Antigenic Phenotype of Malignant Mesotheliomas and Pulmonary Adenocarcinomas

An Immunohistologic Analysis Demonstrating the Value of Leu M1 Antigen

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To evaluate the usefulness of an immunohistologic approach to the differential diagnosis of mesothelioma and pulmonary adenocarcinoma, the authors studied paraffin-embedded, fixed tissue sections from 50 primary adenocarcinomas of the lung and 28 mesotheliomas of the pleura by using a panel of monoclonal antikeratin, anti-human milk fat globule (HMFG-2), anti-Leu M1, and monoclonal anticarcinoembryonic antigen (CEA) antibody; we also used a conventional heterologous anti-CEA antiserum with and without prior absorption with spleen powder to remove antibodies to nonspecific cross-reacting antigen (NCA). Keratin was present in both mesotheliomas and adenocarcinomas and did not help in distinguishing between these two neoplasms. HMFG-2 was detected in 48 (96%), and Leu M1 was positive in 47 (94%) of the adenocarcinomas, but not in any of the mesotheliomas. By using conventional rabbit antiserum, the authors detected CEA in the majority of adenocarcinomas (96%), but also in two cases of mesothelioma. When the anti-CEA antiserum was absorbed with NCA, the number of positively reacting adenocarcinomas decreased considerably to 76%; however, after this treatment, none of the mesotheliomas gave positive reactions. The monoclonal anti-CEA antibody was reactive in 36 of the adenocarcinomas (72%), but in none of the mesotheliomas. Our results indicate that, in addition to HMFG-2 and CEA, the expression of Leu M1 antigen by most primary pulmonary adenocarcinoma (94%) and its absence in mesothelioma could be used as a valuable marker for primary adenocarcinoma of the lung that involves the pleura and permits its differentiation from mesothelioma. (Am J Pathol 1986, 123:212–219)

HISTOLOGIC criteria for the diagnosis of mesothelioma are well established, but the distinction of mesotheliomas from adenocarcinomas that involve the pleura may be extremely difficult.[1-5] Electron-microscopic and histochemical studies can be helpful, but they do not always provide a definitive solution for this diagnostic dilemma.[1-5] Recently, we[2] and others[3-5] showed that an immunohistologic approach is valuable in the differential diagnosis of diffuse malignant mesothelioma and adenocarcinoma invading the pleural surface. In a previous study, we evaluated the expression of keratin, human milk fat globule (HMFG-2), and carcinoembryonic antigen (CEA) (using conventional heterologous rabbit antiserum) in 12 mesotheliomas and 100 adenocarcinomas derived from various organs, including breast and lung.[2] Keratin was present in both mesotheliomas and adenocarcinomas and, therefore, had no value for the distinction of mesotheliomas from adenocarcinomas. On the other hand, the CEA was detectable in the majority of adenocarcinomas (65%), but only in rare cases of mesothelioma. Similarly, HMFG-2 was discovered in most of the adenocarcinomas (85%), but in none of the mesotheliomas. Based on these observations, we concuded that positivity for CEA, and particularly for HMFG-2, can help to confirm the diagnosis of carcinoma when mesothelioma is a part of the differential diagnosis.

In another recent study,[6] we evaluated the expression

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of Leu M1, which is primarily a myelomonocytic antigen but also is expressed in other cell lines, including in Reed-Sternberg cells in Hodgkin's disease,[8,9] in a series of 400 hematopoietic and nonhematopoietic neoplasms, including adenocarcinomas and mesotheliomas. Leu M1 was detectable in most of the adenocarcinomas of various organs (58%), including all 6 cases of primary pulmonary adenocarcinoma in the series. None of the mesotheliomas evaluated in that study were immunoreactive with anti-Leu M1. This unexpected finding stimulated us to continue investigating the significance of Leu M1 positivity as a possible diagnostic discriminator in the differential diagnosis between primary pulmonary adenocarcinoma and mesothelioma. Furthermore, because of controversial results in the literature obtained by studying mesothelioma with conventional heterologous anti-CEA, we used a monoclonal anti-CEA antibody and evaluated its specificity, as compared with that of polyclonal anti-CEA, in detecting human CEA antigen.

