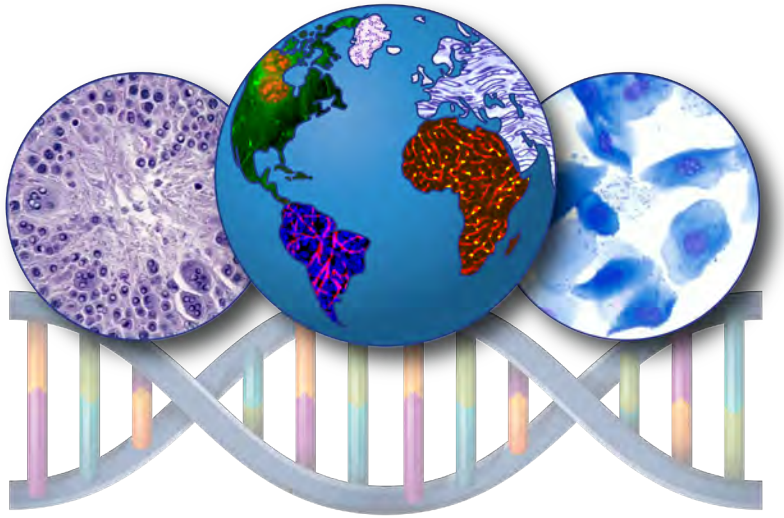
Histology of the Normal Rodent Immune System



Division of Translational Toxicology Global Toxicologic Pathology Training Program

Module outline

- Introduction
- Organs of the adaptive immune system (development, histomorphology, and aging changes)
  - Thymus
  - Bone Marrow
  - Spleen
  - Lymph Node
  - Mucosa-associated lymphoid tissue (MALT)
- Adaptive immunological components of ‘non-immune’ organs
- Tertiary lymphoid tissue

Abbreviations

- AGM – aorta-gonad-mesonephros (early fetal location of hematopoiesis)
- BALT – bronchus-associated lymphoid tissue
- B cells — B lymphocytes
- CD3 — cluster of differentiation 3 (a marker for T lymphocytes)
- CD45RA — cluster of differentiation 45 (used as marker for B cells in rat tissues)
- CMJ — corticomedullary junction (e.g., thymus)
- DCU — deep cortical unit (lymph node)
- ETP — early thymic progenitor cells
- FAE — follicle-associated epithelium (in various MALT structures)
- GALT — gastrointestinal-associated lymphoid tissue
- GD — gestation day
- H&E — hematoxylin and eosin (routine histologic staining procedure for tissue sections)

Abbreviations, continued

- IEL — intraepithelial lymphocytes (of gastrointestinal tract)
- IHC — immunohistochemical (staining procedure for tissue sections)
- LTI — lymphoid tissue initiator cells (in lymph node formation)
- LTO — lymphoid tissue organizer cells (in lymph node formation)
- MALT — mucosa-associated lymphoid tissue
- MZ — marginal zone in spleen
- NALT — nasopharynx-associated lymphoid tissue
- PALS — periarteriolar lymphoid sheath in spleen
- PAS — periodic acid/Schiff (histologic staining procedure for tissue sections)
- PND — postnatal day
- T cells — T lymphocytes

Introduction

- Immunological functioning involves closely coordinated actions between various components of the innate and adaptive immune systems
- The adaptive immune system is concentrated in individual immune system organs, but the effector cells and molecular mediators are disseminated throughout the body
  - By contrast, cellular elements of the innate immune system tend to be distributed throughout the body
- Certain aspects of immune system functioning are visible by microscopic examination of immune system and non-immune organs, but many aspects of immune functioning are not visible microscopically
  - Additional evaluation modalities such as flow cytometry and functional assays are required to evaluate those aspects of immune function
- Treatment-related immunological alterations may include suppression, exacerbation, mis-direction or disorganization of the various elements of immune functions. Of these, immunosuppression is most amenable to microscopic detection.
- Microscopic evaluation of the immune system should be on observations on whole animals or groups rather than observations in a single organ
  - This will allow detection of trends that may not be clearly apparent in individual organs
- Microscopic evaluation should include pathologic alterations in both immune and non-immune tissues that may be a result of altered immune functioning, e.g., infectious or inflammatory processes in various organs

Thymus

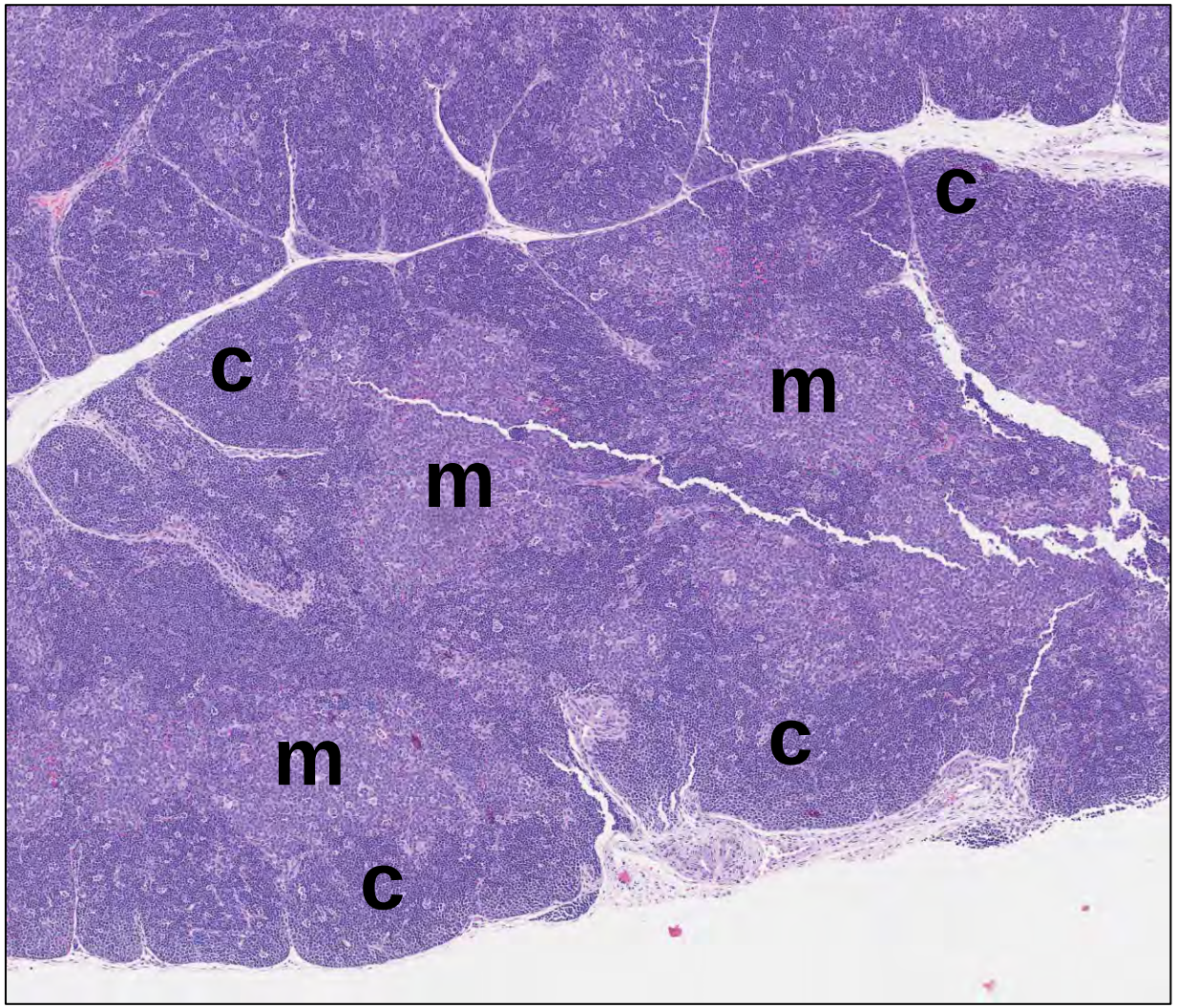
Overview of T cell generation

- The thymus is a primary immune system organ that receives early thymic progenitor (ETP) cells from the bone marrow and guides their proliferation and maturation to become functional naïve T cells that populate secondary immune system organs
  - A major function of the thymus is control of T cell antigenic specificity to largely eliminate self-reactivity
- ETP cells enter the thymus near the corticomedullary junction (CMJ)
  - Then, during maturation and clonal expansion, the maturing thymocytes physically relocate from the CMJ through the cortex out toward the thymic capsule, then migrate downward into the medulla, and finally exit the thymus at the CMJ
  - ETP cells enter the thymus at approximately 2-week intervals
- Thymocyte maturation in the mouse takes approximately 3 weeks
  - Thymocyte developmental stages consist of double negative stages 1-4 (negative for T cell receptor [TCR] and co-receptors for CD4 and CD8) and double positive stages 1 and 2 (CD4 and CD8 positive)
- Positive and negative selection results in apoptosis of approximately 95% of immature thymocytes, producing cellular debris that is engulfed by macrophages, resulting in "tingible-body macrophages”
  - Cells that survive the positive and negative selection exit the thymus as naïve T cells that populate secondary immune system organs
- Upon exposure to specific antigens in secondary immune system organs, naïve T cells undergo clonal expansion and become effector T cells

Thymus

Development

- The thymus is derived from pharyngeal pouches III and IV and migrates caudally along with the thyroid and parathyroid glands
- As a primary immune system organ, development of the thymus proceeds via a genetically determined pathway without influence by external factors
- At postnatal day (PND) 0, the thymus of rats is a bilobed structure that has sharply demarcated cortical (c) and medullary (m) regions
  - The immature cortex is slightly thinner relative to the medulla as compared to the adult proportions
- By PND 14, the cortex and medulla are present in adult proportions
  - After PND 14, the only morphological change in the thymus is increase in size in proportion to the increased size of the rat
- The thymus is different from other organs in that thymic stroma is of epithelial rather than mesenchymal origin

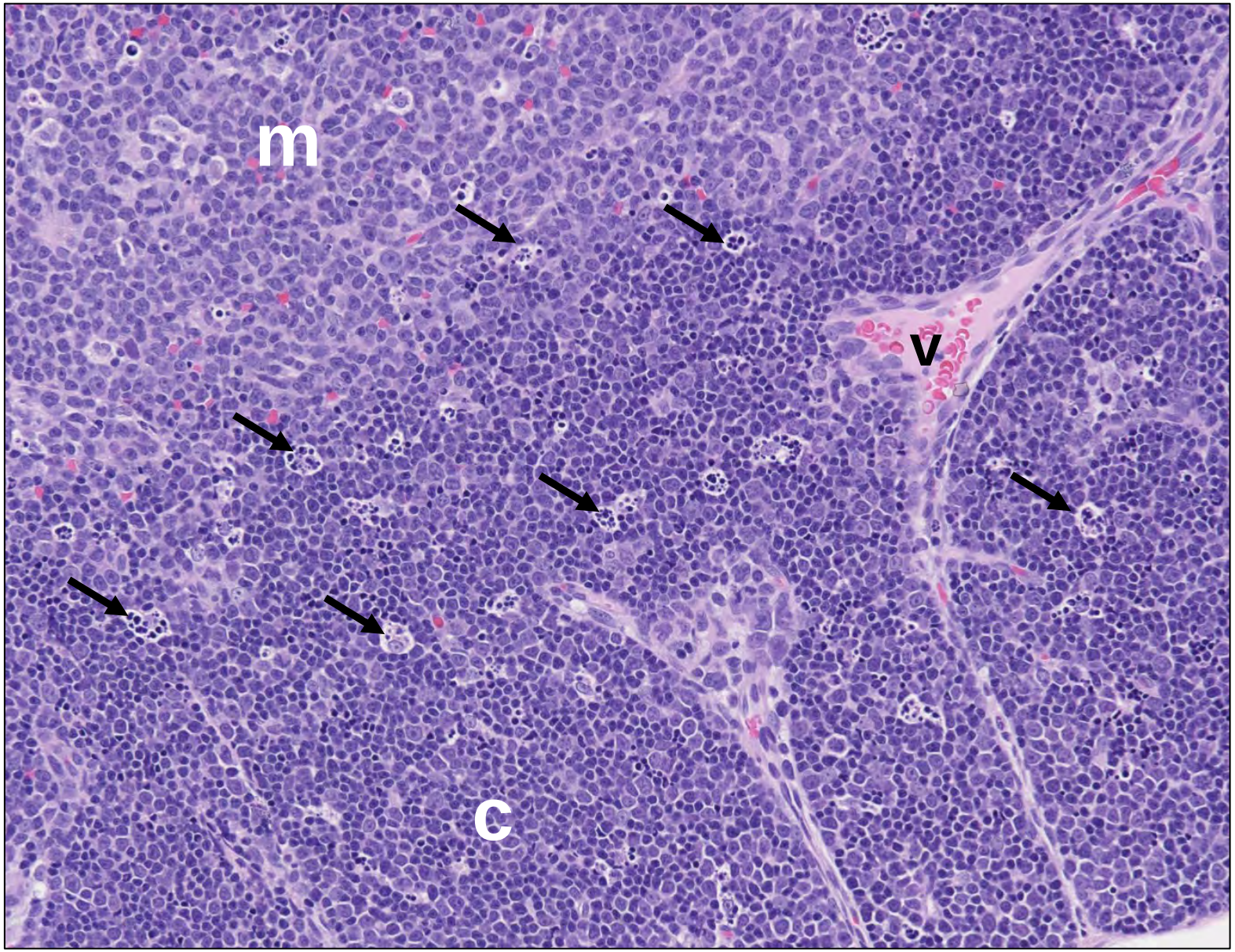


Sprague Dawley rat, male, postnatal day 0

Thymus

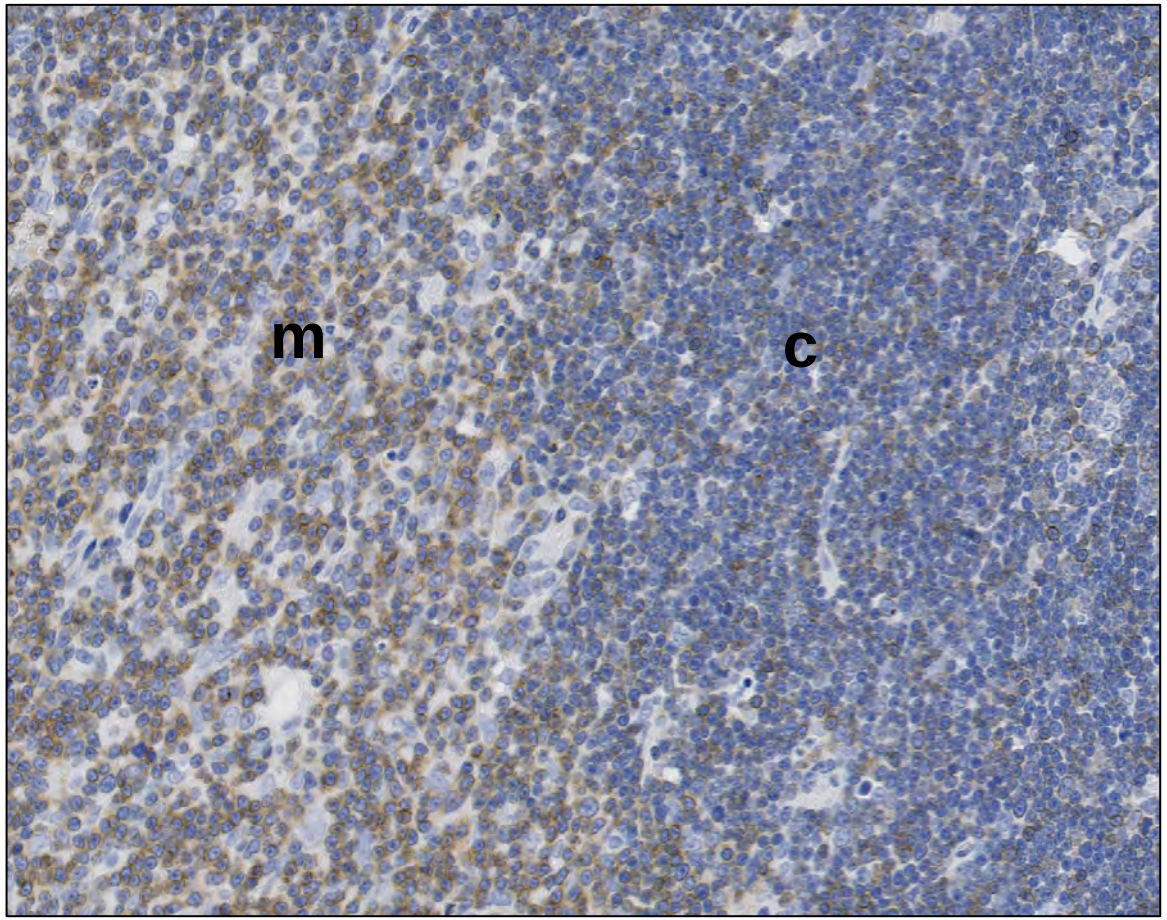
Histomorphology

- At PND 0, the thymus has distinct cellular populations in the cortex (c) and medulla (m), with a well-defined border between the two regions
- Immunologic selective processes in the cortex result in apoptosis of non-selected developing thymocytes
  - Fragments of these apoptotic thymocytes are engulfed by “tingible body macrophages” (arrows)
- Blood vessels (v) near the corticomedullary junction serve as points of entry of early thymic progenitor (ETP) cells from the bone marrow as well as points of exit for naïve T cells destined for localization in secondary lymphoid organs

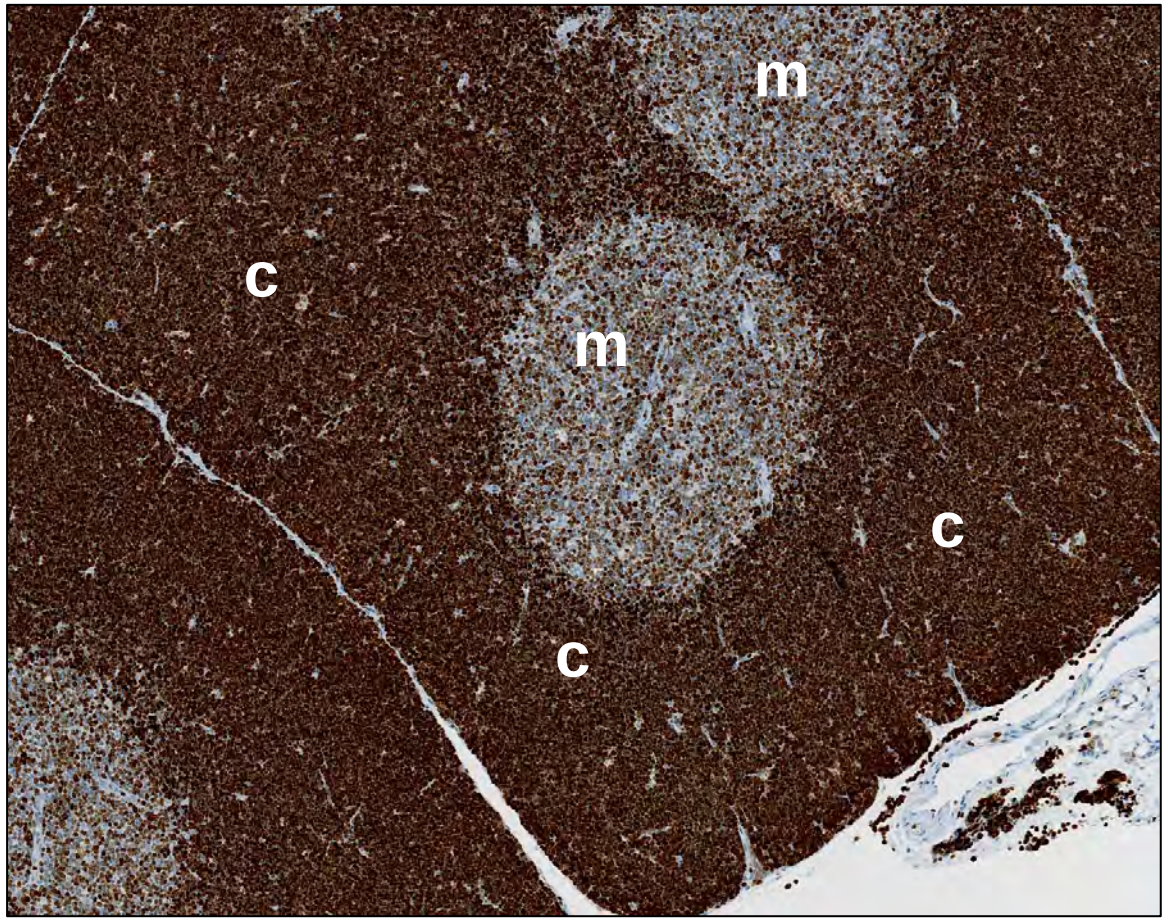


Thymus

Histomorphology: immunohistochemistry



CD3



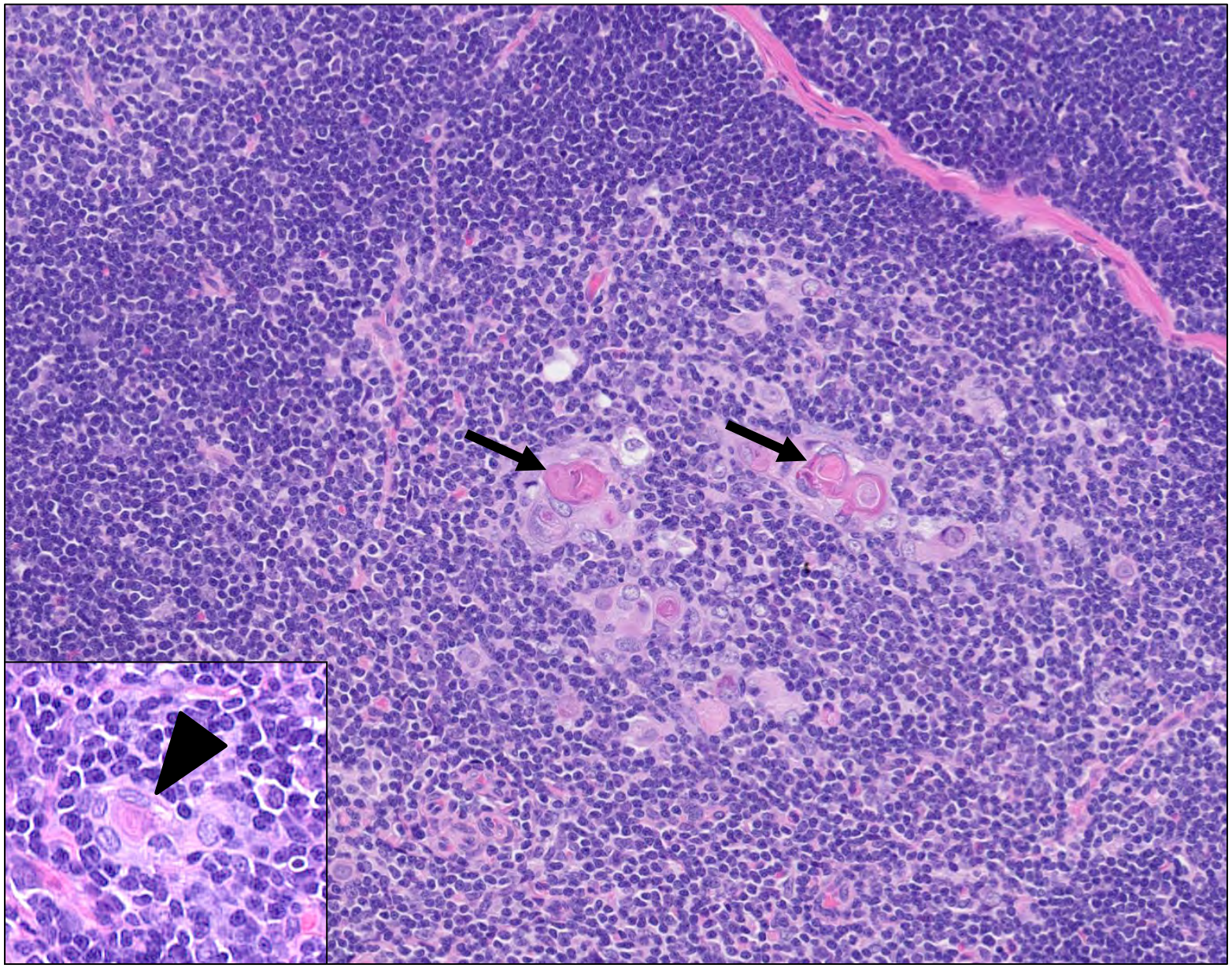
Ki67

Immunohistochemical staining performed on formalin-fixed, paraffin-embedded thymus specimens. LEFT: Brown CD3 staining marks T cells in cortex (c) and medulla (m). RIGHT: Dark brown Ki67 staining indicates dense population of replicating cells in cortex (c), with fewer replicating cells in medulla (m). BOTH IMAGES: Brown staining by 3,3'-diaminobenzidine chromogen indicates target molecules, blue hematoxylin counterstain stains cell nuclei.

Thymus

Histomorphology: Hassall's corpuscles

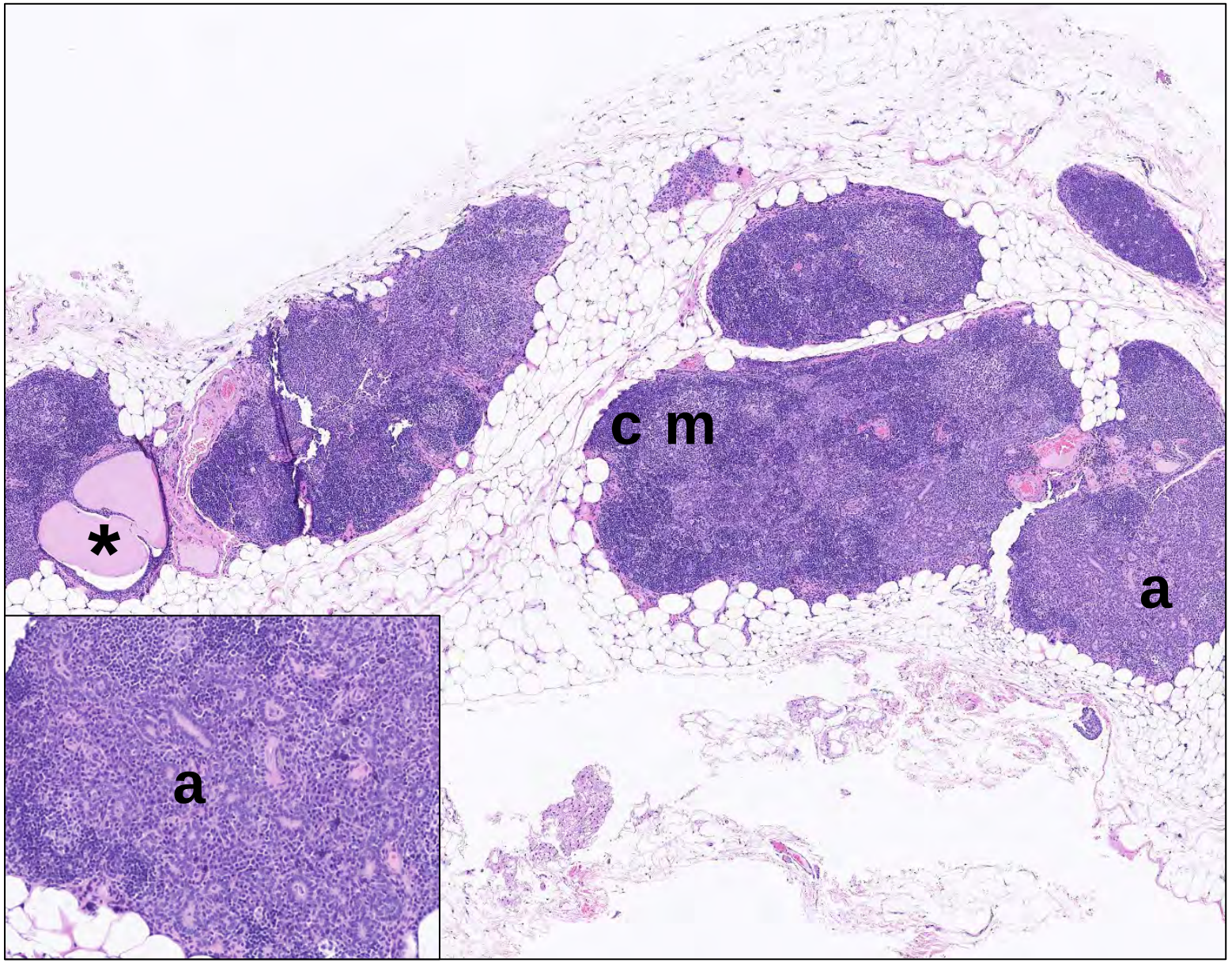
- Hassall's corpuscles are distinct epithelial structures that characterize the thymus of many species, including humans
- Immunohistochemical staining of Hassall's corpuscles reveals the presence of multiple cytokeratins
- In the larger image, Hassall's corpuscles are clearly visible in this cynomolgus macaque thymus (arrows). They can also be seen in the thymus of dogs and pigs. In the inset image, individual keratinized cells in the thymic medulla of older rats (inset arrowhead) may be the rodent equivalent of Hassall's corpuscles.
- Hassall's corpuscles have classically been considered degenerative structures, but more recent studies have shown they are involved in removal of apoptotic thymocytes, negative selection of T cells, and differentiation of regulatory T cells (which help maintain immune tolerance)



Thymus

Aging changes

- Involution is the normal, age-related process whereby the thymus becomes reduced in cellularity and function
- Thymic involution occurs in all mammalian species
- The thymus starts to involute soon after puberty and is substantially reduced in size by the time of termination of a typical two-year carcinogenicity study
- After the thymus involutes, T cell generation takes place in the intestinal lamina propria and the biliary tract
- With the loss of the immunological selective processes of the involuted thymus, there is greater potential for generation of self-reactive T cells. This contributes to the increased incidence of autoimmune pathologic processes in the aged.

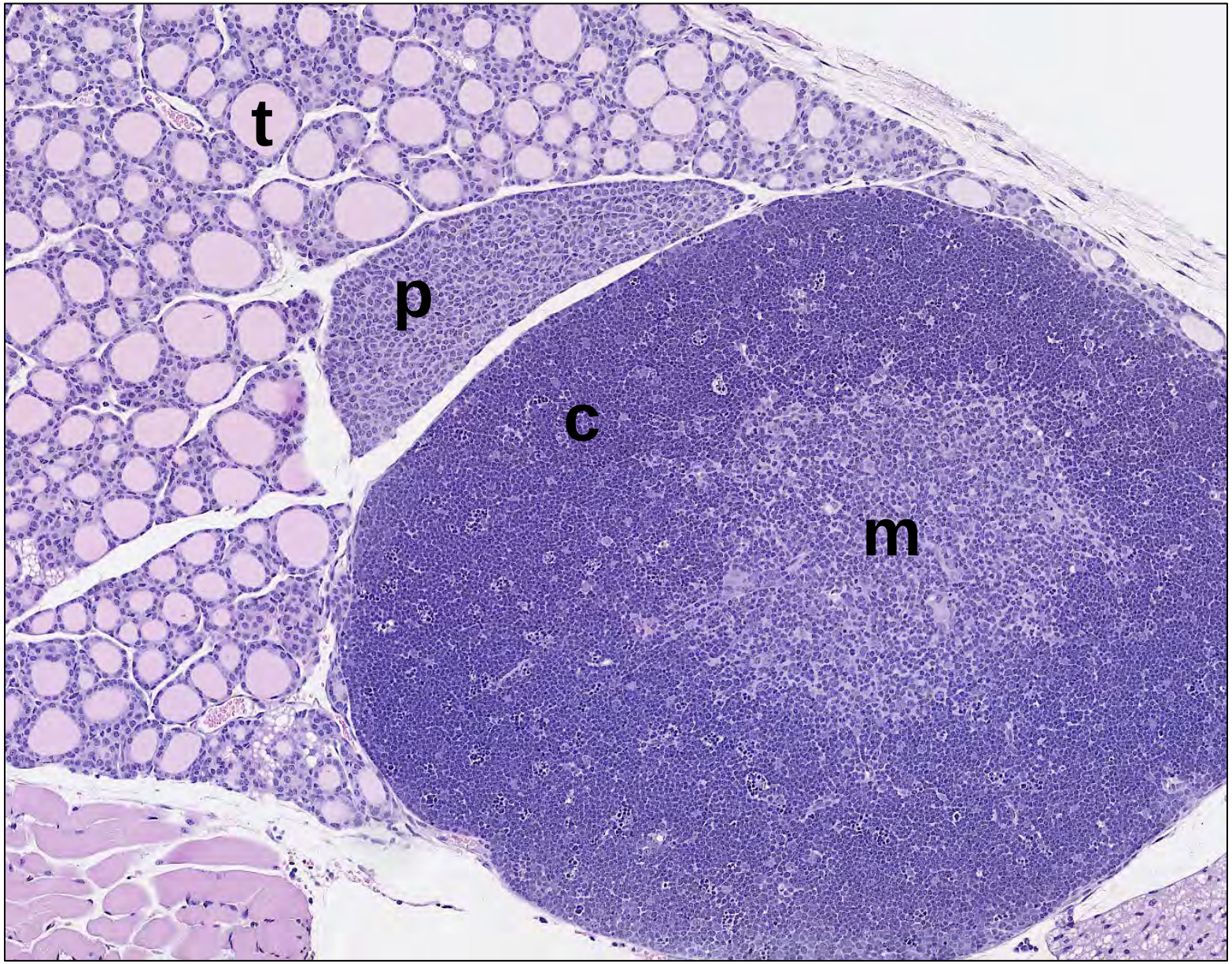


RIGHT: The thymus of an aged female Sprague Dawley rat has reduced cortical cellularity as well as aggregations of epithelial nests and tubules (a) and a single cystic cavity (*). c = cortex, m = medulla

Thymus

Ectopic thymic tissue

- Ectopic (presence of normal tissue occurring in an abnormal location) thymic tissue is most commonly found in histologic sections of thyroid/parathyroid glands but may be found in any tissue along the embryological migration path from the branchial arches down to the anterior mediastinum
- Pathological changes in the primary thymus are commonly manifested in the ectopic thymic structures as well



RIGHT: The image shows ectopic thymic tissue adjacent to thyroid gland (t) of a young adult CD-1 mouse. This ectopic thymic fragment has distinct cortex (c) and medulla (m). Parathyroid gland (p) lies between the thyroid gland and ectopic thymic tissue.

