IMMUNOLÓGIAI ÉS BIOTECHNOLÓGIAI INTÉZET

1st practice: Introduction, structure of lymphoid organs

Basic Immunology

University of Pécs, Clinical Center
Department of Immunology and Biotechnology
Pécs, 2026.

Introducing the subject 1.

- Contact lessons: 2x45 min Lectures (Thursdays 12:00-13:45) and 2x 45 min Practices.
- On the **lectures**, the lecturers give a summary of the most important questions of immunology based on their several years expertise and own research in the field. Unfortunately, due to time limitations, its is impossible to discuss every detail.
- The ppt slides of the presentations will be published in the website of the Department (www.immbio.hu) on the corresponding week. ATTENTION! The lecture slides alone are not suitable for learning the subject Basic Immunology because they are incomplete without the live explanations and additions given on the lectures by our teachers. For the complete understanding of our topics, we expect that students take part on the lectures and make their own notes as well as the use of the recommended textbook.

Introducing the subject 2.

Practices:

- We will record the presence on every week. Maximum 3 absences are allowed.
- Students must attend the practices well prepared from the topics of the previous lectures and practices. This will be monitored on a regular basis.
- The most important theoretical and practical aspects of immunological methods will be covered, paying special attention to the medical diagnostics applications.
- On practical weeks, students will be provided with the required technical protocols in advance, and the steps of the practices will be performed in pairs under the supervision of the tutors.
- Discussion of the lecture topics, involving interactive learning methods.

Introducing the subject 3.

- During the semester, the efficacy of learning will be monitored using the online **moodle test system (moodle.pte.hu)**.
- The advantage of the moodle system is that each student gets randomized questions each time and the answers are also randomized.
- All students will be registered into the system automatically based on the Neptun data. The username and password for logging in will be the same as for the Neptun.
- Please try to login to moodle on the first week of the semester to avoid any problems.
- Should you have any problem write to boldizsar.ferenc@pte.hu

Introducing the subject 4.

Midterm tests: on weeks 7 and 13 students must write a test consisting of 30 questions in 45 minutes from the teaching materials of the previous weeks. Questions can be solved only once, correction or stepping back to previous questions is not allowed.

- For the acceptance of the semester students must reach minimum 15 points (50%) in both tests.
- If somebody has 25 points or more in both tests, he/she will be offered exemption from the final exam with a result of “5” (Excellent).
- There will be make up tests on week 8 and 14 for such students who were absent or did not reach the 15 points.

Exam:

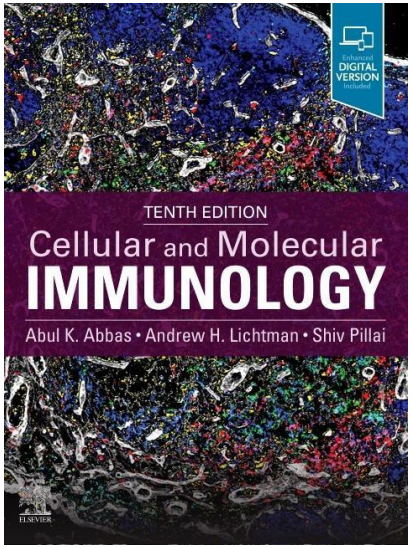
The final exam will be managed through the moodle system, too. The online test will be written in the Faculty’s lecture rooms using the Faculty’s laptops.

There will be 100 questions in 100 minutes. Similarly to the midterm tests, questions can be solved only once, correction or stepping back to previous questions is not allowed.

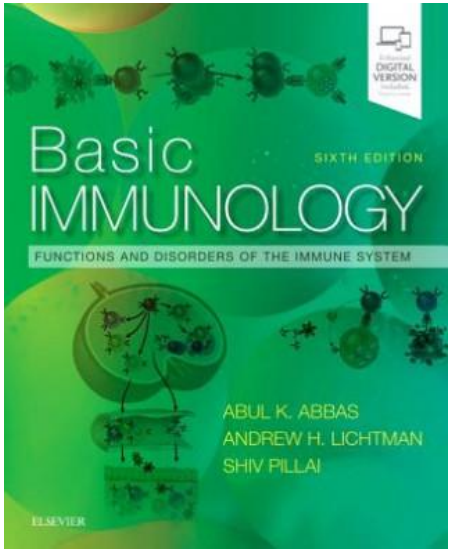
Grades: 0-59: 1; 60-68: 2; 69-75: 3; 76-83: 4; 84-: 5

Introducing the subject 5.

- Books you can learn from:



Abul K. Abbas, Andrew H.H. Lichtman, Shiv Pillai: **Cellular and Molecular Immunology**, 10th edition, 2021.



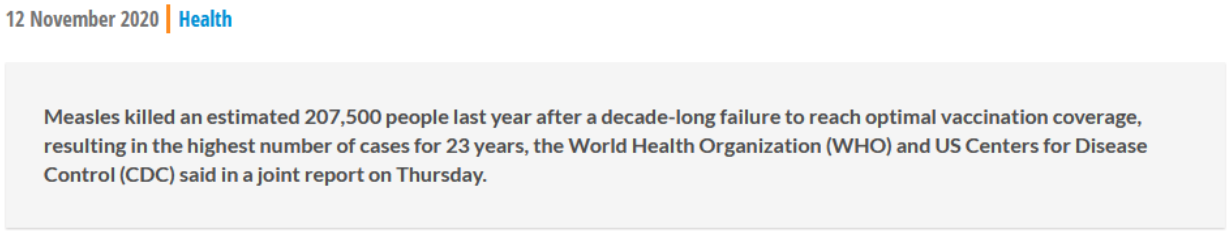
Abul K. Abbas, Andrew H.H. Lichtman, Shiv Pillai: **Basic Immunology**, 6th edition, 2019.

- All the educational material will be available: **www.immbio.hu** and **https://potepedia.aok.pte.hu/en/front-page-english/**

- **Attention!** Our department has never published or lectured any notes for students; therefore, we do NOT recommend that you study from those „unofficial" handouts prepared by previous years students.

Introducing the subject 6.

- **What is the significance of immunology?**
  - The immune system is involved one way or another in almost all human pathological conditions.
  - Many of the laboratory diagnostics are based on immunological methods. (see later)
  - More and more diseases get treatable by manipulating the immune system. (see later)
  - Autoimmune diseases affect 7-8% of the population, they are chronic and generally incurable, yet many can be treated effectively. (see later)
  - The number of immunocompromised patients has increased recently. (therapeutic immunosuppression and HIV, see later)
  - COVID-19/SARS-COV2 pandemy
  - Laypeople also seem to have strong opinions regarding immunology. → Media tends to mix medical facts with quackery and pseudoscience.



12 November 2020 | Health

Measles killed an estimated 207,500 people last year after a decade-long failure to reach optimal vaccination coverage, resulting in the highest number of cases for 23 years, the World Health Organization (WHO) and US Centers for Disease Control (CDC) said in a joint report on Thursday.

Main tasks of the immune system

Preserving the integrity of an organism

Defense against **external pathogens** (e.g. viruses, bacteria, parasites)

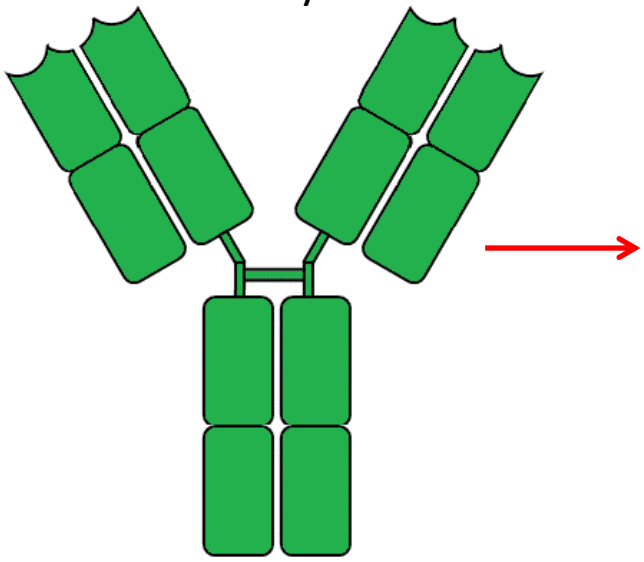
Elimination of one's **pathologically altered cells** (e.g. virally infected cells, cancer cells)

Altered foreign structures must be **recognized** and **distinguished** from the organism's own healthy cells.

IMMUNE RESPONSE (either an aggressive response or immunological tolerance)

Components of the immune system

- The components of immune system can be classified into subsystems (See the lectures for more details):
  - **Innate immunity** (e.g. granulocytes, macrophages, NK cells, complement system)
  - **Natural immunity** (e.g. B1 B cells, γδ T cells)
  - **Adaptive immunity** (e.g. αβ T cells, B2 cells, antibodies)
- The distinction above is artificial, in the organisms these work hand in hand.
- You will mostly learn about the adaptive immunity throughout the semester.



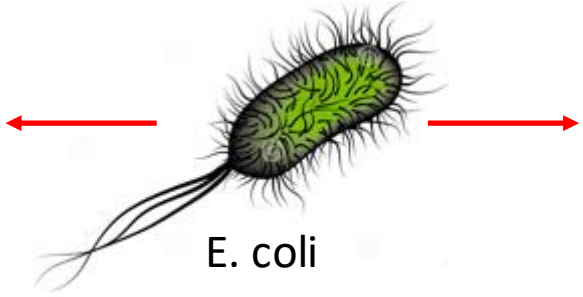
Antibody = Immunoglobulin

Innate vs adaptive immunity

	Innate	Adaptive
Recognition	**Pattern-based (not antigen-specific)**	**Antigen-specific**
Kinetics	**Quick (minutes, hours)**	**Slow (days, weeks)**
Amplification of response	**Linear**	**Exponential**
Immunological memory	**No**	**Yes**

Antigen: Substance recognized by T and B cell receptors (TCR and BCR) which induces either an immune response or immunological tolerance.
Difference between pattern-recognition and antigen-recognition:

