21 Lymph Node

Normal Histology

The normal or benign lymph node is composed of a collection of follicles and interfollicular areas surrounded by sinuses (spaces mostly filled with histiocytes), vessels, and sometimes fat. Lymph flows from the subcapsular sinuses, through the medullary sinuses in the lymph node, and out the hilum. The follicles represent areas of maturing B cells ($CD20^+$), whereas the interfollicular areas are mostly mature T cells ($CD3^+$).

Follicles begin as primary follicles, or aggregates of antigen-naïve B cells. As they mature into secondary follicles they acquire germinal centers, which are visible as targetoid nodules within the follicles (Figure 21.1). The dark outer rim of the follicle is the displaced remains of the primary follicle and is called the *mantle zone*, still composed of antigen-naïve B cells. Once exposed to antigen, the B cells move to the germinal center and become centroblasts, large cells with primitive-looking nuclei. From there they either mature into centrocytes or die through apoptosis. Finally, B cells leave the germinal center genetically altered to circulate as memory B cells, which may ultimately differentiate into plasma cells if they meet their antigen.

Other normal germinal center components include the supporting follicular dendritic cells and tingible body macrophages, which clean up the apoptotic debris. These appear as relatively clear cells within the germinal center, with visible "dust specks" in the cytoplasm (Figure 21.2). Germinal centers may be found in any hotbed of lymphocyte activity outside the lymph nodes, but the morphology and staining pattern are preserved.

The paracortex, or interfollicular area, may occupy most of the lymph node in some cases. This absence of obvious follicles is not necessarily a reason for concern. The benign paracortex should have a mottled appearance due to the scattered pale histiocytes among the T cells.

Lymphoma, Conceptually

The word *lymphoma* means a malignancy of the lymphoid system and usually implies a solid tumor mass. However, remember that many of the lymphomas can have an associated leukemia, which is when the neoplastic cells invade the bone marrow and become circulating (Table 21.1). Among the myeloid leukemias, discussed in Chapter 20, solid tumor disease is uncommon but exists (such as the chloroma, or granulocytic sarcoma).

This chapter covers several major categories of lymphomas:

- Lymphoblastic (neoplasms of precursor cells, or lymphoblasts): These cells resemble myeloblasts and are the solid tumor counterpart to the acute lymphoblastic leukemias.

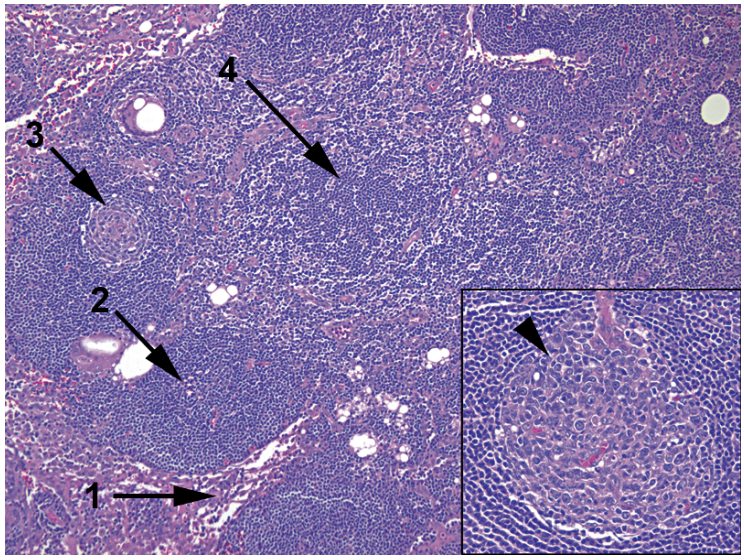

Figure 21.1. Normal lymph node. The sinuses are visible mainly as loose collections of histiocytes (1). Primary follicles (2) are collections of B cells lacking germinal centers. Secondary follicles contain germinal centers (3). The space between the follicles, or paracortex (4), is composed of T cells and shows a characteristic spotted or mottled appearance. **Inset**: A normal germinal center should be polarized, with large centroblasts clustered at one side of the follicle (arrowhead), creating a lopsided appearance.

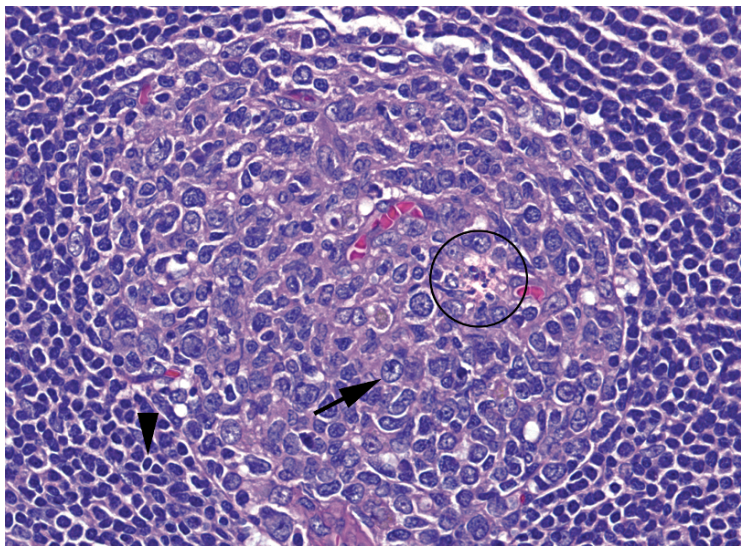

Figure 21.2. Germinal center. Other components of a benign germinal center include tingible body macrophages (circle) and large centroblasts with prominent nucleoli (arrow). The germinal center is surrounded by the mature B cells of the mantle zone (arrowhead).

