Pediatric ALK+ Anaplastic Large Cell Lymphoma With t(3;8)(q26.2;q24) Translocation and *c-myc* Rearrangement Terminating in a Leukemic Phase

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Pediatric ALK-positive anaplastic large cell lymphoma (ALK+ ALCL) is usually associated with a favorable prognosis. ALK+ ALCL associated with a leukemic phase is uncommon, but has been associated with an aggressive clinical course and unfavorable prognosis. Overexpression of c-myc has been shown to be a consistent finding in ALK+, but not ALK-negative ALCL (ALK− ALCL), and the *c-myc* gene is considered a downstream target of deregulated ALK signaling. We describe a pediatric ALK+ ALCL with a leukemic phase at relapse. Similar to other rare cases described in the literature, it followed an aggressive clinical course despite multiple regimens of chemotherapy and bone marrow transplantation. Lymphoma cells showed aberrant ALK expression and c-myc overexpression. In addition to the characteristic t(2;5)(p23;q35) translocation, a t(3;8)(q26.2;q24) translocation was also present, and *c-myc* gene rearrangement was confirmed by FISH analysis. The findings in this case demonstrate the association of peripheral blood leukemic involvement and aggressive clinical course, and suggest that other factors, such as *c-myc* rearrangement, may be responsible for the aggressive clinical behavior in ALK+ ALCL. Am. J. Hematol. 82:59–64, 2007. © 2006 Wiley-Liss, Inc.

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INTRODUCTION

Anaplastic large cell lymphomas (ALCLs) are high-grade lymphomas characterized by a broad spectrum of clinical and morphologic features that commonly express CD30 and most often have a T-cell phenotype. A subset of ALCL, characterized by aberrant expression of anaplastic lymphoma kinase (ALK), is currently recognized in the World Health Organization classification of hematopoietic neoplasms as a distinct clinicopathologic entity [1]. It is, however, unclear whether ALK negative cases comprise a distinct entity. Aberrant expression of ALK, a tyrosine kinase receptor in the insulin receptor superfamily, is due to rearrangements of the *ALK* gene on chromosome 2, most commonly as the t(2;5)(p23;q35) reciprocal chromosomal translocation. Other variant chromosomal abnormalities are also described with less frequency [2–5]. The t(2;5)(p23;q35) results in a chimeric fusion protein of

nucleophosmin (NPM) and ALK with subsequent ligand-independent activation of ALK. This translocation is typically seen in children comprising 20–50% of large cell lymphomas in this age group, and is usually associated with a good response to therapy and good survival [6,7]. Rare cases of pediatric ALK + ALCL with a leukemic peripheral blood involvement have been described in the literature and followed an aggressive clinical course, unlike the typical course of ALK + ALCL in this age group [8–10].

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In a previous study [11], the overexpression of c-myc was demonstrated in pediatric ALK+ but not in ALK− ALCL. The overexpression of c-myc was considered a defining characteristic of ALK+ ALCL and deregulation of this oncogene was suggested to play a role in ALK+ ALCL pathogenesis as a downstream target of ALK signaling. Although no cytogenetic studies documenting any aberrations involving the *c-myc* gene were reported in this study, chromosomal structural alterations, including gains of 8q affecting the *c-myc* gene, were documented in an ALCL cell line [12].

We describe a case of pediatric ALK+ ALCL, which followed an unusually aggressive clinical course terminating in a leukemic phase. Despite multiple intensive chemotherapy regimens and bone marrow transplantation, the patient died of widely disseminated disease. In addition to the characteristic t(2;5)(p23;q35) translocation, a t(3;8)(q26.2;q24) translocation was also detected and rearrangement of the *c-myc* gene was confirmed by FISH analysis. Expression of the c-myc protein was demonstrated by immunohistochemical (IHC) staining. This case suggests an association between leukemic involvement and aggressive clinical course in ALK+ ALCL. In addition, this is the first reported case of ALK+ ALCL with a balanced reciprocal translocation t(3;8)(q26;q24) resulting in *c-myc* rearrangement, as a mechanism for c-myc expression in ALK+ ALCL and possibly poor prognostic indicator.

