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T-cell lymphomas of the stomach: morphological and immunological studies characterizing two cases of T-cell lymphoma

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Summary. Using cytochemical, electron microscopic and immunohistochemical techniques in 20 primary malignant lymphomas of the stomach, we found 18 B-cell and 2 T-cell lymphomas. Primary T-cell lymphoma in the stomach has not been previously reported. The T cells in both cases were reminiscent of T immunoblasts with prominent nucleoli and a basophilic cytoplasm. Case 1 showed a cytological relationship to pleomorphic T-cell lymphoma, large cell type. Case 2 contained in addition some cells not previously described in T-cell lymphomas, resembling immature plasma cells with abundant rough endoplasmic reticulum. Focal positivity to acid phosphatase and dipeptidylaminopeptidase IV suggests the T-cell nature of both lymphomas. In both cases the tumour cells were OKT 11 and OKT 4 positive, and negative for OKT 8. Thus, both cases represent high-grade malignant T-cell lymphomas which correspond phenotypically to T-helper cell lymphoma. Case 2 revealed a further immunohistochemical peculiarity: atypical immunoblasts reacted positively with Ki-1 antibody. Thus, it is a Ki-1 lymphoma of T-cell type.

Key words: T-non-Hodgkin's lymphoma – Gastrointestinal tract – Monoclonal antibodies

Introduction

Most primary non-Hodgkin's lymphomas (NHL) of the gastrointestinal tract, like most primary NHLs of the lymph nodes, are of B-cell origin (Lennert et al. 1975; Lewin et al. 1978; Van den Heule et al. 1979; Isaacson et al. 1979; Filippa et al. 1983). Unlike primary lymphomas arising in the lymph nodes, however, T-cell lymphomas of the gastrointestinal tract have not been described in the literature. This is surprising, since, like the lymph nodes, the lymphatic tissue of the small intestine and appendix (Waksman 1973; Calkins et al. 1975; Parrott 1976), and of the stomach and large intestine (Moubayed et al., unpublished findings) consists of T and B cells. It would be expected, therefore, that T-cell lymphomas would also occur in the gastrointestinal tract. Bearing this in mind, we studied 20 gastric resections from patients with primary tumour manifestation in the stomach using morphological and immunological techniques. Two cases proved to be T-cell lymphomas and form the basis of this study.

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Case reports

Case 1. A 66-year-old woman with chronic stomach pain and weight loss was suspected endoscopically to have a partially ulcerated tumour in the anterior wall of the stomach (Fig. 1 a). Histomorphological examination of biopsy material confirmed a malignant lymphoreticular tumour. Further histomorphological analysis of the resected tissue found a high-grade malignant non-Hodgkin's lymphoma of the stomach; a staging procedure revealed merely local involvement of lymph nodes. The patient received adjuvant polychemotherapy and has been in remission for 4 years.

Case 2. Clinical and endoscopic examination on an 18-year-old male with stomach pains and gastrointestinal bleeding revealed an extensive ulcerated polypoid tumour in the anterior wall with spread to the lesser curvature (Fig. 1 b). Histological analysis of biopsy material suggested a high-grade malignant immunoblastic lymphoma; this diagnosis was later confirmed on resected tissue. Staging analysis found only local involvement of lymph nodes. Adjuvant chemotherapy was given and the patient has been in remission for three years.

Materials and methods

Immediately after resection the stomachs were opened along the greater curvature. Following removal of fresh tissue for immunohistochemical and electron microscopic studies, the stomachs were fixed in formalin. Histological sections were

