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A Malignant Tumor Arising from Interdigitating Cells; Light Microscopical, Ultrastructural, Immuno- and Enzyme-Histochemical Characteristics

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Summary. A tumor in a 37 years old male is described in which the tumor cells appeared to be derived from interdigitating cells normally found in the T-cell area of lymph nodes.

The patient presented with superior vena caval obstruction due to a mediastinal mass, followed by lymph node enlargement and skin lesions leading to death within 4 months.

The tumor cells lacked immune markers for lymphocytic cells. They showed Ia-like antigens and high adenosine triphosphatase activity, while acid phosphatase and α-naphthyl acetate esterase activity was absent. Their fine morphology was strikingly similar to that of interdigitating cells.

A combination of these data led us to the conclusion that this tumor represents a specific subtype of the tumors derived from the mononuclear phagocyte system, namely a sarcoma of interdigitating cells.

Key words: Interdigitating cells – Histiocytoma – Lymphoma – Microscopy – Electron – Fluorescent antibody technic

Introduction

Of the lymph node tumors that do not originate from lymphoid cells, those tumors derived from genuine histiocytic cells are the most extensively studied (Byrne and Rappaport 1973; Warnke et al. 1975; Lampert et al. 1978; Huhn and Meister 1978; Lennert and Mohri 1978; Vilpo et al. 1980). Malignant histiocytic cells are characterized by the presence of certain lysosomal enzymes (non-specific esterase and acid phosphatase), usually by evidence of phagocytosis, and by their fine structure. These criteria can, however, be quite variable, probably as a result of differing degrees of differentiation of the tumor cells (Huhn and Meister 1978; Lombardi et al. 1978; Vilpo et al. 1980).

Histiocytes belong to the mononuclear phagocyte system (Van Furth et al. 1972). Other cells belonging to this system are monocytes, Langerhans cells of the

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skin, interdigitating cells (IDC) of the thymus-dependent areas and some other cells (Veerman 1974; Hoefsmit 1975; Kamperdijk et al. 1978; Van Furth 1980). Neoplastic diseases of monocytes, histiocytes and Langerhans cells are well described entities, but to our knowledge a malignant tumor composed of cells with characteristics of IDC has not been described before. The IDC has a distinct combination of morphological, immunochemical and enzyme histochemical characteristics (Veldman 1970; Müller-Hermelink and Lennert 1978), and resembles in many respects the Langerhans cell of the skin (Janossy et al. 1980; Lampert et al. 1980; Van Furth 1980).

In our multidisciplinary study on human non-Hodgkin's lymphoma we were confronted with one case, that was diagnosed at first by light microscopy as a malignant fibrous histiocytoma. However, a multiparameter study revealed a combination of qualities which could not be matched with those generally accepted in fibro-histiocytic malignancy, malignant histiocytosis or in any of the lymphoid lymphomas. In the present paper the light and electron microscopic appearances, and the immunochemical and enzyme histochemical findings in this tumor will be discussed in view of its probable origin.

Case History

A 37-year-old Caucasian male entered hospital in January 1978 complaining of fatigue; he was found to have superior vena caval obstruction. One month later swelling on both sides of the neck developed and in March a large mediastinal tumor was detected. Biopsies of this tumor were examined by light microscopy and a preliminary diagnosis of malignant fibrous histiocytoma was made. The tumor consisted of two components: spindle cells resembling fibroblasts and larger rounded cells. The latter had abundant clear cytoplasm, and pale-staining nuclei with frequent mitotic figures. The nuclei were generally bean-shaped, but greatly enlarged pleomorphic nuclei with deep identations were also present (Fig. 1a, b). The patient was transferred to the hospital of the Netherlands Cancer Institute at the end of March 1978, where an axillary lymph node was removed for multidisciplinary examination. An iliac crest biopsy showed no abnormality. An initial improvement in his condition followed treatment with radiotherapy and chemotherapy (CHVP) but in April 1978 he developed skin lesions on the thorax and a high fever; he died some weeks later. No autopsy was performed.

