# 1 SPECIMENS AND STUDIES

Lymph node specimens may include fine-needle aspiration biopsies, needle core biopsies, excisional lymph node biopsies, including sentinel lymph node biopsies, and lymph node dissections.[1–6] The first three types of specimens are typically used for lymphoma diagnosis, while sentinel lymph node biopsies and lymph node dissections are performed in the assessment and treatment of metastatic neoplasm and are discussed later. Fine-needle aspiration biopsies may be very useful but in general should be interpreted by only those with adequate training in their interpretation. They are most useful in ruling out neoplastic disease or in staging or determining recurrence of disease. Nonetheless, in conjunction with ancillary studies, they may be used by experienced practitioners to establish an initial diagnosis of malignant lymphoma. These ancillary studies commonly include flow cytometry, immunohistochemical studies performed on cytospin preparations, fluorescence in situ hybridization (FISH) studies performed on the smears, and/or microbiologic culture, as appropriate. Needle core biopsies are fast replacing excision biopsies as the primary means of lymphoma diagnosis. Multiple cores may be obtained, with some cores fixed in formalin or other fixatives for light microscopic examination and paraffin section immunohistochemical studies; snap-frozen for possible molecular studies, immunohistochemical studies (rare antibodies), or microbiologic studies, as appropriate; and touch preparations saved for possible FISH studies.

Excisional lymph node biopsies still provide the best material for primary lymphoma diagnosis. Optimally, the largest abnormal lymph node should be excised. The specimen should be received fresh if possible, in a capped empty container, but should be placed in a small amount of sterile saline if greater than 1 hour between excision and processing is expected. Specimens should not be placed on sponge, gauze, or any material that can desiccate or introduce other artifacts into the tissue. The specimen should be bread-loafed into thin slices, with the first slices removed sterile for possible cytogenetic and/or microbiologic studies, as appropriate. Touch or preferably scrape preparations should be made for assessment of cytologic detail and possible FISH studies. A portion of the specimen should be sent for flow cytometry studies, and another portion should be snap-frozen for possible molecular studies or rare immunohistochemical studies that do not work well in paraffin sections. The remainder of the tissue should be fixed and paraffin embedded. Most laboratories prefer formalin fixation, which is sufficient if the tissue is sliced thin and adequate time for fixation is allowed. Some laboratories also employ a second fixative; although governmental

regulations generally do not permit the use of metal-based fixatives any more, reasonable surrogate commercial alternatives are available. I do not recommend fixation in glutaraldehyde for cases of suspected lymphoma, as there is no good role for electron microscopy in the diagnosis and classification.

The Association of the Directors of Anatomic and Surgical Pathology (ADASP) has made a series of recommendations for the handling of lymph node specimens with possible metastatic disease. They recommend that, in the absence of gross tumor, a lymph node biopsy be cut into 3- to 4-mm slides and entirely submitted, processing different surfaces for microscopic examination. Furthermore, they recommend the examination of several levels with hematoxylin and eosin (H&E). For lymph node dissections, they recommend fresh processing. They do not believe that clearing of adipose tissue is necessary, although others have found that this technique may be necessary for finding the smallest lymph nodes. Every lymph node should be submitted for microscopic examination, again submitting each node in its entirety, unless it shows grossly evident tumor. Levels are not recommended for lymph node dissections. For sentinel lymph node biopsies, they recommend levels on each block, if tumor is not seen grossly or at the time of frozen section. They provide no recommendations on the performance of immunostains, although it is my laboratory's practice to routinely perform keratin and S-100 stains for carcinoma and malignant melanoma, respectively.

Ancillary studies are as important for lymphoma diagnosis as routine light microscopic evaluation. A list of paraffin section immunohistochemical markers useful in the workup of cases of suspected malignant lymphoma is given in Table 1. Flow cytometry studies can supplement immunohistochemical analysis, particularly in several areas: determination of specific kappa:lambda ratios and determination of surface marker antigen expression that may be weak or not detected in paraffin sections due to sensitivity issues (Table 2). Nonetheless, there are potential pitfalls to the interpretation of flow cytometry studies, including gating on the wrong population, the obvious inability to assess antigen expression in an architectural context, the relative inability to detect nuclear antibodies, the inability to determine kappa:lambda ratios when the neoplastic population lacks surface immunoglobulin expression, and the inability to detect abnormal antigen expression in rare cell populations. Detection of antigen receptor gene rearrangement studies is most often determined using polymerase chain reaction (PCR) studies as opposed to the older (and much slower) Southern blot studies. While the detection of clonal gene rearrangements is not synonymous with malignancy, and their absence is not synonymous with benignity, their detection or lack of detection may be very helpful, when interpreted in the proper context and in conjunction with the results of other studies. Finally, a variety of specific recurring chromosomal translocations have been associated with various subtypes of malignant lymphoma and may be helpful in both diagnosis and classification of lymphoma.

