Virchows Arch [Pathol Anat] (1985) 408:229–240

Virchows Archiv A

Lectin I of ulex europaeus as a marker for a subset of histiocytic tumours of the lymph node*

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Summary. We describe four lymph node based tumours in which numerous neoplastic cells and some mitotic figures were characterized by staining affinity for Lectin I of Ulex europaeus (UEA-I). The patients had no vascular or epithelial tumours and presented symptoms suggestive of a systemic lymphoproliferative disease. Histologically, the tumours were composed of large, cohesive, cells which were mainly located in the paracortex. UEA-I reactivity was more evident in the Golgi area and was present in large mononucleated cells often arranged to delimit vascular-like spaces. The neoplastic cells were weakly muramidase-positive in one case, and were ANAE+/AP+ in two other cases. Large dots of UEA-I reactivity were detected in S-100+/muramidase-negative Langerhans-like cells present in one case of Letterer-Siwe disease. UEA-I staining was consistently negative in 20 cases of B cell- or T cell lymphoma and in 9 other cases of histiocytic lymphoma. It is suggested that UEA-I+ tumours of the lymph nodes are part of a distinct subset of histiocytic malignancies whose neoplastic cells present some morphological and phenotypic properties normally associated with endothelial cells.

Key words: Histiocytic tumours – Ulex europaeus – Lectins – Lymph node – Immunohistochemistry

Introduction

Cells belonging to the histiocytic and accessory cell lineages can be divided into subsets according to their immunological and enzymatic determinants. Monocyte/macrophages, interdigitating reticulum cells, follicular dendritic reticulum cells, fibroblastic reticulum cells and Langerhans cells are all char-

* Supported by CNR contract N. 84.00440.44 Progetto Finalizzato Oncologia

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acterized by distinctive phenotypic properties allowing their specific recognition (Mason and Taylor 1975; Takahashi et al. 1981; Naiem et al. 1983; Beckstead 1983; Fithian et al. 1981). Histiocytic malignancies reflect this heterogeneity and may show morphological and phenotypic differentiation towards each of the specialized subsets of histiocytes or accessory cells (Mendelsohn et al. 1980; van der Valk et al. 1982; Watanabe et al. 1983; Ruco et al. 1984; Turner et al. 1984).

Lymph node sinuses are lined by a thin layer of flattened endothelial-like cells which are able to ingest particulate material and which are particularly rich in lysosomal enzymes (Fossum and Ford 1985); the concomitant presence in these cells of phenotypic traits which are normally ascribed either to macrophages or to endothelial cells has led to their ambiguous designation as "endothelial macrophage" (Moe 1964). The advent of immunohistochemistry has further substantiated the concept of endothelial macrophage. It has been demonstrated recently that the sinusoidal lining cells of the spleen have a "hybrid" phenotype being Factor VIII-RA positive, like endothelial cells, and ANAE/AP/muramidase positive, like macrophages (Buckley et al. 1985). On the basis of this evidence, we have investigated the expression of an endothelial cell marker, the Lectin I of Ulex europaeus (UEA-I) (Holthofer et al. 1982), in 13 cases of lymph node based histiocytic malignancies. Our results have demonstrated that in 4 cases some of the neoplastic cells were stained by the lectin and were characterized by endothelial-like appearance. These observations suggest the existence of a further subset of histiocytic tumours whose neoplastic cells present strict morphological and phenotypic analogy with the sinus lining cells of lymphoid tissues.

Materials and methods

Patients. All patients were admitted to the Haematology Section of the Department of Human Biopathology, University "La Sapienza", Rome, Italy. Patient 1 was a 14-year-old white male who presented with fever, bone pains, swelling of the left wrist and right ankle, and cervical lymphadenopathy. X-rays revealed two osteolytic areas in the left radius and in the right tibia. Chest x-rays showed mediastinal enlargment and right basal pleural effusion. The haemogram was in the normal range. Histological examination of the biopsy of the osteolytic radial lesion revealed bone involvement by malignant histiocytosis. Bone marrow aspirate and a needle iliac crest biopsy were negative. A right cervical lymph node was biopsied.

