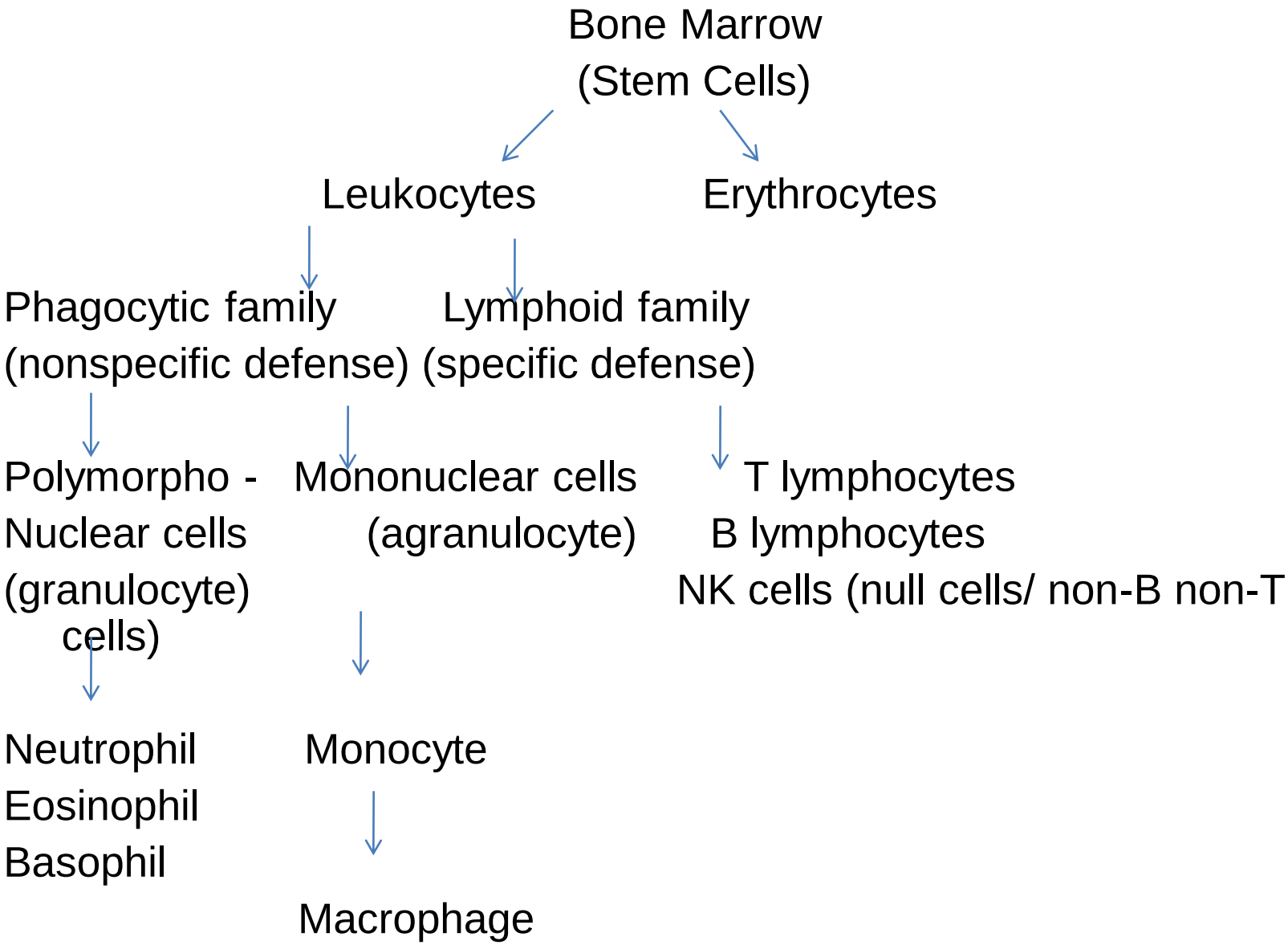
IMMUNOLOGY

Components of Immune System

CC – 13/ Unit - 2

- Derived from *latin* term “immunis” means to exempt.
- Immunity - is the ability of an organism to resist infection by pathogens or foreign organisms or substances.
- Array of cells, tissues and organs carrying out this activity altogether constitute the immune system.

- Immunology - is the science concerning with immune response to foreign challenges.


Bone Marrow
(Stem Cells)
Leukocytes
Erythrocytes
Phagocytic family
(nonspecific defense)
Lymphoid family
(specific defense)
Polymorpho -
Nuclear cells
(granulocyte)
cells)
Mononuclear cells
(agranulocyte)
T lymphocytes
B lymphocytes
NK cells (null cells/ non-B non-T
Neutrophil
Eosinophil
Basophil
Monocyte
Macrophage

Hematopoietic Stem Cells (HSCs)

- Have self-renewal property (maintain their population level by cell division)
- Pluripotent (able to differentiate in various ways to generate different cell types)
- Have enormous proliferative capacity
- Generate all blood cells (hematopoiesis)

- They are difficult to study for their scarcity & difficulty in growing *in vitro*
- Early in hematopoiesis, a pluripotent stem cell differenciates forming either a lymphoid or a myeloid progenitor stem cell depending upon the types and amounts of growth factor present in the microenvironment
- Upon differenciation into progenitor cell, lymphoid or myeloid stem cells lost self-renewal capacity and are committed to a particular cell lineage

Site of Hematopoiesis

- It begins in the embryonic yolk sac during the first week of development
- In the 3rd month of gestation, HSCs migrate from the yolk sac to the fetal lever and then to spleen
- Fetal lever & spleen play major roles in hematopoiesis from 3rd to 7th months of gestation
- After that HSCs start differentiating in the Bone Marrow and becomes the major factor in hematopoiesis
- By birth, there is little or no hematopoiesis in the liver and spleen

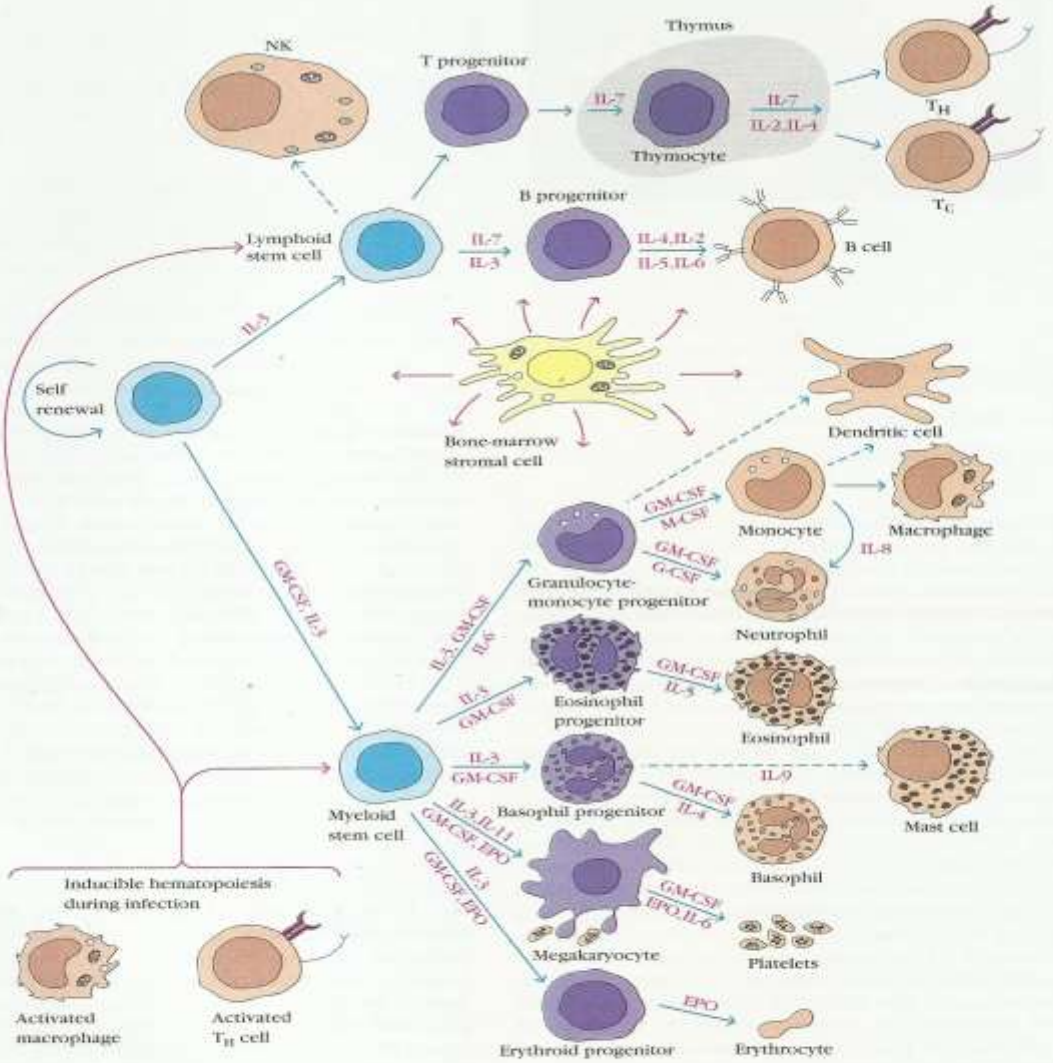


FIGURE 2-1 Regulation of hematopoiesis by cytokines that stimulate the proliferation and/or differentiation of various hematopoietic cells. In the absence of infection, bone-marrow stromal cells are the major source of hematopoietic cytokines (red arrows). In the presence of infection, cytokines (red arrows) produced by activated macrophages and T_H cells induce additional hematopoietic activity, resulting in rapid expansion of the population of white blood cells that fight infection. IL = interleukin; CSF = colony-stimulating factor; EPO = erythropoietin, NK = natural killer cell. Stem cells are shown in blue; progenitor and immature cells in purple; stromal cell in yellow; and mature differentiated cells in tan. Dashed lines indicate likely, but still hypothetical pathways.

Hematopoietic Growth Factors

- These are hematopoietic cytokines
- Regulate survival, proliferation, differenciation and maturation of HSCs
- Identified by studying their ability to stimulate the formation of hematopoietic cell colonies in bone-marrow cultures.
- Erythropoietin

  A glycoprotein produced by kidney that induces the terminal development of erythrocytes and regulates the production of RBCs

- Colony Stimulating Factors (CSF) -

a family of acidic glycoproteins that induce the formation of distinct hematopoietic cell lines –

a) Multilineage CSF (IL-3)
b) Macrophage CSF (M-CSF)
c) Granulocyte CSF (G-CSF)
d) Granulocyte-macrophage CSF (GM-CSF)

Genes Regulating Hematopoiesis (Transcriptional Factors)

- Development of HSCs into different cell types is dependent on the expression of different sets of lineage-determining and lineage-specific genes at appropriate time and in correct order
- These genes are identified by studying mice in which a gene has been inactivated or “knocked out” by targeted disruption. Therefore, production of the protein that the gene encodes is blocked
- GATA-2 essential for the development of lymphoid, erythroid, myeloid lineage

  Animals die during embryonic development in which this gene is disrupted

- Ikaros essential for the development of lymphoid lineage

  Ikaros knockout mice survive embryonic development but are immunocompromised and die of infections at an early stage
- Bmi-1 essential for the self-renewing capacity of HSCs

  In its absence animals die within 2 months of birth because of the failure to repopulate their RBCs and WBCs
- Notch 1 regulates the choice between T & B lymphocyte lineages

- Hematopoiesis is regulated in 4 stages –

1. Controlling levels & types of cytokines produced by BM stromal cells
2. Controlling production of cytokines by activated T lymphocytes and macrophages
3. Regulating the expression of receptors for hematopoietically active cytokines in stem cells and progenitor cells
4. Regulating apoptosis of some cells

Clinical Uses of HSCs

- Transplantations of HSCs has 3 major applications –

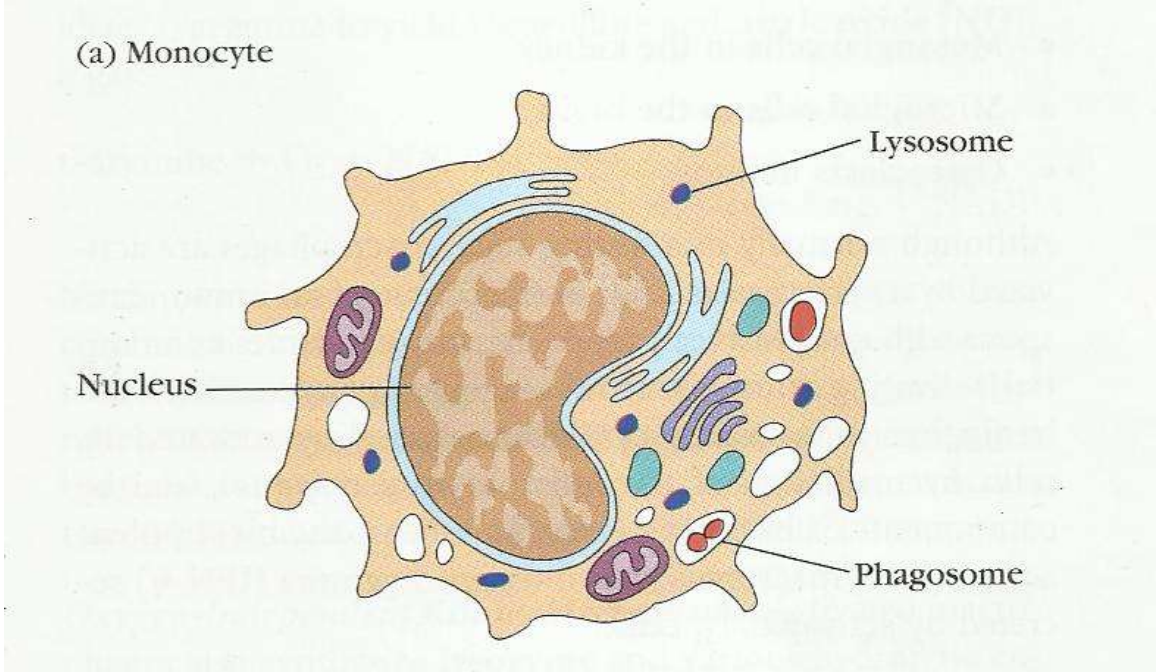
1. Provides a functional immune system to individuals with genetically determined immunodeficiencies, like Severe Combined Immunodeficiency (SCID)
2. Replaces a defective hematopoietic system with a functional one to cure patients having sickle cell anemia or thalassemia (nonmalignant genetic disordres in hematopoiesis)
3. Restores hematopoietic system of cancer patients after chemotherapy

Stromal Cells

- Non- hematopoietic cells of bone marrow that support the growth and differenciation of HSCs.
- In adult BM, HSCs grow & mature on a meshwork of stromal cells
- They provide a hematopoietic-inducing microenvironment (HIM) consisting of a cellular matrix and growth factors (membrane bound or diffusible) to influence the differenciation of HSCs
- Stromal cells include fat cells, endothelial cells, fibroblasts and macrophages

Monocyte

- Large agranulocyte
- Slightly lobed nuclei
- Small spherical cell with few projections, abundant cytoplasm and many granules
- Circulate in the blood stream before migrating into tissues where it become macrophages or dendritic cells


(a) Monocyte
Lysosome
Nucleus
Phagosome

Macrophage

- Phagocytes derived from blood monocytes
- Functions – to remove foreign objects (bacteria, fungi, spores, dust, dead body cells)
- They also process Ag ,can stimulate immune response, secrete cytokines that amplify immune response (IL-1, IL-6, IL-12, TNF-α), can cause fever and are responsible for many of the clinical signs of infection
- Have receptors for Abs or complement –

