

INTRODUCTION TO IMMUNOLOGY

Lecture 1 & 2

Introduction

- In basic terms, the immune system has two lines of defense: innate immunity and adaptive immunity. Innate immunity is the first immunological, non-specific (antigen-independent) mechanism for fighting against an intruding pathogen. It is a rapid immune response, occurring within minutes or hours after aggression, that has no immunologic memory.

Introduction

- Adaptive immunity, on the other hand, is antigen-dependent and antigen-specific; it has the capacity for memory, which enables the host to mount a more rapid and efficient immune response upon subsequent exposure to the antigen.
- There is a great deal of synergy between the adaptive immune system and its innate counterpart,
- Any defects in either system can provoke illness or disease, such as autoimmune diseases, immunodeficiency disorders and hypersensitivity reactions.

IMMUNOLOGY AND THE IMMUNE SYSTEM

- Immunology
 - Study of the components and function of the immune system
- Immune System
 - Molecules, cells, tissues and organs which provide non-specific and specific protection against
 - Microorganisms
 - Microbial toxins
 - Tumor cells
 - Crucial to human survival

THE IMMUNE RESPONSE AND IMMUNITY

- Immune response
 - Innate (non-specific)
 - Adaptive (specific)
 - Primary
 - Secondary
- Immunity
 - State of non-specific and specific protection
- Acquisition of Immunity
 - Natural
 - Artificial

Innate Immunity	Acquired immunity
Depends of pre-formed cells and molecules	Depends on clonal selection, i.e. growth of T/B cells, release of antibodies selected for antigen specificity
Fast (starts in mins/hrs)	Slow (starts in days)
Limited specificity- pathogen associated, i.e. recognition of danger signals	Highly specific to foreign proteins, i.e. antigens
Cells involved: <ul style="list-style-type: none"> - Neutrophils (PMN) - Macrophages - Natural killer (NK) cells 	Cells involved : <ul style="list-style-type: none"> - T lymphocytes - B lymphocytes - Dendritic cells - Eosinophils - Basophils/mast cells
Soluble factors involved <ul style="list-style-type: none"> - Acute-phase proteins - Cytokines - Complement 	Soluble factors involved <ul style="list-style-type: none"> - Antibodies

Cutaneous immune system

- **Epidermis** contains keratin cells that produce IL-1, 6 and TNF during inflammation; and IL-10, TGF- β during healing
 - **Dermis** contains **fibroblasts** that produce collagen, remove apoptotic cells
-

NATURALLY ACQUIRED IMMUNITY

- Active
 - Antigens enter body naturally with response of
 - Innate and adaptive immune systems
 - Provides long term protection
- Passive
 - Antibodies pass from mother to
 - Fetus across placenta
 - Infant in breast milk
 - Provides immediate short term protection

ARTIFICIALLY ACQUIRED IMMUNITY

- Active
 - Antigens enter body through vaccination with response of
 - Innate and adaptive immune systems
 - Provides long term protection
- Passive
 - Antibodies from immune individuals injected into body
 - Referred to as
 - Immune serum globulins (ISG)
 - Immune globulins (IG)
 - Gamma globulins
 - Provides immediate short term protection

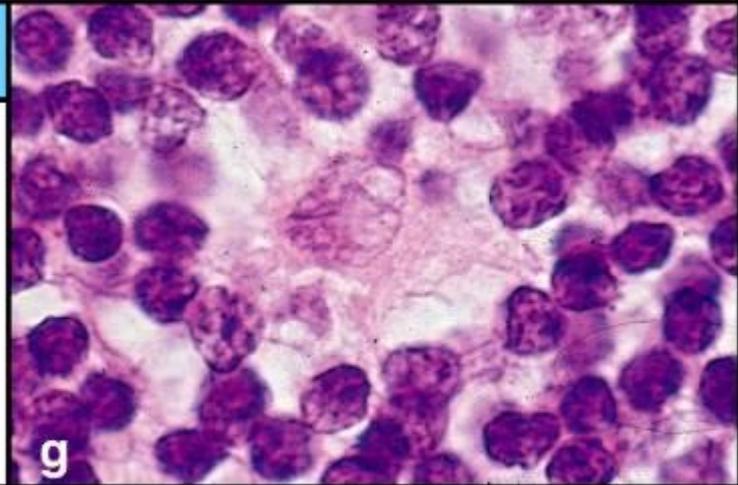
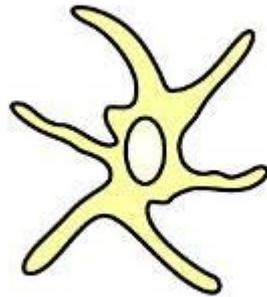
PRINCIPAL FUNCTION OF THE IMMUNE SYSTEM

- To protect humans from pathogenic microorganisms
- Pathogenic microorganisms (Pathogens)
 - Microorganisms capable of causing infection and/or disease
- Infection
 - Ability of pathogen to enter host, multiply and stimulate an immune response
- Disease
 - Clinical manifestations associated with infection

CELLS OF INNATE AND ADAPTIVE IMMUNITY

- Myeloid lineage
 - Dendritic cells
 - Cells with dendriform (star shaped) morphology
 - Interdigitating reticular cells (synonym)
 - Capture and present antigens to T lymphocytes
 - Mast cells
 - Located in mucous membrane and connective tissue throughout body
 - Major effector cell in allergy
 - Modulation of initial immune response

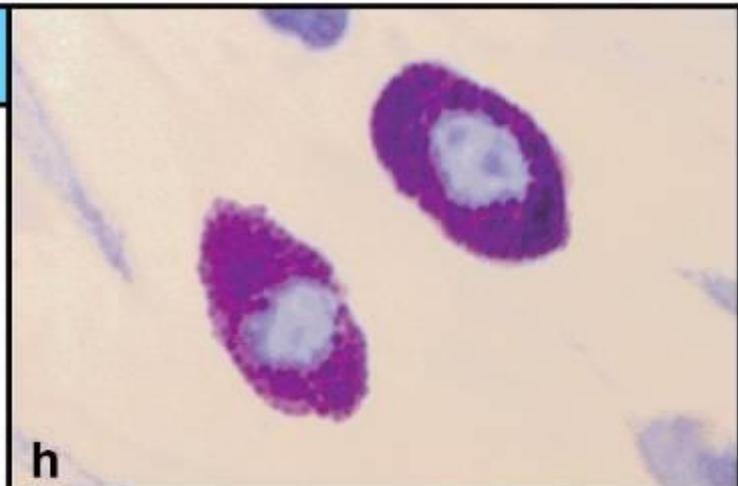
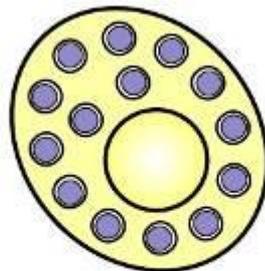
Dendritic cell



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Activation of T cells and initiation of adaptive immune responses

Mast cell



h

Expulsion of parasites from body through release of granules containing histamine and other active agents

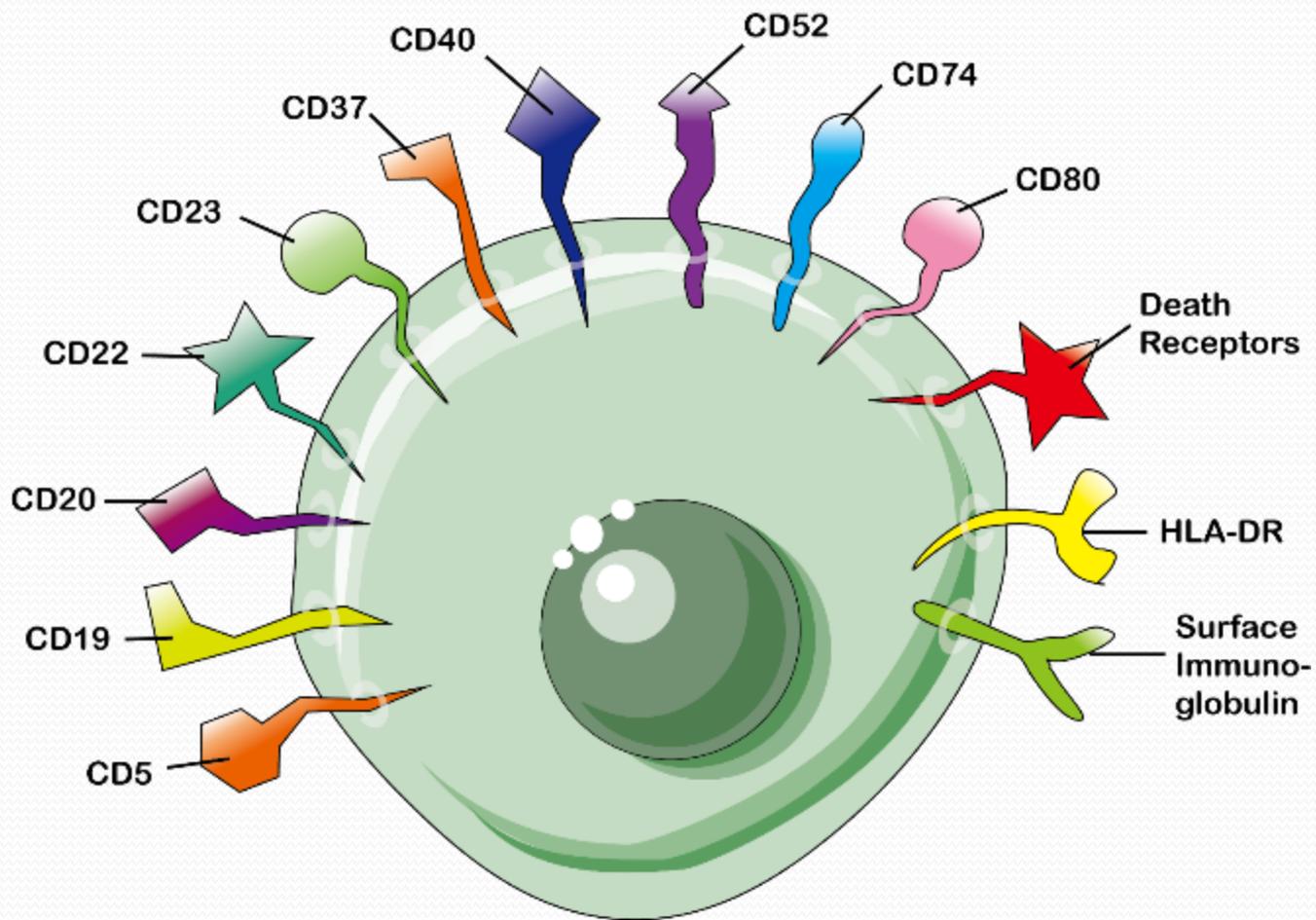


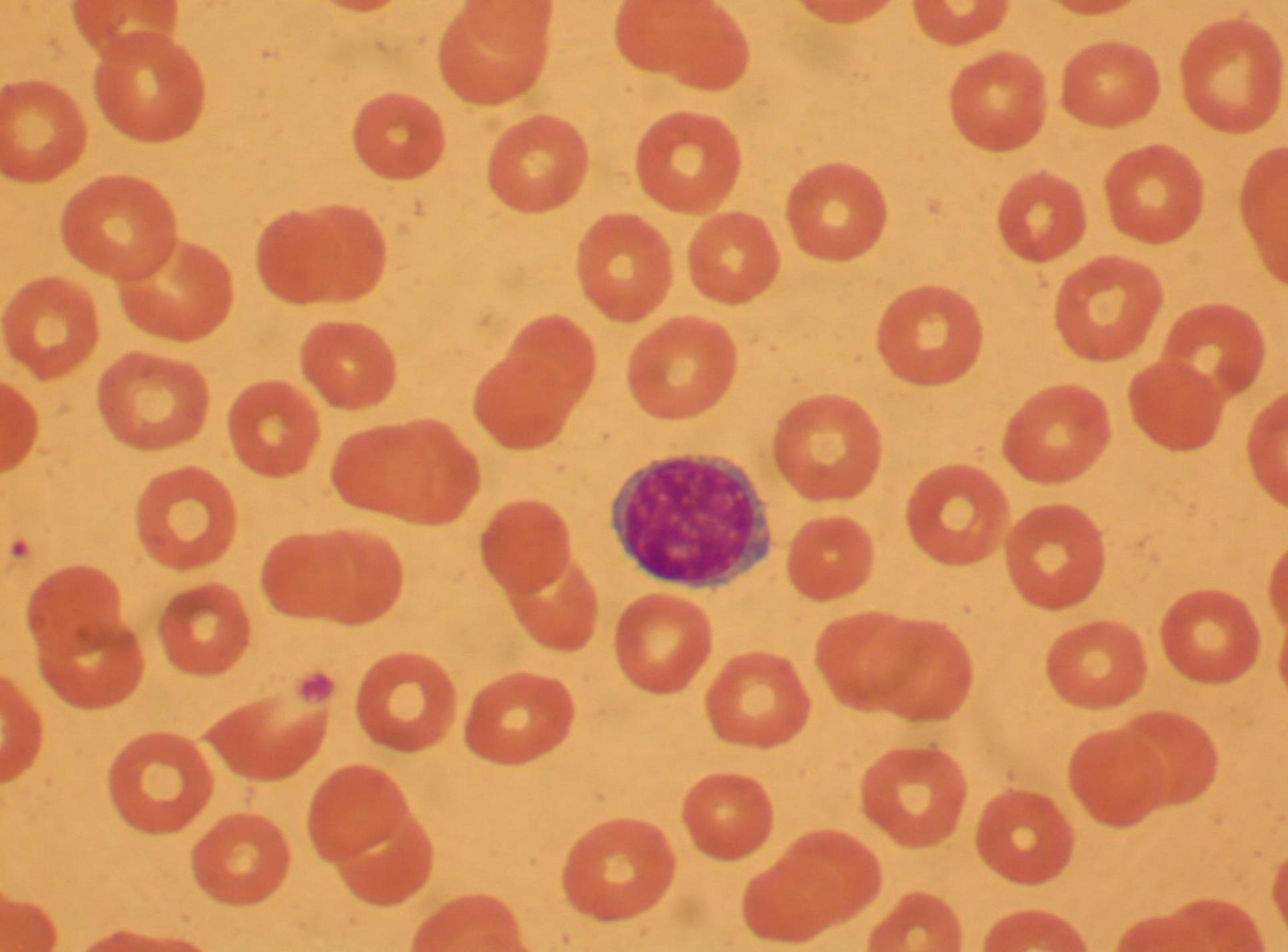
CELLS OF INNATE AND ADAPTIVE IMMUNITY

- Lymphoid Lineage
 - Large lymphocytes (large granular lymphocytes)
 - Natural killer (NK) cells (CD16, CD56)
 - Innate immunity to viruses and other intracellular pathogens
 - Participate in antibody-dependent cell-mediated cytotoxicity (ADCC)
 - Small lymphocytes
 - B cells (CD19)
 - T cells (CD3, CD4 or CD8)
 - Adaptive immunity
 - Lymphocytes refers to small lymphocytes

THE CLUSTER OF DIFFERENTIATION (CD)

- A protocol for identification and investigation of cell surface molecules
- CD antigens are molecules originally defined as being present on the cell surface of leucocytes and recognized by specific antibody molecules,
- CD nomenclature established in 1982
 - 1st International Workshop and Conference on Human Leukocyte Differentiation Antigens (HLDA)





Cell type	Proportion of leukocytes (%)
Neutrophil	40–75
Eosinophil	1–6
Basophil	<1
Monocyte	2–10
Lymphocyte	20–50

Figure 1-12 The Immune System, 2/e (© Garland Science 2005)

COMPLETE BLOOD COUNT WITH DIFFERENTIAL (CBC WITH DIFF)

References Ranges

Erythrocytes (RBC)	4.0 to 5.4	M/uL
Thrombocytes (Platelets)	145 to 400	K/uL
Leukocytes (WBC)	4.8 to 10.8	K/uL
Neutrophils	40 to 74	%
Band neutrophils	0 to 9	
Eosinophils	0 to 6	
Basophils	0 to 1	
Lymphocytes	15 to 47	
Monocytes	0 to 12	

LYMPHOCYTES, LYMPHOID TISSUES AND ORGANS

- Lymphocytes originate in bone marrow
- Lymphoid tissues and organs
 - Primary
 - Development and maturation of lymphocytes
 - Bone Marrow (B cells) and thymus gland (T cells)
 - Secondary
 - Mature lymphocytes meet pathogens
 - Spleen, adenoids, tonsils, appendix, lymph nodes, Peyer's patches, mucosa-associated lymphoid tissue (MALT)

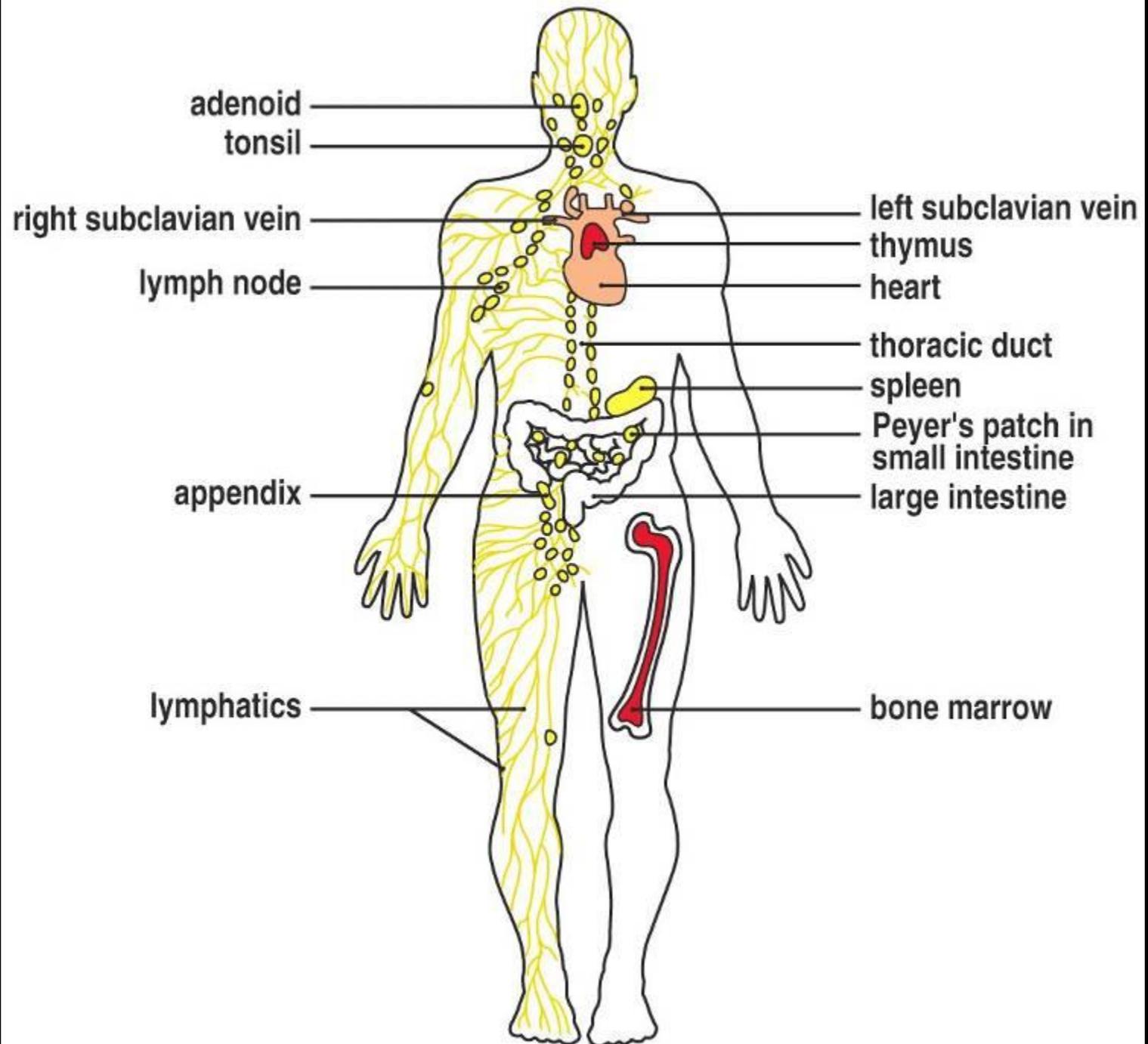


Figure 1-15 The Immune System, 2/e (© Garland Science 2005)

THE LYMPHATIC SYSTEM

- Lymph
 - Fluid and cells in lymphatic vessels
- Lymphatic vessels
 - Collect and return interstitial fluid to blood
 - Transport immune cells throughout body
 - Transport lipid from intestine to blood
- Lymph nodes
 - Kidney shaped organs at intervals along lymphatic vessels
- Other secondary lymphatic tissues and organs

LYMPHOCYTES AND THE LYMPH NODES

- Naïve lymphocytes circulate between blood, lymph and secondary lymph nodes
- Pathogens from infected tissue sites are picked up by lymphatic vessels and arrive at closest lymph node
- T and B cells congregate at specific regions of nodes
- Architecture and size of nodes change in response to activation of lymphocytes

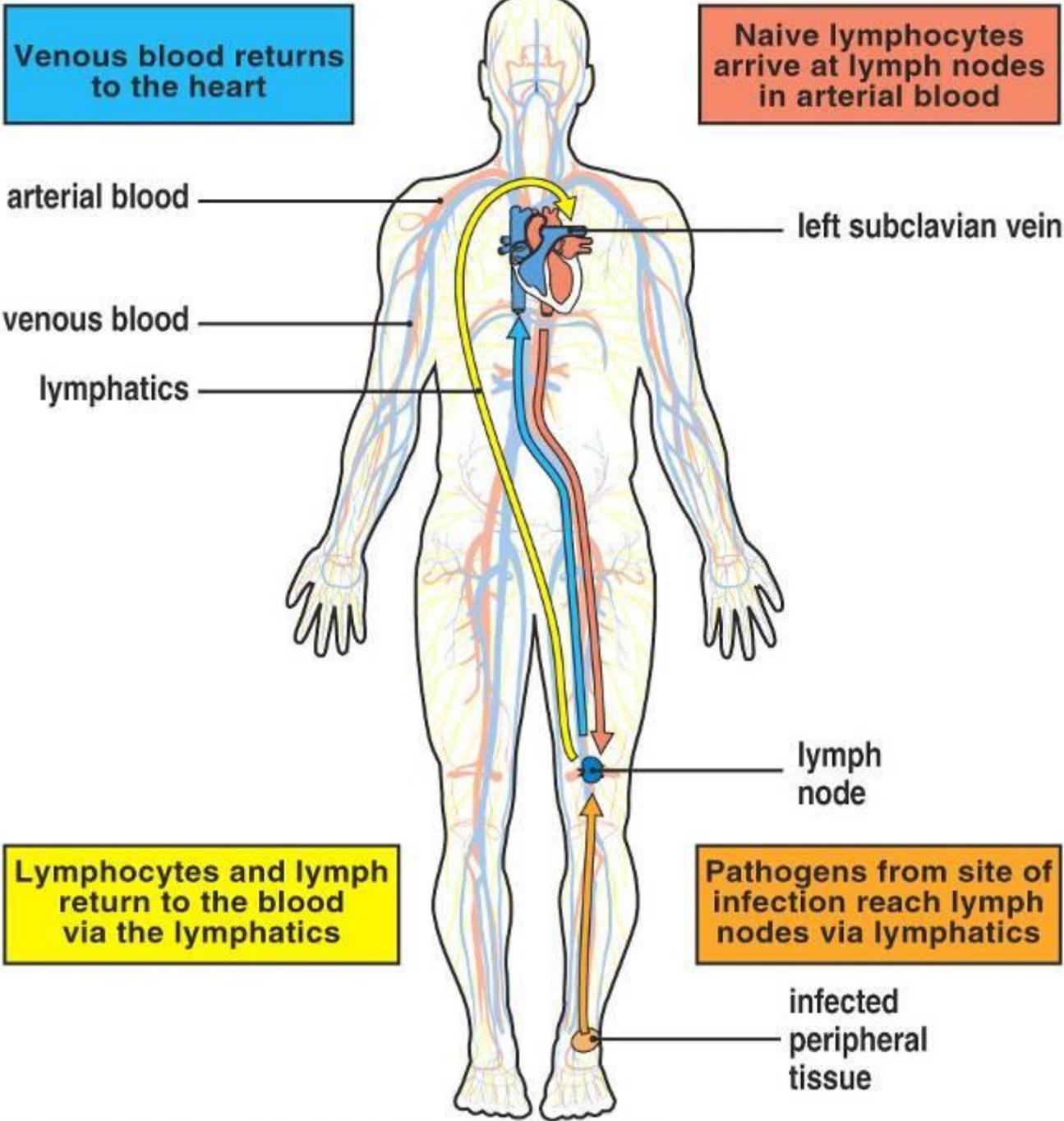


Figure 1-16 The Immune System, 2/e (© Garland Science 2005)

The lymph node

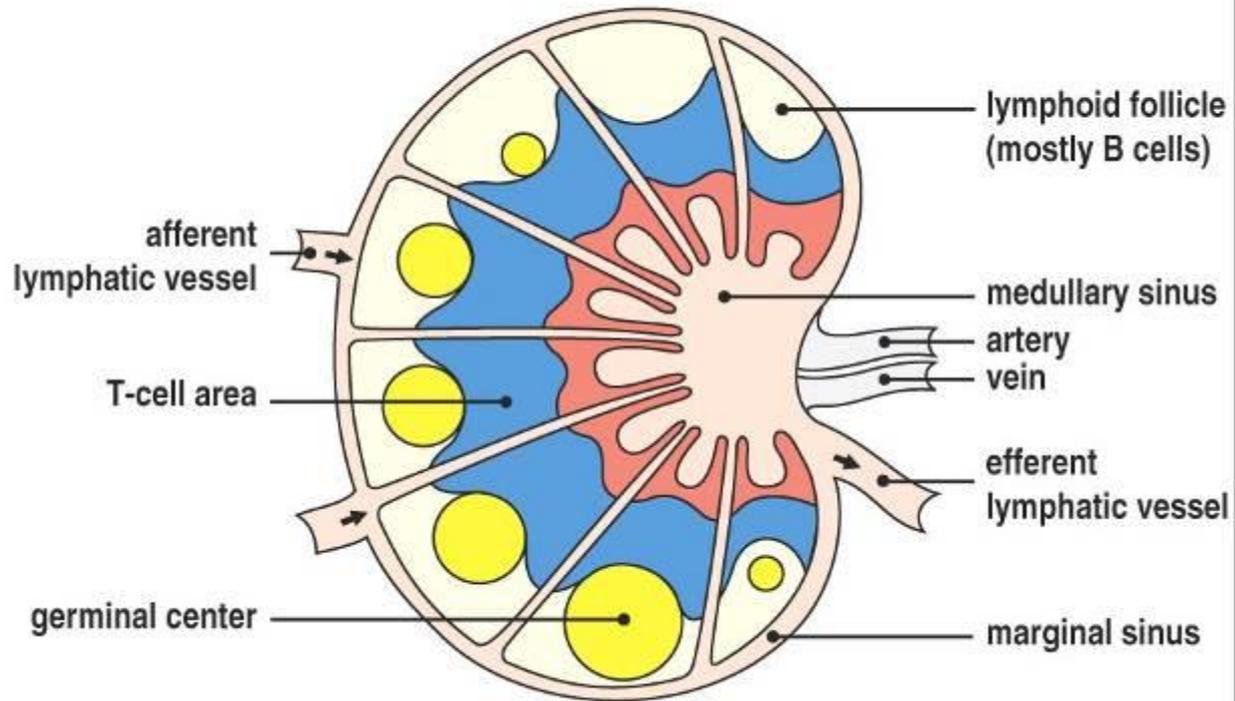
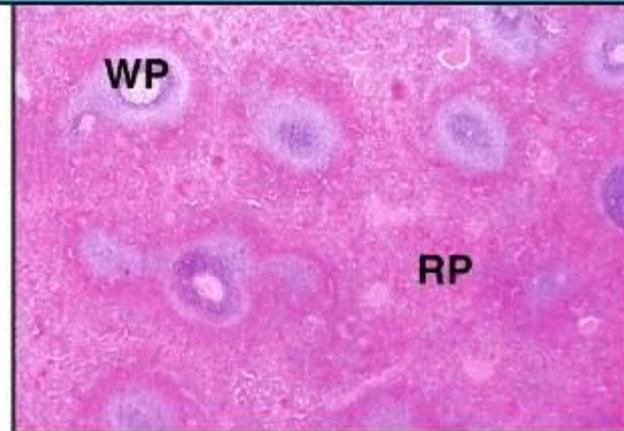
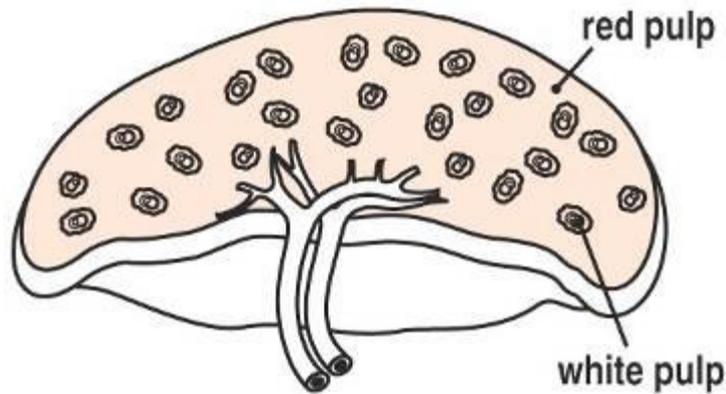


Figure 1-17 The Immune System, 2/e (© Garland Science 2005)

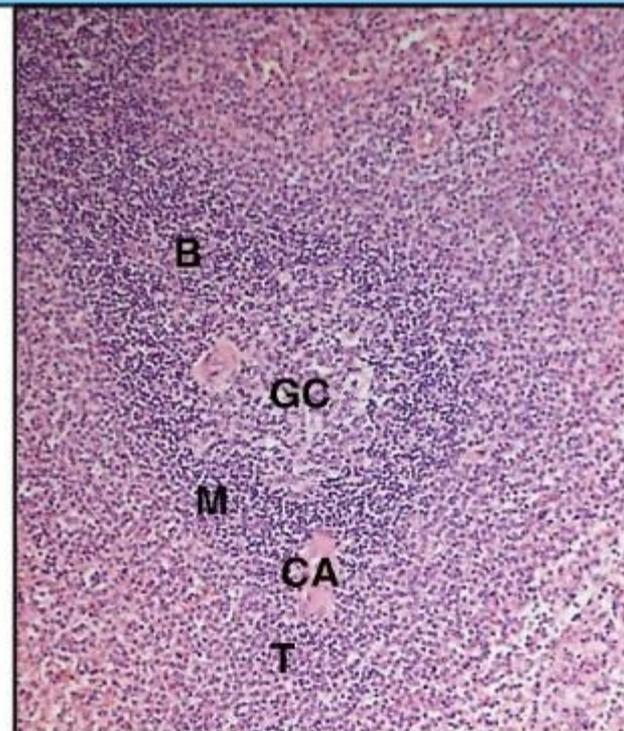
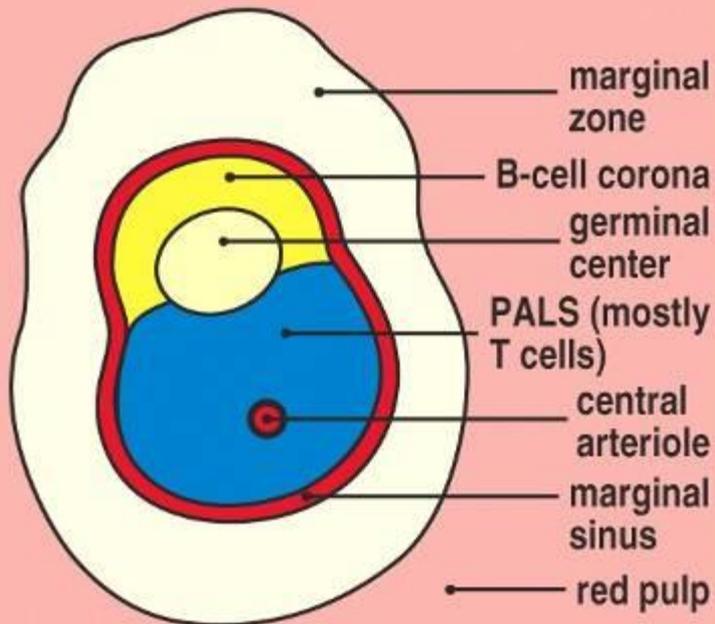
LYMPHOCYTES AND THE SPLEEN

- Spleen
 - Lymphoid organ in upper left abdomen
 - Functions
 - Remove damaged or old erythrocytes
 - Activation of lymphocytes from blood borne pathogens
- Architecture of Spleen
 - Red pulp
 - Erythrocytes removed
 - White pulp
 - Lymphocytes stimulated

The spleen



Transverse section of white pulp of spleen



SECONDARY LYMPHOID TISSUES ASSOCIATED WITH MUCOUS MEMBRANES

- Primary portals of entry for pathogens
 - Respiratory tract
 - Gastrointestinal tract
- Secondary lymphoid tissues
 - Bronchial-associated lymphoid tissue (BALT)
 - Gut-associated lymphoid tissues (GALT)
 - Tonsils, adenoids, appendix, Peyer's patches
- Pathogens are directly transferred across mucosa by "M" cells

Gut-associated lymphoid tissue

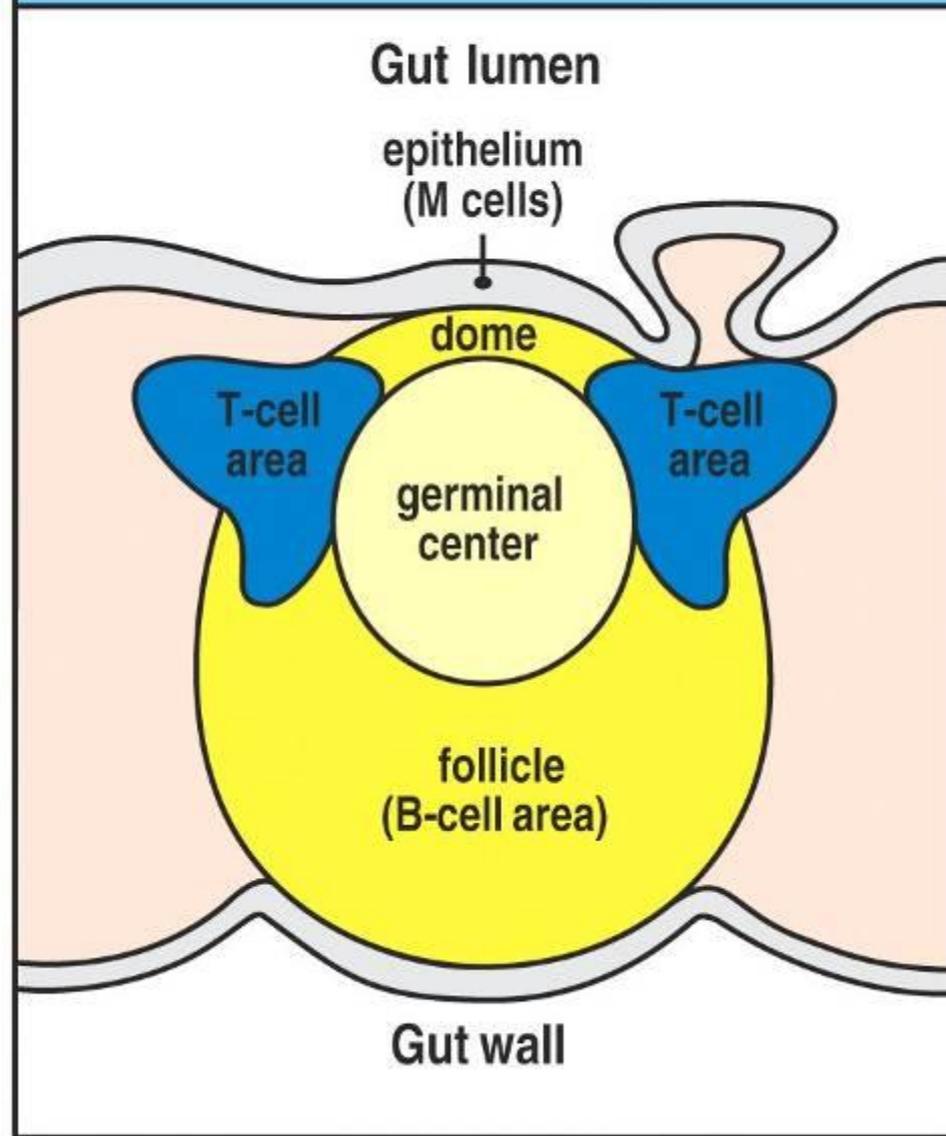


Figure 1-20 The Immune System, 2/e (© Garland Science 2005)

THE NATURE OF ANTIGENS

- Historically named as antibody generators
 - Molecule which stimulates production of and binds specifically to an antibody
- Contemporary view distinguishes between
 - Antigen
 - Molecule which can bind to specific antibody but cannot elicit adaptive immune response
 - Immunogen
 - Molecule which can stimulate adaptive immune response
- Best immunogens are proteins with
MW > 10,000

THE NATURE OF ANTIGENS

- Carbohydrates, nucleic acids and lipids are also potential antigens / immunogens
- Hapten
 - Small (low MW) molecule unable to elicit immune response
 - Combines with larger carrier molecule which together function as immunogen
 - Antibody may react independently with hapten following hapten/carrier adaptive immune response
 - Example
 - Penicillin G (MW of 372)
 - Albumin (MW of 66,000)

THE NATURE OF ANTIBODIES

- Antibodies are glycoproteins
- Exist as monomers, dimers or pentamers of basic structure
- Basic antibody structure has 4 polypeptide chains
 - 2 identical light chains
 - 2 identical heavy chains
- Regions of heavy and light chains
 - Variable
 - Constant

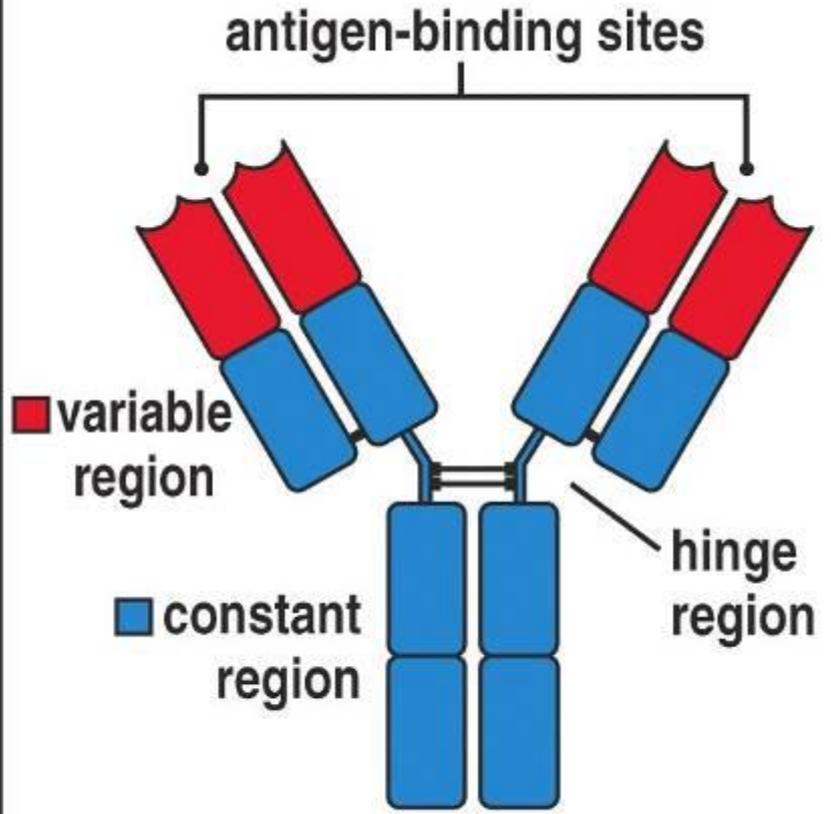
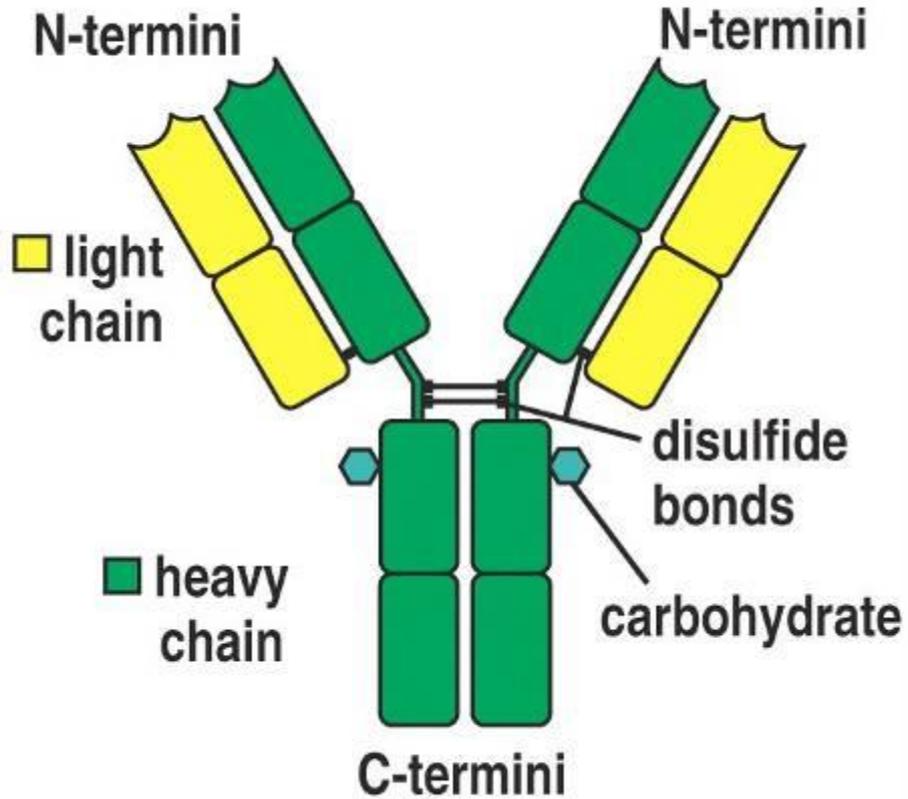


Figure 2-2 The Immune System, 2/e (© Garland Science 2005)

THE NATURE OF ANTIBODIES

- Also referred to as
 - Immune globulins / Immunoglobulins (IG)
 - Immune serum globulins (ISG)
 - Gamma globulins
- Contemporary immunology
 - Antibody
 - Secreted form of IG made by plasma cells
 - Immunoglobulin
 - Antigen binding molecules of B cells
 - (B cell antigen receptors)

CLASSIFICATION OF ANTIBODIES (IMMUNOGLOBULINS)

- Five (5) classes (isotypes)
 - Immunoglobulin A (IgA)
 - Immunoglobulin G (IgG)
 - Immunoglobulin M (IgM)
 - Immunoglobulin D (IgD)
 - Immunoglobulin E (IgE)
- Based on structural differences in constant regions of heavy chains
- Classes have specialized effector functions

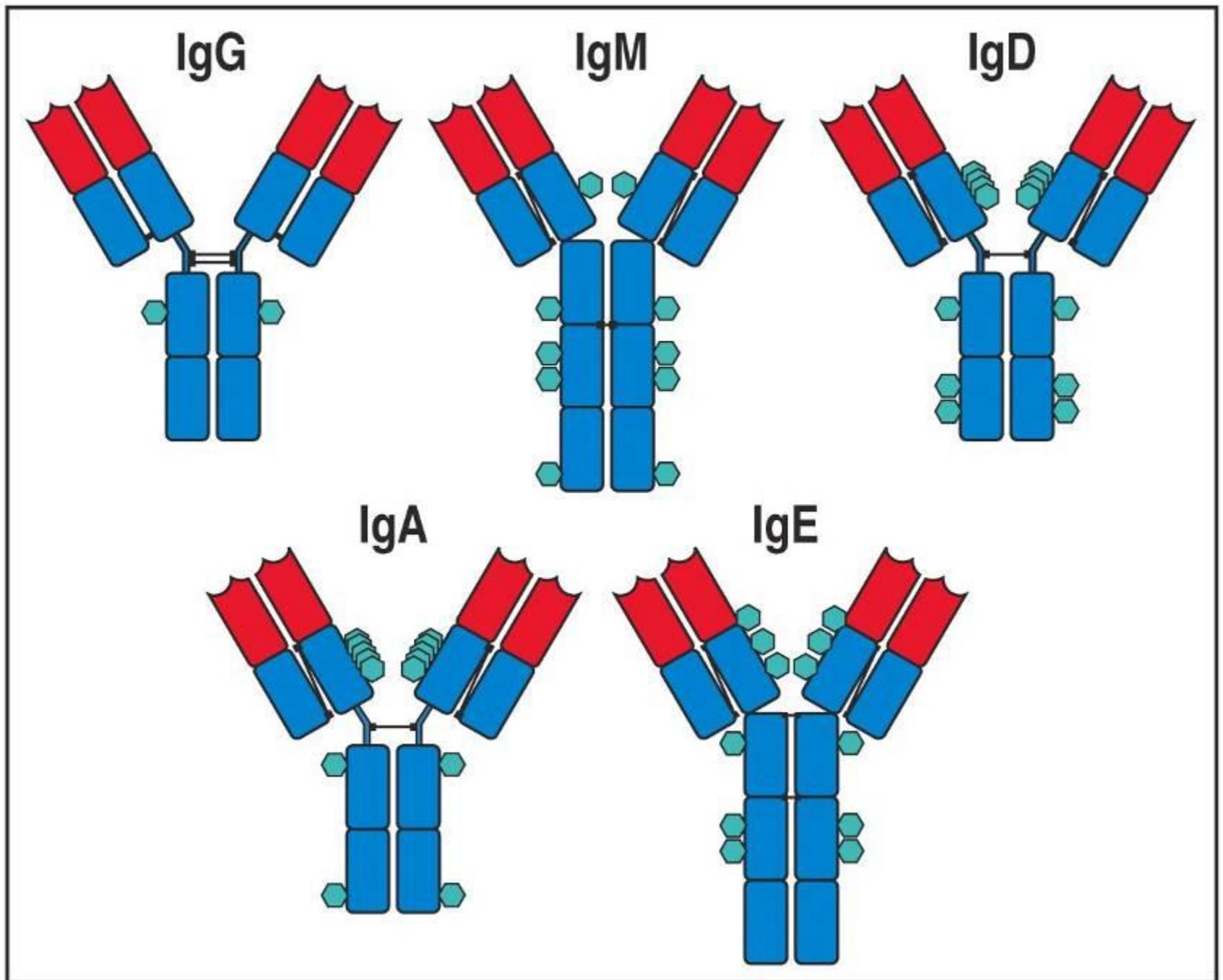


Figure 2-4 The Immune System, 2/e (© Garland Science 2005)

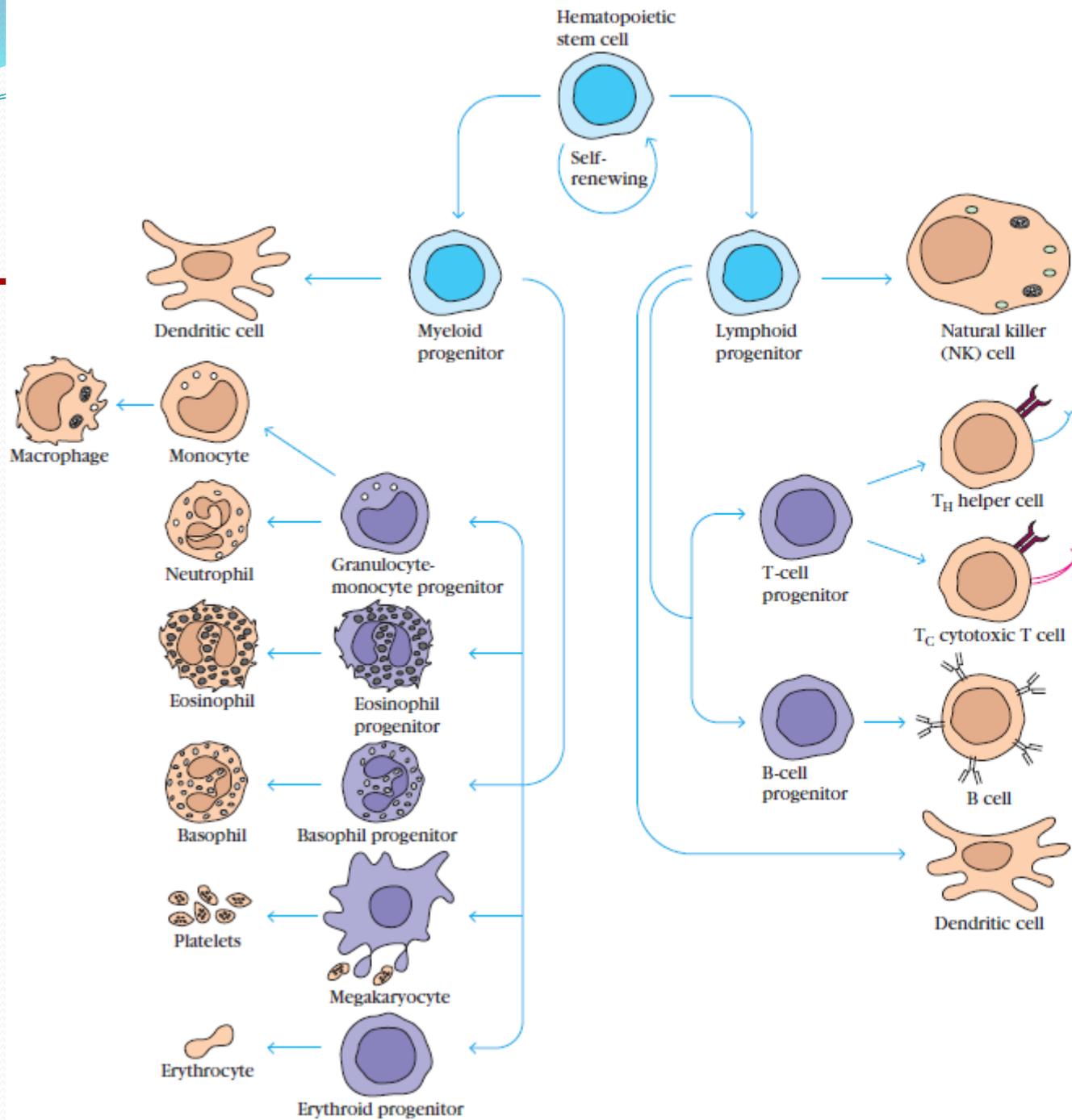


Macrophages, T and B cells,

Lecture 3 & 4

Macrophages

- Terminal stage of monocyte-macrophage line differentiation
- Monocyte-macrophage cells differentiate from myeloid precursor (developed from pluripotent stem cell bearing CD34) in bone marrow
- Matured monocytes are released to peripheral blood stream, then move in organs and develop into tissue macrophages



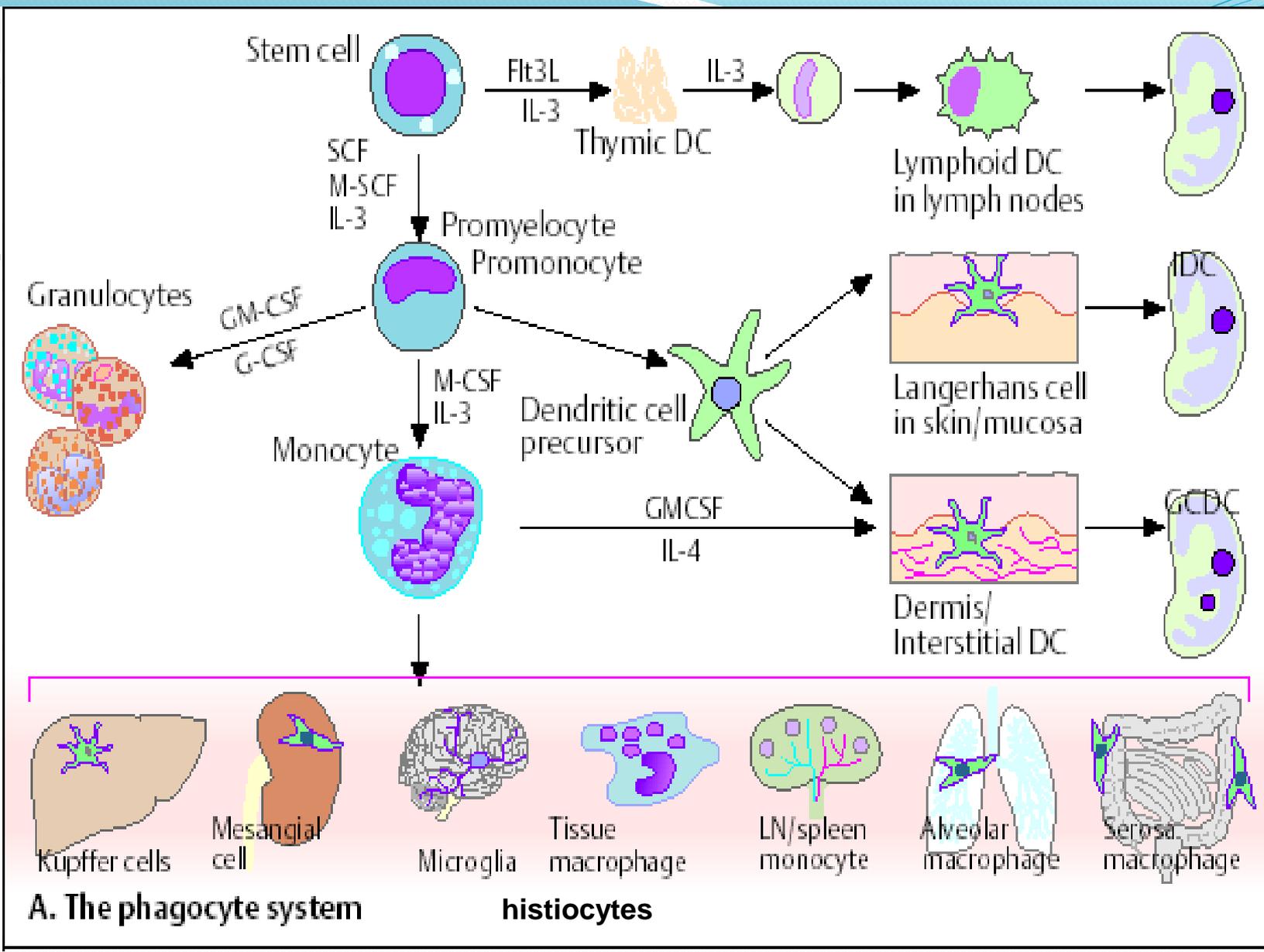
Development

of monocytes and macrophages is affected by various cytokines:

- **SCF**(stem cell factor): produced by stromal cells → activation of stem cell
- **GM-CSF** (granulocyte-monocyte colony stimulating factor): produced by bone marrow (BM) stromal cells, lymphocytes → stimulation of monocyte production
- **M-CSF** (monocyte colony stimulating factor): produced by stromal cells, lymphocytes, endothelial and epithelial cells → production and maturation of monocytes
- **IL-3**: produced by lymphocytes → production of monocytes (and other blood cells)

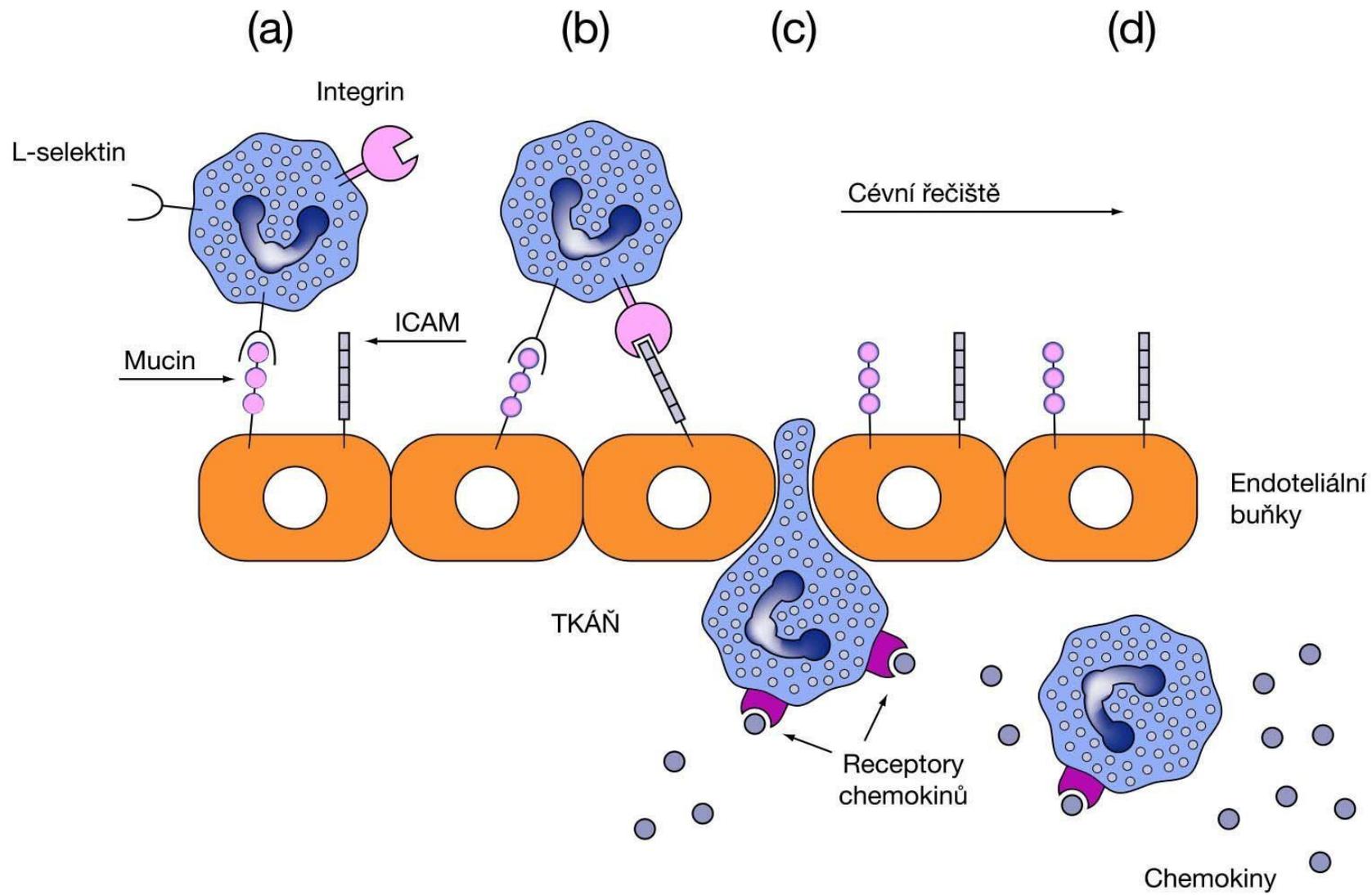
Macrophages- development

- **Monocytes**- in the blood (7%) and the rest in BM
- **Macrophages** - in tissues



Macrophages

- a monocyte enter damaged tissue through the endothelium of a blood vessel
- a monocyte is attracted to damaged site by chemokines, triggered by stimuli including damaged cells, pathogens and cytokines released by macrophages
- after migration of monocytes to the tissues, they differentiate into different forms of macrophages
- macrophages survive several months



Macrophage surface molecules

- **MHC gp I, II** assist in the presentation of antigen to T lymphocytes
- **CD 35** - complement receptor 1 (**CR 1**), binds complement C₃b
- **Receptor** for the Fc portion of IgG
- **CD 14** - receptor for bacterial lipopolysaccharides

Cytokines produced by macrophages

- **IL-1 α , β** - stimulate both T and B cells, Ig synthesis, activation of other macrophages, sensitizing cells to IL-2 and IFN
- **TNF- α** - similar in function to IL-1
- **IL-8** - secreted by activated macrophages
 - chemokine attracting neutrophils and T cells
- **IL-12** - promotes induction of Th1 cells, inhibits Th2 cells
- **IFN- α** - **activates** host cells to induce enzymes inhibiting viral replication; **increases** expression of MHC gp I on host cells; **activates** NK cells, T cells, other macrophages

Functions of macrophages

- Phagocytosis
- Production of cytokines
- Presentation of epitops with MHC gp II
- Presentation of epitops with MHC gp I

Phagocytosis

a foreign substances are **ingested**

microbes are **killed** and **digested**

follows **processing** of antigenic epitopes and their presentation on the cell membrane

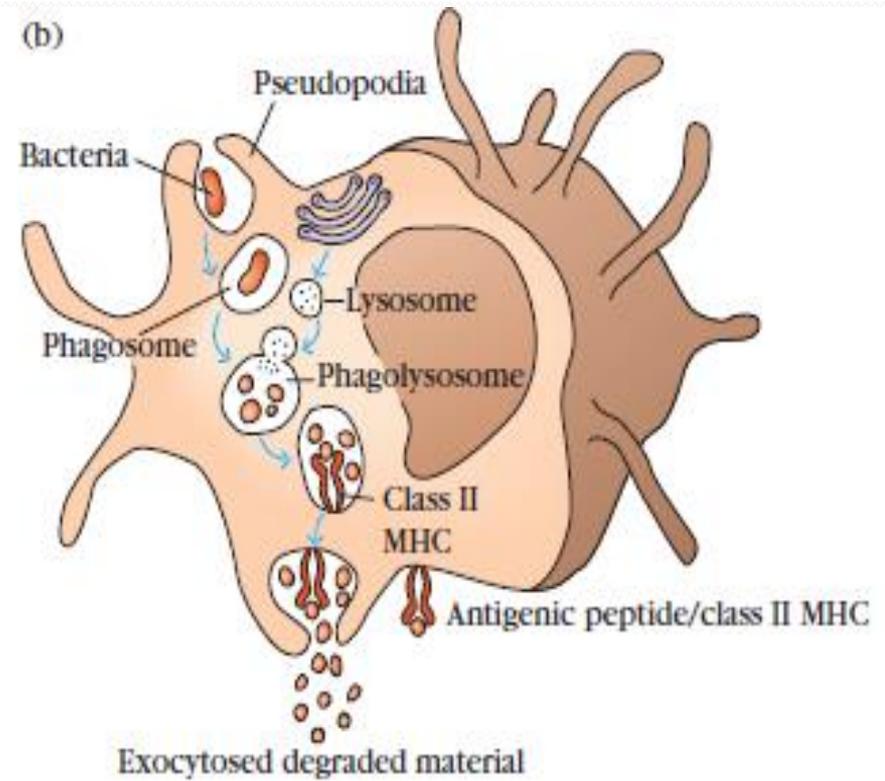
Macrophage - functions

- Macrophages provide defense against tumor cells and human cells infected with fungi or parasites.
- T cell becomes an activated effector cell after recognition of an antigen on the surface of the APC → release chemical mediators → stimulation of macrophages

(a)



(b)



Presentation of epitopes with MHC gp II

- After **endocytosis** and **degradation** of the antigen, presentation of its epitopes follows
- epitope is connected to MHC gp II → cell surface → presentation to Th cells
- **MHC** (Major Histocompatibility Complex) = complex of genes that governs the production of the major histocompatibility antigens - in humans termed HLAs (Human Leukocyte Antigens)

Presentation epitopes with MHC gp I

- intracellular parasites are **hydrolyzed** in proteasomes of macrophages
- their **peptides** are connected to **TAP** (Transporters Associated with antigen Processing molecules 1,2), that carry the epitope and **MHC gp I** → presentation on the cell surface to Tc cells

Antigen presentation

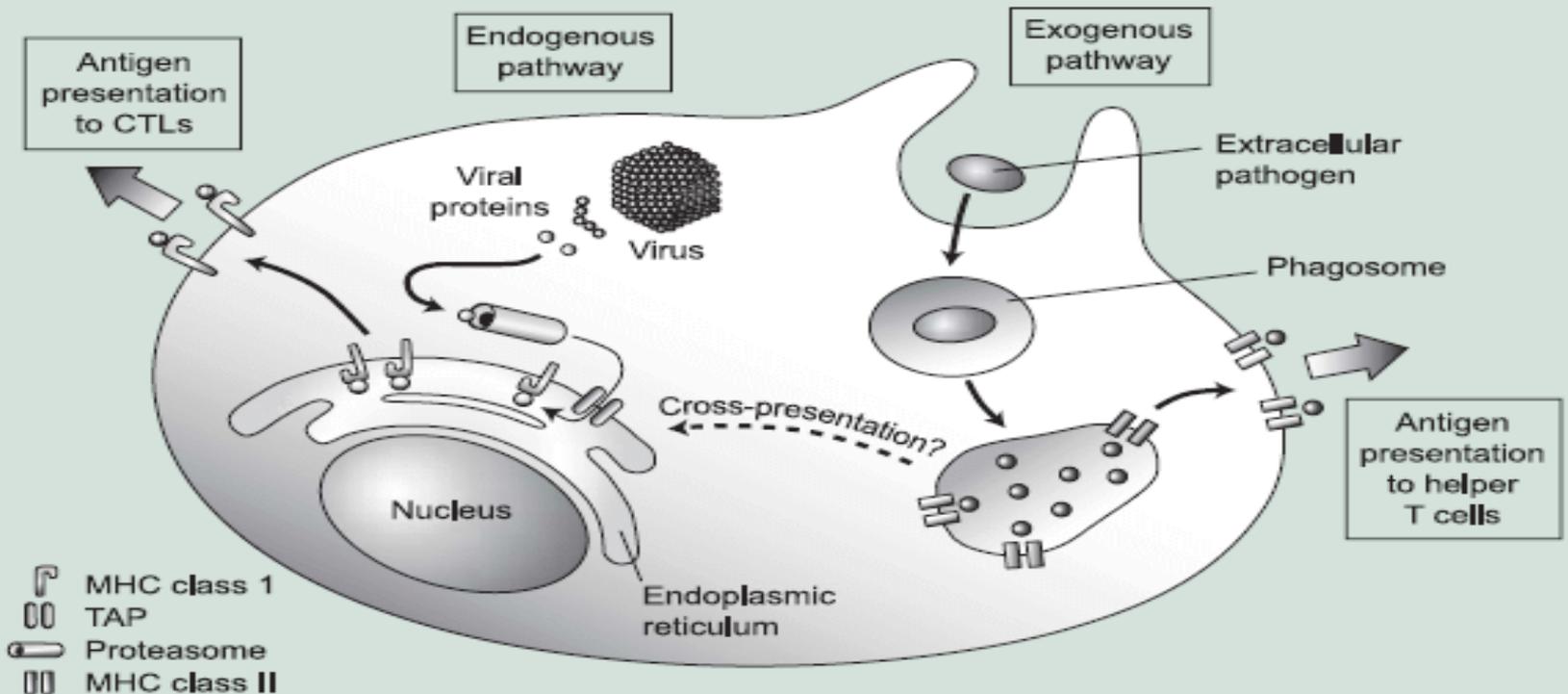
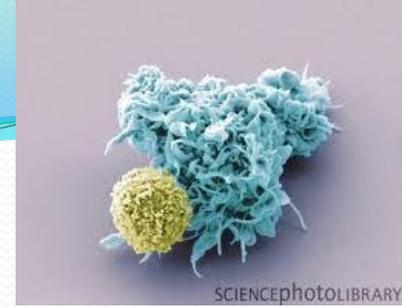


Figure 1. Two ways of antigen processing and presentation. Typically, antigens from endogenous pathogens, such as a virus, are processed by the endogenous pathway, in which the proteasome degrades the antigen into smaller peptides, which are then transported to the endoplasmic reticulum (ER) via TAP (transporter associated with antigen processing). In the ER, the peptide is attached to a major histocompatibility complex (MHC) class I molecule and delivered to the cell's surface for presentation to a CD8⁺ T cell. Exogenous antigens are taken into the cells by a phagosome, joined to an MHC class II molecule, and taken to the cell's surface for presentation to a CH4⁺ T cell. Some exogenous antigens, however, are presented to CD8⁺ T cells via cross-presentation. Adapted with permission from Roy CR. Professional secrets. *Nature*. 2003;425:351-352. Copyright © 2003 Nature Publishing Group.

