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Original Article

The Use of Monoclonal Antibody 44-3A6 in Cell Blocks in the Diagnosis of Lung Carcinoma, Carcinomas Metastatic to Lung and Pleura, and Pleural Malignant Mesothelioma

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Monoclonal antibody (MoAb) 44-3A6 recognizes a glandular differentiation-associated antigen and has been used to identify exocrine differentiation in pulmonary carcinomas. The authors assessed its value in the diagnosis of lung carcinomas metastatic to lung/pleura and pleural malignant mesothelioma (MM), using cell blocks derived from cytologic specimens. Sixty-three primary lung carcinomas, 31 metastatic adenocarcinomas (ACs) (from breast, gastrointestinal tract, or genitourinary tract), and 36 MMs were immunostained with 44-3A6, Leu-M1, and anti-carcinoembryonic antigen (CEA). The results confirm the value of 44-3A6 in identifying ACs but do not allow distinction between those of pulmonary, breast, GIT, or ovarian mucinous derivation. Endometrial, ovarian serous, and renal ACs are essentially nonreactive, as are almost all MMs. The occurrence of one positive MM predicates caution in interpreting 44-3A6 positivity in isolation, but, judiciously used with other discriminating antibodies such as Leu-M1 and anti-CEA, 44-3A6 is of value in the differential diagnosis of ACs and MMs. Further, its applicability to cytologic specimens may obviate the need for more invasive diagnostic procedures and lead to rapid, accurate diagnosis. (Key words: Monoclonal antibody 44-3A6; Leu-M1; Carcinoembryonic antigen; Cytology; Adenocarcinoma; Malignant mesothelioma) Am J Clin Pathol 1991;95:322–329

The assessment of pleuropulmonary malignant neoplasms in routine biopsy or cytologic material may pose differential diagnostic problems, which have important therapeutic and prognostic implications. These problems relate mainly to the subclassification of pulmonary carcinomas (particularly identifying neuroendocrine *vs.* nonneuroendocrine carcinomas), distinction between primary and secondary carcinomas in the lung, and distinction between pleural malignant mesothelioma (MM) and metastatic adenocarcinoma (AC).

Recourse to ancillary specialized diagnostic modalities such as immunohistochemistry (IH) and electron microscopy (EM) is often helpful in the resolution of such problems, particularly in the distinction between MM and AC. In this differential diagnosis the expression of carcinoembryonic antigen (CEA) still remains one of the most reliable aids to diagnosis (see Johnson and associates[1] and Whitaker and associates[2] for review). More recently, several novel monoclonal antibodies (MoAbs), reactive with mucin-type glycoproteins or related proteins, have been raised and are of potential value in the differential diagnosis of pleuropulmonary malignancies. These include Leu-M1[3–8] and the analogous 624A12,[9,10] 44-3A6 (see below), B72.3,[3,8,11] and A-80.[12]

The mouse MoAb 44-3A6 is an IgG1 antibody raised against the National Cancer Institute A549 human pul-

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monary AC cell line.[13] It detects a 40-kD cell surface antigen that is preserved in formalin-fixed, paraffin-embedded tissue sections.[13] It has been shown to be of value in the differential diagnosis of pulmonary carcinomas, particularly in the identification of those showing exocrine differentiation,[14-17] and also in the distinction between pleural MM and AC.[9] With one exception,[14] these studies have been performed in surgically obtained, routinely-fixed and processed tissue sections. Because material obtained initially for diagnostic purposes is often, and sometimes solely, cytologic in nature, the successful application of immunohistochemical studies on such material may obviate the need for additional invasive biopsy procedures and lead to rapid, accurate diagnosis.

The goals of this study are to (1) assess the utility of the antibody 44-3A6, applied to cell blocks from cytologic specimens obtained from primary and secondary pleuropulmonary malignant neoplasms, and (2) compare the results obtained with 44-3A6 with those obtained with the use of Leu-M1 and CEA staining, in the differential diagnosis of pleural MM and metastatic AC.

