Follicular Lymphoma with PAS-Positive Amorphous Material: Report of Two Cases

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Abstract. Extracellular, PAS-positive material has been described in various lymphoproliferative disorders, but is rare in malignant lymphomas. We report two such cases of grade 2 follicular lymphoma that were investigated by routine H&E histology, immunohistochemistry, flow cytometry, and molecular pathology. Ancillary studies confirmed the monoclonality in each case and the antigenic similarity to the neoplastic cells, with the exception of bcL-2. One possible pathogenic mechanism for this extracellular material may be the exocytosis of overproduced cell membranes.

Keywords: bcL-2, follicular lymphoma, amorphous PAS-positive material, immunohistochemistry

Introduction

Accumulation of amorphous, hyaline, non-amyloid substances is an occasional finding in lymphoproliferative B-cell disorders such as reactive follicular hyperplasia [1], follicular lymphoma [2,3], plasma cell dyscrasias [4,5], and dysimmune disorders [6]. These disorders (particularly the signet ring variant of follicular lymphoma) may also contain PAS-positive intracytoplasmic inclusions [7]. These associations suggest that the extracellular material may represent abnormal products of the immune reaction. The deposits commonly show the same monoclonal immunoglobulin [4], or share the same immunophenotype [3], as the accompanying lymphoma. The deposits might lead the observer mistakenly to diagnose a benign process such as reactive follicular hyperplasia. In addition, the extracellular material may resemble Hassell’s corpuscles, as seen in benign mediastinal lymph node hyperplasia [8,9], or in a lymphoid hamartoma [10].

We report two cases of documented follicular lymphoma with abundant extracellular accumulation of PAS-positive material. These cases were studied by H&E histology, immunohistochemistry, flow cytometry, and molecular techniques.

Case Reports

Case 1. The patient is a 50-yr-old male whose problems began in June 1999 when he noticed a lump in the right axilla. His primary care physician chose to observe this lump. Two months later, the patient developed right posterior cervical lymphadenopathy, which was treated with antibiotics and improved following therapy. In September, the patient developed left preauricular and left submandibular lymphadenopathy. Biopsy of a left axillary lymph node biopsy was performed. Computed tomography scans revealed bilateral cervical and axillary lymphadenopathy. The bone marrow was uninvolved. At a 1-mo follow-up visit, the patient did not have any new lymphadenopathy.

Case 2. The patient is a 54-yr-old woman with a medical history significant for anemia of chronic disease, lymphadenopathy, and splenomegaly.
June 1999, she noticed pain in her left arm and shoulder region. Her internist obtained a chest X-ray, which was interpreted as negative. The patient subsequently developed night sweating, low-grade fever, and body weight loss of 9 kg during 2 mo. Computed tomography scans in July showed bilateral axillary, paratracheal, mesenteric, and hilar lymphadenopathy. Splenomegaly was also noted. Two separate bone marrow biopsies were performed; both showed paratrabecular lymphoid aggregates. A left axillary lymph node was also biopsied. At a 7-mo follow-up visit, the patient complained only of bone and joint pain. She was completing her fifth cycle of chemotherapy with chlorambucil and prednisone.

Material and Methods

Flow cytometric analysis. Flow cytometric immunophenotyping was performed on a single cell suspension from the lymph nodes, using antibodies to CD5, CD10, CD19, CD20, CD23, CD38, CD7, CD25, HLA-DR, CD1, CD2, CD45, CD3, CD4, CD8 (all from Coulter/Immunotech, Kendall, FL), kappa light chain, and lambda light chain (both from DAKO, Carpenteria, CA). Data was collected and analyzed using a Coulter Epics XL-MCL flow cytometer (Coulter, Miami, FL).

Immunohistochemical analysis. Immunophenotyping was performed on paraffin sections of formalin-fixed tissue with an automated immunostainer (Ventana Biotech Medical Systems, Tucson, AZ). Reactivity was detected by an avidin-biotin immunoperoxidase method with diaminobenzidine-tetrahydrochloride dihydrate (Ventana Biotech Medical Systems) as the chromogen. Antigen retrieval in a pressure cooker was used. The antibodies used were L-26 (CD20), polyclonal CD3, kappa light chain, lambda light chain, and bcL-2.

Southern blot analysis. High molecular weight DNA was isolated from the lymph node using a PureGene kit (Gentra). For T-cell analysis, DNA (10 µg) was digested with 10 units/µg DNA of EcoRi, BamHI, and HindIII (Promega, Madison, WI) in 1x buffer (Promega) and bovine serum albumin (100 µg/ml, Sigma) for 60 min at 37°C. For B-cell analysis, BamHI/HindIII, BglII, and Xbal were used. The DNA digests were separated by 0.8% agarose gel electrophoresis. The DNA was depurinated with 0.1 M HCl for 15 min, denatured with 0.5 M NaOH/1 M NaCl for 30 min, neutralized with 1.5 M NaCl/0.5 M Tris (pH 7.4), for 30 minutes, and pressure blotted to a nylon membrane (Strategen). The membrane was baked (2 hr, 80°C), and hybridized in hybridization buffer overnight at 60°C (DAKO). Post-hybridization stringent washes and chemiluminescent detection was performed according to the manufacturer’s protocol (DAKO).

