Clinicopathologic Features and Treatment Outcome of Children With Large-Cell Lymphoma and the t(2;5)(p23;q35)


The t(2;5)(p23;q35) chromosomal rearrangement has been observed in cases of large-cell non-Hodgkin lymphoma (NHL) defined by the National Cancer Institute (NCI) Working Formulation as large-cell, immunoblastic (polymorphous subtype) and by the Kiel classification system as anaplastic (coherent sheets of cells containing abundant cytoplasm, indented lobulated nuclei, and prominent nucleoli, invading lymph node sinuses). Among patients with large-cell NHL, there exists an association between the presence of the (2;5) translocation and expression of CD30, an activation antigen first identified in Reed-Sternberg cells. CD30 is expressed in approximately 40% of pediatric large-cell NHL cases as defined by the NCI Working Formulation and in about 90% of those with anaplastic features (Kiel)6,11,12; however, the frequency of CD30 expression among large-cell NHL cases with the t(2;5) has yet to be determined. Phenotypically, the majority of CD30+ large-cell NHLs exhibit T-cell antigens, although usually in an incomplete or aberrant fashion; the tumor cells also express epithelial membrane antigen and leukocyte common antigen.15

CD30+ large-cell NHLs exhibit a bimodal age distribution (resembling that of Hodgkin’s disease) and frequent involvement of extranodal disease sites, including skin, lung, bone, soft tissue, and the gastrointestinal tract. Preliminary data suggest that for children with large-cell NHL, CD30 expression does not influence event-free survival, but may be associated with a better overall survival for patients with advanced-stage disease. Less is known about the prognostic significance of the t(2;5) among children with large-cell NHL.

The partially overlapping nature of CD30+ large-cell NHLs (NCI Working Formulation), anaplastic large-cell NHLs (Kiel), and large-cell NHLs containing the t(2;5) has created some confusion as to the distinction between these entities. Do they, in fact, represent one disease or a spectrum of one disease type? To address this issue, we have studied the histologic features, frequency of CD30 expression, clinical features, and treatment outcome of 9 children with t(2;5) containing large-cell NHLs treated at St Jude Children’s Research Hospital, in addition to a review of the literature.

PATIENTS AND METHODS

Patient characteristics. One hundred fifteen children with biopsy-proven large-cell NHL (NCI Working Formulation) were evaluated at St Jude Children’s Research Hospital from 1975 through 1993. Of these, 57 did not have tissue submitted for cytogenetics, 21 had insufficient quantity of tissue submitted, 18 had morphologically noninvolved bone marrow (BM) samples evaluated in which no chromosomal abnormality was identified, and 18 had tissue submitted (4 in BM and 14 in tumor samples) in which a karyotypic abnormality was found. The t(2;5)(p23;q35) chromosomal abnormality was identified in 9 of the 18 cases with abnormal karyotypes (50%). The six boys and three girls had a median age at diagnosis of 10 years (range, 2 to 16 years). Their lymphomas were staged and classified according to the St Jude system as previously described.12,23

Immunophenotyping. Antibodies were used to detect the CD30 antigen (Ber H2; Dako, Santa Barbara, CA), and the T- and B-cell associated antigens, which included CD45 (leucocyte common antigen; Dako), CD3 (Dako), CD43 (M-1; Biotest, Danville, NJ), CD45Ro (UCHL-1; Dako), CD20 (L26; Dako), MB-2 (Biotest), and CD45Rα (4KB5; Dako).

Primary antibodies were detected using biotinylated secondary antibodies (Vector Laboratories, Burlingame, CA), followed by alkaline phosphatase-conjugated Streptavidin (Biogenex, San Ramon, CA). The chromogen was developed with Fast Red TR and tissues were counterstained with hematoxylin. Tryptsin digests were performed before CD3 and CD30 analysis. After deparaffinization, mercury pigmentation was removed from B-5–fixed tissue; this step was omitted for Ber H2 reactions. All procedures were performed on an automated stainer (Code-On; Instrumentation Labs, Lexington, MA).

