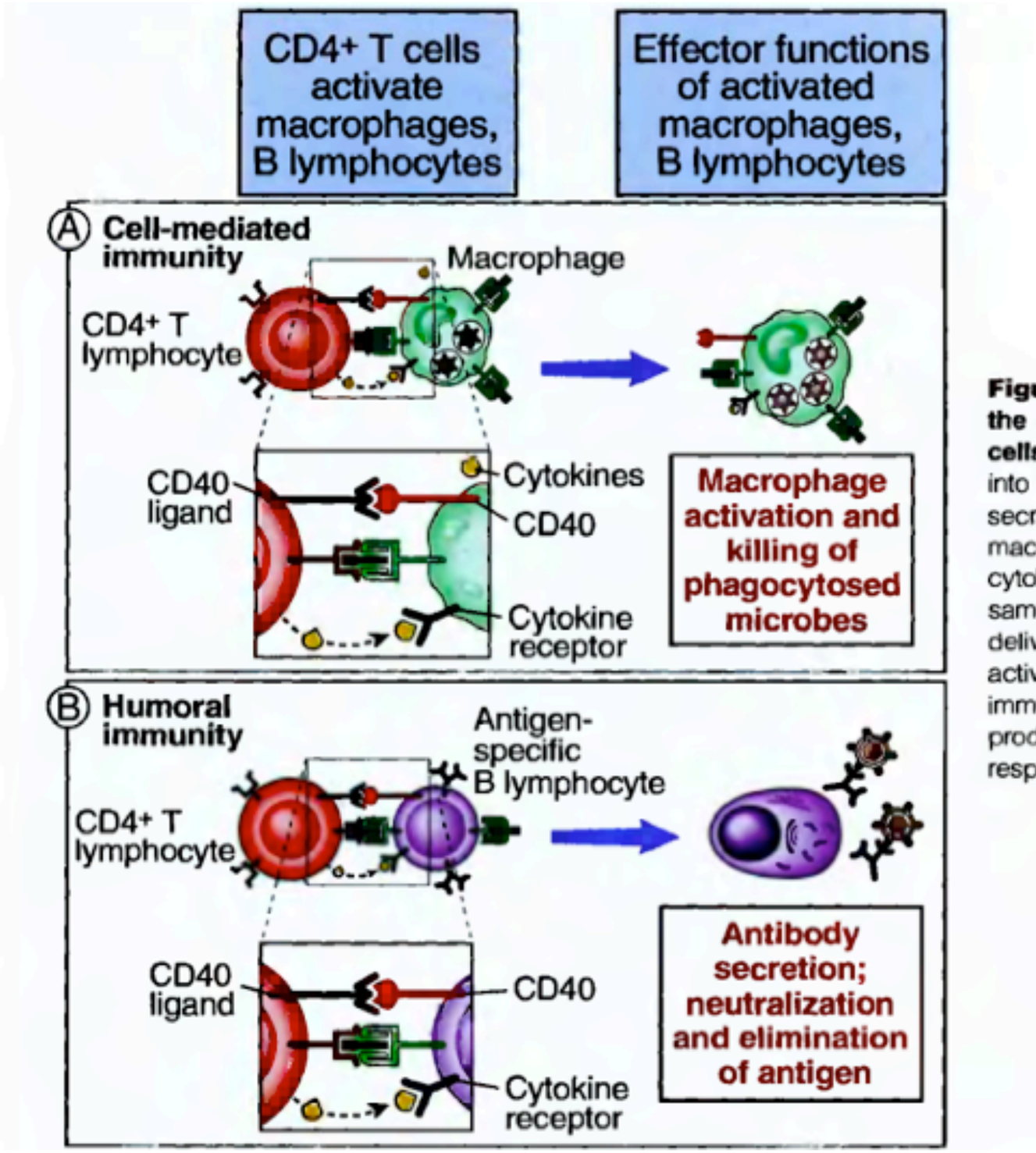
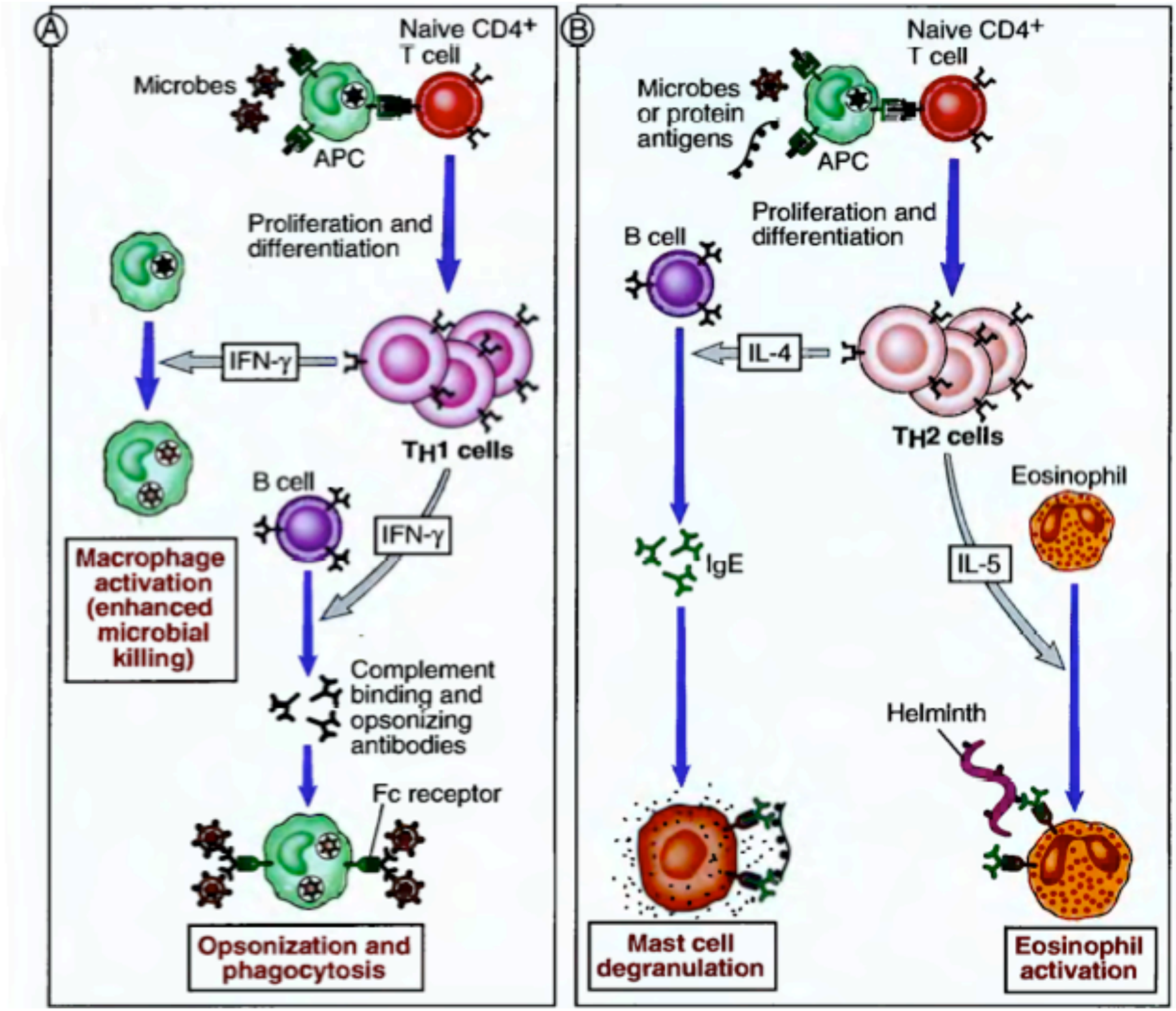


Figure 5–10 The molecules involved in the effector functions of CD4+ helper T cells. CD4+ T cells that have differentiated into effector cells express CD40L and secrete cytokines. CD40L binds to CD40 on macrophages or B lymphocytes, and cytokines bind to their receptors on the same cells. The combination of signals delivered by CD40 and cytokine receptors activates macrophages in cell-mediated immunity (A) and activates B cells to produce antibodies in humoral immune responses (B).

DIFFERENTIATION OF NAIVE T-CELLS INTO EFFECTOR CELLS

- CD4+ helpers: differentiate into effector cells that respond to antigen
  - surface molecules and cytokines production to activate macrophages and B-lymphocytes
  - differentiate into subsets of effector cells that produce distinct sets of cytokines that perform different functions (e.g. ***TH1 &TH2*** cells)

TH1 & TH2 SUBSETS OF CD4+ T LYMPHOCYTES



TH1: stimulate phagocyte-mediated ingestion and killing of microbes (IFN-g)

TH2: stimulate phagocyte-independent, eosinophil-mediated immunity (IL-5;parasitic)

NOTE: TH2 may produce cytokines (IL-4, IL-10 and IL-13) that inhibit macrophage activation and suppress TH1 CMI = Balancing between activation in response to microbes

DIFFERENTIATION OF NAIVE T-CELLS INTO EFFECTOR CELLS

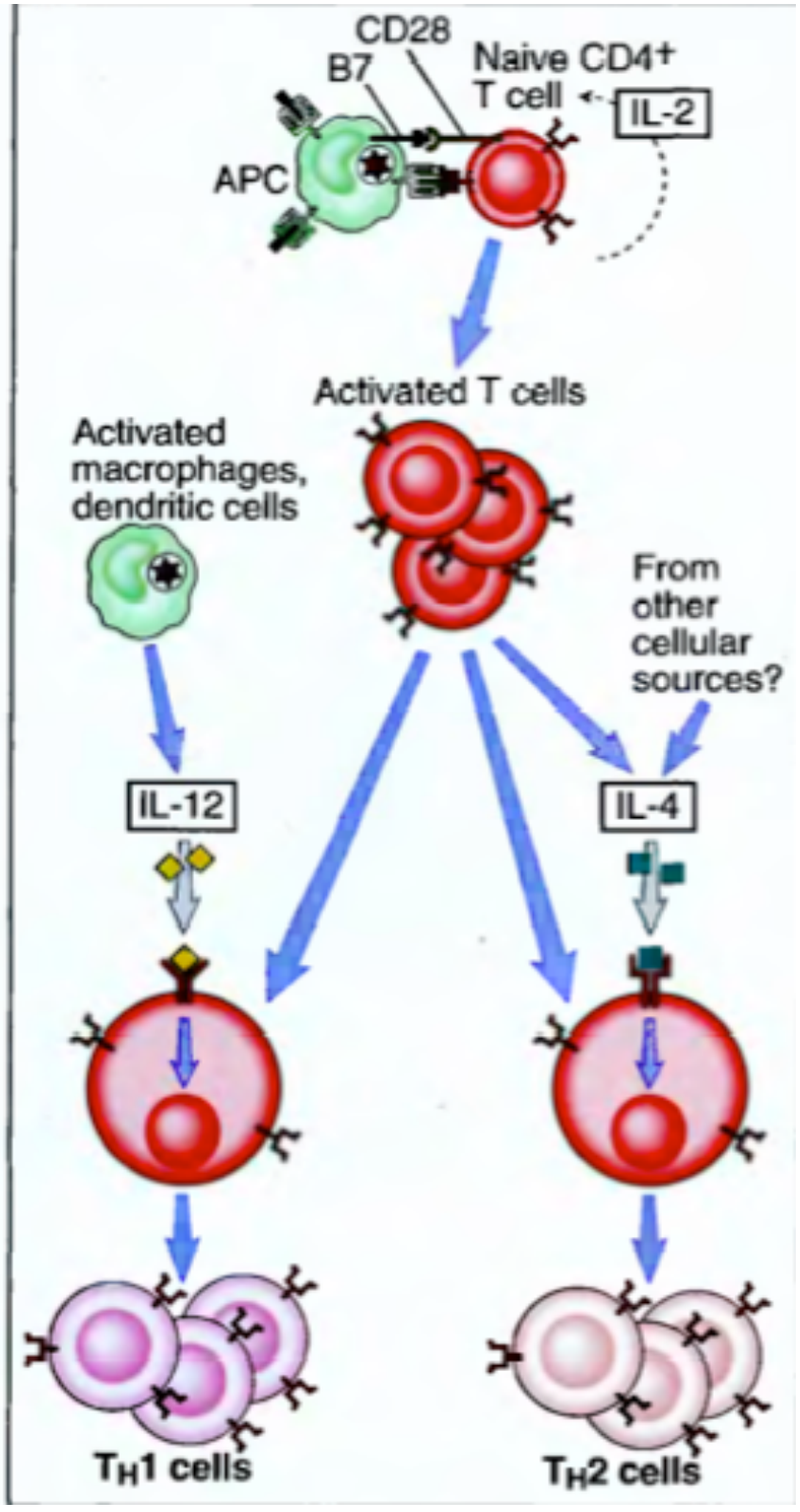
- e.g. ***INTERFERON-g (IFN-g)*** = cytokine that inhibited or interfered with viral infection
  - potent activator of macrophages
  - stimulates production of antibody isotypes that promotes phagocytosis of microbes
    - ***NOTE: these antibodies bind directly to phagocyte Fc receptors = activate complement***

TH1 & TH2 SUBSETS OF CD4+ T LYMPHOCYTES

© Property	T_H1 subset	T_H2 subset
Cytokines produced		
IFN-γ, IL-2, TNF	+++	-
IL-4, IL-5, IL-13	-	+++
IL-10	+/-	++
IL-3, GM-CSF	++	++
Cytokine receptor expression		
IL-12R β chain	++	-
IL-18R	++	-
Chemokine receptor expression		
CCR3, CCR4	+/-	++
CXCR3, CCR5	++	+/-
Ligands for E- and P- selectin	++	+/-
Antibody isotypes stimulated	IgG2a (mouse)	IgE; IgG1 (mouse)/ IgG4 (humans)
Macrophage activation	+++	-

Figure 5–11, Cont'd C. The main differences between T_H1 and T_H2 subsets of helper T cells are summarized. Note that many helper T cells are not readily classified into these distinct and polarized subsets. The chemokine receptors are called CCR or CXCR because they bind chemokines classified into CC or CXC chemokines based on whether key cysteines are adjacent or separated by one amino acid. Different chemokine receptors control the migration of different types of cells. These, in combination with the selectins, determine whether T_H1 or T_H2 cells dominate in different inflammatory reactions in various tissues.

DIFFERENTIATION OF CD4+ T-CELLS INTO TH1 & TH2 EFFECTOR CELLS



The development of TH1 & TH2 cells is regulated by the stimuli that naive CD4+ T-cells receive when they encounter microbial pathogens

IL-12: promotes differentiation of T-cells into the TH1 subset

IL-4: induces differentiation towards the TH2 subset

DIFFERENTIATION OF NAIVE T-CELLS INTO EFFECTOR CELLS

- CD8+ T-lymphocytes activated by antigen and costimulators differentiate into CTLs that are able to kill infected cells expressing the antigen

- ***HOW?***

  - secretes proteins that create pores in the membranes of the infected cells and induce DNA fragmentation and apoptosis of these cells

- differentiation of naive CD8+ T-cells into effector CTLs is accompanied by the synthesis of the molecules that kill infected cells

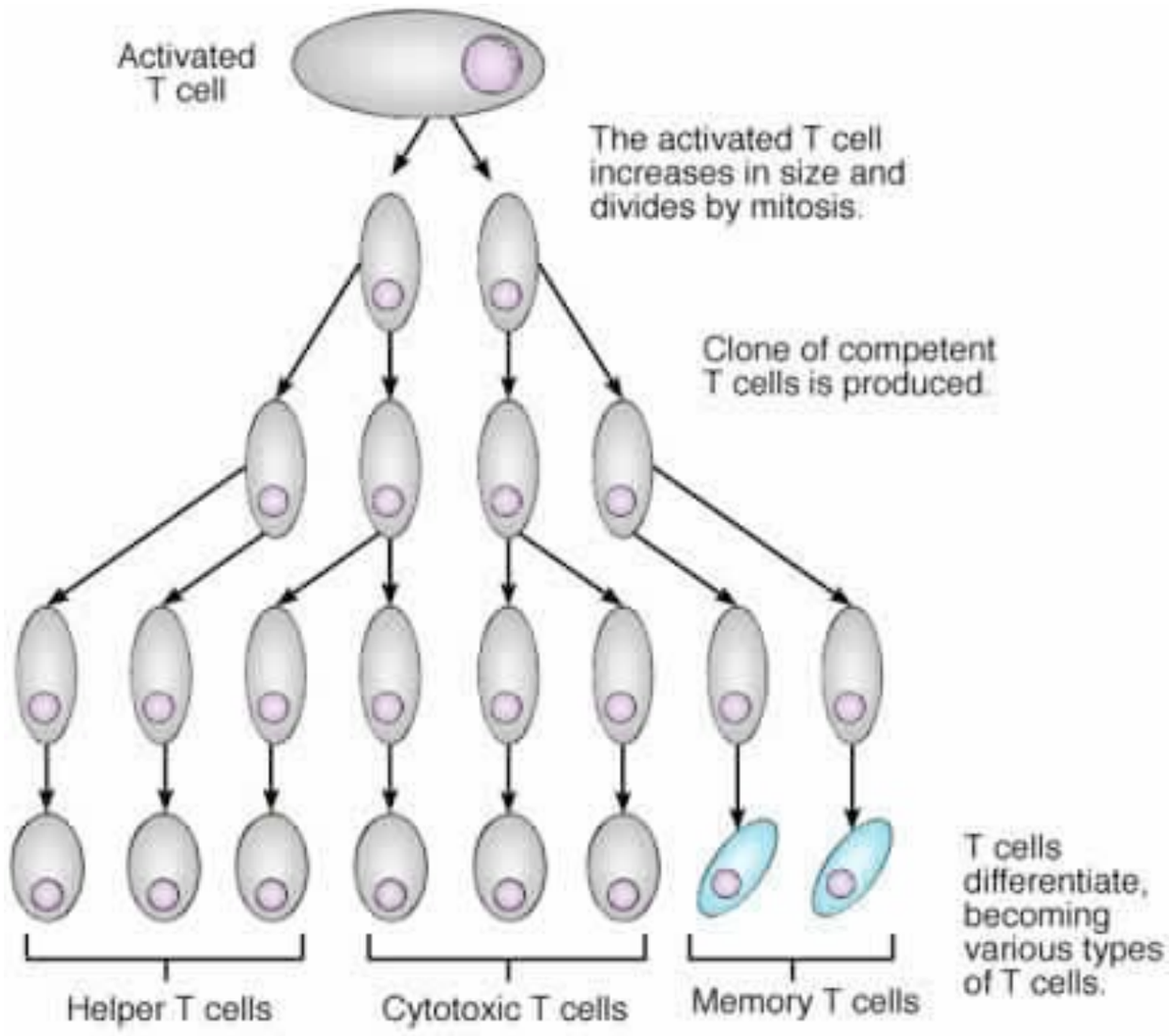
DEVELOPMENT OF MEMORY T-LYMPHOCYTES

- a fraction of antigen-activated T-lymphocytes differentiates into long-lived memory T-cells (survives even after the infection is eradicated)

- found in lymphoid tissues, mucosal barriers and in circulation

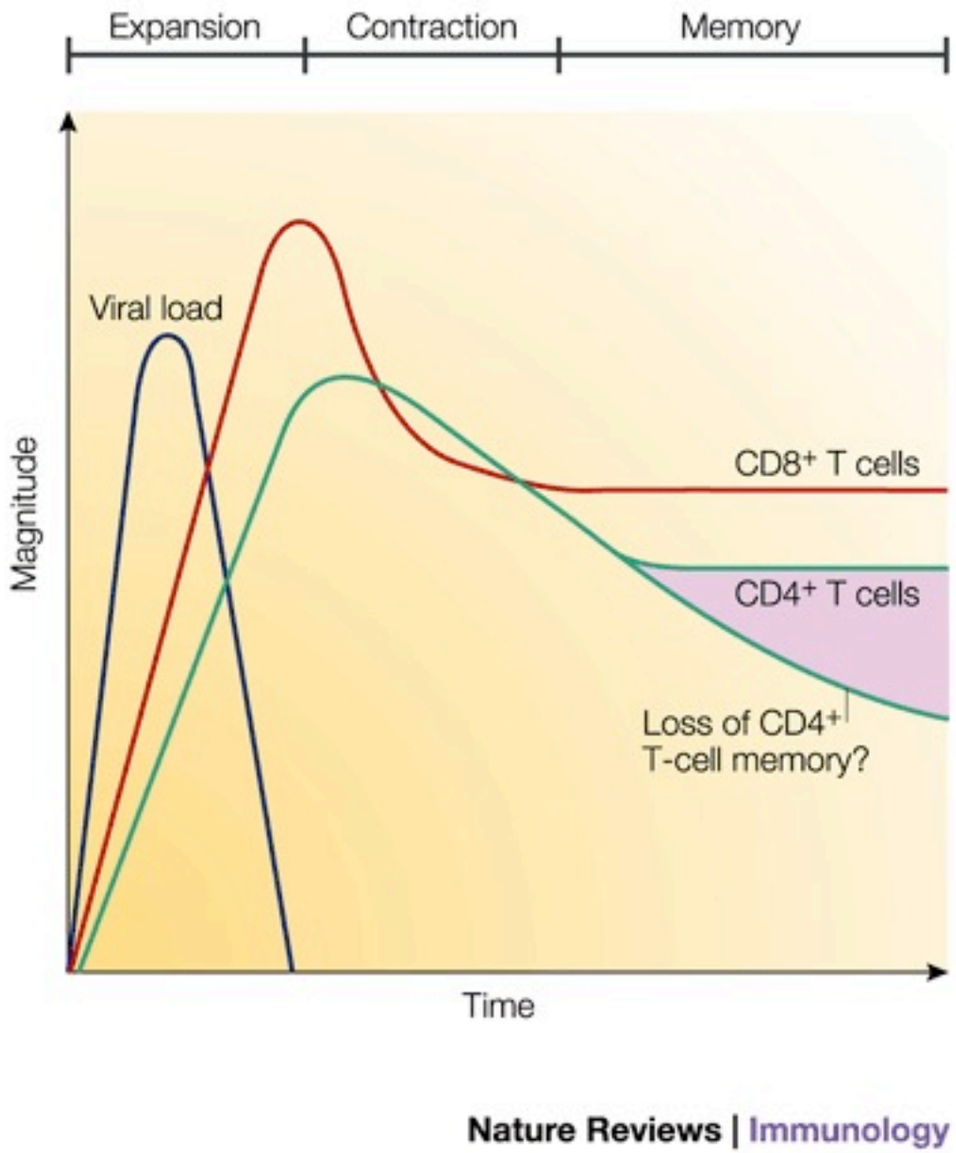
- do not continue to produce cytokines or kill infected cells but upon encounter with antigen (that they recognize) they will rapidly differentiate into effector cells

- ***"lymphocytes in waiting"***



DECLINE OF IMMUNE RESPONSE

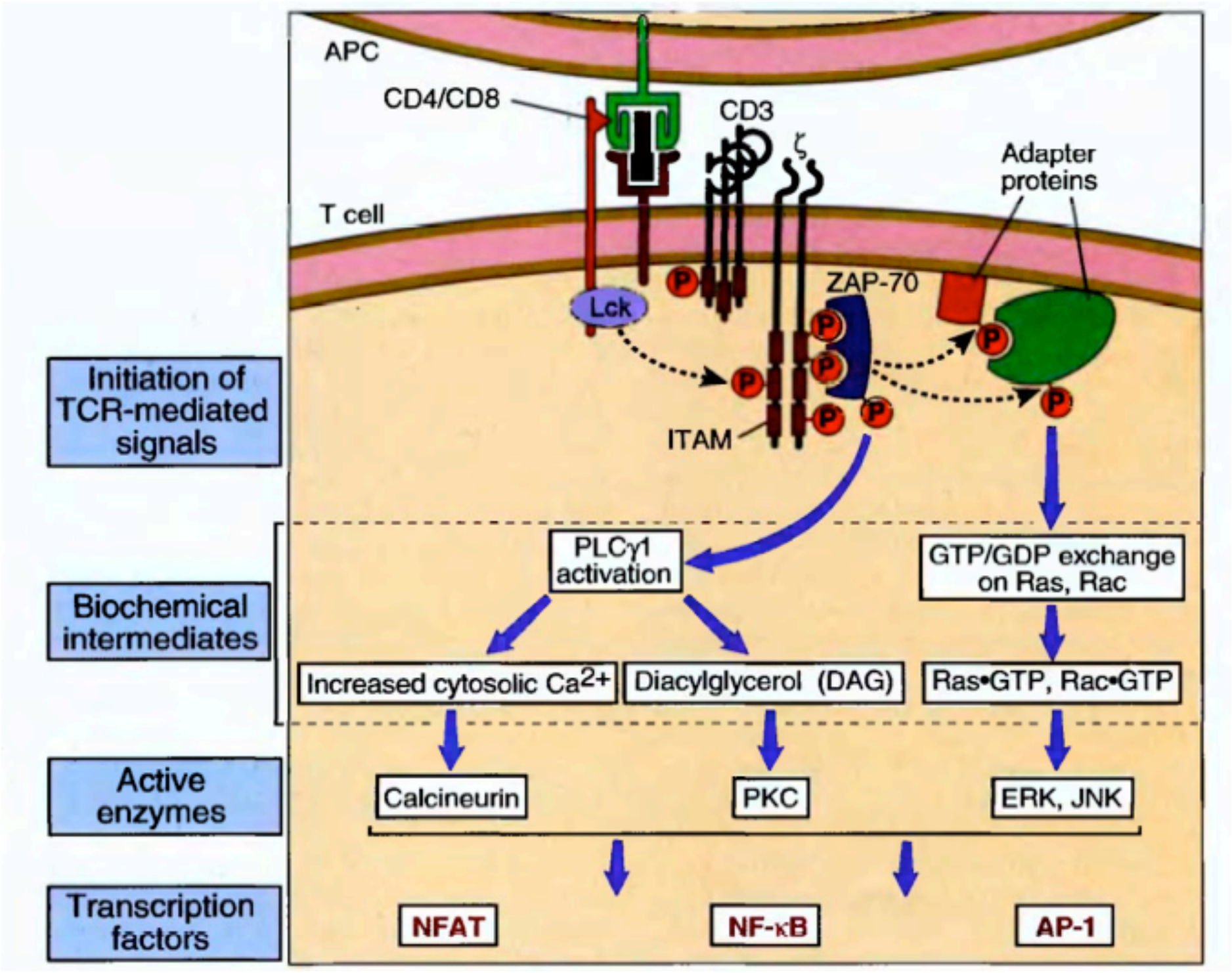
- As the infection is cleared and the stimuli for lymphocyte activation disappear, many of the cells that had proliferated in response to antigen are deprived of survival factors
    - As a result, these cells die via apoptosis (programmed cell death)
- Response subsides within 1 or 2 weeks after the infection is eradicated (only sign that a T cell-mediated immune response had occurred is the pool of surviving memory lymphocytes)



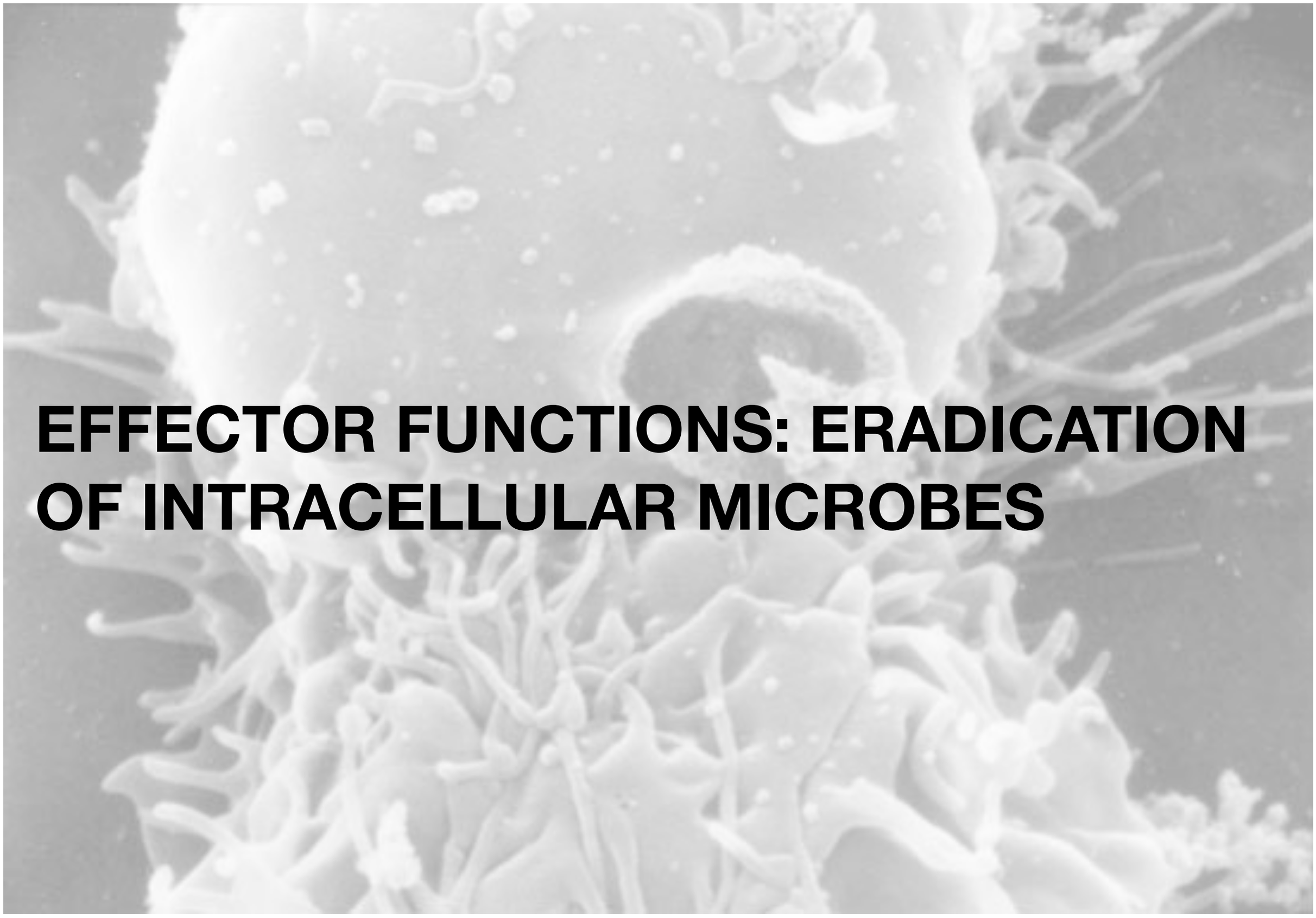
IT'S NOT AN EASY TASK to be a T-cell...

PROBLEMS	SOLUTIONS
Naive T-cells have to find the antigen	APCs concentrate in lymphoid organs where naive T-cells recirculate
Which type of T-cell will respond?	Specificity of CD4 and CD8 co-receptors for class I and II MHC
Can antigen-bearing APCs hold on long enough for the T-cell to be activated?	Adhesion molecules stabilizes T-cell binding to APC for sufficiently long contacts
Should respond to microbial antigens and not to harmless proteins	Co-stimulators are required for T-cell activation induced by APC-microbe
From small numbers to a large pool of effector cells	Amplification mechanisms induced by microbes and activated T-cells

SIGNAL TRANSDUCTION PATHWAYS IN T-LYMPHOCYTES



The biochemical signals triggered in T-cells by antigen recognition result in the ***activation of various transcription factors*** that ***stimulate the expression of genes*** encoding cytokines, cytokine receptors, and other molecules ***involved in T cell responses***

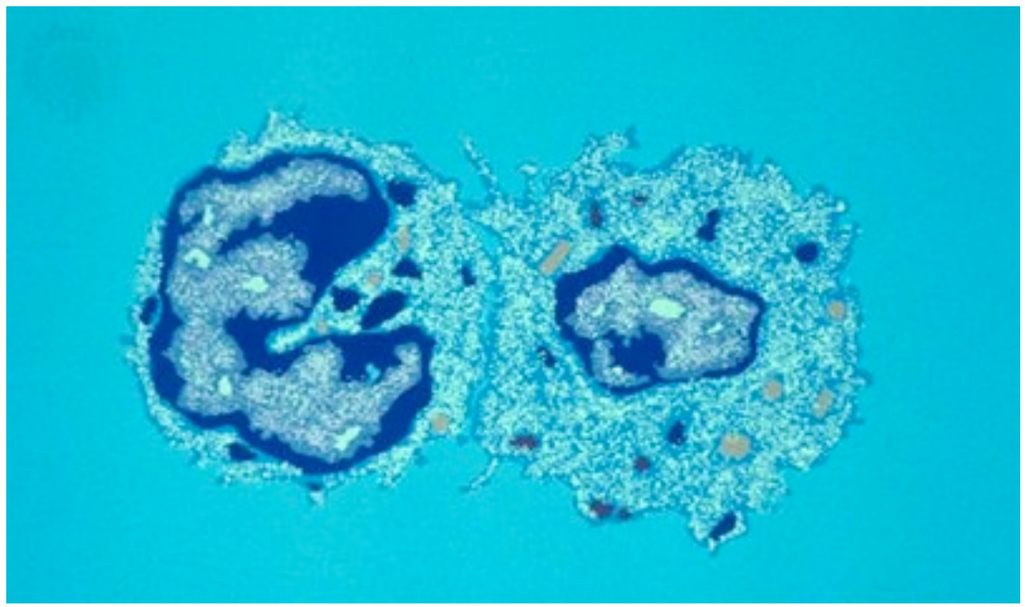


EFFECTOR FUNCTIONS: ERADICATION OF INTRACELLULAR MICROBES

IMPORTANT POINTS

- ***<u>WHAT WE KNOW:</u>***
  - CMI function to eradicate **INTRACELLULAR** microbes
  - Effector phase: T-lymphocytes
  - NOTE: antibodies play **NO** role in eradicating infections by microbes living inside the host
  - **PHASES**: 1) activation; 2) proliferation; 3) differentiation; 4) elimination by effector T-cells

- ***<u>WHAT IS LEFT TO UNDERSTAND</u>***:
  - How do effector T-lymphocytes ***<u>locate</u>*** intracellular microbes at any site in the body:
  - How do effector T-lymphocytes ***<u>eradicate</u>*** infections by these microbes?



CELL-MEDIATED IMMUNITY AGAINST INTRACELLULAR MICROBES

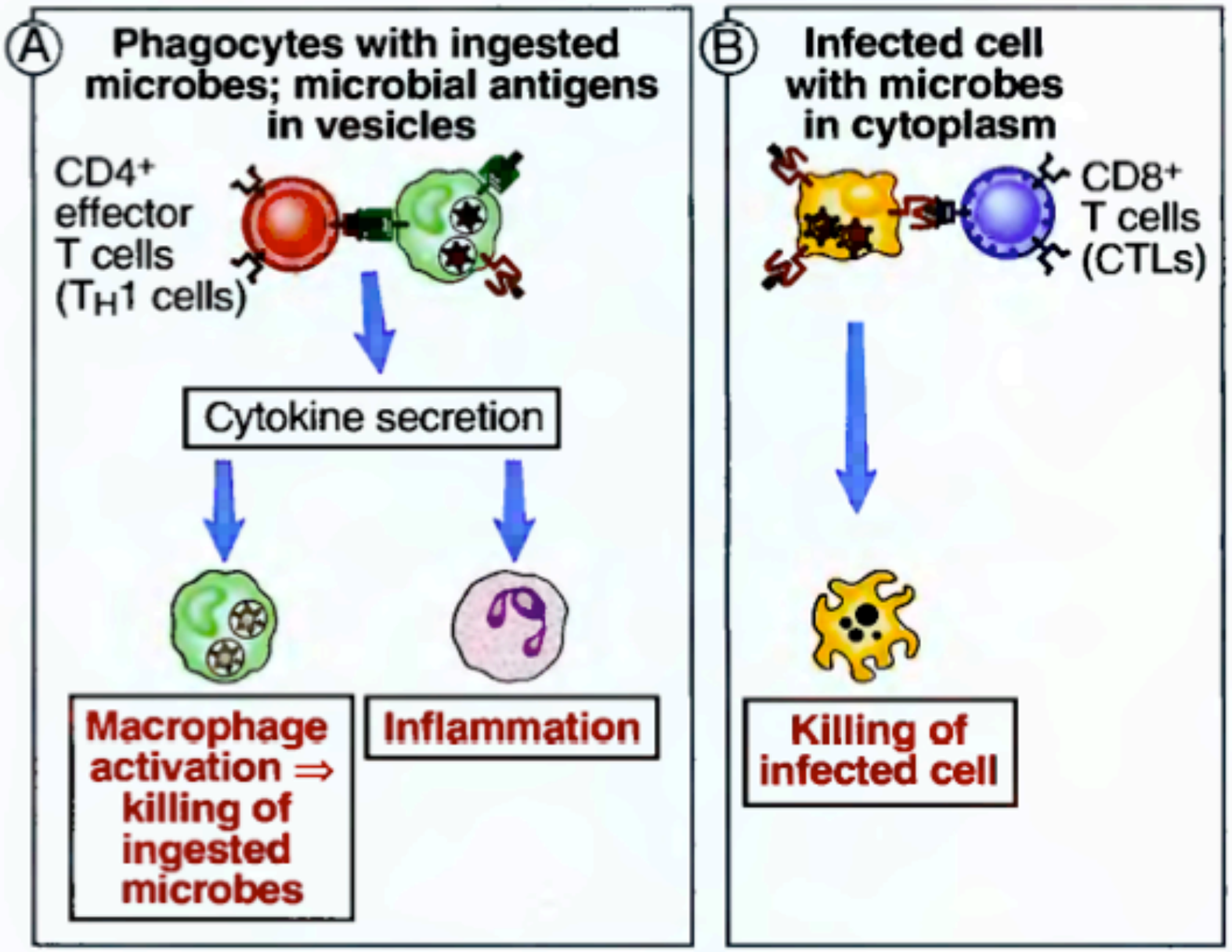
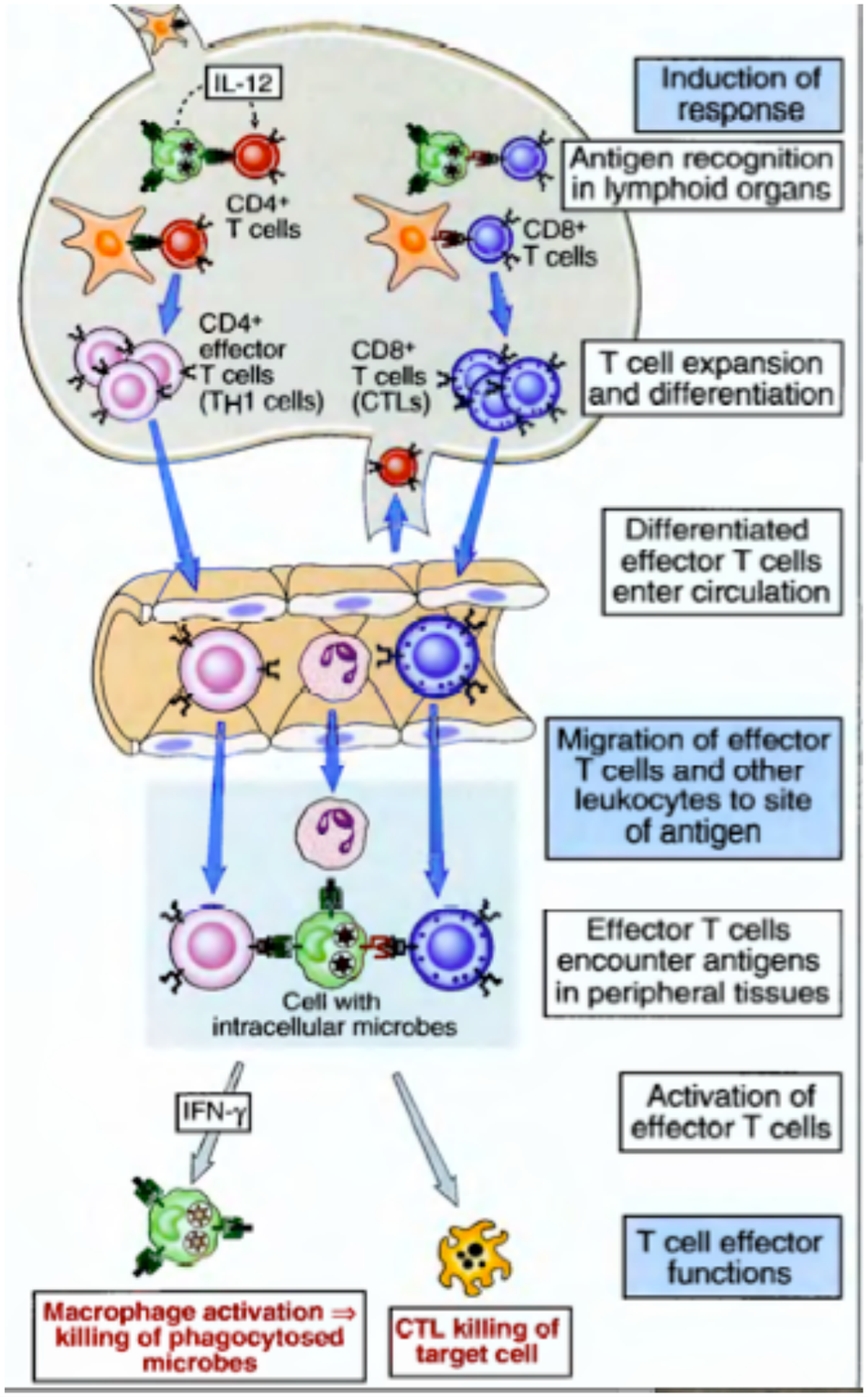


Figure 6–1 Cell-mediated immunity against intracellular microbes. A. Effector T cells of the CD4+ T_H1 subset recognize the antigens of microbes ingested by phagocytes and activate the phagocytes to kill the microbes and induce inflammation. Phagocyte activation and inflammation are responses to cytokines produced by the T cells (discussed later). CD8+ T lymphocytes also produce cytokines that elicit the same reactions, but CD8+ T cells recognize microbial antigens in the cytoplasm of infected cells (not shown). B. CD8+ CTLs kill infected cells with microbes in the cytoplasm. CTLs, cytolytic T lymphocytes.

INDUCTION AND EFFECTOR PHASES OF CMI

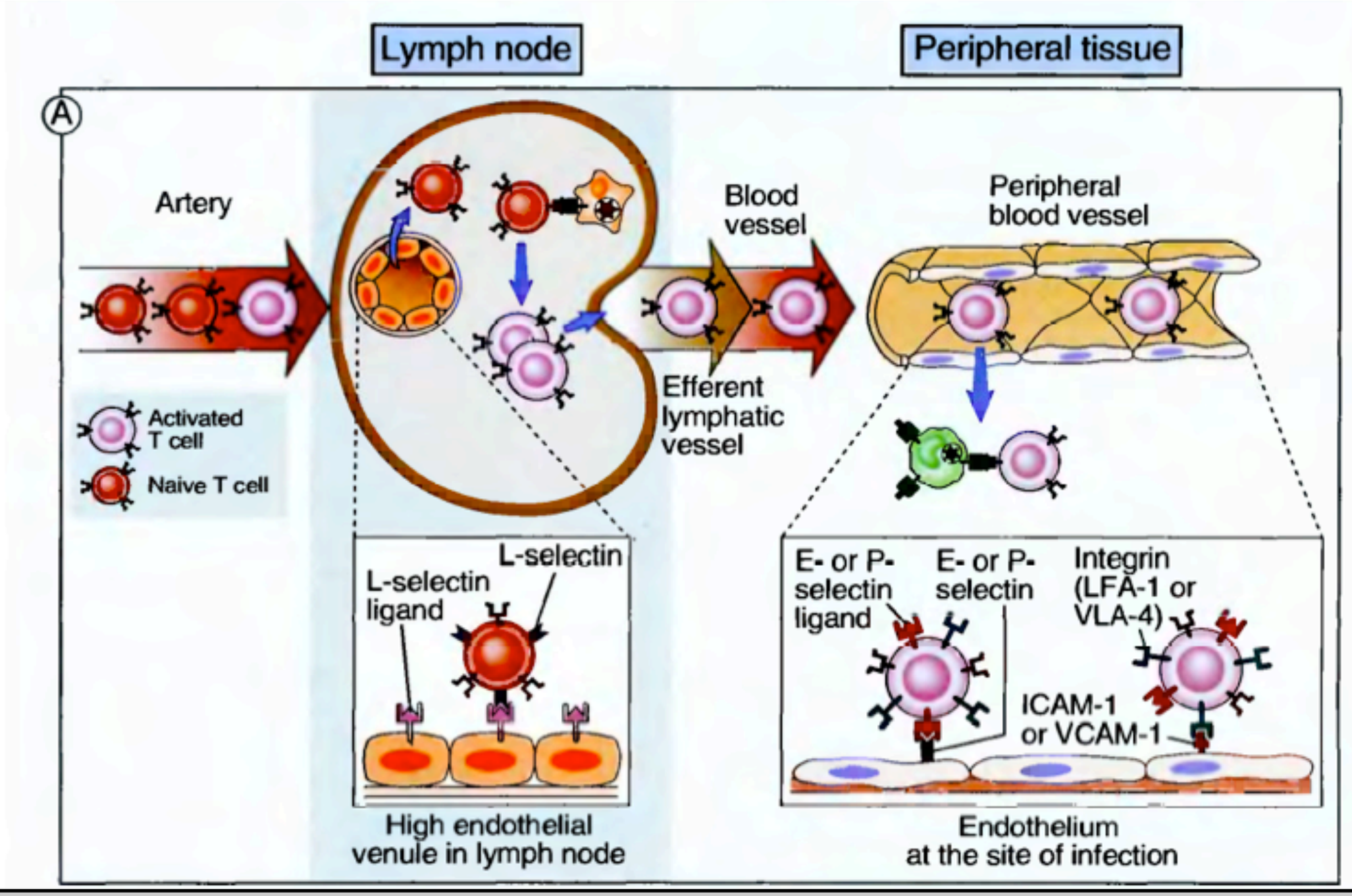


2 STAGES IN PROTEIN ANTIGEN RECOGNITION (CMI)

1. Naive T cells ***recognize*** antigens in lymphoid tissues and respond by ***proliferating*** and by ***differentiating*** into effector cells

2. Effector T-cells recognize the same antigens anywhere in the body and respond by ***eliminating*** these microbes

MIGRATION OF NAIVE AND EFFECTOR T-LYMPHOCYTES



Effector T cells migrate to sites of infection because these lymphocytes express high levels of adhesion molecules that bind to ligands that are expressed on endothelium *on* exposure to microbes and because chemoattractant cytokines are produced at the infection site

MIGRATION OF NAIVE AND EFFECTOR T-LYMPHOCYTES

Ⓑ T cell homing receptor	Ligand on endothelial cell	Function of receptor: ligand pair
Naive T cells L-selectin	L-selectin ligand	Adhesion of naive T cells to high endothelial venule in lymph node
Activated (effector and memory) T cells E- and P-selectin ligand	E- or P-selectin	Initial weak adhesion of effector and memory T cells to cytokine-activated endothelium at peripheral site of infection
LFA-1 (β2 integrin) or VLA-4 (β1 integrin)	ICAM-1 or VCAM-1	Stable arrest on cytokine-activated endothelium at peripheral site of infection

MIGRATION OF NAIVE AND EFFECTOR T-LYMPHOCYTES

"HOMING" or MIGRATION

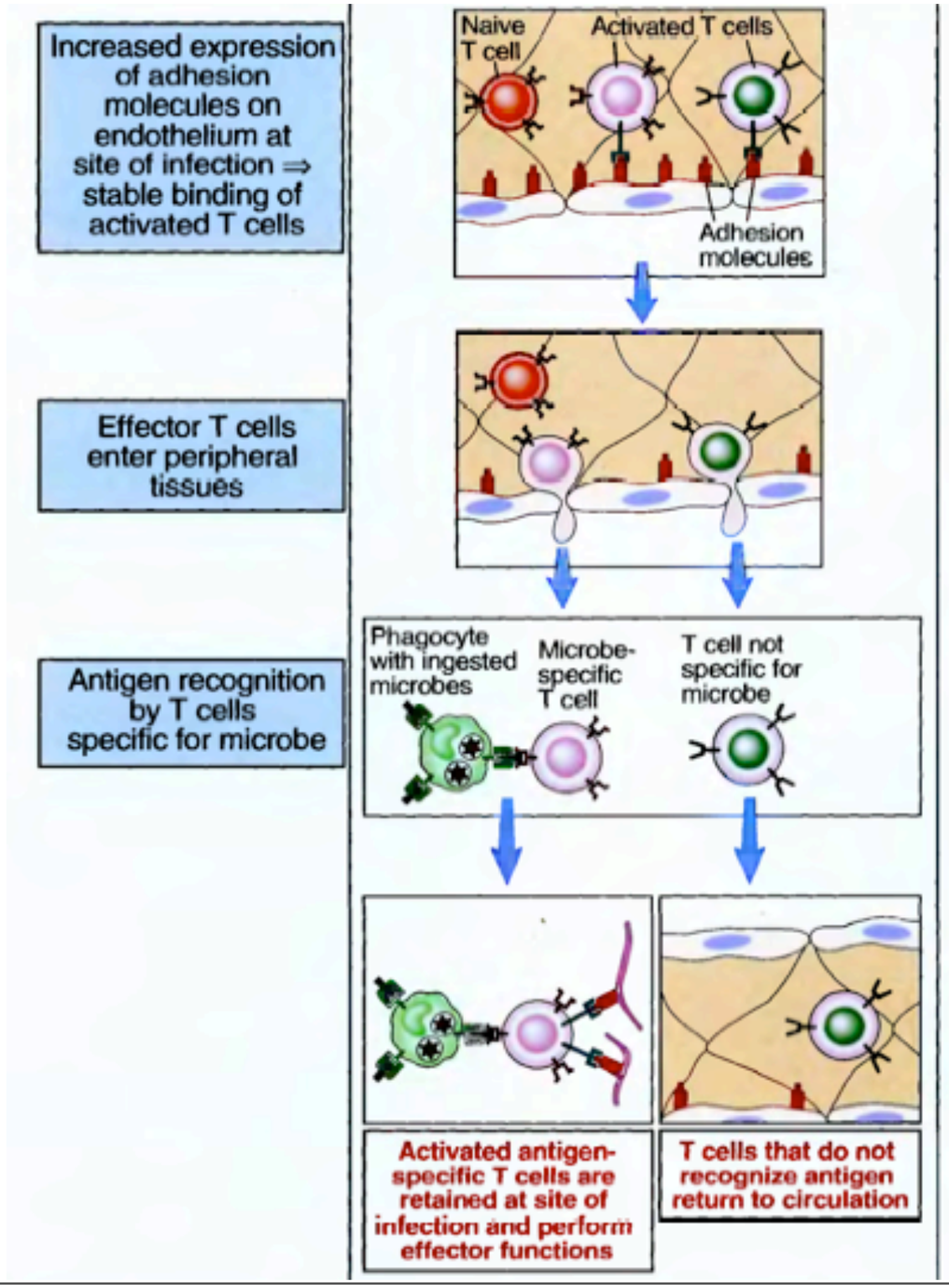
Chemokines (attract and stimulate)

*** displayed on endothelial cells bound to cell surface proteoglycans (increase the affinity of their integrins for endothelial ligands)

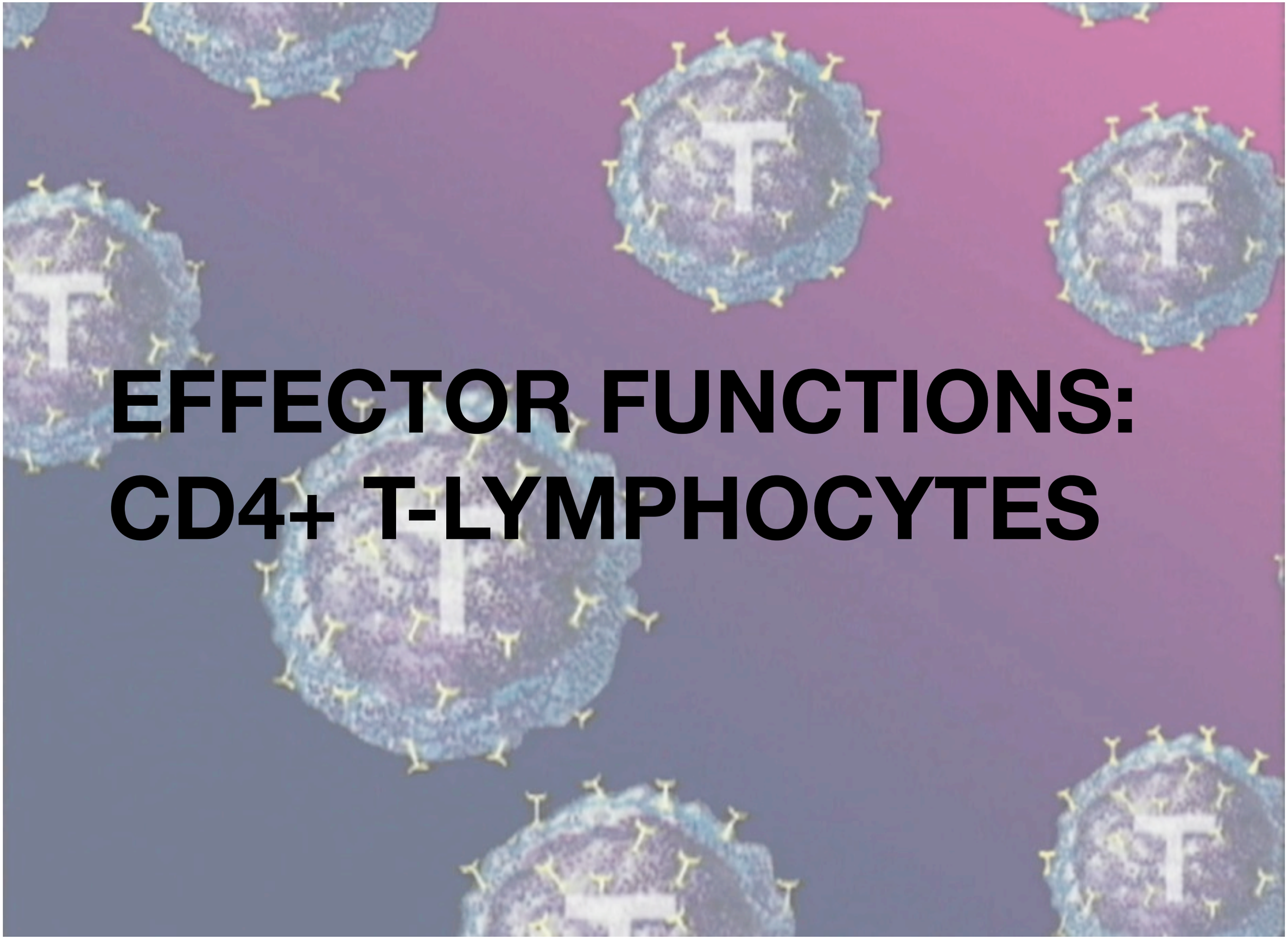
*** high local concentration near the site of infection

*** produced at the extravascular infection site by leukocytes that are reacting to the infectious microbe, and this creates a concentration gradient of chemokines toward the infection (stimulate the motility of these cells)

MIGRATION & RETENTION OF EFFECTOR T-CELLS AT SITE OF INFECTION



The homing of effector T-cells to a site of infection is independent of antigen recognition, but lymphocytes that recognize microbial antigens are preferentially retained at the site


EFFECTOR FUNCTIONS:
CD4+ T-LYMPHOCYTES

1. Type 4: Delayed-type Hypersensitivity/DTH (CD4 T-cells TH1 subset)

2. Cytotoxic T-lymphocytes/CTLs (CD8 T-lymphocytes)

CMI VERSUS *LISTERIA*

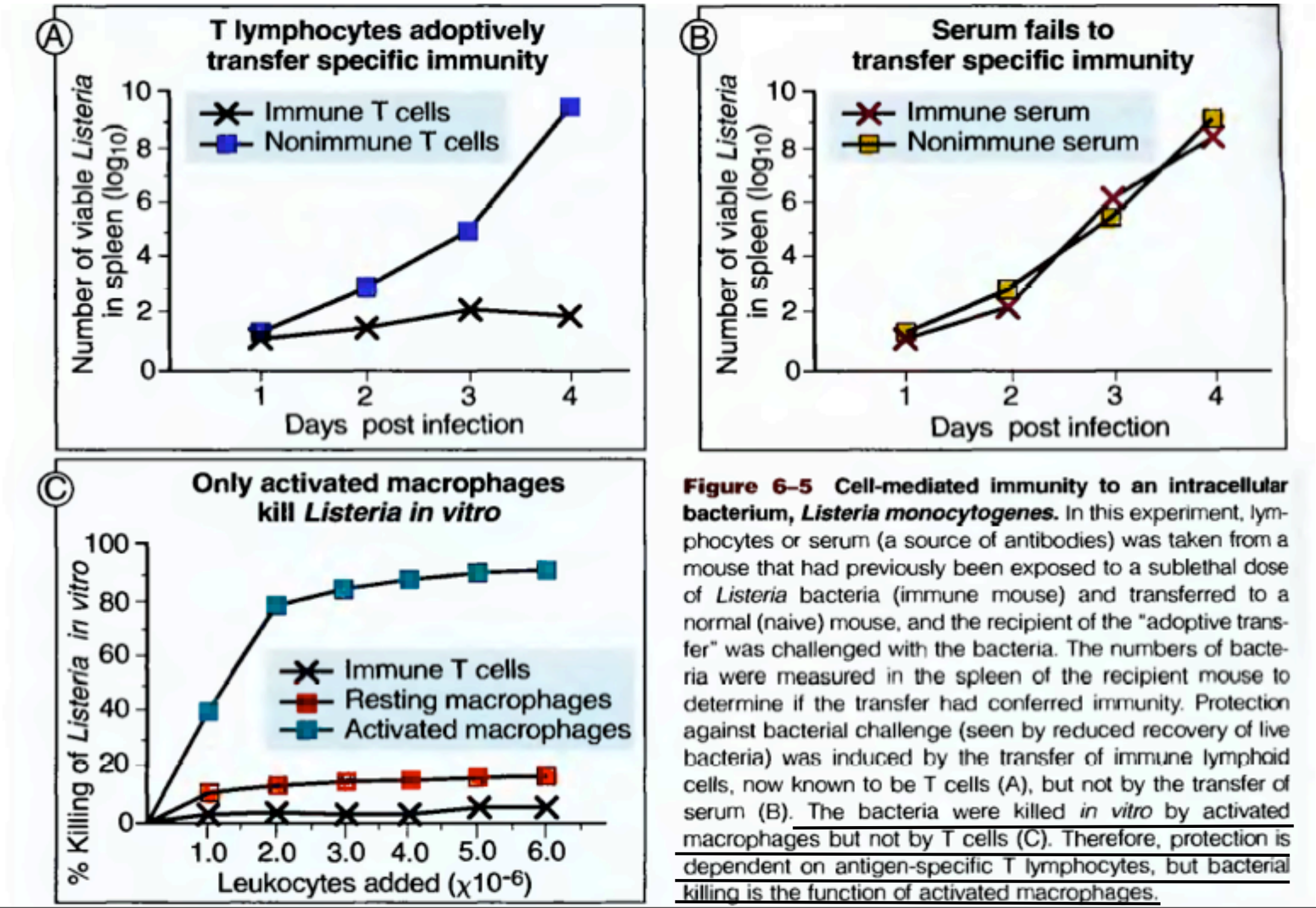
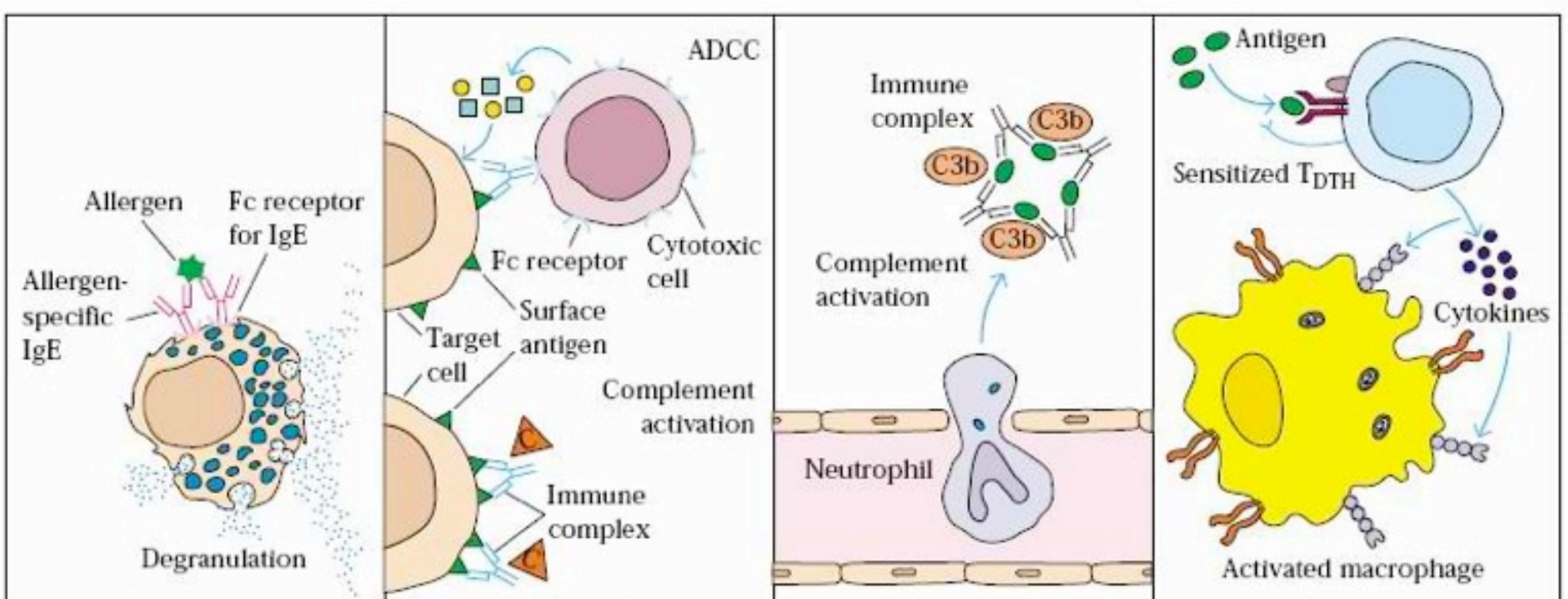


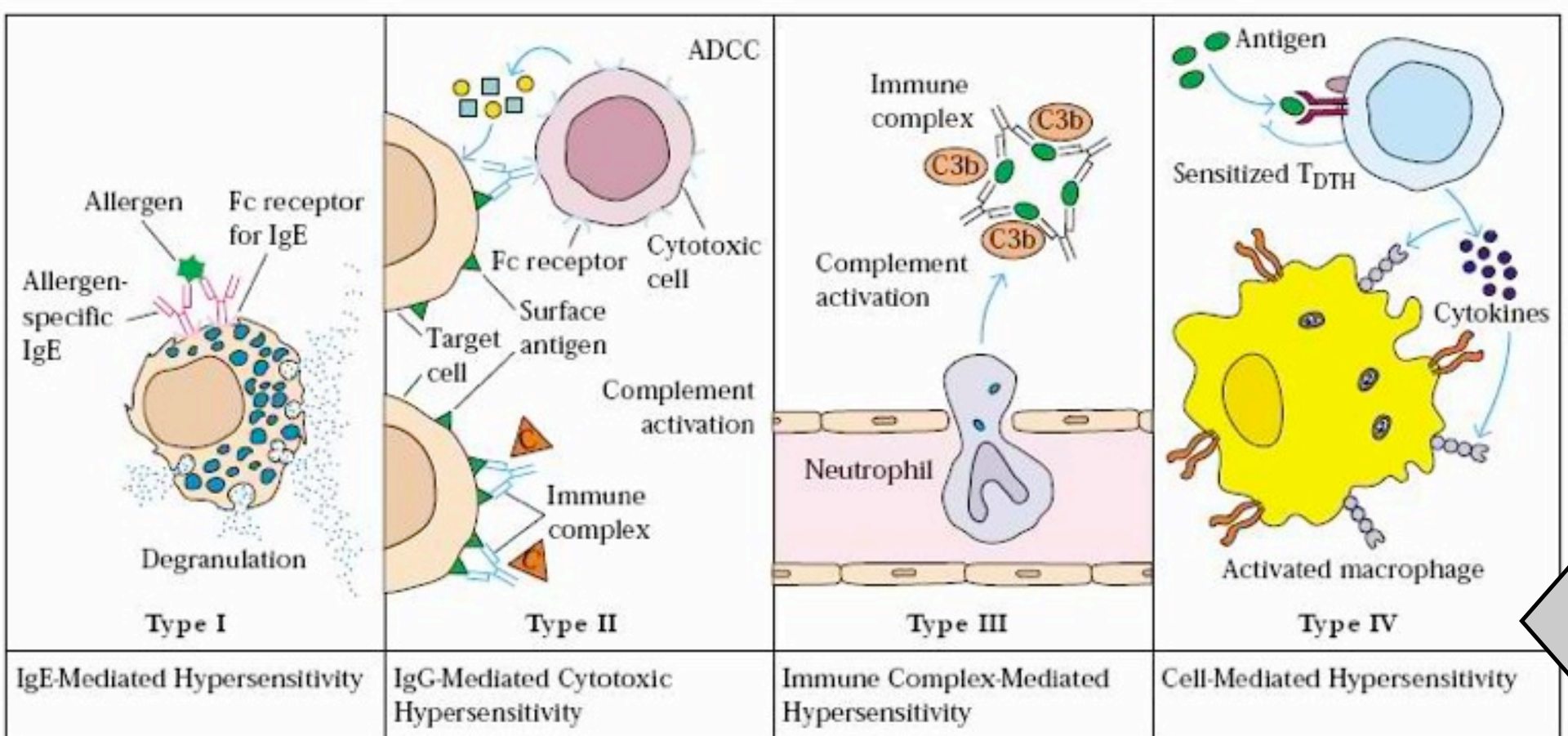
Figure 6–5 Cell-mediated immunity to an intracellular bacterium, *Listeria monocytogenes*. In this experiment, lymphocytes or serum (a source of antibodies) was taken from a mouse that had previously been exposed to a sublethal dose of *Listeria* bacteria (immune mouse) and transferred to a normal (naive) mouse, and the recipient of the "adoptive transfer" was challenged with the bacteria. The numbers of bacteria were measured in the spleen of the recipient mouse to determine if the transfer had conferred immunity. Protection against bacterial challenge (seen by reduced recovery of live bacteria) was induced by the transfer of immune lymphoid cells, now known to be T cells (A), but not by the transfer of serum (B). The bacteria were killed *in vitro* by activated macrophages but not by T cells (C). Therefore, protection is dependent on antigen-specific T lymphocytes, but bacterial killing is the function of activated macrophages.

HYPERSENSITIVITY TYPES



Type I	Type II	Type III	Type IV
IgE-Mediated Hypersensitivity	IgG-Mediated Cytotoxic Hypersensitivity	Immune Complex-Mediated Hypersensitivity	Cell-Mediated Hypersensitivity
Ag induces crosslinking of IgE bound to mast cells and basophils with release of vasoactive mediators	Ab directed against cell surface antigens meditates cell destruction via complement activation or ADCC	Ag-Ab complexes deposited in various tissues induce complement activation and an ensuing inflammatory response mediated by massive infiltration of neutrophils	Sensitized T_H1 cells release cytokines that activate macrophages or T_C cells which mediate direct cellular damage
Typical manifestations include systemic anaphylaxis and localized anaphylaxis such as hay fever, asthma, hives, food allergies, and eczema	Typical manifestations include blood transfusion reactions, erythroblastosis fetalis, and autoimmune hemolytic anemia	Typical manifestations include localized Arthus reaction and generalized reactions such as serum sickness, necrotizing vasculitis, glomerulnephritis, rheumatoid arthritis, and systemic lupus erythematosus	Typical manifestations include contact dermatitis, tubercular lesions and graft rejection

HYPERSENSITIVITY TYPES



Type I	Type II	Type III	Type IV
IgE-Mediated Hypersensitivity	IgG-Mediated Cytotoxic Hypersensitivity	Immune Complex-Mediated Hypersensitivity	Cell-Mediated Hypersensitivity
Ag induces crosslinking of IgE bound to mast cells and basophils with release of vasoactive mediators	Ab directed against cell surface antigens meditates cell destruction via complement activation or ADCC	Ag-Ab complexes deposited in various tissues induce complement activation and an ensuing inflammatory response mediated by massive infiltration of neutrophils	Sensitized T_H1 cells release cytokines that activate macrophages or T_C cells which mediate direct cellular damage
Typical manifestations include systemic anaphylaxis and localized anaphylaxis such as hay fever, asthma, hives, food allergies, and eczema	Typical manifestations include blood transfusion reactions, erythroblastosis fetalis, and autoimmune hemolytic anemia	Typical manifestations include localized Arthus reaction and generalized reactions such as serum sickness, necrotizing vasculitis, glomerulnephritis, rheumatoid arthritis, and systemic lupus erythematosus	Typical manifestations include contact dermatitis, tubercular lesions and graft rejection

TYPE 4: DELAYED-TYPE HYPERSENSITIVITY

- occurs 24-48 hours after an immunized individual is challenged by a microbial protein (reflects an **increased sensitivity** to antigen challenge)

- Circulating effector T-lymphocytes to:
    - home to the site of antigen challenge
    - respond to the antigen at this site
    - induce a detectable reaction

DELAYED-TYPE HYPERSENSITIVITY

- ***Manifestations:***
  - infiltrates of T-cells and monocytes into the tissues
  - edema
  - fibrin deposition
- ***Causes:***
  - increased vascular permeability in response to cytokines produced by CD4+ T-cells
  - tissue damage induced by the products of macrophage activated by T-cells

DELAYED-TYPE HYPERSENSITIVITY

- ***Manifestations:***
  - infiltrates of T-cells and monocytes into the tissues
  - edema
  - fibrin deposition

Note: clinical use of DTH reactions = purified protein derivative/PPD skin test to detect past or active mycobacterial infection

- ***Causes:***
  - increased vascular permeability in response to cytokines produced by CD4+ T-cells
  - tissue damage induced by the products of macrophage activated by T-cells

DELAYED-TYPE HYPERSENSITIVITY (DTH)

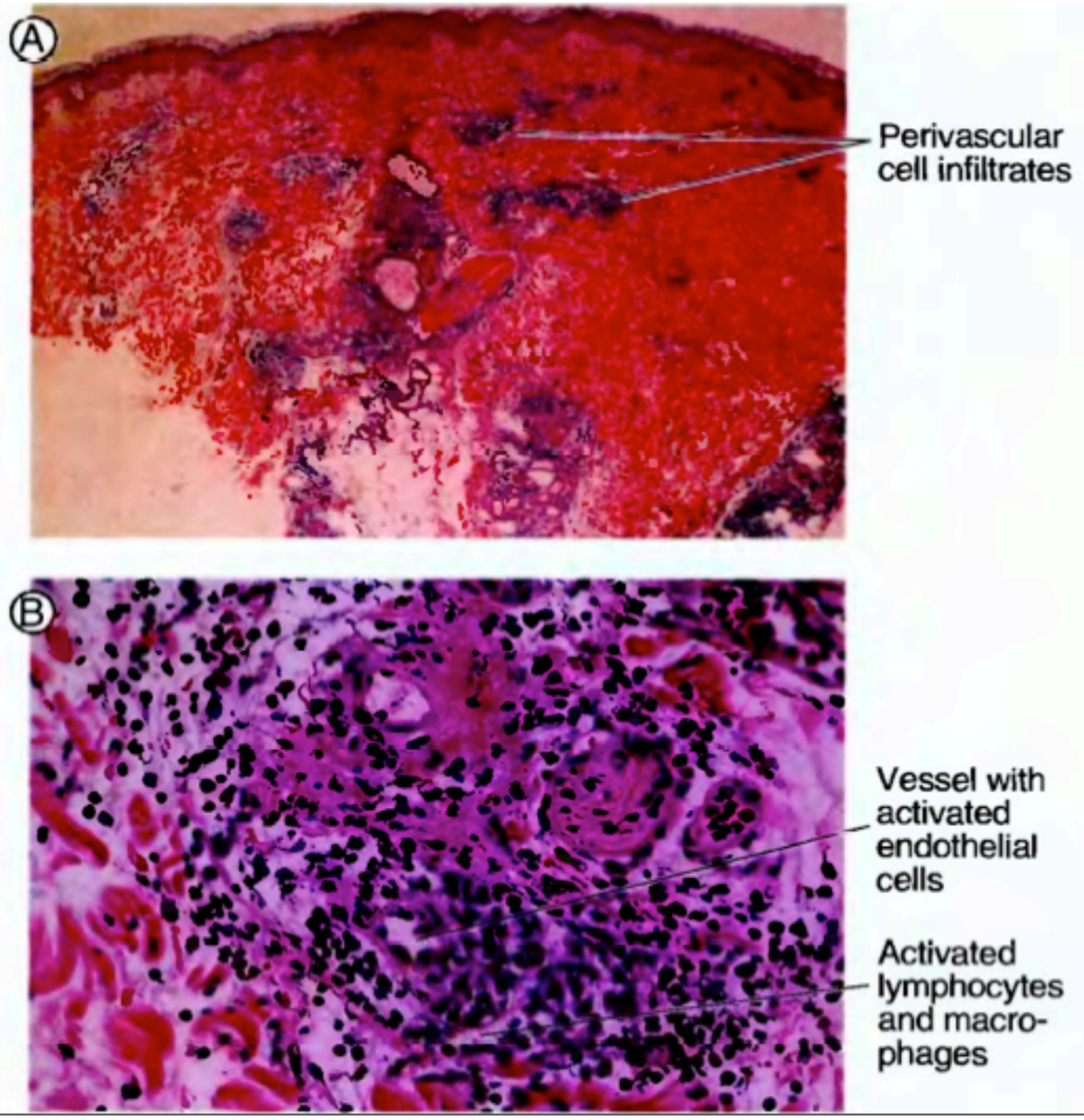
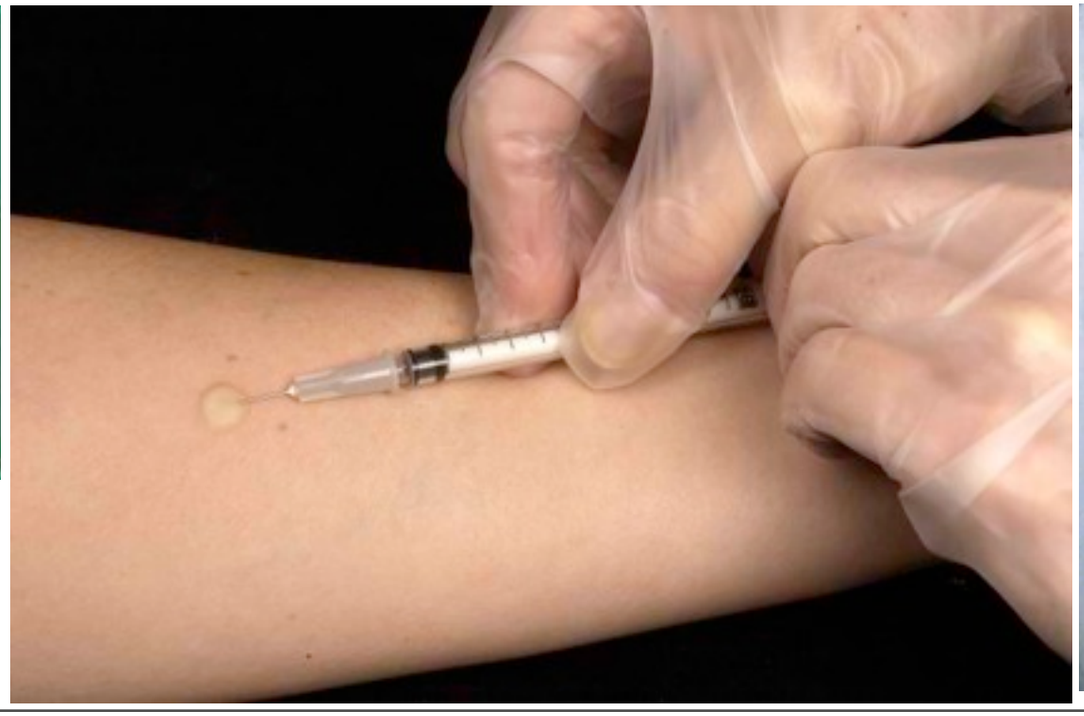
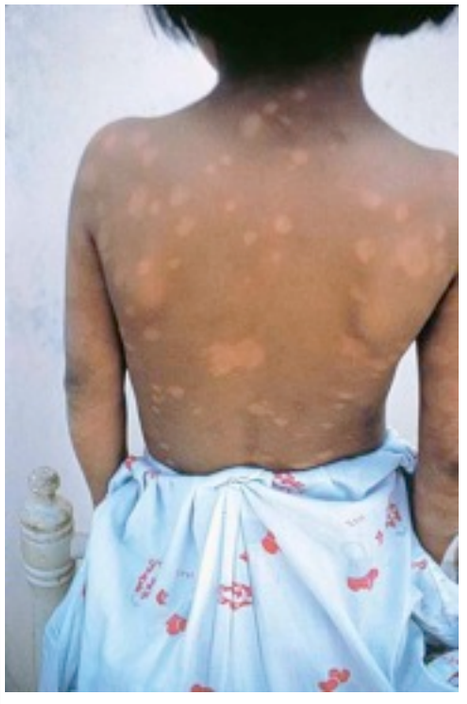


Figure 6–6 The morphology of a delayed-type hypersensitivity (DTH) reaction. In an individual previously exposed to an antigen, skin challenge with that antigen elicits a DTH reaction. Histopathologic examination of the reaction shows perivascular mononuclear cell infiltrates in the dermis (A). At higher magnification, the infiltrate is seen to consist of activated lymphocytes and macrophages surrounding small blood vessels in which the endothelial cells are activated (B). (Courtesy of Dr. J. Faix, Department of Pathology, Stanford University School of Medicine, Palo Alto, CA.)

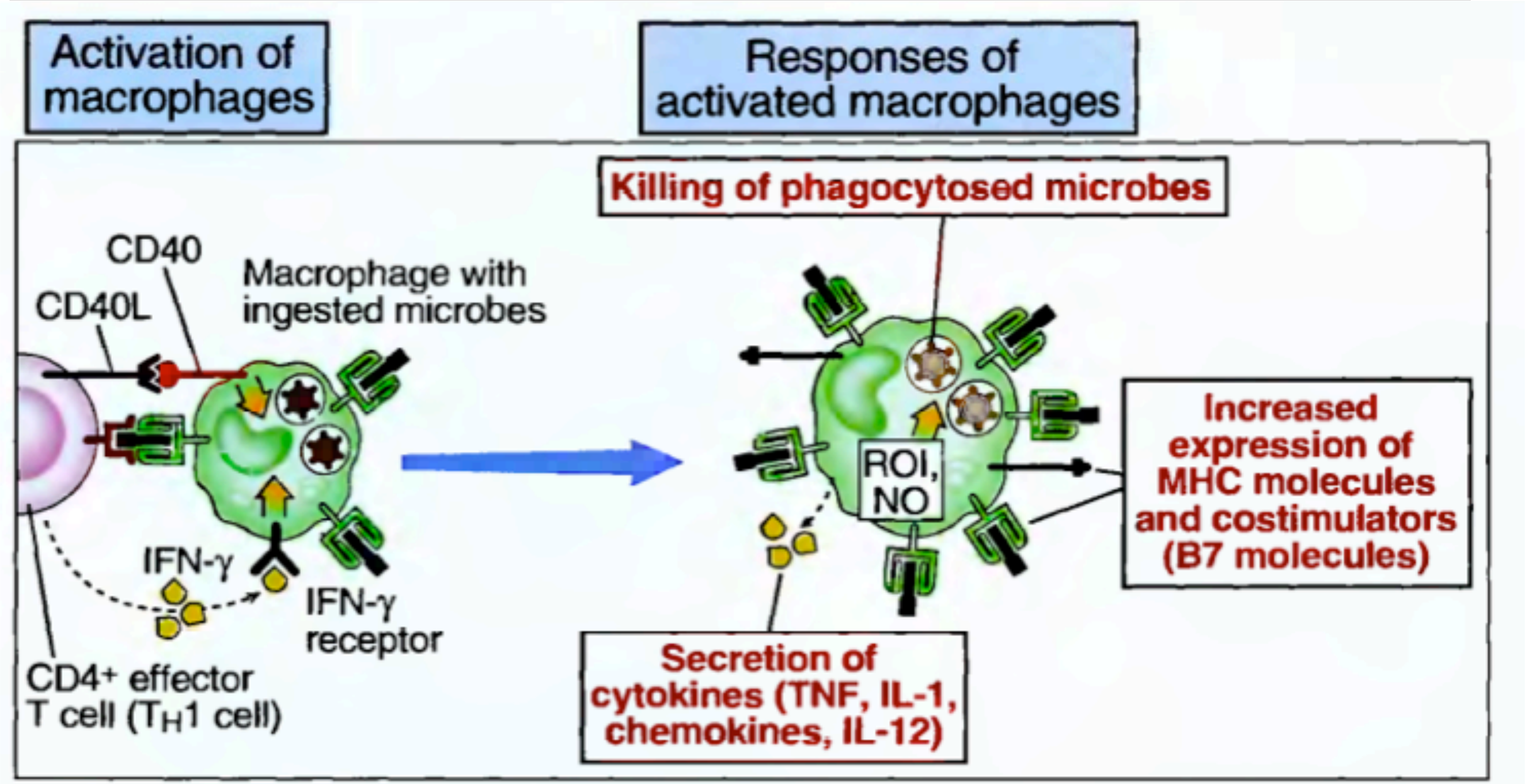
EXAMPLES OF DTH

type	reaction time	clinical appearance	histology	antigen and site
contact	48-72 hr	eczema	lymphocytes, followed by macrophages; edema of epidermis	epidermal (organic chemicals, poison ivy, heavy metals, *etc.*)
tuberculin	48-72 hr	local induration	lymphocytes, monocytes, macrophages	intradermal (tuberculin, lepromin, *etc.*)
granuloma	21-28 days	hardening	macrophages, epitheloid and giant cells, fibrosis	persistent antigen or foreign body presence (tuberculosis, leprosy, *etc.*)





ACTIVATION OF MACROPHAGES BY T-LYMPHOCYTES



Effector T-lymphocytes of the ***TH1 subset*** that recognize macrophage-associated antigens activate macrophages:
a) by CD40 ligand-CD40 interactions
b) by secreting the macrophage-activating cytokines IFN-g

CYTOKINE-MEDIATED INTERACTIONS BETWEEN T-LYMPHOCYTES & MACROPHAGES IN CMI

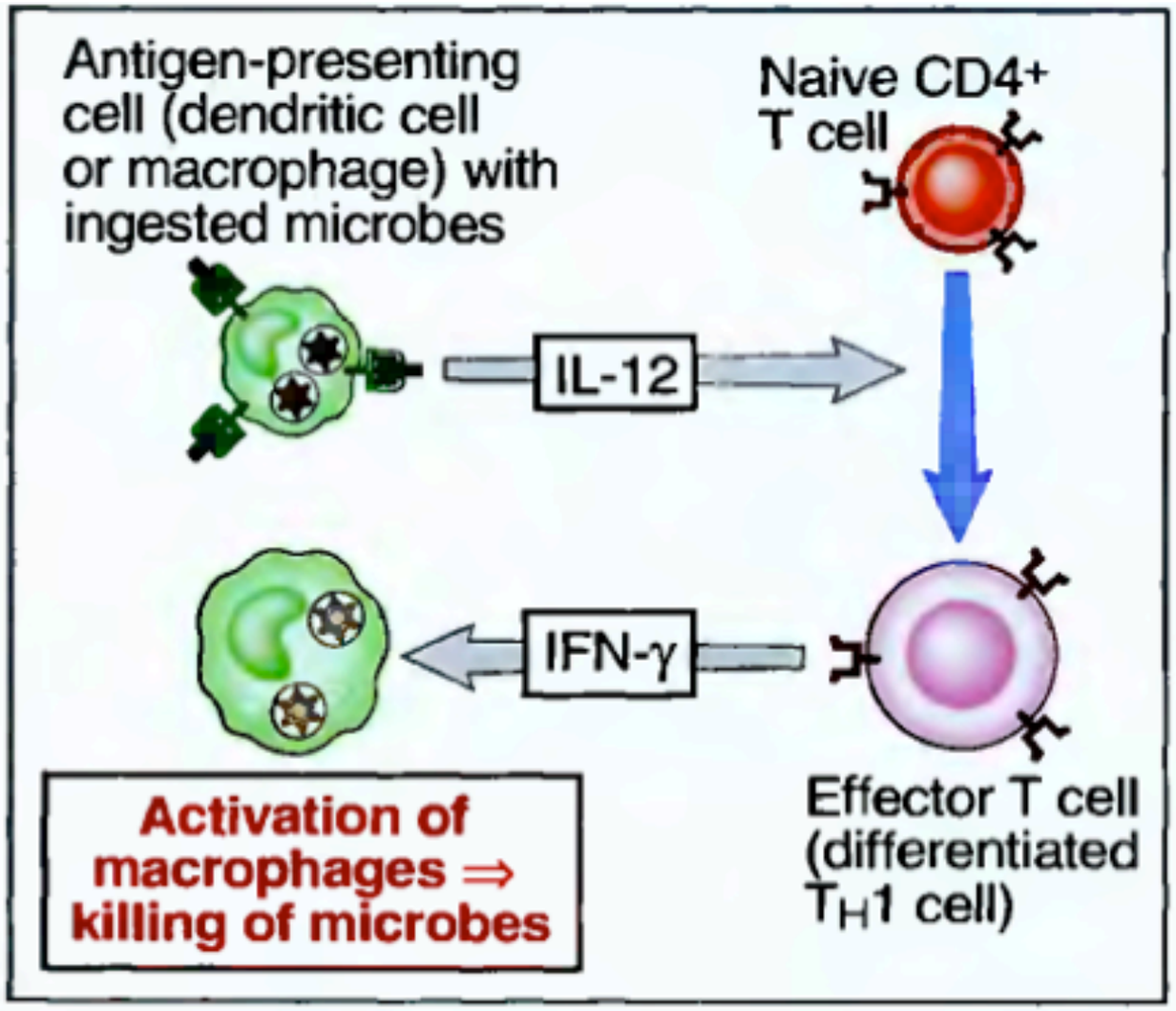


Figure 6–8 Cytokine-mediated interactions between T lymphocytes and macrophages in cell-mediated immunity. Macrophages that encounter microbes secrete the cytokine IL-12, which stimulates naive CD4+ T cells to differentiate into IFN-γ–secreting T_H1 cells and enhances IFN-γ production. IFN-γ activates the macrophages to kill ingested microbes.

BIDIRECTIONAL INTERACTIONS between macrophages and T-lymphocytes

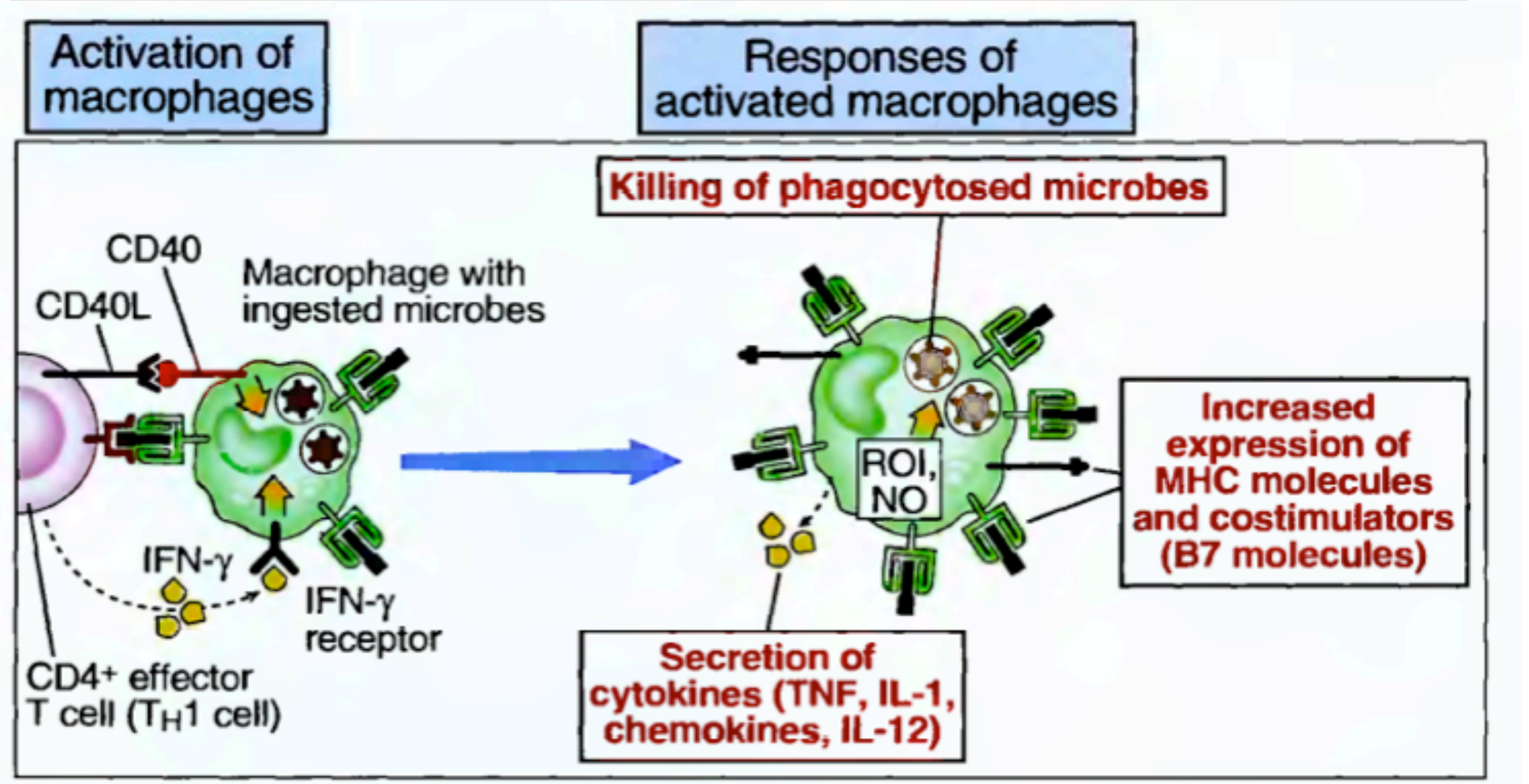
= macrophages that have phagocytosed microbes produce cytokine IL-12

= IL-12 stimulates the differentiation of CD4+ T cells to TH1 subset

= TH1 subset produces IFN-g

=IFN-g activates the phagocytes to kill ingested microbes

HOW ACTIVATED MACROPHAGE ELIMINATE MICROBES



Macrophage activation leads to the expression of ***enzymes*** that catalyze the production of ***microbicidal substances*** in phagosomes and phagolysosomes:

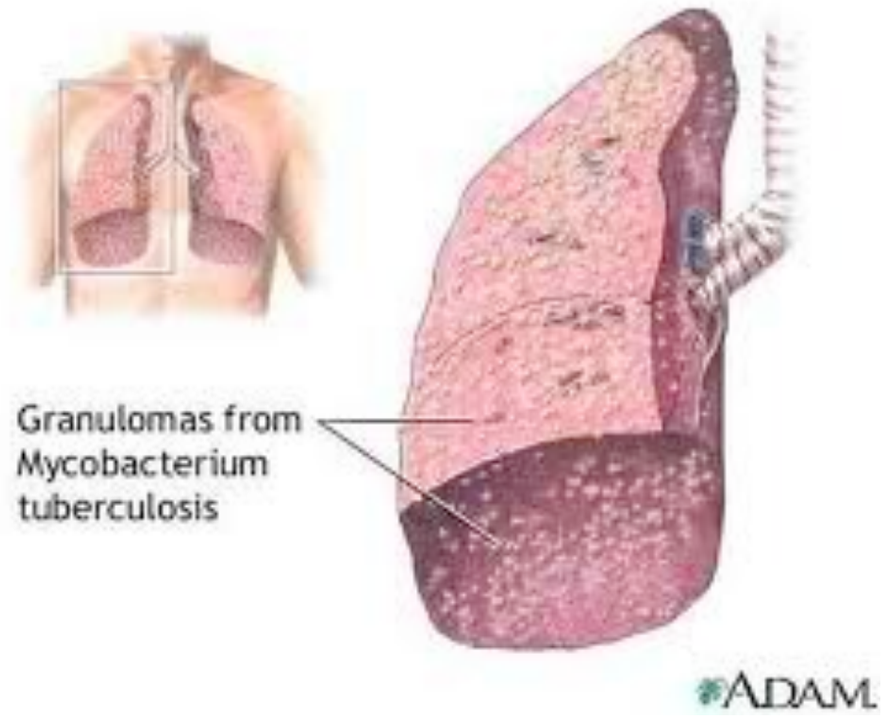
1. reactive oxygen intermediates (ROIs)
2. Nitric Oxide (NO)
3. proteolytic enzymes

CMI CRITICAL FOR HOST DEFENSE

- ***INNATE:*** macrophage killing activated when they encountered microbes
- **ADAPTIVE**: TH1 subset activates the same mechanism
- CRITICAL IN TWO SITUATIONS;
  - When macrophages are not activated by the microbes themselves (ineffective innate immunity)
  - When pathogenic microbes have evolved to resist te dfeense mechanisms of innate immunity
- ***HOW?:*** the additional macrophage activation by T-cells changes the ***balance*** between microbes and host defense in favor of the macrophages = ***eradicate*** intracellular infections

CONSEQUENCE OF PROLONGED MACROPHAGE ACTIVATION

- substances that are toxic to microbes may ***injure*** normal tissues if they are released into the extracellular milieu because these substances ***do not distinguish between microbes and host cells***



- ***RESULT:*** tissue injury in DTH reactions during prolonged macrophage activation (chronic CMI) leading to considerable injury to adjacent normal cells

- e.g. ***mycobacterial infections*** = sustained T-cell and macrophage response leads to ***granuloma formation*** = collections of activated lymphocytes and macrophages with fibrosis and tissue around the microbe

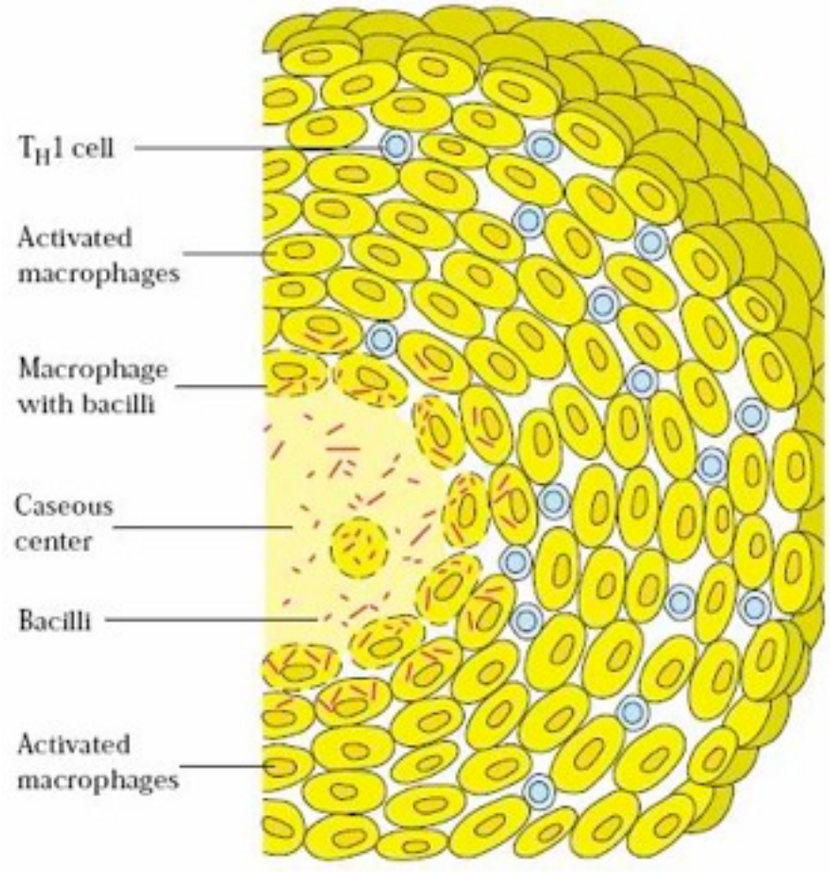
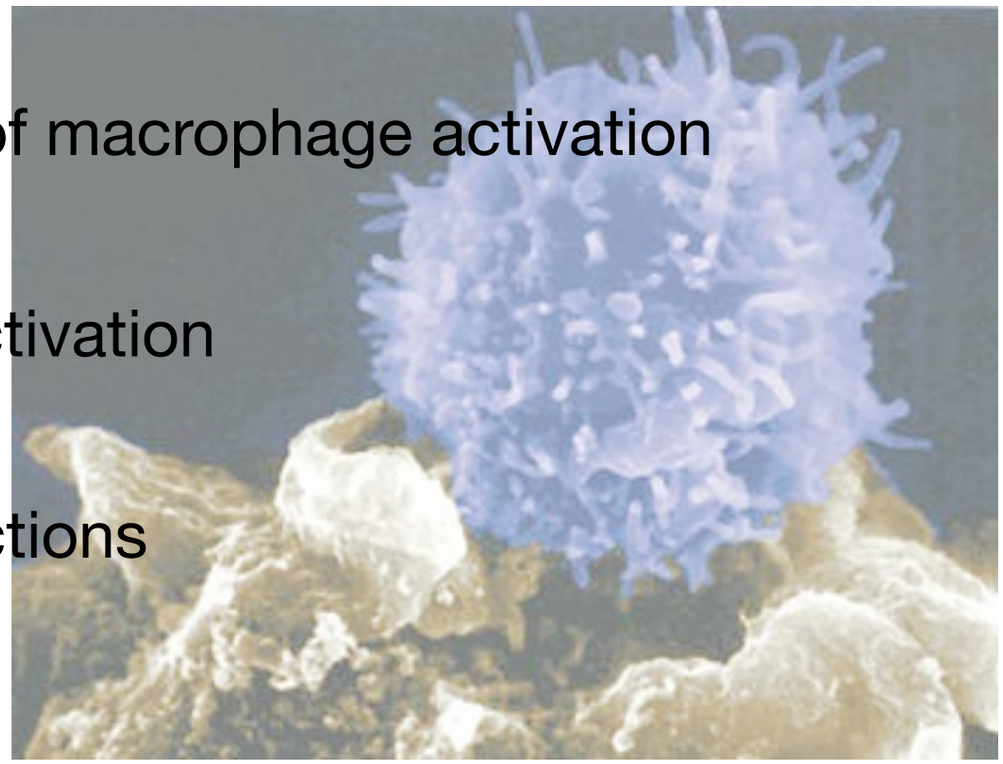


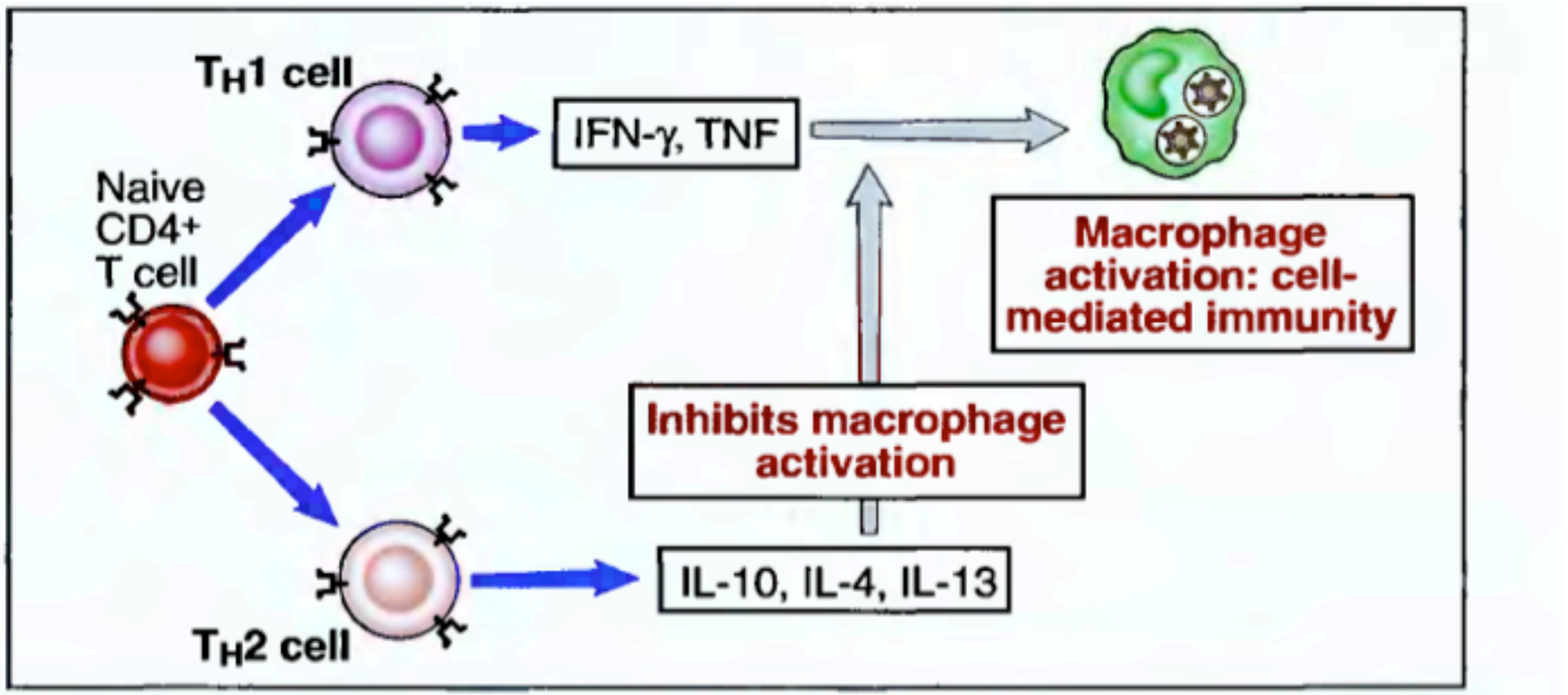
FIGURE 17-10 A tubercle formed in pulmonary tuberculosis.

THE ROLE OF TH2 subset of CD4 T-CELLS IN CMI

- stimulates eosinophil-rich inflammation (defense against helminthic infections)
    - ***IL-4***: stimulates the production of IgE antibody while ***IL-5:*** activates eosinophils
    - eosinophils bind to IgE-coated helminths and the helminths are killed by granule proteins of eosinophils
- functions to limit the injurious consequences of macrophage activation
    - IL-4, IL-10, IL-13: inhibit macrophage activation
    - TH2 terminates TH1-mediated DTH reactions
    - limits the tissue injury

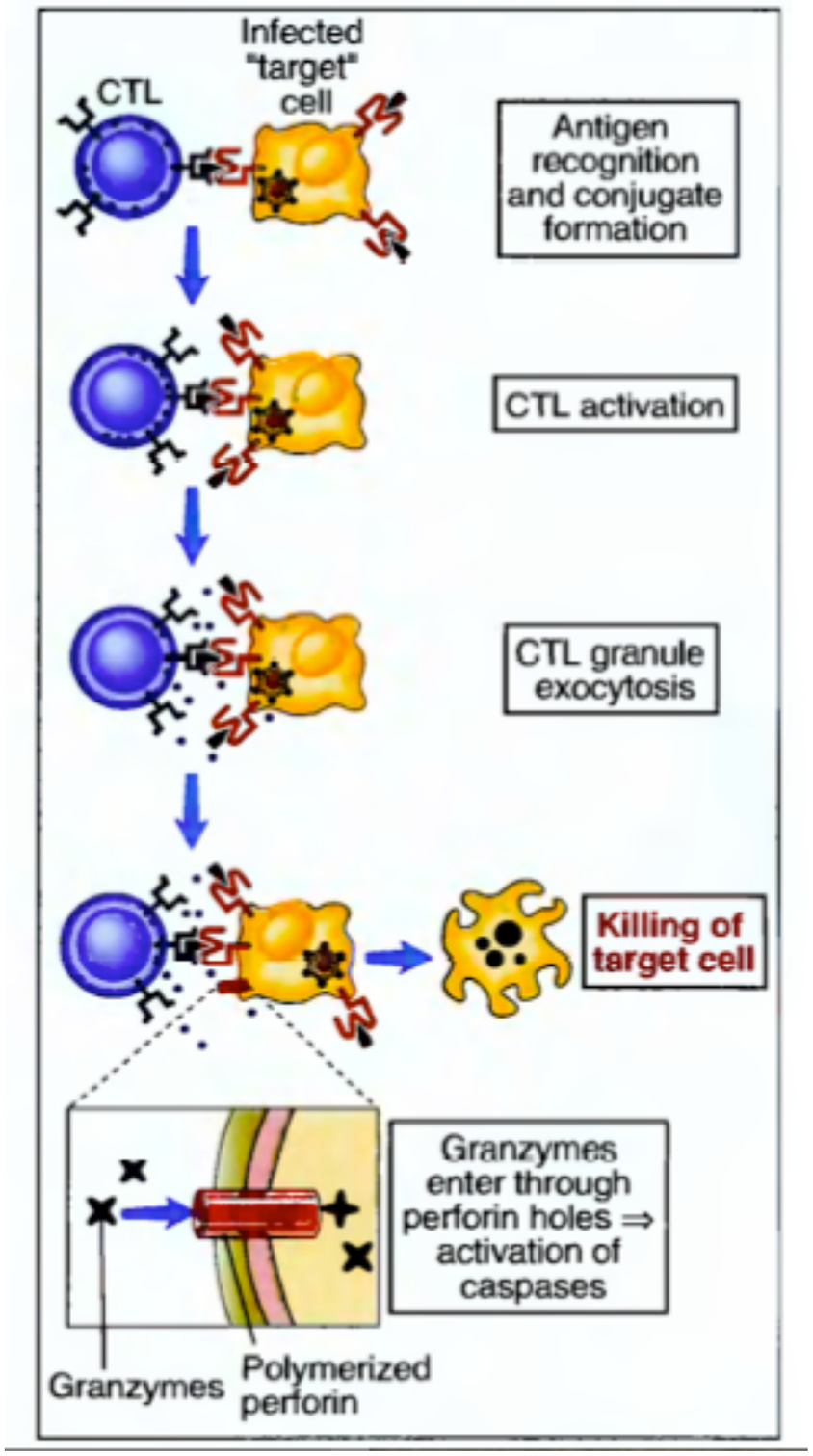


THE BALNCE BETWEEN TH1 & TH2 CELL ACTIVATION DETERMINES THE OUTCOME OF INTRACELLULAR INFECTIONS



Infection	Response	Outcome
Leishmania major	Most mouse strains: T_H1 ⇒ BALB/c mice: T_H2 ⇒	Recovery Disseminated infection
Mycobacterium leprae	Some patients: T_H1 ⇒ Some patients: Defective T_H1 or dominant T_H2 ⇒	Tuberculoid leprosy Lepromatous leprosy (high bacterial count)

MECHANISM OF KILLING INFECTED CELLS BY CD8+ CTLs



PROBLEM: activated macrophages are best at killing microbes that are confined to vesicles...BUT.....microbes that directly enter the cytoplasm (e.g. virus) or escape from phagosomes into the cytoplasm (e.g. phagocytosed bacteria) are relatively resistant to the microbial mechanisms of phagocytes

SOLUTION: cytolytic T-lymphocytes (CTLs)

COOPERATION: CD4+ & CD8+ T-CELLS IN ERADICATION OF INTRACELLULAR INFECTIONS

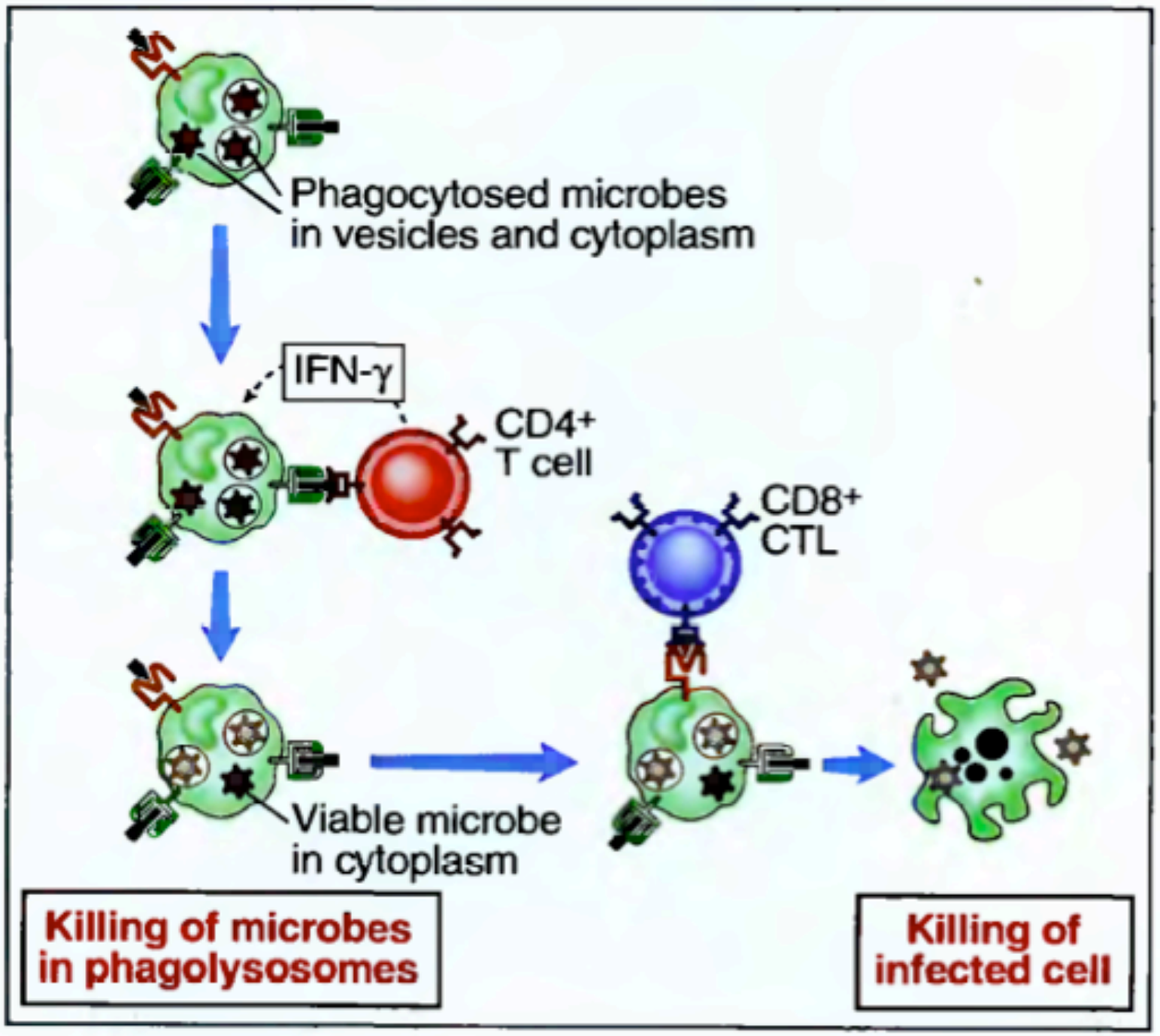
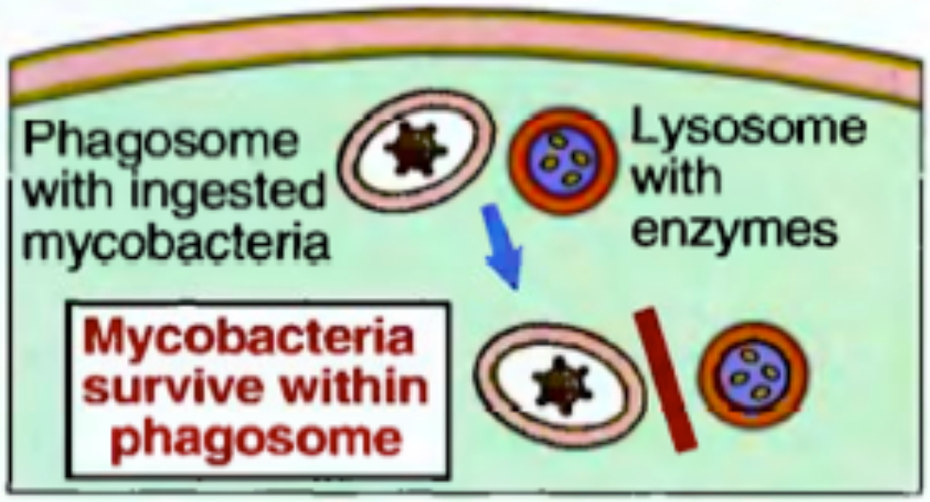
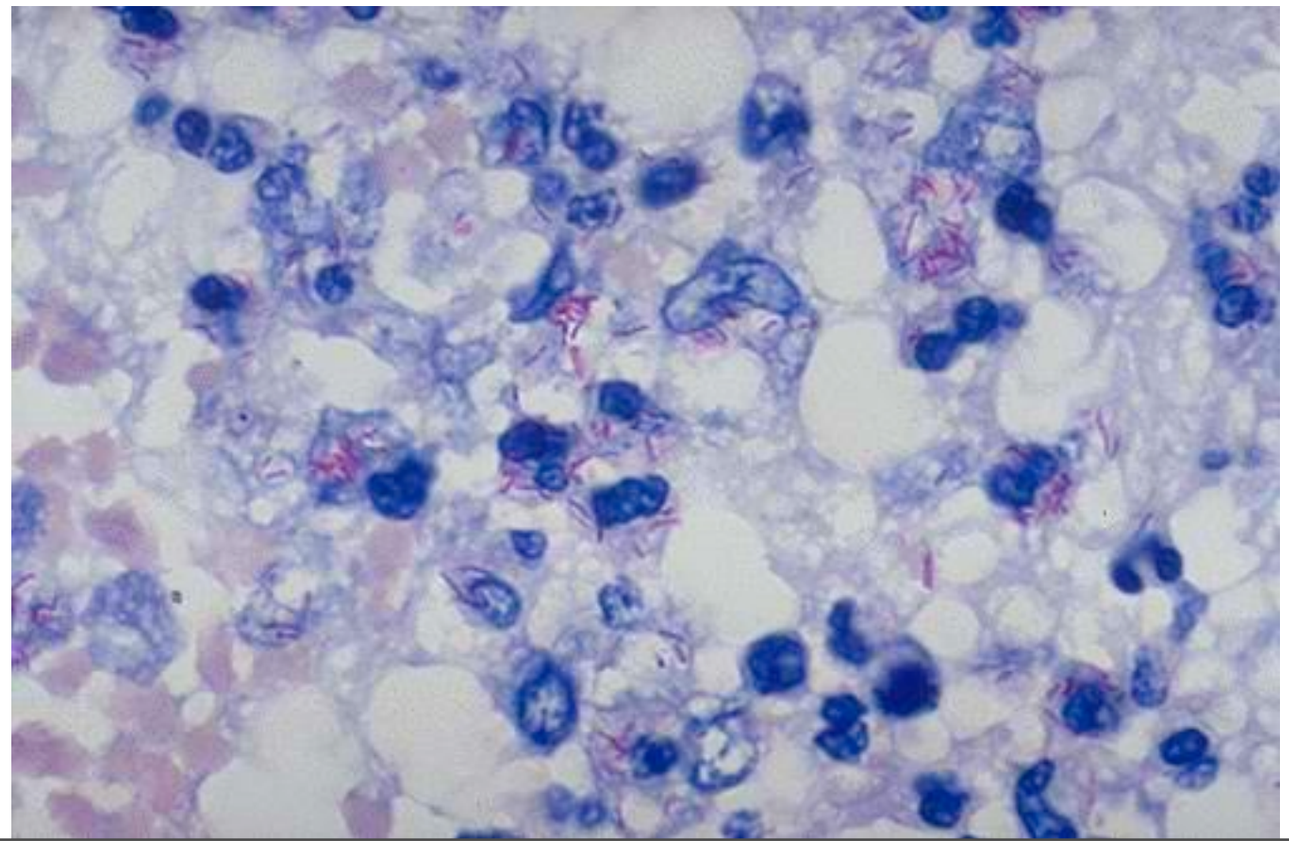


Figure 6–11 Cooperation between CD4⁺ and CD8⁺ T cells in the eradication of intracellular infections. In a macrophage infected by an intracellular bacterium, some of the bacteria are sequestered in vesicles (phagosomes) and others may escape into the cytoplasm. CD4⁺ T cells recognize antigens derived from the vesicular microbes and activate the macrophage to kill the microbes in the vesicles. CD8⁺ T cells recognize antigens derived from the cytoplasmic bacteria and are needed to kill the infected cell, thus eliminating the reservoir of infection.