Dendritic Cells (DC)



- DC mature after a contact with pathogen, then migrate to lymph nodes where antigen-specific immune response develops
- DC are equipped with numerous cytoplasmic processes, allowing contact with up to 3000 T cells
- In lymph nodes, the expression of MHC gp I and co-stimulatory molecules (CD80, CD86) on DC increases

Types of Dendritic Cells

Myeloid DC

- similar to monocytes

Plasmacytoid DC

- looks like plasma cells, but have certain characteristics like myeloid cells
- production of huge amounts of interferons

Function of DCs

DCs are **the most important APC**

DCs can be easily infected by viruses → processing of viral proteins → their presentation in complex with MHC gp I → **activation of Tc**

DCs can ingest extracellular viral particles → their presentation in complex with MHC gp II → **activation of Th2 cells** → help for B cells → production of antiviral antibodies

DCs can also be activated by apoptotic cells

Antigen Presenting Cells (APC)

Dendritic cells, macrophages, B cells

Antigen processing and its **presentation** to T cells in the complex with HLA class I or II

Providing additional signals to T cells which are necessary for their activation (CD 80, CD 86)

T lymphocytes - ontogenesis

Stem cell in BM gives rise to lymphoid precursor cell which matures into 3 types of lymphocytes:

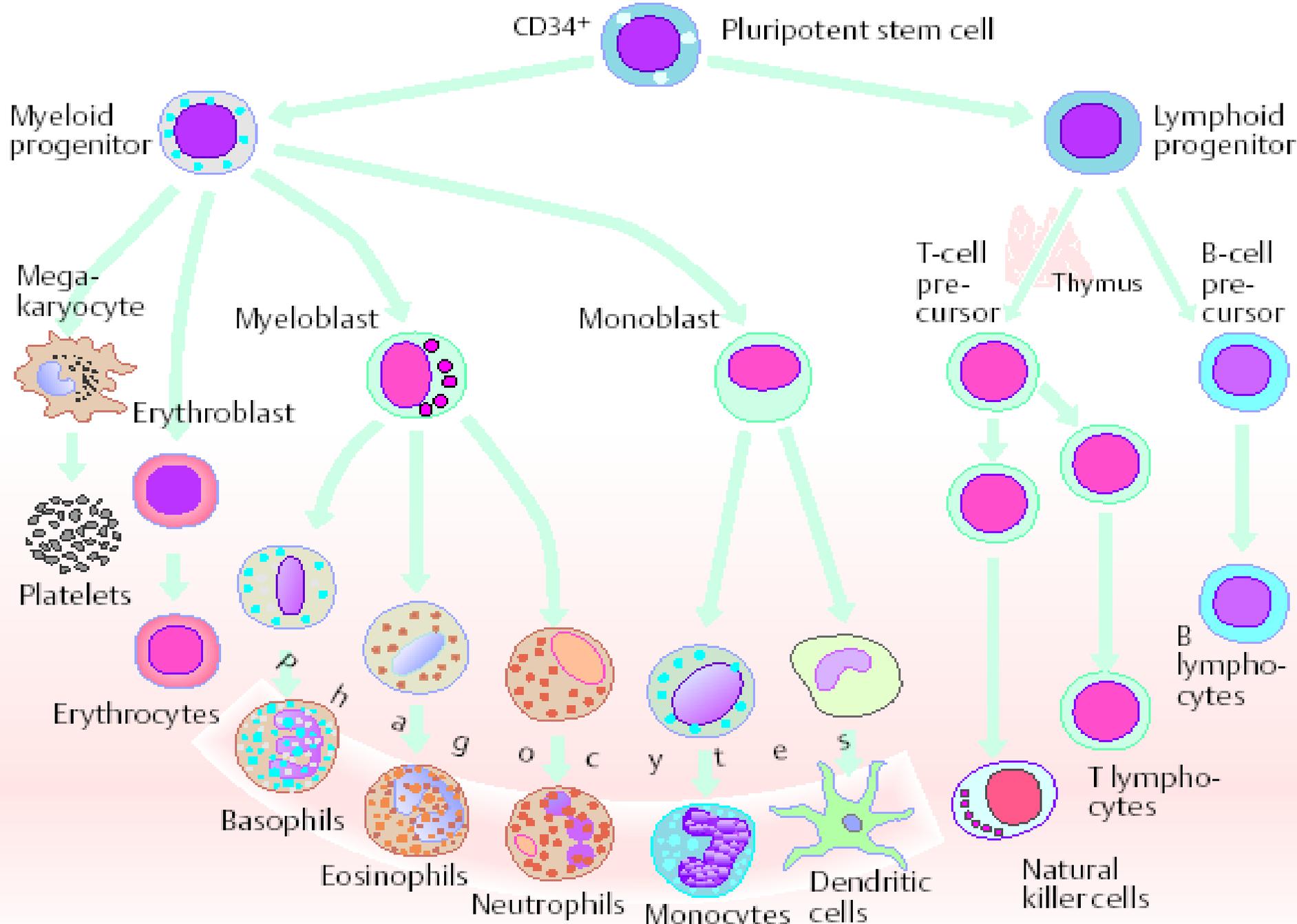
T lymphocytes

B lymphocytes

Natural killer (NK) cells

Pro-thymocytes move to the thymus where continue the maturation into T lymphocytes

Maturation of B lymphocytes continues in BM



A. Origin of cells of the immune system

Surface markers of T cells

CD (Cluster of Differentiation) proteins - molecules on the cells membrane, they allow the identification of cells

TCR - receptor for antigen

MHC gp I

CD proteins

allow an identification of T-cell subsets

CD 2 = adhesion molecule

CD 3 = important in intracellular signaling (initiation of immune response); closely associated with TCR

CD 5,7

CD 4,8 = are expressed on subclasses of mature T cells; CD4 reacts with MHC gp II, CD8 reacts with MHC gp I on macrophages

CD 28 – molecule that provides co-stimulatory signals, binds CD80 and 86

Maturation of T lymphocytes

Consist of three types of processes:

Proliferation of immature cells

Expression of antigen receptors genes

Selection of lymphocytes

TCR (T Cell Receptor)

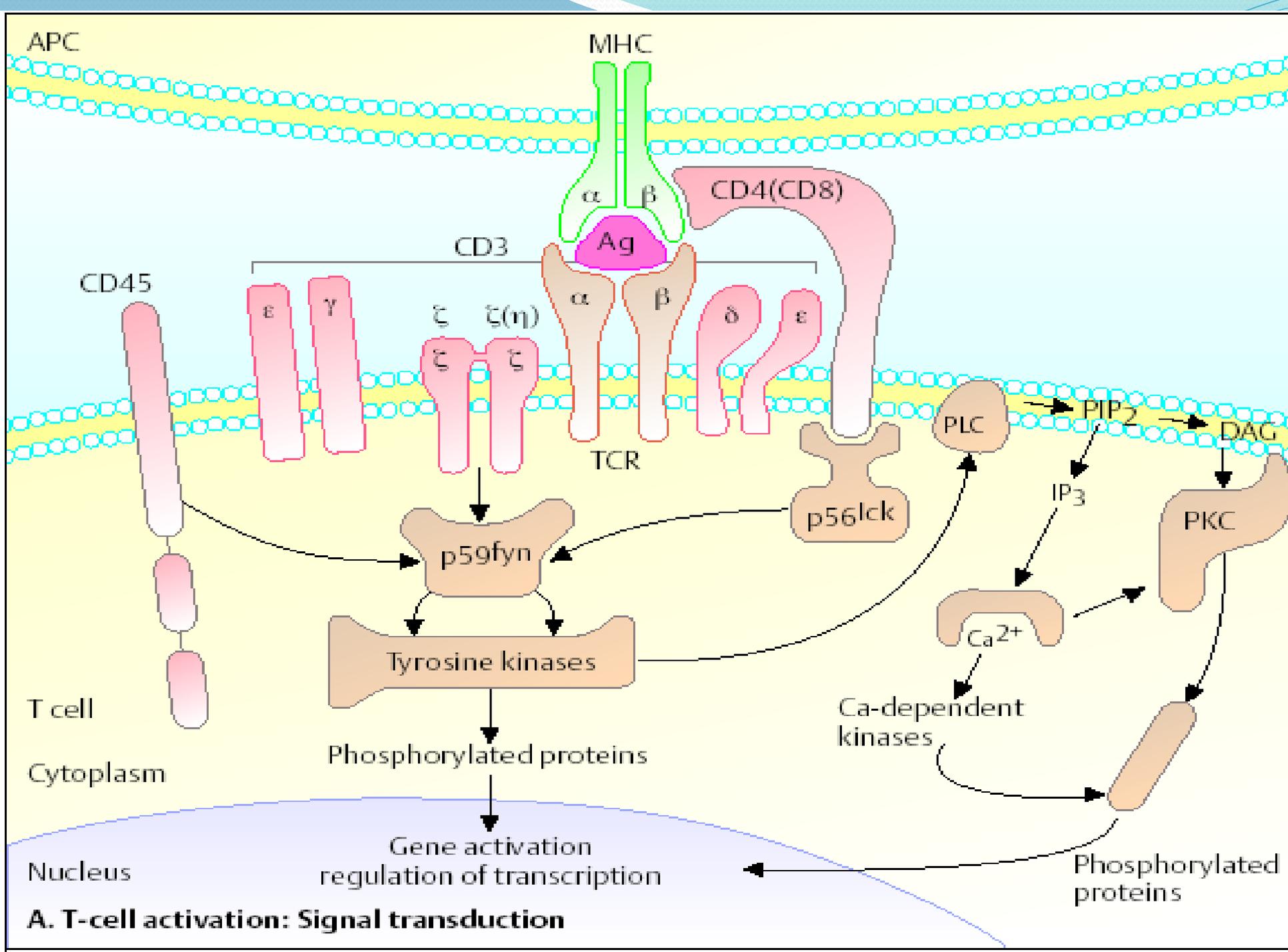
Antigen receptors are encoded by several gene segments that **recombine** during lymphocyte maturation

Heterodimer consisting of 2 non-identical polypeptide chains linked together by disulfide bonds

> 95% T cells express the $\alpha\beta$ heterodimer, 5% $\gamma\delta$

TCR heterodimer is non-covalently associated with the γ,δ,ϵ chains of the CD3 molecule

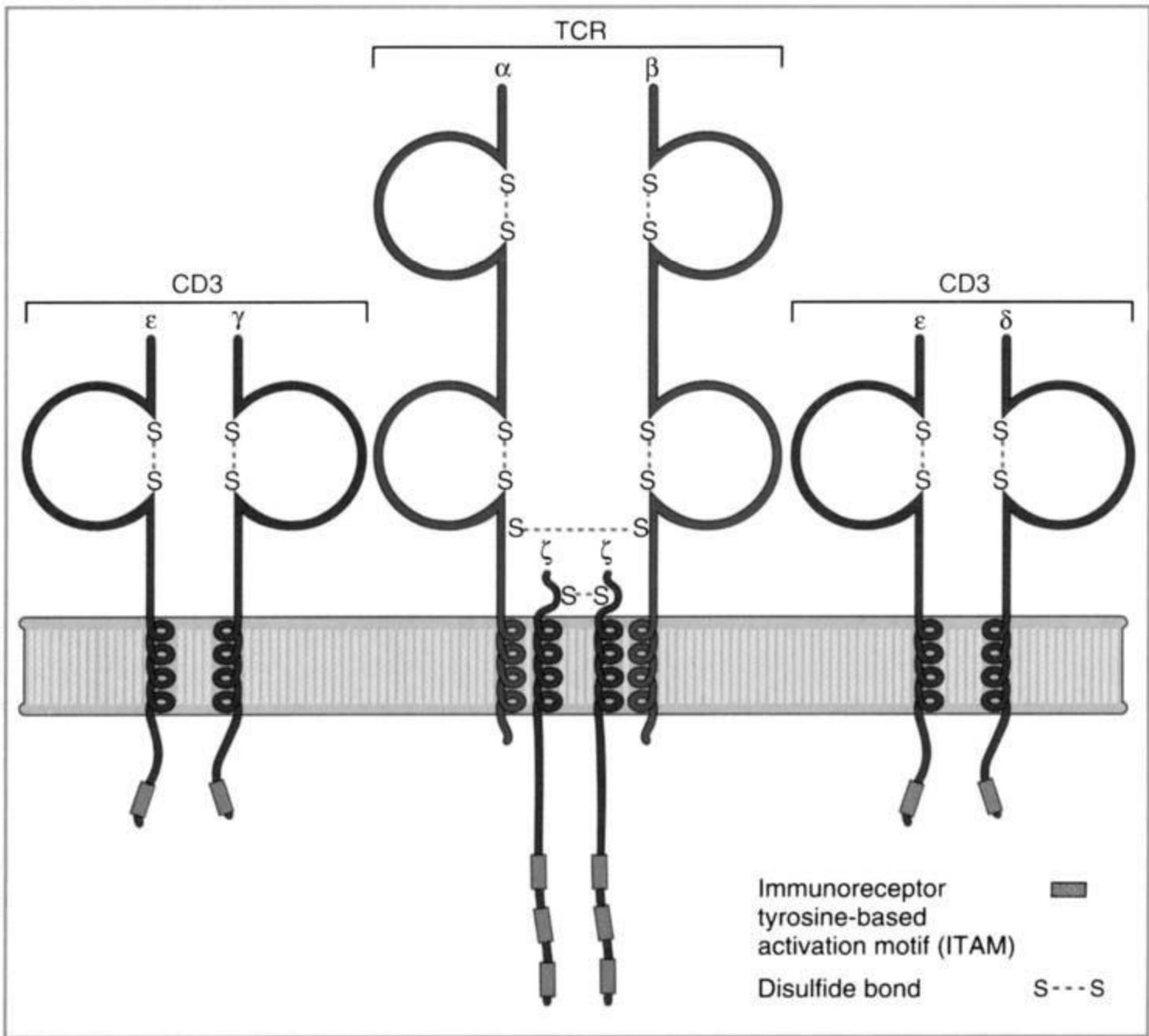
complex **TCR-CD3** makes contact with both the **Ag** and **MHC gp**



Extracellular space

Plasma membrane

Cytoplasm



Recognition of antigen by T cells

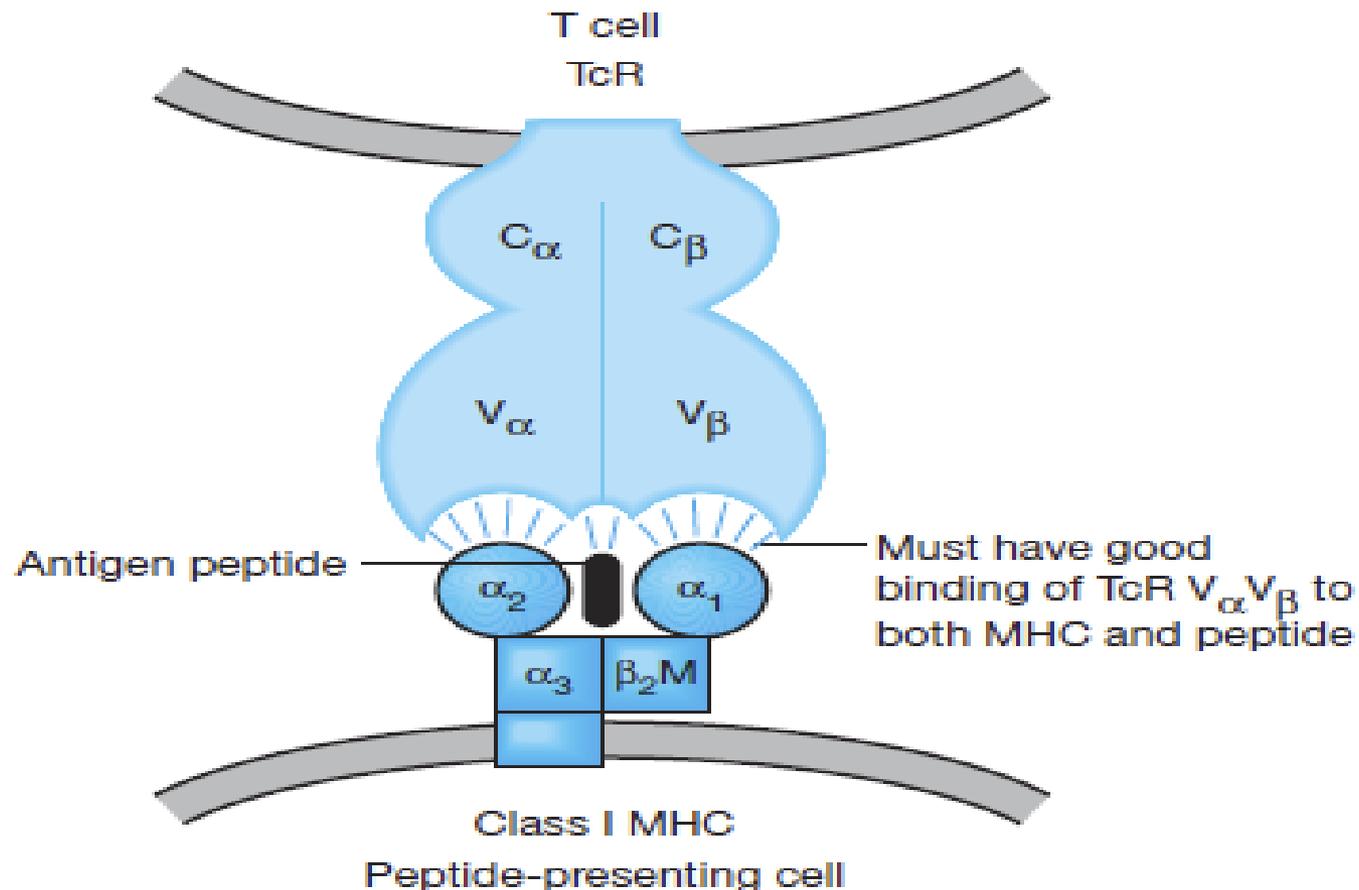


Figure 4.6 Interaction of the TcR with antigen/class I MHC. The antigen-binding site of the TcR is made up of parts of the V_α and V_β chains. Parts of the binding site interact with the antigenic peptide bound in the groove of the MHC molecule and parts of the binding site interact with the MHC molecule.

Are there any structures that the CD3 and ζ chains recognize?

No. The CD3 and ζ chains have no V-regions and thus they cannot recognize antigens. Moreover, there are no natural ligands or counter-structures that these chains can interact with. Their sole function is to “sense” the triggering of the TCR by its specific antigen, and then to transduce the signal that accompanies such a triggering.

T cells and Cell-Mediated Immunity

- **Types of T cells**
- **TH (Helper T cells):** activated by antigen in Class II MHC, respond by secreting cytokines to influence other immune cells
- **TH₁:** activate cells related to cell-mediated immunity (TC and Macrophages)
- **TH₂:** activate B cells to make antibodies (T-dependent antigens)
- **TC (Cytotoxic T cells):** activated by antigen in Class I MHC, respond by secreting perforin and lysing the target cell. This often requires pre-activation of the TC by a TH₁ cell. (Cells expressing foreign antigens in Class I MHC are likely to be infected with virus or are cancerous and thus are quickly destroyed.)
- **TS (Suppressor T cells):** regulate the immune response, inhibit T and B cell activity when antigen levels decline

T suppressor lymphocytes (Tsup;CD8+)

- Cause lysis of target cell; active against tumors, virus-infected cells, transplanted allogeneic tissue

release TNF → decrease of proteosynthesis

recognize the foreign epitope in association with **MHC gp I** molecules

Destroy target cells by perforins (create pores in the cell membrane → cell lysis) and granzymes (degradation of essential macromolecules)

T helper lymphocytes (Th; CD4+)

recognize the epitopes in association with MHC gp II
help for B cells to produce antibodies and help for
phagocytes to destroy ingested microbes
subsets of Th cells: Th₁, Th₂ cells

Regulatory T cells

Express CD4, CD25, FoxP₃

Regulate the activation or effector function of other T cells

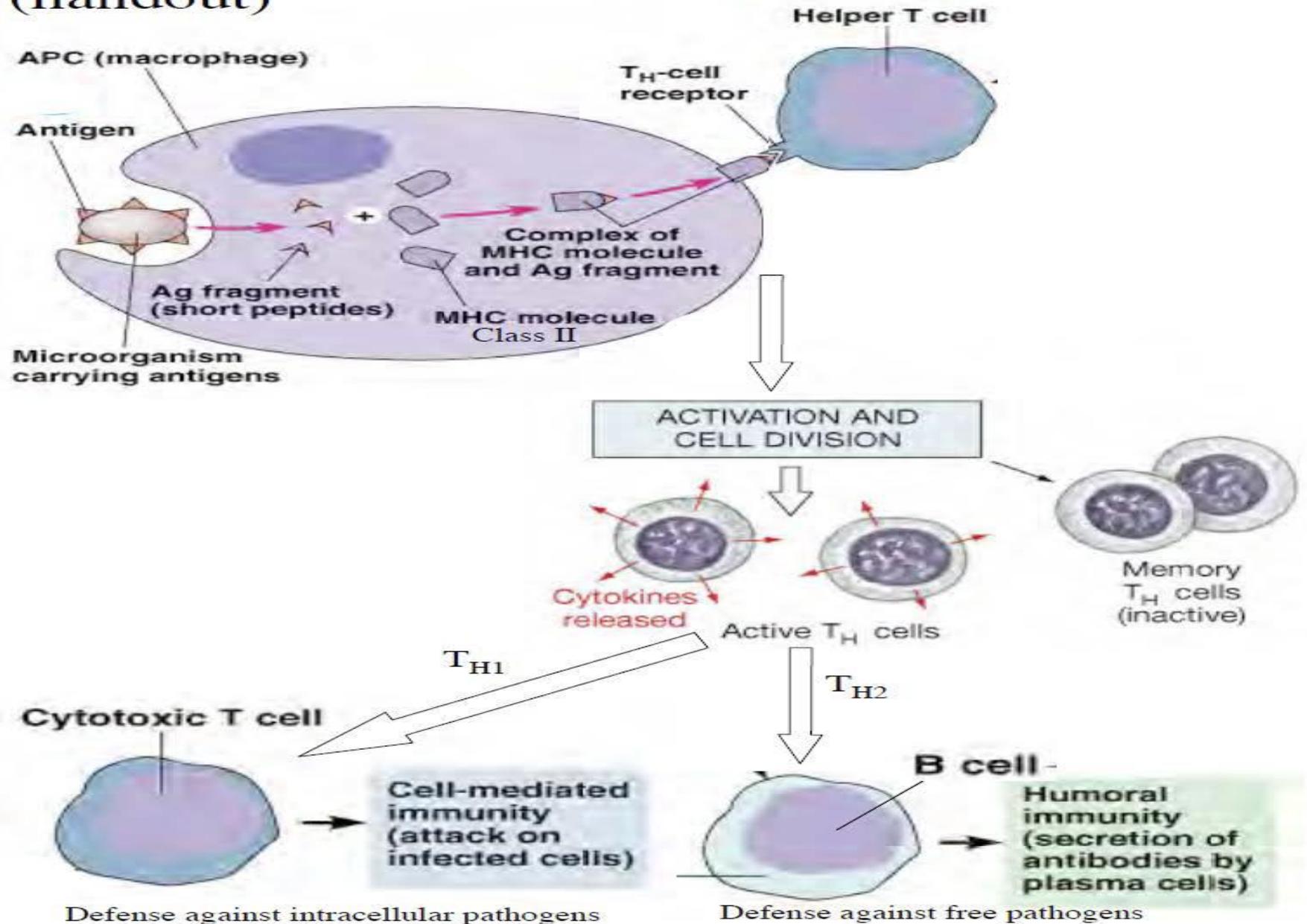
Are necessary to maintain tolerance to self antigens

Production of IL-10, TGF- β

B cell

- 1. IgD antibody receptor on B cell binds its specific antigen/epitope
- 2. B cell is activated and undergoes clonal selection: the B cell proliferates and differentiates into two types of cell populations --> Memory B cells and Plasma Cells
- 3. Plasma cells secrete antibodies specific for the original epitope (2000 antibody molecules per second) for 3-5 days [Time from initial antigen binding to antibodies appearing in the blood is 7-10 days]
- 4. Upon second exposure to the same antigen/epitope, memory cells bind antigen and are triggered to differentiate into plasma cells and secrete antibodies. [Time from initial antigen binding to antibodies appearing in the blood is 3-5 days]

(handout) Activation of Helper T Cell



Natural Killer Cells (NK cells)

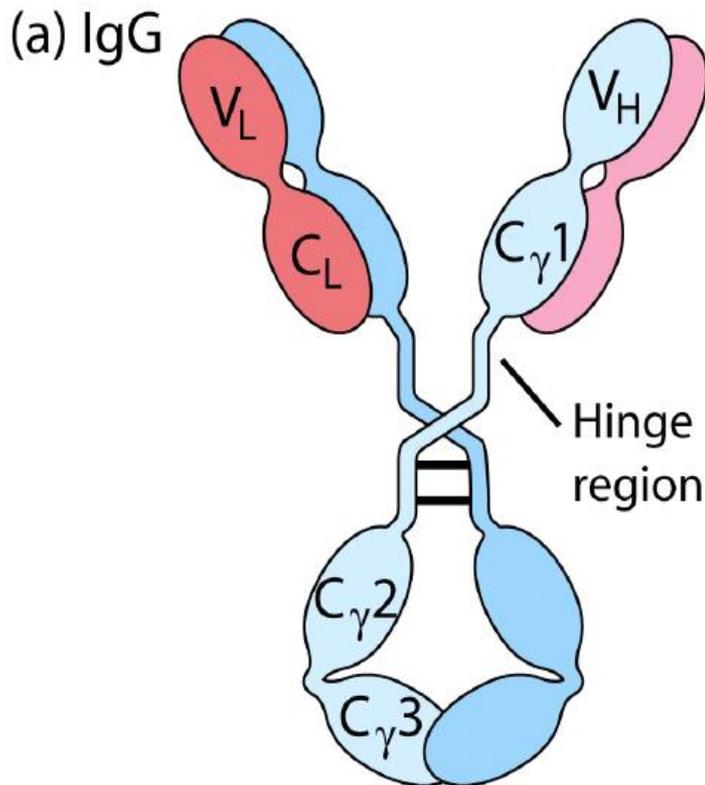
- not immunologically specific
- attack any abnormal antigen on eukaryotic cells: virus-infected, cancer, large parasites
- lyse target cell by releasing perforins to disrupt membrane
- **Inter-relationship of Cell-Mediated and Antibody-Mediated Immunity**
- T-dependent antigens:
- more common
- protein epitopes
- require TH2 cells to signal B cells to produce antibodies

- **Activation of B cells (T-dependent Antigen). Epitope tends to be protein, produces stronger immune response than T-independent Antigen)**
- B cell binds specific antigen in the IgD receptor and internalizes it.
- B cell transfers antigen to a Class II MHC receptor and return antigen now bound to MHC back to the surface of the cell. B cell is now sensitized.
- A Th2 cell specific for the antigen recognizes and binds to the antigen in the Class II MHC and becomes activated.
- The activated Th2 cell secretes cytokines on the B cell.
- Cytokines activate the B cell.
- The activated B cell undergoes clonal selection producing Memory B and plasma cells.
- Plasma cells secrete antibodies that are specific for the original antigen. Memory cells wait for second exposure.

Antibody

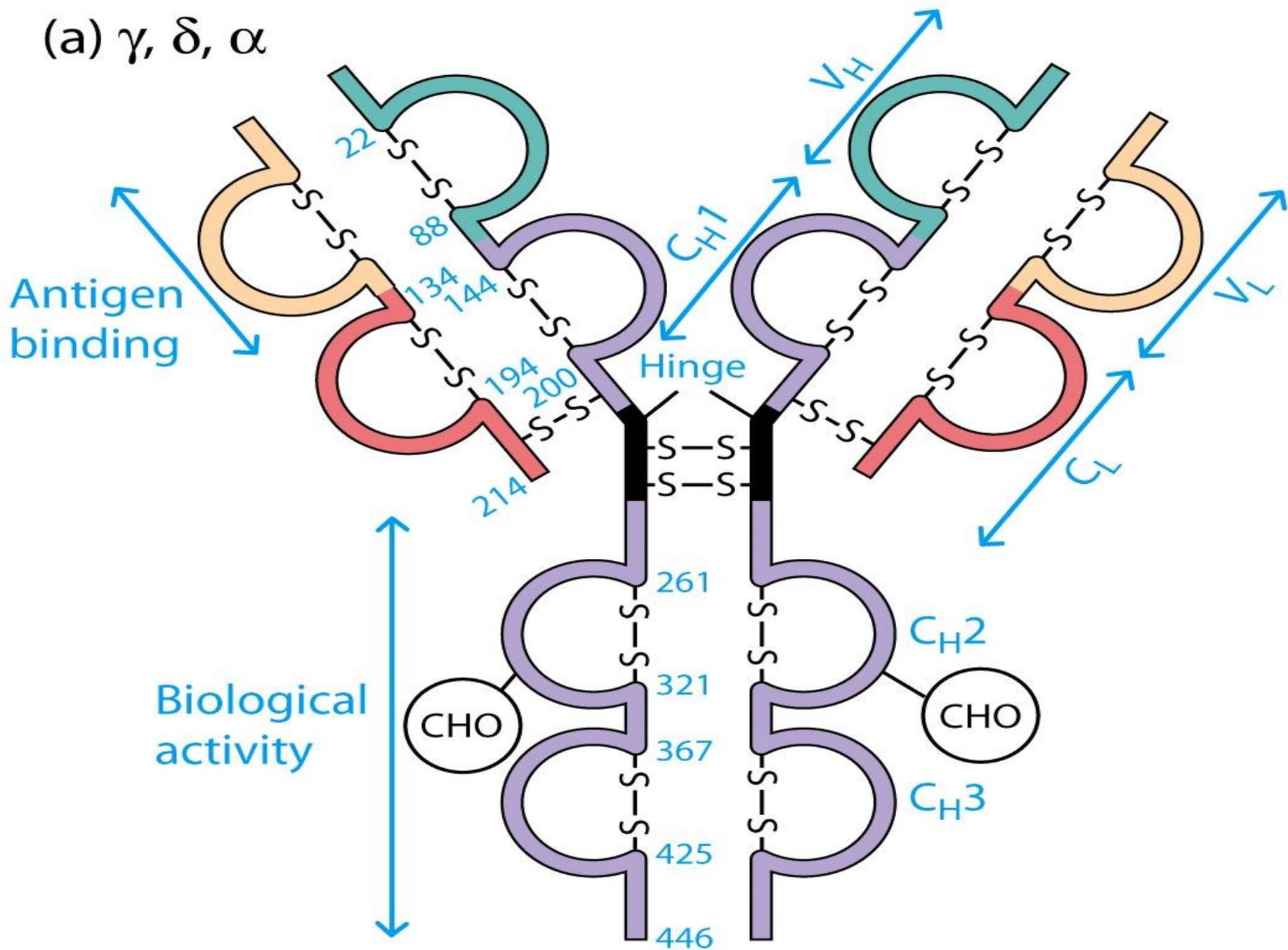
Lecture 5

Antibody Classes: IgG



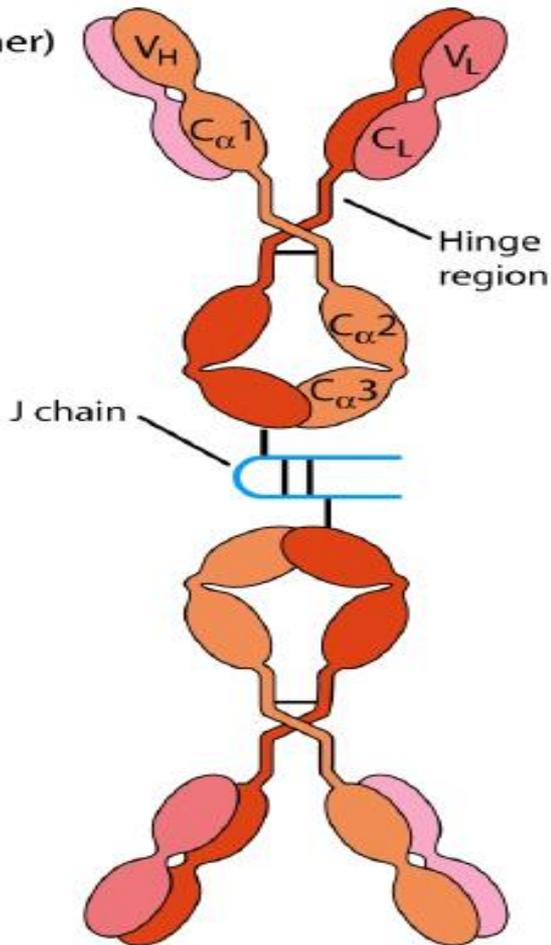
- ~80% of total serum Ab
- 2γ H chains + 2 κ or 2λ L chains
- 4 subclasses due to variation in γ chain aa seq
- Variation affects bio activity
- #’d according to prevalence (IgG₁>IgG₂>IgG₃>IgG₄)
- IgG_{1,3,4} readily cross placenta
- 3 effective complement activator
- 1,3 bind effectively to Fc receptor on phago cells

(a) γ , δ , α



Antibody Classes: IgA

(d) IgA (dimer)



- 10-15% of total serum Ab; predom in secretions
- Monomeric form in blood
- Di/tri/tetrameric forms in secretions (sIgA)
- “secretory component” of epith cells aids in uptake/release to surfaces
- Daily prod of IgA > than any other class (5-15 g/day) in mucus

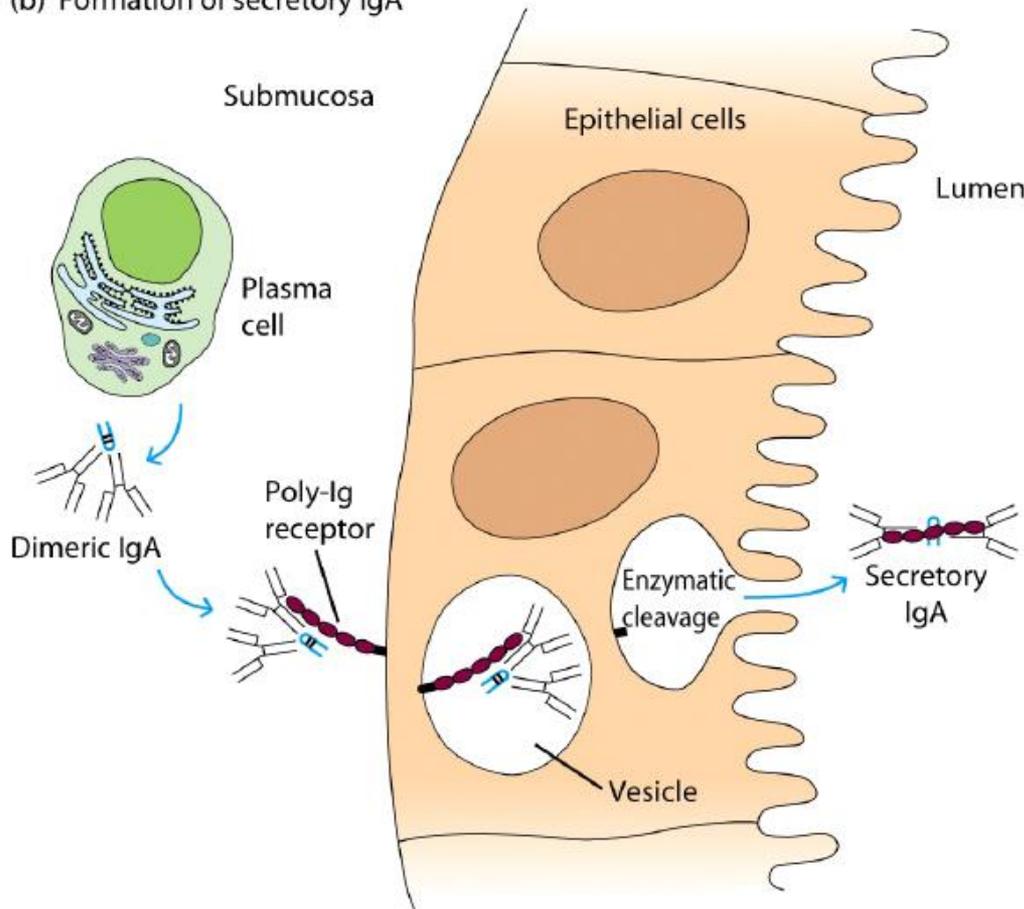
Antibody Classes: IgA

IgA plasma cells

“home” to subepithelial tissue → release IgA which binds to **poly-Ig receptor** → endocytosis → cleavage of receptor to become “**secretory component**” for sIgA

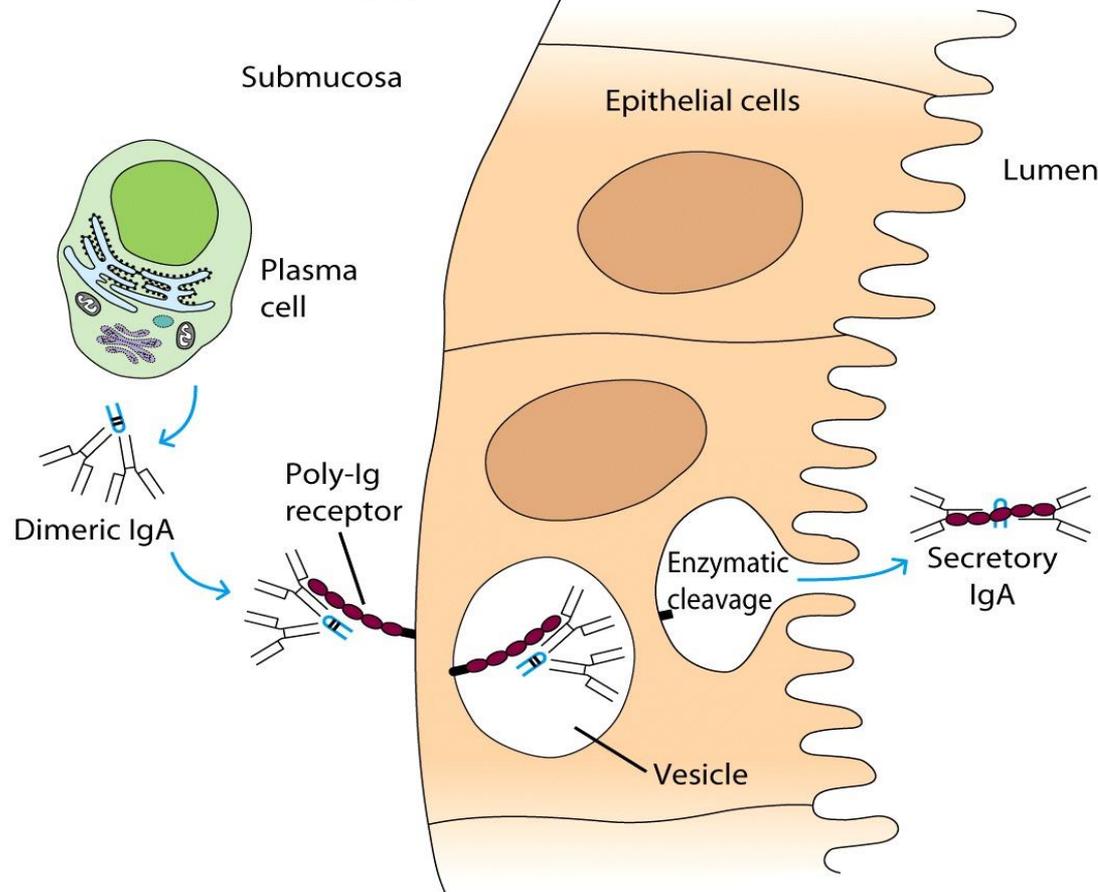
*Important effector function! at these locations

(b) Formation of secretory IgA



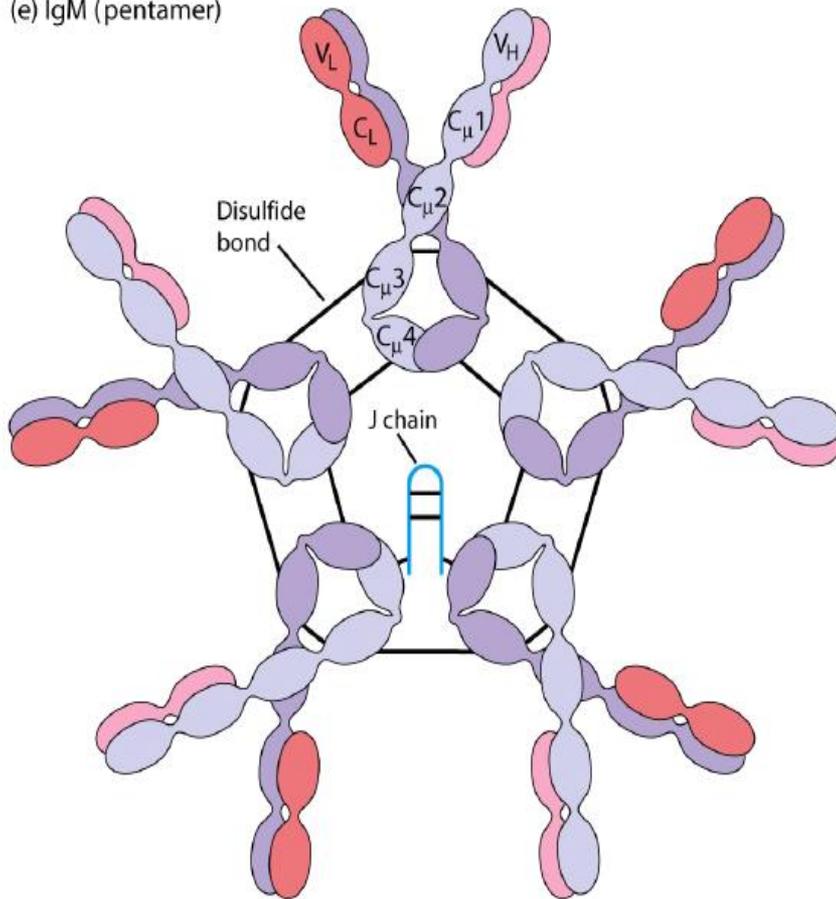
IgA Antibody Transport Across Cell (Transcytosis)

(b) Formation of secretory IgA



Antibody Classes: IgM

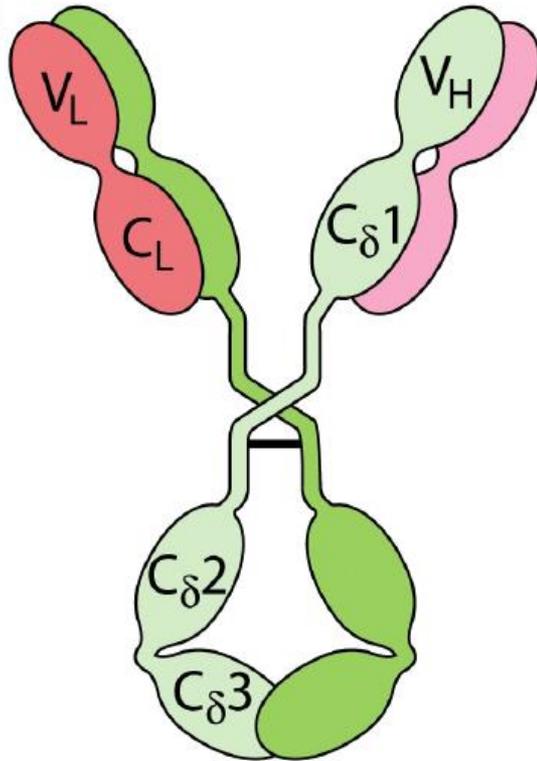
(e) IgM (pentamer)



- 5-10% of total serum Ab
- Monomeric IgM as mIgM on B cells
- Pentameric IgM is secreted (sIgM)
- Joined by J chain of glycoprotein
- 1st Ig class of 1^o IR
- Most efficient at Ag-binding
- Most efficient at complement activation
- Can be secreted to mucosal surfaces (2nd to sIgA)

Antibody Classes: IgD

(b) IgD



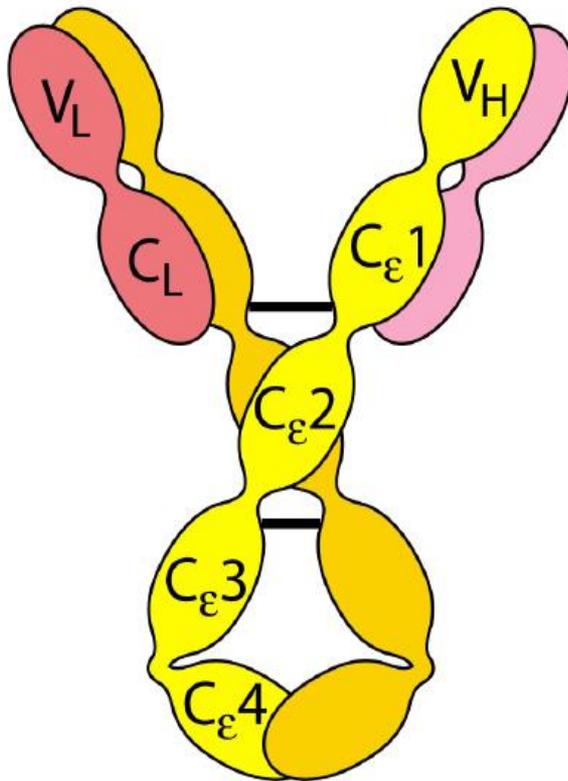
- ~0.2% of serum Ab
- Effector function is unclear
- Receptor on B cells

Antibody Classes And Biological Activities

- IgD
 - Expressed on B-cell Surface
- IgM and IgD, Expressed on B-cell Surface
- We Do Not Know Any Other Biological Effector Activity
- Low serum concentrations, $\sim 30\mu\text{g/mL}$

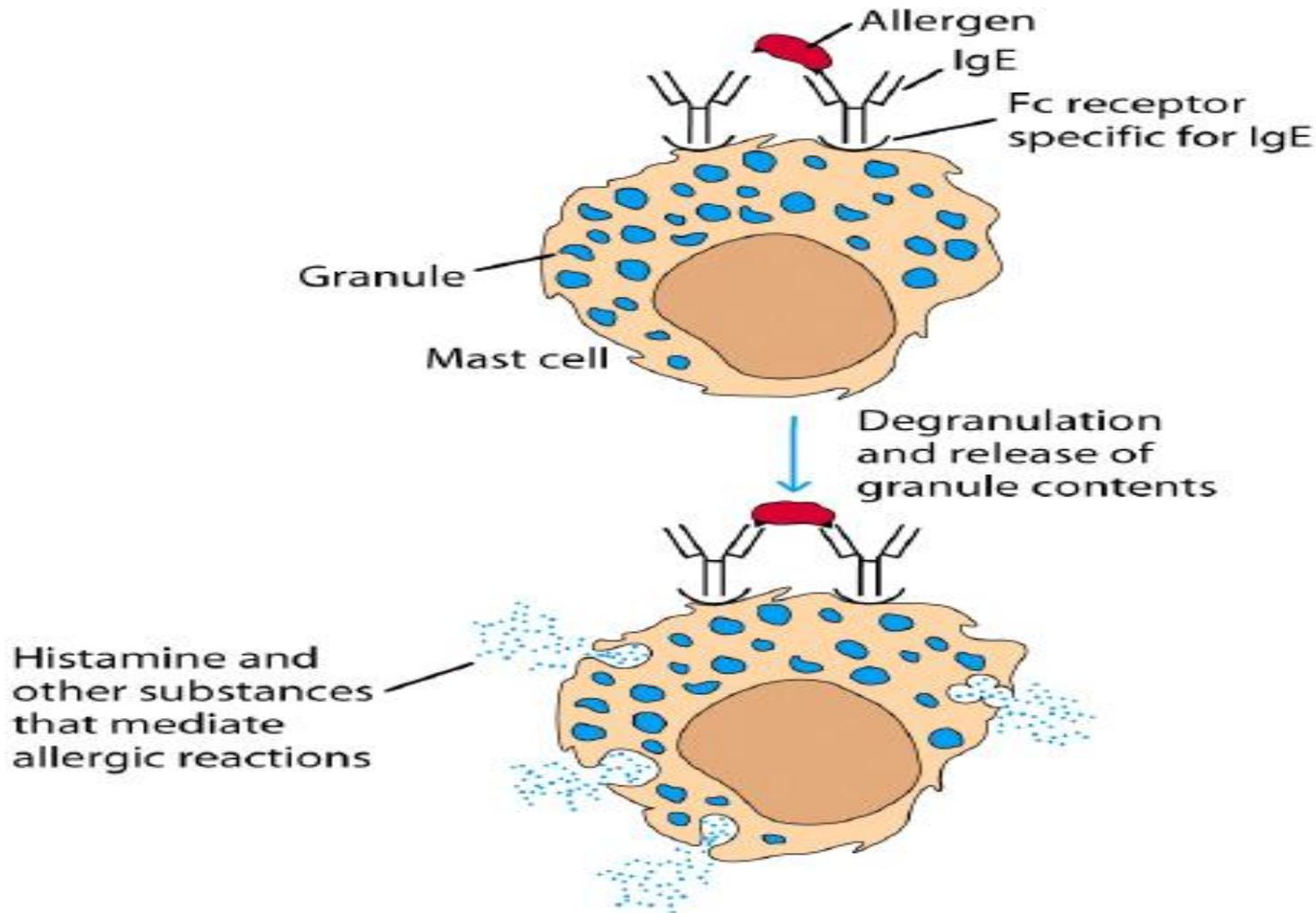
Antibody Classes: IgE

(c) IgE



- <0.01% of serum Ab
- Named for “E antigen” of ragweed pollen
- Involved in allergic hypersensitivity rxns (hayfever, hives, asthma, anaphylactic shock)
- Bound to surface of basophils/mast cells

Allergen cross-linkage of receptor-bound IgE



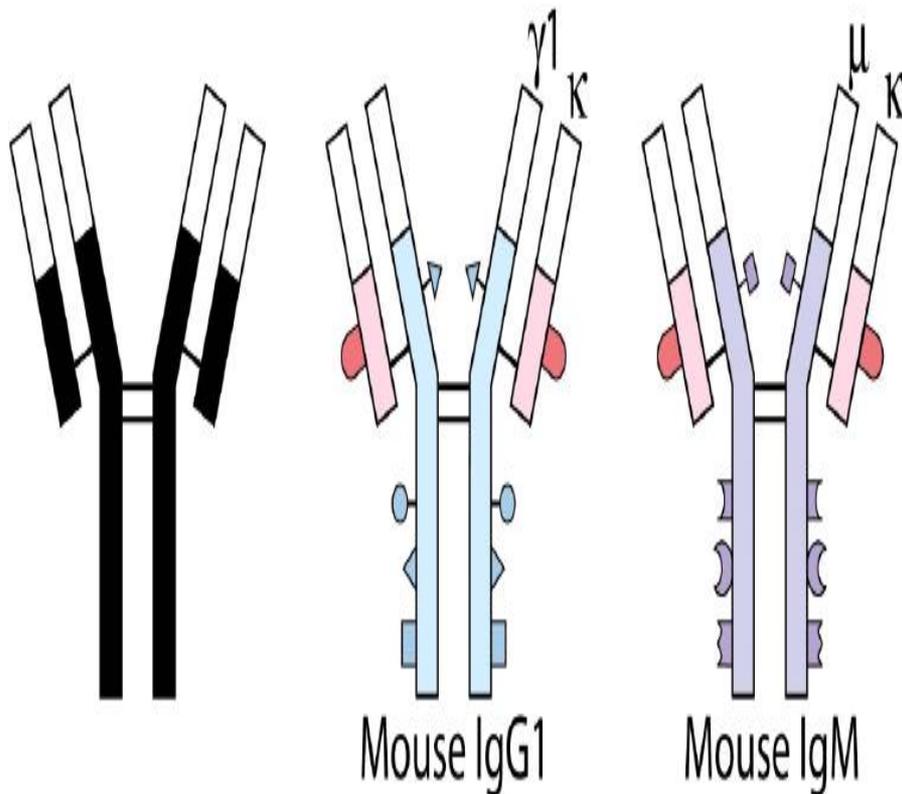
Antibody as immunogens

- Since Ig's are glycoproteins, they can stimulate an IR and stimulate anti-Ig Ab's
- Epitopes on Ig's fall into 3 categories:
 - Isotypic
 - Allotypic
 - Idiotypic

