EMBRYOLOGY

Definition

- Spleen is hematopoietic and lymphoid organ derived from embryonic mesoderm
  - Largest organ of reticuloendothelial (lymphatic) system
  - Largest secondary lymphoid organ in body

Splenic Morphogenesis

- Recognized as bulge in dorsal mesogastrium at 33-41 days of gestation
  - Condensation consists of long strip of mesenchymal cells adjacent to forming stomach above developing pancreas
  - Dorsal to greater curvature of stomach
    - Embryo at around 8 mm of crown to rump length
- Vessels are observed at around 33 days
  - Arises initially from branches of dorsal aorta
- Mesenchymal cells are differentiated from dorsal mesogastrium
  - Mesenchymal cells eventually differentiate to form capsule, trabeculae, and reticular framework
- Hematopoietic cells becomes detectable after 44 days
- Splenic sinus formation starts around 49 days
- Splenic hilum formation becomes evident after 49 days
- Spleen becomes apparent after 49 days of embryonic age
  - Parallel arteries and veins are observed around 56 days
  - Splenic lobules eventually fuse, and spleen acquires smooth, nonlobulated appearance
- Rotation of stomach carries spleen to left side of stomach
  - Situated between origin of root of mesentery from anterior aspect of left kidney and stomach itself
- Initially, spleen has role in hematopoiesis, then in immune system development
  - Begin in 2nd month of fetal development
  - Functions as hematopoietic center until late fetal life
  - Cells required for hematopoietic function arise from yolk sac wall and near dorsal aorta
  - Hematopoiesis in spleen stops toward 3rd trimester and then moves to bone marrow
  - T and B lymphocytes migrate to spleen and populate white pulp associated with trabeculae
- Later, spleen plays significant role in immune system

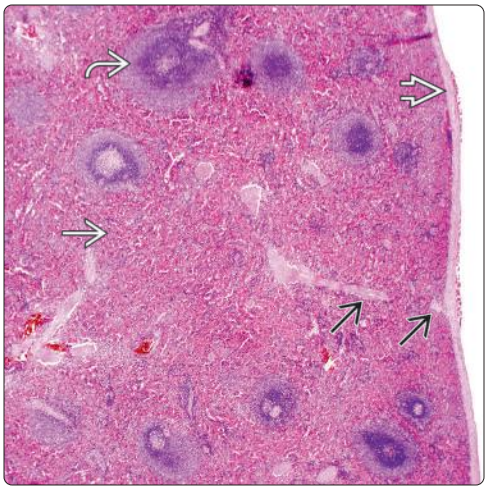
Congenital Abnormalities

- Common congenital anomalies
  - Splenic lobulation/lobulated spleen
  - Accessory spleen (spleniculi)
    - Small-sized functional spleen in presence of otherwise normal-sized spleen
    - Common locations include hilum of spleen and splenorenal and gastrosplenic ligaments
    - Other locations include tail of pancreas, jejunal wall, mesentery, and omentum
    - Found in 10-30% of patients at autopsy
- Rare congenital anomalies
  - Wandering spleen
  - Polysplenia
    - Often associated with other developmental abnormalities
      - Cardiac abnormalities, including dextrocardia, ventricular septal defect, ostium primum defect
      - Gastrointestinal abnormalities, including intestinal malrotation, duodenal atresia, biliary atresia
      - Abnormal left-right orientations of thoracic and abdominal organs (heterotaxia)
      - Spleen may be present on right side and divided into multiple small pieces
    - Polysplenia can occur alone without other congenital abnormalities
  - Splenogonadal fusion
    - Abnormal fusion of splenic and gonadal primordia during prenatal development
- Asplenia
  - Rare
  - Associated with cardiovascular abnormalities

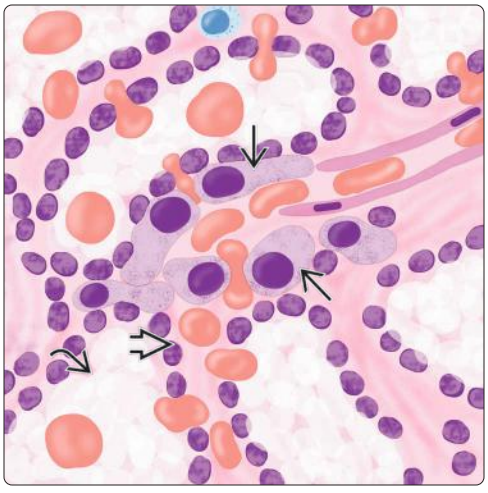
Anatomy

- Located in upper left abdominal cavity, just beneath diaphragm, and posterior to stomach

Low-Power View of Normal Splenic Histology



Graphic of Filtering Unit of Spleen



(Left) *Section of a normal spleen viewed at low power shows white pulp and red pulp. A thin splenic capsule with slivers of branching trabeculae is also noted.* **(Right)** *The functional filtering unit of the spleen is illustrated. Red cells pass from the sheathed capillaries that are lined by macrophages into the cords and then into the sinuses.*

- Lies against diaphragm in left hypochondriac region at level of 9th and 10th ribs
- Adjacent to greater curvature of stomach and within omentum
- Superior to left colic flexure
- Anterior to left kidney
- Deep to stomach fundus

GROSS

Macroscopic Anatomy

- Bean-shaped organ surrounded by fibrous capsule that extends inward to divide organ into lobules
  - Roughly triangular in cross section
  - Similar to lymph node in shape and structure but much larger
- Dark red to blue-black in color
- Consists of 2 types of tissue
  - White pulp
    - Numerous small nodules (1-2 mm) of lymphoid tissue
  - Red pulp
    - Venous sinuses filled with blood and cords of lymphatic cells (e.g., lymphocytes and macrophages)
- Blood supply via splenic artery
  - Splenic artery enters at hilum and branches in spleen
- Spleen weight can be important in evaluation
  - Typically 150-200 g
  - Varies with sex, age, height, and weight
  - Size and weight may show considerable variation related to amount of blood
- Splenic hilum
  - Entry point for splenic vessels and nerves
  - Exit point for efferent lymphatics
  - Attachment point for gastrosplenic ligament
  - Located at L1 vertebral level along transpyloric plane
- Relation to peritoneum
  - Lies within 2 leaflets of dorsal mesentery
  - Completely enclosed by peritoneum except at hilum
- Small fragments of spleen may be present following trauma (splenosis)

