The (t;2;5) Chromosomal Translocation Is Not a Common Feature of Primary Cutaneous CD30+ Lymphoproliferative Disorders: Comparison With Anaplastic Large-Cell Lymphoma of Nodal Origin

By John F. DeCoteau, Janet R. Butmarc, Marsha C. Kinney, and Marshall E. Kadin

Primary cutaneous CD30+ lymphoproliferative disorders (LPDs), including lymphomatoid papulosis (LyP), anaplastic and nonanaplastic CD30+ large-cell lymphoma, and borderline cases, comprise a clinical and histologic spectrum. Primary cutaneous and primary nodal CD30+ anaplastic large-cell lymphomas (ALCLs) are distinct clinical entities that have identical morphologic features but differ in age of onset, immunophenotype, and prognosis. It can be difficult to distinguish primary cutaneous from nodal ALCLs that secondarily involve the skin, which is important because these diseases differ significantly in response to treatment and clinical outcome. The (t;2;5) chromosomal translocation is highly associated with primary CD30+ ALCL of nodal origin. The possible occurrence of (t;2;5) in primary cutaneous CD30+ LPDs has not been studied extensively, and it remains to be determined if expression of this translocation can be used to distinguish primary cutaneous ALCL from nodal ALCL that secondarily involves the skin. To address these issues, we studied 43 cases of cutaneous and nodal CD30+ LPDs using reverse transcriptase-polymerase chain reaction (RT-PCR) and/or immunohistochemistry. We found no evidence for the (t;2;5) translocation in 14 cases of primary cutaneous CD30+ LPDs, which included 10 cases of LyP, three cases of primary cutaneous CD30+ ALCL, and one borderline case. These findings were in marked contrast to CD30+ ALCL of nodal origin, in which 19 of 29 (66%) cases were positive for (t;2;5), including all five cases with secondary skin involvement. Our results support the hypothesis that (1) primary cutaneous CD30+ LPDs (including LyP) and primary nodal ALCL are distinct diseases that differ in clinical behavior and pathogenesis and (2) differential expression of (t;2;5) can help to distinguish between primary cutaneous CD30+ LPDs and ALCL of nodal origin.

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the NPM/ALK fusion, it is believed to be a reliable marker of the p80 fusion protein that results from the t(2;5) translocation, because ALK expression has not been detected in the hematopoietic system. In support of this, Shiota et al., in a study of 50 lymphomas, found positive p80-staining reactions exclusively in cases found to have the t(2;5) by cytogenetics and RT-PCR analysis.

The possible occurrence of the t(2;5) chromosomal translocation in primary cutaneous CD30+ LPDs has not been studied extensively, and it remains to be determined if differential expression of the t(2;5) chromosomal translocation can be used to distinguish primary cutaneous ALCL from nodal-based ALCL that secondarily involves the skin. To address these issues, we studied 43 cases of cutaneous and nodal CD30+ LPDs using RT-PCR and/or immunohistochemistry.

