Primary Cutaneous B Cell Lymphoma

— A Case Report —

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We report a case of B-cell lymphoma primarily involving the skin in a 12-year-old boy. The histopathologic findings were compatible with those of small lymphocytic type of non-Hodgkin's lymphoma. A cutaneous lesion was the sole manifestation of his disease without any other organ involvement. Immunophenotypic studies and immunoglobulin gene rearrangement with Southern blot analysis determined its lineages and monoclonality with result of B-cell lineage neoplasm, i. d. CD20+, CD23+, CD35+ and rearranged band on JH probe. We treated him with surgical excision and CVP regimen of chemotherapy (cyclophosphamide, vincristine, prednisolone). There is no recurrence or metastasis during the last six months. (Ann Dermatol 6(2) 249-255, 1994)

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Non-Hodgkin's lymphomas develop not infrequently at extranodal sites. The skin ranks third among the most common sites for extranodal non-Hodgkin's lymphomas1. Most cutaneous non-Hodgkin's lymphomas originate from the T-cell line, but about one third of them belong to B-cell lymphomas which undoubtedly include primary B-cell lymphomas on the skin. Considering that skin is not a normal homing site for the B-lymphocytes and that cutaneous B-cell lymphomas may later spread to lymph nodes and other organs, the possibility that primary cutaneous B-cell lymphomas do exist is still a controversial theory.3

Nevertheless, some cases of primary cutaneous B-cell lymphomas, e.g. primary cutaneous follicular center cell lymphomas, are characterized by a strikingly favorable clinical course, a locoregional extension and a very good response to local treatment. Dermatologists are faced with difficulties in distinguishing cutaneous lymphomas from pseudolymphomas when the skin lesion is present as a solitary plaque of short duration and showing the histology of diffuse lymphoid infiltrates and germinal center-like structures in the dermis and subcutaneous tissue.5 Although we can use immunophenotyping to make a more correct diagnosis of cutaneous B-cell lymphomas, such pitfalls as a lack of immunoglobulin expression and admixed reactive B or T cells necessitate our performance of immunoglobulin gene rearrangement analysis for elucidating specific cellular lineage and demonstrating monoclonality.6 We experienced a child case of B-cell lymphoma primarily existing in the skin without any other organ involvement and encountered a clinical difficulty in determining its benignity or malignancy and in predicting its prognosis including treatment regimens. We performed immunophenotypic studies and im-
munoglobulin (Ig) gene rearrangement analysis with the biopsied sections in addition to routine staging work-up procedures.

REPORT OF A CASE

A 12-year-old boy was seen in August, 1992 with a violaceous and smooth surfaced plaque on his left upper arm present since April, 1992. He had been in good health and had not taken anticonvulsant drugs nor had any insect bites or tattoos. Initially the skin lesion was a $2 \times 4$ cm sized oval macule and then progressed into a $4 \times 6.5$ cm sized violaceous infiltrating plaque after 4 months (Fig. 1). He had no fever, malaise, night sweats, weight loss, peripheral lymphadenopathy or hepatosplenomegaly associated with his skin lesion. We performed a punch biopsy whose specimen revealed diffuse lymphoid cells aggregated around blood vessels and skin appendages within the mid- and deep dermis in a bottom-heavy pattern sparing the subepidermal zone of collagens (Fig. 2A, B). We subsequently performed total excision of the skin lesion and systemic survey for detection of any metastasis to other organs, including CBC profile, LFT, immunoglobulin series, peripheral blood smear, bone marrow aspiration and biopsy, radioisotope scans (bone, liver, spleen) and abdominal CT scan without any positive results or abnormal findings. An excisional biopsy showed histologic findings similar to the previous punch biopsy that massive diffuse dermal infiltrates extended into the subcutaneous tissue and most cells were relatively monomorphic small lymphoid cells with a small round or oval nucleus and dense chromatin (Fig. 2C, D). There were germinal center-like foci which contained centroblasts and centrocytes (Fig. 2E). The lymphomatous cells showed mitotic figures in some proportions and were associated with endothelial venules.

Immunophenotypic Studies

We performed immunohistochemical staining procedures with the specimens stored in frozen or paraffin-embedded state. Five $\mu$m-cut sections were stained with their respective antibodies by the standard avidin-biotin complex immunoper oxidase technique or direct immunofluorescence techniques, especially for anti-Ig A, anti-Ig M, and anti-Ig G antibodies (See Table 1). The lymphoid infiltrates reacted with leukocyte common antigen (LCA), CD20 (a pan B cell marker) and CD23 (a marker for early activation B cells) and UCHL1 positive cells appeared mostly in peripheral areas of massive lymphoid infiltrates (Fig. 3A, B). None of the sections expressed immunoglobulins, light chains, CD19 (a pan-B cell marker) or CD35 (a marker for C3b receptor).