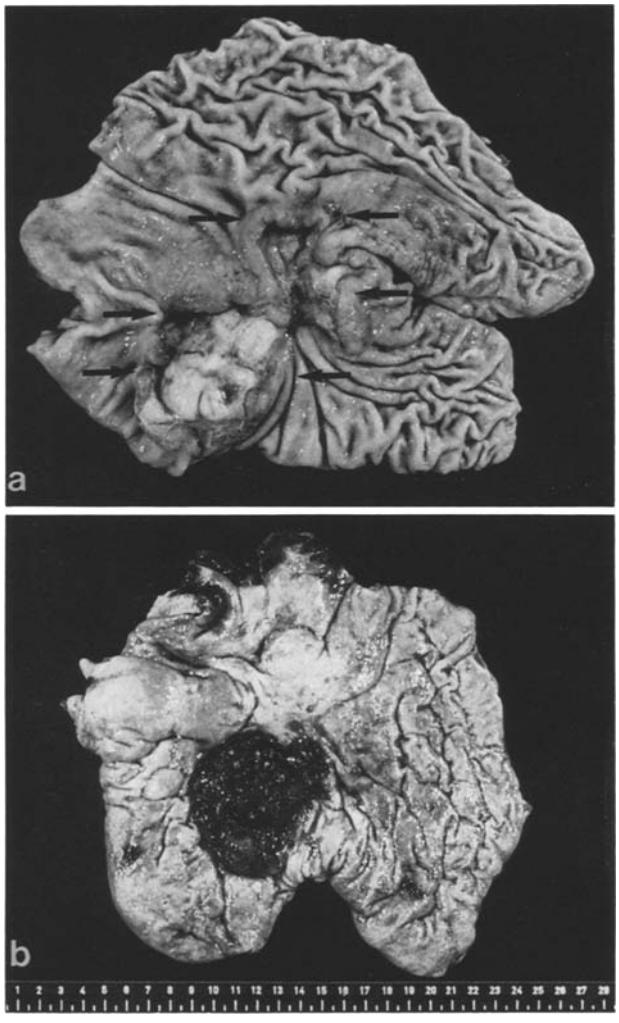

Fig. 1. a (*Case 1*) A partially ulcerated, high-grade malignant NHL of the antrum and intermediate zone of the stomach. The adjoining mucosa shows spreading of the mucosal fold pattern (*arrows*). **b** (*Case 2*) Resected stomach with extensive haemorrhage of polypoid high-grade malignant NHL (tumour average diameter approximately 6 cm)

stained with Giemsa, Gomori and periodic acid tumour Schiff (PAS). Imprints of unfixed tumor tissue were stained with Pappenheim (May-Grünwald-Giemsa) and PAS. The following reactions were used for cytological analysis of imprints: acid alpha-naphthyl-acetate-esterase, acid phosphatase, dipeptidylaminopeptidase IV (DAP IV), alkaline phosphatase, and naphthol AS-D-chloroacetate esterase.

For electron microscopy tissue was cut into 1 mm^3 pieces and fixed in a 4% glutaraldehyde phosphate buffer at 4° C for at least 2 h. Postfixation was done in 1% OsO_4 (Rhodin buffer) for 2 h at 4° C, followed by embedding in Araldite. Ultrathin sections were stained with uranylacetate and lead citrate. Electron micrographs were made on a Siemens Elmiscope I. Immunohistochemical analysis on paraffin embedded tissue using peroxidase-antiperoxidase (PAP) was done using the technique of Mepham et al. (1979). Sections were investigated with the following monospecific antisera: Rabbit anti-human serum (Dako, Copenhagen, Denmark); IgA (1:400), IgM (1:200), IgG (1:400), Ig-Kappa (1:400), Ig-Lambda (1:500), Lysozyme (1:200), Alpha-1-antitrypsin (1:100), Alpha-1-antichymotrypsin (1:100), J-chain (1:100); (Nordic, Bochum, FRG).

Albumin tests were used as a control. Immunohistochemical analyses were done on fresh tissue using the immunoperoxidase method of Sternberger et al. (1970) as modified by Stein et al. (1980). The following monoclonal antibodies were used:

OKT 11	(Sheep erythrocyte receptor)	1:500	Ortho Diagnostic, Systems, Heidelberg, FRG
OKT 4	(helper/inducer T-cells)	1:500	Ortho Diagnostic, Systems, Heidelberg, FRG
OKT 8	(T suppressor/ cytotoxic cell)	1:500	Ortho Diagnostic, Systems, Heidelberg, FRG
Ki-1		1:2000	Schwab et al. 1982
Tü 35	(HLA-DR/DQ)	1:10	Ziegler et al. 1982
R4/23	(DRC)		Naiem et al. 1983
3C4	(myeloid cells)		Schienle et al. 1982

Surface immunoglobulin: IgA (1:100), IgM (1:50), IgG (1:25) Ig-Kappa (1:50), Ig-Lambda (1:200) (DAKO, Copenhagen, Denmark).

