Clinicopathologic, Immunophenotypic, and Molecular Characterization of Primary Cutaneous Follicular B-Cell Lymphoma

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Objective: To determine the clinicopathologic, immunophenotypic, and molecular characteristics of primary follicular cutaneous B-cell lymphoma (CBCL) as defined by the revised European-American lymphoma classification.

Design: A retrospective survey of the medical records, an immunohistochemical study of archival biopsy specimens, and molecular studies of preserved DNA of all patients with follicle center lymphoma-follicular (FCL-F) primary CBCL from 1987 to 1997.

Setting: A single-center outpatient specialty clinic at an academic medical center.

Patients: Twenty-one patients (68% of all new primary CBCL cases), including 14 men and 7 women (age range, 33-88 years; mean, 55 years).

Results: The head and neck region was the most frequent primary site. Following treatment, recurrences were relatively frequent, but the overall mortality rate during 1.0 to 11.3 years (mean, 6.3 years) of follow-up was 4.8%. Immunohistochemical analysis for B- and T-cell lineages was helpful in enhancing the folliclelike structures. CD10, bcl-2, and CD43 were expressed by the neoplastic cells in 9 (47%) of 19 cases, 4 (21%) of 19 cases, and 2 (13%) of 16 cases, respectively. Immunohistochemical detection of cytoplasmic immunoglobulin light chains, using steaming in EDTA as the antigen-retrieval technique, was successful in 12 (71%) of 17 cases. The Ig heavy-chain gene rearrangements, using the Southern blot technique, detected clonality in 17 (94%) of 18 cases. The bcl-2 gene rearrangements were detected in only 2 (13%) of 15 of the primary cutaneous FCL-F cases, compared with 9 (75%) of 12 of the primary nodal FCL-F cases (P=.002).

Conclusions: Primary cutaneous FCL-F is a relatively common subtype of CBCL, with a relatively indolent course. It has many features in common with primary nodal FCL-F, except for low rates of bcl-2 expression and bcl-2 gene rearrangements.

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Primary cutaneous lymphomas represent a heterogeneous group of T- and B-cell lymphomas that show considerable variation in clinical presentation, histological features, immunophenotype, and prognosis.1,2 Immunohistochemical and genotypic studies have suggested that primary cutaneous B-cell lymphomas (CBCLs) represent a distinct biological entity among B-cell non-Hodgkin lymphomas, characterized by a favorable clinical prognosis.3 Before the introduction of immunohistochemistry, the vast majority of cutaneous follicular lymphoid infiltrates were thought to represent benign processes, eg, pseudolymphomas.4 With the advent of monoclonal antibodies, however, follicular CBCLs were shown to exist.5 More recently, primary cutaneous marginal zone lymphoma (MZL), which is related to the low-grade B-cell lymphoma of mucosa-associated lymphoid tissue (MALT),6 has been described.6-10 It is often characterized histologically by the presence of reactive follicles, in addition to the neoplastic cells.6-10 The distinction of follicular

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CBCL from reactive follicular lymphoid hyperplasia is often difficult. Although several histological and immunohistochemical means have been suggested to distinguish these 2 entities, the presence or absence of immunoglobulin (Ig) light-chain restriction, and/or Ig gene rearrangement, is currently thought to distinguish between a neoplastic monoclonal and a reactive polyclonal lymphoid infiltrate.11 While in the past, the reliable detection of
PATIENTS, MATERIALS, AND METHODS

PATIENTS

All the case files from 1987 to 1997 of the Department of Dermatology at the Mayo Clinic, Rochester, Minn, were searched for newly diagnosed cases of CBCL. The histological slides, the pathology and immunopathology reports, and the medical record of each patient were reviewed. Thirty-one cases satisfied the following inclusion criteria: (1) histological findings compatible with CBCL; (2) B-cell lineage, determined by immunohistochemical studies; (3) Ig light-chain restriction demonstrated on frozen sections and/or Ig gene rearrangements using a Southern blot technique as previously described; and (4) no extranodal involvement detected by history and physical examination, complete blood cell count, liver function tests, radiography of the chest, computed tomography of the abdomen and pelvis, and bone marrow aspiration and biopsy. The lymphomas were classified based on the morphological and phenotypic criteria outlined by the International Lymphoma Study Group.26,29 Follow-up information was obtained by chart review or by letter or telephone call to the patient.