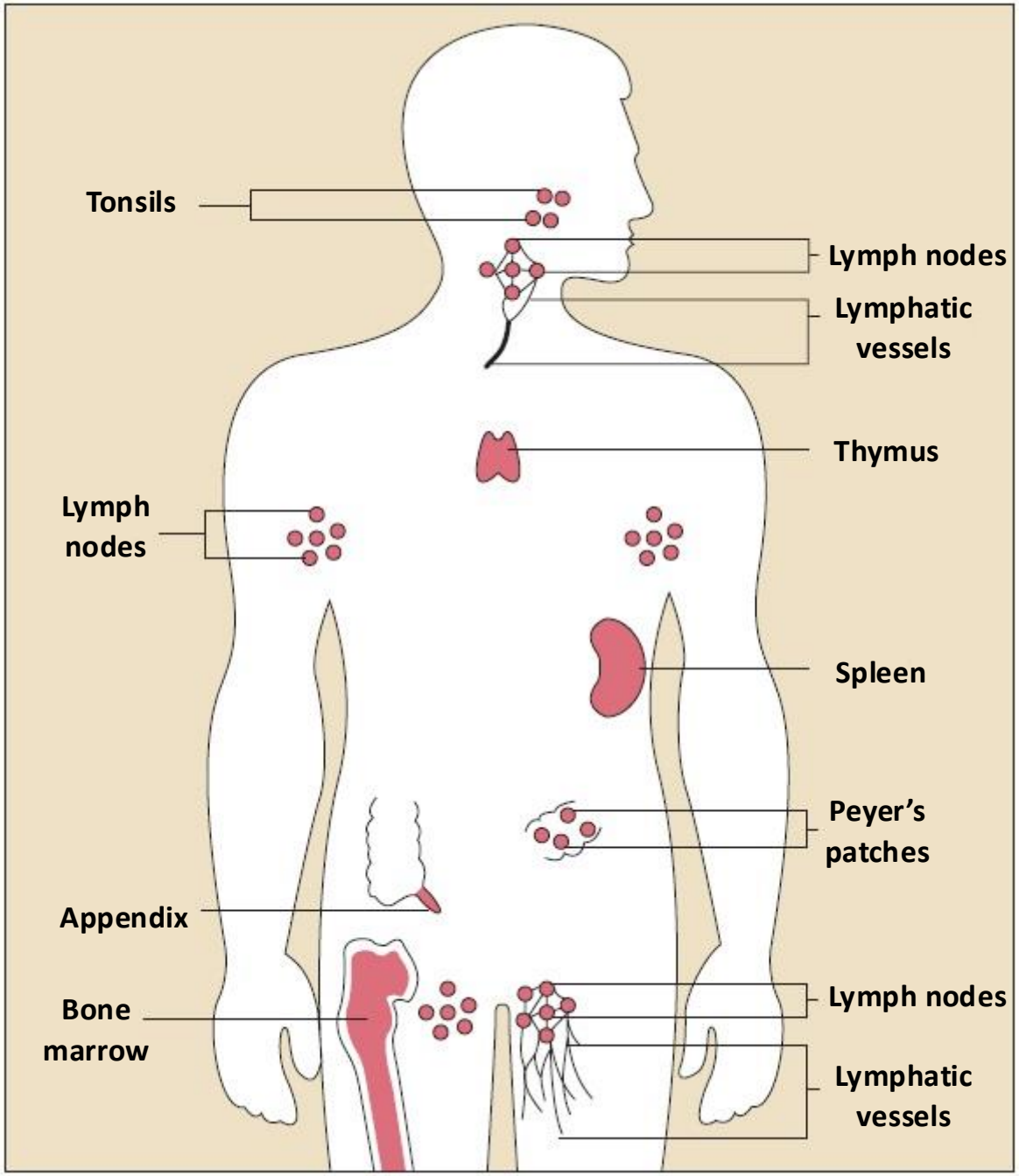
Innate: „The cell surface is full of carbohydrates usually found on bacteria - it must be some kind of bacteria."


E. coli

Adaptive: „This is the 45-60 amino acid segment of *E. coli* flagellin."

Organs of the immune system

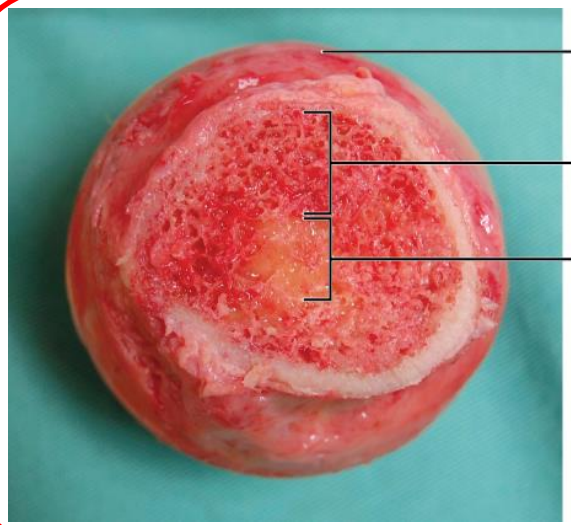
- The immune system is organized into a **network** of cells and organs. (the entire body must be protected from pathogens)
- Lymphoid organs:
  - Primary (production of immune cells)
    - **Bone marrow, thymus**, embryonic liver (+bursa of Fabricius in birds [nomenclature: „B" lymphocytes originating from the bursa and „T" cells from the thymus[1.]])
  - Secondary (site of antigen recognition, immune response)
    - **Lymph nodes, spleen, MALT** (mucosa-associated lymphoid tissue), **SALT** (skin-associated lymphatic tissue)
  - Tertiary (pathological, usually due to chronic inflammation)
    - E.g. ectopic (=at an abnormal site) lymphoid follicles

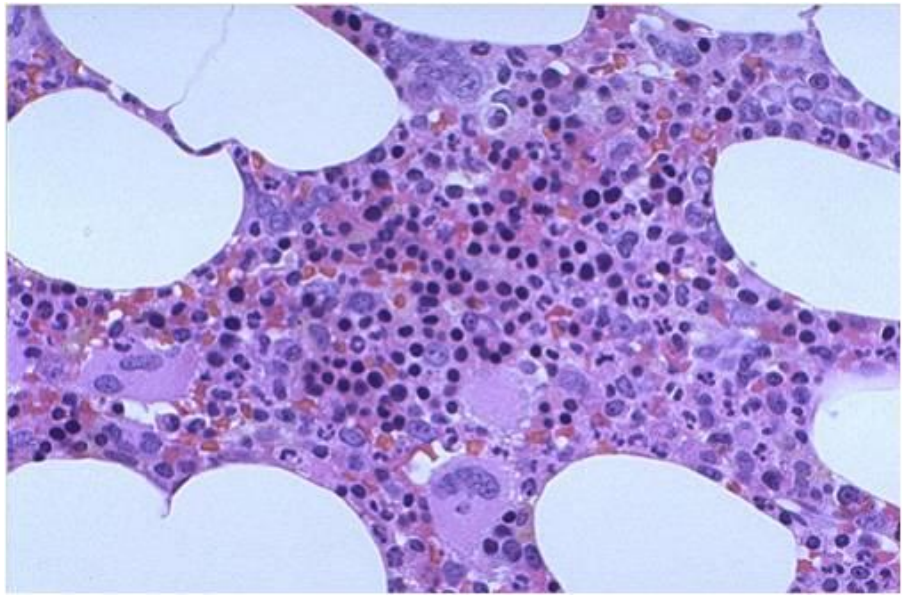

Tonsils
Lymph nodes
Lymphatic vessels
Thymus
Lymph nodes
Spleen
Peyer's patches
Appendix
Bone marrow
Lymph nodes
Lymphatic vessels

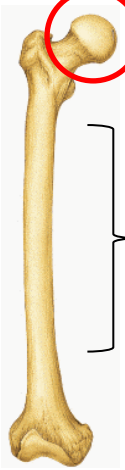
Bone marrow (medulla ossium)

- Spongiform tissue found within bones which constitutes 4-5% of the total body weight in adults. (≈2,6 kg)[2.]
- Red bone marrow (medulla ossium rubra):
  - Found **in short and flat bones** (sternum, ribs, clavicle, scapula, pelvis, vertebrae, skull) and the **epiphysis of long bones** (e.g. femur)
  - Role: **Producing blood cells** (hematopoiesis) → 10^{11} new cells daily of neutrophils alone[3.] (the human body is made of approx. $3{,}7 \times 10^{13}$ cells)[4.]
- Yellow bone marrow (medulla ossium flava):
  - Found in the diaphysis of long bones
  - Mainly composed of adipocytes, can turn into red bone marrow when needed (e.g. after blood loss)

Structure of the red bone marrow



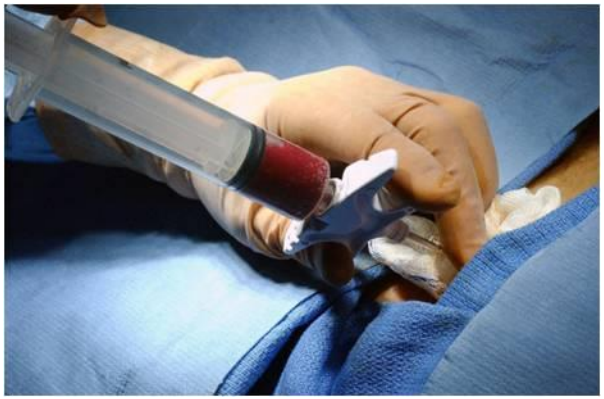


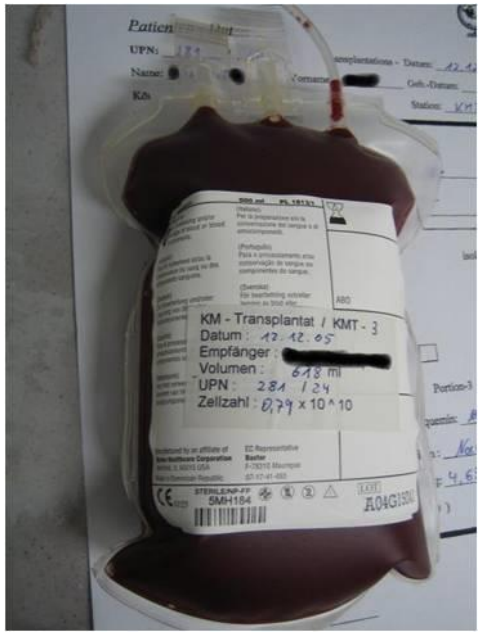


- Spongy bone tissue with sinusoids, spaces are filled with cells of various lineages undergoing hematopoiesis (see later), stromal cells and adipocytes.[2.]
- Mature and naive B cells leave the bone marrow, whereas T cells produced by the bone marrow are still immature and must undergo further maturation in the thymus.
- **Mature**: capable of recognizing an antigen
- **Naive:** haven't yet encountered an antigen

Clinical significance of the bone marrow

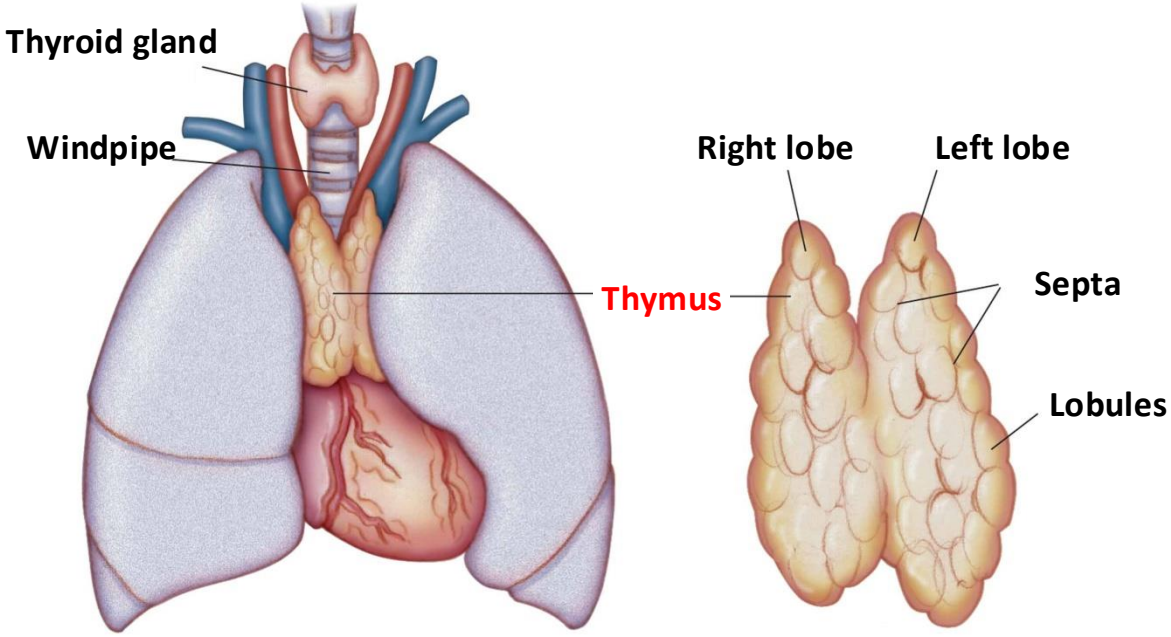
- Bone marrow biopsy or aspiration for histological or cytological assessment in case of hematological diseases (e.g. leukemias, aplastic anemia, etc.)
  - Performed from: **iliac crest** or **sternum**[5.]
- Collecting hematopoietic stem cells (HSC) to perform bone marrow transplantation
  - Usually gathered from the peripheral blood after cell mobilization[6.]