Patient 2 was a 59-year-old white male who presented with fever, pruritus, generalized supra- and sub-diaphragmatic lymphadenopathy and papulo-nodular reddish skin lesions of the neck and of the left mammary region. Bilateral needle iliac crest biopsy was negative. A left axillary lymph node was biopsied.

Patient 3 was a 39-year-old white male who presented with fever, weight loss and inguinal, para-aortic and iliac lymphadenopathy. Abdominal ultrasonography revealed hypoecogenic nodular areas in the liver and splenome-

galy. The haemogram demonstrated leucopaenia (total leucocytes 2×10^9/l) and thrombocytopaenia (70×10^9/l). Bilateral needle iliac crest biopsy was negative. A left inguinal lymph node was biopsied.

Patient 4 was a 28-year-old white female who presented with lymph node enlargement in the left groin and lymphoedema of the left lower limb. Abdominal CT scan revealed bilateral iliac and para-aortic lymph node enlargements. Bilateral iliac crest biopsy was negative. The haemogram was normal. A left inguinal lymph node was biopsied.

Histology and immunohistochemistry: Routine sections of formalin-fixed lymph nodes were stained with haematoxylyn-eosin and Giemsa. Immunoperoxidase staining for cytoplasmic immunoglobulins, muramidase, α1-anti-trypsin, α1-anti-chymotrypsin, S-100 protein and Factor VIII-related antigens were performed using rabbit polyspecific sera purchased from Dakopatts, Denmark. Paraffin sections were incubated with optimal dilutions (1:500–1:1000) of the specific serum for 12 h; at the end of the incubation, the sections were washed three times with PBS and were then incubated with a 1:50 dilution of a peroxidase-conjugated swine anti-rabbit serum (Dakopatts, Denmark) for 30 min. Sections were washed and incubated with 0.03% H_2O_2 and 0.06% 3-3-diaminobenzidine (BDH Chemicals, England) for 3–5 min. Negative controls were performed using normal rabbit serum in place of the specific antiserum or omitting the specific antiserum.

Ulex europaeus I lectin (UEA-I) and a goat derived anti-UEA-I serum were purchased from Vector Laboratories, Burlingame, CA. For detection of the lectin binding sites, paraffin sections were incubated with a 1:500 dilution of 1.0 mg/ml UEA-I in PBS solution for 12 h; at the end of the incubation the sections were washed three times with PBS for 10 min and were then incubated with 1:50 dilution of the goat anti-UEA-I serum for 30 min. The sections were washed three times with PBS and then incubated with a 1:50 dilution of a peroxidase-conjugated rabbit anti-goat serum (Dakopatts, Denmark) for 30 min. After three further washes the sections were incubated with 0.03% H_2O_2 and 0.06% 3-3-diaminobenzidine for 3–5 min.

Peroxidase-conjugated Peanut agglutinin (PNA) was purchased from Sigma, St. Louis, Mo. Paraffin sections were incubated with a 1:20 dilution of 0.25 mg/ml PNA in PBS solution for 30 min; sections were washed three times with PBS and were incubated with 0.03% H_2O_2 and 0.06% 3-3-diaminobenzidine for 3-5 min. Cryostat sections of fresh lymph node fragments were available in cases 1 and 3, and were used for detection of Factor VIII related antigens by indirect immunperoxidase technique. Cryostat sections were also stained for acid phosphatase and α-naphtyl acetate esterase.

Results

The lymph nodes are moderately to markedly enlarged. The capsule and the perinodal tissue are not infiltrated. The sub-capsular sinus is invaded by the tumor in cases 1, 2 and 4, and is compressed in case 3; the medullary

