Nuclear Protein Dysregulation in Lymphoplasmacytic Lymphoma/Waldenström Macroglobulinemia

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Key Words: Lymphoplasmacytic lymphoma/Waldenström macroglobulinemia; PAX5; CD138; Nuclear transcription factor expression

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Upon completion of this activity you will be able to:
• describe the immunophenotypic characteristics of clonal B lymphocytes and plasma cells in lymphoplasmacytic lymphoma/Waldenström macroglobulinemia (LPL/WM);
• recognize the clinicopathologic findings that distinguish LPL/WM from other B-cell lymphomas and plasma cell neoplasia;
• describe immunophenotypically aberrant cell populations in LPL/WM and some cases of plasma cell myeloma that show evidence of persistence of the B-cell gene expression program.

Abstract

Waldenström macroglobulinemia (WM) is characterized by monoclonal gammapathy, usually IgM, in association with lymphoplasmacytic lymphoma (LPL). Little is known of the expression of nuclear proteins involved in B-cell development in LPL/WM. In this study, the expression patterns of PAX5/BSAP, MUM1/IRF4, and PRDM1/BLIMP1 were analyzed in plasma cells and lymphocytes in 29 cases of newly diagnosed LPL/WM by double immunohistochemical staining with CD138 and CD22. These patterns were compared with the expression profiles seen in normal bone marrow samples, reactive tonsils, and cases of plasma cell myeloma and marginal zone lymphoma. The median percentage of plasma cells coexpressing CD138 and PAX5 was significantly higher in LPL/WM compared with benign tissues (P < .001), marginal zone lymphoma (P < .002), and plasma cell myeloma (P < .0001), whereas the median percentage of plasma cells coexpressing CD138 and MUM1 was lower in LPL/WM than plasma cells in benign tissues (P = .02), marginal zone lymphoma (P = .001), and plasma cell myeloma (P = .0002). These findings show that a subset of plasma cells in LPL/WM demonstrates a nuclear protein expression pattern characteristic of the B-cell developmental program. Thus, the results better define the immunophenotypic profile of the neoplastic cells in LPL/WM.
immunohistochemistry have been developed for each of these nuclear proteins. Ultimately, these expression patterns could be useful not only in the diagnosis of LPL/WM but also in possibly understanding its pathogenesis.

In this study, we used double immunohistochemical staining to evaluate the expression profiles of nuclear proteins PAX5 and MUM1 and surface antigens CD138 and CD22 in neoplastic plasma cells and B cells, respectively, in 29 cases of LPL/WM at diagnosis. These expression profiles were then compared with normal bone marrow samples, reactive tonsils, and cases of plasma cell myeloma and marginal zone lymphoma. A subset of cases was also analyzed for dual expression of BLIMP1 and CD138. Our finding of deregulated PAX5 and MUM1 expression in LPL/WM plasma cells and, to a lesser extent, B lymphocytes supports aberrant persistence of the B-cell developmental program in LPL/WM plasma cells. These findings better define the immunophenotypic profile of the neoplastic cells in LPL/WM.

Materials and Methods

Patients and Pathologic Specimens

Following Northwestern University Institutional Review Board approval (including Health Insurance Portability and Accountability Act requirements), 29 LPL/WM tissue specimens were identified retrospectively and obtained from the Department of Pathology of Northwestern Memorial Hospital (Chicago, IL). LPL/WM tissues included 25 pretreatment diagnostic bone marrow biopsy specimens, as well as 3 lymph nodes and 1 spleen (from patients in the LPL/WM bone marrow biopsy group). Control tissues included 2 bone marrow core biopsy specimens (conducted for lymphoma staging) and 3 reactive tonsils. Nineteen plasma cell myelomas (15 medullary and 4 extramedullary) and 6 marginal zone lymphomas (4 medullary and 2 extramedullary) were also examined. The cases of marginal zone lymphoma were selected based on the presence of monocytic plasma cells. Because of challenges inherent in staining B5 fixed, decalcified bone marrow biopsy specimens with BLIMP1, double staining for CD138 and BLIMP1 was conducted in formalin-fixed non-decalcified tissues. These tissues included 6 LPL tissues (5 lymph nodes, 1 spleen), 3 reactive tonsils, 5 plasmacytomas from patients with plasma cell myeloma, and 5 marginal zone lymphomas.

