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Differentiation of Adenocarcinoma of the Lung from Mesothelioma

Periodic Acid-Schiff, Monoclonal Antibodies B72.3, and Leu M1

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The immunohistochemical reactivity of 38 mesotheliomas and 44 adeno-carcinomas or large cell carcinomas of the lung with monoclonal antibodies (MAb) B72.3 and Leu M1 was compared with their reactivity with the routine histochemic stains periodic acid-Schiff with diastase digestion (PAS-D) and alcian blue ± hyaluronidase. Both MAbs reacted selectively with carcinomas when a positive test was set at ≥10% reactive tumor cells. However, MAb B72.3 reacted with significantly more of the carcinomas (86%, chi-square test, $P < 0.01$) and bound to a greater percentage of tumor cells (47 ± 28%; mean ± SD, *t*-test, $P < 0.001$) than Leu M1 (57% and 25 ± 28%, respectively). The similar reactivities of surgically resected tumor specimens and post mortem tissues with both antibodies confirmed antigen stability and suggested broad clinical utility. PAS-D stained 61% of the carcinomas. Using the markers for carcinomas (PAS-D, B72.3, and Leu M1), the tumors were classified into the correct group in 80 of 82 (98%) cases (95% confidence level: >92% accuracy). The alcian blue stain was useful to confirm a diagnosis of dimorphic or epithelial mesothelioma (48% were positive). (Am J Pathol 1988, 133: 30–38)

THE DIFFERENTIATION of carcinoma from mesothelioma in certain cases remains a matter of judgement despite advances in diagnostic techniques. Histochemical stains for neutral mucin (periodic acid-Schiff after diastase digestion, PAS-D) in adenocarcinomas and for acid mucins (alcian blue with and without hyaluronidase pretreatment, AB ± H) in mesotheliomas have been considered diagnostic, but they are negative in many tumors.[1-5] Electron microscopy also is not always definitive.[6-8] Many investigators have studied the usefulness of immunohistochemical techniques to aid in the diagnosis.[5,9-15] To date no single test has been shown to distinguish reliably and reproducibly between the two types of tumor; however, recently two new monoclonal antibodies (MAb) have shown promise in delineating lung carcinoma from mesothelioma.[16-18]

MAb B72.3, derived from immunizing with a membrane-enriched fraction of human breast cancer, reacts with a >1,000,000 dalton glycoprotein termed TAG-72 (tumor-associated glycoprotein).[19,20] It is widely reactive with different types of carcinomas and has shown no reactivity with neural, hematopoietic, or mesenchymal neoplasms or the vast majority of benign adult tissues.[21] MAb B72.3 has demonstrated selective reactivity with carcinoma cells and lack of affinity for reactive mesothelium in cytologic studies of human effusions.[22] It has been shown to be a useful adjunct for the diagnosis of carcinoma in fine-needle aspiration biopsies[23,24] and also has been used to distinguish malignant mesothelioma from adenocarcinoma.[17,18]

MAb Leu M1 (MMA), originally produced by immunizing with a human-derived monocytic cell line,

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reacts with a differentiation antigen on myelomonocytic cells.[25] Empirically, it has been found to react with lymphomas and a number of different carcinomas.[15,26-29] Almost all mesotheliomas tested have shown no reactivity with either antibody in early studies.[16-18,28] This study confirms these specificities, examines the concordance of MAb B72.3 and Leu M1 reactivity, and compares these with the results of the PAS-D and AB ± H stains.

Materials and Methods

Specimen Selection

Paraffin-embedded, formalin-fixed tissues from adenocarcinomas and large cell undifferentiated carcinomas of the lung as well as mesotheliomas of the pleura or peritoneum were obtained from the consultation files of one of the authors. One paraffin block was selected. The area of the tumor examined varied considerably, but all of the tissue sections had at least an equivalent of two low power fields of tumor. Both surgical and autopsy specimens were used. Primary lung carcinomas were classified by light microscopy according to the World Health Organization criteria[30] into the categories adenocarcinoma, large cell carcinoma, or combined adenocarcinoma and other carcinoma. The adenocarcinomas were subdivided into the categories papillary, well-differentiated, poorly differentiated, or bronchioloalveolar carcinomas. Subjects with metastatic cancer to the lung were excluded.