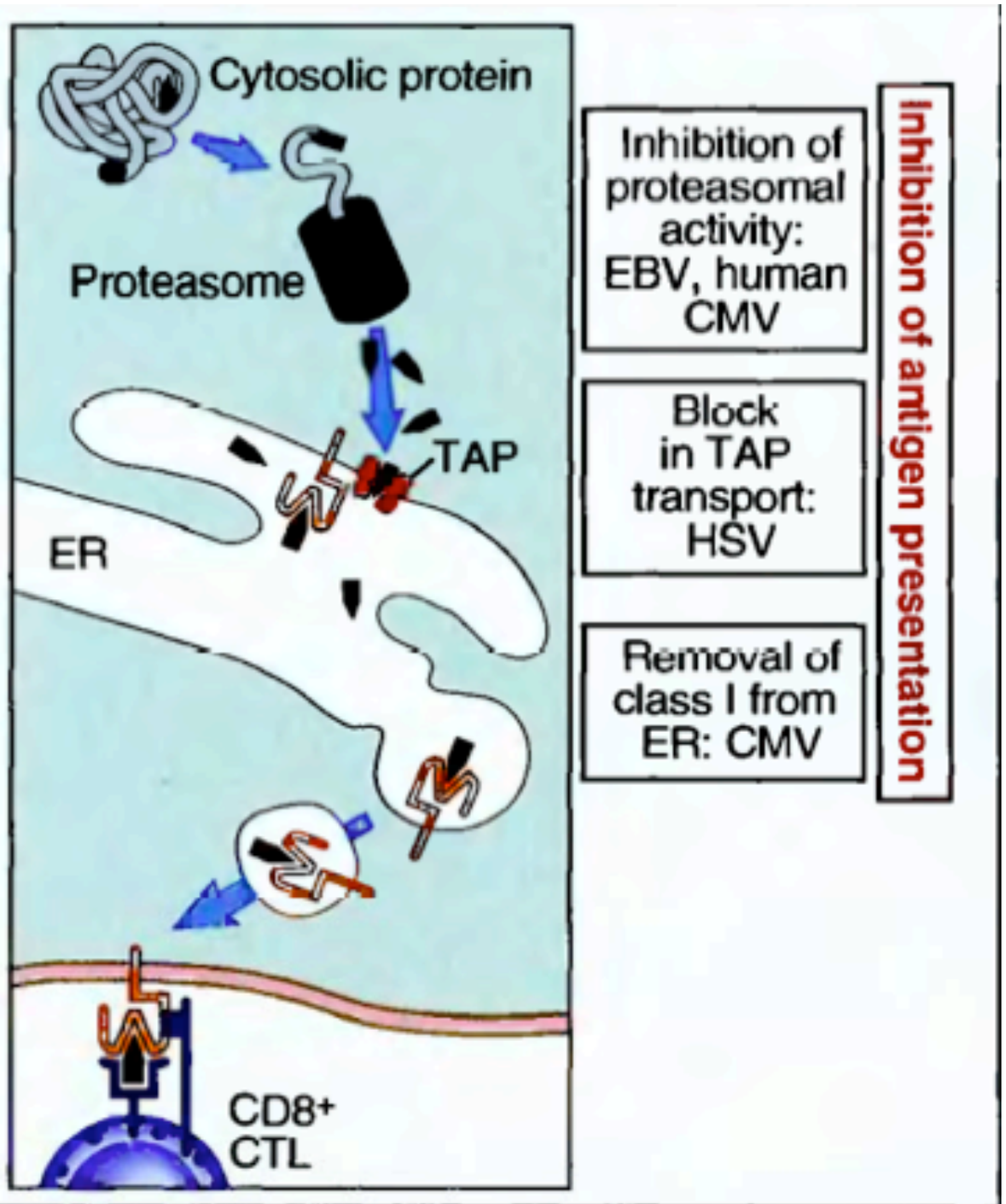
EVASION OF CMI

Microbe	Mechanism	
Mycobacteria	Inhibition of phagolysosome fusion	

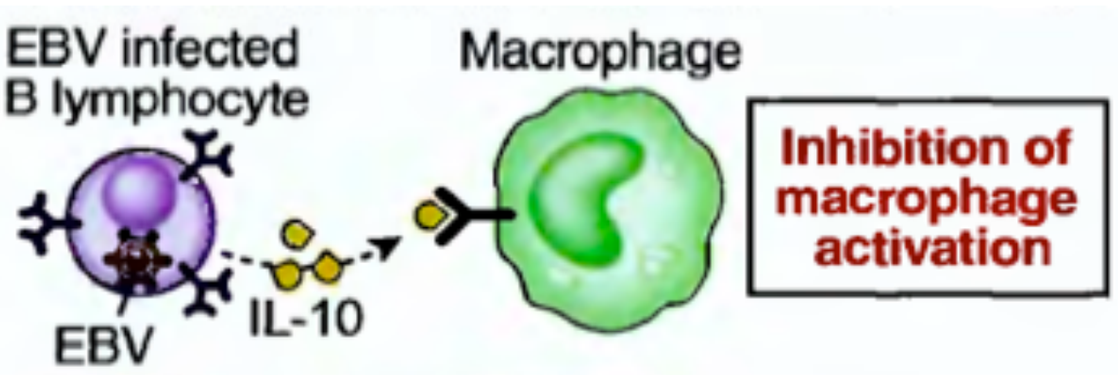
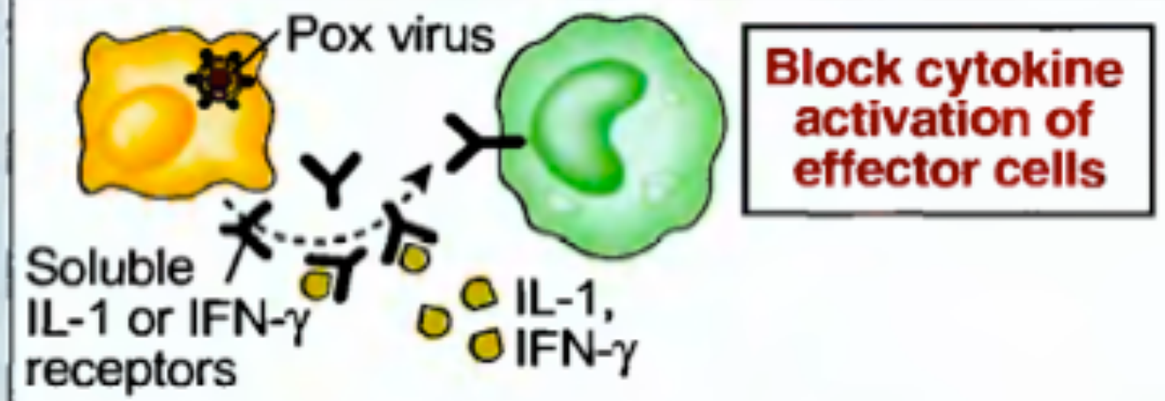


EVASION OF CMI

Herpes simplex virus (HSV)	Inhibition of antigen presentation: HSV peptide interferes with TAP transporter
Cytomegalovirus (CMV)	Inhibition of antigen presentation: inhibition of proteasomal activity; removal of class I MHC molecules from endoplasmic reticulum (ER)
Epstein-Barr virus (EBV)	Inhibition of antigen presentation: inhibition of proteasomal activity



EVASION OF CMI

Epstein-Barr virus (EBV)	Production of IL-10, inhibition of macrophage activation	
Pox virus	Inhibition of effector cell activation: production of soluble cytokine receptors	

NEXT MEETING:
HUMORAL IMMUNITY

Chapter 7

The Complement System

Dr. Capers

IMMUNOLOGY

Kindt • Goldsby • Osborne

Kuby IMMUNOLOGY

Sixth Edition

Chapter 7

The Complement System

Complement System

- **Major effector branch of humoral immune system in vertebrates**
  - However, invertebrates possess proteins related to complement system

Functions of Complement

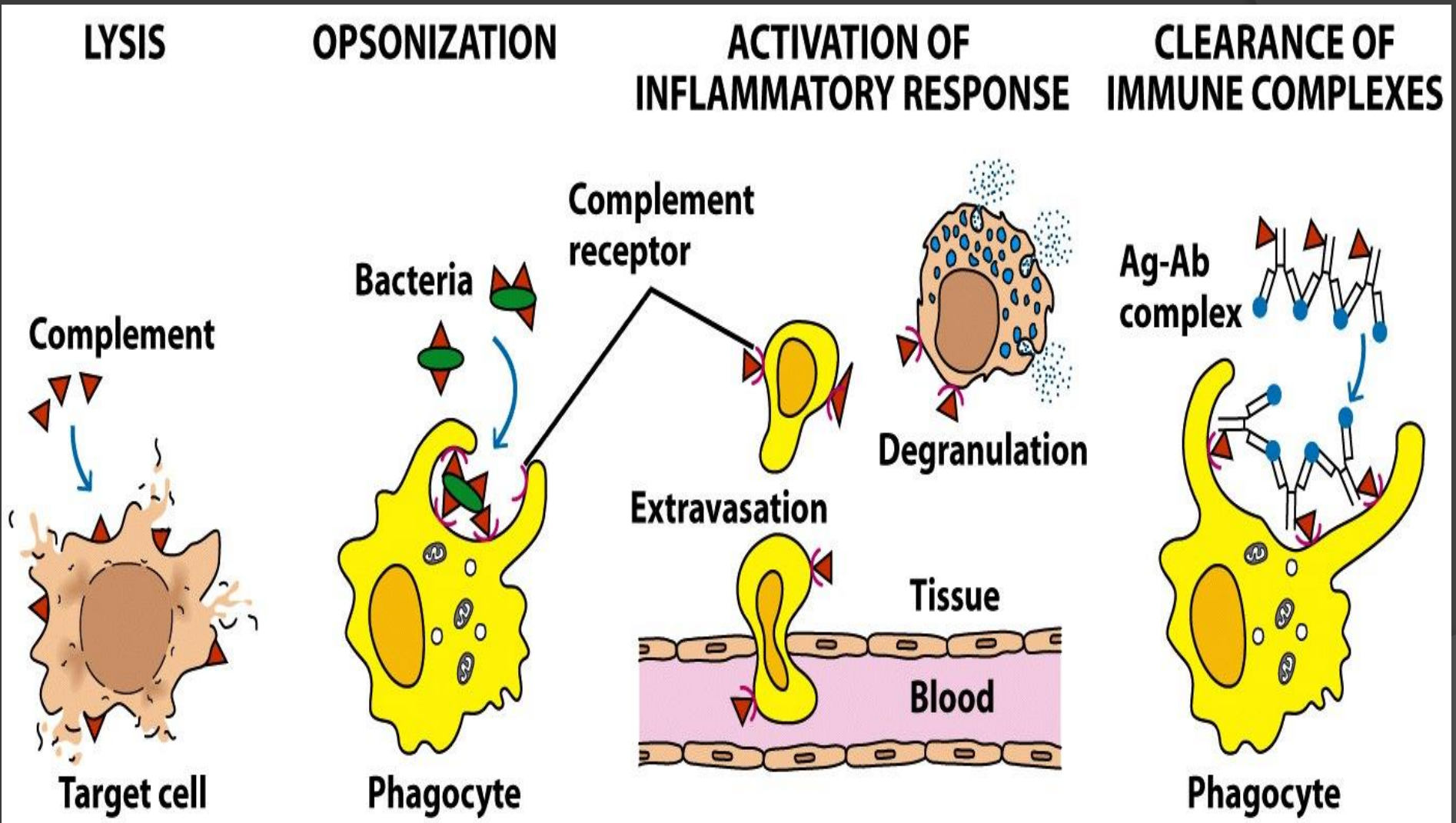


Figure 7-1
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Components of Complement

- **Soluble proteins and glycoproteins**
  - Synthesized mainly by liver hepatocytes and other cell types
  - 5% of serum globulins
    - Circulate as inactive proenzymes – proteolytic cleavage removes inhibitory fragment and exposes active site

Components of Complement

- **Designated by numerals, letter symbols, or trivial names**
  - Examples: C1-C9, factor D, homologous restriction factor
- **Peptide fragments made by activation**
  - "a" for smaller fragment – C3a
  - "b" for larger fragment – C3b
- Complexes with enzymatic activity have bar on top – $\overline{C4b2a}$

Complement Activation

- **Early steps – resulting in C5**
  - **Can occur by 3 pathways:**
    - Classical
    - Alternative
    - Lectin
- **Final steps leading to membrane-attack complex (MAC) are identical in all 3 pathways**

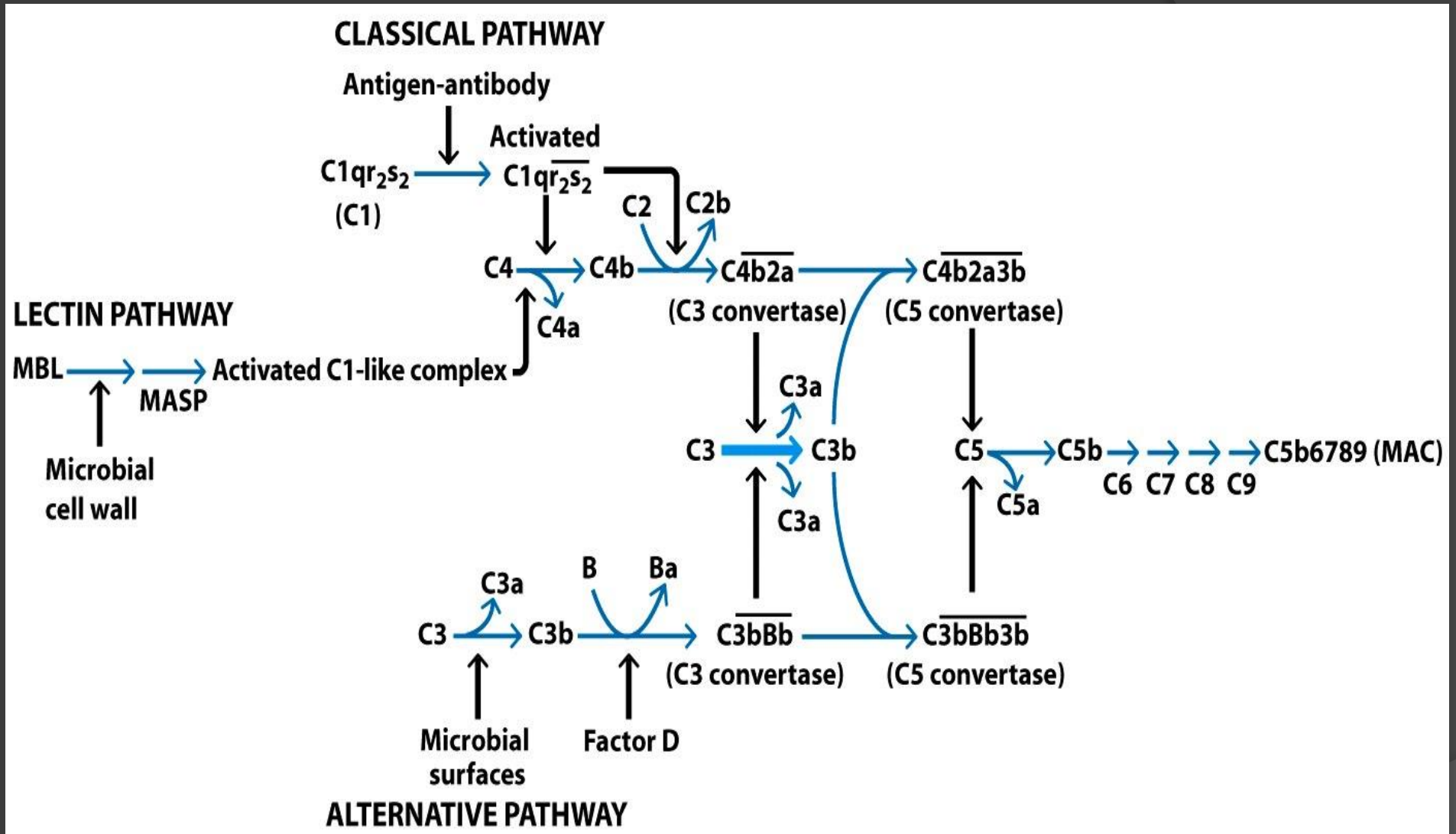

Figure 7-2
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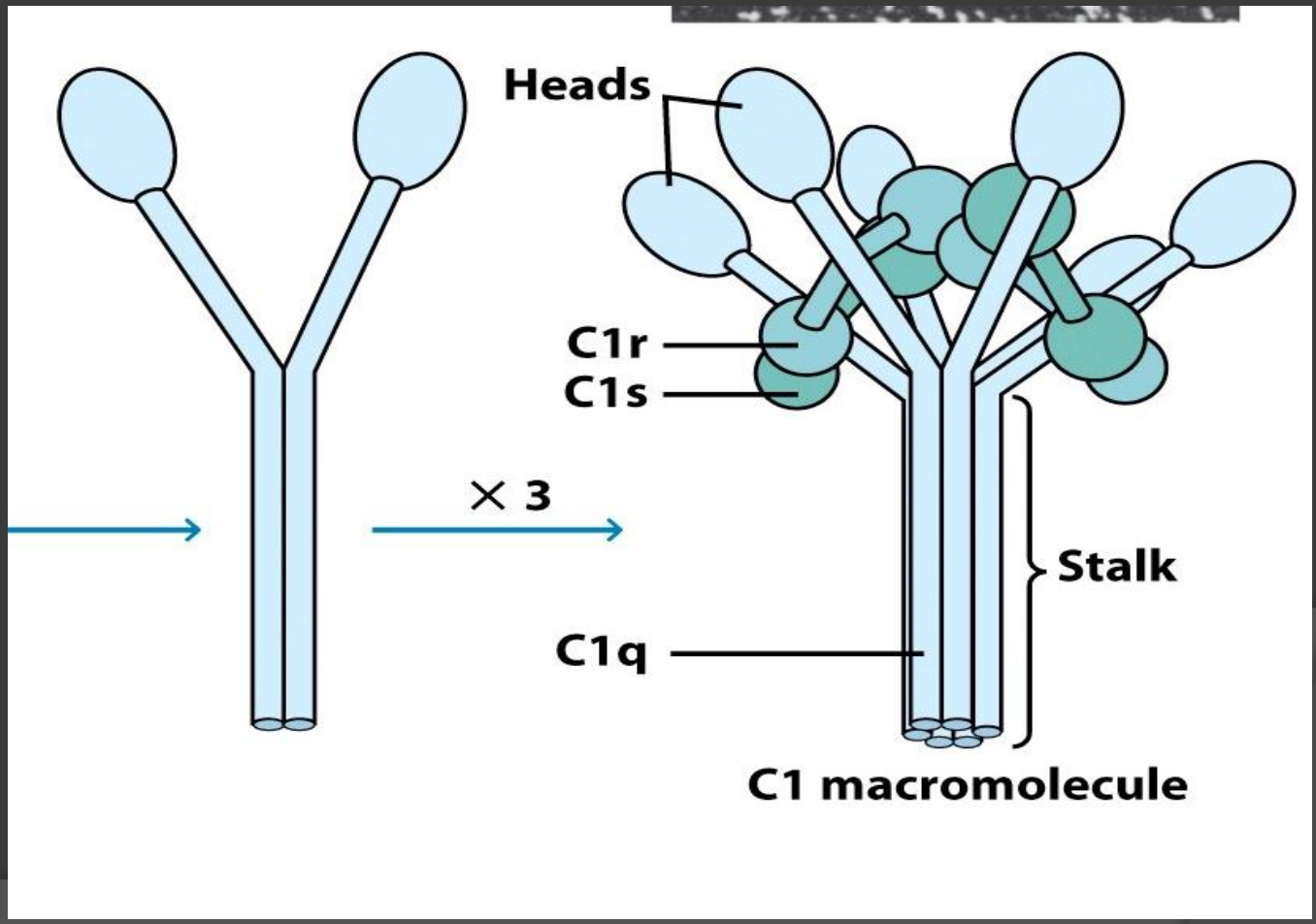
Classical Pathway

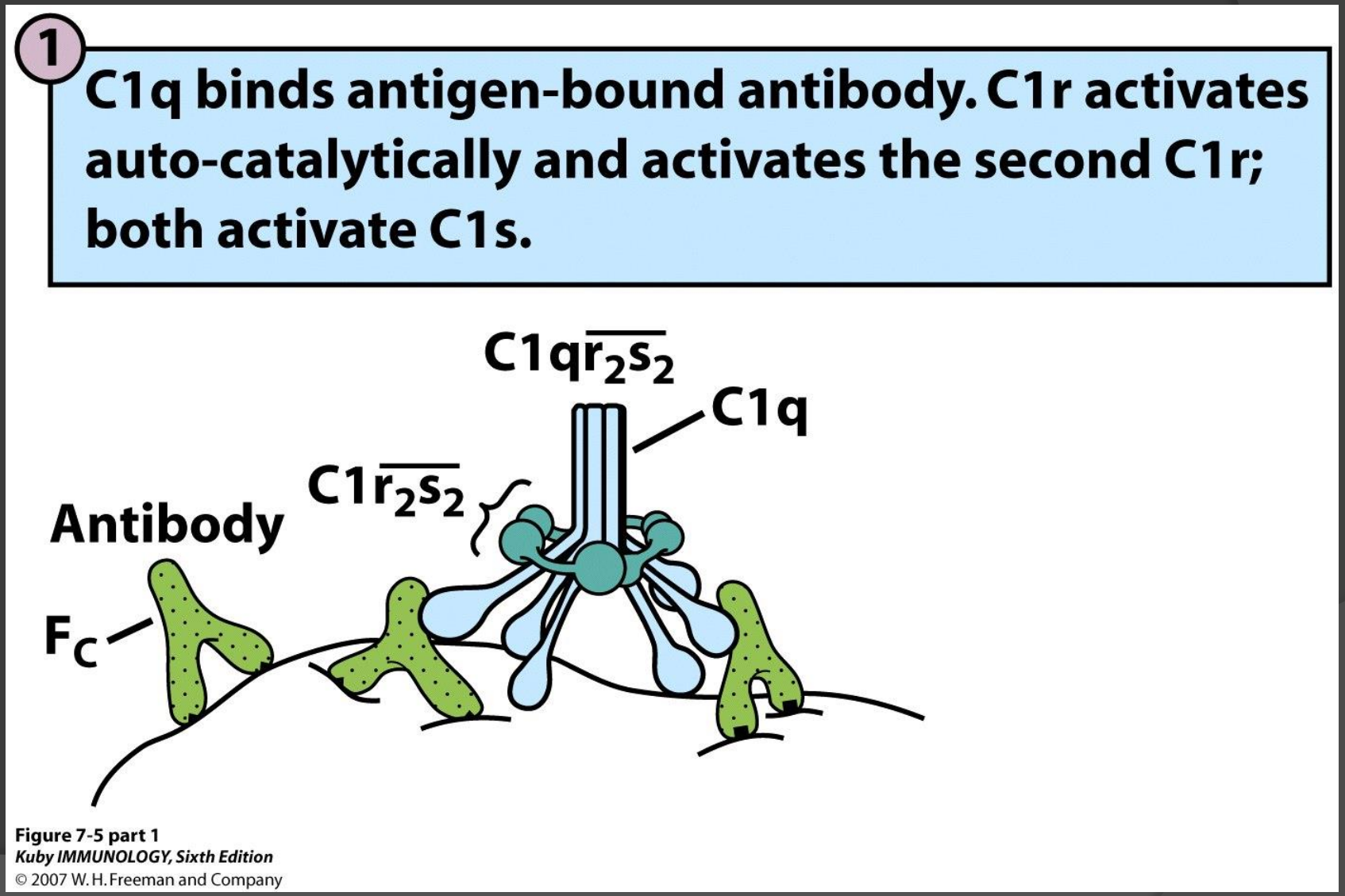
- **Antibody Dependent**
  - Activated by Ag-Ab complex (most commonly IgM and IgG)
  - Early stages involve C1, C2, C3, and C4

Classical Pathway

- What C1 looks like



Classical Pathway



Classical Pathway

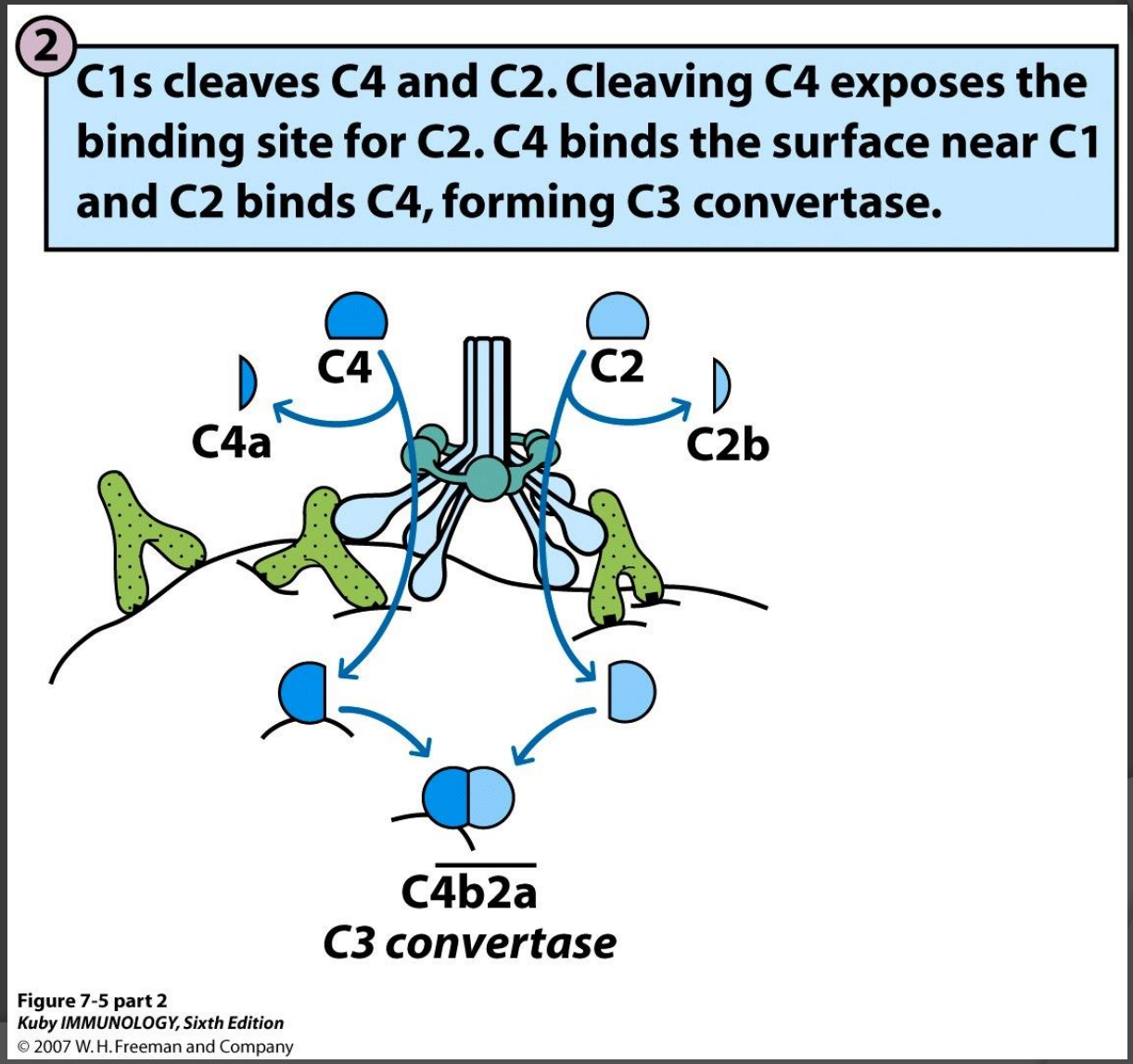


Figure 7-5 part 2
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Classical Pathway

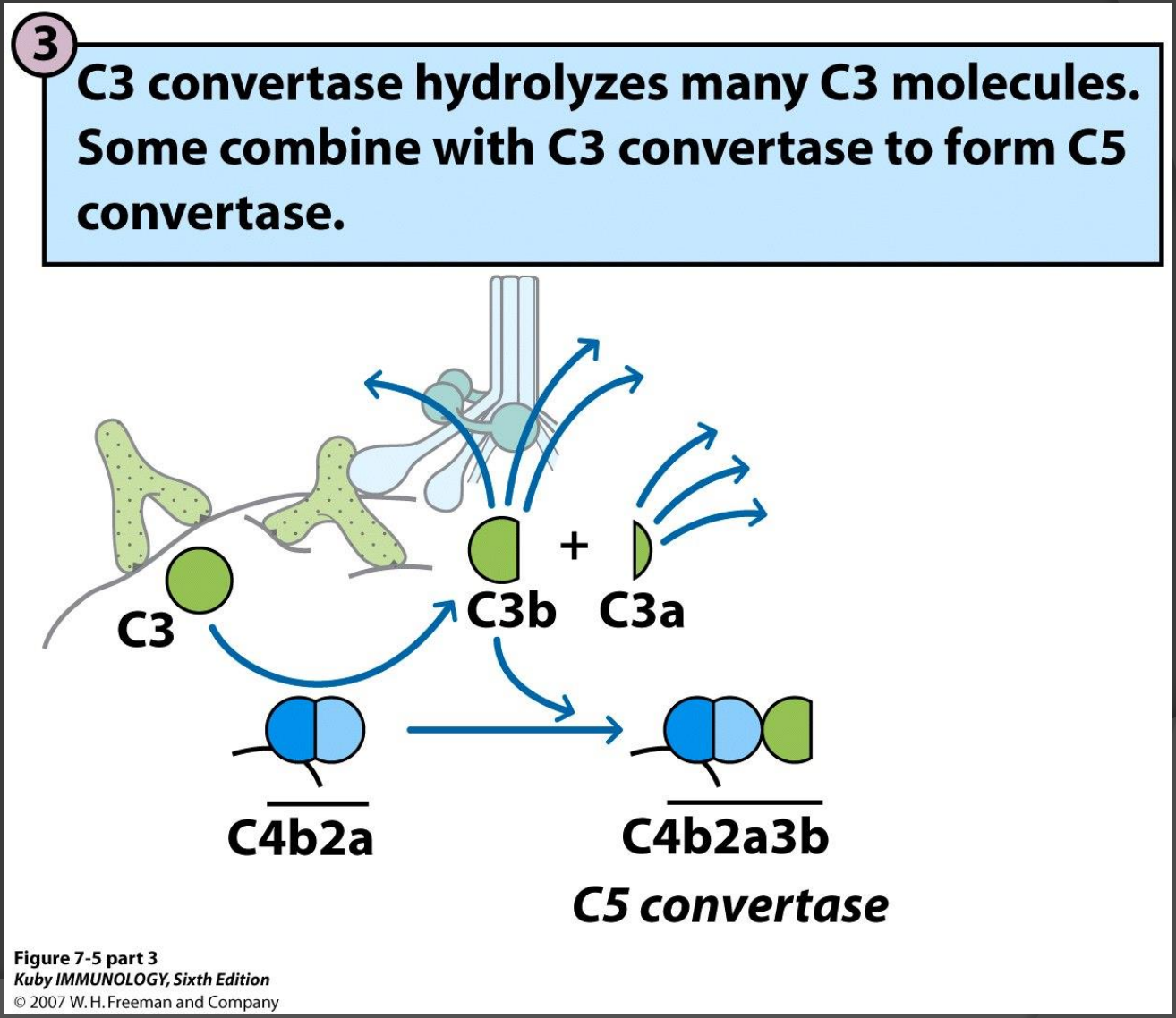

Figure 7-5 part 3
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Classical Pathway

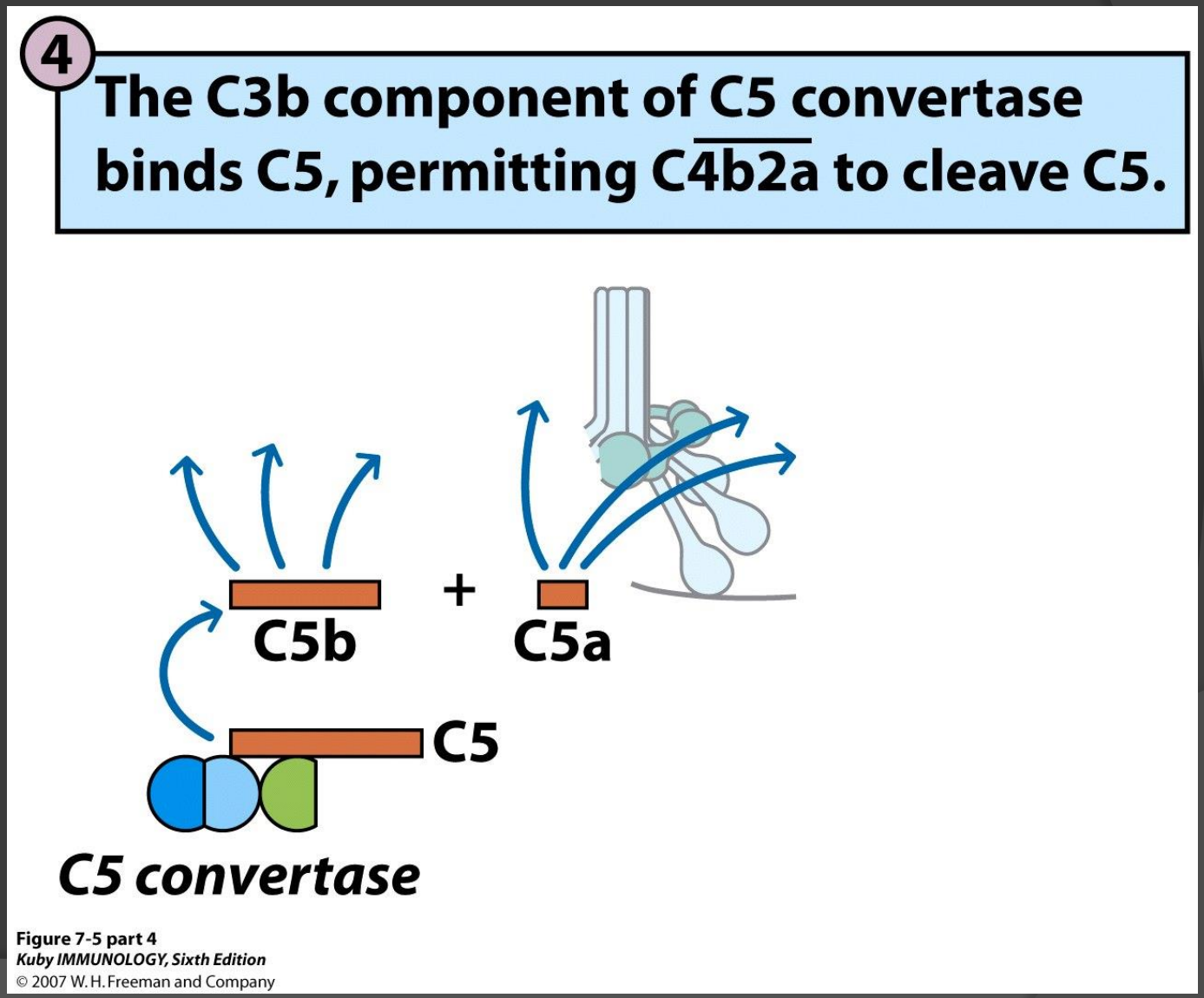

Figure 7-5 part 4
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Classical Pathway

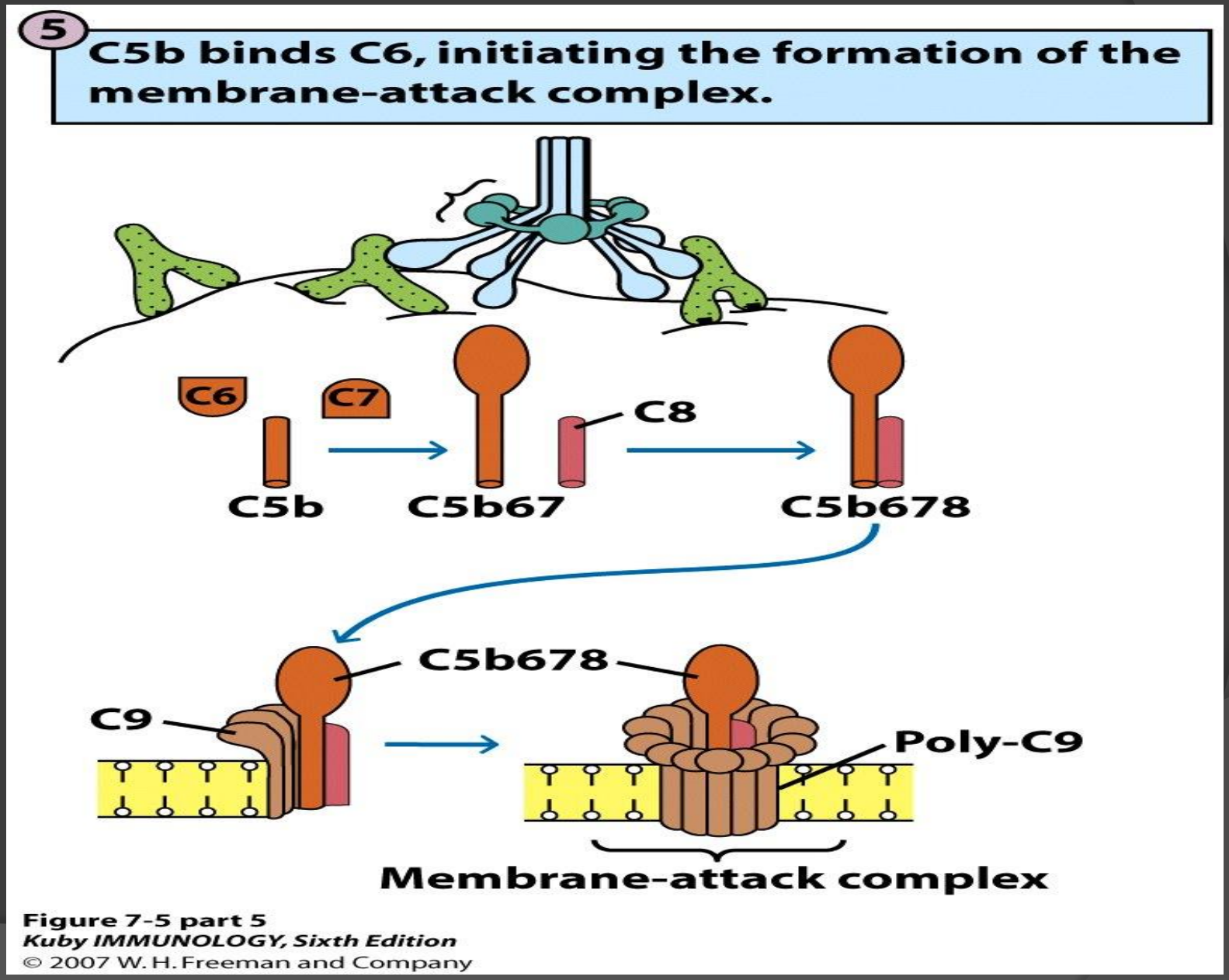

Figure 7-5 part 5
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Alternative Pathway

- **Antibody-Independent**
  - Component of innate immune system
  - Early stages involve C3, factor B, factor D, and properdin
- **Initiated by cell surface constituents foreign to host**
  - For example – Gram - and Gram+ bacteria

TABLE 7-1 Initiators of the alternative pathway of complement activation

PATHOGENS AND PARTICLES OF MICROBIAL ORIGIN
Many strains of gram-negative bacteria
Lipopolysaccharides from gram-negative bacteria
Many strains of gram-positive bacteria
Teichoic acid from gram-positive cell walls
Fungal and yeast cell walls (zymosan)
Some viruses and virus-infected cells
Some tumor cells (Raji)
Parasites (trypanosomes)
NONPATHOGENS
Human IgG, IgA, and IgE in complexes
Rabbit and guinea pig IgG in complexes
Cobra venom factor
Heterologous erythrocytes (rabbit, mouse, chicken)
Anionic polymers (dextran sulfate)
Pure carbohydrates (agarose, inulin)

SOURCE: Adapted from M. K. Pangburn, 1986, in *Immunobiology of the Complement System,* G. Ross, ed., Academic Press, Orlando.

Table 7-1
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Alternative Pathway

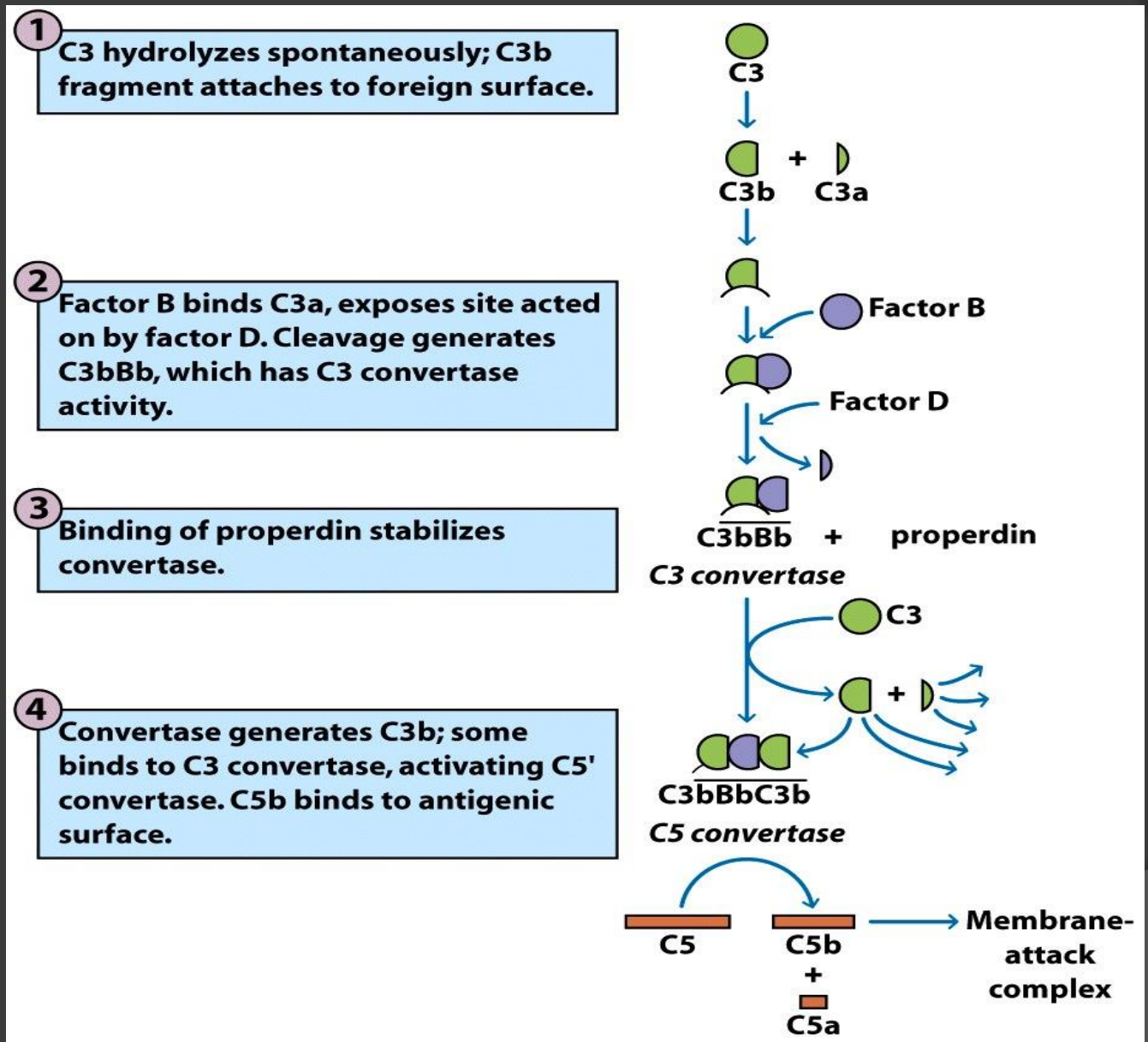


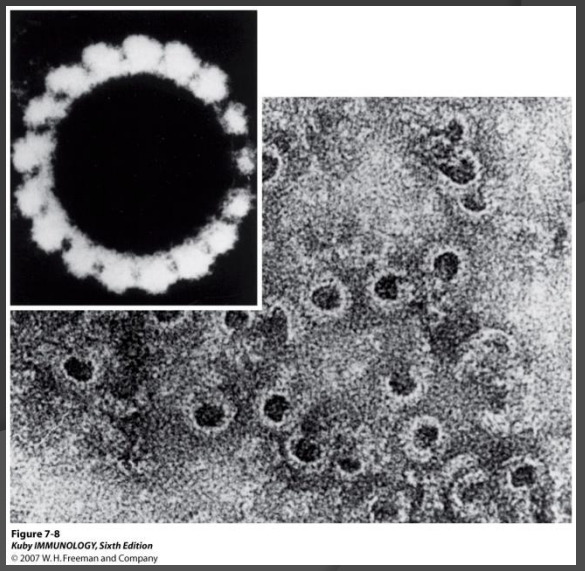
Figure 7-7
Kuby IMMUNOLOGY, Sixth Edition

Lectin Pathway

- Antibody-Independent
  - However, proceeds more like classical pathway
    - Uses C4 and C2
- Activated by binding of mannose-binding lectin (MBL) to mannose residues on glycoproteins or carbs on surface of microorganisms

Membrane Attack Complex (MAC)

- Forms pores in cell membrane
- Ions and small molecules can freely pass through pores
- Cell cannot maintain osmotic stability



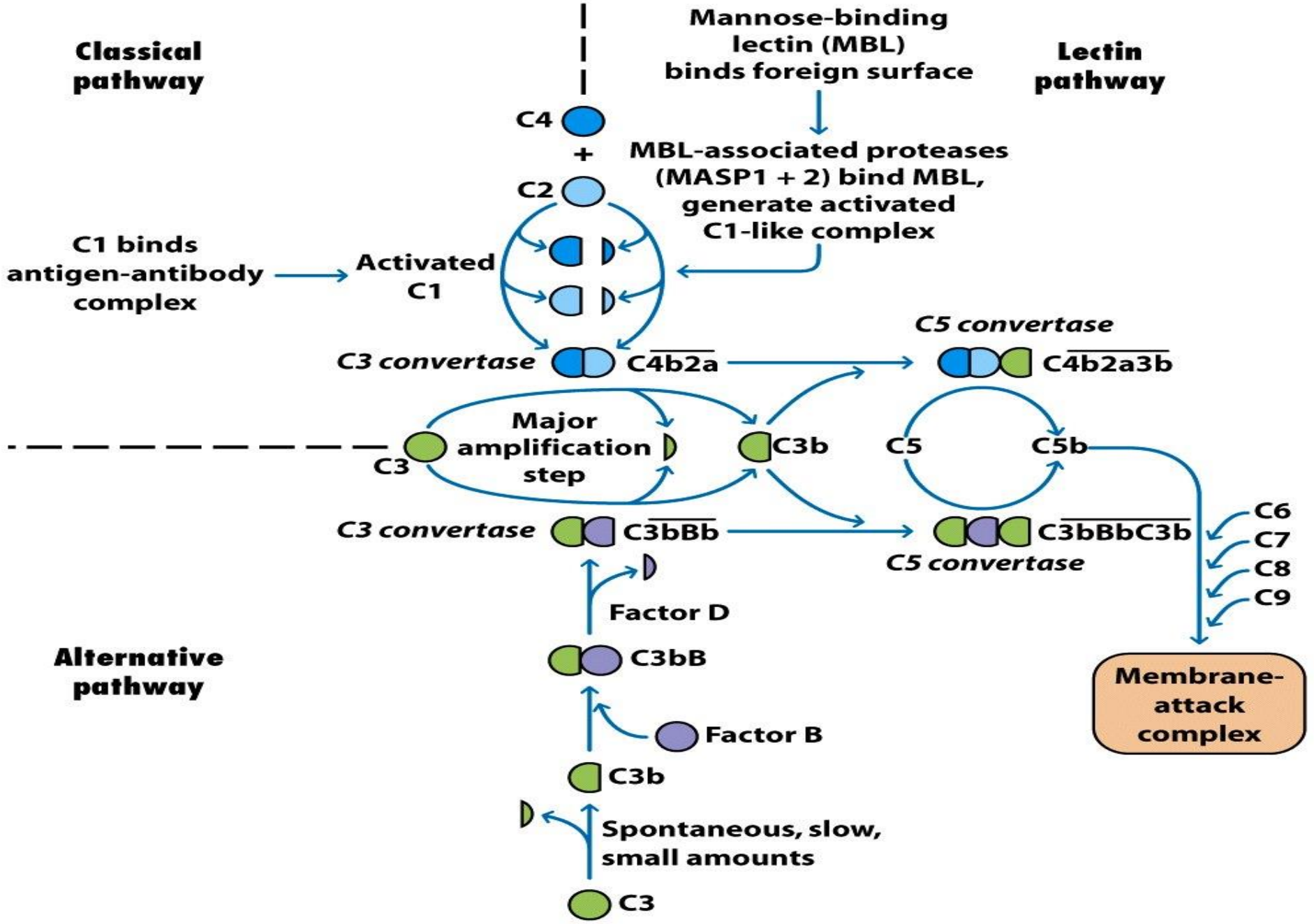


Figure 7-9
Kuby IMMUNOLOGY, Sixth Edition

Regulation

- Components are capable of attacking host cells
- Components undergo spontaneous inactivation if they are not stabilized with other components
- C3 convertase is major amplification step in all 3 pathways
  - Regulatory proteins are present that control C3 convertase

TABLE 7-2 Proteins that regulate the complement system

Protein	Type of protein	Pathway affected	Immunologic function
C1 inhibitor (C1Inh)	Soluble	Classical	Serine protease inhibitor: causes $C1r_2s_2$ to dissociate from C1q
C4b-binding protein (C4bBP)*	Soluble	Classical and lectin	Blocks formation of C3 convertase by binding C4b; cofactor for cleavage of C4b by factor I
Factor H*	Soluble	Alternative	Blocks formation of C3 convertase by binding C3b; cofactor for cleavage of C3b by factor I
Complement receptor type 1 (CR1 or CD35)* Membrane-cofactor protein (MCP or CD46)*	Membrane bound	Classical, alternative, and lectin	Block formation of C3 convertase by binding C4b or C3b; cofactor for factor I–catalyzed cleavage of C4b or C3b
Decay-accelerating factor (DAF or CD55)*	Membrane bound	Classical, alternative, and lectin	Accelerates dissociation of $C\overline{4b2a}$ and $C\overline{3bBb}$ (classical and alternative C3 convertases)
Factor I	Soluble	Classical, alternative, and lectin	Serine protease: cleaves C4b or C3b using C4bBP, CR1, factor H, DAE, or MCP as cofactor
S protein	Soluble	Terminal	Binds soluble C5b67 and prevents its insertion into cell membrane
Homologous restriction factor (HRF), also called membrane inhibitor of reactive lysis (MIRL or CD59)*	Membrane bound	Terminal	Bind to C5b678 on autologous cells, blocking binding of C9
Anaphylatoxin inactivator	Soluble	Effector	Inactivates anaphylatoxin activity of C3a, C4a, and C5a by carboxypeptidase N-catalyzed removal of C-terminal Arg

*An RCA (regulator of complement activation) protein. In humans, all RCA proteins are encoded on chromosome 1 and contain short consensus repeats.

Table 7-2
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Regulation of the Complement System

(a) Before assembly of convertase activity

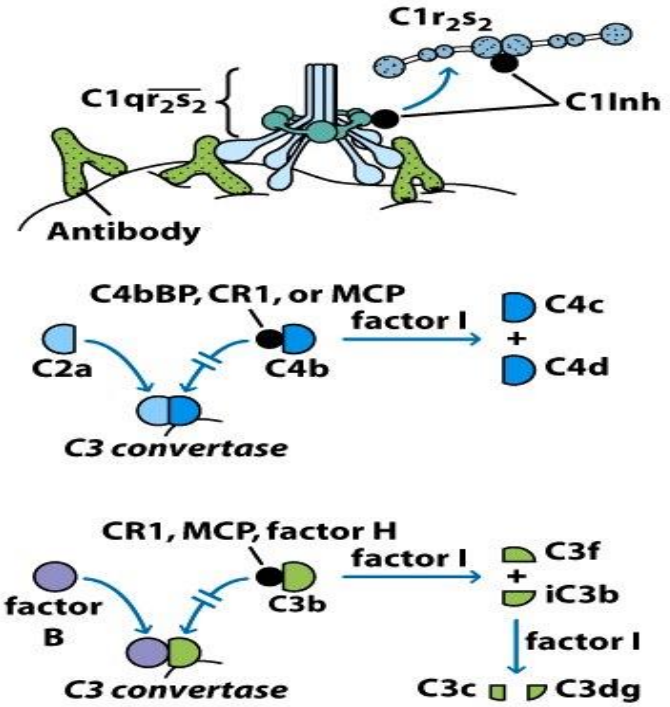
1. C1 inhibitor (C1Inh) binds $C1r_2s_2$, causing dissociation from C1q.

2. Association of C4b and C2a is blocked by binding C4b-binding protein (C4bBP), complement receptor type I, or membrane cofactor protein (MCP).

3. Inhibitor-bound C4b is cleaved by factor I.

4. In alternative pathway, CR1, MCP, or factor H prevents binding of C3b and factor B.

5. Inhibitor-bound C3b is cleaved by factor I.



(b) After assembly of convertase

C3 convertases are dissociated by C4bBP, CR1, factor H, and decay-accelerating factor (DAF).

(c) Regulation at assembly of membrane-attack complex (MAC)

1. S protein prevents insertion of C5b67 MAC component into the membrane.

2. Homologous restriction factor (HRF) or membrane inhibitor of reactive lysis (MIRL or CD59) bind C5b678, preventing assembly of poly-C9 and blocking formation of MAC.

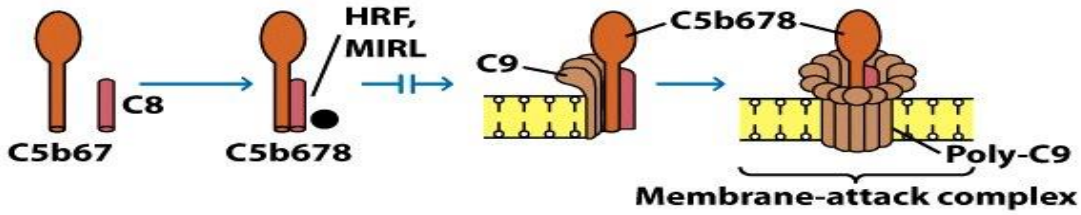


Figure 7-10
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Biological Consequences of Complement Activation

- Amplifies humoral response and causes it to be an effector response
  - Lyse cells
  - Participate in inflammatory response
  - Opsonization of antigen
  - Clearance of immune complexes

Cell Lysis

- MAC and lyse broad spectrum of cells
- Gram+ bacteria generally more resistant because of thick peptidoglycan
- Some have developed ways to evade MAC

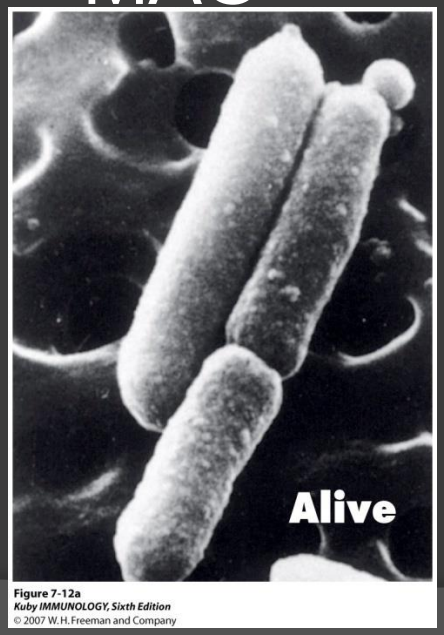

Figure 7-12a
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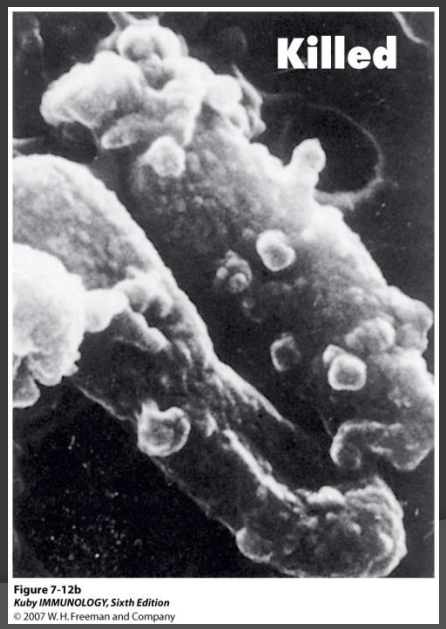

Figure 7-12b
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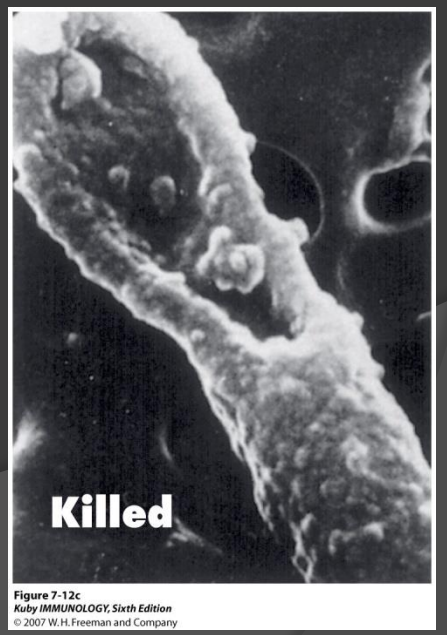

Figure 7-12c
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TABLE 7-5 Microbial evasion of complement-mediated damage

Microbial component	Mechanism of evasion	Examples
	GRAM-NEGATIVE BACTERIA	
Long polysaccharide chains in cell wall LPS*	Side chains prevent insertion of MAC into bacterial membrane*	Resistant strains of *E. coli* and *Salmonella*
Outer membrane protein	MAC interacts with membrane protein and fails to insert into bacterial membrane	Resistant strains of *Neisseria gonorrhoeae*
Elastase	Anaphylatoxins C3a and C5a are inactivated by microbial elastase	*Pseudomonas aeruginosa*
	GRAM-POSITIVE BACTERIA	
Peptidoglycan layer of cell wall	Insertion of MAC into bacterial membrane is prevented by thick layer of peptidoglycan	*Streptococcus*
Bacterial capsule	Capsule provides physical barrier between C3b deposited on bacterial membrane and CR1 on phagocytic cells*	*Streptococcus pneumoniae*
	OTHER MICROBES	
Proteins that mimic complement regulatory proteins	Protein present in various bacteria, viruses, fungi, and protozoans inhibit the complement cascade	Vaccinia virus, herpes simplex, Epstein-Barr virus, *Trypanosoma cruzi*, Candida *albicans*

*LPS = lipopolysaccharide; MAC = membrane-attack complex; CR1 = complement receptor type 1.

Table 7-5
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Mediating Inflammation

- Cleavage products of complement components mediate inflammation
  - Smaller fragments bind to basophils and mast cells
  - C3a and C5a (anaphylatoxins) induce smooth muscle contraction and increase vascular permeability

Opsonization

- C3b and C4b have opsonizing activity – cause phagocytosis

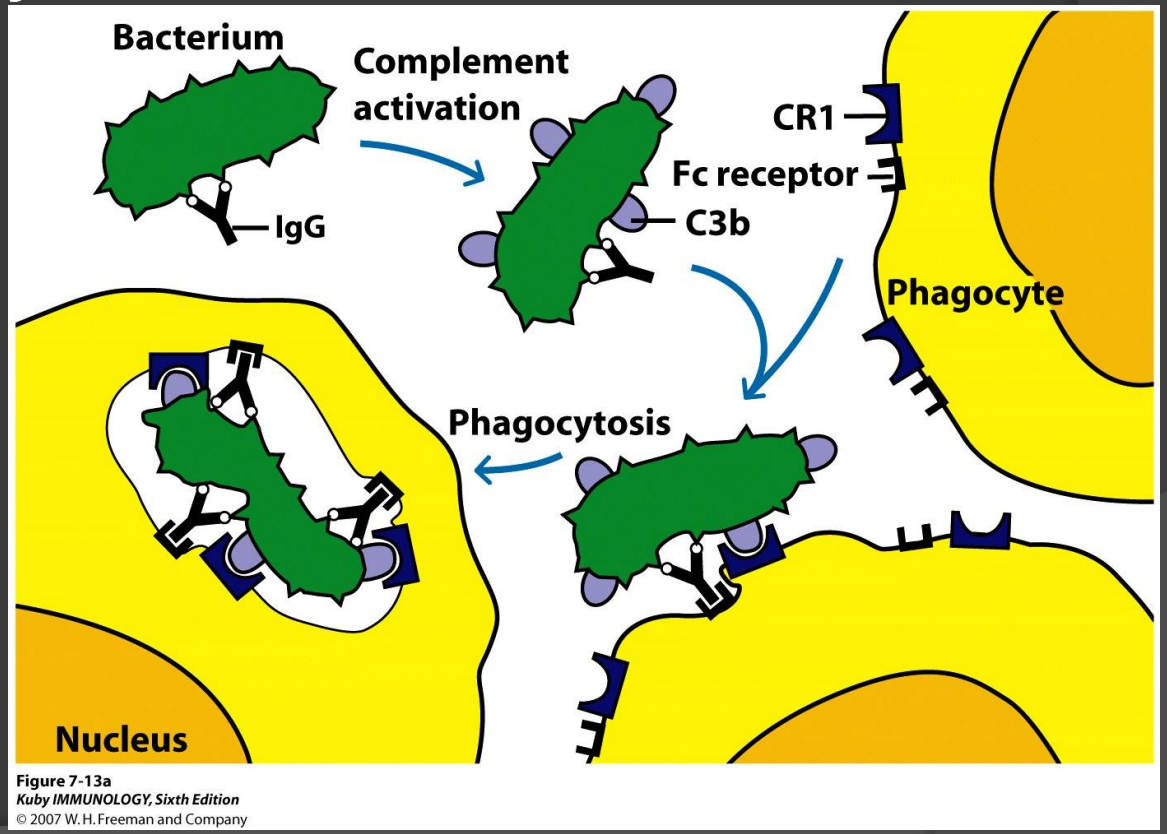

Figure 7-13a
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Viral Neutralization

- Binding of antibody and complement to viruses blocks attachment to susceptible host cells

Clearing of Immune Complexes

- Tissue damage can result from build up of immune complexes
- C3b coats immune complexes
  - RBC have capability of binding C3b coated complexes and carrying them to liver and spleen to be cleared
  - Deficiencies with any of complement may result in improper binding of C3b and loss of clearing may occur

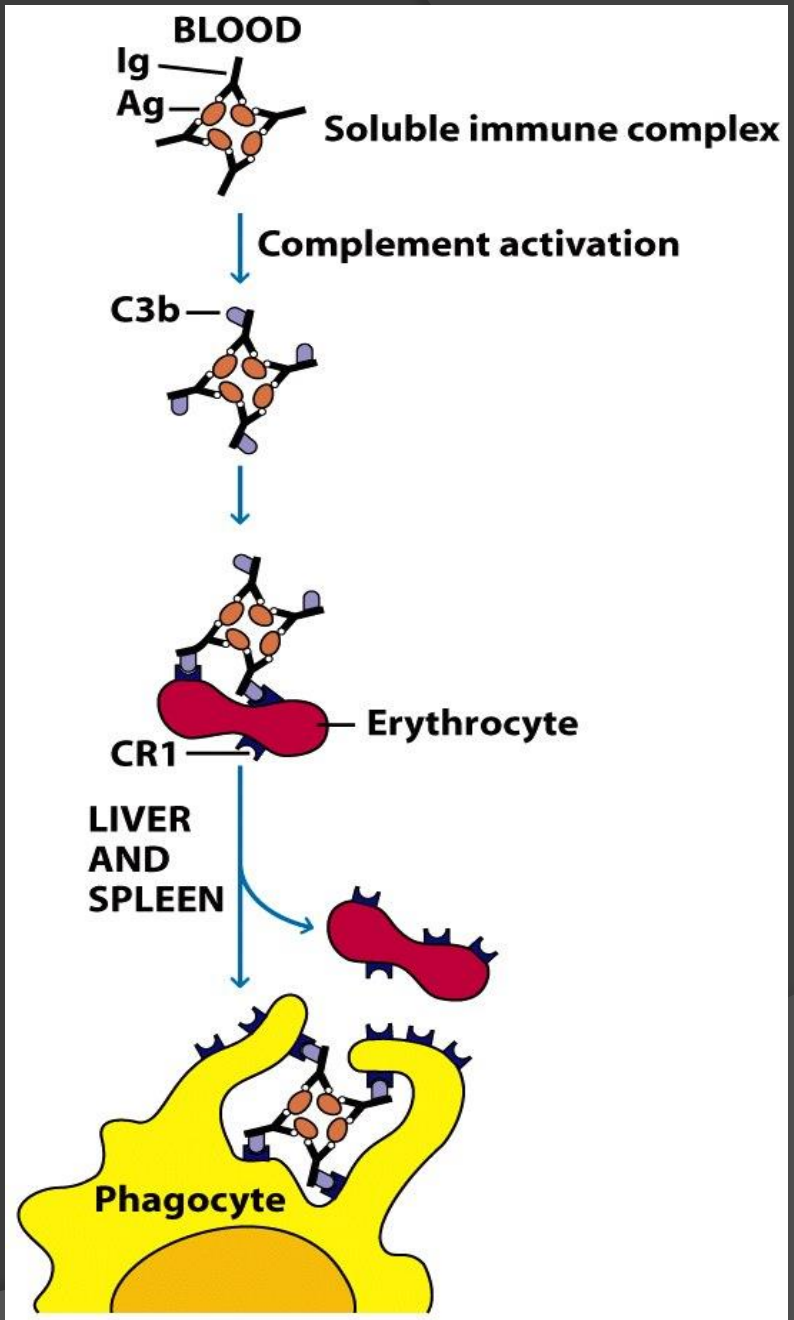

Figure 7-15
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TABLE 7-3 Summary of biological effects mediated by complement products

Effect	Complement product mediating*
Cell lysis	**C5b–9, the membrane-attack complex (MAC)**
Inflammatory response	
Degranulation of mast cells and basophils†	**C3a, C4a, and C5a (anaphylatoxins)**
Degranulation of eosinophils	**C3a, C5a**
Extravasation and chemotaxis of leukocytes at inflammatory site	**C3a, C5a, C5b67**
Aggregation of platelets	**C3a, C5a**
Inhibition of monocyte/macrophage migration and induction of their spreading	**Bb**
Release of neutrophils from bone marrow	**C3c**
Release of hydrolytic enzymes from neutrophils	**C5a**
Increased expression of complement receptors type 1 and 3 (CR1 and CR3) on neutrophils	**C5a**
Opsonization of particulate antigens, increasing their phagocytosis	**C3b, C4b, iC3b**
Viral neutralization	**C3b, C5b–9 (MAC)**
Solubilization and clearance of immune complexes	**C3b**

*Boldfaced component is most important in mediating indicated effect.

†Degranulation leads to release of histamine and other mediators that induce contraction of smooth muscle and increased permeability of vessels.

Table 7-3
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TABLE 7-4 Complement-binding receptors

Receptor	Major ligands	Activity	Cellular distribution
CR1 (CD35)	C3b, C4b	Blocks formation of C3 convertase; binds immune complexes to cells	Erythrocytes, neutrophils, monocytes, macrophages, eosinophils, follicular dendritic cells, B cells, some T cells
CR2 (CD21)	C3d, C3dg,* iC3b	Part of B-cell coreceptor; binds Epstein-Barr virus	B cells, follicular dendritic cells, some T cells
CR3 (CD11b/18) CR4 (CD11c/18)	iC3b	Bind cell adhesion molecules on neutrophils, facilitating their extravasation; bind immune complexes, enhancing their phagocytosis	Monocytes, macrophages, neutrophils, natural killer cells, some T cells
C3a/C4a receptor	C3a, C4a	Induces degranulation of mast cells and basophils	Mast cells, basophils, granulocytes
C5a receptor	C5a	Induces degranulation of mast cells and basophils	Mast cells, basophils, granulocytes, monocytes, macrophages, platelets, endothelial cells

*Cleavage of C3dg by serum proteases generates C3d and C3g.

Table 7-4
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FLUORESCENCE – ACTIVATED CELL SORTING

Ana Luisa Caetano

Unidade de Citometria de Fluxo
Instituto de Medicina Molecular

INSTITUTO DE
MEDICINA MOLECULAR

Fluorescence – Activated Cell Sorting

1) How does a FACS sort cells?

2) Examples of cell sorting experiments.

3) The future of FACS and other cell sorting technologies.

4) How to do cell sorting at the IMM.

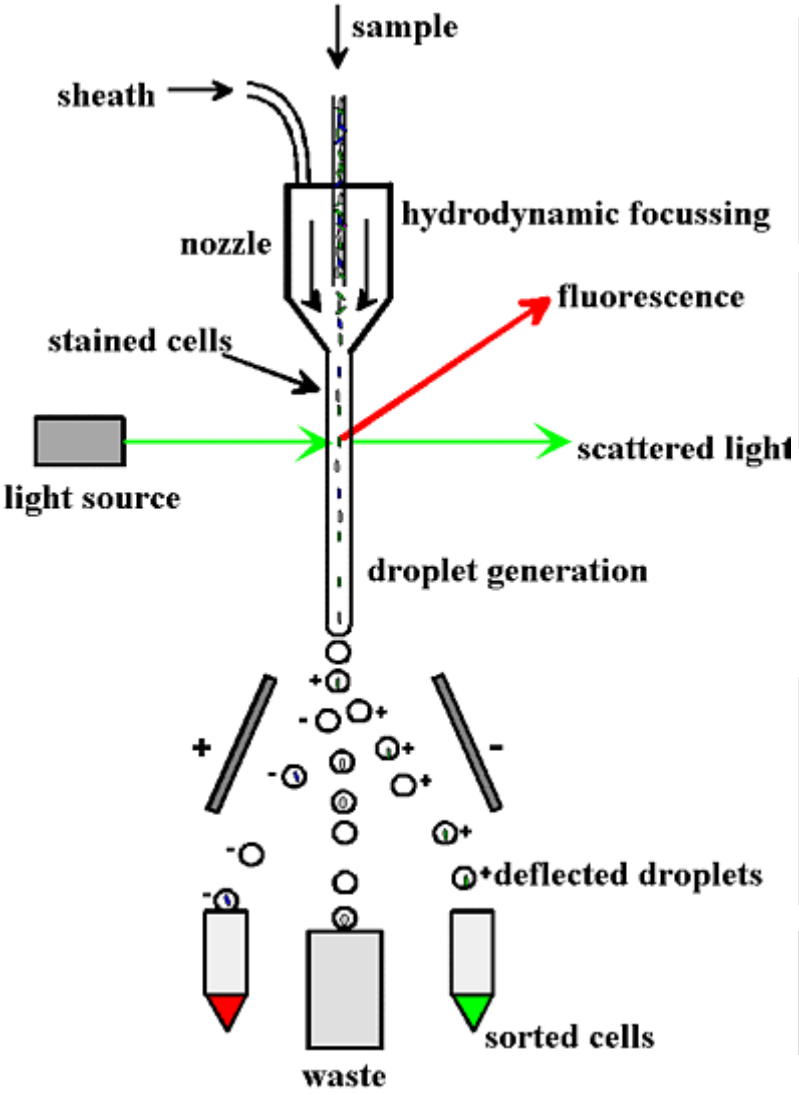
How does a FACS sort cells

- Aligns the particles in a continuous laminar fluid flow.
- Detects the "physical" properties and fluorescence of the particles as they flow past a light source (laser).
- Records and displays the measured parameters.
- Applies regions and gates to define sub-populations.

and then…

SORTERS have the ability to physically isolate these sub-populations.

Flow Cytometry Sorting Schematic



The nozzle/flow cell is vibrated by a transducer (converts electrical energy into mechanical energy) so it produces a stream breaking into droplets.

Laser interrogation and signal processing followed by sort decision: sort right, sort left, or no sort.

Electronic delay until cell reaches break off point. Then the stream is charged : + or -.

Charged droplets deflected by electrostatic field
from plates held at high voltage (+/-3000 volts).

Besides tubes can sort onto slides or multi-well plates.

Basic Theory of Sorting

Sort Setup:

1. Droplets are created by vibrating the Nozzle at a very precise amplitude and frequency.
2. The "drop delay" is calculated which defines the time duration for a particle to travel from the interrogation point at the laser to the "last attached drop".
3. Sort decisions are defined by the user in the Analysis Software.

The Sort:

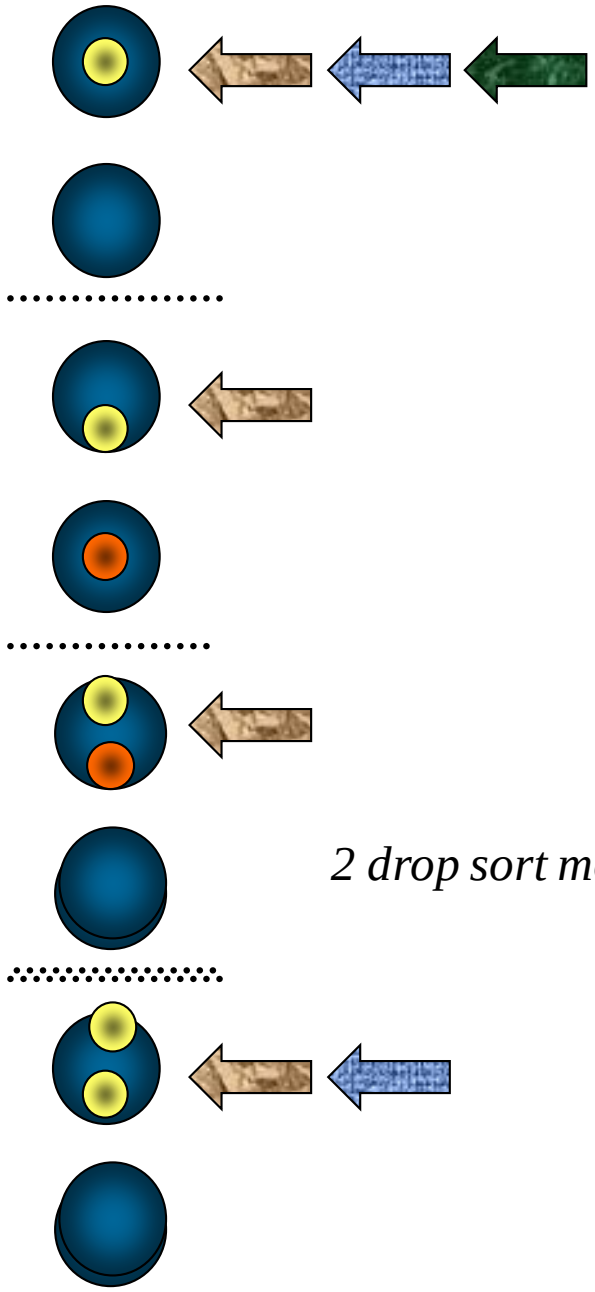
4. A decision is made at the interrogation point whether to sort a cell or abort a cell.
5. If a cell is to be sorted, the electronics waits for the "Drop Delay" time when the cell will be contained in the "Last Attached Drop".
6. A charge is sent down the sheath/sample stream.
7. The "Last Attached Drop" breaks off carrying the charge which can be positive or negative depending on the direction to be sorted.
8. As the charged droplet falls between the electrical field created by the charged Sort Deflection Plates, it is deflected into the proper sorting tube for collection.

4-way Sorting

It is possible to sort up to 4 populations at once by applying 4 different charges to the droplet (+1,+0.5,-0.5,-1) for each of the different populations.

Sort Aborts

- It may happen that within the drop envelope charge that has your wanted cell there may also be an unwanted cell.
- The sorter knows to stop sort if unwanted cell is so close to wanted cell that it would be included in sort window.
- This is termed a Sort Abort.
- Can operate sorter in different ways to deal with aborts.



2 drop sort mode

Sort Aborts

Positive event

Negative event

Enrich mode

All positive events sorted.

Purify mode

All negative events aborted.

Single mode

All negative events aborted and must only have one positive cell.

Sort Modes

Mode	Characteristic	General Application
Enrich	Tries to capture all positive events regardless of the presence of negative events	High Recovery
Purify	Sorts positives only in the absence of a negative event	High Purity
Single	Same as Purify mode except will only accept one positive event per sort decision	Single Cell Deposition
Mixed Mode	Sorts Purify Mode left and all the aborts to the right	High Purity one direction while recovering all aborted positive events the other direction

What Effects the Sort Abort rate?

- At a cell analysis rate of 10 000/sec average of 1 cell every 9 drops.
- Increasing the analysis frequency (while drop drive frequency remains same) will increase the chance of aborts.
- Sort abort rate also affected by what proportion of the population you are sorting: the lower the proportion the more likely that it will be an unwanted cell close to your wanted cell.
- To decrease aborts need to trade off between analysis rate and how long you want to sort for.

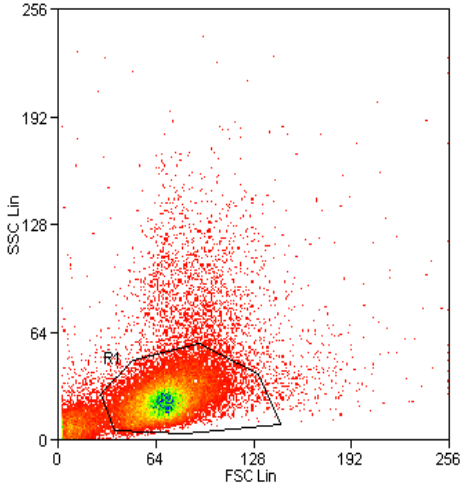
Yield: Proportion of cells wanted in sample actually sorted.
Purity: Proportion of wanted cells in the sorted population.

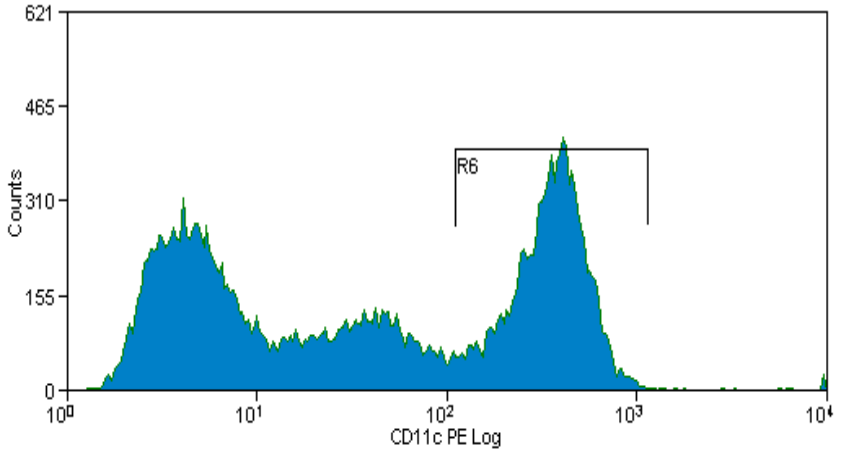
Preparing Samples for Sorting

1. Need to have a clean, single cell suspension.
2. Need to know how many sorted cells you require.
3. Need to ensure you have adequate number of cells to get the number of sorted cells you want. To cover for losses during sample preparation and sorting best to have at least double the amount from basic calculation.
4. Need to know what you want to sort the cells into (tubes, plates, slides, type of media).

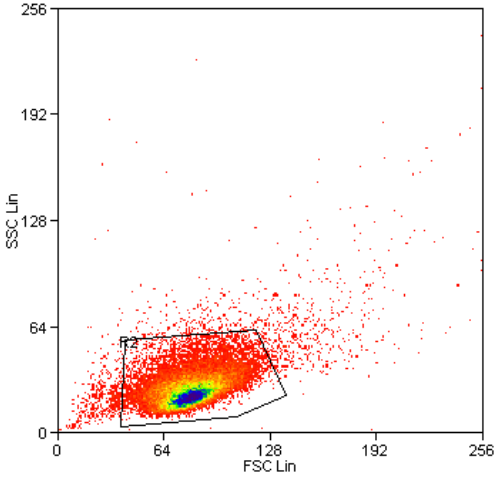
SPLEEN DENDRITIC CELL SORT

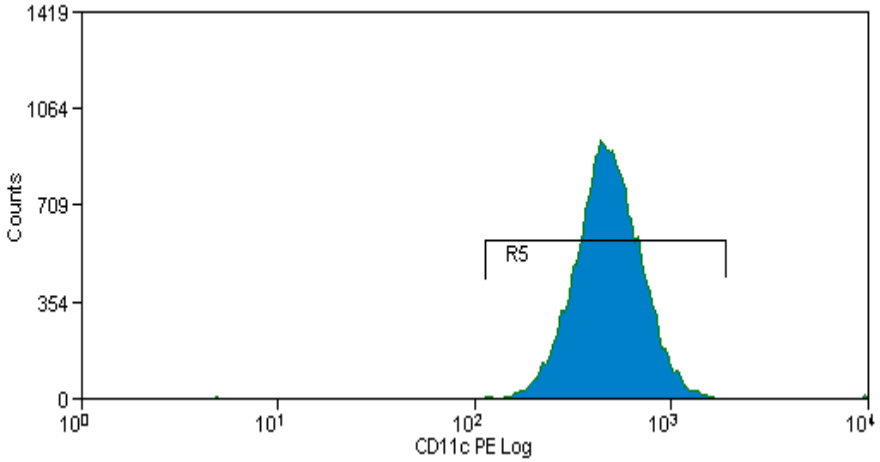
BEFORE





AFTER





Sorting rules

- Always filter the sample as last step of cell preparation to prevent clogs.
- Adjust cell concentration so you can run at correct analysis rate.
- Use color gating to ensure the populations are gated correctly.
- Use Doublet Discrimination for optimum results.
- Always double-check the Sort Logic and Mode before starting a sort.
- Before performing a re-analysis of a sorted population, backflush and check for the absence of any carry over.

Other Cell Sorting Technologies

Magnetic beads (MACS):

Coated with antibody, run past magnet to remove unwanted cells or enrich wanted cells.

Panning:

Coat tissue culture plate with antibody and then incubate cells on it, can remove unwanted cells or enrich wanted cells.

Mechanical catch (FACScalibur or Partec):

Wanted cells physically isolated by a movable catch (slow).

Combine beads and flow; do pre-enrichment by MACS or panning.

INSTITUTO DE MEDICINA MOLECULAR

FLUORESCENCE-ACTIVATED CELL SORTING

At the IMM we have 3-laser FacsAria. It can do 4-way sorting and sort into plates as well as tubes.

For booking please contact myself or Ana Luisa Caetano on ext 47222 /47224 (IMM 21 799 9530).

At moment booked up to 2 months in advance so need to book early!

Humanized Antibody

Submitted to: Dr. Amber

Submitted by: Mahnoor Khawaja

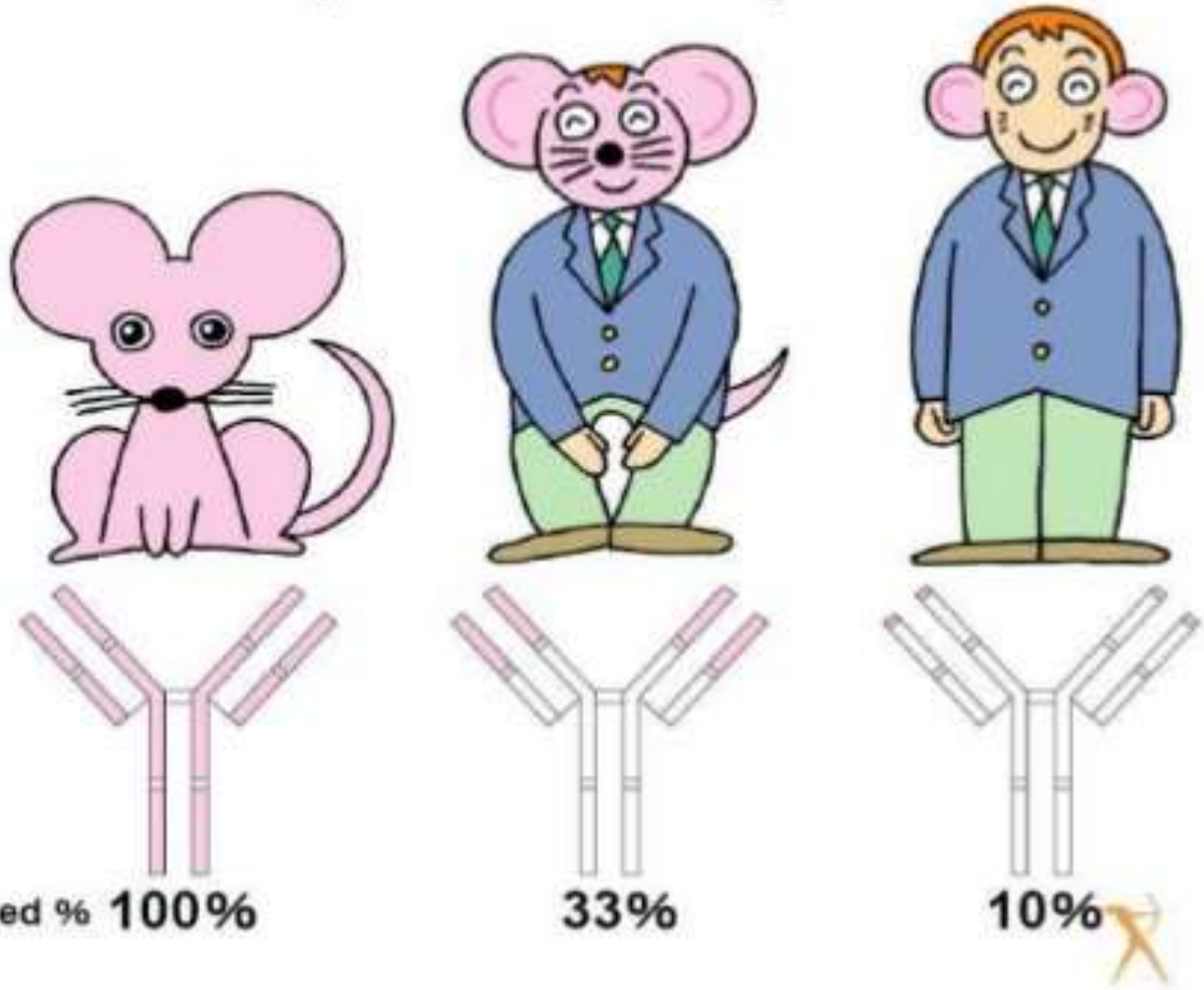
Definition

- **Humanized antibodies** are antibodies made from non-human species whose protein sequences have been modified to increase their similarity to antibody variants produced naturally in humans.

Era of humanized antibody

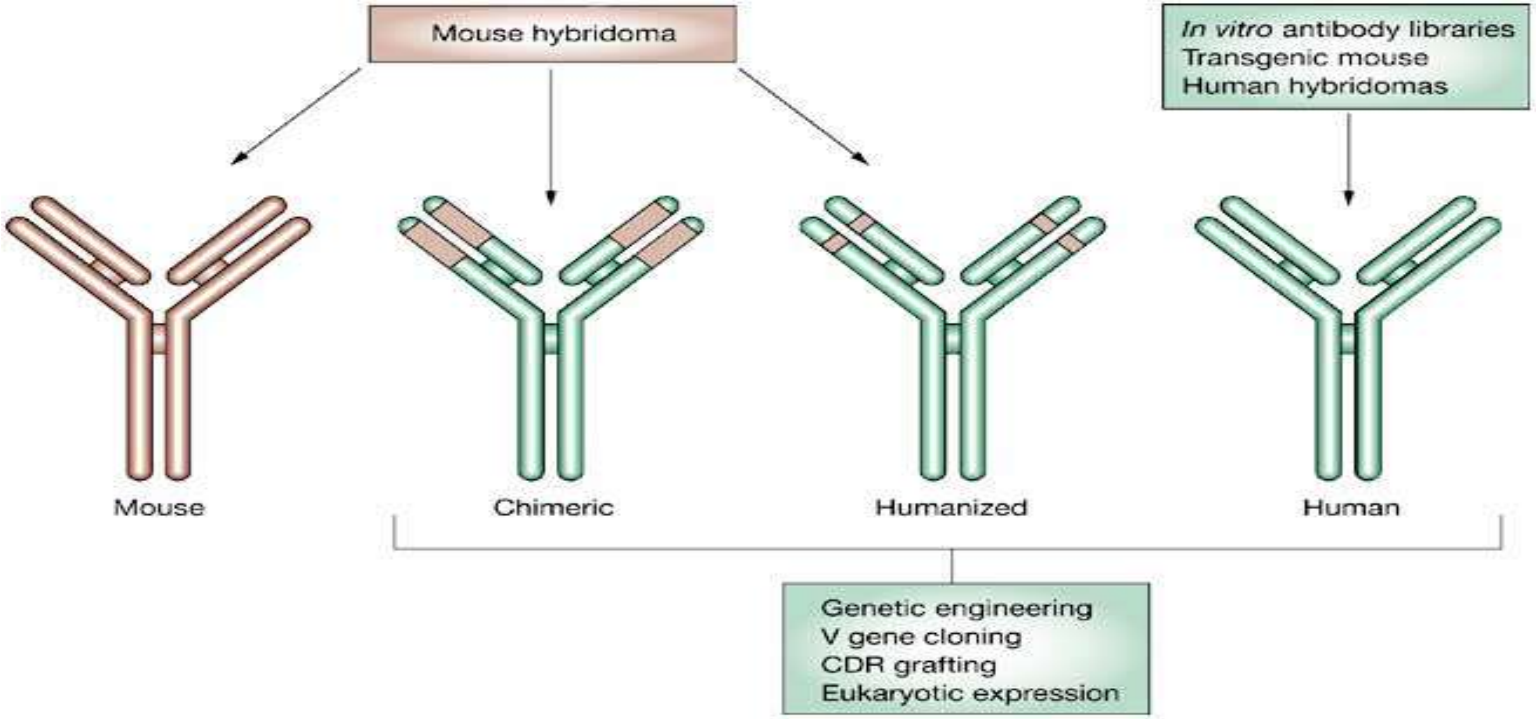
- Murine
- Chimeric
- Humanized
- Human or Fully Human

Differences in antibodies



Human or fully human

- A human antibody is one of which both chain types, and the J chain in the case of polymeric antibodies, are of human origin.



Preparation

- Strains used

Xeno mouse

HuMab Mouse

TC Mouse

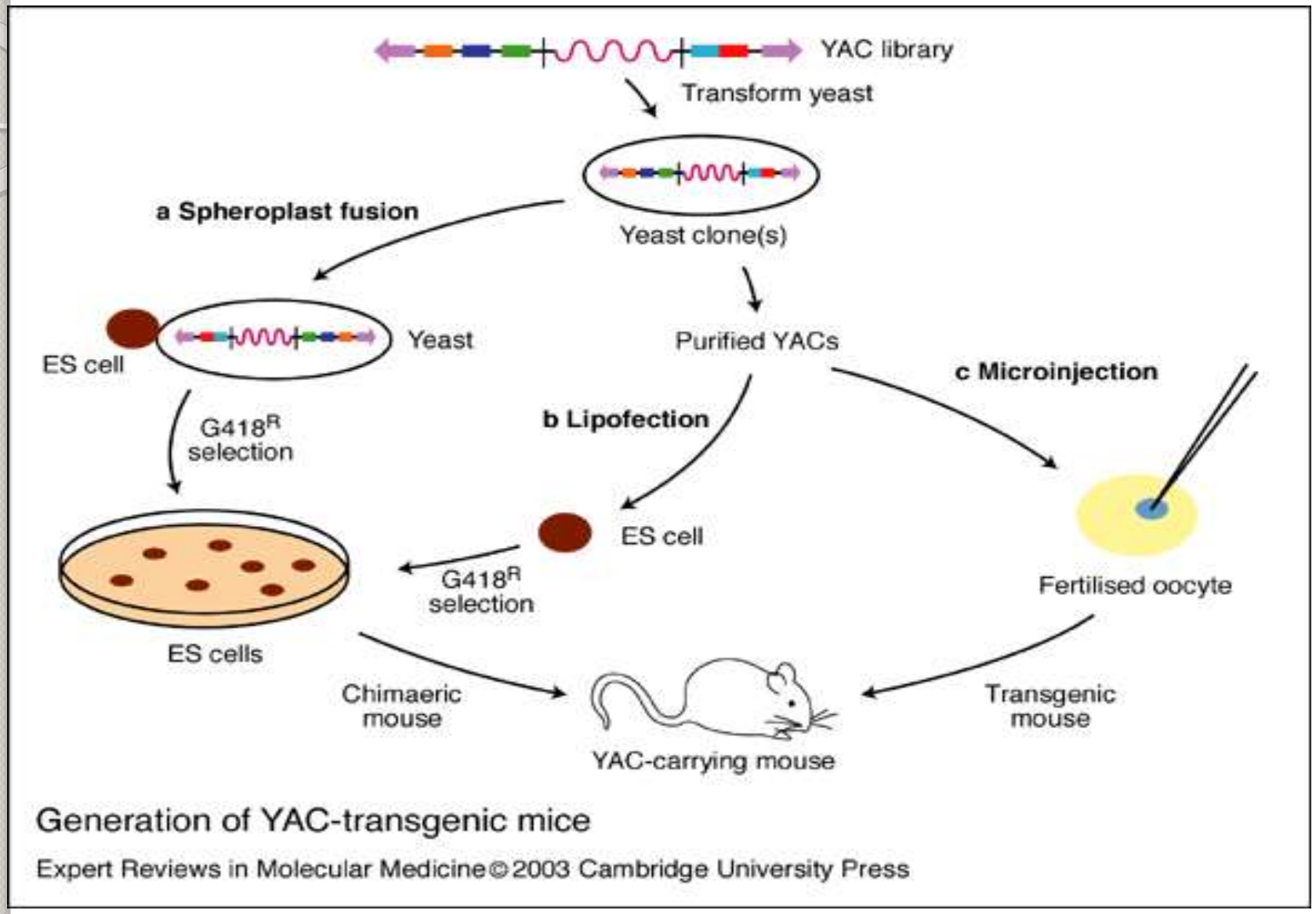
Technologies used

Phage Display

Transgenic Mice

Primarily human system

Transgenic mice



Generation of YAC-transgenic mice

Phage display

- The genes for the V regions of antibody heavy and light chains can be inserted separately into these phages, and they display the proteins on the surface.

❑ Gene isolated from;

(a) Naïve B-cells

(b) Immunized, antigen-specific memory B-cells.

Selection

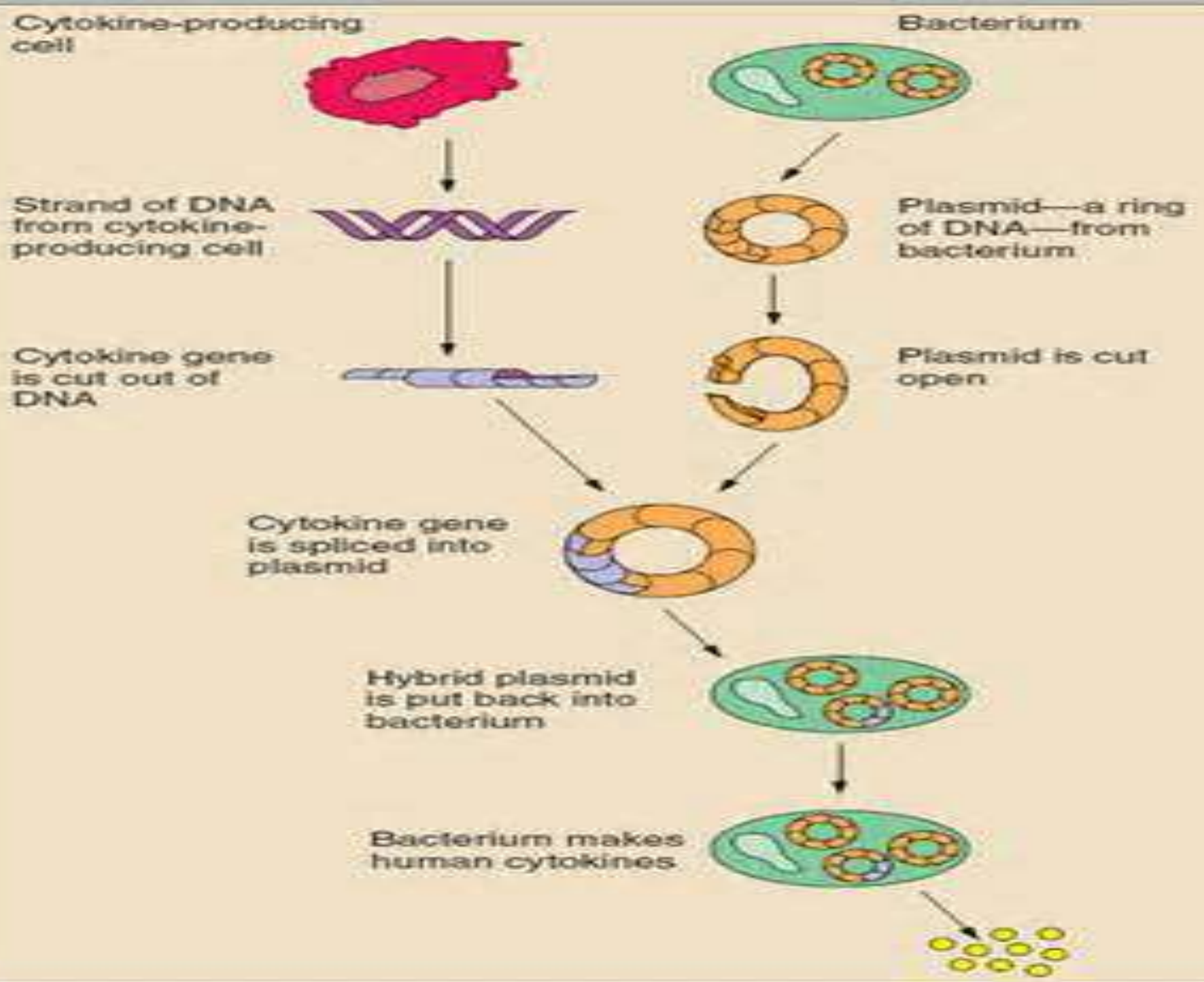
- The proteins may be selected by screening against the antigen of choice, and the desirable proteins may be produced and secreted by bacteria infected by the specific phage.
- The secreted proteins can be reassembled in vitro to make functional antibodies.

Primarily human system

- The best approach so far for producing antibody .
- Strategies used ;

Mouse myeloma cell line transfected with human immunoglobulin genes

Fusion of human B cells with a mouse-human hybrid 'heteromyeloma'


Cytokine-producing cell
Bacterium
Strand of DNA from cytokine-producing cell
Plasmid—a ring of DNA—from bacterium
Cytokine gene is cut out of DNA
Plasmid is cut open
Cytokine gene is spliced into plasmid
Hybrid plasmid is put back into bacterium
Bacterium makes human cytokines

Chapter 5

Innate Immunity

Dr. Capers

IMMUNOLOGY

Kindt • Goldsby • Osborne

Kuby IMMUNOLOGY

Sixth Edition

Chapter 3

Innate Immunity

- Vertebrate are protected by 2 systems of immunity
  - Innate Immunity
  - Adaptive Immunity
    - Takes time but has memory
- Innate Immunity can be found in all multicellular plants and animals
- Adaptive Immunity evolved in jawed vertebrates

TABLE 3-1 Innate and adaptive immunity

Attribute	Innate immunity	Adaptive immunity
Response time	Minutes/hours	Days
Specificity	Specific for molecules and molecular patterns associated with pathogens	Highly specific; discriminates even minor differences in molecular structure; details of microbial or nonmicrobial structure recognized with high specificity
Diversity	A limited number of germ line–encoded receptors	Highly diverse; a very large number of receptors arising from genetic recombination of receptor genes
Memory responses	None	Persistent memory, with faster response of greater magnitude on subsequent infection
Self/nonself discrimination	Perfect; no microbe-specific patterns in host	Very good; occasional failures of self/nonself discrimination result in autoimmune disease
Soluble components of blood or tissue fluids	Many antimicrobial peptides and proteins	Antibodies
Major cell types	Phagocytes (monocytes, macrophages, neutrophils), natural killer (NK) cells, dendritic cells	T cells, B cells, antigen-presenting cells

Table 3-1
Kuby IMMUNOLOGY, Sixth Edition

Innate Immunity

- Innate Immune System:
  - Physical/Anatomical Barriers
    - Skin and mucous membranes
  - Chemical Barriers
    - Acidity of stomach, antimicrobial molecules
  - Cellular Barriers
    - Cells with sensitive receptors that can detect microbial invaders

Innate Immunity

- Antimicrobial Proteins
  - Psoriasin – produced by skin
    - Antibacterial activity to *E. coli*
  - Help when skin is scratched or cut to prevent infection
  - Saliva, tears, and mucous membranes help to wash invaders away as well as contain antimicrobial peptides

Innate Immunity

- Normal flora
  - Help to out-compete pathogens for space and nutrients

Organ or tissue	Innate mechanisms protecting skin/epithelium
Skin	Antimicrobial peptides, fatty acids in sebum
Mouth and upper alimentary canal	Enzymes, antimicrobial peptides, and sweeping of surface by directional flow of fluid toward stomach
Stomach	Low pH, digestive enzymes, antimicrobial peptides, fluid flow toward intestine
Small intestine	Digestive enzymes, antimicrobial peptides, fluid flow to large intestine
Large intestine	Normal intestinal flora compete with invading microbes, fluid/feces expelled from rectum
Airway and lungs	Cilia sweep mucus outward, coughing, sneezing expel mucus, macrophages in alveoli of lungs

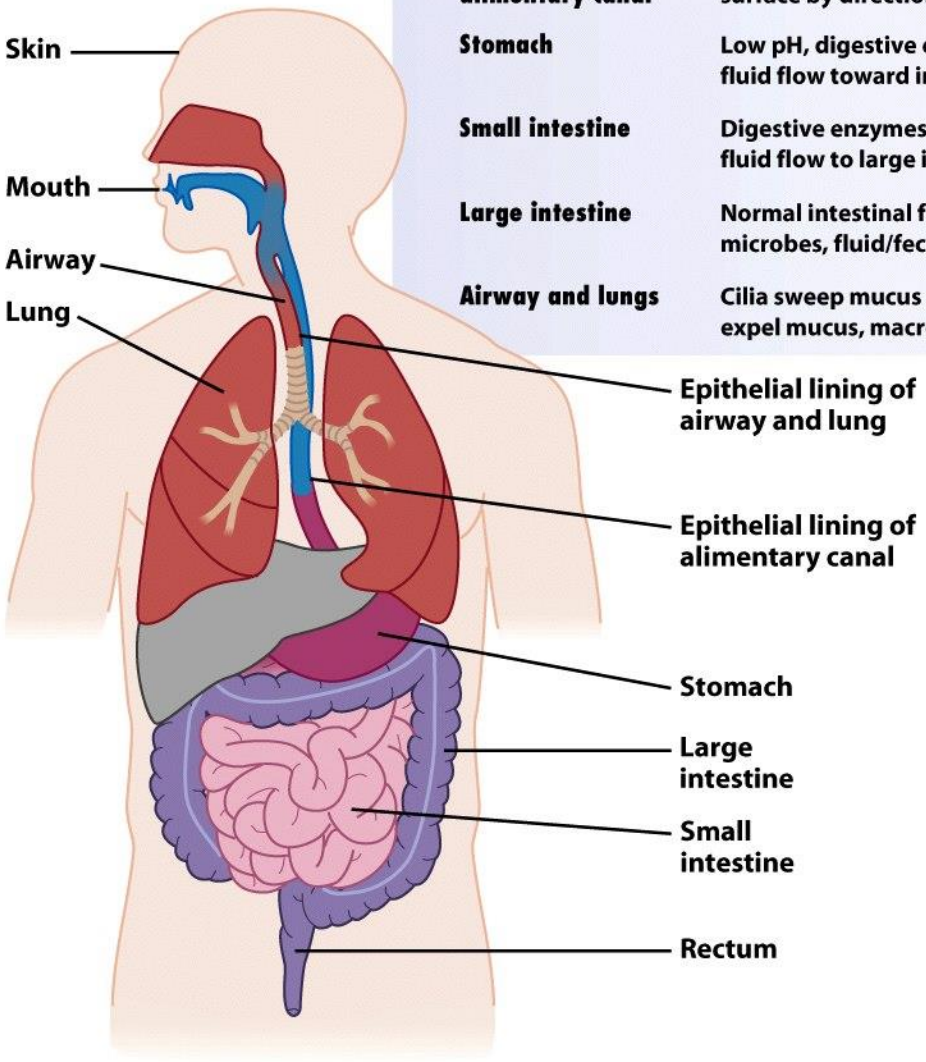


Figure 3-1
Kuby IMMUNOLOGY, Sixth Edition

Also includes:
Urogenital tract

Salivary, lacrimal, and Mammary glands

- Connection between adaptive and innate immunity
  - Pathogens may get past anatomical barriers
  - Interact with membrane-bound molecules (sensors) that recognize broad structural motifs of microbial species
    - Pattern Recognition Receptors (PRRs)
    - On pathogen it is called Pathogen-Associated Molecular Patterns (PAMPs)

- Pattern Recognition Receptors (PRRs)
  - In contrast, antibodies and T cell receptors recognize finer details of molecular structure
  - If PAMPs are detected, complement system will be activated

- Complement System
  - One part is a collection of proteins that form aggregates that punch holes in pathogen's cell membrane causing lysis
  - Include serum glycoproteins that promote uptake of pathogens by phagocytes (opsonization)
  - Complement system ties innate and adaptive immunity

- Dendritic cells and Macrophages have variety of receptors
  - Toll-like receptors – detect microbial products
- Activated macrophages will secrete cytokines
  - Hormone or growth-like factors to induce specific cell activities (upregulation of B and T cells); again tying innate and adaptive immunity

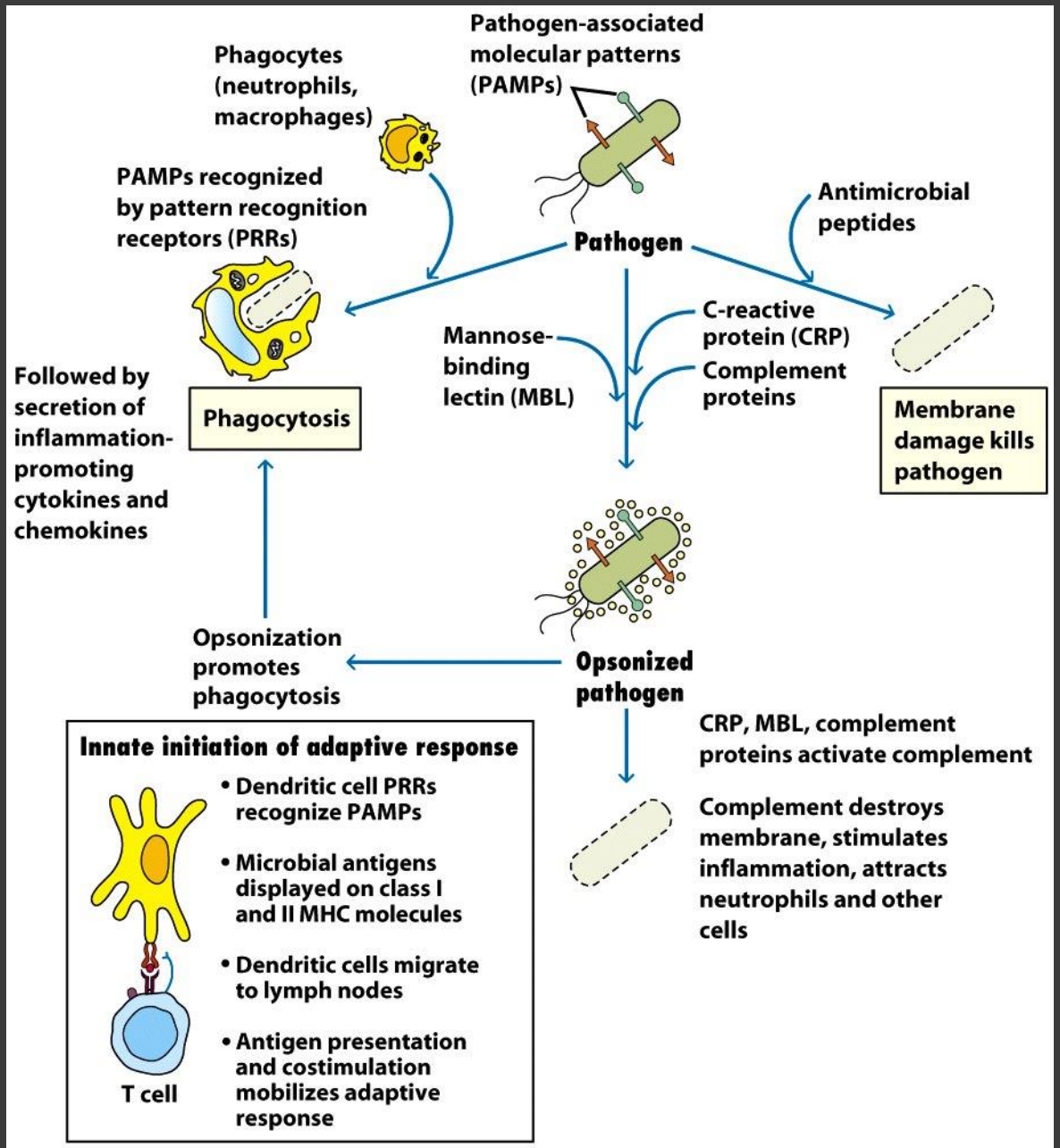


Figure 3-4
Kuby IMMUNOLOGY, Sixth Edition

Inflammation

- Hallmarks
  - Swelling
  - Redness
  - Heat
  - pain

Inflammation

- Within minutes of tissue injury:
  - Vasodilation – rise of blood volume to area
  - Vascular permeability increases – accumulation of fluid
    - Edema
  - Leukocytes adhere to endothelial cells and pass through walls of capillaries into tissues - extravasation

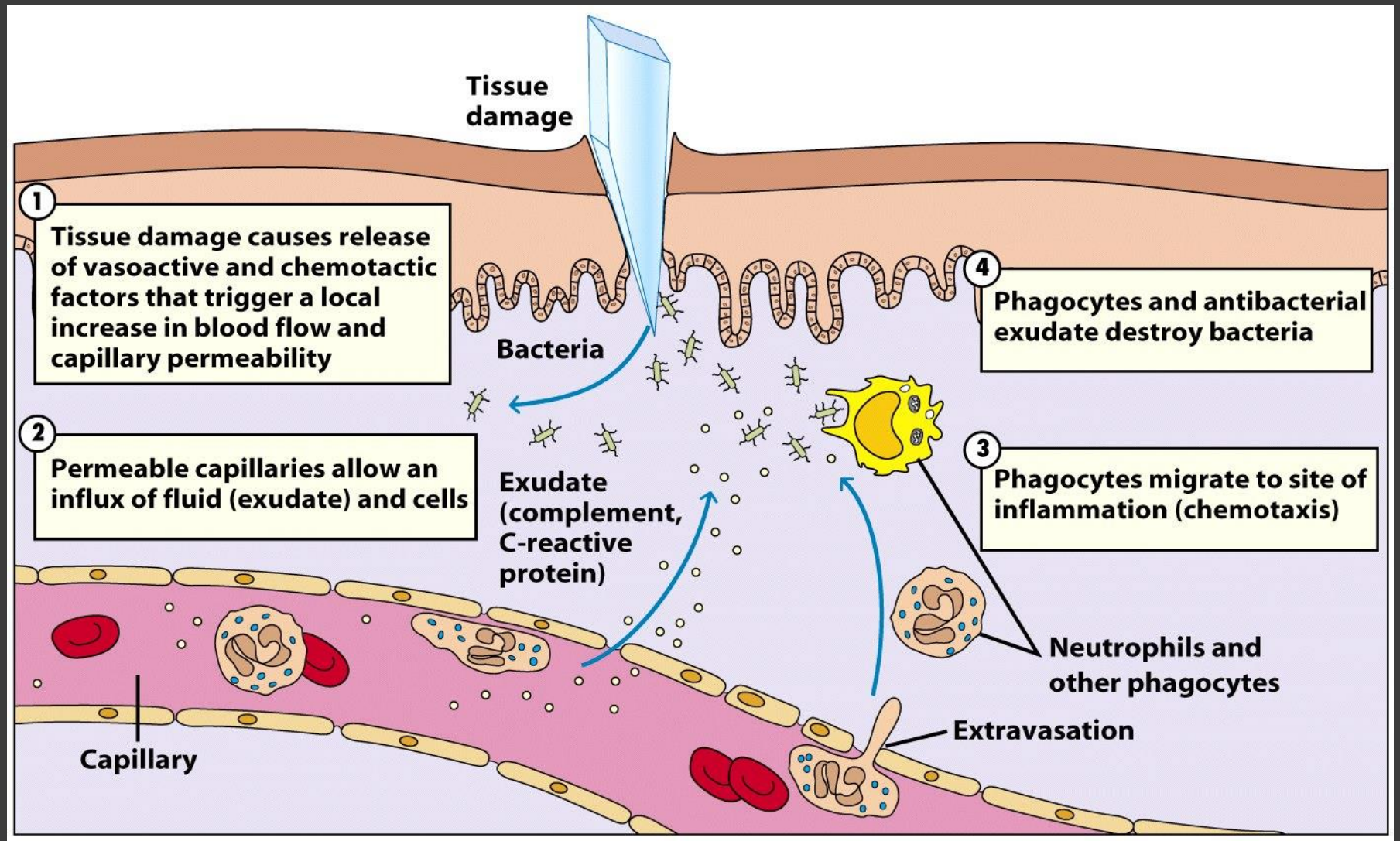


Figure 3-5
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Inflammation

- Extravasation
  - Inflammatory response develops – various cytokines and inflammatory mediators act on endothelium of blood vessels
    - Increased expression of Cell Adhesion Molecules (CAMs)
    - Cells, such as neutrophils, adhere to endothelium using these CAMs strongly enough not to be swept away by flowing blood
    - Then they must penetrate the wall of the vessel to move into the tissue

Inflammation

- Neutrophil Extravasation
  - Rolling
  - Activation of chemoattractant stimulus
  - Arrest and adhesion
  - Transendothelial migration into tissue

Rolling and extravasation

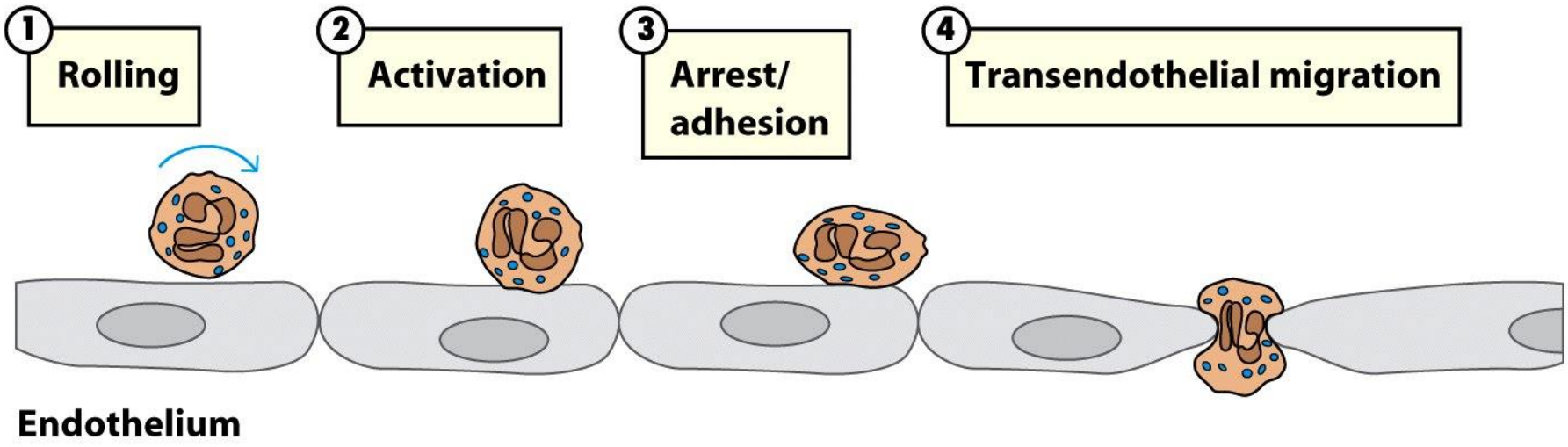

Figure 3-7a
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Initiation of extravasation

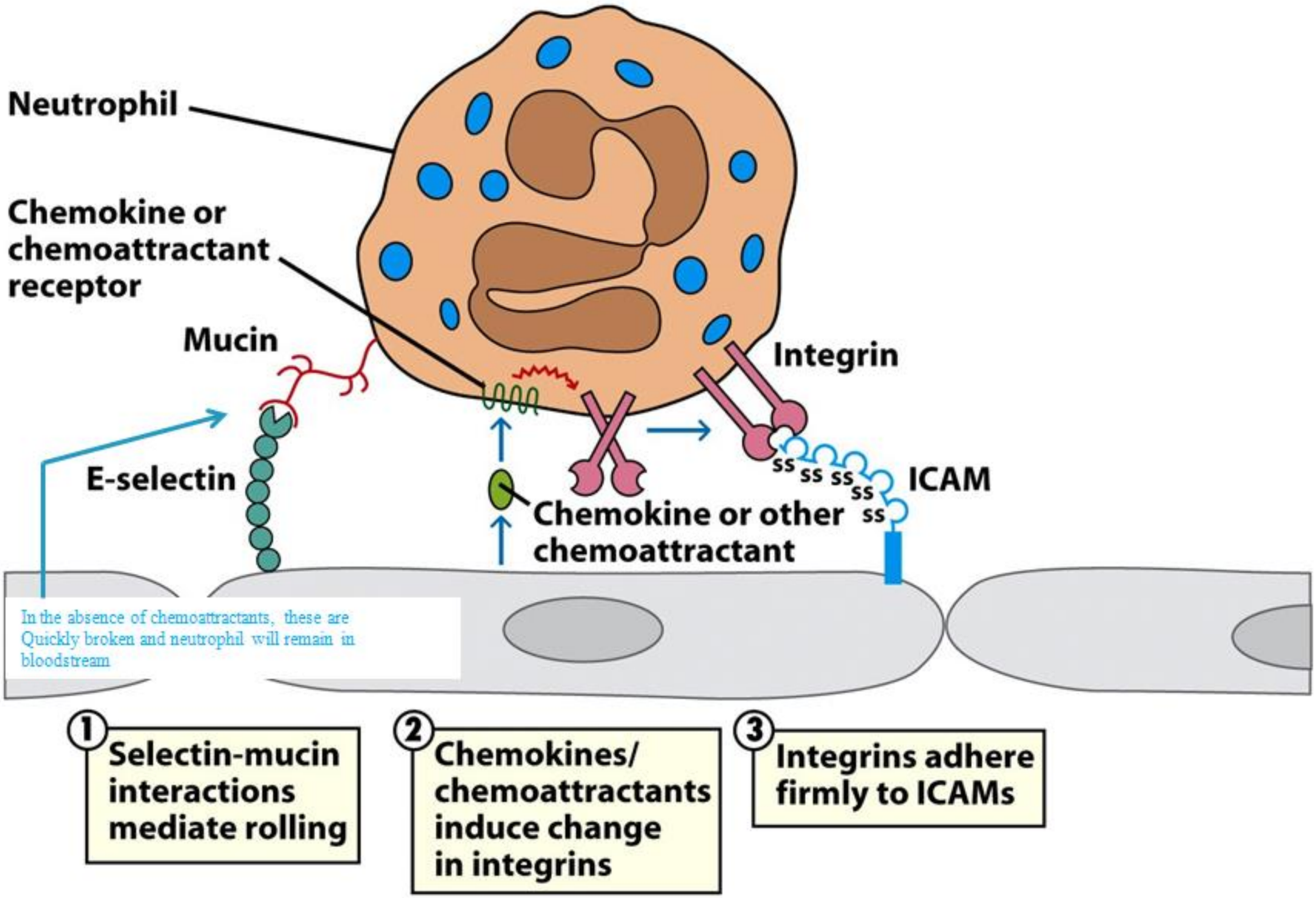

Figure 3-7b
Kuby IMMUNOLOGY, Sixth Edition

TABLE 3-2 Some antimicrobial peptides

Peptide	Typical producer species*	Typical microbial activity*
Defensin family α-Defensins	Human (found in paneth cells of intestine and in cytoplasmic granules of neutrophils)	Antibacterial
β-Defensins	Human (found in epithelia and other tissues)	Antibacterial
Cathelicidins	Human, bovine	Antibacterial
Magainins	Frog	Antibacterial; antifungal
Cercropins	Silk moth	Antibacterial
Drosomycin	Fruit fly	Antifungal
Spinigerin	Termite	Antibacterial; antifungal

*In many cases, production of the indicated antimicrobial peptide or family is not limited to the typical producer but is produced by many different species. Also, some members of the indicated peptide or family may have broader antimicrobial activity than the typical one indicated.

