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Interdigitating cell sarcoma (ICS)

Evidence of interdigitating cell origin, immunocytochemical studies with monoclonal anti-ICS antibodies*

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Summary. Three independent mouse monoclonal antibodies (mAbs) ID1 (IgG3), ID2 and ID3 (IgM) were raised against whole cells of a surgically resected human interdigitating cell sarcoma (ICS). In immunoperoxidase staining, these mAbs strongly stained the cytoplasm of ICS neoplastic cells as well as interdigitating cells in normal lymphoid tissues. These mAbs also detected monocyte/macrophages and dendritic cells, although their staining was highly variable depending on tissue distribution of the cells. Additional immuno-histological and enzyme histochemical study revealed that the neoplastic cells of ICS had cytoplasmic acid phosphatase and membranous alkaline phosphatase activity, and also possessed S100β protein, Ki-1 antigen, DAKO-macrophage antigen, and weak vimentin activity. Neither rearrangement of immunoglobulin heavy chain gene nor of T-cell receptor genes was detected in the DNA of ICS by Southern hybridization. These observations provide further confirmation of our previous finding (Nakamura et al. 1988, 1989) that the origin of ICS is interdigitating rather than lymphoid cell, and indicate that our mAbs could be useful as a cellular differentiation marker of interdigitating cells and for diagnosis of ICS.

Key words: Interdigitating cell sarcoma – Monoclonal antibodies – Gene rearrangements – Ki-1 antigen

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Introduction

Interdigitating cells are morphologically defined chiefly on the basis of their distinctive ultrastructural properties (Lasser 1983; Van Furth 1982; Tew et al. 1982; Wood et al. 1985). Recently, several investigators have described interdigitating cell sarcoma (ICS), a rare malignant tumour that shares many morphological features with interdigitating cells (Chan and Zaatari 1986; Daum et al. 1985; Feltkamp et al. 1981; Nakamura et al. 1988; Rabkin et al. 1988; Turner et al. 1984). Diagnosis is based on a combination of ultrastructural, enzyme- and immunohistochemical features, in particular detection of S100 protein. These rare cases resemble interdigitating cells more than any other known cell type. However, diagnosis of interdigitating cell sarcoma should be regarded as tentative, because our understanding of cell lineage relationships between monocyte/macrophages and dendritic cells is still evolving (Gaudernack and Bjercke 1985; Buckley et al. 1984, 1987; Franklin et al. 1986; Hancock et al. 1983; Radzun et al. 1984; Van den Oord et al. 1985; Wood et al. 1985), an also because there are a variety of lymphomas with morphological features of malignant histiocytosis, which require differential diagnosis (Isaacson et al. 1985; Kadin et al. 1981; Osborne et al. 1980; Weiss et al. 1985). The main obstacle in elucidating the origin of ICS is lack of adequate cellular markers identifying interdigitating cells and their precursors unequivocally. To obtain such markers, we generated a series of mouse mAbs against a human ICS and found three particularly useful mAbs. By immunohistochemistry with these re-

agents, we analyzed the properties of both normal and malignant interdigitating cells and dendritic cells, and report additional data on ICS.

Materials and methods

The case of ICS has been reported previously (Nakamura et al. 1988, 1989). The tumour was growing slowly and the patient was still alive in May, 1989, more than 6 years after onset. A small tissue specimen was taken from the surgically resected tumour of the jejunum and mechanically disrupted in RPMI1640 medium (GIBCO, Grand Island, New York) to prepare the cell suspension. The cells were stored frozen in liquid nitrogen.

Ten-week-old female BALB/cCr mice (Charles River Japan, Inc., Atsuki) were immunized by 4 i.p. injections with 5×10^6 ICS cells at 3 week intervals. Four days after the last immunization, the spleen was taken out for cell fusion.

Fusion of spleen cells from immunized mice and NS-1 myeloma cells and subsequent selection of cell hybrids in hypoxanthine-aminopterin-thymidine (HAT) medium were performed as described (Köhler and Milstein 1975; Kaneshima et al. 1987). Clones producing antibodies against ICS were isolated by a two-step selection procedure: (a) culture supernatants of hybridoma clones were screened for mouse immunoglobulins (Igs) in an enzyme-linked immuno-solid phase assay (ELISA) and (b) the Ig-positive supernatants were subsequently screened for antibodies to ICS by immunohistochemistry. Positive hybridomas were further cloned by cycles of limiting dilution. To obtain hybridoma ascites, pristane-primed BALB/c mice were implanted i.p. with 5×10^6 hybridoma cells, and the ascites fluid was collected after 2–3 weeks.

Ig isotypes of the hybridoma antibody were determined by Ouchterlony's double diffusion test with rabbit anti-mouse Ig heavy chain-specific antisera (Miles Scientific, Naperville, Illinois).

The following human tissues including malignant lymphomas were taken from biopsy specimens of patients undergoing standard operative procedures: lymph node, tonsil, lung, kidney, colon, appendix, skin, ileum, stomach, and liver. Thymus, brain, and bone marrow were obtained at autopsy within 2 h after death from adults less than 40 years old. Tissue specimens were mounted in Tissue Tek mounting medium (Miles Lab, Naperville, Illinois) and frozen on dry ice. Cryostat sections, 5 μm thick, were air-dried with a fan for 30 min, and fixed in cold acetone for 10 min. Cytocentrifuge smears of peripheral blood were air-dried and fixed in cold acetone for 30 s.

All lymphomas were classified according to morphological, enzyme cytochemical, and immunohistological criteria as described previously (Lukes and Collins 1975; Lennert et al. 1978; Suchi et al. 1987). In all cases, clinical findings were consistent with morphological diagnosis.

