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Short Communication

The Monoclonal Antibody ALK1 Identifies a Distinct Morphological Subtype of Anaplastic Large Cell Lymphoma Associated with 2p23/*ALK* Rearrangements

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Anaplastic large cell lymphoma (ALCL) is a heterogeneous group of diseases by morphology, phenotype, genotype, and clinical presentation. Using a new monoclonal antibody (ALK1) that recognizes the native anaplastic lymphoma kinase (ALK) protein as well as the fusion product of the t(2;5)(p23;q35), nucleophosmin (*NPM*)/*ALK*, we investigated for ALK expression cases diagnosed as ALCL as well as lymphoproliferative disorders possessing overlapping features with ALCL. Thirteen cases showed cytoplasmic staining of the neoplastic cells. These cases were characterized by a fairly uniform morphology and occurred in children and young adults as a systemic disease. All other cases comprising T or null ALCL (17 cases), B ALCL (8 cases), Hodgkin's disease (HD) (15 cases), HD-like ALCL (23 cases), and lymphomatoid papulosis (9 cases), were negative for ALK expression. Translocation t(2;5)(p23;q35) was found by classical cytogenetics or interphase fluorescence *in situ* hybridization in 8 of the ALK1-positive cases and by reverse transcription-polymerase chain reaction in 1 other case. Two additional ALK1-positive cases with an abnormal karyotype, but without t(2;5)(p23;q35), showed by fluorescence *in situ* hybridization analysis a cryptic *NPM/ALK* gene fusion caused by an insertion of *ALK* near *NPM* in one case and a translocation of *ALK* to 2q35 as a result of an indiscernible inv(2)(p23q35) in the other. The latter variant translocation points to a localization of an unknown gene at 2q35 that, like *NPM*, might deregulate *ALK* and be involved in the pathogenesis of ALCL. In summary, immunohistochemistry with ALK1 antibody allows the identification of a distinct subgroup within the ALCL of T or null phenotype that is associated with 2p23 abnormalities and lacks the marked histological pleomorphism described in ALCL in general. Whereas immunostaining is the most sensitive method to identify this group, it does not help to additionally clarify the relationship among ALCL, HD, and HD-like ALCL. ***(Am J Pathol 1997, 151:343–351)***

Anaplastic large cell lymphoma (ALCL) represents a subgroup of non-Hodgkin's lymphomas characterized by a specific morphology and a particular phenotype.[1] Although by definition the neoplastic cells should be large and pleomorphic,[2,3] several histological variants have been described; eg, a common variant, pleomorphic and monomorphic,[4] a lymphohistiocytic (macrophage-rich) variant[5] or a giant cell-rich variant,[1] a Hodgkin's disease (HD)-like variant,[6,7] and a small cell variant.[8] The vast majority of cases are characterized by CD30 expression and EMA positivity.[2,9,10] According to the original descriptions, the neoplastic cells express T-cell, null-cell, hybrid, or B-cell markers.[2] However, only T-cell or null-

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cell proliferations are now identified as ALCL in the International Lymphoma Study Group proposal.[11]

The translocation t(2;5)(p23;q35) that results in the fusion of the nucleophosmin (*NPM*) gene on chromosome 5q35 and the anaplastic lymphoma kinase (*ALK*) gene on chromosome 2p23 [12] has been detected mostly by using reverse transcription-polymerase chain reaction (RT-PCR) technique in 16 to 80% of ALCL cases.[13–19] Cases with the translocation have been associated frequently with T- or null-cell phenotype, although in rare instances a B-cell phenotype has been reported.[19–21] A few authors have noted a more frequent occurrence of the *NPM/ALK* fusion in a particular subset of cases,[8,13] while others have observed the translocation in several of the morphological variants of ALCL[15,20,22] or in lymphoid neoplasms other than ALCL.[19,21,23]

Based on clinical presentation, a primary cutaneous disorder and a systemic disease with nodal and extranodal involvement have been identified, characterized by different clinical behaviors and responses to treatment.[17,24–26] Within systemic ALCL two additional distinct subgroups are emerging: one subgroup affecting young patients with good responses to therapy[27,28] and frequently documented by t(2;5) and a second subgroup occurring at a later age that displays a more aggressive behavior.

