Exercise 23

The Lymphatic System

Laboratory Objectives

On completion of the activities in this exercise, you will be able to:

- List the main functions of the lymphatic system.
- Explain the functional relationship between the lymphatic system and the cardiovascular system.
- Identify the locations of lymphatic organs and nodules in the human body.
- Describe the anatomical relations of lymphatic organs and nodules to adjacent structures.
- Describe the microscopic structure of important lymphatic structures.
- Describe the structure of an antibody molecule.
- Use immunodiffusion (ID) to observe an antigen–antibody reaction.

Materials

- Anatomical models
  - Midsaggital section of the head and neck
  - Human torso
- Compound light microscopes
- Prepared microscope slides
  - Thymus
  - Spleen
  - Lymph node
  - Tonsils
  - Small intestine (ileum)
  - Lymphatic vessels
- Petri dishes divided into three compartments
- Pipettes with rubber bulbs
- Micropipettes with rubber bulbs
- Beakers
- Graduated cylinders
- Hot plates
- Wax marking pencils
- 1% agar, containing 8% sodium chloride (NaCl)
- Animal sera containing natural concentrations of albumins
  - Bovine (cow) serum
  - Horse serum
  - Swine (pig) serum
- Goat antisera containing antibodies for albumins
  - Goat anti-bovine albumin
  - Goat anti-horse albumin
  - Goat anti-swine albumin
- Physiological saline

The lymphatic system consists of an extensive network of **lymphatic vessels** and various **lymphatic organs** and **lymphatic nodules** distributed throughout the body. Functionally, this system is closely connected to the cardiovascular system in the following ways.

- Lymphatic capillaries provide drainage for accumulating fluid that leaks from blood capillaries into the surrounding tissue spaces. This fluid, known as **lymph**, is similar in composition to blood plasma, and contains many **lymphocytes** and smaller numbers of other white blood cells such as neutrophils and monocytes. Lymph is transported along a system of lymphatic vessels and is ultimately returned to the bloodstream.
- After a meal, dietary lipids and lipid-soluble vitamins are absorbed by the small intestine and packaged within vesicles called chylomicrons. Lymphatic vessels transport the chylomicrons, and the fats they contain, to the blood circulation.
- Lymphocytes are found in both the lymphatic and cardiovascular systems. These cells initiate specific immune responses that provide protection for the body against disease and infection.

WHAT'S IN A WORD The word *lymph* is derived from the Latin word *lympha*, which means "clear spring water." Typically, lymph is a clear fluid, although in the small intestine, it has a milky appearance due to the high fat content.

Gross Anatomy of the Lymphatic System

An overview of the lymphatic system is illustrated in Figure 23.1. The system includes the following components.

- Lymphatic organs are organized areas of lymphoid tissue surrounded by a connective tissue capsule. These structures include the **lymph nodes**, **spleen**, and **thymus**.
- Lymphatic nodules are concentrated regions of lymphoid tissue that lack a connective tissue capsule. They are found in the walls of the respiratory, digestive, and urinary tracts. Examples include the **tonsils** in the wall of the pharynx, **aggregated lymphoid nodules (Peyer's patches)** in the wall of the small intestine, and the nodules located in the **appendix**.
- The **lymphatic circulation**, which transports lymph, includes **lymphatic capillaries**, **lymphatic collecting vessels**, **lymphatic trunks**, and **lymphatic ducts**.

ACTIVITY 23.1 Examining the Gross Anatomical Structure of the Lymphatic System

Lymphatic Organs

1. Obtain a torso model and remove the anterior body wall to expose the organs in the thoracic and abdominopelvic cavities.
2. Identify the following lymphatic organs.

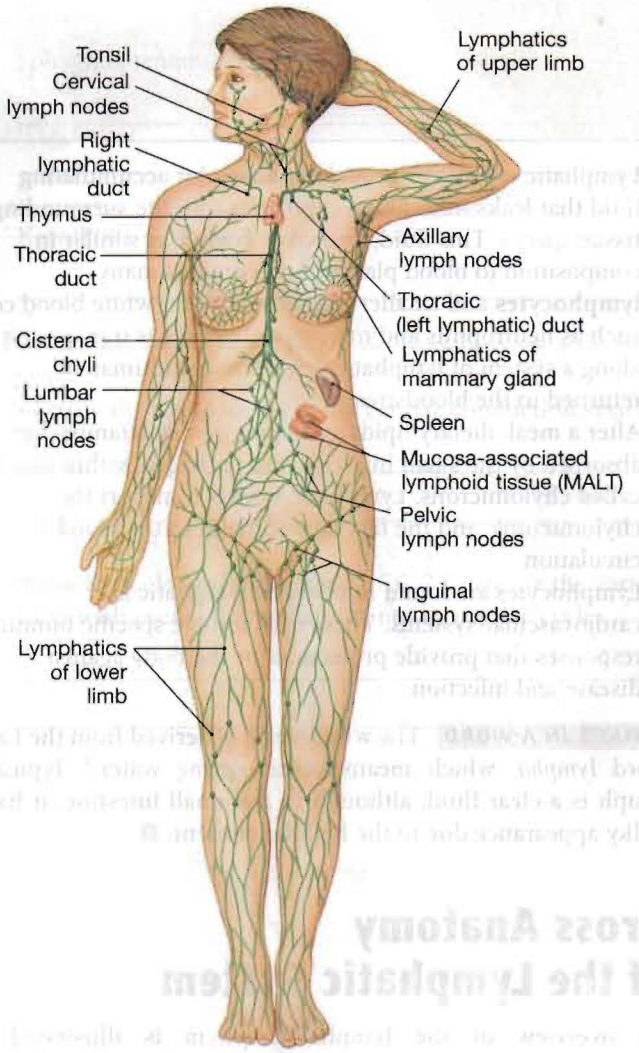

Figure 23.1 Overview of the lymphatic system. Components of the lymphatic system include lymphoid organs, lymphoid nodules, and lymphatic vessels.

- The thymus gland is a bilobed organ located in the mediastinum. If present on the models in your lab, observe how it covers the superior portion of the heart and the great vessels. Also notice that it extends superiorly into the base of the neck, where it blankets the inferior end of the trachea (Figure 23.2a). As you learned earlier when you studied the endocrine system (Exercise 18), the thymus produces a group of hormones called **thymosins** (thymic hormones). Lymphoid stem cells from bone marrow migrate to the thymus and, under the influence of these hormones, become mature **T lymphocytes (T cells)**. From the thymus, mature cells are transported by the bloodstream to the spleen, lymph nodes, and other lymphoid structures, thus establishing T-cell populations throughout the body. These populations are vital for coordinating the body's immune response against diseases and infections. The thymus is most active during childhood and adolescence. Consequently, it is relatively large in newborns and young children. Shortly after puberty, however, the organ becomes less active and begins to atrophy. As the thymus progressively degenerates, glandular tissue is gradually replaced by fat and fibrous connective tissue.

