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Mediastinal large-cell lymphoma with sclerosis

Genotypic analysis establishes its B nature*

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Summary. The Southern blot hybridization technique has been applied to study the configuration of immunoglobulin and T-cell receptor genes in 6 cases of the so called mediastinal large cell lymphoma with sclerosis. This lymphoma has been recently recognized as a separate entity among non-Hodgkin lymphomas mainly affecting young adult patients. The B-cell origin of this neoplasm was suggested by means of immunohistochemical analysis. However, the immunophenotypical B-cell related markers used do not always exhibit lineage fidelity. The Southern blot analysis demonstrated the presence of unique heavy and k-light chain immunoglobulin gene rearrangements, establishing genotypically their B-cell origin.

Key words: Mediastinal lymphoma – Immunoglobulin deficient B cell lymphoma – Gene rearrangement – Immunoglobulin genes – T cell receptor gene

Introduction

A mediastinal large cell lymphoma with sclerosis has been recognized recently as a separate entity among non-Hodgkin lymphomas (Yousem et al. 1985; Addis and Isaacson 1986; Menestrina et al. 1986; Möller et al. 1986). This lymphoma is characterized by primary occurrence in the upper anterior mediastinum, young adult age of patients, no involvement of bone marrow, secondary spreading to unusual sites such as kidney and aggressive clinical behaviour. On morphological grounds this peculiar lymphoid malignancy might be easily misdiagnosed as seminoma, thymoma or undifferentiated carcinoma (Addis and Isaacson 1986; Menestrina et al. 1986; Möller et al. 1986).

Evidence for the B-cell origin of these lymphomas has been provided by the positive staining of neoplastic cells with a variety of monoclonal antibodies (MoAbs) which recognize B-cell related antigens such as BA1 (CD24), TO15 (CD22), anti-C3dR (CD21) and B1 (CD20) (Yousem et al. 1985; Addis and Isaacson 1986; Möller et al. 1986; Menestrina et al. 1986). However, these immunophenotypical markers do not always exhibit lineage fidelity (Knowles II et al. 1985). In addition, monotypic surface light chain expression has been reported only in a minority of these cases (Yousem et al. 1985; Addis and Isaacson 1986; Menestrina et al. 1986; Möller et al. 1986).

We have studied the configuration of immunoglobulin (Ig) and T-cell receptor (TcR) genes in six cases of mediastinal large cell lymphoma with sclerosis, establishing genotypically their B-cell origin.

Materials and methods

Tissue samples were obtained at mediastinoscopy or thoracotomy from 6 patients with a mediastinal mass. Fresh tissue fragments were divided into three parts. One portion was fixed in 10% formol-saline and processed for conventional histology. The other portions were snap-frozen in liquid nitrogen for immunohistochemistry and DNA analysis.

Immunohistochemical analysis was performed on cryostat sections as previously described (Men-

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Table 1. Patients and results of immunologic studies

Patient	age	sex	Antibodies[a]:					
			HLe-1 CD45	Ig	HLA-DR	Leu 14 CD22	C3dR CD21	Leu 4 CD3
1	30	F	+	−	−	++	−	−
2	30	F	+	−	−	+	+	−
3	23	M	+	−	++	−	+	−
4	30	F	+	−	+	+	++	−
5	30	M	+	−	+++	+	+	−
6	38	M	+	−	−	+	−	−

[a] All antibodies were purchased from Becton Dickinson

Ig = surface and cytoplasmic immunoglobulins

estrina et al. 1986). Briefly, 5 μm tissue sections were fixed in cold acetone and immunostained using a peroxidase-anti-peroxidase (PAP) technique. The antibodies used in this study are listed in Table 1.