All located at different locations of the Ab molecule

Isotype epitopes

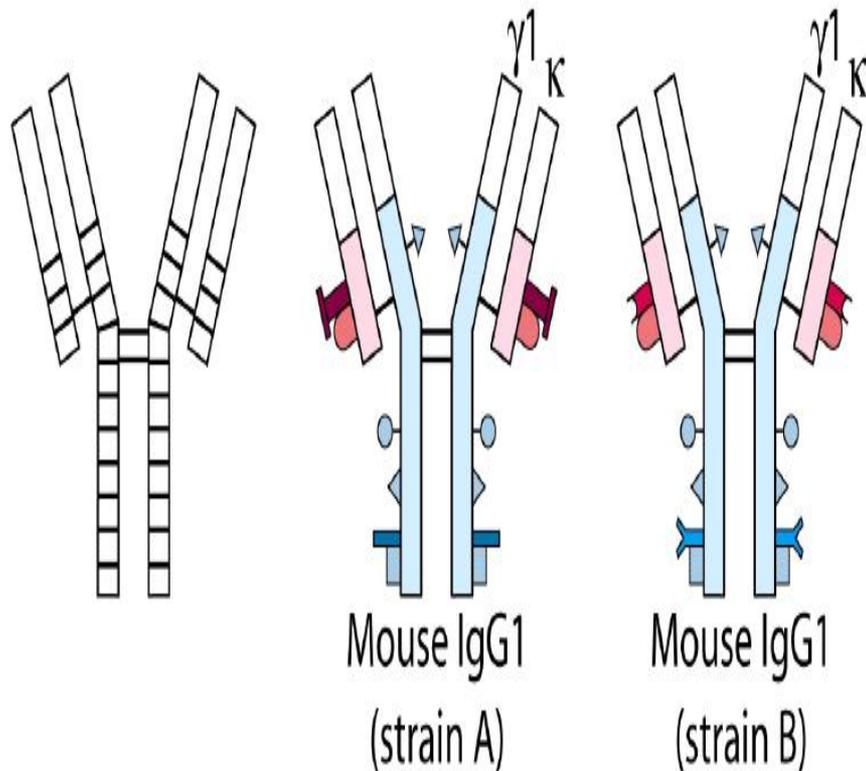
(a) Isotypic determinants



- (C)onstant region epitope for each H chain class, and L chain type/subtype
- Each ind of a species produces their own isotypes
- **differences in C regions will be recog as foreign
- Anti-isotype Ab used for research and diagnostic tests (incl. ELISA)

Allotype epitopes

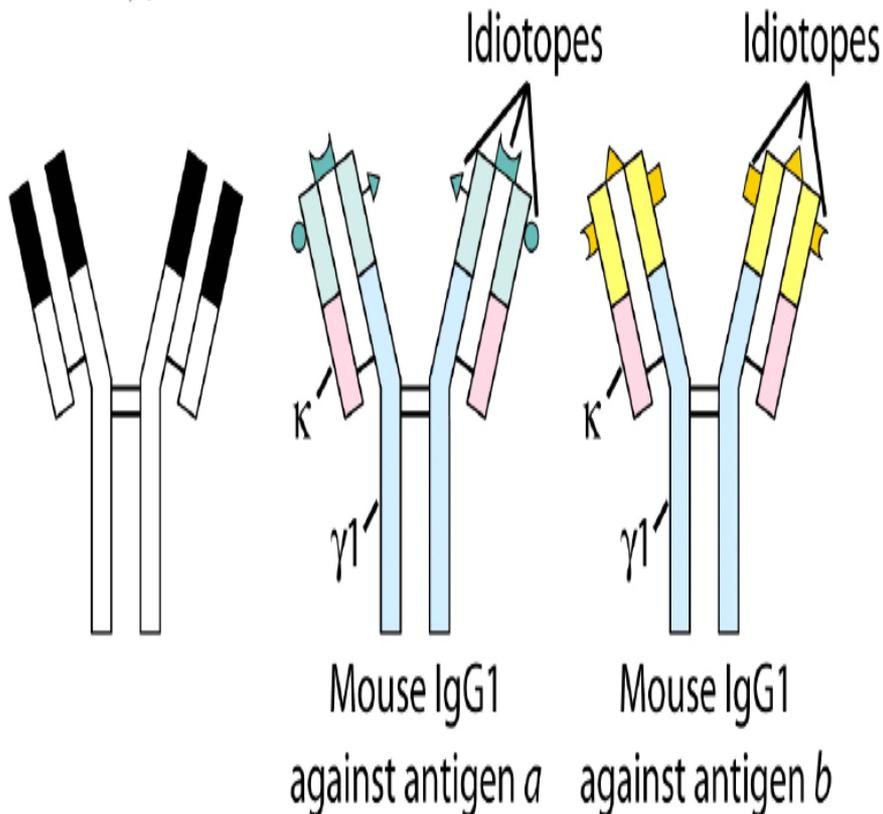
(b) Allotypic determinants



- Specific regions along the C region (isotype) are called allotypes
- Ab's to allotypic epitopes can arise in ♀ vs paternal allotypes on fetal Ig's
- Can also occur in blood transfusions

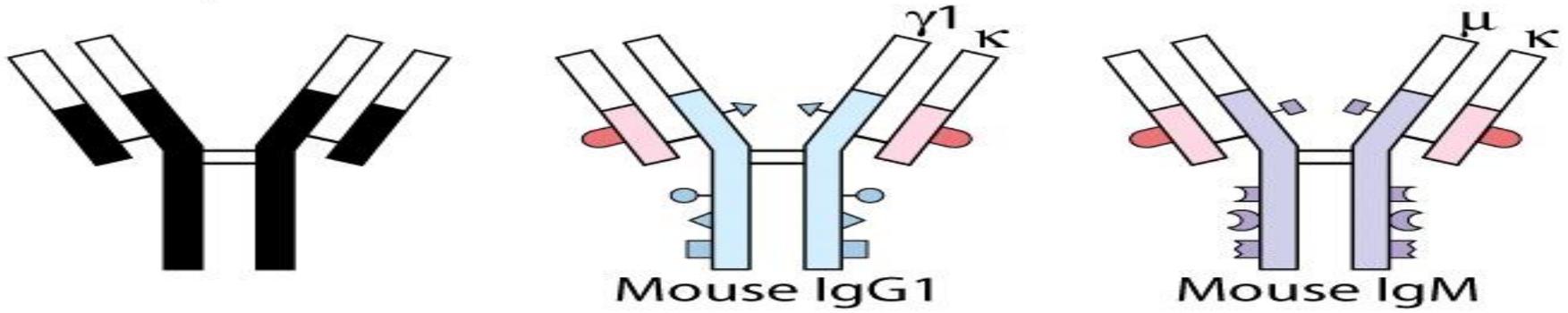
Idiotypic epitopes

(c) Idiotypic determinants

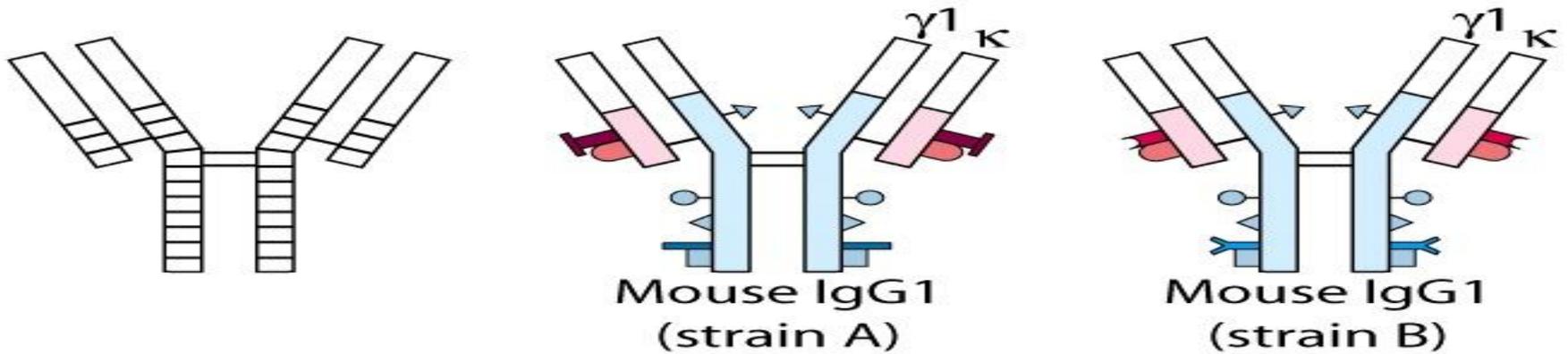


- Arise from aa seq of V regions of H + L chain
- Either at the CDR or at another location in V region
- Each Ab contains multiple idiotypes; collectively referred to as the idiotype of the Ab
- Plasma B cells produce Ab's all with the same idiotype

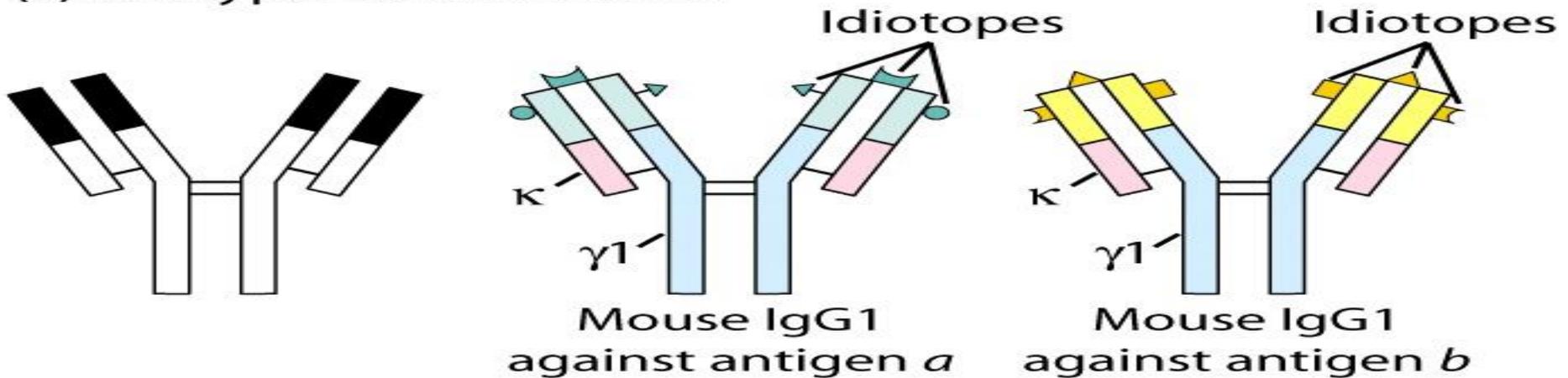
(a) Isotypic determinants



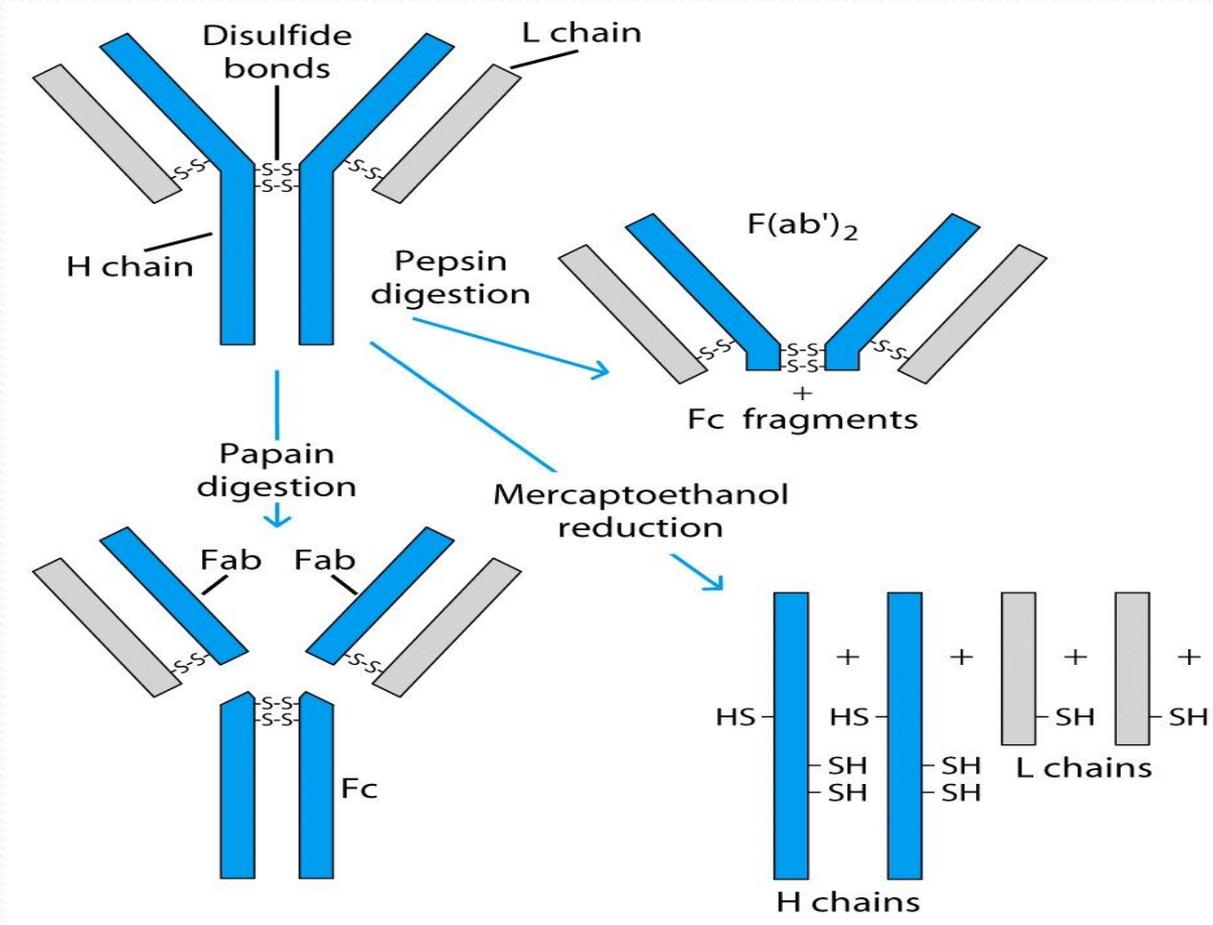
(b) Allotypic determinants



(c) Idiotypic determinants



Enzymatic Digestion Of Antibodies



- Digestion With Papain Yields
 - 3 Fragments
 - 2 identical Fab and 1 Fc
 - Fab Because Fragment That is Antigen Binding
 - Fc Because Found To Crystallize In Cold Storage
- Pepsin Digestion
 - $F(ab')_2$
 - No Fc Recovery, Digested Entirely
- Mercaptoethanol Reduction (Eliminates Disulfide Bonds).

Sequencing Of Heavy Chains

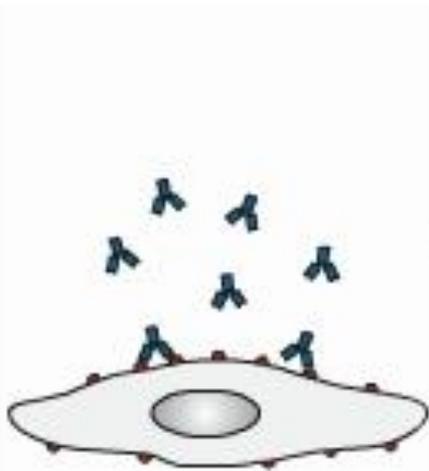
- Sequencing Of Several Immunoglobulins Revealed
 - 100-110 Amino Terminus, Highly Variable (V)
 - Five Basic Sequence Patterns
 - $\alpha, \gamma, \delta, \epsilon, \mu$
 - IgA, IgG, IgD, IgE and IgM
 - The Above Classes Are Called Isotype
 - Each class can have either κ or λ light chains
 - Minor Differences Led To Sub-classes For IgA and IgG
 - IgA₁, IgGA₂ and IgG₁, IgG₂, IgG₃, IgG₄

Immunoglobulin classes and biological activities

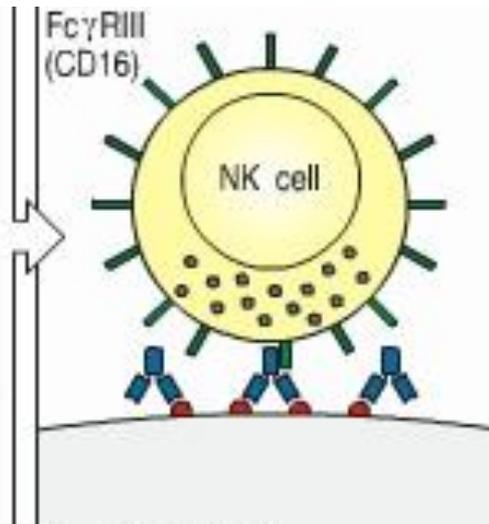
Structure	IgG	IgM	IgA	IgD	IgE
% of Total serum antibody	80%	5-10%	10-15%	0.2%	0.002%
Location	Blood, lymph, intestine	Blood, lymph, B-cell surface	Secretion-tears, saliva, mucus, milk	Blood, lymph, B-cell surface	Bound to mast and basophil cells
M.Wt.	150,000	970,000	405,000	175,000	190,000
Complement fixation	yes	yes	No	No	No
Placental transfer	yes	No	No	No	No
Known function	Enhance phagocytosis, neutralize toxin and virus, protect foetus and new born	First Ab produced in response to initial infection	Localized protection on mucosal surface	Serum function is not known	Allergic reaction

Antibody-Dependent Cellular Toxicity (ADCC)

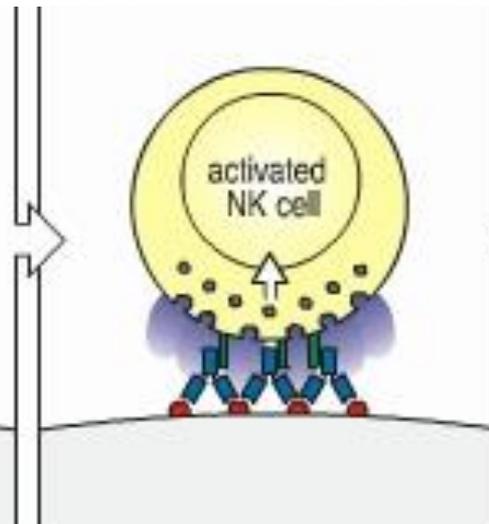
Antibody binds
antigens on the
surface of target cells



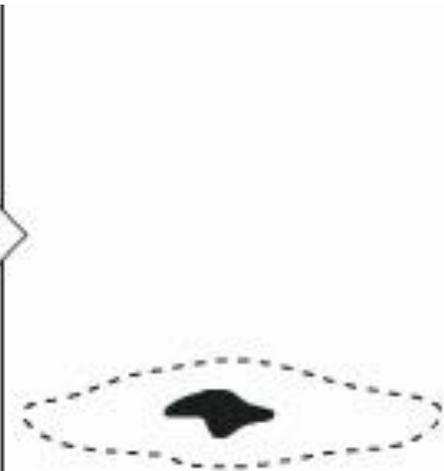
Fc receptors on
NK cells recognize
bound antibody



Cross-linking of Fc
receptors signals the
NK cell to kill
the target cell



Target cell dies by
apoptosis and
membrane damage



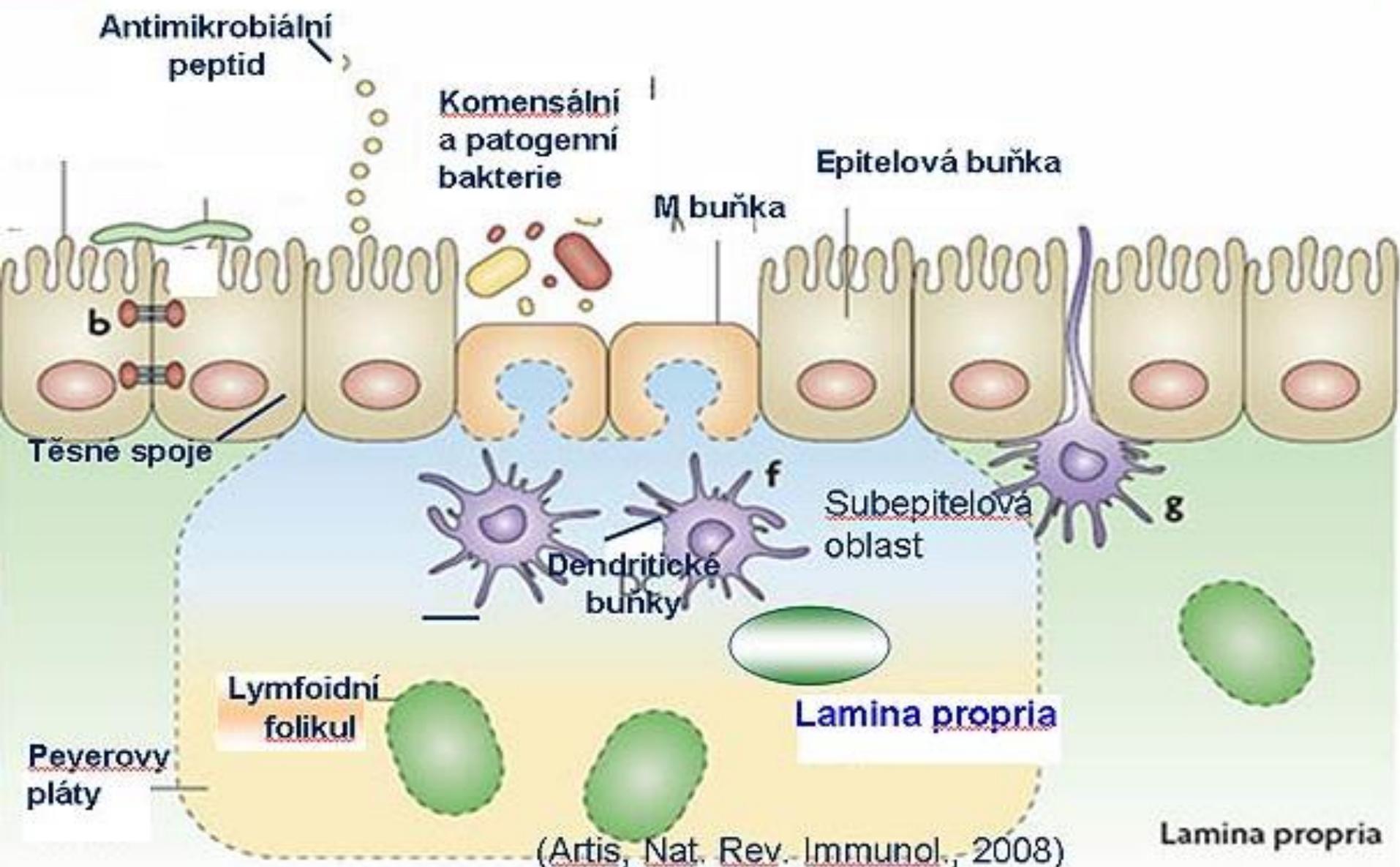
M cells

- are epithelial cells that are specialized for the transport antigen from the lumen of the respiratory, GIT, and urogenital tracts to the underlying MALT.
- contain a characteristic pocket filled with B cells, T cells, and macrophages
- are found at inductive sites that overlie organized lymphoid follicles in the lamina propria
- antigens are endocytosed and transported within vesicles from the luminal membrane to the pocket membrane, where the vesicles fuse and deliver their contents to antigen-presenting cells

Secretory IgA

- daily production of secretory IgA into mucus secretions exceeds that of any other class of immunoglobulin (5-15 g each day)
- is an important line of defense for mucosal surfaces against bacteria
- binding of secretory IgA to bacteria and viruses also prevents attachment to mucosal epithelial cells, thereby inhibiting infection and colonization

Slizniční bariera střeva



ANTIGENS

Lecture 6
Immunology

Definition

Antigen is defined as a substance that is recognized by the immune system:

- An organism, a molecule or part of molecule
- Simple, complex, protein, carbohydrate, or synthetic

Epitopes:

Are the antigenic determinants or the smallest part of the antigen recognized by lymphocytes

Antigens are divided into three functional sub types

1. **Immunogen:** A substance that induces a specific immune response.
2. **Hapten:** A substance that is non-immunogenic but can react with the products of a specific immune response. Haptens are small molecules which could never induce an immune response when administered by themselves

3. Tolerogen: A substance that produces immunological tolerance (a state of specific immunological unresponsiveness to subsequent challenging doses of that antigen)

IMMUNOGENICITY

The ability to elicit an immune response

FACTORS INFLUENCING IMMUNOGENICITY:

1. Foreignness:

The immune system normally discriminates between self and non-self (only foreign molecules are immunogenic)

2. Size: Proteins greater than 10 kDa are more immunogenic

3. Chemical Composition: the more complex the substance is chemically the more immunogenic it will be.

4. Degradability

Antigens that are easily phagocytosed are generally more immunogenic.

5. Method of Administration :Dose, route (SC is better than IV& oral)

Chemical nature of immunogens:

- 1. proteins: (glycoproteins or lipoproteins)**the vast majority of Immunogens are proteins
- 2. polysaccharides:** good immunogens
- 3. Nucleic acids:** usually poorly immunogenic, become immunogenic if single stranded or are complexed with proteins
- 4. Lipids:** in general are non-immunogenic but may be haptens

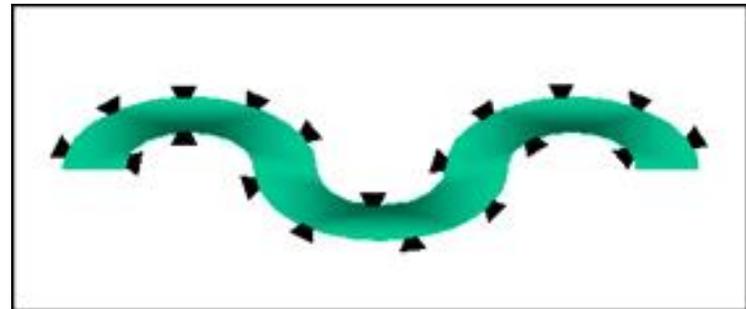
IV. TYPES OF ANTIGENS:

A. T-independent Antigens: are antigens which can directly stimulate the B cells to produce antibody without the requirement for T cell help e.g. polysaccharides

Properties of T-independent antigens:

1. Polymeric structure

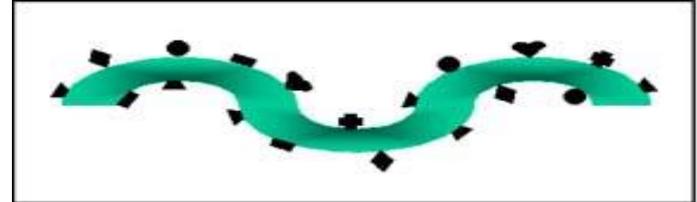
These antigens are characterized by the same antigenic determinant repeated many times.



2. Polyclonal activation of B cells: can activate B cell clones specific for other antigens (polyclonal activation).

3. Resistance to degradation

T-independent antigens are generally more resistant to degradation and thus they persist for longer periods of time and continue to stimulate the immune system.



B. T-dependent Antigens:

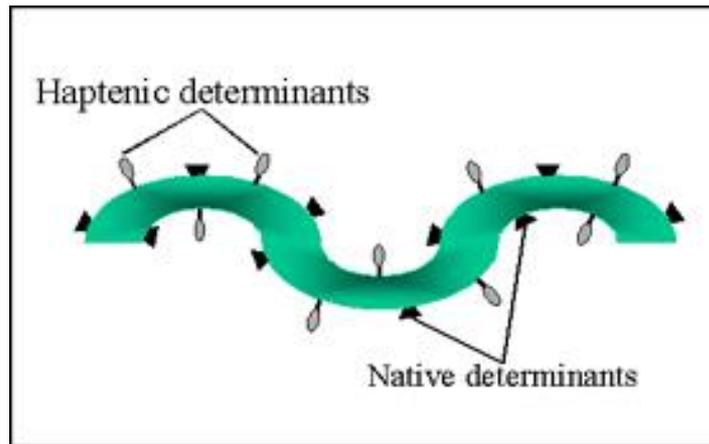
◆ T-dependent antigens are those that do not directly stimulate the production of antibody without the help of T cells (***Proteins are T-dependent antigens***)

◆ characterized by a few copies of many different antigenic determinants

HAPTEN-CARRIER CONJUGATES:

are immunogenic molecules to which haptens have been covalently attached

(The immunogenic molecule is called the carrier)



ANTIGENIC DETERMINANTS:

A. Determinants recognized by B cells

- ◆ are small and are limited to approximately 4-8 residues.
(amino acids and or sugars).

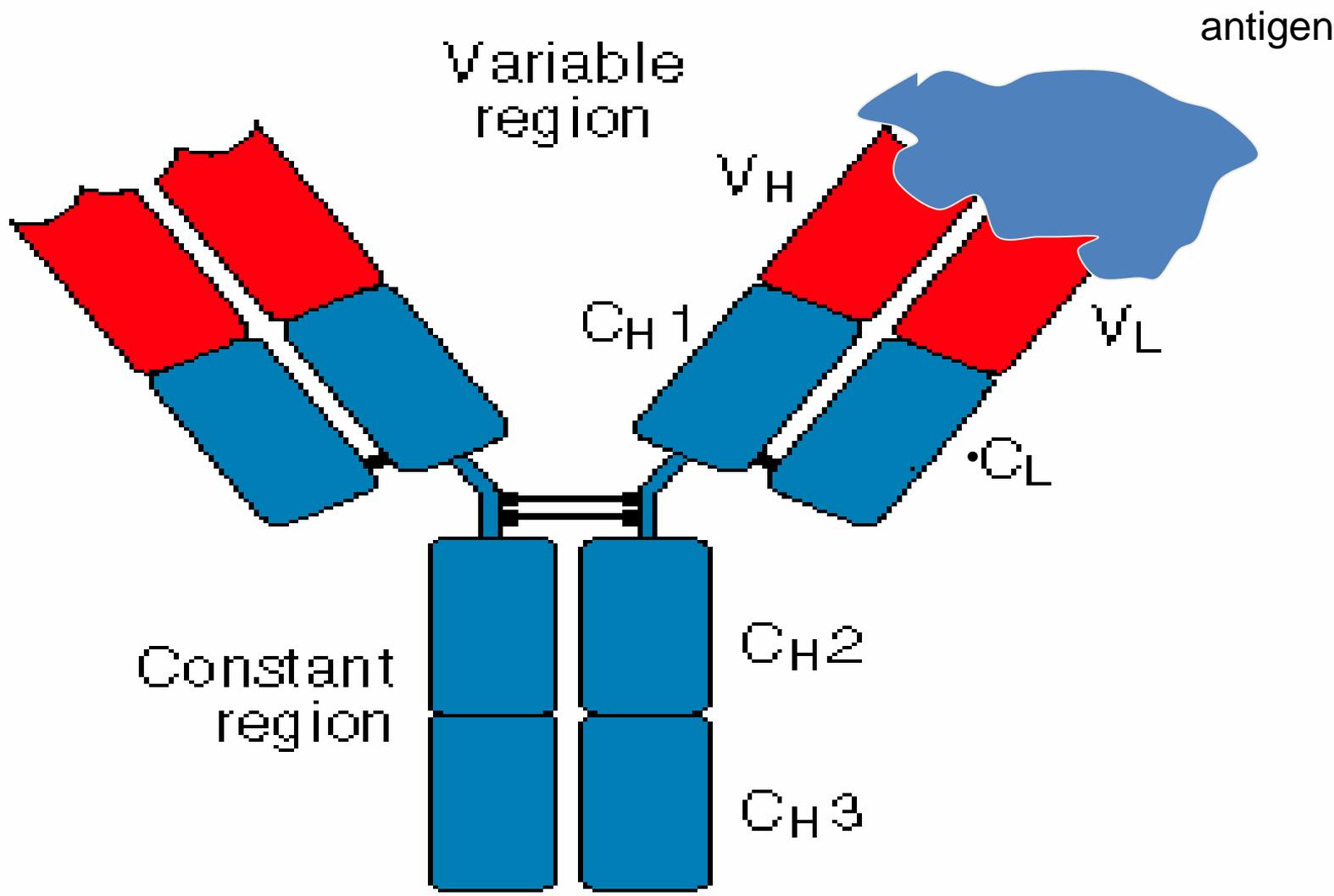
- ◆ are either:

Linear(primary sequence residues)

Conformational(secondary, tertiary or quaternary)

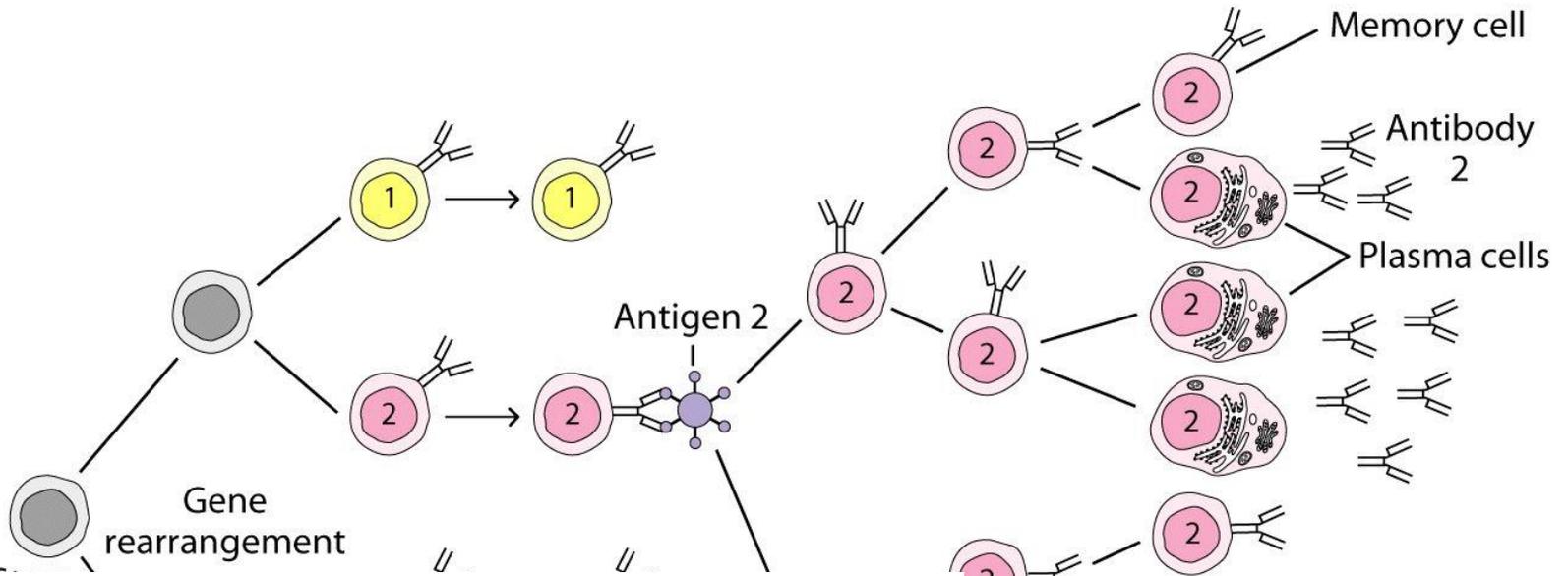
B. Determinants recognized by T cells:

- ◆ are limited to approximately 8-15 residues
- ◆ Only primary sequence residues

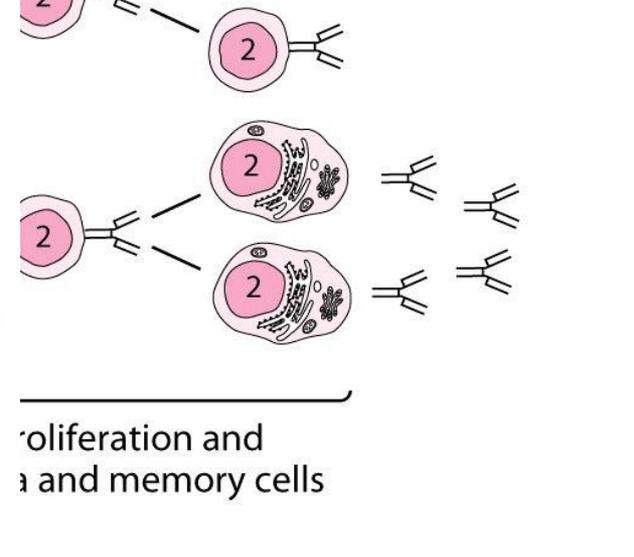
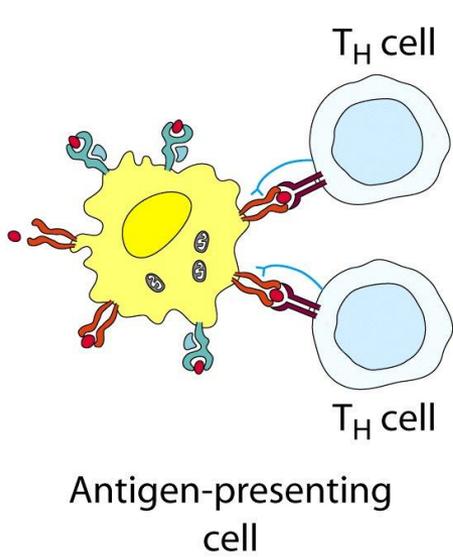
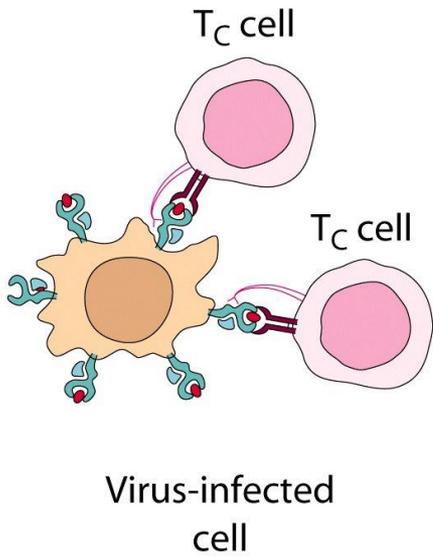


Bone marrow

Peripheral lymphoid tissue



- Antigenic peptide
- Class I MHC
- Class II MHC
- T cell receptor
- CD8
- CD4



Proliferation and differentiation into memory cells and plasma cells

The antigen receptor of B cells (antibody) • binds directly to antigen. Antibody exists in both a transmembrane receptor and secreted form.

The antigen receptor of T cells (TCR) • binds processed antigen (peptide) on the surface of an antigen presenting cell. TCR exists only as transmembrane form.

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

Kuby IMMUNOLOGY, Sixth Edition

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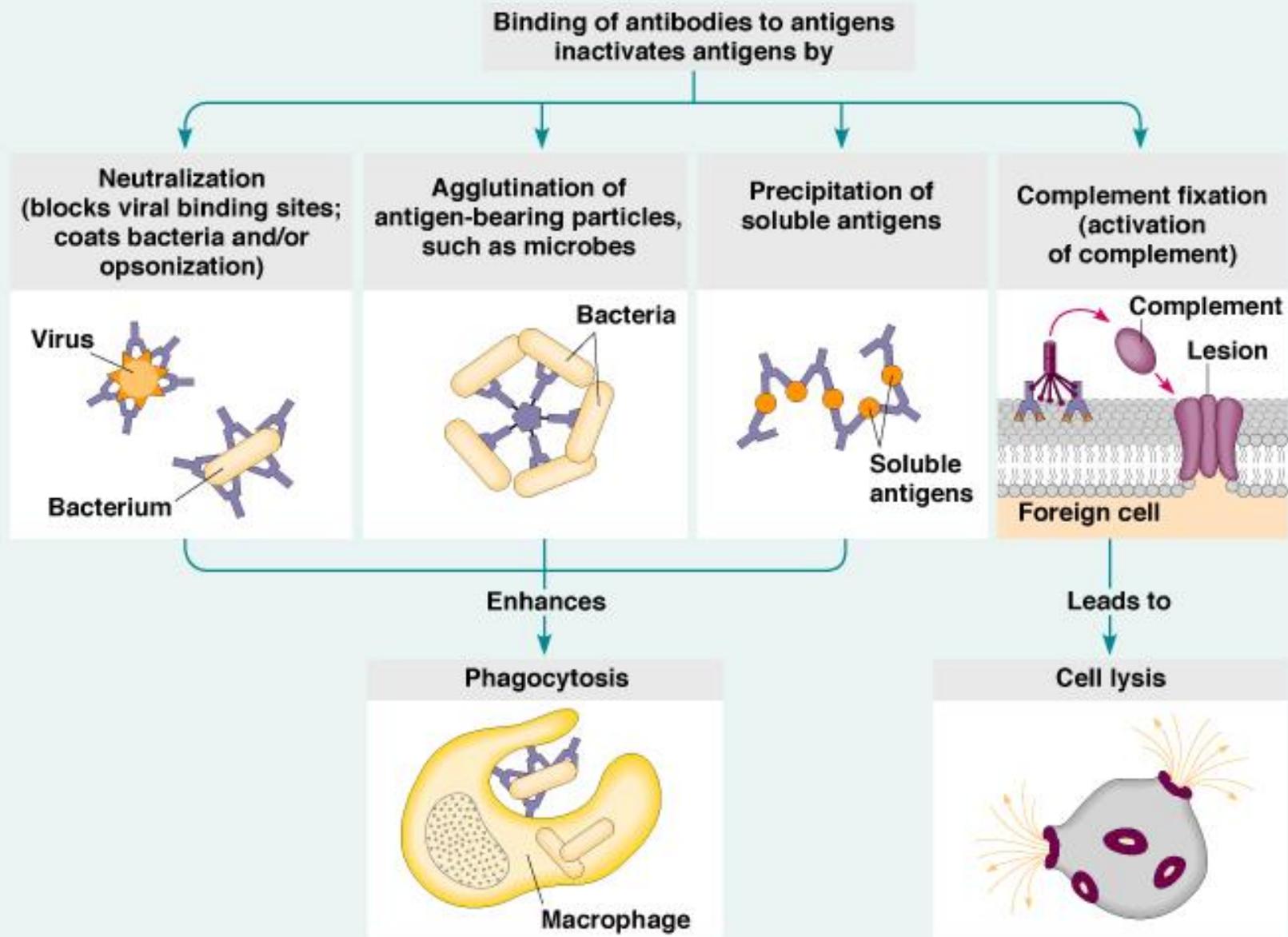
Antigen-antibody reactions

LECTURE 7

Types of Antigen-antibody reactions:

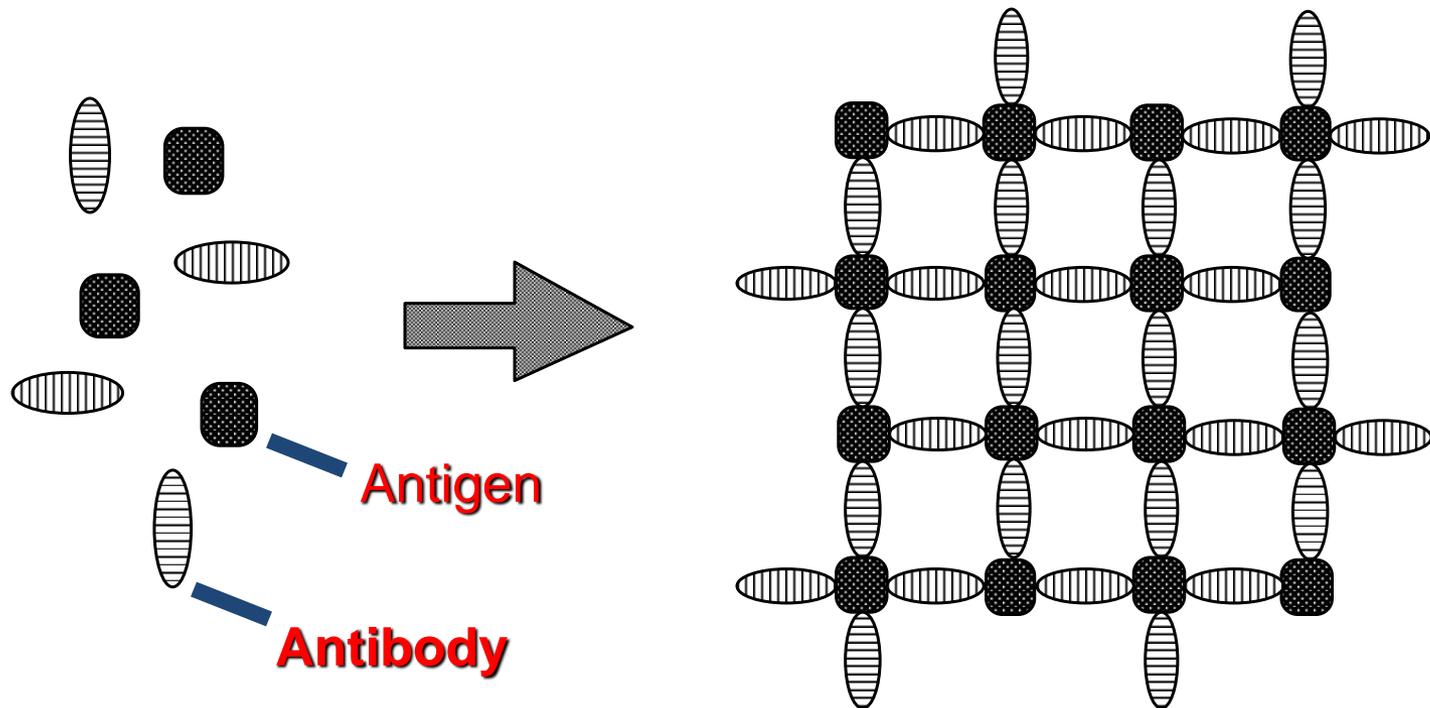
- **Precipitation**
- **Agglutination**
- **Neutralization (Antitoxins)**
- **Opsonization**
- **Antibody-dependant cell-mediated cytotoxicity**
- **The complement activation Membrane attack complex**

Consequences of Antibody Binding



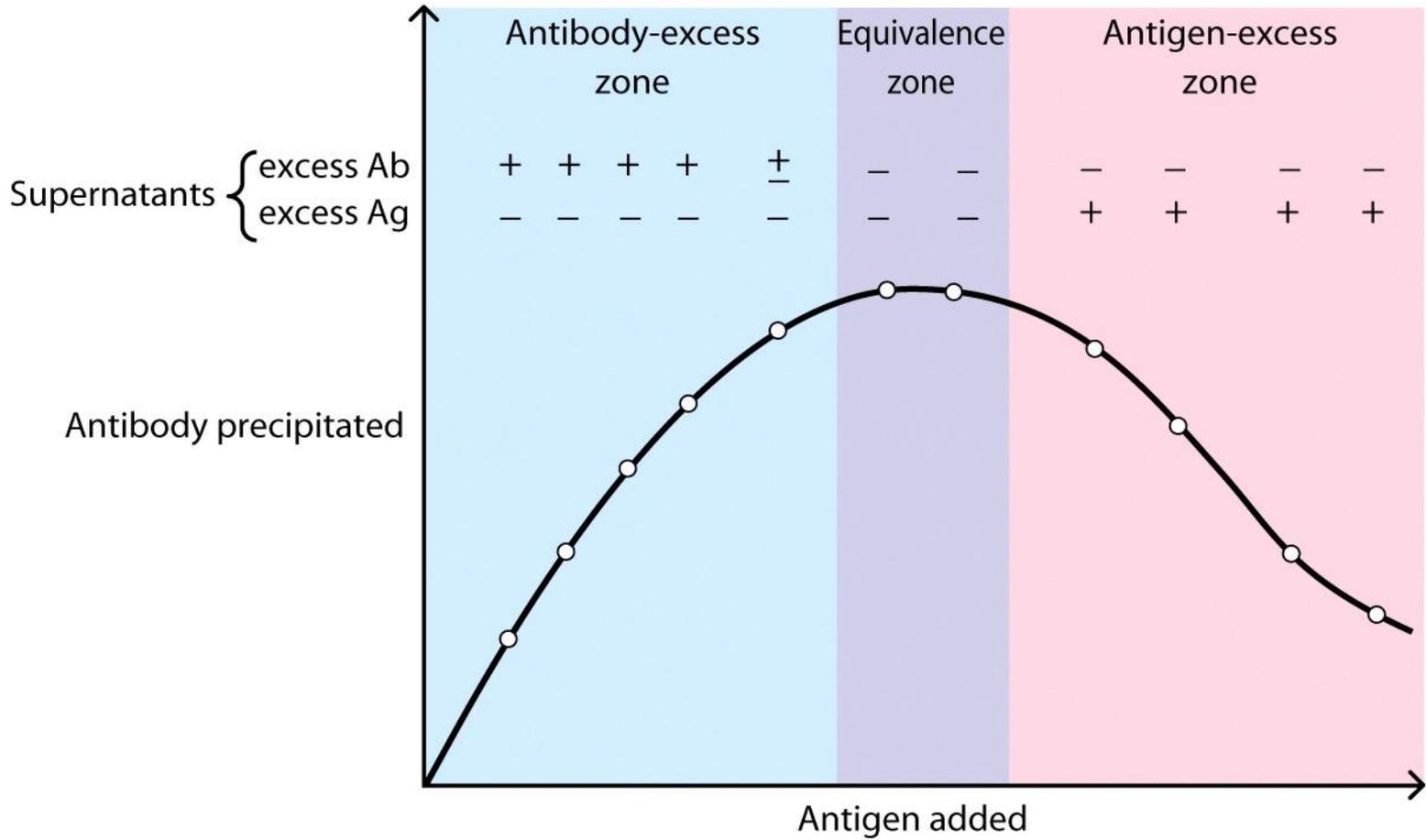
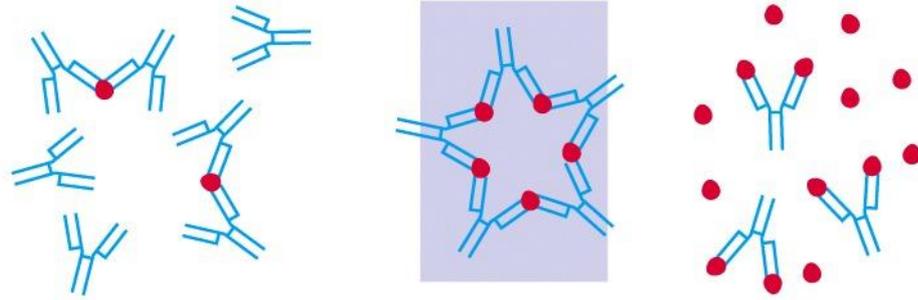
PRECIPITATION

- Is the reaction of soluble Ag with soluble Ab.
- The reaction results in the formation of Ag-Ab complexes (lattices)



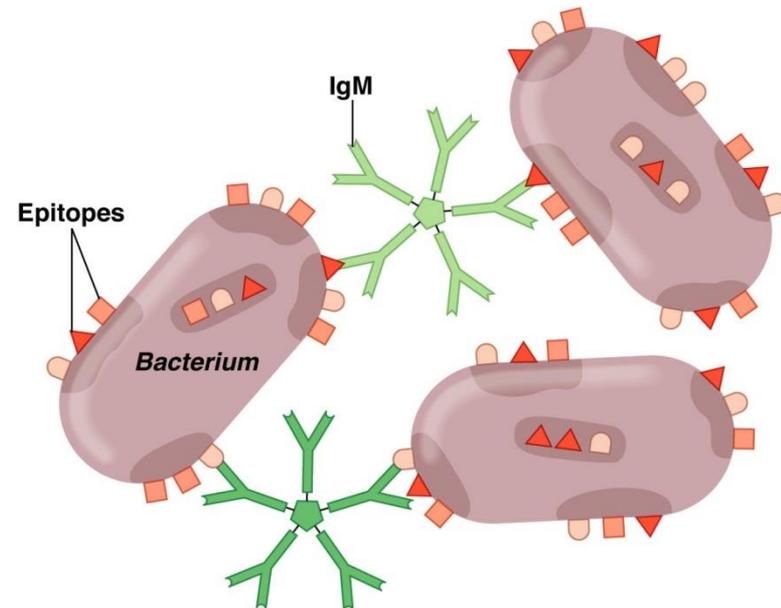
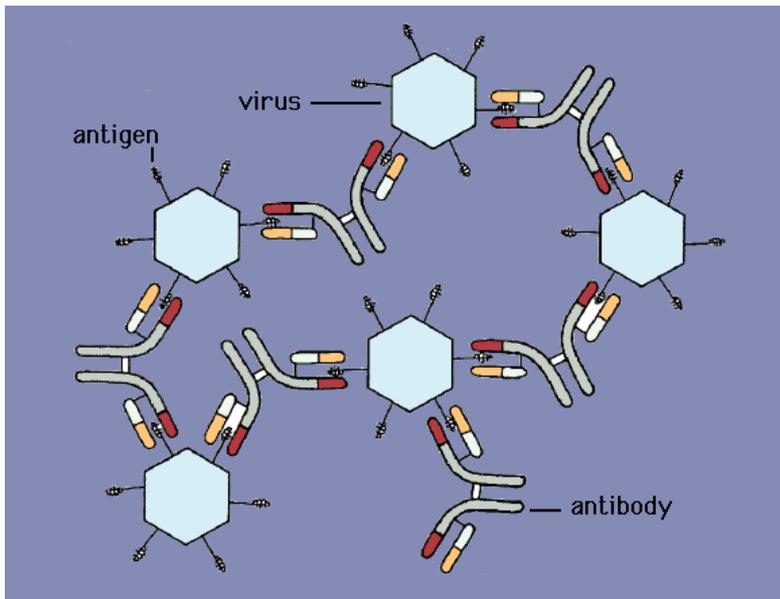
The Quantitative Precipitation Reaction:

- ❖ Varying amounts of Ag are mixed and incubated with Constant volume of antisera
- ❖ Precipitate is measured, amount of precipitate depends on :
 - the ratio of Ag : Ab
 - The Ab avidity
- ❖ Plot in a curve, three zones are detected:
 - i. Zone of Ag excess : insufficient Ab → too small complexes to precipitate
 - ii. Equivalence zone : large lattice is formed → visible precipitates
 - iii. Zone of Ab excess : not enough Ag → too small complexes to precipitate

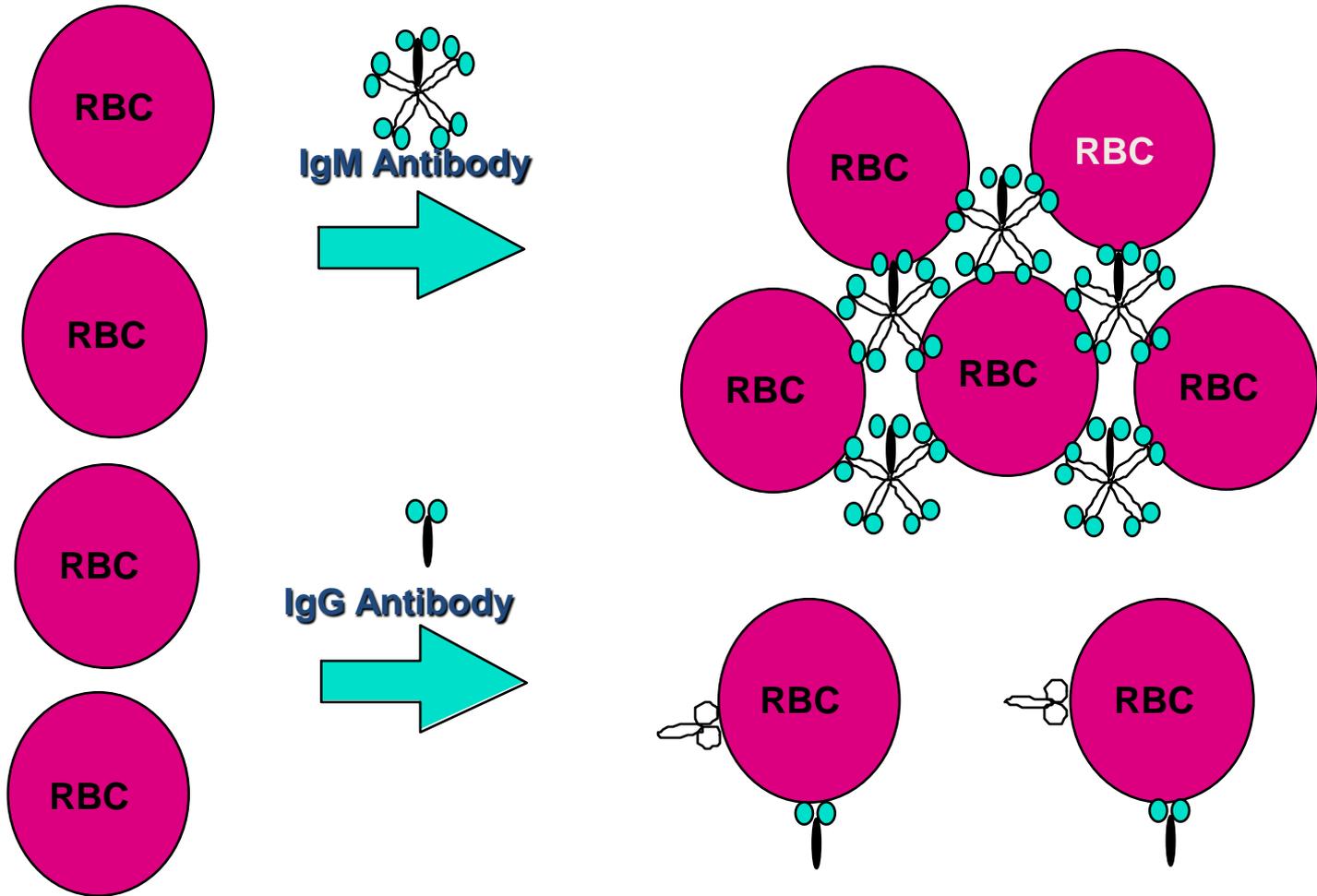


AGGLUTINATION

- ❖ Abs can bind and cross-link cells or particles ⇒ aggregate formation
- ❖ Entrap microbial invaders
- ❖ IgM & IgA are the most suitable (*IgG in sufficient amounts can agglutinate cells*)



Agglutination



Applications of Agglutination

1. Agglutination/Hemagglutination:

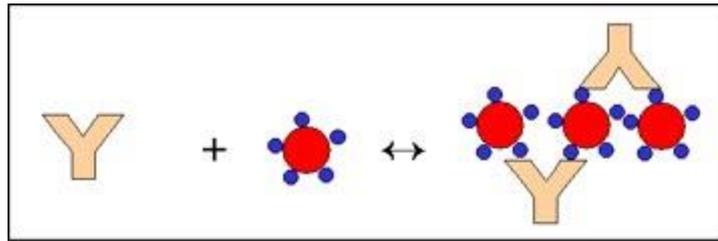
a. Qualitative agglutination test

Determination of blood types or antibodies to blood group Ags

b. Quantitative agglutination test

Agglutination tests can also be used to measure the level of antibodies to particulate antigens. (*titration*)

2. Passive hemagglutination: erythrocytes are coated with a soluble antigen (e.g. viral antigen, a polysaccharide or a hapten) and use the coated red blood cells in an agglutination test for antibody to the soluble antigen



3. Coomb's Test (Antiglobulin Test)

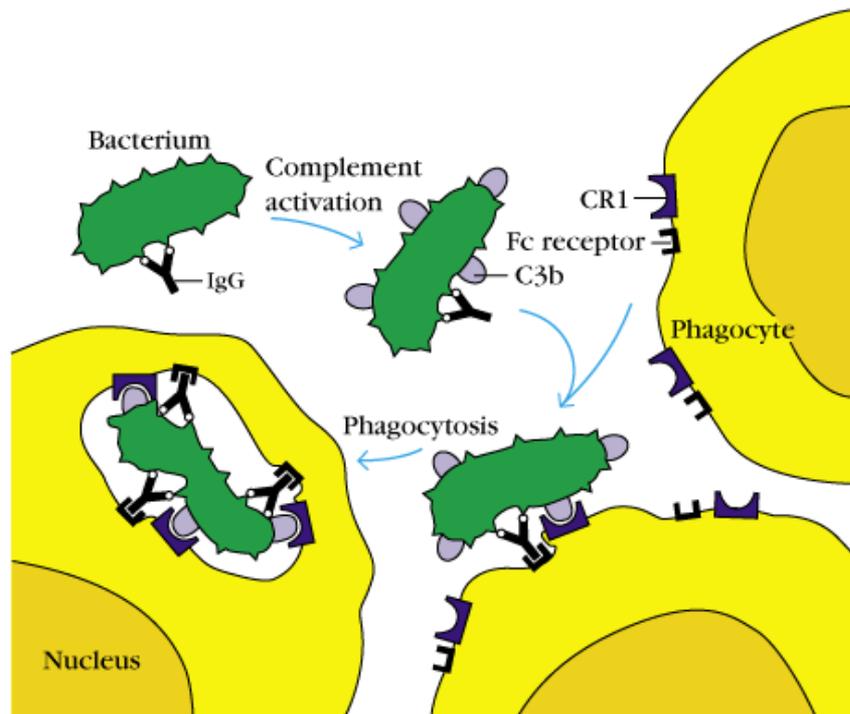
NEUTRALIZATION

- ◆ Is the binding of Ab to microbial epitopes or soluble molecules(e.g. toxins) which inhibits their binding to host cells.
- ◆ Abs are mostly IgG & IgA
- ◆ Used to identify toxins and viruses

