Malignant Mesotheliomas

Improved Differential Diagnosis From Lung Adenocarcinomas Using Monoclonal Antibodies 44-3A6 and 624A12

INCHUL LEE, JAMES A. RADOSEVICH, GREGORIO CHEJFEC, YIXING MA, WILLIAM H. WARREN, STEVEN T. ROSEN, and VICTOR E. GOULD

From the Department of Pathology, Rush-Presbyterian-St. Luke's Medical Center, The Department of Medicine, Section of Medical Oncology, Northwestern University/VA Lakeside Medical Center, The Department of Pathology, Hines VA Hospital/Loyola University Medical School and The Department of Thoracic Surgery, Rush-Presbyterian-St. Luke's Center, Chicago, Illinois

Forty-three malignant pleural mesotheliomas and 10 known metastatic pulmonary adenocarcinomas to the pleura were studied by immunohistochemistry using monoclonal antibodies 44-3A6 and 624A12. Monoclonal antibodies 44-3A6 and 624A12 were raised against human pulmonary carcinoma cell lines; they recognize a membrane-associated protein of 40,000 mol wt and a specific sugar sequence of lacto-N-fucopentose III, respectively. Samples were also studied with a broad-spectrum anti-keratin antibody and a polyclonal antibody to carcinoembryonic antigen (CEA). These investigations were performed on formalin-fixed and paraffin-embedded tissues. The mesotheliomas comprised only grossly evident, pleurectomized, or pneumonectomized cases; they included 22 epithelial, 15 biphasic, and 6 spindle cell types. Electron-microscopic study was also done on 9 cases. None of the mesotheliomas was immunoreactive to 624A12, while 9/10 metastatic pulmonary adenocarcinomas were convincingly immunoreactive. Monoclonal antibody 44-3A6 immunostained all of the metastatic adenocarcinomas strongly, whereas only 10/43 mesotheliomas were focally and weakly immunoreactive. The latter included 5 epithelial and 4 biophasic mesotheliomas and 1 spindle cell mesothelioma; the immunoreaction was confined to scattered single cells, and the staining pattern was readily discernible from that of adenocarcinomas. Forty of 43 mesotheliomas were strongly immunoreactive with the broad-spectrum anti-keratin antibody, whereas 8/10 metastatic pulmonary adenocarcinomas showed focal and rather weak staining. Seven of 10 metastatic adenocarcinomas were immunoreactive to anti-CEA antibody, while only 15/43 mesotheliomas displayed weak immunoreactivity. It is concluded that monoclonal antibodies 44-3A6 and 624A12 are excellent phenotypic markers of metastatic pulmonary adenocarcinomas to the pleura and thus are useful for the differential diagnosis of pleural mesotheliomas. Given conventionally fixed and processed tissues, it appears that the combined use of these monoclonal antibodies may be more effective for that differential diagnosis than anti-CEA and anti-keratin antibodies. (Am J Pathol 1986, 123:497-507)

MALIGNANT MESOTHELIOMAS may be regarded as carcinomas, given their consistent cytokeratin expression.[1] They are classified into three histologic subtypes: epithelial, spindle cell, and biphasic (mixed). Given only a small biopsy, epithelial mesotheliomas may pose serious diagnostic problems because they may form glandular or tubular structures which are often difficult to distinguish from pulmonary adenocarcinomas extending to or metastatic to the pleura. Many attempts have been made to define conventional morphologic criteria or phenotypic markers that may lead to the unequivocal diagnosis and identification of mesotheliomas. These include various electron-microscopic criteria (for reviews see Bolen and Thorning[2] and Warhol et al[3]), histochemical reactions for acidic or neutral mucopolysaccharides, and immunohistochemical staining for various antigens such as CEA,[4-9] certain "milk

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Address reprint requests to Inchul Lee, MD, Department of Pathology, Rush-Presbyterian-St. Luke's Medical Center, 1753 W. Congress Parkway, Chicago, IL 60612.

fat globule" antigens,[8] cytokeratin,[6-10] and unspecified "mesothelial antigens."[11]

It has been established that pulmonary adenocarcinomas as well as neuroendocrine carcinomas express "simple epithelium type" cytokeratins as their single or predominant intermediate filament cytoskeleton.[12,13] Recently, it was demonstrated by immunofluorescence and two-dimensional gel electrophoresis that these "simple epithelium type" cytokeratins can be found in all types of mesotheliomas, most of which, however, also express additional cytokeratins such as polypeptides 5 and, sometimes, 4, 6, 14, or 17.[1] Nevertheless, considerable amounts of freshly frozen tissue are required for immunohistochemical and biochemical analysis, and these procedures are too complex and time-consuming for conventional diagnostic pathology laboratories.

In an attempt to establish simple and consistent diagnostic criteria for the differential diagnosis of mesotheliomas from metastatic pulmonary adenocarcinomas, we have studied their immunohistochemical patterns using two novel monoclonal antibodies (Mab). Mab 44-3A6 was developed against a human pulmonary adenocarcinoma cell line, A549[15]; it recognizes a membrane-associated protein antigen of 40,000 mol wt.[15] Mab 624A12 was raised against a human pulmonary "small cell carcinoma" cell line NCI-H69,[14,16] and it recognizes a specific sugar sequence found in lacto-N-fucopentose III.[17] Both antigens are expressed by human pulmonary adenocarcinomas as well as certain normal tissues.[18,19] The antigens are well preserved after conventional formalin fixation and paraffin embedding. We have found that these Mab's preferentially immunostain metastatic pulmonary adenocarcinomas to the pleura, discriminating them from mesotheliomas; therefore, we suggest that they may be useful adjunct tools for this difficult differential diagnosis.

