**LYMPHOID ORGANS HISTOLOGY**

 In adults, stem cells for all lymphocytes are located in the red bone marrow, but cells of the major lymphoid lineages mature and become functional in two different central or **primary lymphoid organs**. Cells destined to become B lymphocytes remain and differentiate further in the **bone marrow**. Progenitors of T lymphocytes move via the circulation into the developing **thymus**.

After maturation in these primary structures, B and T cells circulate to the peripheral **secondary lymphoid organs**, which include the **MALT**, the **lymph nodes**, and the **spleen**. Lymphocytes do not stay long in the lymphoid organs; they continuously recirculate through the body in connective tissues, blood, and lymph. Because of the constant mobility of lymphocytes and APCs(Antigen Presenting Cells), the cellular locations and microscopic details of lymphoid organs differ from one day to the next. However, the relative percentages of T and B lymphocytes in these compartments are relatively steady.

Lymphoid tissue is usually **reticular** connective tissue filled with large numbers of lymphocytes. It can be either diffuse within areas of loose connective tissue or surrounded by capsules, forming discrete (secondary) lymphoid organs. Because lymphocytes have prominent basophilic nuclei and very little cytoplasm, lymphoid tissue packed with such cells usually stains dark blue in Hematoxylin and Eosin (H&E)– stained sections.

 In all secondary lymphoid tissue the lymphocytes are supported by a rich **reticulin fiber** network of type III collagen. The fibers are produced by fibroblastic **reticular cells**, which extend numerous processes along and around the fibers. Besides lymphocytes and reticular cells, lymphoid tissue typically contains various APCs and plasma cells.

**THYMUS**

While immature B lymphocytes emerge from the bone marrow, the primary or central lymphoid organ in which T cells are produced is the **thymus**, a bilobed structure in the mediastinum. A main function of the thymus is induction of **central tolerance**, which along with regulatory T cells prevents autoimmunity. The organ originates from the embryo’s third pair of pharyngeal pouches (endoderm), with precursor lymphoblasts circulating from the bone marrow to invade and proliferate in this unique thymic epithelium during its development.

Fully formed and functional at birth, the thymus remains large and very active in T-cell production until puberty, during which it undergoes **involution,** decreasing greatly in size and activity and becoming largely filled with adipose tissue. The thymus has a vascularized connective tissue capsule that extends septa into the parenchyma, dividing the organ into many incompletely separated lobules. Each lobule has an outer darkly basophilic **cortex** surrounding a more lightly stained **medulla**. The staining differences reflect the much greater density of lymphoblasts and small lymphocytes in the cortex than the medulla.

The thymic cortex contains an extensive population of T lymphoblasts (or thymocytes), some newly arrived via venules, located among numerous macrophages and associated with the unique thymic epithelial cells (**TECs**) that have certain features of both epithelial and reticular cells. These cells usually have large euchromatic nuclei but are morphologically and functionally diverse.

There are three major types of TECs in the cortex of the thymus that form the following:

**1-** **blood-thymus barrier** preventing unregulated exposure of thymocytes to antigens.

**2-** form a **cytoreticulum** to which macrophages and developing lymphocytes attach instead of to reticulin fibers.

**3-**Importantly, these cells are **APCs,** expressing MHC class II molecules in addition to MHC class I. They also secrete numerous cytokines for T-cell development.

**4-**contributing to a functional **corticomedullary barrier.**

The more lightly stained thymic medulla contains fewer and larger, more mature lymphocytes. Three related types of medullary TECs form the following:

**1-** A second layer of the **boundary** between cortex and medulla.

**2-** A **cytoreticulum** that (1) supports less densely packed T lymphocytes, dendritic cells, and macrophages, and (2) expresses many specialized proteins specific to cells of other organs.

**3-** Large aggregates of TECs, sometimes concentrically arranged, called **Hassall corpuscles** or (thymic corpuscles) are unique to the medulla. Their cells secrete several cytokines that control activity of local dendritic cells, including factors that promote development of regulatory T cells for peripheral tolerance.

**Role of the Thymus in T-Cell Maturation & Selection**

**Positive selection** occurs in the **cortex** and allows survival only of T cells with functional TCRs (Thymic Cell Receptors) that recognize MHC class I and class II molecules. **Negative selection** occurs in the **medulla** and allows survival only of T cells that do *not* tightly bind self-antigens presented on dendritic cells there.

**MUCOSA-ASSOCIATED LYMPHOID TISSUE**

Secondary lymphoid structures, where most lymphocytes are activated by antigen presentation, include the mucosaassociated lymphoid tissue (MALT), the lymph nodes, and the spleen.