All tissues were obtained from routine diagnostic evaluations performed between 1997 and 2009. Clinical data for LPL/WM cases were obtained from the electronic medical record. Previously reviewed tissues, results of prior routine immunophenotypic studies (both flow cytometric and immunohistochemical), and clinical information were reviewed by 3 authors (M.J.R., A.C., and L.C.P.) to confirm the initial diagnoses using criteria outlined in the 2008 World Health Organization’s WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.

Immunophenotypic Analysis

In 23 of 25 LPL/WM patients with a bone marrow biopsy specimen, flow cytometric immunophenotyping was performed to characterize the malignant cells as a part of the initial workup (n = 22) or in a subsequent bone marrow specimen (n = 1) per methods described by Cao et al. A monotypic B-lymphocyte population positive for CD19 and CD20 was identified in all 23 patients. These cells were CD5– and CD10– in 20 cases, CD5+ and CD10– in 2 cases, and CD5– and CD10+ in 1 case, as has been described previously. In all cases, the surface κ or λ immunoglobulin light chain expressed by the neoplastic B lymphocytes was also expressed by the plasma cells (intracytoplasmic) whether examined by flow cytometry (7 cases) or immunohistochemistry (16 cases). In the 7 cases evaluated by flow cytometry, dim CD45+ to CD45–, CD138+, and CD38+ plasma cells expressed monotypic cytoplasmic immunoglobulin. These cells were CD20+ and CD56– (4 cases), CD20– and CD56– (2 cases), and CD20– and CD56+ (1 case).

Sections from B5 fixed and decalcified bone marrow core biopsy specimens (n = 44) or formalin-fixed tissues (n = 21) were immunostained using antibodies to CD138 (B-A38; AbD Serotec, Oxford, UK), CD22 and PAX5 (FCP1, 1EW; Novocastra Laboratories, Newcastle upon Tyne, UK), MUM1 and κ and λ immunoglobulin light chain (MUM1p, polyclonal κ, polyclonal λ; DAKO North America, Carpinteria, CA), and PRDM1/BLIMP1 (3H2E8, provided by Giovanna Roncador, Monoclonal Antibodies Unit, Spanish National Cancer Center, Madrid, Spain [described in Garcia et al]).

To visualize membrane and nuclear staining in the same cells, a red chromogen (alkaline phosphatase) was used for membrane staining (CD22, CD38), and a brown chromogen (3,3′-diaminobenzidine [DAB]) was used for nuclear staining (PAX5, MUM1, BLIMP1). Double immunohistochemical staining combinations included CD138/PAX5 and CD138/MUM1 for plasma cell analysis and CD22/PAX5 and CD22/MUM1 for B-lymphocyte analysis. The CD138/BLIMP1 staining combination was performed on non–bone marrow specimens only.

Immunohistochemical staining was performed on a Leica Bond-Max automated immunostainer (Leica Biosystems, Newcastle upon Tyne, UK) except as noted below. Tissue sections were deparaffinized and subjected to endogenous peroxidase inactivation. For the first (nuclear) antibody, heat-induced epitope retrieval with the Bond Epitope Retrieval (ER2) solution (Leica Biosystems) was conducted at 100°C for 20 minutes (PAX5, MUM1) or 30 minutes (BLIMP1). Following retrieval, sections were sequentially incubated with the primary antibody followed by Bond Polymer Refine

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Detection (Leica Biosystems) and developed with DAB for 10 minutes. Secondary antibody incubation was followed by the Bond Polymer Refine Red Detection System (Leica Biosystems). Single immunohistochemical staining for BLIMP1 was performed on the Ventana Benchmark Ultra immunostainer (Ventana Medical Systems, Tucson, AZ) per manufacturer protocols. Single immunostaining for \( \kappa \) and \( \lambda \) immunoglobulin light chains was performed using the Ventana Benchmark XT immunostainer (Ventana Medical Systems) on a subset of LPL/WM and marginal zone lymphoma cases to confirm the presence of monotypic plasma cells. The remaining 7 cases had monotypic plasma cells identified at the time of diagnosis by flow cytometric immunophenotyping.

