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Ultrastructural study of 4 cases of Ki-1 positive large anaplastic cell malignant lymphoma

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Summary. The ultrastructural morphology of 4 cases of large anaplastic cell malignant lymphoma (Ana ML) is reported. Three cases were primary Ana ML and one pleomorphic large T cell lymphoma with some Ki-1 positive cells. All were confirmed by immunohistochemistry on frozen and paraffin sections. The Ki-1 and EMA positive tumour cells had an abundant cytoplasm, with no differentiation and large pale nuclei with multiple compact or dispersed nucleoli. The morphology is that of an activated cell engaged in protein synthesis and/or in the mitotic cycle. These tumour cells resemble to the Hodgkin's and monolobated Reed-Sternberg cells described in Hodgkin's disease.

Key words: Large anaplastic cell lymphoma – Ki-1 lymphoma – Ultrastructural pattern

Introduction

Large anaplastic cell malignant lymphoma (Ana ML) is a new type of lymphoma of high grade malignancy. It is defined by morphological and immunohistochemical criteria (Lennert et al. 1986; Stein et al. 1985; Suchi et al. 1987). Some Ana ML are primary, others are secondary to different types of ML. We report here the ultrastructural morphology of the large anaplastic cells of 4 cases. In three, peripheral lymph nodes were involved and in the fourth, there was lymphomatous involvement of the ileum, with mesenteric lymph node and splenic involvement.

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Material and methods

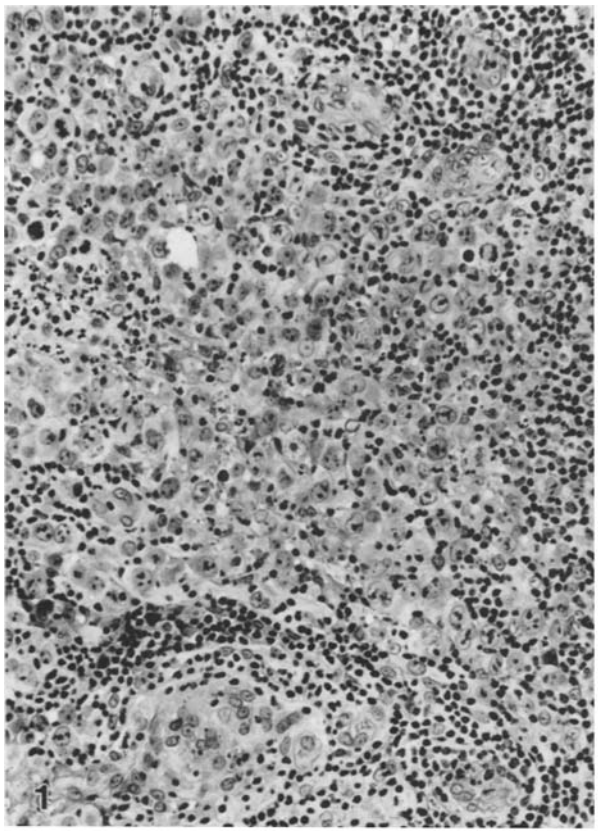
The most important clinical and biological data are summarized in Table 1. The diagnosis was made on paraffin embedded tissue sections stained with haematoxylin-eosin, PAS, Giemsa and silver staining according to Gordon and Sweet.

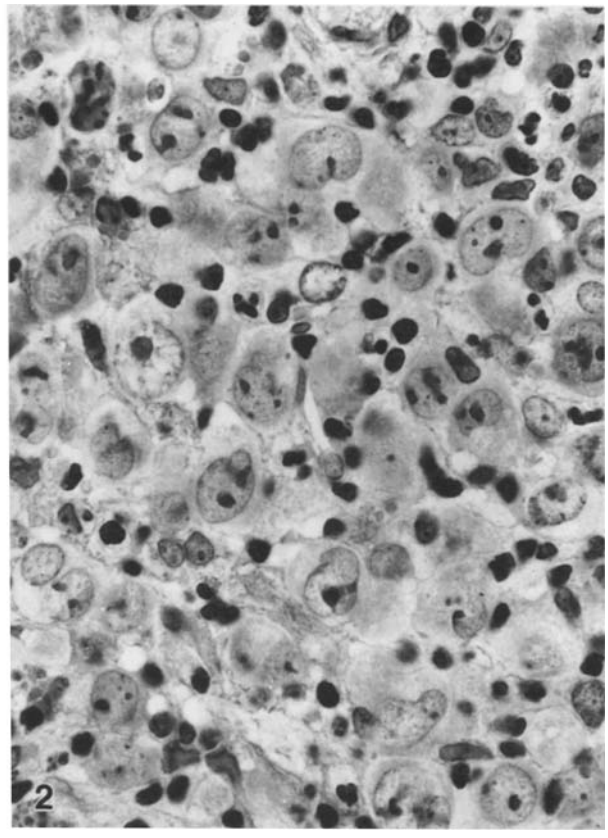
The immunolabelling study was performed on frozen and paraffin sections with monoclonal antibodies using an ABC peroxidase labelling procedure (Vectastain ABC Kit) revealed with aminoethyl carbazole (AEC) and H_2O_2.