To further complicate the issue, CD30 expression, the hallmark of ALCL, is also present in the Reed-Sternberg cells of HD. Together with the histology, this finding points towards a gray zone between HD and ALCL and has resulted in the description of a lesion with overlapping features, namely HD-like ALCL.[7] Although by classical cytogenetics the t(2;5) has not been associated with HD, by RT-PCR the fusion transcript has been identified in some cases.[29,30] However, numerous other studies have failed to confirm these data.[13,15,18,22,31–33]

A polyclonal antibody, p80, was raised against the kinase domain of the ALK protein,[34] and in several subsequent studies its reactivity correlated well with the presence of the *NPM/ALK* fusion product.[15,22,27] Variant translocation involving 2p23 was also identified by staining with this antibody.[15] More recently a monoclonal antibody (designated ALK1) against the ALK protein was developed.[35] The specificity of this antibody for ALK proteins has allowed reliable immunostaining of tumors to be performed.[35] With the acquisition of this new tool for immunohistochemistry, which can be applied on archival material (paraffin-embedded), we sought to determine whether the detection of ALK protein, in conjunction with cytogenetics and fluorescence *in situ* hybridization (FISH) analysis, could help in the delineation of a specific entity within the heterogeneous group of ALCL and histologically related disorders.

Materials and Methods

Tissue Samples and Immunohistochemistry

All cases diagnosed as ALCL of T-cell or null-cell phenotype,[25] ALCL B-cell phenotype,[4] and consecutive cases diagnosed as HD,[15] HD-like ALCL,[23] and lymphomatoid papulosis[9] were selected from the files of the Department of Pathology at the University of Leuven, Belgium. In addition, 5 cases of ALCL with T- or null-cell phenotype and 4 cases of ALCL B-cell phenotype were retrieved from the Hospital Clinico Provincial of Barcelona. All cases were documented by B5 or formalin-fixed paraffin-embedded material and in 62 cases with freshly frozen material. Immunophenotyping was performed with a panel of monoclonal antibodies to CD2 (OKT11), CD3, CD3 (Leu4), CD4 (Leu3a/OKT4), CD5 (Leu1), CD7 (Leu9), CD8, CD15 (LeuM1), CD19 (Leu12), CD20 (L26), CD22 (Leu14), CD30 (BerH2), CD45, CD45RO (UCHL1), CD68, and EMA using a streptavidin-biotin-peroxidase (ABC) three-stage technique. The peroxidase reaction was developed using 3,3′-diaminobenzidine tetrahydrochloride (DAB) (Dako, Glostrup, Denmark) and hydrogen peroxide, 0.01% v/v. The tetrahydrochloride immunohistochemical labeling with ALK1 antibody was performed on paraffin sections as previously described.[35] For the latter immunostaining an antigen retrieval system was used by placing the deparaffinized slides in 10 mmol/L citrate buffer solution, pH 6.0, and heating them in a 750-W microwave oven for 10 minutes. The slides then were removed and allowed to cool at room temperature for 30 minutes before beginning the staining procedure with a three-stage technique using the ABC method. The Leu series of monoclonal antibodies was purchased from Becton Dickinson, San Jose, CA, the OKT series from Ortho Diagnostic Systems, Raritan, NJ, and the remaining antibodies from Dako.

Cytogenetics and FISH

One-day cultures of lymph node cells were used for cytogenetic and FISH analysis. Three to 25 G-banded karyotypes were analyzed for each case and classified according to the International System for Human Cytogenetic Nomenclature.[36]

Two-color FISH with a digoxigenin-11-dUTP-labeled *ALK* P1 clone 2639 and biotin-16-dUTP-labeled three cosmids (clones 13, 15–2, and 47C12) for the 5q35 region centromeric to the *NPM* locus was performed as previously described.[37] In case 13 additional FISH studies with a library 2 (Cambio, Cambridge, UK), two probes for the subtelomeric regions of 2p and 2q, namely 68J13 (PAC) and 210E14 (P1),[38] and a chimeric YAC 745D10 hybridizing to 2p21 and 5q35,[39] were performed. For interphase FISH, between 200 and 250 cells were studied for each case. In 3 cases (8, 12, and 13) in which metaphase cells were available, 6 to 25 cells were analyzed. Preparations were examined with a cooled black and white CCD camera (Photometrics, Tucson, AZ) run by Smart Capture software (Vysis, Stuttgart, Germany).

Reverse Transcription-Polymerase Chain Reaction

Total RNA was obtained from the frozen tissue of 6 cases (5 T ALCL and 1 B ALCL) from Barcelona by guanidine