The immunophenotypic profile of LPL/WM plasma cells and lymphocytes was evaluated by counting 200 (or as many present) CD138+ plasma cells and 200 (or as many present) CD22+ lymphocytes for each double-stain combination using PAX5, MUM1, or BLIMP1 nuclear stains. The number of double-positive cells per CD138+ or CD22+ cells counted was reported as “percent double-positive cells.” Plasma cells were morphologically defined as small to medium, round to oval-shaped cells with eccentrically placed nuclei. Lymphocytes were morphologically defined as a small cell with a round shape and centrally located nucleus.

All LPL/WM bone marrow biopsy specimens and lymph nodes, marginal zone lymphomas, and normal tissues examined contained scattered CD138+, MUM1+ cells with lymphocyte-like morphology. In serial bone marrow sections, these cells lacked CD22, consistent with a population of activated T cells. In tonsils, rare germinatal center cells expressed MUM1 and CD22, consistent with germinatal center B cells committed to plasma cell differentiation. Both of these CD138−, MUM1+ populations have been described previously.

Microscopic analysis was performed using an Olympus BH2 microscope (Olympus America, Center Valley, PA). Photographic images were obtained with an Olympus BX45 microscope with an Olympus DP71 camera attachment using DP Controller software version 3.2.1.276 (Olympus America).

Statistical Analysis

Statistical analysis was performed using Stata software (version 10.0, StataCorp, College Station, TX). Median values for percent double-positive cells in LPL/WM cases were compared with benign tissues, plasma cell myeloma cases, and marginal zone lymphoma cases using the Wilcoxon rank-sum test. B lymphocytes from LPL/WM were not compared with plasma cell myeloma cases owing to the lack of clonal B cells in plasma cell lesions. Data are represented in box-and-whisker plots with median, first and third quartiles, values closest to the upper and lower fence (upper hinge + [1.5 × H-spread]), and outliers. P values of .05 or lower were considered statistically significant for these comparisons.

Results

Clinical and Pathologic Features of LPL/WM Patients at Diagnosis

The clinical and pathologic features in 25 of the 29 LPL/WM cases with diagnostic bone marrow specimens are shown in Table 1. The male to female ratio was 1.6 to 1. The median age was 69 years (range, 39-85 years). Five of 25
patients had lymphadenopathy, 1 patient had splenomegaly at initial diagnosis, and no patients had lytic bone lesions. The median serum IgM level was 2,600 mg/dL (range, 477-13,500 mg/dL; normal, 46-304 mg/dL). Serum viscosity was elevated in 12 of 13 patients tested.

LPL/WM cells involved a median of 50% of the inter trabecular space with a range of 10% to 95% involvement. LPL/WM had variable patterns of infiltration, including focal nodular, paratrabecular, interstitial, and diffuse, with mixed patterns seen in all cases. The relative percentages of B lymphocytes and plasma cells in LPL/WM infiltrates varied considerably at the time of diagnosis, although lymphocytes usually predominated over plasma cells. Immunohistochemical staining showed that CD138+ plasma cells comprised between <5% and 50% (median, 30%) of cells, whereas PAX5+ B lymphocytes comprised between 50% and 95% of the cells in the infiltrates (median, 70%).

Comparison of the Immunophenotypic Profile of Neoplastic Cells in LPL/WM With the Neoplastic Cells in Plasma Cell Myeloma, Marginal Zone Lymphoma, and Benign Tissues

Most CD138+ plasma cells in LPL/WM, plasma cell myeloma, marginal zone lymphoma, and benign tissues were negative for PAX5 (Figure 1). However, a subset of plasma cells coexpressing CD138 and PAX5 was identified in all cases of LPL/WM. The median percentage of plasma cells coexpressing CD138 and PAX5 was significantly lower than in LPL/WM.

### Table 1
Clinical and Bone Marrow Findings in 25 Cases of LPL/WM at Diagnosis

<table>
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<tr>
<th>Case No.</th>
<th>Age, y</th>
<th>Sex</th>
<th>IgM, g/dL</th>
<th>Ig Light Chain</th>
<th>Involvement, %</th>
<th>CD138+ Plasma Cells, %</th>
<th>PAX5+/CD138– Lymphocytes, %</th>
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<td>F</td>
<td>2,740</td>
<td>κ</td>
<td>30</td>
<td>20</td>
<td>90</td>
</tr>
</tbody>
</table>

LPL, lymphoplasmacytic lymphoma; NA, not available; WM, Waldenström macroglobulinemia.

