Molecular diagnostics. L4-5
Lecturer: Zhussupova A.I.

What is adaptive immunity?

What is innate immunity?

Terminology

Pathology:

The study of the structural and functional changes leading to disease in:

- Cell
- Tissue
- Organs

Pathophysiology:

Is the abnormal function of organs or systems due to disease

Tools:

- Molecular
- Microbiological
- Immunological
- Morphological

Terminology

Pathology is divided

- General
- Special or systemic

General pathology:

Basic reaction of cells and tissue to normal stimuli

Specific pathology:

Specific response special organs to well defined stimuli

Four aspects of disease process

Aetiology

Pathogenesis

Morphological changes

Clinical significance

1.Aetiology(Cause)

- A) Determining cause
  - Specifically known to be the soul cause of disease such pathogenic organism e.g. HIV
- B) Predisposing causes
  - Leading indirectly to disease such as genetic predisposition

2.Pathogenesis

- Is the mechanism by which a certain aetiological factor causes disease (In Greek: *pathos* = disease, *genesis* = development).
- Some forms of pathogenesis are:
- Inflammation
- Malignancy
- Tissue breakdown

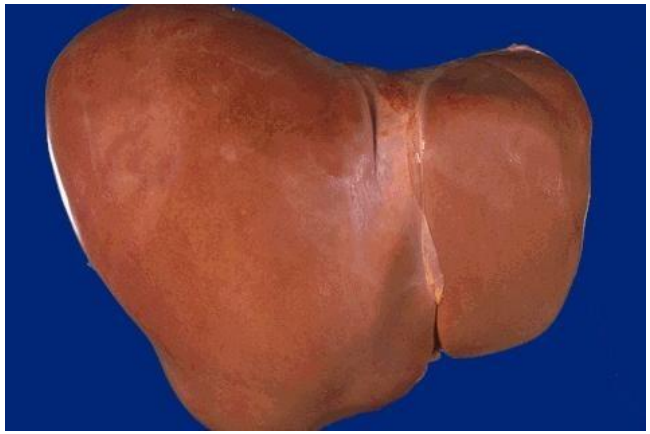
2.Pathogenesis

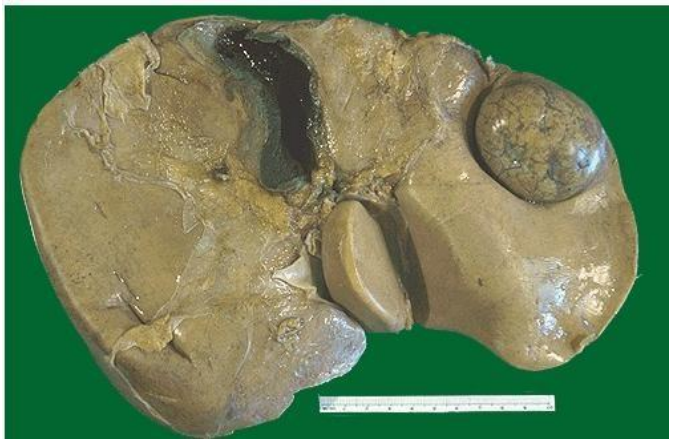
- The pathogenesis process leads to the formation of lesion
- *Lesion* is derived from the Latin word "laesio" which means "injury."
- Lesions are a result of damage to tissues. For example:
  - A cancerous tumor is an example of a lesion
  - The surrounding tissue damaged by a tumour is also termed a lesion.

3.Morphological changes

- Are the changes that occur in the cell tissue or organ as a result of the pathological process

- These changes can be Morbid:
  - Macroscopic appearance visible to the naked eye

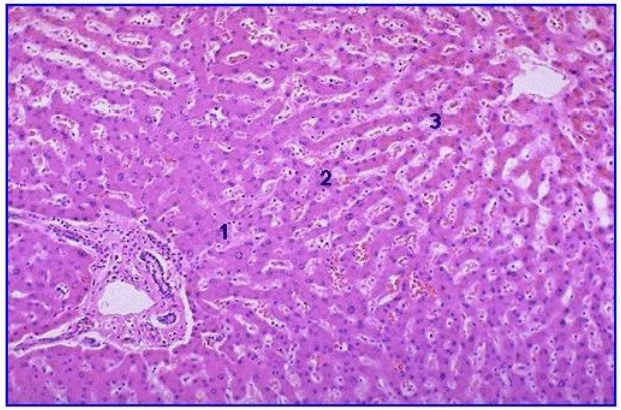


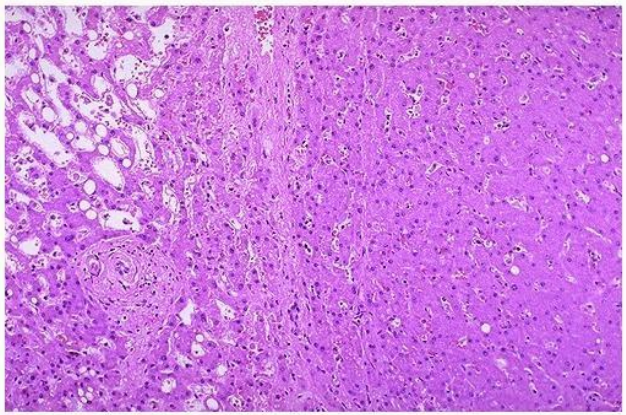


3.Morphological changes

- Are the changes that occur in the cell tissue or organ as a result of the pathological process

- Or Histological :
  - Microscopic appearance only visible under the microscope





4.Clinical significance

- What impact do these changes have on the patient?

Progression of a disease

- Complete cure
- Death
- Complication
  - Additional pathological changes which may occur during or after the course of any disease

Pathological investigation

- During life
  - Surgical biopsy
  - Fine needle aspiration biopsy (FNAB)
  - Cytopathology
  - Molecular techniques
- After death
  - Autopsy

Exposure to stress (irritant)

- Mild irritant
- Moderate

} A) inflammation
B) Degeneration

- Severe irritant → Necrosis

Types of irritants

- Living irritant:
  - Bacteria
  - Pathogenic fungi
  - Parasite
  - Virus
- Non-living irritant:
  - Physical
    - Trauma, Burns, Radiation
  - Chemical
    - Acids, Alkalies
  - Immunological
    - Ag-Ab reaction
    - Hypersensitivity reaction

Cardinal signs of inflammation

- Redness
- Hotness
- Swelling (edema) due to inflammatory exudate
- Pain: due to pressure of edema on nerves and irritation of nerve ends by metabolites
- Loss of function: this is to make the inflamed part of tissue rest and heal.

Inflammation

- Greek root + -itis
- complex protective reaction
- caused by various endo- and exogenous stimuli
- injurious agents are destroyed, diluted or walled-off
- without inflammation and mechanism of healing could organism not survive
- can be potentially harmfull

Mechanisms

- local - in cases of mild injury
- systemic
- 3 major:
- 1. alteration
- 2. exsudation - inflammatory exsudate
  - liquid (exsudate)
  - cellular (infiltrate)
- 3. proliferation (formation of granulation and fibrous tissue)
- usualy - all 3 components - not the same intensity

Classification

- several points of view
- length:
  - acute × chronic (+ subacute, hyperacute)
- according to predominant component
  1. alterative (predominance of necrosis - diphtheria)
  2. exsudative (pleuritis)
  3. proliferative (cholecystitis - thickening of the wall by fibrous tissue)

Classification

- according to histological features
  - nonspecific (not possible to trace the etiology) - vast majority
  - specific (e.g. TB)

- according to causative agent
  - aseptic (sterile) - chemical substances, congelation, radiation - inflammation has a reparative character
  - septic (caused by living organisms) - inflammation has a protective character

Acute inflammation

- important role in inflammation has microcirculation!
- supply of white blood cells, interleukins, fibrin, etc.

Local symptomatology

- classical 5 symptoms (Celsus 1st c. B.C., Virchow 19th c. A.D.)
- 1. calor - heat
- 2. rubor - redness
- 3. tumor - swelling
- 4. dolor - pain
- 5. functio laesa - loss (or impairment) of function

Systemic symptomatology

- fever (irritation of centre of thermoregulation)
  - TNF, IL-1
  - IL-6 – high erythrocyte sedimentation rate
- leucocytosis
  - bacteria – neutrophils
  - parasites – eosinophils
  - viruses - lymphocytosis
- leucopenia
  - viral infections, salmonella infections, rickettsiosis
- immunologic reactions - increased level of some substances (C-reactive protein)

Vascular changes

- vasodilation
- increased permeability of vessels due to widened intercell. junctions and contraction of endothelial cells (histamin, VEGF, bradykinin)
- protein poor transudate (edema)
- protein rich exsudate

- leukocyte-dependent endothelial injury
- proteolysis – protein leakage
- $\rightarrow$ platelet adhesion $\rightarrow$ thrombosis

Cellular events

- leukocytes margination → rolling → adhesion → transmigration
- emigration of:
  - neutrophils (1-2 days)
  - monocytes (2-3 days)
- chemotaxis
  - endogenous signaling molecules - lymphokines
  - exogenous - toxins
- phagocytosis - lysosomal enzymes, free radicals, oxidative burst
- passive emigration of RBC - no active role in inflamm. - hemorrhagic inflammation

Phagocytosis

- adhesion and invagination into cytoplasm
- engulfment
- lysosomes - destruction
- in highly virulent microorganisms can die leucocyte and not the microbe
- in highly resistant microorganisms - persistence within macrophage - activation after many years

Outcomes of acute inflammation

1. resolution - restoration to normal, limited injury
   - chemical substances neutralization
   - normalization of vasc. permeability
   - apoptosis of inflammatory cells
   - lymphatic drainage
2. healing by scar
   - tissue destruction
   - fibrinous inflammtion
   - purulent infl. $\rightarrow$ abscess formation (pus, pyogenic membrane, resorption - pseudoxanthoma cells - weeks to months)
3. progression into chronic inflammation

Chronic inflammation

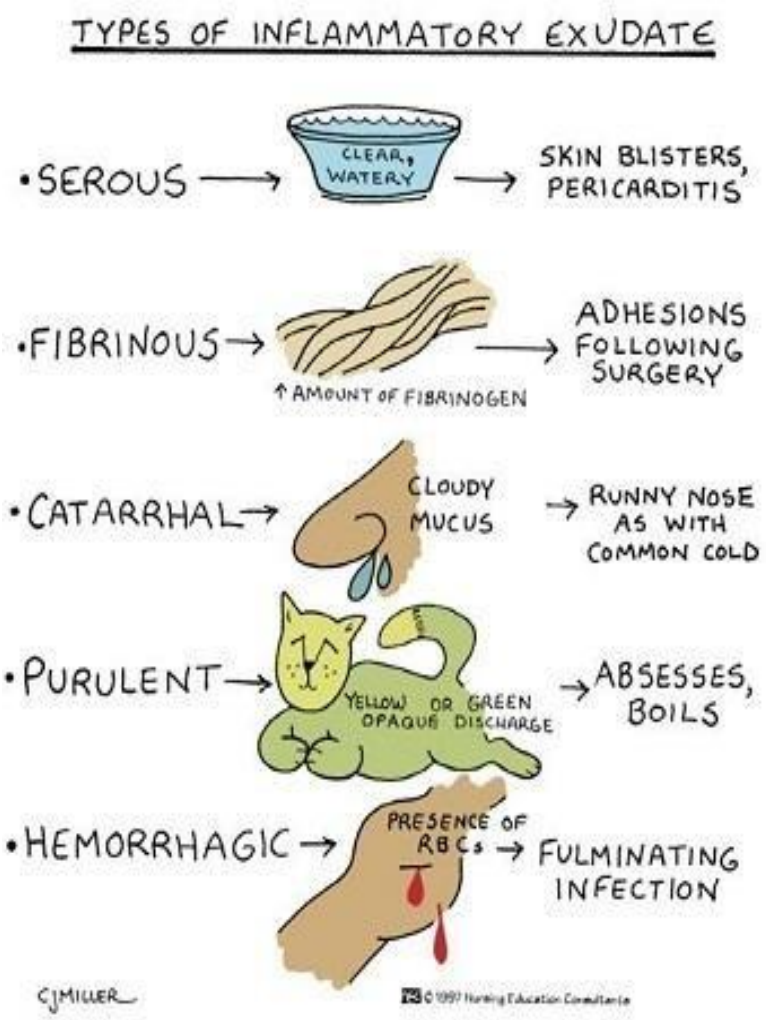
- reasons:
  - persisting infection or prolonged exposure to irritants (intracell. surviving of agents - TBC)
  - repeated acute inflamations (otitis, rhinitis)
  - primary chronic inflammation - low virulence, sterile inflammations (silicosis)
  - autoimmune reactions (rheumatoid arthritis, glomerulonephritis, multiple sclerosis)

Chronic inflammation

- chronic inflammatory cells ("round cell" infiltrate)
  - lymphocytes
  - plasma cells
  - monocytes/macrophages activation of macrophages by various mediators - fight against invaders
- lymphocytes → plasma cells, cytotoxic (NK) cells, coordination with other parts of immune system
- plasma cells - production of Ig
- monocytes-macrophages-specialized cells (siderophages, gitter cells, mucophages)

Types of acute inflammation (based on type of exudates)

1. **Catarrhal inflammation**:
2. **Serous inflammation**:
3. **Fibrinous inflammation**:
4. **Membranous inflammation**:
5. **Hemorrhagic inflammation**:
6. **Gangrenous inflammation**:
7. **Allergic inflammation**:
8. **Suppurative or purulent inflammation**:



Name	Occur in	Characterized by
Catarrhal	Mild inflammation in mucous membrane of respiratory or alimentary tracts e.g. common cold and catarrhal appendicitis	Exudates rich in mucous
Serous	Mild inflammation in serous surface such as pleural cavity, joint cavity where no damage in endothelium ex. Tuberculosis pleurisy and Common blisters	Extensive watery low protein exudates
Fibrinous	Outpouring of exudates with high protein and less volume ex. in lobar pneumonia due to *Streptococcus pneumonia* & pericardium inflammation	Exudates rich in fibrinogen
Membranous	Fibrinous inflammation in which network of fibrin entangling inflammatory cells and bacteria forms pseudo-membrane. Example: Diphtheria , Bacillary dysentery.	Yellowish grey pseudo membrane rich in fibrin, polymorphs & necrotic tissues
Hemorrhagic	In blood vessels e.g. in plague	Exudates rich RBCs
Gangrenous	Acute appendicitis	Necrotic tissues resulting from thrombi or emboli
Allergic	Result to Ag – Ab reaction Hypersensitivity	Presence of edema & increase in vascularity.
Suppurative	Caused by pyogenic bacteria and is characterized by pus formation Example: Abscess.	Large amount of Pus & Purulent exudates produced

healing

Tissue repair involves replacement of damaged tissue with new healthy living tissue when resolution cannot occur

Types

Usually involves two separate but coordinated components

A) **Regeneration:**

healing by the same type of tissue cells from surrounding healthy living cells, this occurs with in small damages of labile cells and stable cells for examples liver cirrhosis and bone fractures

B) Fibros (scar tissue):

healing by granulation tissue (fibroblast with new capillaries formed) which mature a vascular fibrous tissue (scar), this occurs in the healing process of permanent cells and stable cells with high damage. for example myocardial infraction and wounds

Sequence of events in healing

Initial phase - Hemostasis

- Following vasoconstriction, platelets adhere to damaged endothelium and discharge adenosine diphosphate (ADP), promoting thrombocyte clumping, which dams the Wound
- The inflammatory phase is initiated by the release of numerous cytokines by platelets.
- Fibrinogen is cleaved into fibrin and the framework for completion of the coagulation process is formed. Fibrin provides the structural support for cellular constituents of inflammation.
- This process starts immediately after the insult and may continue for a few days

Sequence of events in healing

Second phase - Inflammation

- Within the first 6-8 hours, the next phase of the healing process is underway, with polymorphonuclear leukocytes (PMNs) or PNLs engorging the wound
- These cells “cleanse” the wound, clearing it of debris. The PMNs attain their maximal numbers in 24-48 hours and commence their departure by hour 72
- As the process continues, monocytes also exude from the vessels. These are termed macrophages. The macrophages continue the cleansing process and manufacture various growth factors during days 3-4.
- Many factors influencing the wound healing process are secreted by macrophages. These include TGFs, cytokines and interleukin-1 (IL-1), tumor necrosis factor (TNF)

Sequence of events in healing

Third phase - Granulation

This phase consists of different subphases. These subphases do not happen in discrete time frames but constitute an overall and ongoing process. The subphases are:

- fibroplasia
- matrix deposition
- angiogenesis
- and re-epithelialization

- In days 5-7, fibroblasts have migrated into the wound, laying down new collagen of the subtypes I and III

- The wound is suffused with GAGs and fibronectin that are bonded covalently to a protein core and contribute to matrix deposition

- Angiogenesis is the product of parent vessel offshoots. The formation of new vasculature requires extracellular matrix and basement membrane degradation followed by migration, mitosis, and maturation of endothelial cells

- Re-epithelization occurs with the migration of cells from the periphery of the wound and adnexal structures. This process commences with the spreading of cells within 24 hours. Division of peripheral cells occurs in hours 48-72, resulting in a thin epithelial cell layer, which bridges the wound.

This succession of subphases can last up to 4 weeks in the clean and uncontaminated wound.

Sequence of events in healing

Fourth phase - Remodeling

After the third week, the wound undergoes constant alterations, known as remodeling,

- This can last for years after the initial injury occurred. Collagen is degraded and deposited in an equilibrium-producing fashion
- The collagen deposition in normal wound healing reaches a peak by the third week after the wound is created.
- Contraction of the wound is an ongoing process resulting in part from the proliferation of the specialized fibroblasts termed myofibroblasts, which resemble contractile smooth muscle cells.

Stages of wound healing

Resolution/ Remodeling

Vessel regression, Collagen remodeling

Proliferation

Reepithelialization, Angiogenesis, Fibrogenesis,

Inflammation

PMNs, Macrophages, Lymphocytes

Hemostasis

Fibrin clot, platelet deposition

Time after injury

We are going to look at Immune System

1. Organs
2. Cells
3. Molecules

Role of the immune system

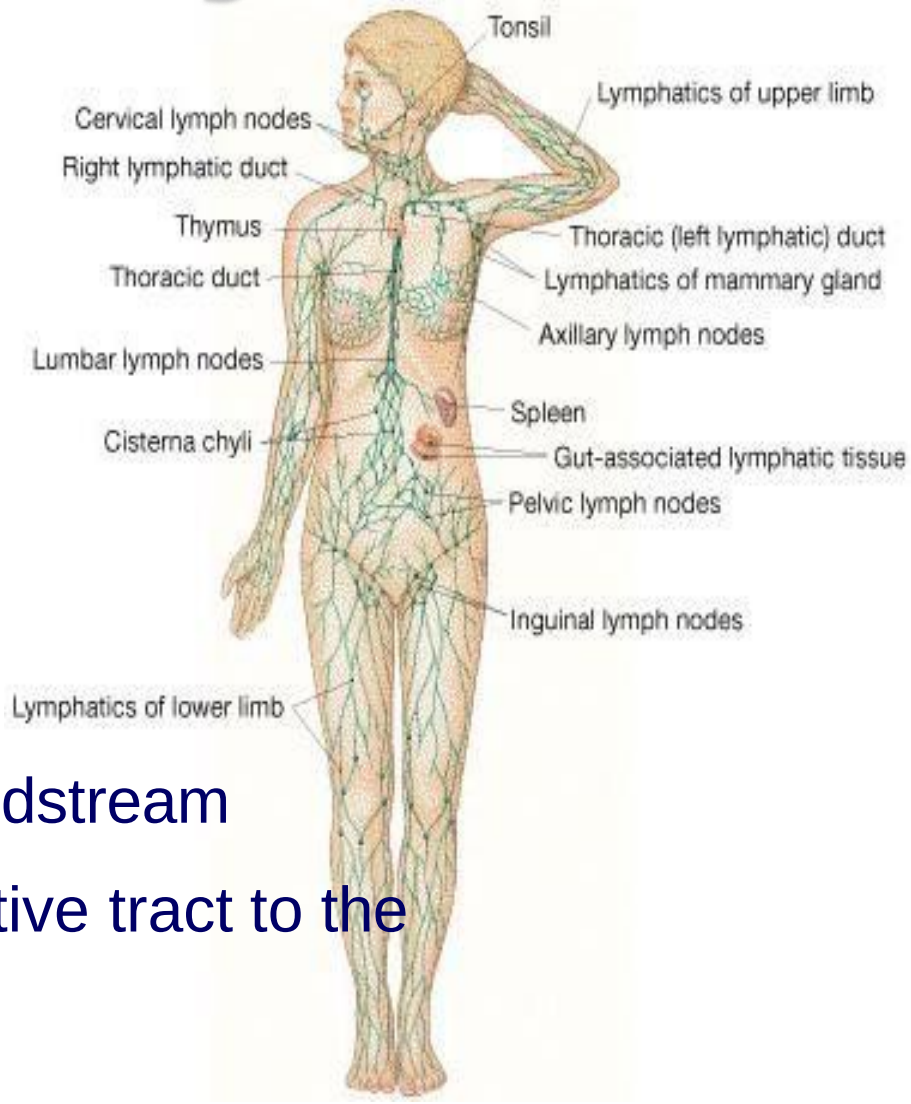
- Defense against microbes
- Defense against the growth of tumor cells
  - kills the growth of tumor cells
- Homeostasis
  - destruction of abnormal or dead cells
    (e.g. dead red or white blood cells, antigen-antibody complex)

Immune System: (1) organs

- Tonsils and adenoids
- Thymus
- Lymph nodes
- Spleen
- Payer’s patches
- Appendix
- Lymphatic vessels
- Bone marrow