MATERIALS AND METHODS

Patients. Patient biopsy material to be studied was identified by reviewing the files of Beth Israel Hospital (Boston, MA) and Vanderbilt Medical Center (Nashville, TN). Patients with CD30+ lymphoma who had frozen tissue available for RNA extraction were included in the study. Additional cases to be studied by immunohistochemistry only were selected on the basis of large-cell morphology and available correlative immunophenotypic and clinical data. Immunophenotypic data were obtained using standard immunoperoxidase techniques performed on Formalin-fixed, paraffin-embedded sections. Monoclonal antibodies used included CD45 (LCA; Dako), CD30 (Ber-H2; Dako), CD45RO (UCHL-1; Dako), CD3 (Dako), CD43 (Leu 22; Becton Dickinson, San Jose, CA), and CD20 (L26; Dako). Cases were defined as T lineage if they reacted with one or more antibodies directed against the T-cell antigens, CD45RO, CD3, and CD43, and lacked reactivity for the B-cell–associated antigen, CD20. A B-cell lineage was assigned if the opposite pattern of reactivity was observed, and a null phenotype was assigned to cases that did not express either T- or B-cell–associated markers. Childhood cases were defined as occurring in patients 18 years of age or younger. The primary cutaneous CD30+ LPDs included 10 cases of LyP, three cases of primary cutaneous CD30+ ALCL without associated lymphadenopathy, and one borderline case. Borderline cases were defined as lesions with the clinical behavior of LyP but having histologic features of CD30+ LCL. These lesions were characterized by clusters of large atypical CD30+ cells, an inflammatory background, pseudopitheliotomatous hyperplasia, and absence of extension into the subcutis. All of the cutaneous CD30+ LPD cases, with the exception of one LyP case, were from adult patients. Twenty-nine cases of nodal-based CD30+ ALCL were studied, including 11 adult cases and 18 childhood cases. In two of the adult cases and three of the childhood cases, patients manifested skin lesions in addition to lymphadenopathy. We compared these cases with 14 childhood cases and one adult case of Ki-1-negative LCL of nodal origin, five adult cases of Hodgkin’s disease, and five adult cases of mycosis fungoides. Statistical differences were analyzed by Fisher’s exact test.

RT-PCR. Total cellular RNA was isolated by the RNeasy B method according to the manufacturer’s instructions (Cinna/Biotex, Friendswood, TX). Single-stranded cDNA was then prepared from total cellular RNA by reverse transcription according to the manufacturer’s recommendations (Perkin-Elmer, Branchburg, NJ). The cDNA was amplified in the presence of 1 U Taq polymerase (Cetus, Emeryville, CA) and the appropriate 5’ and 3’ primers in a total volume of 20 µL. The amplification conditions were denaturation at 95°C for 1 minute, annealing at 54°C for 45 seconds, and extension at 72°C for 2 minutes. At the end of 35 cycles, PCR products were electrophoresed through 1.6% agarose gels containing ethidium bromide in Tris-borate-EDTA (TBE) buffer. Gels were then visualized with UV light and photographed. Amplification of the fusion message of the t(2;5)(p23;q35) translocation used primer sequences reported by Morris et al. 5’NPM, 5’-TCCCTGGGGGCTT-TGAATAAACCC-3’, and 3’ ALK, 5’-CGAGGTGCGGAG-CTTGCTCAGC-3’. In all cases, amplification of human β-actin mRNA was performed in parallel using commercially obtained primers (Clontech, Palo Alto, CA) to control for integrity of the cDNA and PCR reactions. Positive controls included cDNA obtained from a CD30+ ALCL cell line, JB-6, shown by cytogenetics to be positive for the t(2;5) translocation. Negative controls to exclude contaminating genomic DNA as a source of false-positive reactions were prepared by performing the cDNA synthesis reaction in the absence of RT. In addition, lymph node biopsy specimens from three cases of follicular lymphoma and one case of reactive lymph node hyperplasia were used as negative patient controls.

Identification of the p80 protein by immunohistochemistry. A three-stage avidin-biotin immunoperoxidase method was used to detect p80, the fusion protein product of the chimeric NPM-ALK gene. The anti-p80 antibody, a rabbit polyclonal IgG that recognizes an epitope of the kinase domain of the p80 fusion protein, was a kind gift from Dr Shigeo Mori (Institute of Medical Science, University of Tokyo, Tokyo, Japan). Details of the generation of this antibody have been previously published.17 Formalin-fixed, paraffin-embedded sections were microwaved at high power for 8 to 10 minutes in 0.01 mol/L citric acid solution, pH 6.0. Sections were overlaid with 200 µL anti-p80 antibody diluted 1:10,000 in PBS/BSA and incubated for 1 hour at room temperature. Sections were then washed in PBS and overlaid with 200 µL biotin-conjugated swine antirabbit Ig antibody diluted 1:200 in PBS and incubated for 1 hour at room temperature. Sections were then washed repeatedly in PBS and incubated with 200 µL of a 1:400 dilution of streptavidin-horseradish peroxidase conjugate for 1 hour at room temperature. Slides were stained with 0.2 mg/mL 3,3’-diaminobenzidine tetrahydrochloride in PBS containing 0.01% H2O2 for 5 minutes. Sections were washed and counterstained with 2% Methylgreen that had been chloroform-extracted. Positive controls included sections from cases of CD30+ ALCL known to bear the t(2;5) translocation. Negative controls included lymph node biopsies from five adult cases of Hodgkin’s disease, including four cases of the syncytial variant of nodular sclerosis and one case of mixed cellularity, and skin biopsies from five adult patients with mycosis fungoides.

