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Antibody Specific to Muscle Actins in the Diagnosis and Classification of Soft Tissue Tumors

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A series of soft tissue tumors, melanomas, carcinomas, and lymphomas were studied immunohistochemically for the presence of muscle actins (MA) with the monoclonal antibody HHF-35, and for the presence of desmin for comparison. In nonneoplastic tissues, MA immunoreactivity was present in skeletal and smooth muscle cells, in the pericytes of small vessels, and in the myoepithelial cells. Desmin immunoreactivity had a similar distribution, except that the pericytes of small vessels and myoepithelial cells were negative. All 17 rhabdomyosarcomas were positive for both MA and desmin. Of leiomyosarcomas, 31/32 were positive for MA, and 29/32 for desmin. In pleomorphic undifferentiated sarcomas (malignant fibrous histiocytomas) MA and desmin-positive cells were present in 9/35 and 5/35 cases, respectively. Three of five pleomorphic liposarcomas showed MA-positive tumor cells, which were also desmin-positive in one case. Desmoid tumors often showed a moderate number of both desmin- and MA-positive cells. Hemangiopericytoma, Kaposi's sarcoma, and endometrial stromal sarcoma showed MA-positive staining only in the pericytes and not in the neoplastic cells. In various types of carcinomas, melanomas, and lymphomas, MA- or desmin-positive neoplastic cells were not identified. MA, but not desmin, was present in the desmoplastic stroma in many carcinomas. Both MA and desmin are good markers for muscle differentiation and especially serve to identify rhabdomyosarcomas and leiomyosarcomas. These markers are also present in some sarcomas currently regarded as nonmuscle tumors. This may suggest that some of these tumors have differentiation properties related to true myosarcomas. The absence of muscle actin, a pericytic marker, in hemangiopericytoma does not confirm the concept of pericytic nature of this tumor. (Am J Pathol 1988, 130:205–215)

ANTIBODIES to the cytoskeletal intermediate filament proteins have been widely and successfully applied in the diagnosis and classification of human tumors during the past five years.[1–5] Muscle differentiation of neoplasms has been evaluated with antibodies to desmin, the intermediate filament protein typical of striated and smooth muscle cells; and desmin has been found to be a good marker for also poorly differentiated rhabdomyosarcomas[6–9] and for leiomyosarcomas.[10]

Actins are protein constituents of the microfilaments, the ubiquitous cytoskeletal elements present in most cells.[11,12] In analogy to the intermediate filament protein family, actins can be divided into several closely related, although biochemically and immunologically distinguishable subtypes, which are expressed in muscle and nonmuscle types of cells in a way somewhat resembling the cell type-specific expression of intermediate filament proteins.[13,14]

Actins can be biochemically and immunologically divided into three main subsets: α-actins are present in muscle tissues, and β- and γ-actins are present in nonmuscle cells; in addition, a minor subset of γ-actins is probably present muscle cells.[13,14] Therefore, antibodies selective to subsets of actins would be of interest in the diagnosis and classification of human tumors. Recently, a monoclonal antibody reactive with muscle actins, α actins, and γ actins of smooth muscle cells has been described.[15] In this report, the monoclonal HHF-35 antibody to muscle cell actins has been evaluated as a differentiation marker in the diagnosis and taxonomic studies of soft tissue tumors.

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Table 1—Immunostaining Results for Muscle Actin (MA) and for Desmin in Soft Tissue Tumors

Tumor Type	MA	Desmin
Rhabdomyosarcoma, alveolar	7/7	7/7
Rhabdomyosarcoma, embryonal + botryoid (1)	10/10	10/10
Fibrous histiocytoma, skin	5/10	0/10
Dermatofibrosarcoma protuberans (2)	0/7	0/7
Monophasic synovial sarcoma, spindle cell type (3)	0/3	0/3
Desmoid tumor (5)	9/15	15/15
Fibrosarcoma (1)	0/6	0/6
Leiomyoma, vascular, skin	10/10	10/10
Leiomyoma, uterine	13/13	13/13
Renal angiomyolipoma (2)	3/3	3/3
Leiomyosarcoma (1)	31/32	29/32
Liposarcoma, myxoid (1)	0/7	0/7
Liposarcoma, pleomorphic (1)	3/5	1/5
Malignant endothelioma (1)	0/5	0/5
Kaposi sarcoma	0/7	0/7
Glomus tumor	15/15	1/15
Hemangiopericytoma (2)	0/8	0/8
Schwannoma, benign	0/4	0/4
Schwannoma, malignant (2)	0/8	0/8
Granular cell tumor	0/6	0/6
Endometrial stromal sarcoma	0/7	0/7
Clear cell sarcoma (1)	0/4	0/4
Malignant melanoma (3)	0/12	0/12
Epithelioid sarcoma	0/3	0/3
Ewing's sarcoma (2)	0/3	0/3
Alveolar soft part sarcoma	0/1	0/1
Spindle cell sarcoma, NOS (5)	5/39	2/39
Pleomorphic sarcoma, NOS (7)	9/35	5/35
Squamous carcinoma of lung (7)	0/7	0/7
Mammary ductal carcinoma (11)	0/11	0/11
Gastrointestinal adenocarcinoma (6)	0/10	0/10
Serous papillary cystadenocarcinoma	0/2	0/2
Renal adenocarcinoma	0/3	0/3
Thyroid carcinomas, different types (9)	0/9	0/9
Other adenocarcinomas (5)	0/5	0/5
Malignant mesothelioma (2)	0/2	0/2
Malignant lymphomas (10)	0/10	0/10
Number of cases together	344	344

The number of cases studied in frozen sections is shown in parentheses. The results are expressed as the number of positive cases/number of studied cases

Materials and Methods

Tumor Material

Sections from 285 well-characterized soft tissue tumors, 47 carcinomas of different types, 2 malignant mesotheliomas, and 10 lymphomas were examined in this study. Mainly formaldehyde-fixed and paraffin-embedded tissues were used; frozen sections were available from 40 soft tissue tumors and 50 tumors of the other groups. The diagnosis of soft tissue tumors was based on the standard histopathologic criteria.[16] Strict criteria were applied when making specific tumor-type diagnoses. Many of the rhabdomyosarcomas (9/17), leiomyosarcomas (18/32), and pleomorphic liposarcomas (3/5) were verified by electron microscopy by their characteristic ultrastructural features.[17,18]

