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Evidence for neoplastic cell differentiation in mediastinal T lymphoblastic lymphoma*

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Summary. Immunostaining of frozen sections from a mediastinal T lymphoblastic lymphoma (T-LL) revealed the existence of two neoplatic cell populations characterized by different degrees of maturation. Several large nodules of 3A1+/T11+/T9+ T6−/T4−/T8−/T3− lymphoid cells, resembling normal early thymocytes, were surrounded by 3A1+/T11+/T9+/T6+/T4+/T8+/T3− cells resembling normal cortical thymocytes. The junctional area between early and cortical lymphocytes was occupied by numerous Leu-M3+/PAM-1+/DR+ reticular macrophages which were also characterized by J5 reactivity. Cytokeratin+/keratin+ epithelial cells were absent. Immunostaining of paraffin sections and of cytocentrifuge smears obtained from tumour cell suspensions revealed that a consistent percentage (8%) of neoplastic lymphoblasts were S-100+. Our findings are consistent with a cortical T-LL presenting areas of dedifferentiated cells or, alternatively, with an early T-LL whose cells were able to differentiate into cortical thymocytes, perhaps through the interaction with a specialized subset of J5+ macrophages.

Key words: Lymphoblastic lymphoma – Immunohistochemistry – Cell differentiation – Histiocytes – Lymphocytes

Introduction

Neoplastic transformation of haemopoietic cells is often associated with maturation arrest and uncoupling of proliferation and differentiation (Greaves et al. 1986). The maturation block, however, is not always irreversible since it has been demonstrated that a variety of stimuli from different sources can induce terminal differentiation of acute leukemia cells in vitro (Gabrilove et al. 1985); this evidence suggests that the genetic material regulating maturation is not always compromised by the transformation event.

T Acute Lymphoblastic Leukaemia (T-ALL) and T Lymphoblastic Lymphomas (T-LL) are composed of lymphoid cells having enzymatic and antigenic properties similar to those of normal thymocytes at different maturation stages (Reinherz et al. 1980). Moreover, immunological characterizations of T-ALL/T-LL have demonstrated that the neoplastic cell populations are uniformly arrested at a definite maturation stage allowing tumour classification as early, cortical or medullary type (Greaves et al. 1981; Bernard et al. 1981; Weiss et al. 1986).

In the present paper we describe a case of mediastinal T-LL in which two neoplastic cell populations, one resembling early thymocytes and the other similar to cortical thymocytes, were coexisting within the tumour. The possibility is discussed that this composite T-LL is derived from neoplastic cells having the capacity to progress along the maturation pathway in a way similar to that of normal thymocytes.

Materials and methods

Fresh tissue specimens obtained from an anterior mediastinal mass were processed to obtain acetone-fixed cryostat sections, formalin-fixed paraffin-embedded sections and single cell suspensions.

Cryostat sections were stained with one of the following antibodies: OKT3, OKT4, OKT6, OKT8, OKT9, OKT10, OKT11, OKT16, OKM-1, OKIa-1, anti-Cytokeratin A, anti-Keratin B (Ortho Pharmaceutical, Raritan, N.J., USA), Leu-

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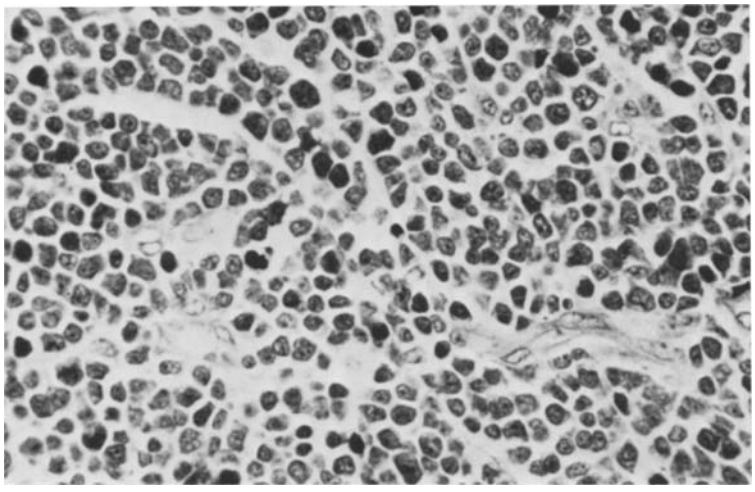

