Use of an anti-ALK antibody in the characterization of anaplastic large-cell lymphoma of childhood


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Summary

Background: Anaplastic lymphoma kinase (ALK) is a tyrosine kinase inappropriately expressed in lymphoid tissue involved by CD30+ anaplastic large-cell lymphomas (ALCL) with the translocation t(2;5)(p23;q35), which juxtaposes the nucleophosmin gene (NPM) with that encoding ALK, resulting in a hybrid (NPM-ALK) message.

Patients and methods: A polyclonal antibody against residues of the kinase portion of NPM-ALK (designated anti-ALK 11) was tested for clinical utility in paraffin sections of 44 cases of pediatric large-cell lymphoma (LCL) and 17 additional lymphoma cases, by streptavidin-biotin-alkaline phosphatase method.

Results: Nineteen of 20 CD30+ cases (the majority exhibiting anaplastic morphology) labeled with anti-ALK 11, and 5/28 CD30- cases were also ALK+ (3 T cells, 1 null cell, and 1 B cell). Sixteen of 17 B-cell pediatric LCLs were negative, as were 6/6 cases of Hodgkin's disease and 7/7 cases of adult B-cell lymphoma. In pediatric LCLs with adequate follow-up (24/44 ALK+), there was no significant association between ALK expression and two-year event-free survival, similar to the finding reported previously for CD30 expression in these cases.

Conclusion: We conclude that the majority of pediatric CD30+ ALCLs show ALK overexpression, consistent with the presence of the (2;5)-encoded NPM-ALK fusion, but that the clinical significance of this entity remains unproven.

Key words: adolescence, ALK antigen, anaplasia, anaplastic large-cell lymphoma, CD30 antigen, child, diagnosis, Ki-1 large-cell lymphoma, non-Hodgkin's lymphoma

Background

Non-Hodgkin's lymphoma occurring in childhood and adolescence differs from that in adults by its restriction to diffuse aggressive types with relative susceptibility to current chemotherapies and subsequently high cure rates [1-4]. Histologic types are distributed almost equally among lymphoblastic, Burkitt's, and large-cell categories [4-6]. The former two lymphoma types have analogs in the leukemias, T-cell and B-cell acute lymphoblastic (Burkitt's), and have been more extensively characterized than the large-cell types. Until relatively recently, it was generally assumed that pediatric large-cell lymphoma (LCL) was analogous to LCL occurring in adults, that is, diffuse B-cell lymphoma [7]. With the advent of immunohistochemical techniques and monoclonal antibodies optimized for paraffin sections of fixed tissue, it became apparent that B-cell follicle center-cell lymphomas are not a majority of pediatric LCLs [8]. A substantial proportion, if not the majority, are of a histology designated by the Working Formulation as polymorphous immunoblastic [9] and by the updated Kiel classification as anaplastic large cell [10]. These usually exhibit T-cell or null-cell phenotypes when examined by immunohistochemistry or molecular techniques and sometimes exhibit monocyte/macrophage-associated immunologic markers. Many of these cases would have in the past been diagnosed as malignant histiocytosis and some as metastatic undifferentiated tumors or Hodgkin's disease. They frequently express the Hodgkin's disease-derived marker CD30 and molecular techniques and sometimes exhibit monocyte/macrophage-associated immunologic markers. Many of these cases would have in the past been diagnosed as malignant histiocytosis and some as metastatic undifferentiated tumors or Hodgkin's disease. They frequently express the Hodgkin's disease-derived marker CD30 and are now generally categorized as CD30+ anaplastic large-cell lymphoma [11-14].

This lymphoma was described by Stein and colleagues, following studies on the immunologic features of Hodgkin's disease and related tumors [15]. The histologic features of nodal or systemic disease include a predilection for lymph node, sinus, and paracortical (perifollicular) involvement by sometimes cohesive sheets of anaplastic large cells with abundant cytoplasm and indented or multilobated nuclei, including Hodgkin-like cells in many cases [16] (Figure 1A). Both the distribution of cells and the cytologic features are quite variable, however, with some cases exhibiting uniformly round nuclei while others mimic Hodgkin's disease and sometimes even show bands of sclerosis [17-22].