RESULTS

Analysis of the t(2;5) translocation by anti-p80 staining is shown in Fig 1, and by RT-PCR in Fig 2. The results are summarized in Table 1. None of the cases of primary cutaneous CD30+ LPD were positive for t(2;5). This included 10 cases of LyP studied by RT-PCR and p80 immunostaining, three cases of primary cutaneous CD30+ ALCL, two of which were studied by RT-PCR and p80 immunostaining, and one of which was studied by p80 immunostaining alone, and a borderline case studied by p80 immunostaining alone. The 11 adult cases of CD30+ ALCL of nodal origin included 10 of T-cell phenotype and one null phenotype. Of these, two cases were found to express t(2;5) by RT-PCR and immunohistochemistry, five cases studied by p80 immunohistochemistry alone were positive, three cases were negative by both immunohistochemistry and RT-PCR, and one
case was negative by immunohistochemistry alone. Within this group of adult patients, each of two with secondary skin involvement were positive for p80 when studied by immunohistochemistry alone. Of seven positive adult cases, three were prototypic ALCL and included the single null phenotype case, one was a small-cell variant of ALCL, and three were monomorphic variants of ALCL. The childhood cases of CD30+ ALCL consisted of 17 cases of T-cell phenotype and one case of null phenotype. The t(2;5) translocation, inferred from expression of p80 by immunohistochemistry, was identified in the tumors of 12 of 18 (67%) children with CD30+ ALCL of nodal origin. One of these cases positive by immunohistochemistry was studied by RT-PCR and found to express the NPM-ALK fusion transcript. The positive cases included all three children who had skin lesions in addition to lymphadenopathy and three of five cases of the monomorphic variant of ALCL in lymph nodes and the single case of null phenotype. Three of 14 childhood cases of CD30+ LCL, all of nodal origin and B-cell phenotype, were positive for p80, while the single adult case of nodal CD30− LCL was p80-negative. In all cases positive for p80 protein by immunohistochemistry, the staining reaction was limited to the cytoplasm of the cytologically atypical malignant cells. The five cases of Hodgkin’s disease and the five cases of cutaneous T-cell lymphoma were negative when studied by immunohistochemistry, and the three cases of follicular lymphoma and the single case of reactive lymphoid hyperplasia were negative when studied by RT-PCR.

DISCUSSION

The association of nonrandom chromosomal abnormalities with biologically and clinically distinct subgroups of lymphomas has been clearly established. Several studies of the t(2;5) chromosomal translocation in non-Hodgkin LCLs have suggested that LCLs containing t(2;5) may represent a distinct genetic subgroup that has variable histologic and immunophenotypic characteristics. Although t(2;5) is most frequently associated with CD30+ ALCL of nodal origin, it has also been identified in other LCL subtypes, including CD30− LCL. An important question that

<table>
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<tr>
<th>Disease</th>
<th>p80-Positive</th>
<th>RT-PCR-Positive</th>
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<tr>
<td>Lymphomatoid papulosis</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>Primary cutaneous CD30+ ALCL</td>
<td>0/3</td>
<td>0/2</td>
</tr>
<tr>
<td>Borderline</td>
<td>0/1</td>
<td>ND</td>
</tr>
<tr>
<td>Nodal CD30+ ALCL</td>
<td>19/29</td>
<td>4/7</td>
</tr>
<tr>
<td>Nodal CD30− LCL</td>
<td>3/15</td>
<td>ND</td>
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Abbreviation: ND, not determined.
has not been resolved in previous studies of the t(2;5) translocation is whether this genetic abnormality identifies a clinically relevant subtype of non-Hodgkin’s lymphoma.