The samples were reacted with hybridoma supernatants or diluted ascites fluid (1:300 to 1:5000) for 2 h at room temperature, washed, and reacted with the labelled second reagent. To visualize the bound mAbs, we employed several second reagents: Vectastain ABC Kit, Pk 4002 including biotin-conjugated horse anti-mouse IgG and avidin-biotin-peroxidase complex (Vector Lab., Burlingame, California) (Hsu et al. 1981); and either fluorescein isothiocyanate (FITC) or horseradish peroxidase (HRP) conjugated goat anti-mouse monoclonal IgG and/or IgM antiserum (TAGO, Burlingame, California). The sections were counterstained with methyl green or haematoxylin. For negative control, mAbs absorbed with ICS cells were used as the first antibody.

To survey other cell markers, cryostat and paraffin-embedded sections were stained with the following antisera: mAbs to Macrophages (DAKO-Macrophage), Ki-1 antigen (DAKO-Reed-Sternberg Cell), leukocyte common antigen (LCA), epithelial membrane antigen (EMA), vimentin and Ki-1 like antigen (Ber-H2) (DAKO, Copenhagen, Denmark); rabbit anti S100alpha and beta protein antisera (Haimoto et al. 1987), rabbit anti desmin (Bioscience Products, Emmenbreucke, Switzerland), carcino-embryonic antigen (CEA) and keratin (DAKO, Copenhagen, Denmark). Staining procedures were previously described (Hsu et al. 1981). The antigenic determinants tested and their antibodies are listed in Table 2.

Histochemical demonstration of acid and alkaline phosphatase of ICS were performed as described by Beckstead et al. (1981). DNA Extraction and Southern Blot Analysis. High-molecular-weight DNA extracted from the frozen ICS tumour was digested with restriction endonucleases; EcoRI, BamHI, or XbaI (Boehringer Mannheim Biochemicals, Indianapolis, Indiana). Possible rearrangements in genes of immunoglobulin heavy chain and T-cell receptor beta- and gamma-chain gene were examined by Southern blot hybridization analysis using DNA probes specific to each gene as previously described (Suzuki et al. 1986, 1887).

Results

Three independent hybridoma clones secreting mAb ID1 (IgG3), ID2 (IgM) and ID3 (IgM) were established from the fusion of spleen cells of ICS immunized mice and NS-1 cells. In immunohistochemistry, these mAbs intensely stained the cytoplasm of ICS cells (Fig. 1).

Data obtained by immunoperoxidase staining of cryostat sections of normal human tissue are summarized in Table 1. Tissues containing monocytes/macrophages and dendritic cells were invariably stained but the control sections stained with mAbs absorbed with ICS cells were uniformly negative. In lymph nodes, spleen and Peyer's patches the monoclonal antibodies ID1, ID2 and ID3 reacted with interdigitating cells in the T cell areas of the three sites. However, positively stained cells with ID3 were sparser than with ID1 and ID2 (Figs. 2–4). The follicular dendritic cells in the germinal centers of these organs were stained, but less intensely (Fig. 5). In spleen, only ID2 stained the sinusoidal lining cells uniformly and intensely, and the staining resulted in sharp delineation of splenic sinusoids. Except for splenic sinusoidal lining cells, none of the mAbs reacted with endothelial cells of blood vessels and lymphatics in the organs examined. ID1 weakly labelled mantle zone B cells in the lymphoid organs, whereas ID2 and ID3 did not stain any lymphocytes at the light microscopic level. In the tonsil, with ID1 and ID3, a few scattered positively stained dendritic cells were detected throughout the germinal centers and interfollicular zones (Fig. 6). ID2 reacted with tonsillar epithelium and a very small number of dendritic cells (Fig. 7). In the thymus stromal cells stained

Table 1. Reactivity of ID1, ID2 and ID3 on normal human lymphoid and nonlymphoid tissues

