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Blastic OKT6-positive proliferation preceding malignant histiocytosis*

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Summary. A 45-year old male presented latero-cervical lymphoadenopathy. Biopsy revealed a malignant proliferation of immature "lymphoid" cells bearing T6 antigen and HLA-DR but negative for other lymphoid markers, suggesting a phenotype similar to Langerhans cells. The patient did not receive any therapy and six months later developed a histologically typical malignant histiocytosis, involving spleen and liver. Other reported cases of lymphoid malignancies (two bearing the T6 antigen on blast cells) preceding malignant histiocytosis were found and compared with ours. Most of these cases were characterized by the pediatric age of the patients and were presented as acute leukemias exhibiting, in at least some of them, reliable T-cell markers.

Our case appears to represent, on the other hand, a blastic proliferation of precursors of both histiocytes and Langerhans dendritic cells which eventually progressed to malignant histiocytosis. The relevance of this observation in the debate on the origin of Langerhans cells and the relationships existing between macrophages and dendritic cells is discussed.

Key words: Malignant histiocytosis - Immature "Langerhans" phenotype - Immunohistochemistry

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Introduction

Recently, dendritic cells with a low phagocyting capacity but extremely efficient as "accessory" antigen-presenting cells in cell-mediated immunity have been recognized (Steinman, 1981). These cells share some functional and phenotypical characteristics with macrophages and appear to derive from bone-marrow as do macrophages. Nevertheless, the ontogenetic relationships between these two cell types have not been fully elucidated. The detection and characterization of neoplastic proliferations of macrophages and dendritic cells can be of value for a better definition of the relationships occurring between these important cell types.

We report here the case of an adult patient who developed a typical malignant histiocytosis (MH) six months after the diagnosis of lymph-node involvement by an immature cell proliferation which was originally thought to be a lymphoblastic T-cell lymphoma on the basis of morphology. However, when immunohistochemical analysis of the lymph node was performed, a reliable characterization of the neoplastic cells revealed the presence of pan-leukocyte (2D1), HLA-DR and OKT6 (CD1) antigens, and the lack of important T-lymphoblastic markers such as terminal deoxynucleotidyl transferase (TdT), Leu-9 (CD7), and Leu-l (CD5), this phenotype not being compatible with a lyrnphoblastic proliferation.

On the basis of these data it is reasonable to consider the two cell populations as deriving from a unique neoplastic event, the first neoplasia being composed by OKT6+ histiocyte-precursors (possibly related to Langerhans cell precursors) and the latter MH cells being a more differentiated form.

Several cases of association between acute leukemia preceding MH have been reported (Starkie et al., 1969; Clar and Dawson, 1969; Skarin et al., 1972; Shreiner, 1975;

Warnke et al., 1975; Chen et al., 1976; Karcher et al., 1978). We compare these findings with ours, suggesting more than a fortuitous analogy.

Materials and methods

Clinical history

A 45 yr old male presented, in December 1983, right latero-cervical lymphoadenopathy. Routine laboratory tests were all within normal limits. The lymph-node revealed, on histological examination, an infiltration of immature "lymphoid" cells and a diagnosis of lymphoblastic lymphoma was suggested on morphological grounds (see histology). Immunohistochemical analysis with a panel of monoclonal antibodies (Table 1) showed a phenotype similar to Langerhans cells, based on the presence of T6 antigen, the absence of T-cell markers including the Leu-9 antigen which is also present in very immature T cells and related neoplasms, and the absence of TdT (see "immunohistochemistry" for details).

No therapy was given at that time due to lack of a precise diagnosis of malignancy, a monthly follow-up being decided.

After four months (March 1984) the patient presented systemic symptoms characterized by fever, asthenia, weakness, weight loss and nocturnal sweating, associated with hepato-splenomegaly and lymphoadenopathy in left axillary and left latero-cervical stations.