Mesotheliomas were classified as definite or probable. Definite mesotheliomas had a typical pleural or peritoneal distribution at surgical exploration or autopsy, no other primary site identified, a typical histologic pattern,[31] presence of intracellular acid mucin indicated by alcian blue reactivity removed or substantially reduced by pretreatment with bovine testicular hyaluronidase, and absence of reaction with PAS-D. Cases classified as probable mesothelioma lacked only a positive reaction with AB ± H. The patient's exposure to asbestos was assessed by review of the clinical record or by an interview with the patient or next of kin as described previously.[32]

Monoclonal Antibodies

MAb B72.3, isotype IgG_1, purified according to previously published techniques,[19,20] was obtained from Dr. Jeffrey Schlom, National Institutes of Health, Bethesda, MD. As a negative control, isotypic identical MAb MOPC-21 (IgG_1) (Sigma, St. Louis, MO) was used on serial sections under similar conditions. MAb Leu M1 was obtained from Becton Dickinson, Mountain View, CA.

Immunoperoxidase Methods

The reactivities of MAb B72.3 and Leu M1 were studied by a modification of the ABC immunohistochemical method described by Hsu et al.[26] Five-micrometer tissue sections cut from paraffin blocks were mounted on clean uncoated slides and heated at 60 C for 1 hour. The sections were deparaffinized in xylene and rehydrated in graded alcohols.

For MAb Leu M1 all reactions were performed at room temperature, and all dilutions were made in calcium- and magnesium-free phosphate buffered saline. The procedure described by Szpak et al[17] was followed, using a 1:50 dilution of Leu M1 for 30 minutes as the primary antibody. Control sections of each tumor were treated in the same way except that a 10% solution of horse serum was substituted for the primary antibody.

The procedure for MAb B72.3 was as outlined[17] using a concentration of 0.04 mg/ml with an incubation time of 12 hours at 4 C before treatment with the biotinylated antibody. Control sections were incubated with MAb MOPC-21 using the same concentration and conditions. The test for Leu M1 was repeated on a different day, and the test for B72.3 was performed once for each tumor. Control tissues with known reactivity for each MAb were included in all assays to ensure interassay reproducibility.

Interpretation of MAbs

The slides were coded and read by each of the authors separately. Blinding as to tumor type was not absolute, however, because of the characteristic histologic features of many of the tumors. After some practice sessions together, each of the three authors estimated the percentage of positive, viable tumor cells in the whole slide. Both cytoplasmic and membrane staining were counted, but very faint staining was ignored. The percentages were then averaged for the case. As in the past, ≥10% reactive cells was required to define a positive reaction.[17] The authors also noted whether the staining was diffuse or patchy. Control slides were evaluated by each author at the same time as the test slides. Controls were negative in all cases.

Histochemical Procedures

To detect acid mucins the alcian blue stain was performed at pH 2.5.[33] Absence or attenuation of blue

