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Short Communication

Frequent Expression of the NPM-ALK Chimeric Fusion Protein in Anaplastic Large-Cell Lymphoma, Lympho-Histiocytic Type

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The revised European-American lymphoma classification recognizes a subtype of anaplastic large-cell lymphoma (ALCL), termed lympho-histiocytic because of its peculiar cytological composition. As in the case of classical ALCL, this tumor usually occurs in young patients and shows an excellent response to chemotherapy, but some authors have suggested that in reality this is a nonanaplastic T-cell lymphoma rich in histiocytes. In this paper, we show that three of five cases of lympho-histiocytic ALCL stain with anti-ALK antibodies and can therefore be presumed to express the chimeric NPM/ALK protein secondary to (2;5) translocation. These findings further support the inclusion of this as a type of ALCL and not among the nonanaplastic peripheral T-cell lymphomas. Furthermore, they indicate that staining for ALK proteins is a powerful tool for the diagnosis of lympho-histiocytic ALCL, the recognition of which may be difficult on morphological grounds. (Am J Pathol 1997, 150:1207–1211)

After the first report by Stein et al[1] in 1985, studies of anaplastic large-cell lymphoma (ALCL) have flourished in the literature and have contributed to its clinicopathological definition.[2–9] This tumor type was incorporated first in the updated Kiel classification[10] and more recently in the revised European-American lymphoma (REAL) classification.[11] In the latter scheme, the term anaplastic refers to a specific category of large-cell tumors, of peripheral T-cell or null phenotype, which arise as primary nodal or extranodal neoplasms or as secondary tumors in non-ALCL or Hodgkin's disease (HD).[11] Furthermore, the REAL classification[11] incorporates most of the concepts that emerged from two workshops on ALCL, held in Berlin in 1987 and 1988.[5] Thus, four subtypes of the tumor are recognized: common, giant-cell-rich, lympho-histiocytic (LH), and Hodgkin-related/Hodgkin's-like.[5–9,11,12] ALCL-LH represents a rare form, observed mostly in the first two decades of life, which is extremely rich in reactive histiocytes.[12]

In the late 1980s, several papers reported that a characteristic reciprocal translocation t(2;5)(p23;q35) is associated with ALCL.[13–16] In 1994, Morris et al[17] succeeded in cloning the relevant breakpoint regions. From this[17] and from the independent studies of Shiota et al,[18,19] it emerged that the nucleophosmin gene (*NPM*) fuses with a novel receptor

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tyrosine kinase gene, termed *ALK* (anaplastic lymphoma kinase) to produce a chimeric protein in which 40% of the amino-terminal portion of NPM is linked to the entire intracytoplasmic portion of ALK. Recently, a reverse transcriptase polymerase chain reaction (RT-PCR)[17] and antibodies specifically directed against fixation-resistant epitopes on the intracytoplasmic portion of ALK[18–20] have become available, thus allowing the detection of *NPM-ALK* transcripts or product in bioptic samples. Expression of the *ALK* gene does not occur in normal lymphoid tissue and among lymphoid neoplasms; it appears to be essentially restricted to cells carrying the t(2;5) or other aberrations involving chromosomal region 2p23.[17]

The *NPM-ALK* fusion gene and its protein product have been found in approximately 40% of the ALCL common tumors tested,[19–23] but there has been no report of the frequency with which it is found in the lympho-histiocytic subtype.

In the present paper, we report that neoplastic cells in the majority of LH ALCLs show strong positivity with antibodies specific for ALK protein, and hence are likely to carry the t(2;5) anomaly found in other types of ALCL.