Results

In Case 1 light microscopy revealed a lymphatic tumour with pleomorphic cytology. The lymphatic blast cells were polymorphic with indented or cerebriform, often vesicular nuclei, prominent nucleoli and chiefly a broad, strongly basophilic rim of cytoplasm (Fig. 2a, b). A few histiocytes and small clusters of epithelioid cells and eosinophilic granulocytes were also found. A fine network of reticulum fibers had developed around the tumour cells. Many capillary vessels, occasionally with a swollen endothelium, were also present.

On electron microscopy, polymorphous blasts three to five times larger than the scattered lymphocytes predominated. The blasts had large irregular nuclei with prominent, usually centrally located nucleoli. The nuclei were occasionally so deeply indented as to appear segmented (Fig. 3). The heterochromatin was marginally condensed. The cytoplasm contained numerous ribosomes and polyribosomes, was very electron dense and had small, electron-transparent vesicles and a few short tubules of ergastoplasm. Swollen mitochondria and, near the nuclear invaginations, a large Golgi apparatus with numerous vesicles are visible.

In Case 2 light microscopy showed infiltration of the stomach wall by clusters of medium-sized to large blasts (Fig. 4a). The tumour cells had predominantly round, occasionally slightly gyriform or indented, peripherally located nuclei with finely condensed nuclear chromatin and a basophilic, of-

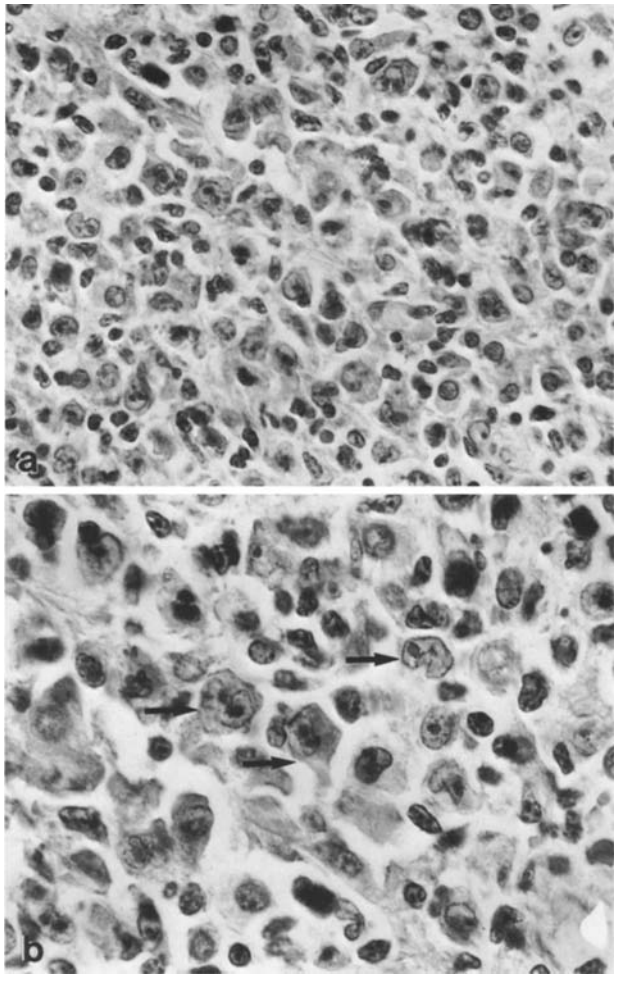

Fig. 2. Pleomorphic T-cell lymphoma, large cell type (*Case 1*). Large pleomorphic tumour cells showing irregular, occasionally deeply indented nuclei with prominent nucleoli and a moderate rim of cytoplasm. (PAS, **(a)** ×448, **(b)** ×880)