WHAT'S IN A WORD The term *thymus* is the Latin word for *sweetbread*. If you go to a restaurant and order sweetbread from the menu, you will probably be eating the thymus gland.

CLINICAL CORRELATION

The primary target for the **human immunodeficiency virus (HIV),** the virus that causes AIDS, is a type of T lymphocyte called a **T-helper cell.** These cells are needed to stimulate the actions of all other immune functions. As HIV disease progresses to AIDS, the T-helper cell population declines and the body's immune system is suppressed. As a result, an individual's ability to fight off infections (opportunistic infections) is compromised. AIDS-related deaths occur when individuals succumb to infections that a suppressed immune system can no longer control.

- Locate the spleen in the upper left quadrant of the abdominal cavity. It is posterolateral to the stomach and is positioned between ribs 9 and 11 along the posterior body wall (Figures 23.1 and 23.3a). Remove the stomach to expose the anterior or visceral surface of the spleen. Along the visceral surface, locate the **hilus**, an area where blood vessels, lymphatic vessels, and nerves enter and exit (Figure 23.3b). With the stomach removed, notice that the spleen is just lateral to the left kidney and the tail of the pancreas (Figure 23.3a).

  The spleen performs the following important functions that define its close relationship to the blood.

  - It acts as a "blood filter." Macrophages and monocytes in the spleen will attack and eliminate bacteria, viruses, or other infectious agents that are present in the blood. In addition, lymphocytes are activated to begin a specific immune response.
  - Splenic macrophages break down old red blood cells and platelets, and the components are ultimately recycled.
  - It produces lymphocytes in the adult and red blood cells during fetal development.
  - It serves as a blood reservoir by storing red blood cells and platelets that can be added to the circulation when needed.
- On a torso model, identify lymph nodes in the axillary or inguinal regions. Notice how they are positioned along the course of lymphatic collecting vessels, like beads on a string (Figures 23.1 and 23.4a). Lymph nodes are ovoid structures that are typically less than 2.5 cm in length. They are found deep and superficially throughout the body, but are aggregated into clusters in specific body regions such as the axilla (axillary lymph nodes) and groin (inguinal lymph nodes). Lymph is constantly passing through lymph nodes. As the fluid percolates through the network of lymph sinuses, macrophages attack any foreign microorganisms that might be present. In addition,

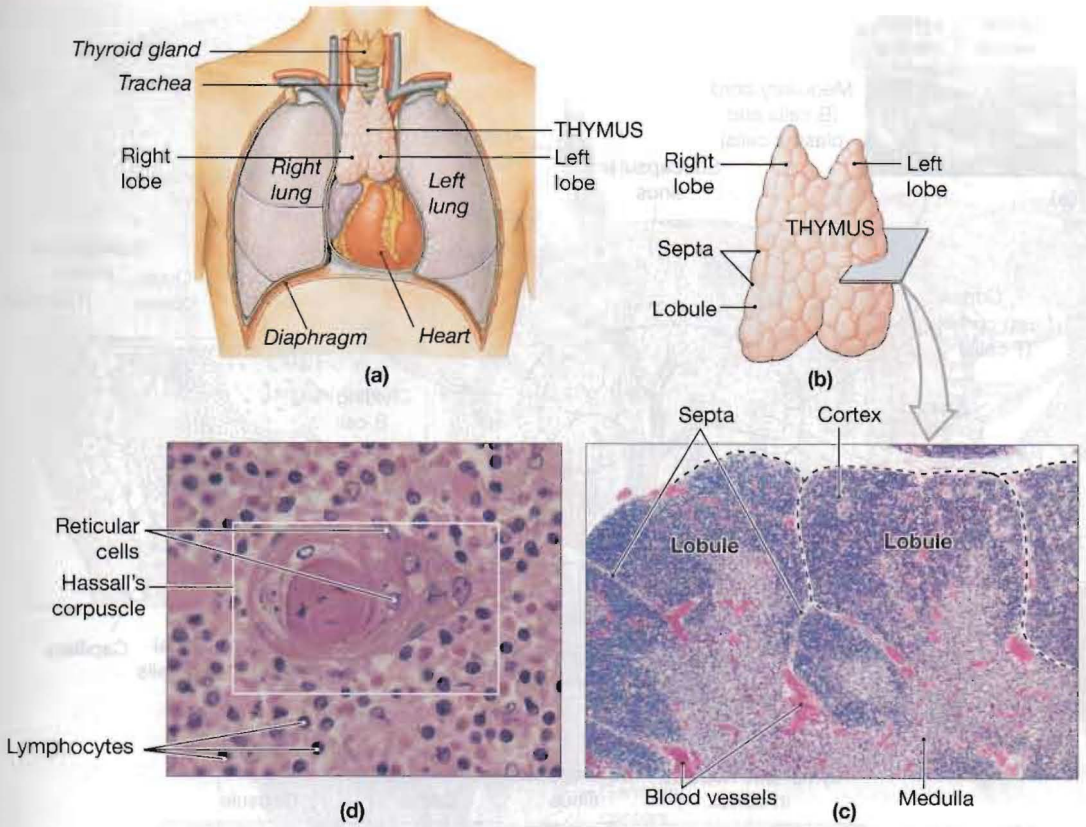

Figure 23.2 Anatomy of the thymus. a) Position of the thymus in relation to the heart and trachea; **b)** the thymus, in isolation, illustrating its bilobed appearance; **c)** low-power light micrograph illustrating the microscopic structure of the thymus (LM × 40); **d)** high-power light micrograph showing a Hassall's body surrounded by lymphocytes (LM × 400).

