Virchows Arch [Pathol Anat] (1984) 404:351–358

Virchows Archiv A

S-100+ lymph node neoplasm

Report of a case with histological AL and immunological features intermediate between T cell lymphoma and malignant histiocytosis

Luigi P. Ruco, Antonella Stoppacciaro[1], Paola Barsotti[2], Domenico Vitolo[1], Marina Mirolo[1], Anna Maria Cassano[1], Cesare Guglielmi[3], Franco Mandelli[3], Stefania Uccini[1], and Carlo D. Baroni[1]

[1] 4th Chair of Pathological Anatomy, Department of Biopathology, Sections of Immunopathology,
[2] Ultrastructural Pathology and
[3] Haematology, University of Rome "La Sapienza", Italy

Summary. A 16-yr-old white female was affected by continuous fever, pancytopenia with relative increase of T-8 lymphocytes, severe bone marrow hypoplasia, generalized lymphadenomegaly and splenomegaly. A first lymph node biopsy, obtained at the onset of the disease, was involved by a paracortical tumor with some S-100+ "lymphocyte-like" cells in the neoplastic areas; in the cell suspension, 70-80% of cells were E4+/E37+ lymphocytes with prevalent expression of the T-8 phenotype (52%). A second lymph node biopsy, obtained five months later, was involved by a diffuse proliferation of S-100+ cells with high mitotic activity; in the cell suspension, the majority of cells were E-/T-11+/T-3+/T-8+. At the TEM level, the neoplastic cells were characterized by regular or indented nuclei with finely dispersed chromatin and by regular or indented nuclei with finely dispersed chromatin and by irregular cytoplasmic profiles with thick pseudopodia-like projections. The possibility is discussed that this neoplasm may share some similarities with the T-γ lymphoma being part of a poorly described group of tumors with intermediate features between T cell lymphoma and malignant histiocytosis.

Key words: Accessory – Cell – Lymph mode – Tumour

Introduction

The proliferative lesions of the lymphoid system are now believed to result from an uncontrolled proliferation of lymphoid cells which are recognizable

Offprint requests to: Dr. Luigi P. Ruco, 4th Chair of *Pathological Anatomy*, Viale Regina Elena 324, 00161 Roma, Italy

Supported by CNR contract N. 83.00724.96, Progetto Finalizzato "Controllo della crescita neoplastica"

as the malignant counterparts of morphological stages of differentiation and of maturation of both T and B lymphocytes and of the accessory cells related to the monocyte-macrophage system (Berard et al. 1978; Lennert and Mohri 1978; Lukes and Collins 1974). The two main classifications of lymphoid tumours (Lukes and Collins 1975; Lennert 1978) provide histological criteria for identification of T and B non-Hodgkin's lymphomas and of neoplastic proliferations deriving from the accessory cell system. This system is highly heterogeneous being composed of several non-lymphoid cells which can be distinguished by their morphological, enzymatical and phenotypical properties. A sub-population of accessory cells is represented by the Interdigitating Reticulum (IDR) cells which, in lymphoid tissues, are specifically recognized by anti-S-100 antisera (Takahashi et al. 1981). This report describes the case of a young patient with a peculiar type of non-Hodgkin's lymphoma whose cells were S-100+. Despite the S-100 reactivity of the neoplastic cells, the histology and some of the immunological data were suggestive for a possible T cell origin of the neoplasm.

Case report

The patient, a 16-yr-old white female, was admitted to the Division of Heamatology of the University of Rome in February 1983 because of rapidly progressing illness with fever, weight loss and general debilitation. No useful information was obtained from the patient's past case history. At the physical examination, generalized lymphadenomegaly (supra and sub-diaphragmatic) and splenomegaly were discovered. Results from routine laboratory investigations revealed a marked pancytopaenia (Haemoglobin 5.9 gr/dl; Leukocytes $2.0 \times 10^9/l$; Thrombocytes $79 \times 10^9/l$). The iliac crest needle biopsy was characterized by severe cellular depletion and by extensive replacement of the intertrabecular marrow spaces with fatty tissue. The chest x-ray results were normal, without mediastinal enlargement. A cervical lymph node was excised and a diagnosis of non-Hodgkin's lymphoma was made. The patient was then treated with intensive inductive polychemotherapy and experienced an initial and partial improvement of the symptoms. Despite therapy, the patient's disease progressed and she died seven months after symptom onset of supervening infection. A second cervical lymph node biopsy was taken few days before death. The autopsy was not performed.