Materials and Methods

Monoclonal antibodies 44-3A6 and 624A12 were made against cell lines A549 and NCI-H69, respectively, using the well-defined hybridoma techniques[20]; details of the procedure have been described elsewhere.[15]

For immunohistochemical staining of monoclonal antibodies, 7-μ sections of tissue were prepared. Following deparaffinization, immunohistochemical staining was performed with the avidin–biotin complex method (Vector Laboratories, Burlingame, Calif) as previously described.[18,19,21] The Mab's were used at a concentration of 1 μg/ml. Slides were then counterstained with hematoxylin for 1.5 minutes, dehydrated, and mounted. Negative controls were performed by omitting the primary antibodies and substituting with nonimmune serum.

The peroxidase–antiperoxidase technique was used for immunohistochemical staining of CEA and keratin. The anti-keratin antibody was generously provided by Dr. I. Virtanen, University of Helsinki, Helsinki, Finland. This is a monoclonal antibody currently marketed by Labsystems Inc., Helsinki, Finland; it was raised against cytokeratin of canine renal epithelium of the MDCK cell line,[22] and it recognizes a broad spectrum of cytokeratin polypeptides.[13] It was used at a 1:200 dilution. The polyclonal anti-CEA antibody was obtained from the Dako Corporation (Santa Barbara, Calif). It was used at a 1:200 dilution. After conventional deparaffinization, sections were treated with a solution of 0.05 g of trypsin in 50 ml of 0.1% calcium chloride for 30 minutes. Subsequently, they were incubated with primary antibodies for 30 minutes and washed. The goat anti-rabbit bridging antibody (Bionetics Laboratory, Charleston, SC) was used at a 1:20 dilution. The peroxidase–antiperoxidase (rabbit, Dako Corporation) was used at 1:100 dilution. 3'3-Diaminobenzidine (Aldrich Chemical Company, Danvers, Mass) was used as chromogen. Positive controls for CEA and keratin were provided by immunostaining colon carcinomas and oral mucosa respectively. Negative controls were provided by substituting nonimmune goat serum for the primary antibody. The slides were graded as "focal" when the immunostained cells were less than 30% of the whole neoplastic cells. The immunoreaction was graded from + to +++ according to the intensity of staining.

Samples of malignant pleural mesotheliomas were formalin-fixed and paraffin-embedded; they were obtained from the surgical pathology file of the Rush-Presbyterian St. Luke's Medical Center, Chicago, for the period 1965–1985. Only grossly evident, pneumonectomized or pleurectomized specimens were included so that questionable or controversial cases could be avoided. They included 22 epithelial, 15 biphasic, and 6 spindle cell types. Patients comprised 36 males and 7 females ranging from 36 to 93 years of age at the time of diagnosis. No significant difference related to age was noted in different types of mesothelioma groups. The overall perioperative mortality was 13.5%. Thirty seven patients died, with a maximal survival of 60 months; 6 patients are still alive at from 3 to 43 months after surgery.

Ten metastatic pulmonary adenocarcinomas to the pleura were also studied; these cases were selected on the basis of a previously established diagnosis of primary pulmonary adenocarcinoma and the known absence of another primary adenocarcinoma. Patients ranged from 47 to 64 years of age. Seven patients died, with a maximal survival of 15 months; 3 patients are alive at from 4 to 18 months after the diagnosis. Tumor samples included either pulmonary resections or

pleural biopsies; both pleural metastases and the original lung primaries were studied.

Ultrastructural studies were performed on 5 epithelial, 2 biphasic, and 2 spindle cell type mesotheliomas. At the time of resection, random samples of tumor were diced, fixed in *s*-collidine-buffered 2% glutaraldehyde, washed in tris buffer, postfixed in 1% osmium tetroxide, dehydrated in graded alcohols, and embedded in Epon. Thin sections were stained with uranyl acetate and lead hydroxide; they were examined with a Philips EM 300 electron microscope.

Results

Light and Electron Microscopy

Twenty-two epithelial mesotheliomas were examined (Table 1). Grossly, the pleura was diffusely involved by the tumors (Figure 1a). Some mesotheliomas extended into the subjacent lung parenchyma. By light microscopy, no spindle cell areas were noted. Cells were arranged in solid, tubular, papillary, or reticular patterns (Figure 1b). Nuclei were ovoid, usually vesicular, centrally located, and variable in size; the nucleoli were generally single. The cytoplasm was pale to eosinophilic and variable in amount. Mitotic activity varied. Focal necrosis was frequent and was more prominent in tumors with a high mitotic rate. However, necrosis was rarely extensive. Four cases presented with lymph node metastases. They included periaortic, mediastinal, and carinal lymph nodes. Metastatic mesotheliomas in lymph nodes displayed morphologic features similar to those of the primaries. Ultrastructurally, the epithelial mesotheliomas consisted of cells linked by frequent and prominent desmosomes (Figure 1f). Basal lamina was frequently present, with occasional reduplication. The nuclei were round to ovoid, with coarse chromatin pattern; nucleoli were prominent, with a frequent nucleonema pattern. The cytoplasm was abundant; it contained abundant intermediate filaments and occasional microfilaments and microtubules. Undulating bundles of intermediate filaments (tonofilaments) were also seen. Rough endoplasmic reticulum, mitochondria, and lysosomes were occasionally present. Microvilli were prominent; their shape and distribution were variable.