OPSONIZATION

Is the process by which a pathogen is marked (tagged) for ingestion and destruction by phagocytic cells

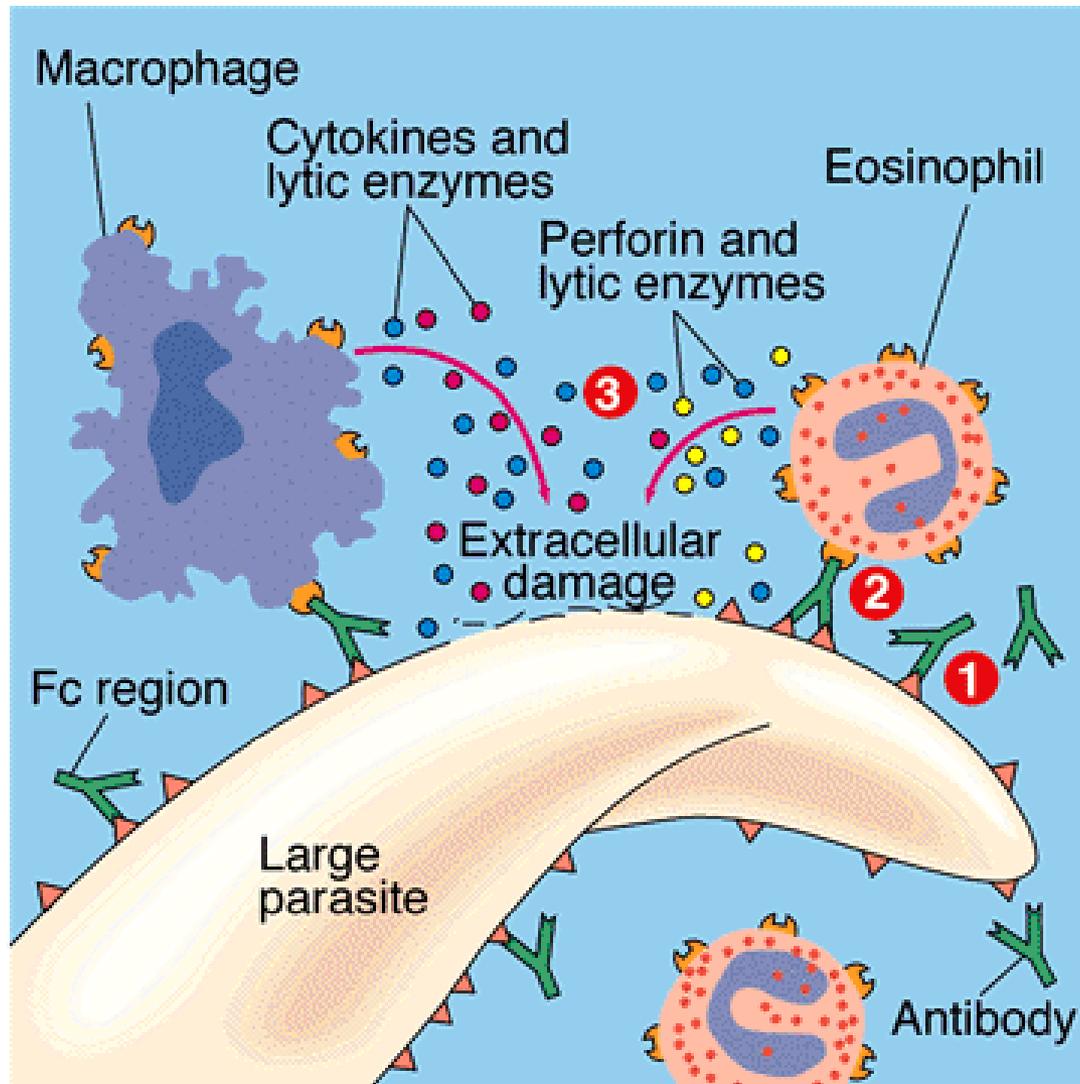
(a)



Antibody-dependant cell-mediated cytotoxicity

- Coating of an organism can attract phagocytic cells as well as *other cytolytic cells(NK cells, eosinophils)*
- The organism may be: bacteria, protozoa, parasitic worms
- These cells use cytolytic mechanisms to kill those organisms

Destruction of Large Parasites by ADCC



Primary and Secondary Immune Response

Lecture 8, 9

THE INNATE IMMUNE RESPONSE

Mediated (initiated) by phagocytes, NK cells and soluble proteins

Phagocytes

Cells specialized in the process of phagocytosis

Macrophages

Reside in tissues and recruit neutrophils

Neutrophils

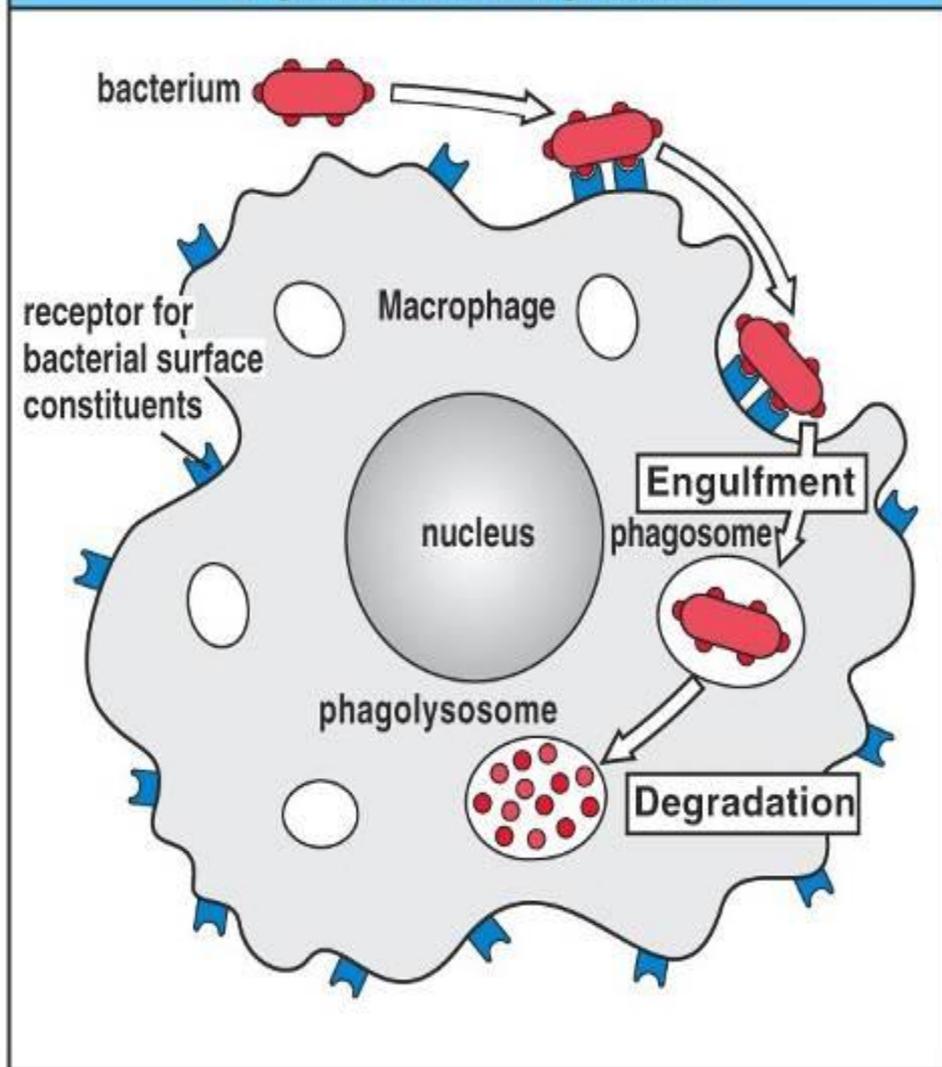
Enter infected tissues in large numbers

Recognize common molecules of bacterial cell surface using a few surface receptors

Phagocytosis

Capture, engulfment and breakdown of bacterial pathogen

Bacteria binding to endocytic receptors of macrophages induce their engulfment and degradation



Bacterial components binding to signaling receptors of macrophages induce the synthesis of inflammatory cytokines

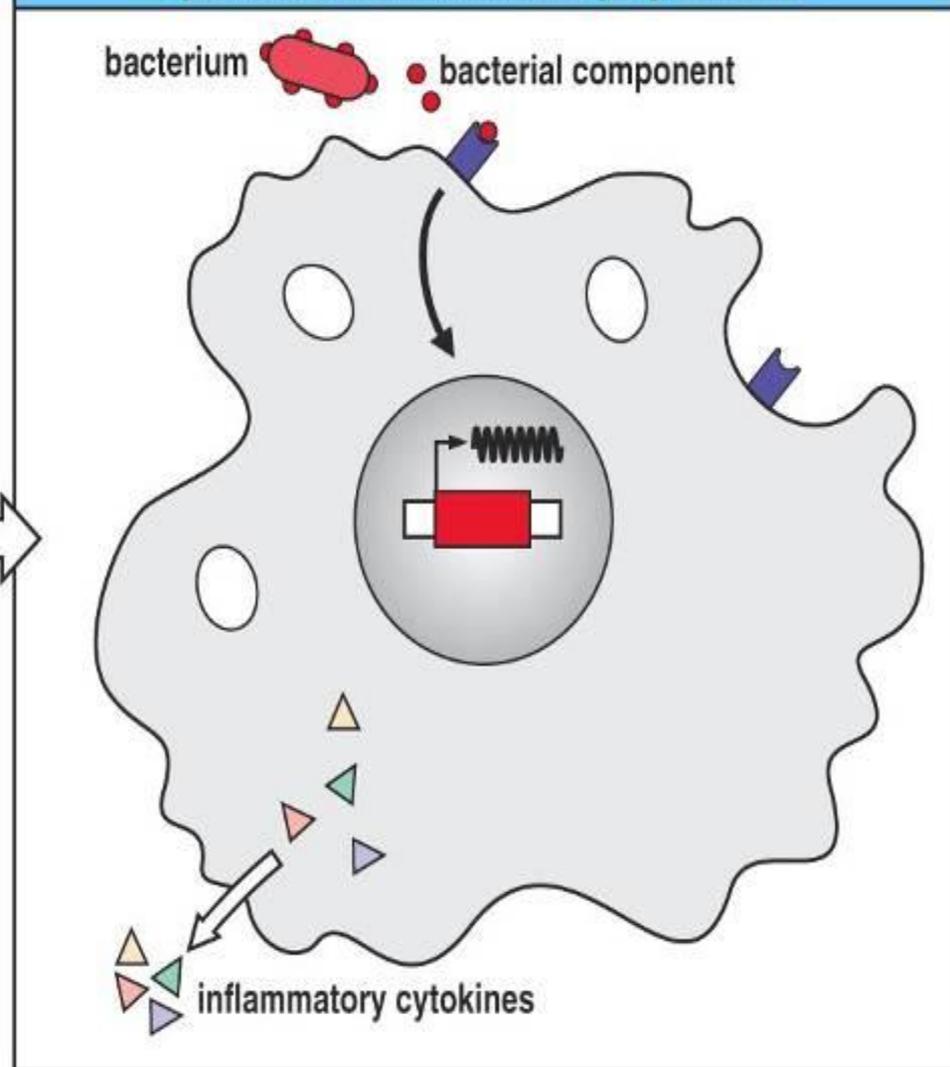


Figure 1-14 The Immune System, 2/e (© Garland Science 2005)

THE INNATE IMMUNE RESPONSE

Inflammatory response enhances phagocytosis through acute phase proteins

Mannose-binding lectin (MBL)

Binds to bacterial surface with particular spatial arrangement of mannose or fucose

C-reactive protein (CRP)

Binds to phosphorylcholine on bacterial surface

Complement

Set of proteins which bind to bacterial surface

Inflammatory response

Accumulation of fluid and cells at infection site (swelling, redness, heat and pain)

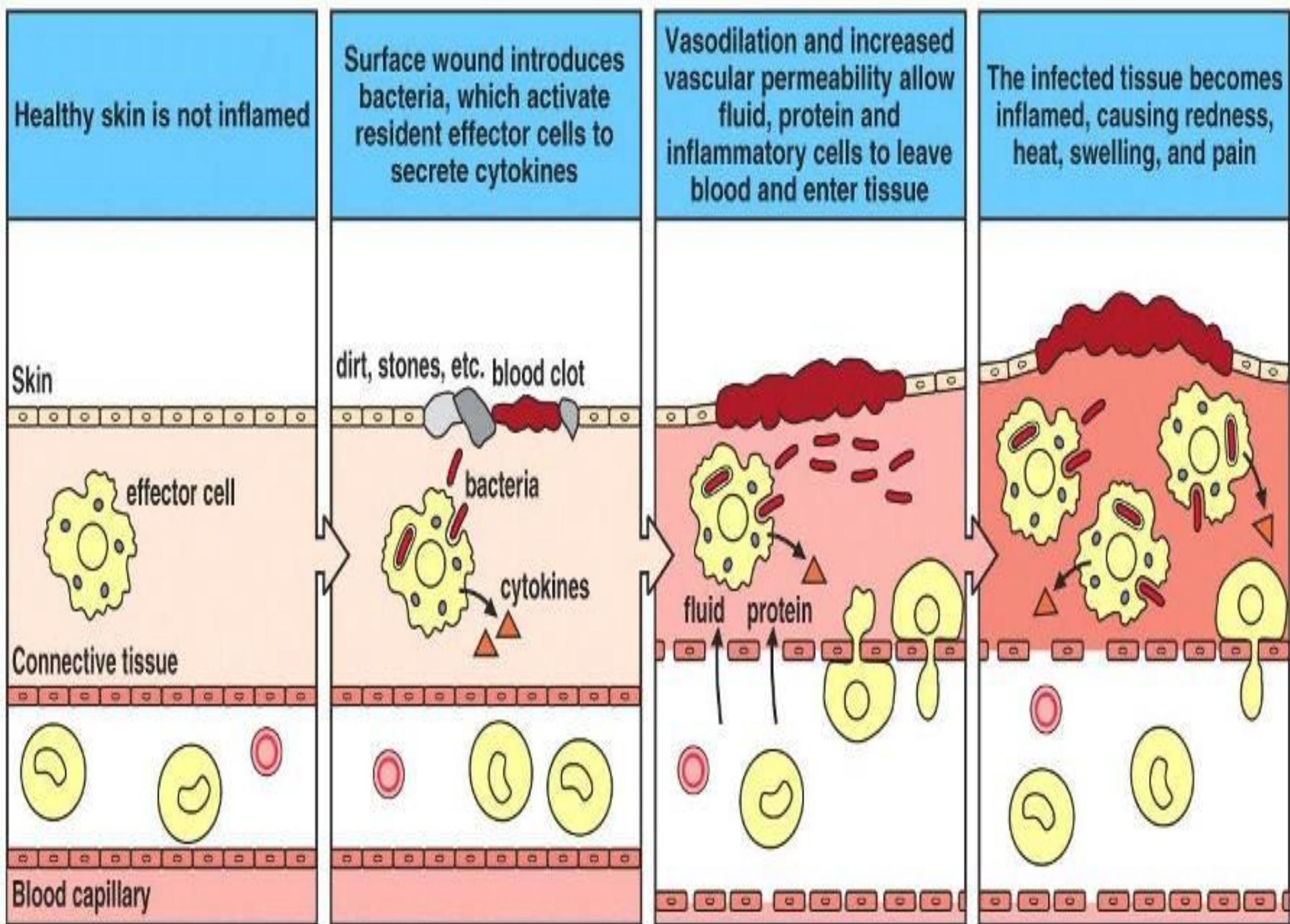


Figure 1-6 The Immune System, 2/e © Garland Science 2005)

THE ADAPTIVE IMMUNE RESPONSE

Creates millions of different B and T cells for specific antibody-mediated and cell-mediated immunity

Antibody-Mediated Immunity (AMI)

Involves B lymphocytes, plasma cells and antibodies

Humoral immunity

Name derives from antibodies found in body fluids (humors - old medical term)

Cell-Mediated Immunity (CMI)

Involves T lymphocytes, antigen-presenting cells and MHC (major histocompatibility complex) molecules

Cellular immunity

ANTIBODY-MEDIATED (HUMORAL) IMMUNITY

Directed against extracellular microorganisms and toxins

B-lymphocytes (B cells)

Differentiate into plasma cells which produce antibodies

Function as antigen-presenting cells (APC's)

Classification of Antibodies (Immunoglobulins)

Immunoglobulin M (IgM)

Immunoglobulin G (IgG)

Immunoglobulin A (IgA)

Immunoglobulin D (IgD)

Immunoglobulin E (IgE)

CELL-MEDIATED IMMUNITY (CMI)

Directed against intracellular microorganisms
Non-phagocytic cells and phagocytic cells

T-lymphocytes (T cells)

Differentiate into effector cells following antigen presentation by antigen presenting cells (APC's)

Functional types of T cells

Helper (CD4 T cells)

TH1 and TH2 cells

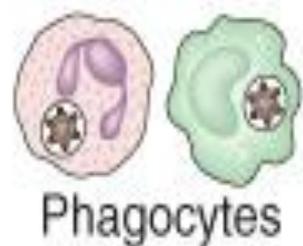
Cytotoxic (CD8 T cells)

Regulatory

CD4 and CD8 Tregs •

Microbe

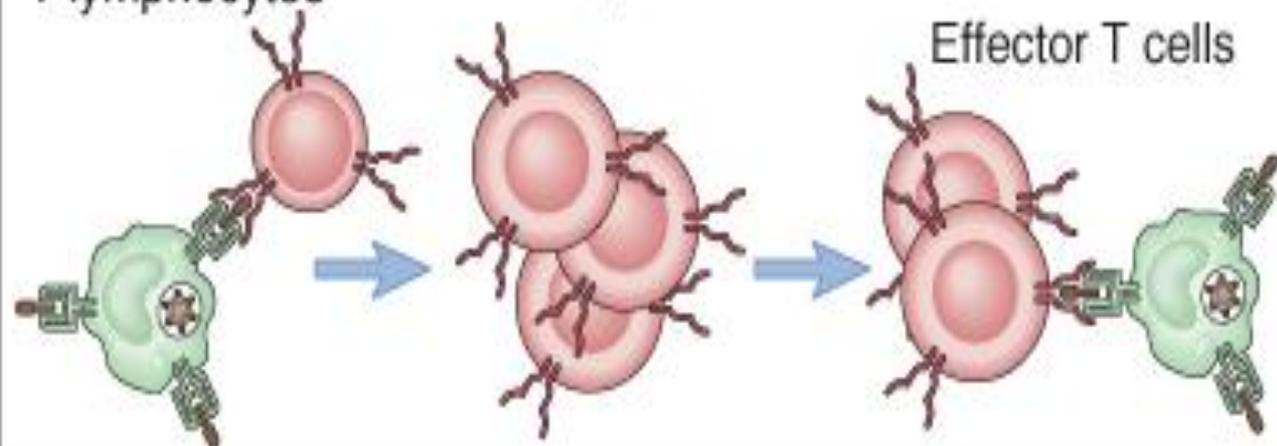
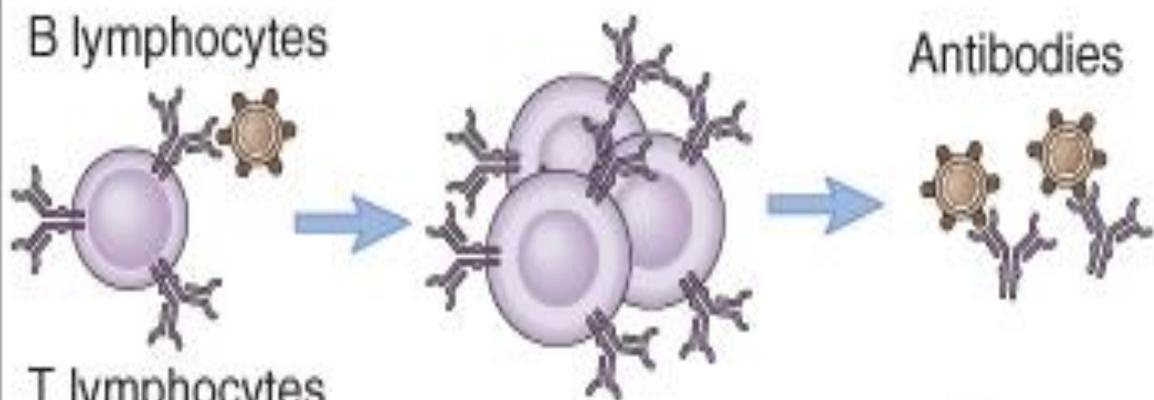
Innate immunity



Hours

0 6 12

Adaptive immunity



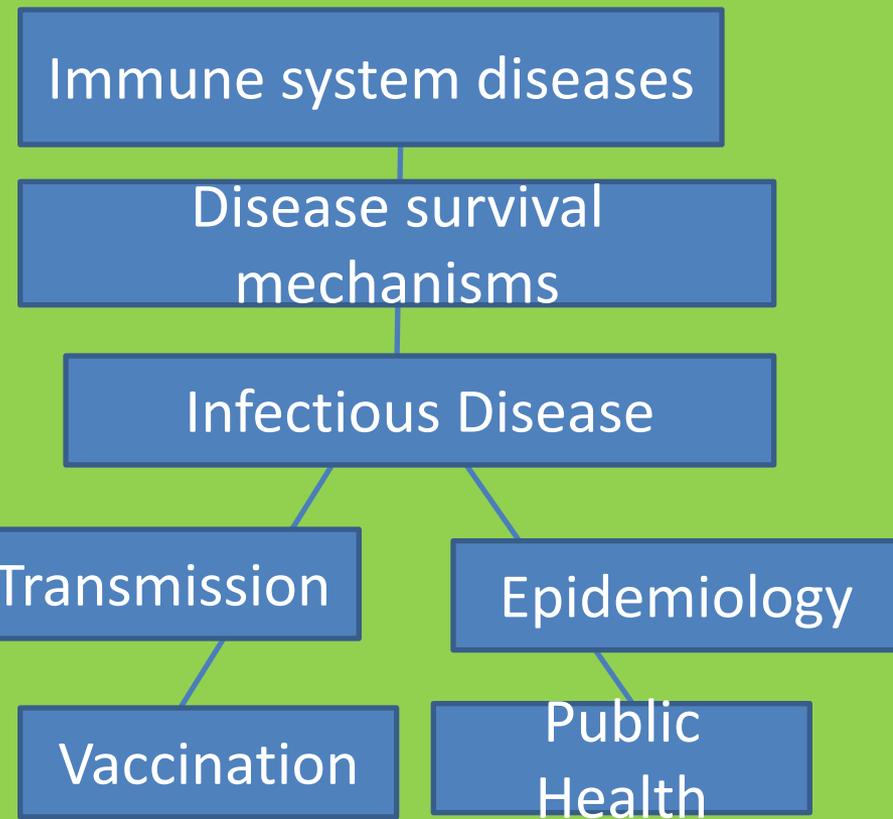
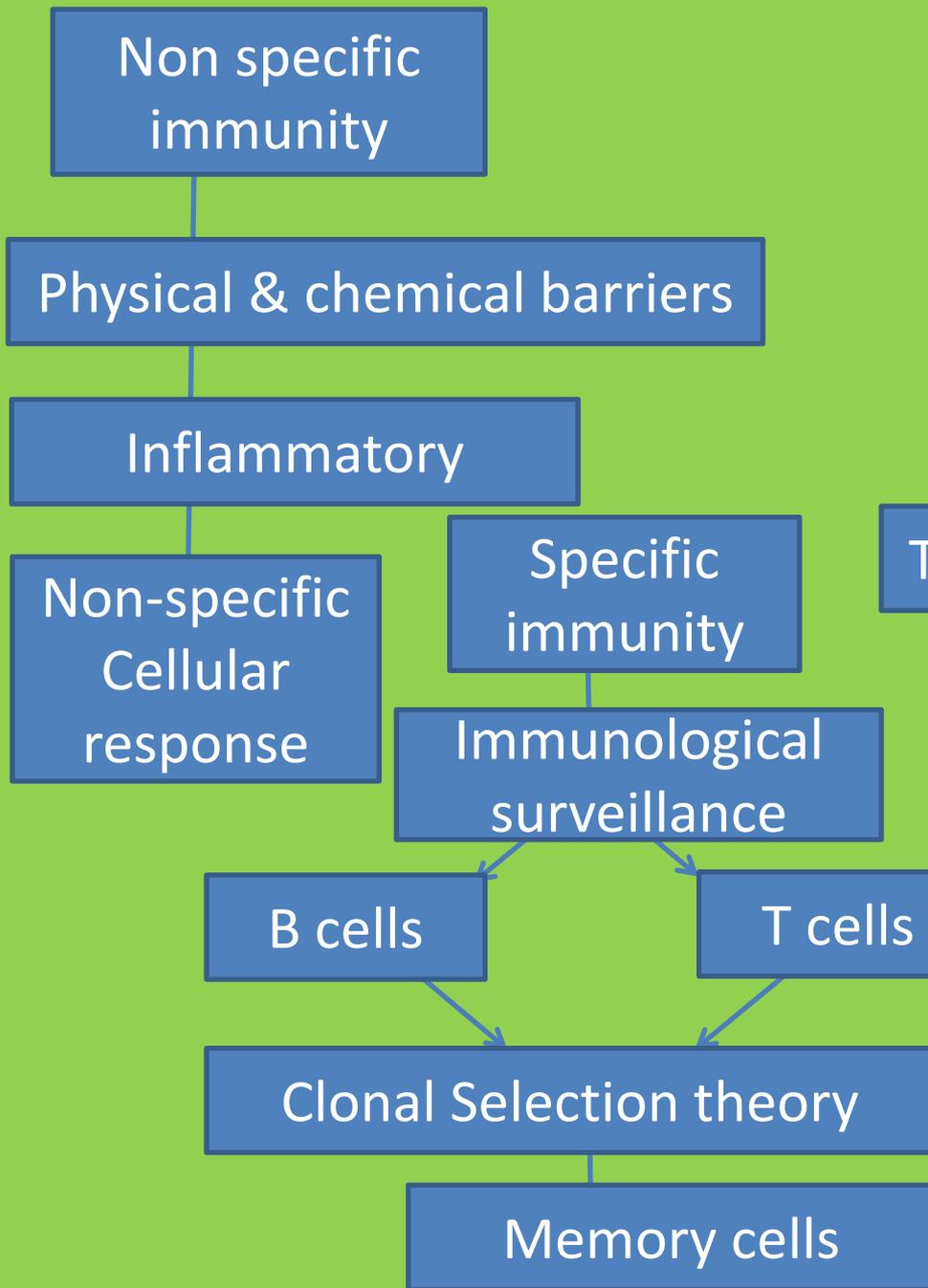
Days

1 3 5
Time after infection →

CELL comparison

STARTER

Cell	Natural Killer Cell	B Lymphocyte	T Lymphocyte
Part of immune system	Non-specific	Specific made in bone marrow	Specific made in thymus
Types? / Subspecies?	N/A	Activated plasma cell or memory B cell	Cytotoxic or helper T cells
Works on	Virus/ cancer cells	External pathogen	Virus/ cancer cells Support other cells
Mode of action?	Perforins, signal molecules switch on genes which make degradative enzymes triggering cell apoptosis	Clonal selection of 1 specific antibody receptor, proliferate and make antibodies, trap/neutralise for phagocytes or cause cell lysis. Memory cells remain for subsequent attack.	Clonal selection, helper T cells produce cytokines for other cells to be activated. Killer cells see antigen, then clonally selected and can induce apoptosis. Memory cells remain for subsequent attack.



BIG PICTURE

Antigen (1st exposure)

Free antigens
directly activate

Engulfed by

Antigens displayed by
infected cells activate

Macrophage

Stimulates

Stimulates

Stimulates

B cell

Helper T cell

Suppressor T cell

Gives rise to

Gives rise to

Memory helper T cell

Stimulates

Stimulates

**Antigen
(2nd exposure)**

Plasma cells

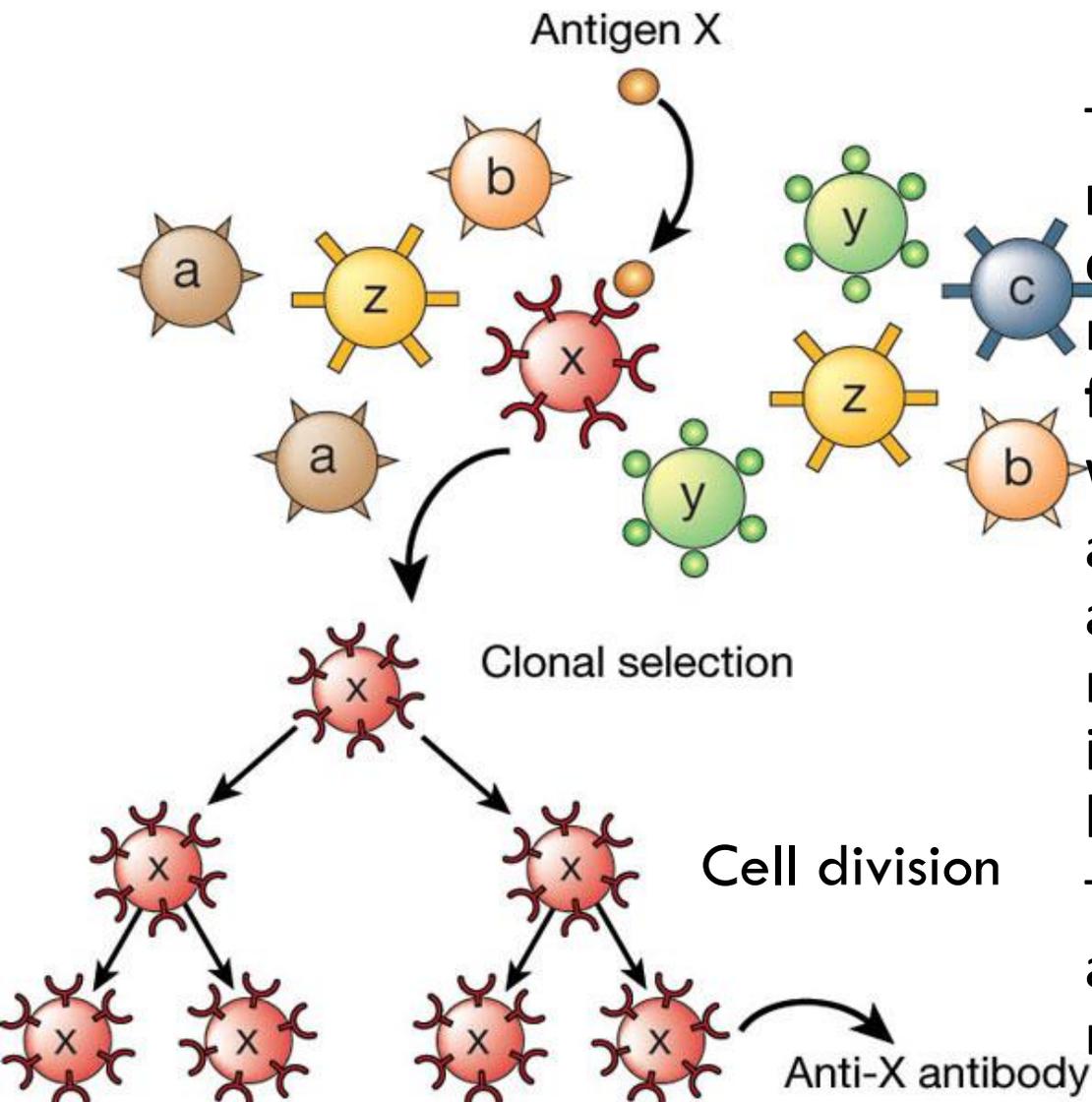
Memory B cells

Memory T cells

Active cytotoxic T cells

Antibodies

Clonal selection theory

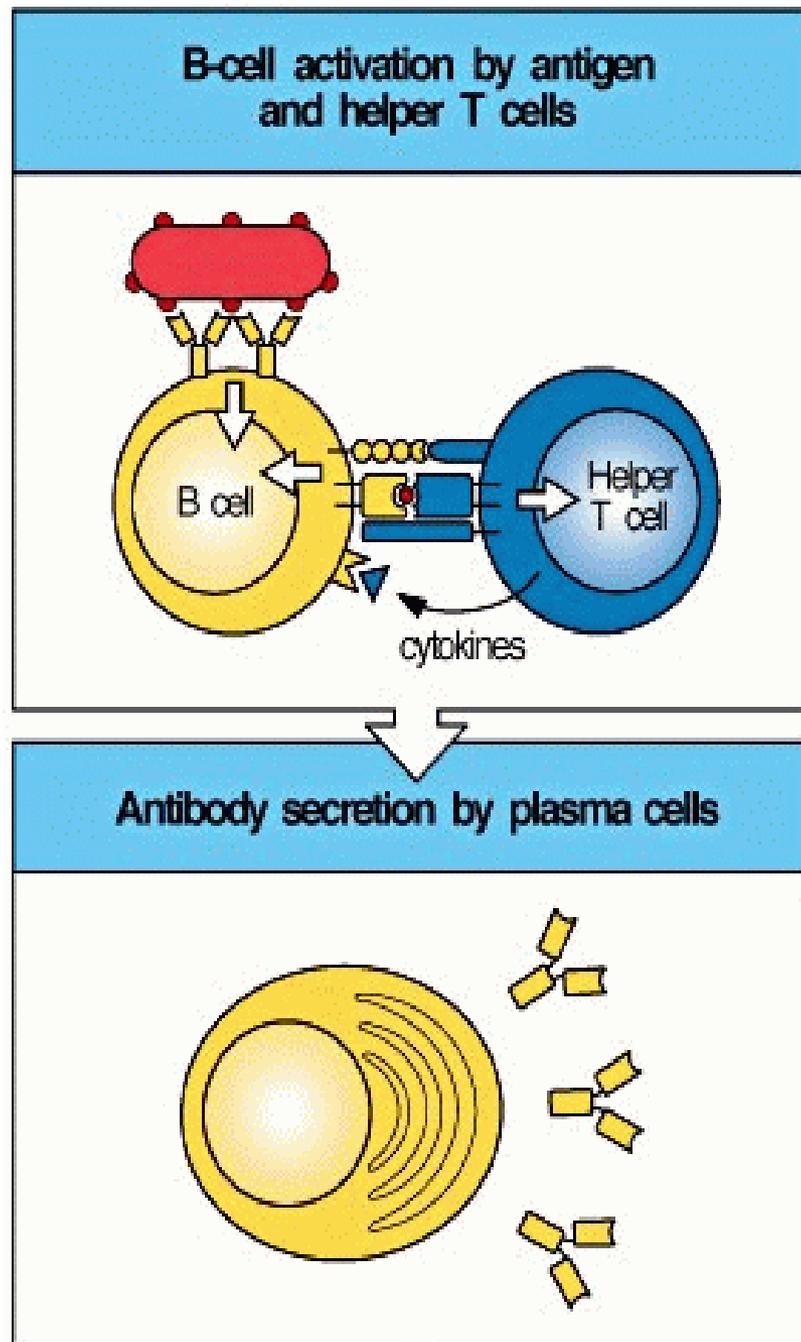


- The body has a **large number of lymphocytes** each with a **single type** of membrane receptor **specific for one antigen**.

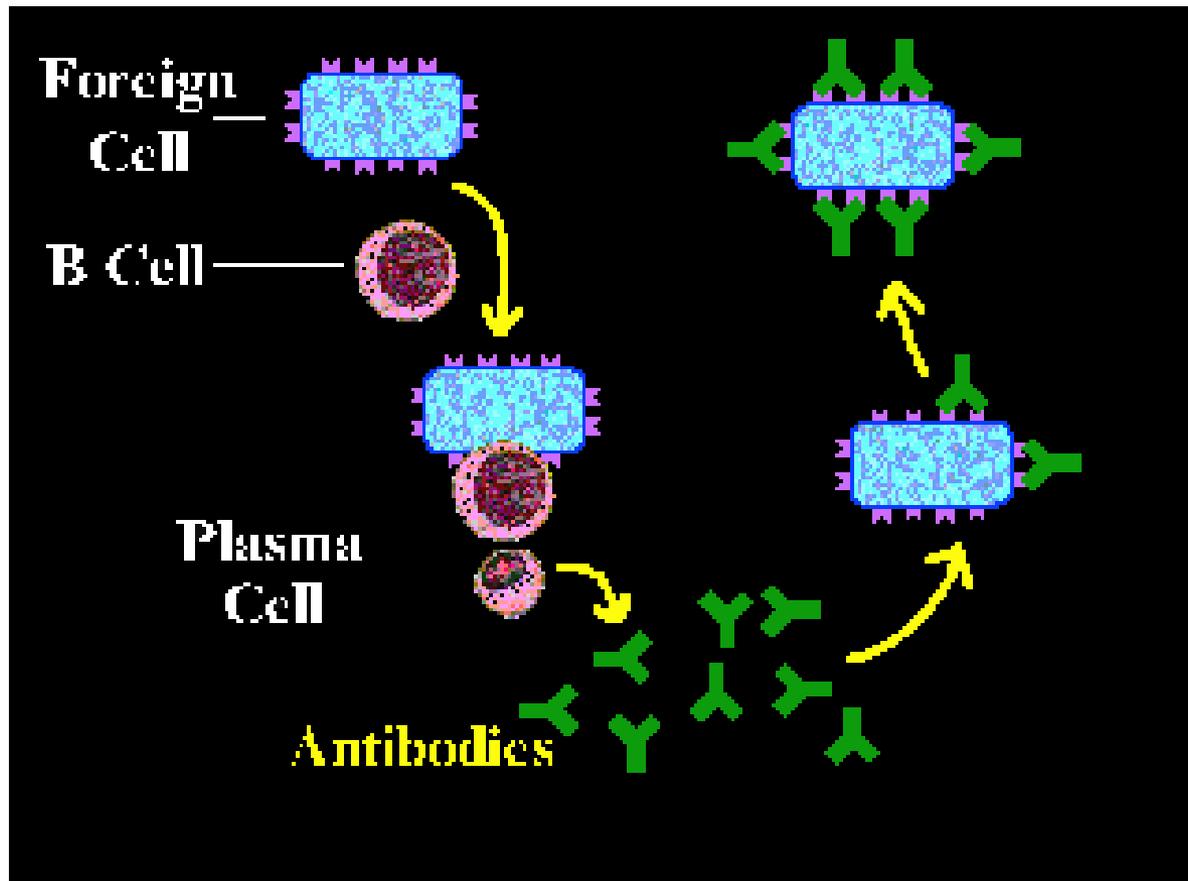
- When a receptor is activated by the binding of an antigen, the lymphocyte repeatedly divides resulting in a clonal population of lymphocytes.

- To become fully activated – antigen presenting cells are needed

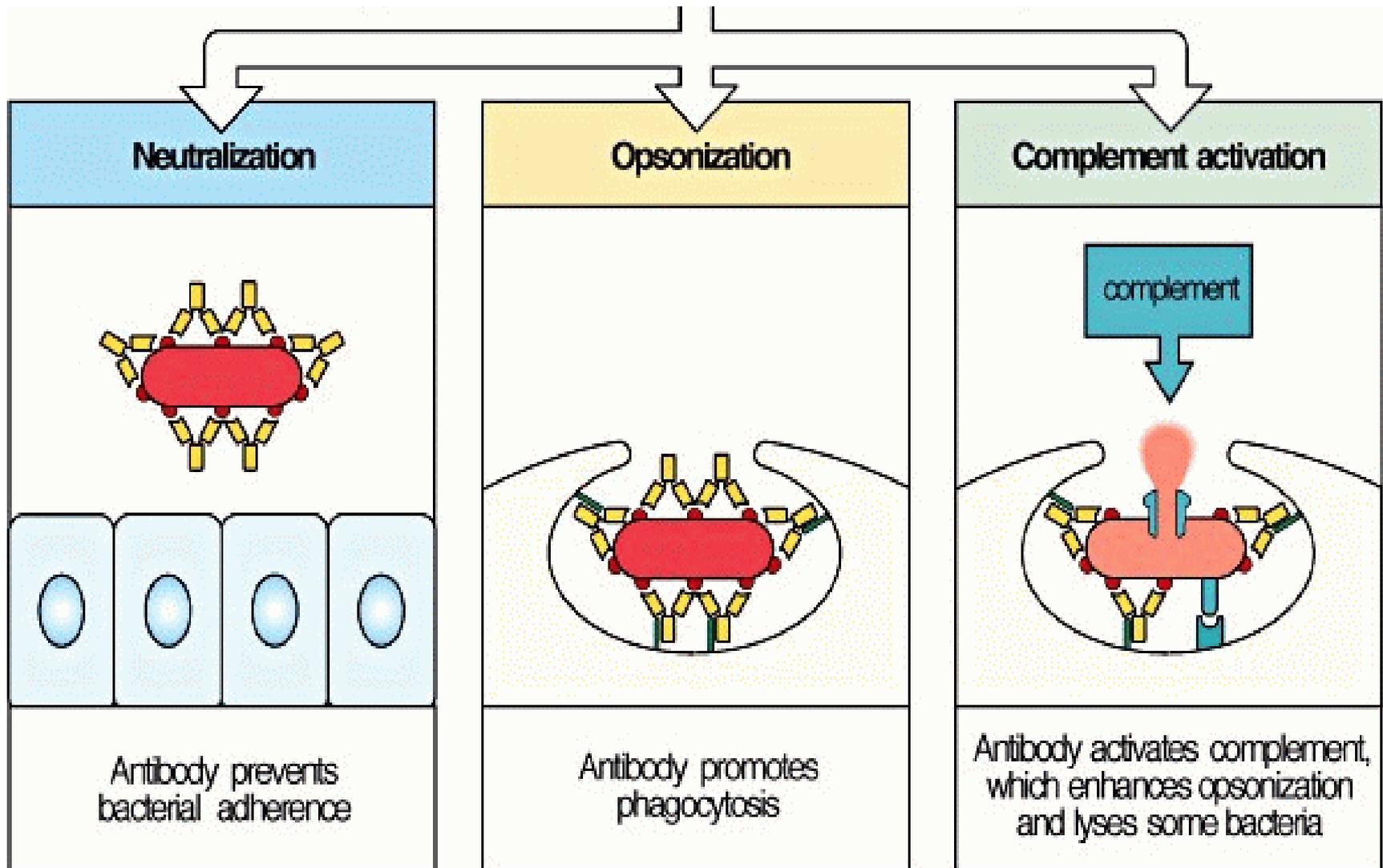
The Antibody Response



Humoral Immune Response



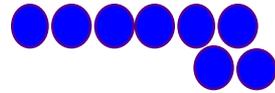
Antibody Protection of the Host



Immunologic

Memory

Virgin lymphocyte pool



PRIMARY RESPONSE

effector cells



memory cell pool

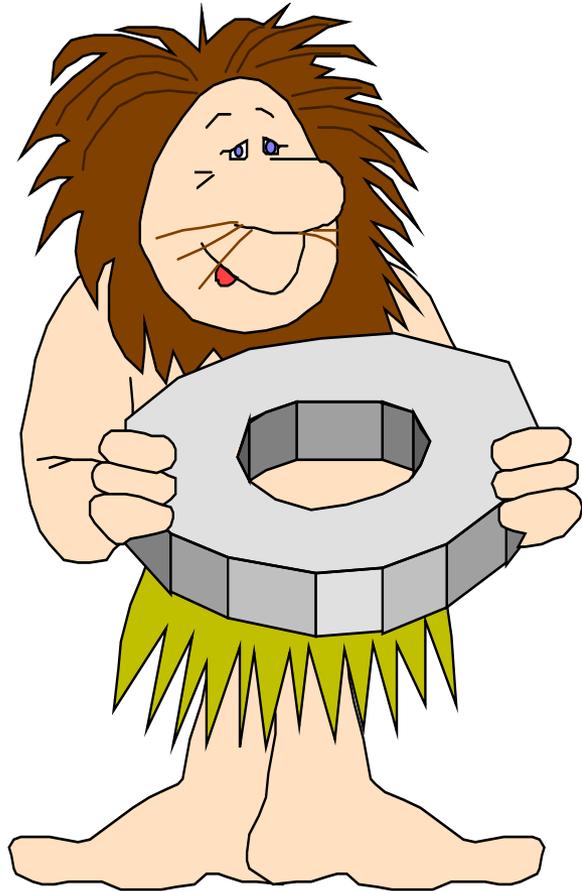
SECONDARY RESPONSE

effector cells



memory cell pool

Immunologically Naive

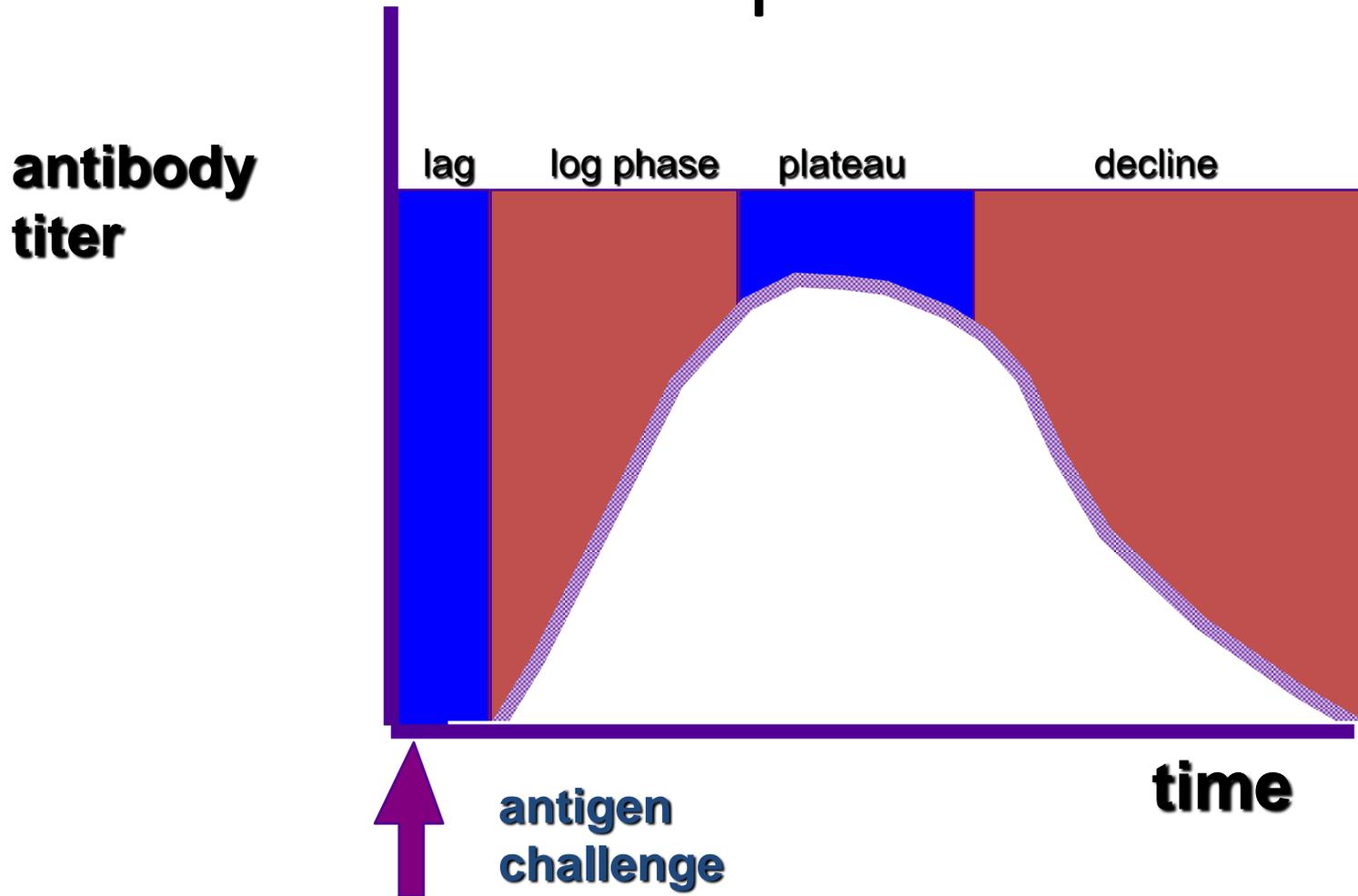


**No previous
experience**

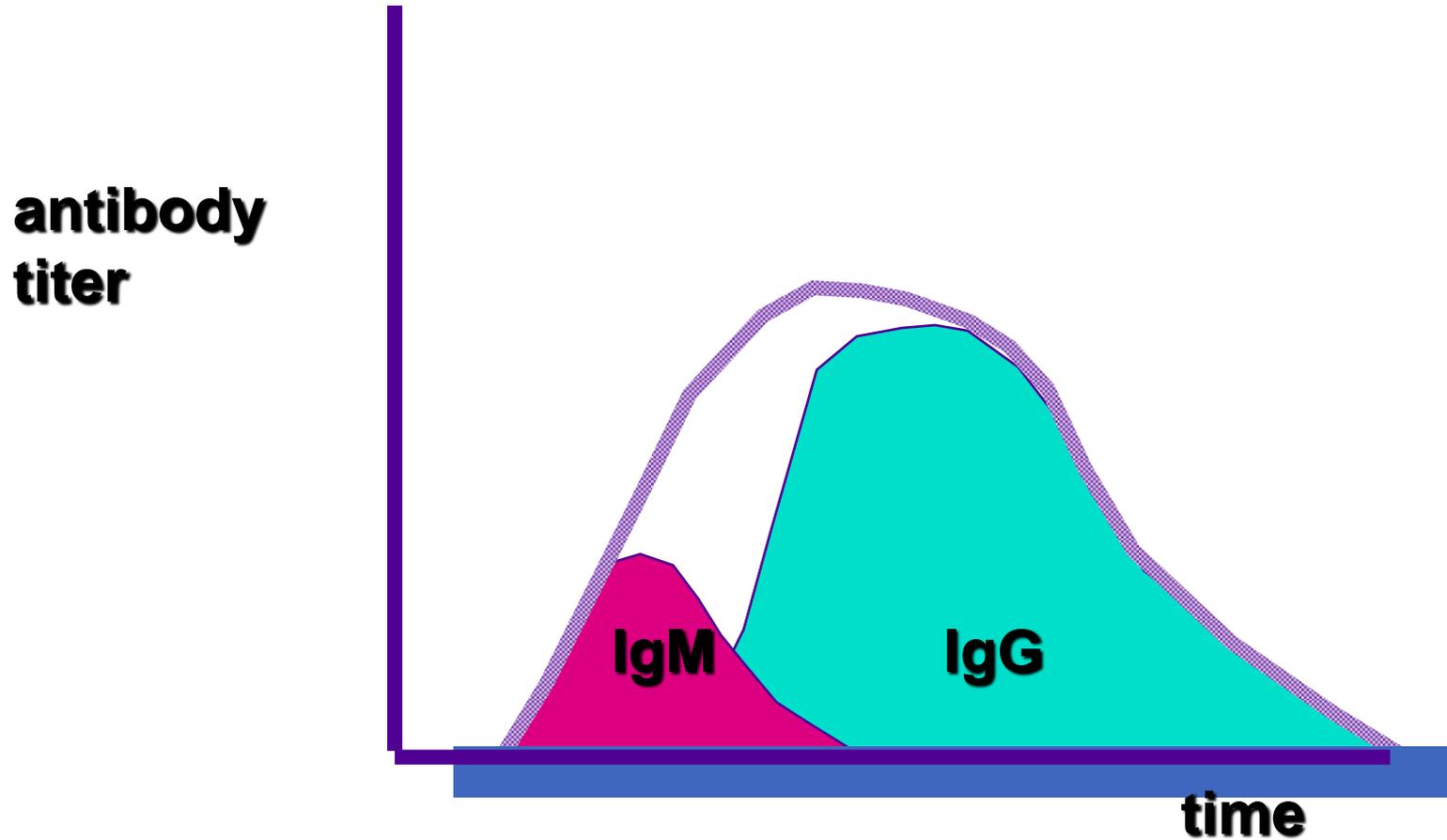
No memory

Must be educated

The Primary Immune Response



Class Switching



Dynamics of Antibody Production

Primary immune response

Latent period

Gradual rise in antibody production taking days to weeks

Plateau reached

Antibody level declines

Dynamics of Antibody Production

Antibody production

Initial antibody produced in IgM

Lasts 10-12 days

Followed by production of IgG

Lasts 4-5 days

Without continued antigenic challenge antibody levels drop off, although IgG may continue to be produced.

Four phases of the primary response

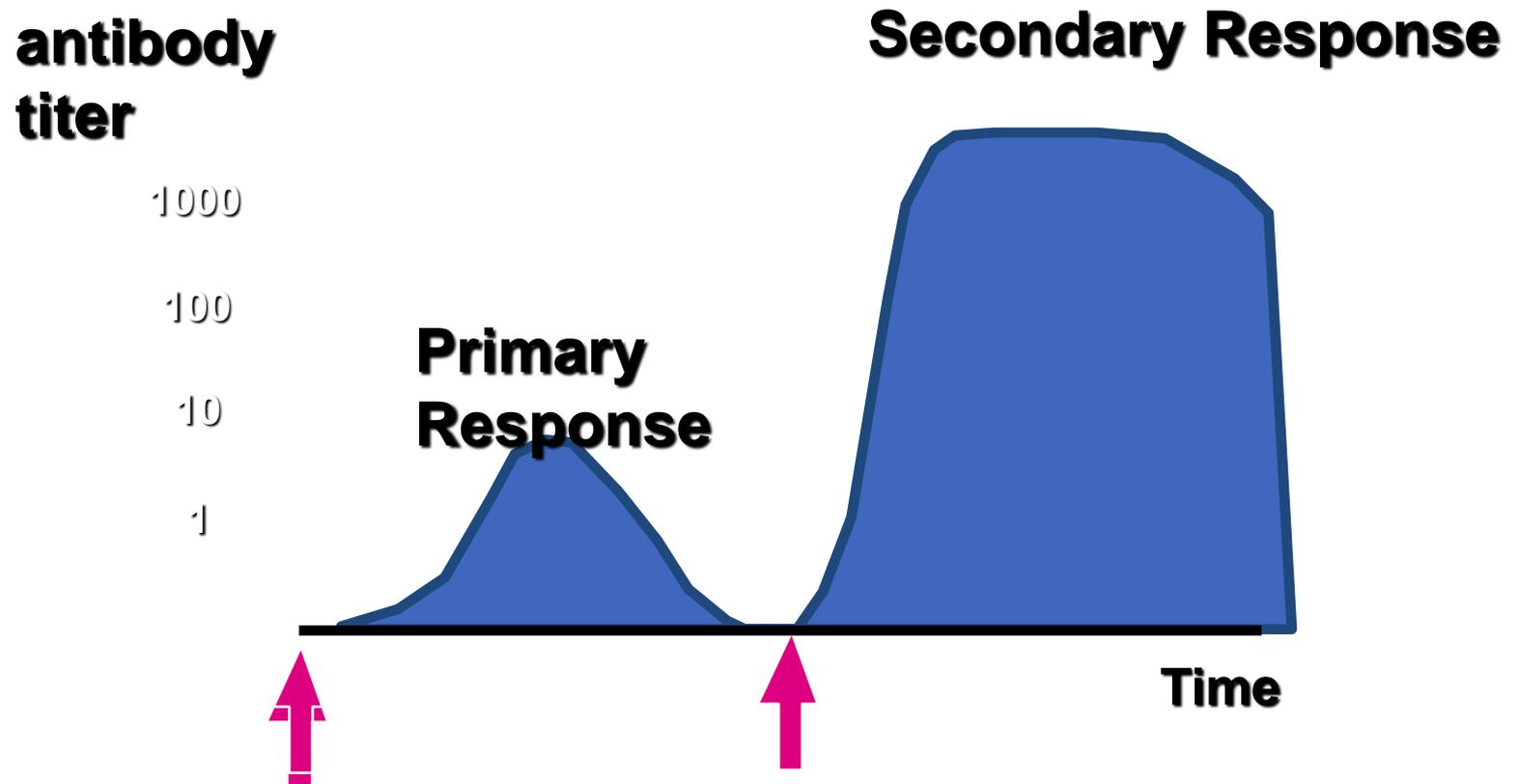
a *lag phase* where no antibody is detected

a *log phase* in which the antibody titer rises logarithmically

a *plateau phase* during which the antibody titer stabilizes

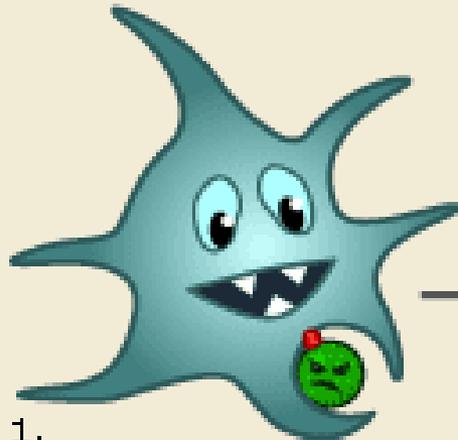
a phase (*decline*) during which the antibody is cleared or catabolized

The Anamnestic Immune Response

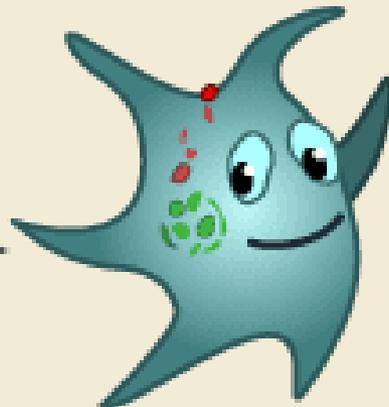
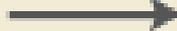


Antigen Presentation

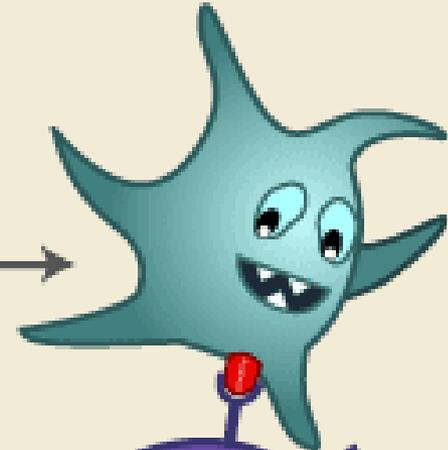
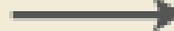
dendritic cell



1.
A phagocyte "eats"
a bacteria.



2.
Parts of the bacteria
(antigen) goes to the
surface of the phagocyte



3.
The phagocyte
presents the antigen
to a helper T cell



helper T cell

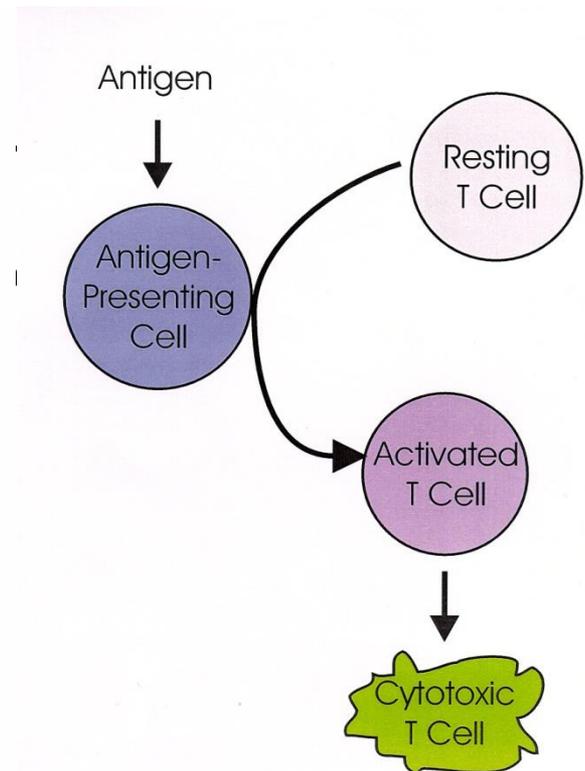
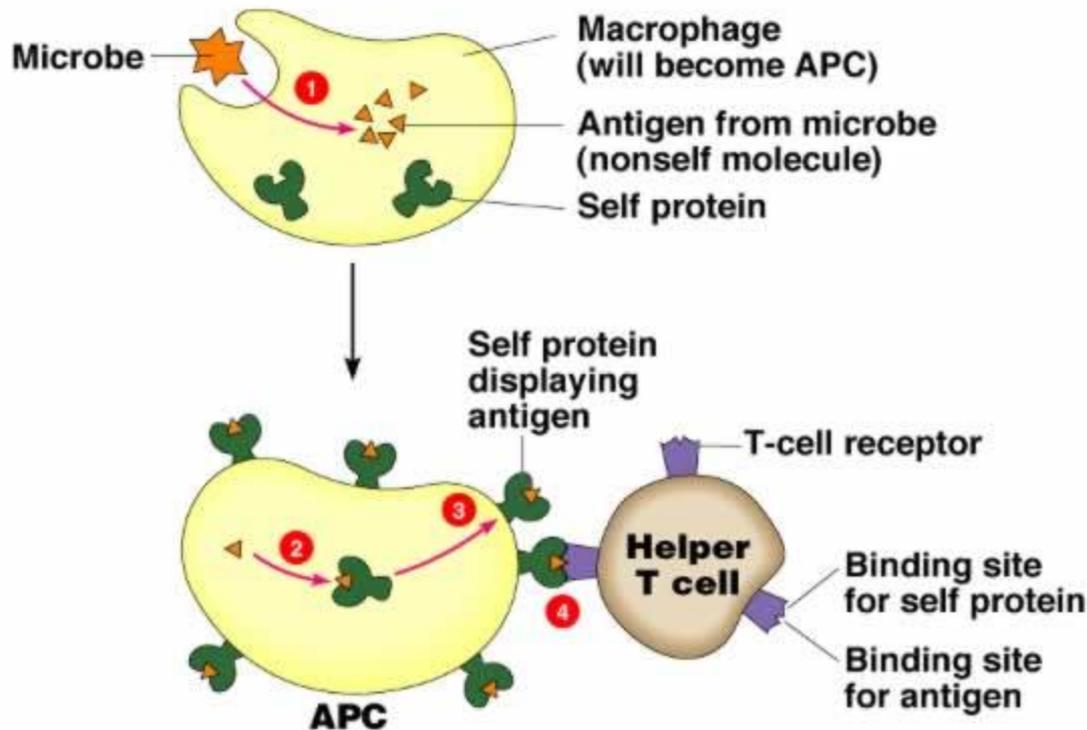


activated
helper T cell

4.
The helper T cell
is activated.

Antigen presenting cells

Ingested pathogens (phagocytes like • macrophages) or infected cells display fragments of the pathogen on the surface of the cell

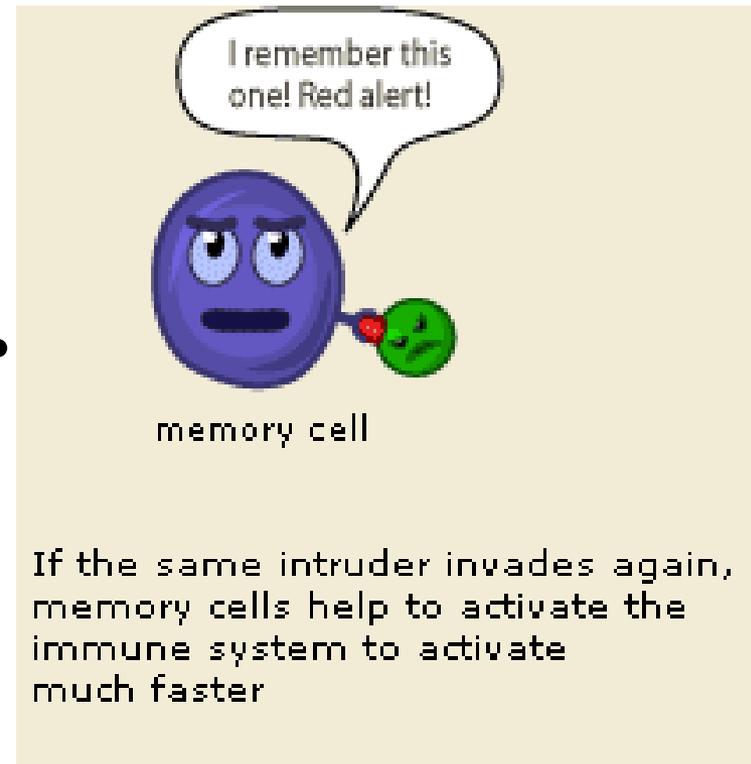


Memory cells



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.**



Primary .vs. Secondary 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.

