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TITLE: Lymph Node Structure and Function

PRESENTER: Teresa S. Kraus, MD

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Hello, my name is Teresa Kraus, and I am an assistant professor and medical director of the clinical hematology laboratory at the University of Oklahoma Health Sciences Center. Welcome to this Pearl of Laboratory Medicine on "Lymph Node Structure and Function."

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Primary lymphoid tissues are sites of foreign antigen-independent lymphoid differentiation. B cell precursors originate, and undergo the early stages of differentiation, in the bone marrow, and enter the circulation as mature naïve B cells. T cell progenitors originate in the bone marrow and migrate to the thymus where they undergo selection and mature into naïve T cells, which express either CD4 or CD8.

The secondary lymphoid tissues include the lymph nodes, spleen, and mucosa-associated lymphoid tissue (MALT). At these sites, naïve B and T cells encounter foreign antigens and undergo antigen-dependent maturation.

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Lymphatic vessels are present throughout most of the body, and drain excess interstitial fluid from tissues, eventually returning the fluid to the circulation via the subclavian veins. Lymph nodes are present at multiple points along the lymphatic network, and are particularly frequent along major vessels; in the neck, axillae, and groin; the mediastinum, and mesentery.

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Lymph nodes are small, bean-shaped structures, usually measuring between 0.2 and 2 cm, and are surrounded by a fibrous capsule. Fibrous trabeculae projecting from the capsule lend structural support to the lymph node. The lymph node can be separated into three cellular compartments: the cortex, paracortex, and medulla. The cortex contains lymphoid follicles composed mostly of B cells; in the paracortex, T cells predominate. The medulla consists of the medullary sinuses, and the medullary cords, which contain lymphocytes, plasma cells, and macrophages.

Lymphatic fluid containing antigens from tissues enters the lymph node via the afferent lymphatics, and flows into subcapsular, intermediate, and medullary sinuses, before exiting through the efferent lymphatics. The blood supply to the lymph node is derived from arteries that enter through the hilum of the node and branch into capillary loops that drain into postcapillary high endothelial venules in the paracortex. High endothelial venules are specialized vessels lined by cuboidal endothelial cells, and express lymphocyte adhesion molecules that facilitate extravasation of circulating lymphocytes through the vessel wall into the lymph node.

The cortex of an unstimulated lymph node consists of primary follicles composed of naïve B cells, with an underlying meshwork of follicular dendritic cells. The naïve B cells in primary follicles are small, mature lymphocytes with condensed chromatin and scant cytoplasm.

After exposure to antigen, there is a rapid proliferation of B cells. Germinal centers form in the center of B cell follicles; during this process, primary follicle cells are pushed to the periphery, where they form a mantle zone around the germinal center. The mantle zone also contains some memory B cells. A secondary follicle is made up of a germinal center and surrounding mantle zone.

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In this low-power image of a lymph node, the hilar vessels are visible on the left side of the photograph. The cortex is seen on the right side of the image, and is composed of B cell follicles. In this lymph node, the majority of the follicles are secondary follicles; the pale germinal centers are surrounded by a darker mantle zone.

The paracortex contains T cells and dendritic cells. Rare scattered B immunoblasts may be seen in the paracortex. These immunoblasts are large in size, with vesicular chromatin and a single prominent nucleolus. Immunoblasts may increase in number in some viral infections and other reactive conditions.

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The medulla consists of the medullary cords, which contain lymphocytes, macrophages, and plasma cells. The medullary sinuses are contiguous with the efferent lymphatics, and contain lymph, macrophages, plasma cells, and mast cells. Macrophages in the sinuses help “filter” the lymph by removing foreign material.

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The secondary follicle consists of a germinal center with a surrounding mantle zone. Uncommonly, a marginal zone composed of mature lymphocytes with moderate amounts of pale cytoplasm is seen outside the mantle zone. Marginal zones are more frequently encountered in the spleen and in mesenteric lymph nodes.

Fully developed germinal centers are polarized into a dark zone, which faces the T cell-rich zone toward the center of the lymph node, and a light zone, which is oriented toward the lymph node capsule.

Germinal center B cells can be subdivided into centroblasts and centrocytes based on their morphologic features. Centroblasts are large cells with high nuclear:cytoplasmic ratios, scant basophilic cytoplasm,

vesicular nuclei with smooth nuclear contours, and 1-3 small peripheral nucleoli. Centrocytes are small to large in size, with irregular to cleaved nuclear contours, scant cytoplasm, and condensed chromatin.

Centroblasts are concentrated in the dark zone of the germinal center. The centroblasts downregulate expression of surface immunoglobulin, and undergo rapid division and somatic hypermutation of their immunoglobulin genes. They then differentiate into centrocytes, express surface immunoglobulin, and migrate to the light zone, where they are selected for based on their affinity for antigens displayed on follicular dendritic cells. Centrocytes with the appropriate antigen affinity are selected to become memory B cells or plasma cells based on their interactions with dendritic cells and T cells in the light zone. Centrocytes with ineffective immunoglobulin mutations receive pro-apoptotic signals and are removed. Tingible body macrophages scattered throughout the germinal center help clear the apoptotic cells.

The classical view of the germinal center reaction suggested a unidirectional progression of germinal center B cells from the dark zone to the light zone. More recent cellular imaging studies have suggested that there is more bidirectional migration of cells between the light and dark zones than previously thought.