  $F_{C\gamma}RI$ (CD 64) – binds to F_C region of antibody in both free and Ag-bound form

  $F_{C\gamma}RII$ (CD 32) & $F_{C\gamma}RIII$ (CD 16) – additional antibody receptors found in human macrophage

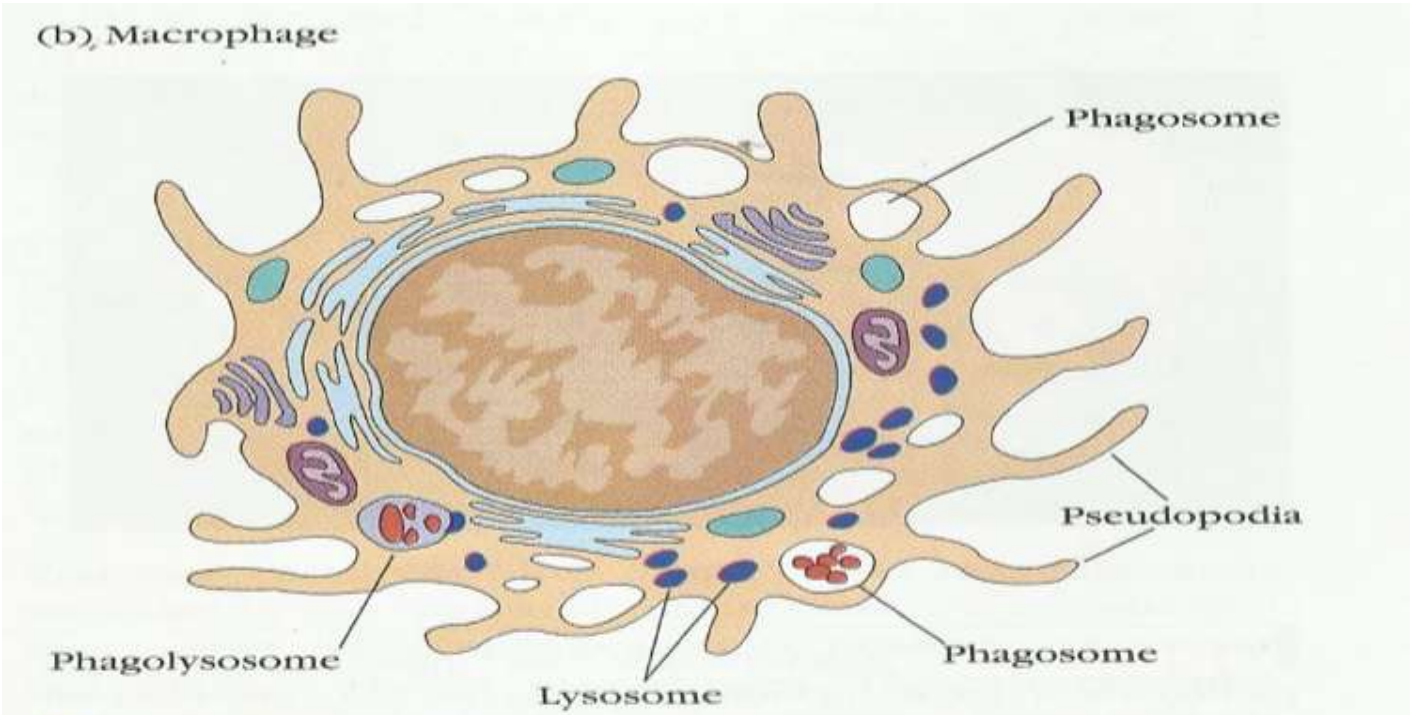


FIGURE 2-8 Typical morphology of a monocyte and a macrophage. Macrophages are five- to tenfold larger than monocytes and contain more organelles, especially lysosomes.

- Wandering macrophages - travel throughout the body including extracellular spaces and perform scavenger function
- Monocytes differenciates to a variety of histolytic forms that are fixed macrophages –

1. Kupffer cells - large cells with many cytoplasmic projections: liver
2. Alveolar macrophages – lungs
3. Splenic macrophages – white pulp (surrounds branches of splenic artery)
4. Peritoneal macrophages – free floating in the peritoneal fluid
5. Osteoclasts – bone
6. Mesangial cells – kidney
7. Microglial cells – central nervous tissue
8. Histiocytes – connective tissue

Oxidative Killing Mechanism of Macrophage
1. By Reactive Nitrogen Species

Macrophage + Foreign Ag

↓

Induction of nitric oxide synthatase

↓

Oxidizes arginine to citrulline & nitric oxide (NO)

(other reactive oxides NO_2, NO_2^-, N_2O_3, NO_3^-, nitrosamines, nitrosothiols may be produced)

↓

Form complexes with iron containing enzymes and inhibit oxidative metabolism of ingested bacteria and intracellular parasites

2. By Reactive Oxygen Species

Phagocytosis

↓

Respiratory burst (metabolic process)

↓

Activates membrane bound oxidase

↓

Catalyzes reduction of oxygen to superoxide anion

↓

Superoxide anion generates hydrogen peroxide & hydroxyl radicals (oxidizing agents)

Fusion of lysosome with phagosome

Production of hypochlorite by myeloperoxidase from hydrogen peroxide & chloride

Non-Oxidative Killing Mechanism of Macrophage

Mediated by - 1. lysozyme

2. various hydrolytic enzymes

3. TNF- α (cytotoxic for some tumor cells)

4. defensins – a group of antimicrobial and cytotoxic, cystein rich peptides containing 29-35 amino acids. They kill bacteria by forming ion-permeable channels in bacterial cell membranes.

It can kill Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Haemophilus influenzae etc..

Epithelioid Cells:

- When foreign material persists for long time within the body, macrophages may accumulate in large numbers around it and look like epithelium on histological examination.
- These cells are called epithelioid cells that may fuse to form multinucleated giant cells when attempting to enclose particles too large to be ingested by a single cell.

Opsonin:

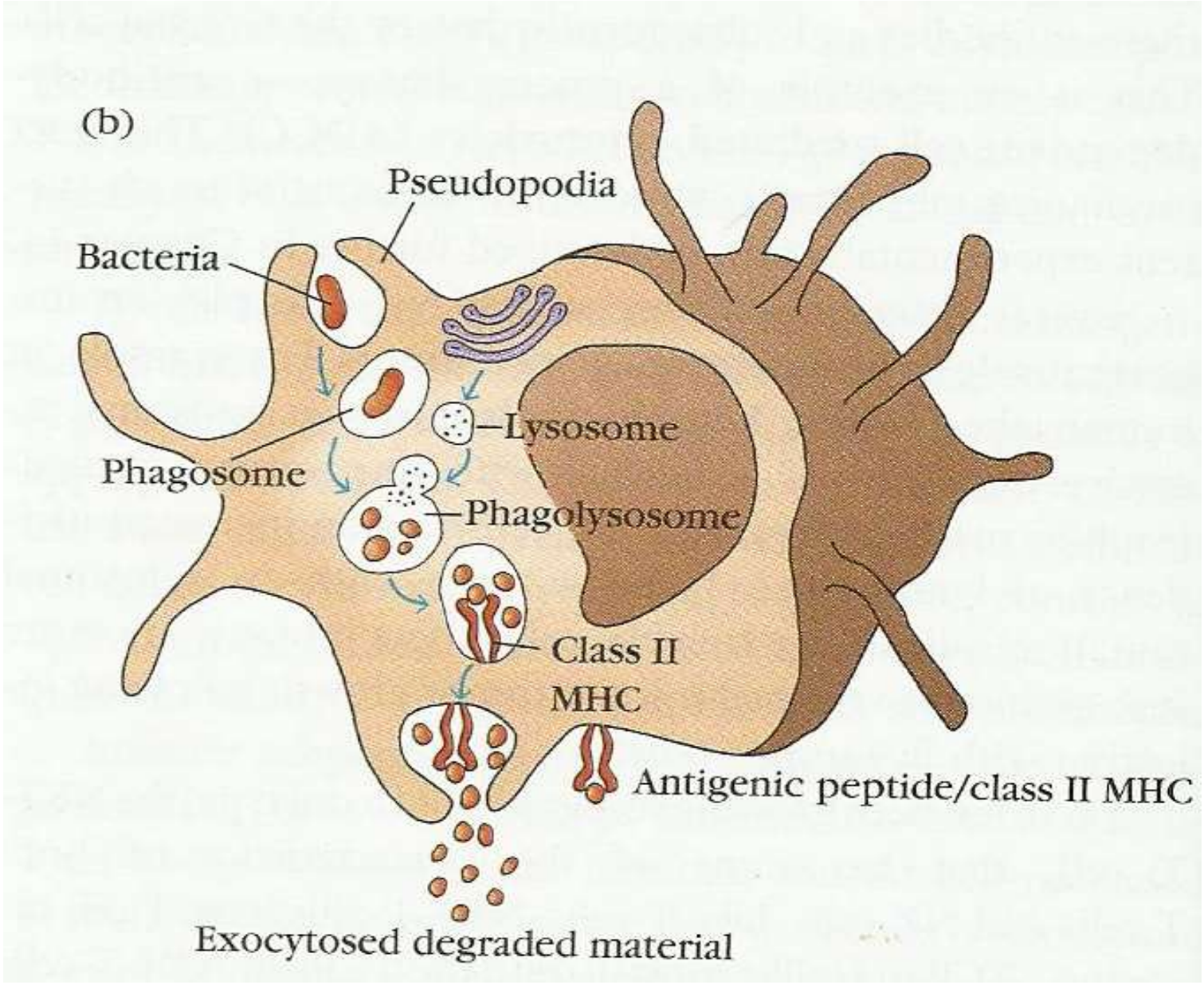
- Molecules (Ab, complement) that bind to both Ag and macrophages and enhance phagocytosis.
- The process by which particulate Ags becomes more susceptible to phagocytosis is called opsonization.

Phagocytosis

Uptake of particulate materials (whole microorganisms, insoluble particles, injured or dead cells, cellular debris, activated clotting factors) by engulfment

1. Macrophages are attracted by & move towards a variety of substances generated in an immune response in a process called chemotaxis.
2. Ags adhere to the cell membrane of macrophage.
3. Membrane protrusions – pseudopodia- are induced by adherence and it extend around the attached material.
4. Fusion of pseudopodia encloses the material within a membrane-bound structure – phagosome.

5. Then phagosome moves toward the cell interior and fuses with lysosome to form a phagolysosome.
6. Lysozyme & other hydrolytic enzymes in lysosome digest the ingested material.
7. The digested contents are then eliminated by exocytosis.
8. Most of the ingested and digested material are exocytosed but some peptide products interact with class-II MHC molecule forming complexes that move to cell surface and present the Ag to T_H cells.


(b)
Pseudopodia
Bacteria
Lysosome
Phagosome
Phagolysosome
Class II
MHC
Antigenic peptide/class II MHC
Exocytosed degraded material

Dendritic Cell

- Originates from BM
- It is covered with a maze of long membrane extension that resemble the dendrites of nerve cells and hence got the name
- Specialized for Ag presentation to T_H cells
- Express high levels of MHC class-II molecules which acquire Ag by phagocytosis, then process & present it to T_H cells
- 5 types –

1. Langerhans cells: epidermis (skin) & mucous membrane; bears F_C receptor

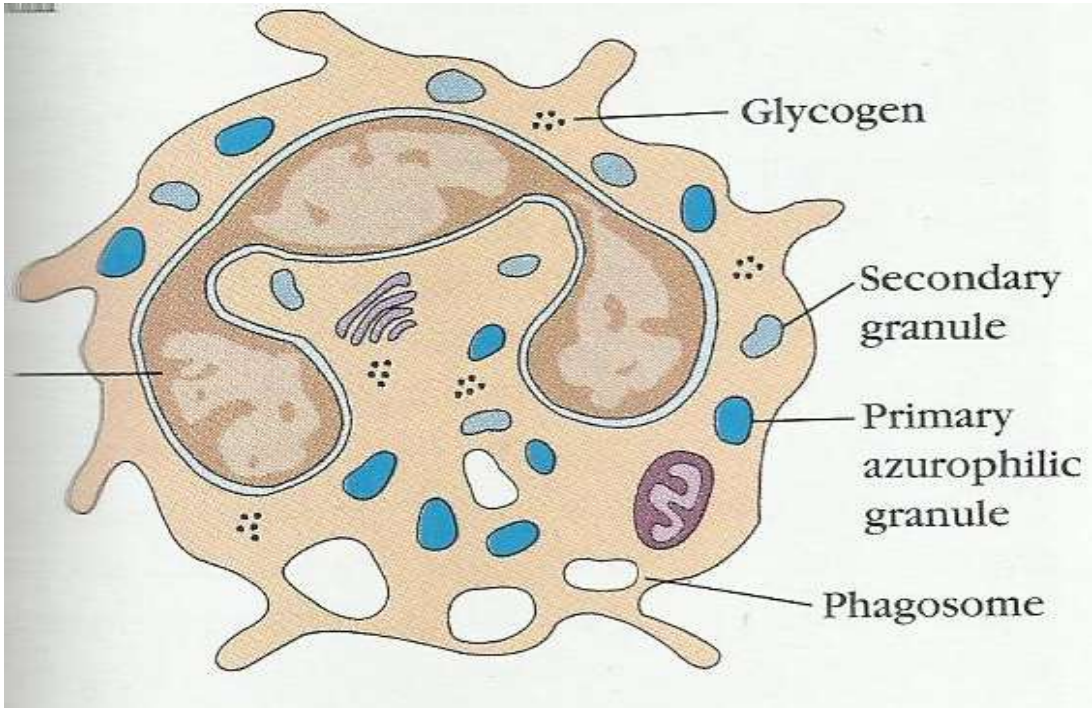
A morphologic variation moving from site of Ag acquisition to secondary lymphoid tissues are called Veiled cells (circulating dendritic cells)

2. Interstitial dendritic cells: heart, lungs, liver, kidney, GI tract
3. Myeloid dendritic cells:
4. Lymphoid dendritic cells:
5. Interdigitating dendritic cells: T cell areas of secondary lymphoid tissue & thymic medulla

- Follicular Dendritic Cells:
- Cell with extensive dendritic extensions found in follicles of lymph nodes does not arise from BM
- Has a different function from Ag presenting dendritic cells
- Do not express MHC class-II molecules
- Have receptors for Ab allowing the binding of Ag-Ab complexes
- They maintain follicular and germinal center structure in lymph node and present antigens to B cells

Neutrophil

- Produced by hematopoiesis in BM
- Most abundant leukocyte in blood (60-75%)
- Has multilobed nucleus & granulated cytoplasm
- Cytoplasm stains with both acid & basic dyes
- Round cells of 12 micrometer diameter
- Known as polymorphonuclear leukocyte (PMN) for its multilobed nucleus


Glycogen
Secondary granule
Primary azurophilic granule
Phagosome

- Neutrophils first arrive at the site of inflammation
- In response to many types of infection, more than usual number of neutrophils are released, resulting in increase in number of circulating neutrophils – leukocytosis (medical indication of infection)
- They secrete proteins having antimicrobial activity & tissue remodelling capacity
- Movement of circulating neutrophils in tissue – extravasation - are as follows:

1. Adherence of cells to vascular endothelium

1. Penetration into the gap between adjacent endothelial cells of vessel wall lining
2. Penetration into the vascular basement membrane and moving out into the tissue space

- Substances generated in inflammatory reaction & promoting accumulation of neutrophils at the inflammatory site are called chemotactic factors (complement components, blood-clotting system components, cytokines secreted by activated T_H cells & macrophages)
- Acts in a similar way to macrophages
- Exception – here lytic enzyme & bactericidal substances are contained in primary and secondary granules