Fifteen biphasic mesotheliomas comprised both epithelial and spindle cell components (Table 2). The number of spindle cells varied, constituting 10 to 70% of the entire cell population. They were irregularly intermixed with epithelial cells (Figure 2a). The collagenous stroma was dense and abundant, as compared with the epithelial mesotheliomas. Many spindle cells displayed nuclear pleomorphism and hyperchromatism. The cytoplasm was eosinophilic and variable in amount. There were also frequent "transitional" cells which could not be readily classified as either fusiform or epithelial cells. Mitoses were readily found. Necrosis was frequent and often extensive. Ultrastructurally, the biphasic mesotheliomas consisted of tightly apposed round and spindle cells (Figure 2f). The round cells displayed ultrastructural characteristics similar to those of the cells in epithelial mesotheliomas. However, microvilli were

Table 1—Epithelial Mesothelioma

Patient	Age/Sex	Survival	Operation	44-3A6	624A12	Keratin	CEA
1	32/M	5 months	Pn	–	–	+ +	–
2	58/M	13 months	Pn	–	–	+ +	+F
3	57/M	4 months	Pn	–	–	+	–
4	53/F	59 months	Pn	–	–	+	+F
5	46/F	60 months	Pn	–	–	+	–
6	57/M	57 months	Pl	–	–	+	–
7	55/M	5 months	Pl	–	–	+	–
8	49/M	2 days	Pn	–	–	+F	+F
9	57/M	13 months	Pn	+F	–	+ + +	+F
10	56/F	Alive 43 months	Pn	–	–	+	–
11	56/F	13 months	Pn	–	–	+ +	+
12	69/M	5 months	Pn	–	–	+ +	+F
13	62/M	Lost	Pl	–	–	+ +F	+F
14	58/M	12 months	Pn	–	–	+	+F
15	46/F	Alive 24 months	Pn	–	–	+	–
16	64/M	21 months	Pn	–	–	+	–
17	55/M	12 months	Pl	+F	–	+F	–
18	55/M	55 months	Pl	+F	–	+	–
19	71/M	Alive 3 months	Pl	–	–	+ + +	–
20	52/M	Alive 3 months	Pn	–	–	+F	–
21	38/M	5 months	Pn	–	–	+F	–
22	51/M	12 months	Pl	–	–	+F	–

Pl, pleurectomy; Pn, pneumonectomy; +, weak immunostaining; + +, moderate immunostaining; + + +, strong immunoreaction; F, focal immunoreaction (less than 30% immunostained cells).

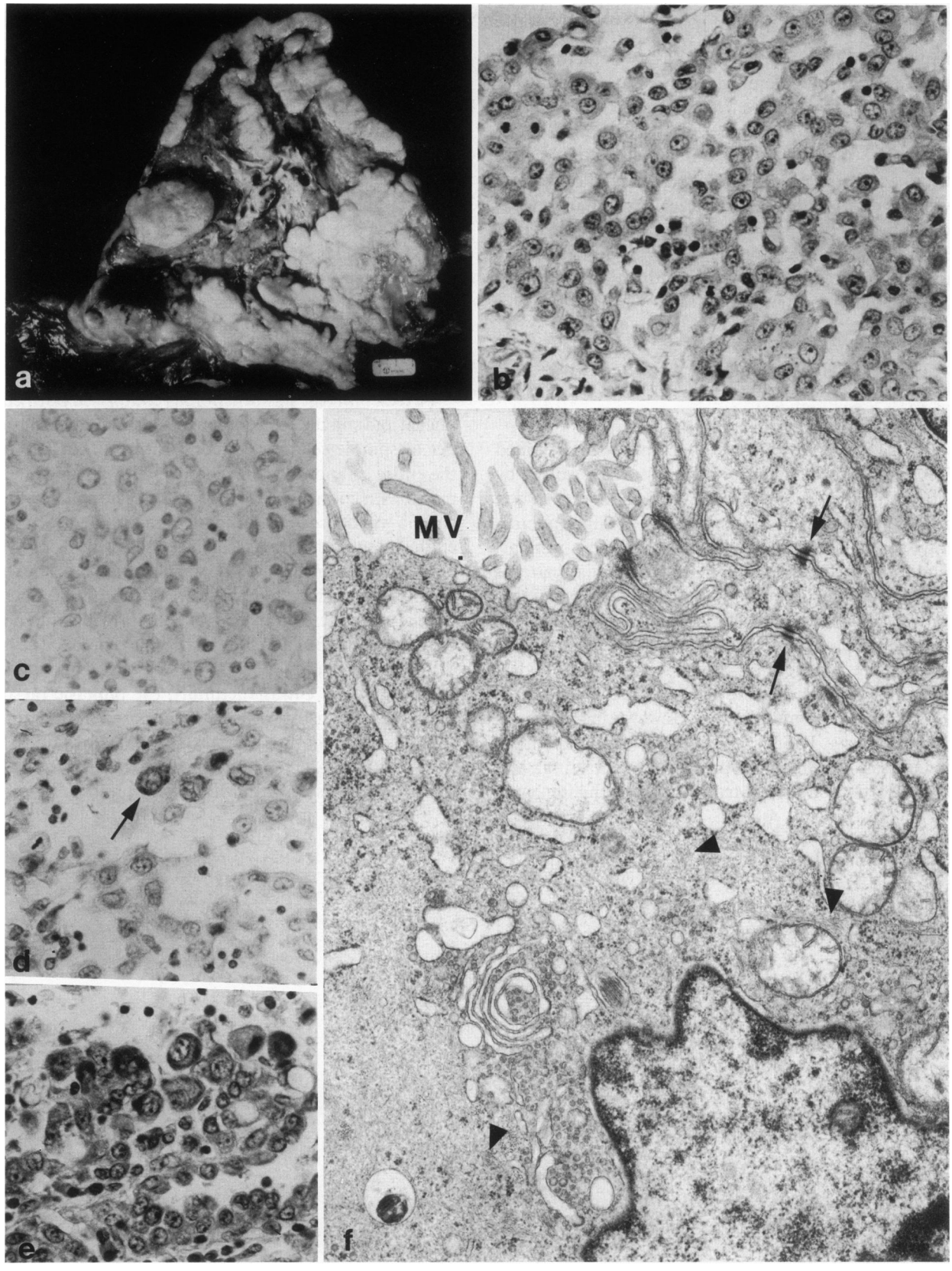

Figure 1—Epithelial mesothelioma. **a**—Pneumonectomized specimen; note diffuse tumor involvement of pleural surfaces and obliteration of the interlobar septa. **b**—Note irregular reticular arrangement. (H&E, ×250) **c**—Note lack of immunostaining with 624A12. (×250) **d**—Mab 44-3A6 immunostains occasional, single cells (*arrow*), but most cells are not stained. (×250) **e**—Papillary area displaying richly immunoreactive cells for keratin. (×250) **f**—Electron micrograph displaying complex microvilli (*MV*), desmosomes (*arrows*), and scattered intermediate filaments (*arrowheads*). (×16,200)

