Part I

Normal Lymph Node

Normal Lymph Node Architecture and Function

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Lymph nodes are part of the immune system and play a critical role in innate and adaptive immune responses [1, 2]. Lymphatic vessels channel lymph to nodes spread throughout the body. As lymph enters nodes through afferent channels, it percolates through the subcapsular sinus into delicate sinusoidal vasculature until it exits through the nodal medulla into efferent lymphatics. As lymph traverses the nodal parenchyma, antigens are brought in contact with the effector cells of the adaptive immune system begin a cascade of immune processes that allow recognition and ultimately neutralization of foreign antigens and pathogens. Blood enters and exits the lymph node through hilar arterioles and venules, respectively. Within the substance of the lymph node, blood passes through specialized vascular segments called high-endothelial venules. Lymph nodes are part of the so-called secondary lymphoid system, which also includes the spleen and mucosa-associated lymphoid tissue. Primary lymphoid organs are the bone marrow and thymus, the sites of B-cell and T-cell production.

The microarchitecture of the lymph node is divided into three areas: cortex, paracortex, and medulla. The cortex mainly contains the lymphoid follicles, which can be primary or secondary. Primary lymphoid follicles are antigen-naïve round structures composed of uniform small lymphocytes. Antigenic stimulation results in the formation of secondary lymphoid follicles, which contain lymphocytes at various stages of functional maturation. The secondary follicles consist of three principal compartments: marginal zone, mantle zone, and germinal center. The lifecycle of secondary lymphoid follicles includes an involution stage in which the three major follicular components, especially the marginal zone, are diminished. B-cells predominate in primary and secondary follicles. In contrast, the paracortex is rich in T-cells, which may be small or large depending on their maturation stage. The medulla contains lymphocytes, plasmacytoid lymphocytes, plasmablasts, and mature plasma cells; it represents the primary maturation site of antibody-producing plasma cells.

While all lymph nodes share common histologic characteristics, variations exist in different anatomic regions of the body. For instance, mesenteric lymph nodes have prominent marginal zones, medullary chords, and sinuses; whereas peripheral nodes tend to have larger and more numerous secondary follicles with germinal centers, especially when draining areas of active antigenic stimulation [1].

Immunohistochemistry is a helpful tool to highlight the various lymph node components (Tables 1.1 and 1.2). B-cell markers (CD19, CD20, PAX5) are positive in cortical structures primarily. Germinal centers are characteristically positive for CD10 and BCL6, and they are negative for the anti-apoptotic protein BCL2. T-cell markers (CD2, CD3, CD4, CD5, CD7, and CD8) are positive in paracortical structures primarily. A subset of T-cells called *follicular helper T-cells* is localized within the germinal center.

Approaches for lymph node sampling in clinical practice includes fine needle aspiration, core biopsy, and open biopsy. Fine needle aspiration and core biopsy sampling may be optimized through image guidance (computerized tomography or ultrasound). Each of these sampling options offers advantages and entails inherent drawbacks. Fine needle aspiration is minimally invasive, provides superior cytomorphologic details, and the material obtained can be used to perform any standard ancillary studies. However, fine needle aspiration cytology provides no information about tissue architecture. Core biopsy sampling is generally considered the method of choice for lymph node sampling in most clinical circumstances because it is minimally invasive and provides information about tissue architecture. Although open (incisional/excisional) biopsy generally provides the most adequate sampling option from a diagnostic standpoint, it is an invasive surgical procedure.

Regardless of the sampling method used, triaging a lymph node biopsy from a patient suspected of having primary lymph node pathology should prioritize histologic evaluation and multicolor flow cytometry immunophenotyping. Procurement of fresh tissue for cytogenetic evaluation and molecular diagnostics has become less important since fluorescence in situ hybridization and molecular tests can be adequately performed on formalin-fixed paraffin-embedded tissue material.