Methods

Light Microscopy. Representative samples of the axillary lymph node were fixed in buffered formalin (pH 7) and embedded in paraplast. Sections (4 μm thick) were stained with haematoxylin and eosin, reticulin, PAS, Giemsa and methyl green-pyronin. Imprint smears stained with Giemsa were compared with the histological sections.

Electron Microscopy. A cross section (1 mm thick) of the lymph node was fixed in 2.5 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3), cut into small wedge-shaped blocks for proper orientation, postfixed in 1 % OsO_4 in the same buffer and embedded in a mixture of Epon 812 and Araldite. Thin sections, stained with uranyl acetate and lead hydroxide were examined with a Philips EM 301 electron microscope.

Immunohistochemistry. The presence of IgG, IgM, IgA, IgD, IgE, $\varkappa$, λ, T-cell antigen and lymphocyte antigen was studied by immunofluorescence on cryostat sections of frozen tissue as described previously (Van Heerde et al. 1980). In addition the cryostat sections were studied for the presence of Ia-like antigens (Ia Ag) with an antiserum raised in a rabbit by the intravenous administration of peripheral blood lymphocytes from a patient with B-cell chronic lymphocytic leukemia in a blastic phase. This anti-Ia Ag serum stained B cells weakly, whereas Langerhans cells in the epidermis, IDC in the thymus-dependent areas in lymphatic tissue, and reticulo-epithelial cells in the thymus were stained intensely.