- Low-grade B cell lymphomas (neoplasms of mature B cells): Like the chronic leukemias, these are indolent and simmering and are diseases of adults. They have a low mitotic rate and are therefore not susceptible to most chemotherapy.
- High-grade (aggressive) lymphomas (neoplasms of activated B cells and T cells [activated means exposed to the target antigen] or that resemble activated cells): These have a high mitotic rate but are therefore potentially curable through chemotherapy.

Table 21.1. Lymphomas and associated leukemias.

	Myeloid line	Lymphoid line	
Cell of origin	Leukemias	Lymphomas	Leukemias
Blasts	Acute myeloid leukemia, most types	Pre-B lymphoblastic lymphoma[1,2] T lymphoblastic lymphoma[1]	Pre-B lymphoblastic leukemia[1,2] T lymphoblastic leukemia[1]
Immature precursors	Acute promyelocytic leukemia	Burkitt's lymphoma[2]	
Mature cells	Chronic myeloid leukemia and myeloproliferative disorders	Low-grade lymphomas: Follicular[2] Small lymphocytic[3] Mantle cell[3] Marginal zone Lymphoplasmacytic Mycosis fungoides	Chronic lymphocytic leukemia[3] Myeloma T-cell large granular lymphocytic leukemia Sezary syndrome
Activated cells		Diffuse large B cell[4] Anaplastic large cell[4] Hodgkin's lymphoma[4]	Prolymphocytic leukemia

Markers go in batches: [1]TdT, CD34+; [2]CD10+; [3]CD5+; [4]CD30+.

- Hodgkin's lymphomas: As a group, these are neoplasms in which the neoplastic cells are a minority population, with a variable mixed inflammatory background. The prototypical tumor cell is the Reed-Sternberg cell, of which there are many variants.
- Plasmacytoma: Plasmacytomas are neoplasms of plasma cells
- Other: Other lymphomas include T-cell neoplasms and non-B, non-T cell types.

Many of the lymphomas can be placed into categories by nuclear morphology, and learning to recognize the "look" of each group is important. The lymphoblastic lymphomas have immature chromatin, which means the texture is very fine grained, with small nucleoli and an indistinct nuclear membrane, much like the myeloblasts in acute myeloid leukemia. On H&E stain, they may be mistaken for small cell carcinoma. The low-grade neoplasms resemble normal lymphocytes, with small condensed nuclei. The high-grade lymphomas show very carcinoma-like nuclei: they are large (compared with lymphocytes) and pleomorphic, with prominent nucleoli and coarse nuclear membranes. Hodgkin's lymphomas are the hardest to identify, usually, as the diagnostic cells (Reed-Sternberg cells and variants) may be few and far between. However, they do resemble the high-grade lymphoma nuclei in terms of the chromatin pattern.

Recognizing a lymphoma in an extranodal site, especially a tumor of unknown origin, takes practice. Clues to lymphoma include a relatively homogeneous, sheet-like growth of malignant cells; a lack of cell-to-cell cohesiveness or architecture; nuclei that are highly irregular in shape or contour; and an accentuation of cell density around vessels (especially in the central nervous system; Figure 21.3). Most should stain for CD45, the common leukocyte antigen, or for specific B or T markers. Positive staining for melanoma markers or cytokeratins rules out lymphoma. Sarcoma markers should be used with caution, though, as many of the familiar stains (CD117, CD34, etc.) also stain hematopoietic elements.

Diffuse large B-cell lymphoma

As the most common lymphoma, you will see diffuse large B-cell lymphoma (DLBCL) frequently. DLBCL is essentially a final common pathway in lymphoma; although it can arise spontaneously, it can also arise from the setting of any other low-grade B-cell lymphoma or from Hodgkin's lymphoma. The "diffuse" is used here as an opposite of follicular or nodular, and it implies sheet-like growth. The "large" should be interpreted with caution—what is large in hematopathology may still be fairly small next to a squamous cell.