Materials and Methods

Patient Material

The reactivity of anti-keratins, HMFG-2, anti-Leu M1, and anti-CEA antibodies was evaluated in a series of 50 primary pulmonary adenocarcinomas and in 28 mesotheliomas of the pleural cavity. The diagnosis of mesothelioma in most of these cases was confirmed by histochemical and transmission electron microscopic studies. In addition, the presence of hyaluronic acid in most cases was demonstrated by electrophoresis of glycosaminoglycans.[2] Sections of benign reactive mesothelial tissue and normal lung were used as controls. Specimens from patients with well-characterized Hodgkin's disease were used as positive controls for Leu M1 reactivity, and immunologically documented cases of B-cell non-Hodgkin's lymphoma were included as negative controls. All cases were derived from the files of the Sylvia Cowan Surgical Pathology Laboratory at the City of Hope National Medical Center and from the consultation files of one of us (H.B.). The expression of keratins, HMFG-2, Leu M1, and CEA was evaluated in all cases.

Tissue Preparation for Histologic Examination

The tissue sections were fixed in buffered formalin and B5 solution, embedded in paraffin, and stained with hematoxylin and eosin for routine histologic examination.

Tissue Preparation for Immunohistologic Study

The tissues were paraffin-embedded and fixed as has been described in detail previously[2] for immunohistochemical studies with all of the antibodies. Sections were cut at 6 μ, and two sections were placed on a glass slide. The sections were studied after deparaffinization and rehydration. The sections prepared for identification of cytokeratin were pretreated with 0.1% trypsin (ICN Nutritional, Cleveland, Ohio) at 37 C for 60 minutes.

Immunohistologic Reagents

The antibodies, their reactivity, their commercial sources, and relevant references are listed in Table 1. The monoclonal anti-CEA antibody used, designated CEA.41C12.1.1.1, was produced by Dr. John Shively in the Division of Immunology at the Beckman Research Center, City of Hope National Medical Center, as previously described.[10] An aliquot of the heterologous rabbit anti-CEA was treated with overnight incubation with spleen acetone powder for removal of nonspecific cross-reacting antigen.

Immunohistologic Techniques

A modification of the avidin-biotin complex (ABC) technique was used for identification of keratins, Leu M1, CEA, and HMFG-2 antigens as previously described.[11] Briefly, the cryostat cut frozen sections were fixed in graded acetone for five minutes. The primary antibody was placed on one of two sections at a dilution of 1:50 and allowed to incubate for 30 minutes. After removal of excessive primary antibody by brief washing in modified PBS, sections were overlaid with biotinylated, affinity-purified anti-mouse antibody (Vector Laboratories, Burlingame, Calif) at a dilution of 1:200 for 20 minutes. Subsequently, a preformed complex of avidin and biotinylated horseradish peroxidase (Vector Laboratories) at a dilution of 1:100 was applied for 15 minutes. After removal of excessive reagent from the tissue surface with an isotonic buffer system, the substrate color reaction product was developed with diaminobenzidine (DAB) obtained from Sigma Chemical Co. (St. Louis, Mo).

Quality Control

Primary antibody was added to only one of the two sections on each slide; the second section served as a control for endogenous peroxidase activity. An additional control in each case was the substitution of primary antibody by mouse ascitic fluid or nonimmune serum.

Sections were considered positive when we could clearly identify positively stained neoplastic cells, which were easily distinguishable from the adjacent stroma on low-power examination.

Table 1—Antibody Panel Used

Antibody	Predominant immunoreactivity	Source	Reference
Monoclonal			
Anti-keratin (AE-1)	Cytoskeletal keratin	Boehringer Mannheim, Indianapolis, Indiana	2
Anti-leu M1	Monocytes, myeloid cells, epithelioid cells, R-S cells	Becton-Dickinson Labs, Mountain View, California	7, 8, 9
Anti-CEA	CEA	John Shively, PhD, Division of Immunology, City of Hope, National Medical Center	10
HMFG-2	HMFG-related antigen	Seward Laboratories, London, England	2, 5, 20, 23, 24
Polyclonal			
Rabbit anti-CEA	CEA, NCA, NFA, BGP-1	DAKO Corporation, Santa Barbara, California	2
NCA absorbed rabbit anti-CEA	CEA	See text	

R-S, Reed–Sternberg; CEA, carcinoembryonic antigen; NCA, nonspecific cross-reacting antigen; NFA, normal fetal antigen; BGP-1, biliary glycoprotein.