Hematopoiesis

Overview

- The earliest hematopoiesis (blood cell formation) in mammals consists of hemangioblasts located outside the embryo in the yolk sac and inside the embryo in the para-aortic splanchnopleure (part of the mesoderm that gives rise to the circulatory system, heart, and other visceral organs)
- Primitive progenitor cells generated by the hemangioblasts first colonize the aorta-gonad-mesonephros (AGM), then enter the bloodstream and colonize multiple sites, including the liver and spleen
- In the rat, hepatic hematopoiesis starts at gestation day (GD) 12-13; and, by GD 15, hematopoiesis accounts for 37% of liver volume
  - As long bones of the fetus mature, hematopoiesis migrates to the marrow cavities
  - By the time of birth of the rat pup, hepatic hematopoiesis accounts for only 10% of liver volume
- Splenic hematopoiesis in the rat commences at approximately GD 17 and is most pronounced at postnatal days (PND) 0-14, after which hematopoiesis diminishes to adult levels that persist throughout life
- The population of lymphocytes in the rat bone marrow increases from approximately 20% at PND 7 to approximately 45% at PND 14 through 42
  - This population of lymphocytes includes naïve immunocyte (cells involved in immune functions) progenitors that seed distant lymphoid organs

Hematopoiesis

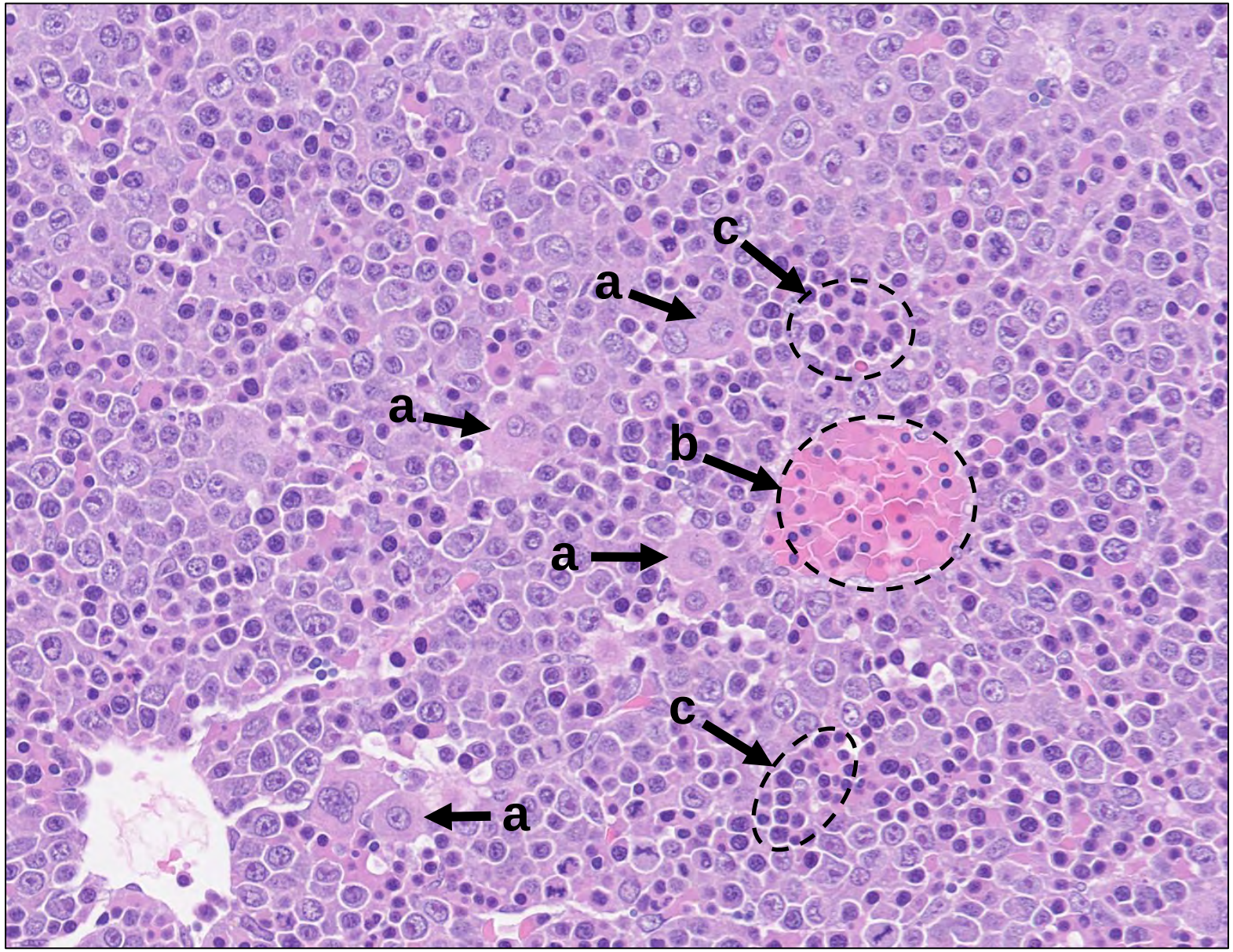
Rat, liver, gestation day 15

In the fetal rat, hematopoietic cells constitute 37% of the liver volume at gestation day (GD) 15.

a = individual hepatocytes

b = intravascular nucleated erythrocytes

c = hematopoietic cells



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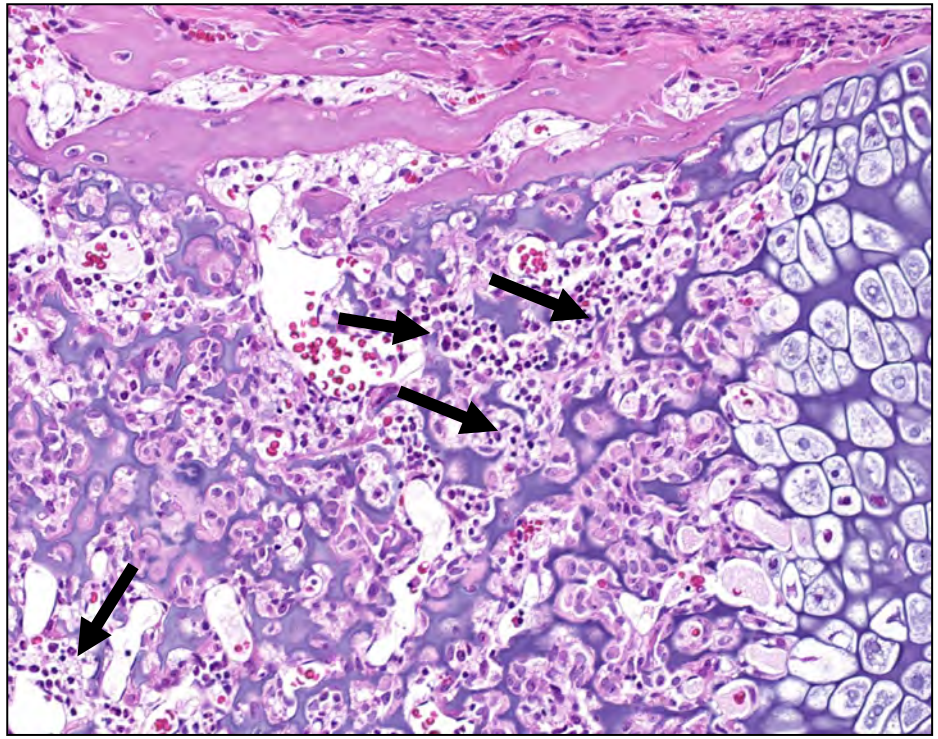
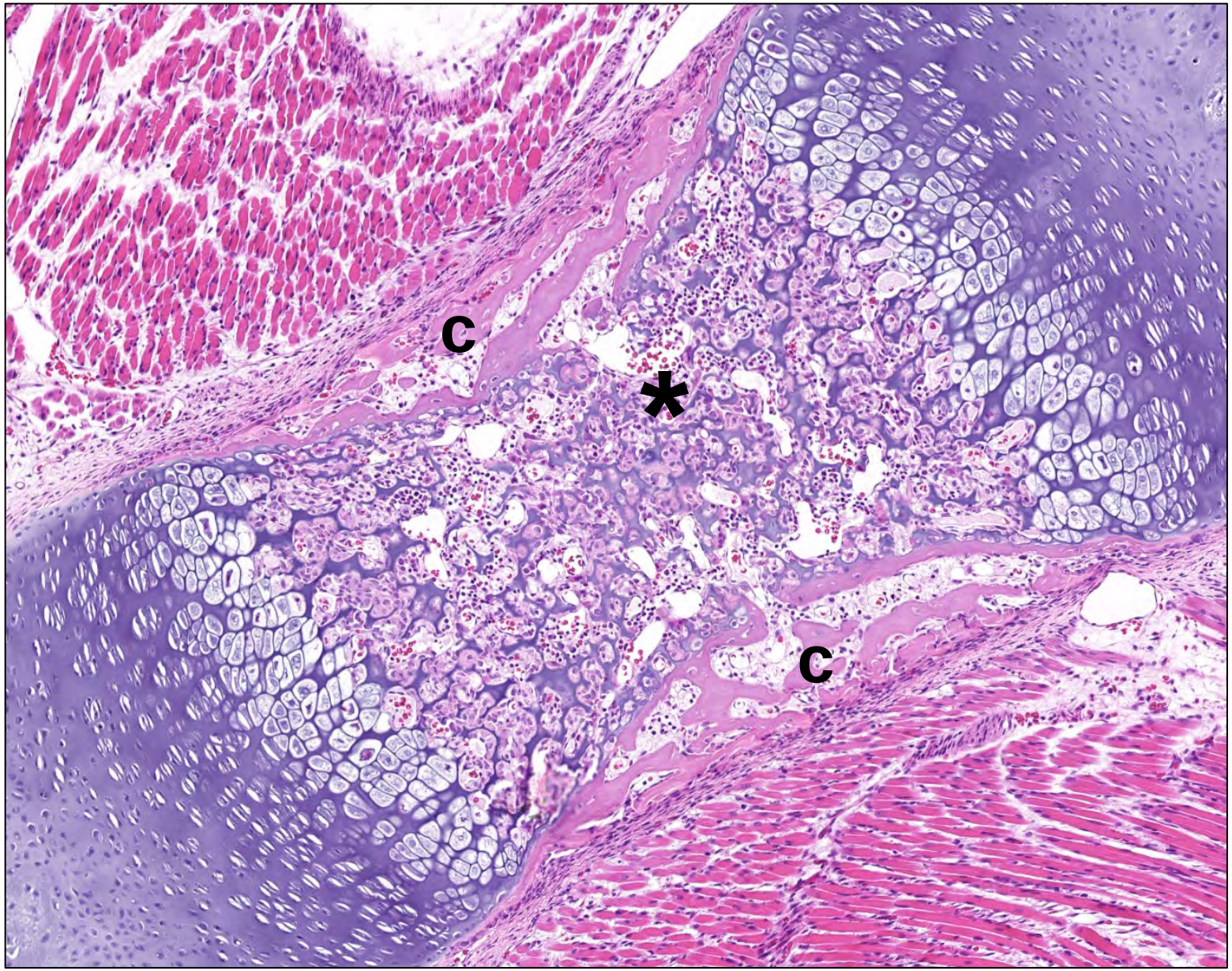
Bone Marrow

Development

Marrow cavities form in long bones near the time of birth. As marrow cavities are formed, blood cell formation (hematopoiesis) migrates from the liver to the marrow cavities of bones, which are the primary site of hematopoiesis throughout adulthood.

At PND 1, the cortex (c) of a Sprague Dawley rat consists of nonmineralized bone. The incompletely formed marrow cavity (*) contains clusters of hematopoietic cells (arrows).





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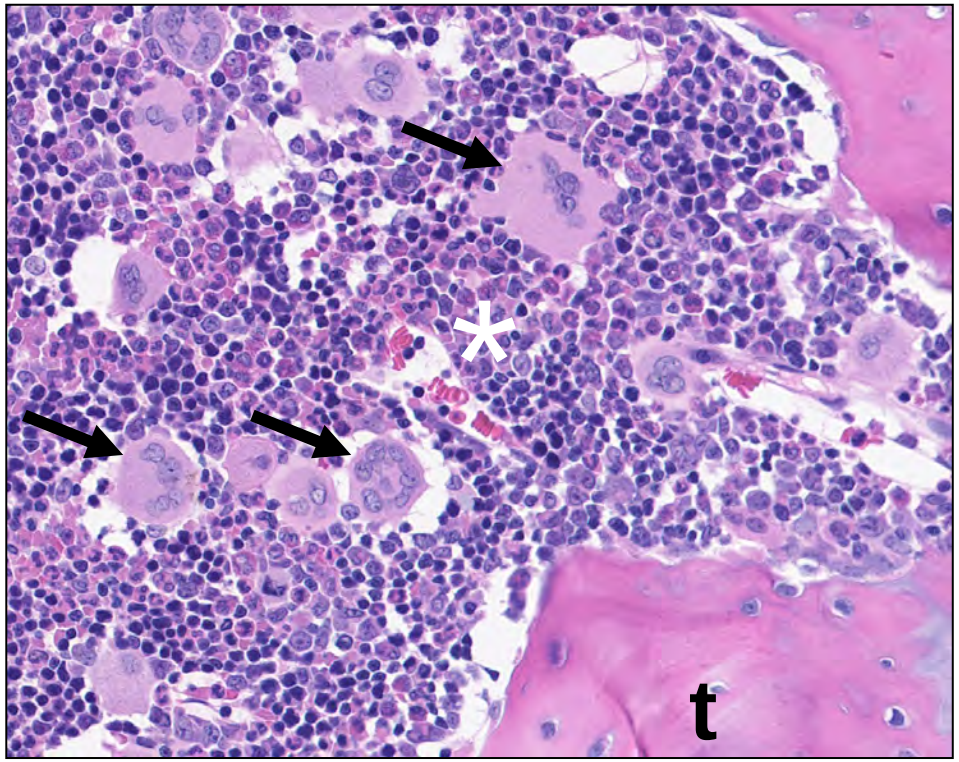
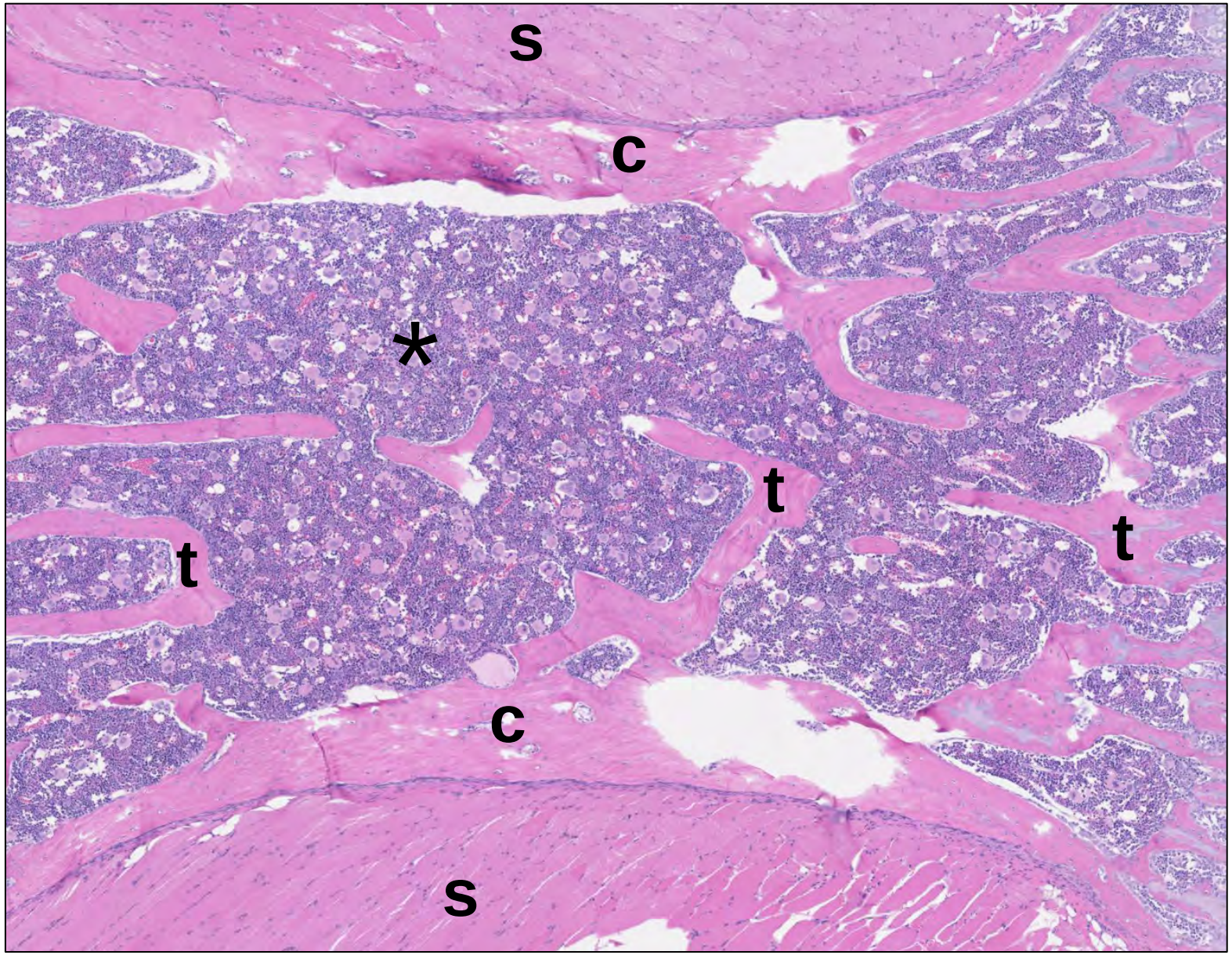
Bone Marrow

Histomorphology

By PND 42 (young adult), the sternal marrow (*) of this Sprague Dawley rat has the dense cellularity that is characteristic of the adult. Later in life, the bone marrow elements are partially replaced by fat cells (adipocytes). Arrows = megakaryocytes; c = sternal cortex; t = bone trabeculae; s = skeletal muscle



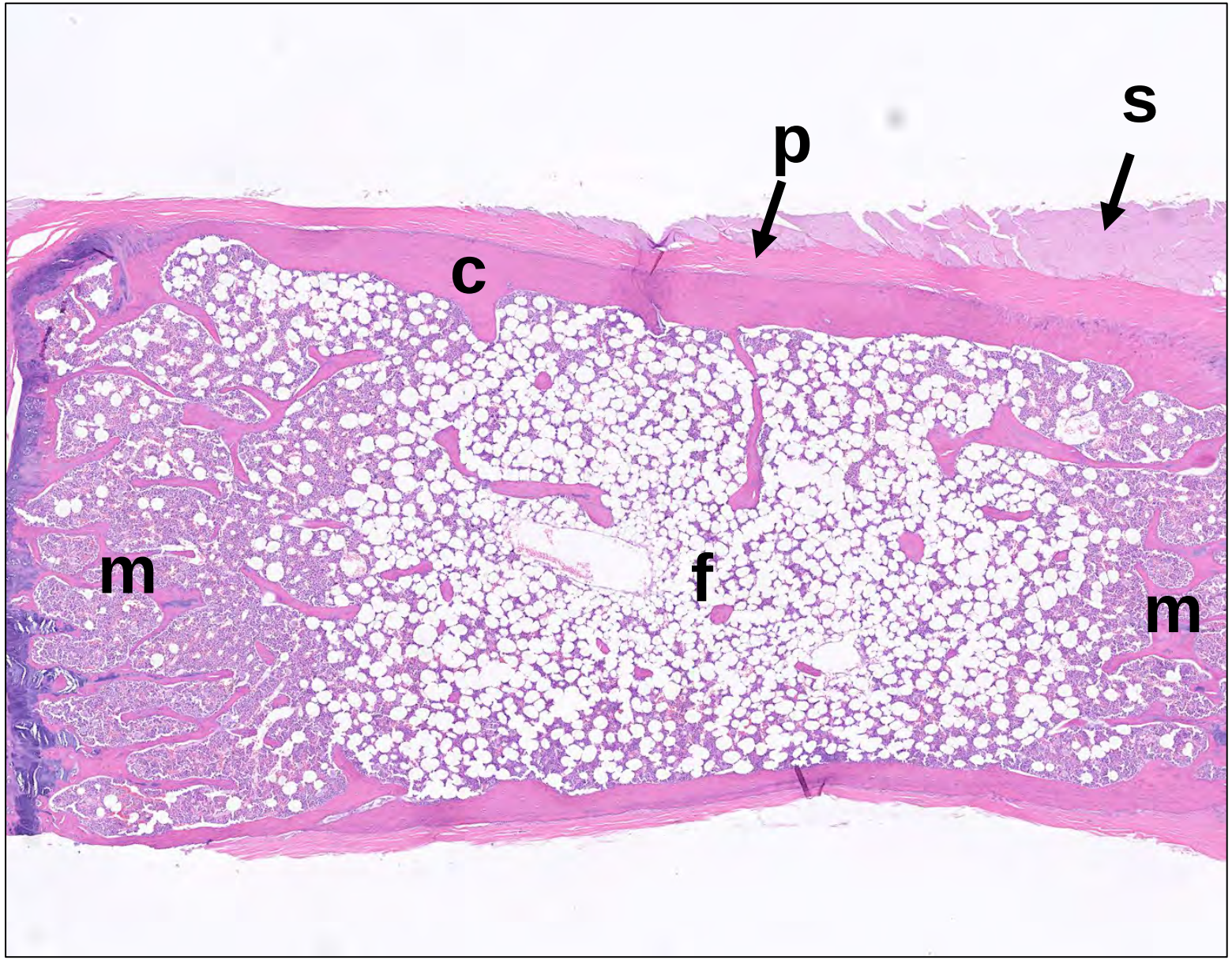


Bone Marrow

Bone marrow changes with aging

- With aging, the marrow cavity is gradually replaced by adipocytes. This process typically starts in the center of the sternal marrow cavity (f).
- Stress response in the rat causes decreases in hematopoietic cell populations and increases the conversion of preadipocytes to adipocytes.
- Stress response should be considered when excessive sternal adipocytes are encountered in young rats (less than 6 weeks of age) from juvenile toxicity studies or in the young adult rats (6-14 weeks of age) that are commonly used in short-term toxicology studies.

RIGHT: Sternum with bone marrow from aged female Sprague Dawley at the end of a 2-year carcinogenicity study. m = highly cellular bone marrow at ends of marrow cavity; f = clear round spaces indicate fat-containing adipocytes in center of marrow cavity; c = sternal bone cortex; p = dense fibrous periosteum; s = skeletal muscle



Spleen

Spleen overview

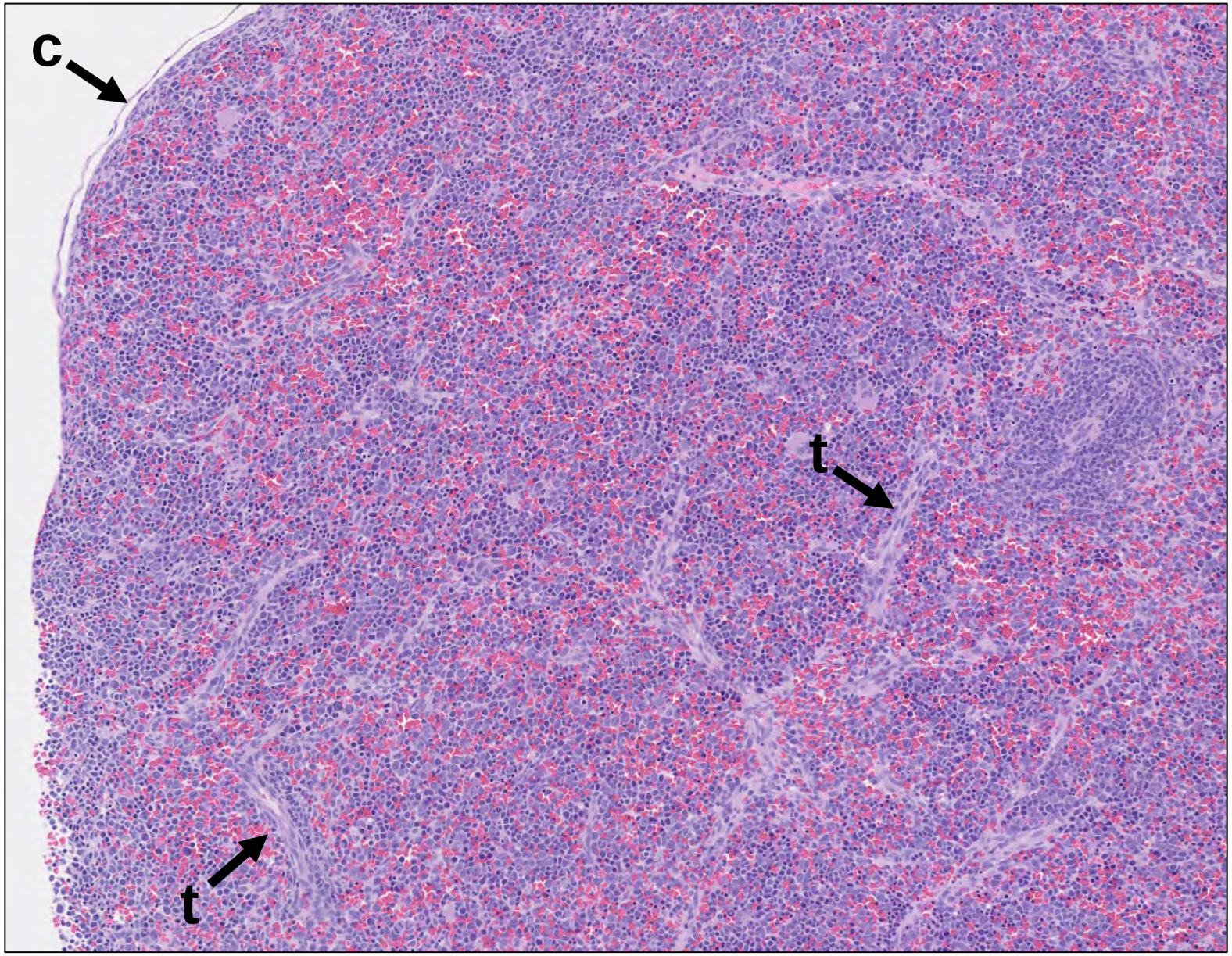
- The fetal spleen of the mouse arises as 5 (typically) aggregates of mesodermal tissue that fuse to form the intact spleen
  - Spleen embryogenesis in rats is similar to mice
- The spleen has 3 major functions: blood storage and filtration, hematopoiesis, and immunological functions.
  - Blood storage and hematopoiesis occur in the red pulp, and immunological functions occur in the white pulp.
- Extramedullary (outside of the bone marrow) hematopoiesis is normal in the spleen of rodents and is more pronounced in mice than rats. Microscopic detection of increased hematopoiesis is largely a subjective determination. The cross-sections of spleen that are typically prepared in toxicology studies have a triangular profile. Macroscopically visible bulging of the triangular profile with rounding of the corners is a consistent indication of increased internal cellularity, commonly due to an increased level of hematopoiesis.
- The white pulp has three compartments: the periarteriolar lymphoid sheaths (PALS), follicles, and marginal zone
  - The spleen is organized into T cell-rich PALS that branch throughout the spleen
  - In rats, there is a prominent marginal zone located immediately exterior to the PALS
  - Lymphoid follicles with predominant B cell populations are located at the margin of PALS. As the B cells of lymphoid follicles respond to antigen exposure, they undergo clonal expansion to form germinal centers within follicles.
  - The T cell and B cell populations are readily identified by immunohistochemical (IHC) staining for T and B cell markers. Proliferating cells in germinal centers are readily identified by IHC staining for cell proliferation, e.g., Ki-67.

Spleen

Development

- As a secondary immune system organ, the lymphoid component of the spleen is poorly formed at PND 1. During the period between PND 1 and PND 42 the lymphoid component undergoes progressive maturation to result in adult-appearing histomorphology.
- Periarteriolar lymphoid sheaths (PALS) and marginal zones develop in advance of lymphoid follicles with germinal centers, the latter being present at approximately PND 35.

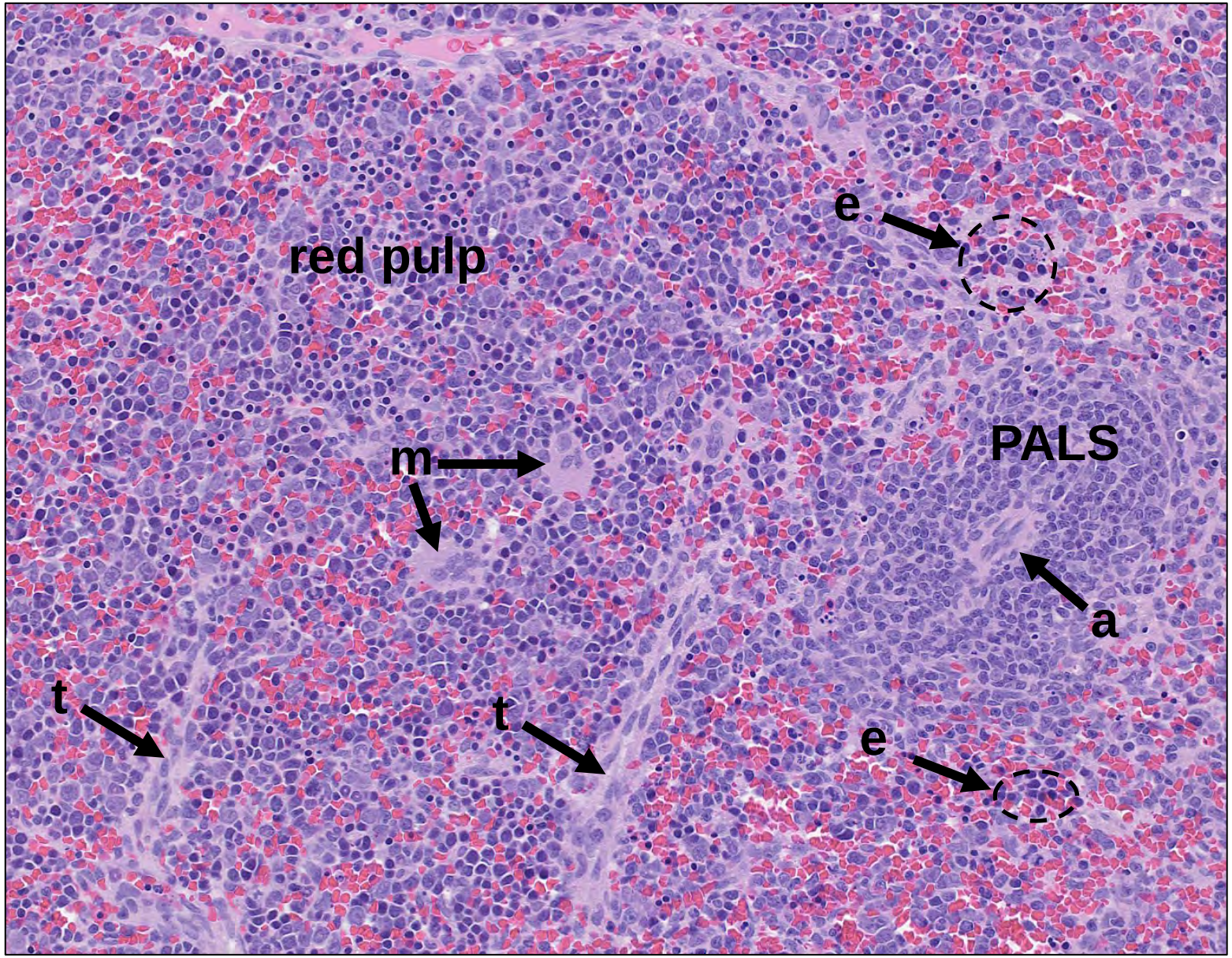
RIGHT: At PND 0 (day of birth), the Sprague Dawley rat spleen has little internal substructure, consisting of a sheet of individualized mononuclear cells supported by smooth muscle trabeculae (t). The thin superficial capsule (c) readily expands with changes in the internal cellularity.