Materials and Methods

Tissue, Reagents, and Immunostaining

Paraffin-embedded blocks from five cases of LH ALCLs were retrieved from the files of the Hematopathology Section of Bologna University. In all cases, tissue had been fixed in 10% buffered formalin and embedded in Histologie noncaking paraffin for histology (Merck, Darmstadt, Germany). The diagnosis was based on the morphological examination of 4-μm-thick sections stained with hematoxylin and eosin (H&E), Giemsa, and Gomori silver impregnation and extensive immunophenotyping using the alkaline phosphatase anti-alkaline phosphatase technique[24] and antibodies directed against one or more of the following molecules: CD30 (Ber-H2; Prof. Stein), CD15 (C3D-1; Dako, Glostrup, Denmark), CD45 (2B11+PD7/26; Dako), CD3 (polyclonal; Dako, Denmark), CD43 (DFT1; Dako), CD45R0 (UCHL1; Dako), CD20 (L26; Dako), CD79a (JCB117; Dr. Mason), CD21 and CD68/KP-1 (Dako), CD68/PG-M1 (Prof. Falini), EMA (E29; Dako), lysozyme (polyclonal; Dako), protein S-100 (polyclonal; Dako), Ki-67 (MIB-1; Prof. Gerdes), and LMP-1 (CS1-4; Dako). The clinical findings at presentation and during the course of the disease were also reviewed.

Routine sections from these cases were stained for ALK protein using a polyclonal antibody produced by immunizing rabbit with a synthetic peptide[18,19] and a monoclonal antibody (ALK1) raised against recombinant ALK protein.[20] Sections were collected on silane-coated slides, dried overnight at 37°C, dewaxed in Histo-Clear, rehydrated in graded alcohols, and finally rinsed twice in running water for 5 minutes. Rehydrated sections in metal racks were immersed in a boiling retrieval solution consisting of 1 mmol/L NaOH EDTA (pH 8.0)[25] in a pressure cooker. The pressure cooker was then sealed and brought to its full pressure (15 psi) after which sections were boiled for 90 seconds (for the polyclonal antibody) or 150 seconds (for the monoclonal reagent). Slides were then cooled, washed twice in 0.1 mmol/L Tris-buffered saline (pH 7.2), and immunostained at room temperature as follows: 1) incubation with fetal calf serum diluted 1:5 for 20 minutes, 2) incubation with the primary antibody for 30 minutes (the polyclonal being diluted 1:60, the monoclonal at 1:2), 3) repeated washes in Tris-buffered saline, 4) incubation with rabbit anti-mouse antibody diluted 1:30 (Dako, code number Z0259) for 30 minutes, 5) repeated washes as above, 6) incubation with alkaline phosphatase anti-alkaline phosphatase complexes from mouse diluted 1:35 (Dako, code number D0651) for 30 minutes, and 7) repeated washes as above. Steps from 4 to 7 were repeated twice. The reaction was developed according to Cordell et al.[24] Slides were finally counterstained with Gill's hematoxylin (Sigma Chemical Co., St. Louis, MO; code number GHS-2-16) and mounted in Kaiser's glycerol gelatin (Merck, code number 1.09242.0100). When the polyclonal antibody from rabbit was used as primary reagent, an additional step was added between steps 3 and 4, ie, incubation in a mouse anti-rabbit monoclonal antibody diluted 1:30 (Dako, code number M0737) for 30 minutes, followed by repeated washes in buffer solution.

Results

The five cases of LH ALCL that formed the basis of this study all occurred in males (four children who were 5, 6, 13, and 14 years old and one adult who was 58 years old), presented in stages III and IV, and entered complete remission in response to aggressive chemotherapy (median follow-up, 54.5 months; range, 13 to 118 months). All cases of LH ALCL had been diagnosed in lymph node biopsies and were characterized by the complete effacement of the normal structure by a diffuse infiltrate, composed