Lymphatic System

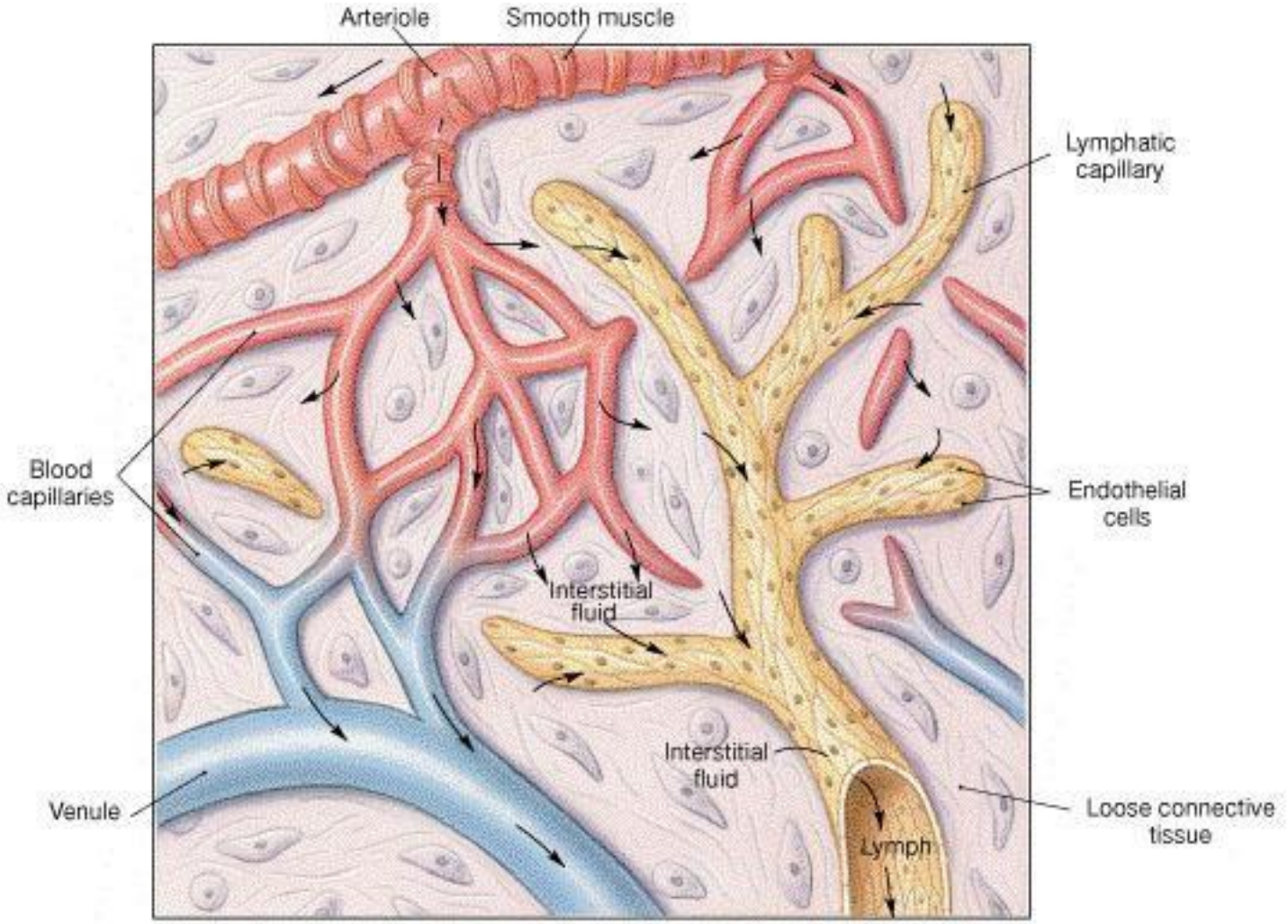
- Components
  - **Lymph** is the fluid
  - Vessels – **lymphatics**
  - Structures & organs
- Functions
  - Return tissue fluid to the bloodstream
  - Transport fats from the digestive tract to the bloodstream
  - Surveillance & defense



Lymphatics

- Originate as lymph capillaries
- Capillaries unite to form larger vessels
  - Resemble veins in structure
  - Connect to lymph nodes at various intervals
- Lymphatics ultimately deliver lymph into 2 main channels
  - Right lymphatic duct
    - Drains right side of head & neck, right arm, right thorax
    - Empties into the right subclavian vein
  - Thoracic duct
    - Drains the rest of the body
    - Empties into the left subclavian vein

Lymph Capillaries

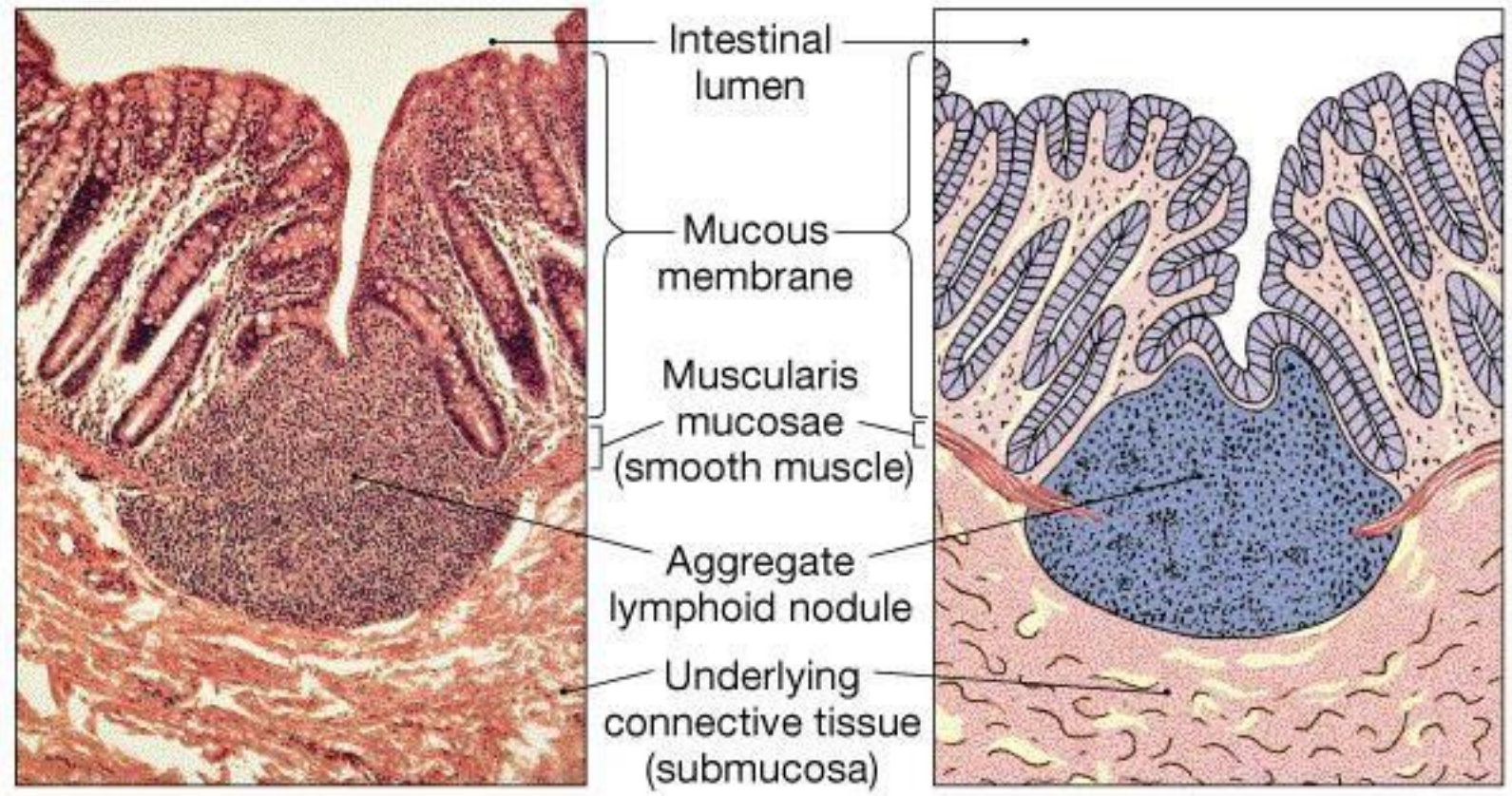


(a) Association of blood capillaries, tissue, and lymphatic capillaries

Lymph Tissue

- 3 types
  - Diffuse lymphatic tissue
    - No capsule present
    - Found in connective tissue of almost all organs
  - Lymphatic nodules
    - No capsule present
    - Oval-shaped masses
    - Found singly or in clusters
  - Lymphatic organs
    - Capsule present
    - Lymph nodes, spleen, thymus gland

Lymph Nodules

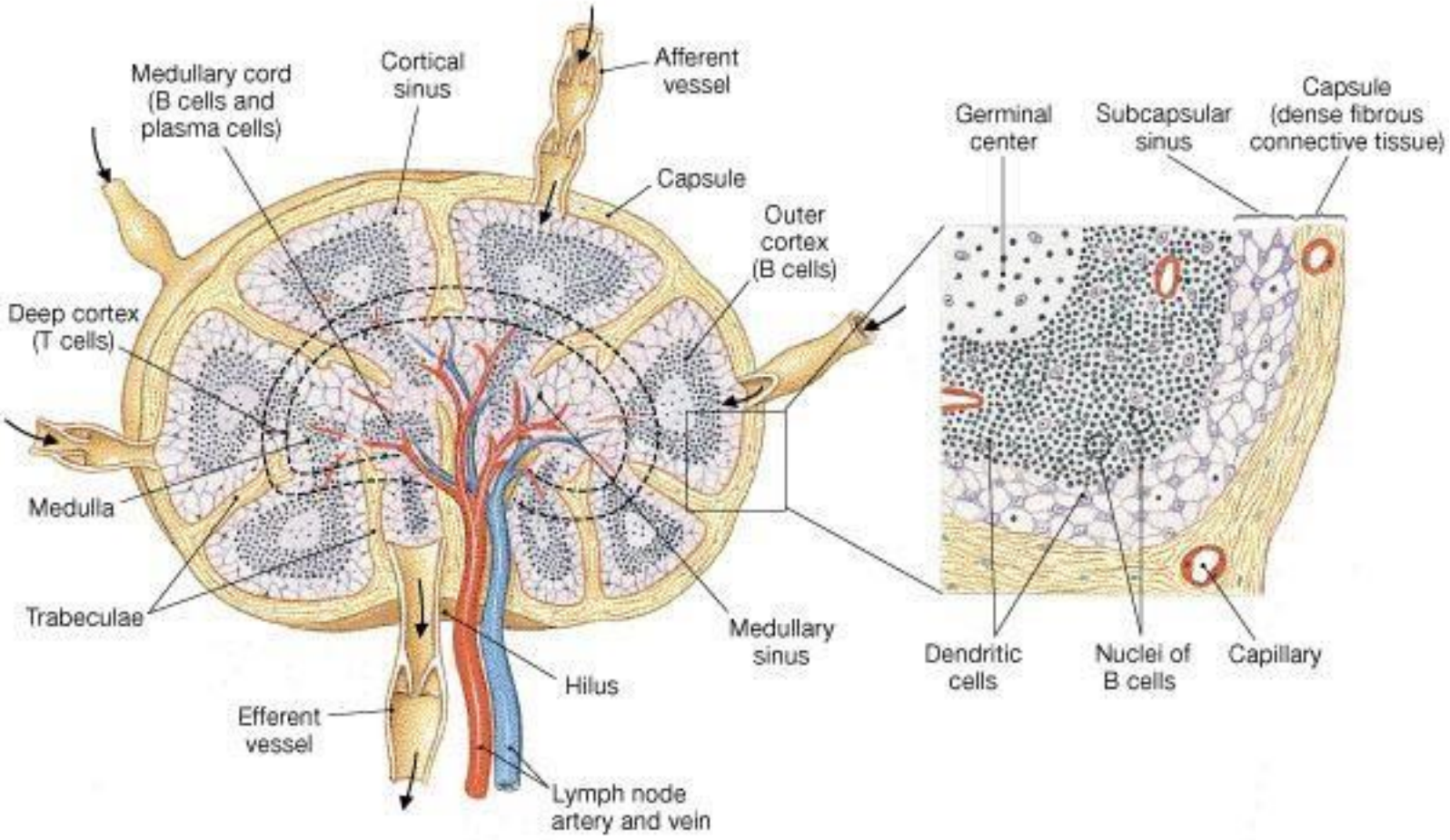


(a) Lymphoid nodule

Lymph Nodes

- Oval structures located along lymphatics
- Enclosed by a fibrous capsule
- Cortex = outer portion
  - Germinal centers produce lymphocytes
- Medulla = inner portion
  - Medullary cords
- Lymph enters nodes through afferent lymphatics, flows through sinuses, exits through efferent lymhpatic

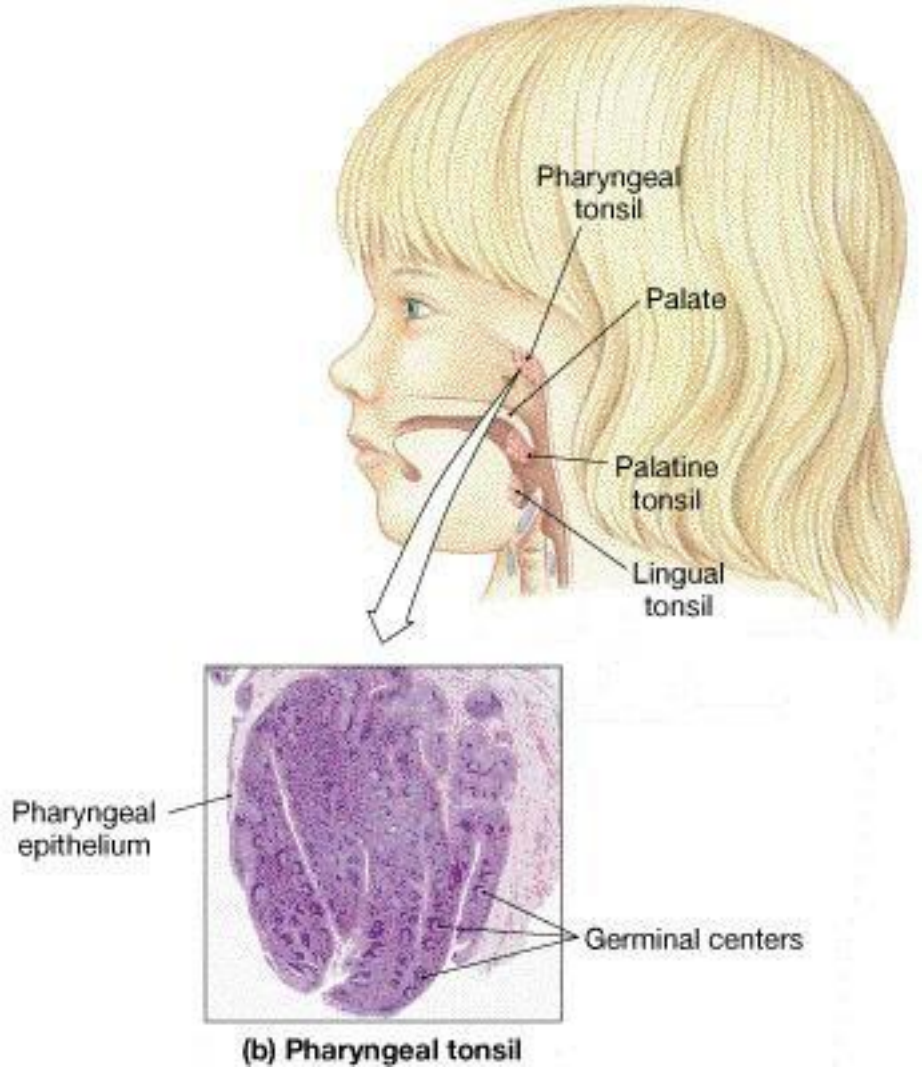
Lymph Node


Medullary cord
(B cells and
plasma cells)
Cortical
sinus
Afferent
vessel
Capsule
Outer
cortex
(B cells)
Deep cortex
(T cells)
Medulla
Trabeculae
Medullary
sinus
Hilus
Efferent
vessel
Lymph node
artery and vein
Germinal
center
Subcapsular
sinus
Capsule
(dense fibrous
connective tissue)
Dendritic
cells
Nuclei of
B cells
Capillary

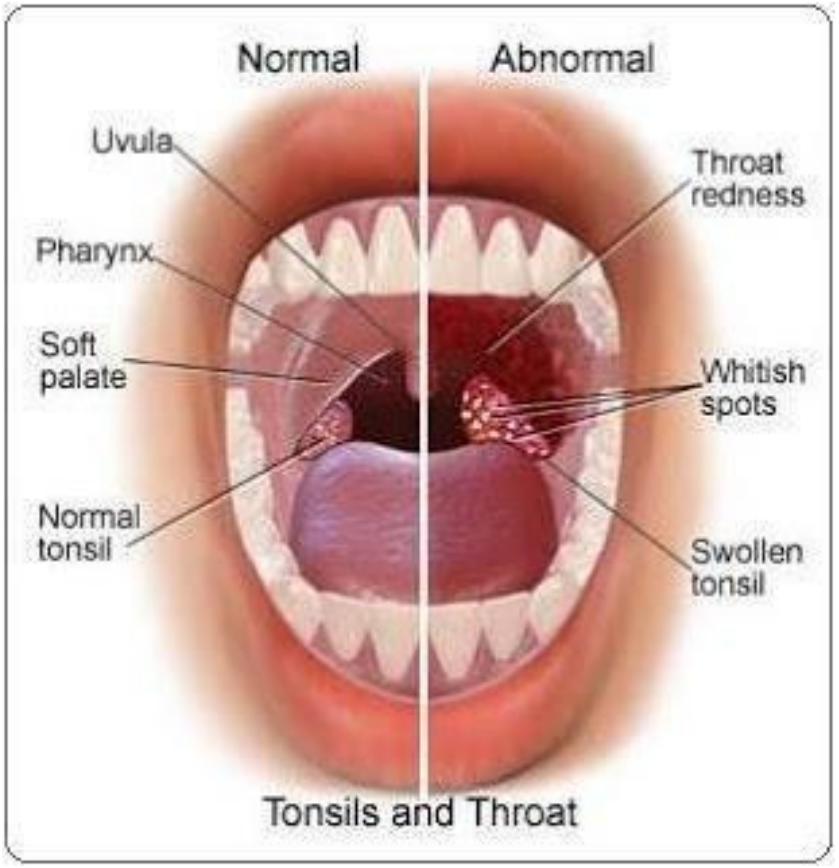
Tonsils

- Multiple groups of large lymphatic nodules
- Location – mucous membrane of the oral and pharyngeal cavities
- Palatine tonsils
  - Posterior-lateral walls of the oropharynx
- Pharyngeal tonsil
  - Posterior wall of nasopharynx
- Lingual tonsils
  - Base of tongue

Tonsils


Pharyngeal tonsil
Palate
Palatine tonsil
Lingual tonsil
Pharyngeal epithelium
Germinal centers
(b) Pharyngeal tonsil

The *adenoid*, also known as a pharyngeal tonsil or nasopharyngeal tonsil, is the superior-most of the tonsils



Functions of the Lymphatic System

1. Monitor body surfaces and fluid compartments (e.g. epidermis, mucosae*, interstitium)
2. React to the presence of potentially harmful antigens recognized as "non-self"
3. Autoimmune diseases (rheumatoid arthritis, type I diabetes, etc.)

Lymphatic System consists of:

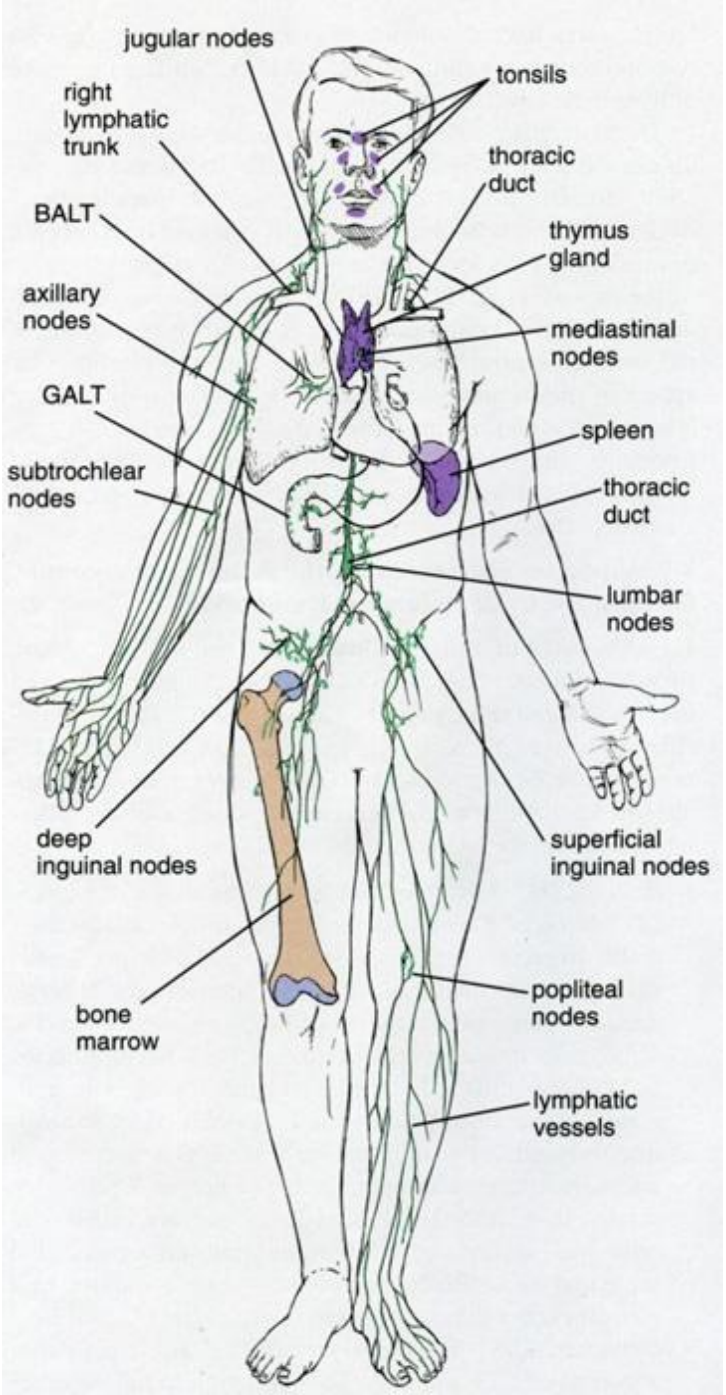
A. Cells
   1. Lymphocytes (B,T, natural killer)
   2. Antigen-presenting cells (dendritic cells, Langerhans' cells & macrophages)

B. Lymphatic "tissue" –diffuse and nodular

C. Lymphatic "organs" (lymph nodes, spleen, thymus)

D. Lymphatic vessels that carry the cells and fluid

*Mucosae refers to lining tissue of the body cavities, e.g. GI tract, respiratory tract, genitourinary tract



Lymphoid organs are classified as:

Primary lymphoid organs

- Thymus
- Bone marrow
- Lymphatic nodules of the distal intestinal tract (e.g. ileum and appendix)

Secondary (effector) lymphoid organs/tissue

- Spleen & lymph nodes (organs)
- Mucosal associated lymphoid tissue (MALT), e.g. lymphocytes and lymphatic nodules in the lamina propria

Primary Lymphoid Organs:

The bone marrow and the thymus and the Gut-Associated Lymphoid Tissue (e.g. appendix, terminal ileum) are the initial "education centers" of the immune system

In these organs, lymphocytes (T cells in the thymus, B cells in bone marrow and gut) differentiate into **immunocompetent** cells
(i.e. they can recognize "self" vs. "nonself")

This differentiation is said to be antigen-***independent***

The lymphocytes then enter the blood and lymph to populate:

- epidermis and mucosae
- connective tissue
- secondary lymphoid organs

Secondary Lymphoid Organs:

The lymph nodes, lymphatic nodules, tonsils, spleen are the secondary "education centers" of the immune system

In these organs, immunocompetent lymphocytes differentiate into immune effector and memory cells that undergo antigen-***dependent*** activation and proliferation in these organs.