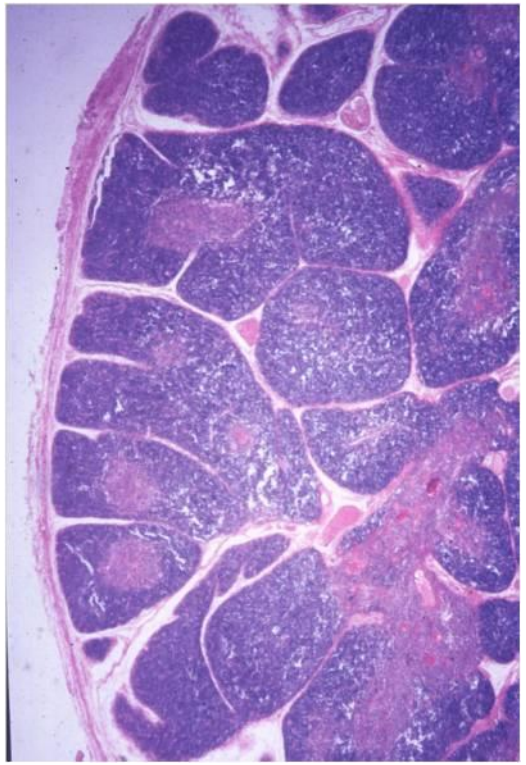




Thymus

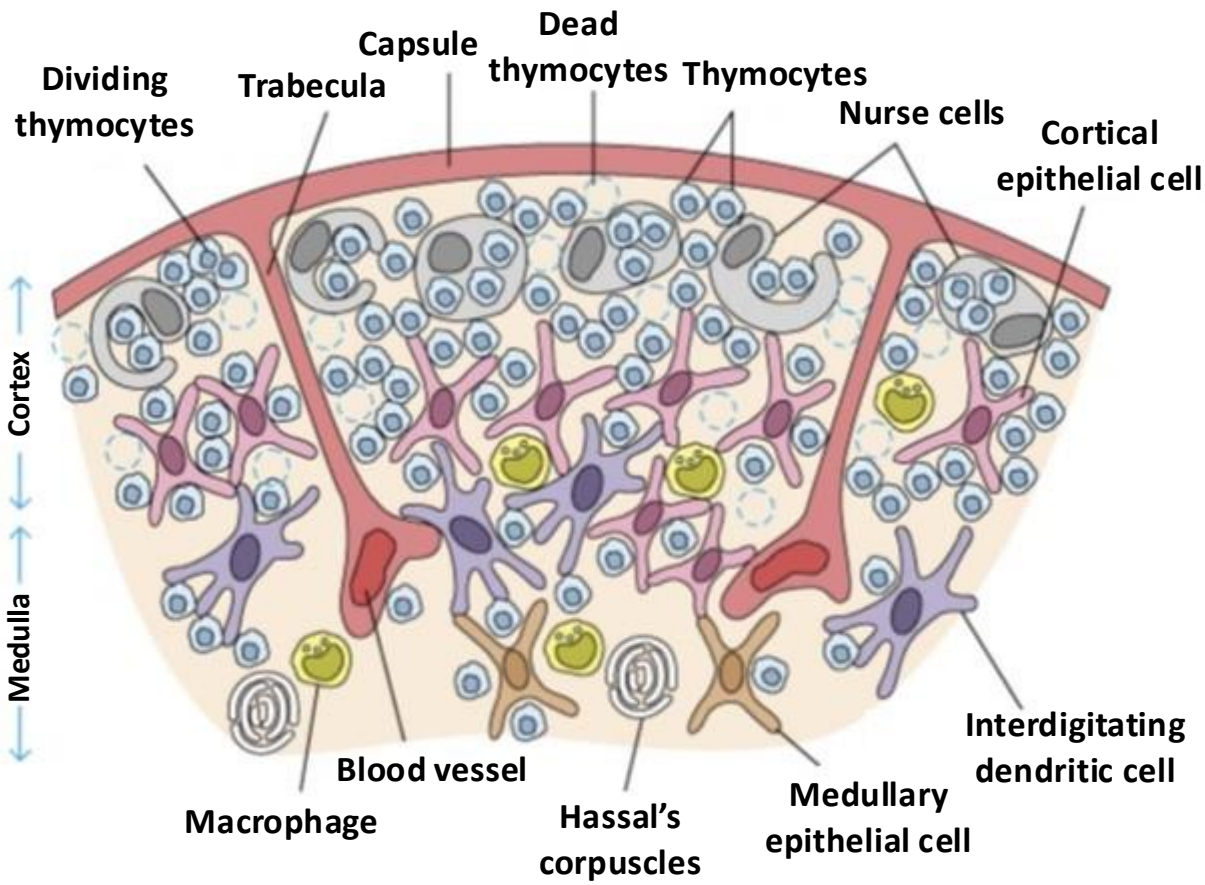
- A lobulated organ located in the superior mediastinum, it is the **primary site of T cell maturation**.
- Consists of **2 lobes** further divided into **lobules** separated by connective tissue septa. The inner layer of the lobules is called **medulla**, the peripheral layer is the **cortex**.

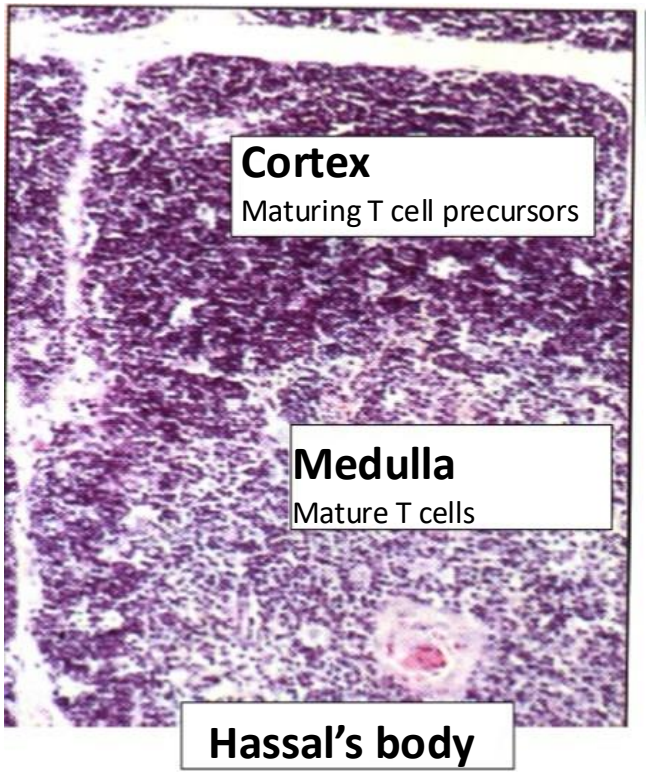




Thymus (H&E staining): the peripheral, basophilic layer is the cortex. The inner medulla seems more eosinophilic because it contains less cell nuclei.

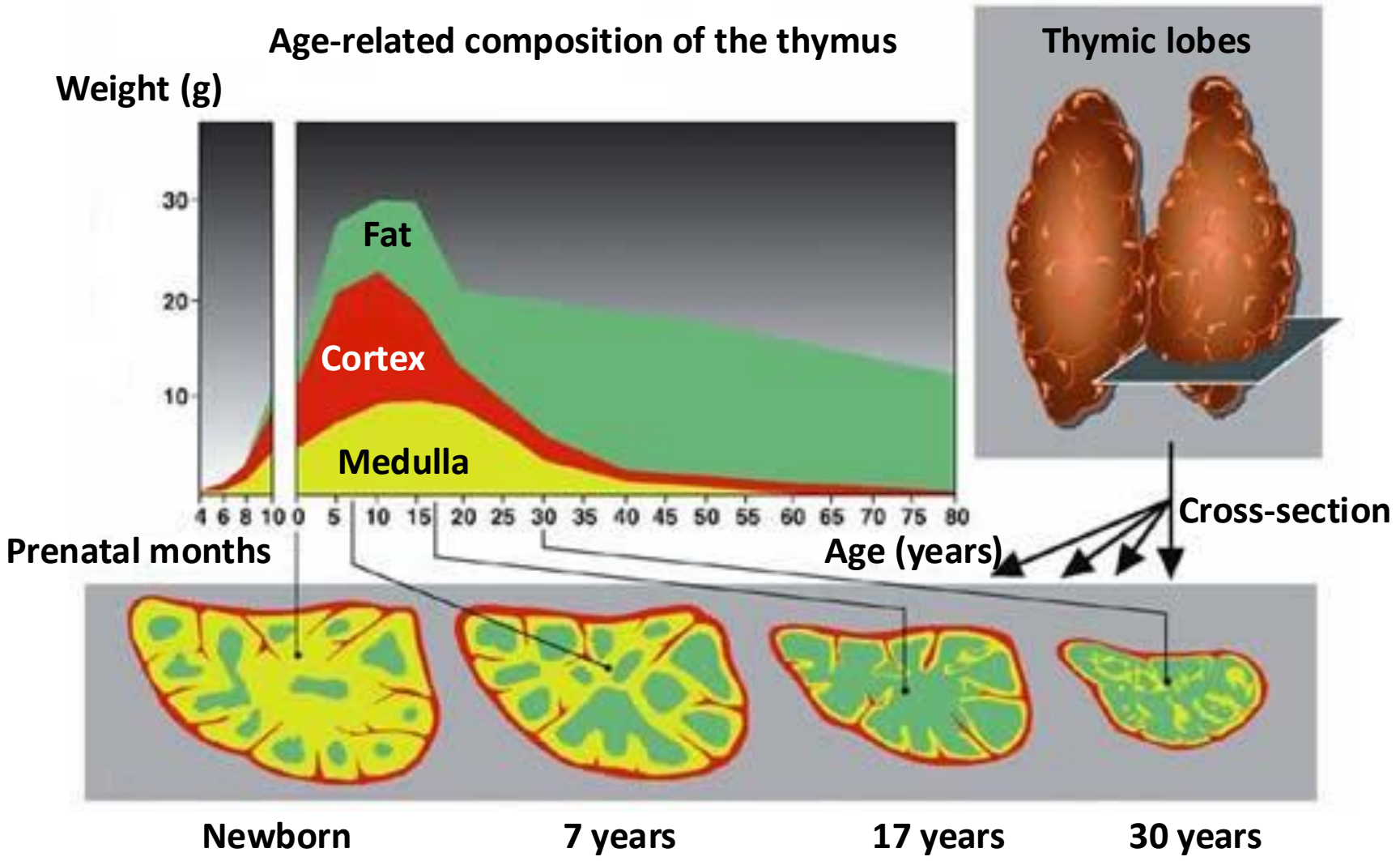
Histology of the thymus





- T-cell precursors (=immature cells) produced by the bone marrow enter the thymus through blood vessels → **MATURATION** (see later) → Mature and naive T cells leave the thymus and enter circulation
- **Main cellular components of the thymus: T cells (thymocytes), thymus epithelial cells, dendritic cells, macrophages, epithelioreticular cells**[7.]