**Table 1.** Commonly Used Major Leukocyte Antigens Detectable in Paraffin Sections

| *Antibody* | *Predominant Hematolymphoid Cell Expression* |
|---|---|
| ALK | Anaplastic large-cell lymphomas with ALK translocation |
| Bcl-2 | Non–germinal center B-cells, most T-cells, most follicular lymphomas, many low-grade and some higher grade B-cell lymphomas |
| Bcl-6 | Germinal center B-cells, lymphomas of follicular origin |
| BOB.1 | B-cells and B-cell lymphomas, nodular lymphocyte-predominant Hodgkin lymphoma |
| Cyclin D1 (Bcl-1) | Mantle cell lymphoma, hairy cell leukemia |
| DBA.44 | Hairy cells, B-cells |
| Elastase | Myeloid tumors, leukemia |
| Epithelial membrane antigen | Plasma cells and plasma cell neoplasms, many cases of nodular lymphocyte predominance, anaplastic large-cell lymphoma, and T-cell–rich B-cell lymphoma |
| EBV latent membrane protein | Some EBV-infected cells, including EBV+ Hodgkin cells, posttransplantation lymphoproliferative disorders, and EBV-associated infectious mononucleosis |
| Fascin | Dendritic cells, Reed-Sternberg cells |
| Granzyme B | NK-cells and cytotoxic T-cells |
| Hemoglobin A | Nucleated erythroid cells (benign and neoplastic) |
| HLA-DR | B-cells, interdigitating cells, Langerhans cells, immature granulocytes, and erythroid cells |
| Immunoglobulin light and heavy chains | Plasma cells, plasma cell and plasmacytoid neoplasms, some follicular and marginal zone lymphomas |
| Ki-67 (MIB-1) | Proliferating cells |
| Langerin | Langerhans cells |
| Lysozyme | Histiocytes–monocytes and myeloid cells (benign and neoplastic) |
| Myeloperoxidase | Myeloid cells (benign and neoplastic) |
| MUM-1/IRF | Plasma cells, classical Hodgkin lymphoma, subset of diffuse large B-cell lymphomas |
| OCT-2 | B-cells and B-cell lymphomas and nodular lymphocyte-predominant Hodgkin lymphoma |
| PAX-5 | B-cells and neoplasms |
| Perforin | Cytotoxic T-cells and NK-cells |
| Tartrate-resistant acid phosphatase | Hairy cell leukemia |
| TdT | Thymic lymphoid cells, lymphoblastic neoplasms, and some myeloid neoplasms |
| TIA-1 | Cytotoxic T-cells and NK-cells |
| Tryptase | Mast cells |
| ZAP-70 | Subset of small lymphocyte leukemia/small lymphocytic lymphoma |
| CD1a | Thymocytes, some T-lymphoblastic lymphomas, and Langerhans cells |
| CD2 | T-cells and T-cell lymphomas |
| CD3 | T-cells and many T-cell lymphomas |
| CD4 | Histiocytes and histiocytic neoplasms, T-helper cells, and many T-cell lymphomas |
| CD5 | T-cells and many T-cell lymphomas, B chronic lymphocytic leukemia/small lymphocytic lymphoma, mantle cell lymphoma |
| CD7 | T-cells, some T-cell neoplasms, some myeloid leukemias |
| CD8 | T-cytotoxic suppressor cells, some T-cell lymphomas |
| CD10 (CALLA) | Precursor B-cells and B-lymphoblastic neoplasms, lymphomas of follicular origin, Burkitt lymphoma |
| CD15 | Myeloid cells, Hodgkin lymphoma, rare non-Hodgkin lymphomas |