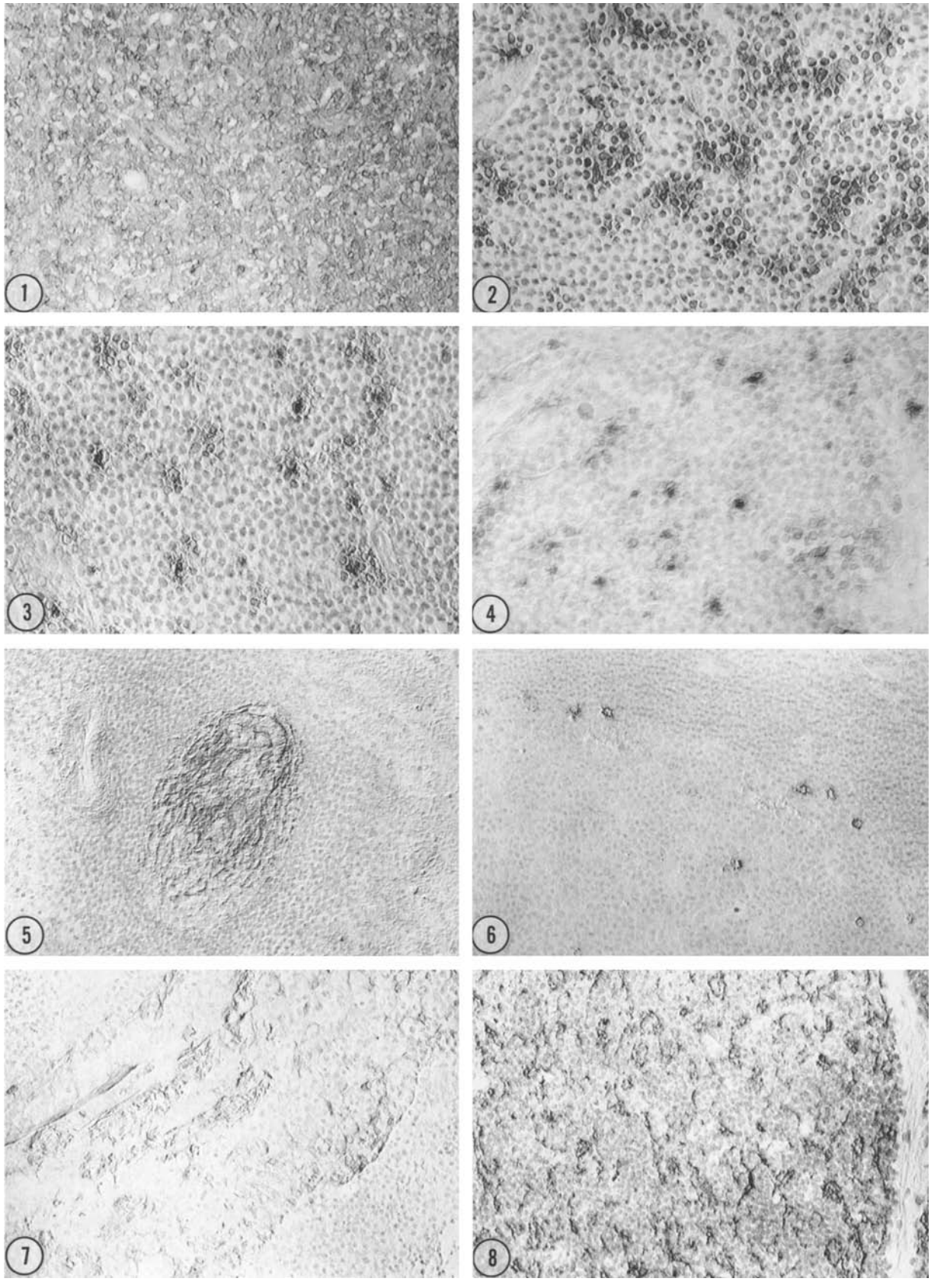
Tissues	ID1	ID2	ID3
Lymph node			
Interdigitating cells	++	++	+*
Follicular dendritic cells	+	+	+*
Sinus histiocytes	+*	+*	+*
Paracotical T cells	−	−	−
Mantle zone B cells	−~±	−	−
Germinal center B cells	na	−	−
Plasma cells	−	−	−
Endothelium	−	−	−
Spleen			
Interdigitating cells	++	++	+*
Follicular dendritic cells	+	+	+*
Red pulp sinus macrophages	+	−	−
Sinusoidal lining cells	−	+	−
Thymus			
Dendritic cells of the cortex	++	+	+*
Dendritic cells of the medulla	++	+	+*
Lymphocytes	−	−	−
Tonsil			
Dendritic cells	++	±*	+
Tonsilar epithelium	−	+	−
Liver			
Kupffer cells	−	−	−
Portal dendritic cells	±*	−	−
Brain			
Perivascular cells*	++	+*	−
Microglia	±*	+	−
Neurons and other glial cells	−	+	−
Skin			
Dendritic cells of the epidermis	++	−	−
Dendritic cells of the dermis	++	−	−
Kidney			
Mesangial cells	±	−	−
Interstitial cells	+	−	−
Small intestine			
Interstitial cells	+	−	−
Bone marrow			
Megakaryocytes	−	−	−
Granular series	−	−	−
Erythroid series	−	−	−
Monocytes	+*	+*	+*
Reticulum cells	+	−	−
Peripheral Blood			
Monocytes	+*	±*	±*

Intensity of immunoperoxidase staining from − to ++; +*, some; ±*, few
na: not assessed (staining reactions in germinal centers being obscured by labelling of follicular dendritic cells.)

* Perivascular cells, Perivascular microglial cells?

with ID1 and ID2 were distributed throughout the cortex and medulla (Fig. 8). Positive staining of ID3 was seen in part of the subcapsular zone, cortico-medullary junction and medulla, but not in the cortex (Fig. 9). None of our mAbs was reactive with Hassall's corpuscles. ID1 labelled the dendritic cells in the epidermis and dermis (Fig. 10), but ID2 and ID3 did not. Epidermal cells were weakly stained with ID2 but not with the others. In the liver a small number of dendritic cells in Glisson's capsule were stained with ID1, whereas Kupffer cells and parenchymal cells were negative with all anti-ICS mAbs. In the kidney and gastrointestinal tract scattered interstitial cells were labelled by ID1, but not by ID2 and ID3. ID1 also reacted with the cells surrounding blood vessels, probably the pericytes (Fig. 11), and some cells in Auerbach's plexus (Fig. 12). ID2 reacted with neurites within Auerbach's and Meissner's plexus (Fig. 13). ID1 stained alveolar macrophages lying free in the air space, whereas ID2 and ID3 did not. With ID1, cells closely associated with the cerebral capillaries were positively stained (Fig. 14). These cells, located beside the capillary endothelium in tangential section, possessed the morphology of perivascular microglial cells. ID2 labelled a number of neurons and glial cells, including the perivascular microglias (Fig. 15). With ID3, no positive staining was observed in the brain. In bone marrow and peripheral blood all 3 mAbs labelled some monocytes in the bone marrow. Similar staining was observed using FITC-labelled second reagent (Figs. 16, 17). In this staining, however, the cells with morphology of bone marrow reticular cells were visualized with ID1, which hardly detected them in immunoperoxidase staining (Fig. 16). In peripheral blood, all 3 mAbs stained a small number of the monocytes, although the number of positive cells was sparser than with ID1.