Results

Morphologic findings. Histologic sections from the submitted lymph nodes from both patients showed similar findings. Both nodes had a predominantly follicular pattern with effacement of the normal architecture by enlarged follicles with attenuated mantle zones. Some back-to-back molding of the follicles was evident. The follicles were composed of both large non-cleaved lymphocytes (centroblasts) and small cleaved lymphocytes (centrocytes); they lacked tingible-body macrophages. There were 6 to 15 centroblasts/high power field, which corresponds to grade 2 by “Berard” criteria. Many follicles contained amorphous eosinophilic extracellular material. This material was PAS-positive and diastase-resistant and was negative with Congo red. The sections of lymph nodes from Case 1 also contained scattered karyorrhectic nuclear debris.

Immunophenotypic findings. Immunohistochemistry was only performed on Case 1. The malignant lymphocytes in the germinal center were positive for CD20 and over-expressed bcL-2. Interestingly, the amorphous extracellular material was also positive for CD20, but was negative for bcL-2. Staining for kappa and lambda light chains was noncontributory. Flow cytometric immunophenotyping revealed a population of CD19-positive B-cells that were light chain restricted. They were also positive for CD20, CD45, CD38, HLA-DR, and they co-expressed CD10.
Fig. 1. (Case 1). [A] Follicular hyperplasia is evident with an amorphous infiltrate present in the hyperplastic follicles (4X, H&E stain). [B] Higher magnification (20X) of a single follicle showing a mixture of small (centrocyte) and larger (centroblast) lymphocytes with an interstitial pattern of infiltration of the amorphous material. [C] The amorphous infiltrate stains positively with periodic-acid-Schiff (PAS) stain. [D] On immunohistochemical staining, the follicles are positive for bcl-2 protein, consistent with follicular lymphoma.
Molecular genetic findings. Southern blot studies for the joining region of the immunoglobulin heavy chain, performed on DNA extracts from snap-frozen tissue, demonstrated non-germline fragments in all digests of Case 2. The T-cell gene receptor rearrangement (TCR) was germline.

Discussion

The differential diagnosis between follicular hyperplasia and follicular lymphoma has in the past been of great concern to pathologists. Now with ancillary techniques such as bcL-2 immunostaining, flow cytometry, and molecular clonality studies, this differential diagnosis is more easily resolved. However, it has been suggested that the number of malignant, atypical, follicular center cells may be reduced due to extracellular accumulation of PAS-positive material, potentially leading to an incorrect benign diagnosis. Most studies have found no correlations between the amount of precipitate and the clinical course of

Fig. 2. (Case 2). [A] Follicular hyperplasia is evident with an amorphous infiltrate present in the hyperplastic follicles (4X, H&E stain). [B] The amorphous infiltrate stains positively with PAS. The follicles were also positive for bcL-2 protein by immunohistochemical staining (not shown).
the disease, the cytologic type of lymphoma, or its anatomic location [2]. These deposits must be differentiated from amyloid deposits found in follicular lymphoma [11], and from stromal reactions variously described as hyalinosis, fibrosis, or sclerosis [12-14].

One of the first reports of such extracellular accumulation of amorphous hyaline material was by Cooper et al [15] in 1969. They studied the material by histochemistry and immunofluorescence and concluded that the precipitate was composed of collagen and plasma glycoprotein. This was confirmed by Talerman and Platenburg [14].

Rosas-Uribe et al [2] further characterized this phenomenon in 1973 in 13 cases of follicular lymphoma. They concluded that it was different from fibrin, reticulin, collagen, or amyloid. Electron microscopy in one of their cases showed clusters of extracellular fibrillar material. Since this patient had a polyclonal IgM hypergammaglobulinemia, the authors speculated that the material might represent an antigen-antibody complex.

Also in 1973, Dorfman [16] noted that nodular lymphomas may contain amorphous eosinophilic material that is PAS-positive and stains blue with Masson’s trichrome stain. Based on ultrastructural findings, the material did not appear to be collagen, but rather an accumulation of membranous profiles.

In previous studies, ultrastructural observations have revealed extracellular accumulations of fibrillar, amorphous non-fibrillar, or microvesicular material. The signet ring variant of follicular lymphoma has also subjected to intense ultrastructural scrutiny [3]. The signet ring phenomenon appears to be due to retentive intracellular accumulation of microvesicular membranous structures with bound immunoglobulin. Grogan et al [17] presented evidence that intracellular vacuole formation is probably a reflection of deregulated cell wall synthesis with endocytosis of these vesicles.

The extracellular material commonly appears to be immunohistochemically identical to the neoplastic follicle center cells (ie, both express B-cell antigens and monotypic immunoglobulin). It has been proposed that the extracellular accumulation of deposits antigenically identical to the neoplastic cells is a reversal of the process seen in signet ring follicular lymphomas. Chittal et al [3] propose that this material most likely derives from exocytosis or externalization of sequestered plasma membranes due to abortive overproduction of cell membranes.

In one of our cases, karyorrhectic nuclear debris was noted adjacent to the amorphous material. Therefore, it is impossible to exclude the possibility of some degenerative process within the neoplastic cells. CD20/L26 positivity of the precipitate was noted in one case, which suggests that the material may be remnants of cell wall membranes. While the neoplastic B-cells within the germinal centers were positive for bcl-2, the extracellular material was clearly bcl-2 negative. The reason for this discrepancy is unclear and bcl-2 reactivity has not been previously used to characterize this material.

Unfortunately, the light chain immunohistochemical results were non-contributory (apparently polyclonal). Both of our patients had a Grade 2 follicular lymphoma and are doing well at last reported follow-up visits. The prognostic significance of this amorphous material is difficult to assess, given the paucity of reported cases and the generally indolent nature of follicular lymphomas.

References