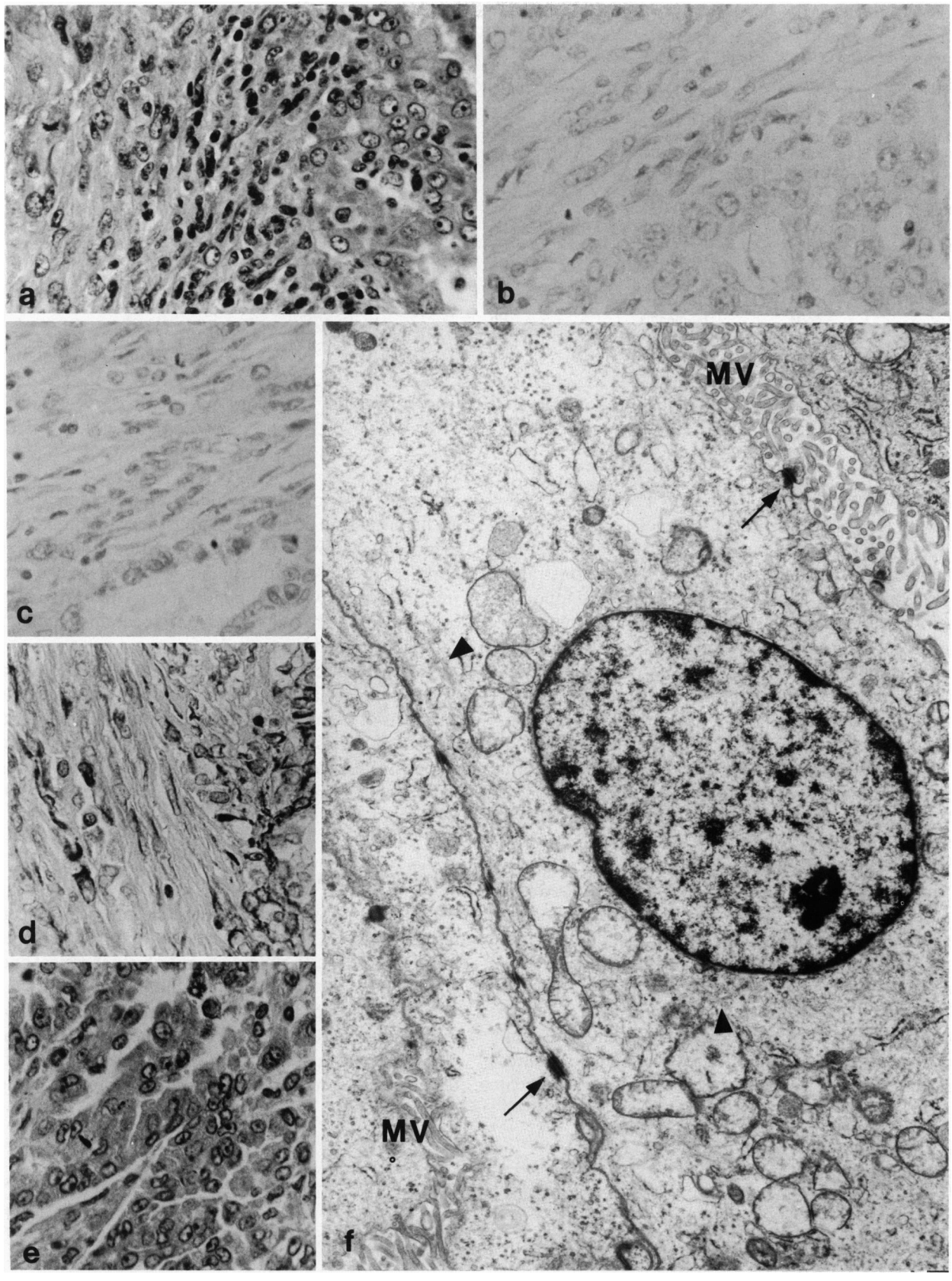

Figure 2—Biphasic mesothelioma. **a**—Note coexistence of epithelial and spindle cells. (H&E, ×250) **b**—The cells are not immunostained with 624A12. (×250) **c**—The cells are not immunoreactive with 44-3A6. (×250) **d**—Immunostaining for keratin; note that both epithelial cells and occasional fusiform cells are stained. (×250) **e**—Immunostaining for CEA displaying moderate immunoreactivity. (×250) **f**—Electron micrograph displaying elongated cells with frequent desmosomes (*arrows*), microvilli (*MV*), and scattered intermediate filaments (*arrowheads*). (×6600)

Table 2—Biphasic Mesothelioma

Patient	Age/Sex	Survival	Operation	44-3A6	624A12	Keratin	CEA
1	54/M	3 months	Pn	–	–	–	–
2	65/M	5 days	Pl	+F	–	+F	–
3	48/M	30 months	Pn	–	–	++	–
4	60/M	11 months	Pn	–	–	++	–
5	73/M	11 months	Pl	–	–	–	–
6	59/M	6 months	Pn	–	–	++	–
7	67/M	7 months	Pn	–	–	++	+
8	36/M	8 months	Pn	–	–	+	–
9	72/M	2 months	Pn	+F	–	+	+
10	72/M	8 months	Pl	–	–	+	+
11	63/F	Op death	Pl	–	–	+	–
12	44/M	60 months	Pn	–	–	+	–
13	53/M	3 months	Pn	+F	–	+F	–
14	66/F	Alive 5 months	Pl	+F	–	+F	+F
15	65/M	14 months	Pl	–	–	–	–

Pl, pleurectomy; Pn, pneumonectomy; +, weak immunostaining; ++, moderate immunostaining; F, focal immunoreaction (less than 30% immunostained cells).

distinctively less well developed. The spindle cells showed elongated, pleomorphic nuclei with a coarse chromatin pattern and inconspicuous nucleoli. The epithelial nature of the fusiform cells was demonstrated by the presence of occasional desmosomes and tonofilaments. Rough endoplasmic reticulum and free ribosomes were occasionally present. There were occasional cells displaying focally thickened plasma membrane domains reminiscent of the attachment plaques seen in smooth muscle cells; however, these cells did not display other characteristics of leiomyocytes.