IMMUNOHISTOCHEMICAL STUDIES

Immunohistochemical studies were performed only if sufficient tissues remained in the blocks. Immunoperoxidase stains were performed on paraffin sections of the skin specimens using previously described methods13 and antibodies to CD3, CD20, bcl-2 protein, and k and l light chains (Dako Corp, Carpenteria, Calif), CD10 (Novoceastra, Vector Laboratories, Inc, Burlingame, Calif), and CD43 (Becton Dickinson Corp, San Jose, Calif). During antigen retrieval, the slides were steamed in either 1-mmol/L citrate buffer (pH, 6.0) or 1-mmol/L EDTA (pH, 8.0). All cases were stained using automated instruments.

Ig light-chain restriction required frozen-section immunohistochemical analysis, new methods using paraffin sections have been introduced. This methodology has allowed the study of archival material and has made it easier to distinguish between nonneoplastic and neoplastic cells.12,14

Chromosomal translocation (14;18), which juxtaposes the bcl-2 gene with the Ig heavy-chain gene in chromosome 14, resulting in overexpression of the bcl-2 oncprotein, is believed to be linked to the pathogenesis of B-cell lymphoma via the prevention of apoptotic cell death.15 The bcl-2 gene rearrangements have been demonstrated in two thirds of follicular lymphomas and in 10% to 40% of high-grade non-Hodgkin lymphoma of the lymph nodes.16-20 In contrast, molecular studies of t(14;18) in CBCL using the Southern blot technique yielded negative results20 or positive findings in only 2% of the cases.9 With the use of the highly sensitive polymerase chain reaction (PCR) technique on DNA retrieved from paraffin-embedded tissues, t(14;18) was detected in only 1 (7%) of 14 CBCLs, compared with 3 (38%) of 8 primary nodal B-cell lymphomas with cutaneous infiltration.22

CD10 antibodies recognize a 90- to 100-kd cell surface glycoprotein that is present in a variety of cell types, and CD10 is considered to be a marker of early B-cell activation.23 CD10 expression has commonly been demonstrated in nodal follicular lymphomas but is rarely present or is absent in most cases of lymphoplasmacytoid and marginal zone lymphomas.14,24 In 2 large series of CBCLs, CD10 was not found to be expressed.3,24 This lack of CD10 reactivity, as well as the very low rate of bcl-2 gene rearrangements and the relatively favorable outcome, has led some authors to suggest that CBCL is related to MALT or MZL B-cell lymphoma rather than to follicle center cell lymphomas.10,11,25

The revised European-American lymphoma (REAL) classification proposed by the International Lymphoma Study Group in 1994,26 and largely adopted recently by the proposed World Health Organization (WHO) classification,27 has outlined several B-cell lymphomas that seem to be pertinent to the skin. The present study was

bcl-2 GENE REARRANGEMENT

Studies of bcl-2 rearrangement were performed in all 15 cases of FCL-F CBCL in which cryopreserved DNA obtained from fresh lesional biopsy specimens was available, as well as in 12 cases of primary nodal FCL-F. DNA was prepared from skin biopsy specimens using proteinase K digestion and phenol/chloroform extraction, according to standard procedures. The PCR conditions and primers for amplification of the bcl-2 major breakpoint region (MBR) and the minor cluster region (MCR) rearrangements were based on previously published protocols.20,21 Briefly, the primer sequences were as follows: MBR, 5'-TTA GAG AGT TGC TTT ACG TG-3'; MCR, 5'-GAC TCC TTT ACG TGC TGG TAC C-3'; and JH consensus primer, 5'-ACC TGA GGA GAC GGT GAC G-3'. Additional primers to the β-globin gene were used to verify the presence of amplifiable DNA. The PCRs for MBR and MCR were set up separately, and reaction mixtures contained 1 PCR buffer, 200-nmol/L deoxynucleotide triphosphates, 5 pmol of each primer, 1.25 U of Taq gold, either 2.0-mmol/L magnesium (for MBR) or 2.5-mmol/L magnesium (for MCR), and 250 ng of DNA in a reaction volume of 25 µL. All reagents are commercially available (PE Applied Biosystems, Foster City, Calif). Amplification conditions for the MBR reactions were 10 minutes at 95°C (hot start), 30 cycles at 94°C for 1 minute, 60°C for 1 minute, and 72°C for 1 minute, ending with a final extension at 72°C for 10 minutes, followed by a 5°C hold. Conditions for the MCR reactions were the same, except that the annealing temperature was increased to 65°C. A thermal cycler (Perkin Elmer PE Applied Biosystems) was used for all reactions.