These lymphocytes then carry out their functions in the:

- connective tissue
- secondary lymphoid organs
- mucosal surfaces lining epithelia

They participate in:

- Cell mediated immunity (mostly "cytotoxic" T cells)
- Humoral responses (production of antibody) (B cells, also requires "helper" T cells.

Spleen

- Largest lymphatic organ
- Located between the stomach & diaphragm
- Structure is similar to a node
  - Capsule present
  - But no afferent vessels or sinuses
- Histology
  - Red pulp contains all the components of circulating blood
  - White pulp is similar to lymphatic nodules

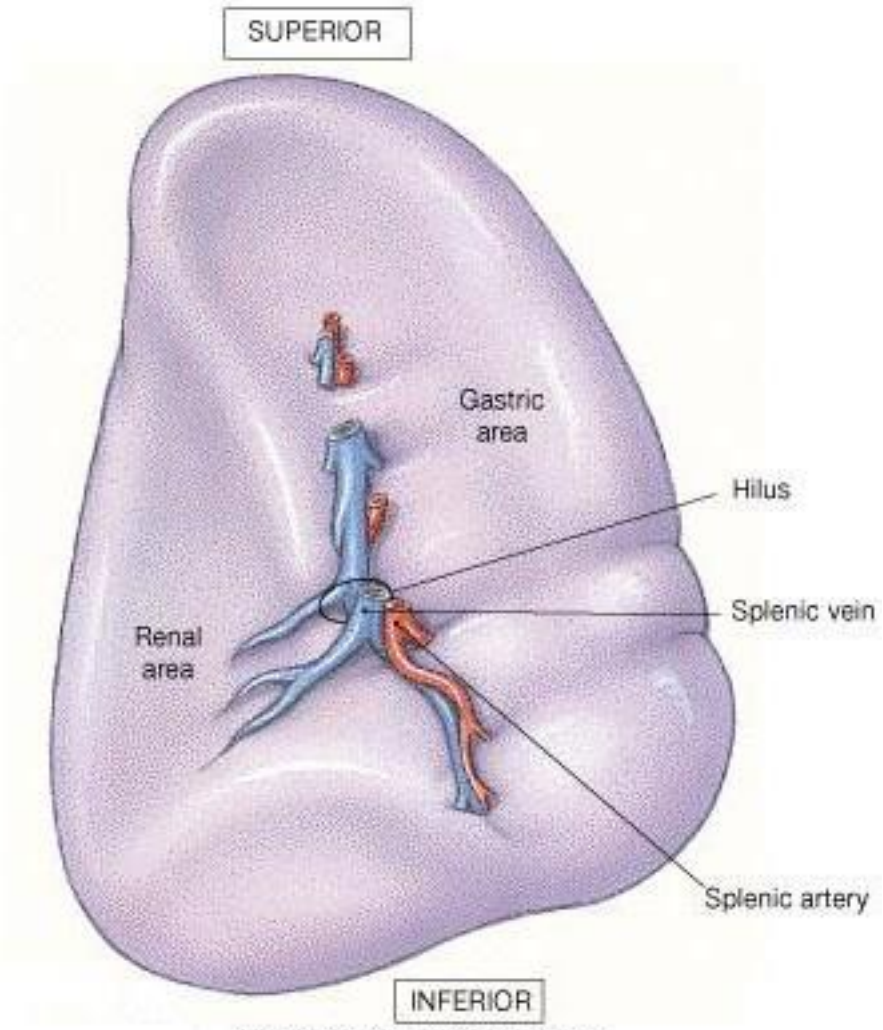
Immune Functions Of the Spleen

- Monitoring antigens in blood
- Proliferation of lymphocytes
- Production of humoral antibodies

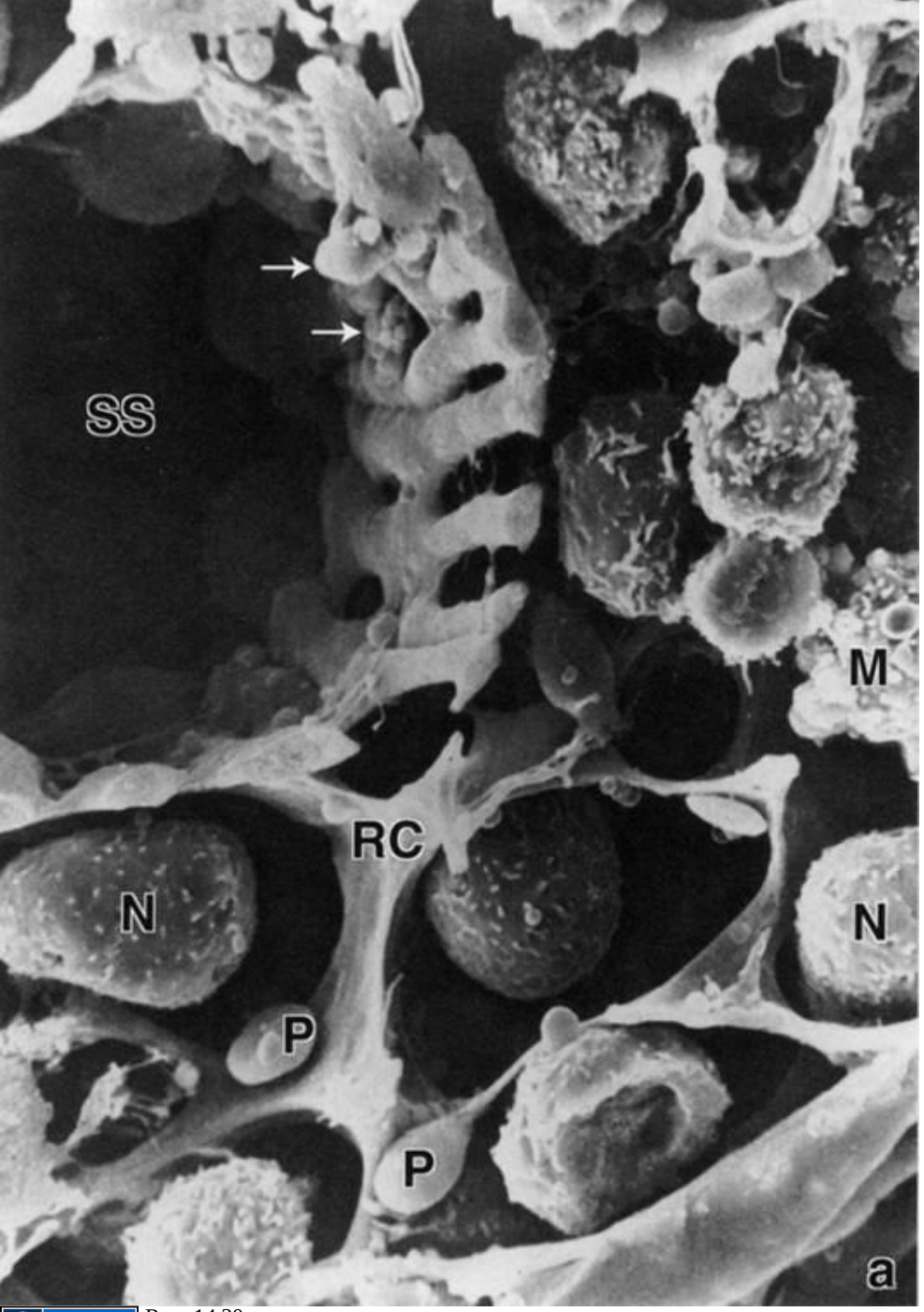
Hematopoietic Functions Of the Spleen

- Formation of blood cells in fetal life
- Removal and destruction of RBCs & platelets
- Retrieval of iron from RBC hemoglobin
- Storage of RBCs and platelets (more so in non-human species)

Spleen



(b) Visceral surface of spleen



Scanning EM of a Splenic Sinus (SS) and Cord of Billroth

The cords contain, RBCs, neutrophils (N), macrophages (M), blood platelets (P)

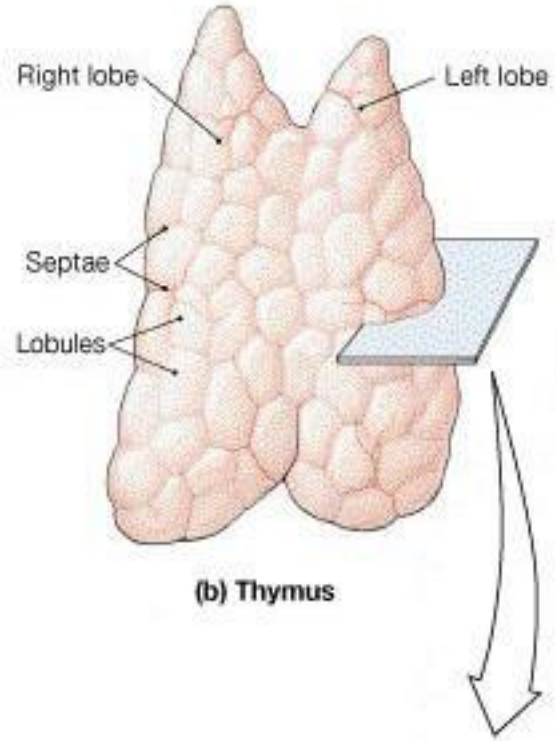
A reticular cell framework (RC) supports the cord. The sinus is bounded by the epithelial cells that form the basket-like structure of the sinus (VS)

Ross 14.30a

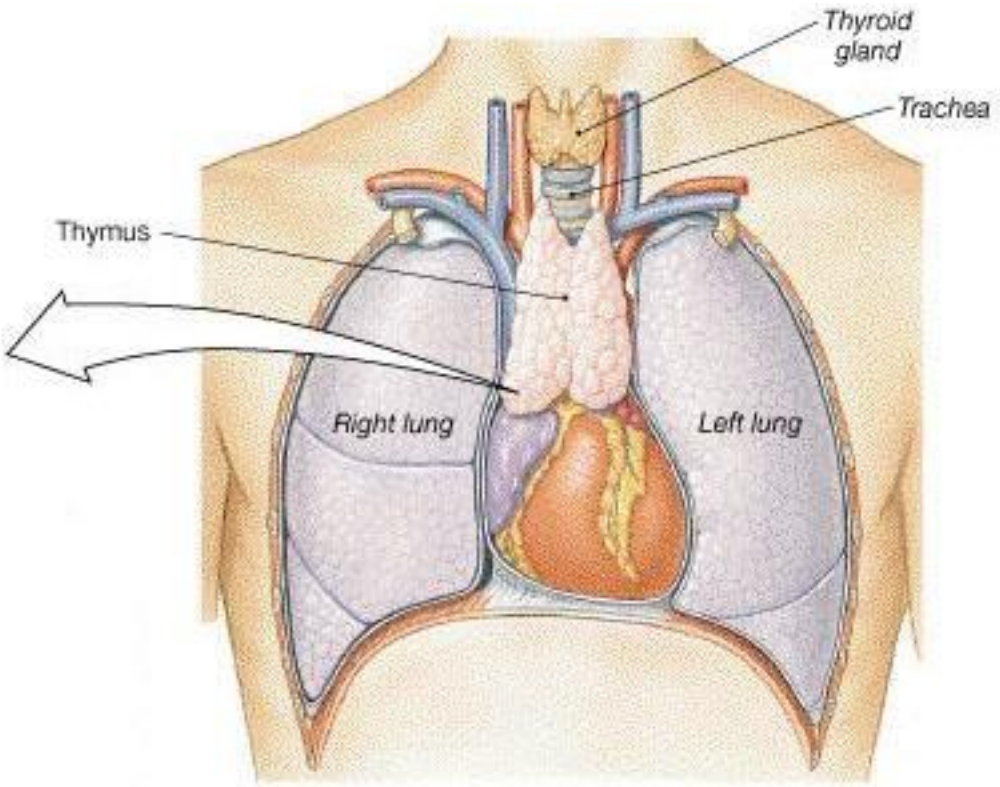
Thymus Gland

- Location – behind the sternum in the mediastinum
- The capsule divides it into 2 lobes
- Development
  - Infant – conspicuous
  - Puberty – maximum size
  - Maturity – decreases in size
- Function
  - Differentiation and maturation of T cells

Thymus Gland



(b) Thymus

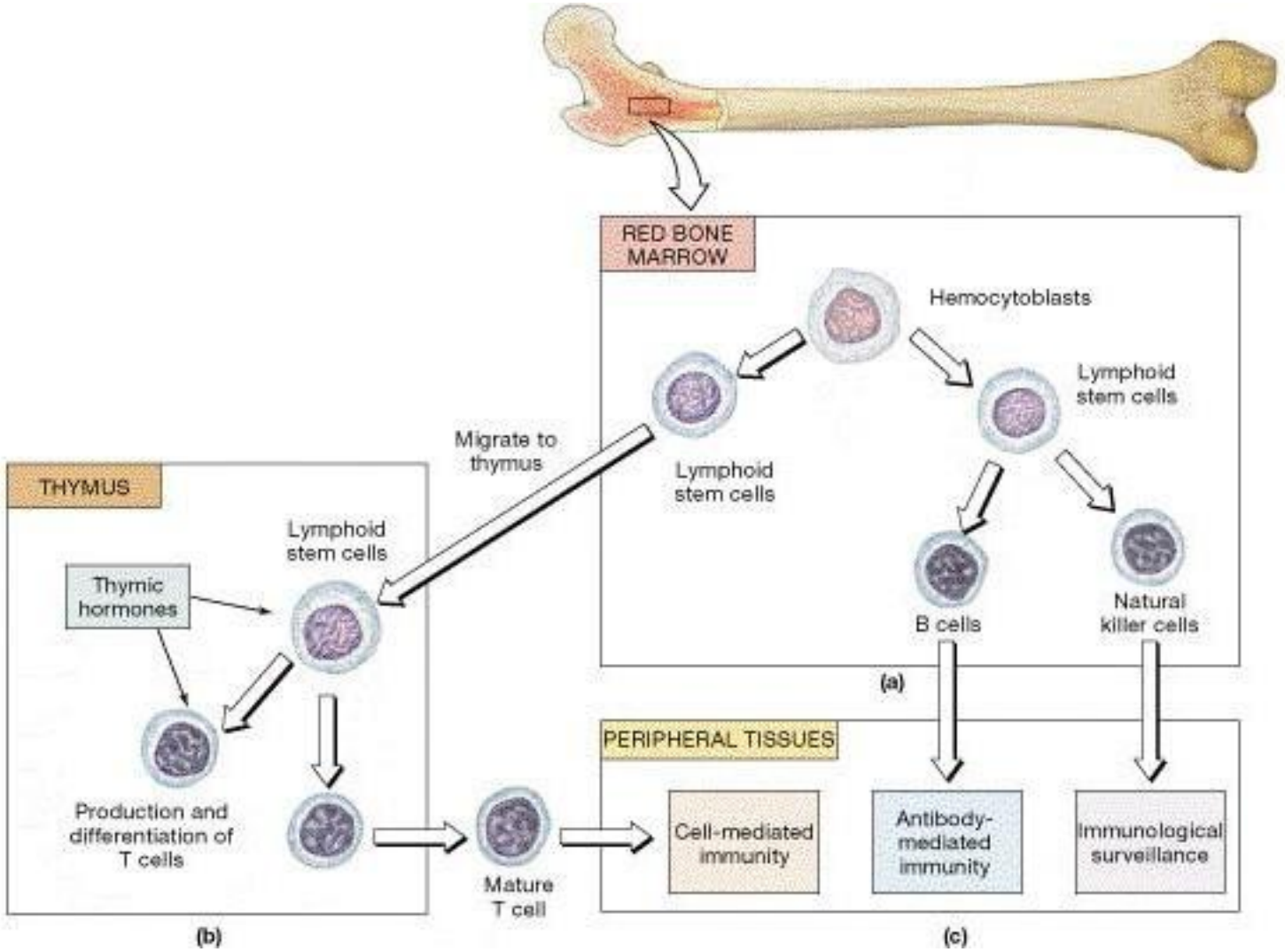


(a) Location of thymus within thoracic cavity

Function of the Lymphatic System

- Defense against harmful organisms and chemicals
- 2 types of defense
  - Nonspecific
  - Specific
- Specific defense = immunity
  - Humoral immunity involves B cells that become plasma cells which produce antibodies that bind with specific antigens.
  - Cell-mediated immunity involves T cells that directly destroy foreign cells

Derivation and Distribution of Lymphocytes

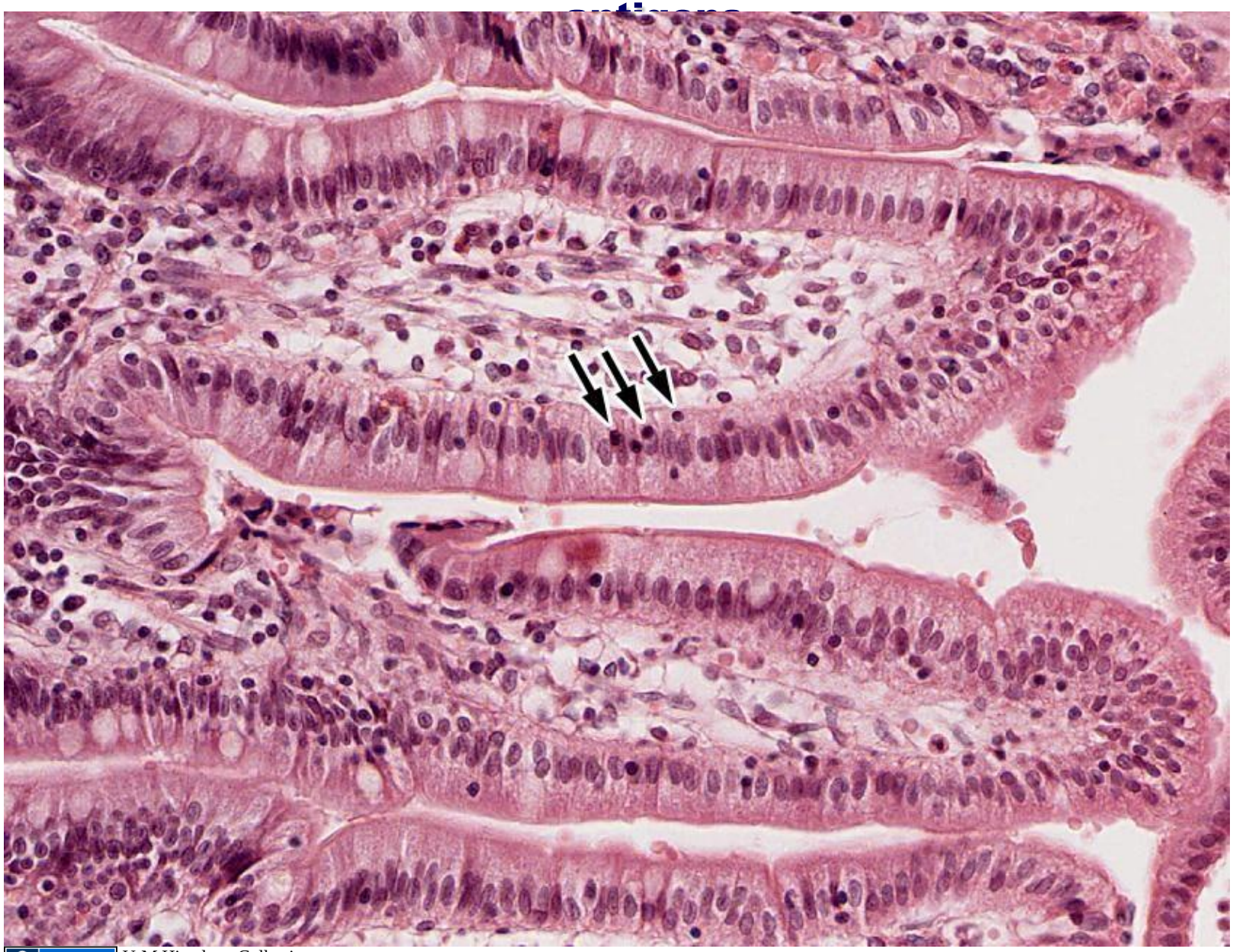


Immune system: (2) cells

- Lymphocytes
  - T-lymphocytes
  - B-Lymphocytes, plasma cells
  - natural killer lymphocytes
- Monocytes, Macrophage
- Granulocytes
  - neutrophils
  - eosinophils
  - basophils

MALT: intraepithelial lymphocytes:

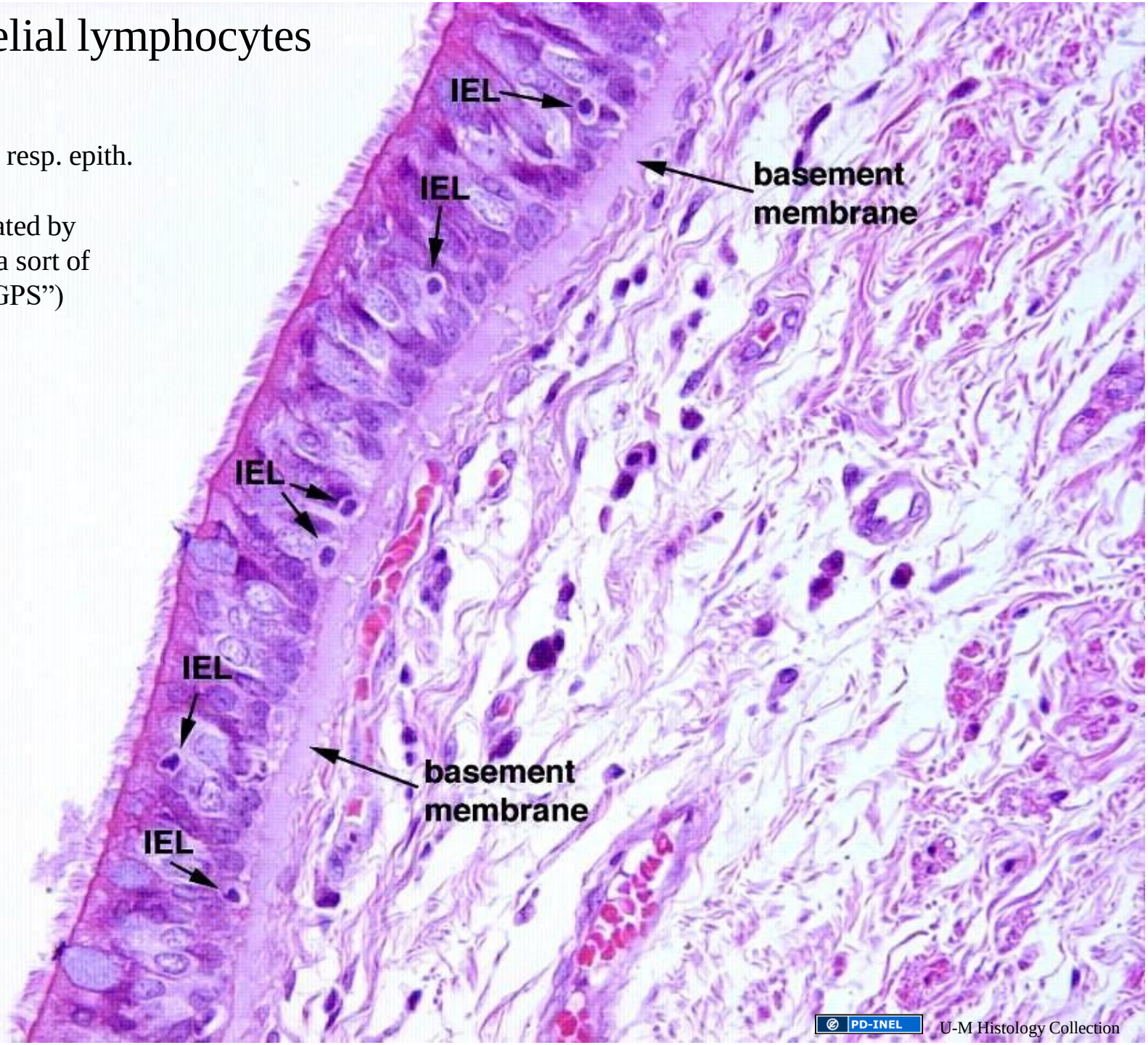
γδT-cells (neither helper nor cytotoxic): first to see antigens



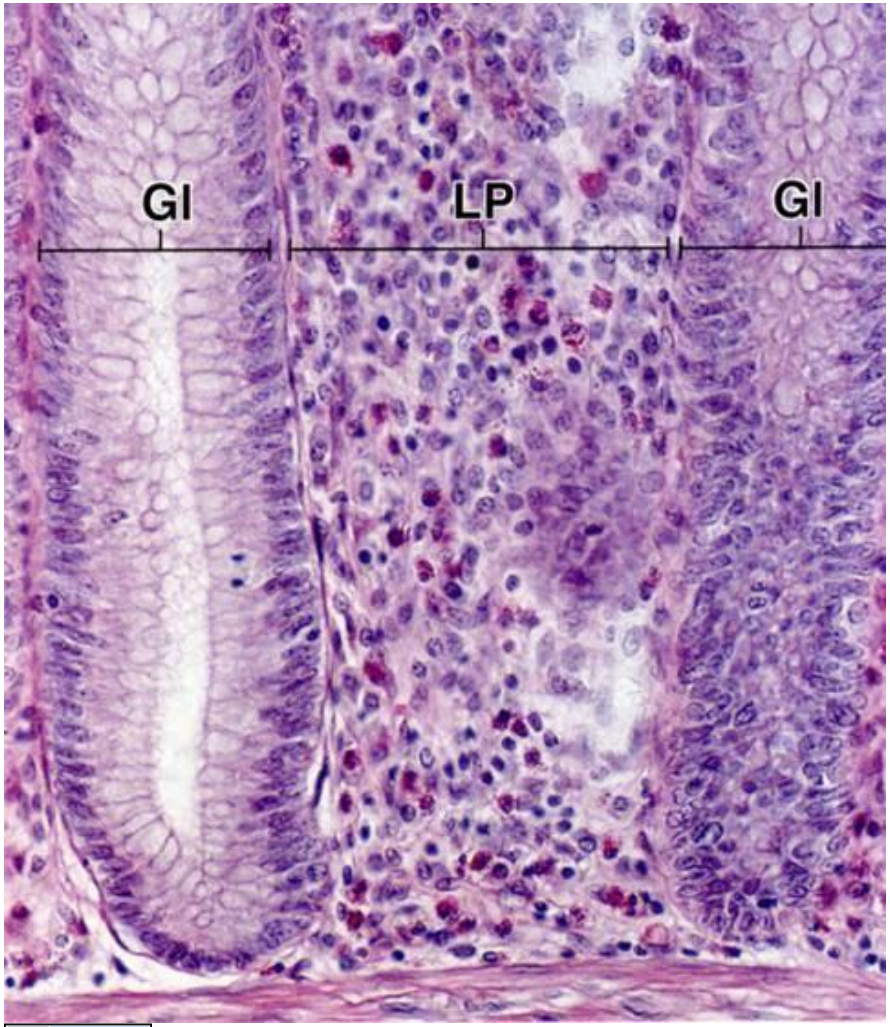
Intraepithelial lymphocytes

Shown here in resp. epith.

Homing mediated by "addressins" (a sort of lymphocyte "GPS")



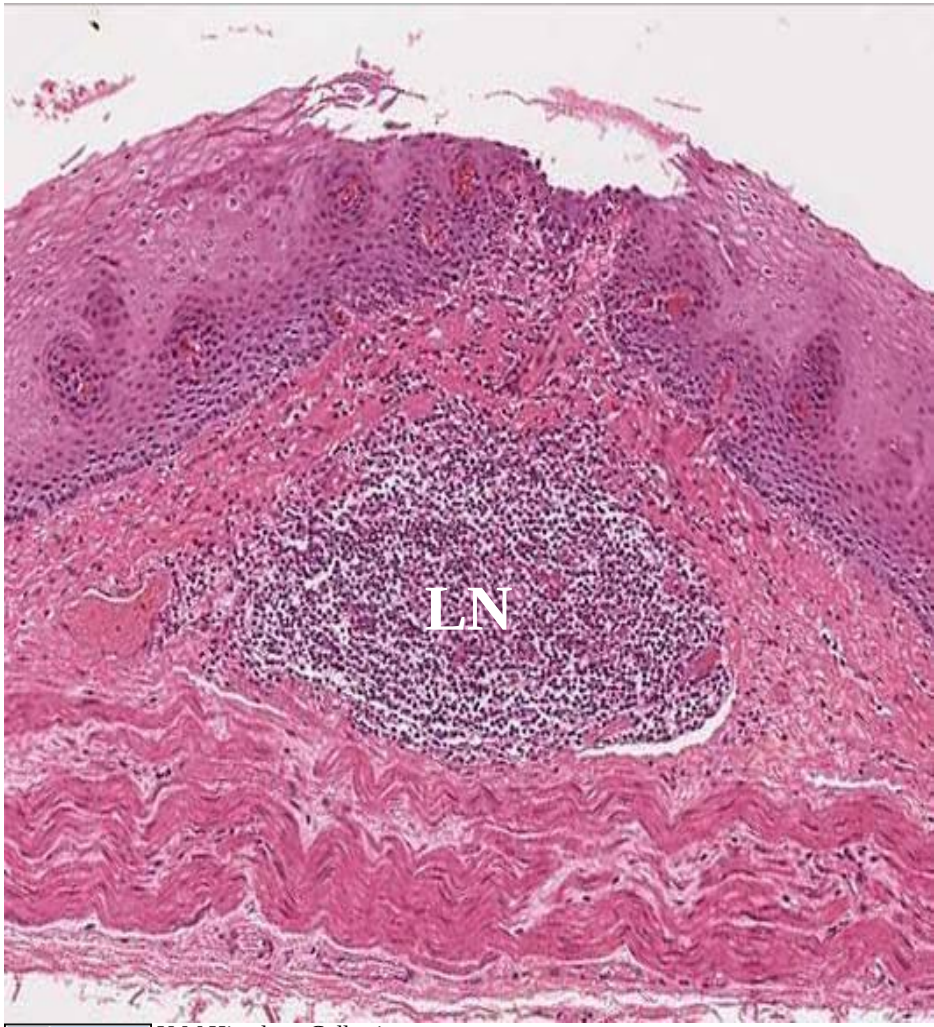
LYMPHOCYTES IN CONNECTIVE TISSUE:
MALT = mucosa-associated lymphoid tissue



PD-INEL Ross and Pawlina, *Histology: A Text and Atlas*

Diffuse lymphoid tissue

Lamina propria (LP) of gut shown here, but can be found associated with mucosae anywhere in the gut, respiratory, and genitourinary tracts.

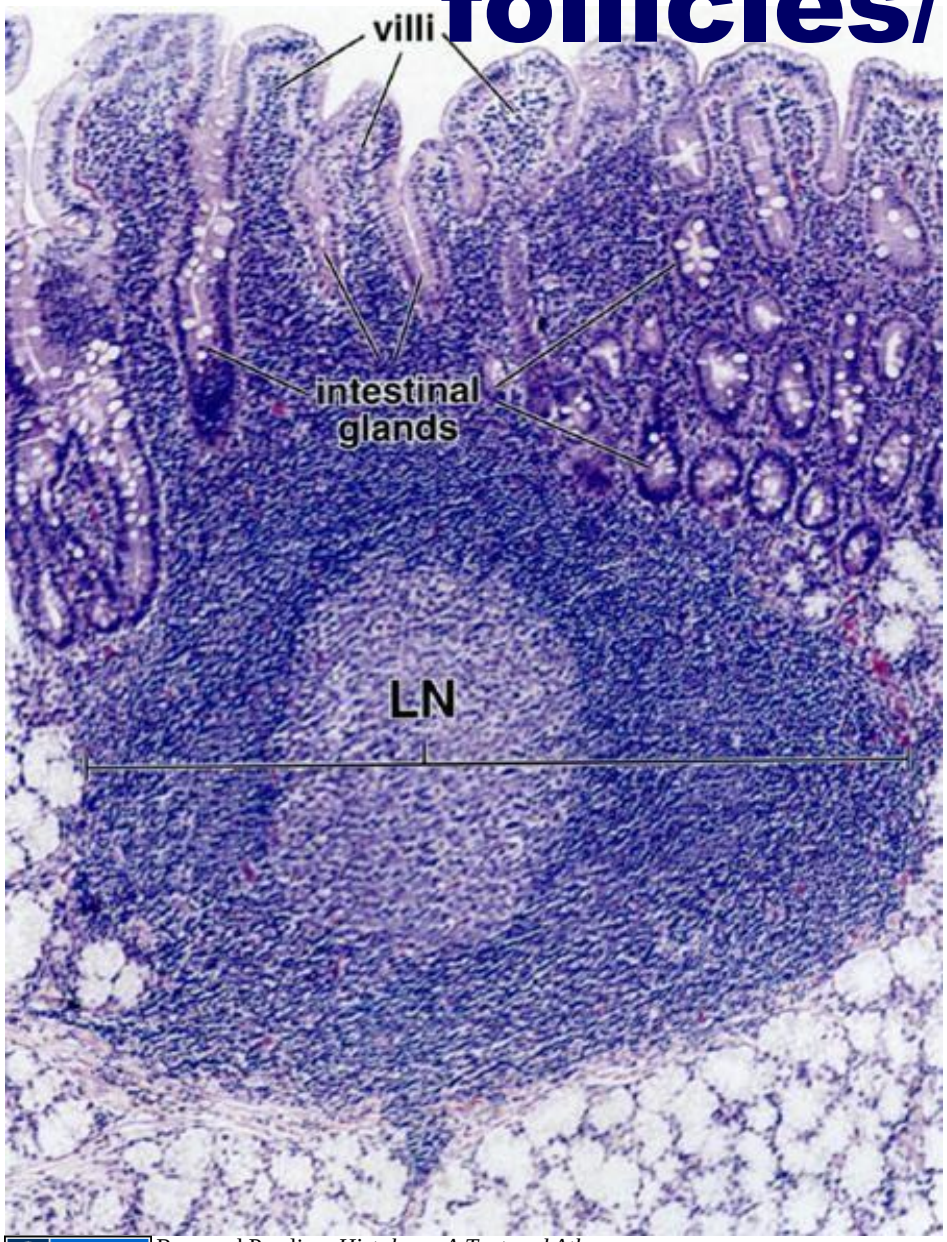


PD-INEL U-M Histology Collection

Primary lymphatic nodule/follicle (LN)

Aggregation of lymphocytes in lamina propria or submucosa

Secondary follicles/nodules

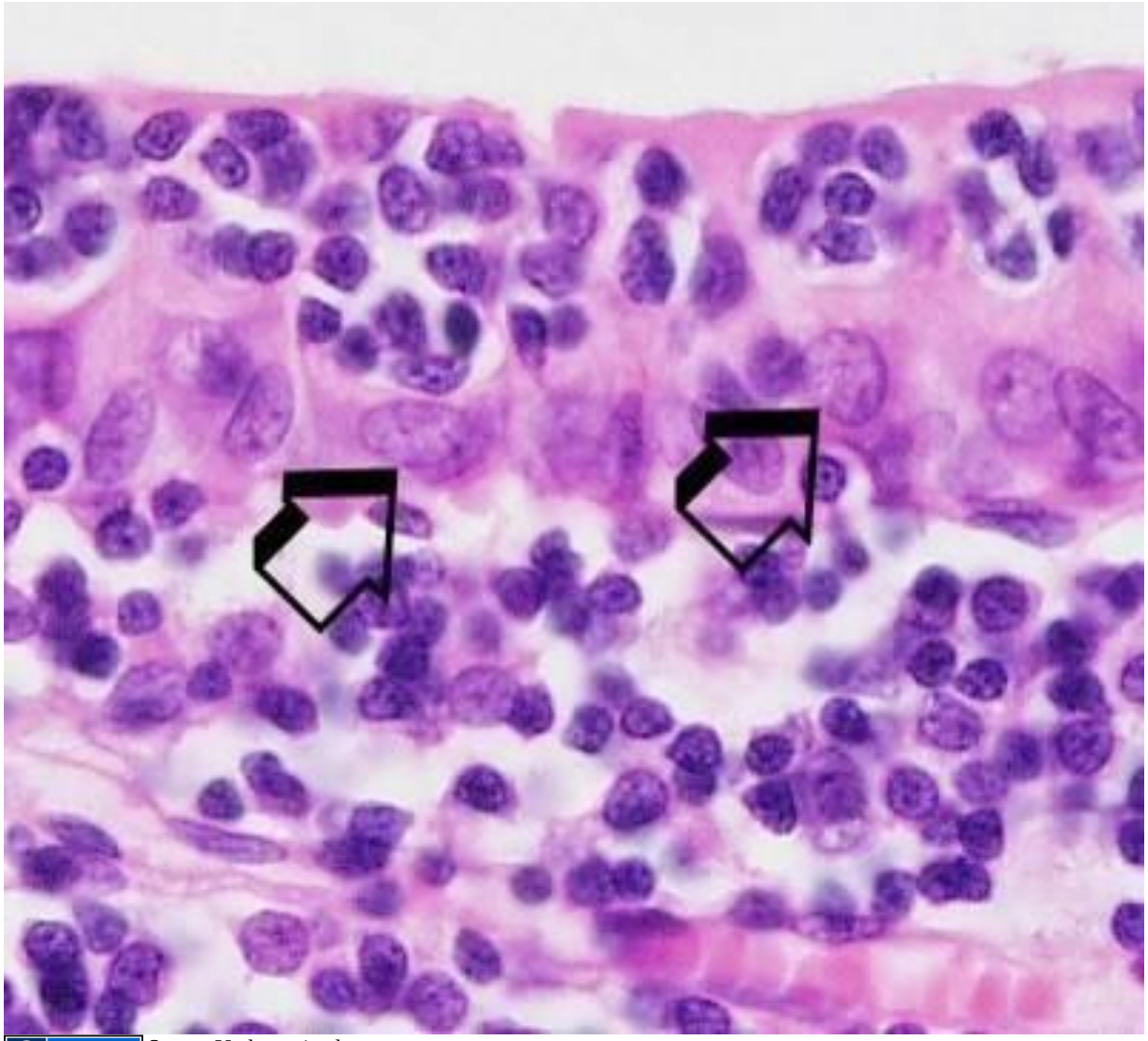


- Contain germinal centers
- Arise when B-lymphocytes are presented with appropriate antigen, receive T-cell help, and then begin proliferating as lymphoblasts
- Lymphoblasts differentiate into plasma cells or memory cells; aberrant lymphoblasts undergo apoptosis.

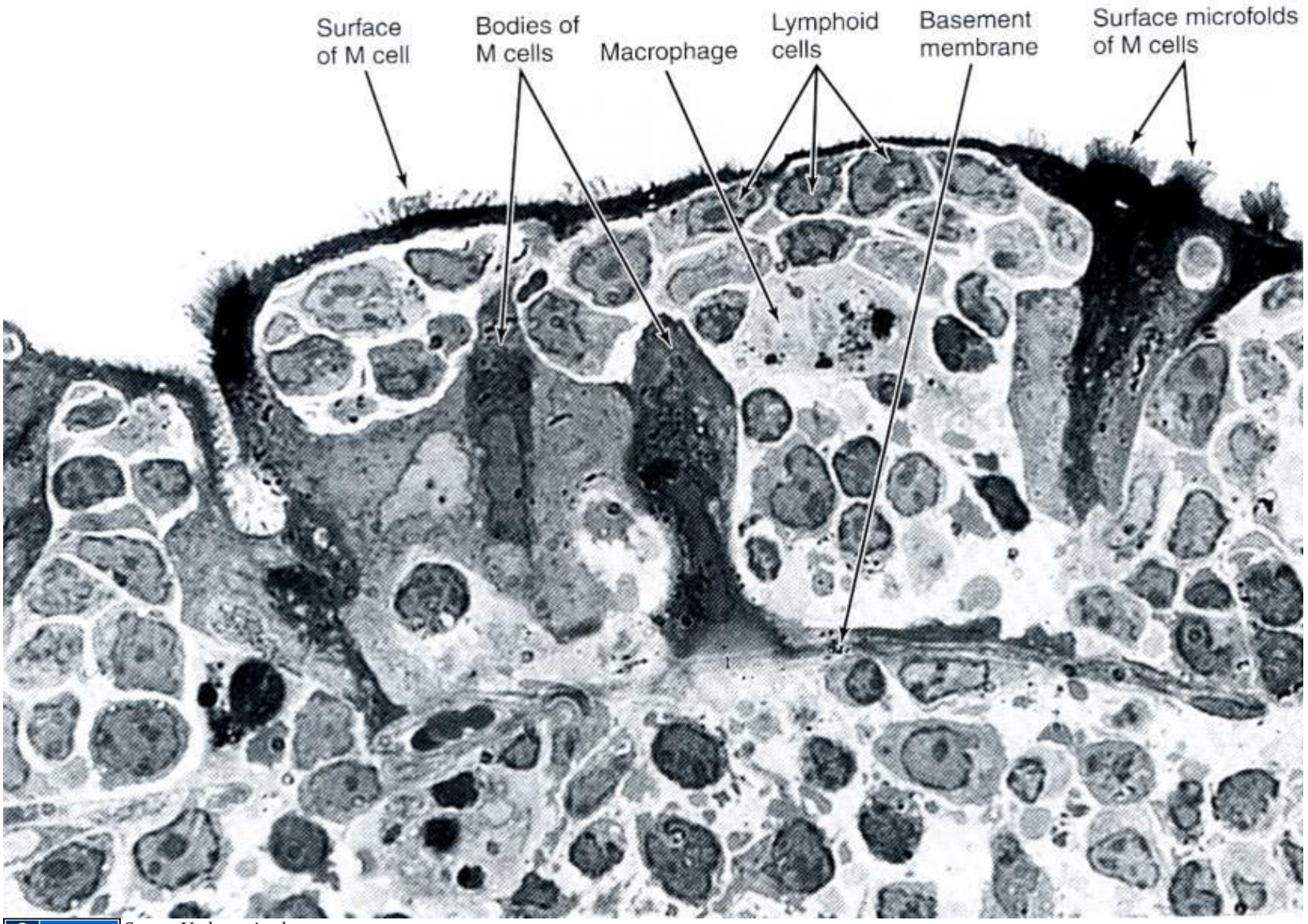
 Ross and Pawlina, *Histology: A Text and Atlas*

Microfold, or “M” CELLS

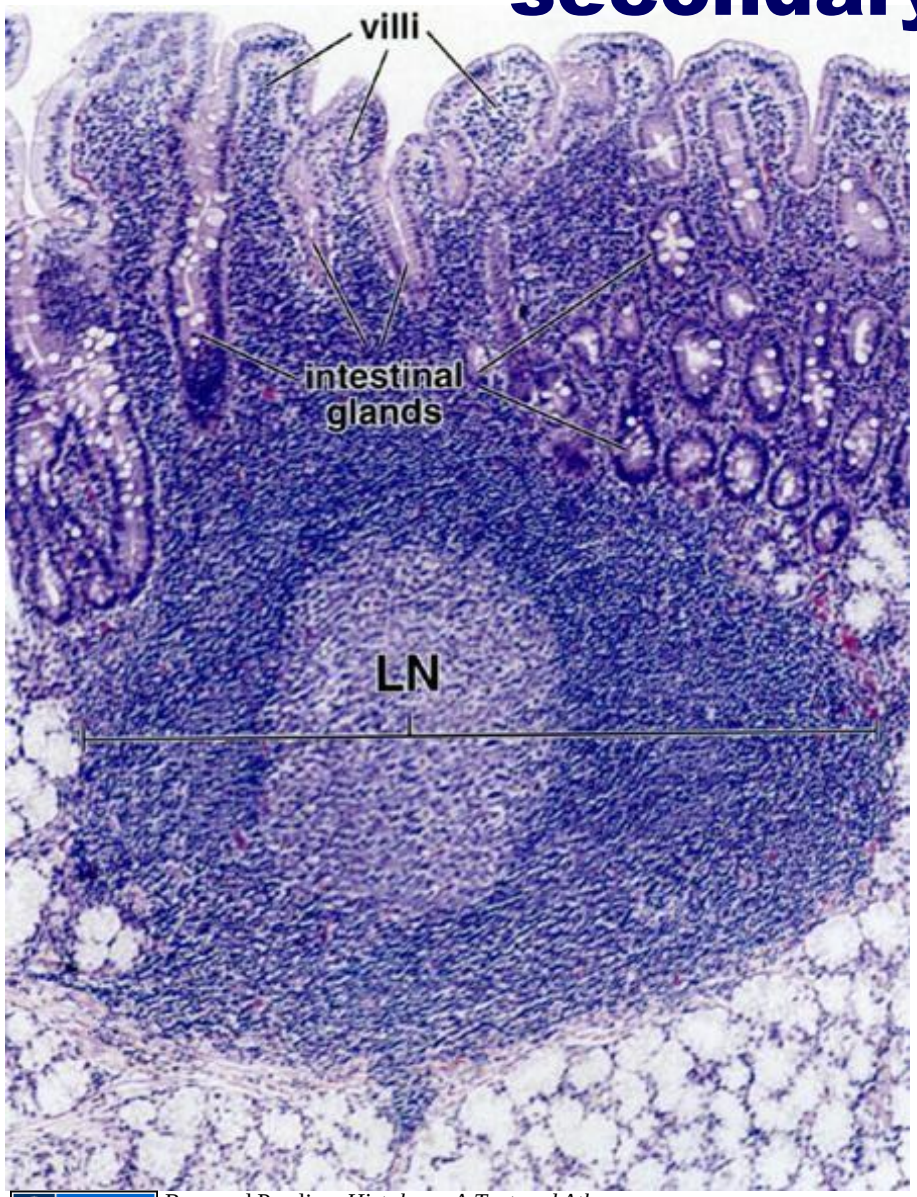
Modified intestinal epithelial cells that assist in antigen presentation by conveying macromolecules from the intestinal lumen to underlying compartments housing lymphocytes and macrophages.