Samples were interpreted as CD30+ if the majority of the tumor cells was CD30+.
cases in adequately fixed portions of the specimen displayed a membrane and/or Golgi pattern of staining. Cases were determined to be either T-cell NHL if one or more T-cell-associated antibodies (UCHL-1, CD3, or MT-1) reacted and B-cell antibodies did not, or, conversely, were determined to be B-cell if one or more B-cell-associated antibodies (L26, 4K5, or MB-2) reacted and antibodies to T-cell markers did not. In rare instances, MB-2 reacted with normal BM in addition to lymph node samples. The abnormal karyotype in 1 patient (case no. 2 through 7 and 9) was identified in BM cells; no lymphoid tissue was available for study. Two patients (cases no. 3 and 9) had karyotypic analysis performed in morphologically normal BM in addition to lymph node samples.

Cyogenetics. The lymph node or tumor mass suspensions were processed immediately or after culture for 24 to 48 hours. Briefly, the cell suspensions were exposed to colcemid for 45 minutes, to two or more changes of hypotonic solution for 7 to 10 minutes, and to 1% acetic acid in 10% ethanol for 5 minutes each, and then G-banded by trypsin and Wright's stain. The chromosome abnormalities are described according to the International System for Human Cytogenetic Nomenclature (ISCN, 1991).26

RESULTS

The complete karyotypes of these patient's tumor samples are listed in Table 1. Each patient shared an identical reciprocal translocation involving the short arm of chromosome 2 band p23 and the long arm of chromosome 5 band q35. The modal chromosome number for these cases was diverse; 3 were diploid (46), 2 near tetraploid, and modal numbers of 45, 47, 49, and 66 were found in the other cases. The karyotypes of BM samples taken from patients no. 3 and 9 were normal. In most cases, the karyotypes were very complex and the only discernable common features among them other than the t(2;5) were breakpoints at 1p22, 3q29, and 19q13. The complete karyotypes of these patient's tumor samples were listed in the Table 1. Each patient shared an identical reciprocal translocation involving the short arm of chromosome 2 band p23 and the long arm of chromosome 5 band q35. The modal chromosome number for these cases was diverse; 3 were diploid (46), 2 near tetraploid, and modal numbers of 45, 47, 49, and 66 were found in the other cases. The karyotypes of BM samples taken from patients no. 3 and 9 were normal. In most cases, the karyotypes were very complex and the only discernable common features among them other than the t(2;5) were breakpoints at 1p22, 3q29, and 19q13 identified in two cases each.

Table 2 summarizes the clinical features and the heterogeneous histopathologic and immunophenotypic features of these cases. According to the NCI Working Formulation, 2 were diffuse large-cell and 7 large-cell, immunoblastic. According to the Kiel classification, 6 were anaplastic large-cell, 2 immunoblastic large-cell, and 1 centroblastic. Expression of CD30 was present in 6 of 8 cases tested; CD30 expression in 1 case was focally positive. One patient (case no. 1) was not studied for CD30 expression because of an insufficient quantity of tumor sample. Sites of disease included lymph node (n = 9), bone (n = 4), spleen (n = 3), skin (n = 3), mediastinum (n = 1), testicle (n = 1), and BM (n = 1). None had involvement of the central nervous system. The mean serum lactate dehydrogenase (LDH) level at the time of diagnosis was 240 U/L (range, 118 to 478 U/L).

TREATMENT

Complete remission was achieved in all patients, although case no. 5 failed to respond completely to CHOP therapy and required involved-field radiation and the DHAP regimen (dexamethasone–cisplatin–high-dose cytarabine) to attain complete remission (CR). Although 3 children suffered a relapse, 2 remain alive in second remission for 58+ and 80+ months after DHAP and autologous BM transplantation (BMT); 1 achieved a short second complete remission with methotrexate, VP-16, and ifosfamide followed by autologous BMT, but subsequently died of recurrent disease.

