Malignant Fibrous Histiocytoma

Expression of Monocyte/Macrophage Differentiation Antigens Detected With Monoclonal Antibodies

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Monoclonal antibodies were used in an investigation of the histogenesis of malignant fibrous histiocytoma (MFH), a neoplasm with morphologic features of both fibroblastic and histiocytic differentiation. In 4 cases of MFH studied, the tumor cells were found to react uniformly with antibodies to determinants expressed on monocyte macrophages (T-200, Ia, MoS-1, MoS-39, MoR-17). Both spindle and histiocyte-like tumor cells expressed these markers. In contrast, in 8 non-MFH soft tissue tumors, tumor cell reactivity was not observed. The reactivity of the spindle cells of MFH for determinants of monocyte/macrophages favors their origin from tissue histiocytes (facultative fibroblasts). The results support the view that MFH is a tumor of the mononuclear phagocyte system. (Am J Pathol 1986, 124:303–309)

MALIGNANT fibrous histiocytoma (MFH) is a soft tissue neoplasm, the cells of which characteristically express features of both fibroblasts and histiocytes.[1–3] Features of the former include spindle cell growth pattern and collagen formation. Features of the latter include xanthoma cells and histiocytelike giant cells. Histogenesis of these tumors is therefore understandably controversial, with proponents of origin from both true tissue histiocytes (macrophages)[4,5] and from primitive soft tissue mesenchymal cells.[6–8] Histiocytes (tissue macrophages) are a part of the mononuclear phagocyte system (MPS) and generally considered to be derived from circulating blood monocytes, which are in turn derived from the bone marrow.[9–11] The recent development of monoclonal antibodies recognizing specific differentiation antigens with selective expression on cells of monocyte macrophage lineage[12–15] permits a new approach to the investigation of the histogenesis of these neoplasms of possible MPS origin. In the present study a panel of monoclonal antibodies recognizing antigens expressed by cells of the MPS was utilized for study of MFHs in comparison with other soft tissue tumors. The results support the view that MFH is derived from the cells of the MPS.

Materials and Methods

Tumor Tissue

Tissue was obtained from patients undergoing surgical biopsy or excision of soft tissue tumors at The Mount Sinai Hospital. Tissue was obtained fresh, and a representative sample was snap-frozen and stored at −70 C for immunologic studies. Tissue for histologic studies was fixed in 10% neutral buffered formalin. The diagnosis and classification was established by standard histologic criteria.[2]

Monoclonal Antibodies

The following monoclonal antibodies were utilized throughout the study. T-200 (Hybritech, Inc., San Diego, Calif), recognizes a 200,000 mol wt glycoprotein expressed on all hematopoietic cells, including granulo-

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cytes, lymphocytes, and macrophages, but not epithelial or mesenchymal cells. S-34, produced by one of the authors (A.D.B.), detects a nonpolymorphic Ia-like antigen (HLA-DR). This reagent reacts with 100% of B lymphocytes and monocytes and 1–2% of peripheral T lymphocytes. MoS-1, MoS-39, and MoR-17, produced by one of the authors (A.D.B.), detect antigens expressed on monocyte macrophages at various stages of development. Briefly, MoS-1 and MoS-39 detect antigens expressed on a major fraction of circulating monocytes and fluid macrophages; whereas MoR-17 detects antigens expressed predominantly on tissue-localized macrophages.[15–17]

Immunohistochemical Staining

Immunoperoxidase staining with monoclonal antibodies was performed on acetone-fixed fresh-frozen sections with the use of the avidin-biotin-peroxidase complex technique.[18] Briefly, cryostat sections (5-μ) were cut and placed on gelatinized slides and fixed in acetone. Sections were washed in Tris buffer (0.05 M Tris pH 7.6) and incubated with normal horse serum (Vector Laboratories, Inc., Burlingame, Calif) to block nonspecific binding. Sections were incubated with the unlabelled mouse monoclonal antibody at 1:30 dilution for 30–60 minutes and washed with Tris buffer. Sections were then incubated with biotinylated horse antimouse immunoglobulin (Vector) for 30 minutes followed by freshly prepared avidin–biotin–horseradish peroxidase complex (Vector) for 30 minutes. Staining reaction was developed with diaminobenzidine and hydrogen peroxide. Quenching of endogenous peroxidase was not performed. Sections were lightly stained with hematoxylin for cellular identification.