The staining reactivity of ID1, ID2 and ID3 with human tumours and benign lesions of the lymph node are shown in Table 2. All mAbs stained the cytoplasm of the interdigitating cells, follicular dendritic cells, sinus histiocytes and epithelioid cells in benign lesions of the lymph node in varying degrees, but not tingible body macrophages. ID1 and ID2 also weakly stained epithelioid cells found in the Hodgkin's disease lymph nodes. However, at the higher magnification (Fig. 18), the Hodgkin's cells and Reed-Sternberg cells were negative for ID1 and ID2 despite the strong positivity of the interdigitating cells in the surrounding areas. ID1 stained positively the cell surface of non-Hodgkin's lymphoma cells of B cell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type (Fig. 19), whereas ID2 and ID3 did not. None of our mAbs stained the neoplastic cells of selected human carcinomas and sarcomas (Table 2).

We next studied several other differentiation markers in ICS as summarized in Table 3. The tumour cells expressed cytoplasmic acid phosphatase activity (Fig. 20). Membranous alkaline phosphatase was also detected in 30–50% of the neoplastic cells, and was sensitive to Levamisole (Fig. 21). The neoplastic cells were positive for DAKO-Macrophage, DAKO-Reed-Sternberg Cell and anti-S100beta protein. Some of the neoplastic cells were positive with Ber-H2. Its staining pattern was membrane-associated or in the perinuclear area, probably the Golgi area (Fig. 22). Vimentin was weakly positive in the tumour cells. CEA, EMA, LCA, S100alpha protein, desmin and keratin were not demonstrated.

In order to exclude the possibility of T- or B-cell origin for ICS, tumour DNA was surveyed for rearrangements in genes of immunoglobulin heavy chain, T-cell receptor beta and gamma by Southern blot analysis using DNA probes specific to each gene. These genes in the ICS tumour were found in germline configuration (Fig. 23). Therefore, T- or B-cell origin of ICS is not likely.

Discussion

Our present study describes three independent mouse mAbs (ID1, ID2 and ID3) raised against an ICS. In immunohistochemistry, they stained the cytoplasm of the ICS intensely as well as normal interdigitating cells.

Dendritic cells, including interdigitating cells, are distributed widely in various organs, in particular lymphoid tissues. With our mAbs, the intensity of staining varied considerably among dendritic cells populations of different anatomical locations. Similar observations have been reported by others (Buckley et al. 1987; Franklin et al. 1986; Hancock et al. 1983; Wood et al. 1985). This indicates that the dendritic cells in different tissue compartments have distinct profiles of antibody binding, and probably represent subpopulations.

The spectrum of staining with our mAbs casts new light on cell lineage relationships among cells of lymphoid tissues. Follicular dendritic cells share structural features and several immunological markers with the interdigitating cells (Franklin et al. 1986; Tew et al. 1982; Wood et al. 1985). There was weak but definitive staining of the follicular dendritic cells with our mAbs, further supporting a close cell lineage relationship between interdigitating cells and follicular dendritic cells. Some authors assumed that tissue dendritic cells are derived from monocyte-like precursors circulating in the blood (Bhoopat et al. 1988; Parwaresh et al. 1983a, 1983b; Radzun et al. 1984), since their mAbs raised against blood monocytes reacted with dendritic cells. Our mAbs, raised against ICS, labelled a small fraction of blood monocytes and some dendritic cells. However, our staining spectrum does not completely coincide with previous reports. For example, Kupffer cells in the liver are not stained with anti-ICS mAbs. Therefore, both categories of cells have some antigens in common, but it would be premature to conclude a lineage relationship between them. Finally, ID1 and ID2

Figs. 1–19. Immunohistochemical staining reactivity of monoclonal antibodies ID1, ID2 and ID3 on cold acetone-fixed frozen sections (refers to Figs. 1 to 15, all of which were counterstained with methyl green)

Fig. 1. Interdigitating cell sarcoma stained for ID1. The tumour cells are strongly reactive with ID1. × 200

Fig. 2. Lymph node stained for ID1. ID1 strongly stains the interdigitating cells in the paracortical area, and weakly labels the follicular dendritic cells in the germinal center of the follicle. × 400

Fig. 3. Lymph node stained for ID2. ID2 stains the interdigitating cells and weakly the folliuclar dendritic cells. × 400

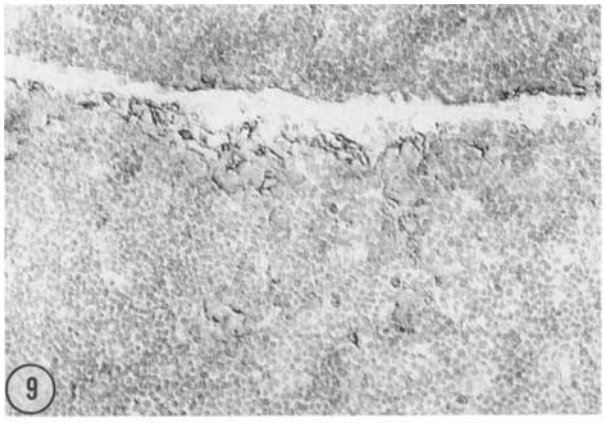
Fig. 4. Lymph node stained for ID3. Some interdigitating cells are reactive with ID3. × 400