Table 1. Overall Data

Group	Cases	Histology	Males/ Females	Age median (range in yr)	Involved sites*	ALK1 staining	Phenotype of the neoplastic cells					
							T	B	Null	CD30	CD15	EMA
I	1–13	ALCL	10/3	21 (9–37)	Ln 5, ex 2, both 6	Positive	7/12	0/8	5/12	12/13	0/3	8 /11
	14–30	ALCL	11/6	50 (16–73)	Ln 5, ex 3, both 6	Negative	6/15	0/14	9/15	16/17	1/8	4 /8
	31–38	ALCL	3/5	54 (8–78)	Ln 2, ex 1, both 1	Negative	0/6	8/8	0/6	7/8	1/5	0 /2
II	39–53	HD	4/11	47 (19–70)	Ln 13, ex 1, both 1	Negative	0/11	0/13	11/11	6/11	12/15	ND
	54–76	HD-like ALCL	12/11	26 (10–86)	Ln 18, ex 4, both 1	Negative	0/11	0/19	11/11	14/20	16/21	0 /4
	77–85	Lymphomatoid papulosis	6/3	47 (36–71)	Skin 9	Negative	ND	ND	ND	6	ND	ND

Abbreviations: *Ln, lymph node involvement; ex, extranodal involvement; both, lymph node and extranodal involvement; ND, not done.

isothiocyanate extraction and cesium chloride gradient centrifugation. The RT-PCR technique was performed using the primers and protocol previously described.[12] Total RNA from the Karpas 299 t(2;5)-positive cell line was used as positive control.

Results

Clinical and Histological Data

Clinical data and the results of the immunohistochemical studies of ALCL cases (group I) and related disorders (group II) are summarized in Table 1.

Group I: Cases Diagnosed as ALCL

Thirty of these cases were of null- or T-cell phenotype and 8 were of B-cell phenotype. A partial expression of T-cell markers, namely CD3, CD5, and/or CD7, was documented in 7 cases. In 12 cases a proliferation of medium- to large-sized cells was noted often replacing the entire lymph node (cases 1 to 12), whereas in 1 case (case 13) the neoplastic cells invaded the marginal sinus forming tight clusters. These cells seemed to be morphologically similar despite a certain degree of variation in their size and shape. The neoplastic cells displayed amphophilic cytoplasm and a fairly round nucleus with fine chromatin (Figure 1A). Although nucleoli were inconspicuous in some cases, they were prominent and eosinophilic in others. Occasionally few larger cells were present, but in general Reed-Sternberg-like cells were not seen. Frequent mitoses were noted, as was single cell necrosis accompanied by tingible-body macrophages, giving rise to a "starry sky" appearance in 2 cases. A large number of histiocytes were noted in 2 cases (case 1 and 5).

In the remaining 17 cases (cases 14 to 30) the neoplastic cells grew in a cohesive manner, either infiltrating the marginal sinus or forming solid masses replacing the entire lymph node. In the majority of these cases the atypical cells were large with an abundant amphophilic cytoplasm and pronounced nuclear pleomorphism with prominent inclusion-like nucleoli (Figure 1C). Reed-Sternberg-like cells were seen (Figure 1D). Eight additional cases of B-cell ALCL (cases 31 to 38) with a similar pleomorphic morphology showed CD20 expression.

Cases 1 to 13 were reactive for ALK protein; the neoplastic cells showed a cytoplasmic labeling (Figure 1B) without background staining of normal cells. All but one case were CD30-positive. By contrast, all other 25 cases (cases 14 to 30 and 31 to 38) were unlabeled by ALK1 antibody, and CD30 immunostaining was found in 21 of these cases.

Group II: Cases Showing Overlapping Features with ALCL

Fifteen cases were diagnosed as HD either of nodular sclerosing type (10 cases) or mixed cellularity type (5 cases). None of these cases stained with ALK1.

Twenty-three cases were diagnosed as HD-like ALCL. These cases showed capsular and parenchymal fibrosis with the frequent presence of dense collagenous bands. The neoplastic population was composed of a huge number of tightly packed atypical cells that formed nodules of varying sizes. Giant or multinucleated cells with inclusion-like nucleoli were seen. Areas of necrosis were regularly observed, as were accompanying inflammatory cells, mainly eosinophils and plasma cells. None of these cases stained with ALK1. The expression of CD30, detected in 16 of 20 cases, was less intense when compared with cases of typical ALCL.

Nine cases were diagnosed as lymphomatoid papulosis. Most of the cases showed typical features consisting of a band-like dense infiltrate extending from the papillary dermis along the adnexa into the deeper dermis. This infiltrate was composed predominantly of small lymphocytes with a variable number of large pleomorphic cells. In addition, epidermal hyperplasia with focal lymphoid infiltration (epidermotropism) and edema of the papillary dermis were seen. All cases, regardless of the number of atypical cells, were negative for ALK1 immunostaining. The number of CD30-positive cells varied from case to case, but in 4 cases a large number of large CD30-positive atypical cells were found.