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**Table 1.** *continued*

| | |
|---|---|
| CD16 | NK-cells and neoplasms, some myeloid cells |
| CD20 | B-cells and B-cell lymphomas, nodular lymphocyte-predominant Hodgkin lymphoma |
| CD21 | Follicular dendritic cells and neoplasms, mantle and marginal zone B-cells |
| CD23 | Follicular dendritic cells, mantle zone B-cells, and most B chronic lymphocytic leukemia/small lymphocytic lymphoma |
| CD25 | Cells expressing IL-2R, classical Hodgkin lymphoma, human T-cell leukemia/lymphoma, subset of other T-cell lymphomas |
| CD30 | Activated lymphoid cells, classical Hodgkin lymphoma, anaplastic large-cell lymphoma |
| CD34 | Progenitor cells, some myeloid and lymphoblastic neoplasms |
| CD35 | Follicular dendritic cells and neoplasms |
| CD43 | T-cells, myeloid cells, mast cells, T-cell lymphomas, some B-cell lymphomas, myeloid leukemia, mast cell neoplasms |
| CD45/CD45RB | All hematolymphoid cells, nodular lymphocyte-predominant Hodgkin lymphoma; relatively low expression in anaplastic large-cell lymphoma and lymphoblastic neoplasms; not on Reed-Sternberg cells |
| CD45RA | B-cells and subset of T-cells, B-cell lymphomas, nodular lymphocyte-predominant Hodgkin lymphoma |
| CD45RO | Most T-cells, histiocytes, myeloid cells, T-cell lymphomas |
| CD56 | NK-cells and subset of T-cell lymphomas |
| CD57 | Subset of T-cells and NK-cells, subset of T-cell lymphomas |
| CD61 | Megakaryocytes (including dysplastic and neoplastic forms) |
| CD68 | Histiocytes, myeloid cells, mast cells and neoplasms, some non–Hodgkin lymphomas |
| CD79a | Immature and mature B-cells and lymphomas, plasma cells and plasma cell neoplasms |
| CD99 | Lymphoblastic lymphoma/leukemia |
| CD117 | Immature myeloid cells |
| CD138 | Plasma cells, plasma cell lesions |
| CD163 | Histiocytes |

**Table 2.** Flow Cytometry Markers Not Typically Performed in Paraffin Sections

| | |
|---|---|
| FMC-7 | Conformational epitope on CD20; expressed in most B-cell lymphomas except chronic lymphocytic leukemia/small lymphocytic lymphoma |
| CD11c | Hairy cell leukemia, acute myeloid leukemia, some chronic lymphocytic leukemia/small lymphocytic lymphoma |
| CD14 | Macrophages |
| CD19 | B-cells and B-cell lymphomas |
| CD22 | B-cells and B-cell lymphomas |
| CD33 | Cells of monocytic/myeloid lineage |
| CD38 | Activated lymphocytes, subset of cases of chronic lymphocytic leukemia/small lymphocytic lymphoma |
| CD52 | Mature lymphocytes (protein targeted by alemtuzumab) |
| CD103 | Mucosal intraepithelial lymphocytes, enteropathy-associated T-cell lymphoma, hairy cell leukemia |

# 2 NORMAL LYMPH NODE



## STRUCTURE AND CELLS[7,8]

Since the lymph nodes participate in immune reactions, there is no fixed or archetypical normal lymph node architecture; the specific histologic appearance of each lymph node reflects its level of stimulation by antigens. Nonetheless, one can discern major features that are shared by most lymph nodes. Lymph nodes are composed of a cortex, paracortical region, medullary cords, and sinuses, covered by a capsule. Afferent lymphatic and a few afferent blood vessels enter the lymph node at multiple points through the capsule, while most afferent blood vessels and all efferent blood vessels and lymphatics enter and leave through a depressed central area of the capsule called the hilum. Trabeculae composed of fibrosis tissue branch off the capsule to partially penetrate the lymph node parenchyma. The lymph node structure is further supported by a network of fibroblastic reticulum cells. These cells represent a heterogeneous mixture of spindled cells that may express vimentin, desmin, myosin, the isoform of alpha-actin specific for smooth muscle, desmoplakin I, desmoglein, or keratin.

The cortex essentially consists of primary and secondary follicles. Primary follicles are composed predominantly of naive and memory B-lymphocytes expressing a high density of surface immunoglobulin composed of mu and delta heavy chains combined with polytypic light chains (Figure 1). They express bcl-2 protein, but lack CD10 and bcl-6 protein (Figure 2). Secondary follicles contain germinal centers and a mantle zone. The mantle zone is composed of the same B-cells that comprise the primary follicle and may be thick or thin; thin or seemingly absent mantle cells may be particularly common in children. The germinal center consists of a mixture of B-cells, T-cells, antigen-processing cells, and tingible-body macrophages (Figure 3). The B-cells usually predominate and are a mixture of small cleaved and large cleaved cells (centrocytes) as well as large noncleaved cells (centroblasts). They are bcl-2 protein negative and express both CD10 and bcl-6 protein. They have low levels of surface immunoglobulin, but have cytoplasmic immunoglobulin, usually of mu–heavy chain type. The T-cells