Results

Histology

Four cases of MFH were studied. All were of soft tissue origin. All four corresponded to the storiform-pleomorphic subtype as defined by Enzinger.[2] Three (Cases 1–3) were predominantly pleomorphic; 1 (Case 4) was predominantly storiform-fibroblastic. Eight various examples of other benign and malignant soft tissue tumors were also studied for comparison (Table 1).

Table 1—Cases Studied

1	MFH	Soft tissue
2	MFH	Soft tissue
3	MFH	Soft tissue
4	MFH	Soft tissue
5	Nodular fasciitis	Subcutaneous tissue
6	Leiomyoma	Uterus
7	Hemangioendothelioma	Spleen
8	Liposarcoma	Soft tissue
9	Leiomyosarcoma	Lung (metastatic)
10	Ewing sarcoma	Soft tissue and rib
11	Malignant schwannoma	Soft tissue
12	Malignant schwannoma	Soft tissue

Monoclonal Antibody Staining

All 4 cases of MFH demonstrated strong staining with monoclonal antibodies to specific macrophage differentiation antigens (MoS-1, MoS-39, and MoR-17). Staining of the three cellular constituents of these tumors was observed: histiocytelike cells, spindle cells, and pleomorphic giant cells (Figures 1 and 2). Staining of the spindle cells of Case 4 was particularly striking (Figure 2). Staining was diffusely distributed (80–90% of the cells) and predominantly cytoplasmic. Staining was similar with each of the monoclonal antibodies. In contrast, staining of tumor cells was not observed in any of the non-MFH tumors, although a small number of reactive macrophages stained positively in each. These were scattered or in small clusters and readily distinguished from the diffuse staining observed in MFH (Figure 3).

T-200 Staining

All 4 examples of MFH demonstrated intense staining for T-200 antigen (Table 2). Staining appeared both membranous and cytoplasmic, and in each case the overwhelming majority of tumor cells (80–90%) stained (Figures 1 and 2). Staining of all three cellular constituents was observed: histiocytelike cells, spindle cells, and some pleomorphic giant cells. In Case 4, the storiform spindle cells stained strongly. In contrast, staining of tumor cells was not observed in any of the 8 other soft tissue tumors. Scattered inflammatory cells and macrophages in these tumors were stained, but this pattern was readily distinguishable from the diffuse cellular staining observed in fibrohistiocytic neoplasms (Figure 3).

S-34 Staining

Expression of the Ia antigens as detected by the S-34 monoclonal reagent was distributed similarly to T-200 (Table 2). All 4 examples of MFH stained strongly and diffusely, including the spindle cells of Case 4. In the non-MFH tumors only scattered inflammatory cells and macrophages stained; the tumor cells were uniformly unstained.

