Hematopathology / Follicular Hyperplasia, Follicular Lysis, and Progressive Transformation of Germinal Centers

Follicular Hyperplasia, Follicular Lysis, and Progressive Transformation of Germinal Centers

A Sequential Spectrum of Morphologic Evolution in Lymphoid Hyperplasia

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Abstract

We studied mantle B-cell and T-cell ingression in hyperplastic follicles (HFs), follicular lysis (FL), and progressive transformation of germinal centers (PTGC) in 19 paraffin-embedded, H&E-, bcl-2–, CD20–, and CD3-stained lymph nodes. We enumerated the T cells (CD3+) and mantle B cells (bcl-2+/CD3–) per 100 cells in 5 high-power fields of each entity (mean ± SD). Compared with HF, FL had increased numbers of T cells migrating into germinal centers (39.8 ± 10.0 vs 25.8 ± 7.8; P < .0001) and a mild increase of mantle B cells (12.3 ± 11.4 vs 2.1 ± 1.6; P < .001). PTGC showed an increase of T-cell ingression compared with HF (36.5 ± 12.1 vs 25.8 ± 7.8; P < .0001) and more migration of mantle B cells into the follicle than FL (41.0 ± 22.5 vs 12.3 ± 11.4; P < .0001).

T cells and mantle B cells ingress in FL and PTGC, although the mantle B-cell component predominates in the latter, suggesting that follicular hyperplasia, FL, and PTGC constitute an evolutionary spectrum in resolution of lymphoid hyperplasia with sequential ingression of T cells followed by mantle B cells. The maintenance of bcl-2 expression in mantle B cells in PTGC may cause differential diagnostic pitfalls in florid PTGC vs follicular lymphoma, particularly the so-called floral variant.

Progressive transformation of germinal centers (PTGC) is a morphologic feature of reactive hyperplasia initially described by Lennert and Müller-Hermelink1 as “germinal centers that are lost in a mass of lymphocytes.” PTGC is seen most commonly in lymph nodes in association with reactive follicular hyperplasia.2-4 Overall, 3.5% to 10% of reactive lymph nodes with chronic nonspecific lymphadenitis will contain 1 or more areas of PTGC.2-4 Persistent or recurring PTGC can occur, particularly in pediatric cases (up to 50%), and usually is located in the same lymph node region.4,5 PTGC also may occur as a predominant pattern in lymph node hyperplasia, designated as florid PTGC. This manifestation is described classically in children or in young males in their second to third decade of life who have an asymptomatic, solitary, enlarged lymph node (cervical most frequent followed by inguinal and then axillary).5-7 PTGC also has been described in association with Hodgkin disease. It has been reported that 16% to 20% of cases of PTGC are associated with Hodgkin disease, mostly lymphocyte predominant Hodgkin disease (LPHD).5-7 Poppema8 and Poppema et al9 suggested an association between PTGC and LPHD based on similar morphologic features and the observation that both entities may occur together in the same lymph node. PTGC may coexist or precede LPHD or be present in subsequent lymph node biopsy specimens following a diagnosis of lymphoma.10,11 However, in prospective studies, the identification of a significant component of PTGC in a lymph node was not associated definitively with an increased risk of developing LPHD.4,5,10 PTGC usually is identified at low magnification as well-defined nodules that are larger than adjacent germinal
centers. High power reveals the nodules as composed of a polymorphous mixture of predominantly small lymphocytes with morphologic features suggestive of mantle B cells and T lymphocytes and scattered residual follicle center cells.10,12 The mantle B cells are small with round to slightly irregular nuclear contours, and they express surface IgM and surface IgD, characteristics of mantle B cells.12 However, tingible body macrophages and mitoses typically are absent.

Although PTGC has long been believed to be part of the spectrum of lymphoid hyperplasia, the potential relationships between hyperplastic follicles (HF), follicular lysis (FL), and PTGC has not been well illustrated. Only a few studies have suggested that PTGC is caused by the ingression of T cells and mantle B cells.10,12 These studies are limited further by the small numbers of cases studied and by the use of immunohistochemical analysis on frozen tissue sections, which do not provide optimal morphologic features for evaluation.