Cytogenetic and Molecular Data

Cytogenetic analysis was performed in 40 of 85 cases, and chromosomal abnormalities were found in 20 cases. The remaining cases showed either a normal karyotype (7 cases) or the absence of mitosis (13 cases). Translocation t(2;5)(p23;q35) was found in 4 (cases 1 to 4) of the ALK1-positive ALCL cases (Table 2). The remaining cytogenetically abnormal cases showed complex and fre-

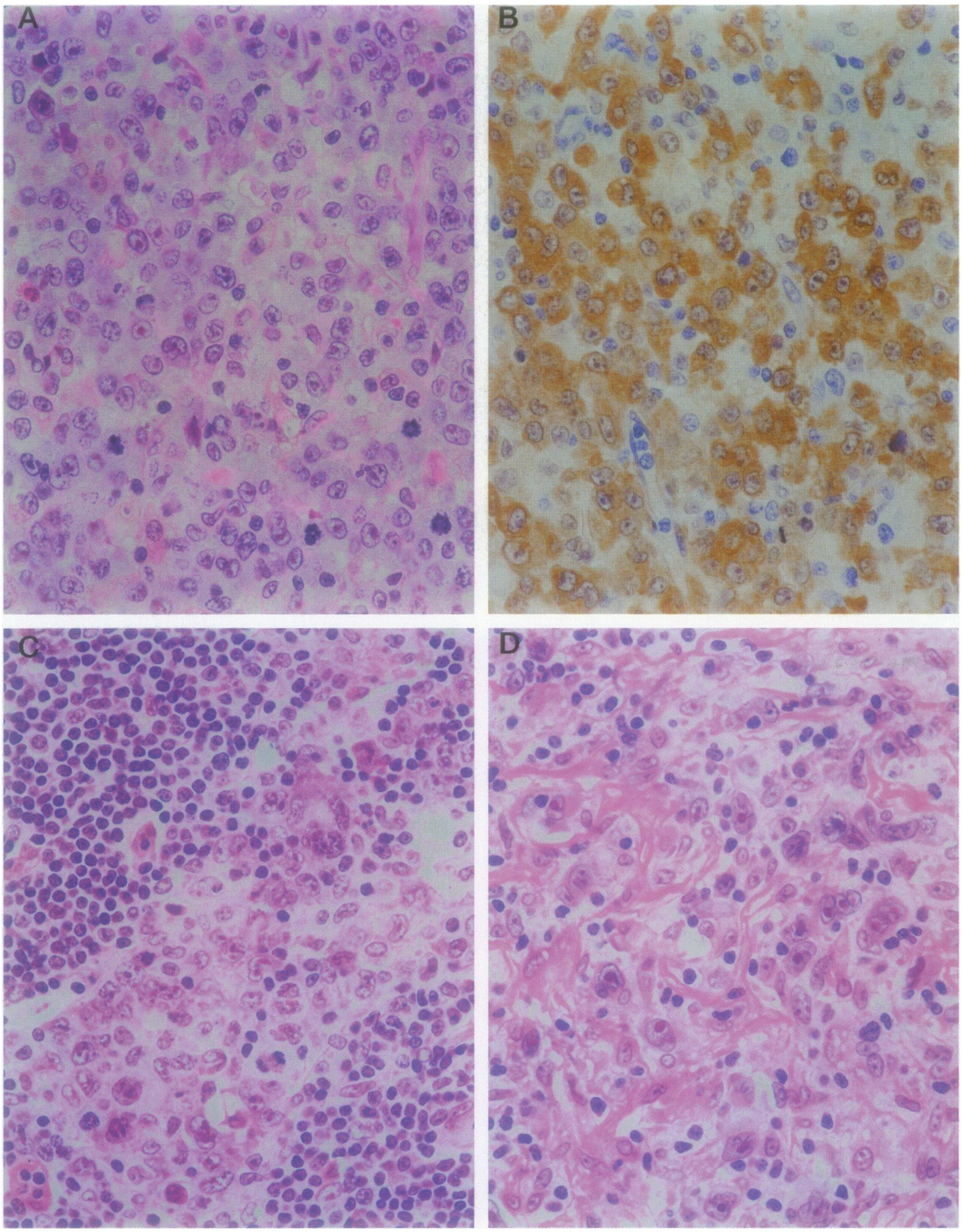

Figure 1. A: Section of a paraffin-embedded lymph node from an ALCL ALK1-positive case (case 1). The neoplastic cells only show a certain degree of nuclear variability and large amphophilic cytoplasm; occasionally prominent nucleoli are seen. Reed-Sternberg-like cells are absent (Hematoxylin & eosin staining). **B:** Immunostaining with monoclonal antibody ALK1 of a B5-fixed paraffin-embedded lymph node. The neoplastic cells are clearly labeled with an intense cytoplasmic staining, whereas the reactive cells are negative. **C** and **D:** Sections of paraffin-embedded lymph nodes from ALCL ALK1-negative cases (cases 14 and 17). In contrast in these cases the neoplastic cells show marked nuclear pleomorphisms and prominent eosinophilic nucleoli. Reed-Sternberg-like cells are easily identified.