MATERIALS AND METHODS

A total of 130 cases of pulmonary carcinomas, nonpulmonary carcinomas metastatic to pleura or lung, and pleural MMs (all epithelial in morphologic characteristics) were used for the study (see Table 1). These cases were retrieved from the files of the Pathology Department, Sir Charles Gairdner Hospital, except for two cases of MM originating from the Repatriation General Hospital (Hollywood, Western Australia). Case selection was on the basis of a cell block containing adequate cellular material being available for immunostaining. Patient case records and any relevant pathologic material, including surgical biopsy and postmortem tissue, or tissue examined ultrastructurally were reviewed. If there was any doubt regarding the primary or secondary nature of any pulmonary carcinoma, it was excluded from the study. All cases of MM were thoroughly documented clinically, morphologically, and immunohistochemically, and in 24 cases ultrastructural confirmation was obtained. The cytologic material was derived mainly from pleural fluid (99 cases) and the remainder from fine-needle aspiration specimens of lung (31 cases). Cell blocks were prepared with the use of standard techniques, fixed in formol sublimate, and processed routinely to paraffin blocks.

Immunostaining was performed on 6-μm sections by the avidin–biotin complex (ABC) technique.[19] The following antibodies were used: 44-3A6 (tissue culture supernatant[13] at a concentration of approximately 1 μg/mL); Leu-M1 (Becton-Dickinson, Mountain View, CA; 1:50 dilution); polyclonal anti-CEA (DAKO, Carpinteria, CA; 1:400 dilution); and monoclonal anti-CEA (Bio-Genex, San Ramon, CA, prediluted). Sections were incubated with the primary antibodies overnight at 4 °C. Slides were counterstained lightly with hematoxylin. Negative controls were performed by omitting the primary

TABLE 1. IMMUNOSTAINING OF LUNG CARCINOMAS, METASTATIC CARCINOMAS, AND MALIGNANT MESOTHELIOMA IN CELL BLOCKS OF EFFUSIONS AND FINE-NEEDLE ASPIRATES

Tumor Type	*Number of Cases*	*Number of Positive Cases*			
		44-3A6	*Leu-M1*	*CEA (p)*	*CEA (m)*
Adenocarcinoma					
Primary lung	24 (18, 6)*	24	19	24	18
Metastatic					
Breast	10 (10, 0)	9	9	10	8
Colon	5 (3, 2)	5	5	5	4
Stomach	3 (3, 0)	3	2	3	3
Pancreas	2 (2, 0)	2	0	2	2
Ovary	7 (7, 0)	5†	5	6	2
Endometrium	2 (1, 1)	1‡	1	1	0
Kidney	1 (1, 0)	0	0	0	0
Large cell anaplastic carcinoma, lung	7 (1, 6)	6	6	7	3
Squamous carcinoma					
Primary lung	8 (2, 6)	1	1	4	3
Metastatic cervix	1 (0, 1)	0	0	1	0
Small cell anaplastic carcinoma, lung	24 (15, 9)	4‡	6	14	7
Pleural malignant mesothelioma	36 (36, 0)	3	0	2	3§

* The figures in parentheses refer to specimens derived from pleural fluid and fine-needle aspiration, in that order.

† Of the ovarian carcinomas, two were mucinous and five were poorly differentiated serous. Both mucinous carcinomas were diffusely and strongly 44-3A6$^+$, while only rarely were positive cells seen in three of five serous carcinomas.

‡ Only rare 44-3A6$^+$ cells.

§ The pattern of reaction in these cases, seen only with the monoclonal anti-CEA, was clearly distinct from the usual pattern of diffuse cytoplasmic and/or membrane-accentuated stain seen with polyclonal anti-CEA in adenocarcinomas. It consisted of discrete, coarsely granular staining in the cytoplasm, localized to the Golgi and rough endoplasmic reticulum by immunoultrastructural studies (this is the subject of another report[18]).

antibody and substituting a nonreactive antibody of the same isotype. As a positive control, a cell block of a known metastatic breast AC, strongly immunoreactive with 44-3A6, Leu-M1, and anti-CEA, was used. In one case of MM containing 44-3A6–positive cells, for which additional surgical and/or postmortem tissue sections were available, these were also stained for comparison with cell block material. In some cases of MM, sequential sections of the cell blocks were stained with 44-3A6, Cam 5.2 (Becton-Dickinson; 1:20 dilution), and anti–epithelial membrane antigen (EMA) (DAKO; 1:100 dilution) to confirm the mesothelial nature of isolated cells that may have been confused with histiocytes.