Fig. 1. Anterior mediastinal mass from a 16 year old female patient. The tumor is characterized by a diffuse proliferation of small and medium-sized lymphoid cells with convoluted nuclei (Giemsa, × 352)

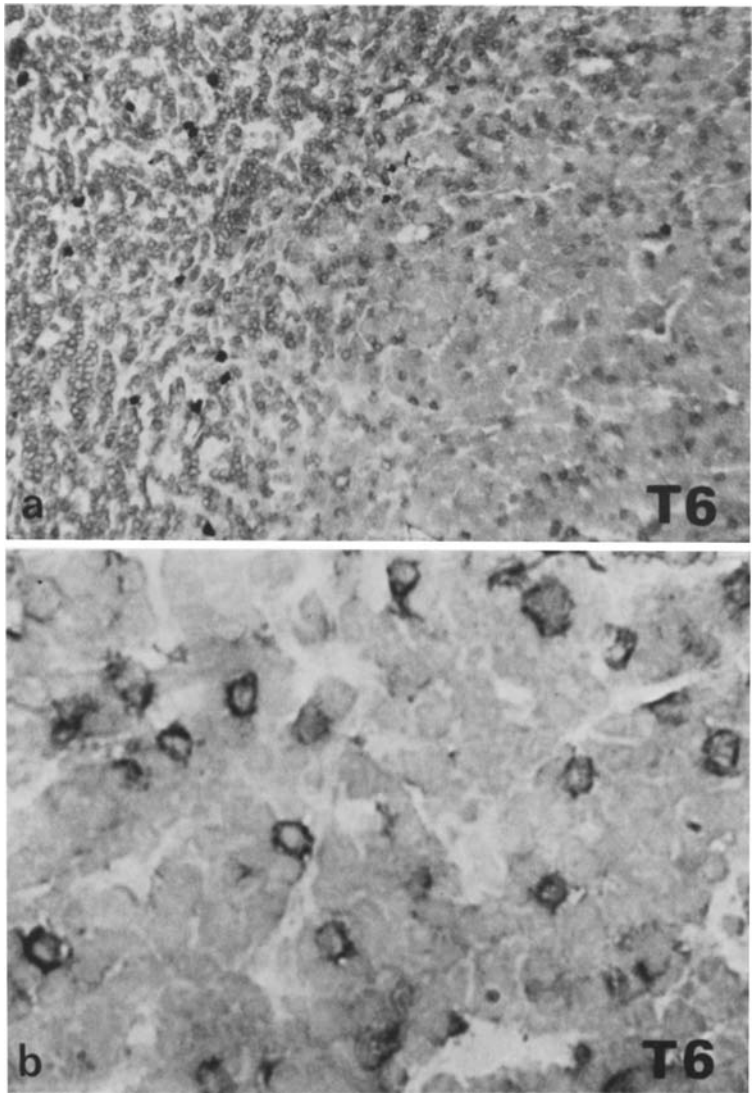

Fig. 2. Mediastinal T lymphoblastic lymphoma. Immunostaining of frozen sections showing the phenotypic heterogeneity of the neoplastic cell population. **a** A large nodule of T6− cells is surrounded by numerous T6+ cells with scattered eosinophils (× 100). **b** Higher magnification of the transition area showing the intimate commixture of negative and positive lymphoid cells (× 400). (Avidin-biotin immunoperoxidase, counterstained with Haematoxylyn)