M cells: TEM



After antigen presentation and T-cell help, activated B-cells set up germinal centers in secondary follicles



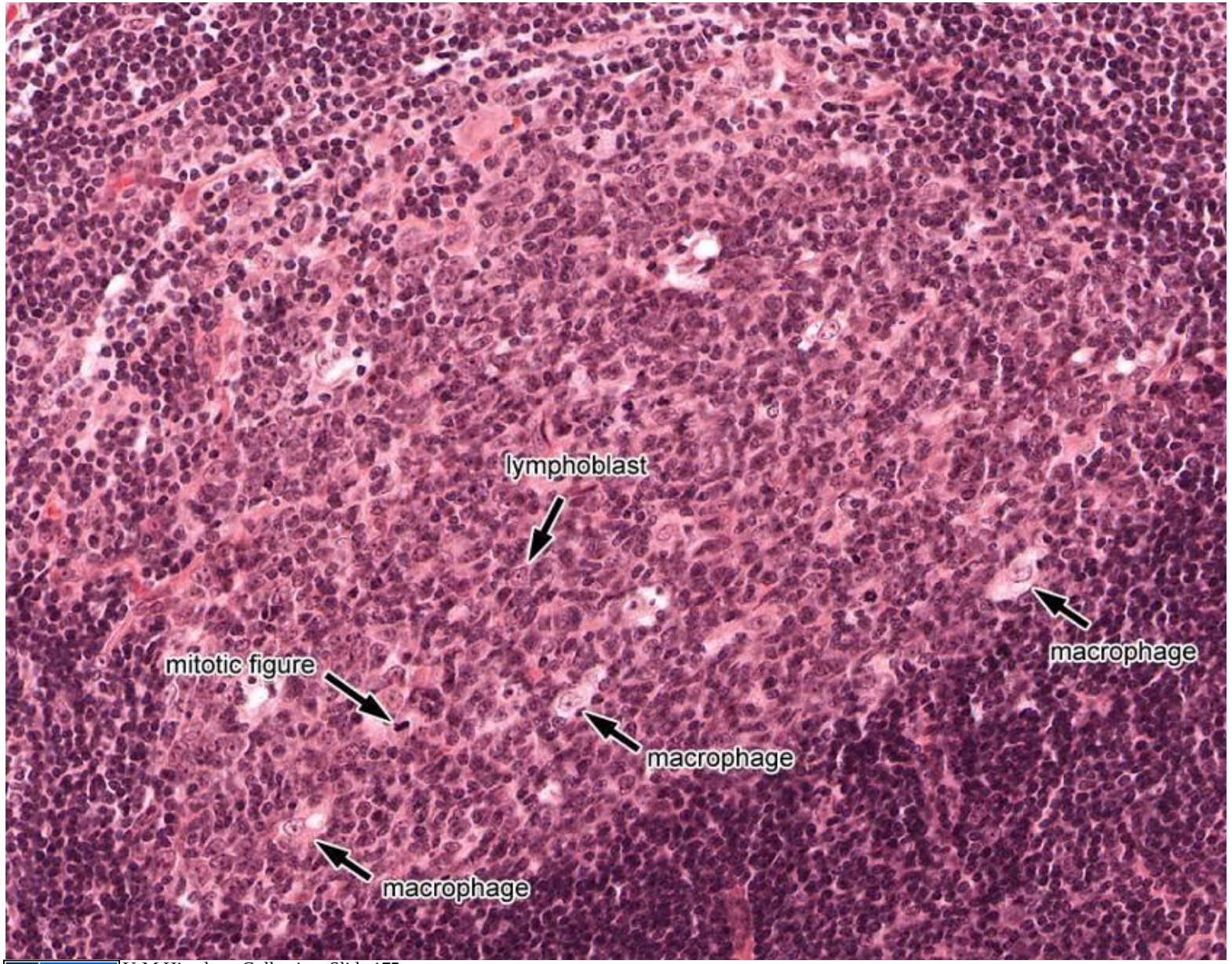
 Ross and Pawlina, *Histology: A Text and Atlas*

Secondary follicle germinal centers

- Arise when B-lymphocytes are presented with appropriate antigen, receive T-cell help, and then begin proliferating as lymphoblasts

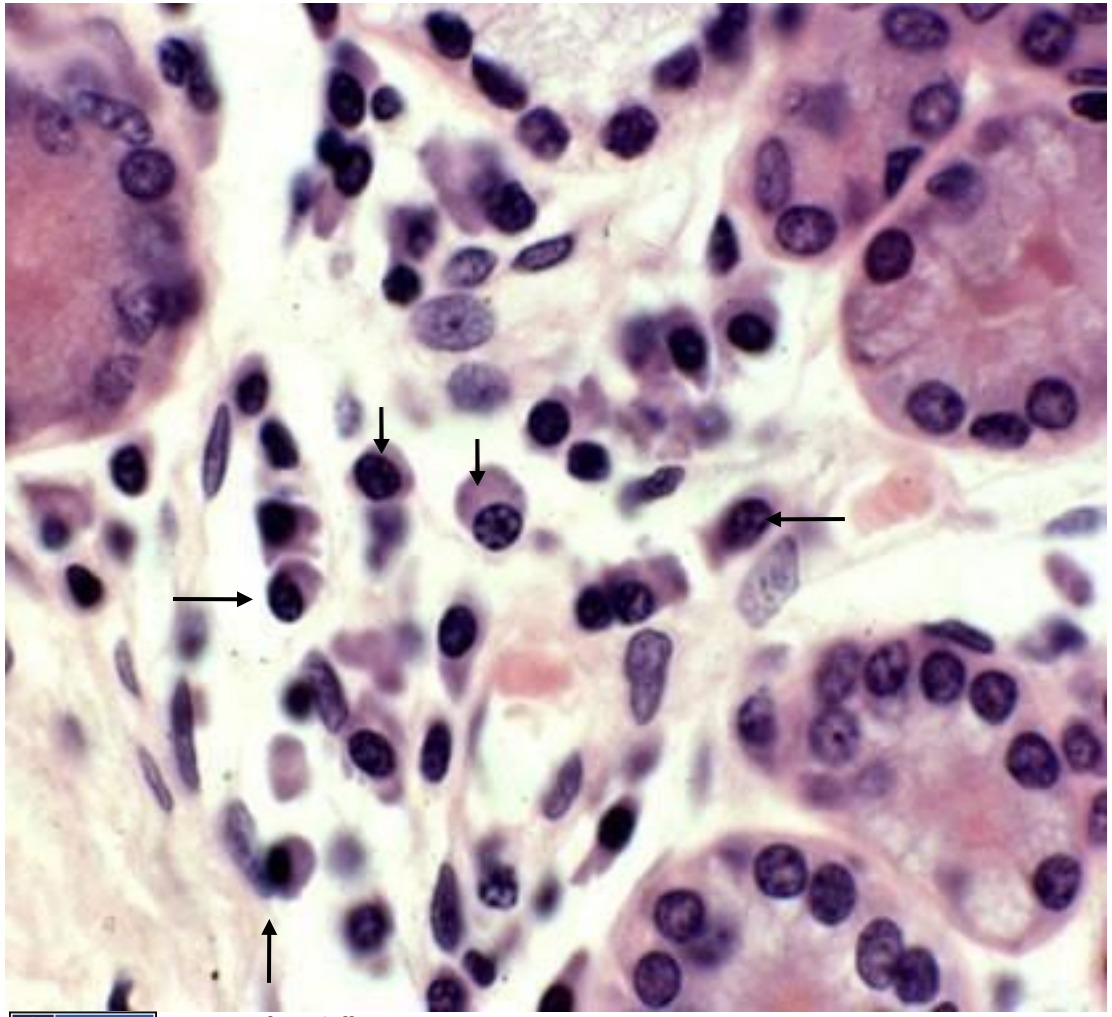
- Lymphoblasts differentiate into plasma cells or memory cells; aberrant lymphoblasts undergo apoptosis.

Germinal center: high magnification



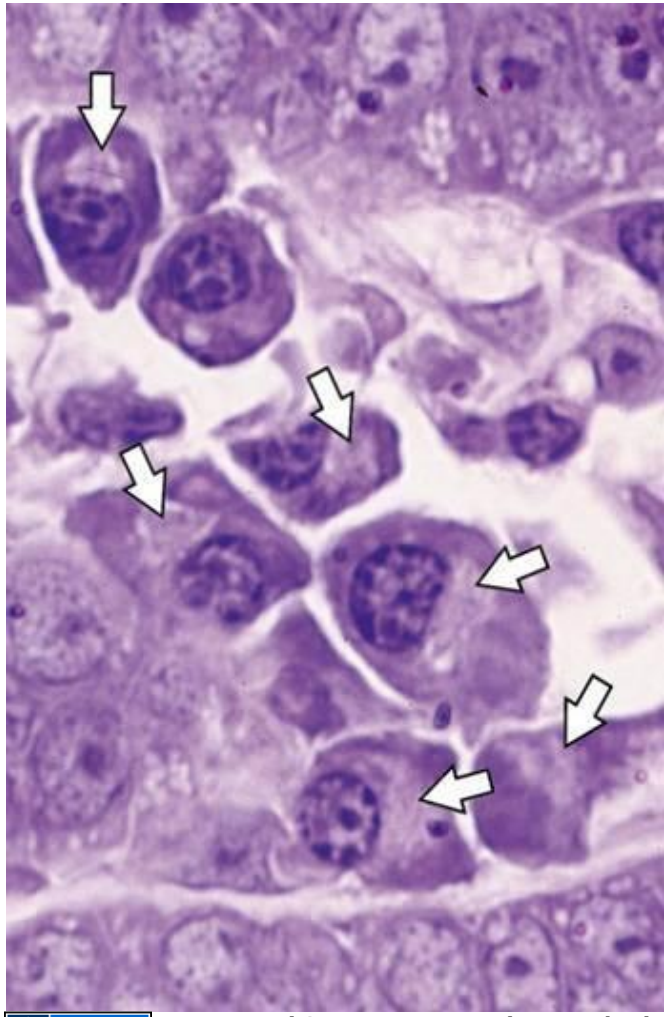
 U-M Histology Collection. Slide 175.

Plasma Cells are mature B lymphocytes



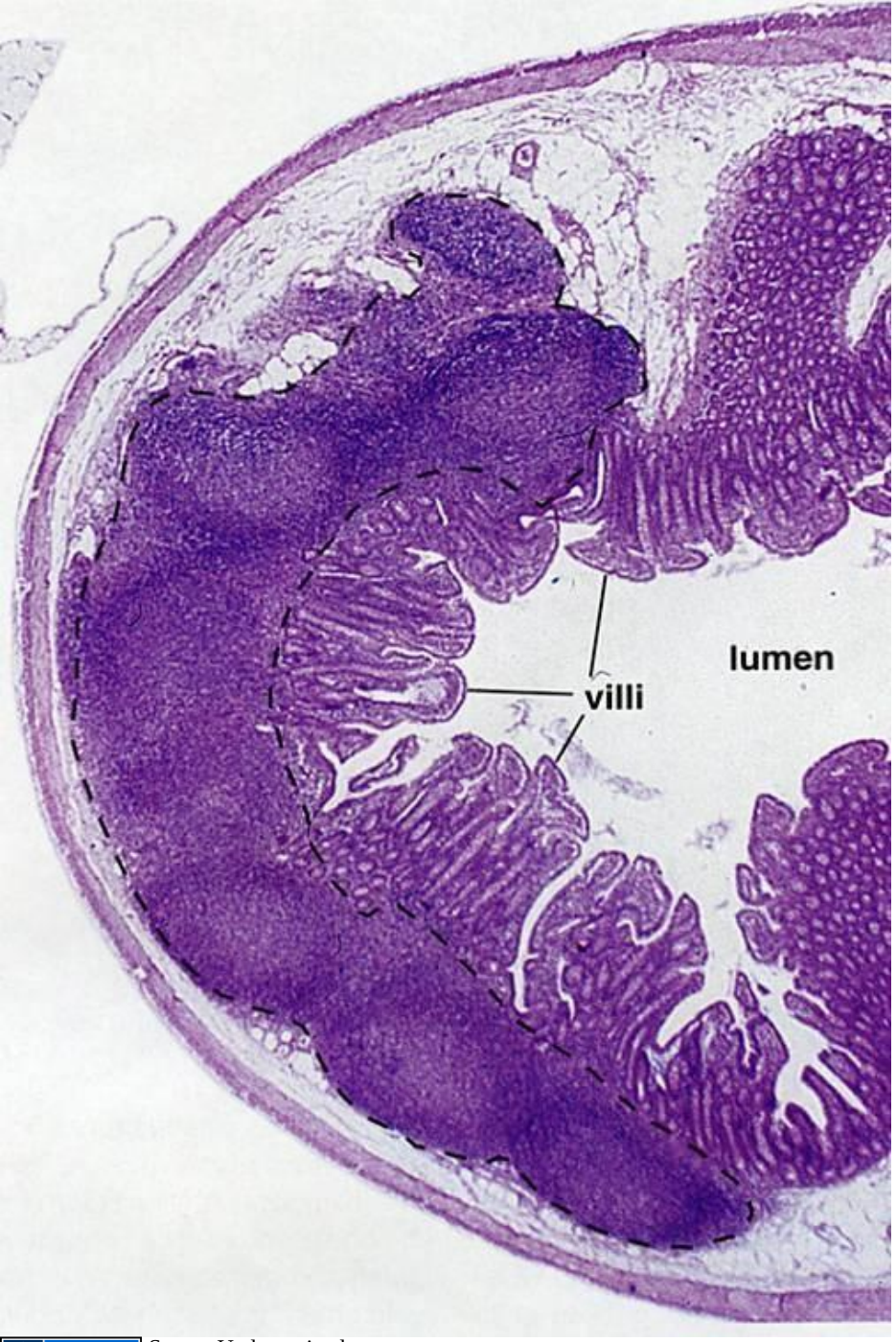
PD-INEL U-M Histology Collection

Black arrows indicate several plasma cells



PD-INEL Junquiera and Carneiro. Basic Histology. Tenth Ed. 2003

White arrows = Golgi regions



So, associated with just about any mucosa (GI, respiratory, genitourinary), you may see:

- Intraepithelial lymphocytes (T-cells)
- Diffuse lymphoid tissue:
  - B-cells
  - T-cells
  - APCs
- Primary nodules
- Secondary nodules
  - Germinal center with lymphoblasts and mphages

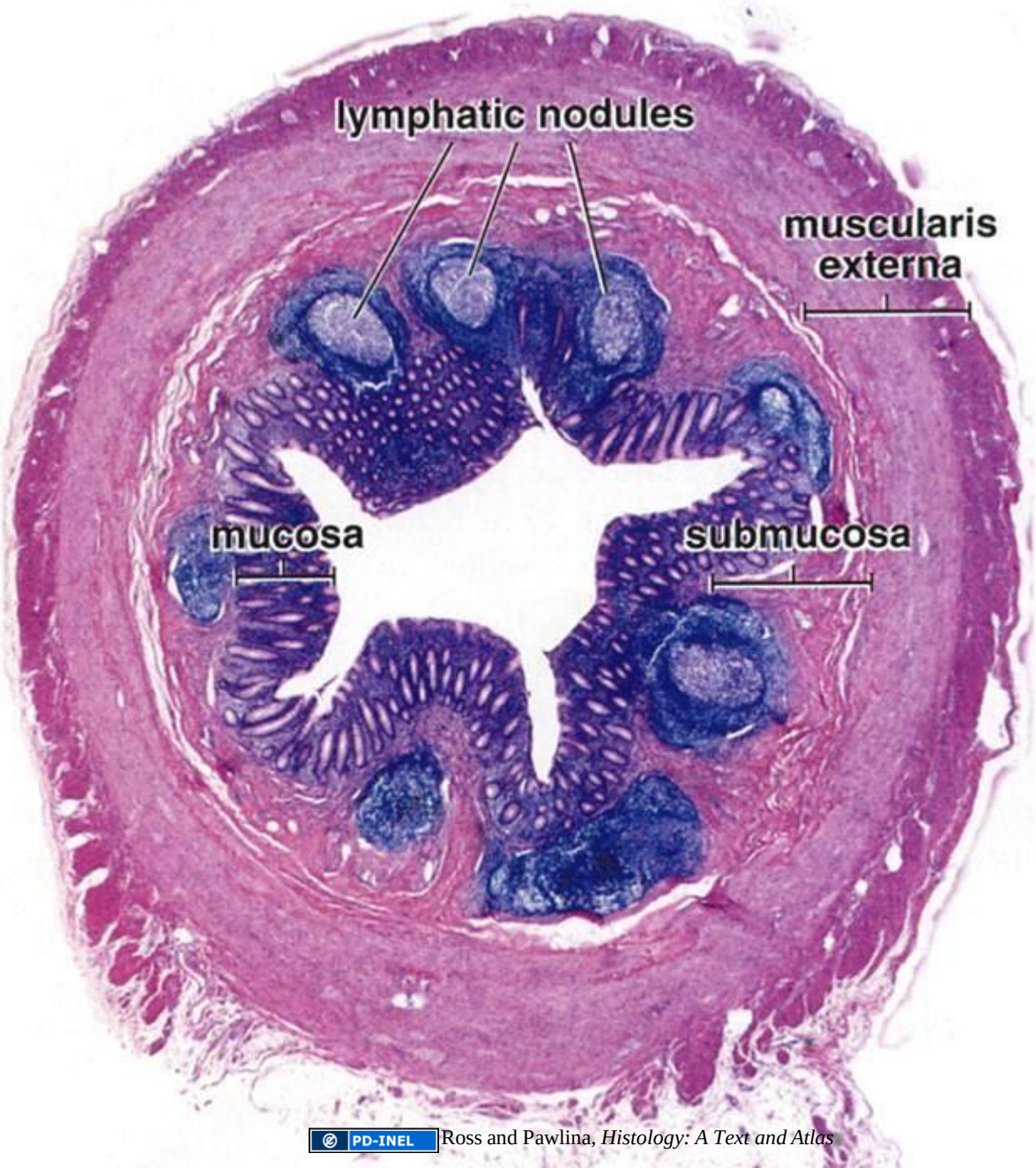
Regions of extensive lymphoid infiltration: Peyer's patches

Aggregates of lymphoid follicles in the ileum.