Materials and methods

Histopathology. Routine sections of formalin-fixed lymph nodes were stained with haematoxilin-eosin and with Giemsa. Immunoperoxidase staining for cytoplasmic immunoglobulin light chains, muramidase, α-l-anti-trypsyn (Dakopatts, Denmark) and for S-100 protein were carried out using the previously described PAP technique (Sternberg et al. 1970). Two different anti-S-100 antisera were used; the first was a rabbit hyperimmune serum (Cocchia and Michetti 1981); the second was purchased from Dakopatts. Enzyme-histochemistry was performed on cryostat sections for detection of α-naphtyl-acetate esterase (Horwitz et al. 1977), acid phosphatase (Li et al. 1970). Bone marrow histology was studied an haematoxylin-eosin stained sections from plastic embedded iliac crest needle biopsy.

Tissue fragments for electron microscopy were prepared and fixed in buffered paraformaldehyde, post-fixed in osmium-tetroxide and embedded in Epon. Ultrathin sections from selected blocks were treated with uranyl-acetate and lead citrate.

Cell suspension study. Mononuclear cells were separated by Ficoll-hypaque technique from peripheral blood, bone marrow and lymph nodes. SMIg+ cells were enumerated by direct immunofluorescence with anti-light chain (kappa and lambda) polyspecific antisera (Nordic Immunology, The Netherlands). The percentage of E-rosetting cells was evaluated with

neuraminidase-treated sheep erythrocytes at 4° C and at 37° C. Indirect immunofluorescence was used to enumerate the proportions of mononuclear cells expressing antigens detected by the following monoclonal antibodies: OKT-3, OKT-4, OKT-6, OKT-8, OKT-10, OKT-11, OKM-1 and OKIa-1 (Ortho Diagnostic, Raritan, NJ), HNK-1 (Becton Dickinson, Sunnyvale, CA). Cytocentrifuge smears were stained with Giemsa or for detection of neutral-esterase, acid-esterase and acid-phosphatase.

Results

Pathology findings. Lymph node n.1: The general architecture of the node is partially effaced by a proliferation of small-medium size cells mainly located in the paracortical areas (Fig. 1). Few small follicles are still present in the subcapsular zone. The lymphoid cell population is mainly composed of two types of cells (Fig. 1; Inset 1): a) Lymphocyte-like cells characterized by irregular or indented nuclei, by a thin rim of cytoplasm and by a coarse pattern of nuclear chromatin. b) Large cells, occasionally binucleated, characterized by fairly regular nuclei with finely dispersed chromatin. Interdigitating reticulum cells and some reactive cells, including plasma cells and histiocytes, are scattered among lymphoid cells. A prominent vascular component is present in the paracortical areas. Capsule, peripheral and medullary sinuses are not infiltrated. Numerous mitotic figures (110/20 HPG) are present. The immunohistochemistry study revealed the presence of S-100+