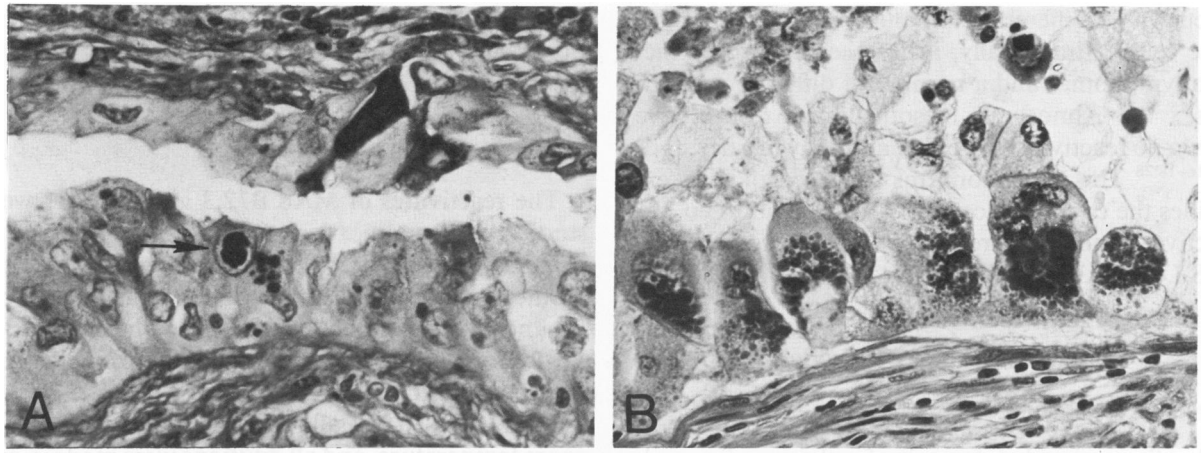

Figure 1A—Adenocarcinoma stained with PAS-D showing a characteristic, positive-staining, target-like intracellular mucin droplet (arrow). (×330) **B**—Adenocarcinoma stained with PAS-D showing predominantly infranuclear, positive-staining hyaline droplets that should not be confused with mucin. (×300)

staining after pretreatment of a serial section with bovine testicular hyaluronidase (0.5 mg/ml, cat. no. H-2376 Type IV, Sigma, St. Louis, MO) overnight at 4 C was required to define a positive test.

To detect neutral mucins diagnostic of adenocarcinomas staining with PAS-D was performed.[33] One section from each tumor was scanned serially with overlapping fields at a magnification of ×450 when a positive reaction was not readily visible. Two types of staining were considered positive. Tumors with apparent intracytoplasmic vacuoles, 4 μ in diameter or larger, with a sharply circumscribed red rim and a central reticular network or a central red mass surrounded by a clear halo (Figure 1A) were counted as positive if more than 25 were present in each section. The number 25 was chosen arbitrarily to avoid equivocal reactions. Tumors with definite luminal secretion within nests of tumor cells were also counted as positive. This staining was often accompanied by apical fine secretory granules in tumor cells. These scattered or clustered fine PAS-positive granules, which also occurred in some mesotheliomas, were not counted as positive unless accompanied by luminal secretion, however. Other types of PAS-D positive cytoplasmic inclusions to be distinguished from mucin included hyaline droplets (Figure 1B), lysosomes (Figure 2), and phagocytized cellular debris.

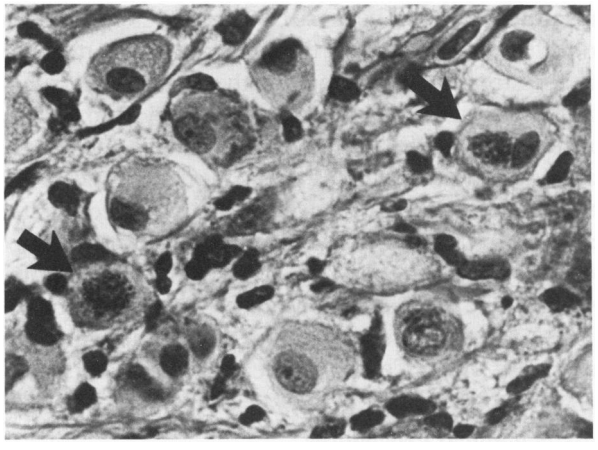

Figure 2—Peritoneal mesothelioma stained with PAS-D showing cells with clustered, fine positive cytoplasmic granules (arrows) often adjacent to the nucleus. These do not signify mucin. (×400)

Statistical Methods

Analysis of variance, the *t*-test, and the chi-square test were used where appropriate.[34] A P value of <0.05 was taken to indicate a significant difference. To determine the ability of the tests to discriminate between carcinoma and mesothelioma, false positive, false negative, and misclassification rates were evaluated. Because the classification rule was prespecified and not determined from these data, the simple misclassification rates are applicable. Confidence intervals for these rates were computed using the asymmetric intervals of Fleiss,[35] except that the exact method[36] was used when the rate was 100%. Because the rates were all high, one-sided lower bounds were used for the confidence intervals.