RESULTS

Primary Lung ACs

The 24 pulmonary ACs were all stained with 44-3A6. In most instances, most cells were stained strongly, in a diffuse, coarsely granular cytoplasmic-like pattern (Fig. 1), often with apparent membranous accentuation (Fig. 2). In any one case the staining pattern was heterogeneous in that both cytoplasmic-like and/or membranous-like staining could be found, and in a minority of cells there was no reaction. In cells containing cytoplasmic mucinous vacuoles, staining was observed in the cytoplasm apparently around the vacuoles, without staining of the contents of the vacuoles (Fig. 3). In only two ACs was the staining weak or moderate in intensity. In an additional case, only approximately 30% of tumor cells were stained, but strongly so. All 24 ACs stained with the polyclonal anti-CEA. The reaction product appeared to be located within the cytoplasm, and often concentrated along the cell membrane. Generally, the staining intensity was similar or stronger than that obtained with 44-3A6. Fewer cases (75%) stained with the monoclonal anti-CEA, giving a weaker reaction but a cleaner background than that obtained with the polyclonal antibody. Leu-M1 stained 19 cases, giving a membrane-like and/or localized (? Golgi-related) or diffuse cytoplasmic pattern of reaction (Fig. 4). In no case was the staining for Leu-M1 more widespread or stronger than that obtained with 44-3A6. The 44-3A6$^+$, LeuM1$^+$, CEA$^+$ was demonstrated by 79% of the ACs.

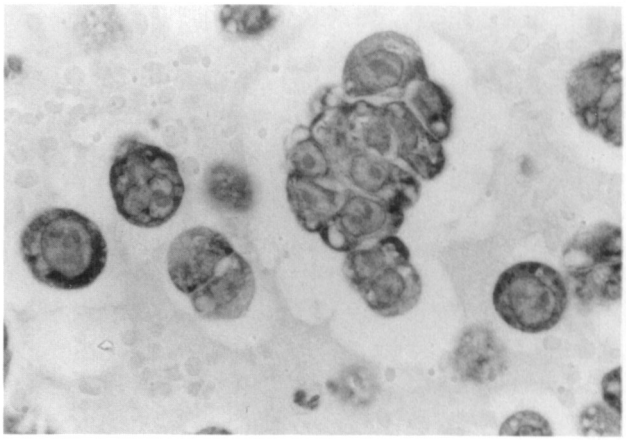

FIG. 2. Metastatic lung adenocarcinoma immunostained with MCA 44-3A6. Peripherally accentuated staining is seen in the central cluster of cells. Other single cells display more diffuse cytoplasmic staining. Hematoxylin counterstain (×625).

Metastatic Carcinomas

Twenty-five of the 30 carcinomas metastatic from primary sites other than lung also stained with 44-3A6, with the same staining patterns as observed in pulmonary AC. There were no features that enabled distinction between

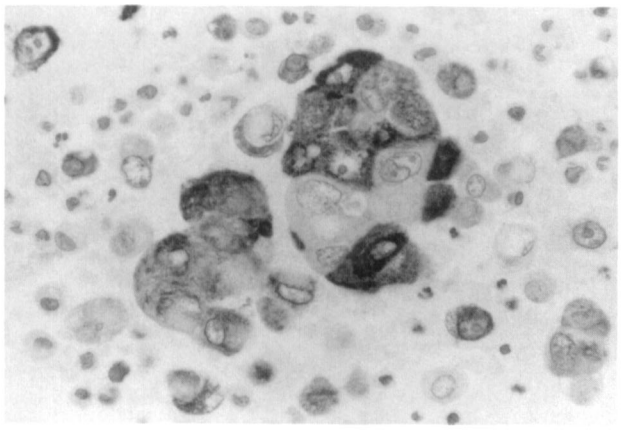

FIG. 1. Metastatic lung adenocarcinoma immunostained with MCA 44-3A6. Coarsely granular, diffuse cytoplasmic staining is evident in most tumor cells. Hematoxylin counterstain (×400).

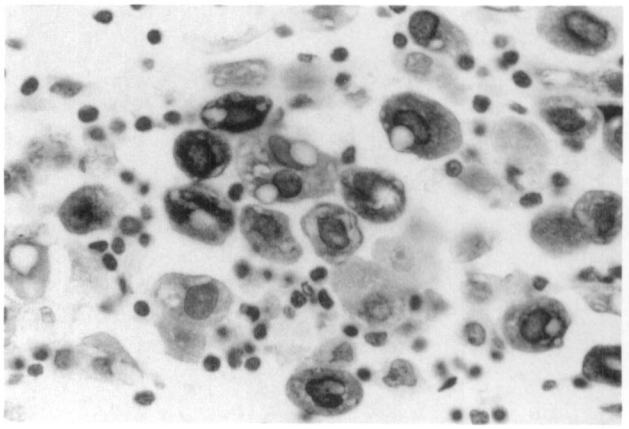

FIG. 3. Metastatic lung adenocarcinoma immunostained with MCA 44-3A6. There is diffuse cytoplasmic staining, but the mucinous vacuoles do not show any reaction product. Hematoxylin counterstain (×625).

