Immunology Lecture note
for
Degree program Medical laboratory sciences students (Year-II, Second Semester)

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Immunology

Immunology

Chapter 1: An Overview of the Immune System

1.0 Learning Objectives for Immunology Overview

Upon completion of this lecture and exercises the student will be able to:

- Define the terms immunity, immunology
- Describe major historical events in the development of immunology
- Differentiate innate and adaptive immunity in terms of components and type of immune response.
- Explain the major defenses of innate immunity
- Describe the mechanisms used by the body to defend itself in an innate response.

1.1 Definition of terms

- **Immunology**
  - The study of immune system or immunity
  - the study of all aspects of host defense against infection and of adverse consequences of immune responses.
  - The study of the physiological mechanisms which enable the body to recognize materials as foreign and to neutralize, metabolize or eliminate them without injury to the host tissue.
- **Immunity**
  - State of protection from infectious diseases

1.1 Definition of terms

Immune system

- A remarkably versatile defense system that has evolved to protect animals from invading pathogenic microorganisms and cancer.
- It is able to generate an enormous variety of cells and molecules capable of specifically recognizing and eliminating an apparently limitless variety of foreign invaders.

1.2. History of immunology

- Its principles among the earliest written observations;
- Individuals recovering from certain disease rarely contracted that same disease again.
- observation promoted deliberate attempts to induce immunity
- Athens plague as of Thucydides in 430BC (recovered people only nurse sick one)

1.2 History….. *Cont*

- Chinese(1500A.D) custom of inhaling crusts from smallpox lesions to prevent development of small pox in later life.
- Injecting materials from crusts or fluid from smallpox blisters (“variolation”), used through out the eastern world, in 1718 was introduced into western medicine by British ambassador’s wife, to Turkey, had her children so treated.
    - **Note-** The virus used could be transmitted => protection by variolation was hazardous to the community at large!!

1.2 History *Cont*

- **In 1798, Jenner's** work on vaccination, describing a related, y*et safe procedure.*
  - Noted people, who had **cow pox,** were spared in small pox epidemics,
  - inoculated boy with pus from milk maid with cow pox, and
  - re-inoculated same boy with infectious pus from a patient in the active small pox.
  - No disease state followed these inoculations, and experiment was repeated several times with great success!

Louis Pasteur- demonstrating that it was possible to **attenuate,** or weaken, a pathogen and administer the attenuated strain as a vaccine.

In 1885, Pasteur administered his first vaccine to a human, a young boy who had been bitten repeatedly by a rabid dog

Wood engraving of Louis Pasteur watching Joseph Meister receive the rabies vaccine. [*From Harper's Weekly* ***29:836;*** *courtesy of the National Library of Medicine.]*

1.2 History ….*Cont*

- Jenner`s provided first clear evidence that **active immunization** could be used safely to prevent an infectious disease.
- **Almost 70 Years later, Pasteur** - introduced **pasteurization also**
  - Recognized and exploited the **general principle underlying vaccination**
- **At about 1900**,
  - Role of phagocytes and cellular immunity were elucidated

1.2 History …*Cont*

- Killed vaccines were introduced
- Complement was described

- **In 20th century,**
  - *Acquired immunity* resulted from both cellular and humoral elements were demonstrated.
  - *Opsonization* was described
  - The term *antigen* came in to regular use

1.2 History …*Cont*

Noble prize winners for immunologic research

- 1901 Emil von Behring, Serum antitoxins
- 1905 Robert Koch, Cellular immunity to tuberculosis
- 1908 Elie Metchnikoff, Role of phagocytosis
- 1908 Paul Ehrlich, antitoxins in immunity
- 1913 Charles Richet, Anaphylaxis
- 1919 Jules Border, Complement-mediated bacteriolysis
- 1930 Karl Landsteiner, Discovery of human blood groups

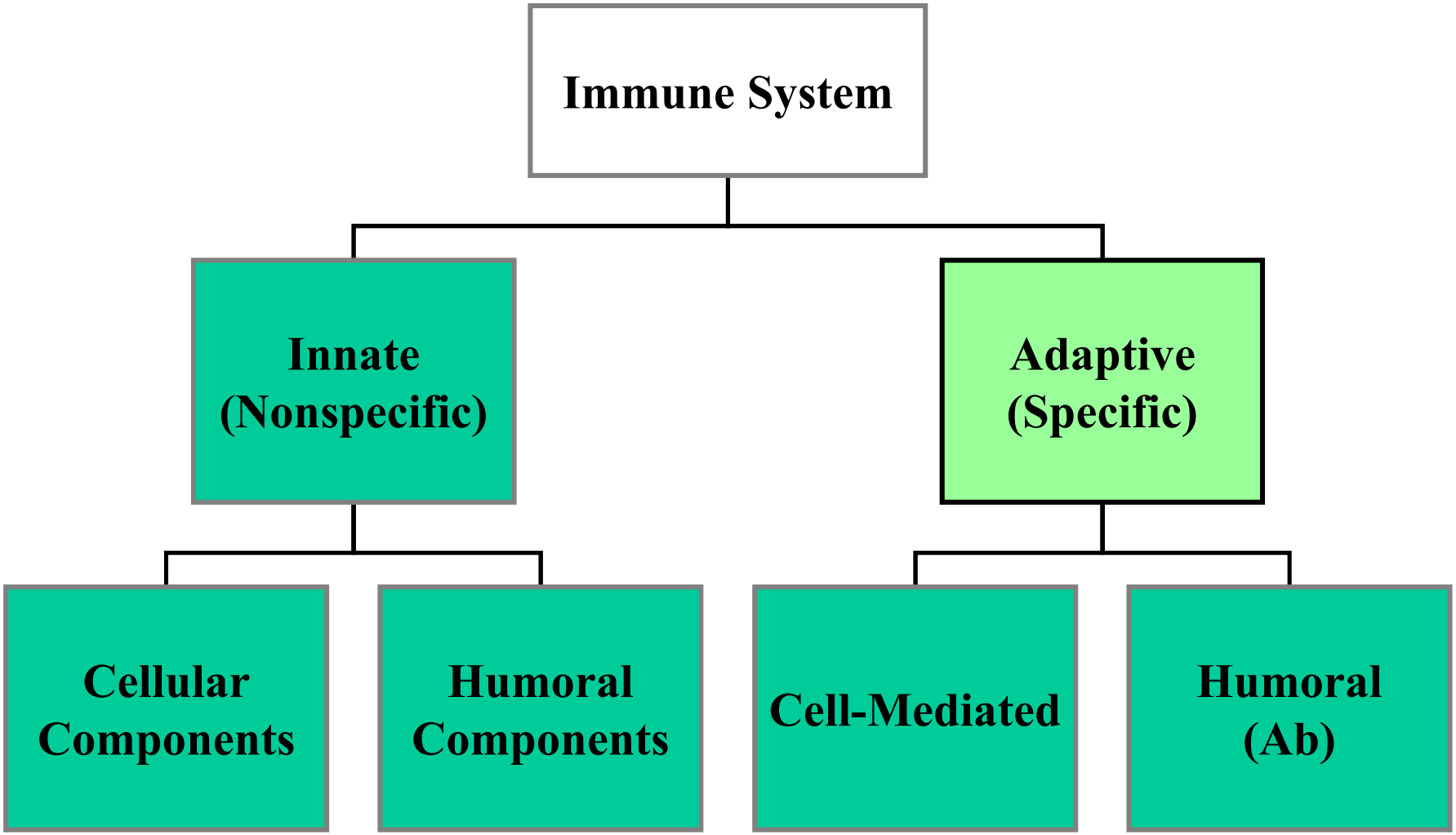
1.2 History …*Cont*

- 1951 Max Theiler, Development of yellow fever vaccine
- 1957 Daniel Bovet ,Antihistamines
- 1960 F. Macfarlane Burnet and Peter Medawar, Discovery of acquired immunological tolerance
- 1972 Rodney R. Porter and Gerald M. Edelman, Chemical structure of antibodies
- 1977 Rosalyn R. Yalow, Development of radioimmunoassay
- 1980 George Snell, Jean Dausscт and Baruj Benacerraf Major histocompatibility complex

1.2 History …*Cont*

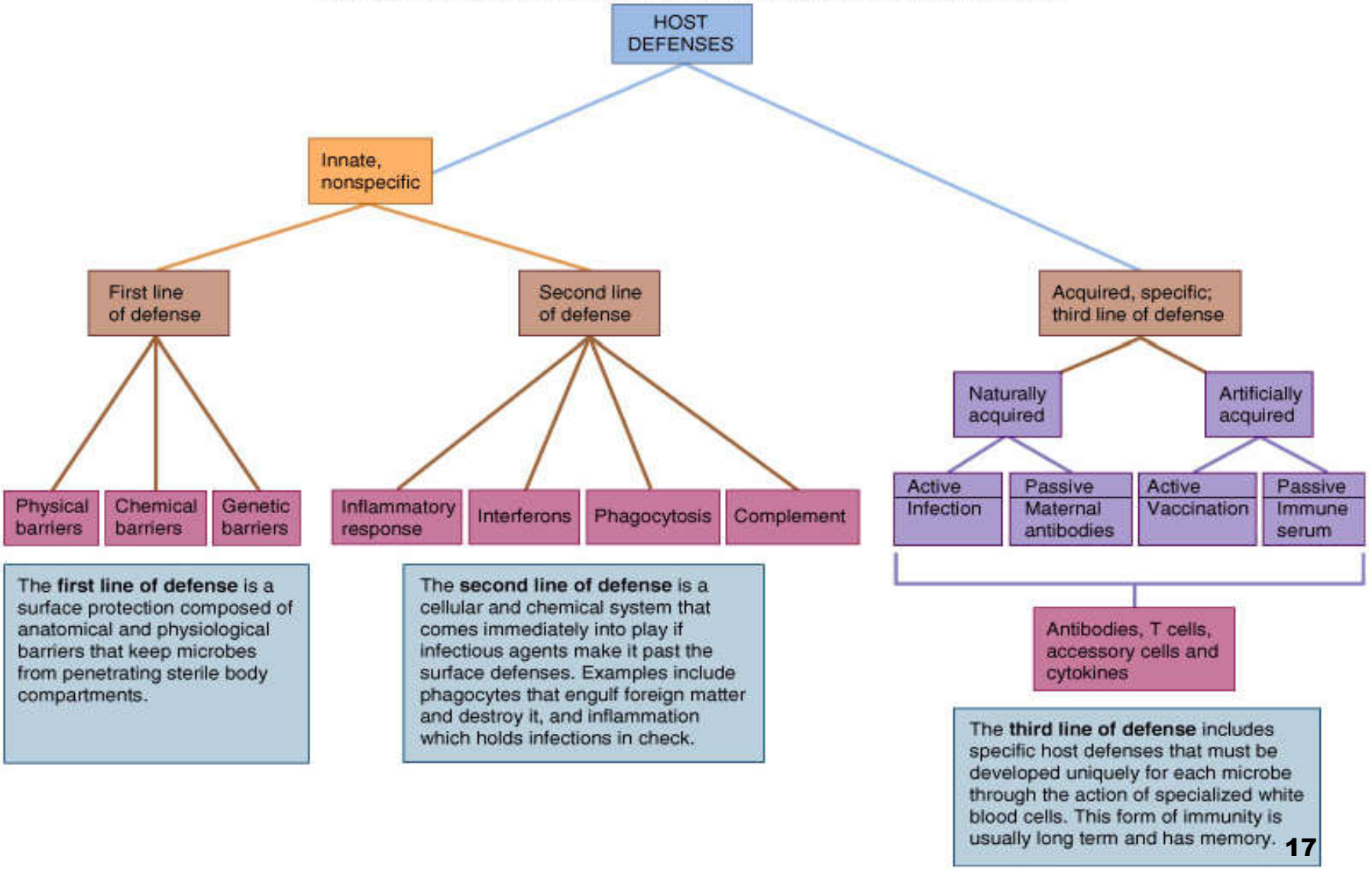
- 1984 Cesar Milstein and Georges E. Köhler, Monoclonal antibody
- 1984 Niels K. Jerne, Immune regulatory theories
- 1987 Susumu Tonegawa, Gene rearrangement in antibody production
- 1991 E. Donnall Thomas and Joseph Murray Transplantation immunology
- 1996 Peter C. Doherty, Role of major histocompatibility complex
- 1996 Rolf M. Zinkernagel, in antigen recognition by by T cells

1.3. The immune system



1.3. The immune system

Overview of the Immune System



1.4 The Innate immunity

Natural immune system (Innate Immunity)

- ✓ Non – specific
- ✓ First line of defense
- ✓ Repeated exposure - no augmentation

- Components

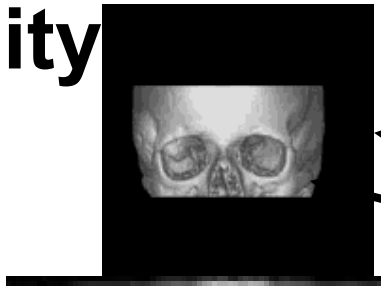
✓Biochemical

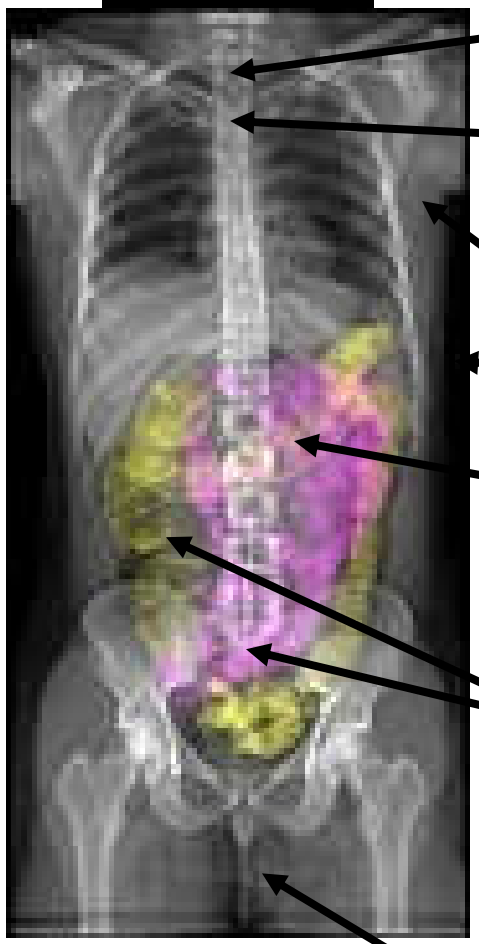
✓Physical

✓Cells

1.4 Innate Immunity

1. Components
   a. Biochemical
      - enzymes, C', etc.
      - secretions
      - pH
   b. Physical
      - skin
      - cilia
   c. Cells
      - Phagocytes, NK
2. Example
   a. Burn response





Lysozymes

Mucus

Cilia: trachea

Sebaceous glands

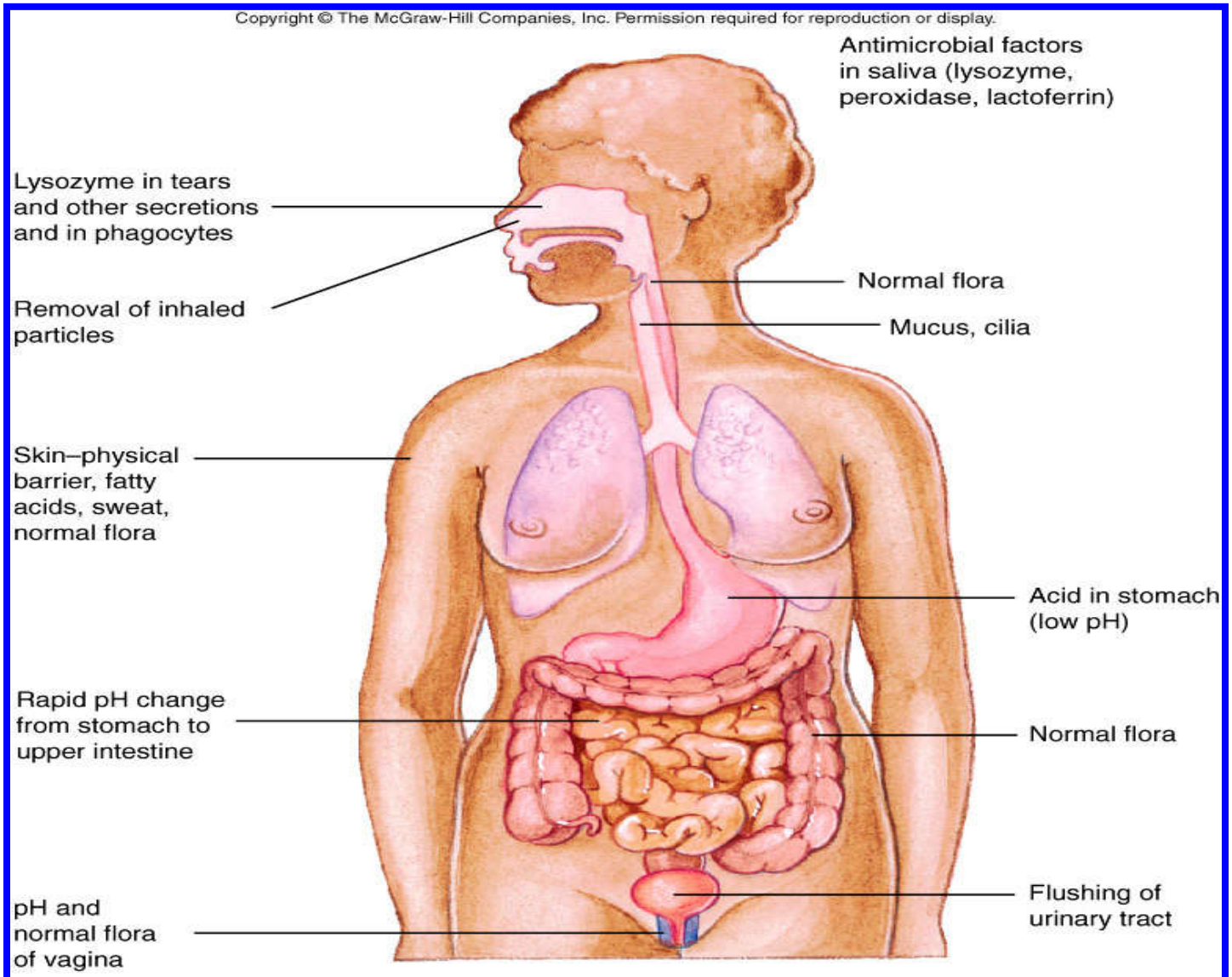
Skin

Acid in stomach

Commensal organisms in gut & vagina

Spermine in semen

1.4 Innate Immunity



1.4 Innate Immunity

- Overall non-specific reaction of body to injury or invasion – starts immediately with infection or trauma
  - Reactants may initiate, expand, or sustain the response
  - Can be acute (short duration) or become chronic (prolonged duration)
- Has 4 cardinal signs: **heat, pain, redness, loss of function** resulting from:

1.4 Innate Immunity

- Increased blood and plasma flow to the area
- Increased capillary permeability by retraction of endothelial cells
- mediated by vaso active agents such as **histamine and prostaglandins.**
  - derived from injured cells and later from cells that infiltrate the area.
- Migration of leucocytes, particularly Neutrophils and macrophages, from the capillaries to the site of injury is due to a process called *chemotaxis.*

1.4 Innate Immunity

- Migration of white cells, especially early migration of neutraphils then macrophages to the area
- Increased release of mediators such as histamine from damaged mast cells – furthering capillary dilation
- Increased concentration of acute phase reactants that can amplify and/or control the response

- **Complement** – a series of enzymes normally circulating in an inactive form may be activated resulting in lysis or enhanced phagocytosis of cells

1.4.1 External Innate Defense Systems

- Prevent entrance:
  - Structural barriers – effective with most microorganisms
    - Skin - epidermis = layers of tightly packed epithelial cells. Outer layer is dead cells and keratin, waterproofing protein
    - Inner layer skin - dermis = blood vessels, hair follicles, sweat glands, and sebaceous glands that produce an oily secretion called **sebum**
    - Cilia and cough reflex – helps expel microbe containing mucous
    - Sneeze

1.4.1 External Innate Defense Systems

- Mucus - conjunctivae, alimentary, respiratory, and urogenital tracts
  - saliva, tears, and mucous secretions wash away invaders and contain antibacterial or antiviral substances.
  - acidity (pH 5.6) of sweat, sebaceous glands, vagina (pH 5) and stomach (pH 1) – unfriendly to many microorganisms
  - enzymes present in the skin and stomach, tears
- Normal flora - out compete pathogens for attachment sites on the epithelial cell surface and for necessary nutrients.

1.4.1 Internal Innate Defense System

- To prevent expansion of penetration
  - Recognize carbohydrates not normally present on cells such as mannose
  - May cause nonspecific activation of white cells
    - Phagocytosis – by neutraphils, eosinophils, basophils, or macrophages, mast cells, and dendritic cells
  - Clotting mechanism which entraps organisms in fibrin clots
  - Complement System can lyse cells or enhance phagocytosis

1.4 Innate Immunity

Physiologic Barriers

- Soluble factors contribute to innate immunity, they are collectively known as acute phase reactants.
- Normal serum components, non-specific responders to inflammation
- Increase because of infection, injury, trauma
- Produced mostly by liver in response to inflammation and cytokine stimulation
  - Cytokines: IL-1, IL-6 and TNF alpha which are produced by macrophages and monocytes at inflammatory site are activators

1.4 Innate Immunity

- Acute phase reactants are chemically varied and include:
  - C-reactive protein,
  - serum amyloid A,
  - mannose binding protein,
  - alpha-1 anti-trypsin,
  - haptoglobulin,
  - fibrinogen,
  - ceruloplasmin,
  - alpha-1 acid glycoprotein
  - Complement

1.4 Innate Immunity

- **Complement** – a series of enzymes normally circulating in an inactive form
  - May be activated by the classical or alternate pathways
  - Can result in lysis or enhanced phagocytosis of cells

- **Lysozyme,** a hydrolytic enzyme in mucous secretions and in tears, can cleave the peptidoglycan layer of bacterial cell wall.
- **Interferon,** proteins produced by virus-infected cells. Has many functions including ability to bind to nearby cells and induce a generalized antiviral state.

1.4 Innate Immunity

C-Reactive Protein

- Normally trace levels in serum
- **Early acute inflammation indicator**:
  - increases within 4-6 hrs of infection or trauma
  - 100 to 1000 fold increase serum concentration
  - concentration drops rapidly in serum when stimulus removed
- Enhances opsonization, agglutination, precipitation, and classical pathway complement activation – enhances removal of irritant

1.4 Innate Immunity

Phagocytosis

- Phagocytic cells Chemotaxins such as
  - Complement components
  - Coagulation cascade proteins
  - Bacterial and viral products
- Attract phagocytic cells including:
  - Mast cell, lymphocyte, macrophage, neutrophil products
- Physical contact between phagocytic cell and foreign object results in
  - Formation of phagosome
  - Formation of phagolysosome
  - Digestion
  - Release of debris

1.4 Innate Immunity

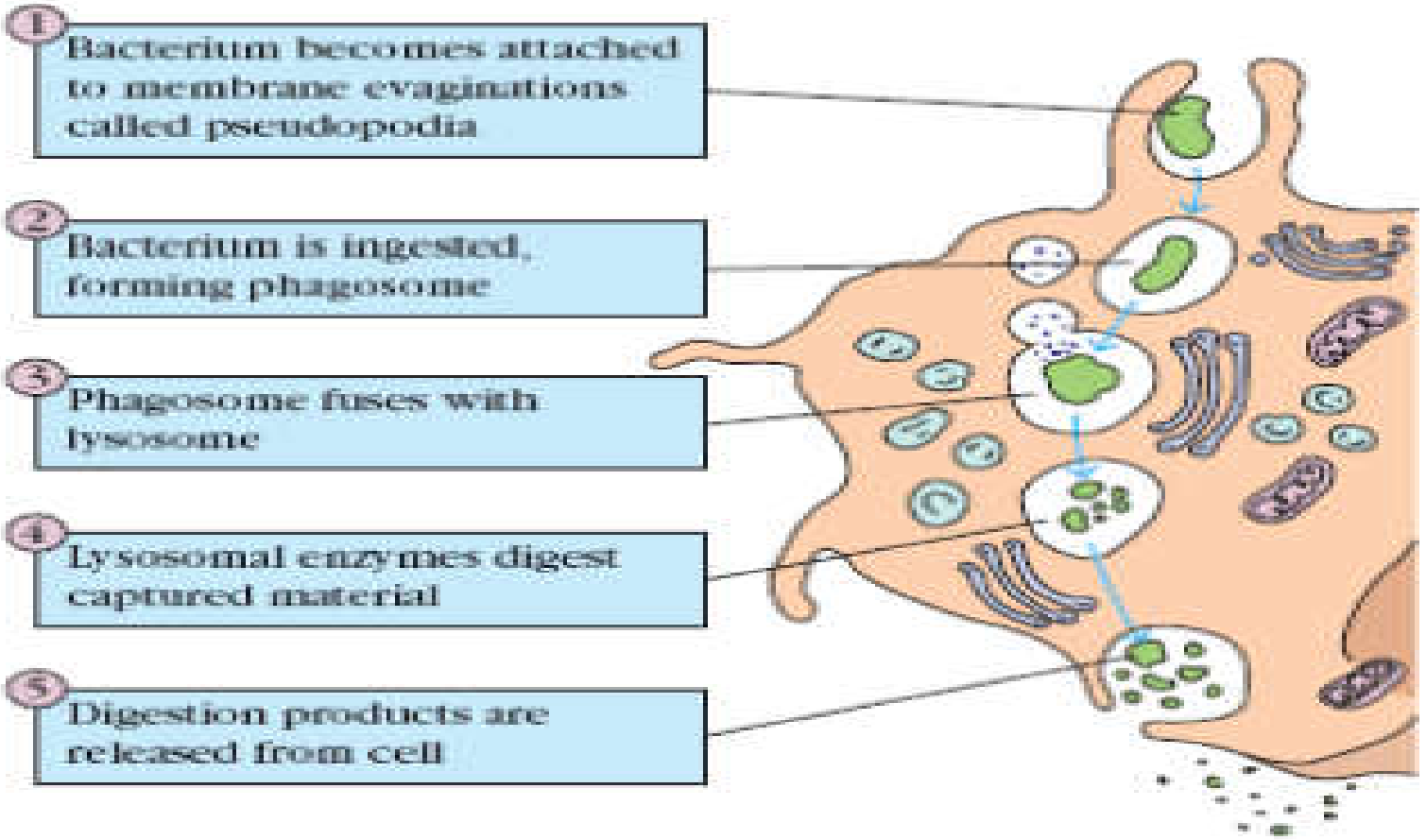
- Phagocytosis
  - Is a form of endocytosis.
  - Important body defense mechanism is process in which specialized cells engulf and destroy foreign particles such as microorganisms or damaged cells.
  - Macrophages and segmented Neutrophiils are the most important phagocytic cells.
- Can be divided in to several stages:
  - chemotaxis – attraction of leukocytes or other cells by chemicals
  - Movement of neutraphils is influenced by chemotaxins – chemical messangers
    - Complement, proteins from coagulation,
    - Products from bacteria and viruses,
    - Secretions from mast cells, lymphocytes, macrophages, and other neutraphils

1.4 Innate Immunity

- Phagocytosis ...
  - Adherence – binding of organism to the surface of phagocytic cell.
  - Engulfment:- is the injestion of m/os and formation of phagosomes.
  - Digestion – after the foreign particle or m/os is ingested, cytoplasm lysosome fuse with phagosome The enzymes of lysosome then contribute to microbial killing and lysis.

1.4 Innate Immunity

Phagocytosis ...



1.5 Adaptive Immunity

- Specific
- Second line of defense
- Repeated exposure - augmented – memory
- Faster response
- More vigorous response
- Longer lasting response
- Anamnestic

Components

Classic Immune System

- Cells (Cell mediated) =CMI
- Soluble Factors (Humoral immunity) = HI

1.5 Adaptive immune system

- Capable of recognizing and selectively eliminating specific foreign microorganisms and molecules(i.e., foreign antigens).
- Unlike innate immune responses, adaptive immune responses are reactions to specific antigenic challenges
- Different populations of lymphocytes and their products are the major actors together with accessory cells – Antigen presenting cells (APCs)
- Cardinal features are :
  - Specificity
  - Diversity
  - Memory

1.5 Adaptive immune system

Cardinal Features of adaptive Immune Responses

- **Specificity** –
  - specific for distinct antigen, and
  - for different structural components of a single complex protein, polysaccharide, or other macromolecules.
  - Portions of such antigens recognized by individual lymphocytes are called *determinants or epitopes*.
  - This fine specificity exists because individual lymphocyte express membrane receptors able to distinguish subtle (slight) differences in structure between distinct antigens.

1.5 Adaptive immune system

- **Diversity**- total number of antigenic specificities of the lymphocytes in an individual, called *the lymphocyte repertoire,* is extremely large.
  - estimated mammalian immune system can discriminate 10^9 to 10^{11} distinct antigenic date ruminants.
  - This property of the lymphocyte repertoire is called diversity. It is the *result of variability in the structures of antigen- binding sites of lymphocyte receptors for antigens.*
- **Memory-** Exposure of the immune system to foreign antigen:
  - enhances its ability to respond again to that antigen.
  - Responses to second and subsequent exposure to the same antigen, called *secondary immune responses*, are usually more rapid and larger than the first or primary immune response.

Adaptive immune continued

- An effective immune response involves three major groups of cells: Cellular Immunity (*T lymphocytes), Humoral Immunity (B cells),* and Accessory cells (*antigen-presenting cells).*
- The two major populations of lymphocytes—**B lymphocytes (B cells) of Humoral immunity** and **T lymphocytes (T cells) of Cellular Immunity provide us with our specific adaptive immunity**

Adaptive immune continued

- **Specialization** –the immune system responds in distinct and special ways to different microbes*, maximizing the efficiency* of antimicrobial defense mechanisms. Thus, humoral immunity and cell mediated immunity are elicited by different classes of microbes or by the same microbe at different stages of infection (extra cellular & intra cellular)
- **Self –limitation-** All normal immune responses returning the immune system to its resting or basal state with time after antigen stimulations, process called **homeostasis**.

Summary of innate and adaptive immunity

Comparison of Innate and Adaptive Immunity	
Innate Immunity	**Adaptive Immunity**
• No time lag	• A lag period
• Not antigen specific	• Antigen specific
• No memory	• Development of memory

CHAPTER 2

Cells and organs of immune system

Cells and organs of immune system

Learning Objectives

Upon completion of this lesson the student will be able to:

- Describe cells and organs of immune system
- Describe lymphoid tissue by primary or secondary, locations of specialized tissues, cells produced and key role in immunity.
- Describe the morphology, source and role of macrophages, natural killer cells, cytotoxic, helper, suppressor or B lymphocytes and plasma cells.
- Discuss the role of surface markers in cells involved with immunity, referring to specific markers used to differentiate T and B lymphocytes.

2.1. Organs of the immune system

- The immune system consists of many different organs and tissues that are found throughout the body.
- These organs can be classified functionally into two main groups.
    - The primary lymphoid organs provide appropriate microenvironments for the development and maturation of lymphocytes. This includes Thymus, Bone marrow, Fetal liver

Organs of the immune system *cont*..

- The *secondary lymphoid organs* trap antigen from defined tissues or vascular spaces and are sites where mature lymphocytes can interact effectively with that antigen.
- Blood vessels and lymphatic systems connect these organs, uniting them into a functional whole.
- These are
  - Lymph nodes, Spleen,
  - Mucosa Associated Lymph tissue (MALT)
  - Tonsils, Peyers patches, lamina propria (largest amount of lymphs), appendix collectively known Gut associated lymhoid tissue (GALT)
  - Bronchial associated lymphoid tissue (BALT)

Organs of the Immune System Continued

tertiary lymphoid tissues

- which normally contain fewer lymphoid cells than secondary lymphoid organs,
- Can import lymphoid cells during an inflammatory response.
- Most prominent of these are
  - cutaneous-associated lymphoid Tissues (CALT).

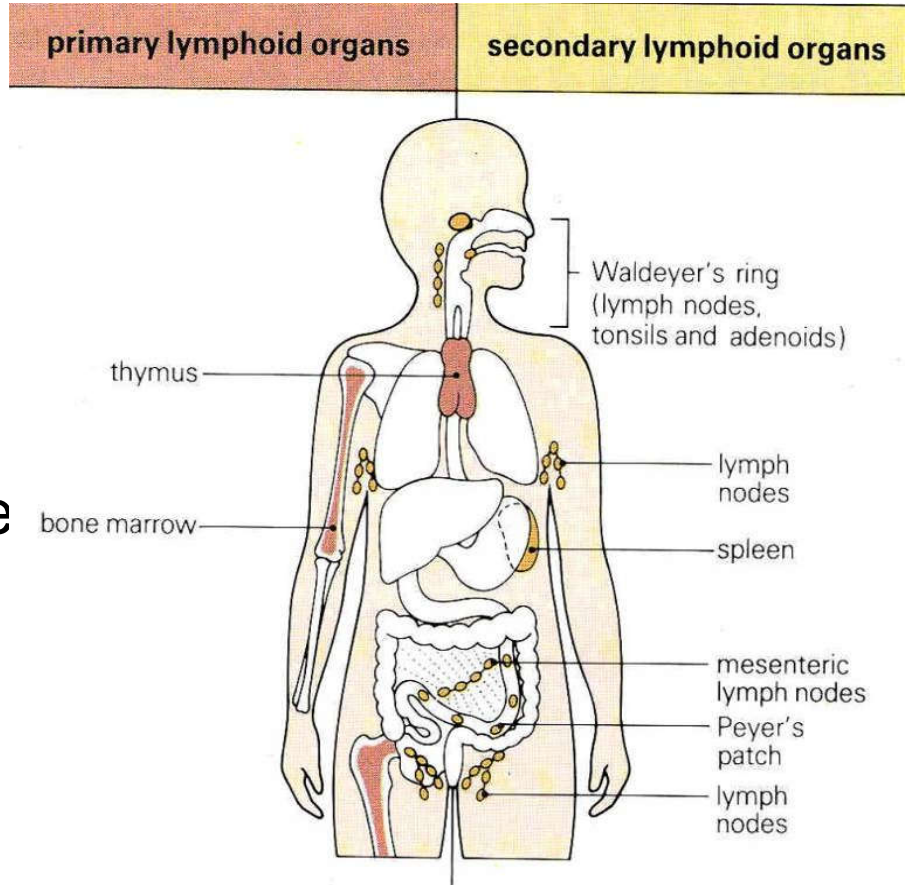
Organs of the Immune System …..Continued

- Once mature lymphocytes have been generated in the primary lymphoid organs, they circulate in the blood and **lymphatic system,** a network of vessels that collect fluid that has escaped into the tissues from capillaries of the circulatory system and ultimately return it to the blood.

2.2.1 Lymphoid System

Classification-Residence

1. Primary lymphoid tissue primary diff/maturation
    1. Thymus
    2. Bone marrow
    3. Fetal liver
2. Secondary Lymphoid tissue - Ag exposure and final differentiation
    1. Lymph nodes, Spleen
    2. Mucosa Associated Lymph tissue (MALT)



Organs of the Immune System … continued

THYMUS

- the site of T-cell development and maturation.
- flat, bilobed organ situated above the heart.
- each lobe is surrounded by a capsule and is divided into lobules.
- each lobule is organized into two compartments:
- the **outer** compartment, or *cortex,* is densely packed with immature T cells, called thymocytes.
- the **inner** compartment, or *medulla,* is sparsely populated with thymocytes.

Organs of the Immune System …continued

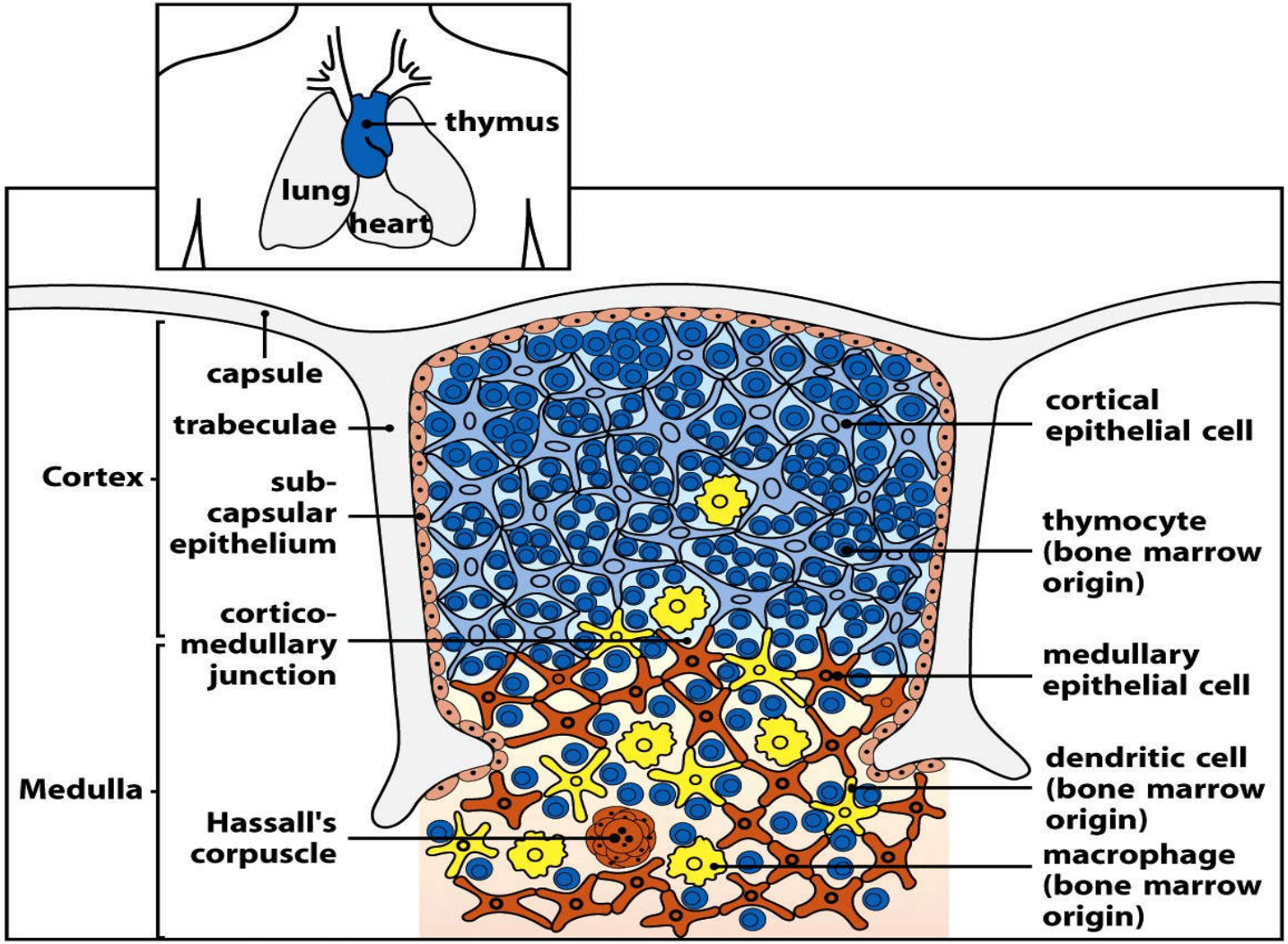
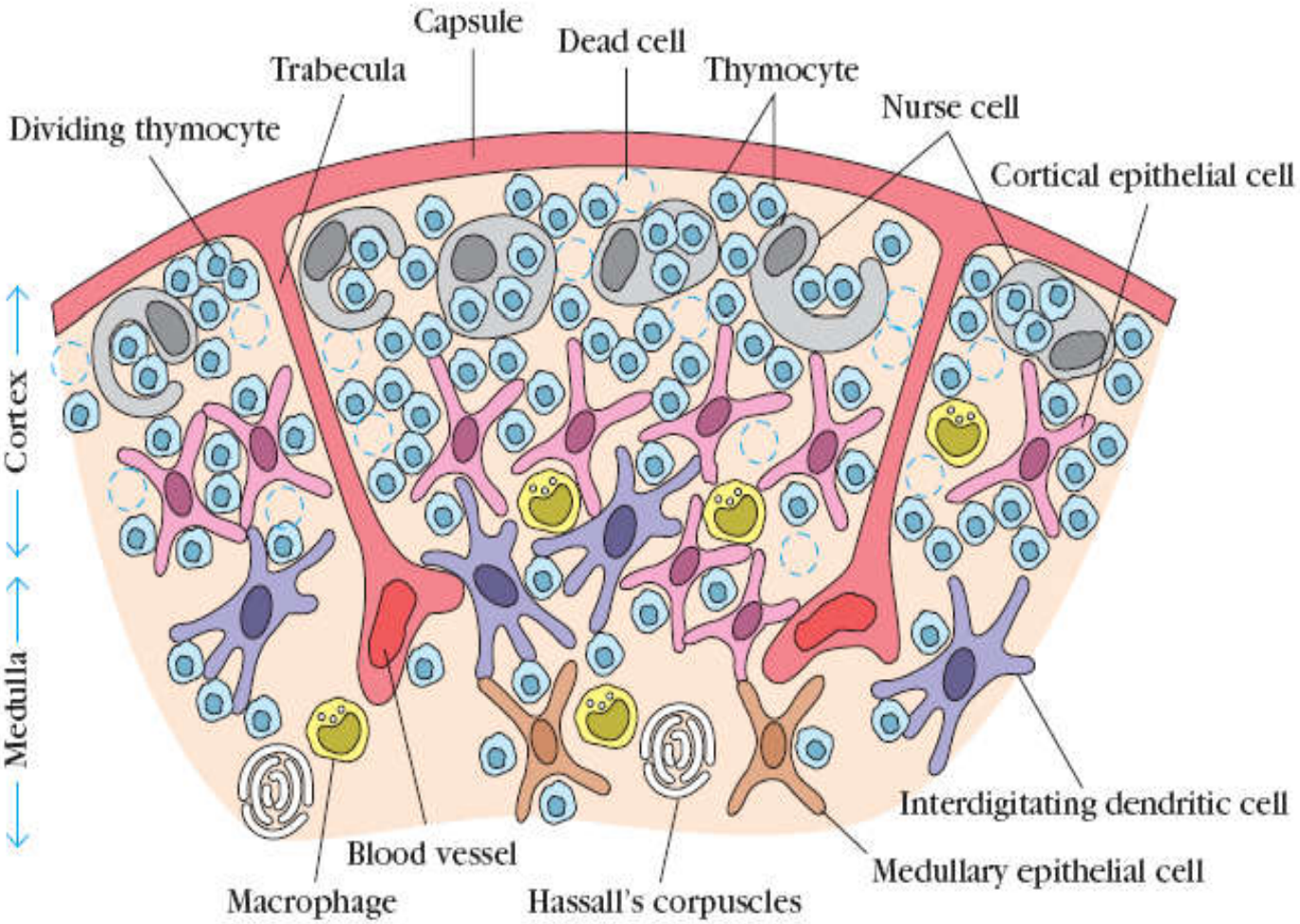


Figure 7-15 part 1 of 2 Immunobiology, 7ed. (© Garland Science 2008)

Organs of the Immune System …continued



Organs of the Immune System …continued

- Both the cortex and medulla are criss crossed by a three-dimensional stromal-cell network composed of
  - epithelial cells,
  - dendritic cells, and
  - macrophages, which make up the framework of the organ and contribute to the growth and maturation of thymocytes (T cells)

Organs of the Immune System continued

Function of Thymus

- to generate and select a repertoire of T cells that will protect the body from infection.
- As thymocytes develop, an enormous diversity of T-cell receptors is generated by a random process that produces some T cells with receptors capable of recognizing antigen-MHC complexes.

Organs of the Immune System continued

- most of the T-cell receptors produced by this random process are incapable of recognizing antigen-MHC complexes and a small portion react with combinations of self antigen-MHC complexes.
- More than 95% of all thymocytes die by apoptosis in the thymus without ever reaching maturity.

Organs of the Immune System Continued

BONE MARROW

- the site of B-cell origin and development.
- Immature B cells proliferate and differentiate within the bone marrow, and stromal cells within the bone marrow interact directly with the B cells and secrete various cytokines that are required for development.
- selection process within the bone marrow eliminates B cells with self-reactive antibody receptors.

Organs of the Immune System Continued

LYMPH NODES

- These are sites where immune responses are mounted to antigens in lymph.
- are encapsulated bean shaped structures containing a reticular network packed with
  - lymphocytes,
  - macrophages, and
  - dendritic cells

Organs of the Immune System Continued

LYMPH NODES

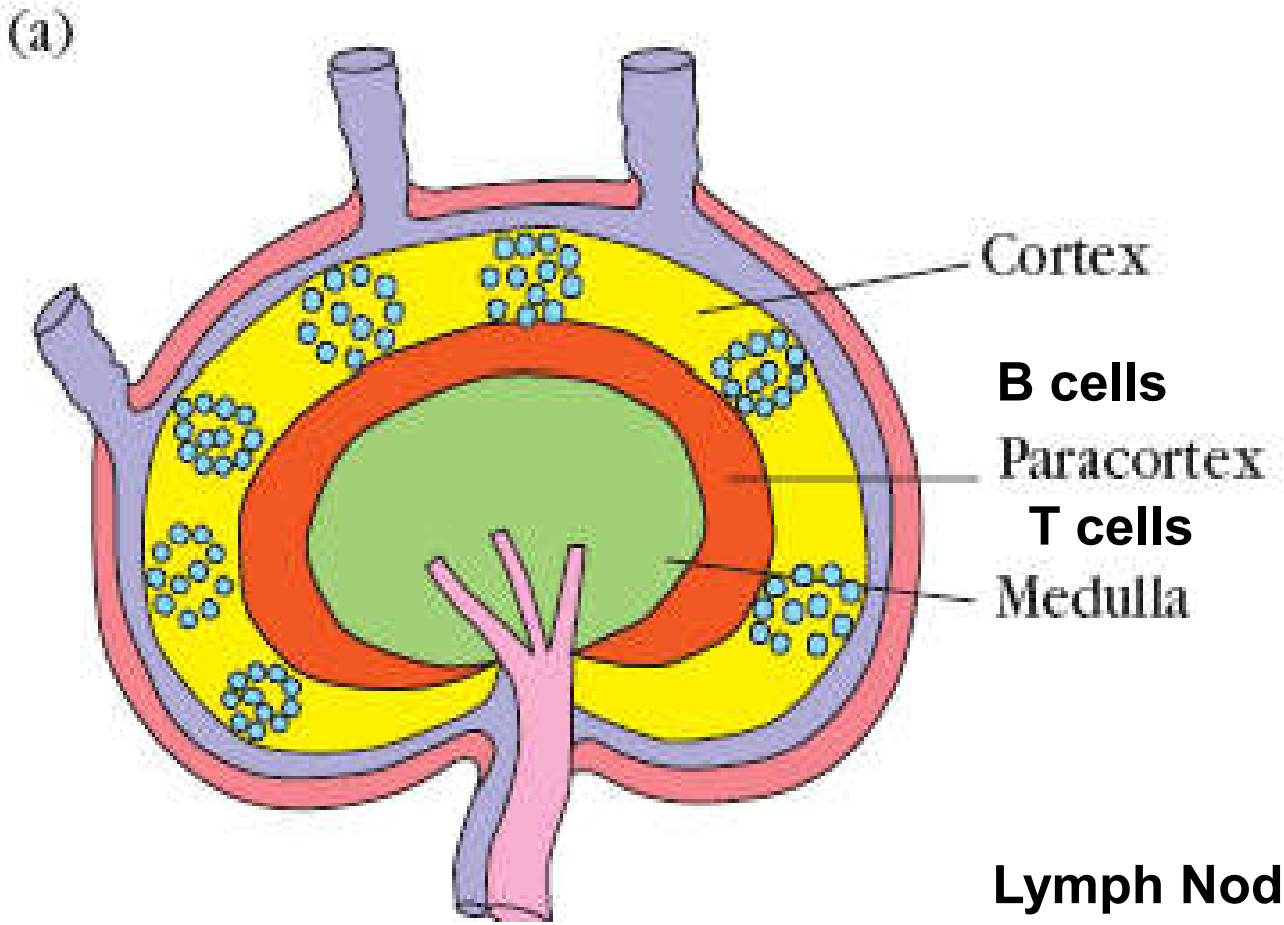
- the first organized lymphoid structure to encounter antigens that enter the tissue spaces.
- **Morphology**

  lymph node can be divided into three
- the **cortex**
  - The outermost layer,,
  - Contains lymphocytes (mostly B cells), macro-phages, and follicular dendritic cells arranged in primary follicles.
  - After antigenic challenge, the primary follicles enlarge into secondary follicles, each containing a germinal center.

Organs of the Immune System Continued

- the **paracortex**
  - Beneath the cortex.
  - populated largely by T lymphocytes and
  - contains interdigitating dendritic cells
  - These interdigitating dendritic cells express high levels of class II MHC molecules, which function as APC .
- the **medulla**
  - The innermost layer .
  - more sparsely populated with lymphoid-lineage cells; of those present, many are plasma cells actively secreting antibody molecules.

Organs of the Immune System Continued



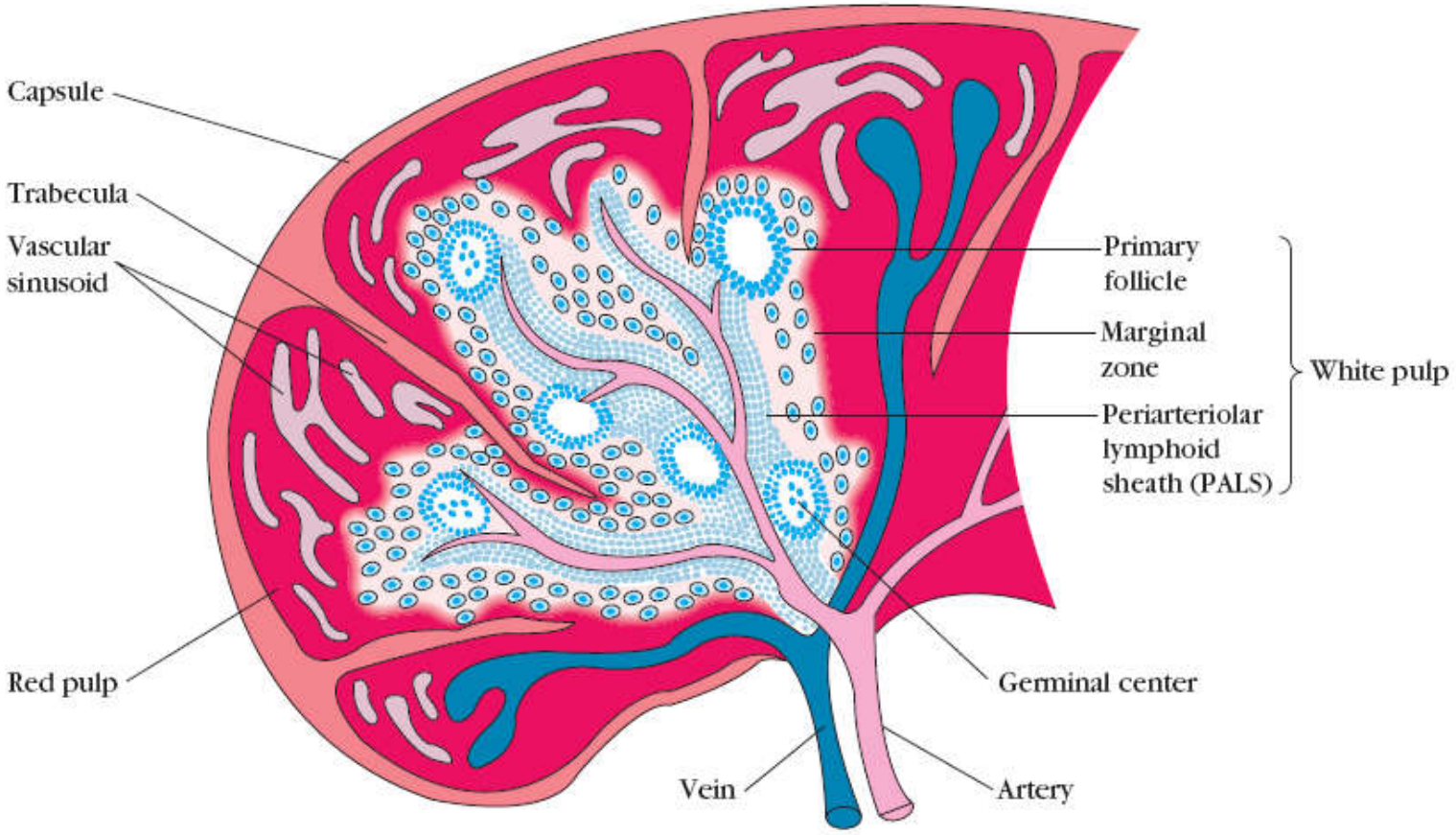
Lymph Node

Organs of the Immune System Continued

SPLEEN

- plays a major role in mounting immune responses to antigens in the blood stream.
- large, ovoid situated high in the left abdominal cavity.
- specializes in filtering blood and trapping blood-borne antigens;
- can respond to systemic infections.
- two compartments
  - the red pulp populated by MØs, RBCs and few lymphocytes
    - A site where old and defective RBCs are destroyed and removed
  - white pulp, is primarily populated by T cells and B cells

Organs of the Immune System Continued



Structure of SPLEEN

Organs of the Immune System Continued

Cutaneous-Associated Lymphoid Tissue

- The skin is barrier to the external environment.
- important in nonspecific defenses.
- epithelial cells the outer layer of the skin (keratinocytes) secrete a number of cytokines that may function to induce a local inflammatory reaction.

Organs of the Immune System Continued

- keratinocytes can be induced to express class II MHC molecules and may function as APC.
- The Langerhans cells migrate from the epidermis to regional lymph nodes, where they differentiate into interdigitating dendritic cells.
- These cells express high levels of class II MHC molecules and function as potent activators of naive TH cell

Summary

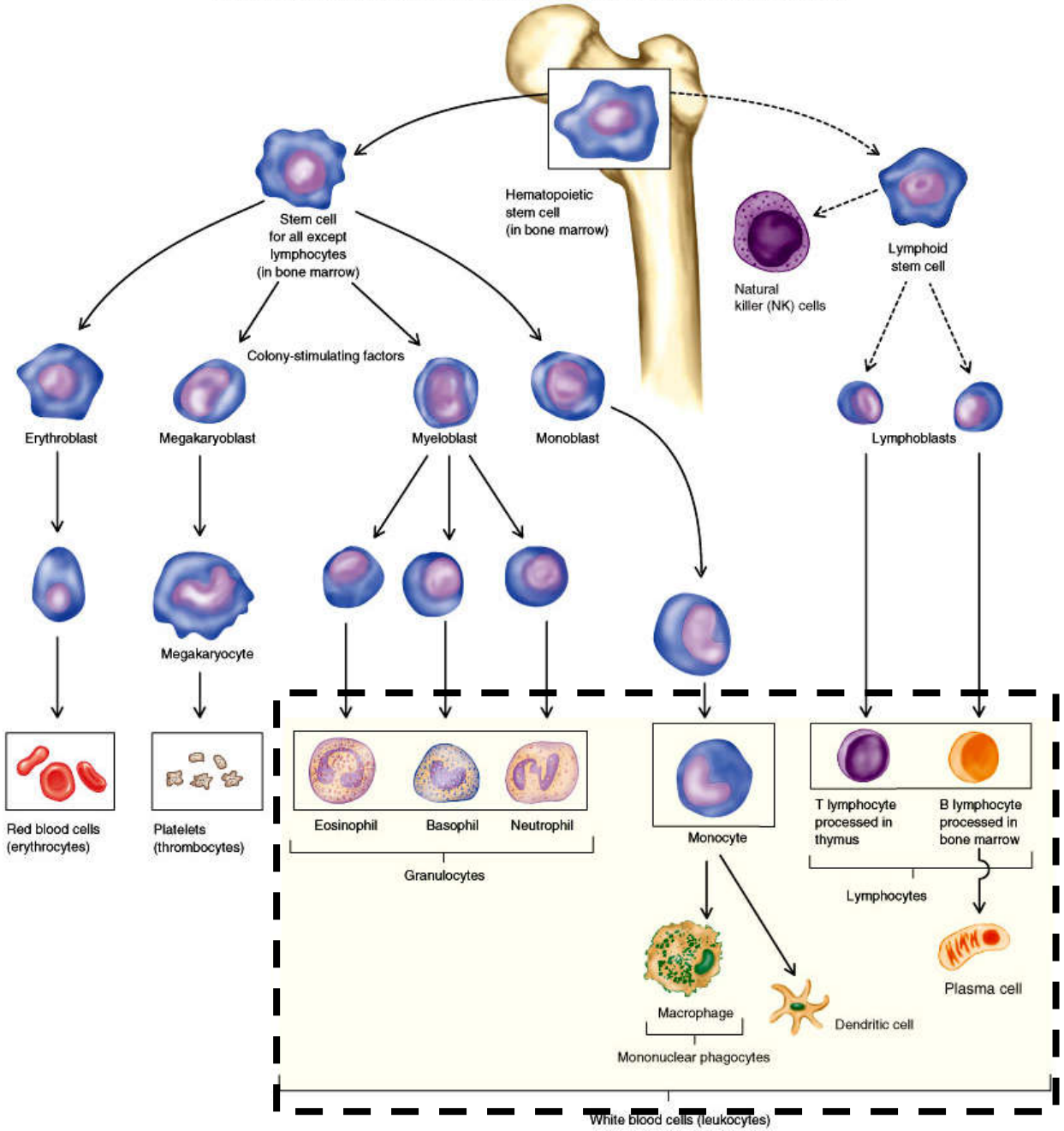
- The cells that participate in the immune response are white blood cells.
- The lymphocyte is the only cell to possess the immunologic attributes of specificity, diversity, memory, and self/non self recognition
- The primary lymphoid organs provide sites where lymphocytes mature and become antigenically committed
- Secondary lymphoid organs capture antigens and provide sites where lymphocytes become activated by interaction with antigens.

Development of Immune cells

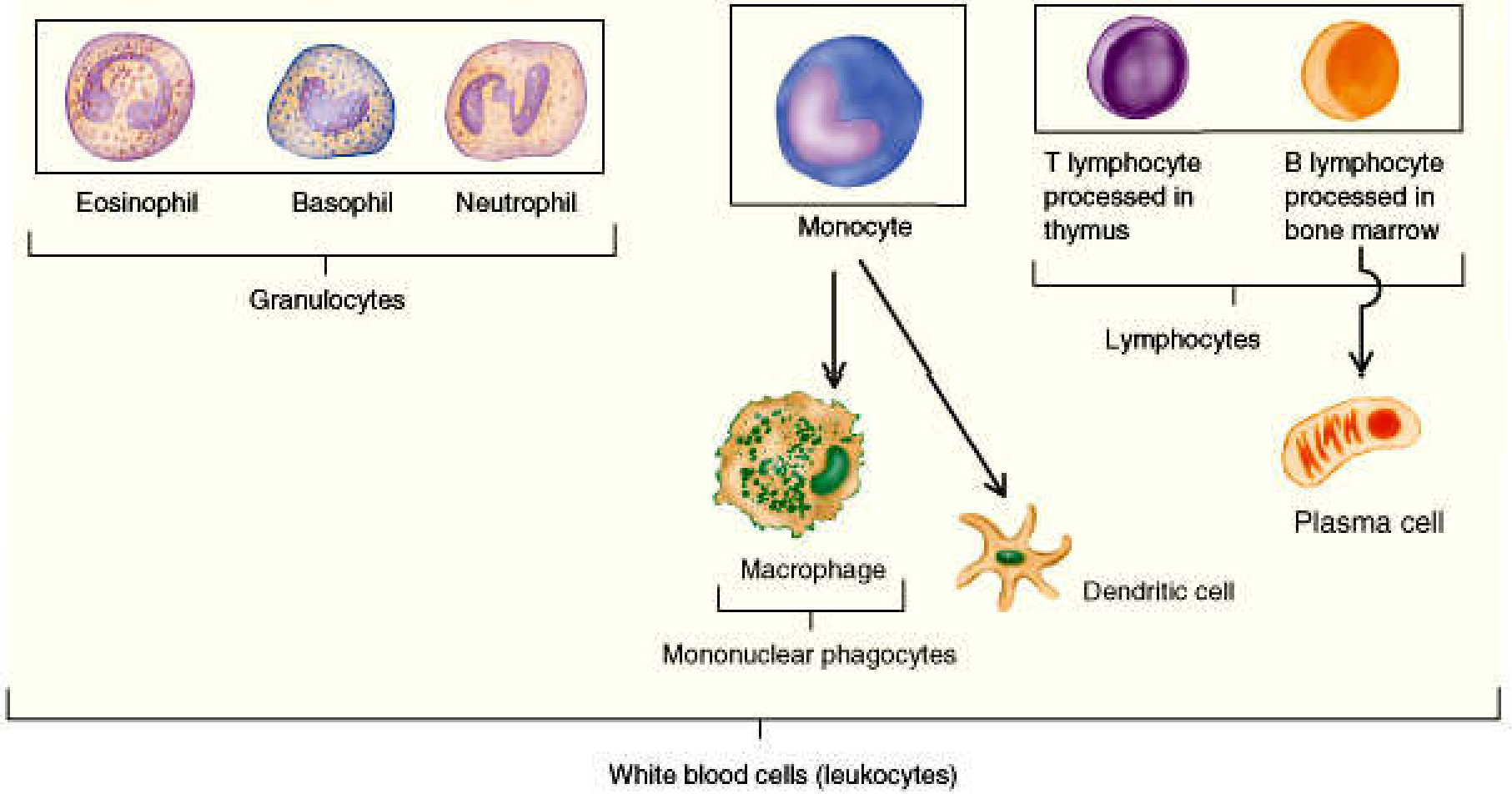
Hematopoiesis

- All blood cells arise from a type of cell called the **hematopoietic stem cell (HSC).**
- **Stem cells** are cells that can differentiate into other cell types; they are self-renewing-they maintain their population level by cell division.
- In humans, **hematopoiesis,** the formation and development of red and WBCs, begins in the embryonic yolk sac during the first weeks of development.