Extravasation (Getting out of the Blood Stream) of Neutrophils

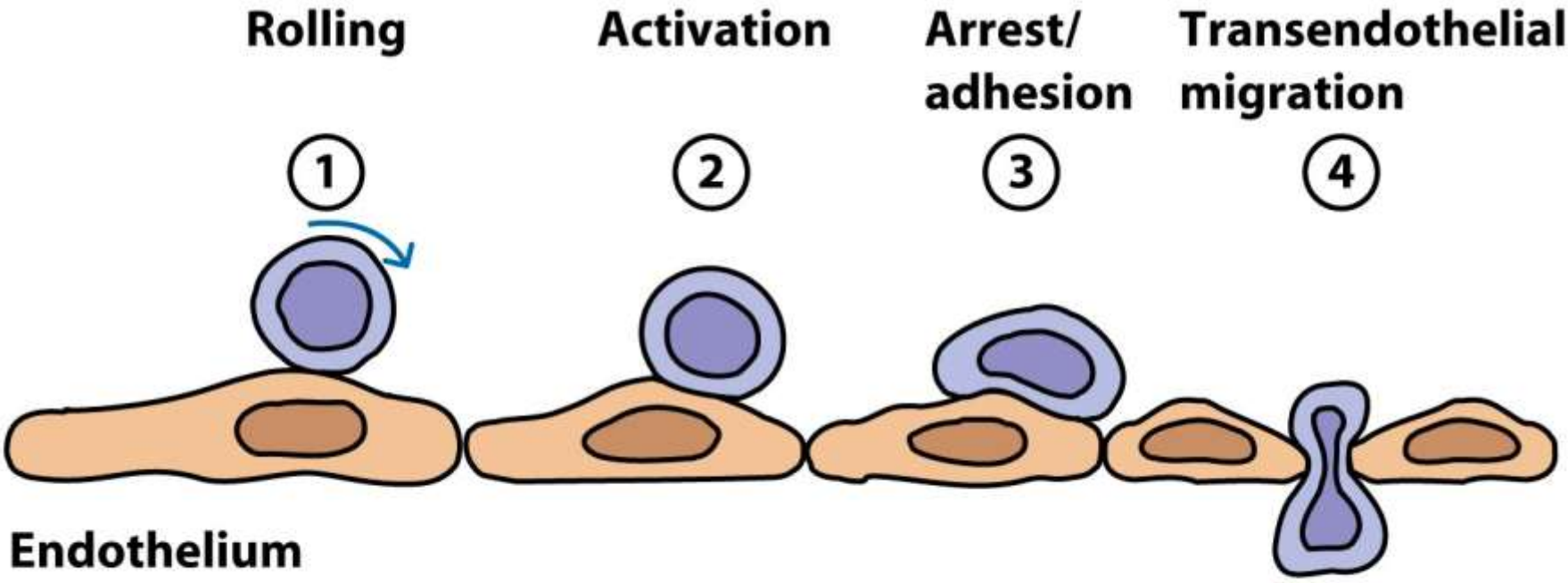
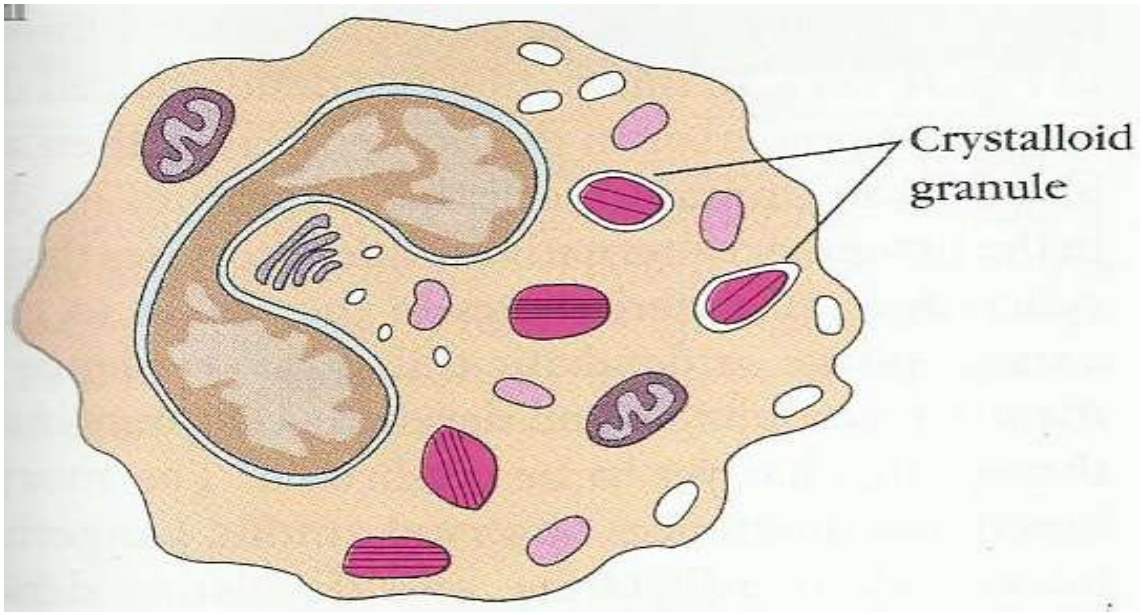


Figure 13-4
Kuby IMMUNOLOGY, Sixth Edition

- Primary granules: larger, denser primary granules are a type of lysosome & contains peroxidase, lysozyme, other hydrolytic enzymes
- Secondary granules: smaller granules containing collagenase, lactoferrin & lysozyme
- Both the granules fuse with phagosome and digest and eliminate its content
- Neutrophils exhibit a larger respiratory burst and express higher levels of defensins

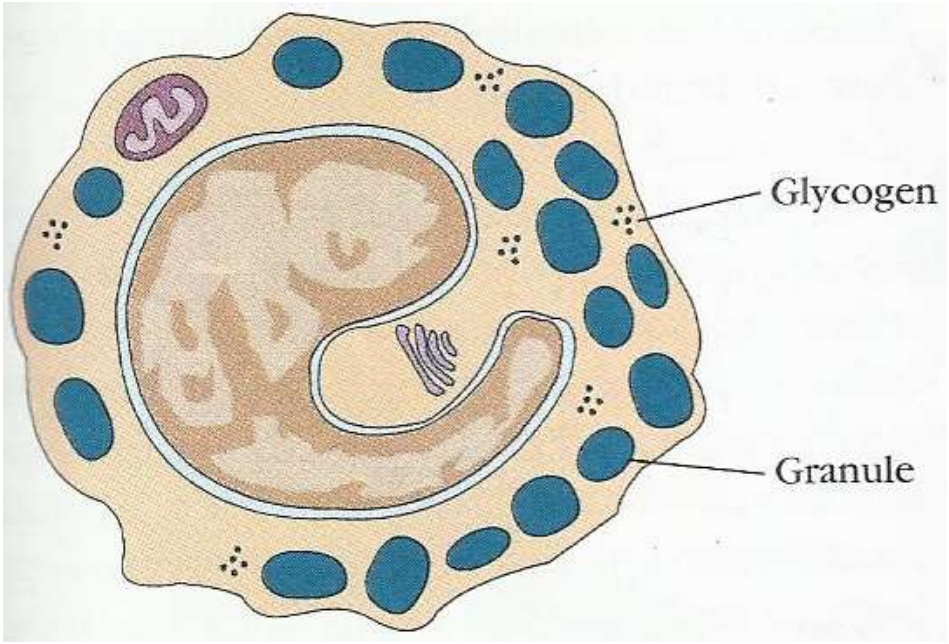
Eosinophil

- Bilobed nucleus & granulated cytoplasm that stains with acidic dye eosin red
- Normal value is 2-5% in healthy humans
- Motile cells that can migrate from blood into tissue spaces
- Their phagocytic role is insignificant
- They provide defense against multicellular parasitic organisms (helminths or worms)
- Eosinophilic granules secrete acid phosphatase and peroxidase that damage parasite membrane
- Large eosinophilic granules contain a protein – major basic protein (MBP) – highly toxic for parasitic worms


Crystalloid
granule

Basophils

- Lobed nucleus & a heavily granulated cytoplasm that stains with basic dyes methylene blue, hematoxylin
- Non- phagocytic granulocyte playing major role in certain allergic reactions by releasing pharmacologically active substances from their granules
- Provoke inflammation as their granules contains vasoactive amines like histamine, serotonin
- Play critical role against parasites (helminths or worms)
- Neutrophil, eosinophil & basophil secrete cytokines that modulate adaptive immune response


Glycogen
Granule

Mast Cells

- Precursor of basophils; originate from BM by hematopoiesis
- Released into blood as undifferenciated cells
- They differenciate after entering tissue
- Found in skin, connective tissues of various organs, mucosal epithelial tissues of respiratory, genitourinary and digestive tract
- Have large numbers of cytoplasmic granules containing histamine and other pharmacologically active substances
- Mast cells together with circulating basophils play major role in the development of allergies

- Megakaryocytes:

- Large myeloid cell at BM
- Give rise to thousands of platelets by cytoplasmic budding
- Platelets are very small cells or cell fragments, devoid of own nuclei, circulating in blood and participate in blood clotting

- Erythroid Cells:

- Contains high concentration of hemoglobin
- Circulate through blood vessels & capillaries delivering oxygen to surrounding cells and tissues
- Damaged RBCs release free radicals that induce innate immune response
- In mammals, RBCs are anuclear; their nucleated precursor (erythroblasts) extrude nuclei in BM
- Almost all non-mammalian vertebrates retain nuclei

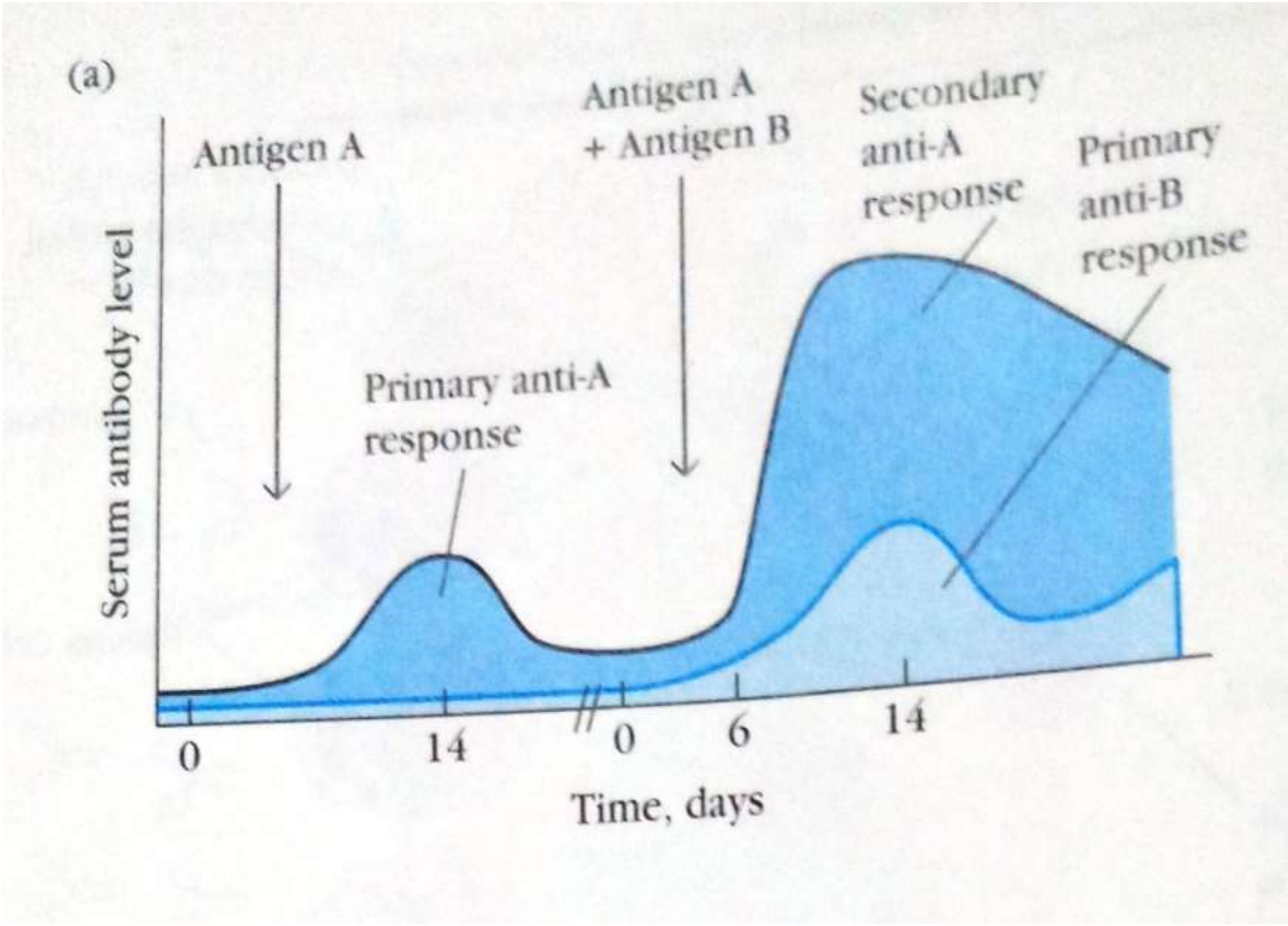
Cells of Lymphoid Family

- Lymphocytes are the major cells in adaptive immune response
- Represent 20%-40% of circulating WBCs and 99% of the cells in lymph
- Broadly subdivided into 3 types on the basis of phenotypic and functional differences – B lymphocytes, T lymphocytes and NK (Natural Killer) Cells
- Since, T and B cells are very difficult to distinguish morphologically, they are often distinguished on the basis of some cell surface proteins they express – known as Cluster of Differentiation or Cluster of Designation (CD molecules)

- In addition to CD surface molecules or markers, each B or T cell also expresses antigen-specific B cell receptor (BCR)and T cell receptor (TCR), respectively
- After lymphocytic division, the two newly formed daughter cells both bear antigen receptors with antigenic specificities identical to each other and to the parental cell from which they arose. The resulting population, all arising from the same founding lymphocyte, is a clone
- Naïve cells – mature T and B cells that do not encounter antigen
- Contact with antigen makes naïve cells to be primed and to proliferate and differentiate into effector cells (carry out specific functions to combat pathogen) and memory cells (upon rechallenge with the same antigen mediate a quicker and hyped secondary response)

Differences between Primary Response and Secondary Response

Property	Primary Response	Secondary Response
B cell type involved	Naive B cell	Memory B cell
Lag period after Ag administration	4-7 days	1-3 days
Time of peak response	7-10 days	3-5 days
Magnitude of peak Ab response	Depends on Ag	Generally 100-1000 times higher than primary response
Antibody type involved	IgM	IgG
Antigen	Both thymus dependent and thymus independent	Thymus dependent
Antibody Affinity	Lower	Higher


(a)
Antigen A
Antigen A
+ Antigen B
Secondary
anti-A
response
Primary
anti-B
response
Primary anti-A
response
Serum antibody level
0
14
0
6
14
Time, days

B Lymphocytes

- Derive its letter designation from its site of maturation – *b*ursa of Fabricius in Birds or *b*one marrow in humans, mice, other mammals
- Bone Marrow is their production and maturation site
- B cells have CD5 (unknown function), CD21, CD35 (receptor for complement component), CD32 (receptor for Fc region of IgG), CD40, CD45, CD19 (signal transduction)
- Mature B cells display a membrane-bound immunoglobulin (antibody) molecule as BCR that binds to antigen
- After interacting with antigen, B cells differentiate into antibody-secreting plasma cells and memory cells
- They are the only cell types capable of producing antibody molecules and are therefore the central cellular component of Humoral Immune Response
- B cells can also act as Antigen Presenting Cells (APCs)