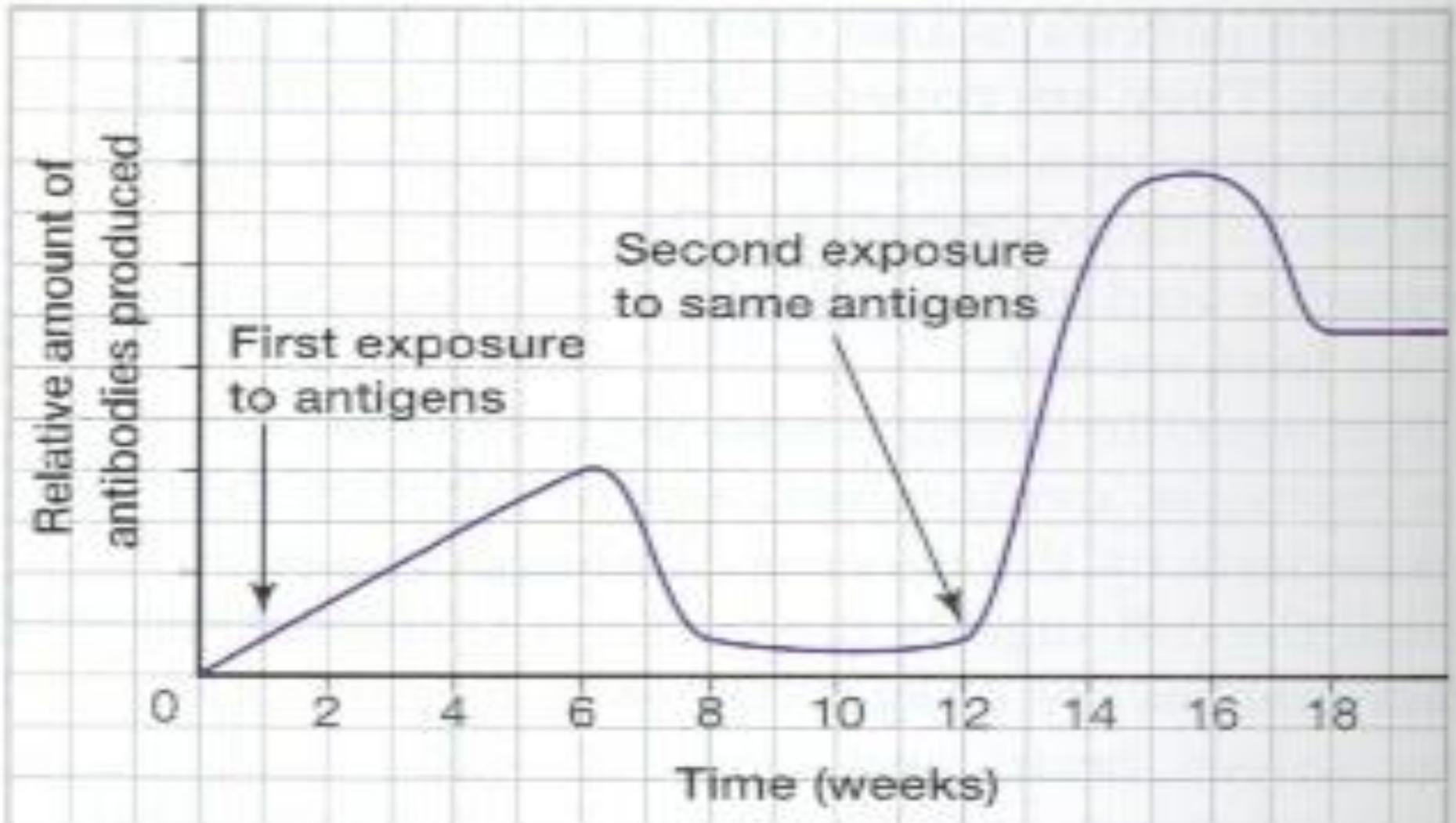
Next 10 – 15 days antibody production grows steadily

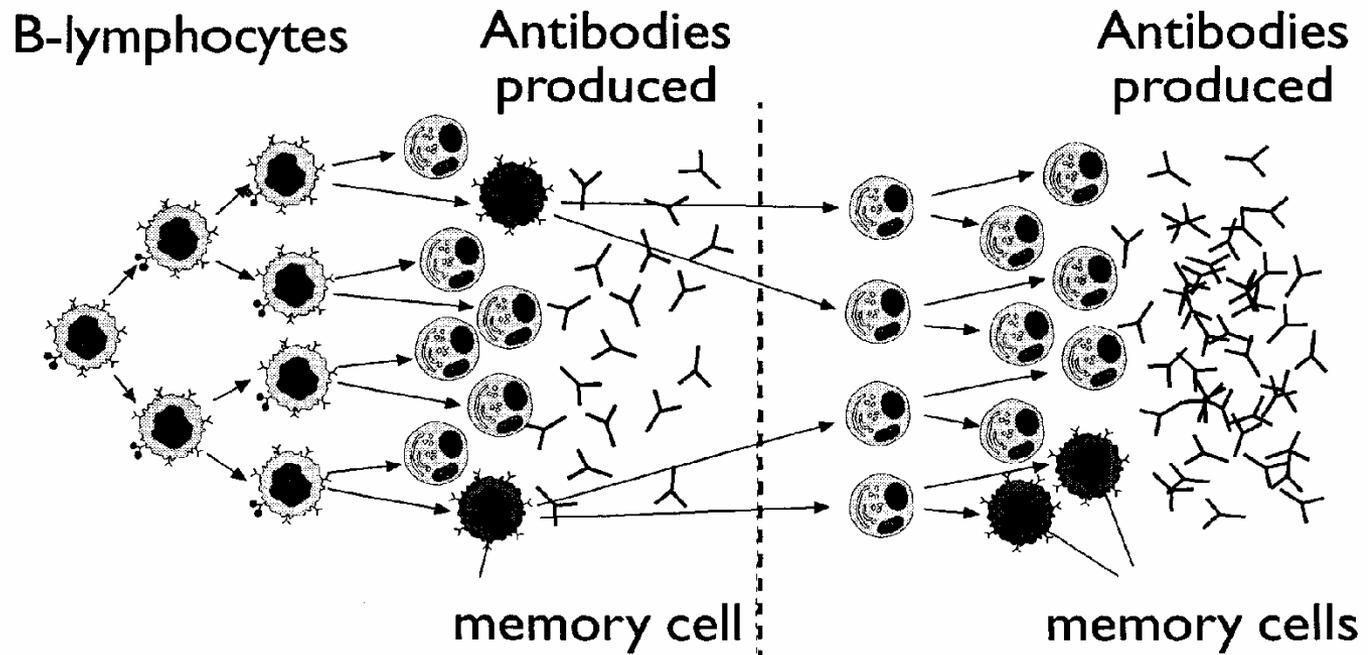
Secondary Immune Response

A more rapid response to an invader the 2nd time it invades the body.

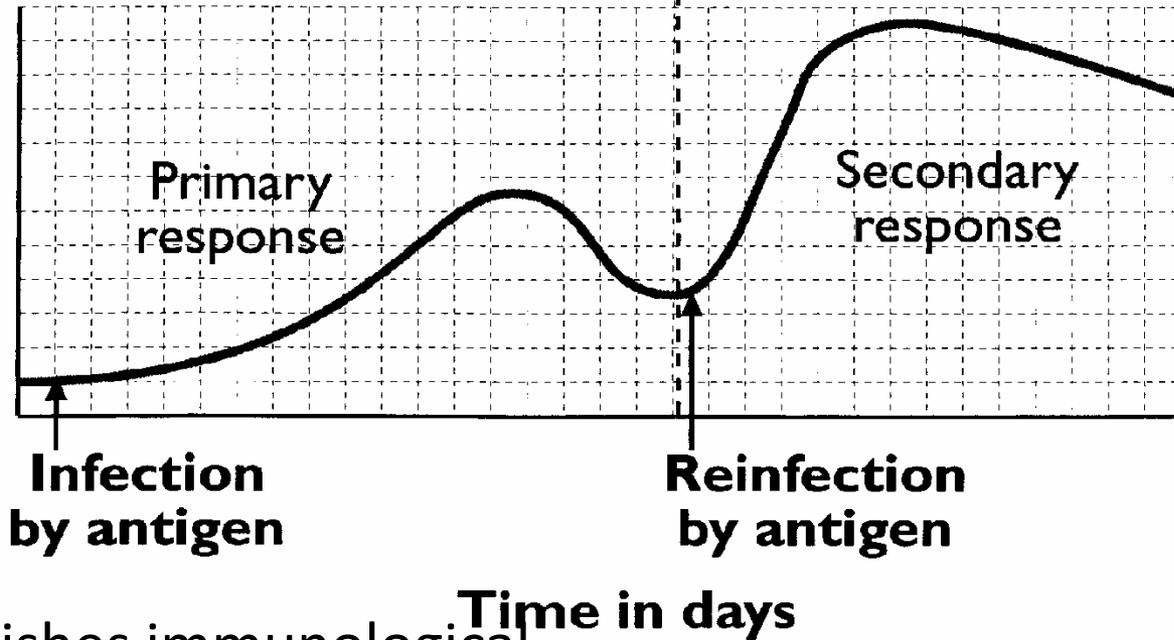
Antibody production increases dramatically and in a much shorter time period..

Primary .vs. Secondary Immune Response





Concentration of antibodies in plasma



Primary – establishes immunological memory

Primary and secondary responses

Annotate your graph of the primary and secondary responses by adding the following labels:

- First dose of antigen
- Second dose of antigen
- Time between 1st and second exposure
- Primary response
- Secondary response

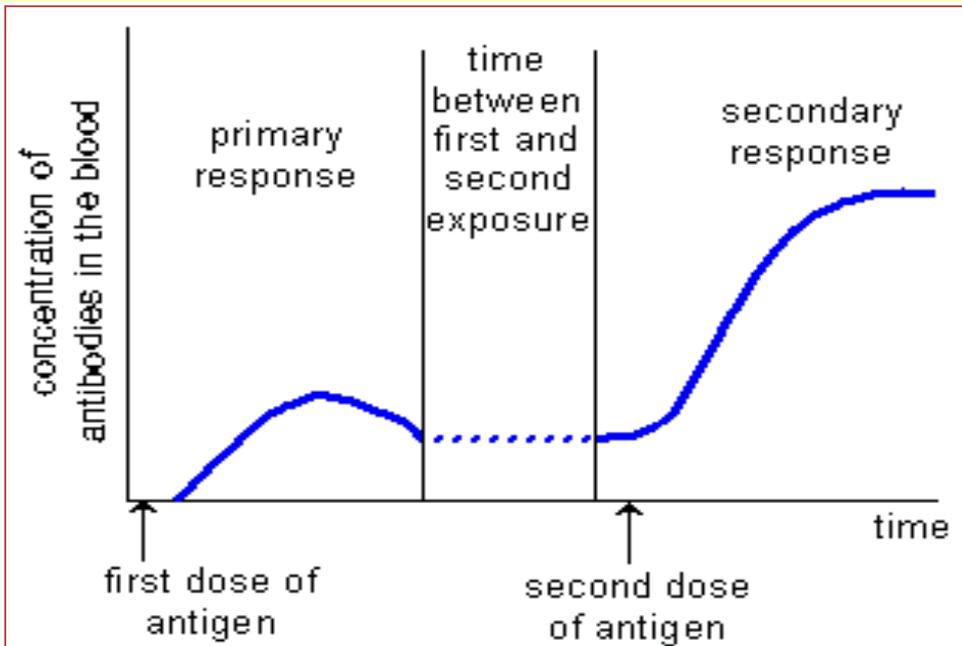
Questions:

- 1) Compare the primary and secondary responses in terms of time taken, decline of antibodies and antibodies produced.
- 2) Memory cells are produced in the primary response, how else could these be produced without the person being infected?

Extra challenge: What causes the secondary response to occur more rapidly?

LO:

Antibody Concentration – Primary and Secondary Response



Primary Response

1. Infection (Ag)
2. Lag phase
3. Antibodies produced
4. Antibody level rises to combat infection
5. Ag dealt with
6. Ab level declines – short lived

Secondary Response

After the primary response, Ab's do not stay in blood – the **level declines**

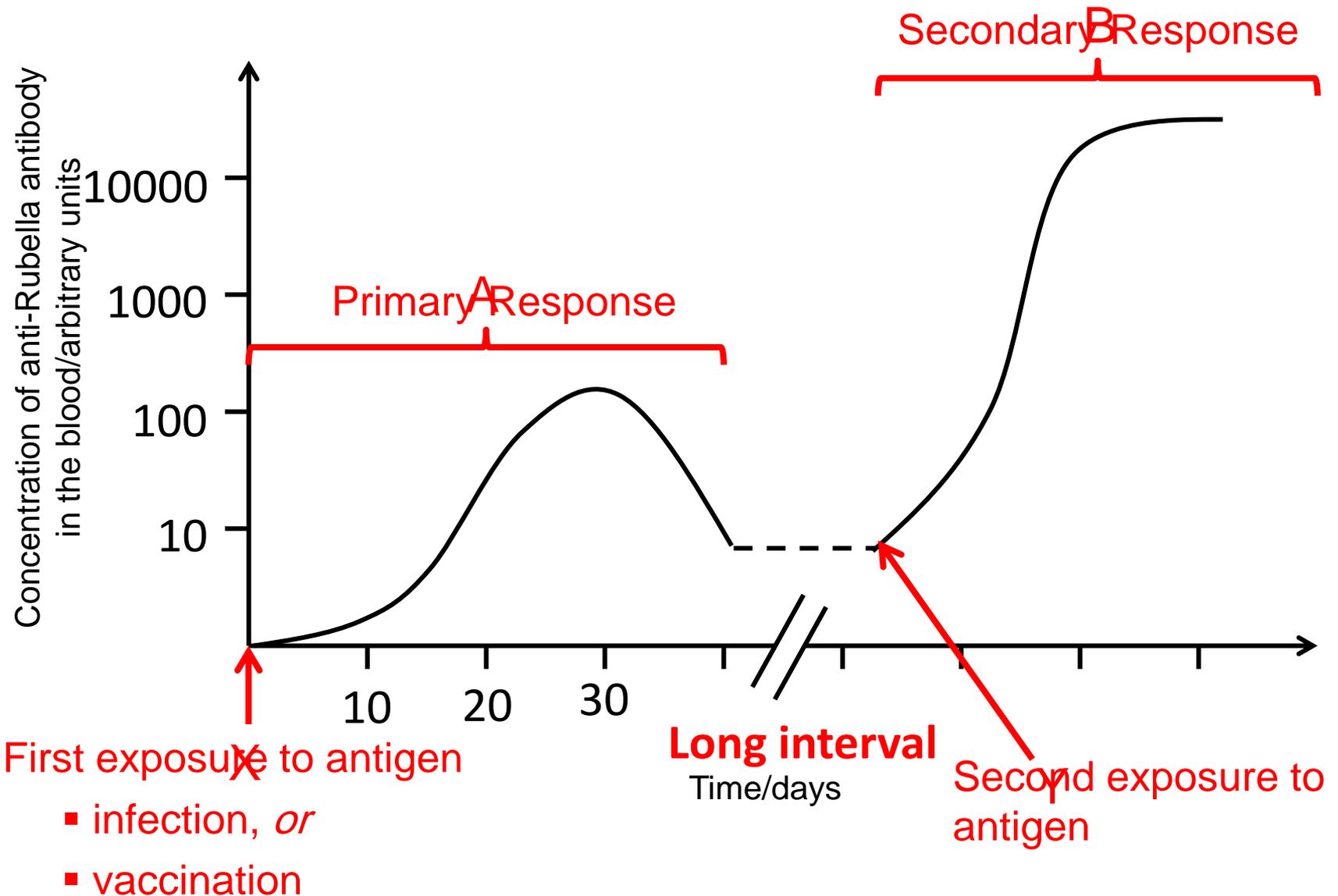
If the body is infected by the same Ag a second time Ab's must be made again

Re-infection causes **much more rapid** and a **stronger** immune response – concentration of Ab's rises sooner- reaches a higher concentration – more plasma cells than in 1^o response – more cells to respond to Ag; less time to produce same number of plasma cells –hence, a greater [Ab] compared to 1^o response; increased affinity of Ab for Ag.

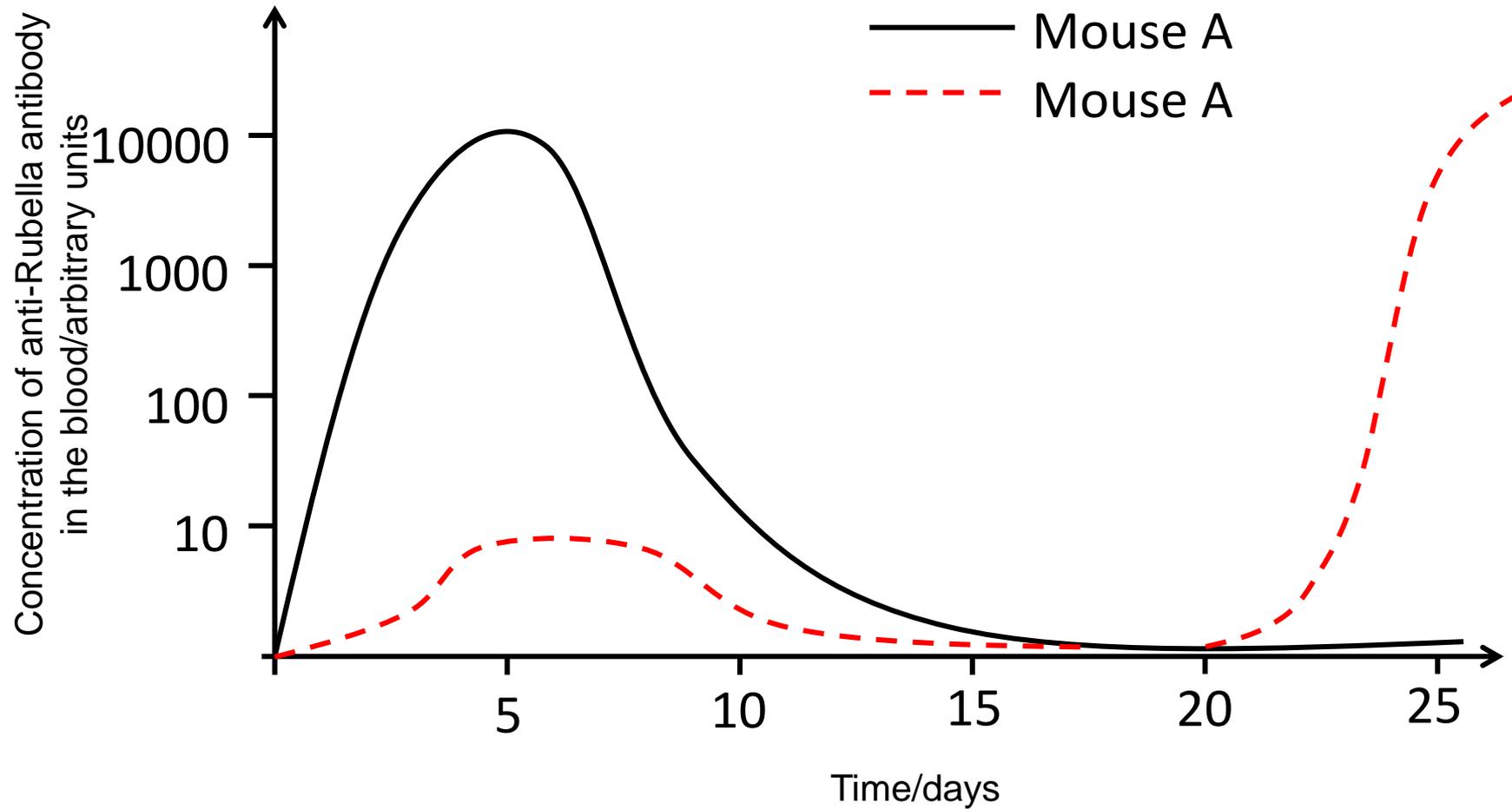
This is due to the presence of **memory cells** (made during the primary response) – no need for antigen presentation and clonal selection

Long-lived; basis of vaccination

The graph below shows the levels of anti-Rubella (German Measles) in the blood



The graph below shows the immune response of two mice exposed to a pathogen. Both mice were exposed on day 0 of the experiment



Plenary: True or False?

- 1) Antibodies are produced by memory cells.
- 2) Antibodies can detect any antigen.
- 3) A antigen-antibody complex is formed when an antibody binds to a pathogen's antigens.
- 4) Agglutination makes it easier for phagocytes to engulf pathogens.
- 5) Neutralisation only occurs with viruses.
- 6) Opsonisation causes phagocytes to swell and burst.
- 7) Antibodies can stop viruses entering host cells.
- 8) The primary immune response produces memory cells.
- 9) The secondary immune response is caused by vaccination.
- 10) The secondary immune response is quicker.

Cellular Events

Antigen is “processed” by T lymphocytes and macrophages.

Possess special receptors on surface.

Termed “antigen presenter cell” APC.

Antigen presented to B cell

Cellular Immune Response

Important in defending against: fungi, parasites, bacteria.

Responsible for hypersensitivity, transplant rejection, tumor surveillance.

Thymus derived (T) lymphocytes

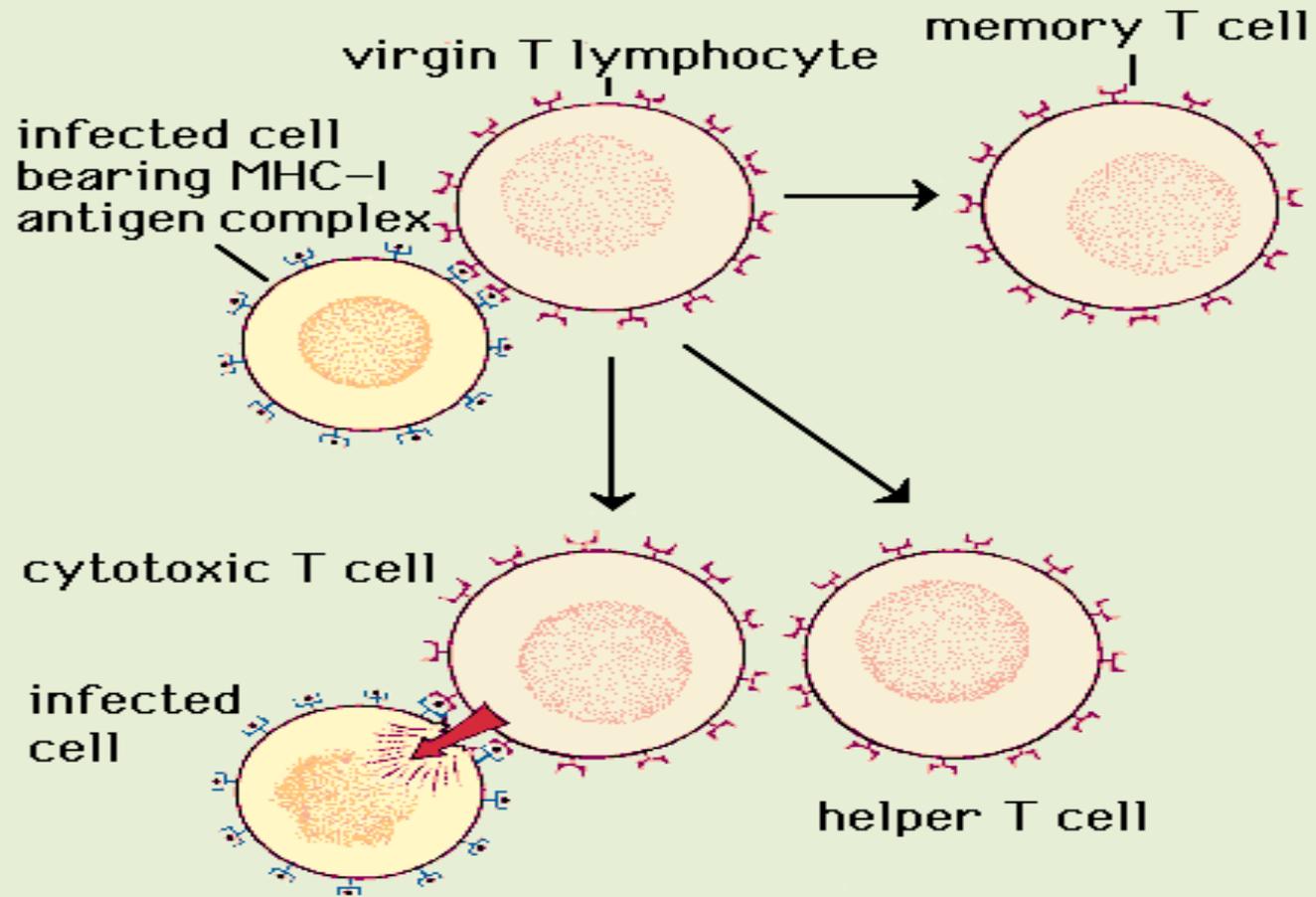
Cell Mediated Reaction

Helper T cells – turn on immune response

Suppressor T cells – turn off immune response

Cytotoxic T cells directly attack antigen

Cell Mediated Immunity



Secondary Response

- Second exposure to SAME antigen.
- Memory cells are a beautiful thing.
- Recognition of antigen is immediate.
- Results in immediate production of protective antibody, mainly IgG but may see some IgM

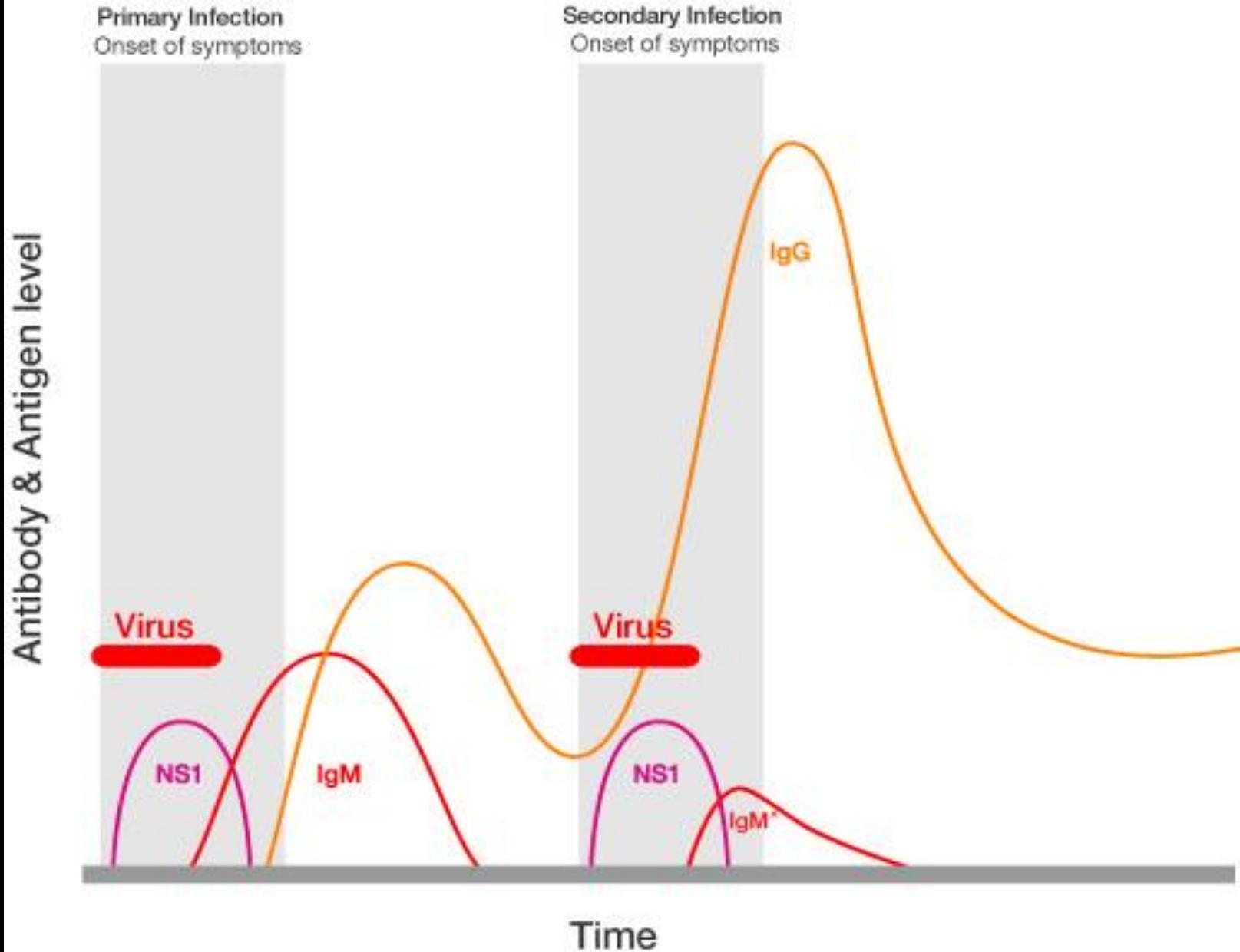
Comparison of Primary and Secondary Responses

- Time course
- Antibody titer
- Antibody class
- Antibody affinity

PRIMARY AND SECONDARY ANTIBODY RESPONSE

- Primary Response
 - Following exposure to an antigen, there is a slow rise in IgM followed by a slow rise in IgG
- Secondary Response
 - Following exposure to previously encountered antigen, there is a rapid rise in IgG and slow or no rise in IgM
 - Memory or anamnestic response

Dengue infection: immune response



T LYMPHOCYTES AND CELL-MEDIATED IMMUNITY

- Originate from stem cells in bone marrow followed by migration to thymus gland
- Maturation takes place in thymus gland followed by migration to secondary lymphoid tissue
- Respond to antigens on the surface of antigen presenting cells (APC's)
- Antigen presenting cells (APC's)
 - Macrophages
 - Dendritic cells
 - B lymphocytes

T LYMPHOCYTES AND CELL-MEDIATED IMMUNITY

- Antigen presenting cells (APC's)
 - Ingest and process antigens then display fragments (short peptides) on their surface in association with molecules of major histocompatibility complex (MHC)
- Major histocompatibility (MHC) molecules
 - MHC class I molecules
 - Present antigens to CD8 T cells
 - MHC class II molecules
 - Present antigens to CD4 T cells
- T cells which encounter antigen differentiate into effector T cells

MHC class I

MHC class II

peptide

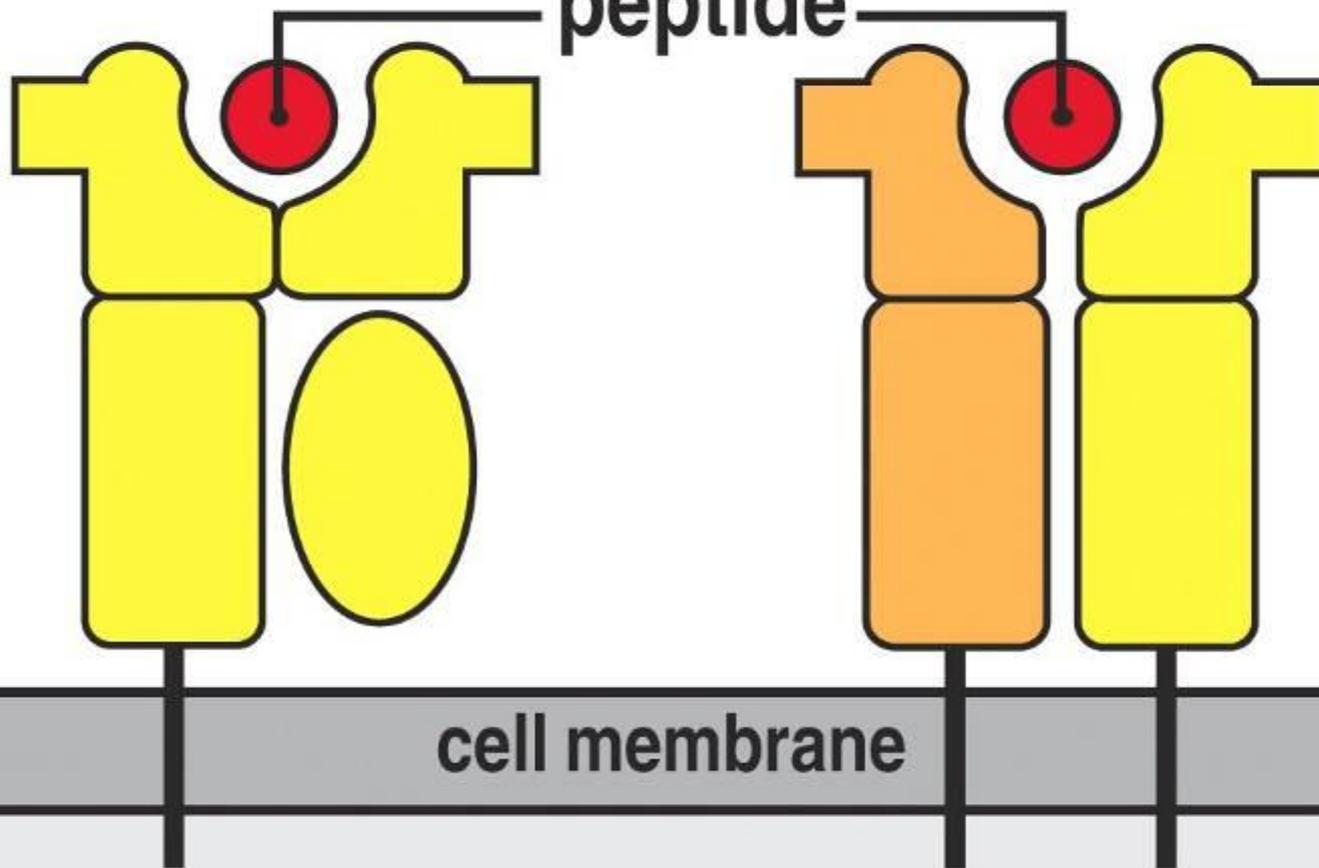


Figure 1-25 The Immune System, 2/e (© Garland Science 2005)

ROLES OF EFFECTOR T CELLS IN IMMUNE RESPONSE

- CD8 cytotoxic T cells
 - Enter bloodstream and travel to infection site
 - Kill cells infected with viruses and other intracellular microorganisms
- CD4 TH₁ helper T cells
 - Enter blood stream and travel to infection site
 - Help activate macrophages
- CD4 TH₂ helper T cells
 - Work within secondary lymphoid tissues
 - Help activate B cells

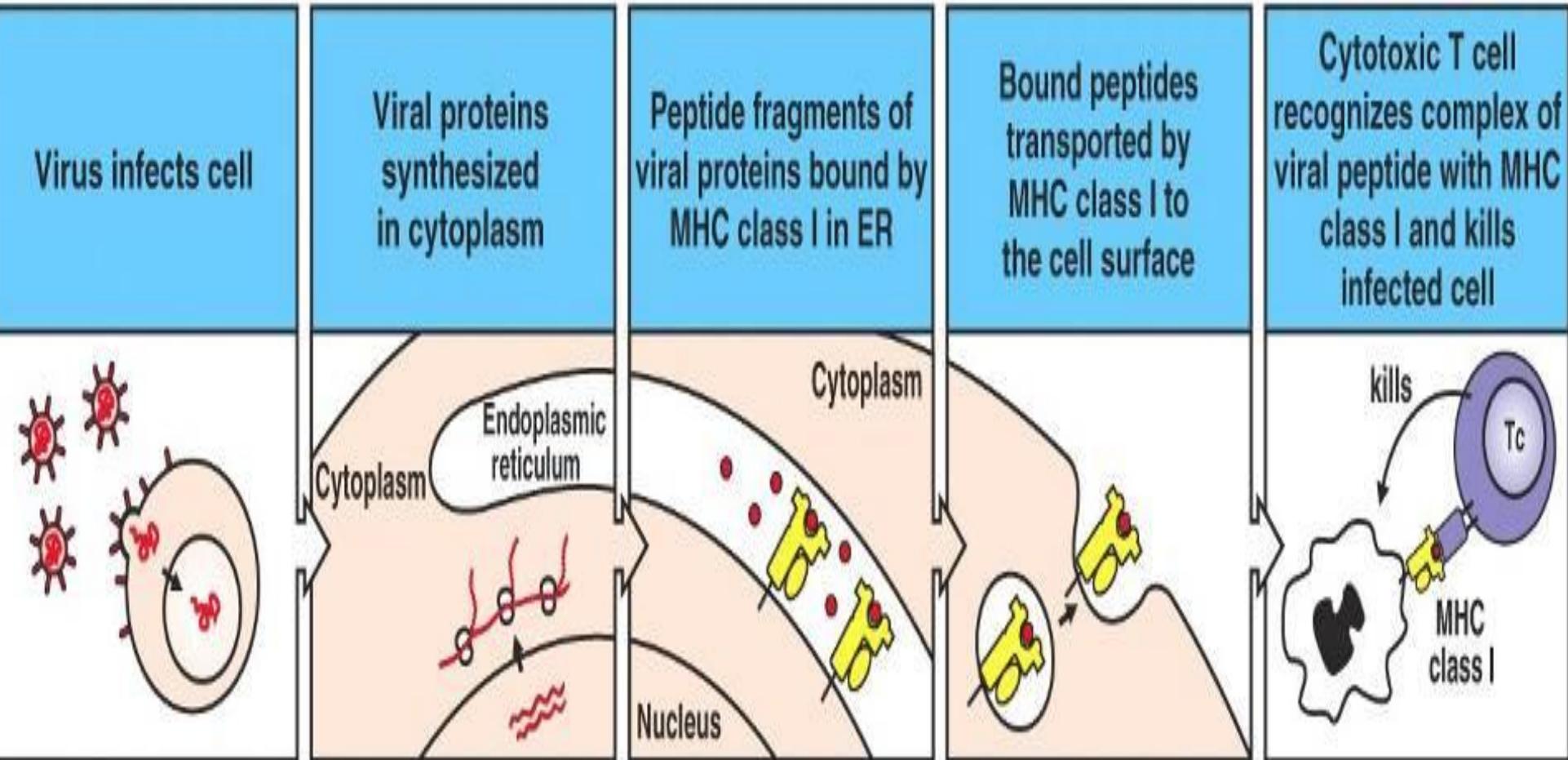


Figure 1-26 The Immune System, 2/e (© Garland Science 2005)

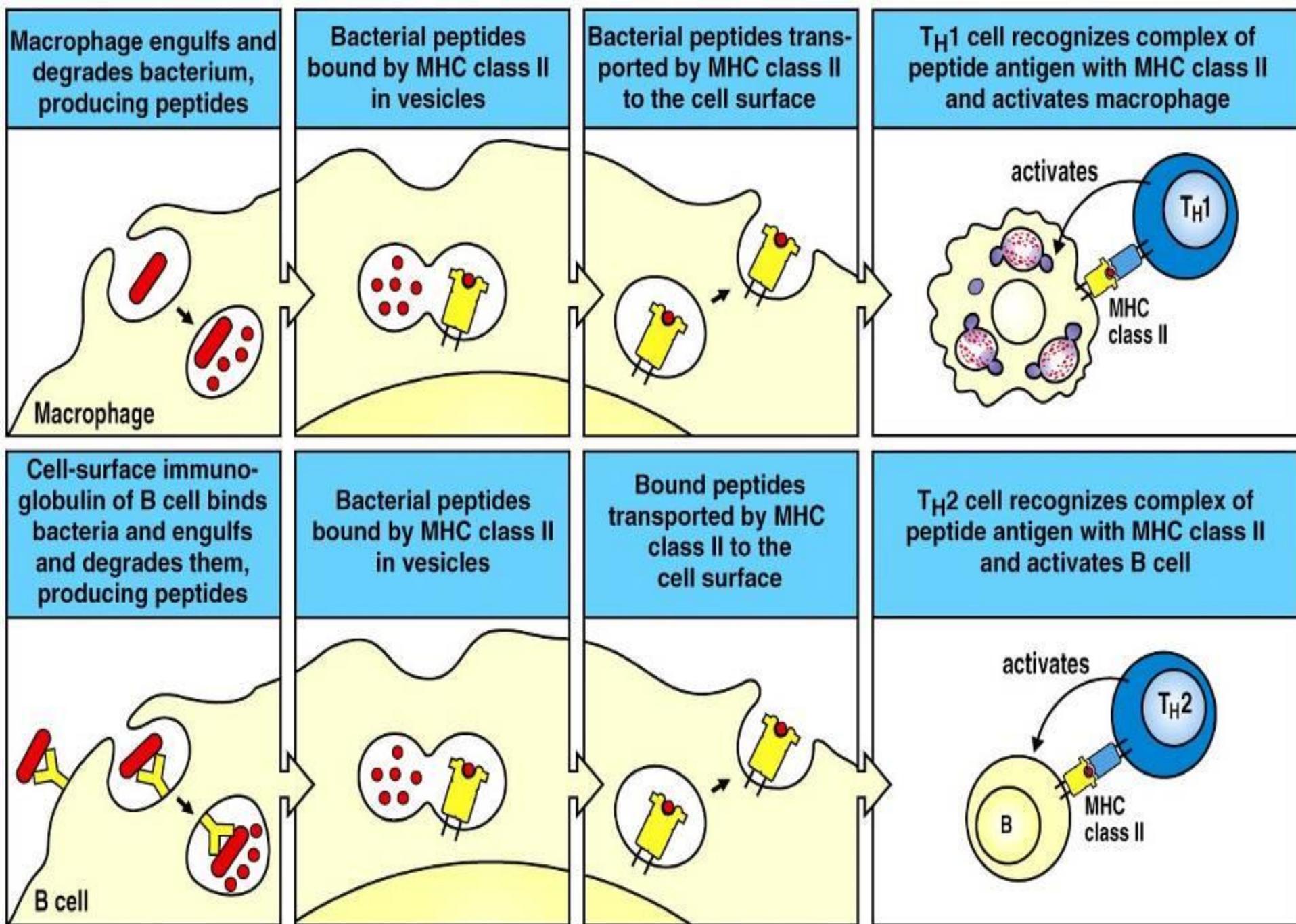


Figure 1-27 The Immune System, 2/e (© Garland Science 2005)

Two types of immune response:

- 1. Humoral / Antibody-Mediated Immunity
- involves antibodies produced by B cells to confer immunity
- best against bacteria, toxins, and virus that are free in body fluids
- 2. Cell-Mediated Immunity
- involves T cells that act against foreign organisms or tissues
- works best on bacteria- or virus-infected cells, fungi, protozoa, tissue grafts and cancer

B cells and Humoral Immunity

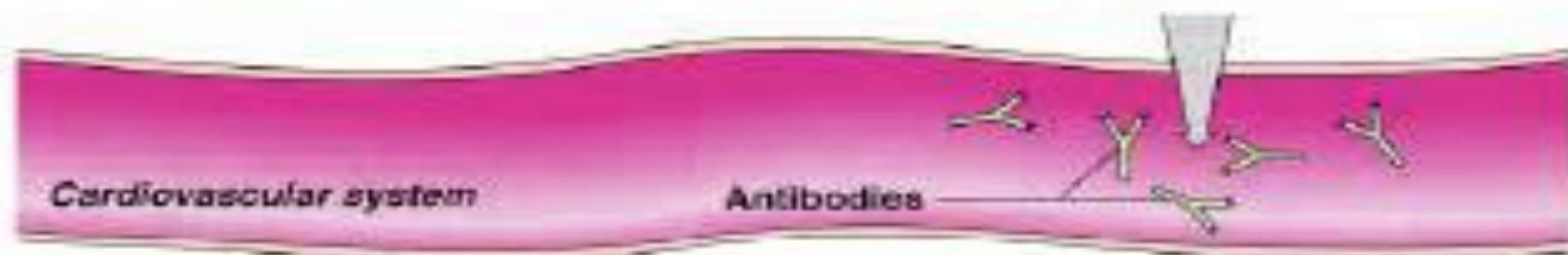
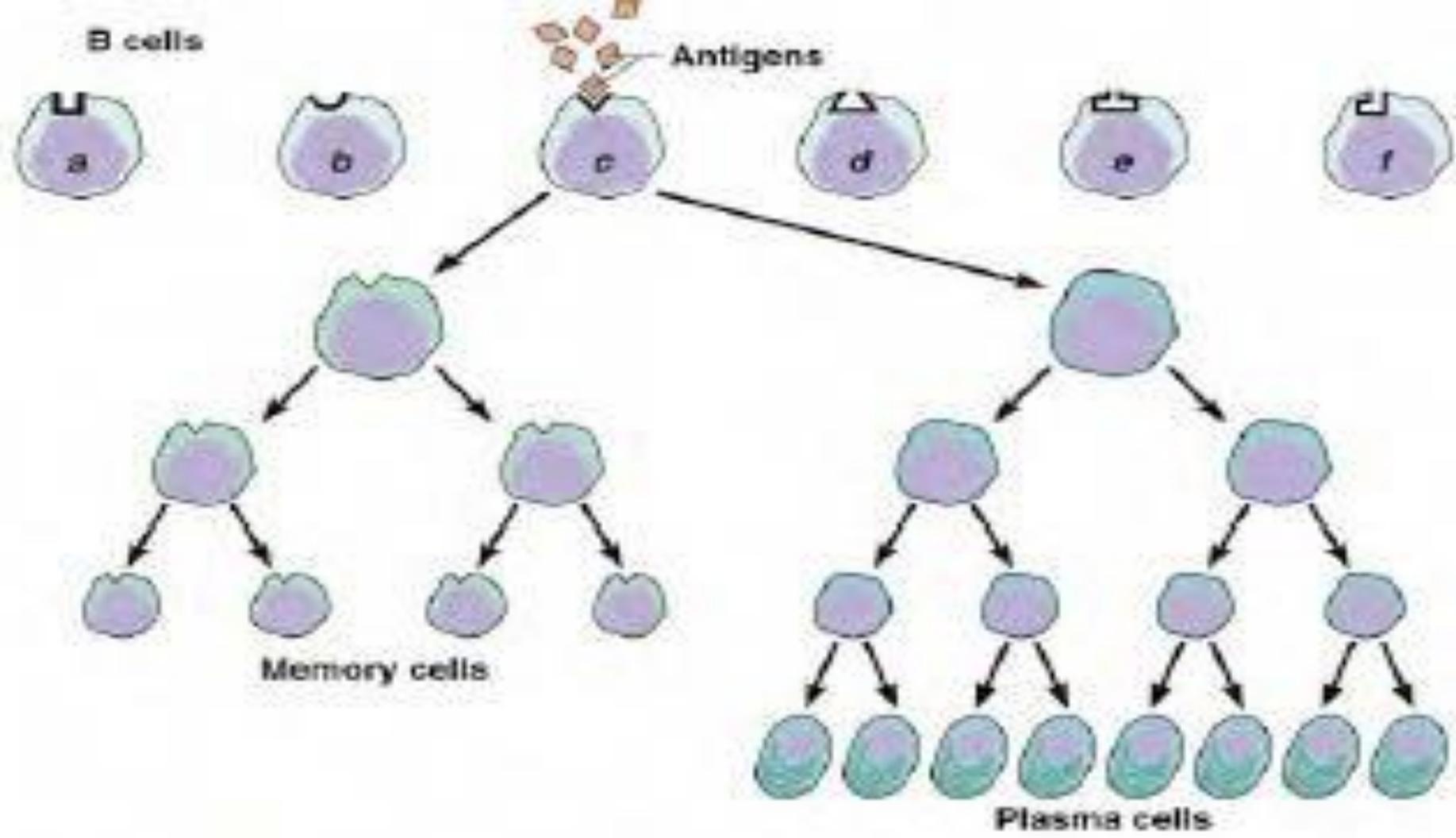
- • B cells produce antibodies = humoral / antibody-mediated immunity
- • B cells arise from stem cells in bone marrow
- • when mature, migrate to lymphoid tissue
- • wait to recognize antigen and be stimulated to produce antibodies

• **Activation of B cells by clonal selection:**

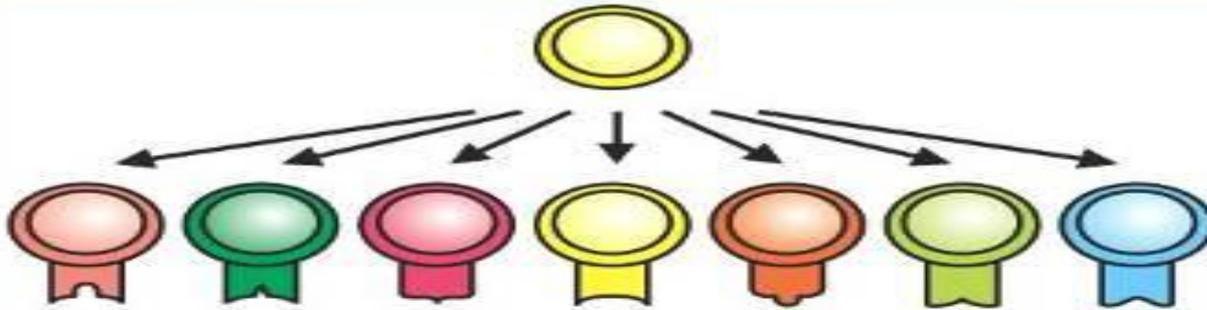
- each B cells produces only one antibody against one antigen/epitope
- • recognizes antigen/epitope via IgD on cell surface (receptor)
- • when activated it will divide to produce clones.