MICROSCOPIC

Capsule

- Fibroelastic capsule surrounded by peritoneum, except at hilum

Trabeculae

- Connective tissue extensions of capsule into parenchyma that carry arterial and venous branches

Splenic Vasculature

- Blood enters spleen via splenic artery, which then branches into trabecular arteries
- Splenic cuff
  - Branching trabeculae composed of dense connective tissue
  - Surrounds branching arteries, veins, and lymphatics
- Trabecular arteries
  - Emerge from connective tissue and become arterioles of white pulp (central arterioles), which are surrounded by periarteriolar lymphatic sheath
- Central arterioles
  - Continue into follicles (follicular arterioles)
- Follicular arterioles
  - Become smaller and terminate in marginal zone or form vascular tuft of capillaries in red pulp
- Arteries, arterioles, and capillaries are lined by endothelial cells
- Capillaries in red pulp end as sheathed capillaries
- Sheathed capillaries
  - Lack direct communication to sinuses
  - Lined by concentrically arranged macrophages and reticular fibers that become continuous with reticular network (stroma) of red pulp (cords)
  - Red cells enter adjacent sinuses via sheathed capillaries and cords
  - In conjunction with cords, function as filtering unit of spleen
- Spleen lacks afferent lymphatic vessels
  - May at least partially explain low frequency of epithelial-derived metastasis to spleen

Red Pulp

- Loose reticular network of capillaries, penetrating venous sinuses, and cords
- Venous sinuses collect blood and are lined by discontinuous layer of cuboidal littoral cells
  - Littoral cell: Type of endothelial cell that stains with both histiocytic and some, but not all, endothelial markers
- Splenic cords (cords of Billroth) represent tissue between venous sinuses
  - Contain reticular cells, macrophages, and plasma cells

Perifollicular Zone

- Area adjacent to follicles and T-cell compartments
- Indicated by numerous erythrocytes directly adjacent to lymphoid cells
- Capillaries and sheathed capillaries are also present in this zone

White Pulp

- Consists of B- and T-cell compartments
- **Follicles**
  - B-cell compartments may be seen as primary follicles (unstimulated) or as secondary follicles (antigen stimulated with germinal center formation)
  - Follicles are surrounded by rim of mantle zone B cells and outer rim of marginal zone B cells
  - Mantle zone
    - Tightly packed B cells with minimal cytoplasm
  - Marginal zone
    - Loosely packed B cells with folded nuclei and more abundant cytoplasm (monocytoid)
    - Unique region of spleen situated at interface of red pulp and follicles
    - Considered by many to be separate compartment rather than part of white pulp
- **Periarteriolar Lymphatic Sheath**
  - T-cell compartment that lies adjacent to arterioles
  - Irregular areas composed primarily of CD4(+) T cells

Age Variation

- White pulp does not contain well-formed follicles until birth

- Maturing hematopoietic precursors are commonly seen in fetal spleen
- Secondary follicles are more common in patients < 20 years of age
- Patients > 20 years of age typically have fewer secondary follicles
- Hyalinization of vessels is common in both old and young patients
- Extramedullary hematopoiesis in adult spleen is associated with pathologic conditions (e.g., primary myelofibrosis)

Pitfalls/Artifacts

- Splenic red pulp is extremely vulnerable to autolysis
- Additional stains may be helpful to visualize splenic architecture (e.g., reticulin, periodic acid-Schiff)

IMMUNOARCHITECTURE OF SPLEEN

White Pulp

- Primary follicle
  - Composed of mature B lymphocytes
  - Expresses pan-B-cell markers (CD19, CD20, CD79a, PAX5, and BCL2)
- Secondary follicle (contains active germinal center)
  - Composed of mature stimulated B lymphocytes
  - Expresses pan-B-cell markers (CD19, CD20, CD79a, PAX5, and CD10)
  - IgM(+)/IgD(+)
  - Lacks BCL2
  - Ki-67 shows polarized pattern of nuclear reactivity
  - Mantle zone
    - Expresses CD19, CD20, DBA.44, BCL2, and IgD
    - Negative for CD10 and CD23
  - Marginal zone B cells
    - Express CD19, CD20, BCL2, CD22, and CD27
    - Typically IgM(+)/IgD(-)
    - Negative for CD5, CD10, CD23, CD43, and DBA.44
- Follicular dendritic cells
  - Express CD21, CD23, and CD35
- Periarteriolar lymphatic sheath
  - Composed of mature T cells
    - Predominantly CD4(+) T-helper subset; expresses pan-T-cell antigens (CD2, CD3, CD4, CD5, CD7)
    - Few cytotoxic CD8(+) T cells may also be present; express similar pan-T-cell antigens

Red Pulp

- Sinus endothelial cells (littoral cells)
  - Express some endothelial markers (CD31, FVIII, ERG) and CD8
    - Sinus endothelial cells (littoral cells) are CD34(-)
  - Expression of CD8 by splenic sinus lining cells is unique
    - Lymph node and bone marrow sinuses are CD8(-)
  - May express CD68 and CD21
- Endothelial lining of arteries, arterioles, and capillaries
  - Express endothelial markers (CD31, CD34, FVIII, ERG) and WT1

FUNCTION

Overview

- Largest secondary immune organ
  - Initiates and regulates immune reactions to blood-borne and polysaccharide antigens
- Filters blood of foreign material and old or damaged red blood cells
- Carried out by white pulp and red pulp (2 main compartments of spleen)