MATERIALS AND METHODS

Morphological assessment was performed on Wright-Giemsa-stained smears and cytospins from peripheral blood and pleural fluids, and standard H&E-stained sections from formalin-fixed, paraffin-embedded tissue. Immunophenotyping was performed using combined IHC and flow cytometric analysis. Flow cytometry was performed on peripheral blood and pleural fluid specimens using directly conjugated antibodies (Becton Dickinson, San Diego, CA) and analyzed on a 4-color FACSCalibur flow cytometer (Becton Dickinson) using the CellQuest software (Becton Dickinson). IHC analysis was performed on formalin-fixed, paraffin-embedded sections using the Dako Envision plus system for detection (DAKO, Carpinteria, CA) and commercially available antibodies. Cytogenetic analysis was performed by conventional G-banded chromosome analysis and fluorescence in situ hybridization (FISH) using the LSI *c-myc* probe (VYSIS, Downer's Grove, IL) hybridized using standard methods. Hybridization signals were scored on Nikon Eclipse 600 microscope attached to CytoVision imaging system (Applied
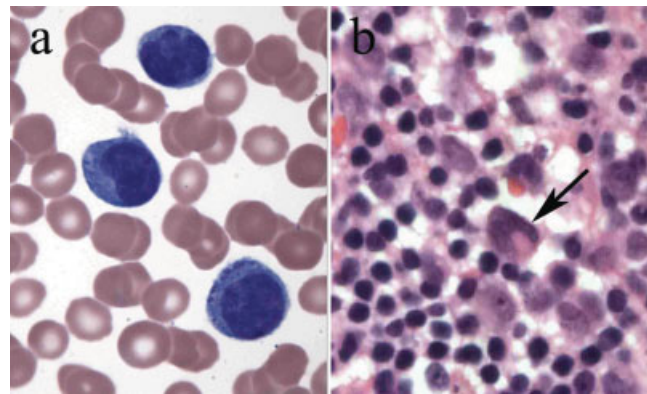
Fig. 1. (a) Peripheral blood smear with abundant neoplastic cells, some showing highly irregular, convoluted nuclei (Wright-Giemsa, 1000X). (b) Lymph node biopsy with a rare hallmark cell (arrow) and medium-sized neoplastic cells in prominent sinusoidal and para-cortical distribution (H&E stain, 400X). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Imaging, Santa Clara, CA). SKY was performed on metaphase preparations using human SKYPaint kit obtained from Applied Spectral Imaging (Carlsbad, CA) according to the manufacturer's protocol. SKY images were acquired with SD200 Spectra cube mounted on Nikon Eclipse 800 microscope by using SKY optical filter (Chroma Technology, Brattleboro, VT) analyzed using the SKY View software.

Molecular analysis of the T-cell receptor gamma (TCR γ) gene was performed using polymerase chain reaction (PCR)-heteroduplex analysis with polyacrylamide gel electrophoresis (PAGE) and V1, V9, V10/11, J1/2, JP, and JP1/P2 primers. EBV infection status was investigated by in situ hybridization (ISH) for EBV-encoded RNAs (EBER 1-2, Ventana INFORM EBER, Tucson, AZ).