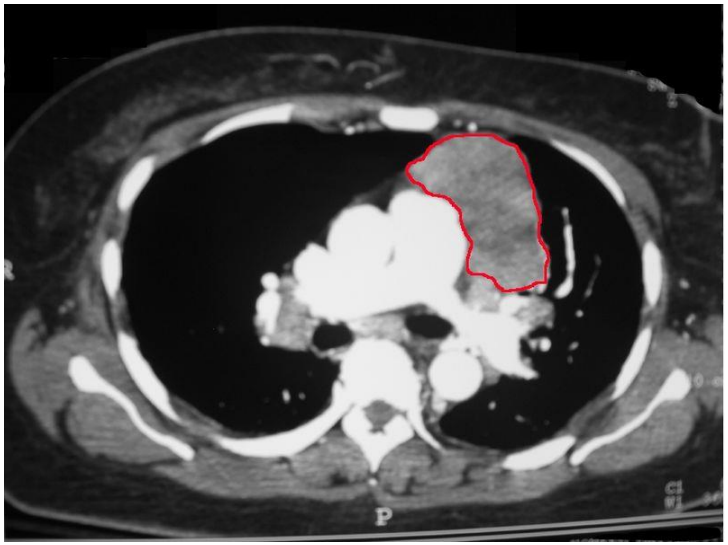
Involution of the thymus



Clinical significance of the thymus

- Congenital abnormalities (e.g. ectopic thymus or thymic aplasia [=absence of thymus] for instance in DiGeorge syndrome → **immunodeficiency**)

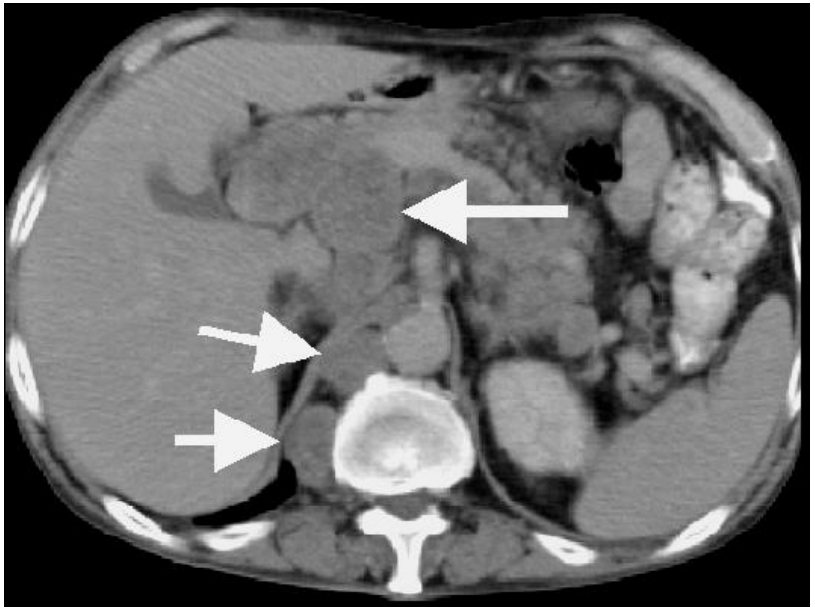
- Tumors (thymoma, thymus carcinoma)[8.]
  - May be associated with autoimmune disorders such as myasthenia gravis (see later)
  - Might compress nearby structures (e.g. superior vena cava syndrome, dysphagia, see later in the clinical phase of your studies)



Thoracic CT angiography (dye seen in blood vessels): The red line marks a thoracic mass later confirmed to be a thymoma by histological evaluation.

Lymph node (nodus lymphaticus)

- They act as **filters of the lymph:** lymph reaching the node through the afferent lymphatic vessels is filtered for **pathogens** and **cancer cells**. (one of the organs where the adaptive immune cells can meet with antigens the first time)
- This is the place where the antigens that entered the lymphatic system will be **recognized** by the adaptive immune cells followed by cell **proliferation** and **differentiation**.
- **Tremendous clinical significance**: Infectious agents and cancer cells may spread via the lymphatic system.

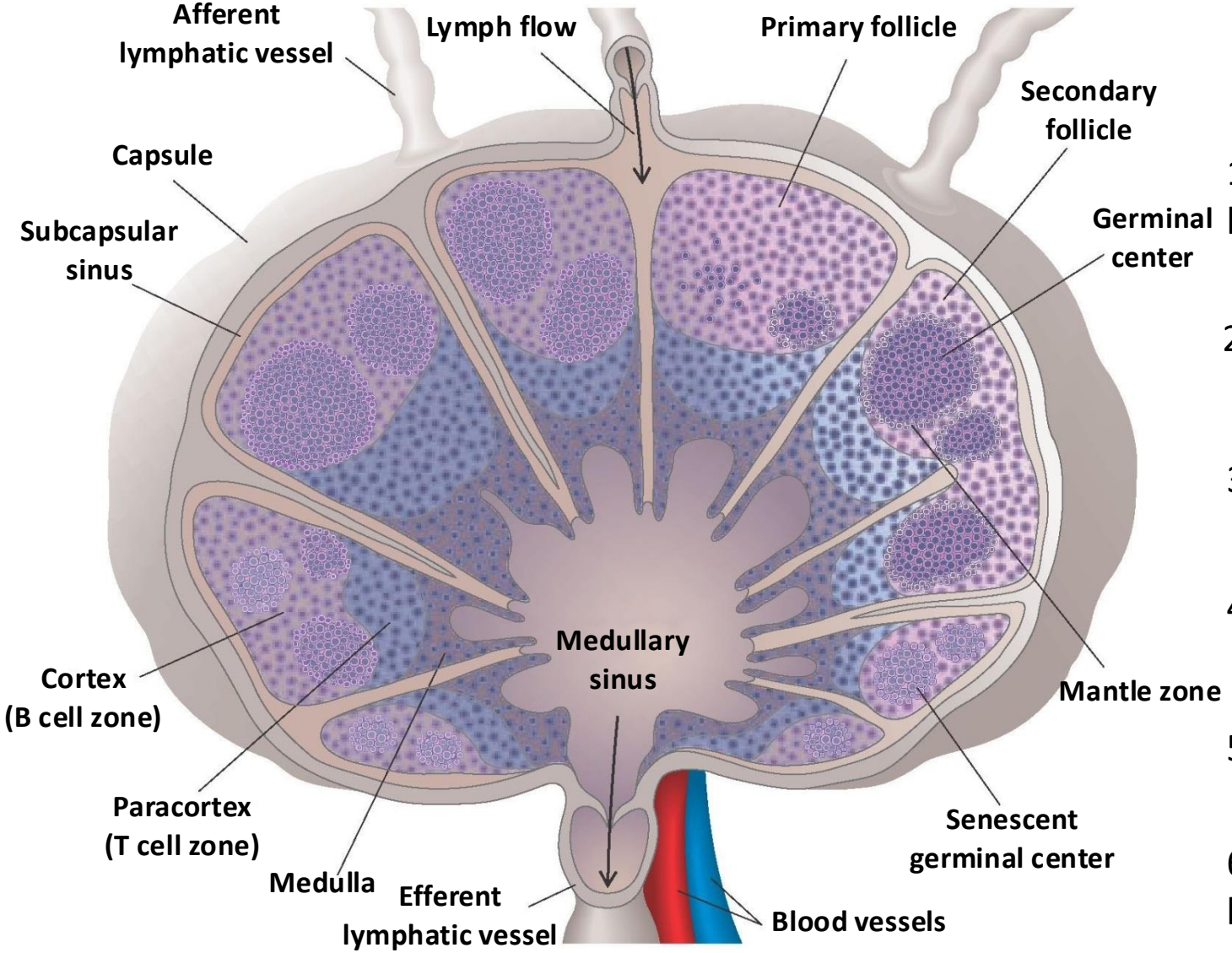


Retroperitoneal lymphadenomegaly (=enlarged lymph nodes) seen on a CT scan image. Arrows mark enlarged lymph nodes.

Structure of lymph nodes 1.

- Have outer fibrous capsule from which trabeculae radiate towards the inner part of the organ.
- Layers from outermost to innermost: **cortex**, **paracortex** and the **medulla**.
- Afferent lymphatic vessels enter through the convex surface; the efferent lymphatic vessels and blood vessels (artery and venule) are located at the hilum.
- Reticular connective tissue forms the frameworks of the lymph nodes.
- Sites where immune cells enter:
  - From the bloodstream: **high endothelial venules** (HEV)
  - From the lymphatic system: afferent lymphatic vessels
- Cellular zones:[9.]
  - Cortex: **B cells** organized into **follicles**, cells that recognized an antigen proliferate and form germinal centers
  - Paracortex: **T cells** and **dendritic cells** diffusely
  - Medulla: mainly antibody-producing **plasma cells**

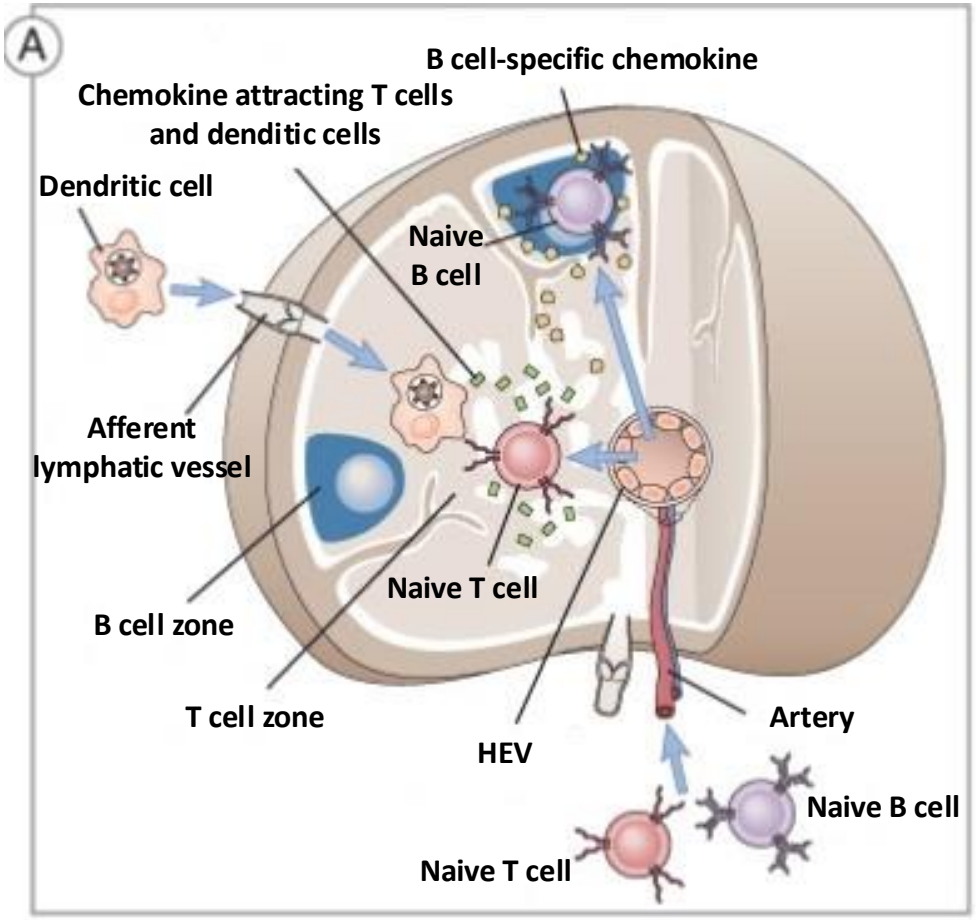
Structure of lymph nodes 2.