The mucosa or inner lining of the digestive, respiratory, and genitourinary tracts is a common site of invasion by pathogens because their lumens open to the external environment.

To protect against such invaders mucosal connective tissue of these tracts contains large and diffuse collections of lymphocytes, IgA-secreting plasma cells, APCs, and lymphoid nodules, all of which comprise the MALT.

Lymphocytes are also present within the epithelial lining of such mucosae. Most of the immune cells in MALT are dispersed diffusely in the connective tissue; others are found in aggregates that form large, conspicuous structures such as the **tonsils**, the **Peyer patches** in the ileum, and the **appendix.** Collectively the MALT is one of the largest lymphoid organs, containing up to 70% of all the body’s immune cells. Most of the lymphocytes here are B cells; among T cells, CD4+ helper T cells predominate.

Tonsils are large, irregular masses of lymphoid tissue in the mucosa of the posterior oral cavity and nasopharynx where their cells encounter antigens entering the mouth and nose. Named by their location these masses are the palatine, lingual, and pharyngeal tonsils. In all tonsils the lymphoid tissue is closely associated with the surface epithelium. Other features include the following:

■ **Palatine tonsils**, located posteriorly on the soft palate, are covered by stratified squamous epithelium. The surface area of each is enlarged with 10-20 deep invaginations or **tonsillar crypts** in which the epithelial lining is densely infiltrated with lymphocytes and other leukocytes. The lymphoid tissue is filled diffusely with lymphocytes, with many secondary lymphoid nodules around the crypts. This tissue is underlain by dense connective tissue that acts as a partial capsule.

■ **Lingual tonsils** are situated along the base of the tongue, are also covered by stratified squamous epithelium with crypts, and have many of the same features as palatine tonsils but lack distinct capsules.

■ The single **pharyngeal tonsil** is situated in the posterior wall of the nasopharynx, is covered by pseudostratified ciliated columnar epithelium, and has a thin underlying capsule. The mucosa with diffuse lymphoid tissue and lymphoid nodules is invaginated with shallow infoldings but lacks crypts.

Diffuse MALT extends from the pharynx along the entire gastrointestinal tract but becomes very well-developed again in the mucosa and submucosa of the ileum. Here large aggregates of lymphoid nodules comprise the **Peyer patches**, each containing dozens of nodules with no underlying connective tissue capsule.

Another significant collection of MALT occurs in the mucosa of the **appendix**, a short, small-diameter projection from the cecum. Typically the mucosa of the appendix is almost completely filled with lymphoid tissue, effacing the glands otherwise found in the large intestine wall. The lumen contains the normal bacterial flora of the large intestine and may serve to retain some of these beneficial bacteria there during diarrheal illnesses.

**LYMPH NODES**

Lymph nodes are bean-shaped, encapsulated structures, generally only 10 mm by 2.5 cm in size, distributed throughout the body along the lymphatic vessels. A total of 400 to 450 lymph nodes are present, most abundantly in the axillae (armpits) and groin, along the major vessels of the neck, and in the thorax and abdomen, especially in mesenteries.

The nodes constitute a series of in-line filters of lymph that defend against the spread of microorganisms and tumor cells and provide enclosed environments that facilitate production of plasma cells secreting non-IgA antibodies. Before merging with the bloodstream, all lymph is filtered and has antibodies added by at least one lymph node.

Embedded in loose connective tissue, a lymph node has a convex surface where **afferent lymphatics** enter and a concave depression, the **hilum**, where an **efferent** **lymphatic** leaves and where an artery, vein, and nerve penetrate the organ. A dense connective tissue capsule surroundsthe lymph node, extending trabeculae internally throughwhich the blood vessels branch. Valves in the lymphaticsensure that lymph flow is unidirectional.

The most abundant cells of lymph nodes are lymphocytes of all types, plasma cells, dendritic cells, macrophages, and other APCs. FDCs(Follicular Dendritic Cells) are present within lymphoid nodules.

All of these cells are arranged in a stroma of reticulin fibers and reticular cells to form three major regions:

an outer **cortex,** a central **medulla**, and a smaller area between these two called the **paracortex**. These regions are not physically compartmentalized like those of the thymus.

The **cortex** includes the following components:

■ A **subcapsular sinus**, immediately inside the capsule, receives lymph from the afferent lymphatics. From this space **cortical sinuses** (or trabecular sinuses) branch internally among the lymphoid nodules along trabeculae. These sinuses are lined by a very thin, discontinuous endothelium penetrated by reticulin fibers and processes of dendritic cells. Lymph containing antigens, lymphocytes, and APCs passes through these sinuses and percolates easily into the surrounding lymphoid tissue.