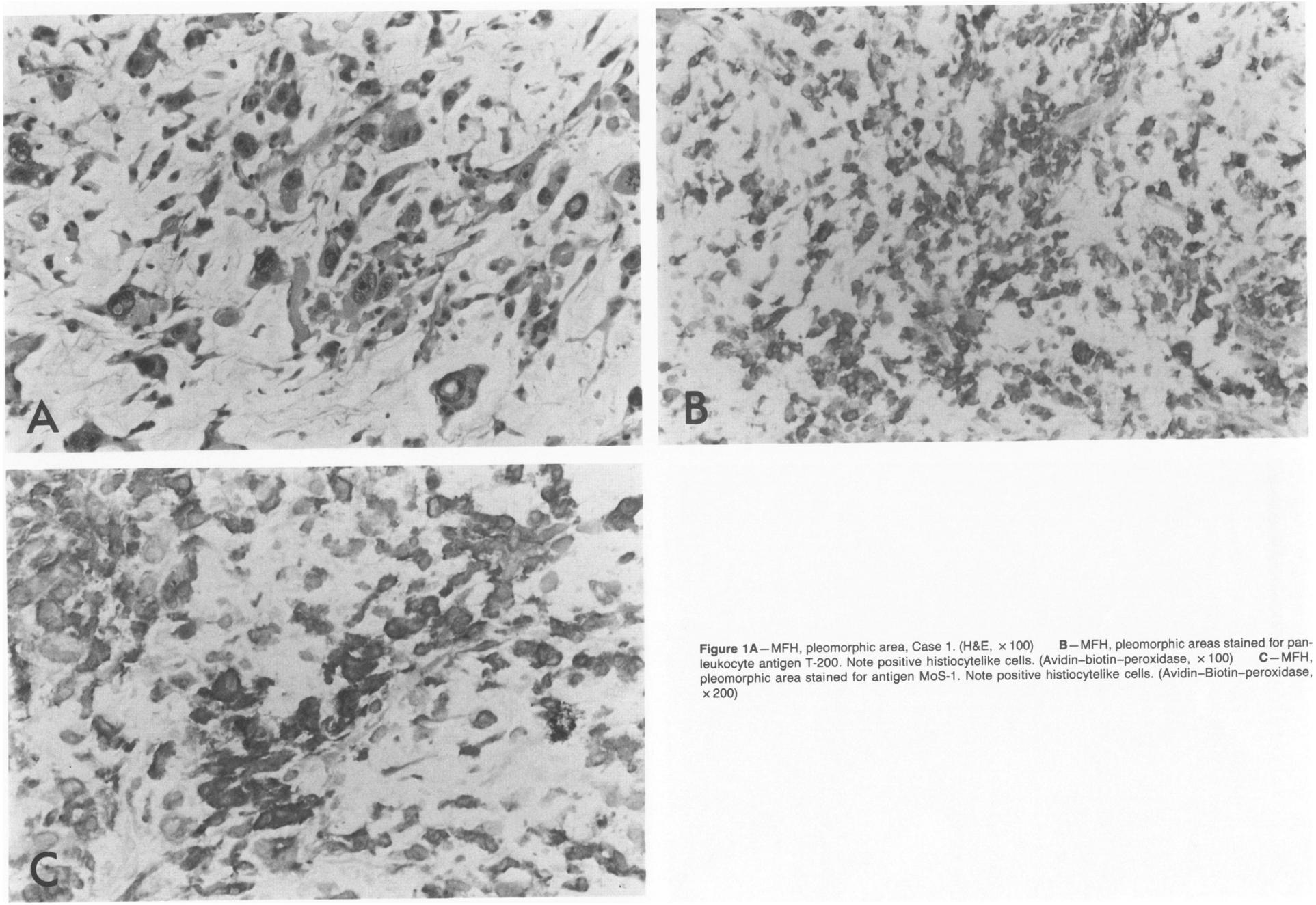
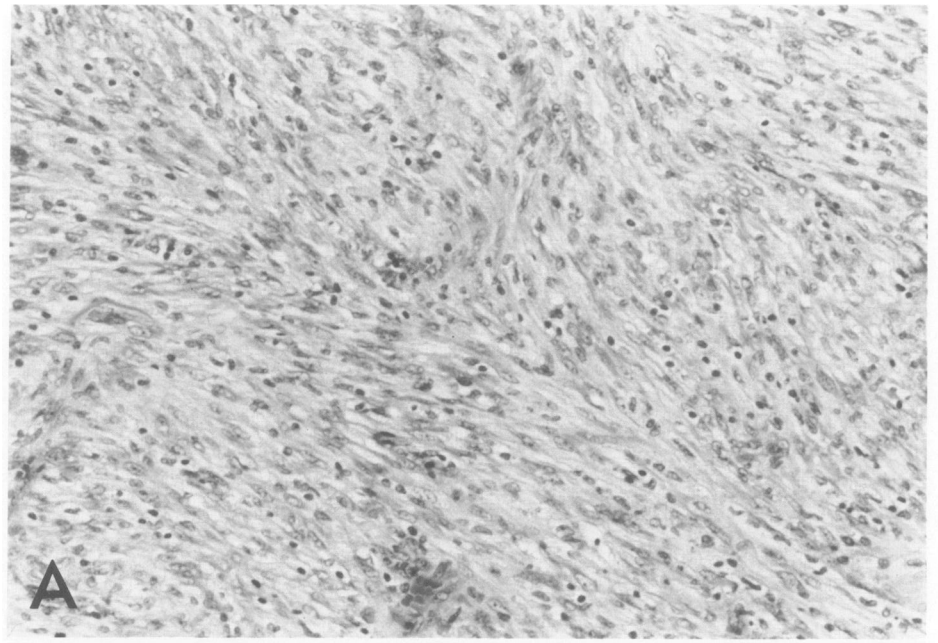