Results

Table 2 summarizes the results of the immunohistologic studies on the 50 pulmonary adenocarcinomas and 28 mesotheliomas.

Pulmonary Adenocarcinoma

Keratins

Specimens from all 50 adenocarcinomas showed positive reaction to the anti-keratin antibody, characterized by intensely granular cytoplasmic immunoreactivity. No immunostaining was obtained in the negative control sections, including sections of non-Hodgkin's lymphomas.

Human Milk Fat Globule

Forty-eight of the 50 adenocarcinomas (96%) showed a strong immunoreactivity to anti-MFG-2. The immunostaining was intense and predominantly cytoplasmic (Figure 1), although occasional cases had surface membrane staining in addition to the cytoplasmic reaction. Two cases in which the immunoreactivity was limited to surface membranes were interpreted as negative.

Leu M1

Forty-seven of the 50 primary adenocarcinomas (94%) exhibited diffuse or focal, finely to coarsely granular, predominantly cytoplasmic immunoreactivity to anti-Leu M1. In general, the intensity of immunostaining was greater in well-differentiated than in the poorly differentiated adenocarcinomas (Figure 2A).

Carcinoembryonic Antigen

Rabbit Heterologous Anti-CEA Antiserum

Expression of CEA was identified in 96% of the adenocarcinomas, but the intensity of the immunoreactivity in the neoplastic cells was variable. The histiocytes and leukocytes also showed intensely positive immunoreactivity.

Rabbit Heterologous Anti-CEA Antiserum Absorbed With NCA

When the anti-CEA antiserum was absorbed with spleen powder for removal of NCA reactivity, the number of positive adenocarcinomas was considerably diminished (to 76%). Histiocytes and leukocytes showed no immunoreactivity.

Table 2—Antigenic Phenotype of 50 Primary Pulmonary Adenocarcinomas and 28 Mesotheliomas

Antibody	Adenocarcimona	Mesothelioma	Reactive mesothelioma	Normal lung
Monoclonal				
Anti-keratin	50/50 (100%)	28/28 (100%)	+	+
HMFG-2	48/50 (96%)	0/28 (0%)	–	+
Anti-Leu M1	47/50 (94%)	0/28 (0%)	–	+
Anti-CEA	36/50 (72%)	0/28 (0%)	–	–
Polyclonal				
Rabbit anti-CEA	48/50 (96%)	2/28 (7%)	–	–
Absorbed rabbit anti-CEA	38/12 (76%)	0/28 (0%)	–	–

+, reactive; –, nonreactive; (%), percentage of positive cells.