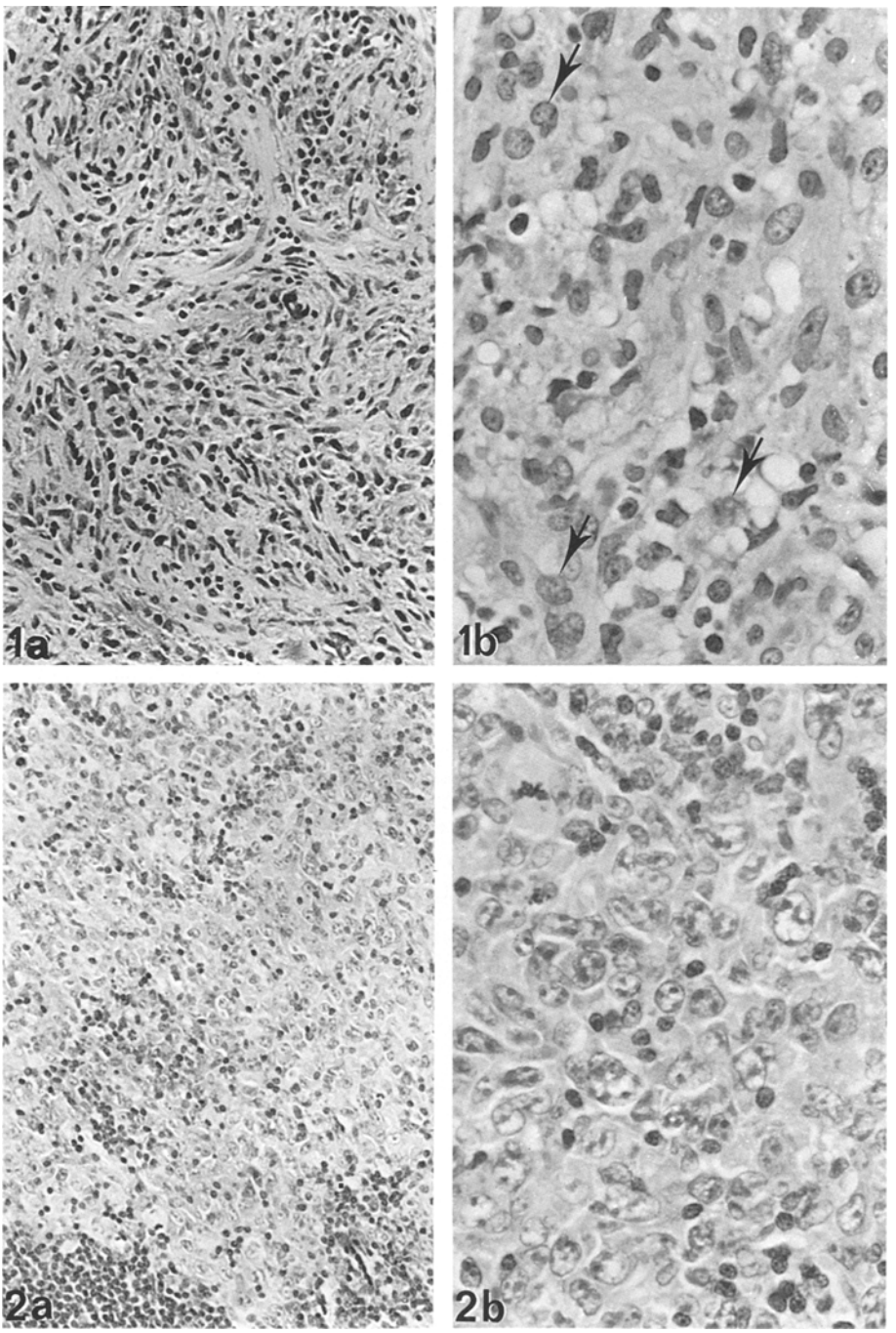

Fig. 1 a, b. Mediastinal tumor of case 1. Note the sarcomatous aspect with spindle cells (**a**) and large cells with lobulated nuclei (**b**, *arrows*). (H & E, a ×200; b ×500)

Fig. 2 a, b. Interdigitating cell sarcoma in a lymph node (case 1). **a** Absence of distinct spindle cells. **b** Large field of pale-staining cells. (H & E, a ×200; b ×500)

Enzyme Histochemistry. Cryostat sections (8 μm) were fixed in formadex prior to incubation. The following enzyme reactions were performed as described by Van Heerde et al. (1980): alkaline phosphatase, acid phosphatase, α-naphthyl acetate esterase, naphthol AS-D chloro-acetate esterase, adenosine mononucleotidase and adenosine triphosphatase.

Results

Light Microscopy

The normal architecture of the lymph node is destroyed by a diffuse proliferation of large to very large polymorphic cells (Fig. 2a, b, 3a). A spindle cell component, as seen in the mediastinal tumor, is not a feature. There is a slight increase of venules with cuboidal endothelium similar to those seen in the paracortical area of a normal lymph node. Many mitoses are present. The abundant cytoplasm of the tumor cells is pale and sometimes foamy, with some diffuse PAS-positivity. No obvious pyroninophilia is detected. The nuclei are pale, generally bean-shaped or strongly lobulated, often with enlarged nucleoli (Fig. 3b). Some binuclear giant cells resemble Sternberg-Reed cells. Reticulin fibres can be detected between the tumor cells (Fig. 3c). In a small remnant of normal lymph-node tissue, solitary tumor cells are present within the sinuses. Cytologic examination of imprints shows a very similar morphology in the tumor cells. No signs of phagocytosis are seen, and no lipids can be demonstrated by fat staining.

Electron Microscopy

Most tissue blocks observed consist predominantly of large cells with a very low electron density of the cytoplasm and nucleus (Fig. 4). The cells have a smooth general outline with short blade-like indentations and are surrounded by broad cellular protrusions of similar low electron density (Fig. 4b, c). Slender microvilli characteristic of epithelioid cells are absent and cell contacts are not observed. The nuclei are often very bizarre with deep invaginations; some cells seem to be multinucleated, but regular ovoid nuclei are also seen. However, both the latter phenomena may be due to thin sectioning. The finely dispersed chromatin is condensed in a thin rim against the nuclear membrane. The nucleoli vary from one or two large profiles to numerous small nucleonemata, generally in contact with the nuclear membrane. The cytoplasm contains dispersed ribosomes and a few polysomes; only a few ER profiles are present in the form of long sheets or closed rings; mitochondria are often concentrated into one area. The cells occasionally contain a few granules and lysosomes, no Langerhans granules and only very few fat droplets. The most striking feature of the cells is the architecture of the elaborate Golgi apparatus, which consists of many narrow tubules and vesicles and only a few broader cisternae (Fig. 4d).