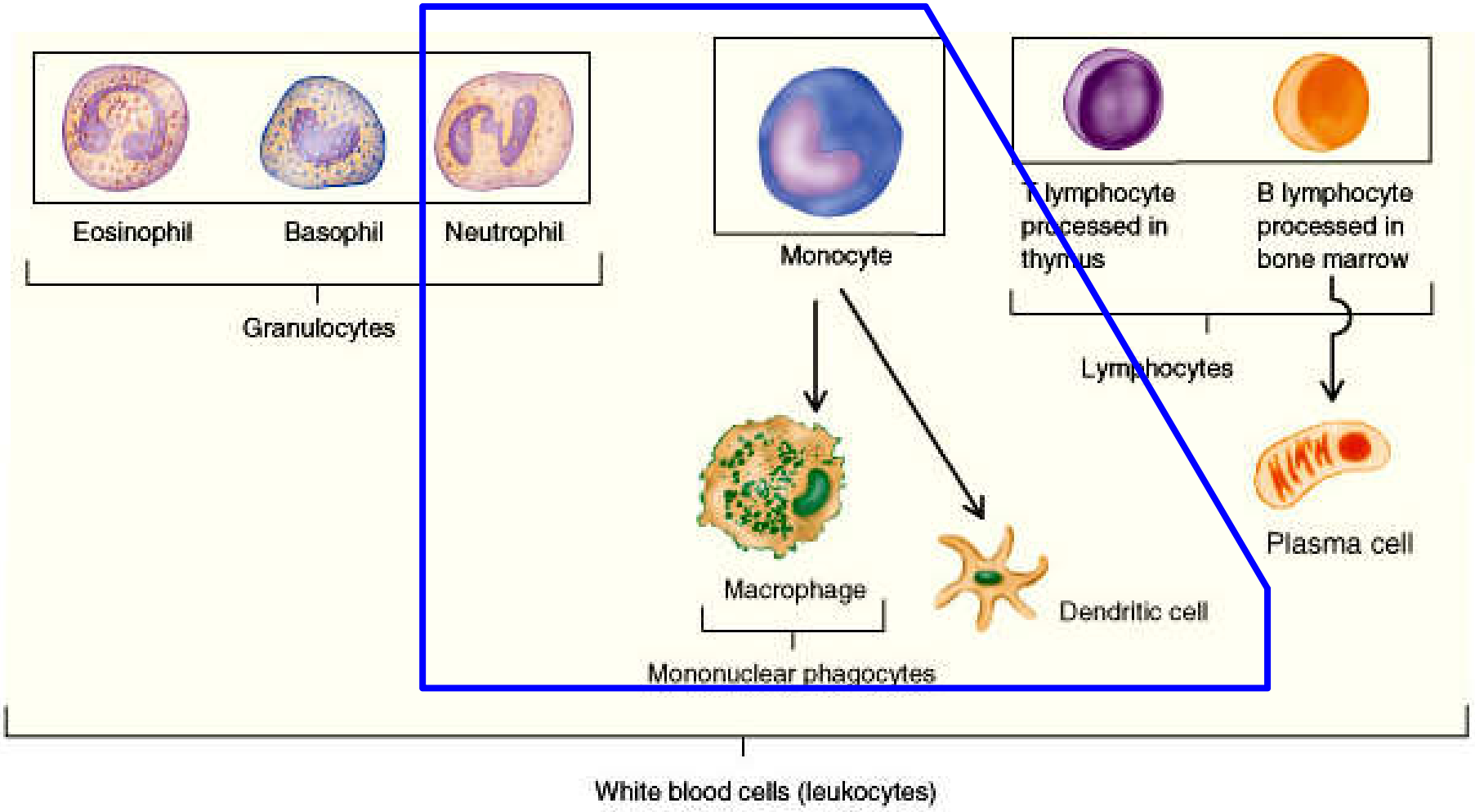
Development of immune Cells



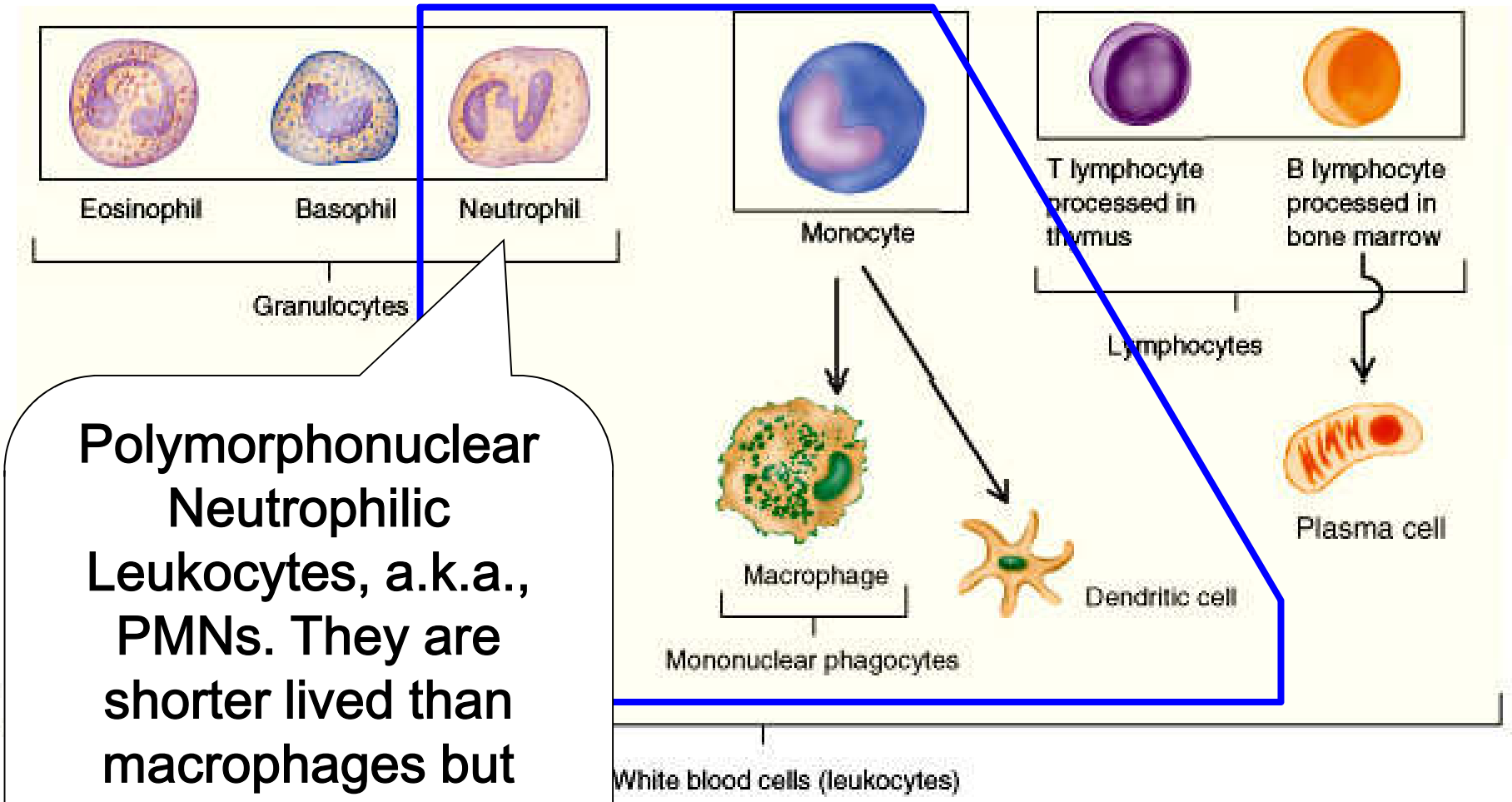
Cells of the immune system



Cells of the immune system

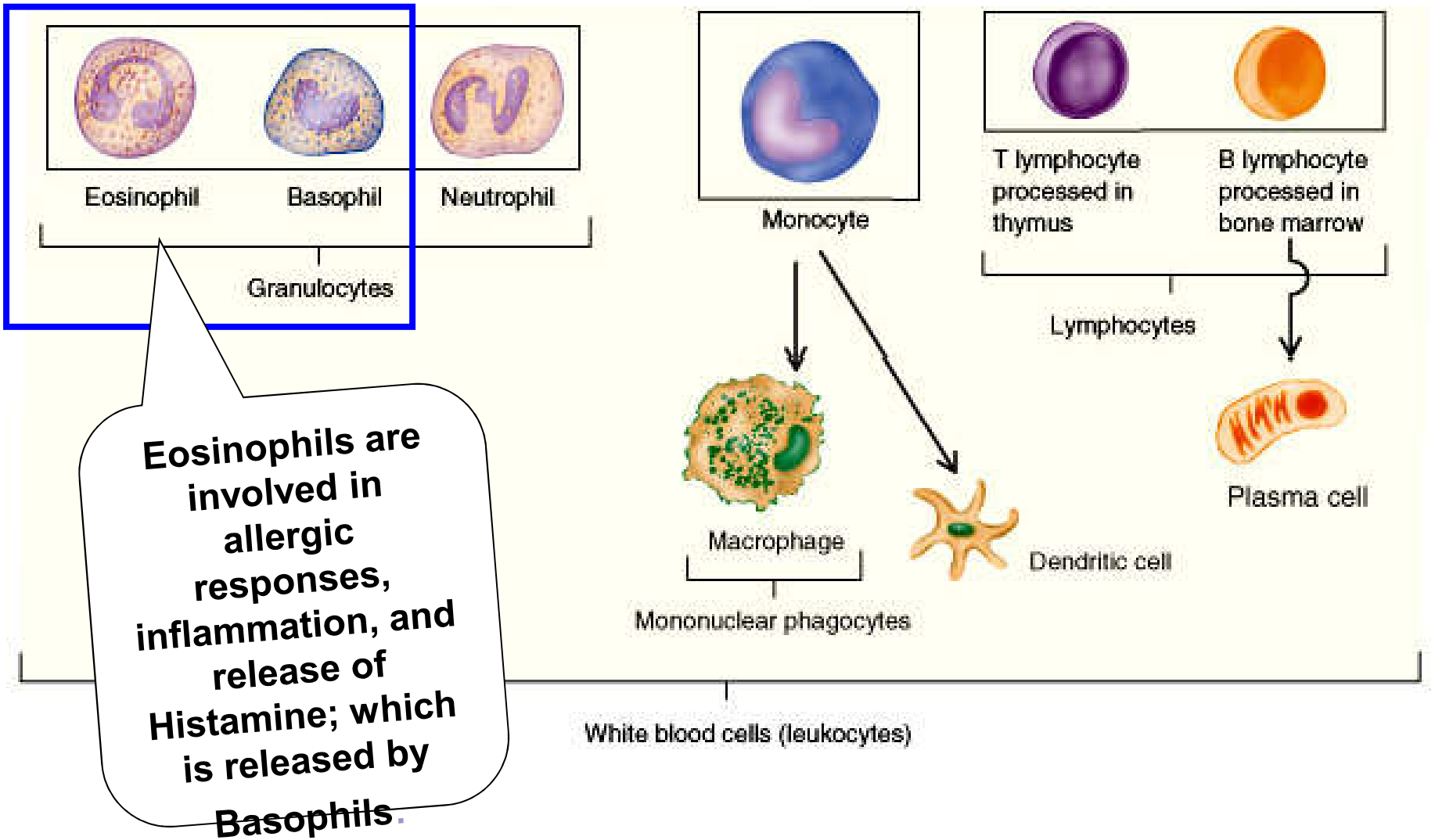


Cells of the immune system



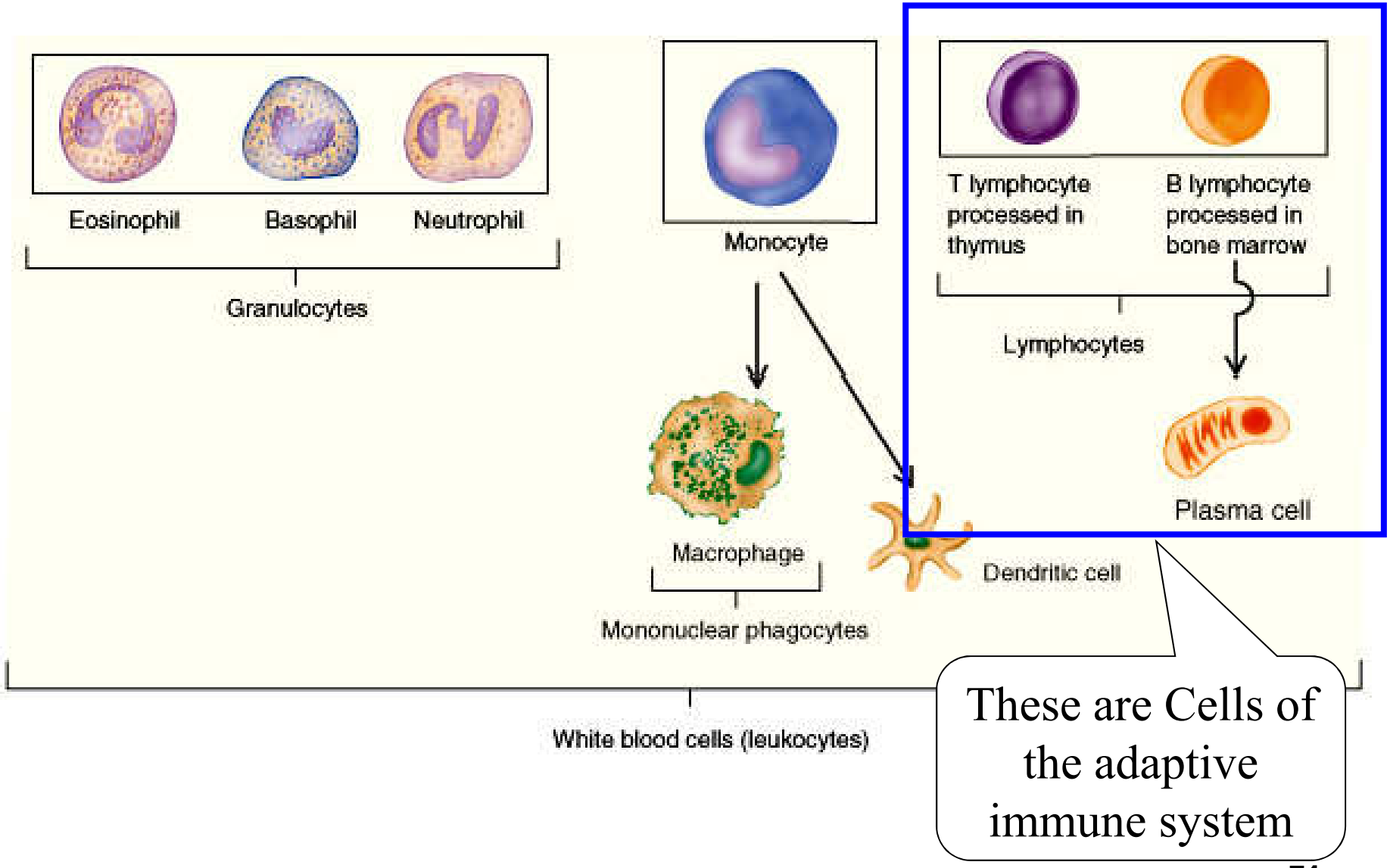
Polymorphonuclear Neutrophilic Leukocytes, a.k.a., PMNs. They are shorter lived than macrophages but have greater killing power.

Cells of the immune system



Eosinophils are involved in allergic responses, inflammation, and release of Histamine; which is released by Basophils.

Cells of the immune system



These are Cells of the adaptive immune system

2.1. Cells of the immune system

- Lymphocytes: (B cells and T cells)

  Lymphocytes are the central cells of the immune system, responsible for adaptive immunity and the immunologic attributes of diversity, specificity, memory, and self/non-self recognition.
- The other types of white blood cells play important roles, engulfing and destroying microorganisms, presenting antigens, and secreting cytokines such as Natural Killer cells (NKs), Nutrophils, Macrophage, Eosinophils, Basophilis, Mast cells and Dendritic cells.

Cells of Innate Immunity

Natural Killer Cells

- Natural killer cells (NKs), described in 1970s, were shown to be a small population of large, granular lymphocytes that display cytotoxic activity against a wide range of tumor cells in the absence of any previous immunization with the tumor.
- NK cells play an important role in host defense both against tumor cells and against cells infected with some though not all, viruses.

Cells of Innate Immunity

Natural Killer Cells Continued

- do not express the membrane molecules and receptors that distinguish T- and B-cell lineages.

- Although NK cells do not have T-cell receptors or immunoglobulin incorporated in their plasma membranes, they can recognize potential target cells in two different ways:

Cells of Innate Immunity

Natural Killer Cells Continued

- NK cells may employ receptors that distinguish abnormalities, notably a reduction in class I MHC molecules and the unusual profile of surface antigens displayed by some tumor cells and cells infected by some viruses.
- NK cells recognize potential target cells depends upon the fact that some tumor cells and cells infected by certain viruses display antigens against which the immune system has made an antibody response, so that antitumor or antiviral antibodies are bound to their surfaces

Cells of Innate Immunity

- **Mononuclear Phagocytes**
  - The mononuclear phagocytic system consists of:
    - **Monocytes**
    - **Macrophages all sorts**
- **Polymorphonuclear phagocytic cells (PMNs)**
  - PMNs are capable of ingesting and digesting exogenous antigens, such as whole microorganisms and insoluble particles, and endogenous matter.

Dendritic cells (DCs)

- Derived from myeloid progenitor (some lymphoid)

Immature DCs:

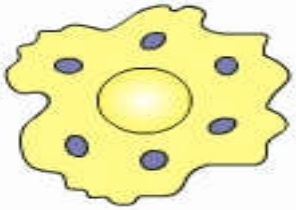
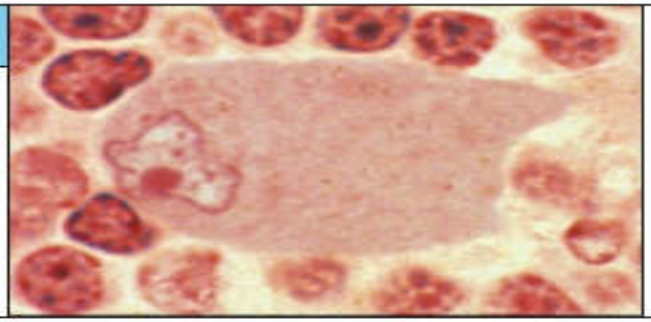
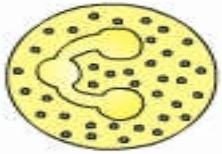
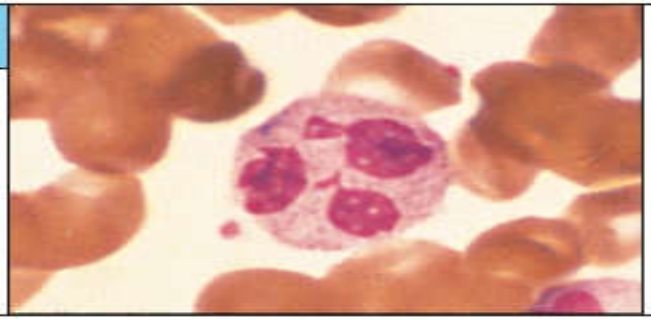
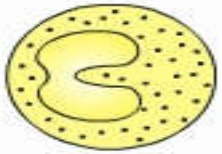
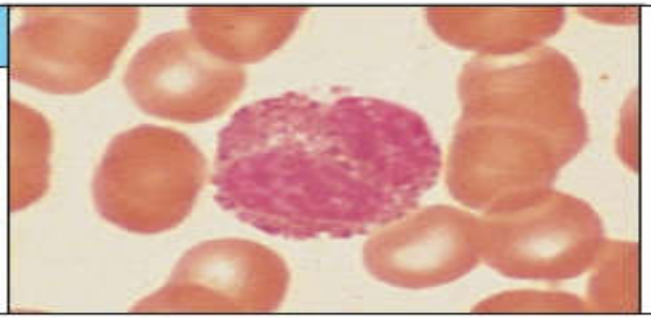
- migrate from blood to reside in tissues and are both phagocytic and micropinocytic (ingest large amount of the surrounding ECF)
- continuously migrate from the tissues bearing self Ags and induce tolerance as they lack co-stimulatory molecules
- Upon encountering a pathogen, they readily mature, express co-stimulatory molecules, and migrate to lymph nodes

Dendritic cells (DCs) cont'd

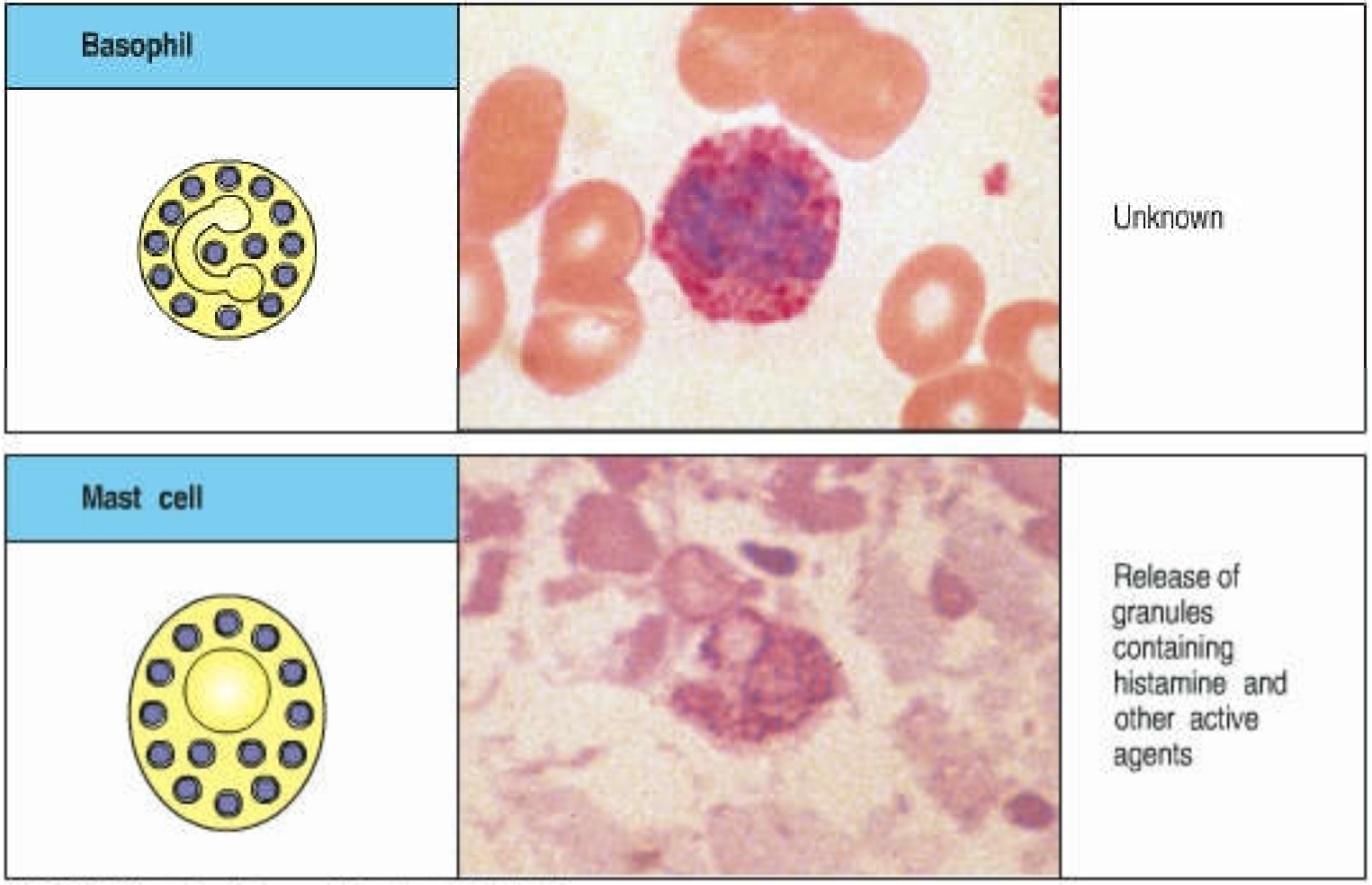
Mature DCs:

- specialized to take up Ag, process it, and display it for recognition by T Lymphocytes i.e., act as APCs to T cells initiating adaptive IR (express co-stimulatory molecules when encountering pathogen)
- classified by location as follows:
  - langerhans cells (epidermis/skin and mucous membranes)
  - interstitial DCs (organs: heart, lungs, liver, kidney, GIT)

Cells of Innate Immunity

Cell		Activated function
Macrophage 		Phagocytosis
Neutrophil 		Phagocytosis and activation of bactericidal mechanisms
Eosinophil 		Killing of antibody-coated parasites

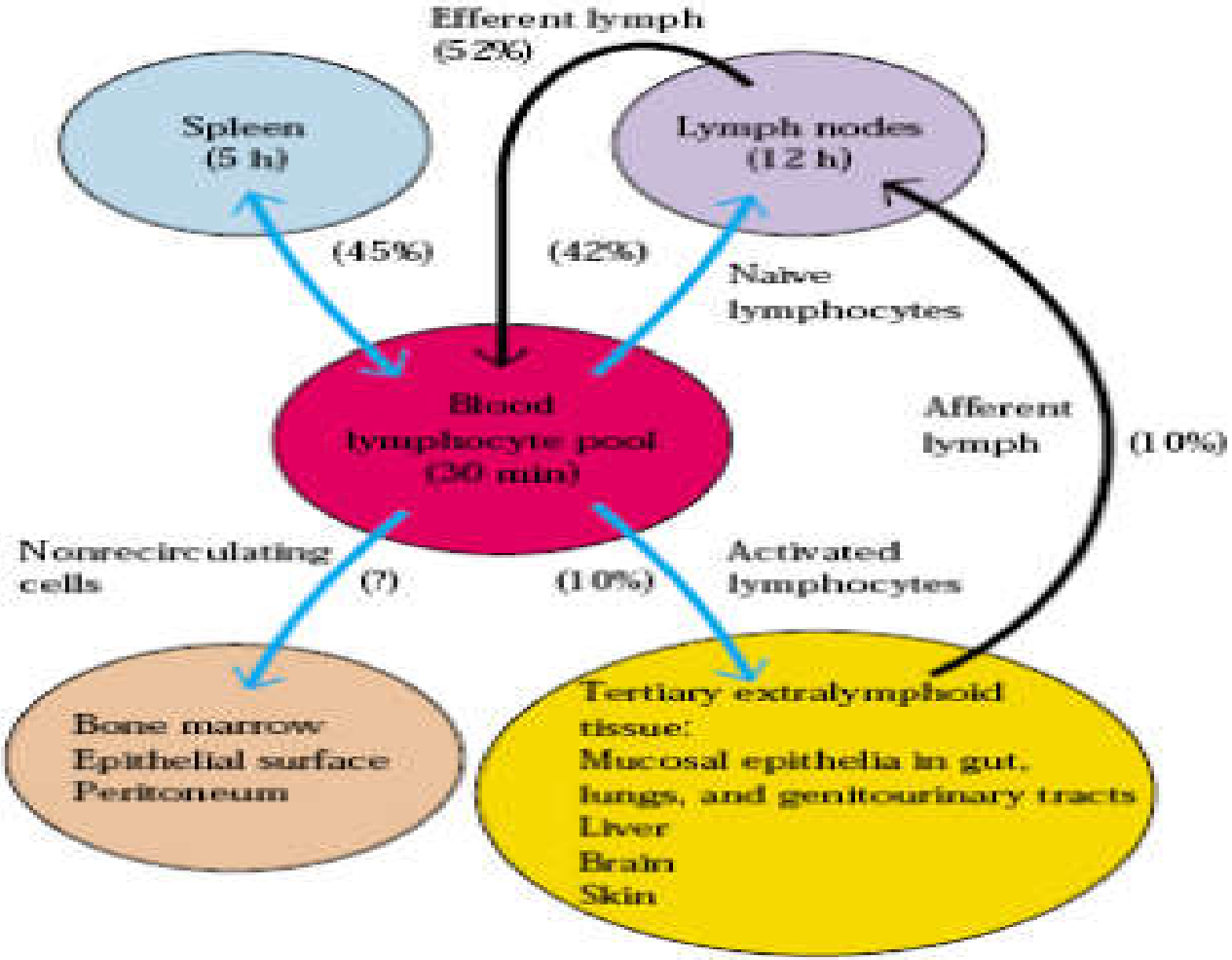
Cells of Innate Immunity



Lymphocyte Recirculation

- Lymphocytes are capable of recirculation, continually moving through the blood and lymph to the various lymphoid organs
- Lymphocytes migrate from the blood into lymph nodes through specialized areas in post-capillary venules called high-endothelial venules (HEVs).

Lymphocyte recirculation



Cell-Adhesion Molecules

- The vascular endothelium regulate the movement of
  - blood-borne molecules and
  - leukocytes into the tissues.
- In order for circulating leukocytes to enter inflamed tissue or peripheral lymphoid organs, the cells must adhere to and pass between the endothelial cells lining the walls of blood vessels, a process called **extravasation.**
- Endothelial cells express leukocyte-specific **cell adhesionmolecules (CAM)**

Cell-Adhesion Molecules

- Recalculating leukocytes bear receptors that bind to CAMs on the vascular endothelium,
  - enabling these cells to extravasate into the tissues.
- CAMs on leukocytes also serve to increase the strength of the functional interactions between cells of the immune system.

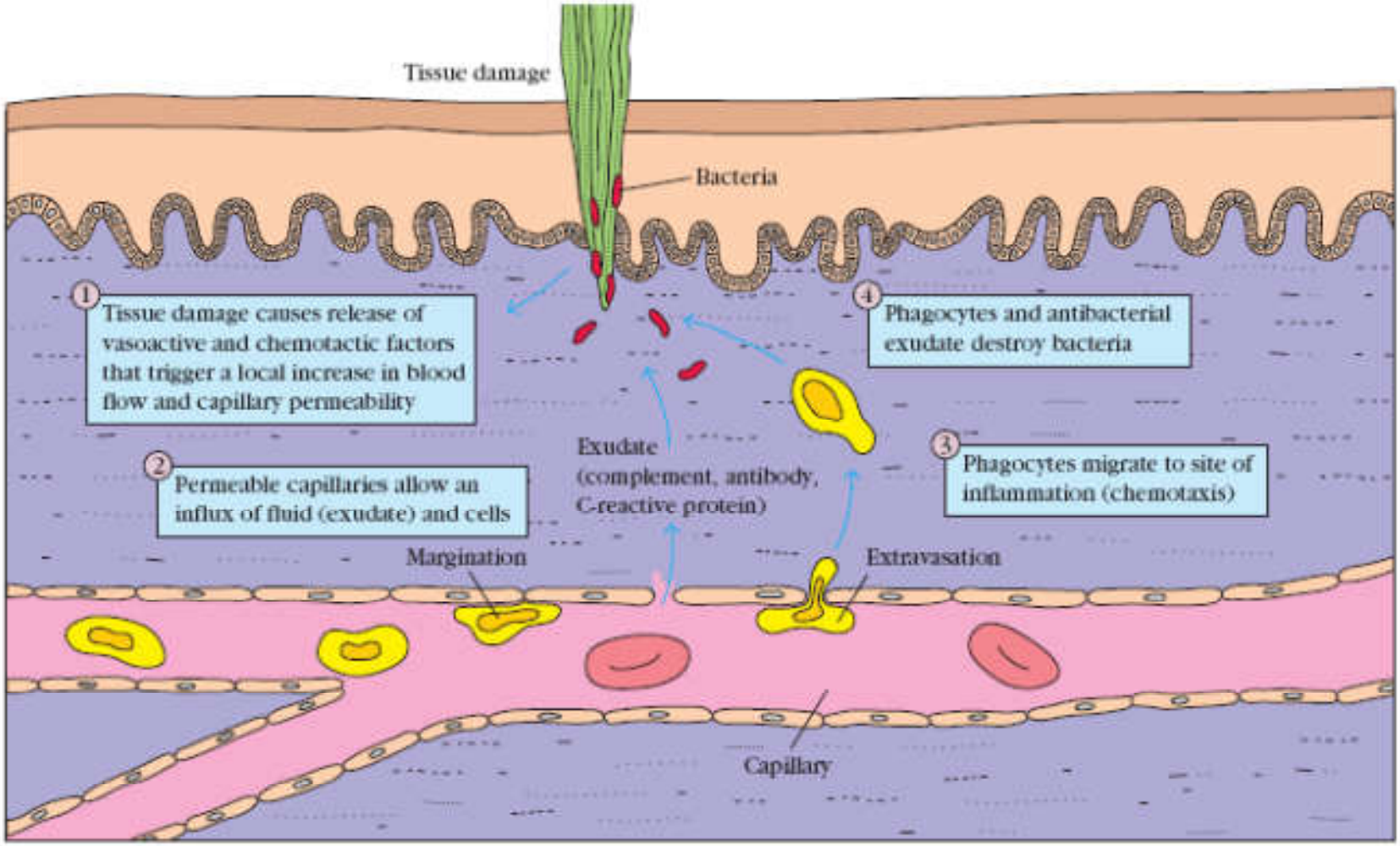
Cell-Adhesion Molecules

- ?????

Neutrophil Extravasation

- Neutrophils first bind to inflamed endothelium and extravasate into the tissues.
- Neutrophils recognize the inflamed endothelium and adhere .
  - so that they are not swept away by the flowing blood.
- The bound neutrophils then penetrate the endothelial layer and migrate into the underlying tissue.
- Monocytes and eosinophils extravasate by a similar process,

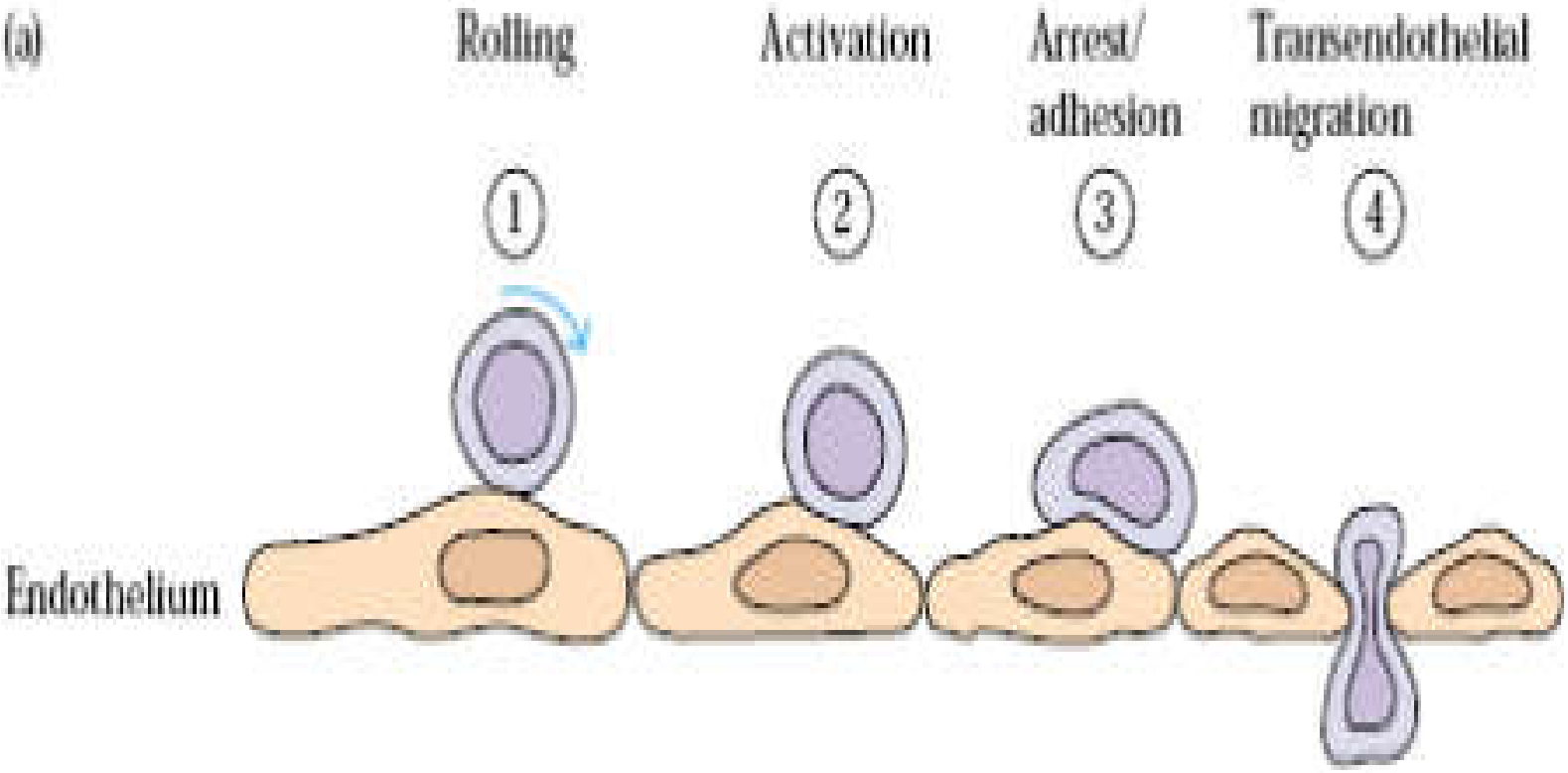
Neutrophil Extravasation



Neutrophil Extravasation

- Neutrophil extravasation has four sequential steps:
  - rolling,
  - activation by chemoattractant stimulus,
  - arrest and adhesion, and
  - trans endothelial migration.

Neutrophil Extravasation



Lymphocyte Extravasation

- Lymphocytes exhibit directed extravasation at
  - inflammatory sites and
    - secondary lymphoid organs.
- The recirculation of lymphocytes is carefully controlled
  - to ensure appropriate populations of B and T cells are recruited into different tissues.

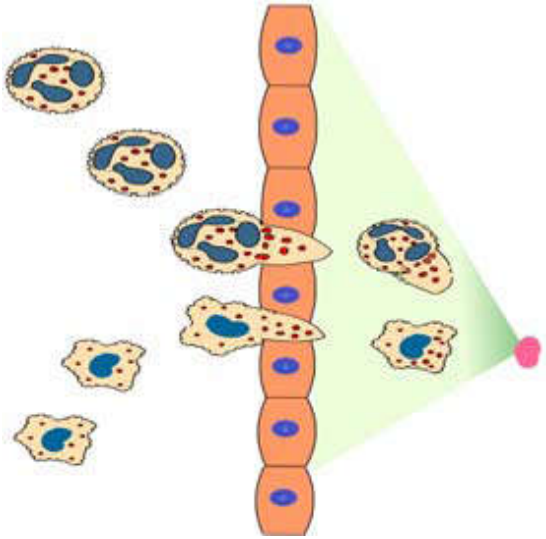
Lymphocyte Extravasation continued

- Extravasation of lymphocytes involves interactions among a number of cell-adhesion molecules.
- Some regions of vascular endothelium in postcapillary venules of lymphoid organs are composed of specialized cells with a plump, cuboidal (“high”) shape; such regions are called **high-endothelial venules,** or **HEVs** .

Lymphocyte Extravasation continued

- Each of the secondary lymphoid organs, with the exception of the spleen, contains HEVs.
- HEVs express a variety of cell-adhesion molecules.
- Unlike neutrophils, various lymphocyte populations exhibit differential extravasation into various tissues.
- Trafficking, or homing receptors on lymphocytes interact with tissue-specific adhesion molecules, called vascular addressins, on high endothelial venules (HEVs) in lymphoid organs and on the endothelium in extra lymphoid tissues

Lymphocyte Extravasation continued



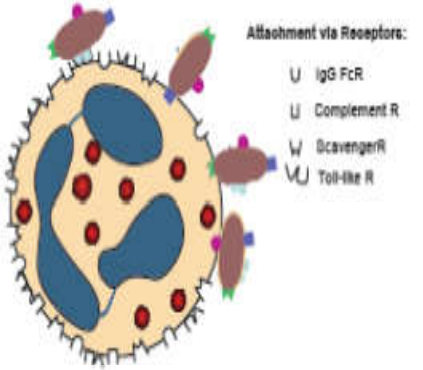


Figure 5. Phagocytic Receptors

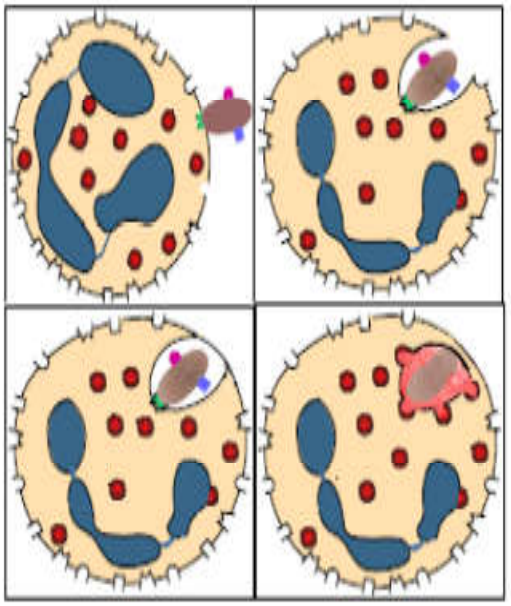


Figure 6. Phagocytosis

The Inflammatory Process

- Is a physiologic response to a variety of stimuli
  - such as infections and tissue injury.
- An acute inflammatory response
  - has a rapid onset
  - lasts a short and
  - accompanied by a systemic reaction known as the acute-phase response, which is

  -characterized by a rapid alteration in the levels of several plasma proteins.

The Inflammatory Process continued

- chronic inflammation results in,
  - persistent immune activation
  - pathologic consequences
  - lead to formation of a **granuloma**
- The accumulation and activation of macrophages is the hallmark of chronic inflammation.

The Inflammatory Process continued

- Two cytokines in particular, IFN-γ and TNF-α, play a central role in the development of chronic inflammation.
- TH1 cells,NK cells, and TC cells release IFN- γ, while activated macrophages secrete TNF-α.

The Inflammatory Process continued

- Inflammatory Responses May Be Localized or Systemic
- The hallmarks of a localized acute inflammatory response, are
  - swelling (*tumor*)
  - redness(*rubor*)
  - heat (*calor*)
  - pain (*dolor*), and loss of function

The Inflammatory Process continued

- The local inflammatory response is accompanied by a systemic response known as the **acute-phase response ,** marked by
  - the induction of fever,
  - increased synthesis of hormones such as ACTH and hydrocortisone,
  - increased production of white blood cell (leukocytosis),
  - production of a large number of **acute-phase proteins** (C-reactive protein) in the liver.

The Inflammatory Process continued

- Many systemic acute-phase effects are due to the combined action of IL-1, TNF- and IL-6.

Summary of Cells of the immune system

insert a good diagram

CHAPTER 3

Complement

Learning Objectives

Upon completion of this lesson the student will be able to:

1. Understand different pathways of complement activation
2. Identify the enzymatic and non-enzymatic mechanisms if complement activation
3. Discuss the biologic properties of C activation products
4. Describe the significance of Complement system in host resistance, inflammation and damage to cells
5. Discuss the mechanism of regulating complement activation and its products

3.0 Introduction to Complement

- Complement component are proteins and glycoproteins, about 5% of serum proteins
  - Synthesized mainly by liver hepatocytes, blood monocytes, tissue macrophages, plus epithelial cells of the gastrointestinal and genitourinary tracts.
- Complement components are designated by
  - numerals (C1–C9),
  - letter symbols (e.g., factor D), or
  - trivial names (e.g., homologous restriction factor).
- Peptide fragments formed by activation of a component are denoted by small letters.

3.0 Introduction to Complement

- These proteins are important in inflammation
- Are normally present in the circulation in an inactive state – once activated show enzymatic action on subsequent components and finally target antigen
- The two major pathways of complement activation are
  - the **classical pathway** which is activated by certain isotypes of Abs bound to Ags and the **alternative pathway** which is activated on microbial cell surfaces in the absence of antibody.
- At least 30 are activated in the classical pathway by antibody

Overview of Complement cascade

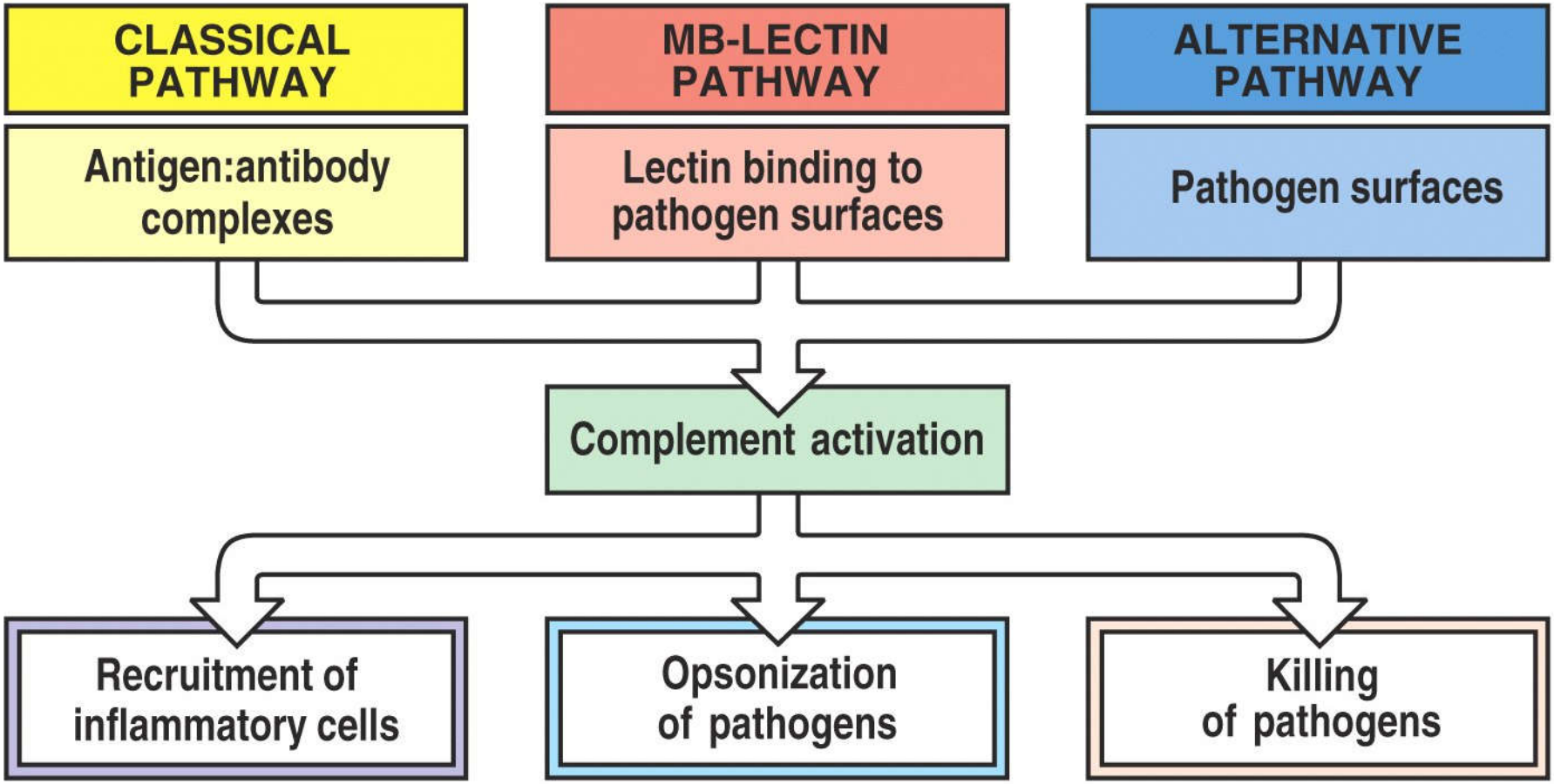


Figure 2-18 Immunobiology, 6/e. (© Garland Science 2005)

3.1 Immune Functions of Complement

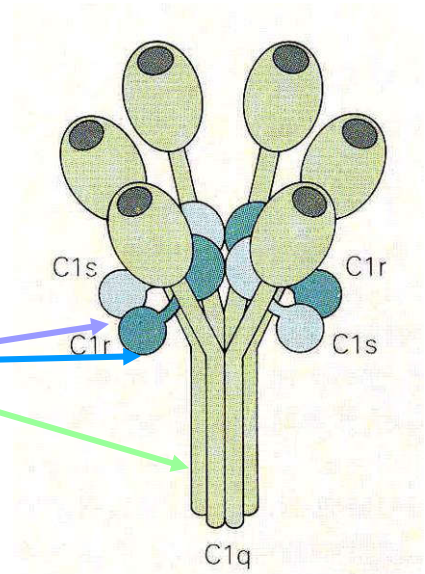
- **Opsonization and phagocytosis**
  - Aids inflammation by opsonizaton of antigens and causing membrane damage to pathogens.
  - As complement is *activated* antigens *become coated* with C3b, iC3b or C4b and are phagocytosed by attaching to specific receptors on macrophage and neutrophils.
- **Expansion of inflammatory responses**
  - Complement fragments C5a, C4a, and C3a induce powerful anaphylatic changes, which can be systemic.
- **Complement- mediated cytolysis**
  - Membrane Attack Complex (MAC) permits complement-mediated lysis of foreign organism. This appears to be a important defense against only a few types of microbes such as Nisseria because genetic defects in MAC components do not reduce desruction of other pathogens

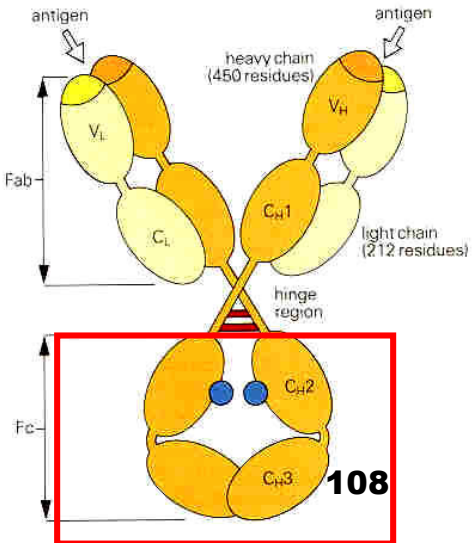
Other Functions of the Complement System

- By binding to antigen-antibody complexes, complement proteins promote the solublizing of these complexes and their clearance by phagocytes.
  - The later function is achieved by the binding of immune complexes with attached C3b to CR1 on erythrocytes, and complexes are cleared by phagocytosis in reticuloendothelial system.
- The C3d protein generated by C3b cleavage binds to CR2 on B cells, activates the B cells, and provides a signal for inactivating humoral immune responses.
- Viral neutralization

Classical Pathway of Activation

- Is initiated by binding complement protein C1 to antibody (IgG or IgM) molecules that have attached to foreign antigens.
- C1 is composed of 3 parts: C1q which binds antibody while C1r and C1s are proteases.
- The C1q subunit, an umbrella like radial array of six chains, each of with a globular head connected by a collagen like arm to a central stalk. C1q performs the recognition function and binds specifically to the Fc regions of γ and μ heavy chains that have bound to antigen sites.

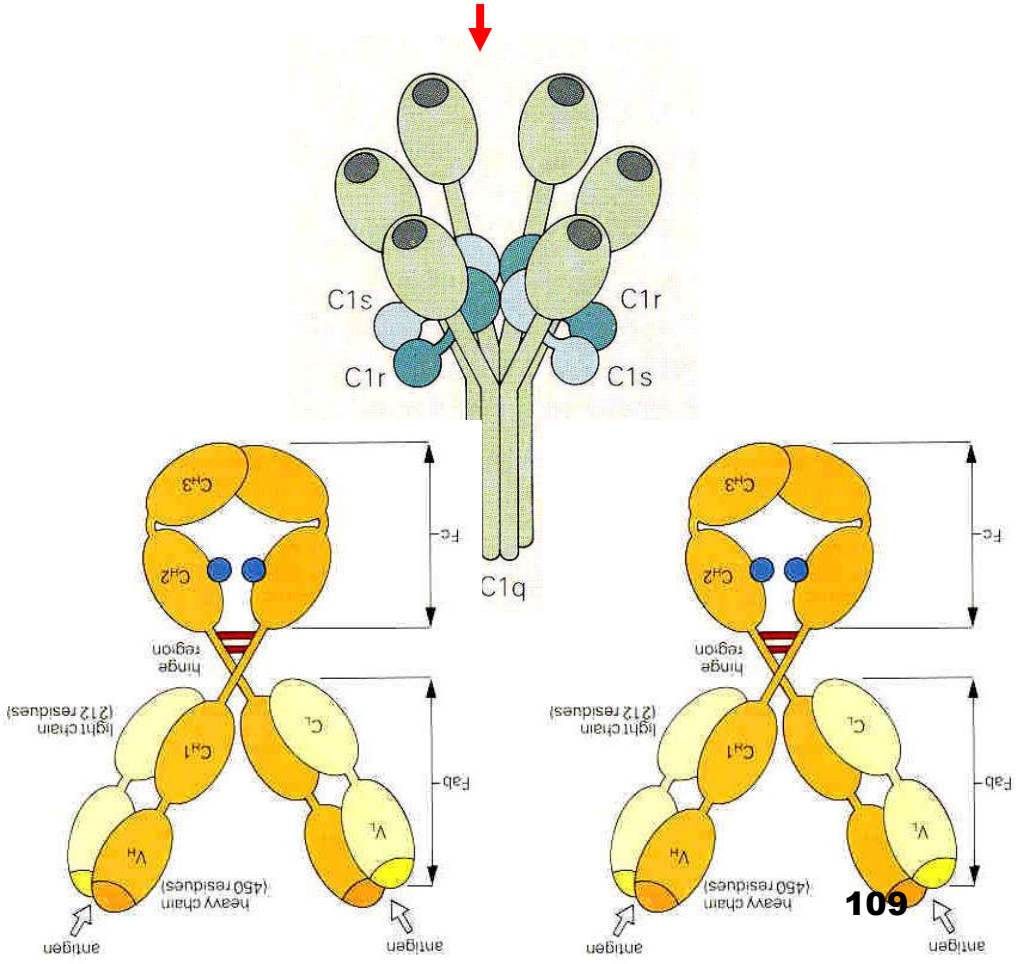




Classical Pathway of Activation

- 1C1g-binding site to 1 Ig Fc region and C1q molecule needs 2 binding sites to be activated
- IgG molecules has only one Fc region so must have at least 2 IgG molecules close together before C1q can bind.
- Globular heads of C1q bound to the Fc regions of IgG or IgM enzymatically activate of the associated C1r which cleaves and activates C1s.

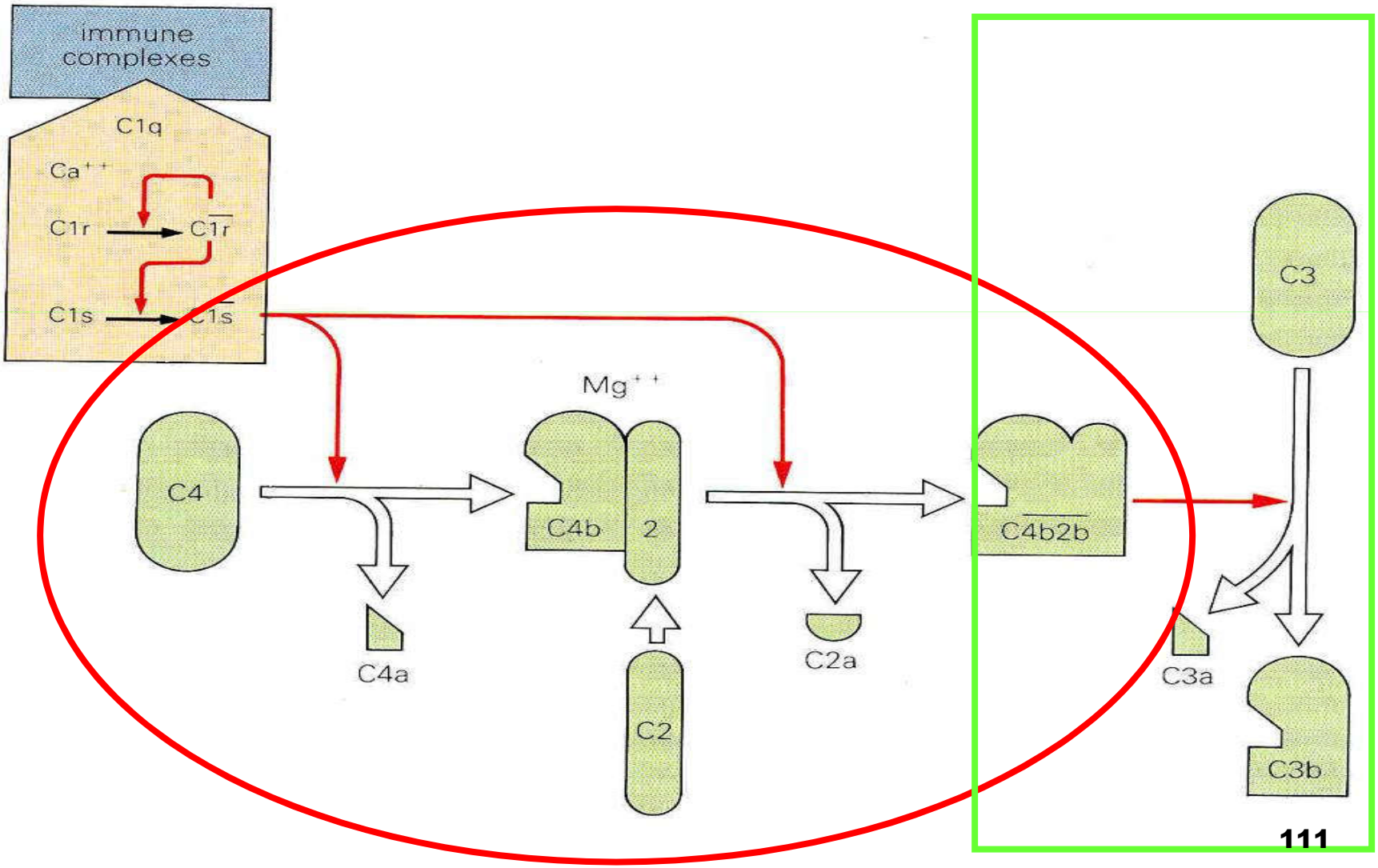
C1q bound to 2 IgG sites – activated C1r then C1s



Classical Pathway of Activation

- Activated C1s cleaves C4 to form C4b (C4a is released.) C4b binds it self with the antigen antibody complex or with the adjacent surface of a cell.
- C2 then complexes with the cell surface-bound C4b and is cleaved by a near by C1s molecule to generate a soluble C2a fragment and a larger C2b that remains physically associated with C4b on the cell surface.

Classical Pathway of Activation

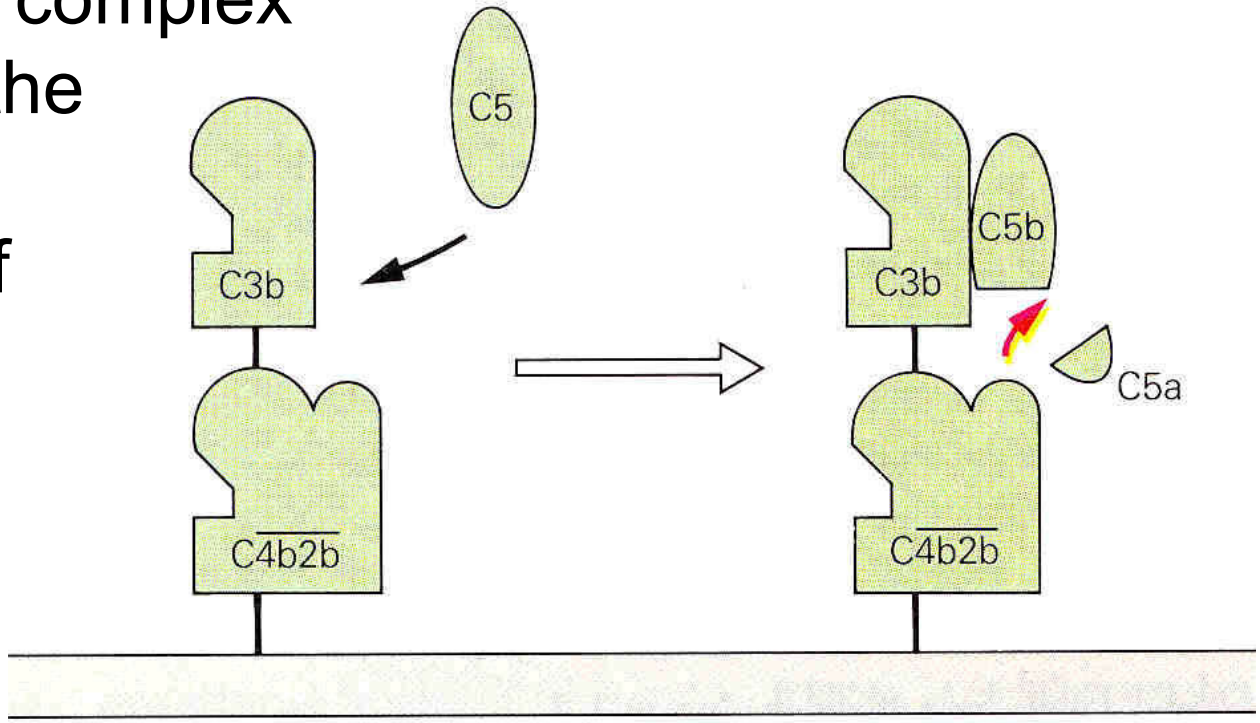


Classical Complement Activation

- In the green box on the previous slide, C4b2b complex, C3 convertase, is binding to and proteolytically cleaving C3
  - The C4b actively binds the C3 and C2b catalyzes proteolysis of the C3 making it active for the next step.
- Activation of C3b is an critical point of expanding the complement activation
- Proteolysis of C3 cleaves
  - a small C3a fragment,
  - leaving C3b's that may remain bound with the C4b2b on the antibody or form covalent bonds with the cells surface near the antigen/antibody complex.

Classical Complement Activation

- The C4b2b3b complex functions as the classical C5 convertase of complement activation



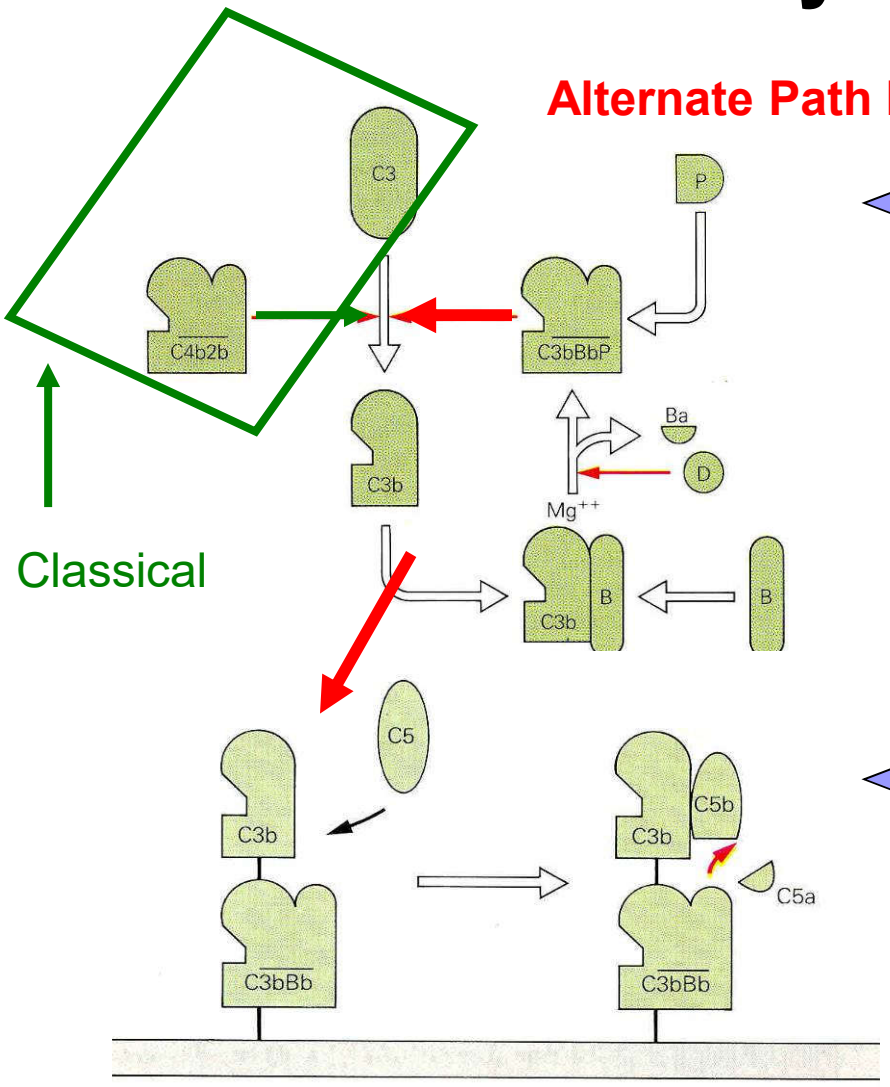
Alternate Pathway of Complement Activation

- The alternative pathway activation results in proteolysis of C3 and stable attachment of its breakdown product C3b to microbial surfaces without a role for antibody.
  - Microbial surfaces (viral, parasite and fungal surface antigens) and lipopolysaccharides can accomplish this.
  - Normally small amounts of C3b are activated in the absence of antigen/antibody reactions or by the alternate pathway.

Alternate Pathway of Complement Activation continued

- The surface bound C3b binds Factor B, a plasma protein
- Bound Factor B is cleaved by Factor D, a plasma serine protease to generate a fragment called Bb that remains attached to C3b forming C3bBb (and Ba is released).
  - C3bBb has a short active life (5 min.) unless stabilized by Properidin, a serum component, – this extends the active life up to 30 minutes.
- The C3bBb complex is the alternative pathway's C3 convertase, and it functions to cleave more C3 molecules amplifying the reaction.

Alternative Pathway



Amplification loop

D = Factor D
P = Properdin
B = Factor B

Feeds into Classical complement pathway from here

Complement Continued

- The C3 convertase activity of C3bBb generates the C3bBb3b complex, which exhibits C5 convertase activity, analogous to the C4b2a3b complex in the classical pathway.
- The nonenzymatic C3b component binds C5, and the Bb component subsequently hydrolyzes the bound C5 to generate C5a and C5b.

The Lectin Pathway of Complement Activation

- Lectins are proteins that recognize and bind to specific carbohydrate targets. (Because the lectin that activates complement binds to mannose residues, some authors designate this the MBLectin pathway or mannan-binding lectin pathway.)
- The lectin pathway, like the alternative pathway, does not depend on antibody for its activation.
- However, the mechanism is more like that of the classical pathway, because after initiation, it proceeds, through the action of C4 and C2, to produce a C5 convertase

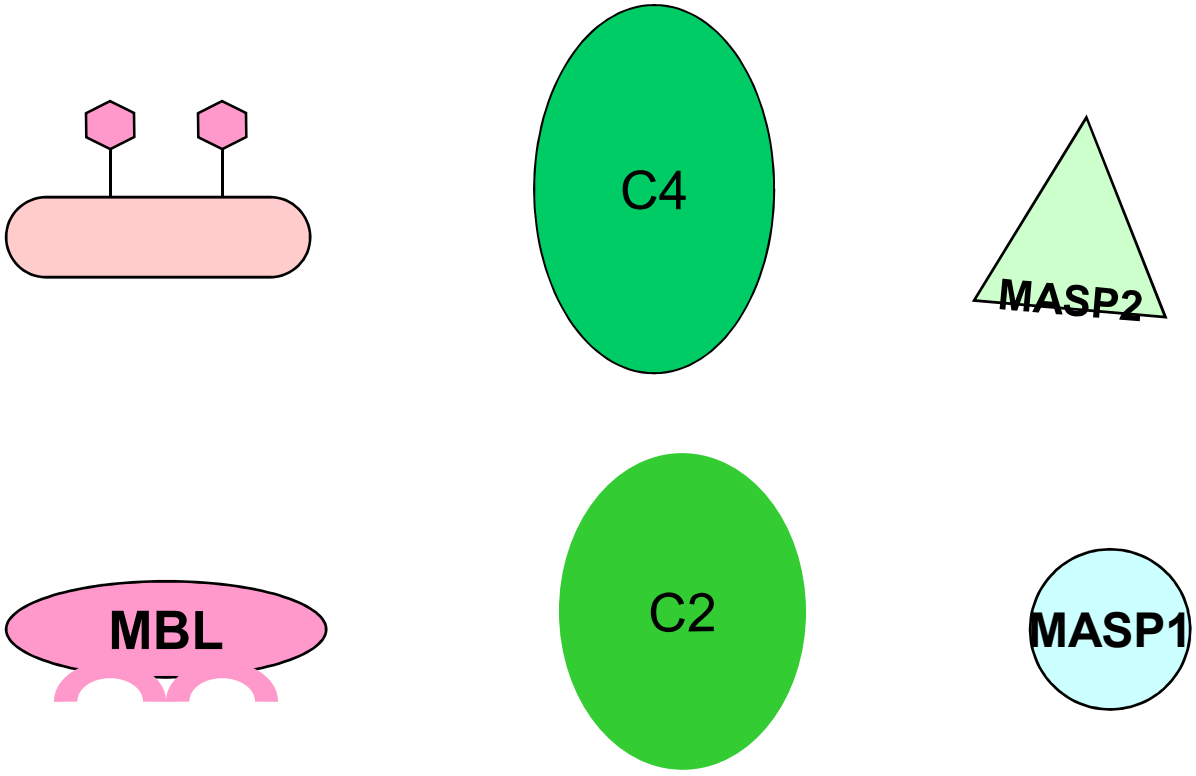
Lectin Pathway Continued

- The lectin pathway is activated by bound mannose-binding lectin (MBL) to mannose residues found on microorganisms including certain *Salmonella, Listeria,* and *Neisseria* strains, as well as *Cryptococcus neoformans* and *Candida albicans.*
- MBL, an **acute phase protein,** functions in the complement pathway similarly to C1q, which it resembles in structure.
- After MBL binds to the surface of a cell or pathogen, MBL-associated serine proteases,MASP-1 and MASP-2, bind to MBL.

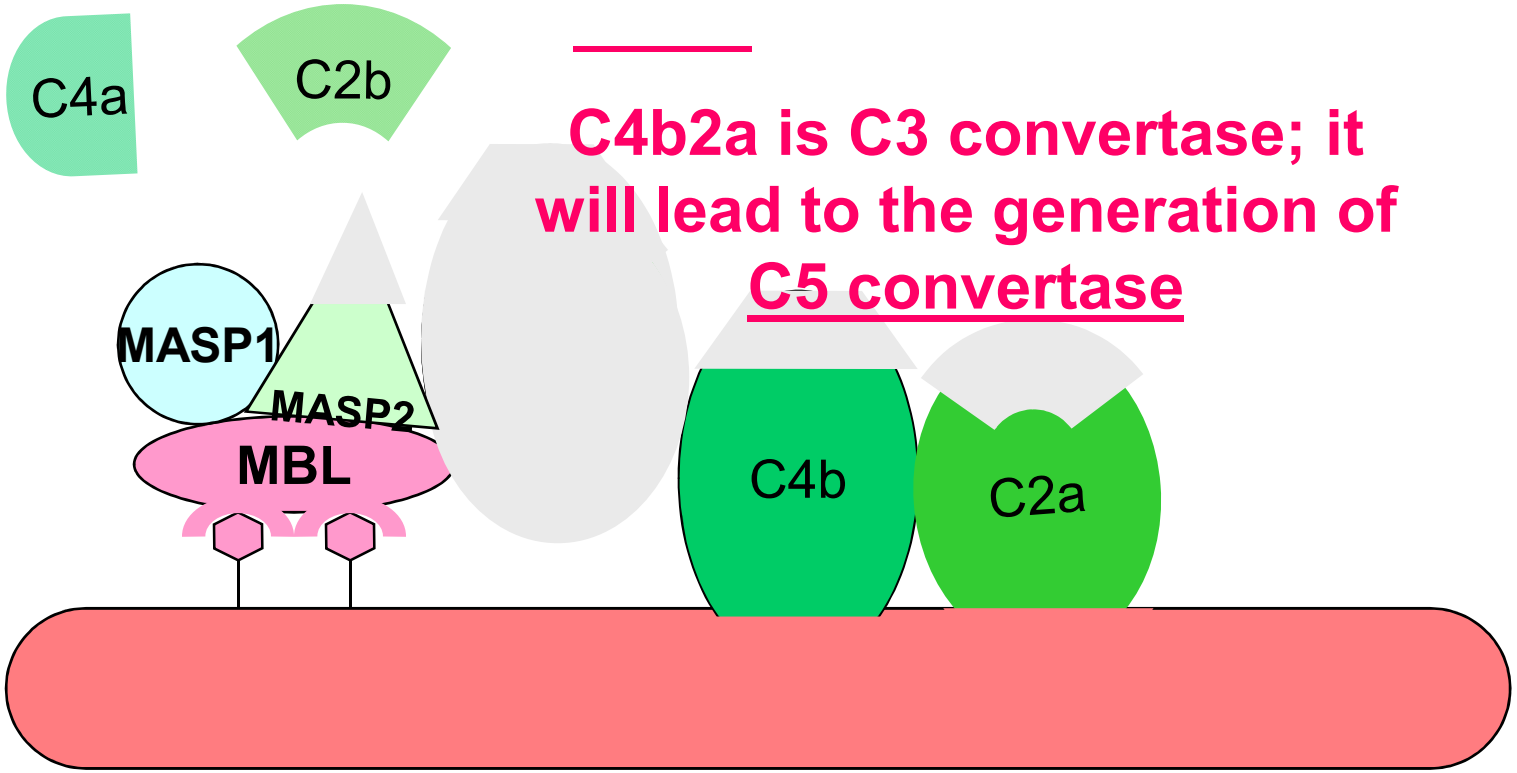
Lectin Pathway Continued

- The active MBL/MASP-1/MASP-2 complex cleaves and activates C4 and C2. The MASP-1 and -2 proteins, structurally similar to C1r and C1s, mimic their activities.
- This means of activating the C2–C4 components to form a C5 convertase without need for specific antibody binding represents an important innate defense mechanism comparable to the alternative pathway, but utilizing the elements of the classical pathway except for the C1 proteins.