T Lymphocytes

- Derive its letter designation from its site of maturation – *t*hymus
- They are produced in Bone Marrow but mature in the thymus
- Mature T cells express a unique antigen-binding receptor known as TCR
- Unlike BCR, TCR recognizes processed pieces of antigen bound to cell membrane proteins called Major Histocompatibility Complex (MHC) molecules
- T cells do not produce antibody but perform various functions like, elimination of cancer cells, virus-infected cells or grafts, when APCs bring antigens into the secondary lymphoid organs

- There are 2 major types of T cells – T Helper cells (T_H; CD4+) and T cytotoxic cells (T_C; CD8+)
- In normal mouse and humans CD4+ : CD8+ ratio is 2:1 any change in this ratio is an indicator of immunodeficiency disease (HIV), autoimmune disease etc..
- All T cells express CD3 (signal transduction). In addition T_H cells have CD2, CD4, CD45 (signal transduction), CD5 (unknown), CD28 (receptor for B7 molecules on APCs). T_C cells have CD2, CD3, CD5, CD8 (signal transduction), CD28, CD45
- Naïve CD8+ T cells browse the surfaces of APCs with TCR. When they bind to an MHC-peptide complex, they become activated, proliferate and differentiate into an effector cell - Cytotoxic T Lymphocytes (CTL). To proliferate and differentiate optimally, they need help from mature TH cells

- CTL monitors body cells and eliminates cells displaying foreign antigen complexed with class I MHC, such as, virus-infected cells, tumor cells, cells of foreign tissue graft
- Naïve CD4+ cells browse the surfaces of APCs with their TCR and when they recognize an MHC-peptide complex, they become activated and proliferate and differentiate into a variety of effector T cells – TH1 cells (combat intracellular pathogens), TH2 cells (combat extracellular pathogens), TH17 (secrete IL17, play an important role in CMI and combat fungal infection), T Follicular Helper Cells (TFH: regulate B cell development in germinal centers and play an important role in HI)
- These effector TH cell subpopulations produce different types of cytokines that in turn activate B cells, TC cells, macrophages etc..
- Another CD4+ & CD25+ subpopulation, Regulatory T cells (T REG), inhibits immune response. They limits our normal T cell response. However, they help us to eliminate autoreactive responses

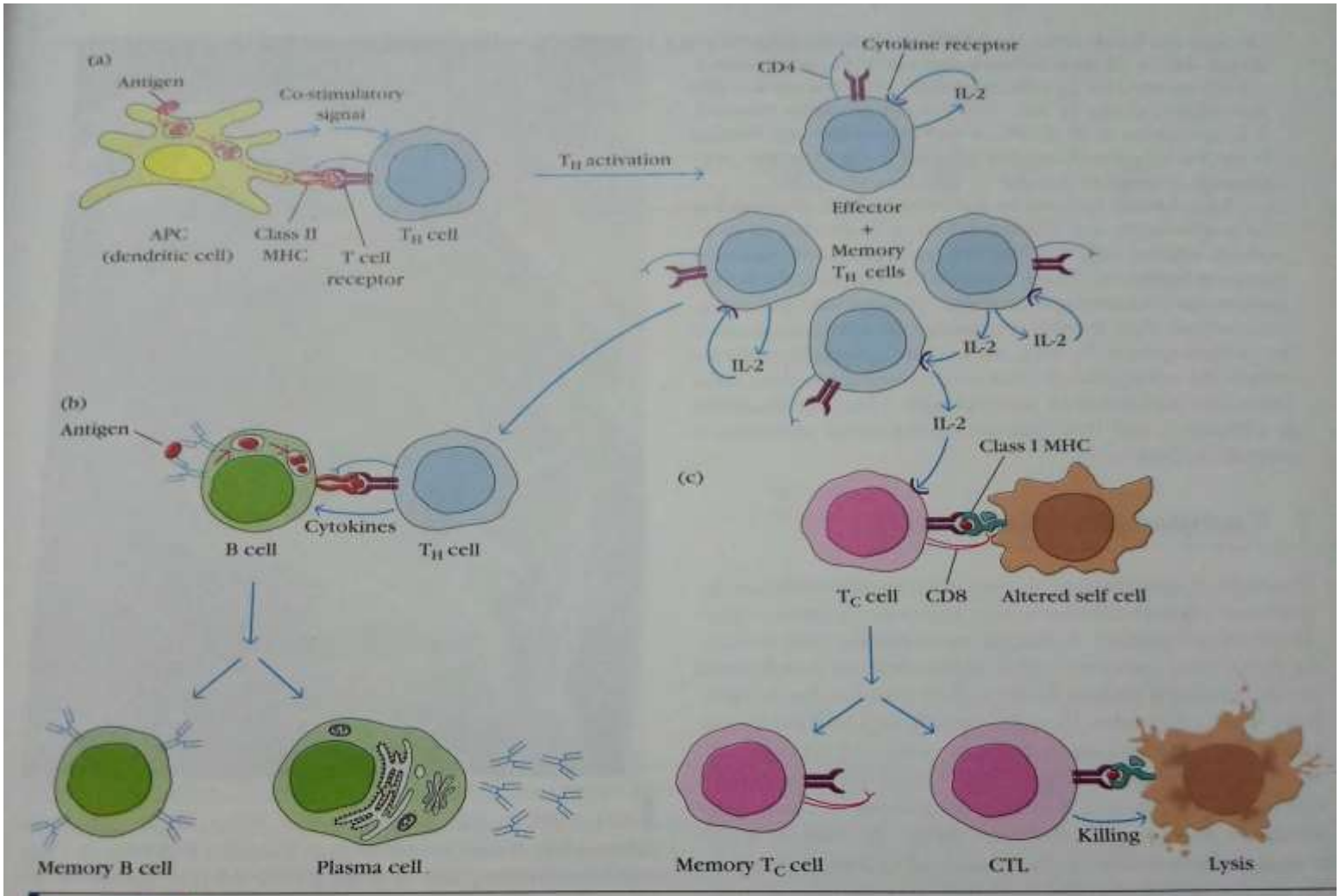
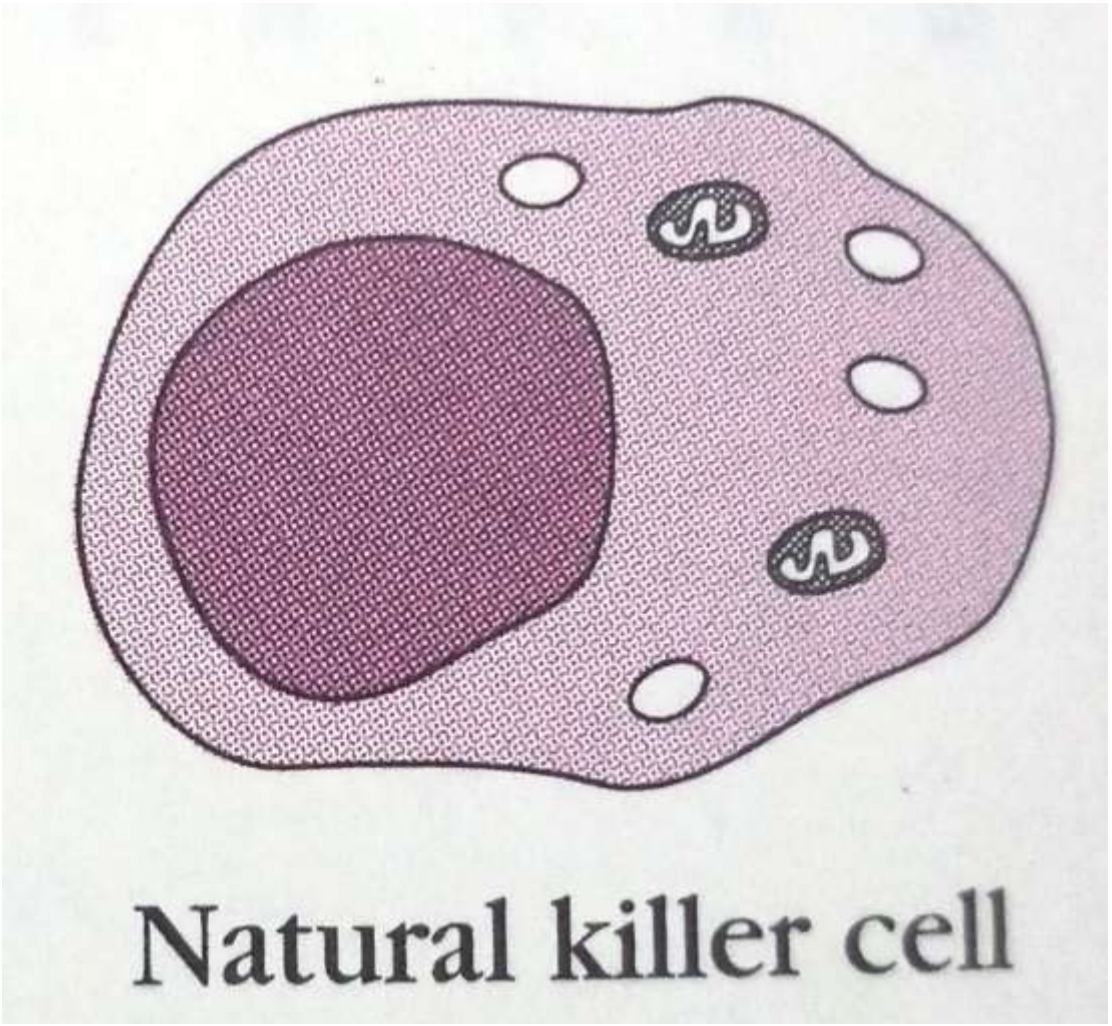


FIGURE 1-13 Cellular interactions involved in induction of immune responses. Activation (APC = antigen-presenting cell) and proliferation of T_H cells (a) is required for generation of a humoral response (b) and a cell-mediated response to altered self-cells (c).

NK Cell

- Constitute 5-10% of lymphocyte population
- Have enormous cytoplasm than a naïve lymphocyte
- Its cytoplasm is full of granules that are used to kill target cells
- They kill cells infected with intracellular pathogens
- They are non- phagocytic cells
- They destroy target cells by releasing biologically potent molecules
- Like TC cells, NK Cells destroy target cells by using perforin and granzyme
- Unlike TC Cells, NK Cells destroy target cells in absence of a specific antigen



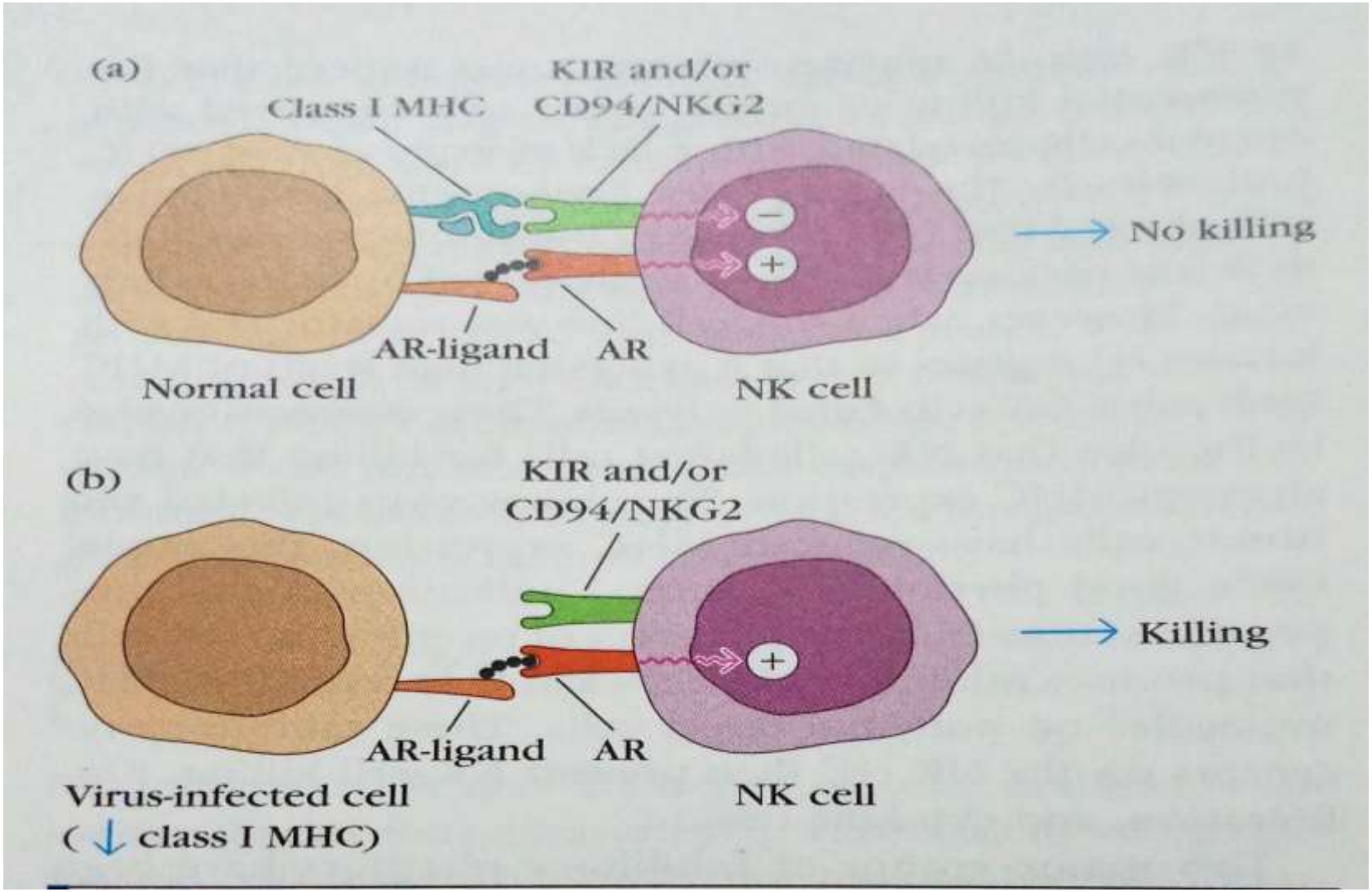
Natural killer cell

- NK cells are Null cells :
- 1. They do not express membrane molecules and receptors that distinguish T and B cells
- 2. They do not synthesize immunoglobulin and incorporate it into their plasma membrane
- 3. They lack immunologic specificity and memory as they do not produce antigen-binding receptors

Antibody Dependent Cell-mediated Cytotoxicity (ADCC)

- Some tumor and virus-infected cells display antigens against which immune system has already generated an antibody response
- Therefore, antitumor or antiviral antibodies are bound to cell surfaces
- NK cells bear CD16 which recognizes the F_C region of IgG molecules
- Through CD16 NK cells attach to these antibodies and destroy target cells

- NK cells can function in another way –
- An Activation Receptor (AR) on NK cell interacts with its ligand on normal as well as infected cells, inducing an activation signal for killing
- NK cells have a set of special Class I receptors (KIR or CD94/ NKG2) which recognize Class I MHC proteins of normal cells
- This binding deactivates the lytic mechanism
- In tumor or virus-infected cells, this MHC Class I protein is altered
- Therefore, NK cells can not recognize and bind to altered Class I MHC proteins on infected cells
- In absence of binding, the lytic mechanism remains active and NK cell kills the unrecognized target cells