White Pulp

- Lymphoid follicle
  - Involved in antigen presentation
  - If follicular center cells have surface immunoglobulin that binds to presented antigen, they proliferate and form germinal center reaction
    - Pool of B cells expands in response to antigen and differentiates into plasma cells and new memory B cells
- Marginal zone
  - Marginal zone macrophages are important in clearance of microorganisms and viruses
    - Designed to screen systemic circulation for antigens and pathogens
    - Important role in antigen processing
  - Marginal zone B cells bind polysaccharide antigens
    - May then migrate into germinal center, where they can present antigen to germinal center B cells
  - Marginal zone B cells in association with cytokines elaborated by T cells
    - Can induce differentiation into plasma cells
    - Can induce synthesis and secretion of antigen-specific immunoglobulin
- Periarteriolar lymphatic sheath
  - Area of intense phagocytic activity

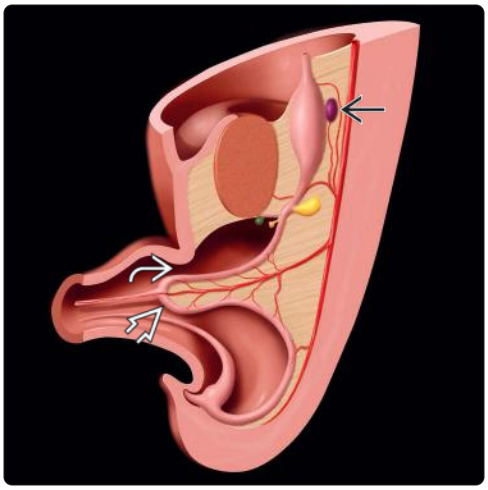
Red Pulp

- Blood filter that removes foreign material as well as damaged &/or old erythrocytes
  - Macrophages of sheathed capillaries and red pulp cords along with littoral cells comprise filtering unit of spleen
  - Red cell inclusions and red cells that are too inflexible &/or have low osmotic resistance are removed
  - Bacteria, antigens, and immune complexes are also removed
- Storage site for FVIII, iron, platelets, and erythrocytes
  - Up to 240 mL of red blood cells are stored within spleen and released in hypovolemia cases

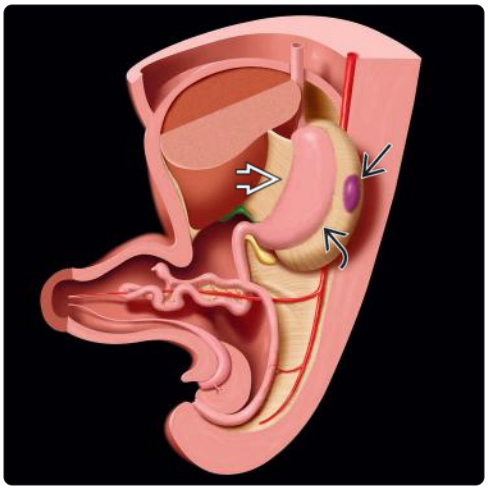
SELECTED REFERENCES

1. Chen G et al: Splenogonadal fusion: a case report and review of the literature. BMC Urol. 21(1):16, 2021
2. Borch WR et al: Practical applications in immunohistochemistry: an immunophenotypic approach to the spleen. Arch Pathol Lab Med. 143(9):1093-105, 2019
3. Lewis SM et al: Structure and function of the immune system in the spleen. Sci Immunol. 4(33), 2019
4. Endo A et al: Morphogenesis of the spleen during the human embryonic period. Anat Rec (Hoboken). 298(5):820-6, 2015
5. Varga I et al: Congenital anomalies of the spleen from an embryological point of view. Med Sci Monit. 15(12):RA269-76, 2009
6. Steiniger B et al: Fetal and early post-natal development of the human spleen: from primordial arterial B cell lobules to a non-segmented organ. Histochem Cell Biol. 128(3):205-15, 2007
7. Cesta MF: Normal structure, function, and histology of the spleen. Toxicol Pathol. 34(5):455-65, 2006
8. Kurtin PJ et al: Marginal zone B cells, monocytoid B cells, and the follicular microenvironment determinants of morphologic features in a subset of low-grade B-cell lymphomas. Am J Clin Pathol. 114(4):505-8, 2000

Embryonic Spleen Arising as Mesodermal Condensation

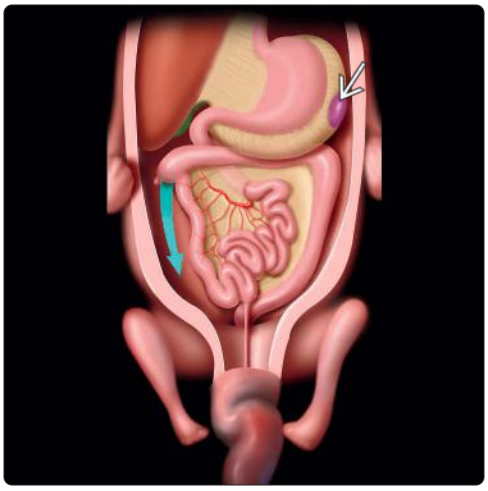


Rotation of Embryonic Spleen to Left

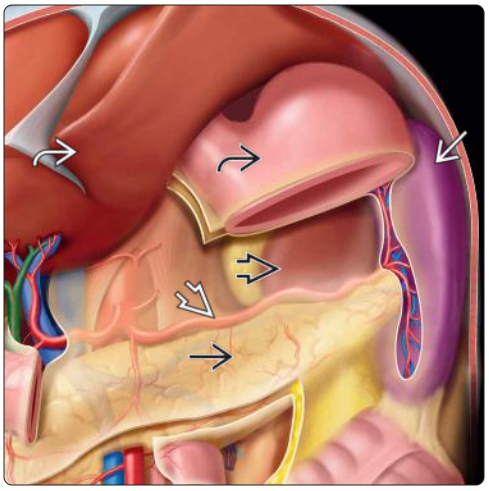


(Left) *Graphic of the embryonic abdomen (sagittal section, lateral view) displays the spleen as it arises as a mesodermal condensation in the dorsal mesogastrium. The midgut loop and intestinal portal are also highlighted.* **(Right)** *Graphic demonstrates the embryonic abdomen (sagittal section, lateral view). In this stage of development, the stomach and spleen have rotated to the left. The dorsal border of the stomach has elongated to form the greater curvature.*