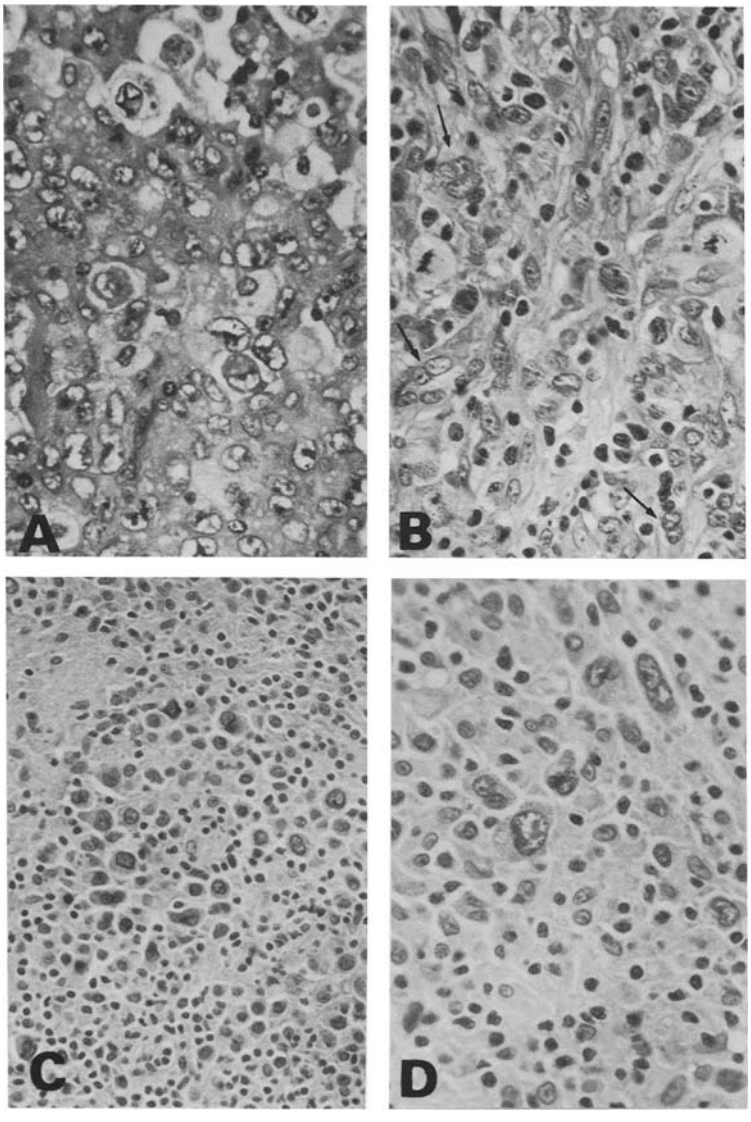

Fig. 1 A–D. Lymph node. **A** In case 1, neoplastic cells are large and highly cohesive; the cytoplasm is abundant and weakly basophilic; the nuclei are irregular with vesicular chromatin and prominent nucleoli (H & E, ×250). **B** In case 2, neoplastic cells are often elongated and arranged in a meshwork containing several lymphoid cells. The nuclei are round, oval or irregular with finely dispersed chromatin and distinct nucleoli. Several neoplastic cells are either binucleated (*arrows*) or present deep indentations of the nucleus (H & E, ×250). **C, D** In case 3, neoplastic cells are poorly cohesive and variable in size and shape; they are either aggregated in small clusters or individually scattered (H & E, ×100 and ×250)

