Flow Cytometric Findings in the Floral Variant of Follicular Lymphoma

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The floral variant of follicular lymphoma (FL-F) is an unusual variant of follicular lymphoma. The malignant "follicles" have an unusual "floral" appearance due to the proliferation of surrounding and infiltrating small lymphocytes. This variant of malignant lymphoma has been supported by immunohistochemical immunophenotyping and gene-rearrangement studies; however, the flow cytometric findings

have not previously been described. We report the flow cytometric findings in two cases of FL-F. The surrounding and infiltrating small lymphocytes represented mantle cells which, in one case, obscured monoclonality in the lymphocyte region. The monoclonal B-cells were CD10+, supporting a follicular center cell origin. *J. Clin. Lab. Anal.* 12:310–314, 1998.

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INTRODUCTION

The so-called "floral variant" of follicular lymphoma (FL-F) is considered an unusual variant of follicular lymphoma. Although morphologically it has been described as mimicking progressive transformation of germinal centers (PTGC) (1), immunophenotypic and/or genotypic support for malignant lymphoma has been demonstrated in the majority (80%) of cases studied by Goates, et al. (2). These cases were studied by immunohistochemistry and/or gene-rearrangement studies. The flow cytometric findings have not previously been described in this entity.

We report the flow cytometric findings in two cases of FL-F. The findings support a follicular center cell origin. However, in one case, the proliferation of mantle cells obscured the presence of monoclonality in the lymphocyte region.

CASE HISTORIES/GROSS PATHOLOGIC FINDINGS

Case 1

An 89 year-old "healthy" white male presented with an enlarged right upper neck lymph node. He had no constitutional or B symptoms. No additional clinical follow-up was available. The cervical lymph node measured 2.0 × 1.0 × 1.0 cms in greatest dimensions. The cut surface was homogenous, tan-white, and of fish-flesh consistency.

Case 2

A 70 year-old white male presented with a "chest wall mass." He had presented 12 years previously with a "scalp

lesion" which apparently had been present for many years and was diagnosed as "progressive transformation of germinal centers." The pathology report of the scalp lesion described extensive lymphoid infiltration of the dermis and subcutis. The lymphoid infiltrate was described as composed primarily of small cells. Ill-defined follicular centers were described as being composed primarily of small cleaved cells with some "transformed" large cleaved and noncleaved cells and "rare" tingible body macrophages. Infiltration of several follicles by mantle zone lymphocytes was also described. The slides were not available for review and flow cytometric immunophenotyping was not performed on the scalp lesion.

The chest wall mass measured 1.4 × 0.8 × 0.5 cm in greatest dimensions. The cut surface was firm and light tan. A subsequent bone marrow biopsy showed lymphomatous involvement, characterized by a single paratrabeular infiltrate.

MATERIALS AND METHODS

Lymph node and chest tissue were fixed in 10% nonbuffered formalin, sectioned at 4 µm, and stained with hematoxylin and eosin (HE) and the following immunoperoxidase stains using the DAKO LSAB-2 (labelled streptavidin biotin) peroxidase system (Carpinteria, CA): L-26 (CD20), CD23, CD3, kappa, bcl-2 (DAKO); CD5 (Vector, Burlingame, CA);

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lambda (Biogenex, San Ramon, CA), and cyclin D1 (cocktail of mouse monoclonal, Immunotech, Westbrook, ME; and mouse monoclonal, Vector). Control tissue was tonsil (L-26, CD23, CD3, kappa, bcl-2, CD5, lambda) and a case of mantle cell lymphoma (cyclin D1). Antigen retrieval techniques were used for all the antibodies except for kappa and lambda. Lymph node and chest tissue were also prepared for a single-cell suspension in RPMI medium (Cellgro Mediatech Tissue Culture Media, Fisher Scientific, Pittsburgh, PA) by grating the tissue on a fine wire mesh with a scalpel blade and analyzed on an Ortho Cytofluor absolute flow cytometer (Ortho Diagnostic Systems, Raritan, NJ) for various antigens using standard techniques and the following commercially-available monoclonal antibodies: CD3, CD4, and CD45 (Becton-Dickinson, San Jose, CA); CD5 and CD8 (Gen Trak Inc., Wayne, PA); CD10, CD11c, CD14 (MY4), CD19, and CD20 (Coulter Clone, Coulter Immunology, Hialeah, FL); CD23 and CD25 (Dako Corporation, Carpinteria, CA); HLA-DR (Ortho Diagnostic Systems); and immunoglobulin (Ig)A, IgD, IgG, IgM, and kappa and lambda light chains (Kallestad Inc., Chaska, MA). Dual staining was performed as follows: CD3/

