LABORATORY 16 - LYMPHATIC ORGANS - (second of two laboratory sessions)

OBJECTIVES: LIGHT MICROSCOPY: Recognize the structure of lymph node, spleen and thymus and their subcomponents. Structural features that should be observed where appropriate include: connective tissue capsule, cortex and medulla, hilus, trabeculae, sinuses, afferent and efferent lymphatic vessels, primary and secondary nodules and distribution of T and B lymphocytes as well as other specific characteristics for each organ. Know how the function of each organ relates to the details of its structure. Understand the significance and distribution of the macrophage system.

ELECTRON MICROSCOPY: Recognize micrographs of cells of the immune system, e.g. lymphocytes, plasma cells and macrophages etc.

ASSIGNMENT FOR TODAY'S LABORATORY

GLASS SLIDES

SL 77 Lymph node
SL 78 Lymph node (mainly cortex)
SL 66A Spleen
SL 66B Spleen
SL 79 Spleen
SL 32 Spleen (reticular fibers)
SL 80 Thymus
SL 81 Thymus
SL 82 Thymus
SL 34 Thymus
SL 170 (To be distributed in laboratory) Demonstration slide of tonsil - T and B Lymphocytes

Macrophage System
SL 67 Rat liver
SL 59 Lung
SL 65 Lymph node
SL 84 Lung with abscess
SL 125 Skin with inflammation

POSTED ELECTRON MICROGRAPHS

S-51 Spleen
S-52 Spleen
Lab 16 Posted EMs; Lab 16 Posted EMs with some yellow labels

HISTOLOGY IMAGE REVIEW - available on computers in HSL

Chapter 10. Lymphoid System
Frames: 647-676, 681-693

SUPPLEMENTARY ELECTRON MICROGRAPHS

Rhodin, J. A.G., An Atlas of Histology
Copies of this text are on reserve in the HSL.
Lymphoid tissue pp. 212 – 222

A. LYMPH NODES

1. SL 77 - (J. 14-20 to 14-26; W. 11.8 to 11.15). By gross observation (scan) compare this slide with Fig. W. 11.8-11.9. Note cortex, medulla and that a portion of the hilus may be present. Scan your slide with the scanning objective. Note that the lymphoid tissue is not as dense as depicted in the Atlas.

   (a) Locate the cortex where numerous, mostly secondary nodules (red circles) are located.

   (b) Observe medullary region with "cords" of lymphoid tissue separated by wide medullary sinuses (med, high) (cords within blue lines, sinuses within red lines) (W. 11.2).

   (c) An intermediate region, the paracortical zone, is not as well defined as shown in W. 11.9). One should note (W. 11.14) that this region is the location of high endothelial post-capillary venules (within blue circle). It is unlikely that you will be able to delineate these on your slide.

   (d) In tracing the pathway of lymph find the capsule that is quite thin on this slide. Small spaces within and just outside the capsule are mostly afferent lymphatics (blue arrows). Just beneath the capsule is a space that contains a network of stromal reticular cells, the subcapsular sinus (enclosed within blue line). Scattered strands of c.t., trabeculae, that enter the cortex between nodules, may be evident. The sinuses accompanying the trabeculae are often difficult to find. The cortical sinuses empty into wide sinuses of the medulla, and these collect to form the efferent lymphatics (red arrows) that are present in any section that includes a portion of the hilus.

   (e) Other features - note reticular cells and macrophages in the sinuses (red arrow, macrophage; blue arrows reticular cells) (W. 11.11). Many slides will show plasma cells in the medullary cords (W. 11.15). Elements of the blood supply may be evident scattered throughout.

2. SL 78 – Lymph node sectioned so that mainly cortex is present. Compare to above.

3. What is the functional role of cortical nodules and paracortical zones? (J. p. 280)

B. SPLEEN

Much of the description of the spleen that appears in texts relies on the use of animal material as well as specialized techniques. There is a great deal of variability in routine H & E preparations, but they do allow determination of the basic organization. Details of blood and lymphatic flow and physiologic subdivisions must be obtained from your textbooks.