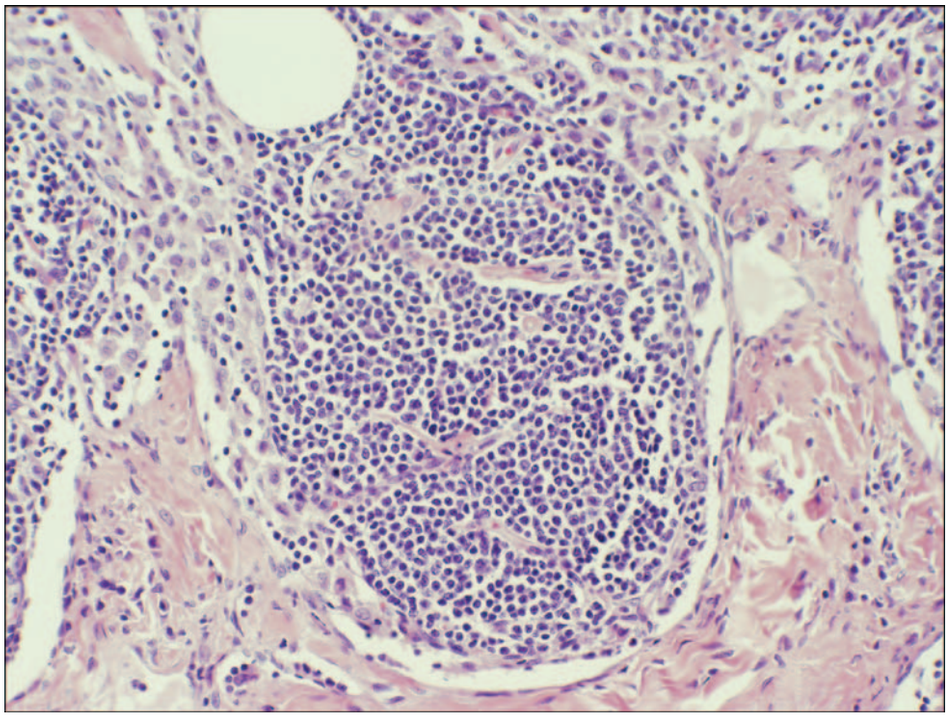

Figure 1. Primary follicle. The follicle consists of a round collection of small mature lymphocytes. No germinal center is seen and small and large cleaved cells are absent.

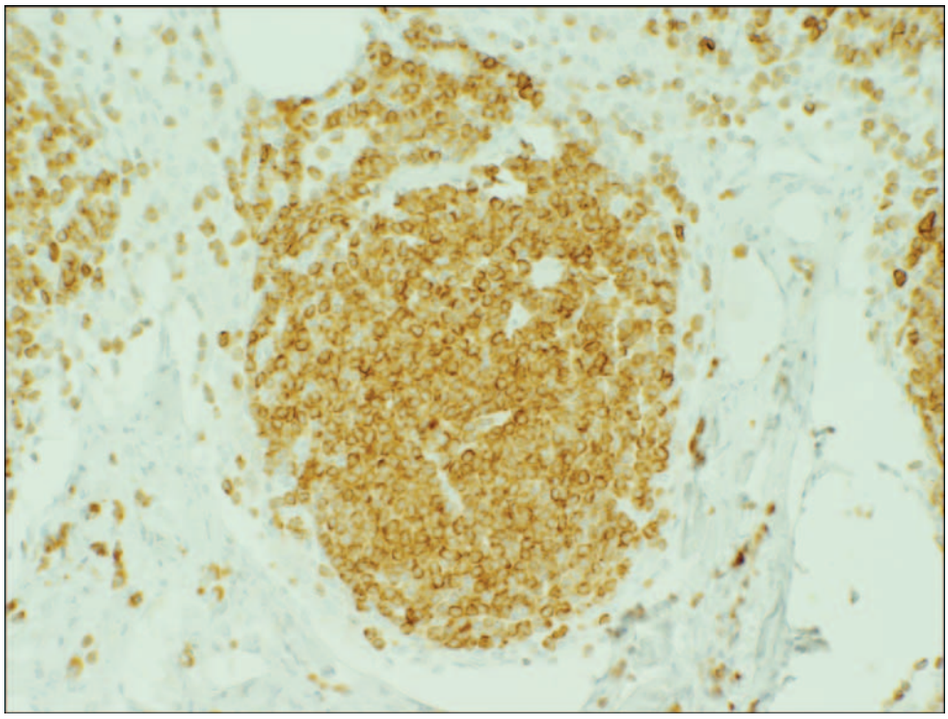

Figure 2. Primary follicle, bcl-2 stain. The cells are bcl-2 positive. This bcl-2 staining of follicles cannot be presumed to be evidence of follicular lymphoma, until the possibility of primary follicles has been eliminated. Primary follicles lack the cleaved cells characteristic of follicular lymphoma.

are CD4-positive/CD8-negative helper/inducer cells, and a subset is CD57 positive. The antigen-processing cells are mostly follicular dendritic cells. These cells express an array of antigens, suggesting that they are a specialized form of myofibroblasts and may derive from bone marrow stromal cell progenitors. They are usually histologically inapparent, although one can identify them by