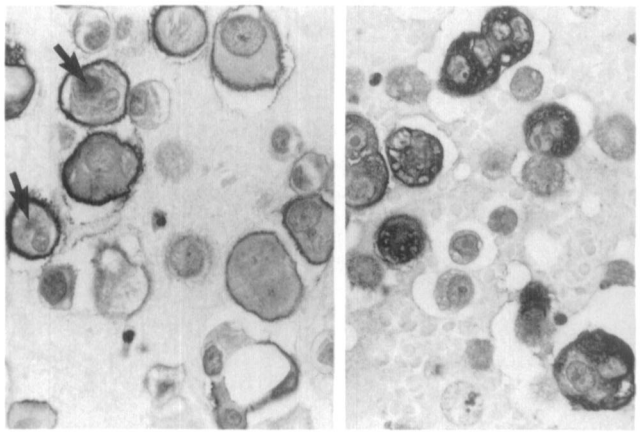

FIG. 4. Metastatic adenocarcinoma immunostained with MCA Leu-M1. The *left panel* illustrates peripheral, presumably membranous, reaction product, and focal paranuclear (?Golgi-related) staining in two cells (*arrows*). The *right panel* shows diffuse cytoplasmic staining. Hematoxylin counterstain (×400).

primary or secondary ACs. Nineteen of the 20 ACs originating from breast, colon, stomach, and pancreas were 44-3A6 positive (Fig. 5). Typically, most malignant cells stained strongly, but in two of the breast carcinomas and both of the pancreatic ACs less than 30% of the cells were 44-3A6 positive. One breast AC was completely negative. These 20 carcinomas all stained strongly with the polyclonal anti-CEA, and 75% also stained with Leu-M1 with the same heterogeneous pattern as seen in the pulmonary ACs (Fig. 6). In general, fewer cells were Leu-M1 positive compared with 44-3A6, apart from two metastatic breast carcinomas.

Of the seven metastatic ovarian carcinomas, both mucinous carcinomas were strongly and diffusely 44-3A6 positive and CEA positive (Fig. 7); in both cases, fewer

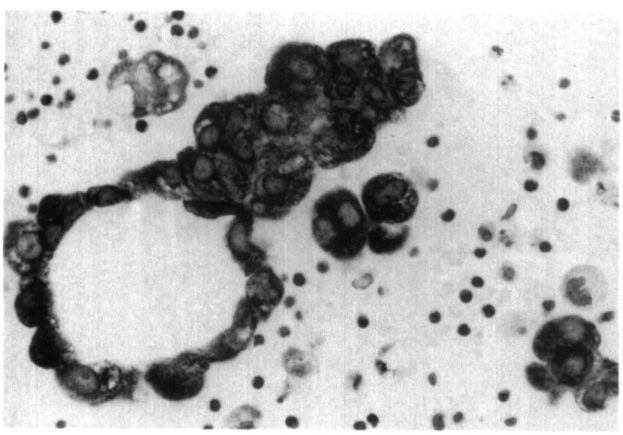

FIG. 5. Metastatic breast carcinoma in pleural fluid immunostained with MCA 44-3A6. There is strong diffuse cytoplasmic staining of all tumor cells. Hematoxylin counterstain (×400).

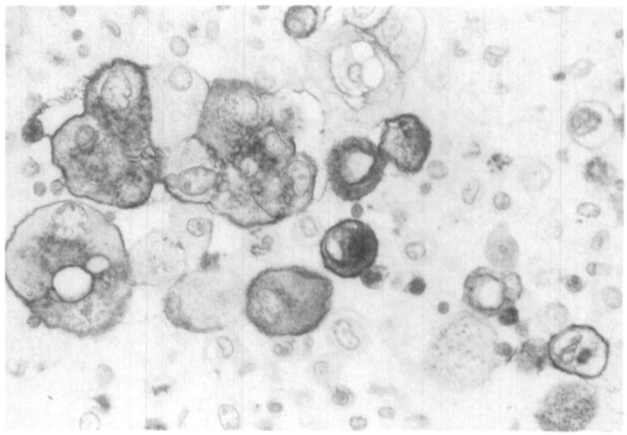

FIG. 6. Metastatic breast carcinoma in pleural fluid immunostained with MCA Leu-M1. There is peripheral membrane staining and weaker cytoplasmic staining. Hematoxylin counterstain (×400).