Laboratory tests showed increased lactic dehydrogenase, altered hepatic functions and slight hypoproteinemia. Bone marrow biopsy and peripheral blood examinations were unremarkable. CT-Scan confirmed hepatosplenomegaly and made evident a retroperitoneal lymphoadenopathy. Hepatic and splenic biopsies, performed during laparatomy, showed focal involvement by atypical histiocytes, diagnostic of MH (see histology).

After two cycles of chemiotherapeutic regimen CHP (cyclophosphamide, adriamicine, vincristine, prednisone), no improvement was observed and splenomegaly increased.

Laparotomy with splenectomy was decided and performed in June 84. Involvement by MH was evident in spleen, liver and of splenic hilus lymph-nodes on histological examination.

After splenectomy the patient's general conditions slightly improved and systemic symptoms disappeared.

The platelet count rose to 550 000/mc. Chemotherapy was changed to Pro MACE/MOPP. In spite of chemotherapy the disease progressed: multiple nodular skin lesions at neck, trunk, and anus appeared and increased in size. Suggestions of lung and stomach involvement were found by radiology. A Diabetes Insipidus Syndrome, without radiological signs of involvement of the sella turcica, appeared.

In November 84 severe anemia and trombocytopenia developed. In spite of a further course of chemotherapy the disease progressed and the patient died (November, 84). No autopsy was performed.

Specimens (including first biopsy: latero-cervical lymph-node and second biopsies: spleen, hilus lymph-nodes, liver) were received fresh from O.R. A portion was fixed in 10% formalin for 24 h and routinely processed for histology. A second fragment of each biopsy was snap frozen in liquid nitrogen and used to prepare 4 μm thick cryostat sections. The sections were placed onto albuminized glass slides, fixed in a cold 1:1 chloroform to acetone mixture for 3 minutes, air-dried and used for immunohistochemical analysis. The sections used for enzyme histochemistry were fixed in cold 10% buffered formalin (10 minutes).

Immunohistochemistry and enzyme histochemistry

The type and source of antibodies used to investigate the cell phenotype are listed in Table 1. The antibodies, opportunely diluted in buffered saline (PBS), were used as first layer on sections using an indirect immunoperoxidase technic. Peroxidase-conjugated rabbit anti-mouse immunoglobulin (from KPL) was applied on sections as second layer when monoclonal antibodies (MoAbs) were used. When rabbit antisera were employed, the peroxidase-anti-peroxidase (PAP) method (Sternberger, 1974) was followed. Histochemical demonstration of peroxidase was performed with diaminobenzidine and hydrogen peroxidase as previously described (Chilosi et al., 1981a). Acid phosphatase and nonspecific esterase staining were performed as also previously described (Chilosi et al., 1981b).

Results

Histology and immunohistochemistry

Sections of the first biopsy showed four small lymph-nodes. The structure of two of them was completely obliterated by a monomorphic population of medium-size cells expanding beyond the capsule and invading the perilymphonodal fat. The other two lymph-nodes were only partially involved by the same cell population, clustering in peripheral sinuses. These cells, densely packed, exhibited discrete ill-defined cytoplasm and nuclei with a monocytoid appearance, ovoid or indented with incospicuous nucleoli, and having mitotic activity (3-4 mitoses x HPF). Small aggregates of residual lymphocytes were present while other cells, such as macrophages, granulocytes and plasmacells, were absent (Fig. 1).

As summarized in Table 1, the neoplastic cell population in the first biopsy (latero-cervical lymph-node) was slightly positive for acid phosphatase but negative for nonspecific esterase. In addition, a strong positive reaction was observed when tested with OKT6 and anti-HLA-DR monoclonal antibodies. Other antigens analysed, including B-cell markers (IgM, K, λ, Leu14, BA1), T-cell markers (OKT1, OKT4, OKT8, Leu-9) and monocytic-histiocytic markers (such as Leu-M1, Leu-M3, Leu-M4, S-100 protein, Lysozyme, anti-chymotrypsin) were negative (Fig. 2).

On the other hand sections of the second group of biopsies (including spleen, liver and spleen hilus lymph-nodes) obtained six months later, showed involvement by large atypical cells with abundant cytoplasm, a large nucleus often nucleolated and having mitotic activity (Fig. 3).