Spleen

Development

- Sprague Dawley rat spleen at PND 0 has initial stage of periarteriolar lymphoid sheath (PALS) surrounding an arteriole (a)
- The highly cellular red pulp has extensive blood cell formation (hematopoiesis), including the megakaryocytes (m) that form blood platelets
- Blood cell types tend to occur in clusters; Erythroid precursors (e) have densely basophilic nuclei, while granulocytic precursors have slightly larger, less basophilic nuclei
- Smooth muscle trabeculae (t) provide overall structure to the spleen

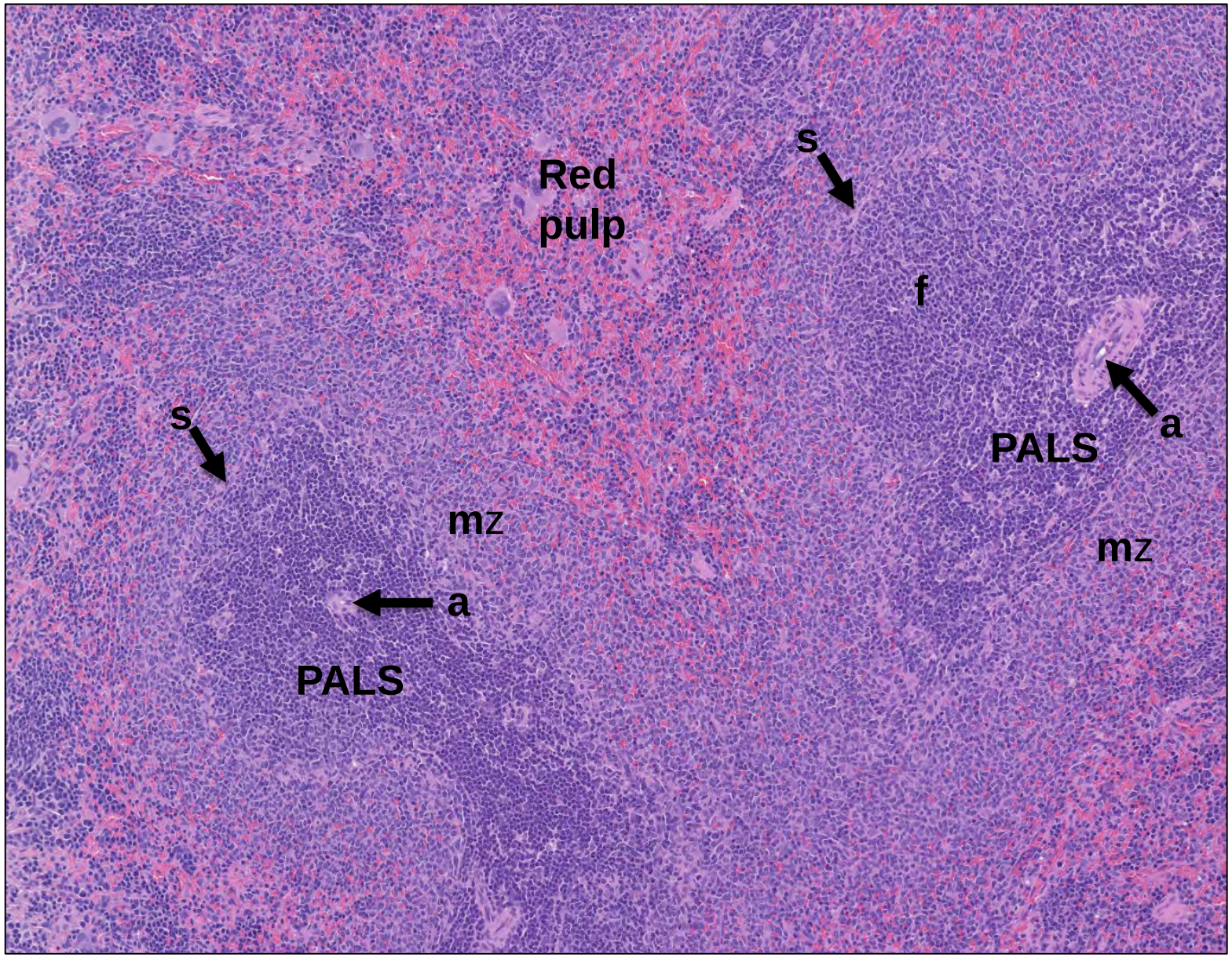


Spleen

Histomorphology

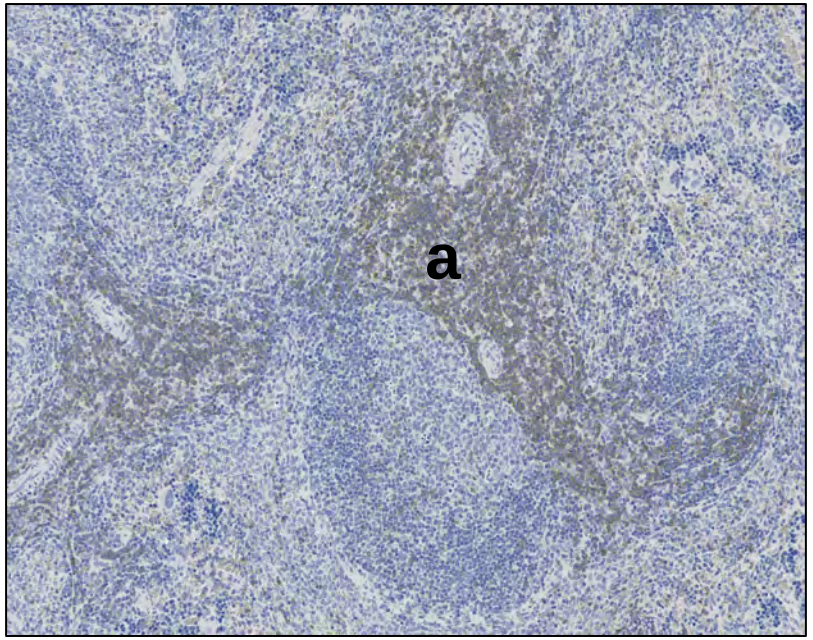
- By PND 42 the Sprague Dawley rat spleen has adult histomorphology with fully developed lymphoid components
- The marginal zone of rodents, particularly rats, is more prominent than in other species
- The marginal zone serves to introduce blood-borne antigens to the immunocytes contained in the marginal zone sinus and periarteriolar lymphoid sheaths (PALS)
- The inner aspect of the marginal zone contains specifically adapted marginal zone metallophilic macrophages that are important in clearance of circulating microorganisms and viruses
- The red pulp contains an additional population of macrophages that are focused on detection and destruction of obsolescent erythrocytes and recycling of erythrocyte components, resulting in the brown hemosiderin pigment that is seen microscopically

RIGHT: a = arteriole; PALS = periarteriolar lymphoid sheath; s = marginal sinus; f = follicle; mz = marginal zone

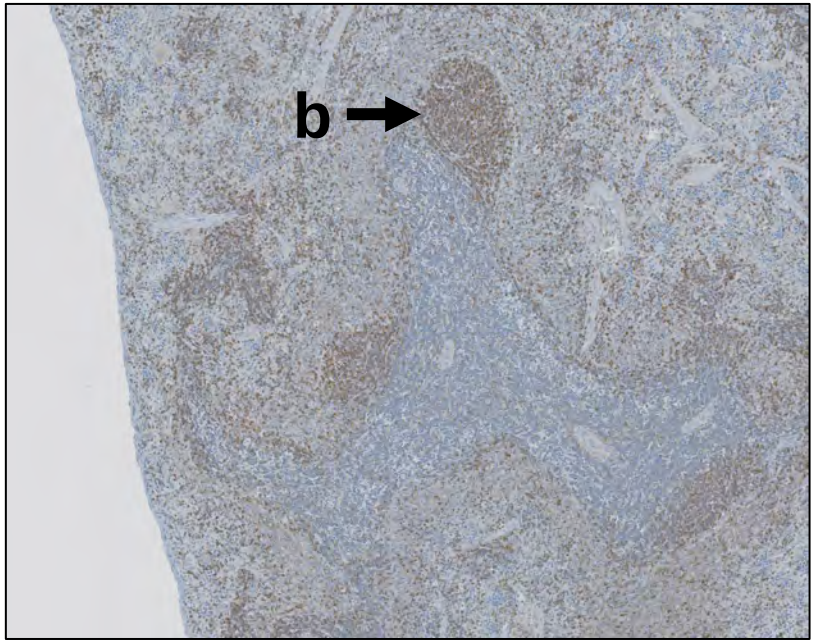


Spleen

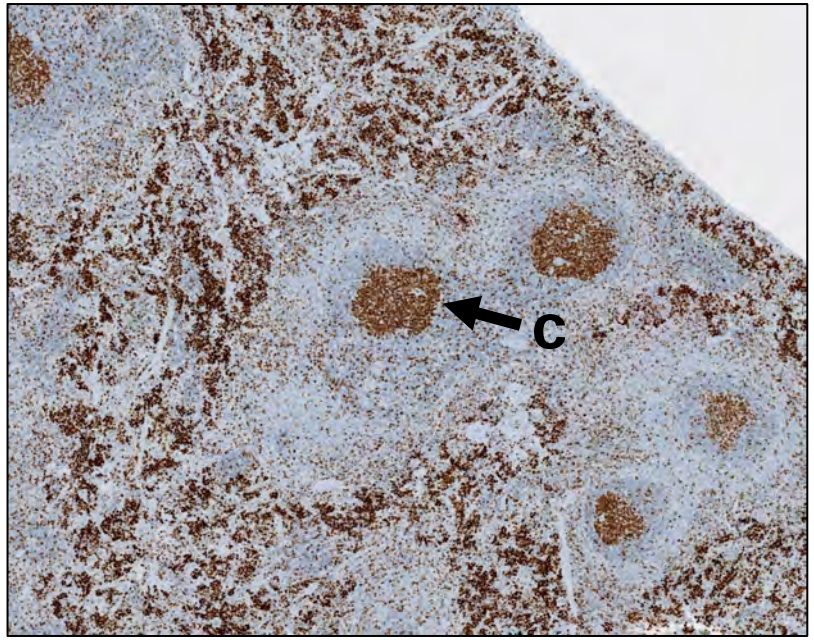
Histomorphology: immunohistochemistry



CD3



CD45RA



Ki67

Immunohistochemical staining performed on formalin-fixed, paraffin-embedded specimens of spleen from a Sprague Dawley rat at PND 42. LEFT: Brown CD3 staining (a) marks T cells in the periarteriolar lymphoid sheaths (PALS). MIDDLE: Brown CD45RA staining (b) marks B cells in follicles. RIGHT: Brown Ki67 staining (c) marks proliferating cells in germinal centers of follicles. ALL IMAGES: Brown staining by 3,3'-diaminobenzidine chromogen indicates target molecules, blue hematoxylin counterstain stains cell nuclei.

Spleen

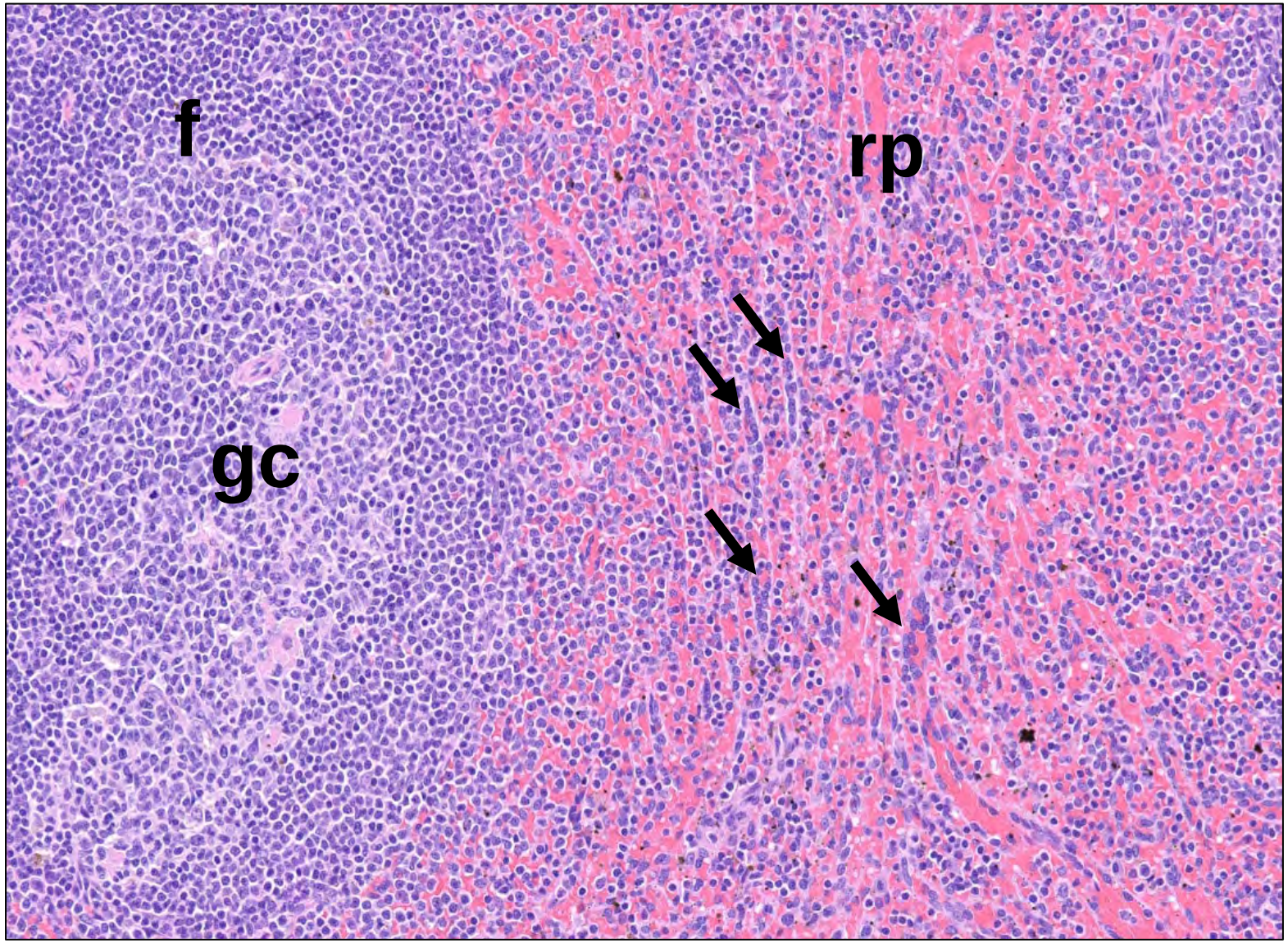
Spleen, cynomolgus macaque: ellipsoids

- Ellipsoids are cellular nodules with a centrally located capillary that is present in humans, nonhuman primates, other mammals, birds, reptiles, and fish, but they are not present in rodents.

- Marginal zones of rodents and ellipsoids of various other species serve the same function, i.e., presentation of blood-borne antigens to the immune system components.

RIGHT: Linear ellipsoids (arrows) in the spleen of a normal cynomolgus macaque consist of linear arrays of cells within the red pulp.

f = follicle, gc = germinal center, rp = red pulp



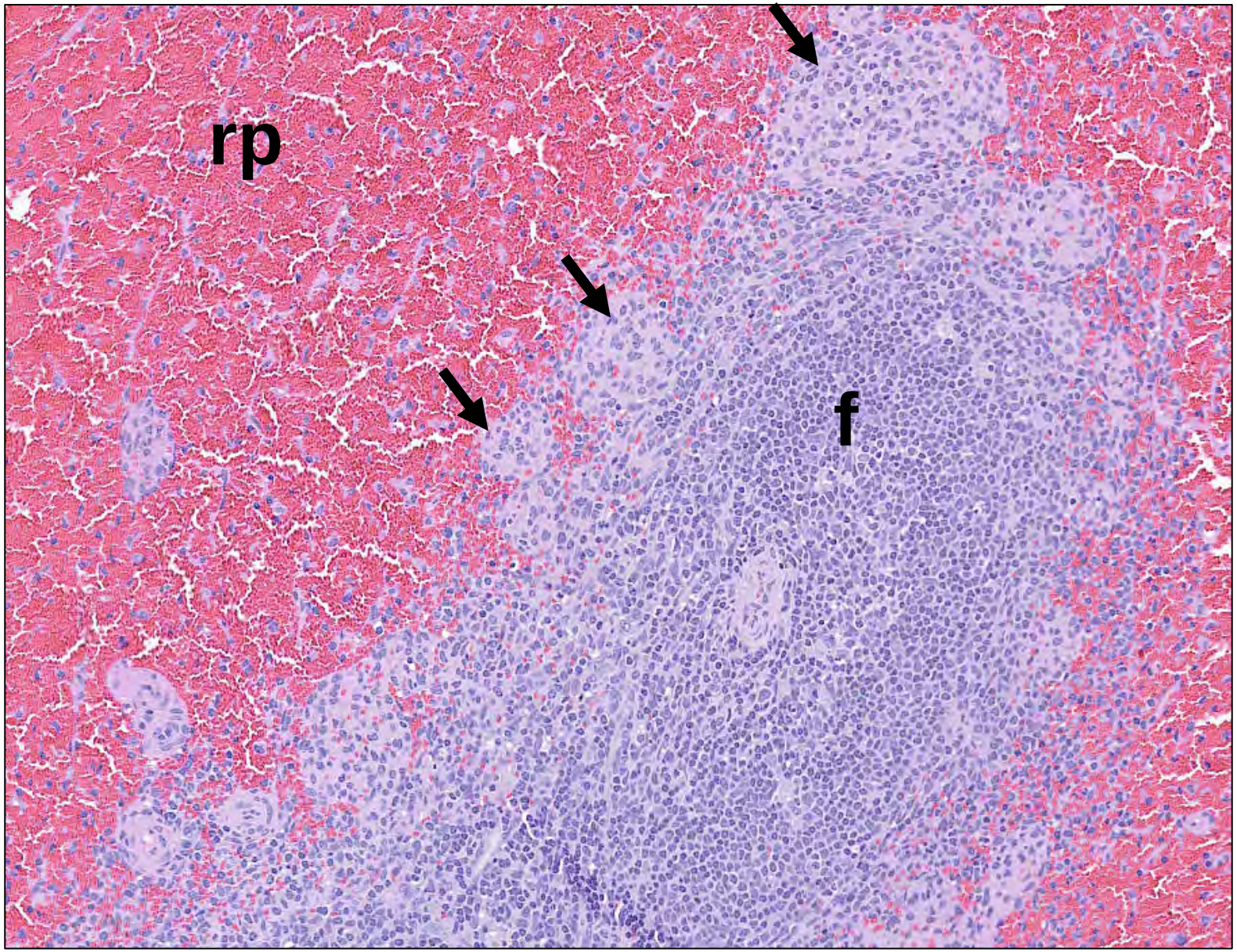
Spleen

Spleen, pig: ellipsoids

In pig spleens, ellipsoids are composed of reticular cells, lymphocytes, and abundant macrophages.

RIGHT: The microscopic appearance of ellipsoids (arrows) in the spleen of pigs is different from the morphology of ellipsoids in nonhuman primates and marginal zones of rodents, but the basic function of both ellipsoids and marginal zones is the interrogation of blood for detection of blood-borne antigens and subsequent antigen presentation.

f = follicle, rp = red pulp

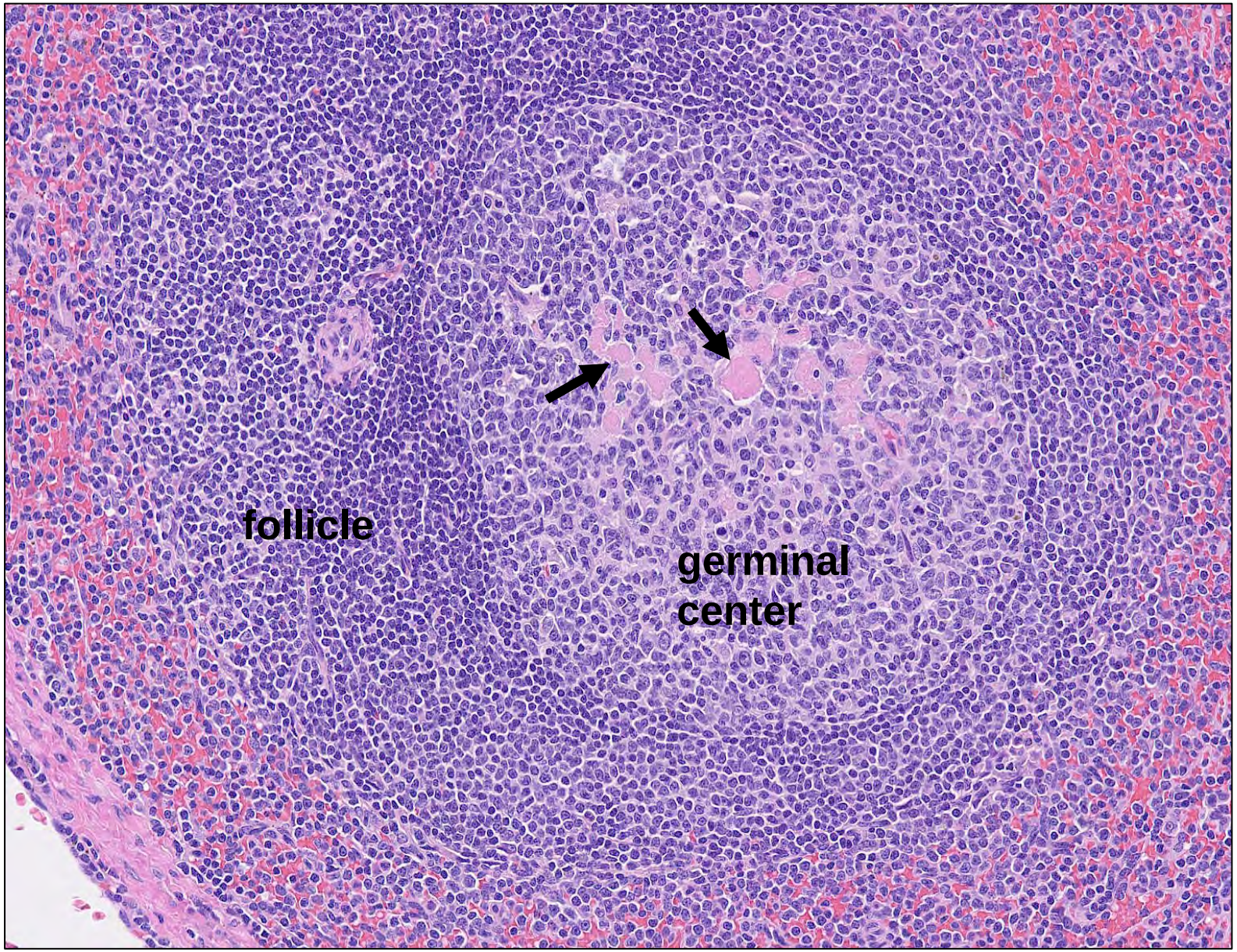


Spleen

Histomorphology

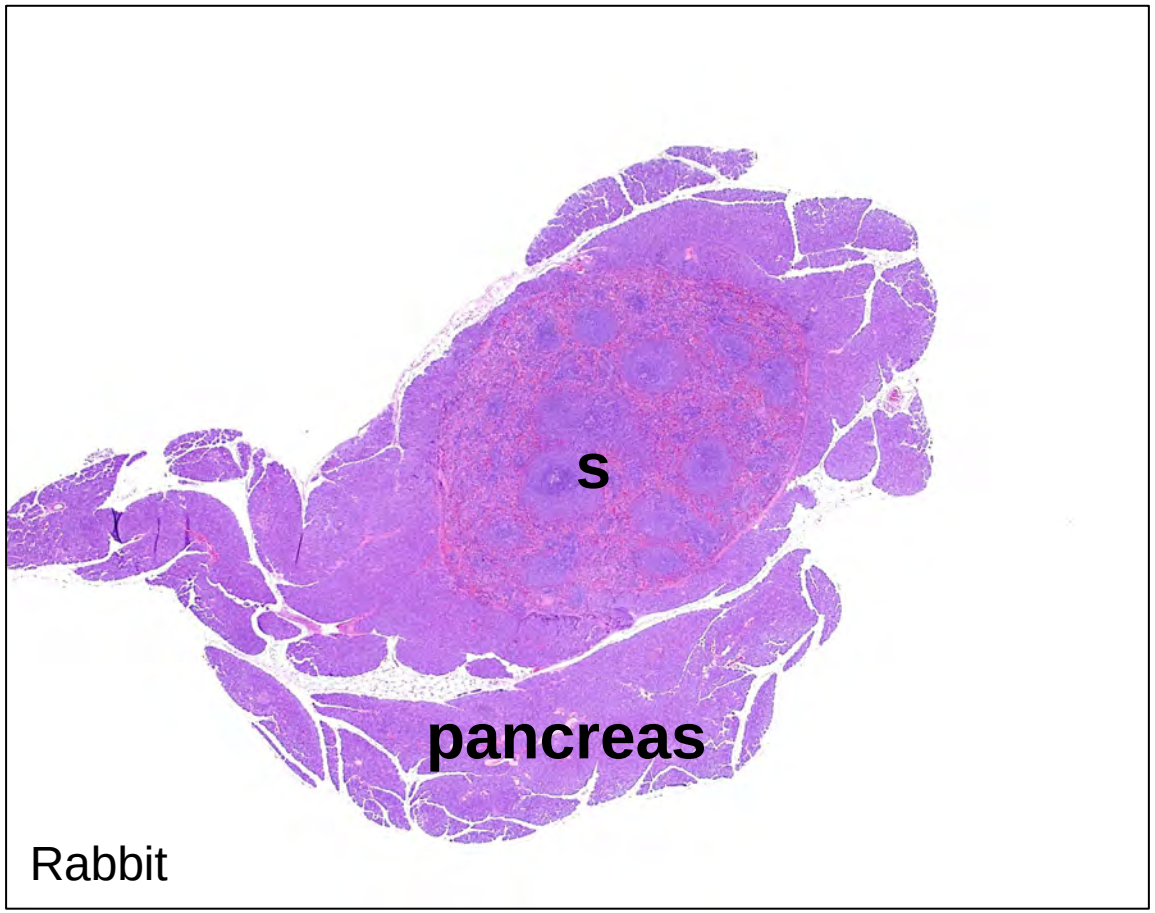
- Active germinal centers in follicles in the spleen and lymph nodes of nonhuman primates commonly contain amorphous acidophilic material (arrows)
- Microscopic features of the material are consistent with amyloid, but special stains for amyloid are negative. The material stains positively with periodic acid/Schiff (PAS) stain.
- The material often stains positively for IgM (immunoglobulin M, the antibody produced during the initial response to an antigen or microbe) with immunohistochemistry (IHC) staining and may stain positively for kappa and lambda immunoglobulin light chains
- The exact nature and genesis of this material is uncertain, but it is presumed to be a product of immune reactions such as antigen-antibody complexes

RIGHT: Spleen of young adult cynomolgus macaque from a short-term safety assessment study with amorphous acidophilic material (arrows) in a germinal center

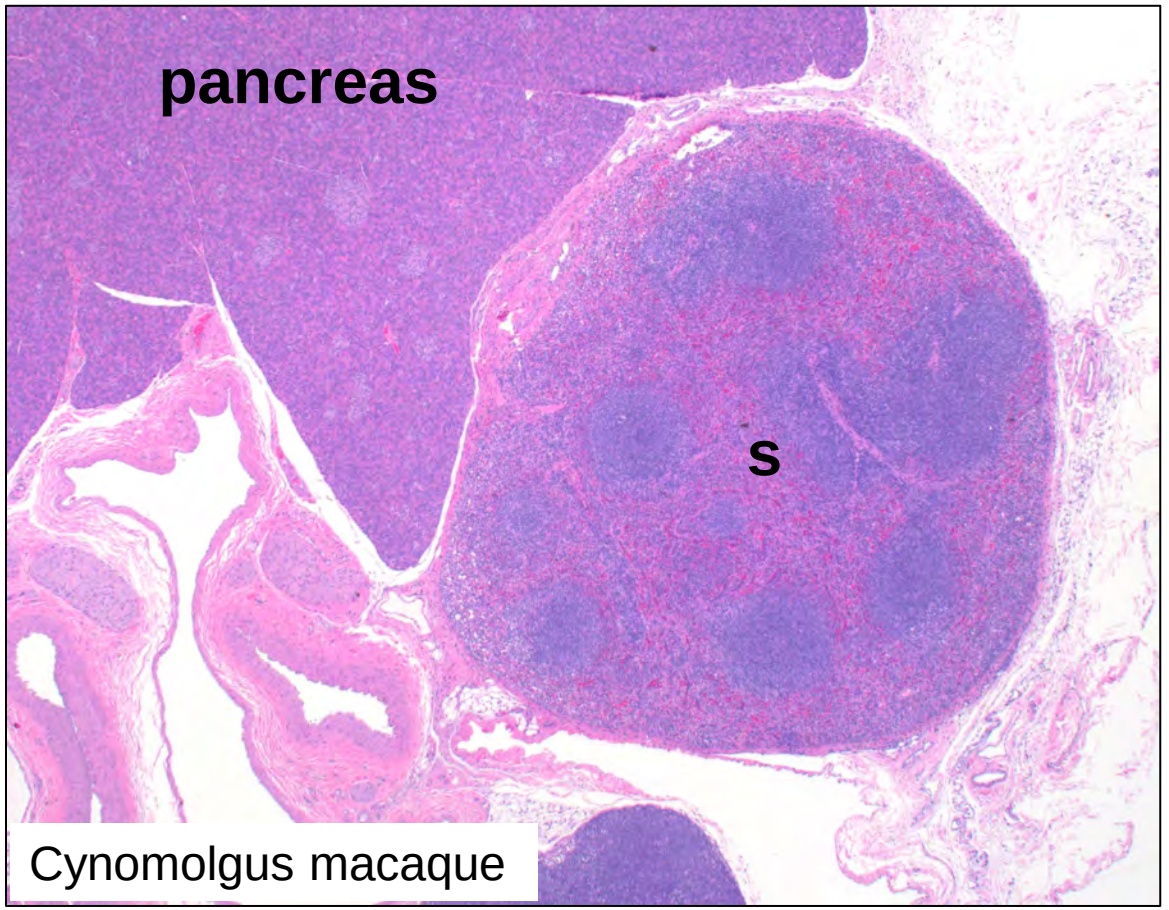


Spleen

Ectopic spleen tissue



Rabbit



Cynomolgus macaque

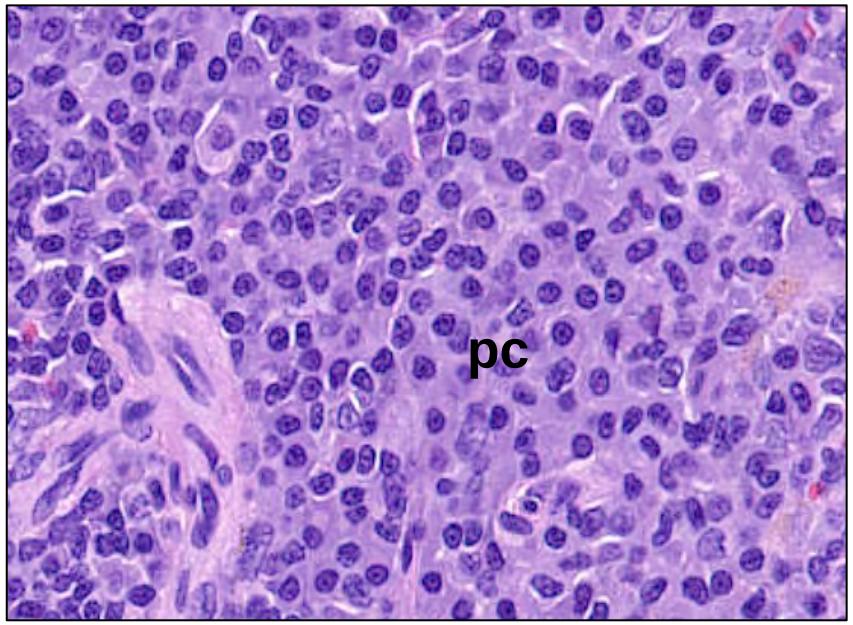
Failure of fusion of the splenic primordia results in ectopic splenic tissue within adjacent tissues, commonly the pancreas. Ectopic splenic tissue in various areas of the abdominal cavity may result from abdominal trauma with rupture of the spleen, but such events are uncommon in toxicology studies. s = ectopic splenic tissue

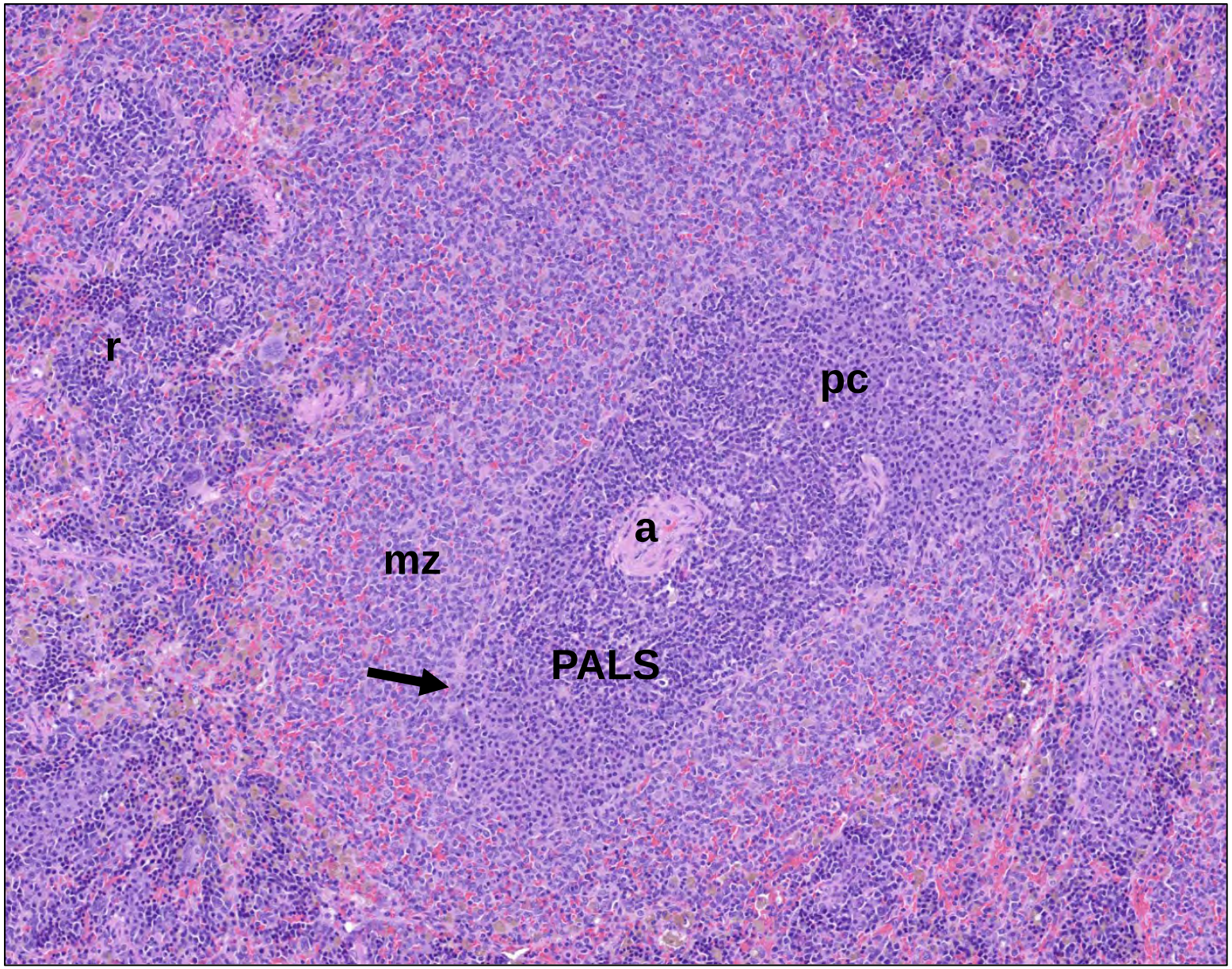
Spleen

Aging changes

The spleen from an aged male rat has normal-appearing periarteriolar lymphoid sheath (PALS) and marginal zone (mz) surrounding an arteriole (a), but the spleen has no visible follicles or germinal centers.

The arrow indicates the location of the marginal zone sinus. The red pulp (r) has abundant hematopoietic activity. An aggregation of plasma cells (pc) is present at the periphery of the PALS.