Components of mannose-binding lectin pathway



Mannose-binding lectin pathway



C4b2a is C3 convertase; it will lead to the generation of C5 convertase

Terminal Sequence Shared by ALL Pathways

- The terminal sequence of complement activation involves C5b, C6, C7, C8, and C9, which interact sequentially to form a macromolecular structure called the **membrane-attack complex (MAC).**
- This complex forms a large channel through the membrane of the target cell, enabling ions and small molecules to diffuse freely across the membrane.
- The end result of activating the classical, alternative, or lectin pathway

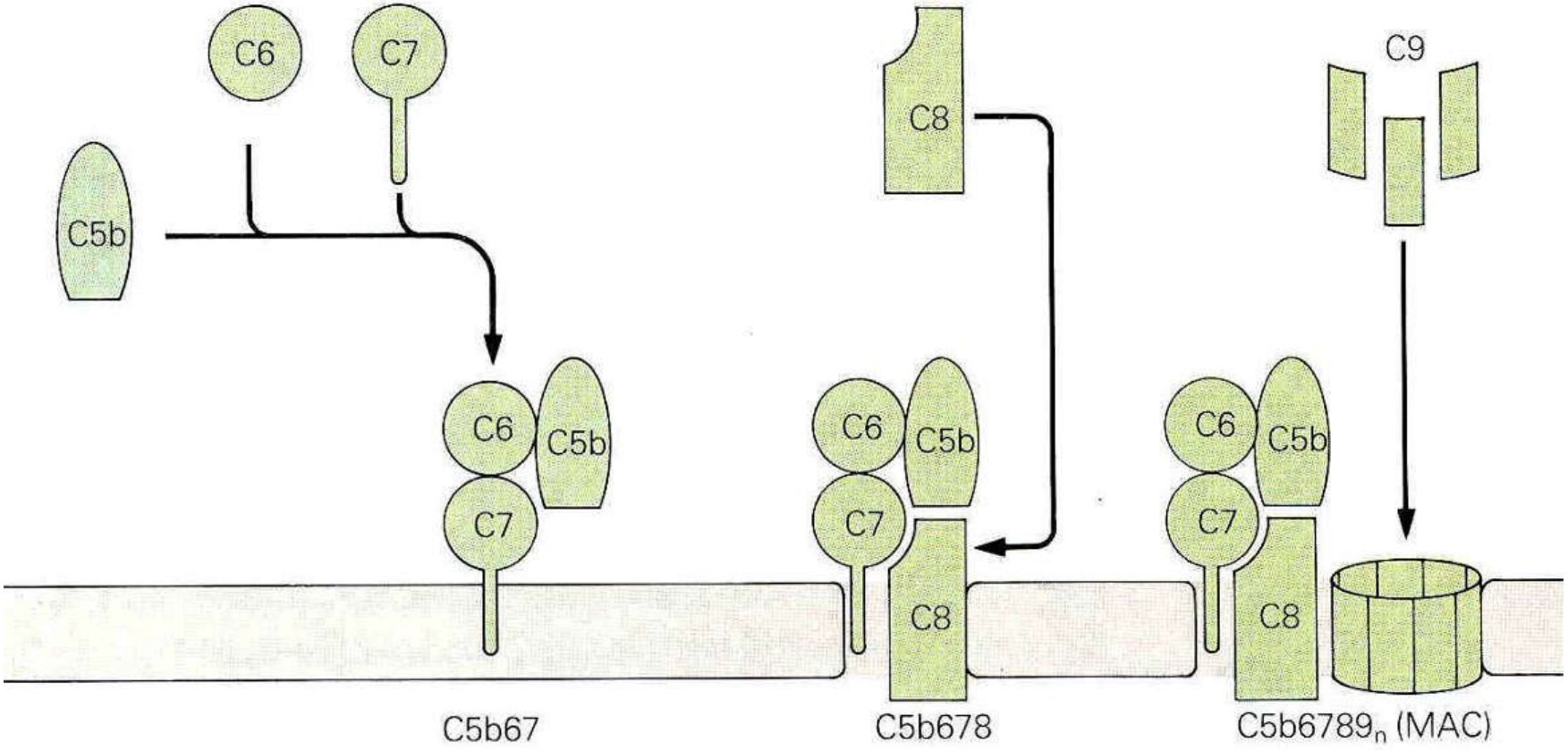
Terminal Steps in Complement Activation Continued

- C5 convertase cleave C5 into a small C5a fragment that is released and the large C5b fragment, which binds to the surface of the target cell and provides a binding site for the subsequent components of the membrane-attack complex
- The C5b component is extremely labile and becomes inactive within 2 minutes unless C6 binds to it and stabilizes its activity.
- C6, C7, C8, and C9, are structurally related proteins with out enzymatic activity.
- As C5b6 binds to C7, the resulting complex undergoes a hydrophilic-amphiphilic structural transition that exposes hydrophobic regions, which serve as binding sites for membrane phospholipids.

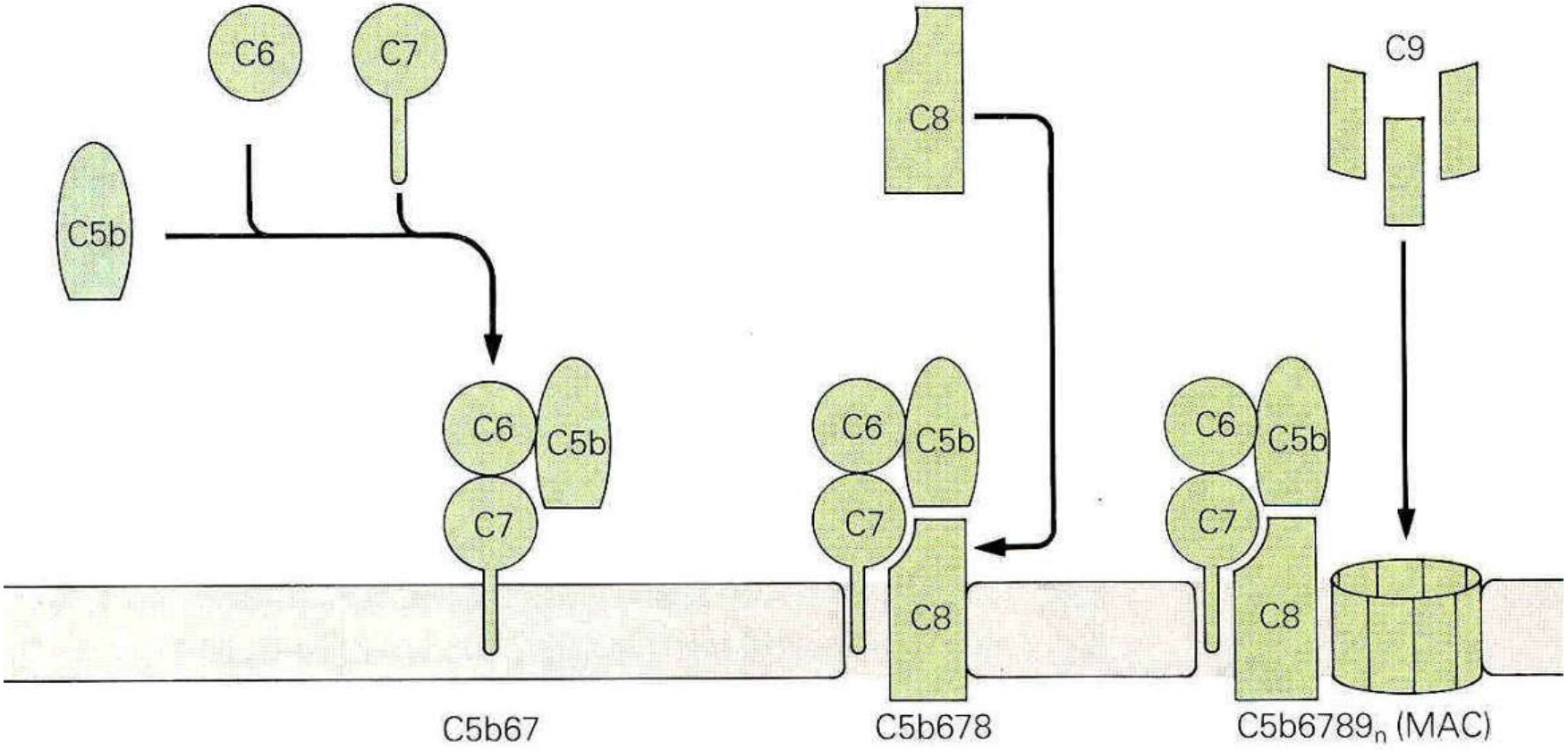
Terminal Steps in Complement Activation Continued

- If the reaction occurs on a target-cell membrane, the hydrophobic binding sites enable the C5b67 complex to insert into the phospholipid bilayer. This complex has limited ability to lyse cells.
- The formation of a fully active MAC is accomplished by the binding of C9, the final component of the complement cascade, to the C5b-8 complex.
- C9 is a serum protein that polymerizes at the site of the bound C5b-8 to form pores in plasma membranes.

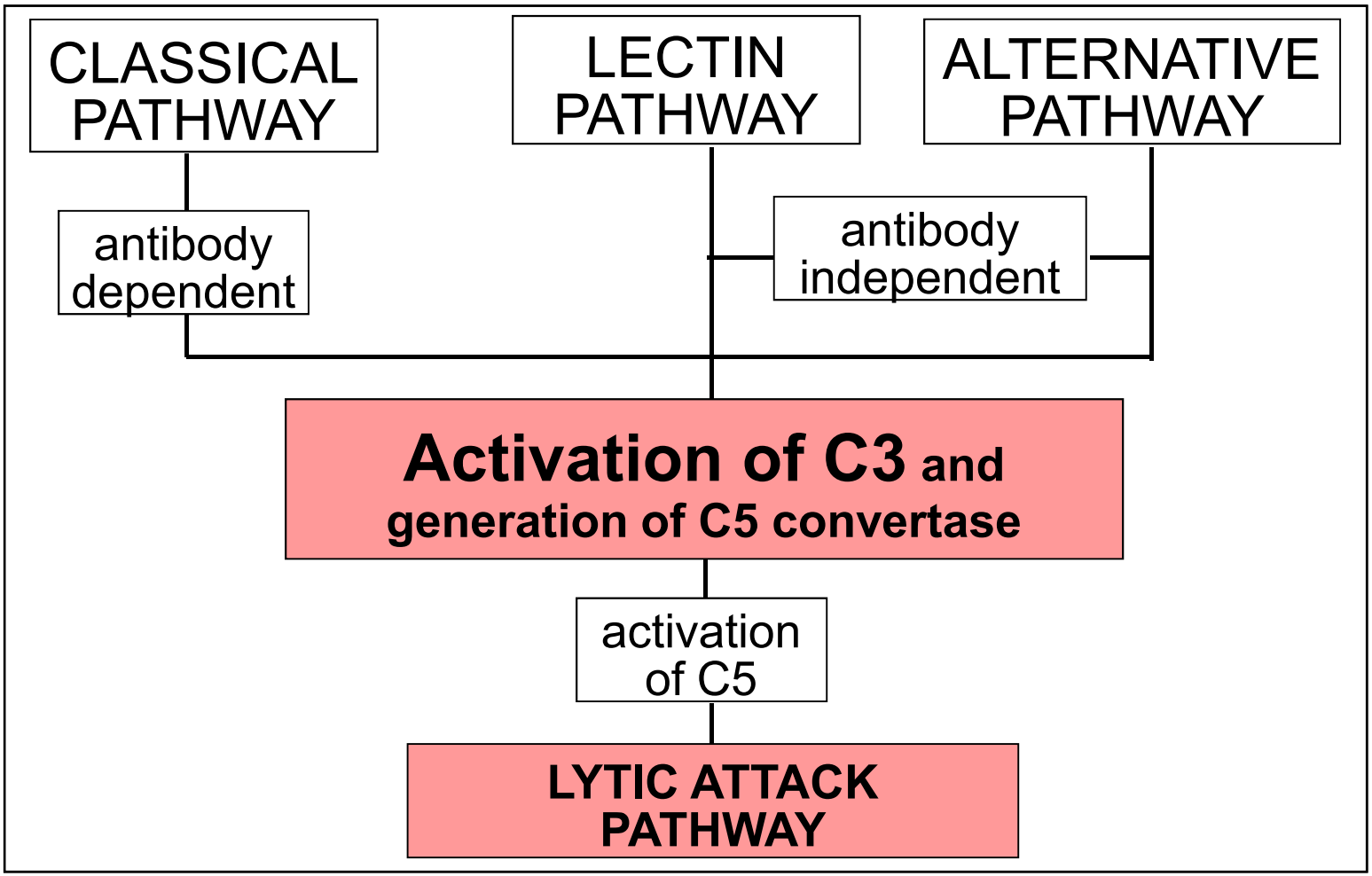
Terminal Steps in Complement Activation Continued



Terminal Steps in Complement Activation Continued



Pathways of complement activation



Regulation of the Complement System

Protein	Distribution	Interacts With	Function
C1 inhibitor (C1INH)	Plasma Protein	C1r, C1s	Serine protease inhibitor; binds to C1r & C1s & dissociates them from C1q
Factor I	Plasma Protein	C4b, C3b	Serine protease; cleaves C3b & C4b by using Factor H, MCP, C4BP or CR1 as a cofactor
Factor H	Plasma protein	C3b	Binds C3b & displace Bb Cofactor for factor I-mediated C3b cleavage

Regulation of the Complement System Continued

C4-binding protein (C4BP)	Plasma Protein	C4b	Binds C4b and displaces C2b
Membrane cofactor protein (MCP, CD46)	**Leukocytes, epithelial cells, endothelial cells**	**C3b, C4b**	**Cofactor for Factor I-mediated cleavage of C3b & C4b**
Decay-accelerating factor (DAF)	Blood cells, Endothelial cells, Epithelial cells	C4b2b, C3bBb	Displaces C2b from C4b and Bb from C3b.
CD59	Blood cells, Endothelia & Epithelial cells	C7, C8	Blocks C9 binding & prevents formation of the MAC

Laboratory Issues in Complement

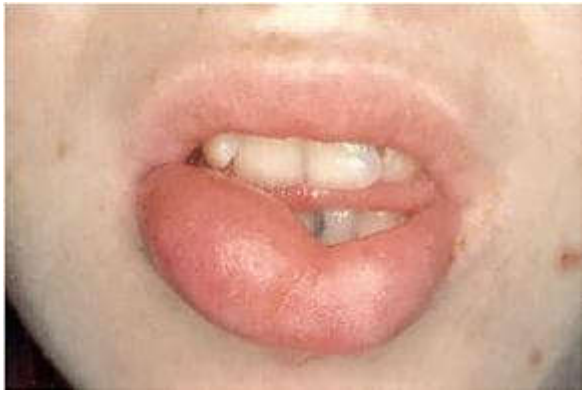
- Why measure?
  - Complement over utilization (decreases)
    - Infection (bacterial, etc)
    - Autoimmune (indication of flare or active disease)
    - Long list
  - Complement increases
    - Acute phase proteins
    - Any disease associated with increase in acute phase proteins (ie. Rheumatoid Arthritis, Diabetes, ulcerative colitis)
  - Complement deficiencies (rare)

Laboratory Issues in Complement

- What to measure?
  - CH_{50} (Hemolytic complement Assay)
    - Good screen for clinically relevant levels
      - Decreased levels/Function
      - All parameters taken into account
    - Assay description - cautions (labile protein)
  - C3, C4 Quantitative Measurements
    - Classical vs Alternative pathway indication
      - C3 common to both, C4 only Classical
    - Highest serum conc. - easy to measure
    - Follow disease course - sensitive
  - Other components
    - Rarely C2, C1q inh quantitative & functional
    - C5-9 Extremely rare

Complement Disorder

- Hereditary Angioedema - C1Q inhibitor deficiency
  - Clinical pathology
    - unregulated production of protease enzymes and mediators of inflamation
    - increased vascular permeability and exudation of fluid
  - What Lab assays can be useful ???
    - Quantitative
    - Functional



Complement summary

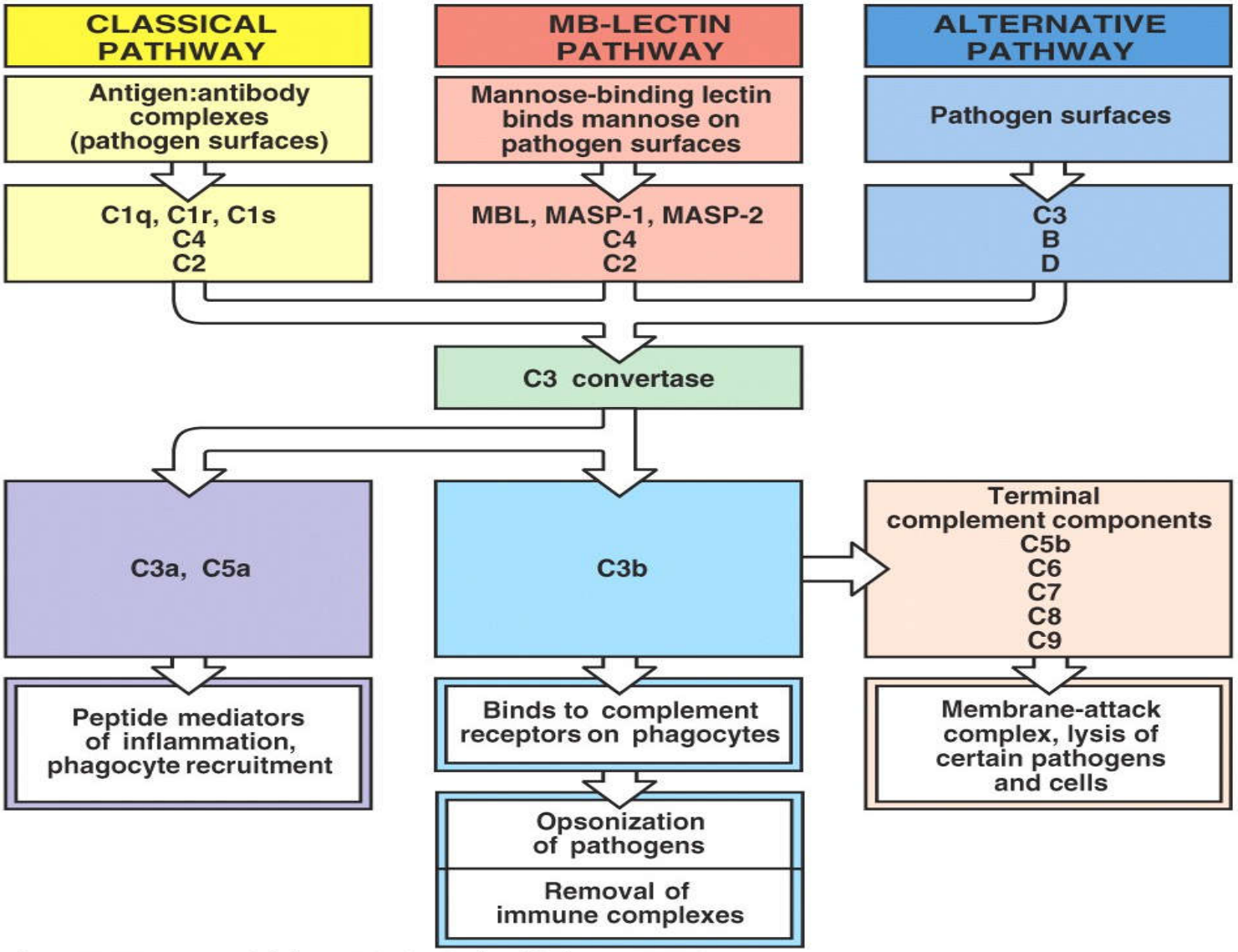


Figure 2-19 Immunobiology, 6/e. (© Garland Science 2005)

CHAPTER 4

Cytokines

Learning Objectives

- Upon completion of this lesson the student will be able to:

1. List innate, adaptive and haematopoietic cytokines
2. Identify cytokines according to their function
3. Describe properties of cytokines
4. Discuss cytokine receptors on different cells
5. Differentiate immunological role of cytokines
6. List cytokine related diseases
7. Discuss effect of cytokines on T helper cells

4.1 Properties of Cytokines

- Cytokines, proteins, secreted by cells of innate and adaptive immunity.
- Cytokines, produced in response to microbes and other antigens, and other cytokines.
- Cytokines determine and stimulate many diverse responses of cells involved in immunity and inflammation.
- During immune response activation phase, cytokines stimulate the growth and differentiation of lymphocytes,

Properties of Cytokines Continued

- In the effector phases of innate and adaptive immunity, they activate a different effector cells to eliminate microbes and other antigens.
- They also stimulate the development of hematopoietic cells.
- In clinical medicine, cytokines are important as therapeutic agents or as targets for specific antagonist in numerous immune and inflammatory disease

Properties of Cytokines Continued

- The term cytokine is a general term used to describe a large group of proteins but there are other terms that are commonly used to describe particular kinds of cytokines. These include:

1) monokines, cytokines produced by mononuclear phagocytic cells;
2) lymphokines, cytokines produced by activated lymphocytes, especially Th cells; and
3) interleukins, cytokines that act as mediators between leukocytes.

Properties of Cytokines Continued

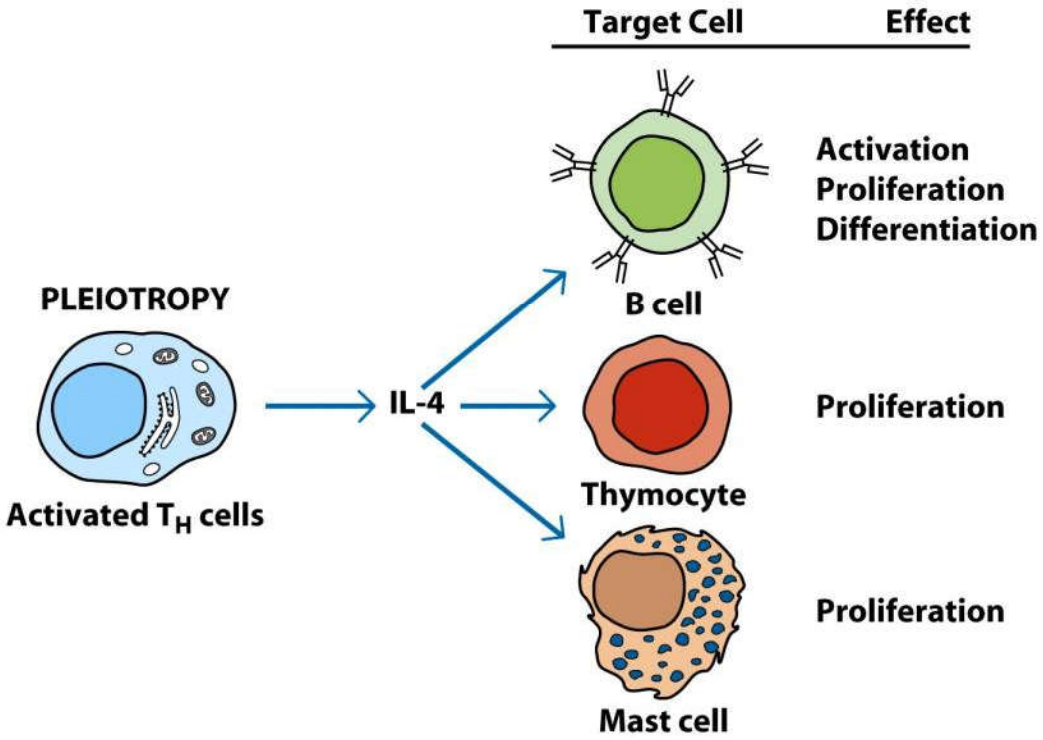
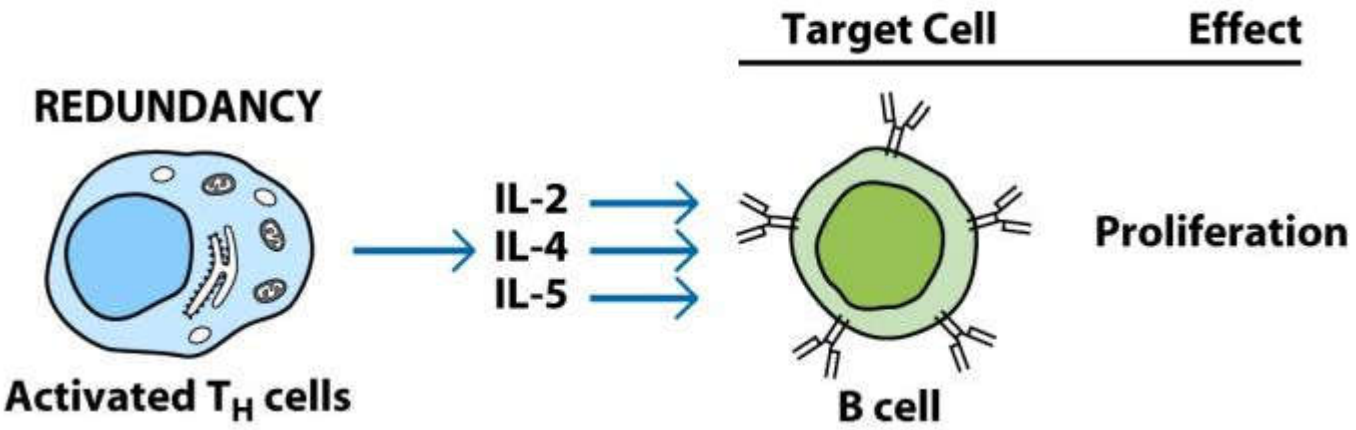


Figure 12-2a part 1
Kuby IMMUNOLOGY, Sixth Edition

Properties of Cytokines Continued



Properties of Cytokines Continued

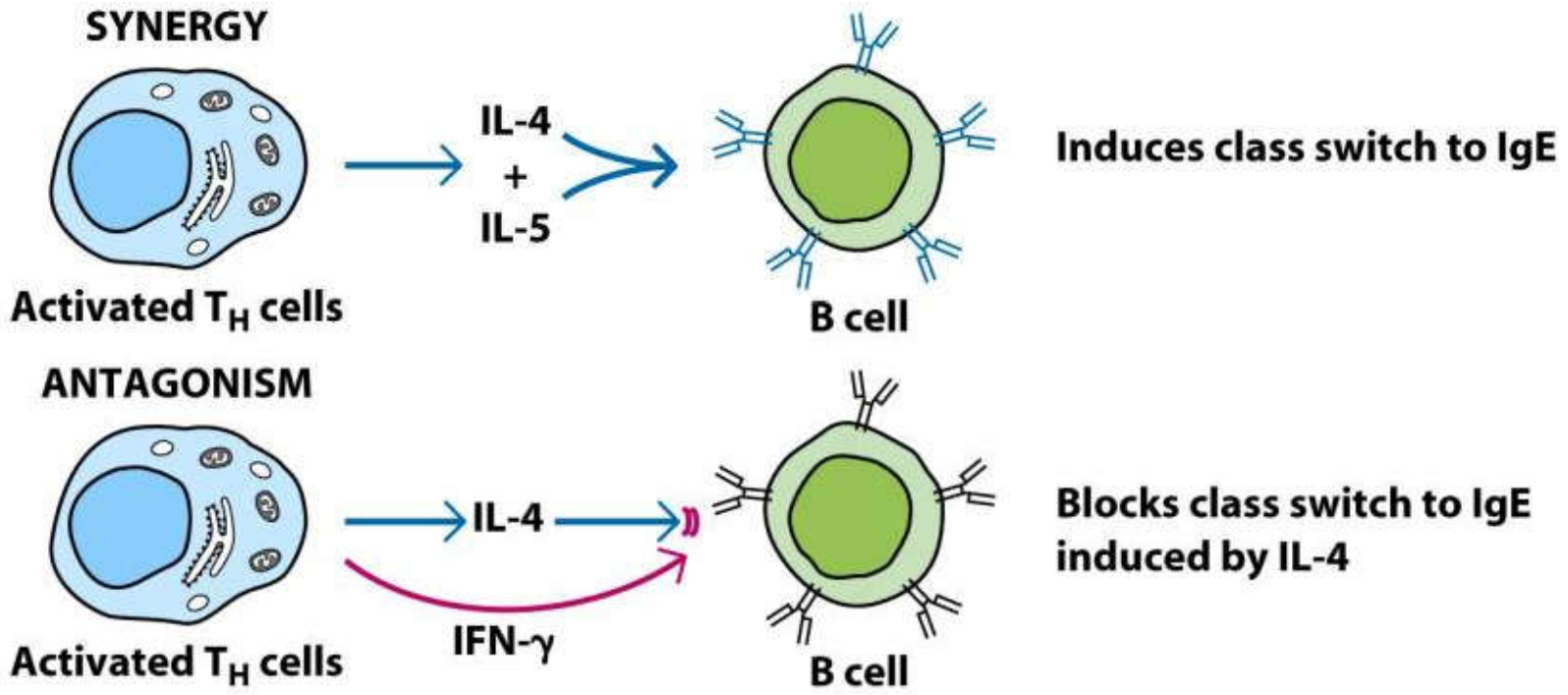


Figure 12-2a part 2
Kuby IMMUNOLOGY, Sixth Edition

Cytokine Antagonist

- A number of proteins inhibit biological activity of cytokines.
- These proteins act in one of two ways:
  - bind directly to a cytokine receptor but fail to activate the cell,
  - bind directly to a cytokine, inhibiting its activity.
- Best-characterized inhibitor is IL-1 receptor antagonist (IL-1Ra), which binds to the IL-1 receptor but has no activity.
  - This receptor blocks binding of IL-1's
  - Production of IL-1Ra is thought to play in regulating intensity of inflammatory response.
- These inhibitors are in both blood and extracellular fluids.

Cytokine Antagonists continued

- **Soluble antagonists** arise from enzymatic cleavage of the extracellular domain of cytokine receptors.
- Soluble cytokine receptors that have been detected are those for IL-2, -4, -6, and -7, IFN TNF, and LIF.
- Proteolytic cleavage forms a soluble IL-2 receptor. This shed receptor binds IL-2 and prevent its interaction with the membrane-bound IL-2 receptor.

Cytokine Antagonists continued

- Some viruses also produce **cytokine-binding proteins or cytokine mimics**.
- Molecules produced by viruses that mimic cytokines allow the virus to manipulate the immune response in ways that aid the survival of the pathogen.

Cytokine Secretions by TH1 or TH2 cells

- Differences in cytokine-secretion patterns among TH-cell subsets produce different responses to different types antigens in immune response.
- CD4+ TH cells exert most of their helper functions through secreted cytokines
  - acting on the cells, or
  - modulating the responses of other cells.

Cytokine Secretions by TH1 or TH2 cells continued

- Two CD4+ TH-cell subpopulations designated TH1 and TH2, can be distinguished in vitro by the cytokines they secrete.
  - Both subsets secrete IL-3 and GM-CSF but differ in the other cytokines they produce
- TH1 and TH2 cells are characterized by the following functional differences:

TH1 subset

- Responsible for
  - many cell-mediated functions (e.g., delayed-type hypersensitivity and activation of TC cells),
  - production of opsonization-promoting IgG antibodies (i.e. antibodies binding to phagocyte high-affinity Fc receptors and interact with the complement system), and
  - the promotion of excessive inflammation and tissue injury.

TH1 subset

- Secretes the important cytokine, IFN-γ
  - Activates macrophages, to increase microbicidal activity,
  - up-regulate the level of class II MHC, and
  - Induces antibody-class switching to IgG classes enhancing phagocytosis and fixation of complement.
- Secretes IL-12, inducing TH cells to differentiate into the TH1 subset.
- Secrete TNF-β and IFN-γ mediating inflammation, especially TH1's activity in delayed hypersensitivity.
- Produce IL-2 and IFN-γ cytokines promoting differentiation of fully cytotoxic TC cells from CD8+ precursors.
  - This cytokine makes the TH1 subset particularly suited to respond to viral infections and intracellular pathogens.
- IFN-γ inhibits the expansion of the TH2 population.

TH2 subset

- Responsible for
  - eosinophil activation and differentiation,
  - providing help B cells that specifically produce increased amounts of IgM, IgE, and non-complement-activating IgG isotypes, and also
  - supports allergic reactions.

TH2 Subset continued

- The secretion of IL-4 and IL-5 induces production of IgE and supports eosinophil-mediated attack on helminth (roundworm).
- IL-4 promotes class switch from IgM to IgG subclass that does not

  activate the complement pathway.
- IL-4 also increases class switch from IgM to IgE.
- Combined IL-4 and Il-5 action increases Fc receptors on eosinophils.

TH2 Subset continued

- IL-4 and IL-10 suppress the expansion of TH1 cell populations.
- Many helper T cells do not show either a TH1 or a TH2 profile;
  - individual cells have shown striking heterogeneity in the TH-cell population.
  - One of these is the TH0 subset, which secretes IL-2, IL-4, IL-5, IFN-, and IL-10, as well as IL-3 and GM-CSF.

Comparison of TH1 And TH2 Cytokine functions

Cytokine/function	TH1	TH2
CYTOKINE SECRETION		
IL-2	+	–
IFN-	++	–
TNF-	++	–
GM-CSF	++	+
IL-3	++	++
IL-4	–	++
IL-5	–	++
IL-10	–	++
IL-13	–	++

FUNCTIONS

	TH1	TH2
■ Help for total antibody production	+	++
■ Help for IgE production	–	++
■ Help for IgG2a production	++	+
■ Eosinophil and mast-cell production	–	++
■ Macrophage activation	++	–
■ Delayed-type hypersensitivity	++	–
■ TC-cell activation	++	–

SOURCE: Adapted from F. Powrie and R. L. Coffman, 1993, *Immunol. Today* **14:**270.

Cytokines by Functional Category

- Mediators of natural/innate immunity
  - Type I IFN
  - TNF-α
- Regulators of lymphocytic growth, activation and differentiation
  - IL-2, IL-4, IL-5, IL-12, IL-15

Cytokines by Functional Category continued

- Activators of inflammatory cells
  - Type II IFN
    - IFN-γ
- Stimulators of hematopoiesis
  - IL-3, GM-CSF, IL-7

Cytokine-Related Diseases

- Defects in the complex regulatory networks governing the expression of cytokines and cytokine receptors have been implicated in a number of diseases.

Bacterial Septic Shock

- The role of cytokine overproduction in pathogenesis can be illustrated by bacterial septic shock
- It develops because bacterial cell-wall **endotoxins** stimulate macrophages to overproduce IL-1 and TNF- to levels that cause septic shock

Cytokine Activity Is Implicated in Lymphoid and Myeloid Cancers

- Abnormal production of cytokines or their receptors have been associated with some types of cancer. For example.
- In myeloma cells, IL-6 appears to operate in an autocrine manner to stimulate cell proliferation.
- When monoclonal antibodies to IL-6 are added to in vitro cultures of myeloma cells, their growth is inhibited

CHAPTER 5

Antigens

Learning Objectives

Upon completion of this lesson the student will be able to:

- Describe definition and classification of antigens
- Describe difference between antigenecity and immunogensity
- Describe factors that influence immunogensity
- Describe pattern-recognition receptors
- Describe Antigen-Presenting Cells and their role
- Describe how antigens Processed and Presented to T-cells

5.1 Definitions

- **Antigen (Ag)** :
  - is substance which when introduced parentally into the body stimulates the production of an antibody with which it reacts specifically and in an observable manner.
  - The word originated from the notion that they can stimulate **anti**body **gen**eration
- **Immunogen**:
  - A substance that induces a specific immune response.
- **Epitope or Antigenic Determinant:**
  - That portion of an antigen that combines with the products of a specific immune response.

5.1 Definitions

- **Tolerogen**: antigen that induce Immunologic tolerance Immunologic tolerance is unresponsiveness to an antigen that is induced by prior exposure to that antigen.
- **Allergen**: antigen that induce Anaphylaxis (severe immediate hypersensitivity reaction occurring as a result of rapid generalized mast-cell granulation) Allergen: some medicine, flower powder, seafood

5.1 Definitions

- **Tumor antigens - are those antigens that are presented by the MHC I molecules on the surface of tumor cells. These antigens can sometimes be presented only by tumor cells and never by the normal ones. In this case, they are called tumor-specific antigens (TSAs) and typically result from a tumor specific mutation.**

5.1 Definitions

- **Autoantigens** - is usually a normal protein or complex of proteins (and sometimes DNA or RNA) that is recognized by the immune system of patients suffering from a specific autoimmune disease.

  These antigens should under normal conditions not be the target of the immune system, but due to mainly genetic and environmental factors the normal immunological tolerance for such an antigen has been lost in these patients.

5.1 Definitions

Vaccines: are antigen preparations that induce a protective immune response against microbes and are used to prevent diseases.

The preparations could be :

- Killed vaccine: Rubella virus,
- Attenuated vaccine: Measles
- Toxoid :Tetanus

- **Antibody (Ab):**
  - A specific protein which is produced in response to an immunogen and which reacts with an antigen

5.2 Immunogenicity Versus Antigenicity

- **Immunogenicity** is the ability of a molecule/microbe or cell to be recognized by hosts immune cells and elicit an immune response.
- While
  - The ability of a molecule to bind/react with the products of an immune response (antibodies or lymphocytes) is called antigenicity
- Not all antigens are immunogens while all immunogens are antigens.

5.3 Classification of Antigen (Ag)

Basis for classification:

- Complete and Incomplete
- According to source/origin of Ag
- According to whether need the help of T cells when B cells produce Ab
- Based on chemical nature

Classification of Antigen cont…

- ***Incomplete antigens*** (hapten)***:***
  - A substance that is non-immunogenic but which 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 but which can when coupled to a carrier molecule.
  - Free haptens, however, can react with 2 products of the immune response after such products have been elicited.
  - Haptens have the property of antigenicity but not immunogenicity.

Classification of Antigen cont…

- **Complete antigens:**
  - Are usually proteins or porteinous in nature, large in molecular size and are capable of stimulating an immune response by them selves
  - Majority human pathogens/microbes and their toxins are examples of complete antigens/ immunogens

Classification of Antigen cont…

- **Exogenous antigens:**
  - Are antigens that have entered the body from the outside, for example by inhalation, ingestion, or injection. By endocytosis or phagocytosis, these antigens are taken into the antigen-presenting cells (APCs) and processed into fragments.

Classification of Antigen cont…

- **Endogenous antigens:**
  - **Are antigens that have been generated within the cell, as a result of normal cell metabolism, or because of viral or intracellular bacterial infection.**

Example

- Autoantigens - is usually a normal protein or complex of proteins (and sometimes DNA or RNA).
- Tumor antigens - are those antigens that are presented by the MHC I molecules on the surface of tumor cells.

Classification of Antigen cont…

- Based on chemical nature antigens classified into:
  - **Protein antigens**:- The vast majority of immunogens are proteins. These may be pure proteins or they may be glycoproteins or lipoproteins. In general, proteins are usually very good immunogens
  - **Polysaccharide antigens**:- Pure polysaccharides and lipopolysaccharides are good immunogens.

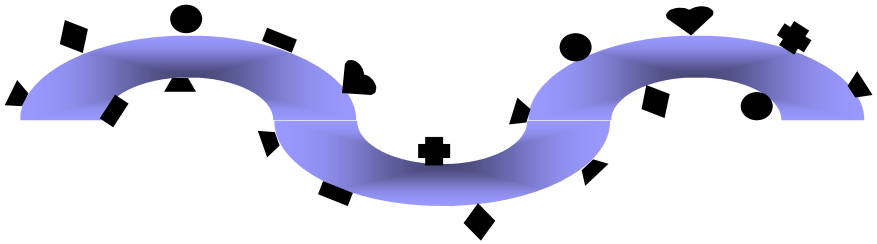
Classification of Antigen cont…

- Based on chemical nature antigens classified into:
  - **Nucleic acid antigens**:- Nucleic acids are usually poorly immunogenic. However they may become immunogenic when single stranded or when complexed with proteins.
  - **Lipid antigens**:- In general lipids are non-immunogenic, although they may be haptens. Some glycolipids and phospholipids can stimulate T cells and produce a cell-mediated immune response.

Classification of Antigen cont…

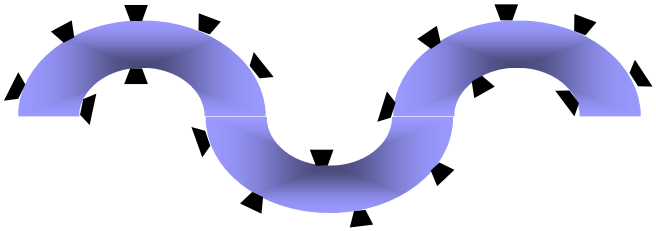
- **T-dependent-** antigens:
  - Do not directly stimulate the production of antibody without the help of T cells.
  - Proteins are T-dependent antigens.

- Structurally these antigens are characterized by a few copies of many different antigenic determinants
  - Examples
    - Microbial proteins
    - Non-self or Altered-self proteins



Classification of Antigen cont…

- **T-independent antigens:**
  - Can directly stimulate the B cells to produce antibody without the requirement for T cell help.
  - Characterized by the production of almost exclusively IgM Ab and no secondary response.
- Properties
  - Polysaccharides
  - Polymeric/repetitive structure
  - Resistance to degradation
  - Monoclonal B cell activation
- Examples
  - Pneumococcal polysaccharide, lipopolysaccharide, Flagella



Classification of Antigen cont…

- **Superantigens:**
  - Are potent T lymphocyte mitogens and simultaneously bind to class II MHC molecules. Superantigen stimulate the production Polyclonal T cell response
- Conventional Antigen stimulate the production Monoclonal/Oligoclonal T cell response
- Examples
  - Staphylococcal enterotoxins
  - Staphylococcal toxic shock toxin
  - Staphylococcal exfoliating toxin
  - Streptococcal pyrogenic exotoxins

5.4 Factors That influence Immunogenicity

Factors that influence immunogenicity could be :

- Those related to the antigen/ foregin substance
- Those related to the host/ biologic system

I. Factors related to the immunogen/antigen are:

1. **Foreignness** - The immune system normally discriminates between self and non-self such that only foreign molecules are immunogenic.

Factors cont.

2. **Size** - There is not absolute size above which a substance will be immunogenic. However, in general, the larger the molecule the more immunogenic it is likely to be.
   - Most potent immunogens have a molecular weight greater than
     50×10^3 Daltons (Da)
   - Few immunogens have mole wt between 10×10^3 -50×10^3 Da
   - A few immunogens are known to have mole wt lessthan 10,000 Da

Factors continued

3. **Chemical Composition** - In general, the more complex the substance is chemically the more immunogenic it will be. Complex proteins are potent immunogens

4. **Physical form** - In general particulate antigens are more immunogenic than soluble ones and denatured antigens more immunogenic than the native form.
   - Particulate > Soluble
   - Denatured > Native

Factors continued

5. Degradability - Antigens that are easily phagocytosed are generally more immunogenic. This is because for most antigens (T-dependant antigens) the development of an immune response requires that the antigen be phagocytosed, processed and presented to helper T cells by an antigen presenting cell (APC).

Factors continued

Contribution of the host/biological System

1. **Genetic Factors -** The species or individuals may lack or have altered genes that code for the receptors for antigen on B cells and T cells or they may not have the appropriate genes needed for the APC to present antigen to the helper T cells.
2. **Age -** Age can also influence immunogenicity. Usually the very young and the very old have a diminished ability to mount an immune response in response to an immunogen.

Factors continued…

- **Method of Administration**
  1. **Dose -** The dose of administration of an immunogen can influence its immunogenicity. There is a dose of antigen above or below which the immune response will not be optimal.
  2. **Route -** Generally the subcutaneous route is better than the intravenous or intra gastric routes. The route of antigen administration can also alter the nature of the respons
  3. **Adjuvants -** Substances that can enhance the immune response to an immunogen are called adjuvants.

5.5 Determinants Recognized by the Innate Immune System

- The innate immune system functions by recognizing highly conserved sets of molecules

- **PAMPs** – **Pathogen Associated Molecular Patterns**

  Are structural molecules on the surface of microbes or secreted; that are recognized by the host innate immune molecules.

- **PRRs** – **Pattern Recognition Receptors**

  Are molecules on the cells and or molecules of the immune system that are capable of recognizing foreign substances

Determinants Recognized by the Innate Immune System . . .

- These molecular structures are specific to the microbes (pathogen-associated molecular patterns, or PAMPs) through a limited set of germ line encoded receptors called pattern-recognition receptors (PRRs)
- There are several distinct classes of PRRs, each of which is involved in performing specific tasks
- These include opsonization, activation of complement cascade, phagocytosis, etc.

Determinants Recognized by the Innate Immune System . . .

- First, PRRs recognize microbial components, known as pathogen-associated molecular patterns (PAMPs), that are essential for the survival of the microorganism and are therefore difficult for the microorganism to alter
- Second, PRRs are expressed constitutively in the host and detect the pathogens regardless of their life-cycle stage
- Third, PRRs are expressed on all cells of a given type, and independent of immunologic memory

Determinants Recognized by the Innate Immune System . . .

- Different PRRs react with specific PAMPs, show distinct expression patterns, activate specific signaling pathways, and lead to distinct antipathogen responses
- The basic machineries underlying innate immune recognition are highly conserved among species, from plants and fruit flies to mammals
- A class of PRRs called Toll-like receptors (TLRs) has the ability to recognize pathogens or pathogen-derived products and initiate signaling events leading to activation of innate host defenses

Determinants Recognized by the Innate Immune System . . .

- Toll-like receptors (TLRs) are a family of pattern recognition receptors that are activated by specific components of microbes and certain host molecules.
- Signaling by TLRs initiates acute inflammatory responses by induction of anti-microbial genes and inflammatory cytokines and chemokines
- Subsequent events, such as recruitment of neutrophils and activation of macrophages, lead to direct killing of the microbes

Determinants Recognized by the Innate Immune System . . .

- The notion of TLRs is primary sensors of pathogens and responsible for orchestrating the innate responses
- TLRs contribute significantly to activation of adaptive immune responses
- There are 10 TLRs, named TLRs 1–10, known in mammals
- These receptors recognizes molecules derived from a unique class of microbial agents
- See table below for examples of PAMPs, PRRs and their biologic effects

Determinants Recognized by the Innate Immune System . . .

PAMP	PRR	Biological Consequence of Interaction
Microbial cell wall components	Complement	Opsonization; Complement activation
Mannose-containing carbohydrates	Mannose-binding protein	Opsonization; Complement activation
Polyanions	Scavenger receptors	Phagocytosis
Lipoproteins of Gram + bacteria Yeast cell wall components	TLR-2 (Toll-like receptor 2)	Macrophage activation; Secretion of inflammatory cytokines

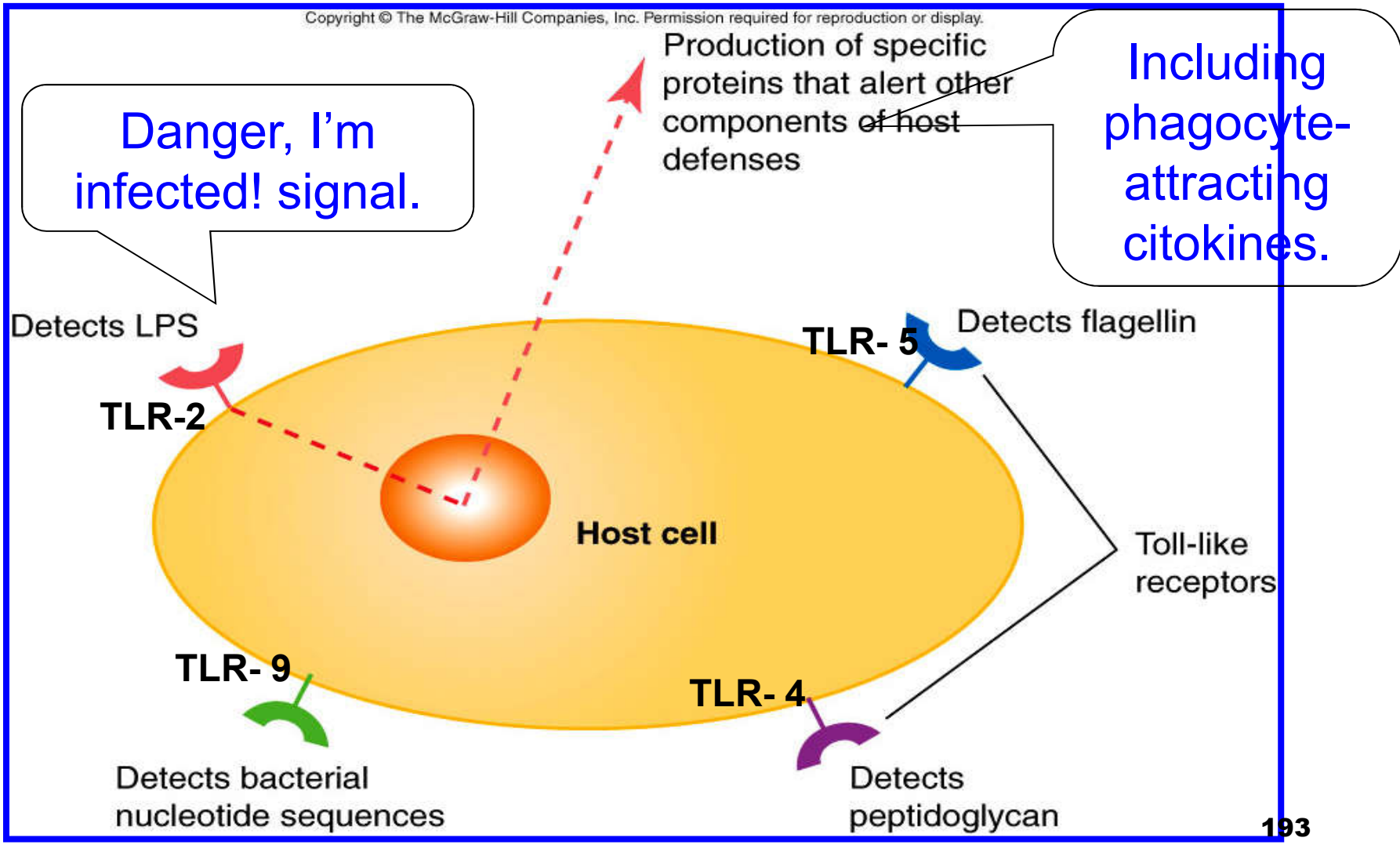
Determinants Recognized by the Innate Immune System . . .

PAMP	PRR	Biological Consequence of Interaction
Double stranded RNA	TLR-3	Production of interferon (antiviral)
LPS (lipopolysaccharide of Gram – bacteria	TLR-4	Macrophage activation; Secretion of inflammatory cytokines
Flagellin (bacterial flagella)	TLR-5	Macrophage activation; Secretion of inflammatory cytokines

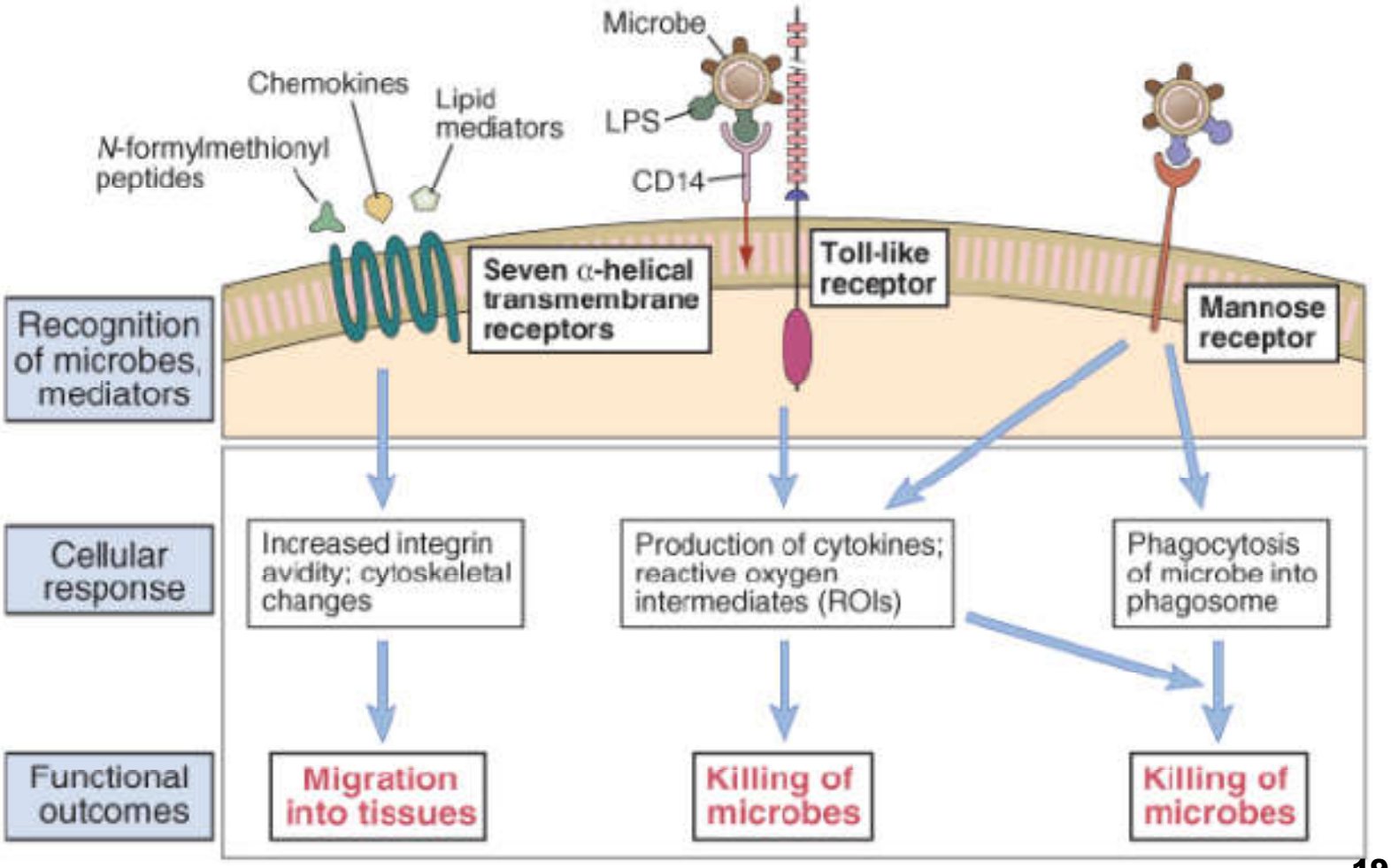
Determinants Recognized by the Innate Immune System . . .

PAMP	PRR	Biological Consequence of Interaction
U-rich single stranded viral RNA	TLR-7	Production of interferon (antiviral)
CpG containing DNA	TLR-9	Macrophage activation; Secretion of inflammatory cytokines

Toll-Like Receptors



Innate immunity
Pattern-recognition receptors



Innate immunity
Pattern-recognition receptors

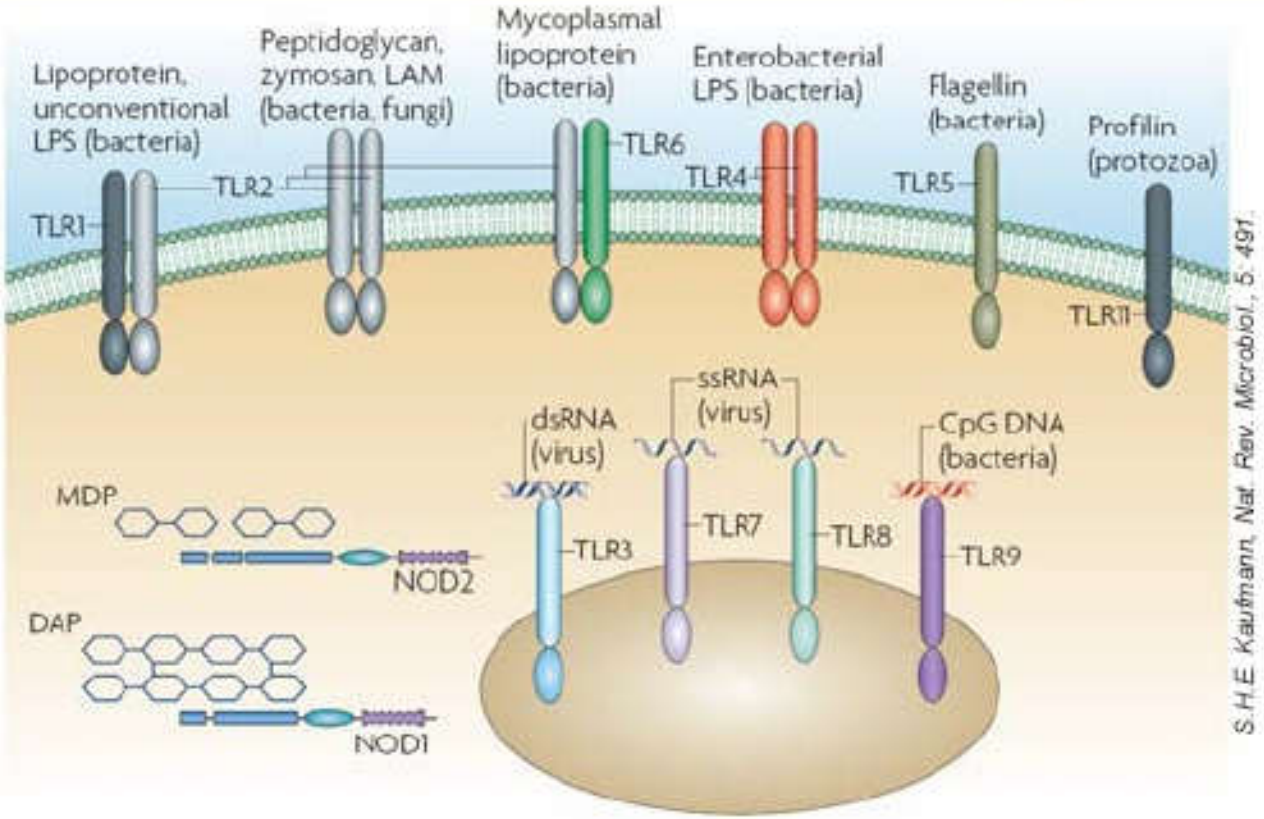


Figure 2 | **Pattern-recognition receptors: TLRs and NODs.** The figure focuses on the better-known pattern-recognition receptors — Toll-like receptors (TLRs) and NODs — leaving out the more recently described members of the expanding Nod-like receptor (NLR) family. The different specificity of each receptor is discussed in the main text. DAP, diaminopimelic acid; ds, double-stranded; MDP, muramyl dipeptide; LPS, lipopolysaccharide; LAM, lipoarabinomannan; ss, single-stranded.

5.6 Antigen Processing and Presentation

- Antigen processing involves the interaction of PAMPs and PRRs followed by digestion of the foreign substance by host phagocytic cells.
- Antigen presentation is the process of displaying peptide antigens associated with MHC molecules to a T cell.
- The path leading to the association of protein fragments with MHC molecules differs for class I and class II MHC.
- MHC class I molecules present degradation products derived from intracellular (endogenous) proteins in the cytosol.
- MHC class II molecules present fragments derived from extracellular (exogenous) proteins that are located in an intracellular compartment.

Antigen Processing cont…

- Class I MHC Pathway:
  - All nucleated cells express class I MHC.
  - Proteins are fragmented in the cytosol by proteosomes (a complex of proteins having proteolytic activity) or by other proteases.
  - The fragments are then transported across the membrane of the endoplasmic reticulum by transporter proteins. (The transporter proteins and some components of the proteosome are encoded by genes in the MHC complex).

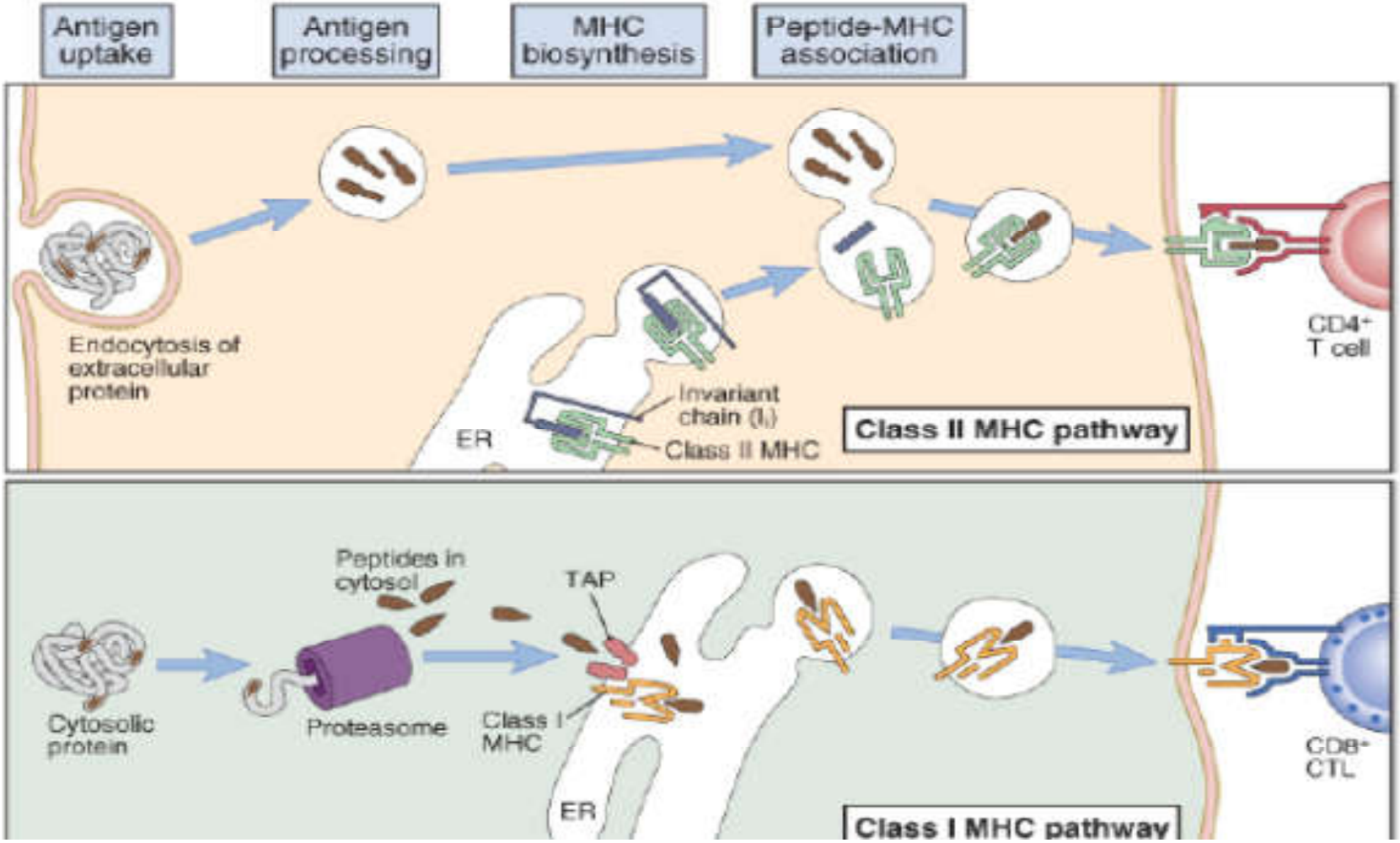
Antigen Processing cont…

- Class II MHC pathway
  - Only a limited group of cells express class II MHC, which includes the antigen presenting cells (APC).
  - The principal APC are macrophages (MΘ) , dendritic cells (Langerhans cells) (DCs), and B cells. Often known as professional APCs
  - The expression of class II MHC molecules is either constitutive or inducible, especially by interferon-gamma in the case of macrophages.
  - exogenous proteins taken in by endocytosis are fragmented by proteases in an endosome.