Apart from these cells only few small lymphocytes and histiocytic cells of normal morphology with many lysosomes are seen.

Immunofluorescence

The tumor cells were negative with anti-human lymphocyte (ALS) and anti-human T cell (HTLA) antisera, and no surface nor cytoplasmic immunoglobulins were

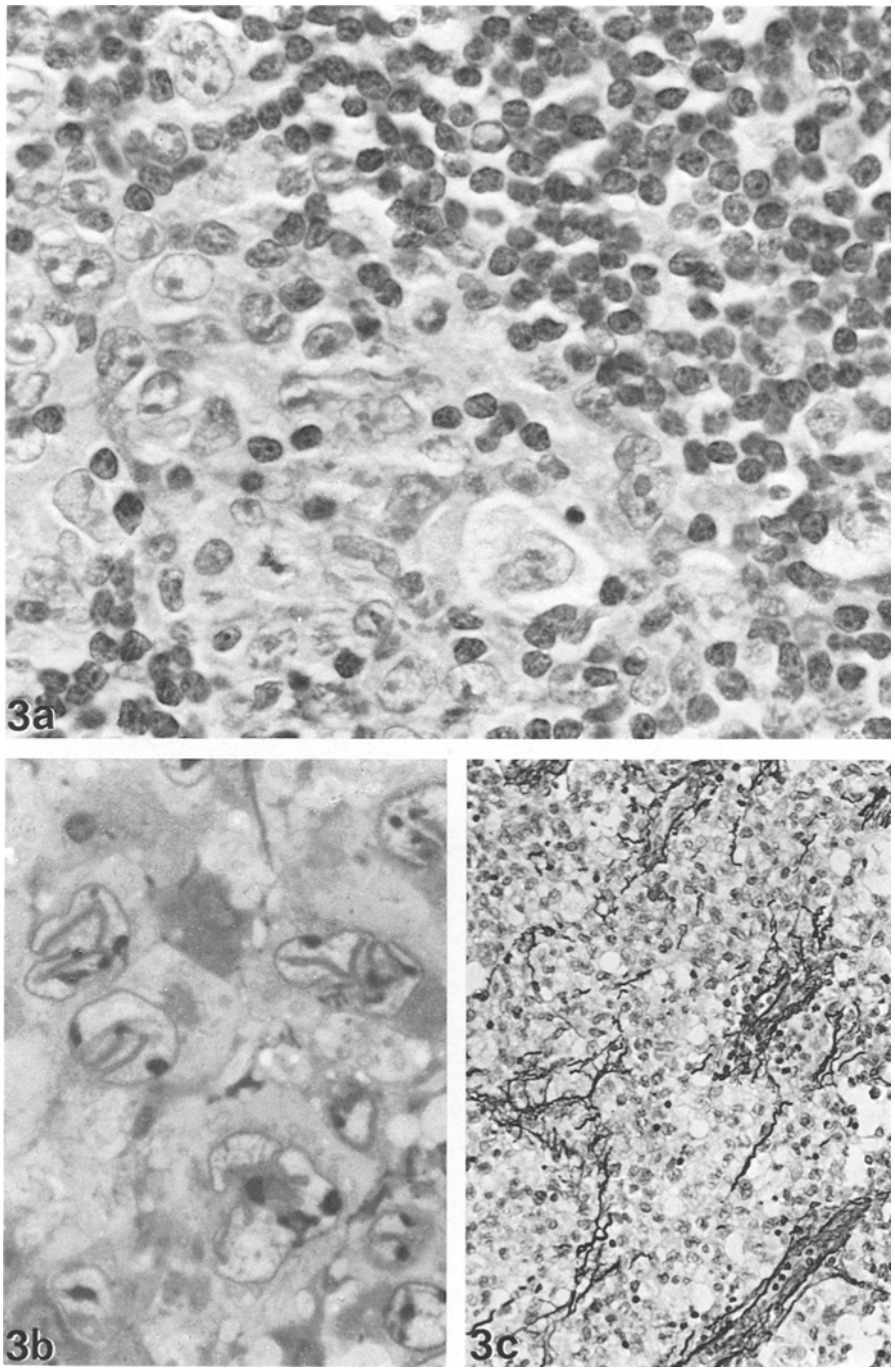

Fig. 3a–c. Interdigitating cell sarcoma in a lymph node (case 1). **a** Large cells with irregular nuclei between preexisting lymphoid tissue. **b** Bizarre indented nuclei with large nucleoli. **c** Reticulin stain shows epithelioid venules and focally fibers between tumor cells. (a H&E ×800; b plastic 1 μ section, toluidin blue ×1,250; c reticulin ×200)