Results

Demographic Data: Mesotheliomas

Sections of tumor tissue (9 surgical specimens; 29 autopsy specimens) from 38 subjects with mesothelioma were examined. The histochemical and immu-

Table 1—Histochemical and Immunochemical Activity of Mesotheliomas by Type

Type of case	Histochemical reactivity		Immunochemical reactivity (≥10%)		Histologic type	Location
	PAS-D	AB*	B72.3 +/−	Leu M1 +/−	Epith/Di/Sarc†	PlR/L/Per‡
Definite (14)	0/14	14/14	0/14§	0/13§	7/7/0	8/4/2
Probable (24)	0/23	0/23	0/24‖	0/24§	5/10/9	15/6/2

* Alcian blue ± hyaluronidase. PAS-D and AB ± H were not performed on one sarcomatous mesothelioma.
† Epithelial/Dimorphic/Sarcomatous.
‡ Pl R, Right pleura; L, Left pleura; Per, Peritoneum. The origin of one probable pleural tumor was unknown.
§ One tumor had 5% reactivity. Leu M1 was not run on one mesothelioma because of insufficient tissue.
‖ One tumor had 5% and one had 2% reactivity. Reactivity with one of the antibodies was never accompanied by reactivity with the other.

nohistochemical reactivity, and type and location of tumors classified as definite or probable are listed in Table 1. The average age of the 31 autopsied subjects was 64 ± 7 (mean ± SD) years. Thirty-five subjects were men and 3 were women. All but one subject had a known history of exposure to asbestos. Twelve tumors were purely epithelial, 9 were sarcomatous, and 17 were dimorphic. No autopsy was performed in four subjects (three probable tumors), and three subjects are alive (one probable tumor).

Histochemical Data: Mesotheliomas

None of the tumors had PAS-positive cytoplasmic vacuoles after diastase digestion although scattered fine granules, presumably lysosomes, (not diagnostic for mucin, see Materials and Methods) were present occasionally and sometimes were numerous (Figure 2). Fourteen (48%) of 29 epithelial or dimorphic tumors had alcian blue-positive vacuoles in the cytoplasm. Usually the blue stain formed a rim at the edge of the vacuole with faint staining centrally. This staining, which was removed completely or attenuated by hyaluronidase pretreatment in all 14 instances, conferred a definite diagnosis of mesothelioma. Stromal staining, not specific for mesothelioma,[2,31] was ignored. The remaining tumors were considered probable mesotheliomas for this study.

Immunohistochemical Reactivities: Mesotheliomas

The monoclonal antibodies B72.3 and Leu M1 reacted with less than 10% of the malignant mesothelial cells in all cases (Table 1). Five percent of tumor cells showed reactivity with B72.3 in one definite dimorphic and one probable epithelial mesothelioma. Two percent of tumor cells showed reactivity in another probable epithelial mesothelioma. The reactivity with B72.3 in the mesotheliomas that stained was predominantly cytoplasmic, but had some membrane reactivity. Five percent of cells showed reactivity with Leu M1 in one definite epithelial and one probable dimorphic mesothelioma. The Leu M1 reactivity, when present, showed a localization similar to that of B72.3. Stroma and ground substance were uniformly negative with both antibodies, but normal respiratory and glandular epithelium sometimes showed reactive membranes and apical granules, and reactive type II epithelial cells also were sometimes positive with MAb B72.3. The immunohistochemical reaction with Leu M1 was more difficult to interpret than the reaction with B72.3 because of the reactivity of neutrophils or mononuclear phagocytes. These cells frequently invaded tumor, were sometimes phagocytosed by tumor cells, or were present within vessels in the tumor.

Demographic Data: Carcinomas

Tumor tissue from 44 subjects with adenocarcinoma or large cell carcinoma was examined. Twenty-five tumors were from patients undergoing surgical resections, and 19 were obtained at autopsy. The average age of the autopsied subjects was 62 ± 10 years. Thirty of the tumors were from men and 14 from women.

Histochemical and Immunohistochemical Data: Carcinomas

The tumor type, histologic differentiation, and PAS-D reactivity for surgical specimens (Table 2) and autopsy specimens (Table 3) were indistinguishable (chi-square test).[34] The PAS-D stain was positive (≥25 typical droplets present, Figure 1A) in 27 of 44 (61%) of the tumors. When the PAS-D-stained slides with fewer than 25 positive droplets by scanning serially across the tumor at a magnification of ×450, were reviewed, 10–24 unequivocal droplets were found in three cases, 1–9 in four cases and none in ten cases.