Table 3-2
Kuby IMMUNOLOGY, Sixth Edition

Antimicrobial Peptides

- Isolated from humans, frogs, flies, nematodes, plants
- Range from 6-59 amino acids long
- Good source in humans is the neutrophil
- Work by disrupting microbial membrane
  - How do they discriminate between microbial and host membrane?
  - Big area of research

C Reactive Protein

- Recognizes ligands on surface of microbes
  - Helps in phagocytosis
  - Activates complement-mediated attack

Pattern Recognition Receptors – Toll-like Receptors

- 1980s
  - Toll in flies
    - Important in fly development
- 1996
  - Toll in fruit flies
    - Mutation caused susceptibility to infection of fungus
- 1997 (Janeway)
  - Found that Toll-like receptor activated expression of immune response genes
    - Made of leucine-rich repeat sequences

TABLE 3-3 Receptors of the innate immune system

Receptor (location)	Target (source)	Effect of recognition
Complement (bloodstream, tissue fluids)	Microbial cell wall components	Complement activation, opsonization, lysis
Mannose-binding lectin (MBL) (bloodstream, tissue fluids)	Mannose-containing microbial carbohydrates (cell walls)	Complement activation, opsonization
C-reactive protein (CRP) (bloodstream, tissue fluids)	Phosphatidylcholine, pneumococcal polysaccharide (microbial membranes)	Complement activation, opsonization
Lipopolysaccharide (LPS) receptor;* LPS-binding protein (LBP) (bloodstream, tissue fluids)	Bacterial lipopolysaccharide (gram-negative bacterial cell walls)	Delivery to cell membrane
Toll-like receptors (cell surface or internal compartments)	Microbial components not found in hosts	Induces innate responses
NOD† family receptors (intracellular)	Bacterial cell wall components	Induces innate responses
Scavenger receptors (SRs) (cell membrane)	Many targets; gram-positive and gram-negative bacteria, apoptotic host cells	Induces phagocytosis or endocytosis

* LPS is bound at the cell membrane by a complex of proteins that includes CD14, MD-2, and a TLR (usually TLR4).

† *N*ucleotide-binding *o*ligomerization *d*omain.

Table 3-3
Kuby IMMUNOLOGY, Sixth Edition

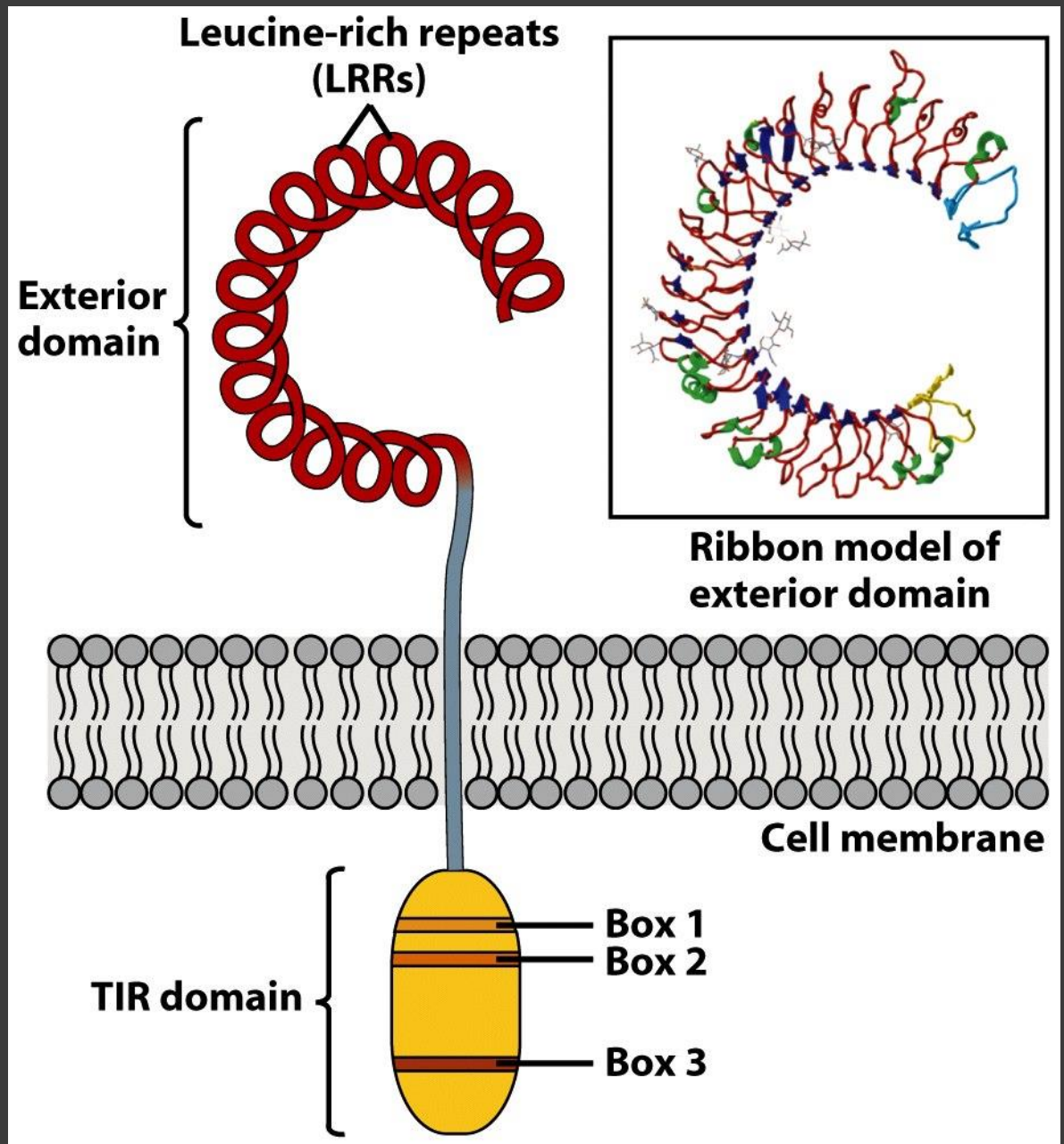

Figure 3-10
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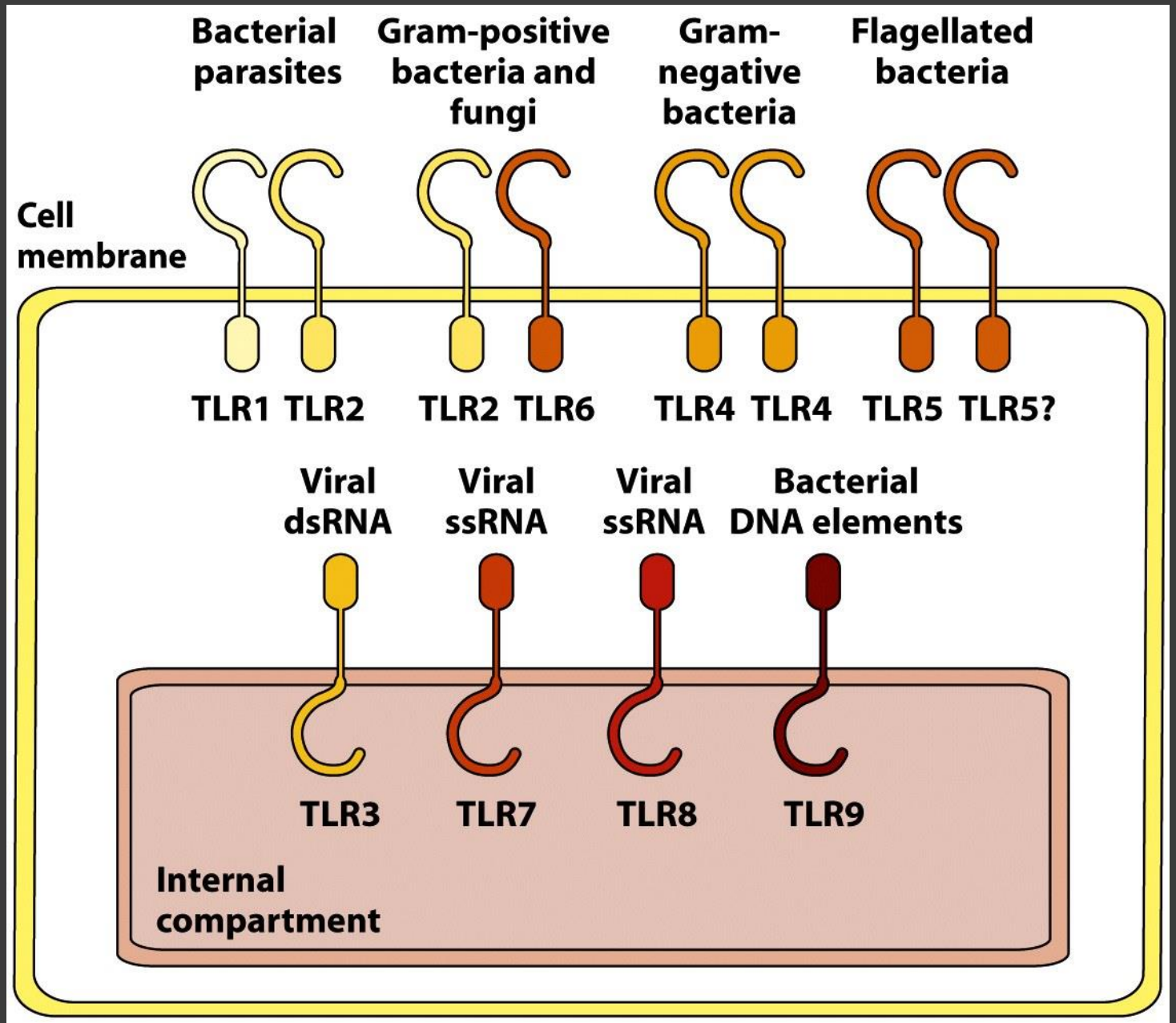

Figure 3-11 part 1
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TLRs	Ligands	Target microbes
TLR1	Triacyl lipopeptides	Mycobacteria
TLR2	Peptidoglycans	Gram-positive bacteria
	GPI-linked proteins	Trypanosomes
	Lipoproteins	Mycobacteria
	Zymosan	Yeasts and other fungi
TLR3	Double-stranded RNA (dsRNA)	Viruses
TLR4	LPS	Gram-negative bacteria
	F-protein	Respiratory syncytial virus (RSV)
TLR5	Flagellin	Bacteria
TLR6	Diacyl lipopeptides	Mycobacteria
	Zymosan	Yeasts and fungi
TLR7	Single-stranded RNA (ssRNA)	Viruses
TLR8	Single-stranded RNA (ssRNA)	Viruses
TLR9	CpG unmethylated dinucleotides	Bacterial DNA
	Dinucleotides	
	Herpesvirus infection	Some herpesviruses
TLR10,11	Unknown	Unknown

Figure 3-11 part 2
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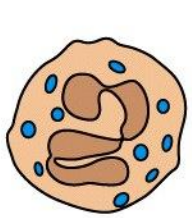
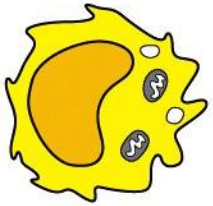
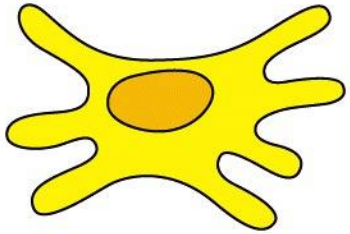
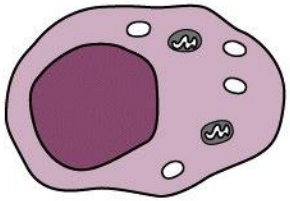
Cell Types of Innate Immunity

Cell type	Neutrophils	Macrophages	Dendritic cells	Natural killer cells
Function	Phagocytosis Reactive oxygen and nitrogen species Antimicrobial peptides	Phagocytosis Inflammatory mediators Antigen presentation Reactive oxygen and nitrogen species Cytokines Complement proteins	Antigen presentation Costimulatory signals Reactive oxygen species Interferon Cytokines	Lysis of viral-infected cells Interferon Macrophage activation

Figure 3-12
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Monocytes have many of the same functions
As macrophage

Signal Transduction Pathways

- Signal
- Receptor
- Signal Transduction
- Effector Mechanism

- Microbial product
- Extracellular portion of TLR
- Interactions of intracellular molecules – phosphorylation; signal transduction pathway – promotes phosphorylation of transcription factors in nucleus
- Cell differentiation, inflammation, antigen-presentation, etc

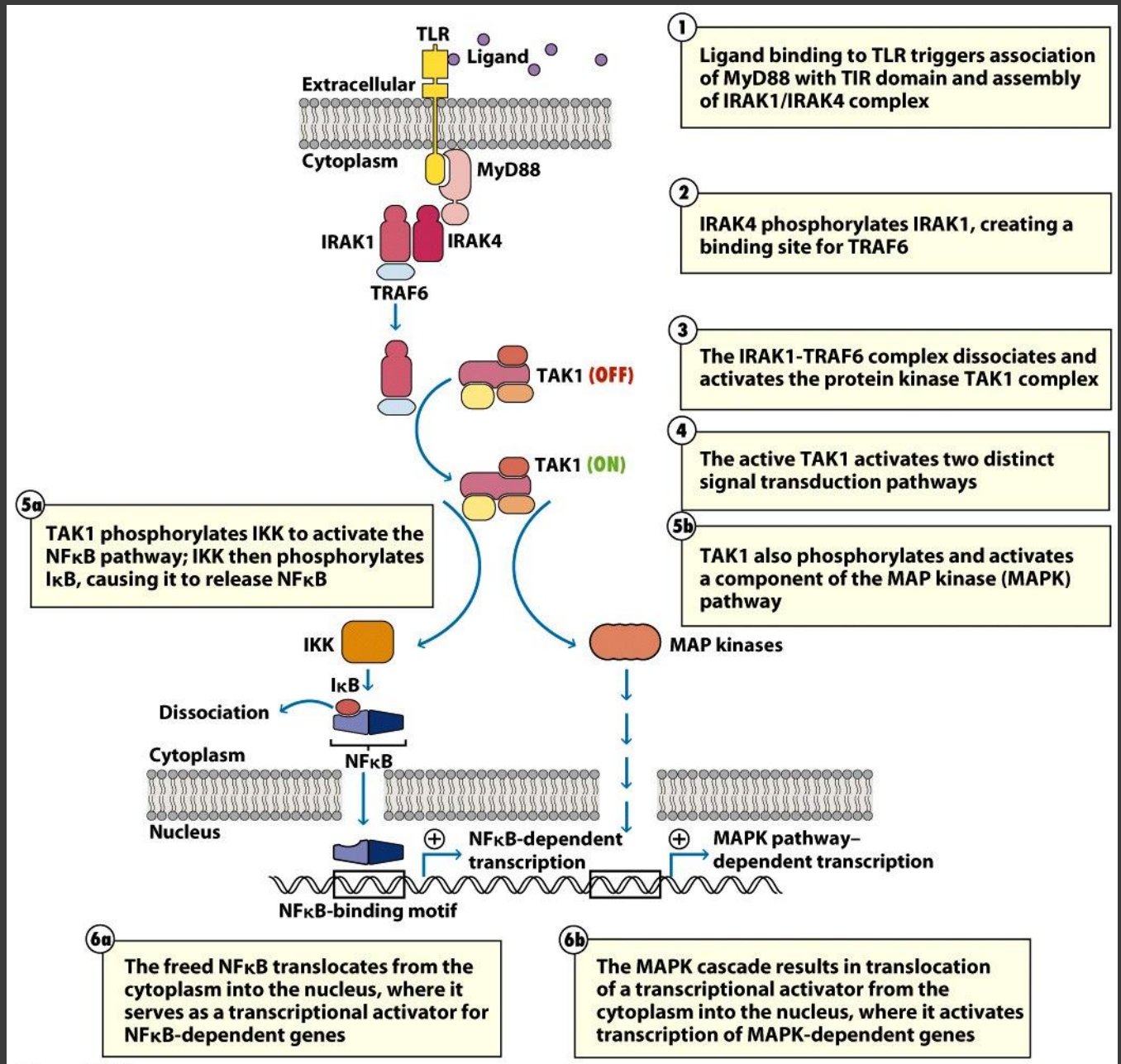

Figure 3-14
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- Adaptive Immunity tends to be found in vertebrates
- However, do find innate immunity in nonvertebrates, even plants
  - Sea squirt (chordate) – complement, TLRs
  - Fruit Fly – TLRs, antimicrobial proteins
  - Tomato – oxidative bursts, enzymes that digest fungi, plant can isolate infection by strengthening cell walls

TABLE 3-4 Immunity in multicellular organisms

Taxonomic group	Innate immunity (nonspecific)	Adaptive immunity (specific)	Invasion-induced protective enzymes and enzyme cascades	Phagocytosis	Anti-microbial peptides	Pattern recognition receptors	Graft rejection	T and B cells	Anti-bodies
Higher plants	+	−	+	−	+	+	−	−	−
Invertebrate animals									
Porifera (sponges)	+	−	?	+	?	?	+	−	−
Annelids (earthworms)	+	−	?	+	?	?	+	−	−
Arthropods (insects, crustaceans)	+	−	+	+	+	+	?	−	−
Vertebrate animals									
Elasmobranchs (cartilaginous fish; e.g., sharks, rays)	+	+	+	+	Equivalent agents	+	+	+	+
Teleost fish and bony fish (e.g., salmon, tuna)	+	+	+	+	Probable	+	+	+	+
Amphibians	+	+	+	+	+	+	+	+	+
Reptiles	+	+	+	+	?	+	+	+	+
Birds	+	+	+	+	?	+	+	+	+
Mammals	+	+	+	+	+	+	+	+	+

KEY: + = definitive demonstration; − = failure to demonstrate thus far; ? = presence or absence remains to be established.

SOURCES: M. J. Flajnik, K. Miller, and L. Du Pasquier, 2003, "Origin and Evolution of the Vertebrate Immune System," in *Fundamental Immunology*, 5th ed., W. E. Paul (ed.), Lippincott, Philadelphia; M. J. Flajnik and L. Du Pasquier, 2004, *Trends in Immunology* **25**:640.

Table 3-4
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Chapter 5

Innate Immunity

Dr. Capers

IMMUNOLOGY

Kindt • Goldsby • Osborne

Kuby IMMUNOLOGY

Sixth Edition

Chapter 3

Innate Immunity

- Vertebrate are protected by 2 systems of immunity
  - Innate Immunity
  - Adaptive Immunity
    - Takes time but has memory
- Innate Immunity can be found in all multicellular plants and animals
- Adaptive Immunity evolved in jawed vertebrates

TABLE 3-1 Innate and adaptive immunity

Attribute	Innate immunity	Adaptive immunity
Response time	Minutes/hours	Days
Specificity	Specific for molecules and molecular patterns associated with pathogens	Highly specific; discriminates even minor differences in molecular structure; details of microbial or nonmicrobial structure recognized with high specificity
Diversity	A limited number of germ line–encoded receptors	Highly diverse; a very large number of receptors arising from genetic recombination of receptor genes
Memory responses	None	Persistent memory, with faster response of greater magnitude on subsequent infection
Self/nonself discrimination	Perfect; no microbe-specific patterns in host	Very good; occasional failures of self/nonself discrimination result in autoimmune disease
Soluble components of blood or tissue fluids	Many antimicrobial peptides and proteins	Antibodies
Major cell types	Phagocytes (monocytes, macrophages, neutrophils), natural killer (NK) cells, dendritic cells	T cells, B cells, antigen-presenting cells

Table 3-1
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Innate Immunity

- Innate Immune System:
  - Physical/Anatomical Barriers
    - Skin and mucous membranes
  - Chemical Barriers
    - Acidity of stomach, antimicrobial molecules
  - Cellular Barriers
    - Cells with sensitive receptors that can detect microbial invaders

Innate Immunity

- Antimicrobial Proteins
  - Psoriasin – produced by skin
    - Antibacterial activity to *E. coli*
  - Help when skin is scratched or cut to prevent infection
  - Saliva, tears, and mucous membranes help to wash invaders away as well as contain antimicrobial peptides

Innate Immunity

- Normal flora
  - Help to out-compete pathogens for space and nutrients

Organ or tissue	Innate mechanisms protecting skin/epithelium
Skin	Antimicrobial peptides, fatty acids in sebum
Mouth and upper alimentary canal	Enzymes, antimicrobial peptides, and sweeping of surface by directional flow of fluid toward stomach
Stomach	Low pH, digestive enzymes, antimicrobial peptides, fluid flow toward intestine
Small intestine	Digestive enzymes, antimicrobial peptides, fluid flow to large intestine
Large intestine	Normal intestinal flora compete with invading microbes, fluid/feces expelled from rectum
Airway and lungs	Cilia sweep mucus outward, coughing, sneezing expel mucus, macrophages in alveoli of lungs

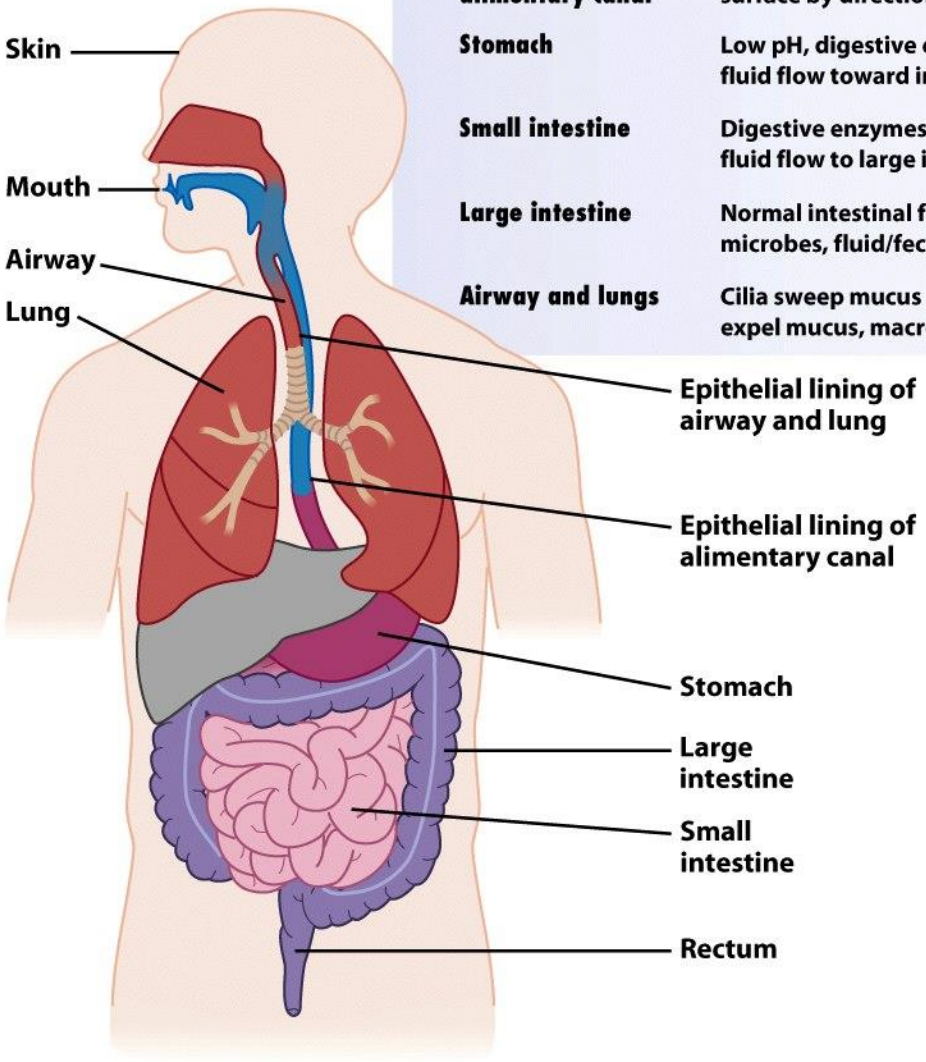

Figure 3-1
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Also includes:
Urogenital tract

Salivary, lacrimal, and Mammary glands

- Connection between adaptive and innate immunity
  - Pathogens may get past anatomical barriers
  - Interact with membrane-bound molecules (sensors) that recognize broad structural motifs of microbial species
    - Pattern Recognition Receptors (PRRs)
    - On pathogen it is called Pathogen-Associated Molecular Patterns (PAMPs)

- Pattern Recognition Receptors (PRRs)
  - In contrast, antibodies and T cell receptors recognize finer details of molecular structure
  - If PAMPs are detected, complement system will be activated

- Complement System
  - One part is a collection of proteins that form aggregates that punch holes in pathogen's cell membrane causing lysis
  - Include serum glycoproteins that promote uptake of pathogens by phagocytes (opsonization)
  - Complement system ties innate and adaptive immunity

- Dendritic cells and Macrophages have variety of receptors
  - Toll-like receptors – detect microbial products
- Activated macrophages will secrete cytokines
  - Hormone or growth-like factors to induce specific cell activities (upregulation of B and T cells); again tying innate and adaptive immunity

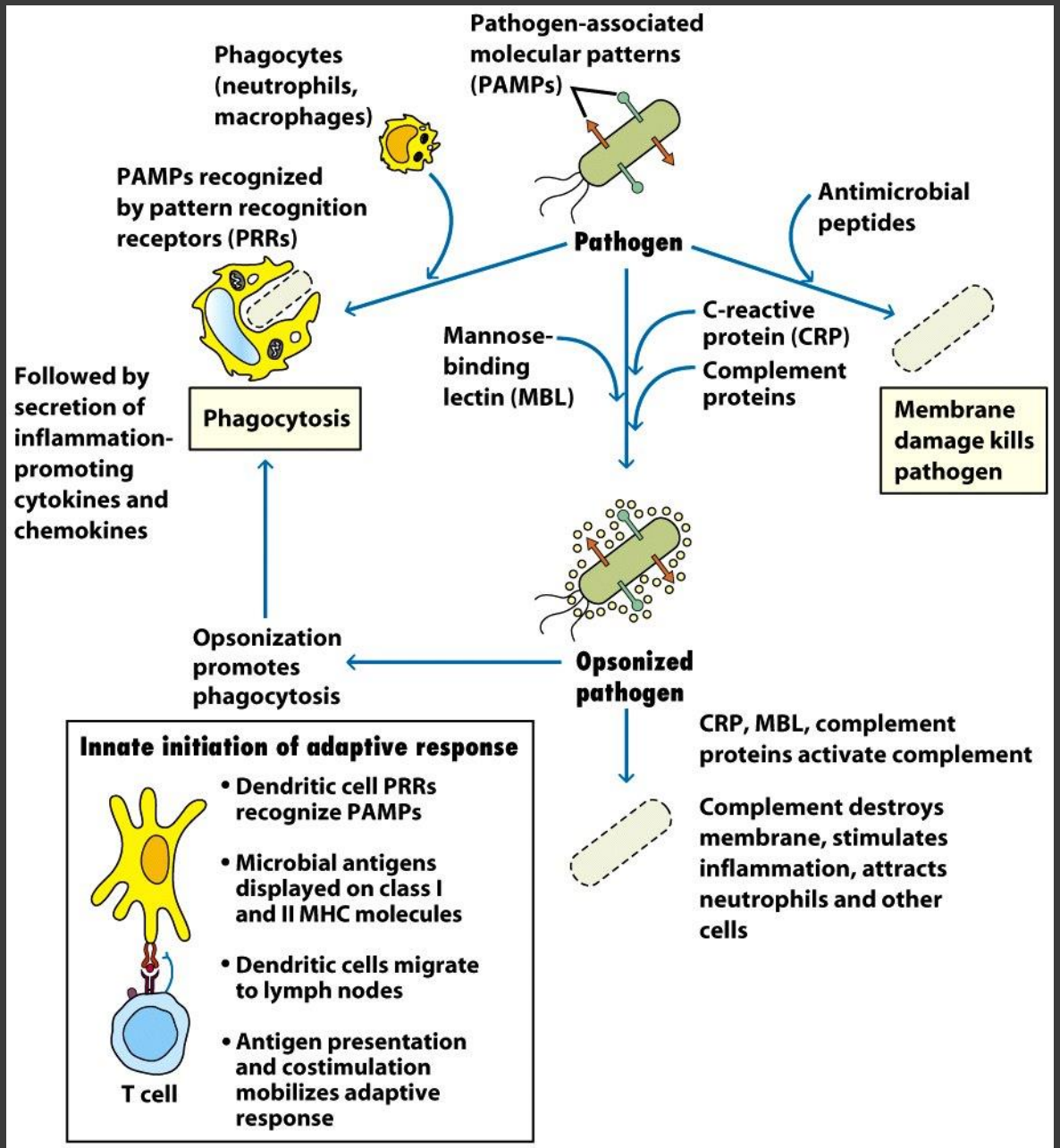

Figure 3-4
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Inflammation

- Hallmarks
  - Swelling
  - Redness
  - Heat
  - pain

Inflammation

- Within minutes of tissue injury:
  - Vasodilation – rise of blood volume to area
  - Vascular permeability increases – accumulation of fluid
    - Edema
  - Leukocytes adhere to endothelial cells and pass through walls of capillaries into tissues - extravasation

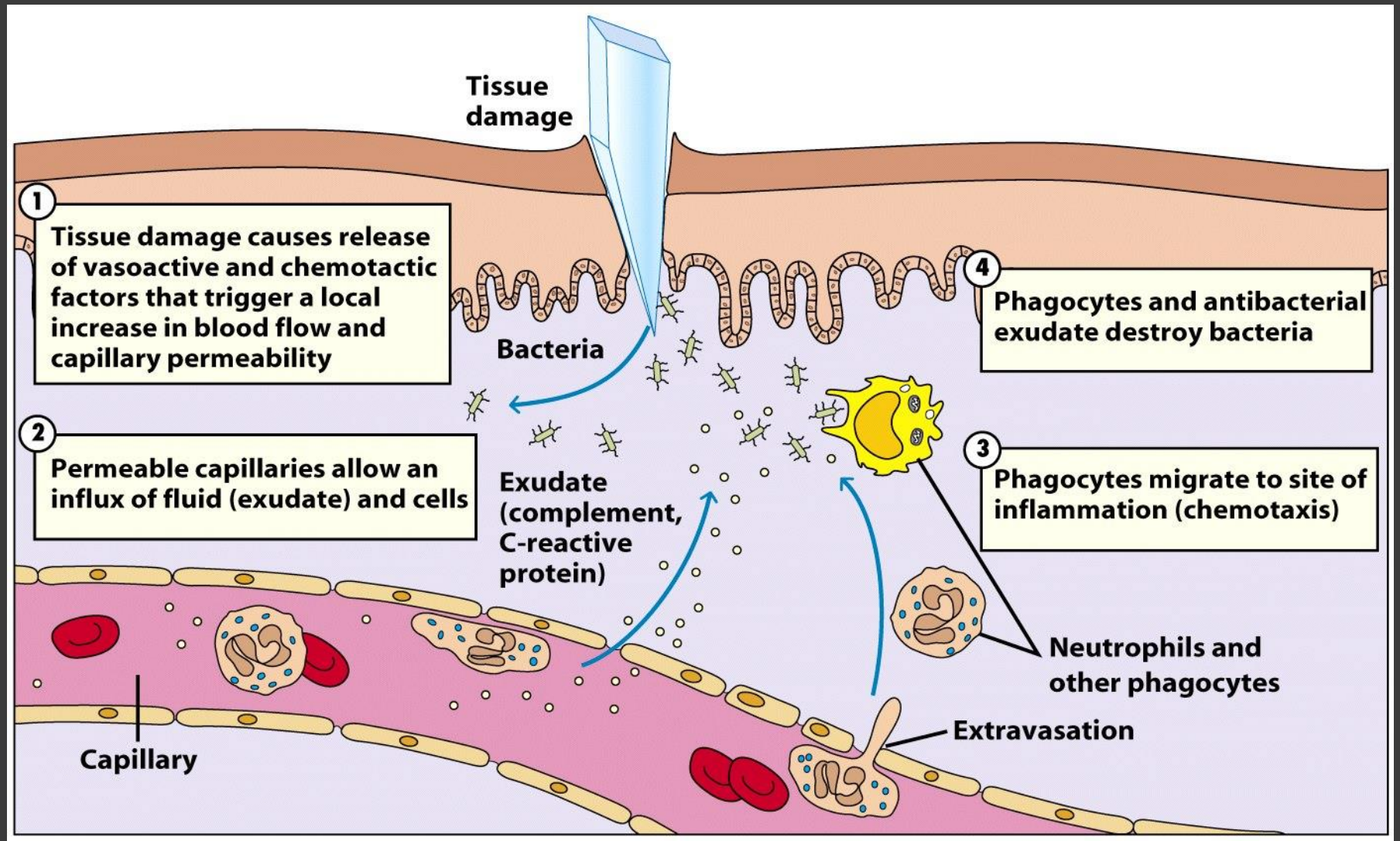

Figure 3-5
Kuby IMMUNOLOGY, Sixth Edition

Inflammation

- Extravasation
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    - Cells, such as neutrophils, adhere to endothelium using these CAMs strongly enough not to be swept away by flowing blood
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Rolling and extravasation

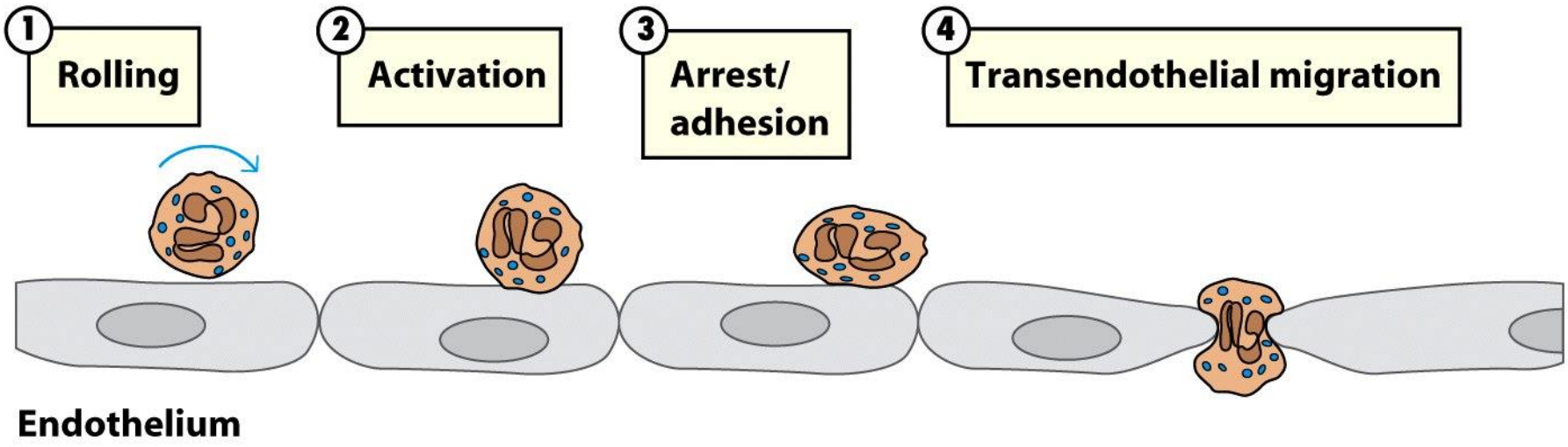

Figure 3-7a
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Initiation of extravasation

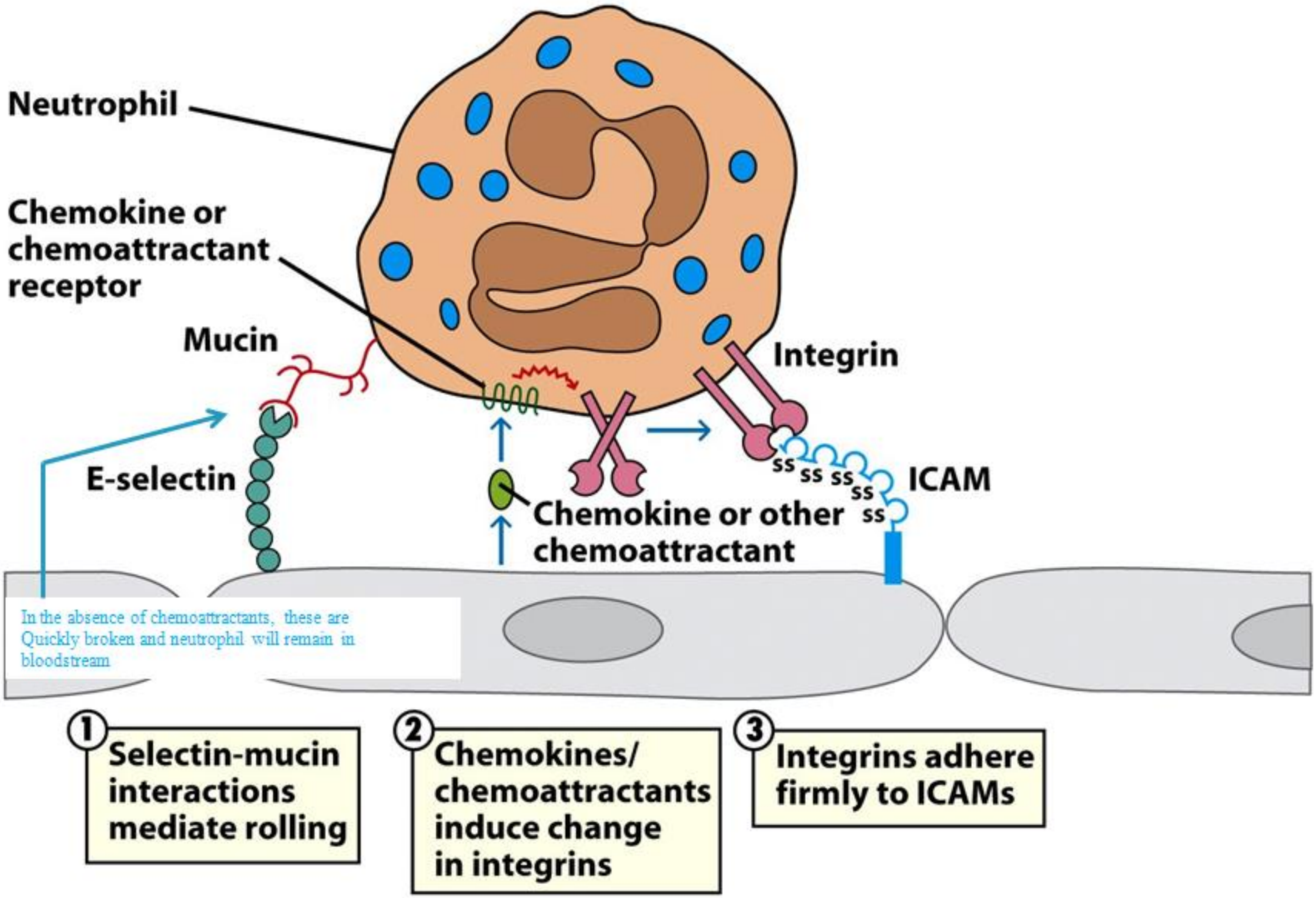

Figure 3-7b
Kuby IMMUNOLOGY, Sixth Edition

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Kuby IMMUNOLOGY, Sixth Edition

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Scavenger receptors (SRs) (cell membrane)	Many targets; gram-positive and gram-negative bacteria, apoptotic host cells	Induces phagocytosis or endocytosis

* LPS is bound at the cell membrane by a complex of proteins that includes CD14, MD-2, and a TLR (usually TLR4).

† *N*ucleotide-binding *o*ligomerization *d*omain.

Table 3-3
Kuby IMMUNOLOGY, Sixth Edition

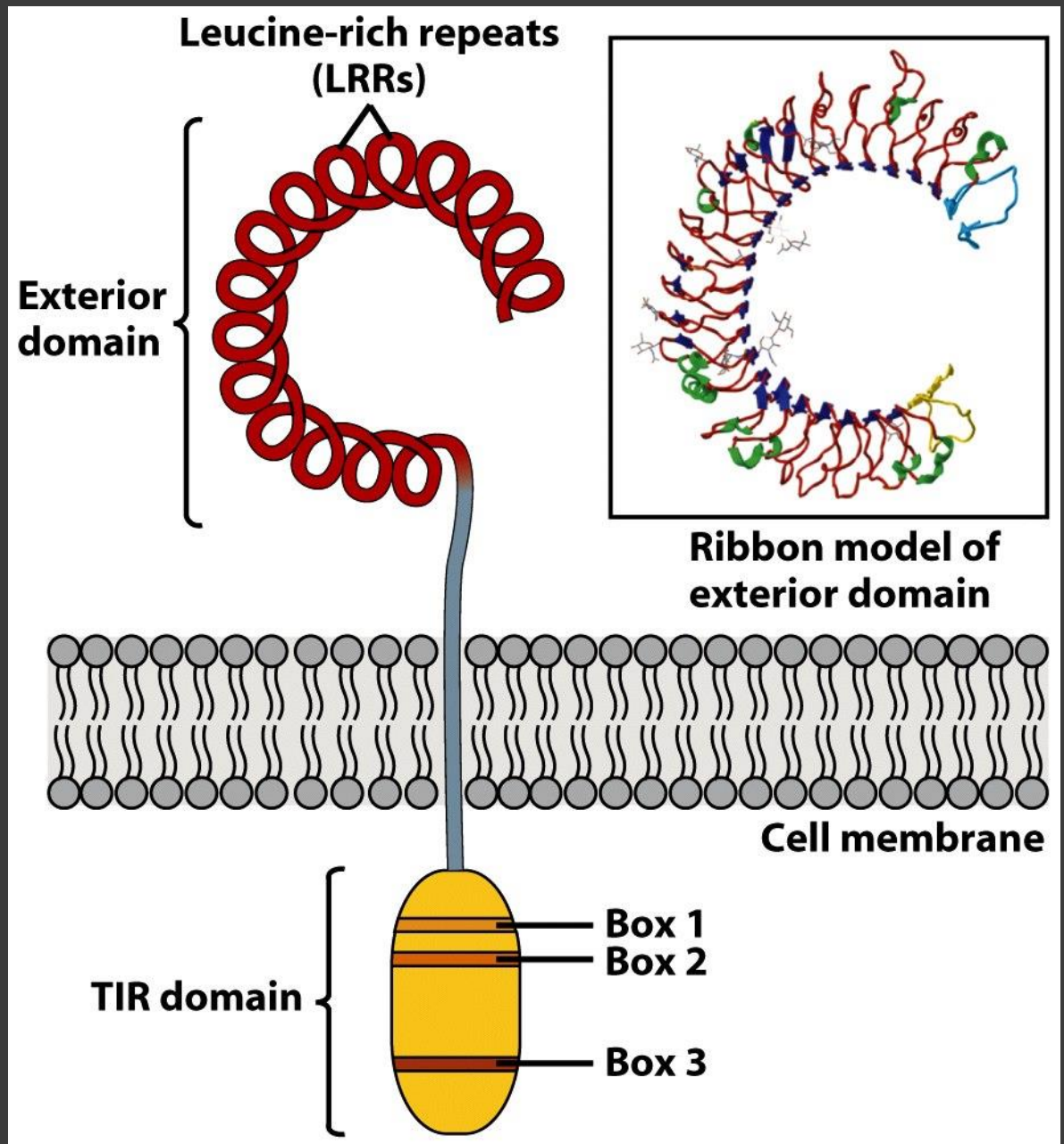

Figure 3-10
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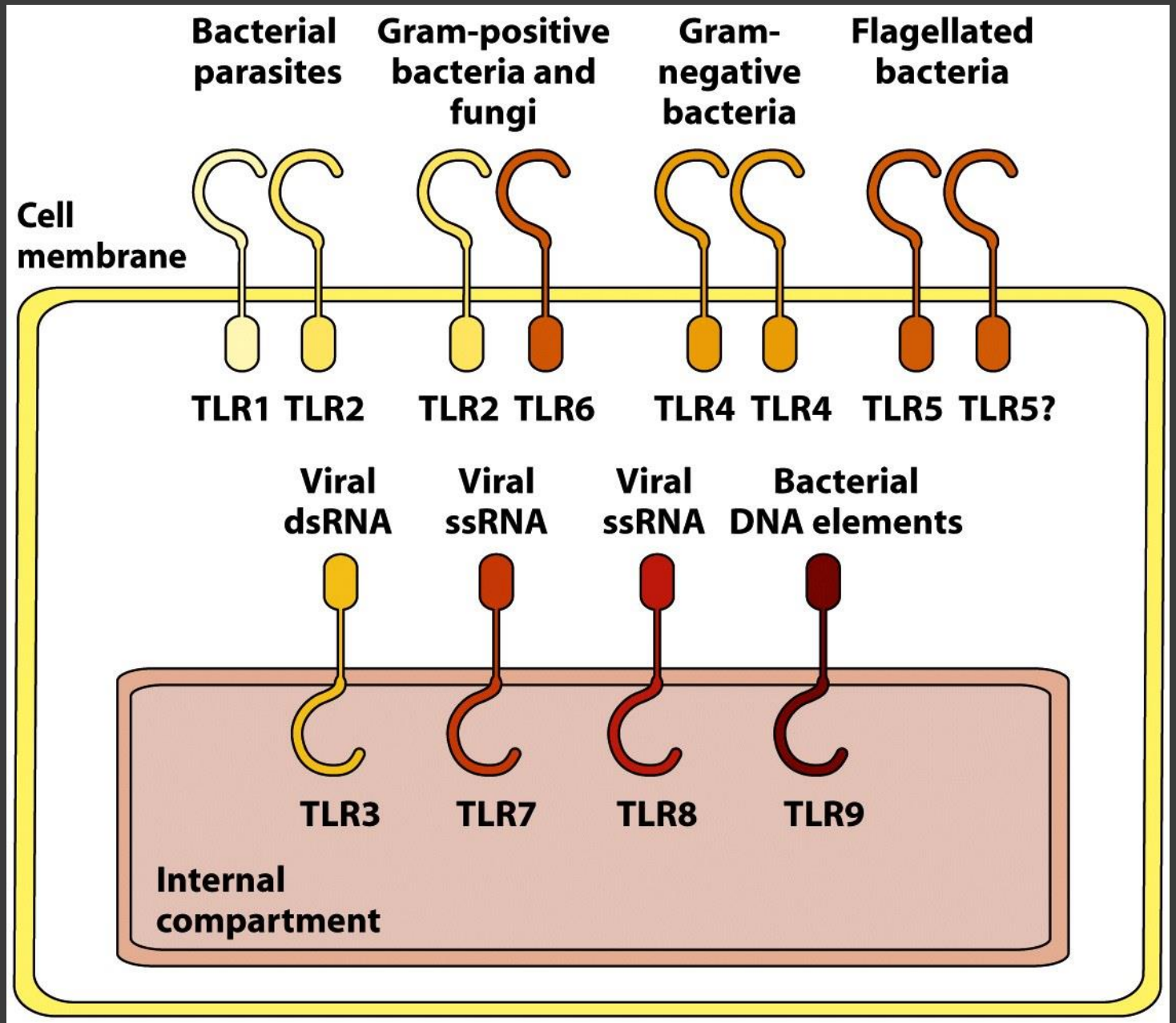

Figure 3-11 part 1
Kuby IMMUNOLOGY, Sixth Edition

TLRs	Ligands	Target microbes
TLR1	Triacyl lipopeptides	Mycobacteria
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TLR6	Diacyl lipopeptides	Mycobacteria
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TLR7	Single-stranded RNA (ssRNA)	Viruses
TLR8	Single-stranded RNA (ssRNA)	Viruses
TLR9	CpG unmethylated dinucleotides	Bacterial DNA
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	Herpesvirus infection	Some herpesviruses
TLR10,11	Unknown	Unknown

Figure 3-11 part 2
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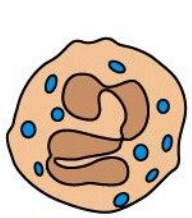
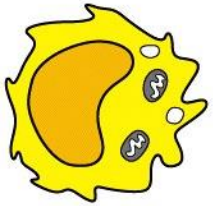
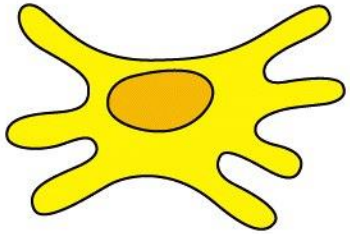
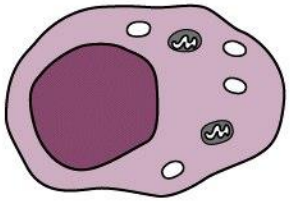
Cell Types of Innate Immunity

Cell type	Neutrophils	Macrophages	Dendritic cells	Natural killer cells
Function	Phagocytosis Reactive oxygen and nitrogen species Antimicrobial peptides	Phagocytosis Inflammatory mediators Antigen presentation Reactive oxygen and nitrogen species Cytokines Complement proteins	Antigen presentation Costimulatory signals Reactive oxygen species Interferon Cytokines	Lysis of viral-infected cells Interferon Macrophage activation

Figure 3-12
Kuby IMMUNOLOGY, Sixth Edition

Monocytes have many of the same functions
As macrophage

Signal Transduction Pathways

- Signal
- Receptor
- Signal Transduction
- Effector Mechanism

- Microbial product
- Extracellular portion of TLR
- Interactions of intracellular molecules – phosphorylation; signal transduction pathway – promotes phosphorylation of transcription factors in nucleus
- Cell differentiation, inflammation, antigen-presentation, etc

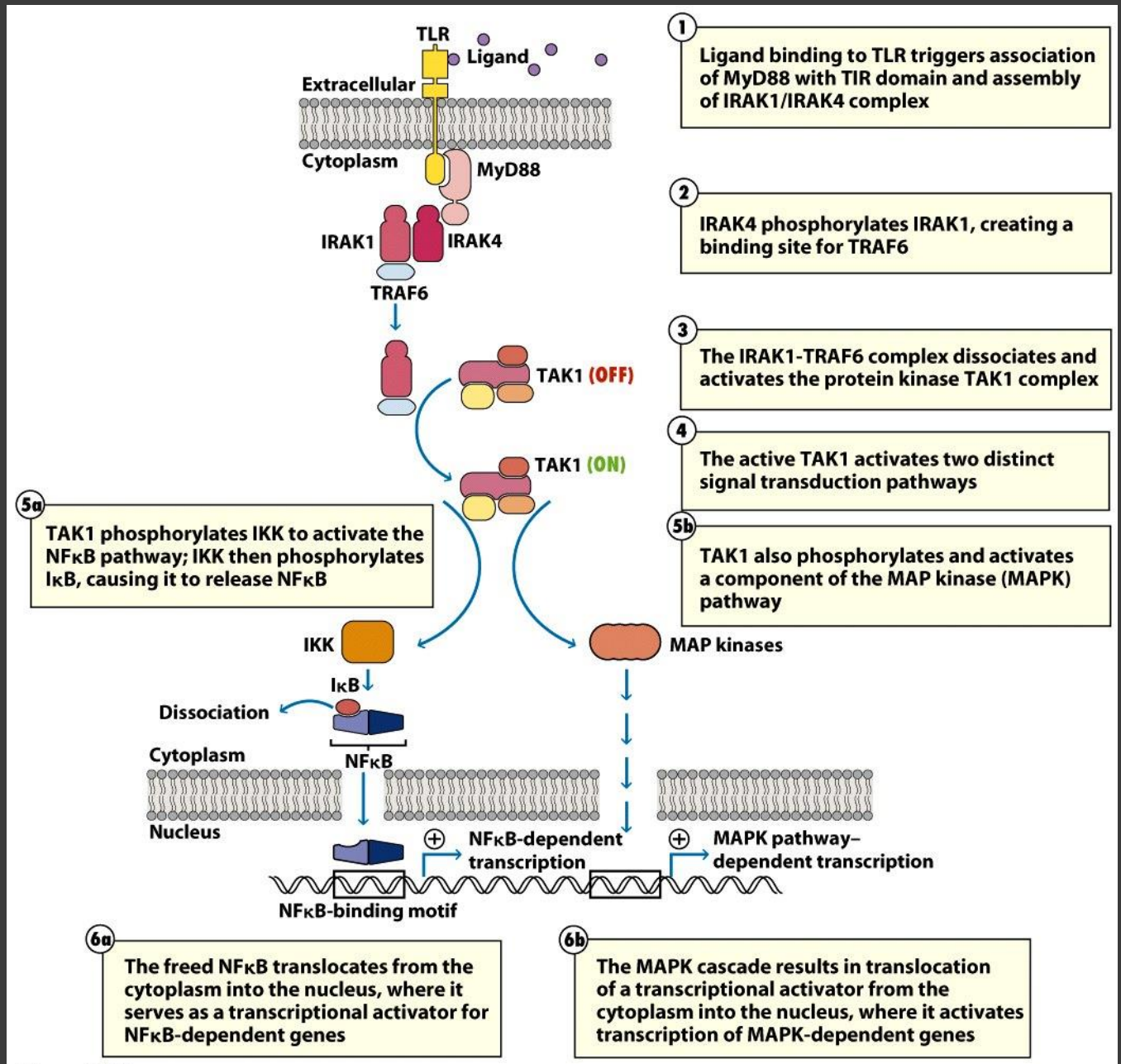


Figure 3-14
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- Adaptive Immunity tends to be found in vertebrates
- However, do find innate immunity in nonvertebrates, even plants
  - Sea squirt (chordate) – complement, TLRs
  - Fruit Fly – TLRs, antimicrobial proteins
  - Tomato – oxidative bursts, enzymes that digest fungi, plant can isolate infection by strengthening cell walls

TABLE 3-4 Immunity in multicellular organisms

Taxonomic group	Innate immunity (nonspecific)	Adaptive immunity (specific)	Invasion-induced protective enzymes and enzyme cascades	Phagocytosis	Anti-microbial peptides	Pattern recognition receptors	Graft rejection	T and B cells	Anti-bodies
Higher plants	+	−	+	−	+	+	−	−	−
Invertebrate animals									
Porifera (sponges)	+	−	?	+	?	?	+	−	−
Annelids (earthworms)	+	−	?	+	?	?	+	−	−
Arthropods (insects, crustaceans)	+	−	+	+	+	+	?	−	−
Vertebrate animals									
Elasmobranchs (cartilaginous fish; e.g., sharks, rays)	+	+	+	+	Equivalent agents	+	+	+	+
Teleost fish and bony fish (e.g., salmon, tuna)	+	+	+	+	Probable	+	+	+	+
Amphibians	+	+	+	+	+	+	+	+	+
Reptiles	+	+	+	+	?	+	+	+	+
Birds	+	+	+	+	?	+	+	+	+
Mammals	+	+	+	+	+	+	+	+	+

KEY: + = definitive demonstration; − = failure to demonstrate thus far; ? = presence or absence remains to be established.

SOURCES: M. J. Flajnik, K. Miller, and L. Du Pasquier, 2003, "Origin and Evolution of the Vertebrate Immune System," in *Fundamental Immunology*, 5th ed., W. E. Paul (ed.), Lippincott, Philadelphia; M. J. Flajnik and L. Du Pasquier, 2004, *Trends in Immunology* **25**:640.

Table 3-4
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Chapter 8

Major Histocompatibility Complex

Dr. Capers

IMMUNOLOGY

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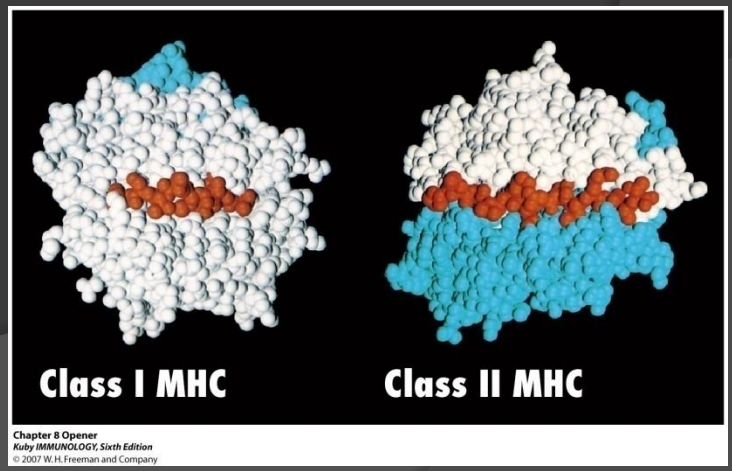
Chapter 8

The Major Histocompatibility Complex and Antigen Presentation

The Major Histocompatibility Complex

- Antibodies can't recognize antigen alone
- T-cell receptors can only recognize antigen that has been processed and presented by Major Histocompatibility Complex (MHC)
  - **Involves:**
    - Antigen processing
    - Antigen presentation



Inheritance of MHC

- MHC coded by cluster of genes
  - Rejection of foreign tissue is due to immune response against cell surface molecules, histocompatibility antigens

Inheritance of MHC

- Collection of genes on chromosome 6 in humans (HLA complex) and chromosome 17 in mice (H-2 complex)
  - Class I MHC genes
    - Encode glycoproteins expressed on all nucleated cells
  - Class II MHC genes
    - Encode glycoproteins expressed on antigen-presenting cells (macrophages, B cells, dendritic cells)
  - Class III MHC genes
    - Encode various products involved in complement and inflammation

Inheritance of MHC

Region of chromosome

Mouse H-2 complex

Complex	H–2						
MHC class	I	II		III		I	
Region	K	IA	IE	S		D	
Gene products	H–2K	IA αβ	IE αβ	C′ proteins	TNF-α TNF-β	H–2D	H–2L*

*Not present in all haplotypes

Region of chromosome

Human HLA complex

Complex	HLA							
MHC class	II			III		I		
Region	DP	DQ	DR	C4, C2, BF		B	C	A
Gene products	DP αβ	DQ αβ	DR αβ	C′ proteins	TNF-α TNF-β	HLA-B	HLA-C	HLA-A

Figure 8-1
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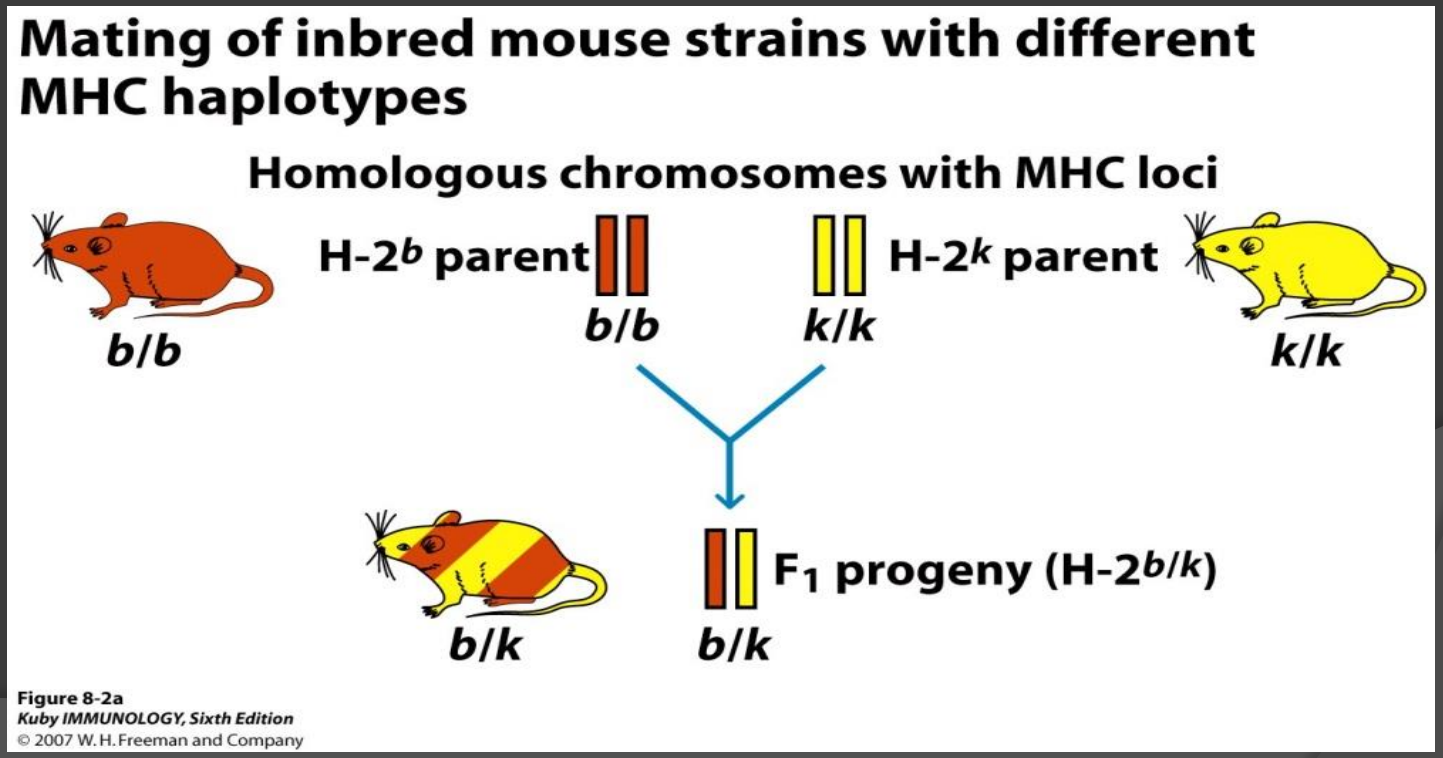
Inheritance of MHC

- Many different alleles exist at each locus among the population
  - Each set of alleles is called a **haplotype**
  - Genes of MHC lie close together so crossing over during meiosis occurs infrequently
  - Individual inherits one haplotype from mom, one from dad
    - Many in the population are heterozygous
    - Alleles are codominant so expressed simultaneously

Inheritance of MHC

- Inbred strains will express identical haplotypes – homozygous
  - Inbred mice are solid colors



Skin transplantation between inbred mouse strains with same or different MHC haplotypes

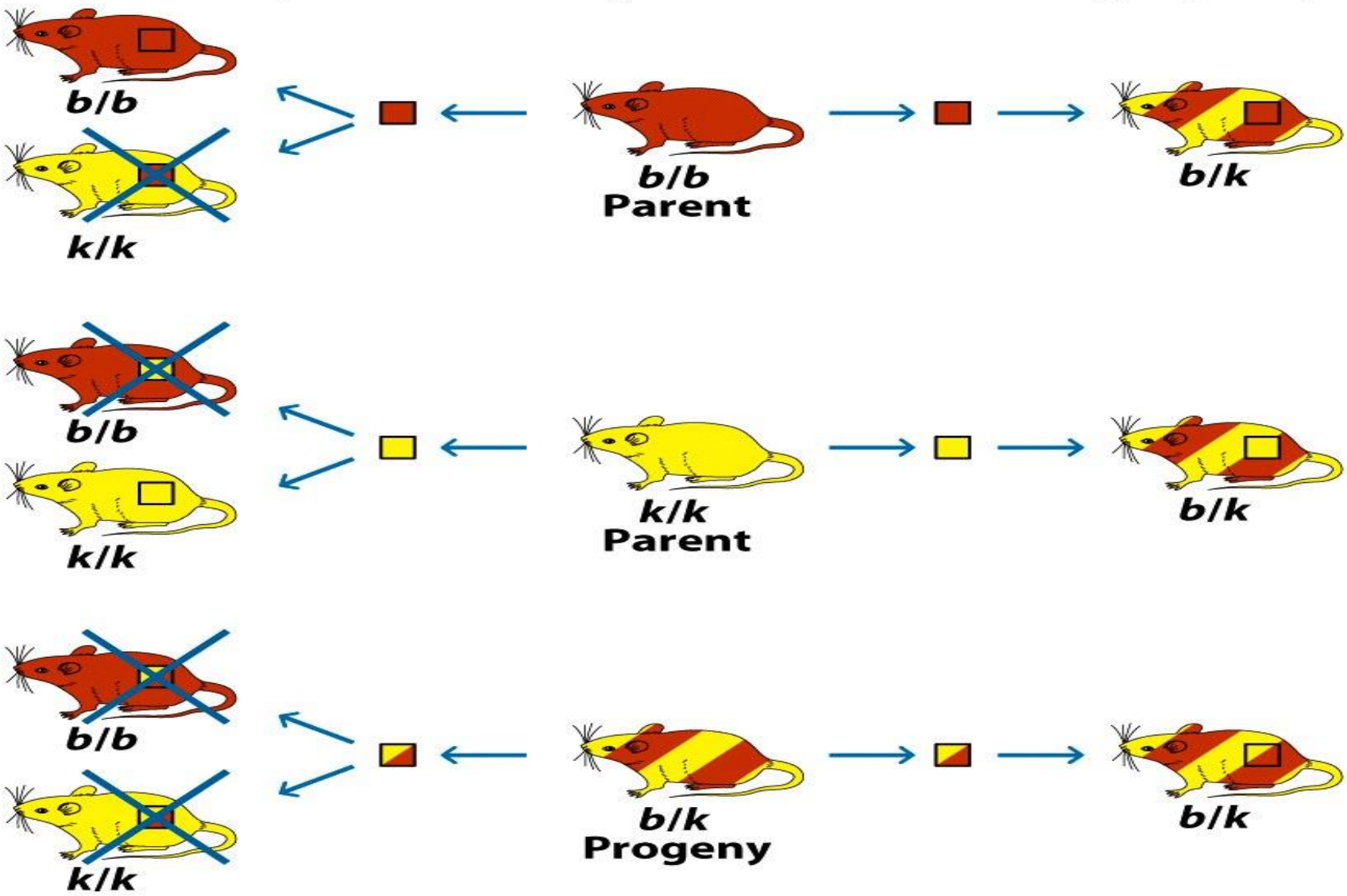


Figure 8-2b
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Inheritance of MHC

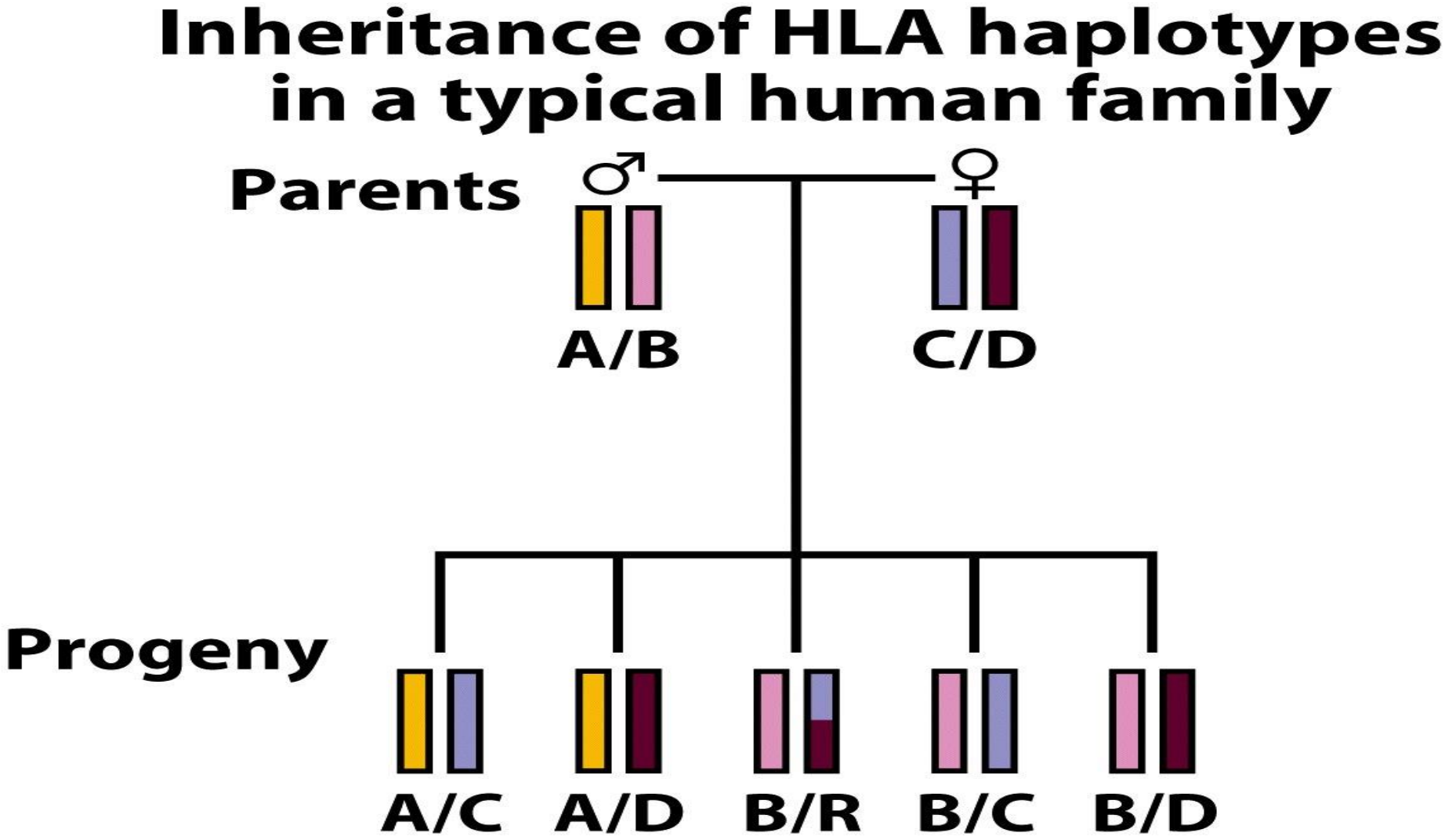

Figure 8-2c
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MHC molecules

- Both Class I and Class II are membrane-bound glycoproteins
  - Antigen-presenting molecules

- **Class I MHC**
  - Alpha α chain
    - Transmembrane
    - Encoded by A, B, and C regions in human MHC complex
  - Beta β_2-microglobulin
    - Encoded by highly conserved gene on different chromosome

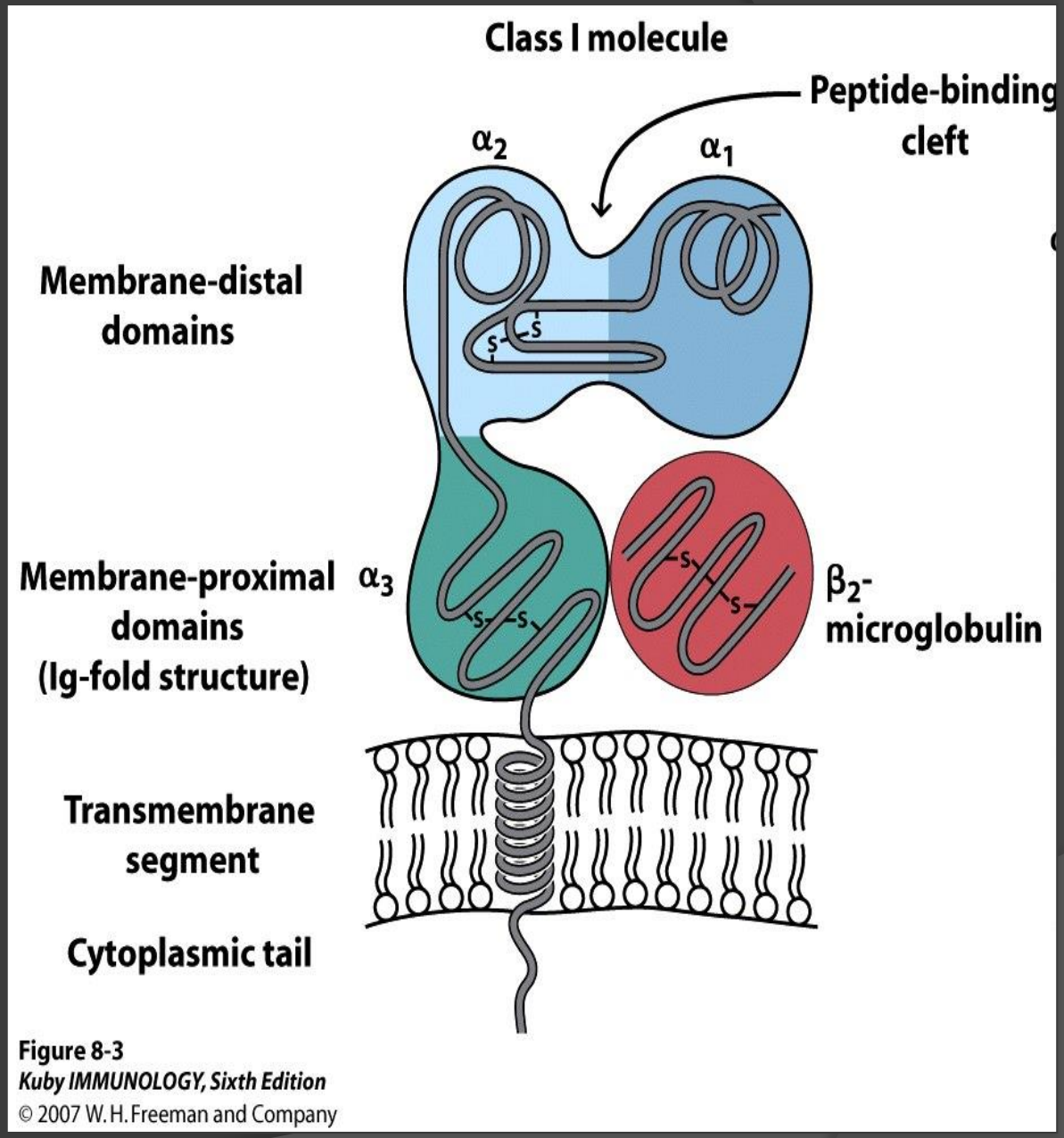

Figure 8-3
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- **Class II Molecule**
  - α_1 and α_2 chain
    - Transmembrane
  - β_1 and β_2 chain
    - transmembrane

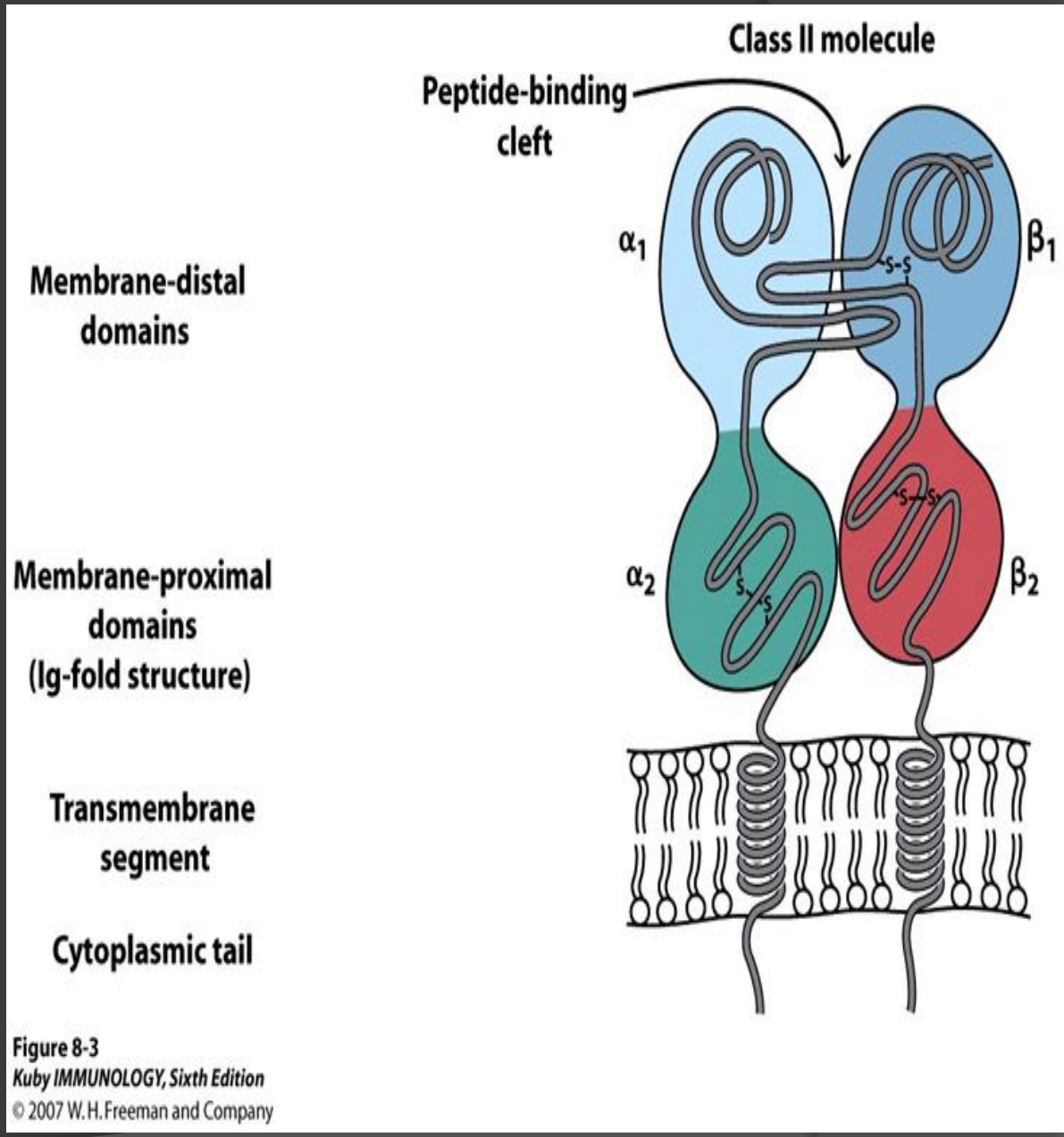

Figure 8-3
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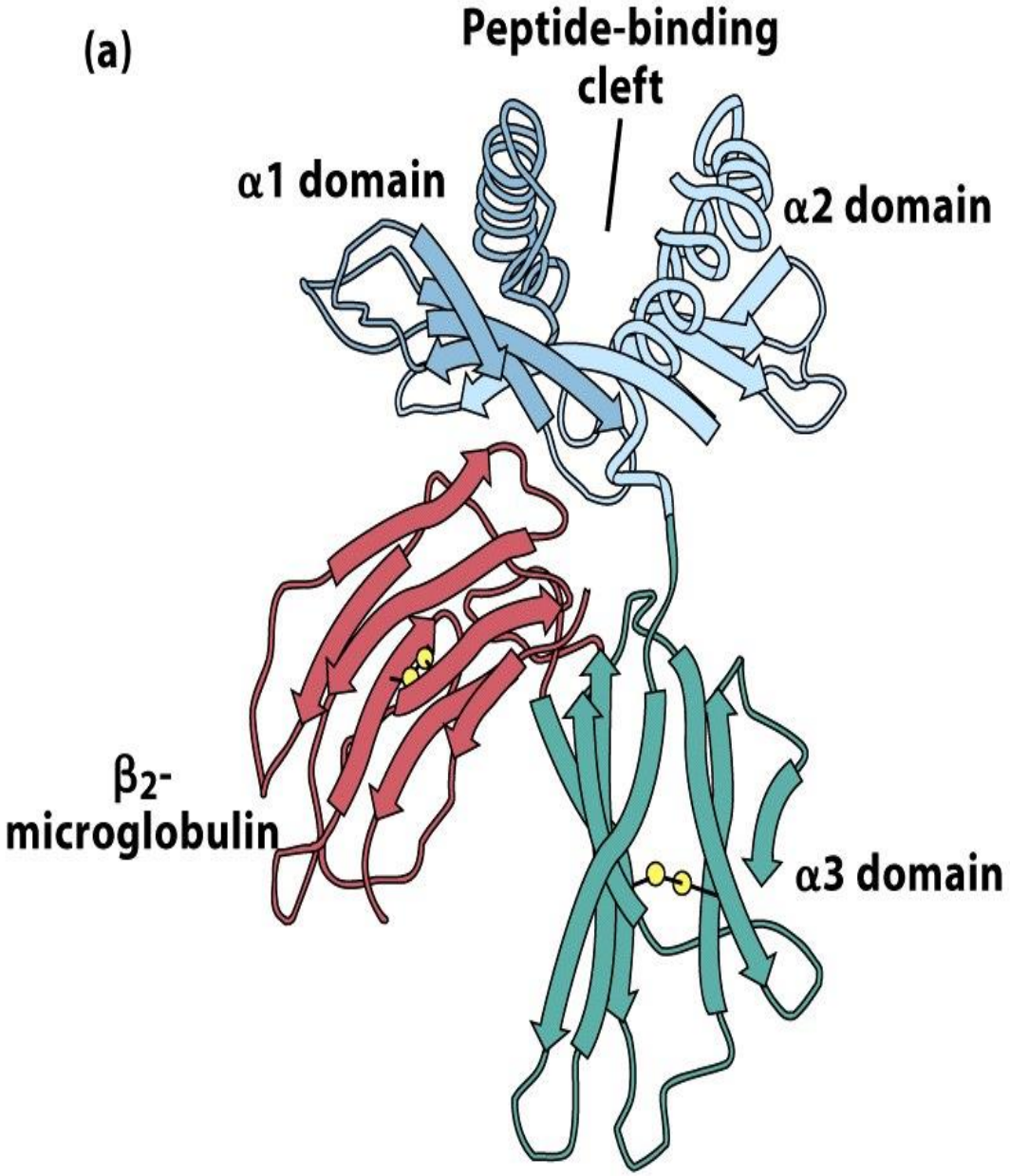

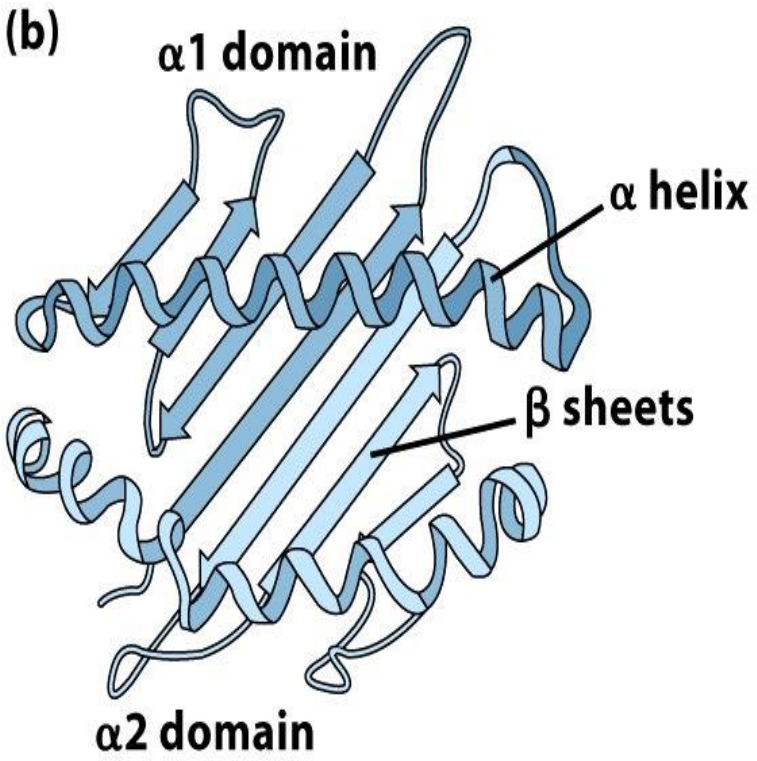

Figure b shows top View of peptide cleft

Figure 8-4
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- **Every individual expresses small number of different Class I and Class II**
  - Limited number of MHC must be able to present enormous array of different antigens
  - MHC does not display specificity of Antibodies
  - MHC is “promiscuous” ☺

Peptide Interactions with MHC

TABLE 8-2 Peptide binding by class I and class II MHC molecules

	Class I molecules	Class II molecules
Peptide-binding domain	α1/α2	α1/β1
Nature of peptide-binding cleft	Closed at both ends	Open at both ends
General size of bound peptides	8–10 amino acids	13–18 amino acids
Peptide motifs involved in binding to MHC molecule	Anchor residues at both ends of peptide; generally hydrophobic carboxyl-terminal anchor	Anchor residues distributed along the length of the peptide
Nature of bound peptide	Extended structure in which both ends interact with MHC cleft but middle arches up away from MHC molecule	Extended structure that is held at a constant elevation above the floor of MHC cleft

Table 8-2
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- Generation of B-cell receptors (antibodies) and T-cell receptors is dynamic, changing over-time
  - Gene rearrangement
- In contrast, MHC molecules are fixed in the genes
  - Differences in population due to large number of alleles
    - In humans, ~370 A alleles, 660 B alleles, 190 C alleles

Location of genes

- **Human**
  - Class I MHC are red
    - Telomeric end of HLA complex
  - Class II MHC are blue
    - Centromeric end of HLA complex

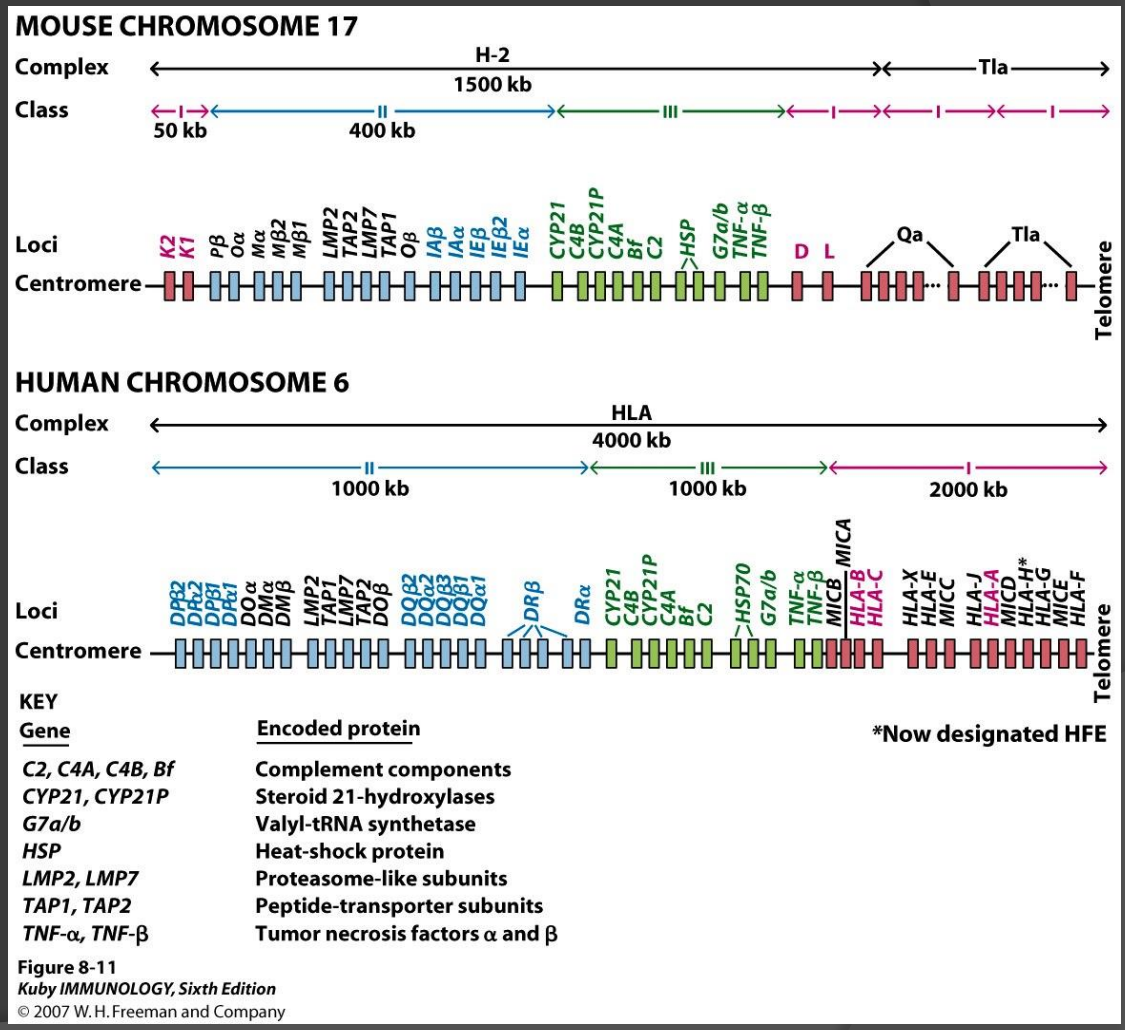

Figure 8-11
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Cellular Expression of MHC

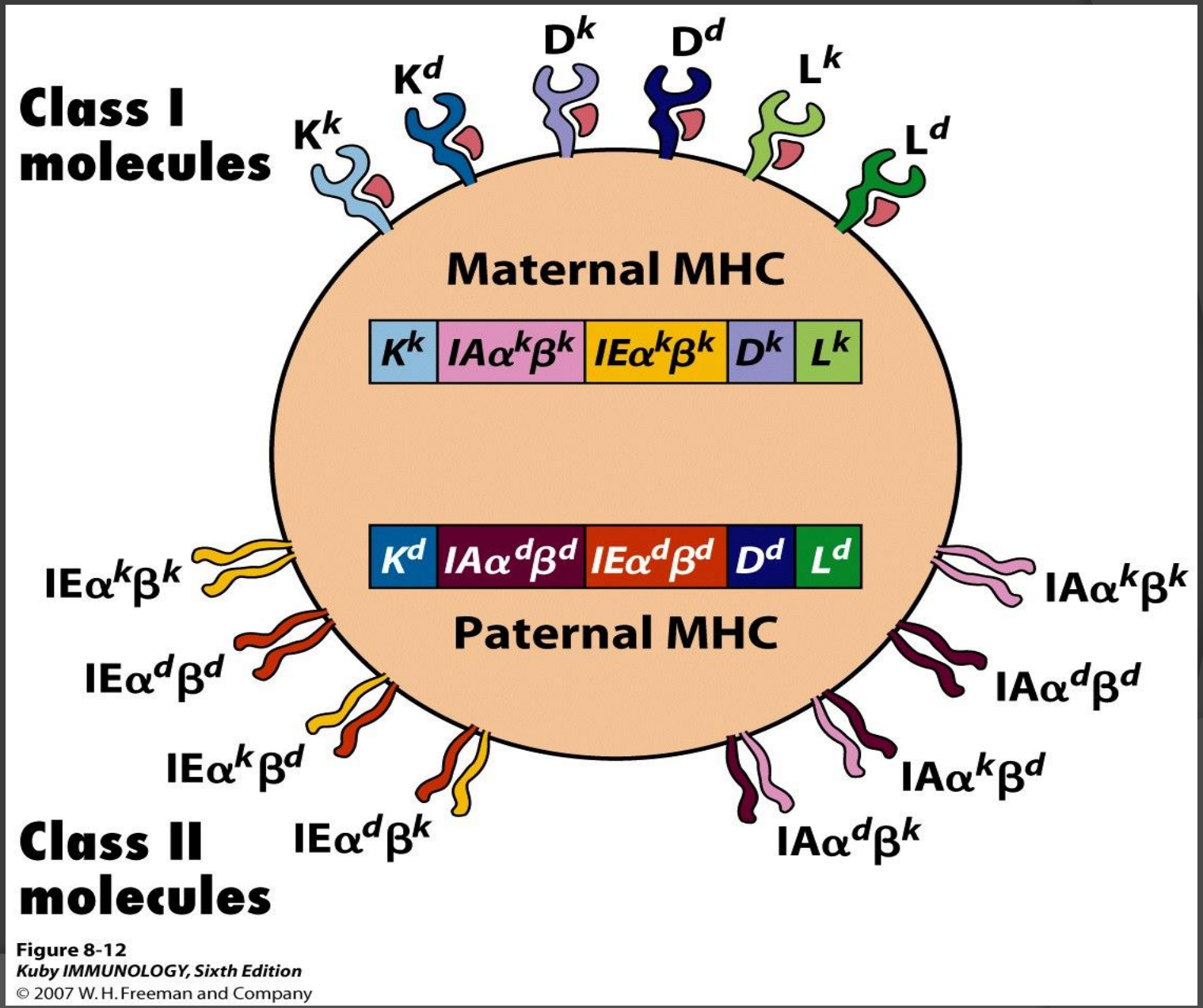

Figure 8-12
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- MHC Diversity
  - Diversity (polymorphism) helps to protect a species from wide range of infectious diseases
    - Certain alleles make individuals more susceptible to diseases
    - Example, polymorphism in cheetah is limited, due to bottleneck effect

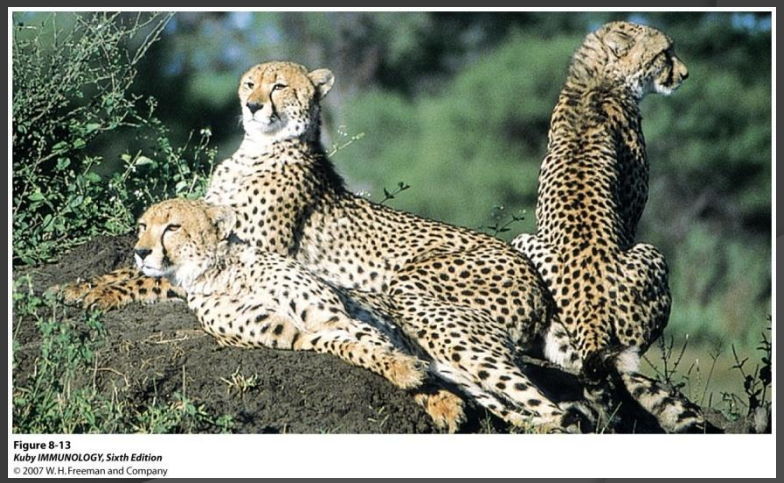

Figure 8-13
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MHC Restriction

- **CD8+ T_c cells are MHC Class I restricted**
  - Can only recognize antigen presented by MHC Class I molecules
  - Therefore, cells with MHC Class I are called “taget cells”, killed by cytotoxic T cells
- **CD4+ T_H cells are MHC Class II restricted**
  - Cells with MHC Class II are called antigen-presenting cells (APCs)

MHC Restriction

- Mice immunized with lymphocytic choriomenigitis virus (LCM)
- Animal's spleen cells were extracted (containing Tc cells)

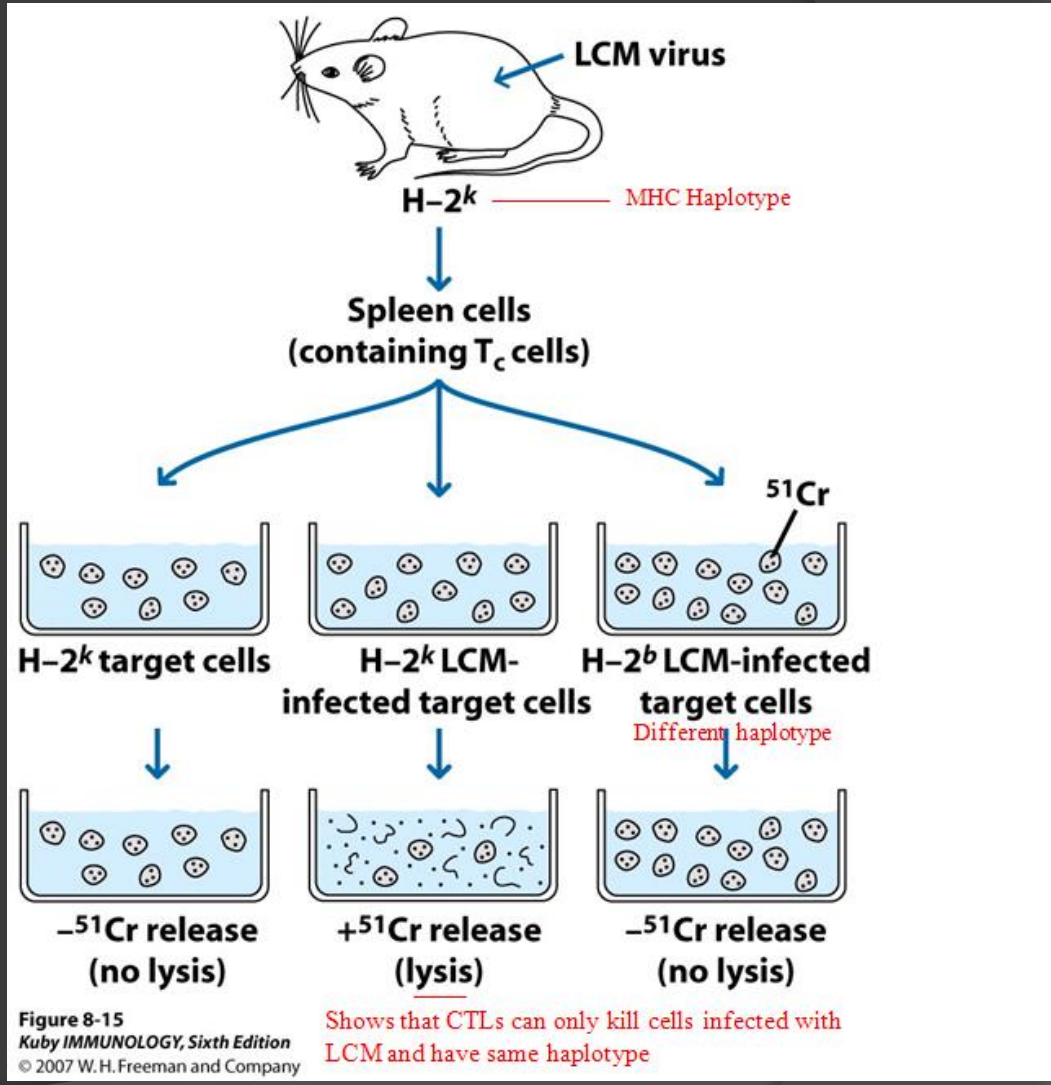

Figure 8-15
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Antigen Presenting Pathways

- **Cystolic Pathway**
  - Endogeneous antigens – produced in cell, in infected cell
  - Antigens presented on MHC Class I to T_c cells
- **Endocytic Pathway**
  - Exogeneous antigen – taken in by endocytosis by antigen-presenting cells and presented to T_H cells by MHC Class II

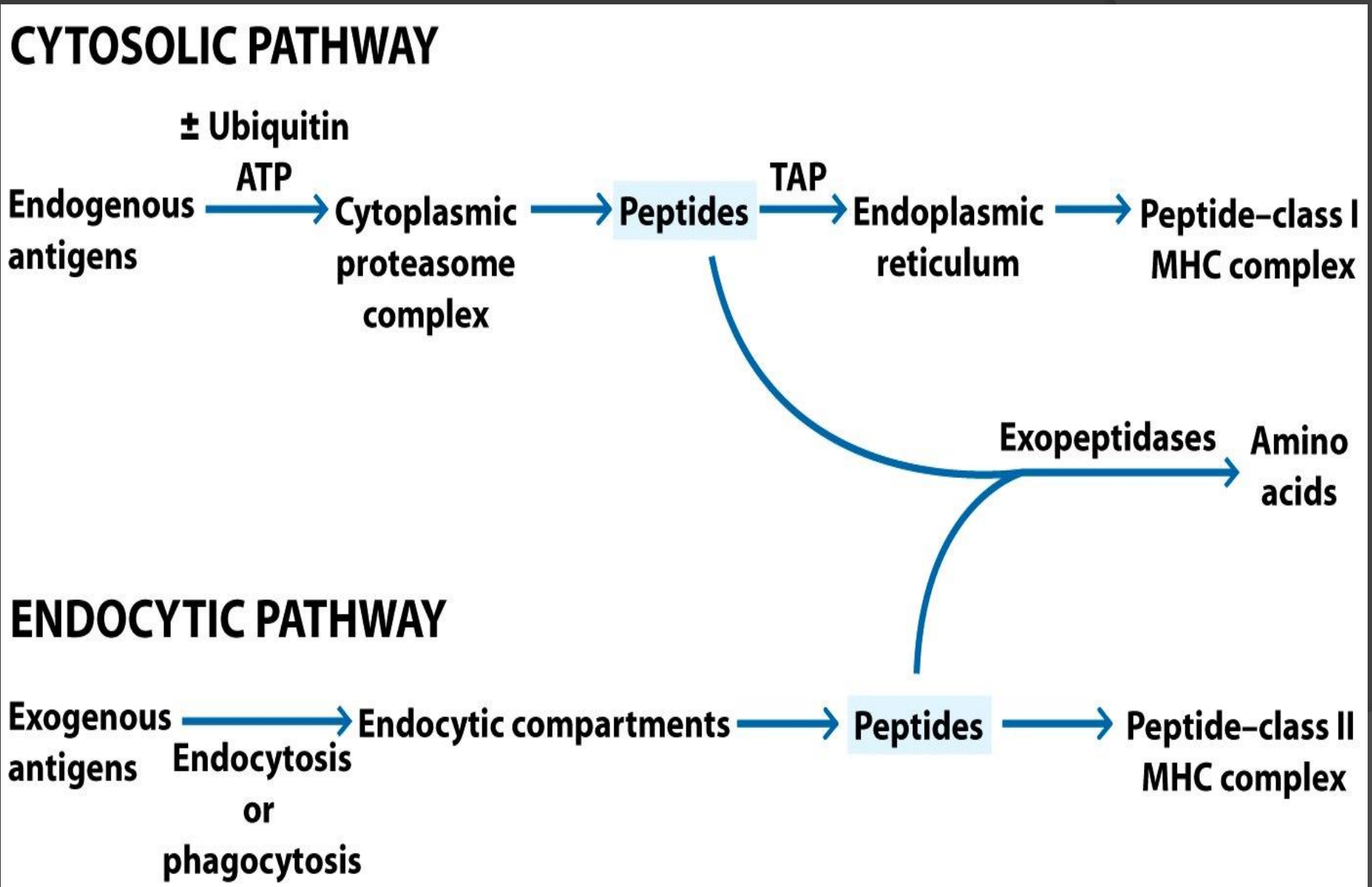

Figure 8-17
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Cystolic Pathway

Endogenous

- Figure below:
  - (a) shows degradation of misfolded protein
  - (b) shows intact proteins linked to ubiquitin to be degraded

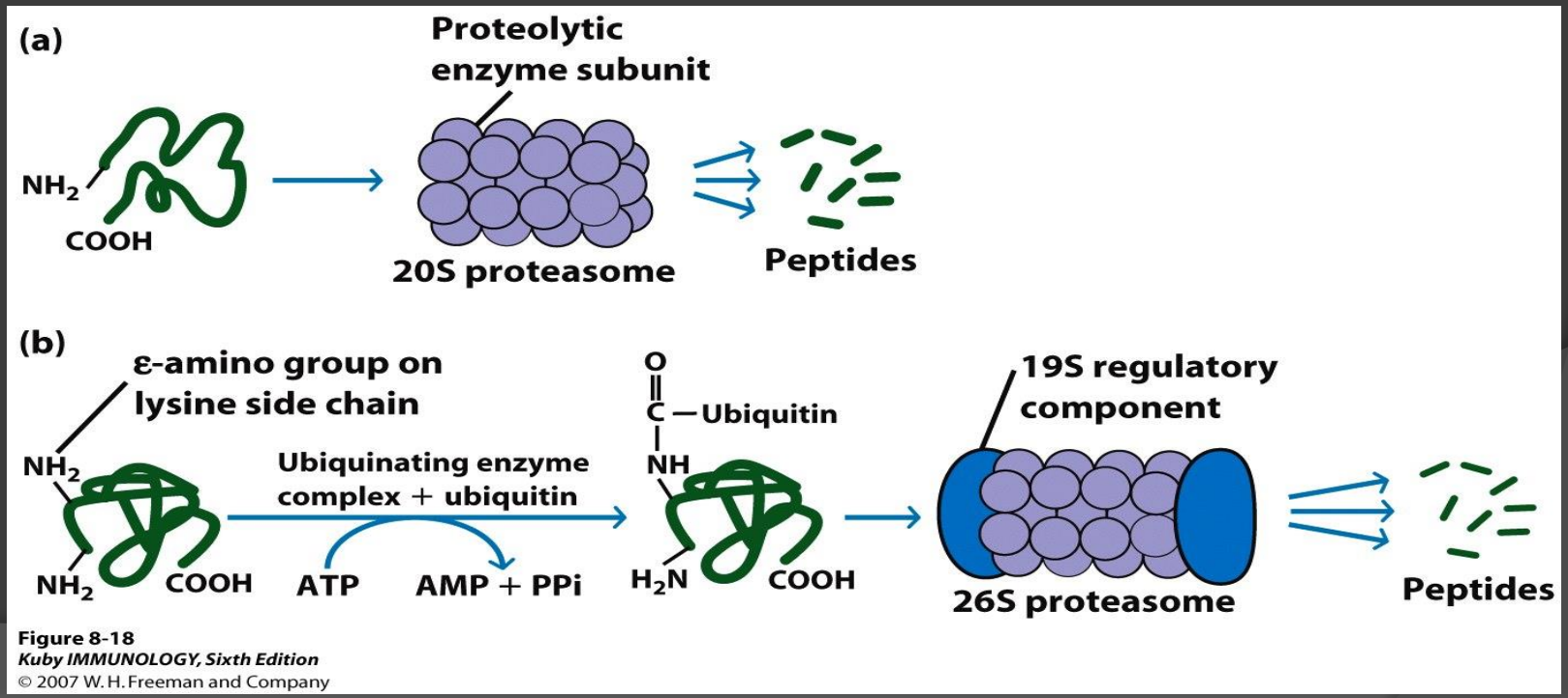

Figure 8-18
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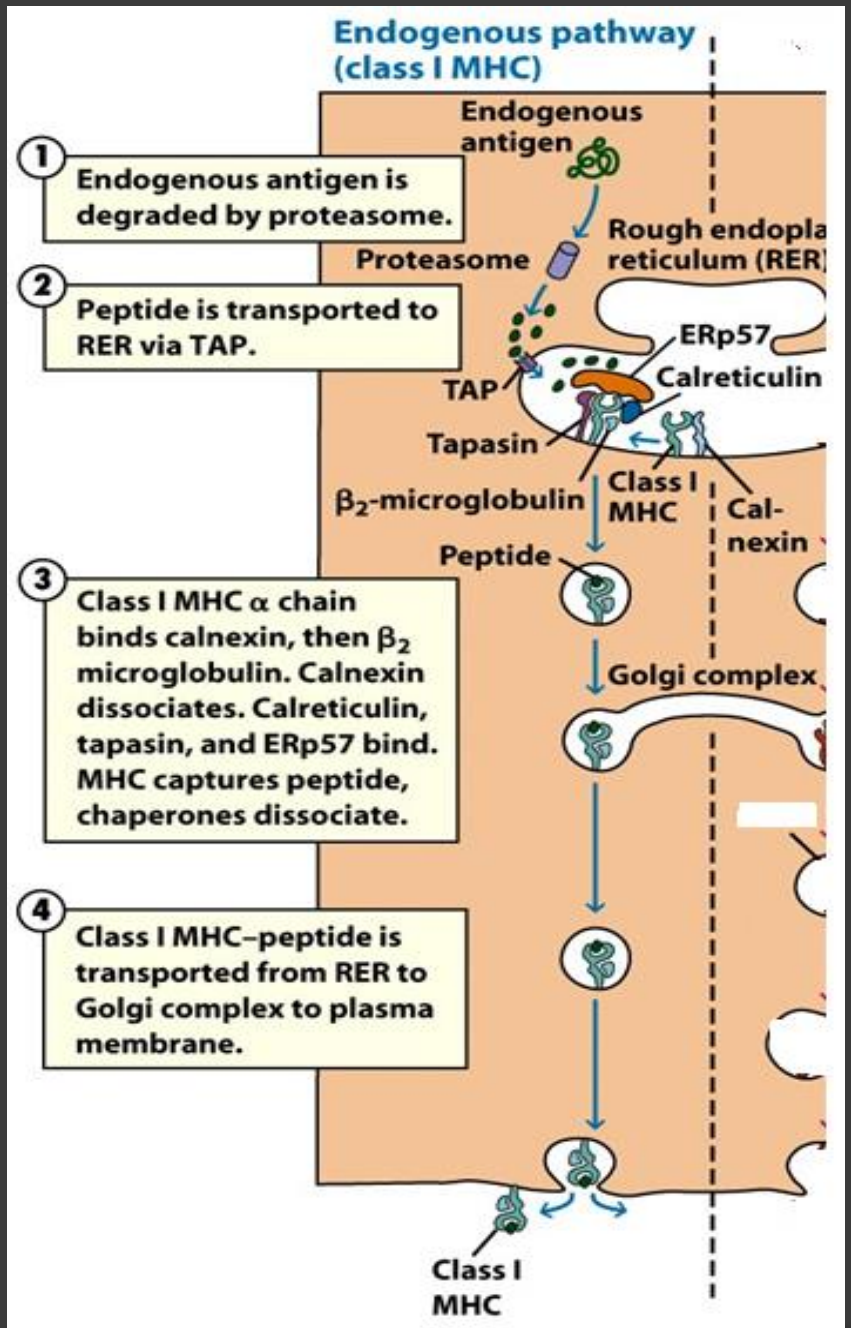

Figure 8-23
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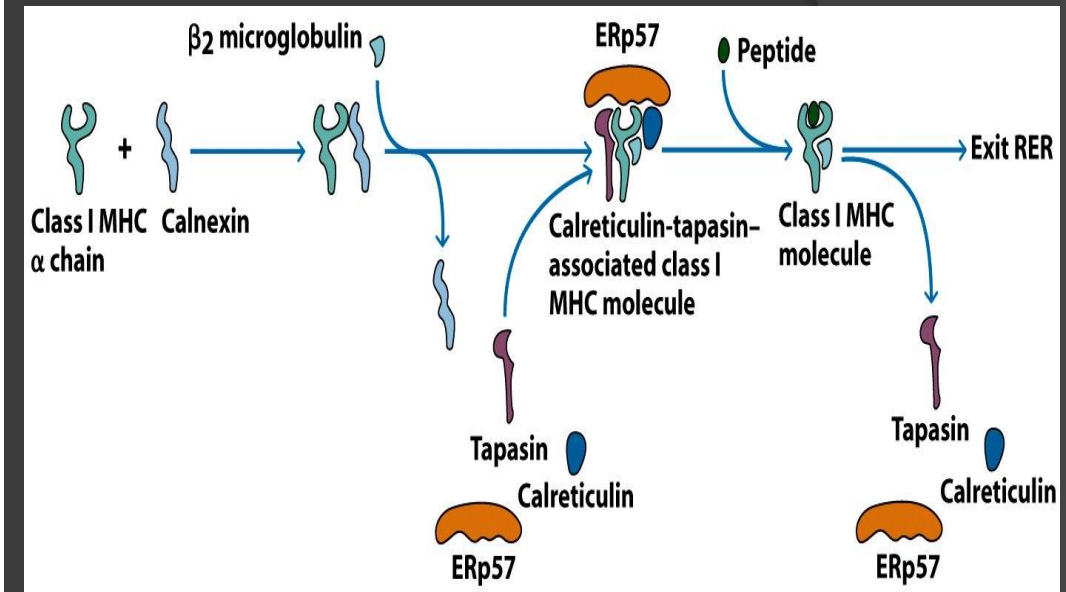

Figure 8-20
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Endocytic Pathway

Exogenous

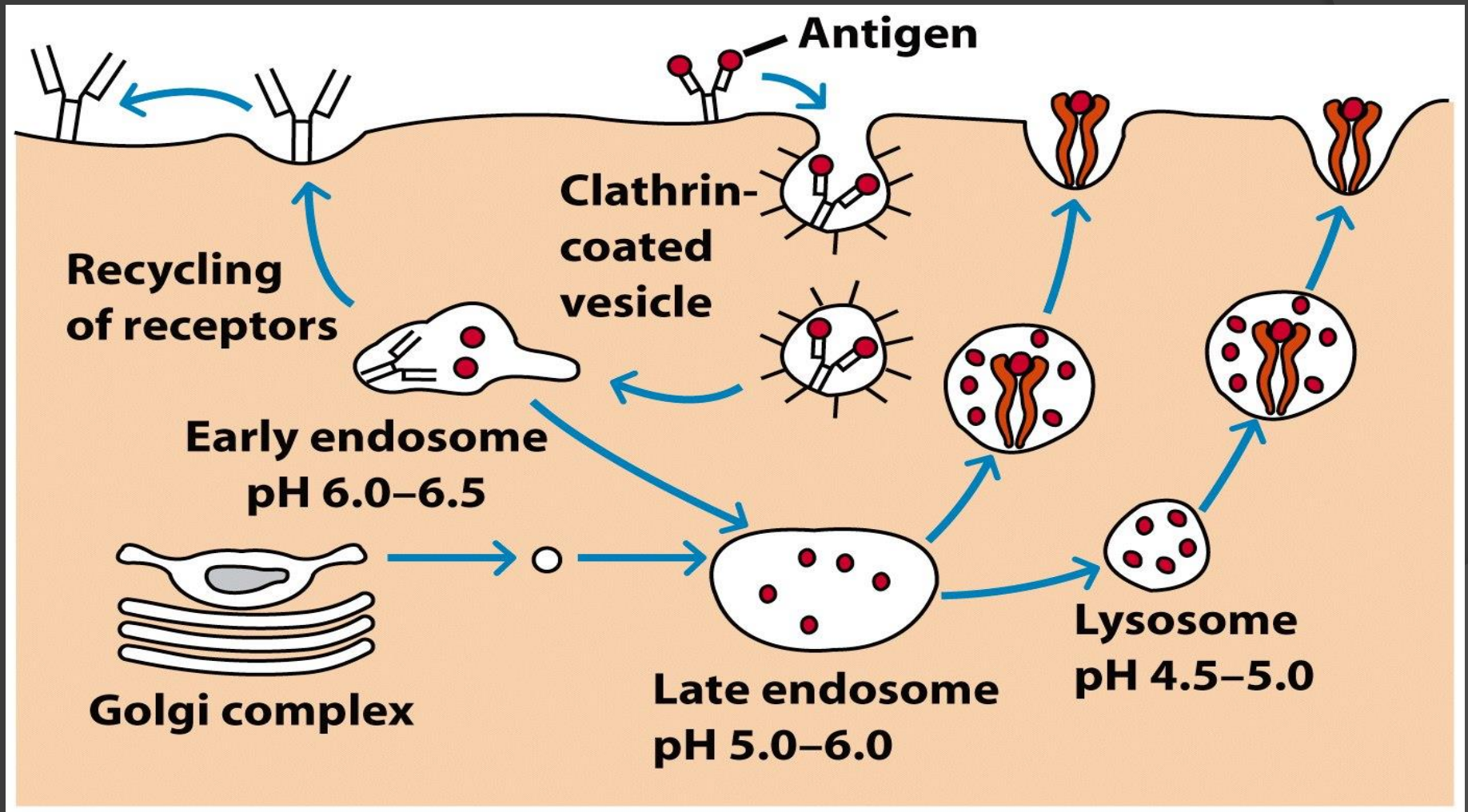

Figure 8-21
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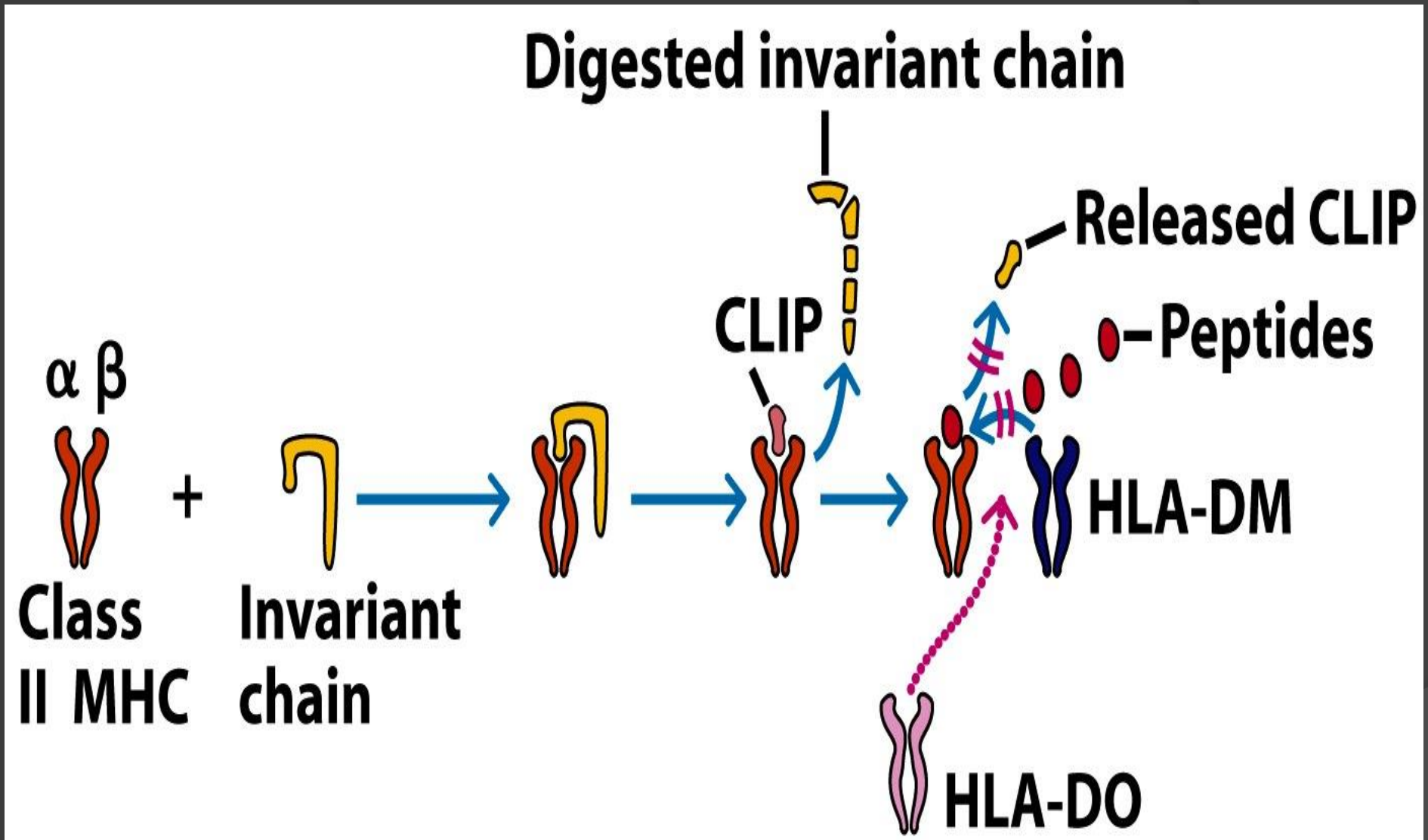

Figure 8-22a
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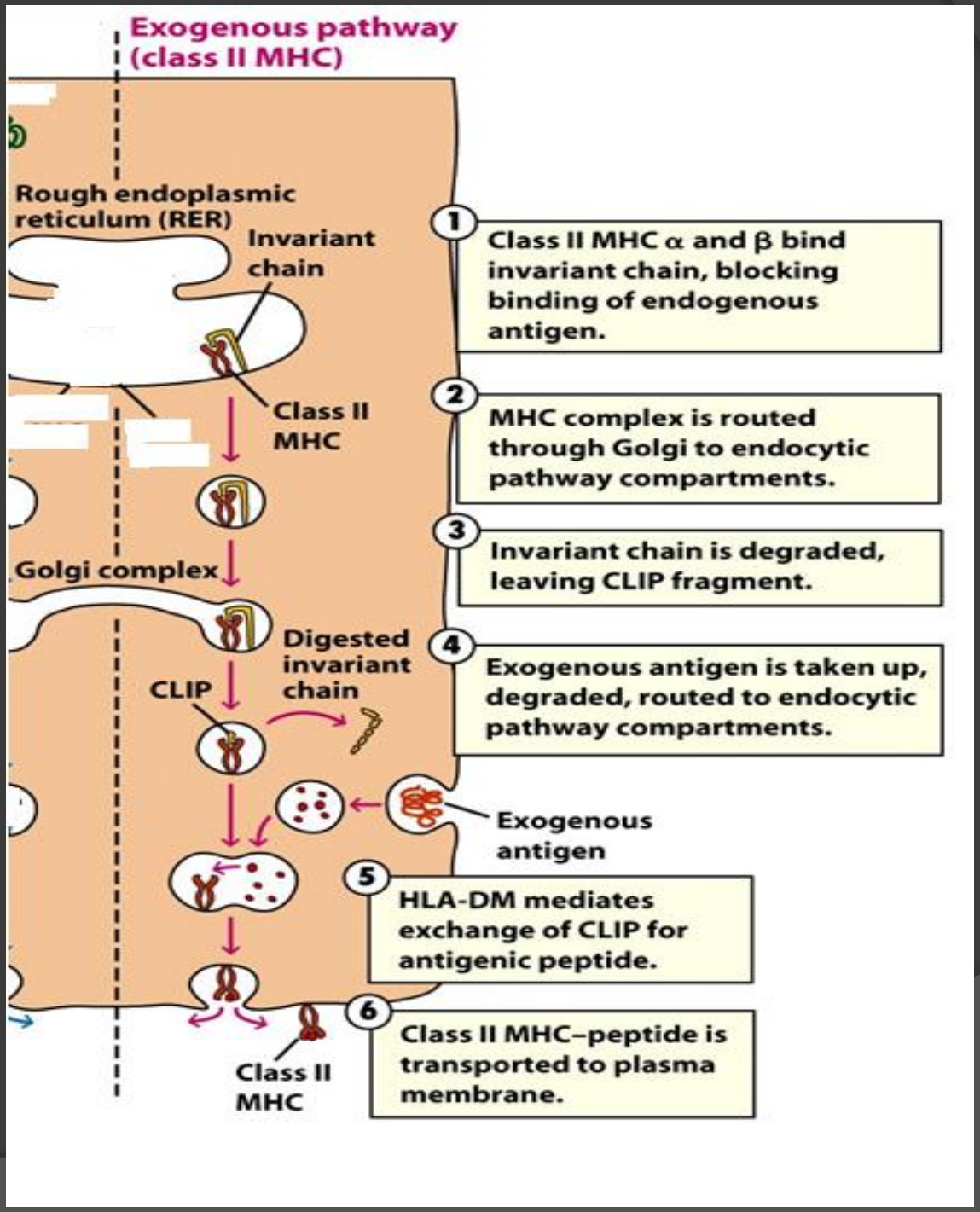
Exogenous pathway
(class II MHC)
Rough endoplasmic reticulum (RER)
Invariant chain
Class II MHC
Golgi complex
Digested invariant chain
CLIP
Exogenous antigen
Class II MHC
1
Class II MHC α and β bind invariant chain, blocking binding of endogenous antigen.
2
MHC complex is routed through Golgi to endocytic pathway compartments.
3
Invariant chain is degraded, leaving CLIP fragment.
4
Exogenous antigen is taken up, degraded, routed to endocytic pathway compartments.
5
HLA-DM mediates exchange of CLIP for antigenic peptide.
6
Class II MHC–peptide is transported to plasma membrane.