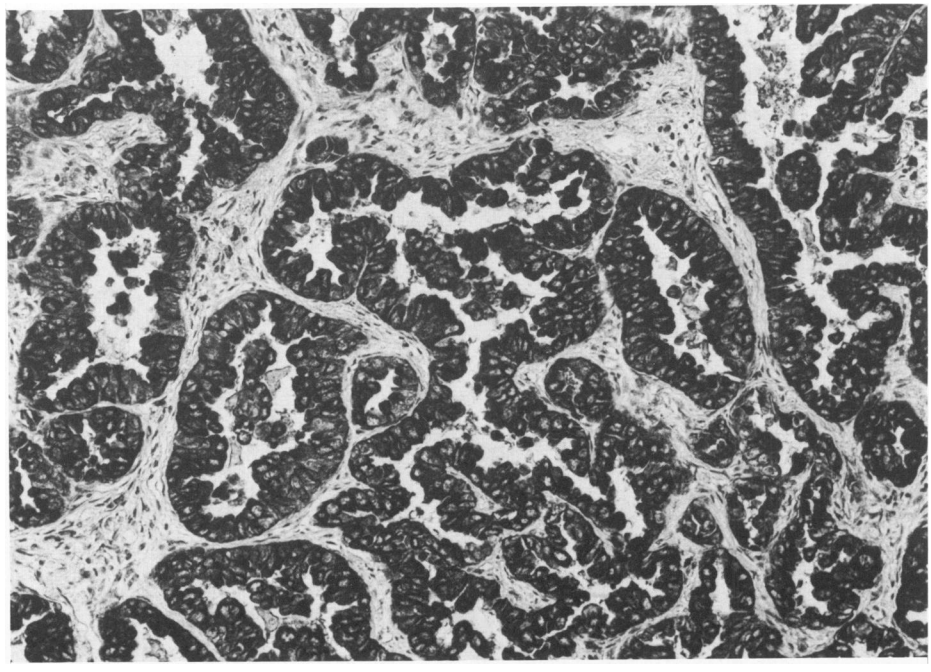

Figure 1—Primary adenocarcinoma of lung, stained with anti-HMFG-2. The neoplastic cells show strong cytoplasmic staining. (B5-fixed, hematoxylin-counterstained, ×250)