Appendix

Blind sac extending from the caecum

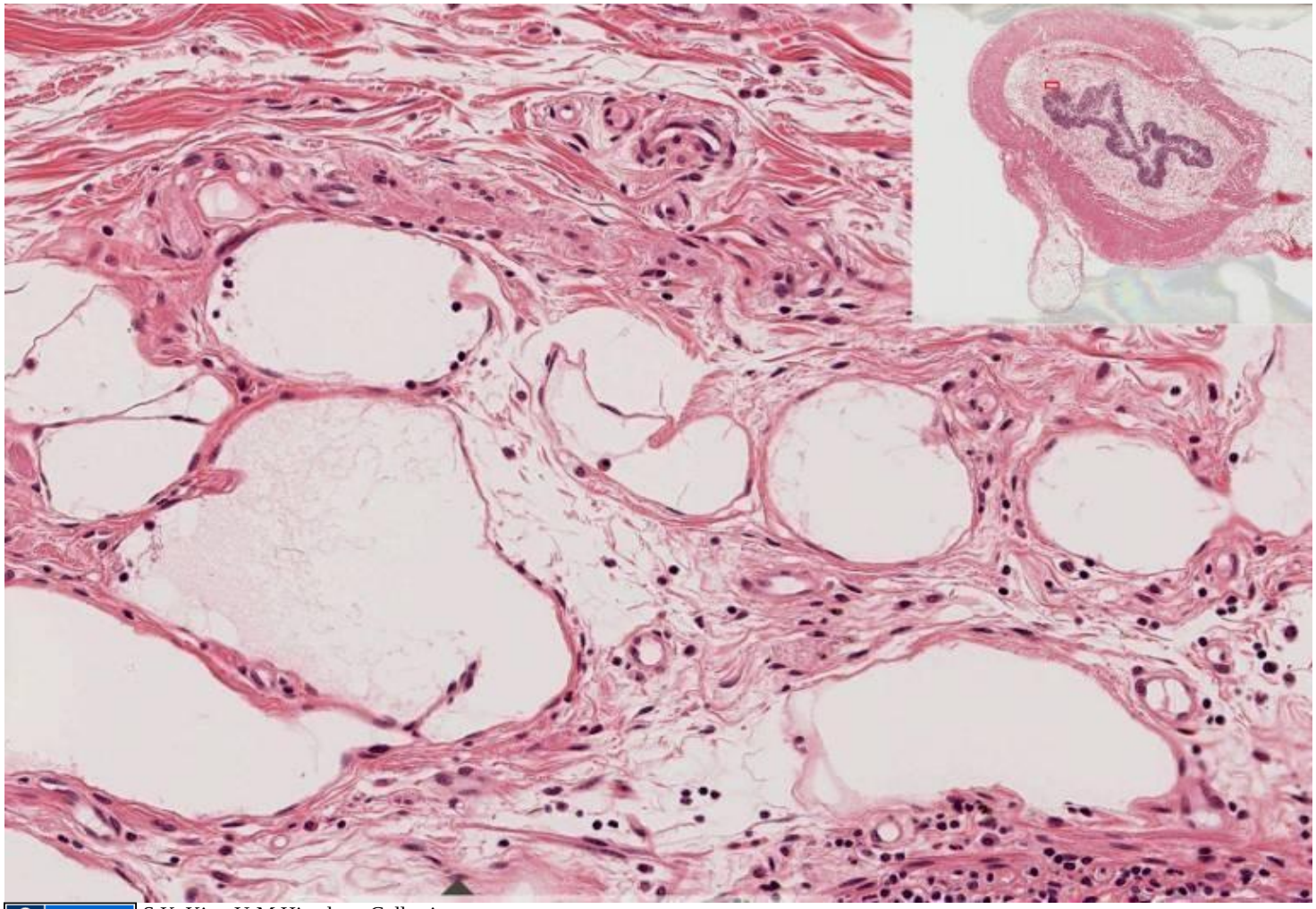
- primary and secondary follicles in lamina propria and submucosa
- So, clearly a secondary lymphoid organ…
- However, also a site of antigen-INDEPENDENT differentiation (similar to Bursa of Fabriscus is birds)
- So, also a primary lymphoid organ



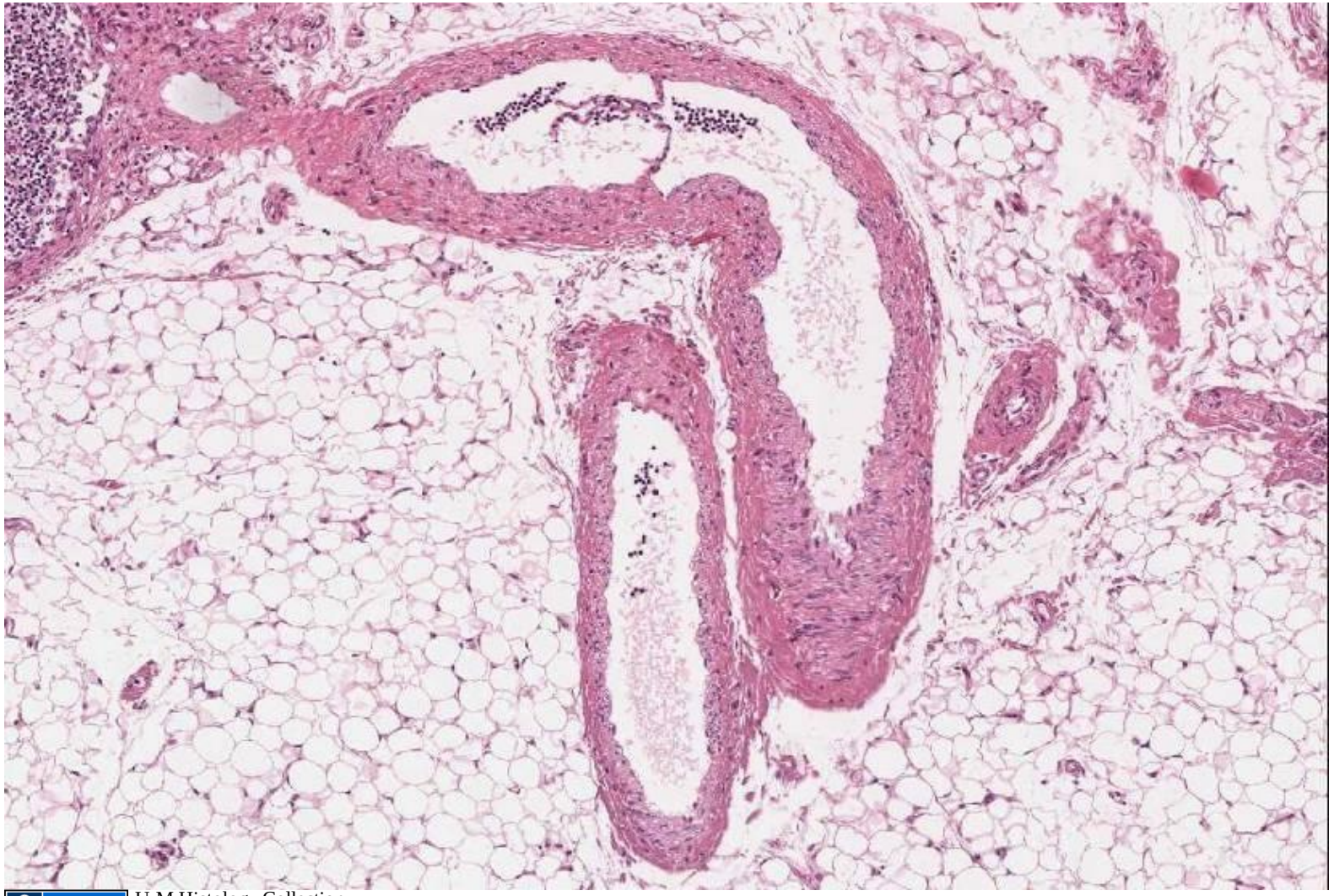
 Ross and Pawlina, *Histology: A Text and Atlas*

Wanderlust: lymphocytes don't just stay in one place

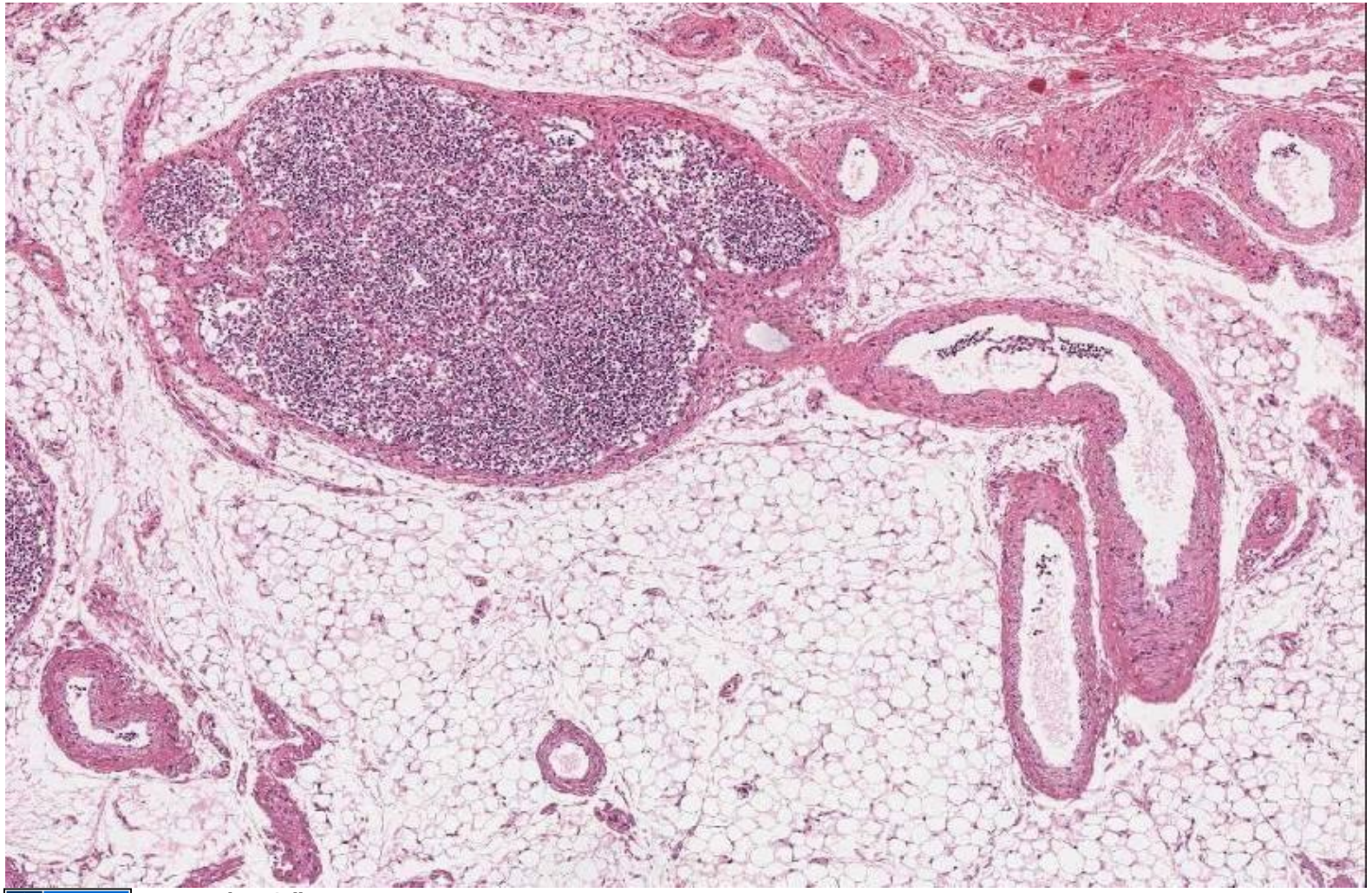
From the MALT, lymphocytes can squeeze into lymph vessels…



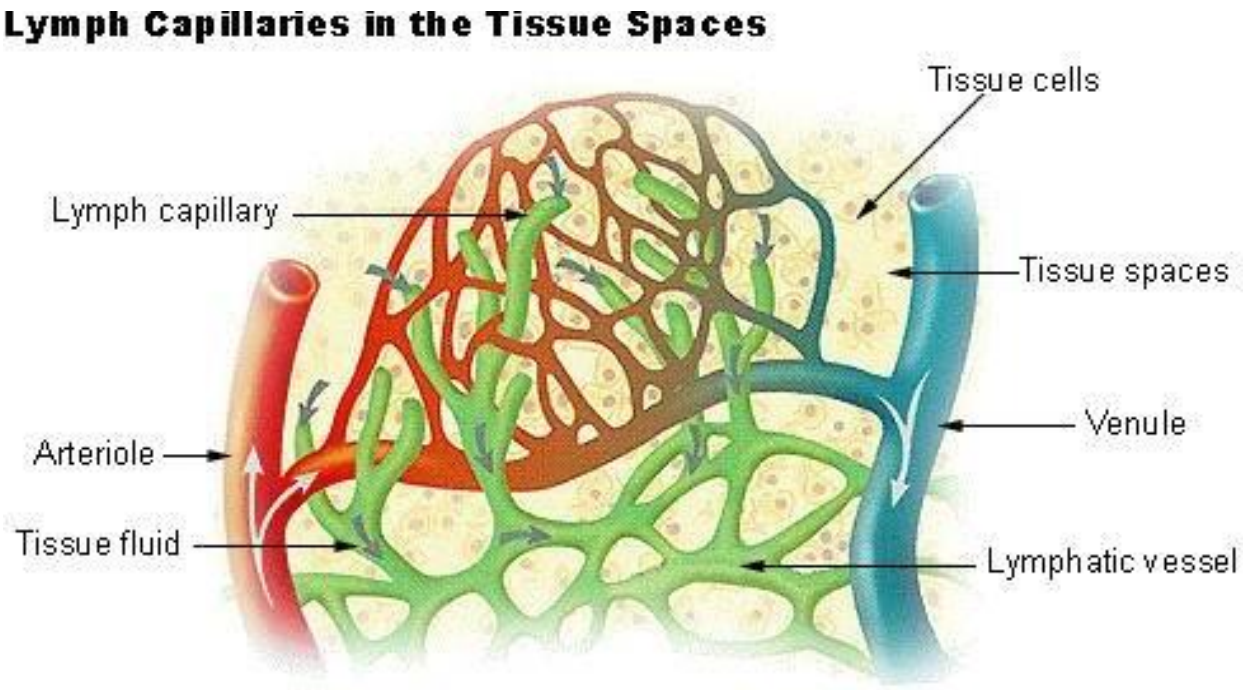
..go through larger lymphatic channels in the mesentery…



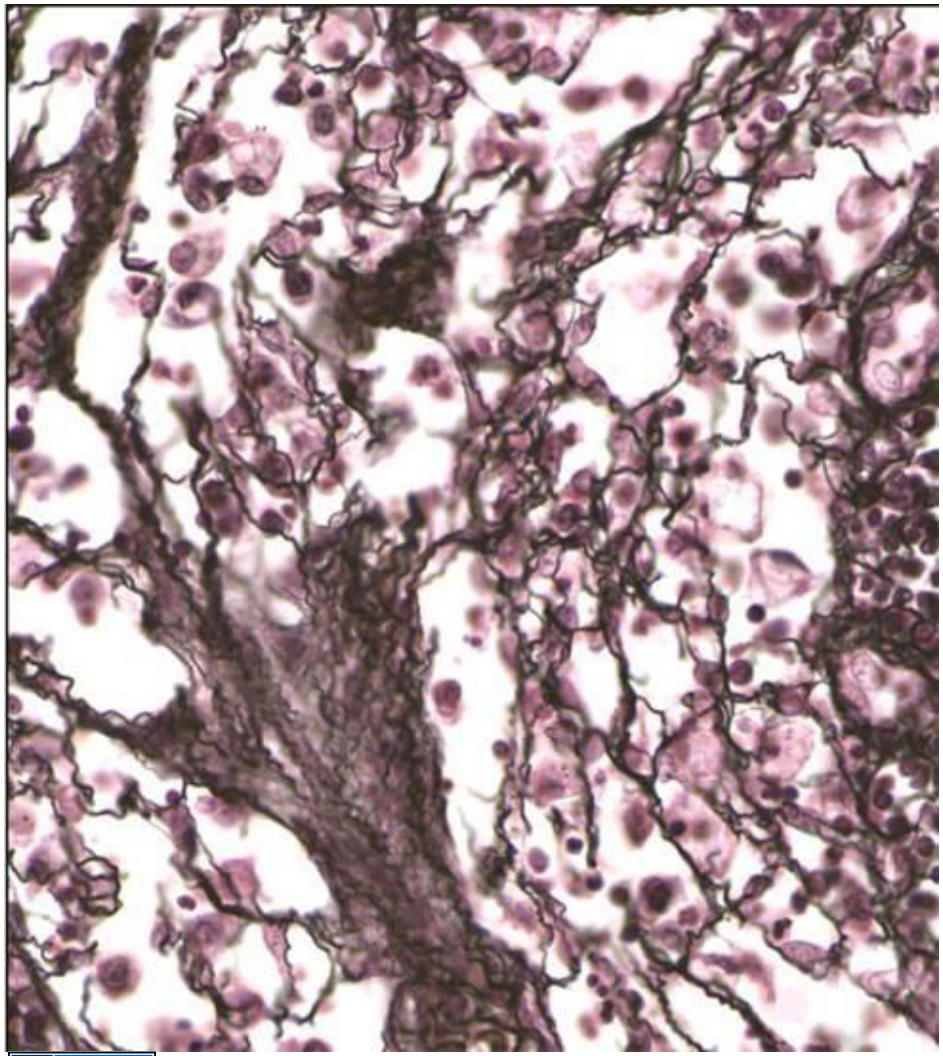
..and end up at a LYMPH NODE.



Lymphoid circulation in the body takes place in both the blood stream and the **lymphatic vessels,** a separate vessel system that carries cells of the lymphoid system and their products (cytokines, antibodies, etc.).



Reticular (Reticulin) Fibers

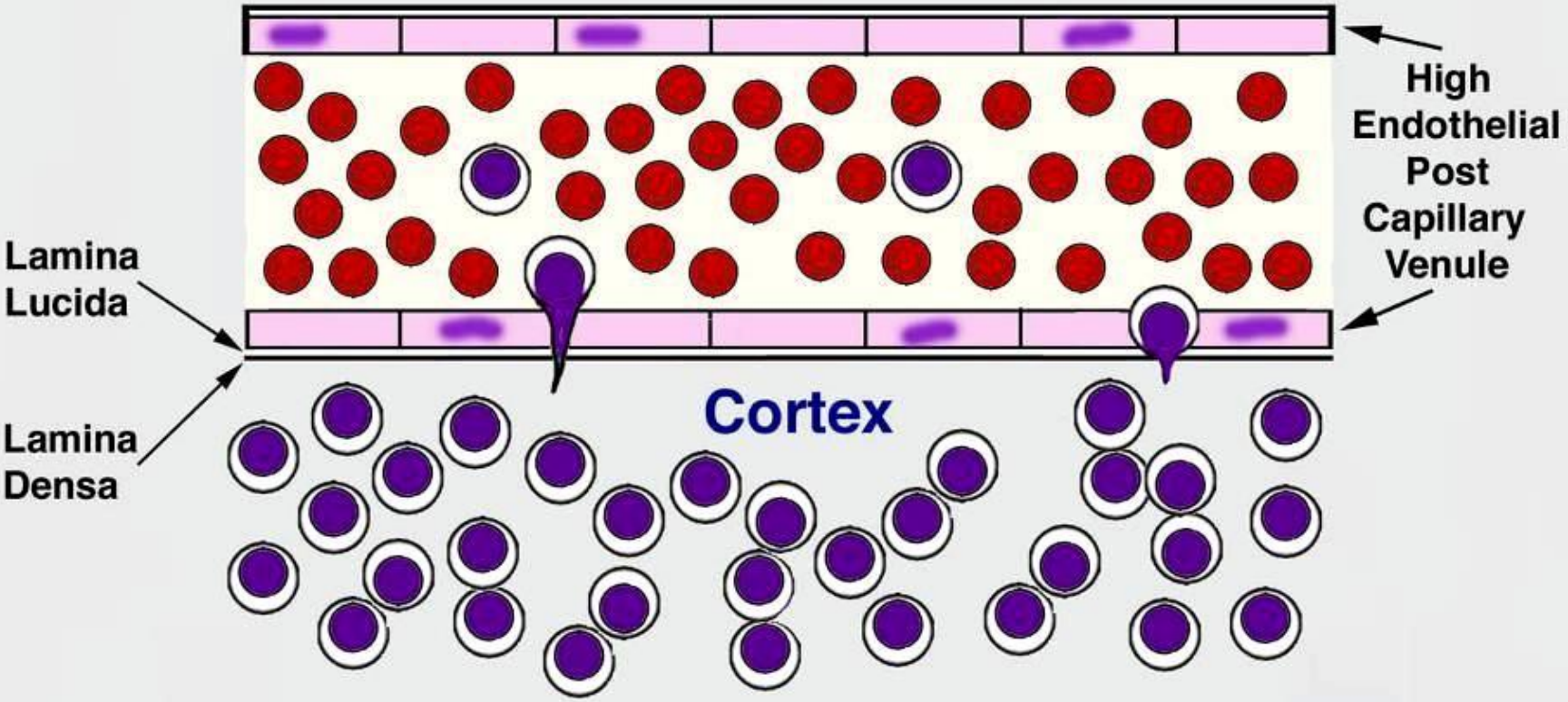


PD-INEL Source Undetermined

- Form a delicate supporting framework for highly cellular tissues (endocrine glands, lymph nodes, liver, bone marrow, spleen, smooth muscle).
- Composed mainly of Type III collagen, with a carbohydrate moiety that reduces Ag+ to metallic sliver = argyrophilic.
- Special stain: silver impregnation to visualize.
- Thinner than type I collagen (Type III fibrils are 30-40 nm diameter; type I fibrils are ~200 nm diameter)

Lymphocyte Homing -

Extravasation by T and B Cells in the High Endothelial Postcapillary Venules of the Lymph Node Cortex



Immune system: (3) molecules

- Antibodies
- Complement
- Cytokines
- Interleukines
- Interferons

Two types of immunity

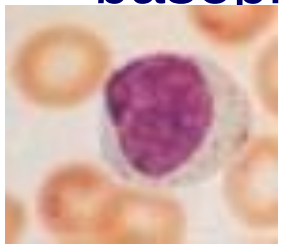
1. Innate (non-adaptive)
   - first line of immune response
   - relies on mechanisms that exist before infection
2. Acquired (adaptive)
   - Second line of response (if innate fails)
   - relies on mechanisms that adapt after infection
   - handled by T- and B- lymphocytes
   - one cell determines one antigenic determinant

Innate immunity

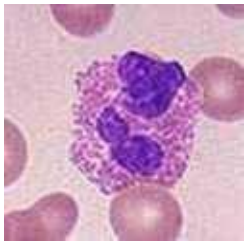
- Based on genetic make-up
- Relies on already formed components
- Rapid response: within minutes of infection
- Not specific
  - same molecules / cells respond to a range of pathogens
- Has no memory
  - same response after repeated exposure
- Does not lead to clonal expansion

Innate immunity: mechanisms

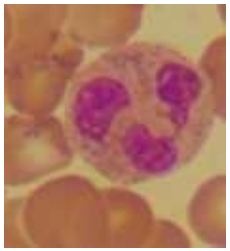
- Mechanical barriers / surface secretion
  - skin, acidic pH in stomach, cilia
- Humoral mechanisms
  - lysozymes, basic proteins, complement, interferons
- Cellular defense mechanisms
  - natural killer cells neutrophils, macrophages, mast cells, basophils, eosinophils



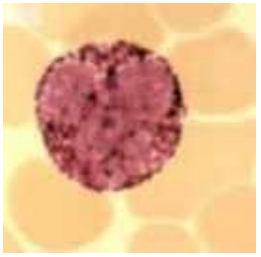
NK Cell



Eosinophils



Neutrophil



Basophils & Mast cells

Monocyte Macrophage

Adaptive immunity: second line of response

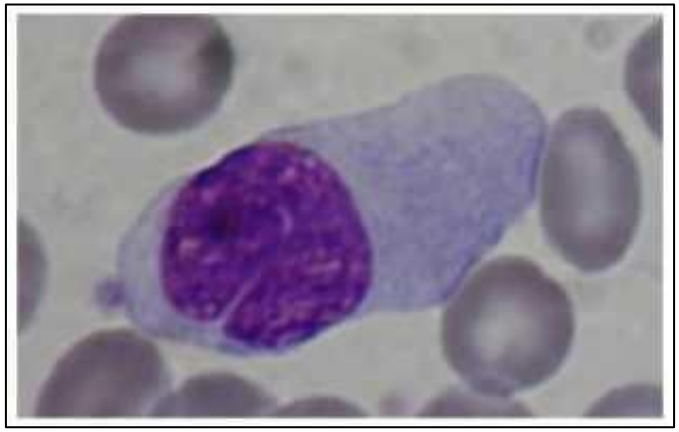
- Based upon resistance acquired during life
- Relies on genetic events and cellular growth
- Responds more slowly, over few days
- Is specific
  - each cell responds to a single epitope on an antigen
- Has anamnestic memory
  - repeated exposure leads to faster, stronger response
- Leads to clonal expansion