1. SL 66A and SL 66B (scan) (J. 14-27 to 14-35; W. 11.18 to 11.21). As an introduction to the structure of the spleen survey this section at low magnification to determine the distribution of Red and white pulp (white pulp enclosed within blue line). (As is the case in sections derived from older individuals, there is some degree of hyaline thickening of the walls of arteries that is a nonspecific pathologic condition). Then use higher magnification to observe the differences in the two regions.

   (a) Histologically, note the thick capsule on one edge. Trabeculae composed of connective tissue are distributed throughout the spleen. The larger trabeculae (red arrows) often contain branches of the splenic artery and vein. In addition, nerves and lymphatics may be present.

(b) Observe areas of white pulp, these may be identified as concentrations of lymphocytes. Observe areas of red pulp that may be recognized as the regions between concentrations of white pulp. They consist of loosely arranged tissue with numerous spaces. The spaces are the lumens of the venous sinusoids [(c) below].

(c) White pulp - Observe that there are many lymph nodules, and associated with each is a small artery. The artery is located at the periphery of the nodule, but is referred to as a central artery (arteriole). In the vicinity of the nodules, single or groups of smaller arterioles may be seen, some with associated white pulp, others extending out into the perinodular region. These are the penicillar arterioles (central artery within red circle, penicillar arterioles black arrows).
Note that the nodules are sites of B cell concentration and activity. Part of the lymphoid tissue immediately around the central artery in these areas of nodules and the other concentrations of lymphocytes scattered around smaller arterioles represent the T cell rich compartment, generally referred to as the periarterial lymphoid sheath (PALS).

(d) Red pulp - (1, 2) Primarily composed of venous sinusoids separated by splenic cords (of Bilroth) (venous sinusoids within red lines, cords are between sinusoids). The sinusoids may contain blood and are lined by elongated endothelial cells. When cut in cross section these endothelial cells have a round profile and round nucleus. The splenic cords between the sinusoids contain macrophages, RBC^s, lymphocytes and other cells of the immune system. Reticular fibers (type III collagen) are distributed throughout the organ forming a supportive network that is especially evident at the periphery of the venous sinusoids (see below).

(e) The descriptions above are general features. Other specific items to consider:
(1) In the red pulp, one may find terminations of penicillar arteries called sheathed capillaries. These capillaries may be recognized as rows of flattened endothelial cells. Sheathed capillaries are difficult to identify definitively.
(2) Observe that the contents of the trabeculae vary. Some trabeculae have no artery or vein, some have veins only, and some have both.
(3) You should be able to find trabecular veins that have tributaries that extend into the red pulp. These pulp veins have little connective tissue, and carry blood away from the sinusoids and into the trabecular veins.
(4) The origin of lymphatics in the white pulp cannot be seen.

(f) SPLEEN, some slides have sections cut through the hilus. SL 79. Briefly review structures identified above.

(g) SPLEEN stained for reticular stroma. SL 32. Note reticular fibers in red pulp outline the margins of the sinusoids (adjacent to blue lines and between blue arrows). The tissue filling in between the sinusoids represents the splenic cords (of Bilroth).

C. THYMUS

1. THYMUS, 4 year old child. SL 80 (low). (J. 14-14, 14-16, 14-17, 14-18, 14-15, 14-19; W. 11.5 to 11.7).

(a) Observe the general architecture. Note the densely packed lymphoid tissue of the cortex, the less dense medullary region, capsule (high) and c.t. septa.

(b) The thymus is made up of lobules of various sizes with a central core of medulla extending into the various lobules. Some lobules are cut so that the continuity of the medulla with the central core is not seen, showing a central area of medulla and outer rim of cortex.

(b) In the medulla - note the characteristic eosinophilic Hassall's Corpuscles of varying size. Note also the cells of the stroma, identifiable by their elongated nuclei, and remember these are epithelial reticular cells, differing in origin from the stromal cells of other lymphoid organs.

(d) Blood vessels are scattered throughout and there are no lymph sinuses as in nodes.

2. The following series of slides illustrates variations in thymic structure that occur due to age or stressful events, referred to as age or accidental involution. Note alterations in architecture, primarily the replacement of lymphoid tissue with fat (J. 14-19).
   THYMUS, premature baby. SL 81.
   THYMUS, 17-year-old male. SL 82.
   THYMUS, 36-year-old female. SL 34.