High molecular weight DNA from 6 cases of mediastinal large cell lymphoma was purified by sodium dodecyl sulfate (SDS)-proteinase k digestion, extraction with phenol chloroform and ethanol precipitation (Maniatis 1982). The concentration and purity were determined by spectrophotometry. DNAs were digested with appropriate restriction enzymes (see below), electrophoresed in 0.8% agarose gel, denatured, neutralized and transferred to a nitrocellulose filter according to Southern (1975). The filters were hybridized at 68° C for 18 h with nick translated (Rigby et al. 1977) ^{32}P radioactive probe ($1–2 \times 10^6$ cpm/ml) in 6× concentrated SSC (1 × SSC: 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0), containing 0.5% SDS, 0.02% w/v of each of Ficoll, bovine serum albumin and polyvinylpyrrolidone, 200 μg/ml denatured salmon sperm DNA. ^{32}P dCTP was purchased from Amersham (Amersham UK); all the other reagents were obtained from Sigma (St. Louis, MO, USA).

Filters were washed to a final stringency of 0.2 × SSC and 0.1% SDS, pH 7, at 68° C for 2 h and exposed for 2–7 days at −70° C to Kodak X-AR-5 films with intensifying screens.

The DNA probes employed in this study were specific for the heavy chain joining region (JH) (Ravetch et al. 1981) and the constant region of the kappa light chain (Ck) genes (Hieter et al. 1980) [gift of Dr. Philip Leder, Harvard Medical School, Boston, USA]. The T-cell receptor gene rearrangement has been evaluated with a specific probe for the β subunit of the receptor recognizing both regions (Cβ1 and Cβ2) of the Tβ constant gene (Yoshikai et al. 1984) [kindly supplied by Dr. Tak Mak, Ontario Cancer Institute, Canada].

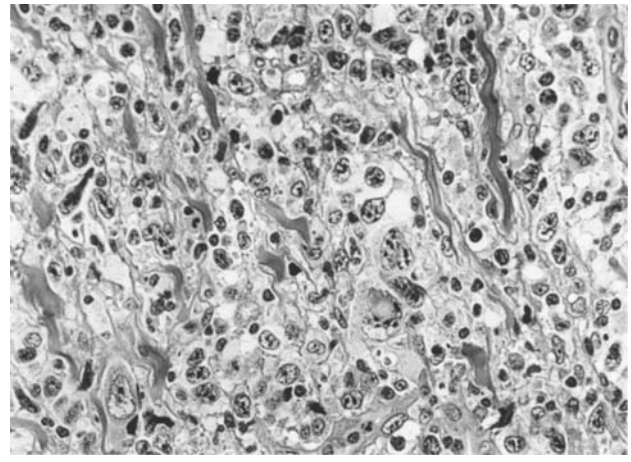

Fig. 1. Histological appearance of mediastinal large cell lymphoma (case # 2): Large, epithelial-like cells with abundant, clear cytoplasm separated by thin collagen bands. H & E ×250

DNA samples were digested with restriction enzymes Eco RI and Bam HI before hybridization with JH and TcR β chain gene probes; with Bam HI before Ck hybridization.

A rearrangement was scored by the appearance of bands other than the germline ones.

Results

In all cases the histological examination showed a morphology very unusual for a lymphoma, as previously described (Menestrina et al. 1986) (Fig. 1). Large epithelial-like neoplastic cells were arranged in nests and ribbons frequently compartmentalized by thin collagen bands.

The immunohistochemical analysis confirmed the lymphoid nature of the neoplastic cells, which expressed panleukocyte antigen (CD 45) and at

Table 2. Results of gene analysis

Patient	J_H	K	T_β
1	+/−	+/+	−/−
2	+/−	+/−	+/−
3	+/−	+/−	−/−
4	+/+	+/−	−/−
5	+/−	+/+	−/−
6	+/−	+/−	−/−

+ rearranged allele
− germline allele

least one B-cell related marker (Table 1). In no one case surface immunoglobulins could be detected.

We analyzed the organization of Ig and TcR genes in all these cases by Southern blot hybridization using probes representative of different portions of Ig and Tβ loci. In several control cases (normal lymphoid and non-lymphoid tissues) the DNA examined with the JH probe, after digestion with Eco RI or Bam HI restriction enzymes, showed a single band in the Southern blot autoradiogram at 18 and 17 kb respectively. These bands are defined as germline bands.

