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Ki-1 lymphomas in childhood: immunohistochemical analysis and the significance of epithelial membrane antigen (EMA) as a new marker

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Summary. Two cases of Ki-1 lymphomas in childhood were analyzed immunohistochemically and immunoelectron microscopically. They expressed Hodgkin's disease associated antigen, Ki-1, interleukin-2 receptor (IL2R), OKT9, and HLA-DR. Histologically, the tumour cells were large in size with abundant cytoplasm and atypical nuclei. Lymph node involvement was characterized by parafollicular and marginal sinus infiltration. These features were identical to those reported in Ki-1 lymphomas. Electron microscopically tumour cells had abundant cytoplasmic organelles with pleomorphic nuclei but had no specific granules. Some tumour cells had marked interdigitation of cell membrane. Immunoelectron microscopically Ki-1 was positive on cell membrane. Tumour cells had no T-cell or B-cell antigens except for Leu-3 (T4). Unexpectedly they expressed epithelial membrane antigen (EMA) strongly. EMA was positive on cell membrane and in the cytoplasm. EMA was detected effectively in paraffin-embedded sections. Among the malignant lymphomas in childhood tested, two cases were EMA-positive. The pattern of EMA-reactivity and the histology were very similar to Ki-1 lymphomas. These results strongly suggest that Ki-1 lymphomas in childhood may arise from non-lymphoid haematopoietic cells and that EMA can be used as a new marker to distingish certain type of Ki-1 lymphomas in childhood.

Key words: Malignant lymphomas – Monoclonal antibodies – Hodgkin's disease – Immunoperoxidase techniques

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Introduction

Ki-1 antigen was first reported by Schwab et al. (1982) as a Reed-Sternberg or Hodgkin cell specific antigen in Hodgkin's disease. A rescent study, however, showed Ki-1 antigen as an activation-related molecule on lymphoid cells since the antigen can easily be induced on peripheral blood mononuclear cells by human lymphotropic virus II or by plant mitogens (Stein et al. 1985). Stein et al. (1985) has recently reported that the Ki-1 positive anaplastic large cell lymphomas previously considered to be anaplastic carcinoma are derived from activated lymphoid cells. From the typical histological appearance and the infiltration pattern these Ki-1 positive lymphomas have been considered to represent a clearly recognizable lymphoma category. However there have been only a few reports on this particular type of human neoplasm. In this report we describe two Ki-1 positive malignant tumours in childhood whose characteristics are comparable to those of Ki-1 positive lymphomas. Furthermore, we describe that the epithelial membrane antigen (EMA) can be used as an another antigen to characterize this tumour.

Materials and methods

Case 1 was a 8-year-old male who had a fever and cervical lymph node swelling. Lymph nodes were removed surgically and were analyzed. Case 2 was a 3-year-old male who developed a low grade fever, abdominal pain and inguinal and cervical lymph node swelling. The lymph nodes from both regions were surgically removed. Swelling of the right testis was also noticed and biopsy material of the testis was also analyzed in this study.

Surgically removed specimens were stored frozen in OCT-compound (Miles Scientific, Naperville, USA) at −80° C until use. One part of the tissue was also stored frozen following