Antibodies and Immunostaining

Monoclonal mouse hybridoma antibody specific for muscle actin(s) (MA) (clone designation HHF-35, Enzo Biochem, New York, NY) has been previously characterized.[15] In immunoblots, this antibody reacts with a band of 42,000, which corresponds to α-actins and a muscle cell-specific fraction of gamma actins as studied by combined biochemical and immunohistochemical analysis.[15] Monoclonal anti-desmin antibody, (Amersham Ltd., Little Chalfont, UK) was used in this study. The documentation of this antibody has been previously published.[19]

The immunostaining was performed with the immunoperoxidase technique by using the avidin-biotin amplification system as described by Hsu et al.[20] When we were using paraffin sections, the endogenous peroxidase was blocked by immersing the slides in water containing 0.5% hydrogen peroxide for 5 minutes. Frozen sections were fixed in acetone, air-dried, and rehydrated. The endogenous peroxidase was not blocked, but a section in which the primary antibody was omitted served as a control. The primary antibody was incubated overnight at +4 C. The antibody to MA was diluted to 1:400–1:1000, and the antibody to desmin was diluted to 1:10–1:20. Biotinylated rabbit-antimouse immunoglobulin antiserum (Dakopatts, Copenhagen, dilution 1:300) and avidin combined *in vitro* with biotinylated horseradish peroxidase complex (Dakopatts, dilution for both 1:150) were sequentially applied to the sections for 30 minutes at room temperature. The color was developed with 3-amino-9-ethylcarbazole (0.2 mg/ml in 0.05 N acetate buffer, pH 5.0, 20 minutes, at room temperature). The slides were lightly counterstained with Mayer's hematoxylin and mounted in an aqueous mounting medium.

The HHF-35 antibody to muscle actins worked in formaldehyde-fixed and paraffin-embedded tissues, but if the sections were deparaffinized only, the immunostaining was often weak and the contrast between negative and positive structures often appeared unsharp, especially in the smooth muscle layer of the arteries and in the myoepithelial cells. In addition, there was some spurious staining of epithelia that was not observed in frozen sections. Therefore, three alternative pretreatment protocols were tested: 1) pepsin, 0.05% crude enzyme preparation (Merck & Co, Darmstadt, FRG) in HCl, pH 1.8, for 20 minutes at +37 C; 2) trypsin, 0.1% crude enzyme preparation (Difco, Detroit, Michigan) in phosphate-buffered sa-

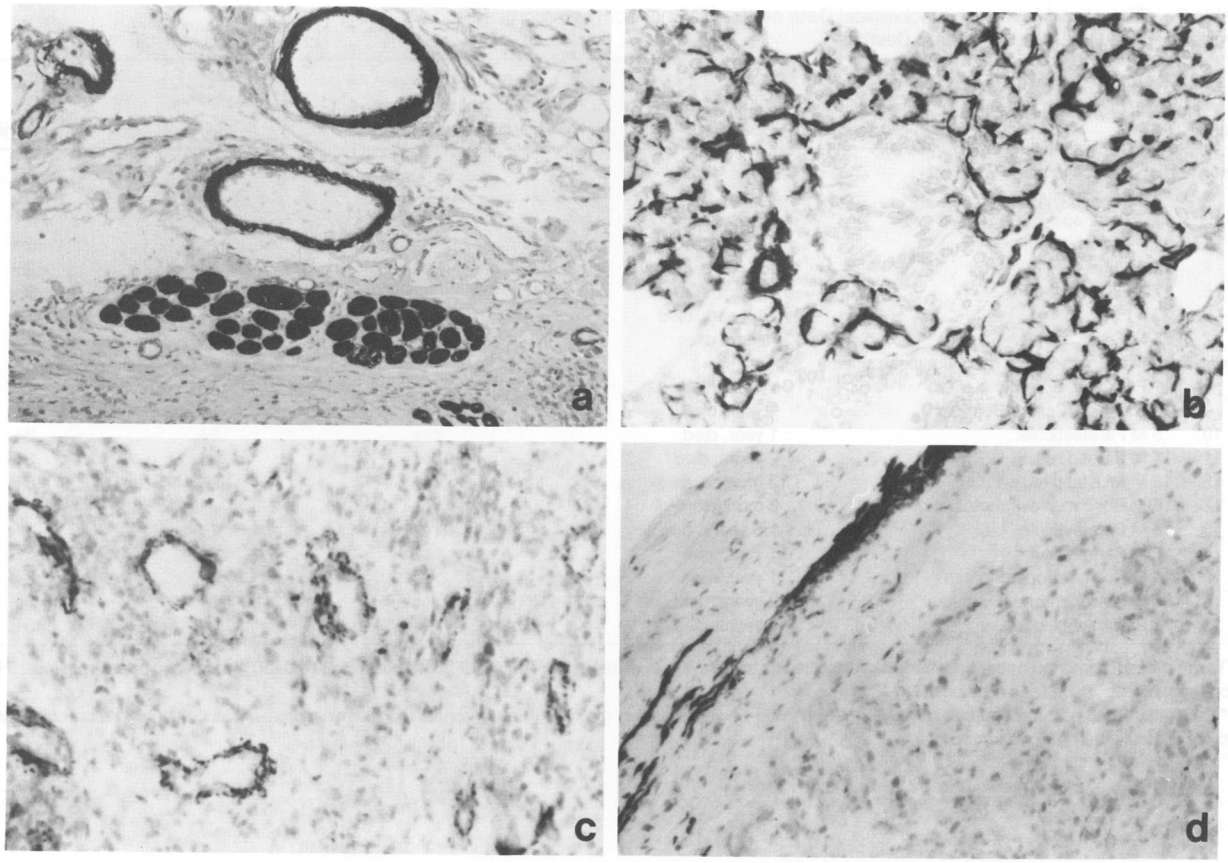

Figure 1—MA immunoreactivity is seen in the striated muscle cells and in the vascular smooth muscle cells (**a**). The myoepithelial cells in the periphery of the parotid gland acini are positive for MA, but the acinara cells and the intercalated ducts are negative (**b**). In loose vascular connective tissue, MA positivity is seen in the pericytes of small vessels (**c**); pericytes are negative for desmin and only the muscular layer of a larger vessel (*upper left*) is positive (**d**). (Immunoperoxidase, a light counterstain with hematoxylin. **a** and **b** ×150; **c** and **d**, ×300)

line (PBS), pH 7.6, for 10 minutes at +37 C; 3) pronase (Calbiochem, San Diego, Calif), 0.01% solution in PBS, pH 7.6, for 10 minutes at +37 C. Sections of normal skin, breast, and salivary gland and a representative sample of soft tissue tumors was tested. The treatment with pepsin, trypsin, or pronase appeared equally effective to produce results comparable to those obtained in frozen sections of normal tissues. A sharp contrast between negative and positive structures was observed, and the spurious staining of epithelial structures, sometimes observed in paraffin sections, was clearly reduced. Therefore, a mild protein digestion (pepsin), as specified above, was employed in the immunostaining of paraffin sections in the whole material.