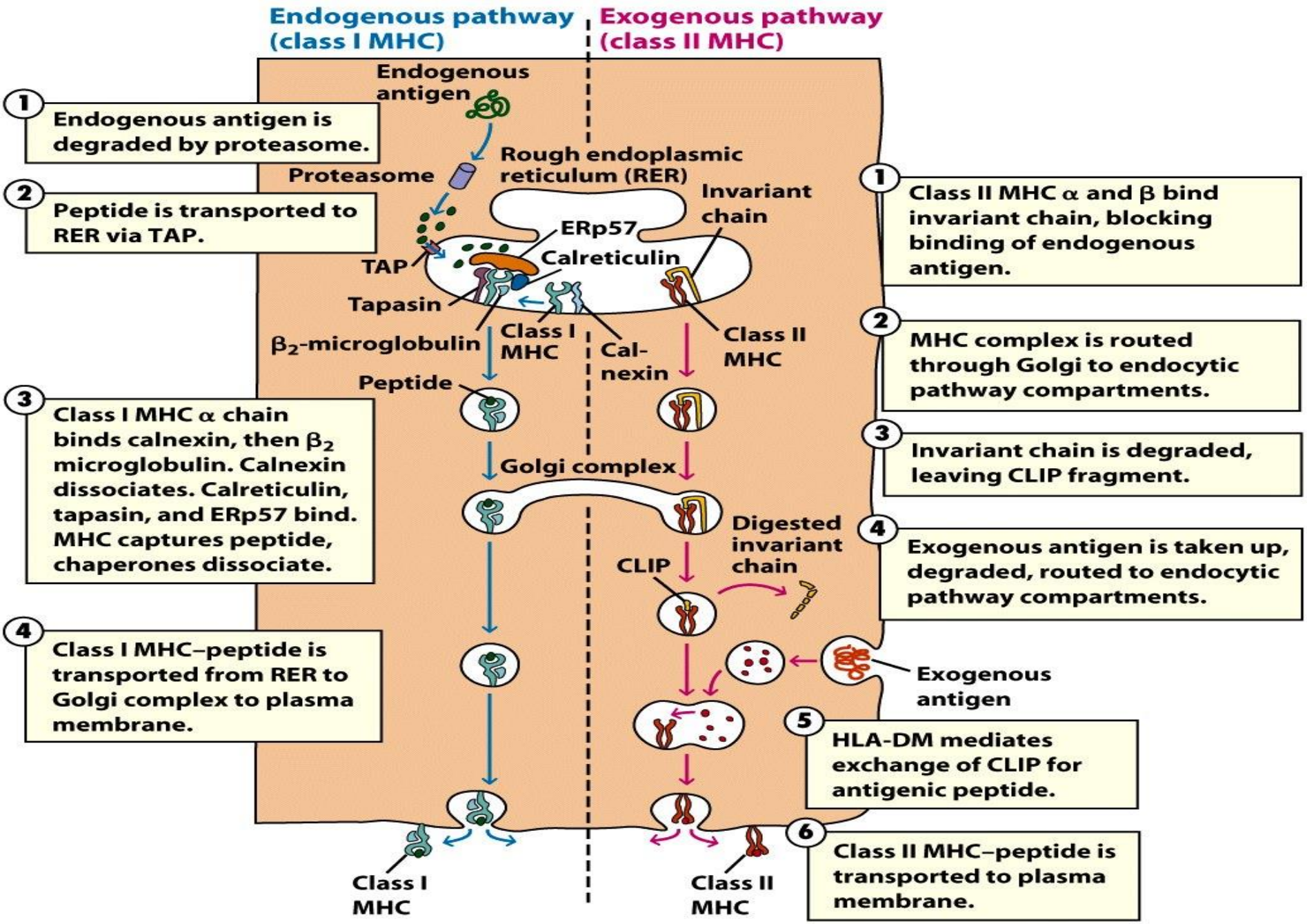

Figure 8-23
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Chapter 15

Hypersensitivity Reactions

Dr. Capers

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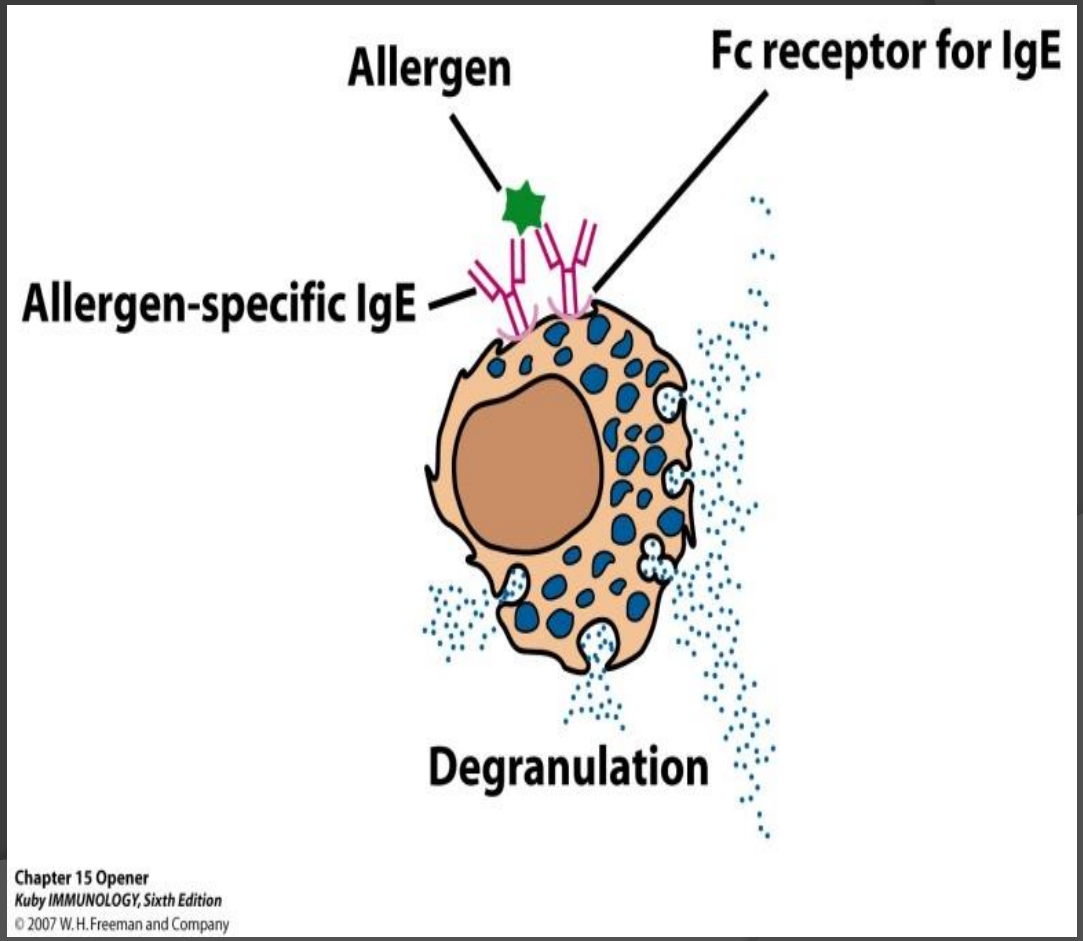
Chapter 15

Hypersensitivity Reactions

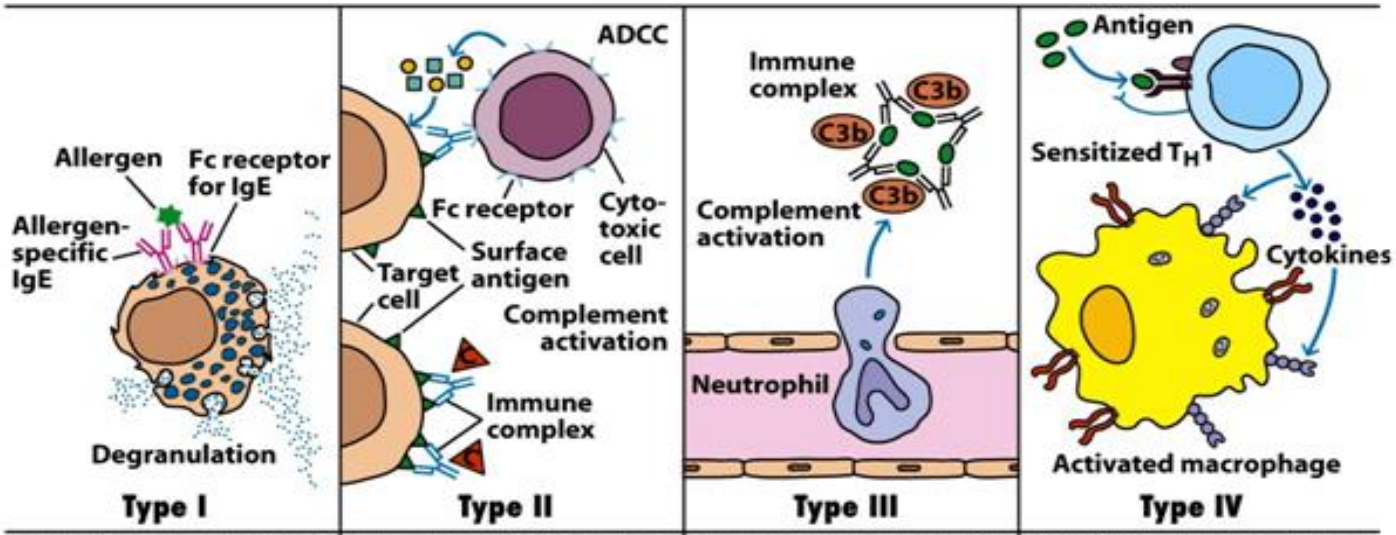
- **Hypersensitivity** – responding inappropriately to an antigen
- Inflammatory response can have deleterious effects
  - Tissue injury
  - Disease
  - death



Hypersensitivity Reactions

- May develop in course of humoral OR cell-mediated response
  - **Immediate hypersensitivity**
    - Anaphylactic
    - Antibody-antigen complexes
    - Manifests in minutes
  - **Delayed-type hypersensitivity**
    - May occur in days

4 types of hypersensitive reactions



Type I	Type II	Type III	Type IV
IgE-Mediated Hypersensitivity	IgG- or IgM-Mediated Cytotoxic Hypersensitivity	Immune Complex–Mediated Hypersensitivity	Cell-Mediated Hypersensitivity
Ag induces cross-linking of IgE bound to mast cells and basophils with release of vasoactive mediators.	Ab directed against cell surface antigens meditates cell destruction via complement activation or ADCC.	Ag-Ab complexes deposited in various tissues induce complement activation and an ensuing inflammatory response mediated by massive infiltration of neutrophils.	Sensitized T_H1 cells shown above release cytokines that activate macrophages or T_C cells that mediate direct cellular damage. T_H2 cells and CTLs mediate similar responses.
Typical manifestations include systemic anaphylaxis and localized anaphylaxis such as hay fever, asthma, hives, food allergies, and eczema.	Typical manifestations include blood transfusion reactions, erythroblastosis fetalis, and autoimmune hemolytic anemia.	Typical manifestations include localized Arthus reaction and generalized reactions such as serum sickness, necrotizing vasculitis, glomerulonephritis, rheumatoid arthritis, and systemic lupus erythematosus.	Typical manifestations include contact dermatitis, tubercular lesions, and graft rejection.

Figure 15-1
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Humoral | Cell-mediated

Type I – Ig E-Mediated Hypersensitivity

- Induced by antigens referred to as allergens
- Induces humoral response but induces high secretion of IgE
  - Fc portion of IgE binds with Fc receptors on mast cells and basophils
  - Degranulation occurs

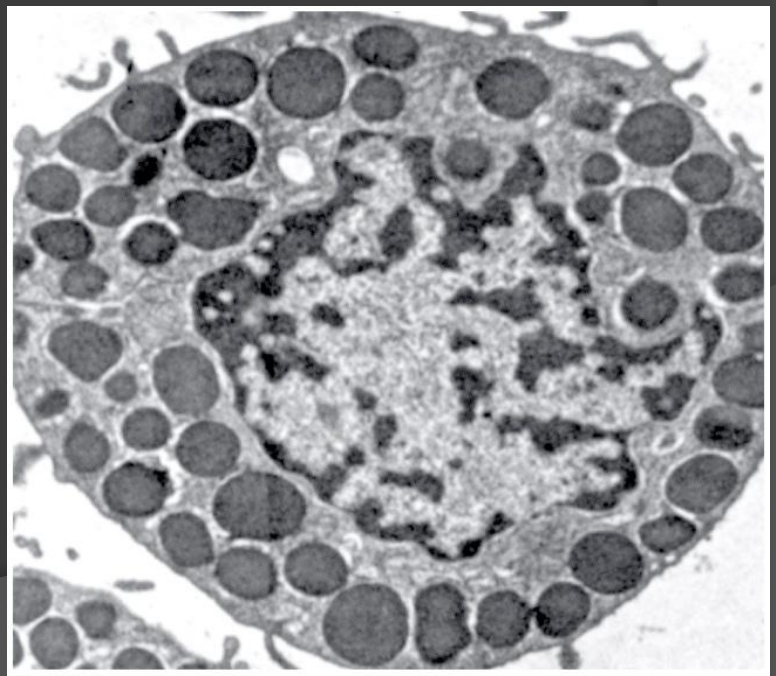

Figure 15-3a
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Type I

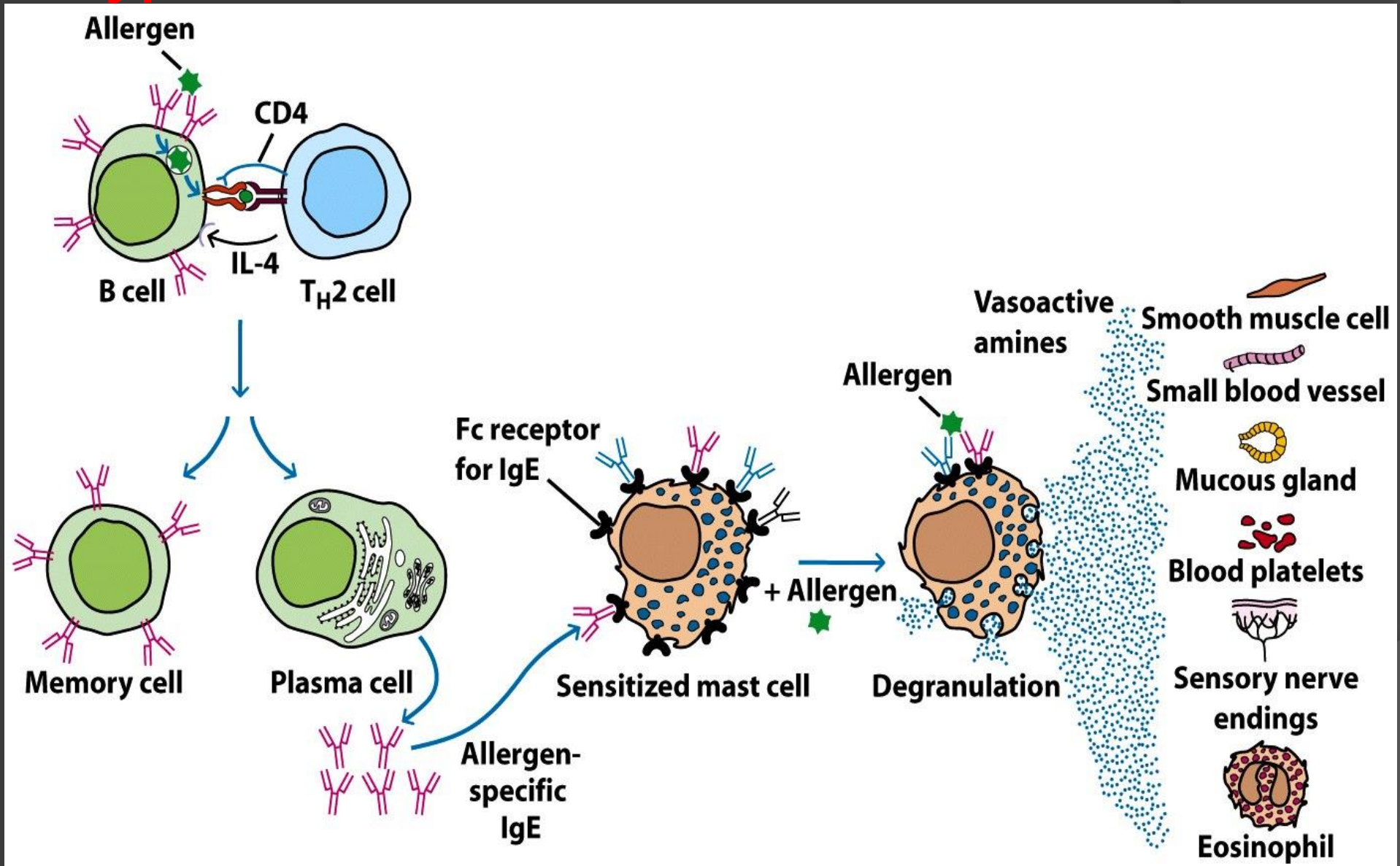

Figure 15-2
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Type 1

Common components

- **Allergens**
  - Atopy – hereditary predisposition to development of immediate hypersensitivity reactions to common antigens
    - Allows nonparasitic antigens to induce IgE response
- **IgE**
  - Normally lowest of all antibody classes in serum
  - Half-life is 2-3 days but once bound to mast cells or basophils, can last for weeks
- **Mast cells and basophils**
- **IgE binding receptors**
  - High affinity
  - Low affinity
    - Atopic individuals have higher amount of soluble IgE receptor that has been shown to increase IgE production by B cells

TABLE 15-1 Common allergens associated with type I hypersensitivity

Proteins
- Foreign serum
- Vaccines

Plant pollens
- Rye grass
- Ragweed
- Timothy grass
- Birch trees

Drugs
- Penicillin
- Sulfonamides
- Local anesthetics
- Salicylates

Foods
- Nuts
- Seafood
- Eggs
- Peas, beans
- Milk

Insect products
- Bee venom
- Wasp venom
- Ant venom
- Cockroach calyx
- Dust mites

Mold spores

Animal hair and dander

Latex

Table 15-1
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IgE cross-linkage initiates degranulation

- Once cross-linkage of antigen has occurred, intracellular signaling result in mast cell degranulation
  - Cooperation among protein and lipid kinases, phosphatases, rearrangement of the cytoskeleton

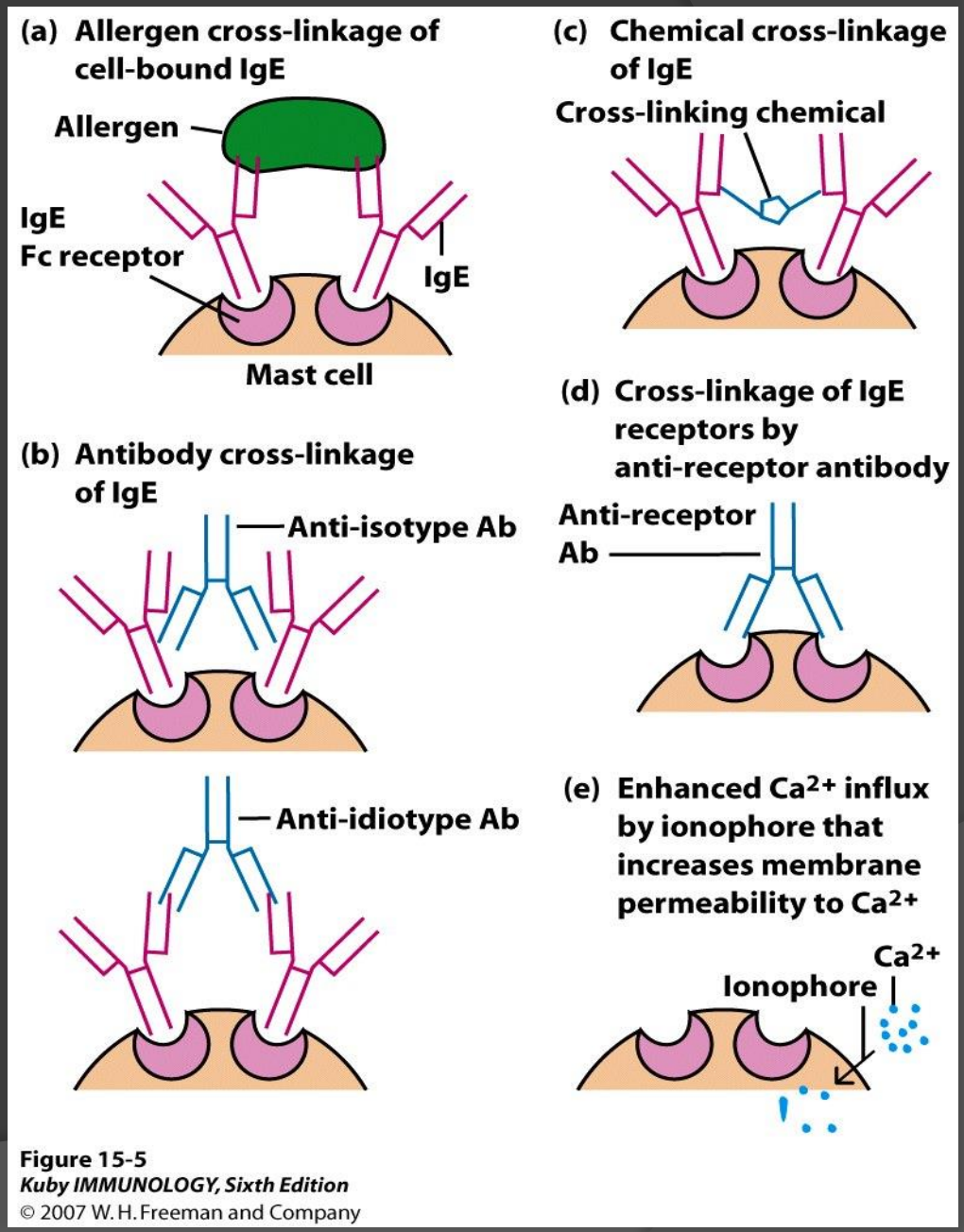

Figure 15-5
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Pharmacologic agents that mediate Type I

- **Primary mediators**
  - Made before and stored in granules
  - Histamine, proteases, eosinophil chemotactic factor, heparin
- **Secondary mediators**
  - Synthesized after
  - Platelet-activating factor, leukotrienes, prostaglandins, bradykinins, some cytokines and chemokines

TABLE 15-3 Principal mediators involved in type I hypersensitivity

Mediator	Effects
	PRIMARY
Histamine, heparin	Increased vascular permeability; smooth muscle contraction
Serotonin (rodents)	Increased vascular permeability; smooth muscle contraction
Eosinophil chemotactic factor (ECF-A)	Eosinophil chemotaxis
Neutrophil chemotactic factor (NCF-A)	Neutrophil chemotaxis
Proteases (tryptase, chymase)	Bronchial mucus secretion; degradation of blood vessel basement membrane; generation of complement split products
	SECONDARY
Platelet-activating factor	Platelet aggregation and degranulation; contraction of pulmonary smooth muscles
Leukotrienes (slow reactive substance of anaphylaxis, SRS-A)	Increased vascular permeability; contraction of pulmonary smooth muscles
Prostaglandins	Vasodilation; contraction of pulmonary smooth muscles; platelet aggregation
Bradykinin	Increased vascular permeability; smooth muscle contraction
Cytokines	
IL-1 and TNF-α	Systemic anaphylaxis; increased expression of CAMs on venular endothelial cells
IL-4 and IL-13	Increased IgE production
IL-3, IL-5, IL-6, IL-10, TGF-β, and GM-CSF	Various effects (see Table 12-1)

Table 15-3
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Histamine

- Formed by decarboxylation of amino acid Histidine
- Major component of granules
- Effects observed in minutes
- Contraction of smooth muscle (intestinal and bronchial), increase permeability of venules, increased mucus secretion by goblet cells

- **Leukotrienes and prostaglandins**
  - Effects longer to become apparent
  - Effects longer lasting than histamine
  - Bronchoconstriction, vascular permeability, mucus production

Type 1 can be systemic or localized

- **Systemic anaphylaxis**
  - Quick, can be fatal
  - Respiration labored, blood pressure drops, bronchiole constriction, edema, shock
  - Epinephrine treats, relaxes smooth muscle and increases cardiac output (prevents vascular collapse)

Type 1 can be systemic or localized

- **Localized Hypersensitivity Reactions (Atopy)**
  - **Allergic Rhinitis**
    - Most common, "hay fever"
  - **Asthma**
    - Triggered like hay fever but doesn't happen in nasal cavity, happens in lower respiratory tract
  - **Food allergies**
    - Hives, vomiting
  - **Atopic dermatitis**
    - Allergic eczema

Asthma

- Inflammatory disease
- Induce expression of adhesion molecules on endothelial cells for eosinophils and neutrophils
  - Cause significant injury because of toxic enzymes, cytokines
  - Notice sloughing of the pseudostratified ciliated columnar epithelial cells lining the bronchiole

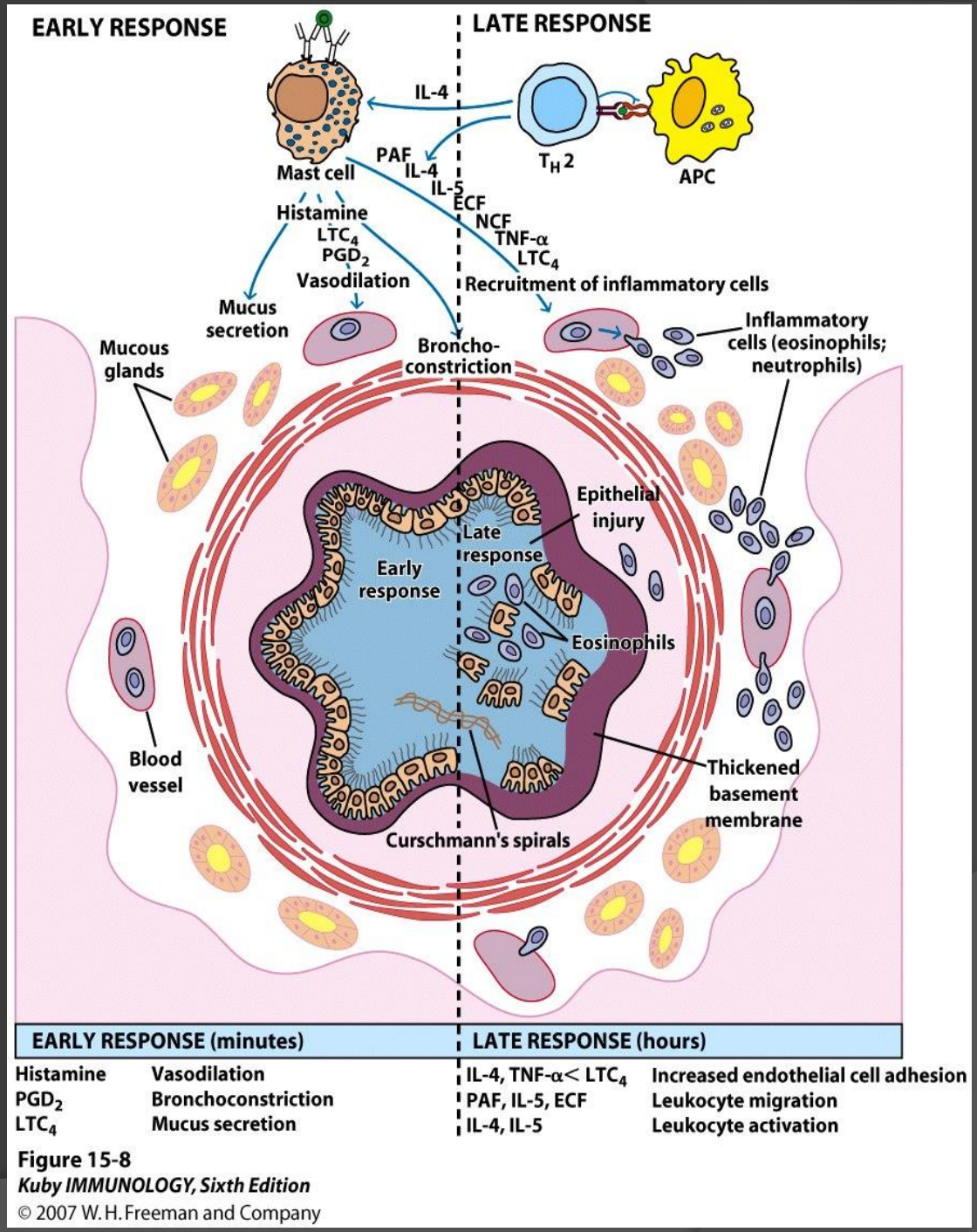

Figure 15-8
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Clinical Methods to detect Type 1

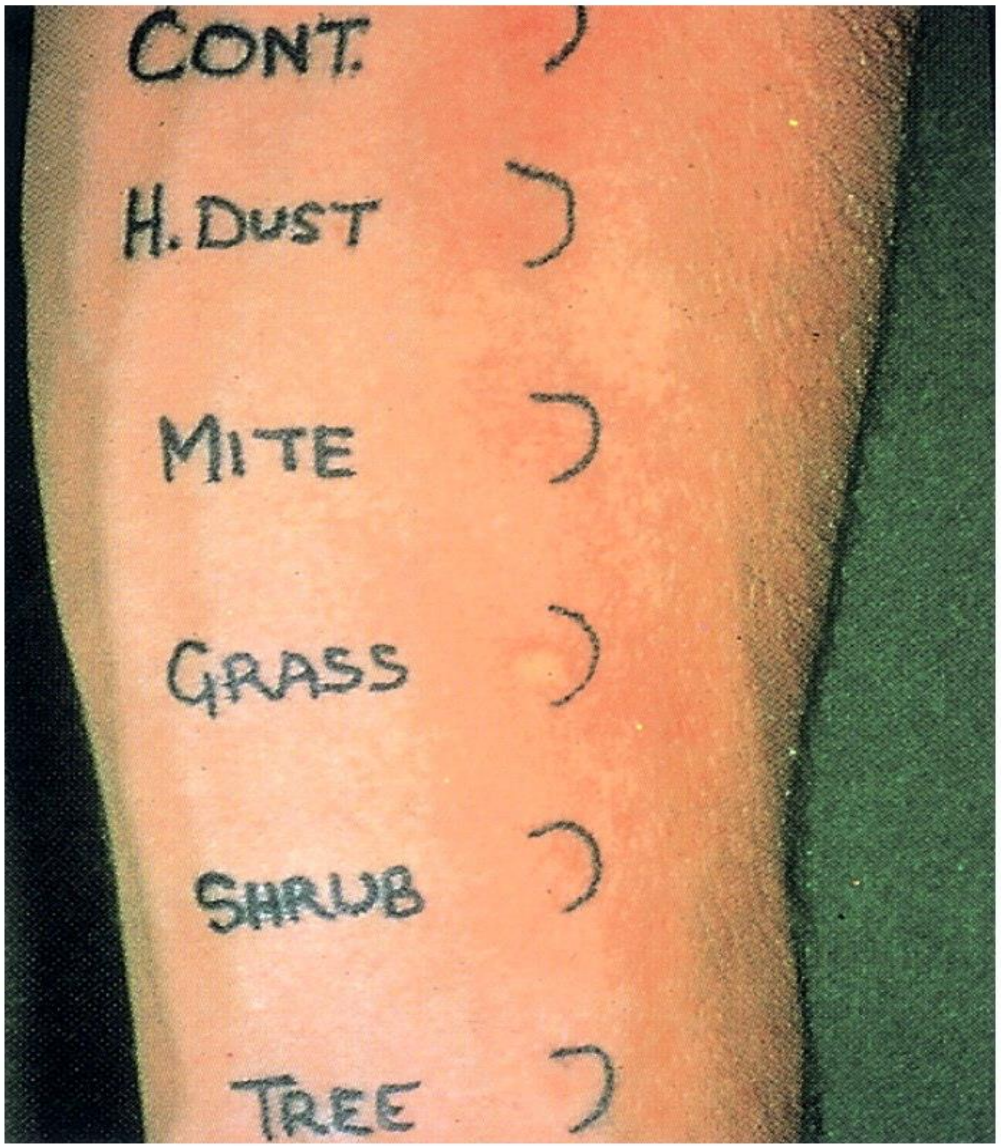

Figure 15-10
Kuby IMMUNOLOGY, Sixth Edition

- Skin testing
- Checking serum level of IgE

Control of Type 1

- **Avoiding contact**
- **Immunotherapy**
  - Subcutaneous injections of allergens
  - Causes shift to IgG production instead of IgE
  - Monoclonal anti-human IgE
- **Drug therapies**

TABLE 15-4 Mechanism of action of some drugs used to treat type I hypersensitivity

Drug	Action
Antihistamines	Block H_1 and H_2 receptors on target cells
Cromolyn sodium	Blocks Ca^{2+} influx into mast cells
Theophylline	Prolongs high cAMP levels in mast cells by inhibiting phosphodiesterase, which cleaves cAMP to 5′-AMP*
Epinephrine (adrenaline)	Stimulates cAMP production by binding to β-adrenergic receptors on mast cells*
Cortisone	Reduces histamine levels by blocking conversion of histidine to histamine and stimulates mast-cell production of cAMP*

*Although cAMP rises transiently during mast-cell activation, degranulation is prevented if cAMP levels remain high.

Table 15-4
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Type II – Antibody-Mediated Cytotoxic Hypersensitivity

- Transfusion Reactions
  - Due to exposure to microorganisms in gut, individuals have antibodies to blood types not their own
  - Antibody attaches to RBC and initiates complement system to lyse RBC
  - After lysis:
    - Hemoglobin detected in plasma, starts to filter through kidneys and found in urine (hemoglobinuria)
    - Hemoglobin converted to bilirubin – toxic at high levels
    - Fever, chills, blood clotting

Type II – Antibody-Mediated Cytotoxic Hypersensitivity

- Hemolytic disease of newborn
  - Rh^+ fetus, Rh^- mother
  - IgG antibodies cross placenta
    - Some of these antibodies may be anti-Rh antibodies
      - Can have severe consequences
    - Antibodies against ABO blood groups produce less consequences, can be easily treated
  - Rhogam shot
    - Given to mother
    - Anti-Rh antibodies bind to fetal cells that might have entered mother's system during birthing process, facilitates clearing before there is a B cell response

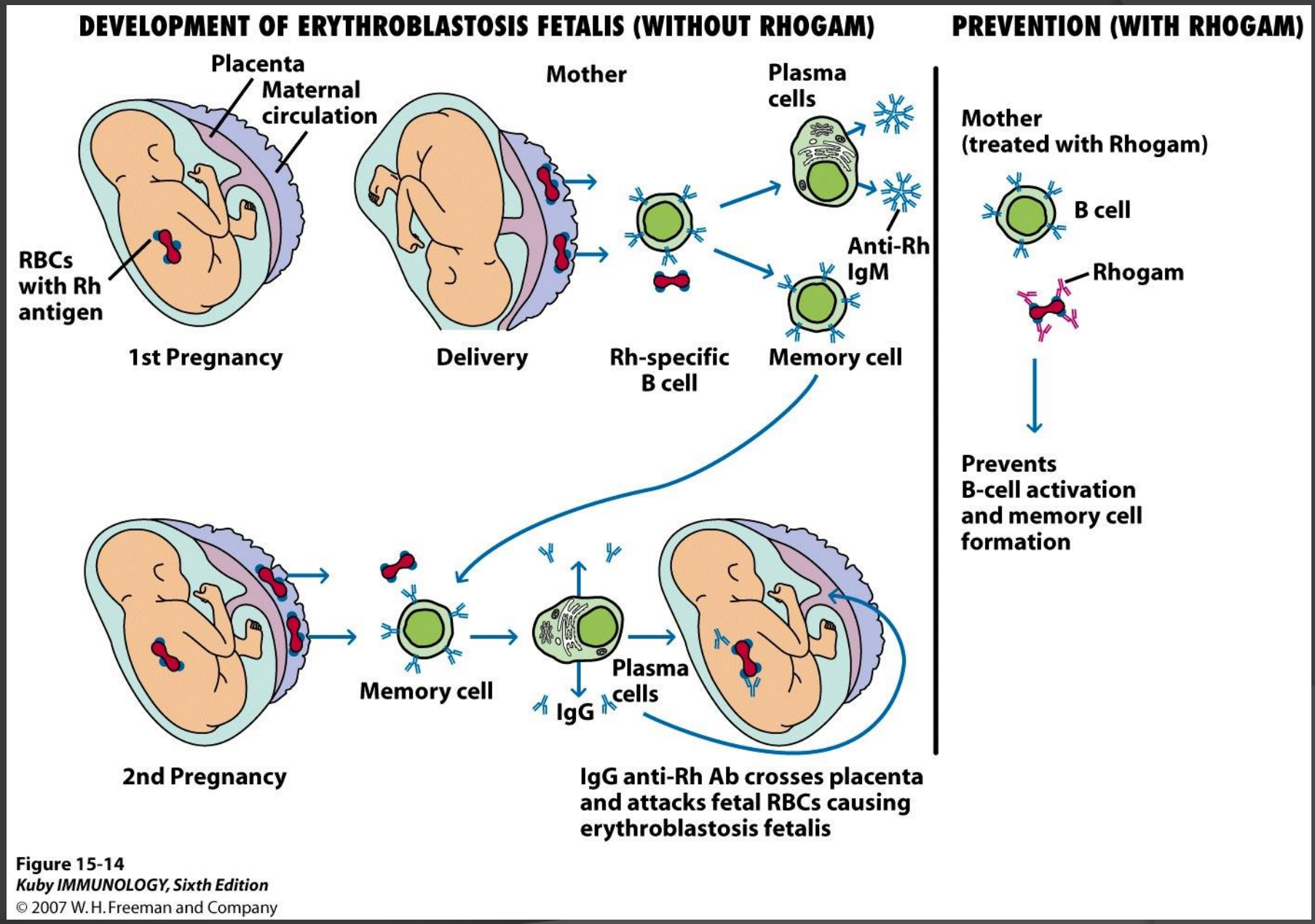

Figure 15-14
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Type III – Immune complex-mediated hypersensitivity

- Complexing of antigen plus antibody facilitates phagocytosis and clearing of antigen
- Large amounts of these complexes can lead to tissue damage

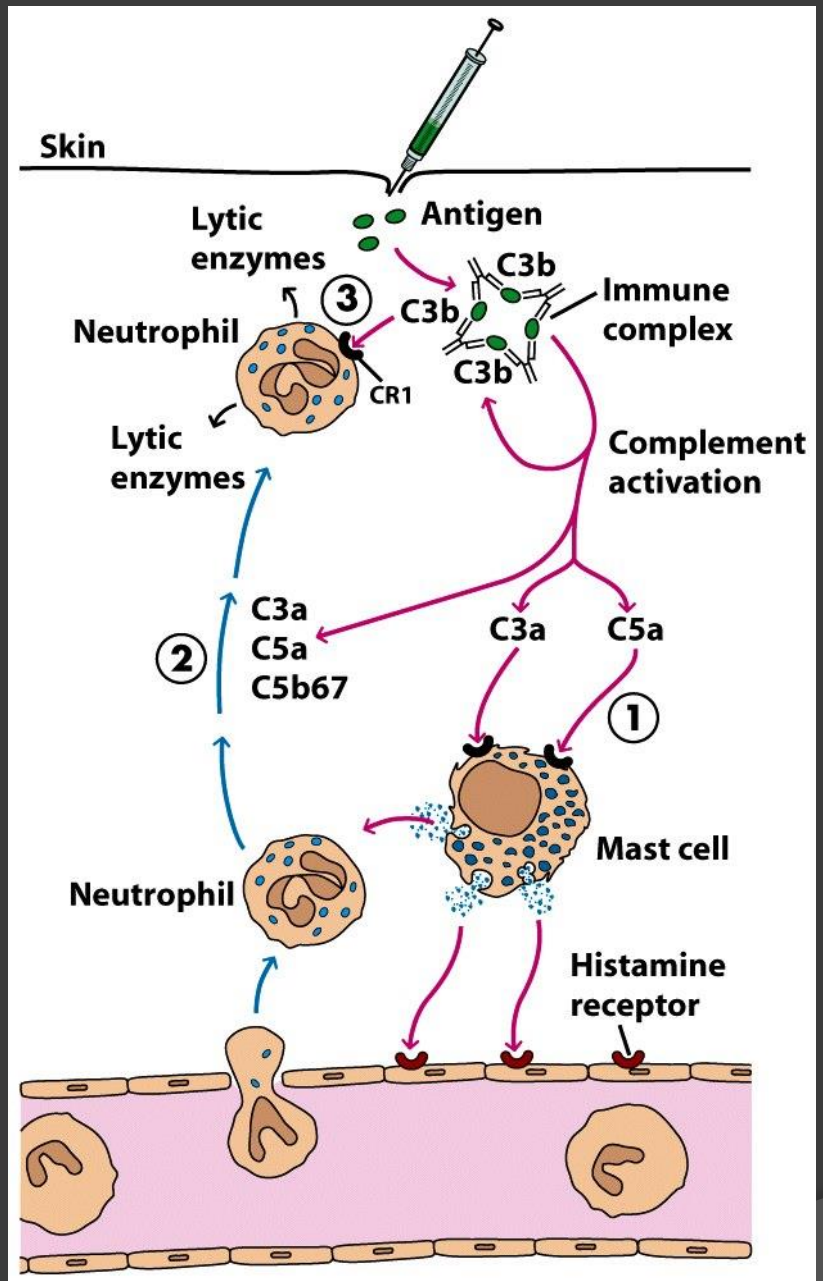

Figure 15-15
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Type III can be localized

- Injection of antigen intradermally or subcu into animal that has high level of antibody for that antigen
  - Arthus reaction
  - Bug bites

Type III can be generalized

- Serum sickness
  - After receiving antiserum (serum from another animal that may contain antitoxins for treatment)
- Use of monoclonal antibodies for use of cancer treatment
  - Patient developed antibody against mouse monoclonal antibody
- Autoimmune diseases
  - Lupus, Rheumatoid arthritis
- Drug reactions
  - Penicillin, sulfonamides
- Infectious disease

Type IV – Delayed-type Hypersensitivity

- Some subpopulations of T_H cells encounter antigen, secrete cytokines and induce localized inflammatory response
- Most cases are not detrimental

TABLE 15-6 Intracellular pathogens and contact antigens that induce delayed-type (type IV) hypersensitivity

Intracellular bacteria	**Intracellular viruses**
Mycobacterium tuberculosis	Herpes simplex virus
Mycobacterium leprae	Variola (smallpox)
Listeria monocytogenes	Measles virus
Brucella abortus	
Intracellular fungi	**Contact antigens**
Pneumocystis carinii	Picrylchloride
Candida albicans	Hair dyes
Histoplasma capsulatum	Nickel salts
Cryptococcus neoformans	Poison ivy
Intracellular parasites	Poison oak
Leishmania sp.	

Table 15-6
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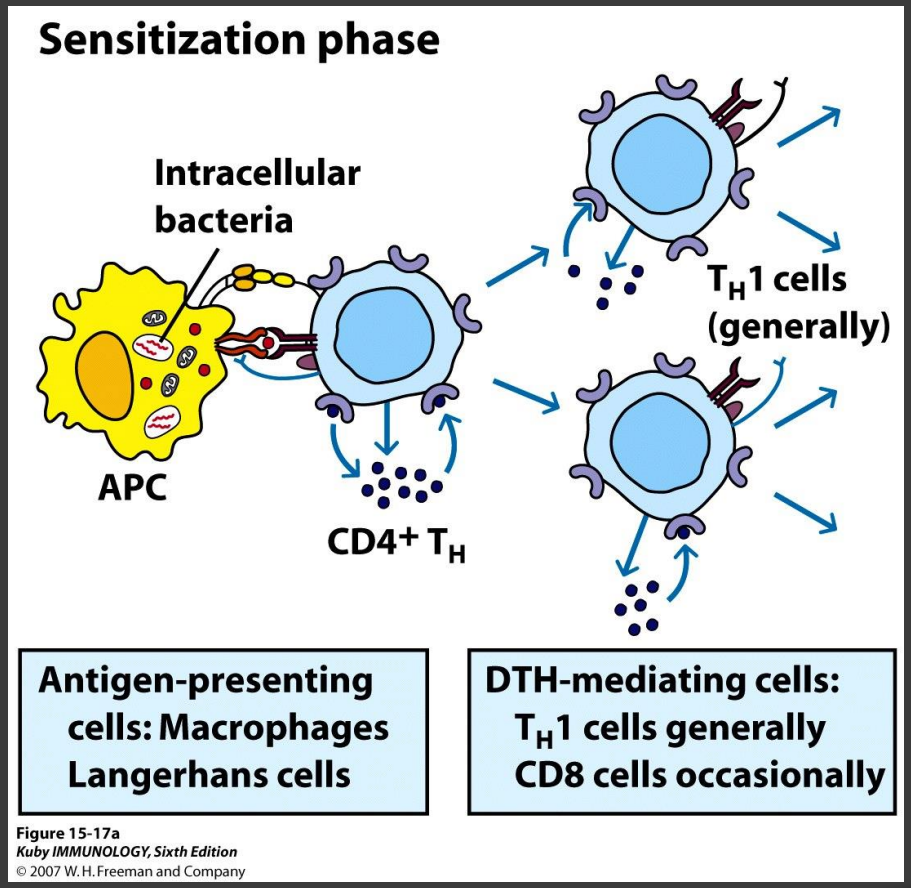
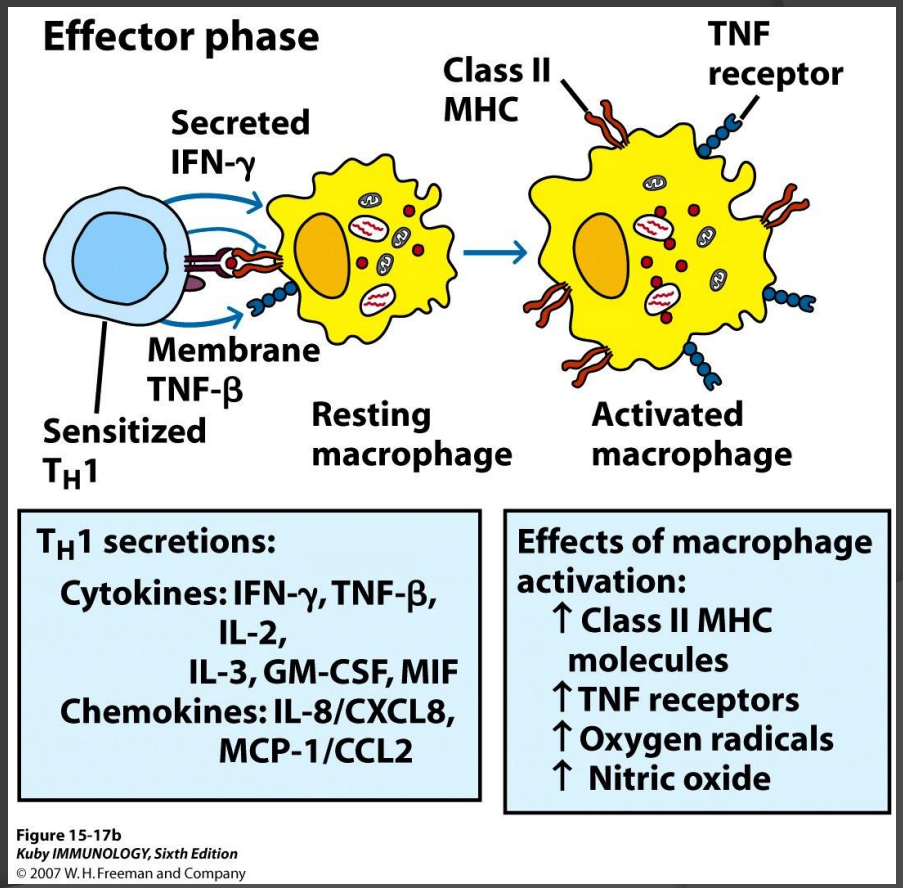
Type IV
Sensitization phase and Effector phase of DTH





Prolonged DTH can lead to formation of granuloma

Tuberculosis test is done this way

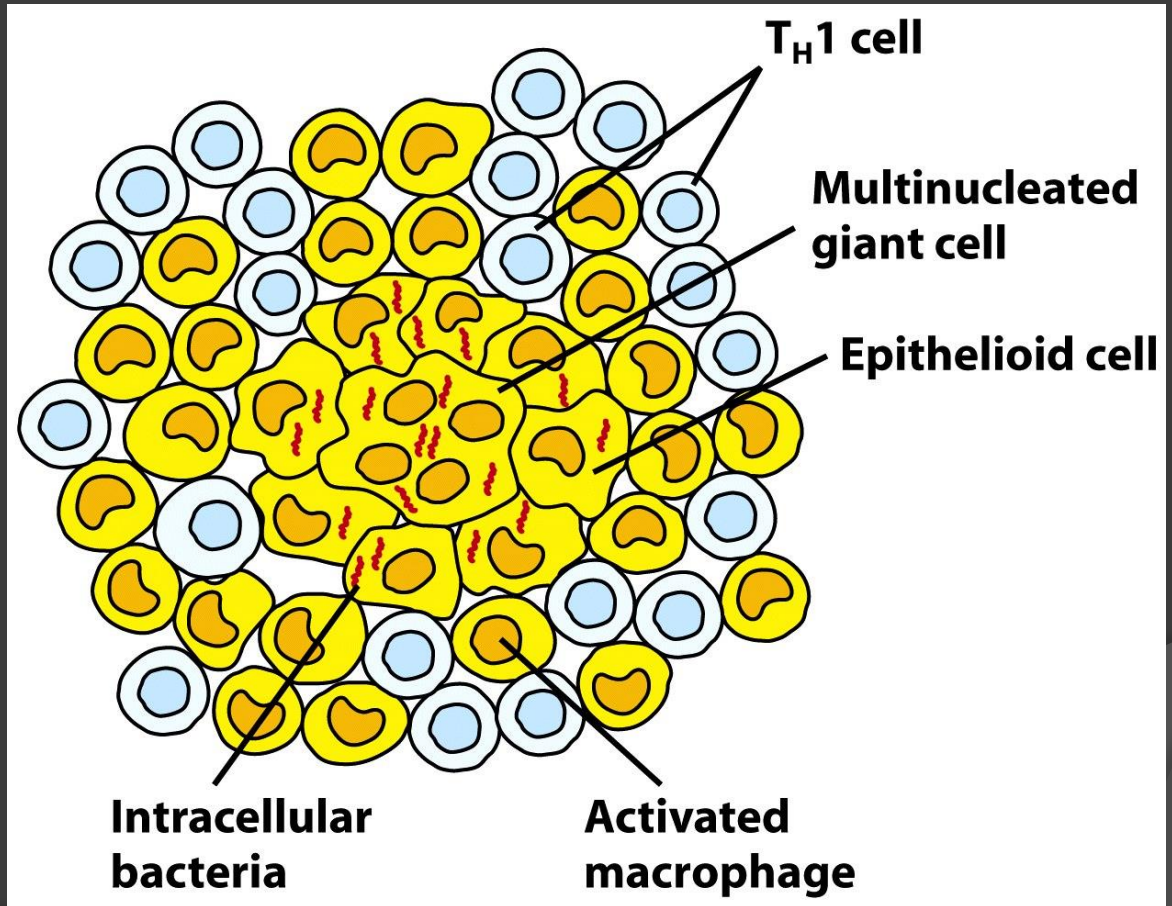


Figure 15-18
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Type IV – contact dermatitis

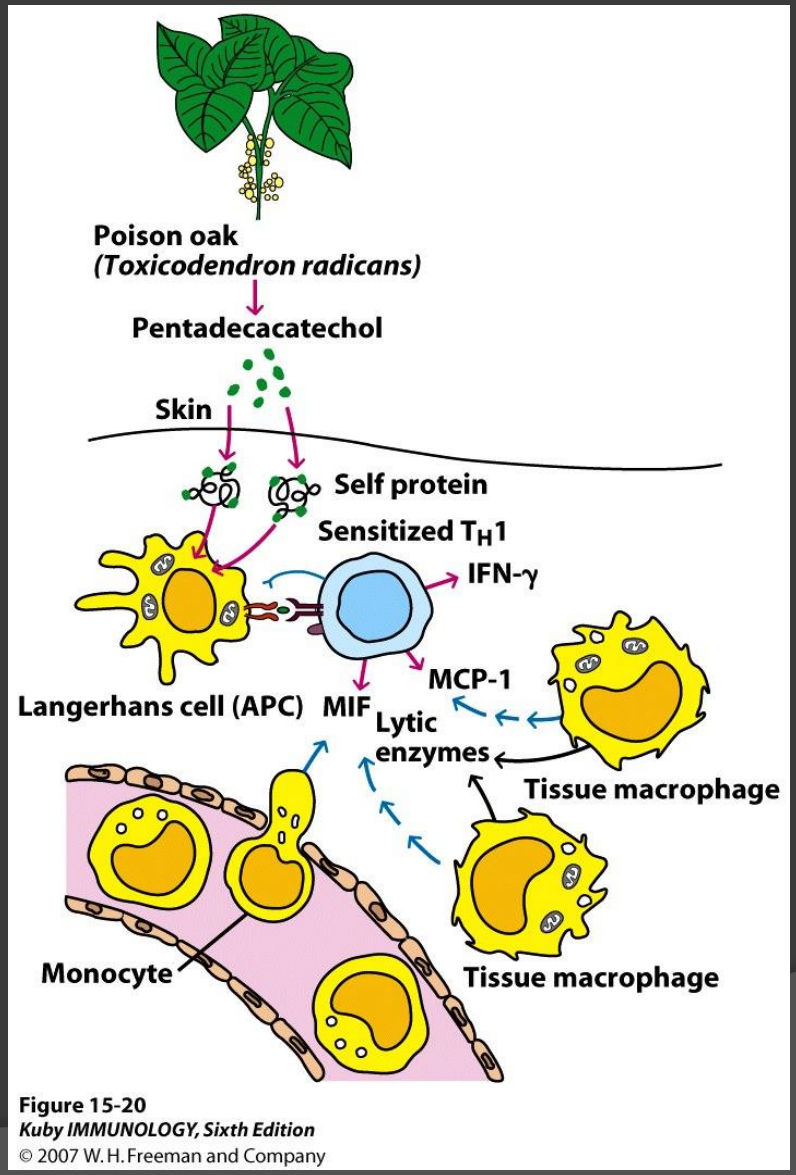

Figure 15-20
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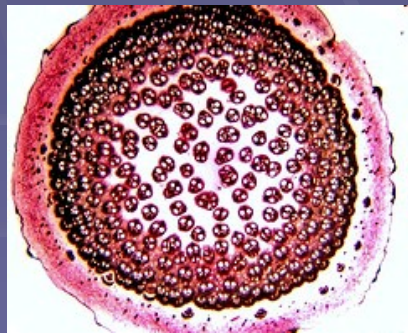
Stem Cells

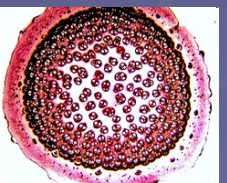
Clinical Applications

Dr T.V.Rao MD

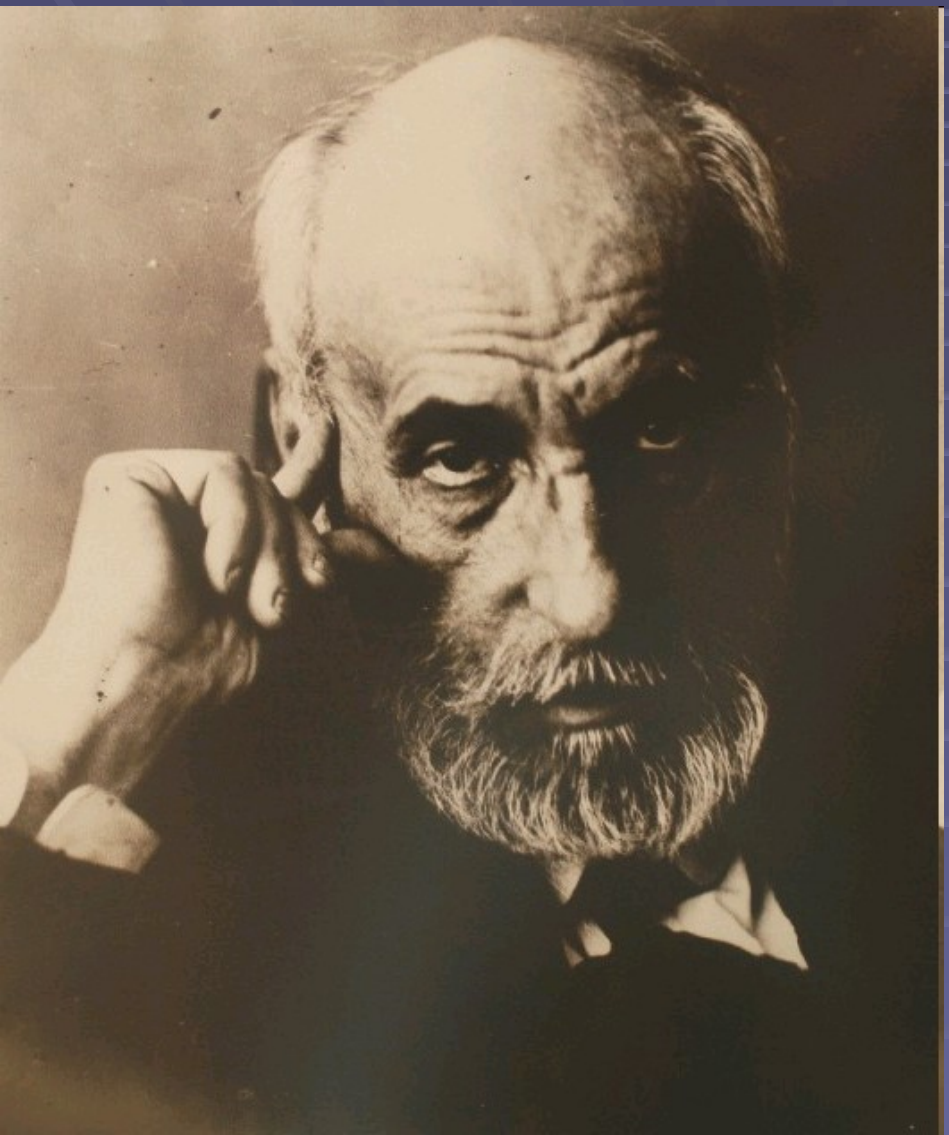


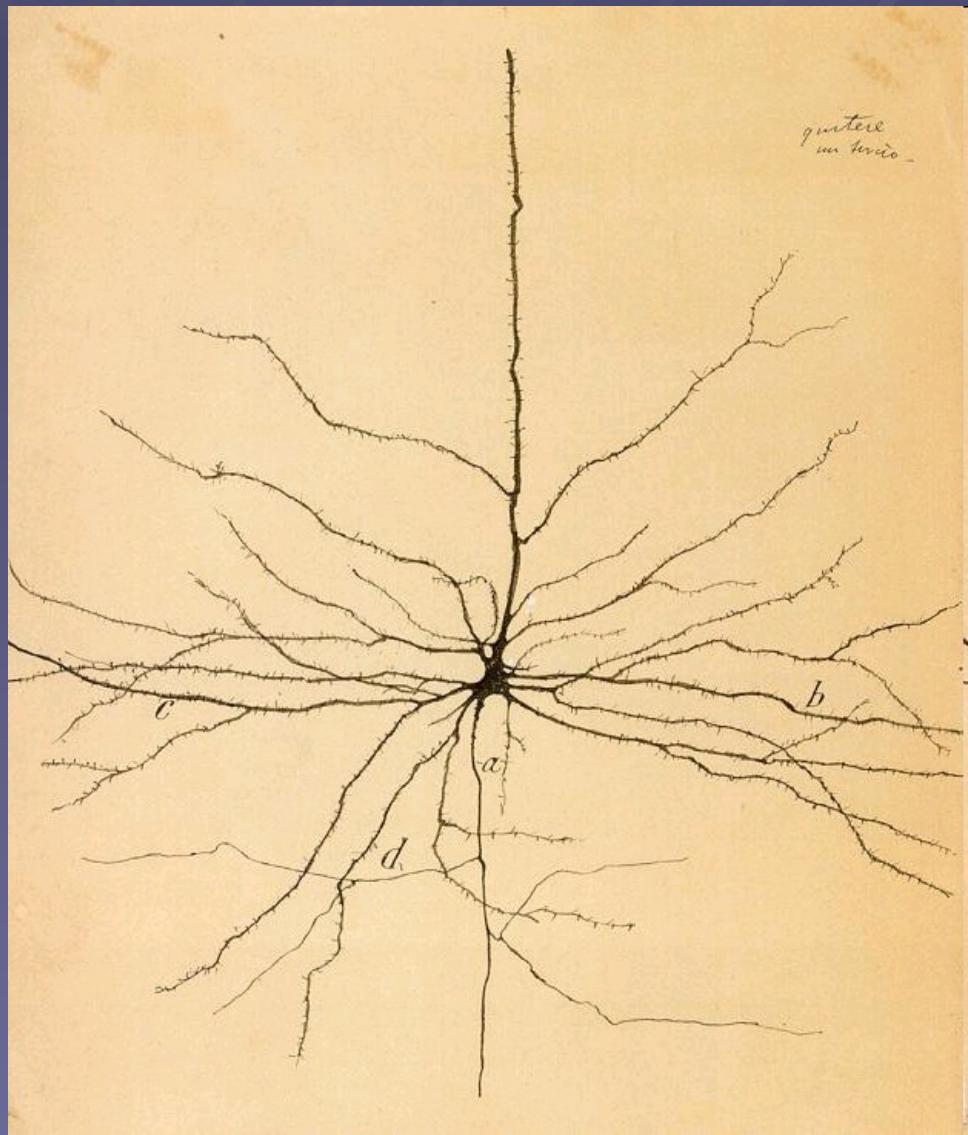
Nerve Cell Cannot Regenerate ?

- In 1913 the great Spanish neuroscientist ***Santiago Ramón y Cajal*** pronounced "that in adult centres the nerve paths are something fixed, ended, immutable. Everything may die, nothing may be regenerated". For many years neuroscientists believed not just that brain damage was irreparable, but also that no process to replace lost neurons existed in our brain.

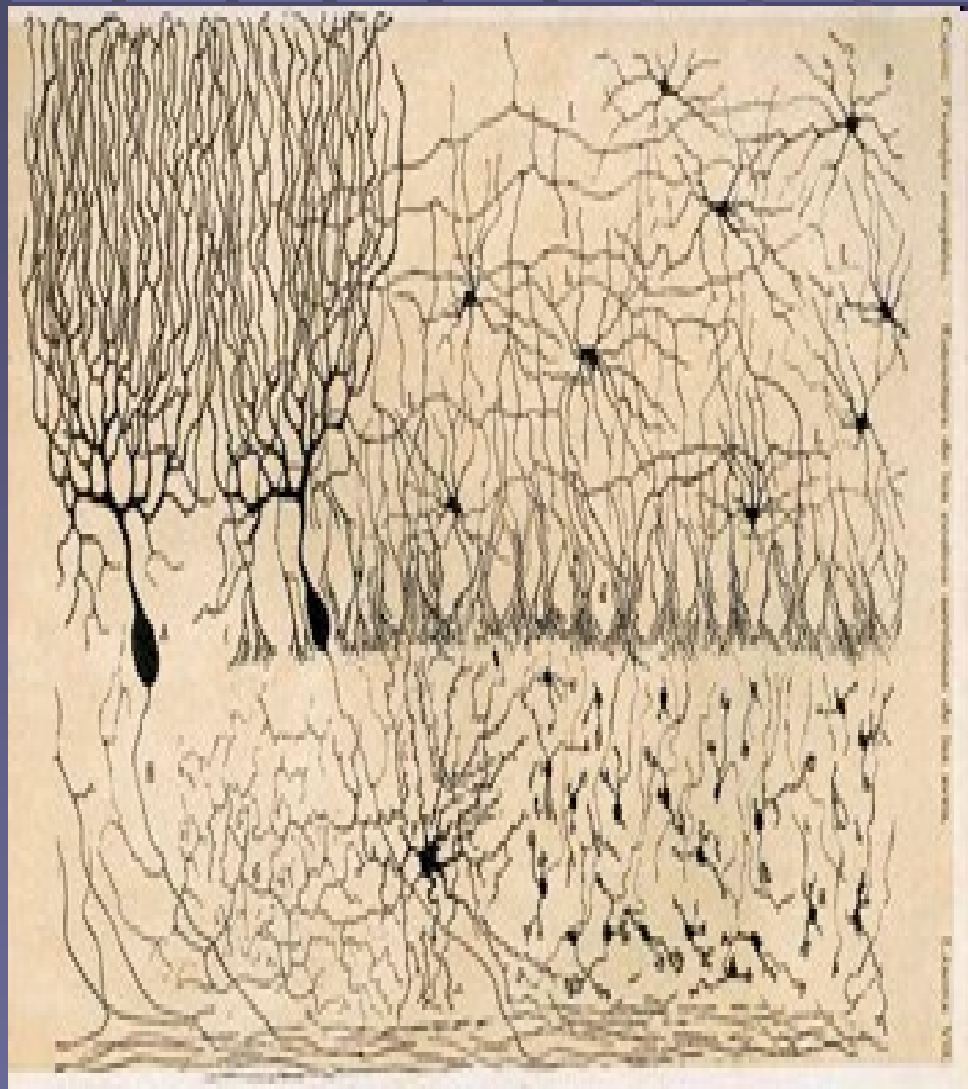


antiago Ramón y Cajal (1852-1934)



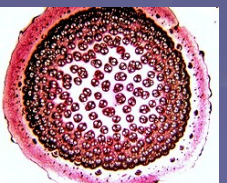


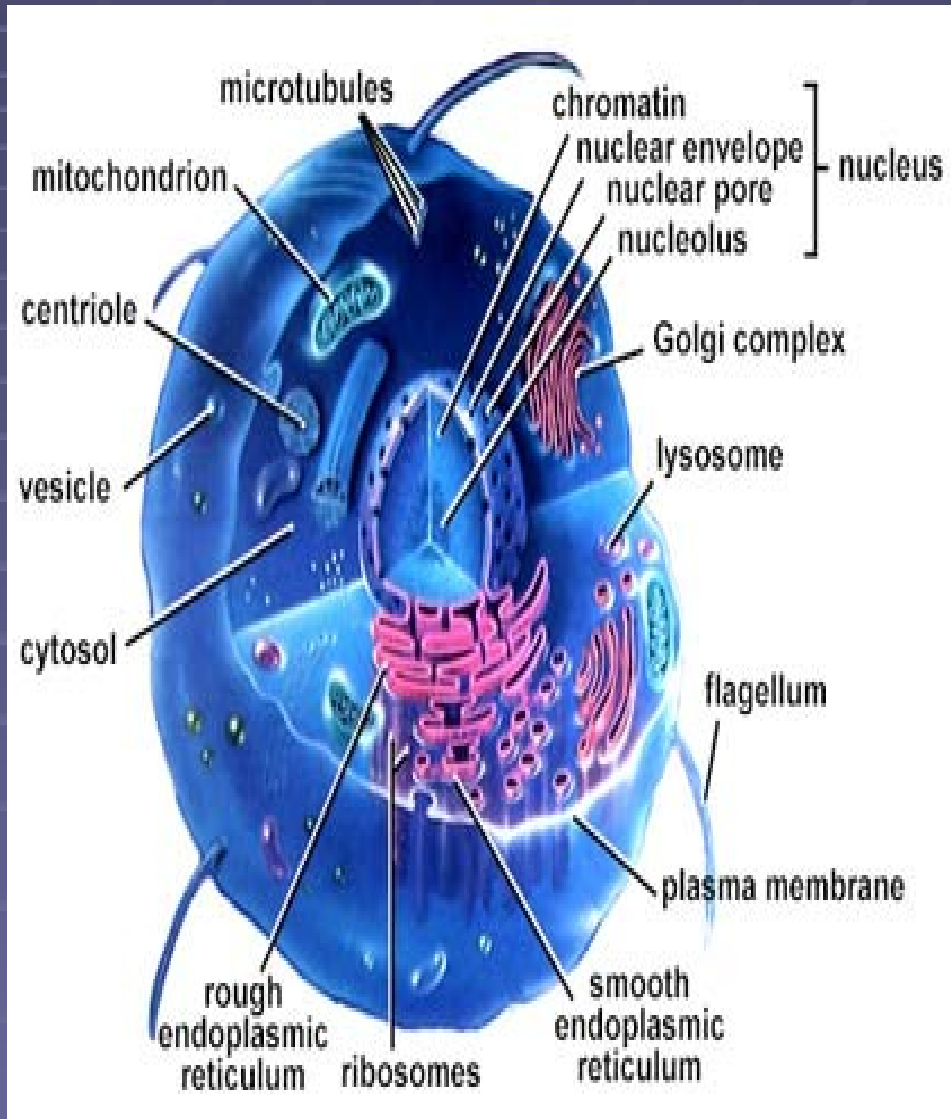
Both beliefs turned out to be false as several types of cells can regenerate



Cell – A complex Organ

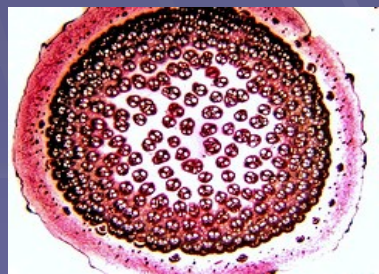
- Cell Theory: all living things are composed of one or more cells
- Cells fall into two basic types prokaryotic and eukaryotic.
- Prokaryotic cells are smaller and lack much of the internal **compartmentalizin and complexity of eukaryotic cells.**



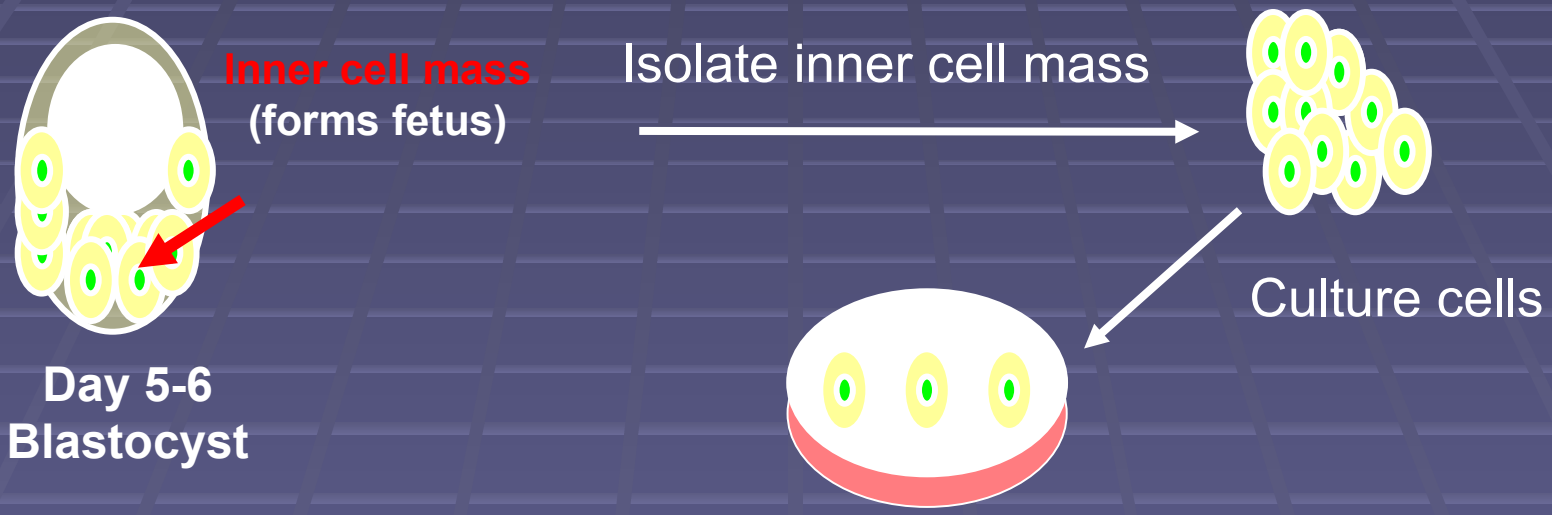


History of Adult Stem Cell Research

- Since the 1970's, **bone marrow** transplants have been used for treatment of Immunodeficient and **leukemia.**



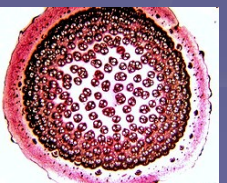
History of Human Embryonic Stem Cell Research



- In 1998, **James Thomson (University of Wisconsin-Madison)** isolated cells from the inner cell mass of the blastocyst, and developed the first human embryonic stem cell line in culture.

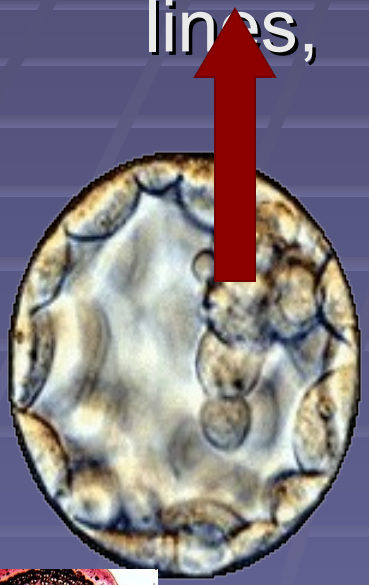
Stem Cell History

- 1998 - Researchers first extract stem cells from human embryos
- 1999 - First Successful human transplant of insulin-making cells from cadavers
- 2001 - President Bush restricts federal funding for embryonic stem-cell research
- 2002 - Juvenile Diabetes Research Foundation International creates $20 million fund-raising effort to support stem-cell research
- 2003?? - California ok stem cell research
- 2004 - Harvard researchers grow stem cells from embryos using private funding
- 2004 - Ballot measure for $3 Billion bond for stem cells

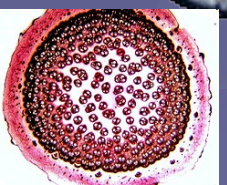


History of Human Embryonic Stem Cell Research

- In 1998, James Thomson (University of Wisconsin-Madison) isolated cells from the inner cell mass of the early embryo, and developed the first human embryonic stem cell lines,

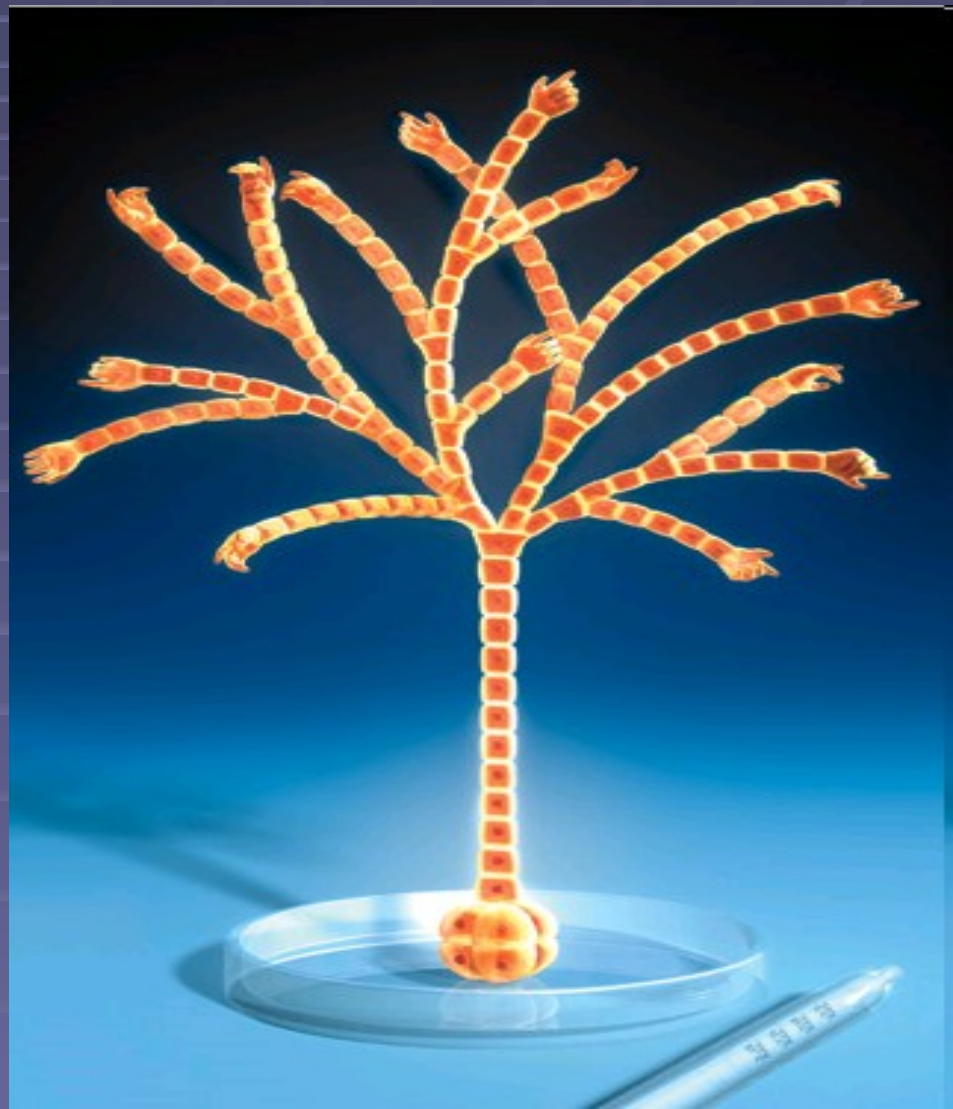


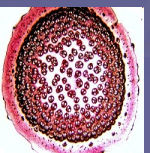
- **In 1998, John Gearhart (Johns Hopkins University) derived human embryonic germ cells from cells in fetal gonadal tissue (primordial germ cells).**
- **Pluripotent stem cell "lines" were developed from both sources**



Stem Cell/Cloning Topics

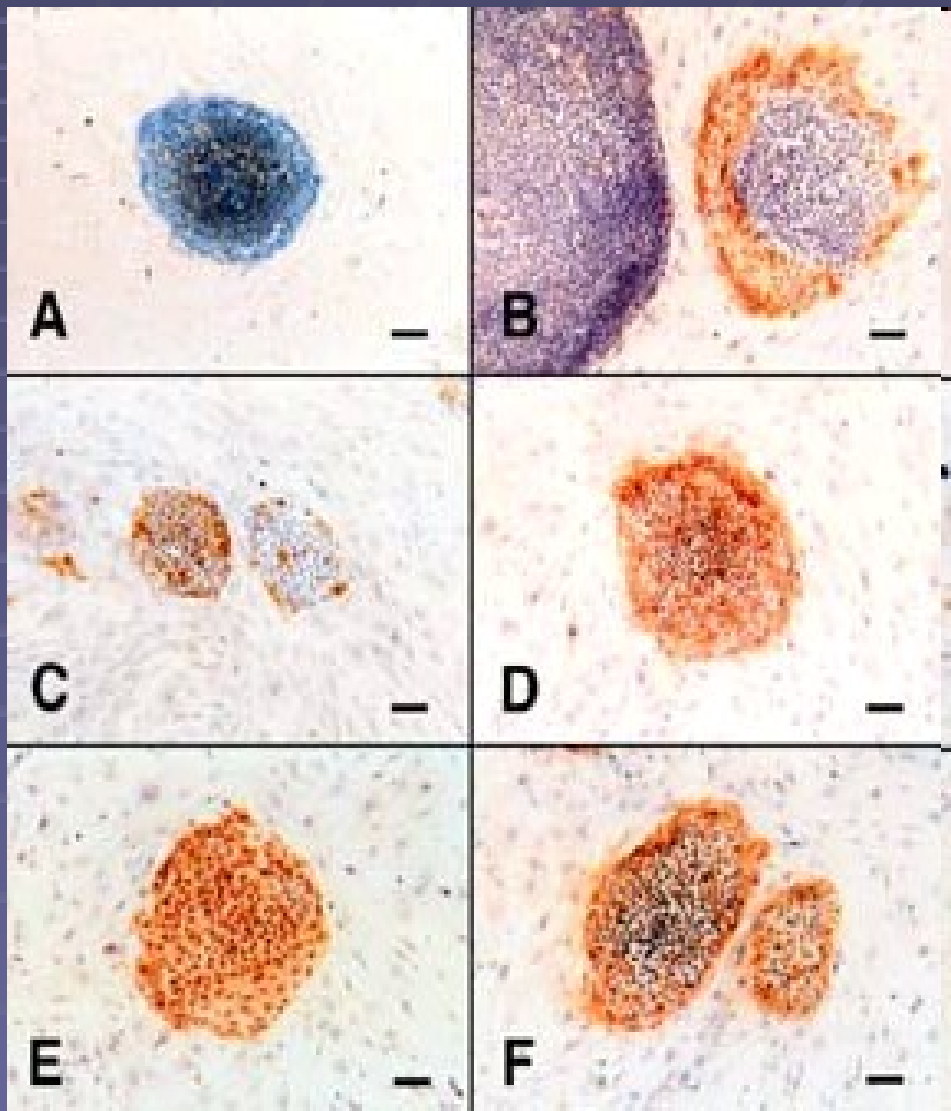
- What are stem cells?
- History of stem cell/cloning research
- Possible uses of the technology
- Current status/knowledge
- Questions and known problems
- Legal considerations
- Politics
- Moral considerations

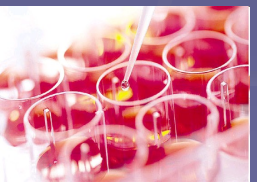




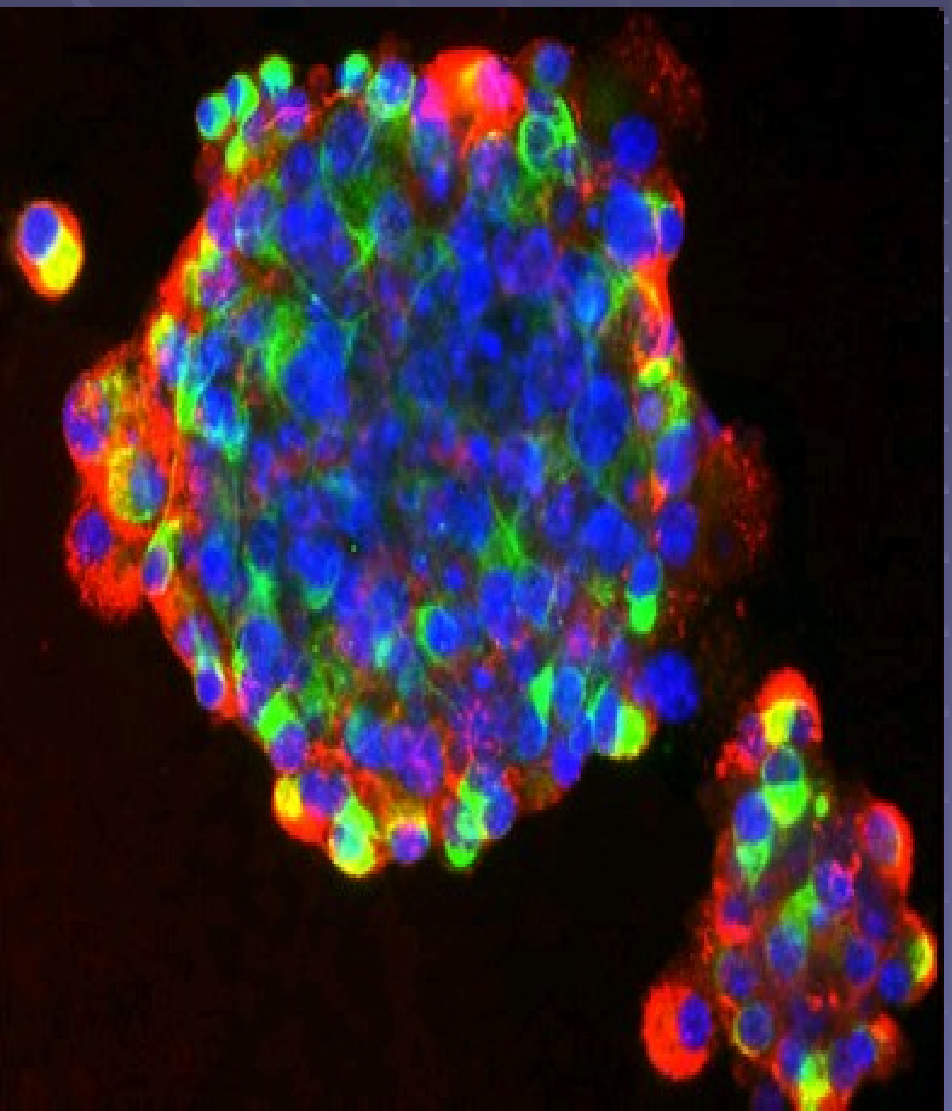
What Are Stem Cells?

- Stem cells are the raw material from which all of the body's mature, differentiated cells are made. Stem cells give rise to brain cells, nerve cells, heart cells, pancreatic cells, etc.





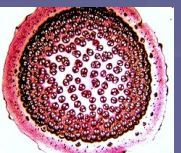
Stem Cell – Definition

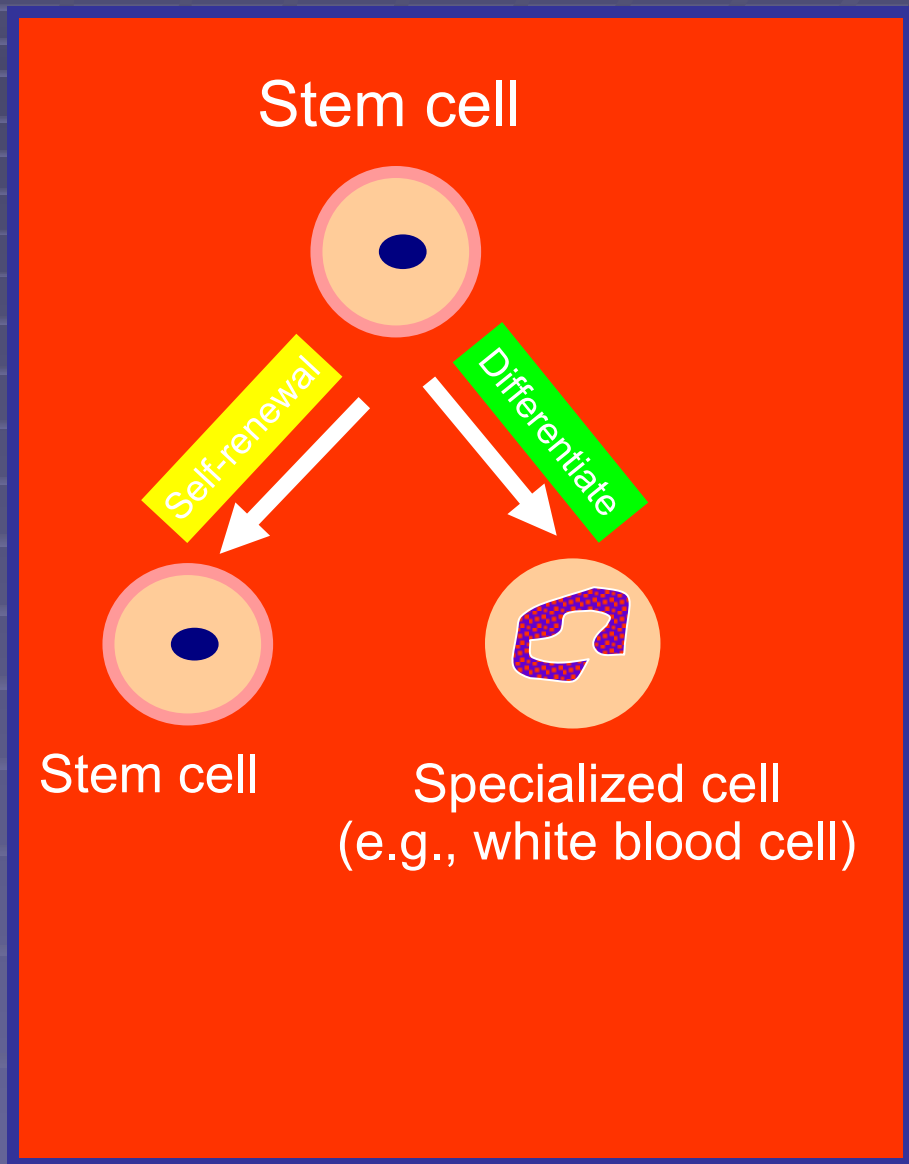


- A cell that has the ability to continuously divide and differentiate (develop) into various other kind(s) of cells/tissues

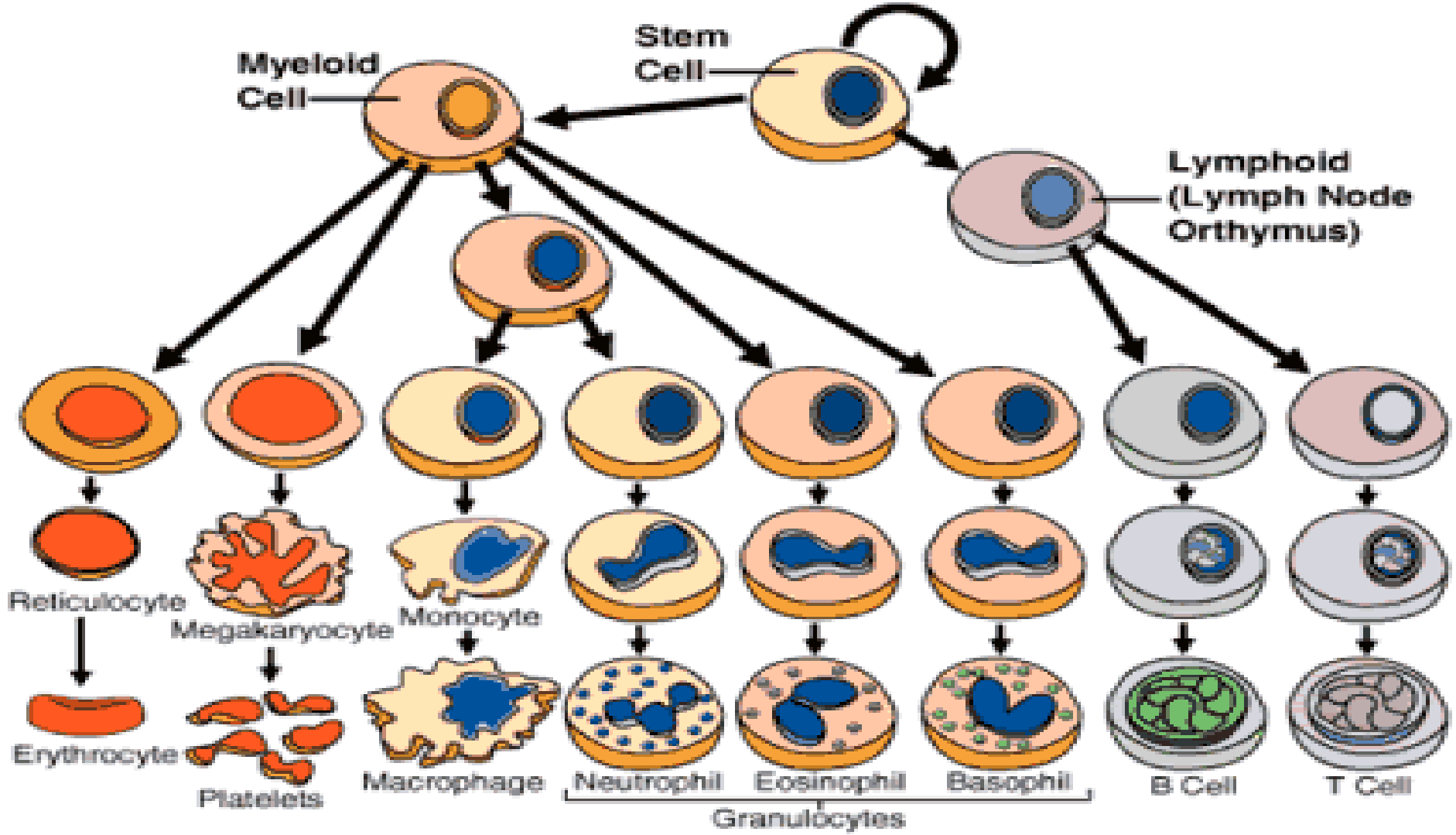
Stem Cell – are Dynamic

- Are undifferentiated "master" cell that do not yet have a specific function
- Can change to one or several different cell types (differentiate) under proper conditions
- Can undergo unlimited cell division, self-renewal)



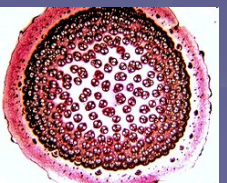


One Cell - Several lineages

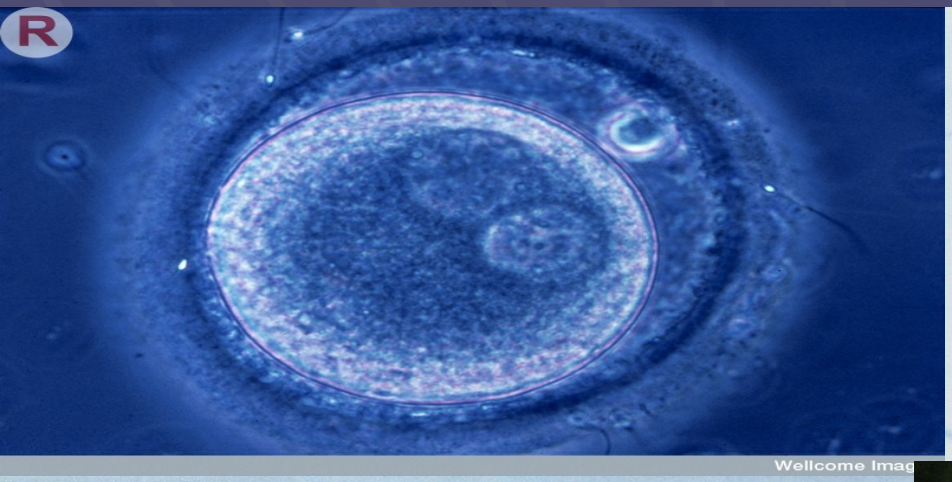


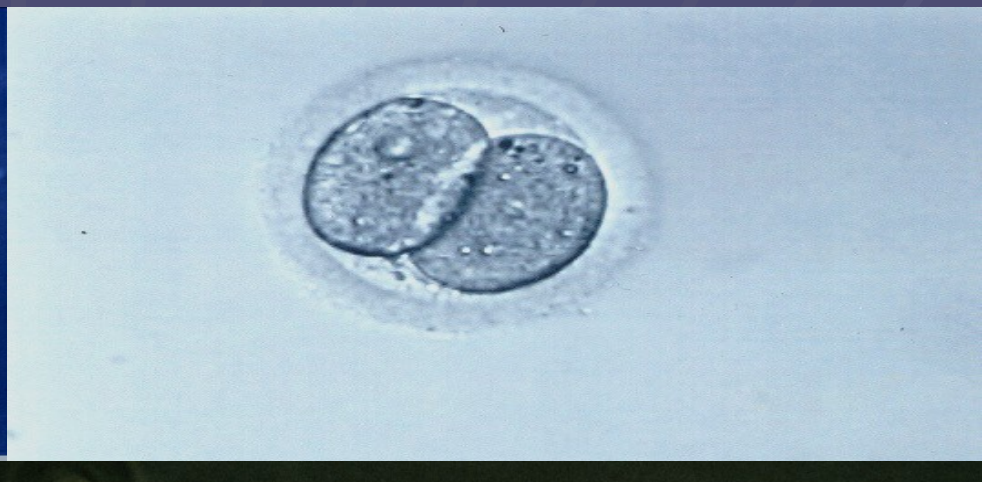
Embryogenesis and Differentiation

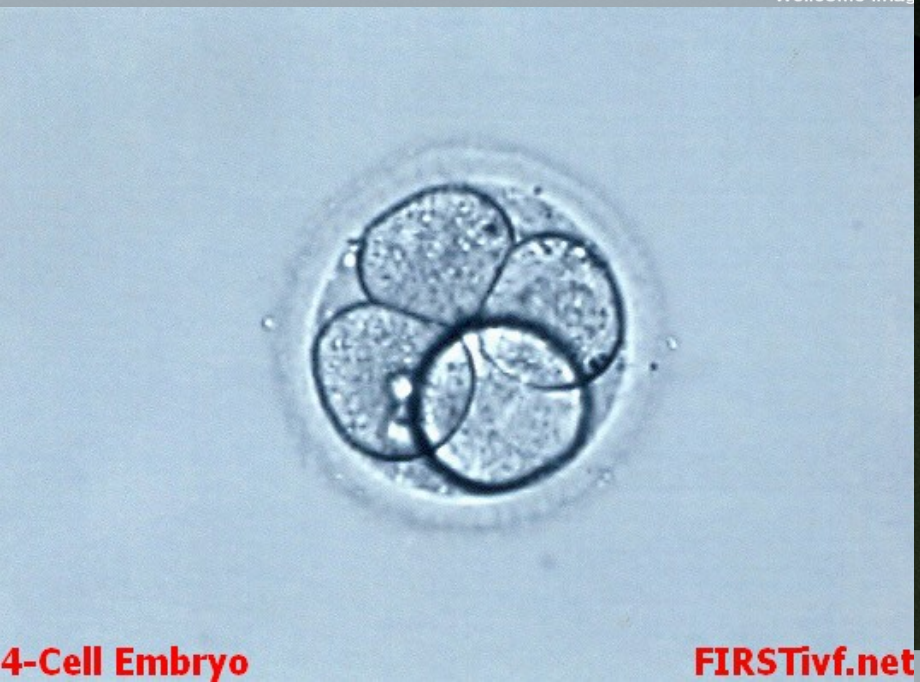
- Specific regions of the embryo give rise to the specific organ systems
  - **Ectoderm** generates the outer layer of the embryo and produces the surface layer (epidermis) of the skin and forms the nerves
  - **Endoderm** becomes the innermost layer of the embryo and produces the digestive tube and its associated organs (including the lungs)
  - **Mesoderm** becomes sandwiched between the ectoderm and endoderm and generates the blood, heart, kidney, gonads, bones, and connective tissues.

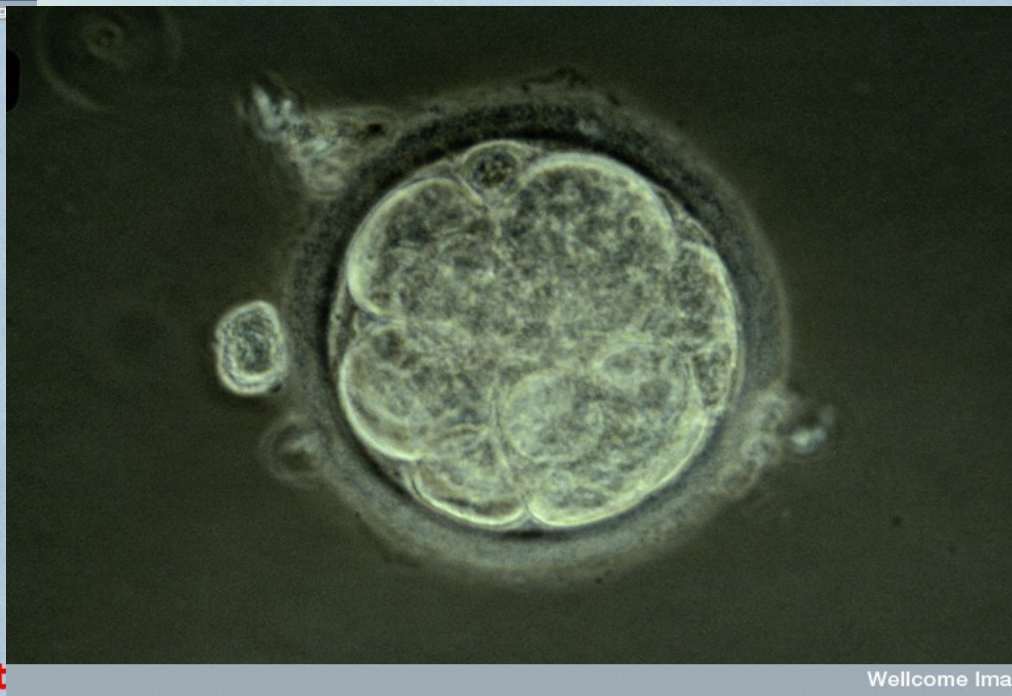


Stages of Development

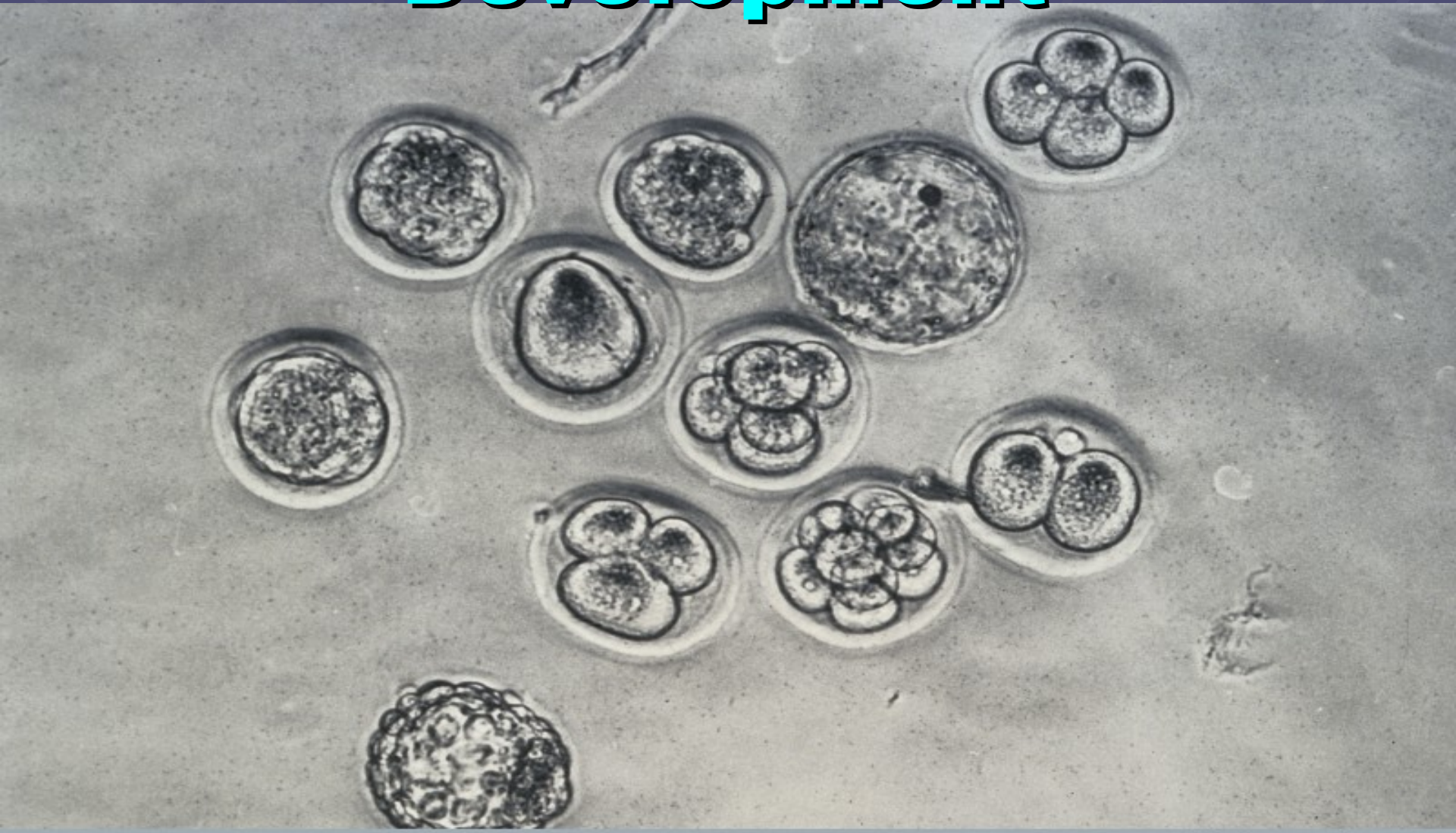








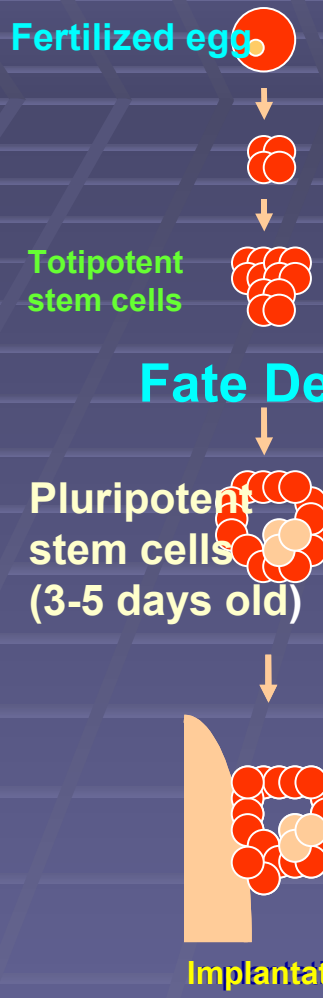
Early Human Development



Wellcome Images

An Overview of Early Development

modeled with Play-Dough



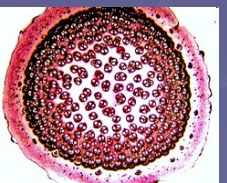
Totipotent: Can become any cell in body or placenta

Pluripotent: Can become any cell in body

Multipotent: Can become any cell within a specific germ layer or cell lineage

Embryonic stem cells come from inner cell mass of blastocyst.