Southern Blot Analysis For Immunoglobulin (Ig) Gene Rearrangement

We performed standard Southern blot analysis for detection of Ig gene rearrangement on DNA extracts from frozen tissues of the skin biopsy. As the control, we used paraffin blocks of normal lymph nodes and intestinal lymphoma specimens obtained from other clinical sources. From these, DNA was extracted from tissue specimens by a phenol-chloroform method, digested with each restriction enzyme as indicated for respective probes (Bam HI + Hind III for JH probe; Eco RI for Ck probe; Eco RI + Hind III for Ck probe), size-fractionated by electrophoresis on a 0.8% agarose gel, transferred to a nylon membrane, and hybridized with each $^{32}$P-labelled probe. The probes used in this study were a DNA fragment specific for the joining region of the Ig heavy chain gene (JH); a DNA fragment specific for the constant region of the $\kappa$ light chain gene (Ck); and a DNA fragment specific for the constant region of the light chain gene (Cl). All probes were provided by Prof. KW Kim (Pusan National University, Pusan). Results were determined by autoradiography. Southern blot analysis using the JH probe revealed Bam HI + Hind III digest which contained one rearranged nongerm-line band (Fig. 4). Another restriction enzyme digests hybridized with Ck or Cl probes revealed only germ-line bands (Table 2).

Treatment and Clinical Course

We concluded that the patient had a malignant lymphoma of small lymphocytic type with low histologic grade. For treatment, we performed 6 months of chemotherapy with CVP regimen (cyclophosphamide, vincristine, prednisolone) fol-
Fig. 1. A violaceous and smooth surfaced infiltrating plaque on extensor of left upper arm.

Fig. 2. A, Diffuse cellular infiltrates aggregate around blood vessels and appendages within the mid- and deep dermis in a bottom-heavy pattern sparing subepidermal zone (H&E, × 40).

Fig. 2. B. Most of the infiltrating cells were small lymphoid cells with a monomorphic pattern (H&E, × 200).

Fig. 2. C, Massive diffuse infiltrates extended into the subcutaneous fat tissue (H&E, × 40).

DISCUSSION

Although uncommon in non-Hodgkin's lym-
Fig. 2. D, Most of the infiltrating cells have a small round or oval nucleus with dense chromatin and some show mitotic figure (H&E, × 200).

Fig. 2. E, A proliferating center is composed of large cells with vesicular nuclei and prominent nucleoli (H&E, × 100).

Table 1. Details of antibodies used in immunohistochemical staining procedures and results of their reactivity

<table>
<thead>
<tr>
<th>Antibodies (Dako, USA)</th>
<th>Specificity</th>
<th>Sections</th>
<th>Methods</th>
<th>Results</th>
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<tr>
<td>CD 45</td>
<td>LCA</td>
<td>paraffin</td>
<td>immunoperoxidase</td>
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<td>anti-kappa</td>
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<td>immunoperoxidase</td>
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</tr>
<tr>
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<td>α heavy chain</td>
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<tr>
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<td>μ heavy chain</td>
<td>frozen</td>
<td>direct IF</td>
<td>-</td>
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<tr>
<td>anti-Ig G</td>
<td>γ heavy chain</td>
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<td>direct IF</td>
<td>-</td>
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<td>frozen</td>
<td>immunoperoxidase</td>
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<tr>
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<td>UCHEL 1</td>
<td>pan T cells and some B-cell lymphomas</td>
<td>paraffin</td>
<td>immunoperoxidase</td>
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Table 2. Probes and their restriction enzymes used in southern blot analysis and results

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Probes</th>
<th>JH Restriction enzymes</th>
<th>Cα Restriction enzymes</th>
<th>Cκ Restriction enzymes</th>
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<tbody>
<tr>
<td>Cutaneous lymphoma</td>
<td>Rearranged</td>
<td>Bam HI + HindIII</td>
<td>Germline</td>
<td>Germline</td>
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<tr>
<td>Lymph nodes (control)</td>
<td>Germline</td>
<td>EcoRI + HindIII</td>
<td>Germline</td>
<td>Germline</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Eco RI</td>
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Fig. 3. A, The lymphoid infiltrates react with CD20 (PAP, × 100).
B, The lymphoid infiltrates react with CD23 (PAP, × 100).

B-cell lymphomas, there is increasing recognition that cutaneous B-cell lymphomas do exist. Whether primary in the skin or secondary to nodal involvement, cutaneous B-cell lymphomas show some clinical, histopathologic, immunophenotypic, and genotypic features that in most cases allow differentiation from cutaneous T-cell lymphomas. The prognosis for primary cutaneous B-cell lymphomas is reasonably good, whereas it is much poorer for cases with nodal disease with secondary skin involvement.

When dermatologists are faced with histologic sections of patchy lymphocytic infiltration in the dermis, we are apt to presume five Ls in their differentiation. Cutaneous B-cell lymphomas are particularly difficult to distinguish from pseudolymphomas histologically. To make the correct diagnosis, it is emphasized that more detailed techniques should be used including immunophenotyping and Southern blot analysis of immunoglobulin and T-cell receptor gene rearrangements.