ACTIVATION OF ANTIBODY PRODUCING CELLS BY CLONAL SELECTION

- Proliferation of activated cells is followed by differentiation into
 - Plasma cells
 - Life span of
 - 4 to 5 days
 - 1 to 2 months
 - Produce 2,000 antibody molecules / second
 - Memory cells
 - Life span of years to decades
 - Differentiate into plasma cells following stimulation by same antigen



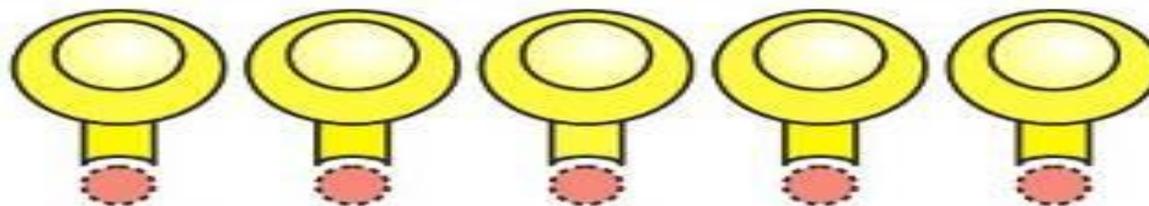
During development, progenitor cells give rise to large numbers of lymphocytes, each with a different specificity



Pool of circulating small lymphocytes



Proliferation and differentiation of pathogen-activated lymphocytes to form a clone of effector cells



Effector cells eliminate pathogen

Major Histocompatibility Complex (MHC)

Lecture 10 & 11

MHC

- Major Histocompatibility Complex
 - Cluster of genes found in all mammals
 - Its products play role in discriminating self/non-self
 - Participant in both humoral and cell-mediated immunity
- MHC Act As Antigen Presenting Structures
- In Human MHC Is Found On Chromosome 6
 - Referred to as HLA complex

MHC

- Genes Of MHC Organized In 3 Classes
 - Class I MHC genes
 - Glycoproteins expressed on all nucleated cells
 - Major function to present processed Ags to T_C
 - Class II MHC genes
 - Glycoproteins expressed on $M\Phi$, B-cells, DCs
 - Major function to present processed Ags to T_H
 - Class III MHC genes
 - Products that include secreted proteins that have immune functions. Ex. Complement system, inflammatory molecules

Class I, II and III MHC

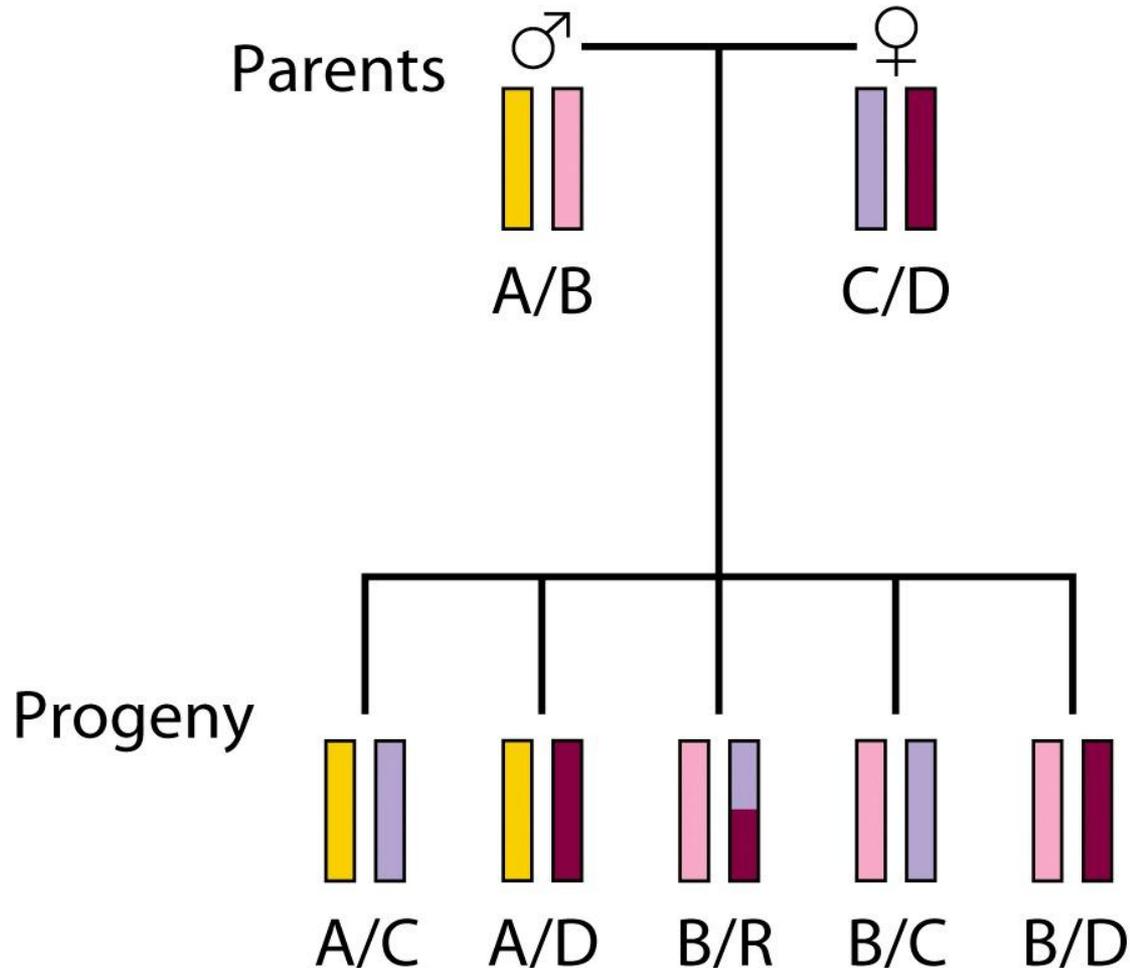
- Class I MHC Genes Found In Regions A, B and C In Humans (K and D In Mice)
- Class II MHC Genes Found In Regions DR, DP and DQ (IA and IE In Mice)
- Class I and Class II MHC Share Structural Features
 - Both involved in APC
- Class III MHC Have No Structural Similarity To Class I and II
 - Ex. TNF, heat shock proteins, complement components

MHC Genes Are Polymorphic

- MHC Products Are Highly Polymorphic
 - Vary considerably from person to person
- However, Crossover Rate Is Low
 - 0.5% crossover rate
 - Inherited as 2 sets (one from father, one from mother)
 - Haplotype refers to set from mother or father
- MHC Alleles Are Co-dominantly Expressed
 - Both mother and father alleles are expressed

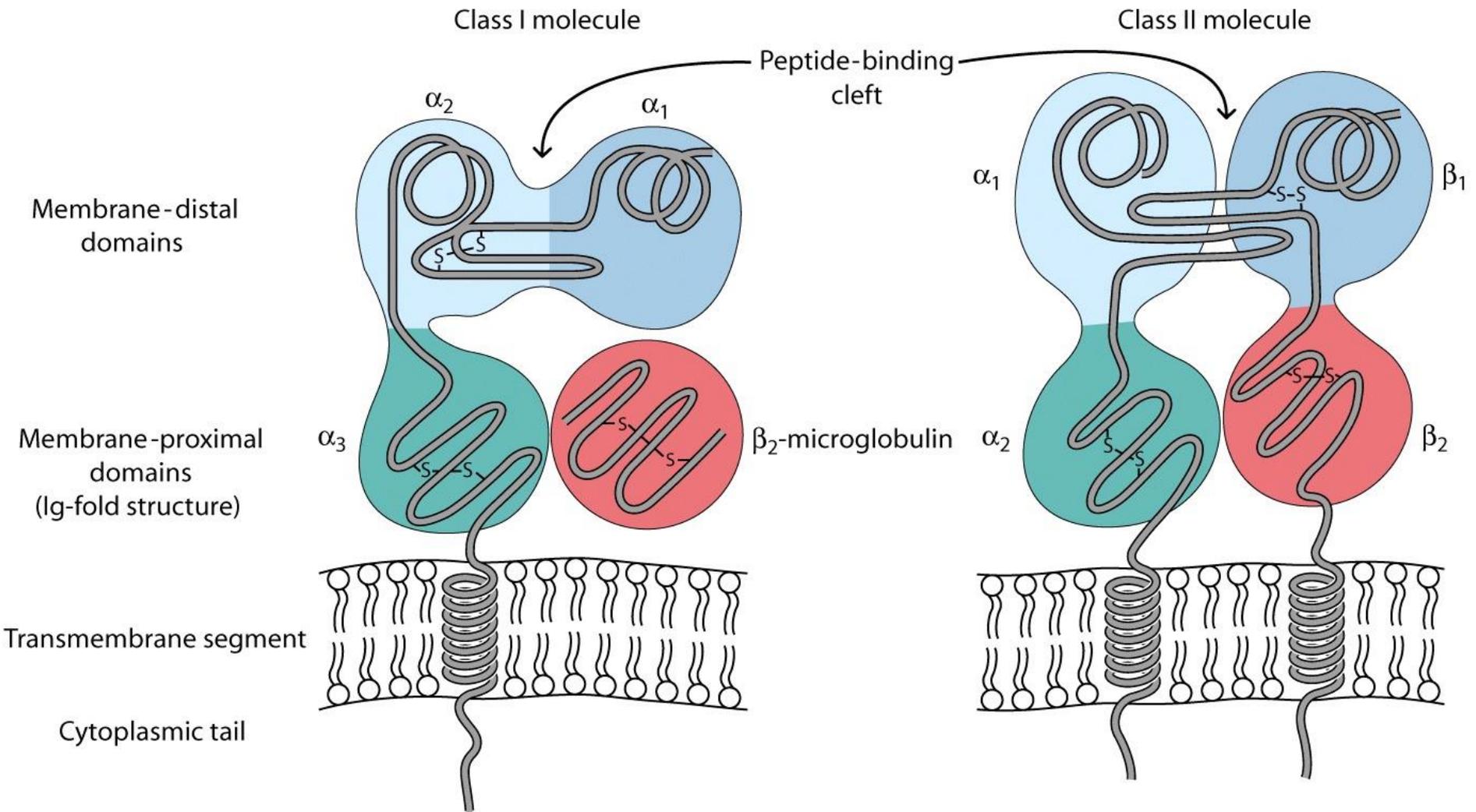
Inheritance Of HLA Haplotypes

(c) Inheritance of HLA haplotypes in a typical human family



Class I MHC Molecule

- Comprised of 2 molecules
 - α chain (45 kDa), transmembrane
 - β_2 -microglobulin (12 kDa)
 - Non-covalently associated with each other
- Association Of α Chain and β_2 Is Required For Surface Expression
- α Chain Made Up Of 3 Domains (α_1 , α_2 and α_3)
- β_2 -microglobulin Similar To α_3
- α_1 And α_2 Form Peptide Binding Cleft
 - Fits peptide of about 8-10 a/a long
- α_3 Highly Conserved Among MHC I Molecules
 - Interacts with CD8 (T_C) molecule



Class II MHC Molecule

- Comprised of α and β chains
 - α chain and β chain associate non-covalently
- α and β chains Made Up Of Domains
 - α_1 and α_2 (α chain)
 - β_1 and β_2 (β chain)
- α_1 and β_1 Form Antigen Binding Cleft
- α and β Heterodimer Has Been Shown To Dimerize
- CD4 Molecule Binds α_2/β_2 domains

Class I And II Similarity

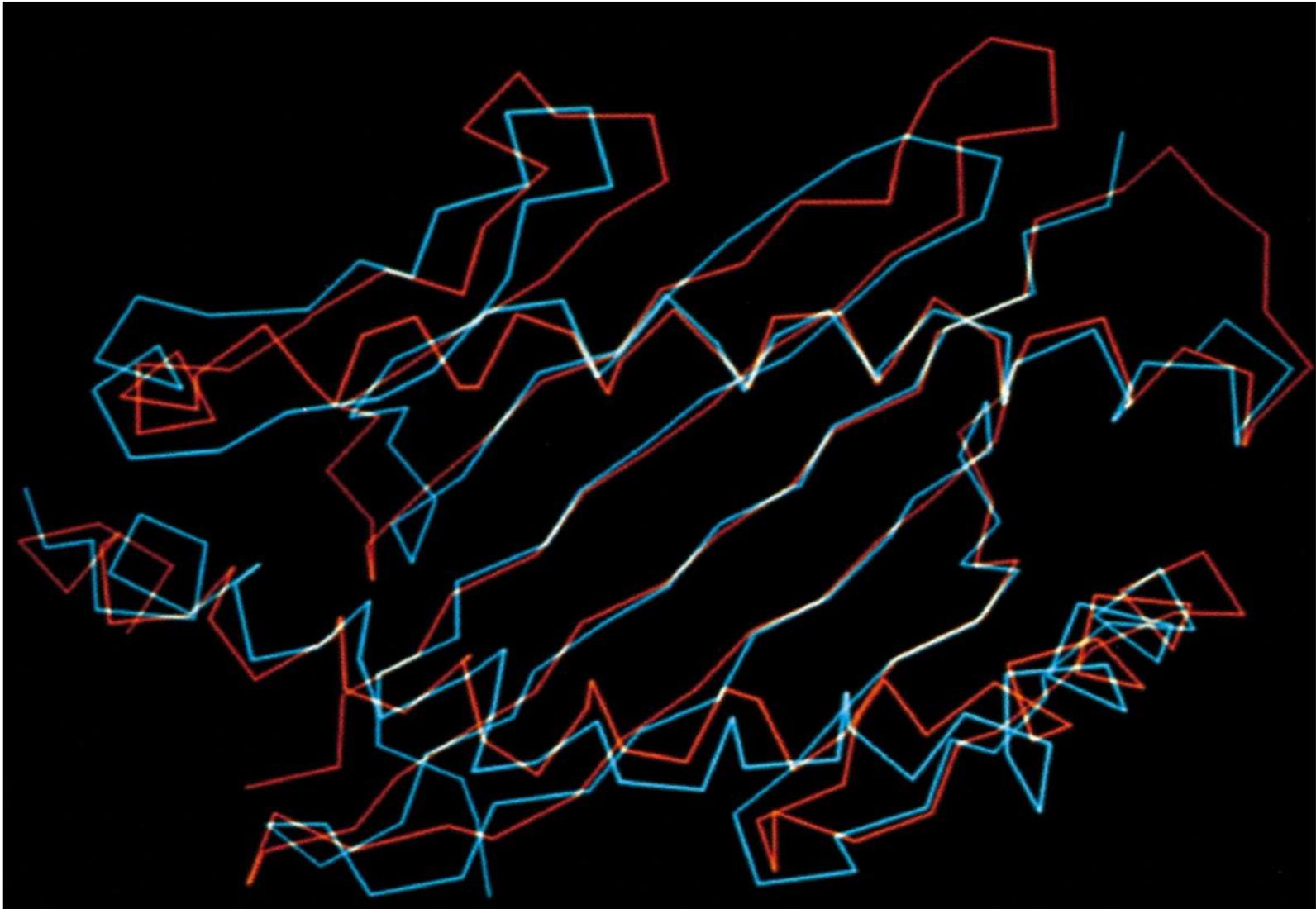


TABLE 7-2

Peptide binding by class I and class II MHC molecules

	Class I molecules	Class II molecules
Peptide-binding domain	$\alpha 1/\alpha 2$	$\alpha 1/\beta 1$
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

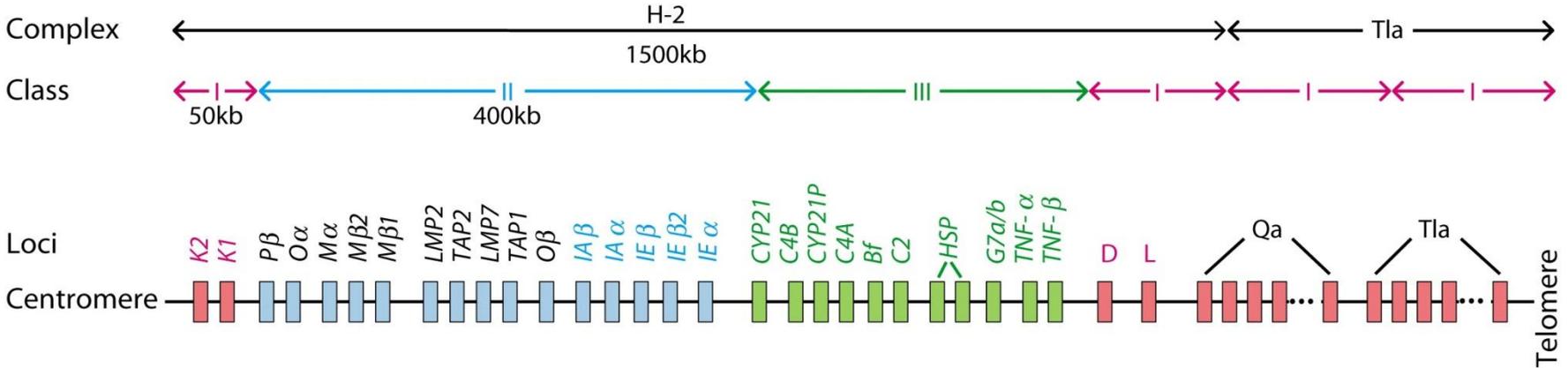
Class I And II Specificity

- Several Hundred Allelic Variants Have Been Identified In Humans
- However, up to 6 MHC I And 12 MHC II Molecules Are Expressed In An Individual
- Enormous Number Of Peptides Needs To Be Presented Using These MHC Molecules
- To Achieve This Task MHC Molecules Are Not Very Specific For Peptides (Unlike TCR and BCR)
- Promiscuous Binding Occurs
 - A peptide can bind a number of MHC
 - An MHC molecule can bind numerous peptides

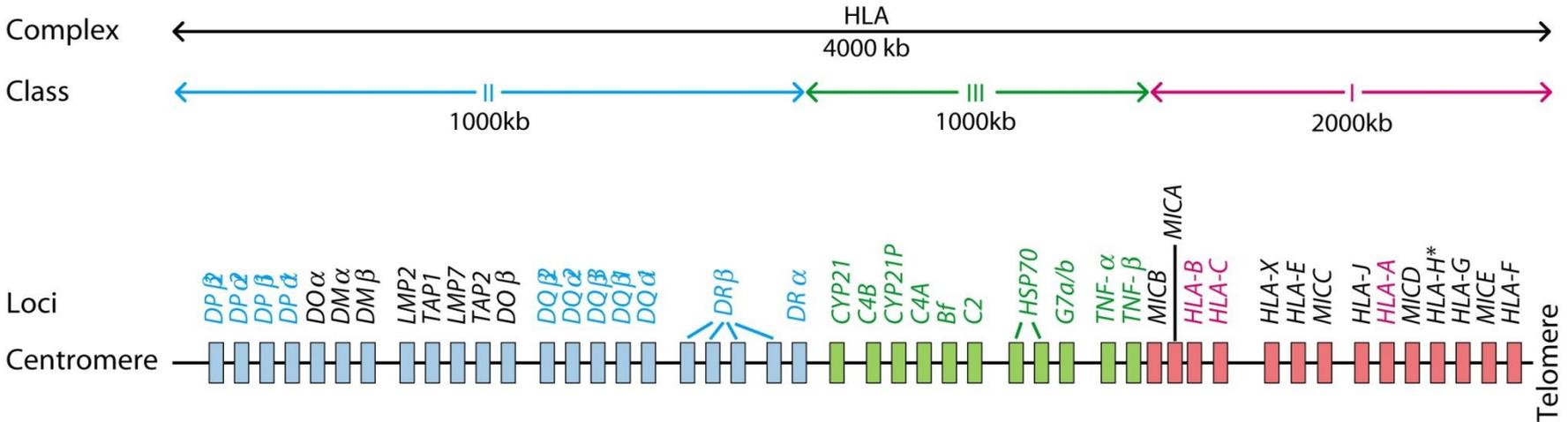
Class I And II Diversity And Polymorphism

- MHC Is One Of The Most Polymorphic Complexes Known
- Alleles Can Differ Up To 20 a/a
- Class I Alleles In Humans: 240 A, 470 B, 110 C
- Class II Alleles In Humans: HLA-DR 350 β , 2 α !
- HLA-DR
 - β genes vary from 2-9 in different individuals!!!,
 - 1 α gene (α can combine with all β products increasing number of APC molecules)
- DP (2 α , 2 β) and DQ (2 α , 3 β)

MOUSE CHROMOSOME 17



HUMAN CHROMOSOME 6



KEY

Gene	Encoded protein
C2, C4A, C4B, Bf	Complement components
CYP21, CYP21P	Steroid 21-hydroxylases
G7a/b	Valyl-tRNA synthetase
HSP	Heat-shock protein
LMP2, LMP7	Proteasome-like subunits
TAP1, TAP2	Peptide-transporter subunits
TNF-α, TNF-β	Tumor necrosis factors α and β

*Now designated HFE

Class I MHC Peptides

- Peptides Presented Thru MHC I Are Endogenous Proteins
- As Few As 100 Peptide/MHC Complex Can Activate T_{sup}
- Peptide Features
 - size 8-10 a/a, preferably 9
- Peptides Bind MHC Due To Presence Of Specific a/a Found At The Ends Of Peptide. Ex. Glycine @ Position 2

Class II MHC Peptides

- Peptides Presented Thru MHC II Are Exogenous
 - Processed thru endocytic pathway
- Peptides Are Presented To T_H
- Peptides Are 13-18 a/a Long
- Binding Is Due To Central 13 a/a
- Longer Peptides Can Still Bind MHC II
 - Like A long hot dog
- MHC I Peptides Fit Exactly, Not The Case With MHC II Peptides

MHC Expression

- Expression Is Regulated By Many Cytokines
 - IFN α , IFN β , IFN γ and TNF Increase MHC expression
- Transcription Factors That Increase MHC gene Expression
 - CIITA (Trans-activator), RFX (Trans-activator)
- Some Viruses Decrease MHC Expression
 - CMV, HBV.
- Reduction Of MHC May Allow For Immune System Evasion

MECHANISM FOR PROCESSING ANTIGENS FROM EXTRACELLULAR PATHOGENS

- Extracellular microorganisms and toxins engulfed by phagocytosis / endocytosis in
 - Phagosomes / endosomes
- Phagosomes fuse with lysosomes (proteases/hydrolases) forming phagolysosome
- Peptides produced bind with MHC class II molecules within vesicular system
- Peptide:MHC class II complexes transported to cell surface

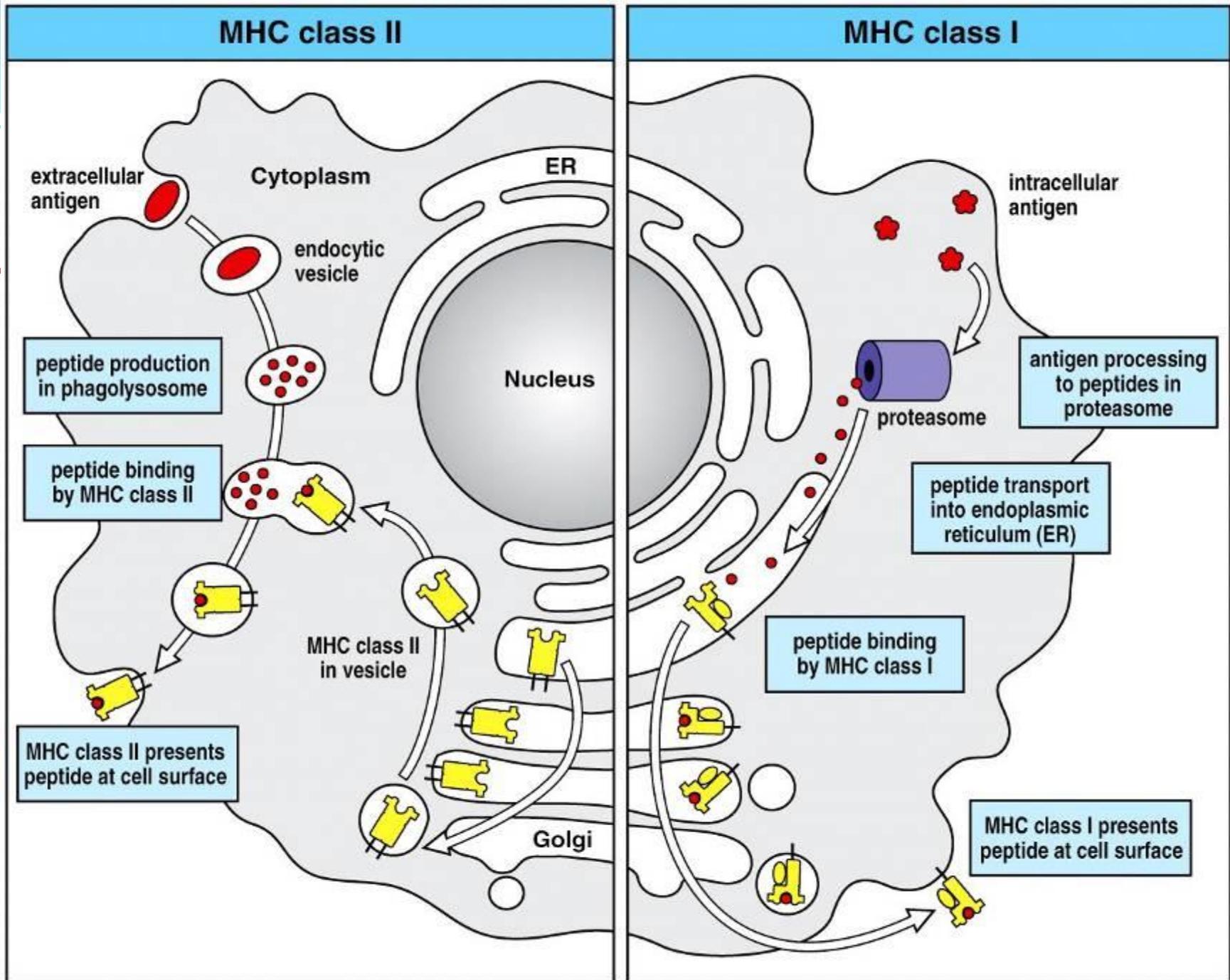


Figure 3-19 The Immune System, 2/e (© Garland Science 2005)

MECHANISM FOR PROCESSING ANTIGENS FROM EXTRACELLULAR PATHOGENS

- MHC class II alpha and beta chains transported into ER
- In ER, associated with “invariant chain” which functions
 - Prevent peptide binding
 - Chaperones MHC II molecules to endosomes
- In endosomes, invariant chain degraded by
 - Cathepsin L
- Degradation results in small fragment which covers MHC II peptide binding site
 - Class II associated invariant chain peptide (CLIP)

MECHANISM FOR PROCESSING ANTIGENS FROM EXTRACELLULAR PATHOGENS

- CLIP removal associated with
 - Interaction of MHC II and endosome membrane glycoprotein
 - HLA-DM
- HLA-DM
 - Similar structure to MHC II
 - Does not bind peptides or appear on cell surface
- MHC II quickly binds peptide or is degraded
- Peptide:MHC II transported to cell surface for recognition by specific T-cell receptor

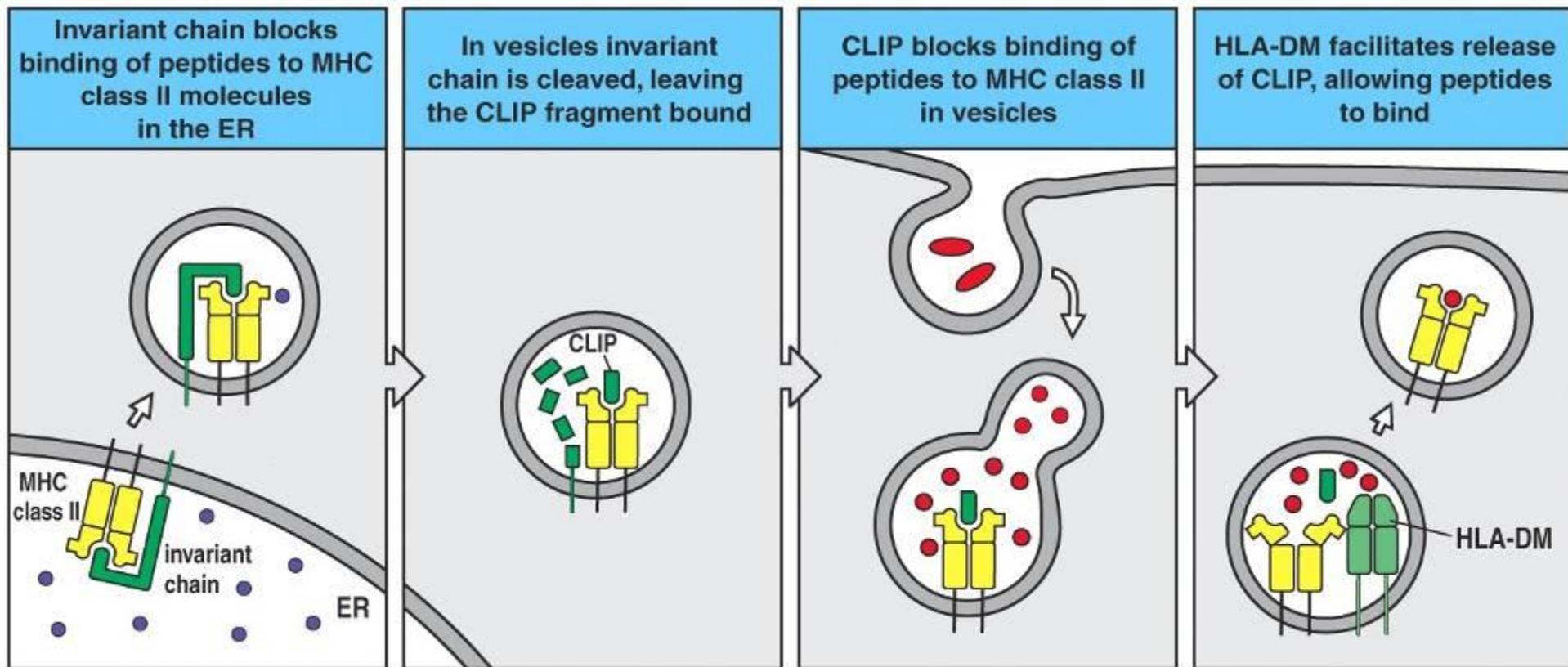


Figure 3-20 The Immune System, 2/e (© Garland Science 2005)

EXPRESSION OF MHC I AND MHC II ON HUMAN CELLS

- MHC class I
 - Guard the intracellular territory
 - Constitutive expression on virtually all cells
 - Comprehensive surveillance by CD8 T-cells
- MHC class II
 - Guard the extracellular territory
 - Constitutive expression only on APC's
 - Macrophages
 - B lymphocytes
 - Dendritic cells (immature)

Tissue/cell	MHC	
	class I	class II
Hematopoietic		
T cells	+++	+*
B cells	+++	+++
Macrophages	+++	++
Dendritic cells	+++	+++
Neutrophils	+++	-
Erythrocytes	-	-
Non-hematopoietic		
Thymic epithelium	+	+++
Liver hepatocytes	+	-
Kidney epithelium	+	-
Brain	+	-†

Figure 5.23 The Immune System, 3ed. (© Garland Science 2009)

EXPRESSION OF MHC I AND MHC II ON HUMAN CELLS

- Antigen uptake by APC's
 - Macrophages
 - Phagocytosis and pinocytosis in all tissues
 - B lymphocytes
 - Internalize antigens bound to surface IG
 - Receptor-mediated endocytosis
 - Dendritic cells (immature)
 - Phagocytosis and macropinocytosis in all tissues
- Cytokine upregulation of MHC I and II in immune response
 - Interferons

MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)

- Named MHC following identification of region responsible for rejection of tissue or organ transplant
- MHC molecules encoded by a number of closely linked genes on chromosome 6
 - Conventional gene configuration
- Large number of variants in human population
- Variants responsible for
 - Host versus graft
 - Graft versus host

MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)

- Complex also called
 - Human leukocyte antigen (HLA) complex
 - Antibodies originally used to identify MHC molecules react with leukocytes
- HLA I genes and HLA II genes
 - Located on short arm of chromosome 6
- Beta-2-microglobulin (C-15) and invariant chain (C-5) not located in HLA region

MECHANISMS OF DIVERSITY IN MHC MOLECULES

- Polygeny (polygenic)
 - Multiple genes encode alpha chain of MHC I molecules
 - Multiple genes encode alpha and beta chains of MHC II molecules
- Polymorphism (polymorphic)
 - Multiple alternative forms of MHC I and MHC II genes in human population
 - Alternative gene forms called “alleles”

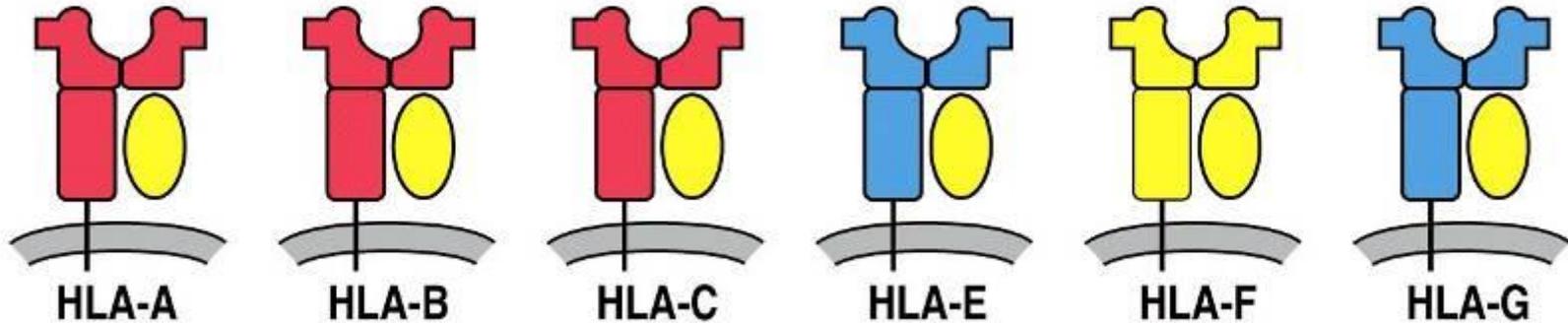
POLYGENY AND POLYMORPHISM IN HUMAN MHC CLASS I MOLECULES

- Polygeny (multiple genes)
 - 3 genes for alpha chain
 - HLA-A, HLA-B and HLA-C
- Polymorphism (multiple alleles)
 - Alleles
 - HLA-A (506)
 - HLA-B (872)
 - HLA-C (274)

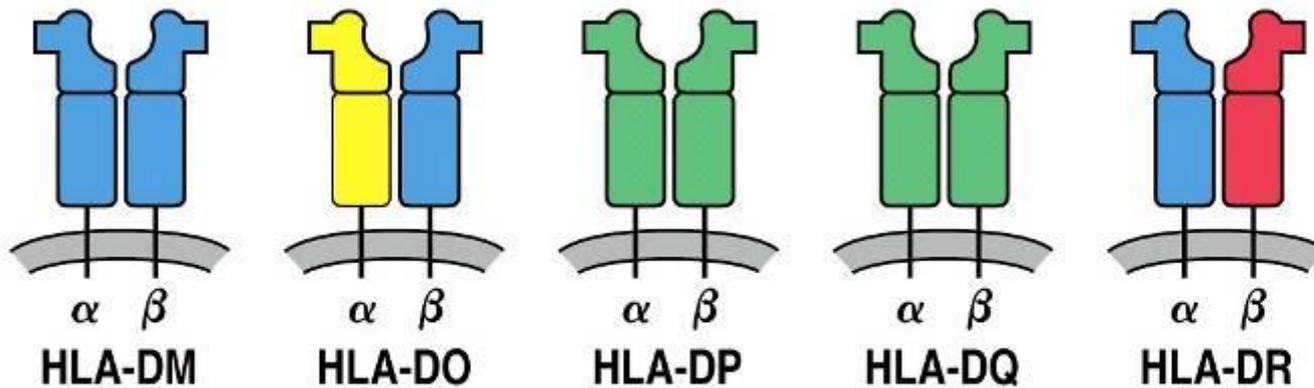
POLYGENY AND POLYMORPHISM IN HUMAN MHC CLASS II MOLECULES

- Polygeny (multiple genes)
 - HLA-DP
 - 1 gene for each alpha and beta chain
 - HLA-DQ
 - 1 gene for each alpha and beta chain
 - HLA-DR
 - 1 gene for alpha chain
 - DRA
 - 4 genes for beta chain
 - DRB₁, DRB₃, DRB₄, DRB₅
- Polymorphism (alleles)
 - Multiple alleles for all genes except DRA

Human MHC class I isotypes



Human MHC class II isotypes



■ highly polymorphic ■ polymorphic ■ oligomorphic ■ monomorphic

Figure 3-23 The Immune System, 2/e (© Garland Science 2005)

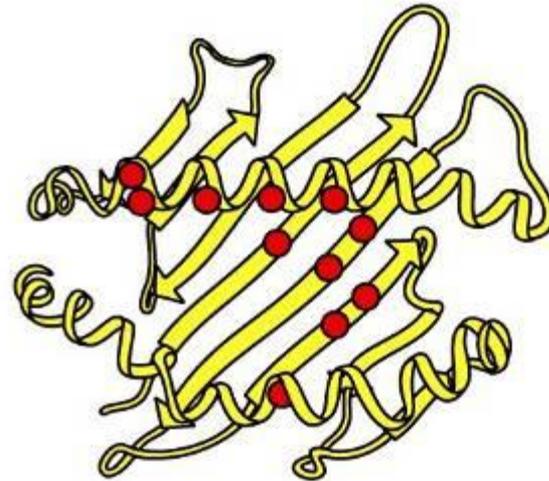
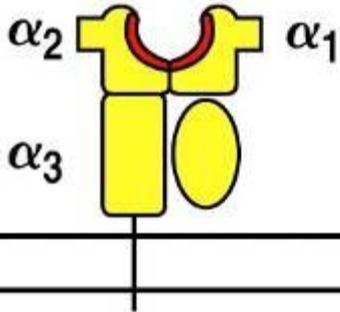
HLA polymorphism		
MHC class	HLA locus	Number of allotypes
MHC class I	A	506
	B	872
	C	274
	E	3
	F	4
	G	10
	MHC class II	DMA
DMB		7
DOA		3
DOB		4
DPA1		15
DPB1		114
DQA1		25
DQB1		66
DRA		2
DRB1		466
DRB3		37
DRB4		7
DRB5		15

Figure 5.25 The Immune System, 3ed. (© Garland Science 2009)

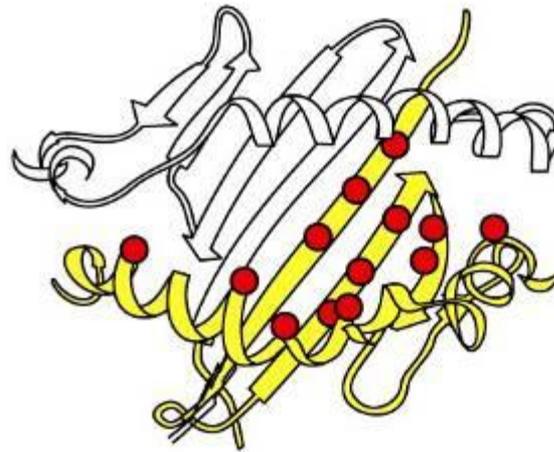
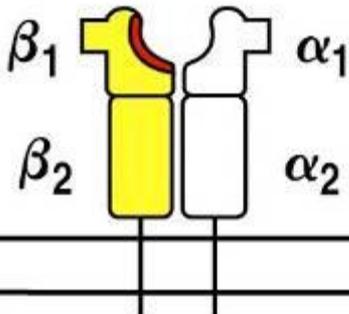
MHC POLYMORPHISM AND REJECTION OF TRANSPLANTED TISSUES AND ORGANS

- MHC molecules primary reason for transplant rejection
- Allogeneic
 - Genetic differences between two members of same species
- Alloantigens
 - Antigens which differ between members of same species
- Alloreaction
 - Immune response to alloantigens
- MHC allotype variation is clustered in peptide binding site

MHC class I variability



MHC class II variability



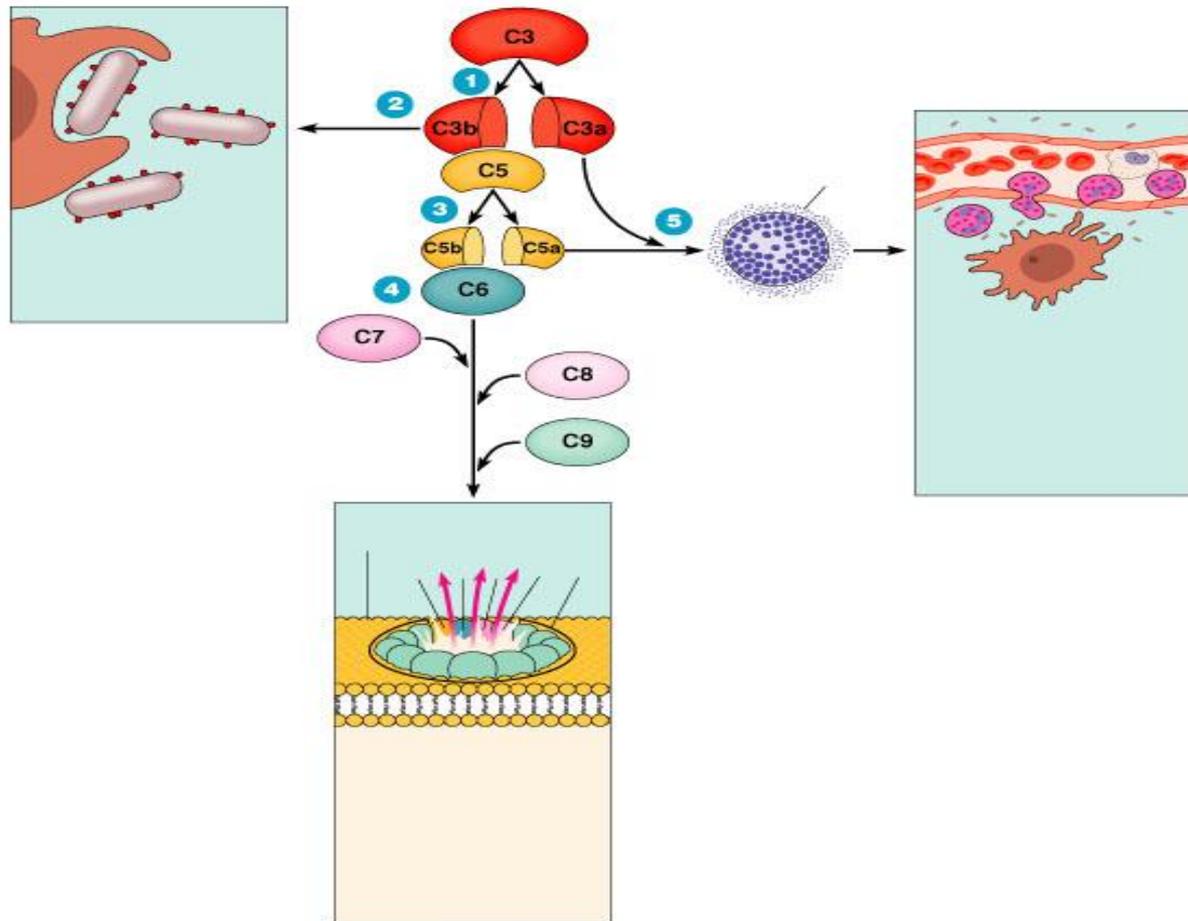
HUMAN LEUKOCYTE ANTIGEN (HLA) COMPLEX

- HLA type
 - Combination of HLA class I and HLA class II allotypes
- HLA typing in medicine
 - Selection of donors and recipients for transplantation
- Transplantation of organs
 - Problem of graft rejection by recipient
 - HLA mismatches overcome using immunosuppressive agents
- Transplantation of bone marrow
 - Problem of alloreaction of graft against recipients tissues

The Complement system

Lecture 12

The complement system



The complement system

- A defensive system consisting of over 30 proteins produced by the liver and found in circulating blood serum.
- Complement kills microbes in three different ways
 - 1. opsonization
 - 2. inflammation
 - 3. Cytolysis

A Cascade system

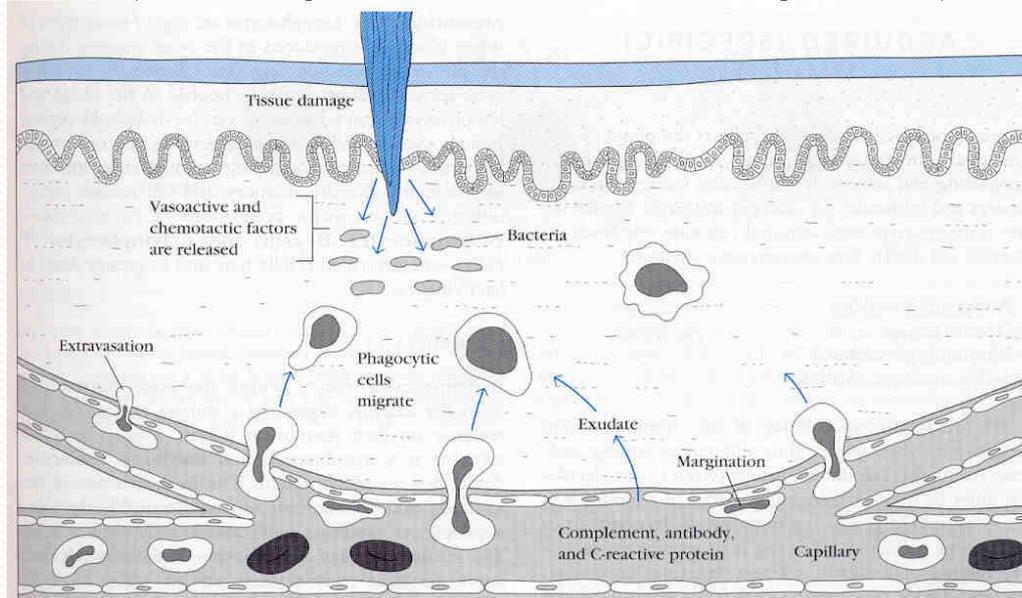
- The complement works as a cascade system.
 - Cascade is when one reaction triggers another reaction which trigger others and so on. These types of systems can grow exponentially very fast.

Cascade activation

- Complement proteins are often designated by an uppercase letter C and are inactive until they are split into products.
 - Example: C₁
- When the products are split they become active. The active products are usually designated with a lower case a or b.
 - Example: C_{1a} and C_{1b}

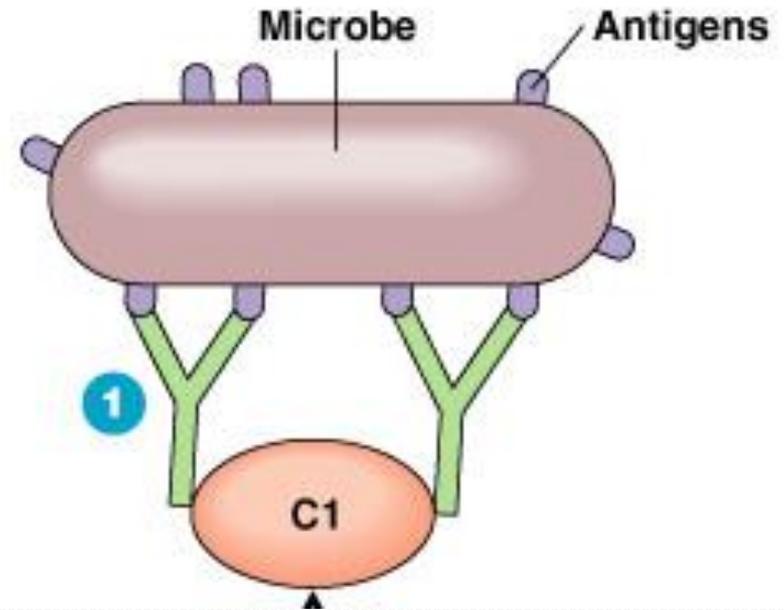
Two Pathways

- The complement pathway can be activated by either of two different pathways.
 - Classical pathway (specific immune system)
 - alternative (non-specific immune system)



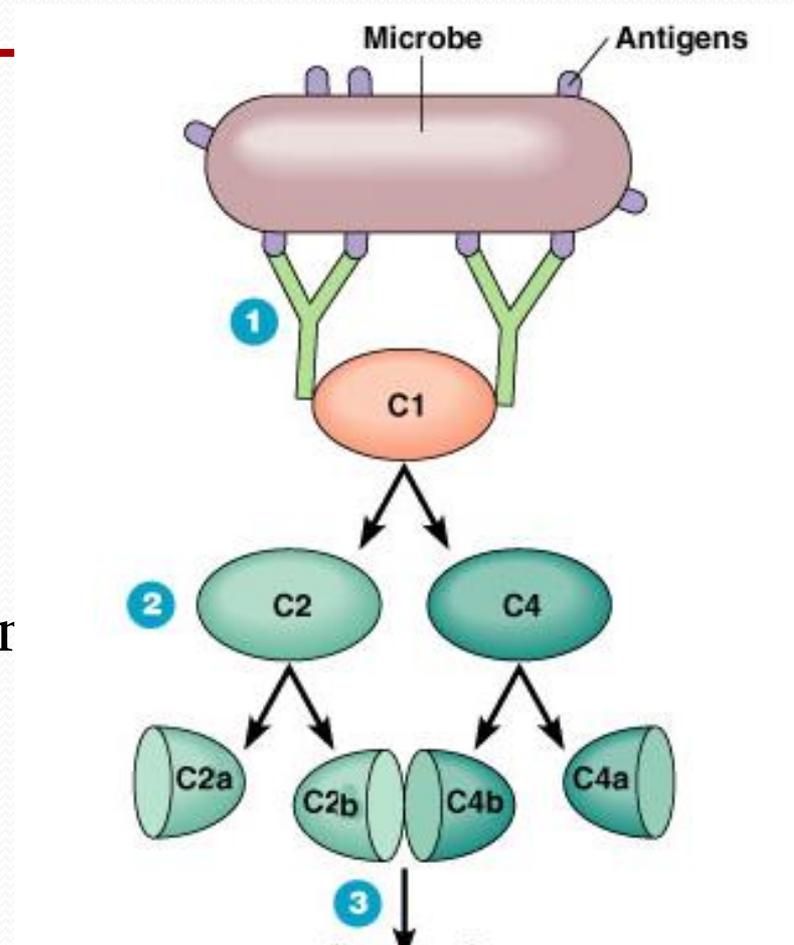
The Classical Pathway

- The classical pathway is considered to be part of the specific immune response because it relies on antibodies to initiate it.
- C1 becomes activated when it binds to the ends of antibodies



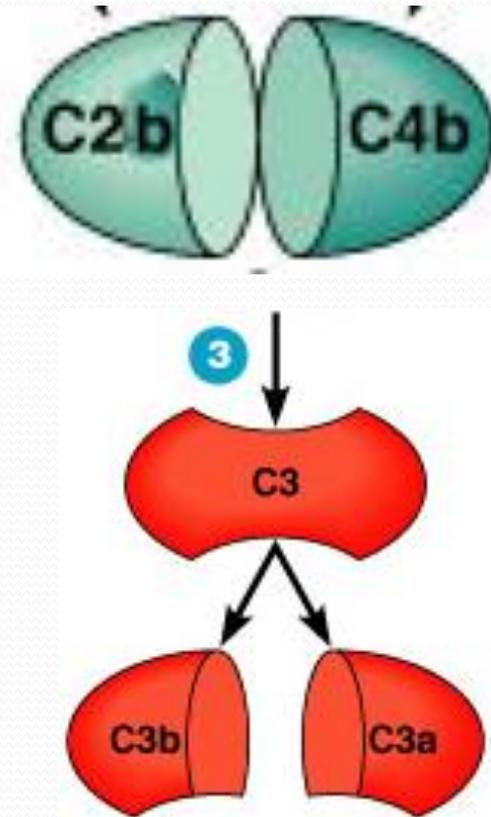
The building of a C3 activation complex

- Once C1 is activated, it activates 2 other complement proteins, C2 and C4 by cutting them in half
- C2 is cleaved into C2a and C2b
- C4 is cleaved into C4a and C4b
- Both C2b and C4b bind together on the surface of the bacteria
- C2a and C4a diffuse away



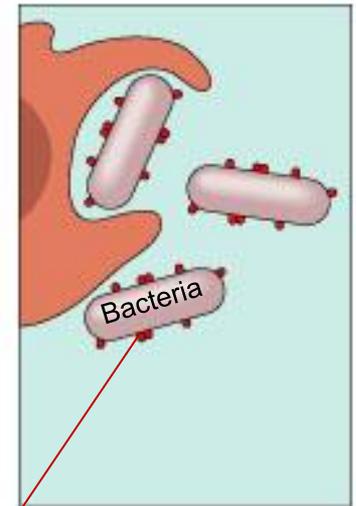
C3 Activation complex

- C2b and C4b bind together on the surface to form a **C₃ activation complex**
- The function of the C₃ activation complex is to activate C₃ proteins.
 - This is done by cleaving C₃ into C₃a and C₃b



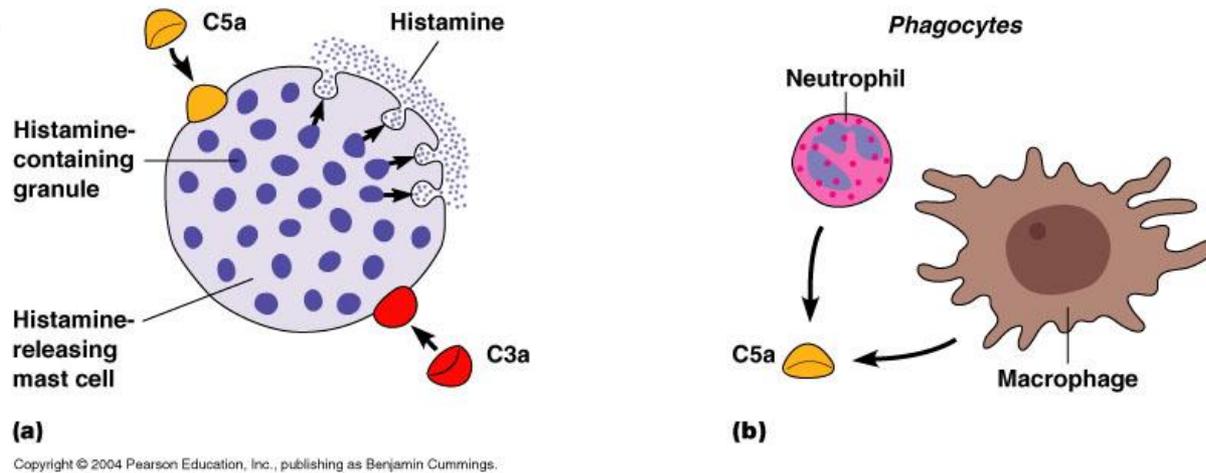
C3b

- Many C3b molecules are produced by the C3 activation complex.
- The C3b bind to and coat the surface of the bacteria
- C3b is an opsonin
 - Opsonins are molecules that bind both to bacteria and phagocytes
 - Opsonization increases phagocytosis by 1,000 fold.



Opsonins

C3a



C3a increases the inflammatory response by binding to mast cells and causing them to release histamine

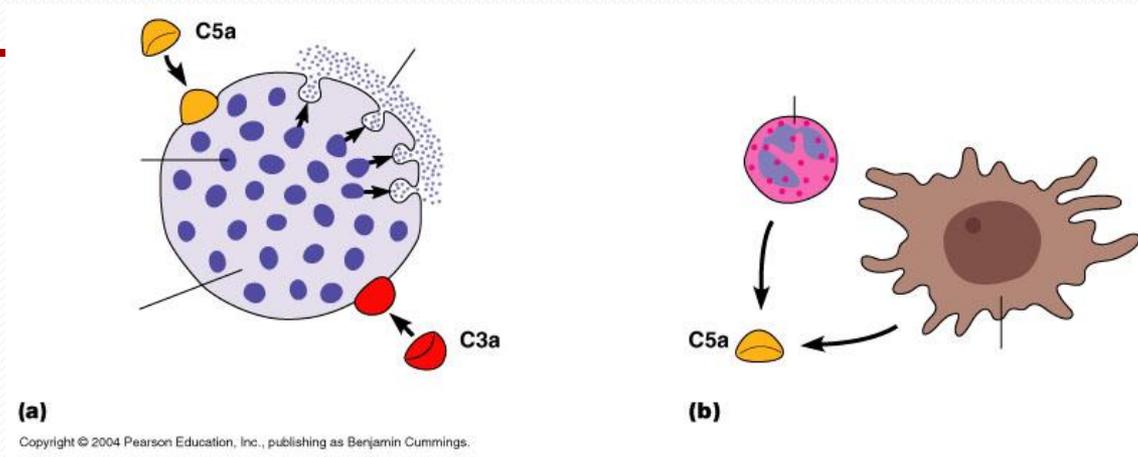
Building the C5 activation complex

- Eventually enough C3b is cleaved that the surface of the bacteria begins to become saturated with it.
- C2b and C4b which make up the C3 activation complex has a slight affinity for C3b and C3b binds to them
- When C3b binds to C2b and C4b it forms a new complex referred to as the C5 activation complex

The C5 activation complex

- The C5 activation complex (C2b, C4b, C3b) activates C5 proteins by cleaving them into C5a and C5b
- Many C5b proteins are produced by the C5 activation complex. These C5b begin to coat the surface of the bacteria.

The function of C5a



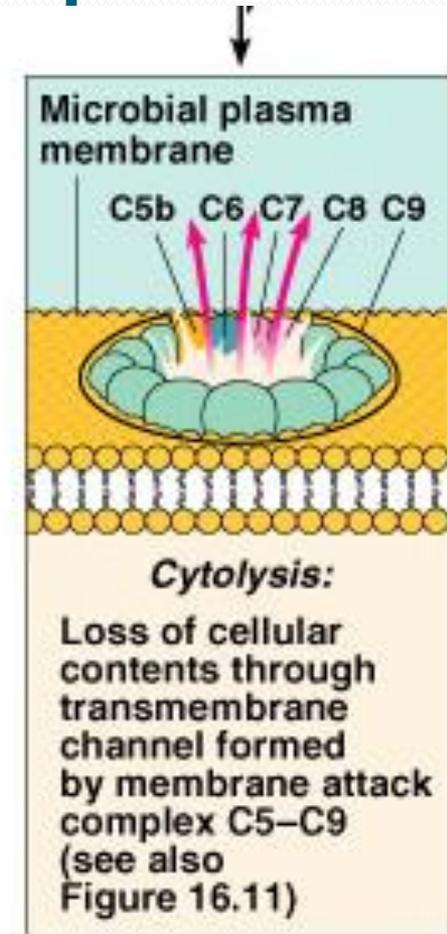
- C5a disperses away from the bacteria.
 - Binds to mast cells and increases inflammation.
 - Most powerful chemotactic factor known for leukocytes

Building the Membrane Attack complex

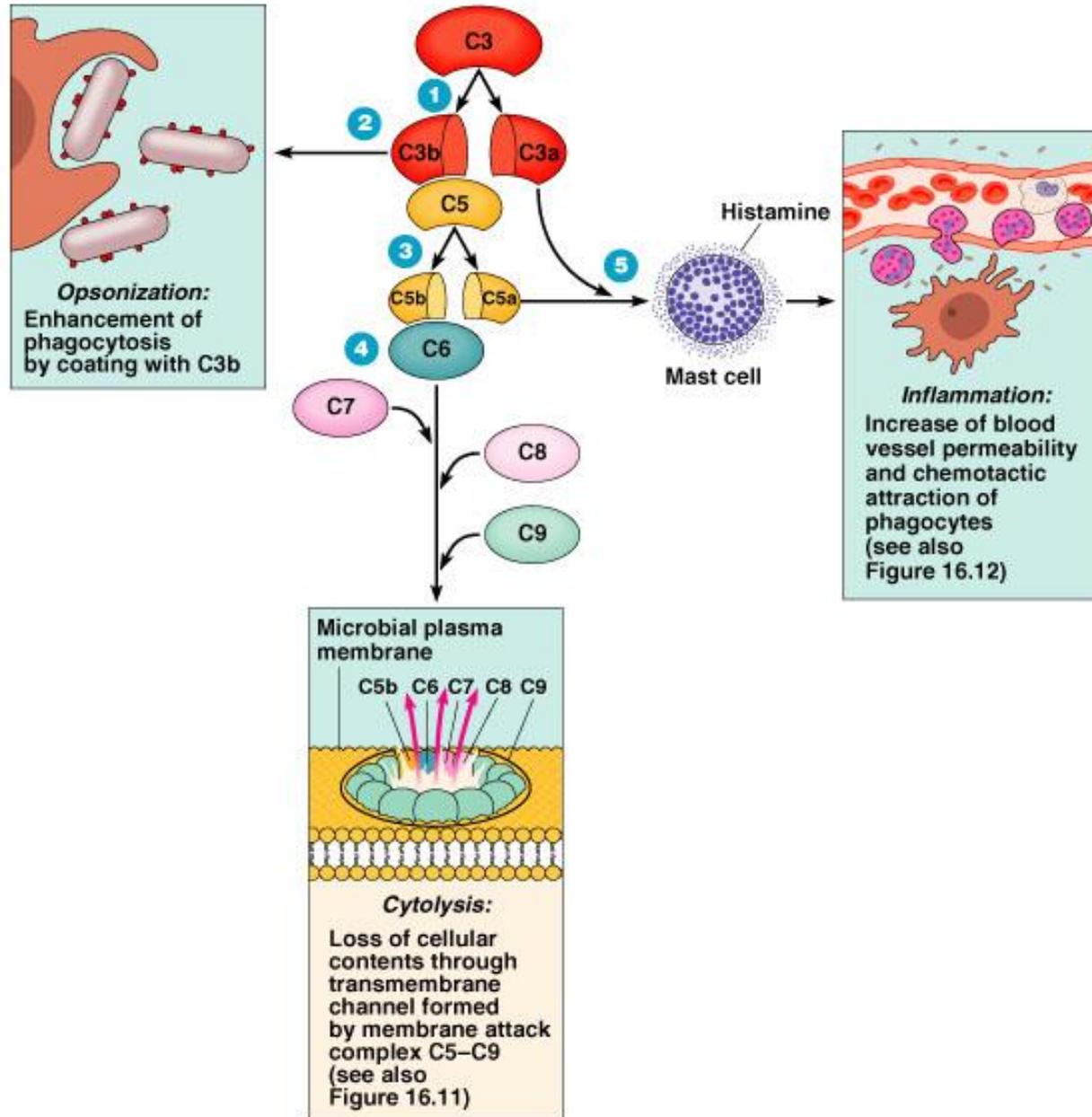
- C5b on the surface of bacteria binds to C6
- The binding of C6 to C5b activates C6 so that it can bind to C7
- C7 binds to C8 which in turn binds to many C9's
- Together these proteins form a circular complex called the Membrane attack complex (MAC)

Membrane Attack complex

- The MAC causes Cytolysis.
 - The circular membrane attack complex acts as a channel in which cytoplasm can rush out of and water rushes in.
- The cells inner integrity is compromised and it dies
- [Animation of the classical pathway](#)



Overview

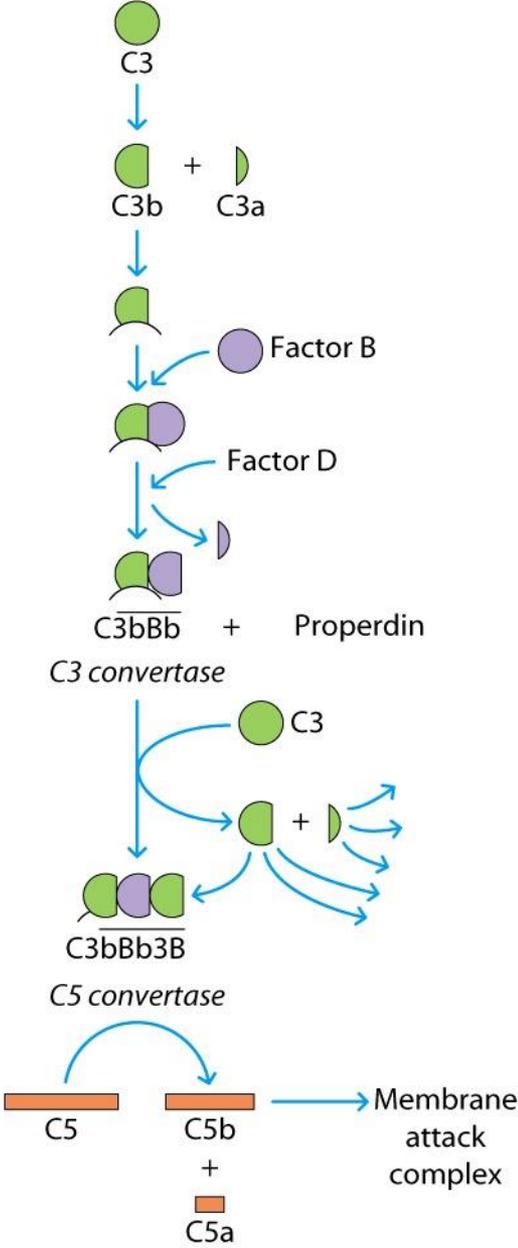


The alternative pathway

Properdin Pathway

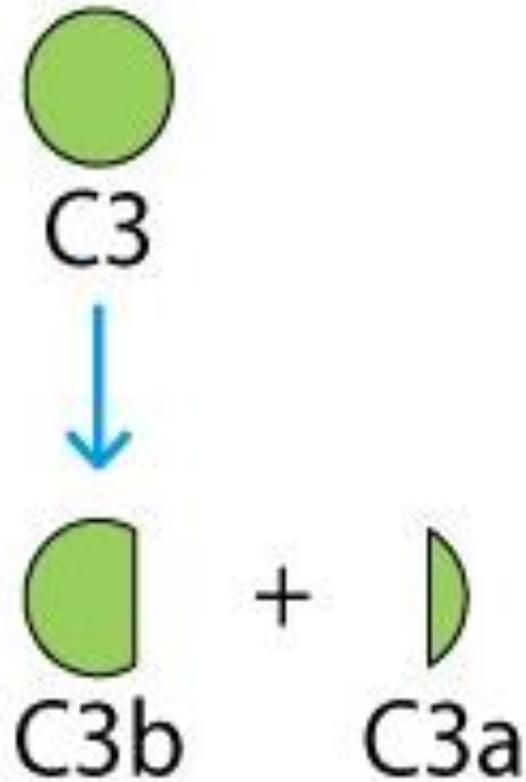
- The alternative pathway is part of the non-specific defense because it does not need antibodies to initiate the pathway.
- The alternative pathway is slower than the Classical pathway

The Alternative complement pathway



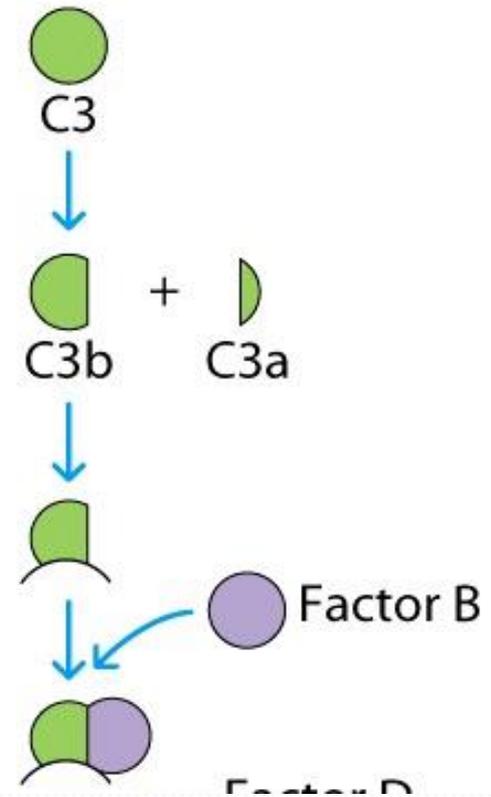
Initiation of The Alternative pathway

- C₃ contains in unstable thioester bond.
- This unstable bond makes C₃ subject to slow spontaneous hydrolysis to C₃b and C₃a
- The C₃b is able to bind to foreign surface antigens.
- Mammalian cells contain sialic acid which inactivates C₃b



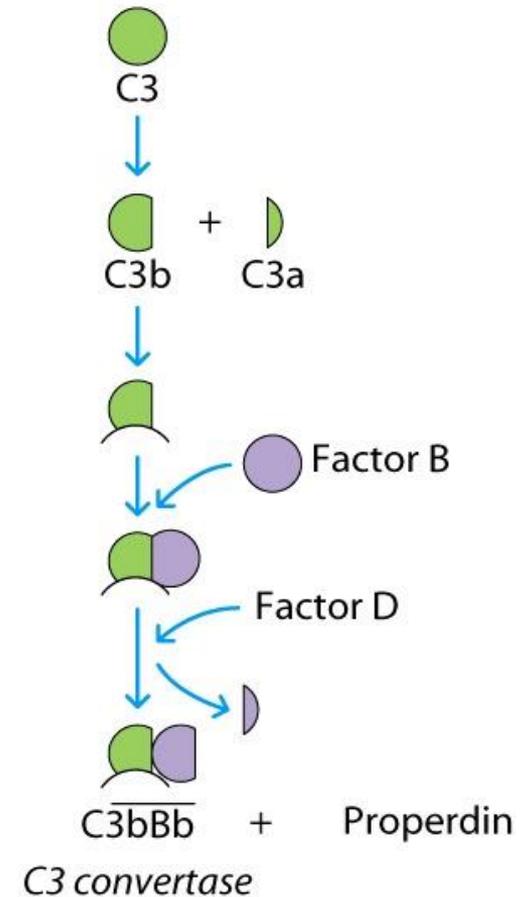
Factor B

- C3b on the surface of a foreign cells binds to another plasma protein called factor B

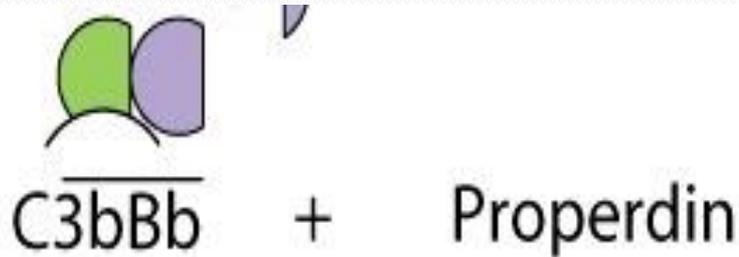


Factor D

- The binding of C₃b to factor B allows a protein enzyme called Factor D to cleave Factor B to Ba and Bb.
- Factor Bb remains bound to C₃b while Ba and Factor D disperse away.