Results

Normal Tissues

The striated muscle fibers of the myocardium and the skeletal muscle, the muscular layer of arteries (Figure 1a), the smooth muscle layers of the entire gastrointestinal tract, and smooth muscle tissue of the myometrium and the prostate reacted intensely with both anti-MA and anti-desmin antibodies. In addition to muscle cells, the anti-MA, but not anti-desmin, reacted with the myoepithelial cell layer around the mammary ducts, sweat glands, and salivary glands (Figure 1b). The outer single cell layer in the wall of small arteries, virtually representing pericytes, was reactive with anti-MA but not with anti-desmin (Figure 1c and d). Vascular endothelial cells and fibroblasts were negative for both MA and desmin.

The immunostaining results for MA and desmin in 344 tumors has been summarized in Table 1.

Rhabdomyosarcoma (RMS)

The clinical and immunohistochemical data on RMS have been summarized in Table 2. There were 7 alveolar and 10 embryonal RMSs, including two bo-

Table 2—Clinical and Immunohistochemical Data on the 17 Rhabdomyosarcomas Studied for the Presence of Muscle Actin (MA) and Desmin

Case	Age/Primary location sex	Follow-up	Electron-microscopic documentation	MA	Desmin
Alveolar rhabdomyosarcomas					
1.	5M Mediastinum	2 years, died		3+	3+
2.	6 F Foot, sole	1 year, died		2+	2+
3.	11 F Periorbital	3 years, died		2+	2+
4.	16 F Vulva	1,5 years, died	Yes	2+	2+
5.	17 F Foot	2 years, died		3+	3+
6.	22 F Foot	1,5 years, died		2+	2+
7.	25 M Foot	Recent case		2+	2+
Embryonal, including botryoid rhabdomyosarcomas					
8.	1 F Vagina, Botryoid	1,5 years, died		1+	1+
9.	1 F Vagina/Cervix, Botryoid	1 year, died		2+	2+
10.	5 M Paratesticular	1 year, died	Yes	3+	3+
11.	11 M Parotid region	2 years, died	Yes	3+	3+
12.	16 M Prostatic region	3 months, died	Yes	3+	3+
12a.	16 M Bone marrow, metastatic	3 months, died	Yes	2+	1+
13.	16 M Paratesticular	1,5 years, died	Yes	1+	1+
14.	17 M Paratesticular	1 year, alive		2+	2+
15.	25 F Thigh, retroperitoneum	1 year, alive	Yes	3+	3+
16.	34 M Retroperitoneum, metast*	2 years, died	Yes	3+	3+
17.	72 F Urinary bladder	2 years, died	Yes	3+	3+

The number of immunoreactive cells has been classified as follows: 1+, less that 10% of neoplastic cells positive; 2+ 10–30% of neoplastic cells positive; 3+, more than 30% of neoplastic cells positive.

*This patient had had a round cell sarcoma in the parotid 11 years earlier; the new retroperitoneal rhabdomyosarcoma was considered a probable late recurrence from the previous tumor.

tryoid tumors, one of the alveolar and 8 of the embryonal RMSs were verified by electron microscopy and showed distinguishable sarcomeric structures. In alveolar RMS, the number of MA-positive cells sometimes exceeded that of desmin-immunoreactive cells and varied in individual cases from 20% to 80% of the tumor cells. The number of immmunoreactive cells was in positive correlation with the differentiation level (Figure 2a and b). Especially in alveolar rhabdomyosarcomas, the reactive stromal spindle cells were often positive for MA (Figure 2a) but not for desmin. In embryonal botryoid RMS, MA and desmin immunoreactivity was seen in the elongated differentiated cells, but not in the majority of the undifferentiated round cells (Figure 2c and d). Paratesticular RMS showed varying numbers of MA- and desmin-positive cells. Typically, the large differentiated rhabdomyoblasts were positive, whereas only a minor portion of the undifferentiated-appearing round or spindle cells were positive. A spindle cell embryonal RMS in the parotid region (Figure 2e) was strongly positive for MA (Figure 2f) and desmin (not shown); this tumor was verified as a RMS by electron microscopy on the basis of the content of thick and thin filaments which often formed distinguishable sarcomeric units with Z-bands (Figure 2g).

Leiomyomas and Leiomyosarcomas (LMS)

Vascular leiomyomas of the skin and uterine leiomyomas were consistently positive for MA and desmin. All three renal angiomyolipomas showed both MA and desmin in many, but not in all, spindle cells. Of malignant tumors, only those that satisfied the typical light-microscopic[16] or ultrastructural criteria of LMS[17] were included in this category. The tumors were located as follows: retroperitoneum, 14; skin, 8, deep soft tissues of extremities, 5; inferior vena cava, 2; liver, metastasis?, primary unknown, 2. These tumors typically showed elongated cells with cigar-shaped nuclei and variably eosinophilic cytoplasm. Eighteen of the LMS were ultrastructurally verified by the content of cytoplasmic and cell membrane-associated densities of microfilaments, and variably developed basal laminas. Almost all LMS (31/32) showed MA immunoreactivity, most usually in the majority of the tumor cells; desmin was present in 29/32 cases. Two spindle cell sarcomas and two highly pleomorphic tumors (Figure 3a) were reclassified as leiomyosarcomas by their prominent MA (Figure 3b) and desmin immunoreactivity (not shown) and by electron-microscopic evidence of smooth muscle differentiation (Figure 3c).