cells stained with Leu-M1 than with 44-3A6. In three of the five poorly differentiated serous carcinomas, only rarely were tumor cells 44-3A6 positive. In three of the serous carcinomas, relatively more cells (but still less than 20% overall) stained with Leu-M1 and for CEA than with 44-3A6. Of the two endometrial carcinomas, one showed rare 44-3A6–positive and Leu-M1–positive cells, whereas no cells stained for CEA. The second case contained only occasional CEA-positive cells and no cells staining for Leu-M1 or 44-3A6. The solitary metastatic renal carcinoma did not stain with any of the conjugates.

Other Primary Lung Carcinomas

Six of the seven large cell anaplastic carcinomas (LCACs) of lung were 44-3A6 positive (Fig. 8). In five of these cases, more than 50% of the cells were positive, with

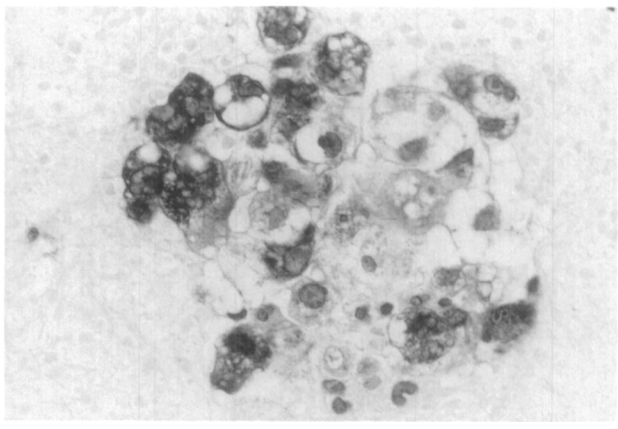

FIG. 7. Metastatic ovarian mucinous carcinoma in pleural fluid. Immunostaining with MCA 44-3A6 reveals cytoplasmic staining in the majority of cells but spares cytoplasmic mucinous vacuoles. Hematoxylin counterstain (×400).

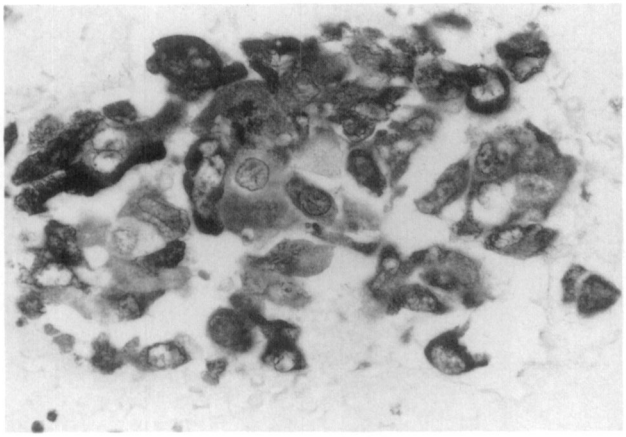

FIG. 8. Metastatic large cell anaplastic carcinoma of lung immunostained with MCA 44-3A6. Strong, diffuse cytoplasmic reaction product is present in almost all tumor cells. Hematoxylin counterstain (×400).

staining patterns similar to those seen in ACs. Only one of nine squamous carcinomas showed very focal 44-3A6 staining. Of the 24 small cell anaplastic carcinomas, in only 4 cases were isolated cells 44-3A6 positive; most tumor cells did not stain.

Malignant Mesothelioma

Of the 36 cases of MM, 33 did not show any staining with 44-3A6. In two cases, a few single tumor cells were stained (confirmed as mesothelial in sequential sections stained with Cam 5.2 and anti-EMA); in a third case, more than 50% of the tumor cells were strongly stained (Fig. 9). The latter patient had a contralateral right pleural MM one year previously, which also stained diffusely with 44-3A6. The 44-3A6–positive cells in these three cases showed a membranous and/or cytoplasmic-like pattern of reaction. All MMs were Leu-M1 negative. Two cases displayed isolated 44-3A6–negative, CEA-positive (polyclonal) tumor cells with apparent surface membrane and sometimes cytoplasmic-like staining. In three cases, unusual coarsely granular cytoplasmic staining was obtained with the monoclonal anti-CEA only. In no case did staining with both 44-3A6 and anti-CEA occur.