Fig. 5. Normal spleen stained for ID3. Note the meshwork of the follicular dendritic cells in the germinal center. × 200

Fig. 6. Tonsil stained for ID3. Stain for ID3 is reactive with scattered dendritic cells. × 400

Fig. 7. Tonsil stained for ID2. Stain for ID2 is reactive with the epithelium, but not dendritic cells. × 200

Fig. 8. Thymus stained for ID1. Stromal cells labelled with ID1 are distributed throughout the cortex and medulla of the thymus. × 200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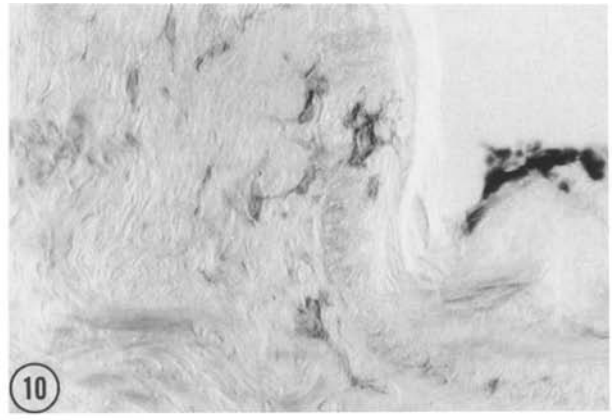
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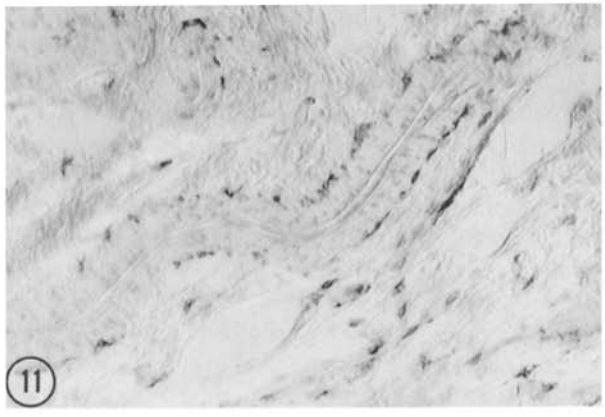
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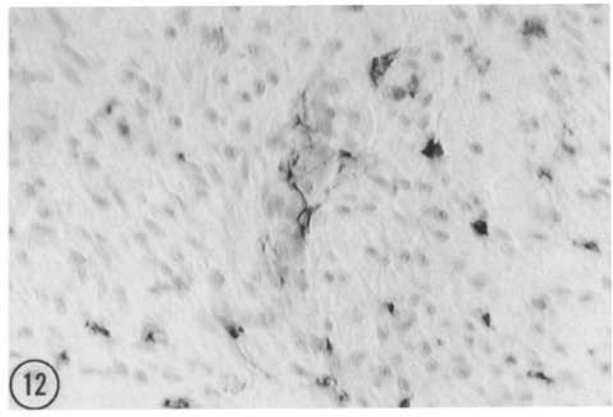
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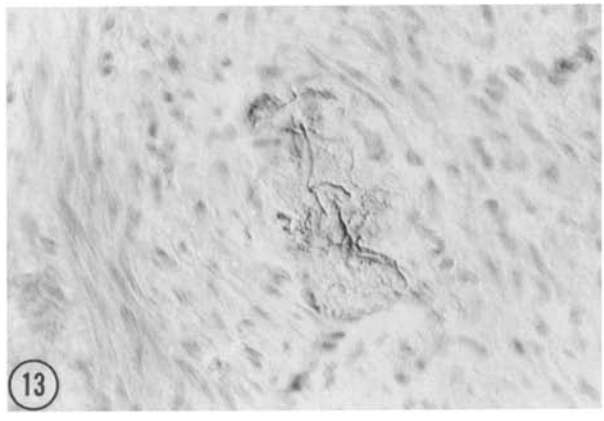
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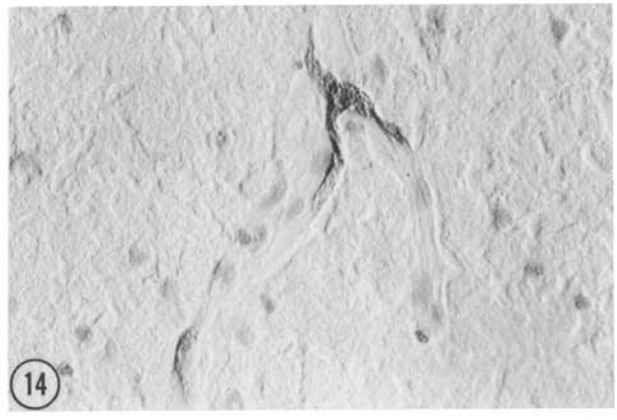
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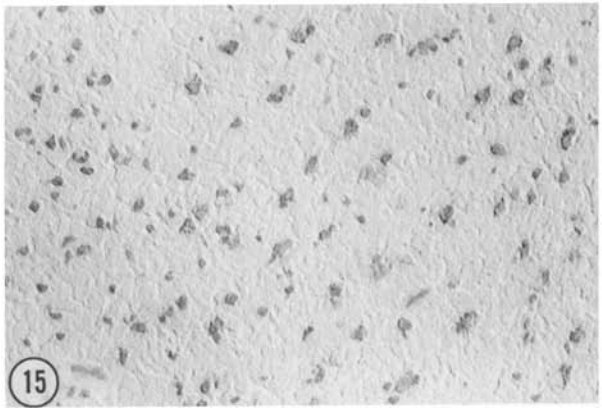
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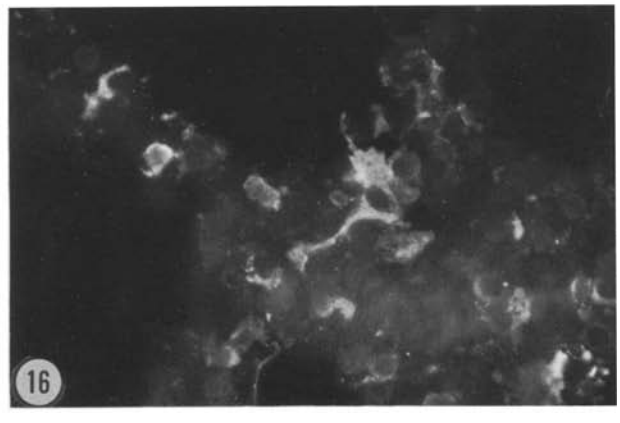
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Table 2. Reactivity of ID1, ID2 and ID3 with benign lymphoid lesions and human tumours