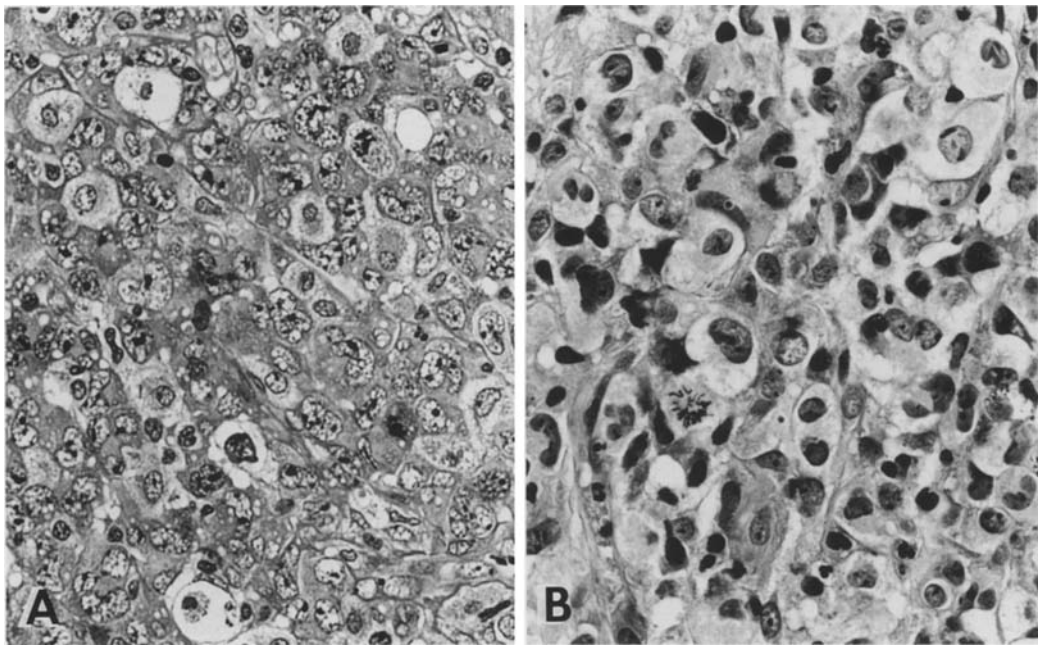

Fig. 1. Histology of Ki-1 lymphomas. A: Lymph node of Case 1 (H & E stain, ×170), B: Right testis of Case 2 (H & E stain, ×170)

fixation by 4% paraformaldehyde according to the method described (Hata et al. 1980). For electron microscopy tissue was fixed by 2.5% glutaraldehyde in a sodium phosphate buffer and processed according to the method described previously (Hata et al. 1980). Routine paraffin sections were also made and histological diagnosis was done by Working Formulation Classification (1982).

Monoclonal and polyclonal antibodies used in this study are listed below. Monoclonal RSC-1 which detects Ki-1 antigen, anti-interleukin 2 receptor (IL2R), anti-leukocyte common antigen (LC), anti-epithelial membrane antigen (EMA) were purchased from Dakopatts, Copenhagen, Denmark. Antibodies reactive with T cells (L1, L2, L3, L6, Leu-1, Leu-2, Leu-3, Leu-4, Leu-5, Leu-6, and Leu-9), B cells (B4, B1, and L26) and macrophages/histiocytes (OKM1, Leu-M1, My4, My7, and My9) were also used. L series antibodies and anti-HLA-DR framework antibody were developed in Department of Pathology, Sapporo Medical College, Japan (Ishii et al. 1983; Ishii et al. 1984; Takei et al. 1985). L1, L2, L3, L6 correspond to Leu-1, Leu2, Leu-3 and Leu-6, respectively. Leu series antibodies, OK series antibodies, My and B series antibodies were purchased from the respective company. MT-1 which detects T cell related antigen on paraffin section was obtained from Bio-Science Products, Emmenbruck, Switzerland. Polyclonal antibodies, anti-S100 protein, anti-lysozyme, anti-alpha-1-antitrypsin and anti-human immunoglobulins, and anti-keratin were obtained from Dakopatts.

Frozen sections were cut by Cryostat (Tissue-Tek II, Miles Scientific) and placed on slide glasses. These were air-dried and fixed by cold acetone for 15 min and used for immunostaining. Paraffin-embedded sections were deparaffinized and used for immunostaining. The procedure for immunostaining were done by indirect immunoperoxidase method already described elswhere (Ishii et al. 1987). Immunoelectron microscopic observation was performed according to the method previously described (Hata et al. 1980).