Gastrulation (day 14) leads to Primary Germ Cells
Endoderm (inner)→digestive tract, resp. track
Mesoderm (middle)→bones, blood cells, heart
Ectoderm (outer)→skin, CNS



Blastula

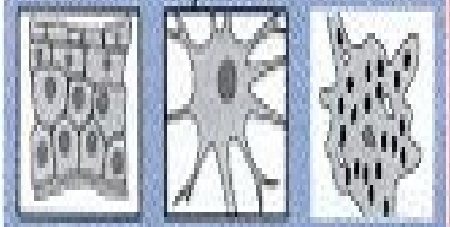
Gastrula

Ectoderm (outer layer)

Outer surface

Central nervous system

Neural crest



Skin cells of epidermis

Neuron of brain

Pigment cell (melan-ocyte)

Mesoderm (middle layer)

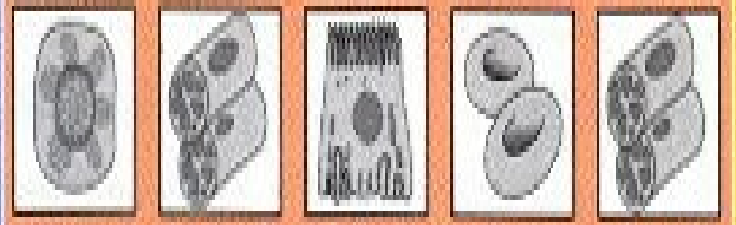
Dorsal

Paraxial

Intermediate

Lateral

Head



Noto-chord

Skeletal muscle cells

Tubule cell of the kidney

Red blood cells

Facial muscle

Endoderm (internal layer)

Digestive tube

Pharynx

Respir-atory tube

Pancreatic cell

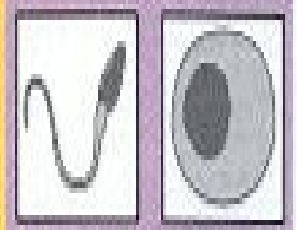
Thyroid cell

Lung cell (alveolar cell)

Germ cells

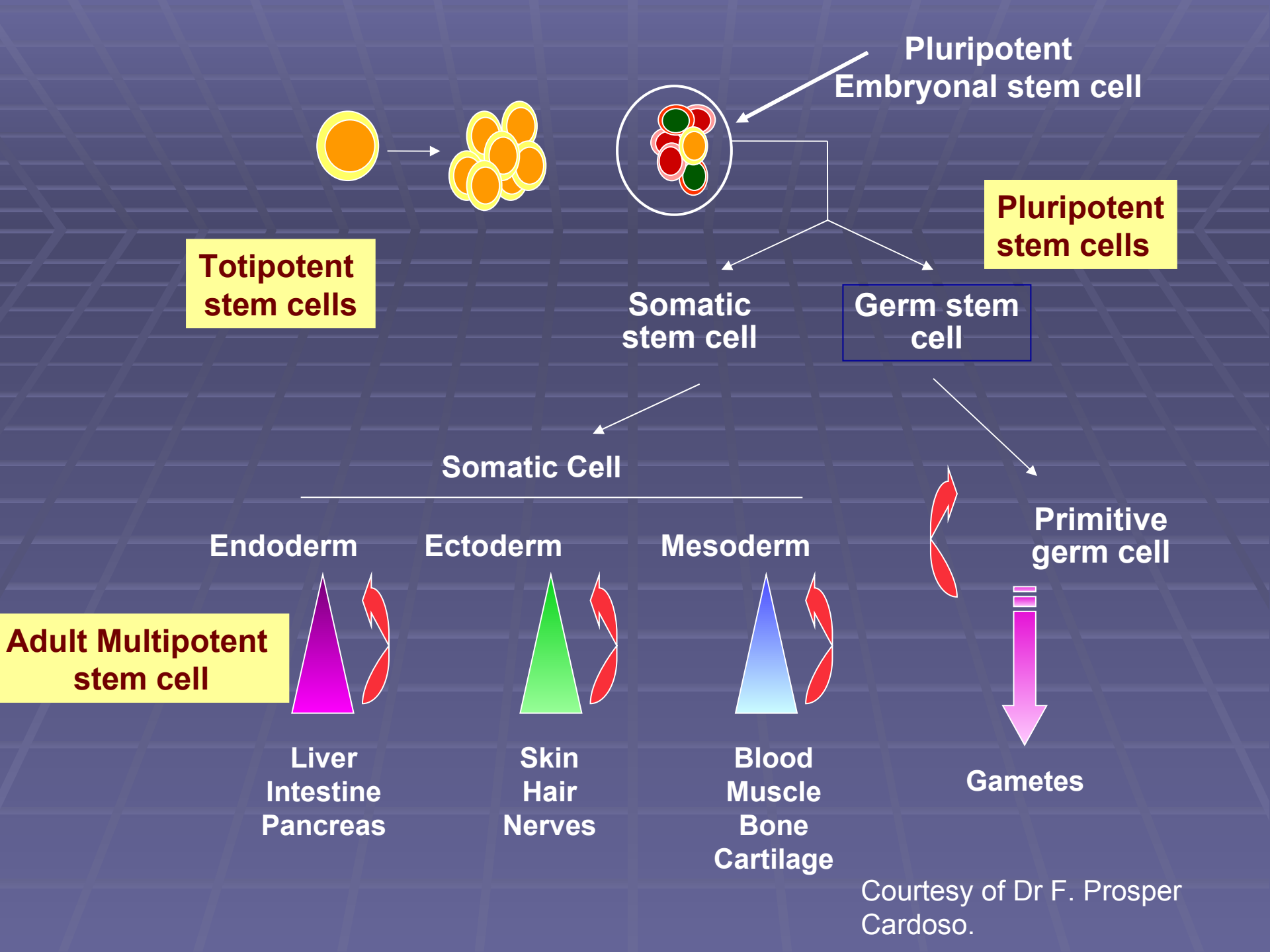
Male

Female



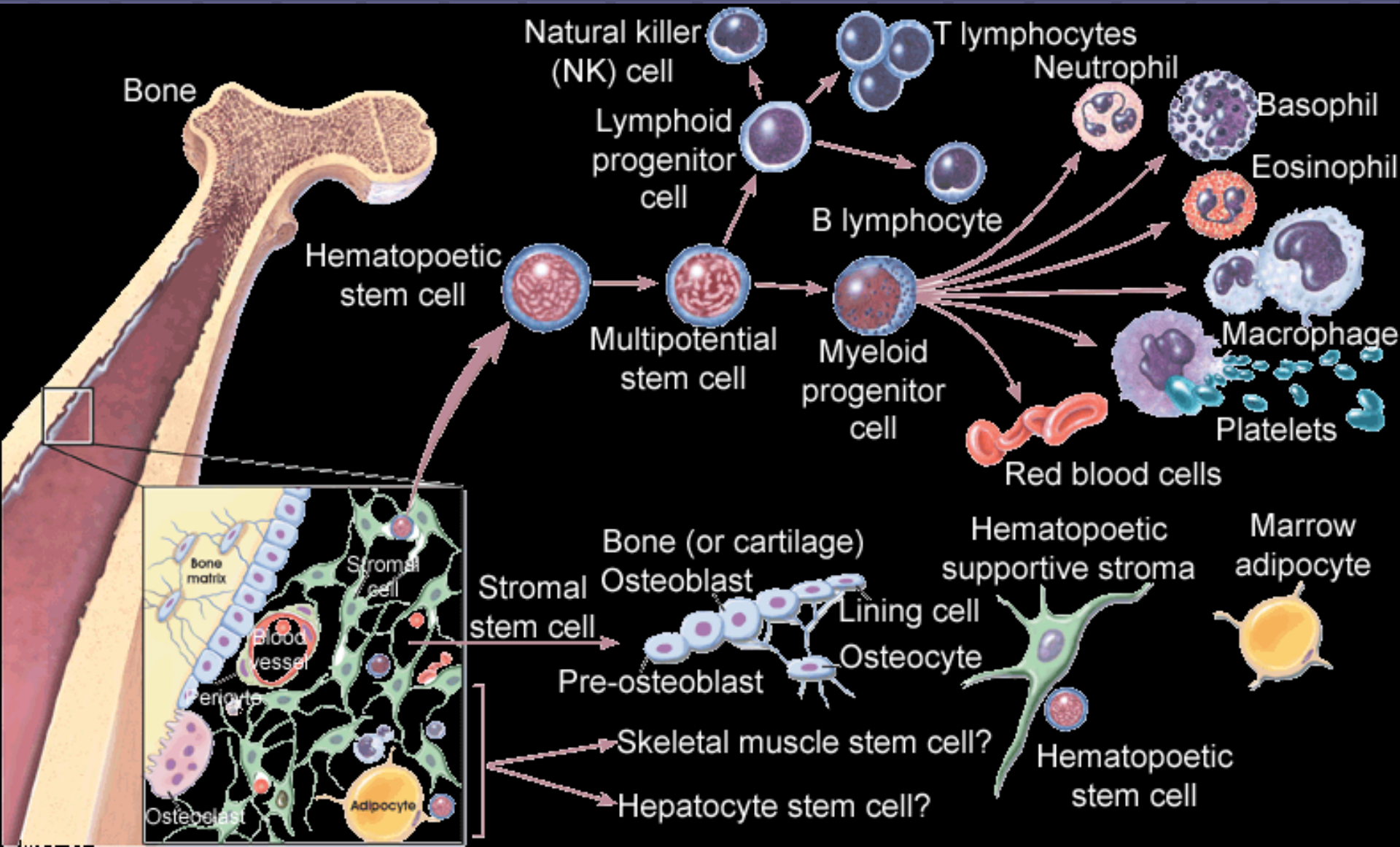
Sperm

Egg



Courtesy of Dr F. Prosper Cardoso.

Bone Marrow Stem Cells

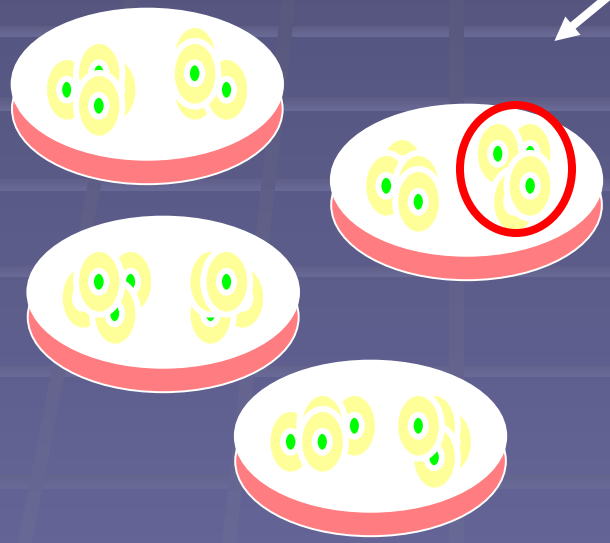


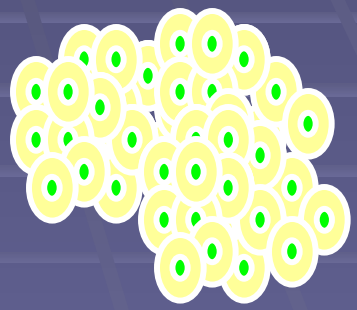
How to Derive an Embryonic Stem Cell Line?

Day 5-6
Blastocyst

Isolate inner cell mass
(destroys embryo)
ETHICS?

Culture cells



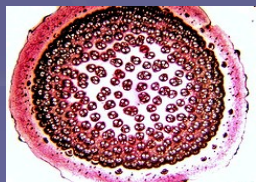


An embryonic stem cell clone

A stem cell line is composed of a population of cells that can replicate themselves for long periods of time *in vitro* (out of the body)

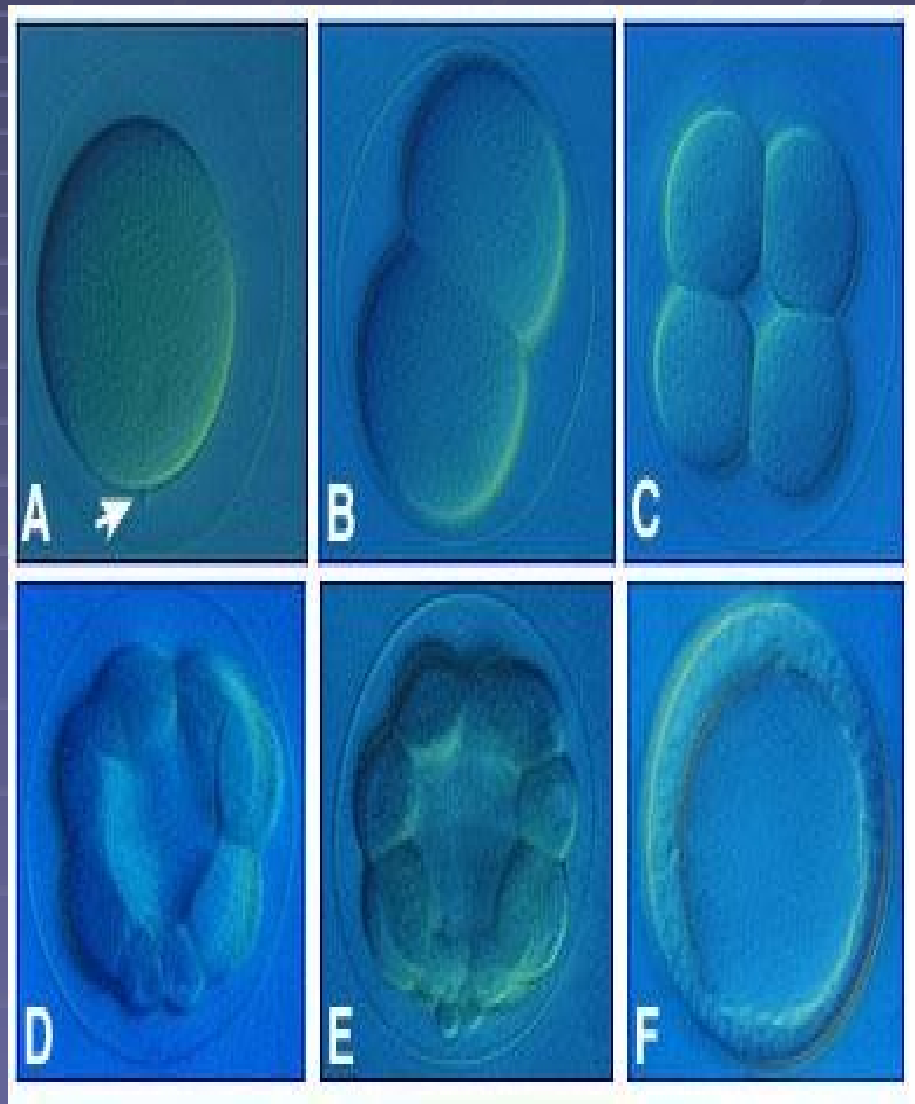
The Science of Stem Cells

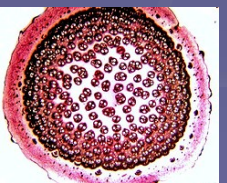
- Stem cells have the ability to continually reproduce themselves while maintaining the capacity to give rise to other more specialized cells.
- Stem cells are found at all stages of development, from embryonic stem (ES) cells that can differentiate into all specialized cells found in the human body, to adult stem cells capable of regenerating their tissue of origin.
- **Stem cells occur from the earliest stages of development and provide the starting material for every organ and tissues**.



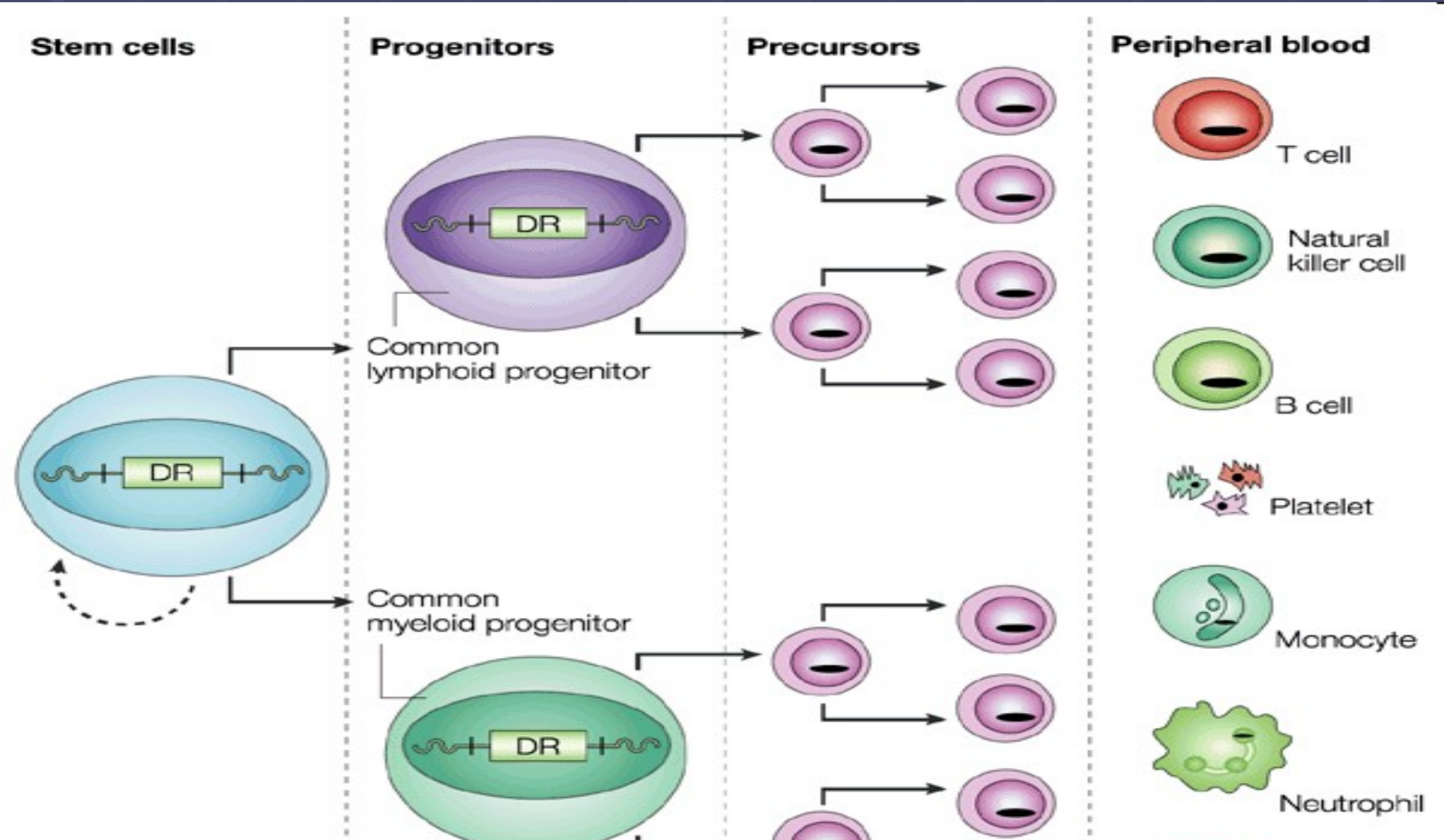
Embryonic stem (ES) cells

- ES cells are found at the blastocyst stage, four to five days after the union of the sperm and egg, before the embryo implants in the uterus.

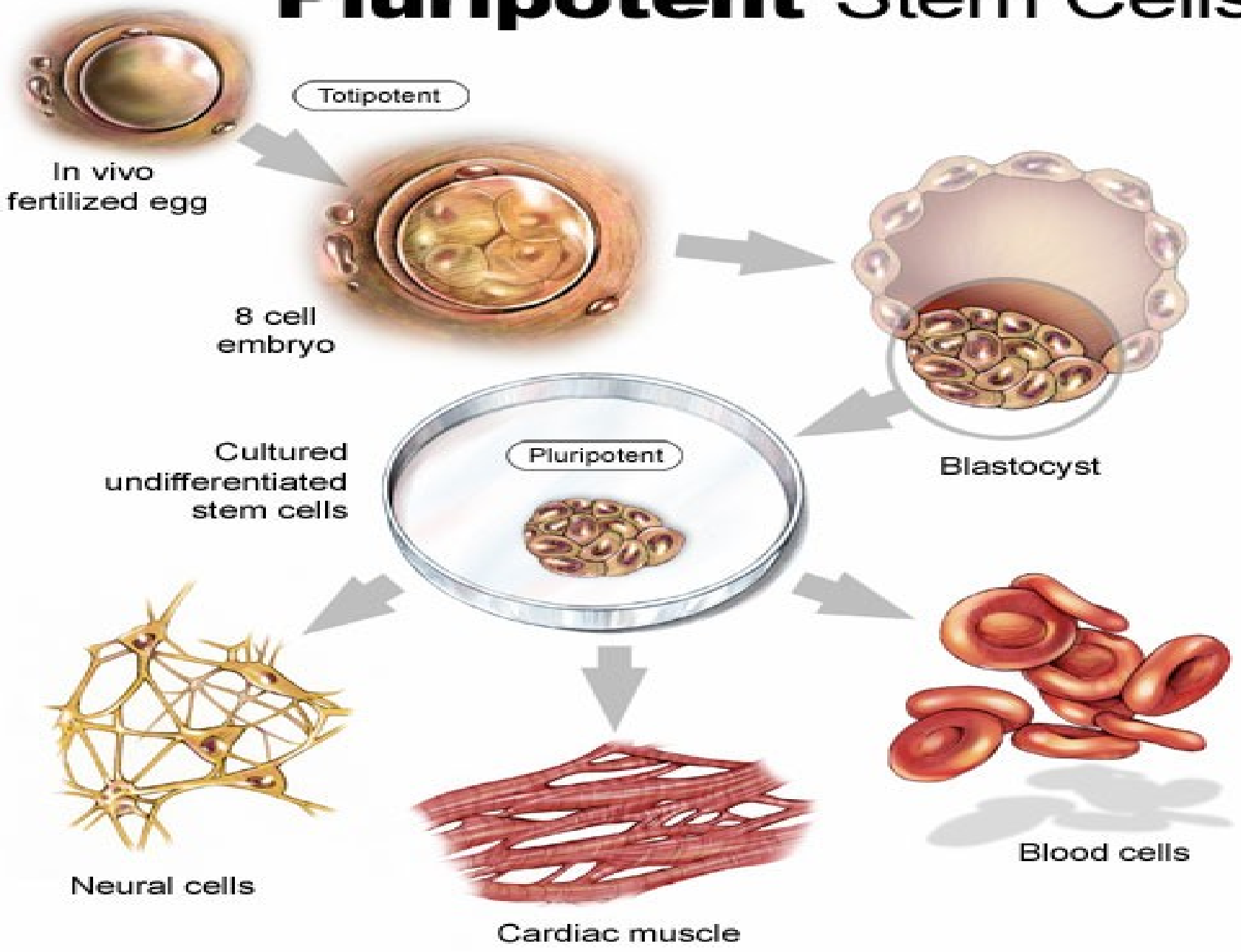




ES Cells are "pluripotent" - i.e. capable of forming embryonic tissues

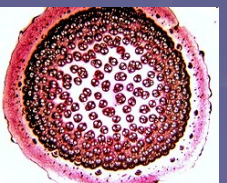


Pluripotent Stem Cells



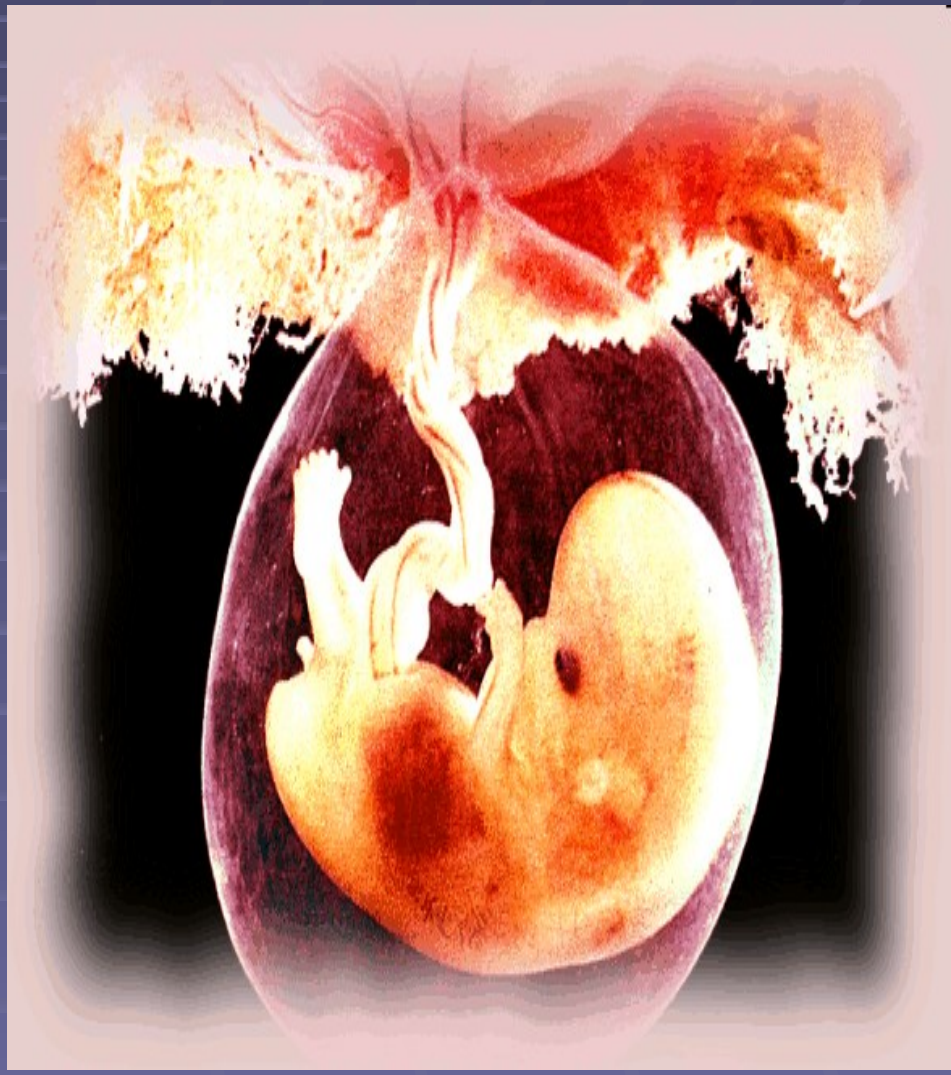
Source of Stem cells

- Stem cells may be derived from autologus, allogeneic or xenogenic sources. Histocompatability is prerequisite for transplantation of allogeneic stem cells. Fatal tissue is the best current tissue source for human neural stem cells, however ethical issues are a major concern.

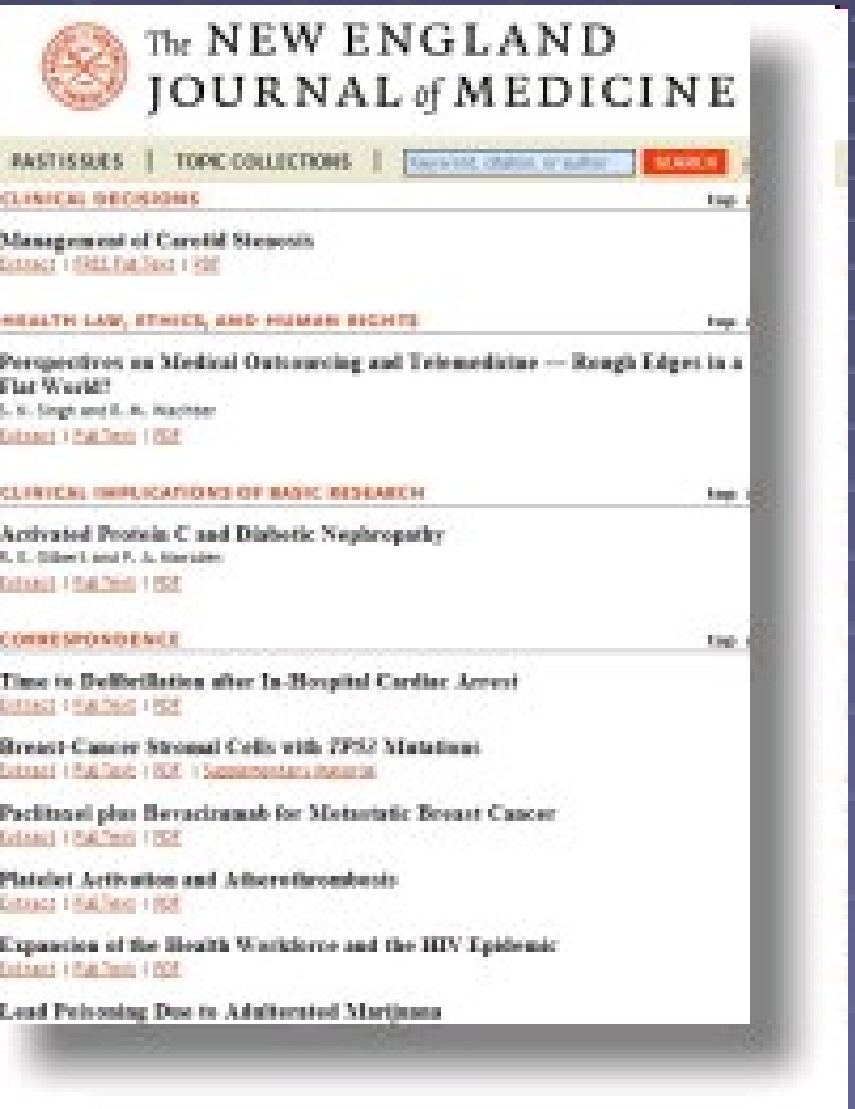


Placenta a Source of Stem Cells

- Placental stem cells, like umbilical cord blood and bone marrow stem cells, can be used to cure chronic blood-related disorders such as sickle cell disease, Thalasemia, and leukaemia.

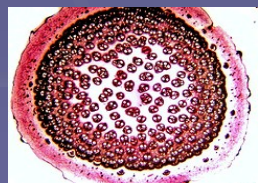


Placental Blood as a Source of Hematopoietic Stem Cells for Transplantation into Unrelated Recipients



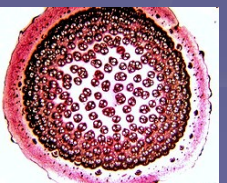
- Report of preliminary results of transplantation using partially HLA-mismatched placental blood from unrelated donors.

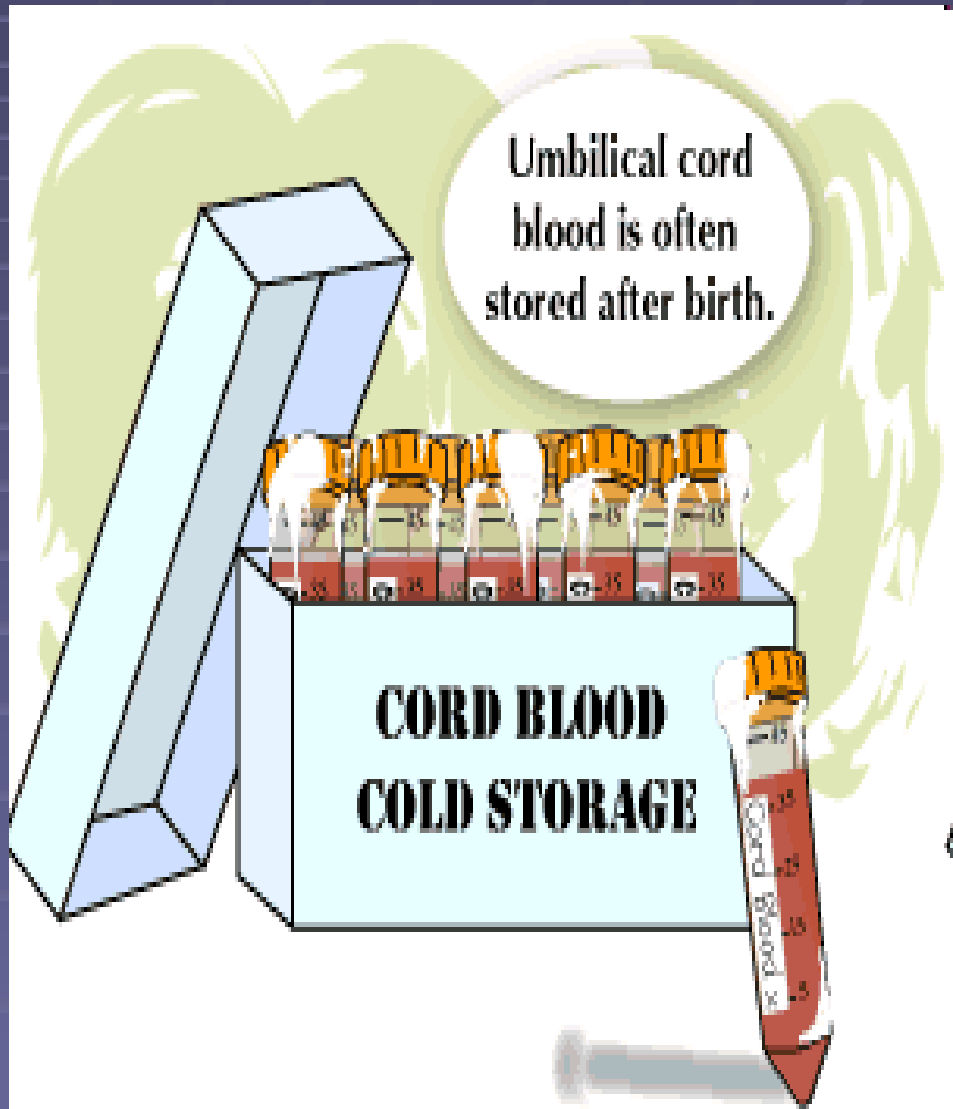
  Joanne Kurtzberg, M.D.et al



Umbilical Cord Blood Stem Cell Transplant

- Umbilical cord blood stem cell transplants are less prone to rejection than either bone marrow or peripheral blood stem cells. This is probably because the cells have not yet developed the features that can be recognized and attacked by the recipient's immune system



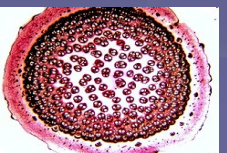


Kinds of Stem Cells

Stem cell type	Description	Examples
Totipotent	Each cell can develop into a new individual	Cells from early (1-3 days) embryos
Pluripotent	Cells can form any (over 200) cell types	Some cells of blastocyst (5 to 14 days)
Multipotent	Cells differentiated, but can form a number of other tissues	Fetal tissue, cord blood, and adult stem cells

What's So Special About Stem Cells?

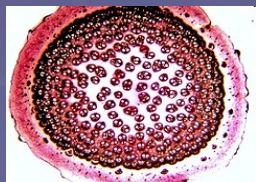
- They have the potential to replace cell tissue that has been damaged or destroyed by severe illnesses.
- They can replicate themselves over and over for a very long time.
- Understanding how stem cells develop into healthy and diseased cells will assist the search for cures.



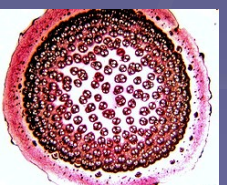
Two Kinds of Stem Cells

- Embryonic (also called “pluripotent”) stem cells are capable of developing into all the cell types of the body.

- Adult stem cells are less versatile and more difficult to identify, isolate, and purify.



Stages of Embryogenesis



Day 1
Fertilized egg

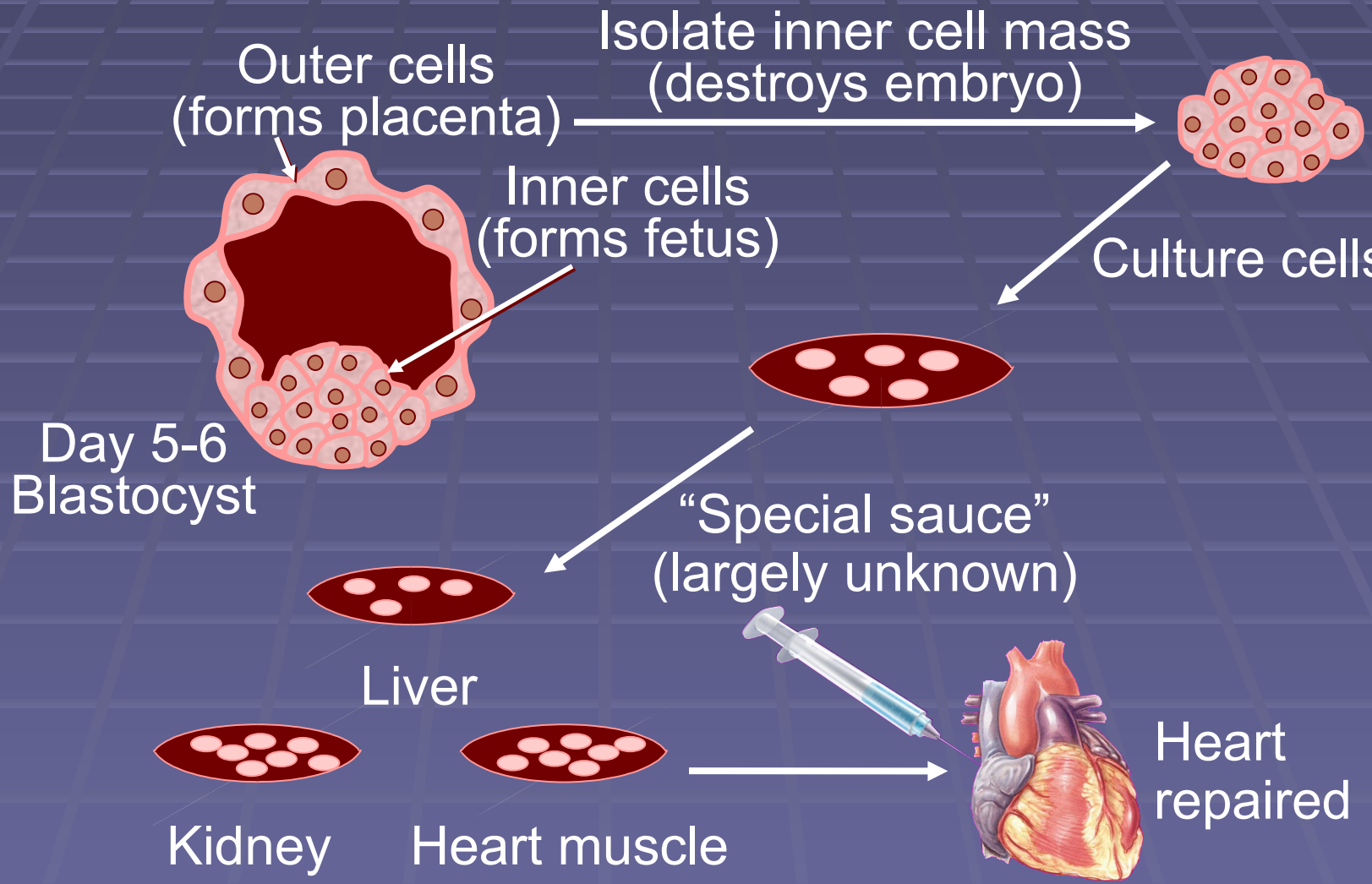
Day 2
2-cell embryo

Day 3-4
Multi-cell embryo

Day 5-6
Blastocyst

Day 11-14
Tissue Differentiation

Derivation and Use of Embryonic Stem Cell Lines

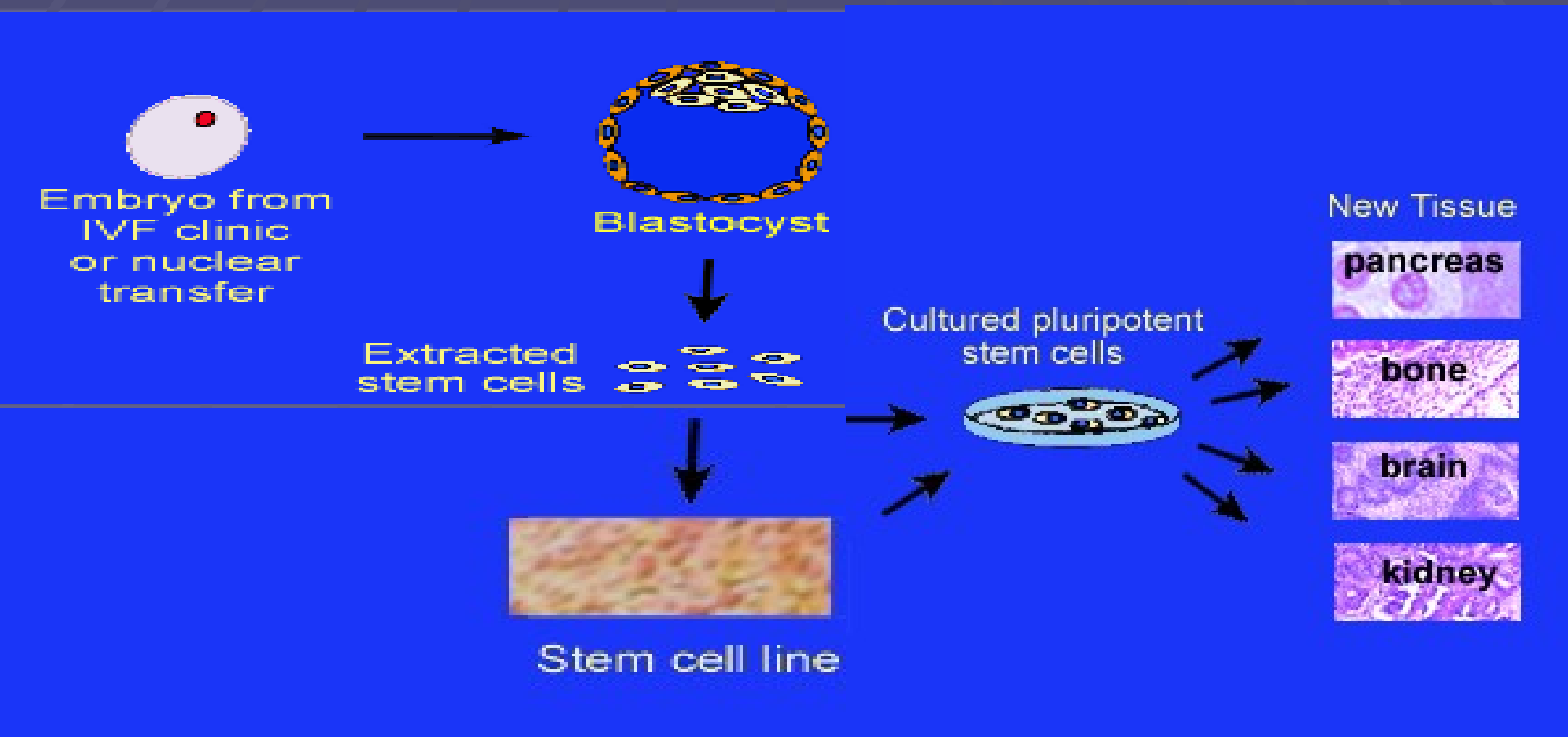


Embryonic Stem Cells:

Researchers extract stem cells from a 5-7 days old blastocyst.

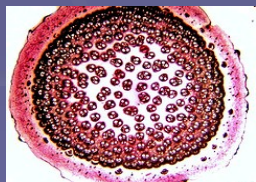
Stem cells can divide in culture to form more of their own kind, thereby creating a stem cell line.

The research aims to induce these cells to generate healthy tissue needed by patients.



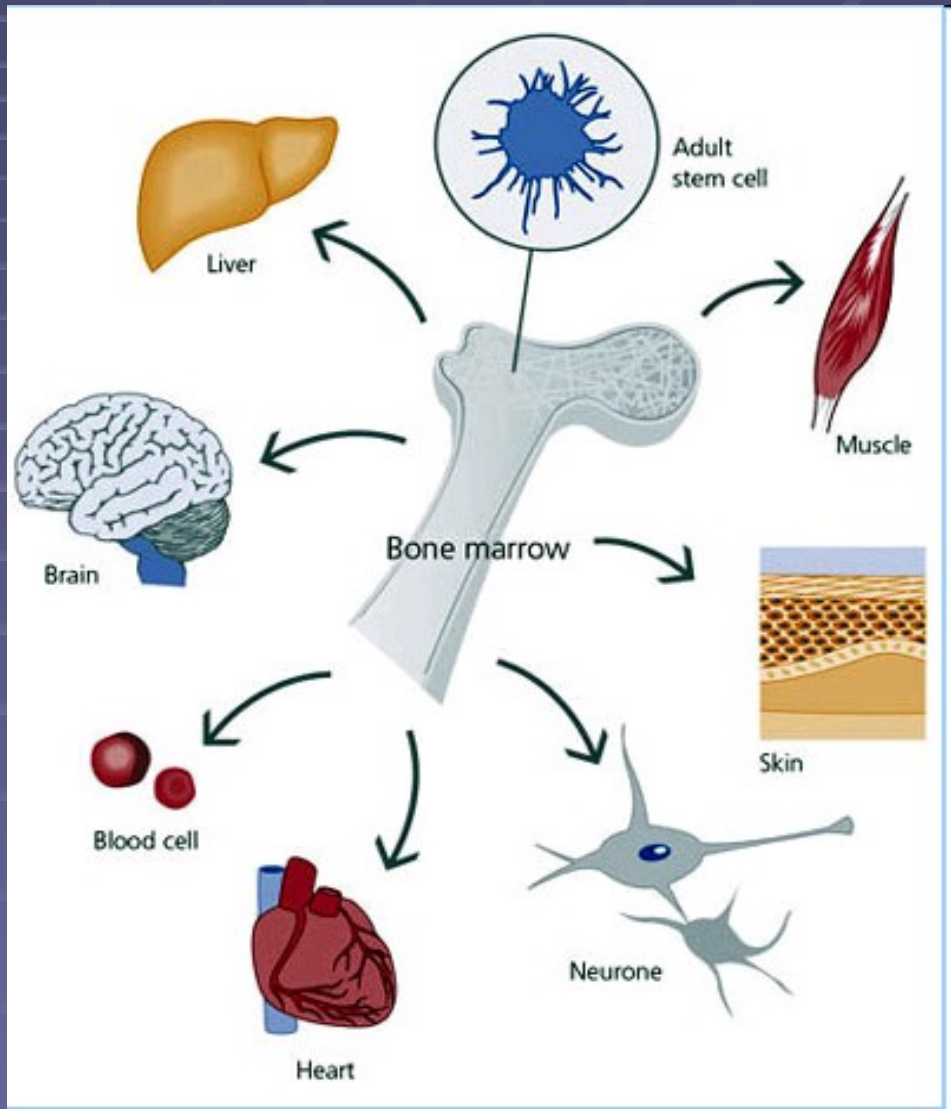
How Many Human Embryonic Stem Cell Lines are There?

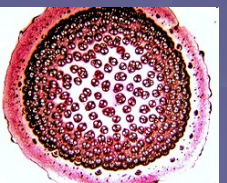
- The actual number of human embryonic stem cell lines is a matter of some debate.
- To date, **more than 100** human embryonic stem cell lines have been derived worldwide.
- However, most of those lines are not adequately characterized yet.
- Only 22 cell lines are eligible for federal funding in the USA.



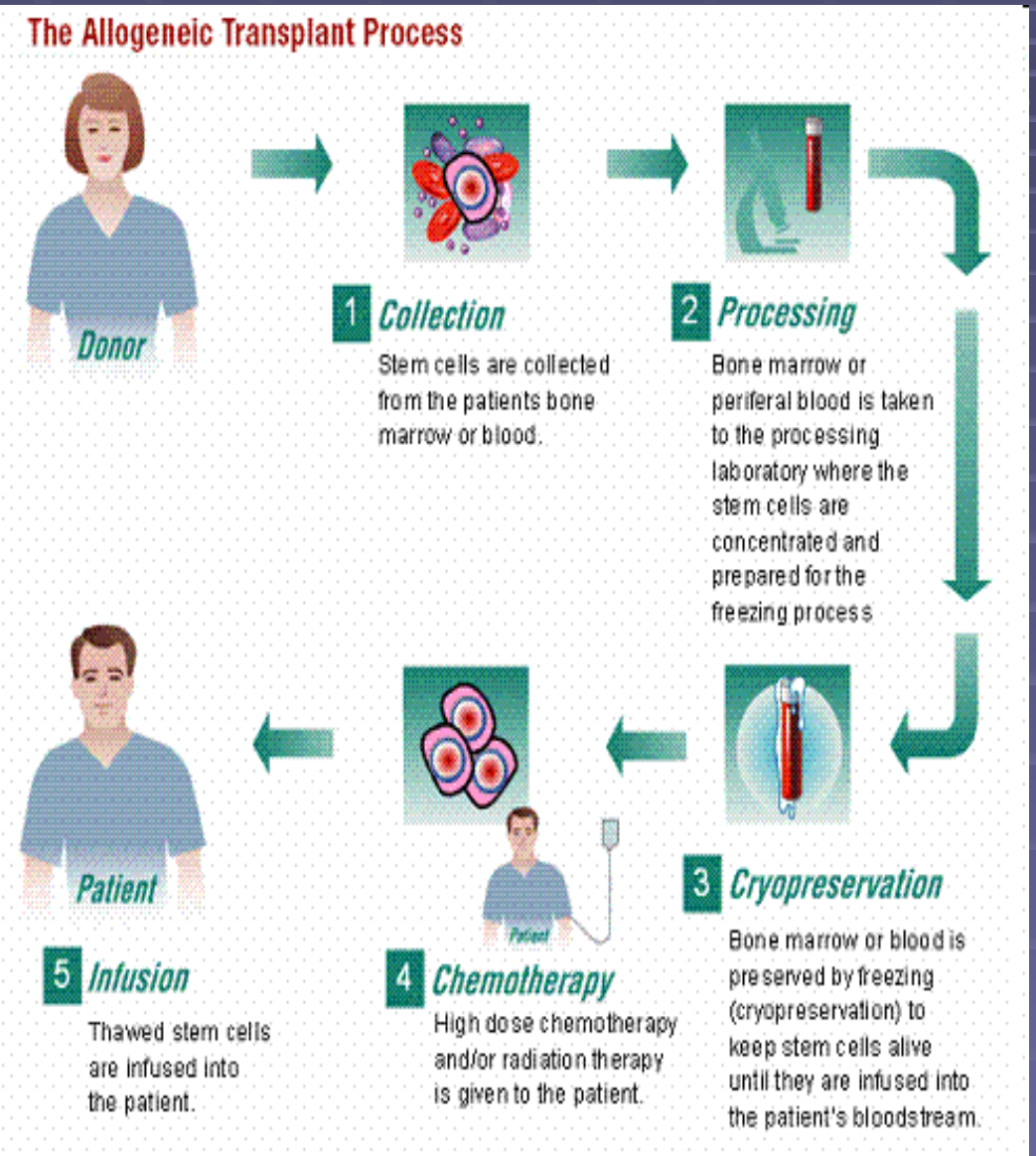
Autologous – Stem Cells

- Sources of the patient's own stem cells (autologous) are either the cells from patient's own body or his or her cord blood. For autologous transplants physicians now usually collect stem cells from the peripheral blood rather than the marrow
- This procedure is easier, unlike a bone marrow harvest, it can take place outside of an operating room and the patient does not have to be under general anaesthesia.





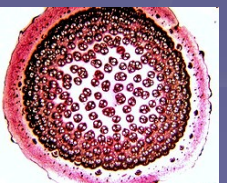
Allogeneic – Stem Cells

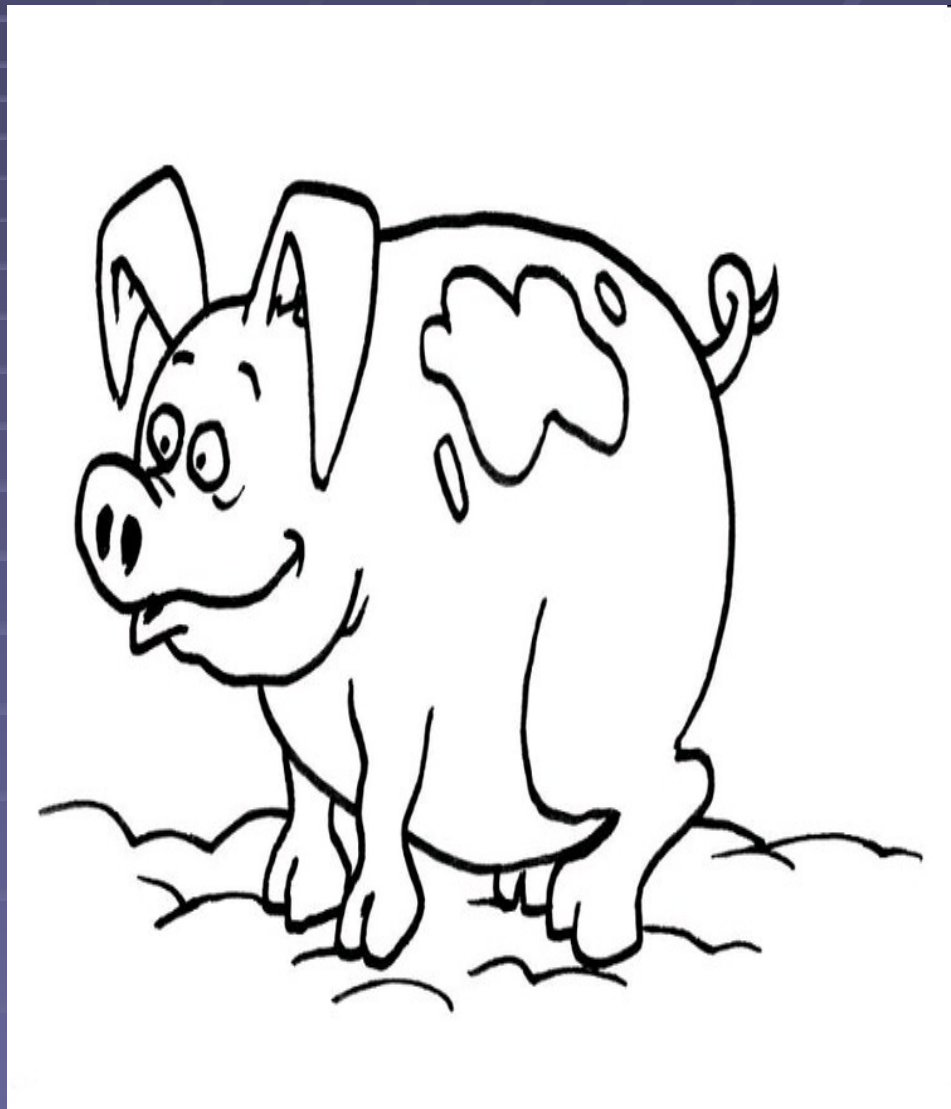


Sources of stem cells from another donor (allogeneic) are primarily relatives (familial-allogeneic) or completely unrelated donors (unrelated-allogeneic). The stem cells in this situation are extracted from either the donor's body or cord blood

Xenogenic - Stem Cells

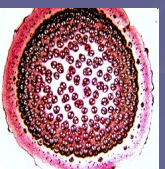
- In this stem cells from different species are transplanted, e.g. striatal porcine fetal ventral mesencephalic (FVM) xenotransplants for Parkinson's disease. This has no major ethical concerns and a large amount of tissue is available, however life long immunosupression and risk of rejection are the major limitations

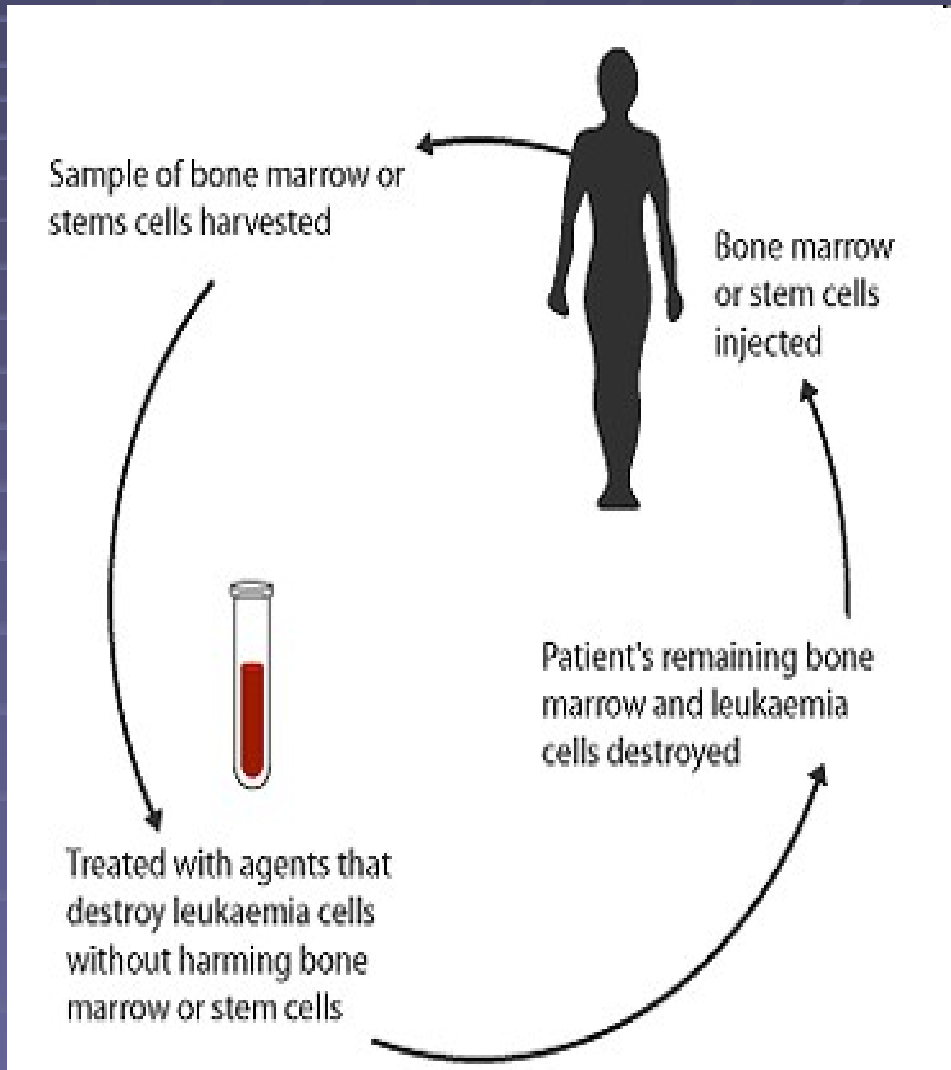




How Does Cell Therapy Work?

- Stem cells can be used to generate healthy and functioning specialized cells, which can then replace diseased or dysfunctional cells.
- It is similar to the process of organ transplantation only the treatment consists of **transplanting cells instead of organs**.

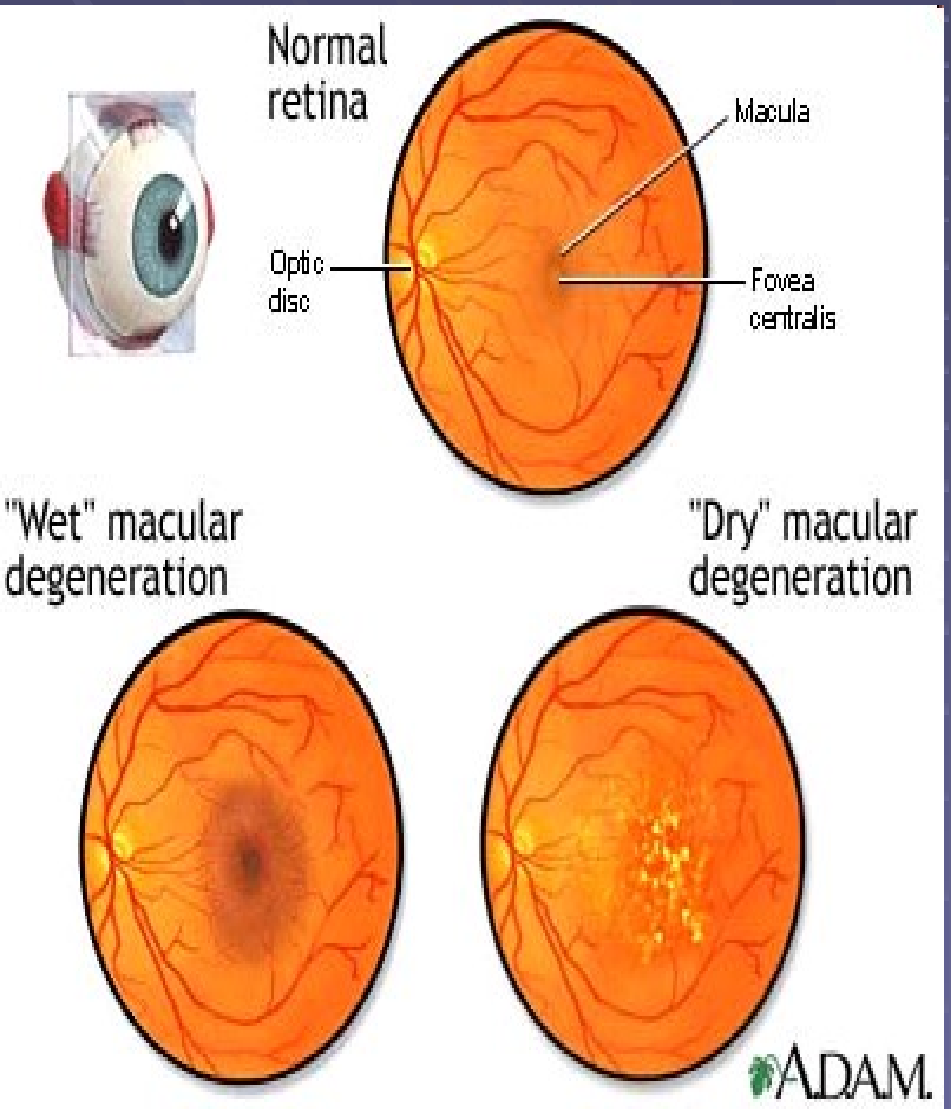




How Does Cell Therapy Work?

- **Bone marrow transplants** are an example of cell therapy in which the stem cells in a donor's marrow are used to replace the blood cells of the victims of leukemia.
- Cell therapy is also being used in experiments to **graft new skin cells** to treat serious burn victims, and to **grow new corneas** for the sight-impaired.
- **In all of these uses, the goal is for the healthy cells to become integrated into the body and begin to function like the patient's own cells.**

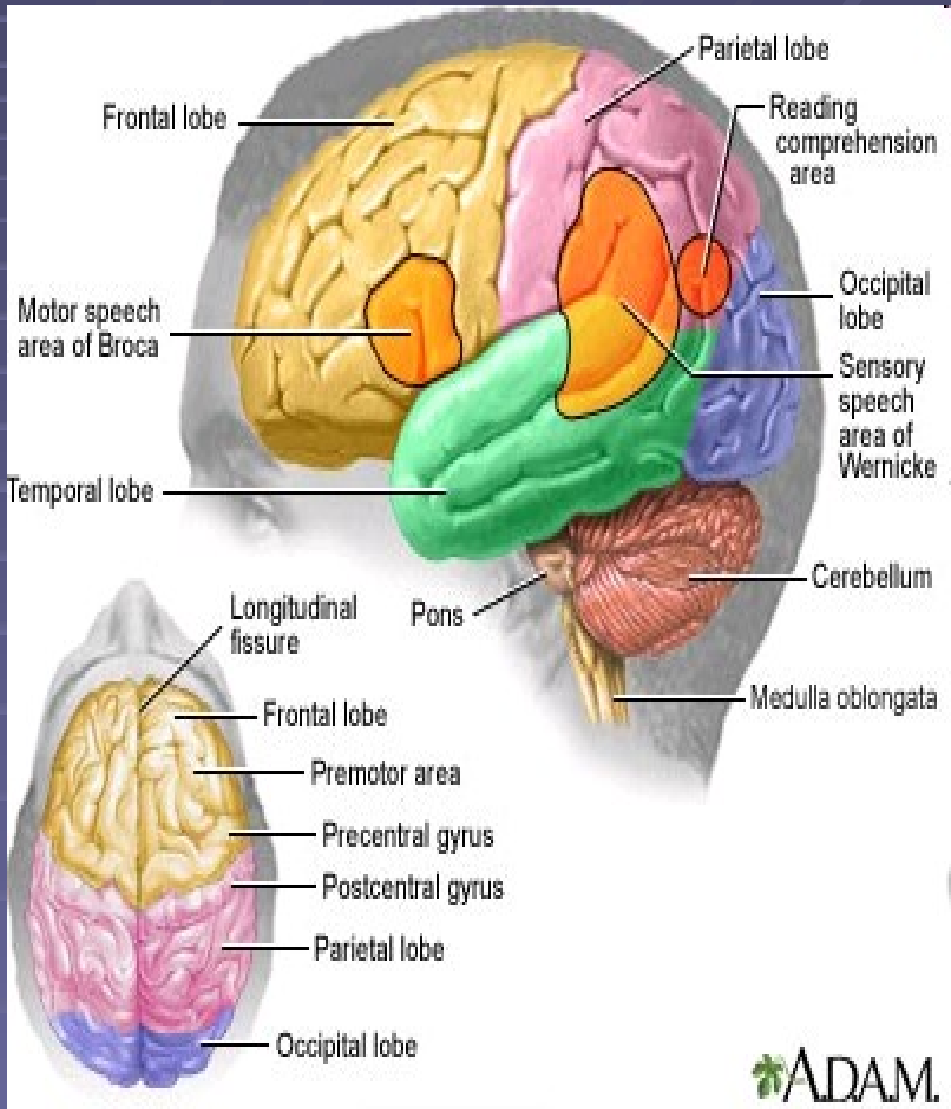
What Diseases Can be Cured by Stem Cell Therapies

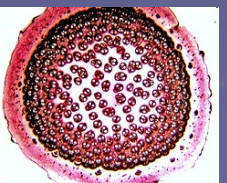


- **Any disease in which there is tissue degeneration can be a potential candidate for stem cell therapies**

Major Progress in Several Important Health problems

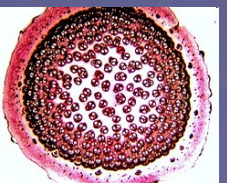
- *Alzheimer's disease*
- Parkinson's disease
- *Spinal cord injury*
- Heart disease
- *Severe burns*
- Diabetes

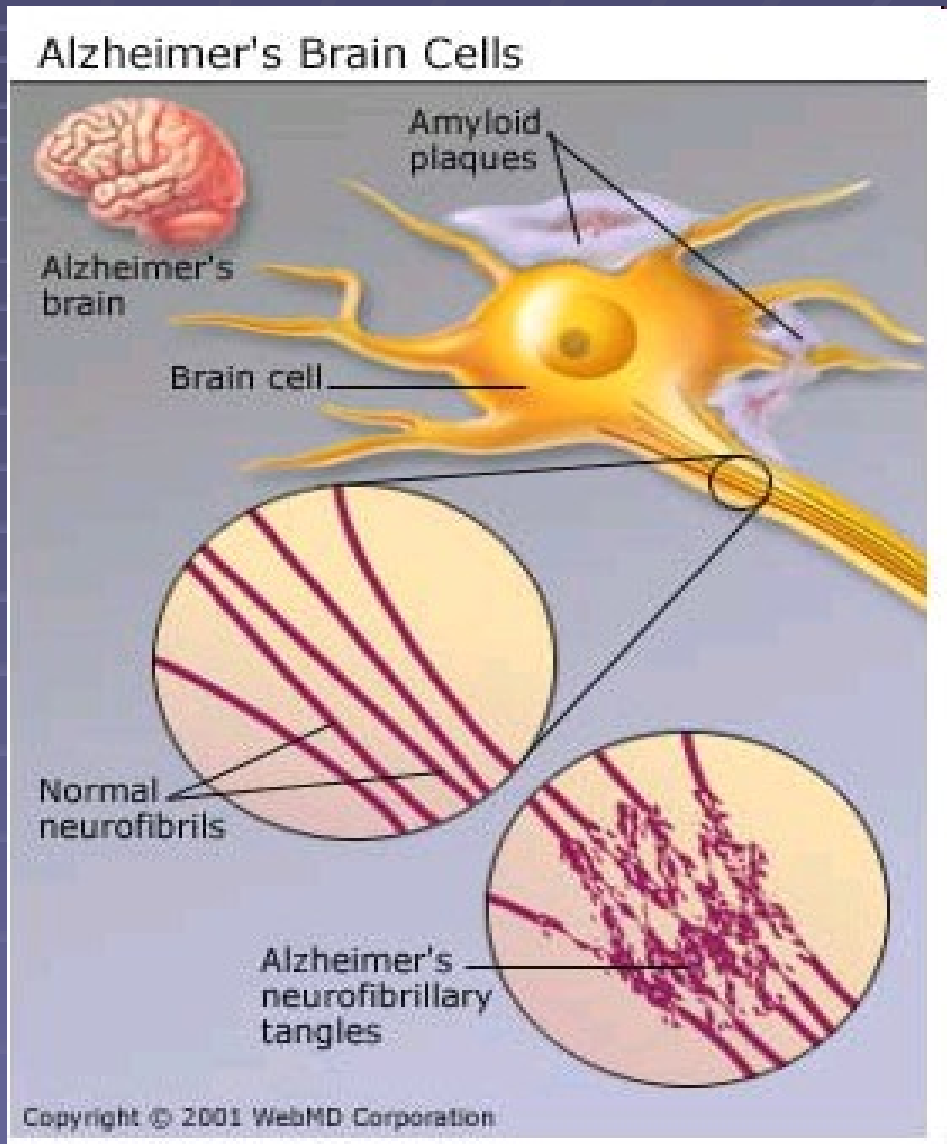




Alzheimer's disease and can stem cells help?

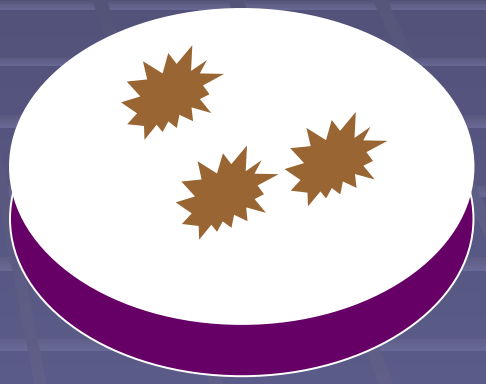
- Stem cells could, however, be genetically modified so as to deliver substances to the Alzheimer brain, to stop cells from dying and stimulate the function of existing cells. A recent clinical trial (Phase I) has shown this approach to be of some benefit to patients with Alzheimer's disease, by slowing down the progression of the disease.

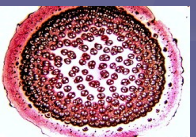




Drug Testing

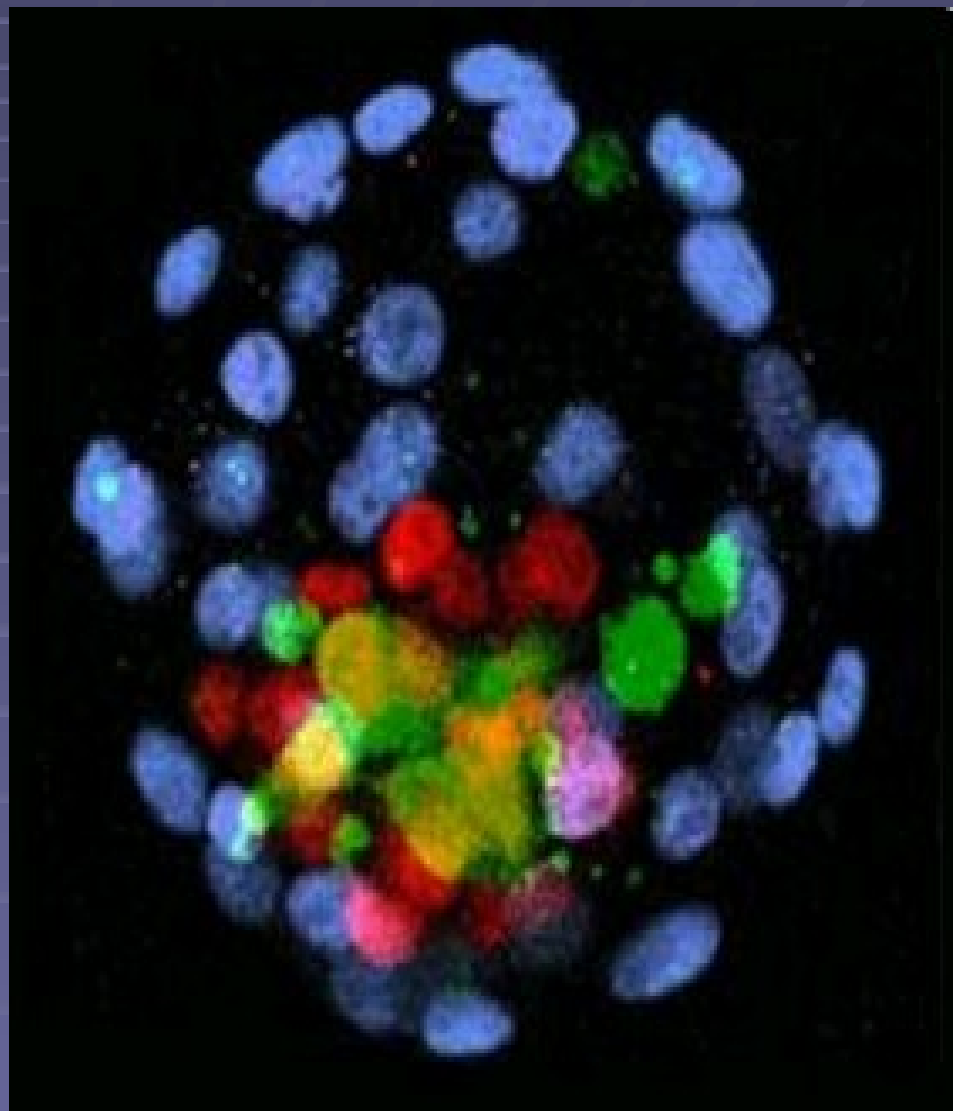
- Stem cells could allow scientists to test new drugs using human cell line which could speed up new drug development.
- Only drugs that were safe and had beneficial effects in cell line testing would graduate to whole animal or human testing.
- It would allow quicker and safer development of new drugs.

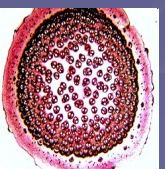




Major types of Stem Cells

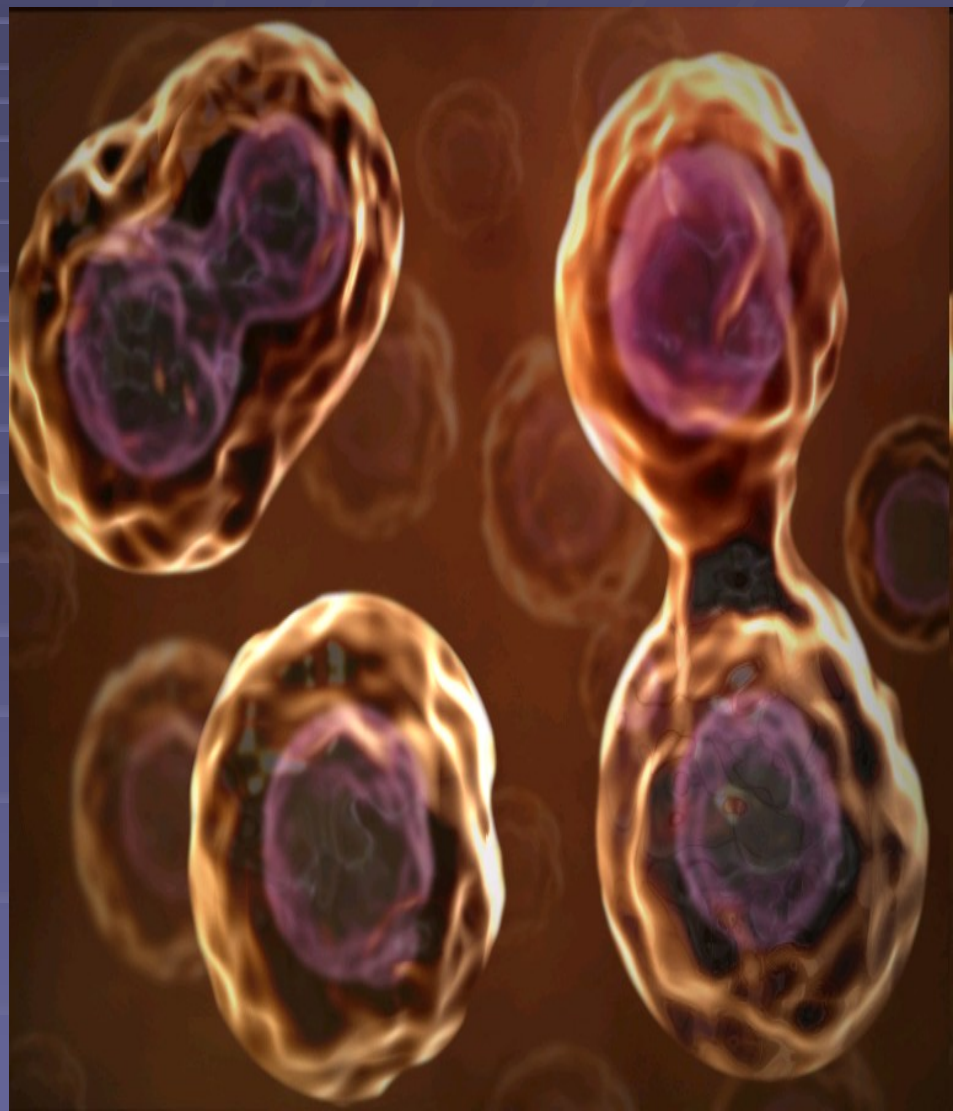
- The two broad types of mammalian stem cells are: **embryonic stem cells** that are isolated from the inner cell mass of blastocysts, and **adult stem cells** that are found in adult tissues. In a developing embryo, stem cells can differentiate into all of the specialized embryonic tissues. I

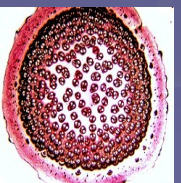




Stem cells act as Progenitor cells

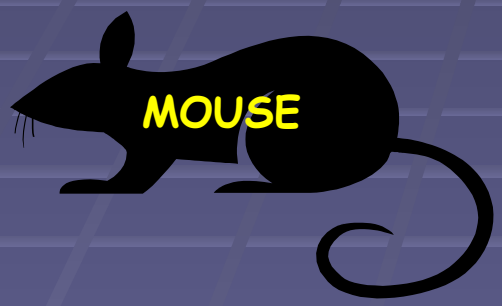
- In adult organisms, stem cells and **progenitor cells** act as a repair system for the body, replenishing specialized cells, but also maintain the normal turnover of regenerative organs, such as blood, skin or intestinal tissues.

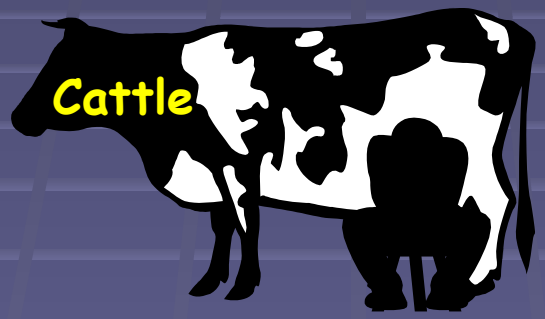


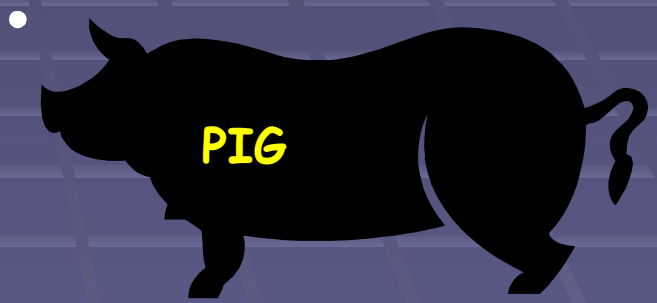


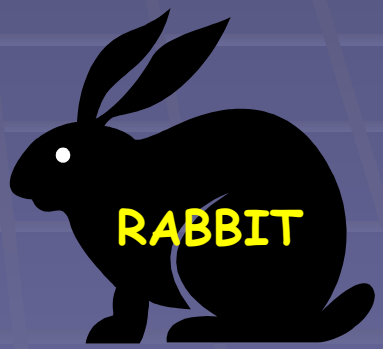
History of Animal Cloning

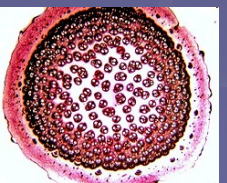
- Since then, animals including mice (1998), cows (1998), pigs (2000), cats (2001), and rabbits (2002) were successfully cloned.











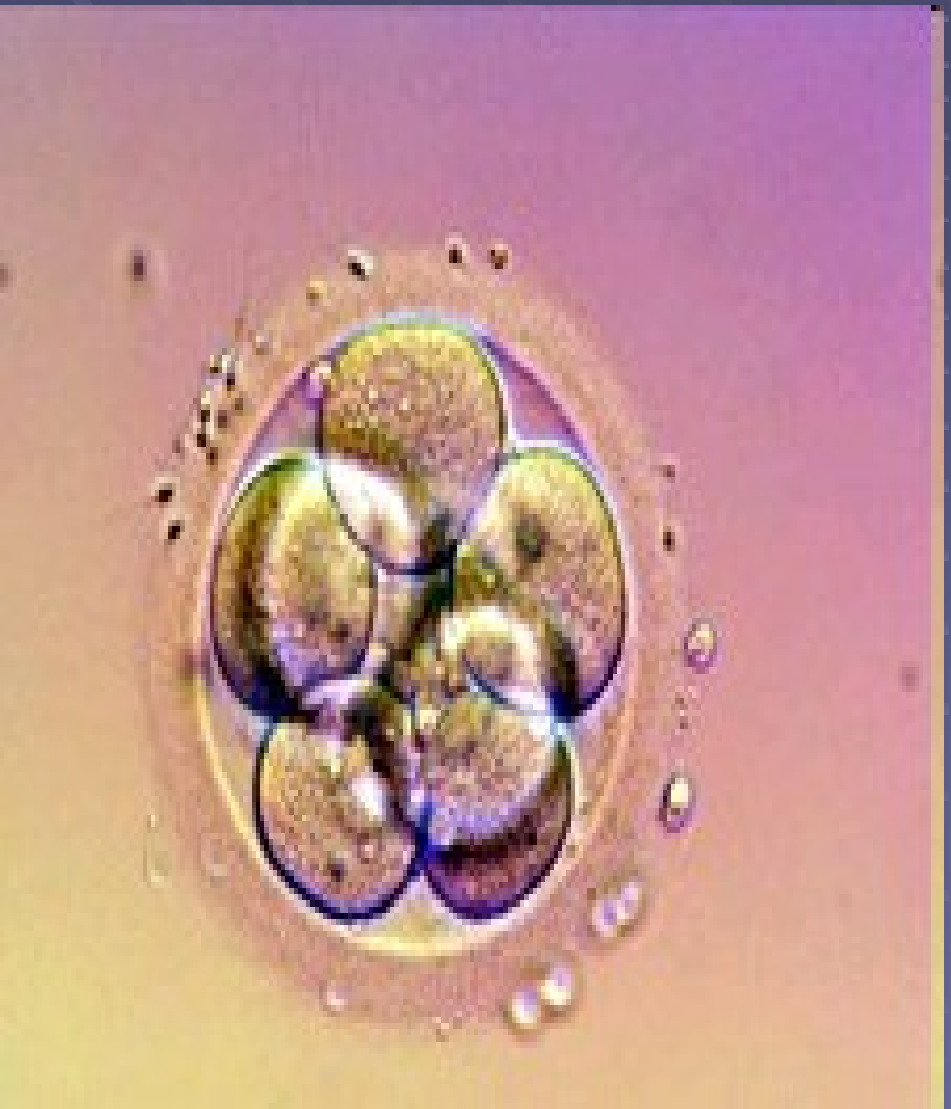
How Successful Was Animal Cloning? Very low (~1-3%)

Dolly (sheep)	1 live birth out of 29 cloned embryos	3%
Cloned mice	31 live births out of 2468 cloned embryos	1%
Cloned pigs	5 live births out of 335 cloned embryos	1%
Cloned goats	3 live births out of 85 cloned embryos	3%
Cloned cattle	30 live births out of 496 cloned embryos	6%
Cloned cat	1 live birth out of 87 cloned embryos	1%
Cloned rabbits	6 live births out of 371 of cloned embryos	1%

First Success of Human Embryo Cloning

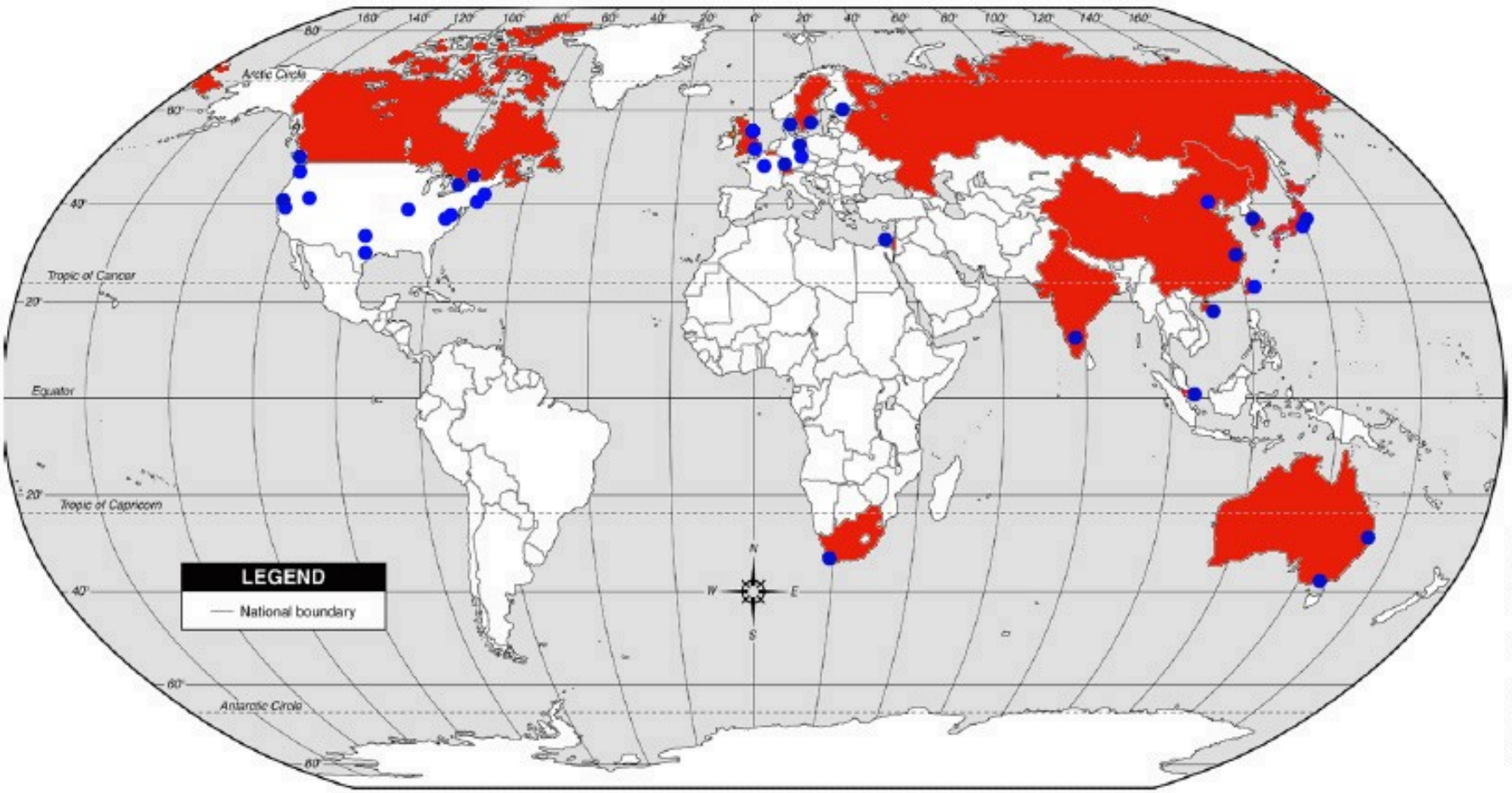
- **On February 12, 2004, South Korean scientists**, Dr. Woo Suk Hwang and Dr. Shin Young Moon of Seoul National University, reported the successful creation of **30 cloned human embryos developed** to the **blastocyst stage** and then destroyed by stem cell extraction, **yielding one embryonic stem cell line**.

Source of Stem Cells for Medical therapies



- Tens of thousands of frozen embryos are routinely destroyed when couples finish their treatment.
- These surplus embryos can be used to produce stem cells.
- Regenerative medical research aims to develop these cells into new, healthy tissue to heal severe illnesses.

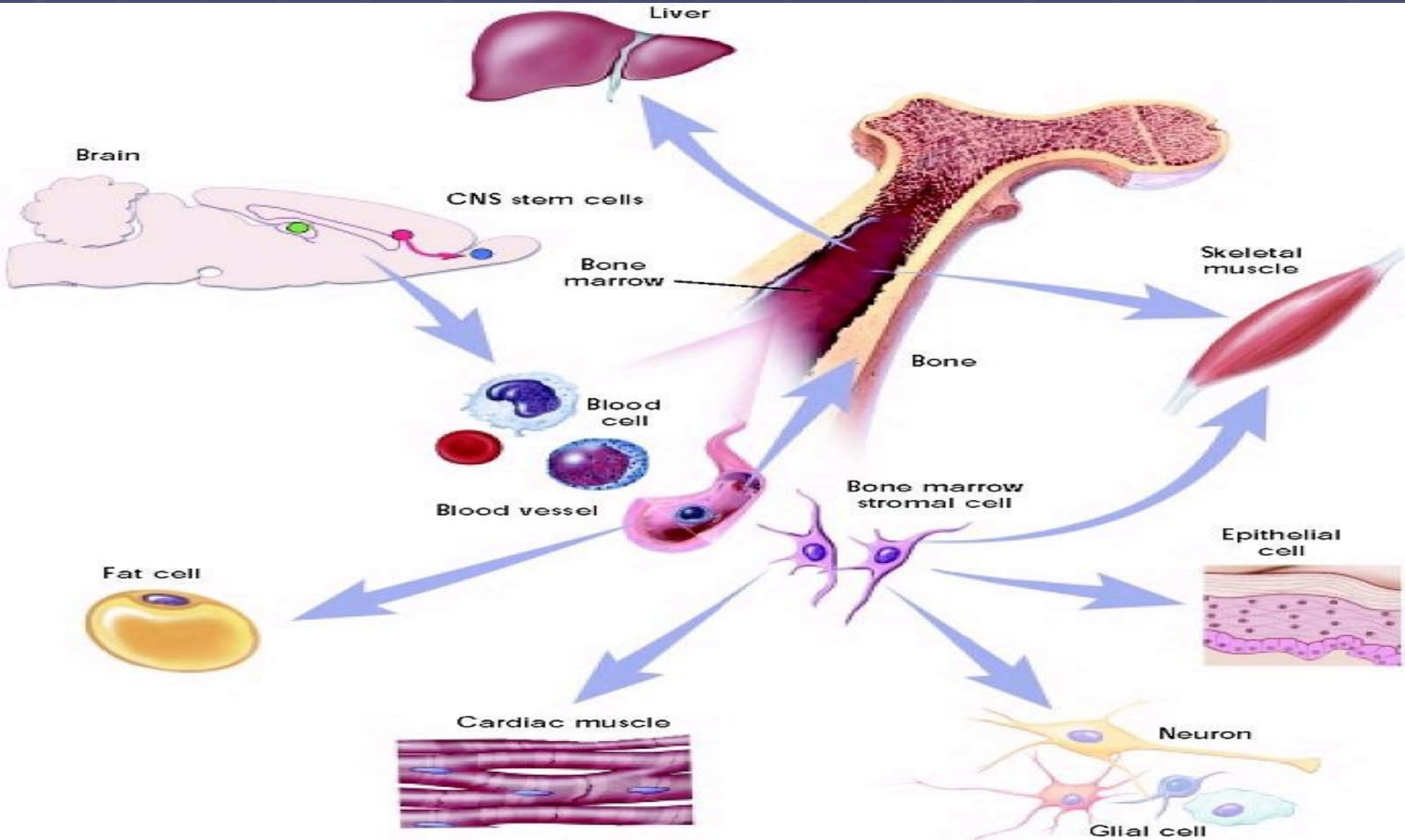
Stem Cell Research Worldwide



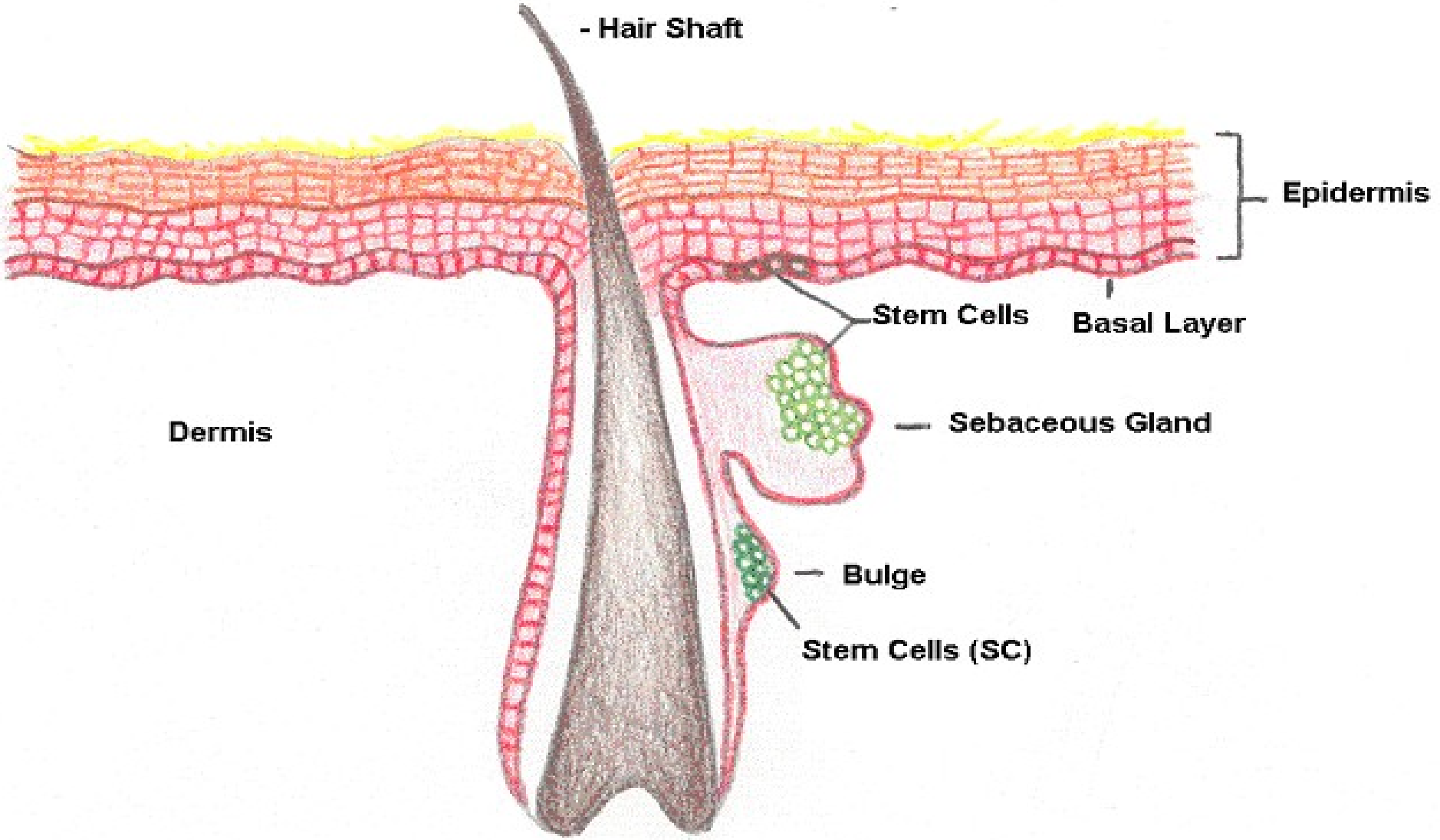
Countries with a permissive or flexible policy on embryonic stem cell research (in red)

- Denotes Genome Sequencing Center

Adult **multipotent** stem cells

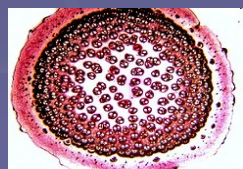


Adult Stem Cells

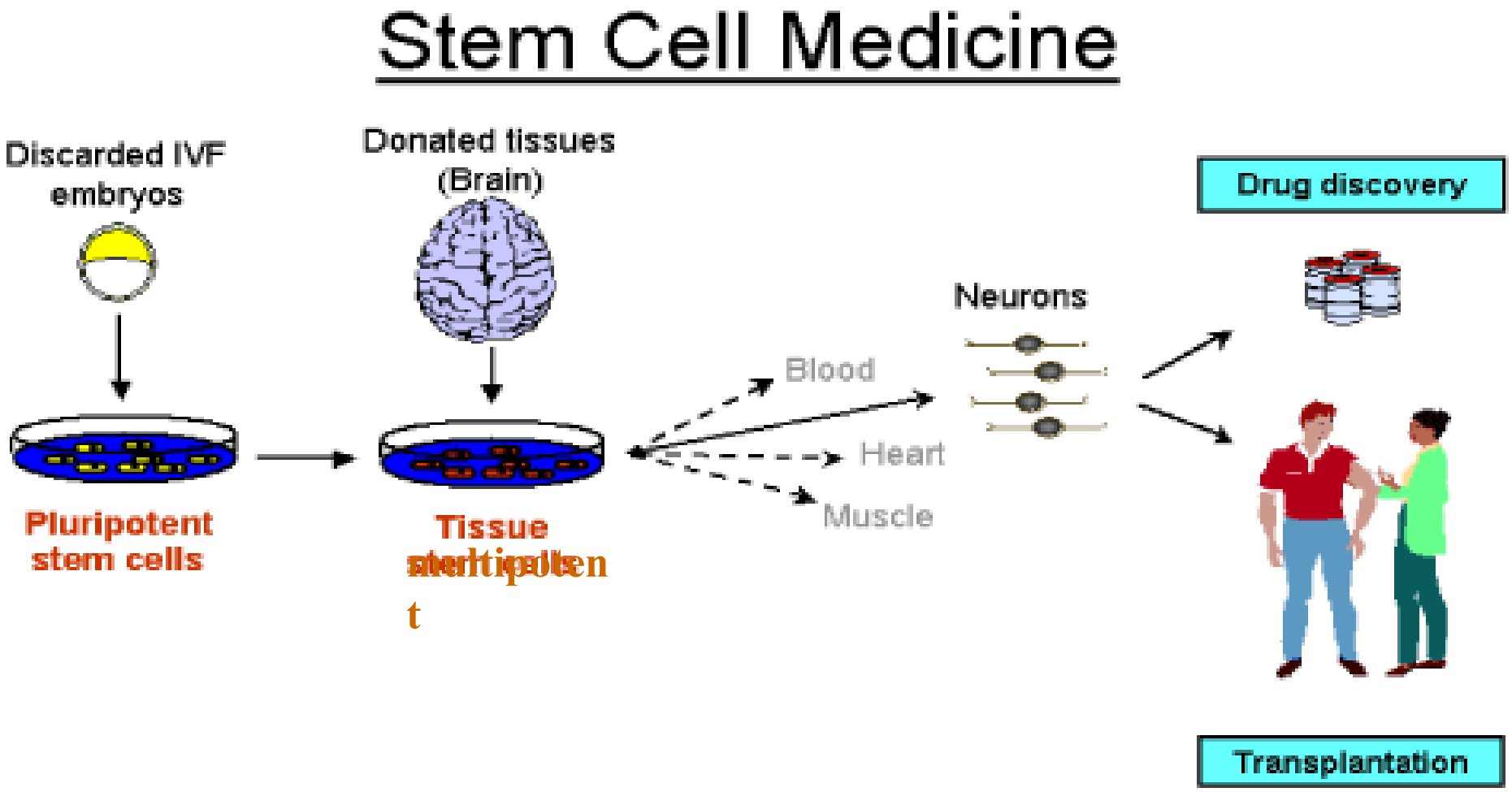


Autologus – Stem Cells

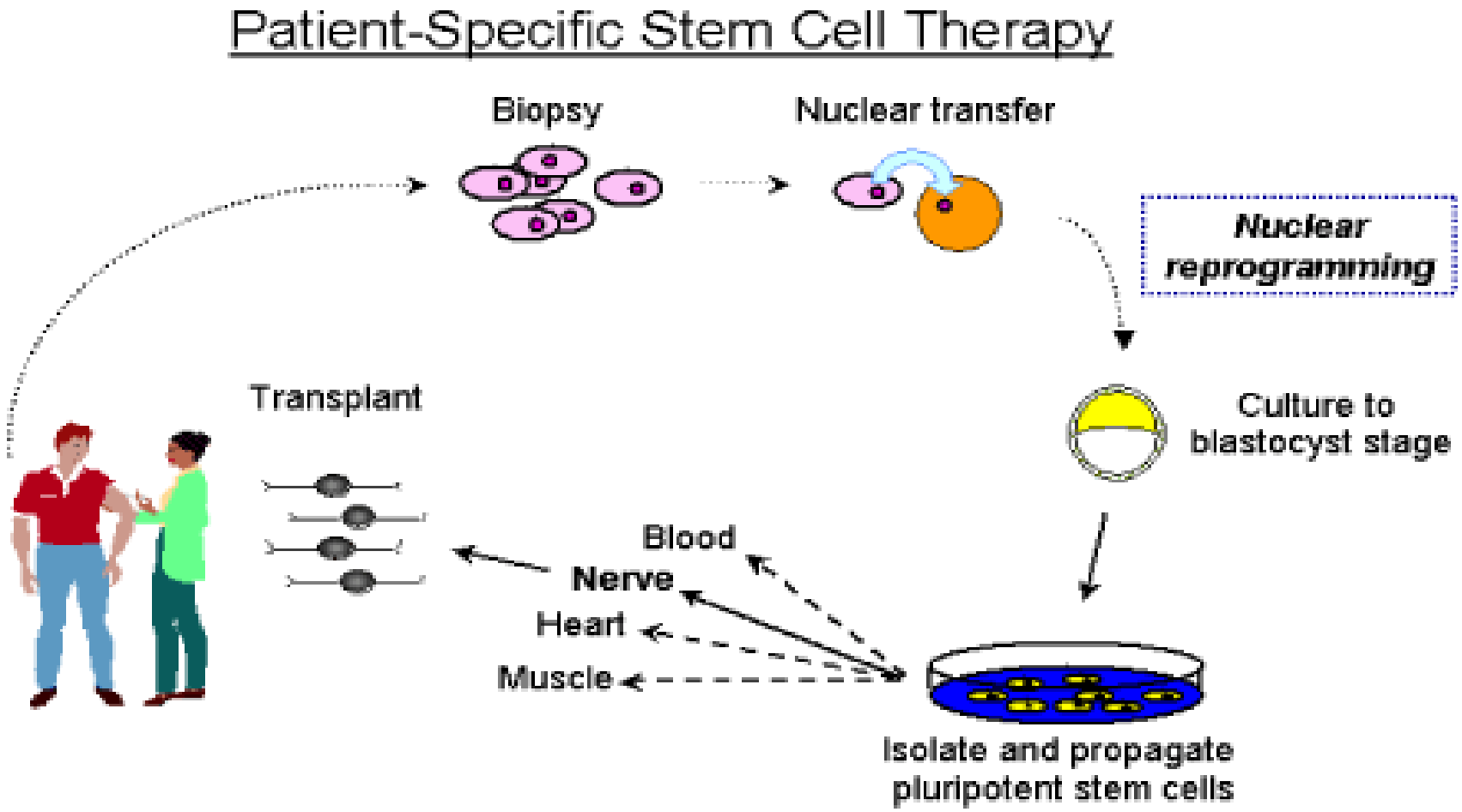
- While most blood stem cells reside in the bone marrow, a small number are present in the bloodstream. These multipotent peripheral blood stem cells, or PBSCs, can be used just like bone marrow stem cells to treat leukaemia, other cancers and various blood disorders



Speculation

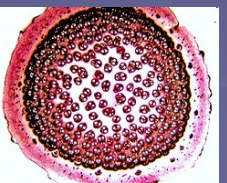


Treatments becomes specific



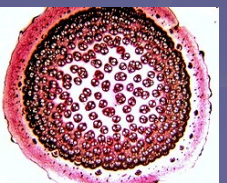
Applications of Stem Cells

- Cell Replacement Therapies
  - Cells could be stimulated to develop into specialized cells that represent renewable sources of cells and tissue for transplantation.
  - Cell replacement therapy could treat injuries and various genetic and degenerative conditions including muscular dystrophies, retinal degeneration, Alzheimer disease, Parkinson's disease, arthritis, diabetes, spinal cord injuries, and blood disorders such as hemophilia.



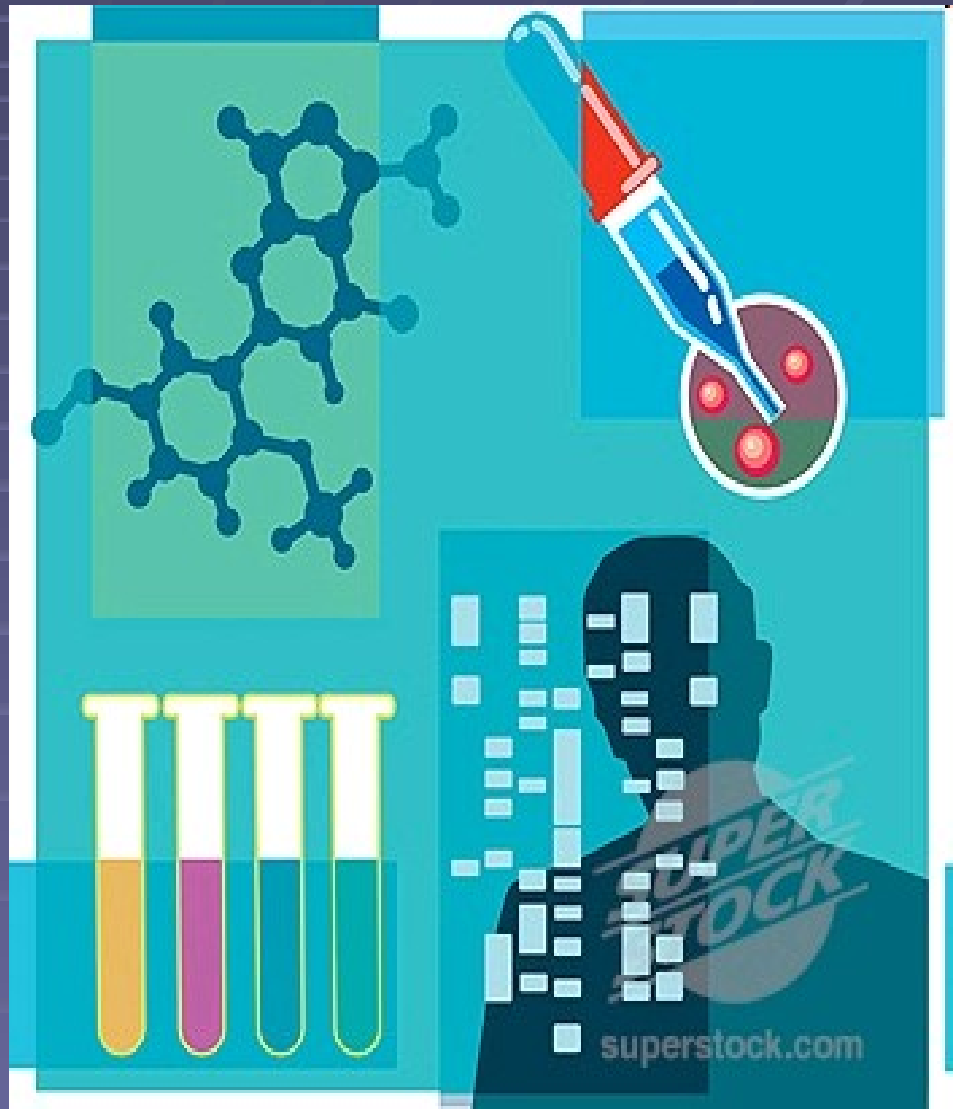
Understanding Cell Specialization

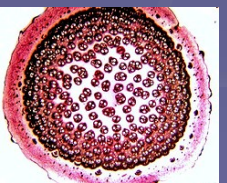
- Studying human pluripotent stem cells can lead to the identification of factors responsible for differentiation of stem cells into specialized cell types.
  - these factors may ultimately be used to drive tissue regeneration and repair if administered therapeutically.
- This work will provide basic knowledge on cell determination and differentiation, human development, genomic imprinting and somatic cell aging.



Development and Testing of Drugs

- Researchers could study the beneficial and toxic effects of new medications on human pluripotent stem cells that have been developed to mimic the disease processes.





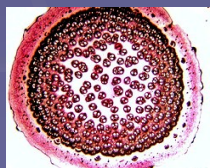
Can Sex Make difference in Stem cell Therapy ?

- Are there sex-specific differences in the biology of stem cells? (short-long term
- • How do sex-specific differences play out in terms of self-renewal and differentiation? (mid-long term)
- Is there existing evidence that the sex of stem cells affects success of the transplant?

XX

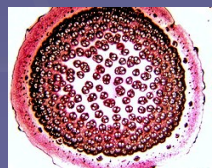
vs.

XY



Stem cells – Blindness

- In clinical trials at Moorfields Eye Hospital in London, surgeons restored eye sight for six patients who lost their sight after chemical accidents and genetic diseases. The patients went under successful stem-cell transplant.