Ultrastructural study was performed on samples of tissue fixed in 1.5% glutaraldehyde in PBS buffer, pH 7.4, post-fixed in 2% osmium tetroxide and included in Epoxy resin (LX 12). The ultrathin sections were stained with uranyl acetate and lead citrate. They were observed using a Siemens Elmiskop 101 electron microscope.

Table 1. Clinical data

	Age Sex	Weight loss/ asthenia/fever	Adenopathy	Hepato-megaly	Spleno-megaly	Other	E.M. Study
Case 1 GIO...	32 F	0	Mesenteric	0	+	Ileal occlusion in a renal transplant recipient	• Ileon • Mesenteric lymph node • Spleen
Case 2 LUT...	52 M	+	Multiple; superficial area	+	+	0	Inguinal lymph node
Case 3 HUA...	20 F	+	Multiple; superficial and deep area	0	0	0	Axillary lymph node
Case 4 SAI...	39 F	0	Multiple; superficial area and mediastinal	0	0	0	Sub. clav. lymph node





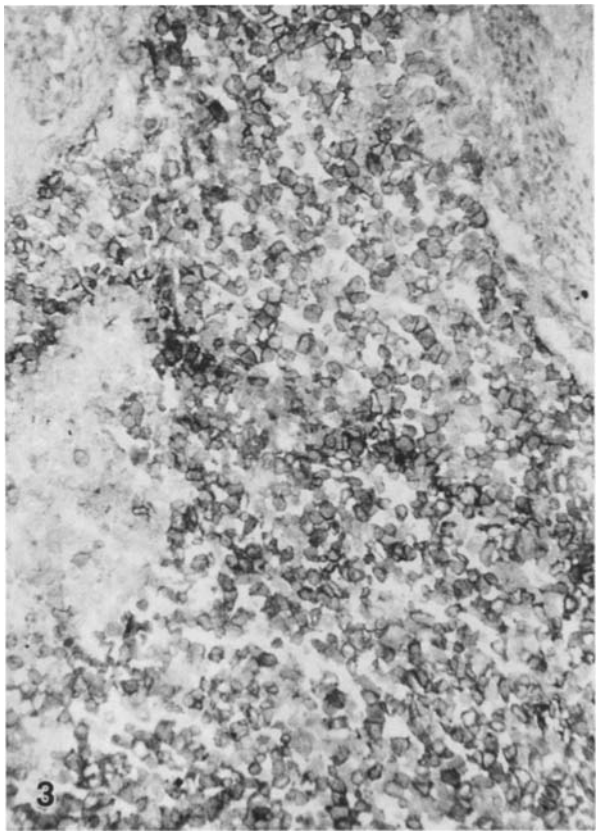


Fig. 1. Lymph node, case 2: (Haematoxylin-eosin G = 225 ×). Large sheets of tumour cells with sinusoidal extension

Fig. 2. Same case: (Haematoxylin-eosin, G = 780 ×). Notice the large pale cytoplasm, the irregular clear nuclei with nucleoli of variable size. Reactive lymphocytes can be seen between the tumour cells

Fig. 3. Lymph node, case 3: (Immunoperoxidase ABC procedure, Ki-1 (CD30) monoclonal antibody, G = 160 ×). All the tumour cells express cytoplasmic and/or membrane positivity

Table 2. Results of immunohistochemistry on frozen sections (1)

	CD30 (Ki-1)	CD25 RiL2	CD26	IOT9 Transf. Recept.	Ki-67	CD2	CD3	CD4	CD5	CD7	CD8	CLA PD7/26 2B11
Case 1	+ 100%	+	+ −	ND	40%	−	−	+	−	−	−	+
Case 2	+ 100%	+	+ −	+	30%	−	−	−	−	−	−	+ −
Case 3	+ 100%	+	+ −	+	10%	+	−	−	+ −	+	+	+
Case 4	+ 10%	−	+ −	+	50%	+	+	+	−	+	−	+

Results

Histologically, the tumour cells are large with a clear irregular nucleus and have abundant cytoplasm (Fig. 1). Large nucleoli are present. The cytoplasm is pale or irregularly basophilic on Giemsa staining (Fig. 2). Some cells resemble Reed-Sternberg cells. These tumour cells form small clusters or large sheets destroying the normal tissue (Fig. 1). In the lymph nodes, some sinuses are filled with such cells. This morphology is typical of large anaplastic cell lymphoma. In case 3, a first lymph node biopsy showed a marked hyperplasia of histiocytes with erythrophagocytosis and a small number of large anaplastic cells.