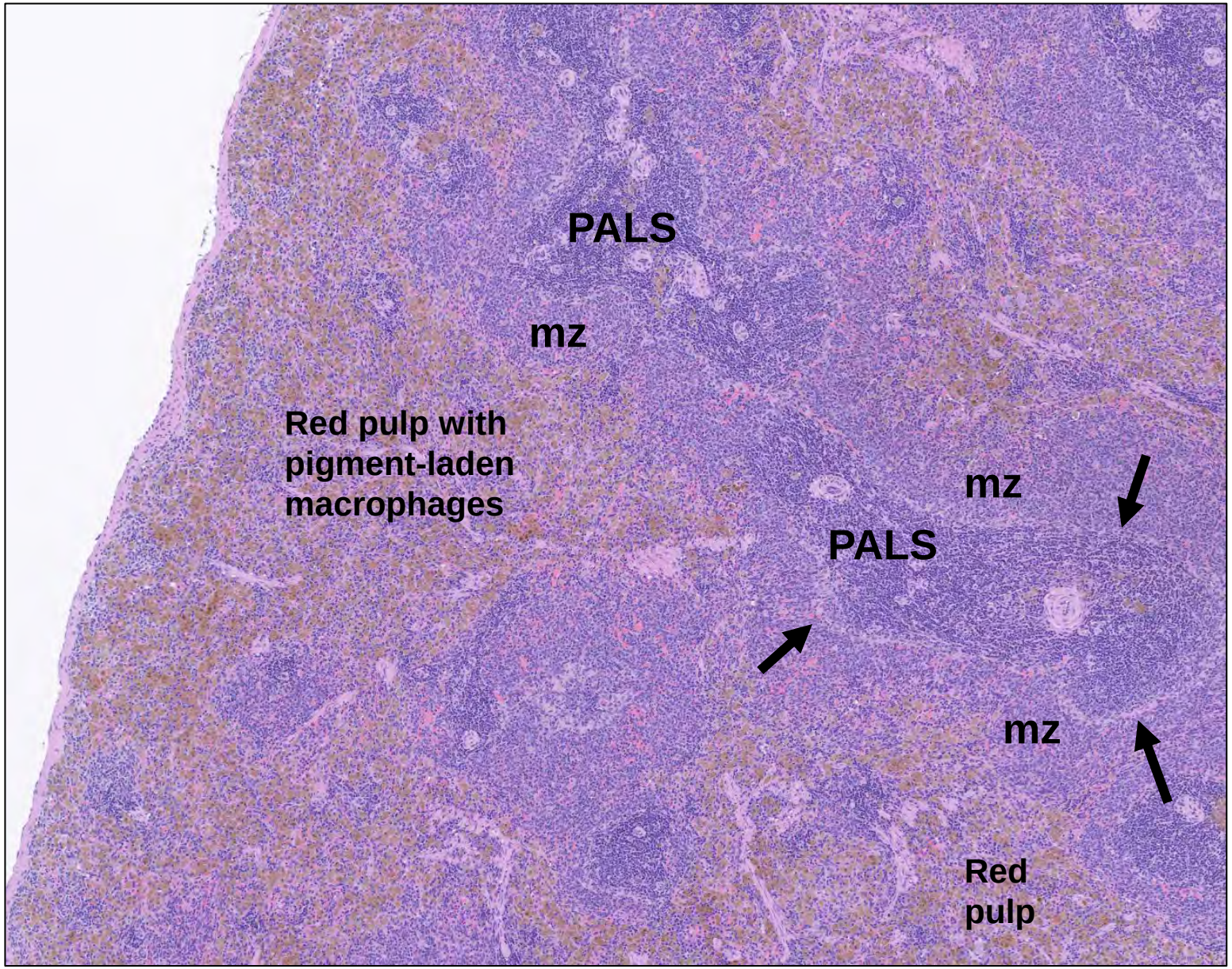




Spleen

Aging changes

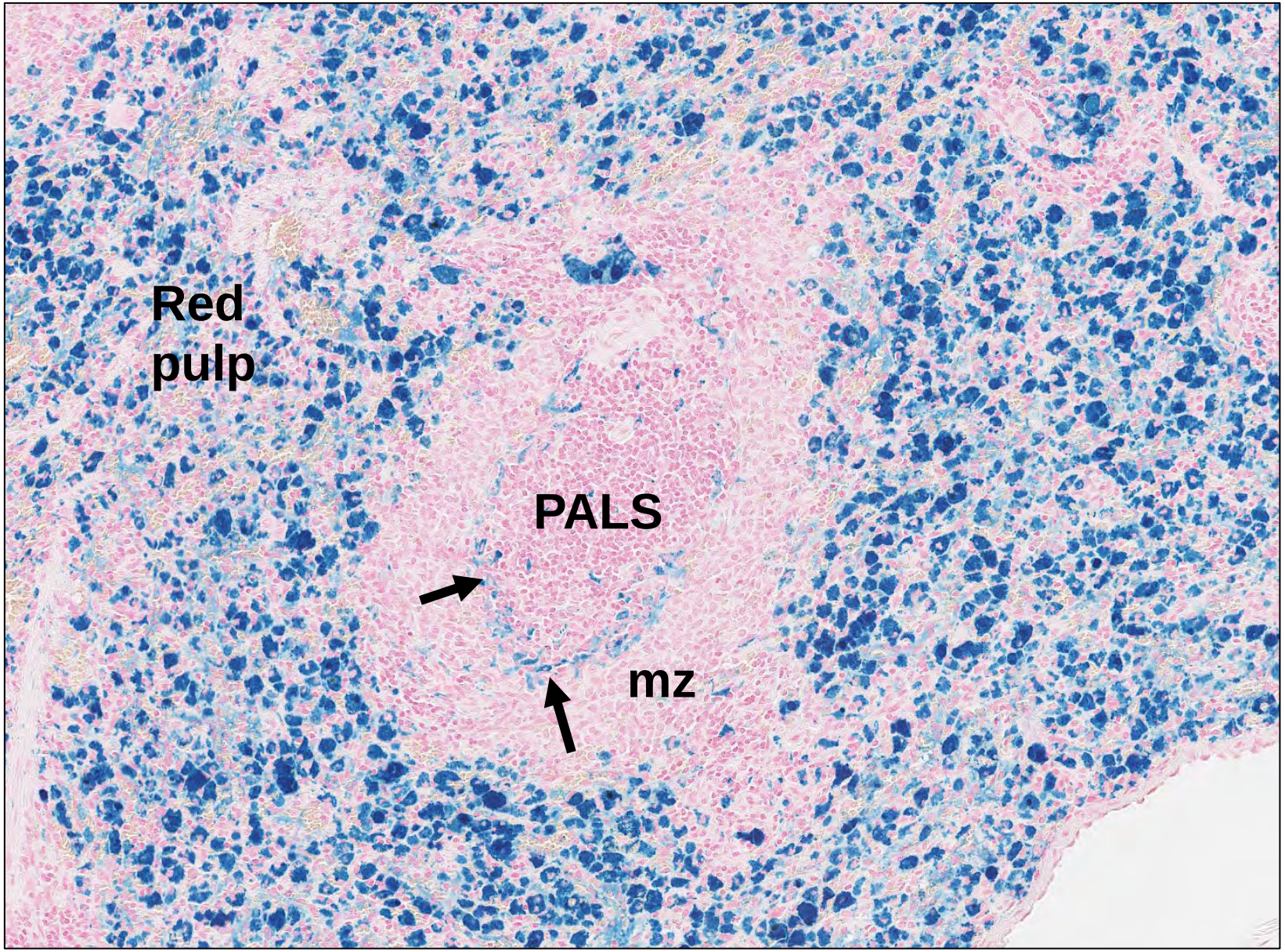
- Aged rats, particularly females, tend to accumulate blood breakdown pigment in the spleen, which reflects a major function of the spleen in removing senescent erythrocytes from the circulation.
- Macrophages in the red pulp contain an abundance of golden-brown hemosiderin pigment that results from erythrocytic hemoglobin degradation.
- The spleen of an aged (~24 months) female Wistar rat has prominent periarteriolar lymphoid sheaths (PALS) and marginal zones (mz) but no visible follicles or germinal centers.
- The thin line (arrows) between the PALS and marginal zone of the spleen indicates the location of the marginal zone sinus with its specialized macrophage populations.



Spleen

Aging changes

- In this section of spleen from an aged male Wistar rat stained with Prussian blue, the red pulp has an abundance of blue-stained, iron-laden macrophages that function in removal and degradation of obsolete circulating erythrocytes
- The thin line (arrows) of blue-stained cells represents macrophages in the marginal zone sinus, which is located between the periarteriolar lymphoid sheath (PALS) and the inner aspect of the marginal zone (mz)



Lymph Node

Overview

- Embryogenesis of lymph nodes involves interactions between lymphoid tissue initiator (LTI) cells (of bone marrow origin) and lymphoid tissue organizer (LTO) cells (of preadipocyte origin)
- Embryogenesis of somatic (in areas other than head, neck, or visceral regions) lymph nodes commonly occurs within fatty tissue at points of venule bifurcation
  - Postnatal maturation of intestine-associated lymph nodes occurs earlier than maturation of somatic lymph nodes
- As secondary immune system organs, lymph nodes are poorly formed at birth
  - Subsequent maturation is driven largely by antigenic stimulation of the lymph node
  - The “mature” microscopic appearance of lymph nodes is dependent on continued antigenic stimulation
  - Without that stimulation, lymph nodes revert to an “immature” histomorphology that may be confused with immunotoxicity
- Lymph nodes consist of multiple conjoined units, each of which consists of B cell-predominant follicles and T cell-predominant deep cortical units (DCU)

Lymph Node

Collection

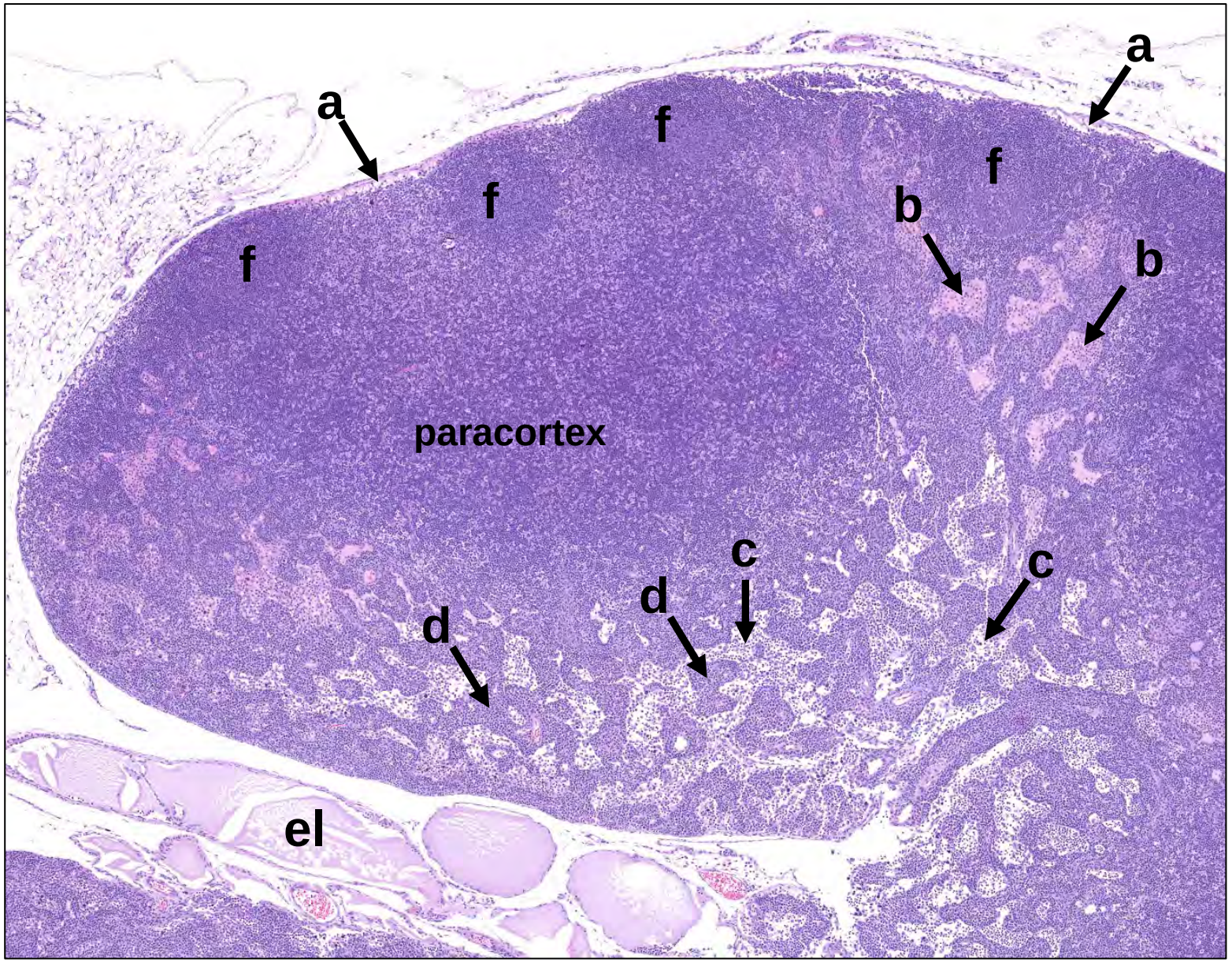
- Mandibular and mesenteric lymph nodes are routinely collected and evaluated from toxicology studies
  - These lymph nodes are highly influenced by ingested antigens or by ingested immunomodulatory molecules
- Ideally, specimens of lymph nodes elsewhere in the body (axillary, popliteal, inguinal, etc.) are also evaluated to provide data on systemic effects on the immune system
- Additional lymph nodes may be collected for specific routes of test article administration, e.g. regional (draining) lymph nodes for subcutaneous injections
- For additional information on lymph node location and terminology, see Tilney (1971) Patterns of lymphatic drainage in the adult laboratory rat. J Anat 109: 369-83. Adapted from Sanders & Florey (1940) The effects of the removal of lymphoid tissue. Br J exp Path 21: 275-287

Lymph Node

Histomorphology

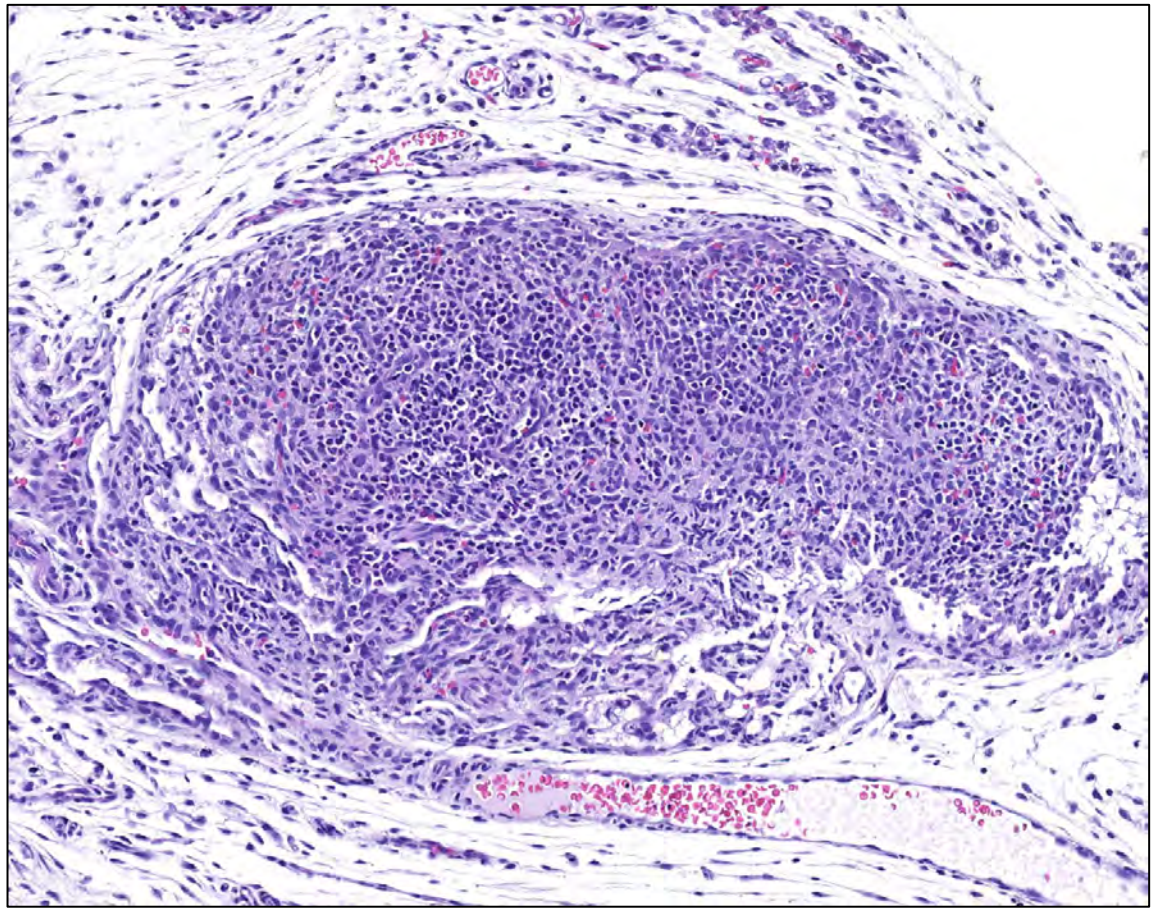
- Lymph fluid enters the lymph node via the subcapsular sinuses (a), travels down through the paracortical sinuses (b) to the medullary sinuses (c), and exits the lymph node via the efferent lymphatics (el)
- The immunoglobulin-producing cells are concentrated in the medullary cords (d)
- B cell areas are concentrated in the cortical follicles (f), while T cells are concentrated in the paracortex
- Antigenic peptides in the lymph interact with naïve B cells in the follicles, and interaction with helper T cells in the paracortex results in an expansion of immunoglobulin-producing B cells and plasma cells

RIGHT: A mesenteric lymph node of a male Sprague Dawley rat at PND 42

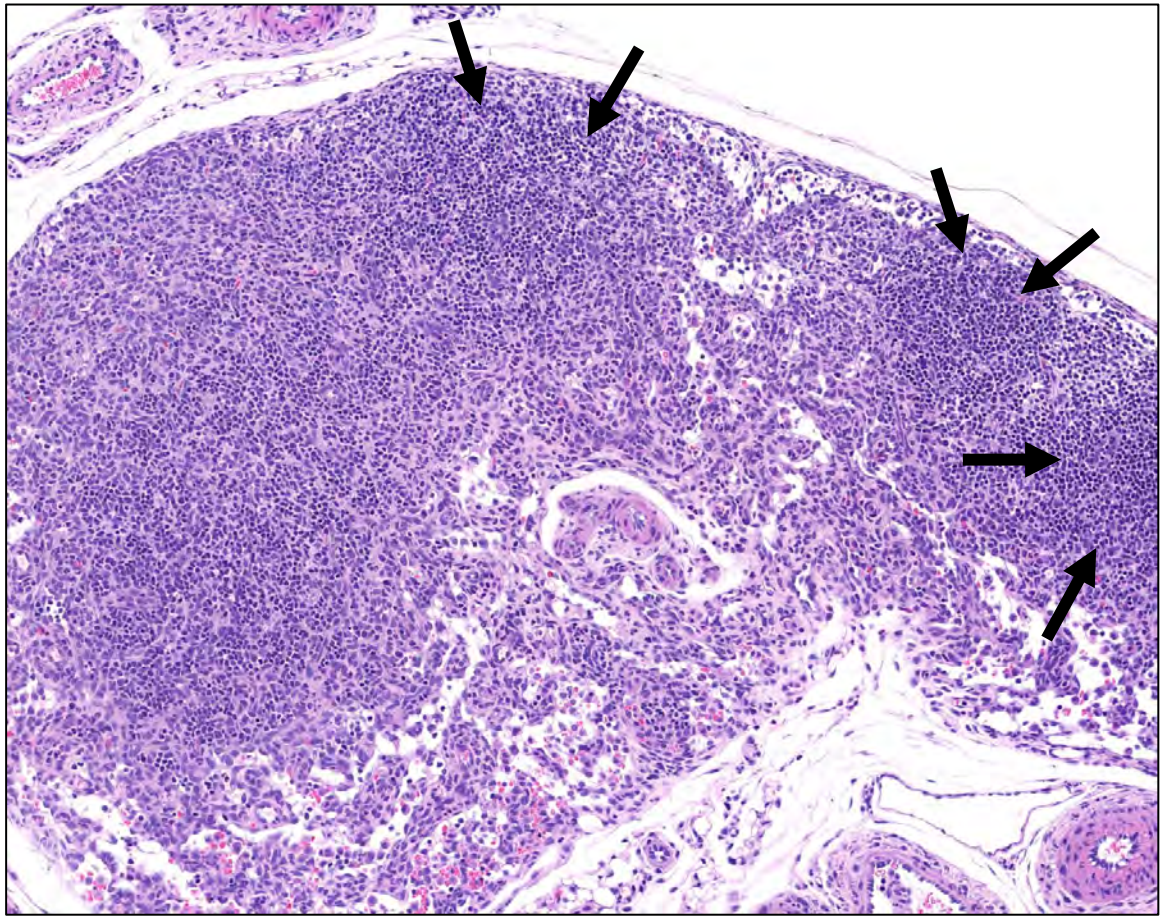


Lymph Node

Postnatal development



Mandibular lymph node of rat at PND 0 has a sparse population of lymphocytes with no indication of follicle development



Axillary lymph node at PND 7 has more intense lymphocyte population with suggestions of follicle development (arrows)

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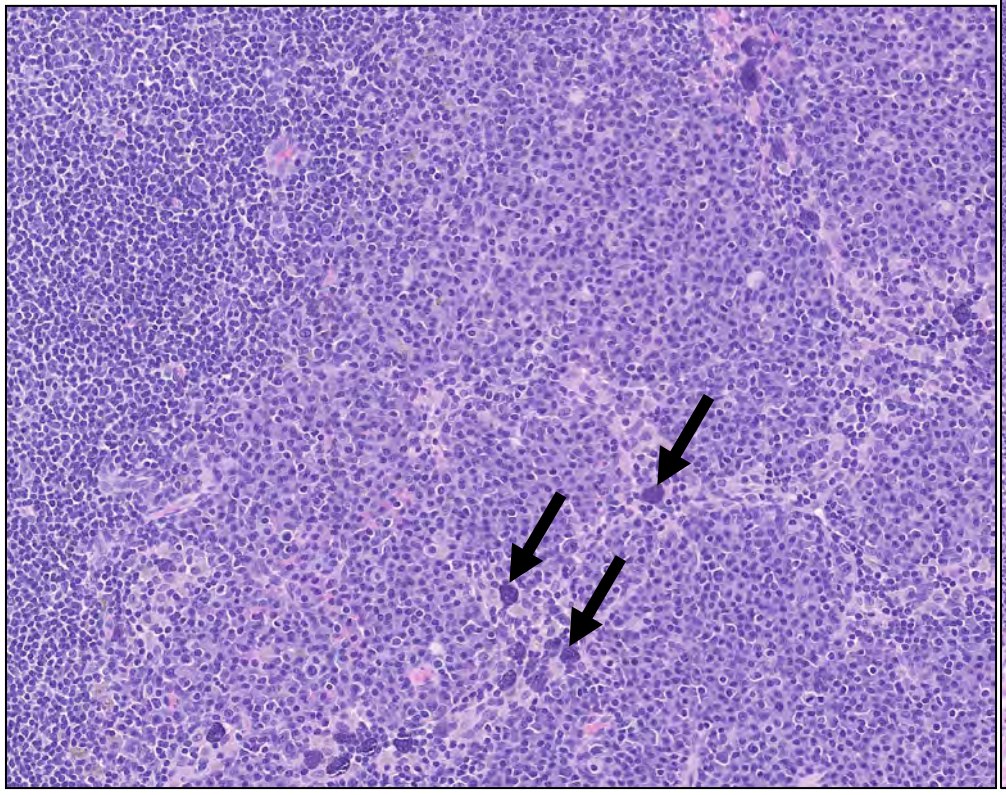
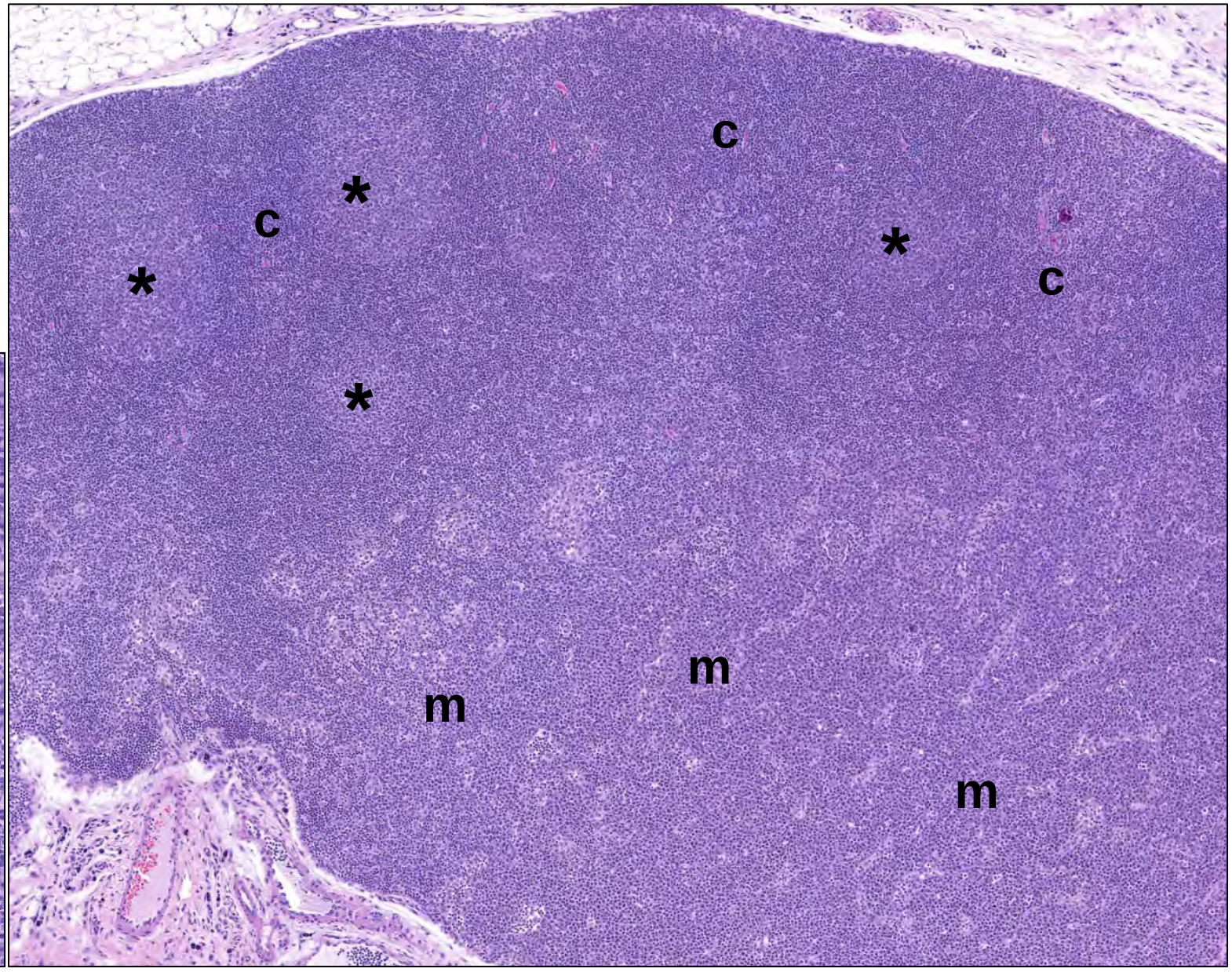
Lymph Node

Histomorphology

The mandibular lymph node has highly cellular cortex (c) and medulla (m), with prominent germinal centers (*). The mandibular lymph node of young rats commonly has a pronounced plasma cell population and often has a mast cell population (arrows).

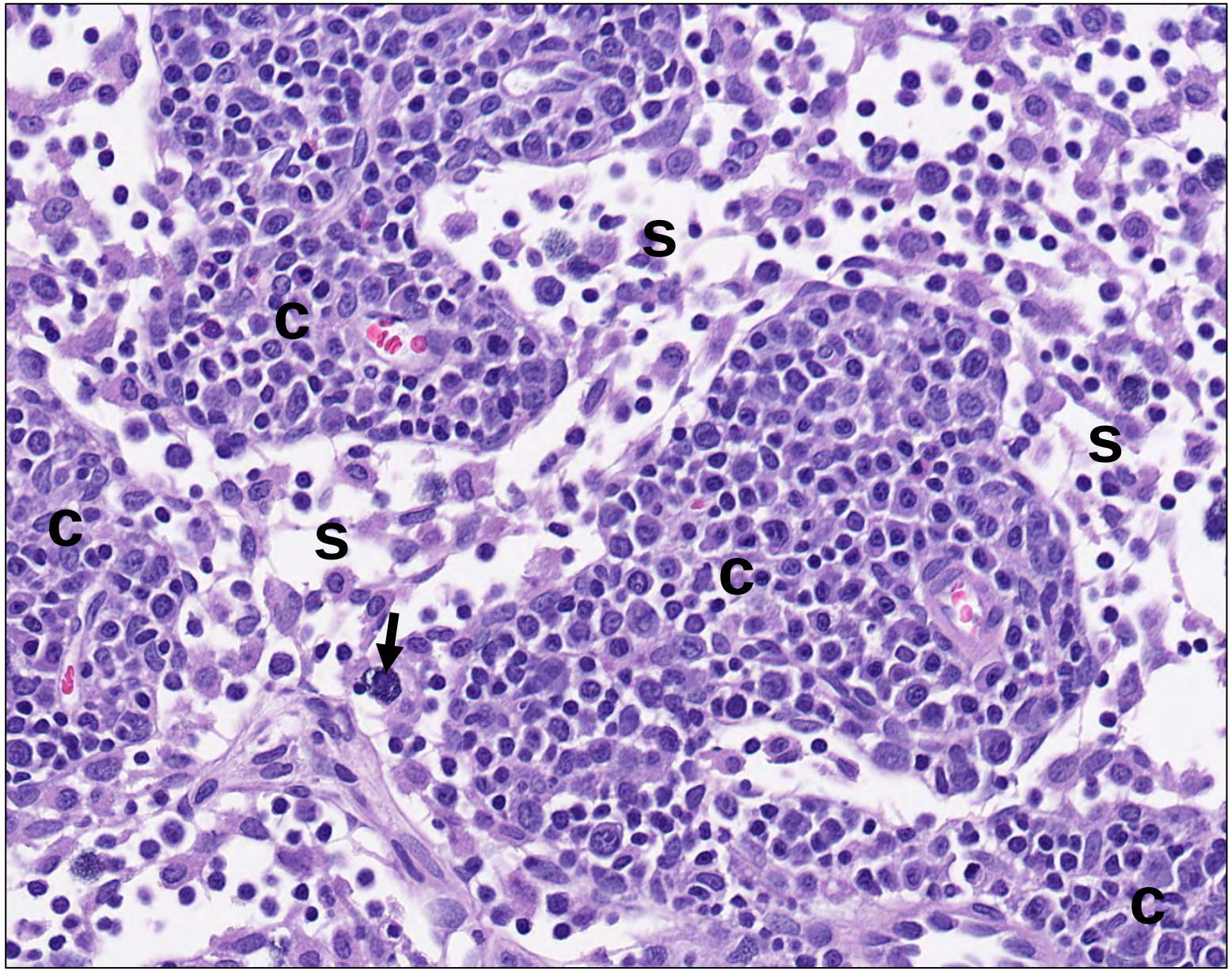




Lymph Node

Histomorphology

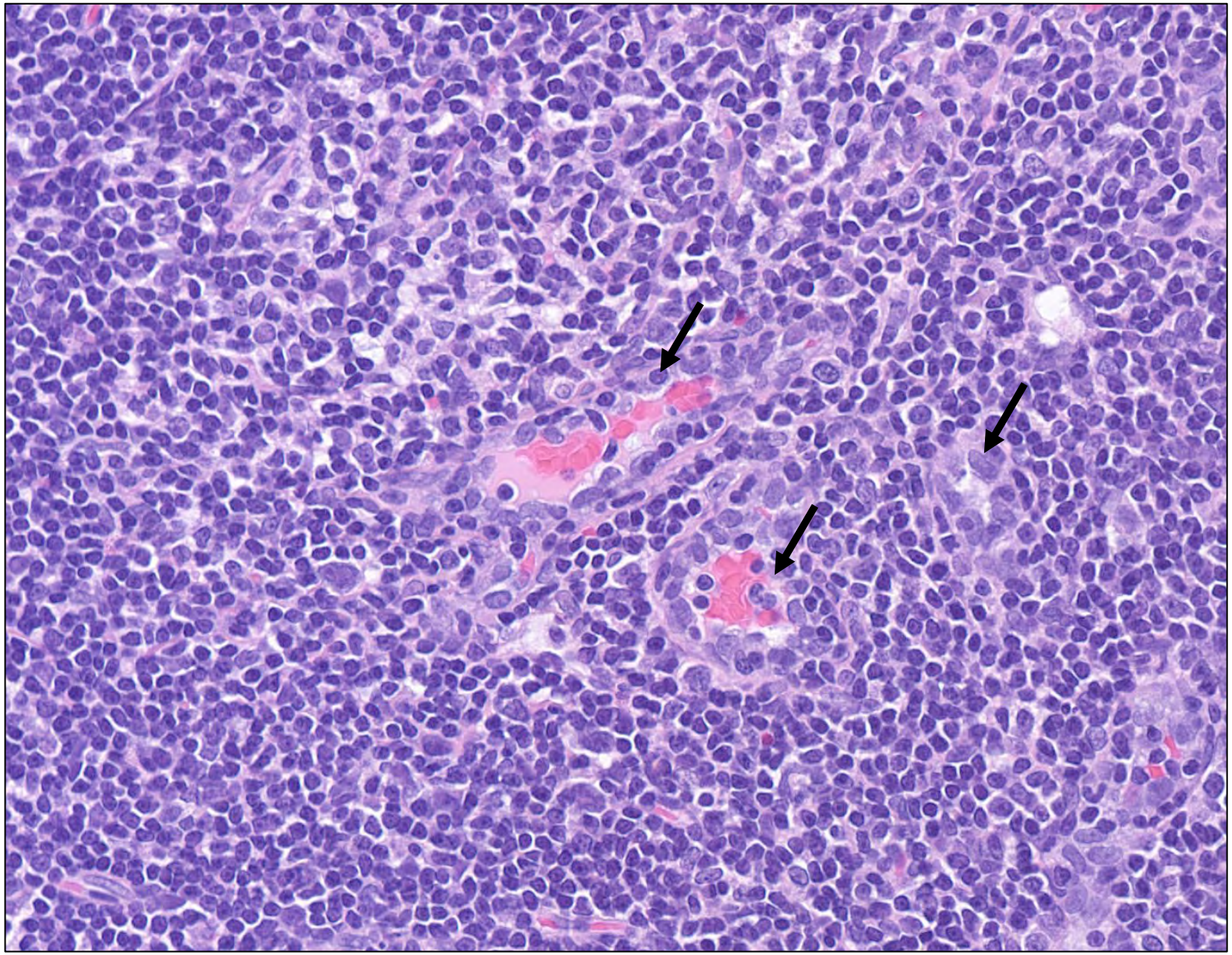
- The medullary region of the mesenteric lymph node from a Sprague Dawley rat at PND 42 has microscopically distinctive medullary cords (c) and sinuses (s), each having distinctive cell populations depending on the immunoreactive status of the lymph node
- Medullary cords contain numerous plasma cells and lymphocytes that are exiting the lymph node
- Medullary sinuses contain macrophages and lymphocytes that are percolating through the sinusoidal system of the lymph node; occasional mast cells (arrow) are present



Lymph Node

Histomorphology: high-endothelial venules

- High endothelial venules (HEV) (arrows) in the paracortex of lymph nodes serve as portals of entry for circulating lymphocytes, including antigen-specific naïve lymphocytes received from the bone marrow
- Emigration of naïve B and T cells from the blood involves progressive steps of rolling, adhesion and transmigration, each mediated by specific signaling and effector molecules
- Fibroblastic reticular cells (FRC) form a 3-dimensional framework that supports movement of naïve B and T cells within the lymph node, as guided by additional signaling and effector molecules
- Presence of an increased number or increased prominence of HEVs may be an indication of elevated immunoreactivity



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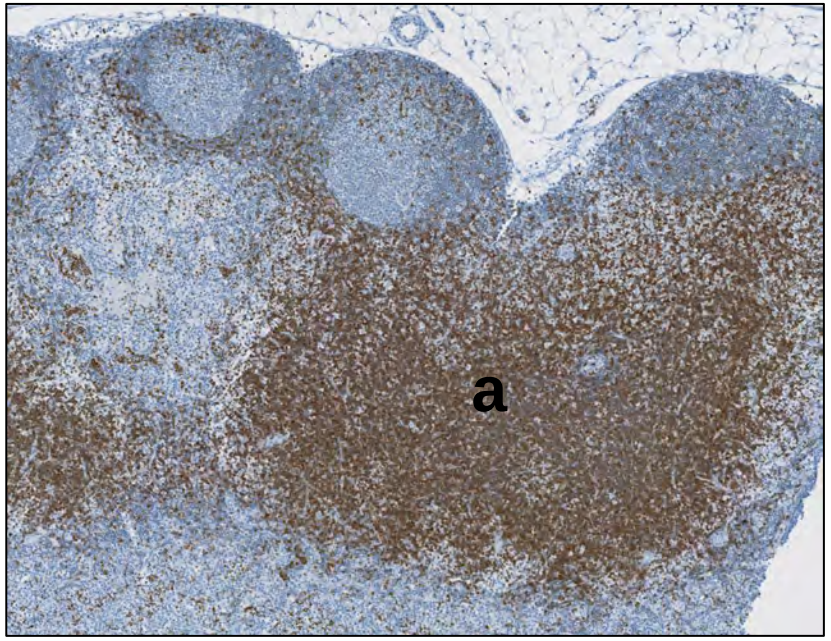
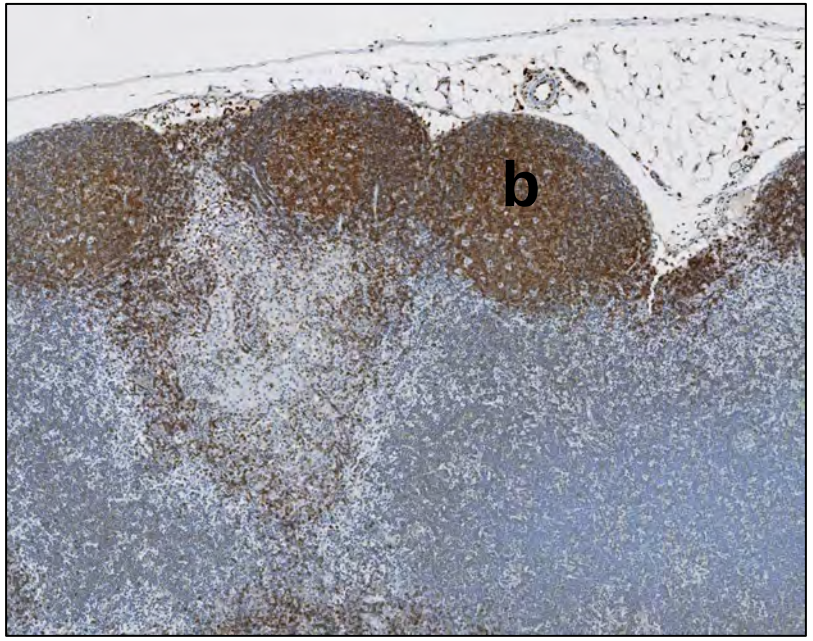
Lymph Node