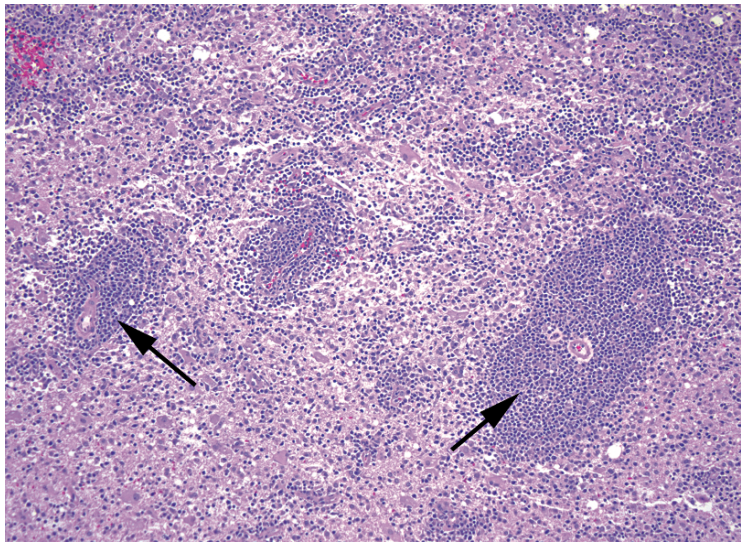

Figure 21.3. Diffuse large B-cell lymphoma in the central nervous system. The tendency of the malignant cells to cluster around blood vessels (arrows) is typical of lymphoma within the brain.

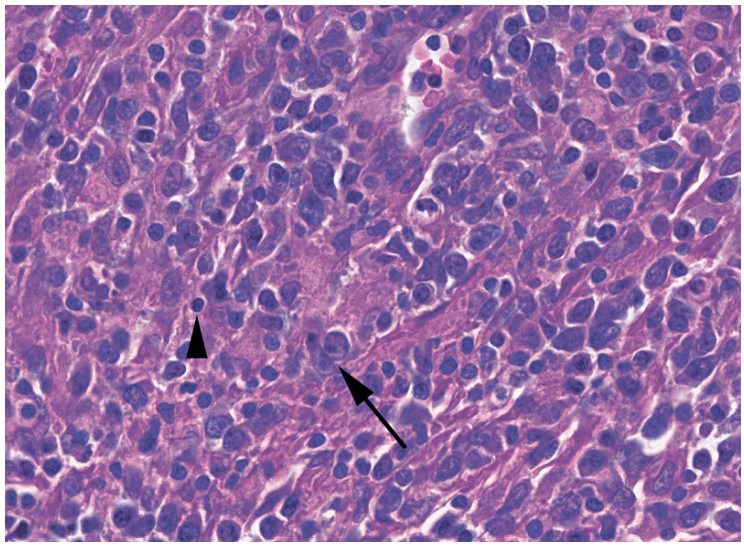

Figure 21.4. Diffuse large B-cell lymphoma. The usual appearance is that of sheets of discohesive cells that do not form any recognizable architectural patterns (such as glands or trabeculae). The cells typically have large nuclei, irregular and prominent nuclear membranes, and nucleoli (arrow). Compare the cell size to a background lymphocyte (arrowhead).

Diffuse large B-cell lymphoma is not usually mistaken for a benign entity; the nuclei are too abnormal. However, it may be mistaken for other types of malignancy, especially given its tendency to crop up in extranodal sites. As described earlier, the nuclei are very irregular in contour, with cleared-out or vesicular chromatin leaving a prominent nucleolus and thick nuclear rim (Figure 21.4). Folded, or cleaved, nuclei are common. The cells may have more cytoplasm than lymphocytes, and therefore a lower nuclear/cytoplasmic ratio.

Anaplastic large cell lymphoma is the T-cell equivalent of DLBCL. It is known for having even more elaborately folded nuclei, described as *cerebriform*, but still must be differentiated by T-specific markers.

Although at the time of this writing, DLBCL is largely a single category in the World Health Organization classification, the splitters are gaining on it. One emerging division is between those DLBCLs that are of germinal center cell origin, such as follicular lymphoma gone bad, and those of activated B-cell origin. The latter have the worse prognosis.

Follicular Lymphoma

Follicular lymphoma is the second-most common non-Hodgkin's lymphoma. Together DLBCL and follicular lymphoma account for over half of the non-Hodgkin's lymphomas. Follicular lymphoma is defined by a translocation in which bcl-2 (an anti-apoptotic factor) is abnormally upregulated. bcl-2 usually turns *off* in germinal centers, making the centroblasts and centrocytes susceptible to apoptosis. Abnormal retention of bcl-2 leads to cells that do not die, more or less, hence the malignancy. Follicular lymphoma appears as a nodular proliferation of back-to-back neoplastic follicles that fill the lymph node (Figures 21.5 and 21.6). Within these follicles are a mixture of neoplastic centrocytes (smaller) and centroblasts (larger); the relative proportion determines the grade of the lymphoma. Follicular lymphoma can also have areas of diffuse growth (the opposite of nodular), can spread to the marrow, and can transform to DLBCL. When circulating as a leukemia, the folded (cleaved) centrocyte nucleus has been compared to a "baby's butt."