DNAs from the 6 mediastinal lymphomas yielded one or two bands in addition to the germline band (Table 2). The appearance of these new bands indicates the presence of uniform rearrangements in the DNA of one or both alleles of Ig heavy chain gene within monoclonal populations.

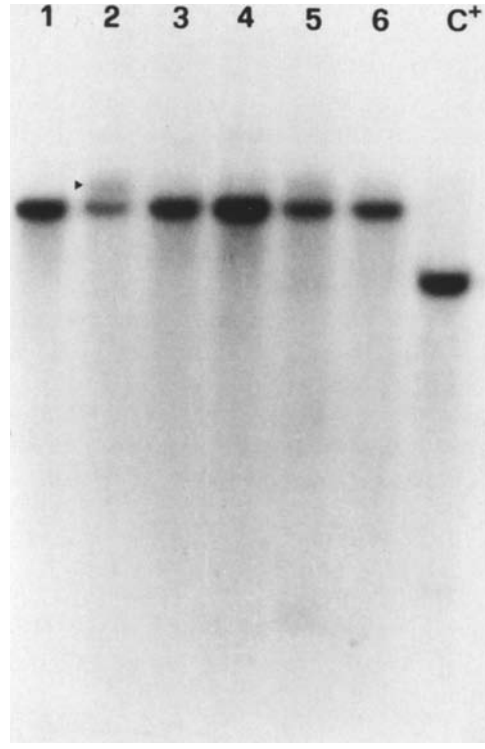

Fig. 3. Autoradiographic analysis of β T-cell receptor gene rearrangements in mediastinal large cell lymphomas, after digestion with BamHI restriction endonuclease. Numbers correspond to cases listed in Table 1. All genes are in germline configuration (23 kb bands) except case n° 2 that shows a rearranged band (*arrowhead*). C^+ is a positive control (T-lymphoma) that shows the deletion of the germline band and the appearance of a new rearranged band

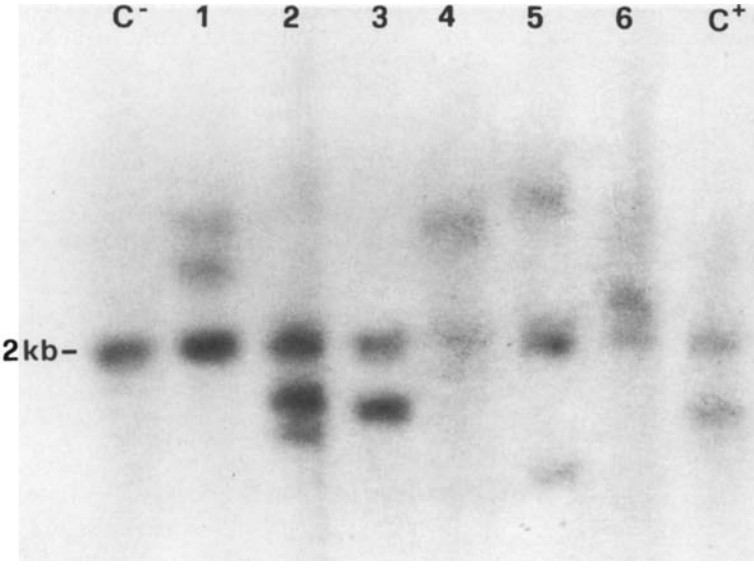

Fig. 2. The figure shows the results obtained when DNAs from six mediastinal large cell lymphoma (1–6; numbers correspond to cases listed in Table 1) were analyzed with a probe specific for the constant region of K-light chain gene, after digestion with BamHI restriction endonuclease. Any band in positions other than that of germline configuration (12 kb) indicates a uniform rearrangement in the DNA within a monoclonal population. C^- is a negative control (T-CLL) and shows a germline configuration after Bam HI digestion of the Ck gene. C^+ is a positive control (B-lymphoma, k-monoclonal) and shows a new band in Bam HI digest indicating rearrangement of the Ck gene

DNAs from all cases were also analyzed with a probe specific for the k-light chain Ig gene, after Bam HI digestion. All mediastinal lymphoma specimens showed one or two rearranged bands other than the germline band at 12 kb (Fig. 2). In cases 1 and 5, the presence of two rearranged bands of the same intensity, suggests that both Ck alleles are rearranged.