Immunohistochemical analysis usually, although not invariably, reveals surface and cytoplasmic (Golgi) presence of CD30 (Figure 1B) and reactivity with epithelial
membrane antigen (EMA) [12, 21, 23–27]. T-cell-associated markers (i.e., CD45Ro and CD3) are often also present. ALCL may also arise in the evolution of other previously diagnosed conditions, including Hodgkin’s disease, lymphomatoid papulosis, cutaneous T-cell lymphoma (mycosis fungoides), and other lymphomas [28]. Lymphomas with similar appearance and CD30 expression also may arise in the setting of immunosuppression and Epstein–Barr virus (EBV) infection, often with B-cell phenotype [29–35], and at least occasionally in association with human T-lymphotrophic virus type 1 (HTLV-1) [36, 37]. Stein et al. originally noted that CD30 expression may be induced in cultured lymphoid cells by EBV, HTLV-1, and other mitogens [15], and it has been noted in EBV infectious mononucleosis [38].

The cytogenetic abnormality t(2;5)(p23;q35) was identified in cases of CD30+ ALCL and is felt to be a specific marker for the entity of ALCL [39–43]. This abnormality was characterized at the molecular level by Morris and colleagues [44] and was found to result in fusion of the nucleophosmin (NPM) nucleolar phosphoprotein gene on 5q35 to a tyrosine kinase gene for anaplastic lymphoma kinase (ALK) on 2p23, and expression of a hybrid protein of which the amino terminus of NPM is fused to the catalytic domain of ALK.

Further cytogenetic and molecular studies of ALCL have revealed heterogeneity [28, 45–52], with many ALCLs, particularly those in adults and/or secondary ALCLs, lacking evidence of the translocation. It appears, however, that pediatric cases of ALCL more often show t(2;5) [28, 47, 48]. Thus, it is entirely possible that the histopathologic features of ALCL represent a common endpoint to a variety of tumorigenic mechanisms, of which t(2;5) and unscheduled ALK expression is one, and that this primary disorder is more frequent in children. The distinction of an entity of primary ALCL has implications for comparison of therapeutic results, selection of therapy, and prognostication.

Patients and methods

Unstained paraffin sections (mounted on glass slides) of B-5 and/or formalin-fixed diagnostic biopsy tissues were available for 44 patients treated for large-cell non-Hodgkin’s lymphoma on one of two protocols of the Pediatric Oncology Group for localized (no. 8719) or advanced (no. 8615) large-cell lymphoma. Features considered indicative of anaplastic large-cell morphology included the presence of large cells with abundant cytoplasm and indented, lobated, or multiple nuclei. These tumors had also been analyzed by immunohistochemistry for T- and B-cell-associated markers and for CD30 [56]. Seventeen additional cases (4 pediatric B-cell LCL, 7 adult B-cell lymphoma, and 6 Hodgkin’s disease) were also selected from the recent files of SUNY-HSC, as well as routine histologic samples from a complete non-neoplastic adult autopsy. Immunocytochemical methods were as previously described [56] and reactions scored by light microscopy. The antibody was prepared in the laboratory of S. W. Morris by ligating a 303 bp Alcl1/PstI ALK cDNA fragment that encodes residues 1359–1460 of the protein (corresponding to residues 419–520 of the chimera NPM-ALK protein) in-frame into the pQE bacterial expression vector (Qiagen Express System, Qiagen, Chatsworth, CA) to produce an ALK polypeptide containing an amino-terminal tag of six consecutive histidine residues for binding to nickel-nitrilotriacetic acid agarose. This protein was used to immunize rabbits. The resulting polyclonal ALK antibody was designated ‘anti-ALK 11’ [58]. Positive tissue controls consisted of paraffin sections of fixed and embedded cells of the SUP-M2 ALCL cell line, which contains t(2;5)(p23;q35) and expresses ALK.