(a)
KIR and/or
CD94/NKG2
Class I MHC
−
+
No killing
AR-ligand
AR
Normal cell
NK cell
(b)
KIR and/or
CD94/NKG2
+
Killing
AR-ligand
AR
Virus-infected cell
(↓ class I MHC)
NK cell

Tissues and Organs of Immune System

- Primary Lymphoid Organs : Formation and Maturation of all leukocytes, eg. – Bone Marrow, Thymus
- Secondary Lymphoid Organs : Trap antigens and provide sites for mature lymphocytes to interact with that antigen, eg. – Lymph node, spleen, various Mucosa-associated lymphoid tissue (MALT) like Gut-associated lymphoid tissue (GALT), Nasal-associated lymphoid tissue (NALT) etc.
- Tertiary Lymphoid Organs : Import lymphoid cells during an inflammatory response, contains fewer lymphoid cells than secondary organs, eg. – lungs, liver, brain

Bone Marrow

- Bursa of Fabricious (eg of GALT) is a primary lymphoid organ first discovered in Birds
- Consists of lymphoid centers containing epithelial cells and B lymphocytes
- Site for B cell maturation
- Mammals do not have Bursa, they have Bone Marrow which has an equivalent function to Bursa
- In primates and rodents (mammals), B cell maturation takes place in regions of BM and other lymphoid tissues
- In cattle and sheep, early fetal spleen and a large specialized Peyer's patch (found in the intestinal wall) called Ileal Peyer's patch are the sites of B cell maturation and proliferation
- Rabbit uses appendix (eg of GALT) for the proliferation and diversification of B cells

- B cells differentiate from HSCs in fetal Lever
- After birth, this function moves to bone marrow for the whole life span
- The long bones (femur, humerus), hip bones (ileum) and sternum are the most active sites of hematopoiesis
- Function – a) supports self-renewal and differentiation of HSCs into mature blood cells

  b) maintains the pool of HSCs throughout the life

  c) Stores mature antibody-secreting plasma cells (B cell)
- Adult bone marrow contains several cell types to coordinate HSC development including –

- ❖ Osteoblast – generates bone and controls the differentiation of HSC
- ❖ Endothelial cells – present in blood vessel lining and regulate HSC differentiation
- ❖ Reticular cells – connects cells to bones and blood vessels by sending processes
- ❖ Sympathetic neurons – control the release of HSCs from bone marrow
- Bone marrow is tightly packed with stromal cells and hematopoietic cells
- With age, fat cells replace 50% or more of BM compartment and hematopoietic efficiency decreases

- There are 2 distinct areas within BM –

1) Endosteal niche – area directly surrounding bone and in contact with bone-producing osteoblasts
2) Vascular niche - area directly surrounding blood vessels and in contact with endothelial cells

- Immature cells are associated with the endosteal niche; as they mature, they migrate towards the vascular niche. Fully differentiated cells exit the marrow via blood vessels

Thymus

- Site of T cell maturation
- Progenitor cells from BM migrate into Thymus gland and differentiate into T cells
- It is a flat, bilobed organ situated above heart
- Each lobe is surrounded by a capsule and is divided into lobules
- Lobules are separated from each other by strands of connective tissue called Trabeculae
- Each lobule has 2 compartments –

  1) Cortex – the outer compartment, filled with immature T cells

2) Medulla – the inner compartment

- The area between Cortex and Thymic capsule – the Subcapsular Cortex – is a site of youngest thymocyte proliferation
- Cortex and medulla are criss-crossed by a 3D stromal cell network composed of epithelial cells, intedigitating dendritic cells and macrophages
- Some epithelial cells in the outer cortex have long membrane extensions surrounding about 50 thymocytes and forming large multinuclear complex known as Nurse cell

- Other cortical epithelial cells having long interconnecting cytoplasmic extensions interact with numerous thymocytes as they traverse the cortex
- Interdigitating Dendritic cells of cortico-medullary junction also have long extensions that interact with developing thymocytes
- T cells mature in cortex and migrate to medulla
- In medulla they encounter macrophages and dendritic cells
- Here T cells undergo thymic selection resulting in development of mature, functional T cells

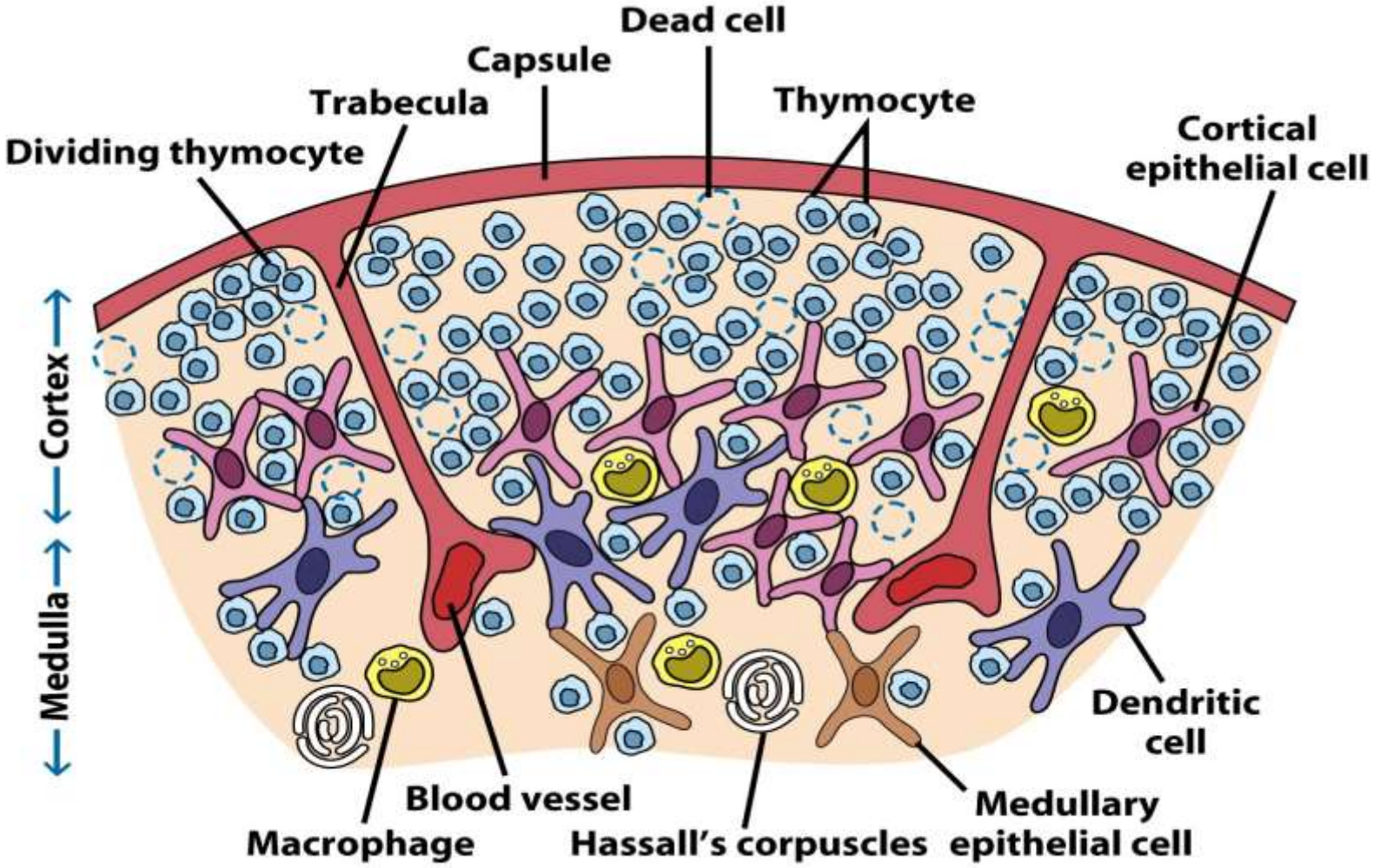


Figure 2-12
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- Mature T cells then leave and enter peripheral blood circulation to reach secondary lymphoid organs
- Importance of thymus in immune system was studied by neonatal thymectomy (surgical removal of thymus from newborn mice) that results in decrease in circulating T lymphocytes and an absence of cell-mediated immunity
- Failure in the development of thymus in mice (nude mice) and humans results in DiGeorge's Syndrome
- In this disease, circulating T cells and cell-mediated immunity are absent and chances of infectious disease are increased
- Aging reduces thymic function
- Thymus reaches its maximal size at puberty
- It weighs 70gms in infants and in elderly people it weighs only 3kgs due to decrease in both cortical and medullary cells and an increase in the total fat content of the organ

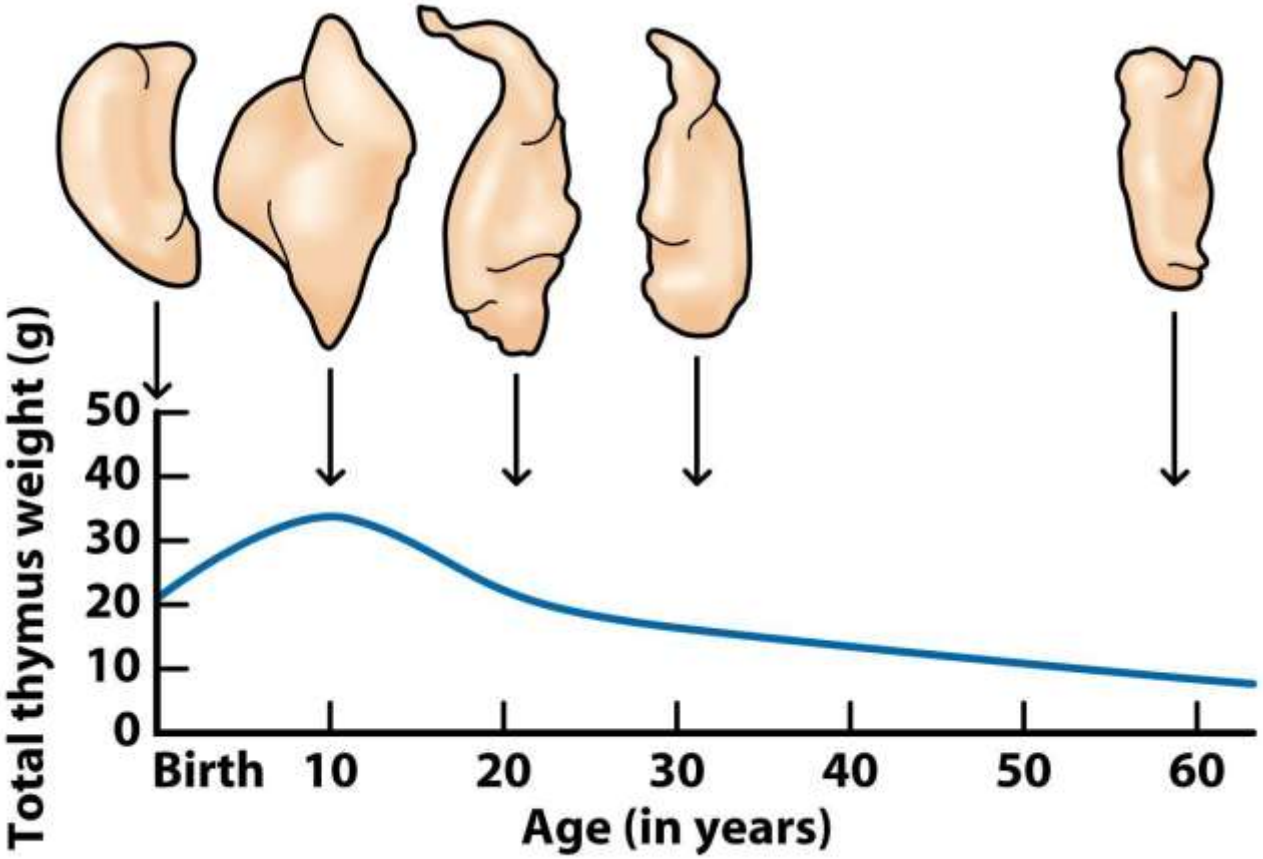

Figure 2-13
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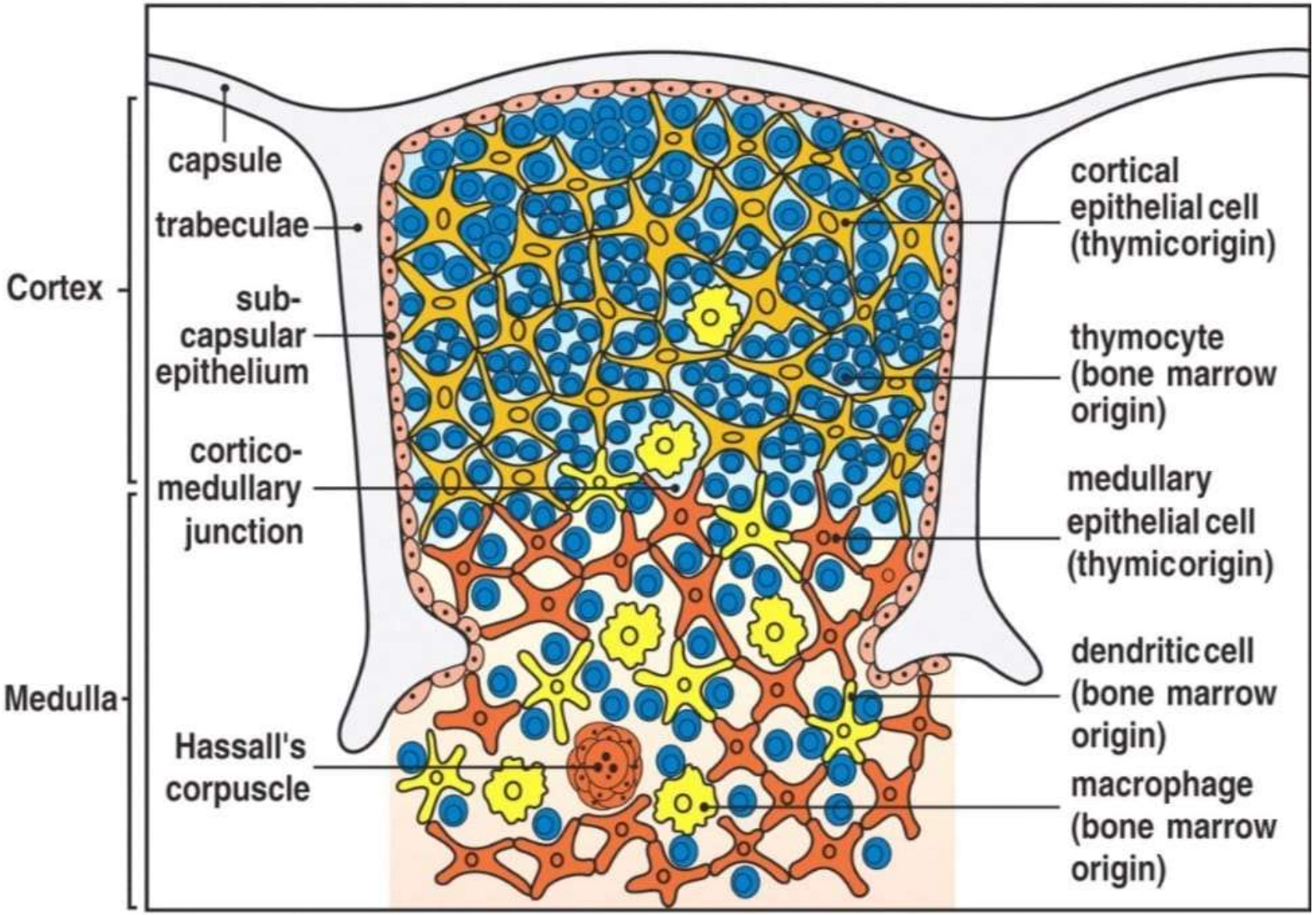

