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Lymphatic Histology

M1 – Immunology Sequence

J. Matthew Velkey, Ph.D.
Learning Objectives

Text: Ross, 5th ed., pp. 396-441

1. Understand the distinction between PRIMARY and SECONDARY lymphoid organs

2. Be able to describe the organization and function of:
   - Mucosa-associated lymphoid tissue
     - Diffuse and nodular lymphoid tissue, also including regions of extensive lymphoid infiltration such as Peyer’s patches, appendix, and tonsils.
   - Lymph nodes
   - Spleen
   - Thymus

3. Be able to identify the regions rich in B and T lymphocytes in each organ and explain the cellular processes, relevant to immune functions, that are taking place in these regions.

4. Know the homing patterns of B & T lymphocytes.
**Functions of the Lymphatic System**

1. Monitor body surfaces and fluid compartments (e.g. epidermis, mucosae*, interstitium)

2. React to the presence of potentially harmful antigens recognized as “non-self”

3. Autoimmune diseases (rheumatoid arthritis, type I diabetes, etc.)

Lymphatic System consists of:

A. Cells
   1. Lymphocytes (B,T, natural killer)
   2. Antigen-presenting cells (dendritic cells, Langerhans’ cells & macrophages)

B. Lymphatic “tissue” – diffuse and nodular

C. Lymphatic “organs” (lymph nodes, spleen, thymus)

D. Lymphatic vessels that carry the cells and fluid

*Mucosae refers to lining tissue of the body cavities, e.g. GI tract, respiratory tract, genitourinary tract*
Lymphoid organs are classified as:

**Primary lymphoid organs**
- Thymus
- Bone marrow
- Lymphatic nodules of the distal intestinal tract (e.g. ileum and appendix)

**Secondary (effector) lymphoid organs/tissue**
- Spleen & lymph nodes (organs)
- Mucosal associated lymphoid tissue (MALT), e.g. lymphocytes and lymphatic nodules in the lamina propria
Primary Lymphoid Organs:

The bone marrow and the thymus and the Gut-Associated Lymphoid Tissue (e.g. appendix, terminal ileum) are the initial “education centers” of the immune system.

In these organs, lymphocytes (T cells in the thymus, B cells in bone marrow and gut) differentiate into immunocompetent cells (i.e. they can recognize “self” vs. “nonself”).

This differentiation is said to be antigen-independent.

The lymphocytes then enter the blood and lymph to populate:

- epidermis and mucosae
- connective tissue
- secondary lymphoid organs
Secondary Lymphoid Organs:

The lymph nodes, lymphatic nodules, tonsils, spleen are the secondary “education centers” of the immune system.

In these organs, immunocompetent lymphocytes differentiate into immune effector and memory cells that undergo antigen-dependent activation and proliferation in these organs.

These lymphocytes then carry out their functions in the:

- connective tissue
- secondary lymphoid organs
- mucosal surfaces lining epithelia

They participate in:

- Cell mediated immunity (mostly “cytotoxic” T cells)
- Humoral responses (production of antibody) (B cells, also requires “helper” T cells.)
Lymphocytes in peripheral blood smear

These are B and T-cells that have undergone antigen-INDEPENDENT differentiation and are trafficking through the bloodstream on their way to lymphoid organs/tissue.
Resting Lymphocyte
Diapedesis: it’s not just for the Normans and the Saxons…

Cytokines and chemokines (along with selectins and integrins) mediate EXTRAvasation of lymphocytes into tissues.

Tether  Roll  Arrest  Migrate

Blood flow

cytokines

chemokines

APCs and other cells

L. Stoolman
MALT: intraepithelial lymphocytes: γδT-cells (neither helper nor cytotoxic): first to see antigens
Intraepithelial lymphocytes

Shown here in resp. epith.