*Of the neoplastic infiltrate.

### Table 2
Median Percentages of Double-Positive Plasma Cells and Lymphocytes in Lymphoplasmacytic Lymphoma/Waldenström Macroglobulinemia Compared With Normal Tissues, Plasma Cell Myeloma, and Marginal Zone Lymphoma

<table>
<thead>
<tr>
<th>Phenotype Cell Type</th>
<th>Double-Positive Cells, Median % (Range %)</th>
<th>P Values *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LPL (n = 29)</td>
<td>NL (n = 5)</td>
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<tr>
<td>CD138+/PAX5+ plasma cells</td>
<td>15 (1-22)</td>
<td>0.5 (0-2)</td>
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<td>CD138+/MUM1+ plasma cells</td>
<td>93 (72-100)</td>
<td>99 (95-100)</td>
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<tr>
<td>CD138+/BCLIM1+ plasma cells</td>
<td>92 (n = 6)</td>
<td>94 (n = 3)</td>
</tr>
<tr>
<td>CD22+/PAX5+ lymphocytes</td>
<td>98 (92-100)</td>
<td>95 (87-98)</td>
</tr>
<tr>
<td>CD22+/MUM1+ lymphocytes</td>
<td>2 (0-14)</td>
<td>1.5 (1-6)</td>
</tr>
</tbody>
</table>

LPL, lymphoplasmacytic lymphoma; NL, normal tissue; PCMI, plasma cell myeloma; MZL, marginal zone lymphoma; NA, not available.

*Wilcoxon rank-sum test.

**Lymph node only; number of cases in parentheses.
Image 1: Representative images from bone marrow specimens involved by lymphoplasmacytic lymphoma/Waldenström macroglobulinemia at diagnosis. A, Bone marrow aspirate and bone marrow (B) core biopsy specimens show increased numbers of lymphocytes, plasmacytoid lymphocytes, and plasma cells. C, Immunostaining for CD20 shows that the lymphocytes are predominately B cells, whereas immunostaining for the (D) k and (E) λ immunoglobulin light chains shows that the cells express the λ light chain but are λ negative.
higher in LPL/WM (15%; range, 1%-50.5%) compared with benign tissues (0.5%; range, 0%-1.5%; \( P = .001 \)), plasma cell myeloma (0%; range, 0%-79.5%; \( P < .0001 \)), and marginal zone lymphoma (1%; range, 0%-3%; \( P = .002 \)) (Table 2). Of the 19 plasma cell myeloma cases studied, 17 had no identifiable CD138/PAX5 double-positive cells. The 2 myeloma cases with CD138/PAX5 double-positive plasma cells (5.5% and 79.5% of plasma cells) were the only CD20+ (dim) by flow cytometry; these cases were both IgG \( \kappa \) positive, t(11;14) negative but were trisomy 11 positive. To determine whether CD20+ plasma cells expressed PAX5 to a greater extent than CD20− plasma cells in plasma cell myeloma, 10 additional CD20+ plasma cell myeloma cases were examined for dual expression of CD138 and PAX5, and none of these cases showed greater than 1% double-positive cells. A small number of cells with lymphocyte-like morphology coexpressing CD138 and PAX5 was also seen in LPL/WM (median, 2%; range, 0%-13%) but not in normal tissues (0%) or marginal zone lymphoma (0%).

The percentage of CD138+ plasma cells that coexpressed MUM1 in plasma cells was lower in LPL/WM (93%; range, 72%-100%) than in normal tissues (99%; range, 97.5%-100%; \( P = .02 \)), plasma cell myeloma (99%; range, 50%-100%; \( P = .0002 \)), and marginal zone lymphoma (100%;
Double staining for CD138 and BLIMP1 was performed in a subset of formalin-fixed, non-decalcified tissues. The median percentage of CD138+ plasma cells expressing BLIMP1 was lower in the LPL/WM cases (92%; range, 86%-98%) when compared with benign tissues (94%; range, 92%-97%), plasma cell myeloma (98%; range, 88%-99.5%), and marginal zone lymphoma (97%; range, 88%-97%), but none of these values were statistically significant (P = .76, .21, and .34, respectively).