In all the cases, the diagnosis was confirmed by immunohistochemistry (Fig. 3). The results are summarized in Tables 2, 3 and 4. In all 4 cases, the B cell markers (CD19, CD20, CD21, CD22, CD24) and the histiocytic markers (CD11, CDW14) were negative. No heavy or light chains could be detected on the cell membrane or in the cytoplasm. The final diagnosis was primary large anaplastic cell lymphoma in cases 1, 2 and 3 and pleomorphic large T cell lymphoma with some Ki-1 positive cells, according to the classification proposed recently (Suchi et al. 1987), in case 4.

At ultrastructural level, in all the cases, the anaplastic cells have an abundant cytoplasm and large nucleus (Figs. 4 and 5). The cytoplasm contains a variable number of dispersed polyribosomes, some segments of smooth endoplasmic reticulum or of ergastoplasm, without parallel arrangement and without dilatation, a few mitochondria, and rare lipid vacuoles. A few lysosomes can be seen near a well-developed Golgi apparatus. The membrane is smooth, with no cell junctions. The morphology of the cytoplasm indicates that these large anaplastic cells have an active metabolism but do not exhibit any type of differentiation. The nuclei

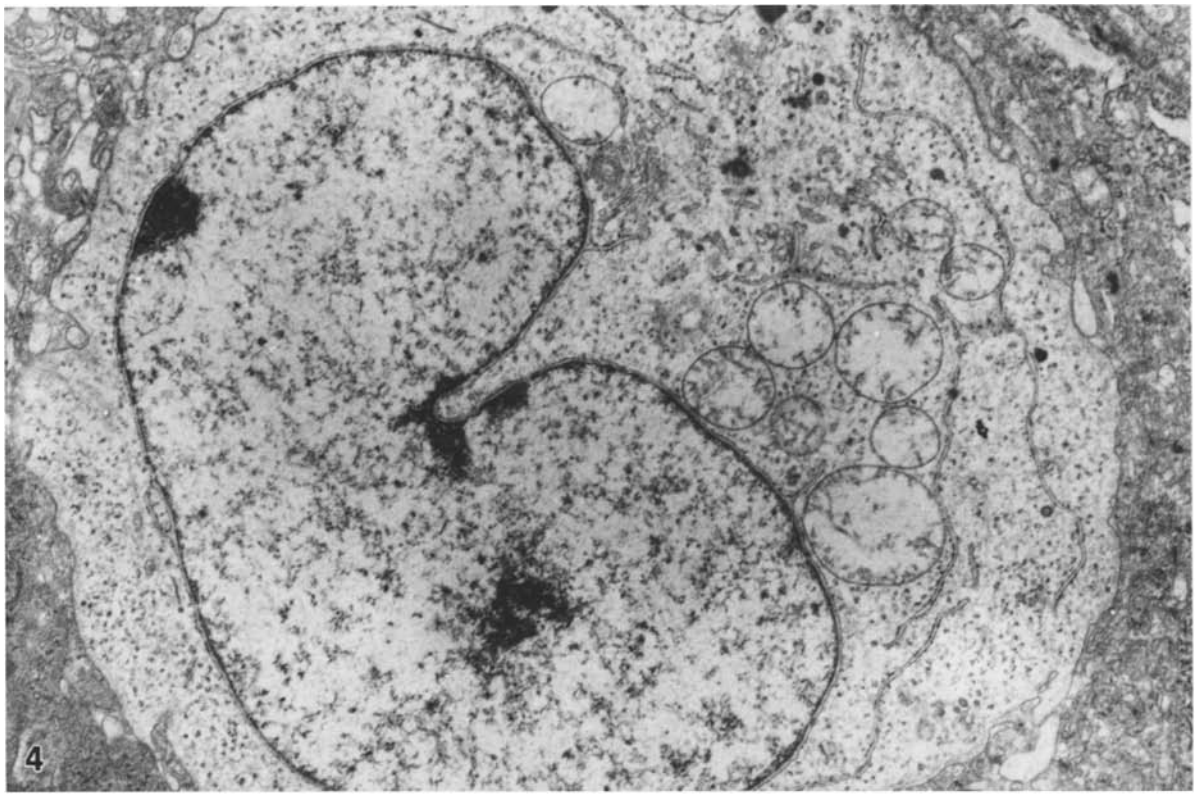
Table 3. Results of immunohistochemistry on frozen sections (2)

• B cell markers	CD19, 20, 22, 23, 24	all negative
• Histiomonocytic markers	CD11, CDW 14	all negative
• No membrane IG		