Table 2. Cytogenetic and Molecular Studies of ALK1-Positive ALCL Cases

Case	Karyotype	FISH		RT-PCR
		Interphase	Metaphase	
1	46,XY,t(2;5)(p23;q35),der(10)t(?5;10)(q14;q25)[10]	*NPM/ALK* Fusion		ND
2	46,XX,t(2;5)(p23;q35),del(9)(q32)[25]			ND
3	48,XY,+X,t(2;5)(p23;q35),add(9)(p24),del(17)(p11),+19[10]			ND
4	47,XX,+X,t(2;5)(p23;q35)[5]/46,XX [11]			ND
5	46,XX [10]	*NPM/ALK* Fusion		ND
6	NA	NA		ND
7	46,XY [8]	*NPM/ALK* Fusion		ND
8	46,X,add(Y)(q12),der(2)t(2;5)(q37;q31)[15/46,X,−Y[2]	ND	46,X,add(Y)(q12),der(2)t(2;5)(q37;q31),.ishdel(2)(p23p23)(ALK P1 2639 −),der(2)(2pter→2q37::5q31→5q35::2p232p23::5q35→5qter)(ALK P1 2639+,cos13/15-2/47C12+,ALK P1 2639+)	ND
9	No mitosis	*NPM/ALK* Fusion		ND
10	NA	NA		Positive
11	NA	NA		Negative
12	46,XY [17]/polyploid [3]	*NPM/ALK* Fusion		ND
13	46,X,−Y,+i(2)(q10) [10]	ND	46,X,−Y,+i(2)(q10),.ish inv(2)(p23q35)(wcp2+,68J13 mv,ALKP1 2639 mv, 745D10+,210E14 mv),ider(2)(q10)inv(2)(p23q35)(wcp2+,68J13 mv, ALK P1 2639 mv, 210E14−)	ND

Abbreviations: NA, not available; ND, not done.
[ ]: Number of cells showing the indicated karyotype.

quently polyploid karyotypes characterized by numerical and structural abnormalities (data not shown).

The presence or absence of *NPM/ALK* fusion was additionally examined by FISH in the ALK1-positive cases in which t(2;5)(p23;q35) was not found by classical cytogenetics (Table 2). By applying a two-color FISH assay, the *NPM/ALK* fusion on the der(5) chromosome can be detected by a "come-together" approach using differently labeled probes specific for *ALK* and for the 5q35 region immediately adjacent to the 5′ portion of the *NPM* locus,[37] as also shown in Figure 2A. In the present study, two-color FISH was performed on interphase cells of 3 cases (cases 5, 7, and 9) in which cytogenetic analysis was unsuccessful and on metaphase cells of 3 cases (cases 8, 12, and 13) with abnormal karyotypes lacking t(2;5). In cases 5, 7, and 9 the hybridization pattern in interphase cells, indicating the presence of the *NPM/ALK* chimeric gene (one red, one green, and one paired red/green signal), was found respectively in 13, 17, and 25% of analyzed interphases. In case 8, which was cytogenetically characterized by a der(2)t(2;5)(q37;q31) (Table 2 and Figure 3), the *ALK* probe hybridized to 2p23 of the der(2) as expected and to the terminal region of this derivative chromosome where 5q31qter fragment was translocated (Figure 2B). Lack of the *ALK* signal on the cytogenetically normal-seeming chromosome 2 and presence of a fusion signal on the der(2)t(2;5)(q37;q31) in the region corresponding to the *NPM* locus indicate a cryptic *NPM/ALK* fusion resulting from the insertion of *ALK* in the vicinity of *NPM*. In case 12 the t(2;5)(p23;q35) was not previously identified because of a poor-quality banding pattern; however, by FISH, a fusion signal was found on 1 or 2 copies of the der(5)t(2;5) (p23;q35) in three polyploid cells. All 15 diploid cells revealed a normal pattern of hybridization. In this case, presence of 5q35 signals on both arms of one chromosome 5 (Figure 2C) indicates an additional rearrangement involving this region. In case 13 with a karyotype described as 46,X,-Y, +i(2)(q10) (Table 2 and Figure 3), four *ALK* hybridization signals were detected (Figure 2D): one at 2p23 of the normal chromosome 2, one at 2q35 of the second cytogenetically normal-seeming chromosome 2, and two at 2q35 on both arms of i(2)(q10). The 5q35 clones hybridized to distal regions of both chromosomes 5 (Figure 2D). These results suggest a previously unrecognized inv(2)(p23q35), or, similarly to case 8, an insertion of the *ALK* gene to 2q35. To clarify this point, additional analysis with the 2p (68J13) and 2q (521E14) subtelomeric probes and YAC hybridizing to 2p21 was performed. The 68J13 probe hybridized to 2ptel region of a normal chromosome 2, 2qtel region of a second chromosome 2, and to both arms of i(2)(q10), whereas the 210E14 probe gave signals on 2q terminal band of normal chromosome 2 and 2ptel region of the second chromosome 2. The 745D10 YAC hybridized to the short arms of both chromosomes 2 (data not shown). These results indicated a pericentric inversion involving distal bands of both arms of chromosome 2, which was overlooked during cytogenetic analysis and excluded the possibility of an *ALK* gene insertion. Chromosome painting with a library 2 showed uniform labeling of both chromosomes 2 and i(2)(q10).