DISCUSSION

The 9 cases reported expand the previously observed association between the (2;5)(p23;q35) translocation and large-cell NHL and suggest that the disease in children shares striking similarity to that observed in young adults.24 We found that, although the t(2;5) was associated with anaplastic morphology, it was not restricted to a specific histologic
Table 2. Clinical and Laboratory Findings in 20 Children With Large-Cell NHL and the t(2;5)(p23;q35) Histologic Classification Survival

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age* (yr)</th>
<th>Sites of Disease</th>
<th>Stage</th>
<th>St Jude</th>
<th>Histologic Classification</th>
<th>NCI Working Formula</th>
<th>Kiel</th>
<th>Phenotype</th>
<th>Primary Chemotherapy</th>
<th>Time to Relapse (mo)</th>
<th>Survival From Diagnosis (mo)</th>
<th>Ref</th>
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<tbody>
<tr>
<td>1*</td>
<td>7</td>
<td>Node (cervical, axillary) Bone Skin</td>
<td>III</td>
<td>LC, immunoblastic</td>
<td>ALCL</td>
<td>T-cell CD30 (NDI)</td>
<td>MACOP-B</td>
<td></td>
<td></td>
<td>7</td>
<td>65+</td>
<td>Present study</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>Node (cervical, supraclavicular, paraaortic) Skin</td>
<td>III</td>
<td>LC, immunoblastic</td>
<td>Immunoblastic</td>
<td>T-cell CD30*</td>
<td>MACOP-B</td>
<td></td>
<td></td>
<td>7</td>
<td>87+</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>Node (cervical, supraclavicular, retroperitoneal) Bone Pleural effusion, mediastinum Spleen</td>
<td>III</td>
<td>Diffuse LC</td>
<td>Centroblastic</td>
<td>B-cell CD30+</td>
<td>MACOP-B</td>
<td></td>
<td></td>
<td>73+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>Bone Node (cervical, axillary, supraclavicular, inguinal, femoral)</td>
<td>III</td>
<td>LC, immunoblastic</td>
<td>ALCL</td>
<td>T-cell CD30*</td>
<td>CHOP</td>
<td></td>
<td></td>
<td>99+</td>
<td></td>
<td></td>
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<tr>
<td>5</td>
<td>10</td>
<td>Nodes (cervical) Skin</td>
<td>II</td>
<td>LC, immunoblastic</td>
<td>Immunoblastic</td>
<td>Null-cell CD30*</td>
<td>CHOP + DHAP + IFRT</td>
<td>MACOP-B</td>
<td></td>
<td>25</td>
<td>Died†</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>Mediastinum, pleural effusion Nodes (axillary, supraclavicular)</td>
<td>III</td>
<td>LC, immunoblastic</td>
<td>ALCL</td>
<td>T-cell CD30*</td>
<td>DAC</td>
<td></td>
<td></td>
<td>21+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>Nodes (inguinal, iliac); pelvic mass</td>
<td>III</td>
<td>LC, immunoblastic</td>
<td>ALCL</td>
<td>Null-cell CD30*</td>
<td>DAC</td>
<td></td>
<td></td>
<td>21+</td>
<td></td>
<td></td>
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<tr>
<td>8</td>
<td>16</td>
<td>Nodes (para-aortic, inguinal); pelvic mass, bone, spleen, bone marrow</td>
<td>IV</td>
<td>Diffuse LC</td>