Table 2—Histochemical and Immunochemical Activity of Carcinomas by Type (Surgical Specimens)

Case no.	Histochemical reactivity PAS-D	Immunochemical reactivity (%) B72.3	Leu M1	Type of tumor Pap/WD/PD/ LCC/BA*
1	+	80	20	WD
2	+	72	10	WD
3	+	63	19	WD
4	+	37	20	WD
5	–	53	22	WD
6	+	89	1	WD
7	+	5	38	WD
8	–	73	1	WD
9	–	97	5	WD
10	+	73	63	WD
11	+	79	90	BA
Mean ± SD		66 ± 26	26 ± 28	
12	+	51	52	PD
13	–	33	70	PD
14	+	35	57	PD
15	–	43	10	PD
16	–	82	2	PD
17	+	47	70	PD
Mean ± SD		48 ± 18	44 ± 30	
18	–	13	0	LCC
19	–	66	4	LCC
Overall mean ± SD		57 ± 25	29 ± 29	

* Papillary/well-differentiated/poorly differentiated/large cell carcinoma/ bronchioloalveolar.

The two tumors that lacked reactivity for both MAbs (nos. 35 and 39, see below) had no unequivocally positive droplets. Artefacts can be confused with mucin droplets, however, and therefore it is recommended that 25 droplets be present to diagnose adenocarcinoma.

The distribution of reactivity for MAbs B72.3 and Leu M1 is shown graphically in Figure 3. As in a previous study,[17] to avoid controversy over positive or negative, ≥10% reactivity was required to consider a specimen positive. MAb B72.3 was positive (at least 10% of cells reactive) in 37 of 43 (86%) tumors, and MAb Leu M1 was positive in statistically fewer tumors (25 of 44 [57%], chi-square test, $P < 0.01$). The average percent cellular reactivity with B72.3 was 47 with a standard deviation of 28. The average Leu M1 reactivity, in comparison, was statistically less (25 ± 28%, *t*-test, $P < 0.001$). The average reactivity for the surgical specimens did not differ from that of the autopsy specimens for either antibody using statistical analysis (analysis of variance[34]). For B72.3, but not for Leu M1, the mean reactivity of the well-differentiated adenocarcinomas was greater than that of the poorly differentiated tumors for both surgical and autopsy specimens, but the differences were not significant (analysis of variance[34]).

The staining for both B72.3 and Leu M1 was diffusely cytoplasmic in most cases although some tumors had predominantly membrane staining, and mixed patterns occurred (Figure 4). Secretions were often positive with both antibodies, but only cellular positivity was counted for the percentages given.

To summarize, PAS-D was positive in 61% of the adenocarcinomas (and large cell cancers) and in none of the mesotheliomas; B72.3 was positive in ≥10% of cells in 86% of adenocarcinomas, and Leu M1 was positive in 57% of adenocarcinomas. Neither MAb was positive with the mesotheliomas. PAS-D and B72.3 together were positive in 42 of 44 (95%) of carcinomas, and PAS-D, B72.3, and Leu M1 together did not increase the rate. The 10% positivity cutoff was predetermined, and no better cutoff emerged in this study. Using these classifications, all tumors were cor-

Table 3—Histochemical and Immunochemical Activity of Carcinomas by Type (Autopsy Specimens)

Case no.	Histochemical reactivity PAS-D	Immunochemical reactivity (%) B72.3	Leu M1	Type of tumor Pap/WD/PD/ LCC/BA/Di*
20	–	88	40	WD
21	+	47	21	WD
22	+	56	58	WD
23	+	20	4	WD
24	–	13	0	WD
25	+	85	0	WD
26	+	–†	38	WD
27	+	85	12	BA
Mean ± SD		56 ± 31	22 ± 22	
28	+	47	27	PD
29	+	50	0	PD
30	–	70	37	PD
31	–	70	83	PD
32	+	10	88	PD
33	+	26	67	PD
34	+	47	5	PD
35	–	4	0	PD
36	+	5	50	PD
37	–	47	0	PD
38	+	30	17	PD
39	–	8	0	PD + SCC
40	+	63	1	PD + SCC
Mean ± SD		37 ± 24	29 ± 33	
41	–	50	0	LCC
42	–	17	0	Pap
43	+	5	0	Pap
44	+	6	5	Di
Overall mean ± SD		40 ± 28	22 ± 28	

* Papillary/well-differentiated/poorly differentiated/large cell carcinoma/ bronchioloalveolar/dimorphic glandular and spindled tumor.