The diagnostic dilemma is that benign, reactive lymphoid hyperplasia can also present as a nodular collection of follicles in an enlarged node. How to distinguish the two? The following are features of *benign follicular hyperplasia*, not seen in follicular lymphoma (see Figures 21.1 and 21.2):

- Germinal centers of variable sizes and cuffed by mantle zones (as opposed to back to back)
- Polarity of germinal centers in which the centroblasts and centrocytes tend to take up opposite positions in the follicle, creating an asymmetry
- Tingible body macrophages
- "Open" sinuses (which are not seen as open, really, but full of histiocytes)
- Abundant mitoses and apoptoses

These features should weed out the most straightforward cases. However, in very tough cases, stains help. The neoplastic follicles of follicular lymphoma can be stained with bcl-2; benign follicles should be negative.

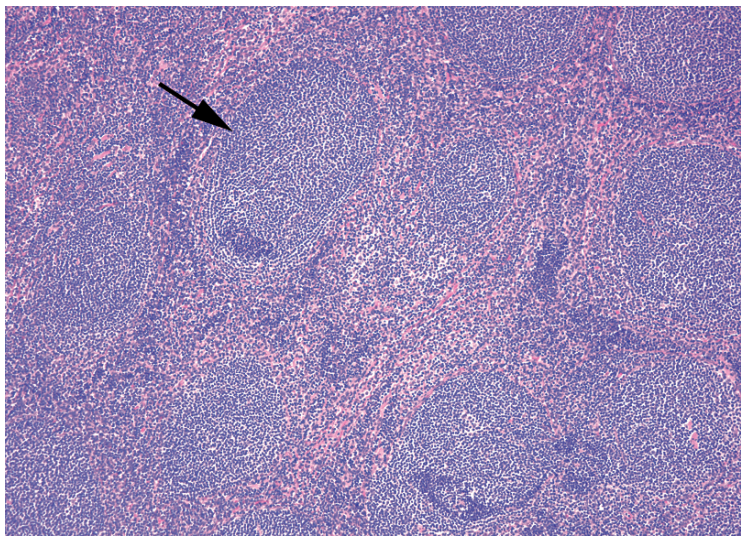

Figure 21.5. Follicular lymphoma. The lymph node is replaced by malignant follicles (arrows), which lack the mantle zones, polarization, and cell heterogeneity of germinal centers.

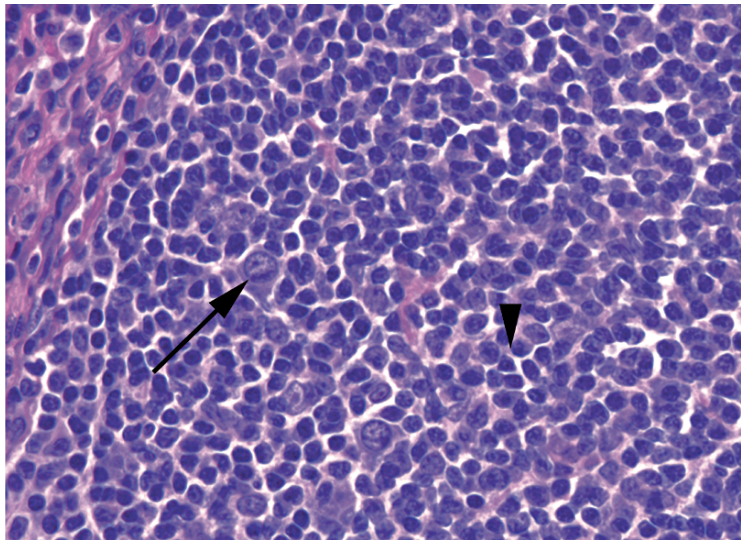

Figure 21.6. Follicular lymphoma, high power. The malignant follicles contain a mixture of small cleaved centrocytes (arrowhead) and large centroblasts (arrow).

Other Low-Grade B-Cell Neoplasms

The other three most common low-grade lymphomas, small lymphocytic lymphoma (SLL), mantle cell lymphoma (MCL), and marginal zone lymphoma (MZL), each make up less than 10% of non-Hodgkin's lymphomas. For these, and actually for most non-Hodgkin's lymphomas, flow cytometry is critical in the diagnosis. Flow cytometry can establish two things:

1. That there is a monoclonal population present. All B cells express either kappa or lambda light chain, so a significant predominance of one or the other implies a large genetically identical population (a neoplasm). A similar test can be done for T-cell neoplasms (T-cell receptor rearrangement studies) but not by flow cytometry.
2. That there are cells with an abnormal phenotype. The power of flow cytometry is that individual cells can be simultaneously tested for multiple markers, for instance, abnormal coexpression of CD20 and CD5. Doing this with immunohistochemistry is much less precise; you can estimate that the $CD5^+$ cells outnumber the normal T cells (identified by CD3) and that they are clustered in areas of B cells (identified by CD20), but you cannot see two markers on a single cell.

The interpretation of flow cytometry is beyond the scope of this chapter, but the major learning point is that saving tissue for flow cytometry will make your life much easier, so set some aside in any lymphadenopathy workup or possible extranodal lymphoma. Formalinized (fixed) tissue cannot be sent for flow cytometric studies.