This neoplastic cell population exhibited varying degrees of cytologic atypia ranging from medium-sized to bizzare giant cells, sometimes resembling Reed-Sternberg cells. Giant cells sometimes showed evidence of phagocytosis. The general histological pattern and the morphological characteristics of neoplastic cells were typical of MH (Fig. 3).

The phenotype of this second neoplastic cell type was different for several markers. In fact the cells, exhibited some well-characterized histiocyte-related markers such as LeuM3 and anti-chymotripsin, together with a strong positivity for acid phosphatase and nonspecific esterase (Fig. 4). HLA-DR antigen was also present on the second neoplastic population whereas OKT6 was absent (Table 1). According to these features a diagnosis of MH was carried out.

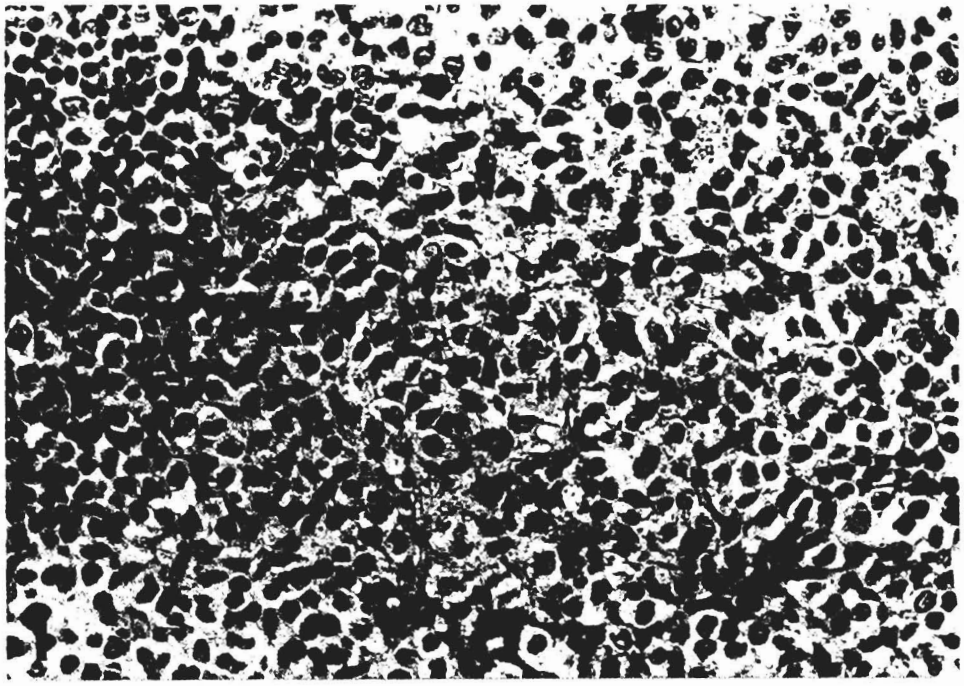


Fig 1. First lymph node biopsy embedded in paraffin and stained with H.E. A diffuse neoplastic proliferation of cells characterized by ovoid, indented nucleus is present, clustering within the lymph node sinuses. On the left (arrow), a lymphoid (non-neoplastic) aggregate is evident. x 400