Presence of ALK message in cultured positive controls was confirmed by in situ hybridization (ISH). A 200 bp XbaI/Kpnl ALK cDNA fragment cloned into the blueprint SK-+ vector was linearized with XbaI digestion and biotinylated with T7 polymerase using the nonradioactive RNA labeling system from GIBCO BRL (Gaithersburg, MD) to generate an antisense probe. A sense probe, used as a negative control, was generated by labeling the Kpnl-digested fragment of the plasmid with T3 RNA polymerase.

Comparison of ALK expression with CD30 expression and anaplastic morphologic features was performed using Fisher’s exact test and the log-rank test [59] to evaluate event-free survival, the time from registration to progression, relapse, death, or second cancer. EFS curves were constructed by the method of Kaplan–Meier [60], with standard errors as reported by Peto et al. [61].

Results

Of all the lymphomas tested, 24 showed ALK reactivity, which appeared as diffuse cytoplasmic and sometimes nuclear staining (Figure 2). Of the CD30+ LCLs treated on POG protocols and sufficiently mature for statistical analysis, 19/20 were ALK+. Sixteen of these showed

**Figure 1.** (A) Anaplastic large-cell lymphoma (Hematoxylin and eosin stain, original magnification 500×). (B) CD30 (Ber-H2) reactivity in cytoplasm (Golgi) and on surface (streptavidin-biotin-alkaline phosphatase stain, original magnification 500×).
Large-cell lymphoma, immunoblastic type in the Working Formulation, with scattered anaplastic cells and strong cytoplasmic reactivity with anti-ALK 11 (streptavidin-biotin-alkaline phosphatase stain, original magnification 500×).

morbidity consistent with anaplastic large-cell lymphoma. Five of 24 CD30− LCLs also showed ALK expression (3 T cells, 1 null cell and 1 B cell); of these, only the 1 null-cell case showed anaplastic features. Nineteen of 24 ALK+ cases were CD30+ and 19/20 ALK− cases were CD30−. None of the Hodgkin's disease, adult B-cell lymphomas, or additional B-cell pediatric lymphomas showed ALK expression. Autopsy tissues showed focal reactivity in peripheral nerve, peribronchial cartilage, and smooth muscle of the small bowel, but no reactivity was seen in lymph node or spleen.

The concordance between ALK and CD30 expression in our patients was highly significant \( P < 0.0001 \), as was that between ALK expression and anaplastic morphology \( P = 0.0001 \). There was no significant difference in two-year event-free survival between ALK+ and ALK− cases \( P = 0.45 \) (Figure 3).

Discussion

Our findings indicate that among patients treated on POG protocols for large-cell lymphoma, expression of CD30, presence of anaplastic morphologic features, and expression of ALK – suggestive of \( t(2:5)(p23;q35) \) – are interrelated and occur in a high proportion of T-cell and indeterminate-lineage cases. This further suggests the relatively frequent occurrence of an entity of CD30+ ALCL in lymphoma patients among the pediatric age group. It does not suggest, however, that this entity shows a different response to therapy than other LCLs, particularly other T-cell or indeterminate-lineage cases. We have previously shown that among a patient group including the statistically analyzed current cases, B-cell immunophenotype is correlated with better event-free survival.

Figure 2. Large-cell lymphoma, immunoblastic type in the Working Formulation, with scattered anaplastic cells and strong cytoplasmic reactivity with anti-ALK 11 (streptavidin-biotin-alkaline phosphatase stain, original magnification 500×).

Figure 3. Event-free survival of 44 cases of pediatric large-cell lymphoma treated on POG protocols 8719 and 8615. No significant difference was found \( P = 0.45 \).