4, CD19/15, CD20/HLA-DR, CD45/10, CD14/23, CD11c/25, and kappa/lambda.

RESULTS

Histology/ Immunohistochemistry

Case 1

Review of an HE-section of the cervical lymph node revealed complete effacement of the normal lymph node architecture. No residual normal lymph node architecture was identified. There was a low-power nodular pattern. Surrounding and infiltrating the nodules was a marked proliferation of small, slightly irregular lymphocytes, corresponding to mantle cells. The infiltration of mantle cells imparted a scalloped, floral pattern to the nodules. The center of the nodules was composed of a mixture of small cleaved and large cleaved and noncleaved cells; the large cells composed greater than 20% but less than 50% of cells in the nodules (Fig. 1). L-26 revealed intense cytoplasmic membrane staining of the great majority of cells within the center of the nodules as well as the mantle cells surrounding and infiltrating the nodules.

Fig. 1. (A) "Floral" appearance of the nodules due to scalloping by mantle cells ($\times 100$). (B) The centers of the nodules were composed of a mixture of

small cleaved, large cleaved, and noncleaved cells which were infiltrated at the edges by mantle cells ($\times 400$).

CD23 intensely stained follicular dendritic cells scattered within the malignant nodules. Kappa and lambda revealed polyclonal cytoplasmic staining of plasma cells and did not stain cells within the malignant nodules. CD3 revealed intense cytoplasmic membrane staining of the majority of cells within the interfollicular regions as well as scattered, predominantly small lymphocytes in the center of the malignant nodules. CD5 showed a similar staining pattern to CD3, but CD5 also stained the mantle cells. Bcl-2 intensely stained the majority of cells within the center of the nodules. Cyclin D1 did not stain the cells within the lymph node.

Case 2

Review of an HE-section of the chest tissue revealed infiltration of fibroadipose tissue by a follicular lymphoma, characterized by back-to-back nodules. In occasional areas, the nodules were infiltrated and scalloped by mantle cells, imparting a floral appearance to the nodules. The centers of the nodules were composed predominantly of small cleaved cells with less than 20% large cells (Fig. 2). The immunoperoxidase stains showed the same pattern as described in Case 1.

Flow Cytometric Immunophenotyping

Case 1

Flow cytometric analysis of the cervical lymph node tissue revealed 89% of cells within the lymphocyte region, 9% within the monocyte region, and 2% within the granulocyte region. Cells within the lymphocyte region were composed of 47% T-cells and 49% B-cells with a kappa:lambda ratio of 2:3. 11% of cells co-expressed CD19 and CD5 (Fig. 3) and 15% and 30% of the B-cells coexpressed CD10 and CD23, respectively. Cells within the monocyte (large cell) region were composed of approximately 46% T-cells and 47% monoclonal B-cells with expression of CD19, CD20, CD10, CD23, HLA-DR, and IgM, lambda of a moderate intensity (Figs. 4 and 5). There was no co-expression of CD5 or CD11c by the monoclonal B-cell population.

Case 2

Flow cytometric analysis of the suspension prepared from the chest tissue revealed 96% of cells within a combined lymphocyte/monocyte region and 4% within the granulocyte region. Cells within the combined lymphocyte/monocyte region

Fig. 2. (A) "Floral" appearance of a nodule due to infiltration by mantle cells (HE stain, $\times 100$). (B) The centers of these nodules were composed predominantly of small cleaved cells ($\times 200$).