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Table 1.1 Summary of the main functional and supportive cellular constituents of the lymph node

Functional cell population	Primary compartment	Broad function	Morphology	Immunophenotype
B-cells	Cortex			CD19+, CD20+, CD79b+
Naïve B-cells	Primary follicle Mantle zone	Unstimulated recirculating B-cells	Small lymphocytes with dense chromatin and scant cytoplasm	IgM+, IgD+
Centroblasts	Germinal center, dark zone	Early activated B-cells	Large noncleaved cells	IgMdim+, CD10+, BCL6+
Centrocytes	Germinal center, light zone	Activated B-cells that begin secretion of immunoglobulins	Small cleaved cells	IgA+, IgG+, CD10+, BCL6+
Memory B-cells	Marginal zone	Memory B-cells	Small round lymphocytes with moderate amount of cytoplasm	IgM+, IgG+
Plasma cells	Medullary area	Immunoglobulin secretion	Eccentric nucleus and basophilic cytoplasm	Cytoplasmic immunoglobulin; CD138+
T-cells	Paracortex	Adaptive cell mediated immunity	Small lymphocytes with clumped chromatin	CD2+, sCD3+, CD5+, CD7+
Helper T-cells	Paracortex	Antigenic processing		CD4+
Follicular helper T-cells	Germinal center	T-helpers in germinal centers		CD4+, PD1+, CXCL13+, BCL6+, CD10+, CXCR5+
Cytotoxic T-cells	Paracortex	Regulatory function	Lymphocytes with cytoplasmic granules	CD8+
NK-cells	Paracortex	Innate immunity	Small lymphocytes	CD2+,CD56+,cCD3+/sCD3–
Follicular dendritic cells	Germinal center	Antigen presentation	Vesicular nuclei appear in pairs	CD21+, CD23+, CD35+, HLA-DR+
Interdigitating dendritic cells	Paracortex	Antigen presentation	Oval, indented vesicular nuclei; moderate amount of cytoplasm	HLA-DR+, CD1a+, S100+
Histiocytes	Germinal center	Antigen presentation; phagocytosis	Oval vesicular nuclei, commonly surrounded by abundant clear or granular cytoplasm	CD68+, CD163+
	Paracortex	Antigen presentation	Vesicular oval nuclei; pink cytoplasm	CD68+
	Sinus			CD68+

IG immunoglobin, *NK* natural killer

Table 1.2 Lymph node structures and immunophenotypic markers

Structure	Positive	Negative
Germinal center	CD10, CD20, BCL6	BCL2
B-cell component	CD10, CD20, BCL6	BCL2
T-cell component	CD10, CD3, BCL6, PD-1	
Follicular dendritic cells	CD21, CD23, CD35	
Mantle zone	CD20, IgD	
Marginal zone	CD20, IgM	
Paracortex	CD2, CD3, CD4	
Sinusoidal cells	CD68, CD163	T- and B-cell markers
Sinusoidal and vascular lining cells	CD34, FVIII-RA	