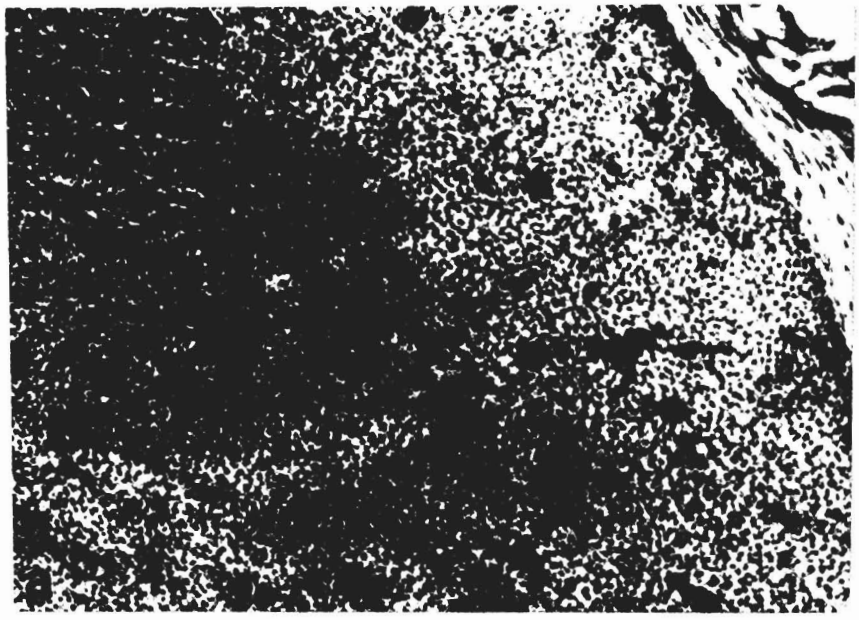
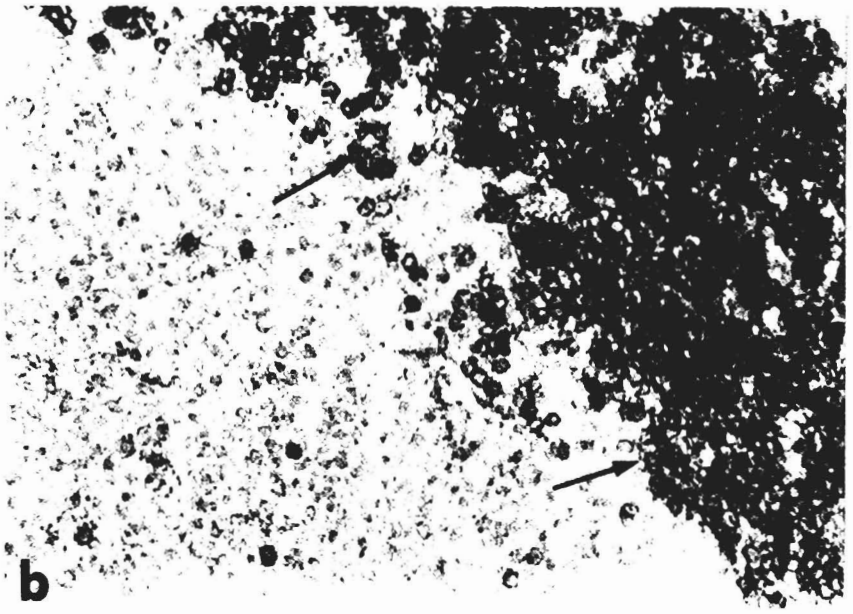