Table 4. Results of immunohistochemistry on paraffin sections

	EMA E.29	CD15 Leu M1	CLA PD7/26 2B11	Cytokeratin KL1 (55–57 KD)
Case 1	+	−	−	−
Case 2	−	+	−	−
Case 3	+	−	−	−
Case 4	−	+	∓	−

are large. Many of them are round or oval (Fig. 6); others are more irregular with one or many deep indentations often facing the Golgi apparatus (Fig. 7). Some nuclei are multilobated and resemble Reed-Sternberg cells (Figs. 7 and 8). In case 3 (Fig. 9) some nuclei exhibit the morphology of "jelly fish" as described in T cell lymphoma (Suchi et al. 1987). The nucleoli are often proeminent, with either a compact or a reticular structure. One or, rarely, two small nuclear bodies can be seen. Depending on the heterochromatin pattern, two different types of nuclei can be described. In cases 2 and 4, the heterochromatin is completely dispersed and the nuclei are electron-lucent (Figs. 4 and 8). In cases 1 and 3, small dots of heterochromatin are dispersed in the nuclei with a spotted aspect similar to that described in some T cell lymphomas (Fig. 9). The morphology of the nuclei suggests that these cells are metabolically active. In case 3, there were very few large anaplastic cells in the first lymph node biopsy. Most of the cells seen around the tumour cells are histiocytes: they



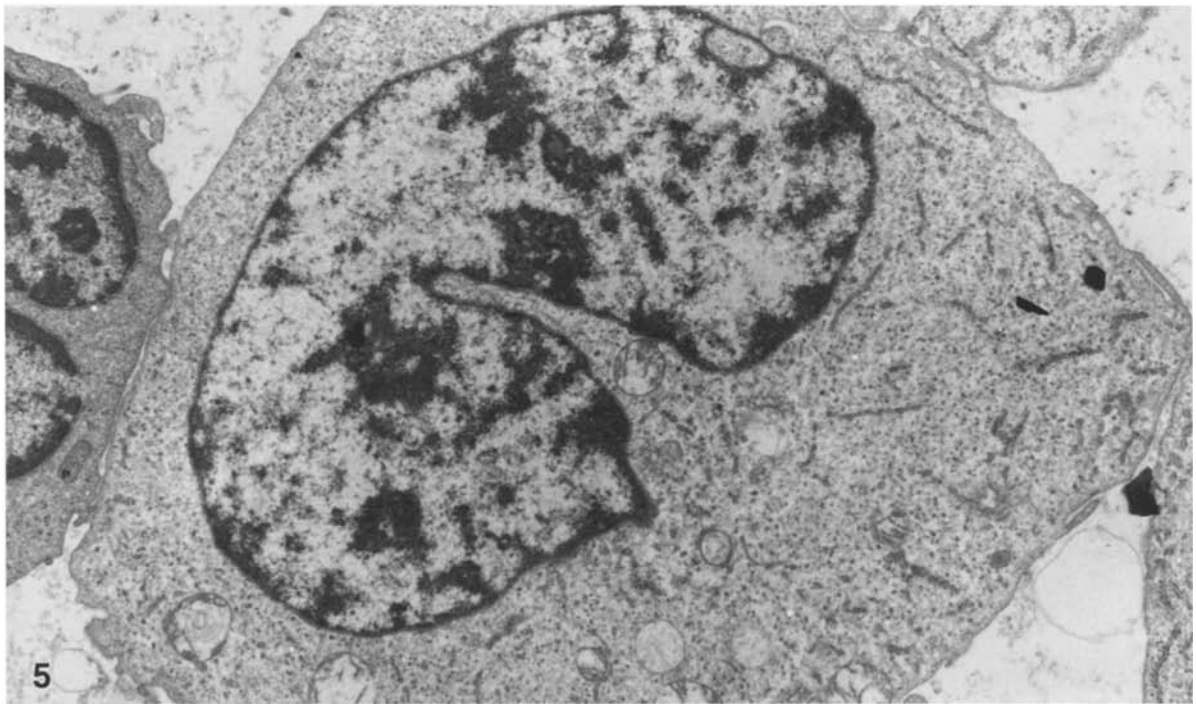


Fig. 4. Lymph node, case 2: (G = 8000 ×). Large clear nucleus with dispersed heterochromatin and one nucleolus facing a nuclear indentation, a well developped Golgi apparatus can be seen. Notice the presence of some segments of endoplasmic reticulum, of many ribosomes and of rare electron dense lysosome-like granules

Fig. 5. Lymph node, case 3: (G = 8000 ×). The cytoplasm exhibit the same morphology as the cell in Fig. 4. The nucleus is also deeply indented. But small dots of heterochromatin are recognizable dispersed in the nucleus, realizing a spotted aspect