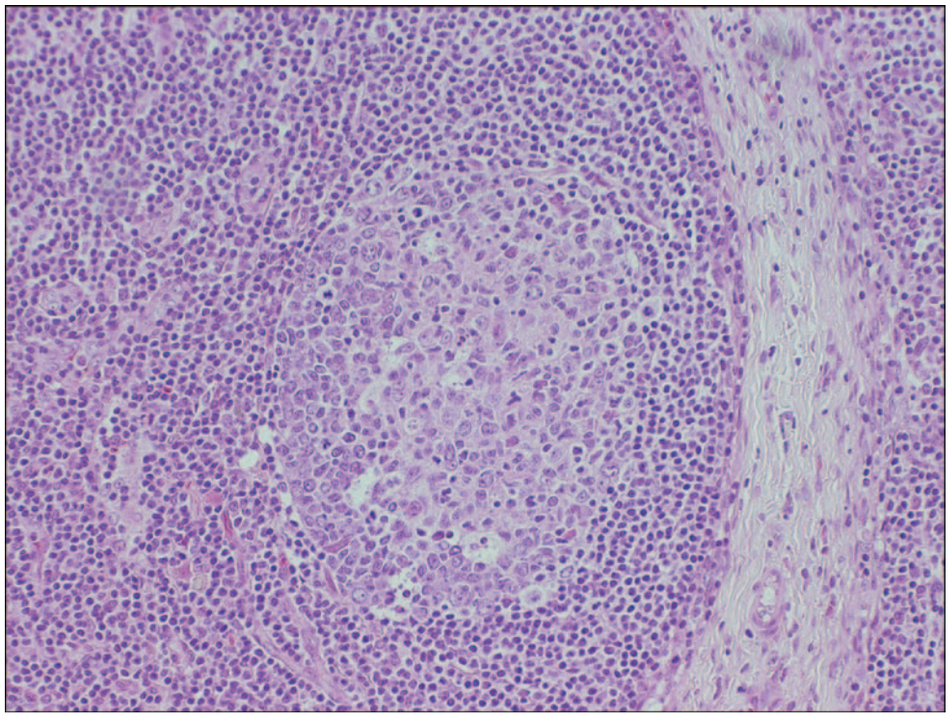

Figure 3. Secondary follicle with germinal center. Notice the polarization, with *light* zone adjacent to the capsule and a *dark* zone with a higher mitotic rate adjacent to the interior of the lymph node. The larger cells predominate in the light zone.

their frequent multinucleation with a relatively fine chromatin pattern with small evident nucleoli and inconspicuous cytoplasm. When highly multinucleated, they have been called polykaryocytes or Warthin-Finkeldey cells (Figure 4). Immunohistochemical studies show expression of CD21, CD35, CD23, and clusterin. In highly stimulated lymph nodes, the germinal centers consist of a light zone (facing the capsule) composed of centrocytes and T-helper/inducer cells and an opposing dark zone (facing the medulla), composed of highly proliferative centroblasts and tingible-body macrophages, so-called polarization. Occasionally, one also sees a marginal zone external to the mantle zone, often best developed in mesenteric lymph nodes. These areas are composed of marginal zone B-cells, cells with nuclear irregularities, and relatively abundant pale cytoplasm.

The paracortical zone varies markedly depending on the degree of immune stimulation. In the unstimulated state, it consists of small mature T-lymphocytes, predominantly of CD4-positive phenotype (Figure 5). It usually comprises the largest area of a stimulated lymph node, consisting of a mixture of T-lymphocytes of both CD4-positive/CD8-negative and CD4-negative/CD8-positive type in widely varying ratios, B-cells, particularly B-immunoblasts, dendritic cells, macrophages, plasma cells, and, depending on the stimulus, eosinophils and plasmacytoid monocyte/dendritic cells (Figure 6). B-immunoblasts are large lymphoid cells, generally with a pale chromatin pattern with a prominent nucleolus and abundant basophilic cytoplasm. The dendritic cells are usually interdigitating dendritic cells (CD1 negative, S-100 protein positive), but may be Langerhans cells (CD1 positive, S-100 protein positive). Plasmacytoid monocytic/dendritic cells are dendritic cells that are slightly larger than resting