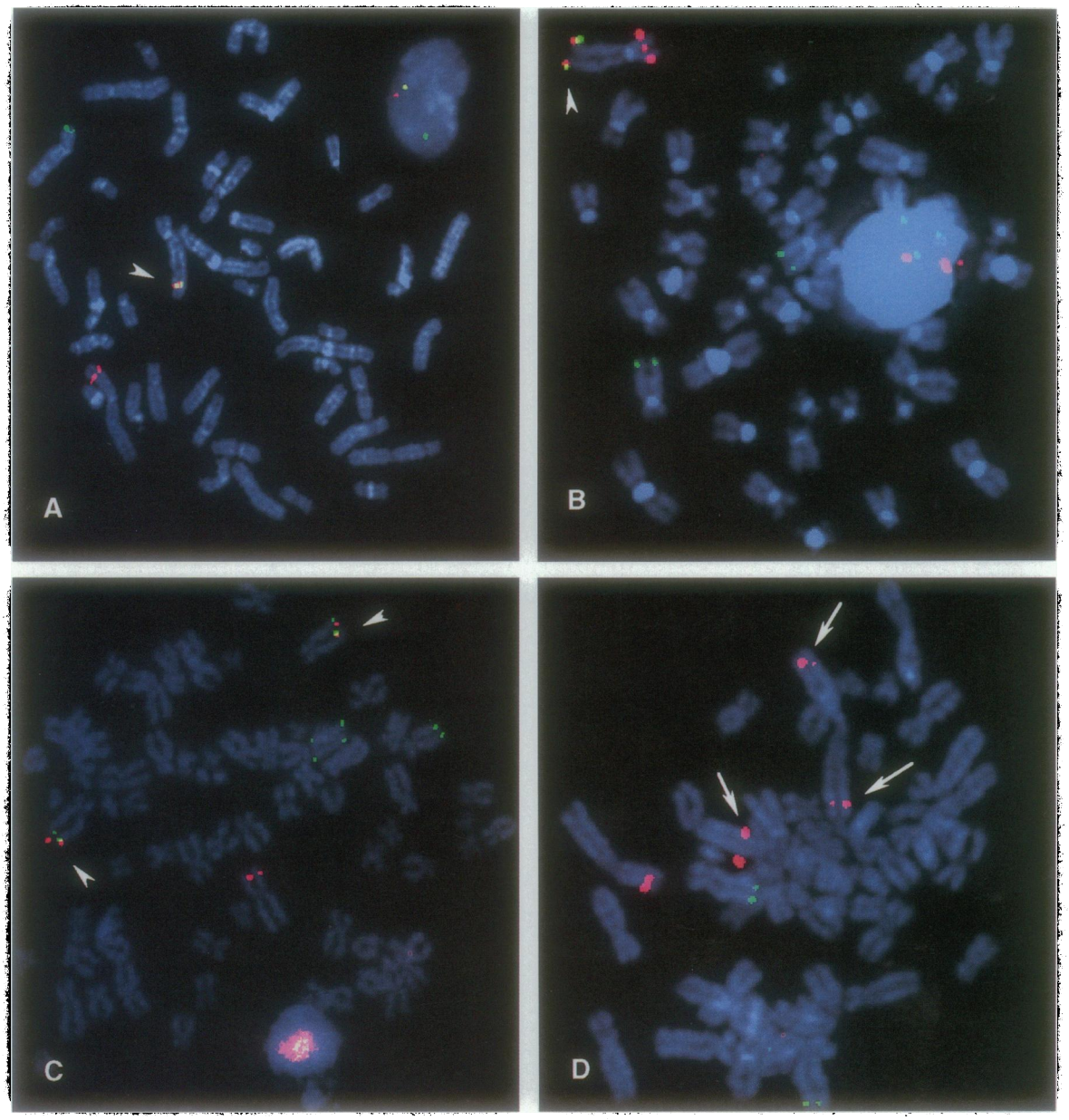

Figure 2. Results of FISH analysis with a digoxigenin-11-dUTP-labeled *ALK* P1 clone (**red signal**) and biotin-16-dUTP-labeled 5q35 cosmids (**green signal**) performed in case 1 (**A**) with cytogenetically demonstrated t(2;5)(p23;q35) and in cases 8 (**B**), 12 (**C**), and 13 (**D**). Fusion signal on the der(5) in cases 1 and 12, and on the der(2) in case 8 is indicated by **arrowheads**. Note the lack of a fusion signal in case 13 and hybridization of the *ALK* probe to 2q35 on one of the two chromosomes 2 and to both arms of the i(2)(q10), as indicated by **arrows**.

Of the two ALK1-positive ALCL cases (cases 10 and 11) analyzed by RT-PCR, only one showed the *NPM/ALK* fusion transcript. No FISH studies could be performed on this *NPM/ALK*-negative case.

One ALK1-positive case (case 6) could not be analyzed by FISH or RT-PCR because of inadequate material.

Discussion

The original definition of ALCL was based on the anaplastic morphology of the tumor cells including large bizarre cells simulating malignant histiocytosis,[2,9] and on the growth pattern that may be cohesive and display sinusoidal involvement mimicking metastatic carcinoma.[10] Whereas CD30 expression was found to be a hallmark of ALCL, its expression is not invariant nor is it restricted to this disease. A lymphocyte activation marker and member of the tumor necrosis factor receptor superfamily,[40,41] CD30 is also expressed in HD and occasionally in other non-Hodgkin's lymphomas, mostly of T-cell type, as well as in embryonal carcinomas.[42]

Approximately 30 to 50% of ALCL cases documented by classical cytogenetics have been associated with the t(2;5)(p23;q35), and therefore this cytogenetic abnormality has been considered as the specific genotypical char-

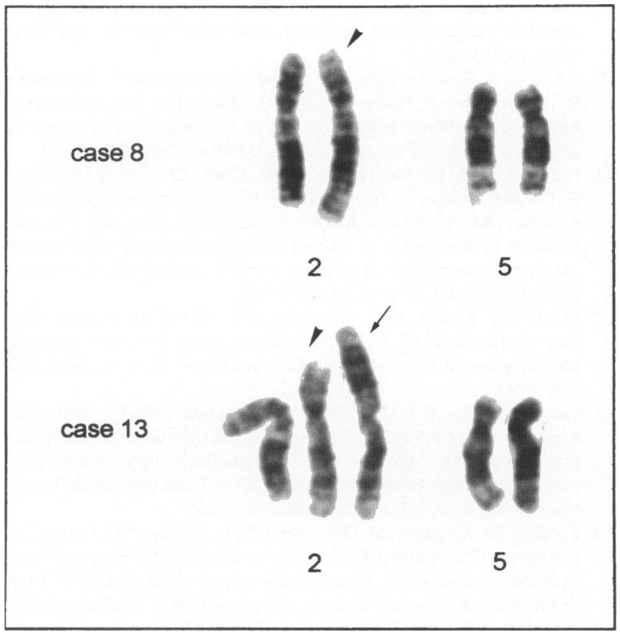

Figure 3. Partial karyotype of two cases with different abnormalities involving 2p23. In each case the normal chromosome 2 is on the left side. **Arrowheads** indicate der(2)t(2;5)(q37;q31) in case 8 and inv(2)(p23q35) in case 13, whereas i(2)(q10) in case 13 is marked by an **arrow**.