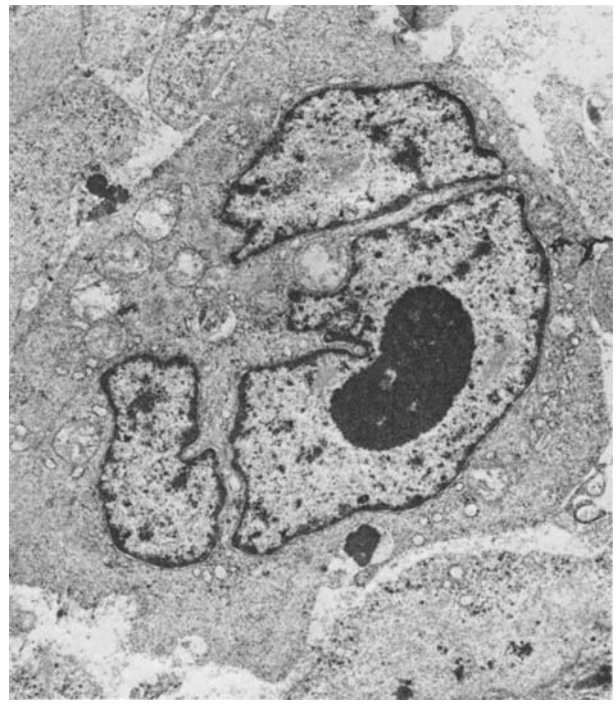

Fig. 3. Pleomorphic T-cell lymphoma, large cell type (*Case 1*). Tumour cell with deeply invaginated nucleolus and a prominent nucleolus. The nucleus is divided into three segments. The electron dense cytoplasm contains swollen mitochondria and a few vesicles (×7000)

ten wide rim of cytoplasm. Many tumour cells showed a perinuclear transparency of the cytoplasm. A large number of tumorous multinucleated giant cells, histiocytes and eosinophilic granulocytes were interspersed between the tumour cells. In addition, there was an elevated number of venules and focal haemorrhage. In imprints some of the tumour cells showed intracytoplasmic azurophilic granules located in the perinuclear area (Fig. 4b). The many tumour cells with peripheral nuclei and strongly basophilic cytoplasm were reminiscent of plasma cells.

Ultrastructurally, the tumour cells appear four to five times larger than the scattered lymphocytes (Fig. 5). They show round or oval nuclei with finely clumped heterochromatin and rare nuclear invaginations. Most of the medium-sized to large nucleoli are centrally located. The cytoplasm contains numerous polyribosomes, a few thin, rough membrane profiles and occasional vesicular rough endoplasmic reticulum. The Golgi apparatus is located near the nucleus. A few lysosome-like, electron-dense granules are present. The surface membranes show very few cytoplasmic inclusions or projections. In addition to the tumour cells, histiocytic cells closely resembling interdigitating reticulum cells can be seen.

Cytochemically, the tumour cells in both cases showed focal reactivity with acid phosphatase and DAP IV (Fig. 6a). Moderate focal acid alpha-naphthylacetate esterase activity was found in tumour cells in Case 1, whereas the tumour cells in Case 2 showed strong focal reactivity for this enzyme. The remaining cytochemical reactions were negative. Immunohistochemically, numerous tumour cells in both cases showed positivity to OKT 11 and OKT 4 (Fig. 6b). No reactivity for OKT 8 was observed. Some cells also reacted positively to HLA-DR antibody. Surface and intracytoplasmic Ig and myeloid antigen (3C4) were not found. The immunohistochemical reactions for lysozyme, alpha-1-antitrypsin and alpha-1-antichymotrypsin in tumour cells were negative. Dendritic reticulum cells (R4/23) were not detected. In Case 2 a positive