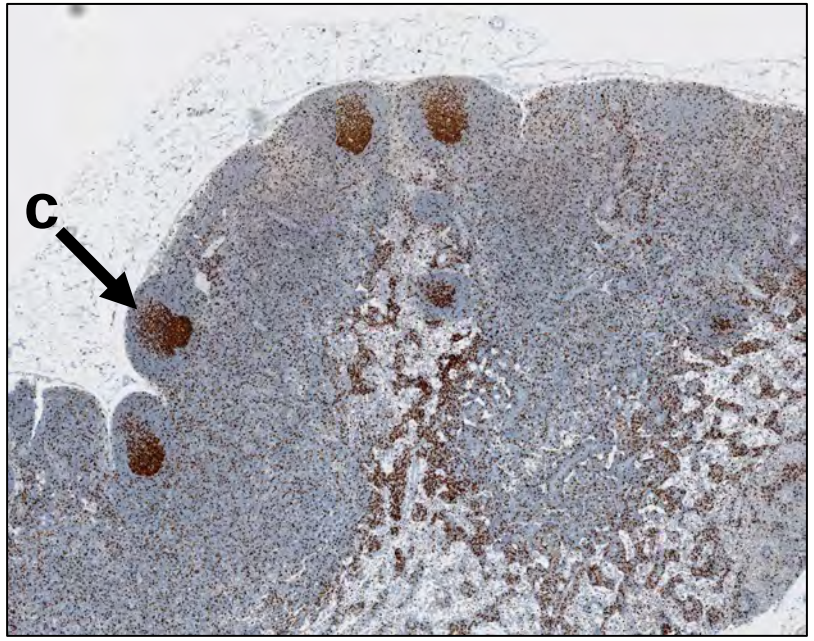
Histomorphology: immunohistochemistry



CD3



CD45RA



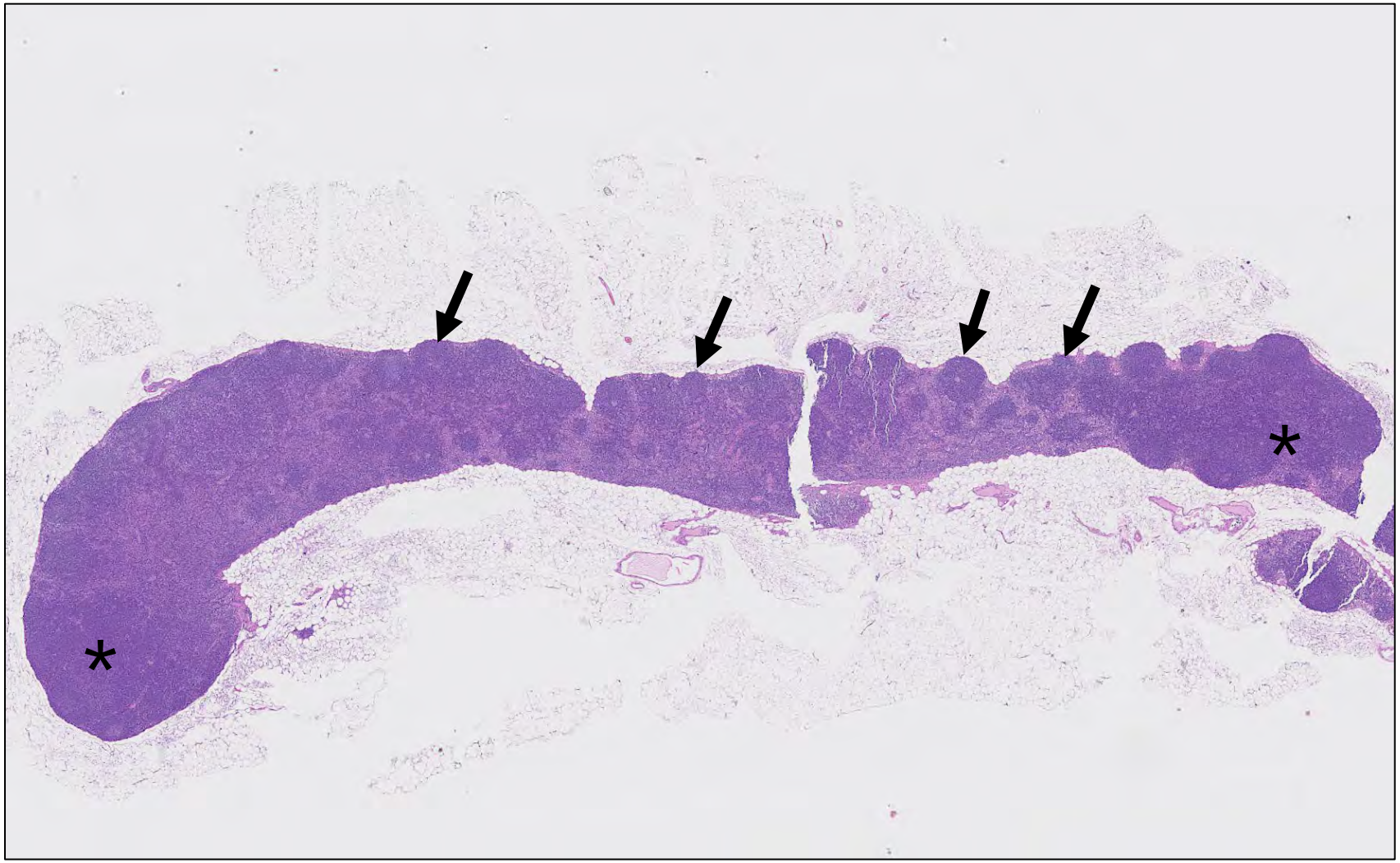
Ki67

Immunohistochemical staining performed on formalin-fixed, paraffin-embedded specimens. LEFT: Brown CD3 staining (a) marks T cells in the paracortex. MIDDLE: Brown CD45RA staining (b) marks B cells in follicles. RIGHT: Brown Ki67 staining (c) marks proliferating cells in germinal centers of follicles. In all images, brown staining by 3,3'-diaminobenzidine chromogen indicates target molecules, blue hematoxylin counterstain stains cell nuclei.

Lymph Node

Histomorphology: multiple functional units

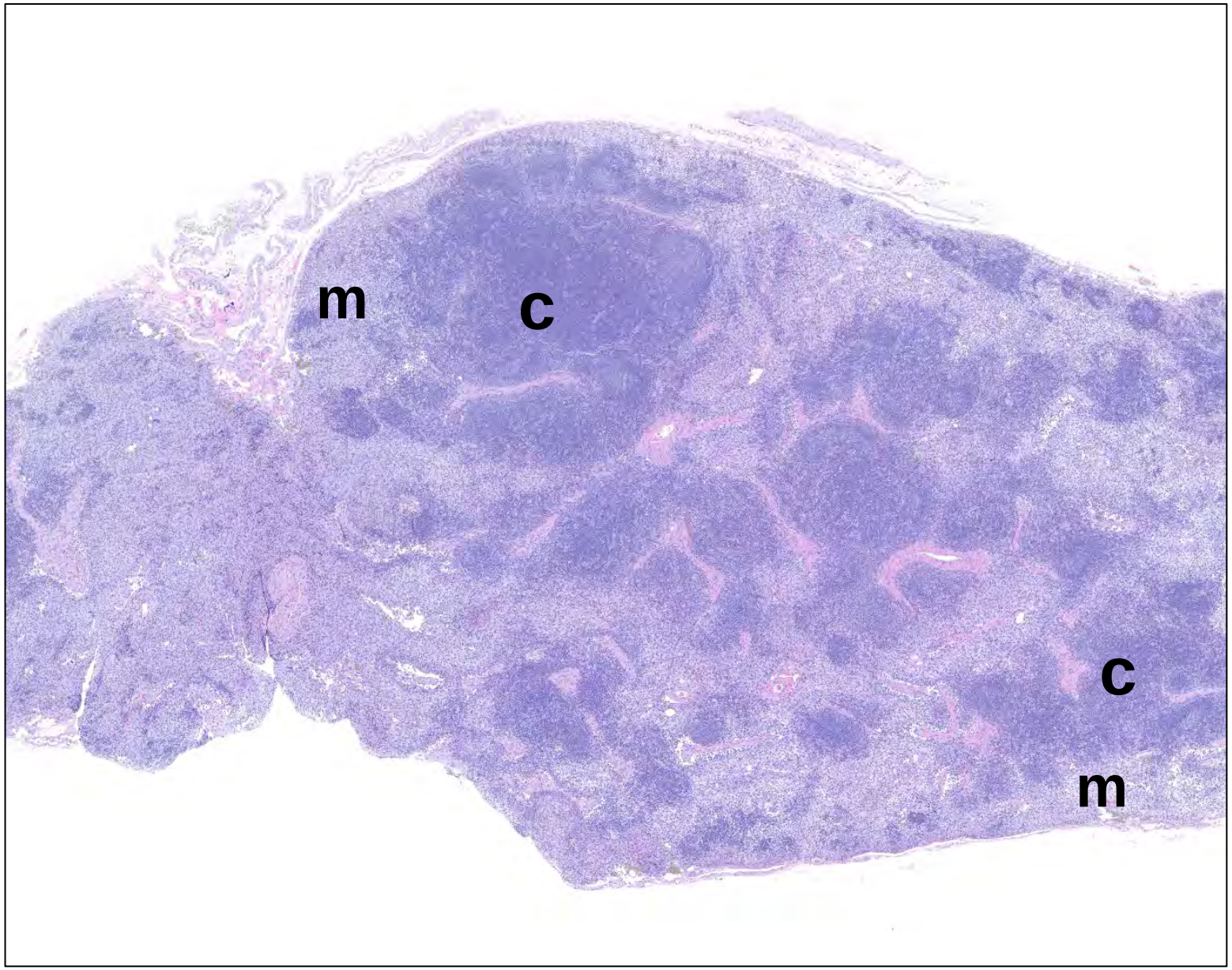
- Lymph nodes consist of multiple functional units fused into single anatomic organs. Functional units may serve different tissue sites and may display different microscopic features, depending on the pathologic processes in the area served.
- This feature is particularly evident in mesenteric lymph nodes of rats, which are typically elongated structures composed of numerous functional units (image on right, longitudinal section). Some areas of the mesenteric lymph node of a Sprague Dawley rat are highly cellular and thickened (*), while other areas are thinner and have small, inactive-appearing follicles (arrows). This morphologic difference reflects pathologic processes in specific regions of the intestine.
- Cross-sections of lymph nodes, particularly mesenteric lymph nodes, may not yield accurate information regarding the full status of the lymph node.



Lymph Node

Histomorphology: minipig

- Lymph nodes of swine, including minipigs, commonly lack the typical lymph node architecture of an external cortex and centrally placed medulla
- Swine are said to have “reversed” microarchitecture in which the densely cellular ‘”cortical” areas (c) are located centrally, and the “medullary” areas (m) are located in the exterior aspect of the lymph node
- Therefore, in swine lymph nodes, the terms “cortical” and “medullary” refer to functional areas of the lymph node, not the physical location of the areas
- Some lymph nodes of swine do not exhibit this “reversal” of microarchitecture, instead having a disorderly mixture of cortical and medullary areas.



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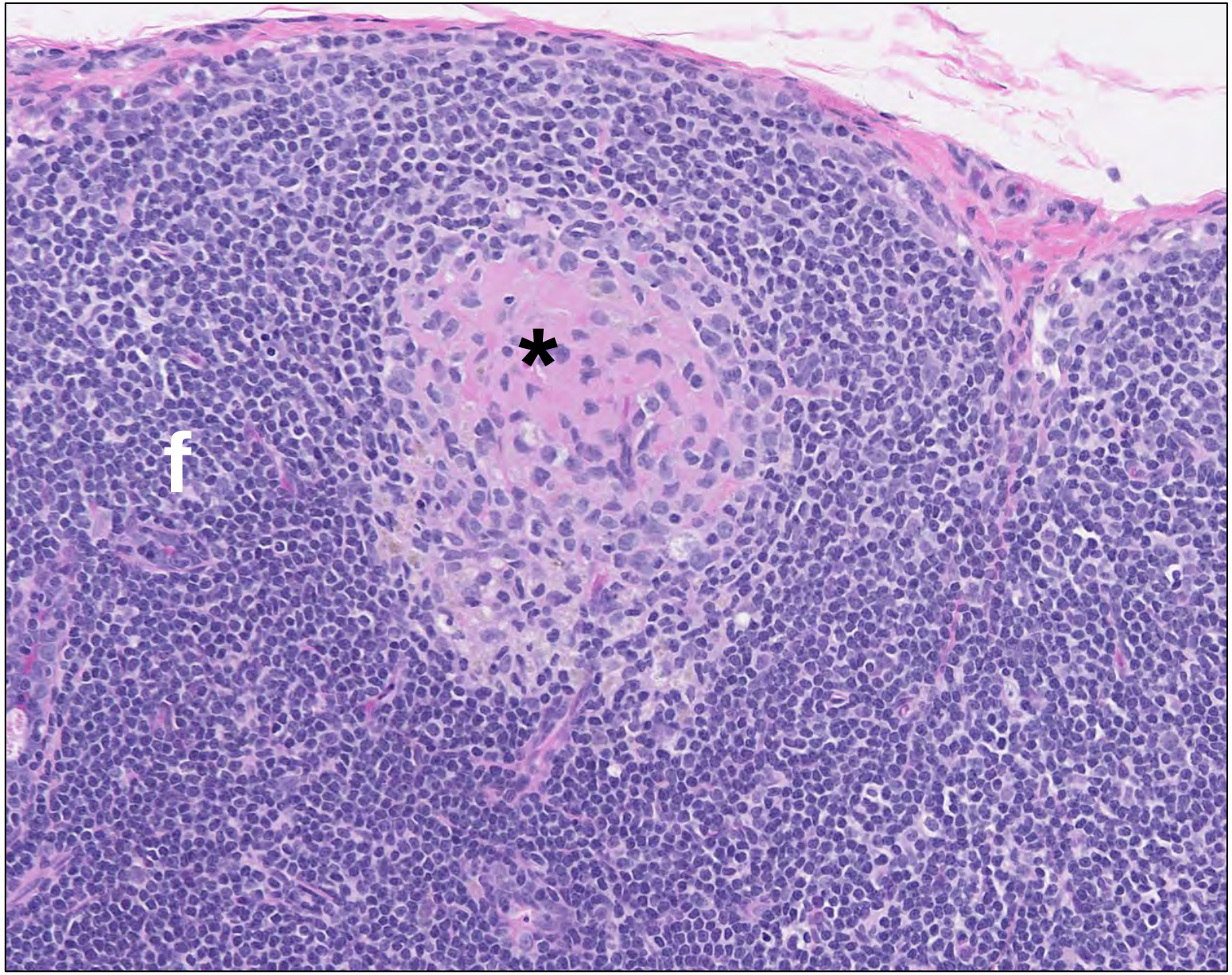
Lymph Node

Histomorphology: nonhuman primate

- Lymphoid follicles (f) of lymph nodes and spleen of nonhuman primates sometimes have accumulations of amorphous proteinaceous material (*) that is of uncertain genesis
- This material appears similar to amyloid deposition on routine H&E staining, but special stains indicate it is not amyloid
- It is important to recognize this species-specific finding as an incidental finding rather than an indication of a pathological process.

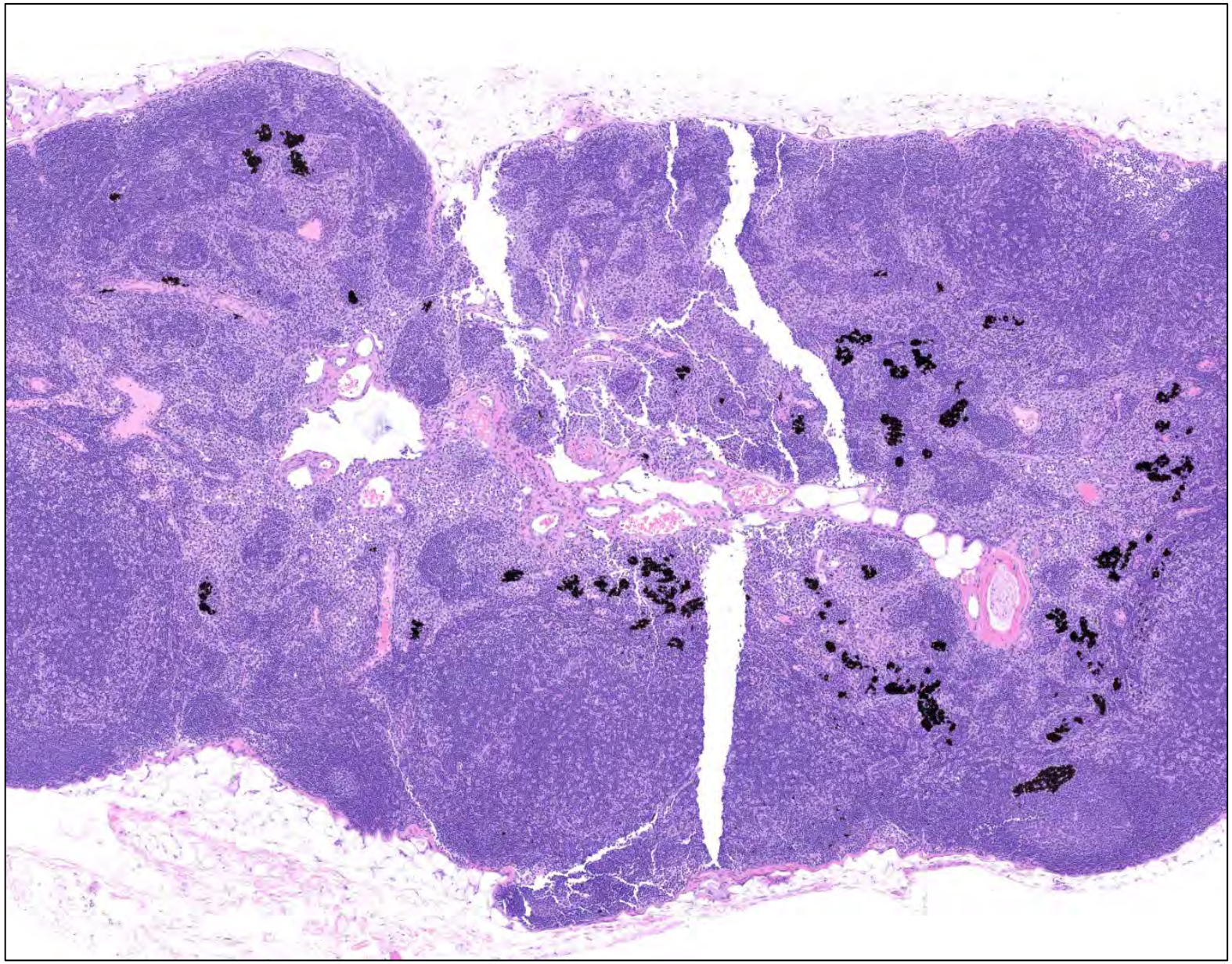
RIGHT: Lymph node from young adult cynomolgus macaque



Lymph Node

Histomorphology: nonhuman primate, tattoo ink

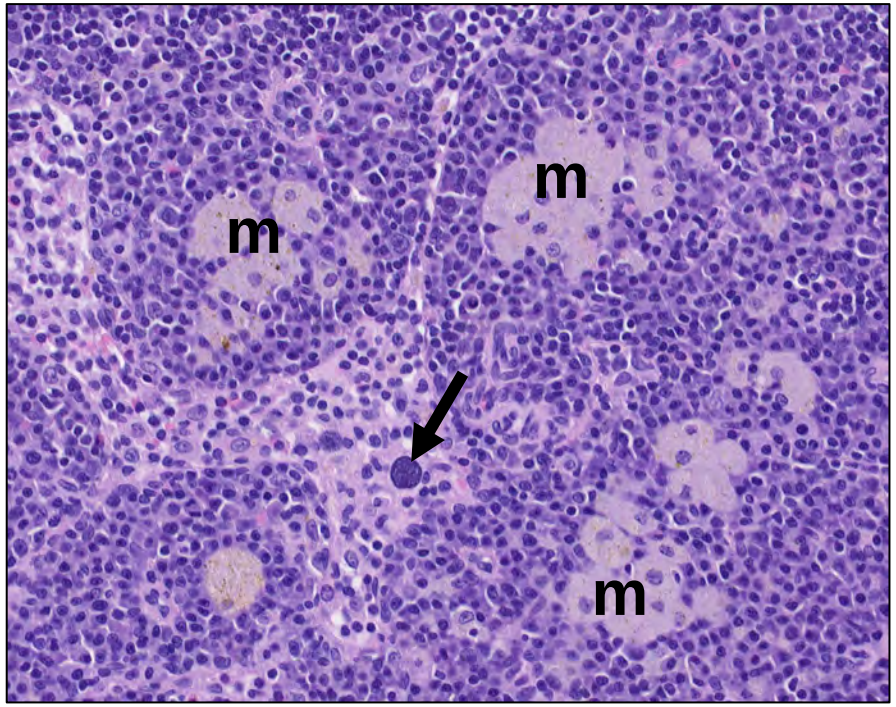
- Nonhuman primates are commonly identified by tattoos on the chest region
- This may result in accumulations of dark pigment in macrophages of the axillary lymph nodes
- This (typically black) pigment must be distinguished from pigmentation resulting from a pathological process

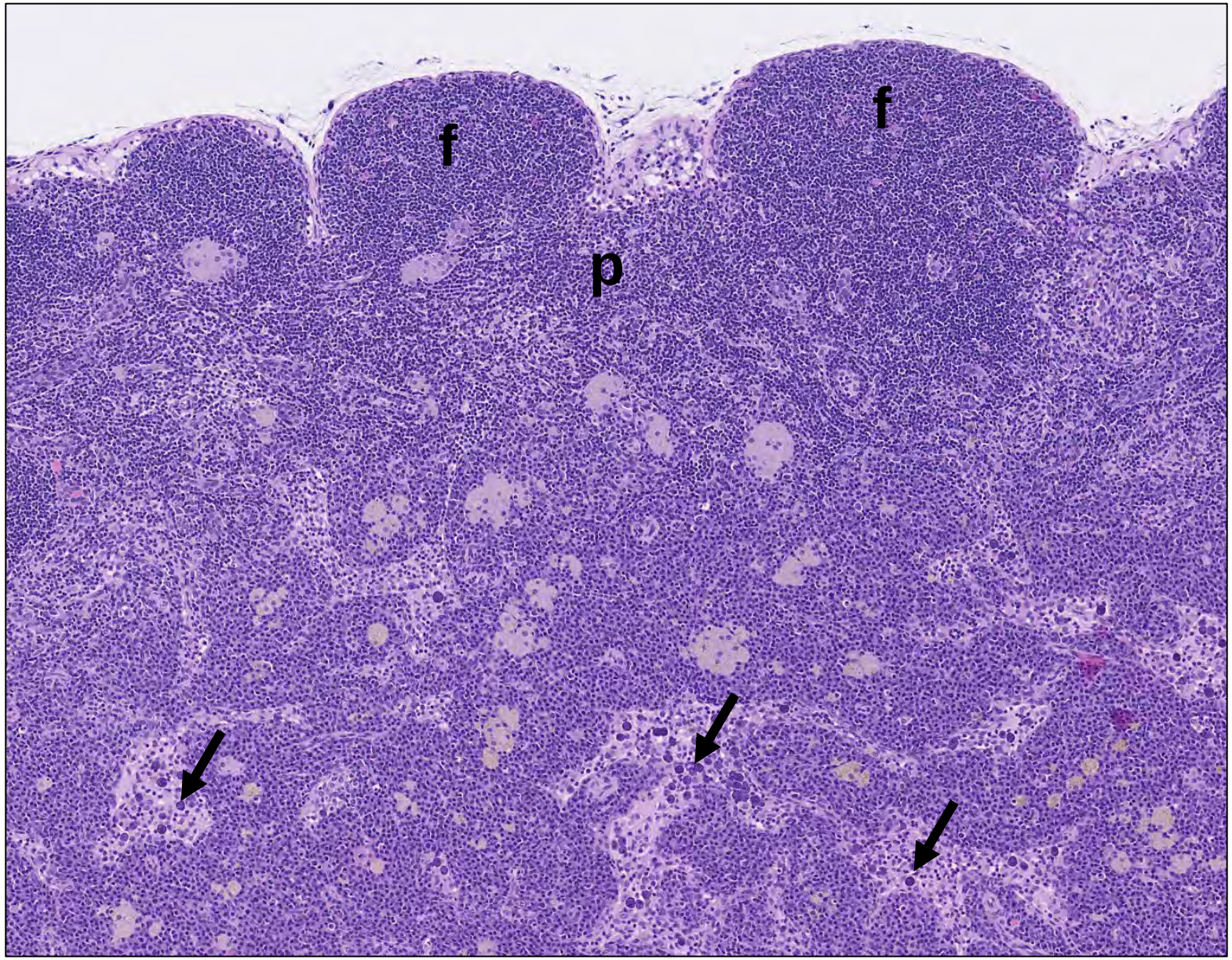


Lymph Node

Rat, mesenteric lymph node, aged male

Age-related microscopic features in lymph nodes include thin paracortex (p), inactive-appearing follicles (f), and aggregates of macrophages (m) laden with brown pigment in the medulla. Note mast cells (arrows).





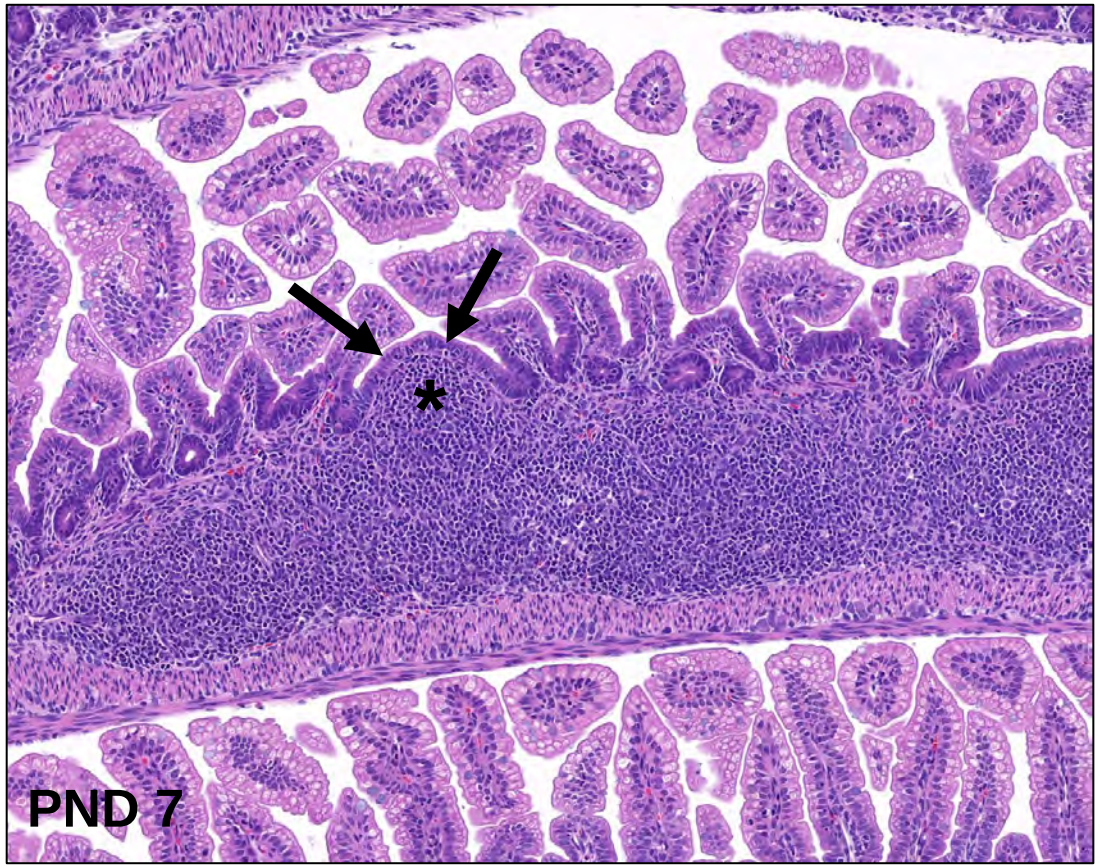
MALT

Mucosa-associated lymphoid tissue (MALT)

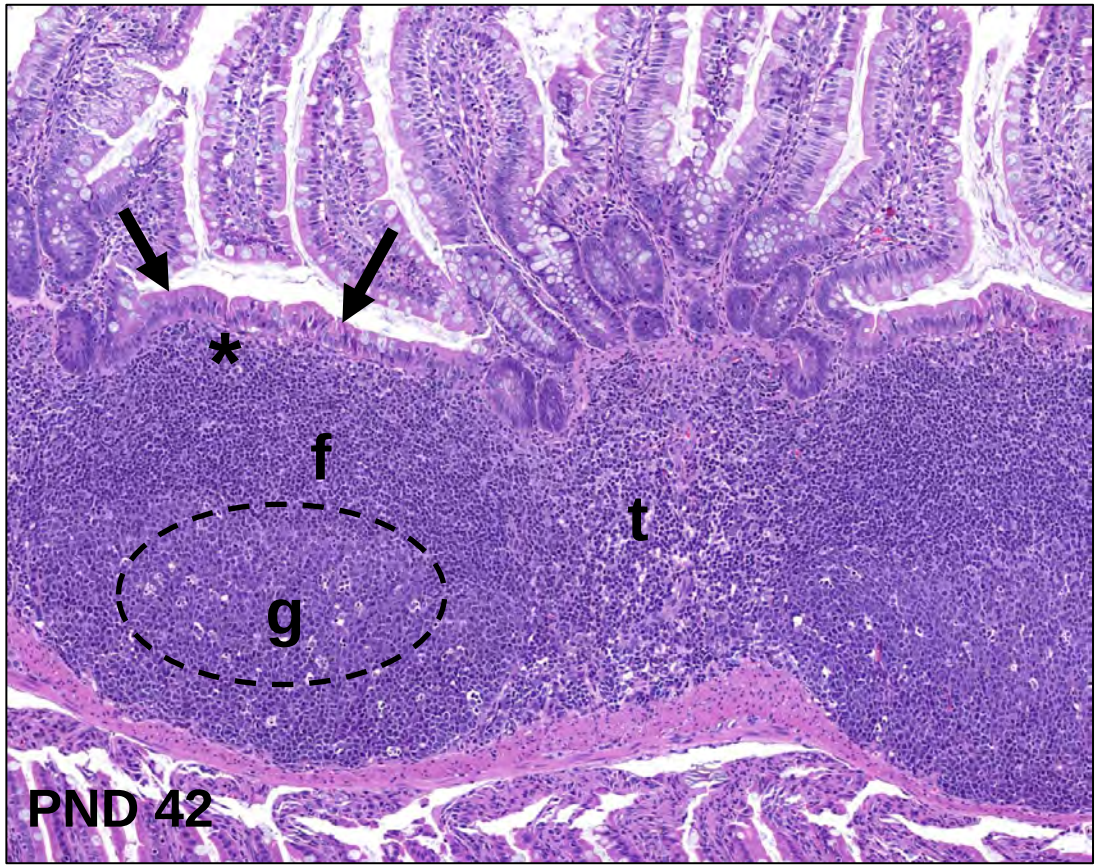
- MALT includes gastrointestinal-associated lymphoid tissue (GALT), bronchus-associated lymphoid tissue (BALT), nasopharynx-associated lymphoid tissue (NALT), etc.
- GALT consists of multiple components:
    - Diffuse intraepithelial lymphocytes of intestine
    - Diffuse lamina propria lymphocytes of intestine
    - Solitary lymphoid follicles of small intestine
    - Peyer's patches of small intestine
    - Lymphocyte-filled villi of small intestine
    - Cryptopatches of small intestine
    - Lymphoglandular complexes of large intestine
- As secondary immune system organs, MALT is poorly developed at birth, and subsequent development is dependent on immunologic stimulation
- BALT may be constitutively expressed (e.g., rat and rabbit) or induced by antigenic stimulation (e.g., mouse and human). Induced BALT is more precisely called iBALT.

MALT

Gastrointestinal-associated lymphoid tissue (GALT): development



At PND 7, the Peyer's patch of a Sprague Dawley rat is clearly visible and has a dome (*) with overlying follicle-associated epithelium (FAE, arrows).

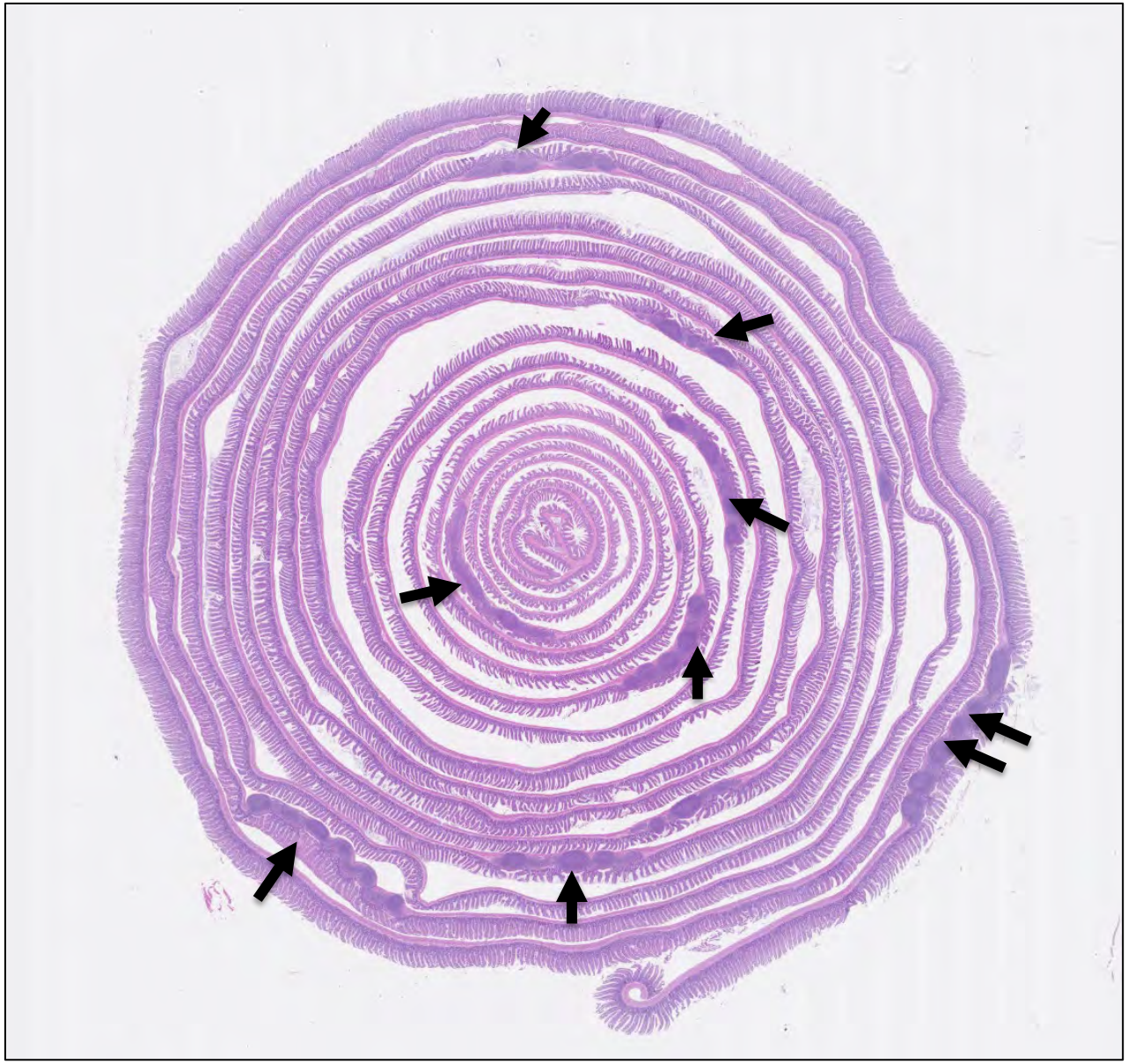


At PND 42, the Peyer's patch has distinct B cell follicles (f) beneath FAE (arrows) and germinal centers (g) with T cell areas (t) between adjacent B cell follicles.