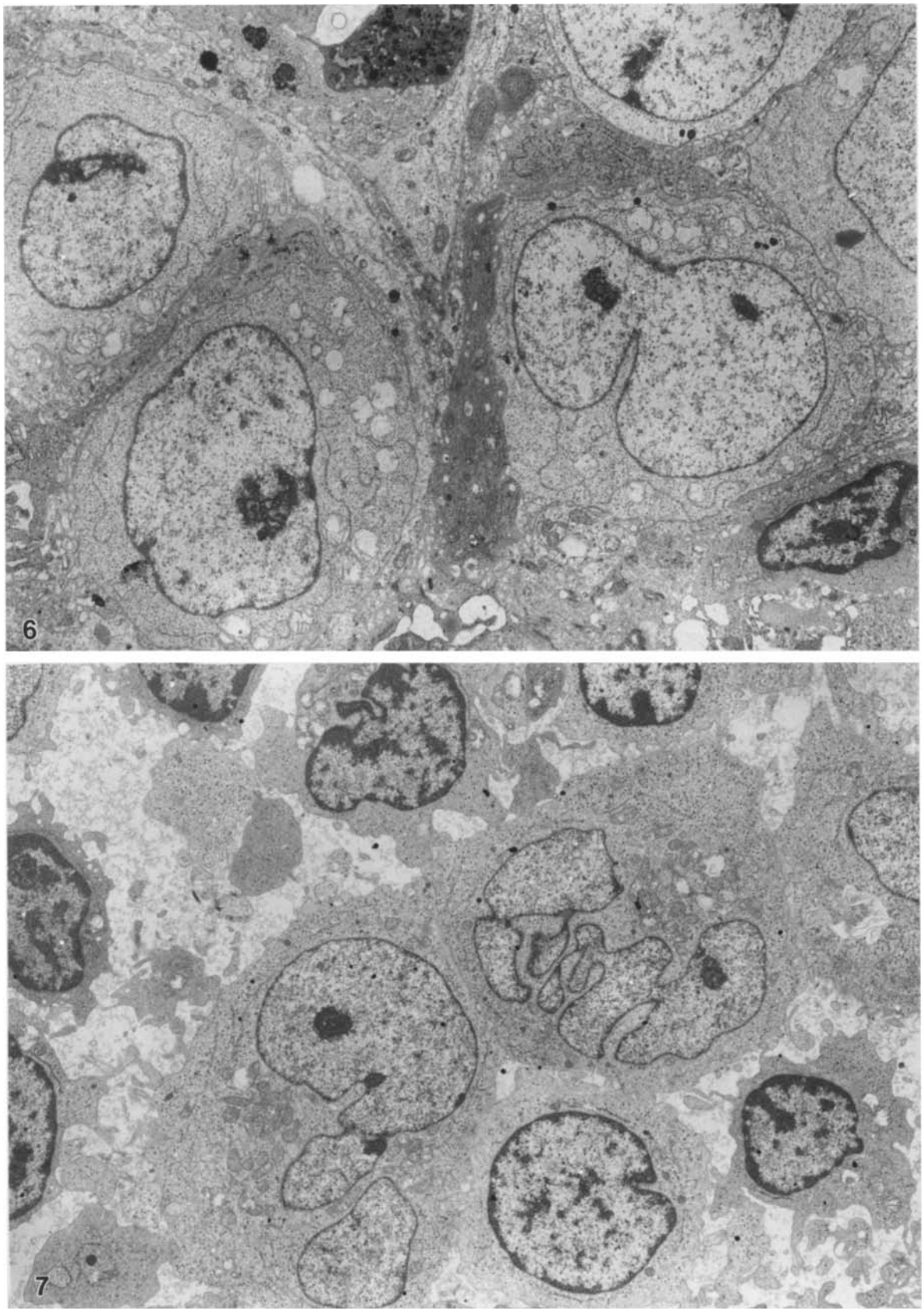
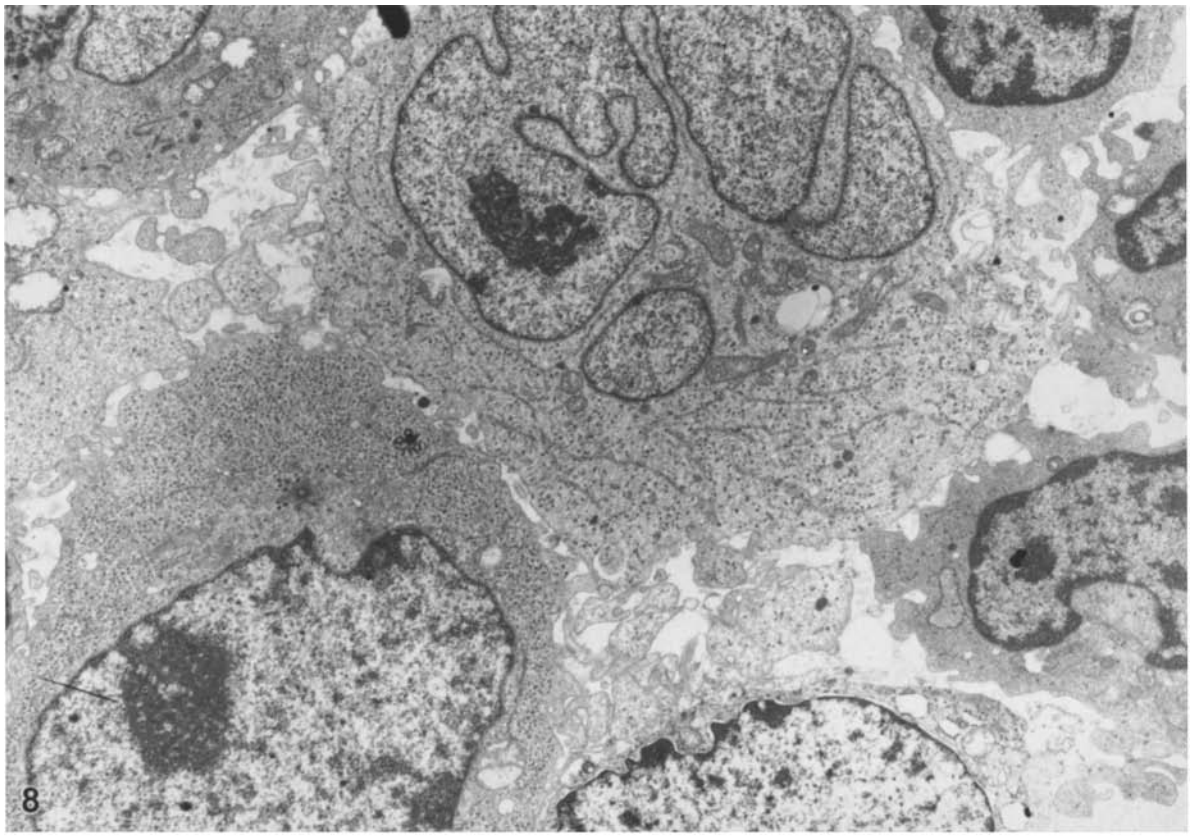