In the low-grade B cell lymphomas, the low-power feature that rings alarm bells is the *effacement of the lymph node*. This means that the normal architecture, the follicles and sinuses and interfollicular areas, have been blurred out or replaced by a rather uniform population of cells. This takes some experience to judge; fortunately, every carcinoma resection comes with some bonus lymph nodes, so take the time to notice what normal looks like.

Small Lymphocytic Lymphoma

Small lymphocytic lymphoma is the solid-phase manifestation of chronic lymphocytic leukemia, and the two are often seen in concert. In lymphoma form, SLL appears at 1× as a

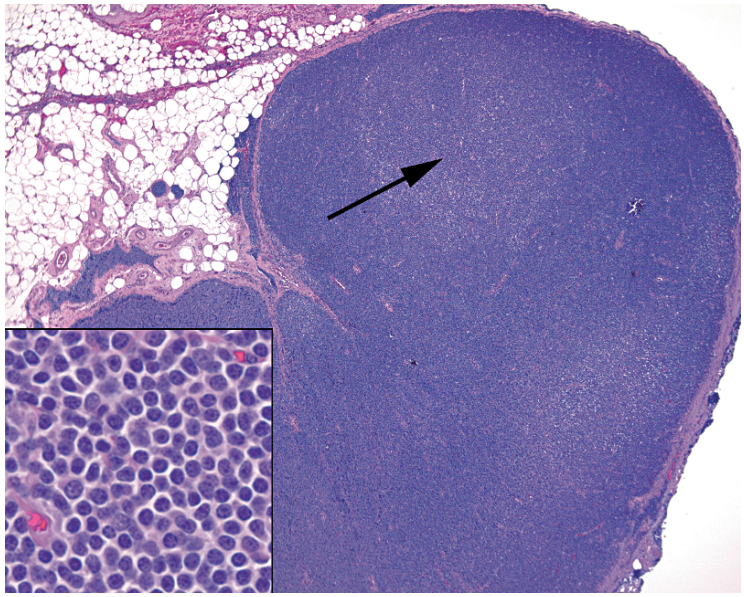

Figure 21.7. Small lymphocytic lymphoma. The lymph node, at low power, is an unnatural flat blue, without the variegation of normal sinuses and follicles. Subtle pale pseudofollicles (arrow) may be seen. **Inset**: The cells are small and nuclei are round and dense, like normal lymphocytes, except the chromatin has a chunky soccer-ball pattern, similar to a plasma cell.

very homogeneous, very blue lymph node. At low power, the follicles, paracortex, and sinuses are replaced by a sheet of what look like normal lymphocytes. There may be a vague suggestion of nodularity, called *pseudofollicles*, containing proliferating cells (Figure 21.7). On high power, the SLL cells usually have chromatin that may remind you of a plasma cell; they look like soccer balls. The nuclei are small, round, regular, and without nucleoli. Small lymphocytic lymphoma cells express CD23 and CD5.

Mantle Cell Lymphoma

Mantle cell lymphoma, although it is in the histologic differential diagnosis for the low-grade lymphomas, actually behaves more aggressively than the others in this group. On low power it is reminiscent of SLL, with sheets of small lymphocytes effacing the node. In a not-entirely-replaced node you may be able to tell that the mantle zones are expanding to engulf the germinal centers. Hyalinized vessels are typical. On high power, the cells of MCL have a chunky dark chromatin similar to the cells of SLL, but the nuclear membranes are more crinkled or angular, with more size variation (Figure 21.8). Cyclin D1 is the marker for MCL, which correlates with the translocation that defines the tumor.

Marginal Zone Lymphoma

The marginal zone of the lymph nodes is named for the more prominent and identifiable marginal zone in the spleen. In the lymph nodes, it is barely visible as a slightly attenuated zone surrounding the mantle. The cells in this zone have a prominent rim of clear cytoplasm, giving them almost a fried-egg appearance, and a pale look at low power. This morphology is called *monocytoid*. This cell type can give rise to at least three distinct lymphomas: splenic MZL (not discussed here), nodal MZL, and extranodal mucosa-associated lymphoid tissue (MALT) lymphoma of gut, salivary glands, and so forth.