Each DNA sample was analyzed in duplicate, with the first PCR containing the specific primers only and the second PCR containing a multiplex of the specific primers and the control β-globin primers. Control reactions included “no DNA” and DNA extracted from documented positive cell lines. After amplification, 5 µL of 0.6× loading buffer were added to each tube, and the PCR products were electrophoresed on a 3% agarose gel for approximately 4 hours and then photographed.
undertaken to determine the clinicopathologic, immunophenotypic, and molecular characteristics of primary follicular CBCL, as recently defined by the REAL/WHO classification schemes.

**RESULTS**

The pertinent clinicopathologic data and the results of the immunophenotyping and molecular studies are summarized in the Table. Thirty-one cases of primary CBCL were identified, 21 (68%) of which were classified histologically as FCL-F, 5 (16%) as diffuse large B-cell lymphoma, and 5 (16%) as MZL.

### CLINICAL FINDINGS

Of the 21 patients with FCL-F CBCL, 14 were male and 7 were female (age range, 33-88 years; mean, 55 years). The duration of the cutaneous disease before the diagnosis of lymphoma ranged from 1 month to 20 years (median, 12 months). The lesions (in descending order of frequency) consisted of red to violaceous nodules, plaques, and papules. The lesions were found in the neck and head areas in 19 patients and in the trunk and limbs in 2 patients. Radiotherapy was the initial treatment in 17 patients, surgical excision in 2 patients, chemotherapy in 1 patient, and hydroxychloroquine plus prednisone in 1 patient. The reason for hydroxychloroquine and prednisone as the initial treatment in the latter patient was the photodistribution of the lesions. CLINICAL follow-up was 1.0 to 11.3 years (mean, 6.3 years). Contact was lost with only 2 patients, after prolonged periods of follow-up (81 and 91 months). The disease recurred in the skin of 12 patients (58%), 3 of whom also had lymph node involvement and 1 of whom also had visceral metastases. The lymph node involvement occurred 2 years after the initial therapy in 2 patients, and retroperitoneal lymph node and visceral metastases to the gut, stomach, and kidneys appeared 8 years after the initial local radiotherapy in 1 patient, who subsequently died of his disease. The latter patient was the only one (4.8%) who died of his disease during the follow-up period.

### HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL FINDINGS

The lymphoid infiltrate was separated from the epidermis by a grenz zone in all cases (Figures 2, 3, 4, 5, and 6), although occasional epidermotropic and folliculotrophic foci of small lymphocytes were observed in a few cases. The lymphoid infiltrate was “top heavy” in 3 cases; “bottom heavy,” with frequent extension into the subcutaneous fat, in 8 cases (Figure 4); and more evenly distributed throughout the dermis (ie, “bottom and top heavy”) in 10 cases. Infiltration of the hair follicle smooth muscle by lymphoid cells was present in 9 cases.

The general architecture of the infiltrate was predominantly nodular. Clear folliculolike structures were frequently observed under low-power magnification (Figure 2). These aggregates were often surrounded by...
incomplete or thin mantle zones (Figures 2 and 3.) In other cases, the folliclelike structures were discernible only under high-power magnification (Figures 4, 5, and 6.) In cases with denser interstitial infiltrates, the basic nodular or folliclelike structures were enhanced by staining for the pan-B antigen CD20. The neoplastic nodules were composed of varying proportions of centrocytes and centroblasts that lacked polarity, and tingible body macrophages were usually absent (Figures 3, 5, and 6). The nodules were frequently infiltrated by large numbers of small lymphocytes, many of which were CD3 positive.

In 9 (47%) of 19 of the cases, cytoplasmic CD10 staining was detected in the neoplastic cells composing the nodular aggregates (Figure 7). Membrane staining for CD43 was observed in all 16 cases in which it was performed, but aberrant CD43 staining, ie, staining of the neoplastic B cells, was identified in only 2 cases (13%). Cytoplasmic bcl-2 staining was detected in the neoplastic cells of only 4 (21%) of 19 of the cases in which it was performed (Figure 8).
The results of the Ig light-chain immunohistochemical studies of the paraffin sections were considered to be successful if the neoplastic cells were stained. In this respect, the results were successful in 12 (71%) of the 17 cases in which the immunohistochemical studies were performed. The site of staining was always cytoplasmic, with frequent perinuclear accentuation. The records of the immunohistochemical studies of the frozen sections performed by several other investigators revealed membranous stainings in 15 (88%) of the 17 cases in which they were performed (Figure 9). The immunohistochemical studies of the paraffin sections demonstrated κ light-chain restriction in all of the cases in which the results were successful, except for 1 case in which no restriction was demonstrated. The positive results of the immunohistochemical studies of the frozen sections demonstrated κ light-chain restriction in 8 cases and no restriction in 7 cases. Of the 9 cases in which both the frozen-section and paraffin-section results were successful, 6 showed concordant κ light-chain restrictions, and 3 showed κ light-chain restriction in the paraffin sections and no restriction in the frozen sections.