We sought to thoroughly examine the morphologic ingression of mantle B cells and T cells into germinal centers to delineate the relationships of HF, FL, and PTGC by using a panel of immunphenotypic markers including CD20 (to highlight the different lesions), bcl-2, and CD3 on paraffin-embedded tissue sections. By defining mantle cells by the immunphenotype of bcl-2+/CD3– and T cells as CD3+, we attempted to define and quantitate the small lymphoid component in HF, FL, and PTGC to gain insight into these processes. Our results document the ingression of T cells and mantle B cells in FL and PTGC, although the mantle B-cell component predominates in PTGC, suggesting that HF, FL, and PTGC constitute a spectrum of evolution in resolution of lymphoid hyperplasia with sequential ingression of T cells followed by mantle B cells. Furthermore, the expression of bcl-2 in mantle B cells is maintained in PTGC. This persistence of bcl-2 expression may cause pitfalls in the differential diagnosis of florid PTGC vs follicular lymphoma, particularly the so-called floral variant.

Materials and Methods

We retrieved 19 paraffin-embedded lymph nodes containing follicular hyperplasia, FL, and PTGC from the pathology files at the University of Utah Health Sciences Center, Salt Lake City, and from our hematopathology consultation service. All cases were reviewed to confirm the diagnosis and the presence of the aforementioned morphologic features. None of the patients had a known history of HIV infection or lymphoma, in particular Hodgkin lymphoma. Of 19 cases, 13 had no known evidence of developing lymphoid malignant neoplasms (range of follow-up period, 2.5 to 9.2 years). The remaining 6 cases were lost to follow-up.

All 19 cases were evaluated for the distribution of mantle B cells and T-cell lymphocytes by immunohistochemical techniques using commercially available monoclonal antibodies (DAKO, Carpinteria, CA) to bcl-2 protein (clone 124; dilution 1:80), CD3 (clone T3-4B5; dilution 1:50), and CD20 (clone L26, pan–B-cell marker; dilution 1:2,000) in sequential sections. All slides were stained using an automated immunostainer (Ventana, Tucson, AZ). The slides underwent heat-induced epitope retrieval using a pressure cooker (CD3 and bcl-2) or microwave irradiation (CD20) in citrate buffer before staining. Staining was detected using a diaminobenzidine chromogen. Appropriate positive and negative controls were used in all cases.

To assess the expression of bcl-2, CD3, and CD20 in HF, FL, and PTGC, evaluation of staining in sequential sections was analyzed. CD20 staining was used to highlight the different lesions. In each lymph node, the number of CD3+ cells (T cells) and bcl-2+ cells (T cells and mantle B cells) per 100 cells in 5 high-power fields (>400) of HF, FL, and PTGC were enumerated by 2 of us (C-C.C. and V.O.). The central areas of HF, FL, and PTGC were selected for the enumerating to avoid counting the mantle zone B cells. This led to enumeration of, in general, 1 high-power field per HF lesion, 1 high-power field per FL lesion, and 1 to 2 high-power fields per PTGC lesion. For each high-power field, CD3+ or bcl-2+ cells were divided by the total number of cells and then multiplied by 100 to obtain the quantity of CD3+ or bcl-2+ cells per 100 cells. There were no significant differences in the counts obtained by the 2 pathologists. The number of mantle B cells per 100 cells was defined as the number of bcl-2+ cells per 100 cells minus the number of CD3+ cells per 100 cells. The numbers (means and SDs of all high-power fields enumerated by the 2 pathologists) of T cells and mantle B cells in each morphologic component then were compared by using the Student t test.

Results

All 19 lymph node sections had a background of reactive follicular hyperplasia showing HF and contained 1 or more PTGC (range, 1-6). None of the cases had clinical or morphologic evidence of LPHD or features of florid PTGC.

Hyperplastic Follicles

H&E examination revealed that HF were composed of germinal centers surrounded by intact and well-defined mantle zones. As shown in Table I and Image IA, CD3 identified a small number of T cells within the germinal centers. Staining for bcl-2 was strongly positive in...
Follicular Lysis

In FL, the germinal center was distorted by tongues and clusters of dark blue mantle cells impinging on and invading the reactive germinal center. The germinal centers were present in greater numbers in FL than in HF (P < .0001; Table 1). In FL, bcl-2+ cells constituted a significantly larger population in the follicle center than did the CD3+ cells, indicating the ingression of mantle B cells (Image 1F). This resulted in a relative increase of the mantle cell component in FL compared with HF (P < .001; Table 1).

Progressive Transformation of Germinal Centers

PTGC was identified as large well-delineated nodules without evident mantle zones or tingible body macrophages. The density of CD3+ T cells in PTGC was similar to that of FL but significantly higher than that of HF (P < .0001; Table 1). There was a marked ingression of mantle B cells, highlighted by the differential between CD3 staining and bcl-2 staining (Image 1H). Image 1I, compared with FL (P < .0001; Table 1). The large numbers of mantle B cells and T cells in PTGC led to the characteristic appearance of diffuse bcl-2 staining in PTGC (Image 1I).