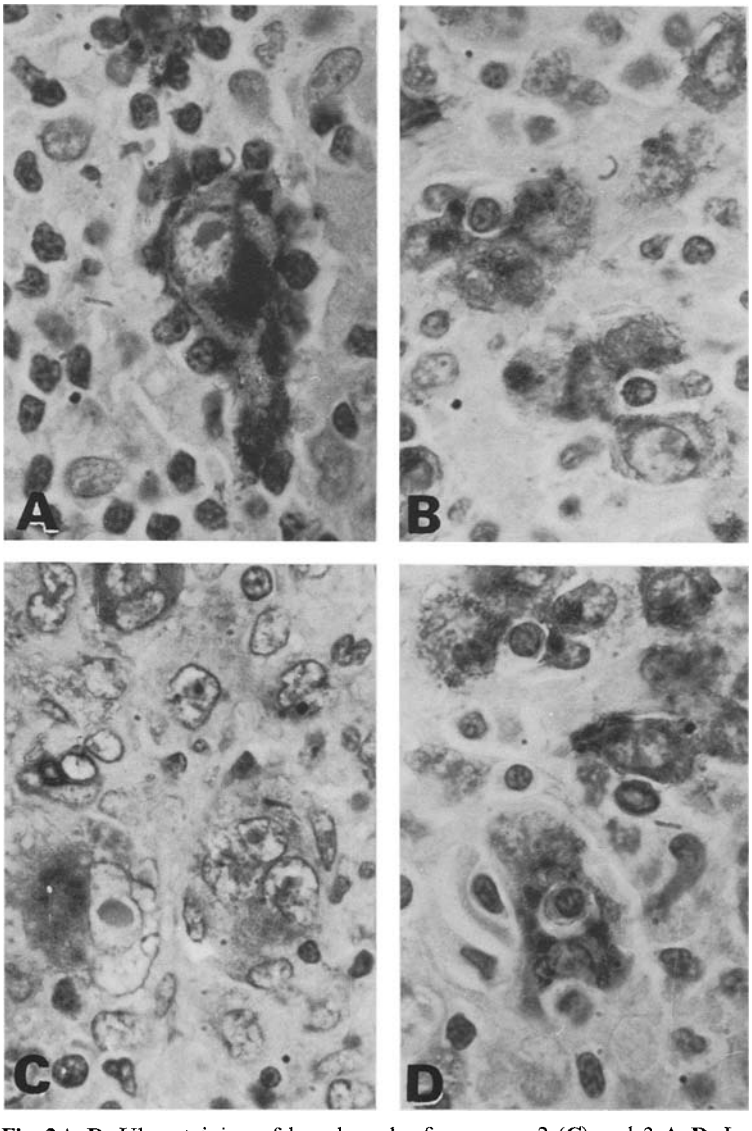

Fig. 2A–D. Ulex staining of lymph nodes from cases 2 (**C**) and 3 **A–D**. Large mononuclear, binucleated and multinucleated cells are stained by the lectin. The reactivity is often more evident in the Golgi area **A, C**. UEA-I+ neoplastic cells either delimit vascular-like spaces containing a lymphocyte **B, D** or show a large cytoplasmic vacuole with a cell inside **D** (Indirect immunoperoxidase technique, counterstained with Haematoxylin, ×1,000)

sinuses are not evident. The neoplasms are mostly located in the paracortical areas and surround few residual lymphoid follicles. The neoplastic cells are cohesive (Fig. 1a) and are arranged in a meshwork in which lymphocytes, plasma cells, eosinophils and erythrocytes are frequently interspersed (Fig. 1b). The meshwork pattern is less evident in case 3 in which the large