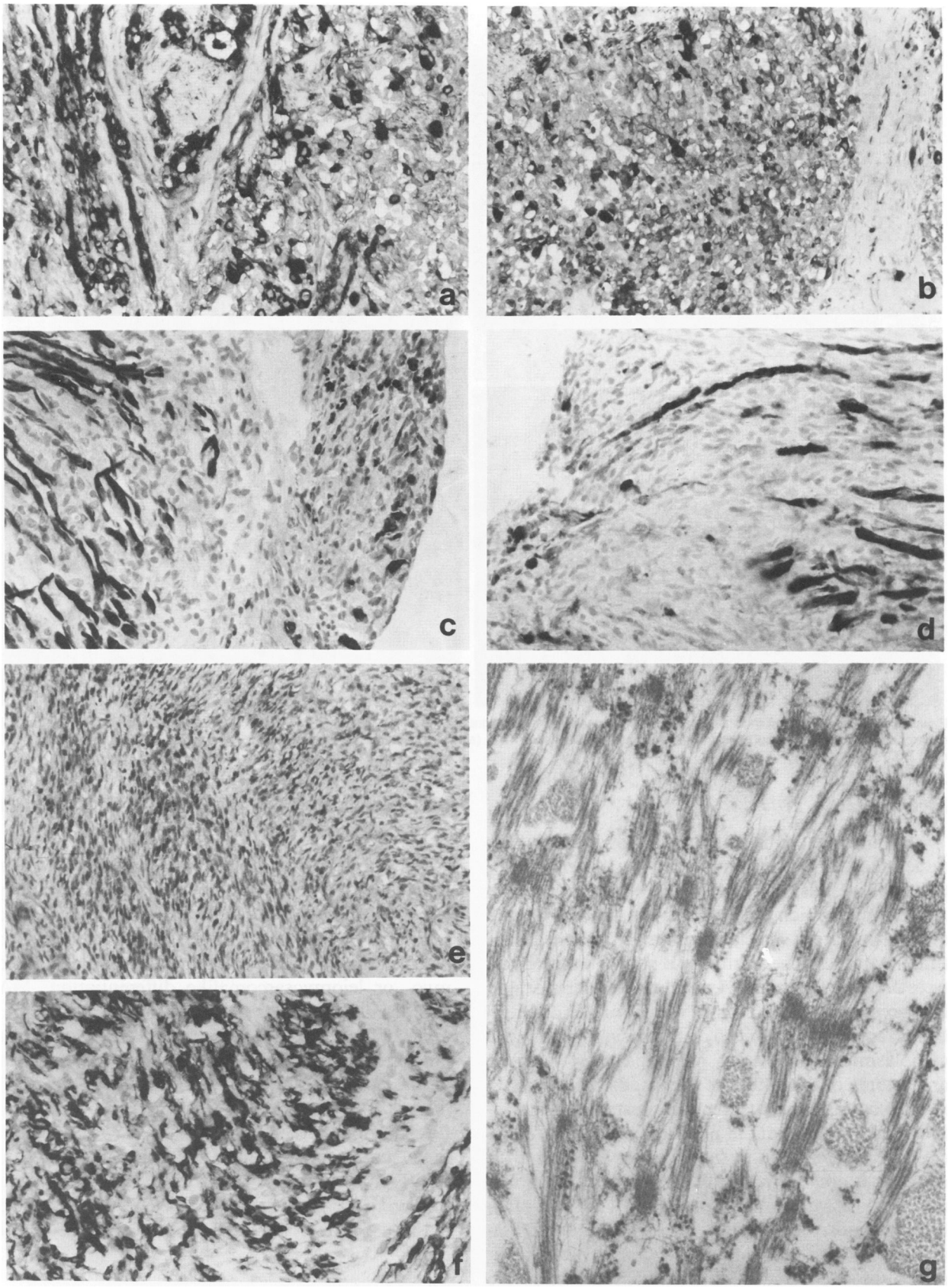

Figure 2—An alveolar rhabdomyosarcoma (RMS) shows MA immunoreactivity in about half of the tumor cells and in the vascular septa and reactive stromal spindle cells (**a**); in the same tumor, desmin positivity is seen in about 30% of tumor cells (**b**). An embryonal botryoid RMS shows MA (**c**) and desmin (**d**) positivity in the elongated rhabdomyoblasts in a similar pattern. A highly cellular spindle cell sarcoma from parotid region (**e**) shows uniform MA positivity (**f**); this tumor is verified as a RMS by the content of sarcomeric structures at electron microscopy (**g**). (**a–d, f,** immunoperoxidase light counterstain with hematoxylin, ×300; H&E, ×150; **g,** ×42,000)