Homing mediated by “addressins” (a sort of lymphocyte “GPS”)

IEL

basement membrane
LYMPHOCYTES IN CONNECTIVE TISSUE: MALT = mucosa-associated lymphoid tissue

Diffuse lymphoid tissue
Lamina propria (LP) of gut shown here, but can be found associated with mucosae anywhere in the gut, respiratory, and genitourinary tracts.

Primary lymphatic nodule/follicle (LN)
Aggregation of lymphocytes in lamina propria or submucosa
Secondary follicles/nodules

- Contain germinal centers

- Arise when B-lymphocytes are presented with appropriate antigen, receive T-cell help, and then begin proliferating as lymphoblasts

- Lymphoblasts differentiate into plasma cells or memory cells; aberrant lymphoblasts undergo apoptosis.
Microfold, or “M” CELLS

Modified intestinal epithelial cells that assist in antigen presentation by conveying macromolecules from the intestinal lumen to underlying compartments housing lymphocytes and macrophages.
M cells: TEM

- Surface of M cell
- Bodies of M cells
- Macrophage
- Lymphoid cells
- Basement membrane
- Surface microfolds of M cells

Source Undetermined
After antigen presentation and T-cell help, activated B-cells set up germinal centers in secondary follicles.

Secondary follicle germinal centers

- Arise when B-lymphocytes are presented with appropriate antigen, receive T-cell help, and then begin proliferating as lymphoblasts.
- Lymphoblasts differentiate into plasma cells or memory cells; aberrant lymphoblasts undergo apoptosis.
Germinal center: high magnification
Lymphoblast viewed by transmission electron microscopy
Germinal Center--Lymphoblasts and Macrophages

L-Blast

Mitotic Figure
Plasma Cells are mature B lymphocytes

Black arrows indicate several plasma cells

White arrows = Golgi regions

Junquiera and Carneiro. Basic Histology. Tenth Ed. 2003
So, associated with just about any mucosa (GI, respiratory, genitourinary), you may see:

- Intraepithelial lymphocytes (T-cells)
- Diffuse lymphoid tissue:
  - B-cells
  - T-cells
  - APCs
- Primary nodules
- Secondary nodules
  - Germinal center with lymphoblasts and mphages
Regions of extensive lymphoid infiltration: Peyer’s patches

Aggregates of lymphoid follicles in the ileum.
Appendix

Blind sac extending from the caecum

- primary and secondary follicles in lamina propria and submucosa

- So, clearly a secondary lymphoid organ…

- However, also a site of antigen-INDEPENDENT differentiation (similar to Bursa of Fabriscus in birds)

- So, also a primary lymphoid organ

Sorry about the various “primary” and “secondary” nomenclature; that’s just the way it is…
Tonsils: MALT of the oropharynx
The palatine tonsils are paired structures made of dense accumulations of lymphatic tissue located in the mucous membrane of the junction of the oropharynx and oral cavity. The tonsils dip down into the underlying CT, forming crypts. There are also lingual tonsils and pharyngeal tonsils (under the roof of the nasopharynx and around the opening of the Eustachian tubes). **Key features:** crypts, abundant nodules, stratified squamous epithelium.
Wanderlust: lymphocytes don’t just stay in one place

From the MALT, lymphocytes can squeeze into lymph vessels…
..go through larger lymphatic channels in the mesentery...
..and end up at a LYMPH NODE.
Lymph Nodes

Main functions:

1. Filter lymph, thereby promoting lymphocyte contact with antigen

2. Provides necessary microenvironment for antigen-dependent differentiation
Lymphoid circulation in the body takes place in both the blood stream and the **lymphatic vessels**, a separate vessel system that carries cells of the lymphoid system and their products (cytokines, antibodies, etc.).
Lymphatic drainage: anatomy

Image of lymphatic drainage anatomy removed

Lymph node structure

Image of lymph node structure removed

Original Image: http://academic.kellogg.cc.mi.us/herbrandsonc/bio201_McKinley/f24-10a_lymph_node_and__c.jpg
Lymphatic Circulation Through a Lymph Node

Lymph nodes filter lymph

1. Afferent lymphatic vessels drain lymph into the Subcapsular Sinus

2. Lymph then passes to the Trabecular sinuses

3. From there, the lymph goes to the Medullary sinuses.

4. Lymphocytes and macrophages pass easily between these sinuses and the tissue of the lymph node.

5. Macrophages in sinuses monitor the fluids. Macrophages phagocytose the antigenic material and present it to T- and B-cells.