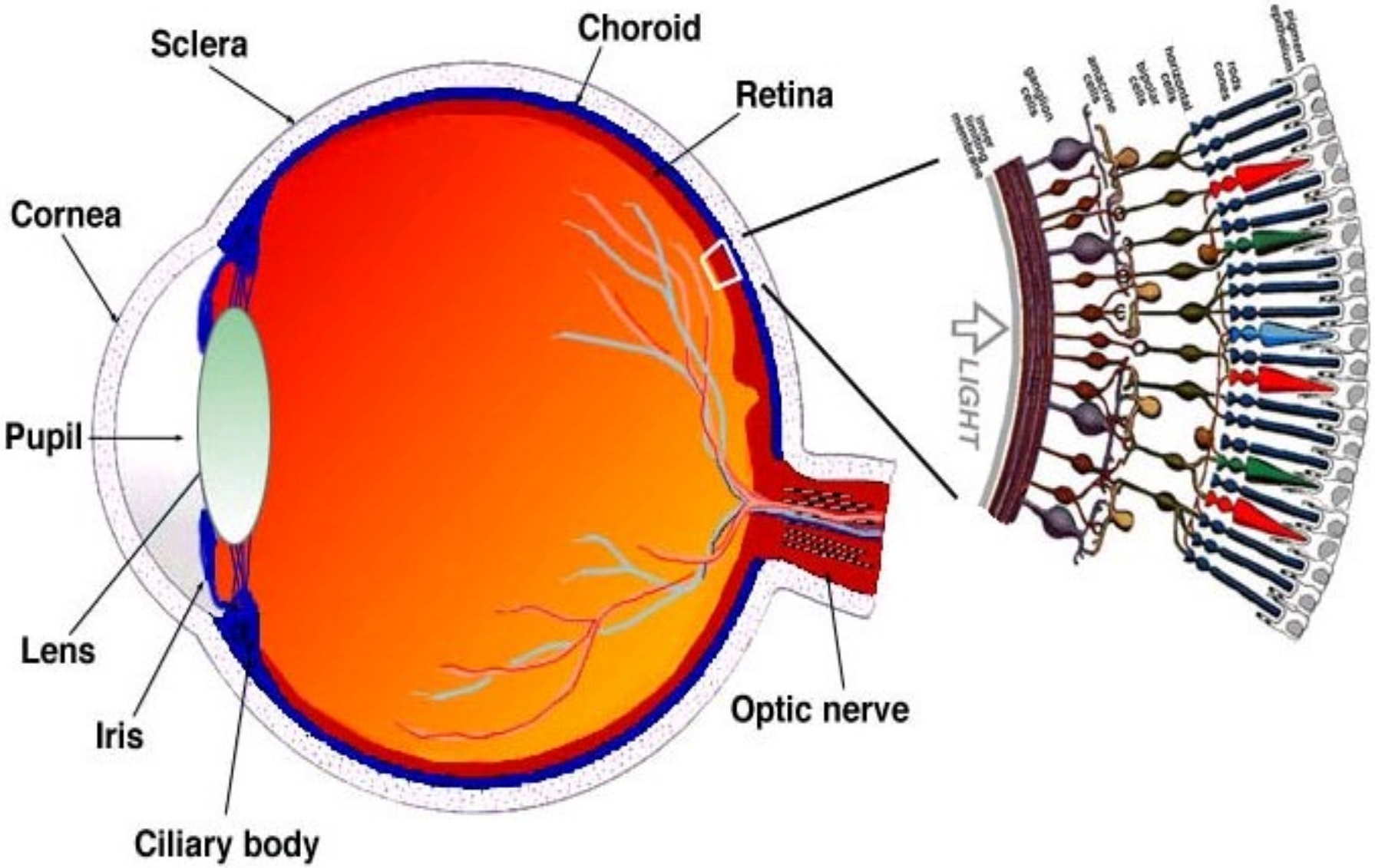
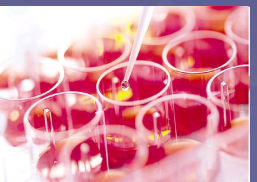
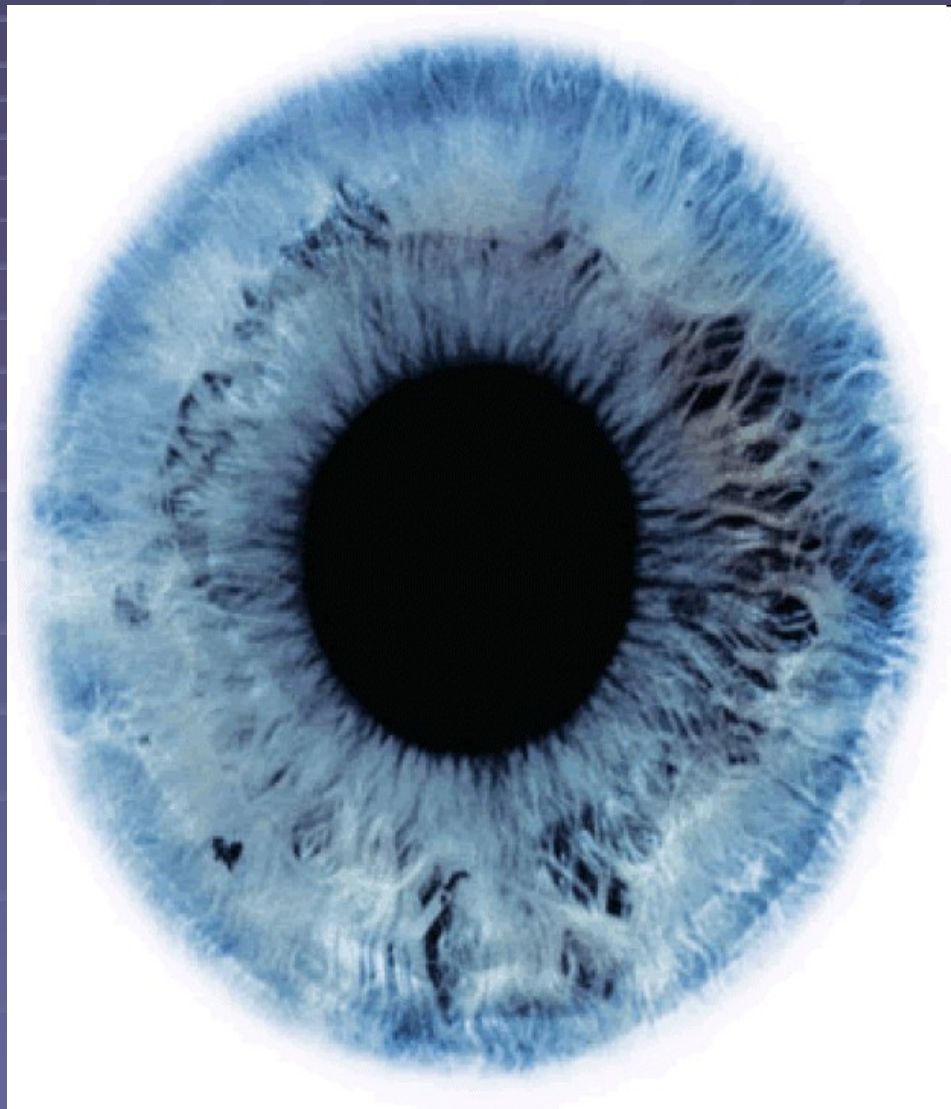


Fig. 1.1. A drawing of a section through the human eye with a schematic enlargement of the retina.

Limbal stem Cell therapy

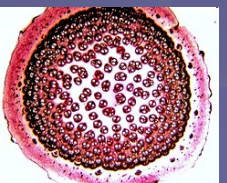
- The treatment is known as limbal stem cell therapy, and the patients who received the treatment suffered from chemical burn or genetic disease know as aniridia
- a By replacing the limbal stem cells, the cornea begins to clear up as the cells are replaced with the healthy transparent layer again.





Current possible uses

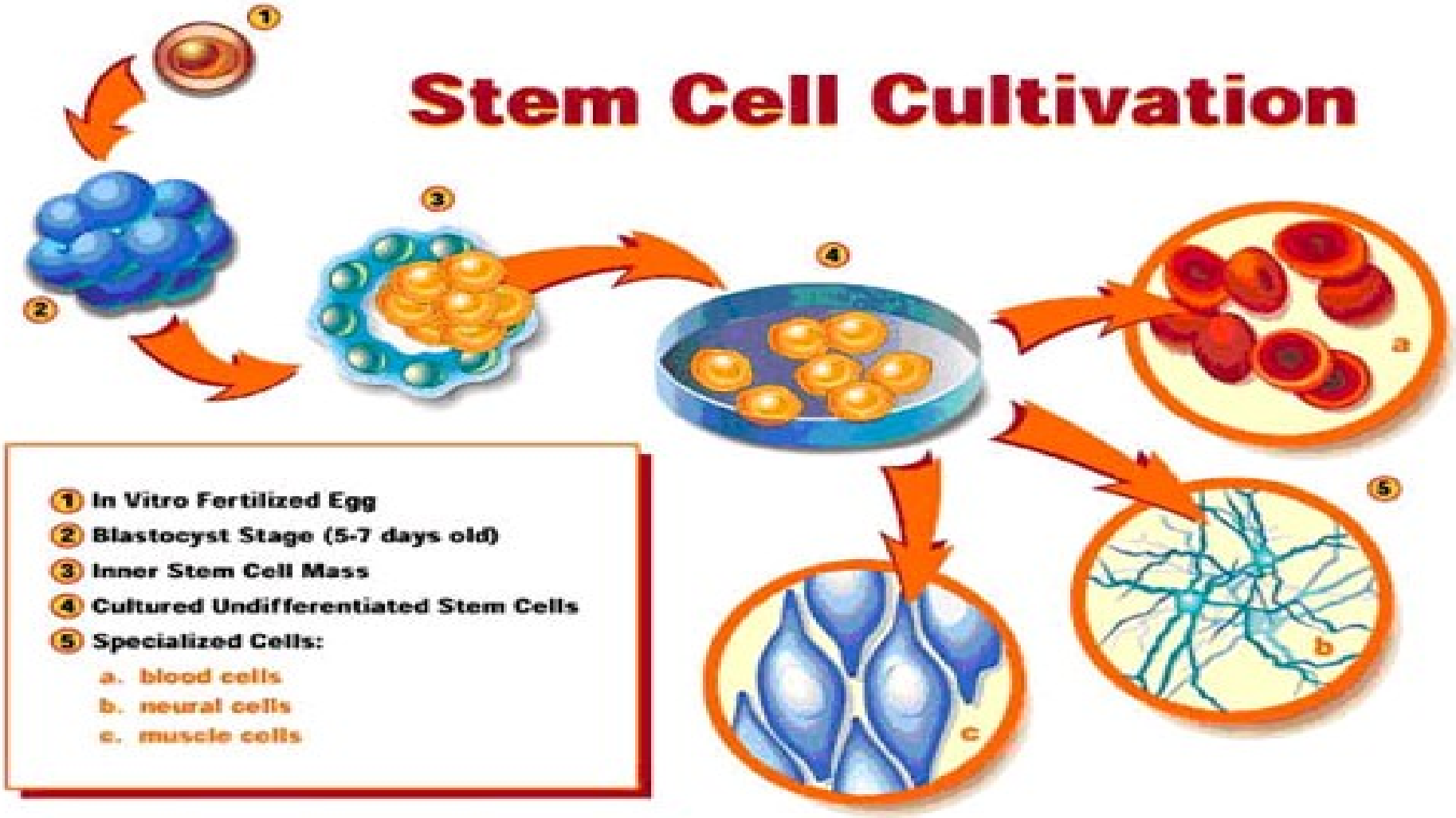
- Research in stem cells has opened up new horizons in the area of treatment of disorders such as stroke, epilepsy, neuro-degeneration and trauma. Current research is aimed at finding the appropriate source of stem cells for a given indication, ways of expanding and perpetuating these cells in culture, best route of administration of these cells and methods to overcome rejection



Possible Uses of Stem Cell Technology

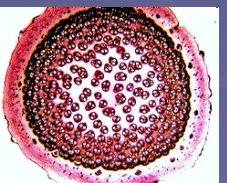
- Replaceable tissues/ organs
- Repair of defective cell types
- Delivery of genetic therapies
- Delivery chemotherapeutic agents

Future –Making cells and replacing the diseased cells ?



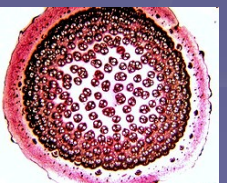
Obstacles of Stem Cell Research

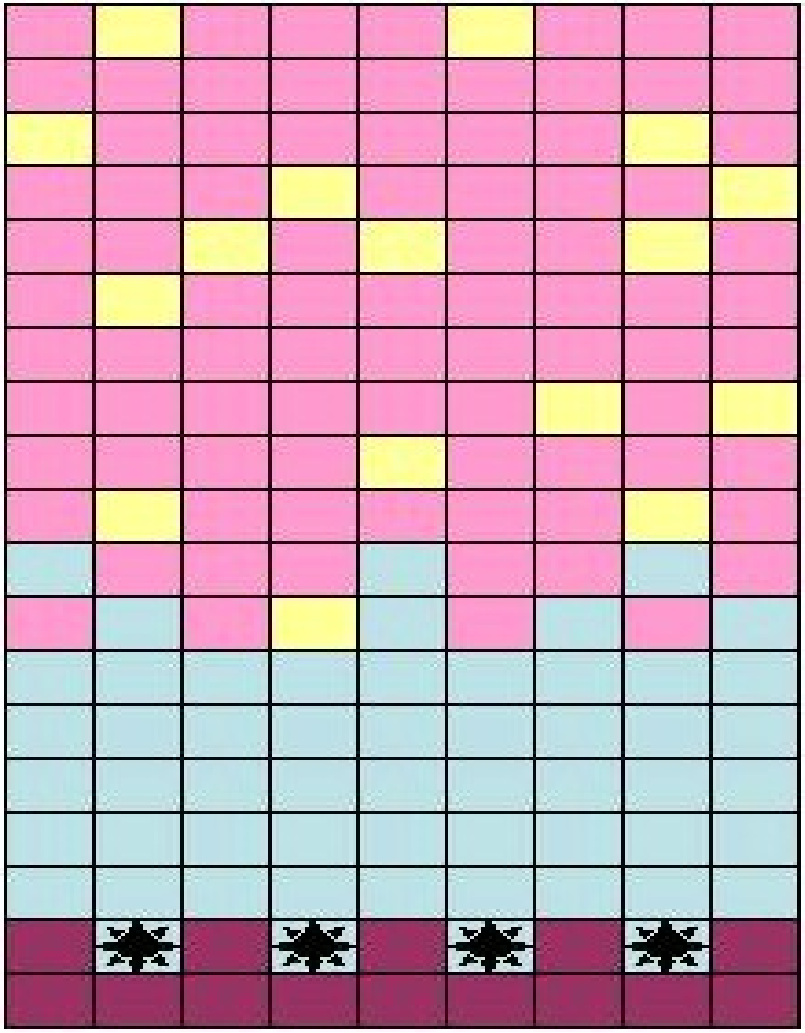
- How to find the right type of stem cells?
- How to put the stem cells into the right place?
- Will the stem cells perform the desired function in the body?
- Differentiation protocols for many cell types have not been developed.



Embryonic Stem Cells are Unstable and Mutate in Culture

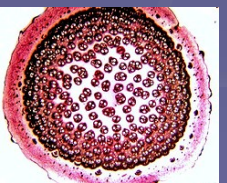
- Like ordinary cells, stem cells accumulate significant numbers of ***mutations*** over time, including several that could cause them to become tumors.





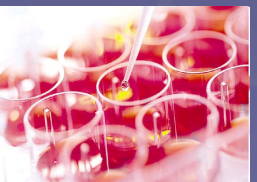
Ethical debate

- Harvesting ES cells destroys the blast cyst
- “This is murder”
- ES cell research requires human cells
- Could create a commercial market for human cells
- “This devalues life”



Destroying life to cure some one – Ethical ?

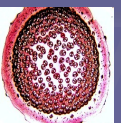
- If stem cells have such potential to relieve suffering, why are so many people so upset about their use? The reason is that the most powerful type of stem cell – embryonic stem (ES) cells – can only be obtained from human embryos. Many people think that it's wrong to create and destroy human embryos to treat disease



Religious Debate over Harvesting Embryonic Stem Cells

- **The pro-life group generally believes that:**
- Personhood happens at, or shortly after, conception.
- Thus, they consider the removal of stem cells from an embryo -- a procedure which kills the stem cells -- to be a form of murder of a human being.
- They argue that no potential health benefits to even hundreds of millions of people can justify the murder of other humans.

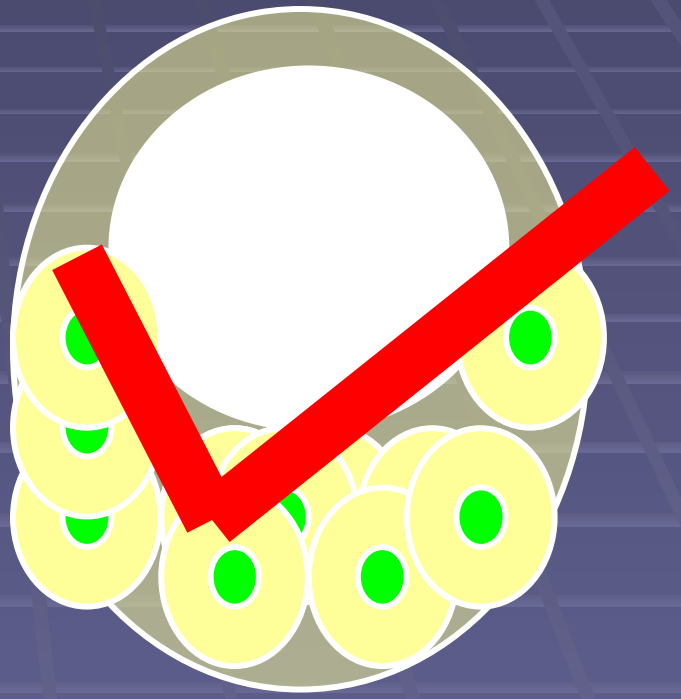
Day 5-6 Blastocyst



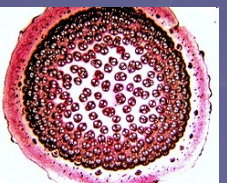
Religious Debate over Harvesting Embryonic Stem Cells

- **The pro-choice group generally believes that:**

- Personhood is attained much later in pregnancy, perhaps when the fetal brain develops consciousness during the third trimester.
- Thus, extracting stem cells from an five or ten-day old pre-embryo is not murder.
- Killing a pre-embryo, which is only a potential human being, is justified if it has the potential to cure diseases and extend the lives of people.

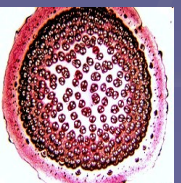


Day 5-6 Blastocyst



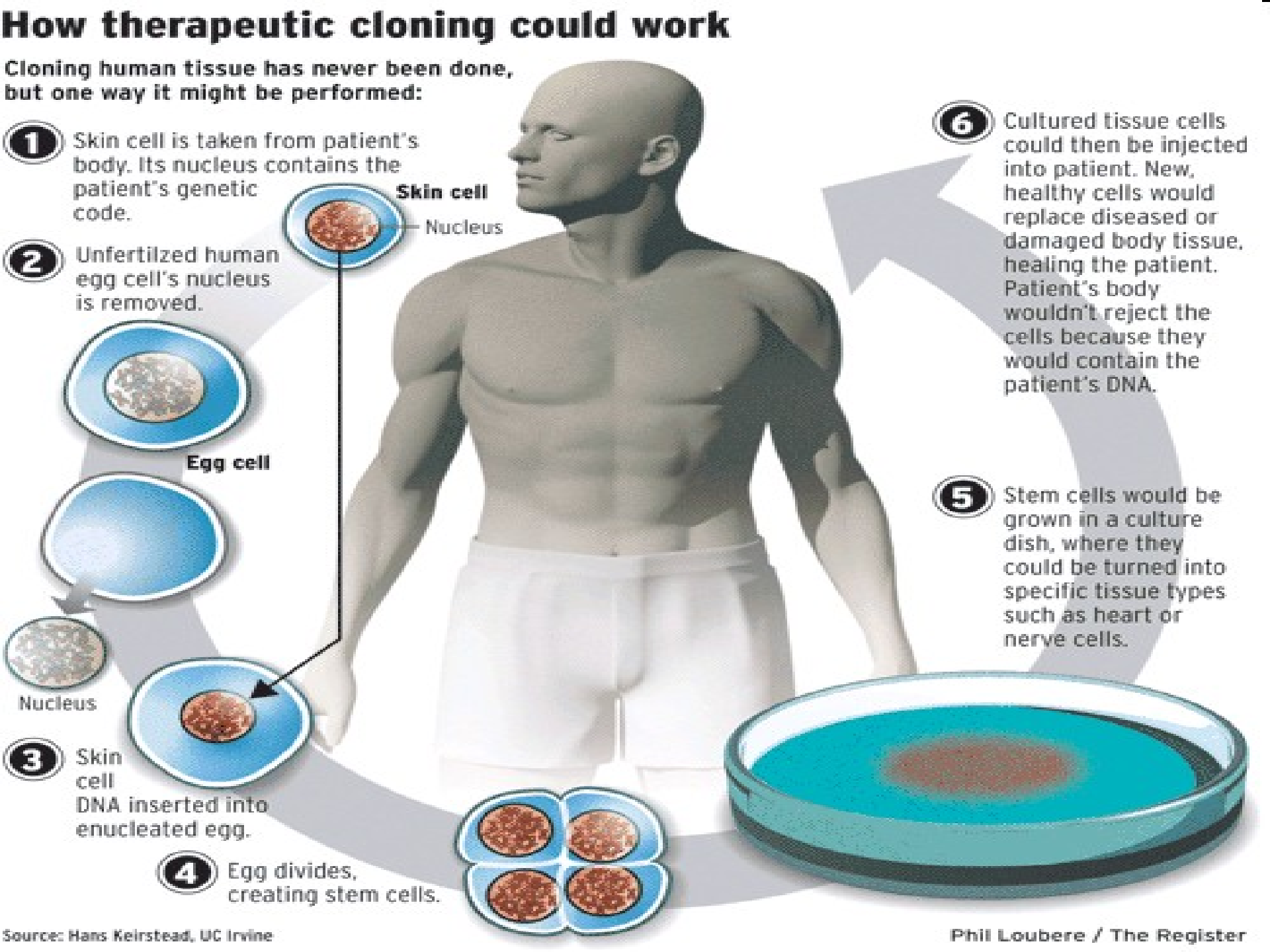
Why we should support
Can help several disabled

- Human embryonic stem cell (HESC) research offers great promise of cures for otherwise incurable conditions: spinal cord injuries, ALS, Alzheimer's, Parkinson's, etc.



How therapeutic cloning could work

Cloning human tissue has never been done, but one way it might be performed:

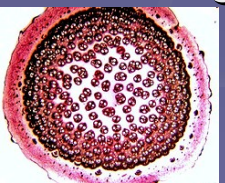


Source: Hans Keirstead, UC Irvine

Phil Loubere / The Register

Shall be Clone Humans ?

- Arguments for and against human cloning research. Should we ban human cloning? Why investors are moving away from human cloning and why human cloning now looks a last-century way to fight disease. Why some people want to clone themselves or even to clone the dead.



Research on Stem Cells is progressing in spite of several restrictions

Created for awareness to Medical and Paramedical Medical Students in Developing World

Dr.T.V.Rao MD

Email

doctortvrao@gmail.com

Hematopoietic stem cell transplantation

HSCT - definition

- **Definition**

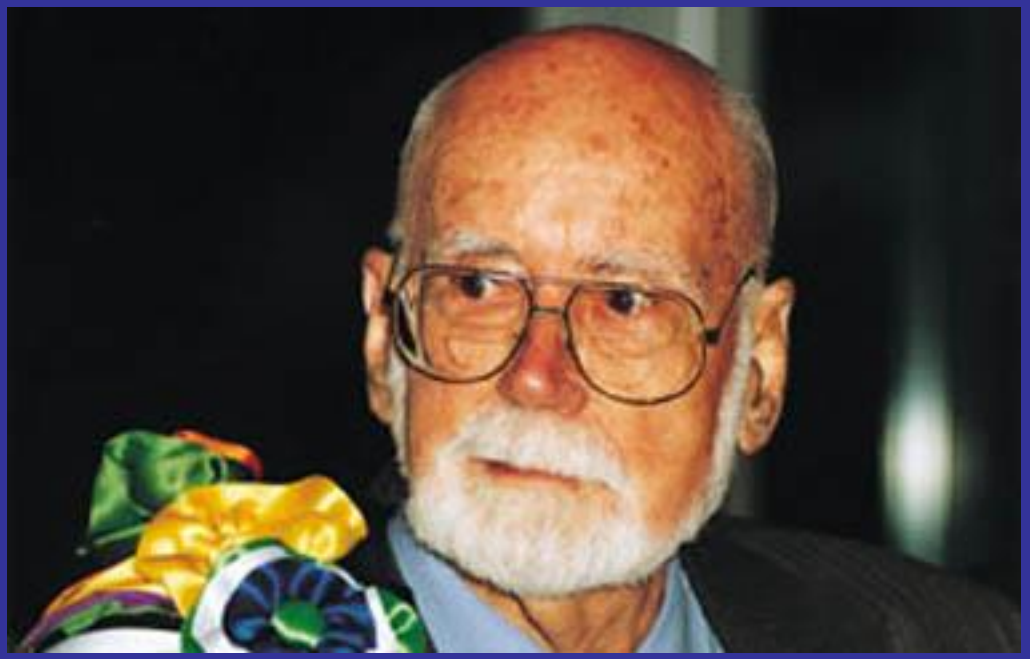
  any procedure where hematopoietic stem cells of any donor and any source are given to a recipient with intention of repopulating/replacing the hematopoietic system in total or in part

History

- **Hematopoietic stem cell transplantation in the mouse**
  - the radiation protection phenomenon (mid-1950s)
- **Hematopoietic stem cell tranplantation in the dog**
- **Hematopoietic stem cell tranlsplantation in human patients**
  - 1959–1963 : first allogeneic HSCT in humans
  - beginning of the Modern Era of HSCT: the end of 1960

The Nobel Prize, 1990

E. Donnall Thomas



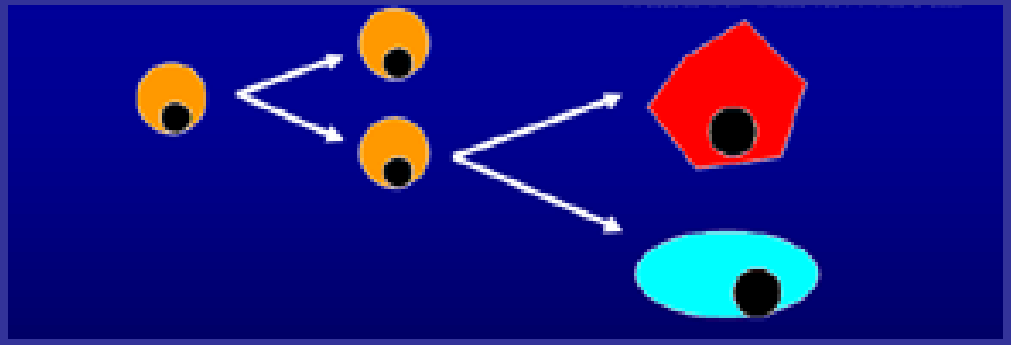
first succsessful HSCT in treatment of acute leukemias

Thomas ED, Lochte HL, Lu WC, Ferrebee JW. Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy. N. Engl. J. Med. 1957; 257: 491.

Stem cells

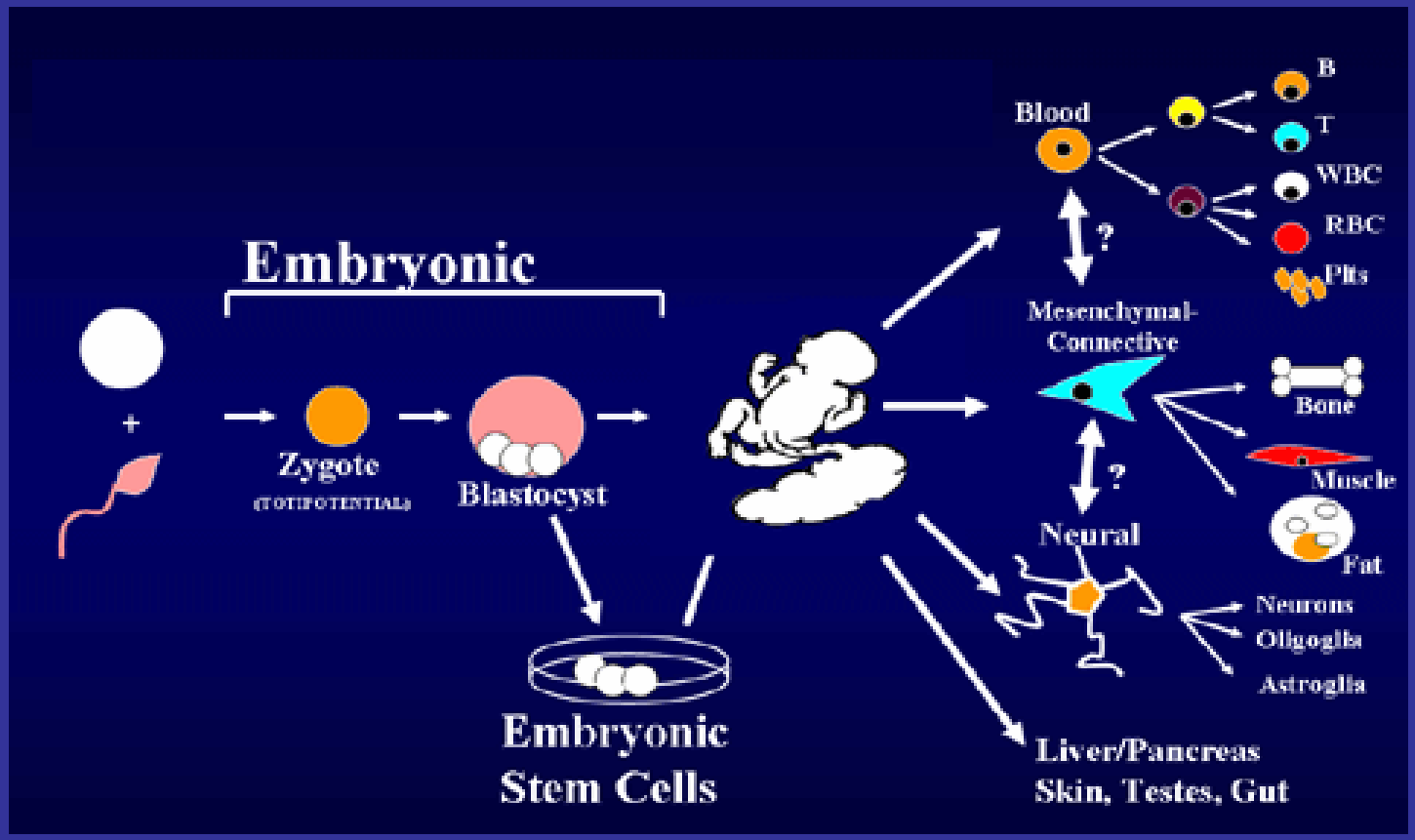
population of undifferentiated cells which are able

- **to divide for indefinite period**
- **to self renew**
- **to generate a functional progeny of highly specialised cells**



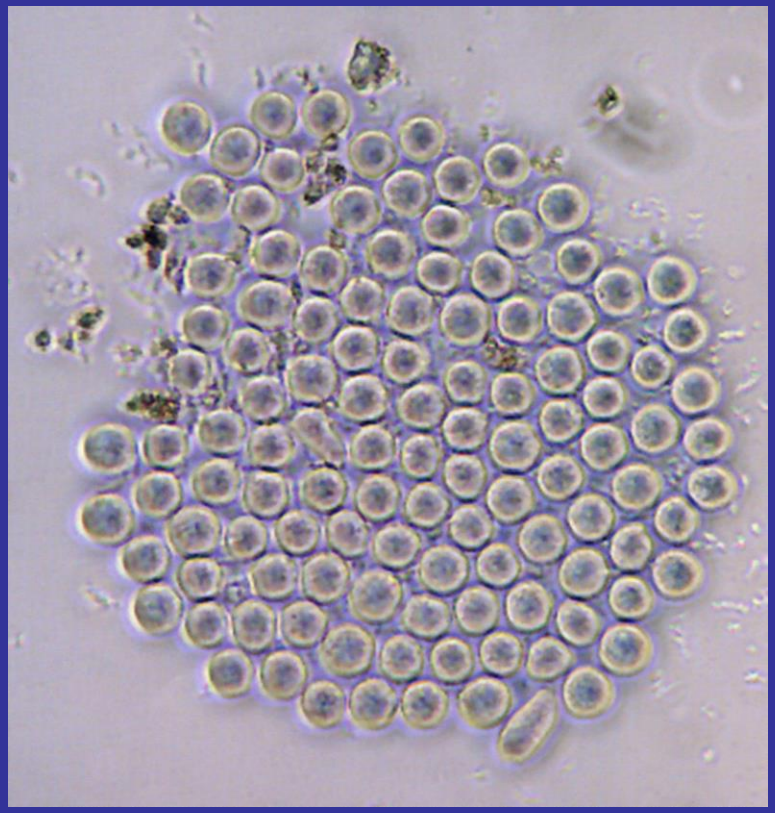
Hierarchy

- Totipotent (fertilised egg)
- Pluripotent (embryonic cell)
- Multipotent (hematopoietic)



Hematopoietic stem cells

1 / 25 000 - 100 000 of bone marrow cells



Blood, 15 Jan 2004

Charakteristic:

- CD34
- CD133
- Lin^-
- C-kit (CD117)
- BCRP

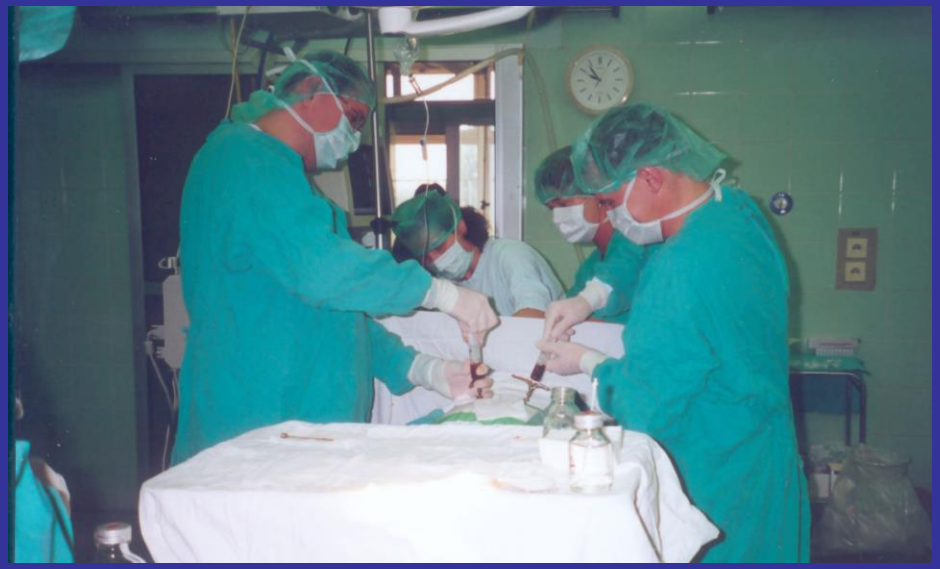
HSCT

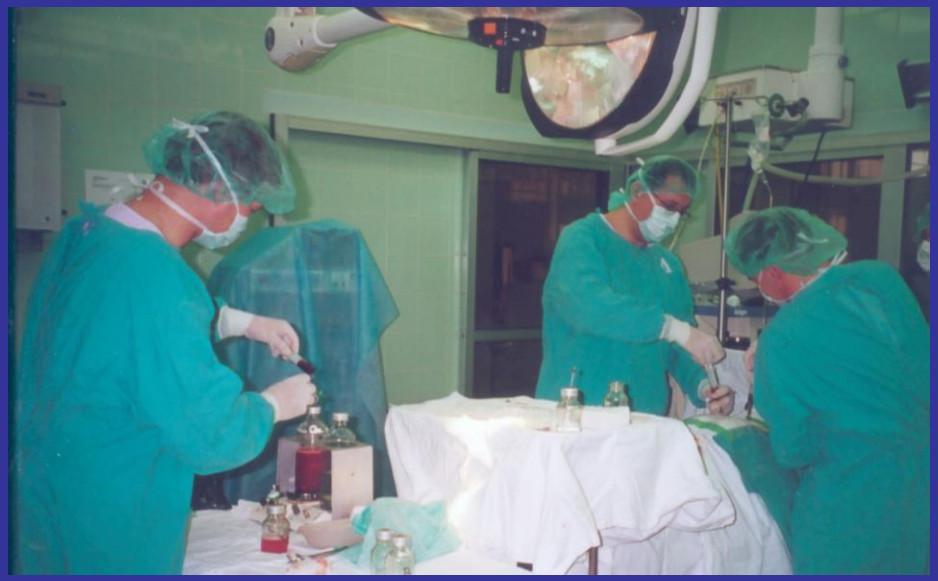
- **Allogeneic HSCT**
  - syngeneic
  - from sibling/related donor
  - from unrelated donor
- **Autologous HSCT**

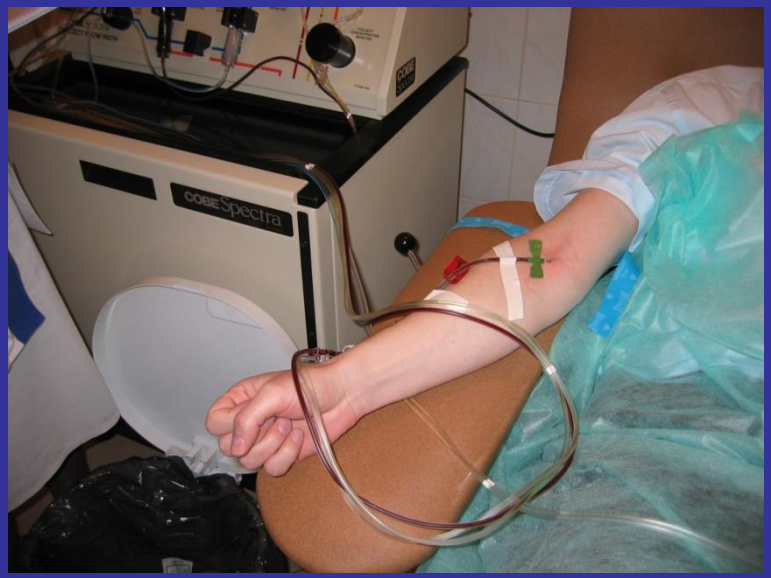
Sources of stem cells

- Bone marrow
- Peripheral blood
- Umbilical cord blood
- Fetus liver

Collection of hematopietic stem cells







bone marrow

peripheral blood

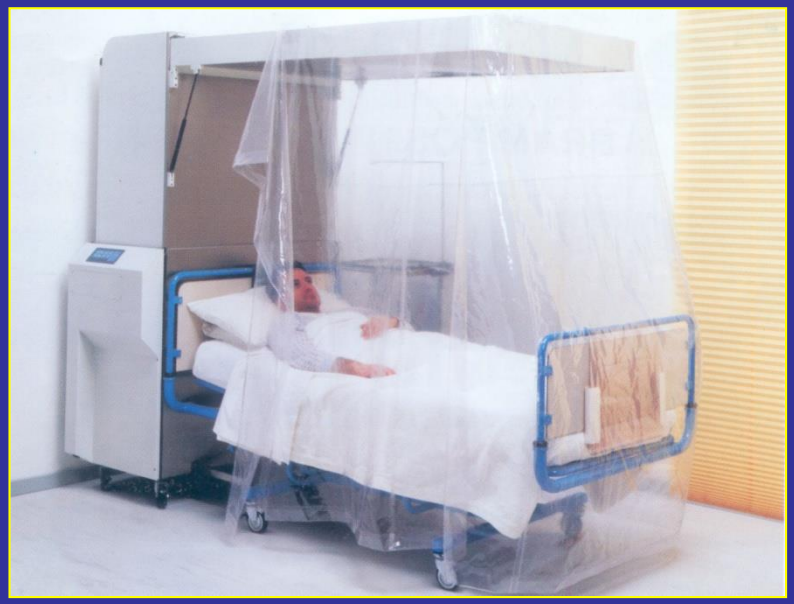
Indication for HSCT

- **Neoplastic disorders**
  - Hematological malignancies
    - Lymphomas (Hodgkin and non-Hodgkin)
    - Leukemias (acute and chronic)
    - Multiple myeloma
    - MDS
  - Solid tumors
- **Non-neoplastic disorders**
  - Aplastic anemia
  - Autoimmune diseases
  - Immunodeficiency
  - Inborn errors of metabolism

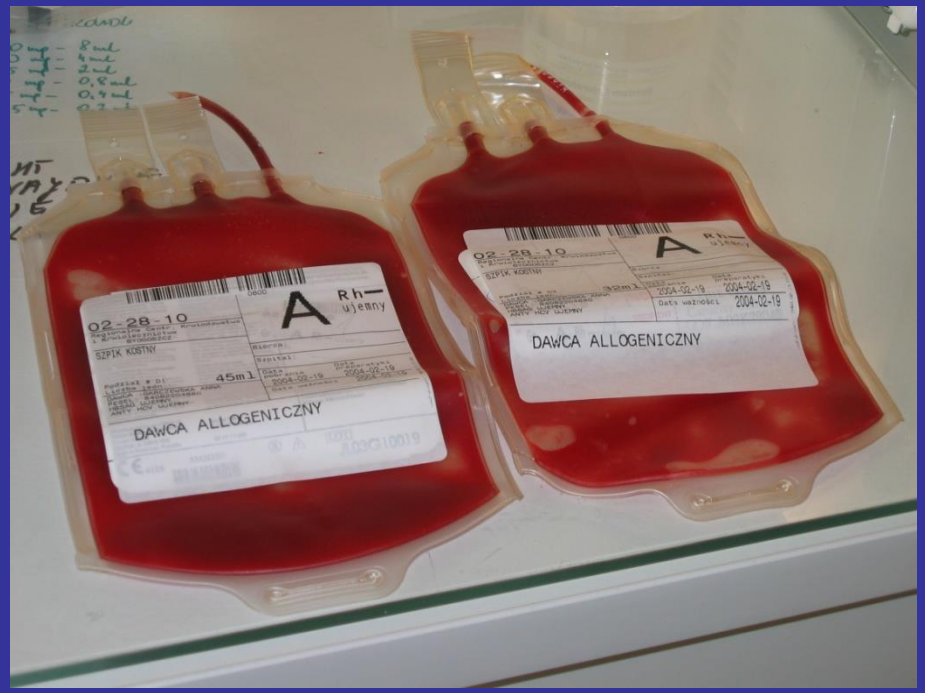
Conditioning regimens

- **Principles**
  - „space-making” (controversial)
  - immunosuppression
  - disease eradication
- **Strategy**
  - Ablative therapy
    - radio/chemo
  - Reduced intensity therapy
    - radio/chemo
  - Non-myloablative therapy
    - radio/chemo

Bone marrow transplantation unit



Hematopoietic stem cell infusion



Factors influencing the outcome of HSCT

- **Disease factors**
  - stage
- **Patient - related factors**
  - Age
- **Donor - related factors**
  - Histopompatibility (HLA)
  - Sex
  - Viral status (CMV positivity)
- **Peri-transplant factors**
  - Conditioning
  - GVHD prevention
  - Stem cell source and content
- **Post-transplant factors**
  - GVHD

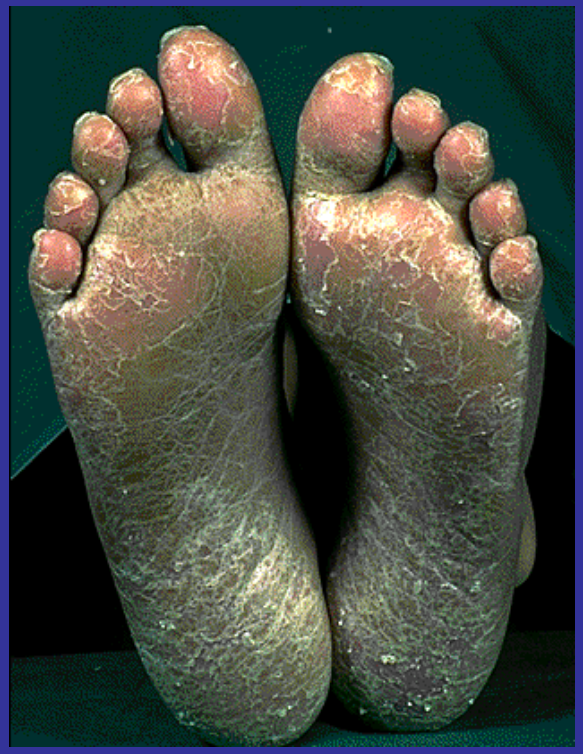
Complication

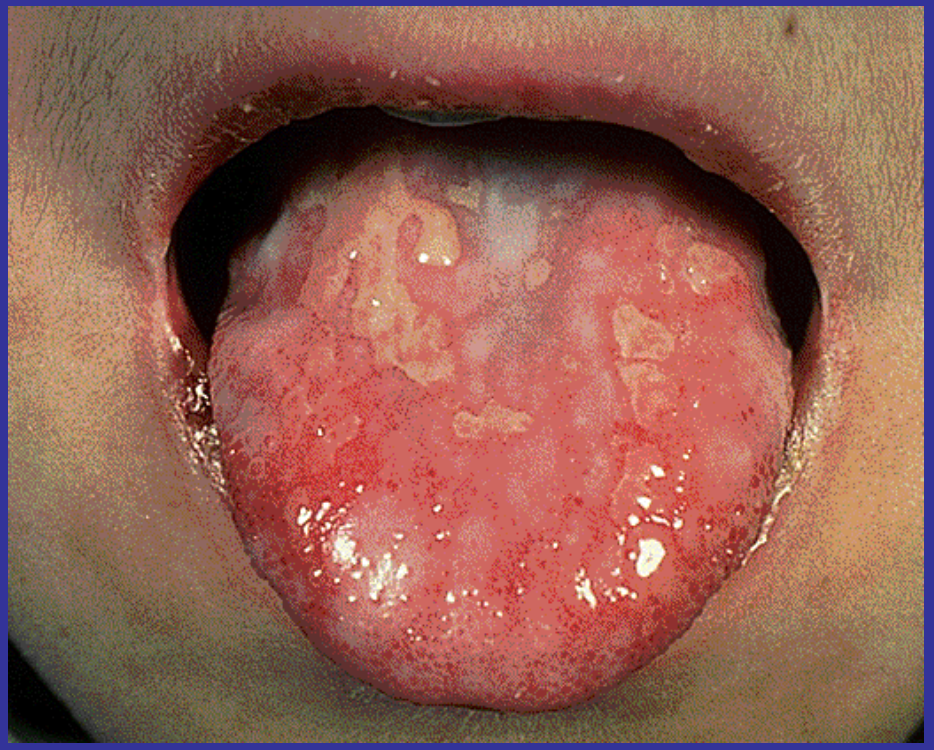
- **Allogeneic**
  - **Early**
    - infection
    - aGVHD
    - bleeding
    - toxicity
    - graft failure
  - **Late**
    - chGVHD
    - infection
    - relapse
    - gonadal failure
    - secondary malignancy
    - toxicity
- **Autologous**
  - **Early**
    - infection
    - bleeding
    - toxicity
  - **Late**
    - relapse
    - infection
    - gonadal failure
    - secondary malignacy
    - toxicity

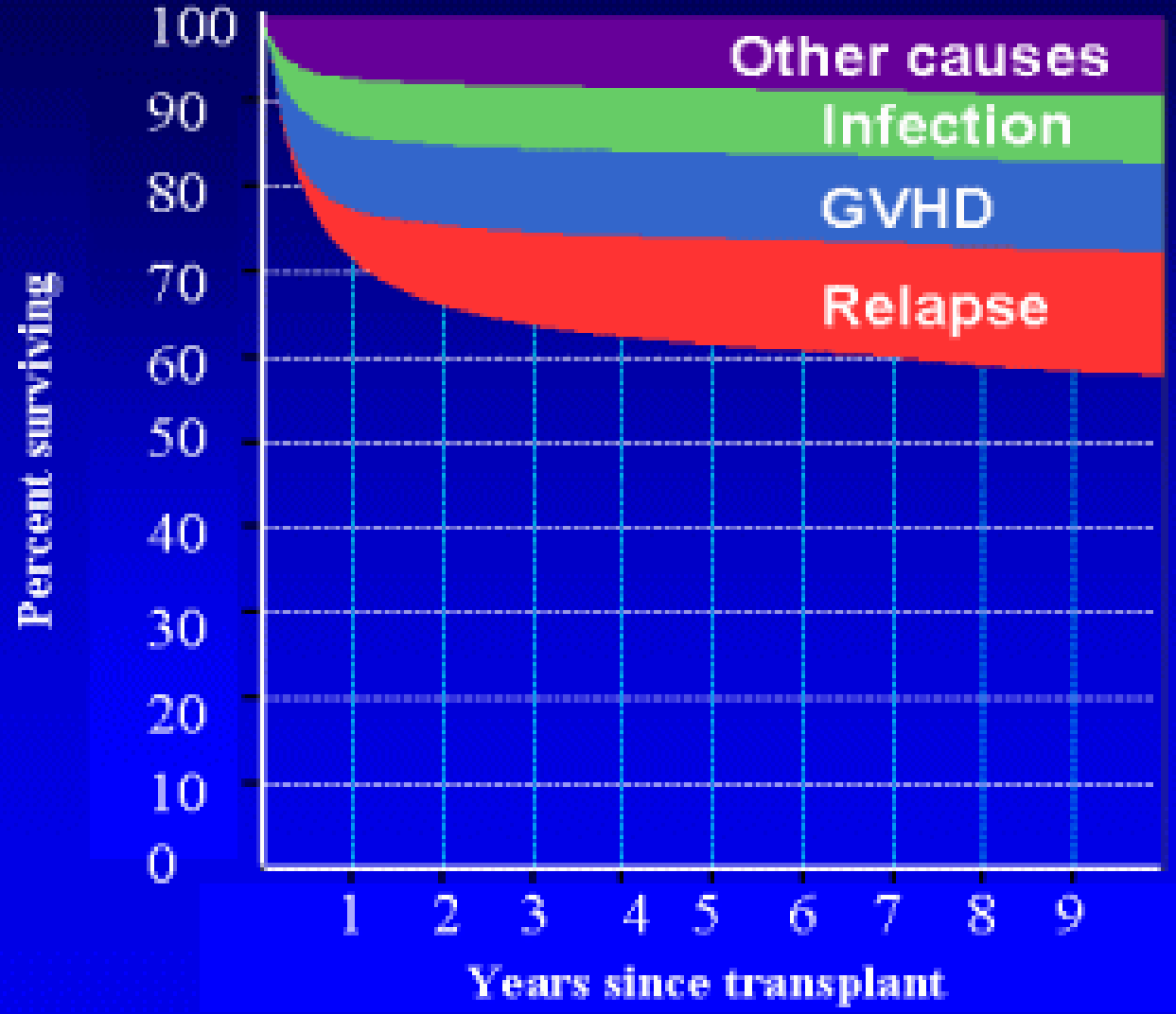
AlloHSCT - graft versus host disease

- **GVHD**
  - Acute (1- 4°)
  - Chronic (limited, extensive)
- **Prophylaxis**
  - Cyclospirine
  - Metotrexate
- **Treatment**
  - Cyclosporine
  - Steroid
  - Mycofenolate mofetil
  - Antithymocytic globuline
  - Anti-TNF alfa, anti-Il 2

Graft versus host disease (GVHD)





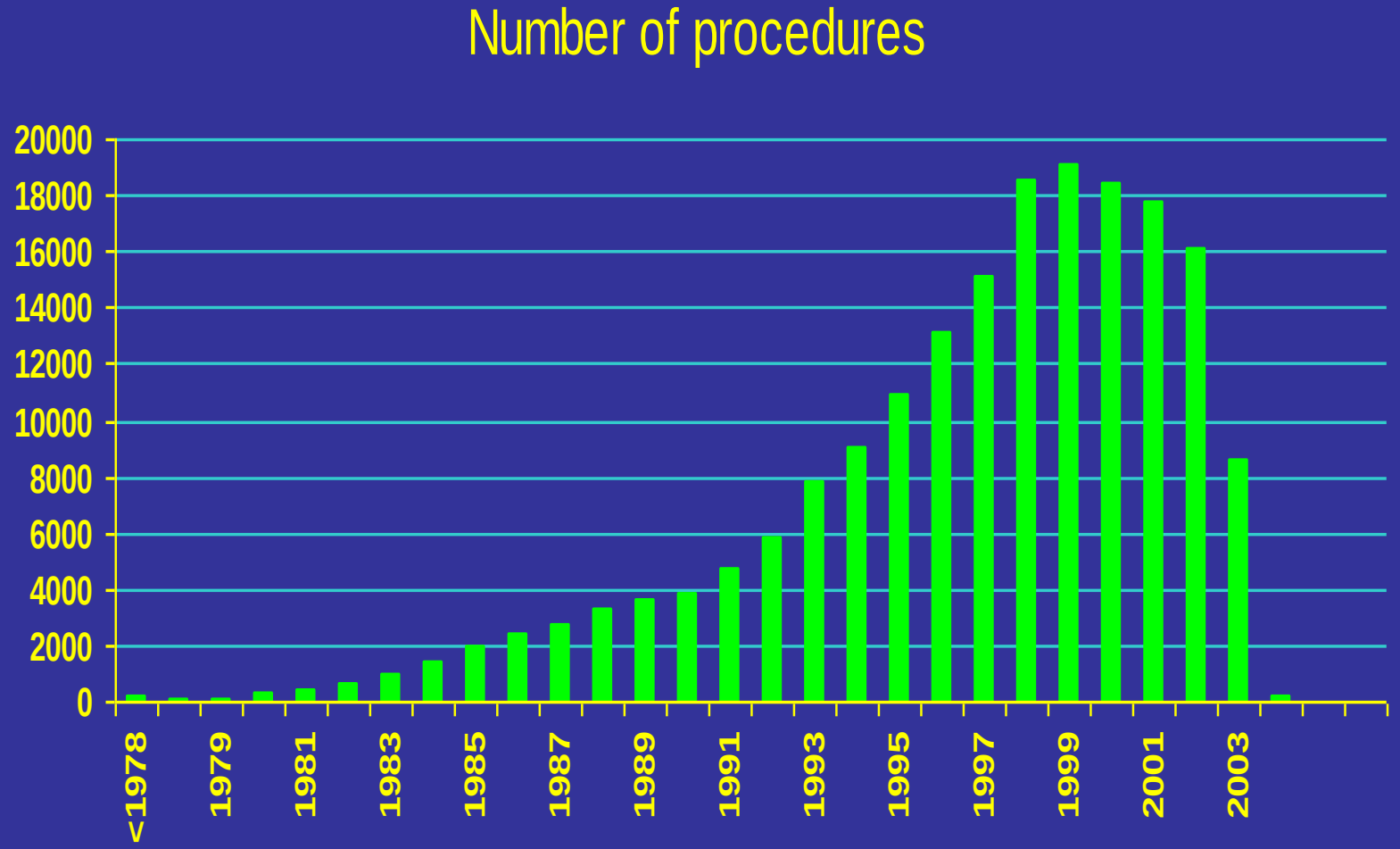

Other causes
Infection
GVHD
Relapse
100
90
80
70
60
50
40
30
20
10
0
Percent surviving
1
2
3
4
5
6
7
8
9
Years since transplant

EBMT Database

Disease	Patients	Procedures
Acute leukaemias: AML	25488	27532
Acute leukaemias: ALL	17328	18521
Acute leukaemias: other/unknown	907	1004
Chronic leukaemias: CML	15344	16526
Chronic leukaemias: CLL	1835	1942
Chronic leukaemias: other/unknown	383	433
Lymphomas: NHL	30399	33109
Lymphomas: Hodgkins	10883	11865
Lymphomas: other/unknown	1592	1863
Mutiple myeloma/Plasma cell disorders	23152	30204
Solid tumours	22973	29430
Myelodysplastic/myeloproliferative	4868	5381
Aplastic anaemias	4478	5012
Immune deficiencies	1687	1938
Other inborn errors	724	818
Autoimmune diseases	405	417
Haemoglobinopathies	2223	2314
Other/unknown	322	394
Total	164991	188703

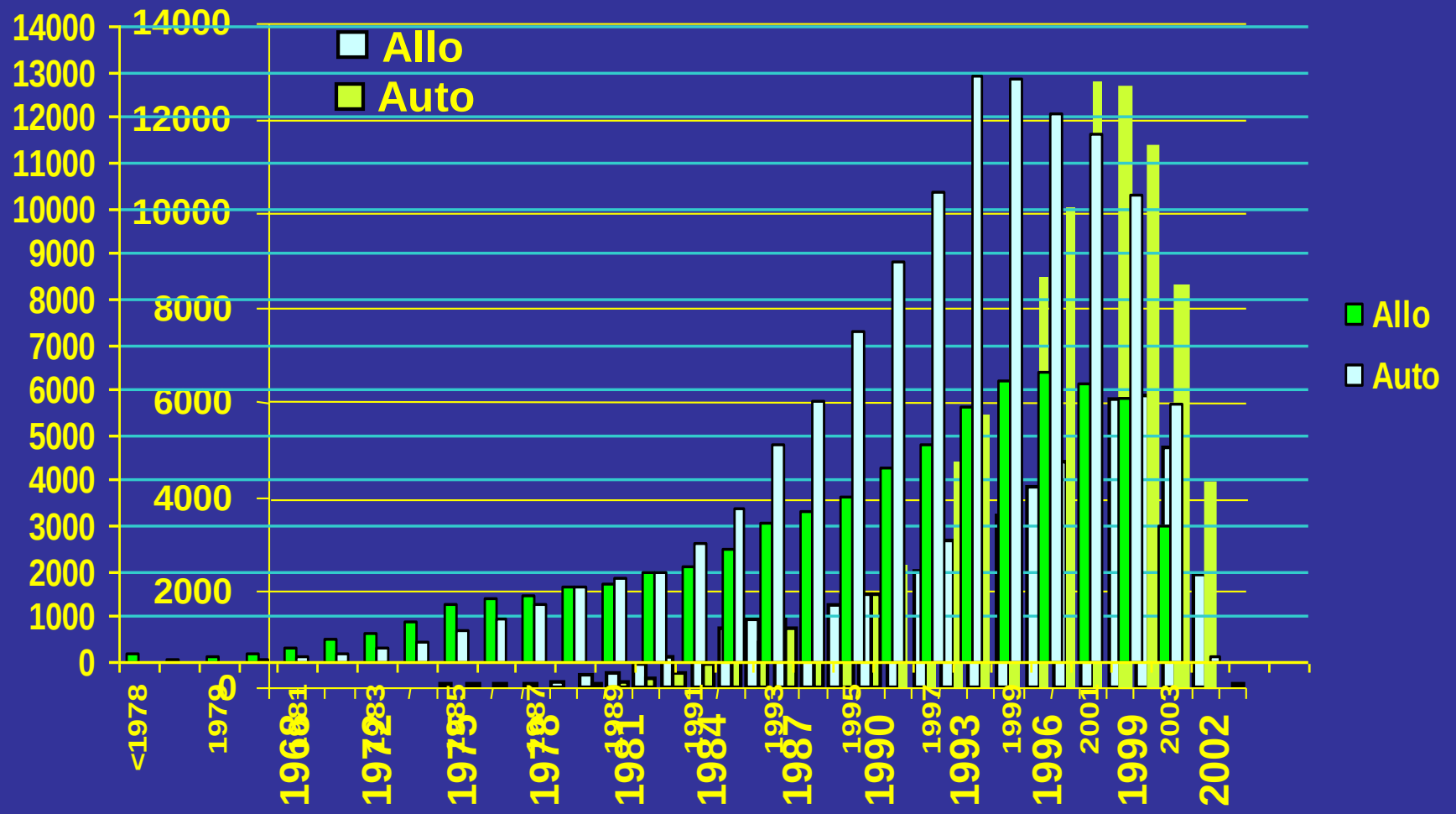
Note: Data reporting is incomplete, in particular for the most recent years

EBMT Database
Number of procedures by year



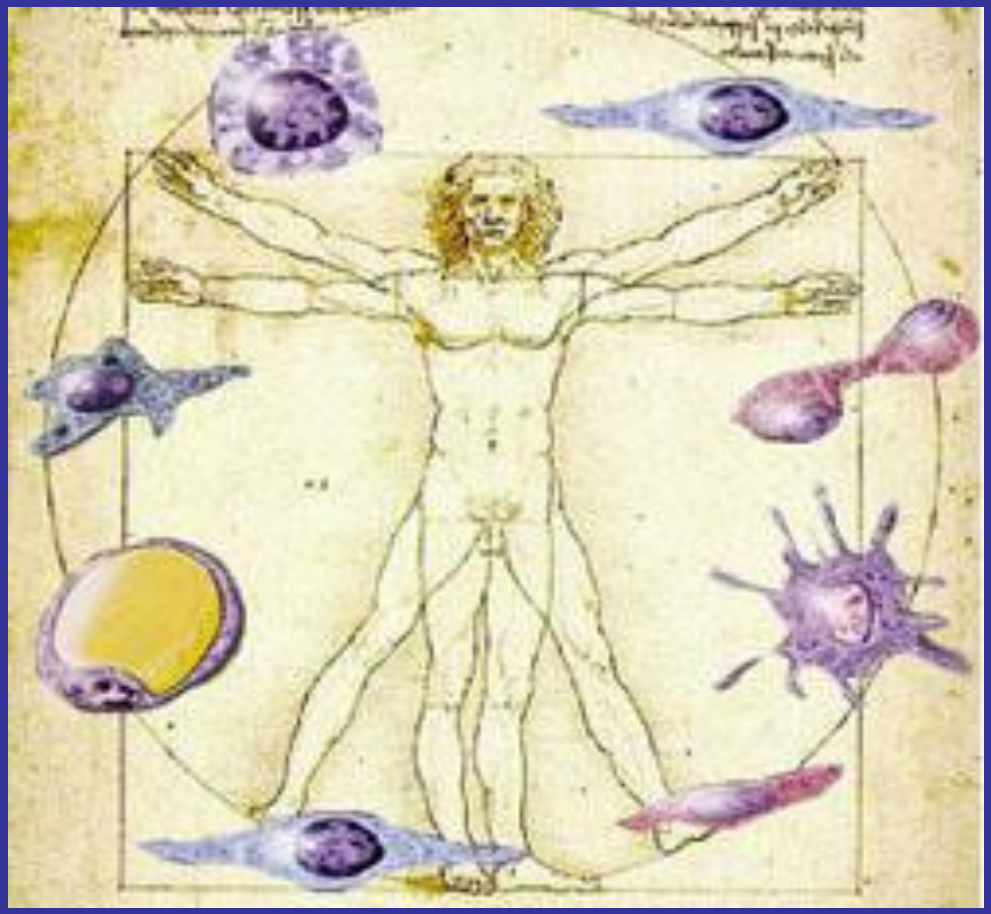
Note: Data reporting is incomplete, in particular for the most recent years

EBMT Database
Number of Procedures by Type of Transplant



Note: Data reporting is incomplete, in particular for the most recent years

Plasticity and transdifferentiate of stem cells: potential clinical impact in regenerative medicine



Chapter 10

T-cell Maturation, Activation, and Differentiation

Dr. Capers

IMMUNOLOGY

Kindt • Goldsby • Osborne

Kuby IMMUNOLOGY

Sixth Edition

Chapter 10

T-Cell Maturation, Activation, and Differentiation

- Progenitor T cells migrate from bone marrow to thymus
- T cells can be grown in vitro in absence of thymic fragments
  - Grown on bone marrow stem cells with Notch protein
    - Notch protein is key in determining T-lineage specification

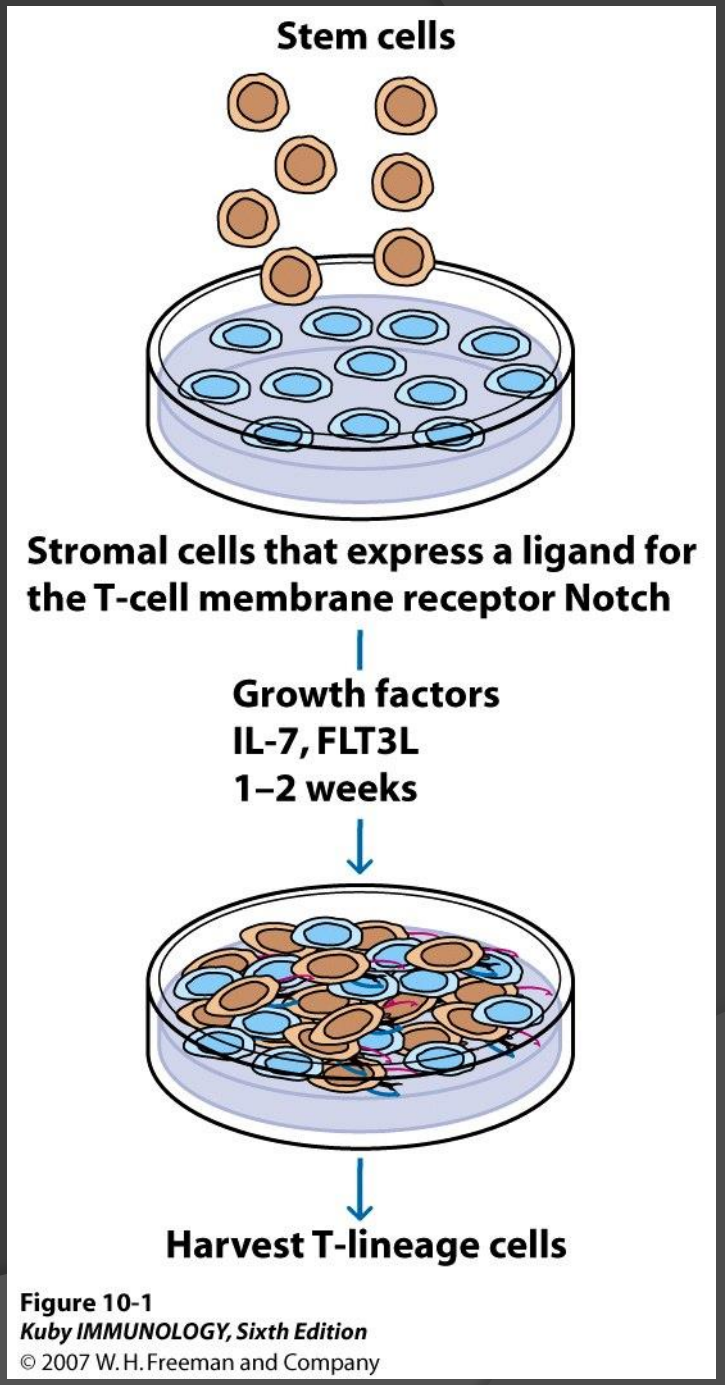


Figure 10-1
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- Progenitor T cells migrate to thymus
  - At about 8th or 9th week of gestation in humans
- T cell maturation involves rearrangements of the germ-line TCR genes
- In thymus, thymocytes proliferate and differentiate

- Selection process in thymus
  - Positive selection
    - Survival of only T cells whose TCRs recognize self-MHC molecules
  - Negative selection
    - Eliminates T cells that react too strongly with self MHC or MHC with self-peptides

T-cell Development

- Begins with arrival of small numbers of lymphoid precursors migrating from blood to thymus
  - When they do arrive in thymus, T-cell precursors don't express signature surface markers (CD3, CD4, and CD8)
  - Do not express RAG-1 or RAG-2 that are necessary for gene rearrangement

T-cell Development

- During 3 week development, differentiating T cells pass through stages of development based on surface phenotypes

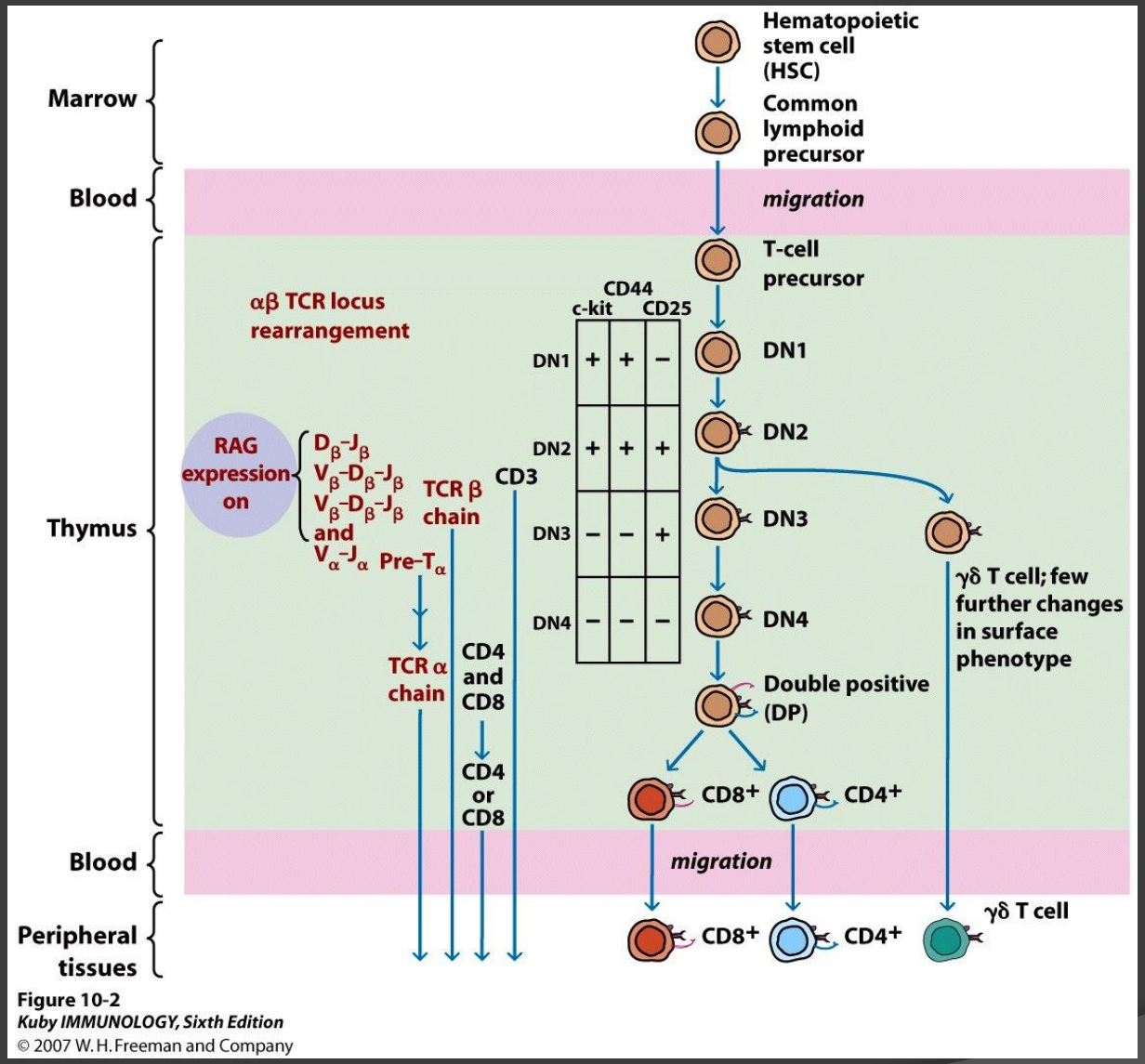

Figure 10-2
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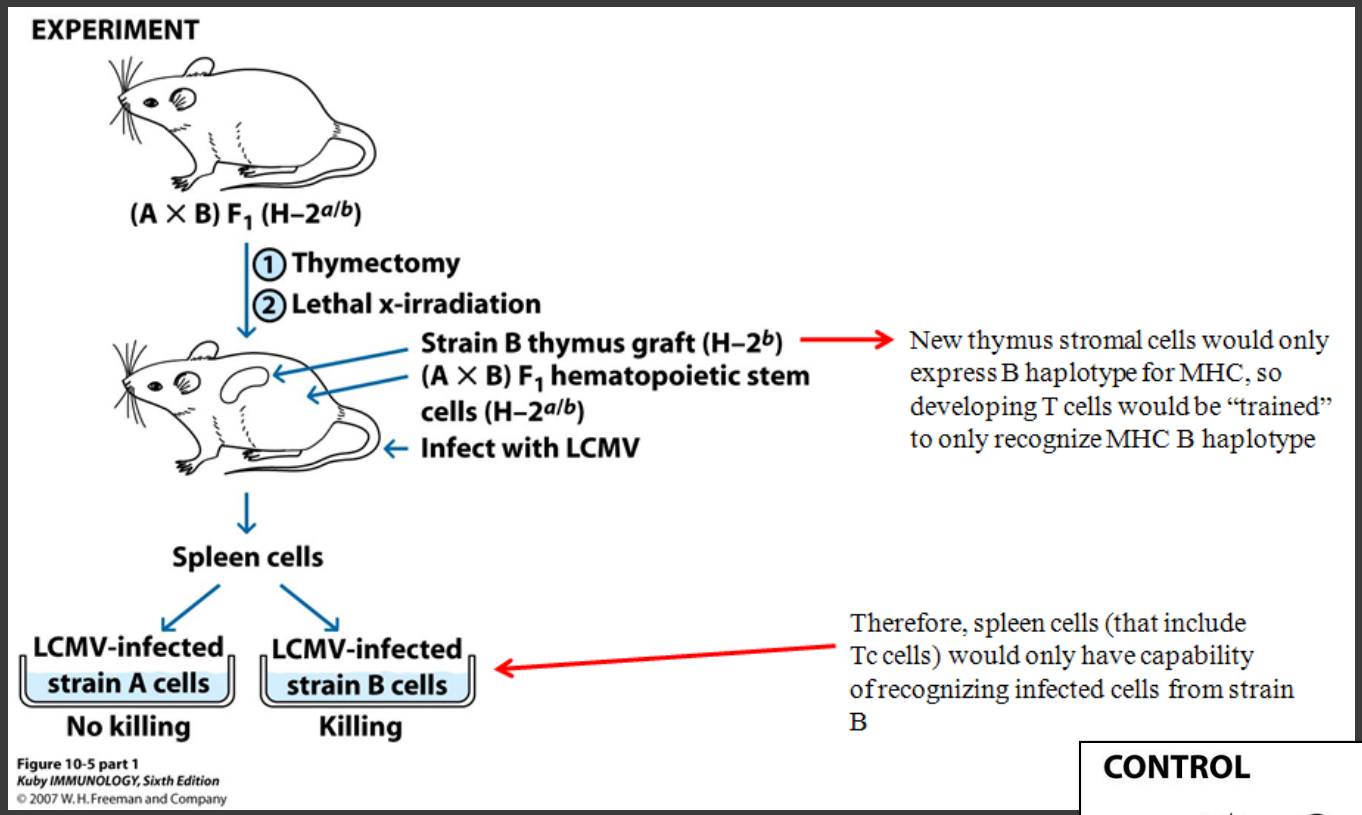
DN = Double negative
CD4- and CD8-

DP = Double positive
CD4+ and CD8+

C-kit – receptor for stem cell growth factor
CD44 – an adhesion molecule
CD25 - alpha chain of IL-2 receptor

- T cell development is expensive for host
  - 98% of all thymocytes do not mature, die by apoptosis within thymus


EXPERIMENT
(A × B) F1 (H–2a/b)
1 Thymectomy
2 Lethal x-irradiation
Strain B thymus graft (H–2b)
(A × B) F1 hematopoietic stem cells (H–2a/b)
Infect with LCMV
New thymus stromal cells would only express B haplotype for MHC, so developing T cells would be "trained" to only recognize MHC B haplotype
Spleen cells
LCMV-infected strain A cells
No killing
LCMV-infected strain B cells
Killing
Therefore, spleen cells (that include Tc cells) would only have capability of recognizing infected cells from strain B
Figure 10-5 part 1
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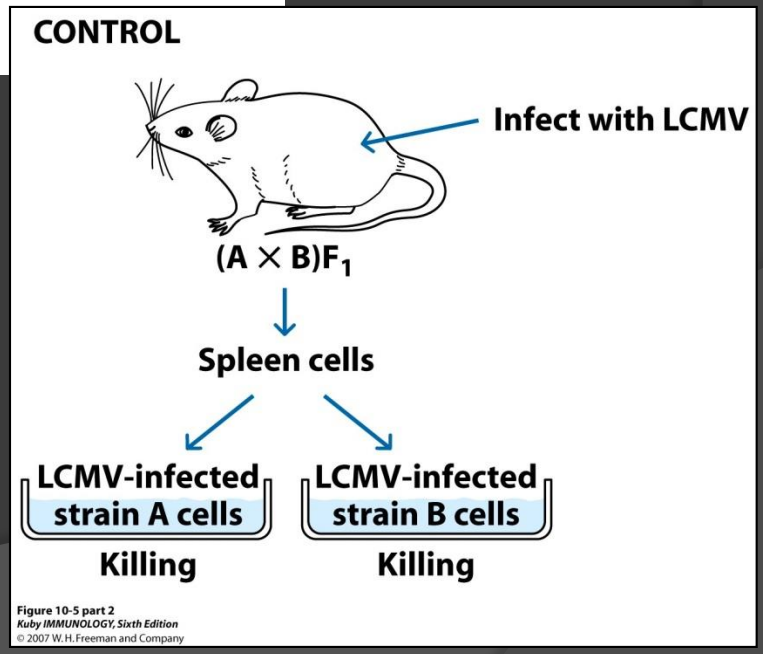
CONTROL
Infect with LCMV
(A × B)F1
Spleen cells
LCMV-infected strain A cells
Killing
LCMV-infected strain B cells
Killing
Figure 10-5 part 2
Kuby IMMUNOLOGY, Sixth Edition
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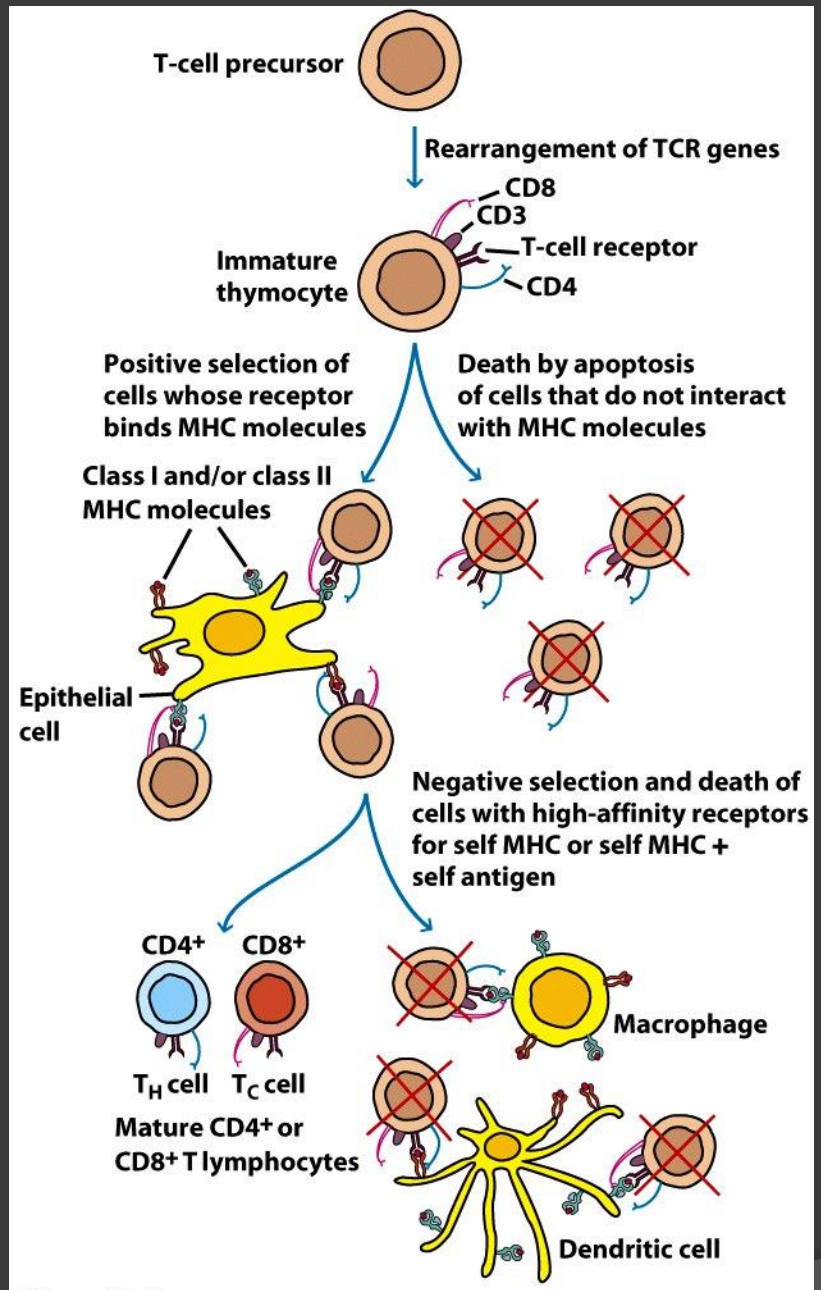

Figure 10-6
Kuby IMMUNOLOGY, Sixth Edition

TABLE 10-1 Effect of class I or II MHC deficiency on thymocyte populations*

Cell type	Control mice	KNOCKOUT MICE: Class I deficient	KNOCKOUT MICE: Class II deficient
$CD4^-CD8^-$	+	+	+
$CD4^+CD8^+$	+	+	+
$CD4^+$	+	+	−
$CD8^+$	+	−	+

*Plus sign indicates normal distribution of indicated cell types in thymus. Minus sign indicates absence of cell type.

Table 10-1
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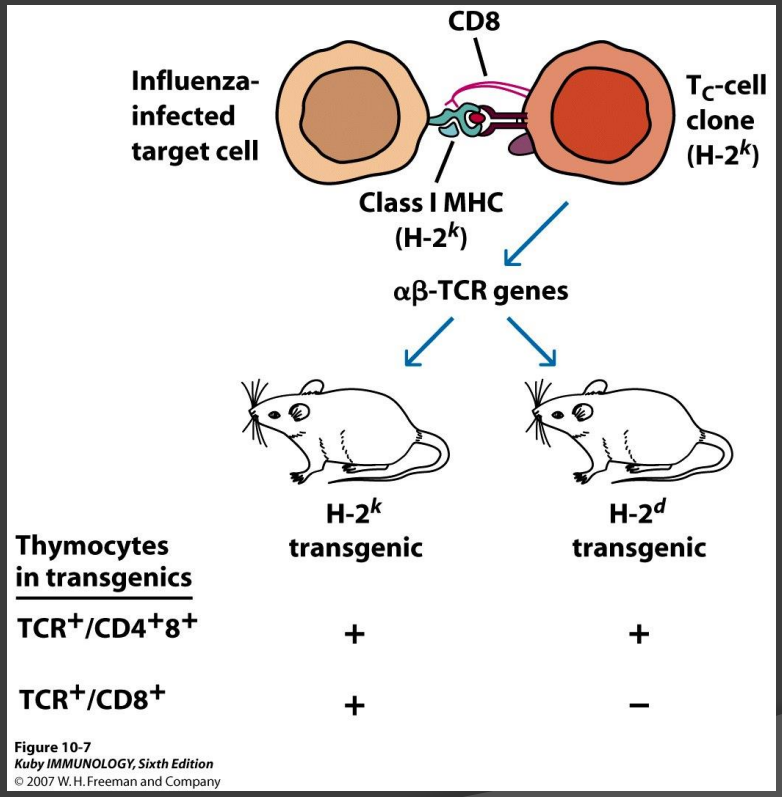


Figure 10-7
Kuby IMMUNOLOGY, Sixth Edition

Insertion of rearranged TCR genes suppress other gene rearrangements in these mice

T cell Activation

- Initiated by TCR-CD3 complex with processed antigen on MHC molecule
  - CD8+ cells with Class I
  - CD4+ cells with Class II
- Initiates cascade of biochemical events
  - Inducing resting T cell to enter cell cycle, proliferate, differentiate into memory and effector T cells

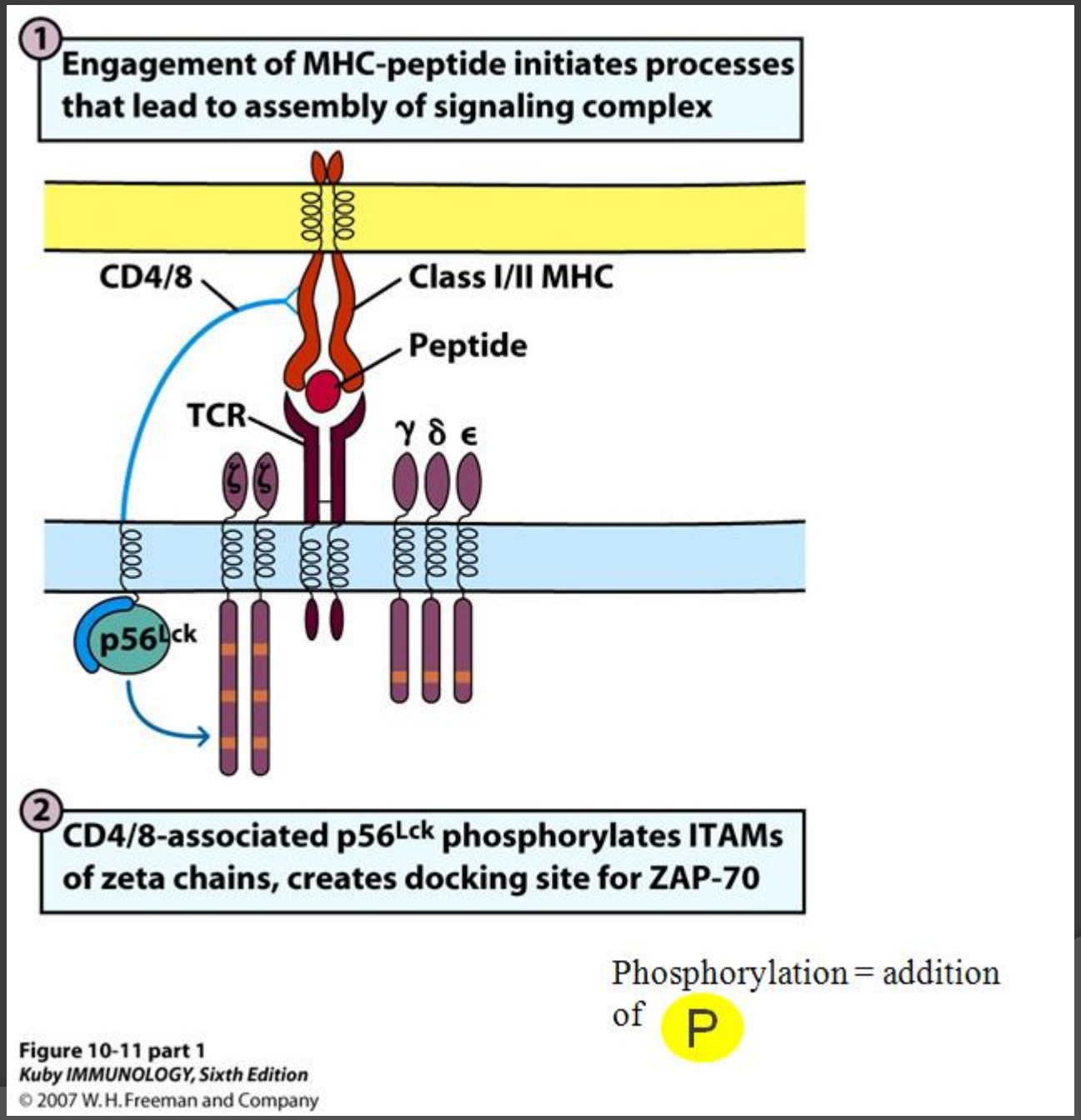
T cell Activation

- Cascade of biochemical events leading to gene expression:
  - Interaction of signal and molecule (example: TCR + MHC and antigen)
  - Generation of “second messenger” that diffuses to other areas of cell
  - Protein kinases and protein phosphatases are activated or inhibitied
  - Signals are amplified by enzyme cascades

Click on link to see example → http://www.youtube.com/watch?v=tMMrTRnFdI4&feature=player_detailpage

T cell Activation

- Gene products after activation
  - Immediate genes (1/2 hour of recognition)
    - Transcription factors (c-Myc, NFAT, NF-κB)
  - Early genes (1-2 hours from recognition)
    - IL-2, IL2R, IL-6, IFN-γ
  - Late genes (more than 2 days later)
    - Encode adhesion molecules



Phosphorylation = addition of P

Figure 10-11 part 1
Kuby IMMUNOLOGY, Sixth Edition

Go onto Next slide

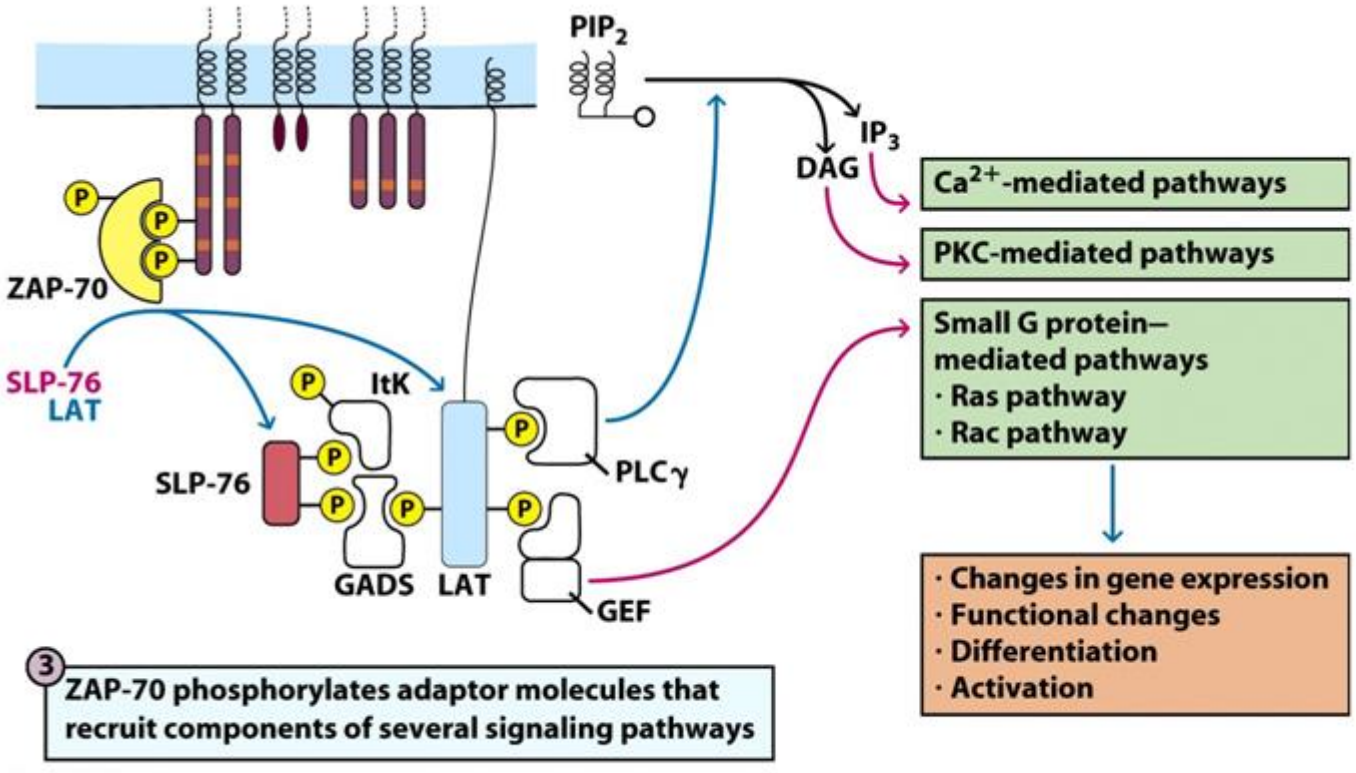


Figure 10-11 part 2
Kuby IMMUNOLOGY, Sixth Edition

Calcium release from ER results in phosphorylation of transcription factors that are required for expression of T-cell-growth-promoting cytokines

Results in activation of transcription factors

Superantigens

- Bind to BOTH the TCR and MHC
- Can cause over-activation
  - Overproduction of T_H-cell cytokines, leading to systemic toxicity
- Exogeneous
  - Variety of exotoxins secreted by some Gram+ bacteria
- Endogeneous
  - Cell membrane proteins encoded by viruses

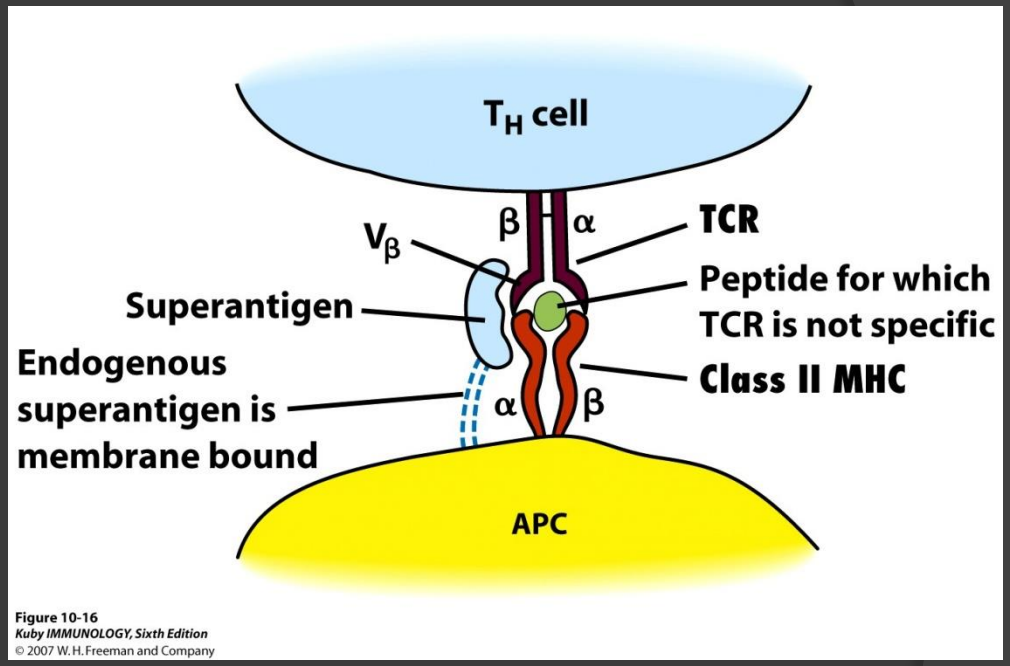


Figure 10-16
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TABLE 10-3 Exogenous superantigens and their V_β specificity

Superantigen	Disease*	V_β SPECIFICITY	
		Mouse	Human
Staphylococcal enterotoxins			
SEA	Food poisoning	1, 3, 10, 11, 12, 17	nd
SEB	Food poisoning	3, 8.1, 8.2, 8.3	3, 12, 14, 15, 17, 20
SEC1	Food poisoning	7, 8.2, 8.3, 11	12
SEC2	Food poisoning	8.2, 10	12, 13, 14, 15, 17, 20
SEC3	Food poisoning	7, 8.2	5, 12
SED	Food poisoning	3, 7, 8.3, 11, 17	5, 12
SEE	Food poisoning	11, 15, 17	5.1, 6.1–6.3, 8, 18
Toxic shock syndrome toxin (TSST1)	Toxic shock syndrome	15, 16	2
Exfoliative dermatitis toxin (ExFT)	Scalded skin syndrome	10, 11, 15	2
Mycoplasma arthritidis supernatant (MAS)	Arthritis, shock	6, 8.1–8.3	nd
Streptococcal pyrogenic exotoxins (SPE-A, B, C, D)	Rheumatic fever, shock	nd	nd

*Disease results from infection by bacteria that produce the indicated superantigens.

Table 10-3
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T-Cell Differentiation

- CD4+ and CD8+ cells leave thymus and enter circulation in G_0 phase
  - Naïve cells (condensed chromatin, little cytoplasm)
  - About twice as many CD4+
- Naïve cell recognized MHC-antigen complex
  - Initiated primary response
  - After 48 hours, enlarges into blast cell and undergoes repeated rounds of cell division
  - Differentiate into:
    - Effector cells – cytokine secretion, B-cell help
    - Memory cells – long lived, respond with heightened activity (secondary response)

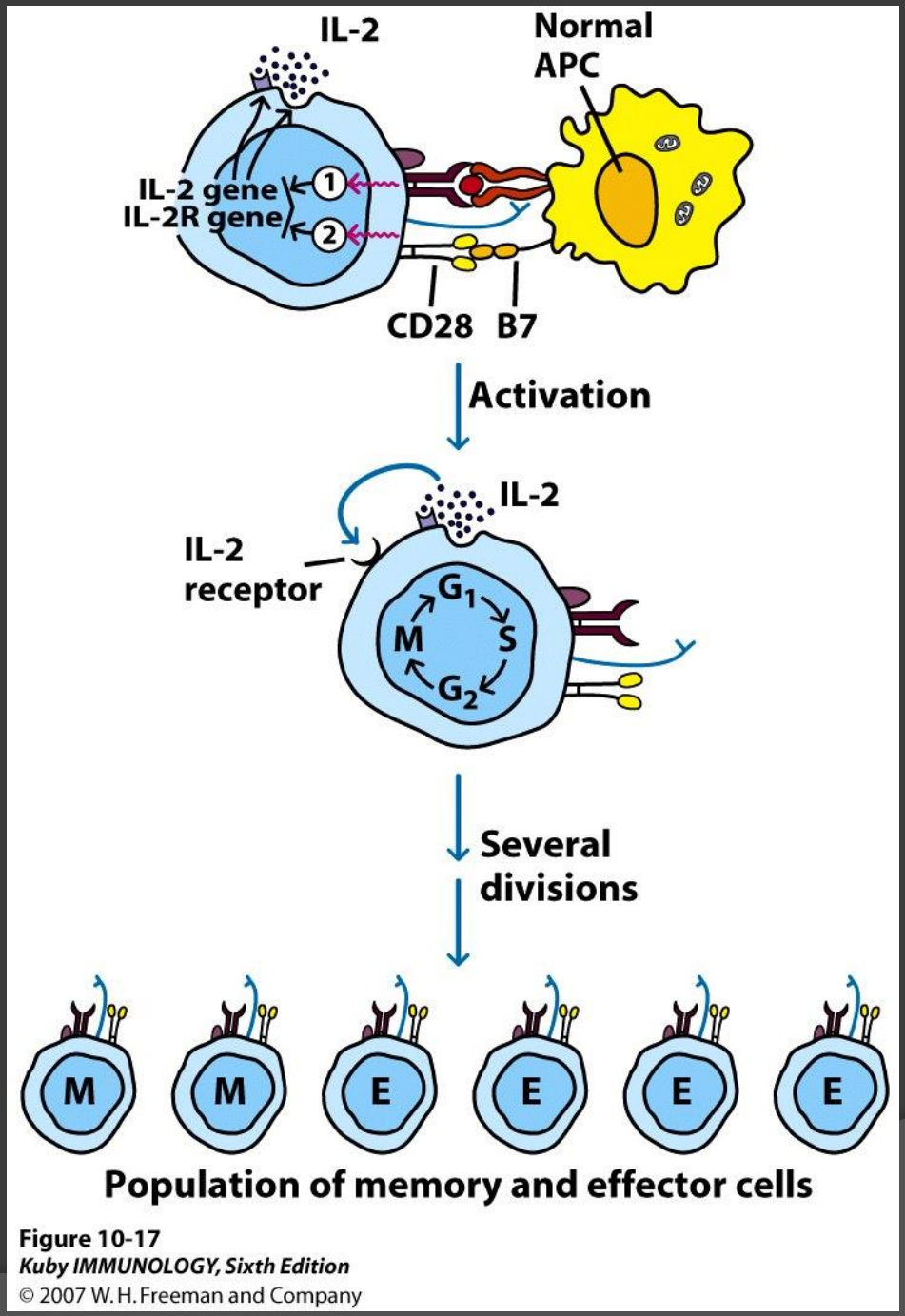

Figure 10-17
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T_{reg} Cells

- Shown to inhibit proliferation of other T cells in vitro
- CD4+CD25+
- Shown to inhibit development of autoimmune diseases

Cell Death and T Cell Populations

- Apoptosis plays critical role
  - Deletion of potentially autoreactive thymocytes
  - Deletion of T cell populations after activation
    - Fas and FasL pathway to induce self death

Chapter 16

Tolerance and Autoimmunity and Transplants

Dr. Capers

IMMUNOLOGY

Kindt • Goldsby • Osborne

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Chapter 16

Tolerance and Autoimmunity

“Horror Autotoxicus”

- Failure of host’s humoral and cellular immune systems to distinguish self from non-self
  - Autoimmunity
  - Can result in tissue and organ damage, can be fatal

TABLE 16-1 Some autoimmune diseases in humans

Disease	Self antigen	Immune response
	ORGAN-SPECIFIC AUTOIMMUNE DISEASES	
Addison's disease	Adrenal cells	Auto-antibodies
Autoimmune hemolytic anemia	RBC membrane proteins	Auto-antibodies
Goodpasture's syndrome	Renal and lung basement membranes	Auto-antibodies
Graves' disease	Thyroid-stimulating hormone receptor	Auto-antibody (stimulating)
Hashimoto's thyroiditis	Thyroid proteins and cells	T_H1 cells, auto-antibodies
Idiopathic thrombocyopenia purpura	Platelet membrane proteins	Auto-antibodies
Insulin-dependent diabetes mellitus	Pancreatic beta cells	T_H1 cells, auto-antibodies
Myasthenia gravis	Acetylcholine receptors	Auto-antibody (blocking)
Myocardial infarction	Heart	Auto-antibodies
Pernicious anemia	Gastric parietal cells; intrinsic factor	Auto-antibody
Poststreptococcal glomerulonephritis	Kidney	Antigen-antibody complexes
Spontaneous infertility	Sperm	Auto-antibodies
	SYSTEMIC AUTOIMMUNE DISEASES	
Ankylosing spondylitis	Vertebrae	Immune complexes
Multiple sclerosis	Brain or white matter	T_H1 cells and T_C cells, auto-antibodies
Rheumatoid arthritis	Connective tissue, IgG	Auto-antibodies, immune complexes
Scleroderma	Nuclei, heart, lungs, gastrointestinal tract, kidney	Auto-antibodies
Sjögren's syndrome	Salivary gland, liver, kidney, thyroid	Auto-antibodies
Systemic lupus erythematosus (SLE)	DNA, nuclear protein, RBC and platelet membranes	Auto-antibodies, immune complexes

Table 16-1
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Tolerance

- # of mechanisms are in place to protect individual from self-reactive lymphocytes
  - Central tolerance – deleting T or B clones before maturity if they have receptors that recognize self-antigens with great affinity
  - Peripheral tolerance – kills lymphocytes in secondary lymphoid tissue
    - Also, life span of lymphocytes regulated by apoptosis

Central tolerance

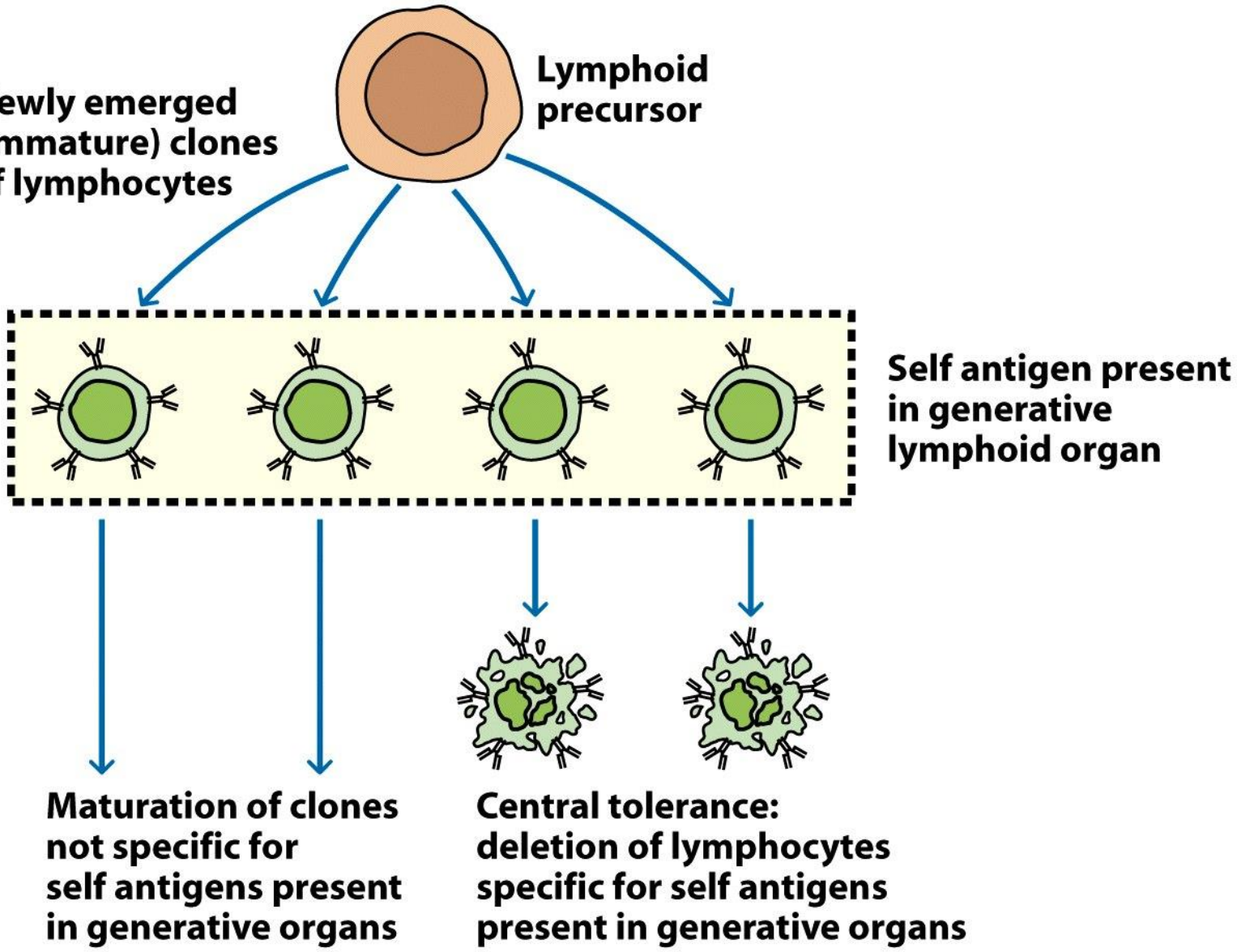

Figure 16-1a
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Peripheral tolerance

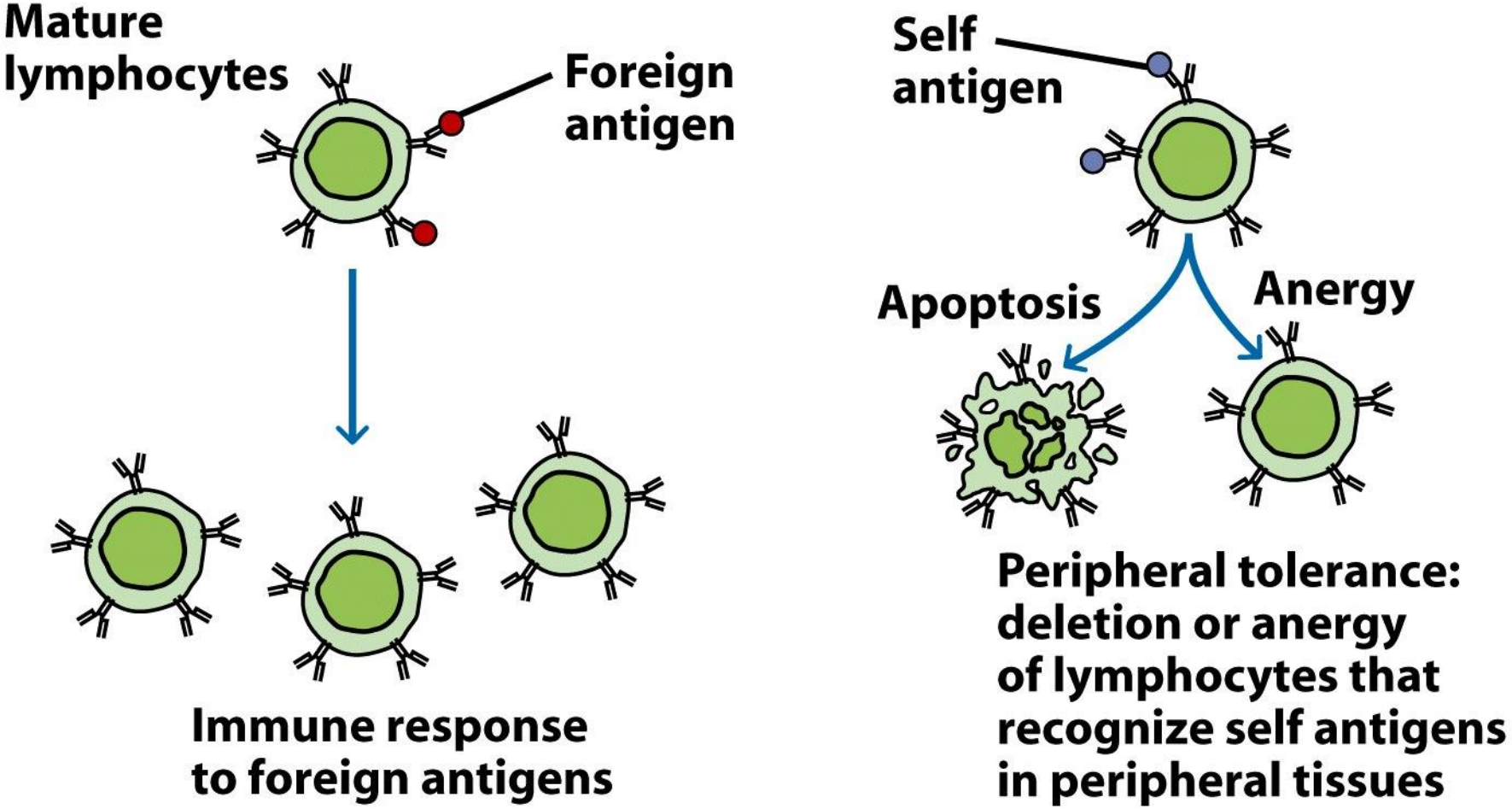

Figure 16-1b
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- Some antigens can produce tolerance
  - Termed tolerogens rather than immunogens
    - High dosages of antigen
    - Persistance of antigen in host
    - IV or oral introduction
    - Absence of adjuvants
    - Low levels of costimulators
      - CD28 will bind to B7 and provide activating signals; however, it was discovered that another receptor, CTLA-4 will bind to B7 and inhibit

- Anergy
  - Unresponsiveness to antigenic stimulus

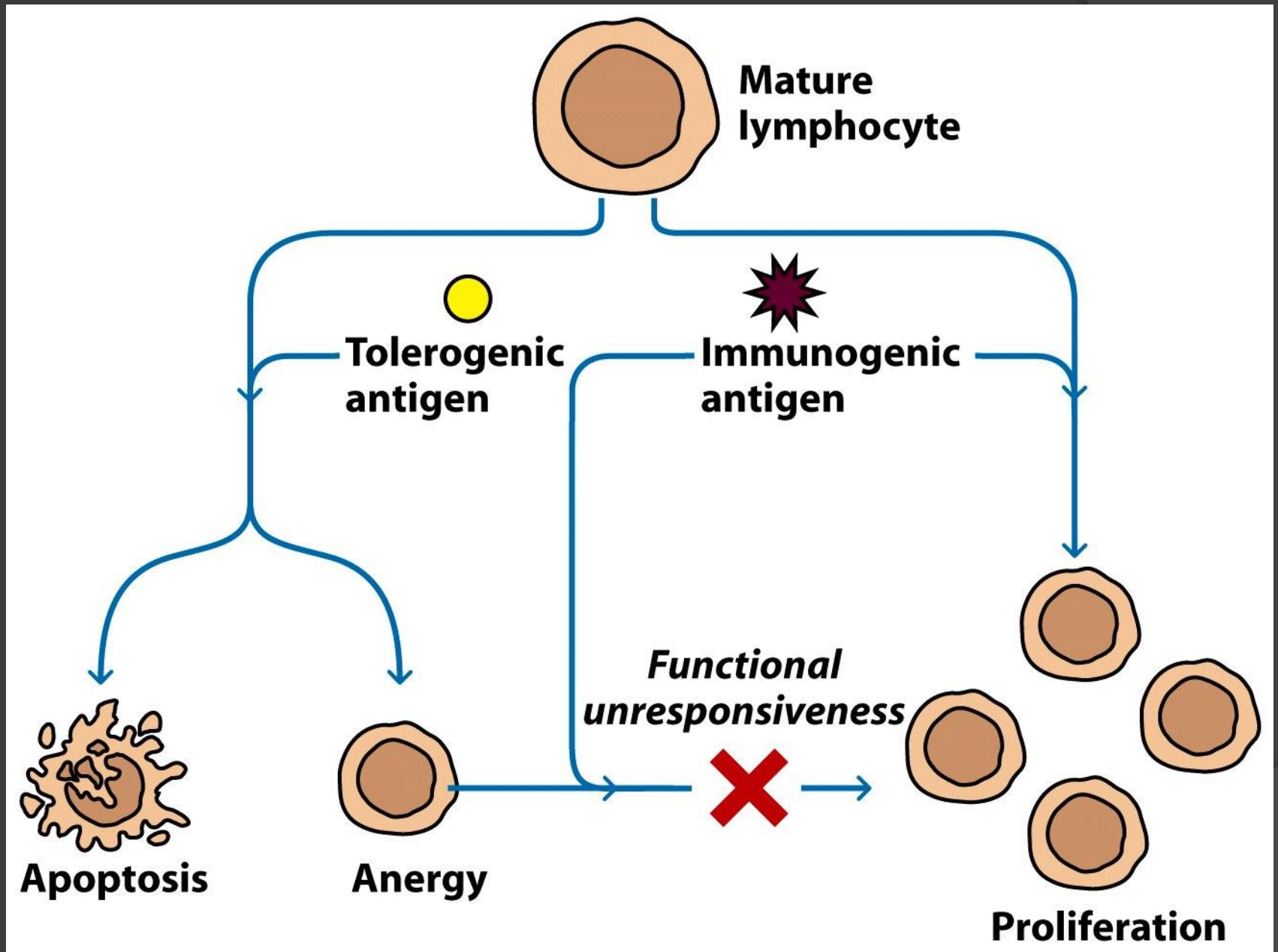

Figure 16-2
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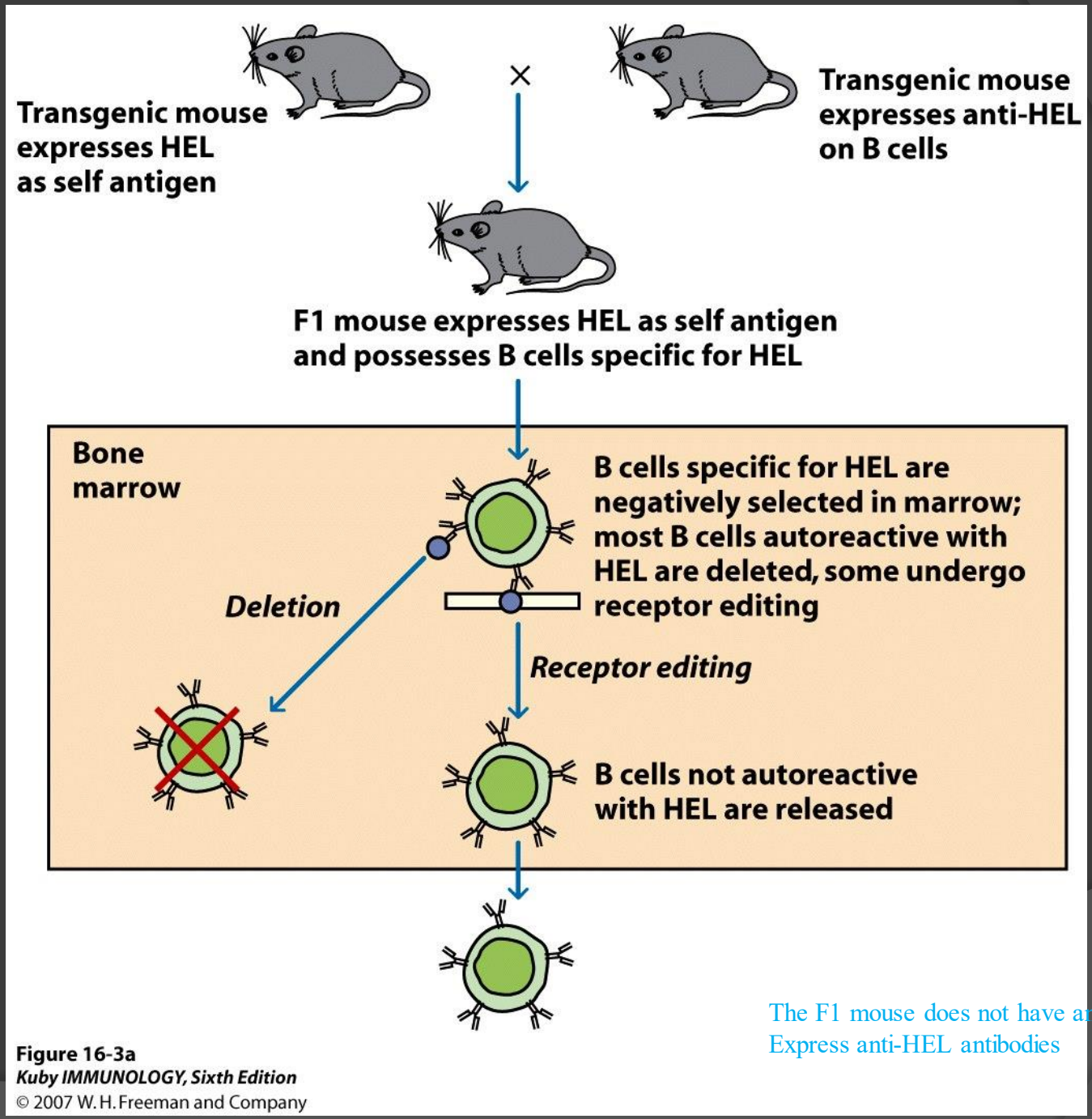