<td>ALCL</td>
<td>T-cell CD30*</td>
<td>DAC</td>
<td></td>
<td></td>
<td>21+</td>
<td></td>
<td></td>
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<tr>
<td>9</td>
<td>11</td>
<td>Nodes (cervical, hilar, axillary, inguinal), spleen</td>
<td>III</td>
<td>LC, immunoblastic</td>
<td>ALCL</td>
<td>Null-cell CD30*</td>
<td>DAC</td>
<td></td>
<td></td>
<td>21+</td>
<td></td>
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<td>10</td>
<td>12</td>
<td>Nodes, pleural effusion</td>
<td>I</td>
<td>NR</td>
<td>ALCL</td>
<td>T-cell CD30*</td>
<td>None</td>
<td></td>
<td></td>
<td>10+</td>
<td>3</td>
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</tr>
<tr>
<td>11</td>
<td>8</td>
<td>Nodes</td>
<td>II</td>
<td>LC, immunoblastic</td>
<td>NR</td>
<td>T-cell CD30*</td>
<td>CHOP</td>
<td></td>
<td></td>
<td>17+</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>9</td>
<td>Nodes</td>
<td>II</td>
<td>LC, pleomorphic</td>
<td>NR</td>
<td>T-cell CD30*</td>
<td>CHOP</td>
<td></td>
<td></td>
<td>7+</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>18</td>
<td>Nodes</td>
<td>I</td>
<td>NR</td>
<td>ALCL</td>
<td>T-cell CD30*</td>
<td>Chemotherapy</td>
<td></td>
<td></td>
<td>NR</td>
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<td>18</td>
<td>Nodes</td>
<td>III</td>
<td>NR</td>
<td>ALCL</td>
<td>T-cell CD30*</td>
<td>ProMACE/ MOPP</td>
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<td></td>
<td>11+</td>
<td>4, 7</td>
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<tr>
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<td>21</td>
<td>Nodes</td>
<td>III</td>
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<td>ALCL</td>
<td>CD30*</td>
<td>M-BACOD</td>
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<td>11+</td>
<td>4, 7</td>
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<tr>
<td>16</td>
<td>NR</td>
<td>Nodes</td>
<td>NR</td>
<td>Diffuse LC</td>
<td>NR</td>
<td>T-cell CD30, NR</td>
<td>NR</td>
<td></td>
<td></td>
<td>NR</td>
<td>NR</td>
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</tr>
<tr>
<td>17</td>
<td>NR</td>
<td>Nodes</td>
<td>NR</td>
<td>NR</td>
<td>ALCL</td>
<td>T-cell CD30, NR</td>
<td>NR</td>
<td></td>
<td></td>
<td>NR</td>
<td>NR</td>
<td>8</td>
</tr>
<tr>
<td>18</td>
<td>NR</td>
<td>Nodes</td>
<td>NR</td>
<td>Diffuse mixed</td>
<td>NR</td>
<td>T-cell CD30*</td>
<td>NR</td>
<td></td>
<td></td>
<td>NR</td>
<td>NR</td>
<td>8</td>
</tr>
<tr>
<td>19</td>
<td>8</td>
<td>Nodes, ascites</td>
<td>IV</td>
<td>Diffuse LC</td>
<td>NR</td>
<td>B-cell CD30*</td>
<td>NR</td>
<td></td>
<td></td>
<td>48+</td>
<td>9</td>
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<tr>
<td>20</td>
<td>12</td>
<td>Soft tissue</td>
<td>NR</td>
<td>Diffuse mixed</td>
<td>NR</td>
<td>T-cell CD30*</td>
<td>NR</td>
<td></td>
<td></td>
<td>NR</td>
<td>NR</td>
<td>10</td>
</tr>
</tbody>
</table>