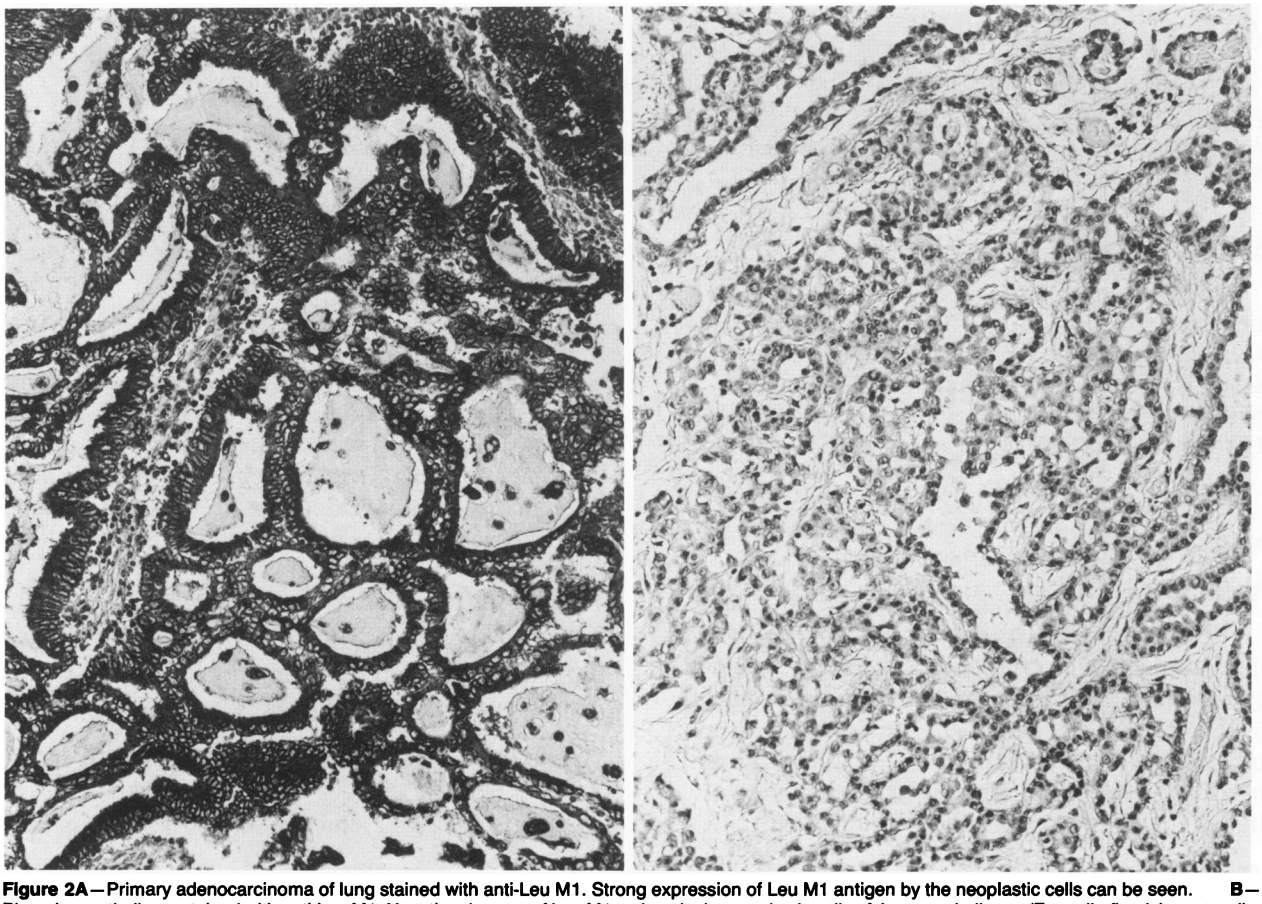

Figure 2A—Primary adenocarcinoma of lung stained with anti-Leu M1. Strong expression of Leu M1 antigen by the neoplastic cells can be seen. **B**—Pleural mesothelioma stained with anti-Leu M1. Note the absence of Leu M1 antigen in the neoplastic cells of the mesothelioma. (Formalin-fixed, hematoxylin-counterstained, ×250)