Fig. 2. Consecutive cryostat sections obtained from a frozen portion of the same lymph node (biopsy I°) shown in fig. 1. In (**a**) the neoplastic cells, within a subcapsular sinus, appear mainly devoid of nonspecific esterase staining (ANAE), whereas a few macrophages are strongly stained (arrows). More deeply in the lymph node a large lymphoid aggregate is present. When immunostained using indirect immunoperoxidase with monoclonal antibodies the neoplastic cells were OKT6 positive (**b**), HLA-DR positive (**c**) and Leu-9 negative (**d**). Cell membrane phenotype (arrows). (a) x 100; (b), (c), (d) x 200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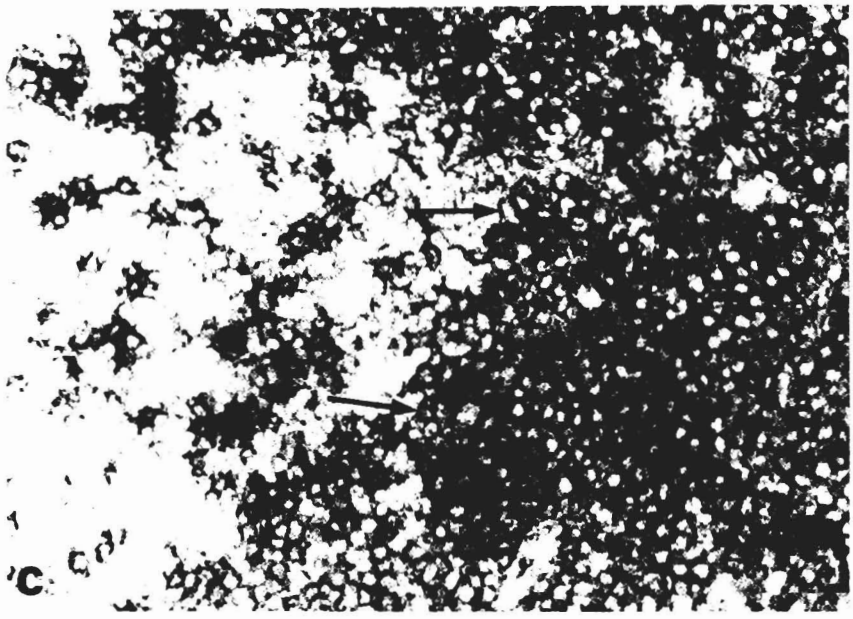
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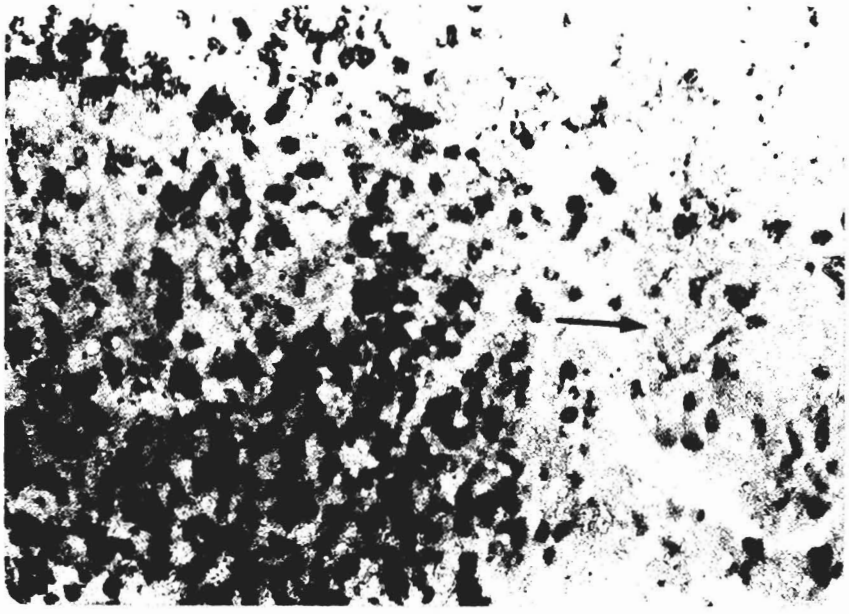