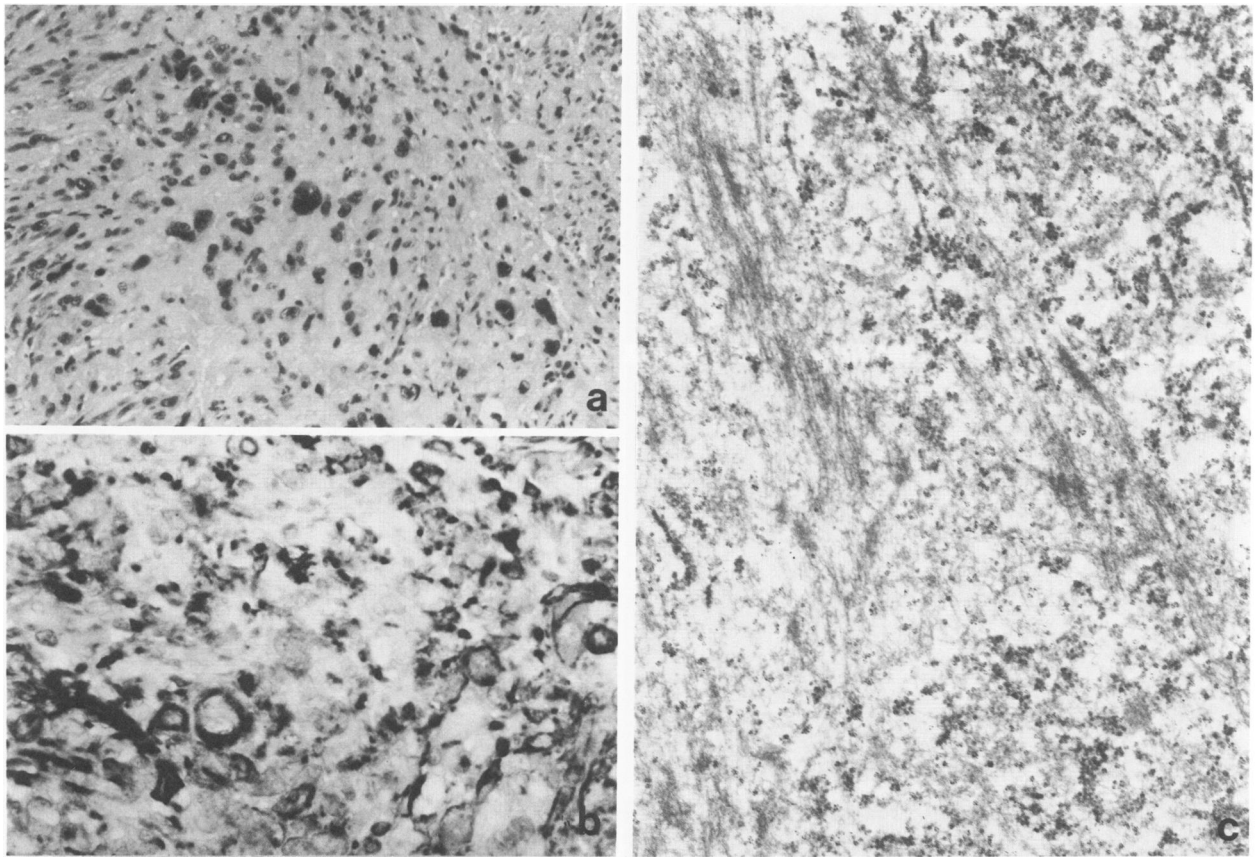

Figure 3—A pleomorphic leiomyosarcoma (**a**) shows muscle actin immunoreactivity in many tumor cells (**b**). This tumor is verified as a leiomyosarcoma by electron microscopy; bundles of actin and many dense bodies are evident (**c**). (**a**, H&E, ×150; **b**, immunoperoxidase, light counterstain with hematoxylin, ×300; **c**, 31,500)

Liposarcoma

The pure myxoid liposarcomas of low-grade malignancy showed MA immunoreactivity in pericytes of the small vessels; these cells were not reactive with anti-desmin, which only labeled the smooth muscle cells in the larger vessels occasionally present in the tumor samples. The tumor cells between the vessels were negative for both MA and desmin. Pleomorphic liposarcomas were tumors in which fat cell differentiation could be recognized on the basis of prominent cytoplasmic vacuolization pattern at least in some areas; three of these tumors were studied by electron microscopy and showed frequent clusters of cytoplasmic lipid droplets but did not show evidence of muscle cell differentiation. A significant proportion (3/5) of these tumors showed cells positive for MA; some desmin-positive cells were present in 1 case. A pleomorphic liposarcoma with cytoplasmic vacuoles (Figure 4a) showed muscle actin immunoreactivity in some tumor cells (Figure 4b). The content of multiple extracted and nonextracted cytoplasmic lipid droplets at electron microscopy (Figure 4c) indicated fat cell differentiation.[17,18]

Pleomorphic Undifferentiated Sarcomas (Malignant Fibrous Histiocytomas)

Thirty-five pleomorphic sarcomas, identified as malignant fibrous histiocytomas by morphology, were studied. Seven cases were studied in frozen section, and all these tumors were also studied by electron microscopy without finding any signs of rhabdomyo- or leiomyosarcomalike differentiation. MA- and desmin-immunoreactive cells were present in 3 and 2 of the 7 frozen section cases. A highly pleomorphic sarcoma with multiple tumor giant cells is shown in Figure 5a. This tumor contained MA-positive cells (Figure 5b) but was negative for desmin (not shown). By electron microscopy this tumor shows undifferentiated mesenchymal cells with fibroblastoid morphology (Figure 5c). Six of the 28 pleomorphic sarcomas studied in paraffin sections showed MA-positive neoplastic cells, and 3 showed desmin-positive neoplastic cells.

Fibroblastic Tumors

Typical fibrosarcomas and dermatofibrosarcoma protuberans were negative for both MA and desmin.