Embryonic Spleen Rotated to Left Into Its Final Anatomic Location

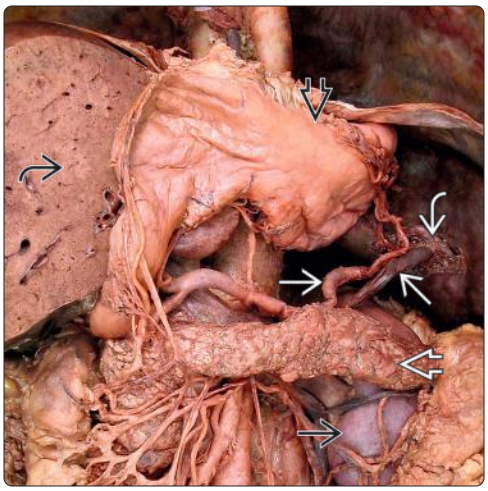


Spleen Relationship to Adjacent Organs

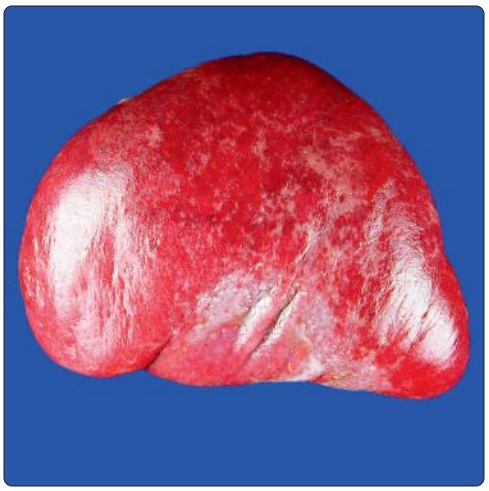


(Left) *The spleen, originally arising as a mesodermal condensation in the dorsal mesogastrium, has rotated left along with the stomach into its final anatomic location. The blue arrow notes the direction of ileocecal rotation.* **(Right)** *Graphic highlights the anatomic location of the spleen and its relationship to adjacent organs (pancreas, liver, stomach, and left kidney). The splenic artery and vein course along the body of the pancreas.*

Spleen in Relation to Neighboring Organs in Cadaveric Dissection of Abdomen

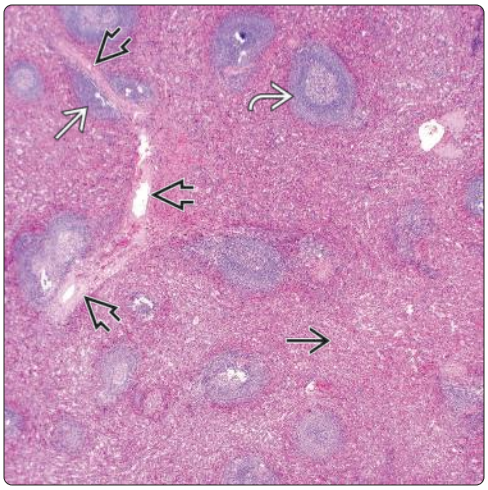


Gross Appearance of Normal Spleen With Intact, Smooth Capsule

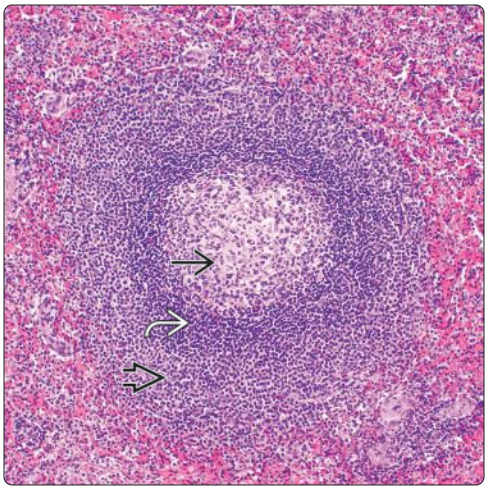


(Left) *Cadaveric dissection of the abdominal cavity highlights the spleen in relation to other neighboring organs and structures (splenic vein and artery, splenic hilum, pancreas, stomach reflected upward, liver, and kidney).* **(Right)** *Gross photograph shows a normal spleen. Note the thin, shimmering, smooth capsule and mottled, gray-red appearance.*