† Not done, insufficient tissue.

rectly classified except for two adenocarcinomas that were classified as mesothelioma.

MAb B72.3 reactivity was usually (32 of 43 cases) diffusely scattered throughout the tumor, but it was patchy in five cases (negative in six cases). In contrast, MAb Leu M1 reactivity was diffuse in only 13 tumors and patchy in 12 (negative in 19 cases). Four tumors (7, 8, 18, and 24) exhibiting patchy staining with one MAb and negative staining with the other might have been misclassified as negative if a biopsy of only two to three low-power fields had been examined.

Discussion

The results of this study show that with a combination of histochemical and immunohistochemical tests, most lung adenocarcinomas can be differentiated from malignant mesotheliomas. The immunochemical reagents used, MAbs B72.3 and Leu M1, were chosen because they have been reported to be reactive with adenocarcinomas but not with mesotheliomas.[16-18] These specificities were confirmed and the results obtained used in conjunction with standard PAS-D and AB ± H tests as a panel to differentiate adenocarcinomas or large cell carcinomas from mesotheliomas.

Compared with reports of other immunohistochemical tests proposed to distinguish adenocarcinomas and mesotheliomas, few studies on B72.3 and Leu M1 have been reported.[16-18,37,38] Leu M1, which reacts with human myeloid cells, a subpopulation of activated T cells,[25] and the neoplastic cells of Hodgkin's disease,[26-28] initially was found to be potentially useful in separating mesotheliomas from adenocarcinomas by Sheibani et al.[28] They found no reactivity in 18 mesotheliomas, but 105 of 179 adenocarcinomas derived from a large variety of organs were positive. Every adenocarcinoma of the lung was focally or diffusely positive. Subsequently they reported no activity in 28 (apparently 10 new[18]) mesotheliomas and reactivity in 47 (94%) of 50 adenocarcinomas.[16] In a more recent study of 19 mesotheliomas and 14 adenocarcinomas, one probable mesothelioma that was not confirmed by electron microscopy and seven of 14 adenocarcinomas were positive with Leu M1.[18] Present results for adenocarcinomas or large cell carcinomas were similar with 57% of tumors being positive (≥10% cellular reactivity).

The same authors also studied B72.3, which reacts with a high molecular weight mucin expressed by many different types of carcinomas.[18] As with Leu M1, they found reactivity in one probable mesothelioma but only seven of 14 adenocarcinomas. In contrast, the present percentage of adenocarcinomas or large cell carcinomas positive with B72.3 was significantly higher (86% *vs.* 50%, $P < 0.025$, chi-square test). They used an unpurified ascites preparation of B72.3 rather than the partially purified antibody that was used here (Battifora H, personal communication). Differences in specimen size, a critical factor when reactivity is focal, antibody dilution (not given), or grading methods may also account in part for the discrepancies in percentage of positive tumors.