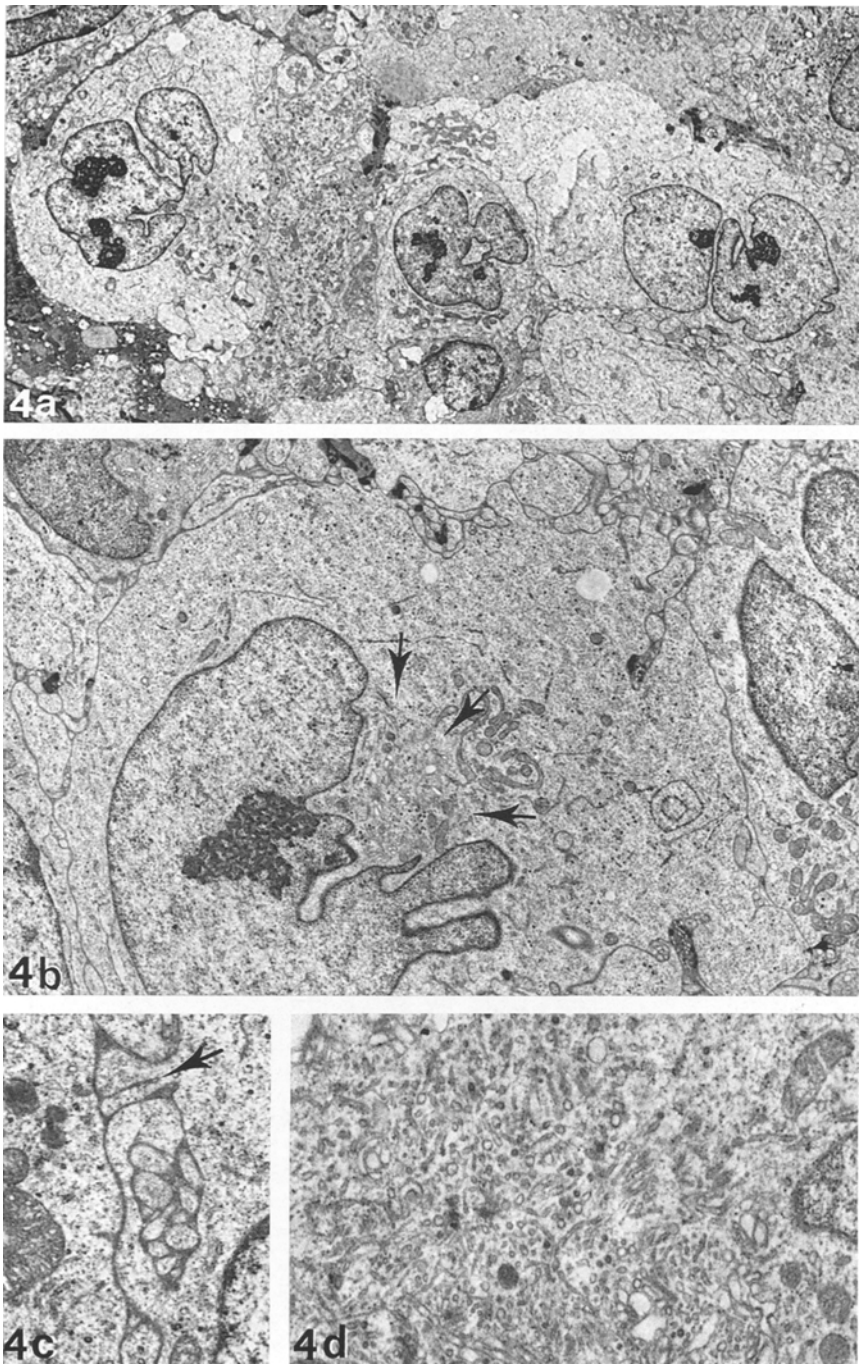

Fig. 4a–d. Electron micrographs of tumor cells in lymph node (case 1). **a** Nuclei are irregular and contain large nucleoli. **b** Cytoplasm contains a distinct Golgi area (*arrows*) and few other organelles. **c** The cell surface shows interdigitations and blade-like invaginations (*arrow*). **d** The Golgi apparatus consists of small vesicles and narrow tubules. (a ×1,800; b ×4,600; c ×9,500; d ×12,000)