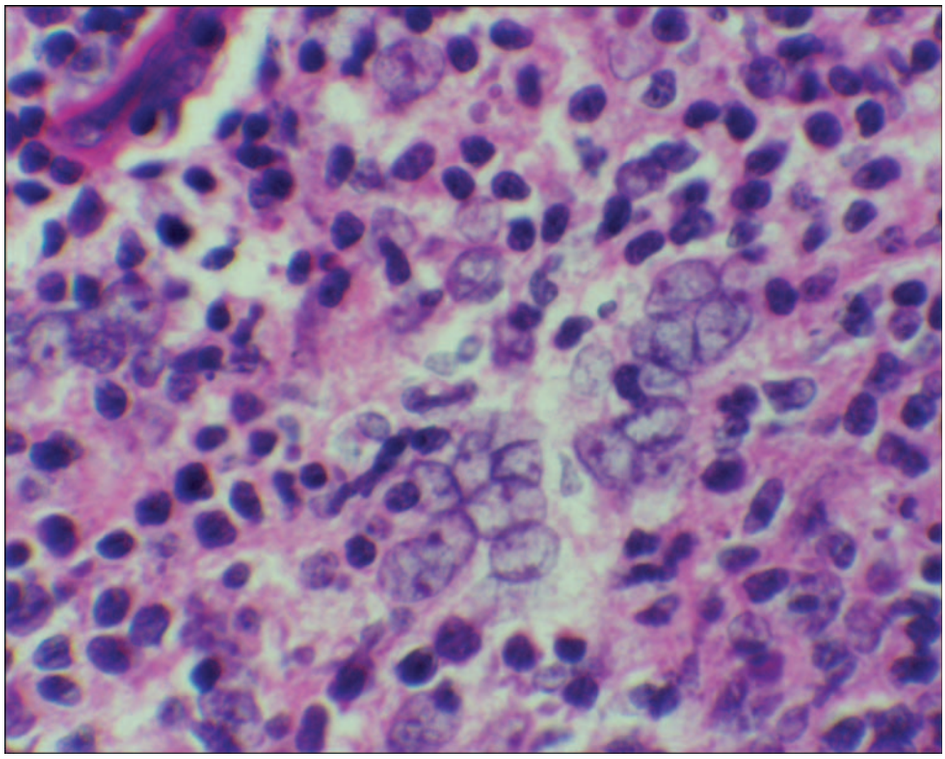

Figure 4. Warthin-Finkeldey cells. The cells have multiple nuclei with a fine chromatin pattern and thin nuclear membranes arranged in a grapelike cluster. The cytoplasm is not discernible.

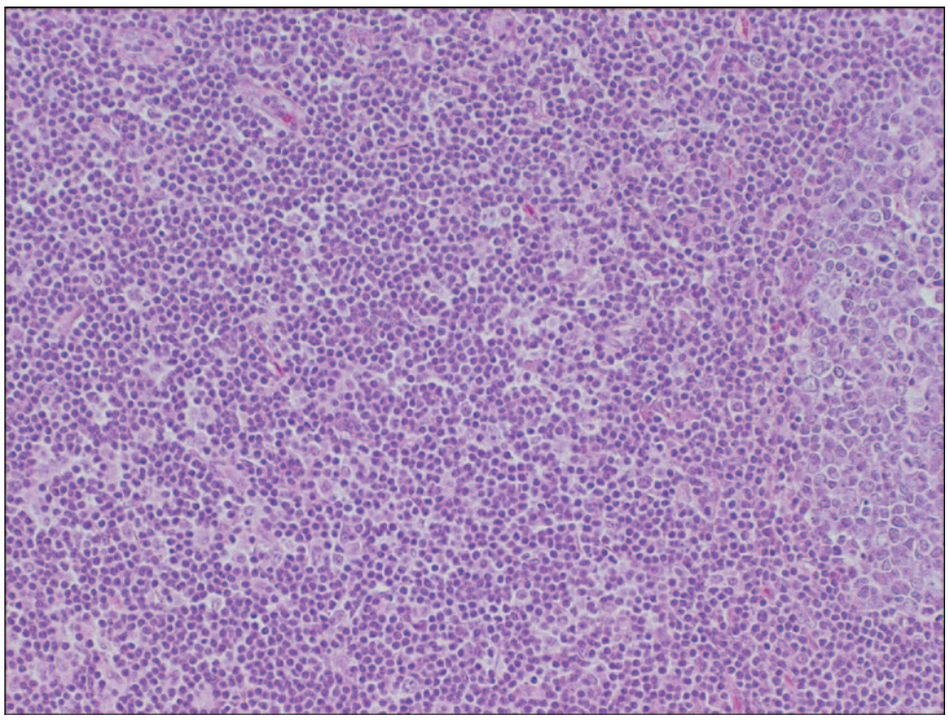

Figure 5. Paracortical region. The paracortical in a typical lymph node consists of a predominance of small mature lymphoid cells, with scattered histiocytes, plasma cells, and large lymphoid cells, with the number depending on the degree of immune stimulation. A germinal center is seen at the right.