MALT lymphomas are discussed in Chapter 7. Like MALT lymphomas, the cells of MZLs are monocytoid in appearance and grow in sheets or clusters, mainly in the interfollicular

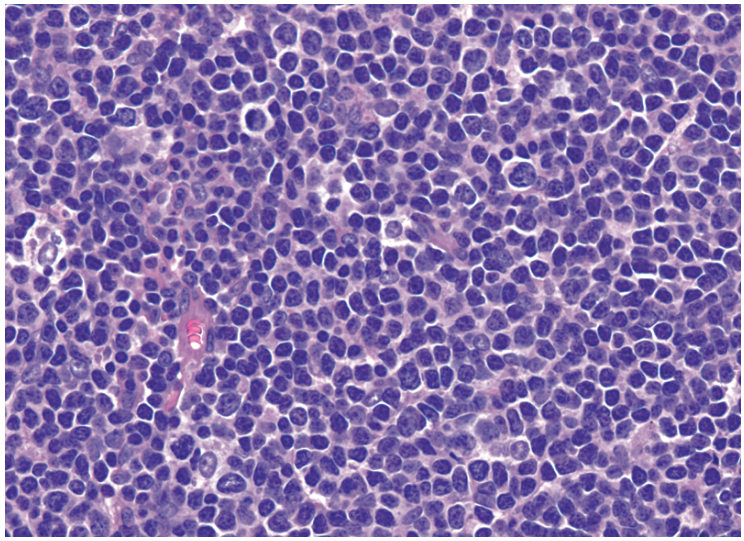

Figure 21.8. Mantle cell lymphoma. The neoplastic cells in mantle cell tend to be more irregular in shape than those of small lymphocytic lymphoma, with slightly angular nuclei. The chromatin pattern, with the soccer-ball splotches, is similar to small lymphocytic lymphoma.

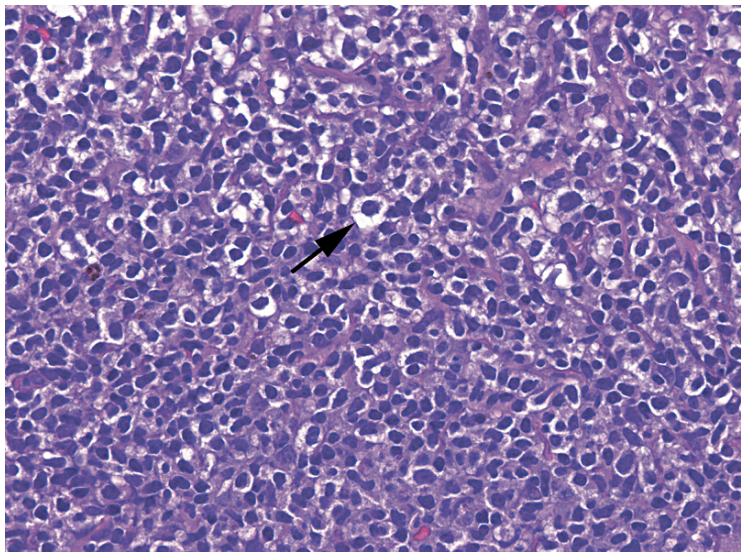

Figure 21.9. Marginal zone lymphoma. The marginal zone cells classically have a monocytoid appearance, meaning there is a distinct thin halo of clear cytoplasm (arrow).

areas (Figure 21.9). They are negative for most of the markers that identify other lymphomas but may sometimes abnormally express CD43.

Markers

Although the low-grade B-cell lymphomas do have histologic features that distinguish them, few pathologists would sign them out without confirming flow cytometry or immunostain studies. Although this book does not otherwise focus on immunostains, it is impossible to discuss the lymphomas without them. The standard panel includes CDs 3 and 20, 5 and 10, and 43, as well as some specific markers discussed earlier. CD3 identifies T cells, and

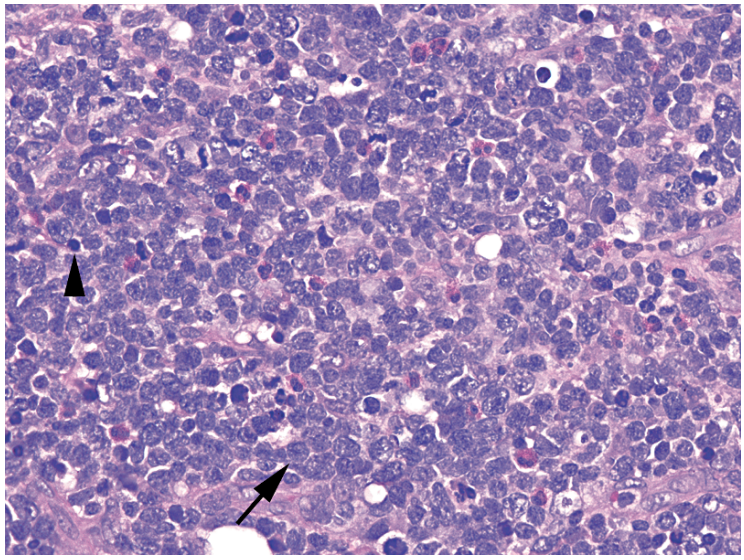

FIGURE 21.10. Acute lymphoblastic lymphoma. The nuclei (arrow) are larger than a normal lymphocyte (arrowhead), and the chromatin is very immature (meaning widely dispersed throughout the nucleus). Unlike large B-cell lymphoma, there are no prominent nucleoli or thick nuclear membranes.

CD20 identifies B cells. The expression of either CD5 or CD43, (T-cell markers), or CD10, (an immature B-cell/germinal center marker), in mature B cells is abnormal, and guides your differential.