Fig. 6. Lymph node, case 2: (G = 5000 ×). The tumour cells have regular nuclei with large nucleoli. The large cytoplasm doesn't present any type of differentiation. Macrophages and small lymphocytes can be seen between the tumour cells

Fig. 7. Lymph node, case 4: (G = 4800 ×). Two tumour cells can be recognized, one with an irregular nucleus resembling those of a Reed-Sternberg cell. Notice the presence around the large cells of small and medium lymphoid cells corresponding to the pleomorphic T lymphomatous cell



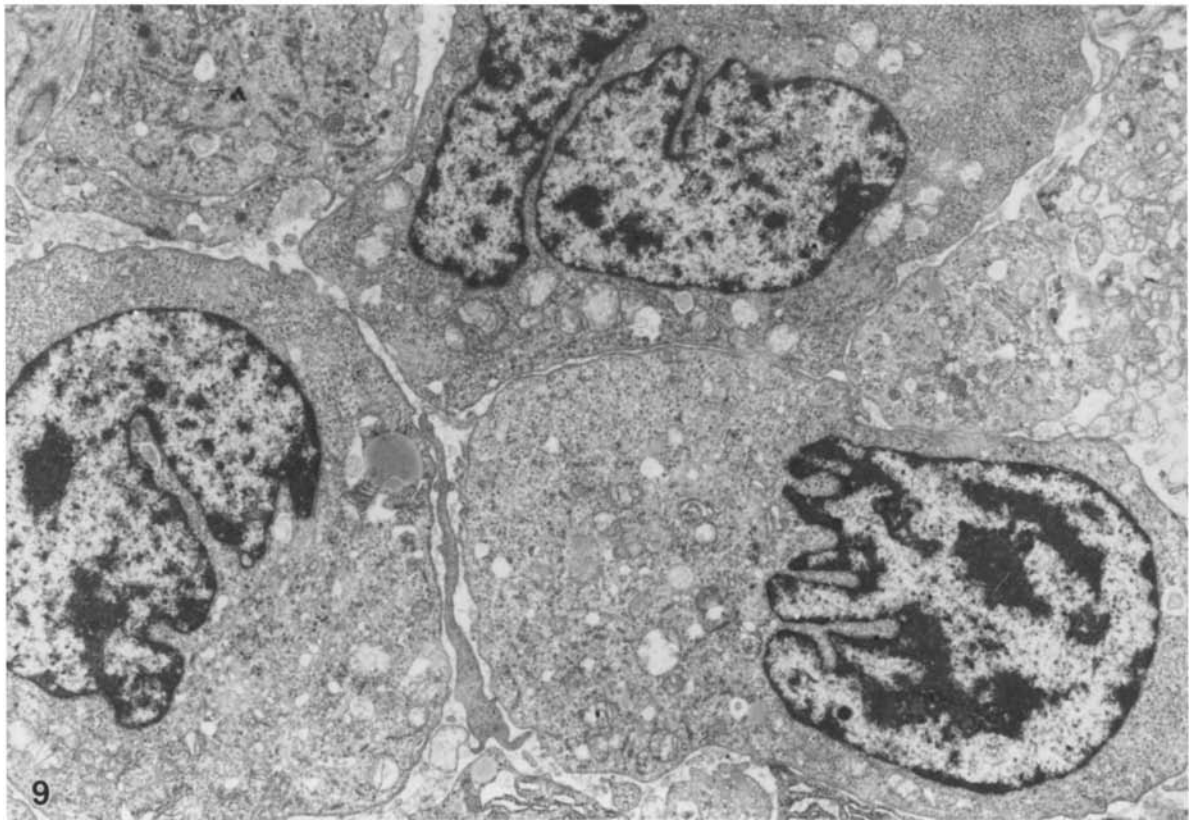