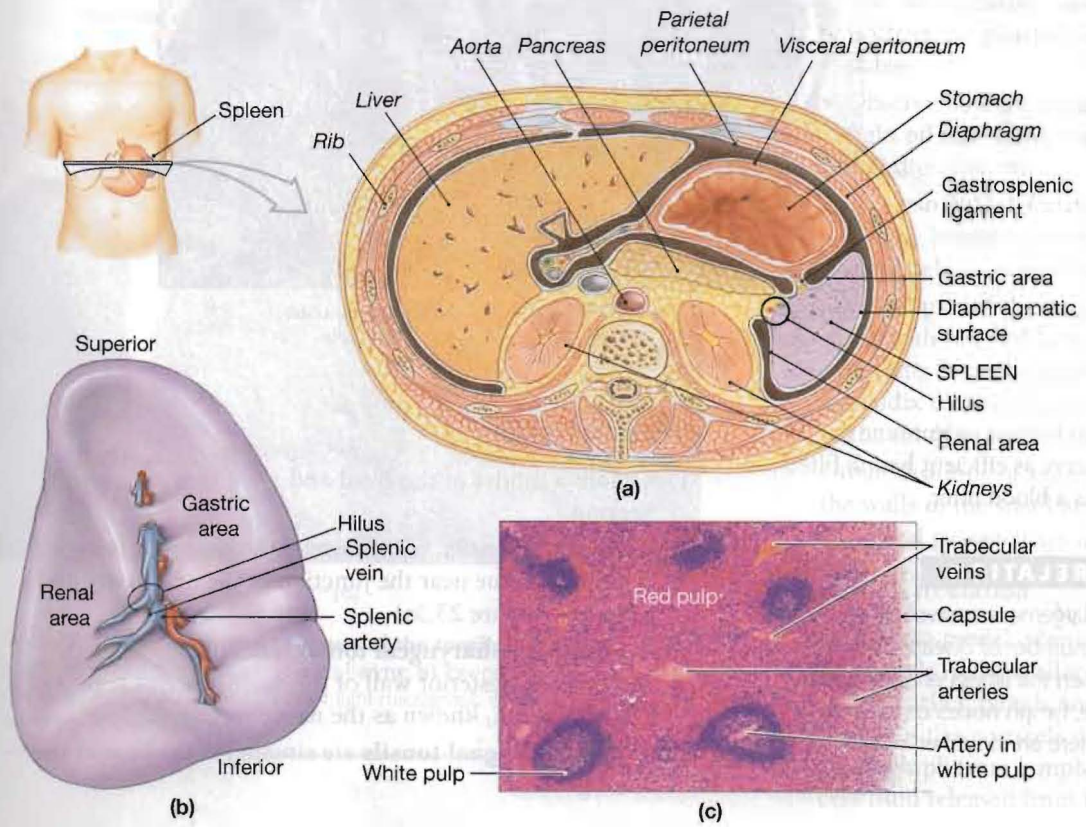

Figure 23.3 Anatomy of the spleen. a) Cross section of the abdominal cavity showing the position of the spleen in relation to adjacent organs; **b)** visceral surface of the spleen showing the location of the hilus; **c)** light micrograph of the spleen illustrating areas of white and red pulp (LM × 40).

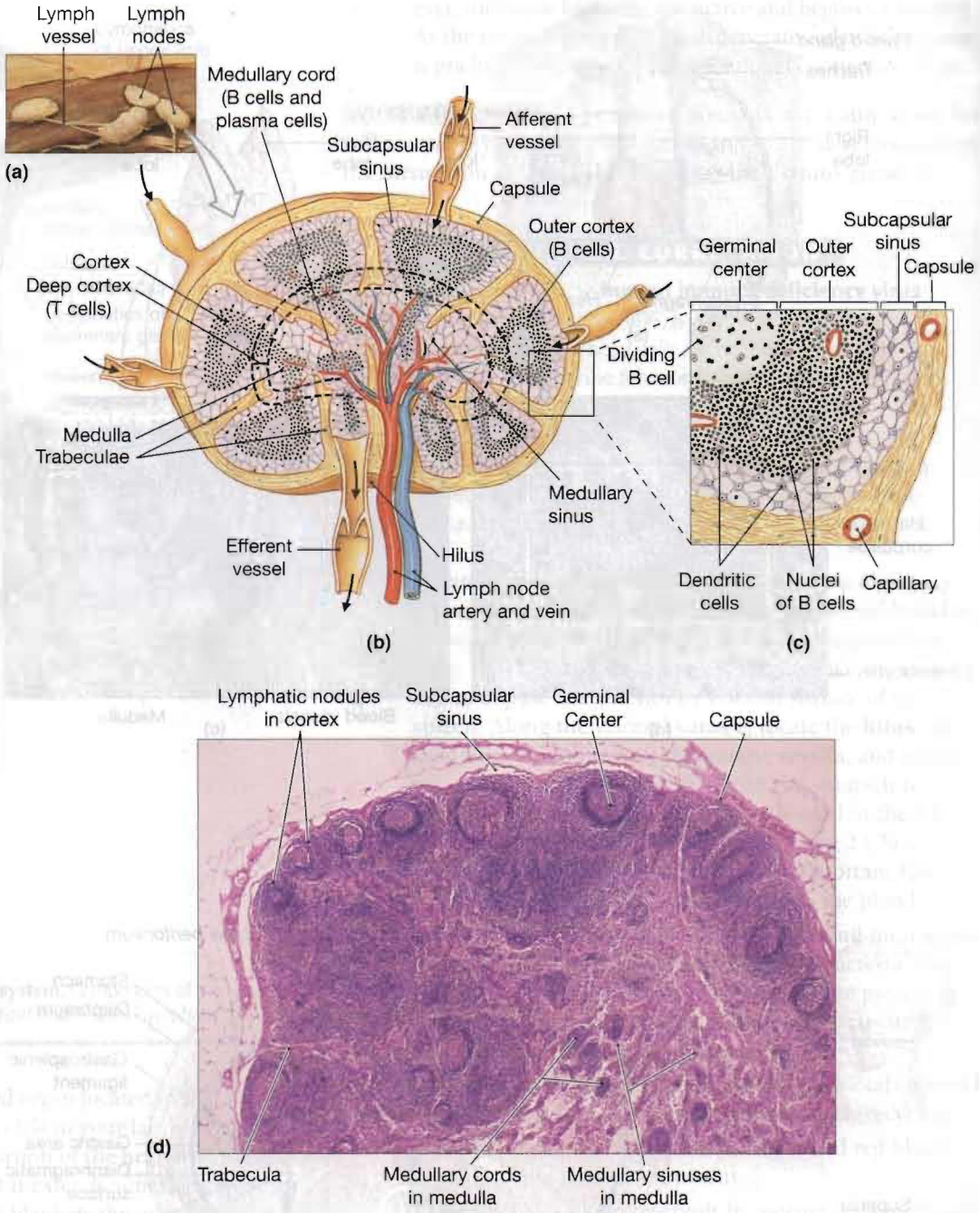

Figure 23.4 Structure of a lymph node. a) Lymph nodes positioned along the course of lymphatic vessels. **b)** Illustration of the microscopic structure of a lymph node. **c)** Illustration of the area enclosed by the box in (b). A closer view of the cortex with part of a germinal center is shown. **d)** Low-power light micrograph of a lymph node illustrating structures in the cortex and medulla (LM × 40).

T lymphocytes are activated to initiate an immune response. Thus, lymph nodes serve as efficient lymph filters, much like the spleen serves as a blood filter.

CLINICAL CORRELATION

Lymphadenopathy refers to an enlargement or swelling of lymph nodes. It can be caused by a number of diseases, and in most cases, the swelling declines when the illness ends. However, for people who are HIV positive, lymph nodes can remain swollen for several months even if there are no other indications or symptoms of a disease.

Lymphatic Nodules

1. Obtain a model of the head and neck in a midsagittal section.
2. Identify the tonsils, which form a discontinuous ring of lymphoid tissue near the junction of the oral cavity and pharynx (Figure 23.5a).
   - The single **pharyngeal tonsil (adenoid)** is located along the posterior wall of the most superior region of the pharynx, known as the nasopharynx.
   - The two **lingual tonsils** are situated at the base of the tongue.

