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Immunohistochemical characterization of Ki-M6 monoclonal antibody in Bouin-fixed, paraffin-embedded sections of normal and neoplastic human tissues

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Summary. A monoclonal antibody (Ki-M6) against the CD 68 antigen, which labels cells of the monocyte/macrophage system, was tested on Bouin-fixed, paraffin-embedded samples of normal, reactive and neoplastic tissues by an avidin-biotin-peroxidase complex method, with the aim of establishing its value in diagnostic pathology. In normal human tissues, Ki-M6 reactivity was confined to the so-called resident macrophages populating normal organs under physiological conditions. Moreover, restricted reactivity against cells of macrophage lineage was observed in reactive and inflammatory lesions. Granulocytes, monocyte/macrophage-related immune accessory cells, and other analysed normal tissue structures did not reveal any reactivity. Ki-M6 was strongly reactive with the cases of benign (4/4) and malignant (15/15) fibrous histiocytomas, in addition to the true histiocytic lymphomas (3/3). Cases of granular cell tumour (2/3) showed strong reactivity with Ki-M6, whereas only few immunoreactive cells, with weak staining, were seen in the other Ki-M6-positive neoplasms [neurofibroma (3/3), benign schwannoma (1/2), ganglioneuroma (1/1), malignant schwannoma (5/9), melanoma (9/28), dermatofibrosarcoma protuberans (1/1), myelomonocytic leukaemia (3/3)]. Among the epithelial malignancies tested (47 cases), Ki-M6 was positive only in renal cell carcinoma (11/14). Malignant lymphomas of the Hodgkin (56 cases) and non-Hodgkin type (67 cases) were uniformly non-reactive. From these data, Ki-M6 appears to be an excellent marker of monocyte/macrophage-related cells and appears to be a reliable indicator for fibrous histiocytomas and true histiocytic malignancies. The availability of this additional antibody capable of staining routinely processed tissue is of practical interest.

Key words: CD 68 – Monoclonal antibody – Immunohistochemistry – Monocyte/macrophage-related cells – Fibrous histiocytoma

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Introduction

The immunohistological identification of monocyte/macrophage-related cells and their derived proliferative disorders has been greatly aided by the availability of a group of monoclonal antibodies which all stain most tissue macrophages selectively (antibodies 24, Y1/82A, Ki-M6, Ki-M7, Y2/131, EBM/11, KP1) (Davey et al. 1988; Hogg and Selvendran 1985; Kelly et al. 1988; Kreipe et al. 1987; Micklem et al. 1989; Parwaresch et al. 1986; Pulford et al. 1989). However, most reagents suffer from the practical limitation of not reacting with routinely processed paraffin-embedded tissue. It has been reported that KP1 (Pulford et al. 1989) monoclonal antibody differs from these other reagents in its ability to stain routinely fixed, paraffin-embedded tissue (Warnke et al. 1989); it recognizes a formalin or B5 fixation-resistant epitope in a variety of tissue macrophages and in granulocyte precursors. Other monoclonal antibodies to human macrophage antigens, such as Ki-M7 (Kreipe et al. 1987), MAC 387 (Flavell et al. 1987), LN4 and LN5 (Bhoopat et al. 1988), react in formalin or B5-fixed, paraffin-embedded tissue sections, but their reactivity is inconsistent (Kreipe et al. 1987) or suffers from a relative lack of specificity (Bhoopat et al. 1988; Flavell et al. 1987).

The monoclonal antibody Ki-M6 showed a restricted reactivity to cells of the monocyte/macrophage system when tested on normal human blood cells and on a wide range of frozen normal human tissues (Parwaresch et al. 1986). This antibody recognizes a glycoprotein which is identical to the molecule detected by KP1 and other described monoclonal antibodies (Micklem et al. 1989). This molecule, which was designated as the CD 68 antigen at the Fourth International Conference on Human Leucocyte Differentiation Antigens (Vienna 1989), is probably the best macrophage marker presently available for immunohistology (Knapp et al. 1989).