CASE REPORT

A 13-year-old boy presented with a 3-week history of left lower quadrant pain and left inguinal lymphadenopathy. Since the lymphadenopathy failed to respond to antibiotics, a lymph node biopsy was performed and diagnosed as small cell variant of ALK+ ALCL. Initial peripheral blood and bone marrow examinations were negative for malignant cells. The patient was initially treated with an induction regimen with Vincristine (1.5 mg/m^2 on days 1, 8, 15, 22, 29), Doxorubicin (75 mg/m^2 on day 1 and 22) and Prednisone (40 mg/m^2 days 1–28). There was an initial clinical response with reduction of lymphadenopathy, but mild lymphadenopathy per-
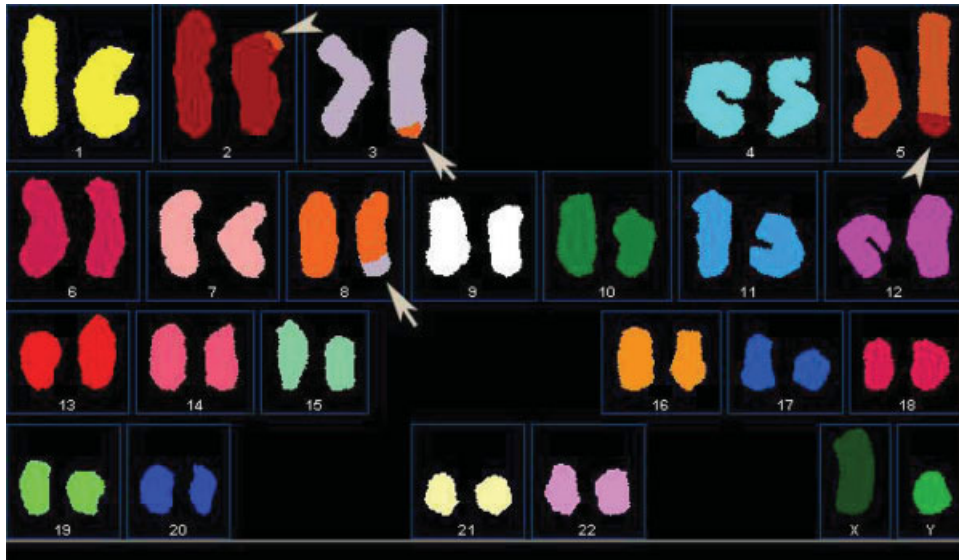
Fig. 2. Spectral karyotype (SKY) shows t(2;5) and t(3;8) translocations (arrows). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
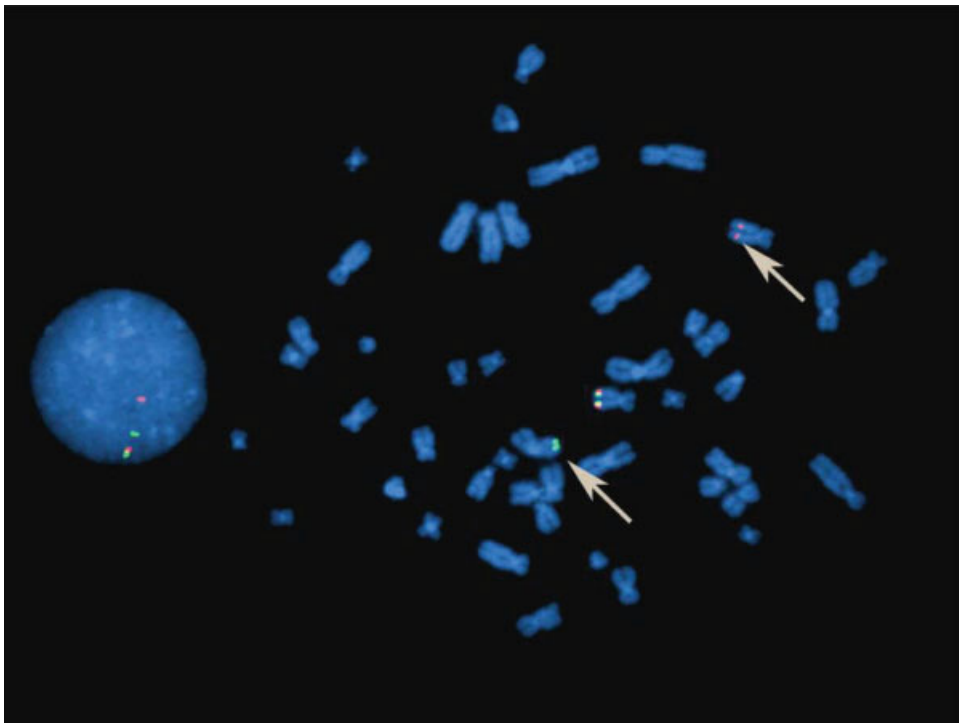

Fig. 3. FISH using the *c-myc* break apart probe shows rearrangement of *c-myc* (arrows). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

sisted and worsened with recovery of peripheral blood counts.