Fig. 8. Case 4 (G=6000×). Two type of cells corresponding to the large anaplastic population are shown, one with a regular nucleus, another with a Reed-Sternberg like nucleus

Fig. 9. Lymph node, case 3. 2nd biopsy: (G=4800×). The large anaplastic cells exhibit nuclei with a stippled pattern. Many indentations can be seen in 2 nuclei, restricted to one part of the nucleus, giving a jelly fish appearance

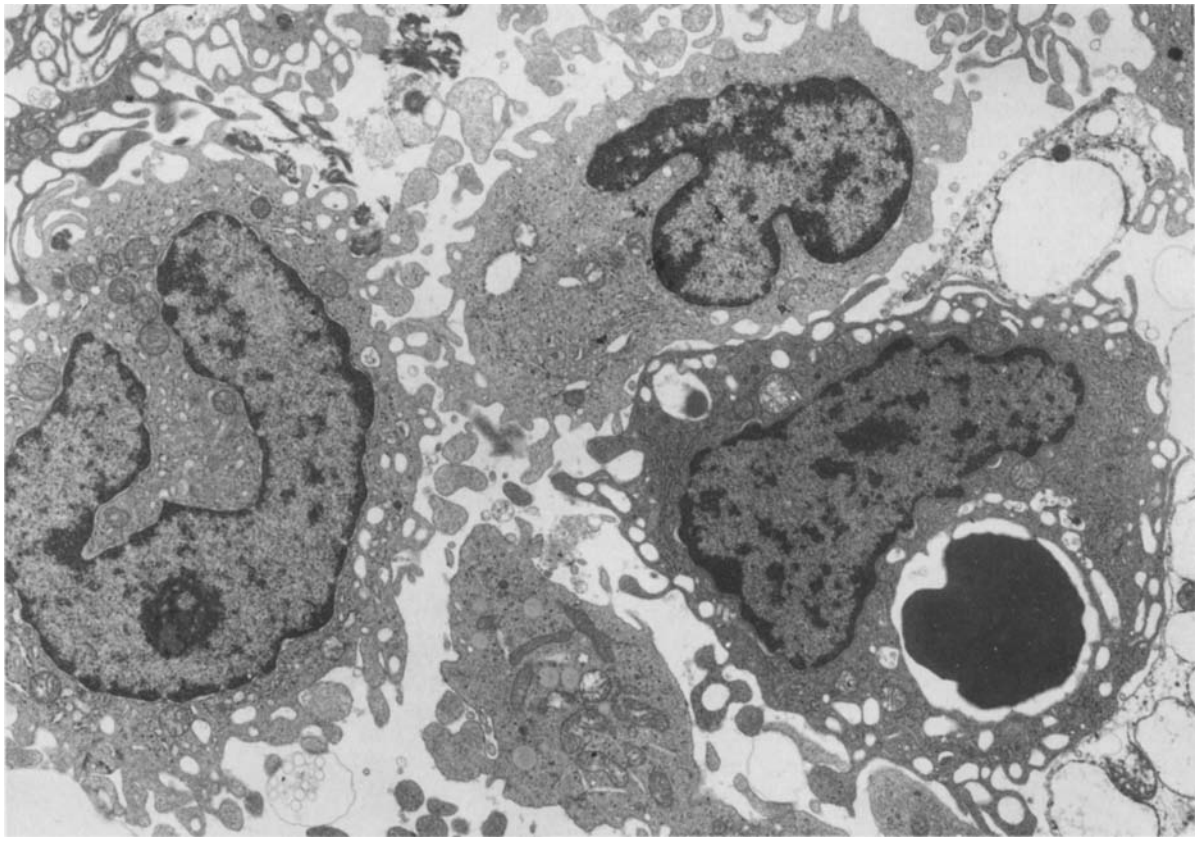


Fig. 10. Lymph node, case 3. 1st biopsy: (G = 8000 ×). In this 1st biopsy, a diffuse infiltrate of reactive histiocytes can be seen; some of them show erythrophagocytosis. No large anaplastic cells can be found in this area