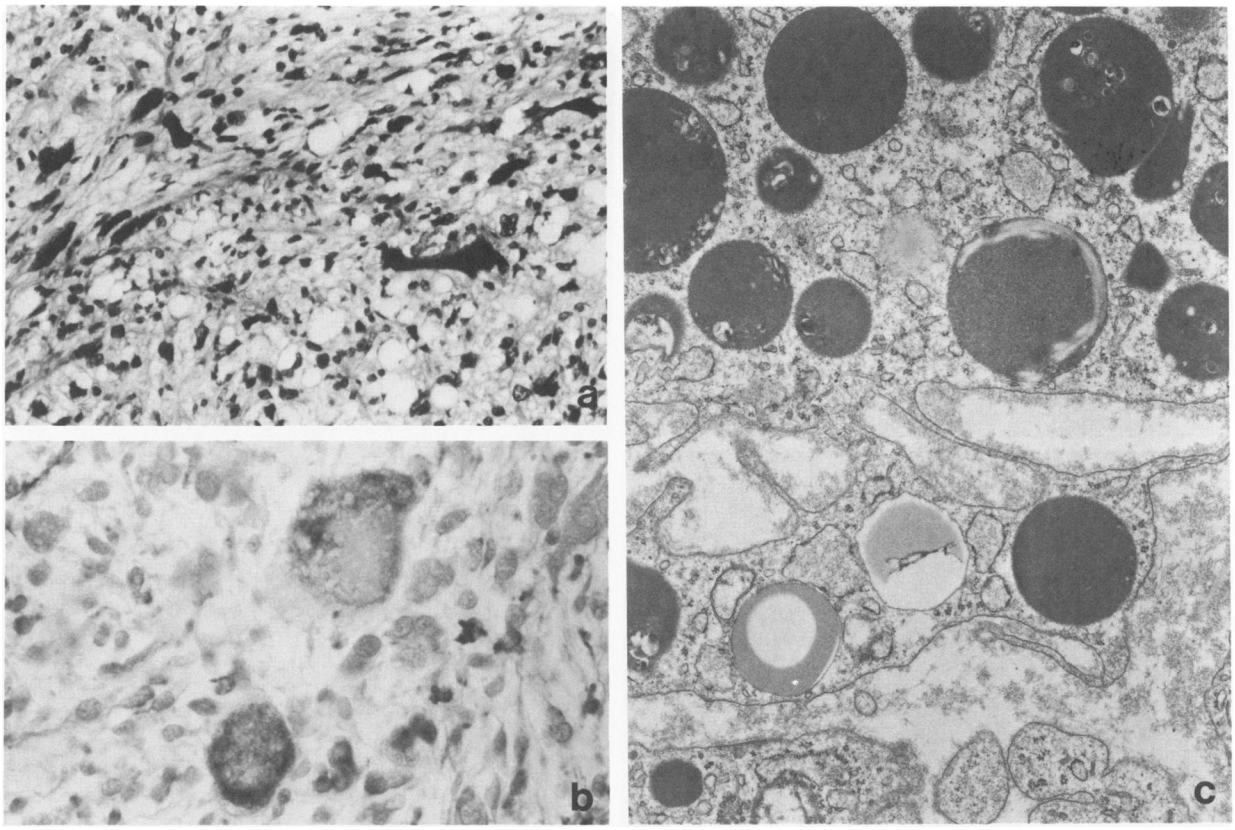

Figure 4—A pleomorphic liposarcoma with many cytoplasmic vacuoles (**a**) shows MA positivity in large atypical cells (**b**). Electron microscopy shows prominent cytoplasmic lipid droplets suggesting fat cell differentiation (**c**). (**a**, H&E, ×240; **b**, immunoperoxidase, light counterstain with hematoxylin, ×240; **c**, ×15,000)

Of the 39 spindle cell sarcomas of high-grade malignancy not obviously belonging to any definite diagnostic categories, MA-positive cells were found in 5 cases and desmin-positive cells in 2 cases; 2 of the 5 (MA^+, $desmin^-$) were pleural sarcomas with tumor cells suggestive of myofibroblastic differentiation; the tumors were cytokeratin-negative, thus giving no evidence of their possible mesothelial nature. Benign dermatofibromas of the skin usually only showed reactivity with abundant vascular smooth muscle cells and pericytes with anti-MA, but a deeper MA-positive spindle cell component was present in 5 of 10 cases; desmin immunoreactivity was not found in these cells. In desmoid tumors, MA-positive tumor cells were found 9/15 cases, and all of these cases showed scattered desmin-positive, clearly neoplastic cells.

Vascular Tumors

Hemangiopericytomas showed MA immunoreactivity in a single cell layer in vessel walls, compatible with the pericytes (Figure 6a), whereas the antibody to desmin showed no reactivity with these cells, but there was positive staining in the walls of larger arteries occasionally present in the specimens. The hemangiopericytoma cells between the vessels were negative for both MA and desmin. Postmastectomy angiosarcomas and malignant endotheliomas of other types showed no reactivity in the tumor cells, but showed staining of the nonneoplastic vessel walls. The neoplastic vascular units in malignant endotheliomas typically lacked the anti-MA-reactive pericytic cell layer (Figure 6b). Glomus tumors, in contrast, showed a strikingly positive staining for MA (Figure 6c), whereas they were usually negative for desmin (Figure 6d); only 1 of them revealed desmin-positive neoplastic cells. Kaposi sarcomas (from elderly patients not having AIDS) showed MA positivity only in the pericytes (Figure 6e).

Other Soft Tissue Tumors

Schwannomas, benign and malignant, endometrial stromal sarcomas, clear cell sarcomas, and malignant melanomas showed no MA or desmin immunoreactivity in the neoplastic cells. Granular cell tumors