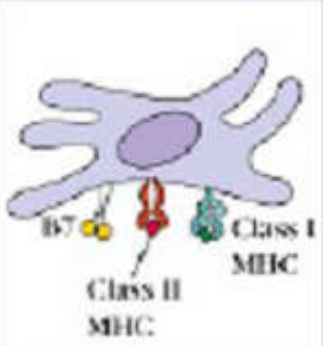
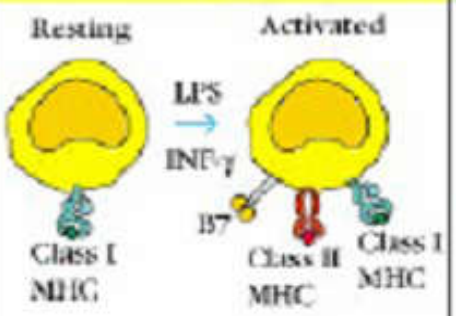
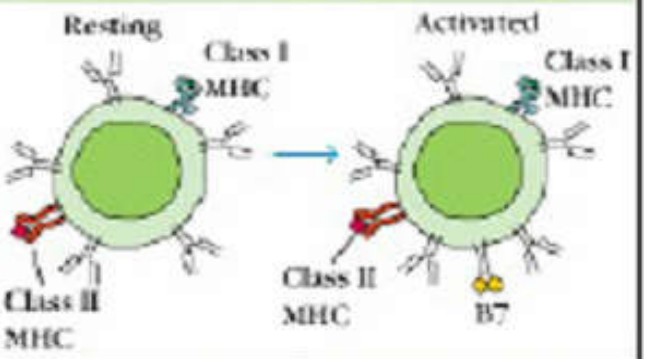
Antigen Processing cont…

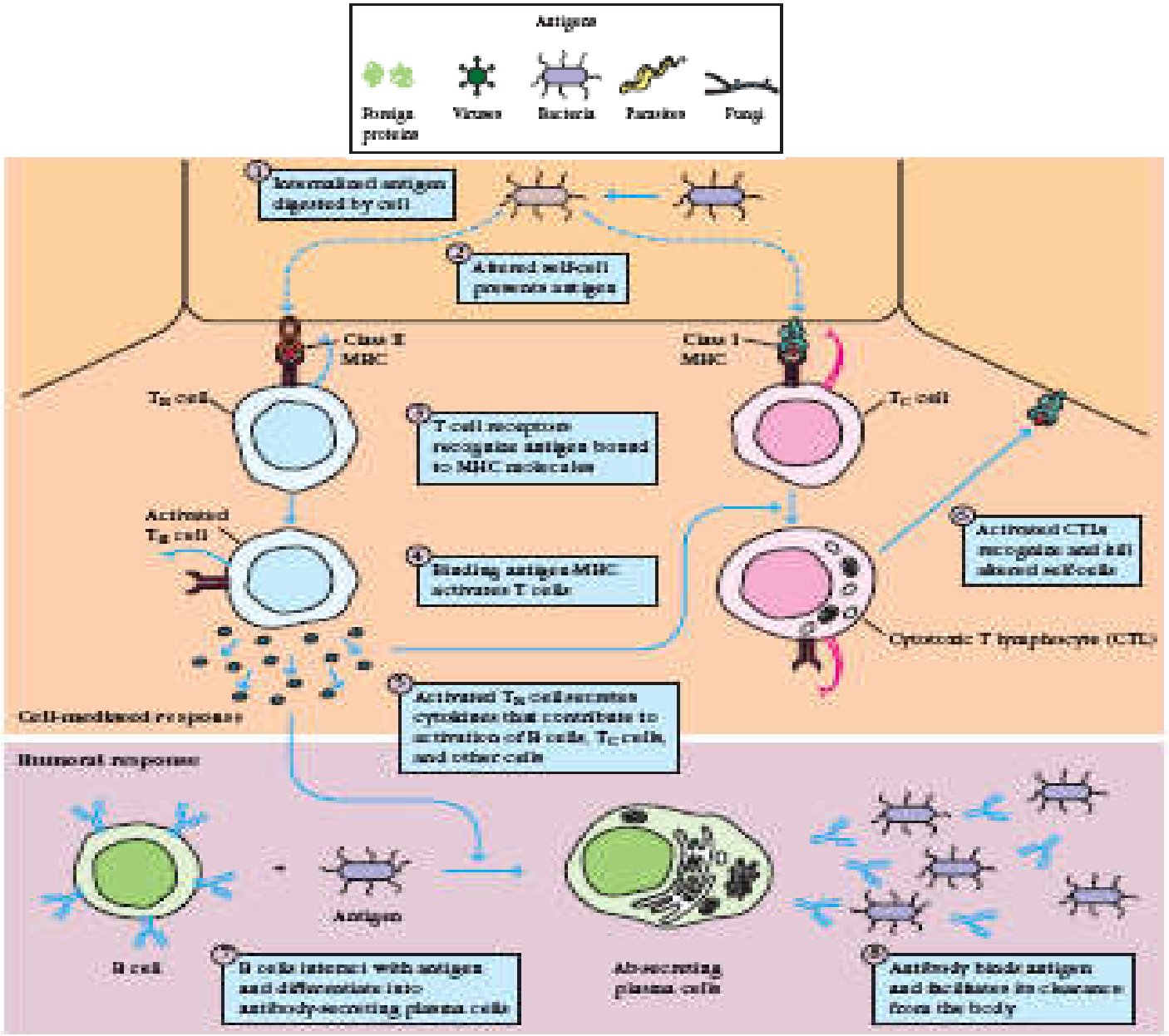
- The alpha and beta chains of MHC class II, along with an invariant chain, are synthesized and assembled in the endoplasmic reticulum
- The invariant chain prevents endogenous peptides from the cytosol from associating with class II MHC molecules.
- The class II MHC molecules with the associated invariant chain are finally transported to the cell surface for presentation to T cells

Antigen processing



5.7 Role of antigen processing cells

Profes sional APCs	Dendritic cell	Macrophage		B Lymphocyte	
					
Antigen uptake	Endocytosis phagocytosis (by Langerhans cells)	Phagocytosis	Phagocytosis	Receptor-mediated endocytosis	Receptor-mediated endocytosis
Class II MHC expression	Constitutive (+++)	Inducible (–)	Inducible (++)	Constitutive (++)	Constitutive (+++)
Co-stimulatory activity	Constitutive B7 (+++)	Inducible B7 (–)	Inducible B7 (++)	Inducible B7 (–)	Inducible B7 (++)
T-cell activation	Naive T cells Effector T cells Memory T cells	(–)	Effector T cells Memory T cells	Effector T cells Memory T cells	Naive T cells Effector T cells Memory T cells


Antigens
Foreign proteins
Viruses
Bacteria
Parasites
Fungi
1 Internalized antigen digested by cell
2 Altered self-cell presents antigen
Class II MHC
Class I MHC
T_H cell
T_C cell
3 T-cell receptors recognize antigen bound to MHC molecules
Activated T_H cell
4 Binding antigen-MHC activates T cells
5 Activated CTLs recognize and kill altered self cells
Cytotoxic T lymphocyte (CTL)
6 Activated T_H cell secretes cytokines that coordinate immune activities of B cells, T_C cells, and other cells
Cell-mediated response
Humoral response
Antigen
B cell
7 B cells interact with antigen and differentiate into antibody-secreting plasma cells
Ab-secreting plasma cells
8 Antibody binds antigen and facilitates its clearance from the body

CHAPTER 6

Major Histocompatibility Complex (MHC)

Objectives

Upon completion of this lesson the student will be able to:

1. Give an overview of the role of the major histocompatibilty complex in immune response
2. Describe the structure and function of class I and class II MHC molecules
3. Discuse the nature of polymorphism in class I and class II MHC molecules

Major Histocompatibility Complex

6.0 Introduction to MHC
6.1 MHC Molecules and Gene
6.2 Cellular Distribution of MHC Molecules
6.3 Regulation of MHC Expression
6.4 MHC and Immune Responsiveness
6.5 MHC and Disease Susceptibility

Introduction to MHC . . .

- Genes in the MHC were first identified as being important genes in rejection of transplanted tissues
- Genes within the MHC were highly polymorphic
- Studies with inbred strains of mice showed that genes within the MHC were also involved in controlling both humoral and cell-mediated immune responses
  - Responder/Non-responder strains

Introduction to MHC . . .

- There were three kinds of molecules encoded by the MHC
  - Class I
  - Class II
  - Class III

- The **major histocompatibility complex (MHC)** products play roles in intercellular recognition and in discrimination between self and nonself.

Introduction to MHC . . .

- The MHC participates in the development of both humoral and cell mediated immune responses.
- While antibodies may react with antigens alone, most T cells recognize antigen only when it is combined with an MHC molecule.
- A peptide must associate with a given MHC of that individual, otherwise no immune response can occur. That is one level of control.

MHC genes encode 3 classes of molecules:

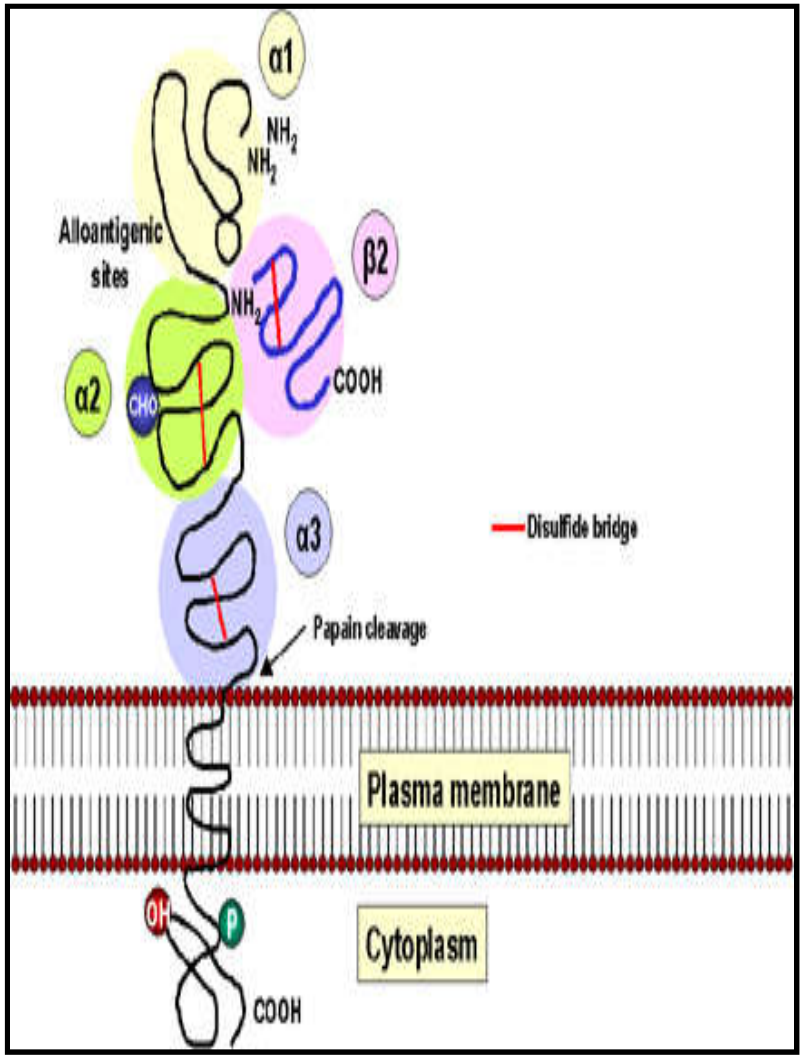
- **Class I MHC genes** encode glycoproteins
  - expressed on the surface of nearly all nucleated cells; but vary in concentration on different cell types
  - Major function of Class I gene products is presenting peptide antigens to TC cells.
  - Class I antigens are produced by the A, B, and C subloci genes
- **Class II MHC genes** encode glycoproteins
  - expressed primarily on antigen-presenting cells (macrophages,
    - dendritic cells, and B cells),
    - where they present processed antigenic peptides to TH cells.
  - Class II antigens are produced by the DP, DQ, and DR subloci genes

MHC genes encode 3 classes of molecules:

- **Class III MHC genes** encode various secreted proteins that have
  - Immune functions, including components of the complement system, and
  - molecules involved in inflammation.

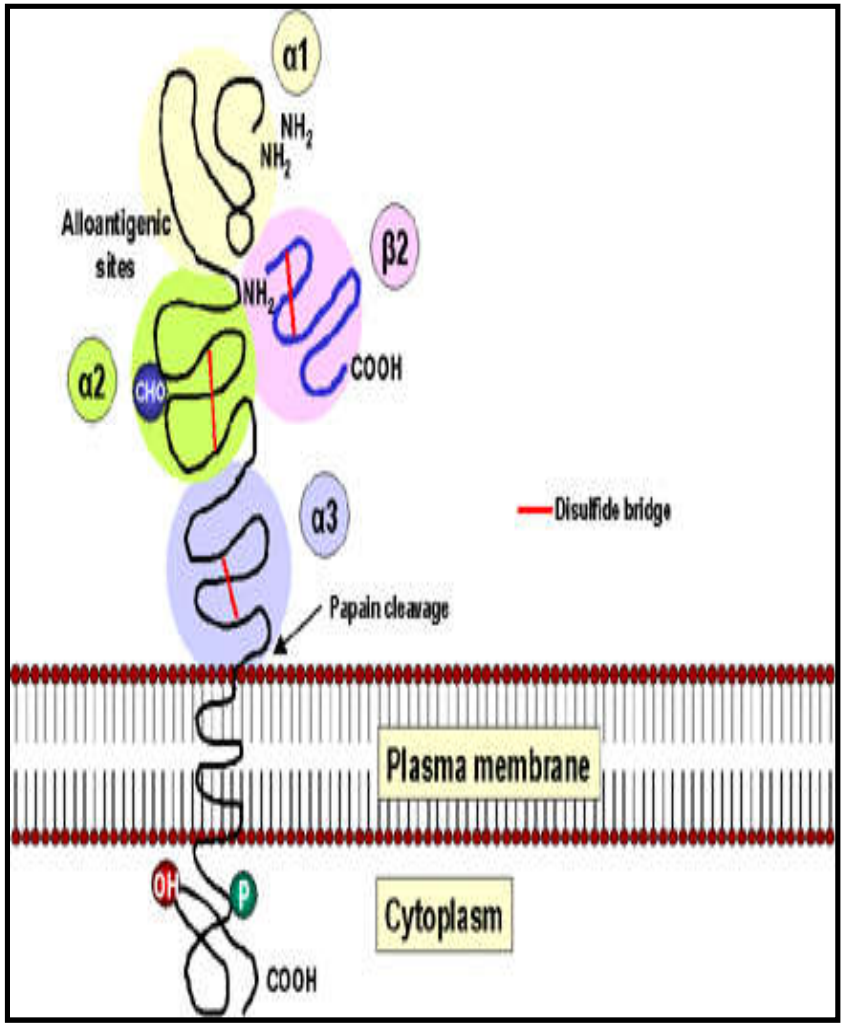
Structure of Class I MHC

- Two polypeptide chains, a long α chain and a short β (β2 microglobulin)
- Four regions
  - Cytoplasmic region containing sites for phosporylation and binding to cytoskeletal elements
  - Transmembrane region containing hydrophobic amino acids



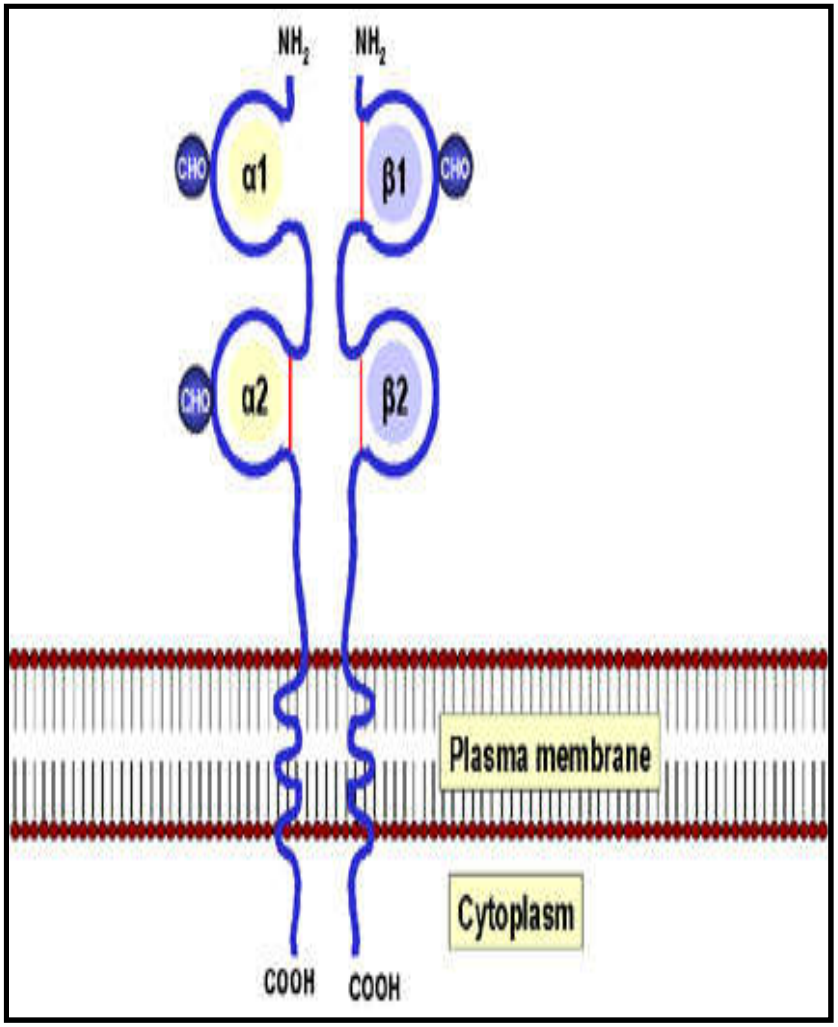
Structure of Class I MHC

- Four regions
  - A highly conserved α3 domain to which CD8 binds
  - A highly polymorphic peptide binding region formed from the α1 and α2 domains
- B2-microglobulin helps stabilize the conformation



Structure of Class II MHC

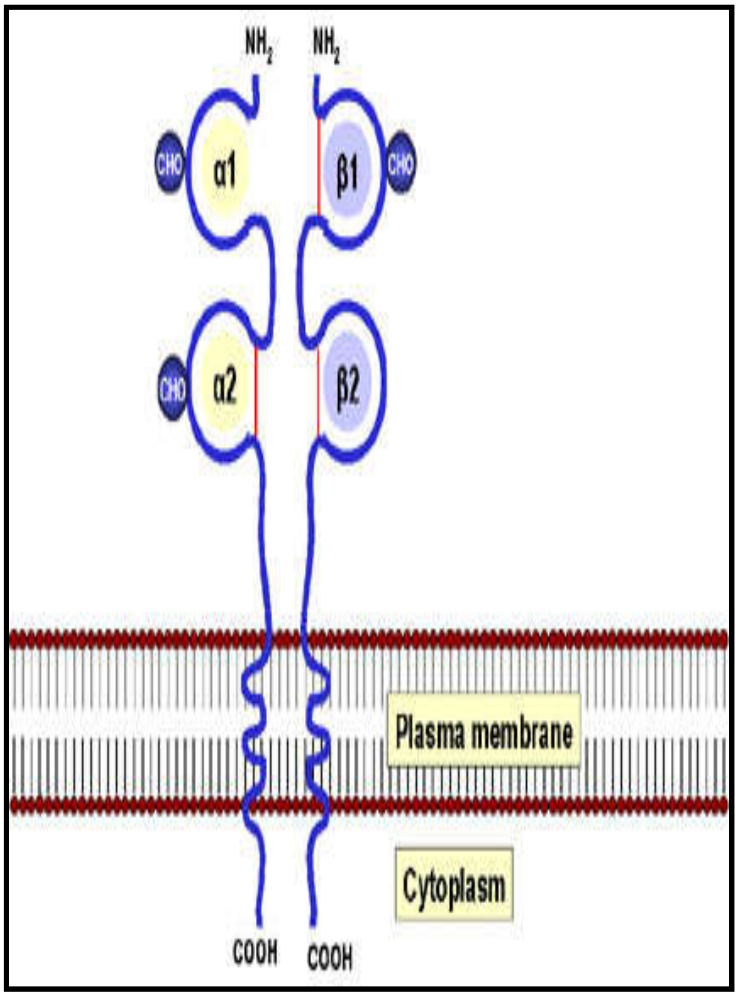
- Two polypeptide chains,α and β, of roughly equal length
- Four regions
  - Cytoplasmic region containing sites for phosporylation and binding to cytoskeletal elements



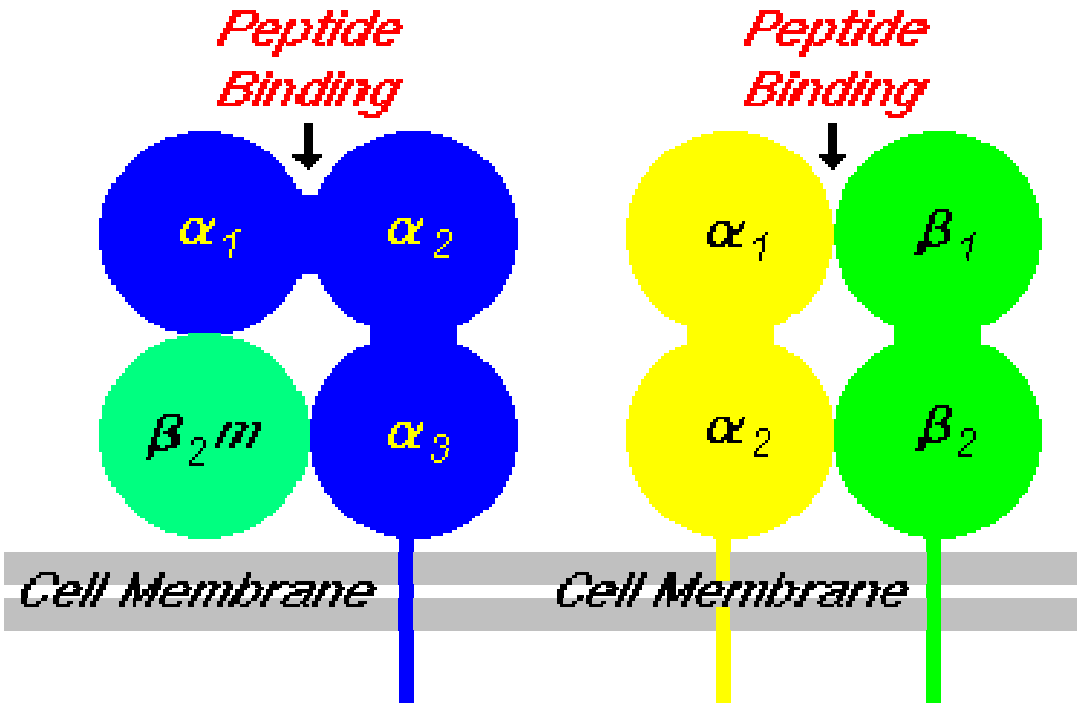
Structure of Class II MHC

Four regions

- Transmembrane region containing hydrophobic amino acids
- A highly conserved α2 and a highly conserved β2 domains to which CD4 binds
- A highly polymorphic peptide binding region formed from the α1 and β1 domains



MHC I Compared to MHC II Structure



MHC I MHC II

MHC Class I polymorphism

Locus	Number of alleles (allotypes)
HLA - A	218
HLA - B	439
HLA - C	96
There are also HLA - E, HLA - F and HLA - G	Relatively few alleles

MHC genes encode 3 classes of molecules:

- Class I and class II MHC molecules are membrane-bound and closely related in both structure and function.
- Both types function as
  - highly specialized antigen-presenting molecules
  - form unusually stable complexes with antigenic peptides, and
  - displaying these antigens on the cell surface for recognition by T cells.

Cellular Distribution of MHC Molecules

- In general, the classical class I MHC molecules are expressed on most nucleated cells, but the level of expression differs among different cell types.
- The highest levels of class I molecules are expressed by lymphocytes
- In contrast, fibroblasts, muscle cells, liver hepatocytes, and neural cells express very low levels of class I MHC molecules.
  - The low level on liver cells may contribute to the considerable success of liver transplants by reducing the likelihood of graft recognition by Tc of the recipient

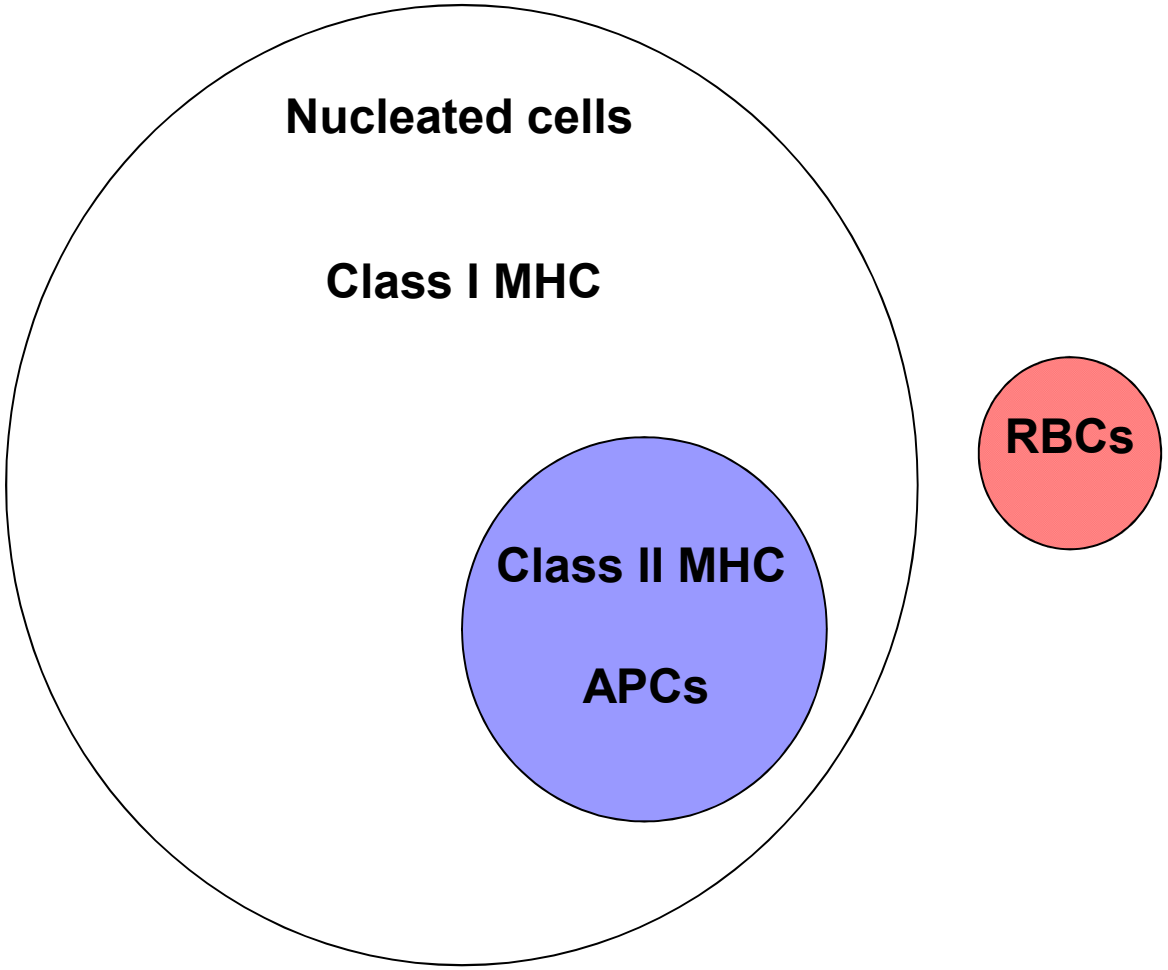
Cellular Distribution of MHC Molecules continued

- In normal, healthy cells, the class I molecules will display self-peptides resulting from normal turnover of self proteins.
- In cells infected by a virus, viral peptides, as well as self peptides, will be displayed

Cellular Distribution of MHC Molecules continued

- Unlike class I MHC molecules, class II molecules are expressed only by
  - antigen-presenting cells, primarily macrophages, dendritic cells, and B cells, and
  - thymic epithelial cells.
- Among the various cell types that express class II MHC molecules,
  - marked differences in expression have been observed,
  - depending on maturation and/or degree of antigenic stimulation.

Introduction to MHC . . .



Regulation of MHC Expression

- The expression of MHC molecules is regulated by various cytokines.
  - interferons (alpha, beta, and gamma) and tumor necrosis factor increase expression of class I MHC molecules on cells.
  - Interferon gamma (IFN-), for example, appears to induce the formation of a specific transcription factor that binds to the promoter sequence flanking the class I MHC genes.

Regulations of MHC Expression continued

- MHC molecules express major self antigens.
- The MHC partly determines the ability of an individual's Tc and Th cells' response to antigens of infectious organisms,
  - it has therefore been implicated in the susceptibility to disease, and
  - in the development of autoimmunity.

Regulation of MHC Expression continued

- Other cytokines influence MHC expression only in certain cell types; for example,
  - IL-4 increases expression of class II molecules by resting B cells.
  - Expression of class II molecules by B cells is down-regulated by IFN-γ
  - MHC expression is decreased by infection with certain viruses, including human cytomegalovirus (CMV), hepatitis B virus (HBV), and adenovirus 12 (Ad12).

MHC Molecules and Immune Responsiveness

- Immune responsiveness's dependence on the class II MHC reflects the importance of class II MHC molecules in presenting antigen to TH cells.
- Absence of an MHC molecule that can bind and present a given peptide, or the absence of T-cell receptors that can recognize a given peptide–MHC molecule complex,
  - may result in the absence of immune responsiveness and
  - account for the observed relationship between MHC haplotype and immune responsiveness to exogenous antigens.

MHC and Disease Susceptibility

- Some HLA alleles occur at a much higher frequency in those suffering from certain diseases than in the general population.
- Diseases associated with particular MHC alleles include
  - autoimmune disorders,
  - certain viral diseases,
  - disorders of the complement system,
  - some neurologic disorders,and
  - several different allergies.

MHC and Disease Susceptibility continued

- The fact that some of the class I MHC alleles are in linkage disequilibrium with the class II MHC alleles makes their contribution to disease susceptibility appear more pronounced than it actually is.
- Allelic differences may yield differences in immune responsiveness arising from variation
  - in the ability to present processed antigen or
  - the ability of T cells to recognize presented Antigen.
- allelic forms of MHC genes may also encode molecules that are recognized as receptors by viruses or bacterial Toxins.

MHC and Disease Susceptibility continued

- Genetic analysis of disease may show that genes at multiple loci must be involved and that complex interactions among them may be needed to trigger disease
- Although some individuals probably will not be able to develop an immune response to a given pathogen and will be susceptible to infection by it,
  - extreme polymorphism ensures that at least some members of a species will be able to respond and will be resistant.
- In this way, MHC diversity appears to protect a species from a wide range of infectious diseases.

CHAPTER 7

B cell generation, Activation and Differentiation

Outline

7.1 B-Cell Maturation and B cell receptor
7.2 B-Cell Activation and Proliferation
7.3 The Humoral Response
7.4 Differentiation
7.5 Regulation of B-Cell Development
7.6 Regulation of the Immune effector response

7.1 B-Cell Maturation

- B-cells arise from progenitor Lymphocytes.
- Generation of mature B cells first occurs in the embryo and continues throughout life, at sites including:
  - yolk sac,
  - fetal liver, and
  - fetal bone marrow are the major sites of B-cell maturation.
- After birth maturation continues in the bone marrow.

B cell maturation and activation

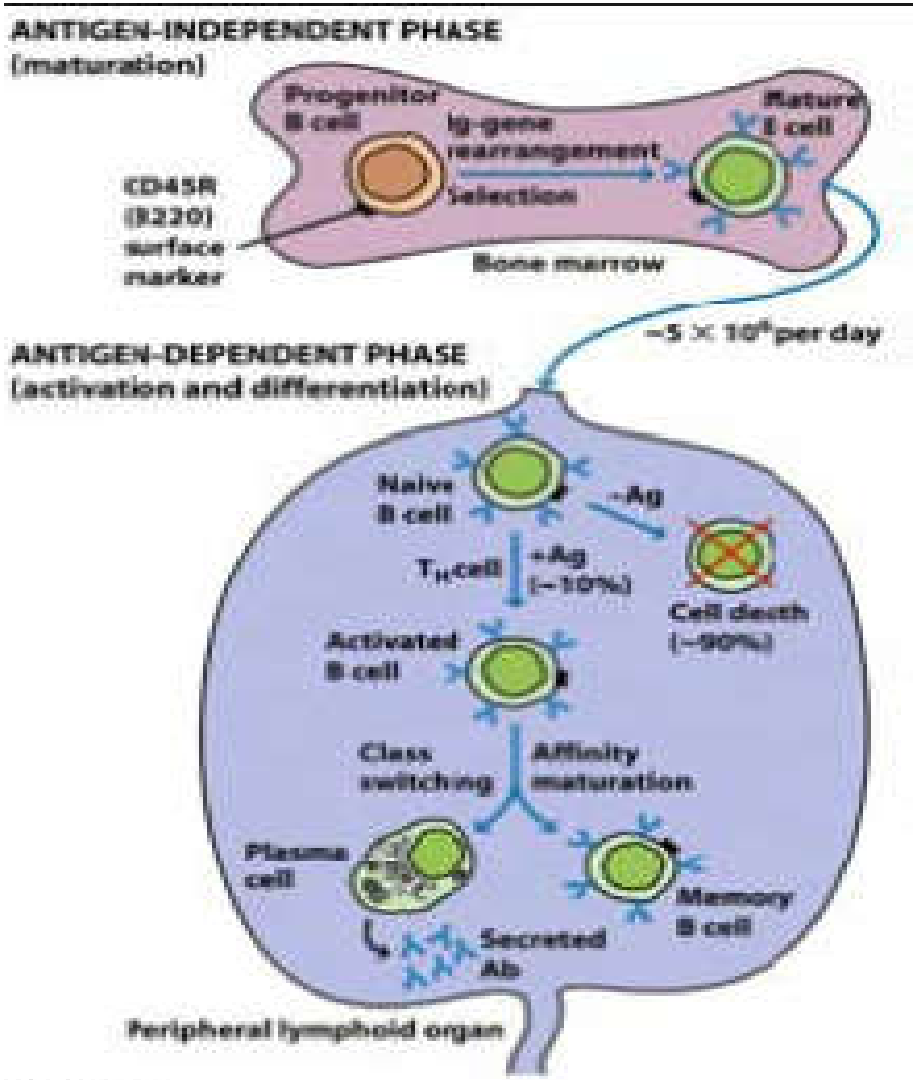


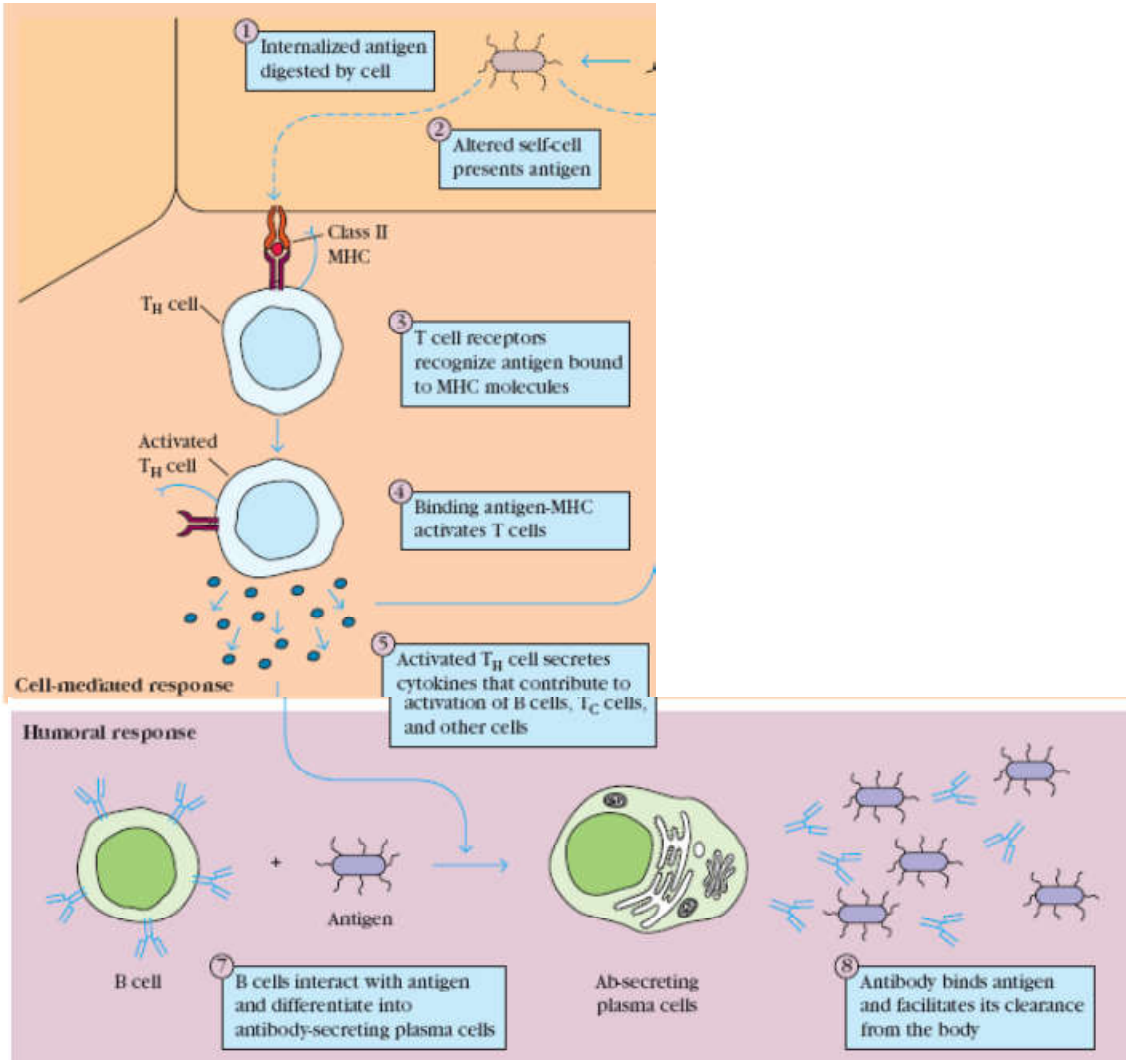
Figure 11-1

Naïve B cells circulate in the blood and lymph and carried to the secondary lymphoid organs such as spleen and lymph nodes

Activation of B cells results in Ag-dependent proliferation (clonal expansion), differentiation into plasma/memory cells, affinity maturation and class switching

Lymphocyte Extravasation continued

- Additional elaboration on B cell maturation

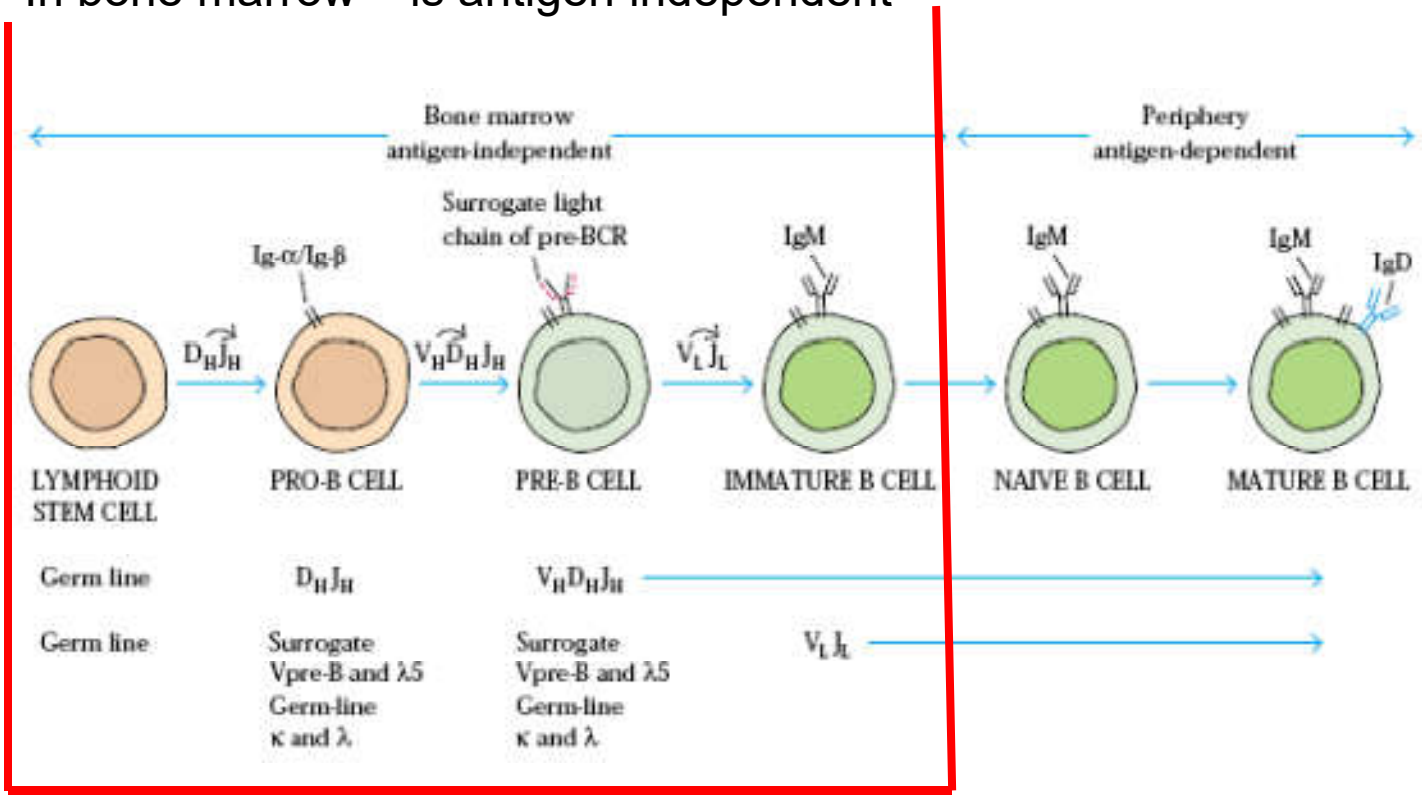

1 Internalized antigen digested by cell
2 Altered self-cell presents antigen
Class II MHC
T_H cell
3 T cell receptors recognize antigen bound to MHC molecules
Activated T_H cell
4 Binding antigen-MHC activates T cells
5 Activated T_H cell secretes cytokines that contribute to activation of B cells, T_C cells, and other cells
Cell-mediated response
Humoral response
Antigen
B cell
7 B cells interact with antigen and differentiate into antibody-secreting plasma cells
Ab-secreting plasma cells
8 Antibody binds antigen and facilitates its clearance from the body

7.1 B-Cell Maturation continued

- Maturation results divided into three broad stages:
  - antigen independent maturation of immunocompetent B cells,
  - activation of mature B cells when they interact with antigen, and
  - differentiation of activated B cells into plasma cells and memory B cells.
- The antigen independent phase of B-cell development
  - maturation directed by the Bone marrow
  - involves orderly, sequential rearrangement of Ig-gene rearrangements
  - progresses in the absence of antigen.

7.1 B-Cell Maturation continued

In bone marrow – is antigen independent

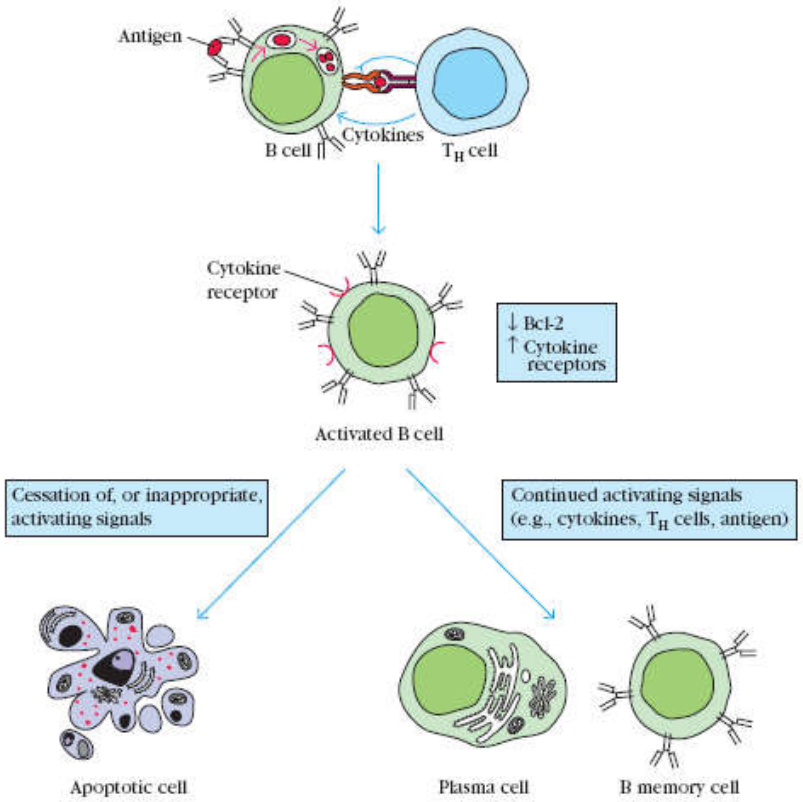


7.1 B-Cell Maturation continued

- A mature B cell leaves the bone marrow expressing membrane-bound immunoglobulin (mIgM and mIgD) with a single antigenic specificity.
  - these B cells are educated to respond to specific antigens, but
  - have not encountered the appropriate antigen – are sometimes referred to as naive

7.2 B-Cell Activation and Proliferation

- B cell activation occurs circulation secondary lymphoid
- B cell proliferation and differentiation occurs in secondary lymphoid organs
- Activation and clonal expansion are driven by encounter with specific foreign antigen, resulting in
  - generation of plasma cells,
  - and memory (with long life span)
- In the absence of antigen-induced activation,
  - naive B cells in the periphery have a short life span,
  - they will die if the appropriate antigen is not encountered,
  - death, within a few weeks, is by apoptosis .


Antigen
Cytokines
B cell
T_H cell
Cytokine receptor
↓ Bcl-2
↑ Cytokine receptors
Activated B cell
Cessation of, or inappropriate, activating signals
Continued activating signals (e.g., cytokines, T_H cells, antigen)
Apoptotic cell
Plasma cell
B memory cell

7.2 B-Cell Activation and Proliferation continued

- The antigen's composition/structure influences which of two maturation routes is followed,
  - **Most antigens are T cell dependent (TD)**:
    - requires direct contact with TH cells, not simply exposure to TH-derived cytokines.
    - examples of these antigens include proteins, lipoproteins,

      and saccharides
    - B cells stimulated by these antigens usually produce IgM and IgG repsonses, with antibodies that are found in the circulation for prolonged time periods
    - activation by this route typically results in Memory B cells

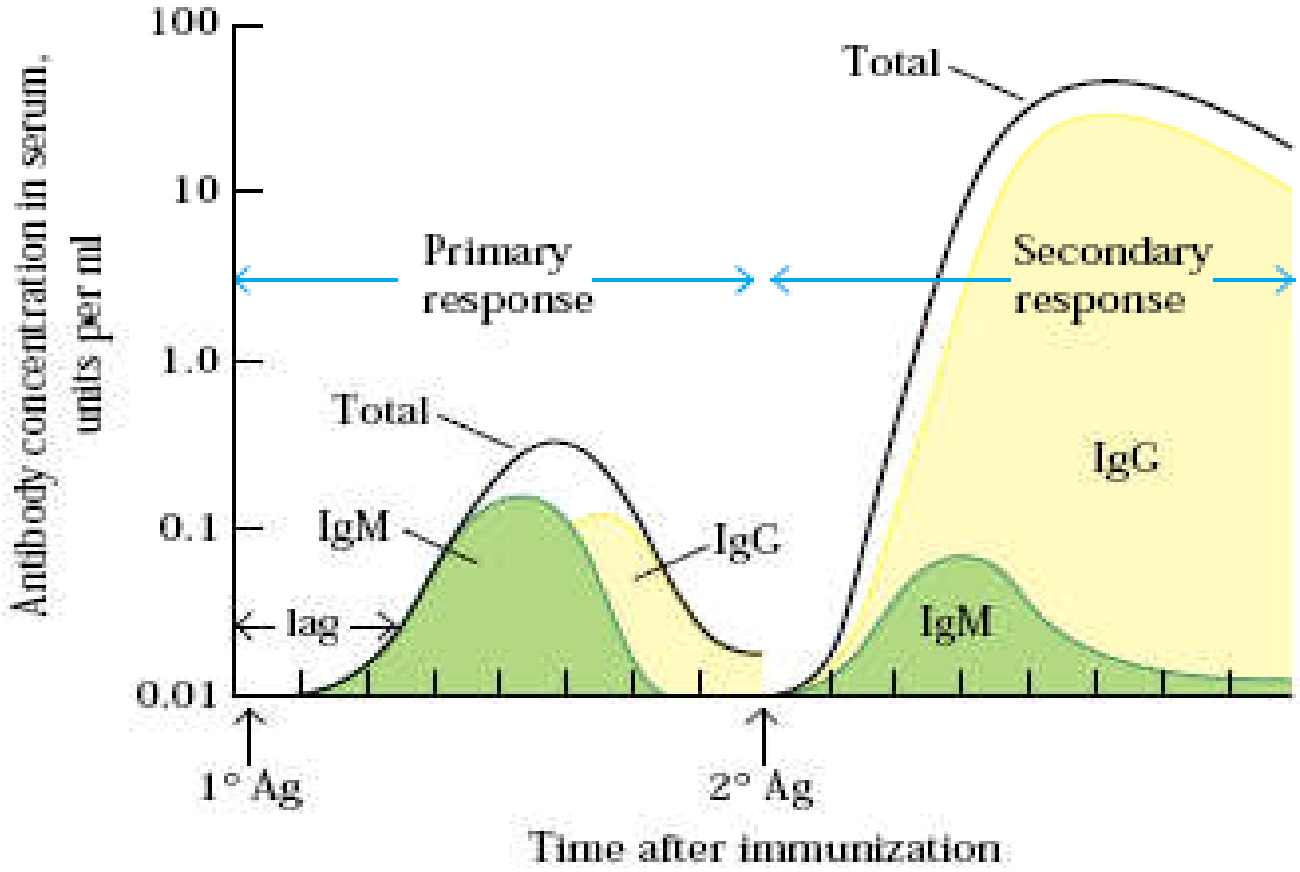
7.2 B-Cell Activation and Proliferation continued

- **T cell-independent (TI) antigens:**
  - can activate B cells without direct participation by TH cells .
  - this response to TI antigen
  - usually involves simple lipids,
  - antibody production is weaker,
  - no memory cells are formed, and
  - IgM is the predominant antibody secreted,
  - reflects low level of class switching.

7.3 The Humoral Response

- humoral response/activation differs between
  - **Primary response** – initial response from activation of naive B cells, and
  - Secondary response – response by memory B cells, previously involved in exposure to the antigen.
- both cases lead to production of secreted antibodies of
  - various isotypes and immunoglobulin classes.
  - variable onset of response and amount of antibody

7.3 Primary and Secondary Response



7.3 Comparison of Primary and Secondary Responses

TABLE 11-4 Comparison of primary and secondary antibody responses

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

7.4 Regulation of B-Cell Development

- Several of transcription factors involved,
- Regulate expression of various gene products at different stages,
- DNA-binding proteins interact with promoter or enhancer sequences,
  - either stimulating, or
  - inhibiting transcription of the associated gene.

7.4 Regulation of B-Cell Development continued

- B-cell transcription factors, a *B-cell–specific activator protein (BSAP)*
  - is a master B-cell regulator.
  - expressed only by B-lineage cells and
  - influences all the B cell stages during maturation
  - required for final differentiation leading to formation of memory B cells and plasma cells.

7.5 Regulation of the Immune Effector Response

- Upon encountering an antigen, immune system can either
  - develop an immune response or
  - **tolerance**, a state of unresponsiveness**.**
  - both responses require specific recognition of antigen by antigen-reactive T or B cells,
- Immune response must be carefully regulated, an inappropriate response—
  - to self antigens or
  - tolerance of a potential pathogen—can have serious and possibly life-threatening consequences

7.5 Regulation of the Immune Effector Response continued

- important regulatory decisions determine :
  - branch of the immune system to be activated,
  - intensity of the response, and its duration.
- competing antigen can regulate the immune response to an unrelated antigen.
- presence of antigen specific-Antibody can suppress B cell response to that antigen.
  - circulating antibody competes with antigen-reactive B cells for antigen - inhibits clonal expansion of those B cells.
  - Binding of antigen-antibody complexes by Fc receptors on B cells reduces signaling by the B-cell-receptor complex

7.5 Regulation of the Immune Effector Response continued

- As the antibody response proceeds,
  - feedback by presence circulating antibody, produces inhibition of the B cell response
  - cytokines from T cells also play important immuno-regulartory role.

CHAPTER 8

Immunoglobulins:
Structure and Function

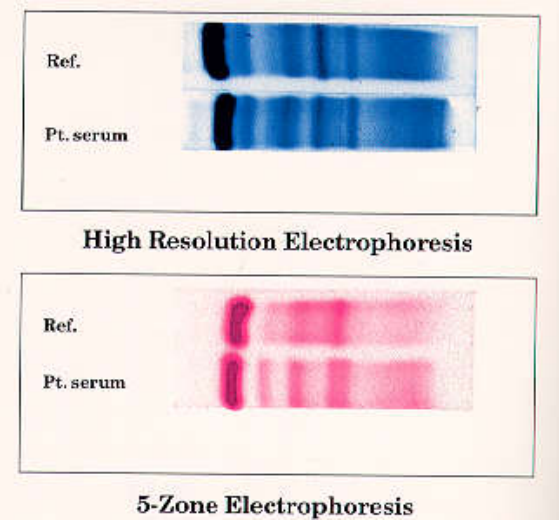
Learning Objectives

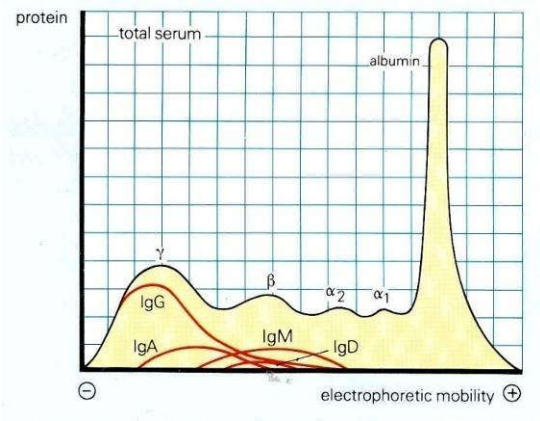
- Up on completion of this chapter the student will be able to :

  1. Discuss the general properties of all immunoglobulins
  2. Describe the basic structure of immunoglobulins
  3. Relate immunoglobulin structure with function
  4. Define immunoglobulin hypervarialble regions
  5. Define and describe immunoglobulin classes, subclasses, types and subtypes
  6. Describe B cell receptor
  7. explain antibody diversity and class switching

Antibodies

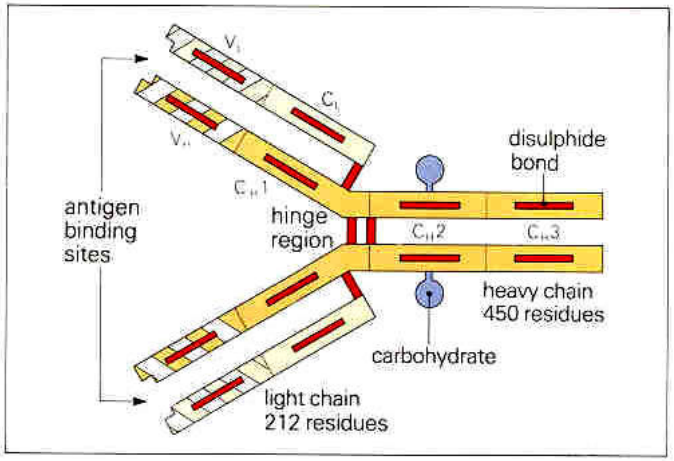
1. Definition - Molecules that are produced by the body in response to a foreign substance and are antagonist to it.
2. They are known as Immunoglobulins when not referring to their specificity
3. Immunoglobulins reside in the globulin fraction of serum.

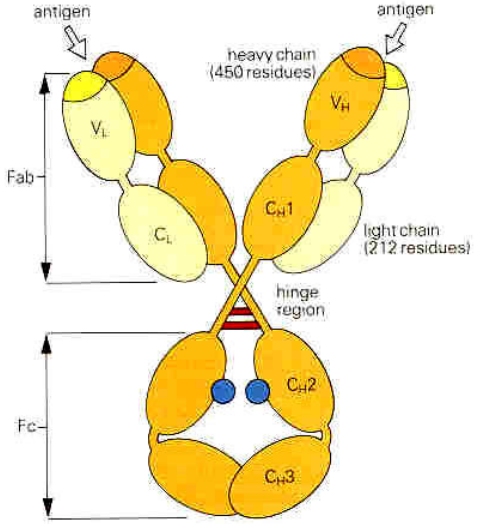




Five Immunoglobulin Classes;

IgG, IgA, IgM, IgD, & IgE

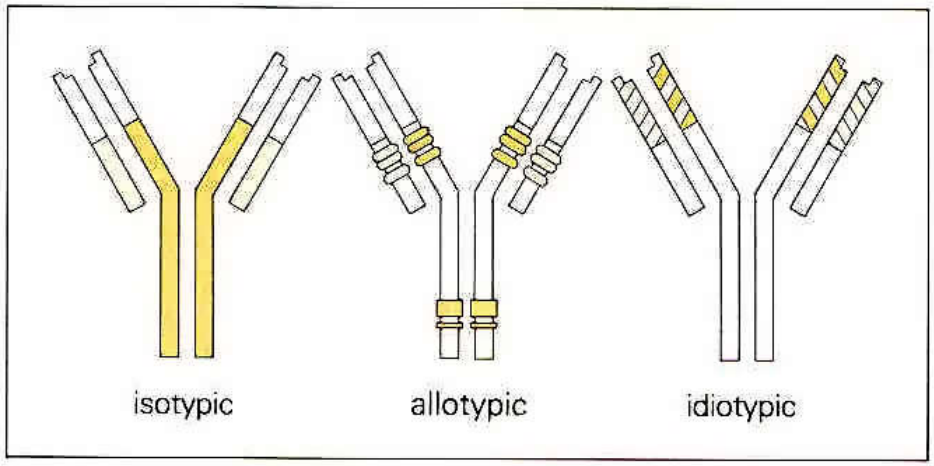




Fab, Disulphide bonds
Hinge, Domains, Variable regions,
Constant regions, COOH end,
NH3 end, Binding site

Antibodies

1. Genetic Variation in Antibody Heterogeneity
   - Isotypes
   - Allotypes
   - Idiotypes
2. Properties
   - C' Fixation
   - Placental transfer



properties of human immunoglobulins

immunoglobulin	IgG1	IgG2	IgG3	IgG4	IgM	IgA	IgD	IgE
complement fixation	++	+	+++	–	+++	–	–	–
placental transfer	+	±	+	+	–	–	–	–

Antibodies-Immunoglobulin Variation

Isotypic variation- immunoglobulin subclasses

Allotypic variation- amino acid substitutions on a given molecule

Idiotypic variation- variation of the amino acid sequence in the antigen binding region given the molecule its antigenic specificity

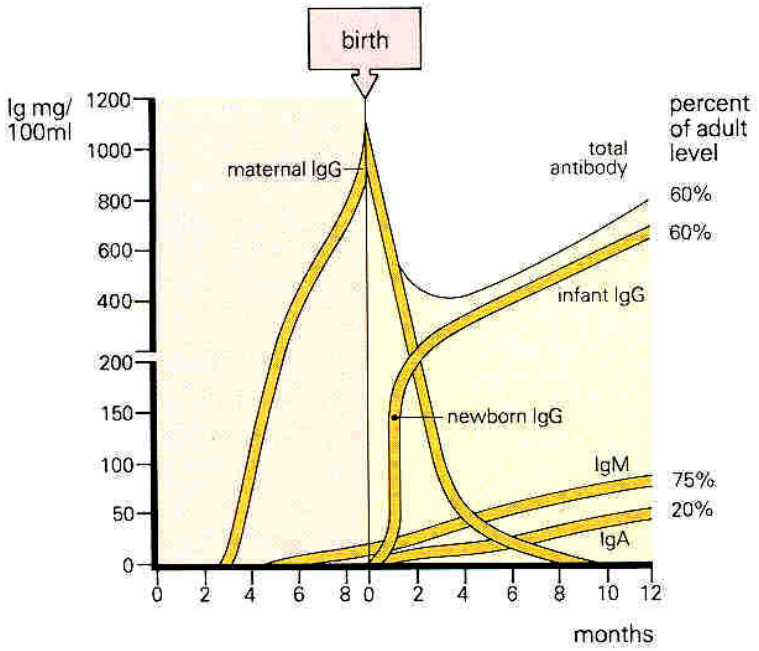
Antibodies - Properties

Extreme importance to Laboratory testing and interpretation

immunoglobulin	IgG1	IgG2	IgG3	IgG4	IgM	IgA1	IgA2	sIgA	IgD	IgE
heavy chain	γ_1	γ_2	γ_3	γ_4	μ	α_1	α_2	α_1 or α_2	δ	ε
mean serum concentration (mg/ml)	9	3	1	0.5	1.5	3.0	0.5	0.05	0.03	0.00005
sedimentation constant	7S	7S	7S	7S	19S	7S	7S	11S	7S	8S
molecular weight	146,000	146,000	170,000	146,000	970,000	160,000	160,000	385,000	184,000	188,000
half-life (days)	21	20	7	21	10	6	6	?	3	2
distribution (% intravascular)	45	45	45	45	80	42	42	trace	75	50
carbohydrate (%)	2–3	2–3	2–3	2–3	12	7–11	7–11	7–11	9–14	12

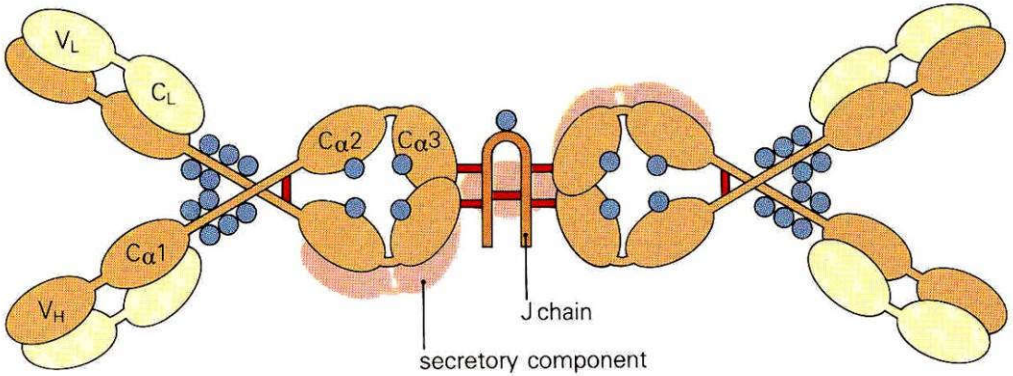
IgG

- Characteristics
  - 150,000 MW
  - Highest serum conc.
  - Transported across placenta via Fc - relevant to Lab diagnosis of ID, Laboratory false positive serologies
  - Four subclasses (IgG1-4)
  - Monomeric in form



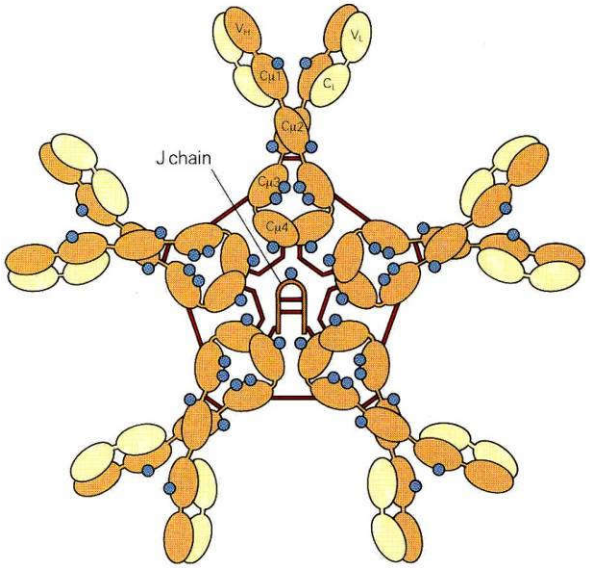
IgA

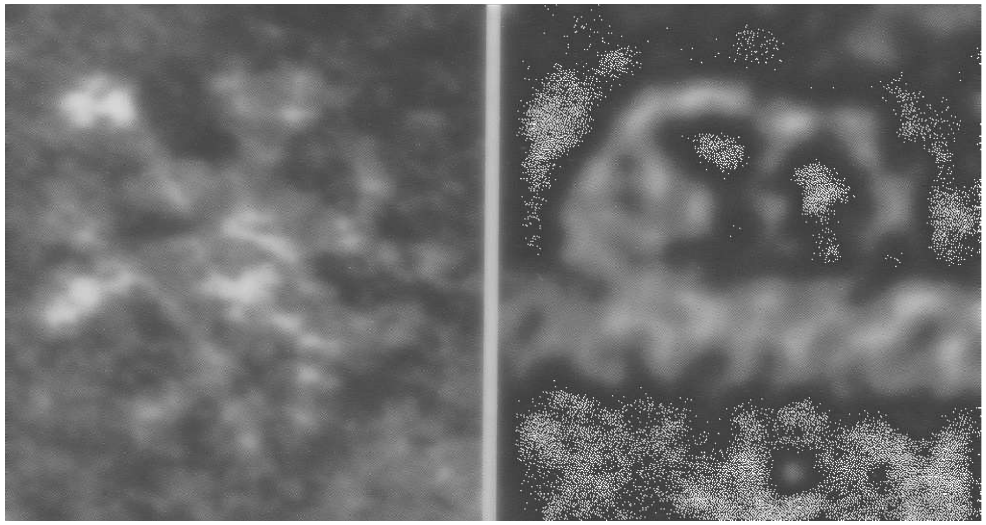
- Characteristics
  - Dimer, monomer
  - Two subtypes (1,2)
  - Secretions
    - Tears, Saliva, GI
    - Secretory IgA has Secretory component made by epithelial cells, that protects it from enzymatic and other chemical degradation
    - It prevents viral and some bacterial attachment to mucosal epithelial cells
  - Dimeric form has J chain - connects



IgM

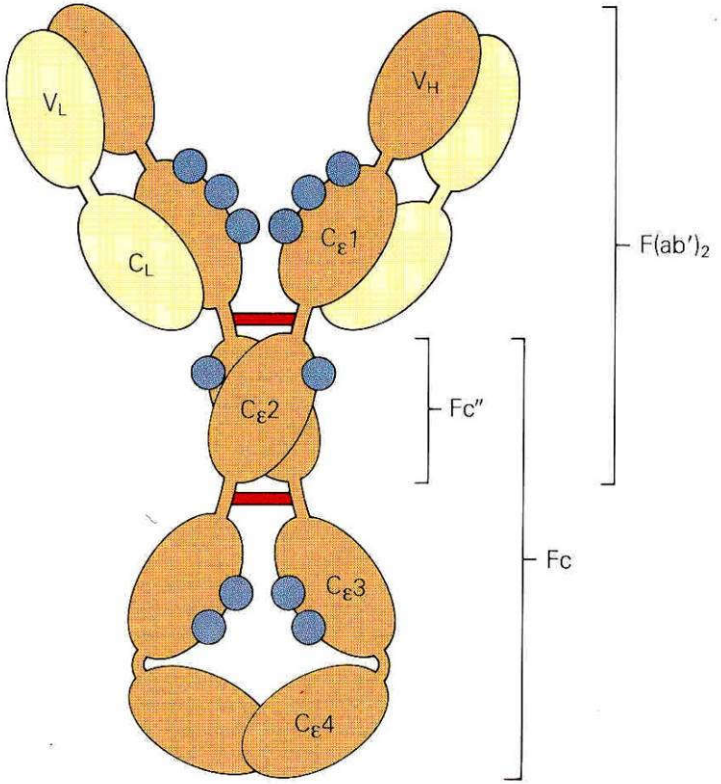
- Characteristics
  - Pentameric form
  - Primary response
    - Role in Lab Diagnostics
  - Does not pass placenta
  - Effective agglutinator
  - Most efficient C' fixation





IgE

- Characteristics
  - Extra Constant region domain (C4)
  - Lowest serum concentration (meaning to diagnostic Lab tests)
  - Binds to Mast cells via Fc - Allergic reactions
  - Protection - Parasitic infections

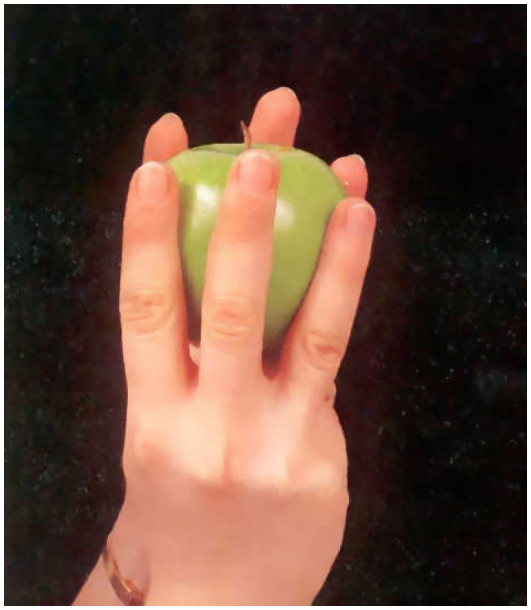


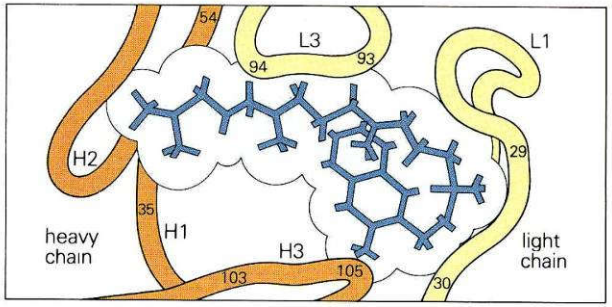
IgD

- Low concentration in serum (<1%)
- Function largely unknown - may act as Antigen receptor for B cells
- No clinical need to measure - clue to physician competence & clerical error

Antibody - Antigen Interactions

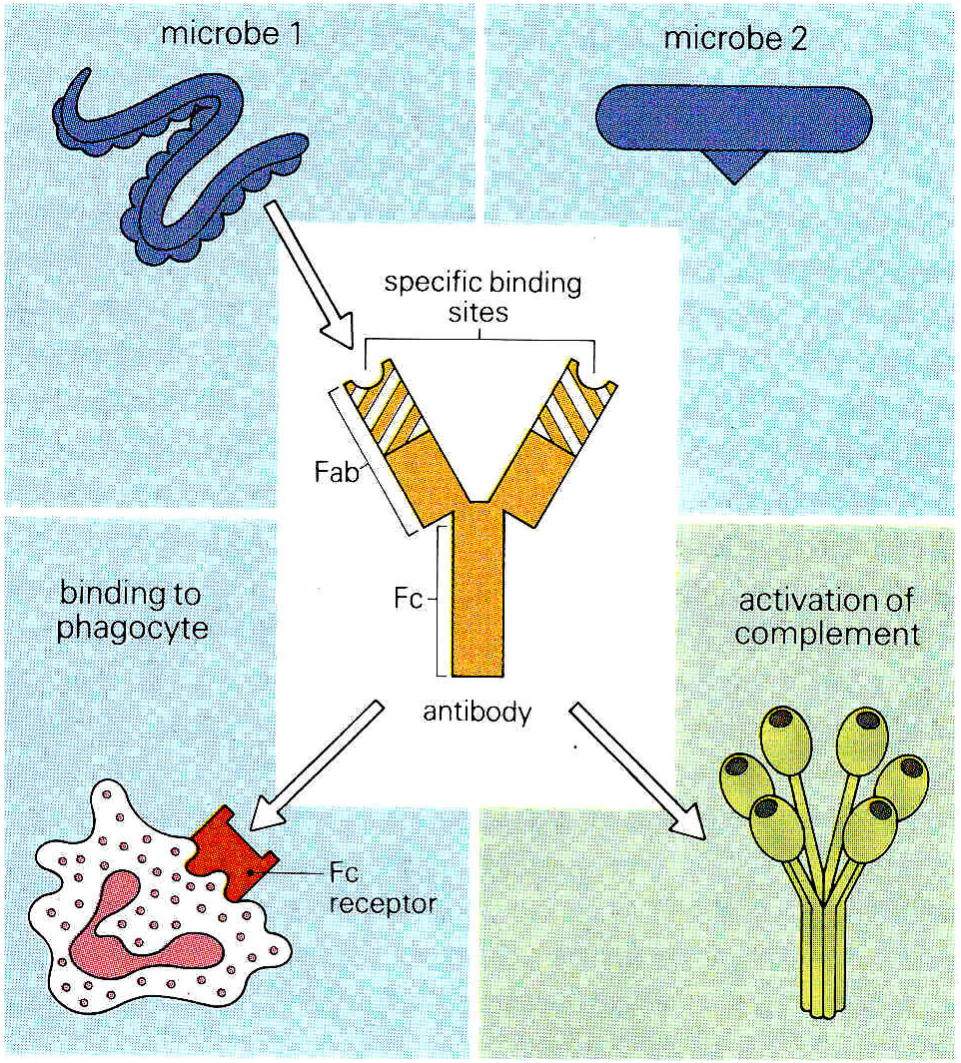
- Antibody binding site
  - Folded structure - forming cleft between Heavy and Light chains - tertiary
  - Apple analogy - best fit
- Antigen binding site
  - Quite small (6 amino acids)
  - Epitope
  - Sequential vs. Conformational - Implications to Lab tests





Outcomes to Antibody Binding

1. Agglutinates Ag
2. Initiates immune response
    1. Cellular
    2. Humoral
3. Activates Complement
4. Cleared/Killed by phagocytic system



Chapter 9

T-Cell Maturation, Activation, and Differentiation

Objectives

Upon completion of this chapter student will be able to:

1. Describe the structure of the T cell receptor for Ag
2. Discuss the genetic basis of diversity in TCR
3. Discuss the role of CD3 complex and co-stimulatory molecules
4. Describe the nature of immunological synapse
5. Discuss the requirements for T cell activation

T-Cell Maturation, Activation, and Differation

- Tcell maturation requires rearrangements of the germ-line, TCR genes, and the expression of various membrane markers.
  - Developing T cells, known as **thymocytes,** proliferate and differentiate along developmental pathways
  - Functionally distinct subpopulations of mature T cells develop...
- Final maturation of most T cells proceed along two different developmental pathways, which generate functionally distinct subpopulations
  - CD4 exhibiting class II MHC restriction, and
  - CD8 exhibiting class I MHC restriction.