have abundant cytoplasm with many lysosomes and phagolysosomes. Erythrophagocytosis can be seen in some cells. All the histiocytes have a large number of cytoplasmic projections. Between these projections, numerous intercellular deposits of fibrin can be detected (Fig. 10).

Discussion

Large anaplastic cell malignant lymphoma (Ana ML) is a new type of high grade malignant lymphoma (Lennert et al. 1986; Stein et al. 1984, 1985). The entity was recognized using the monoclonal antibody Ki-1 (Schwab et al. 1982; Stein et al. 1982). This antibody, recently assigned to CD30, reacts with Reed-Sternberg cells in Hodgkin's disease (Schwab et al. 1982; Stein et al. 1982, 1985). Some large cells of non-Hodgkin's lymphoma of T cells and less frequently, of B cells also react positively. The antigen recognized by CD30 is present on "activated" T and B cells (Gerdes et al. 1986; Schwab et al. 1982; Stein et al. 1985).

The neoplastic cells form sheets of cohesive appearing cells, mimicking anaplastic carcinoma or melanoma. They sometimes involve the lymph node only partially, often in T cell areas. In many cases, sinuses are dilated and filled with the neoplastic cells. Large, actively phagocytic macrophages can be associated in variable numbers, raising the possibility of malignant histiocytosis (Lennert et al. 1987; Schwab et al. 1982; Stein et al. 1984). In Ana ML, every neoplastic cell stains strongly with the Ki-1 antibody (Suchi et al. 1987), as is seen in three of our cases (1, 2, 3). This result suggests only that all the cells are activated, and cannot be compared to positivity found in other tumors with markers of cellular differentiation. However, clusters of Ki-1 positive cells can be found in peripheral T cell lymphoma (Lennert et al. 1986; Suchi et al. 1987) as is the case in our patient n° 4, and may be the origin of secondary large anaplastic cell lymphoma. On immunophenotypic analysis, most of the Ana ML are of T cell origin, with a more or less incomplete phenotype (Lennert et al. 1986; Suchi et al. 1987). Some are of B or, rarely, of monocytic origin (Stein et al.

1984, 1985). A few are null (Stein et al. 1984, 1985). These Ki-1 positive cells express other markers of activated cells, particularly IL-2 receptors (TAC) and HLA class II antigen. They may also express epithelial membrane antigen or EMA (Al Saati et al. 1986), but do not have morphological or clinical singularities.

Three of the 4 reported cases have the clinical, morphological and immunohistochemical criteria of Ki-1 positive Ana ML. The last (case 4) is a pleomorphic T cell lymphoma with a small number of anaplastic Ki-1 positive cells. One case (case 1) occured in a renal transplant with ileal and spleen involvement (Audouin et al. 1988). In case 3, there was large macrophage infiltration of the first lymph node with a very small number of anaplastic cells and the diagnosis was made on the second lymph node biopsy. To our knowledge, the ultrastructural morphology of the Ki-1 positive cells has never been described. The morphology of the cytoplasm and of the nucleus is that of cells engaged in protein synthesis, in accord with the phenotype of these cells which suggests "activated" cells. No differentiation can be found. These cells are similar to those described by Kaiserling in reticulosarcoma (Kaiserling 1978). Cells with the same morphology can also be seen in true Hodgkin's disease of the diffuse lymphohistiocytic, mixed cellularity or nodular sclerosing types (Diebold et al. 1986). Some of the cells described as Hodgkin's cells or Reed-Sternberg cells with monolobated nucleus resemble Ki-1 anaplastic cells. They look like the cells of immunoblastic or intermediary type, as we have reported in Hodgkin's disease (Diebold et al. 1986). Thus Ana ML and Hodgkin's disease are probably lymphomas due to the malignant proliferation of "activated cells" of T or B origin. The behaviour of the lymphoid tissue around the tumour cells perhaps explains the differences in symptomatology, morphology and prognosis of the 2 diseases.

Electron microscopy is useful for diagnosis. The ultrastructural morphology allows carcinoma, melanoma, malignant histiocytosis and also immunoblastic and centroblastic ML to be eliminated. However, it is not possible at the ultrastructural level to distinguish Ana ML from Hodgkin's disease or, sometimes, from undifferentiated tumours of whatever origin. Histological criteria and immunohistochemistry are needed.

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