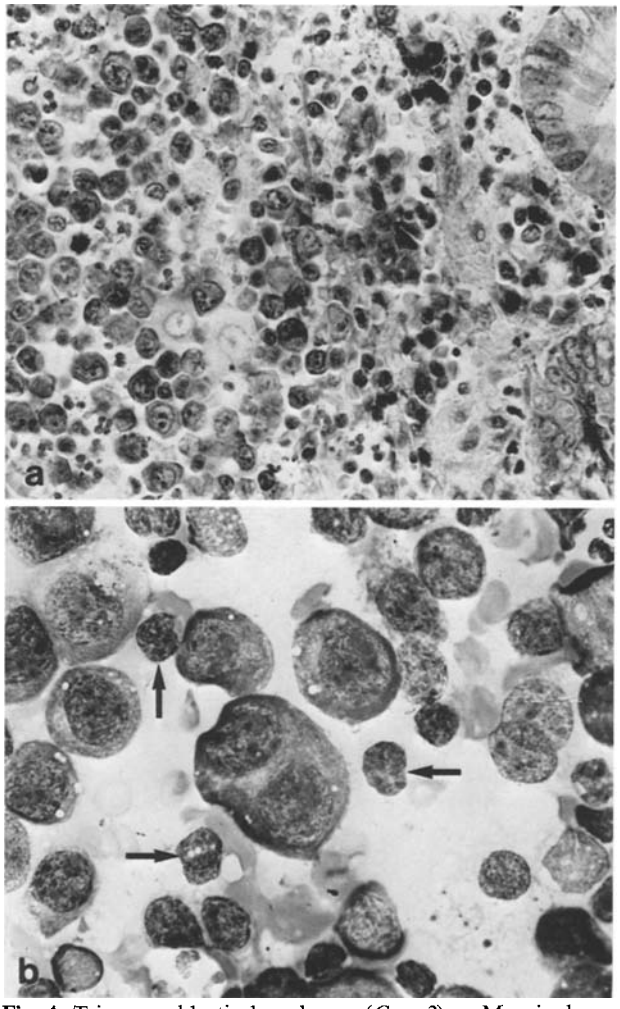

Fig. 4. T-immunoblastic lymphoma (*Case 2*). **a** Marginal section from gastric mucous membrane glands with transition to lymphoma infiltrate. The lymphoma cells show marginal nuclei and relatively wide, dark (basophilic) rims of cytoplasm. Histiocytes and neutrophilic granulocytes can be seen nearby (Giemsa, × 448). **b** In imprint the T-blasts show peripheral nuclei and a basophilic rim of cytoplasm with perinuclear transparency. Small lymphocytes (*arrows*), some with polymorphic nuclei, are visible between the tumour cells (PAS, × 720)

reaction with Ki-1 could be demonstrated in numerous tumour cells.

Discussion

Present knowledge enables easy classification of most cases of malignant non-Hodgkin's lymphomas using conventional methods of light microscopy. This is especially true for NHL's of the lymph nodes, whereas for extranodal lymphomas, particularly those of the gastrointestinal tract, it has only limited validity. As a result, many studies on gastrointestinal lymphomas do not offer a classification as precise as that for nodal tumours. In some studies a relatively high percentage of lymphomas are presented as unclassified (Filippa et al. 1983; Isaacson et al. 1979; Weingard et al. 1982). The spectrum of lymphoreticular neoplasms occurring in the gastrointestinal tract has changed since immunohistochemical analysis on paraffin sections became possible. In our study 18 B-cell and 2 T-cell lymphomas were found.

T-cell lymphomas cannot be identified with monoclonal antibodies once the tissue has been fixed. This may be a reason why no T-cell lymphoma has yet been reported in the gastrointestinal tract. However, this may not be entirely due to technical limitations but also to the relative rarity of T-cell lymphomas. The two cases we studied showed a histological picture which, together with their cytochemistry and ultrastructure, strongly suggests the T-cell nature of these neoplasms.

The irregular nuclear outlines in the tumour cells of Case 1 reveal a clear cytological relationship to pleomorphic T-cell lymphoma, large cell type (Lennert et al. 1985), which is a high-grade malignant T-cell lymphoma. The absence of rough endoplasmic reticulum in this case corresponds well with a diagnosis of T-cell lymphoma. The tumour cells in Case 2 were found to be immunoblast-like upon light and electron microscopy. They showed central, prominent nucleoli and basophilic cytoplasm. Ultrastructurally, the cytoplasm contained abundant polyribosomes, demonstrating a strong similarity to immunoblasts found in reactive lymph nodes or T-immunoblastic lymphomas. On electron microscopy, B-immunoblastic lymphomas often show at least a partial plasma cell differentiation (Stein et al. 1974; Kaiserling 1977).