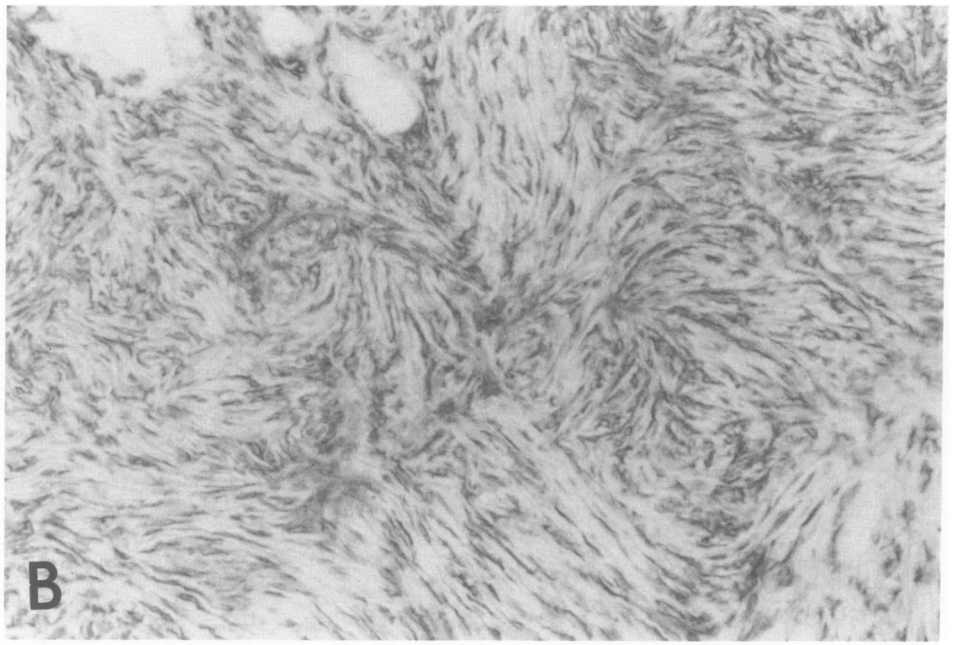