Adaptive Immunity: active and passive

	Active Immunity	Passive Immunity
Natural	clinical, sub-clinical infection	via breast milk, placenta
Artificial	Vaccination: Live, killed, purified antigen vaccine	immune serum, immune cells

Adaptive immunity: mechanisms

- Cell-mediated immune response (CMIR)
  - T-lymphocytes
  - eliminate intracellular microbes that survive within phagocytes or other infected cells
- Humoral immune response (HIR)
  - B-lymphocytes
  - mediated by antibodies
  - eliminate extra-cellular microbes and their toxins

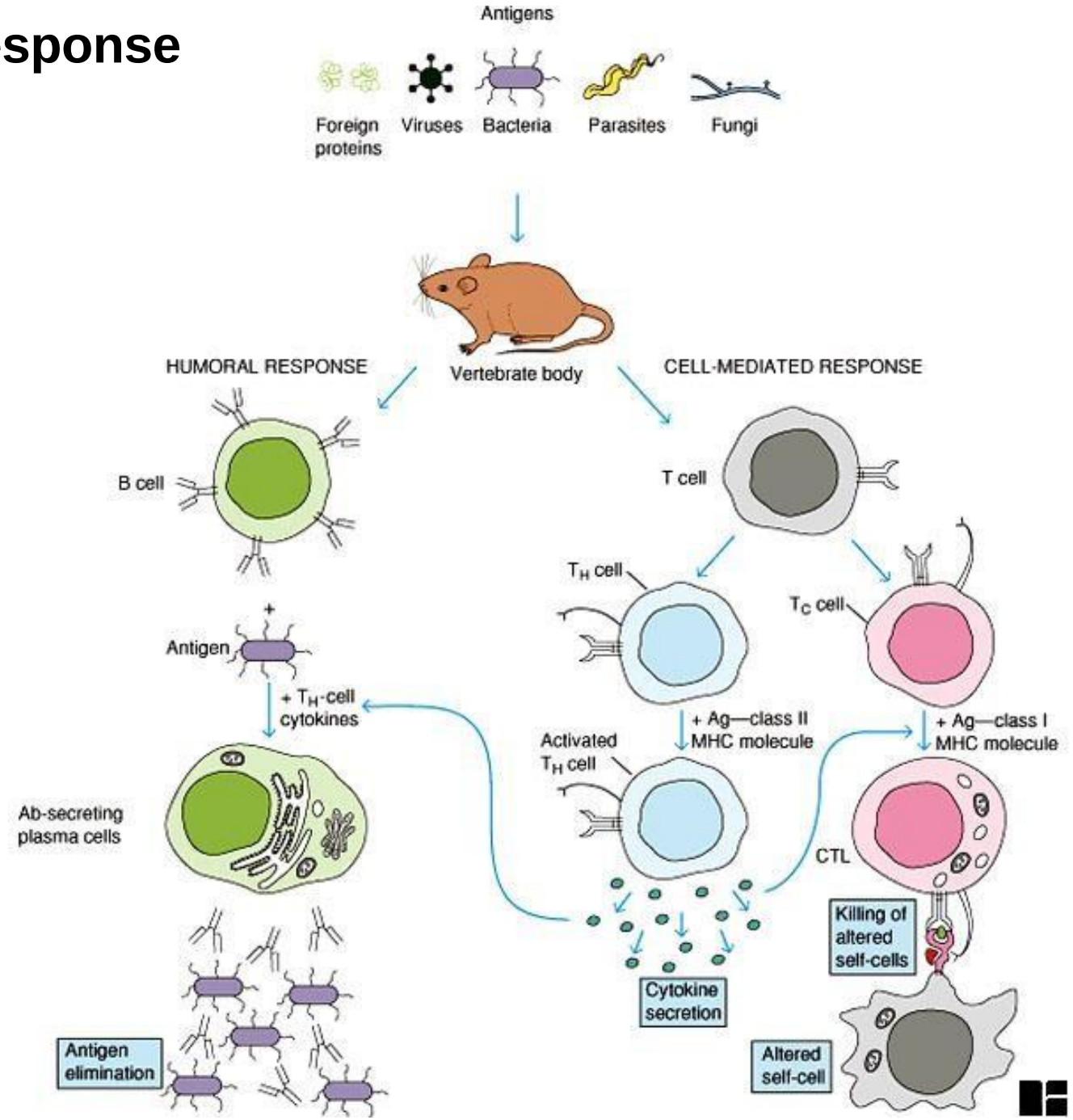


Plasma cell
(Derived from B-lymphocyte, produces antibodies)

The Immune Response

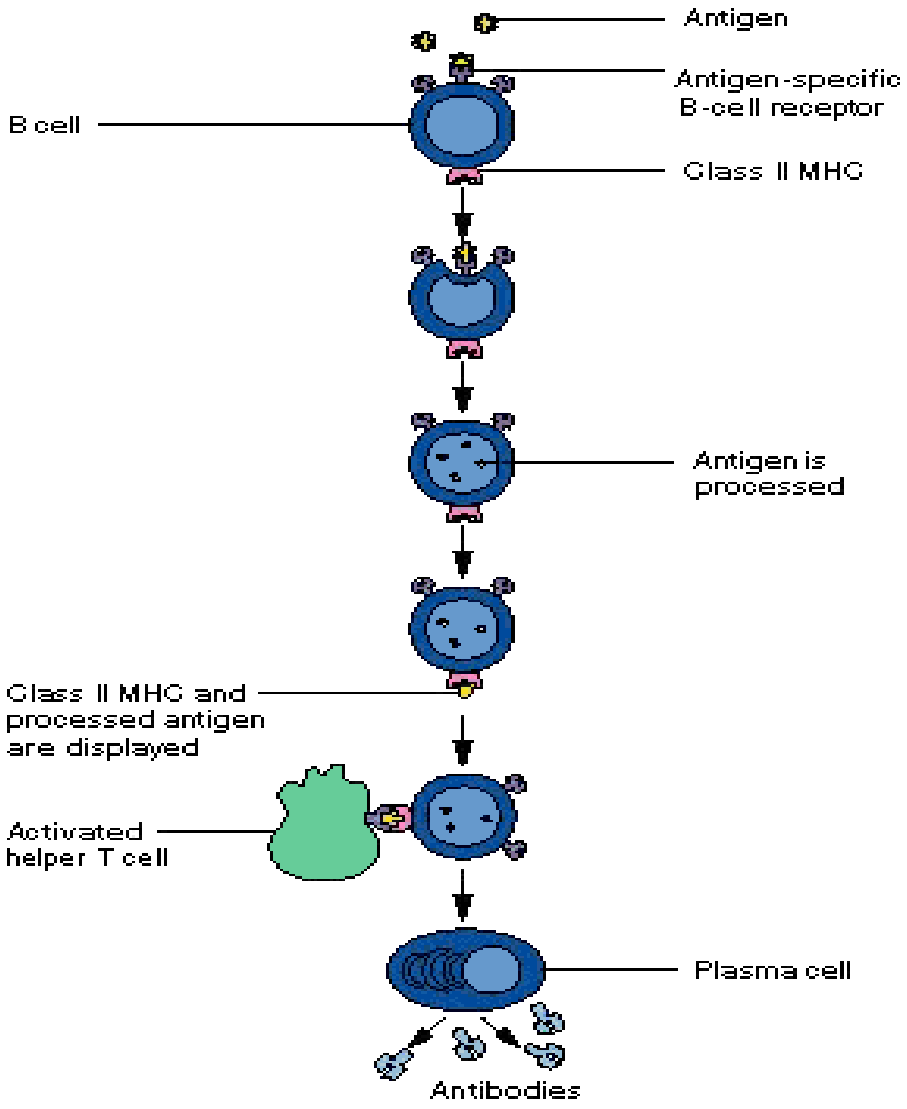
The humoral response involves interaction of B cells with antigen (Ag) and their differentiation into antibody-secreting plasma cells. The secreted antibody (Ab) binds to the antigen and facilitates its clearance from the body.

The cell-mediated responses involve various subpopulations of T cells that recognize antigen presented on self-cells. Helper T cells respond to antigen by producing cytokines. Cytotoxic T cells respond to antigen by developing into cytotoxic T lymphocytes (CTLs), which mediate killing of altered self-cells (e.g., virus-infected cells).



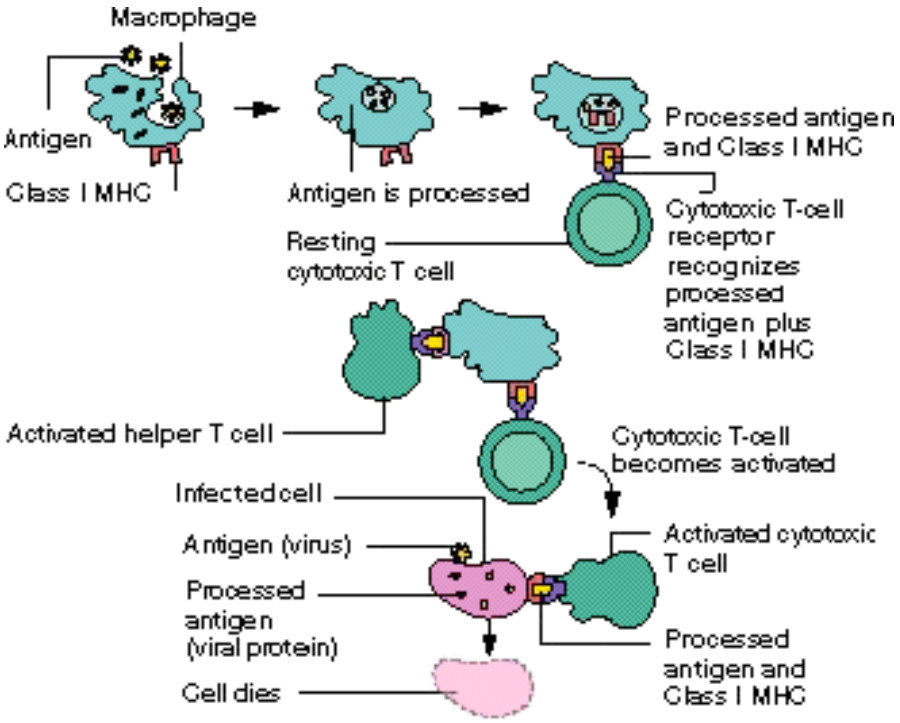
Humoral immune response

1. B lymphocytes recognize specific antigens
   - proliferate and differentiate into antibody-secreting plasma cells
2. Antibodies bind to specific antigens on microbes; destroy microbes via specific mechanisms
3. Some B lymphocytes evolve into the resting state - memory cells



Cell-mediated immune response

1. T-cell
   - recognizes peptide antigen on macrophage in association with major histo-compatibility complex (MHC) class
   - identifies molecules on cell surfaces
   - helps body distinguish self from non-self
1. T-cell goes into effectors cells stage that is able to kill infected cells



T lymphocytes

2 types

- helper T- lymphocytes (CD4+)
  - CD4+ T cells activate phagocytes to kill microbes
- cytolytic T-lymphocyte (CD8+)
  - CD8+ T cells destroy infected cells containing microbes or microbial proteins

Cell mediated immune response

Primary response

- production of specific clones of effector T cells and memory clones
- develops in several days
- does not limit the infection

Secondary response

- more pronounced, faster
- more effective at limiting the infection

Example - cytotoxic reactions against intracellular parasites, delayed hypersensitivity (e.g., Tuberculin test) and allograft rejection

Lymphocytes recognize specific sites on molecules → Ag determinant/epitope

Why is the knowledge of antibody epitopes is so important?

- **Vaccine design (*immunogenicity*,** i.e. ability of vaccine to elicit in the naïve individual the production of pathogen neutralizing antibodies, **is required**)**:**
  - Purified antigen (subunit) vaccines:
    - Inactivated toxins “toxoids”: tetanus toxoid, diphteria toxoid
    - Vaccines composed of bacterial polysaccharide antigens: flu, pneumococcus
  - Synthetic antigen vaccines:
    - hepatitus B (recombinant protein), herpes simplex virus

- **Diagnostic design** *(**antigenicity**,* i.e. ability of synthetic antigen to be recognized by the original antibody, is required*):*
    - Autoimmune diseases: lupus, rheumatoid arthritis
    - Allergic reactions

Strategies for design immunogens that elicit broadly neutralizing antibodies

(from *"HIV vaccine design and the neutralizing antibody problem"* *Nature Immunology, 2004, 5, 233)*

- To produce molecules that mimic the mature trimer Env on the virion surface. These molecules can be recombinant or expressed on the surface of particles such as pseudovirions or proteoliposomes.
- To produce Env molecules engineered to better present NAb epitopes than do "wild-type" molecules.
- To generate stable intermidiates of the entry process with the goal of exposing conserved epitopes to which antibodies could gain access during entry.
- To produce epitope mimics of the broadly NAbs determined from structural studies of antibody-antigen complexes.

Epitope identification

The best precision in identification of antibody epitopes is provided by X-ray crystallography.

Other methods to predict structure and location of antibody epitopes include:

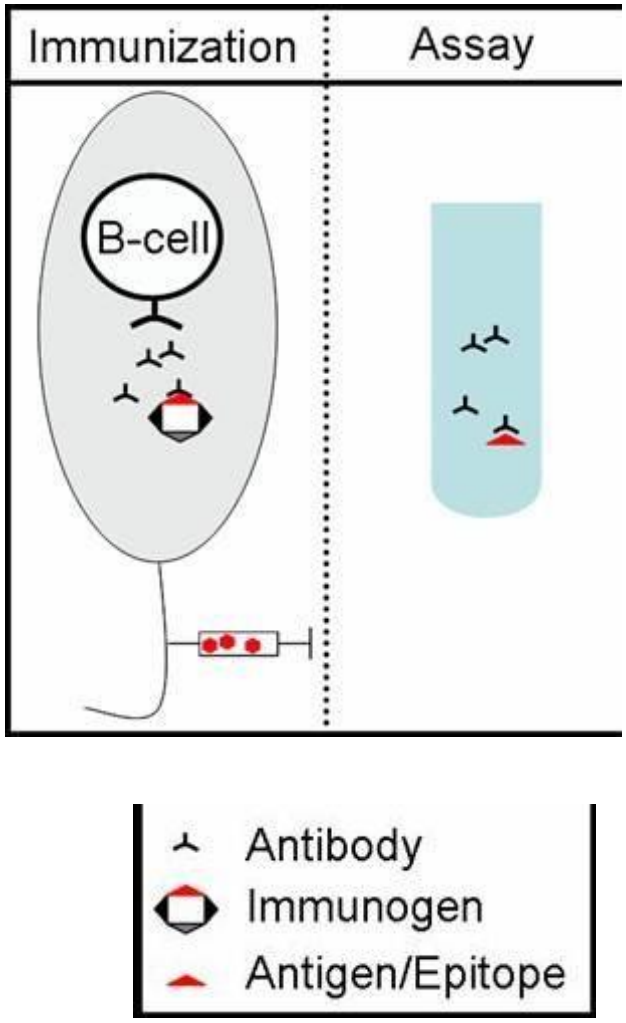
- mass spectrometry combined with immunoaffinity procedures;

-screening of combinatorial phage-display peptide libraries;

-mimitope approach: selection ligands from a library of random combinatorial ligands;

- alanine scan;

- etc.



Methods for antibody epitope prediction

- Sequence-based (*suitable for linear epitopes only*)
  - Amino acid scales: hydrophobicity, secondary structure (beta-turn), polarity, flexibility, solvent accessibility etc.
  - The combination of scales and experimentation with several machine learning algorithms showed little improvement over single scale-based methods.
  - Maximum sensitivity is 59%.
- Structure-based (*antibody binding site prediction for a protein of a given 3D structure*):
  - CEP
  - DiscoTope
- Epitope mapping using peptide libraries

Immune Epitope Database and Analysis Resource - Netscape

File Edit View Go Bookmarks Tools Window Help

http://epitope2.immuneepitope.org/home.do

Home Netscape Search Customize...

Netscape Enter Search Terms Search Highlight Pop-Ups Blocked: 29 Form Fill Clear Browser History

IMMUNE EPITOPE DATABASE AND ANALYSIS RESOURCE

HOME | QUERY | RESOURCES | ACCOUNT | SUPPORT | ABOUT IEDB | Search

The Immune Epitope Database and Analysis Resource (IEDB)

Welcome to the Immune Epitope Database and Analysis Resource (IEDB). The IEDB is a project hosted by scientists at the La Jolla Institute for Allergy and Immunology (LIAI), with support from the National Institute of Allergy and Infectious Diseases (NIAID), a part of the National Institutes of Health (NIH), and Department of Health and Human Services (HHS). While not strictly limited, the current focus is presenting information that facilitates the dissemination of immune epitope information, the generation of new research tools, diagnostic techniques, vaccines and therapeutics for emerging and re-emerging diseases.

The IEDB contains data related to antibody and T cell epitopes for humans, non-human primates, rodents, and other animal species. Curation of data relating to Influenza is currently up-to-date and curation of data relating to NIAID Category A, B, and C priority pathogens is approximately 90% complete and is estimated to be finished before the end of July. The next project on the list will be to complete the curation of NIAID emerging and re-emerging infectious

Summary Metrics (count)

References:	2535
Records:	46813
Distinct Structures:	41464
Distinct Epitopes:	21237

Quick Links

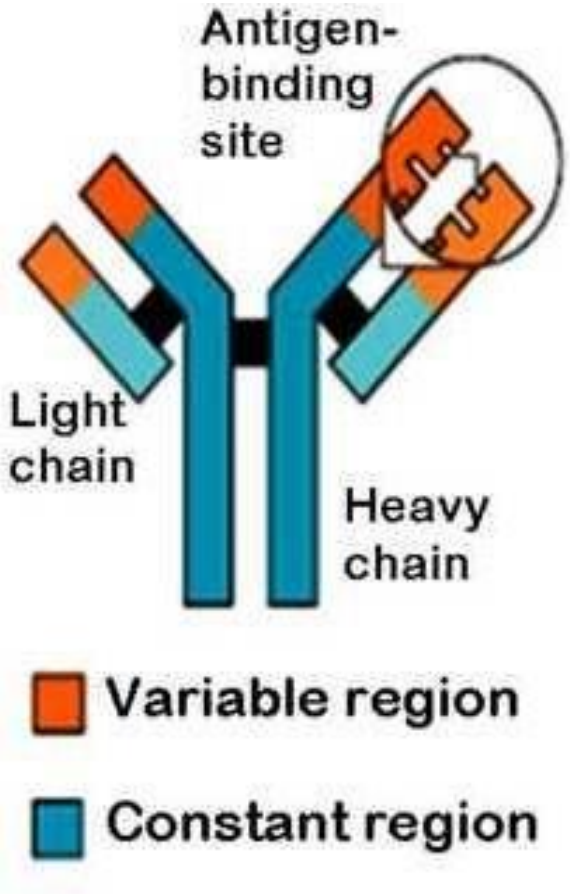
- Perform a Simple Query
- Perform an Advanced Query
- Browse Records by Allele
- Browse Records by Species
- Analysis Tools
- Links
- Forums
- Tour the IEDB
- Register
- Feedback
- Hide Instructions

News / Updates

- 2005 Annual Compendium
- A tool developer resource - Benchmarking MHC-I binding predictions
- July 2006 IEDB Newsletter
- April 2006 IEDB Newsletter
- January 2006 IEDB Newsletter
- October 2005 IEDB Newsletter
- July 2005 IEDB Newsletter
- April 2005 IEDB Newsletter
- January 2005 IEDB Newsletter
- July 2004 IEDB Newsletter

Antibodies (immunoglobulins)

- Belong to the gamma-globulin fraction of serum proteins
- Y-shaped or T-shaped polypeptides
  - 2 identical heavy chains
  - 2 identical light chains
- All immunoglobulins are not antibodies
- Five kinds of antibodies
  - IgG, IgM, IgA, IgD, IgE



IgG

- 70-75% of total immuniglobulin
- Secreted in high quantities in secondary exposures
- Cross the placenta
- Major functions / applications
  - neutralize microbes and toxins
  - opsonize antigens for phagocytosis
  - activate the complement
  - protect the newborn

- 4-fold rise or fall indicates active infection
- A single positive sample indicates past exposure

IgM

- Secreted initially during primary infection
- Cannot cross the placenta
- Major functions / applications
  - secreted first during primary exposure
  - activates the complement
  - used as a marker of recent infection

- Presence in newborn means infection
- Single positive sample in serum or CSF indicates recent or active infection
- Used to detect early phase of infection

IgA

- Monomeric in serum
- Dimeric with secretory component in the lumen of the gastro-intestinal tract and in the respiratory tract
- Major function / application
  - neutralizes microbes and toxins

•Sero-diagnosis of tuberculosis

•Synthicial respiratory virus tests

IgD

- Monomeric
- Major functions / applications
  - present on the surface of B lymphocytes
  - functions as membrane receptor
  - role unclear
    - has a role in antigen stimulated lymphocyte differentiation