In Case 2 we found a cytological feature we have not encountered in other T-cell lymphomas. There were, in addition to tumour cells with the morphology of immunoblasts, a few scattered cells with abundant vesicular ergastoplasm. It is tempting to call these tumour cells immature plasma cells on the basis of their abundant ergastoplasm; however, the total aspect of these cells and the immunohistochemical findings rule this out. In both Case 1 and Case 2 immunohistochemistry detected no tumour cells containing or expressing Ig. It remains unclear how the presence of abundant ergastoplasm is to be interpreted and what it is the tumour cells produce.

In contrast, the cells with abundant ergastoplasm are not identical to the cells of malignant NHL arising from T-associated plasma cells (Kaiserling 1977; Müller-Hermelink et al. 1983; Prasthofer et al. 1985) that can be found in normal

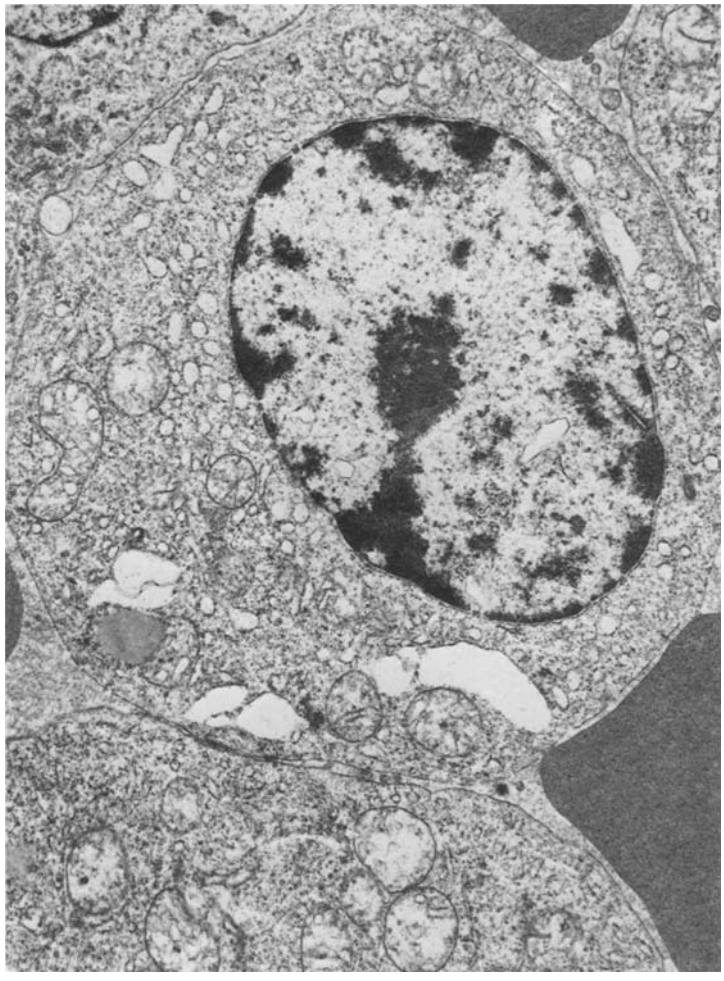

Fig. 5. T-immunoblastic lymphoma (*Case 2*). Tumour cells with oval nuclei. The cytoplasm contains numerous polyribosomes and vesicular ergastoplasm (×11400)

lymphatic tissue (Lennert et al. 1975), reactive lesions of lymph nodes (Vollenweider and Lennert 1983), and various malignant non-Hodgkin's lymphomas (Kaiserling 1977). However, our Case 2 differs morphologically and immunohistochemically from plasmacytoid T-cell lymphoma. Plasmacytoid T-cells, in contrast to the cells with abundant ergastoplasm described in Case 2, show a rough endoplasmic reticulum with short strands lying concentric to the nucleus. In Case 2 the ergastoplasm is vesicular and irregularly distributed. It is notable, however, that both malignant plasmacytoid T-cells and the tumour cells in our Case 2 react positively to OKT 4 and HLA-DR. But, whereas plasmacytoid T cells show negative reactivity for demonstration of sheep erythrocyte receptor (OKT 11), the cells in Case 2 reacted positively. The presence of interdigitating reticulum cells within the tumor of Case 2 speaks for the T-cell nature of this lesion since these cells are specific to the T-region and have been described in various T-cell lymphomas (Goos et al. 1976; Lennert et al. 1975). Cytochemically, the focal reactivity to acid phosphatase, acid alpha-napthylacetate esterase and DAP IV in both cases argues strongly for their T-cell nature. DAP IV is a marker enzyme for demonstration of T-helper lymphocytes (Feller and Parwaresch 1980; Feller et al. 1984).