**Discussion**

In this study, we examined the expression patterns of the nuclear proteins PAX5, MUM1, and BLIMP1 by neoplastic CD138+ plasma cells and CD22+ B lymphocytes in LPL/WM.
at initial diagnosis and compared their expression patterns with those seen in plasma cells and lymphocytes in benign tissues, plasma cell myeloma, and marginal zone lymphoma. Our findings show that PAX5 is aberrantly expressed in a sizable subset of CD138+ plasma cells in LPL/WM, a pattern not seen in benign tissues or in marginal zone lymphoma. We also found that MUM1 is coexpressed by a smaller percentage of CD138+ plasma cells in LPL/WM than in other cases studied.

The finding of relatively increased PAX5 and decreased MUM1 expression in a subset of plasma cells in LPL/WM suggests that components of the B-cell transcriptional program are more active in LPL/WM plasma cells than in other tissues. PAX5 is a B-cell–specific transcription factor with multiple regulatory functions in maintaining B-cell identity and is expressed in both early and mature B lymphocytes. Although PAX5 has been reportedly to be positive in LPL/WM cases studied, the differential expression pattern in B lymphocytes and plasma cells has not been described previously. The presence of PAX5+ plasma cells in LPL/WM was recently shown with single-label immunohistochemical staining by Moreira et al. The addition of a second immunomarker to identify plasma cells (CD138) increases the accuracy of immunostaining and supports our findings that PAX5+ plasma cells are more common in LPL/WM than in benign tissues, plasma cell myeloma, or marginal zone lymphoma. A larger number of cells with lymphocyte morphology were also found to be CD138 and PAX5 double positive in LPL/WM compared with other tissues, although the difference between median values of double expression was small.

Persistence of the B-cell expression program in LPL/WM plasma cells was also supported by comparatively decreased detection of the plasma cell–associated MUM1 protein. In benign tissues, MUM1 is expressed in plasma cells, a small number of germinal center B cells committed to plasma cell differentiation, and a subset of activated T cells. MUM1 has been shown to coordinate isotype switching, downregulation of germinal center BCL6 expression, and BLIMP1 induction prior to subsequent memory B-cell and plasma cell differentiation. MUM1 expression has not been well characterized in LPL/WM, and descriptive studies to date have used single immunohistochemical staining. One study evaluating MUM1 in LPL/WM, and descriptive studies to date have used single immunohistochemical staining by Morice et al. The finding of relatively increased PAX5 and decreased MUM1 expression in a subset of plasma cells in LPL/WM was recently shown with single-label immunohistochemical staining by Moreira et al. The addition of a second immunomarker to identify plasma cells (CD138) increases the accuracy of immunostaining and supports our findings that PAX5+ plasma cells are more common in LPL/WM than in benign tissues, plasma cell myeloma, or marginal zone lymphoma. A larger number of cells with lymphocyte morphology were also found to be CD138 and PAX5 double positive in LPL/WM compared with other tissues, although the difference between median values of double expression was small.

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and exhibited dim positive CD20 expression by flow cytometry. To determine whether plasma cells in CD20+ myelomas showed increased CD138/PAX5 double positivity, we evaluated 10 additional cases of CD20+ myeloma, none of which showed greater than 1% CD138/PAX5 dual-positive cells (data not shown). PAX5 expression in plasma cell myeloma has been previously described by Lin et al., and its presence describes a myeloma clone with B-cell characteristics. It is important to recognize that cases of CD20+ plasma cell myeloma expressing PAX5 can complicate the diagnostic picture, mimicking a B-cell neoplasm such as LPL/WM with plasmacytoid differentiation.

LPL/WM is characterized morphologically by the presence of cells along a continuum of small lymphocytes, plasmacytoid lymphocytes, and plasma cells. Plasmacytic differentiation is not unique to LPL/WM and may be seen in other lymphomas such as marginal zone lymphoma. In this study, we were able to use double immunohistochemical staining to identify a plasma cell population in LPL/WM with aberrant persistence of the B-lymphocyte nuclear expression program. These findings not only better define the immunophenotypic profile of the neoplastic cells in LPL/WM but also identify expression patterns that may assist in determining the cell type that has undergone neoplastic transformation in LPL/WM.

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