Cutaneous CD30+ LPDs comprise a histologic and clinical spectrum of related lymphoma entities in which malignant cells resemble the malignant cells of CD30+ lymphomas of nodal origin. The most aggressive of these cutaneous LPDs, CD30+ ALCL, may be confused with CD30+ ALCL of nodal origin that secondarily involves the skin. It is important to distinguish between these diseases because they vary in response to treatment and clinical outcome. Primary cutaneous CD30+ ALCL can be treated by local excision, radiotherapy, or low-dose methotrexate chemotherapy, whereas nodal and secondary cutaneous CD30+ ALCLs require multiagent chemotherapy. With this in mind, we used RT-PCR and immunohistochemical approaches to compare expression of the t(2;5) chromosomal translocation in cutaneous CD30+ LPDs versus CD30+ ALCLs of nodal origin, including cases with secondary involvement of the skin.

We found no evidence for the t(2;5) translocation in 14 cases of primary cutaneous CD30+ LPDs. These results were in marked contrast to the results of our analysis of CD30+ ALCL of nodal origin, in which 19 of 29 (66%) cases were found to be positive for t(2;5), including all five cases that had secondary skin involvement.

The high frequency of t(2;5)-positive cases in our series of nodal ALCL is in agreement with the results of Wellmann et al., who reported 11 of 17 (65%) positive ALCL cases, but it is much higher than in the study reported by Lopategui et al. A possible explanation is that the fact that our series of CD30+ ALCL, in contrast to that of Lopategui et al, had no cases of B-cell ALCL. In their study, five of 17 T-cell ALCL cases were t(2;5)-positive by PCR, as compared with only one of 15 cases of B-cell ALCL. They also reported two Asian patients with cutaneous lesions that were positive for t(2;5) by RT-PCR, whereas Wellmann et al did not detect t(2;5) in four cases of primary cutaneous ALCL, in agreement with our results. Our finding of none of three cases of primary cutaneous ALCL being positive for p80, as compared with nodal-based ALCL, in which 19 of 29 cases were t(2;5)- and/or p80-positive, is interesting but did not achieve statistical significance (P = .058). A recent Japanese study also detected a low percentage (four of 21) of p80-positive “primary” cutaneous ALCL; LyP was not evaluated. These studies, together with our own, indicate that t(2;5) is absent in LyP and rare, if it occurs at all, in primary cutaneous CD30+ ALCL. Further studies of primary cutaneous CD30+ LPDs are needed to determine the exact frequency of t(2;5) in primary cutaneous ALCL, although it is clearly much lower than in primary nodal ALCL.

In our study, cases of small-cell and monomorphic variants of ALCL were also found to be t(2;5)-positive. In addition, our results support the findings of other studies that have shown that t(2;5) is not limited to ALCL, since three of 15 cases of B-cell LCL in our study were t(2;5)-positive. In conclusion, our results support the hypothesis that (1) primary cutaneous CD30+ LPDs, including LyP, and primary nodal ALCL are distinct diseases that differ in clinical behavior and pathogenesis, and (2) differential expression of t(2;5) can be used to help distinguish between primary cutaneous CD30+ LPDs and ALCL of nodal origin.

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The t(2;5) chromosomal translocation is not a common feature of primary cutaneous CD30+ lymphoproliferative disorders: comparison with anaplastic large-cell lymphoma of nodal origin [see comments]

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