acteristic of ALCL.[1] The identification of the genes involved in the translocation t(2;5), *NPM* and *ALK*, has permitted studies using molecular techniques to be performed on a much larger group of tumors. The incidence of *NPM/ALK* fusion found in these studies varied from 16 to 80% depending on the inclusion and selection criteria adopted.[13,15–19] The distribution of t(2;5)-positive cases within the histological variants of ALCL with T or null phenotype has been reported in some instances,[8,13,15–17] but others have not found any association with specific morphology or phenotype.[19–22]

In the present study, ALK1 immunostaining was confined to 13 cases diagnosed as ALCL. All of these patients had systemic disease with nodal, and often extranodal, involvement. Moreover, the ALK1-positive cases occurred in children and in relatively young patients with no patients older than 37 years of age, whereas the ALK1-negative ALCL cases were distributed over a wider age range. Other studies have reported a high incidence of the t(2;5) in children and young adults,[15,21,27] whereas the occurrence of the t(2;5) in ALCL affecting the older age group is a less common event.[21]

The neoplastic cells in all ALK1-positive cases were relatively uniform, and the presence of a high number of reactive histiocytes was found in two cases. A similar finding was reported also by Shiota and colleagues[27] where the p80-positive cases displayed a less pleomorphic morphology in comparison with the p80-negative cases. Regarding the phenotype, all ALK1-positive cases from the present study, as well as in the study by Pulford and colleagues,[35] were either of T or null phenotype. In contrast to that study only one of the ALK1-positive cases (case 7) lacked CD30 expression; however, despite a normal karyotype a *NPM/ALK* fusion signal was detected by FISH.