The F1 mouse does not have any B cells that Express anti-HEL antibodies

Figure 16-3a
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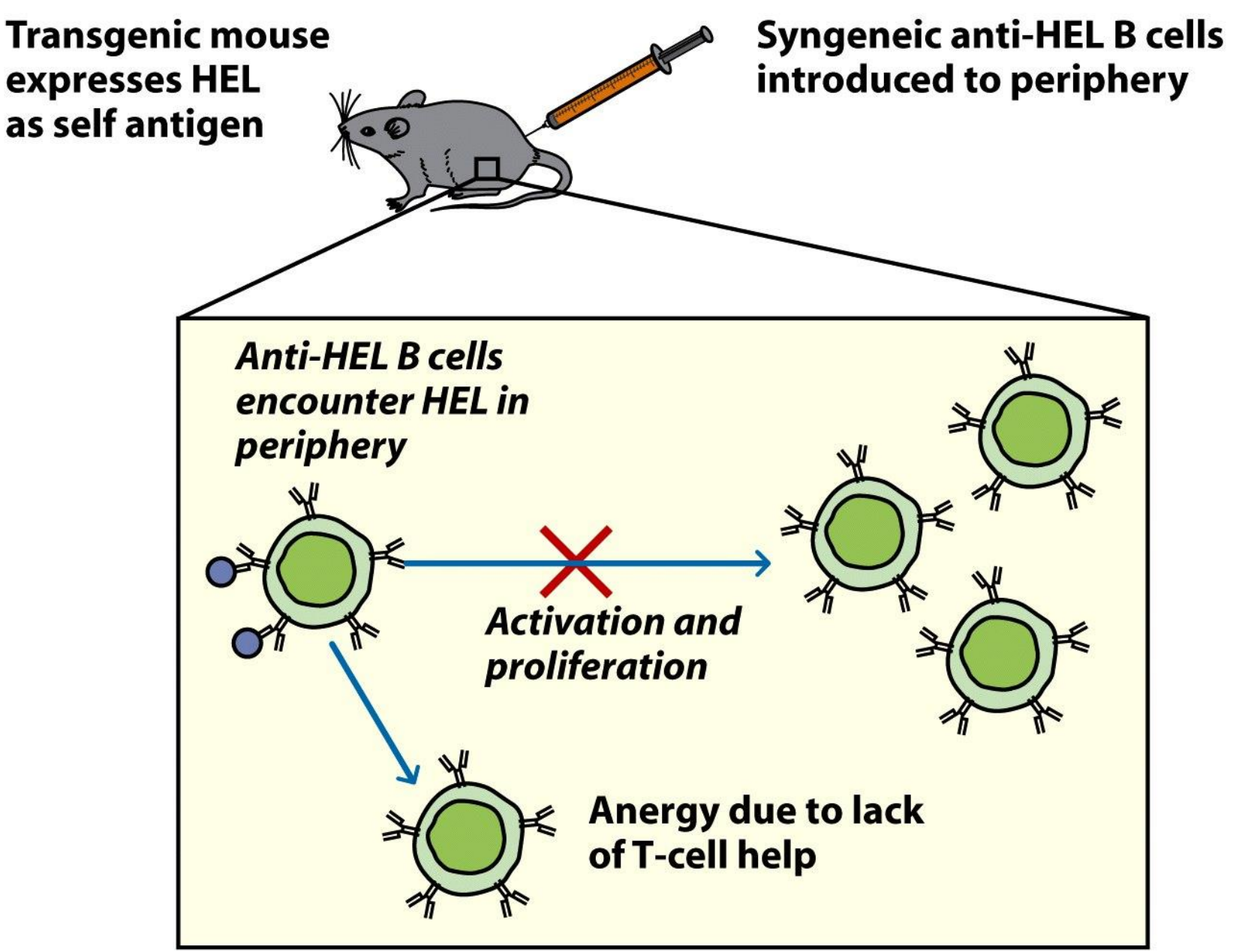

Figure 16-3b
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- Peripheral Tolerance
  - May be induced by T_{reg} cells
    - Unique group of CD4+ T cells
    - Recognize self-antigens on immune system cells and seem to be able to suppress immune system
    - Induce cell death in some immune cells

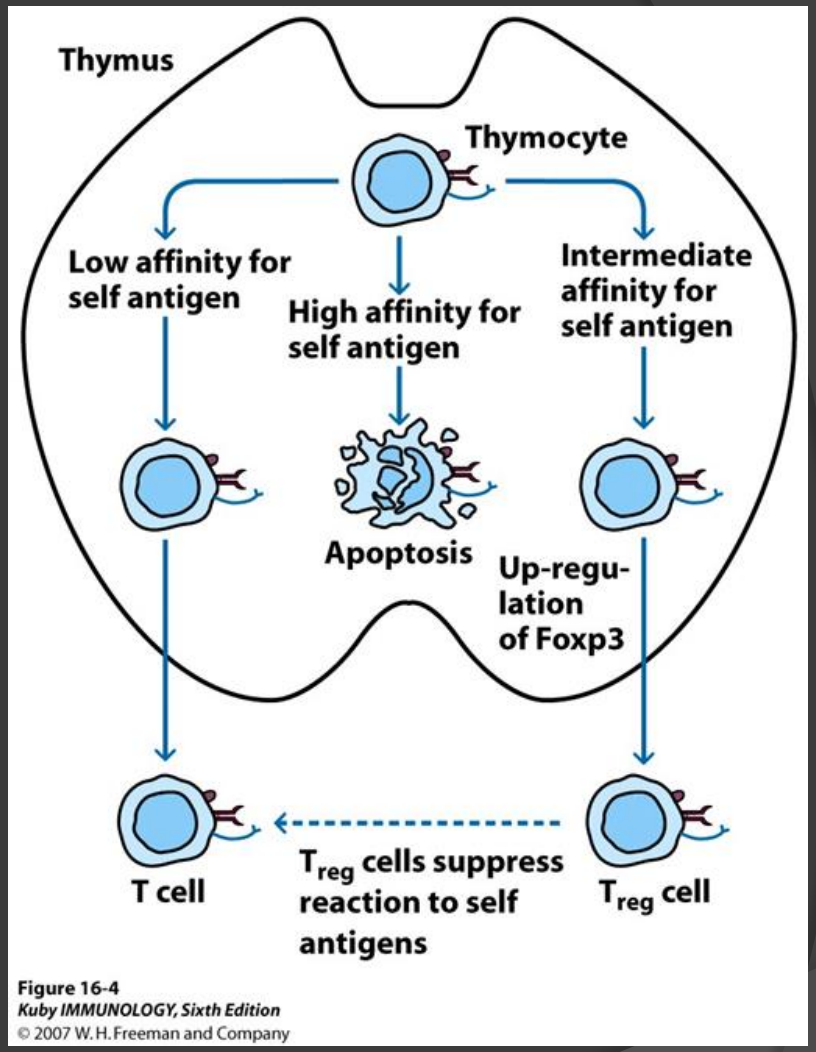

Figure 16-4
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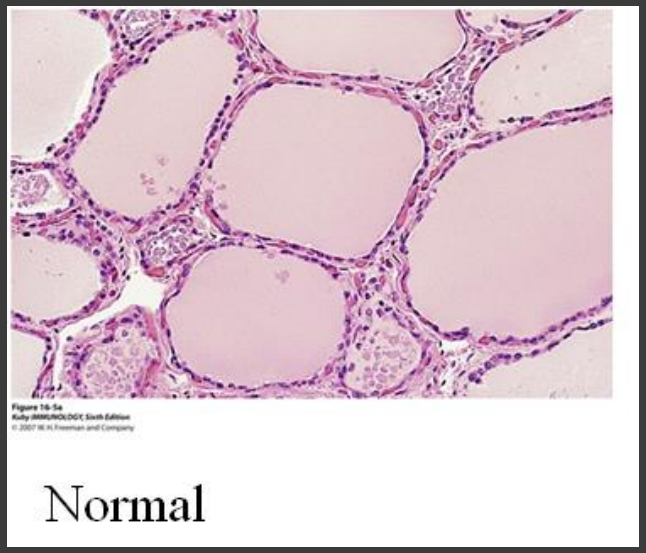
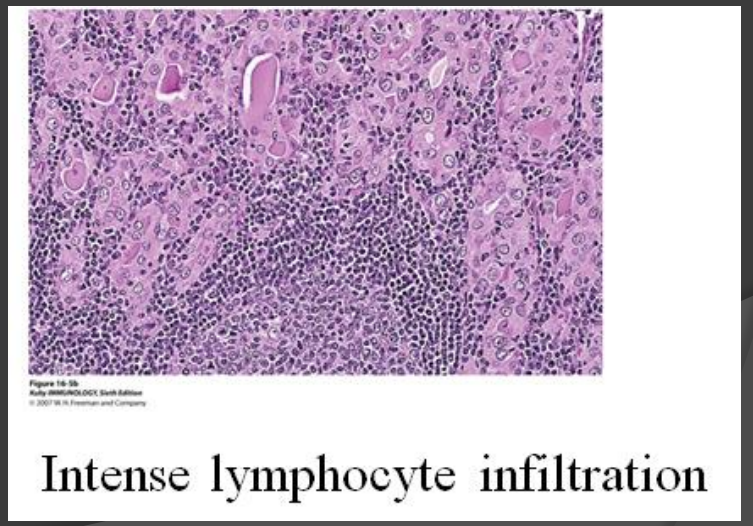
Organ-specific autoimmune diseases

- Target antigen specific to organ or gland
- Cellular lysis and chronic inflammation that can damage organ

- Hashimoto's Thyroiditis
  - Mainly middle-aged women
  - Target is thyroid antigens
  - Goiter can form
  - Hypothyroidism - decrease



Normal



Intense lymphocyte infiltration

- Autoimmune anemias
  - Pernicious anemia
    - Ab against membrane bound intestinal protein that uptakes B_{12} - needed for hematopoiesis
  - Hemolytic anemia
    - Abs to red-blood cell antigens
  - Drug-induced anemia

- Goodpasture's syndrome
  - Abs against basement membranes in glomeruli and aveoli
  - Leads to kidney damage and pulmonary hemmorhage

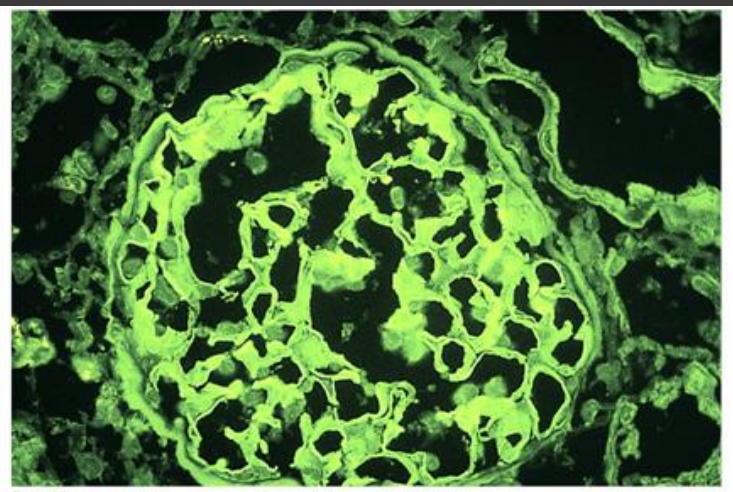


Figure 16-6
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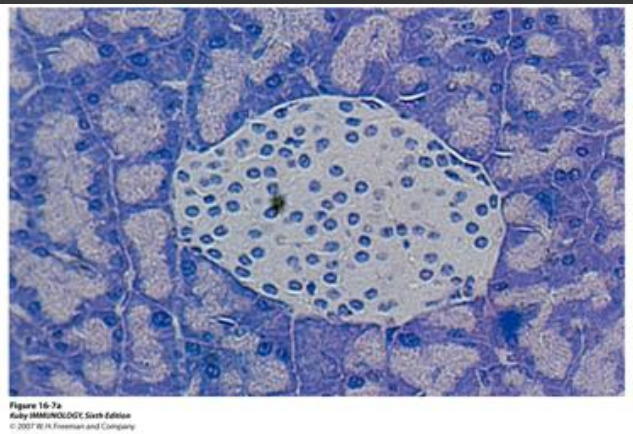
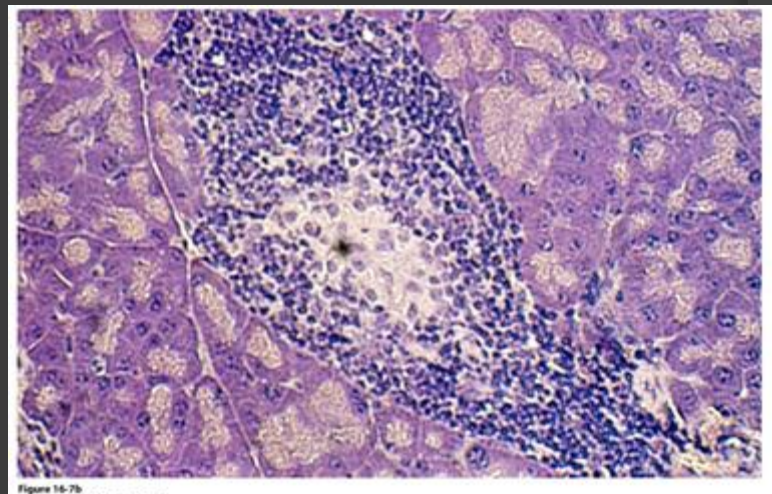
Glomerulus of kidney – fluorescent labeled anti-IgG reveals a large amount of IgG (autoantibodies) attached to glomerulus

- Insulin-Dependent Diabetes Mellitus
  - Abs against beta cells that produce insulin
  - Insulin is needed by cells to uptake glucose needed for cellular respiration



Normal islet with beta cells in pancreas



Islet that is infiltrated by lymphocytes

- In some autoimmune diseases, antibodies act as agonists
  - Bind inappropriately to receptors, resulting in overproduction
    - For example, up-regulating a hormonal response without the presence of that hormone
    - Grave's Disease – auto-Ab binds to receptor for thyroid stimulating hormone resulting in over-stimulation of thyroid
    - Myasthenia gravis
      - Auto-Abs bind acetylcholine receptors on motor end plate of muscles – progressively weakened skeletal muscles

STIMULATING AUTO-ANTIBODIES (Graves' disease)

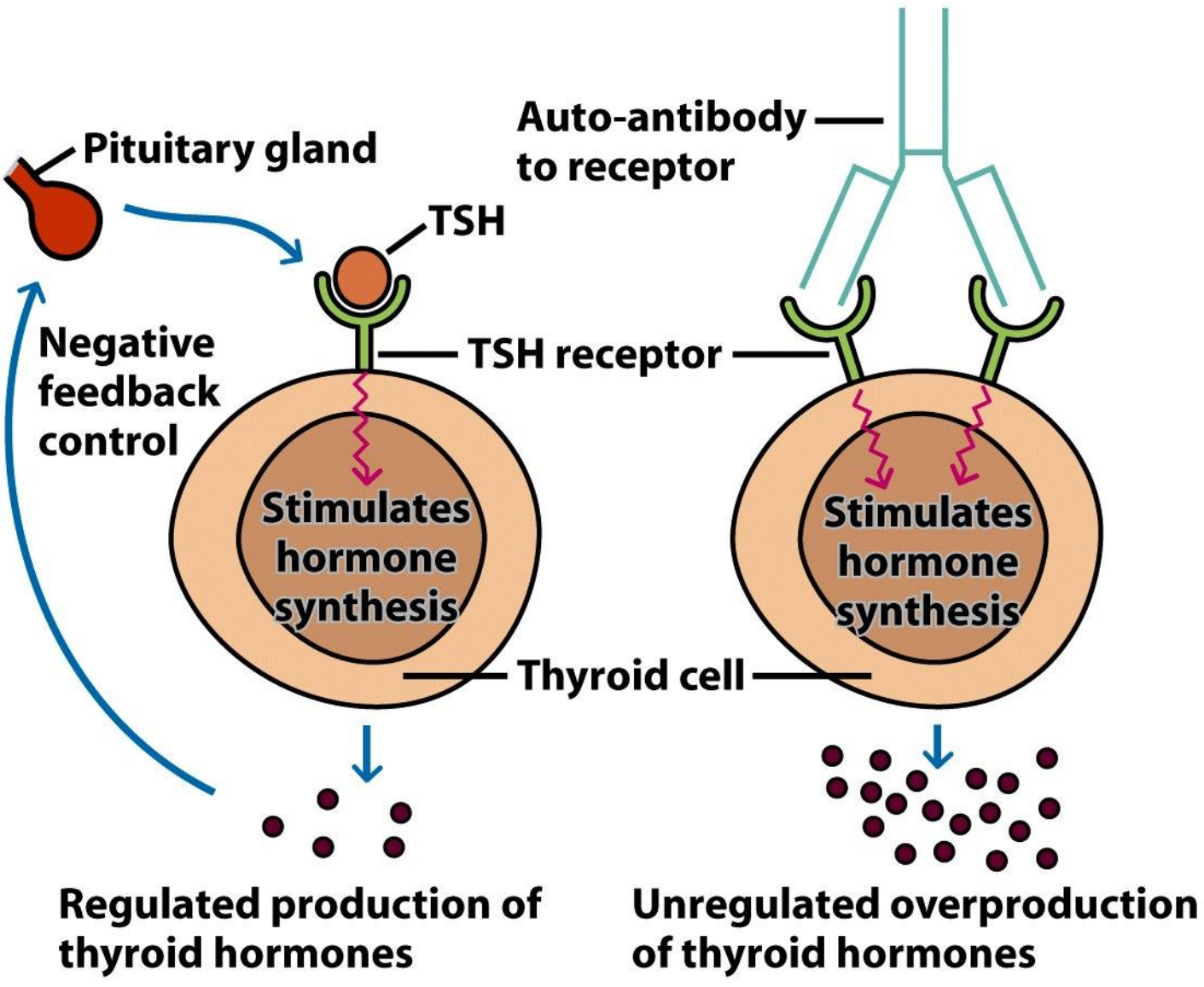


Figure 16-8
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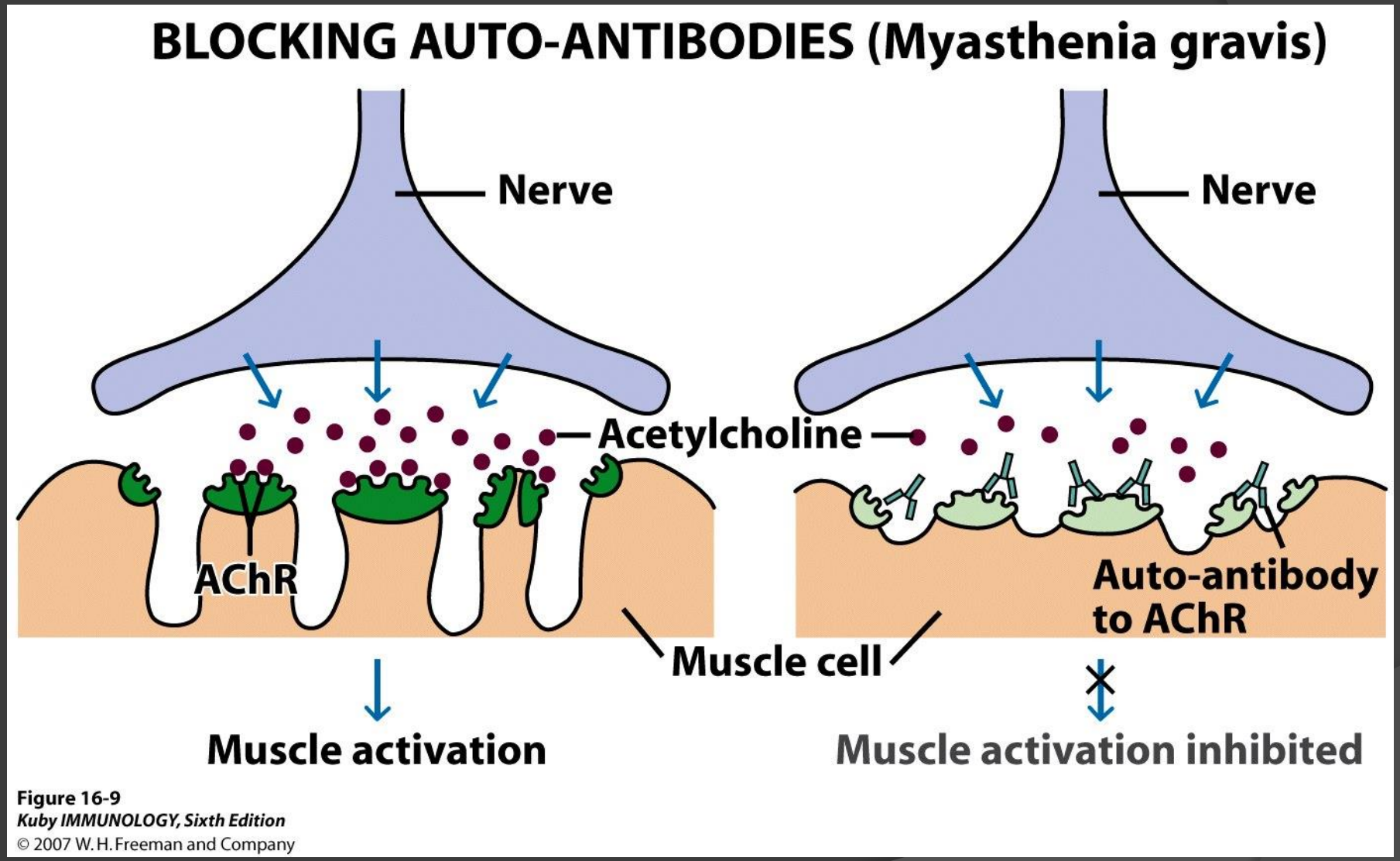

Figure 16-9
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Systemic Autoimmune Diseases

- Response is directed toward wide range of target antigens

- Systemic Lupus Erythematosus
  - Typically middle-aged women
  - Fever, weakness, arthritis, skin rash, kidney problems
  - Produce auto-Abs to DNA, histones, platelets, leukocytes, clotting factors
  - Excessive complement activation

- Multiple sclerosis
  - Numbness, paralysis, vision loss
  - Inflammatory lesions in myelin sheath caused by T cells
  - Epidemiology
    - Frequent in African American and Hispanic women
    - More common in Northern Hemisphere, more common north of 37th parallel
    - Environmental components as well as genetic components

- Rheumatoid Arthritis
  - Chronic inflammation of joints
  - Produce auto-Abs that bind Fc portion of IgG circulating in blood that creates immune complexes

Animal Models

- Autoimmunity develops spontaneously in some lab animals and can be induced with manipulation
  - Rabbits injected with acetylcholine receptors from eels
    - Soon developed muscular weakness as seen with Myasthenia gravis

TABLE 16-2 Experimental animal models of autoimmune diseases

Animal model	Possible human disease counterpart	Inducing antigen	Disease transferred by T cells
SPONTANEOUS AUTOIMMUNE DISEASES			
Nonobese diabetic (NOD) mouse	Insulin-dependent diabetes mellitus (IDDM)	Unknown	Yes
(NZB × NZW) F_1 mouse	Systemic lupus erythematosus (SLE)	Unknown	Yes
Obese-strain chicken	Hashimoto's thyroiditis	Thyroglobulin	Yes
EXPERIMENTALLY INDUCED AUTOIMMUNE DISEASES*			
Experimental autoimmune myasthenia gravis (EAMG)	Myasthenia gravis	Acetylcholine receptor	Yes
Experimental autoimmune encephalomyelitis (EAE)	Multiple sclerosis (MS)	Myelin basic protein (MBP); proteolipid protein (PLP)	Yes
Autoimmune arthritis (AA)	Rheumatoid arthritis	*M. tuberculosis* (proteoglycans)	Yes
Experimental autoimmune thyroiditis (EAT)	Hashimoto's thyroiditis	Thyroglobulin	Yes

*These diseases can be induced by injecting appropriate animals with the indicated antigen in complete Freund's adjuvant. Except for autoimmune arthritis, the antigens used correspond to the self antigens associated with the human disease counterpart. Rheumatoid arthritis involves reaction to proteoglycans, which are self antigens associated with connective tissue.

Table 16-2
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- Animal models have implicated CD4+ T cells to be primary mediator of some autoimmune responses
  - Treatment with anti-CD4 antibodies can help

- Some studies have shown association between expressing particular MHC allele and susceptibility to autoimmunity
  - Individuals that express HLA-B27 have 90 times greater chance of having ankylosing spondylitis (spine inflammation)
    - Interestingly, most of those are male even though women are more likely to suffer from autoimmune disease

- Proposed mechanisms for induction of autoimmunity
  - Release of sequestered antigens
    - Blood-brain barrier, sperm released into tissues during vasectomy
  - Molecular mimicry
  - Inappropriate expression of Class II MHC
    - Non-antigen presenting cells will for some reason express Class II MHC
      - Can be caused by viral infection
    - This allows them to present self antigen to T helper cells – leads to inappropriate reaction

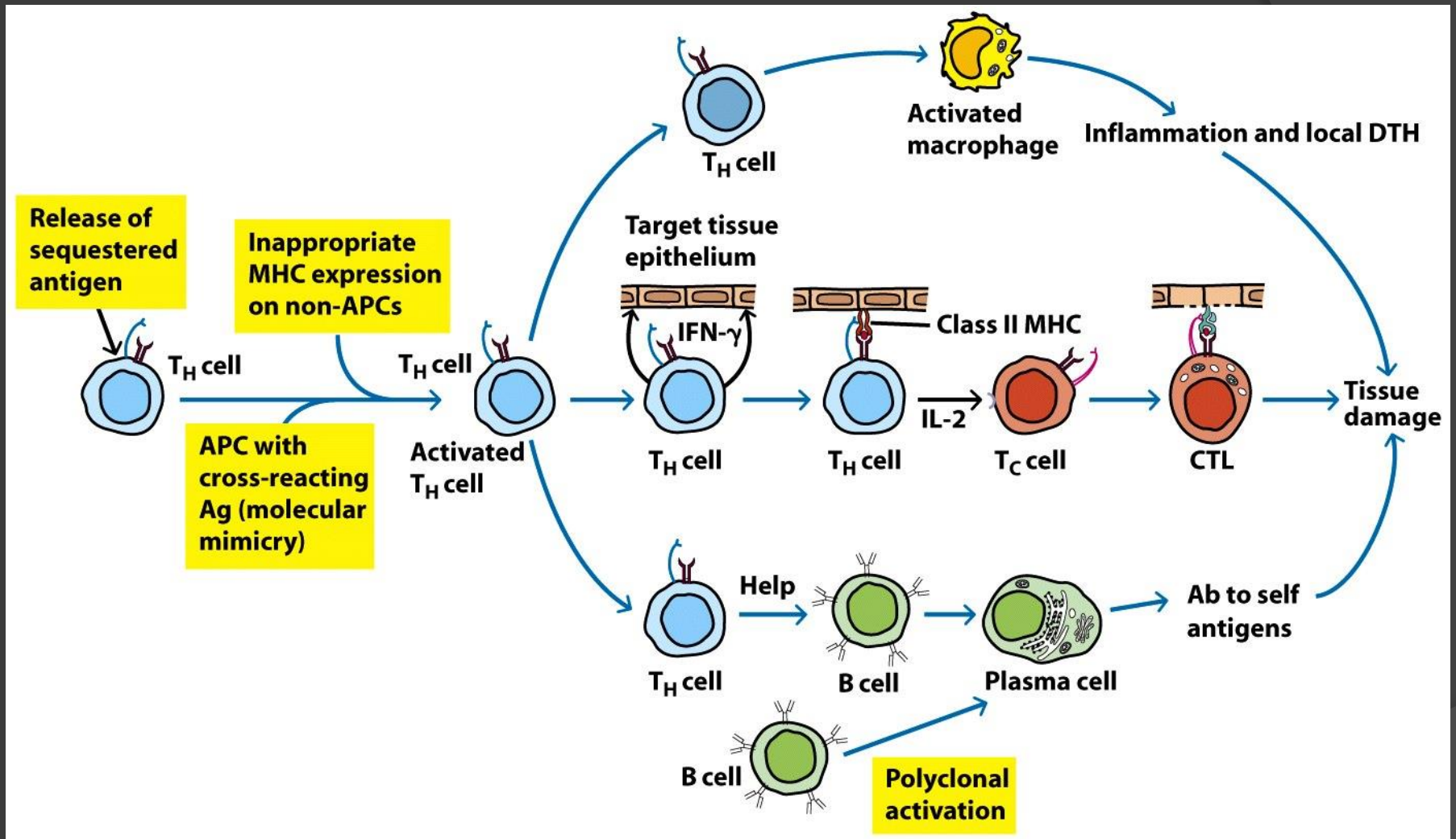

Figure 16-12
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TABLE 16-3 Molecular mimicry between proteins of infectious organisms and human host proteins

Protein*	Sequence†
Human cytomegalovirus IE2	79 P D P L G R P D E D
HLA-DR molecule	60 V T E L G R P D A E
Poliovirus VP2	70 S T T K E S R G T T
Acetylcholine receptor	176 T V I K E S R G T K
Papilloma virus E2	76 S L H L E S L K D S
Insulin receptor	66 V Y G L E S L K D L
Rabies virus glycoprotein	147 T K E S L V I I S
Insulin receptor	764 N K E S L V I S E
Klebsiella pneumoniae nitrogenase	186 S R Q T D R E D E
HLA-B27 molecule	70 K A Q T D R E D L
Adenovirus 12 E1B	384 L R R G M F R P S Q C N
α-Gliadin	206 L G Q G S F R P S Q Q N
Human immunodeficiency virus p24	160 G V E T T T P S
Human IgG constant region	466 G V E T T T P S
Measles virus P3	13 L E C I R A L K
Corticotropin	18 L E C I R A C K
Measles virus P3	31 E I S D N L G Q E
Myelin basic protein	61 E I S F K L G Q E

*In each pair, the human protein is listed second. The proteins in each pair have been shown to exhibit immunologic cross-reactivity.

†Amino acids are indicated by a single-letter code. Identical residues are shown in blue. Numbers indicate amino acid position in the intact protein.

SOURCE: Adapted from M. B. A. Oldstone, 1987, *Cell* **50**:819.

Table 16-3
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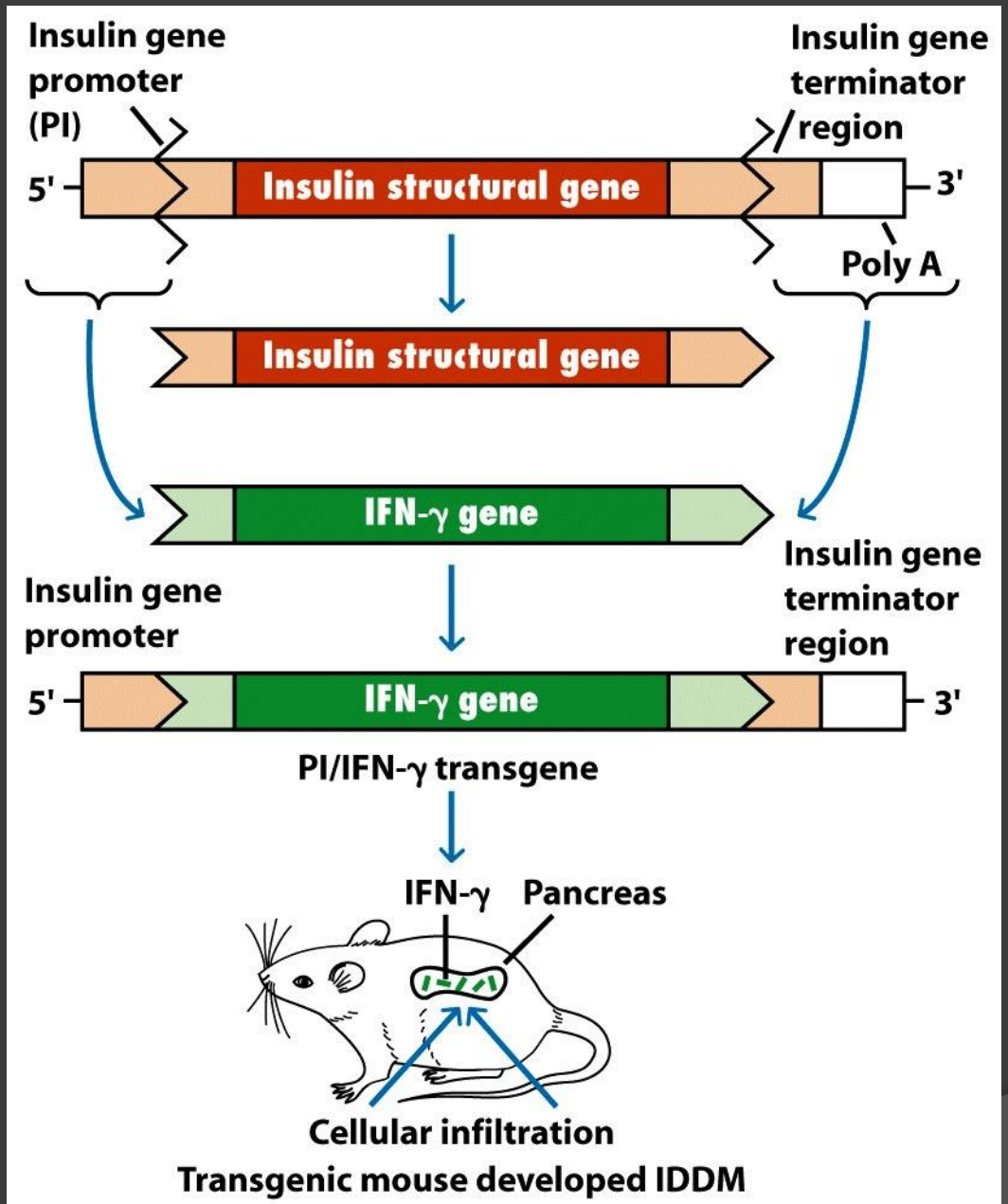

Figure 16-13a
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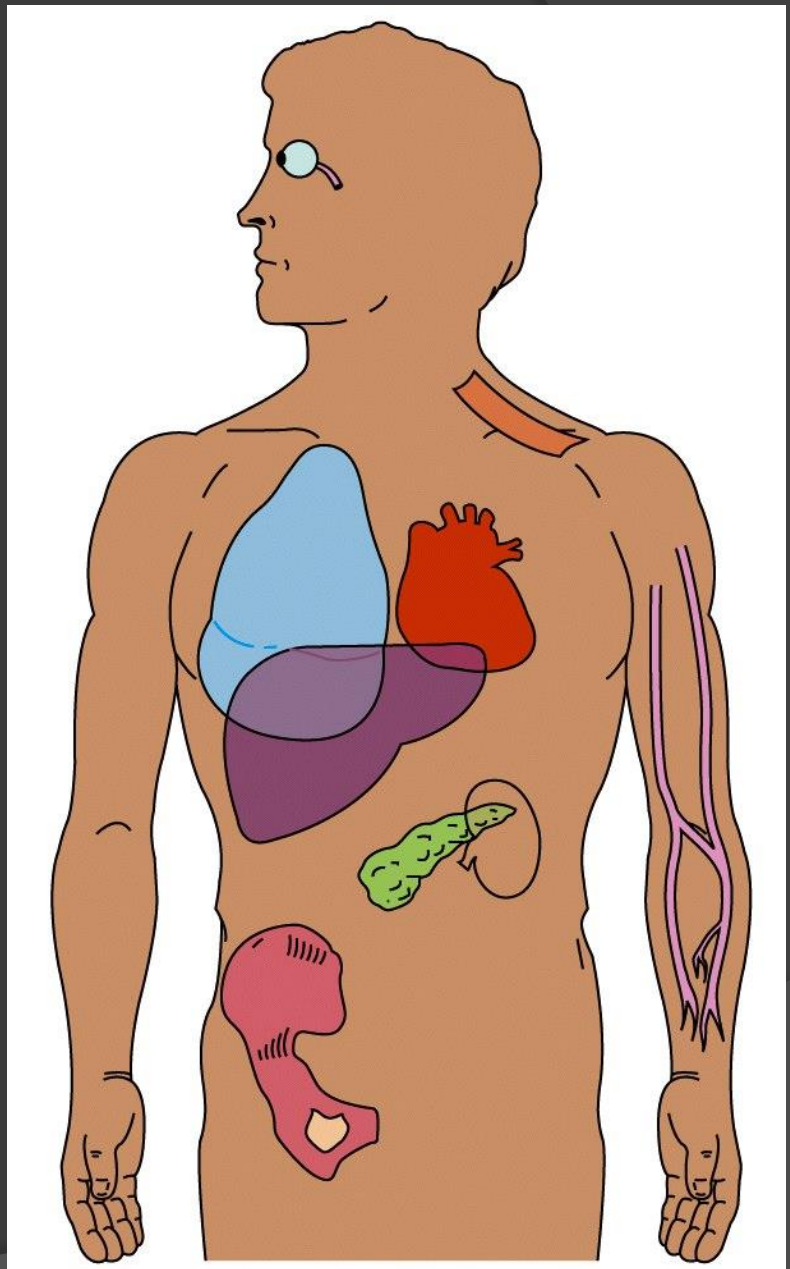
Treatment

- Immunosuppressive drugs
- Removal of thymus (for example, with myasthenia gravis)
- Plasmapheresis – removing plasma and then returning RBCs (removes extra immune complexes)
- Treating the inflammation
- Antigen given orally can induce tolerance

- Transplantation
  - Transfer of cells, tissues, or organs
- 1st human kidney transplant
  - 1935
  - Patient died to mistake in blood typing



Chapter 17 Opener
Kuby IMMUNOLOGY, Sixth Edition

- Immunosuppressive Agents
  - Delay or prevent rejection
  - Majority of these have overall immunosuppressive effect
  - New methods being developed
    - Inducing specific tolerance to graft without suppressing other immune responses

Different types of Transplants

- Autograft
  - Self tissue transferred from one part of body to another
- Isograft
  - Tissue transferred between genetically identical individuals
- Allograft
  - Tissue transferred between genetically different members of same species
    - Most of our transplants
- Xenograft
  - Tissue transferred between different species

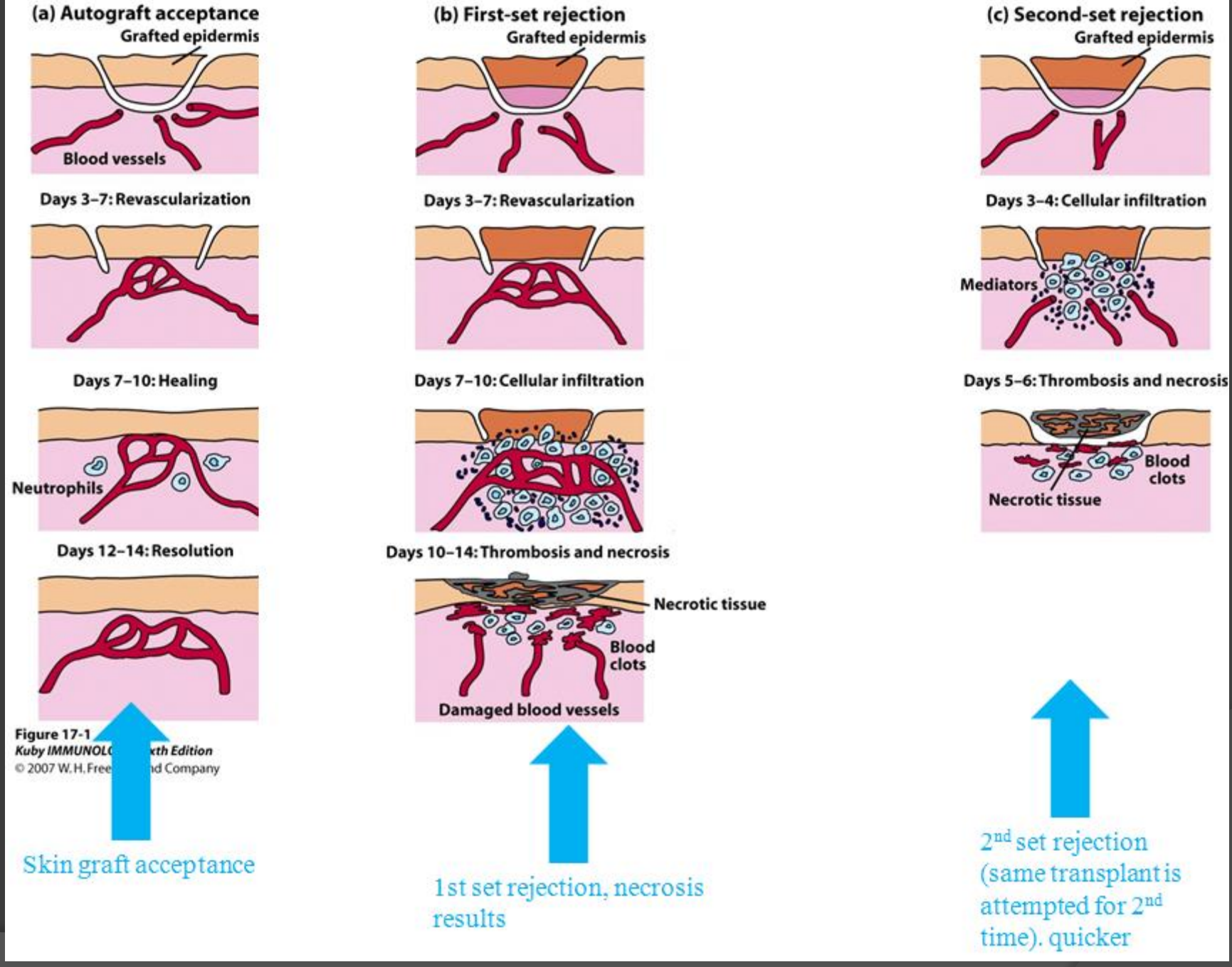


Figure 17-1
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Skin graft acceptance

1st set rejection, necrosis results

2nd set rejection (same transplant is attempted for 2nd time). quicker

- T cells play key role in allograft rejection
  - Both CD4+ and CD8+ populations present

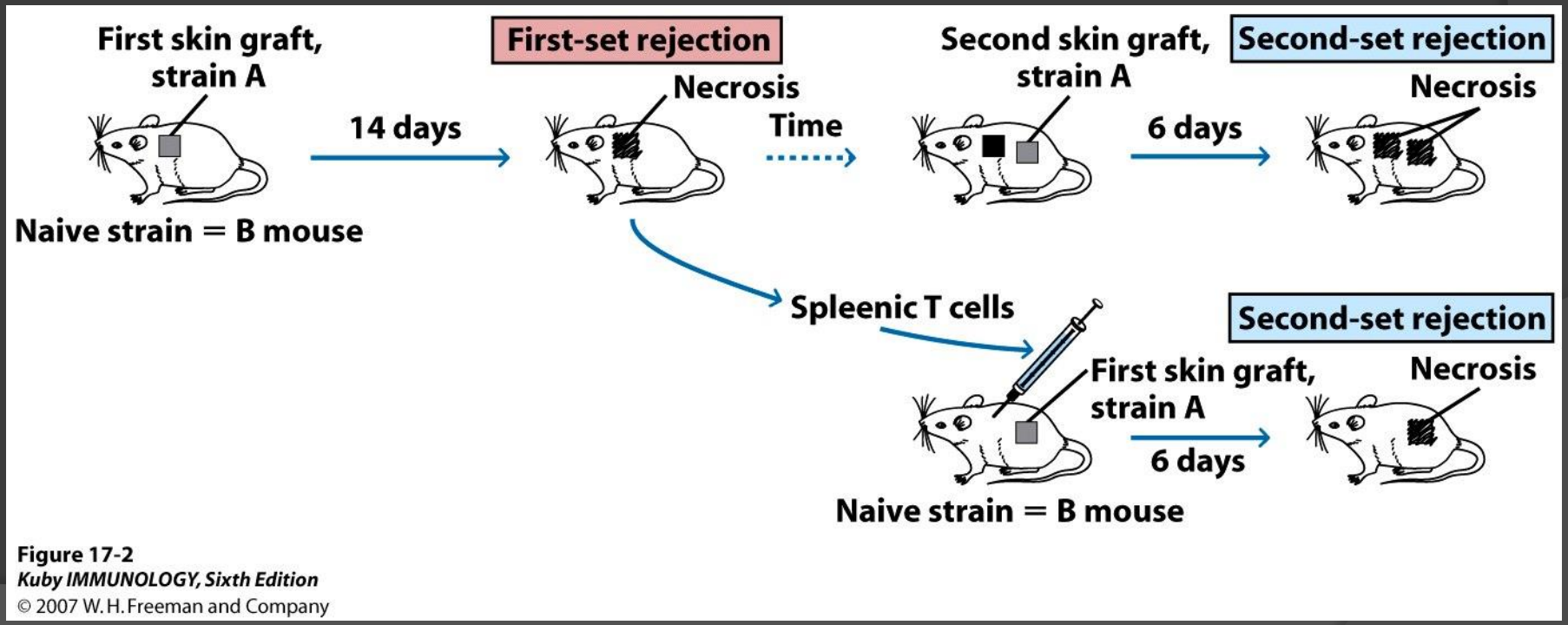

Figure 17-2
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- Tissues that are antigenically similar – *histocompatible*
- Loci most responsible for the most vigorous allograft rejection are within MHC complex
  - Test donors to get matching haplotype
    - Mismatches with Class II are more likely to lead to rejection than mismatches with Class I
  - Also test for blood type

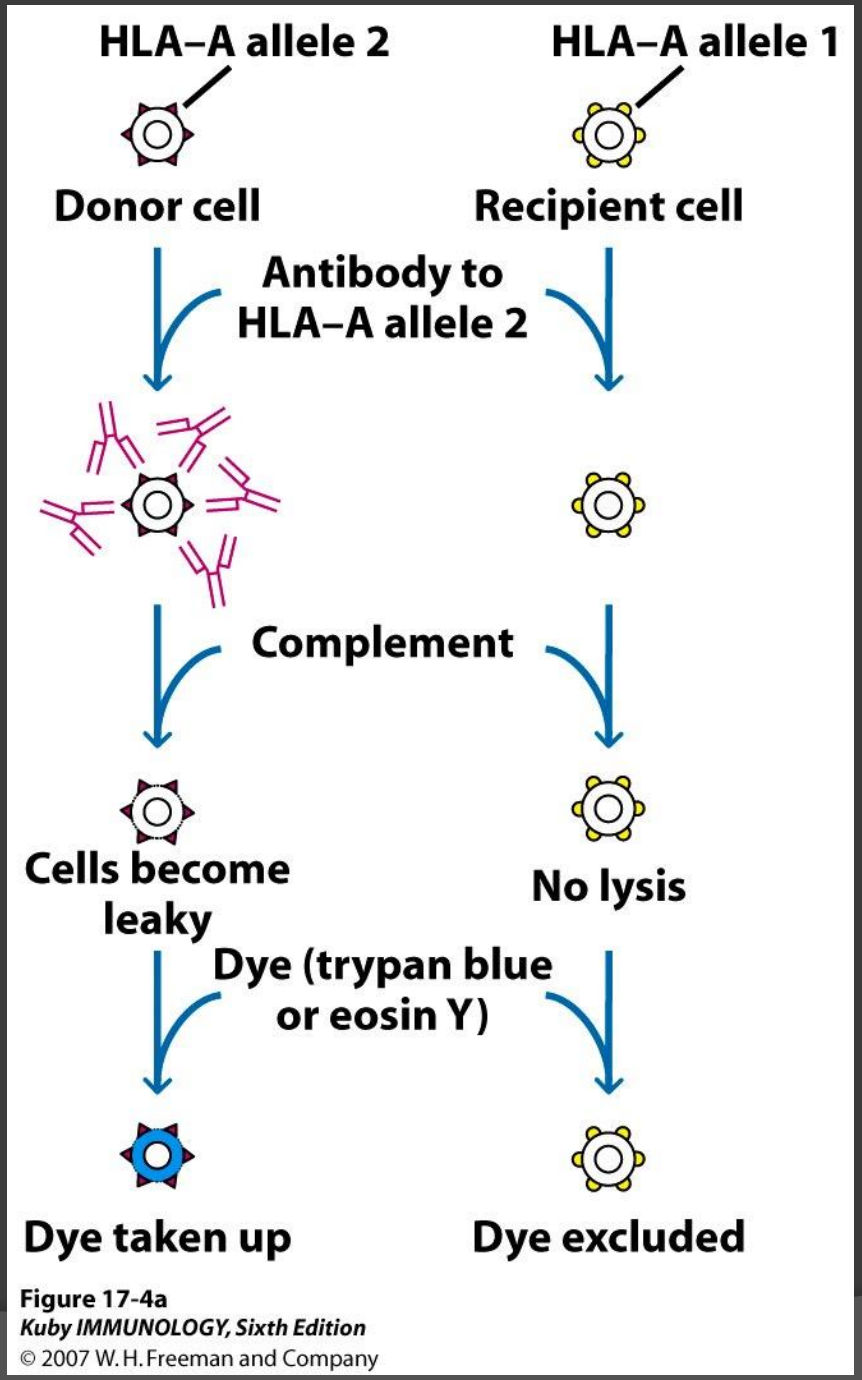

Figure 17-4a
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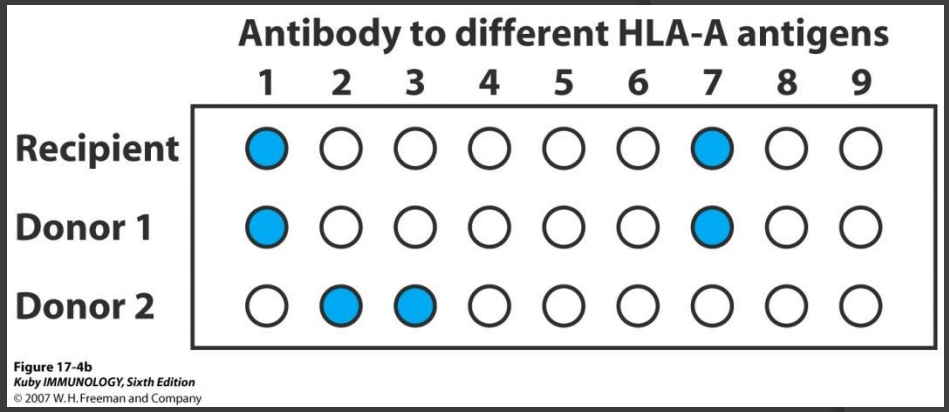

Figure 17-4b
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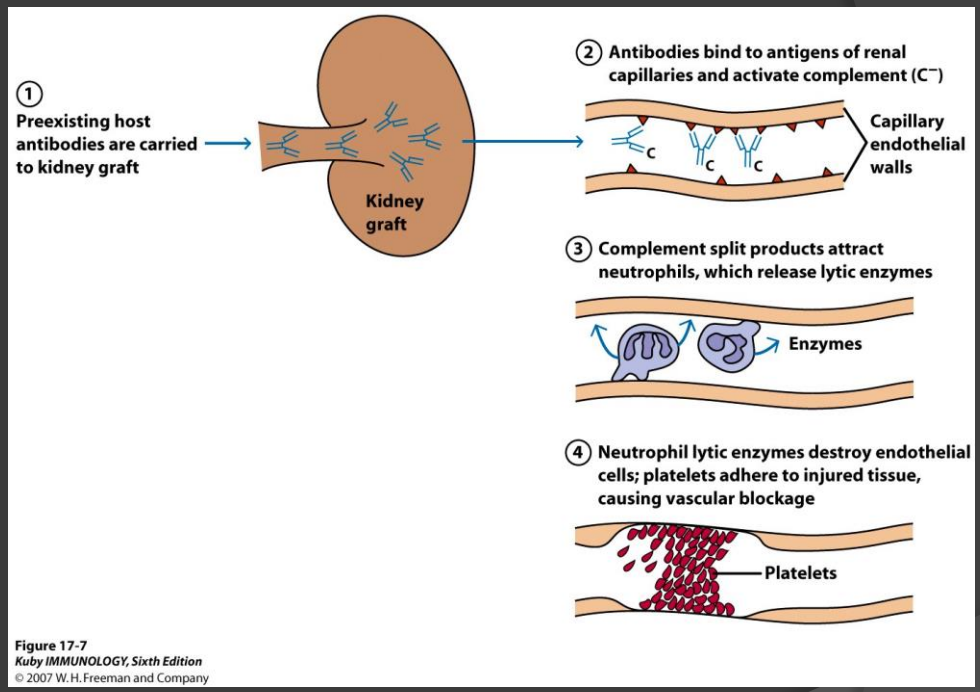
- Microcytoxicity assay for MHC haplotypes
- If antigen is present on cell, complement will lyse it, and it will uptake dye (blue)
- Donor 1 has antigens in common with recepient

Clinical Manifestations of Graft Rejections

- Hyperacute
  - Within hours
- Acute
  - Within weeks
- Chronic
  - Months to years

Clinical Manifestations of Graft Rejection

- Hyperacute
  - Pre-existing recipient antibodies
  - Graft never become vascularized



Immunosuppressive Therapy

- Mitotic inhibitors
  - i.e. Azathioprine
  - Help lower T cell proliferation
- Methotrexate
  - Folic acid antagonist – blocks purine synthesis
- Corticosteroids
  - Reduces inflammation
- X-irradiation of recipient before grafting
- Antibodies specific for immune cells to keep them at lower numbers

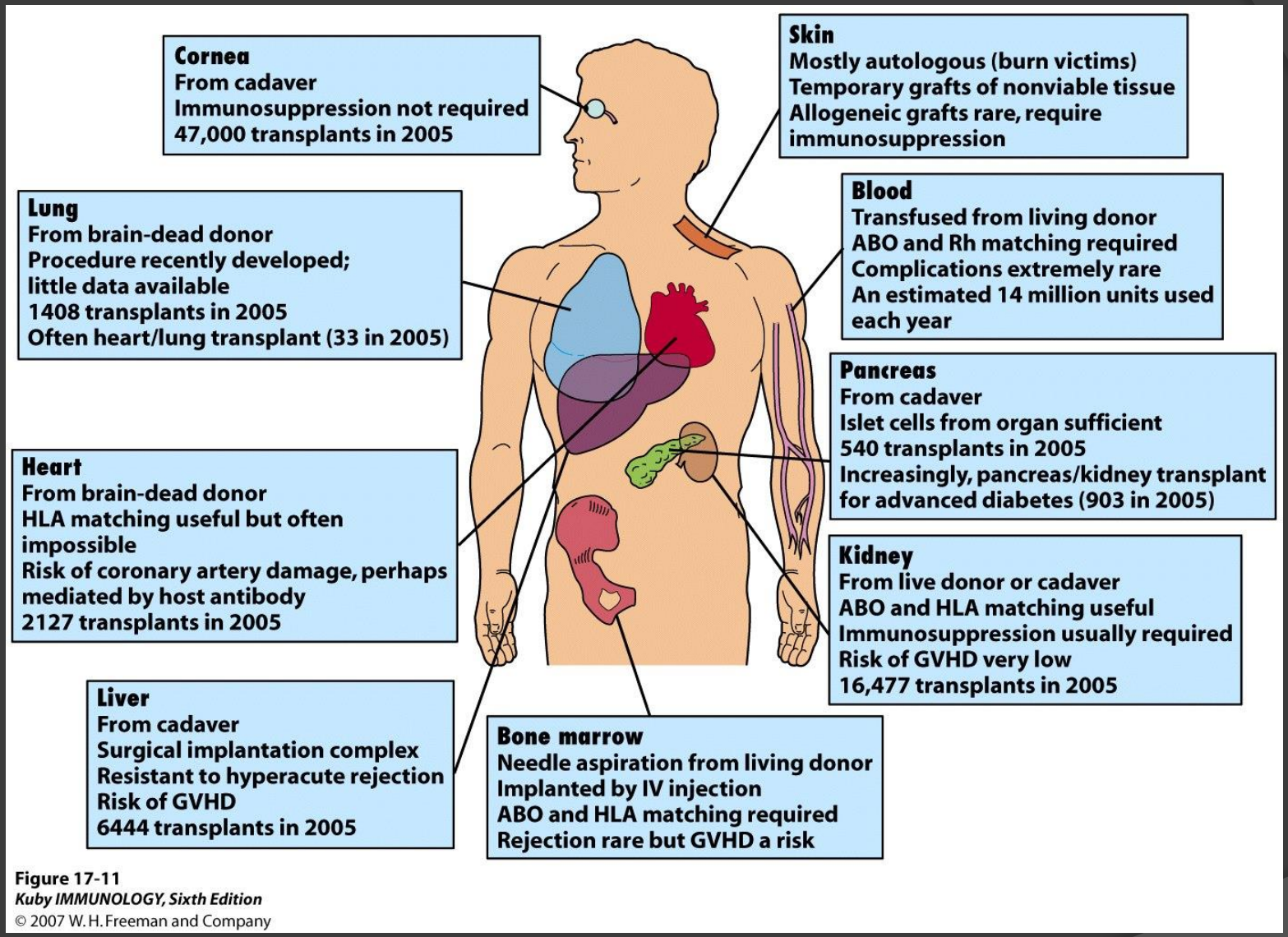


Figure 17-11
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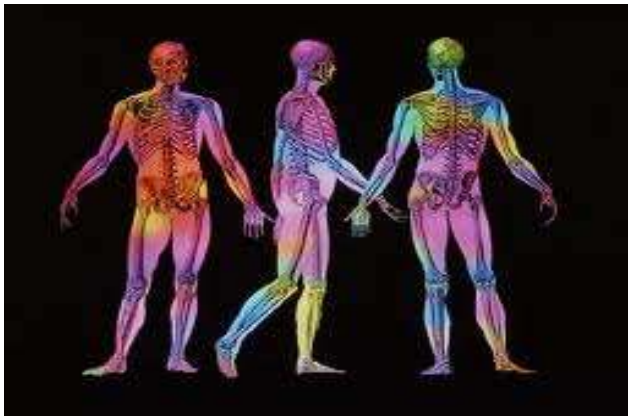
GVHD – Graft versus Host Disease (donor T cells start reacting with host

- Xenotransplantation
  - Shortage of human donors
  - Obstacles with immune system
  - Closely related species have more success
    - However, taking risk of creating new viruses by recombination in graft

TRANSPLANTATION IMMUNOLOGY

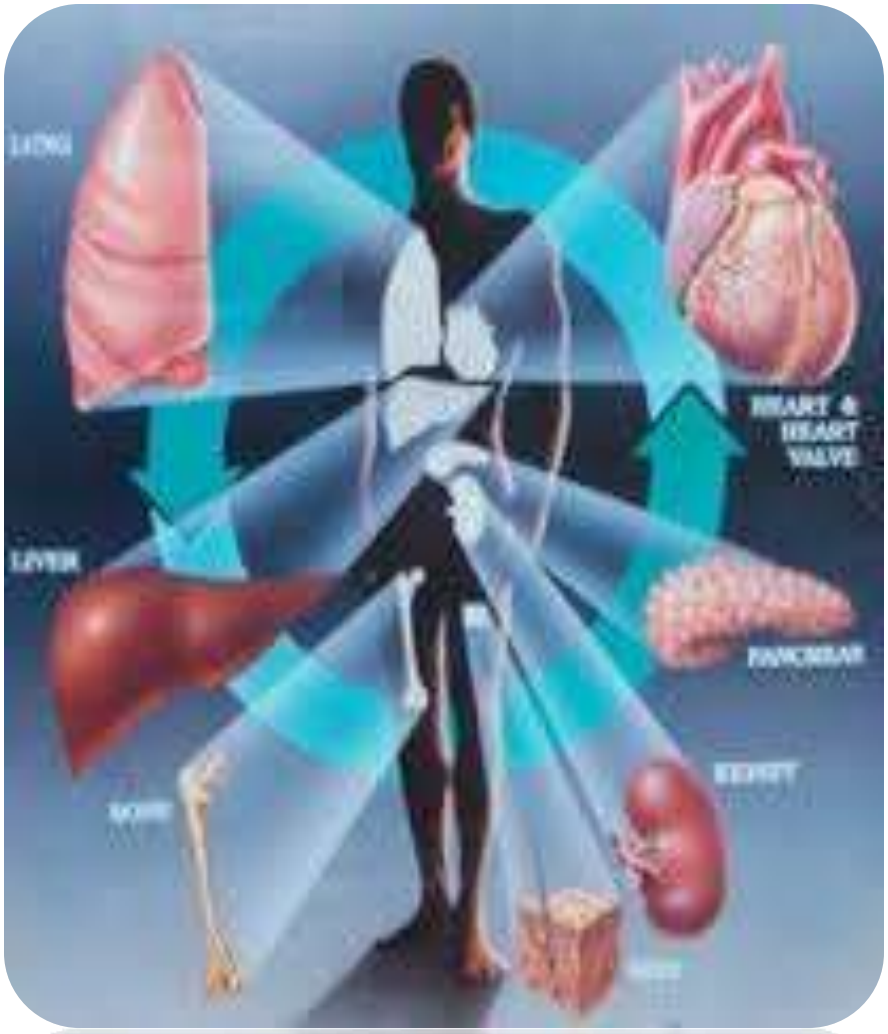
Basics Update

Dr.T.V.Rao MD



Transplantation

- Graft or Transplant: Transfer of living cells, tissues and organs from one part of the body to another or from one individual to another.



Need for Transplantation

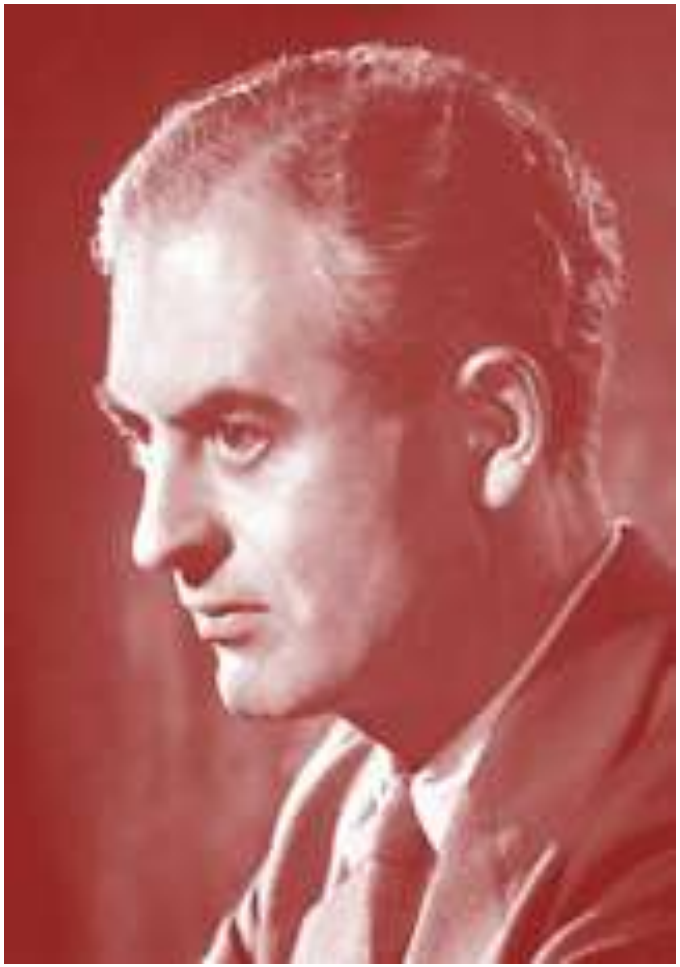
- Many needs in humans
- Damaged organs,
- Non Functional organs

Nobel Prize in Physiology or Medicine 1912

- Alexis Carrel (France)
- Work on vascular suture and the transplantation of blood vessels and organs

Nobel Prize in Physiology or Medicine 1960

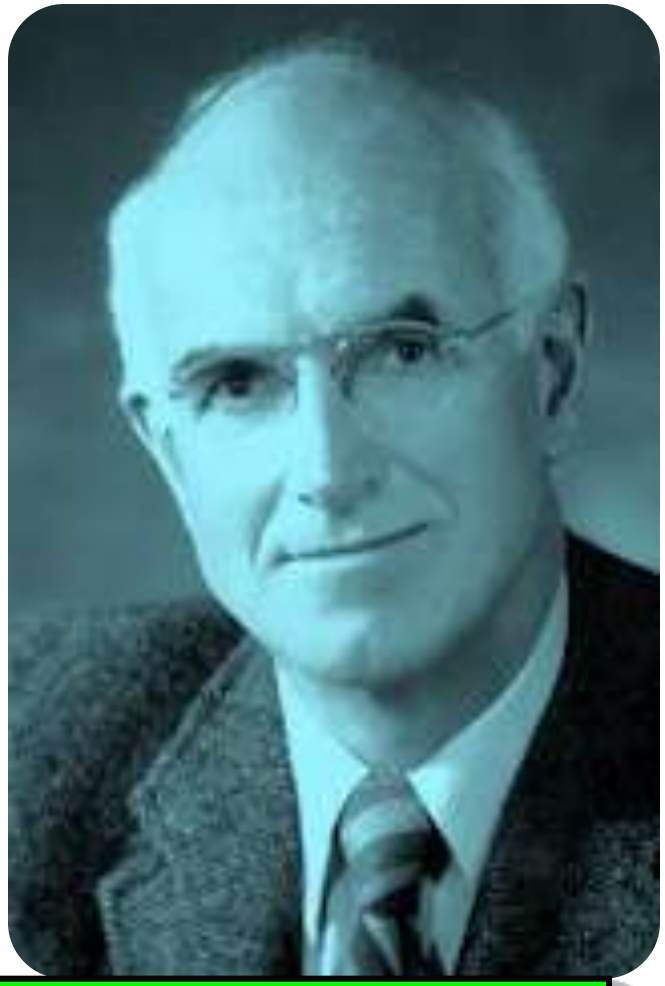
- Peter Brian Medawar (1/2)
- Discovery of acquired immunological tolerance
  - The graft reaction is an immunity phenomenon
  - 1950s, induced immunological tolerance to skin allografts in mice by neonatal injection of allogeneic cells



Great events in history of transplantation

Nobel Prize in Physiology or Medicine 1990

- **Joseph E. Murray** (1/2)
- **Discoveries concerning organ transplantation in the treatment of human disease**
  - **In 1954, the first successful human kidney transplant was performed between twins in Boston.**
  - **Transplants were possible in unrelated people if drugs were taken to suppress the body's immune reaction**



Great events in history of transplantation

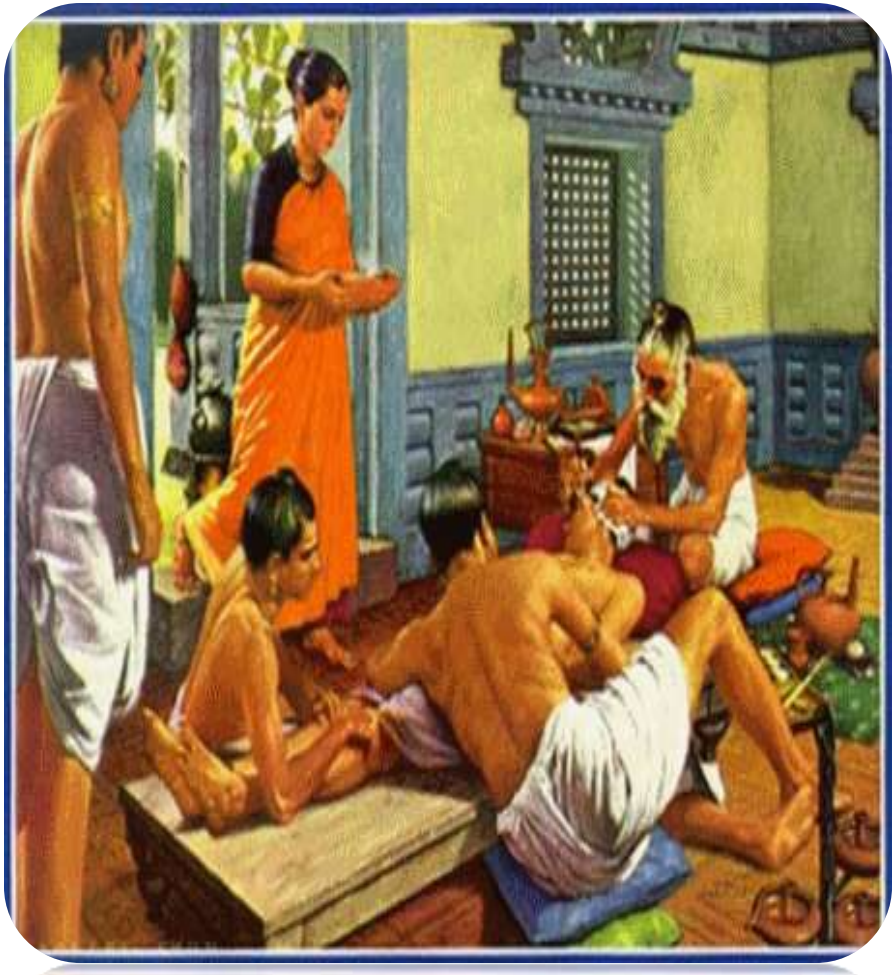
Nobel Prize in Physiology or Medicine 1980

- George D. Snell (1/3), Jean Dausset (1/3)
- Discoveries concerning genetically determined structures on the cell surface that regulate immunological reactions
  - H-genes (histocompatibility genes), H-2 gene
  - Human transplantation antigens (HLA) ----MHC

Great events in history of transplantation

Earliest History

- Skin Grafting for Reconstruction of severed nose
- Done with patients own skin (Sustrutha – Samhita)



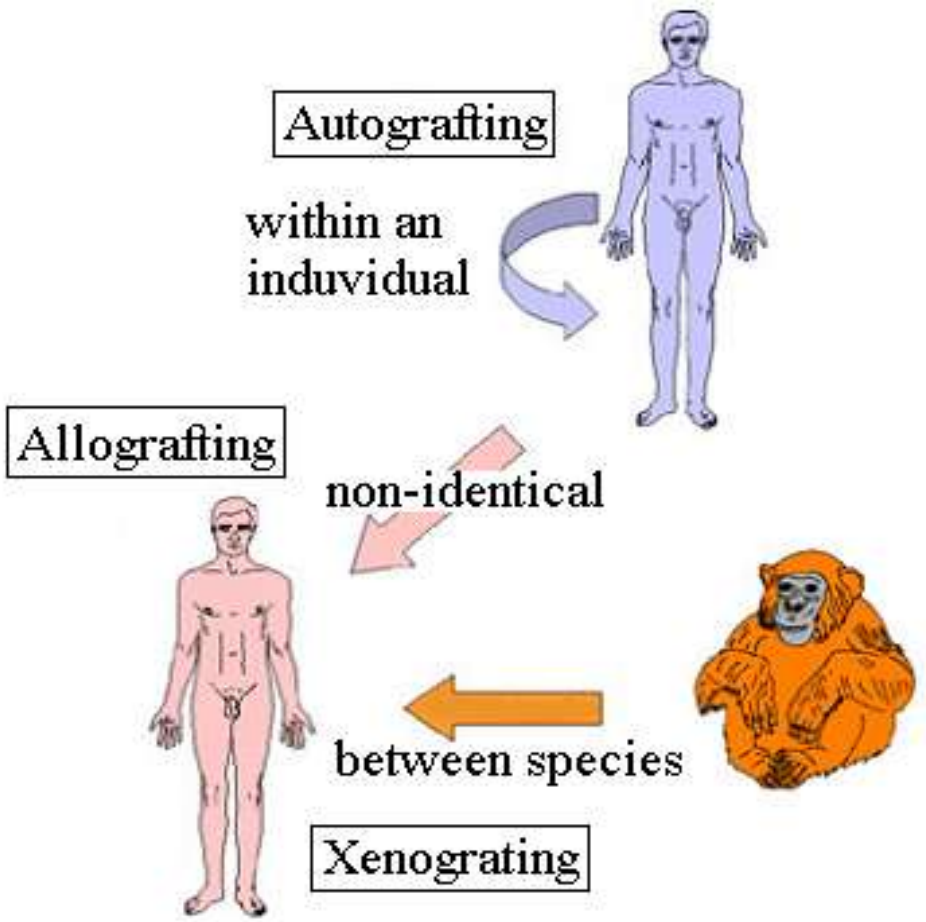
Definition of Transplantation

- Implantation of "non-self" tissue into the body
- The process of taking cells, tissues, or organs called a graft (transplant), from one part or individual and placing them into another (usually different individual).
- donor : the individual who provides the graft.
- recipient or host: the individual who receives the graft.

Methods of Transplantation:

May take place between:

- different parts of the same organism (auto grafting)
- different organisms of the same species (allografting)
- different species (xenografting)



Classification Based on Genetics

- Genetic basis is naming different types of grafts
- Self to Self - Auto graft
- One individual to another – Isograft (Identical twins) both are genetically similar
- Grafts between two genetically non identical members of the same species are called as allograft.

Other names in Terminology

- Can be stored or fresh
- Transplants may be Living or Dead
- Live grafts – Kidney, Hear, also called as Vital grafts.
- Non living – Bone, Artery
- Static or structural grafts.

Classification of Transplants

- Based on nature of organs - Kidney, Liver, Heart, Bone marrow, Skin
- On basis of Anatomical site – Orthotropic,
- Heterotypic
- Orthotropic – Skin graft
- Heterotypic graft 0n abnormal site eg Thyroid gland in subcutaneous region

General information

Immune system rejection

Often a transplanted organ is not identified by the immune system as the tissue of the organism

→ It can be attacked and destroyed.

Against this effect, the patient has to swallow Immunesuppressive which cause symptoms like suffering from AIDS.

In 15-20 minutes the organ dies, unable to withstand the immune system attack.

Rejection of a heart

Allograft: Transplant

- Transplant from one individual to another with a different genetic make-up, within the same species, eg. kidney transplant from one person to any other (except an identical twin).

Allograft

Isograft or syngeneic graft

- **Transplant between genetically identical, monozygotic twins, or between members of an inbred strain of animals.**

Isograft

Auto graft:

- Transplant from one site to another on the same individual, eg. transplanting a blood vessel from the leg to the heart during cardiac bypass surgery. This type of transplant does not require immunosuppressive therapy
- Eg Skin Grafting in burns, destructive injuries.

Auto Graft

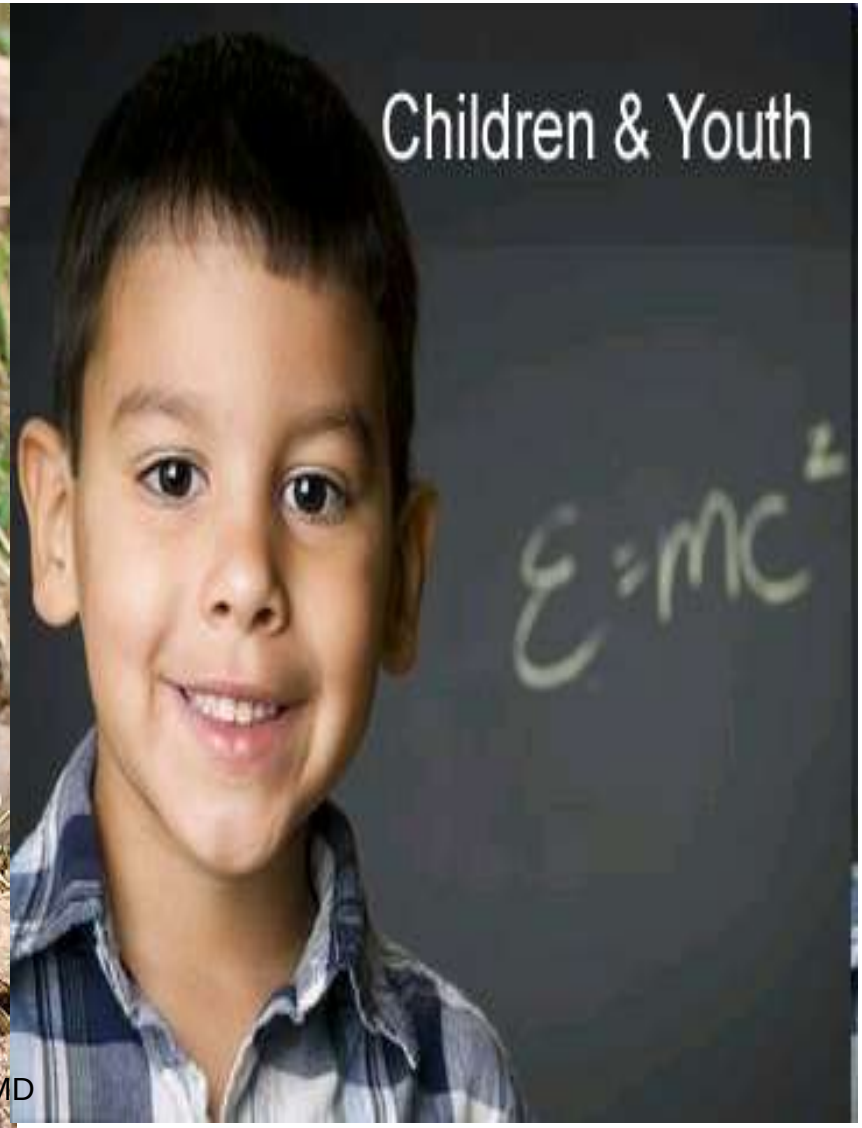
Xenograft:

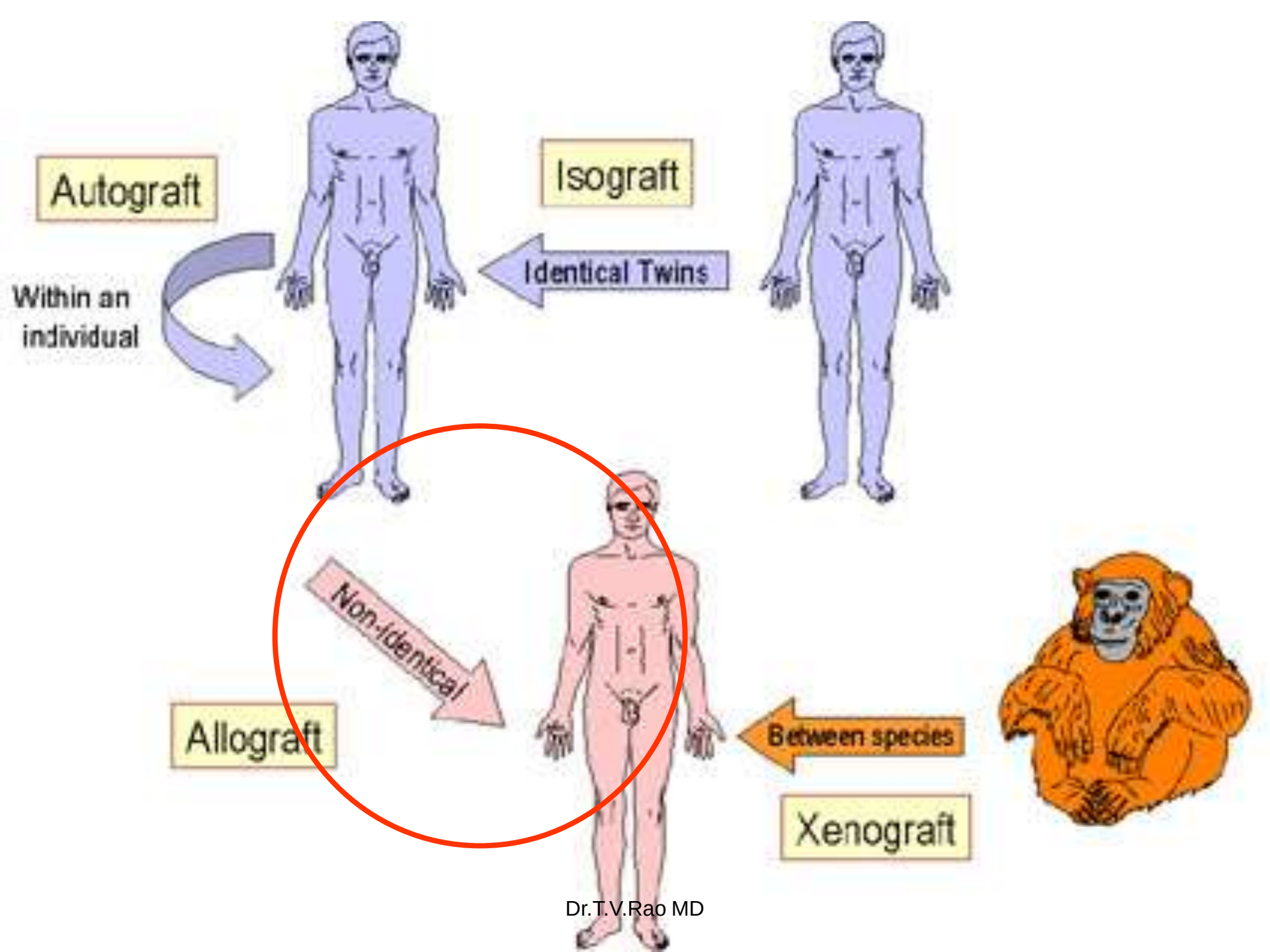
- Transplant across species barriers, eg, transplanting a heart from a baboon to a human. Have a very poor prognosis because of the presence of cross-species reactive antibodies that will induce hyper acute rejection.

Other grafts ...

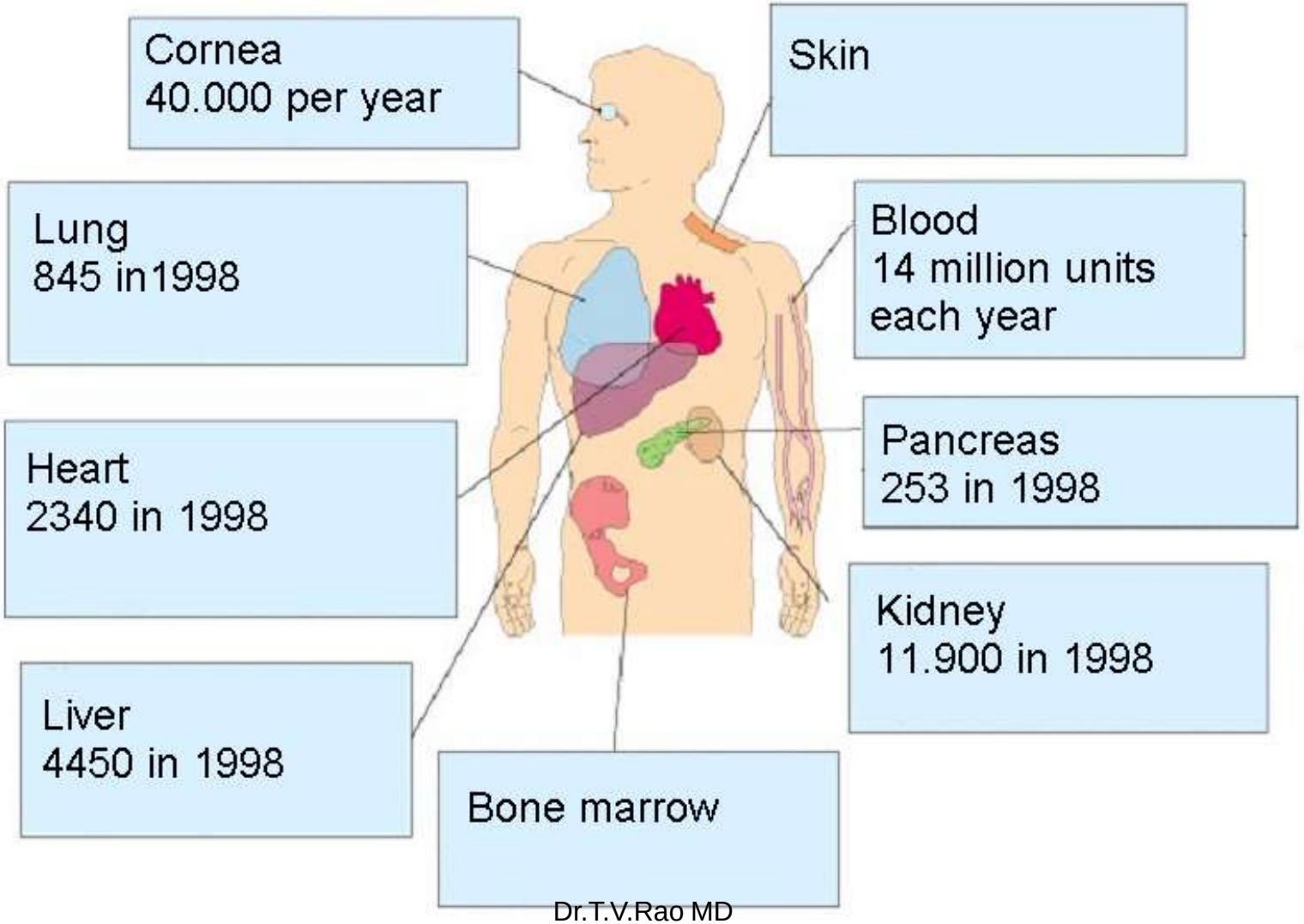
- When grafted between two different species is called as XENOGRAFTS
- Eg From Pig to Humans
- Also called as Heterograft

Xenograft




Autograft
Within an individual
Isograft
Identical Twins
Non-identical
Allograft
Between species
Xenograft

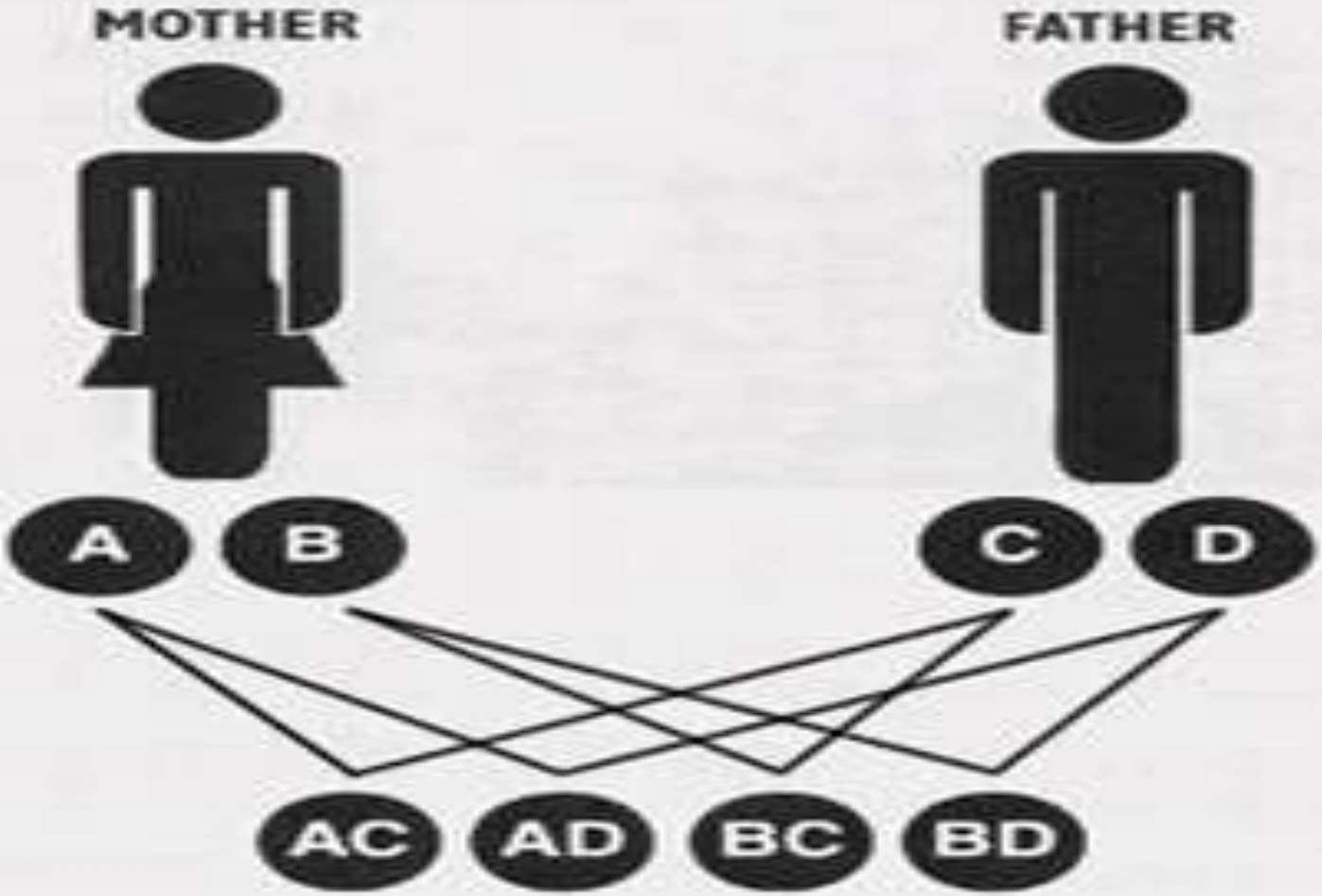
Applications of allografting transplantation



How Grafts are accepted or rejected.

- AA + BB
- F1 hybrid
- AB
- AB can accept graft from both AA or BB
-
- But AA or BB cannot accept the Graft from AB
-

What are the chances of matching HLA siblings?



One set of parents can produce four combinations of offspring. Therefore, each brother or sister has a one-in-four chance of carrying the exact same HLA antigens as the patient.

Classification of Renal Transplantation

- **Auto-RT**
- **Allograft RT**
  - **Living Donor**
    - **Cadaveric**
    - **Living related**
    - **Living unrelated**
- **Xenograft RT (In experimental)**

Transplants from Male to Female

- Male tissues contain xy
- When male tissue with xy grafted to female (xx) as females don't contain y gene
- The grafts may not be accepted
- However grafts done from female to male are accepted.
- The Phenomenon is called as unilateral sex linked Histocompatability is known as **EICHWALD SILMSER EFFECT.**

Eichwald – Silmser Effect
when Male Organs Transplanted to Female

Transplants and the immune system

- Discrimination between self/ nonself
- This is not good for transplants
- At first the only possible transplants were blood transfusions
- Otherwise the grafts were disastrous

Why are blood transfusions tolerated?

MAJOR CONCEPTS IN TRANSPLANT IMMUNOLOGY

- **How does the immune system deal with a transplant, i.e. What are the mechanisms of rejection?**
- **What are the current clinical strategies to block rejection?**
- **What are the new and future strategies to promote specific immune tolerance?**
- **What is the role of xenotransplantation?**
- **What is graft versus host disease?**

Transplantation antigens

Major Histocompatibility Complex (MHC):

– gene complex whose alleles encode polymorphic cell surface glycoproteins involved in antigen recognition and presentation

– MHC-matching between transplant donor and recipient greatly reduces likelihood of rejection

– nomenclature

- HLA: human leukocyte antigen
- SLA: porcine leukocyte antigen
- H-2: mouse MHC
- RT1: rat MHC

Transplantation antigens

Histocompatibility Complex (MHC):

- Class I antigens: constitutively expressed on surface of most cells
- Class II antigens: expressed on cells of lymphoid system
- Expression of MHC molecules can be unregulated by ischemia, etc.
- nomenclature
  - HLA (human) class I: A, B, C; class II: DR, DQ
  - H-2 (mouse) class I: K, D, L; class II: IA, IE

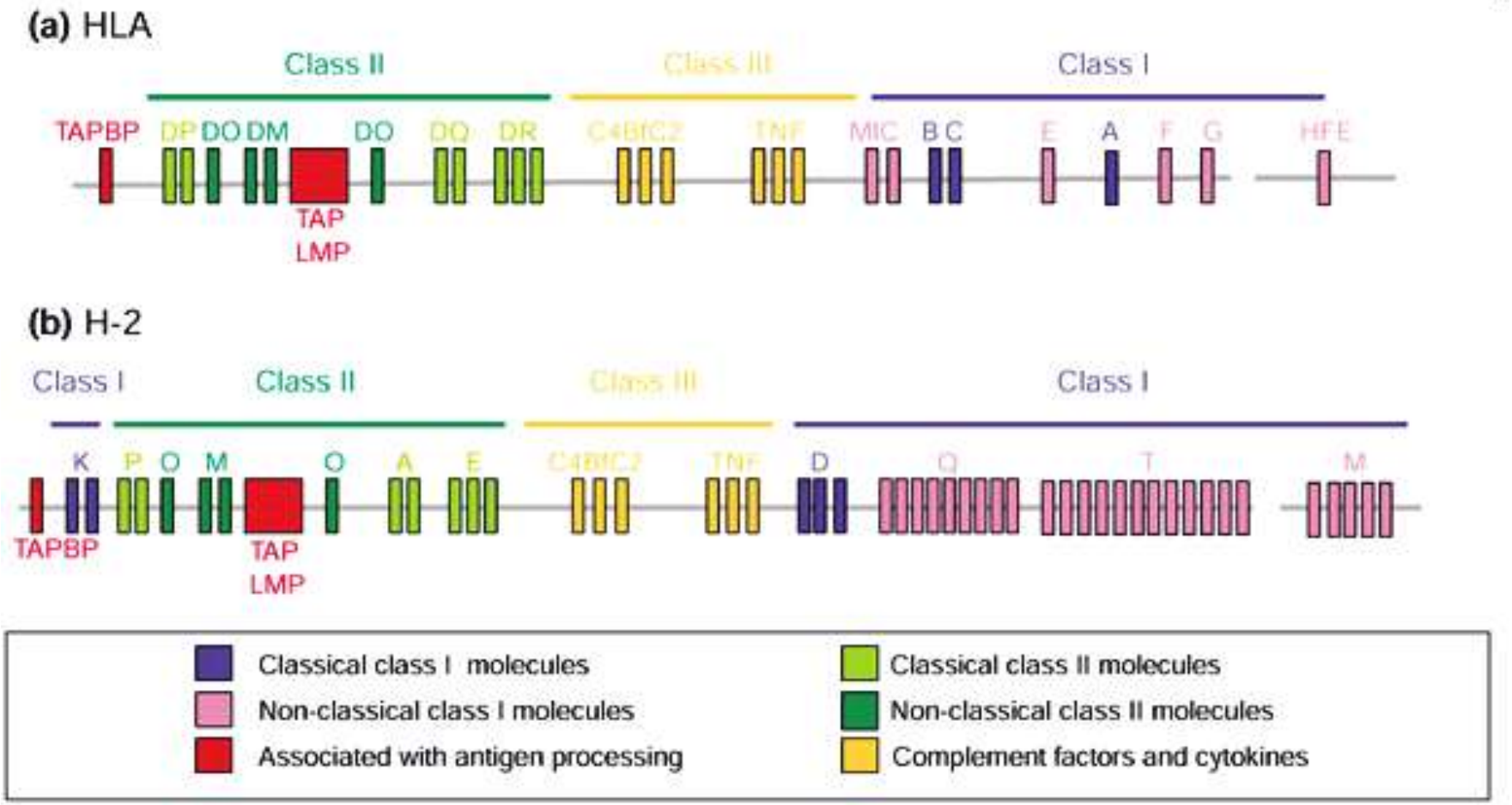
Factors favoring Allograft Survival

- **Blood group compatibility**
- **HLA compatibility**
- **HLA typing and Tissue matching**
- **HLA typing identifies the HLA antigens expressed on the surface of leukocytes.**

Histocompatability Antigens

- Immune response against transplants depends on the presence in the grafted tissue of antigens that are absent in recipient and hence recognized as foreign

HLA system


(a) HLA
Class II
Class III
Class I
TAPBP
DP DO DM
DO
DQ
DR
C4BIC2
TNF
MIC
B C
E
A
F
G
HFE
TAP
LMP
(b) H-2
Class I
Class II
Class III
Class I
K
P O M
O
A
E
C4BIC2
TNF
D
Q
T
M
TAPBP
TAP
LMP
Classical class I molecules
Non-classical class I molecules
Associated with antigen processing
Classical class II molecules
Non-classical class II molecules
Complement factors and cytokines

Identifying MHC polymorphisms ('tissue typing')

- Formerly determined by antibodies against MHC molecules
  - **HLA typing**
  - **MLR**
- Now by DNA testing: allele-specific PCR, sequencing

Tissue typing

- Microcytotoxicity assay
  - Known antibody to WBCs of donor / recipient
  - Complement mediated lysis if Ab present on cell surface
- Mixed lymphocyte culture (MLC)
  - Irradiated donor lymphocytes (stimulants)
  - Incubated with recipient lymphocytes
- Flow cytometry cross typing
- DNA analysis

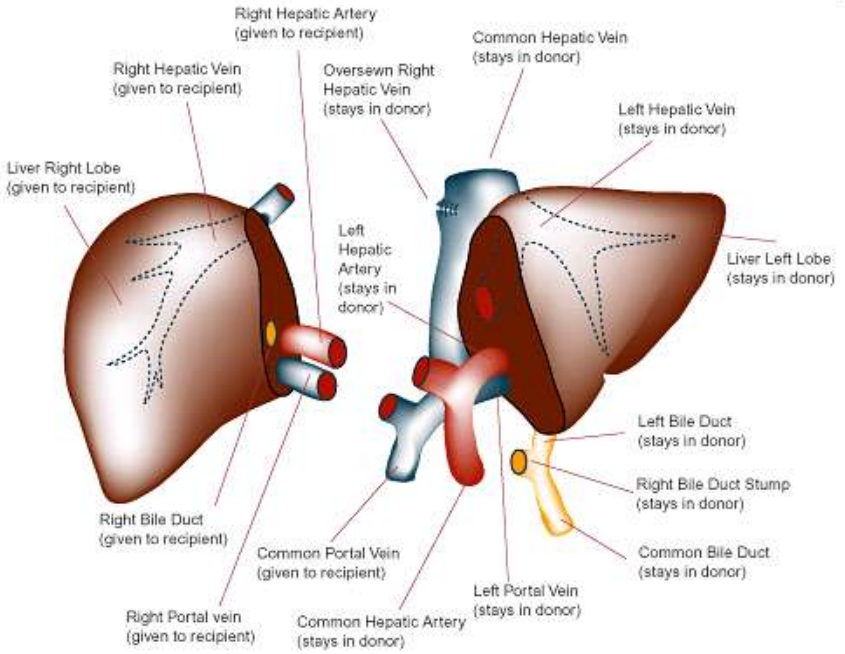
  Genomic typing (very precise, many subtipes)

Clinical phases of rejection

1. **Hyperacute rejection** (minutes to hours)
   - Preexisting antibodies to donor HLA antigens
   - Complement activation, macrophages
2. **Accelerated rejection**
3. **Acute rejection** (around 10 days to 30 days)
   - Cellular mechanism (CD4, CD8, NK, Macrophages)
4. **Chronic rejection** (months to years !!)
   - Mixed humoral and cellular mechanism
   - CHRONIC REJECTION IS STILL HARD TO MANAGE !

Graft acceptance

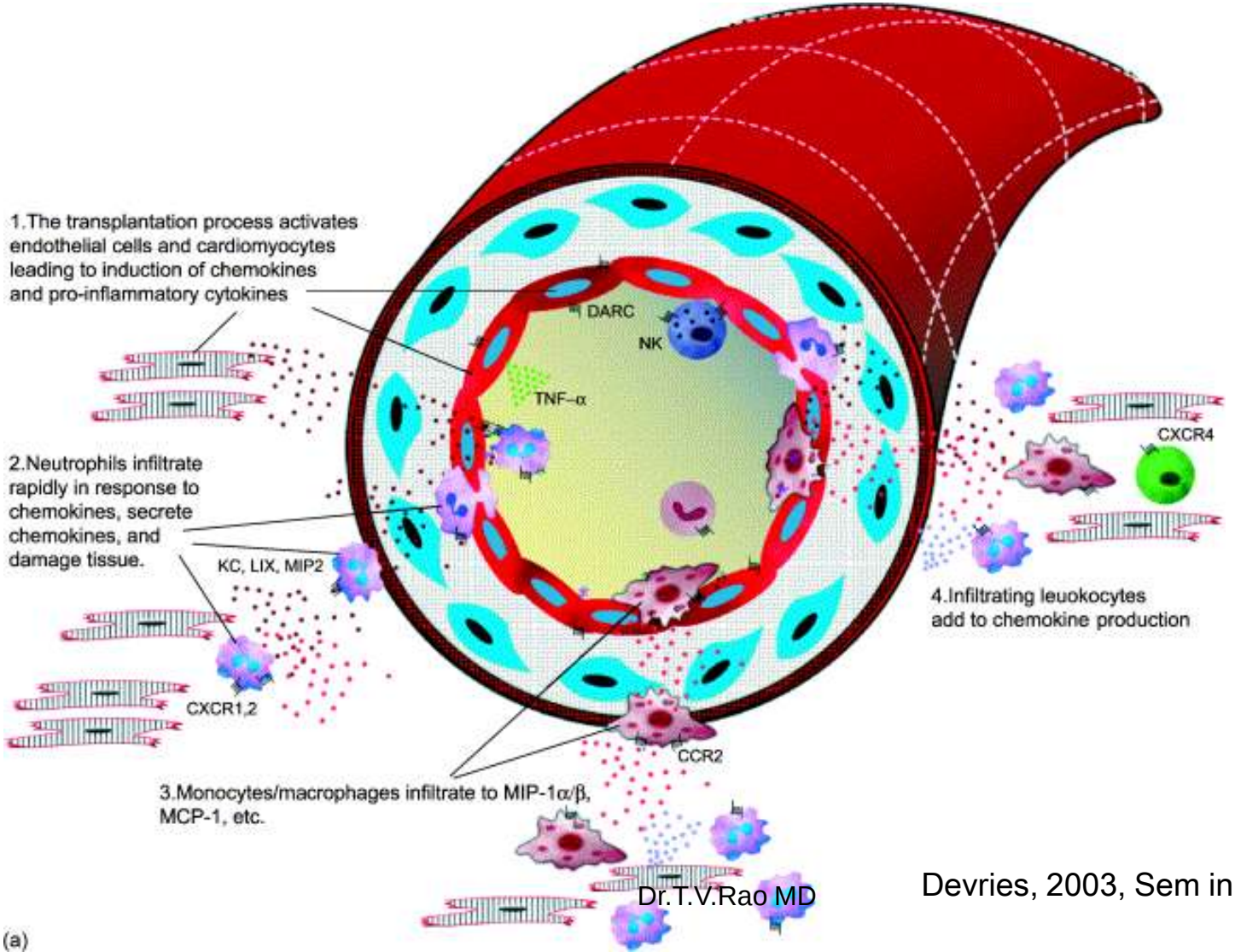
- If the recipient posses all the antigens present in the graft, there will be immune response, and there will be no immune response, and no graft rejection even when the donor and recipient are not syngeneic.



Mechanism of acceptance and rejection

- **The first generation Hybrids between two inbred strains posses antigens representing both the parent strains and will accept grafts from either parent strains and therefore accept grafts from either of the parental strains.**

Peritransplant injury induces chemokine's that increase inflammation and immunity



Devries, 2003, Sem in Imm 15:33-48

Control of Transplant Immunology

.

Transplantation immunity is predominately by cell mediated immunity
First response is mediated by T lymphocytes

Humoral antibody are also produced during Allograft Rejection

What happens after Two to Three days

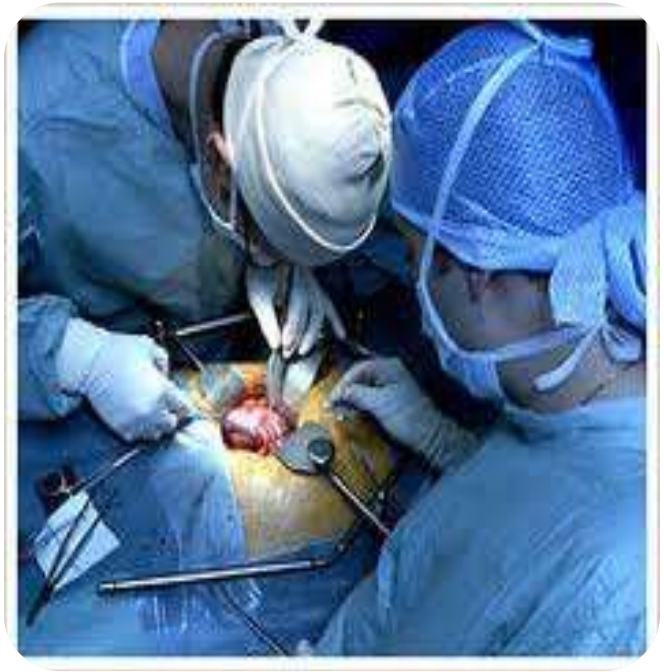
- The site around transplantation is inflamed, invaded by lymphocytes, Macrophages
- Blood vessels occluded by thrombi
- Vascularity to graft diminishes
- Ischemic changes sets in
- Scab like changes appear, sloughs out 10th day
- Above response is called Ist set response

Cellular and Molecular Understandings

•Associated with graft rejections and immunosuppressive therapies

•Rejection has not been eliminated only reduced

- Hyperacute rejection
- Acute rejection
- Chronic rejection



The Allograft Rejection

- What Happens
- Skin from one animal is accepted initially
- Vacularised
- Appears healthy for short period for two or three days
- Inflammation sets in

Hyper acute Rejection

- Occurs within a few minutes to a few hours
- Result of destruction of the transplant by performed antibodies (cytoxic antibodies)
- Some produced by recipient before transplant
- Generated because of previous transplants, blood transfusions, and pregnancies
- Antibodies activate the complement system then platelet activation and deposition causing hemorrhaging and swelling

Cell-mediated immunity is not involved at all in these reactions

When the Graft will be accepted I f

- An allograft will be made acceptable if animal is made immunologically tolerant

Chronic rejection

- Caused by both antibody and cell-mediated immunity
- May occur **months to years** down the road in allograft transplants after normal function has been assumed
- Important to point out rate, extent, and underlying mechanisms of rejection that vary depending on tissue and site
- The recipients circulation, lymphatic drainage, expression of MHC antigens and other factors determine the rejection rate
- **Inflammation, smooth muscle proliferation, fibrosis**
- **Tissue ischemia**

Role of MHC molecules

- When T cells are exposed to foreign cells expressing non-self MHC, many clones are tricked into activation - their TCRs bind to foreign MHC-peptide complex's presented
- T cells are reacting directly with the donor APCs expressing allogeneic MHC in combination with peptide. These donor APCs also have costimulatory activity to generate the second signal for the second reaction to occur
- Minor H antigens are encoded by genes outside the MHC

Laboratory Tests

- ABO Blood typing
- Tissue typing (HLA Matching)
- (Lympho cytotoxicity test)
- (Mixed leukocyte reaction)
- Screening for Presence of Preformed Antibodies to allogeneic HLA
- Crossmatching

Prolonging Allograft Survival

- Anti-inflammatory Agents
- Cytotoxic Drugs
- Agents that interfere with Cytokine production and signaling
- Immunosuppressive Therapies
- New Immunosuppressive strategies

Nobel Prize in Physiology or Medicine 1988

- Gertrude B. Elion (1/3) , George H. Hitchings (1/3)
- Discoveries of important principles for drug treatment
  - Immunosuppressant drug (The first cytotoxic drugs) ----- azathioprine

Great events in history of transplantation

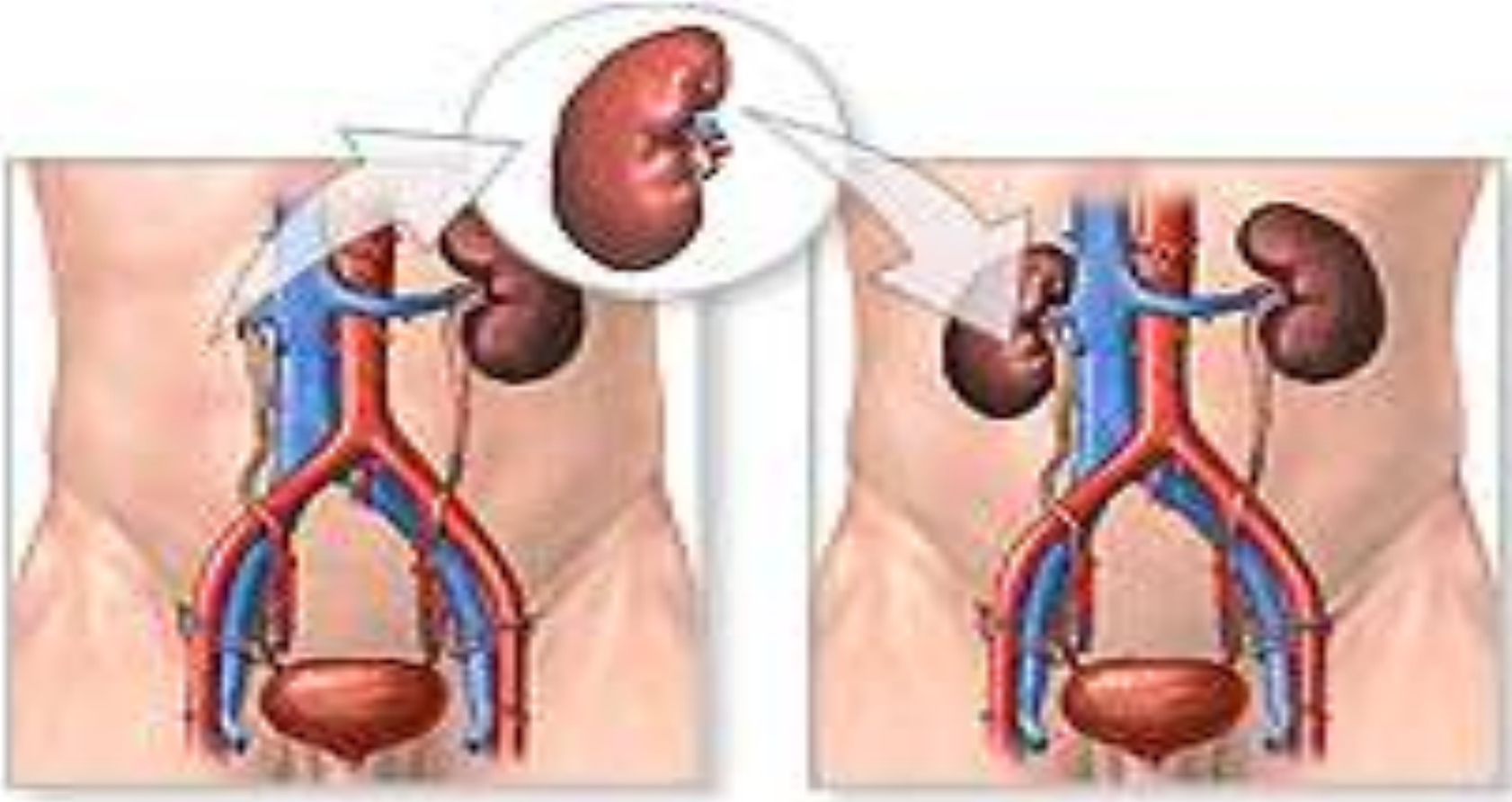
Prolonging Allograft Survival

- Cyclosporine and Tacrolimus (FK-506)
- Azathioprine
- Mycophenolate Mofetil
- Rapamycine
- Corticosteroids
- Anti-CD3, Anti-CD52, Anti-IL-2, Anti–CD25

Does MHC (HLA) ‘matching’ prevent rejection?

- Reduces rejection but there are still ‘minor histocompatibility antigens’ (MiHA)
- MiHA are probably polymorphisms affecting peptides in the grooves
- But we cannot MHC-match most grafts: *too much polymorphism, too little time, too few donors*
- Therefore need immunosuppression

Most Important Organ transplantation



Graft versus Host Reaction (GVHR)

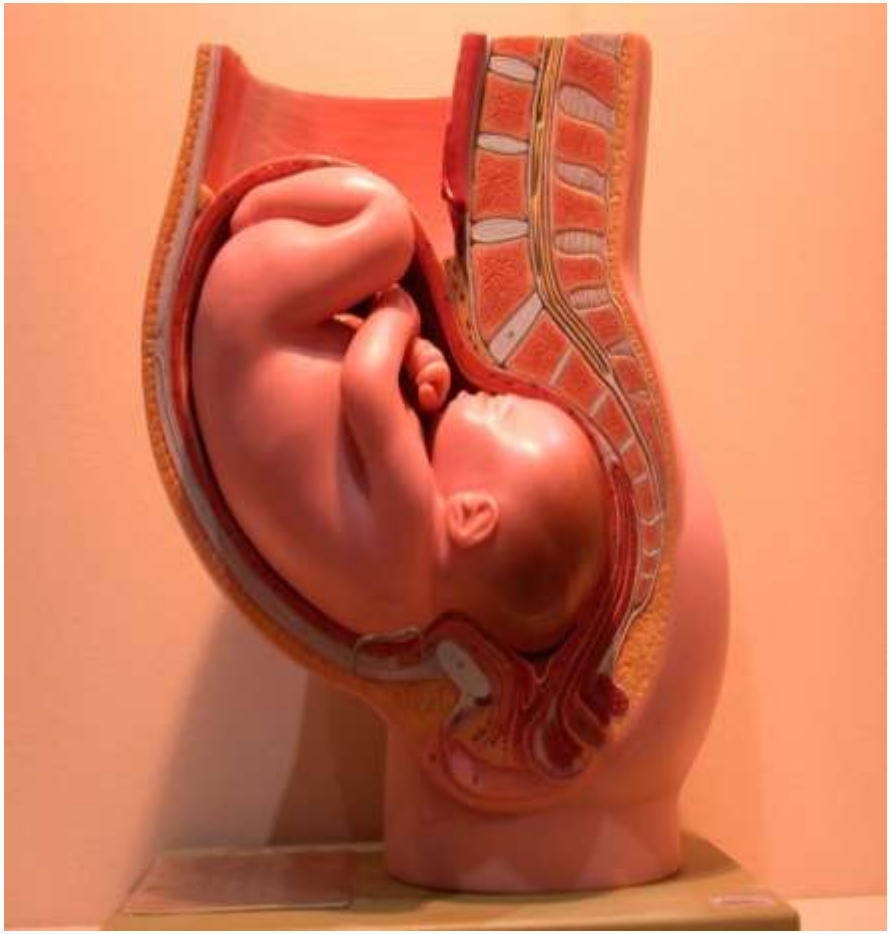
- When grafted tissue has mature T cells, they will attack host tissue leading to GVHR.
- Major problem for bone marrow transplant.
- Methods to overcome GVHR:
  - Treat bone marrow to deplete T cells.
  - Use autologous bone marrow.
  - Use umbilical cord blood.