Six spindle cell mesotheliomas consisted of fusiform cells without a readily identifiable epithelial component (Table 3). The cells frequently formed irregularly intertwined bundles. Many cells had pleomorphic and hyperchromatic nuclei. Nucleoli were occasionally prominent. Mitoses were frequent. The stroma varied in amount and density. Necrosis was frequent and extensive (Figure 3a). Ultrastructurally, spindle cell mesotheliomas consisted of elongated cells with occasional cell junctions (Figure 3f). The cell junctions were mostly rudimentary; however, true desmosomes were noted. Basal lamina was focally present. The nuclei were elongated and often markedly folded, with a coarse chromatin pattern. There were occasional prominent nucleoli. The cytoplasm contained abundant intermediate filaments and occasional parallel arrays of microfilaments and microtubules. There were rare tonofilament bundles. Rough endoplasmic reticulum cisternae and free ribosomes were frequently seen. There were also occasional pinocytotic vesicles and focal thickening of cytoplasmic membranes.

Ten metastatic lung adenocarcinomas to the pleura were examined (Table 4). They displayed irregular glands and nests of atypical cells in a variously fibrotic stroma (Figure 4a); these features were similar to those seen in epithelial mesotheliomas. However, necrosis was more frequent and extensive than in epithelial mesotheliomas.

Immunohistochemistry

By immunohistochemistry, the distinction between immunoreactive and negative cells with our Mab's was readily apparent. Pertinent immunohistochemical and clinical data are summarized in Tables 1–4.

None of the epithelial mesotheliomas was immunoreactive with 624A12 (Figure 1c), whereas 5/22 were focally immunostained with 44-3A6 (Figure 1d). The latter immunostaining was weak and confined to single

Table 3—Spindle Cell Mesothelioma

Patient	Age/Sex	Survival	Operation	44-3A6	624A12	Keratin	CEA
1	65/M	5 days	Pn	+F	–	++	+F
2	93/M	3 months	Pl	–	–	+	+F
3	58/M	3 months	Pn	–	–	++	–
4	63/M	6 months	Pn	–	–	+F	–
5	68/M	1 day	Pn	–	–	+F	+F
6	77/M	Alive 7 months	Pl	–	–	+F	+F

Pl, pleurectomy; Pn, pneumonectomy; +, weak immunostaining; ++, moderate immunostaining; F, focal immunoreaction (less than 30% immunostained cells).