MALT

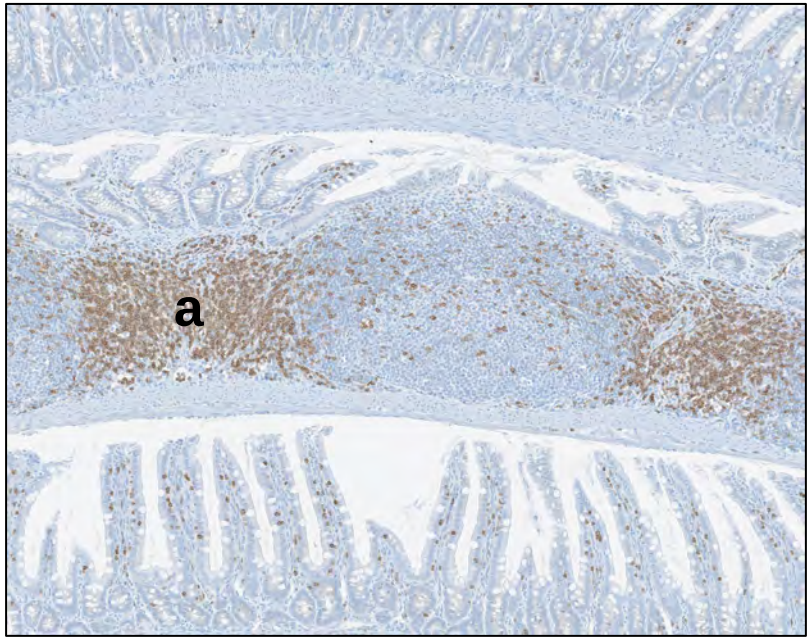
Gastrointestinal-associated lymphoid tissue (GALT): histomorphology

- This Swiss roll preparation contains the entire small intestine of a rat at PND 42. The ileum is at center of the roll.
- Note Peyer's patches (arrows) are scattered throughout the small intestine, including the duodenum (double arrows). Peyer's patches comprise a major component of adaptive immune response to intestinal pathogens.
- Gastrointestinal-associated lymphoid tissue includes Peyer's patches, solitary lymphoid follicles, and cryptopatches of small intestine, lymphoglandular complexes (lymphoid follicles) of large intestine, and diffuse lymphocytic populations throughout the small and large intestine.
- Large intestine lymphoid follicles are not Peyer's patches. The precise term for the large intestinal structures is "lymphoglandular complexes."

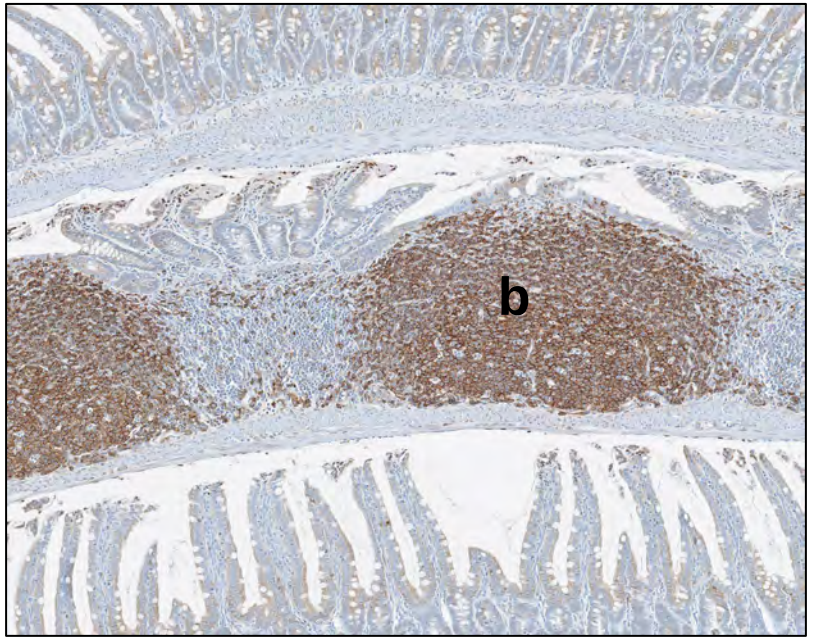


MALT

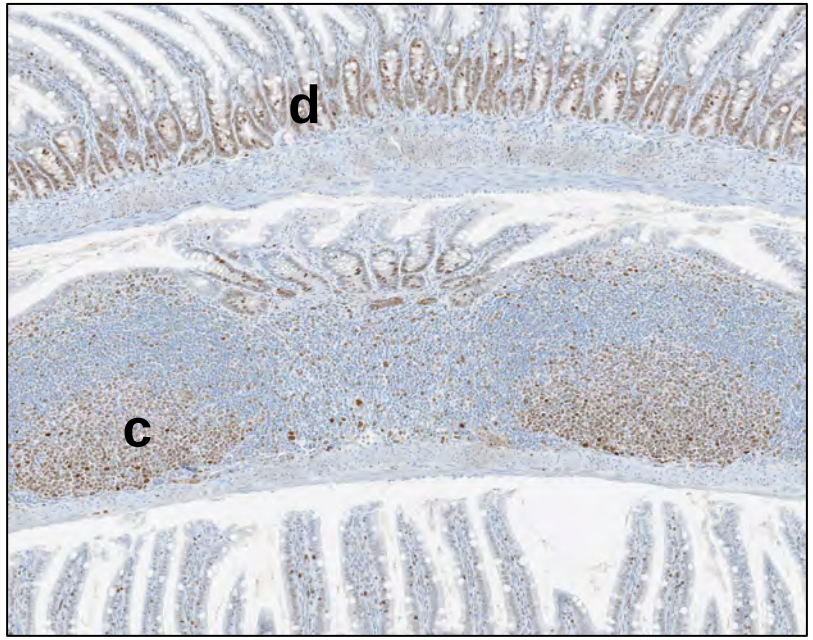
Peyer's patches, histomorphology: immunohistochemistry



CD3



CD45RA



Ki67

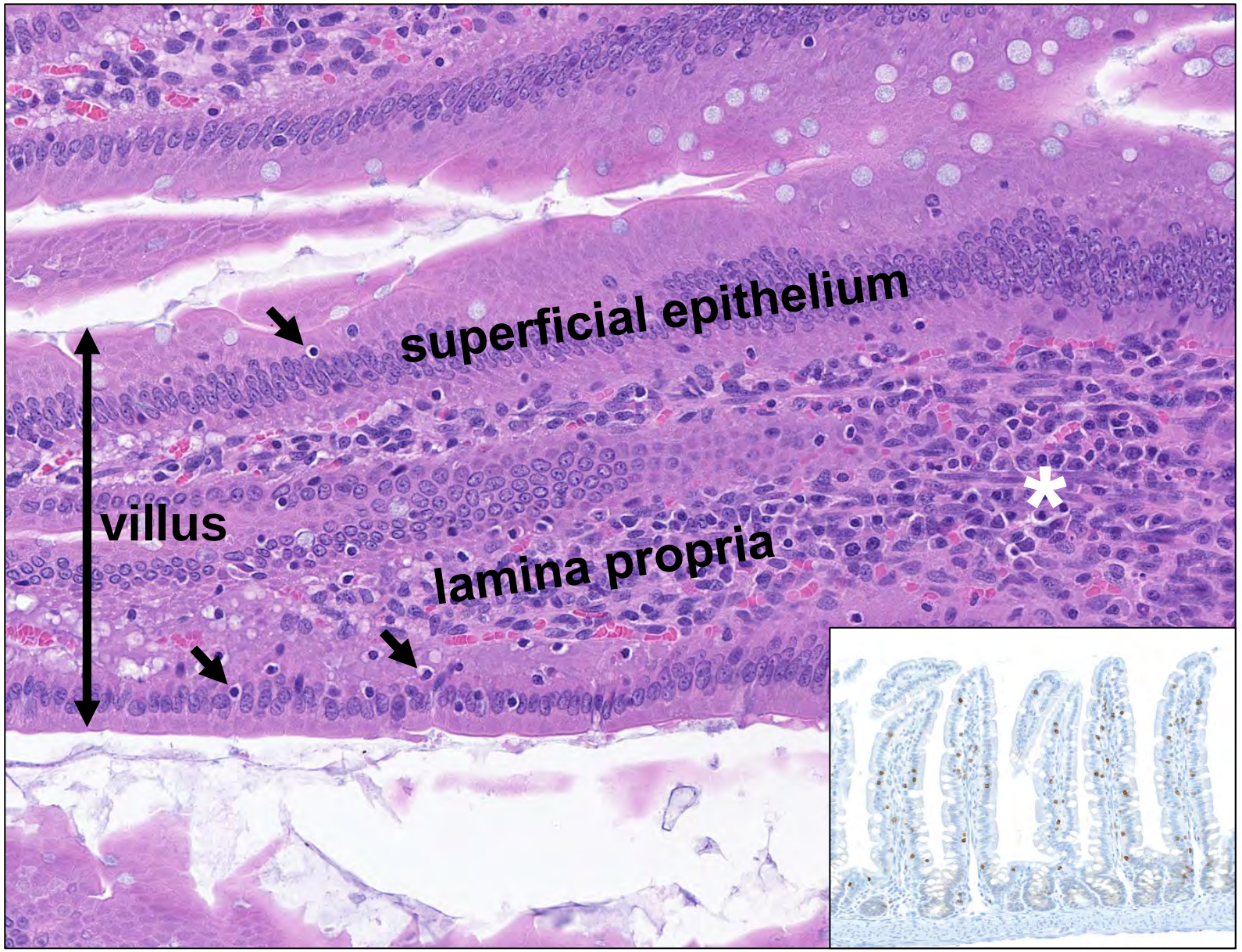
Immunohistochemical staining performed on formalin-fixed, paraffin-embedded specimens. LEFT: Brown CD3 staining (a) marks T cells in the interfollicular areas. MIDDLE: Brown CD45RA staining (b) marks B cells in follicles. RIGHT: Brown Ki67 staining (c) marks proliferating cells in germinal centers of follicles as well as crypt epithelial cells (d). In all images, brown staining by 3,3'-diaminobenzidine chromogen indicates target molecules, blue hematoxylin counterstain stains cell nuclei.

MALT

GALT morphology, small intestine, diffuse lymphocyte populations

- The gastrointestinal-associated lymphoid tissue in the small intestine consists of two distinct cell populations: intraepithelial lymphocytes (IEL) (arrows) and lamina propria immunocytes (*)
- Individual IEL (arrows in primary image) are surrounded by clear spaces and are microscopically similar to halo cells of the epididymis.
- The diffuse intestinal lymphocyte population is very important in mucosal immunity
  - The diffuse lymphocyte population exceeds the population of Peyer's patches
  - Large amounts of immunoglobin A (IgA) are produced, as IgA is an essential component in mucosal protection from commensal bacteria
  - Divalent IgA serves to bind 2 adjacent bacteria, thus hindering bacterial mobility and enhancing phagocytosis by macrophages

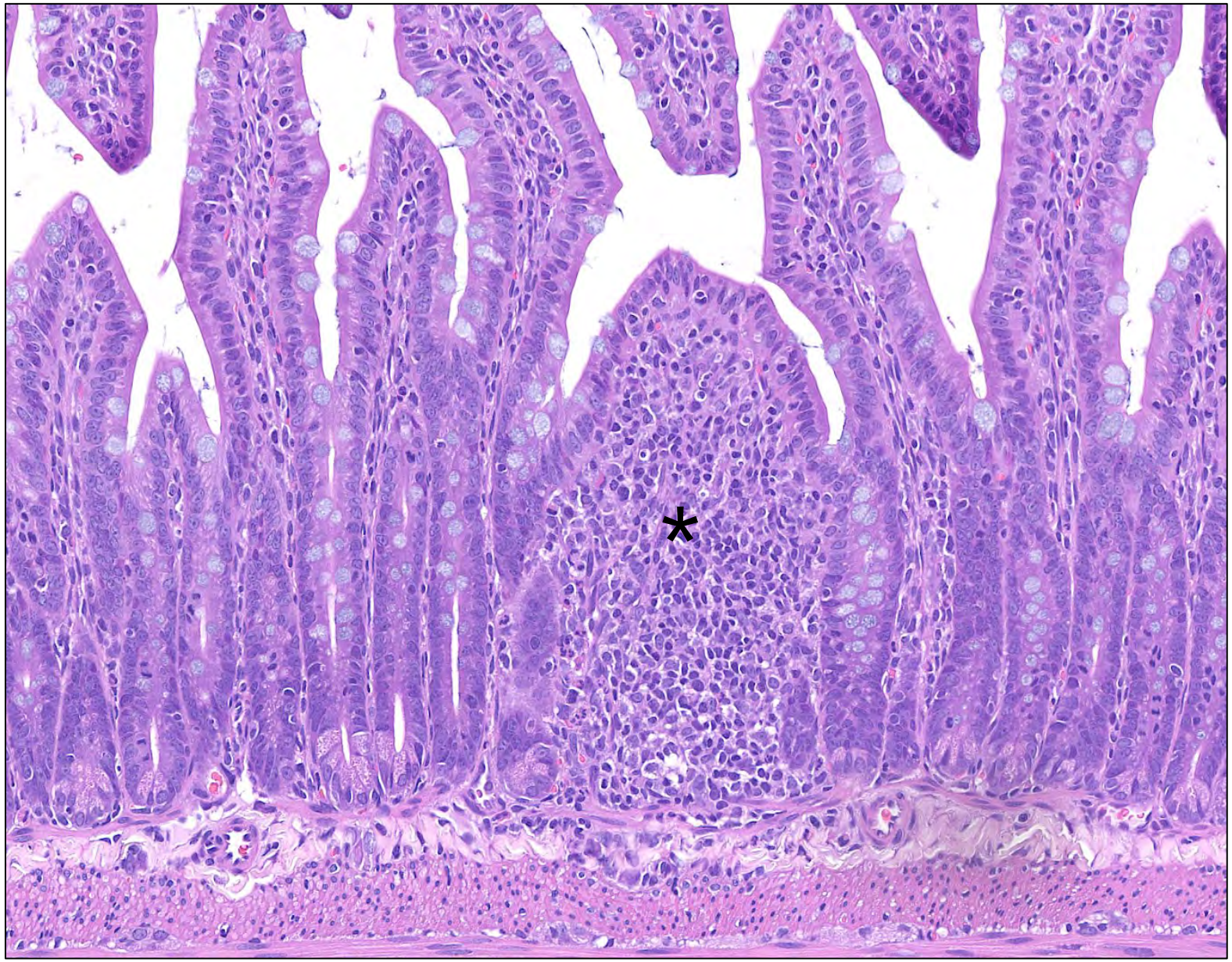
INSET: Brown CD3 IHC positive staining reveals the IEL cells to be T cells, as is also true of some epididymal halo cells.



MALT

GALT morphology, small intestine, lymphocyte-filled villus

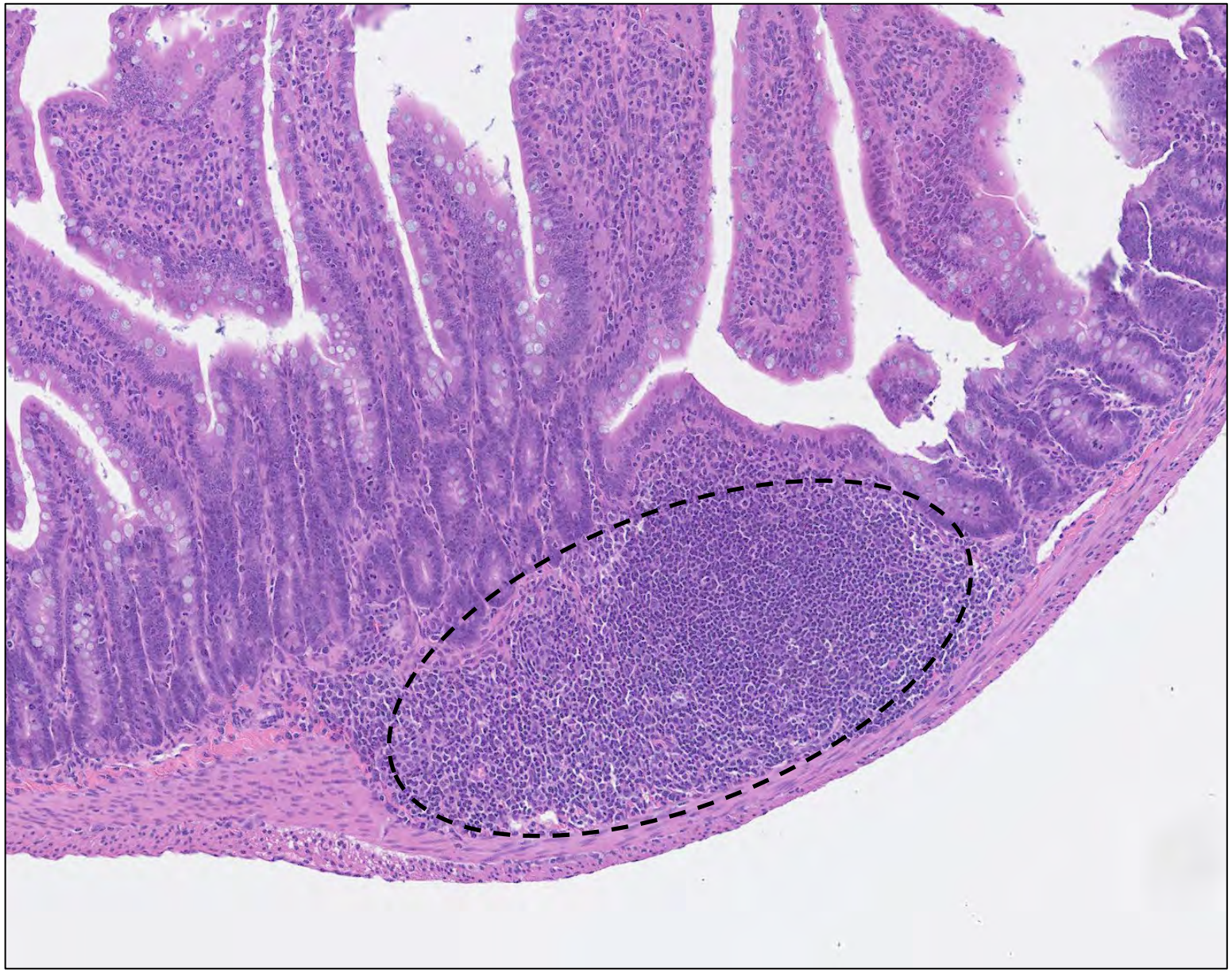
The exact genesis and significance of lymphocyte-filled villi (*) is unknown, but the number of these structures tends to be increased in association with inflammatory conditions of the intestine.



MALT

GALT morphology, small intestine, solitary lymphoid follicle

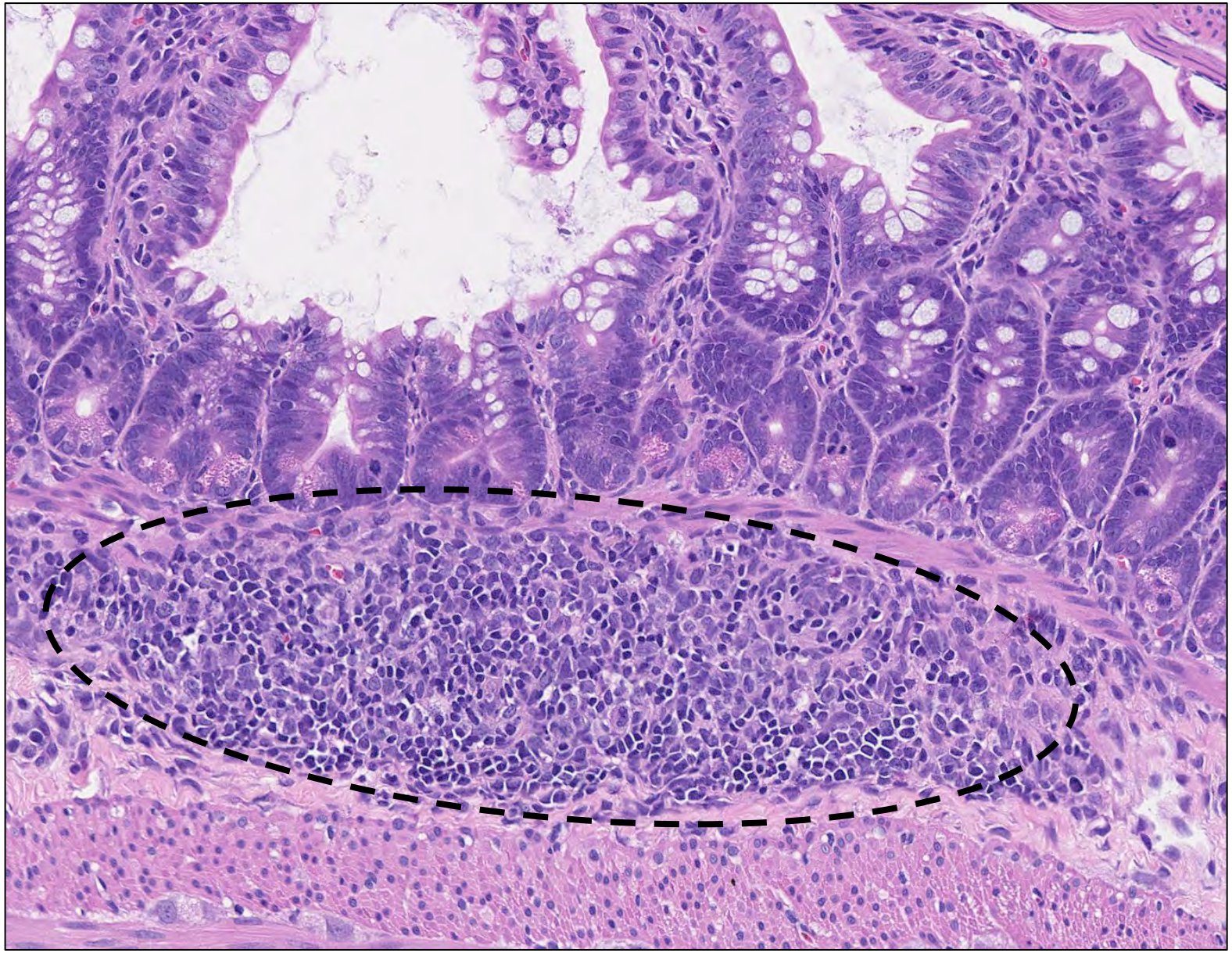
- Solitary lymphoid follicles (oval outline) of the small intestine are morphologically and functionally similar to Peyer's patches
- Peyer's patches consist of linear arrays of individual lymphoid nodules
- When histologic cross-sections of small intestine are prepared, it is not possible to differentiate between Peyer's patches and solitary lymphoid follicles
- When tissue examination lists are prepared for safety assessment study protocols, it is desirable to specify Peyer's patches or solitary lymphoid follicles of small intestine rather than broader terms such as GALT



MALT

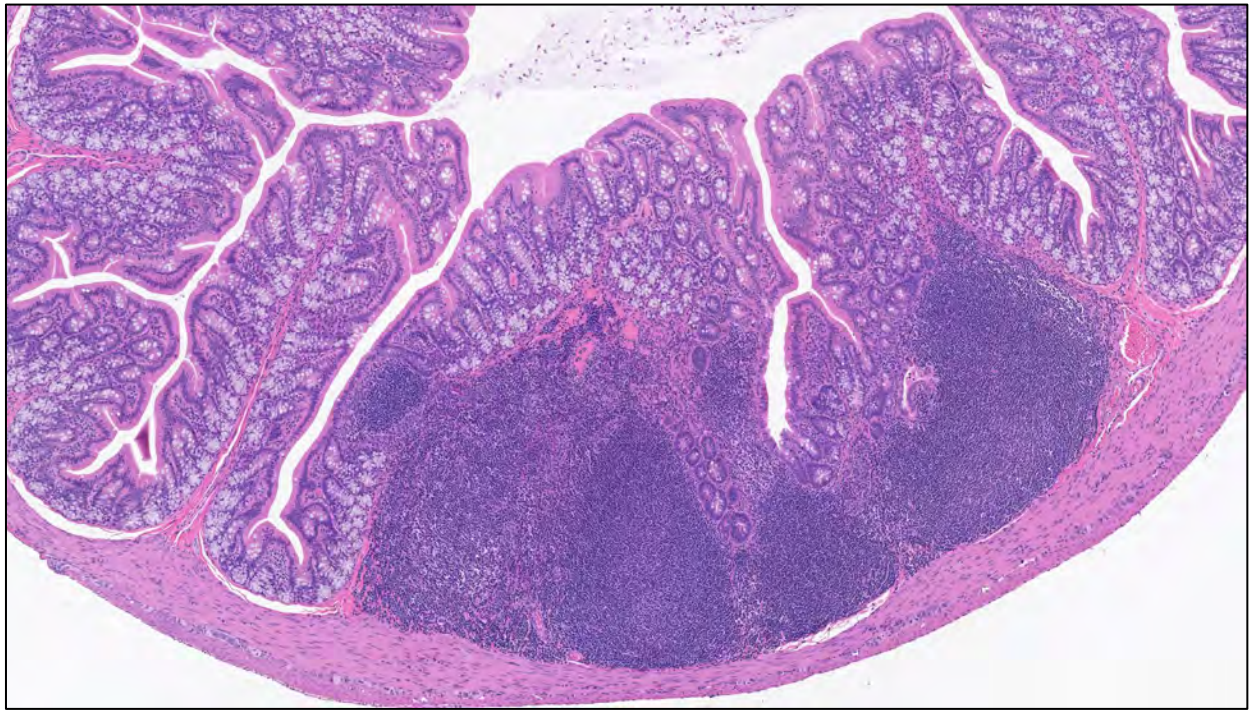
GALT morphology, small intestine, cryptopatch

- Cryptopatches are smaller than solitary lymphoid follicles
- The exact genesis and significance of cryptopatches (oval) is not known
- It is postulated that cryptopatches may represent an early stage in the development of solitary lymphoid follicles

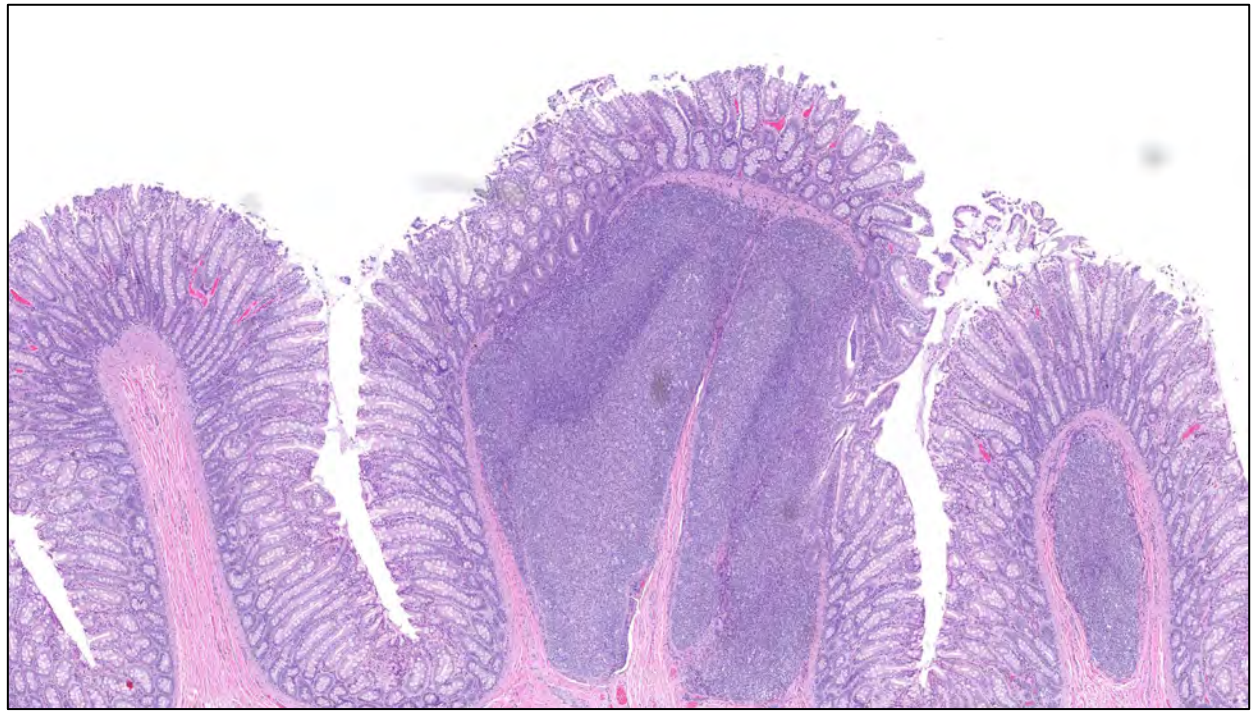


MALT

GALT histomorphology, large intestine, lymphoglandular complexes



Rat colon



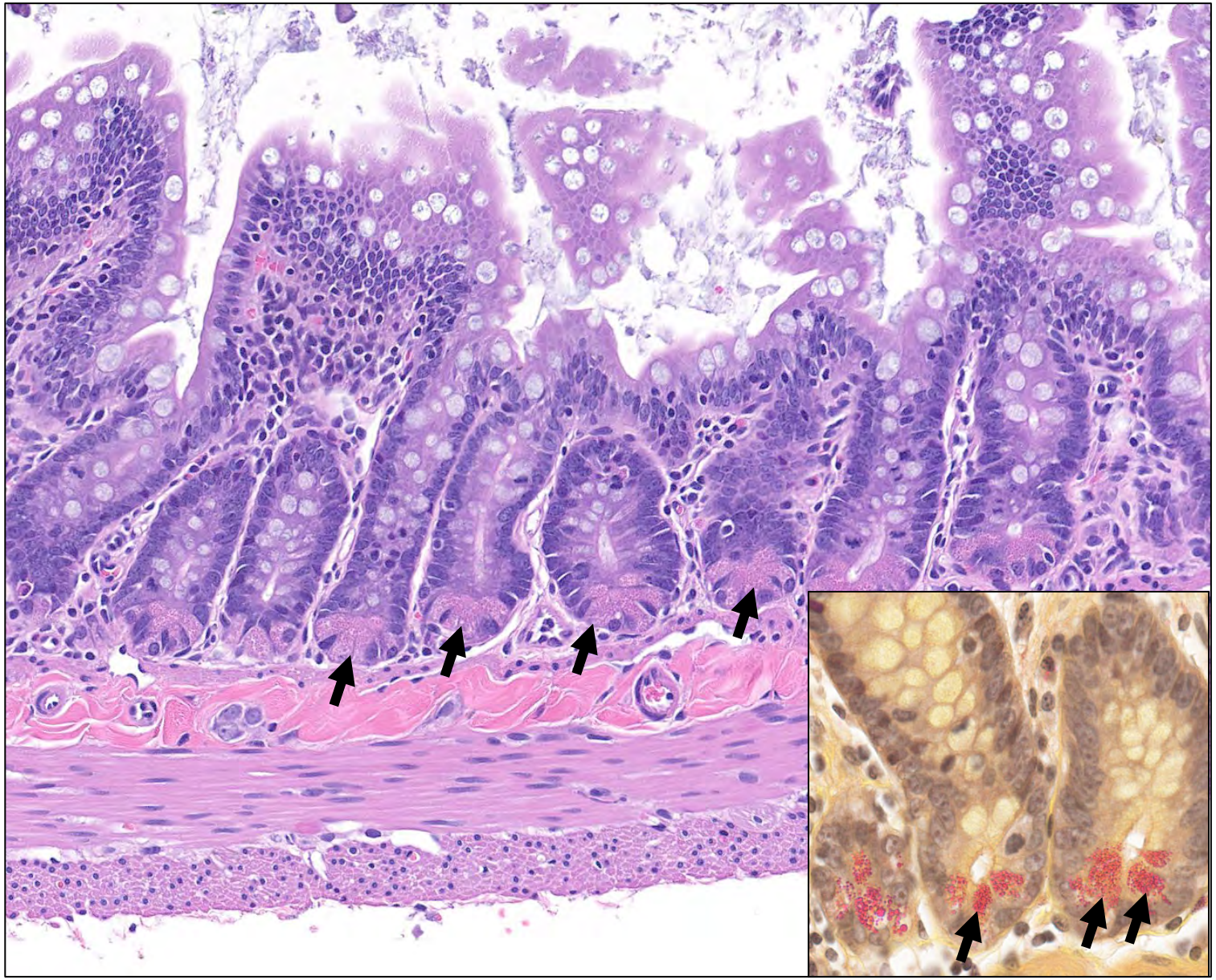
Dog cecum

Lymphoglandular complexes occurring in any region of the large intestine have similar histomorphology. It should be noted that these large intestinal lymphoid structures are not “Peyer’s patches,” which are restricted to the small intestine. The lymphoid structures of the large intestine are structurally and functionally different from the Peyer’s patches and solitary lymphoid follicles of the small intestine.