Results

In two cases (Case 1 and Case 2) the pathological findings were very similar (Fig. 1A and 1B). The tumour cells were remarkably large in size with abundant cytoplasm, pleomorphic nuclei and prominent nucleoli. Abnormal mitotic figures and multinucleated cells were occasionally observed. In Case 1 a sheet of large cells with basophilic cytoplasm and large nuclei were seen (Fig. 1A). The nuclei were pleomorphic with bizarre shape and coarse chromatin. In Case 2 pale-staining tumour cells having atypical nuclei with relatively fine chromatin were observed (Fig. 1B). Remarkable phagocytic activities were often seen, in both cases. The fundamental architecture of the lymph nodes were destroyed by infiltration of tumour cells. Residual follicles and normal lymphocytes were detectable in both cases. Typically in Case 1 focal, often massive infiltration of subcapsular marginal sinuses simulating an appearance of metastatic carcinoma was seen (Fig. 2B). The pattern of infiltration was more clearly demonstrated by immunostaining as is shown in Fig. 2A and 2C (described later). In case 2, the lymph nodes taken from neck and inguinal region were characterized by replacement of fibrois with numerously formed vessels and a few mononuclear cell infiltration. Tumour cells were only occasionally seen in the paracortical areas. Biopsied testicular tissue (Case 2) was sever-

Table 1. The result of immunohistochemistry

Antigens	Case 1	Case 2
Hodgkin's disease associated		
Ki-1	+	+
Activation related		
HLA-DR	+	+
IL2R	+	+
OKT9	+	+
T cell related		
L6, Leu-6	−	−
Leu-5	−	−
Leu-4	−	−
L3, Leu-3	+	+
L1, Leu-1	−	−
Leu-9	−	−
L2, Leu-2	−	−
B cell related		
B4	−	−
B1	−	−
L26	−	−
sIg	−	−
Macrophage/histiocyte related		
OKM1	−	−
Leu-M1	−	−
My4	−	−
My7	−	−
My9	−	−
S-100	−	−
Lysozyme	−	−
alpha-1-AT	−/+ [a]	−/+ [a]
Haematopoietic		
LC	+	+
Epithelial cell related		
EMA	+	+
Keratin	−	−

[a] alpha-1-AT (antitrypsin) was occasionally positive

ely involved by the tumour cells described above. By electron microscopy (Case 1), the tumour cells possessed pleomorphic and vesicular nuclei with peripheral chromatin condensation (Fig. 3) and the cell membrane often showed marked indentation. They contained abundant cytoplasmic organelles such as free ribosomes, rough endoplasmic reticulum, mitochondria and lyosomal granules. No specific granules (including Birbeck's granule) were observed. No apparent developed attachment devices between tumour cells of the types usually seen in epithelial tumours were noted.

Immunostaining on frozen sections was done on these two cases and the results are summarized in Table 1. The pattern of reactivities were identical in both cases. Tumour cells were strongly positive for Ki-1. As is shown in Fig. 2A tumour cells of Case 1 infiltrating into the marginal sinus (upper area) as well as into the medullary sinus (right lower area) were stained with Ki-1. In a higher magnification large atypical tumour cells in the marginal sinus (Fig. 2B) were clearly demonstrated by Ki-1 staining (Fig. 2C). Tumour cells were also positive for IL2R (Fig. 4A), HLA-DR, and OKT9. In contrast to the Reed-Sternberg or Hodgkin cells in Hodgkin's disease, they did not express Leu-M1 antigen (Hsu et al. 1985). Precise localization of Ki-1 antigen were investigated by immuno-electron microscopy. As is shown in Fig. 5A, Ki-1 was located on cell membrane of cells having atypical nuclei, prominent nucleoli and abundant cytoplasm. Interdigitation of tumour cell membrane was clearly demonstrated by Ki-1 immunoelectron microscopy as shown in Fig. 5A (inset). The tumour cells in both cases did not express any T cell specific or B cell specific antigens except that Leu-3 antigen was weakly positive in both cases. The cells did not express S100 and lysozyme although alpha-1-antitrypsin was weakly and granularly positive in some. S100 positive histiocytes and lysozyme positive macrophages were frequently seen. Of note was the finding that epithelial membrane antigen (EMA) was strongly positive in both cases. The pattern of reactivity of this antibody on tissue was identical to those of the Ki-1 antibody. Thus the tumour cells infiltrating in both the paracortical area and in the marginal sinus were strongly positive for EMA. EMA could be detected effectively in paraffin section (Fig. 4B) where the same reactivity as obtained in frozen sections was observed. Localization of EMA in tumour cells was unique. EMA was found to be present not only on cell membrane but also in some cytoplasmic organelles (Fig. 4B).