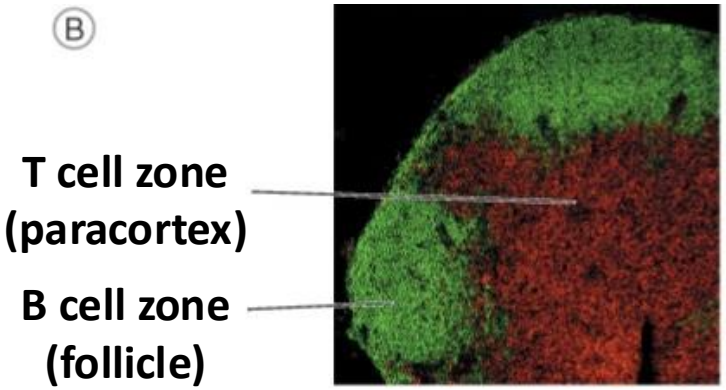
Route of lymph (covered with endothelial cells):

1. Afferent lymphatic vessel
2. Subcapsular sinus
3. Cortical sinus
4. Paracortical sinus
5. Medullary sinus
6. Efferent lympahtic vessel

Structure of lymph nodes 3.

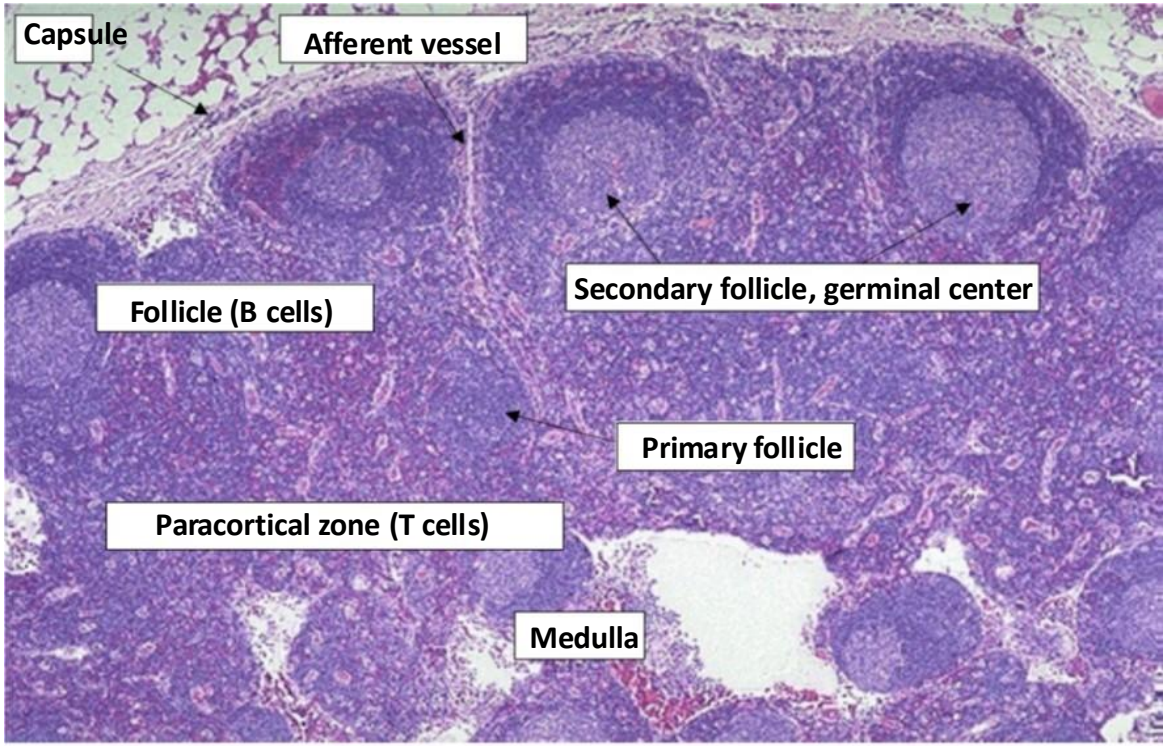


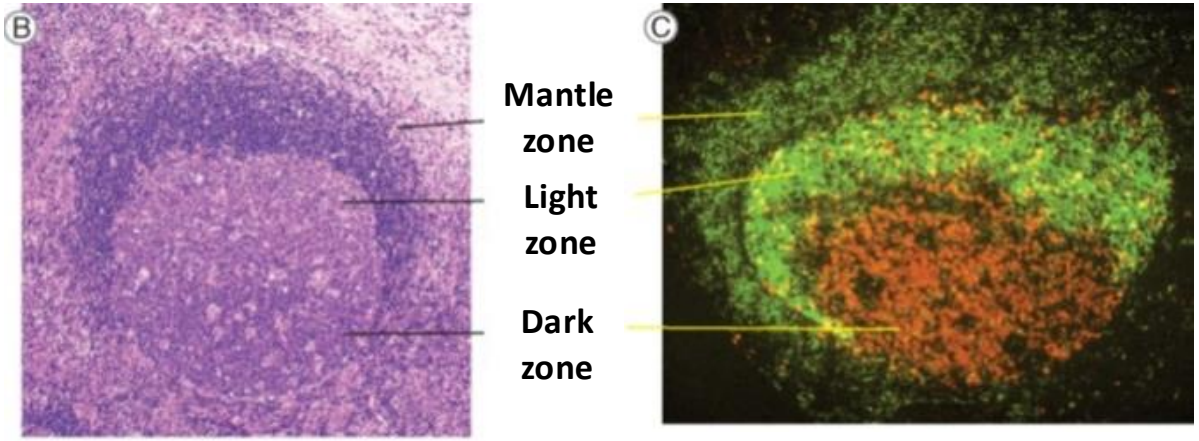
The cellular organization is controlled by **chemokines**. (see later in lectures)



Immunofluorescence microscopy (see later)

Lymphoid follicle (folliculus lymphaticus)





Main cellular components:

B cells, macrophages, **follicular helper T cells**, **follicular dendritic cells** (FDC)

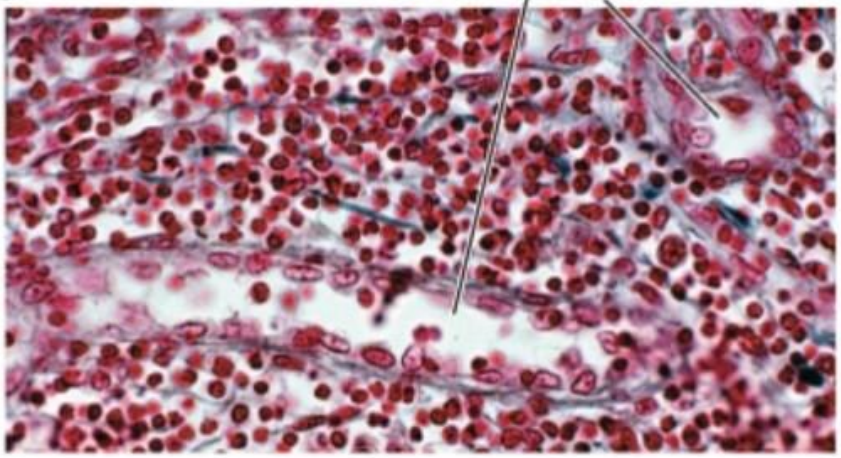
1. **Primary follicle**:
Naive B cells that haven't yet met with an antigen

2. **Secondary follicle** (germinal center):[9.]

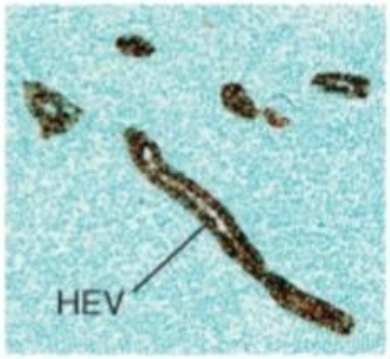
- Dark zone: **centroblasts** (proliferating B cells)
- Light zone: **centrocytes** (B cells undergoing antigen-dependent maturation, see later)
- Mantle zone: transient B cells (=passing through)

High endothelial venules (HEV)

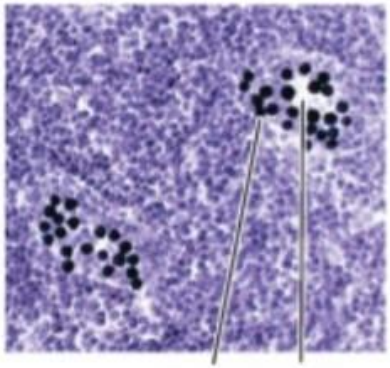
A HEVs in a lymph node



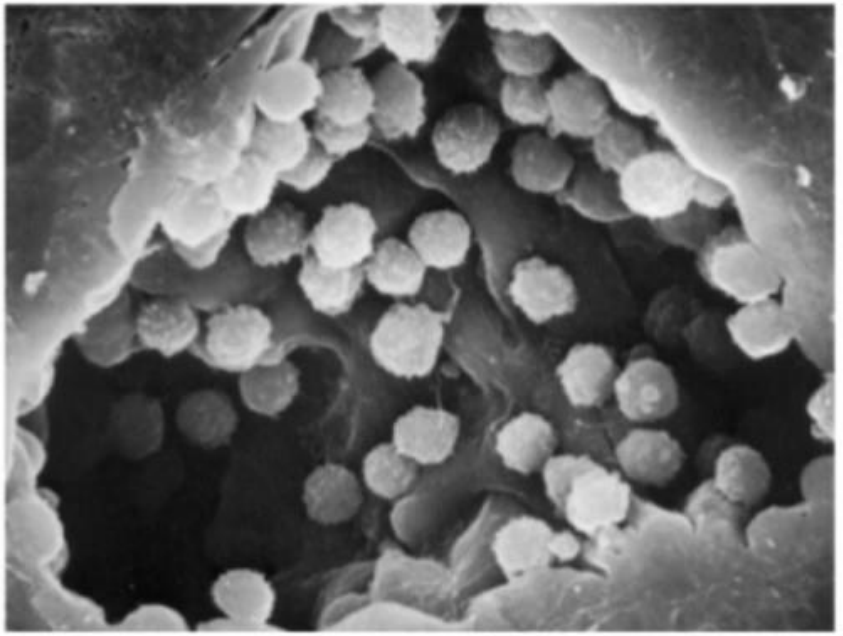
B L-selectin ligand on endothelial cells (IHC)



C T cells binding to HEV (frozen section assay)