MOLECULAR STUDIES

The results of the Ig gene rearrangements were positive in 17 (94%) of the 18 cases in which they were performed. The bcl-2 gene rearrangement studies demonstrated junctional sequences specific for t(14;18) at the MBR in 2 (13%) of 15 of the cutaneous FCL-F cases, compared with 9 (75%) of 12 of the nodal FCL-F cases (P = .002). Both of the CBCL cases with bcl-2 gene rearrangements stained positively for the bcl-2 protein.

COMMENT

The different classification schemes that have been used for CBCL pose difficulties in elucidating the incidence of primary CBCL with a follicular pattern. Kerl and Kresbach screened 178 cases of primary and secondary malignant lymphoma of the skin and diagnosed 13 (17%) cases as germinal center–derived lymphomas. Berti
et al\textsuperscript{33} described 3 histopathological patterns in cutaneous germinal center cell lymphoma: follicular, follicular-diffuse, and diffuse. Approximately 10% of the lymphomas in their file were composed of a pure follicular pattern.\textsuperscript{33} Neri et al\textsuperscript{3} used the updated Kiel classification and classified 10 (19\%) of their 52 cases of primary CBCL as centroblastic-centrocytic follicular/diffuse lymphomas. Other authors use the term \textit{primary cutaneous follicular center cell lymphoma} for nodular or diffuse cutaneous lymphoid infiltrates consisting of centrocytes and centroblasts, irrespective of the histological subclassification.\textsuperscript{34,35} This approach has been largely adopted by the European Organization for Research and Treatment of Cancer classification for cutaneous lymphomas.\textsuperscript{1} Some investigators prefer to use the term primary CBCL for all cases of primary CBCL, without further histological subclassification.\textsuperscript{24} Garcia et al\textsuperscript{4} were able to retrieve 9 cases of primary cutaneous follicular lymphoma from their files and classified them according to the working formation of non-Hodgkin lymphomas.\textsuperscript{36} Burg et al\textsuperscript{37} view follicular center cell lymphoma, with mixed small and large cells (FCL-F according to the REAL classification), as the most common subtype, accounting for approximately 40\% of all CBCLs.

In our study, we used the newly formulated REAL classification and found that as many as 68\% of the newly diagnosed primary CBCL cases during a decade were FCL-F. According to the REAL classification, follicular lymphomas must have a follicular component, although they may have diffuse areas.\textsuperscript{38} Centroblastic lymphomas that have a predominantly follicular growth pattern are also classified as FCL-F lymphoma grade III (predominantly large cell).\textsuperscript{39} The inclusion of a pure follicular pattern along with a follicular pattern with diffuse neoplastic-cell areas, under the heading of FCL-F, may increase the proportion of these lymphomas among CBCLs.

The histological features of primary cutaneous MZL include reactive germinal centers with a proliferation of
marginal zone cells; sheets of intercellular plasma cells, sometimes with Dutcher bodies; and surrounding reactive T lymphocytes with and without lymphoepithelial lesions.\(^7\) The neoplastic marginal zone cells express pan-B-cell antigens and monotypic Ig. The plasma cells also are often neoplastic, expressing monotypic Ig.\(^8\) Histological features that help differentiate FCL-F CBCL from cutaneous pseudolymphoma include bottom-heavy infiltrate, lack of polarity and tangible body macrophages, and thin or absent mantle zones. There are also several immunohistochemically targeted antigens that may be helpful, but the presence or absence of Ig light-chain restriction and/or Ig gene rearrangement is currently thought to distinguish between a neoplastic monoclonal and a reactive polyclonal infiltrate.\(^11\) The monoclonality of the follicular neoplastic cells in follicular lymphomas was also recently demonstrated with microsection and gene rearrangement studies.\(^39\)

The results of previous studies of CD10 expression in CBCLs on frozen sections, or frozen sections and paraffin sections, were negative.\(^3,24\) Since CD10 expression has been reported in almost 60% of cases of nodal follicular lymphomas, and rarely in MALT (MZL) lymphomas,\(^3,24\) it has been suggested that its absence supports a relationship between CBCL and MALT lymphomas rather than with follicular center cell lymphomas.\(^11,22\) However, in our study, CD10 was expressed in 9 (47%) of 19 of the FCL-F lymphoma cases. The large number of commercially available antibodies for CD10\(^23\) and the superiority of paraffin sections over frozen sections for the antibody used in the present study\(^14\) might account for the different results in this study compared with previous ones.\(^24\)

As previously demonstrated in European patients,\(^3,21,22\) we also found, in our group of American patients, a significantly lower frequency of bcl-2 gene rearrangements in the FCL-F cases (13%) than in the nodal follicular lymphomas (75%). Both of our patients with FCL-F and molecular evidence of bcl-2 gene rearrangements had an indolent course indistinguishable from that of the rest of their group.