Histological type of lesion	No. of cases	ID1	ID2	ID3
Benign Lymphoid Lesions				
Piringer's lymphadenitis	1			
Dermatopathic lymphadenopathy	1			
Subacute necrotizing lymphadenopathy	1			
Reactive hyperplasia	2			
Interdigitating cells		++	++	+*
Follicular dendritic cells		+	+	+*
Sinus histiocytes		+*	+*	+*
Epithelioid cells		+*	+*	±*
Tingible body macrophages		−	−	−
Lymphomas of B cell type				
Centroblastic-centrocytic lymphoma	2	+′	−	−
Centroblastic lymphoma	2	+′	−	−
Lymphomas of T cell type				
Pleomorphic (medium, and large cell) lymphoma	2	−	−	−
Angioimmunoblastic (AILD) lymphoma	2	−	−	−
T zone lymphoma	1	−	−	−
Lymphoepithelioid (Lennert's) lymphoma	2	−	−	−
Epithelioid cells		+*	+*	±*
Lymphoblastic lymphoma (thymic)	1	−	−	−
Hodgkin's disease				
Nodular sclerosis	2			
Mixed cellularity	2			
Hodgkin's cells		−	−	−
Epithelioid cells		+*	+*	±*
Adenocarcinoma of stomach	3	−	−	−
Adenocarcinoma of colon	3	−	−	−
Ductal carcinoma of breast	2	−	−	−
Papillary carcinoma of thyroid	1	−	−	−
Leiomyosarcoma of stomach	2	−	−	−

Intensity of immunoperoxidase staining from − to ++
+*, some. ±*, few. +′, reactive with the neoplastic cells on the cell surface

Table 3. Properties of tumour cells from the current case in an additional study

Enzyme histochemistry	
Acid phosphatase (cytoplasmic)	+
Alkaline phosphatase (membrane)	+*
Surface and cytoplasmic markers (antibodies used)	
S100alpha protein	−
S100beta protein	+
Vimentin	(+)
Desmin	−
(DAKO-Macrophage)	+
Ki-1 antigen (DAKO-Reed-Sternberg Cell)	+
Ki-1-like antigen (Ber-H2)	+′

+*, 30–50% of the neoplastic cells, Levamisole sensitive
(+), weakly positive. +′, some positive

stained the cells morphologically resembling perivascular microglial cells in brain. Possible bone marrow origin of perivascular microglial cells has been suggested in mice (Hickey and Kimura 1988).

Immunohistochemical study of human tumours revealed that ID2 and ID3 did not stain the cells of Hodgkin's disease and non-Hodgkin's lymphomas except for epithelioid cells. However, only ID1 showed the positivity with B lymphomas on the cell surface. This finding was intriguing, and indicated that ID1 had a somewhat wider spectrum of staining than ID2 and ID3 in malignant lymphomas. Recently, Bhoopat et al. (1988) also reported the cross-reactivity of the interdigitating cells with B lymphocytes and certain B lymphomas by the use of their mAb. The number of cases as well as spectrum of diseases examined in the present study is still limited. Further studies on histiocyte proliferative lesions such as histiocytosis X and malignant histiocytosis will provide useful information on specificity of our mAbs.

The antigens recognized by the mAbs have not been biochemically characterized in the present

Fig. 9. Thymus stained for ID3. Part of the subcapsular zone is stained. ×200

Fig. 10. Skin stained for ID1. Note the dendritic cells in the epidermis and dermis. ×400

Figs. 11, 12. Small intestine stained for ID1. ID1 stains the interstitial cells, pericytes (**11**. ×200) and some cells in Auerbach's plexus (**12**. ×400)

Fig. 13. Small intestine stained for ID2. Note the neurites within Auerbach's plexus. ×400

Fig. 14. Brain stained for ID1. ID1-stained cells are present around a blood vessel. ×400

Fig. 15. Brain stained for ID2. A number of neurons and glial cells are reactive with ID2. ×200

Fig. 16. Bone marrow stained for ID1. FITC stain reveals reactivity of reticular cells with ID1. ×400