Nine of the 13 ALK1-positive cases showed the presence of the t(2;5)(p23;q35) identified either by classical cytogenetics (4 cases) or by two-color FISH assay (5 cases). In one additional case t(2;5)(p23;q35) was found by RT-PCR. Interestingly, in one case with the *NPM/ALK* gene fusion detected by FISH, lack of a classical t(2;5)(p23;q35) and presence of a der(2)t(2;5)(q37;q31) were found with classical cytogenetic analysis. This case (case 8) was previously studied by FISH with a library 5 and a panel of probes for the 5q31q35 region.[43] The presence of the paired 5q35 and *ALK* signal in the distal band of the der(2), previously shown to be uniformly painted with a library 5, excludes a t(2;5)(p23;q35) and points to the cryptic *NPM/ALK* fusion as a consequence of the insertion of the *ALK* gene from a cytogenetically normal-seeming chromosome 2 in the vicinity of the *NPM* locus on the der(2). These results shed new light on the mechanism of *NPM/ALK* fusion in ALCL. Similar findings were previously reported in chronic myeloid leukemia cases with normal karyotype and *BCR* rearrangement in which an insertion of *BCR* upstream of *ABL* was detected by FISH.[44] Other interesting findings were observed in case 13, in which a new chromosomal abnormality involving the *ALK* gene, namely inv(2)(p23q35), was identified. Expression of the ALK protein in this case and translocation of the *ALK* gene to 2q35 indicate a localization of a new gene that might deregulate *ALK* and be involved in the pathogenesis of ALCL. The presence of three copies of an aberrantly located *ALK* gene in the abnormal cells of this case can also be significant. The inv(2)(p23q35), together with two other variant translocations reported by Sainati and colleagues[45] and Lamant and colleagues,[15] namely t(2;13)(p23;q34) and t(1;2)(q25;p23), indicates that different genes, other than *NPM*, can act as the promoter for the *ALK* gene and drive its transcription and expression. Unfortunately, additional investigations could not be performed on the RT-PCR-negative/ALK1-positive case in which variant rearrangement of the *ALK* gene can be hypothesized. Altogether, our findings lead us to the conclusion that immunostaining with the ALK1 antibody is the most sensitive method for the detection of 2p23 abnormalities in ALCL. Neither two-color FISH assay nor RT-PCR for *NPM/ALK* could identify variant translocations of the t(2;5)(p23;q35), which might be more frequent than expected based on cytogenetic analysis.

Although a morphological spectrum with overlapping histological and immunophenotypic features has been described between ALCL and HD,[46] lack of *NPM/ALK* or ALK protein expression in any of the HD and HD-like anaplastic cases included in our study suggests that HD and HD-like ALCL might be more closely related to each other or to ALK1-negative ALCL cases than to the ALK1-positive subgroup. Also in support of this hypothesis is the lack of t(2;5) in the vast majority of such cases analyzed by RT-PCR.[13,15,18,22,31–33] The relationship among HD, HD-like ALCL, and ALK1-negative ALCL cases awaits the identification of markers more specific for each of these disorders.

Regarding lymphomatoid papulosis, none of the cases were immunostained by ALK1 antibody. These data are in keeping with a majority of prior studies[13,17,33] that did not find the *NPM/ALK* fusion in these cases, which strengthens the distinction between ALCL with cutaneous involvement and lymphomatoid papulosis. It is of interest to note that in the present series, two cases of systemic ALCL with skin involvement showed labeling of the skin lesions with antibody ALK1. This observation may suggest a possible diagnostic application of this immunohistochemical staining in the differential diagnosis of cutaneous involvement by ALCL and lymphomatoid papulosis.

In summary, we demonstrated for the first time, using FISH analysis, that the *ALK* gene can be deregulated by a cryptic *NPM/ALK* fusion or by a variant chromosomal aberration like an inv(2)(p23q35), both not detectable by classical cytogenetics. The ALK1 immunostaining proves to be the most informative technique for the detection of the 2p23 rearrangements associated with a deregulation of the *ALK* gene. The ALK1 reactivity, in combination with morphology and FISH data, strengthens the recognition of a distinct clinicopathological entity in the group of ALCL.

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