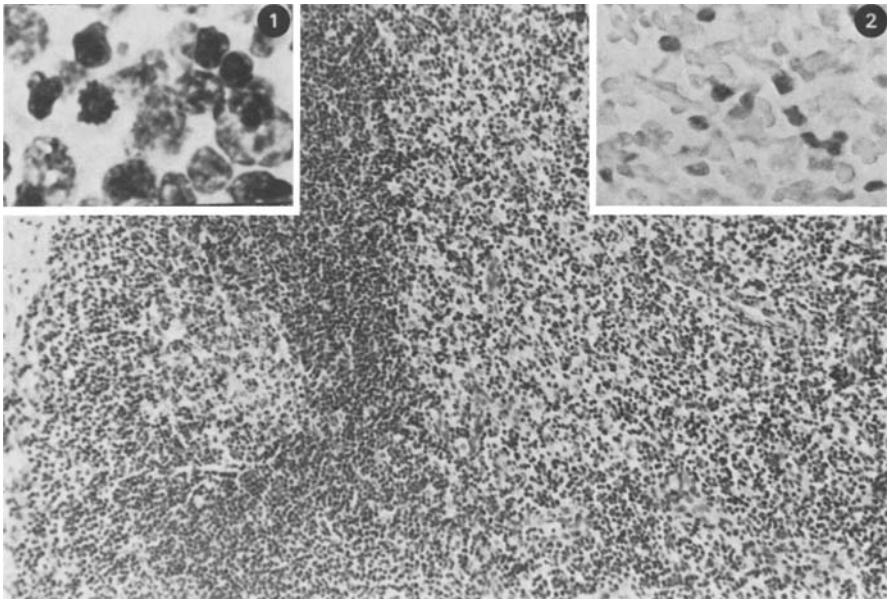

Fig. 1. Histological pattern of the first lymph node biopsy. Note the partial obliteration of the normal architecture with a proliferation of small-large cells in the paracortex. In the cortex a small lymphoid follicle is still recognizible (Haematoxylin and eosin ×75). *Inset 1*: In the paracortex presence of large cells with finely dispersed chromatin intermingled with small lymphocyte-like cells (×1000). *Inset 2*: Immunoperoxidase staining with anti-S-100 serum. Several lymphocyte-like cells are darkly stained (PAP method ×200)

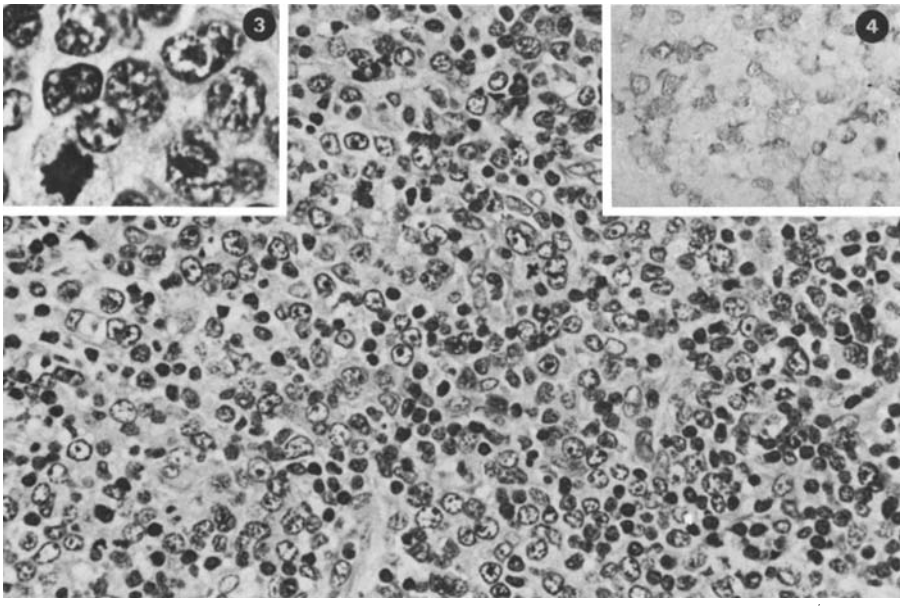

Fig. 2. Histological pattern of the second lymph node biopsy. The normal architecture of the node is completely obliterated by a rather monotonous proliferation of large cells with high mitotic activity (Haematoxylin and eosin ×200). *Inset 3*: The neoplastic cells are characterized by fairly regular nuclei with finely dispersed chromatin and with distinct nucleoli. *Inset 4*: Immunoperoxidase staining with anti-S-100 serum. Numerous large cells are positively stained

IDR cells and of several S-100+ cells with "lymphoid" appearence (Fig. 1; Inset 2); the S-100 reactivity of these cells was confirmed by the use of two antisera obtained from different sources. The anti-light chain reactions confirmed the polyclonal origin of the plasma cells; α-l-anti-trypsin and muramidase stainings were positive in few histiocytic cells and in rare eosinophils.

Lymph node n.2: A second lymph node biopsy was taken five months later. The histology reveals a complete effacement of the normal architecture by a rather monotonous cell population composed of medium-large cells with fairly regular nuclei, with distinct nucleoli and with finely dispersed chromatin (Fig. 2). Lymphocyte-like cells with irregular nuclei are still present, but are a minor component of the tumour. The capsule is not infiltrated. The number of mitoses is higher than in the first biopsy (220/20 HPG). The immunohistochemistry study revealed that the large majority of the cells were diffusely S-100+ (Fig. 2; Inset 4); moreover, in haematoxylin counterstained sections, S-100+ cells were characterized by marked nuclear atypia and by the presence of mitotic figures. Acid phosphatase and α-naphtyl-esterase stainings were positive in a minority of the cells with reticular shape. Muramidase+ cells and plasma cells were virtually absent.