Discussion

In the present study, we revisited the morphologic and immunophenotypic features of PTGC and documented the ingression of mantle B cells and T cells into germinal centers as originally suggested by Lennert and Müller-Hermelink. Most important, by enumerating the T cells and mantle B cells by the combination of bcl-2 and CD3 staining, we illustrated that HF, FL, and PTGC seem to be a sequential morphologic evolution of lymphoid follicular hyperplasia characterized by the ingression of T cells followed by mantle B cells.

The bcl-2 protein is a regulator of lymphocyte apoptosis and has been shown to be expressed normally in mantle B cells and T cells, but it is absent in nonneoplastic follicle center cells. Because of the strong expression of bcl-2 within benign mantle B cells, we used bcl-2+/CD3– to identify and enumerate the mantle B cells and to demonstrate sequentially increased degrees of mantle cell ingression throughout the morphologic spectrum of HF, FL, and PTGC. We further demonstrated that as the mantle B cells began infiltrating the germinal centers, as in FL and PTGC, they retained their bcl-2 expression and, ultimately, together with infiltrating T cells, formed the large bcl-2+ nodule characteristic of PTGC.

The pathogenesis of PTGC remains unclear. Our observation that the amounts of mantle B cells and T cells were increased sequentially from HF to FL to PTGC supports the
concept that PTGC is part of the spectrum of reactive follicular hyperplasia and, possibly, the ultimate fate of a follicular center in response to antigen simulation.4,5,14 This concept would envision a stimulated follicle to undergo sequential steps of follicular hyperplasia, FL, and PTGC as a continuum. The concept is sustained further by a recent study demonstrating that somatic hypermutation, clonal expansion, and selection also can occur in the disorganized PTGC microenvironment, as well as in classic stimulated germinal centers.15

The observation that areas of PTGC are bcl-2+ owing to the marked ingression of mantle B cells and T cells in the present study may have important diagnostic significance. Cases of florid PTGC have been reported,2 as have studies describing follicular lymphoma “mimicking” PTGC, particularly the floral variant of follicular lymphoma.16,17 In the floral variant, more than half of the neoplastic follicles were surrounded by prominent mantle zones that irregularly invaginated the follicle centers, giving the appearance of PTGC.16 An initial diagnosis of atypical hyperplasia or PTGC was made in 21% of these cases.16 It must be emphasized that bcl-2 can be positive in the nodules of both entities, as described in our study, and should not be automatically interpreted as the bcl-2 positivity of follicular lymphoma.

An accurate diagnosis requires a detailed morphologic evaluation and adequate ancillary studies. The diagnosis of the floral variant of follicular lymphoma is supported by the identification of the densely packed nodules of atypical follicular center cells. Extramedullary extension may be present. By contrast, PTGC usually is associated with a background of reactive follicular hyperplasia. A distinctive wreath of epithelioid histiocytes occasionally surrounds the PTGC. Immunohistochemical staining for CD10 can be helpful. Only a few CD10+ follicle center B cells are present in PTGC, while abundant CD10+ cells are observed in the floral variant of follicular lymphoma (unpublished observation). Furthermore, interfollicular CD10+ lymphocytic infiltrates strongly support the diagnosis of follicular lymphoma.18 In difficult cases, molecular studies to detect t(14;18) and clonal immunoglobulin gene rearrangement can be used to confirm the diagnosis of follicular lymphoma.

Our results document the sequential ingression of T cells and mantle B cells in FL and PTGC, with the mantle B cells predominating in PTGC. These findings suggest that HF, FL, and PTGC are a sequential spectrum of lymphoid follicular hyperplasia. We also demonstrated that mantle B cells retain their bcl-2 positivity after ingression into FL and PTGC. Recognizing bcl-2 positivity in PTGC may avoid the potential pitfall of misinterpreting PTGC as follicular lymphoma, particularly the floral variant.

### Table 1
The Quantity of T Cells and Mantle B Cells in Hyperplastic Follicles, Follicular Lysis, and PTGC*

<table>
<thead>
<tr>
<th>Hyperplastic Follicles</th>
<th>Follicular Lysis</th>
<th>PTGC</th>
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<tr>
<td>T cells (CD3+)</td>
<td>25.8 ± 7.8</td>
<td>39.8 ± 10.0</td>
</tr>
<tr>
<td>Mantle B cells (bcl-2+/CD3–)</td>
<td>2.1 ± 1.6</td>
<td>12.3 ± 11.4</td>
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* Data are given as mean ± SD per 100 cells.

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### References