Although we can accept the fact that malignant lymphomas as neoplasms of the immune system often retain the morphologic, functional and migratory characteristics of their normal counterparts, most immunologic criteria for the diagnosis of lymphoid malignancy are putative except for immunoglobulin light-chain restriction as evidence of monoclonality and of B-cell lineage.

The demonstration of clonal immunoglobulin or T-cell receptor gene rearrangements by Southern blot analysis has proved to be a sensitive marker of lineage and clonality in human lymphoid malignancies. However the determination of clonality as detected by an immunoglobulin
gene rearrangement is not, by itself, diagnostic of malignancy, because the Southern blot technique is so sensitive as to detect clonal population at the level of only 1 to 5% of the total cells. Besides this, it must be remembered that heavy chain gene rearrangements may occur in some T cells. Thus without other evidence such as immunophenotypic study, one cannot be absolutely certain that a neoplasm is B-cell type when only a heavy-chain gene is rearranged and all light-chain alleles are germ line.

Our case is very rare in that B-cell lymphoma has occurred primarily in the skin and cutaneous lesion is the sole manifestation of the disease. Although we cannot completely exclude the possibility of later spreading to lymph nodes and other organs, we expect the disease will have an indolent course of long duration because primary cutaneous B-cell lymphoma show a very low tendency to extracutaneous spread despite relatively frequent cutaneous relapses.

Cutaneous B-cell lymphoma is histologically characterized by the so-called B-cell pattern. Infiltrates usually form sharply demarcated perivascular and periappendageal aggreages within the mid- and deep dermis, with frequent sparing of the subepidermal zone of collagen (so-called grenz zone). The B cell pattern alone, however, is not diagnostic of malignancy and may be seen in cutaneous pseudolymphoma.

Small lymphocytic lymphoma in the skin shows the typical B cell pattern and the infiltrates have a small normal or oval nucleus with inconspicuous cytoplasm and dense chromatin. There is no formation of germinal centers in general but the blast cells may be found in small, evenly distributed foci known as pseudofollicles or proliferation centers as in our case.

'Paraimmunoblasts' and 'prolymphocytes' (centrocytes) appear in each proliferative center. The former are rather small with a 'vesicular' nucleus containing a prominent central nucleolus and the latter are intermediate in size and appearance between the lymphocytes and blast cells. The presence of confluent masses of atypical blast cells may signal the transformation of B-lymphocytic lymphoma into a high-grade malignant lymphoma, generally of immunoblastic type (Richter's syndrome). It is still uncertain whether there is a clearly separable entity of B-lymphocytic lymphoma which does not progress to B-type chronic lymphocytic leukemia.

Immunophenotypic criteria for the diagnosis of B-lineage lymphoma can be subdivided into two categories: 1) those that directly reflect clonality (Ig light chain restriction) and 2) those that reflect abnormal antigen expression (light chain-B lineage, Leu 1+ B lineage, 41 H+ proliferating B lineage, loss of pan-B antigens, LFA-1-B lineage). In general, the pan-B antigens such as CD22, CD20, CD19 and MB-1 appear to be coexpressed on all identifiable B cells (vascula cell excluded) in peripheral lymphoid organs and in benign reactive lymphoid proliferations. But this is not the case in B-lineage lymphoma-leukemia, where a considerable numbers of cases lack one or more pan-B antigens without any exception in histologic type. Absence of staining by one or two pan-B reagents does not necessarily rule out the possibility of a B-lineage neoplasm, and therefore multiple pan-B markers as well as plasma cell marker may be required to identify B-lineage differentiation in a given case.

As a analogous counterpart to small lymphocytic lymphoma, chronic B cell leukemia (B-CLL) re-
sembles the small peripheral blood B cells. However, in contrast to peripheral blood B cells, B-CLLs generally do not express CD35(C3b receptor), as seen in our case. When normal B cells are activated by anti-immunoglobulin, they express a variety of B-cell-restricted and associated activation antigens, including B5, Blast-1, CD23, Bac-1, and CD25 without expression of CD5, which are expressed in a subset of B cells stimulated with phorbol ester. These studies suggest that B-CLLs phenotypically resemble a subset of normal in vitro activated B cells16.

There may be a hypothesis that cutaneous pseudo-B-cell lymphomas and primary cutaneous B cell lymphomas are part of a continuous and progressive spectrum of B-cell lymphoproliferative disorders. In fact, differentiating between pseudo-B-cell lymphomas and cutaneous B-cell lymphomas is not always possible by means of gene rearrangement analysis because immunohistochemically defined cutaneous pseudo-B-cell lymphomas often contain occult monoclonal B-cell populations2. In our case, immunoglobulin gene rearrangement study revealed only a rearranged band on JH probe and a germ line band on C\alpha and C\kappa probes. It is impossible to demonstrate B-cell clonality because heavy-chain gene rearrangements may occur in some T-cells. But we concluded B-cell markers such as CD20 and CD23 were determined by immunophenotyping and were considered as complementary evidence to immunoglobulin gene rearrangement study results.

REFERENCES