In a previous work we determined that the antigen recognized by the Ki-M6 antibody was resistant to routine fixative solutions and to conventional paraffin em-

Table 1. Reactivity of Ki-M6 with selected human neoplasms in Bouin-fixed, paraffin-embedded tissue sections

Diagnosis	Number of tested cases	Ki-M6 cell reactivity[a] (number of reactive cases) +	++	+++[b]
Benign neoplasms	**35**			
Fibrous histiocytoma (dermatofibroma)	4	1 (W)	3 (S)	–
Granular cell tumour	3	–	–	2 (S)
Dermal neurofibroma	3	3 (W)	–	–
Schwannoma	2	1 (W)	–	–
Adrenal ganglioneuroma	1	1 (W)	–	–
Leiomyoma	3	–	–	–
Angiolipoma	1	–	–	–
Glomangioma	1	–	–	–
Breast fibroadenoma	3	–	–	–
Parotid pleomorphic adenoma	2	–	–	–
Large bowel villous adenoma	3	–	–	–
Thyroid follicular adenoma	3	–	–	–
Ovary cystadenofibroma	1	–	–	–
Ovary teratoma	2	–	–	–
Testis adenomatoid tumour	1	–	–	–
Cellular blue naevus	1	–	–	–
Dysplastic naevus	1	–	–	–
Malignant neoplasms	**260**			
Malignant fibrous histiocytoma	15	–	9 (S)	6 (S)
Dermatofibrosarcoma protuberans	1	1 (W)	–	–
Leiomyosarcoma	4	–	–	–
Rhabdomyosarcoma	4	–	–	–
Liposarcoma	4	–	–	–
Epithelioid sarcoma	4	–	–	–
Kaposi's sarcoma	4	–	–	–
Chondrosarcoma	2	–	–	–
Malignant schwannoma	9	5 (W)	–	–
Synovial sarcoma	2	–	–	–
Extraskeletal Ewing's sarcoma	3	–	–	–
Melanoma	28	9 (W)	–	–
Seminoma	2	–	–	–
Mesothelioma	2	–	–	–
Breast carcinoma	13	–	–	–
Renal cell carcinoma	14	–	11 (W)	–
Thyroid carcinoma	2	–	–	–
Basal cell carcinoma	3	–	–	–
Squamous cell carcinoma	2	–	–	–
Undifferentiated carcinoma	10	–	–	–
Carcinoid	3	–	–	–
Non-Hodgkin's lymphoma (all Working Formulation categories)	67	–	–	–
Hodgkin's disease (Reed-Sternberg cells)	56	–	–	–
Acute myelomonocytic leukaemia	3	3 (W)	–	–
True histiocytic lymphomas	3	–	3 (S)	–

[a] Staining in tumour cells was present in the cytoplasm
[b] +, Present (few cells); ++, present (several cells); +++, present (most cells); W, weak; S, strong; –, negative

bedding without major technical improvement of the conventional procedure (Gloghini et al. 1990a). Here we describe an evaluation of the reactivity of Ki-M6 antibody on a wide range of routinely processed, Bouin-fixed, paraffin-embedded specimens, including normal tissues, reactive and inflammatory conditions; and in neoplastic disorders. Our aim was to establish its value in diagnostic surgical pathology.

Materials and methods

Fresh human tissues received by the Division of Pathology at Centro di Riferimento Oncologico, Aviano are routinely fixed in Bouin's solution; fixation times usually range from 3 to 12 h. For this study we retrieved from the surgical pathology files paraffin-embedded specimens obtained from normal organs and tissues, reactive and inflammatory lesions and selected benign and malignant neoplasms. Both benign and malignant neoplasms, mostly of non-epithelial origin, were defined according to their conventional histopathological and immunohistological characterization. A total of 295 neoplasms were studied. A complete list of these neoplasms is reported in Table 1.

Reactivity of Ki-M6 antibody was tested on the following normal human tissues and reactive and inflammatory lesions: breast, bronchus, lung, kidney, endometrium, adrenal gland, thyroid, parathyroid, parotid, stomach, gut, skin, prostate, fat, connective tissue, smooth muscle, skeletal muscle, cartilage, bone, vessels, peripheral nerve, villonodular synovitis, gastric ulcer, thyroid cyst, Hashimoto's thyroiditis, chronic prostatitis, gastric polypoid xanthelasma, epidermal cyst, fibrohistiocytic pseudotumour and xanthogranulomatous processes.

Immunostaining was performed on deparaffinized sections by using the avidin-biotin-peroxidase complex method (Hsu et al. 1981) as previously described (Carbone et al. 1986; Gloghini et al. 1990a). Ki-M6 monoclonal antibody (Behringwerke, Marburg, FRG) was applied at the dilution of 1:4000 and incubated overnight at 4° C or for 1 h at room temperature. In cases with low signal we could enhance the intensity of the labelling reaction by applying the monoclonal antibody at a lesser dilution (1:3000); alternatively, the alkaline phosphatase anti-alkaline phosphatase method (Cordell et al. 1984) with the repetitions of the second and third steps was employed as previously described (Gloghini et al. 1990b).