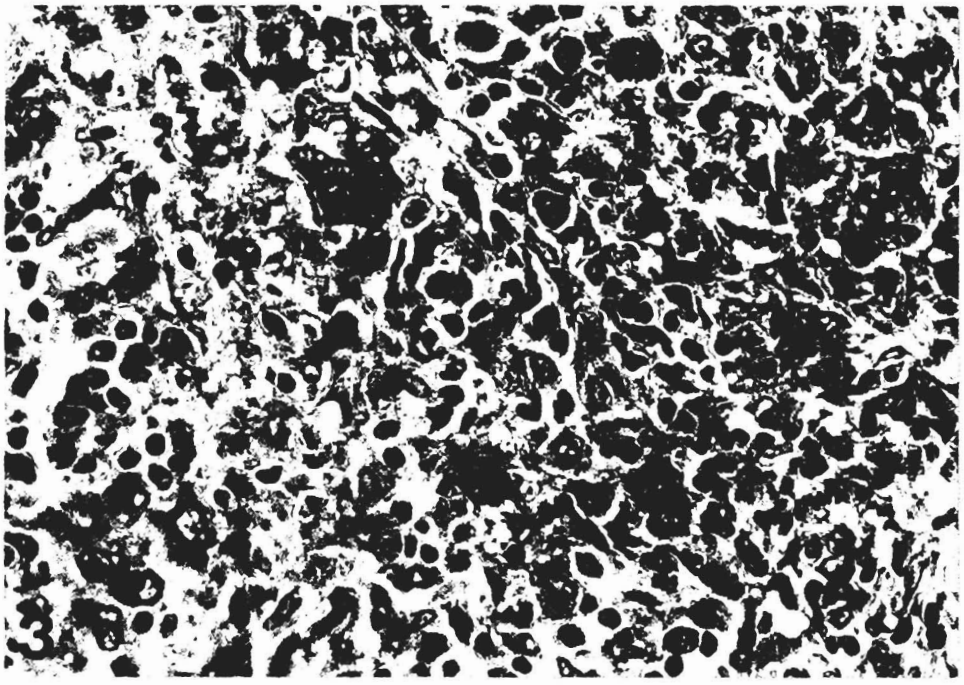

Fig. 3. Second lymph node biopsy embedded in paraffin and stained for H.E. The lymph node structure appears completely effaced by large neoplastic nodules of pleomorphous cells. This histological pattern is suggestive of malignant histiocytosis. x 400

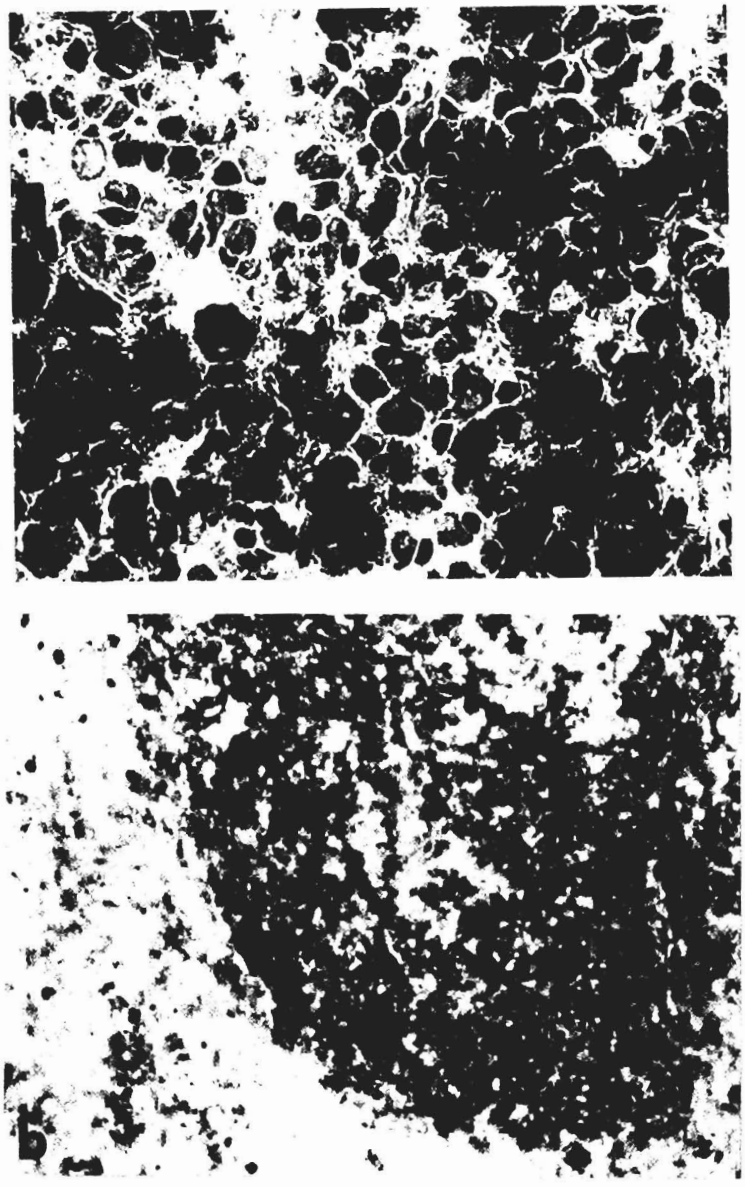

Fig. 4. Consecutive cryostat sections obtained from a different portion of the same lymph node (biopsy II°) shown in Fig. 3. The strong acid phosphatase activity (**a**) and Leu-M3 antigen expression (**b**) on the majority of neoplastic cells confirm the histiocytic nature of the neoplasm. (a) x 400; (b) x 150

