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

Sara Monaco,1 Lawrence Tsao,1 V.V. Murty,1 S.V. Nandula,1 Virginia Donovan,2 J. Oesterheld,3 Govind Bhagat,1 and Bachir Alobeid1*
1 Department of Pathology, Columbia University College of Physicians and Surgeons, New York, New York 10032
2 Department of Pathology, Winthrop University Hospital, Mineola, New York 11501
3 Department of Pediatric Hematology-Oncology, Columbia University College of Physicians and Surgeons, New York, New York 10032

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.

Key words: ALK positive; anaplastic large cell lymphoma; leukemic phase; c-myc; t(3;8)(q26.2;q24) translocation

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].

*Correspondence to: Bachir Alobeid, MD, Department of Pathology, Columbia University, 630 West 168th Street, New York, NY 10032, USA. E-mail: ba2024@columbia.edu

Received for publication 30 January 2006; Accepted 28 June 2006

Published online 5 September 2006 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/ajh.20758

© 2006 Wiley-Liss, Inc.
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)(q26.2;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 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/m² on days 1, 8, 15, 22, 29), Doxorubicin (75 mg/m² on day 1 and 22) and Prednisone (40 mg/m² days 1–28). There was an initial clinical response with reduction of lymphadenopathy, but mild lymphadenopathy per-
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 lymphadenopathy. Pleural fluid and CSF samples were taken, and both were positive for malignant lymphoma cells, confirmed by cytogenetics. Re-induction chemotherapy with COPADM1 as per the CCG 5961 protocol was initiated, followed by autologous stem cell harvest and rescue, leading to stabilization of his...

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 \( c\text{-}myc \) break apart probe shows rearrangement of \( c\text{-}myc \) (arrows). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
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, 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.

American Journal of Hematology DOI 10.1002/ajh
A similar t(3;8)(q26;q24) translocation was recently reported in a small series of patients with myelodysplastic syndromes (MDS) and/or acute myeloid leukemias (AML) [16]. However, the genes involved are unknown. The 3q26 region contains a candidate gene, the EVII (ectotropic 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− ALCLs, 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 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.

REFERENCES

1. Harris NL, Jaffe ES, Stein H, et al. A revised European–American classification of lymphoid neoplasms: A proposal from the International Lymphoma Study Group. Blood 1994;84:1361–1392.
2. Pittaluga S, Wlodarska I, Pulford K, et al. The monoclonal antibody ALK1 identifies a distinct morphological subtype of anaplastic large cell lymphoma associated with 2p23/ALK rearrangements. Am J Pathol 1997;151:343–351.
3. Wlodarska I, De Wolf-Peeters C, Falini B, et al. The cryptic inv(2)(p23q35) defines a new molecular genetic subtype of ALK-positive anaplastic large-cell lymphoma. Blood 1998;92:2688–2695.
4. Rosenwald A, Ott G, Pulford K, et al. t(1;2)(p23;q23) and t(2;3)(p23;q21): Two novel variant translocations of the t(2;5) (p23q35) in anaplastic large cell lymphoma. Blood 1999;94:362–364.
5. Liang X, Meech SJ, Odom LF, et al. Assessment of t(2;5) (p23q35) translocation and variants in pediatric ALK+ anaplastic large cell lymphoma. Am J Clin Pathol 2004;121:496–506.
6. Gascoyne RD, Aoun P, Wu D, et al. Prognostic significance of anaplastic lymphoma kinase (ALK) protein expression in adults with anaplastic large cell lymphoma. Blood 1999;93:3913–3921.
7. Falini B, Pileri S, Zinzani PL, et al. ALK+ lymphoma: Clinicopathological findings and outcome. Blood 1999;93:2697–2706.
8. Onciu M, Behm FG, Raimondi SC, et al. ALK-positive anaplastic large cell lymphoma with leukemic peripheral blood involvement is a clinicopathologic entity with an unfavorable prognosis. Report of three cases and review of the literature. Am J Clin Pathol 2003;120:617–625.
9. Villamor N, Rozman M, Esteve J, et al. Anaplastic large-cell lymphoma with rapid evolution to leukemic phase. Ann Hematol 1999;78:478–482.
10. Dalal BI, Chhanabhai M, Horsman DE, LeHuquet J, Copland R. Anaplastic large-cell lymphoma presenting as acute leukemia. Am J Hematol 2005;79:164–165.
11. Raetz EA, Perkins SL, Carlson MA, Schooler KP, Carroll WL, Virshup DM. The nucleophosmin-anaplastic lymphoma kinase

American Journal of Hematology DOI 10.1002/ajh
fusion protein induces c-Myc expression in pediatric anaplastic large cell lymphomas. Am J Path 2002;161:875–883.

