Histology - HLS

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Lymphatic System

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Spleen
✓ The spleen is an oval-shaped intraperitoneal organ
✓ Approximately 5 inches in height (12-13 cm)
✓ 3 inches in width (7-8 cm)
✓ 1 inch in thickness (2.5 cm)
✓ Weighs 7 ounces (200 gm)
✓ Lies under ribs 9 to 11
✓ Has a notched anterior border.

Functions
✓ Filtration of blood (defense against blood-borne antigens)
✓ The main site of old RBCs destruction.
✓ Production site of antibodies and activated lymphocytes (which are delivered directly into the blood)

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Don’t memorize numbers
The **splenic artery** is the largest branch of the celiac artery. It has a tortuous course as it runs along the upper border of the pancreas. The splenic artery then divides into about six branches, which enter the spleen at the hilum and here it divides into many branches to supply oxygenated blood into spleen.

The **splenic artery** supplies the spleen as well as large parts of the stomach and pancreas.
The **splenic vein** leaves the hilum and runs behind the tail and the body of the pancreas. Behind the neck of the pancreas, the splenic vein joins the superior mesenteric vein to form the portal vein *(portal vein enters the liver through porta hepatis)*.

In cases of portal hypertension, spleen often enlarges from venous congestion.
The parenchyma of the spleen appears in fresh specimen as:

**White pulp** which appears white on gross examination (collection of both B and T lymphocytes)

**Red pulp** which appears red on gross examination (blood filled)

Stroma of spleen is formed by reticular tissue

Condensation of WBCs in particular lymphocytes
The spleen is covered by a **capsule** of dense connective tissue, and have capsular extensions called the **trabeculae** (to divide the spleen into smaller compartments)

Large trabeculae originate at the hilum, on the medial surface of the spleen, and carry branches of the splenic artery, vein, lymphatics, and nerves into the spleen

The spleen is composed of parenchyma and stroma

**Parenchyma:** Splenic pulps

**Stroma:** Reticular tissue (reticular fibers and reticular cells)

There are two types of pulp in the spleen:

- **Red pulp** (rich in blood)
- **White pulp** (lymphatic tissue)

Unlike lymph nodes, the spleen:

1. Has no afferent lymphatics
2. Has no lymphatic sinus system
3. Its lymphatic tissue is not arranged into cortex and medulla
Splenic artery
Divides into trabecular arteries as it enters the hilum

Trabecular arteries
Follow the course of trabeculae

Central arterioles
Are branches of trabecular arteries entering the white pulp. They are surrounded by a sheath of lymphocytes. (Aggregation of T lymphocytes) >> so central arteriole locates at the center of the sheath

Penicillar arterioles  The morphology is like penicillus
Each central arteriole eventually leaves the white pulp and enters the red pulp, losing its sheath of lymphocytes and branching as several short straight penicillar arterioles that continue as terminal capillaries.

Terminal capillaries (Sheathed capillaries)
Some of these terminal capillaries are sheathed with APCs (macrophages) for additional immune surveillance of blood

Blood flow through the splenic red pulp can take either of two routes:

**Open circulation:** the capillaries open into the spaces of the red pulp (splenic cords) and then the blood returns to the venous system through the wall of the splenic sinusoids

**Closed circulation:** the capillaries open directly into the splenic sinusoids (blood is enclosed by endothelium)

Most of the RBCs pass through this route

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White pulp (lymphoid tissue)
✓ Constituting 25% of the spleen, the white pulp is responsible for the immunological (lymphatic) function of the spleen.
✓ The white pulp contains:

**Periarteriolar lymphatic sheaths (PALS):**
- tightly packed T cells arranged in cylindrical sheaths around central arterioles

**Lymphoid follicles:** spherical aggregations of B cells scattered throughout the PALS
- Primary (unstimulated) follicles contain resting (inactive) B cells
- Secondary (stimulated) follicles contain activated B cells in a central region (germinal center)

**Splenic nodules (Malpighian corpuscles)**
- Note: These follicles have the same structural Organization as those found in lymph nodes

**Function:** The lymphocytes and APCs monitor the blood for foreign antigens and respond in a similar way to those in the lymph nodes.