During the second round of chemotherapy, the patient developed pleural effusions with respiratory distress. A chest CT scan showed bilateral pleural effusions with hilar and mediastinal lymphadenopa-thy. Pleural fluid and CSF samples were taken, and both were positive for malignant lymphoma cells, confirmed by cytogenetics. Re-induction chemother-apy with COPADM1 as per the CCG 5961 protocol was initiated, followed by autologous stem cell harvest and rescue, leading to stabilization of his

clinical condition. A reduced intensity allogeneic stem cell transplant from an HLA-matched sibling was performed. Shortly after transplantation, his clinical condition deteriorated with a rapid re-accumulation of the pleural effusions and the development of a significant leukocytosis, WBC 39.5 with >50% neoplastic cells (Fig. 1a). Examination of peripheral blood and bone marrow by morphology, flow cytometric analysis, and cytogenetics confirmed relapsed ALCL. The patient's clinical status continued to deteriorate rapidly and he died of disease soon thereafter (less than 6 months from initial presentation).

RESULTS

Morphologic assessment of H&E-stained sections of the lymph node showed predominantly small to medium-sized lymphoid cells with only rare large "anaplastic" cells in a prominent sinusoidal and para-cortical distribution (Fig. 1b). Wright-Giemsa-stained smears and cytospins of the peripheral blood and pleural fluid, respectively, showed numerous small to medium-sized neoplastic lymphoid cells with irregular, convoluted nuclei accounting for >50% of WBC in the peripheral blood. IHC analysis performed on formalin-fixed, paraffin-embedded sections of the lymph node biopsy showed neoplastic cells with the following phenotype: CD45+, CD30+, EMA+, TIA-1+, CD56+, CD43+, ALK+, and c-myc+. There was no detectable expression of CD15, CD2, CD3, CD5, CD4, CD8, CD20, CD22, CD79a, bcl-6, and Pax5. In situ hybridization for EBER was negative in neoplastic cells. Flow cytometric analysis of the peripheral blood and pleural fluid specimens detected a neoplastic population of cells with an identical phenotype. These histologic and immunophenotypic features are diagnostic of the small cell variant of ALK+ ALCL. Conventional G-banded karyotyping and spectral karyotyping (SKY) showed a diploid chromosome complement (46 XY) with two clonal translocations: t(2;5)(p23;q35) and t(3;8)(q26.2;q24) (Fig.2). FISH analysis confirmed rearrangement of ALK to NPM and rearrangement of c-myc (Fig.3) to an unknown gene partner. Molecular analysis for TCR γ gene rearrangement by PCR-heteroduplex was repeatedly polyclonal.

DISCUSSION

Leukemic peripheral blood involvement is a well-known occurrence in a variety of non-Hodgkin B and T cell lymphomas, especially small lymphocytic lymphoma/chronic lymphocytic leukemia, splenic marginal zone lymphoma, mantle cell lymphoma, adult T-cell leukemia lymphoma, and mycosis fungoides (Sezary syndrome). Presentation of ALCL in a leukemic phase, either at presentation or at relapse, has rarely been reported, mostly as case reports [9,10]. Two small series of four and three pediatric patients with ALK+ ALCL with a leukemic phase were reported by Bayle et al. [13] and Onciu et al. [8], respectively. In the most recent series [8], a review of the literature showed only 12 total reported cases, nine of which were pediatric patients. The majority (75%) of patients had respiratory distress, pleural effusions, or diffuse interstitial lung infiltrates. In 11 of the 12 patients, there was widespread disease with involvement of extranodal sites. Peripheral blood involvement was usually prominent (WBC range 15–151,000 with 5–92% lymphoma cells) with rare and usually minimal involvement of the bone marrow. The most common histologic type reported was the small cell variant (9 of 12), which was previously associated with frequent bone marrow involvement and a worse prognosis [14]. CD30 expression was demonstrated in all cases but the frequency of detecting a T-cell phenotype, either by immunophenotyping or PCR analysis, was variable. In addition, the expression of myeloid associated antigens, CD13 and CD11b, was commonly observed. The classical t(2;5) translocation was documented in most cases, and one case showed a variant t(2,19) translocation. Overall, these cases consistently had a poor prognosis with poor response to therapy and frequent relapses resulting in death from disease.