lymphocytes, with moderately abundant and well-demarcated eosinophilic to faintly basophilic cytoplasm. The nucleus is round, oval, or slightly indented, with finely dispersed chromatin and one or two small nucleoli. They may be identified on light microscopy when they form clusters or may be identified by

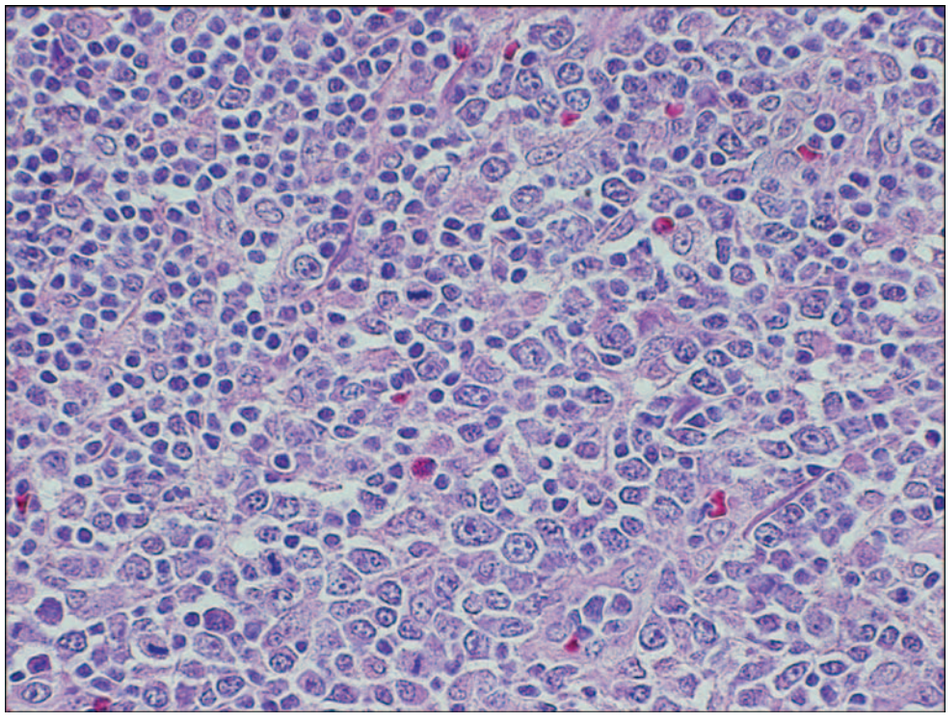

Figure 6. Reactive paracortical hyperplasia. A mottled appearance is imparted by the mixture of small and large cells. There is a mixture of immunoblasts, small lymphocytes, plasma cells, histiocytes, and rare eosinophils in this field. The blood vessels are hard to see, since the nuclei of the high endothelial venules are somewhat vesicular and blend in with the lymphoid cells.

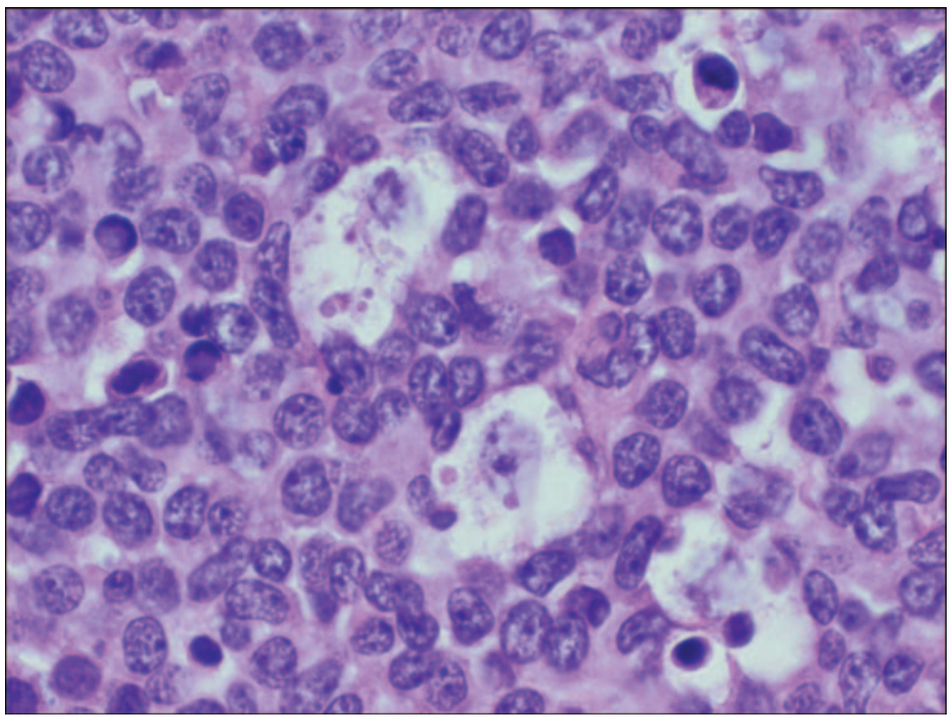

Figure 7. Plasmacytoid dendritic cells. These cells are most easily identified when they are found in clusters. Frequent apoptosis, as seen here, is typical and aids in their identification.