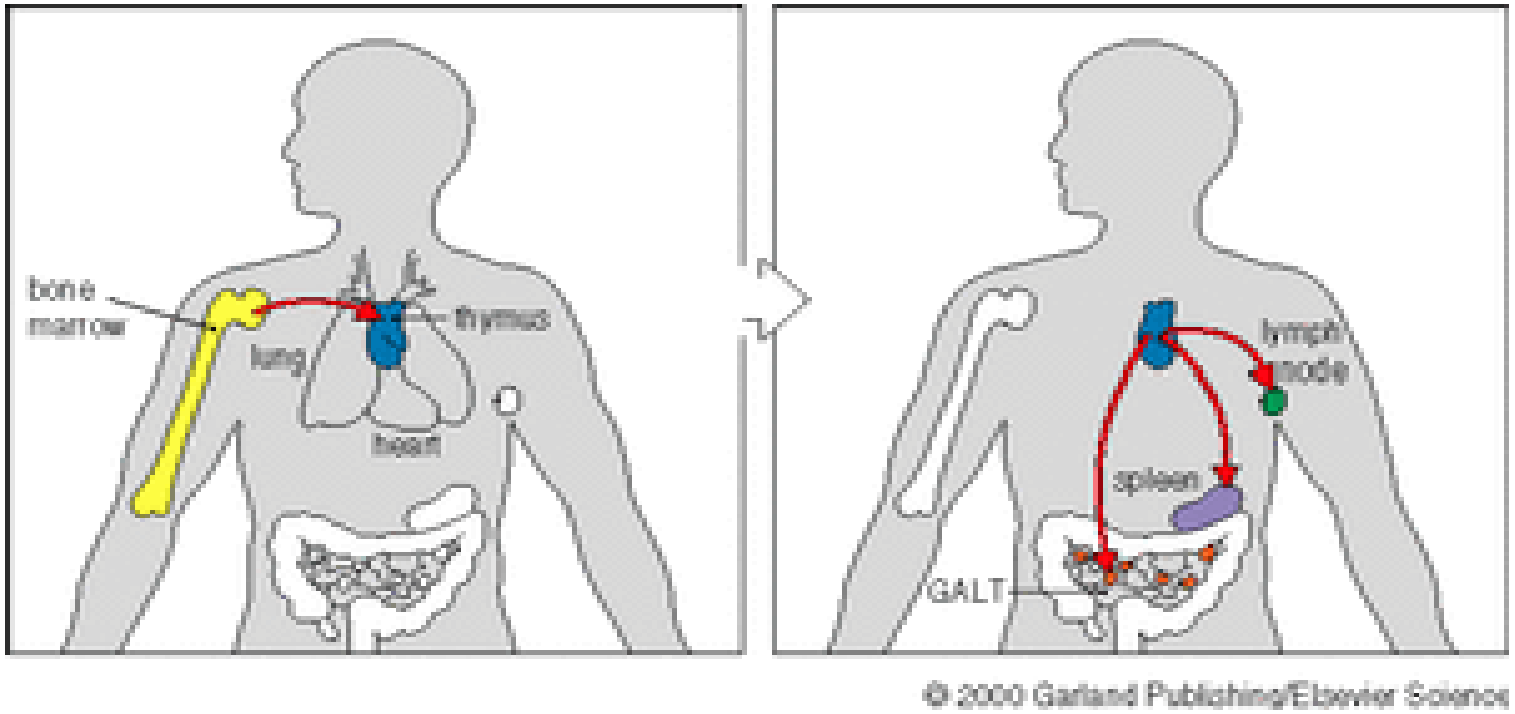
T-Cell Maturation and the Thymus

- Thymus main source of all T cells, it is where T cells diversify and are shaped into an effective primary T-cell repertoire by extraordinary selection processes.
  - **positive selection** permits survival of only T cells whose TCRs are capable of recognizing self-MHC molecules. It is responsible for creation of a self-MHC-restricted repertoire of T cells.
  - **negative selection,** eliminates T cells that react too strongly with self-MHC or with self-MHC plus self peptides. It is an extremely important factor in generating T-cells that are self-tolerant.
- With this maturation the lymphocytes are $CD3^+$

T-Cell Maturation and the Thymus

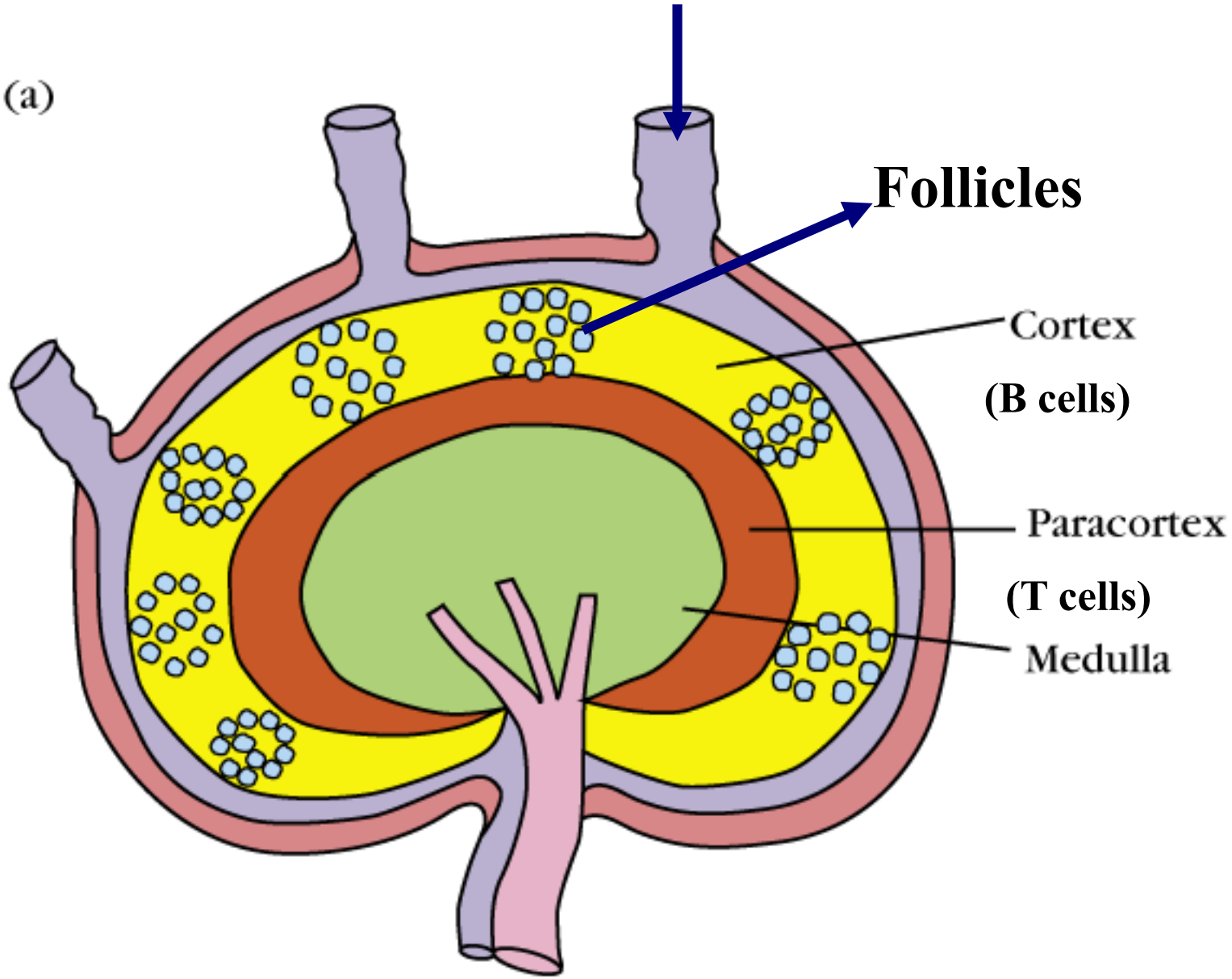
- Interaction of T cell receptor (TCR) with an antigenic peptide in the groove of an MHC molecule begins activation of mature peripheral T cell.
  - Because avidity is low, co-receptors and accessory membranes needed to strengthen the reaction and generate activating signals.
  - Activation leads to the proliferation and differentiation of T cells into various types of effector cells and memory T cells. TCR, its low avidity necessitates the involvement of coreceptors

T cell maturation



Migration of Stem cells to the Thymus early in development and maturation in to thymocytes (T cells)

Migration of Thymocytes to secondary lymphoid organs after maturation for interaction with potential antigens


(a)
Follicles
Cortex
(B cells)
Paracortex
(T cells)
Medulla

Thymic Selection of the T-Cell Repertoire

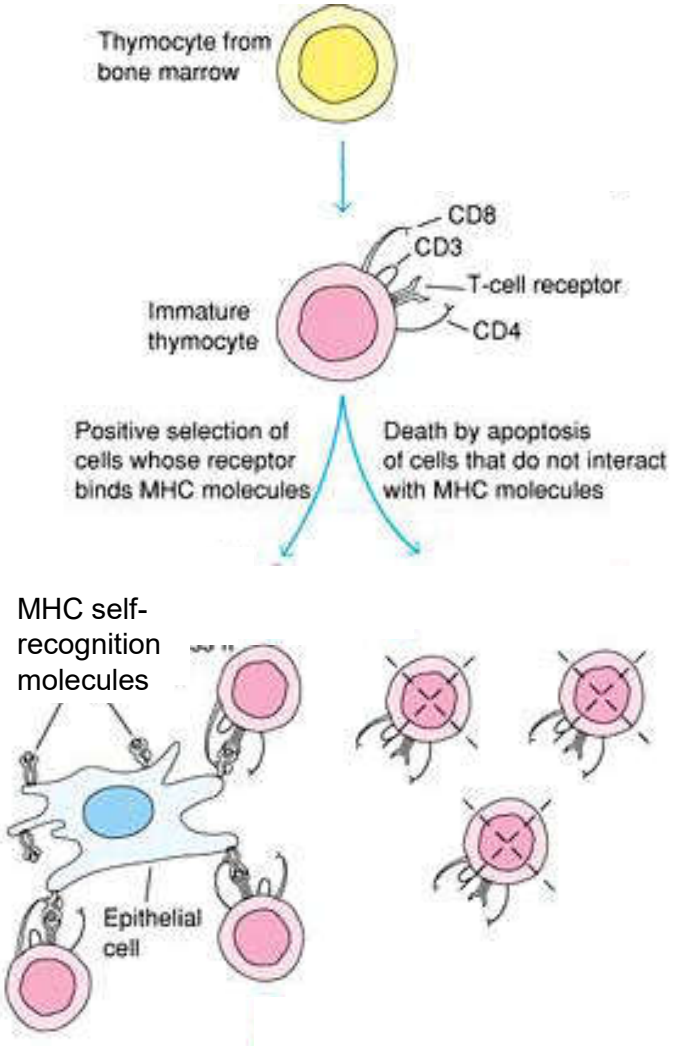
- Random gene rearrangement within TCR germ-line DNA combined with junctional diversity can generate an enormous TCR repertoire,
- with an estimated potential diversity exceeding
  - 10^{15} for the $\alpha\beta$TCR receptor and
  - 10^{18} for the $\gamma\delta$ TCR receptor.
- they should be capable of recognizing
    - Soluble antigen (either foreign or self), self-MHC molecules,or antigen plus a nonself-MHC molecule.
- **the most distinctive property of mature T cells** is that they recognize only foreign antigen combined with self-MHC molecules.

Thymic Selection of the T-Cell Repertoire continued

- Thymocytes undergo two selection processes in the thymus:
  - Positive selection for thymocytes bearing receptors capable of binding self-MHC molecules, which results in **MHC restriction.**
    - Cells that fail positive selection are eliminated within the thymus by apoptosis (≥98% before leave thymus).
  - Negative selection that eliminates thymocytes bearing high-affinity receptors for self-MHC molecules alone or self-antigen presented by self-MHC, which results in **self-tolerance.**

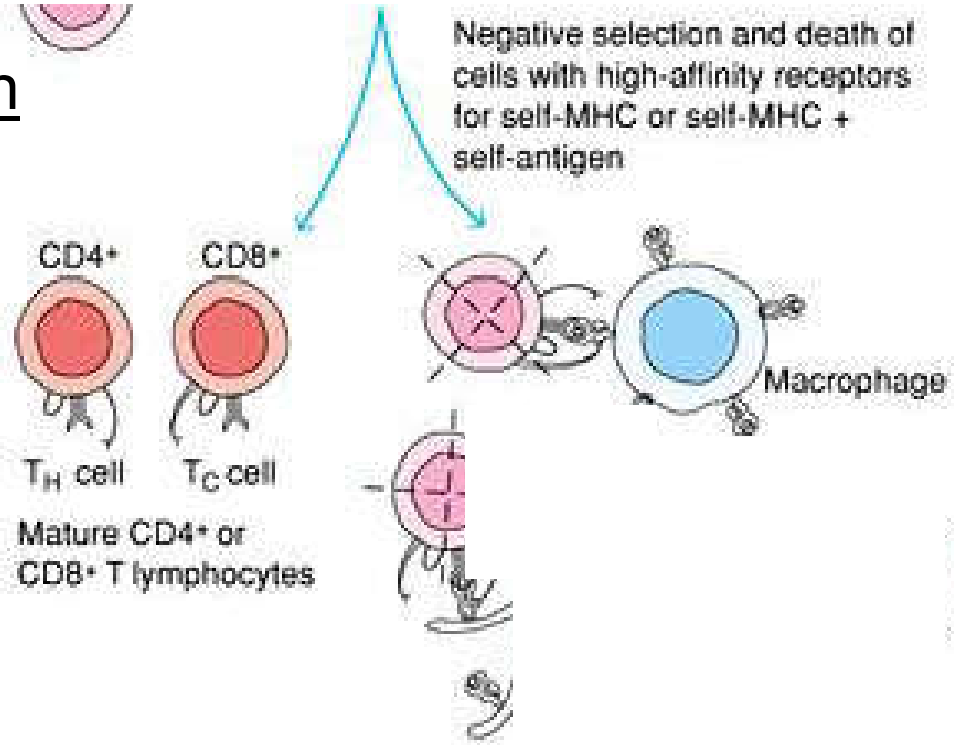
Steps in T cell development

Step 1. Positive selection occurs in the thymic cortex



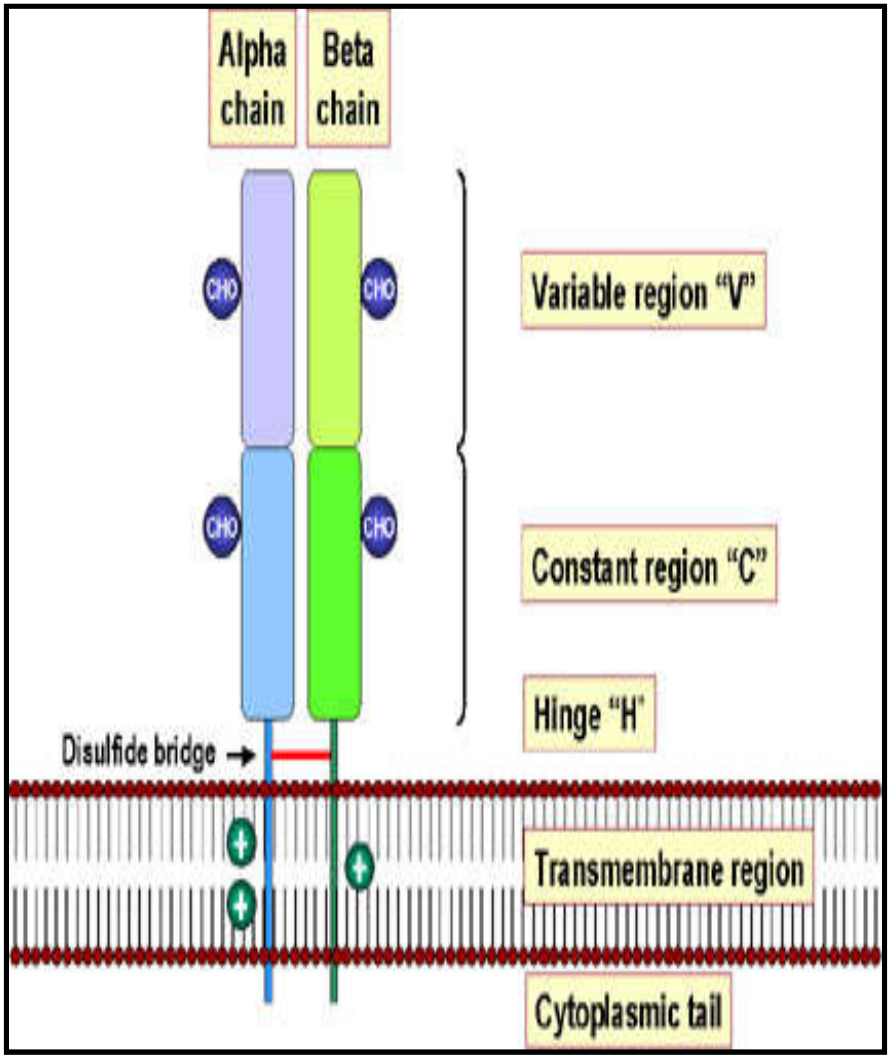
Steps in T cell development (cont'd)

Step 2. Negative selection occurs in the thymic medulla.



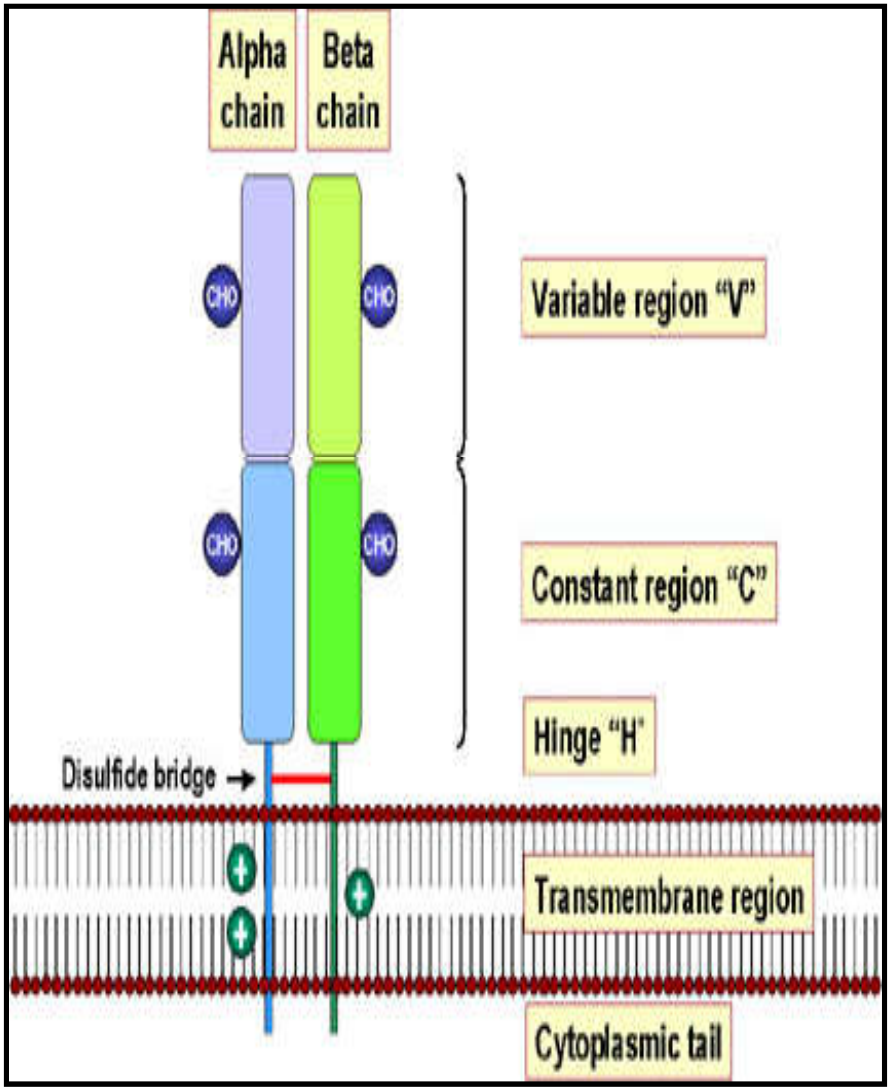
Structure of the T cell Receptor

- Heterodimer with one α and one β chain of roughly equal length
- A short cytoplasmic tail not capable of transducing an activation signal
- A transmembrane region with hydrophobic amino acids



Structure of the T cell Receptor

- Both α and β chains have a variable (V) and constant (C) region
- V regions of the α and β chains contain hypervariable regions that determine the specificity for antigen
- Each T cell bears TCRs of only one specificity (allelic exclusion)



γδ TCR

- Small population of T cells express a TCR that contain γ and δ chains instead of α and β chains
- The Gamma/Delta T cells predominate in the mucosal epithelia and have a repertoire biased toward certain bacterial and viral antigens
- Genes for the δ chains have V, D and J gene segments; γ chains have V and J gene segments
- Repertoire is limited

γδ TCR

- Gamma/Delta T cells can recognize antigen in an MHC-independent manner
- Gamma/Delta T cells play a role in responses to certain viral and bacterial pathogens

T-Cell Receptors

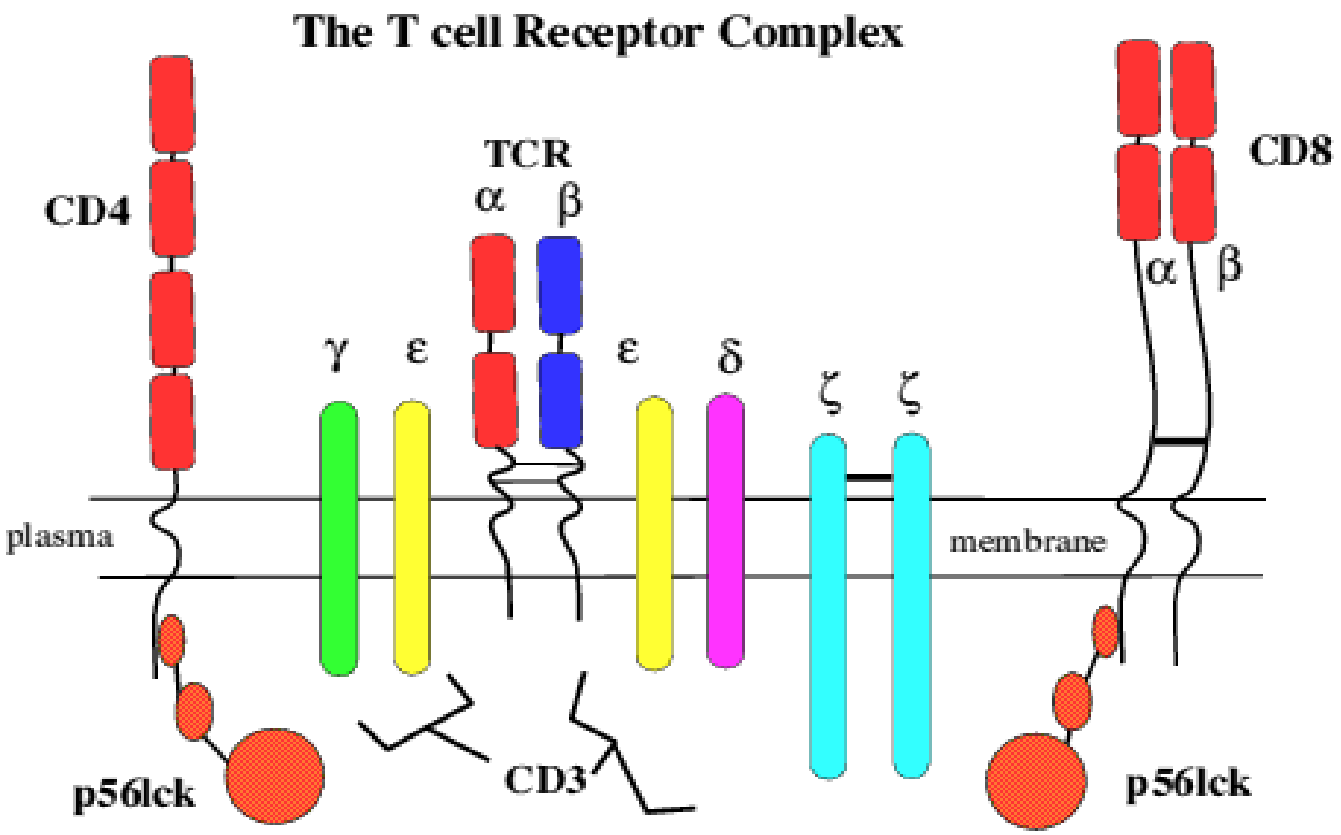
- **The T-cell receptor (TCR) associates with CD3, forming the TCR-CD3 membrane complex.**
- **The accessory molecule participates in signal transduction *after* interaction of T cell with antigen;**
  - it does not influence interaction with antigen.
- CD3 is closely to the $\alpha\beta$ heterodimer and is required for membrane expression of $\alpha\beta$ and $\gamma\delta$ T-cell receptors

T-Cell Receptors

- Each heterodimer forms a complex with CD3 on the T-cell membrane. The T-cell receptor complex can be envisioned as four dimers:
  - the $\alpha\beta$ or $\gamma\delta$ TCR heterodimer determines the ligand-binding specificity,
  - the CD3 dimers ($\gamma\varepsilon$, $\delta\varepsilon$, and $\zeta\zeta$, or $\zeta\eta$) are required for membrane expression of T-cell receptor and for signal transduction

- Class I MHC–specific TCR together with the CD8 co-receptor generates a different signal
- Class II MHC–specific TCR together with the CD4 co-receptor also produces a different signal.

The T Cell Receptor Complex



T-Cell Accessory Membrane Molecules

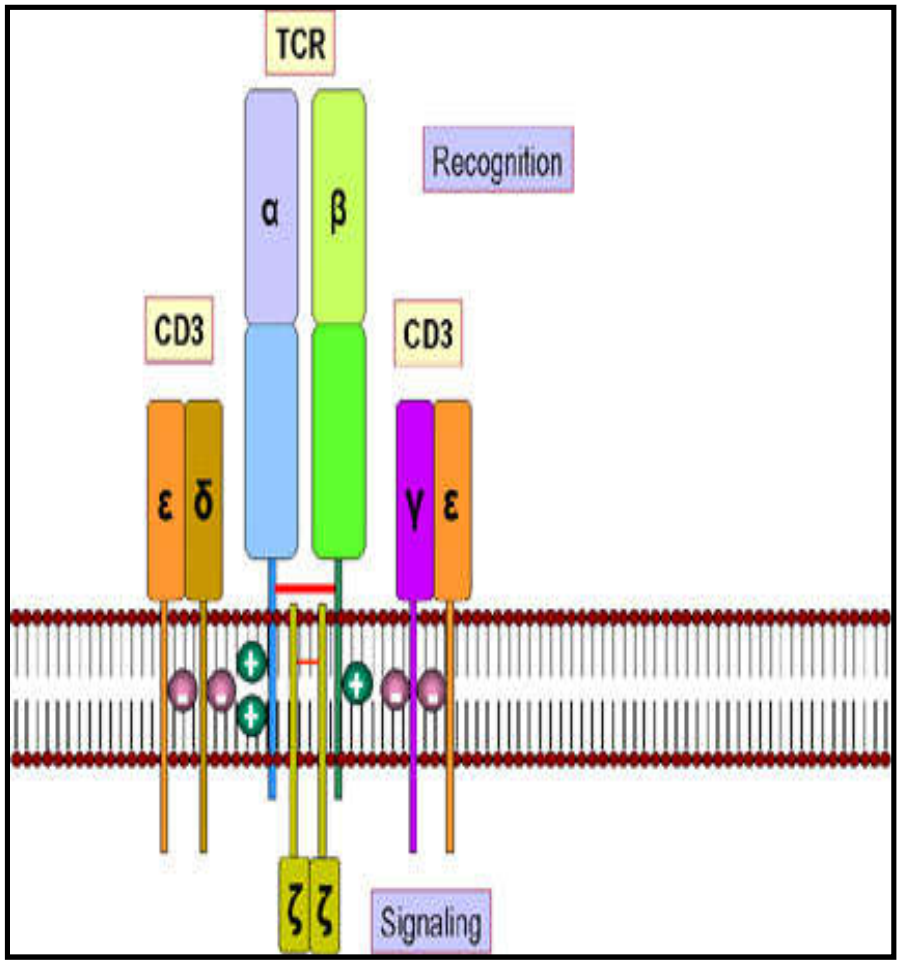
- Recognition of antigen-MHC complexes is mediated solely by the TCR-CD3 complex, **but** other membrane molecules play important accessory roles in antigen recognition and T-cell activation .
- Some of these molecules
  - strengthen the interaction between T cells and antigen-presenting cells or target cells,
  - act in signal transduction, and
  - do both.

T-Cell Accessory Membrane Molecules continued

- CD4 and CD8 are classified as *coreceptors* based on their abilities to recognize the peptide-MHC complex and their roles in signal transduction.
- The extracellular domains of CD4 and CD8 bind to the conserved regions of MHC molecules on antigen-presenting cells (APCs) or target cells.

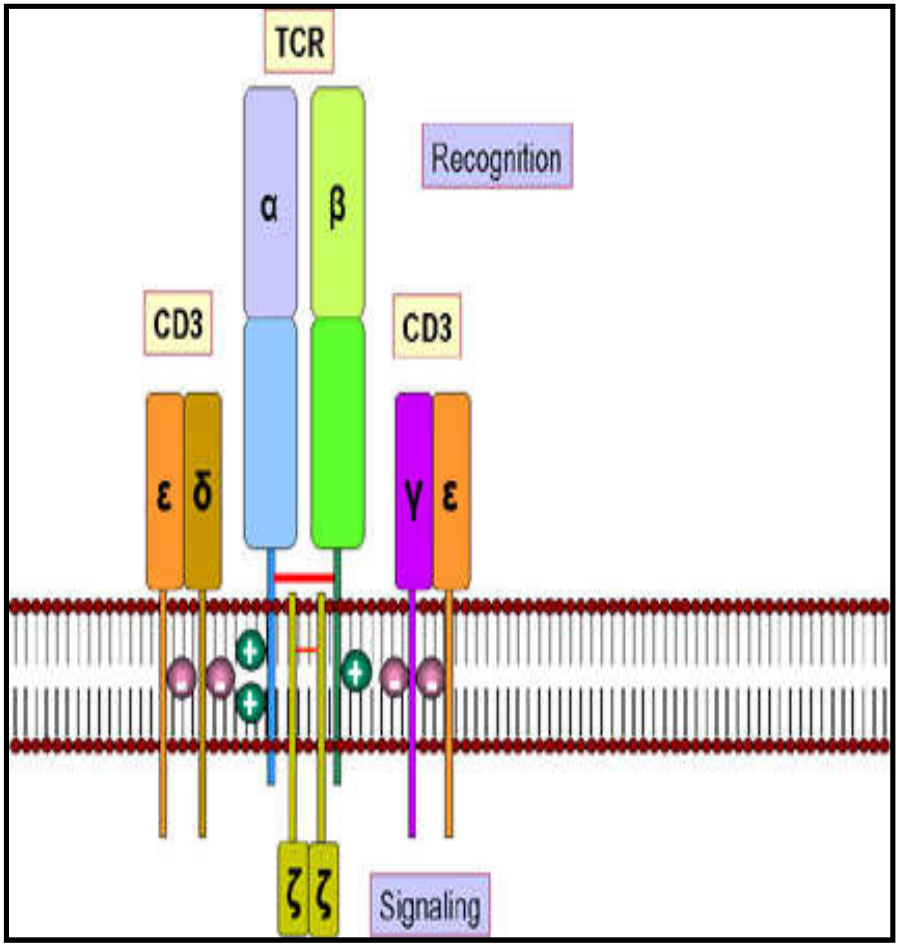
TCR and CD3 Complex

- TCR is closely associated with a group of 5 proteins collectively called the CD3 complex
  - γ chain
  - δ chain
  - 2 ε chains
  - 2 ξ chains
- CD3 proteins are invariant



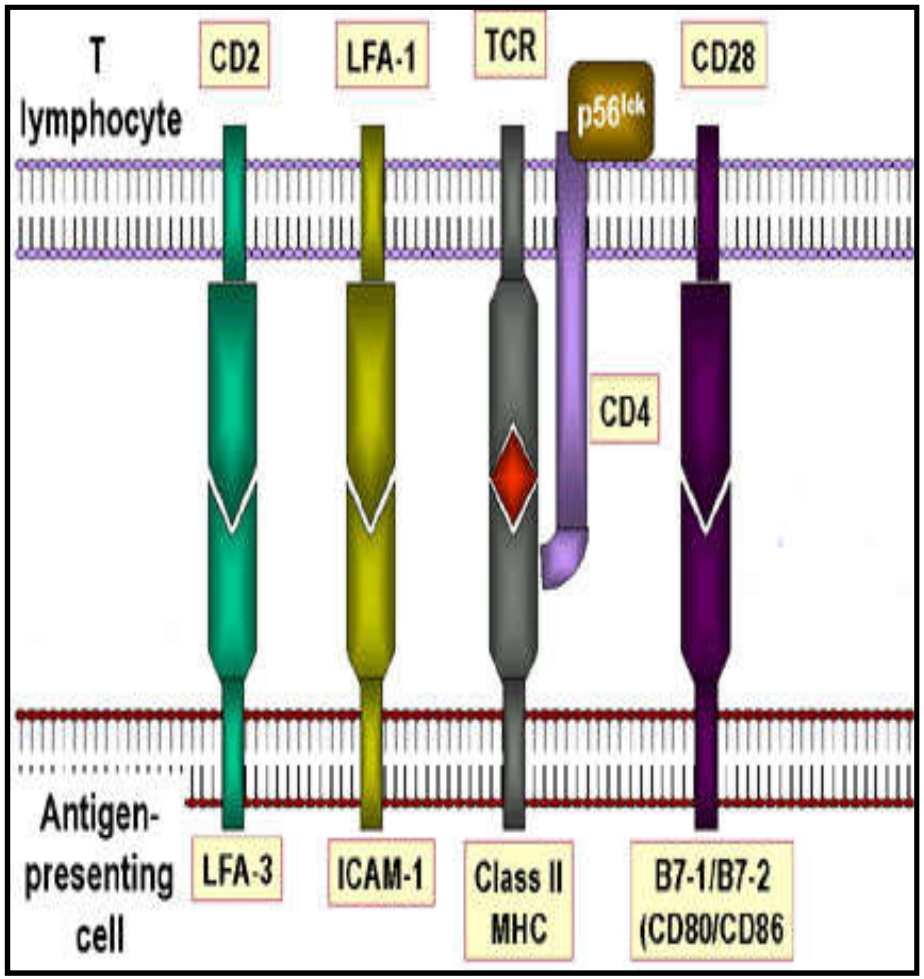
Role of CD3 Complex

- CD3 complex necessary for cell surface expression of TCR during T cell development
- CD3 complex transduces signals to the interior of the cells following interaction of Ag with the TCR



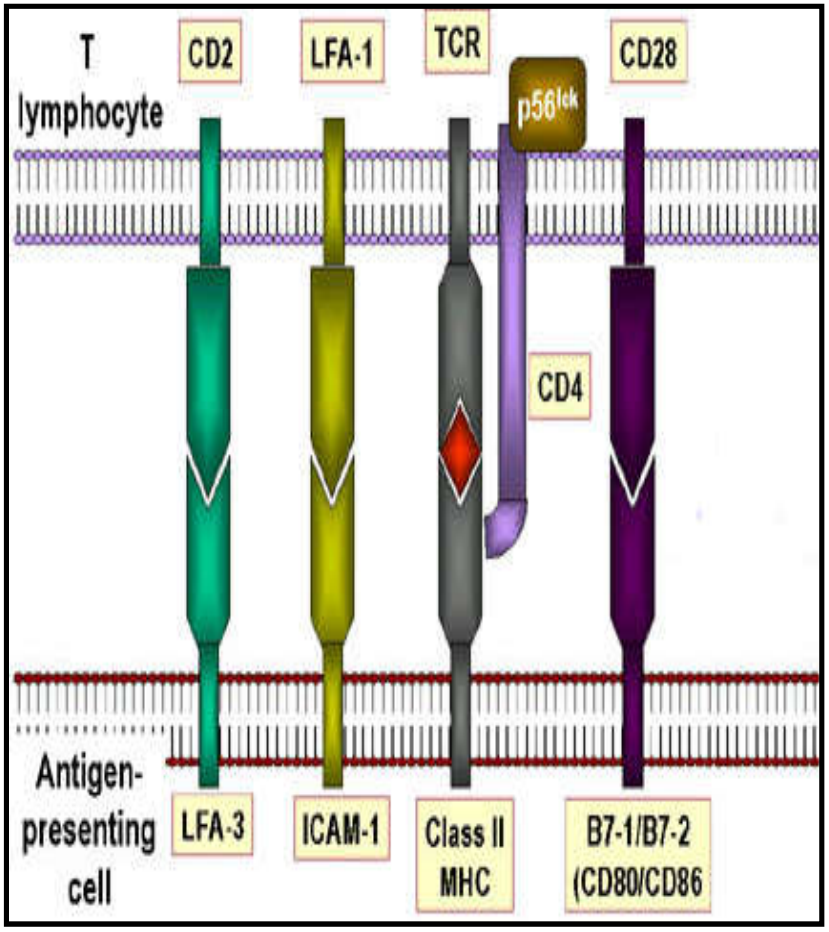
The "Immunological Synapse"

- The interaction between the TCR and MHC molecules are not strong
- Accessory molecules stabilize the interaction
  - CD4/Class II MHC or CD8/Class I MHC
  - CD2/LFA-3
  - LFA-1/ICAM-1



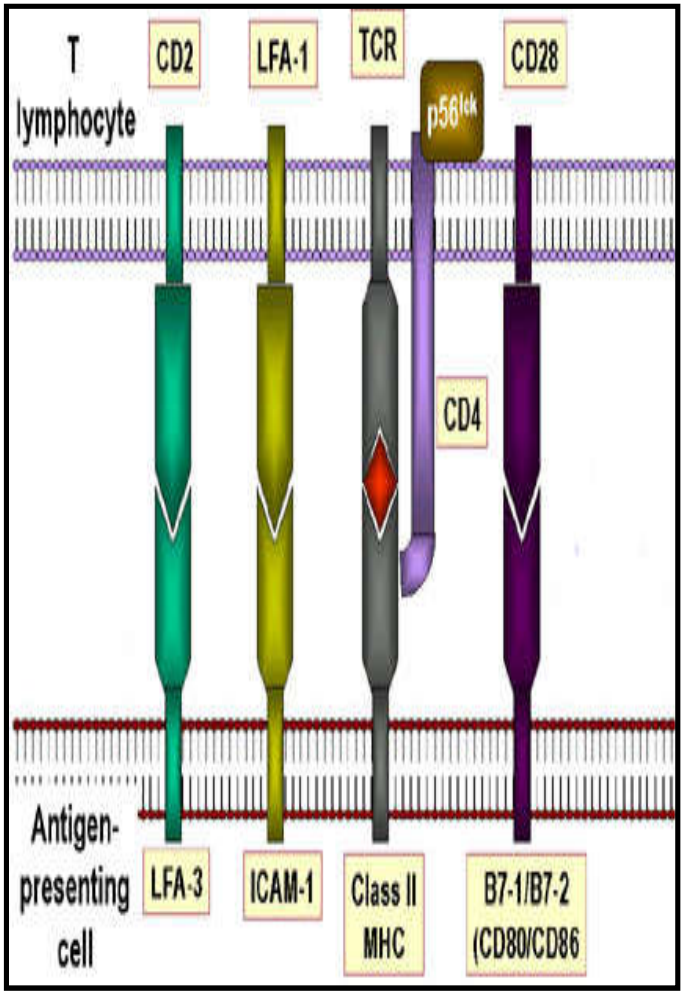
The “Immunological Synapse”

- Specificity for antigen resides solely in the TCR
- The accessory molecules are invariant
- Expression is increased in response to cytokines



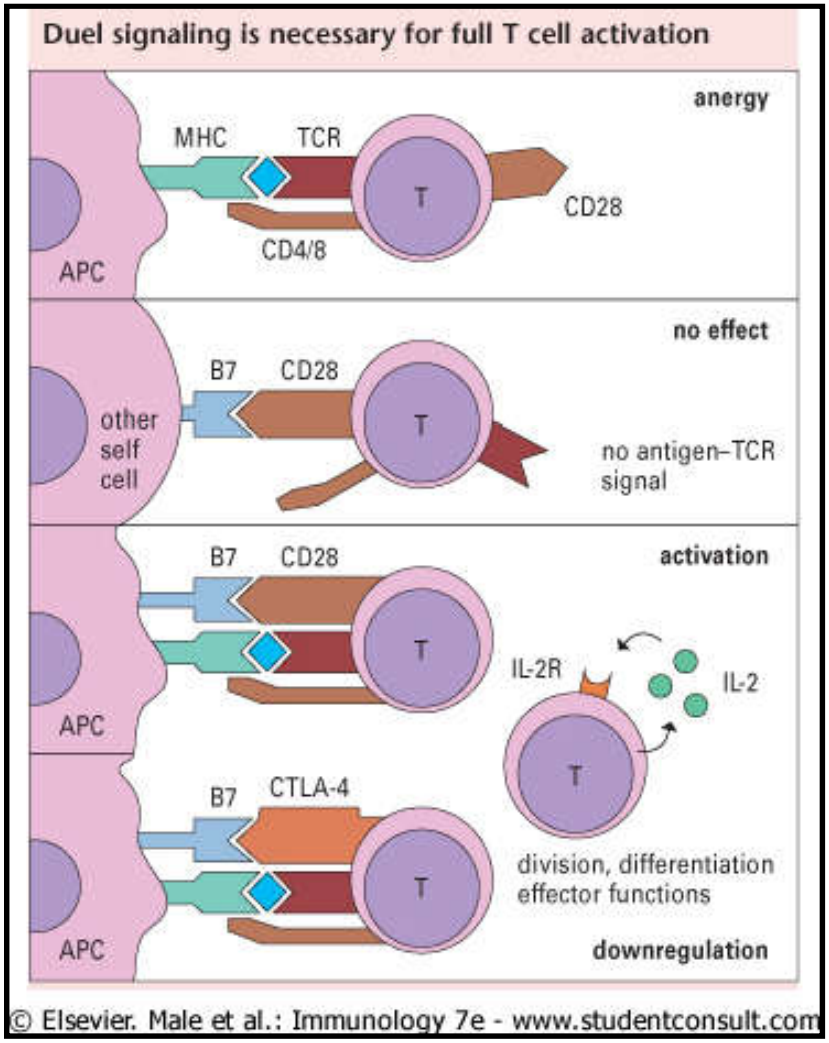
The "Immunological Synapse"

- Engagement of TCR and Ag/MHC is one signal needed for activation of T cells
- Second signal comes from costimulatory molecules
  - CD28 on T cells interacting with B7-1 (CD80) or B7-2 (CD86)
  - Others
- Costimulatory molecules are invariant
- "Immunological synapse"



Costimulation is Necessary for T Cell Activation

- Engagement of TCR and Ag/MHC in the absence of costimulation can lead to anergy
- Engagement of costimulatory molecules in the absenece of TCR engagement results in no response
- Activation only occurs when both TCR and costimulatory molecules are engaged with their respective ligands
- Downregulation occurs if CTLA-4 interacts with B7
  - CTLA-4 send inhibitory signal



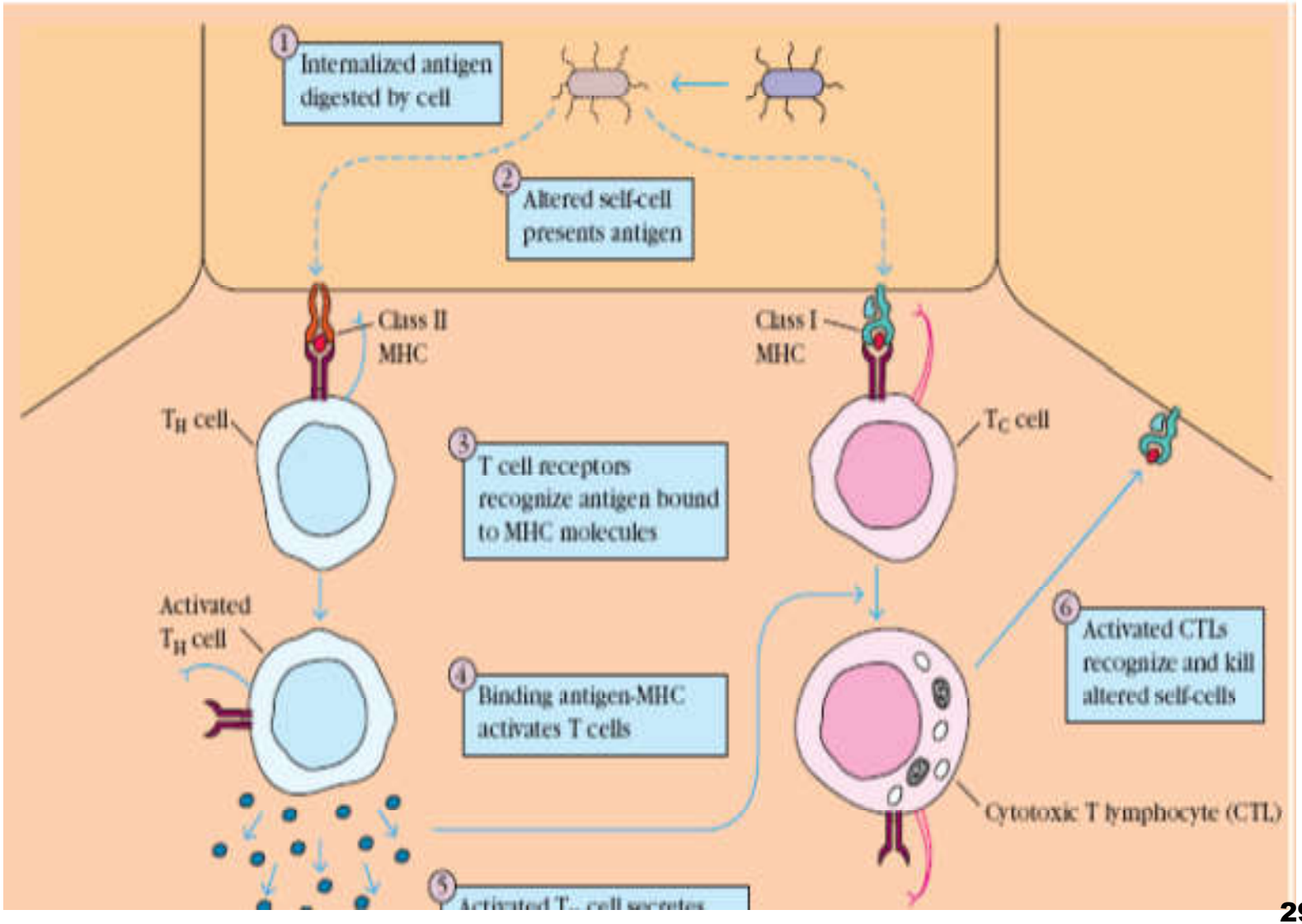
TH-Cell Activation

- Common to starting of both humoral and cell mediated immune responses is the activation and clonal expansion of TH cells.
- TH cell activation is initiated by interaction of the TCR-CD3 complex with a processed antigenic peptide bound to a class II MHC molecule on the surface of an antigen-presenting cell.
- Interaction of a TH cell with antigen initiates a cascade of biochemical events that induces the resting TH cell to enter the cell cycle, proliferating and differentiating into memory cells or effector cells.
- Resulting in release of specific cytokines and forming adhesion molecules.

Key Steps in T cell Activation

- APC must process and present peptides to T cells
- T cells must receive a costimulatory signal
  - Usually from CD28/B7
- Accessory adhesion molecules help to stabilize binding of T cell and APC
  - CD4/MHC-class II or CD8/MHC class I
  - LFA-1/ICAM-1
  - CD2/LFA-3
- Signal from cell surface is transmitted to nucleus
  - Second messengers
- Cytokines produced to help drive cell division
  - IL-2 and others

Activation of T cells



T-Cell Differentiation

- Niave T cells that have not encounted an antigen-MHC complex repeatedly migrate through the circulation and lymphatics.
- When these cells encounter an antigen-MHC complex they undergo repeated cell division producing effector and memory cells.

Cell Death and T-Cell Populations

- is an important feature of lymphocyte homeostasis, returning T- and B-cell populations to their appropriate levels after bursts of antigen-induced proliferation.
- Apoptosis also plays a crucial role in the deletion of potentially autoreactive thymocytes during negative selection and in the removal of developing T cells unable to recognize self (failure to undergo positive selection).

Cell Death and T-Cell Populations

- Outside of the thymus, most of the TCR-mediated apoptosis of mature T cells is mediated by the Fas pathway.
  - Repeated or persistent stimulation of peripheral T cells results in the coexpression of both Fas and Fas ligand, followed by the apoptotic death of the cell.
  - The Fas/FasL mediated death of T cells as a consequence of activation is called *activation-induced cell death* (AICD) .
- The T-cell repertoire is shaped by apoptosis in the thymus and periphery.

CHAPTER 10

Immune response to Infectious Diseases

Objectives

Upon completion of this chapter student will be able to:

- Describe how the body respond to viral, bacterial, fungal, and parasitic infections.
- Describe how viruses, bacteria, fungus and parasites escape host immune response.
- Compare and contrast antigens that stimulate B cells versus T cells

General

- Infection results when organisms grow and exist in or on host tissues
- Disease results from damage to host tissues
- Presence of microbes does not always cause
- Microbes cause disease through two mechanisms
  - Growth of the organism itself
  - Secretion of toxins which cause disease
  - Most organisms are a combination of both

- Defense against microbes is mediated by both innate and adaptive immunity
- The innate response plays a role in determining the nature of specific response
- Immune system responds in specialized ways to specific types of microbes
- Survival & pathogenicity of microbes influenced by their ability to evade or resist protective defenses
- Tissue injury & disease result when microbes overcome host defenses

INFECTION AND IMMUNITY

- **PATHOGENS**
  - ***Extracellular and Intracellular bacteria***
  - ***Viruses***
  - ***Parasites***
  - ***Funguses and yeasts***
- **HOST IMMUNE RESPONSES**
  - ***Innate immunity***
  - ***Adaptive immunity***
- **PATHOGEN/HOST INTERACTIONS**
  - **Mechanisms of Survival and Evasion**

- Distinct effector responses elicited are dependent on
  - *site of entry,*
  - *route of spread,*
  - *tissue specificity, and*
  - *transmission*

- Pathogenicity and survival of the microbes depend on ability to evade or resist host immune responses

- Disease or tissue injury can be the consequence of the
  - Pathogenicity of microbe as well as
  - The host immune response itself

10.1 Immune response to Viral Infections

- A number of specific immune effector mechanisms, together with nonspecific defense mechanisms, are called into play to eliminate an infecting virus.

1. Innate immune response to viral infection

- **Interferon**
  - A group of proteins produced in response to virus infection which stimulates cells to make proteins that block viral transcription, and thus protects them from infection.
  - dsRNA produced during viral replication induce the expression of interferons by the infected cells.
  - Monocytes , macrophages & fibroblasts also synthesize interferons .

Anti-viral activity of interferons (IFNs)

- Virus infected cells produce INF-α;
- INF-α inhibit intracellular replication of viruses
- IFN-α activate NK-cells to kill virus infected cells
- IFNs have no direct effect on extracellular virus
- IFNs act early in viral diseases before antibody
- INFs activity is not specific

- **NK cells**
  - Destroy some virus-infected cells, and are not MHC restricted.
  - Natural killer cells lyse virally infected cells

2. Specific immune response

- **Humoral immunity**
  - Anti-viral antibodies :
    1. prevent spread during acute infection.
    2. protect against reinfection .
    - Virus neutralization:- In viraemic infections, antibodies neutralize virus, preventing its attachment to receptor sites on susceptible.

      cells e.g. Poliovirus, mumps, measles, rubella
    - In superficial non-viraemic infections (infleunza) Secretory IgA neutralizes virus infectivity at the mucous

      surfaces.
  - Antibodies destroy free virus particles directly by:

    i- Aggregation of virus and opsonization

    ii- Complement mediated lysis

    Both mechanisms also act on virus infected cells

- **Cell mediated immunity(CMI)**
  - Cell – mediated immunity is important for control & clearance of viral infections .
  - CMI acts on virus infected cells through:
    - Cytotoxic T-cells (CTLs)
    - NK cells
    - Activated macrophages
  - CTLs kill virus infected cells directly after recognition of viral antigens on cell surface in association with MHC I
  - TH-cells stimulated by viral antigens release cytokines. Cytokines attract and activate macrophages to kill virus infected cells

- Nk-cells destroy virus infected cells early in infection before appearance of antibodies
- Antibody-dependent cell mediated cytotoxicity (ADCC):
  - Antibody binds to virus infected cells such cells are lysed by NK cells, macrophages and polymorphs

Immune evasion by viruses

- Viruses can evade host defenses
  1. Overcome anti - viral effect of INFs blocking the action of protein kinase example Hepatitis C virus .
  2. Reduce surface expression of MHC-I example Adenoviruses & CMV.
  3. Reduce MHC -II levels example Measles ,CMV & HIV
  4. A large no. of viruses cause generalized immnoduppression.example mumps , measles , EBV., CMV.,& HIV.
  5. Antigenic variation example influenza virus

- Antigenic variation in influenza virus

The structure of the virus contain :

  - Hemagglutinins (HA).- 13
  - Neuraminidase (NA).- 9

- **Antigenic drift** – small, ceaseless changes in the genetic structure. New strains continually replace old strains. Gradual minor change in HA & NA.

- **Antigenic shift** – major change, usually occurs when species hosting virus trade viral genes. Novel strain appears without natural immunity in host population. Sudden major change in HA & NA . (new subtype emerge)

Summery on Mechanisms of humoral and cell-mediated immune responses to viruses

Response type	Effector molecule or cell	Activity
Humoral	Antibody (especially, secretory IgA)	Blocks binding of virus to host cells, thus preventing infection or reinfection
	IgG, IgM, and IgA antibody	Blocks fusion of viral envelope with host-cells plasma membrane
	IgG and IgM antibody	Enhances phagocytosis of viral particles (opsonization)
	IgM antibody	Agglutinates viral particles
	Complement activated by IgG or IgM antibody	Mediates opsonization by C3b and lysis of enveloped viral particles by membrane-attack complex
Cell-mediated	IFN-γ secreted by T_H or T_C cells	Has direct antiviral activity
	Cytotoxic T lymphocytes (CTLs)	Kill virus-infected self-cells
	NK cells and macrophages	Kill virus-infected cells by antibody-dependent cell-mediated cytotoxicity (ADCC)

10.2 Immune response to Bacterial Infections

Immunity to extracellular bacteria

1- The innate immunity:

a- Complement activation

b- Phagocytosis

c- The inflammatory response

2- The acquired immune responses:

a- The humoral mechanisms (antibodies) “main role”

b- Cell mediated immune response “less role”

Immunity to extracellular bacteria

i- Antibodies induce immunity through:

a- Neutralization of bacterial toxins

b- Antibodies attach to the surface of bacteria and;

- Act as opsonins, enhance phagocytosis (Opsonization)
- Prevent adherence of bacteria to their target cells e.g. IgA on mucosal surfaces
- Activation the complement leading to bacterial lysis
- Agglutinate bacteria, preventing their spread and facilitating phagocytosis

ii- Cell mediated immune mechanisms:

- Microbes are internalized by APCs and presented to TH
- TH cells are activated and release cytokines which;
  - Activate phagocytosis their microbicidal functions
  - Stimulate antibody production
  - Induce local inflammation

Immunity to intracellular bacteria

- Innate immunity is not very effective against intracellular bacterial pathogens.
- Intracellular bacteria can activate NK cells, which, in turn, provide an early defense against these bacteria.
- Intracellular bacterial infections tend to induce a cell-mediated immune response, specifically, delayed type hypersensitivity.
- In this response, cytokines secreted by CD4+ T cells are important—notably IFNγ, which activates macrophages to kill ingested pathogens more effectively.

- Killing of phagocytosed bacteria as result of macrophage activation by T cell derived cytokines and by direct lysis of infected cells by CD8+ cytotoxic T lymphocytes.
- A pathogenic outcome of chronic T cell and macrophage stimulation to intracellular bacteria can be the formation of granulomas.

MECHANISMS OF ESCAPE OF BACTERIA FROM IMMUNE RESPONSE

- Existence of polysaccharide capsules
- Excretion of toxins (eg.streptolysins toxic for neutrophils)
- Pathogen variability
- Persistence in cells
- Induction of cell apoptosis (*Shigella flexneri)*
- Blockage of cell lysosome action (*Mycobacterium*)
- Inactivation of complement components

MECHANISMS OF ESCAPE OF PATHOGENS FROM IMMUNE RESPONSE Cont…

- Induction of synthesis of actin fibres (*Listeria*, *Shigella*)
- Enzymatic inhibition of active oxygen radicals (*S.aureus*)
- „Hiding" from immune cells in other, such as epithelia
- Ability to interfere with the immune reactions

Host immune responses to bacterial infection and bacterial evasion mechanisms

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

10.3 Immune response to fungal infections

- Fungal infections are normally only a superficial nuisance (e.g. ringworm: top), but a few fungi can cause serious systemic disease, usually entering via the lung in the form of spores
- The outcome depends on the degree and type of immune response, and may range from an unnoticed respiratory episode to rapid fatal dissemination or a violent hypersensitivity reaction

Immune response to fungal infections cont…

- Predominant defense mechanisms differ depending on the specific causative agent
- Immune response to fungi consist mainly of :

1) Innate immunity is mediated by:
   - Neutrophils and macrophages
   - Fungi are readily eliminated by phagocytes
   - Activated neutrophils are critical in the defense against disseminated candidiasis and aspergillosis

2) Acquired immunity (cell mediated immunity)
   - CMI acts in a manner similar to its action against intracellular bacteria
   - Cell-mediated immunity predominates in protection against cryptococcosis, histoplasmosis and mucosal *C. albicans* infection

Immune response to fungal infections cont…

- In general, the survival mechanisms of successful fungi are similar to those of bacteria: antiphagocytic capsules (e.g. Cryptococcus), resistance to digestion within macrophages (e.g. Histoplasma, etc.), and destruction of polymorphs (e.g. Coccidioides).
- Some yeasts activate complement via the alternative pathway, but it is not known if this has any effect on survival.

10.4 Immune response to Protozoan Diseases

- Both humoral and cell-mediated immune responses have been implicated in immunity to protozoan infections.
- Ingeneral, humoral antibody is effective against blood-borne stages of the protozoan life-cycle, but once protozoans have infected host cells, cell-mediated immunity is necessary.

Immune response to Protozoan Diseases cont…

- Protozoa
  - Similar process to that of bacteria
    - Macrophages must be activated by T cells to enhance killing mechanisms
  - Intracellular protozoa like malaria also CMI
  - Larger protozoa utilize antibody mediated responses

Immune response to Protozoan Diseases cont…

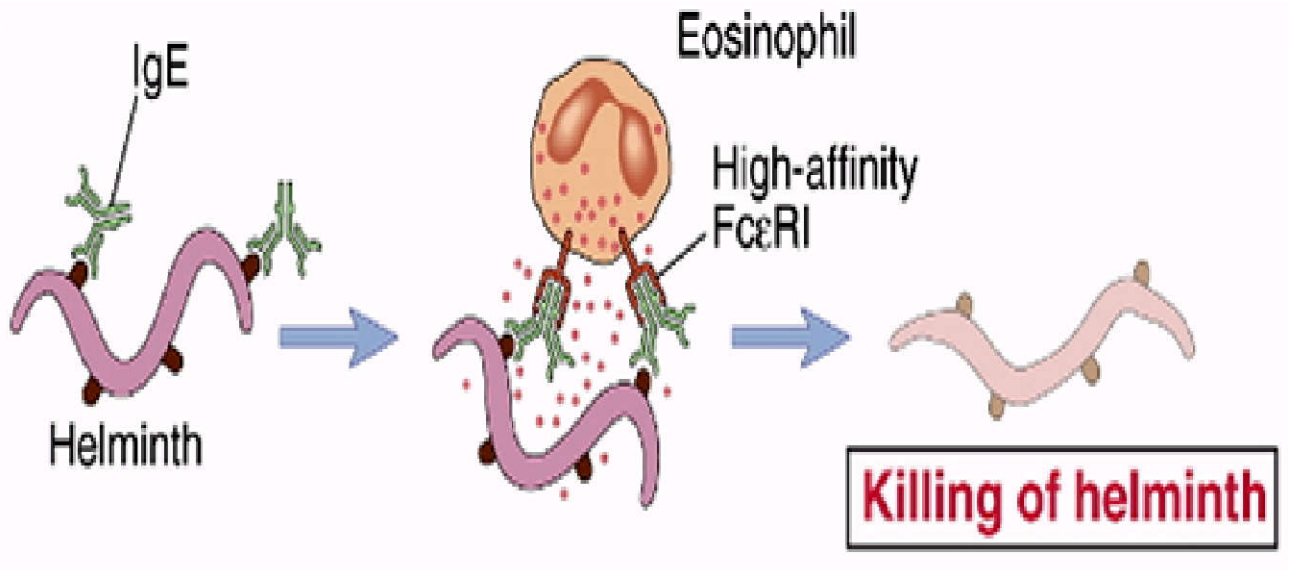
- Protozoans escape the immune response through several mechanisms.
- *Trypanosoma brucei—are covered* by a glycoprotein coat that is constantly changed by a geneticswitch mechanism.
- Others (including *Plasmodium, slough off their* glycoprotein coat after antibody has bound to it.