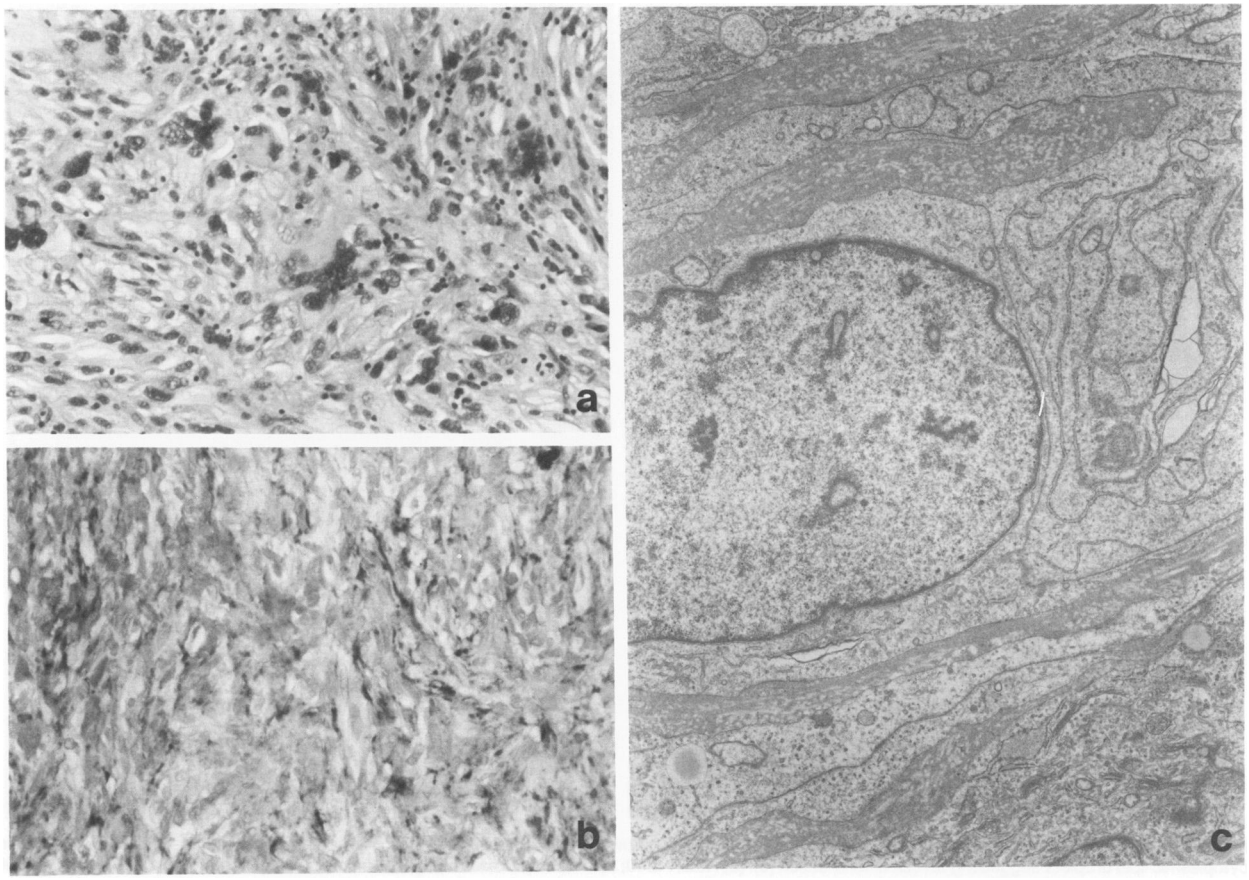

Figure 5—A highly pleomorphic sarcoma with tumor giant cell and atypical mitoses compatible with malignant fibrous histiocytoma (**a**) reveals MA immunoreactivity (**b**). Electron microscopy shows undifferentiated fibroblastoid mesenchymal cells with a prominent rough endoplasmic reticulum (**c**). (**a**, H&E, ×240; **b**, immunoperoxidase, light counterstain with hematoxylin, ×120; **c**, ×6000)

were also negative for both MA (Figure 6f) and desmin.

Carcinomas and Lymphomas

Forty-seven carcinomas of different types were investigated, most of these in frozen sections. The epithelial cells did not react with the antibody to MA in any of the cases, including 31 adenocarcinomas of different types, 7 squamous carcinomas, and 5 undifferentiated carcinomas. Desmin immunoreactivity was not found in carcinomas. The antibody to MA typically reacted with mesenchymal myofibroblast-like cells in the desmoplastic stroma of many of infiltrating carcinomas. These desmoplastic stromal cells were only occasionally desmin-positive. The stromal tissues in infiltrating and metastatic colorectal carcinoma, ovarian serous cystadenocarcinoma, and malignant mesothelioma were especially rich in MA-positive cells, whereas most mammary carcinomas showed a lower number of MA-positive stromal cells. None of the lymphomas showed MA- or desmin-positive neoplastic cells, but occasional slender, desmin- and MA-positive reactive stromal cells were found.

Discussion

In this study, a broad range of soft tissue tumors, carcinomas, and lymphomas were immunohistochemically studied with a MA-specific monoclonal antibody, HHF-35, for evaluation of this marker in the diagnosis of soft tissue sarcomas and the presence of muscle cell differentiation in several specific types of soft tissue tumors. The muscle specificity of the MA antibody is shown by its immunostaining pattern in normal tissue; however, an exception is its reactivity with the myoepithelial cells, as shown by Tsukada et al.[15]

The results showed consistent MA immunoreactivity in all rhabdomyosarcomas of different types, and in almost all leiomyosarcomas, even if formaldehyde-fixed and paraffin-embedded tissue was used. The rare MA-negative leiomyosarcomas may repre-