Negative control experiments were performed by omitting the primary antibody and substituting with phosphate-buffered saline or, in selected cases (i.e. the Ki-M6-positive neoplasms), by incubating the sections with mouse myeloma proteins IgG_1, kappa (MOPC21) (Sigma, St. Louis, MO., USA) or control mouse ascites fluid (NS-1) (Sigma) at the same dilution of the primary antibody. None of the negative control sections was immunostained.

Results

Initial studies with Ki-M6 monoclonal antibody indicated that tissues fixed with formalin or B5 gave some reduction in antigen reactivity when compared with those fixed with Bouin's solution (Gloghini et al. 1990a); thus, only Bouin-fixed specimens were used in this study. In normal human tissues, Ki-M6 reactivity was confined to the so-called resident macrophages populating normal organs under physiological conditions. The antibody reacted with tissue macrophages in a wide range of tissues, including lung macrophages (Fig. 1A), dermal macrophages (Fig. 1B) and lamina propria macrophages (Fig. 1C). Cross-reactions with other analysed tissue structures, such as those of epithelial, endothelial, neural and mesenchymal origin, could not be observed.

A variety of reactive lesions were studied and broad reactivity against cells of macrophage lineage was ob-

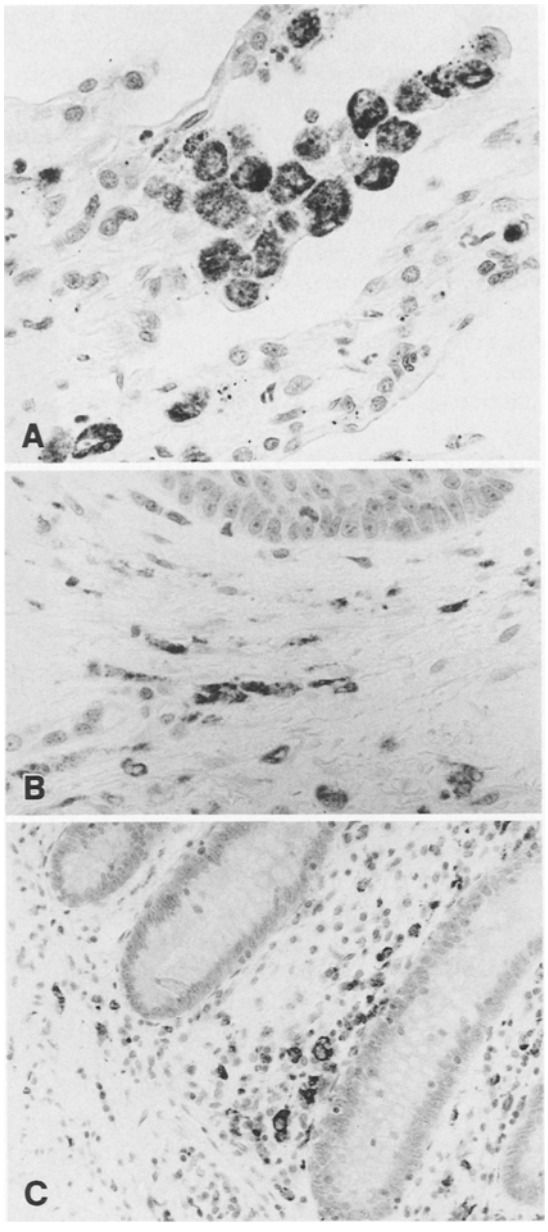

Fig. 1 A–C. Immunohistochemical reactivity of Ki-M6 in normal human tissues. Strong labelling of alveolar (**A**), dermal (**B**) and lamina propria (**C**) macrophages is seen in biopsy specimens of lung (**A**), skin (**B**) and gut (**C**). Immunostained cells display a granular reactivity confined to the cytoplasm. Bouin-fixed, paraffin-embedded sections; ABC; haematoxylin counterstain. **A** ×400; **B** ×300; **C** ×250