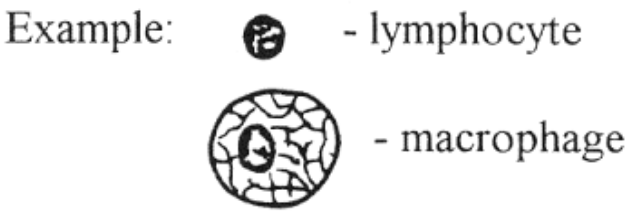
D. THE MACROPHAGE SYSTEM

Macrophages appear in many normal as well as pathologic conditions. One should be able to recognize them in tissue sections.

1. RAT LIVER. SL 67. (W. 15.9). A classical way of demonstrating macrophages is by their ability to phagocytize particulate matter. Here the dye trypan blue was injected into a rat. Some time later the animal was autopsied and sections of liver made. Observe the numerous cells containing blue particles of dye. These are fixed macrophages (red arrows) in the sinusoids of the liver. In the liver these macrophages are called Kupffer cells.

2. LUNG. SL 59 Scattered in this spaces of this tissue, note round cells containing black granules. The granules may be dense enough to obscure the nucleus. These cells are free macrophages, called dust cells, that have taken up carbon particles in the lung (low, high, within red circle) (W. 12.17).

3. LYMPH NODE - H & E. SL 65. Note rounded cells of various sizes with a large proportion of cytoplasm. These cells are numerous in the sinuses (W. 11.11). Some of these macrophages may have RBC or lymphocytes within them that have been phagocytized. Others appear foamy in appearance (red arrows).



4. LUNG WITH TUBERCULAR ABSCESS. SL 84 - Even Desks Only. In the grossly visible dark staining mass, locate multinucleated giant cells (within red circles), the result of fusion of many active macrophages.

5. INFLAMMATORY REACTION IN SKIN. SL 125. (W. 4.19). Review the appearance of all blood cell types as seen in tissue section. Plasma cells, lymphocytes, neutrophils are abundant. Occasionally, eosinophils and macrophages may be present (scan, oil 1, oil 2, (neutrophils, red arrows; plasma cells, blue arrows).

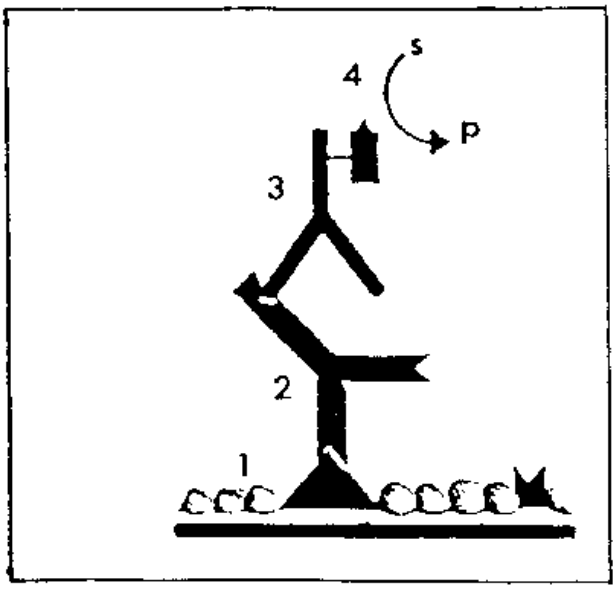
6. DETERMINATION OF THE DISTRIBUTION OF T AND B LYMPHOCYTES BY IMMUNOCYTOCHEMISTRY. SL 170 (W. 11.13)

Although the technique of immunocytochemistry has been available for many years, it was only during the 1980's that the method gained widespread use in research and as a diagnostic tool in pathology. This expansion was due to the recognition and isolation of many marker proteins characteristic for specific cell types.

In the exercise today, we will localize T and B lymphocytes in sections of palatine tonsil. Slides (SL.170) will be distributed (one per lab bench) that contain two sections of palatine tonsil. One section was exposed to an antibody that reacts with a surface marker that is expressed specifically on T lymphocytes (Leu-22) and the other section was exposed to an antibody that is specific for B lymphocytes (L-26). Both antibodies were visualized by the peroxidase method described below that produces a brown precipitate whenever antibody is located. The sections were counter-stained with hematoxylin so that nuclei would be visible throughout the sections.