Low-Power View of Splenic Compartments

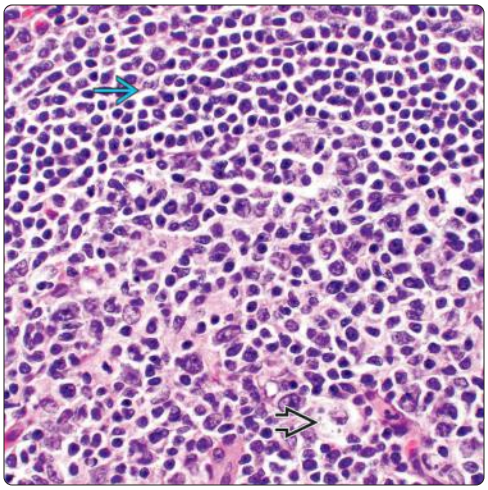


Splenic White Pulp With 3 Distinct Zones

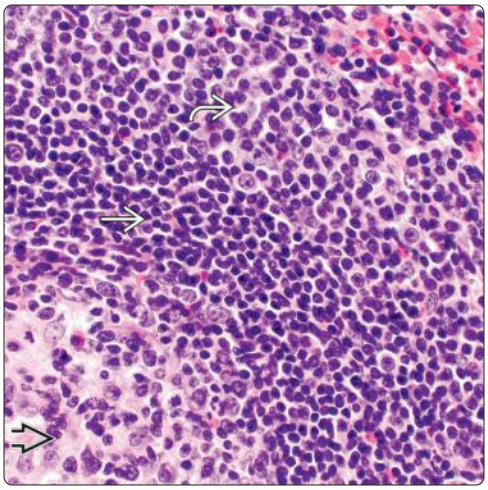


(Left) *Nearly all splenic compartments are illustrated in this image: White pulp (B-cell and T-cell compartments), red pulp, and splenic cuff.* **(Right)** *Medium-power view shows a B-cell follicle that exhibits features of activation, as noted by the germinal center. Note the surrounding rim of dense small lymphocytes and the less dense and more expanded rim of lymphocytes, which represent the mantle and marginal zones, respectively.*

Active Germinal Center of Secondary Follicle With Adjacent Mantle Zone

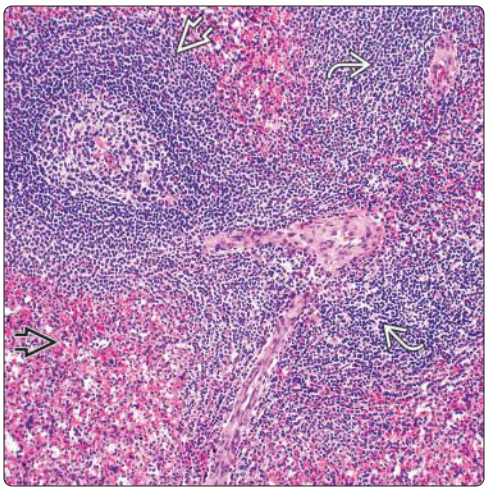


High-Power View of 3 Distinct Zones of Lymphoid Follicle of White Pulp

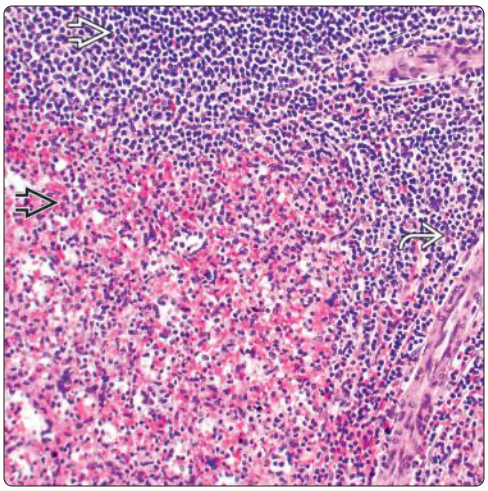


(Left) *Splenic follicles show many of the same features as those of lymph nodes. In this secondary (antigen-stimulated) follicle, a tingible body macrophage is seen within the germinal center. The surrounding mantle zone is also shown.* **(Right)** *High-power view illustrates the mantle zone (dense rim of small B cells with scant cytoplasm), the outer marginal zone (less dense rim of B cells with more abundant cytoplasm), and a small section of germinal center.*

Periarteriolar Lymphatic Sheath

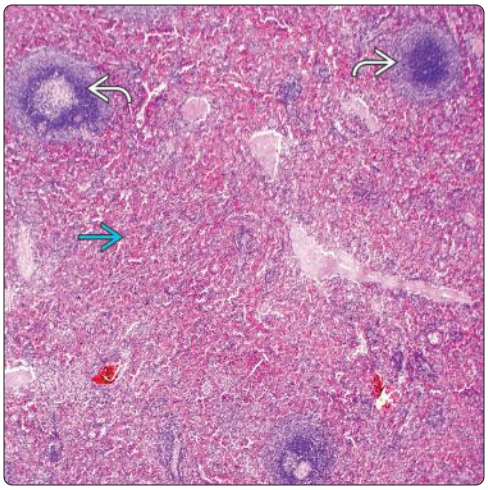


Perifollicular Zone

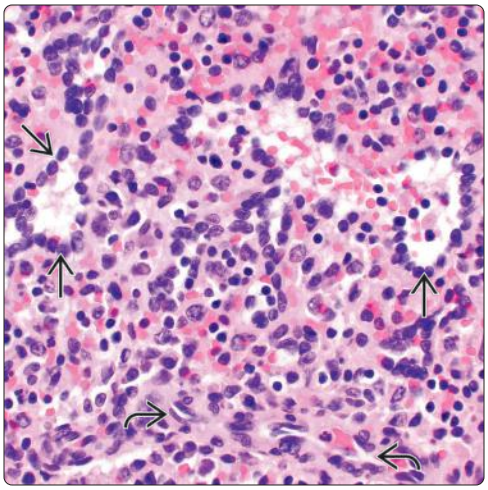


(Left) *T-cell compartment (periarteriolar lymphatic sheath), B-cell compartment (follicle), and perifollicular zone are shown. The T-cell and B-cell compartments together make up the white pulp.* **(Right)** *The perifollicular zone lies adjacent to the follicle and T-cell compartment (periarteriolar lymphatic sheath) and contains numerous erythrocytes in addition to capillaries and sheathed capillaries (not shown).*