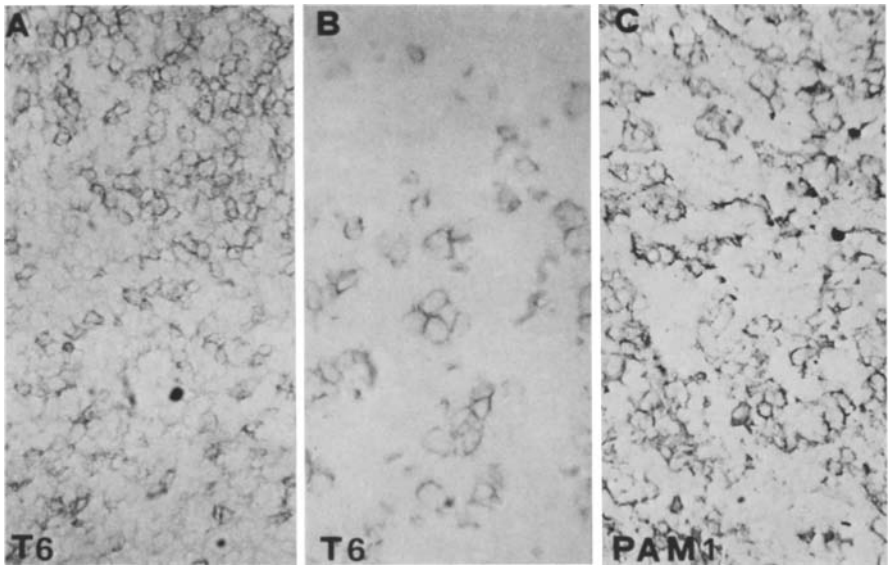

Fig. 3. Mediastinal T lymphoblastic lymphoma. Characterization of the junction area between T6+ cells (**A** *top*) and T6− cells (**A** *bottom* ×250). Small clusters of T6+ cells (**B** ×400) are surrounded by numerous PAM-1+ macrophages (**C** ×250). (Avidin-biotin immunoperoxidase, not counterstained)

M3 (Becton Dickinson, Sunnyvale, CA, USA), J5 (Coulter Clone, Hialeah, FL, USA) anti-Factor VIII Related Antigens (F VIII-RA) and antiproliferating cells (Ki-67) (Dakopatts, Denmark) and PAM-1. PAM-1 monoclonal antibody was raised against pulmonary alveolar macrophages (Biondi et al. 1984) and is highly specific for tissue macrophages (Kobzik et al.). Except for anti F-VIII-RA, which was a rabbit polyspecific serum, slides were incubated with biotin-conjugated horse anti-mouse immunoglobulin antibodies and then with avidin-biotin peroxidase complex (PK 4002; Vector Laboratories, Burlingame, CA, USA). The sections were finally incubated with 0.03% H_2O_2 and 0.06% 3,3′-diaminobenzidine (BDH Chemicals, UK) for 3–5 min. In negative controls the primary antiserum was omitted. Endogenous peroxidase was inhibited by pre-incubation in 1% H_2O_2 in PBS for 30 min. Paraffin sections were stained with haematoxylyn-eosin or were immunostained for S-100 protein, muramidase, α1-antitrypsin (AAT), α1-anti-chimotrypsin (AACT) using rabbit polyspecific sera (Dakopatts, Denmark). Paraffin sections were incubated with optimal dilutions (1:500−1:1,000) of the specific serum for 12 h, then with a 1:50 dilution of a goat anti-rabbit serum (Dakopatts, Denmark) for 30 min, and treated for 30 min with PAP (1:100) (Dakopatts, Denmark). The reaction product was developed as above described.

Cytocentrifuge smears from lymph node cell suspensions were acetone-fixed and were immunostained as previously described for cryostat sections. Cryostat sections and cytocentrifuge smears were also stained for acid phosphatase (AP) and for α-naphtyl-acetate esterase (ANAE).

Results

An anterior mediastinal mass, infiltrating pleura and pericardium, was surgically removed from a 16 year old female. Histologically, the tumour was characterized by a diffuse proliferation of small and medium-sized lymphoid cells with convoluted nuclei (Fig. 1). A starry-sky pattern, due to histiocytes with ingested lymphoid cells, was evident in some areas. Immunostaining of frozen sections revealed that the neoplastic cell population was not homogeneous in terms of surface phenotypes. In fact, several large nodules of 3A1+/T11+/T9+/T6−/T4−/T8−/T3−/DR− cells were surrounded by a population mainly composed of 3A1+/T11+/T9+/T6+/T4+/T8+/T3−/DR− cells (Fig. 2). Both cell populations were highly proliferating as demonstrated by the presence of numerous Ki-67+ cells. The periphery of the nodules was occupied by numerous Leu-M3+/PAM-1+/ANAE+/AP+/DR+ reticular histiocytes (Fig. 3). Histiocytes with a similar antigenic profile were scattered singly in tumour areas composed of cortical thymocytes. J5 staining was detected on T6+ lymphoid cells, as previously described (Greaves et al. 1981; Hsu and Jaffe 1985; Weiss et al. 1986), but was also consistently observed on cells closely resembling reticular histiocytes. The vascular endothelium was FVIII−RA+/DR+. Immunostaining with monoclonal antibodies directed against cytokeratin and keratin failed to demonstrate positive cells. On paraffin sections it was found that tumour associated macrophages were AAT+/AACT+ and that numerous putative neoplastic lymphocytes were characterized by immunoreactivity for S-100 protein. On cytocentrifuge smears, about 70% of cells showed a perinuclear dot of AP reactivity. Immunostaining of cytocentrifuge smears obtained from the tumour cell suspension confirmed the phenotypic heterogeneity of the neoplastic cell population (95% 3A1+/T9+; 30–40% T6+/T4+/T8+) and allowed to establish that 8% of neoplastic lymphocytes were S-100+.