Figure 5-3 part 2 of 2 The Immune System, 2/e (© Garland Science 2005)

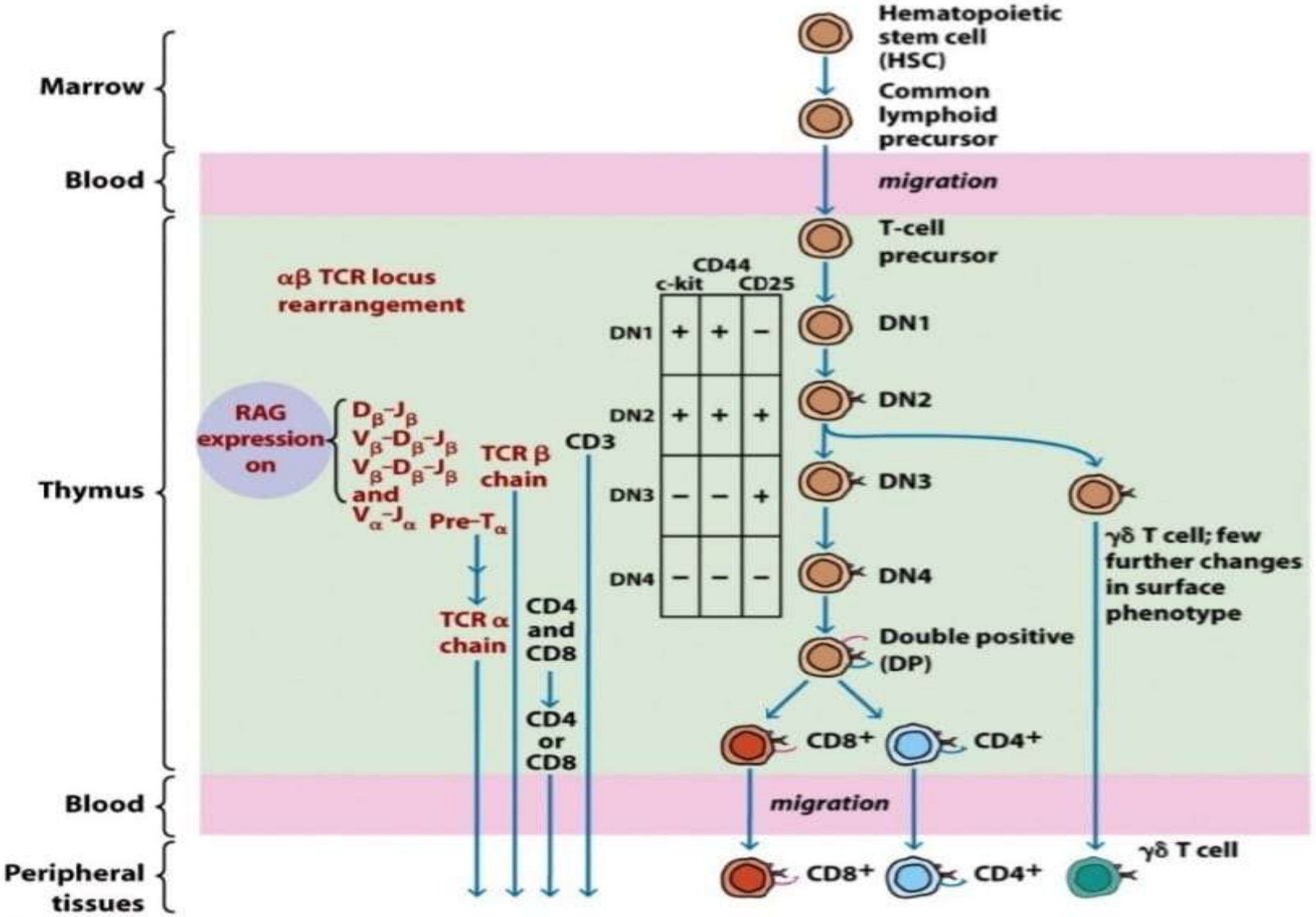

Figure 10-2
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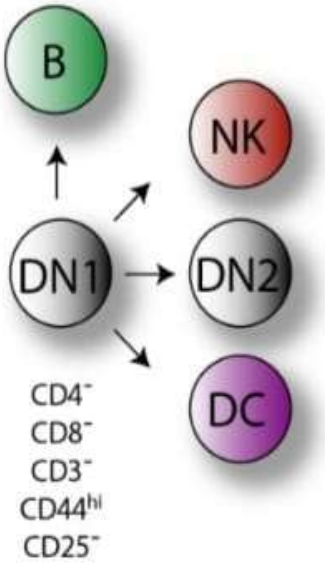
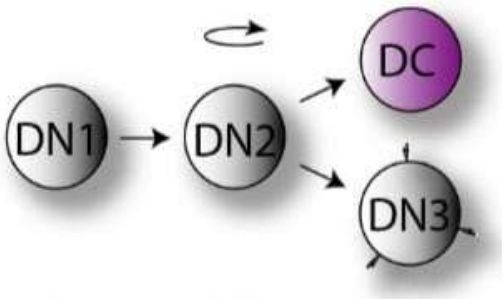
DN1 Cells in the Thymus Are Not Committed to become T Lymphocytes



- TCR loci are in germline configuration
- Cell can differentiate to become a B lymphocyte, Natural Killer cell, or Dendritic cell (Michie et al, JI, 2000; Ikawa et al, JEM, 1999;Sanchez et al, JEM, 1994)

Location: Subcapsular Zone

The DN2 Subset of Thymocytes are More Committed, but Not Quite Exclusive

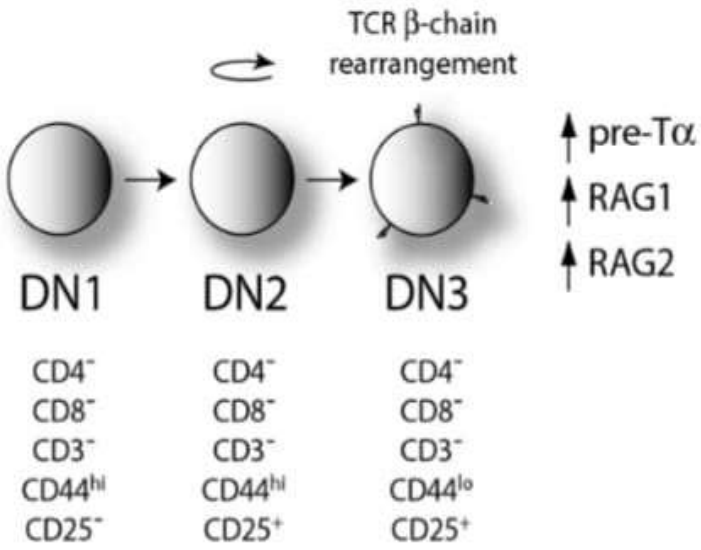


Location: Subcapsular Zone

↻ = proliferation

- TCR loci are in germline configuration
- DN2 thymocytes may give rise to dendritic cell(Moore et al, 1995; Wu et al 1996; Ardavin et al, Nature 1993; Shortman et al, Imm Rev. 1998)
- DN2 thymocytes no longer differentiate to the NK cell or B lymphocyte lineages

DN3 Cells are Committed to the T Lineage



Location: Subcapsular Zone

= proliferation

- Downregulation of CD44 expression
- Upregulation of RAG genes
- V-D-J recombination of TCRβ chain locus
- Expression of pre-TCRα chain

Thymocytes at different developmental stages are found in distinct parts of the thymus

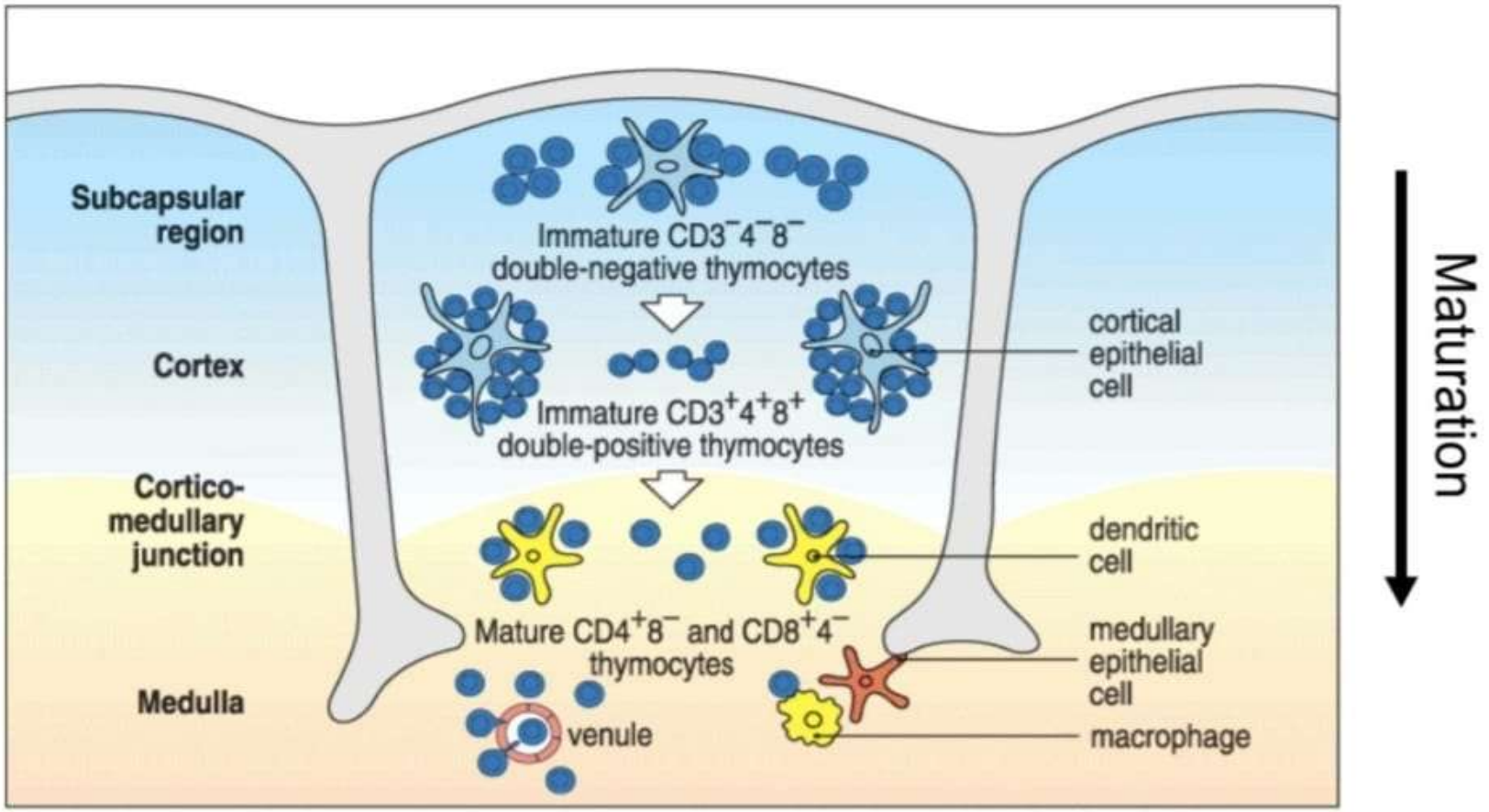


Fig 7.13 © 2001 Garland Science

Secondary Lymphoid Organs

- Lymph nodes and Spleen are the most organized SLOs
- MALT is somewhat less organized SLO and is associated with the linings of multiple organ systems, including the GI tract and respiratory tracts
- MALT includes tonsils, Peyer's patches (small intestine), appendix and numerous lymphoid follicles within the lamina propria of the intestines and in the mucous membranes lining the upper airways, bronchi and gastrointestinal tract
- All SLOs include anatomically distinct regions of T cell and B cell activity and all develop lymphoid follicles
- Lymphoid follicles are highly organized microenvironments that are responsible for the development and selection of antibody producing B cells

Spleen

- It is the largest of the SLOs
- Ovoid shaped, situated high in the left side of the abdominal cavity
- Mounts immune response to antigens in the blood stream
- Spleen specializes in filtering blood and trapping blood borne antigens. Therefore, it is important in response against systemic infections
- Unlike lymph nodes, spleen is not supplied by lymphatic vessels
- Blood-borne antigens and lymphocytes are carried into spleen through the splenic artery and out via the splenic vein

- It is the major organ in the body in which antibodies are synthesized and released into circulation

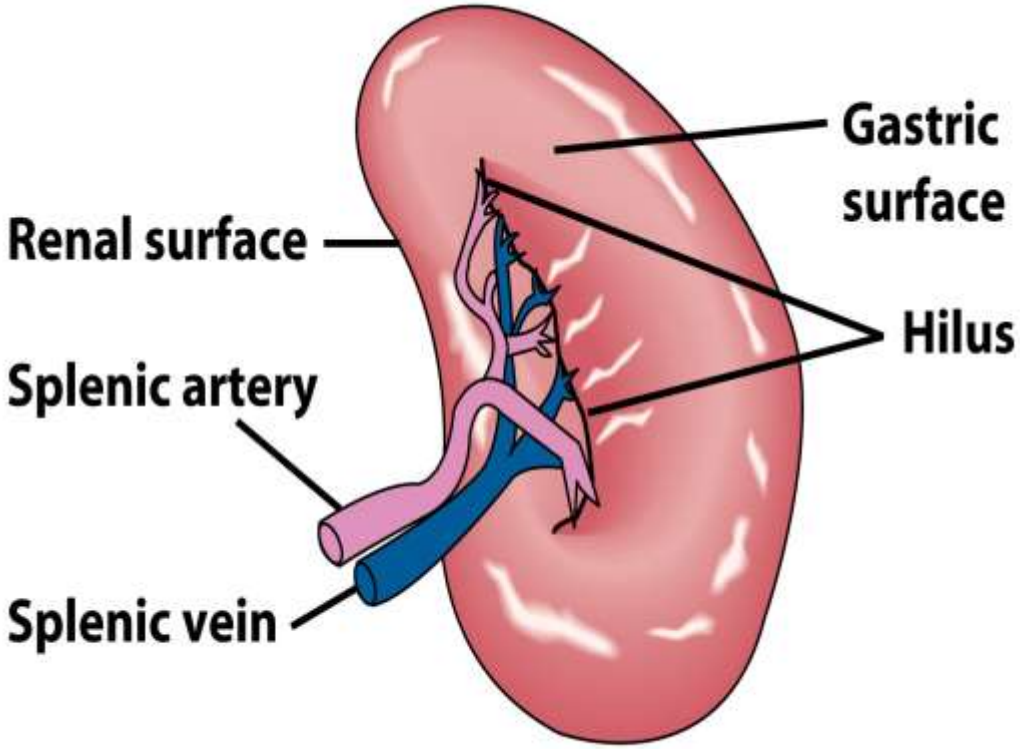


Figure 2-17a
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- Spleen is surrounded by a capsule from which a number of projections, called trabeculae, extend. It gives structural support
- There are 2 main regions : red pulp & white pulp
- These 2 regions are separated by marginal zone. It contains macrophages and B cells which are the first line of defense against certain blood-borne pathogens
- Red pulp consists of RBC, macrophages, some lymphocytes

- Here old and defective RBCs are destroyed and removed
- White pulp is the lymphoid-rich region of spleen
- It consists of Periarteriolar lymphoid sheath (PALS) and surrounds the branches of splenic artery
- PALS contains T cells and B-cell follicles
- Germinal centers are generated within these follicles during an immune response
- Blood-borne antigens and lymphocytes enter spleen through the splenic artery and interact first with the cells at the marginal zone
- Here antigens are trapped and processed by dendritic cells, which then travel to PALS
- Specialized B cells of the marginal zone also bind antigen via complement receptors and convey it to the follicles
- Migrating B and T cells in the blood enter marginal zone and then migrate to the follicles and PALS, respectively