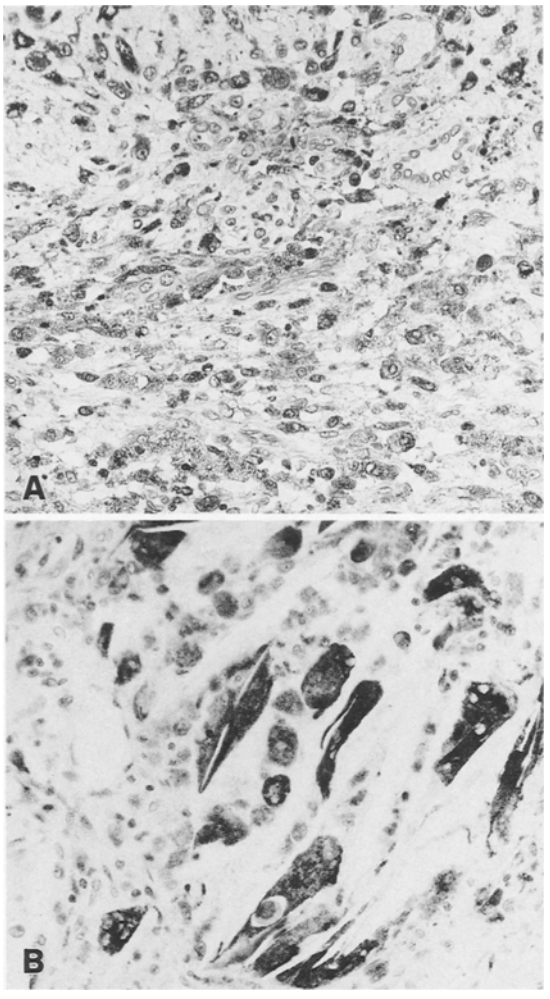

Fig. 2A, B. Immunohistochemical reactivity of Ki-M6 in reactive lesions. Granular cytoplasmic staining is seen in macrophages and foamy cells (**A**), and in foreign body giant cells (**B**). Bouin-fixed, paraffin-embedded sections; ABC; haematoxylin counterstain. **A** ×250; **B** ×320

served (Fig. 2A), epithelioid granulomas and multinucleated cells (particularly foreign-body giant cells) being strongly labelled (Fig. 2B).

Data on the reactivity of Ki-M6 antibody with a variety of benign neoplasms are presented in Table 1 along with the number of cases studied for each tumour type. Ki-M6 was negative in the majority of cases but did show positivity with variable intensity in Bouin-fixed specimens of fibrous histiocytoma (dermatofibroma) (4/4), granular cell tumour (2/3), dermal neurofibroma (3/3), benign schwannoma (1/2) and adrenal ganglioneuroma (1/1). Also the number of stained cells was variable among the positive cases. The pattern of staining in these cases was of the granular type. A strong and extensive granular cytoplasmic staining was seen in most cells of the granular cell tumours (Fig. 3A) and in several fibrous histiocytoma cells (Fig. 3B), whereas faint and/or focal granular staining was seen within a few elongated cells of the dermal neurofibromas, a benign schwannoma and the adrenal ganglioneuroma (see Table 1). Unequivocal differentiation between tumour cells and dendritic interstitial cells, which were abundant in neural tumours, was difficult. Thus it was questionable whether the faint and/or granular focal staining seen within the few elongated cells of the latter tumours really corresponded to Ki-M6-positive tumour cells.

Table 1 shows the data concerning the malignant neoplasms selected for study, together with the number of cases studied for each tumour type. Ki-M6 was strongly positive in only two types of tumour; malignant fibrous histiocytoma and true histiocytic lymphoma. All the cases tested were positively immunostained, though the number of stained cells was variable from case to case