When the lymphatic sheath expands to incorporate the follicles, the central arteriole is displaced to one side and acquires an eccentric position in the follicle but is still called the central arteriole (Follicular arteriole).

If you section the spleen and you see all lymphatic follicles are stimulated so you know that this patient is infected, in particular with an infection caused by blood borne antigens such as capsulated bacteria causing septicemia.

Production of antibodies and activated lymphocytes (which are delivered directly into the blood)
Red pulp (blood filled)
✓ Constituting 75% of the spleen, the red pulp is responsible for the hematological (circulatory) function of the spleen.
✓ The red pulp contains:

**Splenic cords (Billroth’s cords):** consist of all cells between the sinusoids in the red pulp (reticular cells, macrophages, plasma cells, lymphocytes, RBCs, platelets, other leukocytes)

**Splenic sinusoids:** are blood-filled spaces located throughout the red pulp. They have large, dilated, irregular lumens and large pores (spaces between the endothelial cells)

1. The endothelial cells (stave cells) are elongated, fusiform cells that lie parallel to the long axis of the vessel
2. The cells lie side by side around the vessel but not joined by any type of intercellular junctions
3. The endothelial cells are supported by highly discontinuous basal lamina (forms bars and encircles the sinusoid)

**Function:** Destruction of worn-out RBCs and platelets
Note: When B cells in the primary follicles are exposed to Antigen, they proliferate and differentiate to plasma cells and move toward the red pulp.
In this route plasma and all the formed elements of blood must reenter the vasculature by passing through narrow slits between the stave cells into the sinusoids. These small openings present no obstacle to platelets, to the motile leukocytes, or to thin flexible erythrocytes. However, stiff or swollen RBCs at their normal life span of 120 days are blocked from passing between the stave cells and undergo selective removal by macrophages.

Deformed or less pliable RBCs cannot squeeze effectively from the cord into the sinus and upon their mechanical fragmentation are removed by resident macrophages (lie just next to the sinusoids).

Note the wide gaps between endothelial cells which allow for movement of entire cells from cords to sinuses.

- Splenic sinusoid: Large space filled with blood, lined with endothelial cells. These special endothelial cells are arranged longitudinally, and they don’t have cell junctions (desmosomes, tight junctions), and in between these cells we have large intercellular clefts. These endothelial cells are supported by a discontinues layer of basement membrane forming bars, also we have many macrophages that extend their cytoplasmic processes into the lumen to recognize any foreign antigens inside the blood.

- Spaces in between sinusoids where we have blood are called splenic cords which are cords of cells supported by reticular tissue.
Macrophages monitor erythrocytes as they migrate from splenic cords between the endothelial cells into the splenic sinusoids.

Old erythrocytes lose their flexibility, they cannot penetrate the spaces between the endothelial cells and are phagocytosed by macrophages.

Old erythrocytes lose sialic acid from their cell membranes, Galactose exposed, Induce phagocytosis of RBCs.

Hemoglobin is broken into Heme and Globin.

Iron: carried by transferrin to bone marrow (used again)

Bilirubin: excreted by liver bile

As RBC is getting older or abnormal, it will have denaturation of its plasma membrane proteins or submembranous proteins (RBCs don't have organelles, so can't regenerate new proteins), so this old cell will be less flexible (squeezing or twisting is less), it will be slow and undergo membranous fragmentation. Then this cell will be identified by macrophages and getting eaten. We call this test when RBC pass in between the endothelial cells to enter the venous blood twisting or flexibility test.

Fresh and young RBC will pass quickly with high flexibility in between endothelial cells to the venous blood and will not be captured by macrophages.

Some RBCs need more than one circulation to be eliminated (enter the spleen >> not eliminated, then reenter until it will be captured and eliminated).
Schematic view of the blood circulation and the structure of the spleen, from the trabecular artery to the trabecular vein.