■ **Lymphoid nodules**, with or without germinal centers, fill most cortical areas, formed largely by helper T lymphocytes and proliferating B lymphoblasts. Each nodule is organized around the long, interdigitating processes of follicular dendritic cells (FDCs), but these are not readily seen by routine light microscopy. Numerous macrophages are also present for removal of newly formed defective B cells.

The region between the cortex and medulla, the **paracortex** does not have precise boundaries but can be distinguished from the outer cortex by its lack of B-cell lymphoid nodules . Unlike the superficial cortex, the paracortex contains lymphoid tissue rich T cells that can be seen by immunohistochemistry.

Specialized postcapillary venules in the paracortex called **high endothelial venules (HEVs)** represent an important entry point for most (90%) lymphocytes into lymph nodes.

These vessels have an unusual endothelial lining of cuboidal cells, whose apical surface glycoproteins and integrins facilitate rapid diapedesis of lymphocytes out of the blood into the paracortex of the lymph node. HEVs also occur in the large accumulations of MALT discussed previously, but are less well-characterized in those tissues.

The **medulla** of a lymph node has two major components:

■ **Medullary cords** are branched cordlike masses of lymphoid tissue extending from the paracortex. They contain T and B lymphocytes and many plasma cells.

■ **Medullary sinuses** are dilated spaces lined by discontinuous endothelium that separate the medullary cords, the lumens of medullary sinuses include a meshwork of processes from reticular cells, which represent a final lymph filter. These sinuses contain many macrophages and sometimes neutrophils if the lymph node is draining an infected region. They are continuous with the cortical sinuses and converge at the hilum as the efferent lymphatic vessel.

**SPLEEN**

The spleen contains the largest single accumulation of lymphoid tissue in the body and is the only lymphoid organ involved in filtration of blood, making it an important organ in defense against blood-borne antigens. It is also the main site of old erythrocyte destruction.

As is true of other secondary lymphoid organs, the spleen is a production site of antibodies and activated lymphocytes, which here are delivered directly into the blood.

Located high in the left upper quadrant of the abdomen and typically about 12 × 7 × 3 cm in size, the spleen’s volume varies with its content of blood and tends to decrease very slowly after puberty. The organ is surrounded by a capsule of dense connective tissue from which emerge trabeculae to penetrate the parenchyma or **splenic pulp**. 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 splenic pulp.

**Functions of Splenic White & Red Pulp**

The spleen is filled with reticular tissue containing reticular cells and fibers, many lymphocytes and other blood cells, macrophages, and APCs. This splenic pulp has two components:

A-**White pulp** (20% of the spleen). The small masses of **white pulp** consist of:

**lymphoid nodules** and the **periarteriolar lymphoid sheaths (PALS).**

B-**Red pulp**. The **red pulp** consists of:

blood-filled **sinusoids** and **splenic cords**.

Branching from the hilum, small trabecular arteries leave the trabecular connective tissue and enter the parenchyma as arterioles enveloped by the PALS, which consists primarily of T cells with some macrophages, DCs, and plasma cells as part of the white pulp. Surrounded by the PALS, these vessels are known as **central arterioles**.

B cells located within the PALS may be activated by a trapped antigen from the blood and form a temporary lymphoid nodule like those of other secondary lymphoid organs.

In growing nodules the arteriole is pushed to an eccentric position but is still called the central arteriole. These arterioles send capillaries throughout the white pulp and to small sinuses in a peripheral marginal zone of developing B cells around each lymphoid nodule.

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 capillaries. Some of these capillaries are sheathed with APCs for additional immune surveillance of blood.

The red pulp is composed almost entirely of **splenic cords** (of **Billroth**) and **splenic sinusoids** and is the site where effete RBCs in blood are removed. The splenic cords contain a network of reticular cells and fibers filled with T and B lymphocytes, macrophages, other leukocytes, and red blood cells. The splenic cords are separated by the sinusoids. Unusual elongated endothelial cells called **stave cells** line these sinusoids, oriented parallel to the blood flow and sparsely wrapped in reticular fibers and highly discontinuous basal lamina.

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

■**■** In the **closed circulation**, capillaries branching from the penicillar arterioles connect directly to the sinusoids and the blood is always enclosed by endothelium.

■**■** In the **open circulation**, capillaries from about half of the penicillar arterioles are uniquely *open-ended*, dumping blood into the stroma of the splenic cords. 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 effete, swollen RBCs at their normal life span of 120 days are blocked from passing between the stave cells and undergo selective removal by macrophages.