In some of the pleural fluid specimens, histiocytes showed cytoplasmic-like staining with 44-3A6 and polyclonal anti-CEA, but these cells were readily distinguished from carcinoma or mesothelioma cells on morphologic grounds. The possibility that occasional reactive mesothelial cells stained with 44-3A6 could not be entirely excluded. These rare positive cells served as useful controls by indicating that the specimens were properly fixed and processed; any negative case therefore represented a true negative result.

DISCUSSION

Our results indicate that immunohistochemical staining with 44-3A6, Leu-M1, and anti-CEA antibodies can be readily applied to cell blocks prepared from cytologic specimens obtained from pleural effusions or by fine-needle aspiration. Banner and associates[14] have already demonstrated the applicability of 44-3A6 to alcohol-fixed, Papanicolaou-stained smears made from bronchial brushings of pulmonary carcinomas and showed excellent correlation with immunostaining of histologic sections. In our laboratory, cell blocks are routinely prepared from malignant effusions and also in selected fine-needle aspiration specimens. We have found that immunohistochemical staining with a broad range of antibodies is readily performed on these cell blocks, often obviating the need for additional invasive biopsy procedures to obtain more diagnostic material.[7] One important advantage in immunostaining cell blocks as opposed to cytologic smears is that, when addressing a particular differential diagnostic problem, particular cells or cell groups can be studied in parallel sections with the use of panels of antibodies.

Primary Lung Carcinomas

Our cytologic study confirms that 44-3A6 is an excellent marker of exocrine differentiation in pulmonary carcinomas, as demonstrated previously in histologic material.[9,13–17] All primary lung ACs were 44-3A6 positive, with most showing strong staining of most tumor cells. These ACs included two bronchioloalveolar carcinomas (BACs) (one confirmed histologically, the other composed of type II pneumocytes by EM). There has been some

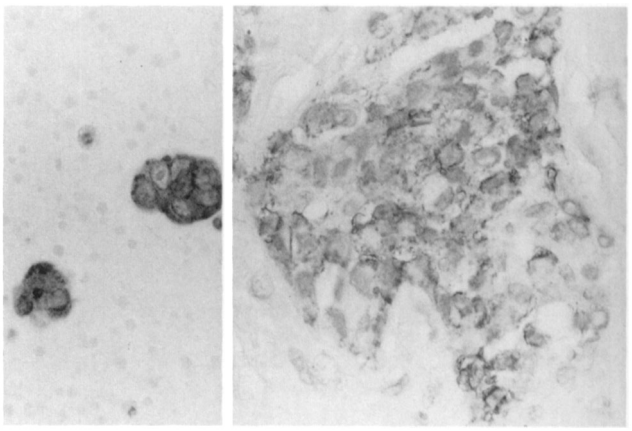

FIG. 9. Pleural malignant mesothelioma immunostained with MCA 44-3A6. In this isolated case, more than 50% of tumor cells showed strong cytoplasmic staining (*left panel*). A contralateral pleural MM from this patient, diagnosed in a pleurectomy specimen, showed similar staining in tumor cells (*right panel*). Hematoxylin counterstain (×400).

variability in the reported 44-3A6 reactivity of BACs. Lee and associates[16] reported that all 12 BACs studied were 44-3A6 negative, whereas, like us, Banner and associates[14] found two cases of BAC to be 44-3A6 positive. These results are consistent with the findings reported on the 44-3A6 reactivity of BAC subsets.[20]

Our results for lung carcinomas other than ACs are broadly similar to those previously reported.[9,14,16,17] Squamous carcinomas and small cell anaplastic (high-grade neuroendocrine) carcinomas are largely nonreactive with 44-3A6; isolated foci of reactivity may indicate focal exocrine differentiation in these neoplasms, such heterogeneity being a well-recognized occurrence in pulmonary carcinomas.[21,22]