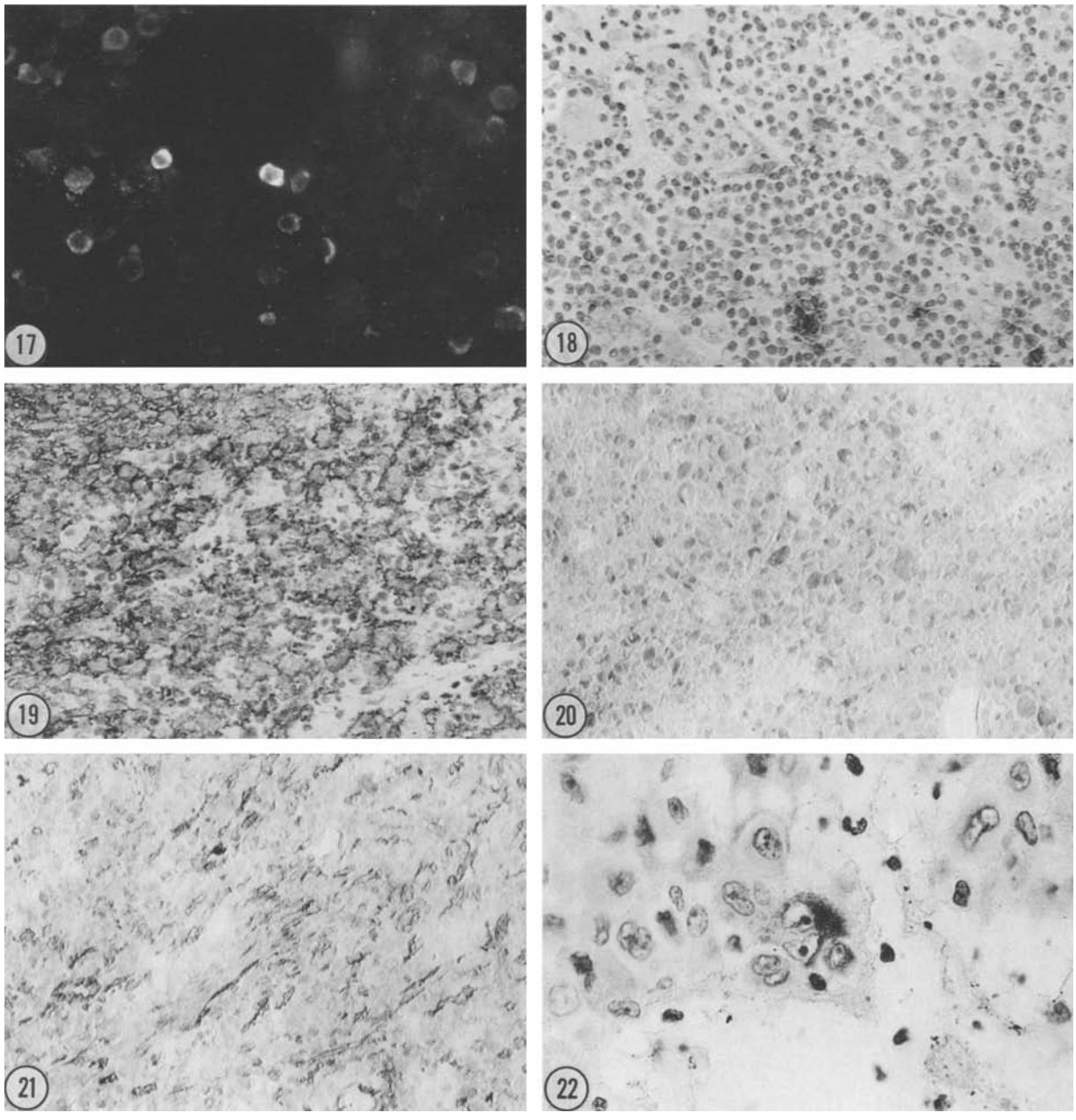

Fig. 17. Bone marrow stained of ID2. FITC stain shows positivity for round-shaped cells, but not reticular cells. × 400

Fig. 18. Hodgkin's disease stained for ID1. Note that the Hodgkin's disease cells at the right upper are unstained. Counterstained with haematoxylin. × 400

Fig. 19. Centroblastic-centrocytic lymphoma stained for ID1. ID1 stains the neoplastic cells on the cell surface. Counterstained with haematoxylin. × 400

Fig. 20. Interdigitating cell sarcoma. Acid phosphatase demonstrates diffuse cytoplasmic staining of many tumour cells. × 200

Fig. 21. Interdigitating cell sarcoma. Alkaline phosphatase stain shows membrane reactivity of some tumour cells. × 200

Fig. 22. Interdigitating cell sarcoma staining with Ber-H2 antibody. The staining pattern is clearly membrane-associated, or positive in the paranuclear area. Counterstained with haematoxylin. × 400