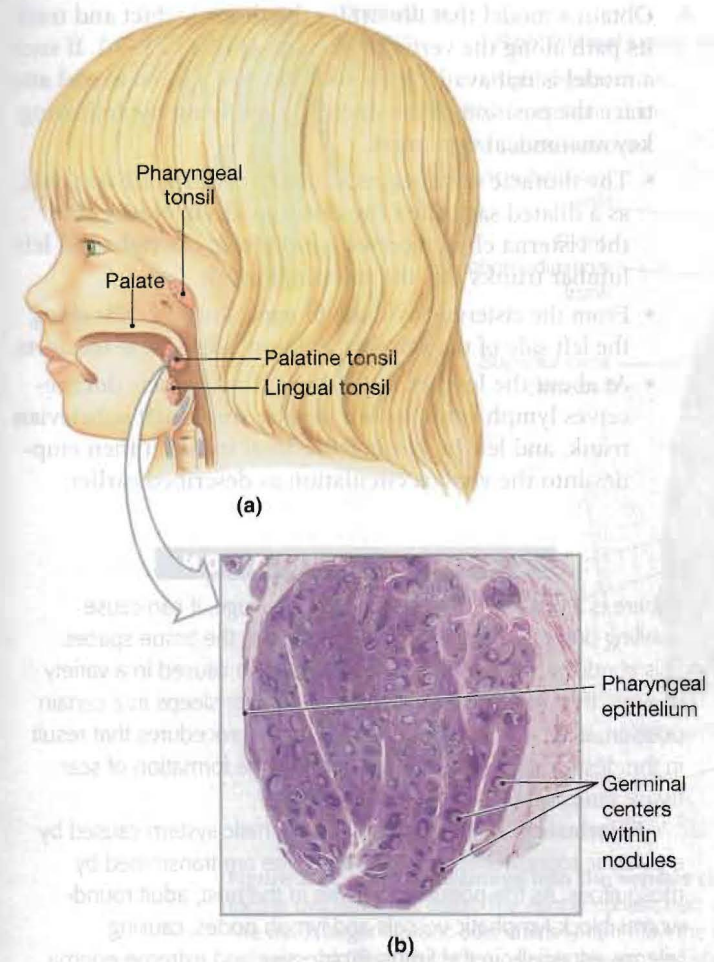

Figure 23.5 Location and structure of the tonsils. a) Illustration showing the positions of the tonsils in the pharynx; **b)** low-power light micrograph of a tonsil (LM × 40); **c)** high-power light micrograph illustrating germinal centers in a tonsil (LM × 100).

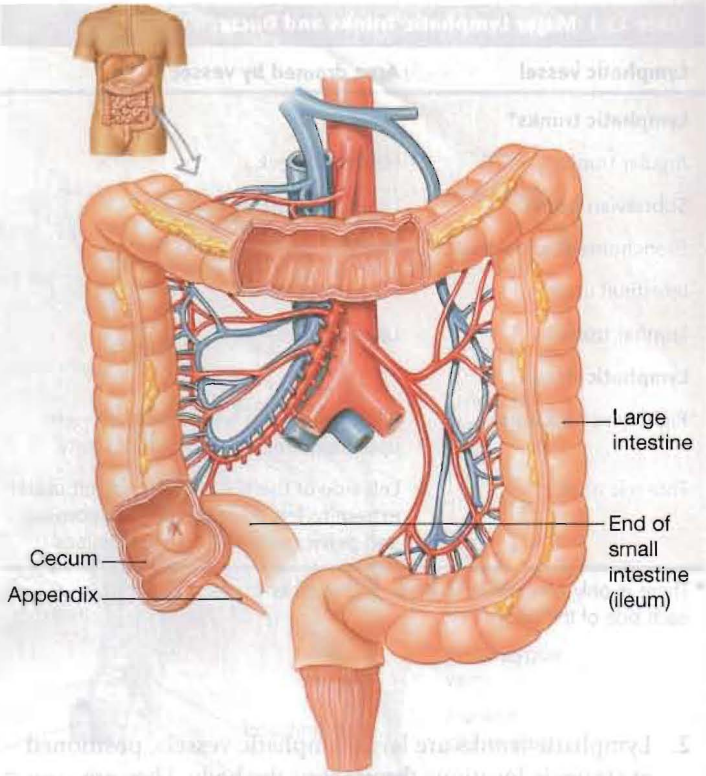

Figure 23.6 Location of the appendix. The appendix extends from the wall of the cecum, near the junction of the small and large intestines.

- The two **palatine tonsils** are found along the lateral walls of the pharynx near the posterior end of the palate.
- Observe how the tonsils are strategically positioned to fight off infections that develop in the upper respiratory and digestive tracts.

3. On a torso model, locate the junction between the small and large intestines in the lower right quadrant of the abdominopelvic cavity (Figure 23.6).
4. The first part of the large intestine is called the cecum. Identify the wormlike appendage, known as the appendix, extending from the wall of the cecum (Figure 23.6). The appendix contains aggregated lymphoid nodules, similar to the tonsils.
5. Other locations of aggregated lymphoid nodules include the walls of the small and large intestines and the bronchial passageways in the lungs.

Lymphatic Circulation

1. On a torso model, identify networks of lymphatic collecting vessels in the axillary or inguinal regions. As you observed earlier, lymph nodes are distributed along the course of these vessels. Lymphatic collecting vessels receive lymph from lymphatic capillaries, which drain excess fluid released from blood capillaries.

Table 23.1 Major Lymphatic Trunks and Ducts

Lymphatic vessel	Area drained by vessel
Lymphatic trunks*	
Jugular trunks	Head and neck
Subclavian trunks	Upper extremities
Bronchomediastinal trunks	Thoracic cavity
Intestinal trunk	Abdominal cavity
Lumbar trunks	Lower extremities
Lymphatic ducts	
Right lymphatic trunk	Right side of the head and neck, right upper extremity, right thoracic cavity
Thoracic trunk	Left side of the head and neck, left upper extremity, left thoracic cavity, abdominal and pelvic cavities, lower extremities

* There is only one intestinal trunk. All the other trunks occur in pairs—one on each side of the body.