Abbreviations: IFRT, involved field radiation therapy; DHAP, dexamethasone-cytarabine-cisplatin; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; MACOP-B, methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone, and bleomycin; DAC, dexamethasone, cytarabine, carboplatin, doxorubicin, 6-mercaptopurine, methotrexate, cyclophosphamide, and L-asparaginase; NR, not reported; LC, large-cell; ALCL, anaplastic large-cell lymphoma.

* Translocation was identified at relapse.
† Died with recurrent disease 40 months from initial diagnosis.
The literature suggests that our observation is supported by the findings of 11 previously reported cases that included a spectrum of NHL histotypes in which the t(2;5) was identified (cases no. 10 through 20, Table 2); however, these case reports did not include both NCI Working Formulation and Kiel classification terminology, making direct comparison difficult.

Interest in CD30 expression has heightened since its recent identification as a new member of the nerve growth factor receptor family, suggesting a potential mechanism for the growth advantage of CD30+ malignant lymphoid cells. CD30 is expressed in approximately 40% of pediatric large-cell NHLs (NCI Working Formulation) and about 90% of pediatric anaplastic NHLs (Kiel). When genetic subtypes of large-cell NHL are considered, CD30 expression is prominent among cases with the t(2;5). CD30 expression was documented in 6 of our 8 cases and in all 9 previously reported cases of large-cell NHL with the t(2;5) for which CD30 expression was determined (Table 2). Although this finding suggests a tight association (about 90%) between CD30 expression and presence of t(2;5), the actual frequency is difficult to define when the small number of cases tested for both features is considered. Interestingly, all the CD30+ cases were either T- or null-cell phenotypes, and the 2 CD30- cases were non-T-cell phenotype (1 MB2+, CD20-, suggesting B-cell; 1 null-cell). CD30 expression was not restricted to any histologic subtype of large-cell NHL as defined by the NCI Working Formulation. According to the Kiel classification, 5 of 6 CD30+ cases had anaplastic morphologies, suggesting an association between CD30 expression and anaplastic morphology among large-cell NHL cases with the t(2;5).

The clinical presentation of our 9 cases was characterized by nodal and extranodal involvement, similar to that of other cases of immunoblastic large-cell lymphomas with the t(2;5) and CD30 expression. The limited number of documented t(2;5)+ large-cell NHL cases in our review precluded a meaningful statistical analysis between them and t(2;5)- cases. However, in our recent study of CD30- versus CD30+ cases of large-cell NHL, there was a significantly higher incidence of skin involvement in the CD30+ group. That the presence of t(2;5) was associated with skin involvement in one-third of our cases is not surprising in light of the apparent association between the presence of the t(2;5) and CD30 expression. Also of note was the wide range of tumor burden at presentation. Our experience suggests that a large proportion of patients have widespread disease. One of our patients presented with stage II disease (head and neck region), whereas the remaining 8 had more advanced stages, including BM involvement in 1 case. Among the 7 case reports for which stage was reported, 4 were limited (stage I or II) and 3 advanced stage (stage III or IV, Table 2). Thus, it appears that the t(2;5) is associated with advanced stage of disease at presentation in children.

The prognostic significance of the t(2;5)(p23;q35) in childhood large-cell lymphomas has yet to be defined. In one review of 10 cases of CD30+ lymphomas, those lacking the t(2;5) had a poorer treatment response. Of the three cases reported by Kaneko et al, one resolved spontaneously, one achieved a partial response, and one had a complete response to chemotherapy (Table 2). In our study, 1 child did not attain a CR with CHOP therapy and 3 patients with stage III disease developed recurrences after completion of therapy. It is noteworthy that 3 of these 4 failures were successfully treated. This suggests that, despite initial treatment failure, children with large-cell NHL containing the t(2;5) remain chemosensitive and potentially salvageable.

In summary, t(2;5) is associated with but not limited to anaplastic histology, a CD30+ T-cell phenotype, advanced stage disease, and nodal (± extranodal) involvement that is responsive to chemotherapy at initial presentation and in relapse. A conceptual schema of our current understanding of the degree of overlap between CD30+ large-cell NHL, anaplastic large-cell NHL, and t(2;5) containing large-cell NHL, as suggested by our cases and those in the literature, is depicted in Fig 1. Recently, Morris et al have cloned the breakpoint of the t(2;5)(p23;q35) and found that the rearrangement results in a fusion of the NPM nuclear phosphoprotein gene on chromosome 5 at q35 to a previously unidentified putative protein-tyrosine kinase gene, termed ALK, that is mapped to chromosome 2 at p23. With probes for these genes now available, polymerase chain reaction screening of tumor samples will facilitate both the identification of t(2;5) containing large-cell NHL cases and our understanding of their relationship to CD30 expression and histopathology.

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