Lymphoblastic Lymphoma

The lymphoblastic lymphomas are not usually diagnosed in lymph nodes. The precursor-B type more commonly presents as a leukemia (acute lymphoblastic leukemia), whereas the precursor-T type is most often seen as a mediastinal mass (remember the immature T cells are found in the thymus; Figure 21.10). Burkitt's lymphoma is also in this category, and the cells are similar in appearance; it may present anywhere, especially in the gut. Most acute lymphoblastic leukemias are now defined by cytogenetics, but they also stain for the blast markers TdT and CD34 (Burkitt's lymphoma not included). As CD45, the usual screening immunostain, is not reliably expressed in these tumors, be aware that a negative CD45 in a pediatric small round blue cell tumor may still be lymphoma. Finally, what looks like a low-grade B-cell lymphoma in a child is much more likely to be a lymphoblastic lymphoma.

Hodgkin's Lymphoma

Hodgkin's lymphoma is common, comprising 30%–40% of all lymphomas. It is now divided into two large groups, classic (most types) versus nodular lymphocyte predominant (NLPHL). Both groups share the histologic features of a dense and effacing mixed inflammatory infiltrate with scattered Reed-Sternberg (or Reed-Sternberg–like) cells. Because of the high benign background population, flow cytometry is not effective in detecting Hodgkin's lymphoma. Making the diagnosis requires either seeing the diagnostic tumor cells or demonstrating them by immunostain. In classic Hodgkin's lymphoma, the Reed-Sternberg cells are CD30 and CD15 positive while negative for CD45 and CD20. In NLPHL, the tumor cells stain exactly the opposite ($45/20^{+}$, $30/15^{-}$). In this sense, NLPHL is really analogous to a DLBCL with an associated inflammatory response.

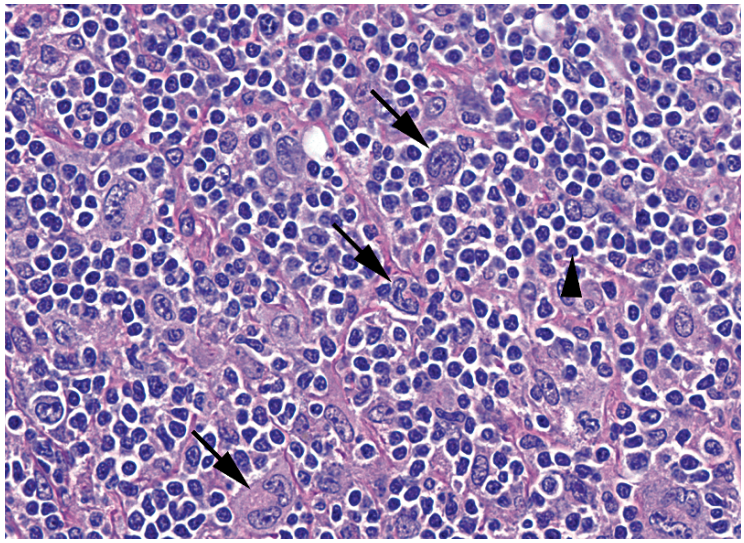

Figure 21.11. Hodgkin's lymphoma. The malignant Reed-Sternberg cells (arrows) are spread out among a background of nonneoplastic inflammatory cells, especially lymphocytes (arrowhead).

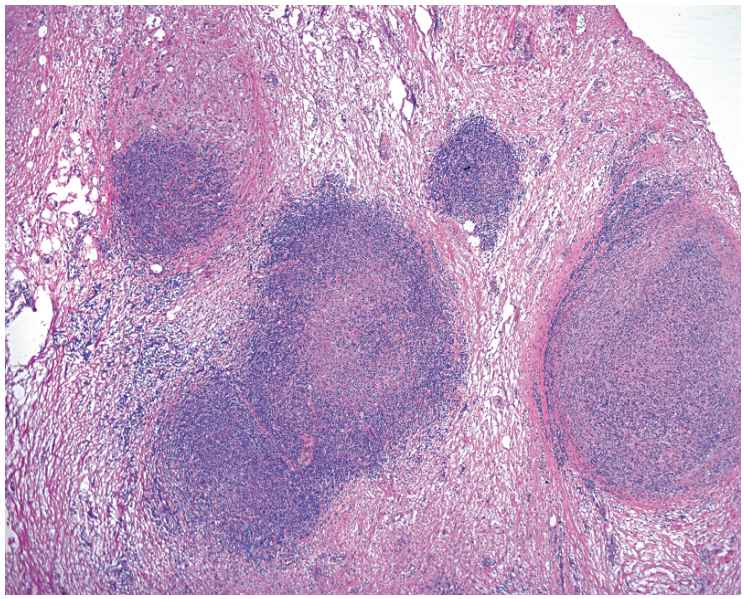

Figure 21.12. Nodular sclerosing Hodgkin's lymphoma. The aggregates of Reed-Sternberg cells and inflammation are separated by broad bands of fibrosis.