2. Lymphatic trunks are large lymphatic vessels, positioned at strategic locations throughout the body. They are formed by the union of several collecting vessels and drain the lymph from a specific region of the body. Review the regions that are drained by each trunk (Table 23.1). Identify three structures from which the following lymphatic trunks will receive lymph.
   - Intestinal trunk _____
   - Jugular trunk _____
   - Lumbar trunk _____
   - Bronchomediastinal trunk _____
   - Subclavian trunk _____
3. The lymphatic trunks unite to form one of two **lymphatic ducts**, which drain lymph into the venous circulation. On each side of a torso model, find the location where the subclavian and internal jugular veins join to form the brachiocephalic vein (Figure 23.7b). On the right side, the **right lymphatic duct** empties into the bloodstream near this venous junction (Figure 23.7b). This duct drains lymph from the upper right quadrant of the body (Figure 23.7a; Table 23.1). The much larger **thoracic duct** has a similar drainage pattern on the left side (Figure 23.7b). It drains lymph from all other body regions (Figure 23.7a; Table 23.1).
4. Obtain a model that illustrates the thoracic duct and trace its path along the vertebral column (Figure 23.7b). If such a model is not available in your lab, use a torso model and trace the position of the duct by identifying the following key anatomical structures.
   - The thoracic duct begins at the level of the L2 vertebra, as a dilated sac called the **cisterna chyli**. Notice that the cisterna chyli receives lymph from the right and left lumbar trunks and the intestinal trunk.
   - From the cisterna chyli, the thoracic duct ascends along the left side of the vertebral column, adjacent to the aorta.
   - At about the level of the clavicle, the thoracic duct receives lymph from the left jugular trunk, left subclavian trunk, and left bronchomediastinal trunk. It then empties into the venous circulation as described earlier.

CLINICAL CORRELATION

If there is a blockage of normal lymph drainage, it can cause swelling due to the accumulation of fluids in the tissue spaces. This condition, referred to as **edema,** can be caused in a variety of ways. If an individual wears tight clothing or sleeps in a certain position, edema will be temporary. Surgical procedures that result in the destruction of lymphatic vessels or the formation of scar tissue can cause a more chronic edema.

Filiariasis is an infection of the lymphatic system caused by a parasitic roundworm. Roundworm larvae are transmitted by mosquitoes. As the population grows in the host, adult roundworms block lymphatic vessels and lymph nodes, causing edema, especially in the limbs. Progressive and extreme edema of the limbs and external genitalia, as a result of this parasitic infection, is called **elephantiasis.**

QUESTIONS TO CONSIDER

1. What would be the effect on the immune system if the thymus was not functioning normally during childhood?

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2. Why is it important to have an abundance of lymphatic nodules located along the respiratory and digestive tracts?

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Microscopic Anatomy of the Lymphatic System

Lymphoid tissue is a specialized form of loose connective tissue that consists of a dense network of **reticular fibers** and a variety of cell types that are largely involved in protecting the body from infection and disease. Lymphocytes are the principal cells found in lymphoid tissue. T lymphocytes are responsible for the **cell-mediated immune response**. They directly attack and destroy

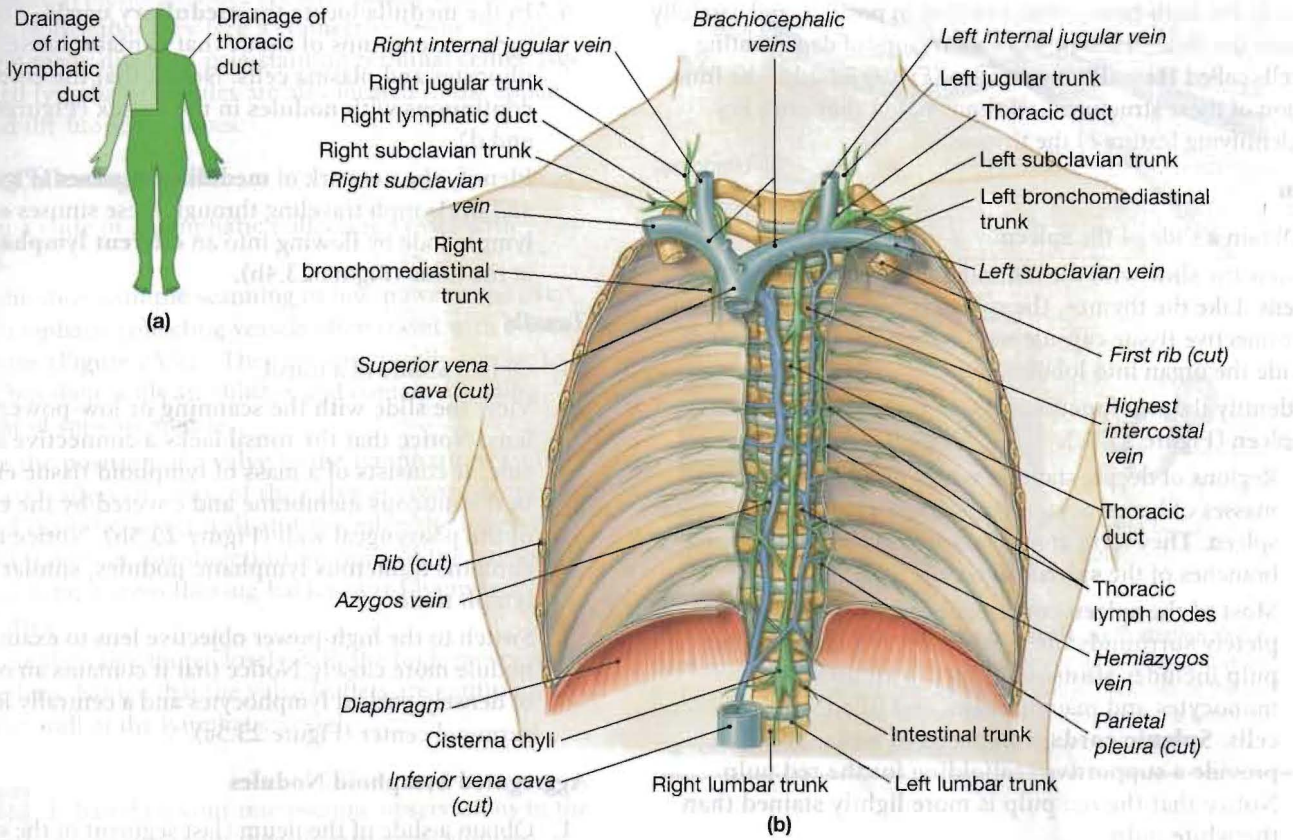

Figure 23.7 Lymph drainage into the venous circulation. a) Illustration of the lymphatic drainage areas for the two lymphatic ducts. The right lymphatic duct drains lymph from the upper right quadrant of the body. The much larger thoracic duct drains lymph from the upper left quadrant and all regions inferior to the diaphragm. **b)** Diagram of the posterior thoracic wall showing the drainage of lymph into the bloodstream. On the right side of the body, the right lymphatic duct empties into the venous circulation near the junction of the subclavian and internal jugular veins. The thoracic duct follows a similar pattern on the left side.

foreign microorganisms by phagocytosis or by releasing chemicals. One type of T lymphocyte, the **T-helper cell**, activates all immune activity. **B lymphocytes** initiate the **humoral immune response** by differentiating into **plasma cells**, which produce **antibodies**. As they circulate through the blood and lymph, antibodies bind to and destroy foreign **antigens**, such as disease-causing microorganisms, certain foods and drugs, or transplanted organs. Other cells found in lymphoid tissue include **macrophages** that attack foreign cells by phagocytosis and activate T lymphocytes, **reticular cells** that produce the reticular fibers, and **dendritic cells** that also play a role in T-lymphocyte activation.