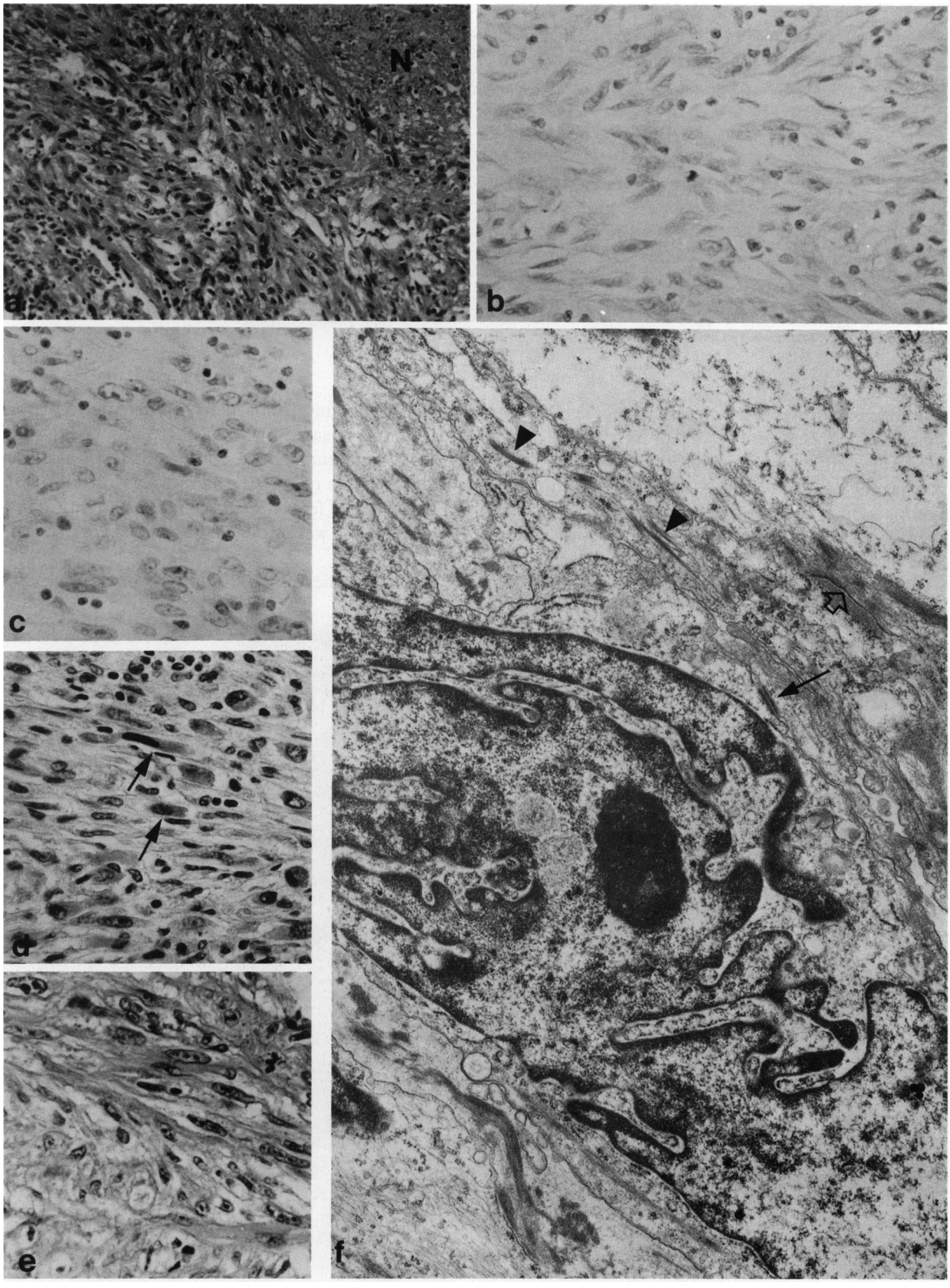

Figure 3—Spindle cell mesothelioma. **a**—Note bundles of fusiform cells and focal necrosis (*N*). (H&E, ×175) **b**—The cells are not immunostained with 624A12. (×250) **c**—The cells are not immunostained with 44-3A6. (×250) **d**—Occasional cells displaying immunoreactivity for keratin (*arrows*). **e**—Focal and weak immunoreactivity for CEA. (×250) **f**—Electron micrograph displaying fusiform cells with markedly folded nucleus, occasional desmosomes (*arrow*), tonofilaments (*arrowheads*), and focal thickening of cell membrane reminiscent of attachment plaques of leiomyocytes or myofibroblasts (*open arrow*). (×5700)

Table 4—Metastatic Lung Adenocarcinoma

Patient	Age/Sex	Survival	Operation	44-3A6	624A12	Keratin	CEA
1	59/M	15 months	Biopsy	+ +F	+ +F	+ +	+
2	49/M	4 months	Pn	+ + +	+ +	+ +	+F
3	49/M	3 months	Biopsy	+ + +	–	+	+ + +
4	40/M	Op death	Biopsy	+ + +	+ +F	+ +	+ +F
5	56/M	3 months	Pn	+ +	+ +F	+ + +F	+ +
6	55/M	13 months	Biopsy	+ + +	+ +	+ +	+ +
7	57/M	Alive 18 months	Biopsy	+ +	+ +	+ +	+ +F
8	47/M	Alive 5 months	Biopsy	+F	+ +	–	–
9	64/M	Alive 4 months	Biopsy	+ +F	+ +	–	–
10	57/M	4 months	Biopsy	+ +	+ +F	+	–

Pl, pleurectomy; Pn, pneumonectomy; +, weak immunostaining; + +, moderate immunostaining; + + +, strong immunoreaction; F, focal immunoreaction (less than 30% immunostained cells).

scattered cells in papillary or reticular areas. No immunoreaction was present in solid or tubular areas. Mab 44-3A6 also immunostained reactive Type II pneumocytes as previously noted.[18] All cases were prominently immunostained with the anti-keratin antibody (Figure 1e), although the extent of the immunoreaction varied. Eight of 22 epithelial mesotheliomas were focally and weakly immunoreactive with the anti-CEA antibody. None of the 4 metastatic mesotheliomas in lymph nodes was immunoreactive with 624A12; however, they were focally immunoreactive with 44-3A6. The primary mesothelioma of 1 of the 4 cases was also focally immunoreactive for 44-3A6; the immunoreaction in the lymph nodes was stronger than in the corresponding primary.