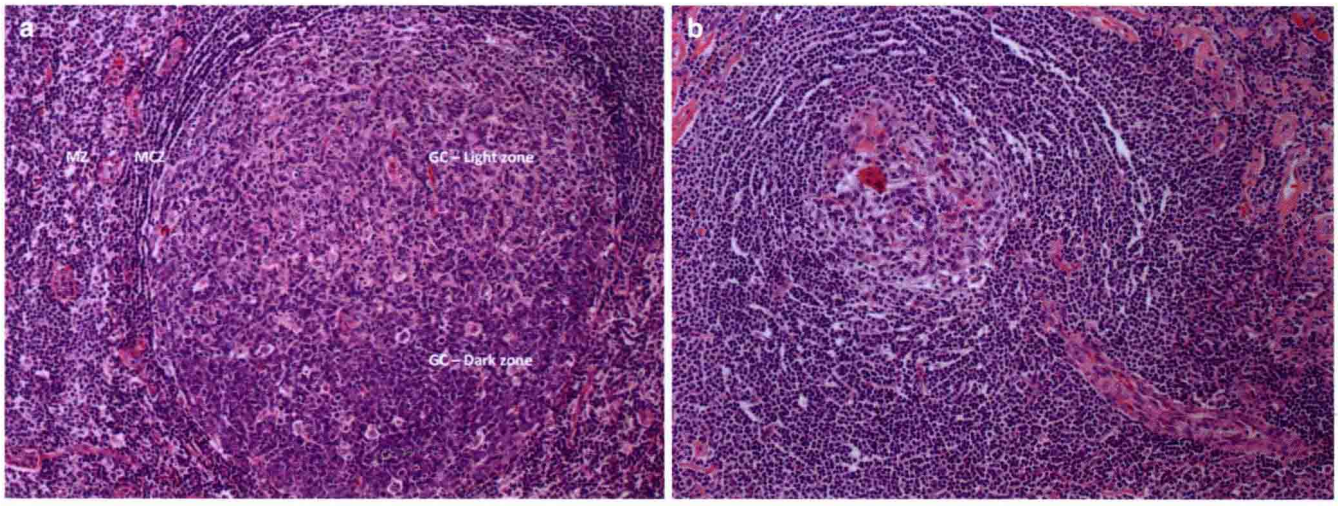

Fig. 1.1 (**a**) Secondary lymphoid follicle consists of a marginal zone (*MZ*), mantle zone (*MCZ*), and a germinal center (*GC*). Mantle zone cells are naïve B-cells that have not been exposed to antigen, whereas marginal zone cells represent lymphocytes that have traversed and survived the germinal center reaction. The germinal center is composed of centrocytes, centroblasts, tingible-body macrophages, and follicular dendritic cells. The distribution of germinal center cells is polarized into a "light zone" and a "dark zone." The GC dark zone is an area of brisk lymphocyte proliferation that is rich in mitotic figures and centroblasts (large noncleaved cells). The light zone is rich in centrocytes (small cleaved lymphocytes) and follicular dendritic cells. (**b**) Involuted germinal center, with depletion of germinal center lymphocytes and depletion of the marginal zone

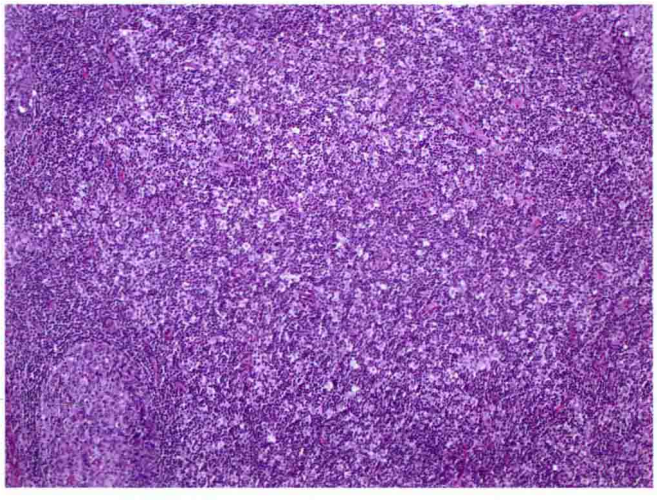

Fig. 1.2 The paracortical area is rich in T-cells. Seen here is an example of paracortical hyperplasia that includes an admixture of small lymphocytes and many histiocytes