CLINICAL CORRELATION

Even when the tissue match between donor and recipient is very close, a transplanted organ is recognized as foreign tissue and it will induce an immune response. To reduce the risk of tissue rejection, patients who receive an organ transplant must take drugs that suppress the immune system. The danger of taking these immunosuppressant drugs is that it increases an individual's vulnerability to disease and infection.

ACTIVITY 23.2 Examining the Microscopic Structure of Lymphatic Structures

Thymus

1. Obtain a slide of the thymus.
2. View the slide with the scanning or low-power objective lens. Notice that the thymus is covered by a connective tissue capsule. The capsule gives rise to connective tissue septa that subdivide each lobe (Figure 23.2b) of the thymus into lobules (Figure 23.2c). Each lobule consists of a dark-staining outer **cortex** with a dense population of lymphocytes, and a light-staining inner **medulla** with far fewer lymphocytes (Figure 23.2c).
3. Use the high-power objective lens to examine the thymus more closely, and identify the dense aggregations of T lymphocytes. In a standard staining preparation, these cells will have round, deeply stained nuclei, surrounded by a lightly stained cytoplasm. Other cells, called **thymocytes**, produce the thymic hormones. They will be difficult to find.

4. Keep the high-power objective lens in position and carefully scan the slide. Attempt to locate regions of degenerating cells called **Hassall's corpuscles** (Figure 23.2d). The function of these structures is unknown, but they are a key identifying feature of the thymus.

Spleen

1. Obtain a slide of the spleen.
2. View the slide with the scanning or low-power objective lens. Like the thymus, the spleen is surrounded by a thin connective tissue capsule with trabeculae (septa) that divide the organ into lobules.
3. Identify the two functional tissue components of the spleen (Figure 23.3c).
   - Regions of deeply staining **white pulp** are compact masses of lymphocytes that are scattered throughout the spleen. They form around **central arteries**, which are branches of the **splenic artery**.
   - Most of the spleen consists of **red pulp**, which completely surrounds the areas of white pulp. The red pulp includes **sinusoids**, lined with numerous monocytes and macrophages, and filled with red blood cells. **Splenic cords**, composed of reticular tissue, provide a supportive scaffolding for the red pulp. Notice that the red pulp is more lightly stained than the white pulp.
4. Move the slide so that an area of white pulp is in the center of the field of view. Switch to the high-power objective lens to examine the region more closely. Identify the dense aggregations of lymphocytes. As in the thymus, these cells can be detected by their deeply stained nuclei.
5. With the low-power objective lens in place, move the slide to an area of red pulp. Switch back to high power and observe the vast network of sinusoids. Most of the important functions of the spleen occur in the red pulp as blood flows through the sinusoids.

Lymph Node

1. Obtain a slide of a lymph node.
2. View the slide with the scanning or low-power objective lens. Locate the connective tissue capsule that surrounds the entire node and the trabeculae that extend inward and form partitions (Figures 23.4b and d). Just beneath the capsule, identify a **subcapsular sinus**. **Afferent lymphatic vessels** deliver lymph to a node by draining into these sinuses (Figure 23.4b).
3. Like the thymus, lymph nodes contain two distinct regions: the outer cortex and the inner medulla (Figures 23.4b and d). Identify these two regions.
4. In the cortex, identify the lymphatic nodules. Each nodule is an aggregation of lymphocytes. In the center are the lighter staining **germinal centers**, where new lymphocytes are produced (Figures 23.4b, c, and d).
5. In the medulla locate the **medullary cords**. These structures are columns of tissue that contain masses of lymphocytes and plasma cells. Notice that the cords are continuous with nodules in the cortex (Figures 23.4b and d).
6. Identify the network of **medullary sinuses** (Figures 23.4b and d). Lymph traveling through these sinuses exits a lymph node by flowing into an **efferent lymphatic vessel** at the hilus (Figure 23.4b).

Tonsils

1. Obtain a slide of a tonsil.
2. View the slide with the scanning or low-power objective lens. Notice that the tonsil lacks a connective tissue capsule. It consists of a mass of lymphoid tissue embedded in the mucous membrane and covered by the epithelium of the pharyngeal wall (Figure 23.5b). Notice that it contains numerous lymphatic nodules, similar to a lymph node.
3. Switch to the high-power objective lens to examine the nodule more closely. Notice that it contains an outer ring of densely packed lymphocytes and a centrally located germinal center (Figure 23.5c).

Aggregated Lymphoid Nodules

1. Obtain a slide of the ileum (last segment of the small intestine) or the large intestine (colon).
2. View the slide with the scanning or low-power objective lens. Scan the slide and locate large masses of deeply stained lymphocytes. These regions are called aggregated lymphoid nodules (Figure 23.8). In the ileum, these structures are called Peyer's patches.

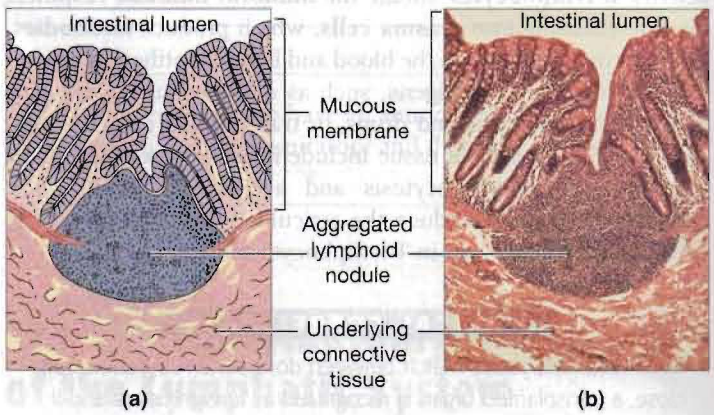

Figure 23.8 Microscopic structure of aggregated lymphoid nodules. **a)** Illustration of a lymphoid nodule in the intestinal wall; **b)** corresponding light micrograph (LM × 20).

3. Aggregated lymphoid tissues are structurally similar to tonsils. Notice that they lack a connective tissue capsule and that many display a pale-staining germinal center. Aggregated lymphatic nodules are also located in the appendix and the bronchial tubes.

Lymphatic Collecting Vessels

1. Obtain a slide of a lymphatic collecting vessel with valves.
2. View the slide with the scanning or low-power objective lens. Lymphatic collecting vessels often travel with arteries and veins (Figure 23.9a). They are structurally similar to veins, but their walls are thinner and contain a smaller amount of smooth muscle.
3. Locate the position of a valve in the lymphatic vessel. The two leaflets or cusps of the valve are more deeply stained than the vessel wall and resemble the letter V. Lymph travels in one direction. Actions of the valves prevent lymph from flowing back toward lymphatic capillaries.
4. Examine the valve more closely with the high-power objective lens. Notice that the valve leaflets are continuous with the wall of the lymphatic vessel.

QUESTIONS TO CONSIDER

1. Based on your microscopic observations in the previous activity, identify structural similarities and differences in the various lymphatic structures. Focus your attention on the arrangement and structure of the lymphoid tissue in each structure.