Image of lymph node circulation removed
- Capsule & subcapsular sinus
- Trabeculae & trabecular sinuses
  sinuses contain lymph, macrophages, and reticular cells
- Cortex:
  • superficial cortex (B-cells)
    - primary follicles/nodules
    - secondary follicles/nodules
      (i.e. with germinal centers)
  • “deep” cortex (T-cells, dendritic cells)
- Medulla:
  • medullary cords (B-cells, plasma cells)
  • medullary sinuses (lymph, more macrophages, plasma cells, and reticular cells)
High magnification view of a sinus (subcapsular sinus shown here)

M=macrophage, Ly=lymphocytes, RF/RC=reticular fiber (and associated reticular cell)
From the sub-capsular sinus, lymph percolates through trabecular sinuses, and finally into medullary sinuses.
Reticular (Reticulin) Fibers

• Form a delicate supporting framework for highly cellular tissues (endocrine glands, lymph nodes, liver, bone marrow, spleen, smooth muscle).

• Composed mainly of Type III collagen, with a carbohydrate moiety that reduces Ag+ to metallic sliver = argyrophilic.

• Special stain: silver impregnation to visualize.

• Thinner than type I collagen (Type III fibrils are 30-40 nm diameter; type I fibrils are ~200 nm diameter).
Reticular Fibers (type III collagen)

made by reticular cells (specialized fibroblasts)

Top left: Ross and Pawlina, Histology: A Text and Atlas. Others: Sources Undetermined
Medullary sinuses drain into EFFERENT lymphatics that exit from the hilum of the lymph node
Blood Circulation Through a Lymph Node

1. Blood enters through an artery at the hilus

2. Arterioles branch from hilar artery to feed into capillary beds

3. Capillary beds are drained by high endothelial venules*

4. HEVs drain into hilar vein

*HEVs are sites where lymphocytes can leave blood stream to enter the lymph node tissue bed.
Slide 27, lymph node, H&E, 10x obj.

medulla

depth cortex

cortex

high endothelial venules

capsule
Slide 27, lymph node, H&E, 40x obj.

high endothelial venules
High Endothelial Venules

Site of:

- Fluid absorption (via aquaporin-1 channels), which causes lymph flow
- EXIT of lymphocytes from bloodstream via diapedesis
Lymphocyte Homing -
Extravasation by T and B Cells in the High Endothelial Postcapillary Venules of the Lymph Node Cortex
Lymphocyte Homing

HE-PCV

HE-PCV

HE-PCV

LY

Source Undetermined
Lymphocyte Homing in the High Endothelial Postcapillary Venule
Summary of lymphocyte traffic in a lymph node

• Solvent drag caused by HEV fluid transport draws lymph in via afferent vessels
  – ~10% of lymphocytes enter this way; mostly memory cells

• HEV endothelial cells express selectins and other receptors for antigen-primed lymphocytes that stimulate them to EXIT bloodstream via diapedesis
  – ~90% enter this way; mostly naïve lymphocytes

• T-cells move to deep cortex; B-cells migrate to superficial cortex; differentiated plasma cells move to medullary cords and secrete IgG into lymph

• Lymphocytes may leave lymph node via EFFERENT lymph vessels (can rejoin bloodstream via thoracic duct, jugular vein, etc.)
The Spleen

Filters the blood
Destroys old red blood cells
Serves as an immune organ

Divided into Red Pulp (RBC/hemoglobin recycling) White Pulp (responsible for immune functions)
Hematopoietic Functions Of the Spleen