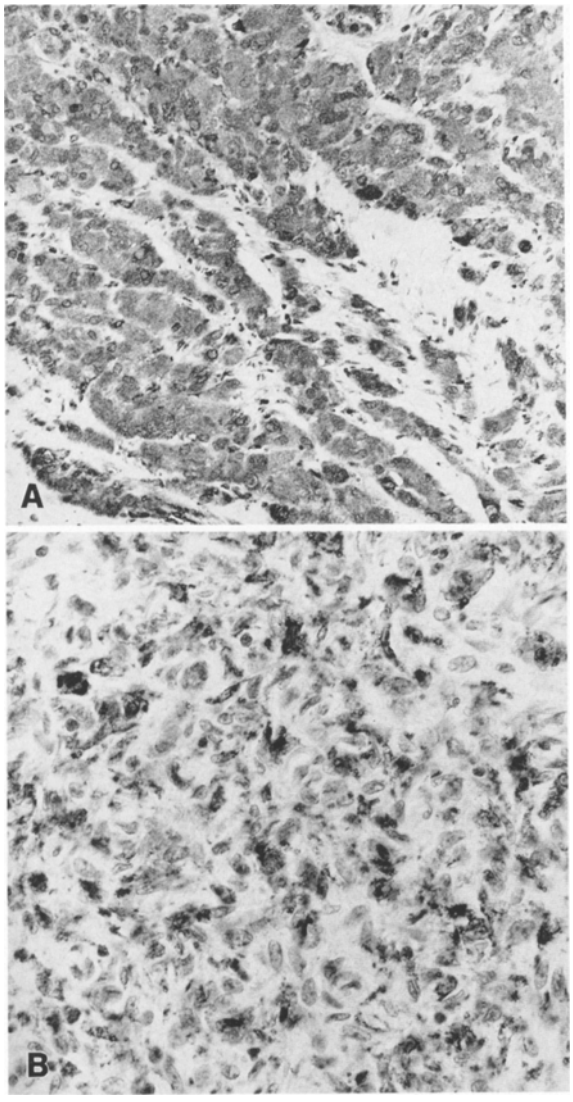

Fig. 3. A Most cells of this granular cell tumour show strong granular staining for Ki-M6 in their large cytoplasm. **B** In this benign fibrous histiocytoma a fraction of the cellularity reacts with Ki-M6. Bouin-fixed, paraffin-embedded sections; ABC; haematoxylin counterstain. **A** ×250; **B** ×400

(Fig. 4A, B). In the single case tested of dermatofibrosarcoma protuberans, few cells were weakly stained.

A wide variety of soft tissue tumours lacked immunoreactivity for Ki-M6; the exceptions to this finding were five of nine malignant schwannomas, where few cells showed a weak granular cytoplasmic positivity.

Mesothelioma, seminoma and malignant melanoma were generally non-reactive; however, in 9 of 28 melanomas, focal, weak, perinuclear granular staining was observed in occasional cells. It is noteworthy that both seminomas were heavily infiltrated with histiocytes; it was thus difficult to distinguish neoplastic from other cells in the tissues.

Among a selection of epithelial malignancies (47 cases), Ki-M6 was positive only in the cases (11/14) of renal cell carcinoma, where faint granular cytoplasmic staining was observed (Fig. 4C).

A total of 67 non-Hodgkin's lymphomas of a wide variety of cell types, according to the Working Formulation (1982), were evaluated. All cases were interpreted as Ki-M6 negative. In some small cell proliferations possible positive staining taking the form of localized dots could be seen in several neoplastic cells. However, this staining was weak and difficult to interpret in terms of its precise, intra- or extracellular, location. Reed-Sternberg cells and variants in 56 cases of Hodgkin's disease (lymphocyte predominance, 7 cases; nodular sclerosis, 38 cases; mixed cellularity, 10 cases; lymphocyte depletion, 1 case) were non-reactive for Ki-M6 (see Table 1). In most cases of nodular sclerosing Hodgkin's disease, however, numerous cells displaying "histiocyte" features were positively immunostained. The 3 cases tested of acute myelomonocytic leukaemia showed few cells labelled by Ki-M6 antibody. The cells showed a weak granular cytoplasmic staining. The biopsy specimens were obtained from the skin.

Discussion

Antibodies Y2/131, EBM/11, Ki-M6, Ki-M7, Y1/82A and KP1, which have recently been designated as CD 68 (Micklem et al. 1989), react with a macrophage-associated antigen for which the gene has been cloned; these antibodies, however, appear to recognize different epitopes (Micklem et al. 1989). With the exception of KP1 monoclonal antibody, which is able to stain routinely fixed, paraffin-embedded tissue (Pulford et al. 1989; Warnke et al. 1989), the reactivity of the other antibodies has been established by testing on frozen tissue sections (Davey et al. 1988; Kelly et al. 1988; Kreipe et al. 1987; Parwaresch et al. 1986).

In this study the Ki-M6 monoclonal antibody fulfils the criteria for a pan-macrophage reagent and has been tested successfully on a wide range of routinely processed, paraffin-embedded tissues. According to previous data from our group (Gloghini et al. 1990a), good immunoreactivity for this antibody was achieved by the use of Bouin's solution as fixative. We are aware that Bouin's solution is not a universally employed fixative; however, the availability of this antibody, which is capable of detecting macrophages and other members of the mononuclear phagocyte lineage in paraffin-embedded tissue, may be of practical interest to surgical pathologists. Many use this picric-acid-containing fixative due to its ability to preserve morphological detail, particularly those of the nucleus in haematoxylin and eosin stained sections.