The ultrastructural study of the second lymph node showed a prominent population of cells with round or variably indented nuclei, slightly condensed chromatin and one or two large trabecular nucleoli (Fig. 3A). The cytoplasm

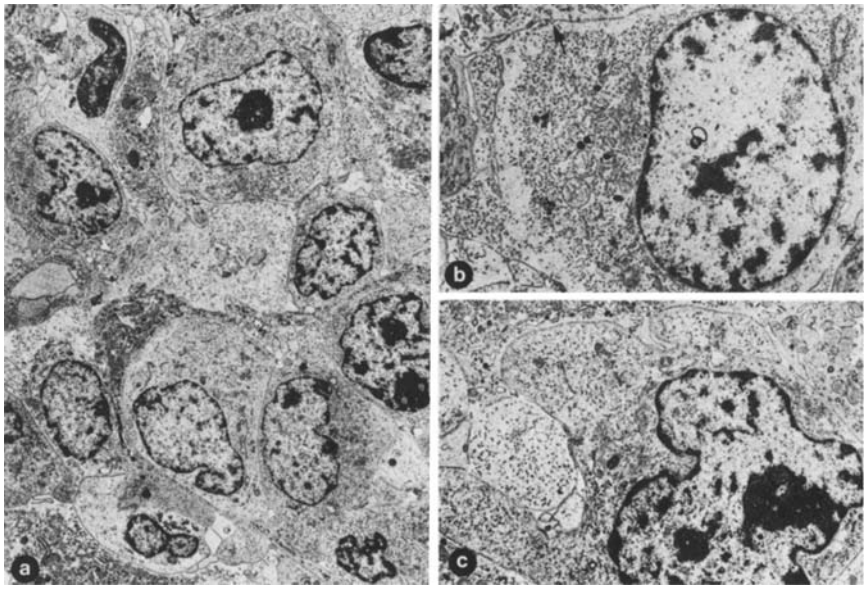

Fig. 3A. Electron microscopy from the second lymph node demonstrates the presence of cells with clear cytoplasm and round or irregularly shaped nuclei. The nuclei contain slightly condensed, marginated chromatin and one or two prominent nucleoli. Ur, Pb ×2400. **B** In this cell electron-dense membrane-bound granules are present near the Golgi area. A coated micropinocytotic vescicle (*arrow*) is also evident. Ur, Pb ×5200. **C** A clear cell showing irregular profiles with blunt cytoplasmic projections. The cytoplasm contains abundant free ribosomes, evident Golgi apparatus, some mithocondria and a few segments of endoplasmic reticulum. The nucleus contains two large granular nucleoli. Ur, Pb ×5200

Table 1. Conventional markers and monoclonal antibody reactivity of lymph node cells, peripheral blood leukocytes and bone marrow cells

Source of cells	Date	SMIg$^+$	Neutral esterase$^+$	E-rosettes		T-11	T-3	T-6	T-10	T-4	T-8	Leu-7	Ia
				4°	37°								
L.N. 1	3- 8-83	20	9	75	73	–	21	8	8	7	52	6	20
L.N. 2	8-10-83	10	10	5	3	72	52	20	8	4	44	–	38
PBL	3- 8-83	–	–	70	59	89	–	–	–	34	91	14	19
PBL	8-10-83	–	–	20	0	56	28	6	8	6	42	–	32
BM	8-10-83	–	–	–	–	30	–	–	–	4	13	–	–

appeared electron-lucent and contained numerous polysomes, evident Golgi apparatus, a few strands of rough endoplasmic reticulum and sparse mithocondria. In a few cells electron-dense, membrane-bound granules were also evident (Fig. 3B). The cells were in close contact each other and showed irregular cytoplasmic profiles with thick pseudopodia-like projections (Fig. 3C) and occasional fuzzy micropinocytotic vescicles.