Table 1. Immunophenotype of neoplastic cells

	I° biopsy	II° biopsy	source	reactivity
HLA-DR	+ +	+ / + +	B&D	B cells, etc.
TDT	–	–	PL	lymphoblasts
LEU-9	–	–	B&D	T-cells
OKT1	–	–	ORTHO	T-cells
LEU-14	–	–	B&D	B-cells
LEU-M3	–	+	B&D	macrophages
LEU-M1	–	–	B&D	polymorphs
OKT6	+ +	–	ORTHO	thymocytes & LC
anti-CHYMOT	–	+ / –	DAKO	macrophages
LYSOZYME	–	–	DAKO	macrophages
S100	–	–	DAKO	dendritic cells
ANAE	–	+ +		macrophages
ACP	+ / –	+ +		macrophages

ANAE = nonspecific esterase (alpha naphtyl acetate)
ACP = acid phosphatase
LC = Langerhans cells

Discussion

Histological diagnosis of MH is based on the finding of large atypical histiocytes, usually exhibiting erithrophagocytosis, often involving the spleen, liver, lymph-nodes and bone marrow. Clinically, patients present fever, weight loss and a downhill course. (Rappaport, 1966; Warnke et al., 1975).

In our case, the clinical and histological features, at the time of the second biopsy, were characteristic of this disease. In addition, the neoplastic cells involving spleen, lymph-nodes and liver, showed a positive reaction when tested for markers of histiocyte-macrophage lineage, such as LeuM3 and anti-chymotripsin.

The peculiarity of the case here described is based on the fact that MH was preceded six months previously by a cell proliferation characterized by a different morphology and phenotype. The strong reactivity for T6 antigen was suggestive of an immature T cell lymphoma. However, more extensive study showing the absence of all T-cell markers including Leu9 (CD7) and TdT which are constantly found in T lymphoblastic proliferations (Peiper and Stass, 1982; Mann et al., 1983) strongly argues against the T lymphoid nature of this first neoplasia. In addition, the positive staining for HLA-DR also suggests a different lineage, HLA-DR being usually negative in T lymphoblastic lymphoma (Peiper and Stass, 1982; Mann et al., 1983).

Several reports have been published concerning the association between acute lymphoblastic leukemia and MH (Starkie et al., 1969; Clark and Dawson, 1969; Skarin et al., 1972; Shreiner, 1975; Warnke et al., 1975; Chen et al., 1976; Karcher et al., 1978). Interestingly, in all previously reported cases where phenotypic analysis was attempted, the neoplastic cells have been considered of T cell origin (Starkie et al., 1969; Chen et al., 1976; Karcher et al., 1978). However, in most of these reports, a convincing proof of the lymphocytic nature of blasts was not given. Some of these cases could be similar to that described in this paper.

The present case shows differences and analogies with these previously reported cases. It is different because our patient was adult while most of the patients who developed MH afterwards were children. Secondly, we did not observe a leukemic spread of neoplastic cells. Nevertheless, this case appears similar in the sequence of events showing an immature "lymphoid" population followed by a typical MH.

In addition, T6 (CD1) cannot be considered, by itself, a T cell restricted antigen, being present in epidermal Langerhans cells, a specialized dendritic cell of bone marrow origin with antigen presenting functions (Katz et al., 1979; Murphy et al., 1981).

Our conclusion is that, in our case, the two phenotypically different neoplasms, one composed of immature cells expressing HLA-DR and T6 antigens, and the other a more mature cell population exhibiting histiocytic features are actually different expressions of the same malignant cell population.