None of the biphasic mesotheliomas was immu-

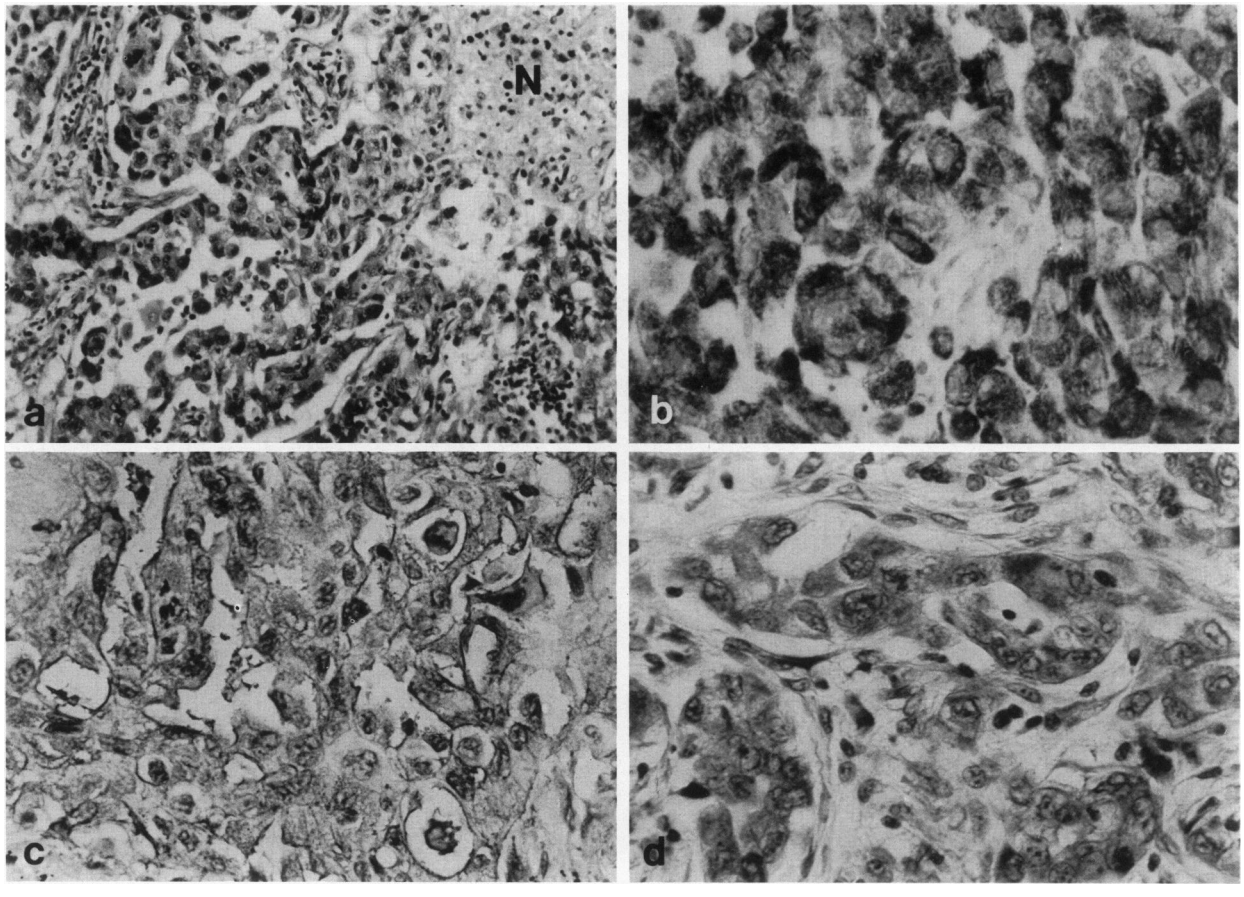

Figure 4—Metastatic pulmonary adenocarcinoma. **a**—The tumor consists of glands and cords of cells with extensive necrosis (*N*). (H&E, ×175) **b**—Strong and diffuse immunoreactivity with 44-3A6. (×250) **c**—Diffuse immunoreactivity with 624A12; note the immunoreaction outlining the cell membranes. (×250) **d**—Diffuse immunoreactivity for CEA. (×250)

noreactive with 624A12 (Figure 2b). Two of 15 cases displayed focal immunoreactivity with 44-3A6. The immunoreaction was weak and confined to occasional single epithelial and/or spindle cells. No solid epithelial cell nests were immunoreactive (Figure 2c). Twelve of 15 cases were immunoreactive with antikeratin antibody either focally or diffusely; the immunoreaction was mostly in the epithelial cells. However, occasional spindle cells were also convincingly immunostained (Figure 2d). Four of 15 cases were focally and very weakly immunostained for CEA (Figure 2e).

None of the spindle cell mesotheliomas was immunoreactive with 624A12 (Figure 3b); only 1/6 cases immunostained focally with 44-3A6 (Figure 3c). The immunoreaction was weak and confined to scattered spindle cells in areas with comparatively loose stroma. All cases were focally immunoreactive with anti-keratin antibody (Figure 3d). Four of 6 cases were focally and weakly immunostained with CEA antibody (Figure 3e).

All 10 metastatic pulmonary adenocarcinomas were strongly immunoreactive with 44-3A6 (Figure 4b); the immunoreaction occurred at the cell membrane and/or in the cytoplasm. All but 1 of the adenocarcinomas were convincingly immunostained with 624A12 either diffusely or focally (Figure 4c). The immunoreaction was usually at the cell membranes; however, occasional large cells also displayed intracytoplasmic immunostaining. Eight of 10 cases were immunostained focally and rather weakly with anti-keratin antibody. Seven of 10 cases immunostained for CEA (Figure 4d); immunostaining was convincing, although the intensity and extent varied.

Discussion

The pathologic differential diagnosis between malignant pleural mesotheliomas, especially the epithelial type, and metastatic pulmonary adenocarcinomas based on conventional microscopic studies is notoriously difficult. The problem becomes more difficult, if not insoluble, when only small tissue fragments are available for pathologic examination. To solve this serious diagnostic dilemma, many adjunct techniques have been advocated, including immunohistochemistry using antikeratin and anti-CEA antibodies as well as electron microscopy.[2-10]

We have studied and compared the patterns of immunoreaction of 43 malignant pleural mesotheliomas and 10 metastatic pulmonary adenocarcinomas to the pleura using two novel Mab's 44-3A6 and 624A12. Mab 44-3A6 was made against a human pulmonary adenocarcinoma cell line A549; it is an IgG1 isotype and recognizes a membrane-associated protein of 40,000 mol wt. The function of this antigen is not known. However, it is distributed in certain exocrine and endocrine cells such as gastric parietal cells, testicular Leydig cells, pancreatic islet cells, and sebaceous glands of skin.[18] MCA 624A12 was raised against a human pulmonary "small cell carcinoma" cell line NCl-H69; it is an IgM isotype and recognizes a sugar sequence found in lacto-N-fucopentose III.[17] In a previous investigation, all of 42 pulmonary adenocarcinomas studied were strongly immunostained with Mab 44-3A6,[18] and all except for one were also convincingly immunoreactive with Mab 624A12 (in preparation). It is noteworthy that the only negative case had been irradiated before surgical removal. It can thus be suggested that Mab's 44-3A6 and 624A12 are effective immunomarkers for pulmonary adenocarcinomas.

In the present study, both monoclonal antibodies 44-3A6 and 624A12 immunostained metastatic adenocarcinomas discriminatively from mesotheliomas. No mesothelioma was immunostained with 624A12, while all but one metastatic pulmonary adenocarcinoma were strongly immunoreactive. In this context, 624A12 was 100% effective for metastatic pulmonary adenocarcinomas. All metastatic adenocarcinomas were strongly immunostained with 44-3A6, whereas only 9/43 mesotheliomas were focally and weakly immunoreactive.