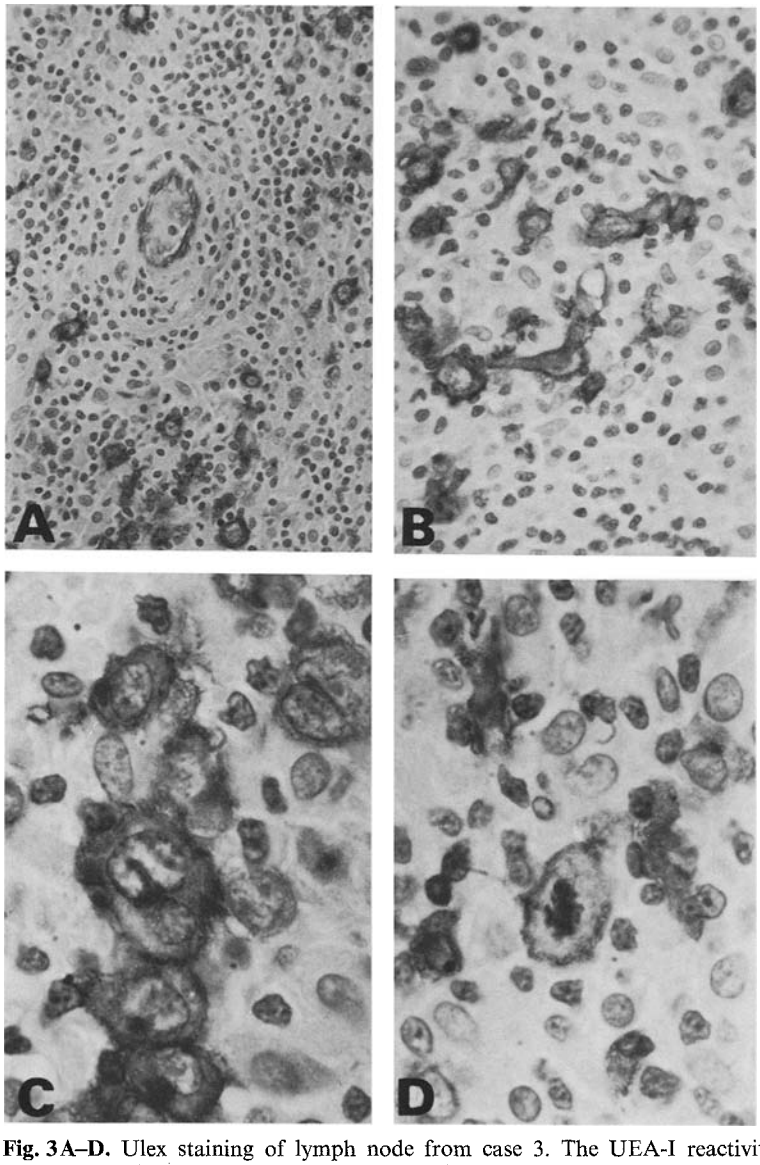

Fig. 3A–D. Ulex staining of lymph node from case 3. The UEA-I reactivity is present on neoplastic cells and on normal vascular endothelium (**A**, ×100). UEA-I+ neoplastic cells have a bizarre elongated shape (**B**, ×250) or are arranged in clusters with evidence of cell to cell contact (**C**, ×1,000). UEA-I+ mitotic figures are also present (**D**, ×1,000) (Indirect immunoperoxidase technique, counterstained with Haematoxylin)

atypical cells are arranged in clusters (Fig. 1c) or are individually scattered among lymphocyte-like cells (Fig. 1d). In case 4, some areas of the tumour are characterized by the presence of dilated vessels containing a few or many neoplastic cells admixed with normal appearing macrophages (Fig. 5). In all cases, the neoplastic cells have abundant, weakly basophilic, cytoplasm