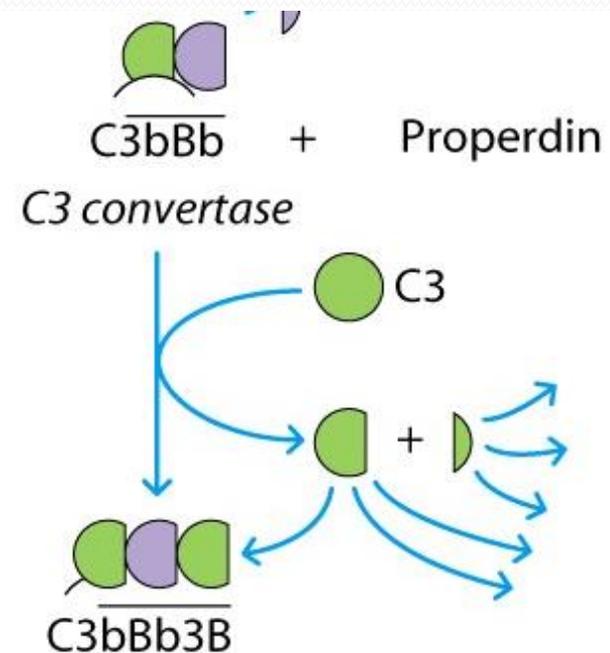
The C3 activation complex



- Properdin, also called factor P, binds to the $C3bBb$ complex to stabilize it.
- $C3bBbP$ make up the $C3$ activation complex for the alternative pathway

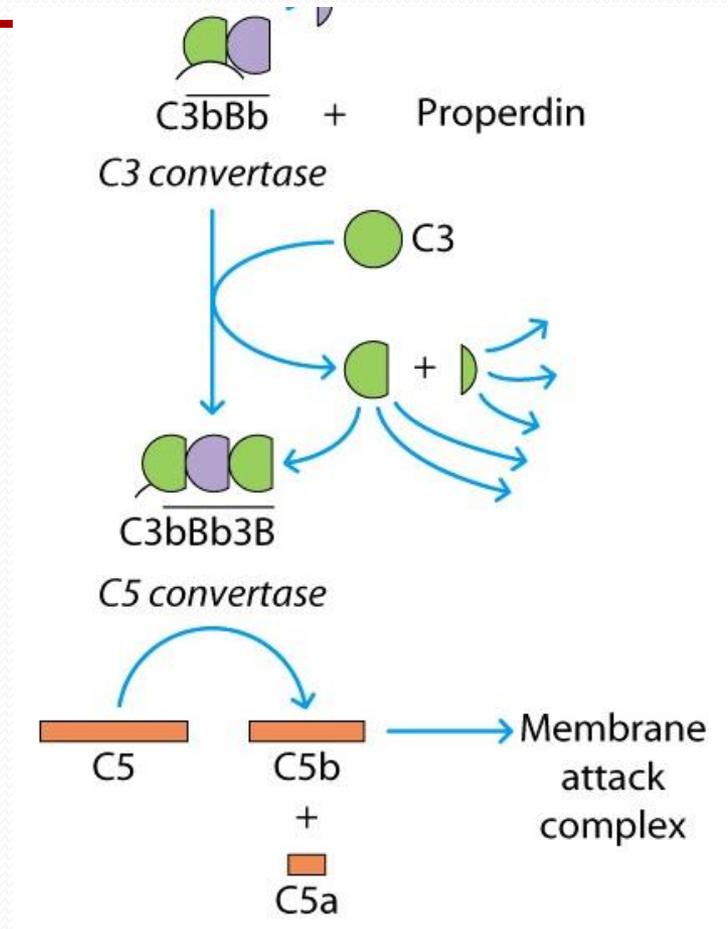
The C3 activation Complex

- The C3 activation complex causes the production of more C3b.
- This allows the initial steps of this pathway to be repeated and amplified
- 2×10^6 molecules can be generated in 5 minutes

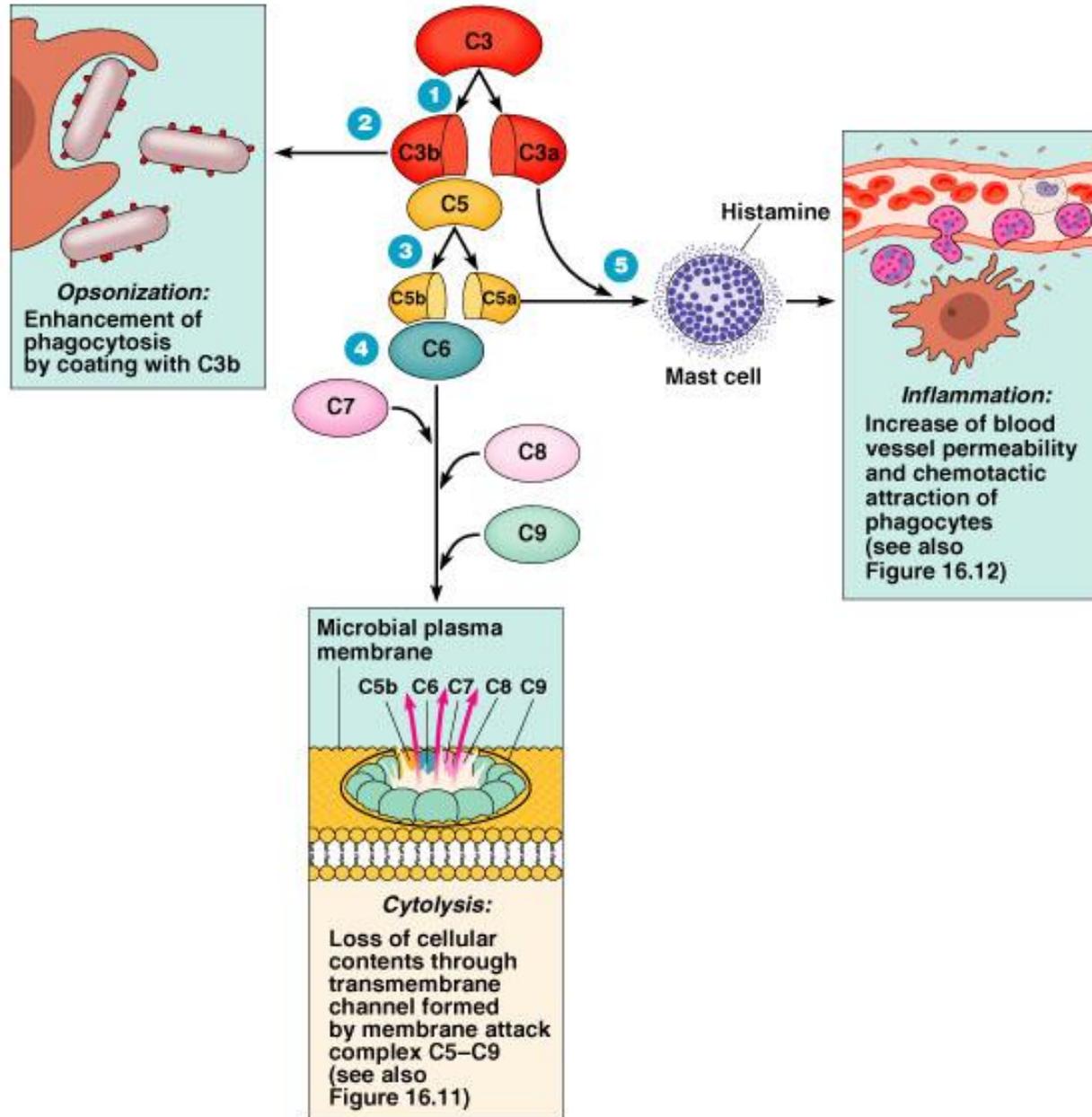


C5 activation complex

- When an additional C3b binds to the C3 activation complex it converts it into a C5 activation complex.
- The C5 activation complex cleaves C5 into C5a and C5b.
- C5b begins the production of the MAC.



Overview



Complement Deficiencies

- Major Pathway components
 - Hereditary deficiency of any component except C9 results in increased susceptibility to infection and delayed clearance of complexes.
 - Deficiency of MBL important during infant transition to making their own antibody, associated with pneumonia, sepsis and meningococcal disease.
 - C3 deficiency most serious, key mediator in ALL pathways. Prone to serious life threatening infections and immune complex disease.
 - Deficiency of terminal components causes increased susceptibility to *Neisseria* infections.

Complement Deficiencies

- Regulatory Factor components
 - PNH – RBCs deficient in DAF, results in hemolysis due to “bystander” affect.
 - Hereditary angiodema results in excess cleavage of C₄ and C₂,
 - keeps classical pathway going
 - Results in increased vascular permeability causing edema.
 - Usually spontaneously subsides but can be life threatening – oropharynx
 - Inhibitors of factors H and I produces constant turnover of C₃ to depletion – recurrent bacterial infections occur.

DISORDERS OF THE IMMUNE SYSTEM

- Hypersensitivity Reactions
 - Over-reaction of adaptive immune response to harmless antigens
 - Four Types of reactions (I- IV)
- Autoimmunity
 - Misdirected adaptive immune response
 - Results from a loss of self-tolerance
 - Three Types (II, III, IV) of reactions
- Immunodeficiencies
 - Components of immune system either absent or defective
 - Genetic or acquired etiology



Cytokines

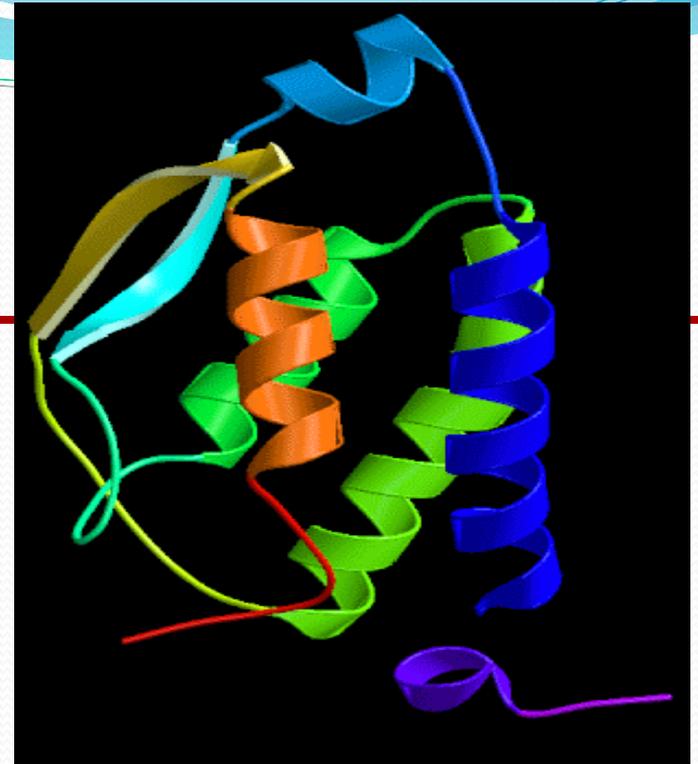
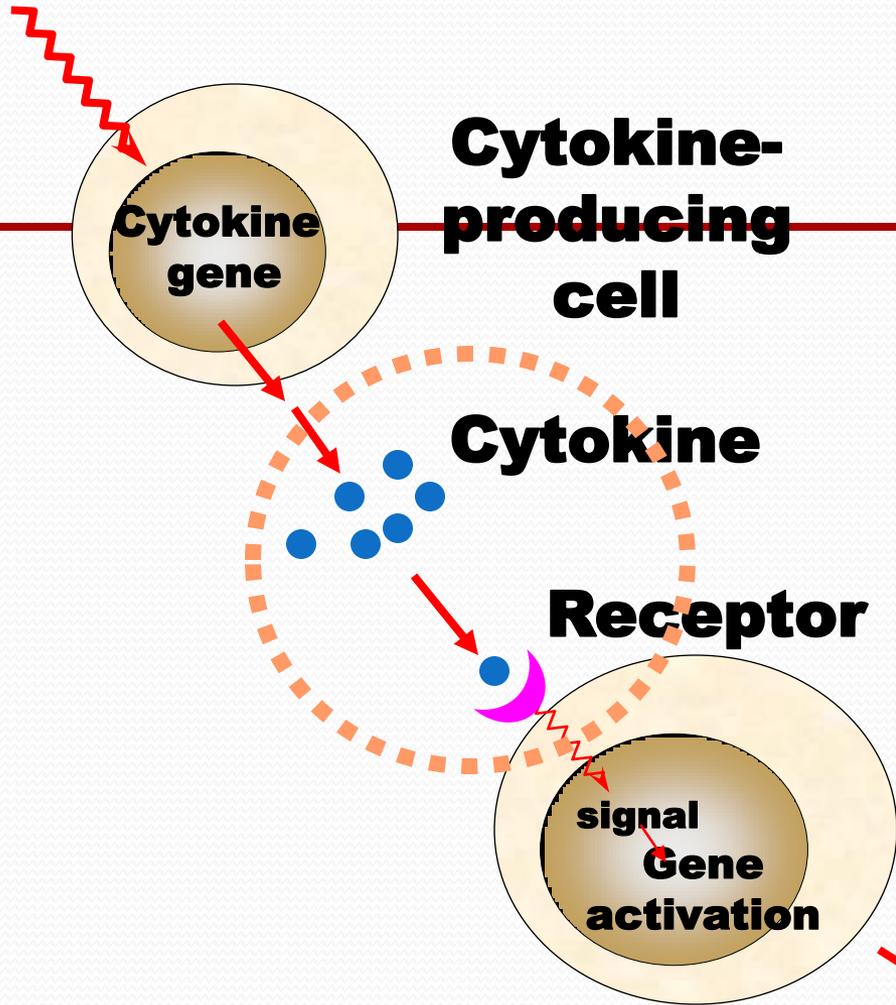
Lecture 13

Introduction to cytokines

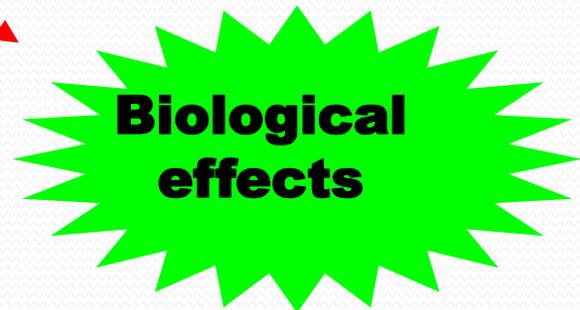
- **Cytokine (CK)**

Cytokines are small proteins (8-80 KD) that are secreted by cells and exert biological activity through specific cell surface receptors.

Inducing stimulus



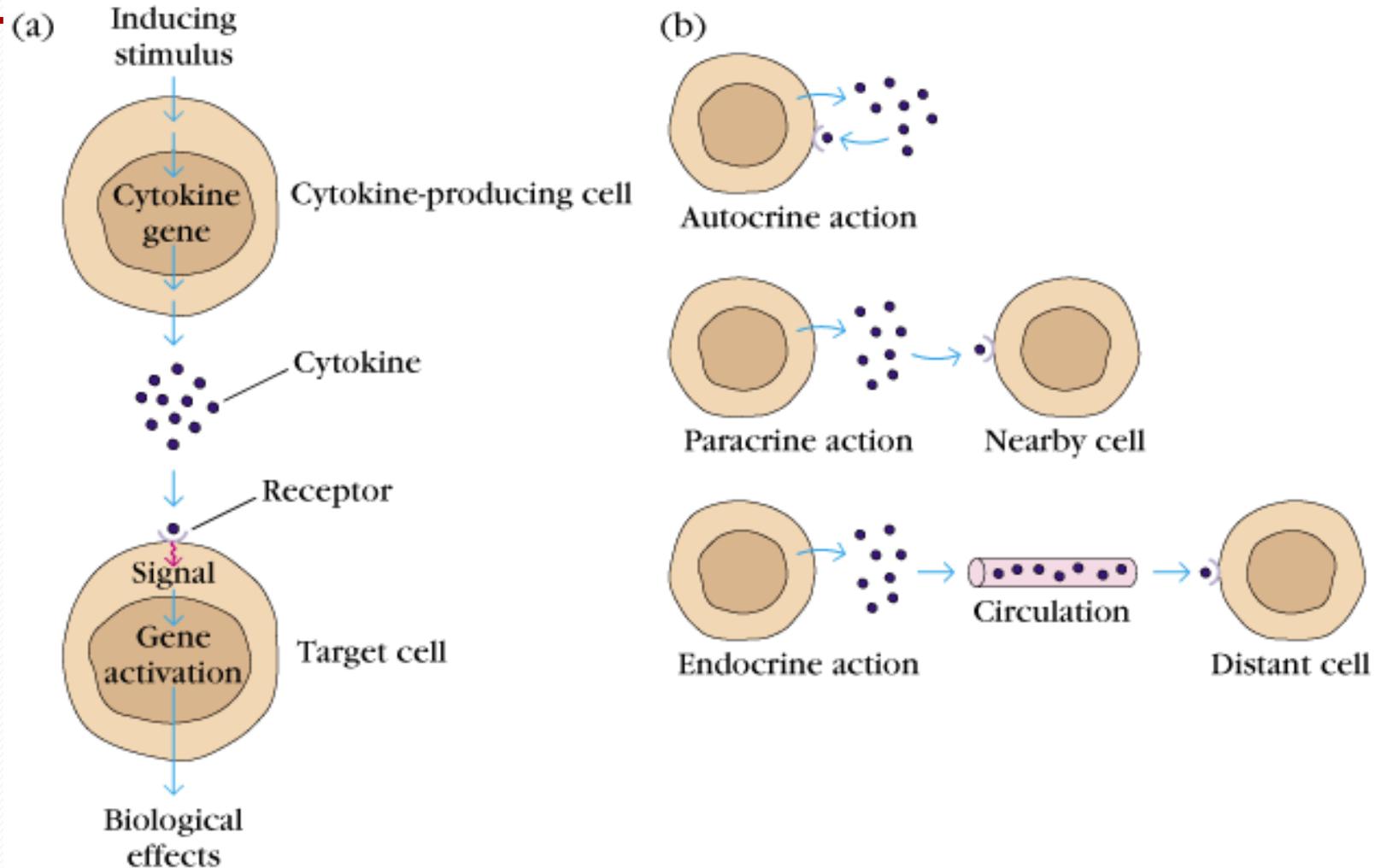
Overview of the induction and function of Cytokine



General properties of CKs

- **Small proteins (MW: approx. 15-80 KD)**
- **Extremely potent, acting at pM or fM**
- **The production is transient and tightly regulated**
- **Autocrine, paracrine or endocrine**
- **Non-specific and non-MHC restricted**

3 MAJOR ROUTES OF CYTOKINE TRAVEL



■ **Cytokine actions**

1) Pleiotropy

Acts on more than one cell type

2) Redundancy

More than one cytokine have the same action

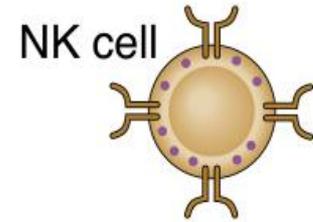
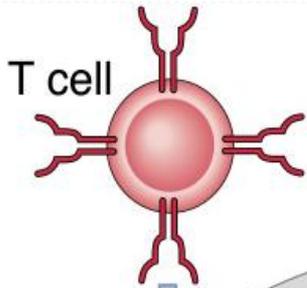
3) Synergy

Two or more cytokines cooperate to produce an effect that is different or greater than the combined effect of the two cytokines when functioning separately

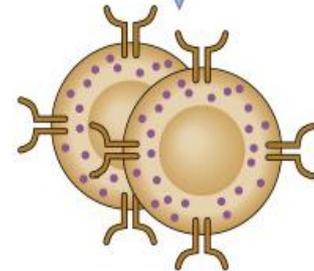
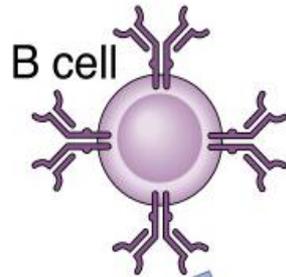
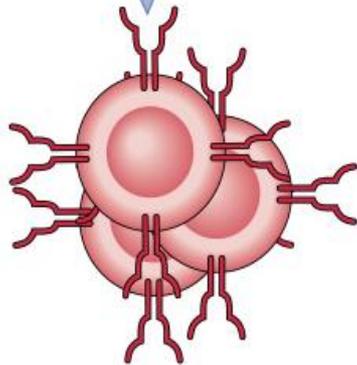
4) Antagonism

Two or more cytokines work against each other

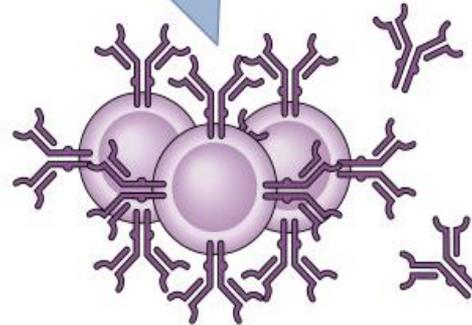
5) The cytokine network



**Interleukin-2
(IL-2)**

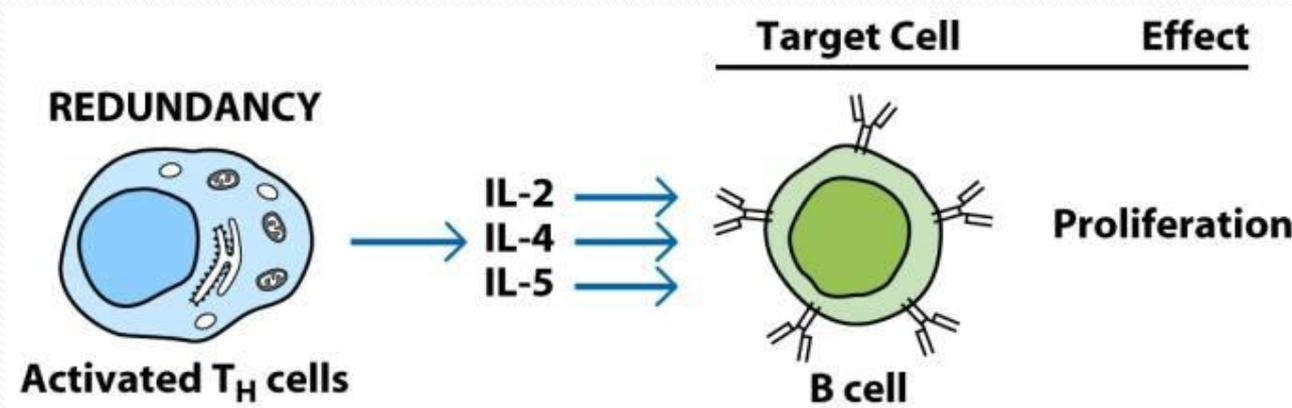


**T cell proliferation,
increased cytokine
production (IL-4, IFN- γ)**



**NK cell
proliferation, increased
cytolytic activity**

**B cell proliferation,
antibody production**



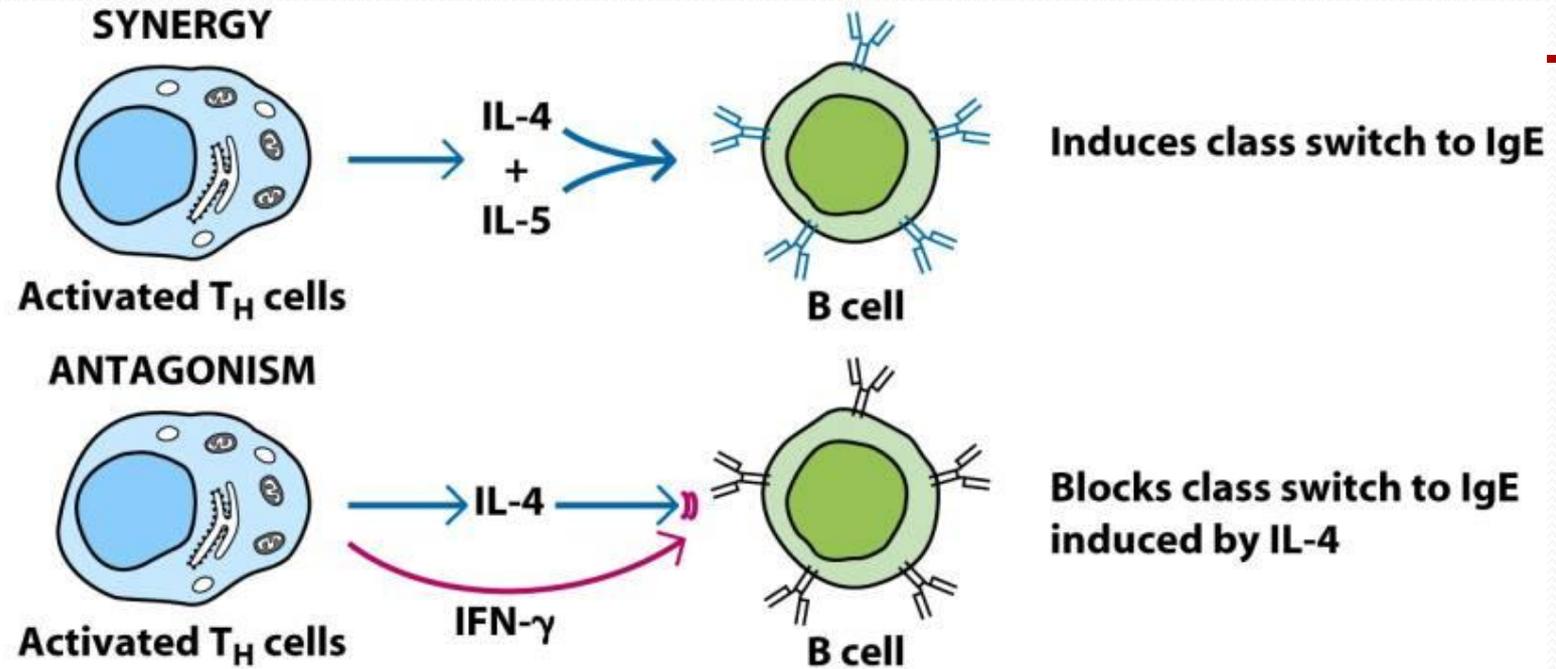
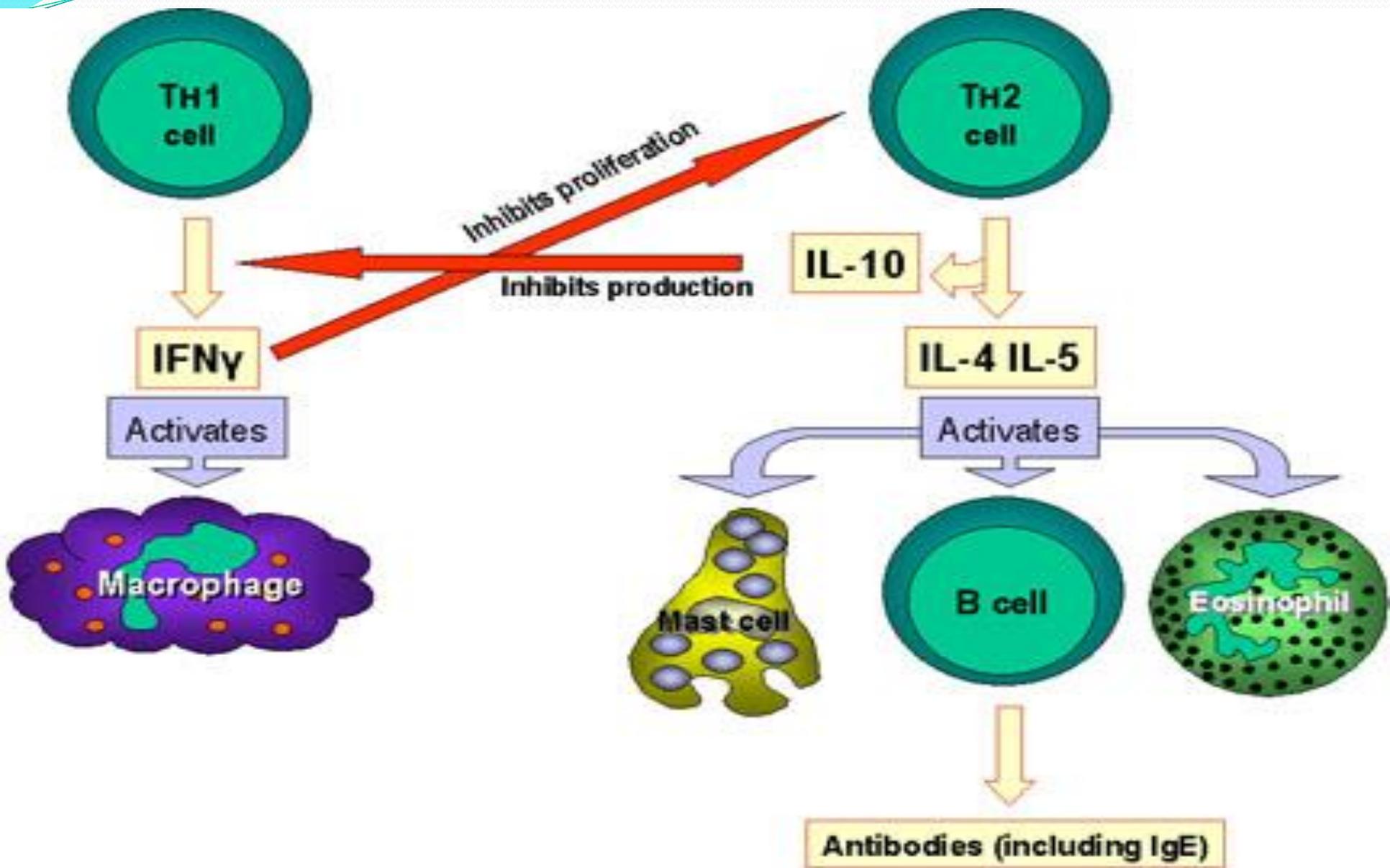


Figure 12-2a part 2
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Categories of CKs

CKs are classified into 6 functional categories

- **Interleukin (IL)**
- **Interferon (IFN)**
- **Tumor necrosis factor (TNF)**
- **Colony stimulating factor (CSF)**
- **Chemokine**
- **Growth factor (GF)**

Interleukin, IL

- IL_{1~38}

IL-1

IL-2

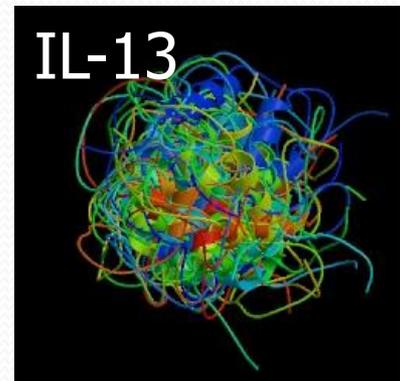
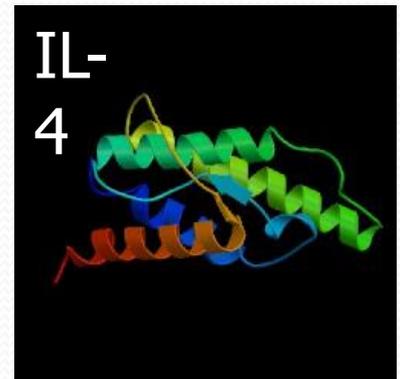
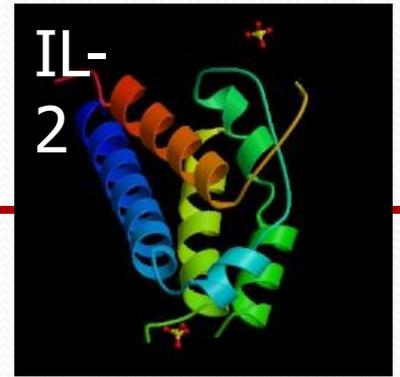
IL-4, IL-5, IL-6, IL-13 (Th₂ type)

IL-8 (belongs to chemokine family)

IL-10 (Tr, Ts type)

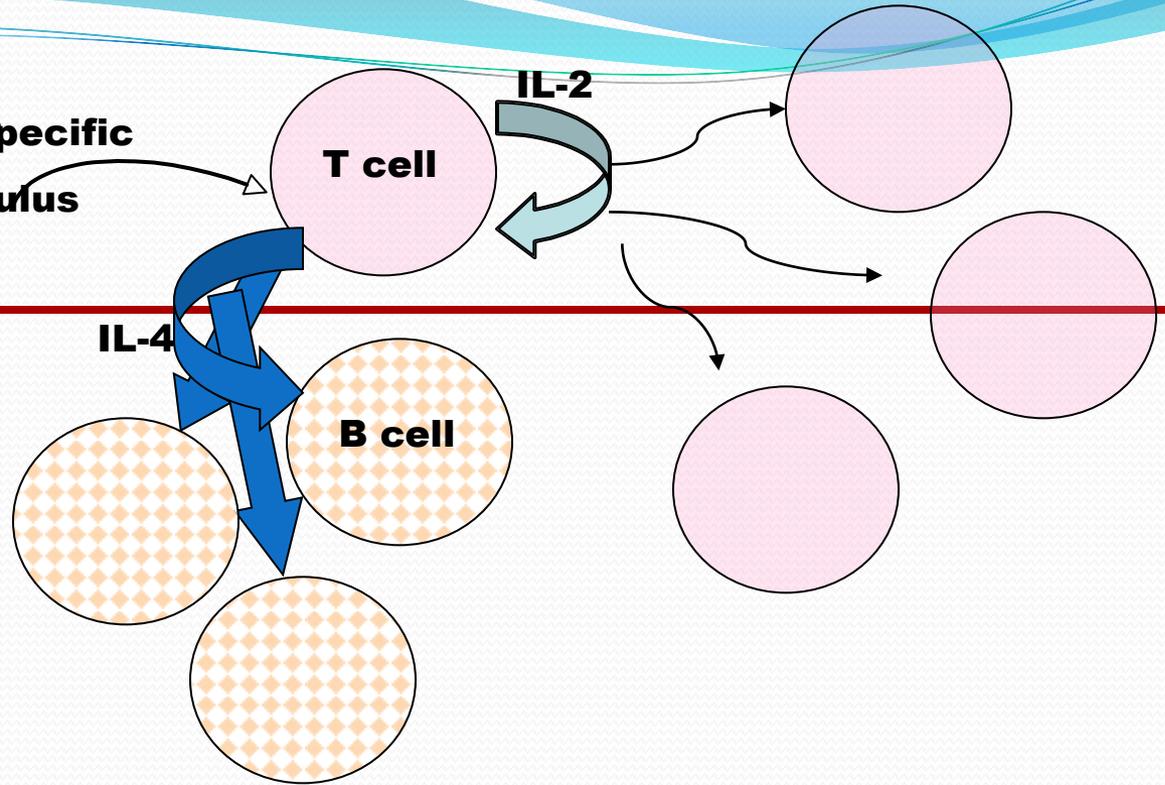
IL-11 (stimulator of platelets)

IL-12 (DC, macrophages, directs Th₁)



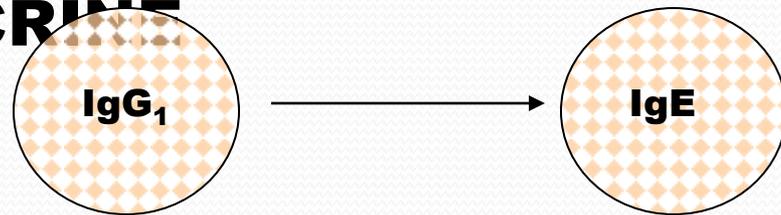
**AUTOCRINE IL-2
DRIVES CLONAL
EXPANSION OF
T CELLS**

Ag-specific
stimulus



**PARACRINE
IL-4
DRIVES
CLONAL
EXPANSION
OF B CELLS**

**AUTOCRINE OR PARACRINE
IL-4 OR IL-13 DRIVES
Ig CLASS SWITCHING
TO IgE**

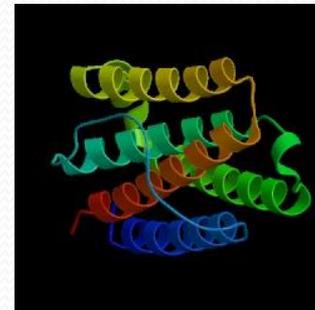
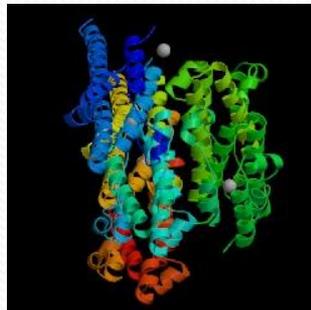


Interferon, IFN

- IFNs are mediators of the innate immune response and Th₁/T suppressor responses.
- Groups

Type I IFN: IFN- α and IFN- β

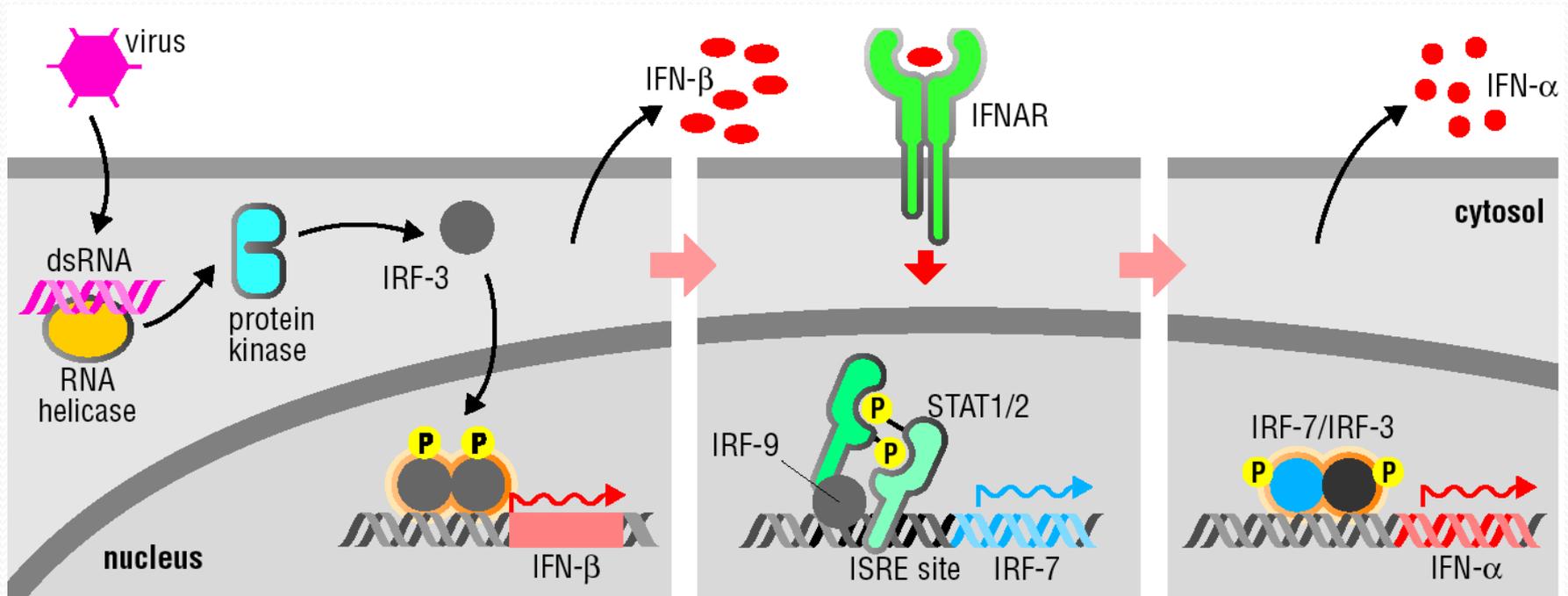
The major source is leukocytes (PDC), fibroblasts and virus infected cells.



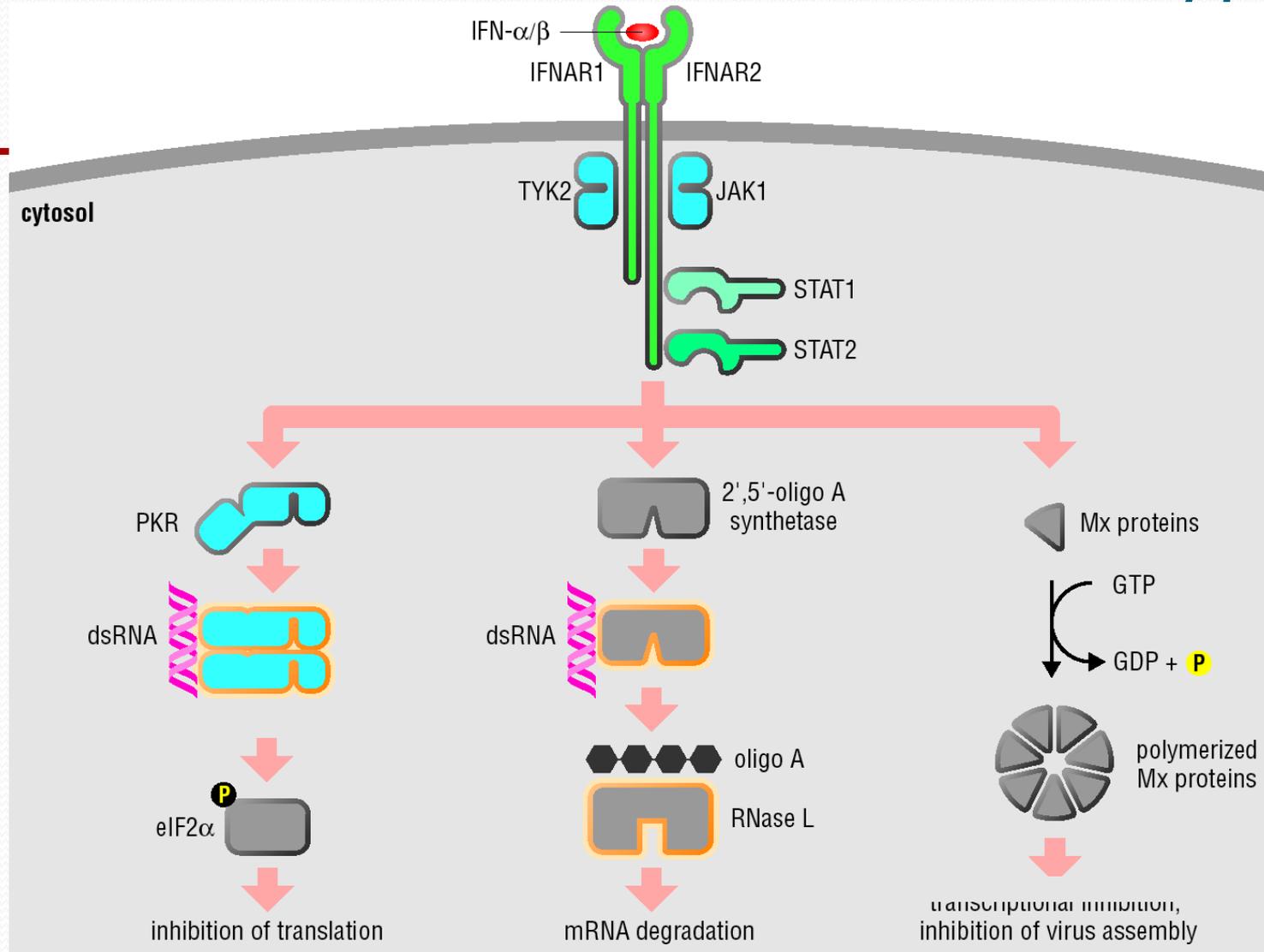
Type II IFN: IFN- γ

IFN- γ is produced by activated T cells and NK cells.

Production of interferon by infected cells

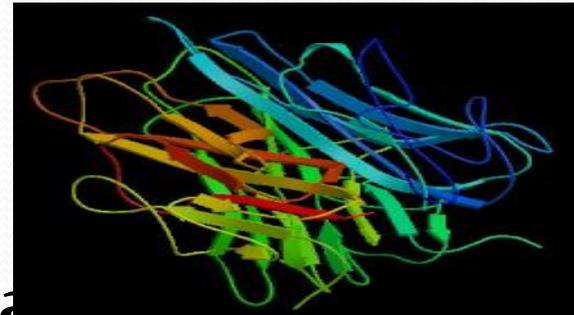


Anti-viral effects of interferon α/β



Tumor necrosis factor, TNF

- TNF was originally identified (and was so named) as a substance that can cause the necrosis of tumors in vivo
- **TNF- α** and **TNF- β**
TNF- α is produced by LPS-stimulated mononuclear phagocytes and activated T cells.



TNF- β is also termed lymphotoxin (LT), and is produced mainly by activated T cells.

TNF

**Low quantities
(plasma conc. $<10^{-9}$ M)**

**Moderate
quantities**

**High quantities
(plasma conc. $\geq 10^{-7}$ M)**

Local inflammation

Systemic effects

Septic shock

Leukocyte



Activation

Brain



Fever

Liver



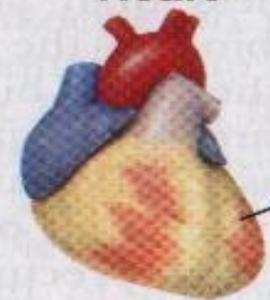
Acute-phase
proteins

Bone marrow



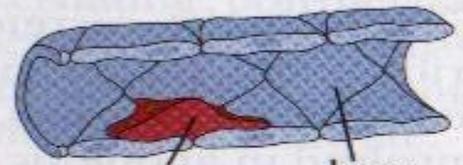
Leukocytes

Heart



Low
output

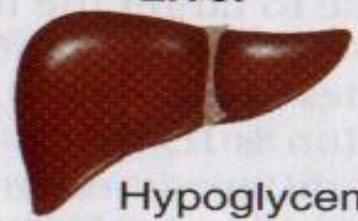
Blood vessel



Thrombus

Low
resistance

Liver



Hypoglycemia

IL-1,
chemokines

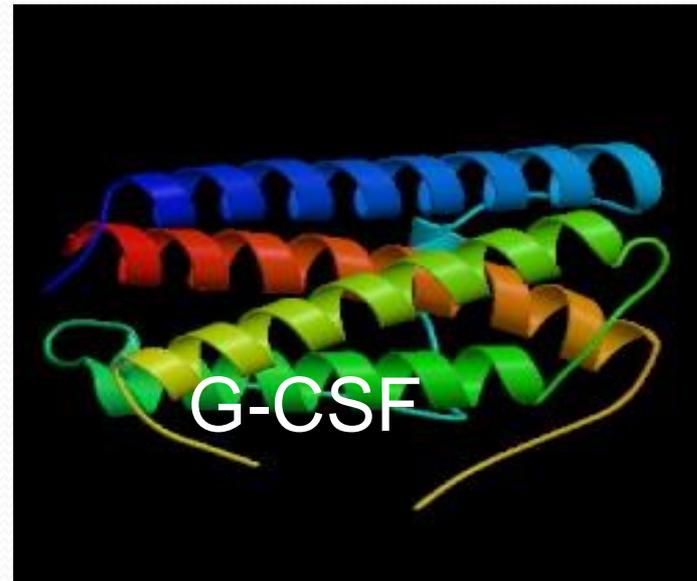
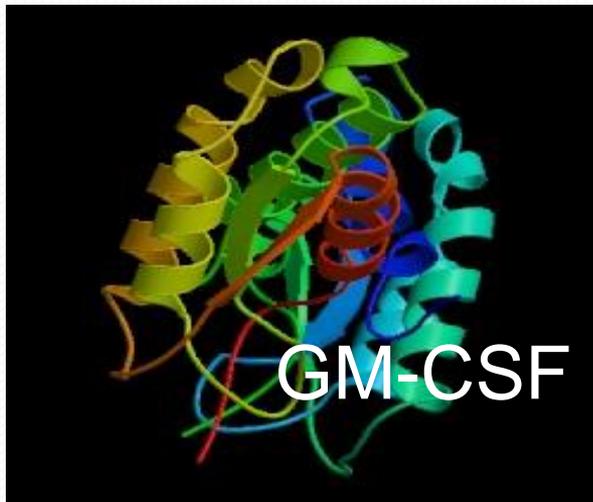
Adhesion
molecule



Endothelial cell

Colony-stimulating factors, CSF

- Stimulates the differentiation and expansion of bone marrow progenitor cells.
- It is assayed by its ability to stimulate the formation of cell colonies in culture.
- Includes IL-3, CSF (G-CSF, M-CSF, GM-CSF), SCF, EPO, TPO, etc .



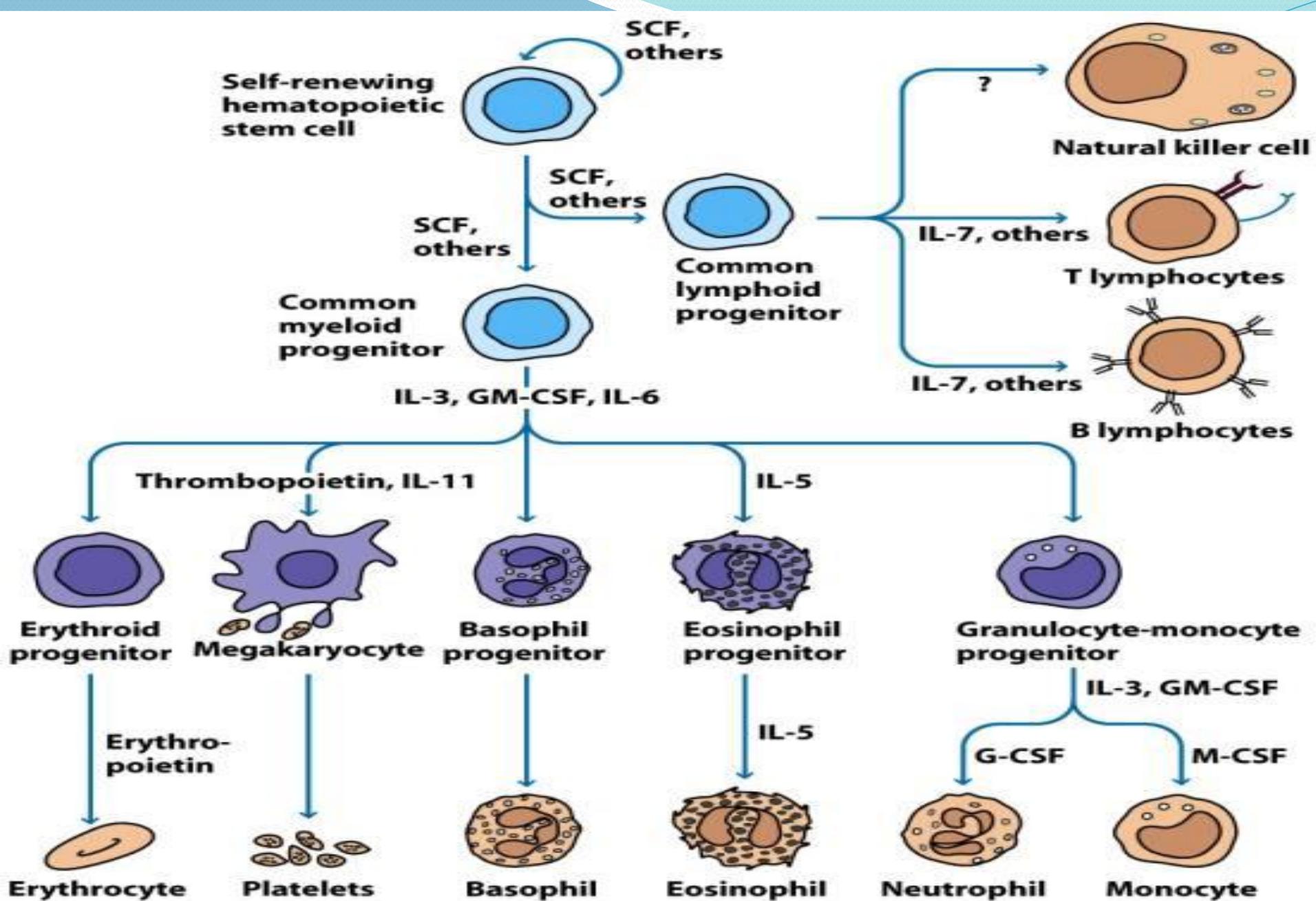
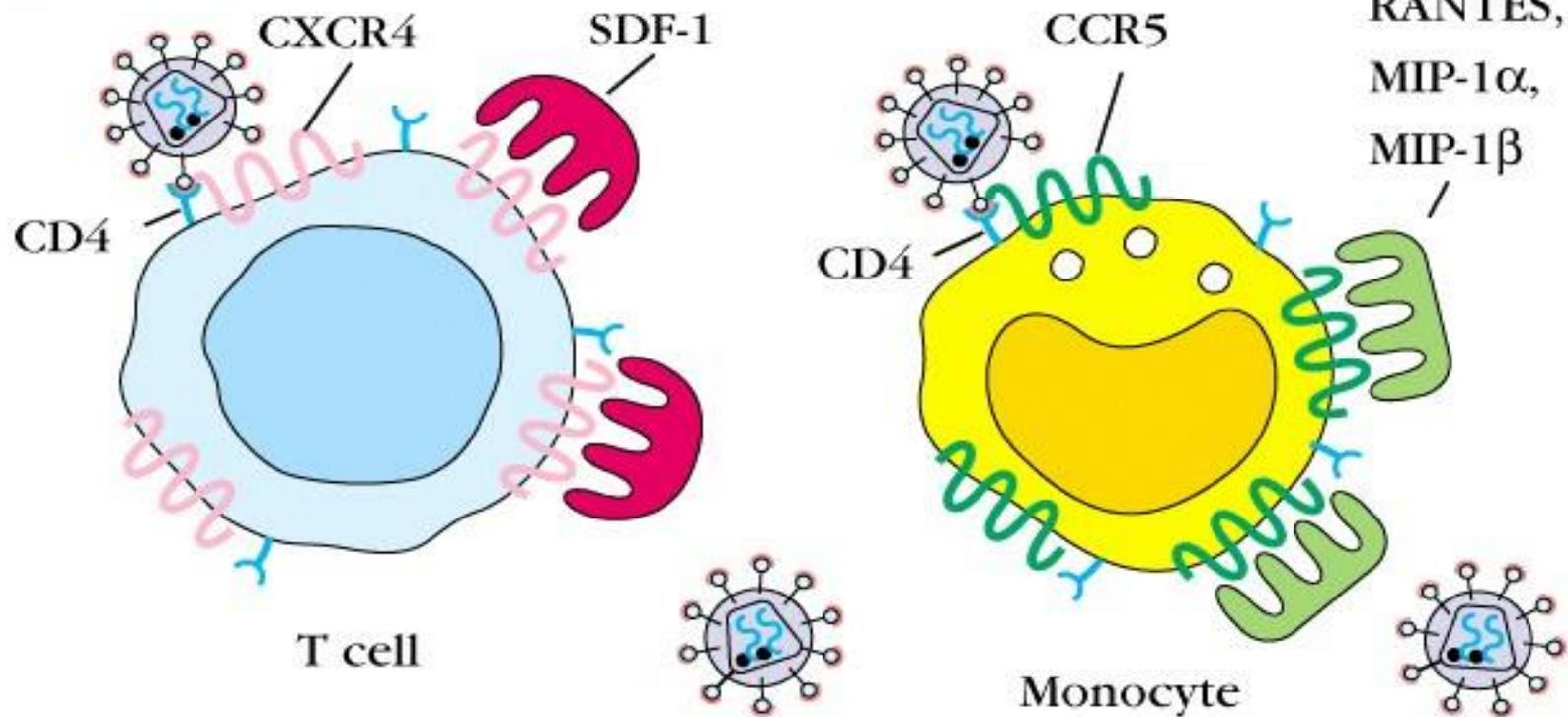


Figure 12-16
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Chemokines (chemotactic cytokines)

- Chemokines are a large family of structurally homologous CKs that stimulate movement and regulate the migration of leukocytes from the blood to tissues and within tissues, and includes about 50 different members.
- Subfamilies: CXC (α), CC (β), C (γ), CX₃C (δ), based on structural characteristics (cysteine residues).