Mab 44-3A6 may be considered rather "oversensitive" for this differential diagnosis. However, the staining pattern of the occasional, immunoreactive mesotheliomas was evidently distinct from that of metastatic pulmonary adenocarcinomas. The immunoreaction in mesotheliomas was weak and confined to foci of poor cellular cohesion; no immunoreaction was observed in solid cellular areas. We may speculate that the 44-3A6 antigen may be expressed or "unmasked" when the mesothelioma cells lose tight cellular apposition. Similarly, Mab 624A12 might be viewed as comparatively "undersensitive" for metastatic adenocarcinoma. We currently regard these two antibodies as complementary for this differential diagnosis.

The value of immunohistochemistry for keratin and CEA for the differential diagnosis between mesotheliomas and metastic adenocarcinomas has been the subject of considerable controversy.[6-10] It has been established that the cytokeratin family consists of at least 19 polypeptides (see reviews [23-25]); cytokeratin subtyping of fresh frozen tissues using various monoclonal antibodies and two-dimensional gel electrophoresis has been successfully applied to diagnostic pathology. For example, all pulmonary adenocarcinomas and neuroendocrine carcinomas express "simple epithelial type" cytokeratins, such as Polypeptides 7, 8, 18, and 19; whereas squamous cell carcinomas express "stratified epithelial type" cytokeratins such as 4, 5, 6, 13, 14, 15, and 17.[12,13] Mesotheliomas are carcinomas because they

express cytokeratins as their predominant class of intermediate filaments; however, they are very special carcinomas, for they express a wide variety of cytokeratin polypeptides simultaneously and frequently coexpress vimentin.[1] Recently, it was demonstrated that spindle and biphasic type mesotheliomas express the "simple epithelial type" cytokeratins, whereas most epithelial mesotheliomas, in addition to the "simple epithelial type" cytokeratins, express additional cytokeratin polypeptides such as Polypeptide 5 and, sometimes 4, 6, 14, and 17.

Although we used a "broad spectrum" cytokeratin antibody in this study, 3 biphasic mesotheliomas and 2 metastatic adenocarcinomas were not immunostained. A number of immunohistochemical studies have been reported on mesotheliomas and adenocarcinomas using various anti-keratin antibodies.[6-10] The results have been variable and apparently contradictory, partly because 1) the immunoreactivity of cytokeratins, especially the "low-molecular-weight polypeptides," may be altered or masked by formalin fixation and other routine histologic procedures and 2) the antibodies used in those studies also varied considerably in their range of recognition of different cytokeratin polypeptides. Prestaining treatment of sections with various proteases may be helpful in antigenic "unmasking." These problems are readily avoided if sufficient amounts of freshly frozen tissue are utilized for two-dimensional gel electrophoresis and for immunohistochemistry using monoclonal antibodies with well-defined antigenic affinities. These procedures are highly reliable but require "ideally" preserved tissue and are often regarded as too complicated to be performed "routinely." Moreover, small biopsies are often insufficient for both biochemical and immunohistochemical analyses.

CEA, originally defined and isolated by Gold and Freedman,[26] is a highly glycosylated protein with a molecular weight of 180 kd. It soon became evident that "CEA" comprises a heterogeneous group of glycoproteins which are different in carbohydrate composition and amino acid sequence, even when isolated from a single tumor.[27,28] Using monoclonal antibodies and two dimensional electrophoresis, Grunert et al demonstrated that CEAs in lung tumors were different from those of breast tumors.[29] In addition to colonic carcinomas, the majority of pulmonary adenocarcinomas are also known to express CEA, whereas mesotheliomas either do not express it or express it weakly. However, the results of immunohistochemical staining of pulmonary adenocarcinomas and mesotheliomas with anti-CEA polyclonal antibodies have varied considerably, partly because of the increasingly recognized heterogeneity of CEA. In this context, it has been shown that preabsorption of polyclonal anti-CEA antibodies with normal tissue antigens reduces the cross-reaction to the "nonspecific cross-reacting antigen."[30,31] In the present study, the aforementioned "preabsorption" technique was not used. This may explain the comparatively frequent albeit weak CEA immunoreactivity shown by some mesotheliomas; however, the negative CEA immunoreactivity in several proven pulmonary adenocarcinomas would still remain unclear.

Electron-microscopic study is helpful in the diagnosis of mesotheliomas. In most cases, the epithelial nature of mesotheliomas is readily demonstrable by features such as desmosomes and glandular lumens displaying complex microvilli. Spindle cells also frequently display such epithelial features in addition to occasional "myofibroblast-like" characteristics. However, since these ultrastructural features may also be present in adenocarcinomas, it is often very difficult to differentiate between the two tumors. Indeed, this confusion is not very surprising, since it is frequently impossible to differentiate a given type of adenocarcinomas from another by electron microscopy. We believe that electron microscopy, while occasionally very helpful for the differential diagnosis of mesotheliomas, can no longer be viewed as a choice adjunct tool. Similarly, studies of acid mucopolysaccharide containing hyaluronic acid demonstrated by histochemistry, while interesting *per se*, are no longer significant diagnostic adjunct tools.[32]

With the simultaneous use of these Mab's, we believe, the differential diagnosis between mesothliomas and pulmonary adenocarcinomas could be consistently made on conventionally fixed and processed paraffin sections of not only pleurectomized or pulmonary resection specimens but also small pleural biopsies. We conclude that both 44-3A6 and 624A12 are excellent adjunct diagnostic tools for the differential diagnosis between mesotheliomas and metastatic adenocarcinomas, and are superior to conventional keratin and CEA antibodies unless two-dimensional gel electrophoresis and/or panels of monoclonal antibodies with well-defined antigenic affinities are applied. However, our current knowledge of immunostaining patterns of carcinomas metastatic to the lung as compared with lung primaries with these Mab's is incomplete; therefore, caution is required in interpreting positive results if the latter issue is in question. That these Mab's can be readily and effectively applied to conventional diagnostic cytologic smears is noteworthy.[33]

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