Specific expression and effective staining of EMA in paraffin sections led us to investigate the expression of EMA in previous cases having similar histological findings. The tumours tested (Table 2) were 5 diffuse large cell lymphomas of B cell origin, 2 diffuse large cell lymphomas of unidentified cell origin, 2 cases of malignant histiocytosis and 3 cases of Hodgkin's disease (mixed type). In two cases which had been previously diagnozed as large cell lymphoma of unidentified origin and malignant histiocytosis, respectively, a positive reaction for EMA was found on paraffin section. In Hodgkin's disease (3 cases of mixed cellularity type) EMA could be detected on a small population of cells different from Reed-Sternberg or Hodgkin cells although their precise cellular origins were not known. In lymph nodes from non-malignant disease, such as reactive lymphadenitis, very small numbers of cells expressed EMA.

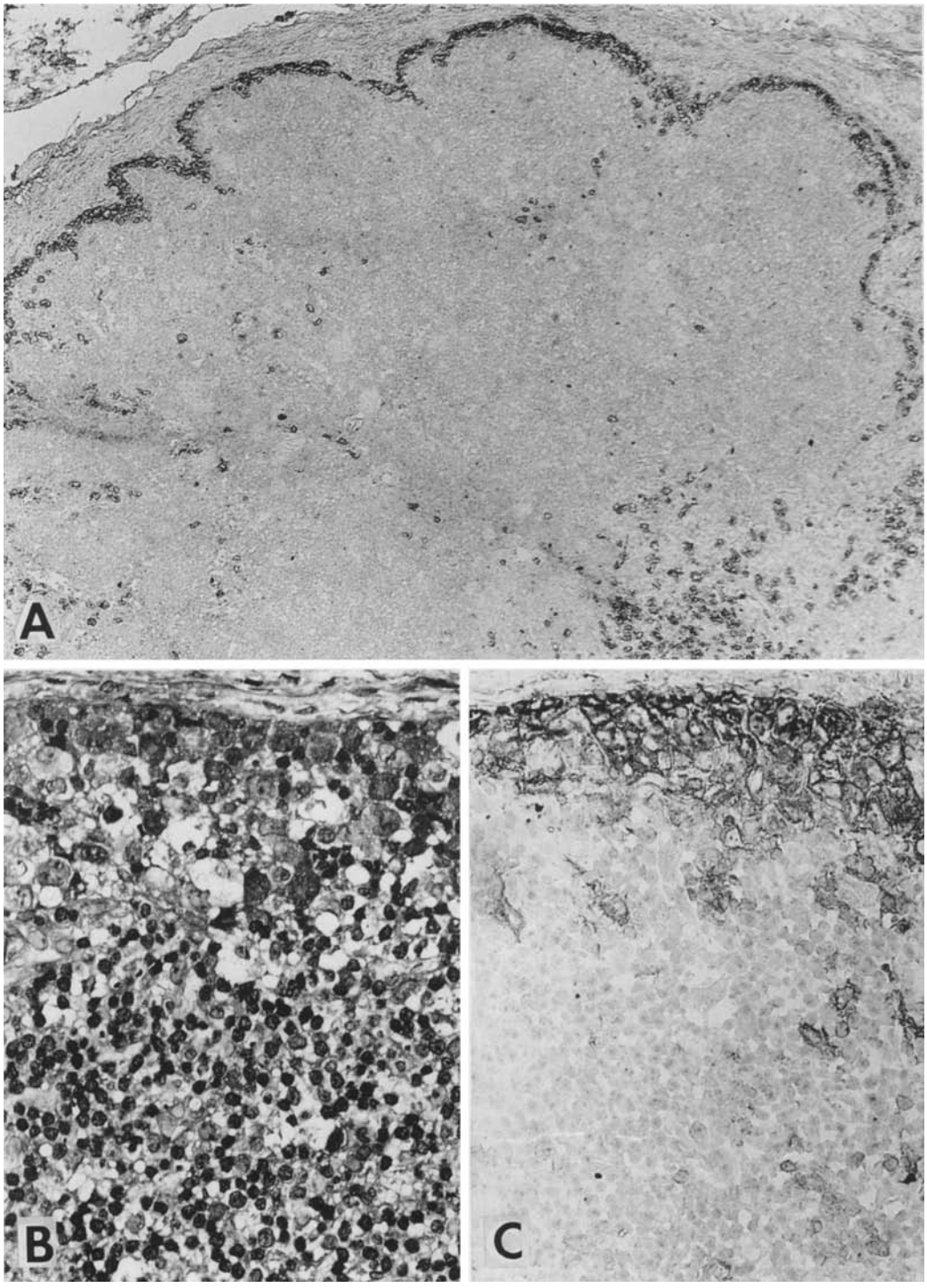

Fig. 2. Ki-1 immunostaining on Case 1. A: Low power view of Ki-1 staining on lymph node (frozen section, ×50). Note the location of Ki-1 positive tumour cells in the marginal sinus (*upper area*) and in the medullary sinus (*right lower area*). B: High power view of tumour cells in the marginal sinus (H & E stain on paraffin section, ×150). C: Ki-1 staining of the tumour cells in the marginal sinus (frozen section, ×150)