This may also explain the variable 44-3A6 reactivity reported in LCAC.[14,16,17] Our finding of strong and diffuse 44-3A6 staining in five cases and focal staining in one case of LCAC parallels the results of Banner and associates.[14] In that study, in which immunohistochemical staining of smears and corresponding tissue sections of various lung carcinomas was performed, the greatest discrepancy in immunostaining between cytologic and histologic material occurred in the group of LCACs, supporting the notion of heterogeneity in this group of tumors. A more recent extensive immunohistochemical study of 52 cases of LCAC,[16] using a panel of antibodies including 44-3A6 and various neuroendocrine markers in tissue sections, showed that most LCACs exhibited exocrine differentiation, even in the absence of overt glandular differentiation by microscopy; fewer showed neuroendocrine differentiation, whereas almost one-fourth exhibited both exocrine and endocrine differentiation. In the tumors exhibiting exocrine differentiation, 44-3A6 staining was diffuse cytoplasmic in more than 50% of cells, often with enhancement at the cell membrane; these results are similar to ours and those of Banner and associates.[14] In the LCAC of mixed phenotype, 44-3A6 reactivity was more variable, ranging from focal staining in some, to predominant staining in other cases. The identification of different immunophenotypic subsets of LCACs may have important therapeutic and prognostic implications.[17]

Metastatic Carcinomas

A recent study reported the reactivity of 44-3A6 in tissue sections from a large number of ACs originating from various nonpulmonary sites.[15] With the exception of hepatocellular, renal cell, and prostatic ACs that were not reactive with 44-3A6, variable staining was obtained in carcinomas originating from the gastrointestinal tract (GIT), including gall bladder and pancreas; breast; female genital tract; and some endocrine and salivary tumors.[15] The reactivity of 44-3A6 in cytologic specimens from ACs of nonpulmonary origin has not been reported previously. Our results with the use of cytologic specimens are similar to those obtained in tissue sections.[15]

Breast and GIT. In our series most carcinomas originating from breast and GIT were 44-3A6 positive, with a heterogeneous staining pattern similar to that seen in primary pulmonary ACs, such that in any individual case distinction between ACs of pulmonary or nonpulmonary origin could not be made on the basis of immunostaining. Similar staining of these carcinomas for CEA with the use of polyclonal antiserum was obtained, but fewer cases were Leu-M1 positive, similar to the experience of others.[3] As found by other investigators, the sensitivity of the monoclonal anti-CEA antibody was less than that of the polyclonal antiserum.[3]

Ovary, Endometrium, and Kidney. There have been few reports on the reactivity of 44-3A6 with ovarian carcinomas.[15] In the absence of known ovarian carcinoma, the finding of papillary clusters of malignant cells in serous effusions from female patients remains a diagnostic problem.[23] Although mucinous carcinomas are readily identified by their content of epithelial mucin, serous carcinomas may contain few mucin-positive cells and, like MMs, are invariably CEA negative. Hence, there is potential for misdiagnosing some metastatic serous carcinomas as MM.

Combs and associates[15] found one serous and one mucinous carcinoma, of a total of five cases, to be focally 44-3A6 positive, with more prominent staining obtained in the mucinous carcinoma. In our study, both mucinous carcinomas showed strong staining of at least 50% of tumor cells with 44-3A6, and for CEA. The pattern of staining was indistinguishable from that obtained in pulmonary ACs. In contrast, rarely were tumor cells from the metastatic serous ovarian carcinomas 44-3A6 positive (it is possible that these rare positive cells could be mucinous in nature, because mucinous cells may occur in varying numbers in serous tumors). This finding may be of value in distinguishing metastatic serous carcinomas from pulmonary ACs but of limited value in distinguishing MMs from ovarian serous carcinomas metastatic to the pleura (see below). A larger number of ovarian carcinomas, of diverse histologic types, needs to be studied to clearly establish patterns of 44-3A6 reactivity. Our finding of isolated 44-3A6–positive cells in one of two endometrial carcinomas is similar to results obtained in tissue sections.[15]

Metastatic renal cell carcinomas also appear to be nonreactive with 44-3A6, as seen in our one case and in those previously reported.[15] In our experience, the distinction between metastatic renal cell carcinoma and MM, es-

pecially in FNA aspirates, may be difficult cytologically and immunohistochemically, because both may share the same phenotype that is epithelial mucin negative, cytokeratin positive, EMA positive, vimentin positive, CEA negative, and 44-3A6 negative. This is an area of diagnostic difficulty that needs to be studied further to identify antibodies that may have discriminatory value.

Malignant Mesothelioma

Our study indicates that 44-3A6 is of value in distinguishing between MM and AC, with the exceptions mentioned above. In both cytologic and histologic specimens, this distinction is often difficult on morphologic grounds alone, and ancillary techniques including IH and EM are often needed to arrive at a diagnosis. In our opinion, there is still no single marker that will always allow a definite distinction between MM and AC; most value is obtained by using a "multimodality" approach to the diagnosis of MM.[24] Because not all laboratories have access to EM, immunohistochemical studies are widely used to aid diagnosis. Differential staining for CEA has proved to be most valuable in this regard,[2,25] but instances of CEA-positive (albeit focal and weak) mesotheliomas are reported,[26-29] hence, in individual difficult cases staining for CEA is not always conclusive.