The area between white pulp and red pulp is called marginal zone.
Marginal zone sinuses
✓ Located between the white and the red pulp
✓ The spaces between these sinuses are wide (2-3µm)

It is here the blood-borne antigens and particulate matter have their first free access to the parenchyma of the spleen

The following events occur at the marginal zone:

1- APCs sample the material travelling in blood searching for antigens

2- Macrophages attack microorganisms present in the blood

Lymphocytes come into contact with APCs, if they recognize their Ag-MHC complex, the lymphocytes initiate immune response within the white pulp

3- The circulating B and T cells leave the blood stream to enter the preferred location within the white pulp
   T cells: PALS
   B cells: lymphatic follicles

- While the blood is passing through the white pulp it’s going to be screened for any antigens or microbes, if there’s an antigen, APC will phagocytose it presenting it on MHC to T lymphocytes which in order will activate B cells and the B cells will become activated and move in the center of the follicle to form germinal center
- Activated B cells form plasma cells, these plasma cells move from the germinal center (white pulp) to populate inside red pulp (splenic cords)
Functions of the spleen:
It has circulatory as well as lymphatic functions.

Blood cell production: During the fetal life, blood cells are produced in the spleen.

Blood storage: A small quantity of blood is stored in the sinusoids of the red pulp.

RBC destruction: Most worn-out or damaged red blood cells are destroyed in the spleen (some in the liver and bone marrow). They are phagocytized by macrophages.

Defense mechanism:
Macrophages phagocytize microbes that have penetrated the blood. Antigens in the blood activate B and T cells residing in the spleen, triggering immune response.

Production of antibodies and activated lymphocytes (which are delivered directly into the blood).

Remember: Patients who had a splenectomy especially at a young age are more prone to septicemia (sepsis).
Under LM we can't differentiate between T and B cells, so how we differentiate PALS area and lymphoid area under LM?

- If you see in the cross section that the central arteriole is located centrally and surrounded by basophilic nuclei >> PALS area
- But if you see aggregations of Lymphocytes and you find an arteriole peripherally located >> area of lymphatic follicle of white pulp

If we have germinal center it's easy to determine the lymphatic follicle

Refer to the video to identify the structure of spleen and lymph nodes
**Histology of lymph node**

- The outermost layer is adventitia

**Histology of spleen**

- The outermost layer is serosa

<table>
<thead>
<tr>
<th>Lymph node</th>
<th>Spleen</th>
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<tbody>
<tr>
<td>Multiple, small</td>
<td>Single, large</td>
</tr>
<tr>
<td>Along the course of lymphatic vessels</td>
<td>Intra-abdominal</td>
</tr>
<tr>
<td>Filters lymph</td>
<td>Filters blood</td>
</tr>
<tr>
<td>Covered by fascia</td>
<td>Covered by peritoneum</td>
</tr>
<tr>
<td>Has afferent vessels</td>
<td>No afferent vessels</td>
</tr>
<tr>
<td>Cortex and medulla</td>
<td>White pulp and red pulp</td>
</tr>
<tr>
<td>Contains Lymphatic sinuses</td>
<td>Contains Blood sinuses</td>
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- Enlarged lymphatic follicles inside the outer cortex with pale centers (might be **bacterial infection** because we fight bacteria by antibodies)

- Normal follicles inside the outer cortex and the area of the paracortex is underdeveloped (abnormal or absence of thymus) >>the patient will have problems in cell mediated immunity

- Underdeveloped lymphoid follicles with normal paracortex >> indicates a problem in B cells >> Can't produce antibodies and fight bacteria

- Underdeveloped paracortex: DiGeorge syndrome

- Normal lymphoid follicles inside the outer cortex but the area of the inner cortex is over developed (might be **viral infection** because we fight viruses by cell mediated immunity) It also could be an Intracellular microbes

- This patient can't handle any type of infection

- Underdeveloped outer cortex: **Bruton's immunodeficiency disease**

- Enlarged paracortex: ex. **Viral infection**

- Both B and T areas are underdeveloped