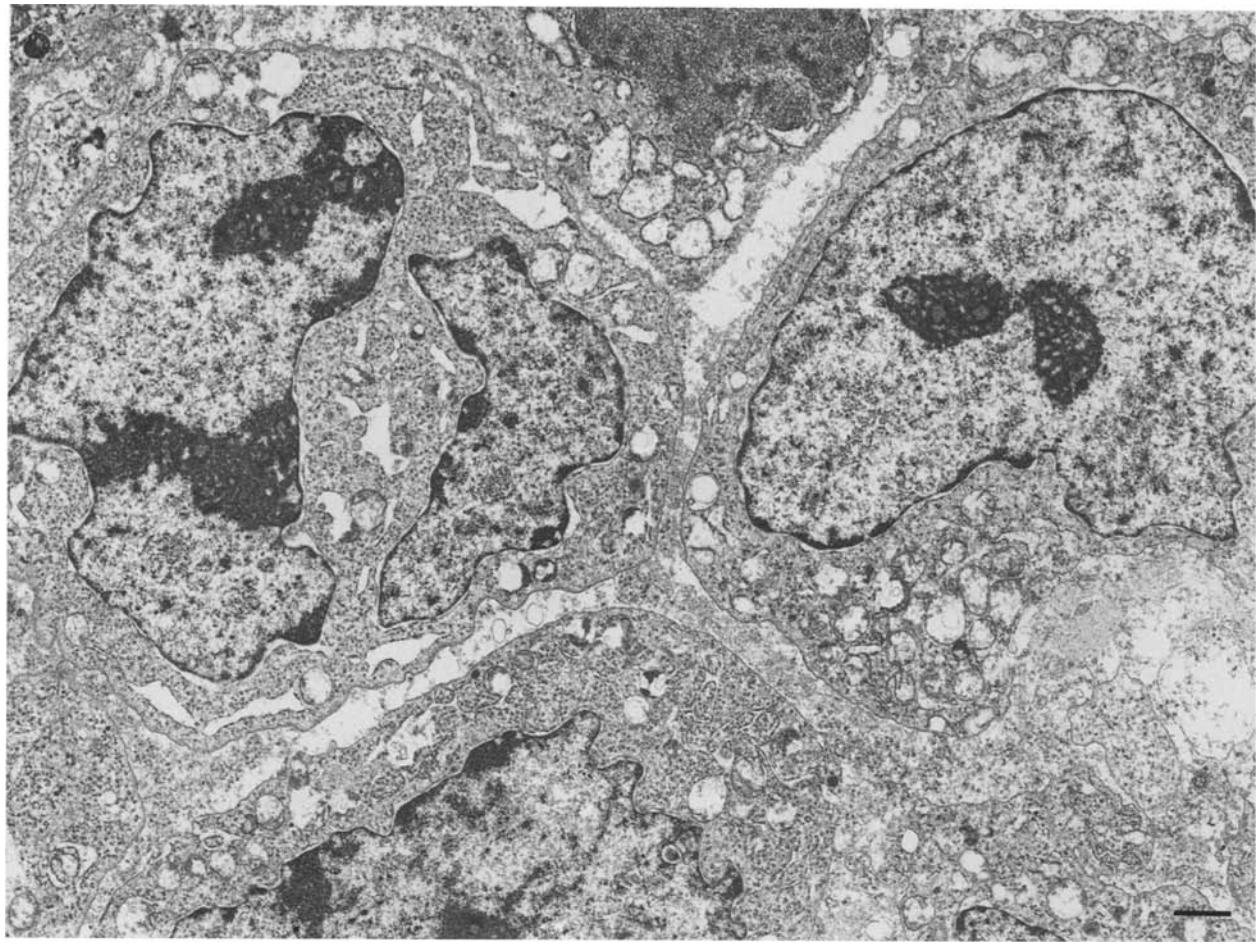

Fig. 3. Electron microscopy of Case 1. (×8000). Bar = 1 micrometer

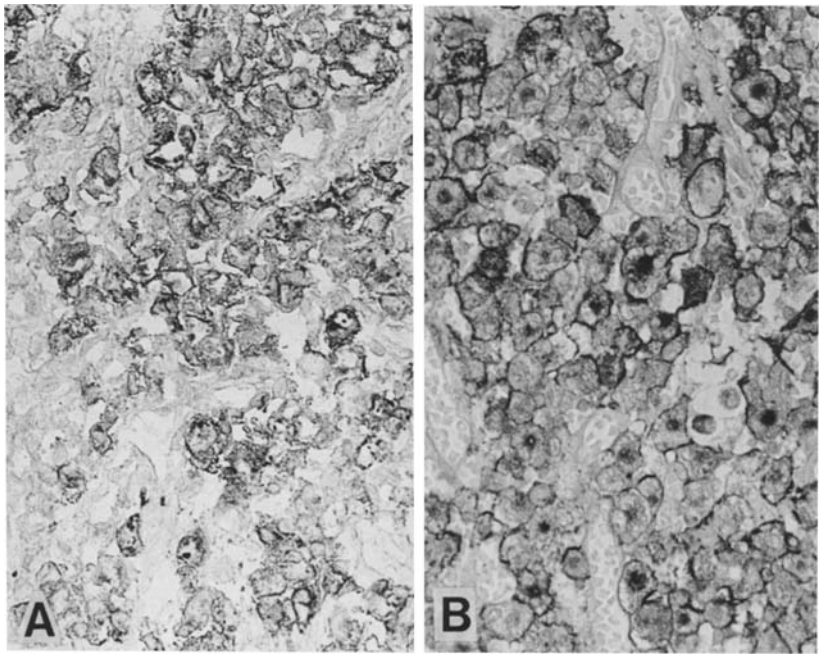

Fig. 4. IL2R and EMA staining on Case 2. A: IL2R staining on the testis of Case 2 (frozen section, ×143). B: EMA staining on the paraffin section of Case 2 (testis, ×143)