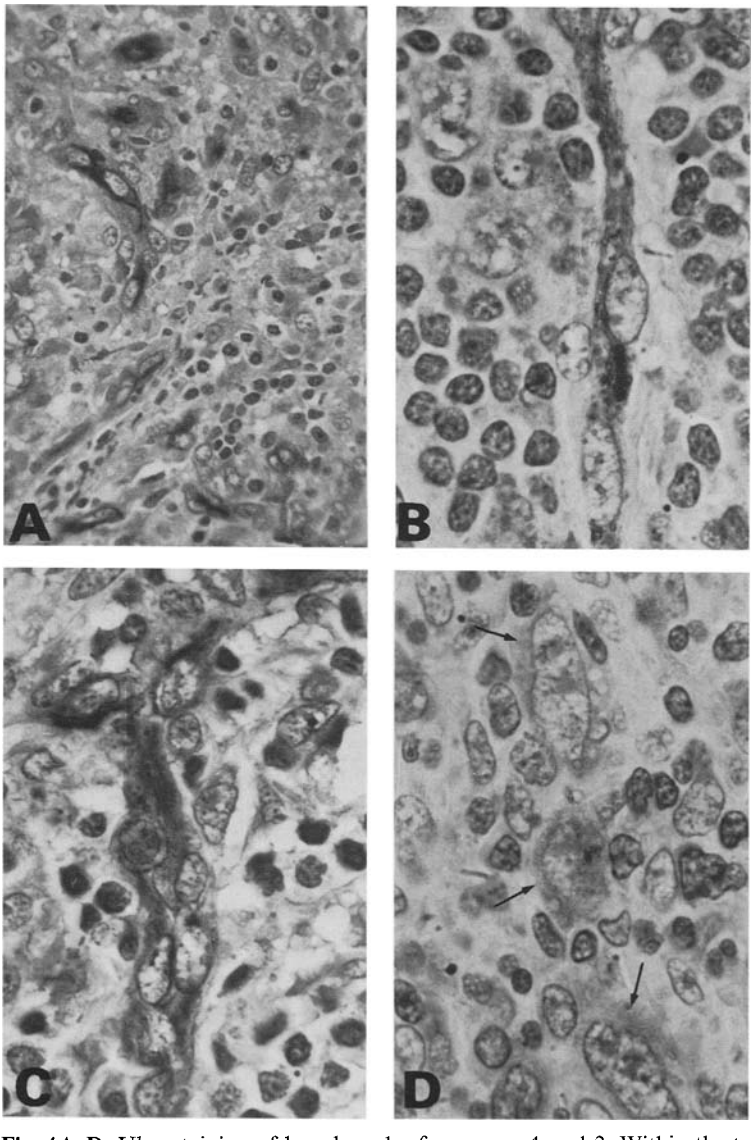

Fig. 4A–D. Ulex staining of lymph nodes from cases 1 and 2. Within the tumour (**A**, ×100), some UEA-I+ cells are almost indistinguishable from endothelial cells of normal vessels (**B, C** ×1,000). Weakly UEA-I+ endothelial cells of possible neoplastic origin (*arrows*) are present (**D** ×1,000) (Indirect immunoperoxidase technique, counterstained with Haematoxylin)

and round, oval or indented nuclei with finely dispersed chromatin and distinct nucleoli. Occasional binucleated and multinucleated cells are present. Mitotic figures are numerous and are often atypical.

UEA-I staining. Reactivity for UEA-I is present on erythrocytes, on vascular endothelium and on numerous neoplastic cells. The UEA-I staining affinity

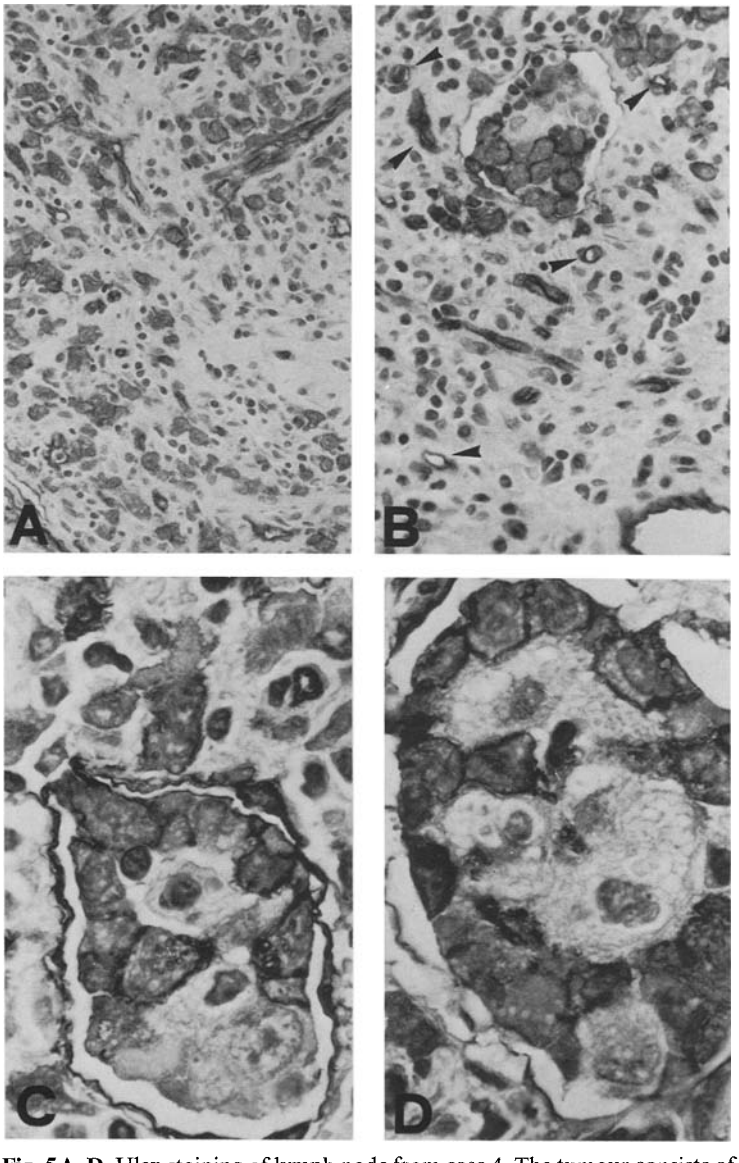

Fig. 5A–D. Ulex staining of lymph node from case 4. The tumour consists of a diffuse proliferation of UEA-I+ cells which are either individually scattered or aggregated in small clusters (**A**, ×100). UEA-I+ slit-like spaces (*arrow*), suggestive of capillary formation, and dilated vessels containing UEA-I+ cells are present (**B**, ×250); the endothelial wall of these latter is discernible in some cases **C** and is indistinct in others **B, D**. The intravascular UEA-I+ cells are admixed with normal appearing foamy macrophages (**C, D** ×1,000) (Indirect immunoperoxidase technique, counterstained with Haematoxylin)