virtue of expression of CD123 (Figure 7). They are probably derived from the lymphoid system and upon stimulation with interleukin (IL)-3 mature into a subset of interdigitating cells in the paracortex. Another feature of the paracortical zone is the presence of high endothelial venules. These are venules lined by cuboidal rather than the typically flattened endothelial cells and have fairly

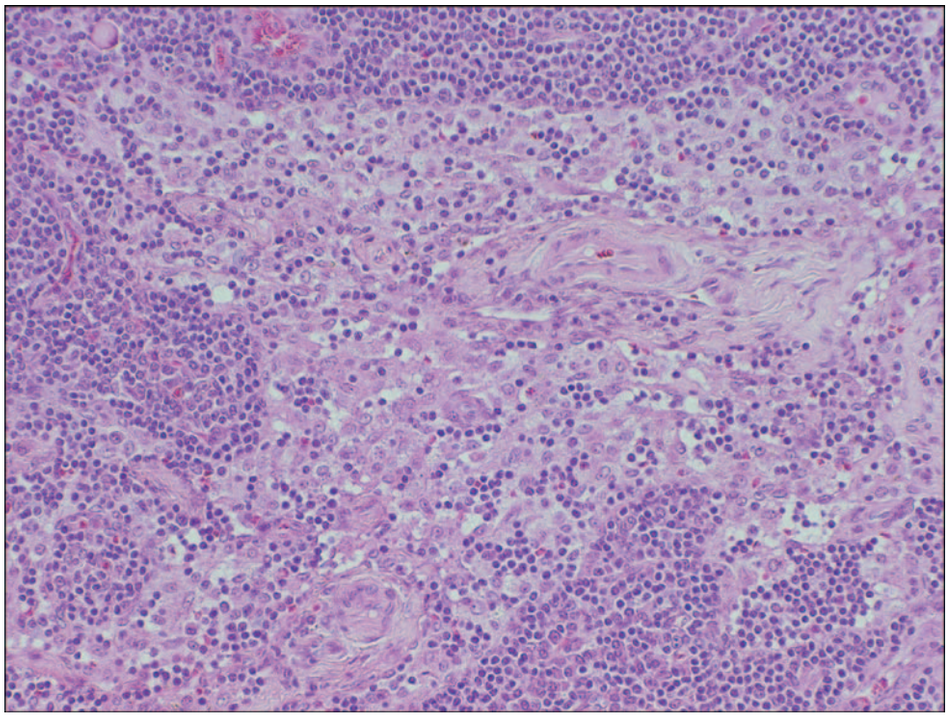

Figure 8. Medullary sinus, with adjacent medullary cords. The sinuses contain loose collections of histiocytes, small lymphocytes, and plasma cells. The cords contain small lymphocytes and plasma cells.

large nuclei. They possess an unusually abundant amount of cytoplasm that appears to almost completely obliterate the vessel lumen.

The sinuses of a lymph node include the subcapsular sinuses, which receive lymph from afferent vessels, and the medullary sinuses, which deliver lymph to the efferent lymphatics (Figure 8). The most frequent cellular component of the sinuses is the sinus histiocyte. Occasionally, the sinuses are filled with collections of monocytoid B-cells. These cells are B-lineage cells that are bcl-2 negative and have nuclear irregularities and a more abundant cytoplasm than naive B-cells (and even more than marginal zone B-cells); there are often admixed neutrophils (Figure 9). The medullary cords are thin to thick cords of lymphoid cells, often with admixed plasma cells, present between the medullary sinuses.

## TRAFFICKING AND THE IMMUNE RESPONSE[9]

T-lymphocytes and, to a lesser extent, B-lymphocytes enter the lymph node via the high endothelial venules, utilizing L-selectin (CD62L) receptors concentrated on lymphocyte microvilli. B-cells may also enter via the subcapsular sinuses, as monocytoid B-cells. Many of the B-cells migrate to primary follicles and the mantle zones of secondary follicles. Antigen enters the lymph node via the afferent lymphatics. Although most antigens are ingested by macrophages within the sinuses, some enter the paracortical and cortical regions and become retained on the cell processes of interdigitating and follicular dendritic cells, respectively. Interdigitating and follicular dendritic cells present these antigens to T-cells and B-cells (in association with class I and II major histocompatibility