For TcR β chain gene analysis, DNA was digested with Eco RI and BamHI restriction enzymes and hybridized to a specific probe that recognizes both (Cβ1 and Cβ2) regions of the Tβ constant gene. Eco RI digestion allows the detection of rearrangements involving only the Cβ1 region of the Tβ gene, whereas Bam HI restriction allows the detection of rearrangements involving both the Cβ1 or Cβ2 regions.

All the TcR β genes of our cases were present in a germline configuration on Eco RI digested DNA (data not shown). However, case 2 displayed a rearrangement of TcR gene on Bam HI restricted DNA (Fig. 3). The results of DNA analysis are summarized in Table 2.

Discussion

In this study we have established at the gene level the B-nature of the so called primary mediastinal large cell lymphoma with sclerosis, using Southern blot hybridization technique in 6 cases.

The results of the genotypic analysis appear to be of relevance since most of these lymphomas lack both cytoplasmic and surface immunoglobulins. These neoplasms have been previously assigned to the B-lineage by the detection of several B-cell related markers including BA1 (CD24), TO15 (CD22), anti-C3dR (CD21) and B1 (CD20). However, such immunophenotypic analysis alone does not always result in a satisfactory lineage assignment (Knowles et al. 1985). In addition, when B-related markers are present, they are variably and heterogeneously expressed among the neoplastic cell population.

The detection of immunoglobulin or T-cell receptor gene rearrangements provides a sensitive marker for both lineage and clonality within lymphoid tumours lacking expression of definitive phenotypes (Korsmeyer et al. 1983; Arnold et al. 1983; Flug et al. 1985; Griesser et al. 1986). The DNA rearrangements produce a change in the location of restriction endonuclease sites that allows the rearranged Ig or TcR gene in a monoclonal expansion of lymphoid cells to be identified by Southern blot analysis using radiolabeled DNA probes, this establishing the B or T nature of otherwise unclassified lymphomas (Isaacson et al. 1985; Knowles et al. 1985; Weiss et al. 1985; O'Connor et al. 1987).

All 6 cases in our study possess unique heavy and k-light chain Ig gene rearrangements. As heavy chain gene rearrangement has been found in occasional non-B-lineage neoplasms (Ha et al. 1984; Rovigatti et al. 1984; Pelicci et al. 1985), light chain rearrangement is more reliable for the assignment to B-lineage, since it is found only in clonal proliferations of B-cells (Korsmeyer et al. 1983; Cleary et al. 1984; Weiss et al. 1985).

Interestingly, one of our cases shows also a TcR gene rearrangement. This finding does not invalidate the assignment of this case to the B-lineage since the occurrence of mixed genotype seems to be fairly frequent in B-cell neoplasms (15% in the series of Pelicci et al. 1985).

According to our results the malignant cells of mediastinal large cell lymphoma appear to be differentiated B cells. They have in fact completed the rearrangements of both heavy- and light-chain Ig genes, yet they fail to produce cytoplasmic or surface Ig. This may reflect a non-functional rearrangement of the Ig genes or an arrested maturation at cellular levels not expressing the gene. This is not an uncommon finding since many B-cell lymphomas of germinal center origin do not express membrane or cytoplasmic immunoglobulins.

The peculiar location in the antero-superior mediastinum and the presence of epithelial thymic remnants observed in a number of cases (Addis and Isaacson 1986; Menestrina et al. 1986) suggest that these large cell lymphomas of B-type arise within the thymus. The normal cell counterpart could be, as for thymic follicular hyperplasia, B lymphocytes belonging to the extraparenchymal compartment of the thymus (Levine and Rosai 1978).

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