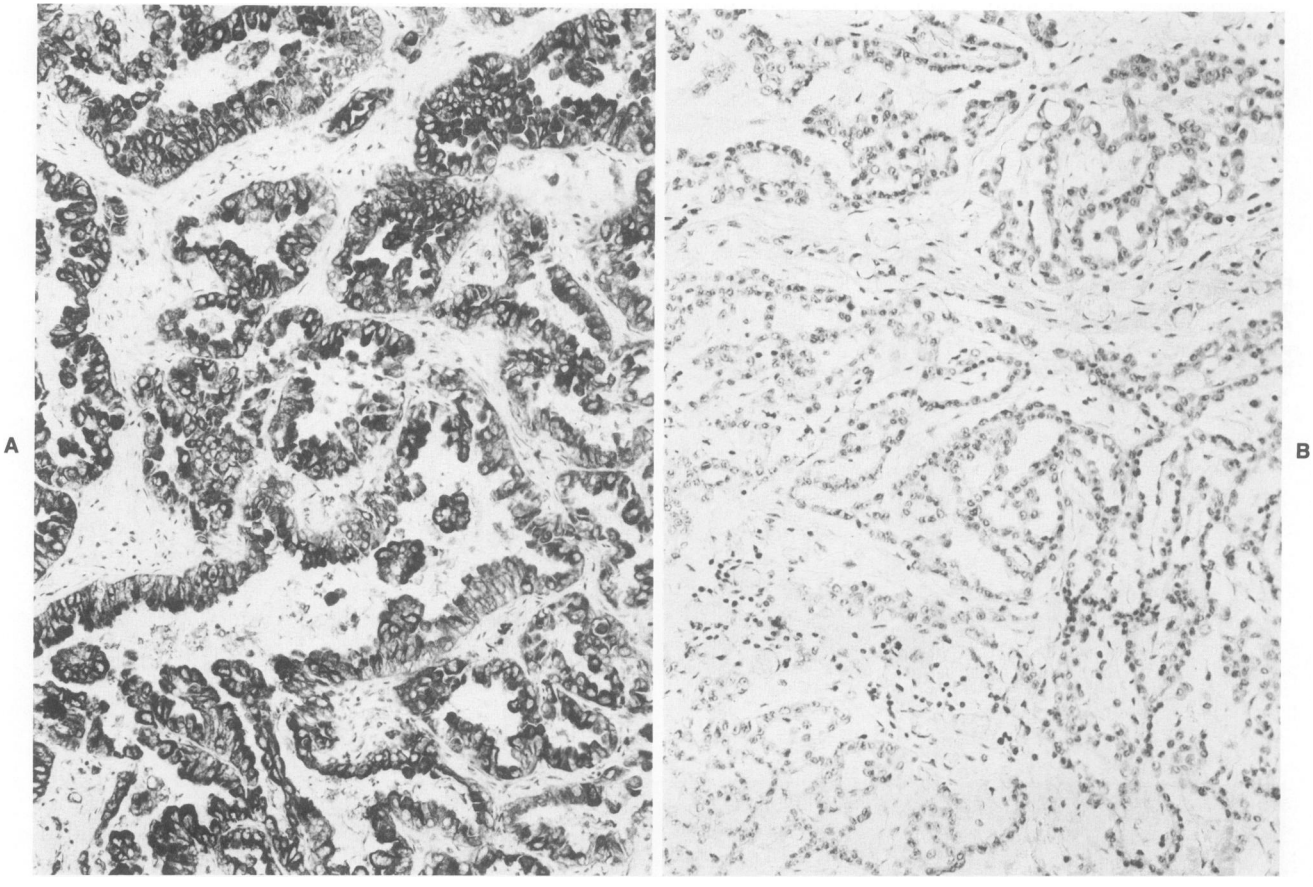

Figure 3A—Primary adenocarcinoma of lung stained with monoclonal anti-CEA. The adenocarcinoma cells were strongly immunoreactive. **B**—Pleural mesothelioma stained with monoclonal anti-CEA. Note the absence of CEA antigen in the neoplastic cells of the mesothelioma. (Formalin-fixed, hematoxylin-counterstained, ×250)

Monoclonal Anti-CEA

Thirty-six cases (72%) were diffusely or focally immunoreactive (Figure 3A). The intensity of immunoreactivity was greater and the background staining considerably less with monoclonal anti-CEA antibody than with rabbit anti-CEA antiserum or was entirely absent. Histiocytes and leukocytes were not reactive.

Mesothelioma

Keratins

All 28 mesotheliomas showed intensely positive immunostaining with antikeratin, in a predominantly cytoplasmic pattern. In some cases, the immunostaining was particularly prominent in the perinuclear areas; but, in general, the intensity and the pattern of positivity of the keratin stain in mesotheliomas were essentially identical to those in adenocarcinomas.

Human Milk Fat Globule

None of the 28 mesotheliomas showed cytoplasmic immunoreactivity with anti-HMFG-2 monoclonal antibody. However, focal staining of the cell membrane was noted in four cases.

Leu M1

In contrast to the adenocarcinomas, none of the 28 mesotheliomas were immunostained with anti-Leu M1 (Figure 2B). Control sections from Hodgkin's disease patients exhibited intense cytoplasmic, predominantly paranuclear immunostaining in Reed–Sternberg cells.

Carcinoembryonic Antigen

Rabbit Heterologous Anti-CEA Antiserum

Of the 28 mesotheliomas, two stained with rabbit anti-CEA antiserum. The immunoreactivity was focal, cytoplasmic, and generally weak.

Rabbit Heterologous Anti-CEA Antiserum Absorbed With NCA

None of the 28 mesotheliomas were immunostained.

Monoclonal Anti-CEA

None of the 28 mesotheliomas were immunostained (Figure 3B).

On the basis of the results of the immunohistologic studies, the predominant antigenic phenotype of pulmonary adenocarcinomas was keratin-positive, Leu M1$^+$, MFG$^+$, CEA$^+$. In contrast, the predominant antigenic phenotype of the mesotheliomas was keratin-positive, Leu M1$^-$, MFG$^-$, CEA$^-$.

Discussion

Surgical pathologists are aware of difficulties in distinguishing pleural mesothelioma from pulmonary adenocarcinoma which infiltrates the pleural surfaces, or from metastatic adenocarcinoma involving the pleura.[1-5] Histochemical studies for the presence of mucin in adenocarcinomas and for the presence of hyaluronic acid in mesotheliomas have been of limited value, because these substances are not consistently detectable in the respective neoplasms.[1,2] Electron microscopy has been successfully used in the diagnosis of mesotheliomas.[12,13] Most cases of mesothelioma have characteristic ultrastructural features, and recent reports suggest that the application of quantitative and qualitative ultrastructural evaluation may permit the distinction of mesotheliomas from adenocarcinomas.[14,15] Not all mesotheliomas, however, may be distinguishable from certain adenocarcinomas at the ultrastructural level.[16] Presently available immunohistochemical procedures, on the other hand, have been shown to be useful in the differential diagnosis of mesothelioma from adenocarcinoma.[2-5] Previous studies in our laboratory demonstrated the importance of an immunohistologic approach for making this distinction.[2] Whereas keratin was present in both mesotheliomas and adenocarcinomas, HMFG-2 and CEA were essentially absent in mesotheliomas. Therefore, positivity for HMFG-2 and CEA favored a diagnosis of adenocarcinoma. Moreover, in a study designed for evaluation of the immunoreactivity of the anti-Leu M1 antibody against a variety of hematopoietic and nonhematopoietic neoplasms, we noted that anti-Leu M1, also, may be useful in differentiating mesothelioma from primary lung adenocarcinoma.[6]