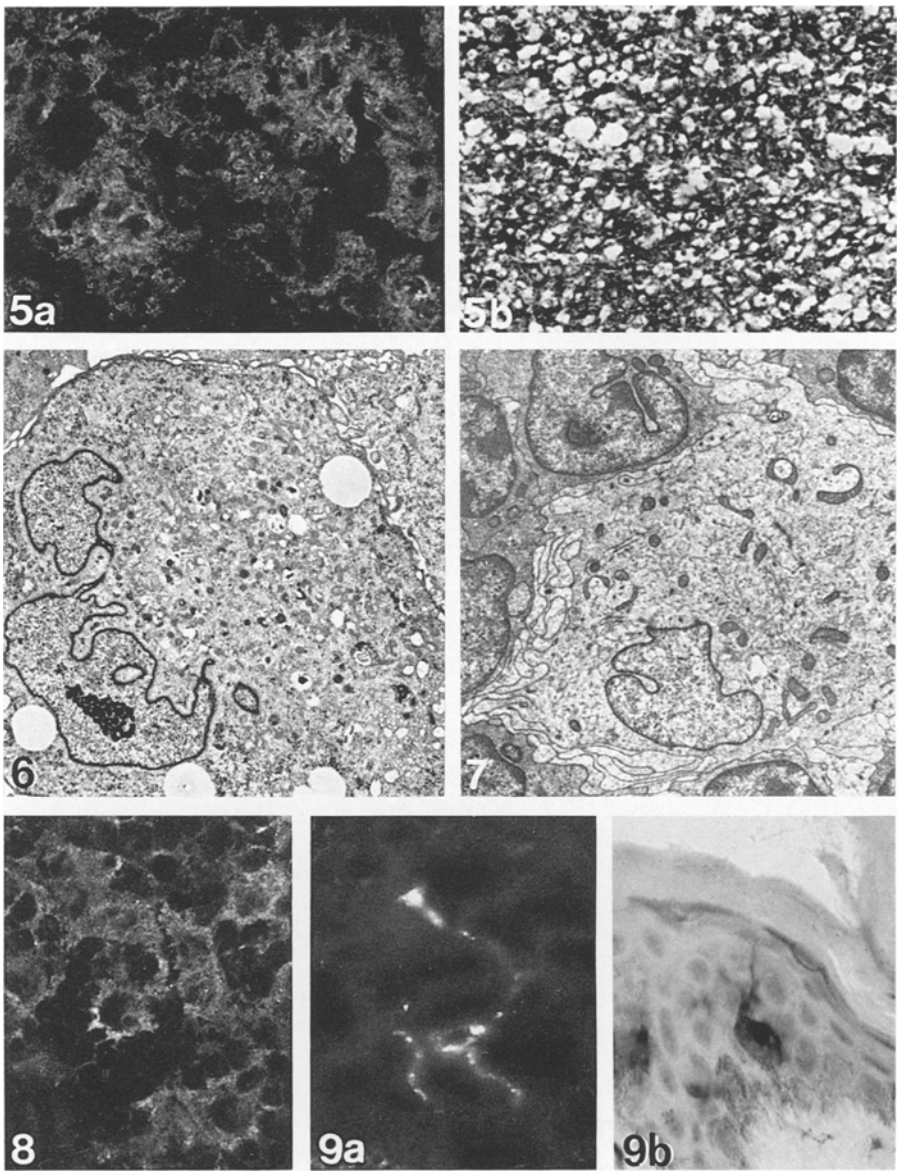

Fig. 5a, b. The tumor cells in the lymph node (case 1) show cytoplasmic fluorescence with anti-Ia Ag serum (**a**) and a strong ATPase reaction (**b**). (a ×400; b ×100)

Fig. 6. Tumor cell of malignant histiocytosis (case 2); irregular nucleus and many lysosomes (×2,800)

Fig. 7. Interdigitating cell in thymus dependent area of lymph node (case 3). Note interdigitating cell surface and practical absence of lysosomes (×4,000)

Fig. 8. Section of thymus-dependent area of lymph node (case 3) incubated with anti-Ia Ag serum (×400)

Fig. 9a, b. Langerhans cells in human skin show strong cytoplasmic Ia Ag fluorescence (**a**) and strong ATPase activity (**b**) (a ×400; b ×250)

Table 1. Immunologic markers and enzyme activities

Case	Cells	ALS	sIg	cIg	Ia-Ag	ATPase	ac. phosph.	α Na est
1. present case	tumor cells	−	−	−	++	+++	−	−
2. malignant histiocytosis	tumor cells	−	−	−	±	−	++	+
3. dermatopathic lymphadenitis	IDC	−	−	−	++	+++	±	++

sIg = surface immunoglobulins; cIg = cytoplasmic immunoglobulins

detected (Table 1, case no. 1). They were clearly positive with the anti-Ia Ag serum (Fig. 5a). Very few B cells and variable numbers of T cells of normal size and appearance were demonstrated.

Enzyme Histochemistry

The tumor cells showed a high ATPase activity (Fig. 5b), but no acid phosphatase and α-naphthyl acetate esterase activity was demonstrated. None of the other enzymes tested (alkaline phosphatase, naphthol AS-D chloro-acetate esterase and adenosine mononucleotidase) showed any reaction (Table 1, case no. 1).

"Controls"

The observations on the tumor cells from this patient (no. 1, Table 1) were compared with our data on biopsies from a patient with malignant histiocytosis (no. 2, Table 1) and those on the paracortical area in a lymph node from a patient with dermatopathic lymphadenitis (no. 3, Table 1).

Patient no. 2 showed the clinical and light microscopical features of malignant histiocytosis. Electron microscopy shows a quite electron dense cytoplasm with many organelles, and a variable number of lysosomes (Fig. 6). The tumor cells were weakly positive for Ia Ag while all other sera tested were negative. High acid phosphatase and α-naphthyl acetate esterase activities were demonstrated, but no ATPase activity could be detected (case no. 2, Table 1).