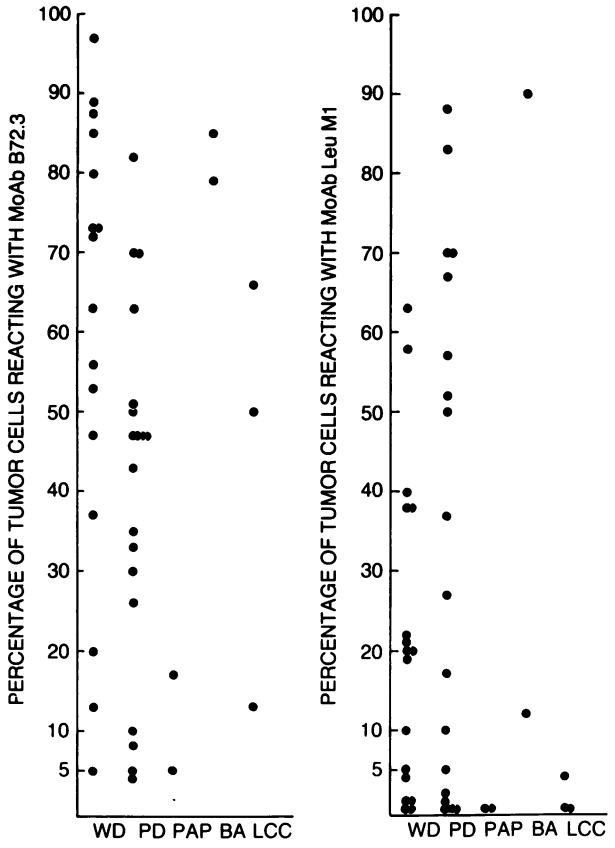

Figure 3—Distribution of reactivity of the carcinomas with MAb B72.3 and Leu M1 by tumor type. The overall average reactivity with B72.3 for 16 well-differentiated tumors was 59 ± 28% and for 19 poorly differentiated tumors was 40 ± 23%. The overall average reactivity with Leu M1 for 17 well-differentiated tumors was 21 ± 20% and for 19 poorly differentiated tumors was 33 ± 32%. WD, well differentiated; PD, poorly differentiated, PAP, papillary; BA, bronchioloalveolar; LCC, large cell cancer.

The present results for the MAb B72.3, however, were similar to those of Szpak et al,[17] who reported reactivity in at least 10% of tumor cells in 19 of 22 (86%) of adenocarcinomas of the lung and none of 20 malignant mesotheliomas. Their average score for the adenocarcinomas was 51, and the present study's was 47 ± 28. Their study also used partially purified ascites MAb B72.3 at 0.04 mg/ml with overnight assays.

The question of reactivity of adenocarcinomas metastatic to the lung, pleura, or peritoneum from else-

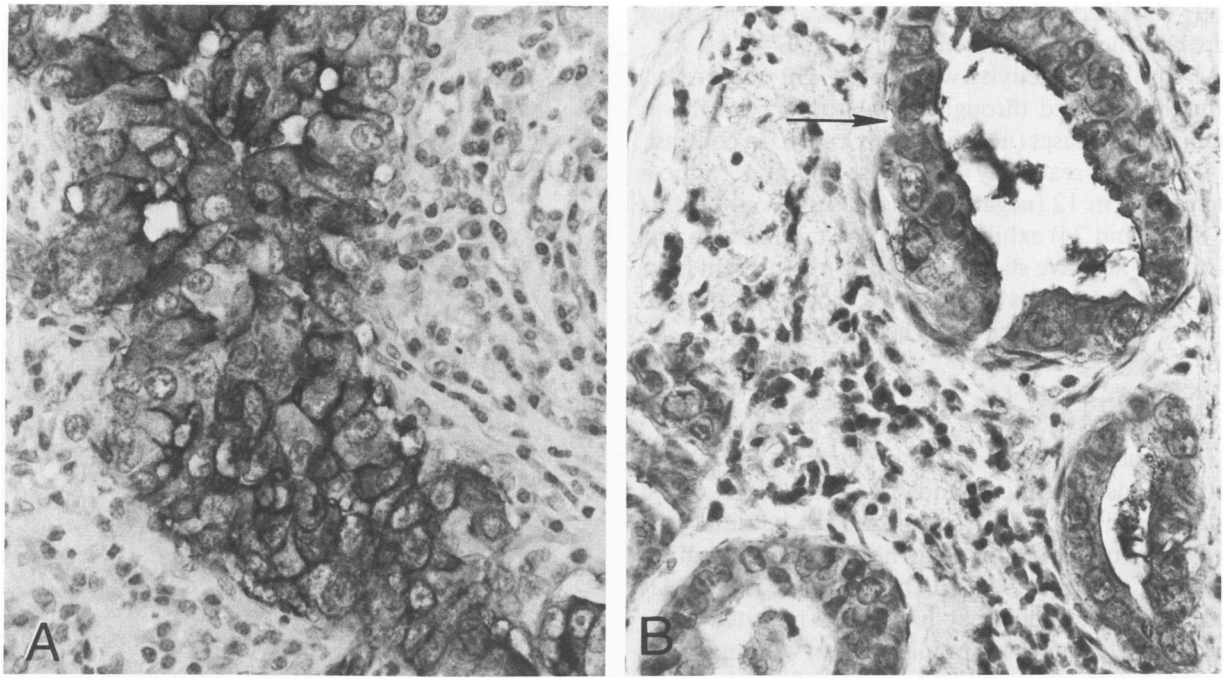

Figure 4A—Adenocarcinoma immunostained with MAb B72.3 showing strong membrane and less strong cytoplasmic stain. (hematoxylin counterstain, ×400) **B**—Adenocarcinoma immunostained with MAb Leu M1 showing predominantly luminal border staining in some (arrow) but not all glands. (hematoxylin counterstain, ×350)