Similarities:

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_____

Differences:

_____

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2. From your microscopic observations of the spleen, you know that the white pulp does not appear white. Why do you think these splenic regions are called "white" pulp?

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3. In the previous activity, you observed lymphatic valves, which prevent the backflow of lymph. Why is it important that lymph not flow back toward lymphatic capillaries?

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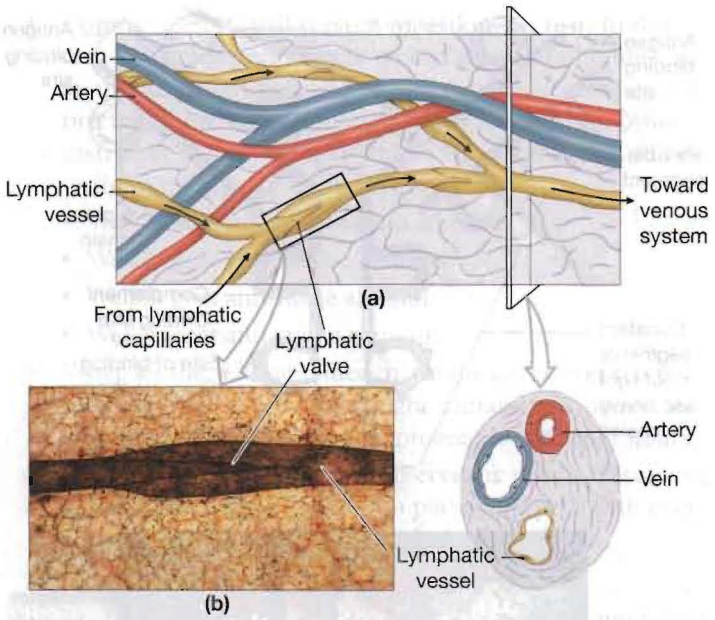

Figure 23.9 Lymphatic collecting vessels. a) Illustration showing relationship of lymphatic collecting vessels with small arteries and veins. The accompanying cross section compares the relative thickness of the walls of an artery, vein, and lymphatic vessel. **b)** Light micrograph of a lymphatic collecting vessel, illustrating the position of a lymphatic valve (LM × 40).

The Antigen-Antibody Reaction

Antibodies are protein molecules known as **immunoglobulins (Ig)** that act to eliminate foreign tissues, cells, or microbes, known as antigens. The typical antibody is a Y-shaped molecule that consists of two large polypeptides, the **heavy chains**, and two small polypeptides, the **light chains** (Figures 23.10a and b). The heavy and light chains contribute to the formation of one **constant segment** and two **variable segments**. The constant segment comprises the base and the first part of each arm on the antibody molecule (Figure 23.10a). The structure of these regions is used as one criterion to categorize antibodies into five distinct classes: IgG, IgE, IgD, IgM, and IgA. Within each class, the amino acid sequences in the constant segments are identical.

The variable segments are located at the ends of each arm on the antibody molecule, and contain the **antigen binding sites** (Figure 23.10a). The changeable structure of these regions is the principal reason for the specificity of the **antigen-antibody reaction**. During an antigen-antibody reaction, antigen binding sites on the antibody bind to **antigenic determinant sites** on the antigen, forming an **antigen-antibody complex** (Figure 23.10c). For each type of antigen that gains entrance into the body, a unique antibody is produced to fight it off. The high degree of specificity is due to the three-dimensional molecular structure at the binding sites that requires a precise fit between antigen and antibody.

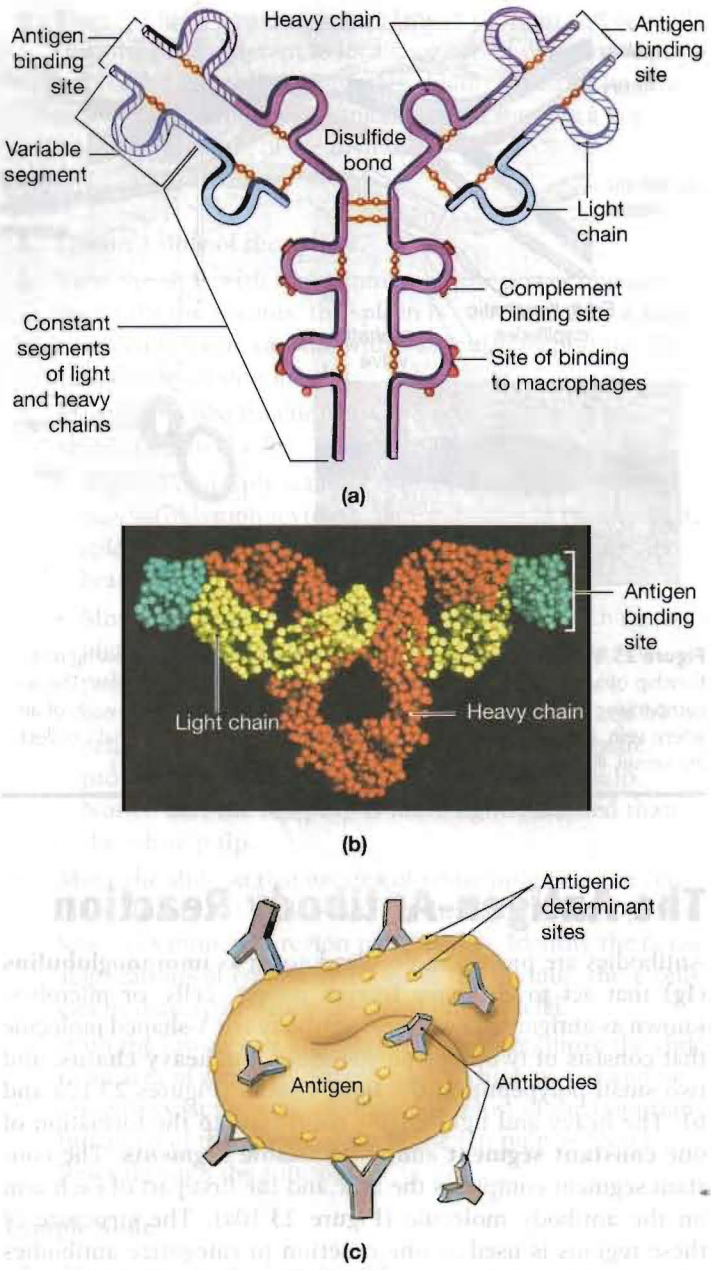

Figure 23.10 Structure of an antibody. a) Diagram showing the Y-shaped configuration of an antibody; **b)** computer-generated image of an antibody molecule; **c)** diagram showing an antigen-antibody complex. Notice that several antibodies can bind to a single antigen.

In the following activity you will use **immunodiffusion** (ID) or the **Ouchterlony technique** to detect the presence of antigens. Antigens and antibodies will be allowed to diffuse toward each other through a saline agar gel in petri dishes. A **line of identity**, or **precipitin line**, will form if a specific antigen-antibody reaction occurs.