The paracortical area in the lymph node of the patient with dermatopathic lymphadenitis (no. 3) was enlarged and contained many HTLA-positive T-lymphocytes, together with large, pale cells with a fine morphology completely compatible with that of IDC. They have an irregular, interdigitating cell surface, a kidney-shaped nucleus and a strikingly clear cytoplasm with a Golgi apparatus consisting of a mass of narrow tubules and small vesicles, and practically no lysosomes (Fig. 7). These non-lymphoid cells were negative with all antisera used except the anti-Ia Ag serum (Fig. 8) and showed ATPase activity, but no acid phosphatase or α-naphthyl acetate esterase activity (case no. 3, Table 1).

Discussion

Histologically, the first biopsy from the present case was considered to be consistent with a malignant fibrous histiocytoma on account of its composition of two cell

types – spindle cells and larger often foamy polymorphic cells. The morphology of both cells conformed to the criteria described by Weiss and Enzinger (1978).

In the lymph node removed later from this patient, a spindle cell component was not evident; a diffuse proliferation of large cells with multilobulated nuclei was found, and a histiocytic origin suggested. However, enzymatic characterization of these cells did not confirm their histiocytic character: they did not show any acid phosphatase or α-naphthyl acetate esterase activity. In nearly all cases of malignant histiocytosis a positive reaction for these enzymes has been found (Huhn and Meister 1978; Lampert et al. 1978; Lombardi et al. 1978). In our hands, the tumor cells of a patient with malignant histiocytosis (case no. 2) showed a high activity of both enzymes. However, since the degree of differentiation of malignant histiocytosis can be variable (Lombardi et al. 1978), an absence of activity of both enzymes is not necessarily incompatible with a histiocytic origin. Another interesting feature was the high ATPase activity found in the tumor cells. In lymph nodes, both B cells and IDC in the T-cell area can show a positive ATPase reaction. A relation between the tumor cells and cells of the B cell lineage is ruled out by the complete absence of any reaction with antilymphocyte serum and the absence of cell surface or cytoplasmic immunoglobulins. In our hands, all lymphoid tumors we have studied, including those consisting of large cells, have reacted with anti-lymphocyte serum, even when other immunologic markers for B or T cells were absent (Van Heerde et al. 1980). In addition, in the present case, the tumor cells showed a clearly positive reaction with anti-Ia Ag serum. In lymph nodes, B cells show a weak reaction, and IDC a strong reaction with anti-Ia Ag serum (Janossy et al. 1980), while a combination of Ia Ag and ATPase activity is found in IDC in lymph nodes and in the closely related Langerhans cells in the skin (Fig. 9a, b). This combination of properties of the tumor cells taken together with their fine structure is strongly in favor of an IDC origin.

Previously, tumors of IDC have not been described with certainty. Lennert and Mohri (1978) made the presumptive diagnosis of an interdigitating cell tumor in the lymph node of a patient with mycosis fungoides. This is in line with the finding of increased numbers of IDC in lymph nodes of patients with mycosis fungoides (Janossy et al. 1980). Huhn and Meister (1978) mention the possibility that one of their cases of malignant histiocytosis could be a proliferation of IDC since very low activities of acid phosphatase and non-specific esterase were found. Kaiserling (1978) indicated the ultrastructural resemblance with IDC in two tumors of malignant histiocytic cells.

Elema and Poppema (1978) stressed the close relationship between interdigitating cells, Histiocytosis X cells and Langerhans cells in the epidermis. They stated that these cells are one group of closely related cells that form part of the mononuclear phagocytic system (Veerman 1974; Hoefsmit 1975; Van Furth 1980; Kamperdijk et al. 1978). Our data are, apart from the absence of so-called Birbeck or Langerhans' cell granules, in agreement with their description of the proliferating cells in Histiocytosis X. On these grounds the present case has to be considered as a tumor of IDC belonging to the group of tumors derived from the mononuclear phagocyte system.

We propose to classify this type of tumor as an interdigitating cell (IDC) sarcoma.

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