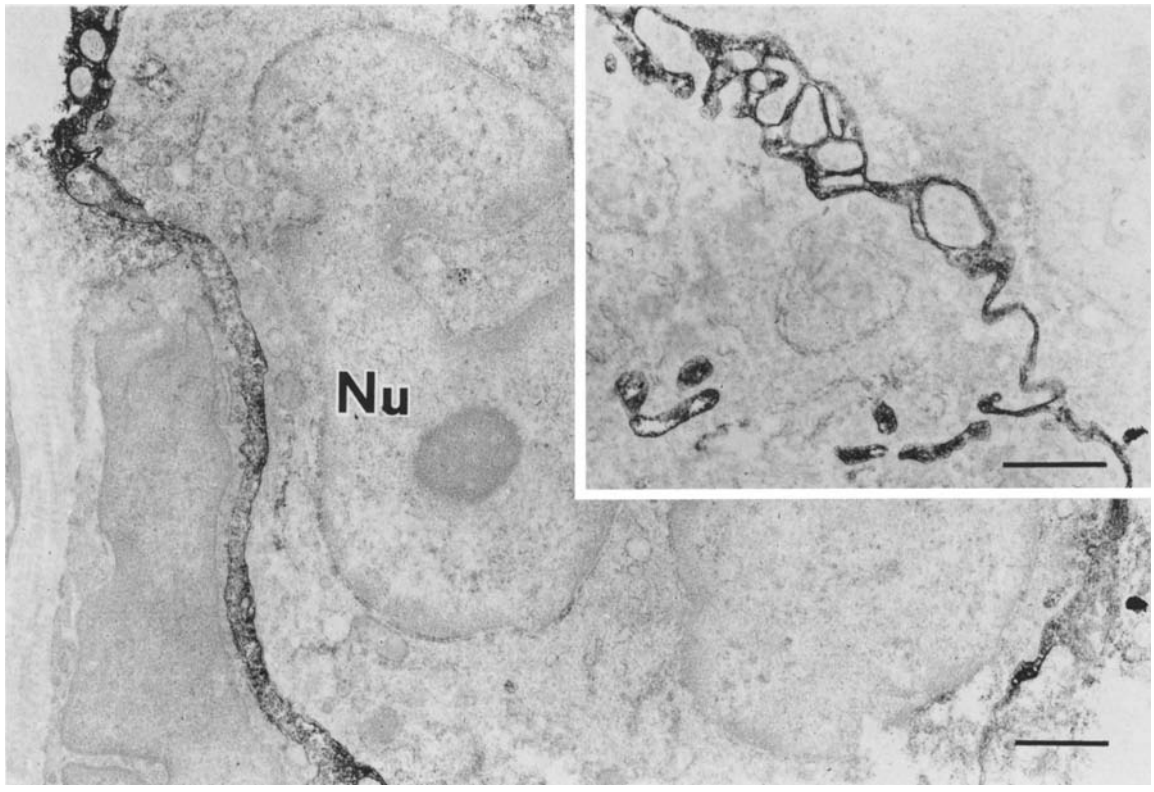

Fig. 5. Immunoelectron microscopy of Case 1. Cell membrane of tumour cell having atypical nucleus was stained with Ki-1 (×12500). Marked interdigitation of cell membrane was also noticed (inset, ×14000). Nu: Nucleus of the tumour cells. Bar = 1 micrometer