10.5 Immune response to Diseases Caused by Parasitic Worms (Helminths)

- Helminths are large parasites that normally do not multiply within cells.
- Because few of these organisms are carried by an affected individual, immune-system exposure to helminths is limited; consequently, only a low level of immunity is induced.
- Although helminths generally are attacked by antibody-mediated defenses, these may be ineffective.
- A cell-mediated response by CD4+ T cells plays a critical role in the response to *Schistosoma.*

Immune response to Diseases Caused by Parasitic Worms cont…

- CMI in response to helminthic parasites is mediated by TH2 cells that stimulate the production of IgE and activation of eosinophils.



Immune response to Diseases Caused by Parasitic Worms cont…

Helminth evasion of immune responses

- **Antigenic disguise** – parasites synthetise host-like antigens to mask their own foreigness. Alternatively they absorb host molecules to their surfaces (Schistosomes)
- **Concomitant immunity or premunition** – worms live in in host for years with no evidence of immune response. The latter however is formed to prevent reinfection of the same worm

CHAPTER 11

Undesirable and altered immunity

Objectives

Upon completion of this chapter the student will be able to:

- Define hypersensitivity, Immunologic tolerance, Autoimmunity and Immunodeficiencie.
- Describe the classification(types) of hypersensitivity reaction, Autoimmunity and Immunodeficiencies.
- Describe diseases and the mechanism of damage associated with hypersensitivity reactions, Autoimmunity and Immunodeficiencies.
- Explaine Laboratory diagnosis of Hypersensitivity reactions Autoimmunity and Immunodeficiencies

11.1 Hypersensitive Reactions

- An Immuneresponse eliminates antigen without extensively damaging the host's tissue.
- Under certain circumstances, however, this response can have deleterious effects, resulting in significant tissue damage or even death.
- This inappropriate immune response is termed **hypersensitivity or allergy.**

Hypersensitive Reactions cont…

- **Although the word *hypersensitivity*** implies an increased response, the response is not always heightened but may, instead, be an inappropriate immune response to an antigen.
- Hypersensitive reactions may develop in the course of either humoral or cell-mediated responses.
- Hypersensitivity reactions require pre-sensitized (immune) state of the host .

11.1.1 Classification Hypersensitivity

- Based on mechanics involved and time taken for the reactions hypersensitivity reactions are Four Types :
  - Type I (Anaphylactic) Reactions
  - Type II (Cytotoxic) Reactions
  - Type III (Immune Complex) Reactions
  - Type IV (Cell-Mediated) Reactions
- The first three occur within the humoral branch and are mediated by antibody or antigen-antibody complexes.
- A fourth type depends on reactions within the cell-mediated branch, and is termed delayed-type hypersensitivity, or DTH(type IV).

11.1.2 Type I (Anaphylactic) Reactions

- It is also known as immediate hypersensitivity.
- The reaction takes 15-30 minutes from the time of exposure to the antigen.
- Type I hypersensitivity is mediated by IgE. The primary cellular component in this hypersensitivity is mast cell or basophil.
- The mechanism of reaction involves preferential production of IgE.

General mechanism underlying a type I hypersensitive reaction

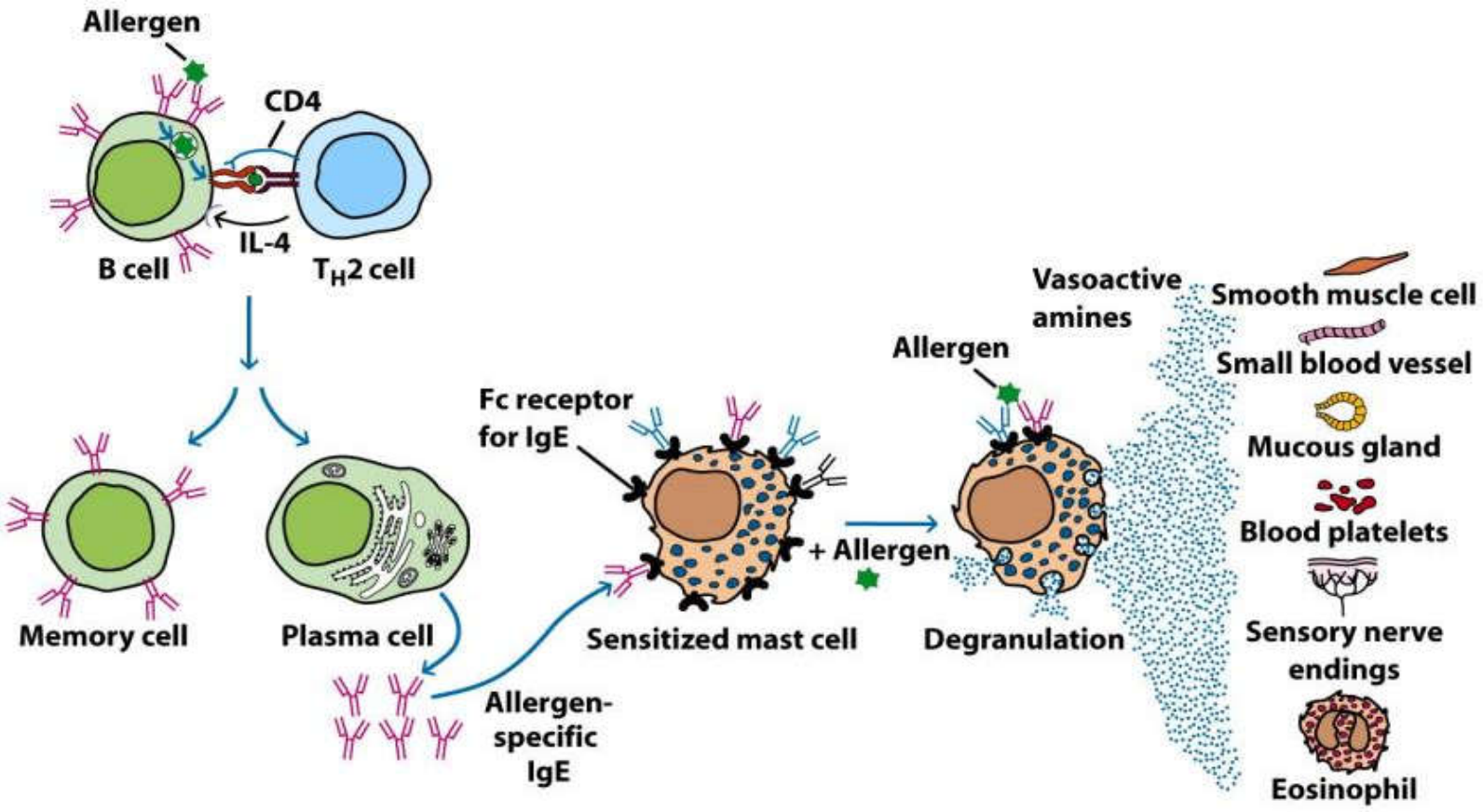


Figure 15-2
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Principal mediators involved in type I hypersensitivity

Mediator	Effects
	PRIMARY
Histamine, heparin	Increased vascular permeability; smooth-muscle contraction
Serotonin	Increased vascular permeability; smooth-muscle contraction
Eosinophil chemotactic factor (ECF-A)	Eosinophil chemotaxis
Neutrophil chemotactic factor (NCF-A)	Neutrophil chemotaxis
Proteases	Bronchial mucus secretion; degradation of blood-vessel basement membrane; generation of complement split products
	SECONDARY
Platelet-activating factor	Platelet aggregation and degranulation; contraction of pulmonary smooth mus
Leukotrienes (slow reactive substance of anaphylaxis, SRS-A)	Increased vascular permeability; contraction of pulmonary smooth muscles
Prostaglandins	Vasodilation; contraction of pulmonary smooth muscles; platelet aggregation
Bradykinin	Increased vascular permeability; smooth-muscle contraction
Cytokines	
IL-1 and TNF-α	Systemic anaphylaxis; increased expression of CAMs on venular endothelial cel
IL-2, IL-3, IL-4, IL-5, IL-6, TGF-β, and GM-CSF	Various effects (see Table 12-1)

Type I Reactions Can Be Systemic or Localized

SYSTEMIC ANAPHYLAXIS

- Is a shock-like and often fatal state whose onset occurs within minutes of a type I hypersensitive reaction.
- A wide range of antigens have been shown to trigger this reaction in susceptible humans, including the venom from bee, wasp, hornet, and ant stings; drugs, such as penicillin, insulin, and antitoxins; and seafood and nuts.
- If not treated quickly, these reactions can be fatal.
- Epinephrine is the drug of choice for systemic anaphylactic reactions.

LOCALIZED ANAPHYLAXIS (ATOPY)

- In localized anaphylaxis, the reaction is
  - limited to a specific target tissue or organ,
  - Often involving epithelial surfaces at the site of allergen entry.
- The tendency to manifest localized anaphylactic reactions is inherited and is called *atopy.*
- Atopic allergies include:
  - Allergic rhinitis (hay fever)
  - Asthma
  - Atopic dermatitis (eczema)
  - Food allergies

ALLERGIC RHINITIS

- Results from the reaction of airborne allergens with sensitized mast cells in the conjunctivae and nasal mucosa to induce the release of pharmacologically active mediators from mast cells.
- The symptoms include watery exudation of the conjunctivae, nasal mucosa, and upper respiratory tract, as well as sneezing and coughing.

ASTHMA

- Airborne or blood-borne allergens, such as pollens, dust, fumes, insect products, or viral antigens, trigger an asthmatic attack (allergic asthma).
- Can be induced by exercise or cold, apparently independently of allergen stimulation (intrinsic asthma)
- The reaction develops in the lower respiratory tract.
- The resulting contraction of the bronchial smooth muscles leads to bronchoconstriction.
- Airway edema, mucus secretion, and inflammation contribute to the bronchial constriction and to airway obstruction.

ATOPIC DERMATITIS

- Is an inflammatory disease of skin that is frequently associated with a family history of atopy.
- The disease is observed most frequently in young children, often developing during infancy.
- The allergic individual develops skin eruptions that are erythematous and filled with pus.
- Unlike a delayed-type hypersensitive reaction, which involves TH1 cells, the skin lesions in atopic dermatitis have TH2 cells and an increased number of eosinophils.

FOOD ALLERGIES

- Various foods also can induce localized anaphylaxis in allergic individuals.
- Allergen cross linking of IgE on mast cells along the upper or lower gastrointestinal tract can induce localized smooth-muscle contraction and vasodilation and thus such symptoms as vomiting or diarrhea.
- Mast-cell degranulation along the gut can increase the permeability of mucous membranes, so that the allergen enters the bloodstream and various symptoms can ensue, depending on where the allergen is deposited.

  For example: asthma, atopic urticaria (hives).

Common allergens associated with type I hypersensitivity

Proteins
- Foreign serum
- Vaccines

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

Drugs
- Penicillin
- Sulfonamides
- Local anesthetics
- Salicylates

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

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

Mold spores

Animal hair and dander

Diagnostic tests for Type I Hypersensitivity Reactions

- Skin testing
- Determine Serum level of total IgE antibody

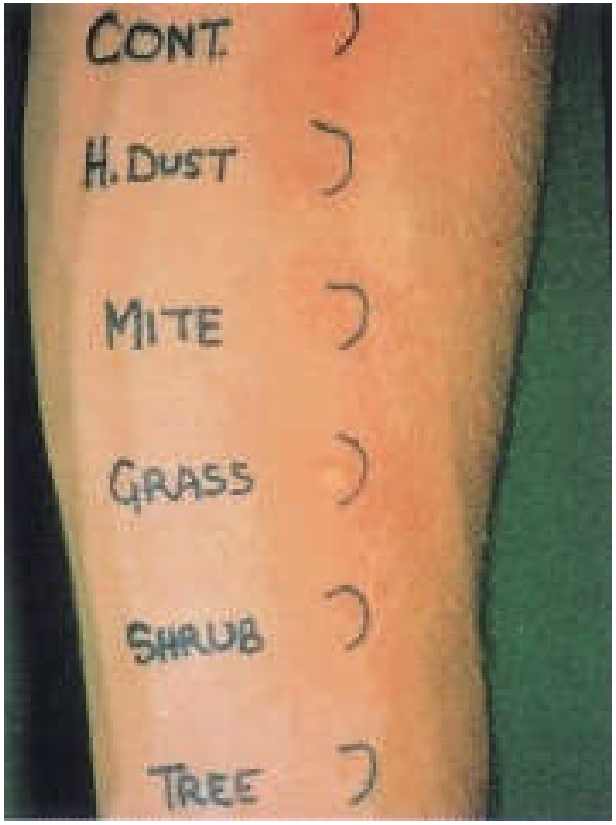
Skin testing

- Small amounts of potential allergens are introduced at specific skin sites either by intradermal injection or by superficial scratching.
- A number of tests can be applied to sites on the forearm or back of an individual at one time.
- If a person is allergic to the allergen, local mast cells degranulate and the release of histamine and other mediators produces a wheal and flare within 30 min. (Fi

- Advantage of skin testing
  - Inexpensive
  - Allows screening of a large number of allergens at ne time
- Disadvantage of skin testing
  - Sometimes sensitizes the allergic individual to new allergens and in some rare cases may induce systemic anaphylactic shock.
  - A few individuals also manifest a late-phase reaction, which comes 4–6 h after testing and sometimes lasts for up to 24 h.

Skin testing by intradermal injection of allergens into the forearm

- In this individual, a weal and flare response developed within a few minutes at the site where grass was injected, indicating that the individual is allergic to grass



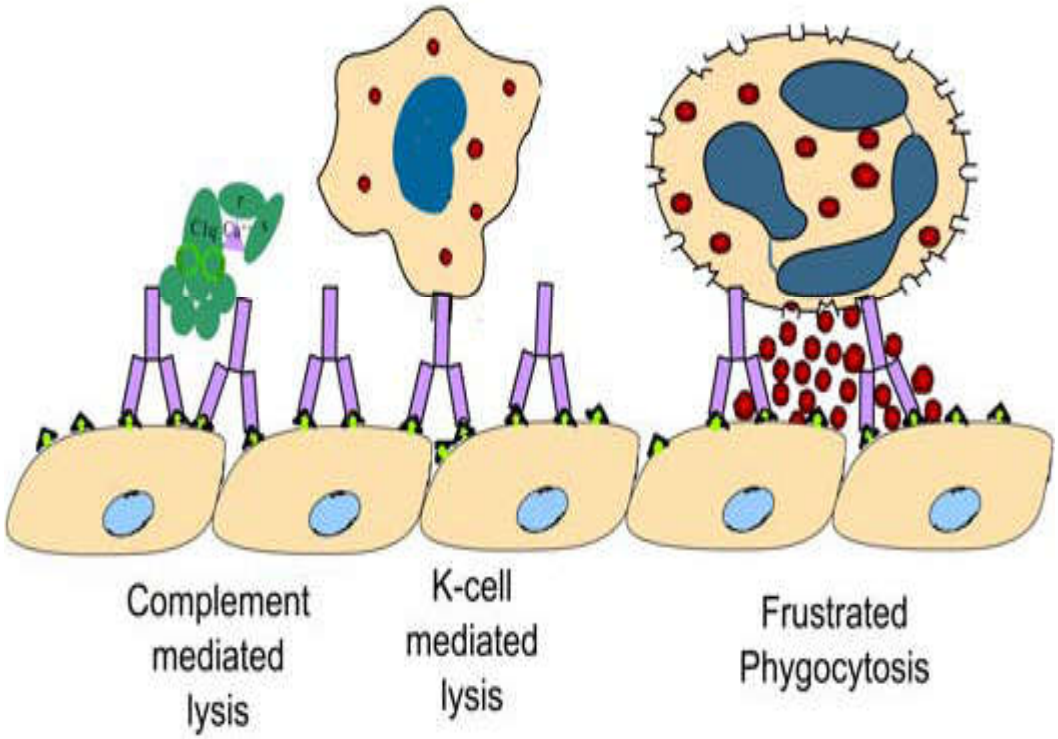
- **Determine the serum level of total IgE antibody by:**
  - Radioimmunosorbent test (RIST)
    - Highly sensitive technique, based on the radioimmunoassay
    - Detect nanomolar levels of total serum IgE.
  - Radioallergosorbent test (RAST)
    - Detects the serum level of IgE specific for a given allergen

11.1.3 Type II (Cytotoxic) Reactions

- Mediated, primarily, by antibodies of IgM or IgG class and complement
- The reaction time is minutes to hours.
- Affect a variety of organs and tissues.
- The antigens are normally endogenous, although exogenous chemicals (haptens) that can attach to cell membranes can also lead to type II hypersensitivity.

Reactions involve antibody-mediated destruction of cells through:

- complement system, or
- Antibodydependent cell-mediated cytotoxicity (ADCC).
- Also can serve as an opsonin, enabling phagocytose the antibody-coated cell



- Examples of type II hypersensitive reactions
  - Transfusion Reactions
  - Hemolytic Disease of the Newborn
  - Drug-Induced Hemolytic Anemia

Transfusion Reactions

- Antibodies to the A, B, and O antigens, called isohemagglutinins are usually of the IgM class.
- Antibodies to other blood-group antigens may result from repeated blood transfusions because minor allelic differences in these antigens can stimulate antibody production.These antibodies are usually of the IgG class.

- The clinical manifestations of transfusion reactions result from massive intravascular hemolysis of the transfused red blood cells by antibody plus complement.

Hemolytic Disease of the Newborn

- Hemolytic disease of the newborn develops when maternal IgG antibodies specific for fetal blood-group antigens cross the placenta and destroy fetal red blood cells.
- Caused by Rh incompatibility

Drug-Induced Hemolytic Anemia

- Certain antibiotics (e.g., penicillin, ephalosporin, and streptomycin) can adsorb nonspecifically to proteins on RBC membranes, forming a complex similar to a hapten-carrier complex.
- In some patients, such drug-protein complexes

  induce formation of antibodies, which then bind to the adsorbed drug on red blood cells, inducing complement mediated lysis and thus progressive anemia.

Laboratory diagnosis of hemolytic disease of the newborn

Hemolytic disease of the newborn caused by Rh incompatibility can be detected by

- Testing maternal serum at intervals during pregnancy for antibodies to the Rh antigen (A rise in the titer of these antibodies as pregnancy progresses indicates that the mother has been exposed to Rh antigens and is producing increasing amounts of antibody).
- The presence of maternal IgG on the surface of fetal red blood cells can be detected by a **Coombs test**.

11.1.4 Type III (Immune Complex) Reactions

- When large amounts of antigen bind to antibody, immune complexes can form.
- If antigen is in excess, small complexes form; because these are not easily cleared by the phagocytic cells, they can cause tissue-damaging
- Antibodies are mostly of IgG class, although IgM may also be involved.
- The antigen may be exogenous (chronic bacterial, viral or parasitic infections), or endogenous (non-organ specific autoimmunity: *e.g., systemic lupus eythematosus-SLE).*

Type III (Immune Complex) Reactions cont…

- When the complexes are deposited in tissue very near the site of antigen entry, a localized reaction develops. example **Arthus reaction**
- When the complexes are formed in the blood, a reaction can develop wherever the complexes are deposited. Example **serum sickness.**

Type III (Immune Complex) Reactions cont…

- Complex deposition in **serum sickness** frequently observed
  - On blood-vessel walls
  - In the synovial membrane of joints
  - On the glomerular basement membrane of the kidney
  - On the choroid plexus of the brain.
- Formation of circulating immune complexes contributes to the pathogenesis of a number of conditions other than serum sickness.

Type III (Immune Complex) Reactions cont…

These include the following:

- *Autoimmune Diseases:*
  - Systemic lupus erythematosus
  - Rheumatoid arthritis
  - Goodpasture's syndrome
- *Drug Reactions:*
  - Allergies to penicillin and sulfonamides

Type III (Immune Complex) Reactions cont…

- *Infectious Diseases*
  - Poststreptococcal glomerulonephritis
  - Meningitis
  - Hepatitis
  - Mononucleosis
  - Malaria
  - Trypanosomiasis

Type III (Immune Complex) Reactions cont…

- The deposition of these complexes initiates a reaction that results in the recruitment of neutrophils to the site.
- The tissue there is injured as a consequence of granular release from the neutrophil.

Development of a localized Arthus reaction (type III hypersensitive reaction)

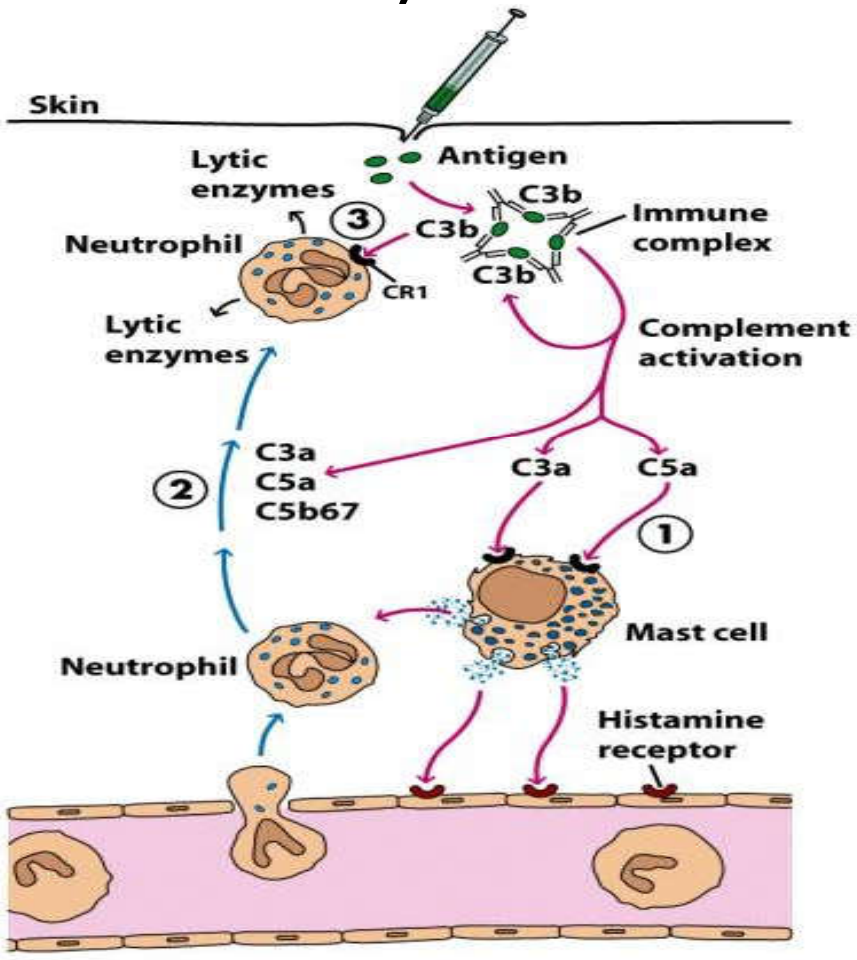


Figure 15-15
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Laboratory diagnosis of type II ???

11.1.5 Type IV (Cell-Mediated) Reactions

- Robert Koch, who observed that individuals infected with *Mycobacterium tuberculosis developed a localized inflammatory* response when injected intradermally with a filtrate derived from a mycobacterial culture.
- He called this localized skin reaction a “tuberculin reaction.” Later, as it became apparent that a variety of other antigens could induce this response its name was changed to delayed-type or type IV hypersensitivity in reference to the delayed onset of the reaction and to the tissue damage (hypersensitivity) that is often associated with it.

Type IV (Cell-Mediated) Reactions cont…

- DTH response does cause extensive tissue damage and is in itself pathologic.
- In many cases tissue damage is limited, and the response plays an important role in defense against intracellular pathogens and contact antigens.
- The hallmarks of a type IV reaction are the delay in time required for the reaction to develop and the recruitment of macrophages as opposed to neutrophils, as found in a type III reaction.
- Macrophages are the major component of the infiltrate that surrounds the site of inflammation.

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

Intracellular bacteria
- *Mycobacterium tuberculosis*
- *Mycobacterium leprae*
- *Listeria monocytogenes*
- *Brucella abortus*

Intracellular fungi
- *Pneumocystis carinii*
- *Candida albicans*
- *Histoplasma capsulatum*
- *Cryptococcus neoformans*

Intracellular parasites
- *Leishmania* sp.

Intracellular viruses
- Herpes simplex virus
- Variola (smallpox)
- Measles virus

Contact antigens
- Picrylchloride
- Hair dyes
- Nickel salts
- Poison ivy
- Poison oak

Phases of the DTH Response

Sensitization phase

- DTH response begins with an initial sensitization phase of 1–2 weeks after primary contact with an antigen.
- During this period, TH cells are activated and clonally expanded by antigen presented together with the requisite class II MHC molecule on an appropriate antigenpresenting cell
- A variety of antigen-presenting cells have been shown to be involved in the activation of a DTH response, including:
  - Langerhans cells
  - Macrophages.

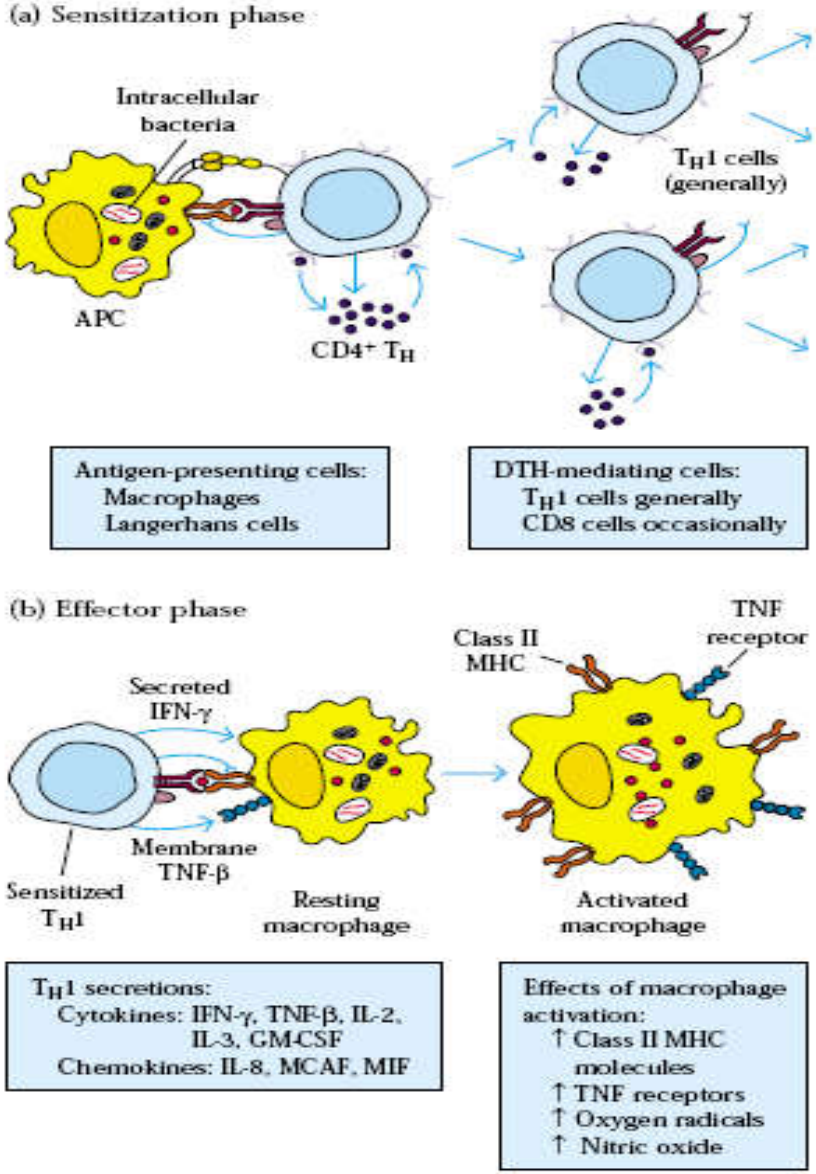
- T cells activated during the sensitization phase are CD4+, primarily of the TH1 subtype.

Efector phase

- A subsequent exposure to the antigen induces the effector phase of the DTH response.
- In the effector phase, TH1 cells secrete a variety of cytokines that recruit and activate macrophages and other nonspecific inflammatory cells.
- A DTH response normally does not become apparent until an average of 24 h after the second contact with the antigen; the response generally peaks 48–72 h after second contact.

- The delayed onset of this response reflects the time required for the cytokines to induce localized influxes of macrophages and their activation
- DTH response is important in host defense against parasites and bacteria that live within cells, where circulating antibodies cannot reach them.
- Generally, the pathogen is cleared rapidly with little tissue damage. However, in some cases, especially if the antigen is not easily cleared, a prolonged
- DTH response can itself become destructive to the host as the intense inflammatory response develops into a visible granulomatous reaction.

Overview of the DTH response



- Examples of DTH
  Tuberculin-type hypersensitivity
  Contact Dermatitis

Contact Dermatitis

- Many contact-dermatitis reactions, including the responses to formaldehyde, trinitrophenol, nickel, turpentine, and active agents in various cosmetics and hair dyes, poison oak, and poison ivy, are mediated by TH1 cells.
- Most of these substances are small molecules that can complex with skin proteins.

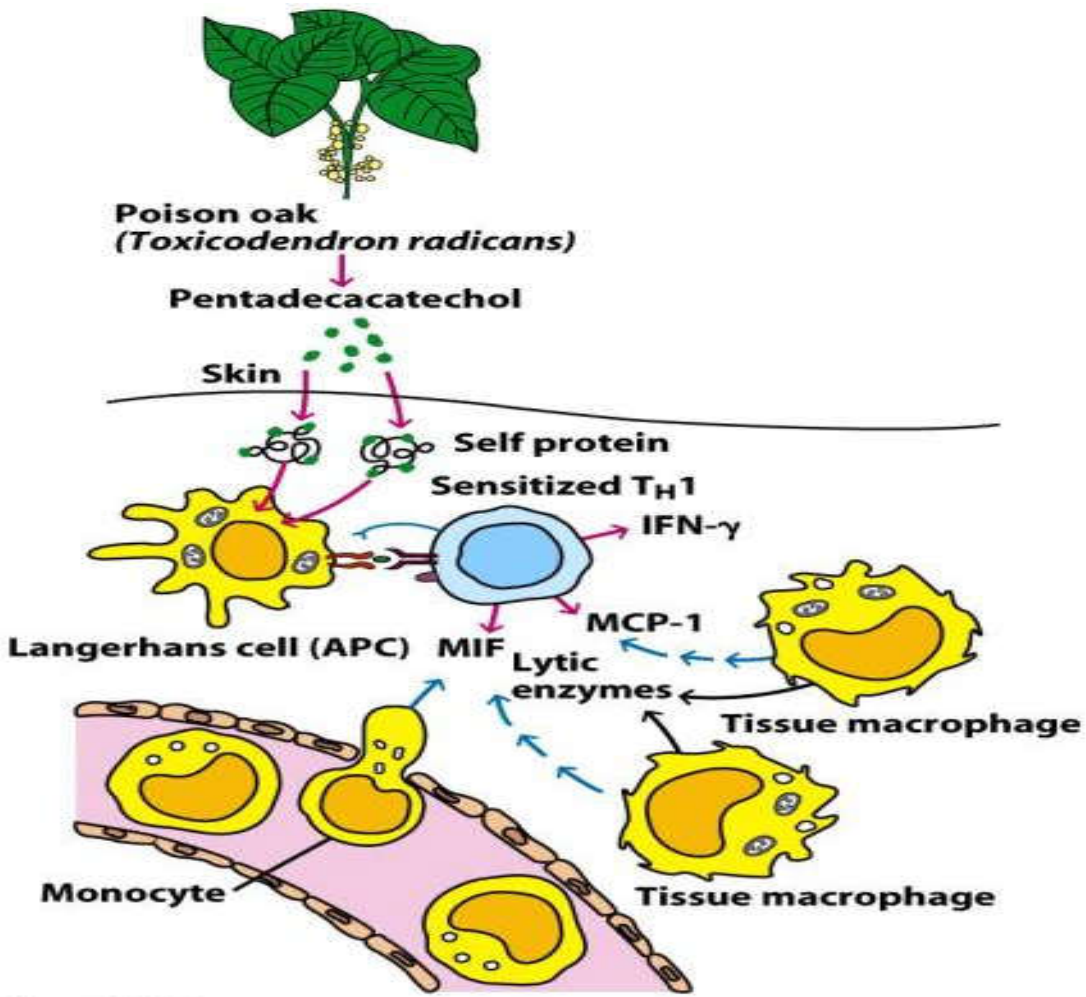


Figure 15-20
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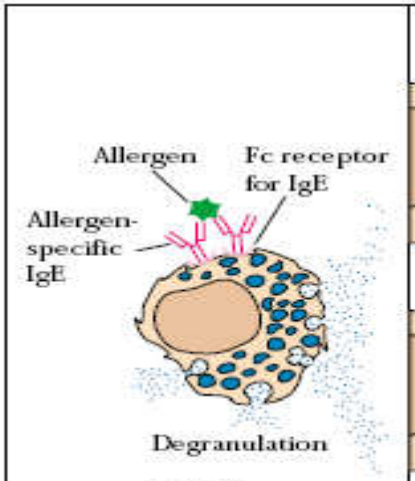
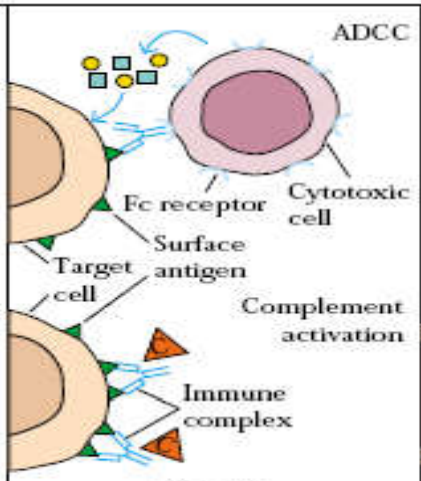
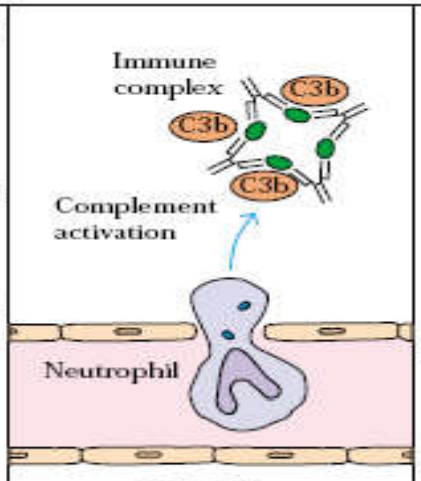
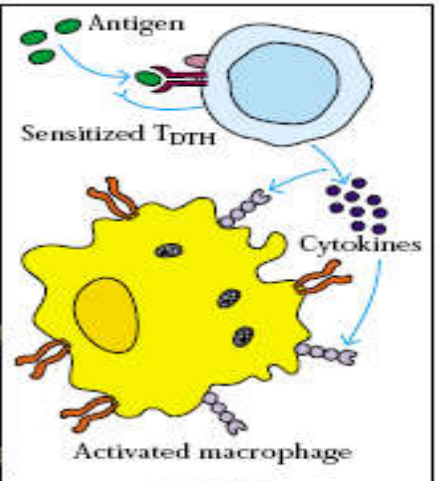
DTH Reaction Is Detected with a Skin Test

- The presence of a DTH reaction can be measured experimentally by injecting antigen intradermally and observing whether a characteristic skin lesion develops at the injection site.
- A positive skin-test reaction indicates that the individual has a population of sensitized TH1 cells specific for the test antigen.
  - For example, to determine whether an individual has been exposed to *M. tuberculosis,* PPD, a protein derived from the cell wall of this mycobacterium, is injected intradermally.
  - Development of a red,slightly swollen, firm lesion at the site between 48 and 72 h later indicates previous exposure.

Summery of The four types of hypersensitive responses

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

Diagnosis of Hypersensitivity reactions

Diagnostic tests

- **In vivo tests:**
  - **Delayed cutaneous reaction (eg. Montoux test)**
  - **Patch test for contact dermatitis**

- **In vitro tests:???**
  - **Mitogenic response**
  - **Lymphocytotoxicty**
  - **IL-2 production**

11.2 Immunologic Tolerance and Autoimmunity

- Objectives:
  - Explain the importance and significance of Tolerance in health.
  - Describe the factors that determine induction of Tolerance
  - Discuss the mechanism of Tolerance induction
  - Explain the theories on etiology of autoimmune disease
  - Compare and contrast the major features of organ-specific versus multi-organ autoimmune diseases
  - Discuss the major features and laboratory tests for the autoimmune diseases presented

Outlines

- Immunologic Tolerance
- Autoimmunity
- Organ specific Autoimmuity
- Systemic Autoimmunity
- CD4+,MHC,and TCR in Autoimmunity
- Mechanisns for induction of Autoimmunity
- Laboratory Diagnosis of Autoimmunity

11.2 Immunologic Tolerance and Autoimmunity

Introduction to **Tolerance**

- Since the late 1970s, much evidence has shown that not all self-reactive lymphocytes are deleted during T-cell and B-cell maturation.
  - Normal healthy individuals have been shown to possess mature, recirculating, self-reactive lymphocytes.
  - The presence of these self-reactive lymphocytes in the periphery does not inevitably result in autoimmune reactions,
  - their activity must be regulated in normal individuals through clonal anergy or clonal suppression.

- A breakdown in regulation can lead to activation of self-reactive clones of T or B cells, generating humoral or cell-mediated responses against self-antigens.
  - reactions can cause serious damage to cells and organs, sometimes with fatal consequences.

11.2 Immunologic Tolerance and Autoimmunity

- **Tolerance** refers to the specific non-reactivity to an antigen from the previous exposure to the same antigen. While the most important form of tolerance is non-reactivity to self-antigens, it is possible to induce tolerance to non-self (foreiegn) antigens. When an antigen induces tolerance it is referred to as a toleragen.

11.2 Immunologic Tolerance and Autoimmunity continued

- Immunologic features of **tolerance**:
  - Tolerance is different from non-specific immuno-suppression and immuno-deficiency.
  - It is an active antigen dependent response in response to the antigen.
  - Like immune response, tolerance is specific and like immunologic memory it can exist in B cells, T cells or both, and
  - like immune memory, tolerance at the T cell level is longer lasting than tolerance at the B cell level.

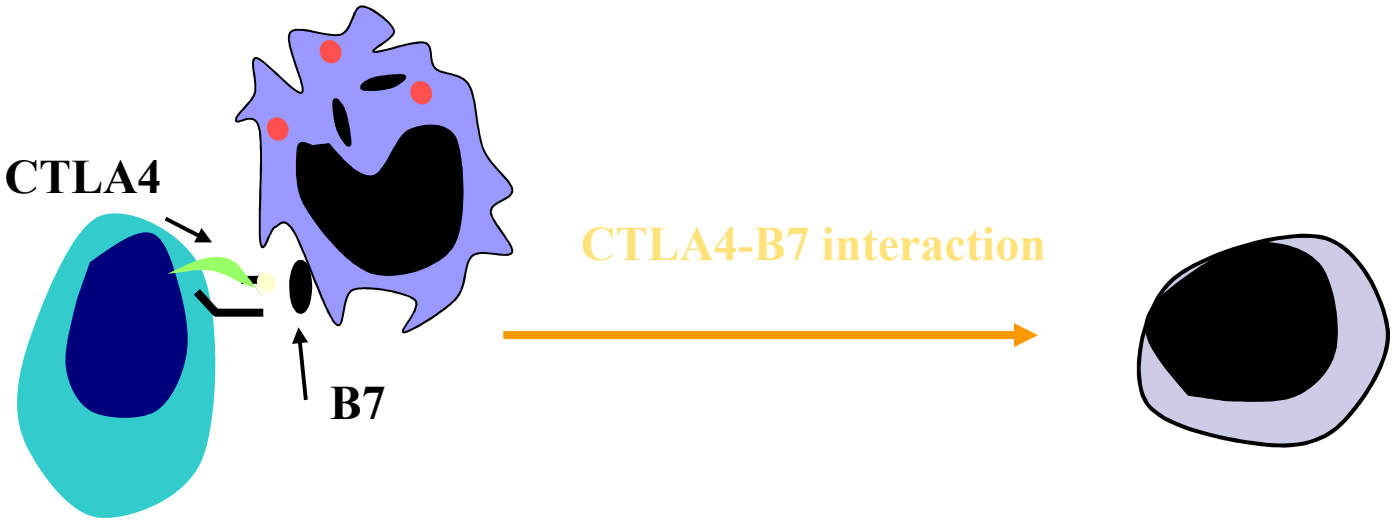
11.2 Immunologic Tolerance and Autoimmunity

- **Definition of Autoimmunity:** immune system response to self component
- **Breakdown in regulation** permits activation of self-reactive clones of
  - T-cells
  - B-cells
  - Resulting in
    - organ-specific autoimmune disease &/or
    - systemic autoimmune disease
    - organ **function may be stimulated**, or
    - **blocked** by autoAb's

Proposed Mechanisms for Induction of Tolerance continued

- **Regulatory T cells (Formerly called suppressor cells):**
  - A distinct population of T cells has been discovered called regulatory T cells.
  - The most well characterized include those that express CD4+ and CD25+.
  - Latest research suggests regulatory T cells are defined by expression of the Foxp3 factor, required for regulatory T cell development and function.
  - The precise mechanism/s through which regulatory T cells suppress other T cell function is not clear.

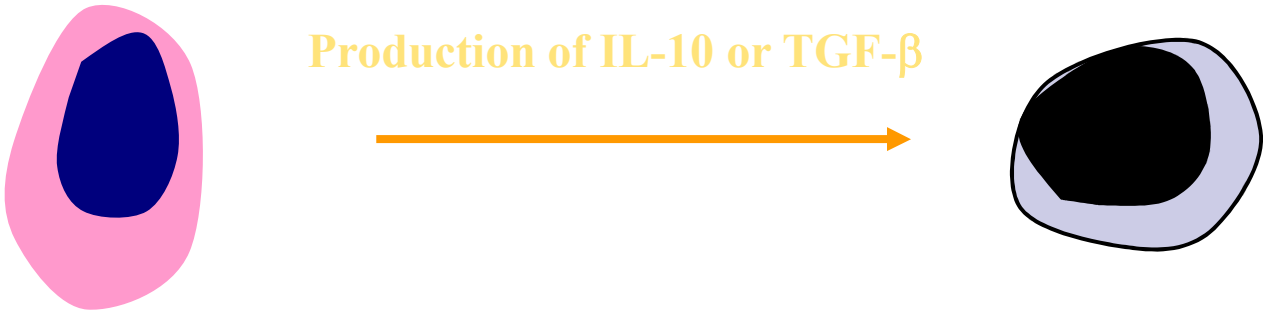
Regulation by CTLA-4



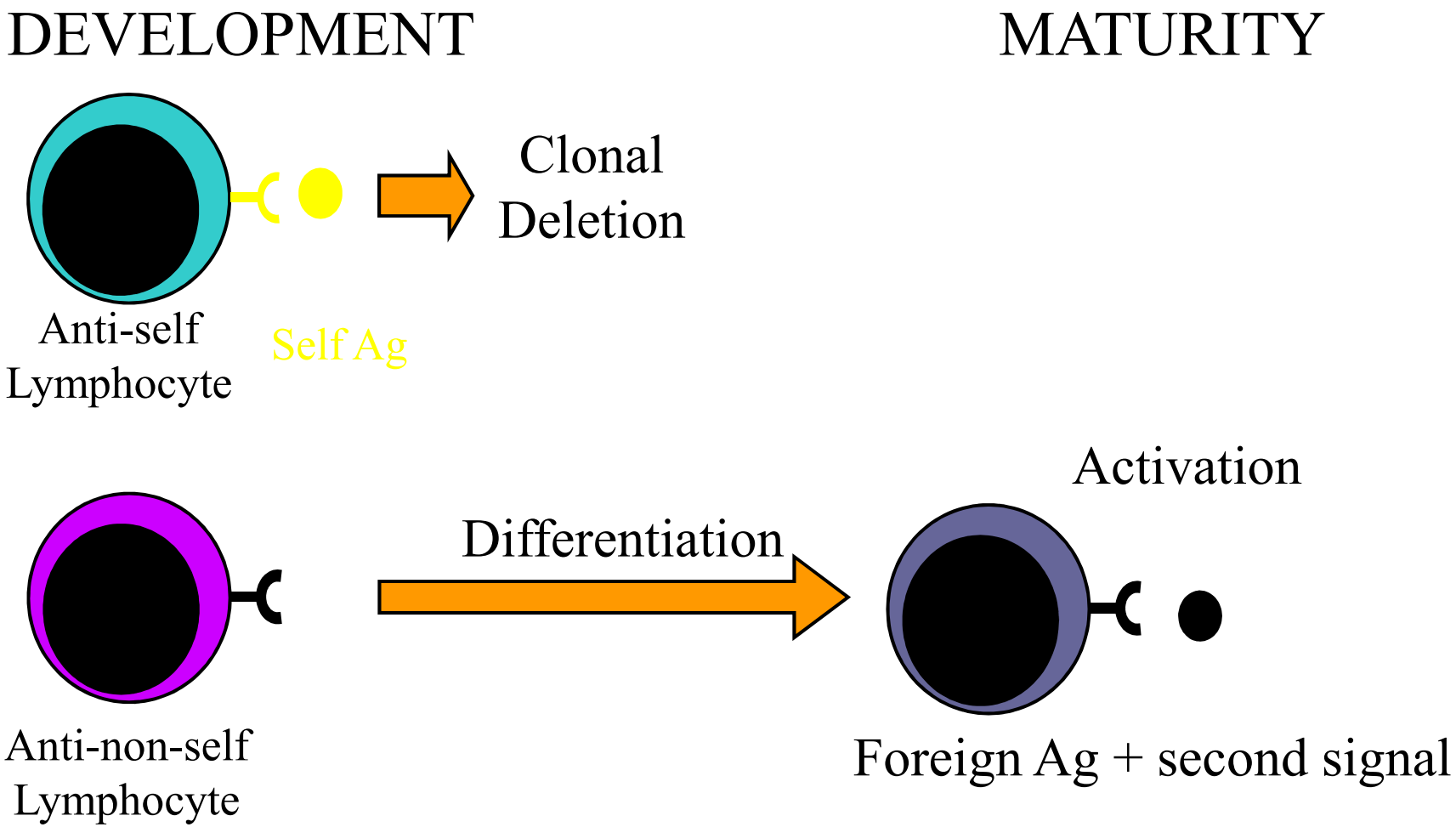
Activated T cell

Functionally Unresponsive (Anergic) T cell

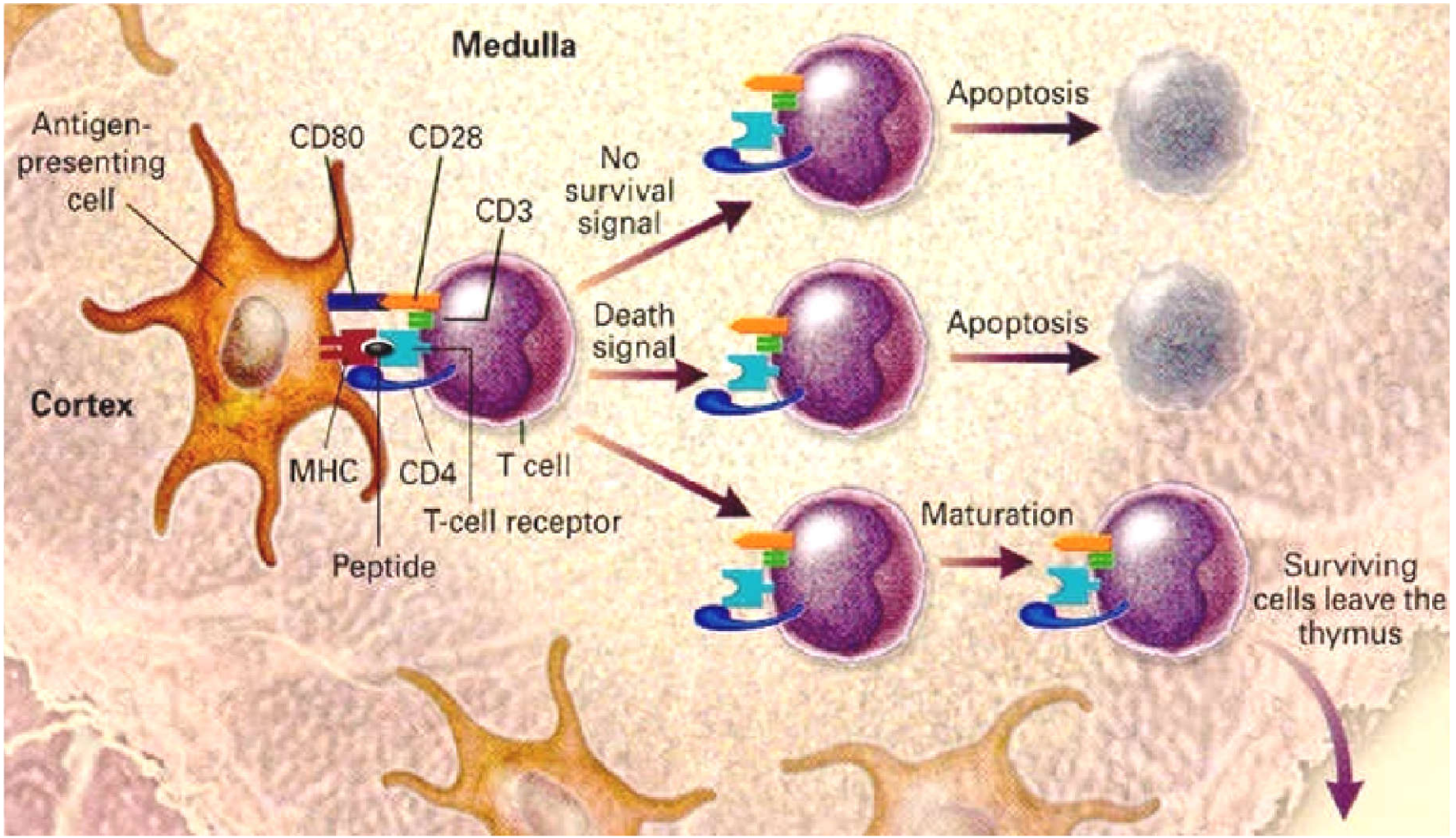
Regulatory T cells



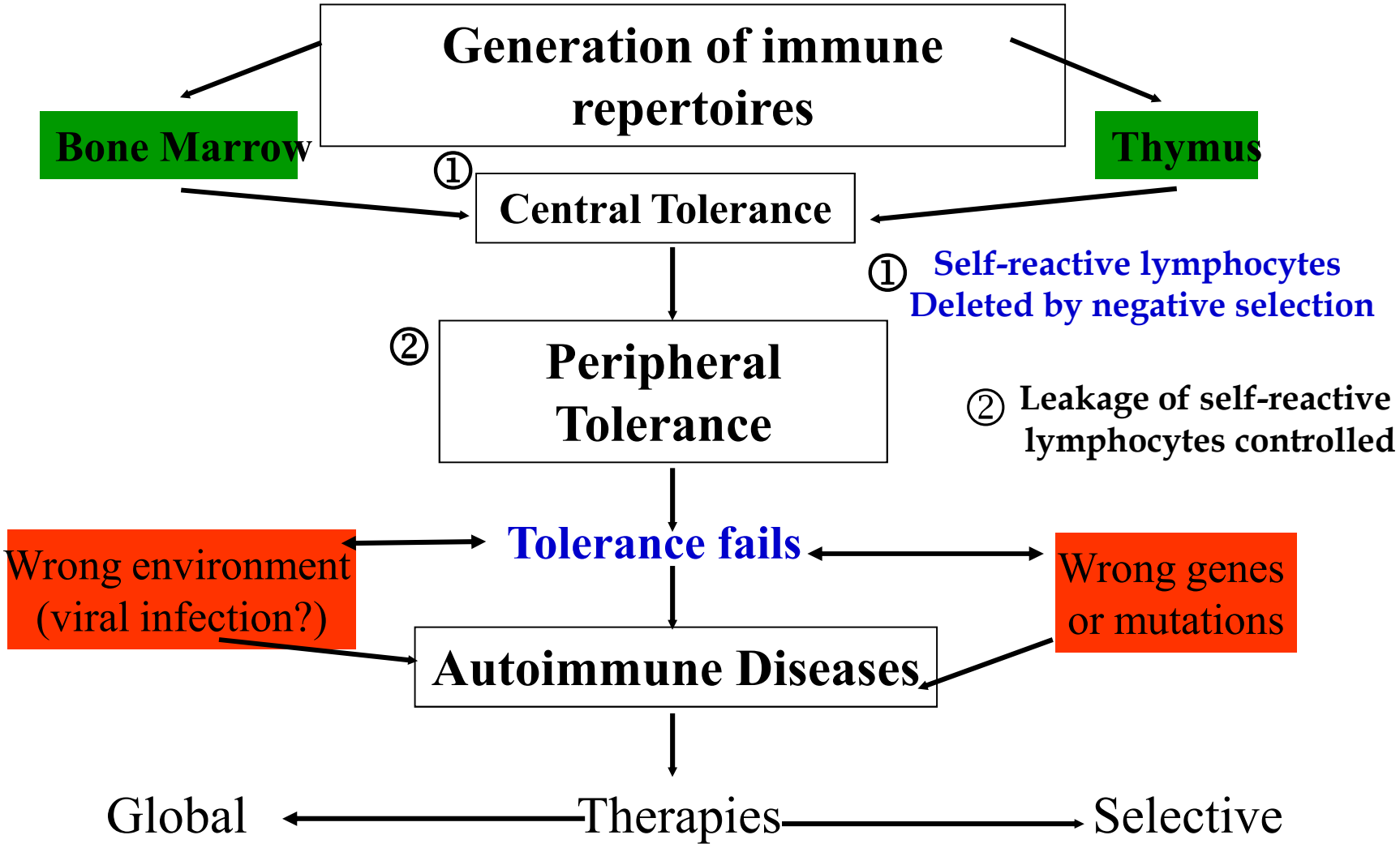
Burnet's Clonal Selection Model: Central Tolerance



Central Tolerance



Tolerance: Establishment and Failure



11.2 Immunologic Tolerance and Autoimmunity - Organ-Specific Diseases

- **Immune response is to an Ag specific to organ or gland -** may result in
  - **direct cellular damage** secondary to lymphocytes or Ab's binding to cell-membrane Ag's
    - cause cell lysis &/or
    - inflammatory response in target organ
    - cellular structures replaced by connective tissue &
    - function decreases, **function may be stimulated**, or
    - **blocked** by autoAb's

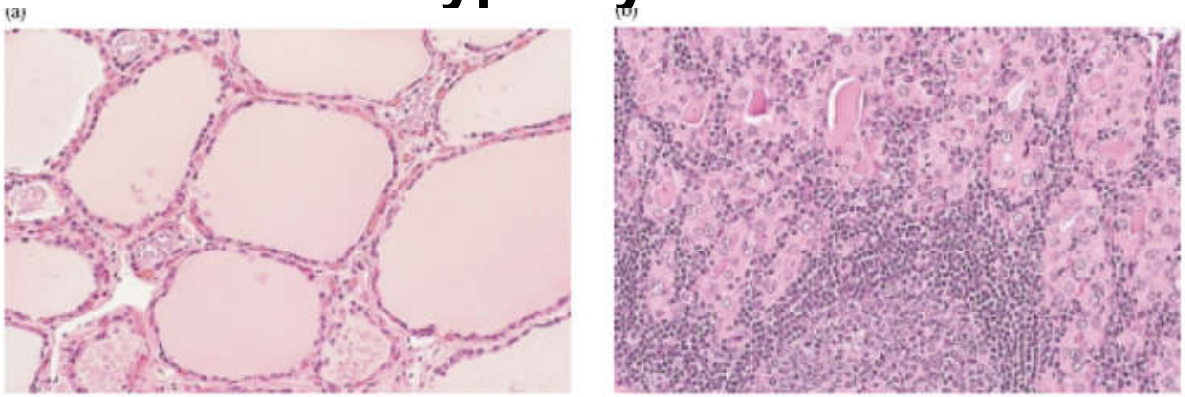
11.2 Immunologic Tolerance and Autoimmunity - Organ-Specific Diseases

- **Direct Cellular Damage Examples**
  - **Hashimoto's Thyroiditis**
    - most common in middle aged females
    - **autoantibodies & sensitized T_{DTH} cells** specific for thyroid Ag's
    - intense **infiltration of thyroid** by
      - lymphocytes, macrophages, &
      - plasma cells form lymphocytic follicles & germinal centers in thyroid

11.2 Immunologic Tolerance and Autoimmunity

- Organ-Specific Diseases

- develop **goiter** or enlarged thyroid
- **Ab's to thyroglobulin & thyroid peroxidase** are proteins involved in iodine uptake
  - prevented by Ab's binding to these proteins
  - decreased production thyroid hormones leads to **Hypothyroidism**



Photomicrographs of (a) normal thyroid gland showing a follicle lined by cuboidal follicular epithelial cells and (b) gland in Hashimoto's thyroiditis showing intense lymphocyte infiltration.

11.2 Immunologic Tolerance and Autoimmunity - Organ-Specific Diseases

- **Pernicious Anemia**
  - auto-Ab's to membrane bound protein called
  - **intrinsic factor is critical to**
    - facilitating uptake of vit. B_{12}
    - needed for hematopoiesis
      - # of mature RBC's decreased

11.2 Immunologic Tolerance and Autoimmunity - Organ-Specific Diseases

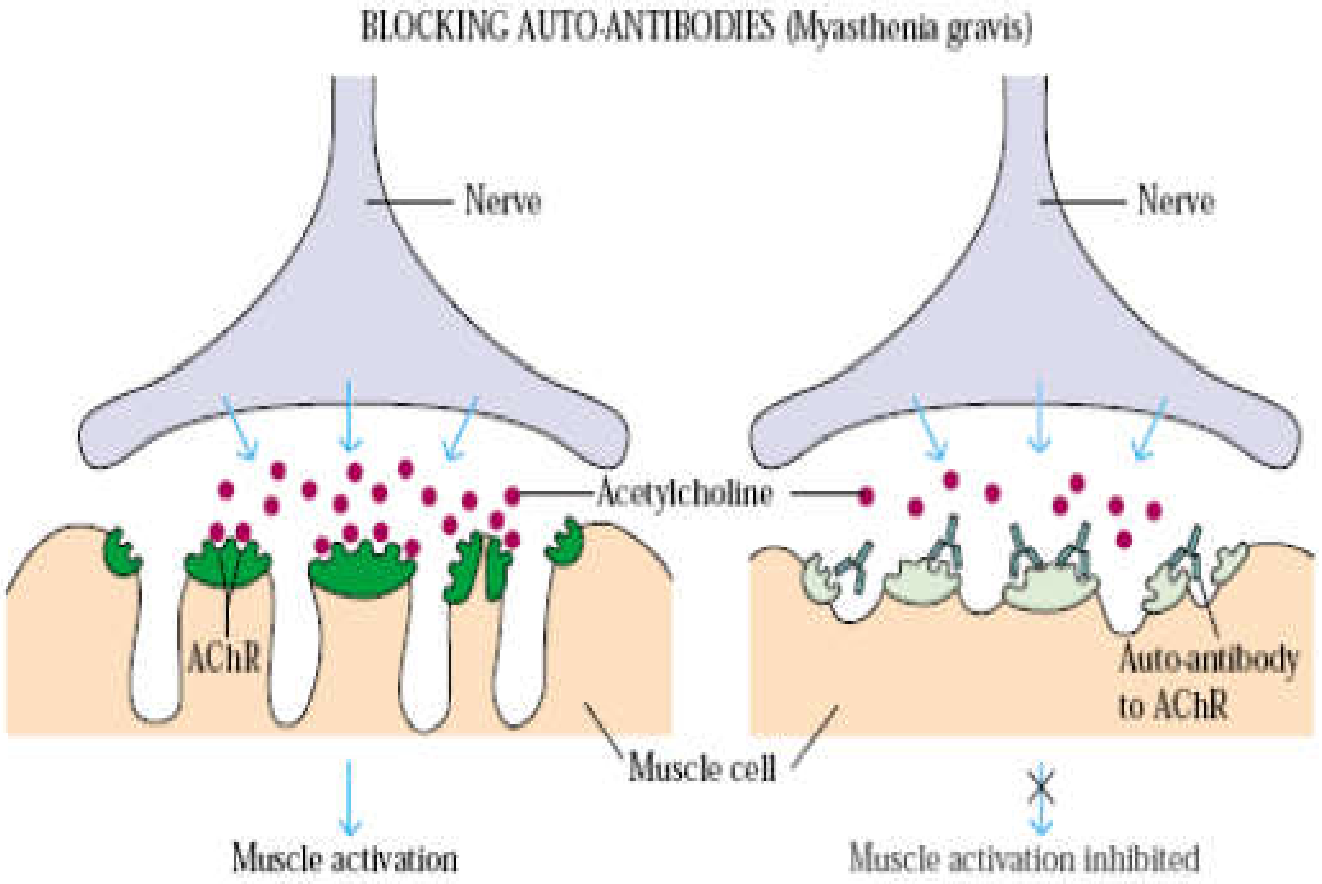
- **Insulin-Dependent Diabetes Mellitus (IDDM)**
  - attacks **beta cells** in **islets of Langerhans**
    - production of insulin decreases and
    - **blood glucose levels increases**
  - develop **insulitis** indicated by
    - T_{DTH} cells infiltrate islets & activate macrophages
    - cytokine mediated destruction of more beta-cells
    - activated macrophages release lytic enzymes causing more destruction
    - Ab-dependent cell-mediated cytotoxicity may be involved

Immunologic Tolerance and Autoimmunity - Organ-Specific Diseases

- **Myasthenia Gravis** - Ex. Of blocking Ab
  - **autoAb's to acetylcholine receptors** on motor end-plates of muscles (C' activation may destroy receptors)
  - prevents binding of acetylcholine, so
  - inhibits muscle activation
  - Loss of both voluntary and then non-voluntary muscle action

Immunologic Tolerance and Autoimmunity

- Organ-Specific Diseases



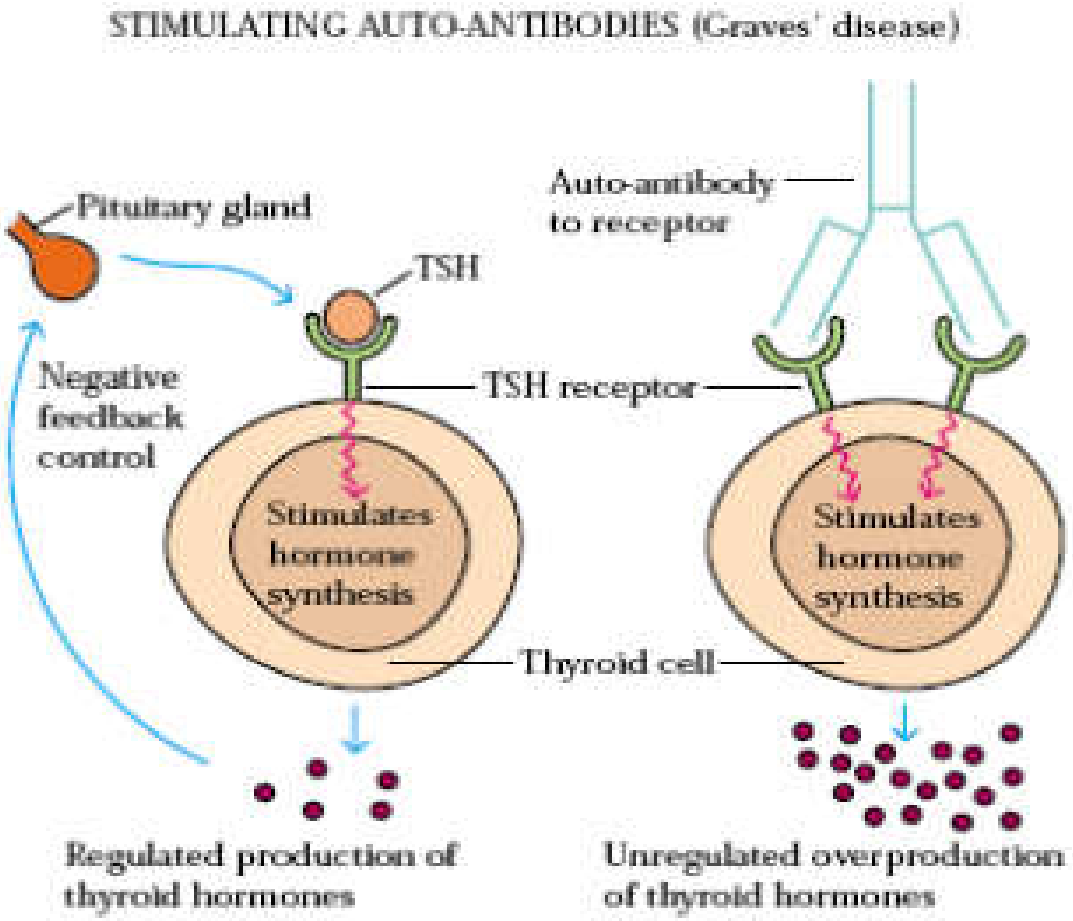
Myasthenia Gravis – Ex. Of blocking receptor sites

11.2 Immunologic Tolerance and Autoimmunity - Organ-Specific Diseases

- **Graves' Disease**
  - thyroid hormone production regulated by thyroid stimulating hormone (TSH) from pituitary gland
  - produce **autoAb to TSH** receptors, mimicing normal action of TSH $\Rightarrow$ cause production of thyroid hormones
    - overstimulate thyroid so called **long-acting thyroid-stimulating (LATS) Ab's**
- **Stimulating or Blocking AutoAb Diseases**
  - **Antagonist** -block receptor function
    - causes gradual atrophy of organ
  - **Agonist** - bind to hormone receptors
    - stimulate overproduction – Ex.: Graves Disease

Immunologic Tolerance and Autoimmunity

- Organ-Specific Diseases



Graves Disease - Ex. Of Ab Stimulating Receptor

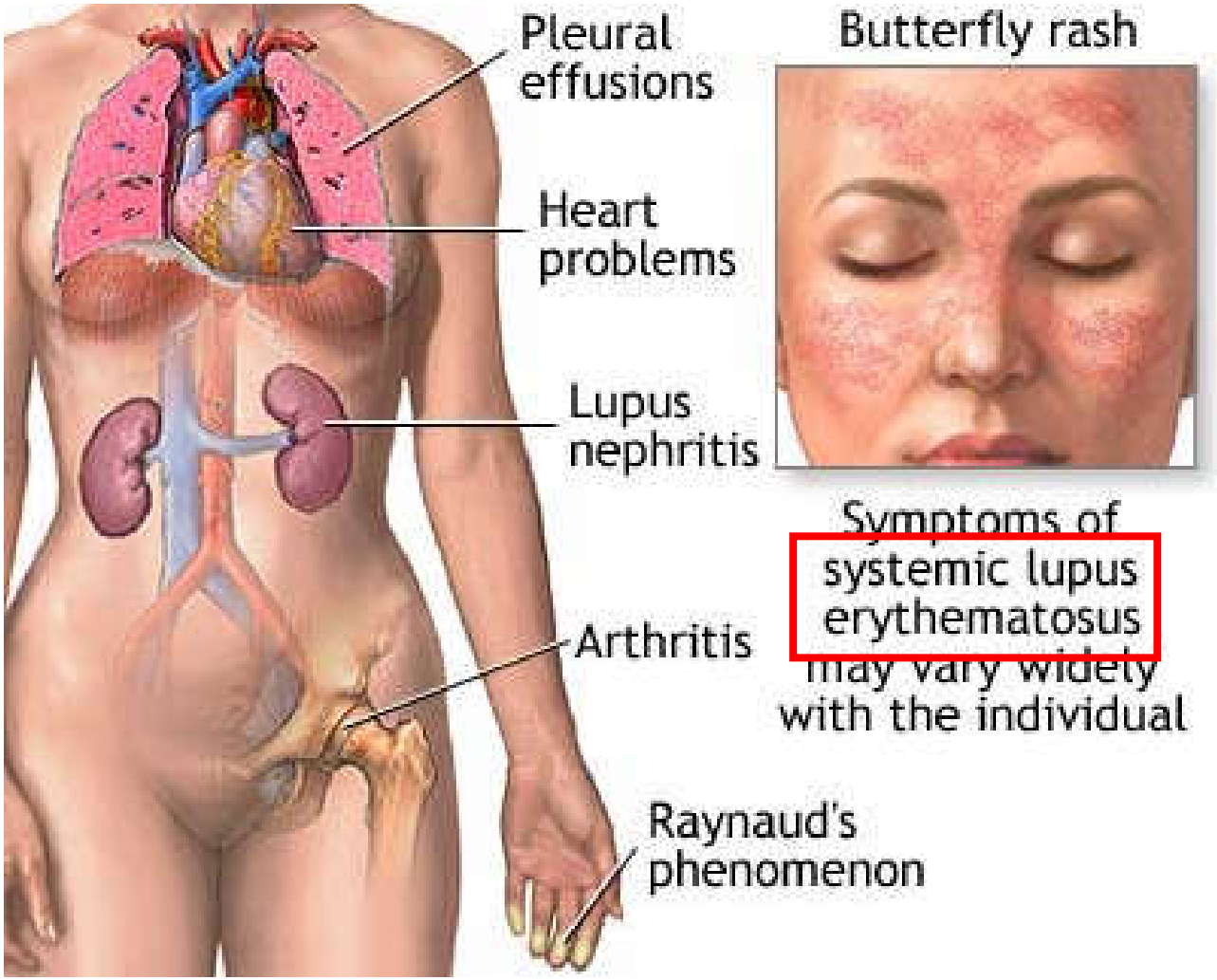
SYSTEMIC AUTOIMMUNE DISEASES

- Generalized hyperactive T & B cells
  - tissue damage wide-spread
  - involves immune complexes
- **Systemic Lupus Erythematosus (SLE)**
  - typical case ⇒ female 20 to 40
  - signs & symptoms
    - fever, weakness, arthritis, skin rash, pleurisy, renal dysfunction
  - autoAb's to
    - **nDNA, histones** ⇒ form immune complexes ⇒ deposit on blood vessel walls, Glomerular Basement Membranes (GBM)

SYSTEMIC AUTOIMMUNE DISEASES

- **RBC's & platelets** ⇒ C' mediated lysis
  - ⇒ ⇑'d circulating C3a & C5a (anaphylotoxins)
  - ⇑'d complement receptor 3 (CR3's) on neutraphils
  - neutraphils aggregate & attach to vascular endothelium
  - neutropenia
    - occlude small vessels ⇒ vasculitis
- **clotting factors**

Examples of Systemic Autoimmunity



SLE

11.2 Immunologic Tolerance and Autoimmunity – Rheumatoid Arthritis (RA)

- The classic patient's **rheumatoid factor** is an IgM antibody .
- Such auto-antibodies bind to normal circulating IgG, forming IgM-IgG complexes that are deposited in the joints.
- These immune complexes can activate the complement cascade:
  - resulting in a type III hypersensitive reaction, which
  - leads to chronic inflammation of the joints.

