2160-3430 Lymphatic system laboratory module

Objectives.
1) Understand the structures in the thymus that support T-cell maturation, and the changes in this gland that occur during aging.
2) Determine how lymph nodes are anatomically organized to expose lymph to B- and T-lymphocytes, and understand the structure of a germinative centre.
3) Follow blood flow through the structures in the spleen to understand how old erythrocytes are removed and blood is exposed to lymphocytes
4) Understand how unencapsulated collections of lymphocytes near epithelia can be exposed to pathogens

Overview
The key concept in studying the histology of lymphoid tissues is that they are organized to provide maximal exposure of lymphocytes to antigens. This is true whether lymphocytes are present as diffuse lymphoid tissue in the walls of hollow organs (gut, respiratory system), under the epidermis of the skin, or in encapsulated lymphoid organs such as lymph nodes and spleen. B-cells, T-cells and killer lymphocytes, representing the three major types of lymphocytes in the body, cannot be distinguished from one another with the normal staining techniques used to stain most of the slides you will examine in the teaching laboratory. However depending on the organ under examination there will be a predominance of B- or T-cells at different sites, as described in the text and your notes.

Encapsulated lymphoid tissue.

Thymus. Even though the thymus is not a location of lymphocyte immunoactivity, it is still considered an encapsulated lymphoid organ because it is the site of T-cell maturation (review the origin of lymphocytes and how they get into the thymus). As an individual ages, fewer T-cells enter the thymus to mature, and this organ degenerates (involution).

42 fetal, 45 infant thymus. Multiple lobules of the thymus gland can be seen on both of these slides; each lobule is surrounded by a capsule (loose connective tissue sheath) that divides it from the other lobules. There may be blood vessels between the lobules. Within each lobule look for the darker-staining cortex and lighter-staining medulla (why do these regions stain differently?). In both regions the long, thin cytoplasmic extensions of reticular cells provide a 3-dimensional meshwork to support the lymphocytes. The nuclei of reticular cells are larger and more lightly stained than lymphocytes; the cytoplasm of reticular cell bodies is also very lightly stained and may appear gray. These cells may be most visible in the medullary region of the lobules on slide 45. Also in the medullary region of some lobules there will be clusters of cells with pink-staining cytoplasm, with the cells organized into a roughly spiral pattern. These are Hassall's corpuscles (also called thymic corpuscles) and are made up of non-functional reticular cell bodies. Hassall's corpuscles become larger and more numerous with age so are more prominent on slide 45 than on slide 42.

54 adult thymus. After puberty the number of T-cells entering the thymus to complete the maturation process decreases rapidly and the space formerly occupied by lymphocytes becomes filled with adipocytes. This process of degeneration is called involution. Compare the number of lymphocytes on this slide with the number of these cells on slides 42 and 45. Hassall's corpuscles will be easily seen on this slide.

Lymph node. Lymph nodes are shaped approximately like kidney beans and vary in size from a few mm to more than 10 mm in length. Multiple afferent lymphatic vessels drain lymph from surrounding tissue into each node where the lymph is exposed to lymphocytes as it moves slowly through the node from the cortex to the medulla. Lymph collects in the medullary sinuses to drain from the node via a single efferent lymph vessel. T-cells in the bloodstream gain access to lymph nodes through the walls of specialized post-capillary venules at the cortico-medullary border. Germinative centres form in the cortex where B-cells have become activated by exposure to antigens in the incoming lymph. Upon activation B-cells divide rapidly and actively secrete antibodies, enlarging into plasma cells which are lighter staining than the inactive B-cells that surround the germinative centre. Together, the germinative center and its surrounding, more darkly staining region, are called a lymph nodule. There are usually a number of nodules in a node.