Discussion

In this report we described two childhood malignant tumours which expressed Ki-1 antigens as well as other activation related molecules, IL2R, HLA-DR and OKT9. Histological characteristics as well as antigen phenotypes were identical to those reported as Ki-1 positive lymphomas originally by Stein et al. (1985) and recently by Kadin et al. (1986). The histological appearance, especially the atypical nuclei and the sinus infiltration pattern, as well as the expression of the above mentioned antigens, have established a distinguishable entity for tumours previously diagnosed as anaplastic large cell lymphomas. Many of the Ki-1 lymphoma cases seem to be derived from T or B cells as judged by the expression of lineage-specific antigens and by the rearrangement of genes for immunoglobulin or T cell receptor. Our cases, however, lacked any T or B cell specific antigens although Leu-3 (T4) antigen, the antigen for helper T cell and monocyte/macrophage (Wood et al. 1983; Stewart et al. 1986) was weakly positive in both cases. The electron and immunoelectron microscopic study revealed the remarkable invagination of cell membrane usually found in interdigitating reticulum cells (Kojima and Imai 1973). These results may suggest that Ki-1 positive cases described in this study are derived from non-lymphoid cell lineage. As described previously there are some Ki-1 positive cases whose cellular origin could not be delineated (Stein et al. 1985). Thus it is highly probable that Ki-1 lymphoma is a heterogenous tumour.

Interestingly, the Ki-1 lymphomas described in this study were positive for EMA. EMA has been used for distinguishing lymphoid and epithelial malignant tumours (Sloane et al. 1981; Cordell et al. 1985) but recent reports described the expression of this antigen on haematopoietic cells and their tumours (Delson et al. 1984; Al Saati et al. 1986; Rabkin and Kjeldsberg 1987; Gonzalez-Crussi et al. 1987). Of note was the report by Al Saati et al. that all the Ki-1 lymphoma cases were EMA-positive (Saati et al. 1986). EMA seems to be one of the activation-related molecules because it can be induced on normal lymphocytes upon activation (Delson et al. 1984). It is therefore not surprising that Ki-1 lymphomas co-expressed EMA because Ki-1, IL2R, and HLA-DR are all

Table 2. EMA positivity in malignant lymphomas in childhood

Histology		Antigens			Site of tested
		L26	MT1	EMA	
Ki-1 lymphoma	Case 1[a]	−	−	+	Lymph node (Neck)
	Case 2[a]	−	−	+	Testis
Large cell lymphoma	9Y, M	−	−	+	Skin
	13Y, M	−	−	−	Lymph node (Neck)
Large cell lymphoma (B cell type)	5Y, M	+	−	−	Small intestine
	6Y, M	+	−	−	Testis
	7Y, M	+	−	−	Lymph node (Neck)
	7Y, F	+	−	−	Lymph node (Neck)
	11Y, F	+	−	−	Lymph node (Neck)
Malignant histiocytosis	8Y, F	−	−	+	Lymph node (Axillary)
	9Y, M	−	−	−	Skin
Hodgkin's disease (mixed type)	14Y, F	−[b]	−[b]	−[c]	Lymph node (Neck)
	15Y, F	−[b]	−[b]	−[c]	Lymph node (Neck)
	15Y, F	−[b]	−[b]	−[c]	Lymph node (Neck)

[a] Described in this study
[b] Non malignant T and B cells were identified
[c] EMA was positive on ill-defined cells

activation-related molecules rather than cell-lineage specific molecules (Stein et al. 1985; Kadin et al. 1986). The advantage of the use of anti-EMA antibody is its utility on paraffin-embedded sections. We identified two more EMA positive childhood cases additionally on paraffin-sections, which were posibly Ki-1 lymphomas by their histology and typical infiltration pattern.

It is obviously necessary to explore more cases for the expression of EMA in Ki-1 positive cases, but the results described in this study suggest that EMA can be used as an another useful marker to distinguish one subtype of Ki-1 positive lymphoma which seems to be heterogenous. Another important aspect is to establish its incidence within the malignant lymphomas in childhood. Finding two fresh Ki-1 positive cases in our two years' immunophenotyping of malignant lymphomas in childhood (30 cases in total) seems to be fairly high incidence. Further accumulation of this special type of tumour is clearly necessary to establish their histogenesis as well as to be aware of their clinical characteristics.

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