More recently, other novel monoclonal antibodies have been used to distinguish between MM and AC, including Leu-M1[3-8] and the analogous 624A12[9,10] and B72.3,[3,8,11] in addition to 44-3A6. The potential utility of these antibodies is enhanced if they are used as a panel together with anti-CEA. Leu-M1, which reacts with a differentiation antigen on myelomonocytic cells,[30] has shown a lack of reactivity with almost all mesotheliomas studied,[3,5,6,8,11] but in some cases staining of few mesothelioma cells has been reported.[3,8] In our study no case of MM stained with Leu-M1, further confirming its usefulness in the differential diagnosis of MM and AC. ACs, in contrast, show variable Leu-M1 positivity.[3-6,8,31] In individual cases with a CEA-negative and Leu-M1–negative phenotype, distinction between MM and AC still may not be made.

In a recent immunohistochemical study of MMs in pleurectomy specimens,[9] 9 of 43 MMs were focally 44-3A6 positive (less than 30% of cells); the staining was weak and occurred mainly in single cells and in foci of poor cellular cohesion rather than in solid tumor areas. Eight MMs were also focally and weakly CEA positive, whereas four showed this reaction with both 44-3A6 and CEA, suggesting that caution needs to be used when interpreting such patterns of reaction. Further, the possibility of false-positive CEA staining in MM, resulting from hyaluronic acid, needs to be kept in mind.[32]

Of our 36 cases of MM, 33 were 44-3A6 negative, all 36 were Leu-M1 negative, and only 2 contained isolated CEA-positive cells with the polyclonal antiserum. The three cases that were 44-3A6 positive were CEA negative and Leu-M1 negative. In two of these, only a few single MM cells were 44-3A6 positive; most MM cells, including cell clusters, did not show any 44-3A6 staining. The third case, confirmed ultrastructurally, showed staining for 44-3A6 in more than 50% of tumor cells. This case was interesting in that, one year previously, a contralateral right pleural MM had been diagnosed in a pleurectomy specimen (confirmed by the Pathology Panel of The Australian Mesothelioma Surveillance Programme). There was no evidence of any primary malignancy elsewhere. The right pleural MM had the typical ultrastructural features of MM and displayed the same 44-3A6–positive, CEA-negative, Leu-M1–negative phenotype as the cells from the left pleural effusion in this study. Apart from this solitary case, the remaining MMs were either totally 44-3A6 negative or, at most, showed very focal staining, unlike that obtained in most ACs. It is important that none of our MMs were both CEA positive and 44-3A6 positive. These results show that 44-3A6 is a useful antibody to use in the differential diagnosis of MM and AC in cytologic material, but we would recommend that it be used in conjunction with other antibodies, such as Leu-M1 and anti-CEA, to minimize any false-negative results.

Metastatic ovarian and renal carcinomas may not be distinguished from MMs with the use of the antibodies used in this study (see above). Metastatic serous ovarian carcinomas in particular (and also endometrial and renal carcinomas) may be indistinguishable from MM cytologically, and a similar 44-3A6–negative, Leu-M1–negative, CEA-negative phenotype may be shared with MM. Hence, other diagnostic modalities need to be used in these situations. Mucinous ovarian carcinomas, from the small number of cases studied so far, are more likely to be 44-3A6 positive, CEA positive, and Leu-M1 positive. Apart from these limitations, in the more common problematic situations in which the differential diagnosis lies between MM and metastatic pulmonary AC, breast carcinoma, or GIT carcinomas, 44-3A6 proves to be a valuable addition to the antibodies available for use in the resolution of these problems.

In summary, we have investigated and demonstrated the value of the monoclonal antibody 44-3A6 in the differential diagnosis of malignant effusions with the use of cell block material. We have confirmed its utility in demonstrating exocrine differentiation in pulmonary carcinomas and have shown that metastatic breast or GIT carcinomas most often show 44-3A6 reactivity similar to that of primary lung ACs. In effusions of MM, 44-3A6 staining,

like that for CEA, is positive or equivocal in only a few cases and suggests that, judiciously used with other discriminating antibodies such as CEA and Leu-M1, 44-3A6 is a valuable antibody in the differential diagnosis of MM and AC in effusions.

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