Cell suspension study. Lymph node cell suspensions were characterized by conventional markers and by a panel of monoclonal antibodies (Table 1). In

the lymph node taken at the onset of the disease, the majority of cells (75–73%) were E4+/E37+ with prevalent expression of the T-8 phenotype (52%). In the subsequent lymph node biopsy, the cell population was composed by 25% small lymphocytes and by 75% medium-large round cells of possible neoplastic origin. At that time, E+ cells were drastically reduced to 5%, but some T cell antigens, including T-11/T-3/T-8, were still detectable on 44-72% of the cells; moreover, a two-fold increase in the percentage of Ia+ and of T-6+ cells was noticed. In both lymph nodes SMIg+ cells were polyclonal and accounted for 10–20% of the cell population; histiocytes, as estimated by neutral esterase staining, were less than 10%. Dot-spot staining for acid phosphatase and for acid esterase were not detected in the lymphoid cells.

The immunological study was also performed on peripheral blood samples and on the bone marrow aspirate (Table 1); even in these tissues, the large majority of lymphocytes were T-11+/T-8+. On cytocentrifuge smears, the lymphoid cells did not show signs of cytological atypia.

Discussion

In non neoplastic lymph nodes, S-100 reactivity is considered a specific marker for IDR cells (Takahashi et al. 1981) and for some "lymphocyte-like" cells located in the paracortical areas (Watanabe et al. 1983a). In neoplastic conditions, S-100+/muramidase- cells were demonstrated in 8/8 cases of malignant histiocytosis and in 2/2 cases of Letterer-Siwe disease (Watanabe et al. 1983b); these observations led to suggest that both these entities were derived from an abnormal proliferation of a particular cell-type defined "T-zone histiocyte". Moreover, since in one of such cases malignant histiocytosis was preceeded by T-ALL, it was also proposed that the T-zone histiocytes were to some extent related to the T cell lineage (Watanabe et al. 1983b).

The data reported in the present paper might support this hypothesis. In fact we have described a paracortical tumour of the lymph node, mainly composed of E37+ T lymphocytes, which was followed, five months later, by a diffuse proliferation of S-100+/muramidase- cells. Because of this "dual" aspect of the neoplastic disease our data are difficult to interpret. It may be suggested that both lymph nodes were involved by a histiocytic tumour able to induce "reactive" alterations in the T cell compartment. However, this interpretation is not supported by the histology of the lymph nodes, which was markedly different from that described in IDR cell sarcoma (Feltkamp et al. 1981) or in malignant histiocytosis (Rappaport 1966), and by the poor content of histiocytic cells in both lymph node cell suspensions.

An alternative explanation might be that both lymph nodes were involved by a T cell neoplasm. This hypothesis is not supported by the S-100 reactivity of the neoplastic cells, but is favored by the following observations: i) The lymph node histology might be reminiscent of a T-zone lymphoma (Lennert and Mohri 1978) evolving into T immunoblastic lymphoma. ii) The large majority of lymph node cells were recognized by T cell markers. iii) The cells

presented some surface abnormalities, including ability to form E-37 rosettes (LN n.1) and dissociation between E-rosetting capacity and T-11 reactivity (LN n.2) which were previously described in other T cell malignancies (Collins et al. 1979; Palutke et al. 1983).

Obviously it is still possible that the S-100 reactivity of neoplastic cells was a fortuitous event related to the malignant transformation; however, other elements, including the presence in the neoplastic cells of an irregular cytoplasmic profile with pseudopodia-like projections and the clinical picture of the disease, contribute to distinguish this case from other T cell neoplasms.

Indeed, some of our findings are similar to those previously described in another peculiar type of T cell lymphoma defined T-γ lymphoma (Kadin et al. 1981). This tumour is characterized by a systemic proliferation of erythrophagocytic T-γ lymphocytes in patients affected by a malignant histiocytosis-like syndrome. The similarities between T-γ lymphoma and our case include the initial paracortical distribution of the tumour, the overlap between T-γ lymphocytes and T-8 lymphocytes (Reinherz et al. 1980) and the clinical picture of the disease.

Finally, the recognition of the T-γ lymphoma was considered to be potentially helpful in providing a rational explanation for the numerous reports dealing with cases of T-ALL terminating as malignant histiocytosis (Kadin 1981). Our observation of a T-γ lymphoma-like evolving in a S-100+ tumour may substantiate this hypothesis and may add further evidence to the existence of a poorly defined group of tumours with intermediate features between T cell lymphoma and malignant histiocytosis.