Splenic Red Pulp

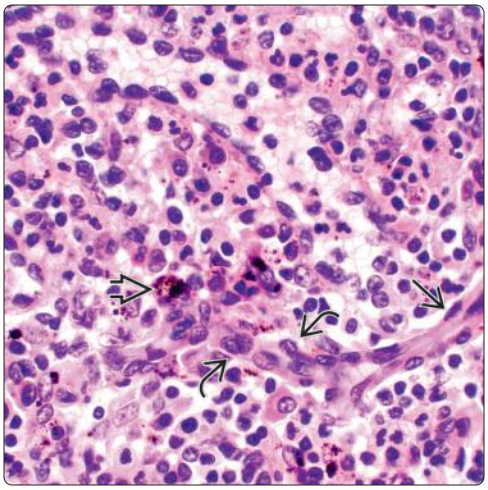


Splenic Sinuses Lined by Littoral Cells

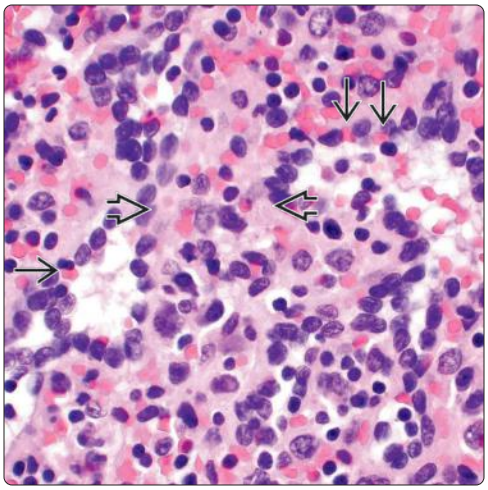


(Left) *Red pulp, seen here at low power, consists of capillaries, venous sinuses, and splenic cords (cords of Billroth) that are visible at higher power. Several follicles along the edges are present.* **(Right)** *Sinuses lined by littoral cells (a type of cuboidal endothelial cells that stain for CD8, some endothelial cells, and histiocytic markers) are shown. Several small capillaries are also noted along the bottom. Note the flat endothelial cells that line the capillaries.*

Sheathed Capillaries and Sinus

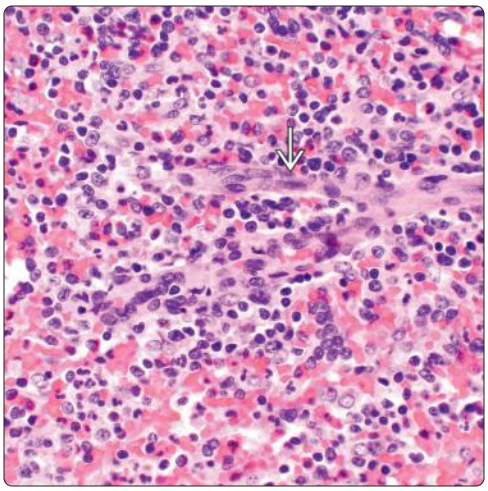


Passage of Red Cells Into Sinuses

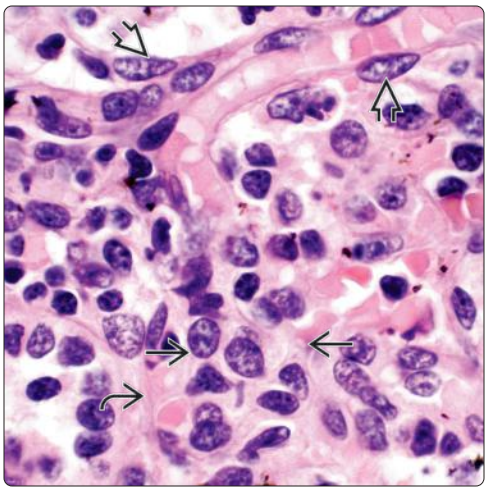


(Left) *Capillaries, sheathed capillaries, and a sinus are shown. Note the abundant hemosiderin in the macrophages that are part of the sheathed capillaries and cords.* **(Right)** *Splenic cords that contain macrophages, reticular cells, and plasma cells represent the tissue that lies between the sinuses. Note the individual red cells that pass from the cords into the sinuses. Old &/or damaged red cells that cannot squeeze through are removed by the spleen.*

Red Pulp Capillaries



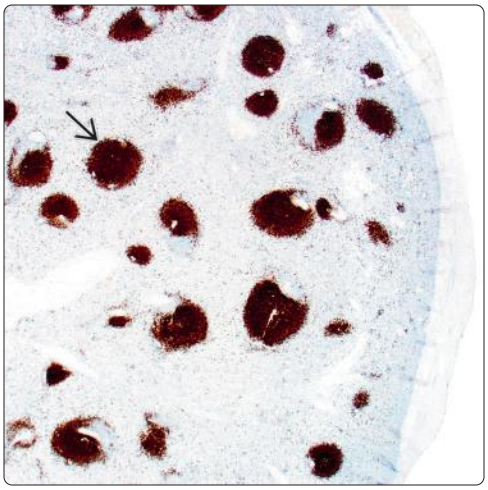
Red Pulp Capillaries



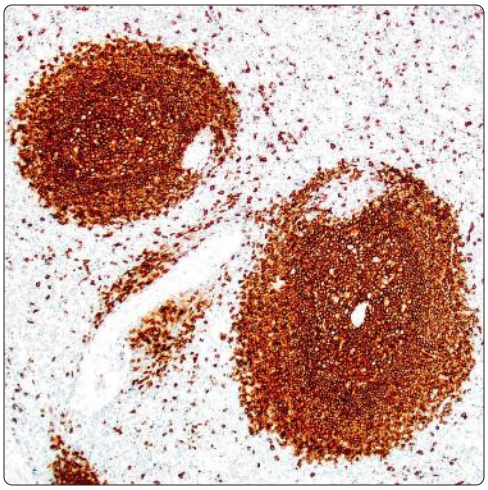
(Left) *A branching capillary lined by flattened endothelial cells is shown. In the spleen, arterioles lead to capillaries, which then lead to sheathed capillaries that are lined by concentrically arranged macrophages.* **(Right)** *A splenic capillary lined by flat endothelial cells precedes the sheathed capillary, which is lined by concentrically arranged macrophages and a network of reticular cells and fibers.*