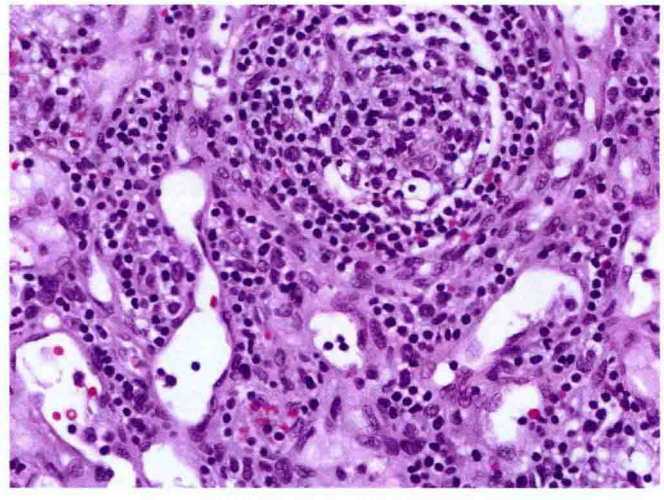

Fig. 1.3 Prominent sinuses in an abdominal lymph node

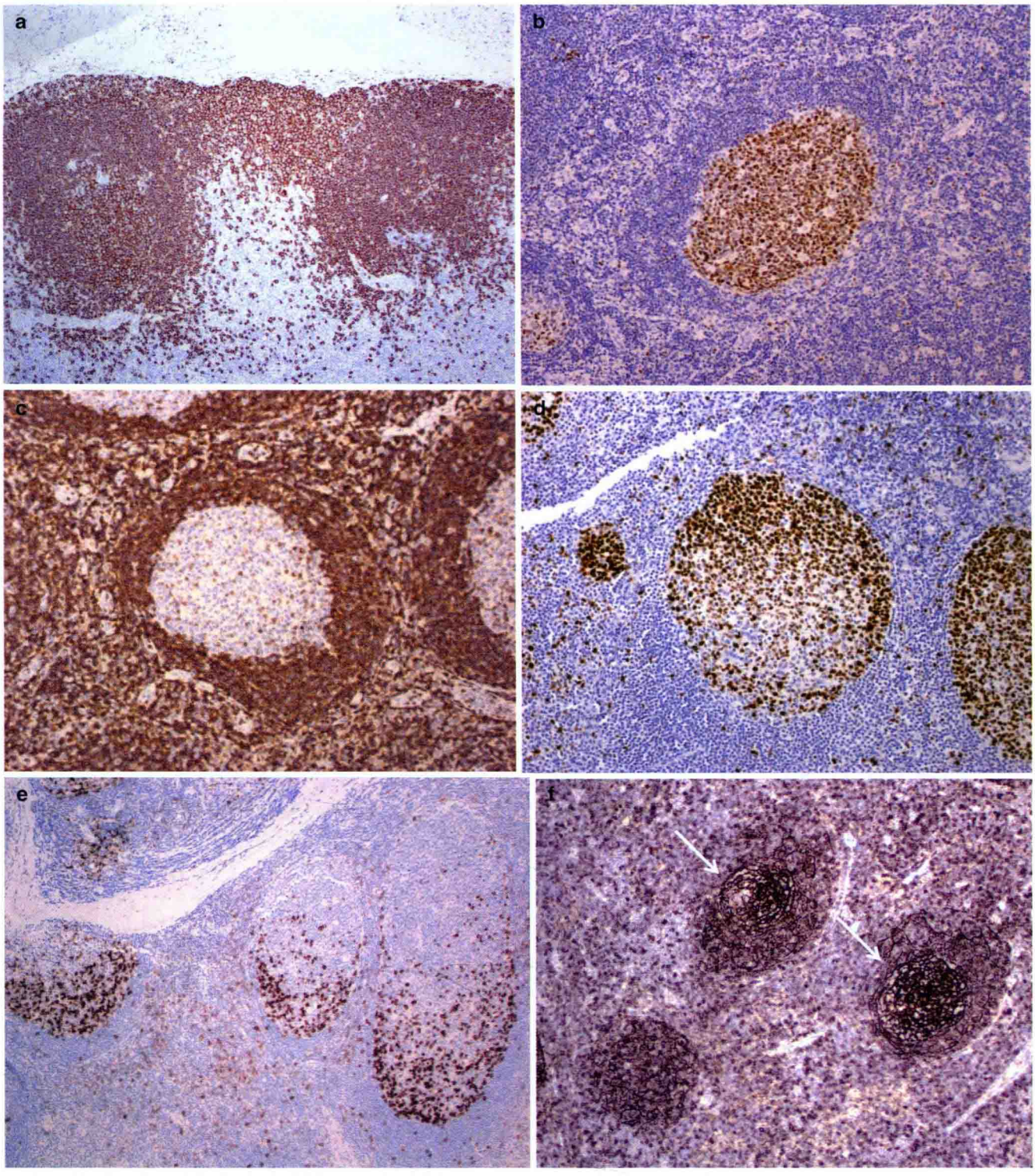

Fig. 1.4 (**a**) CD20 highlights most cells in follicles and scattered paracortical B-cells. Germinal center B-cells are positive for BCL6 (**b**) and negative for BCL2 (**c**). (**d**) Polarization of germinal centers can be further highlighted with an immunostain for the proliferation marker Ki-67, which demonstrates higher numbers of proliferating cells in the germinal center dark zone compared to the light zone. (**e**) Follicular helper T-cells are positive for PD1, and they localize primarily to the light zone of the germinal center. (**f**) Follicular dendritic cells are strongly positive for CD23 (*arrows*). Lymphocytes can also be positive for CD23 in reactive conditions as seen in this example

References

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2. Campo E, Jaffe ES, Harris NL. Normal lymphoid organs and tissues. In: Jaffe ES, Harris NL, Vardiman JW, Campo E, Arber DA, editors. Hematopathology. Philadelphia: Saunders/Elsevier; 2011. p. 97–117.

Part II

Reactive Nonspecific Changes