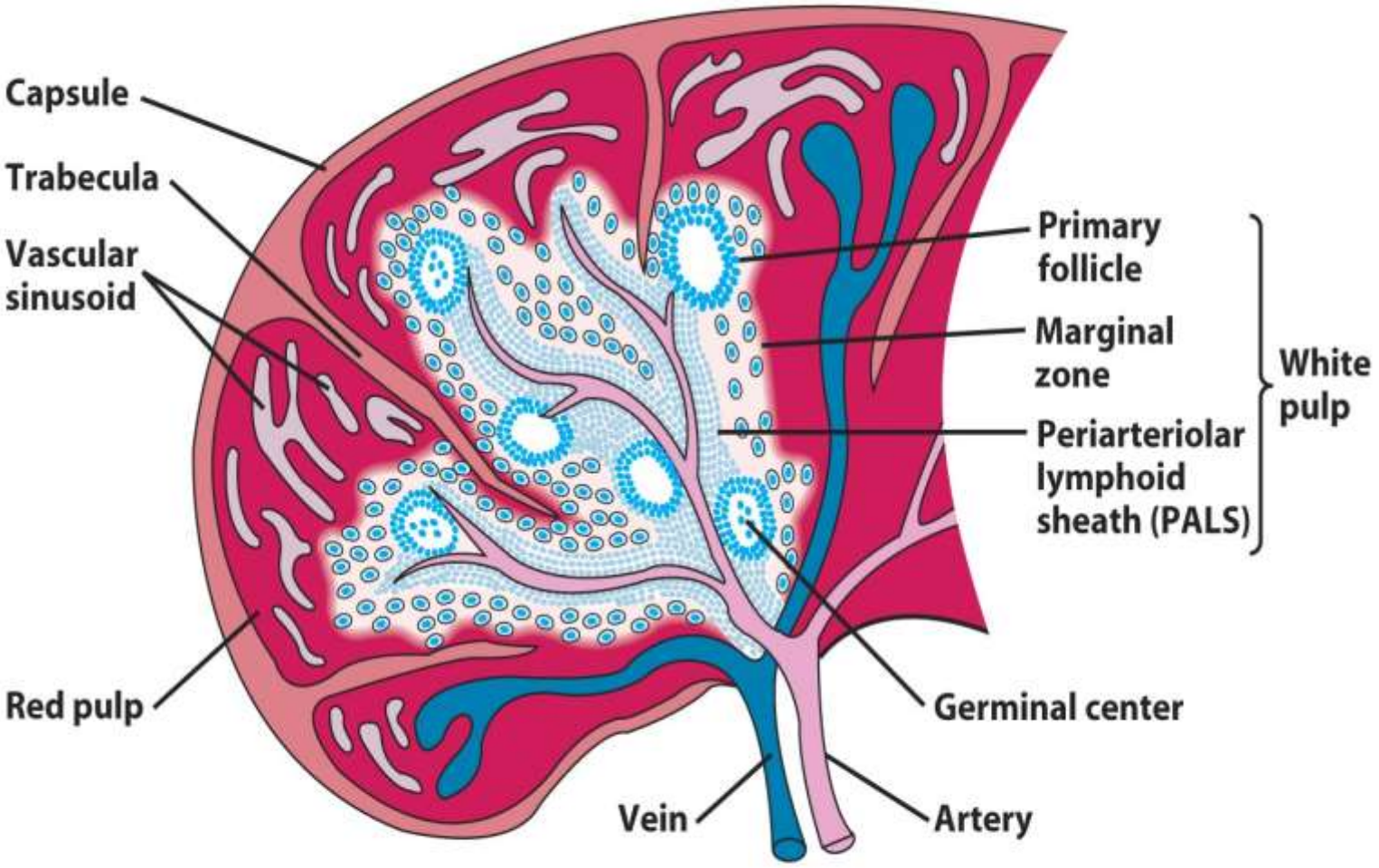

Figure 2-17b
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- Naïve B cells encounter antigen in the follicles and Naïve T cells meet antigen as MHC-peptide complexes on the surface of dendritic cells in the PALS
- Activated CD4+ TH cells then provide help to B cells and CD8+ T cells
- Some activated B cells and TH cells migrate back into follicles and generate germinal centers
- Spleen plays role in iron metabolism, thrombocyte storage, hematopoiesis etc.
- Without a spleen one can lead relatively healthy life
- However, splenectomy leads to an increased incidence of sepsis caused primarily by *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenzae* in children and is known as OPSI, i.e., overwhelming post-splenectomy infection

Mucosa-Associated Lymphoid Tissue

- Mucosal membranes are the major sites of entry for most pathogens and are defended by a group of organized lymphoid tissues collectively known as MALT
- The respiratory epithelium is known as Nasal-associated lymphoid tissue (NALT), the intestinal epithelium is known as Gut-associated lymphoid tissue (GALT)
- GALT includes well-organized structures like tonsils, adenoids, appendix and Peyer’s patch, which are found within the intestinal lining. It contains follicles and T cell zones

- The outer mucosal epithelial layer of intestine contains intraepithelial lymphocytes (IEL), which are mostly T cells
- Lamina propria lies under the epithelial layer and contains B cells, plasma cells, activated T cells and macrophages
- Peyer's patches are nodules of 30-40 lymphoid follicles that are extended into the muscle layers just below lamina propria
- Lymphoid follicles of Peyer's patch can develop into secondary follicles with germinal centers
- Importance of MALT: 1. contain huge number of antibody-producing plasma cells, whose number exceeds that of plasma cells in spleen, lymph nodes and bone marrow combined

  2. epithelial cells of mucous membranes deliver small samples of antigen from respiratory, digestive and urogenital tracts to the underlying MALT

M cells

- M cells transport antigen across epithelium in the digestive tract
- M cells are flattened epithelial cells lacking microvilli
- They have a deep invagination or pocket in the basolateral plasma membrane, filled with a cluster of B cells, T cells and macrophages
- Antigens are endocytosed into vesicles in the intestinal lumen and are transported to pocket membrane
- The vesicles then fuse with pocket membrane delivering antigens to clusters of lymphocytes and APCs (dendritic cells)
- Ultimately B cells are activated and differentiated to produce IgA antibody

- M cells of mucous membranes endocytosed antigen from the lumen of digestive, respiratory and urogenital tracts
- The antigen is transported across the cell and released into the basolateral pocket
- They then activate B cells in the underlying lymphoid follicles
- The activated B cells differentiate into IgA -producing plasma cells which migrate along the lamina propria
- The outer mucosal epithelial layer contains intraepithelial lymphocytes, mainly T cells

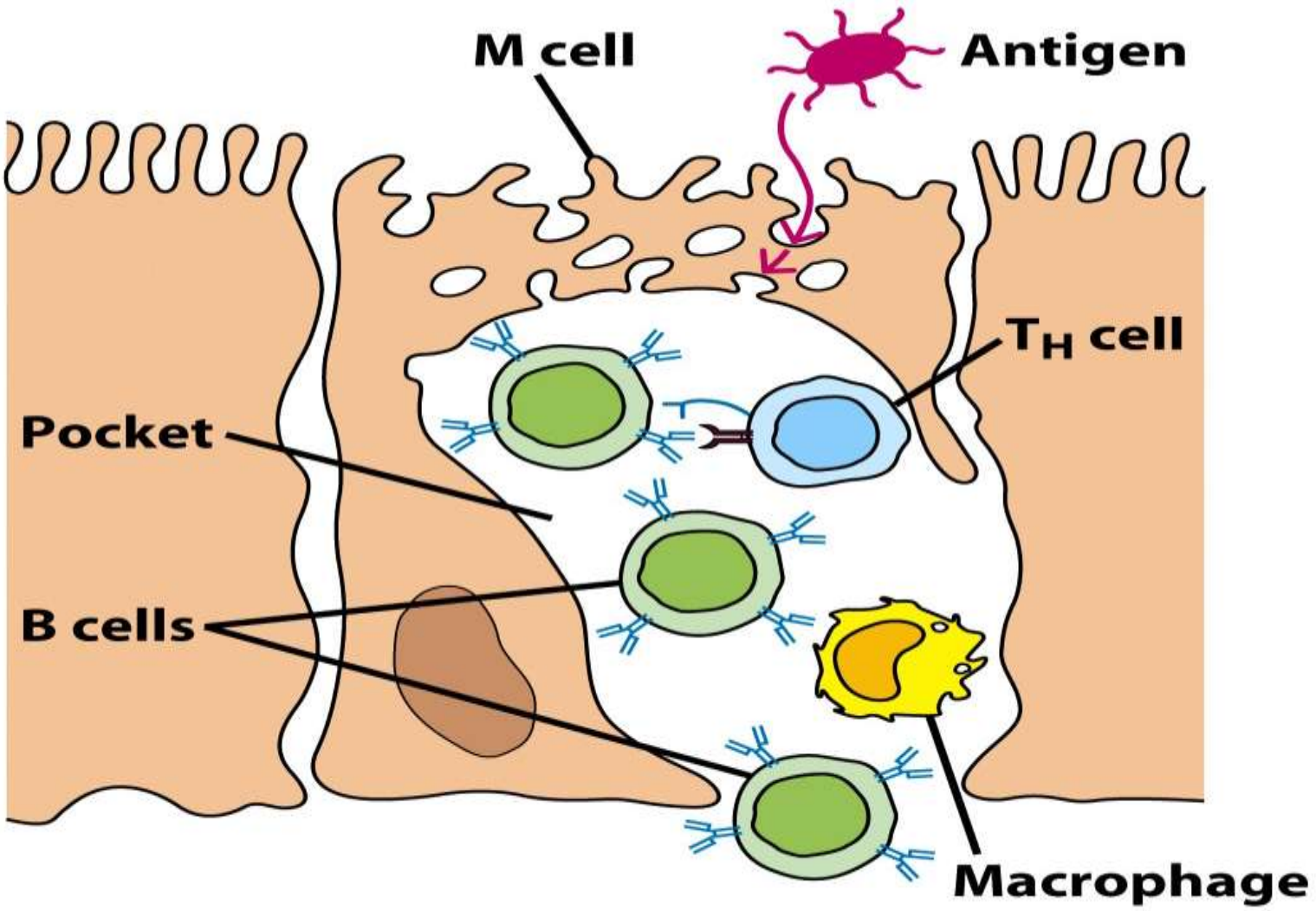

Figure 2-19a
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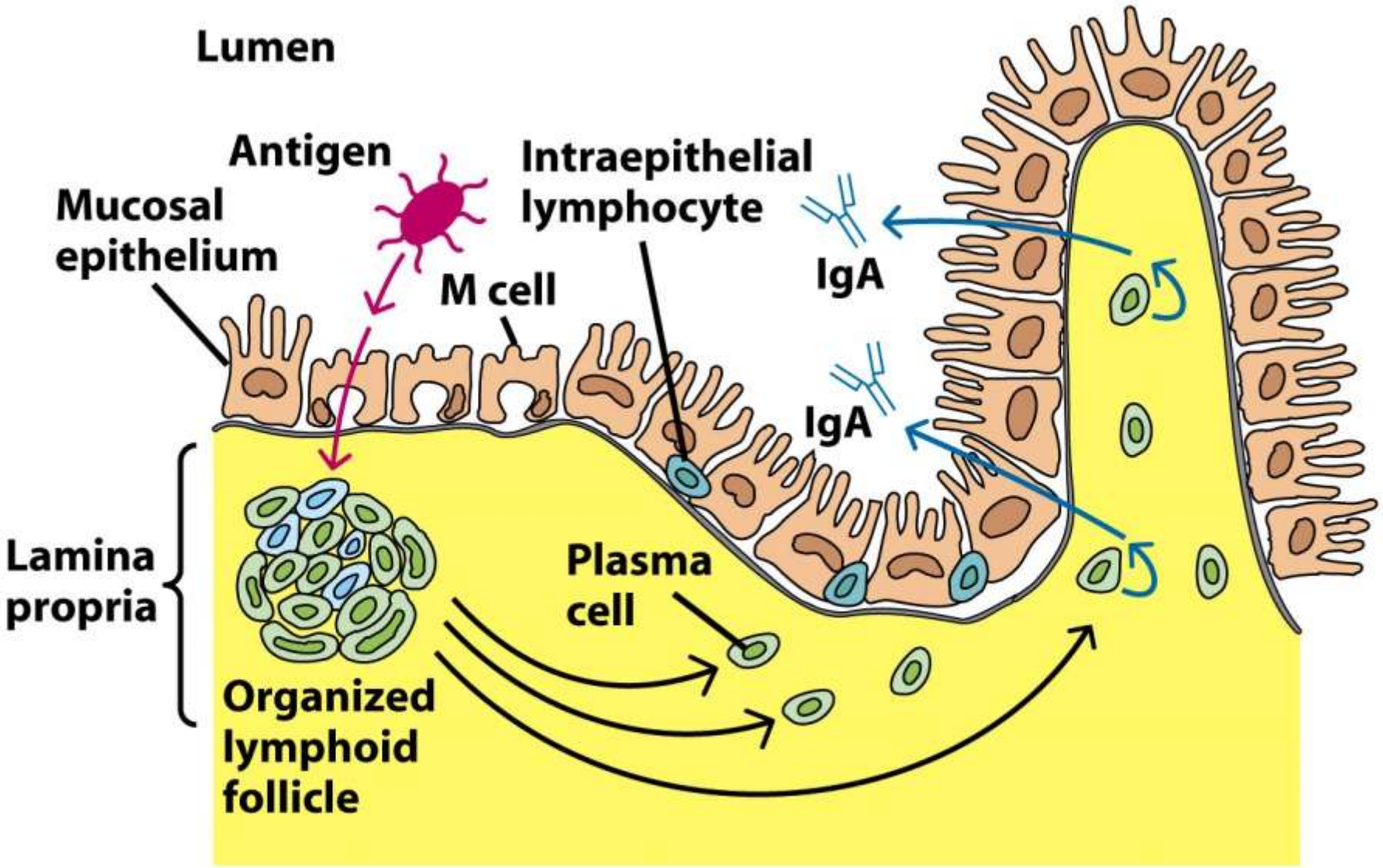

Figure 2-19b
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Lymph node

- Lymph nodes are the most specialized secondary lymphoid organs
- They are encapsulated, bean-shaped structures including networks of stromal cells packed with lymphocytes, macrophages and dendritic cells
- Connected to both blood vessels and lymphatic vessels
- They are the first organized lymphoid structure to encounter antigens entering tissue spaces

- Lymph nodes provide ideal microenvironments for encounters between antigen and lymphocytes and also for organized cellular and humoral immune responses
- It has 3 regions – cortex, paracortex and medulla
- Cortex contains B cells, macrophages and follicular dendritic cells arranged in follicles. B cells are concentrated primarily within follicles and germinal centers
- Paracortex is beneath the cortex and contains T cells, dendritic cells that migrated from tissues to node
- Medulla is the innermost layer and is populated largely by plasma cells. It is the site where cells exit via the efferent (outgoing) lymphatics