(Left) *Section of a spleen that has undergone lymphoid follicular hyperplasia shows scattered, increased, somewhat hyperplastic-appearing lymphoid follicles ⇨ that are highlighted by CD20.* **(Right)** *Higher power view of an immunohistochemical stain using CD20 antibody demonstrates bright expression of CD20 by all 3 zones of the secondary lymphoid follicles of the splenic white pulp.*

CD20 Expressing Follicular Hyperplasia

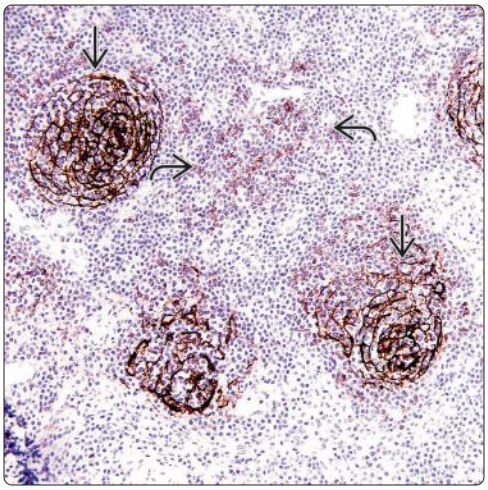


Higher Power View of CD20

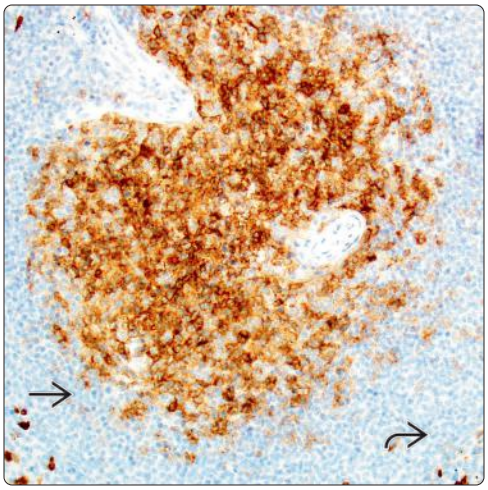


(Left) *CD21 immunohistochemical stain highlights the follicular dendritic meshwork ⇨ of the B-cell follicles. The endothelial cells that line the splenic sinuses ⇨ (littoral cells) may, in some cases, express CD21 (poorly visualized dim reactivity in image).* **(Right)** *CD10 immunohistochemical stain highlights the germinal center cells of a reactive secondary follicle. Note the negative staining of the surrounding mantle zone ⇨ and marginal zone cells ⇨.*

CD21(+) Follicular Dendritic Meshwork

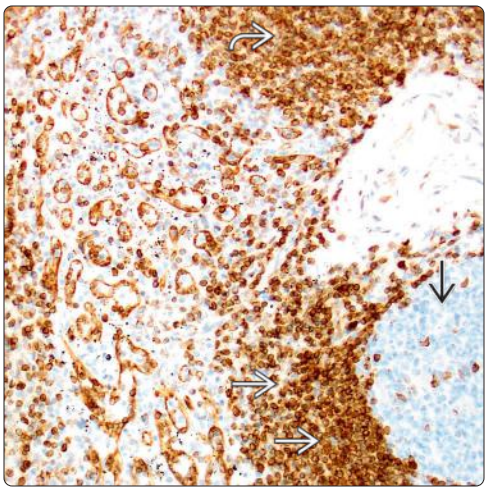


CD10 Expression in Germinal Center Cells of Lymphoid Nodules

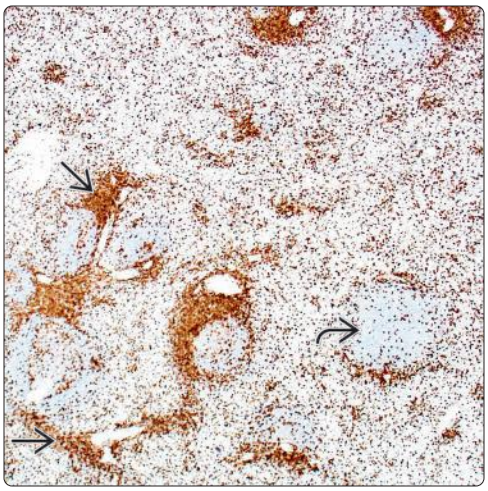


(Left) *Immunohistochemical stain using BCL2 antibody highlights the surrounding mantle and marginal zone B cells ⇨ and periarteriolar T cells ⇨ but is negative in the germinal center of the reactive follicle ⇨.* **(Right)** *The T-cell compartment (periarteriolar lymphatic sheath) is highlighted by CD3 ⇨. Note the negative areas of staining ⇨ that represent the B-cell follicles. Together, these 2 compartments make up the white pulp of the spleen.*

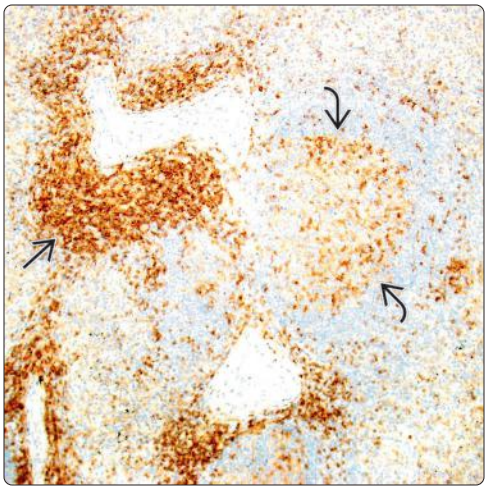
Lack of BCL2 Expression in Reactive Germinal Center