Keratins

All of the mesotheliomas and adenocarcinoma examined in this study showed intense positive immunoreactivity for keratin. Some investigators have reported that the strong immunostaining pattern of cytoplasmic localization of keratin in mesotheliomas may be helpful in distinguishing these tumors from pulmonary adenocarcinomas which, in their experience, express weak keratin staining.[3,4] A strong keratin staining of mesotheliomas with absent or weak expression of CEA in these neoplasms aids in distinguishing them from pulmonary adenocarcinomas.[3] However, in our series, as well as in our previous study (Table 3),[2] the intensity of immunoreactivity and the pattern of immunostaining in mesotheliomas were essentially indistinguishable from those in adenocarcinomas. Similar findings have been reported by others.[17,18] We concluded that expression of cytoskeletal keratin is not helpful in distinguishing mesothelioma from adenocarcinoma. It is possible, however, that reported differences in the keratin immunostaining of mesotheliomas and adenocarcinomas[3,4] result from differences in keratin phenotypes

Table 3—Antigenic Phenotype of Mesothelioma as Determined by Various Investigators

Investigator (date)	Keratin-positive/total	CEA-positive/total	HMFG-2-positive/total
Wang et al[22] (1979)	ND	0/12	ND
Whitaker et al[25] (1981)	ND	0/40	ND
Corson et al[3] (1982)	20/20	3*/20	ND
Kwee et al[1] (1982)	ND	0/37	ND
Loosli et al[5] (1983)	15/15	3/15	9/15
Holden et al[17] (1984)	10/22	8/22	ND
Said et al[4] (1983)	8/8	2†/8	ND
Marshal et al[20] (1984)	ND	0/16	12/16
Battifora et al[2] (1985)	12/12	2/12	0/12
Total	65/77 (85%)	18/182 (10%)	21/43 (48%)

ND, not done; (%) percentage of positive cases.
* In 6 cases reactivity was weak or equivocal.
† Focal positivity.

between these two neoplasms, not detectable with the broad spectrum anti-keratin monoclonal antibody used in our study. Evidence in support of this possibility has been recently reported.[19] It is thus possible that the development of monoclonal antibodies with specificity to individual members of the keratin family might pave the way to this immunohistochemical distinction.

Human Milk Fat Globule

Strong cytoplasmic reactivity for HMFG-2 was observed in all but two of the pulmonary adenocarcinomas evaluated in the present study. In contrast, none of the mesotheliomas showed cytoplasmic positivity with anti-HMFG-2. These results essentially confirm our earlier observations, which were based on a smaller number (12) of cases.[2] In contrast to the results of our studies, those of Loosli and Hurlimann indicated reactivity to anti-HMFG-2 in 9 of 15 mesotheliomas (Table 3).[5] All 9 of the cases in their series showed surface membrane staining. Similarly, 12 of 16 mesotheliomas studied by Marshal et al showed HMFG positivity with the predominant immunostaining pattern being surface membrane, although some cases showed cytoplasmic staining as well.[20] As observed under the conditions of our study, absence of cytoplasmic and membrane staining strongly favored mesothelioma. However, the presence of membrane staining alone was observed in four mesotheliomas and two adenocarcinomas. Strong cytoplasmic staining, often associated with strong membrane staining, however, was typical of adenocarcinoma and was considered to strongly support such a diagnosis. In our present study and in our previous investigations, the criteria for positivity with anti-HMFG-2 antibody required strong cytoplasmic staining.