Graft-vs-host disease

- **Graft-vs-host disease can occur in the special case in which immunocompetent tissue (fresh whole blood, thymus, or bone marrow) is transplanted into an immunocompromised host. T cells from the transplant recognize the host MHC molecules as nonself and attack the host. This is a type IV hypersensitivity reaction; antibody plays no role at all.**

Privileged Sites
Fetus survives

- The placenta acts as immunological barrier
- MHC are present in low density
- Alpha-fetoprotein in blood will help
- Cornea survive because of lack of vascularity



Bone Marrow

- Attempts to use these cells have been around for at least 60 years
- Explored intensely since world war II
- Used for treating blood diseases, severe combined immunodiffency and leukemia
- This type of transplant is also called a form of gene therapy

Immunosuppressive drugs

- Glucocorticosteroids: prednisone
- Small molecule drugs
  - azathioprine
  - calcineurin inhibitors: cyclosporine, tacrolimus
  - target of rapamycin inhibitors: sirolimus (a.k.a rapamycin)
  - IMPDH inhibitors: mycophenolate mofetil
  - lymphocyte recirculation (S-1-P) inhibitors: FTY720
- Depleting antibodies
  - rabbit polyclonal antilymphocyte globulin
  - anti CD52 (Campath-1h), anti CD3
  - B cell depletion: anti CD20
- Non-depleting antibodies and fusion proteins
  - anti CD25
  - CTLA4Ig fusion protein

Source of stem cells for Transplants

- **Peripheral Blood Stem Cells (PBSCT)**
- Stem cells collected peripherally using apheresis (cell separator machine)
    - Less invasive; less discomfort; less morbidity than BM
- Outpatient procedure
- PBSCT results in more rapid hematopoietic recovery than BM
- No difference in treatment outcome
- Quickly replacing traditional BM

  – Using cytokine stimulation (G-CSF injections)
  – BM releases large number CD34 stem cells into circulation
  – Stem cells harvested via peripheral line

Graft – Host reaction

- Graft rejection is due to the reaction of the host to grafted tissue (host – versus- graft response)
- In contrary Graft mounts an immune response against the antigens of the host (GVH)

GVH reaction occurs when

1 The graft contains immunocompetent T cells

2 The recipient possesses transplantation antigens that are absent in the graft

The recipient must not reject the graft

Situation leading for GVH

- Allograft in a recipient in whom specific immunological tolerance has been induced
- Present with clinically
- Retardation of growth
- Diarrhea, Hepatosplenomegaly
- Lymphoid atrophy
- Anemia
- Terminating fatally
- Syndrome is called **Runt disease**

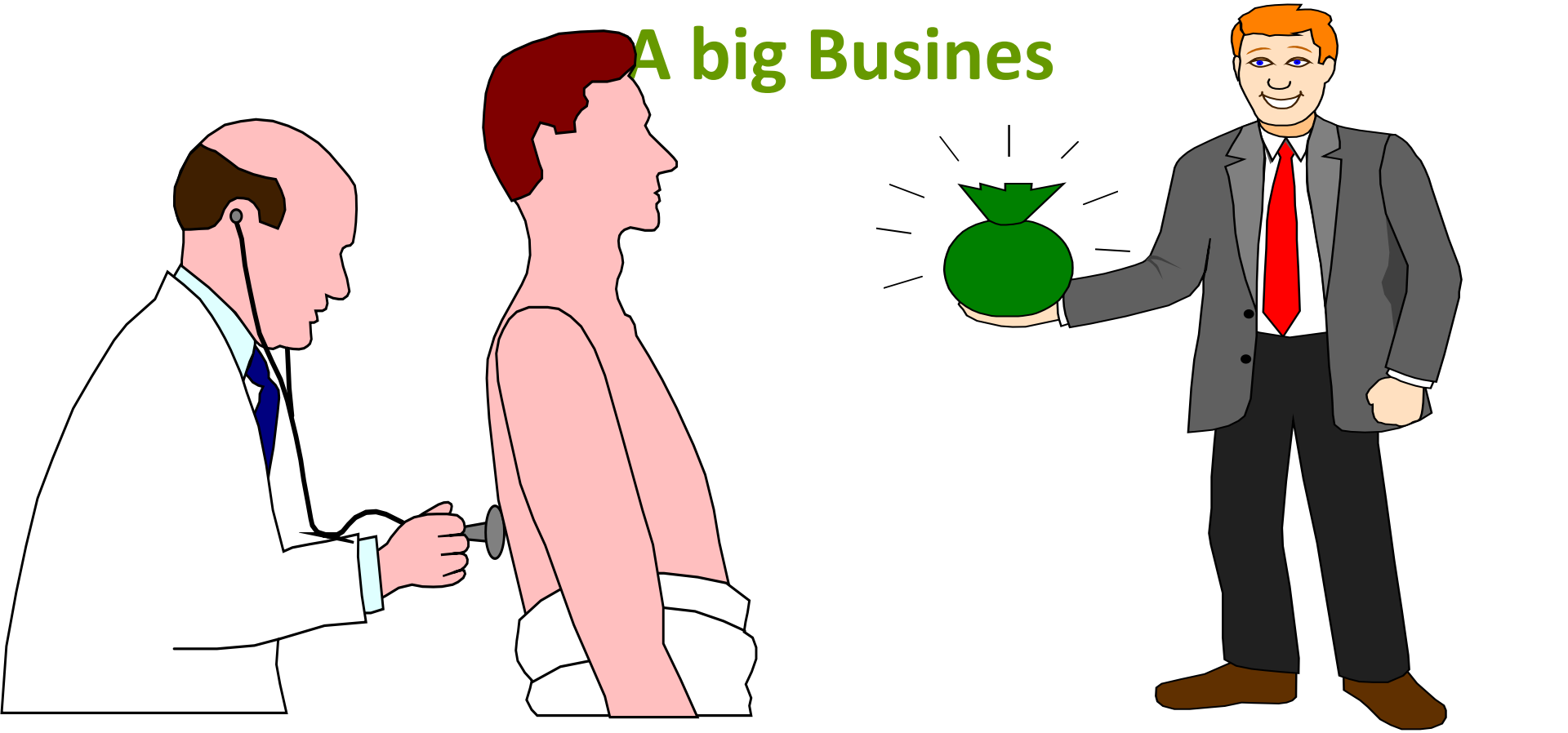
Why is fetus not rejected?

- Progesterone--- hormone--- immunosuppressive.
- Placenta expresses FasL.
- Spontaneous abortions are some times triggered by maternal immune response against fetus.

Ethical aspects

Organs for sale !

A big Busines



Programme Created by Dr.T.V.Rao MD for benefit of Undergraduate Students in Immunology and Microbiology in Developing world

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Chapter 17

Immune Responses to Infectious Disease And Vaccines

Dr. Capers

IMMUNOLOGY

Kindt • Goldsby • Osborne

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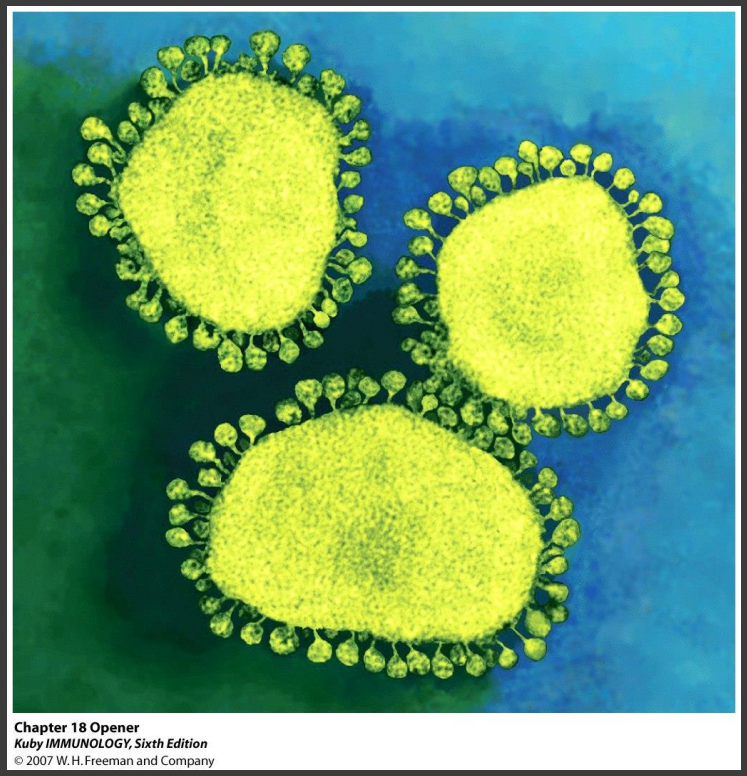
Sixth Edition

Chapter 18

Immune Response to Infectious Diseases



Chapter 18 Opener
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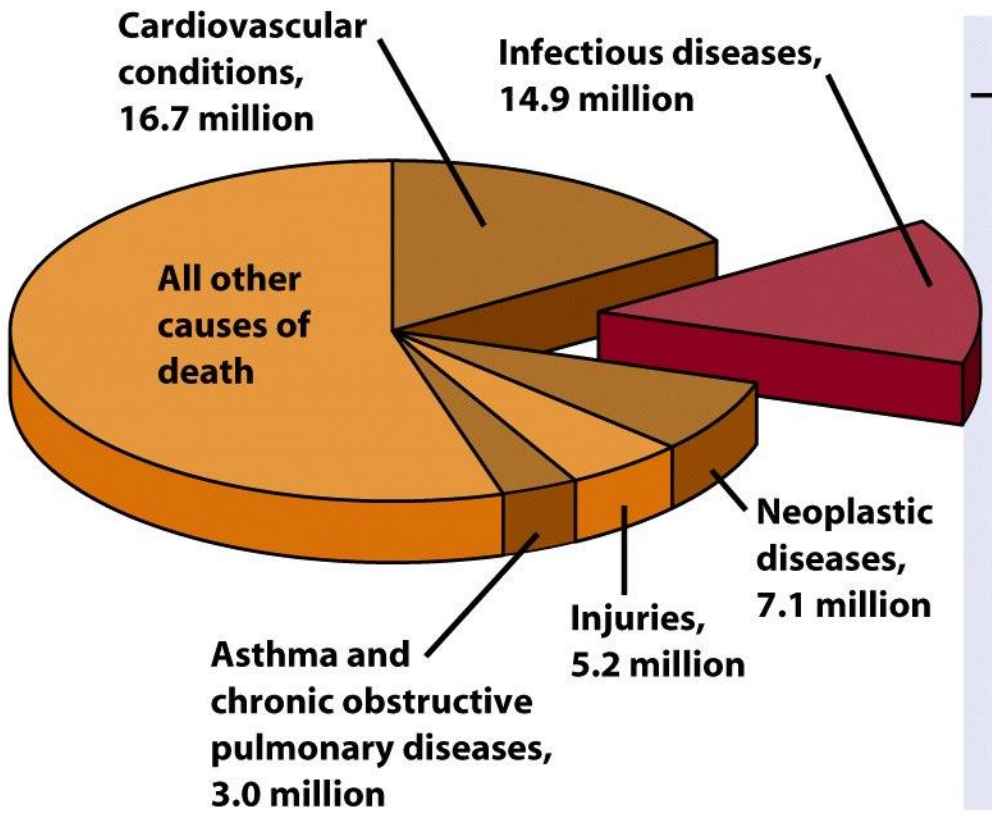
- Pathogens use variety of strategies to escape immune system



Infectious diseases	Annual deaths
Respiratory infections	3.96
HIV/AIDS	2.77
Diarrheal diseases	1.80
Tuberculosis	1.56
Vaccine-preventable childhood diseases	1.12
Malaria	1.27
STDs (other than HIV)	0.18
Meningitis	0.17
Hepatitis B and C	0.16
Tropical parasitic diseases	0.13
Dengue	0.02
Other infectious diseases	1.76

Figure 18-1
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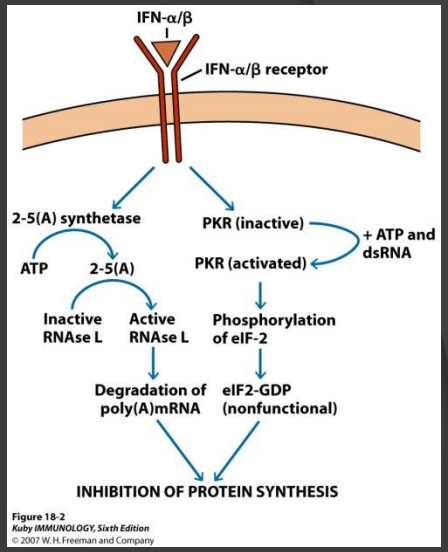
Viral Infections

- Long latency period before severe illness
  - HIV
- Efficient transmission during short illness
  - Influenza
- Life cycle in other host, vectors
  - West nile

Viral Infections



- Activation of NK cells
- Induction of interferons
  - Bind to IFN receptor
  - Activate JAK-STAT pathway
  - Induces transcription of genes of host cell
    - Enzyme that degrades viral RNA
- Can be neutralized by antibodies
- If viral DNA is integrated into host, cell must be killed

TABLE 18-1 Mechanisms of humoral and cell-mediated immune responses to viruses

Response type	Effector molecule or cell	Activity
Humoral	Antibody (especially secretory IgA)	Blocks binding of virus to host cells, thus preventing infection or reinfection
	IgG, IgM, and IgA antibody	Blocks fusion of viral envelope with host cell's plasma membrane
	IgG and IgM antibody	Enhances phagocytosis of viral particles (opsonization)
	IgM antibody	Agglutinates viral particles
	Complement activated by IgG or IgM antibody	Mediates opsonization by C3b and lysis of enveloped viral particles by membrane-attack complex
Cell mediated	IFN-γ secreted by T_H or T_C cells	Has direct antiviral activity
	Cytotoxic T lymphocytes (CTLs)	Kill virus-infected self cells
	NK cells and macrophages	Kill virus-infected cells by antibody-dependent cell-mediated cytotoxicity (ADCC)

Table 18-1
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Viral Infections

- Evading host defenses
  - Block or inhibit production of interferons
  - Inhibition of antigen presentation
  - Evade complement
  - Cause general immunosuppression

Influenza – "Flu"

- Respiratory illness
- Responsible for some of the worse pandemics in history
- Spherical virion surrounded by lipid bilayer acquired from host
  - 2 glycoproteins – hemagglutin (HA) and neuraminidase (NA)
  - Antigenic variation in these (mutations leading to new strains) cause problems in developing sustained immunity in the population

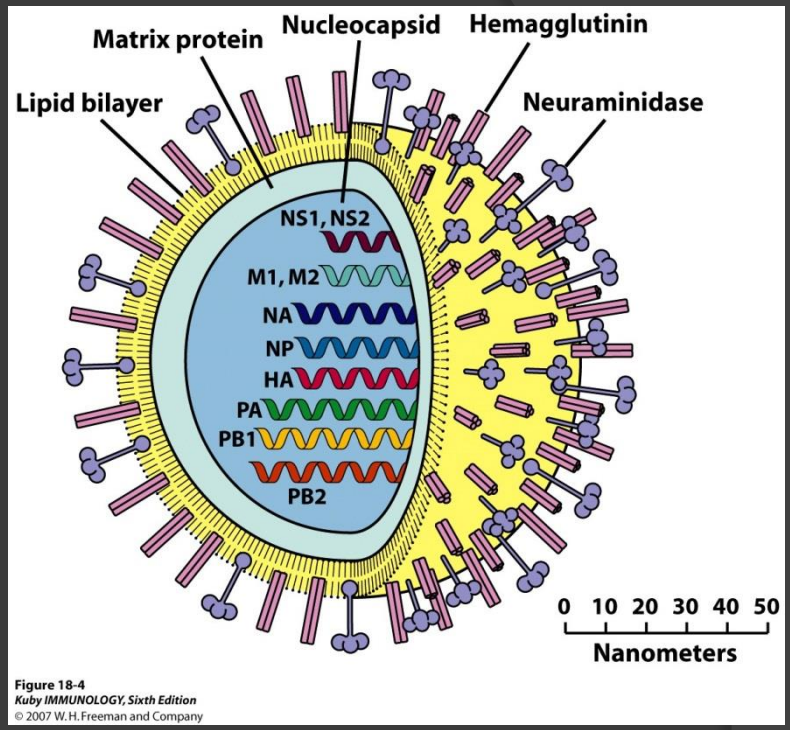

Figure 18-4
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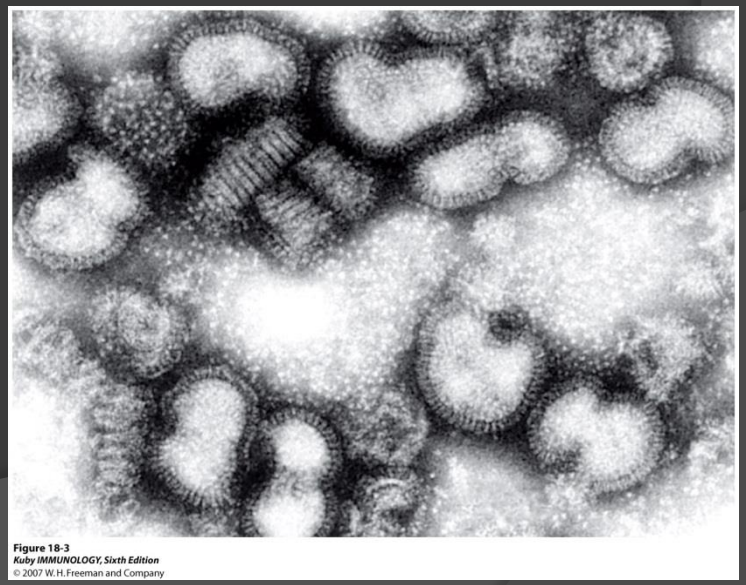
Figure 18-3
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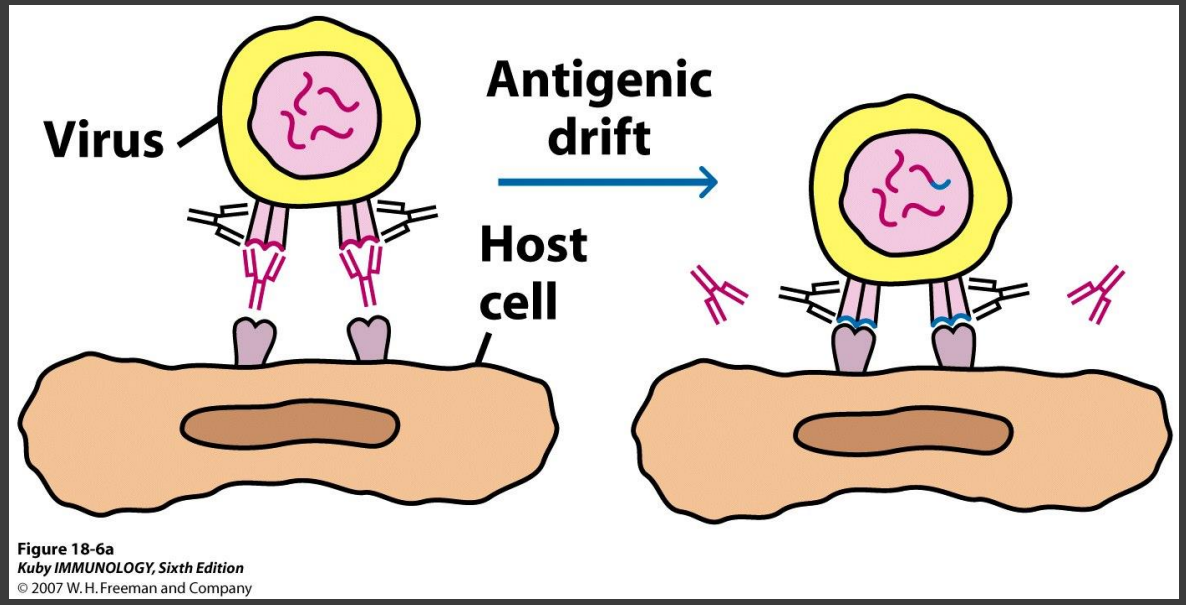

Figure 18-6a
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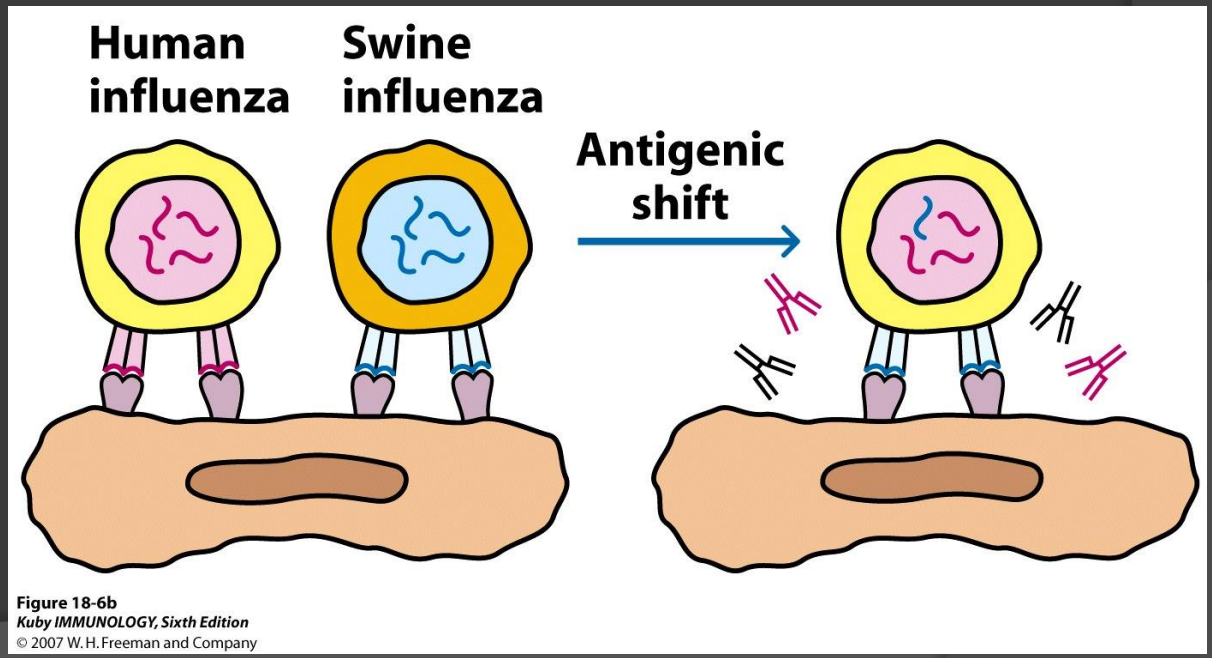

Figure 18-6b
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Bacterial Infections

- Immunity mainly achieved by antibodies
  - Unless bacteria is capable of intracellular growth
- Depending on # of organisms entering and virulence, different levels of host defense enlisted
  - If inoculum size and virulence is low, phagocytes may be able to eliminate the bacteria

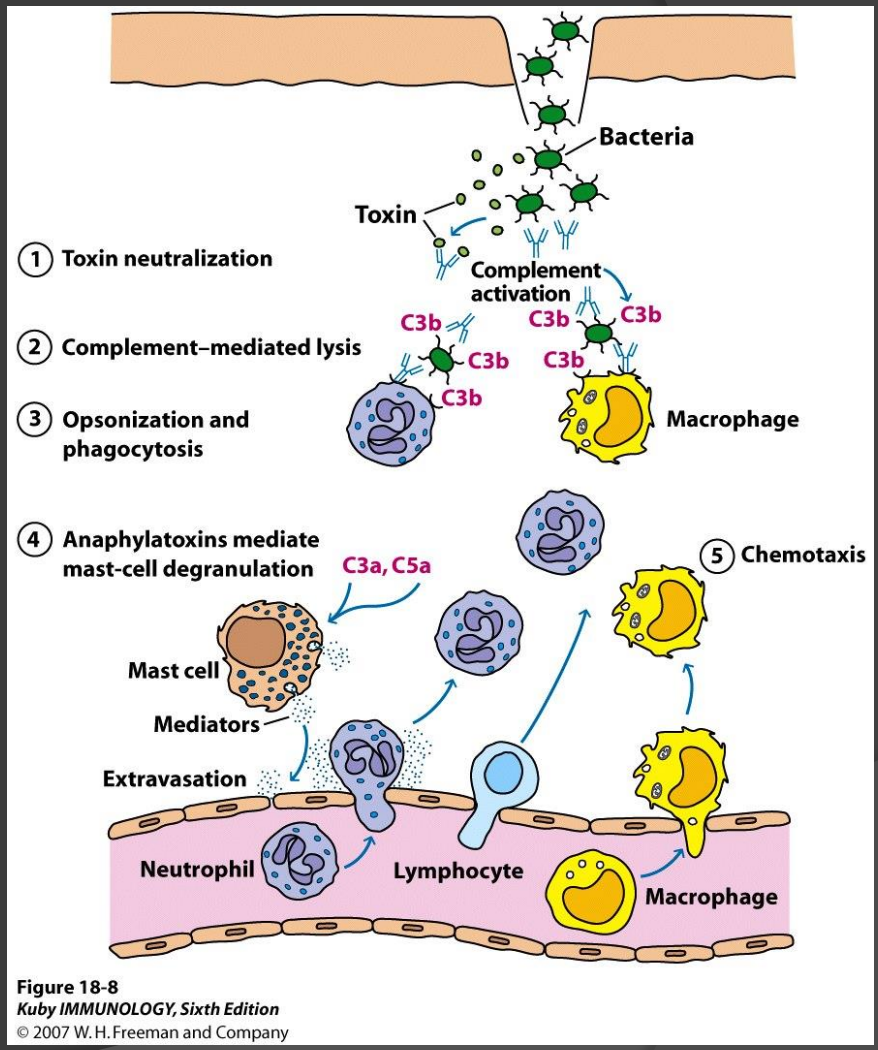

Figure 18-8
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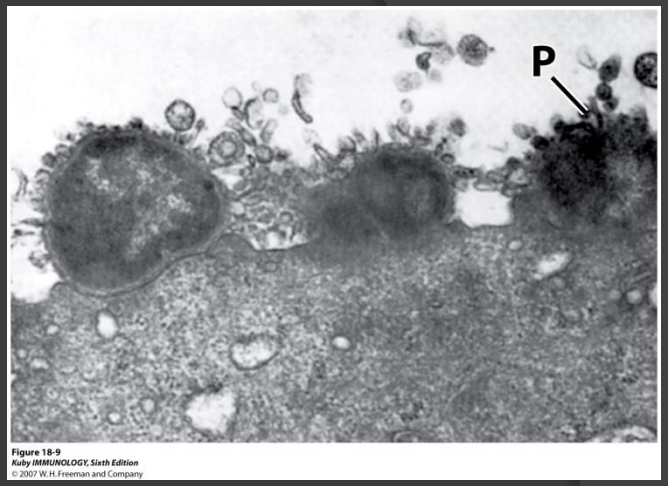
Bacterial Infections



- 4 steps:
  - Attachment to host cells
  - Proliferation
  - Invasion of host tissue
  - Toxin-induced damage to host cells
- Host defenses act at each of these sites, some bacteria have developed ways to avoid

TABLE 18-3 Host immune responses to bacterial infection and bacterial evasion mechanisms

Infection process	Host defense	Bacterial evasion mechanisms
Attachment to host cells	Blockage of attachment by secretory IgA antibodies	Secretion of proteases that cleave secretory IgA dimers (*Neisseria meningitidis, N. gonorrhoeae, Haemophilus influenzae*) Antigenic variation in attachment structures (pili of *N. gonorrhoeae*)
Proliferation	Phagocytosis (Ab- and C3b-mediated opsonization)	Production of surface structures (polysaccharide capsule, M protein, fibrin coat) that inhibit phagocytic cells Mechanisms for surviving within phagocytic cells Induction of apoptosis in macrophages (*Shigella flexneri*)
	Complement-mediated lysis and localized inflammatory response	Generalized resistance of gram-positive bacteria to complement-mediated lysis Insertion of membrane-attack complex prevented by long side chain in cell-wall LPS (some gram-negative bacteria)
Invasion of host tissues	Ab-mediated agglutination	Secretion of elastase that inactivates C3a and C5a (*Pseudomonas*)
Toxin-induced damage to host cells	Neutralization of toxin by antibody	Secretion of hyaluronidase, which enhances bacterial invasiveness

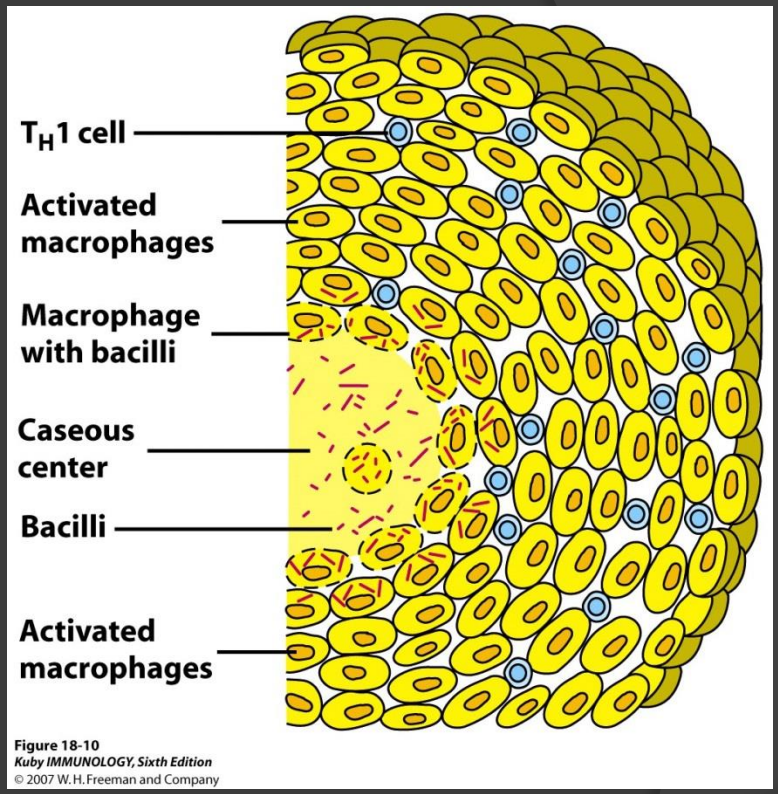
Table 18-3
Kuby IMMUNOLOGY, Sixth Edition

Immune responses can contribute to bacterial pathogenesis

- Overproduction of cytokines
  - Septic shock, food poisoning, toxic shock
- Intracellular bacteria
  - Chronic antigenic activation of CD4+ T cells
  - Leads to tissue destruction
  - Characteristics of delayed-type hypersensitivity
  - Leads to development of granuloma and necrosis

Tuberculosis

- Intracellular bacillus
- CD4+ T cell response
  - Responsible for most of the tissue damage
  - This necrosis can be seen when tested for TB



- Tubercle formed in pulmonary tuberculosis

Parasitic Disease

- Protozoan and helminthic organsims
- Malaria – *Plasmodium*, protozoan
  - Complex life cycle

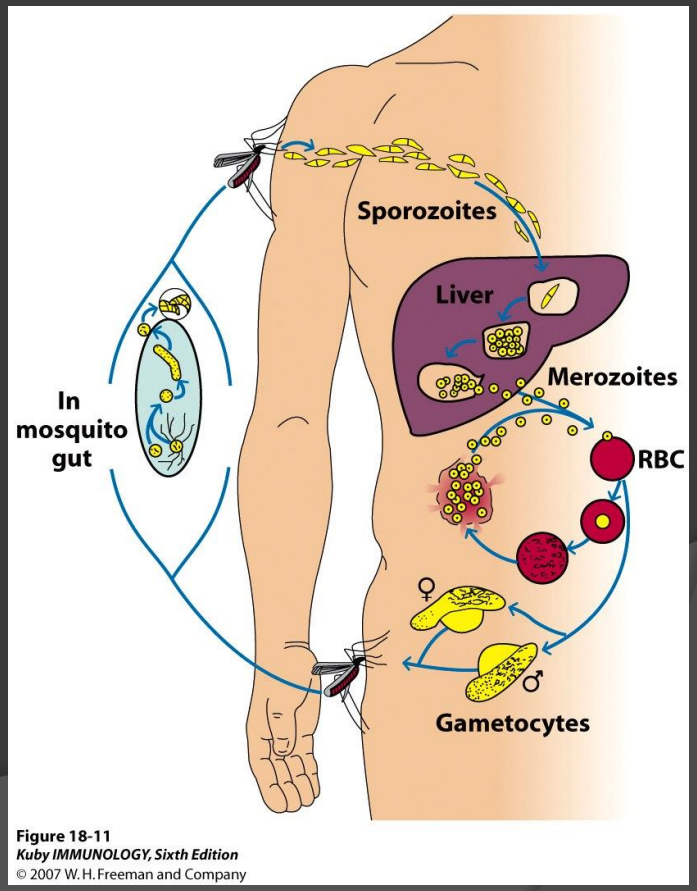


Figure 18-11
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Parasitic Infections

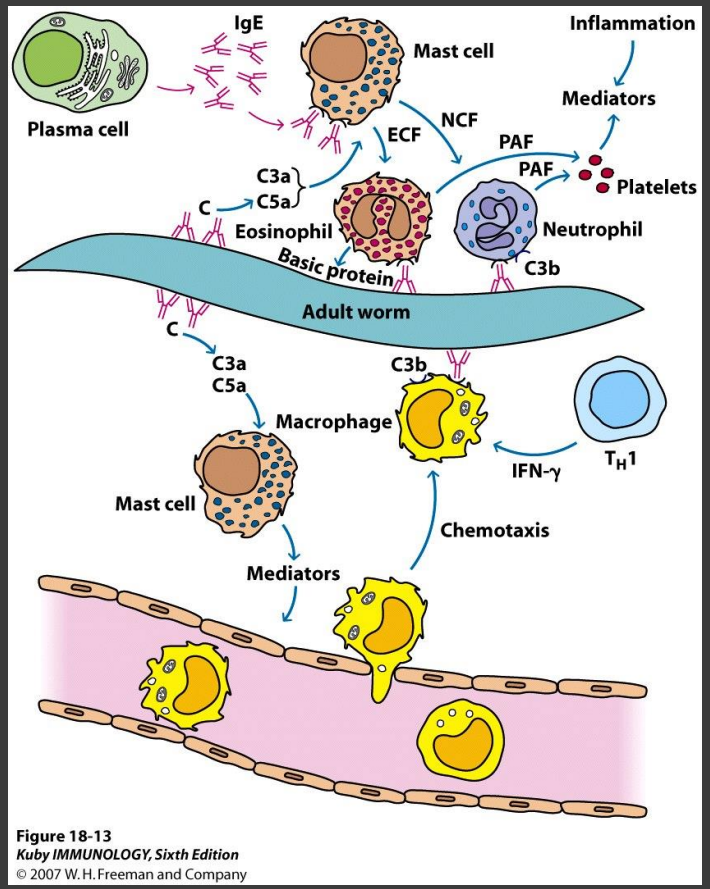

Figure 18-13
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- Helminthes
  - IgE plays big role

Fungal Infections

- Most fungal infections of healthy individuals resolve rapidly
- Barriers of innate immunity control most fungi
- Mannose-binding protein recognizes some major fungal pathogens

TABLE 18-4 Classification of fungal diseases

Site of infection	Superficial	Epidermis, no inflammation
	Cutaneous	Skin, hair, nails
	Subcutaneous	Wounds, usually inflammatory
	Deep or systemic	Lungs, abdominal viscera, bones, CNS
Route of acquistion neous	Exogenous	Environmental, airborne, cutaneous or percuta-
	Endogenous	Latent reactivation, commensal organism
Virulence	Primary	Inherently virulent, infects healthy host
	Opportunistic	Low virulence, infects immunocompromised host

Table 18-4
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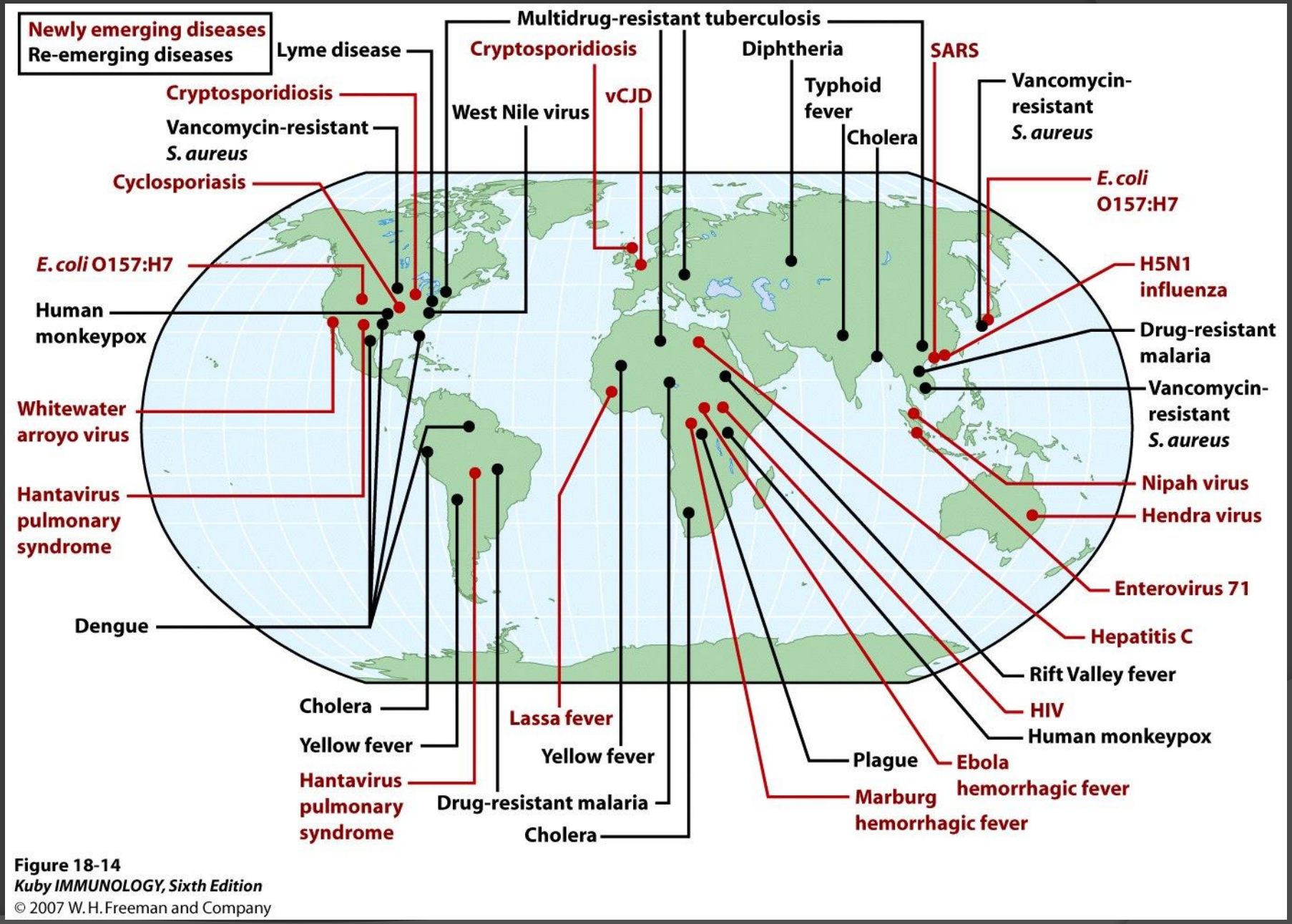

Figure 18-14
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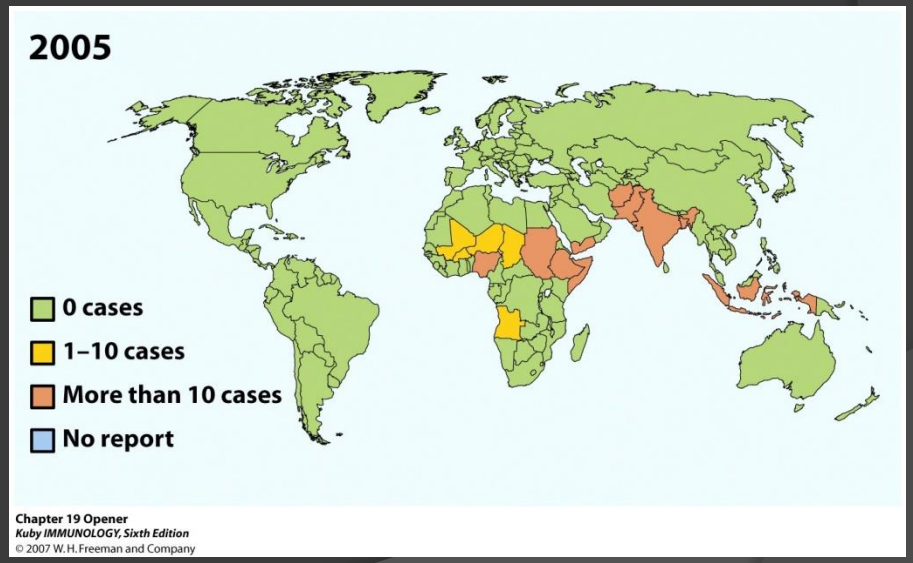
Bioterrorism

- Something to be concerned with….

Unnumbered figure pg 469
Kuby IMMUNOLOGY, Sixth Edition

- Discipline of Immunology
  - Early roots in vaccination trials of Edward Jenner and Louis Pasteur
  - Working vaccines
    - Diptheria
    - Measles
    - Mumps
    - Poliomyelitis
    - Tetanus



Cases of polio have dramatically declined since vaccination

Estimated annual deaths worldwide of children under 5 years of age, by pathogen

Pathogen	Deaths (thousands)
Pneumococcus*	841
Measles	530
***Haemophilus* (strains a–f)†**	945
Rotavirus†	800
Malaria	700
HIV	500
RSV	500
Pertussis	285
Tetanus	201
Tuberculosis	100

*Bold signifies pathogens for which an effective vaccine exists.

†A licensed vaccine is being tested for possible side effects.

SOURCE: Data derived from WHO publications.

Unnumbered table pg 476
Kuby IMMUNOLOGY, Sixth Edition

- Vaccines are still needed against many diseases
- Vaccines that are available need to be administered
  - There are people that are choosing not to vaccinate……could potentially create scary scenario in future

Developing a vaccine

- Lots of research
  - Time consuming, costly
  - Idea is to isolate a component of the organism that proves to be immunogenic….sometimes not possible
- Human trials are strictly regulated
- Might have vaccine developed but there might be adverse side effects – can't be used…

- Immunity can be achieved by active or passive immunization
  - Passive – transfer of preformed antibodies
    - Maternal antibodies to fetus
    - Antibody therapy for bites, immunodeficiency
  - Active – long term protection, immunologic memory, actual exposure
    - Coming into contact with any foreign substance
    - vaccines

TABLE 19-1 Acquisition of passive and active immunity

Type	Acquired through
Passive immunity	Natural maternal antibody
	Immune globulin*
	Humanized monoclonal antibody
	Antitoxin†
Active immunity	Natural infection
	Vaccines‡
	Attenuated organisms
	Inactivated organisms
	Purified microbial macromolecules
	Cloned microbial antigens
	Expressed as recombinant protein
	As cloned DNA alone or in virus vectors
	Multivalent complexes
	Toxoid§

*An antibody-containing solution derived from human blood, obtained by cold ethanol fractionation of large pools of plasma; available in intramuscular and intravenous preparations.

†An antibody derived from the serum of animals that have been stimulated with specific antigens.

‡A suspension of attenuated live or killed microorganisms, or antigenic portions of them, presented to a potential host to induce immunity and prevent disease.

§A bacterial toxin that has been modified to be nontoxic but retains the capacity to stimulate the formation of antitoxin.

Table 19-1
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TABLE 19-2 Common agents used for passive immunization

Disease	Agent
Black widow spider bite	Horse antivenin
Botulism	Horse antitoxin
Cytomegalovirus	Human polyclonal Ab
Diphtheria	Horse antitoxin
Hepatitis A and B	Pooled human immunoglobulin
Measles	Pooled human immunoglobulin
Rabies	Human or horse polyclonal Ab
Respiratory disease	Monoclonal anti-RSV*
Snake bite	Horse antivenin
Tetanus	Pooled human immunoglobulin or horse antitoxin
Varicella zoster virus	Human polyclonal Ab

*Respiratory syncytial virus

SOURCE: *Adapted from A. Casadevall, 1999, *Clinical Immunology* **93**:5.

Table 19-2
Kuby IMMUNOLOGY, Sixth Edition

- There is a chance of side effects in small # of population
  - That is the case with any treatment/drug
  - However, if the benefits to the population out-weigh the risk of side effects, vaccines must be used to protect the majority of the population
  - HERD IMMUNITY

TABLE 19-3 Recommended childhood immunization schedule in the United States, 2006

Vaccine*	Age: Birth	1 month	2 months	4 months	6 months	12 months	15 months	18 months	24 months	4–6 years
Hepatitis B	•	←—•—→			←————	•———→				
Diphtheria, tetanus, pertussis			•	•	•		←—•—→			•
Haemophilus influenzae type b			•	•	•	←—•—→				
Inactivated poliovirus			•	•	←————	•———→				•
Measles, mumps rubella						←—•—→				•
Varicella						←—•—→				
Pneumococcal conjugate			•	•	•	←—•—→				
Influenza				(Yearly)	•	•	•	•	•	•
Hepatitis A				(Two doses at least 6 months apart)		←—•———→				
							←———•—→			

←—•—→ Arrows indicate time range during which an immunization is recommended

*This schedule indicates the recommended ages for routine administration of currently licensed childhood vaccines. Any dose not given at the recommended age should be given as a "catch-up" immunization at any subsequent visit.

SOURCE: Adapted from the CDC Web site; approved by the American Academy of Pediatrics.

Table 19-3
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Designing Effective Vaccine

- Protective immunity must be achieved
  - Must pay attention to how the antigen activates the humoral and cell-mediated branches
- Must produce immunologic memory
  - Vaccine that produces primary response but fails to produce secondary response is not effective

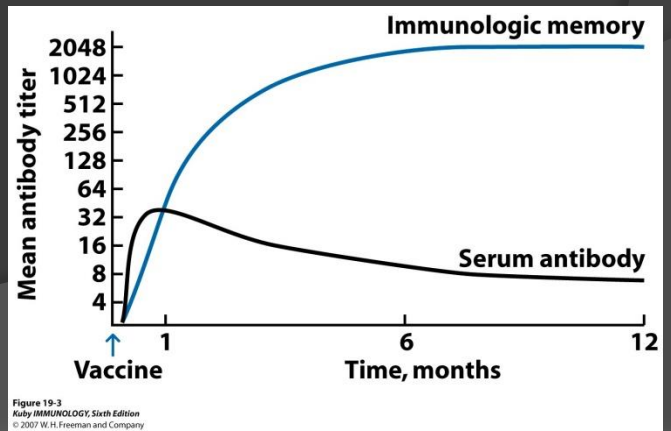

Figure 19-3
Kuby IMMUNOLOGY, Sixth Edition

Live, Attenuated Vaccines

- Microorganisms can be attenuated so that they lose ability to cause significant disease
  - Retain capacity for growth in host
  - Bacteria is grown for prolonged period in adverse conditions
    - Those that survive will not be suited to grow in "better" conditions in host
  - A virus might be grown in cell type that is not normal host
    - Accumulates mutations that might weaken it
  - Measles, mumps, rubella vaccine is example

Live, Attenuated Vaccines

- Advantages
  - Can grow in host therefore producing immunologic memory with only single vaccination
  - Produces memory T cells
  - Good for distribution in Third World countries
- Disadvantages
  - Possibility that it will revert to virulent form
    - Polio – 1 in 2.4 million chance this will happen
  - Complications
    - Measles vaccine – encephalitis
    - Out of 75 million patients between 1970 and 1993, only 48 cases
  - Danger from remaining un-vaccinated and getting disease is much greater than complications to these proven vaccines

TABLE 19-4 Classification of common vaccines for humans

Vaccine type	Diseases	Advantages	Disadvantages
Live attenuated	Measles Mumps Polio (Sabin vaccine) Rotavirus Rubella Tuberculosis Varicella Yellow fever	Strong immune response; often lifelong immunity with few doses	Requires refrigerated storage; may mutate to virulent form
Inactivated or killed	Cholera Influenza Hepatitis A Plague Polio (Salk vaccine) Rabies	Stable; safer than live vaccines; refrigerated storage not required	Weaker immune response than live vaccines; booster shots usually required
Toxoid	Diphtheria Tetanus	Immune system becomes primed to recognize bacterial toxins	
Subunit (inactivated exotoxin)	Hepatitis B Pertussis Streptococcal pneumonia	Specific antigens lower the chance of adverse reactions	Difficult to develop
Conjugate	*Haemophilus influenzae* type B Streptococcal pneumonia	Primes infant immune systems to recognize certain bacteria	
DNA	In clinical testing	Strong humoral and cellular immune response; relatively inexpensive to manufacture	Not yet available
Recombinant vector	In clinical testing	Mimics natural infection, resulting in strong immune response	Not yet available

Table 19-4
Kuby IMMUNOLOGY, Sixth Edition

TABLE 19-5 Risk of complications from natural measles infection compared with known risks of vaccination with a live attenuated virus in immunocompetent individuals

Complication	Risk after natural disease*	Risk after vaccination†
Otitis media	7%–9%	0
Pneumonia	1%–6%	0
Diarrhea	66%	0
Post-infectious encephalomyelitis	0.5–1 per 1000	1 per 1,000,000
SSPE	1 per 100,000	0
Thrombocytopenia	—‡	1 per 30,000§
Death	0.1–1 per 1000 (up to 5%–15% in developing countries)	0

*Risk after natural measles are calculated in terms of events per number of cases.

†Risks after vaccination are calculated in terms of events per number of doses.

‡Although there have been several reports of thrombocytopenia occurring after measles, including bleeding, the risk has not been properly quantified.

§This risk has been reported after MMR vaccination and cannot be attributed only to the measles component.

MMR = measles, mumps, and rubella.

SSPE = subacute sclerosing panencephalitis.

Table 19-5
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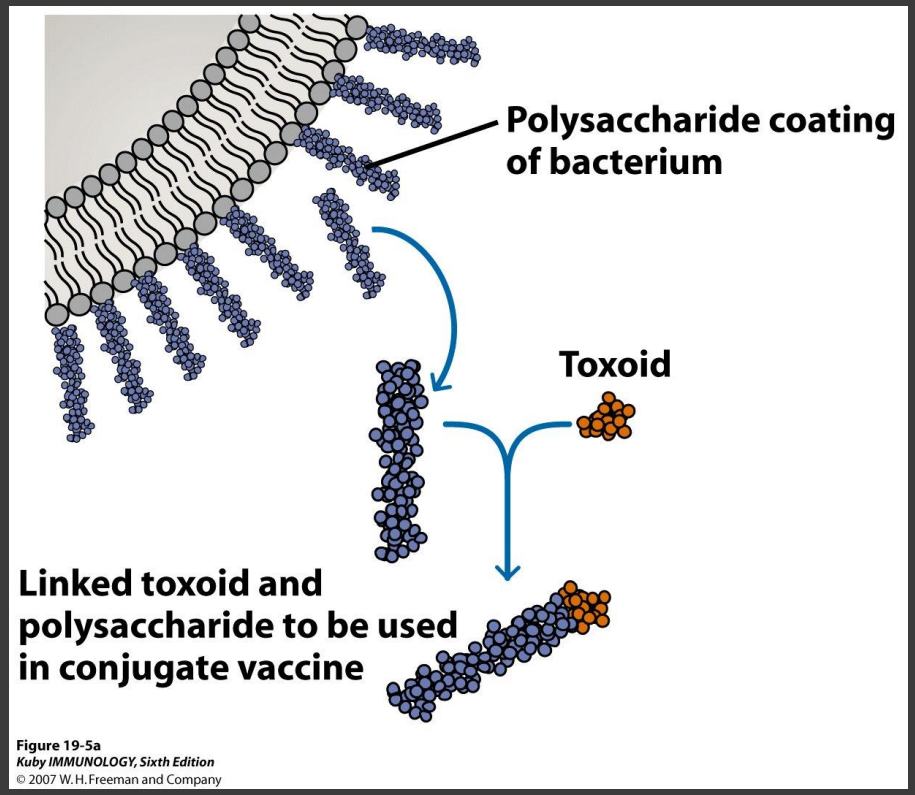
Inactivated or “killed” vaccines

- Inactivation of pathogen by heat or chemical means
  - Not capable of replication in host
  - Epitopes have to be maintained after killing process
- Often require boosters
- Risks
  - Pathogen has to be grown in large #’s prior to inactivation – individuals involved in manufacturing are at risk
  - Some of the pathogen may not be killed
- Pertussis vaccine, typhoid vaccine, flu vaccine

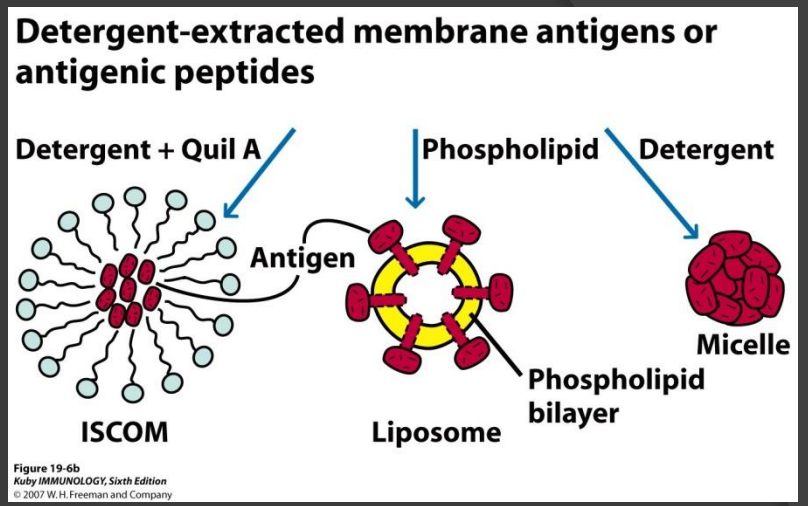
Subunit Vaccines

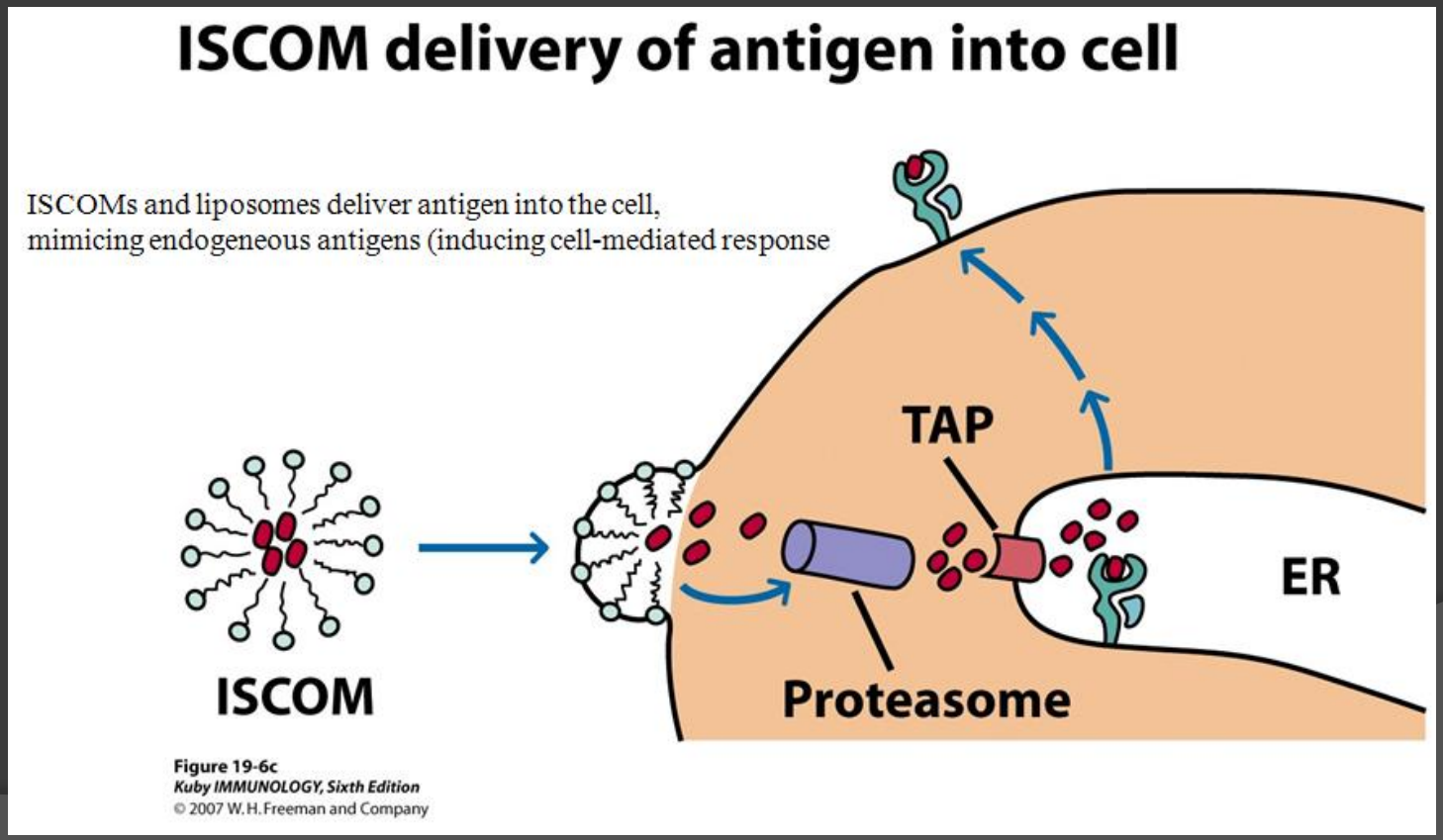
- Purified macromolecules derived from pathogens
- Toxoids
  - Some bacteria are pathogenic because of exotoxins that they produce
  - Purify exotoxin, inactivate it with formaldehyde to form toxoid that can be used to immunize
- Bacterial polysaccharide capsules
- Viral glycoproteins are candidates
  - Little success so far

Conjugate Vaccines



- Polysaccharide vaccines unable to activate TH cells
  - Activate B cells in thymus-independent manner
  - IgM production but no class switching, no memory
- Conjugate to protein carrier that is considerably more immunogenic


Detergent-extracted membrane antigens or antigenic peptides
Detergent + Quil A
Phospholipid
Detergent
Antigen
Micelle
Phospholipid bilayer
ISCOM
Liposome
Figure 19-6b
Kuby IMMUNOLOGY, Sixth Edition
© 2007 W.H.Freeman and Company


ISCOM delivery of antigen into cell
ISCOMs and liposomes deliver antigen into the cell, mimicing endogeneous antigens (inducing cell-mediated response
TAP
ER
ISCOM
Proteasome
Figure 19-6c
Kuby IMMUNOLOGY, Sixth Edition
© 2007 W.H.Freeman and Company

DNA Vaccines

Gene is injected

Protein is expressed inducing humoral and cell-mediated response

Low risk, no refrigeration

But can only be done for antigens that are protein

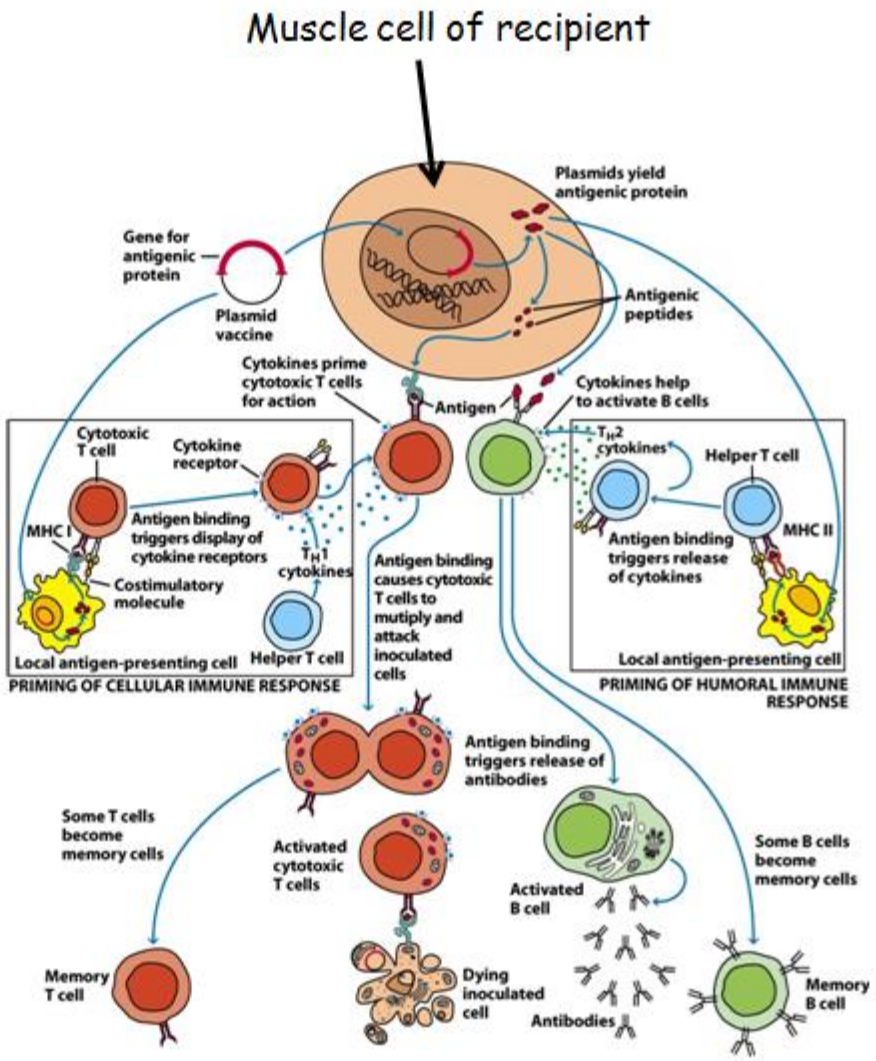


Figure 19-7
Kuby IMMUNOLOGY, Sixth Edition

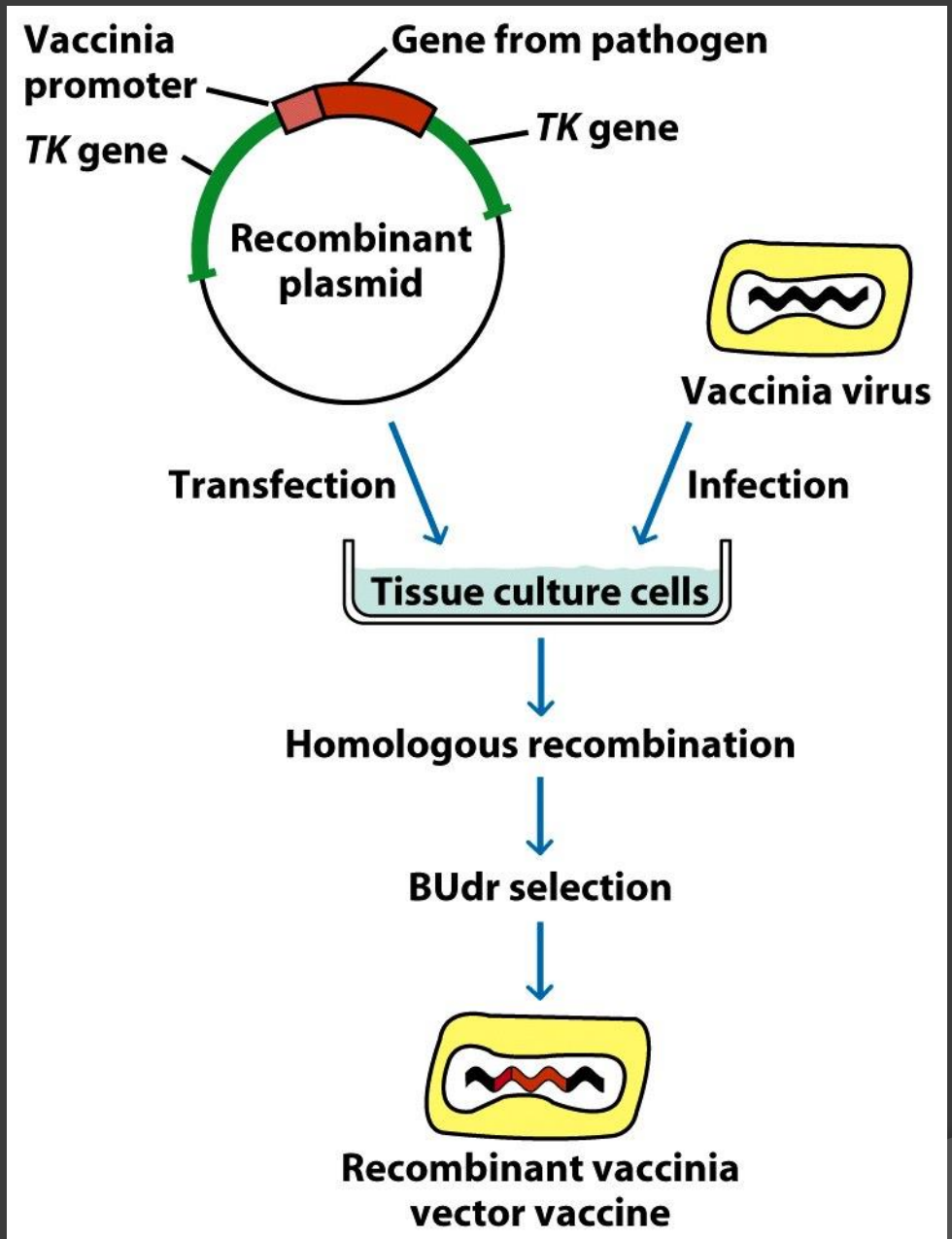


Figure 19-8 part 2
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ABZYMES

SHIJINA.A

BCH 10-05- 04

S2 MSc BIOCHEMISTRY

Introduction

- Antibodies and enzymes share the ability to bind with compounds with great specificity and high affinity.
- This property has been exploited in the development of antibodies with catalytic activity.
- Antibodies have been 1st characterized as proteins produced by the IS for binding with molecules called antigens.
- One basic difference between antibodies and enzymes is that the former binds the complementary structure in its ground state , while enzymes bind in high energy state

- In 1986 , the 1st monoclonal catalytic antibodies termed *abzymes* against a chemically stable analog of the transition state of a reaction were obtained
- Abzymes are catalytic antibodies having structural complementarity for the transition state of an enzyme catalyzed reaction.
- They bind strongly to the transition state with high association constant, enhancing the reaction rate .
- Abzymes reduce rotational entropy .

Sources of Abzymes

- **Abzymes are usually artificial constructs.**
- **They also obtained from human and animal serum.**
- **Found in normal humans and ii patients with autoimmune diseases.**
- **These are capable of hydrolyzing proteins, DNA, RNA, polysaccharides etc**

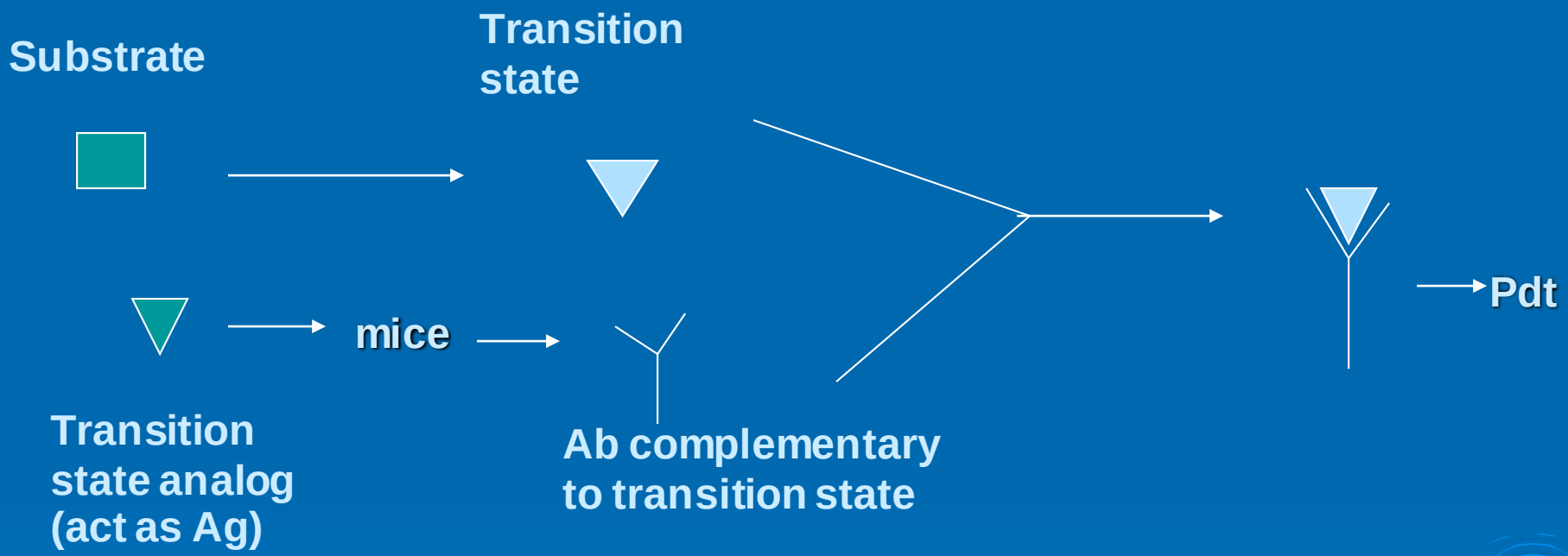
Protabzymes and DNA Abzymes

- Natural abzymes with proteolytic activity are called Protabzymes .e.g.: hydrolysis of specific proteins in patients with autoimmune diseases such as bronchial Asthma ,multiple sclerosis.
- DNA hydrolyzing activity are called DNA abzymes.
- The pathogenic role of DNA abzymes is not quite clear. However they act as a powerful regulator of apoptosis.

Production of abzymes

- **Antibody molecules are produced by the immune system to bind and neutralize foreign substances called antigens**
- **Foreign proteins of bacteria , viruses and some chemical molecules called haptens , act as antigens .**
- **Transition state analogs are molecules which are more stable than the transition state itself , but they mimic its 3D structure .**

- If injected into the blood stream of an animal , transition state analogs act as haptens and elicit antibody production.
- Abs are isolated from the serum of the animal and used as abzymes .
- Theoretically ,if the Ab binds to a transition state molecule, it may be expected to catalyze a corresponding chemical reaction by forcing substrates into transition state geometry.

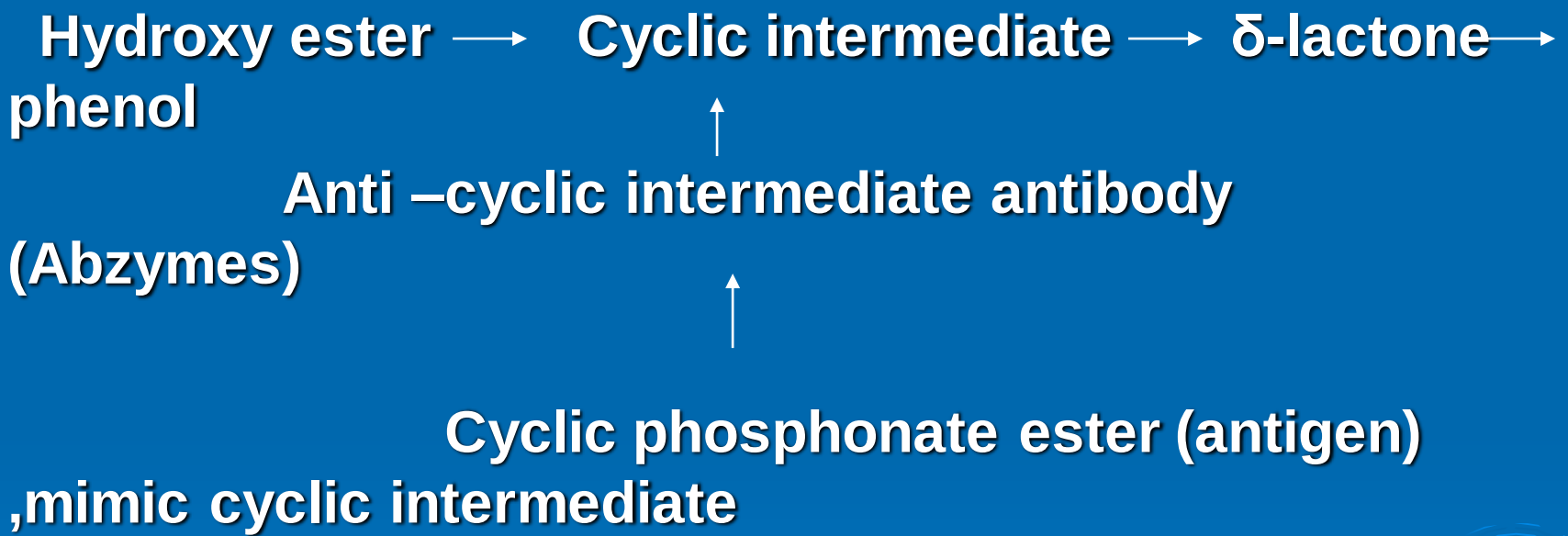

Substrate
Transition state
mice
Pdt
Transition state analog (act as Ag)
Ab complementary to transition state

Examples for abzymes

1. **Hydrolysis of hydroxy ester by abzymes**

   Hydroxy ester forms a cyclic intermediate during hydrolysis.

- **Cyclic phosphonate ester is the structural analog of the cyclic intermediate.**
- **This analog is used as an antigen to elicit antibodies.**
- **These antibodies bind the cyclic intermediate , increasing the reaction rate .**


Hydroxy ester
Cyclic intermediate
δ-lactone
phenol
Anti –cyclic intermediate antibody
(Abzymes)
Cyclic phosphonate ester (antigen)
,mimic cyclic intermediate

2. Hydrolysis of ester by abzymes

- Ester forms a tetrahedral intermediate during hydrolysis
- The phosphate analog of ester mimic this intermediate, used as antigen to elicit antibodies.
- These antibodies recognize and bind to tetrahedral intermediate and stabilize it resulting in rate acceleration.

- Biosynthesis of Heme
  - **It involves introduction of Fe2+** into protophorphyrine by ferrochelatase.
  - This process is called metallation
  - Metallation involves the distortation of pyrole ring by 36ºto create a bent transition state

- This state is apt for the entry Fe2+
- Methyl mesoporphyrin , an analog of the bent transition state , is used as antigen to elicit abzymes.
- These abzymes bind the bent transition state and distorts the porphyrin facilitating metallation rate 2500fold higher

Reactions catalyzed by Abzymes

1. **Amide hydrolysis**
2. **Trans- Esterification**
3. **photo cleavage**
4. **Photodimerization**
5. **Decarboxylation**
6. **Oxidation**
7. **Cyclization**
8. **Reduction of diketone**
9. **Hydrolysis of enol ethers**

Applications

- **Synthesis of simple organic molecules**
- **Drug development**
- **Treat Cancer**
- **Treat allergy**
- **treat viral and bacterial infection**

Reference

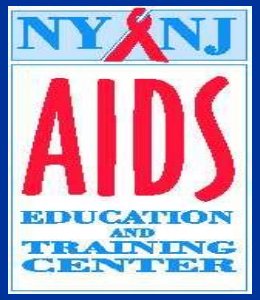
- Enzymology –T. Devasena

THANK YOU

HIV Pathogenesis

Corrections Curriculum Development: Module 2

Albany Medical College
Division of HIV Medicine



Pathogenesis

Definition:

The development of morbid conditions or of disease; more specifically, the cellular events and reactions and other pathologic mechanisms occurring in the development of a disease.

www.rerf.or.jp/eigo/glossary/pathogen.htm

Basic Components of the Immune System

- **Immunology:** cells and tissues involved in recognizing and attacking foreign substances in the body e.g. bacteria, viruses, fungi and parasites.

- **Immunity:** the condition of being immune. Immunity can be innate or the result of a previous exposure.

- **Antigen:** any substance capable of triggering an immune response.

Basic Components of the Immune System

- Of the white blood cell pool, **lymphocytes** primarily drive the immune system.

- Lymphocytes (2 major types which protect host):

  (1) **B cells:** formed in **b**one marrow and produce antibodies after exposure to an antigen.

  (2) **T cells:** processed in the **t**hymus (two subtypes)

  Subtype 1: **Regulator cells** also known as **helper** or **CD4 cells** ("generals" in army of immune system which recognize "invaders" and summon armies of cells to mount a direct attack)

  Subtype 2: **Fighter** or **effector cells** also known as **cytotoxic** or **CD8 cells** (bind directly to antigen and kill it)

Basic Components of the Immune System

- 2 types of CD4 cells:

  (1) **Memory cells:** those programmed to recognize a specific antigen after it has been previously seen

  (2) **Naïve cells:** non-specific responders

- CD4 cells replicate 100 million times a day.

- CD4 cells are the target cells of HIV.

Bartlett, J.: The Johns Hopkins Hospital 2002 Guide to Medical Care of Patients with HIV Infection

Basic Components of the Immune System

- **Lymphatic vessels and nodes:** designed to trap and destroy antigen and play a critical role in fighting all infections including HIV

- **Phagocytes:** "scavengers" of the immune system

  -By digesting/processing antigen, their role is to initiate the immune response by presenting antigen to the lymphocytes.

  -Serve a secretory function critical to mounting the inflammatory response and regulating immune responses

Grimes D. and Grimes R.: AIDS & HIV Infection St. Louis, Mosby, 1994.

HIV Viral Dynamics

- HIV is classified as a **retrovirus**

  -Once HIV enters the host (CD4) cell, it converts its **RNA** (ribonucleic acid) to **DNA** (deoxyribonucleic acid) via its enzyme reverse transcriptase.

- HIV is completely dependent upon CD4 cells for replication and survival.

HIV Viral Dynamics

Replication and survival of HIV occurs through a number of steps:

HIV gains entry into the CD4 cell by **binding onto receptors** on the outside of the CD4 cell and **fusing** with the lipid outer layer of the cell.

Once inside the cell, HIV removes its outer coating, exposing its RNA, and releases **reverse transcriptase enzyme** to convert the HIV RNA to DNA.

HIV DNA then enters the nucleus of the CD4 cell and is **integrated** into the host (CD4) DNA

HIV Viral Dynamics

Replication and survival of HIV (con't)

Once the cellular DNA has been altered in this way, it is known as proviral DNA (part virus/part cell) and begins the process to produce more virus.

The CD4 cell is now programmed to be an 'HIV factory.'

Long viral protein chains are produced which are then cut into the necessary pieces to produce more HIV. This process is activated by the viral **protease enzyme.**

Each step in this process is a target for antiretroviral therapy (to date, reverse transcriptase, protease inhibitors and fusion inhibitors have been approved)

Stages of HIV Disease

- Acute/Early Infection: Following HIV transmission, approximately 50% of individuals will develop a febrile, flu-like illness with some or all of the following conditions:

  - Swollen glands
  - Oral ulcers
  - Sore throat
  - Diarrhea
  - Rash
  - Muscle aches
  - Headache
  - Nausea or vomiting

Stages of HIV Disease

Acute/Early Infection (con't)

- Small % of newly infected individuals will develop liver and/or spleen enlargement
- Onset of illness is generally 1-6 weeks following exposure and can last 1-3 weeks
- "Acute Retroviral Syndrome" is often mistaken for the flu
- An inmate presenting with some or all of the previously mentioned conditions should be questioned about recent potential HIV exposures so that testing can be done:

  - Needle sharing? Tattooing? Unprotected sex/new partner?

Stages of HIV Disease

Acute/Early Infection (con't)

- Testing for HIV antibody may be negative at this time.
- Diagnosis of acute HIV can made by obtaining a quantitative HIV RNA PCR (viral load test) or a pro viral cDNA test.
- A positive HIV antibody usually develops by 4-6 weeks following transmission, but rarely could be up to 12-24 weeks.
- Infection must ultimately be confirmed with an HIV Elisa/Western Blot assay

Stages of HIV Disease

Acute/Early Infection (con't)

- **Window period:** interval between where HIV actually appears, and is ultimately detectable by an antibody test.
- Inmates potentially exposed to HIV must be counseled that a negative antibody test during this period does not guarantee HIV transmission has not occurred.
- If an inmate's HIV test is negative, but suspicion for HIV exposure is high, repeated antibody testing should be performed at **12-26 weeks.**

Stages of HIV Disease

Acute/Early Infection (con't)

HIV Antibody Testing Timeline:

- Baseline
- 6 weeks post-exposure
- 12 weeks post-exposure
- 26 weeks post-exposure

Serocoversion virtually always detected by 6 months

Stages of HIV Disease

Acute/Early Infection (con't)

- Extremely high levels of HIV in the blood during acute infection (hallmark of this disease stage)
- Within days, HIV disseminates into **sanctuary sites** (lymph nodes, central nervous system) where it "hides out" and remains dormant.
- Safer sex practices should be stressed as there is a high risk of spreading infection to others.
- HIV viral levels decrease over the first 4 months post-transmission until plateauing to a **set point** (varies person to person)
- Lower HIV viral setpoint = longer time it will take for an individual's disease to progress over time

Stages of HIV Disease

Intermediate Stage

- T cell destruction by HIV begins to weaken the immune system over time (in contrast to the acute stage, where the immune system "keeps pace" by producing an equivalent amount of CD4 cells).
- In general if untreated, there is an 8-10 year period during which an HIV+ individual undergoes a gradual **decline** in immune function (monitored by laboratory testing of CD4 count) and **increase** in HIV viral load (monitored by laboratory testing of viral load).
- Often no symptoms exhibited during the intermediate disease stage

Stages of HIV Disease

Intermediate Stage (con't)

- Factors which influence how long individuals will remain in this stage before progressing to advanced disease:

  1) How high the viral setpoint is
  2) If and when antiretroviral treatment is initiated

- More than 50% of people do not know they are HIV-infected until they become symptomatic (an indicator of advanced disease).

- As the correctional setting is often an inmate's first interaction with the health care system, a thorough history of risk factors is important and HIV testing should be recommended to all new intakes.

www.thebody.com (HIV testing)

Stages of HIV Disease

Advanced Stage

- Untreated, the rapid replication of HIV will eventually deplete the immune system in most people to such an extent that the patient will lose critical body defenses and can succumb to infections, AIDS and ultimately death.

- Symptomatic HIV can present in a variety of forms.

- Hallmarks of this stage of the disease include:

- Opportunistic infections or malignancies
- Rashes
- Recurrent vaginal candidiasis
- Herpes zoster
- Thrush
- Neuropathy
- Diarrhea
- Recurrent infections
- Cancers
- Anemia

Stages of HIV Disease

Advanced Stage (con't)

- Actual diagnosis of AIDS is made when the CD4 count falls below 200 cells/cmm or when an AIDS-defining condition is diagnosed.

- Once a diagnosis of AIDS has been made, it remains with the patient even if his/her CD4 count returns to above 200 with antiretroviral therapy.

Stages of HIV Disease

AIDS-Defining Conditions

Candidiasis of esophagus, trachea, bronchi or lungs	Herpes simplex with mucocutaneous ulcer for > 1 month or bronchitis, pneumonitis, esophagitis
Cervical cancer, invasive	Histoplasmosis, extrapulmonary
Coccidioidomycosis, extrapulmonary	HIV-associated dementia: disabling cognitive and/or motor dysfunction interfering with occupation or activities of daily living
Cryptococcosis, extrapulmonary	HIV-associated wasting: involuntary weight loss of >10% of baseline plus chronic diarrhea (>2 loose stools/day for >30 days) or chronic weakness and documented enigmatic fever for > 30 days
Cryptosporidiosis with diarrhea for > 1 month	Isoporosis with diarrhea for >1 month
Cytomegalovirus of any organ other than liver, spleen, or lymph nodes	Kaposi's sarcoma in patient younger than 60 (or older than 60 with positive HIV serology)

Stages of HIV Disease

AIDS-Defining Conditions (con't)

Lymphoma of brain in patient younger than 60 (or older than 60 with positive HIV serology)	Pneumocystis carinii pneumonia
Lymphoma, non-Hodgkin's	Pneumonia, recurrent bacterial with positive HIV serology
Mycobacterium avium or M. kansasii, disseminated	Progressive multifocal leukoencephalopathy
Mycobacterium tuberculosis, disseminated	Salmonella septicemia (non-typhoid), recurrent with positive HIV serology
Mycobacterium tuberculosis, pulmonary	Toxoplasmosis of internal organ

Stages of HIV Disease

- The Centers for Disease Control (CDC) has a disease classification system based on immune function and clinical status.
- Each patient is classified with a number which is reflective of CD4 count, and a letter reflective of clinical status.
- This provides prognostic information for providers where a patient fits along the continuum of illness and as to what conditions, if any, he or she may be at risk.

Stages of HIV Disease

CDC Classification of HIV Disease

CD4 Cell Categories (cells/cmm)	**A** Asymptomatic or Acute HIV Infection	**B** Symptomatic (Not A or C)	**C** AIDS Indicator Condition
> 500 (>29%)	A1	B1	C1
200-499 (14-28%)	A2	B2	C2
< 200 (<14%)	A3	B3	C3

Opportunistic Infections

- When CD4 count is in normal range (500-1,600 cells/cmm or 28-50%), the immune system defends itself against most antigens.

- As T-cell count declines with HIV disease progression, the HIV+ patient is at increased risk for infection.

Opportunistic Infections

- When the T-cell count drops below 200 cells/cm (14%), there is increased risk of an AIDS-defining condition occurring.
- Treatment guidelines recommend prophylactic treatment against **pneumocystis carinii pneumonia (PCP)** for patients in this category.
- This is given as TMP-SMZ (Bactrim) 1 DS or 1 SS a day, Dapsone 100 mg a day, **or** Atovaquone (Mepron) 1500 mg at (10 ml)/day.
- Alternate prophylaxis options are listed in the prophylaxis guidelines (Department of Health & Human Services).

Opportunistic Infections

- If the patient develops oral candidiasis (thrush), PCP prophylaxis is recommended, regardless of CD4 count.
- Thrush is an independent risk factor for development of PCP, presumably because it indicates a decline in immune function.
- Primary prophylaxis (treatment in an individual who has never had PCP) can be discontinued if the CD4 count rises above 200 cells/cmm for a period of at least 3-6 months.

Opportunistic Infections

- When the CD4 count falls below 50 cells/cmm, the patient should be started on prophylaxis to protect against **mycobacterium avium complex (MAC)**.
- Lifelong treatment is recommended unless the CD4 count rises above 100 cells/cmm for at least 3-6 months.
- Prophylaxis options include: Azithromycin (Zithromax) 1200 mg/week, Clarithromycin (Biaxin) 500 mg BID, **or** Mycobutin (Rifabutin) 300 mg/day.

Opportunistic Infections

200-500 cells/cmm CD4 count	type
pneumococcal pneumonia	bacterial
pulmonary tuberculosis	bacterial
Kaposi's sarcoma	viral
Herpes zoster	viral
Thrush	fungal
Cryptosporidium	parasitic
Oral hairy leukoplakia	viral
Oro-pharyngeal candida	fungal

Opportunistic Infections

<200 cells/cmm CD4 count	type
pneumocystis carinii pneumonia	fungal (previously thought to be parasitic)
candida esophagitis	fungal
recurrent/disseminated viral herpes simplex	viral
toxoplasmosis	parasitic
histoplasmosis	fungal
Coccidioidomycosis	fungal
progressive multifocal leukoencephalopathy	viral
microsporidiosis	parasitic
extrapulmonary tuberculosis	bacterial

Opportunistic Infections

<50 cells/cmm CD4 count	type
cytomegalovirus	viral
mycobacterium avium complex	bacterial

Resources

- AIDS Education & Training Centers National Resource Center
  www.aids-etc.org/
- AIDS Education Global Information System
  www.aegis.com/
- CDC National Prevention Information Network
  www.cdcnpin.org
- HIV Clinical Resource, New York State Department of Health AIDS Institute
  www.hivguidelines.org
- Johns Hopkins AIDS Service
  www.hopkins-aids.edu

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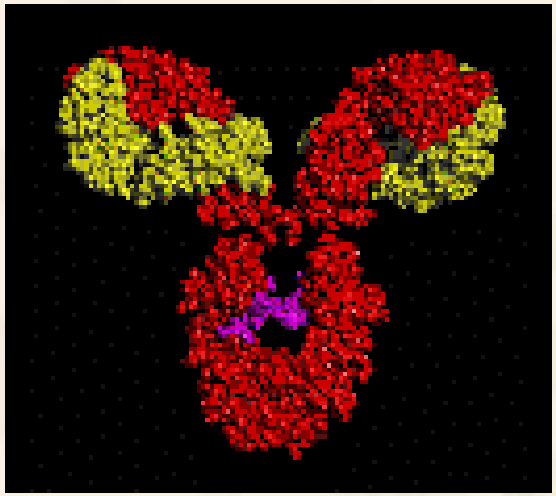
Monoclonal antibodies

Anticancer therapy

Weihua Wu



What is an antibody?

An **antibody** is a protein used by the immune system to identify and neutralize foreign objects like bacteria and viruses. Each antibody recognizes a specific antigen unique to its target.

Monoclonal antibodies (**mAb**) are antibodies that are identical because they were produced by one type of immune cell, all clones of a single parent cell.

Polyclonal antibodies are antibodies that are derived from different cell lines.

Isotypes

According to differences in their heavy chain constant domains, immunoglobulins are grouped into five classes, or isotypes: *IgG*, *IgA*, *IgM*, *IgD*, and *IgE*.

IgG: IgG1 (66%), IgG2 (23%), IgG3 (7%) and IgG4 (4%) , blood and tissue liquid.

IgA:IgA1 (90%) and IgA2 (10%), stomach and intestines

IgM: normally pentamer, ocassionally hexamer, multiple immunoglobins linked with disulfide bonds

IgD:1% of proteins in the plasma membranes of B-lymphocytes, function unknown

IgE: on the surface of plasma membrane of mast cells, play a role in immediate hypersensitive and denfensive for parasite

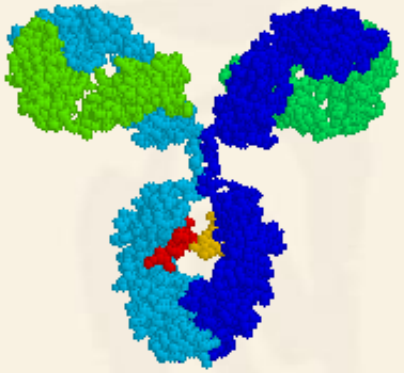
Monoclonal Antibodies

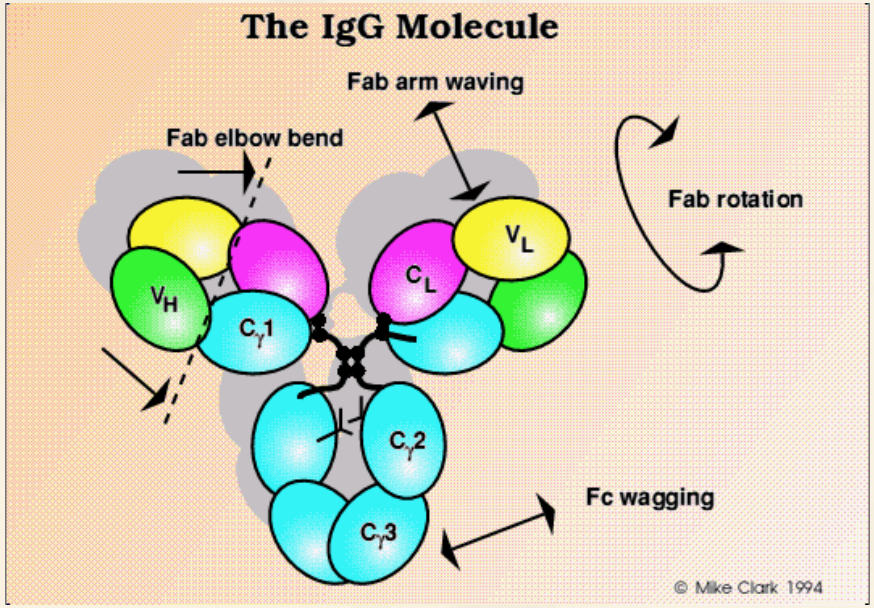
- **Monoclonal antibodies** (**mAb**) are antibodies that are identical because they were produced by one type of immune cell, all clones of a single parent cell. Given (almost) any substance, it is possible to create monoclonal antibodies that specifically bind to that substance; they can then serve to detect or purify that substance. This has become an important tool in biochemistry, molecular biology and medicine.

The IgG Class of Antibodies

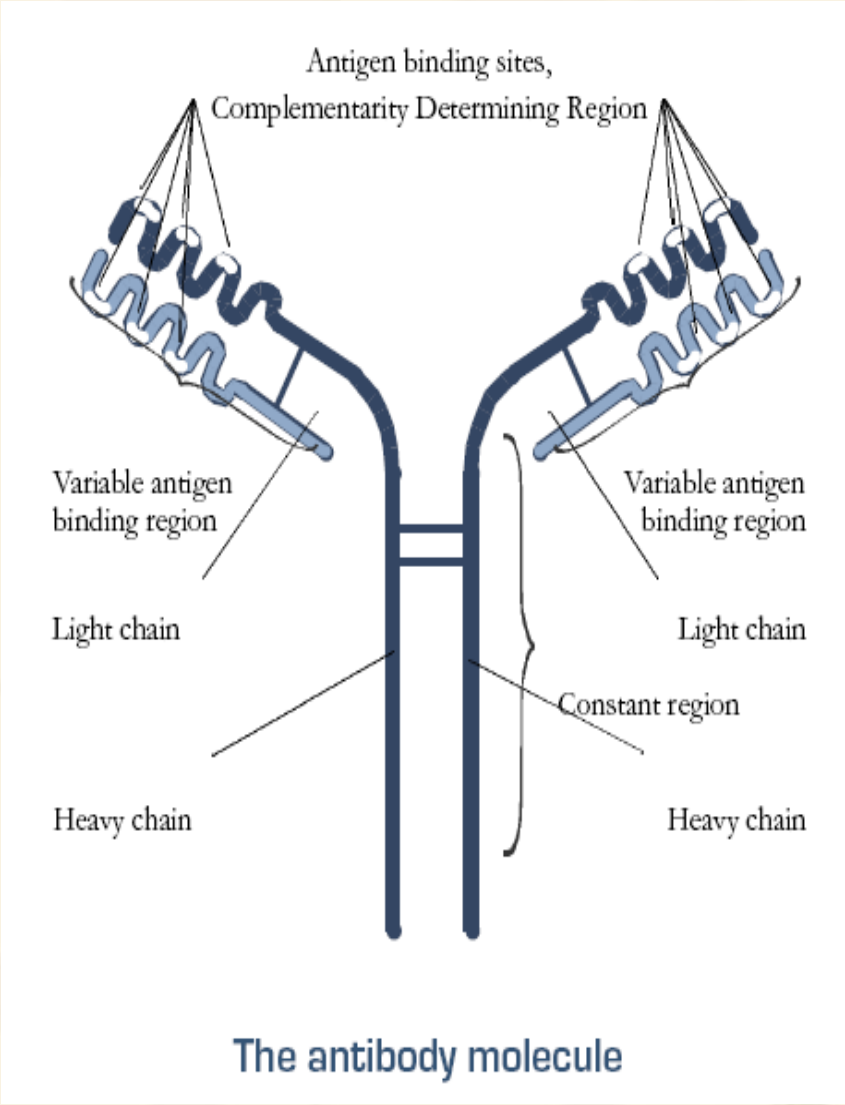
- All current therapeutic antibodies are of the IgG class.
- When the objective of antibody therapy is to directly kill the target cell, the isotype of choice is IgG1, since this isotype is optimal for complement fixation.

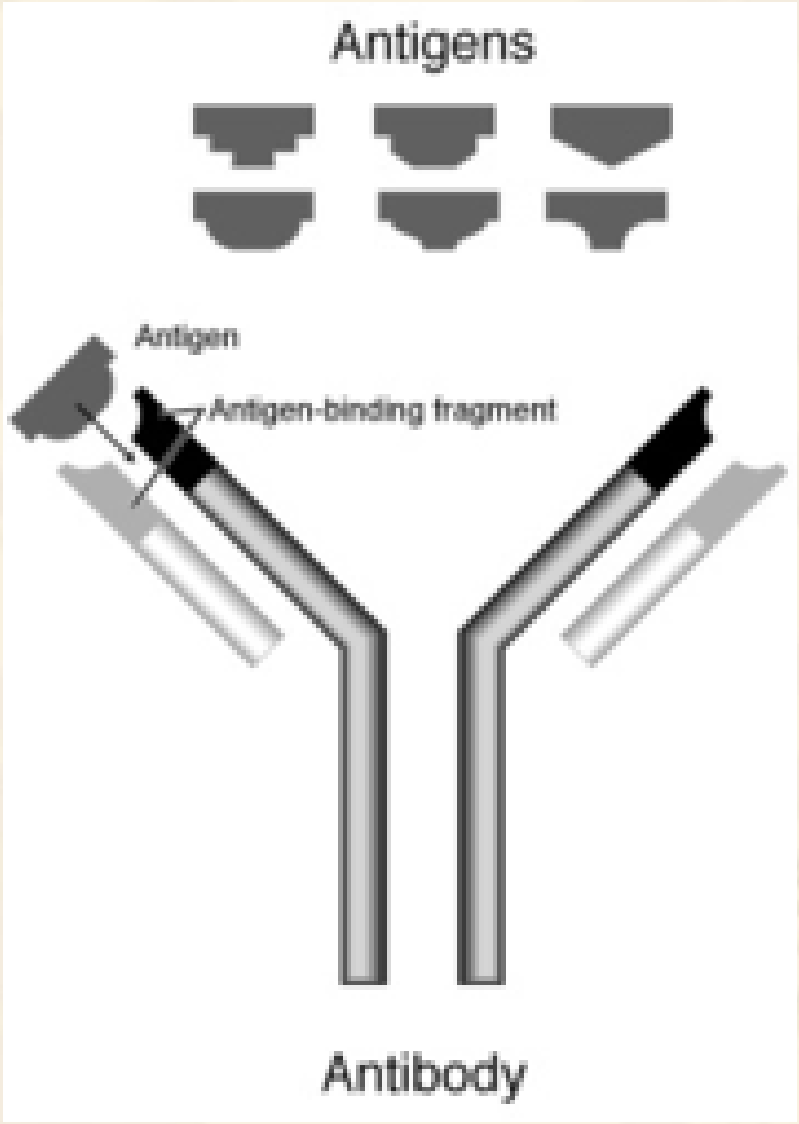
The structure of antibodies





- http://www.path.cam.ac.uk/~mrc7/igs/mikeimages.html




Antigens
Antigen
Antigen-binding fragment
Antibody

Definitions

- Fab = Fragment, antigen binding
- Fc = Fragment, crystalline
- The Fc fragment specifies other biological activities of the molecule. For example, the Fc fragment may determine whether the antibody simply prevents signaling through a receptor, or alternatively, causes the cell's destruction through complement fixation or targeting immune effector cells.

Can Antibodies Destroy Foreign 'Objects'?

- The antibody alone may not be sufficient to destroy a foreign 'object'.
- For example, a common test for HIV is the presence of anti-HIV antibodies in the blood. Obviously, those antibodies alone are not sufficient to protect the host from the virus.

History of Mab development

- 1890 Von Behring and Kitasato discovered the serum of vaccinated persons contained certain substances, termed antibodies
- 1900 Ehrlich proposed the " side-chain theory"
- 1955 Jerne postulated natural selection theory. Frank Macfarlane Burnet expended.
- Almost the same time, Porter isolated fragment of antigen binding (Fab) and fragment crystalline (Fc) from rabbit y-globulin.
- 1964 Littlefield developed a way to isolate hybrid cells from 2 parent cell lines using the hypoxanthine-aminopterin-thymidine (HAT) selection media.
- 1975 Kohler and Milstein provided the most outstanding proof of the clonal selection theory by fusion of normal and malignant cells. This resulted in the first monoclonal antibodies, for which they received the Nobel Prize in 1984.

What Diseases to Target and How?

- Cancer cells express a variety of antigens that are attractive targets for monoclonal antibody-based therapy.
- The development of monoclonal antibodies against specific targets has been largely accomplished by immunizing mice against human tumor cells and screening the hybridomas for antibodies of interest.

Unfulfilled Promise?

- The early promise of the use of antibodies in the treatment of disease initially went unfulfilled (more than two decades) for two reasons:

  1. Early antibodies displayed insufficient activation of human effector functions (i.e. the antibodies did not kill the infecting organism or cell)

  2. The early antibodies were of murine (mouse) origin, and thus triggered the production of human anti-mouse antibodies (HAMA).

Other obstacles to the use of monoclonal antibodies in cancer treatment

- Antigen distribution of malignant cells is highly heterogeneous, so some cells may express tumor antigens, while others do not.
- Tumor blood flow is not always optimal
- High interstitial pressure within the tumor can prevent the passive monoclonal antibody from binding.

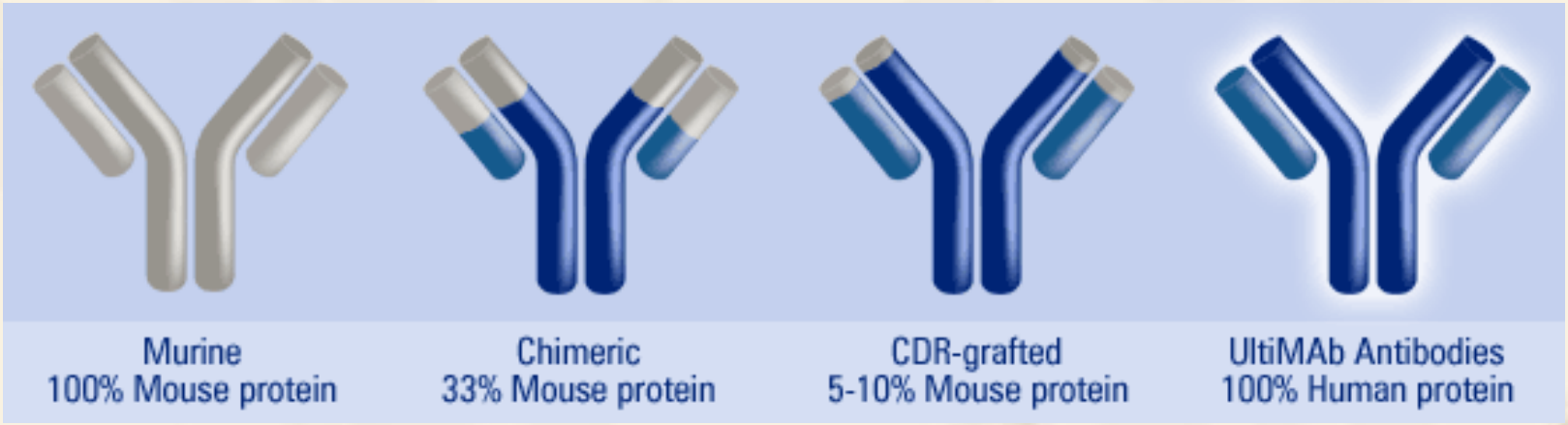
The types of mAb designed

A. Murine source mAbs: rodent mAbs with excellent affinities and specificities, generated using conventional hydrioma technology. Clinical efficacy compromised by HAMA(human anti murine antibody) response, which lead to allergic or immune complex herpersensitivities.

B. Chimeric mAbs: chimers combine the human constant regions with the intact rodent variable regions. Affinity and specificity unchanged. Also cause human antichimeric antibody response (30% murine resource)

C. Humanized mAbs: contained only the CDRs of the rodent variable region grafted onto human variable region framework

Evolution of Therapeutic Antibodies



Nomenclature of Therapeutic Antibodies

- Terminate the name in –*ximab* for chimeric antibodies and –*umab* for humanized antibodies.

Common Chemotherapy in Treatment of Cancer

Shortcomings:

A. Nature of cytotoxin

B. Lack of *in vivo* selectivity

C. The mechanism of anti-proliferation on cells cycle, rather than specific toxicity directed towards particular cancer cell

D. Host toxicity: treatment discontinued, most of them had bad side-effects, such as no appetites, omit, lose hair

Monoclonal antibodies for cancer treatment

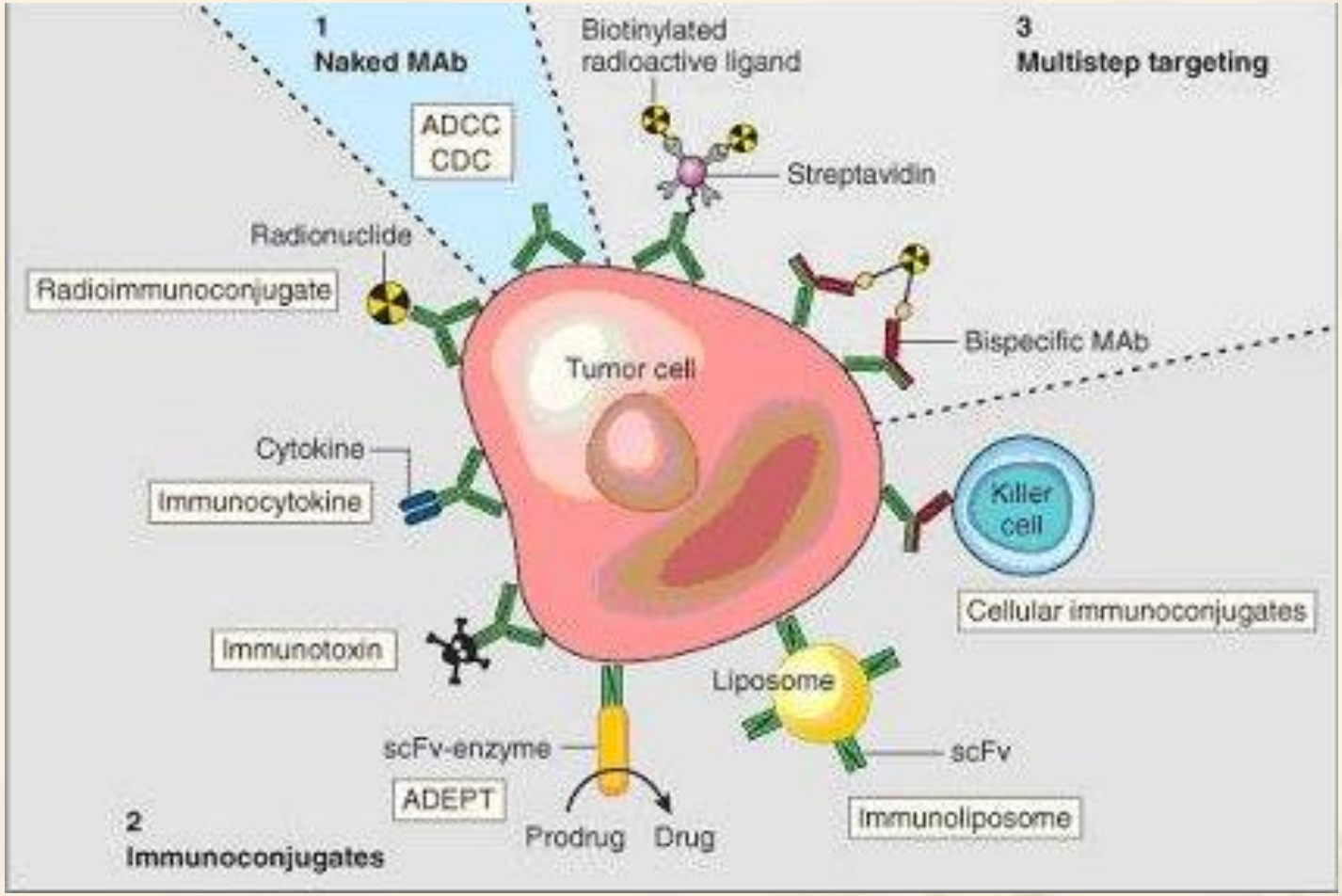
Three mechanisms that could be responsible for the cancer treatment.

A. mAbs act directly when binding to a cancer specific antigens and induce immunological response to cancer cells. Such as inducing cancer cell apoptosis, inhibiting growth, or interfering with a key function.

B. mAbs was modified for delivery of a toxin, radioisotope, cytokine or other active conjugates.

C. it is also possible to design bispecific antibodies that can bind with their Fab regions both to target antigen and to a conjugate or effector cell

mAbs treatment for cancer cells



ADEPT, antibody directed enzyme prodrug therapy; ADCC, antibody dependent cell-mediated cytotoxicity; CDC, complement dependent cytotoxicity; MAb, monoclonal antibody; scFv, single-chain Fv fragment.

Carter P: Improving the efficacy of antibody-based cancer therapies. Nat Rev Cancer 2001;1:118-129

‘Naked’ Monoclonal Antibodies

- ‘Naked’ means these antibodies are not fused to a toxin.
- Naked Monoclonal antibodies can kill cells via a variety of mechanisms, including: Antibody-Dependent Cellular Cytotoxicity (ADCC), Complement-Dependent Cytotoxicity (CDC), and direct induction of apoptosis.
- However, the precise clinical mechanisms often remain uncertain

Antibody-dependent cellular cytotoxicity

From Wikipedia, the free encyclopedia

Antibody-Dependant Cellular Cytotoxicity

ADCC is the least understood of the three mechanisms, it is mediated by either NK cells or CTL. The action of ADCC is dependant on the recognition of the objective cell by antibodies attached on the surface of the effector cell (terminally differentiated leukocyte). This process is part of the adaptive immune response due to the dependence on antibodies and therefore a former anti-body response is required for this mechanism to take effect and be effective against an invading pathogen.

http://www.meds.com/immunotherapy/monoclonal_antibodies.html

Rituximab (Rituxan)

- Rituximab is a chimeric monoclonal antibody that targets the CD20 B-cell antigen.
- This antigen is expressed on 90% of B-cell neoplasms
- The precise biological functions of CD20 are uncertain, but the antibody is believed to function by flagging the B-cells for destruction by the body's own immune system, including ADCC, CDC, and apoptosis.
- This antibody thus leads to the elimination of all B-cells from the body (including cancerous ones), allowing new, healthy B-cells to be produced from lymphoid stem cells.

Trastuzumab (Herceptin)

- Herceptin is an anti-cancer antibody that acts on HER2/neu (erbB2) receptor, which is overexpressed in breast cancer. Only cells overexpressing this receptor are susceptible.
- Such cells, when treated with Herceptin, undergo arrest in the G1 phase of the cell cycle and experience a reduction in proliferation.
- This can reduce the rate of relapse of breast cancer by 50% during the first year.
- The precise mechanism of action is still under investigation.

Monoclonal antibodies which deliver a toxin

- Monoclonal antibodies can be utilized to selectively deliver a toxin to a malignant cell.

Gemtuzumab ozogamicin (Mylotarg)

- This monoclonal antibody is conjugated to the cytotoxic agent calicheamycin
- It is used to treat acute myelogenous leukemia (AML), which is a cancer of the myeloid line of blood cells.
- This monoclonal antibody attacks the CD33 receptor, which is found in most leukemic blast cells, but not in normal hematopoietic stem cells

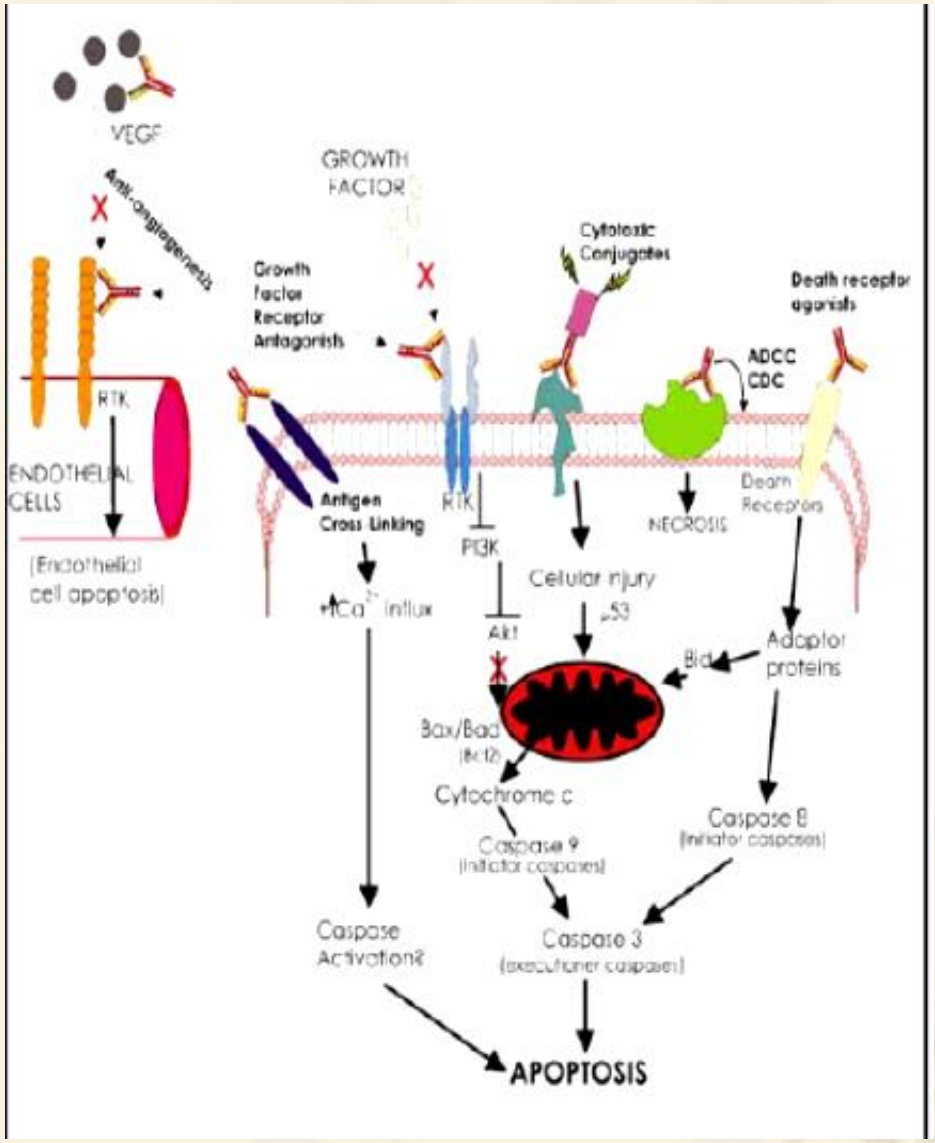
Gemtuzumab ozogamicin (Mylotarg)

- Once bound to CD33, the antibody-calicheamycin complex is transported inside of the AML cells by lysosomes.
- To facilitate selective release inside of the cancer cells, calicheamycin is connected to gemtuzumab by a chemical linker that is stable at physiologic pH but is hydrolyzed in the acidic pH of the lysosomes that transport the antibody-calicheamycin complex into the cell.

Strategy of a direct or in direct induction of apoptosis in targeted cancer cells

1. mAbs target growth factor receptors to exert a direct effect on the growth and survival of the cancer cells by antagonizing ligand-receptor signaling.

2. mAbs can target to cell surface antigens and directly elicit apoptotic signaling.



Dale L Ludwig, etal. Oncogene(2003) 22, 9097-9106

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Until Feb 28, 2005, 18 mAbs were approved by FDA, which were applied in the treatment of organ transplant, Cancer, Asthma, Hematopoietic malignancies and psoriasis.

The first approved mAbs was OKT-3, which is a murine IgGa2 protein to deplete T cells in patients with acute rejection of renal allotransplant.

HAMA response

Jancie, M Recheit, etal. Nature biotechnology, 2005, Sep, Vol. 23, No.9

Stamatis-Nick C. J Allergy Clin. Immunol, Oct. 2005

TABLE I. Humanized antibodies and FPs in clinical trials or introduced into clinical practice

Name	Target antigen-molecule	Application
Antibodies		
OKT3	CD3	Renal transplants
Basiliximab (chimeric)	IL-2 receptor α-chain	Organ transplants
Daclizumab (humanized)	IL-2 receptor α-chain	Organ transplants, noninfectious uveitis
Pavilizumab	Respiratory syncytial virus	Infants with bronchopulmonary dysplasia
Trastuzumab	Receptor tyrosine kinase ERBB2	Cancer
Cetuximab	Receptor tyrosine kinase EGFR	Cancer
Bevacizumab	VEGFR1 and VEGFR2	Cancer (metastatic)
Rituximab	CD20	B-cell lymphomas, autoimmunity
Ibritumomab (yttrium 90 labeled)	CD20	B-cell lymphomas
Tositumomab (iodine 131 labeled)	CD20	B-cell lymphomas
Alemtuzumab	CD52	Hematopoietic malignancies
Epratuzumab	CD22	B-cell lymphomas
Infliximab	TNF-α	RA, CD
Adalimumab	TNF-α	RA, CD
MRA	IL-6 receptor	RA
Anti-IL-2	IL-2	RA
Efalizumab	CD11a	Psoriasis
IDEC-131	CD40L	SLE
Ruplizumab	CD40L	SLE
Omalizumab	IgE	Asthma
FPs		
Etanercept	TNF receptor (p75)	RA
Abatacept	CTLA4	RA, psoriasis

mAbs development

1. Phage display library: construction of V_H and V_L gene libaries and expression of them on a filamentous bacterophage. The phage expressing an antigen-bonding domain specific for a particular antigen to screen the mAbs.

2. Transgenic plants: transgenic tobacco plants to produce IgA.

3. Transgenic animals: transgenic mouse to make humanized IgG. (Abgenix,CA)

Conventional production of mAbs

The hybridoma technology:

spleen cells from immunized mice are fused with the murine myeloma cells. The several process had been developed at large scale.

According to the different cell culture methods, it can calisifed into four fields

1. Robottle cell culture process.
2. Membrane binded cell culture process
3. Microcarrier cell culture process
4. Suspended cell culture process

- Questions?