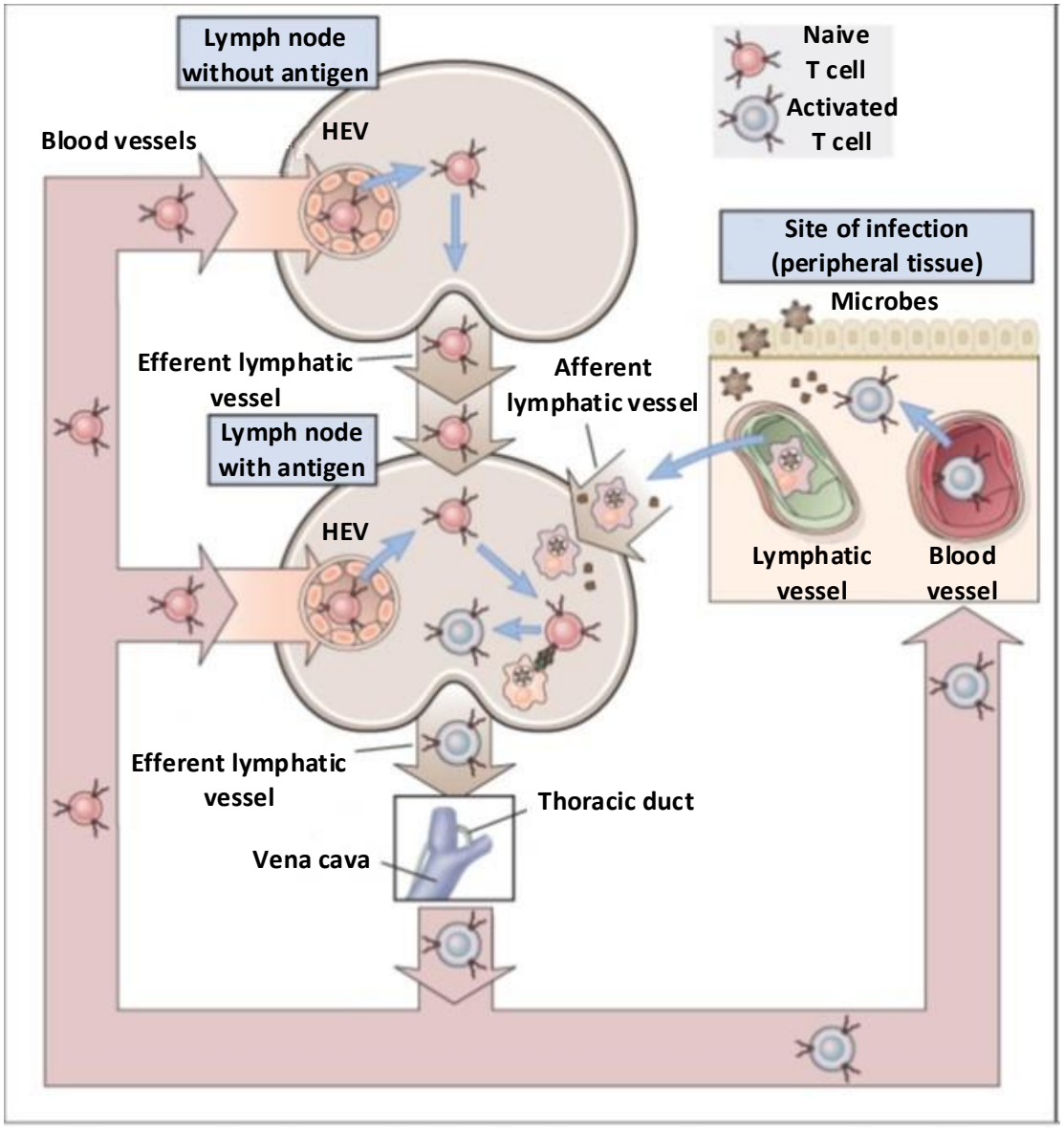


D T cells binding to the luminal surface of a HEV (electron microscopy image)

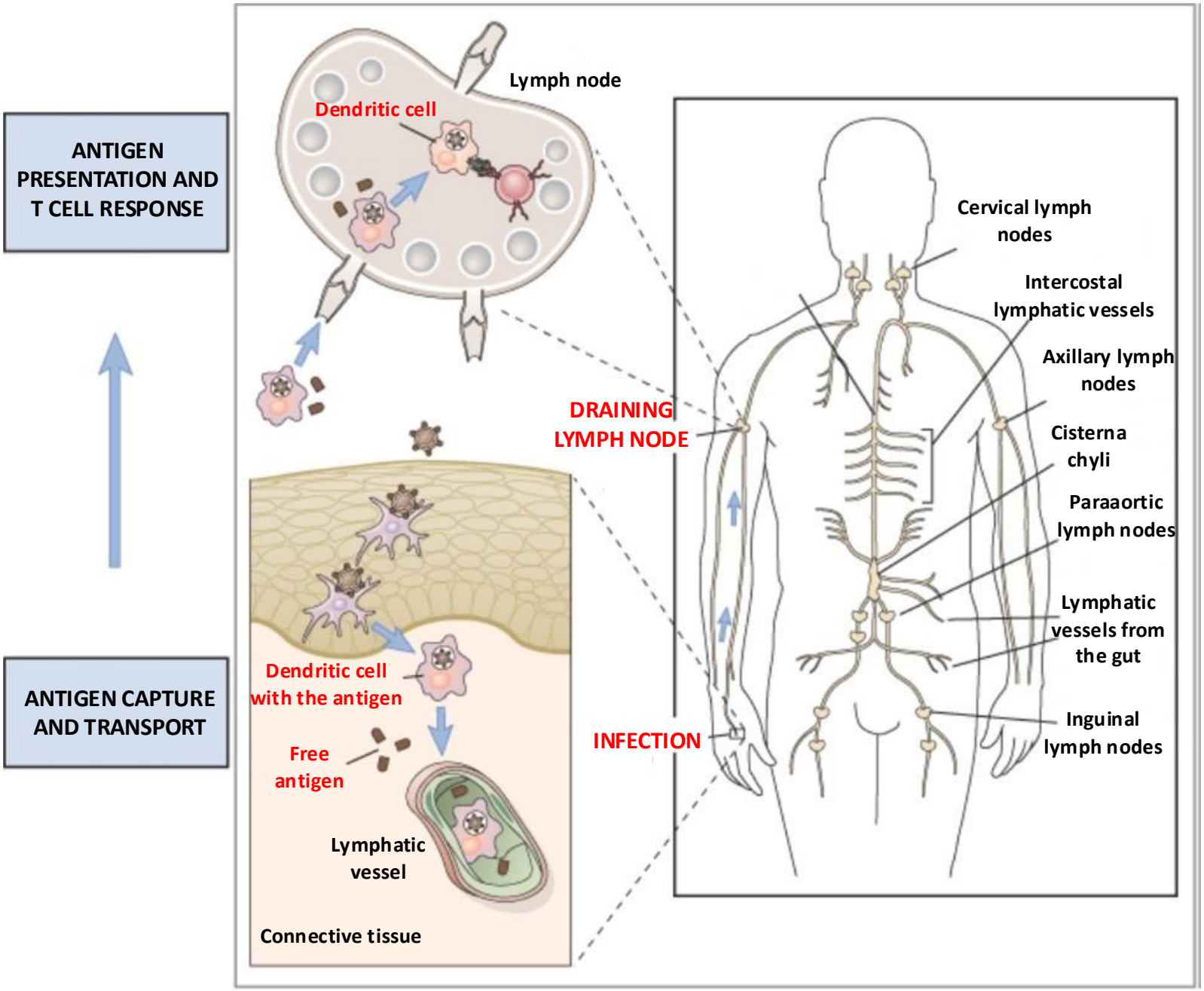


- Lymphocytes use HEVs to enter lymphoid organs. (through L-selectin, see later)
- Found in all secondary lymphoid organs (e.g. lymph nodes, tonsils, Peyer's patches), **EXCEPT THE SPLEEN**[10.]

Filtration of lymph by nodes 1.

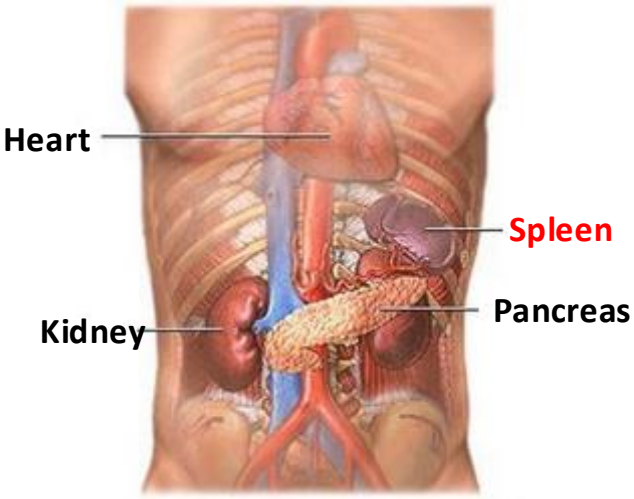


1. Infection on the periphery
2. The same antigen may enter the **lymphatic vessels** in different forms:
   - **Native bound antigen** (e.g. living bacteria)
   - **Native soluble form** (e.g. proteins derived from dead bacteria)
   - **Processed form: dendritic cells** phagocytose the antigen and **present it** as a peptide to **helper T cells** (see later)
3. Lymphocytes enter lymph nodes either through **afferent lymph vessels** or **HEV**s and meet with the antigens (see the lectures for more details)


ANTIGEN PRESENTATION AND T CELL RESPONSE
ANTIGEN CAPTURE AND TRANSPORT
Lymph node
Dendritic cell
DRAINING LYMPH NODE
Cervical lymph nodes
Intercostal lymphatic vessels
Axillary lymph nodes
Cisterna chyli
Paraaortic lymph nodes
Lymphatic vessels from the gut
Inguinal lymph nodes
INFECTION
Dendritic cell with the antigen
Free antigen
Lymphatic vessel
Connective tissue

Spleen (lien or splen)

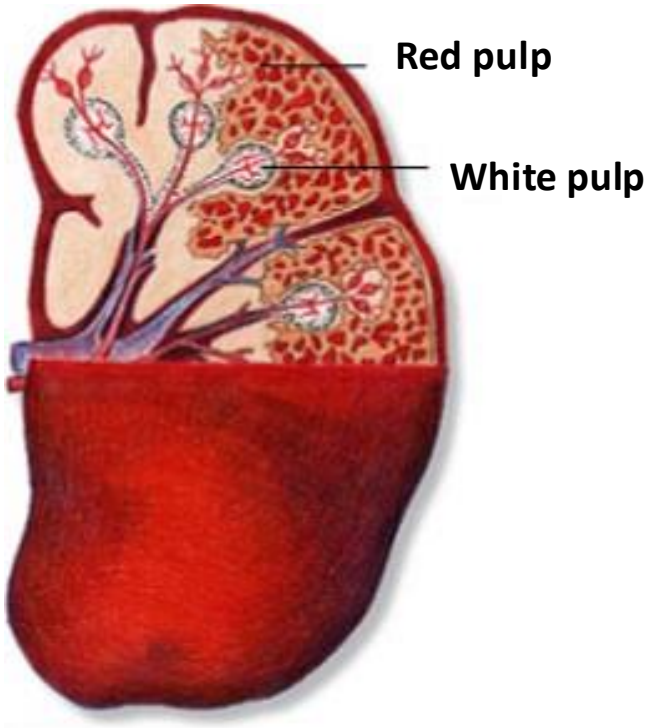
- Located in the left hypochondriac region of the abdomen, weighs approx. 150-200 grams.
- Functions:
  - Immunological: **filtering the blood** for pathogens
  - Hemoglobin metabolism: elimination of aged red blood cells by the reticuloendothelial cells→ formation of bilirubin
  - Site of hematopoiesis in the embryo as in the liver (can produce red blood cells in pathological conditions even in adults)
  - Acts as a storage of red blood cells and platelets (less significant in humans)



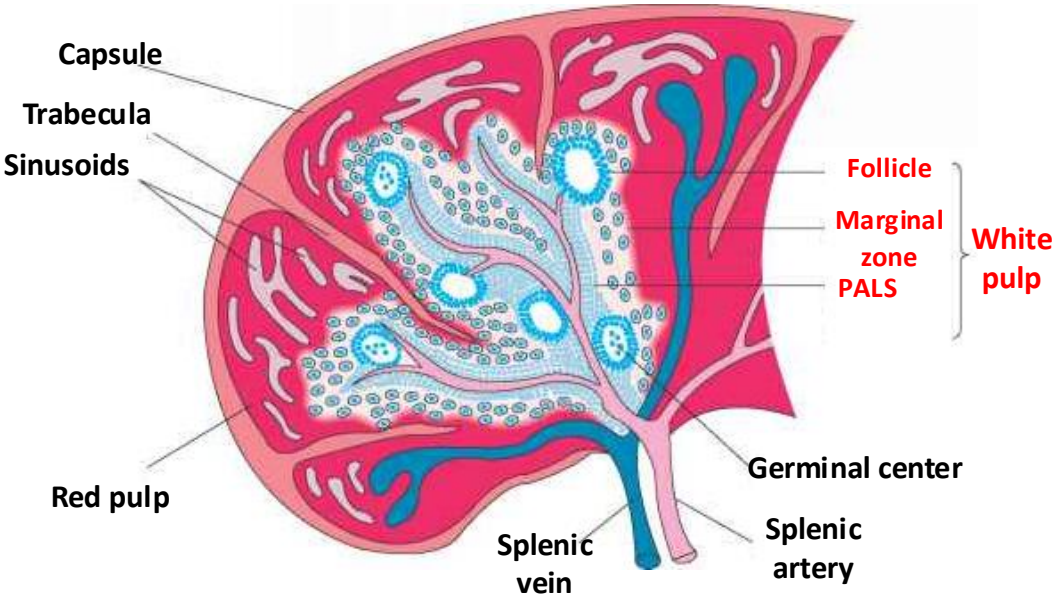
Structure of the spleen 1.