(c)

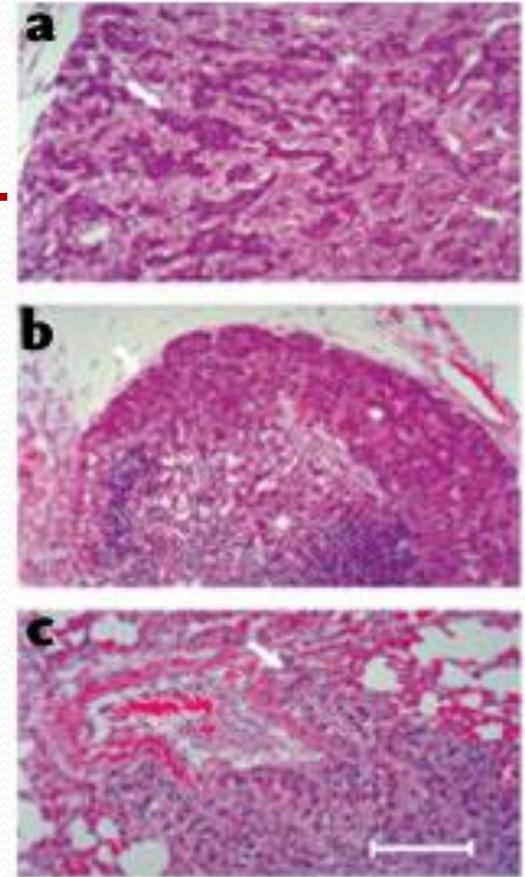
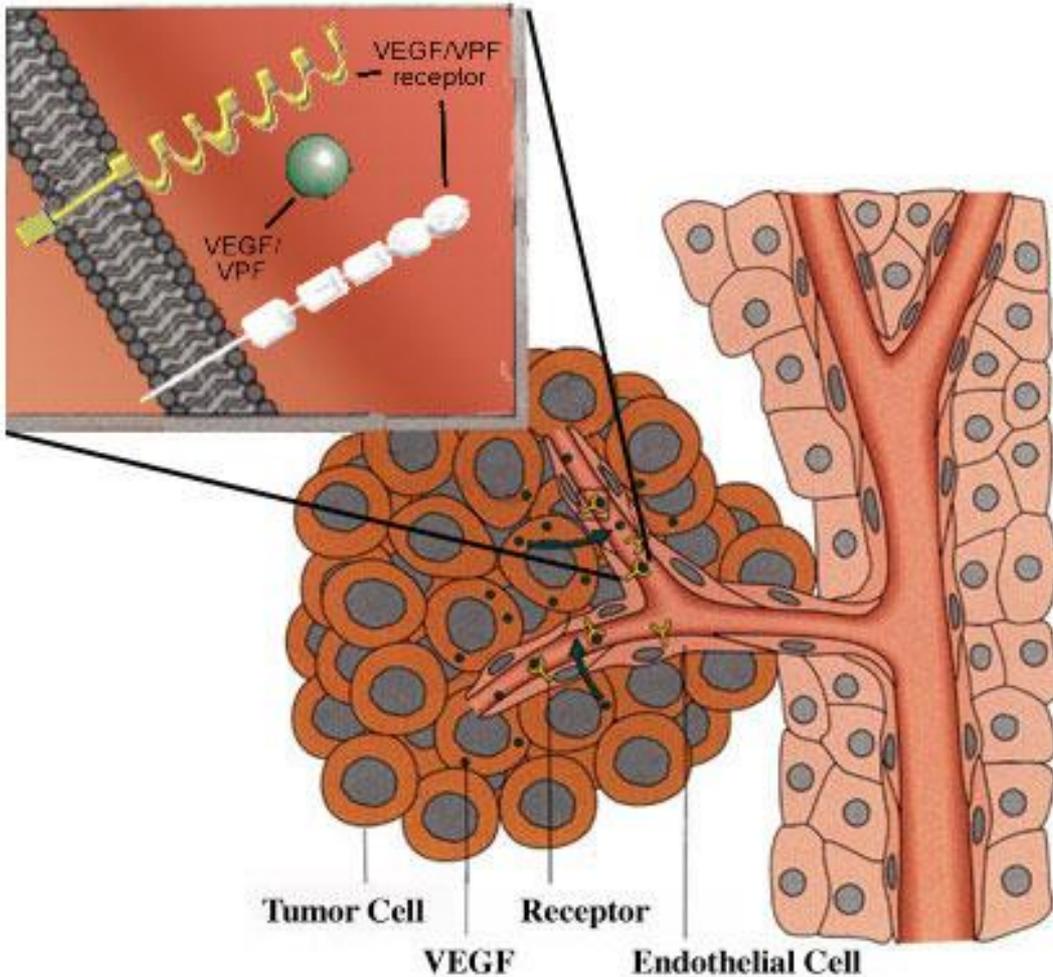


Growth factors, GF

- Promote the proliferation and differentiation of cells.
- Include TGF- β 、EGF、VEGF、FGF、NGF, etc.

**vascular endothelial
growth factor (VEGF)**





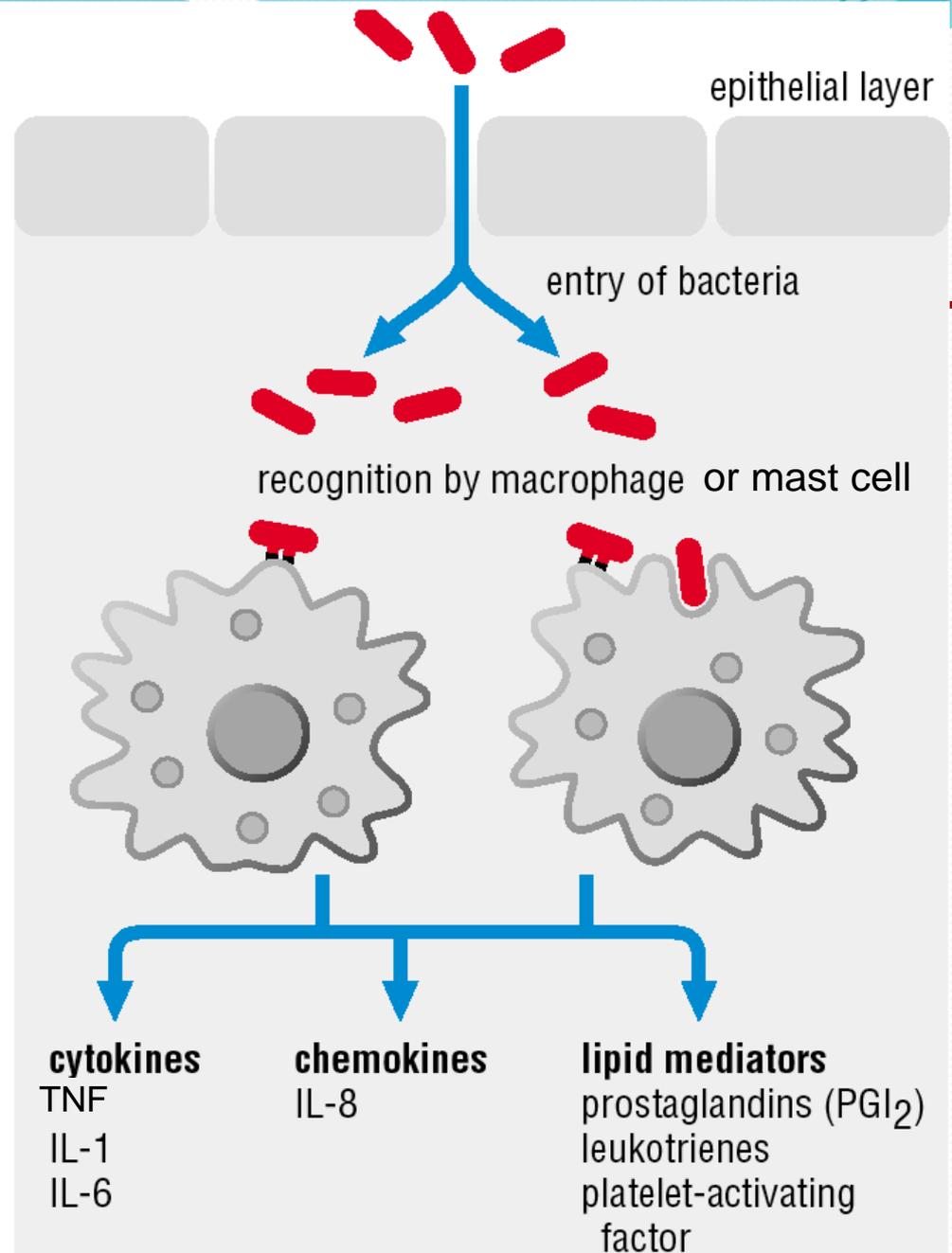
VEGF promotes Tumor Angiogenesis

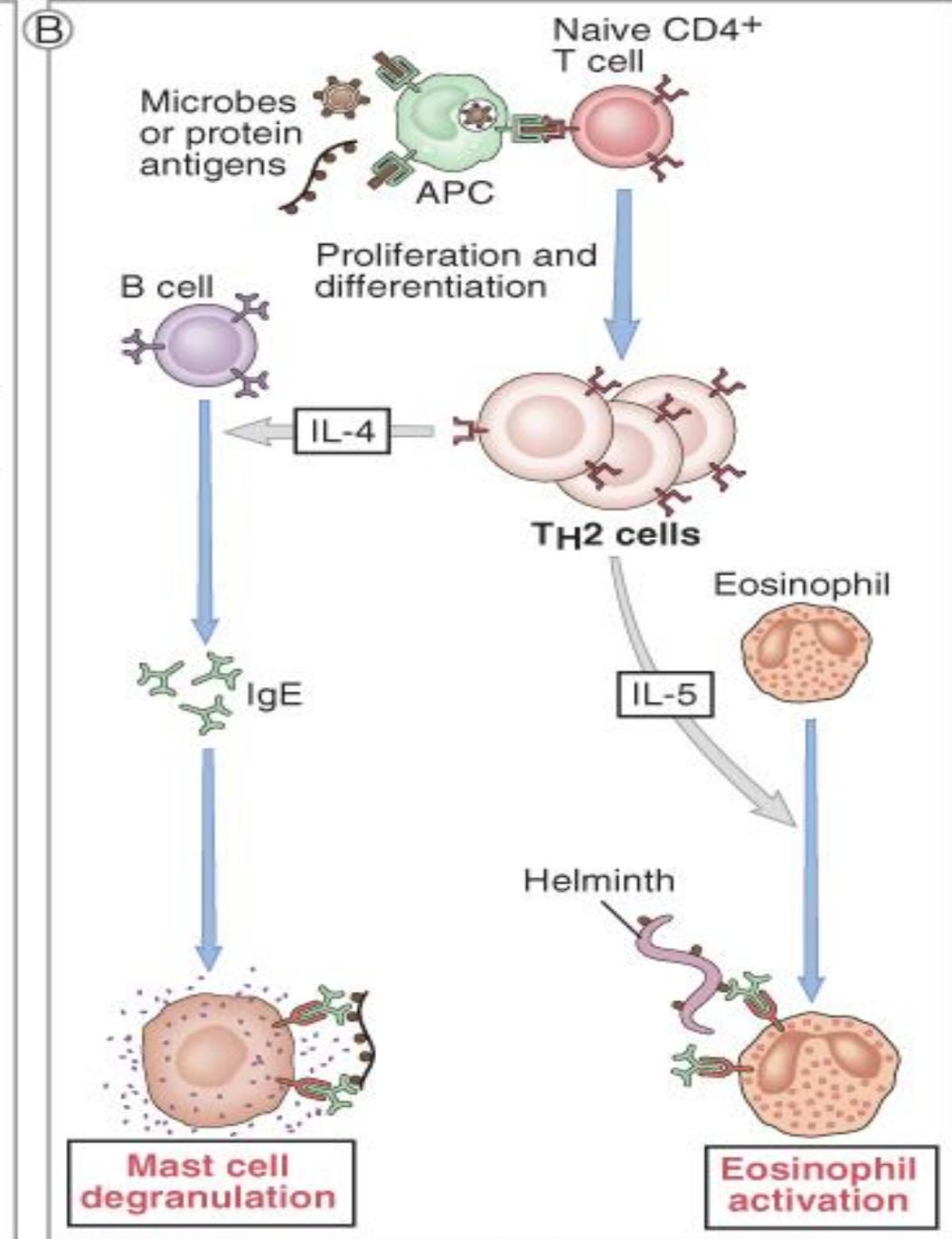
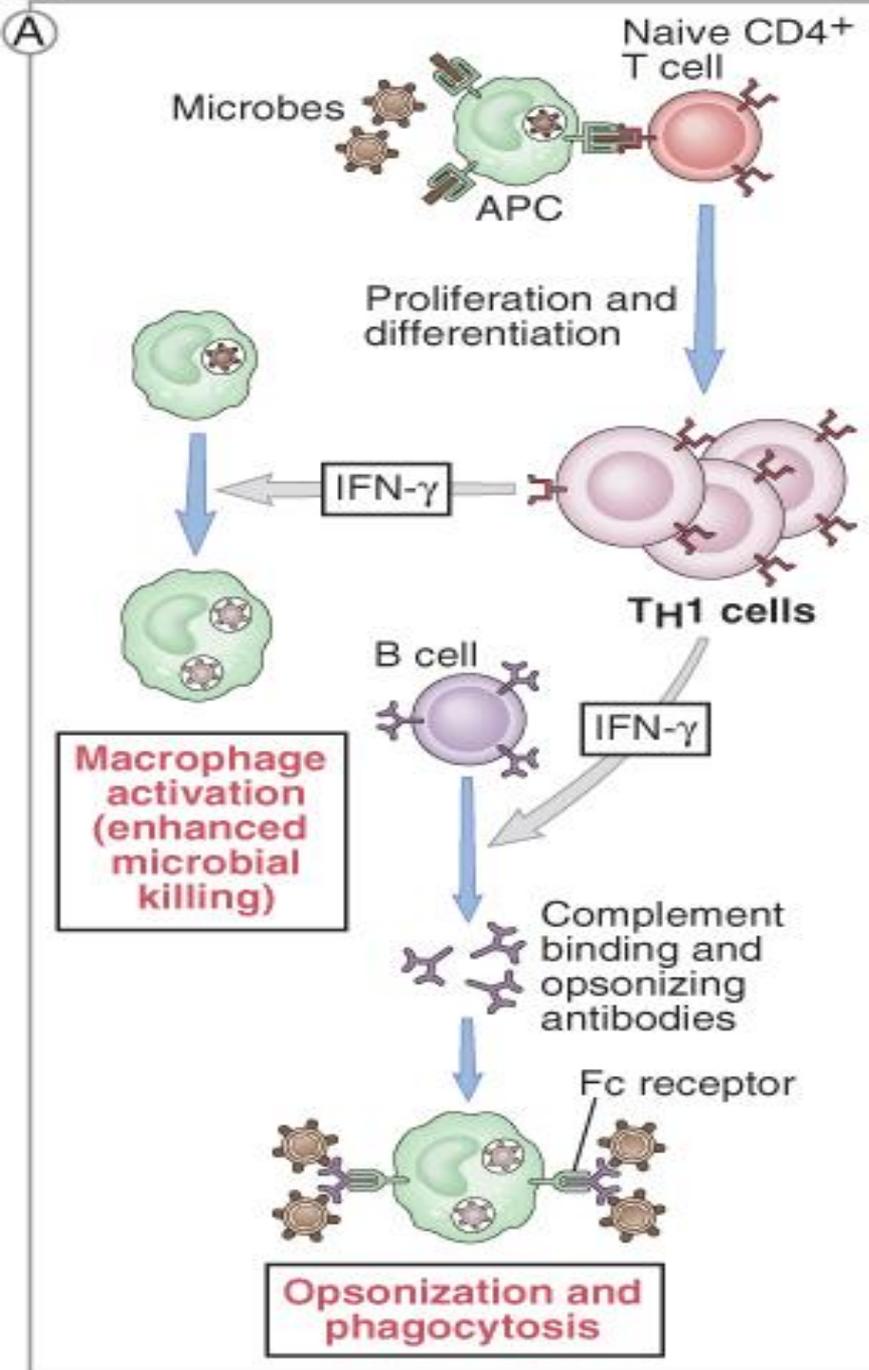
Biologic functions of CKs (1)

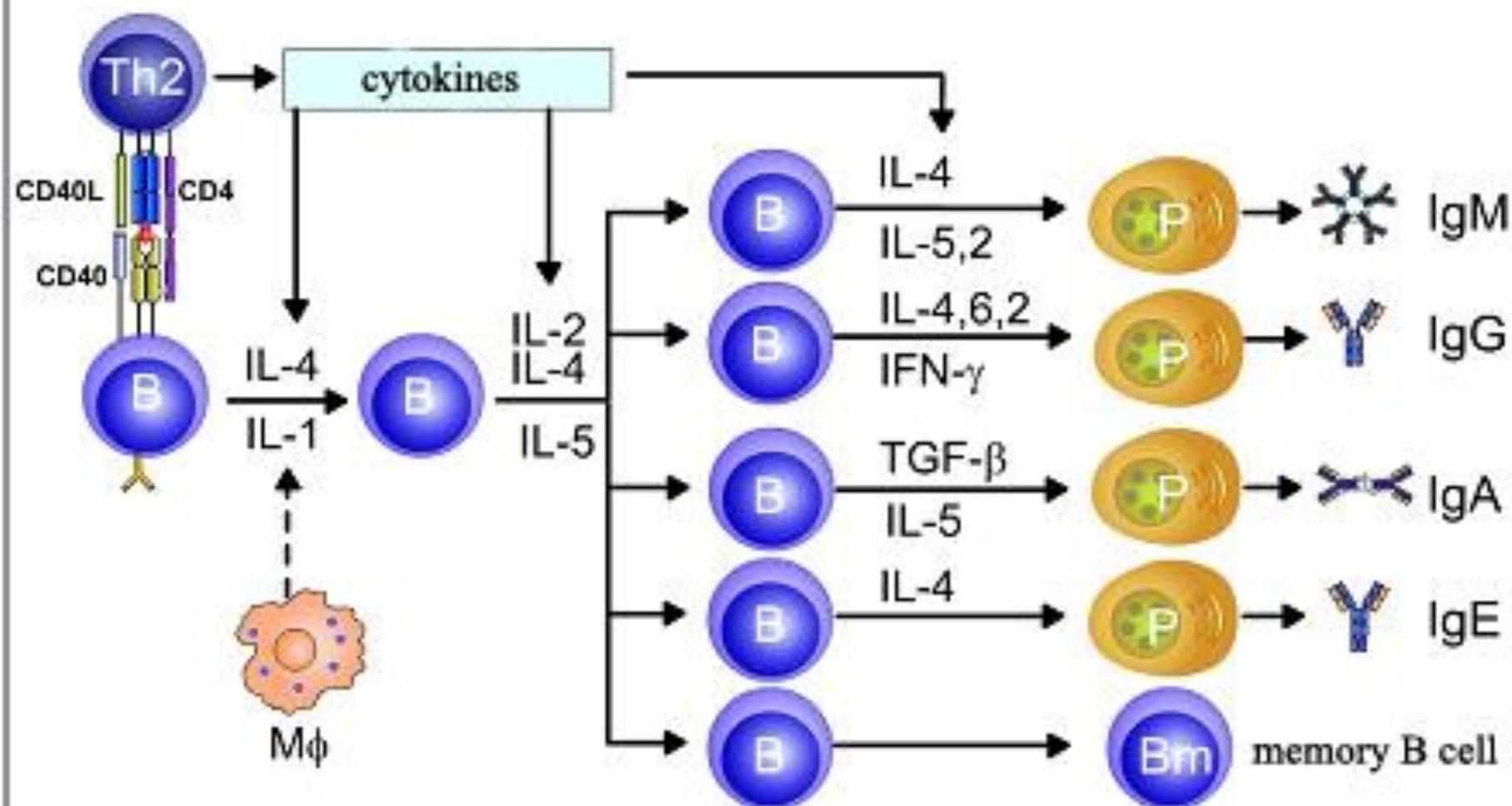
- **Anti-bacteria:** IL-1, TNF, IL-6, IL-8 and IL-12
- **Anti-virus:** type I IFN
- **Mediation and regulation of adaptive immunity:**
 - * **Stimulating the proliferation of lymphocytes:** IFN- γ , IL-2, IL-7, IL-4, IL-5, IL-15.
 - * **Stimulating the development of lymphocytes:** IL-12, IFN- γ , IL-4.
 - * **Enhancing the activity of effector cells:** IFN- γ , IL-2 .
 - * **Inhibiting immune response:** TGF- β , IL-10

Induction of Inflammation following recognition of pathogens

Inflammatory mediators:
Cytokines, chemokines
and lipids







Effects of cytokines on the class switch of Igs

Biologic actions of CKs (2)

- **Stimulation of hemopoiesis**
 - SCF → stem cells
 - CSF → granulocytes and monocytes
 - IL-4 and GM-CSF → dendritic cells
 - EPO → erythrocytes
 - IL-11 and TPO → platelets
 - IL-7, IL-15 → formation of Tm
- **Angiogenesis**
 - IL-8, VEGF, etc.

MONOCLONAL ANTIBODIES

HYBRIDOMA TECHNIQUE

Lecture 14

Monoclonal antibodies (mAb or moAb)

Monoclonal antibodies are:

- ❖ *monospecific* antibodies that are *identical* because they are produced by one type of immune cell (a single parent cell).
- ❖ Antibodies produced from a single clone of B cells.
- ❖ Produced by fusing a B cell secreting the desired antibody with a myeloma cell capable of growing indefinitely in tissue culture.
- ❖ Monoclonal antibodies all have identical antigen-binding sites.
i.e. they all bind to the same epitope with the same affinity.
- ❖ They are all of the same antibody class (isotype).

Polyclonal antibodies

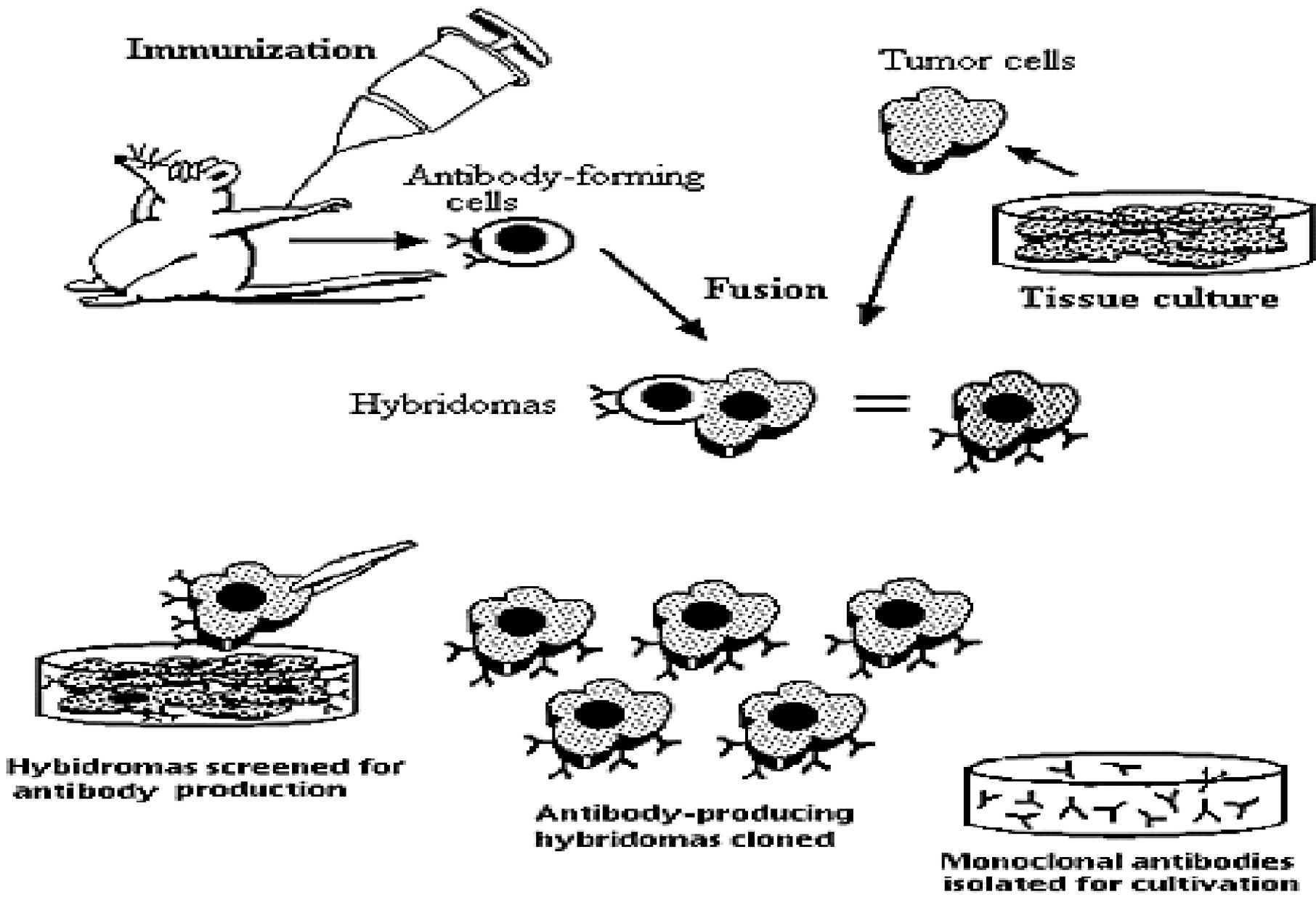
- are a mixture of antibodies with different antigen binding sites that may bind to different epitopes or antigens with varying affinities.
- They may be of different antibody classes.
- The serum obtained from an immunized animal is referred to as a polyclonal antiserum.

Hybridoma

- ❖ are cells that have been engineered to produce a desired antibody in large amounts.
- ❖ To produce monoclonal antibodies, B-cells are removed from the spleen of an animal that has been challenged with the relevant antigen
- ❖ These B-cells are then fused with myeloma tumor cells that can grow indefinitely in culture
- ❖ This fusion is performed by making the cell membranes more permeable.
- ❖ The fused hybrid cells (hybridomas), being cancer cells, will multiply rapidly and indefinitely and will produce large amounts of the desired antibodies

Practical steps in monoclonal antibody production:

- 1) Immunize animal
- 2) Isolate spleen cells (containing antibody-producing B cells)
- 3) Fuse spleen cells with myeloma cells (e.g. using PEG - polyethylene glycol)
- 4) Allow unfused B cells to die
- 5) Add **aminopterin** to culture to kill unfused myeloma cells
- 6) Clone remaining cells (place 1 cell/well and allow each cell to grow into a clone of cells)
- 7) Screen supernatant of each clone for presence of the desired antibody.
- 8) Grow the chosen clone of cells in tissue culture indefinitely.
- 9) Harvest antibody from the culture supernatant.



Monoclonal Antibody Production

Hybridoma Selection

The “HAT Trick”

- ❧ Myeloma cells lack certain enzymes so that they can not use hypoxanthine, aminopterin, and thymidine (**HAT** medium) as a source for nucleic acid biosynthesis and will die in culture.
- ❧ Only B cells that have fused with the engineered myeloma cells will survive in culture when grown in HAT medium.

Purification of monoclonal Antibodies

Cotaminants:

- ❖ Media components: hormones, growth factors, transferrins.....etc
- ❖ Viral, bacterial, endotoxins....etc

Methods of purification:

1. Filtration for larger particles
2. Ultrafiltration esp. for low concentration samples
3. Chromatography:
 - **Ion** exchange chromatography (either *cation* or *anion*) can be used (*Most impurities are usually anions*)
 - *Size exclusion* chromatography

Recombinant monoclonal antibodies

- The term recombinant and especially recombinant DNA refers to an artificial method of producing DNA (synthetic DNA and proteins).
- Recombinant DNA is produced through the addition of relevant DNA into an existing organismal genome, such as the plasmid of bacteria.
- Recombinant antibody engineering involves the use of viruses or yeast to create antibodies, rather than mice.
- A segment of DNA is recombined (recombinant) with the original DNA of the **bacteria**, these genetically modified bacteria **rapidly reproduce** and by that reproduce the DNA segment that was recombined with the original genome.

Uses of monoclonal Abs

Measuring protein and drug levels in serum

Typing tissue and blood

Identifying infectious agents

Identifying clusters of differentiation (CDs) for classification and follow-up therapy of leukemias and lymphomas

Identifying tumor metastasis

Identifying and quantifying hormones

Immunoaffinity Purification

Applications of Monoclonal Antibodies

- Diagnostic Applications
- Therapeutic Applications
 - Transplant rejection
 - Cardiovascular disease
 - Cancer
 - Infectious Diseases
 - Inflammatory disease
- Clinical Applications
 - Purification of drugs, Imaging the target**
- Future Applications
 - Fight against Bioterrorism**

Diagnostic Applications (Immuno-cytochemistry)

1. Tissues and tumors can be classified based on their expression of certain defined markers: e.g. Prostate specific antigen(PSA)
2. Classification of tissues and tumors
3. Distinguishing morphologically similar lesions, like mesothelioma and adenocarcinoma
4. Detecting small quantities of metastatic cancer and MDR

Therapeutic Applications

One possible treatment for cancer involves monoclonal antibodies that bind only to cancer cell-specific antigens and induce an immunological response against cancer cells.

Three mechanisms responsible for the cancer treatment:

1. mAbs act directly when binding to a cancer specific antigens and induce immunological response to cancer cells(apoptosis, inhibiting growth)
2. mAbs can be modified for delivery of a toxin, radioisotope, cytokine or other active conjugates.
3. it is also possible to design bispecific antibodies that can bind with their Fab regions both to target antigen and to an effector cell

Treatment of Cancer

- Cancer cells carry specific tumour-associated antigens (TAA) on their plasma membrane.
- Monoclonal anti-TAA antibodies have been produced.
- Drugs which kill tumour cells or inhibit key proteins in tumour cells are attached to monoclonal anti-TAA antibodies.
- Cancer cells are specifically targeted, avoiding damage to healthy host cells.

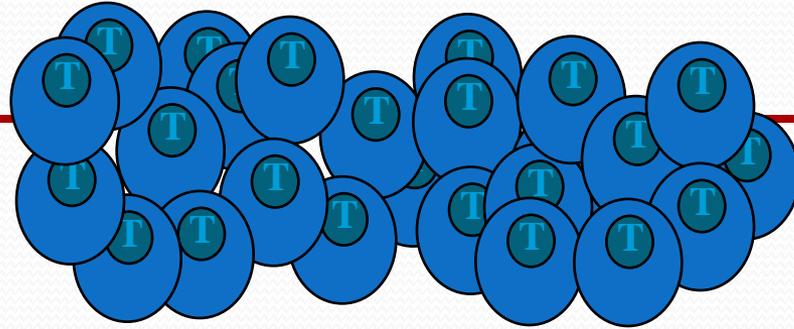
Problems...

- Many patients develop immune response to monoclonal antibodies produced in mice, as these are foreign proteins.
- Genetically engineered antibodies are being perfected to avoid triggering immune response.

T Cell Development, Repertoire Selection and Immune Self Tolerance (Thymus Education)

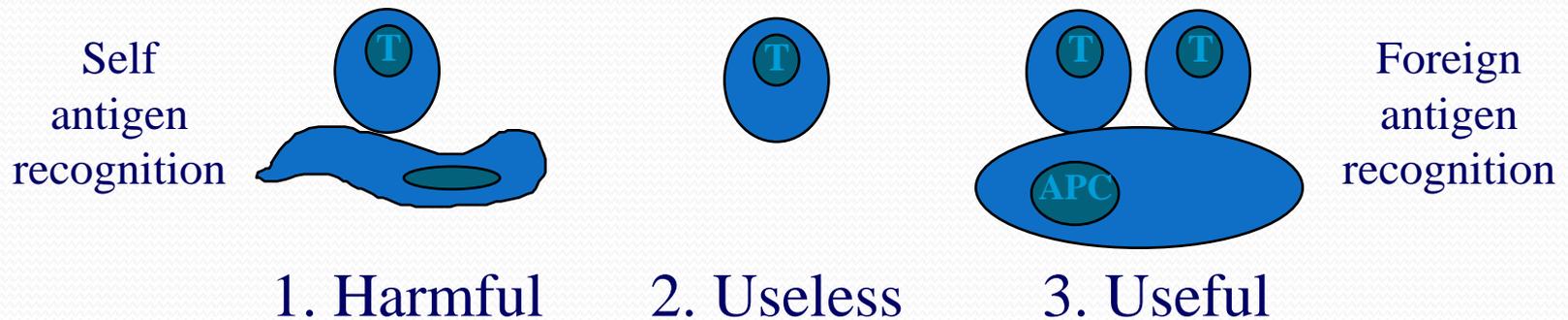
Lecture 15

Why is a mechanism for repertoire selection and self tolerance needed?

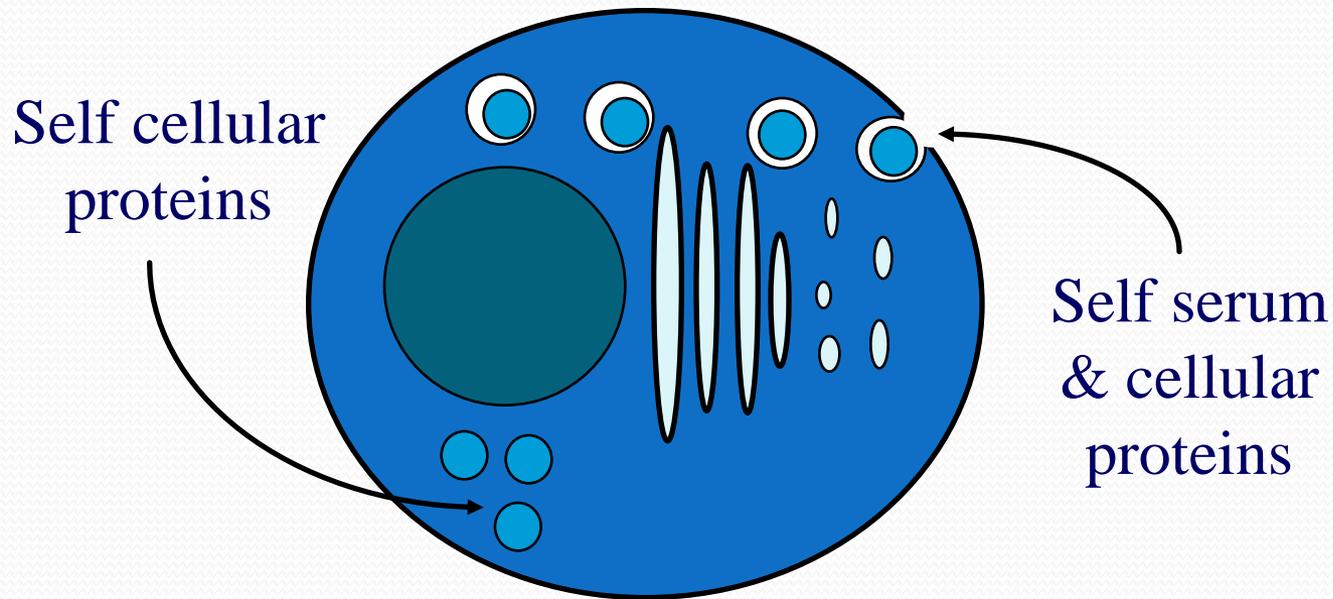


Generation of the TcR repertoire involves many random mechanisms

The specificity of TcR in the immature repertoire is also random & will include cells with receptors that are:



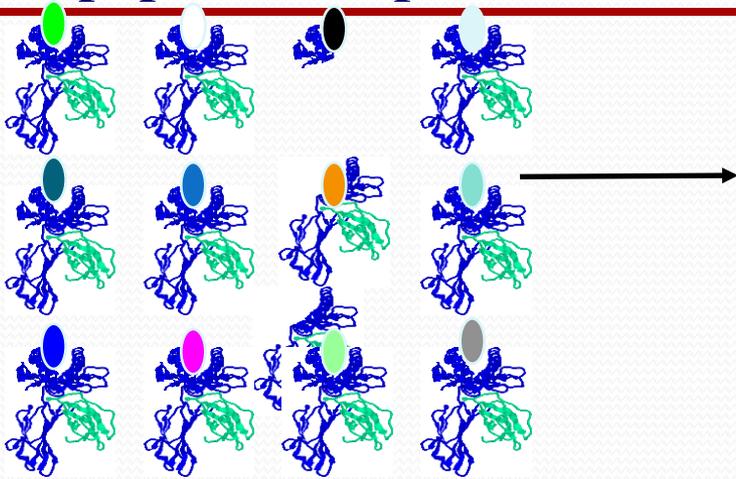
Self proteins enter the endogenous and exogenous antigen processing pathways



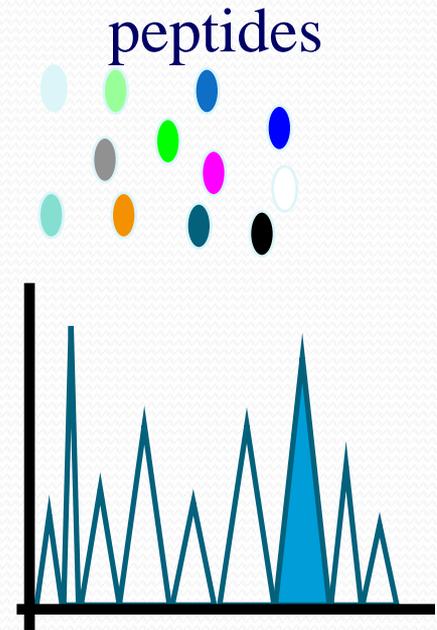
Processing pathways do not distinguish self from non-self

Self peptides load onto MHC class I & II molecules

Purify stable MHC-peptide complexes

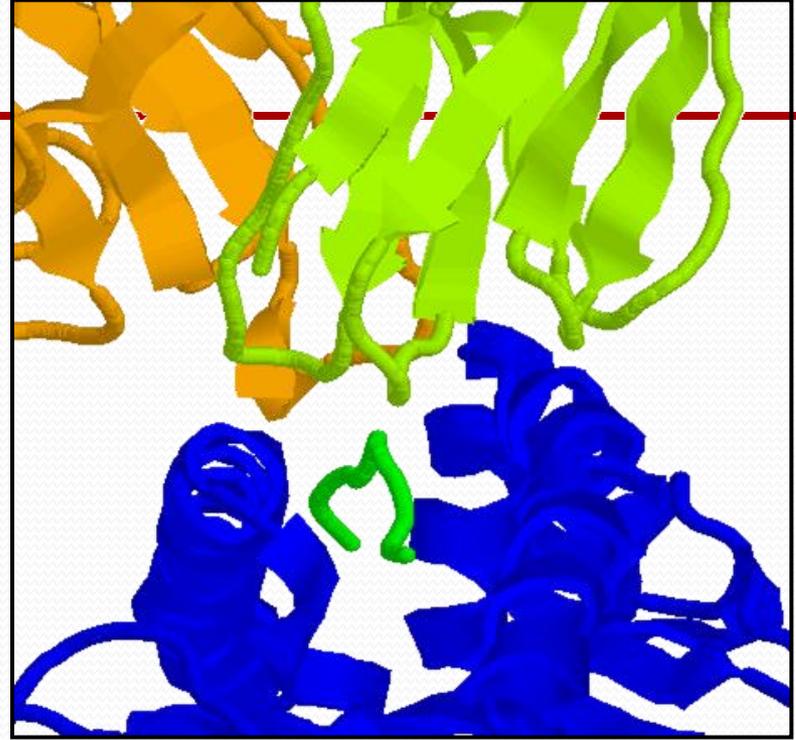
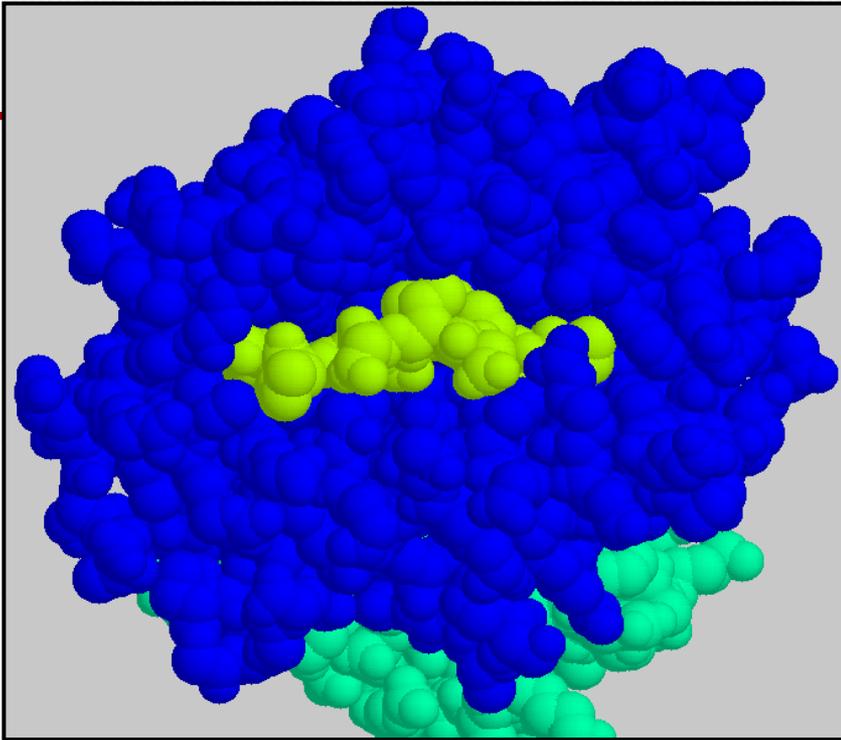


Fractionate and microsequence peptides



>90% of eluted peptides are derived from self proteins
Yet self antigens do not usually activate T cells

The immune system allows a limited degree of self recognition



TcRs recognise the non-self peptide antigen and the self MHC molecule

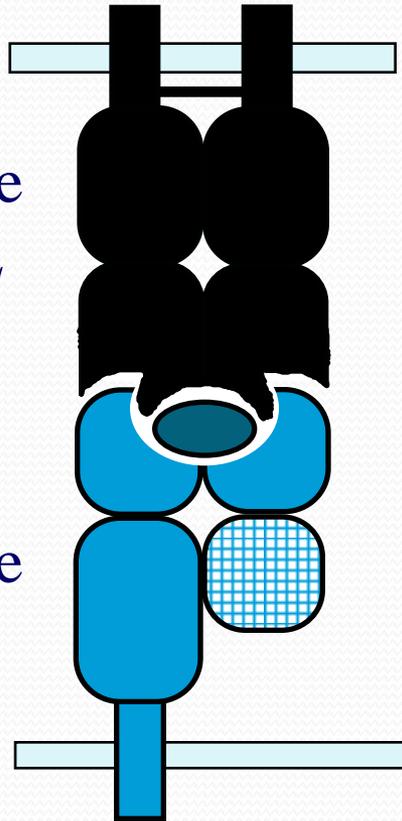
MHC molecules **RESTRICT** T cell activation

But how do T cells learn how much self recognition is acceptable?

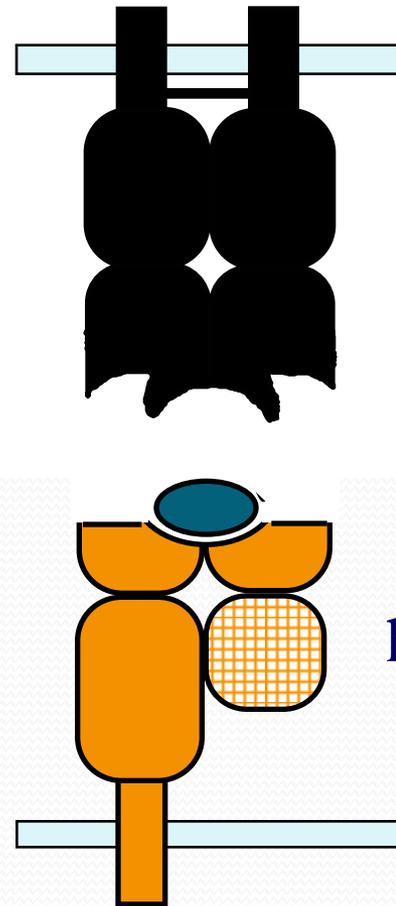
T cells are only allowed to develop if their TcR recognise parts of self MHC

Explains why T cells of MHC haplotype A do not recognise antigen specific presented by MHC B

MHC A
haplotype
T CELL



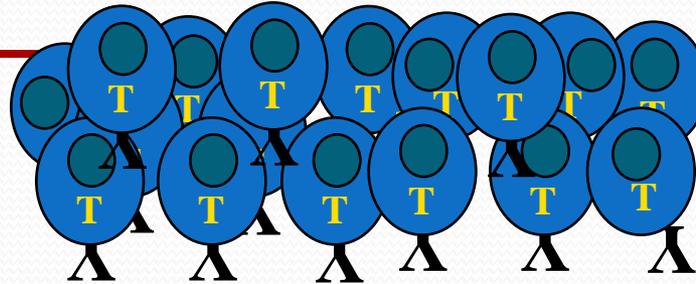
MHC A
haplotype
APC



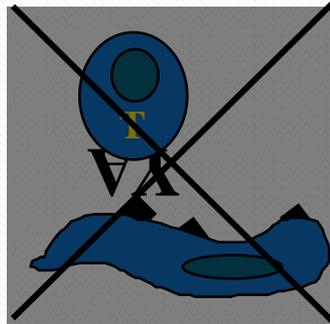
MHC B
haplotype
APC

Wholly self-reactive and useless T cells are removed MHC-restricted are retained

Random TcR repertoire ensures diversity

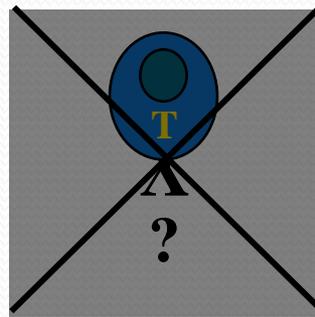


THYMUS



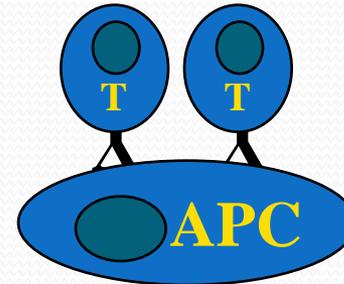
Harmful

Negatively select



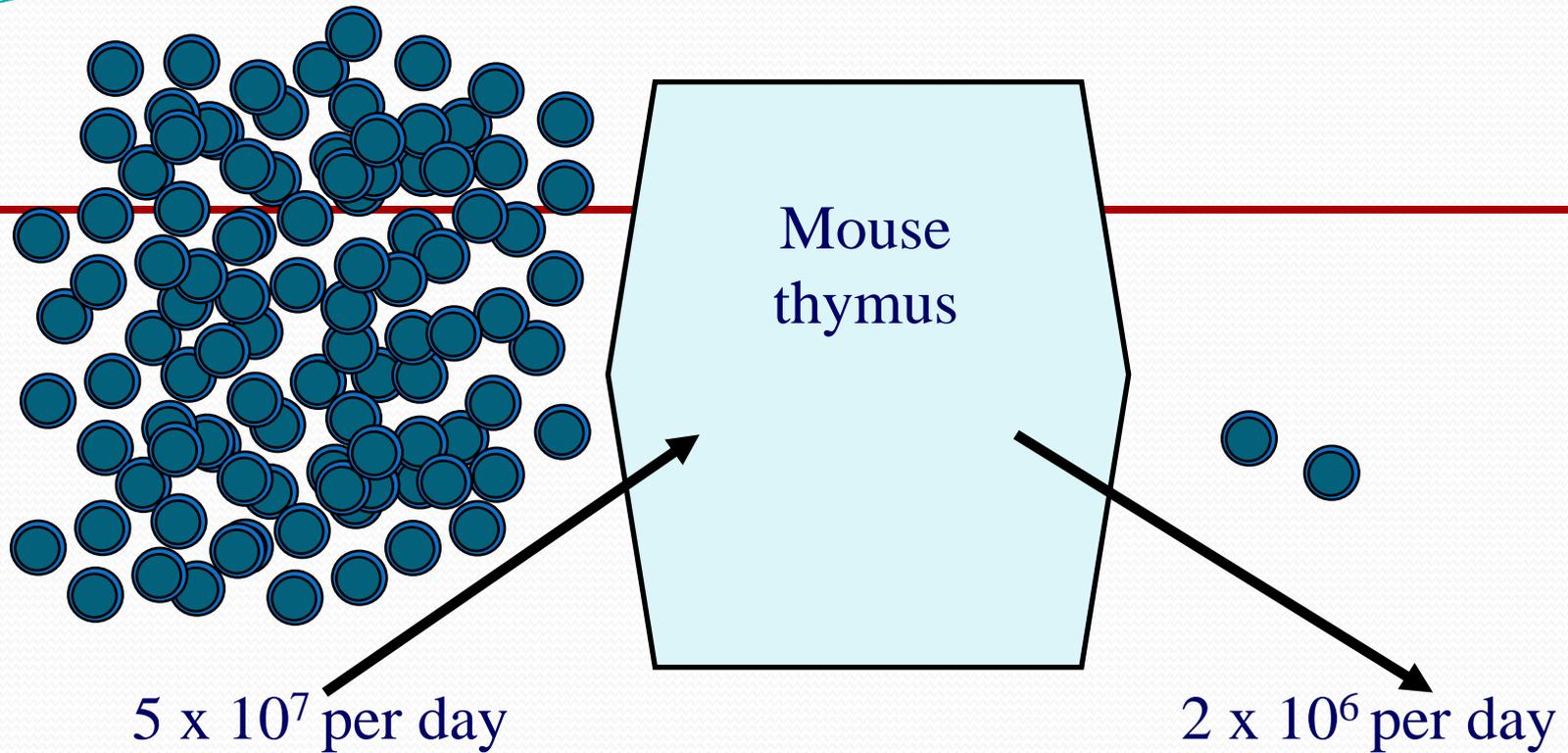
Useless

Neglect



Useful

Positively select



How does the thymus choose which of the cells entering the thymus are useful, harmful and useless

Sorting the useful from the harmful and the useless

Positive selection

Retention of thymocytes expressing TcR that are **RESTRICTED** in their recognition of antigen by self MHC
i.e. selection of the **USEFUL**

Negative selection

Removal of thymocytes expressing TcR that either recognise self antigens presented by self MHC or that have no affinity for self MHC
i.e. selection of the **HARMFUL** and the **USELESS**

MHC restriction

Antigen can be seen by the TcR only in the context of an MHC molecule

TcR will not bind to an MHC molecule unless there is an antigen in the groove

In the presence of antigen, the TcR must have some affinity for the MHC molecule

Summary

Bone marrow chimeras show that MHC restriction is learnt in the thymus

T cells are 'educated' in the thymus to recognise antigens only in the context of self MHC

MHC restriction is learnt in the thymus by positive selection

The MHC haplotype of the environment in which T cells mature determines their MHC restriction element

Negative Selection

Removal of thymocytes expressing TcR that either recognise self antigens presented by self MHC or that have no affinity for self MHC

i.e. selection of the **HARMFUL** and the **USELESS**

Superantigens can be used to probe the mechanisms of negative selection

Nominal antigens & superantigens

Nominal antigens

- Require processing to peptides
- TcR α and β chains are involved in recognition
- >1 in 10^5 T cells recognise each peptide
- Recognition restricted by an MHC class I or II molecule
- Almost all proteins can be nominal antigens

Superantigens

- Not processed
- Only TcR β chain involved in recognition
- 2-20% of T cells recognise each superantigen
- Presented by almost any MHC class II molecule
- Very few antigens are superantigens

Suggests a strikingly different mechanism of antigen presentation & recognition.

Superantigens

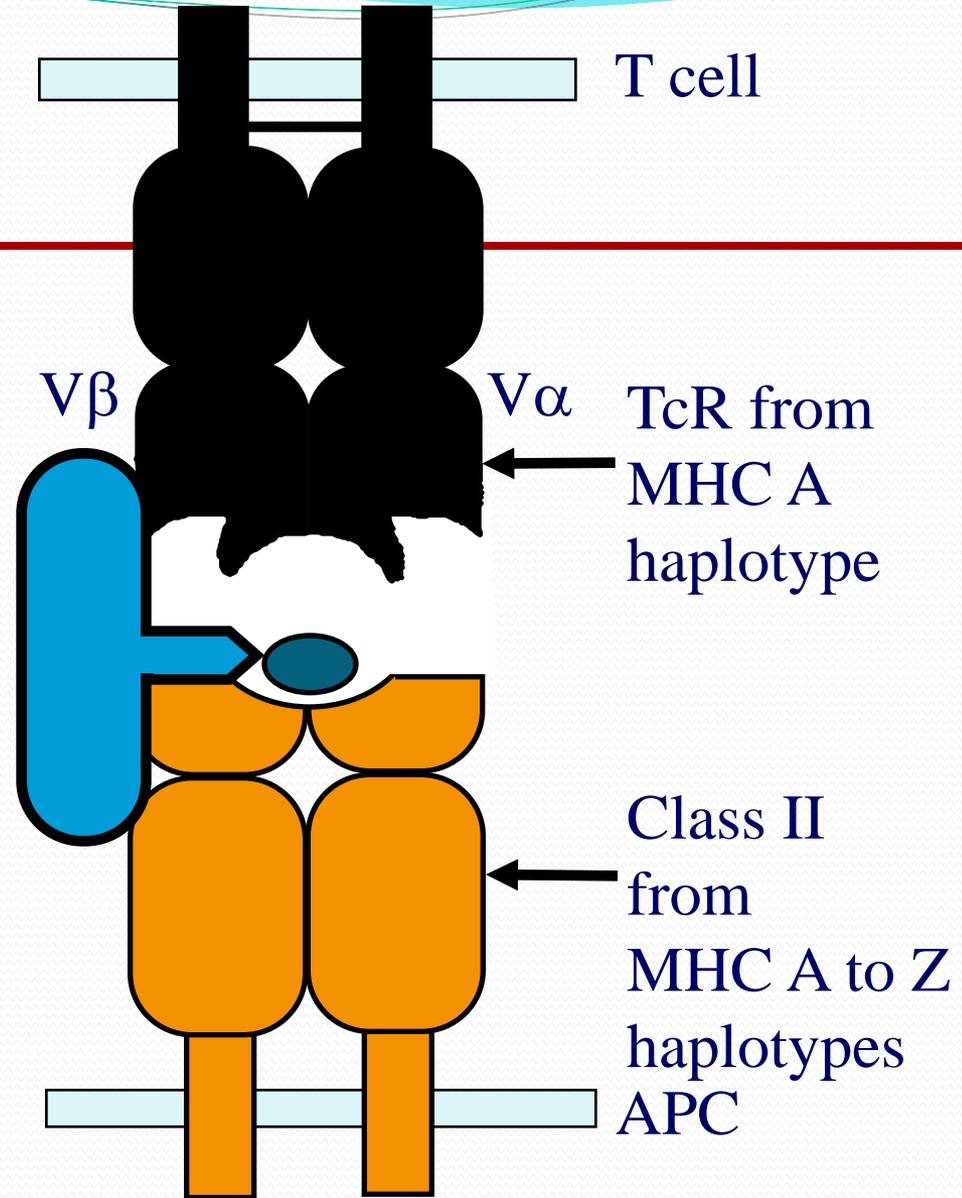
e.g. Staphylococcal enterotoxins

Toxic shock syndrome toxin I
(TSST-1)

Staphylococcal enterotoxins
SEA, SEB, SEC, SED & SEE

Do not induce adaptive
responses, but trigger a massive
burst of cytokines that may cause
fever, systemic toxicity &
immune suppression

*Severe food poisoning Toxic
shock syndrome*



Other exogenous superantigens

Bacterial exoproteins

Staphylococcal exfoliative toxins

Streptococcus pyogenes erythrogenic toxins A & C

(?Streptococcal M protein?)

Yersinia enterocolitica superantigen

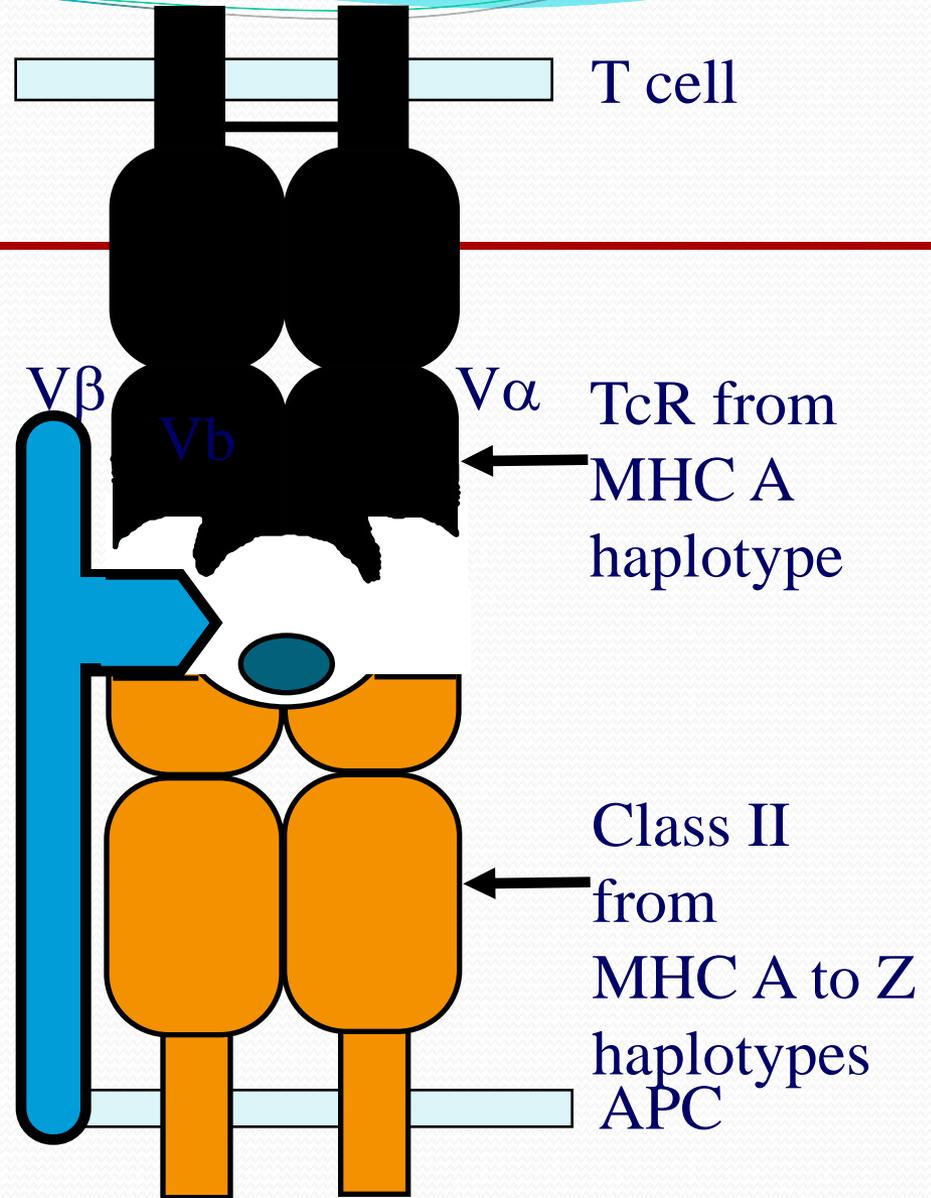
Clostridium perfringens superantigen

Mycoplasma arthritidis mitogen

Superantigens

Mouse mammary tumour viruses (Mtv)

Cell-tethered superantigen encoded by the viral genome



Endogenous superantigens

Mouse mammary tumour viruses (MMTV)

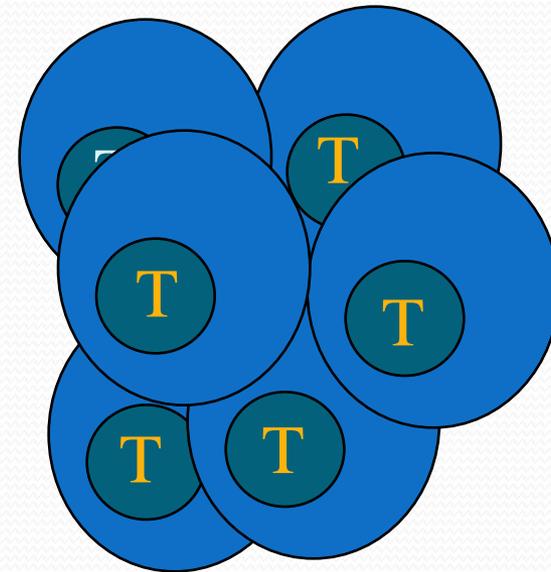
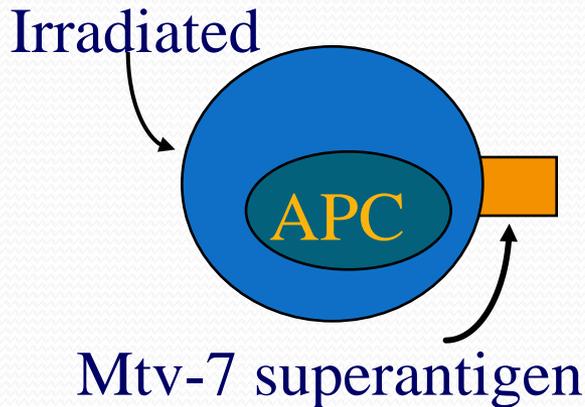
Mtv act in a similar manner to exogenous superantigens in vitro

STIMULATOR CELLS

RESPONDING T CELLS

Mtv-7 +ve

Mtv-7 -ve



Only T cells with TcR containing V β 6, V β 8.1 and V β 9 proliferate
Mtv-7 interacts with V β 6, V β 8.1 and V β 9 and activates
only cells bearing those TcR
Selective expansion of cells bearing certain V β chains

How do pathogens use superantigens?

Unfocussed adaptive immune response activates cells of all specificities as well as those specific for the superantigens

- Reduces the possibility that effective T cell clonal selection can eliminate the pathogen
- Upon resolution, cells activated by the superantigen die, leaving the host immunosuppressed

Transmission of infection