CLINICAL CORRELATION

Typically, the immune system attacks cells and tissues that are foreign to the body. However, if immune function is not operating normally, an **autoimmune response** can be triggered during which antibodies against the body's own tissues are produced. Examples of **autoimmune diseases** include Type I diabetes in which antibodies attack the insulin-producing beta cells in the pancreatic islets, rheumatoid arthritis in which connective tissues around joints are destroyed, and multiple sclerosis in which the myelin sheath is targeted.

ACTIVITY 23.3 Observing the Antigen-Antibody Reaction Using Immunodiffusion

Preparing the Petri Dishes

1. Prepare an 8% sodium chloride (NaCl) solution in distilled water.
2. Use the NaCl solution to make a 1% agar mixture.
3. Place the agar mixture in a gently boiling water bath and stir occasionally until the agar has completely dissolved.
4. Obtain a petri dish that is divided into three compartments. Alternatively, use a petri dish with a single compartment and mark off three sections with a wax marking pencil as illustrated in Figure 23.11. Label the three compartments with the letters A, B, and C.
5. Pour 6 ml of the warm agar mixture equally into the three petri dish compartments.

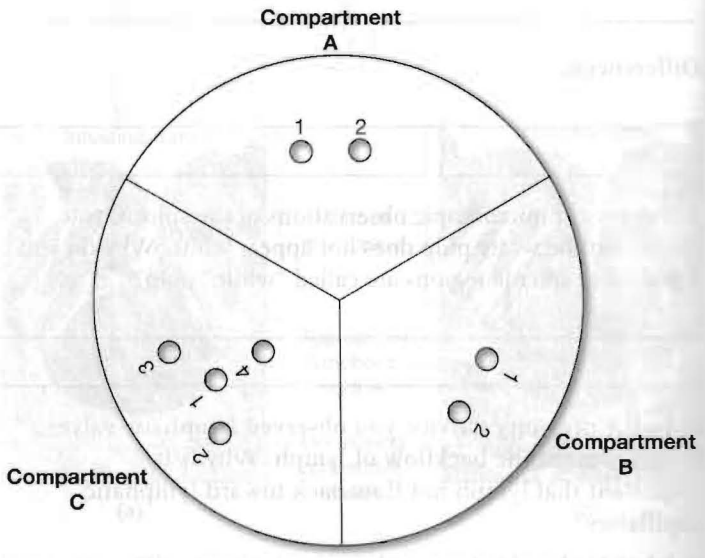

Figure 23.11 Configuration of sample wells for immunodiffusion. Compartments A and B will be used for control tests. Compartment C will be used to determine the identity of an unknown antigen.

6. When the agar has solidified, you will cut out sample wells, 4 mm in diameter, in the agar. Each small circle in Figure 23.11 marks a location where a sample well will be positioned. Place the petri dish over the figure to guide you during this process. Alternatively, you can place a copy of Figure 23.11 on your lab bench and use it as your template.
7. To make the sample wells, use the following microsuction technique.
   a. Squeeze the rubber bulb of a glass pipette or medicine dropper and lower it in a vertical position until the tip is just touching the surface of the agar.
   b. Simultaneously, release the bulb and plunge the pipette into the agar to the bottom of the petri dish. Keep the pipette in a vertical position at all times. As you perform this action, a small amount of agar will be suctioned into the pipette.
   c. Carefully lift the pipette, vertically, out of the newly formed sample well. As you do this, be careful not to damage the sides of the well or to lift the agar off the bottom of the petri dish. If this occurs, there is a risk that your sample will leak into adjacent wells.
8. The petri dish can be prepared in advance and refrigerated in an airtight container. To store for longer periods, place damp paper towels along the bottom of the container. The moisture will extend the storage time.

Preparing the Animal Sera

1. Obtain bovine (cow), horse, and swine (pig) sera, each containing natural concentrations of albumins.
2. Dilute each serum to a 20% solution in physiological saline.
3. The sera can be prepared in advance and refrigerated in covered flasks or bottles until they are used.

Filling the Sample Wells

1. Fine-tipped pipettes or micropipettes should be used when filling the wells. Be sure to use a different pipette for each solution. Fill each well with equal amounts of solution, but avoid overfilling.
2. Compartment A in the petri dish will serve as a **positive control.** In this test, you will expect positive results by observing a precipitin line. Fill the wells according to the following list.
   - Well 1: 20% horse serum albumin
   - Well 2: goat anti-horse albumin
3. Compartment B will serve as a **negative control.** In this test, you will expect negative results. A precipitin line will not appear. Fill the wells according to the following list.
   - Well 1: 20% horse serum albumin
   - Well 2: goat anti-bovine albumin
4. Compartment C will serve as an **unknown test.** In this test, you will determine the type of antigen (albumin) in an unknown serum based on the presence or absence of a precipitin line when it reacts with various antisera. Your instructor will give you an unknown serum to test. Fill the wells according to the following list.
   - Well 1: 20% unknown serum albumin
   - Well 2: goat anti-bovine albumin
   - Well 3: goat anti-horse albumin
   - Well 4: goat anti-swine albumin
5. When all the wells are filled, place the cover over the petri dish and store in a moist, airtight container at room temperature. Allow incubation to proceed for 16 to 24 hours.
6. After the incubation period, observe the results. Precipitin lines will be easier to see if you place your petri dish over a light source. Record your results in Table 23.2.

QUESTIONS TO CONSIDER

1. Why does a precipitin line form (or not form) between two wells?

2. Explain why compartment A in the petri dish is a positive control test, and compartment B is a negative control test.

3. What antigen was present in your unknown serum? How did your results allow you to determine the answer?

Table 23.2 Results of Immunodiffusion to Detect the Presence of Antigens

Test	Presence of a precipitin line (+/−)	Comments
	Between Sample Wells	
A. Positive control	1 & 2: _____	
B. Negative control	1 & 2: _____	
C. Unknown test	1 & 2: _____ 1 & 3: _____ 1 & 4: _____	

Exercise 23 Review Sheet

The Lymphatic System

Name _____

Lab Section _____

Date _____

1. What is the main structural difference between a lymphatic organ and a lymphatic nodule?

2. Explain the meaning of the following statement: *The spleen acts as a blood filter and lymph nodes act as lymph filters.*

3. Explain why normal lymphatic circulation is important.

4. Explain why the thymus is both an endocrine and a lymphatic organ.

5. Briefly explain the difference between the cell-mediated immune response and the humoral immune response.

Questions 6–10: Define the following terms.

6. Aggregated lymphoid nodules

7. Cisterna chyli

8. White pulp versus red pulp

9. Afferent lymphatic vessel versus efferent lymphatic vessel

10. Germinal center

11. Describe the basic structure of an antibody molecule.

12. What is an antigen-antibody complex? Explain why the reaction between an antigen and antibody is very specific.