of neoplastic cells is variable in intensity and is often more evident in the Golgi area as a large dot (Fig. 2a, c). Neoplastic UEA-I positive cells often delimit vascular-like spaces containing a lymphocyte (Fig. 2b); other neoplastic cells show a single large cytoplasmic vacuole containing a lymphocyte (Fig. 2d). In case 3, the atypical cells are either individually scattered or are aggregated in small clusters with evidence of cell to cell contact (Fig. 3a–c). Some cells have bizarre elongated shape (Fig. 3b); UEA-I positive mitotic figures are present (Fig. 3d). In cases 1 and 2, the vascular component of the tumour is more pronounced so that it may be difficult to distinguish between UEA-I positive neoplastic cells and normal vessels with high endothelium (Fig. 4a–d); in both these cases, frankly atypical mononuclear cells, binucleated and multinucleated cells are also stained by the lectin (Fig. 2c). In case 4, the UEA-I positive neoplastic cells are morphologically similar to those of case 3 and are often arranged to delimit slit-like spaces suggestive of capillary formation (Fig. 5a, b). The dilated vessels contain UEA-I positive neoplastic cells admixed with muramidase-positive macrophages; the endothelial wall of these vascular structures is clearly discernible in some instances and is indistinct in others (Fig. 5c, d).

As a control, UEA-I reactivity was investigated in 10 reactive nodes, in 10 B cell lymphomas (5 FCC and 5 immunoblastic), in 10 T cell lymphomas (5 lymphoblastic and 5 immunoblastic), in 9 other cases of true histiocytic lymphoma (muramidase-positive and/or α1-antitrypsin positive) and in one case of Lettere-Siwe disease. Erythrocytes and vascular endothelial cells were consistently UEA-I positive in all cases. The endothelial-like cells which line the intranodal lymphatic sinuses were UEA-I positive in 5/10 reactive nodes and were difficult to evaluate in most of the neoplastic nodes. In all malignancies, the neoplastic cells were consistently UEA-I negative. In Letterer-Siwe disease, most of the S-100 positive muramidase-negative Langerhans-like cells showed a large dot of UEA-I reactivity in the Golgi area.

The large atypical cells are Peanut agglutinin-negative. In case 4, numerous neoplastic cells are weakly, but consistently muramidase positive; in the other cases, only a few cells are immunoreactive for muramidase, α1-anti-trypsin, α1-anti-chymotrypsin, S-100 protein and cytoplasmic immunoglobulins. Frozen sections from cases 1 and 3 were stained for Factor VIII-related antigens (Factor VIII-RA), α-naphthyl-acetate esterase (ANAE) and acid phosphatase (AP); the neoplastic cells are Factor VIII-RA negative, ANAE positive and, to a lesser extent, AP positive.

Discussion

The Lectin I of Ulex europaeus selectively binds to α-L-Fucose (Matsumoto and Osawa 1969). In normal tissues, staining affinity for UEA-I has been detected in vascular endothelial cells, in epithelial cells (Holthofer et al. 1982) and in some endothelial-like cells which line lymph node sinuses (Moller and Lennert 1984). Neoplastic tissues exhibit similar patterns of reactivity since staining affinity for the lectin was documented in benign

and malignant vascular tumours (Miettinen et al. 1983) and in epithelial tumours (Yonesawa et al. 1982). The UEA-I staining has been also employed to investigate the lymph node angiostructure in Hodgkin's disease (Moller and Lennert 1984) and in T cell lymphomas (Kittas et al. 1985); in the course of these studies, neoplastic cells with UEA-I reactivity were not observed.