Discussion

In the present paper we describe a mediastinal T lymphoblastic lymphoma characterized by a composite immunohistochemical pattern. The diagnosis of T-LL was supported by the morphological aspect of the tumour, by the positive staining of neoplastic cells for T lymphocyte markers and by the absence of cytokeratin+/keratin+ epithelial cells which are always present in lymphocytic thymomas of cortical type (Battiflora et al. 1980). On frozen sections it was demonstrated that two T cell populations, with different degrees of maturation but with similar proliferative capacity, coexisted within the tumour. In fact, several large nodules of cells with antigenic profiles resembling those of early thymocytes were surrounded by other neoplastic cells similar to cortical thymocytes. Two interpretations may be proposed to explain our findings: either the tumour was a cortical T-LL containing areas of dedifferentiated cells, or it was an early T-LL in which neoplastic cells retained the capacity to differentiate into cortical thymocytes. The first interpretation seems to be difficult to reconcile with the multicentricity of dedifferentiated areas and with the coherent expression by dedifferentiated cells of surface phenotypes similar to those of early thymocytes. On the other hand, the prominent and constant presence of histiocytes in the junction area between early and cortical cells may support the second interpretation. In fact, it is generally believed that intrathymic maturation of normal thymocytes requires interactions with thymic macrophages and reticulo-epithelial nurse cells (van den Wijngaert et al. 1983); moreover it has been demonstrated that thymic macrophages are able to promote thymocyte differentiation in vitro (Beller and Unanue 1978). Thus, it can be speculated that neoplastic early thymocytes were still able to interact functionally with macrophages and, hence, to further differentiate into cortical thymocytes.

Scattered macrophages are frequently present in T-LL and may confer on the tumour a starry-sky appearance similar to that of Burkitt-type B-LL (Lennert 1978). In our case, we have found that tumour associated macrophages were Leu-M3+/PAM-1+/AAT+/AACT+, like tissue macrophages of other lymphoid organs (Dimitriu-Bona et al. 1983; Kobzik et al. 1985; Motoi et al. 1980). Furthermore, we have presented circumstantial evidence indicating that these cells were also J5+, like normal bone marrow macrophages (Lai et al. 1985). Therefore, it seems possible that J5 expression is restricted to a specialized subset of macrophages which are often associated with immature haematopoietic cells. These observations may further indicate that macrophages in T-LLs are not merely reactive, but play an active role in tumour development.

Immunostaining of tumour sections and cytocentrifuge smears revealed that about 8% of neoplastic lymphoblasts were S-100+. In lymphoid tissues, immunoreactivity for S-100 protein was first recognized on interdigitating reticulum cells (Takahashi et al. 1981), but was then detected in a subpopulation of circulating T8+ lymphocytes (Takahashi et al. 1985) and in lymphocyte-like cells located in the T dependent areas of normal lymphoid tissues (Uccini et al. 1986). Moreover, we have observed a T8+ lymphoma which evolved shortly after in a S-100+ large cell tumour (Ruco et al. 1984). All these findings may indicate that S-100 protein is also a marker for a subset of normal T lymphocytes and may suggest that a minority of neoplastic lymphoblasts retained the capacity to acquire this antigenic specificity.

The demonstration of subpopulations of neoplastic cells expressing different degrees of maturation in the same tumour may help in understanding some clinical aspects of the disease. In fact, it has been reported that a) Neoplastic cells of T Acute Lymphoblastic Leukaemia are often more immature than those of T-LL (Bernard et al. 1981; Weiss et al. 1986). b) Frank leukaemic conversion of T-LL is associated with regression of the differentiative status of neoplastic cells (Bernard et al. 1982). c) Changes in surface antigens on malignant T cells are frequently present in patients at relapse (Bernard et al. 1982). Therefore, it is possible that some of these observations may be related to the different homing capacity of neoplastic subclones which are already present in the primary tumour and which may be further selected by chemotherapy.

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