While some authors favour a diverging cell-lineage for histiocytes with antigen presenting functions (like Langerhans cells and interdigitating dendritic cells) and phagocytic macrophages (Watanabe et al., 1983) others, on the basis of immunophenotypical analysis of monocyte colonies of human bone marrow (Goordyal and Isaacson, 1985) and the occurrence of clinical cases of MH characterized by "mixed" histiocyte-Langerhans phenotypes (Salisbury et al., 1985) suggest a common origin. The finding of neoplastic populations exhibiting mixed phenotypes (Salisbury et al., 1985) or, as in our case, the evolution of a "dendritic-Langerhans" neoplastic population towards histiocytic differentiation supports this latter hypothesis.

References

Chen T.K., Nesbit M.E., McKenna R. and Kersey J.H. (1976). Histiocytic medullary reticulosis in acute lymphoblastic leukemia of T cell origin. Am. J. Dis. Child. 130, 1262-1264.

Chilosi M., Bonetti F. and Iannucci A. (1981a). Carcinogenic 3,3'-diaminobenzidine (letter). Am. J. Clin. Pathol. 75, 638.

Chilosi M., Pizzolo G., Menestrina F., Iannucci A.M., Bonetti F. and Fiore-Donati L. (1981b). Enzyme histochemistry on normal and pathologic paraffin embedded lymphoid tissues. Am. J. Clin. Pathol. 76, 729-736.

Clark B.S. and Dawson P.J. (1969). Histiocytic medullary reticulosis presenting with a leukaemic blood picture. Am. J. Med. 47, 314-317.

Goordyal P. and Isaacson P.G. (1985). Immunocytochemical characterization of monocyte colonies of human bone marrow: a clue to the origin of Langerhans cells and interdigitating reticulum cells. J. Pathol. 146, 189-195.

Karcher D.S., Head D.R. and Mullins I.D. (1978). Malignant histiocytosis occurring in a patient with acute lymphoblastic leukemia. Cancer 41, 1967-1973.

Katz S.I., Tamaki K. and Sachs D.H. (1979). Epidermal Langerhans cells are derived from cells originating in bone marrow. Nature 282, 324-326.

Mann D.L., Haynes B.F., Thomas C., Cole D., Fauci A.S. and Poplack D.G. (1983). Heterogeneity of acute lymphocytic leukemia cell surface markers as detected by monoclonal antibodies. J.N.C.I. 71, 11-17.

Murphy G.F., Bhan A.K. Sato S., Mihm M.C. and Harrist T.J. (1981). A new immunologic marker for Langerhans cells. N. Engl. J. Med. 34, 791-792.

Peiper S.C. and Stass S.A. (1982). Markers of cellular differentiation in acute lymphoblastic leukemia. Arch. Pathol. Lab. Med. 106, 3-8.

Rappaport H. (1966). In: Tumors of the hematopoietc system. Atlas of tumor pathology. Armed Forces Institute of Pathology, Washington D.C., pp 49-63.

Salisbury J.R., Ramsay A.D. and Isaacson P.G. (1985). Histiocytic lymphoma: a report of a case with unusual phenotype. J. Pathol. 146, 99-106.

Shreiner D.P. (1975). Acute lymphoblastic leukemia terminating as histiocytic medullary reticulosis. JAMA 231, 838-840.

Skarin A.T., Karb K. and Reynolds E.S. (1972). Acute leukemia terminating in histiocytic medullary reticulosis. Arch. Pathol. 93, 256-260.

Starkie C.M., Kenny M.W., Mann J.R., Cameron A.H. and Hill F.G. (1969). Histiocytic medullary reticulosis following acute lymphoblastic leukemia. Cancer 47, 537-544.

Steinman R.M. (1981). Dendritic cells. Transplantation 31, 151-155.

Sternberger I.A. (1974). Immunocytochemistry. Prentice-Hall Inc., Englewood Cliffs, N.J.

Warnke R.A., Hun K. and Dorfman R. (1975). Malignant histiocytosis (histiocytic medullary reticulosis): clinicopathological study of 29 cases. Cancer 35, 215-230.

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

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