11.2 Immunologic Tolerance and Autoimmunity

- **Diagnosis of RA is based on signs, symptoms + lab results**
  - **lab results not definitive**, can see low titers of RF test in other autoimmune diseases & can see neg's in RA
  - produce an **Ab most commonly to the Fc portion of IgG** but can also be directed against IgM
    - this autoAb is of the class IgM
    - in the circulation as well as the synovial fluid of the Pt.
    - this Ab is known as the Rheumatoid Factor(RF), it is the UNKNOWN in the RF Test
    - significant Ab titer is usually > 1:20

11.2 Immunologic Tolerance and Autoimmunity

Multiple sclerosis (MS)

- is the most common cause of neurologic disability.
- The symptoms include, numbness in the limbs, paralysis or loss of vision.
- Most people with MS are diagnosed between the ages of 20 and 40.
- Individuals with this disease produce autoreactive T cell that participate in
  - the formation of inflammatory lesions along the myelin sheath of nerve fibers,

11.2 Immunologic Tolerance and Autoimmunity

- The cerebrospinal fluid of active MS contain activated
  - T lymphocyte which infiltrate the brain tissue and cause characteristic inflammatory lesions, destroying the myelin.
  - Serum immunoglobulins that form oligoclonal bands when electrophoresed (serum protein electrophoresis)
- Since myelin functions to insulate the nerve fibers, a breakdown in the myelin sheath leads to numerous neurologic dysfunctions.

11.2 Immunologic Tolerance and Autoimmunity continued

Table 1. Factors which determine induction of immune response or tolerance following challenge with antigen.

determinant	favor immune response	favor tolerance
physical form of antigen	large, aggregated, complex molecules;	soluble, aggregate-free, relatively smaller, less complex molecules, Ag not processed by APC or processed inappropriately
route of Ag administration	sub-cutaneous or intramuscular	oral or sometimes intravenous
dose of antigen	optimal dose	very large (or sometime very small) dose
age of responding animal	older and immunologically mature	Newborn (mice), immunologically immature
differentiation state of cells	fully differentiated cells; memory T and memory B cells	relatively undifferentiated: B cells with only IgM (no IgD), T cells (*e.g.* cells in thymic cortex)

Diagnosis

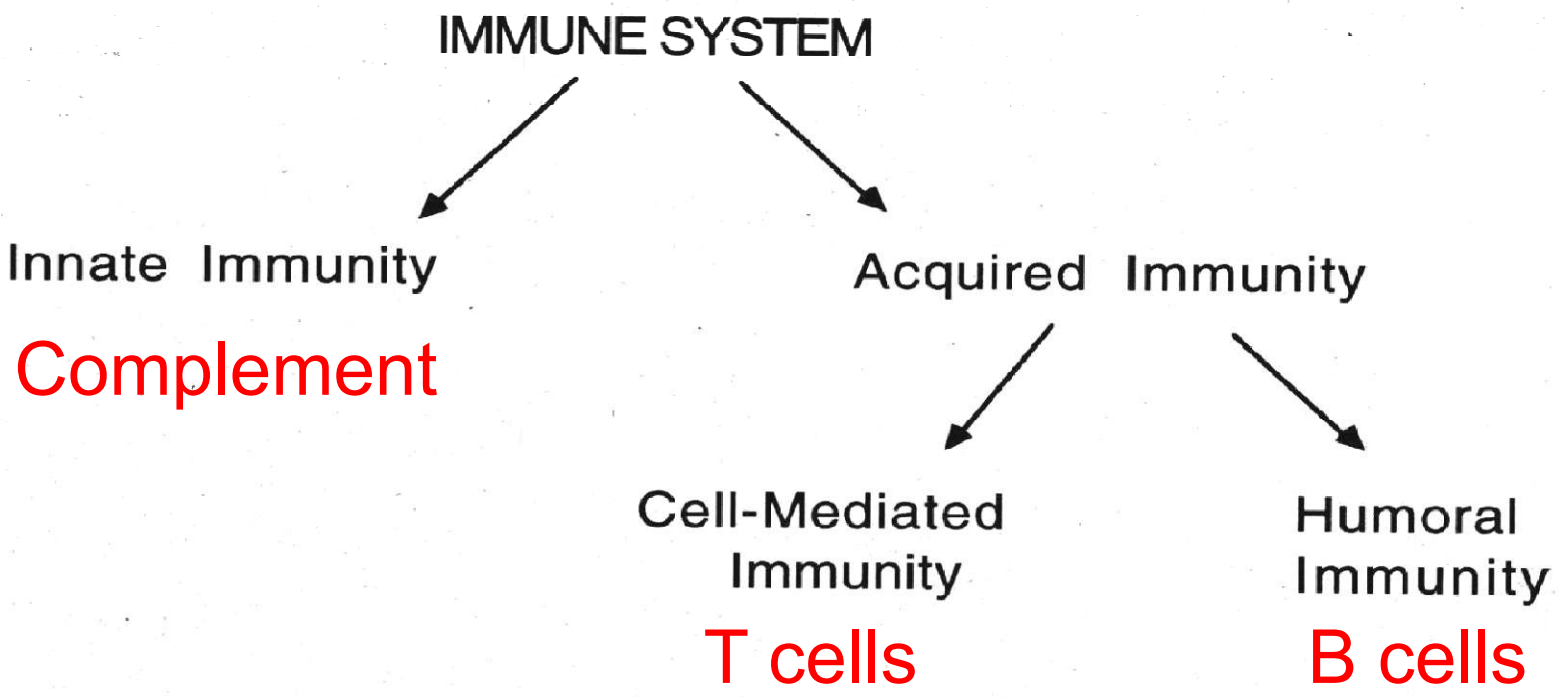
- General tests
  - C Reactive Protein
  - Autoantibody titers (anti DNA, anti phospholipids, etc)
  - Presence of Rheumatoid Factor

- Disease specific tests
  - Neurological exam – MS
  - Fasting glucose – Diabetes
  - Measurement of TSH and Thyroglobulin

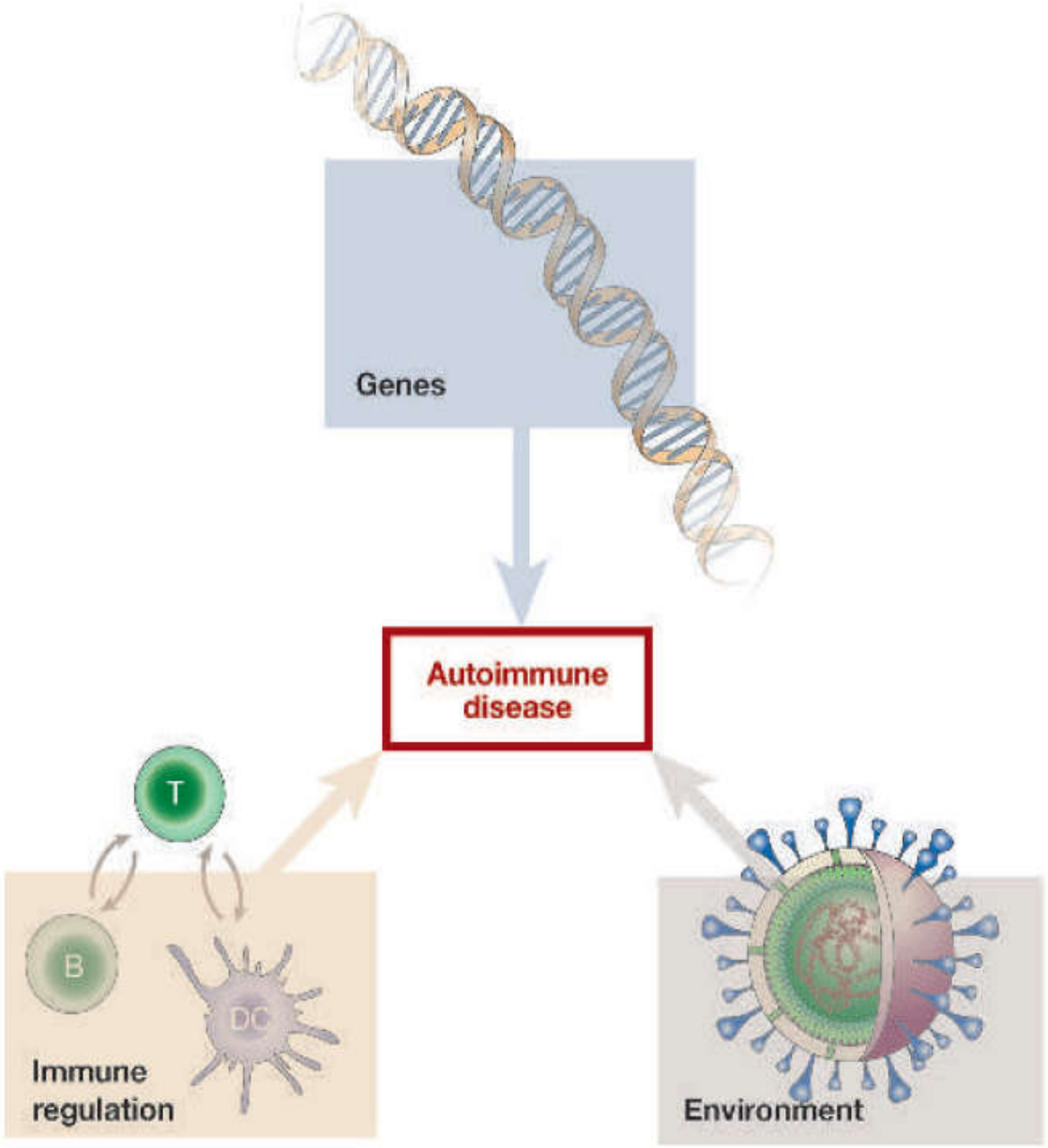
Immune Regulation summary

Autoimmunity is a failure of tolerance!

A defect in any arm of the immune system can trigger autoimmunity



Summary of Autoimmunity



11.3 AIDS and Immunodeficiency

Objectives

Up on completion of this chapter the student will be able to:

1. Differentiate Between Primary and secondary immunodeficiencies
2. Explain Characteristics of B cell, T cell, and Combined Immunodeficiencies
3. Discuss common immune deficiencies
4. Studies on HIV and Development of AIDS
5. Analysis of Strategies for Prevention and Treatment of AIDS
6. Characterize various immunodeficiencies

Outline

- 11.3.1 Definition of Immunodeficiencies
- 11.3.2 Potential causes of immune deficiencies
- 11.3.3 Primary Immune Deficiencies
- 11.3.4 Secondary Immune Deficiencies
- 11.3.5 AIDS and its diagnosis

What is Immunodeficiency?

- ✓ A failing of one or more of the body's defensive mechanisms resulting in morbidity or mortality.
- ✓ Any part of the immune system can be deficient cells, proteins, signalling mechanisms…….
- ✓ The body is susceptible to infection by organisms that meet with little or no resistance.
- ✓ Or, in certain cases, other homeostatic systems in the body will be disrupted by the defect.
- ✓ Severity is variable.
- ✓ Immunodeficiency may be Primary or Secondary.

Immunodeficiency

primary

Secondary

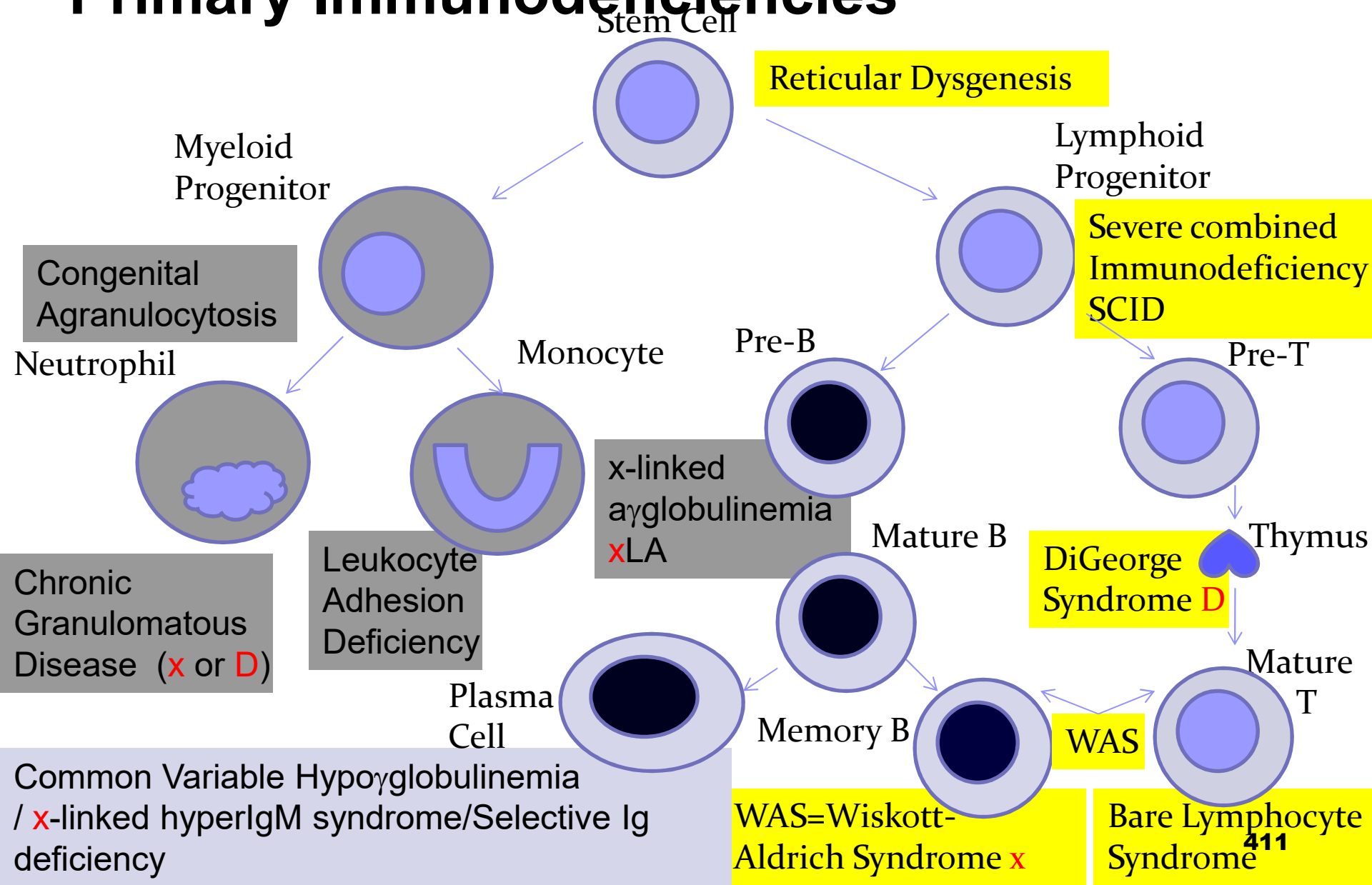
Immunodeficiency

Primary: Inherited genetic defects in immune cell development or function or inherited deficiency on a particular immune molecule.

- Defect in the early hematopoiesis which involves **stem cells** results in **reticular dysgenesis** that leads to general immune defects and subsequent susceptibility to infections. This condition is often fatal.

- **Secondary**: a loss of previously functional immunity as a result of acquired as a consequence of other diseases or environmental factors (e.g. infection, malignancy, aging, starvation, medication, drugs)

Primary Immunodeficiencies



Adaptive Immunity Deficiency

- **T cell deficiency**
  - Susceptible to intracellular bacterial infection
  - Susceptible to viral, parasitic and fungal infection
- **B cell deficiency**
  - Susceptible to extracellular bacterial infection
  - Transplacental/milk transmission of Abs
- **Severe Combined Immunodeficiency Disease (SCID)**
  - T and B cell functions defective
  - Usually fatal
  - Transplacental/milk transmission of Abs
  - TCR gene rearrangement lacking
  - Myeloid and erythroid components intact

Secondary or Acquired Immunodeficiencies

- Agent-induced immunodeficiency: e.g. infections, metaboic disturbance, trauma, corticosteroids, cyclosporin A, radiation, chemotherapy
- Acquired Hypogammaglobulinemia (Low levels of Ig; recurrent infections; treat with Ig)

Secondary Immunodeficiency:

- Infection
- Renal failure, or protein losing enteropathy
- Leukaemia or Lymphoma
- Myeloma
- Extremes of age
- Certain Drug Therapies

Clinical features associated with immunodeficiency

Feature frequency present and highly suspicious:

- ✓ **Chronic infection**
- ✓ **Recurrent infection (more than expected)**
- ✓ **Unusual microbial agents**
- ✓ **Incomplete clearing of infection**
- ✓ **Incomplete response to treatment**

Clinical features associated with immunodeficiency

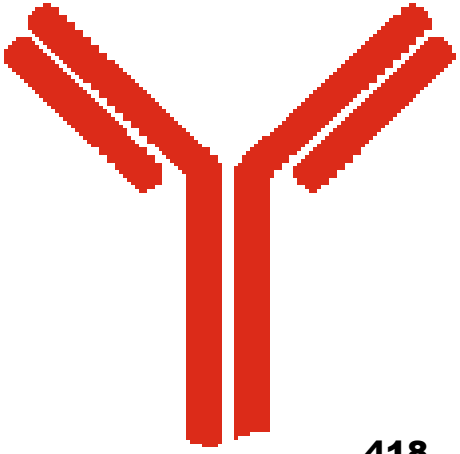
- **Feature moderately suspicious**
  - Diarrhea (chronic)
  - Growth failure
  - Recurrent abscesses
  - Recurrent osteomyelitis
- **Feature associated with specific immunodeficiency disorder**
  - Telangiectasia
  - Partial albinism

Classification of Immunodeficiencies:

- ✓ **Antibody deficiencies**
- ✓ **Cellular deficiencies**
- ✓ **Phagocytic disorders**
- ✓ **Complement deficiencies**

Antibody (B cell) ID

1. X- linked agammaglobulinemia
2. Selective IgA deficiency
3. IgG Subclasses deficiency
4. Hyper – IgM
5. CVID



Evaluation of antibody (B cell)

1. Protein electrophoresis
2. Quantitative of IgG ,IgA, IgM and IgD
3. Isohemagglutinin
4. Specific antibody response
5. B cell quantitation
6. B cell markers (CD19)

T Cell Deficiencies

- **T cell deficiency** affects
  - both cell-mediated and humoral immunity.
  - increases susceptibility to viral, protozoal and fungal infections.
  - Infection with viruses, such as cytomegalovirus or attenuated measles vaccine can be life-threatening in these patients.

T Cell Deficiencies

- 3rd and 4th Pharyngeal pouch syndrome (di George)
- Wiscott Aldrich Syndrome
- Ataxia telangectisia

Evaluation of T cell deficiency

1. Total white blood cell and differential count
2. Quantitation of T cells
3. Quantitation of CD3, 4 and 8 cells
4. Study cytokine profile (ELIspot technique)

- **Phagocytic cell deficency**
  - Congenital Agranulocytosis
  - Leucocyte adhesion deficiency
  - Chronic Granulomatous Disease (x or D)
- Laboratory tests

- **Complement deficiencies**
  - Angioedema (C1 inhibitor deficiency)
  - Homozygous deficiency in any of the early components of Classical pathway (C1q, C1r, C1s, C4,and C2)
  - C3 deficiency
  - Homozoygous deficiency in MAC components
- **Laboratory tests**
  - **C3 and C4 assay**
  - **CH_{50} assay**

Adaptive Immunity Deficiency

- **T cell deficiency**
  - Susceptible to intracellular bacterial infection
  - Susceptible to viral, parasitic and fungal infection
- **B cell deficiency**
  - Susceptible to extracellular bacterial infection
- **Severe Combined Immunodeficiency Disease (SCID)**
  - T and B cell functions defective
  - Usually fatal
  - Transplacental/milk transmission of Abs
  - TCR gene rearrangement lacking
  - Myeloid and erythroid components intact

Table 9–10. Initial Screening Tests for Immunodeficiency

Blood Count
Hemoglobin, white blood cell count, lymphocyte morphology, differential count, platelet estimation or count

Quantitative Immunoglobulins
IgG, IgM, IgA, and IgE levels

Antibody Responses to Previous Vaccines
Tetanus, diphtheria, rubella, *Haemophilus influenzae* titers (for IgG function)

Isoagglutinin (Anti-A and Anti-B) Titers
For IgM function

Total Hemolytic Complement
Tests classical complement pathway

Infection Evaluation
Erythrocyte sedimentation rate, appropriate cultures, appropriate roentgenograms

Secondary or Acquired Immunodeficiencies

- Agent-induced immunodeficiency: e.g. infections, metabolic disturbance, trauma, corticosteroids, cyclosporin A, radiation, chemotherapy
- Acquired Hypogammaglobulinemia (Low levels of Ig; recurrent infections; treat with Ig)

Acquired Immunodeficiency Syndrome (AIDS)

History

- 1950s: Blood samples from Africa have HIV antibodies.
- 1976: First *known* AIDS patient died.
- 1980: First human retrovirus isolated (HTLV-1).
- 1981: First reports of "Acquired Immuno-deficiency Syndrome" in Los Angeles.
- 1983: Virus first isolated in France (LAV).
- 1984: Virus isolated in the U.S. (called HTLV-III and AIDS-Related Virus, ARV).
- 1985: Development and implementation of antibody test to screen blood donors.

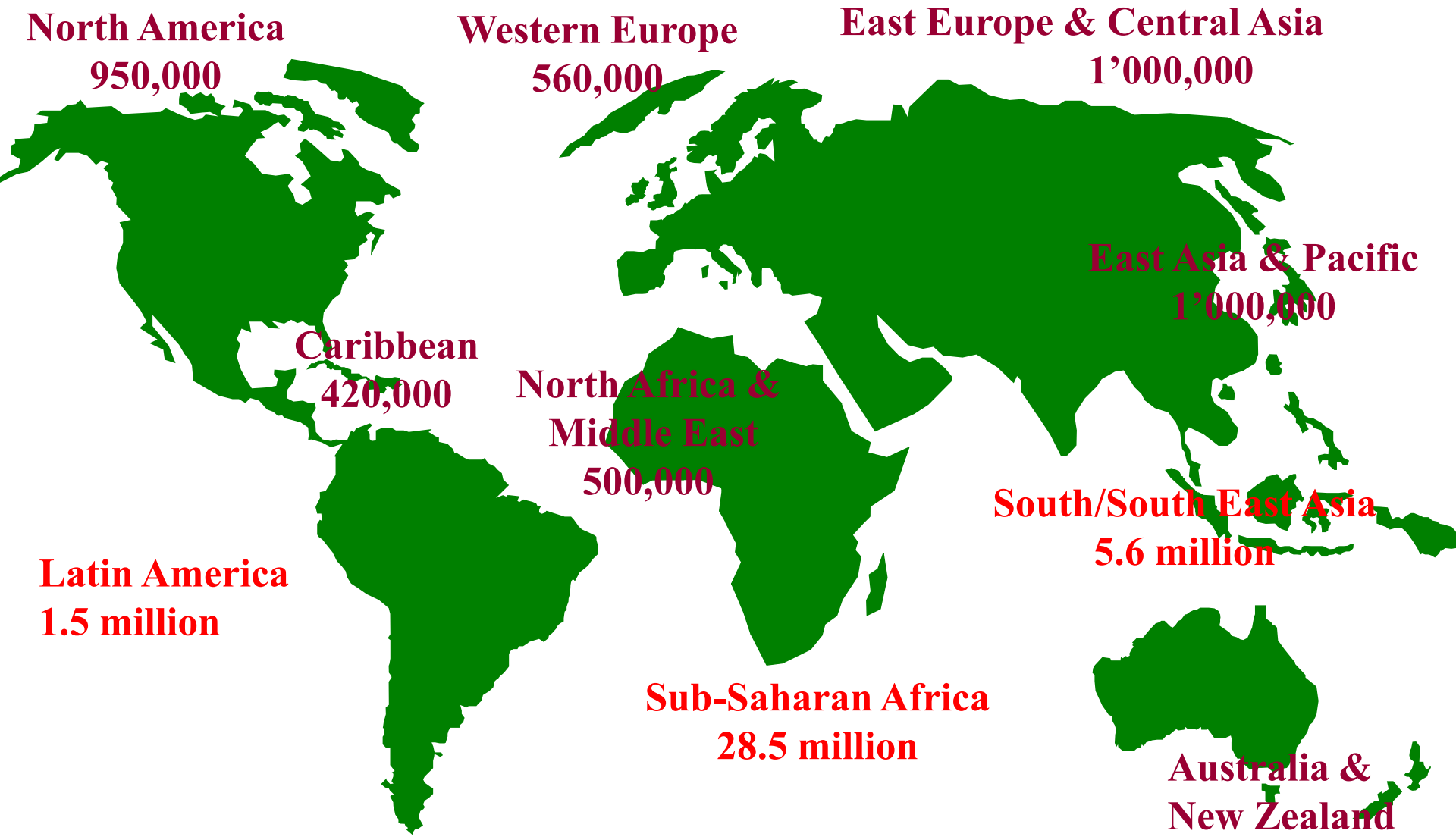
Acquired Immunodeficiency Syndrome (AIDS)

History (Continued)

- 1986: Consensus name Human Immunodeficiency Virus (HIV-1).

  Related virus (HIV-2) identified.
- 1992: AIDS becomes the leading cause of death among adults ages 25-44 in the U.S.
- 1997: Mortality rates of AIDS starts to decline due to the introduction of new drug cocktails.
- 2001: World Health Organization predicts up to 40 million infected individuals. More than 22 million have already died.

People Living with HIV/AIDS by End of 2001



Total: 40 million people

Human Immunodeficiency Virus

- Retrovirus (Lentivirinae genus, family retroviridae)
- HIV-1 and HIV-2
- Patients with low $CD4^+$ T cells
- Homosexual; promiscuous heterosexual, i.v. drug users; transfusion; infants born to infected mothers
- Opportunistic infections with Pnuemocystis carinii, Candida albicans, Mycobacterium avium, etc.
- Kaposi sarcoma

HIV Pathogenesis and Epidemiology

- HIV: the etiologic agent of AIDS
- epidemic proportions in many areas of the world
- no cure, but long term treatment is available
- 4th leading cause of death worldwide
  - leading cause of death in Africa
- accurate diagnosis is important
  - in preventing spread
  - for early intervention

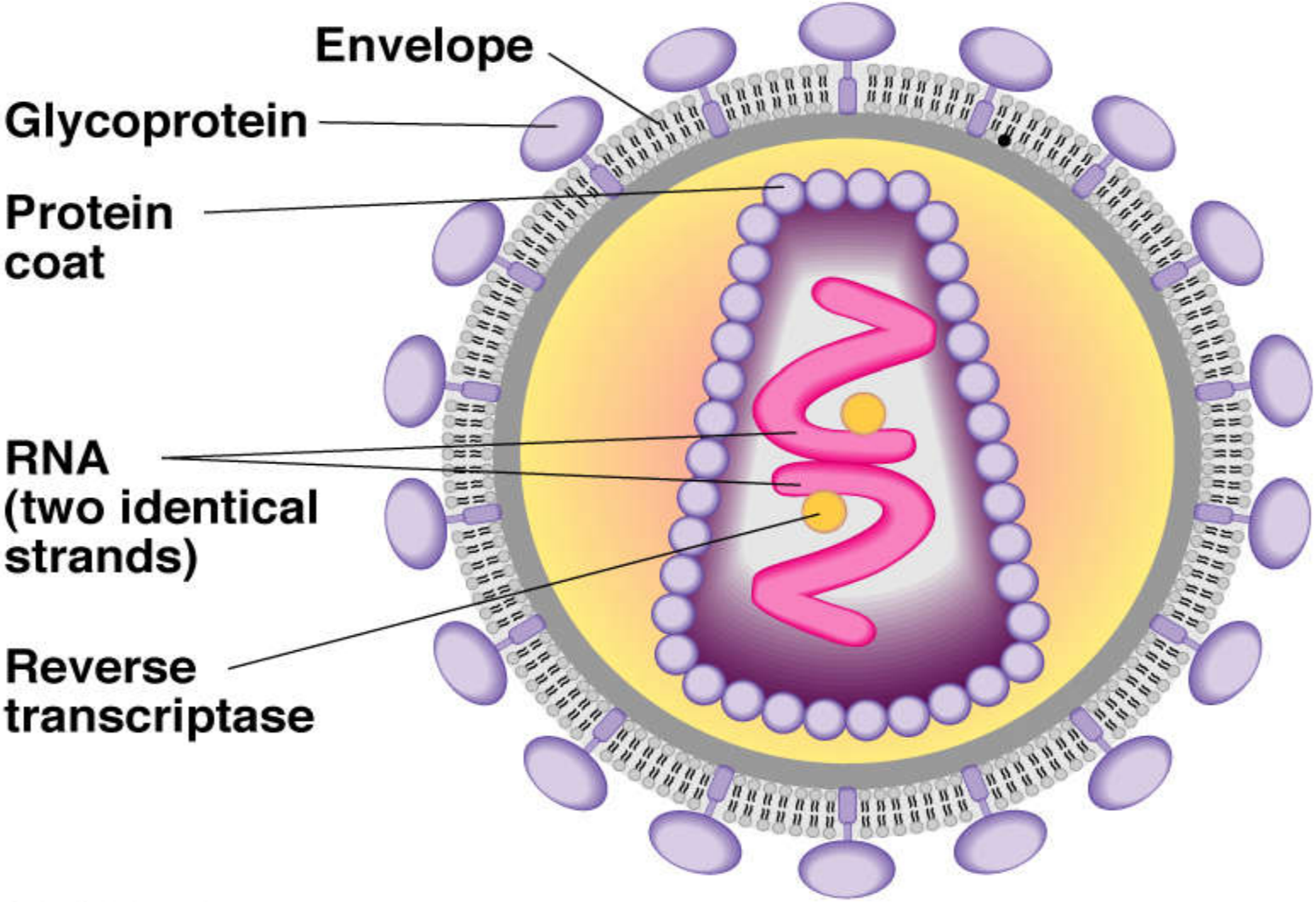
HIV-1 and HIV-2

- Three groups of HIV-1 (virus)
  - Group O- outlier group
  - Group M- main group- worldwide
  - Group N- new group
    - Group M and N mainly West Central Africa
- HIV-2 discovered in 1986
  - related but antigenically distinct virus
  - endemic in West Africa
  - also in some patients in Europe, North America and South America
  - transmission same as HIV-1, as are symptoms
  - Less pathogenic, lower transmission rate

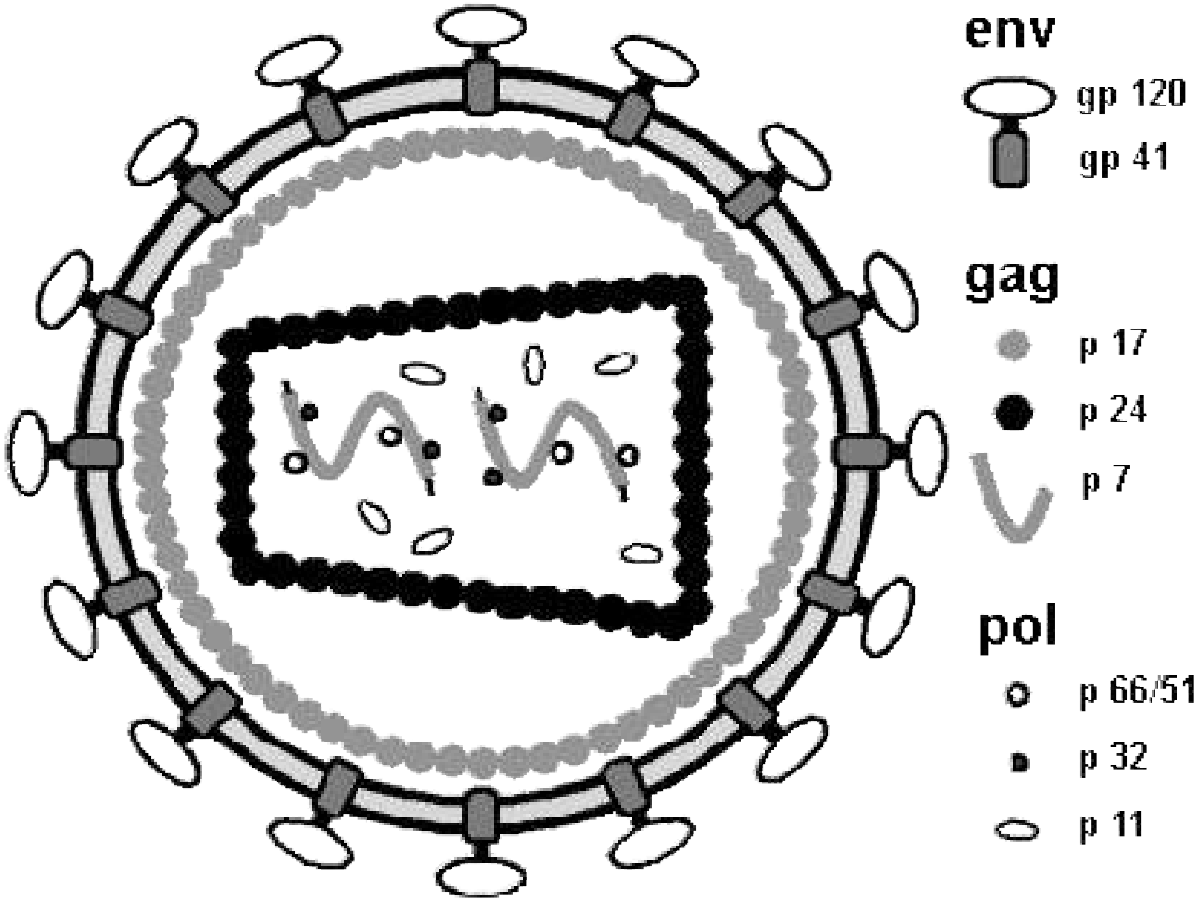
HIV-2

- HIV-2 discovered in 1986
  - related but antigenically distinct virus
  - endemic in West Africa
  - also in some patients in Europe, North America and South America
  - transmission same as HIV-1, as are symptoms
  - Less pathogenic, lower transmission rate

Structure of the Human Immunodeficiency Virus HIV is a Retrovirus

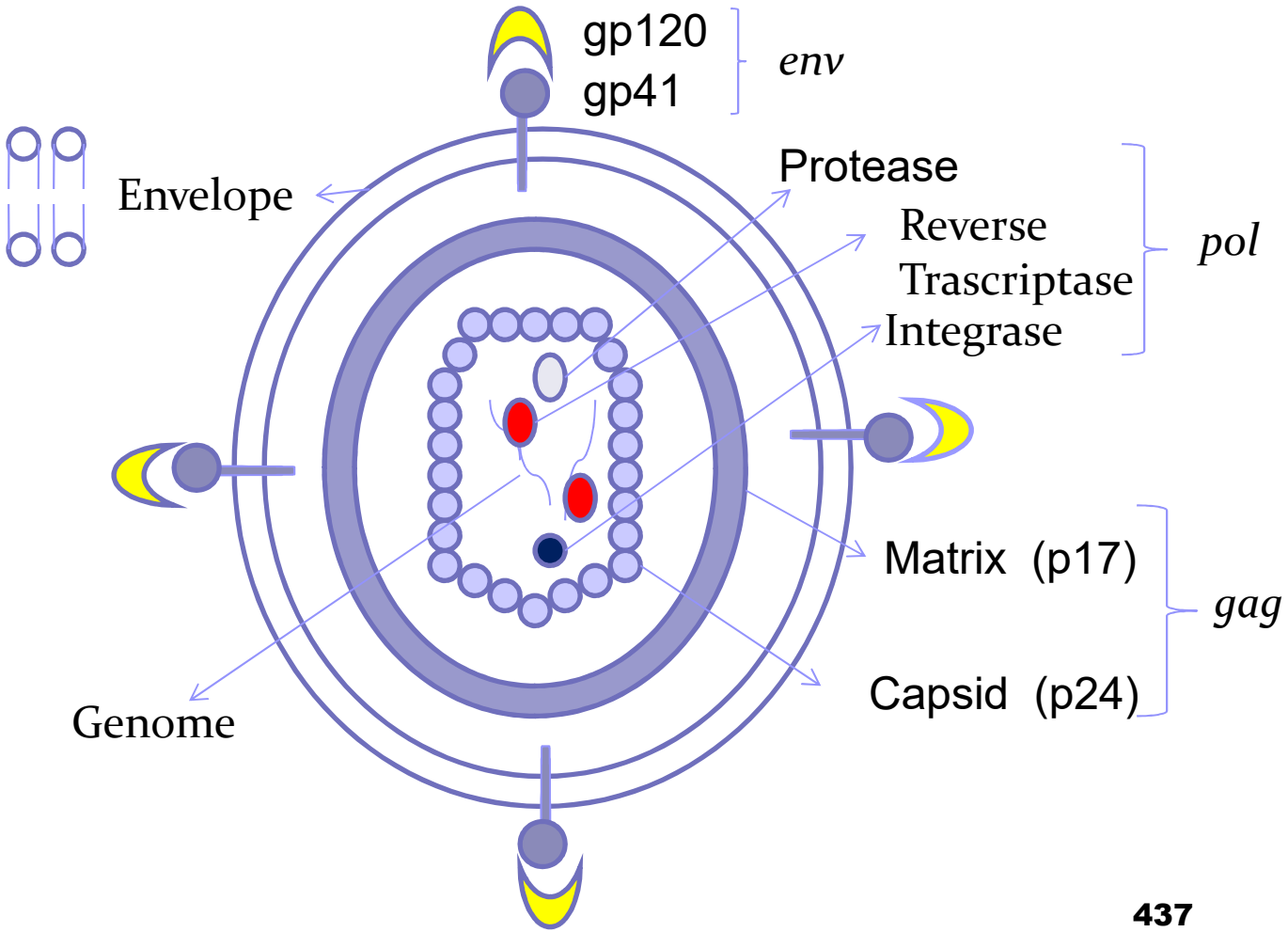


HIV structure

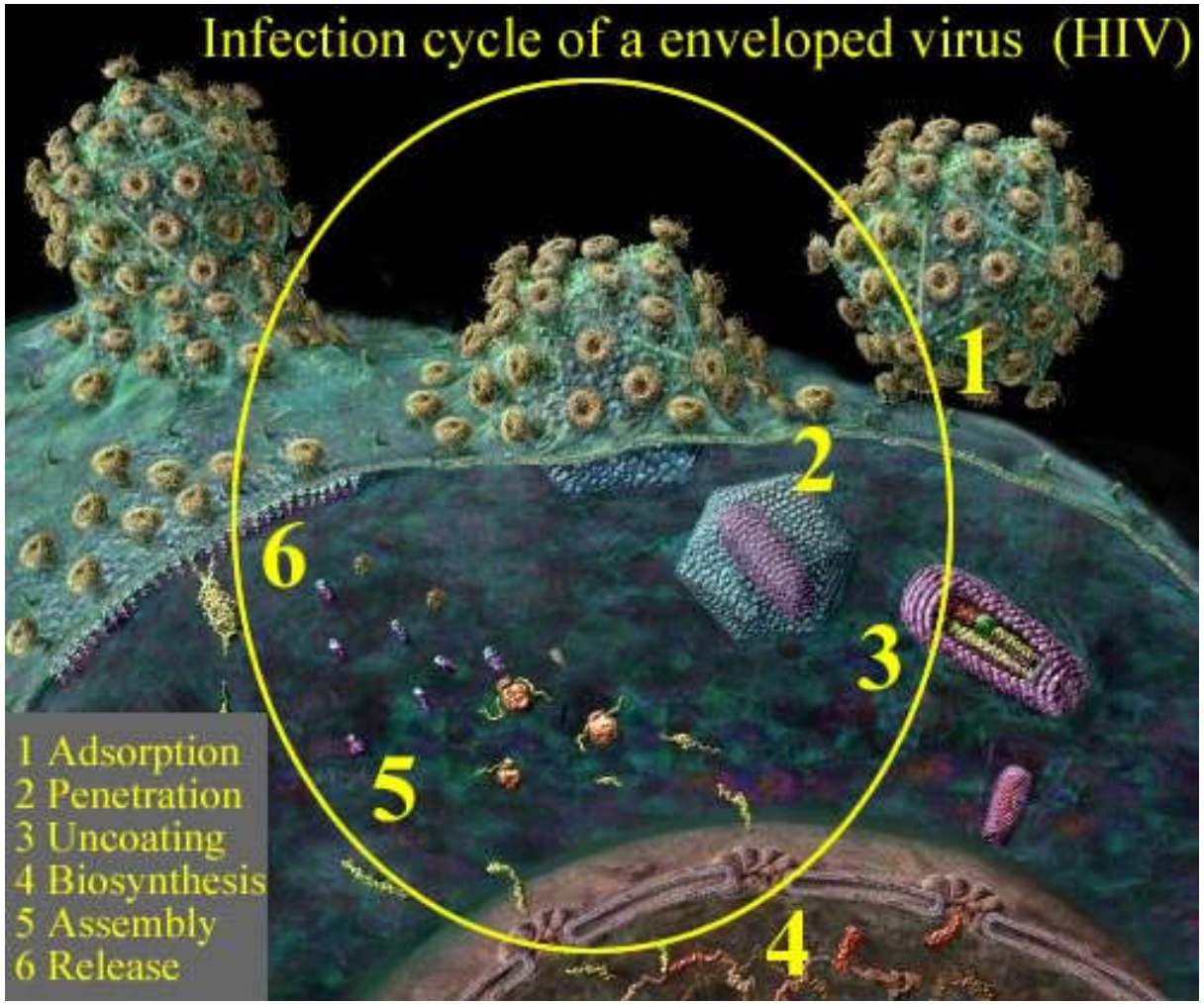


Acquired Immunodeficiency Syndrome (AIDS)

Structure of HIV-1



Viral life cycle

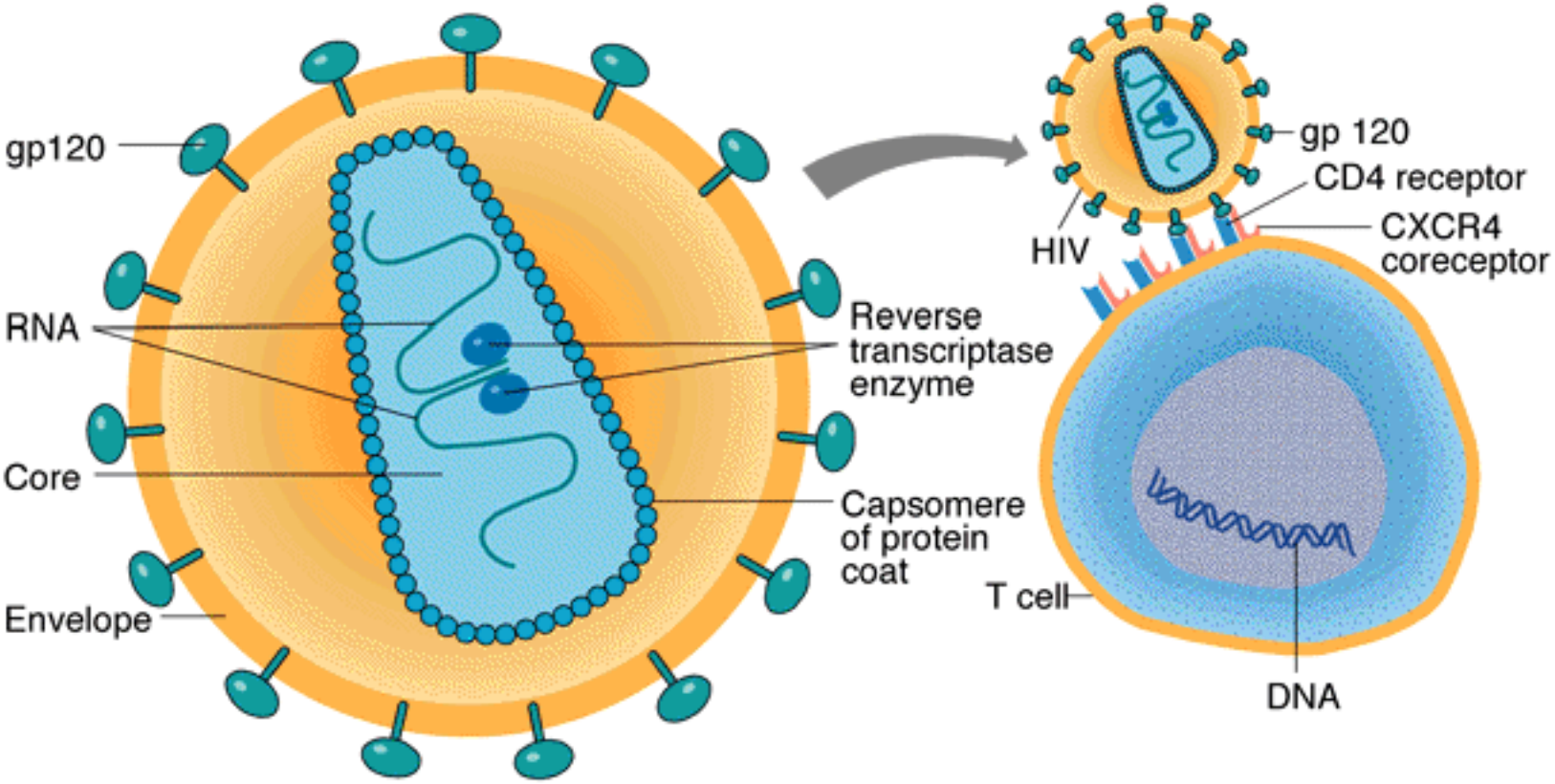


Membrane fusion of the viral particle with the host cell occurs and the viral nucleocapsid enters cell

Life Cycle of HIV

1. Attachment: Virus binds to surface molecule (CD4) of T helper cells and macrophages.
   - Coreceptors: Required for HIV infection.
   - CXCR4 and CCR5 mutants are resistant to infection.
2. Fusion: Viral envelope fuses with cell membrane, releasing contents into the cell.

HIV Life Cycle: Attachment Requires CD4 Receptor plus a Coreceptor

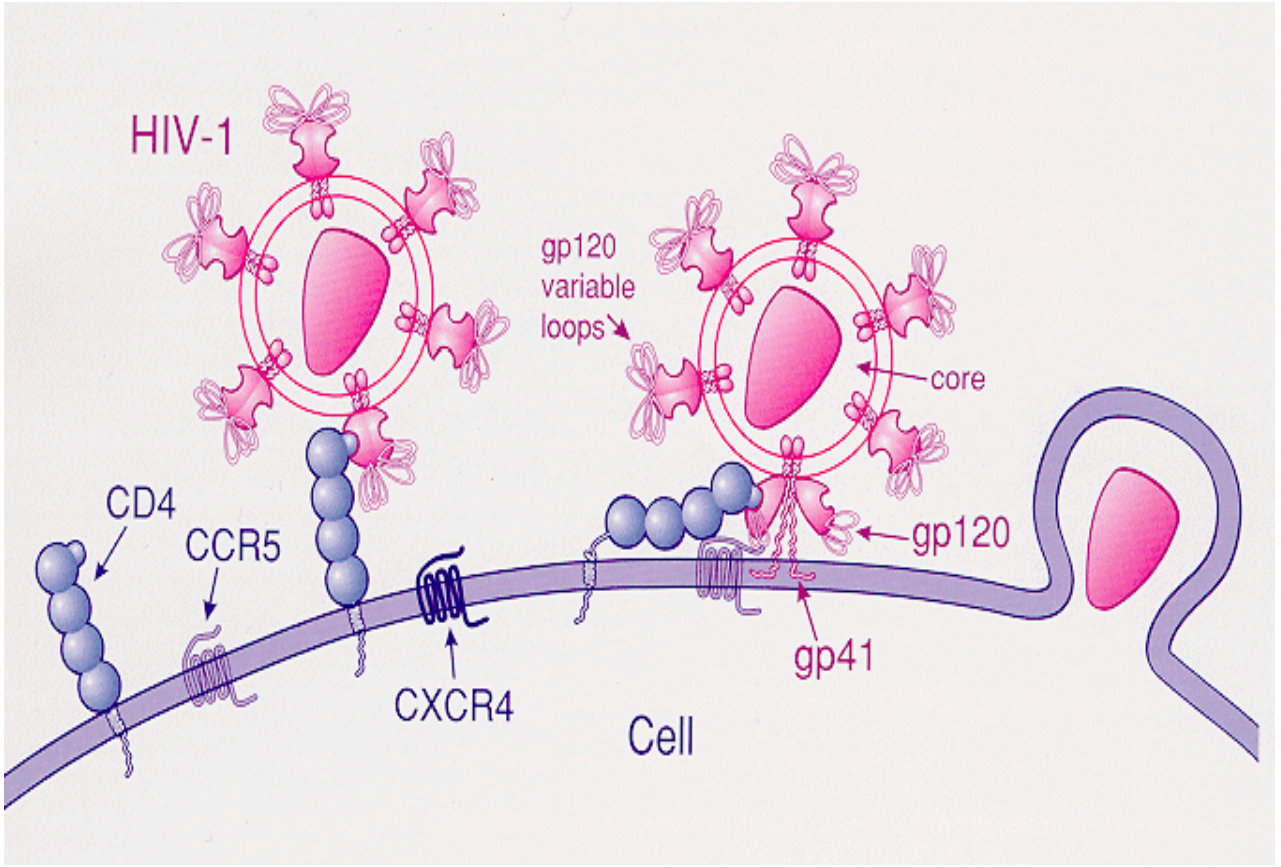


(a) Structure of HIV

(b) HIV infecting a T cell with CD4 receptors, and CXCR4 coreceptors which are distributed over the surface of the cell

Attachment to the Host Cell Membrane

- **CD4 is the primary cell receptor for HIV binding**
- **A chemokine receptor is the co-receptor for binding**



Life Cycle of HIV

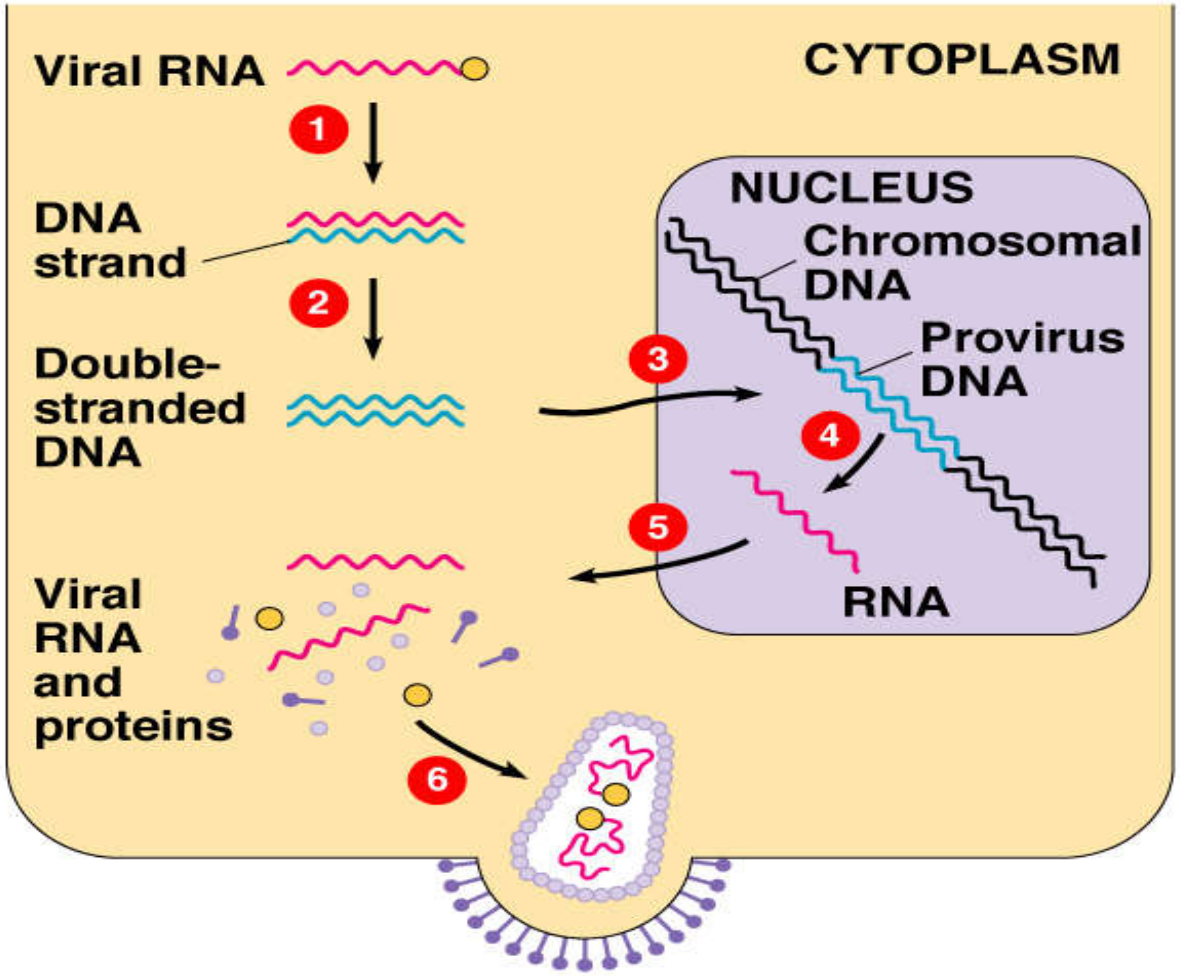
3. Reverse Transcription: Viral RNA is converted into DNA by unique enzyme *reverse transcriptase*.

Reverse transcriptase
RNA ----------> DNA

Reverse transcriptase is the target of several HIV drugs: AZT, ddI, and ddC.

HIV Life Cycle:

Reverse Transcriptase Converts RNA into DNA



Life Cycle of HIV

4. Integration: Viral DNA is inserted into host cell chromosome by unique enzyme *integrase*. Integrated viral DNA may remain latent for years and is called a *provirus*.

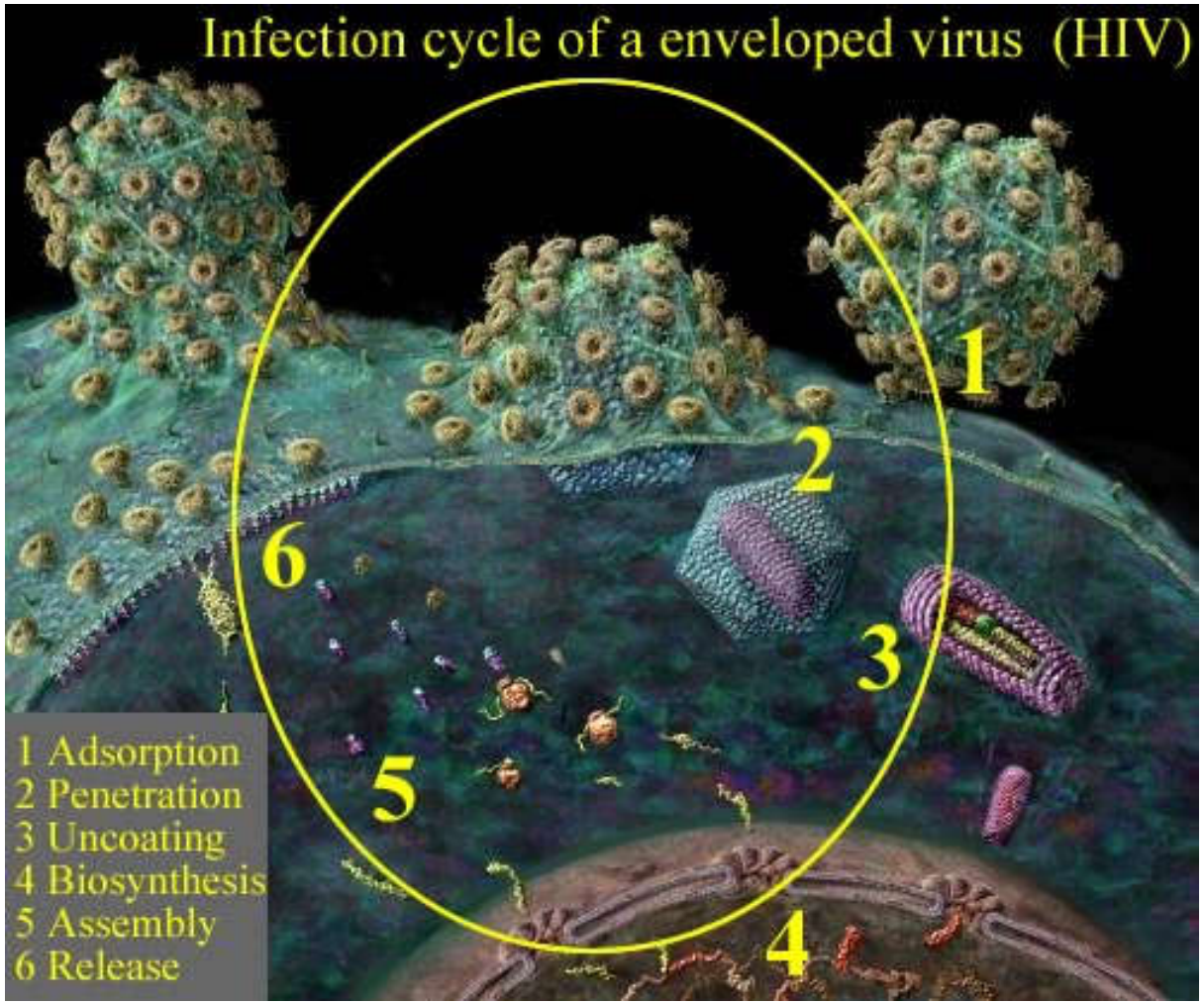
5. Replication: Viral DNA is transcribed and RNA is translated, making viral proteins.

Viral genome is replicated.

6. Assembly: New viruses are made.

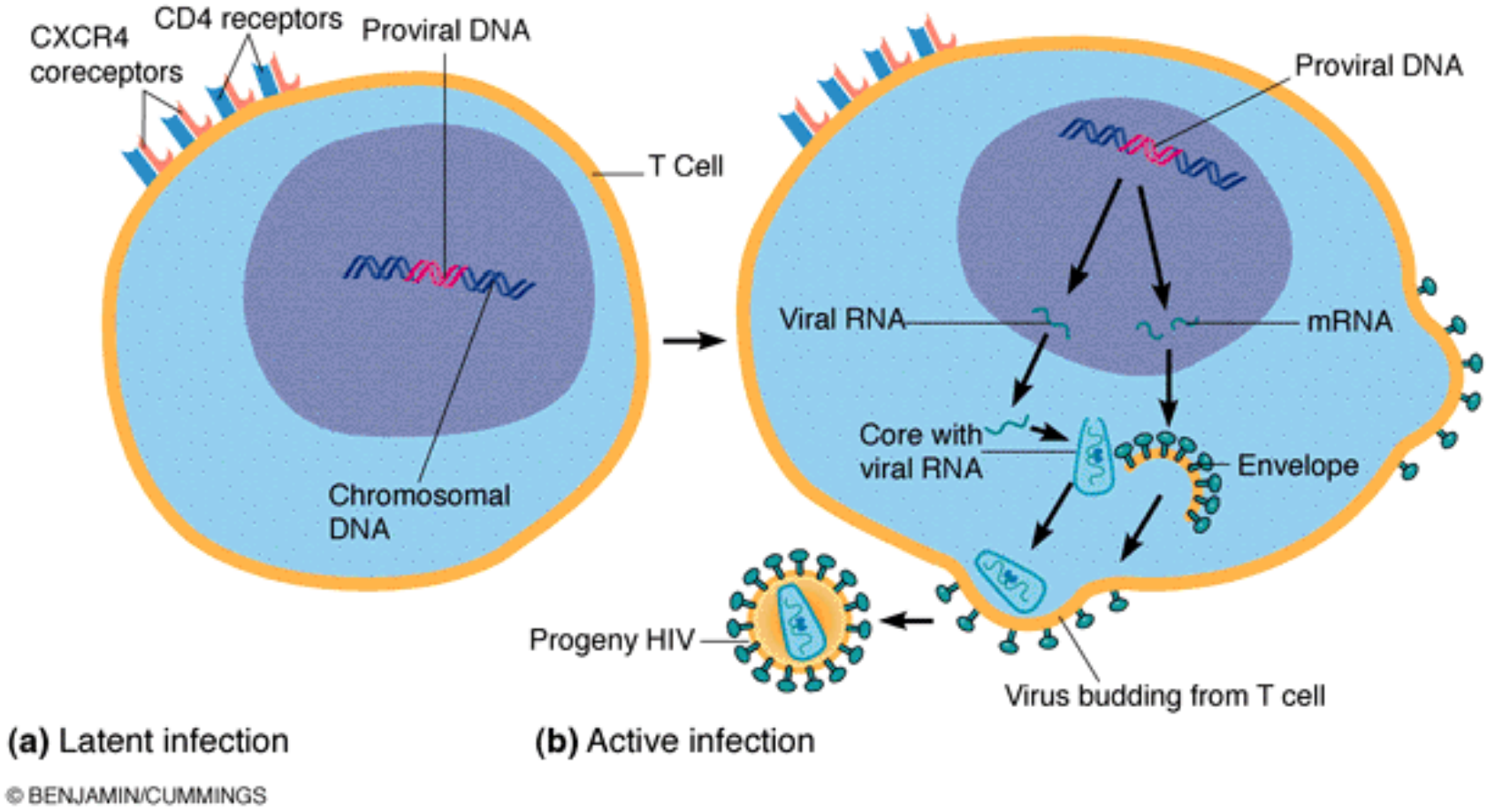
7. Release: New viruses bud through the cell membrane.