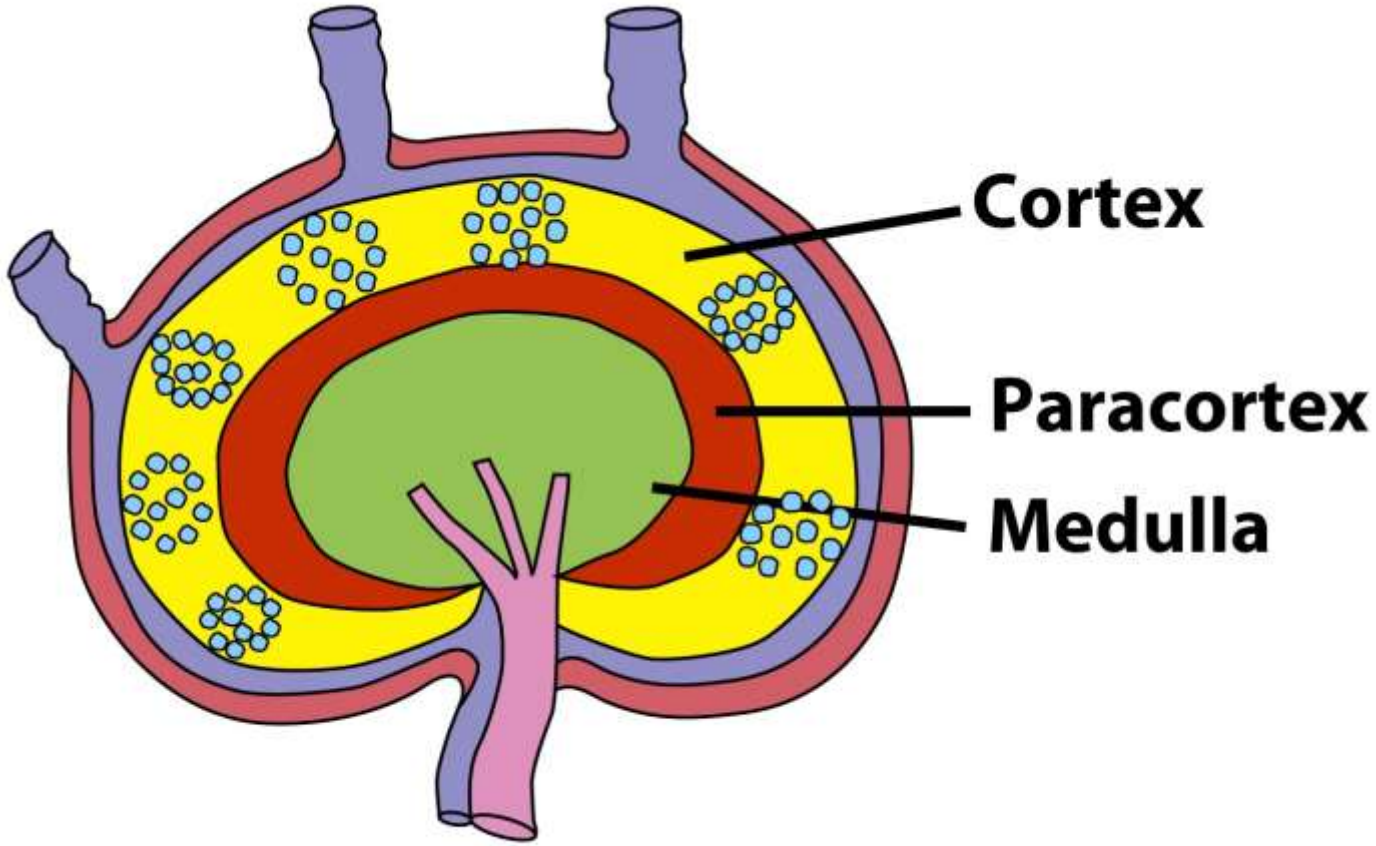

Figure 2-16a
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- Naïve lymphocytes circulating in the blood enter the node via high endothelial venules (HEV) via extravasation
- Antigen, APCs enter via affarent (incoming) lymphatic vessels
- Antigens enter either in particulate form or is processed and presented as peptides on the surface of migrating APCs
- Particulate antigen can be trapped by resident APCs in the subcapsular sinus or cortex and it can be passed to other APCs including B cells
- Otherwise, particulate antigen can be processed and presented as peptide-MHC complexes on cell surfaces of resident dendritic cells present in the T-cell rich paracortex
- All cell exit via efferent lymphatic vessels

T cell in Lymph node:

- After entering lymph node, naïve T cells start browsing MHC-peptide antigen complexes on dendritic cell surfaces in the paracortex
- Every naïve T cell takes about 16-24 hours to browse all the MHC-peptide combinations presented by APCs in a single lymph node
- The paracortex is crisscrossed by processes and conduits formed by fibroblastic reticular cells (FRCs)
- They guide the migration of APCs and T cells, facilitating their interaction
- T cells that browse the lymph node but do not bind MHC-peptide combinations exit via efferent lymphatics in the medulla
- T cells whose TCR bind to an MHC-peptide complex on an APC will stop migrating and take up residence in lymph node for several days
- Here they will proliferate and differentiate into effector cells with various functions

B cells in the Lymph node

- In lymph node, B cells are activated and differentiated to plasma cells
- Their activation requires both antigen engagement by BCR and direct contact with an activated CD4+ TH cell
- B cells circulate through lymph and blood and visit lymph nodes regularly via HEV
- They respond to specific signals and chemokines that draw them to the lymph node follicle
- Follicular dendritic cells assist B cells by presenting antigen to differentiating B cells and by maintaining follicular and germinal center structures
- B cells encounter antigen in the follicle

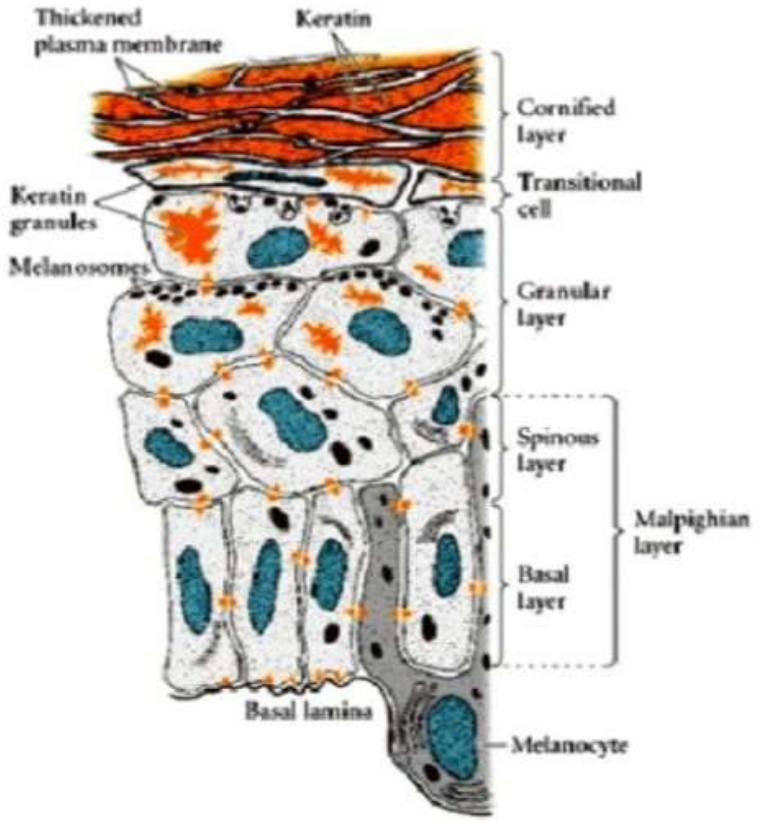
- If BCR binds it, the B cell becomes partially activated and engulfs and processes that antigen and then presents peptide-MHC complexes on their surface to TH cells
- After successfully engaging TH cells, they maintain contact for a number of hours and become fully activated. They then receive signals that induce B cell proliferation
- Some activated B cells differentiate directly into plasma cell but others re-enter the follicle to establish a germinal center
- Follicle without a germinal center is called primary follicle and a follicle that develops a germinal center is known as secondary follicle
- Germinal centers facilitate the generation of B cells with increased receptor affinities

- Here an antigen specific B cell clone will proliferate and undergo somatic hypermutation of the genes coding for their antigen receptors
- Those receptors that retain the ability to bind antigen with highest affinity survive and differentiate into plasma cells
- Plasma cells then travel to the medulla of the lymph node. Some will stay and release antibodies into the bloodstream; others will exit through the efferent lymphatics and take up residence in the bone marrow and release antibodies into circulation
- Initial activation of B cells and establishment of germinal center take place within 4-7 days of the initial infection, but germinal center remains active for 3 weeks or more
- During the first few days of infection, lymph nodes swell visibly and painfully due to an increase in the number of lymphocytes induced to migrate into the node as well as the proliferation of antigen-specific T and B cells within the lobe

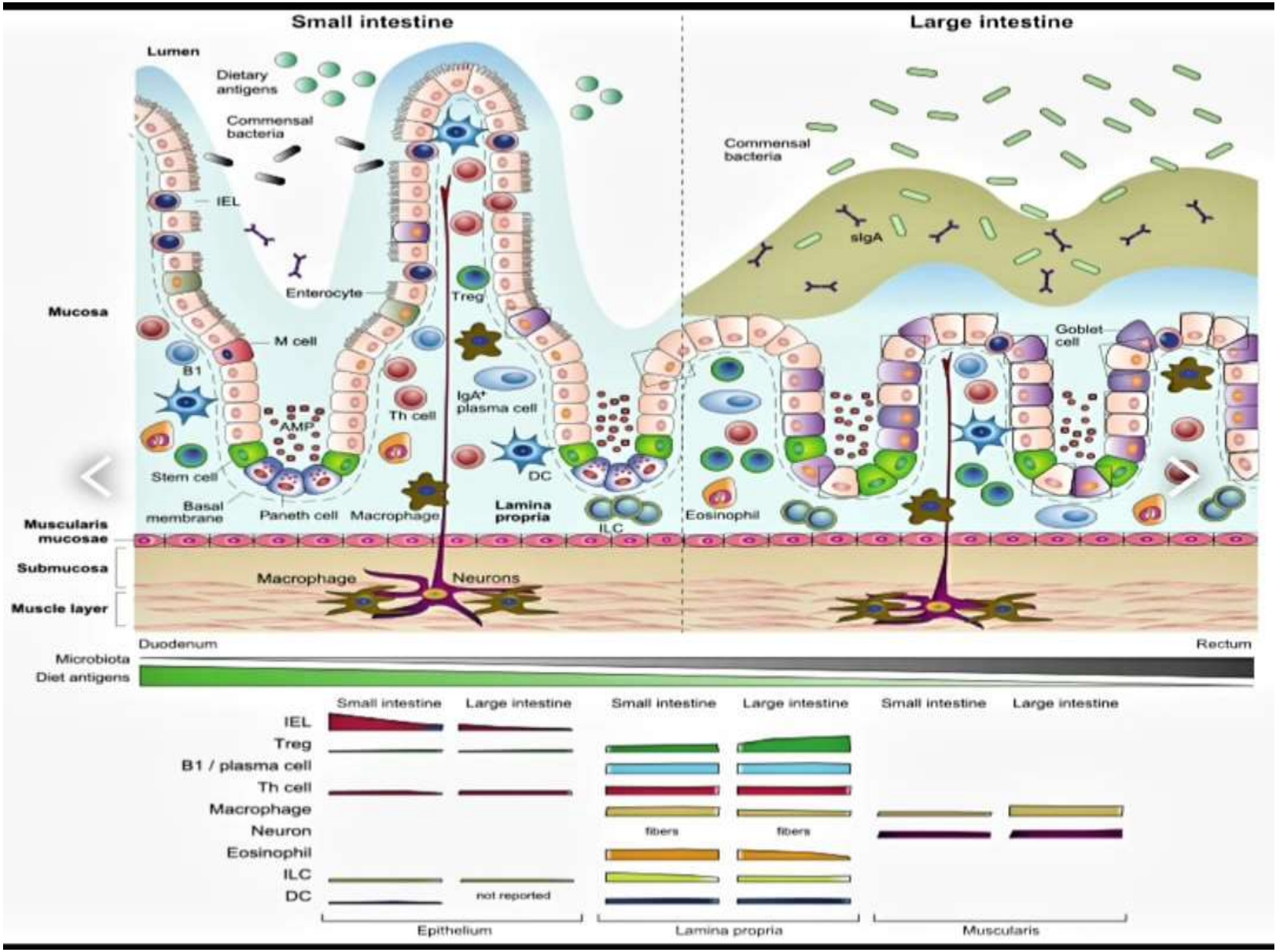
Generation of memory T and B cells in lymph node

- Interactions between TH cells and APCs and between activated TH cells and activated B cells proliferate and differentiate lymphocytes with the generation of memory T and B cells
- Memory T and B cells can take up residence in secondary lymphoid tissues or can exit the lymph node and travel among tissues that first encountered the pathogen
- Memory T cells of secondary lymphoid organs are called central memory cells and are phenotypically and functionally different from effector memory T cells circulating among tissues

Cutaneous Associated Lymphoid Tissue (CALT)



The skin is the largest organ in the body and plays an important role in nonspecific (innate) defences. The epidermal (outer) layer of the skin is composed of specialized cells called keratinocytes. These cells secrete a number of cytokines that may function in local inflammatory reaction. Scattered among the epithelial-cell matrix of the epidermis are Langerhann's cells, atype of dendritic cell, which internalize antigen by phagocytosis or endocytosis. They undergo maturation and migrate from the epidermis to regional lymph nodes, where they function as potent activators of naïve T_H cells. In addition to Langerhans cells, the epidermis also contaions so-called intraepidermal lymphocytes, which are mostly T cells. The underlying dermal layer of the skin also contains scattered T cells and macrophages. Most of these dermal cells appear to be either previously activated cells or memory cells.


Small intestine
Large intestine
Lumen
Dietary antigens
Commensal bacteria
IEL
Enterocyte
Treg
Mucosa
M cell
B1
IgA+ plasma cell
AMP
Th cell
Stem cell
DC
Basal membrane
Paneth cell
Macrophage
Lamina propria
ILC
Eosinophil
Goblet cell
sIgA
Muscularis mucosae
Submucosa
Muscle layer
Neurons
Duodenum
Rectum
Microbiota
Diet antigens
Small intestine
Large intestine
IEL
Treg
B1 / plasma cell
Th cell
Macrophage
Neuron
Eosinophil
ILC
DC
fibers
not reported
Epithelium
Lamina propria
Muscularis

The skin is an important anatomic barrier to the external environment,and its large surface area makes this tissue important in nonspecific(innate)defenses.The epidermal (outer)layer of the skin is composed largely of specialized epithelial cells called keratinocytes.These cells secrete a number of cytokines that may function to induce a local inflammatory reaction.In addition,keratinocytes can be induced to express class II MHC molecules and may function as antigen-presenting cells.Scattered among the epithelial-cell-matrix of the epidermis are Langerhans cells,a type of dendritic cell,which internalize antigen by phagocytosis or endocytosis.The Langerhans cells then 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 Helper cells.

The epidermis also contains so-called intraepidermal lymphocytes.These are similar ti the intraepithelial lymphocytes of MALT in that most of them are CD8 plus cells,which have limited diversity for antigens that enters through the skin and some immunologist believe that they may play a role in combating antigens that enter through skin.The under-laying dermal layer of the skin contains scattered CD4 plus and CD8 plus T cells and macrophages.Most of these dermal T cells were either previously activated cells or are memory cells.

Tertiary Lymphoid Tissue

- Tissues that are the sites of infection are referred to as tertiary lymphoid tissues
- Lymphocytes activated by antigen in the secondary lymphoid tissue can return to these organs (lung, liver, brain) as effector cells and can also reside there as memory cells
- Tertiary lymphoid tissues can generate defined microenvironments that organize the returning lymphoid cells, eg, brain establishes reticular systems that guide lymphocytes responding to toxoplasmosis, a chronic infection caused by protozoa