Figure 1A—MFH, pleomorphic area, Case 1. (H&E, ×100) **B**—MFH, pleomorphic areas stained for panleukocyte antigen T-200. Note positive histiocytelike cells. (Avidin–biotin–peroxidase, ×100) **C**—MFH, pleomorphic area stained for antigen MoS-1. Note positive histiocytelike cells. (Avidin–Biotin–peroxidase, ×200)





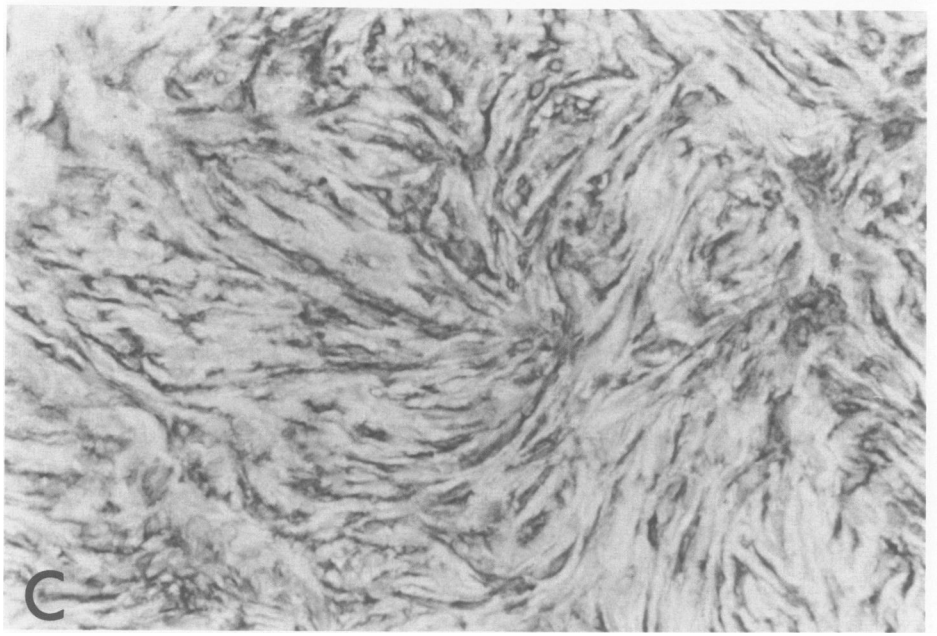
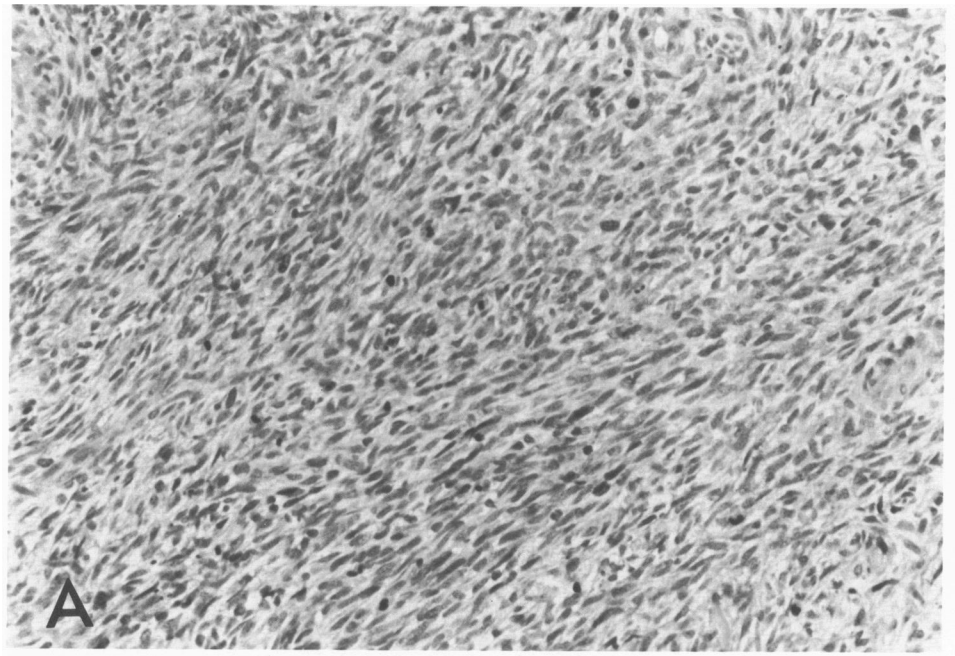
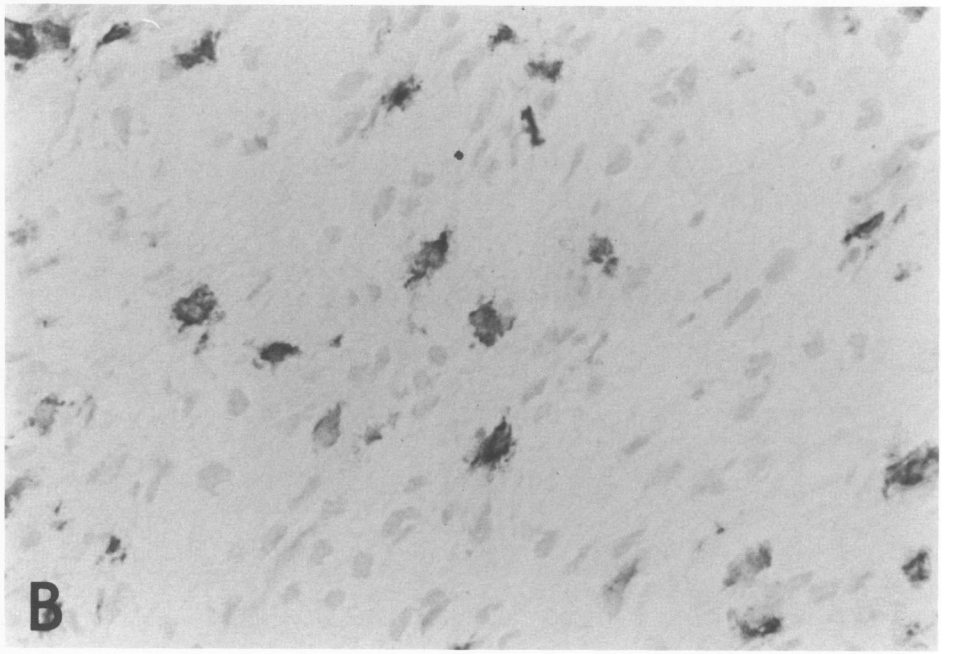