The subtypes of classic Hodgkin's lymphoma include nodular sclerosing, mixed cellularity, and the less common lymphocyte depleted and lymphocyte rich. All should have some variety of the Reed-Sternberg cells, which in classic form have at least two nuclear lobes, each with a prominent, cherry-red nucleolus and thick nuclear membrane (Figure 21.11). However, variants with single or multilobed nuclei may be seen.

In *nodular sclerosing Hodgkin's lymphoma*, at low power, the node is "cirrhotic," with nodules of mixed inflammation divided by broad fibrous bands (Figure 21.12). The node is usually also encapsulated. The Reed-Sternberg cells take the form of lacunar cells, which means the diagnostic nuclei are suspended in a retracted space or halo.

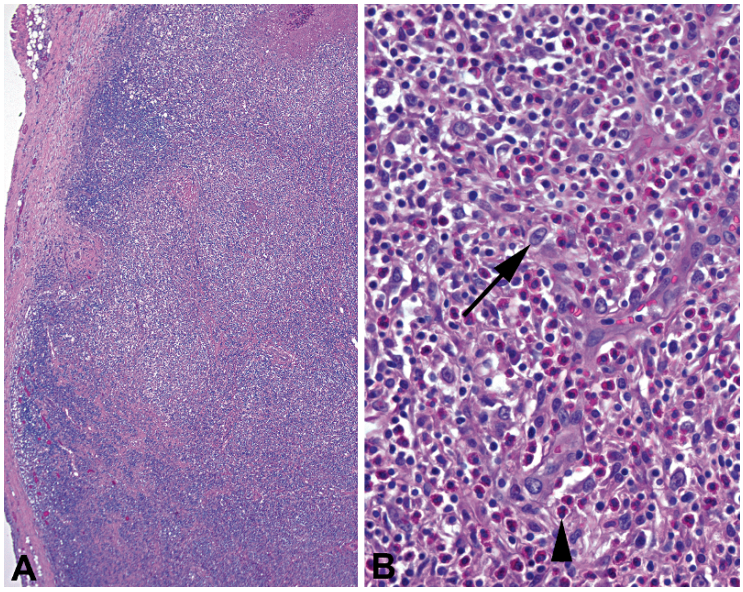

FIGURE 21.13. Mixed cellularity Hodgkin's lymphoma. **(A)** At low power, the lymph node appears to be effaced by a heterogeneous population, giving a slightly pink color to the node (compare to small lymphocytic lymphoma in Figure 21.7). **(B)** The Reed-Sternberg variants are few and far between (arrow), with a dominant population of eosinophils (arrowhead).

At low power, *mixed cellularity Hodgkin's lymphoma* appears "pink" because of the abundant histiocytes and eosinophils found in the background infiltrate (Figure 21.13). Plasma cells and lymphocytes are also common. Very subtle cases may present as granulomatous inflammation.

T-Cell Lymphomas

The incidence of T-cell neoplasms is much lower than that of B cells. Precursor T-lymphoblastic lymphoma has been discussed, as has anaplastic large cell lymphoma. The cutaneous T-cell neoplasms include mycosis fungoides/Sezary syndrome (the solid and circulating phases, respectively) and primary cutaneous anaplastic large cell lymphoma. Of those that may present in a lymph node, peripheral T-cell lymphoma, unspecified, is the most common.

Nonneoplastic Entities

Inflammation in a lymph node? It is only abnormal if it is granulomatous, acute, or necrotizing. Granulomatous inflammation may be nonnecrotizing in sarcoid or caseating in tuberculosis or fungal infection. Both of the latter should have positive findings on bug stains. Other infectious entities include infectious mononucleosis and cytomegalovirus, which can both cause dramatic follicular hyperplasia; cat scratch disease, causing an acute lymphadenitis with neutrophils; and *Toxoplasma*, which causes a follicular hyperplasia with ill-defined granulomatous inflammation. An unusual disease, called *Kikuchi's lymphadenitis*, resembles a granulomatous response, with large swaths of geographic necrosis, but on high power the necrotic areas are paradoxically devoid of neutrophils, showing only apoptotic nuclear debris.

In summary, if you see…	Think of…
Diffuse sheet of small lymphocytes	Small lymphocytic, mantle cell, or marginal zone lymphoma
Prominent nodular pattern	Follicular lymphoma
Diffuse sheet of large atypical cells	Diffuse large B-cell lymphoma
Mitotically active cells resembling small cell carcinoma	Lymphoblastic lymphoma
A pink and/or granulomatous mixed infiltrate	Mixed cellularity Hodgkin's lymphoma
Fibrous bands dividing the node	Nodular sclerosing Hodgkin's lymphoma

Knowledge of the pathway of B cells through the germinal centers, and the various markers that switch on and off, is very helpful in understanding the various B-cell neoplasms that arise from the different stages of maturation. The introductory chapter on mature B-cell neoplasms in the World Health Organization's textbook *Tumours of Haematopoietic and Lymphoid Tissues* is a very good place to start.