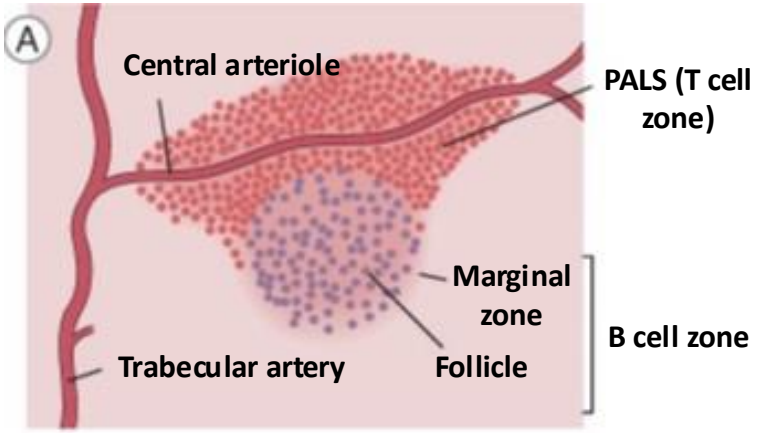
- Has a fibrous capsule and trabeculae.
- **THERE ARE NO** lymphatic vessels and HEVs.
- Tissue architecture:[11.]
  - Red pulp: sinusoids with an open circulation filled with blood: has a reticular framework populated mainly by red blood cells, macrophages, plasma cells and reticular fibrocytes.
  - **White pulp: lymphoid tissue**
    - **PALS** (periarteriolar lymphatic sheath):

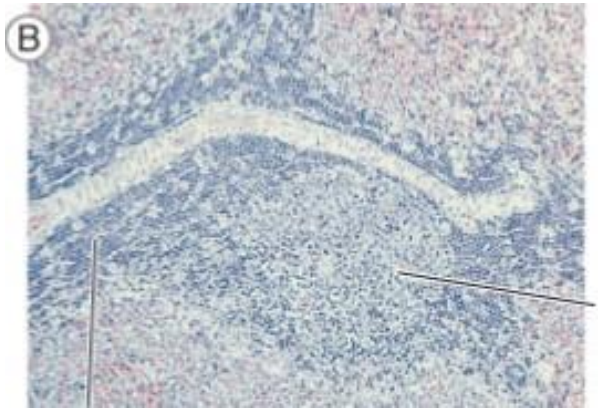
      T cells, dendritic cells
    - **Follicles** (Malpighian follicles)**: B cells and follicular dendritic cells (FDC)**
    - **Marginal zone:** special, **marginal zone B cells** (**MZB**, see later) and **MZ macrophages**



Structure of the spleen 2.

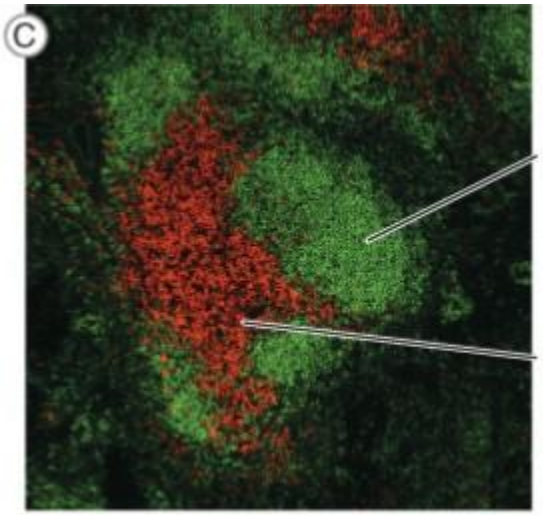






PALS (periarteriolar lymphatic sheath)

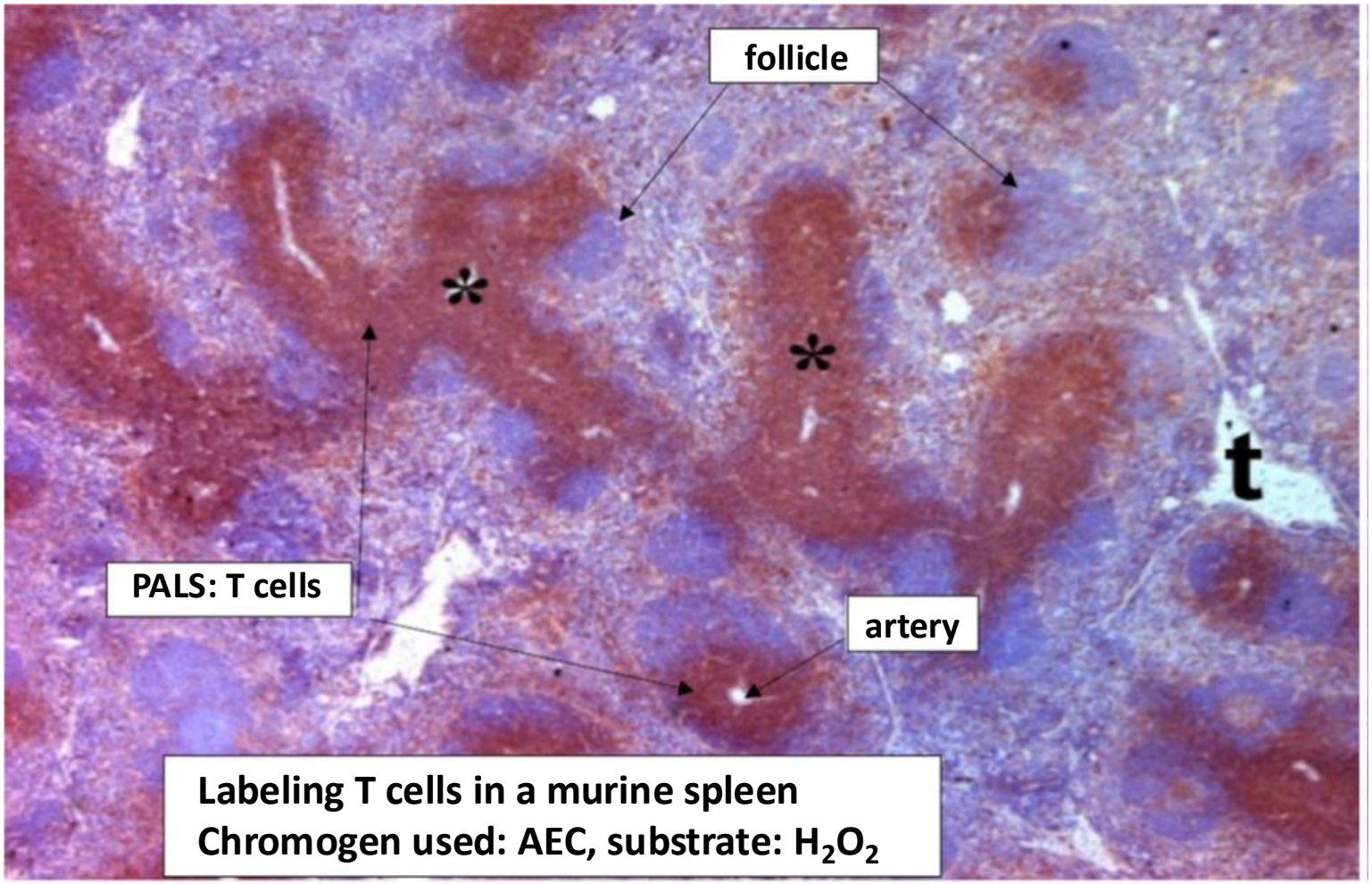
Germinal center (secondary follicle also called Malpighian follicle in the spleen)



B cell zone (follicle)

T cell zone (PALS)

White pulp with immunohistochemistry



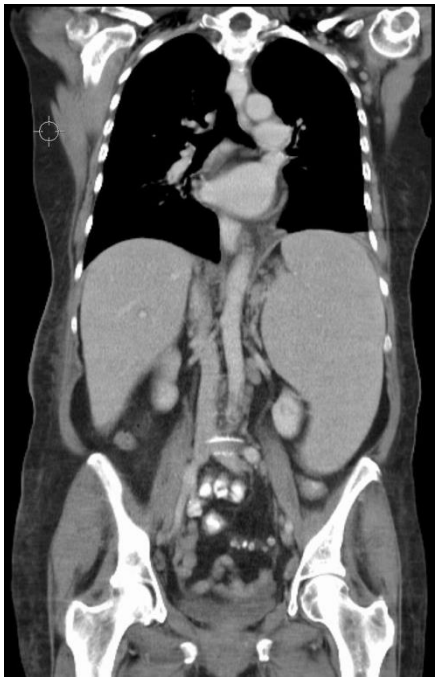
Clinical significance of the spleen

- Splenomegaly (=enlarged spleen):

  Can have several causes such as hematological malignancies, hypersplenism (e.g. hemolytic anemia), increased pressure in the portal veins (cirrhosis), infections (mononucleosis, malaria), storage diseases[12.]
- Splenic rupture (ruptura lienis):

  Caused by trauma or an underlying pathological condition, high risk of intra-abdominal hemorrhage
- Splenectomy (=surgical removal of the spleen):

  Leads to increased vulnerability to polysaccharide encapsulated bacteria (see later)[13.]