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The main cell types in the germinal center are the germinal center B cells (GCBs), which include centroblasts and centrocytes; follicular dendritic cells; follicular helper T cells; and tingible body macrophages. It is important to note that the germinal center reaction involves very complex, and still incompletely understood, interactions between a variety of cell types. Not all of these cell types are listed here, but the listed cells are the major contributors to the germinal center reaction.

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Germinal center B cells express germinal center-associated markers, including CD10 and BCL-6. CD10 is also known as CALLA, or common ALL antigen; it is expressed early in B cell differentiation, but lost as B cells mature. Germinal center B cells re-acquire CD10.

BCL-6 is a transcription factor that plays multiple roles in the germinal center reaction. BCL-6 suppresses p53 expression, protecting the B cell from death due to DNA damage during somatic hypermutation of the immunoglobulin genes. BCL-6 also represses expression of the anti-apoptotic protein BCL-2, helping to make negatively selected GCB cells susceptible to removal by apoptosis.

In a benign lymph node, CD10 and BCL-6 expression should be confined to the germinal center. The presence of significant expression of these antigens outside of germinal centers should raise concern for a B cell lymphoma with germinal center differentiation, such as follicular lymphoma or diffuse large B cell lymphoma.

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Follicular dendritic cells (FDCs) are cells of mesenchymal origin, and are more concentrated in the light zone of the germinal center. On a hematoxylin and eosin stain, follicular dendritic cells are large, often binucleate cells with vesicular chromatin and small nucleoli. FDCs express CD23 (low affinity IgE Fc

receptor FcεRII), and complement receptors CR1 (CD35), CR2 (CD21), and CR3 (CD11b/CD18). FDCs display antigen in the form of antigen complexes via these surface receptors. They also secrete cytokines and chemokines that attract B cells and follicular helper T cells to the germinal center, including CXCL13, the ligand for CXCR5. CXCR5 is expressed by GCB cells and follicular helper T cells.

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Follicular helper T cells are primarily concentrated in the light zones of germinal centers. These are specialized CD4+, CD57+, PD-1+ T cells that express BCL-6 and secrete cytokines that promote B cell proliferation and differentiation. They also play a role in selection of B cells based on affinity for antigens displayed on FDCs. Follicular helper T cells deliver survival signals to GCB cells through a number of different pathways, including CD40-CD40L, PD1-PD1L, and IL-21. The pro-survival signals from follicular helper T cells counteract pro-apoptotic signals from the FAS-FASL pathway. GCB cells that are positively selected through their interactions with FDCs and follicular helper T cells go on to become antibody-producing plasma cells, or long-lived memory B cells that can quickly differentiate into plasma cells if re-exposed to antigen. The B cells that are not selected undergo apoptosis. The apoptotic debris is then phagocytosed by tingible body macrophages.

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The various cell types in the germinal center can be identified by immunohistochemical stains. These immunohistochemical stains can be helpful in defining the lymph node architecture in cases where the morphology is difficult to interpret or partially effaced on routine hematoxylin and eosin (H&E) stains.

CD3, a T cell marker, highlights numerous T cells in the paracortex, as well as a variable but lower number of T cells, including follicular helper T cells, in germinal centers.

CD20, a B cell marker, highlights B cells in the follicles. Both the germinal center B cells and mantle zone B cells are positive for CD20. In a benign lymph node, B cells should be largely confined to follicles. The presence of sheets of B cells outside of well-defined follicles raises concern for B cell lymphoma.

CD10 stains the B cells in germinal centers; it is negative in the mantle zone.

The Ki-67 proliferation index is markedly increased in germinal centers, particularly in the dark zone.

The anti-apoptotic protein BCL-2 is expressed by naïve and memory B cells, and in T cells. BCL-2 is downregulated in the germinal center; benign germinal centers will be negative for this marker. Immunohistochemical staining for BCL-2 is used to help differentiate the malignant follicles of follicular lymphoma from benign reactive germinal centers. The malignant follicles of follicular lymphoma generally coexpress one or more germinal center markers with BCL-2. It is important to note that primary follicles, composed of naïve B cells, will also be positive for BCL-2, but will not express germinal center markers.

Follicular dendritic cells can be highlighted with immunohistochemical stains for CD23, CD21, and CD35.

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In general, the lymph node architecture will be effaced in neoplastic conditions, and preserved (though possibly distorted) in reactive conditions. In reactive or infectious causes of lymphadenopathy, one or more of the lymph node cellular compartments may become hyperplastic. The differential diagnosis can sometimes be narrowed based on the pattern of lymph node hyperplasia. This list provides a few examples of causes of reactive or infectious lymphadenopathy, and their usual patterns of lymph node involvement.

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Assessment of the nodal architecture is a crucial first step in evaluation of a lymph node biopsy. It is important for the pathologist to have a good understanding of normal lymph node structure in order to recognize architectural alterations associated with lymph node disease. In cases where the architectural features are difficult to identify or partially effaced on routine H&E stains, immunohistochemical stains can be of use to highlight the underlying immunoarchitecture.

Slide 15: References

Slide 16: Disclosures

Slide 17: Thank You from www.TraineeCouncil.org
Thank you for joining me on this Pearl of Laboratory Medicine on “Lymph Node Structure and Function.” I am Teresa Kraus.