In the present report we describe four cases of lymph node based tumours in which the neoplastic cells and several mitotic figures showed strong staining affinity for UEA-I. The patients were not affected by vascular or epithelial malignancies and presented symptoms suggestive of a systemic lymphoproliferative disease. The specificity of the UEA-I reaction was indicated by three different lines of evidence: i) Erythrocytes and normal vascular endothelial cells were the only other cell types stained by the lectin. ii) Some UEA-I positive neoplastic cells were characterized by an endothelial-like appearance and were arranged to delimit vascular-like spaces. iii) The same batch of reagent failed to demonstrate UEA-I positive neoplastic cells in a wide range of B cell, T cell and histiocytic tumors.

The clinical picture of the disease and the histological pattern of the lymph nodes were both indicative of malignant histiocytosis. Clinically, the patients presented with fever, pancytopaenia, osteolytic lesions and neoplastic involvement of the skin. Histologically, the lymph nodes were partially or totally effaced by large, cohesive, neoplastic cells with high mitotic activity. Enzyme-immunohistochemistry suggested a histiocytic origin of the tumour in case 4, where most of the cells were weakly muramidase-positive, and in case 1 and 3, where numerous ANAE and AP/positive cells could be detected on frozen sections. Thus, these findings may indicate that UEA-I reactivity is expressed by a subset of histiocytic tumors which are somehow related to malignant histiocytosis. The observation that in a case of Letterer-Siwe disease numerous Langerhans-like cells presented a large dot of UEA-I reactivity further supports this interpretation.

Like others (Moller and Lennert 1984; Kittas et al. 1985), we have observed that staining affinity for UEA-I is present on some flattened, endothelial-like, cells which line the intranodal sinuses. The origin of these cells is still uncertain. Their morphology, the presence of UEA-I reactivity and the observation that the lymphatic collecting vessels are lined by Factor VIII RA positive cells (Svanholm et al. 1984), are all indicative of an endothelial origin; however, other phenotypic traits, including prominent presence of lysosomal enzymes and reactivity with anti-macrophage monoclonal antibodies (Kobzik et al. 1985), suggest a histiocytic origin. This phenotypical ambiguity is probably responsible for the plethora of terms currently used to define sinus lining cells. In fact, definitions like lymphatic endothelial cell, lymphendothelial cell, endothelial macrophage, endothelial-like macrophage and sinus lining histiocyte, clearly reflect a poor understanding of the cell origin. The recent observation that the sinusoidal lining cells of the spleen have a "hybrid" phenotype, inasmuch that they are positive for ANAE, AP, muramidase and Factor VIII RA (Buckley et al. 1985), does not provide any further insight on their histogenesis, but clearly demon-

strates that the lining cells of a lymphoid organ may be characterized by simultaneous expression of both endothelial and histiocytic markers. It is attractive to speculate that these "hybrid" lining cells of the lymphoid tissues might represent the normal counterpart from which the UEA-I positive histiocytic-like tumours originate.

In extralymphoid tissues, malignant histiocytic tumours with a prominent vascular component have already been described. In 1979 Enzinger reported 41 cases of angiomatoid malignant fibrous histiocytoma; this tumour was originally interpreted as a peculiar form of malignant fibrous histiocytoma simulating a vascular neoplasm (Enzinger 1979). Ultrastructural and immunohistochemical characterization of other cases have confirmed the histiocytic origin of most of the neoplastic cells (Wegmann and Heitz 1985), but have also directed attention to the profound morphological abnormalities of the vascular component (Sun et al. 1982). The severity of these abnormalities have led some to suggest that the tumour might originate from a pluripotent mesenchymal cell able of histiocytic and endothelial cell differentiation (Leu and Makek 1982).

In conclusion, evidence is accumulating for the existence of normal and neoplastic cells characterized by simultaneous expression of endothelial and histiocytic traits. These evidences challenge the concept of the Mononuclear Phagocyte System as a complete separate entity from the endothelial system and should provide ground for potential re-evaluation of the concept of reticulo-endothelial system as originally proposed by Aschoff in 1924 (Aschoff 1924).

Acknowledgements. The authors are grateful to Mr. A. Mastracchio and to Mr. M. Macchiolo for skilful technical assistance.

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Accepted August 16, 1985