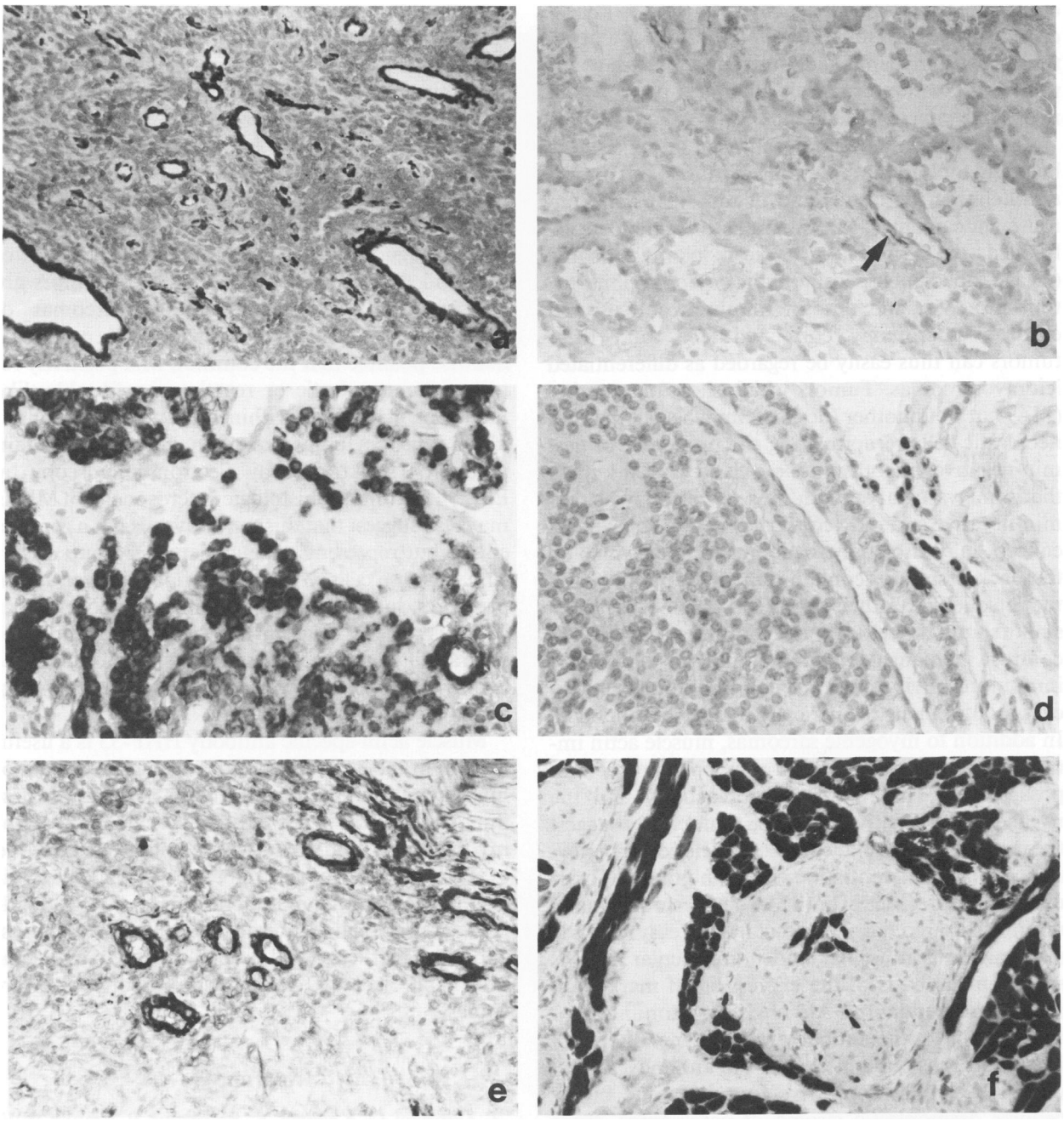

Figure 6—A hemangiopericytoma shows MA positivity only in the pericytes of the vessels (**a**). A malignant endothelioma is negative for MA, but a nonneoplastic vessel shows positive pericytes (*arrow*) (**b**). Glomus tumor cells are strongly positive for MA (**c**), but negative for desmin; only some smooth muscle cells around the tumor are positive (**d**). Kaposi sarcoma shows MA immunoreactivity in the vessel walls, but the spindle cells are negative (**e**). A granular cell tumor is negative for MA, and nonneoplastic striated muscle cells are positive (**f**). (Immunoperoxidase, light counterstain with hematoxylin, **b–e**, ×300; **a–f**, ×150)

sent occasional failures of detection due to unoptimal fixation or tissue processing rather than due to the true absence of MA in these tumors. Thus, muscle actin, like desmin, is conserved in transformed cells. Previously, polyclonal antibodies to actin(s) have also been shown to react with myosarcomas.[21,22] Because of the widespread presence of actins in different cell types,[11,12] however, it is not unexpected that such antibodies may also react with carcinoma cells.[21,22] The monoclonal antibody to muscle actin (HHF-35) seems to be free of this problem, because carcinomas and lymphomas were consistently negative. Recently, a similar conclusion was reached by Tsukada et al[23] when describing the immunostaining for HHF-35 in nearly 300 epithelial and mesenchymal tumors. Notably melanomas and carinomas studied by Tsukada

et al were also HHF-35-negative. An interesting exception is the focal muscle actin immunoreactivity in tumors with myoepithelial differentiation, which fits to the observation of muscle actin positivity of nonneoplastic myoepithelial cells.[23]

Antibodies to MA and desmin can be expected to be useful tools in the taxonomy of muscle cell tumors and defining the borderland of muscular and nonmuscular sarcomas. In tumors that morphologically fulfilled the criteria of typical leiomyosarcoma (cigar-shaped nuclei, eosinophilic or picrinophilic cytoplasm), MA and desmin immunoreactivity and electron microscopy correlated well, and these tumors can thus easily be regarded as differentiated leiomyosarcomas. Tumors which show one marker (MA) but not another (desmin) may be examples of neoplastic cells recapitulating the properties of desmin-negative smooth muscle cells. It is well known that some vascular smooth muscle cells may be desmin-negative and only contain vimentin.[24,25] Such a concept may also apply to glomus tumors, which either lack desmin or contain it in an amount beyond the detection level; their strong MA positivity suggests smooth muscle cell or pericytic differentiation. In contrast, hemangiopericytomas did not show MA immunoreactivity, and thus no immunohistochemical proof for their true pericytic nature was obtained. In addition to myogenic sarcomas, muscle actin immunoreactivity was found in many pleomorphic liposarcomas. The MA immunoreactivity and the usual desmin-negativity in these tumors may suggest the presence of vascular smooth muscle cell or pericytic cell-like differentiation in high-grade liposarcomas. The presence of both MA and desmin in many nonmuscle sarcomas shows that it is not easy to divide sarcomas into muscular and nonmuscular tumors solely on the basis of the expression of markers of muscle differentiation. Although the immunostaining results with the antibody to MA generally paralleled with those obtained with the antibody to desmin, some differences were found. In the highly cellular desmoplastic stroma of carcinomas, there were much more of MA-positive cells than desmin-positive cells. The desmoplastic stroma, present in many invasive carcinomas, is rich in myofibroblasts,[26] cells sharing some functional and ultrastructural properties of both fibroblasts and smooth muscle cells.[27] It is very likely that this positivity can be ascribed to the MA positivity of the stromal myofibroblasts. The present results are in agreement with the previous finding that the myofibroblasts in general are negative for desmin.[28] However, desmoid tumors often showed both desmin and MA-positive cells; they are known to contain a high number of myofibroblasts among typical fibroblasts.[29] This might suggest that a desmin-positive subset of myofibroblasts is present in desmoid tumors.

Many pleomorphic sarcomas, currently most often classified as malignant fibrous histiocytomas (MFHs) by morphology, showed MA and/or desmin-immunoreactive cells. The diagnosis of muscle cell sarcoma was excluded in many of these cases by electron microscopy; thus it was not easy to formulate any alternative diagnosis for these tumors. The presence of MA and desmin may mean that these tumors are examples of very undifferentiated sarcomas of smooth muscle cells (or of striated muscle cells). It is likewise possible that the common MA positivity results from neoplastic or reactive myofibroblastlike nature of many cells within the tumors; malignant fibrous histiocytomas are often known to contain myofibroblastlike cells by electron microscopy by EM.[30] The immunohistologic heterogeneity of MFHs may also suggest that this tumor type is not a genuine entity, but represents a heterogeneous group of sarcomas whose cells may show various differentiation pathways, such as muscle cell differentiation, or that MFH is an end stage in the "dedifferentiation" of several different types of sarcomas, for example, of leiomyosarcomas in occasional cases, as recently suggested by Brooks.[31]

Muscle actin-specific antibody HHF-35 is a useful reagent in the evaluation of the diagnosis of rhabdomyosarcoma and leiomyosarcoma. Similar to desmin, this marker is not entirely specific for rhabdomyosarcomas and leiomyosarcomas, but may be expressed in some other tumors as well. Wider experience with larger materials of sarcomas using antibodies to muscle actin, intermediate filament proteins, and other markers, would probably lead to a biologically more accurate classification of soft tissue sarcomas.

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