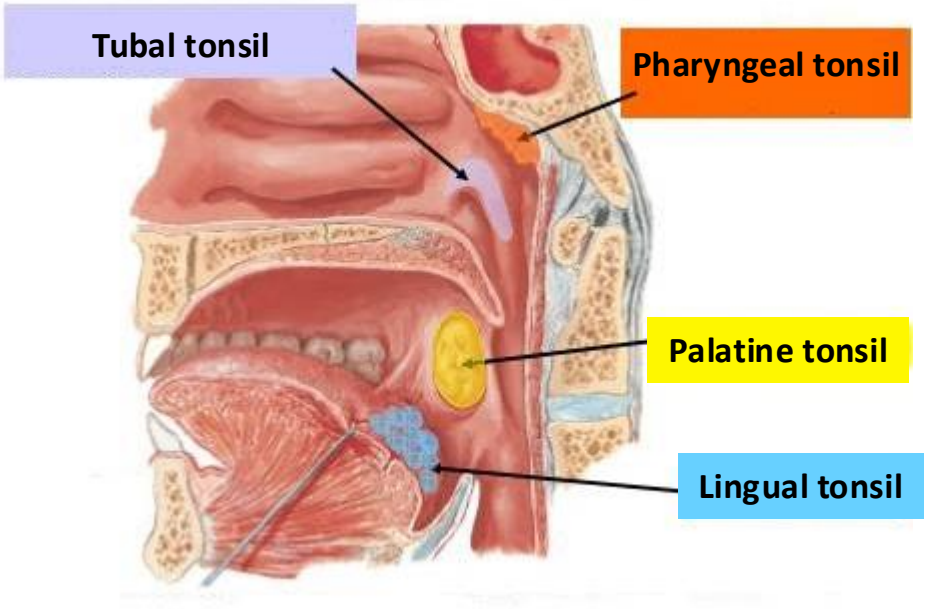
CT scan of a patient with chronic lymphocytic leukemia (CLL) showing massive splenomegaly.

MALT (mucosa-associated lymphoid tissue)

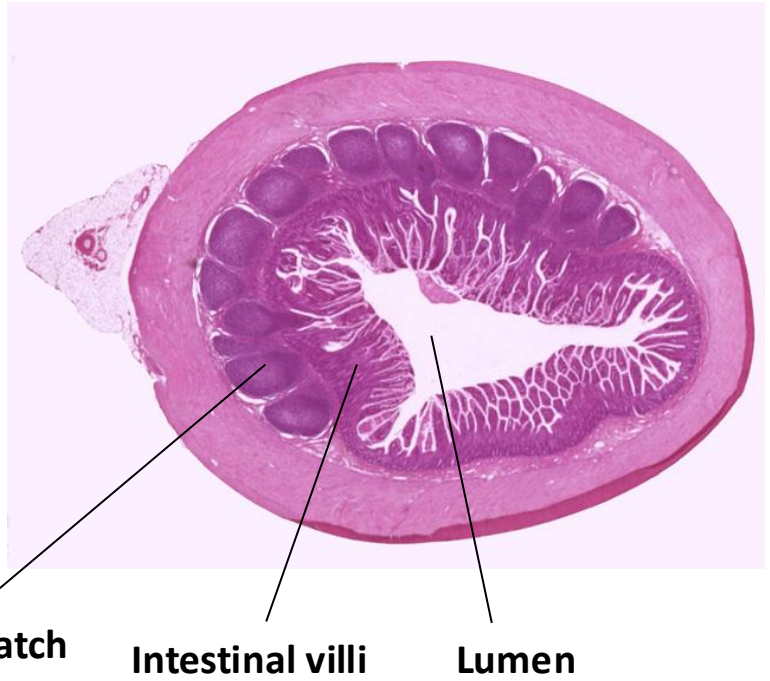
- Mucosa = **enormous surface** for the pathogens to enter the body!
- MALT = The **biggest lymphoid tissue**.
- MALT: can be further classified based on location:[14.]
  - GALT (gut-associated lymphoid tissue) – largest, different from other MALT
  - BALT (bronchus-associated lymphoid tissue)
  - NALT (nasopharynx-associated lymphoid tissue)
- Organized MALT (site of antigen recognition):
  - **Lymphoid cells form organized structures** such as follicles (e.g. tonsils of the Waldeyer-ring, Peyer's patches, cryptopatches, isolated follicles, see in the lectures)
- Diffuse MALT (has effector functions):
  - **Lymphocytes diffusely scattered** in the epithelial layer and lamina propria of mucosal surfaces (IEL=intraepithelial lymphocyte)

Organized MALT

Waldeyer-ring (tonsils):



Peyer's patches in the ileum (H&E, cross-section):

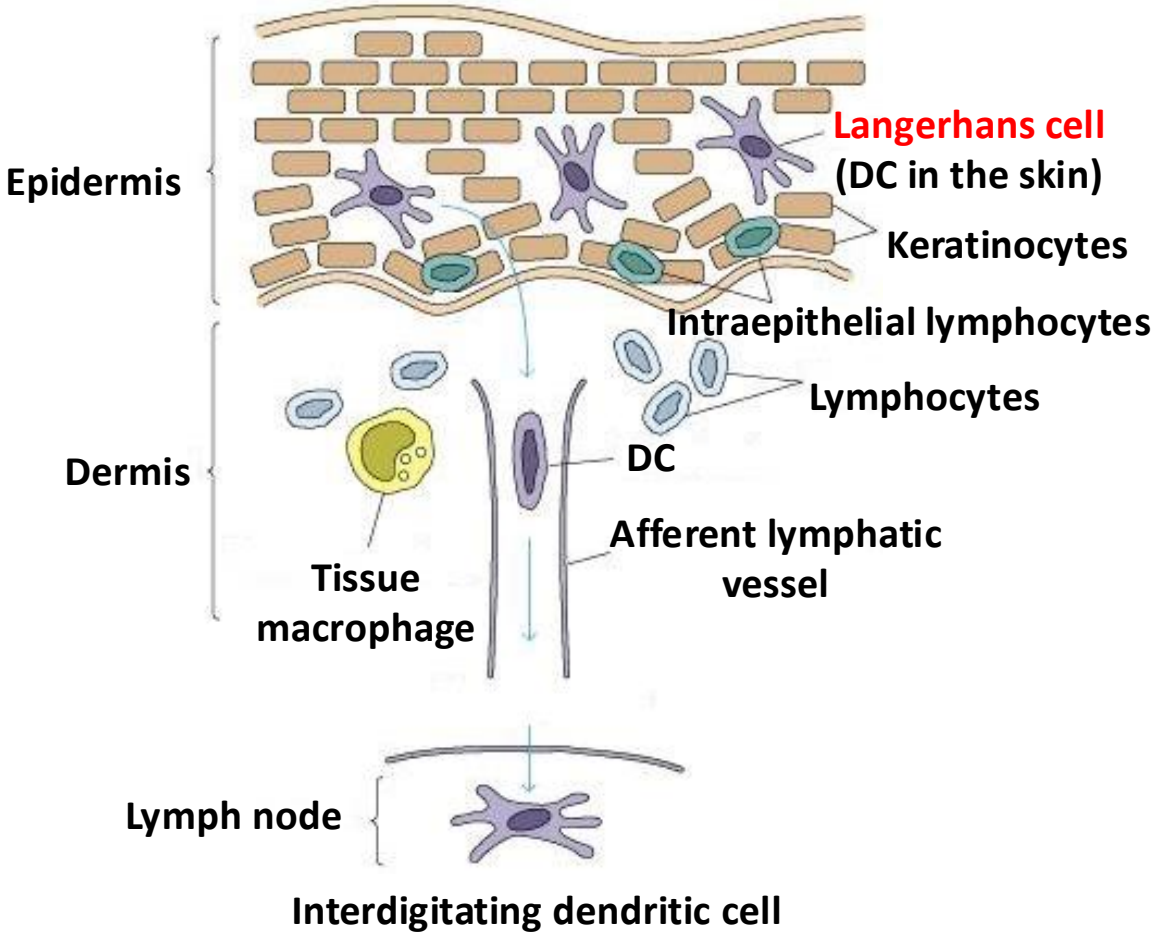


Both tonsils and Peyer's patches have tissue architecture similar to that of lymph nodes (follicles with B cells, separated T cell zones, HEVs), but unlike lymph nodes **they do not have fibrous capsules**.

SALT (skin-associated lymphoid tissue)

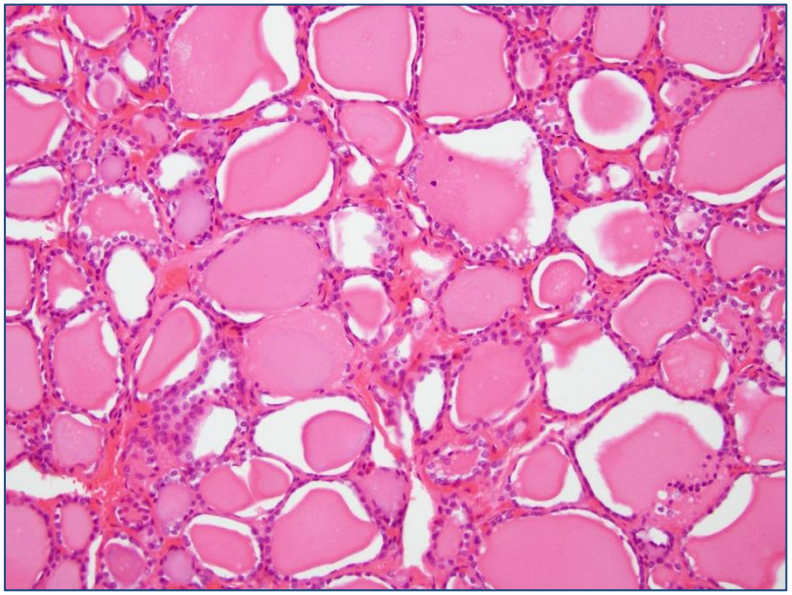
Langerhans cells capture the antigen in the epidermis, then process it and move to the draining lymph node through lymphatic vessels. In the lymph node **they present the processed antigen** to helper T cells.[15.]

Several cell types participate in the immunological defense of the skin. (e.g. keratinocytes, macrophages, γδ T cells, see later)

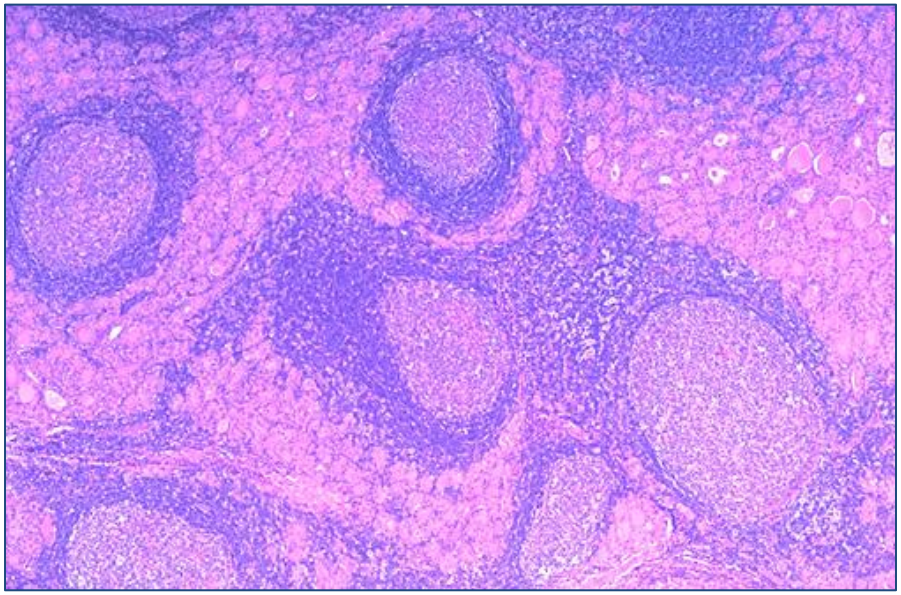


Example for tertiary lymphatic tissue

IT IS PATHOLOGICAL!



Healthy thyroid tissue
(medium magnification)



Ectopic lymphoid follicles in the thyroid gland in Hashimoto's thyreoiditis
(small magnification)

Thank you for your attention!

- Feedback both from students and teachers is highly appreciated, you may send your remarks via email to:

  kohl.zoltan@pte.hu

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