According to previous findings established by testing on frozen tissues (Parwaresch et al. 1986), the Ki-M6 monoclonal antibody showed a highly restricted reactivity to the various tissue-specific phagocytes (resident macrophages) when tested on paraffin-embedded tissues. Moreover, restricted reactivity against cells of macrophage lineage was observed in reactive and inflammatory lesions. Granulocytes, monocyte/macrophage-related immune accessory cells, and other normal tissue structures of epithelial and mesenchymal origin did not reveal any reactivity.

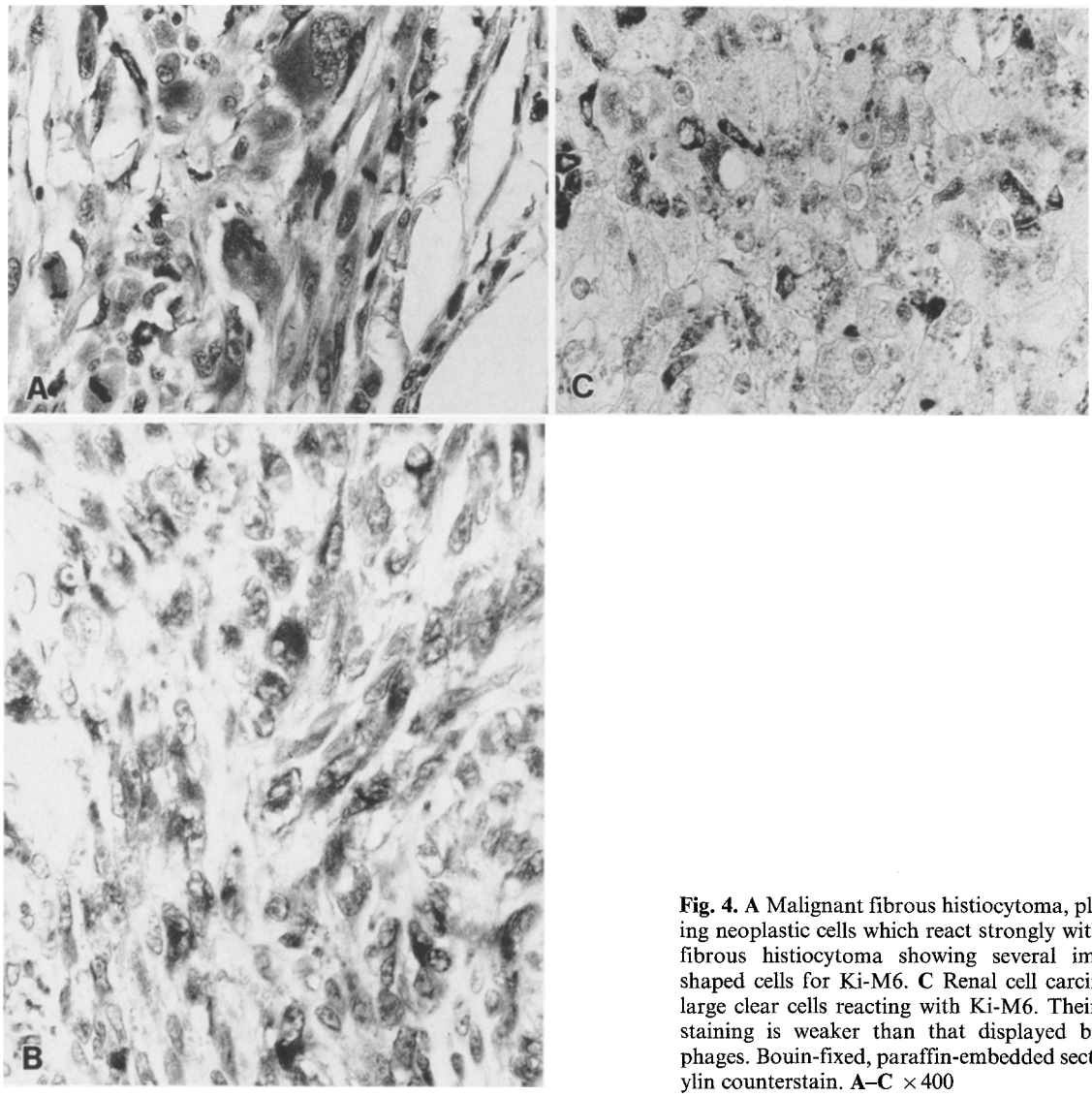

Fig. 4. **A** Malignant fibrous histiocytoma, pleomorphic type, showing neoplastic cells which react strongly with Ki-M6. **B** Malignant fibrous histiocytoma showing several immunostained spindle-shaped cells for Ki-M6. **C** Renal cell carcinoma with a group of large clear cells reacting with Ki-M6. Their granular cytoplasmic staining is weaker than that displayed by interspersed macrophages. Bouin-fixed, paraffin-embedded sections; ABC; haematoxylin counterstain. **A–C** ×400