In our case, leukemic peripheral blood involvement was a late feature, observed during relapse and disease progression. Although the characteristic t(2;5) translocation was present in our case, the neoplastic cells also had an unusual translocation involving the c-myc gene on chromosome 8. In a previous study of ALCLs, approximately 30% were shown to contain abnormalities of the c-myc gene product [15]. However, the ALCLs studied contained both ALK− and ALK+ ALCLs. Chromosomal structural alteration of the c-myc gene containing 8q region have also been identified in an ALK+ ALCL cell line [12]. The expression of c-myc RNA is increased with ALK activation, and c-myc protein overexpression is consistently present in pediatric ALK+ ALCLs [11]. These observations suggest that c-myc may be a downstream effector of the ALK signaling cascade and may play a role in lymphomagenesis. However, the mechanism of c-myc overexpression is still unclear. In our case, a reciprocal translocation involving the c-myc gene and an unknown gene on chromosome 3q26.2 could represent one possible mechanism.

A similar t(3;8)(q26;q24) translocation was recently reported in a small series of patients with myelo-dysplastic syndromes (MDS) and/or acute myeloid leukemias (AML) [16]. However, the genes involved are unknown. The 3q26 region contains a candidate gene, the *EVI1* (ecotropic virus integration site 1) protooncogene, that has been reported to be involved in translocations with multiple partners in cases of AML [17]. However, involvement of *c-myc* has not been reported. Thus, the t(3;8) translocation seen in our case probably involve different loci. Another candidate on chromosome 3 is the human transferrin receptor gene, previously localized to the 3q26.2 region, and associated with various nonlymphoid and lymphoid neoplasms [18,19].

One of the interesting immunophenotypic features of this case is CD56 expression. Although CD56 expression in ALCL has been previously described, the association of CD56 expression with c-myc and ALK is unclear. In a large study comprising of 143 cases of both ALK+ and ALK− ALCL, CD56 expression was found in 18% of the cases and associated with a higher incidence of bone marrow involvement and a poor prognosis [20]. In another study of both ALK+ and ALK− ALCLs, CD56 expression was seen in 36% of ALCLs and TIA-1 expression in 60% of ALCLs [21]. In our case, both CD56 expression and a leukemic peripheral blood involvement were associated with an aggressive clinical course [8,20]. Although previous studies have suggested that alternative ALK abnormalities behave similar to cases with the classic NPM-ALK translocations [7], the behavior of cases with additional cytogenetic abnormalities involving other genes, in our case *c-myc*, is unclear.

Immunophenotypic analysis of ALCL using B- and T-cell antigens has revealed three subsets: T-, null-, and B-cell with the vast majority being of the T- and null-cell types [22,23]. Although many null-cell type ALCLs have shown clonal rearrangement of TCR genes and expression of cytotoxic molecules including TIA-1 [21,24], a minor (10%) subset lack TCR gene rearrangements [24]. In addition, cytotoxic granules including TIA-1 are also expressed by natural-killer cells. The presence of this null-cell ALCL subset with expression of myeloid antigens, CD56, and cytotoxic molecules has led some authors to propose a myeloid-NK-cell origin for a minor subset of ALCLs [25]. However, CD13, CD56, and TIA-1 expression have all been reported in ALCLs of T-cell phenotype [20,24,26]. In addition, ALCLs are generally not associated with EBV, except in occasional cases after solid organ transplantation [27,28]. Our present case shares many similarities with this myeloid-NK-cell-like subset showing a null-cell phenotype, expression of CD13, CD56, and TIA-1. In addition, molecular analyses for TCR γ gene rearrangement by PCR-heteroduplex were repeatedly polyclonal.

In conclusion, we present a case of ALK+ ALCL with an unusual phenotype, an unusually aggressive clinical course with a leukemic involvement of the peripheral blood, and an complex genotype including both NPM-ALK and t(3;8)(q26.2;q24) translocations. This case reinforces the poor prognosis associated ALK+ ALCLs with a peripheral blood involvement. In addition, the presence of aberrant antigen expression can be a diagnostic challenge if comprehensive immunophenotyping is lacking. Finally, it suggests that the presence of c-myc abnormalities in addition to ALK activation may be associated with an unusually aggressive disease course.

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