MALT

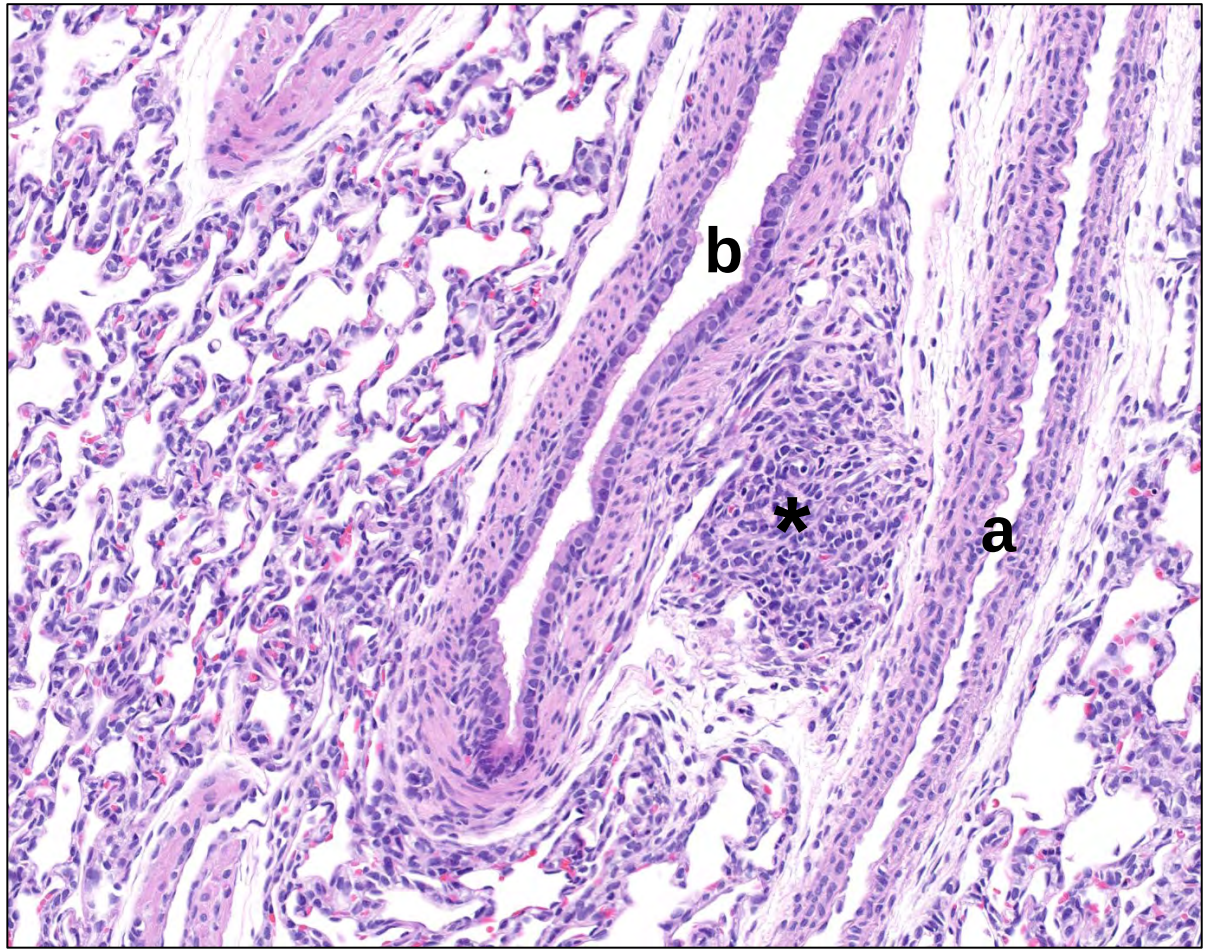
Paneth cells, ileum, rat

- Paneth cells (arrows) located deep in small intestinal crypts are readily identified by the brightly eosinophilic cytoplasmic granules seen with H&E staining (large image on right)
- Paneth cells produce defensins, which have broad-spectrum antimicrobial activity against gram-positive and gram-negative bacteria as well as fungi and viruses
- Three classes of defensins are known: α, β and θ. Only α and β defensins have been identified in mice and humans. In mice the production of α defensin is restricted entirely to Paneth cells.
- Defensins produced by Paneth cells have a direct impact on intestinal microbe populations. Damage to defensins renders the intestinal tract more susceptible to opportunistic infections.
- INSET: Lendrum stain accentuates Paneth cells (arrows) in deep aspect of crypts of a male cynomolgus macaque

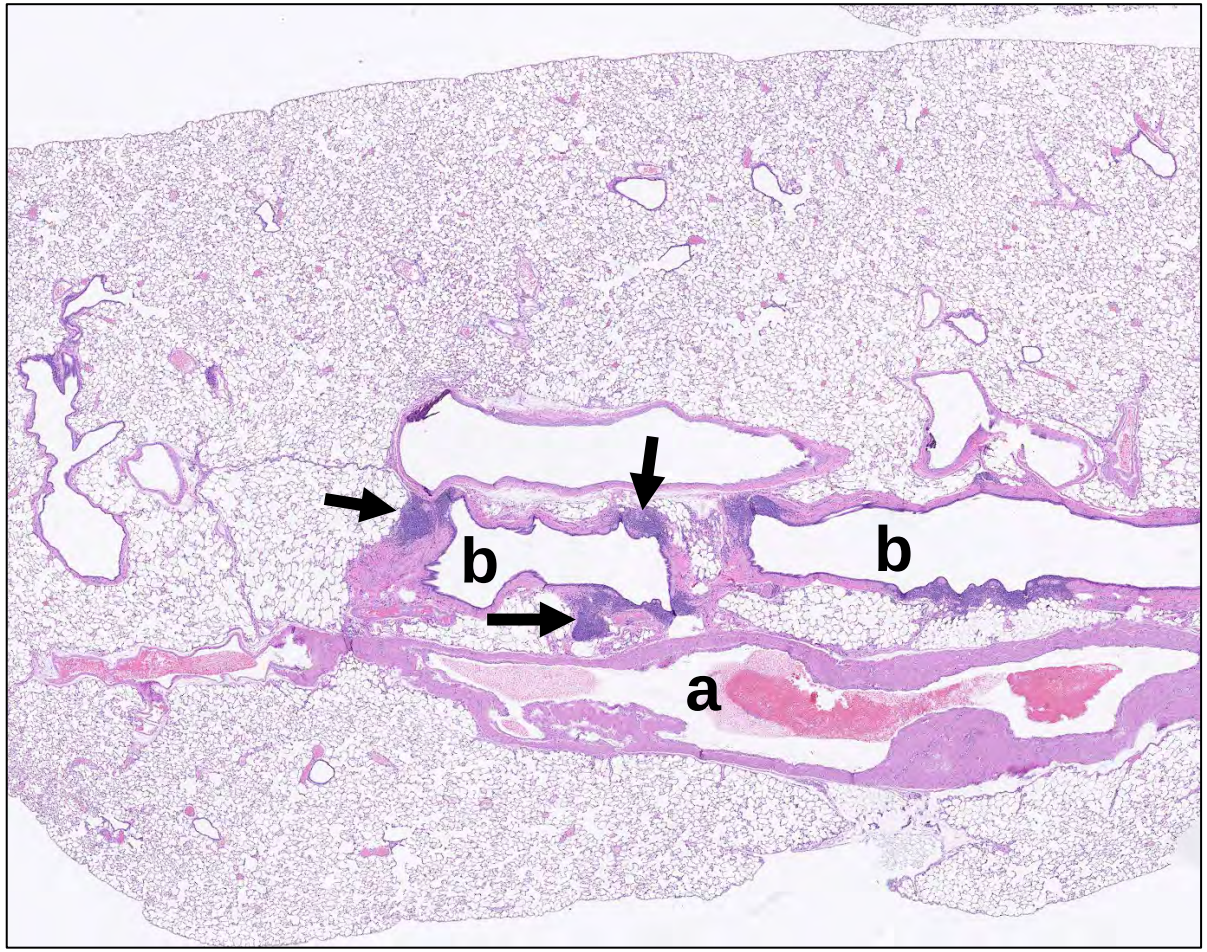


MALT

Bronchus-associated lymphoid tissue (BALT) histomorphology, lung



BALT (*) in the lung is discernible but immature at postnatal day 7 in the Sprague Dawley rat. It is located in close proximity to a bronchiole (b) and arteriole (a).

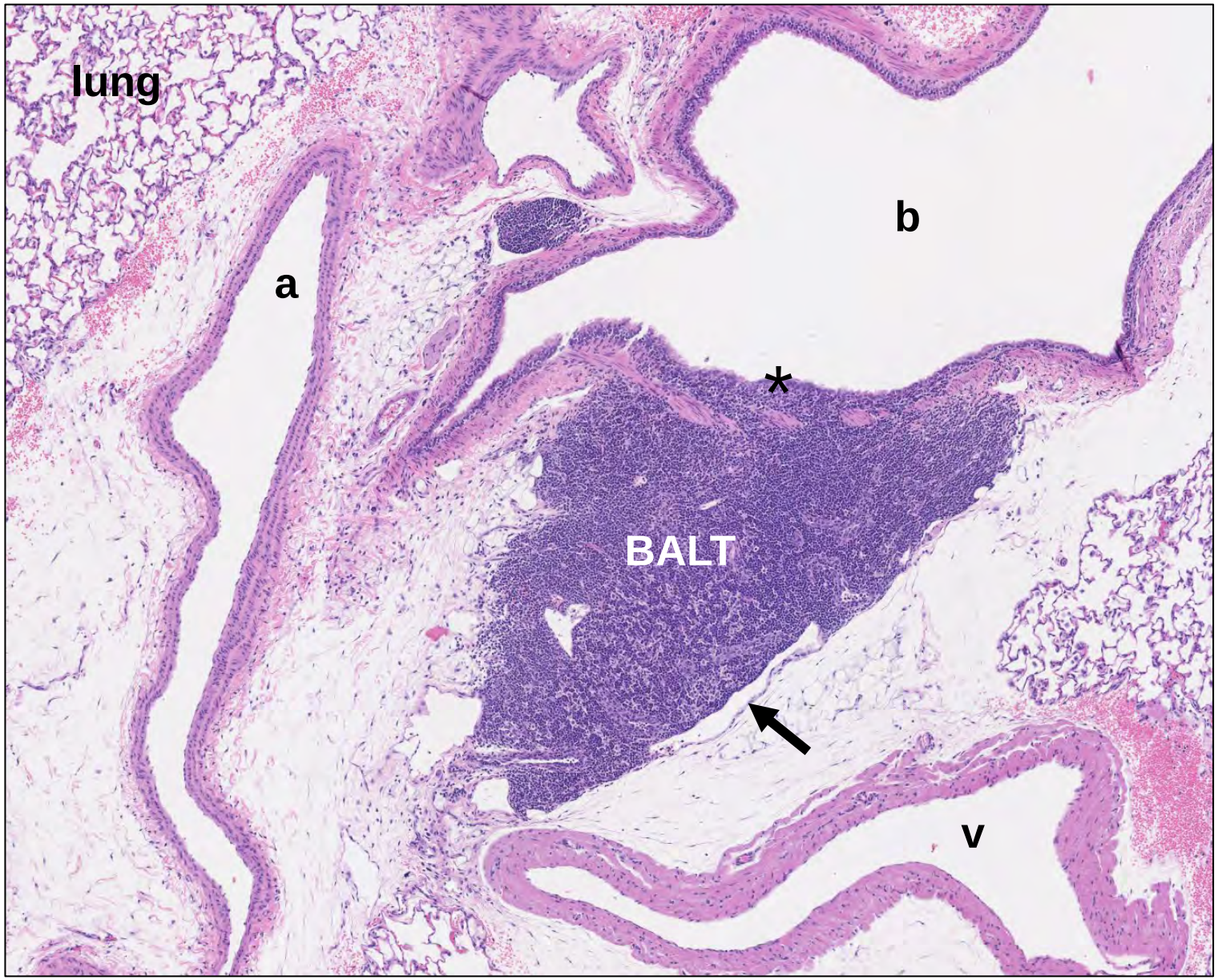


BALT aggregates (arrows) are distributed along the course of a major bronchus (b) in this young adult (approximately 12 weeks of age) Sprague Dawley rat. a = pulmonary artery; b = bronchiole

MALT

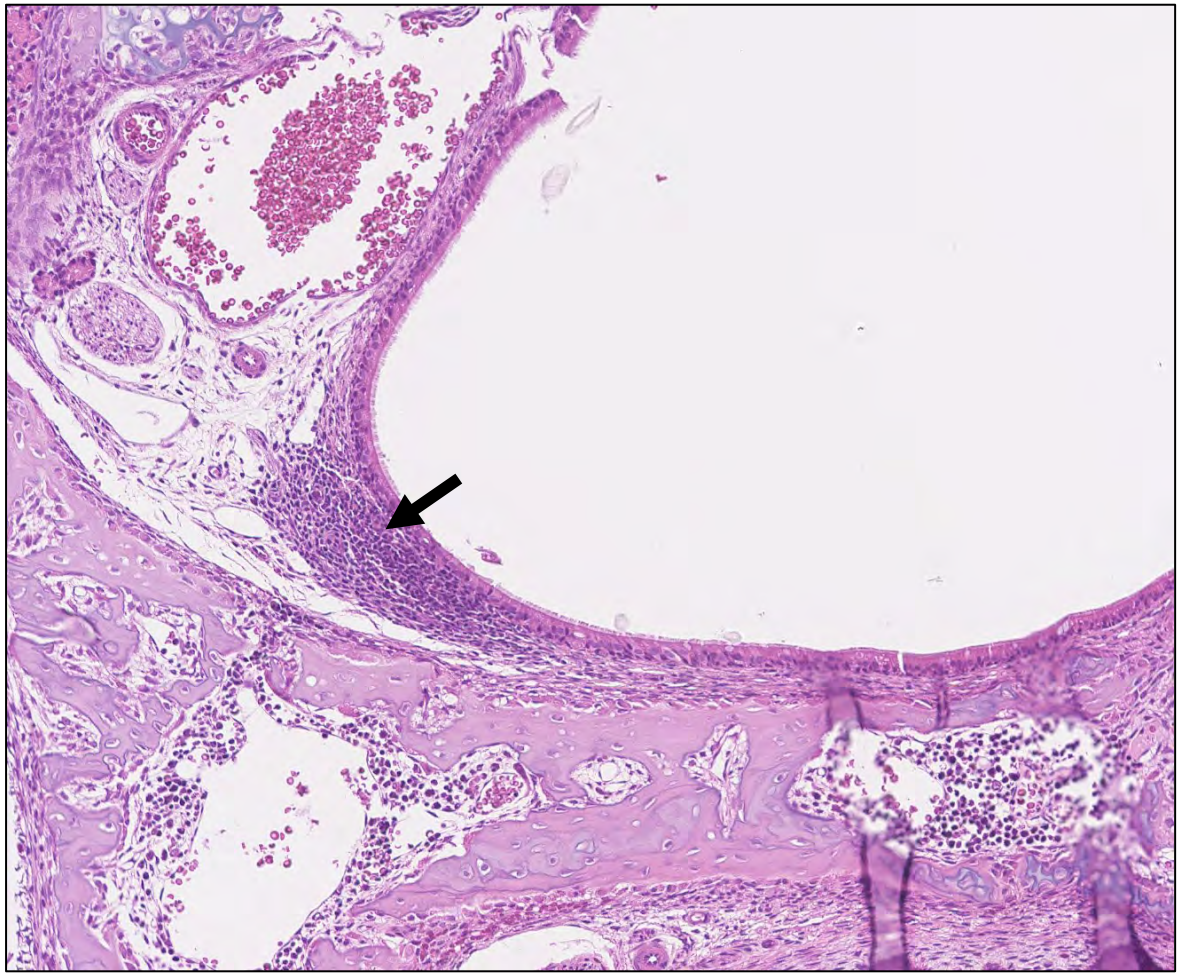
Bronchus-associated lymphoid tissue (BALT) histomorphology, lung

- BALT is constitutively expressed in the lung of rats, as seen in this Sprague Dawley rat at PND 42
- The BALT is in a typical location near an arteriole (a), bronchiole (b) and venule (v). Lymphatics (arrow) are located near the base of the BALT.
- Note the close proximity of the BALT to the bronchiolar epithelium (*). This proximity allows immunological sampling of airborne antigens for reactions by the lymphoid cells of the BALT.

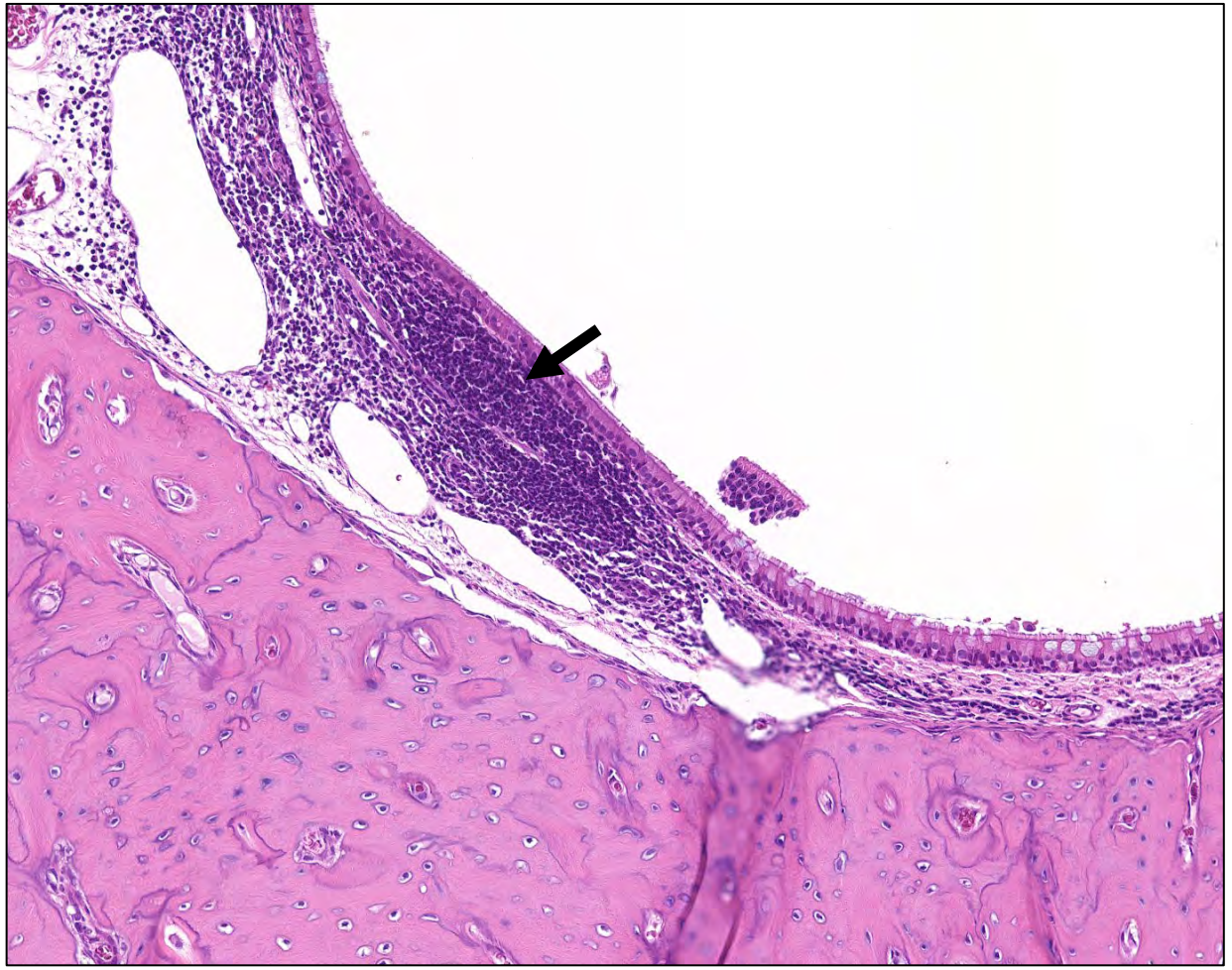


MALT

Nasopharynx-associated lymphoid tissue (NALT) histomorphology



Rat, PND 7



Rat, PND 42

NALT (arrows) is clearly visible at PND 7 in a Sprague Dawley rat and reaches adult morphology by PND 42

Non-Immune Tissues

Immune components of non-immune tissues – overview

- In addition to populations of circulating immunocytes, many organs have specific organ-adapted immunocyte populations or immunological functions, including the following:

  Liver – Kupffer cells, liver-adapted NK cells (“pit cells”), sinusoidal endothelial cells

  Brain – anatomic blood-brain barrier, microglial cells

  Lung – resident alveolar macrophages

  Peritoneal cavity – milky spots

  Skin – resident macrophages (“Langerhans cells”)

  Testis – interstitial macrophages, Sertoli cells, physiological barriers to immune reactivity

  Epididymis – halo cells

  Uterus – metrial gland, immunological protection of the fetus

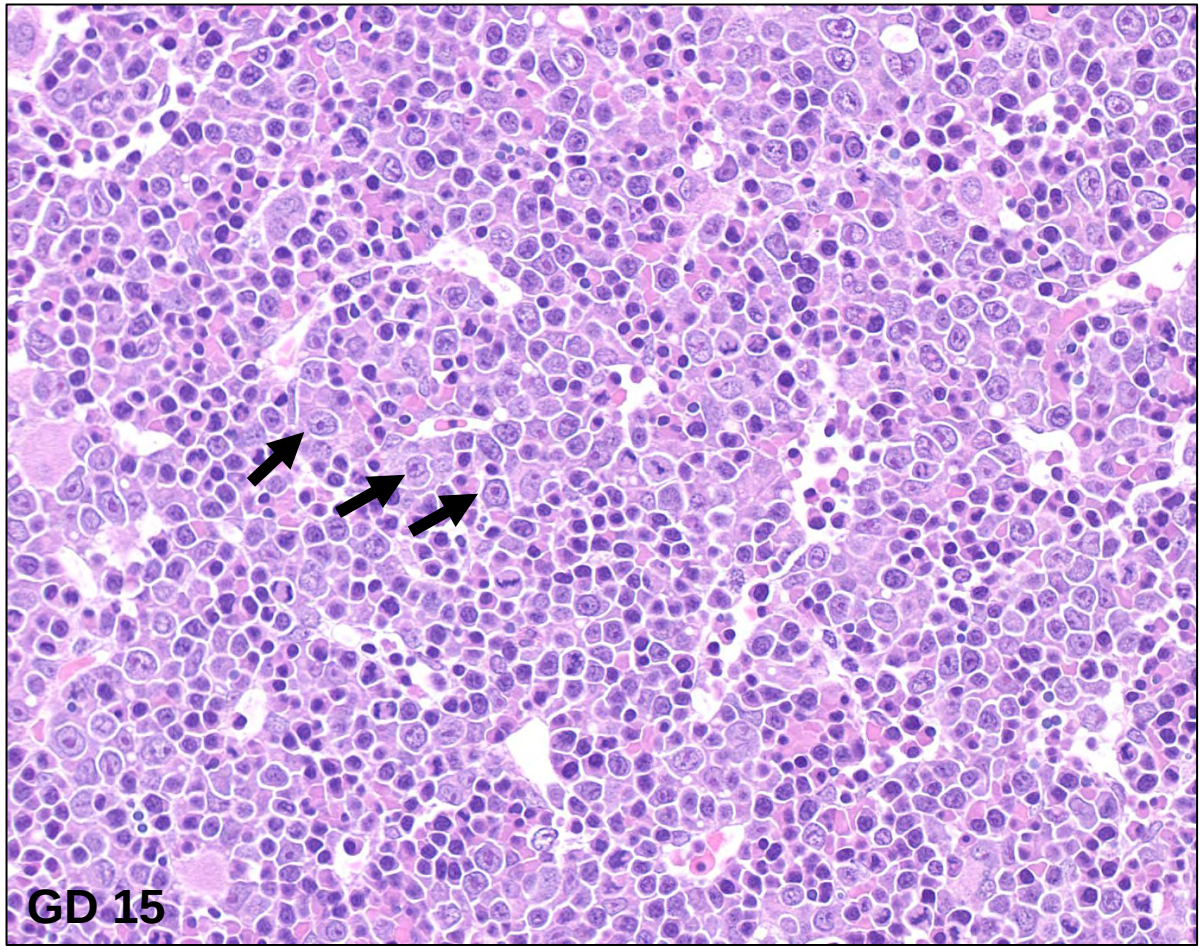
  Ovary – physiological barriers to immune reactivity

  Eye – immunologically privileged site

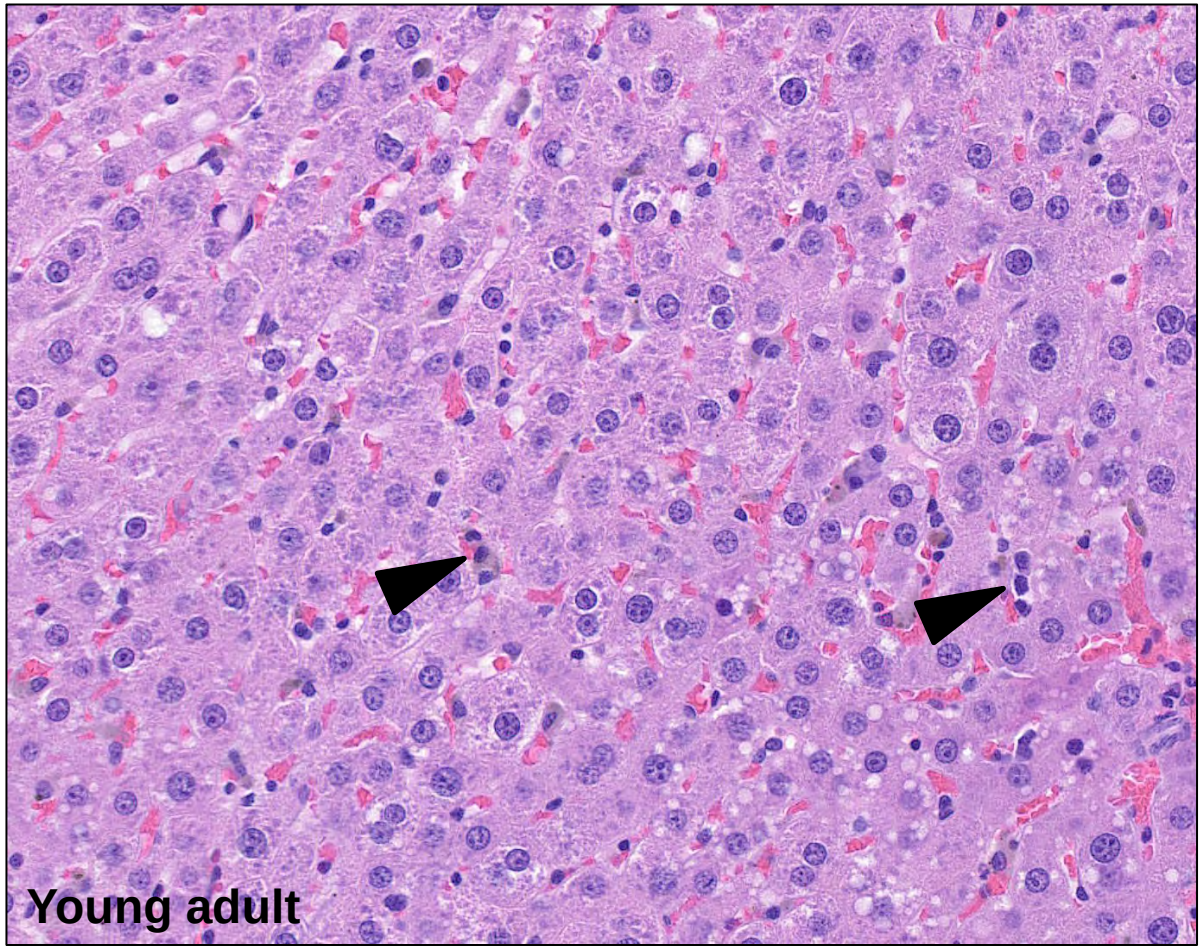
- Tertiary lymphoid tissue consists of lymphoid tissue induced in non-immune organs, typically associated with inflammation in the non-immune organ

Non-Immune Tissues

Liver, rat



At gestation day (GD) 15 the liver of a Sprague Dawley rat is a major center of hematopoiesis with disorganized hepatocytes (arrows) surrounded by numerous hematopoietic cells.



The liver of a young adult rat has a moderate number of Kupffer cells and liver-adapted NK cells known as “pit cells.” Close approximation of Kupffer cells and pit cells (arrowheads) facilitates mutually beneficial cytokine exchange between the cells.

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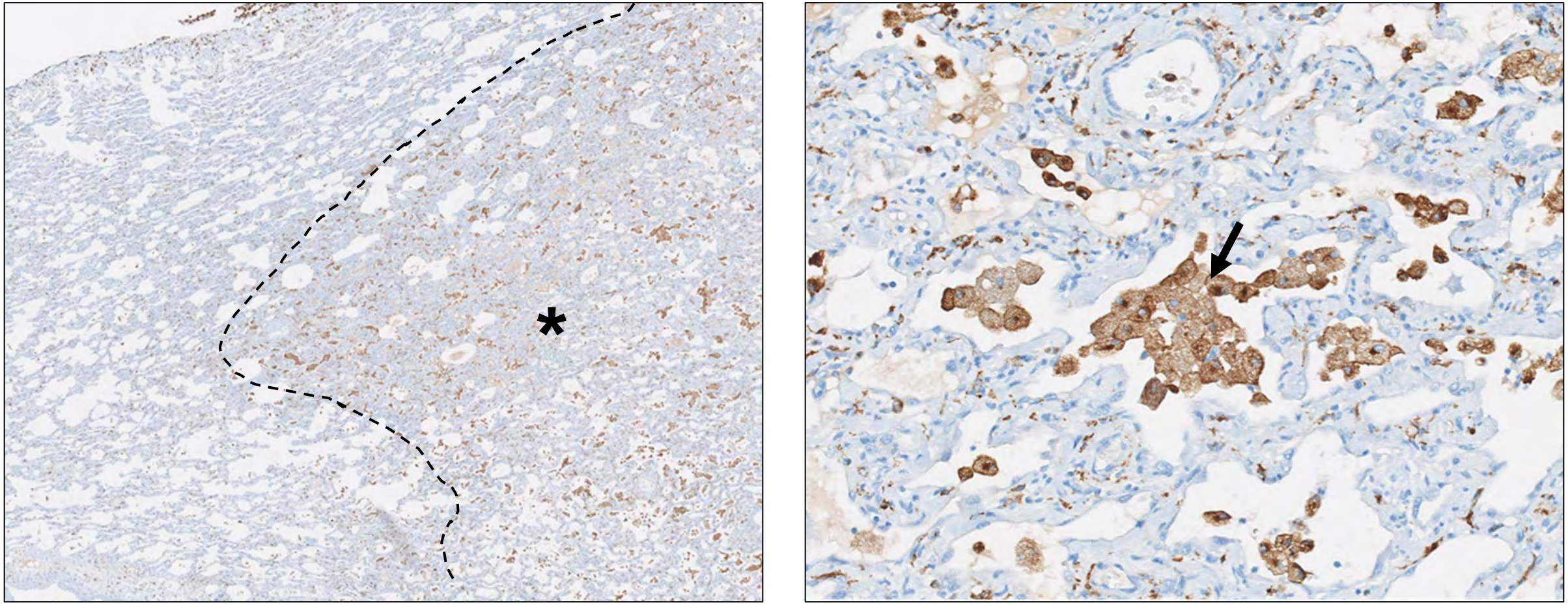
Non-Immune Tissues

Lung, alveolar macrophages at day 177 post-irradiation in rhesus macaque

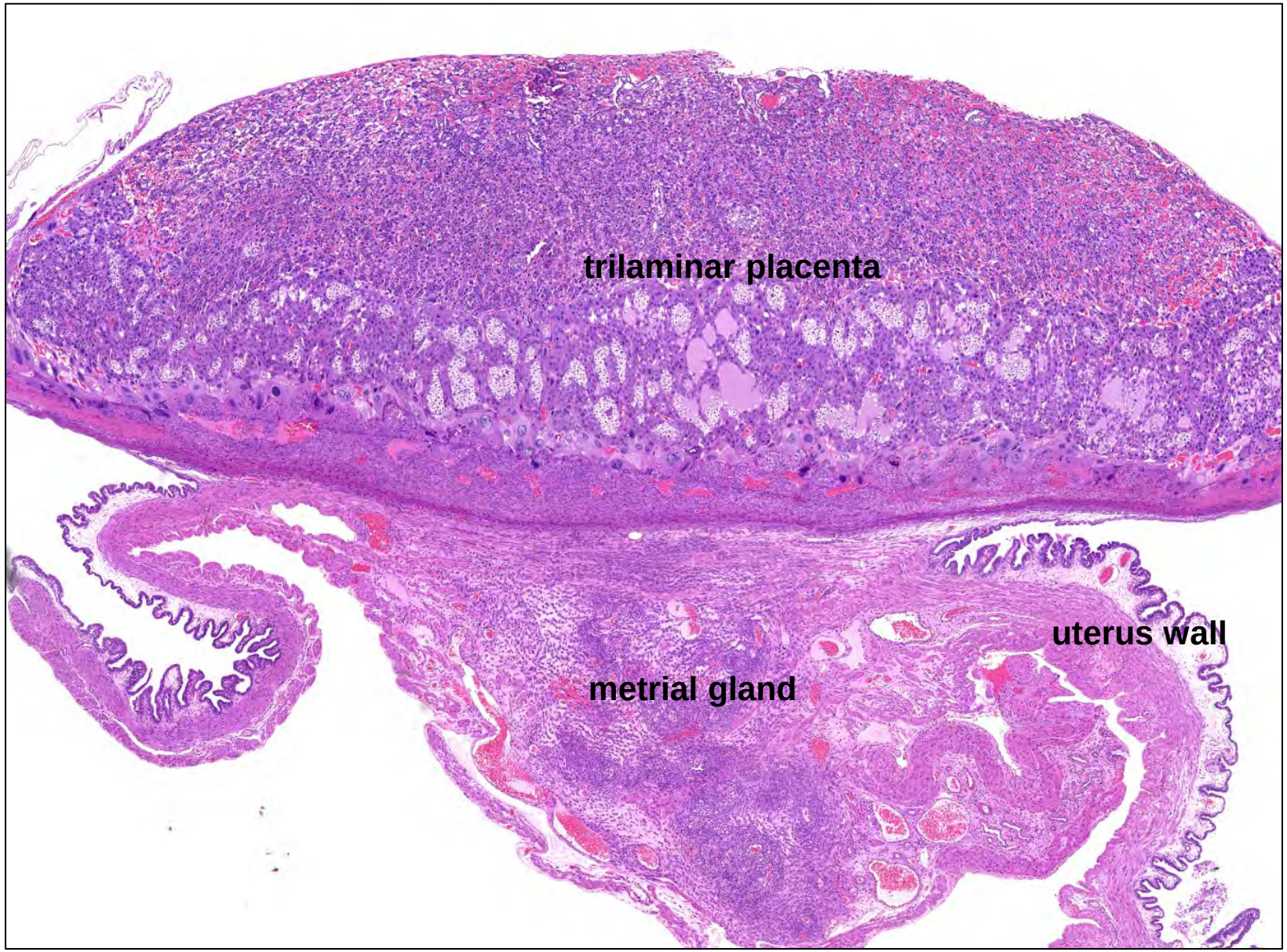


LEFT: Black line delineates area of lung with radiation-induced fibrosis and alveolar macrophage accumulation (*). RIGHT: Brown-stained CD163 marker indicates $CD163^{+}$ macrophages (arrow) that have fibrogenic influence. In both images, brown CD163 immunohistochemical staining by 3,3'-diaminobenzidine chromogen indicates target molecules, blue hematoxylin counterstain stains cell nuclei.