In order to prepare the tissue, the tonsil was fixed, embedded in paraffin and sectioned. Immuno-localization was performed by the sequential addition of a primary monoclonal antibody (either mouse anti-B cell marker or mouse anti-T cell marker) and a horseradish peroxidase-labeled secondary antibody (i.e., peroxidase-labeled rabbit antibodies against mouse immunoglobulin). Between and following incubations with immunoreagents, samples were washed extensively to remove unbound antibody. The complex of secondary antibody bound to primary antibody bound to the appropriate marker, was localized by treating the samples with buffer containing hydrogen peroxide and diaminobenzidine. The horseradish peroxidase reacts with this solution and forms a brown precipitate indicating sites where antibody has bound to the surface marker.

See questions regarding this slide on the next page.



The figure to the right illustrates the methods described. 1. Cell membrane of T or B lymphocyte.2. Primary antibody (mouse anti-B cell marker or mouse anti-T cell marker) has bound to the antigen (cell marker). 3. Secondary antibody against mouse antibodies and labeled with horseradish peroxidase has bound to the primary antibody. 4. Horseradish peroxidase reacts with buffer solution (S) of hydrogen peroxide and diaminobenzidine to form a brown precipitate (P).

1. From the distribution of brown precipitate in each of the two sections and the information given in lecture or your text, can you determine which antibody was used for each section?

2. What results would you expect if these antibodies were used in other lymphoid organs.

3. Would you expect to identify T and B lymphocytes in the thymus?

4. Are there some lymphocytes that express both T and B lymphocyte antigens?

5. In diffuse lymphoid tissue which cell type predominates?

6. Within each lymphoid organ the presence of T and B lymphocytes may alter in response to specific challenges from foreign substances.

OBJECTIVES FOR LABORATORY 16: LYMPHATIC ORGANS

1. Using the light microscope or digital slides, identify:

- Lymph node
  - Hilus
  - Capsule
  - Trabeculae
  - Cortex
    - Subcapsular sinus
    - Trabecular sinus
    - Nodules (including primary and secondary, and cell types; see lab 15)
    - Paracortical zone
  - Medulla
    - Medullary cords
    - Medullary sinuses
  - Afferent lymphatic vessels
  - Efferent lymphatic vessels
  - Distribution of cell types in different regions (esp. T and B cells)
- Spleen
  - Capsule
  - Trabeculae
    - Trabecular arteries and veins
  - White pulp
    - Nodules (including primary and secondary, and cell types; see lab 15)
      - Central arteries
        - Periarteriolar lymphoid sheath (PALS)
      - Penicillar arteries
  - Red pulp
    - Splenic cords
    - Venous sinusoids
    - Pulp veins
  - Distribution of cell types in different regions (esp. T and B cells)
- Thymus
  - Capsule
  - Trabeculae
  - Cortex
  - Medulla
  - Lobules
  - Hassall's corpuscles
  - Epithelial reticular cells
  - Lymphocytes
- Macrophages
  - Kupffer cells (liver)
  - Alveolar macrophages / Dust cells (lung)
  - Macrophage in lymph node
  - Macrophage in inflammation
  - Giant cells (from tubercular abscess)

2. On electron micrographs, identify:

   Be able to interpret electron micrographs of lymphoid organs if given the source of tissue.

3. Understand the procedure, and be able to interpret results from, immunoprecipitation.

REVIEW

1. Fill in chart below + present, - absent

	Afferent Lymphatics	Efferent Lymphatics	Discrete T & B Zones	Hassall's Corpuscles	Many Nodules	Type of Stroma	Type of Sinusoids
Lymph Node							
Thymus							
Spleen							
Tonsil							
Peyer's Patches							

2. This study, added to your prior study of bone marrow, completes the consideration of hematopoiesis. Review aspects of the formation of all blood cells at this time.

3. By this time be able to identify erythrocytes, neutrophils, lymphocytes, monocytes, plasma cells, eosinophils, fibroblasts, and macrophages in tissue sections.

4. What are B and T lymphocytes?

5. Are nodules always permanent features of lymphoid tissue in an organ?