If the reactivity of Ki-M6 antibody is compared with that of KP1, the other CD 68 antibody capable of detecting macrophages in paraffin-embedded tissue, some minor differences are noted. First, KP1 antibody shows reactivity also with granulocytes and their precursors. Second, KP1 antibody reacts with interdigitating reticulum cells in cases of dermatopathic lymphadenitis. Third, B-lineage lymphomas and leukaemias are stained by KP1 antibody, although neoplastic cells exhibit small dots of reactivity in contrast to the strong cytoplasmic staining seen in the neoplasms of myelomonocytic origin (Warnke et al. 1989). Moreover, even if conclusive data on the KP1 reactivity with non-haematopoietic neoplasms have not been published, at least not to our knowledge, Warnke et al. (1989) reported KP1 reactivity with some epithelial neoplasms in Cytospin preparations from fine needle aspirates.

In the present study, non-Hodgkin's lymphomas were generally non-reactive for Ki-M6 antibody; only a weak granular staining was observed in a few cells of the cases of acute myelomonocytic leukaemia. In contrast, this antibody was strongly positive in true histiocytic lymphomas. This finding is of practical importance in diagnostic pathology. In fact, the diagnosis of true histiocytic lymphoma or malignant histiocytosis (Carbone et al. 1981) represents one of the most difficult and controversial areas of lymph-node pathology. The Ki-M6 positivity encountered in the neoplastic cell population of benign or malignant fibrous histiocytomas was expected; hypotheses concerning a primary histiocytic derivation (Ozello et al. 1963) or a dual fibroblastic-histiocytic origin (Magnusson et al. 1983) for these neoplasms have been proposed. Recently, the existence of two different cell types, both fibroblast-like and histiocyte-like, was shown by light and electron microscopy, DNA measurement, and karyotype analysis in malignant fibrous histiocytoma (Genberg et al. 1989).

Interestingly, the other soft tissue tumours analysed in the present study lacked immunoreactivity for Ki-M6, with the exception of those of proven or presumed neuroectodermal cell origin (Harkin and Reed 1969). These Ki-M6-positive neoplasms also included granular cell tu-

mour, a possible heterogeneous entity that has been classified as being of Schwann cell (Armin et al. 1983; Nakazato et al. 1982) or neuronal cell (Rode et al. 1982) origin, based on immunohistochemical findings. Most of these tumours, however, displayed a weak and questionable reactivity confined to a few neoplastic cells.

Among a selection of epithelial neoplasms Ki-M6 reactivity was shown only by renal cell carcinoma. It has been reported that this type of carcinoma also shows reactivity with LN5, a monoclonal antibody which retains its immunoreactivity with human macrophages in B5-fixed, paraffin-embedded tissue (Bhoopat et al. 1988).

In conclusion, this study suggests that Ki-M6 antibody, as a specific reagent for identifying macrophages, may be used effectively to investigate the role of these cells in inflammation and malignancies. Moreover, this antibody seems to be a good indicator for malignant fibrous histiocytomas.

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References

Armin A, Connelly EM, Rowden G (1983) An immunoperoxidase investigation of S-100 protein in granular cell myoblastomas: evidence for Schwann cell derivation. Am J Clin Pathol 79:37–44

Bhoopat L, Turner RR, Stathopoulos E, Meyer PR, Taylor CR, Marder RJ, Epstein AL (1988) Immunohistochemical characterization of two new monoclonal antibodies (LN-4, LN-5) reactive with human macrophage subsets and derived malignancies in B5-fixed, paraffin-embedded tissues. Blood 71:1079–1085

Carbone A, Micheau C, Caillaud J-M, Carlu C (1981) A cytochemical and immunohistochemical approach to malignant histiocytosis. Cancer 47:2862–2871