Figure 2A—MFH, storiform-fibroblastic area, Case 4. (H&E, ×100) **B**—MFH, storiform fibroblastic area stained for pan-leukocyte antigen T-200. Note positive whorled spindle cells. (Avidin–Biotin–peroxidase, ×100) **C**—MFH, storiform-fibroblastic area stained for MoS-1. Note positive whorled spindle cells. (Avidin–biotin–peroxidase, ×200)





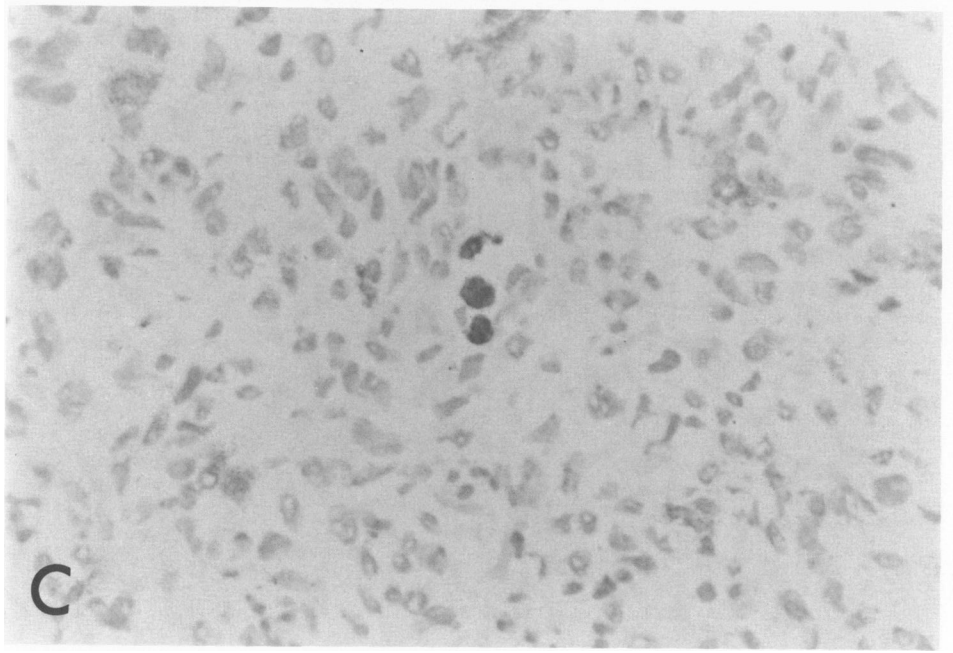


Figure 3A—Malignant schwannoma, Case 12. Patient had documented Von Recklinghausen's disease, (H&E, ×100) **B**—Malignant schwannoma stained for pan-leukocyte antigen T-200. Scattered inflammatory cells are strongly positive. Tumor cells are negative. (Avidin–biotin–peroxidase, ×100) **C**—Malignant schwannoma stained for MoS-1. The tumor cells do not express monocyte/macrophage antigens. Scattered macrophages (*center*) are positive. (Avidin–biotin–peroxidase, ×200)

Table 2—Staining of Tumor Cells With Monoclonal Antibodies

Case	Histology	T-200	HLA-DR	MoS-1	MoS-39	MoR-17
1	MFH	+ + +	+ + +	+ + +	+ + +	+ + +
2	MFH	+ + +	+ + +	+ +	+ + +	+ + +
3	MFH	+ + +	+ + +	+ + +	+ + +	+ + +
4	MFH	+ + +	+ + +	+ + +	+ + +	+ + +
5	Nodular fasciitis	–	–	–	–	–
6	Leiomyoma	–	–	–	–	–
7	Hemangioendothelioma	–	–	–	–	–
8	Liposarcoma	–	–	–	–	–
9	Leiomyosarcoma	–	–	–	–	–
10	Ewing's sarcoma	–	–	–	–	–
11	Malignant schwannoma	–	–	–	–	–
12	Malignant schwannoma	–	–	–	–	–

Table 3—Antibodies Studied

Antibody	Specificity	Distribution
T-200	Pan-leukocyte	Lymphocytes, granulocytes, monocyte/macrophages
S-34	Ia-like	B cells, monocyte/macrophages, activate T cells, others.
MoS-1	MPS	Monocytes, fluid macrophages
MoS-39	MPS	Monocytes, fluid macrophages
MoR-17	MPS	Tissue macrophages

Discussion

Malignant fibrous histiocytoma belongs to a family of fibrohistiocytic neoplasms the cells of which have features of both fibroblasts and histiocytes. Histogenesis of these neoplasms is controversial with proponents of origin from tissue histiocytes (macrophages) and from undifferentiated soft tissue mesenchyme.

The possible histiocytic derivation of MFH was first suggested by the results of in vitro tissue culture.[4,5] The ability of histiocytes to act under some circumstances as fibroblasts ("facultative fibroblasts") is central to this theory of the histogenesis of MFH. Cloning of an MFH in soft agar has resulted in growth of stem cell lines with the features of macrophages.[19] Immunohistochemical studies for histiocytic enzymes has also been applied to these tumors. Although lysozyme has generally not been demonstrated,[20] α_1-antitrypsin and α_1-anti-chymotrypsin have been shown to be focally present.[21] Support for the derivation of MFH from histiocytes also comes from experimental studies in mice. Yumoto and Morimoto demonstrated that tissue implantation of transformed bone marrow histiocytes in mice is followed by the development of transplantable soft tissue storiform-fibroblastic tumors.[22] These tumors were histologically indistinguishable from MFH in humans.