Viral life cycle

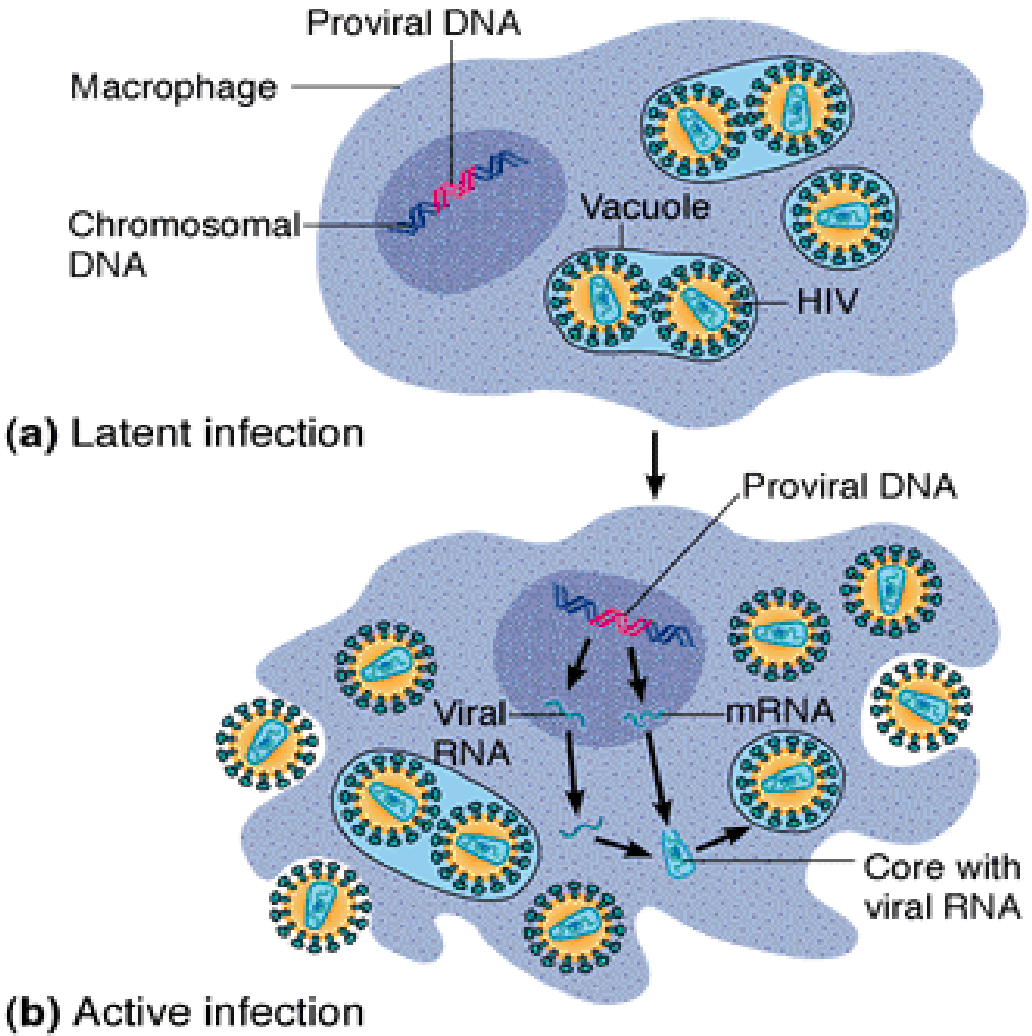


Membrane fusion of the viral particle with the host cell occurs and the viral nucleocapsid enters cell

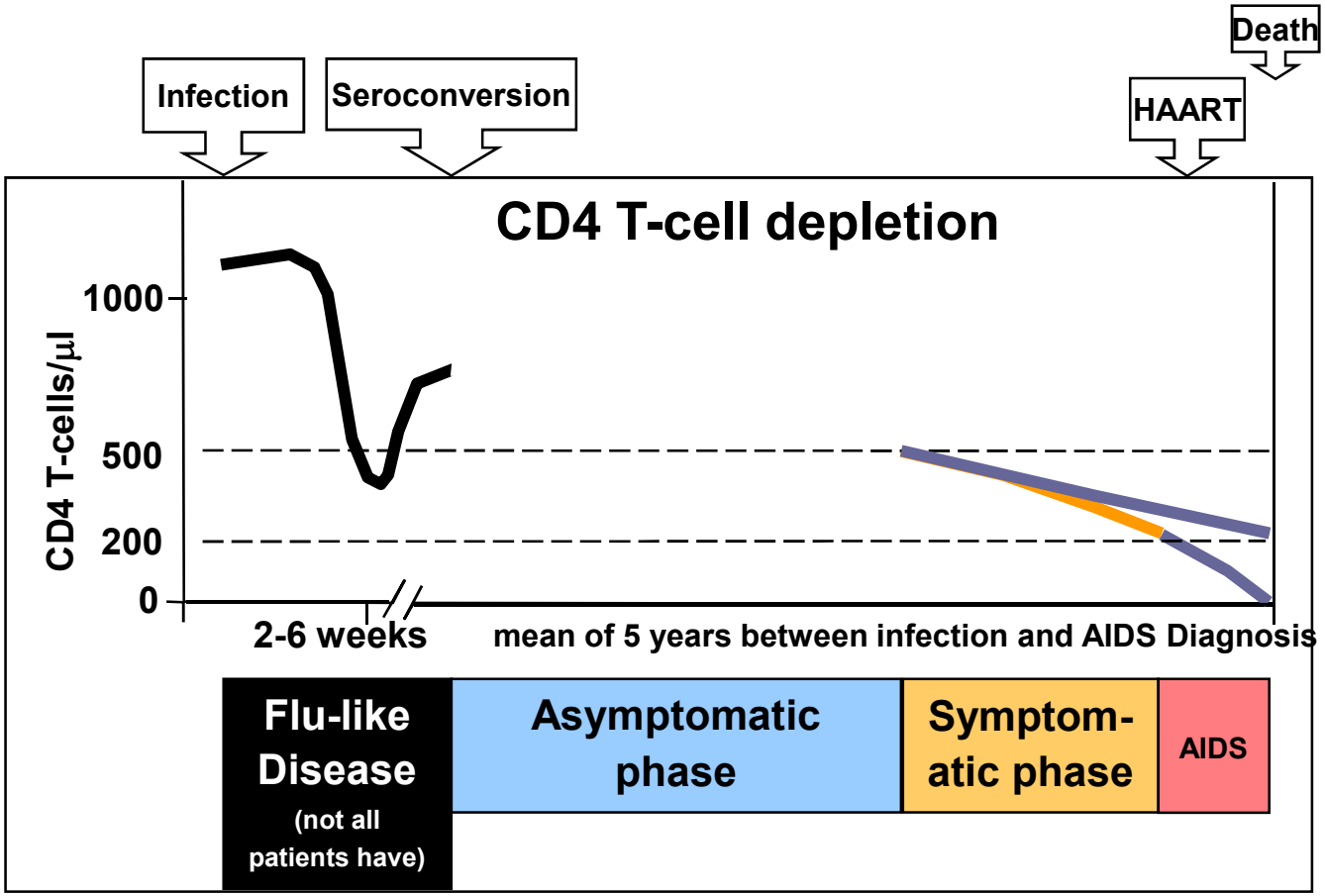
HIV Life Cycle:
Latent versus Active Infection



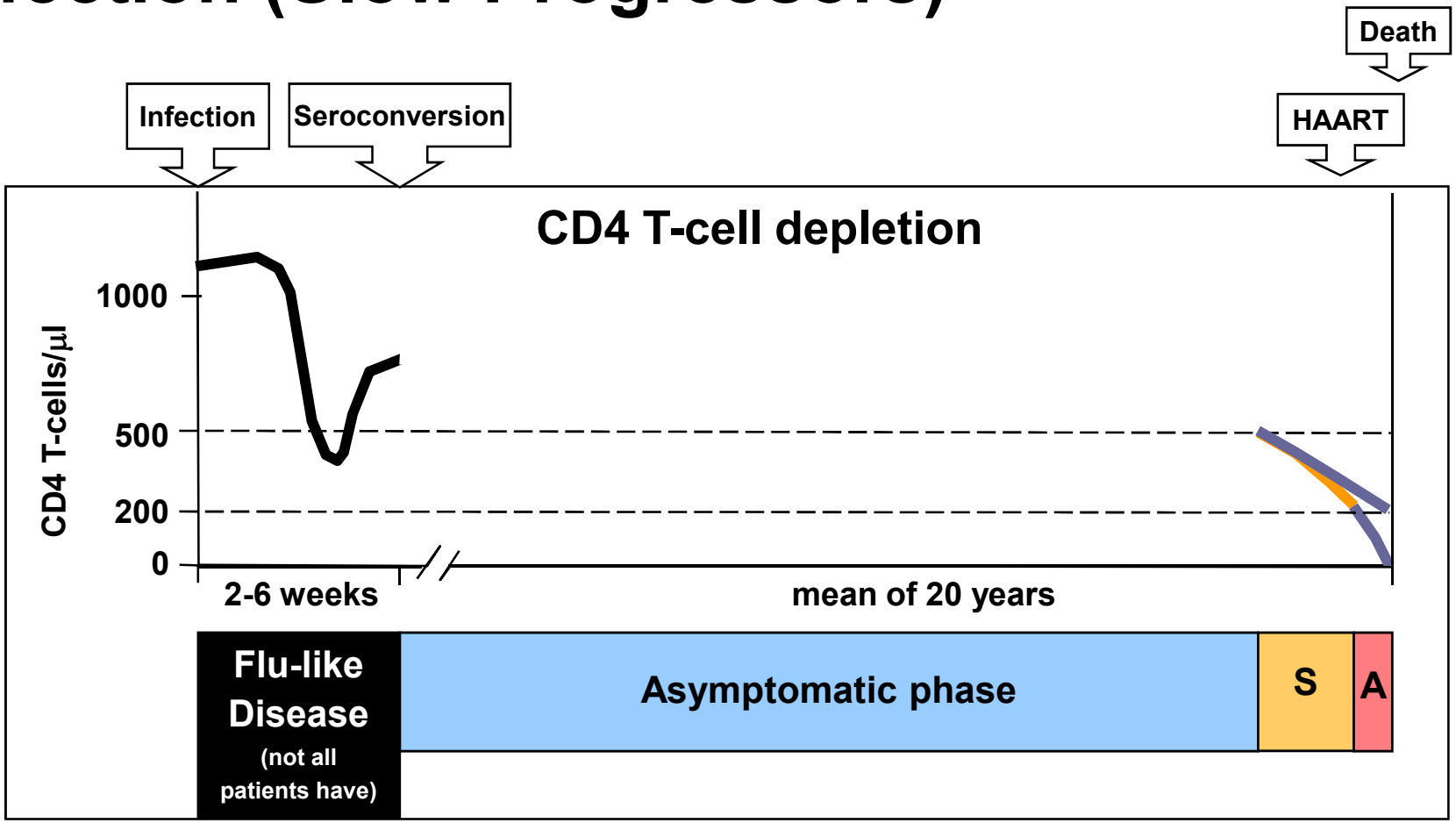
HIV Life Cycle: Latent versus Active Infection in Macrophages



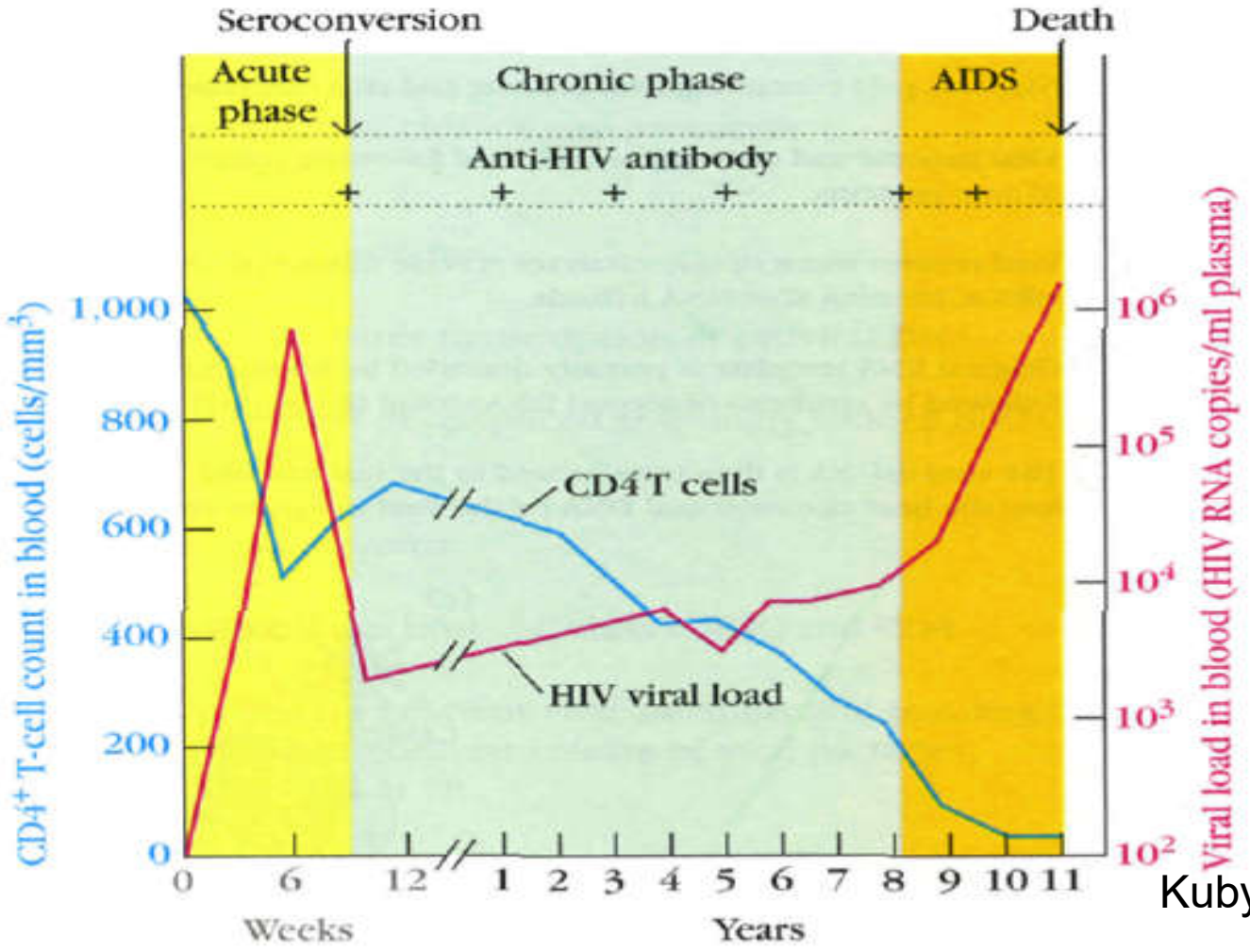
Clinical Consequences of HIV Infection (Fast Progressor)



Clinical Consequences of HIV Infection (Slow Progressors)



Serological Profile



Kuby, 2007

Immunological Abnormalities

- Infection and destruction of dendritic cells, macrophages and Th cells
- Late decrease in Th cell numbers (200/mm^3 blood)
- Resistance to HIV in individuals
  - CCR5D32
  - Some HLA types (HLA-A2 are resistant; HLA-B35 are susceptible)

Virus-induced effects on immune cells

- **Th Cells**
  - Initially control viral load
  - Destruction of infected Th cells by CTL
  - Cytopathic virus
  - Anergy of surviving Th cells
- **CTL**
  - gp120 specific CTL
  - Virus mutation induces resistance to CTL
  - Lack of Th affects CTL activation
  - Resistance to CTL by down regulation of class I MHC on target cells
- Ab
  - Develops after 3 weeks
  - Virus Agic variability
  - Non-neutralizing. Thus, ineffective

Laboratory Diagnosis

- Diagnosis is important for
  - prevention of spread
  - Care efforts
    - Short course of ART decreases spread from infected mothers to infants
    - In tuberculosis patients knowledge of and therapy for HIV can decrease morbidity

Genetic Diversity of HIV Strains Important in Testing

- West Africa
  - HIV-1 CRF_02 and HIV-2 predominate
- Central Africa
  - mixture of subtypes, CRFs, group O and N
- East Africa
  - subtypes A,C and D
- Southern Africa
  - Serotype C
- Important that screening test is sensitive for the HIV found in the area.

Serodiagnosis

- EIA
  - Now at 4th generation tests- sensitivity and specificity very good
  - Solid phase coated with recombinant antigens and/or peptides and similar antigens conjugated to a detecting enzyme
  - IgG and IgM detected (detection of IgM may reduce the 2-4 week window period)
  - Antigens used are mixtures of HIV-1 group M and O and HIV-2

ELISA

- First used viral lysates
  - these contained host HLA Ag,
  - caused some false positive reactions
- Next tests use recombinant or synthetic Ag, but sensitivity less,
- so next used these Ag but used Ag as a capture for patient Ab, then added enzyme labeled Ag
- Fourth generation assays simultaneously detect Ab and p24 antigen

Antibody Detection by ELISA

- Important screening assay
  - easy
  - useful for large sample numbers
  - highly sensitive and specific
  - useful for blood product screening
  - useful for diagnosing and monitoring patients
  - useful for determining disease prevalence
  - useful for research

ELISA

- False negative reactions
  - due to lack of Ab early in disease
  - B cell dysfunction
    - in advanced AIDs
- single sample tested first, then if positive retested in duplicate
  - if 2 of 3 are reactive, confirm results by another method
  - Western blot
  - Other assay

Rapid Assays – 20 minutes or less

- Types
  - Agglutination
  - Immunofiltration
  - Immunochromatographic
- High sensitivity and specificity

HIV Testing Algorithm

- Algorithm 1
  - EIA or other test
  - Confirmed by western blot (WB)
  - Problem –expensive, time consuming
  - WB requires technical expertise
- Algorithm 2
  - Two different assays used- neither is WB
  - First test very sensitive
  - Second test very specific
  - Draw enough sample for both tests

T Cell Enumeration

- First introduce the local normal range or text book if no local info
- CD4 count of less than 500/microliter = immunosuppression
- AIDS diagnosis after count drops below 200
  - lack of CD4+ cell allows in opportunistic infections
- AIDs also if % CD4+Tcells of total lymphocytes is less than 14%

Western Blot Testing

- Confirmatory test
- Antibody profile given to a number of Ag
- Viral lysate separated into components by PAGE electrophoresis

Figure:

Examples of reactions by an HIV-1 Western blot:

1. Positive control (strong)
2. Positive control (weak)
3. Negative control
4. Indeterminate profile
5. Indeterminate profile (highly suggestive)

Western Blot

- Abs to p24, p55 appear first then decrease
- Abs to gp31, gp41, gp120, gp160
  - remain throughout disease
  - reliable disease indicators
- Abs to p51 and p66
- pure HIV Ag important in Western blot assay
  - p17, p24, p31, gp41, p51, p55, p66, gp120 and gp160 must be present for a valid assay.

Interpretation of Western Blots

- Negative no bands at locations of viral Ags
- positives
  - standard is that there must be Ab to two of the major Ags, p24,p31,gp41, gp120/160
  - if a specimen has positive bands but doesn't follow above rule then the result is indeterminate
    - early infections may do this
    - healthy false positive individuals may do this
    - Autoimmune diseases

Detection of HIV Ag

- Research use since 1988
- purpose- to shorten the window period from infection to detection
  - p24 Ag precedes the Ab by several weeks
  - but p24 Ag can only be detected a short time
  - p24 Ag disappears as Ab develops
- Principle of this assay is a sandwich EIA:
  - anti-p24 on solid support. Add patient sera. If Ag binds, then the detecting Ab which is enzyme labeled will bind
  - confirm positives with a neutralization assay
    - add anti-p24 to patients sera before incubating with solid support. This should inhibit the reaction

p24 Ag testing

- Not useful as a primary screening tool
- Its appearance and rate of rise are unpredictable
- It disappears and may not reappear for years
- Low sensitivity
- Useful
  - if early infection is suspected but Ab is negative
  - to detect infection in the newborn
  - to check CSF in patients with dementia
  - monitoring the disease progression
    - Ag reappears when AIDS related infections start
    - Ag-Ab complexes prevent Ag from being detected
    - heat 56 - 60 min to break complexes and improve sensitivity

Polymerase chain reaction (**PCR)**

- can amplify small amounts of viral nucleic acid so they can be detected by hybridization with nucleic acid probes
- mononuclear cells from patient separated by ficoll hypaque gradient centrifugation
- cells are then lysed and double stranded DNA is separated into single strands
- primers are added which are specific for viral DNA
  - addition of DNA polymerase and nucleotides causes amplification of viral DNA
  - alternately heated and cooled to allow for amplification, dissociation reannealing with primers and amplification etc again 30 cycles

PCR continued

- Amplification can result in 1-10 copies of the viral DNA in a million copies being amplified until it is detectable
- Primers are to a highly conserved region present in all samples of HIV
  - gag and pol gene areas
- After amplification the sample is placed on nitrocellulose and is separated into ss again
- A radio labeled or enzyme labeled probe is added, if radioactivity/ color formation is present after a wash step, viral DNA was present

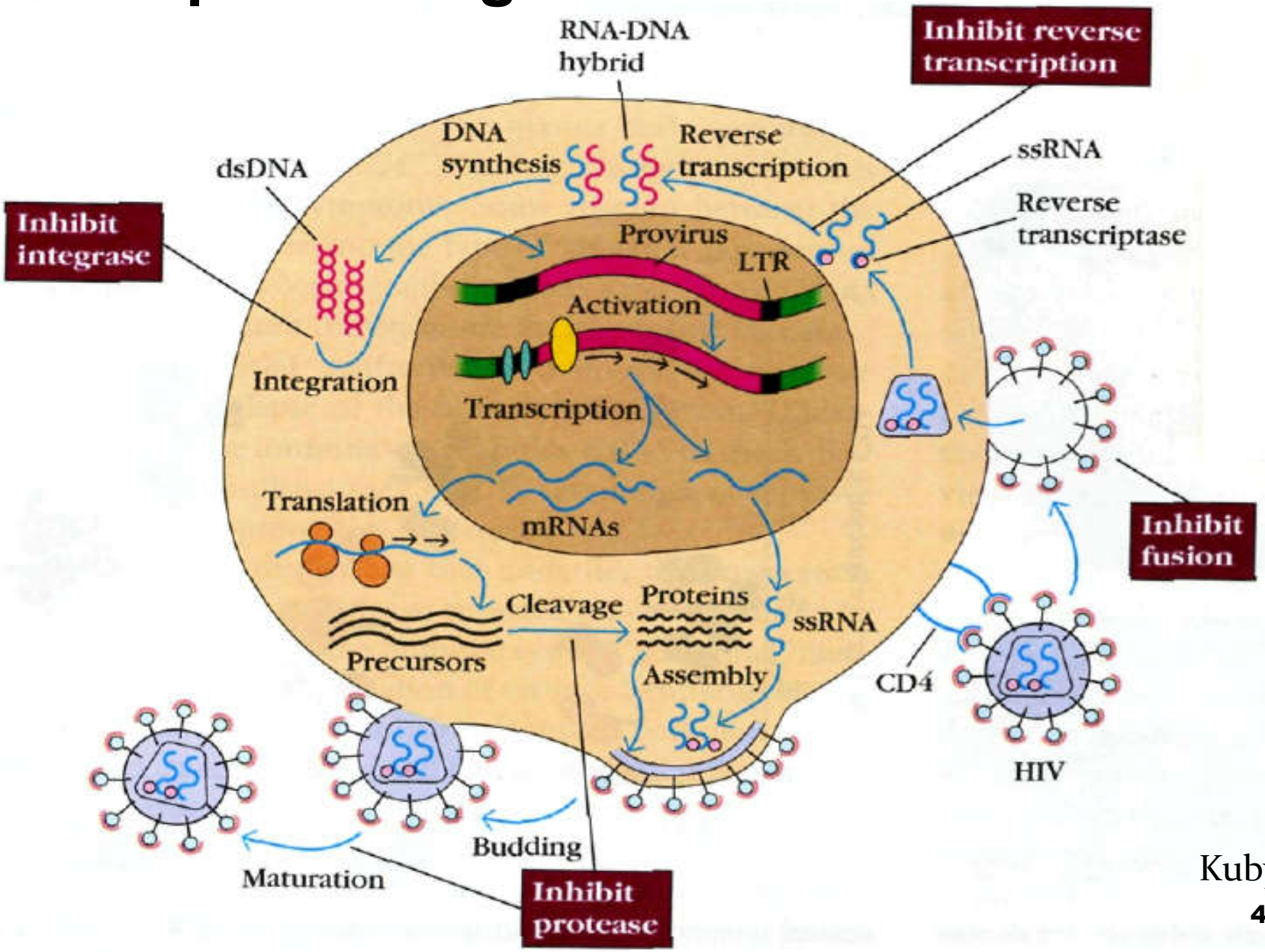
Evaluation of PCR Testing

- Very sensitive
- can detect non replicating viral genomes
- 1-2 ml only needed so ideal for newborns
- useful in window period
- useful for typing HIV-1 and HIV-2
- false positives if carry over from previous samples
  - if Ab negative but PCR positive repeat PCR
  - not used for routine screening
  - used in therapy efficacy measurements

Testing of Neonates

- IgGs in baby are from mother for first few months and don't indicate infection
- Baby will fare better if treated right away
- Detect IgM or IgA after removal of IgG by incubation with beads that bind it
- Use anti-Ig conjugate specific for the heavy chain
- Not sensitive in first 3 months of life
- p24 Ag detection after disrupting Ag-Ab complexes with dilute HCl
- PCR testing - some positive by 4 days, few not positive until 6 months

Therapeutic targets



Kuby, 2007

Therapy

- Reverse Transcription Inhibitors
  - Nucleoside RT inhibitors e.g. Zidovudine or AZT (azidothymidine)
  - Non-nucleoside RT inhibitors e.g. nevirapine (Inhibit RT)
- **Protease inhibitors (proteases cleave precursor proteins into proteins that are needed for virion assembly)**
- **Integrase inhibitors (integration of provirus in the cell DNA e.g. ritonavir)**
- **Entry/Fusion inhibitors e.g. enfuvirtide**
- **HAART (Highly active anti-retroviral therapy) – combination**

Problems:

Anemia

Virus in brain, BBB – not penetrated by drugs

Antigenic variation

High costs

Summary

- Primary immunodeficiencies are inherited
- They can affect hematopoietic stem cells, lymphoid or myeloid cells.
- Secondary immunodeficiencies are due to infections, aging, cancer or chemical exposure
- HIV affects immune system by eliminating CD4+ T cells
- Vaccine development has been hindered by lack of an experimental model, antigenic variation, etc.
- There are promising candidates

CHAPTER 12

Vaccines

12.0 Vaccines

- Learning Objectives
  - Describe how vaccines work
  - List various vaccine development techniques
  - Describe the difference b/n attenuated and inactivated vaccines
  - Differentiate between passive and active immunity
  - Weight the risks of vaccines for the individual against the benefits of vaccinations for society
  - Distinguish traditional and modern vaccine development techniques

12.1 Introduction to Vaccines

- **Vaccines –**
  - biological substances that stimulate the person's immune system
  - to produce an immune response identical to that produced by the natural infection.
- Vaccines can
  - prevent the debilitating and, in some cases, fatal infectious diseases.
  - help to eliminate the illness and disability of polio, measles, and rubella

12.1 Introduction to Vaccines

- Vaccines protect the
  - vaccinated individual,
  - protect society.
- A community with many vaccinated people
  - protects the few who cannot be vaccinated—such as young children.
  - indirectly protects unvaccinated from exposure to disease).= HERD IMMUNITY

- **Aim of an ideal vaccine:**
  - To produce the same immune protection which usually follows natural infection but without causing disease
  - To generate long-lasting immunity
  - To interrupt spread of infection

12.0 Introduction to Vaccine Development

- Differences in epitopes recognized by T cells and B cells has enabled to design vaccines that maximized activation of both immune system arms.
- Differences in antigen-processing pathways became evident, used techniques to design vaccines and to use adjuvants that maximize antigen presentation with class I or class II MHC molecules
- Genetic engineering techniques can be used to develop vaccines to maximize the immune response to selected epitopes and to simplify delivery of the vaccines.

12.2 Criteria for Effective Vaccines

- The World Health Organization (WHO) has stated that the ideal vaccine would have the following properties:
  - Affordable worldwide
  - Heat stable
  - Effective after a single dose
  - Applicable to a number of diseases
  - Administered by a mucosal route
  - Suitable for administration early in life

12.2 Active and Passive Immunization

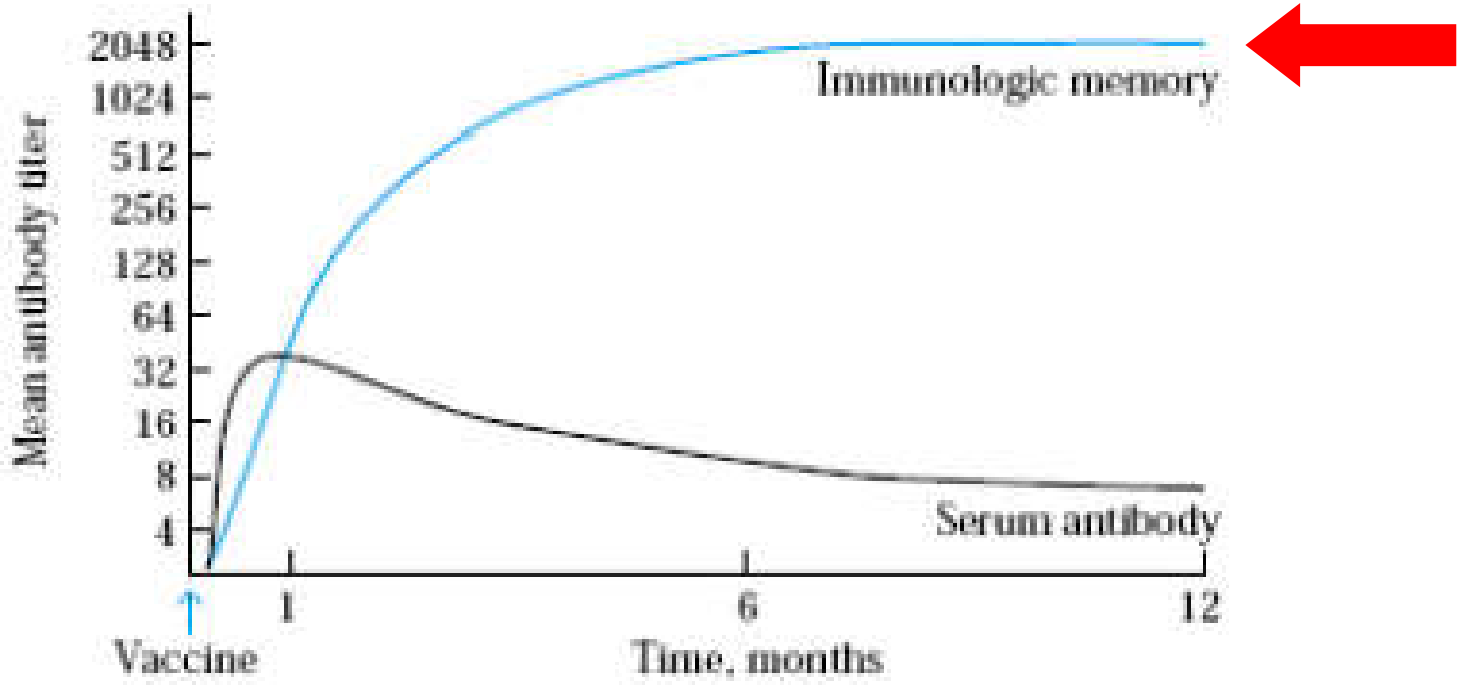
- Many diseases stimulate an immune response in host,
  - those who survive the disease are protected from second infection – **natural acquired active immunization**
  - the risk is many die before becoming immune
- **Vaccinations** uses
  - **Artificially acquired active immunity** - is stimulated by initial exposure to specific foreign macromolecules through the use of vaccines, to artificially establish state of immunity.
  - Individuals, who have not had the disease, can be protected even when exposed at later date

12.2 Active and Passive Immunization continued

- **Limitations of Active immunity**
  - developing an immune response does not = achieving state of protective immunity
  - vaccine can induce primary response but fail to induce memory cells = host unprotected
- Various approaches used to effectively induce humoral and cell-mediated immunity and the production of memory cells.

12.2 Active and Passive Immunization continued

Ideal Vaccination Response



[From M. Zanetti et al., 1987, Immunol. Today 8:18.]

12.2 Active and Passive Immunization continued

- **Artificially acquired passive immunity**;
  the individual receives protective
  - molecules (anti bodies)Tetanus Anti-toxoid or
  - cell (lymphocytes) produced in another individual.

- **Naturally acquired passive immunity**
  refers to antibodies transferred from mother to featus across the placenta and to the newborn in colostrum and breast milk during the first few months of life.

- Passive immunization does not activate the immune system, it generates no memory response and the protection provided is transient

12.3 Designing Vaccines for Active Immunization

- Many common vaccines use
  - **inactivated** (killed), but still antigenic or
  - live/altered – **attenuated** microorganisms.
    - Caused to loose pathogenicity (cultured in abnormal conditions)
  - substance (e.g., protein, polysaccharide) from pathogen, capable of producing an immune response
- **DNA vaccines** currently being tested for human use

12.4 Whole-Organism Vaccines

- Attenuated organisms, for vaccines, lose ability to cause significant disease (pathogenicity) but
  - to attenuate, grow a pathogenic bacterium or virus for prolonged periods under abnormal culture conditions
  - retains capacity for short term growth within inoculated host.
  - capacity for transient growth, permits prolonged immune-system exposure to attenuated epitopes, increased immunogenicity and production of memory cells.
    - As a consequence, these vaccines often require only a single immunization.
    - A major disadvantage is the possibility that they will revert to a virulent form.

12.5 Purified Macromolecules as Vaccines

- Derived from pathogens.
- Are specific, purified macromolecules .
- Avoid some risks associated with attenuated or killed whole organism vaccines.
- Three general forms of such vaccines are in current use:
  - inactivated exotoxins,
  - capsular polysaccharides, and
  - recombinant microbial antigens

12.5 Purified Macromolecules as Vaccines

PURIFIED MACROMOLECULES	
Toxoids	
Diphtheria	Inactivated exotoxin
Tetanus	Inactivated exotoxin
Capsular polysaccharides	
Haemophilus influenzae type b	Polysaccharide + protein carrier
Neisseria meningitidis	Polysaccharide
Streptococcus pneumoniae	23 distinct capsular polysaccharides
Surface antigen	
Hepatitis B	Recombinant surface antigen (HBsAg)

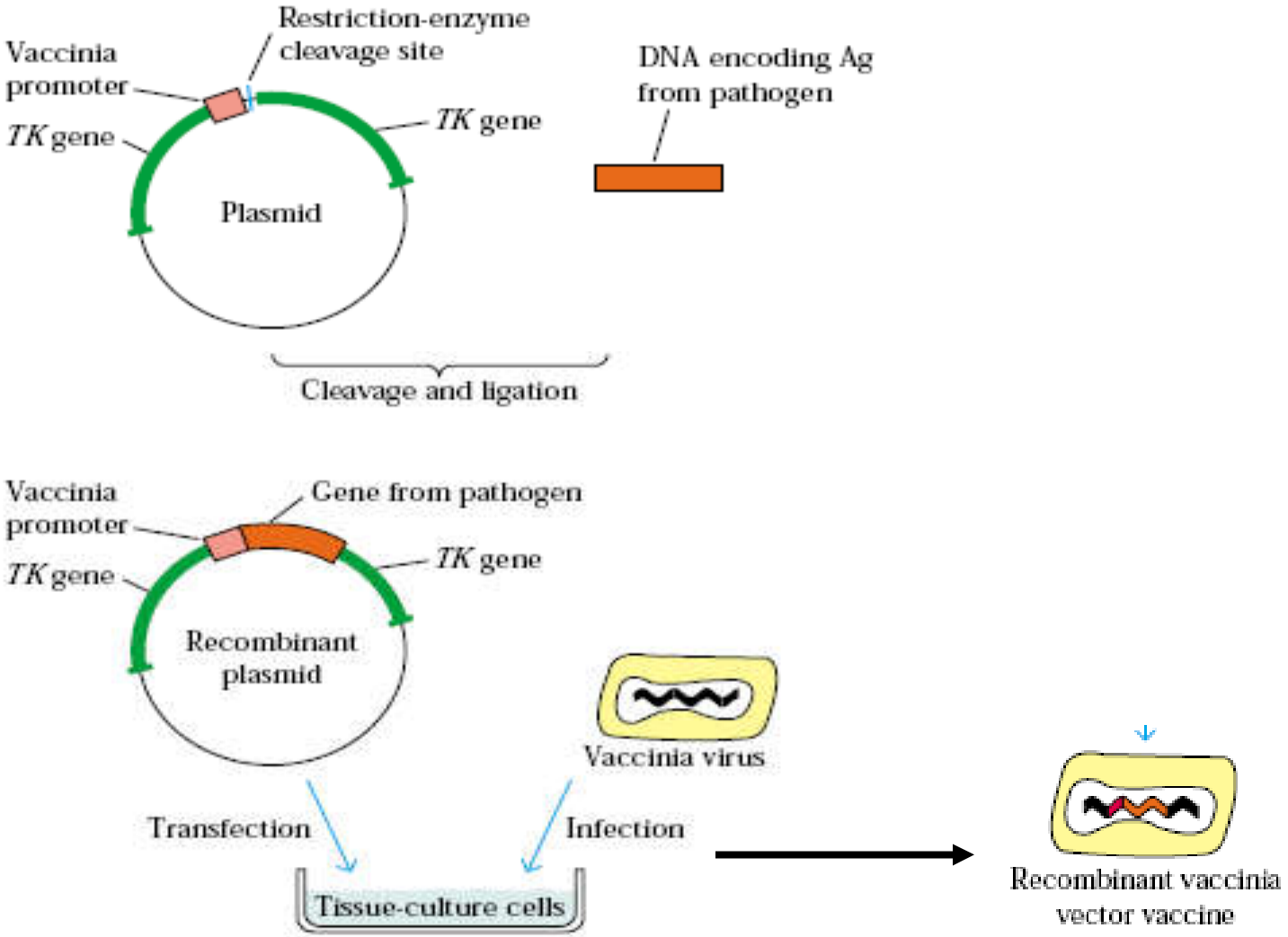
12.5 Purified Macromolecules as Vaccines

- Isolate gene encoding immunogenic protein, clone it, and express/insert in bacterial, yeast, or mammalian cells using recombinant DNA technology.
- Example for human use is hepatitis B vaccine developed by cloning the gene for surface antigen of hepatitis B virus (HBsAg) and expressing it in yeast cells.
- The recombinant yeast cells are grown in large fermenters, and HBsAg accumulates in the cells.
- The yeast cells is disrupted, releases the recombinant HBsAg, which is purified by biochemical techniques.

12.6 Recombinant-Vector Vaccines

- Genetic engineering techniques are a way to attenuate a virus irreversibly by selectively removing genes that are necessary for virulence.
- Genes encoding major antigen of virulent pathogens can be added in high levels to attenuated viruses or bacteria.
- The attenuated organism serves as a vector, replicating within the host and expressing the gene product of the pathogen.
- Vaccinia virus, attenuated vaccine used to eradicate smallpox, was widely employed as a vector vaccine.

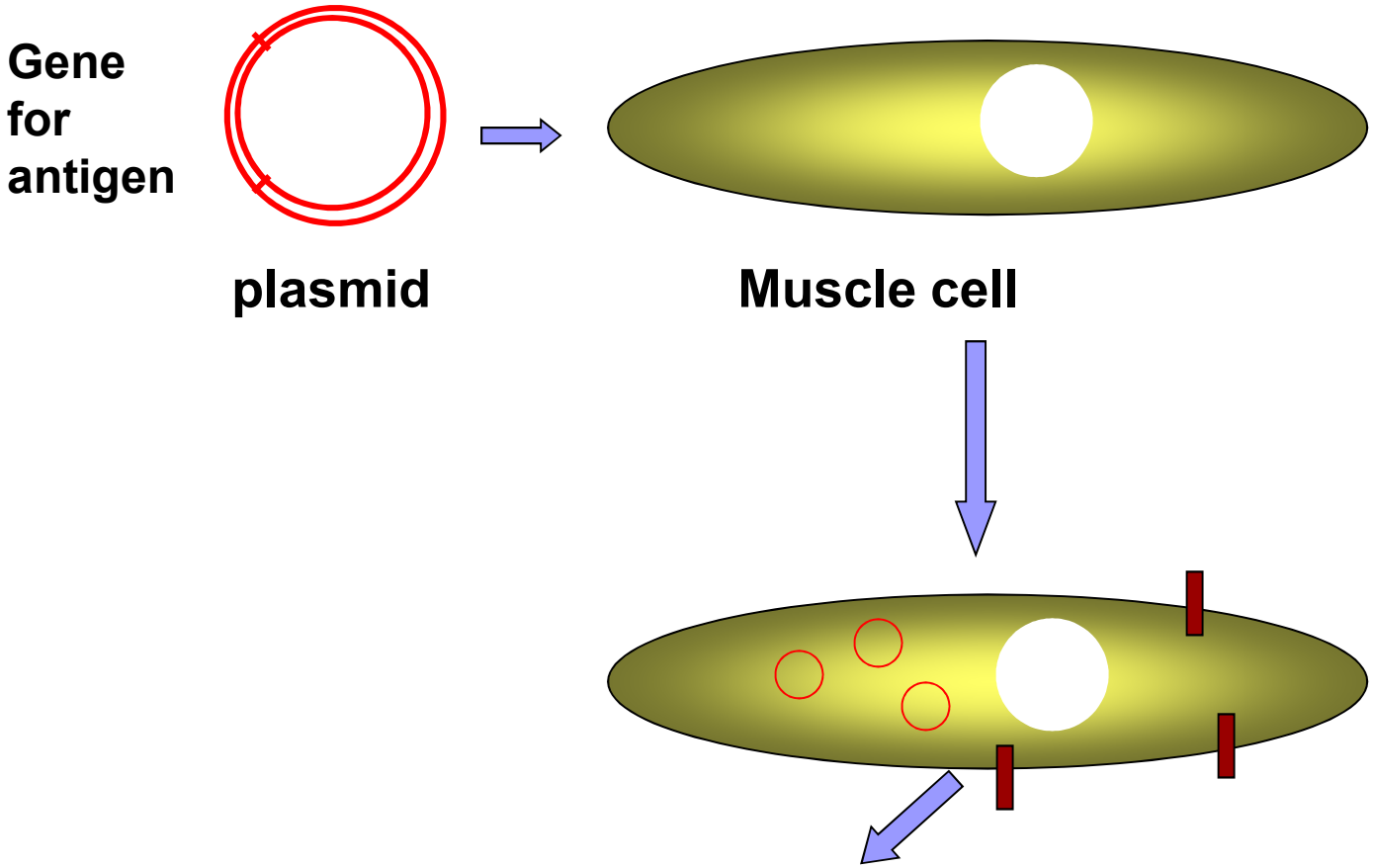
12.6 Recombinant-Vector Vaccines



12.7 DNA Vaccines

- Plasmids are easily manufactured in large amounts
- DNA is very stable, resists temperature extremes so storage and transport are straight forward
- DNA sequence can be changed easily in the laboratory. So can respond to changes in the infectious agent
- The DNA is injected into a person's muscle. It integrates into the sysnthesis of the muscle cell, stimulating a strong Tc cell response with good memory.

12.7 DNA Vaccines



Muscle cell expresses protein; antibody's made; & CTL response

12.7 DNA Vaccines

DNA vaccines produce a situation that reproduces a virally-infected cell

Gives:

- **Broad based immune response**
- **Long lasting CTL response**

Advantage of new DNA vaccine for flu:

CTL response can be against internal protein

In mice a nucleoprotein DNA vaccine is effective against a range of viruses with different hemagglutinins

12.7 DNA Vaccines

- Mixtures of plasmids encoding many different viral protein fragments can produce a broad spectrum vaccine
- plasmid does not replicate. It encodes only proteins of interest
- Vector has no protein component to stimulate an immune response.
- However, there is a CTL response against the pathogen's antigens.
- These CTL responses have advantage of protection against diseases caused by certain obligate intracellular pathogens (e.g. Mycobacterium tuberculosis)

12.7 DNA Vaccines

- **Potential Risks**
  - Potential integration of plasmid into host genome leading to insertional mutagenesis
  - Induction of autoimmune responses (e.g. pathogenic anti DNA antibodies)
  - Induction of immunologic tolerance (e.g. where the expression of the antigen in the host may lead to specific non-responsiveness to that antigen)

12.8 Multivalent Subunit Vaccines

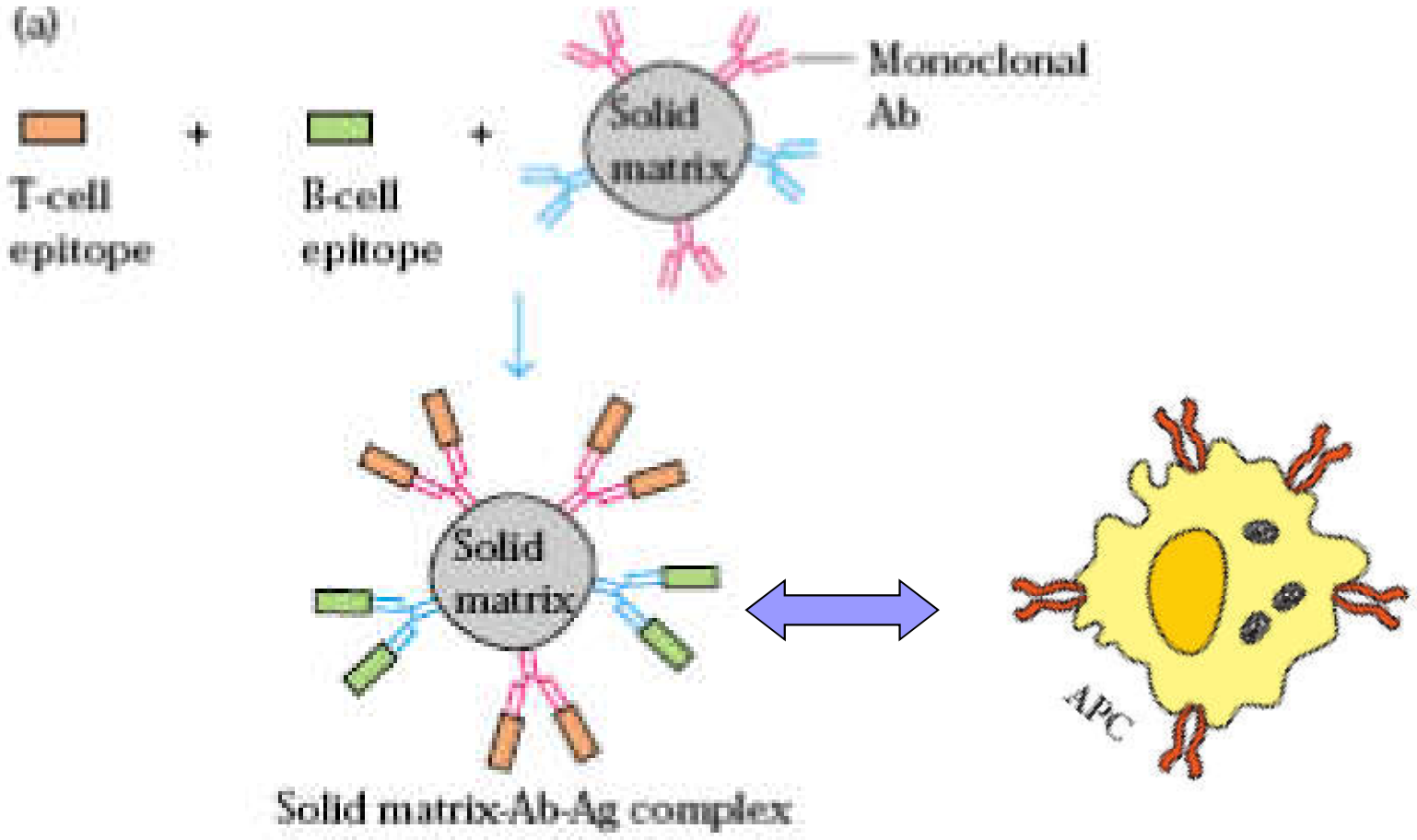
- A method for constructing synthetic peptide vaccines that contain both immunodominant for both
  - B-cell and
  - T-cell epitopes.
- If a CTL response is desired, vaccine must be delivered intra-cellularly so that
  - the peptides can be processed and
  - presented together with class I MHC molecules.

12.8 Multivalent Subunit Vaccines

- Techniques to develop multivalent vaccines that present multiple copies of a given peptide or a mixture of peptides
  - solid matrix–antibody-antigen (SMAA) complexes attaching monoclonal antibodies to particulate solid matrices and
  - then saturating the antibody with the desired antigen.
  - different specificity monoclonal antibodies on solid matrix, permits binding a mixture of peptides or proteins,

    provides immunodominant epitopes for both T cells and B cells,
  - multivalent complexes shown to induce vigorous humoral and cell-mediated responses.

12.8 Multivalent Subunit Vaccines



Vaccines - SUMMARY

- A state of immunity can be induced by passive or active immunization
  - a) Short-term passive immunization is induced by transfer of preformed antibodies.
  - b) Infection or inoculation achieves long-term active immunization.

- Three types of vaccines are currently used in humans:
  - attenuated (avirulent) microorganisms,
  - inactivated (killed) microorganisms, or
  - purified macromolecules.

Vaccines - SUMMARY

- Protein components of pathogens expressed in cell culture may be effective vaccines.
- Recombinant vectors, including viruses or bacteria, engineered to carry genes from infectious microorganisms, maximize cell-mediated immunity to the encoded antigens
- Plasmid DNA encoding a protein antigen from a pathogen can serve as an effective vaccine inducing both humoral and cell-mediated immunity.

Vaccines - SUMMARY

Characteristic	Attenuated vaccine	Inactivated vaccine	DNA vaccine
Production	Selection for avirulent organisms: virulent pathogen is grown under adverse culture conditions or prolonged passage of a virulent human pathogen through different hosts	Virulent pathogen is inactivated by chemicals or irradiation with γ-rays	Easily manufactured and purified
Booster requirement	Generally requires only a single booster	Requires multiple boosters	Single injection may suffice
Relative stability	Less stable	More stable	Highly stable
Type of immunity induced	Humoral and cell-mediated	Mainly humoral	Humoral and cell-mediated
Reversion tendency	May revert to virulent form	Cannot revert to virulent form	Cannot revert

Next Antigen Antibody reactions: Principles and Applications

CHAPTER 13

Antigen Antibody reactions: Principles and Applications

Objectives

Upon completion of this chapter student will be able to:

- Describe the nature of Ag-Ab reaction
- Compare and contrast antibody affinity and avidity
- Describe the basis for antibody specificity and cross reactivity
- Discuss principle of immunological techniques
- Explain applications of immunological techniques
- Compare and contrast the advantages and disadvantages of basic immunological techniques
- Factors affecting imunological techniques (antigen and antibody reaction)

13.1 Strength of Antigen-Antibody Interactions

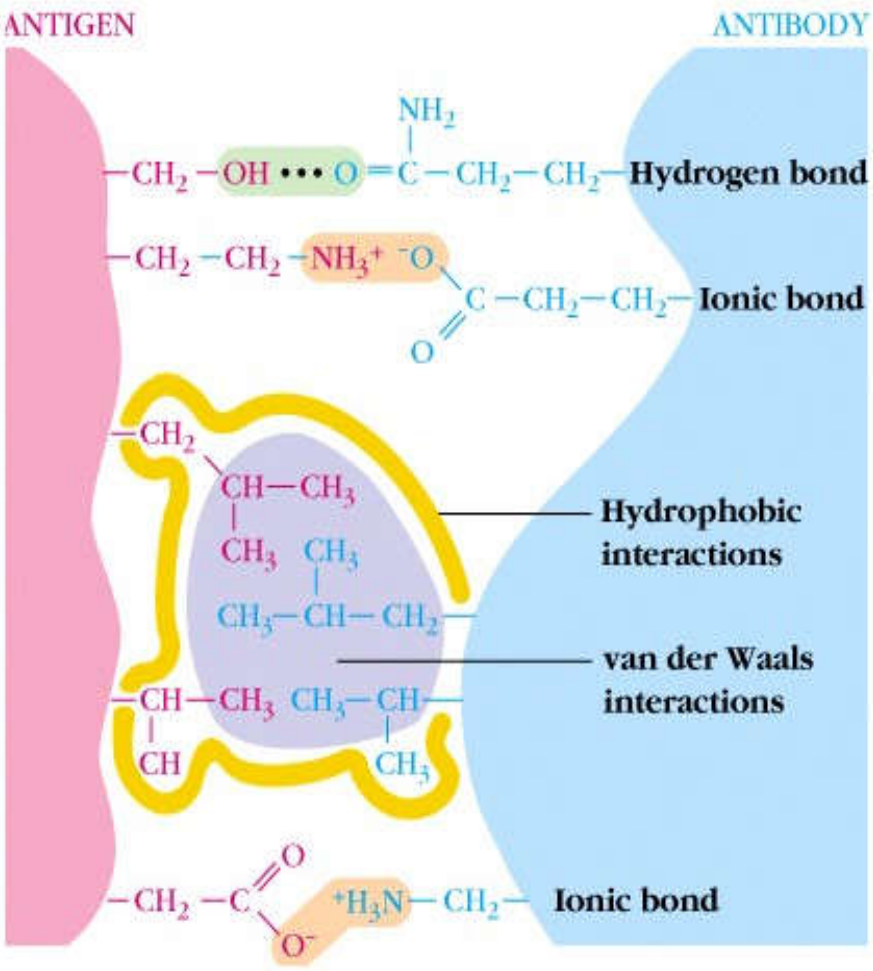
- The Ag-Ab interaction is a biomolecular association similar to enzyme- substrate interaction
- Important distinction:
  - Does not lead to an irreversible chemical reaction i.e. Ag-Ab rxn is reversible.
  - Ag-Ab rxn involves various non covalent interactions between the Ag determinant or epitope of the Ag and the variable domain of Ab mole, particularly the hyper variable region or complementary determining regions (CDRs).

13.1 Strength of Antigen-Antibody Interactions

- The fine specificity of antigen-antibody interactions has led to the development of a variety of immunologic assays, which can be used to detect the presence of either antibody or antigen.
- Immunoassays have played vital roles in:
  - Diagnosing diseases
  - Monitoring the level of the humoral immune response
  - Identifying molecules of biological or medical interest
- These assays differ in their speed and sensitivity.
- Some are strictly qualitative, others are quantitative.

13.1 Strength of Antigen-Antibody Interactions

- The noncovalent interactions that form the basis of antigen –antibody (Ag-Ab) binding include:
  - Hydrogen bonds
  - Ionic bonds
  - Hydrophobic interactions
  - Van der Waals interactions



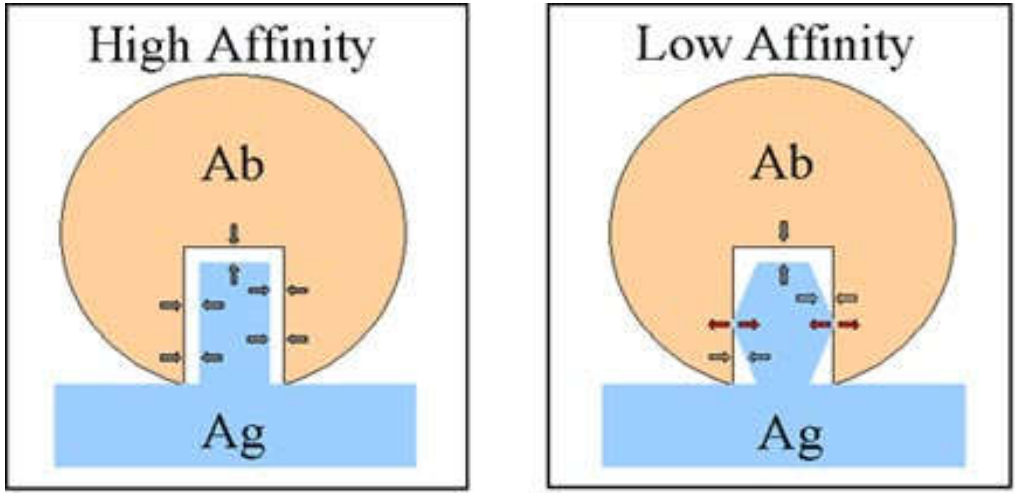
13.1 Strength of Antigen-Antibody Interactions

- These interactions are individually weak (compared with a covalent bond), a large number of such interactions are required to form a strong Ag-Ab interaction.
- These interactions operates over a very short distance, (1 Å=1x10^{7}mm); consequently, a strong Ag-Ab interaction depends on a very close fit between the antigen and antibody.
- This requires a high degree of complementarity and/or specificity between antigen and antibody, that characterizes antigen-antibody interactions.

13.1 Strength of Antigen-Antibody Interactions

Antibody Affinity

- Affinity- strength of the total non covalent interaction between one Ag-binding site and one epitope
- Low affinity Abs bind Ag weakly and tend to dissociate easily, high affinity Abs bind Ag more tightly and remain bound longer.



13.1 Strength of Antigen-Antibody Interactions

Antibody Affinity

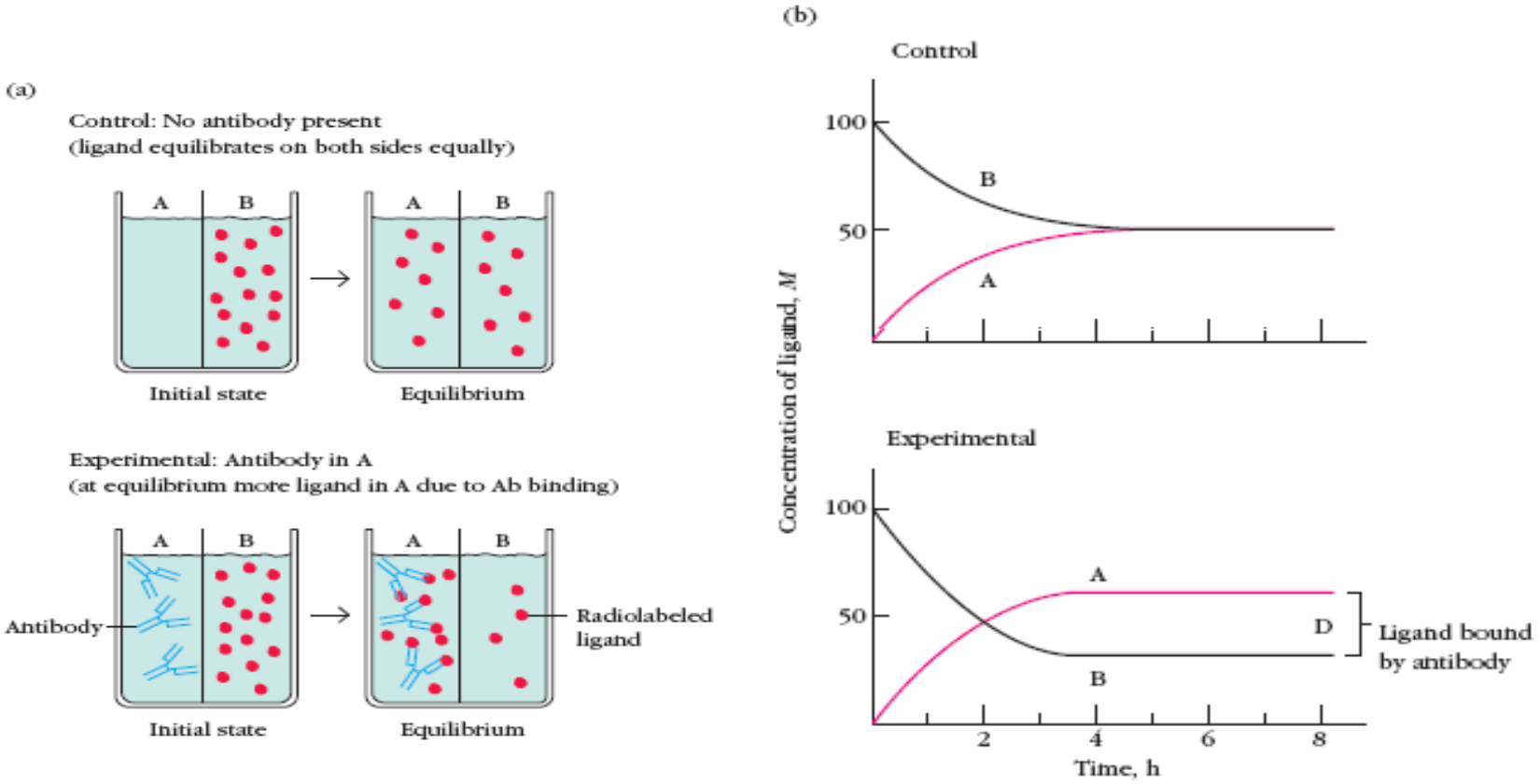
Measured by various methods including

- Equilibrium dialysis,
- Competition assays,
- Microchips (e.g., Biacor)

- The association between a binding site on an antibody (Ab) with a monovalent antigen (Ag) can be described by the equation:

$$\mathrm{Ag} + \mathrm{Ab} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{Ag\text{-}Ab}$$

Determination of antibody affinity by equilibrium dialysis.



13.1 Strength of Antigen-Antibody Interactions

Antibody Avidity

- Affinity at one binding site does not always reflect the true strength of the antibody-antigen interaction.
- When complex antigens containing multiple, repeating antigenic determinants are mixed with antibodies containing multiple binding sites, the interaction of an antibody molecule with an antigen molecule at one site will increase the probability of reaction between those two molecules at a second site.
- The strength of such multiple interactions between a multivalent antibody and antigen is called the **avidity.**

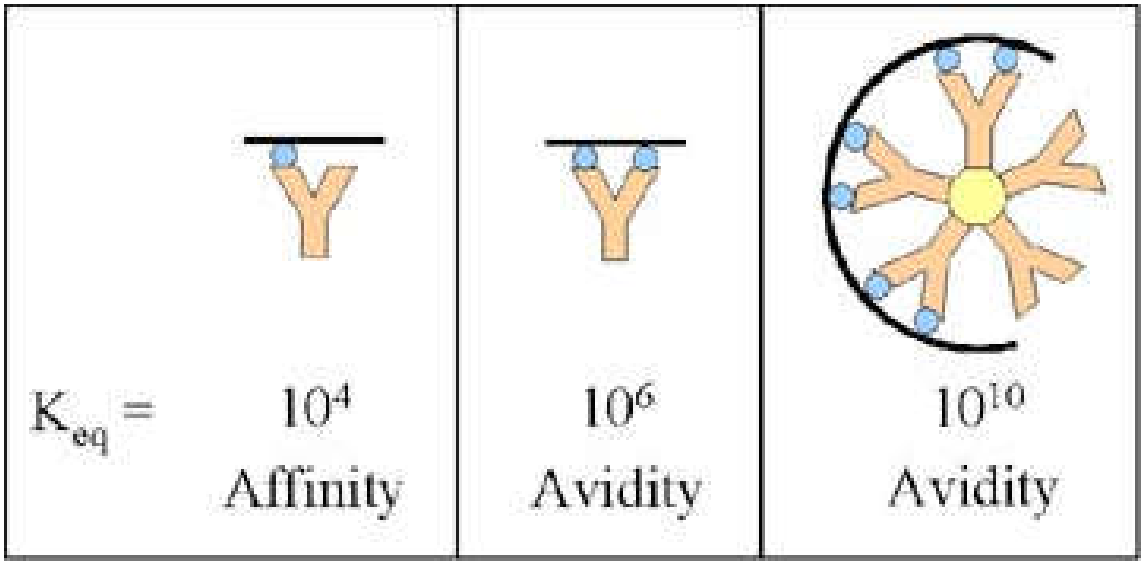
13.1 Strength of Antigen-Antibody Interactions

Antibody Avidity . . .

- The avidity of an antibody is a better measure of its binding capacity within biological systems (e.g., the reaction of an antibody with antigenic determinants on a virus or bacterial cell) than the affinity of its individual binding sites.
- High avidity can compensate for low affinity.
  - For example, secreted pentameric IgM often has a lower affinity than IgG, but the high avidity of IgM, resulting from its higher valence, enables it to bind antigen effectively.

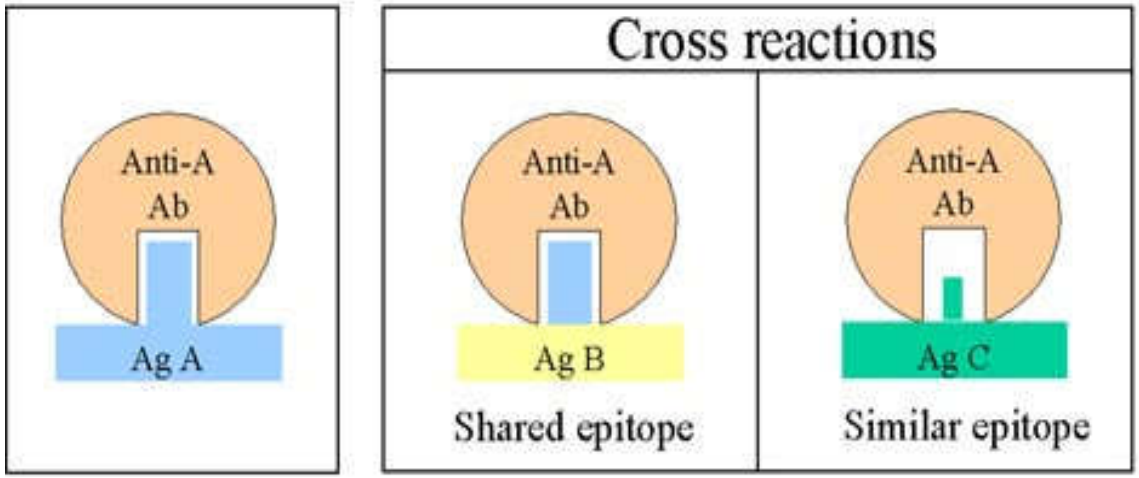
13.1 Strength of Antigen-Antibody Interactions

Antibody Avidity . . .



13.2 Cross-Reactivity

- Although Ag-Ab reactions are highly specific, in some cases antibody elicited by one antigen can cross-react with an unrelated antigen.
- Such cross-reactivity occurs if two different antigens share an identical or very similar epitope.
- Antibody's affinity for the cross-reacting epitope is usually less than that for the original epitope.



- ABO blood-group antigens, for example, are glycoproteins expressed on red blood cells. Subtle differences in the terminal residues of the sugars attached to these surface proteins distinguish the A and B blood-group antigens.
- A number of viruses and bacteria have antigenic determinants identical or similar to normal host-cell components.
- In some cases, these microbial antigens have been shown to elicit antibody that cross-reacts with the host-cell components, resulting in a tissue-damaging autoimmune reaction.

13.3 Primary, secondary and tertiary antigen antibody reactions

- **Three** groups of immunological techniques are used to detect and measure antigen-antibody combination.
  - ***Primary binding tests***
  - ***Secondary binding tests and***
  - ***Tertiary binding tests.***

13.3 Primary, secondary and tertiary antigen antibody reactions

Primary binding tests

- directly measure the binding of antigen and antibody (i.e.; directly measure or visualize the immune complex).

most sensitive techniques in terms of the amount of antigen or antibody detectable.

13.3 Primary, secondary and tertiary antigen antibody reactions

Primary binding tests ….cont

E.g.

- Enzyme linked Immunosorbent assay (ELISA) tests
- Radioimmunoassay (RIA)

- Western blotting
- Northern blotting

- Southern blotting
- Fluorescence tests

13.3 Primary, secondary and tertiary antigen antibody reactions

- **Primary binding tests are performed**
  - by allowing antigen and antibody to combine and then measuring or visualizing the amount of immune complex formed.

  It is usual to use *radioisotopes*, fluorescent dyes, or enzymes as labels to identify one of the reactants.

13.3 Primary, secondary and tertiary antigen antibody reactions

Secondary binding tests

- Secondary binding tests are tests that detect and measure the consequences (secondary effect) of antigen-antibody interaction.

- These consequences include:
  - Clumping (agglutination) of particulate antigens
  - Precipitation of soluble antigens
  - Neutralization of bacteria, viruses, or toxins; and
  - Activation of the complement system.

13.3 Primary, secondary and tertiary antigen antibody reactions

Secondary binding tests . . .

Examples

- Agglutination tests
- Precipitation tests
- Complement fixation tests

- Secondary binding tests are usually;
  - Less sensitive than primary binding tests but
  - Easier to perform

13.3 Primary, secondary and tertiary antigen antibody reactions

Tertiary binding test

- Measures the consequences of immune responses in vivo
- These tests are much more complex than primary and secondary tests but results reflect the practical significance of the immune response
- E.g. measurement of the protective effects of antibody.