where was not addressed. Summarized results of multiple studies of B72.3 with tumors from many sites show a high frequency of reactivity with lung, breast, ovarian, uterine, and gastrointestinal carcinomas and lack of reactivity with lymphomas and sarcomas.[21] This antibody also selectively stains carcinoma cells in effusions, is not reactive with benign mesothelial cells, and is suitable for smears made from fine-needle aspirates.[22–24] Thus, there is evidence that B72.3 reactivity is useful in distinguishing many different types of cancer from mesothelioma and can be used with a variety of tissue specimens. This study demonstrates for the first time antigen preservation in tissues obtained at post mortem examination.

The standard tests for confirming the diagnosis of adenocarcinoma need to be related to the immunohistochemical tests. The PAS-D stain, considered to be diagnostic,[1,2] was positive in 27 of 41 (66%) adenocarcinomas, similar to the 12 of 26 (46%) that were positive in another study.[1] The authors suspect that a search of sections of tumor at a magnification of ×450 serially with overlapping fields yields a higher percentage of positive tumors than does a casual search. Several types of PAS-D positive cytoplasmic inclusions that must be distinguished from those reliably denoting neutral mucin were encountered. The latter consisted of red-rimmed vacuoles with a central red mass and surrounding halo or a central red network. Extracellular PAS-D-positive secretion among tumor cells, when distinguished from necrotic debris, was also diagnostic of adenocarcinomas. Such extracellular secretion was often accompanied by clusters of minute PAS-D-positive granules in the apical cytoplasm of tumor cells. These granules were not used as a criterion of mucin production no matter how frequent they were, because they were indistinguishable from PAS-D-positive lysosomes, which can occur in mesotheliomas as well as adenocarcinomas. Colloid droplets and phagocytized cells, also PAS-D positive, must be distinguished from mucin. Only those tumors with widespread PAS-positivity would be expected to be positive in small biopsies, and hence, other confirmatory tests are often necessary.

The alcian blue stain also required experience for interpretation. A positive result in cytoplasmic vacuoles was usually faint. Stromal staining must be ignored. Many adenocarcinomas showed strong staining with alcian blue in cytoplasmic droplets that also stained with the PAS-D stain. The two tumors differed in that the blue stain was not weakened by pretreatment with hyaluronidase in adenocarcinomas. The alcian blue stain was positive only in mesotheliomas with an epithelial component. The present rate of positivity in 14 of 29 (48%) epithelial or dimorphic mesotheliomas was similar to the 52% reported previously[1] but differed significantly from the 1 in 23 found by

Otis et al ($P < 0.005$, chi-square test).[18] The authors found it useful to distinguish between adenocarcinoma and mesothelioma, similar to Cibas and coworkers, who used it for cell blocks of pleural effusions.[5]

In summary, the number of reported mesotheliomas examined for Leu M1 reactivity, including the present study, is now 92[16,18,28,37,38] and for B72.3 reactivity is 77.[17,18] Only one tumor was reported to be positive with Leu M1 and one with B72.3, but neither tumor had ultrastructural confirmation.[18] Using a combination of B72.3 reactivity and PAS-D positivity, all but two of the lung cancers were distinguished from mesotheliomas, giving a high degree of sensitivity (42 of 44 [95%, 95% confidence level: >86% accuracy]) for carcinomas. Although Leu M1 did not increase the rate of positivity, it showed reactivity with a greater percentage of tumor cells than the B72.3 antibody in 11 cases and therefore may be useful diagnostically in some circumstances in combination with B72.3 and histochemical stains PAS-D and AB ± H. In any case, multiple confirmatory tests should be used when the diagnosis is in question for medical, epidemiologic, or legal reasons. Routine histochemical stains as well as MAb B72.3 and Leu M1 should be performed and interpreted in a consistent manner and should require specific staining patterns and percentage positive cells to be considered diagnostically significant.

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