Non-Immune Tissues

Uterus & placenta with metrial gland, gestation day 16, Rat

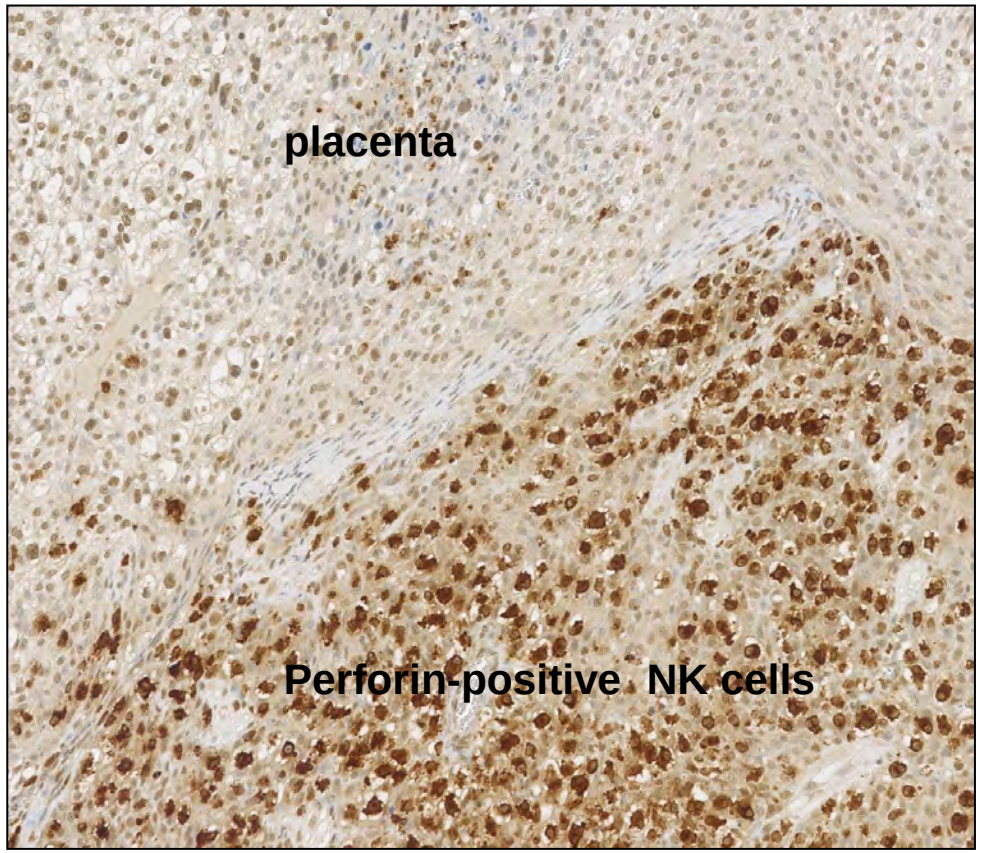
- Metrial gland is the name applied to a functional group of cells that form in the mesometrial attachment to the uterus, which supports the uterus and provides vascular supply
- In mice the metrial gland starts to form at gestation day 8 (GD 8), is very prominent at mid-gestation, and has largely disappeared by the time of parturition
- The metrial gland helps to protect the developing fetus from immune surveillance and destruction by the maternal immune system
- Granulated metrial gland cells (GMGC), the hallmark cells of the metrial gland, are bone marrow-derived, perforin-positive, uterus-adapted natural killer (NK) cells

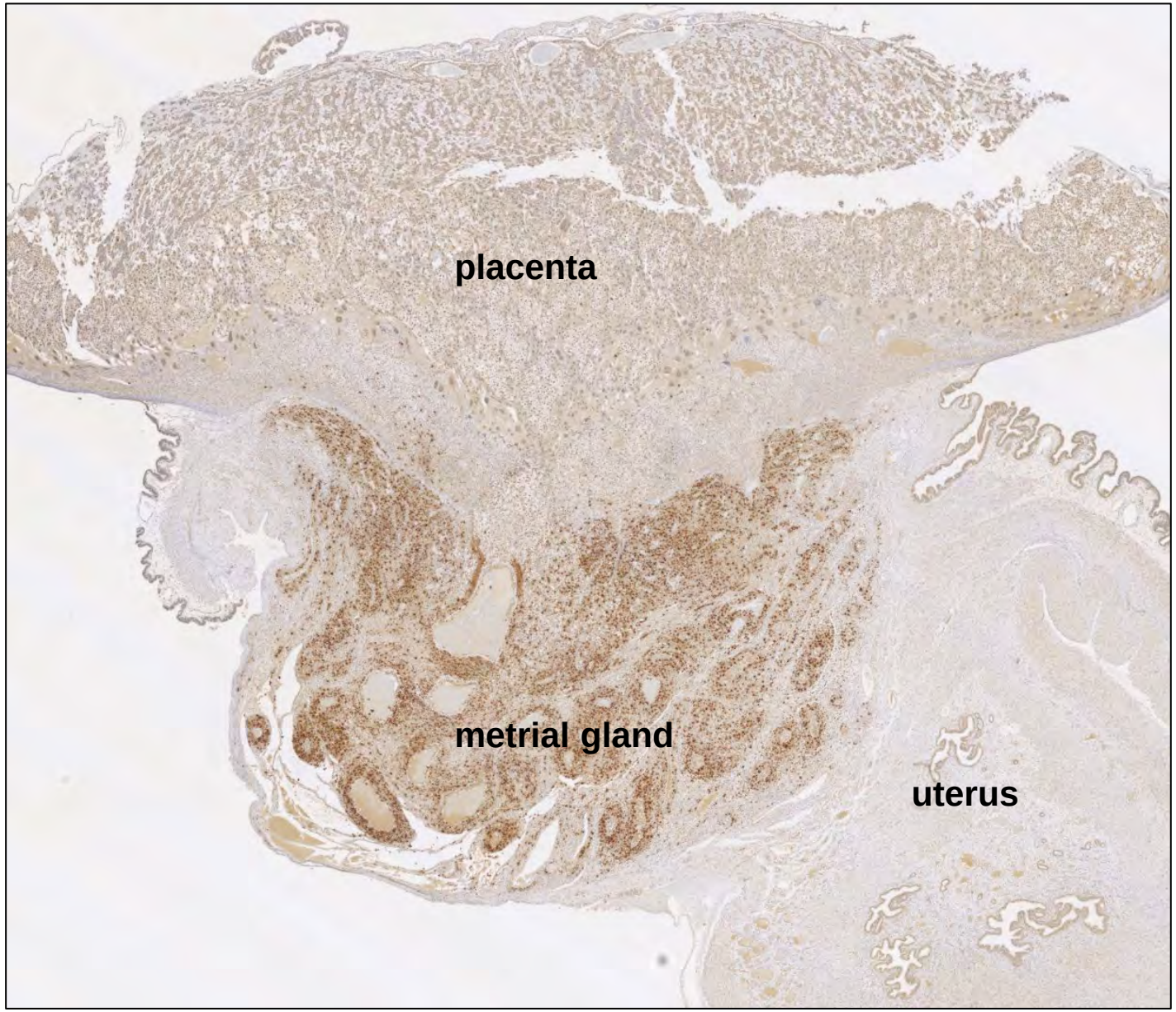


Non-Immune Tissues

Uterus & placenta with metrial gland, gestation day 16, Rat

Immunohistochemical staining for perforin reveals an intense population of perforin-positive, uterus-adapted natural killer (NK) cells in the region of the metrial gland. 3,3'-diaminobenzidine chromogen with hematoxylin counterstain.



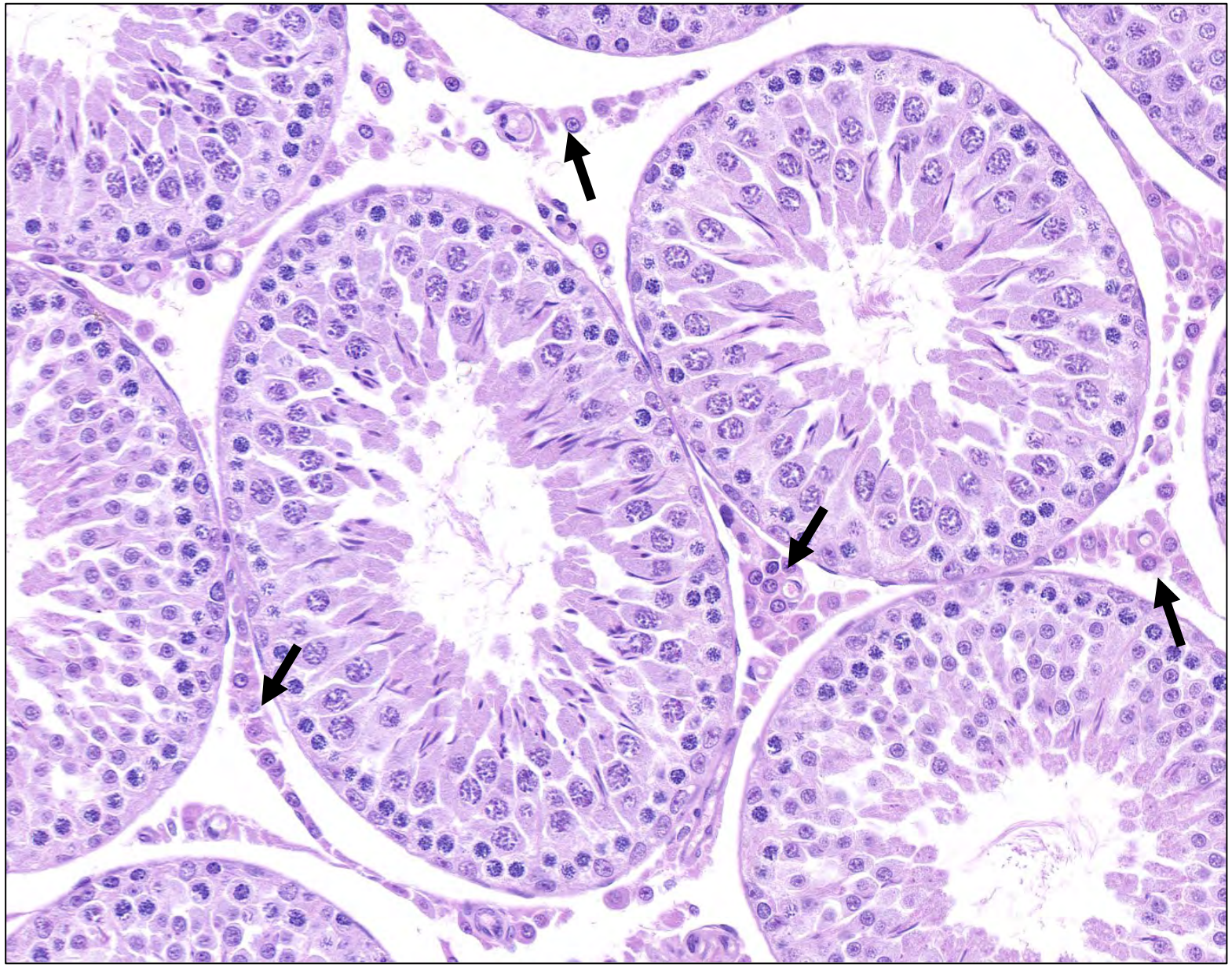


Non-Immune Tissues

Immune systems in the testis

- In the interstitial cell population (arrows) in the testis of rats, approximately 25% of the cells are macrophages rather than Leydig cells
- Sertoli cells make the seminiferous tubules an immunologically privileged site by suppressing T cell and macrophage activation and function, thus suppressing the adaptive immune response
- Sperm are protected from immune-mediated killing by two mechanisms
  - Sperm downregulate classical MHC class I molecules (HLA-A and HLA-B in humans or homologous molecules in other species), hindering killing by cytotoxic T lymphocytes
  - Sperm express non-classical MHC class I molecules, (e.g., HLA-G in humans) which are identified as “self” by NK cells, hindering killing by NK cells

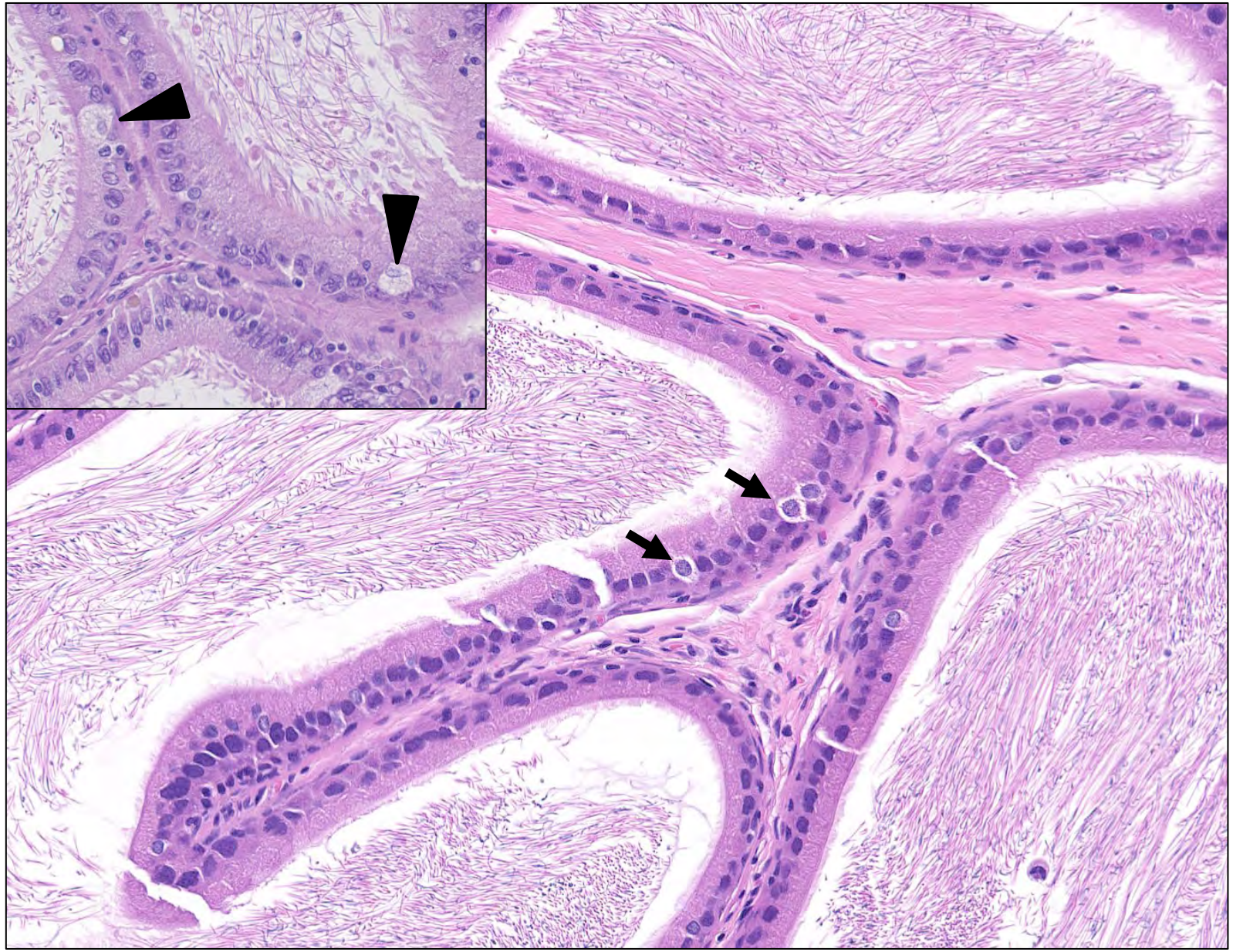
RIGHT: Rat testis at approximately 12 weeks of age, H&E staining



Non-Immune Tissues

Halo cells, epididymis, rat

- Halo cells (arrows in large image to the right) in the epididymis may be monocytes as well as helper or cytotoxic T lymphocytes
- Halo cells appear in the epididymis of the rat at approximately PND 14
- Distribution of halo cells is segment-specific and related to the luminal content of the epididymis
- The halo cell population increases with age and is more prominent in aged rats when smaller numbers of spermatozoa are present in the epididymal lumen
- Halo cells must be distinguished from clear cells (black arrowheads in the upper left image), which are the primary cells responsible for disposing of cytoplasmic droplets released from sperm cells during maturation

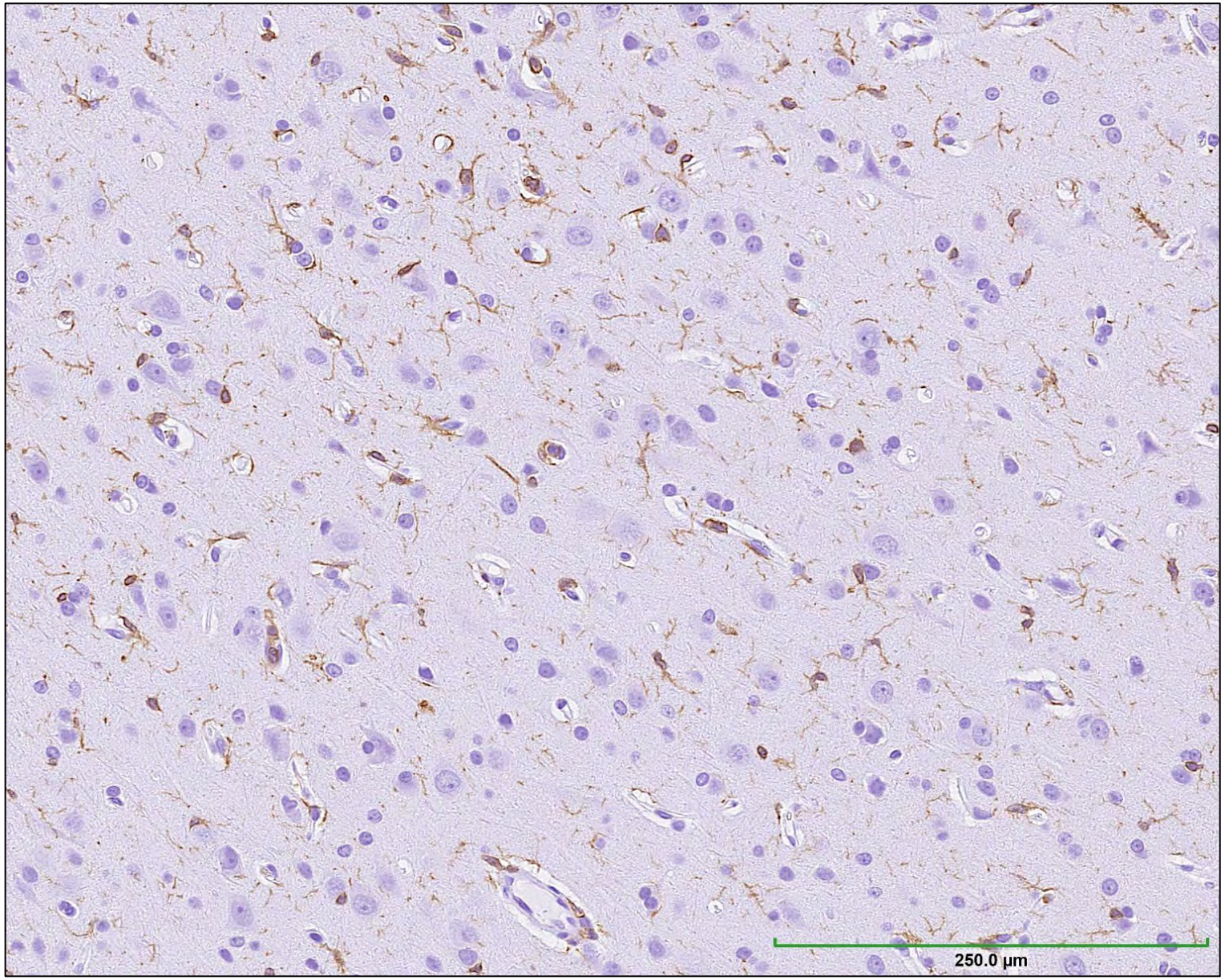


Non-Immune Tissues

Microglia, brain, cynomolgus macaque

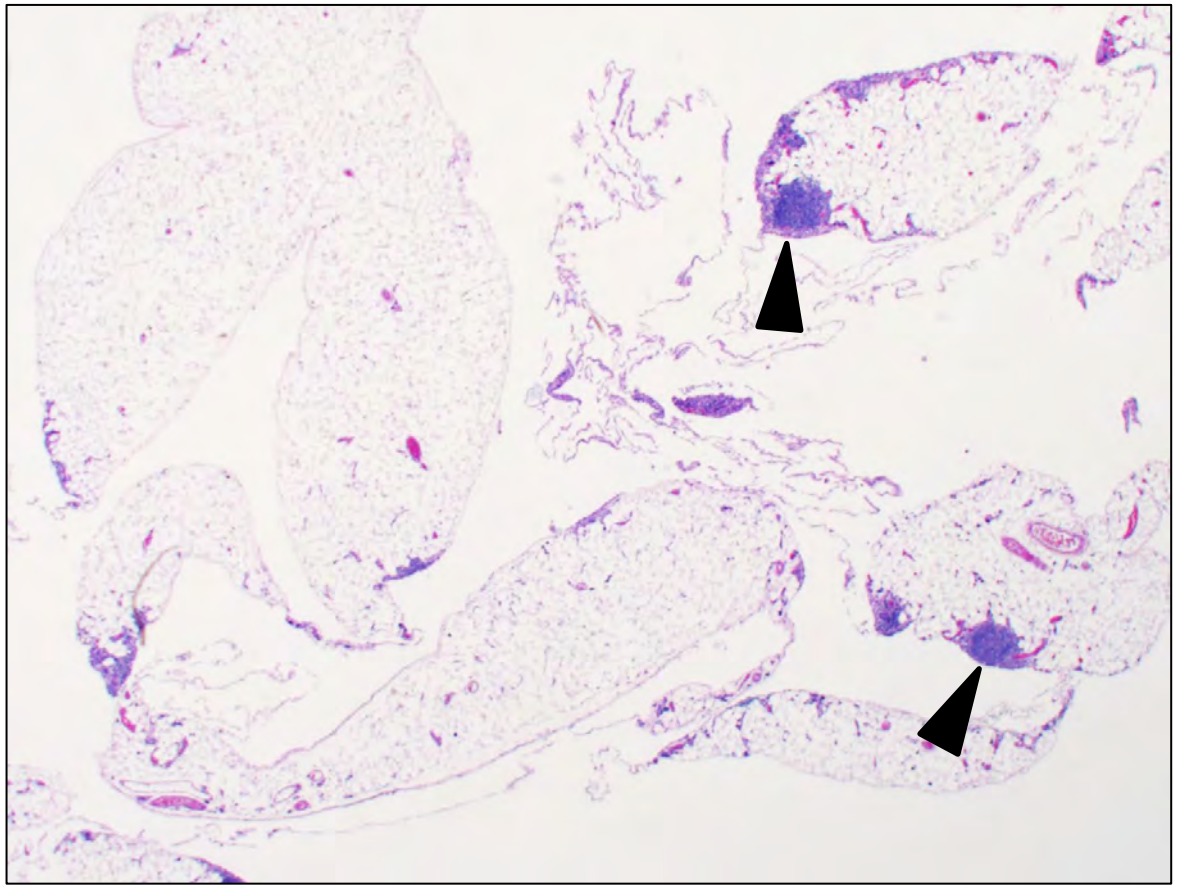
- Microglial cells are the primary immune system cells within the brain, though other cell types may have immunological functions
- Microglia are part of the fixed macrophage system that arises early in development at a time when the hematopoietic system is concentrated in the aorta-gonad-mesonephros (AGM), thus is a very primitive element of the immune system
- Microglia become activated during inflammatory processes in the CNS but may be difficult to visualize in routine H&E-stained histologic sections
- Immunohistochemical staining with IBA-1 reveals the microglial cell bodies as well as the cytoplasmic processes that radiate into the neuropil

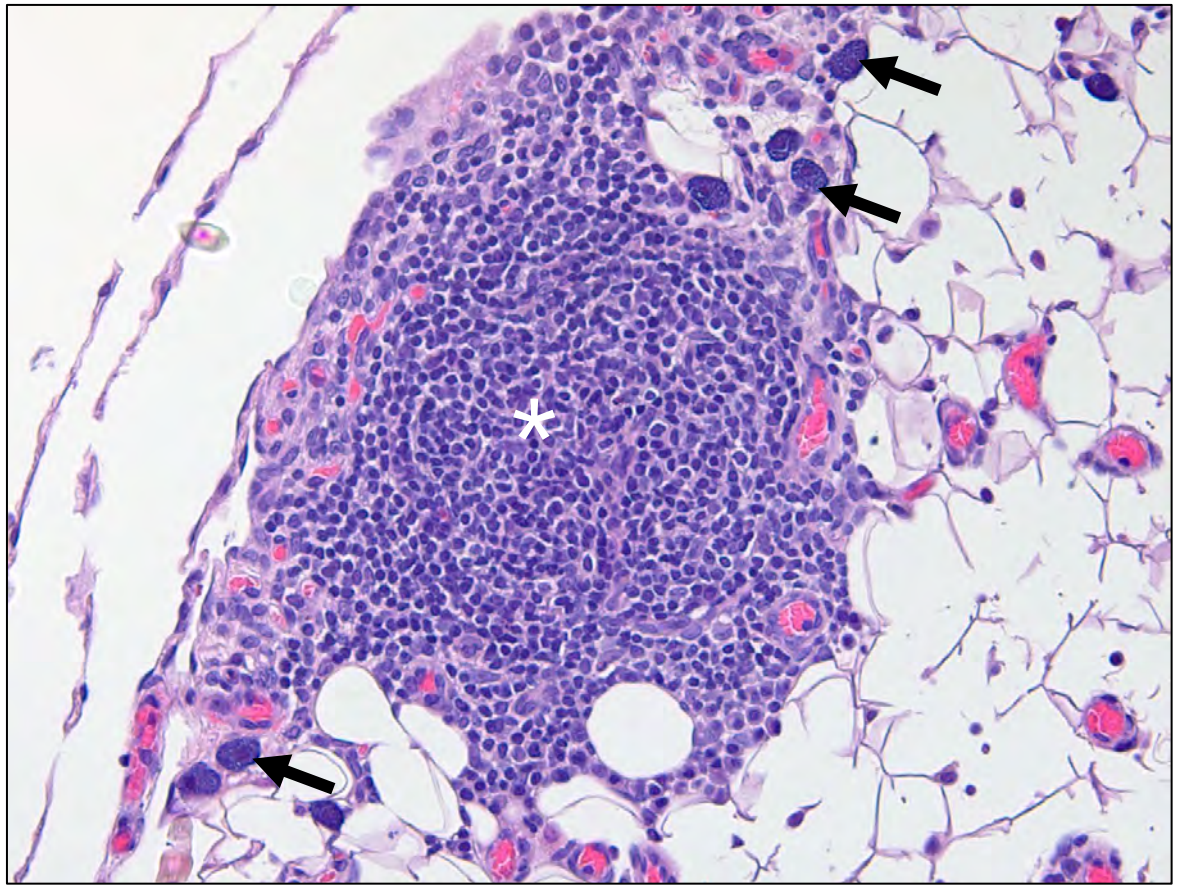
RIGHT: IBA-1 immunohistochemical staining with 3,3'-diaminobenzidine chromogen (brown) and hematoxylin counterstain (blue)



Non-Immune Tissues

Milky spots in mesentery, Rat





Fatty tissue in the peritoneal cavity sometimes includes clusters of lymphoid cells that are grossly visible as “milky spots” (arrowheads in image on left). The specific term for these lymphoid clusters is “fat-associated lymphoid tissue” (FALT). FALT in mouse omentum functions as a secondary lymphoid organ that promotes immunity to peritoneal antigens. Microscopically similar lymphoid clusters may be associated with serosal surfaces (serosa-associated lymphoid tissue, SALT). Note the mast cells (arrows in image on right) associated with the FALT (asterisk, image on right), which are commonly present in many tissues of rats.

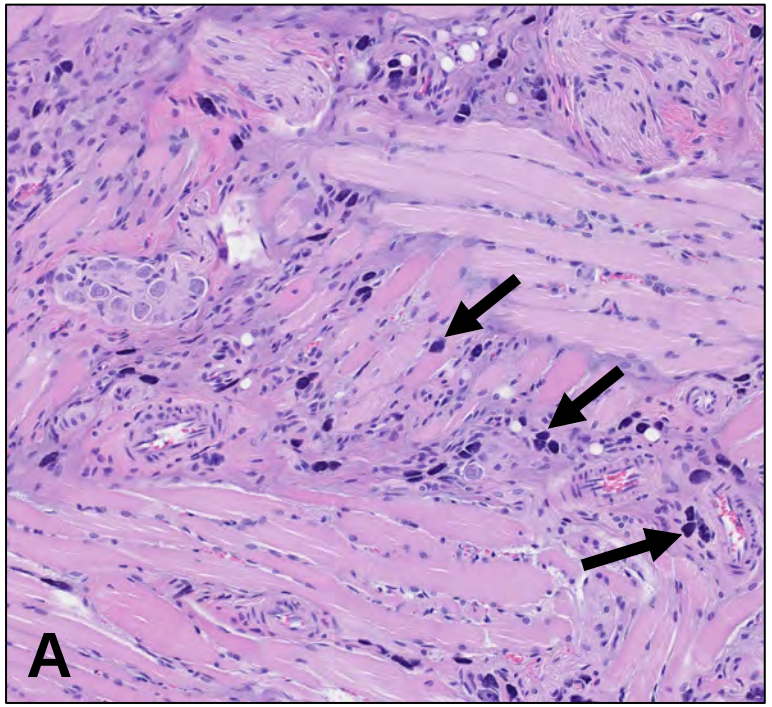
Non-Immune Tissues

Immunologically privileged tissues

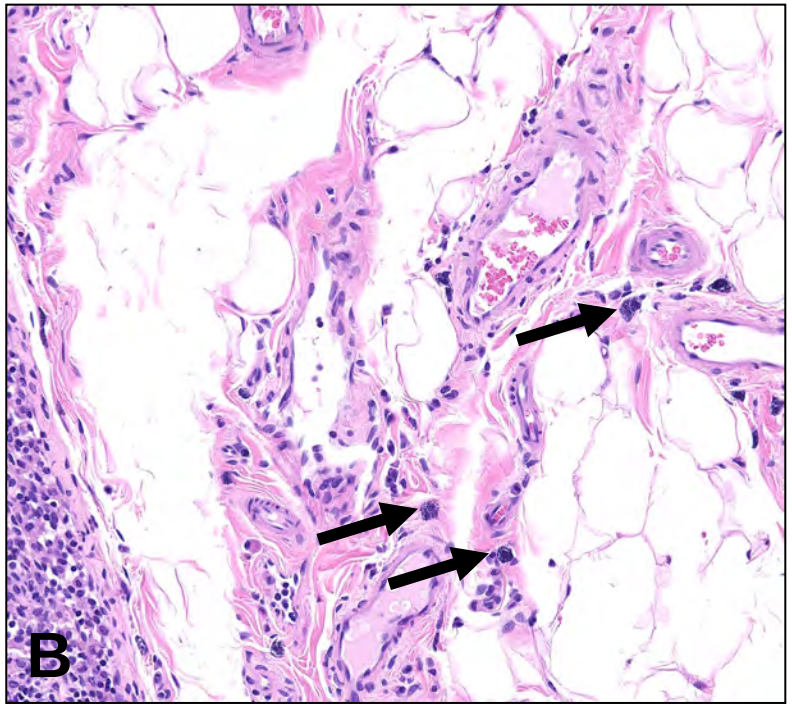
- A number of tissues have local mechanisms for blocking or modifying immune responses
- Brain: The blood-brain barrier blocks access by some immune system cells
- Eye:
  - A blood-ocular barrier in the anterior and posterior chambers prevents the entry of many molecules
  - Lack of MHC expression by ocular cells reduces potential for antigen presentation to the immune system
  - Ocular cells produce a number of molecules that directly suppress immune functions, including Fas ligand (FasL)
  - Fas/FasL binding causes T cells to die of apoptosis, thus T cells are essentially eliminated from the eye
- Testis:
  - Sertoli cells form a blood-testis barrier that block inflow of immunologically active cells and molecules
  - Sertoli cells locally suppress the T cell and macrophage functions that are critical to the adaptive immune response
  - Altered MHC expression prohibits innate immune system killing of sperm
- Ovary: Oocytes avoid killing by natural killer (NK) cells and cytotoxic T lymphocytes (CTL) by expression and secretion of primitive major histocompatibility (MHC) molecules that evade NK- and CTL-mediated killing

Non-Immune Tissues

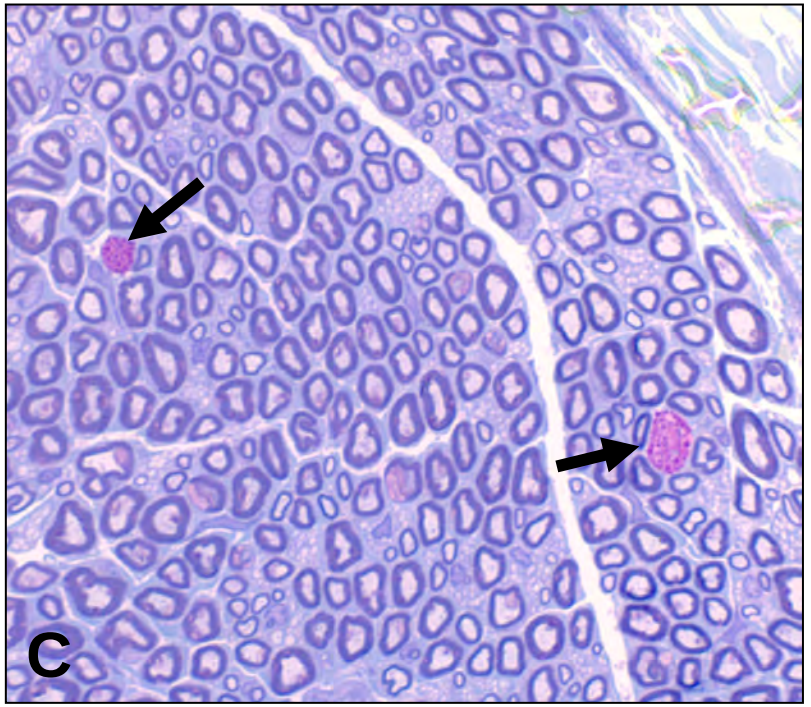
Mast cells in rat tissues



Tongue



Fatty tissue near mandibular lymph node



Tibial nerve

Mast cells (arrows) are commonly observed in many tissues of laboratory rats, as shown in H&E stains on histologic sections of tongue (A), fatty tissue near a lymph node (B), as well as a plastic-embedded thin section of tibial nerve stained with toluidine blue (C). Mast cells tend to be concentrated near blood vessels and near epithelial surfaces but can be present in virtually any location. It should be noted the mast cells that are distinctly visible in formalin-fixed tissue specimens are technically known as connective tissue mast cells. An additional mast cell population, mucosal mast cells, are present in the mucosa of the gastrointestinal and respiratory tracts. Mucosal mast cells have different chemical constituents of the cytoplasmic granules that characterize mast cells, thus **mucosal mast cells are not visible in formalin-fixed tissue specimens**. Special fixatives must be employed if mucosal mast cell populations are of particular interest.

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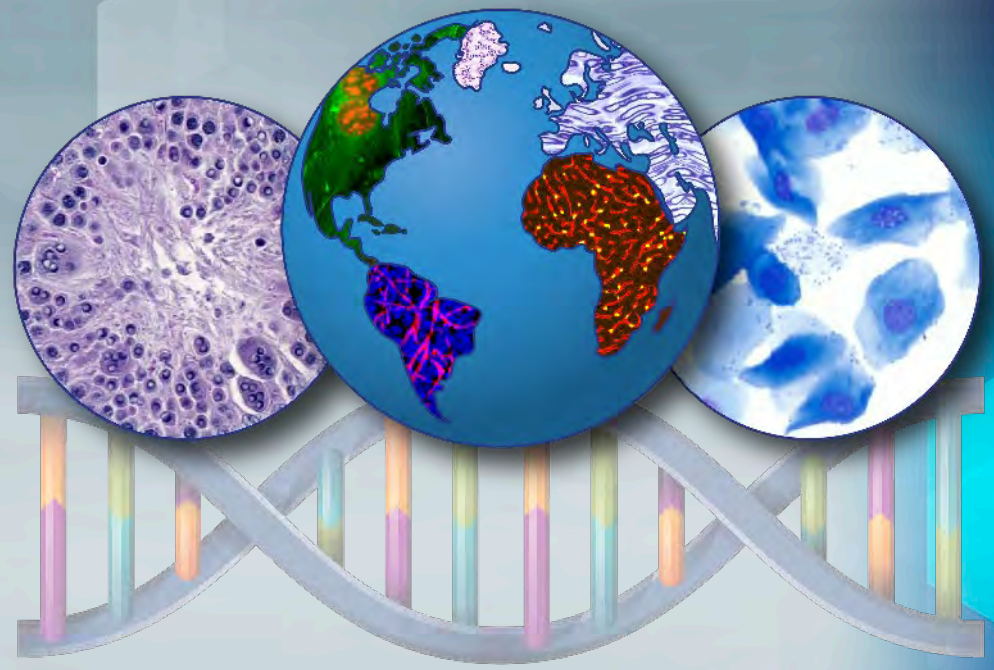
Acknowledgments

- All images presented are from the personal collections of the author unless otherwise noted



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