Immunohistochemically, both tumours showed OKT 11 and OKT 4 positivity and are thus to be considered as T-helper cell lymphomas on the basis of their phenotype. The positive reactivity of some of the tumour cells to HLA-DR receptor does not speak against the T-cell nature since this antigen can be demonstrated on the surface of activated T lymphocytes (Greaves et al. 1981). It also has been found on the cells of peripheral T-cell lymphomas, which belong phenotypically to the group of T-helper cell lymphomas (Lennert et al. 1985; Stein et al. 1984). Furthermore, the positive reaction of numerous tumour cells to Ki-1 antibody in Case 2 is an immunohistochemical peculiarity. This antibody recognizes the membrane antigen of Hodgkin and Sternberg-Reed cells (Schwab

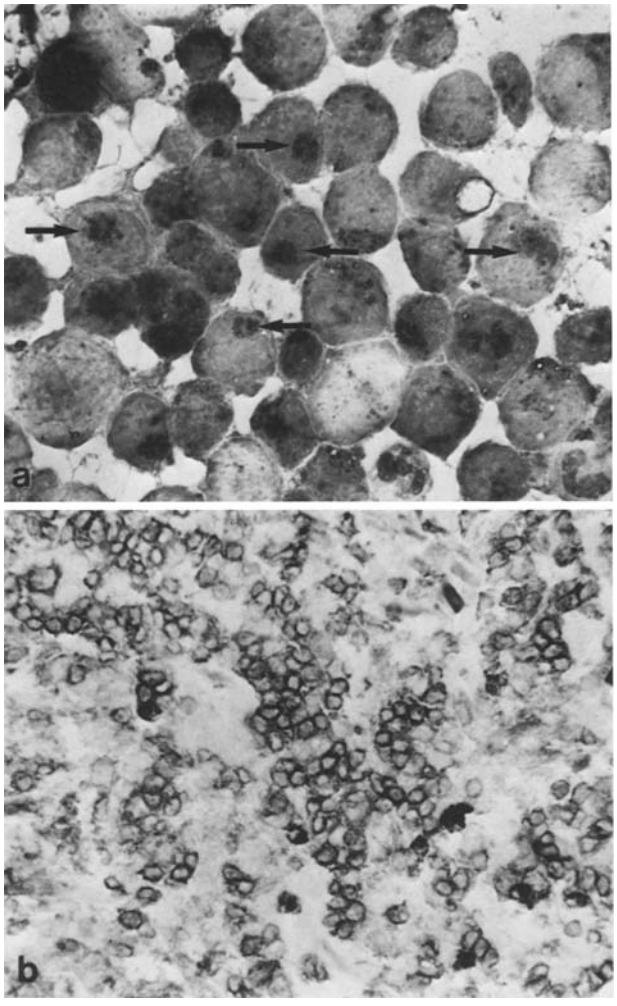

Fig. 6. T-immunoblastic lymphoma (*Case 2*). **a** A strongly positive reaction for DAP IV in neoplastic T-cells (*arrows*) (× 720). **b** Demonstration of sheep erythrocyte receptors on the tumour cells in Cryostat sections using the monoclonal antibody OKT 11. The neoplastic blasts show a ring-shaped, dark-brown reaction. Prominent nucleoli are visible in many blasts. (PAP, × 448)

et al. 1982), which is chiefly seen in Ki-1 lymphomas (Lennert, personal communication; Stein et al. 1982). Our Case 2 corresponds to the T-cell lymphoma belonging to the entity of primary Ki-1 lymphoma demonstrated in a recent study (Lennert, personal communication).

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