Leu M1

In a recent study of more than 400 cases of human neoplasms in which we investigated the distribution of Leu M1 antigen, we observed Leu M1 positivity in the majority of adenocarcinomas (105/179), including all primary pulmonary adenocarcinomas studied.[6] In contrast, all mesotheliomas were negative for Leu M1 antigen.[6] This unexpected finding stimulated us to expand our study to evaluate the usefulness of Leu M1 positivity as an additional aid in the differential diagnosis of mesothelioma versus primary pulmonary adenocarcinoma. We now found that 47 of the 50 adenocarcinomas (94%) stained diffusely or focally with anti-Leu M1. It is curious that of three patients with Leu M1-negative adenocarcinoma, two patients had previous histories of adenocarcinoma of the breast. Although it is possible that the apparent pulmonary adenocarcinomas in these patients may represent metastatic adenocarcinomas of the breast rather than primary adenocarcinomas, the clinical manifestations and the overall morphologic features were more consistent with lung primaries than with metastatic breast carcinomas.

In contrast to the majority of pulmonary adenocarcinomas, none of the 28 mesotheliomas gave a positive reaction. The presence of Leu M1 in the majority of primary pulmonary adenocarcinomas and its absence in mesotheliomas in the present study confirm our previous observations and indicate that Leu M1 positivity is a valuable adjunct for the distinction of adenocarcinoma from mesothelioma. Moreover, because Leu M1 antigen does not deteriorate during routine fixation and embedding processes, it can be detected easily in paraffin-embedded, fixed tissues.

Carcinoembryonic Antigen

The presence or absence of CEA-related antigens in mesotheliomas is a matter of controversy (Table 3).[21] Wang et al reported that CEA was absent in all 12 cases of mesothelioma that they examined,[22] but variable reactivity of mesothelioma to anti-CEA has been noted by other investigators. In a study of 22 cases of malignant mesothelioma, Holden and Churg observed positive immunoreactivity to conventional anti-CEA in 8 cases.[17] We and other investigators have reported the expression of CEA-related antigens by mesotheliomas. However, because of the cross-reactivity of most heterologous anti-CEA antisera with nonspecific cross-reacting antigen (NCA), which has antigenic determinants in common with CEA, the validity of these positive results with CEA in mesotheliomas was questioned.[21] It is recommended that any positive immunoreactivity when anti-CEA antisera are used without preabsorption with NCA should be interpreted cautiously.[21] The anti-NCA reactivity of anti-CEA antisera can be demonstrated by positive staining of macrophages and polymorphonuclear leukocytes; macrophages and polymorphonuclear leukocytes contain NCA, but lack CEA.[21] The monoclonal anti-CEA antibody used in this study does not stain macrophages and leukocytes and presumably is free of cross-reactivity to NCA. In our current study, by using conventional rabbit anti-CEA antiserum without prior absorption with spleen powder, we observed weak focal cytoplasmic CEA reactivity in two of the 28 mesotheliomas. However, no reactivities were noted in any of the mesotheliomas when a preabsorbed heterologous anti-CEA antiserum or a monoclonal anti-CEA antibody was employed. Interestingly, the percentage of lung adenocarcinomas stained with NCA-absorbed heterologous anti-CEA dropped to levels comparable to those obtained by the monoclonal anti-CEA antibody which is free of NCA cross-reactivity. According to these results, when monoclonal anti-CEA antibody or NCA-absorbed heteroantiserum are used, positive immuno-

staining in neoplastic cells effectively rules out a diagnosis of mesothelioma.

In summary, our study indicates the following: 1) Anti-Leu M1 and anti-HMFG-2 are practical and reliable antibodies for distinguishing mesothelioma from primary pulmonary adenocarcinoma. 2) Keratin is expressed by both mesothelioma and adenocarcinoma; thus, contrary to some reports, keratin positivity is not helpful in showing a distinction between these two neoplasms, at least when based in the use of antibodies of broad spectrum against keratins. 3) Because of its greater specificity, the monoclonal anti-CEA antibody is preferable to non-NCA-absorbed polyclonal rabbit anti-CEA antisera and is an additional antibody useful in the discrimination of mesothelioma from primary pulmonary adenocarcinoma.

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