- Formation of blood cells in fetal life
- Removal and destruction of RBCs & platelets
- Retrieval of iron from RBC hemoglobin
- Storage of RBCs and platelets (more so in non-human species)

Immune Functions Of the Spleen

- Monitoring antigens in blood
- Proliferation of lymphocytes
- Production of humoral antibodies

- Formation of blood cells in fetal life
- Removal and destruction of RBCs & platelets
- Retrieval of iron from RBC hemoglobin
- Storage of RBCs and platelets (more so in non-human species)
Spleen: anatomy
Spleen: anatomy

ORGANIZATION OF THE SPLEEN
Splenic Circulation

1. Blood enters via splenic artery at hilus
2. Splenic artery branches into trabecular arteries (which travel within connective tissue trabeculae).
3. Trabecular arteries give off branches known as central arteries which leave the trabecula and enter the substance of the spleen (covered by a peri-arterial lymphatic sheath).
4. Central arteries branch into penicillar arterioles that piece through the lymphatic sheath and spill into splenic cords.
5. Blood percolates through splenic cords and across walls of splenic sinuses.
7. Pulp veins drain into trabecular veins.
8. Trabecular veins drain into splenic vein at the hilus.
Circulation in the human spleen is primarily “OPEN:” blood pours into the red pulp, percolates through red pulp cords, and re-enters the bloodstream at splenic sinuses.

NOTE: NO afferent lymph vessels –not necessary because lymphocytes can easily enter splenic parenchyma via “open” circulation pattern.
Splenic Circulation

Rod Cells

Splenic Cords

1\textsuperscript{o} Fol.

2\textsuperscript{o} Fol.

CA

MZ

PALS

GC

Splenic Sinuses

Splenic Cords

Organization of the spleen: white pulp and red pulp
White pulp: lymphatic aggregations around “central” arteries:
  - periarterial lymphatic sheath (PALS): T-cells
  - lymph nodules: B-cells
Red pulp: cords and sinuses
White pulp function

Blood and antigens pour into red pulp (more on that later)

Antigen presentation takes place in MARGINAL ZONE

T-cells (from PALS) provide “help” to activate mphages and B-cells

- activated mphages stimulated to destroy ingested material (e.g. bacteria)
- activated B-cells set up proliferative germinal centers
As the body is exposed to antigens and the immune system mounts an immune response in the form of antibody production, lymph nodules (w/ germinal centers) appear in the white pulp of the spleen.
PALS w/ secondary follicle

Shown here with “central” artery cut in cross section – note that the CA has been pushed off to the side by the rapid expansion of cells in the germinal center (GC)

RP = red pulp
MZ = marginal zone (antigen presentation)
dashed circle = T-cell rich zone
Scanning EM of a Splenic Sinus (SS) and Cord of Billroth

The cords contain, RBCs, neutrophils (N), macrophages (M), blood platelets (P)

A reticular cell framework (RC) supports the cord. The sinus is bounded by the epithelial cells that form the basket-like structure of the sinus (VS)
Spleen (red pulp) at high power (40x)

- **sinus**
- **cord**

U-M Histology Collection
Percolation of blood into splenic sinuses

Here, you are inside the sinus looking through to the cord, where both a macrophage (M) and a neutrophil (N) are outside the sinus. Note that the endothelial cells have a rodlike appearance.
Splenic sinuses and cords

A. red pulp
B. higher mag of venous sinus and cords of Billroth
C. silver-stained section
D. diagram

discontinuous basement membrane

Image of splenic sinuses and cords removed

SPLEEN: venous sinus showing rodlike endothelial cells
SPLENIC CIRCULATION

Sinuses drain into splenic pulp veins, which, in turn, drain into trabecular veins. Trabecular veins travel within trabeculae and drain into splenic vein at the hilus.
The Thymus

- T-cell education
- Self vs. nonself distinctions
- Cell-mediated immune functions
- Populates effector organs

Lymph nodes
Lymphatic nodules
Spleen
Tonsils

Ross, Fig. 14.1
The Thymus is a Primary Lymphoid (Immune) Organ Responsible For the Education of T-Cells

Located over the great vessels of the heart in the area of the body called the mediastinum.