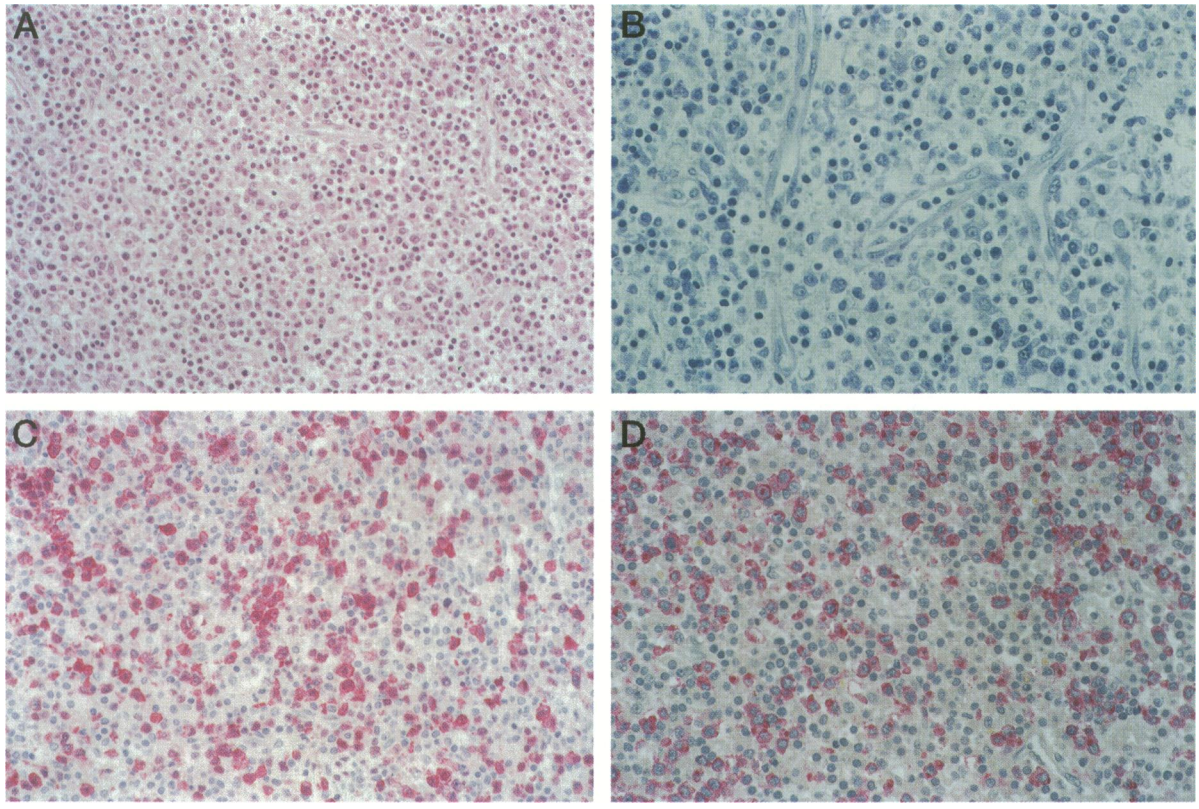

Figure 1. *Anaplastic large-cell lymphoma, lympho-histiocytic type.* a: *At low magnification, the tumor shows a pale appearance due to the very high content of reactive histiocytes with a large acidophilic rim of cytoplasm. H&E; magnification, ×300.* b: *Isolated large anaplastic cells are observed with the reactive histiocytic population. Giemsa; magnification, ×750.* c *and* d: *Large anaplastic cells stained by the monoclonal antibody ALK1 and the Ber-H2/CD30, respectively. APAAP technique; Gill's hematoxylin counterstain; magnification, ×500.*

mainly of uniform looking histiocytes, which had a broad rim of acidophilic cytoplasm and, sometimes, showed a perinuclear clear zone corresponding to the Golgi area (Figure 1, A and B). Their nuclei were eccentric, with dense chromatin. Among these histiocytes, a few small lymphocytes and a varying number of large cells, with typical anaplastic large-cell morphology was present (Figure 1B). The neoplastic cells were usually scattered among the histiocytes or arranged in clusters of a few cells, but on examination of numerous serial sections, large foci of cohesive anaplastic elements were encountered in most cases, which contained numerous mitotic figures and were easily recognized at low power as dark blue areas within the pale macrophage population. The phenotype of the histiocytic and neoplastic elements are summarized in Table 1.

Three of five LH ALCLs displayed a clear-cut reactivity of all neoplastic cells with both of the antibodies against ALK protein, the staining being strongest with the monoclonal reagent (Figure 1C). Positivity paralleled that for CD30 (Figure 1D), so that both antigens clearly picked out the neoplastic cells among the numerous histiocytes. All ALK-positive cases occurred in children and expressed T-cell markers, together, in one case, with LMP-1. One of the negative case was an adult patient with a T-cell tumor phenotype, and the other was a 6 year-old boy with a null tumor.