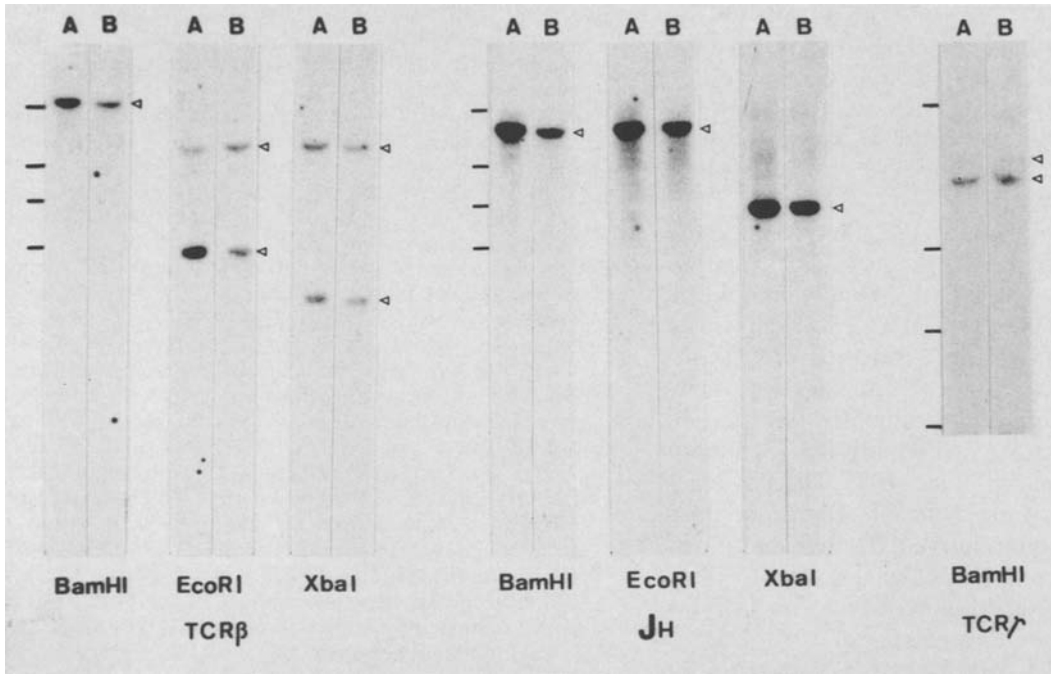

Fig. 23. Aliquots of DNA extracted from the ICS (**A**) and normal human fetal liver as control (**B**) were first digested with either BamHI, EcoRI or XbaI. They were then hybridized according to Southern to DNA probes that detect rearrangements of the immunoglobulin heavy chain (JH), T-cell receptor beta (TCRb) and gamma (TCRr) genes. Dashes on the right indicate positions of the size markers (23.1 kb, 9.4 kb, 6.6 kb, 4.4 kb; kb, kilobase). *Open arrowheads* indicate germ line

study. Nevertheless, the immunoreactivities of the mAbs are probably not due to HLA determinants or to nonspecific binding to Fc receptors. This was indicated by the lack of mAbs binding to Kupffer's cells of liver. These normal macrophage populations are known to express both HLA determinants and Fc receptors (Turner et al. 1987).

We showed that the neoplastic cells of the present case express cytoplasmic acid phosphatase, S100beta protein, vimentin, and antigens recognized by antibodies against macrophages. These data, together with those reported previously (Beckstead 1983; Haimoto et al. 1987; Nakamura et al. 1988, 1989), further support the hypothesis that the origin of ICS is from interdigitating cells. To our knowledge, this is the first report of detection of Ki-1 antigen and Levamisole sensitive alkaline phosphatase in an ICS tumour. This finding, however, further complicates the cytogenesis of ICS. Ki-1 (DAKO-Reed-Sternberg Cell) and Ki-1 like (Ber-H2) antigens is usually found on Reed-Sternberg cells, and also in a minority of non-Hodgkin's lymphomas (Agnarsson and Kadin 1988; Chittal et al. 1988; Herbst et al. 1989; Kadin et al. 1986; Stein et al. 1985). We showed that ICS cells have the same or cross-reacting antigens. Recently, Kadin et al. (1988) and Herbst et al. (1989) reported the detection of T4 and Ki-1 antigens on Reed-Sternberg cells in Hodgkin's disease, and some authors described the expression of Ki-1 antigens in monocyte/macrophage-related cells (Hsu and Hsu 1989; Andreesen et al. 1989). It is noteworthy that both T-cell antigen (T4) and Ki-1 antigens were identified in the present case of ICS. One may argue for association of ICS and Hodgkin's disease. Actually, several investigators have proposed that the cellular origin of Hodgkin's disease is from monocytes or dendritic cells (Kadin 1982; Kaplan and Gartner 1977; Fisher et al. 1983, 1984). However, Hodgkin's disease cells were ID1-, ID2- and ID3-negative in a limited number of the cases examined in the present study, which indicates that this disorder may be antigenically distinct from ICS. Further studies with more cases are needed, as Hodgkin's disease may not represent a disease but a heterogeneous group (Hsu and Hsu 1989; Andreesen et al. 1989; Herbst et al. 1989; Kadin et al. 1988).

Alkaline phosphatase is normally observed in mantle zone B-cells and fibroblastic reticulum cells (FRC) in normal lymph nodes (Beckstead 1983). In the present study, some ICS tumour cells had Levamisole sensitive membranous alkaline phosphatase activity, but lacked immunohistological features of B cell lymphoma. Turner et al. (1984) suggested neoplastic growth of FRC, based on

their enzyme histochemical studies on histiocytic malignancies. Those of our ICS tumour cells which were spindle-shaped have, like FRC, alkaline phosphatase activity. However, the relationship between fibroblastic and dendritic reticulum cells remains obscure (Heuserman et al. 1980; Müller-Hermelink et al. 1981).

Stein and his colleagues (1985) have suggested that many of the neoplasms previously categorized as malignant histiocytosis are actually anaplastic large-cell lymphomas of B- and T-cell origin whose common denominator is the expression of Ki-1 antigen. Furthermore, clonal TCR and IgH gene rearrangements are not infrequently detected in neoplasms morphologically resembling malignant histiocytosis (Isaacson et al. 1985; Weiss et al. 1985). Most likely these tumours represent non-Hodgkin's lymphomas derived from clonal T- or B-cells but not from histiocytes, although they have lost readily detectable markers of cell differentiation, making determination of their lineage or subsets difficult. In our case of ICS, the tumour cells expressed Ki-1 antigen, whereas they lacked markers indicating T- or B-cells and retained the IgH, TCR beta and TCR gamma genes in the germline configuration. Therefore, this tumour may represent a rare example of a neoplasm truly derived from one of the interdigitating cells.

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