Develops from an invagination of EPITHELIUM of the 3rd pharyngeal pouch, so an endodermal organ.

Specialized epithelial cells (called epithioreticular cells) that are joined to one another by long processes with desmosomes on the extremities of the cells (like starfish joined together at the tips) make up the bag-like support for:

Lymphocytes that, when the organ is young, fill this “bag”.

NOTE: There are generally no B cells in the Thymus.
The Young Thymus

Surrounded by a CT capsule; cortex has a lot of lymphocytes, fewer in the medulla. THERE ARE NO GERMINAL CENTERS IN THE THYMUS!
Thymic Cortex and Medulla
Thymic (or Hassall’s) Corpuscles
The Thymus undergoes a process called THYMIC INVOLUTION, as T cells leave the thymus to populate other lymphoid effector organs, the organ shrinks, leaving only the epithelioreticular cells.

The young thymus

Thymus at puberty
Overview of T-cell “education”

1. Naïve T-cells enter medulla via diapedesis across venules
2. Pass into cortex to undergo POSITIVE selection:
   • Presented with MHC molecules and self or non-self antigens by ERCs
   • T-cells that recognize MHCs and self/non-self antigens “pass” this selection process and survive (those that don’t undergo apoptosis)
3. Move into medulla to undergo NEGATIVE selection:
   • T-cells that recognize SELF antigens displayed by self MHCs (i.e. are autoreactive”) are eliminated
4. Differentiate into helper (CD4+) or cytotoxic (CD8+) T-cells and leave medulla via diapedesis across venules
Arterioles & capillaries in the thymic cortex are ensheathed by epithelioreticular cells forming a blood-thymus barrier.
Blood-Thymus Barrier

Education of T-cells must occur in a very controlled environment such that antigens are ONLY presented by epithelial reticular cells.

To ensure that no other cells or free antigens are present, there is a very tight BLOOD-THYMUS BARRIER consisting of:

1. The blood capillary wall
   • endothelial cells
   • endothelial cell basal laminae
   • pericytes

2. Perivascular connective tissue
   • type III collagen
   • macrophages

3. Epithelioreticular cell layer
   • basal lamina of the epithelial reticular cells (type I ERCs)
   • epithelial reticular cells

(Note: T-cells can enter thymus ONLY via bloodstream – NO AFFERENT LYMPH VESSELS!)
Thymic Cortex and Medulla
Thymic (or Hassall’s) Corpuscles
Thymic Epithelial Reticular Cells and Macrophages
Thymocytes Differentiating in the Cytoplasmic Folds of Epithelial Reticular Cells
Formation of Tight Junctions, Tonofilaments, and Desmosomes by Thymic Epithelial Reticular Cells
T-cells that survive selection process allowed to cross venule endothelium (INTRAvasation) to enter circulation.
Hassall’s corpuscles

Type VI ERCs; function not very well known, but produce interleukins (such as IL-4 and IL-7) and so likely influence T-cell differentiation.
In the medulla, epithelioreticular cells form onionized structures called Hassall’s corpuscles – quite prevalent in older thymus.

LM view

EM view
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<thead>
<tr>
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<th>Lymph Node</th>
<th>Splenic White Pulp</th>
<th>Thymus</th>
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<tbody>
<tr>
<td>Follicles</td>
<td></td>
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<td>Germinal Centers</td>
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<tr>
<td>Supporting Meshwork</td>
<td>Reticular Cells/Fibers</td>
<td>Reticular Cells/Fibers</td>
<td>Epithelial Reticular Cells</td>
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</tbody>
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