36, 127, 133 lymph node. Nodes are enclosed in a connective tissue sheath, the capsule, which is made up of Type I collagen, fibroblasts, blood vessels and usually adipocytes. Just under the capsule is the subcapsular sinus, a lymph-filled space continuous with the incoming afferent lymphatic vessels. From the subcapsular sinus, lymph percolates slowly between the lymphocytes in the cortex, then into the medulla. The capsule is lined with a layer of reticular cells making up an epithelium (thin, flat cells) on the inside surface of the capsule. The same cell type is found throughout the cortex and medulla where it makes the precursors of reticular fibres. These reticular fibres form a 3-dimensional meshwork of Type III collagen to support the lymphocytes. Reticular cells can most easily be seen as relatively large, pale-staining cell bodies with pale nuclei scattered between lymphocytes. Lymph nodes are subdivided into compartments internally by trabeculae of connective tissue extending into the cortex. The surfaces of trabeculae are also covered by a layer of reticular cells. At the cortico-medullary border you will find postcapillary venules lined with a specialized epithelium that looks cuboidal (high epithelial venules). Lymphocytes can move between the blood and the lymph node by migrating through the walls of these specialized vessels. These vessels are prominent in the tissue on slide 36. Note germinative centres in the cortex; these are especially numerous in slide 133. In the medulla there are fewer lymphocytes than in the cortex, and these are mostly B-cells and plasma cells. The medullary lymphocytes form sheets of cells and in the spaces between these sheets (medullary sinuses) lymph collects after passing from the cortex. Lymph in these medullary sinuses drains into the efferent lymphatic vessel and returns to the circulation.

Spleen. In the spleen blood is exposed to lymphocytes in the same way as lymph is exposed to lymphocytes in nodes. In addition the spleen acts to remove aging red blood cells from the circulation. Keep in mind the pattern of blood flow through the spleen as you look at the slides below.

60 spleen, reticular fibre stain. This stain shows clearly the 3-dimensional meshwork of reticular fibres supporting the red and white pulp within the spleen. Note also the concentration of reticular fibres in trabeculae and in the walls of blood vessels.

57, 119 spleen. Red and white pulp, trabeculae and large blood vessels can be seen by eye on these slides. Examine under low then high power objectives to identify the capsule and some trabeculae (collagen stained pink). The lymphocytes associated with the central arteries make up the white pulp; this tissue is stained more darkly than the surrounding red pulp. Find central arteries with their sheath of lymphocytes (predominantly T-cells) and the occasional nodule (mostly B-cells). Some nodules may have germinative centres. As the B-cells proliferate at the site of a growing nodule, the mass of these cells pushes the central artery off to one side. Red pulp consists of sheets of lymphocytes with spaces between that are filled with erythrocytes and blood plasma. Within the red pulp are sinusoids in which erythrocytes and plasma collect from the surrounding red pulp (you can differentiate sinusoids from red pulp because sinusoids contain mostly erythrocytes; red pulp contains an abundance of dark-staining lymphocytes as well as erythrocytes).

Unencapsulated and diffuse lymphoid tissue

Collections of lymphocytes are embedded in and distributed throughout most body tissues. There are particularly large numbers of lymphocytes in regions where pathogens may cross epithelia; these include the respiratory system, the gut and the skin. In the inner region of the pharynx, near the entrance to the esophagus and the opening of the trachea, there are large accumulations of unencapsulated lymphocytes, termed tonsils, consisting of many lymph nodules, guarding these openings. However lymph nodules can develop anywhere pathogens breach an epithelium and activate B-cells in the diffuse lymphoid tissue underneath.

34 tonsil. Identify the stratified squamous epithelium covering the tissue on this slide. This epithelium lines the oral cavity. Under the epithelium are numerous germinative centres where B-cells have been activated and form lymph nodules. The lack of a capsule around the lymphoid tissue gives this type of tissue its name.

4 esophagus. The epithelium lining the esophagus is the same type as oral epithelium; look in the loose connective tissue under the epithelium for scattered lymphocytes and plasma cells. This is an example of diffuse lymphoid tissue. There may be a germinative centre in the connective tissue if pathogens have invaded the epithelium in your specimen.

17 tongue. One of the first structures encountering food placed in the mouth is the tongue, and there is a large number of lymphocytes under the epithelium covering this structure. Look for lymphocytes in the loose connective tissue under the epithelium.