IgE

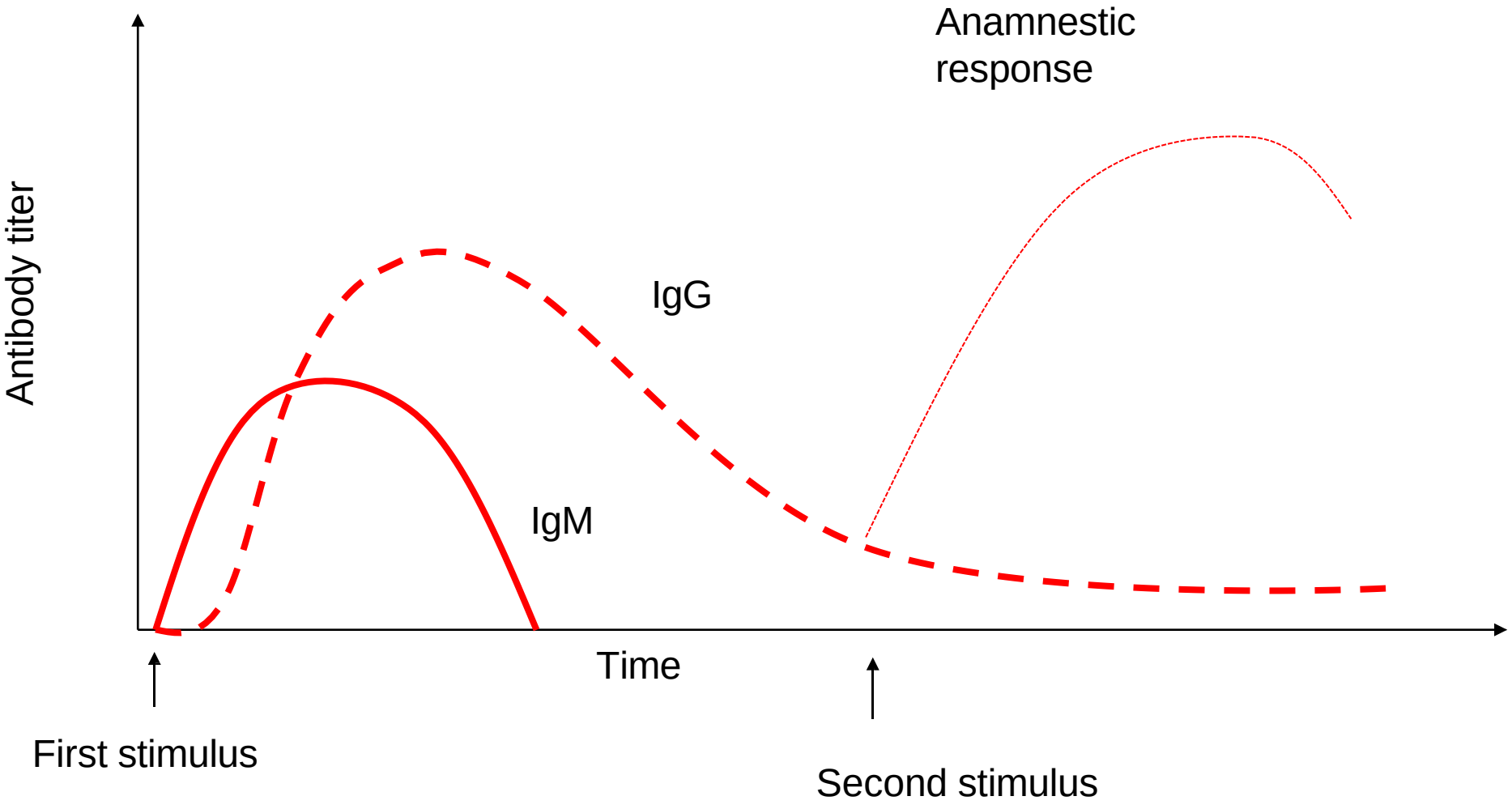
- Mediates type I hypersensitivity
- Monomeric
- Major functions / applications
  - associated with anaphylaxis
  - plays a role in immunity to helminthic parasites

Serodiagnosis of infectious and non infectious allergies (e.g., allergic bronchopulmonary aspergillosis, parasitic diseases)

Sequential IgM-IgG humoral response

- IgM
  - produced as a first response to many antigens
  - levels remain high transiently
- IgG
  - produced after IgM
  - higher levels persist in small amounts throughout life
  - produced in large amounts during secondary response
    - persistence of antigen sensitive ‘memory cells’ after primary response

IgM – IgG sequential response



Failure of immune response

- Immune response helps individuals defend against
  - microbes
  - some cancers
- Immune response can fail
  - hypersensitivity reactions
  - immunodeficiency

Hypersensitivity reactions

- Cause cell damage through excessive immune response to antigens
- Hypersensitivity
  - overreaction to infectious agents
- Allergy
  - overreaction to environmental substances
- Autoimmunity
  - overreaction to self

Immunodeficiency

- Loss or inadequate function of various components of the immune system
- Can occur in any part or state of the immune system
  - physical barrier, phagocytes, B lymphocytes, T lymphocytes, complement, natural killer cells
- The immuno-compromised host
  - has an impaired function of immune system
  - is at high risk of infection

Immunodeficiency

- Congenital (primary) immunodeficiency
  - genetic abnormality
    - defect in lymphocyte maturation
- Acquired (secondary) immunodeficiency
  - results from infections, nutritional deficiencies or treatments
    - AIDS, chronic leukemia

Altered immunity: immuno-compromised

		Disorder	Compromised function
Altered anatomic barrier	Mucus membrane	Reduction in IgA	Microbe binding
	Gastro-intestinal tract	Elevated pH	Bacteria killing
		Change in flora	Colonization resistance
Immune system	Innate immunity	Reduction of complement	Activates phagocytosis Opsonization of bacteria Membrane attack complex
		Neutropenia Monocytopenia	Phagocytosis Bacteria killing
	Adaptive immunity	Reduction of T cells	Activation of macrophages Activation of B lymphocytes
		Hypo-gammaglobulinemia	Neutralizes pathogens and toxins, opsonization, complement activation

Summary (1)

- Innate immunity
  - relies on mechanisms already existing before microbe infects host
  - is the first line of defense
  - has no memory for subsequent exposure
  - relies on non specific mechanisms

Summary (2)

- Adaptive immunity
  - develops following entry of microbe into the host
  - comes into action after innate immunity fails to get rid of microbe
  - has memory to deal with subsequent exposure
  - happens through specific cells
    - T cells (cell mediated)
    - B cells (antibody mediated)

Summary (3)

- Primary immune response
  - short lasting
  - smaller in magnitude
- Secondary immune response
  - longer in duration
  - larger in magnitude
    - develop ‘memory cells’ following primary response
- Failure of immune response can result in:
  - hypersensitivity
  - immunodeficiency

Read about:

- Evolution of blood cells;
- Clonal expansion;
- Cytokines;
- Interleukines;
- Interferons;
- Defensins;
- Cilia (mechanical);
- Complement (humoral)
- Lyzosymes

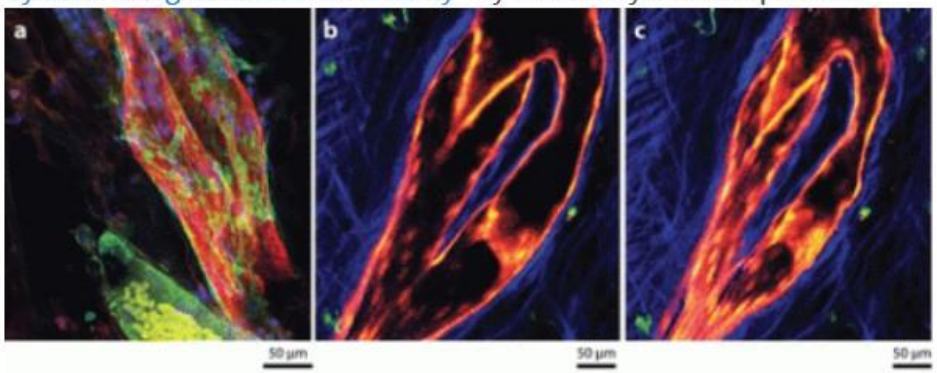
Explain: On which stage of human life each of IS organs plays which role, how did they evolve and develop?

The Complicated Immune Response: Annual Review of Immunology, Volume 35

May 11, 2017 Suzanne K. Moses Volume Annoucement immunol

Browse the *Annual Review of Immunology,* Volume 35 table of contents.

While proofing this volume, I was impressed again by how complicated the immune system is. It seemed so very simple in high school biology! But there are so many related actions that have to happen in precise sequences, and even systems that I thought were disconnected from immunity turn out to be quite important. For instance, see the abstract of "The Lymphatic System: Integral Roles in Immunity" by Gwendalyn Randolph et al.:



Randolph GJ, et al. 2017.
Annu. Rev. Immunol. 35:31–52

The lymphatic vasculature is not considered a formal part of the immune system, but it is critical to immunity. One of its major roles is in the coordination of the trafficking of antigen and immune cells. However, other roles in immunity are emerging. Lymphatic endothelial cells, for example, directly present antigen or express factors that greatly influence the local environment.

Review Article | Published: 05 December 2016

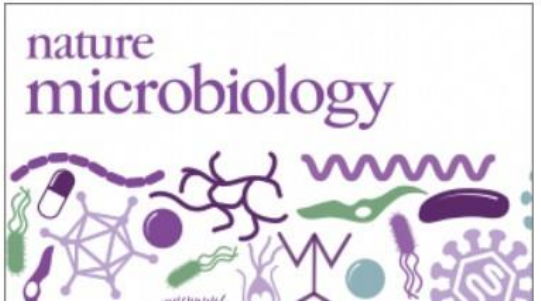
Human immune system variation

Petter Brodin & Mark M. Davis

Nature Reviews Immunology **17**, 21–29 (2017) | Download Citation

Abstract

The human immune system is highly variable between individuals but relatively stable over time within a given person. Recent conceptual and technological advances have enabled systems immunology analyses, which reveal the composition of immune cells and proteins in populations of healthy individuals. The range of variation and some specific influences that shape an individual's immune system is now becoming clearer. Human immune systems vary as a consequence of heritable and non-heritable influences, but symbiotic and pathogenic microbes and other non-heritable influences explain most of this variation. Understanding when and how such influences shape the human immune system is key for defining metrics of immunological health and understanding the risk of immune-mediated and infectious diseases.



GYNECOLOGICAL ENDOCRINOLOGY

http://informahealthcare.com/gye
ISSN: 0951-3590 (print), 1473-0766 (electronic)

Gynecol Endocrinol, Early Online: 1–7
 DOI: 10.3109/09513590.2013.852531

informa
healthcare

REVIEW ARTICLE

The immune system and aging: a review

Camil Castelo-Branco[1] and Iris Soveral[2]

[1]*Faculty of Medicine, Institut Clínic of Gynecology, Obstetrics and Neonatology, University of Barcelona, Barcelona, Spain and*
[2]*Hospital Clinic-Institut d'Investigacions Biomèdiques, August Pi i Sunyer (IDIBAPS), Barcelona, Spain*

Abstract

The concept of immunosenescence reflects age-related changes in immune responses, both cellular and serological, affecting the process of generating specific responses to foreign and self-antigens. The decline of the immune system with age is reflected in the increased susceptibility to infectious diseases, poorer response to vaccination, increased prevalence of cancer, autoimmune and other chronic diseases. Both innate and adaptive immune responses are affected by the aging process; however, the adaptive response seems to be more affected by the age-related changes in the immune system. Additionally, aged individuals tend to

Keywords

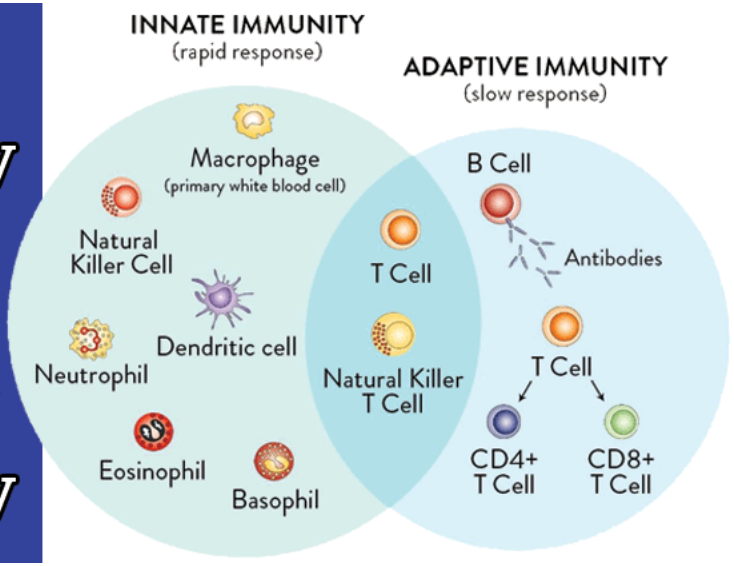
Aging, immune system, immunosenescence, innate and adaptive responses

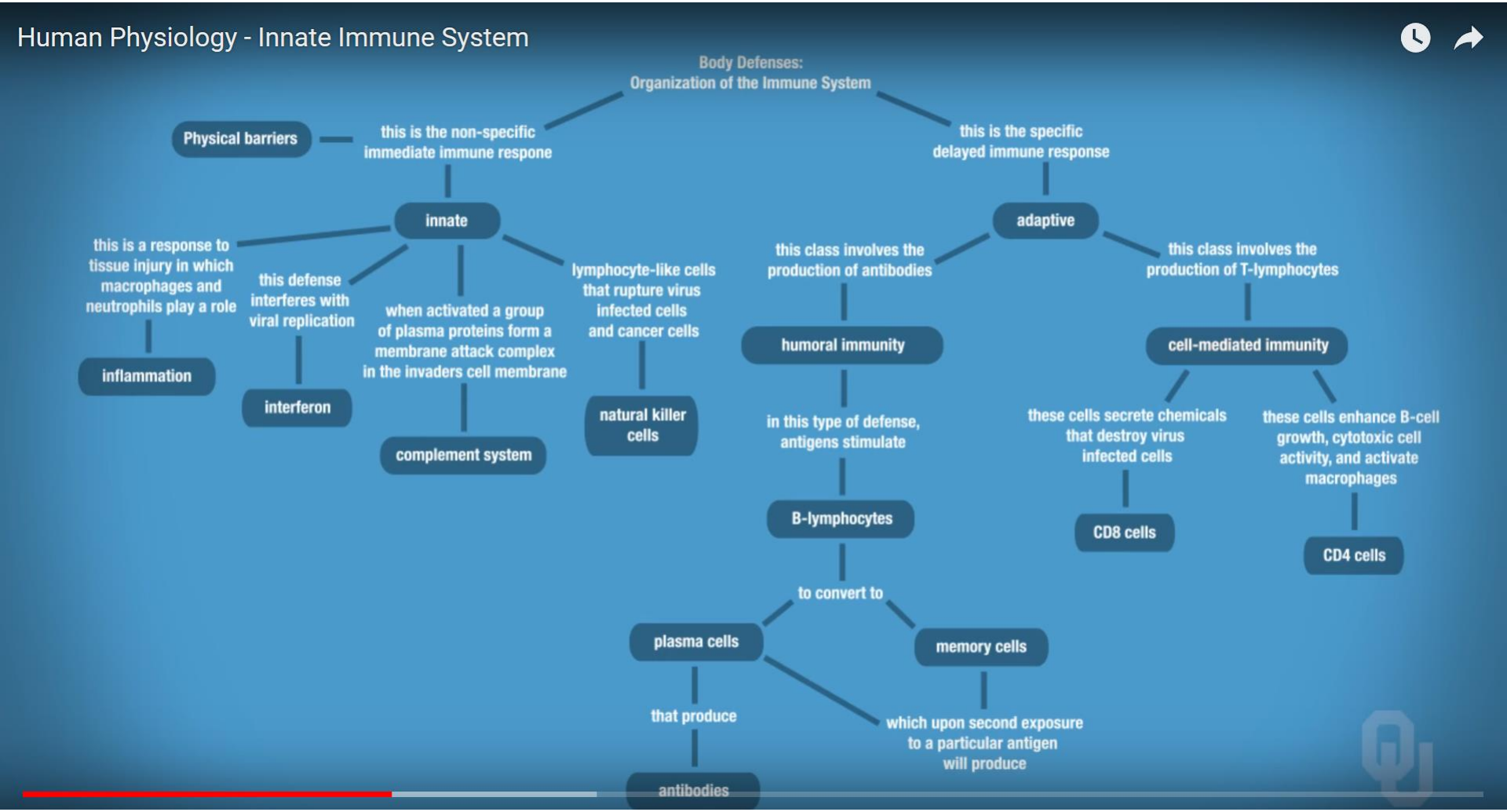
History

Received 8 September 2013
Accepted 3 October 2013

QUESTIONS OR COMMENTS?

Innate Immunity Vs Adaptive Immunity



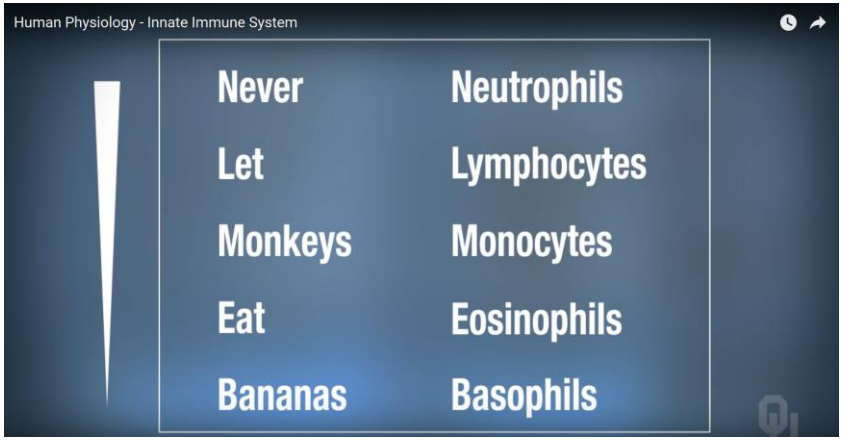


https://www.youtube.com/watch?v=sYjtMP67vyk

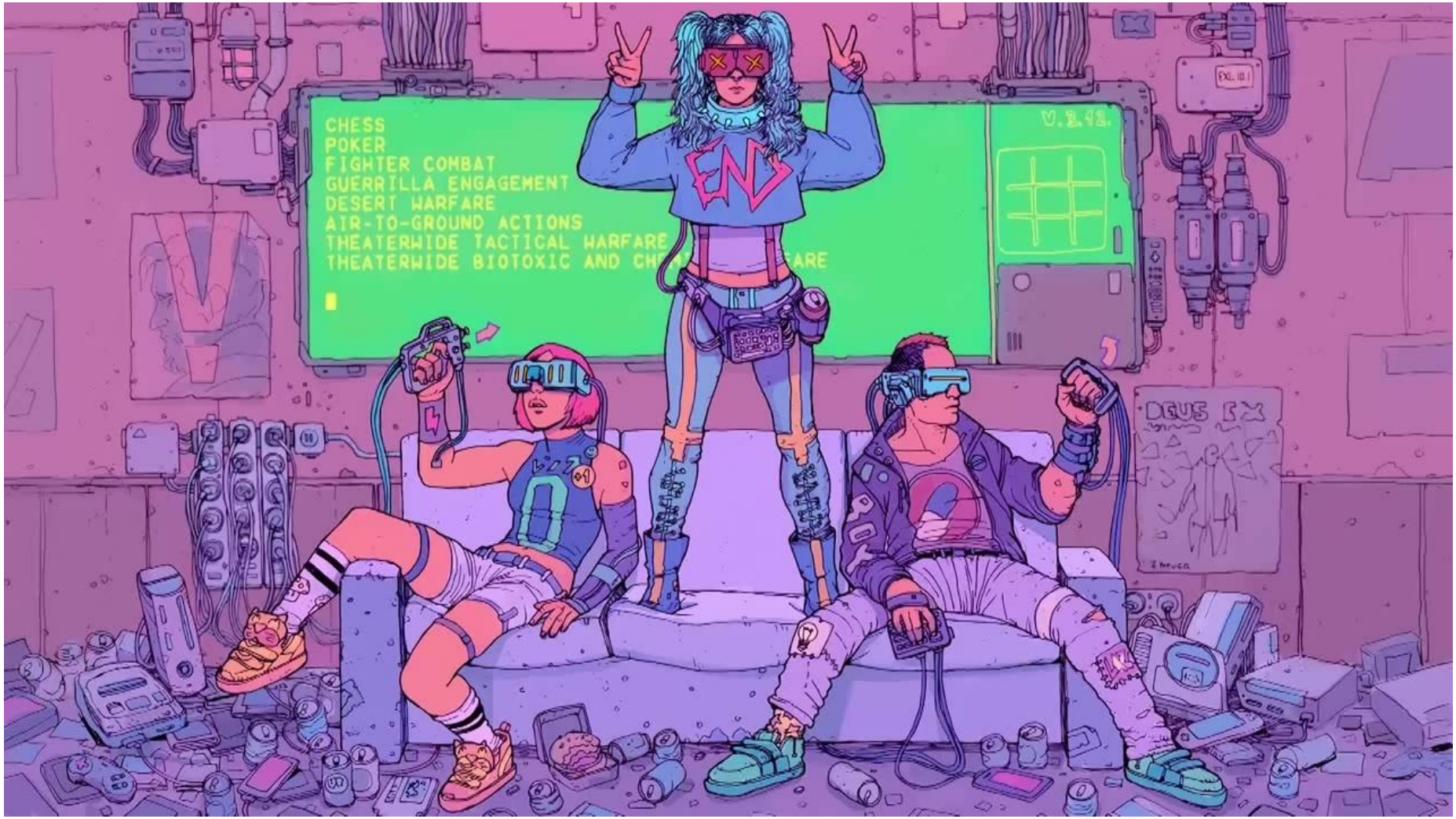
Human Physiology - Innate Immune System

	Component	Amount per microliter (mm^3)	Diameter (μm)	Anatomical features	Primary function
Innate immunity (non-specific)	Erythrocytes	5,000,000	7-8	No nucleus; no organelles; biconcave disk	Transport O_2 and CO_2
	Leukocytes	4,000-10,000			Defend body against pathogens
	Neutrophils	3,000-7,000	10-14	Multilobed nucleus; red and blue staining granules	Phagocytosis of foreign material
	Eosinophils	100-400	10-14	Bilobed nucleus; red staining granules	Kill parasites
	Basophils	20-50	10-12	Multilobed nucleus; blue staining granules	Secrete chemical mediators in inflammation and allergic reactions
	Monocytes	100-700	14-24	Large kidney-shaped nucleus; no granules	Phagocytosis; mature into macrophages in tissues
Acquired (specific or adaptive) immunity	Lymphocytes	1,500-3,000	5-17	Large round nucleus; little cytoplasm; no granules	B cell-secrete antibodies T cells-secrete cytokines that support immune response of other cells; secrete factors that kill infected or tumor cells

https://www.youtube.com/watch?v=sYjtMP67vyk



https://www.youtube.com/watch?v=sYjtMP67vyk


CHESS
POKER
FIGHTER COMBAT
GUERRILLA ENGAGEMENT
DESERT WARFARE
AIR-TO-GROUND ACTIONS
THEATERWIDE TACTICAL WARFARE
THEATERWIDE BIOTOXIC AND CH
V.2.42.
END
DEUS EX