Table 1. *Phenotypic Profiles of Anaplastic Large Cells and Histiocytes in the LH ALCLs of the Present Study*

	Anaplastic large cells	Histiocytic component
CD30	+ (5/5)	− (5/5)
Ki-67	+* (5/5)	− (5/5)
EMA	+ (5/5)	− (5/5)
CD3	+ (4/5)	− (5/5)
CD68 (KP1 AND PG-M1)	− (5/5)	+ (5/5)
Lysozyme	− (5/5)	+ (5/5)
CD45	+ (5/5)	+ (5/5)
CD43	+ (4/5)	+/− (5/5)
CD45R0	+ (4/5)	+/− (5/5)
CD20	− (5/5)	− (5/5)
CD79a	− (5/5)	− (5/5)
CD21	− (5/5)	− (5/5)
Protein S-100	− (5/5)	− (5/5)

*>90% of cell.

Discussion

LH ALCL was first extensively characterized by the authors' group in 1990[12] in a report of 13 examples of the tumor, all occurring in young patients in the first or second decades of life. In each case, typical ALCL cells were greatly outnumbered by reactive histiocytes, which had a distinctive cytological appearance (prominent acidophilic cytoplasm and an eccentric, dense nucleus). The large anaplastic cells expressed CD30 and T-associated antigens and were a proliferating population, as evidenced by mitotic figures and Ki-67 stain, which was not observed in the macrophage component. In the few cases studied by Southern blot analysis, a clonal rearrangement of the genes encoding for the T-cell receptor was detected.

Clinically, all cases treated with aggressive chemotherapy achieved complete remission and tended to maintain it several years after presentation. However, it should be noted that the few cases that did not receive prompt diagnosis, because of the difficulties in the interpretation of the histological features, had a fatal outcome.

This paper[12] had a dual title, which represented a compromise between the authors and one of the referees, who did not accept that the lymphoma was an example of ALCL, and a similar view was further sustained by Lennert and Feller, who considered the neoplasm to be a peripheral T-cell lymphoma analogous to the lympho-epithelioid T-cell lymphoma (Lennert's lymphoma), the difference being in the morphology of the histiocytic component.[26] This interpretation implied that both the large atypical cells and many of the small T lymphocytes were part of the same neoplastic clone, whereas our interpretation was that the small T cells were reactive.

In 1994, we published another series of this neoplasm, which strengthened the concept that it represents a subtype of ALCL.[27] Morphologically, all cases displayed typical features of ALCL as seen previously.[5] Apart from this similarity, some tumours had a null phenotype of the neoplastic cells, three cases occurred in adults, and the small lymphocytes scattered through the growth were of both B- and T-cell nature. The large anaplastic cells in some cases showed genomic integration of Epstein-Barr virus by *in situ* hybridization with EBER-1 and -2 probes.[27] It was on the basis of this study that the tumor was included in the REAL classification as a variety of ALCL.[11]

To the best of our knowledge, this is the first time that the ALK protein has been observed in LH ALCL, although it should be noted that, in their original report on CD30-positive large-cell lymphomas carrying t(2;5), Mason et al[16] included a classical case of ALCL, LH subtype. Although it has not been formally proved that immunostaining reflects the presence of chimeric NPM-ALK protein (as the ALK1 antibody can react with intact ALK[28]), it can be safely assumed. The numbers studied are too small to give a reliable estimation of the frequency of t(2;5) in LH ALCL, but our results suggest that it is comparable to that observed in classical ALCL.

Given that the t(2;5) is accepted as a distinctive feature of ALCL,[13–23] this study provides strong confirmation that LH tumor is indeed a form of ALCL and should not be referred to as a peripheral T-cell lymphoma other than ALCL. Furthermore, our results indicate that positivity for ALK protein may be a very useful tool also in the diagnosis of this tumor. According to our experience with consultation material, cases of LH ALCL are still diagnosed as hyperimmune reactions or atypical lymphoproliferations, and the possibility of detecting the NPM/ALK protein should provide a substantial aid for the recognition of this disease.

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