Acknowledgements. The autors are grateful to Drs. D. Cocchia and F. Michetti for kindly providing their preparation of anti-S-100 serum, and to Mr. A. Mastracchio for skilful technical assistance

References

Berard CW, Jaffe ES, Braylan RC, Mann RB, Nanba K (1978) Immunologic aspects and pathology of malignant lymphomas. Cancer 42:911–921

Cocchia D, Michetti F (1981) S100 antigen in satellite cells of the adrenal medulla and the superior cervical ganglion of the cat. Cell Tissue Res 215:103–112

Collins RD, Waldron JA, Glick AD (1979) Results of multiparameter studies of T cell lymphoid neoplasms. Am J Clin Pathol 72:699–707

Feltkamp CA, van Heerde P, Feltkamp-Vroom TE, Koudstaal J (1981) A malignant tumour arising from Interdigitating cells: light microscopical, ultrastructural, immuno- and enzyme-histochemical characteristics. Virchows Arch (Pathol Anat) 393:183–192

Horwitz DA, Allison AC, Ward P, Kight N (1977) Identification of human mononuclear leukocyte populations by esterase stainings. Clin Exp Immunol 30:289–298

Kadin ME (1981) T gamma cells: a missing link between malignant histiocytosis and T cell leukemia-lymphoma? Human Pathol 12:771–772

Kadin ME, Kamoun M, Lamberg J (1981) Erythrophagocytic T-γ lymphoma: a clinicopathologic entity resembling malignant histiocytosis. N Engl J Med 12:648–653

Lennert K (1978) Classification of non-Hodgkin's lymphomas. In: Uehlinger E (ed) Malignant lymphomas other than Hodgkin's disease. Springer, Berlin Heidelberg New York

Lennert K, Mohri N (1978) Histopathology and diagnosis of non-Hodgkin's lymphomas. In: Uehlinger E (ed) Malignant lymphomas other than Hodgkin's disease. Springer, Berlin Heidelberg New York

Li CY, Yam LT, Lam KW (1970) Acid phosphatase isoenzyme in human leukocytes in normal and pathological conditions. J Histochem Cytochem 18:473–481

Lukes RJ, Collins RD (1974) Immunologic characterization of human malignant lymphomas. Cancer 34 (Suppl 4):1488–1503

Lukes RJ, Collins RD (1975) New approaches to the classification of the lymphomata. Br J Cancer 31 (Suppl II):1–28

Palutke M, Eisenberg L, Kaplan J, Hussain M, Kithier K, Tabacza P, Mirchandani I, Tenenbaum D (1983) Natural killer and suppressor T-cell chronic lymphocytic leukemia. Blood 62:627–634

Rappaport H (1966) Tumours of the hematopoietic system. In: Atlas of tumour pathology, Fasc 8, Sec 3. Armed Forces Institute of Pathology, Washington DC

Reinherz EL, Moretta L, Roper M, Breard JM, Mingari MC, Cooper MD, Schlossman SF (1980) Human T lymphocyte sub-populations defined by Fc receptors and monoclonal antibodies. A comparison. J Exp Med 151:969–974

Sternberg LA, Hardy PH, Cuculis PJ, Meyer HG (1970) The unlabeled antibody enzyme method of immunocytochemistry: preparation and properties of soluble antigen-antibody complex (horseradish peroxidase anti-horseradish peroxidase) and its use in identification of spirochetes. J Histochem Cytochem 18:315–333

Takahashi K, Yamaguchi H, Ishizeki J, Nakajima T, Nakazato J (1981) Immunohistochemical and immunoelectronmicroscopic localization of S-100 protein in Interdigitating reticulum cells of human lymph nodes. Virchows Arch (Cell Pathol) 37:125–135

Watanabe S, Nakajima T, Shimosato Y, Sato Y, Shimizu K (1983a) Malignant histiocytosis and Letterer-Siwe disease. Neoplasms of T-zone histiocyte with S100 protein. Cancer 51:1412–1424

Watanabe S, Nakajima T, Shimosato Y, Shimamura K, Sakuma H (1983b) T-zone histiocytes with S100 protein. Development and distribution in human fetuses. Acta Pathol Jpn 33:15–30

Accepted July 4, 1984