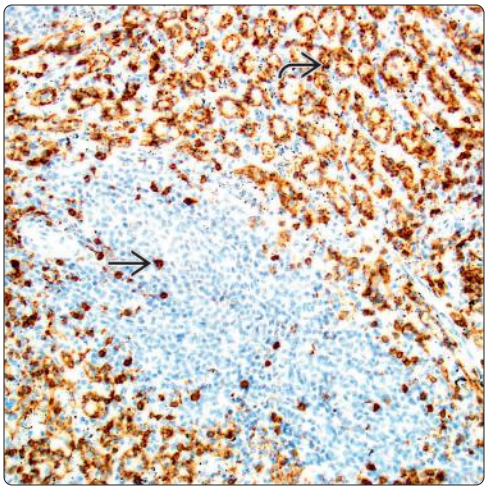
CD3(+) T Cells in Periarteriolar Lymphatic Sheath



CD4(+) T-Helper Subsets

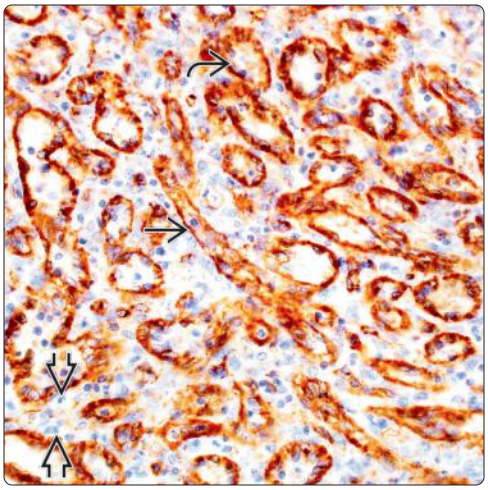


CD8 Expression by Splenic Sinuses

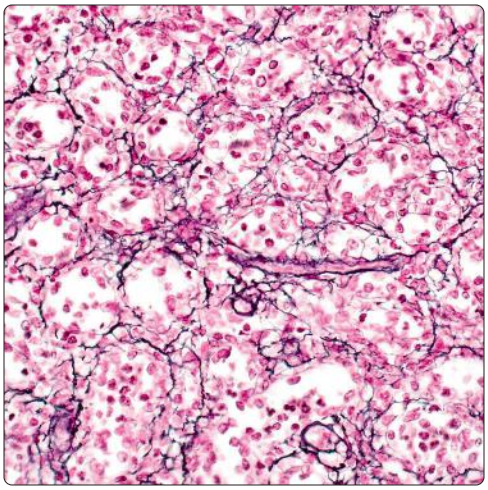


(Left) *The periarteriolar lymphatic sheath (T-cell zone) ⇨ contains predominantly CD4(+) T cells. Scattered CD4(+) T cells are also seen in the germinal center of the reactive lymphoid follicle (follicular T-helper cells) ⇨.* **(Right)** *The periarteriolar lymphatic sheath (T-cell zone) shows only rare CD8(+) T cells ⇨, whereas the endothelial cells lining the splenic sinuses ⇨ stain positively with CD8. The CD8 reactivity is unique to these endothelial cells (littoral cells).*

Factor VIII Immunohistochemical Stain

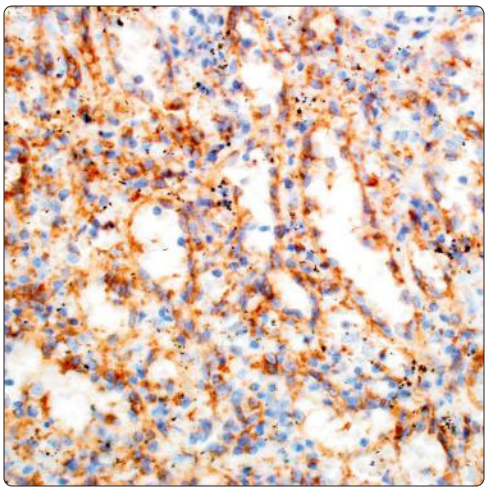


Discontinuous Reticulin Network of Sinuses

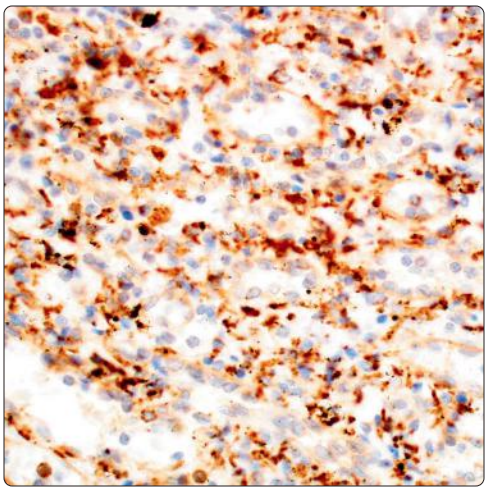


(Left) *The splenic sinuses ⇨ and capillaries ⇨ are highlighted by factor VIII. The space between the sinuses represents the splenic cords (cords of Billroth) ⇨. After passing through the cords into the sinuses, the red cells then progress to venules and veins.* **(Right)** *The reticulin network of sinuses and capillaries is highlighted. The discontinuous reticulin network of the sinuses allows for the passage of cells from the cords into the sinuses.*

CD31 Immunohistochemical Stain



CD68 Immunohistochemical Stain



(Left) *CD31 shows a similar pattern of reactivity to CD68 and factor VIII, highlighting the lining endothelial cells of the sinuses (littoral cells) and capillaries.* **(Right)** *CD68 highlights the cytoplasm of both the macrophages associated with the sheathed capillaries and cords and the littoral cells. Littoral cells are specialized endothelial cells that line the sinuses and stain with CD8, histiocytic markers, and some, but not all, endothelial markers.*