Fig. 3. Flow cytogram of Case 1 showing population of cells dually expressing CD19 and CD5 in upper right box.

were composed of 40% T-cells and approximately 60% monoclonal B-cells with variable expression of CD19, CD20, CD23, CD10, HLA-DR, and IgM, lambda of a strong intensity (Figs. 4 and 5). There was no coexpression of CD5 by the monoclonal B-cell population.

DISCUSSION

The floral variant of follicular lymphoma may be difficult to recognize morphologically as a malignant lymphoma. The floral appearance of the malignant follicles is due to a proliferation of surrounding and infiltrating small lymphocytes, previously described as morphologically mimicking PTGC. However, the centers of the follicles do not morphologically resemble residual reactive germinal centers. They are composed of varying numbers of small cleaved, large cleaved, and noncleaved cells. In addition, immunophenotypic and/or genotypic analysis of the floral variant of follicular lymphoma have supported a diagnosis of malignant lymphoma in the majority of cases. Bcl-2 overexpression has been demonstrated in 8 of 12 (67%) cases studies, supporting a follicular center cell origin. However, the flow cytometric findings have not previously been described in this unusual variant of follicular lymphoma.

Although Sandhaus et al. (3) report a case "consistent with the floral variant of follicular lymphoma" and mimicking PTGC, with flow cytometric analysis, several features cast doubt on the diagnosis. These features include the fact that the diagnosis was based on histopathologic criteria alone without evidence of monoclonality by flow cytometric or immunohistochemical immunophenotyping or by gene rearrangement analysis. Residual normal lymph node was

Fig. 4. Flow cytogram of Case 2 showing population of cells dually expressing CD10 (FL1) and CD45 (FL2) in upper right box.

present. The patient had no additional sites of involvement and no documented disease progression.

The histologic findings in our cases were similar to those described by Goates et al. Of interest, the flow cytometric analysis in Case 1 detected a small population of CD5-positive B-cells which represented the mantle cell proliferation. The monoclonal B-cells had expression of CD10, supporting a follicular center cell origin. It was difficult to appreciate the mono-

Fig. 5. Flow cytogram of Case 2 showing cells expressing kappa (FL1) only (left upper box) versus cells expressing lambda (FL2) only (right lower box); the kappa/lambda ratio was 9:66, indicating monoclonal lambda expression.

clonal B-cell population in the lymphocyte region due to the presence of the mantle cells. This case illustrates the possible difficulty in detecting a monoclonal B-cell population by flow cytometry in this unusual variant of follicular lymphoma since there may be a prominent proliferation of polyclonal mantle cells. In our case, the co-expression of CD10 by B-cells in the lymphocyte region in combination with critical analysis of the monocyte region with dual staining of kappa and lambda helped in reaching a correct diagnosis of malignant lymphoma. However, our case was composed of a mixture of small and large cells. In cases composed predominantly of small cells, the polyclonal mantle cells may pose more of a diagnostic dilemma.

The second case illustrates the usefulness of flow cytometric immunophenotyping in distinguishing progressive

transformation of germinal centers from FL-F. Undoubtedly, the original scalp lesion in this case represented FL-F.

REFERENCES

1. Goates JJ, Kamel OW, LeBrun DP, Benharroch D, Dorfman RF: Follicular variant of follicular lymphoma: Immunological and molecular studies support a neoplastic process. *Am J Surg Pathol* 18(1):37-47, 1994.
2. Osborne BM, Butler JJ: Follicular lymphoma mimicking progressive transformation of germinal centers. *Am J Clin Pathol* 88:264-269, 1987.
3. Sandhaus LM, Voelkerding K, Raska KJ: Follicular lymphoma mimicking progressive transformation of germinal centers: Immunologic analysis of a case [letter]. *Am J Clin Pathol* 90:518-519, 1988.