12. Merz H, Lange K, Gaiser T, et al. Characterization of a novel human anaplastic large cell lymphoma cell line tumorigenic in SCID mice. Leuk Lymphoma 2002;43:165–172.

13. Bayle C, Charpentier A, Duchayene E, et al. Leukaemic presentation of small cell variant anaplastic large cell lymphoma: Report of four cases. Br J Haematol 1999;104:680–688.

14. Kinney MC, Collins RD, Greer JP, Whitlock JA, Sioutos N, Kadin ME. A small-cell-predominant variant of primary Ki-1 (CD30)+ T-cell lymphoma. Am J Surg Path 1993;17:859–868.

15. Inghirami G, Macri L, Cesarmen E, Chadburn A, Zhong J, Knowles DM. Molecular characterization of CD30+ anaplastic large-cell lymphoma: High frequency of c-myc proto-oncogene activation. Blood 1994;83:3581–3590.

16. Lin P, Medeiros LJ, Yin CC, Abruzzo LV. Translocation (3;8)(q26;q24): A recurrent chromosomal abnormality in myelodysplastic syndrome and acute myeloid leukemia. Cancer Genet Cytogenet 2006;166:82–85.

17. Nucifora G. The EVII gene in myeloid leukemia. Leukemia 1997;11:2022–2031.

18. Rabin M, McClelland A, Kuhn L, Ruddle FH. Regional localization of the human transferrin receptor gene to 3q26.2----qter. Am J Hum Genet 1985;37:1112–1116.

19. Yoshida S, Kameita Y, Aoki Y, Seto M, Mori S, Moriyama M. Identification of heterologous translocation partner genes fused to the BCL6 gene in diffuse large B-cell lymphomas: 5′-RACE and LA-PCR analyses of biopsy samples. Oncogene 1999;18:7994–7999.

20. Suzuki R, Kagami Y, Takeuchi K, et al. Prognostic significance of CD56 expression for ALK-positive and ALK-negative anaplastic large-cell lymphoma of T/null cell phenotype. Blood 2000;96:2993–3000.

21. Felgar RE, Salhany KE, Macon WR, Pietra GG, Kinney MC. The expression of TIA-1+ cytolytic-type granules and other cytolytic lymphocyte-associated markers in CD30+ anaplastic large cell lymphomas (ALCL): Correlation with morphology, immunophenotype, ultrastructure, and clinical features. Hum Pathol 1999;30:228–236.

22. Stein H, Mason DY, Gerdes J, et al. The expression of the Hodgkin's disease associated antigen Ki-1 in reactive and neoplastic lymphoid tissue: Evidence that Reed-Sternberg cells and histiocytic malignancies are derived from activated lymphoid cells. Blood 1985;66:848–858.

23. Penny RJ, Blaustein JC, Longtine JA, Pinkus GS. Ki-1-positive large cell lymphomas, a heterogeneous group of neoplasms. Morphologic, immunophenotypic, genotypic, and clinical features of 24 cases. Cancer 1991;68:362–373.

24. Foss HD, Anagnostopoulos I, Araujo I, et al. Anaplastic large-cell lymphomas of T-cell and null-cell phenotype express cytotoxic molecules. Blood 1996;88:4005–4011.

25. Meech SJ, McGavran L, Odom LF, et al. Unusual childhood extramedullary hematologic malignancy with natural killer cell properties that contains tropomyosin 4-anaplastic lymphoma kinase gene fusion. Blood 2001;98:1209–1216.

26. Juco J, Holden JT, Mann KP, Kelley LG, Li S. Immunophenotypic analysis of anaplastic large cell lymphoma by flow cytometry. Am J Clin Pathol 2003;119:205–212.

27. Herling M, Rassidakis GZ, Jones D, Schmitt-Graeff A, Sarris AH, Medeiros LJ. Absence of Epstein-Barr virus in anaplastic large cell lymphoma: A study of 64 cases classified according to World Health Organization criteria. Hum Pathol. 2004;35:455–459.

28. Pitman SD, Rowsell EH, Cao JD, Huang Q, Wang J. Anaplastic large cell lymphoma associated with Epstein-Barr virus following cardiac transplant. Am J Surg Pathol 2004;28:410–415.