The alternative derivation of MFH from undifferentiated mesenchymal cells has been suggested by ultrastructural studies.[6-8] Electron microscopy of MFH has generally shown three cell types: 1) a fibroblastic cell with elongated nuclei, prominent nucleoli, and abundant rough endoplasmic reticulum—some of these cells also demonstrate prominent actinlike filaments and resemble "myofibroblasts"; 2) a histiocytelike cell with oval nuclei, prominent Golgi, and numerous lysosomes and lipid droplets; and 3) undifferentiated mesenchymal cells with scant cytoplasm devoid of organelles. It has been hypothesized that the latter is the neoplastic progenitor which gives rise to the other two cell types. Experimental evidence for the derivation of histiocytelike cells from fibroblasts is available in mice. Culture of murine 3T3 fibroblasts in human serum results in transformation to histiocytelike cells with acid hydrolase content, HLA-DR, and phagocytosis.[23] The significance of this finding for differentiation of macrophage histiocytes *in vivo* is not known.

Histiocytes (or macrophages) are a part of the MPS a network of phagocytic and antigen presenting cells evident in lymph nodes, spleen, and other tissues.[9-11] Most evidence suggests that macrophages are derived from blood monocytes and therefore ultimately from the bone marrow. In recent years, monoclonal antibodies have been developed which recognize differentiation antigens expressed on cells of the MPS.[12-15] These include T-200 (pan leukocytic antigen), Ia-like antigens (HLA-DR), and specific monocyte macrophage antigens MoS1 and MoS39 (expressed predominantly on fluid macrophages) and MoR17 (expressed on tissue macrophages).[16,17] In the present study these monoclonal antibodies were used in the study of tissue from MFH and other soft tissue tumors. MFH was found on a high percentage of cells to express antigens expressed on cells of monocyte macrophage lineage (T-200, Ia, MoS1, MoS-39, and MoR-17). These antigens are expressed on spindle as well as histiocytic-appearing cells, which supports the view that the spindle cells of MFH develop by morphologic alteration of histiocytes (so-called facultative fibroblasts). In contrast, 8 non-MFH tumors did not demonstrate tumor cell staining for these antigens (although in most a population of scattered reactive macrophages was identified within the tumor).

Two other recent studies of MFH have not supported a macrophage origin. Woods et al[24] studied 13 cases of MFH with monoclonal antibodies and noted absent reactivity for HLA-DR, L3 B12 (a common leukocyte antigen) and Leu-M3 (a monocyte macrophage antigen) and favored a fibroblastic derivation. Roholl et al[25] studied 15 cases of MFH with monoclonal antibodies and found positive reactivity for HLA-DR in 8 cases (8 of

11 of storiform-pleomorphic subtype) but negative reactivity for pan-leukocyte antigen (T 29/33) and macrophage antigens (FMC 17, MAC-1, OKM-1, Leu-M1). HLA-DR reactivity was confined to MFH and was not identified in any of 15 other soft tissue tumors. Both studies favored fibroblastic derivation. Our results, however, favor the derivation of MFH from macrophages. The differences observed in these studies may relate to the different antibodies utilized which were not identical. Of note, Roholl et al[25] did find reactivity of MFH with anti-HLA-DR, as we did. HLA-DR reactivity is a feature of macrophage histiocytes, as well as other cell types. The reactivity was observed only in MFH and not other soft tissue tumors in agreement with our findings. Other differences in the studies may be due to case selection. It is possible that only a subset of cases diagnosed morphologically as MFH are truly of histiocytic origin and that the rest represent a final common pathway of dedifferentiation of other soft tissue tumors.

In summary, the cells of 4 cases of MFH were found to express antigens selectively expressed on monocyte macrophages. The study supports the view that MFH is a tumor of the mononuclear phagocyte system. The prominent facultative fibroplasia and resulting sarcoma-like growth pattern of MFH may be related to the biology of soft tissue localized histiocytes.

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