<table>
<thead>
<tr>
<th>Interval</th>
<th>NPM−P (SE)</th>
<th>F</th>
<th>N</th>
<th>NPM+P (SE)</th>
<th>F</th>
<th>N</th>
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<tr>
<td>0 - 1 year</td>
<td>85.0 (8.0)</td>
<td>3</td>
<td>20</td>
<td>91.7 (5.8)</td>
<td>2</td>
<td>24</td>
</tr>
<tr>
<td>1 - 2 years</td>
<td>85.0 (8.5)</td>
<td>0</td>
<td>17</td>
<td>82.9 (7.9)</td>
<td>2</td>
<td>21</td>
</tr>
<tr>
<td>2 - 3 years</td>
<td>85.0 (8.5)</td>
<td>0</td>
<td>15</td>
<td>78.6 (8.8)</td>
<td>1</td>
<td>19</td>
</tr>
<tr>
<td>3 - 4 years</td>
<td>79.3 (10.4)</td>
<td>1</td>
<td>15</td>
<td>73.3 (12.0)</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>4 - 5 years</td>
<td>79.3 (20.8)</td>
<td>0</td>
<td>12</td>
<td>61.1 (17.0)</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>5 - 6 years</td>
<td>61.1 (26.9)</td>
<td>0</td>
<td>3</td>
<td>61.1 (26.9)</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>6 - 7 years</td>
<td>61.1 (38.1)</td>
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<td>2</td>
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<td>2</td>
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<tr>
<td>7 - 8 years</td>
<td>61.1 (46.9)</td>
<td>0</td>
<td>1</td>
<td>61.1 (46.9)</td>
<td>0</td>
<td>1</td>
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survival than T-cell or indeterminate lineage, while CD30 expression and anaplastic features were not correlated with event-free survival [56]. It should be noted that we have not excluded the possibility that ALK expression could in some cases be induced by a mechanism other than a cytogenetic abnormality.

Since ALCL was described, the concept has proven useful in categorization of tumors that were previously difficult to classify, and it has become apparent that they are not rare, constituting 10%–12% of pediatric lymphomas [3]. Several questions persist, however, regarding the definition of ALCL: Is there an entity of ALCL? How should it be defined? and does it have clinical relevance?

Most oncologists and hematopathologists agree that an entity of ALCL exists, as they have seen examples of LCL fitting the histologic and immunologic descriptions, and some unique clinical features have been described [13, 14, 25, 43]. How it should be defined is more difficult, since histologic definitions allow for immunologic and genetic heterogeneity, while biologic definitions allow for a great deal of histologic variability, with multiple morphologic variants described. Whether or not it has clinical relevance is most important, owing to the critical clinical decisions which must be based, at least in part, on pathologic findings.

This study was based on an analysis of uniformly treated pediatric patients with LCL of any histologic subgroup and of any immunophenotype. Pediatric age group, large-cell histology, and enrollment on protocol were our defining features. Prospective histologic analysis was according to the Working Formulation for Clinical Usage, which does not include a category of ALCL. Immunophenotypic examination was retrospective, as was histologic examination for anaplastic features. Anaplastic cells were noted in variable numbers in cases diagnosed as immunoblastic (usually the polymorphous subtype) as well as in cases diagnosed as diffuse large cell, including cases which may be referred to as variants of ALCL. While at variance with the approach of some other investigators, such as Weisenberger and colleagues [28], who require strict histologic definition of ALCL, our approach allowed us to readily analyze antigen expression and morphology separately among uniformly treated patients. The strong concordance found between reactivity with anti-ALK 11 and both CD30 expression and anaplasia is evidence that CD30+ ALCL in pediatric patients is a distinct entity related to NPM-ALK fusion with overexpression, and provides encouragement that this and similar antibodies will be useful for characterizing ALCL.

In summary, our results show that the majority of pediatric CD30+ ALCLs show ALK overexpression, consistent with the presence of the t(2;5)-encoded NPM-ALK fusion. We currently provide no evidence that ALCL behaves differently from other LCLs of T-cell or indeterminate immunophenotype. Further study is required to provide definite conclusions regarding outcome, and we are currently evaluating the results of treatment of LCL by comparing patients in a prospective manner according to morphology, T- versus B-cell immunophenotype, and CD30 and ALK expression.

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