Carbone A, Manconi R, Poletti A, Colombatti A, Tirelli U, Volpe R (1986) S-100 protein, fibronectin, and laminin immunostaining in lymphomas of follicular center cell origin. Cancer 58:2169–2176

Cordell JL, Falini B, Erber WN, Ghosh AK, Abdulaziz Z, MacDonald S, Pulford KAF, Stein H, Mason DY (1984) Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). J Histochem Cytochem 32:219–229

Davey FR, Cordell JL, Erber WN, Pulford KAF, Gatter KC, Mason DY (1988) Monoclonal antibody (Y1/82A) with specificity towards peripheral blood monocytes and tissue macrophages. J Clin Pathol 41:753–758

Flavell DJ, Jones DB, Wright DH (1987) Identification of tissue histiocytes on paraffin sections by a new monoclonal antibody. J Histochem Cytochem 35:1217–1226

Genberg M, Mark J, Hakelius L, Ericsson J, Nistér M (1989) Origin and relationship between different cell types in malignant fibrous histiocytoma. Am J Pathol 135:1185–1196

Gloghini A, Volpe R, Carbone A (1990a) Ki-M6 immunostaining in routinely processed sections of reactive and neoplastic human lymphoid tissue. Am J Clin Pathol 94:734–741

Gloghini A, Volpe R, Carbone A (1990b) Vimentin immunostaining in fibroblastic reticulum cells within human reactive and neoplastic lymphoid follicles. Hum Pathol 21:792–798

Harkin JC, Reed RJ (1969) Tumors of the peripheral nervous system. In: Atlas of tumor pathology, 2nd series, fascicle 3. Armed Forces Institute of Pathology, Washington, D.C.

Hogg N, Selvendran Y (1985) An anti-human monocyte/macrophage monoclonal antibody, reacting most strongly with macrophages in lymphoid tissue. Cell Immunol 92:247–253

Hsu S-M, Raine L, Fanger H (1981) The use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase technique: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29:577–580

Kelly PMA, Bliss E, Morton JA, Burns J, McGee JO'D (1988) Monoclonal antibody EBM/11: high cellular specificity for human macrophages. J Clin Pathol 41:510–515

Knapp W, Rieber P, Dörken B, Schmidt RE, Stein H, Borne AEGKr von dem (1989) Towards a better definition of human leucocyte surface molecules. Immunol Today 10:253–258

Kreipe H, Radzun HJ, Parwaresch MR, Haislip A, Hansman M-L (1987) Ki-M7 monoclonal antibody specific for myelomonocytic cell lineage and macrophages in human. J Histochem Cytochem 35:1117–1126

Magnusson B, Kindblom L-G, Angervall L (1983) Enzyme histochemistry of malignant fibroblastic histiocytic tumors. Appl Pathol 1:223–240

Micklem K, Rigney E, Cordell J, Simmons D, Stross P, Turley H, Seed B, Mason D (1989) A human macrophage-associated antigen (CD68) detected by six different monoclonal antibodies. Br J Haematol 73:6–11

Nakazato Y, Ishizeki J, Takahashi K, Yamaguchi H (1982) Immunohistochemical localization of S-100 protein in granular cell myoblastoma. Cancer 49:1624–1628

National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphomas: summary and description of a Working Formulation for clinical usage (1982) Cancer 49:2112–2135

Ozello L, Stout AP, Murray MR (1963) Cultural characteristics of malignant histiocytomas and fibrous xanthomas. Cancer 16:331–344

Parwaresch MR, Radzun HJ, Kreipe H, Hansmann M-L, Barth J (1986) Monocyte/macrophage-reactive monoclonal antibody Ki-M6 recognizes an intracytoplasmic antigen. Am J Pathol 125:141–151

Pulford KAF, Rigney EM, Micklem KJ, Jones M, Stross WP, Gatter KC, Mason DY (1989) KP1: a new monoclonal antibody that detects a monocyte/macrophage associated antigen in routinely processed tissue sections. J Clin Pathol 42:414–421

Rode J, Dhillon AP, Papadaki L (1982) Immunohistochemical staining of granular cell tumour for neurone specific enolase: evidence in support of neural origin. Diagn Histopathol 5:205–211

Warnke RA, Pulford KAF, Pallesen G, Ralfkiaer E, Brown DC, Gatter KC, Mason DY (1989) Diagnosis of myelomonocytic and macrophage neoplasms in routinely processed tissue biopsies with monoclonal antibody KP1. Am J Pathol 135:1089–1095