It has been shown that the immunohistochemical expression of bcl-2 protein is not limited to lymphoproliferative disorders in which t(14;18) is present, and it may be detected in normal T lymphocytes, mantle zone B lymphocytes, and a variety of lymphomas, including MALT and pure large cell lymphomas.\(^15,40,41\) Nevertheless, follicular lymphoma cells are usually bcl-2-positive, whereas the germinal center cells of benign follicular hyperplasia are consistently negative.\(^15,40,41\) In cutaneous lymphomas, bcl-2 protein expression was found in 12% to 25% of primary CBCLs and in 52% to 75% of secondary CBCLs.\(^24,41\) Triscott et al\(^44\) demonstrated bcl-2 immunoreactivity in 3 (75%) of their 4 follicular lymphomas, whereas Chimenti et al\(^45\) found it in only 1 (9%) of 11 of their follicle center lymphomas. In the present study, bcl-2 protein immunoreactivity was present in the neoplastic cells of only 4 (21%) of 19 of our FCL-F cases. Two of these 4 positive FCL-F cases also demonstrated bcl-2 gene rearrangements, and in the remaining 2 cases it was not performed. All 4 patients had an indolent course, indistinguishable from that of the rest of their group.

Monoclonal antibodies to CD43 normally stain T cells, natural killer cells, myeloid precursors, macrophages, and plasma cells.\(^49\) Aberrant CD43 expression is more helpful in the evaluation of small cell proliferation because it is much more common in low-grade than in high-grade lymphoma.\(^48\) Ritter et al\(^47\) demonstrated aberrant CD43 expression in the skin lesions of some of their patients with primary and secondary cutaneous lymphomas. In the present study, aberrant CD43 expression was demonstrated in 2 (13%) of 16 FCL-F CBCLs in which it was performed. These results do not indicate a major role for aberrant CD43 expression in FCL-F CBCL.

Staining for \(\kappa\) and \(\lambda\) Ig light chains in paraffin sections was one of the earliest applications of immunohistochemistry in diagnostic hematopathology,\(^48\) yet it continues to be a problem to many laboratories.\(^14\) Staining of copious cytoplasmic Ig light chains in plasma cells is relatively easy. However, demonstrating Ig light chains in B-cell lymphomas has been more challenging, because the staining of the lymphoma cells has to be balanced with the background staining of the ubiquitous Ig in the interstitial space.\(^13\) High-quality fixation methods, new antigen-retrieval techniques, proper titering of the primary antisera, utilization of sensitive detection systems, and interpreting as positive only those cells that have distinct cytoplasmic (rather than membrane) staining have greatly improved the sensitivity of Ig light-chain staining of paraffin sections.\(^12,14\) The Ig light-chain antigen retrieval in the present study was performed by steaming the deparaffinized sections in EDTA. The results of immunohistochemical studies performed on frozen sections were more often successful than those performed on the paraffin sections, although both succeeded in more than two thirds of the cases. On the other hand, the immunohistochemical studies performed on the paraffin sections demonstrated light-chain restriction more frequently than those performed on the frozen sections in the same cases. A bias may be attributable to the different investigators who examined the results of frozen- and paraffin-section studies, as well as to the lack of well-preserved frozen sections for reevaluation in most of the cases. However, it seems more likely that the differentiation of positively stained tumor cells from reactive cells is more difficult in frozen sections than in paraffin sections. In this respect, immunohistochemical studies of paraffin sections may have an advantage over those of frozen sections; however, because such studies may fail altogether, both types of stainings should be attempted in each case.

In summary, according to the present study, FCL-F CBCL in American patients seems to be a common subtype of primary CBCL, with a relatively indolent course. Its relatively high frequency among the CBCLs may be attributable in part to the more encompassing definition of follicular lymphomas by the REAL classification. The FCL-F CBCL has many features in common with the primary nodal FCL-F, except for the low frequencies of bcl-2 expression and bcl-2 gene rearrangements. The clinical course in the positive and negative bcl-2 cases is, however, similar. CD43 is usually not aberrantly expressed, and CD10 expression is present much more frequently than has been previously described. Immunohistochemical studies of frozen sections and of paraffin sections using special antigen-


