Immunocytological staining reactions of anti-carcinoembryonic antigen, Ca, and anti-human milk fat globule monoclonal antibodies on benign and malignant exfoliated mesothelial cells

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SUMMARY A panel of three monoclonal antibodies were used in an immunoalkaline phosphatase staining method on a series of serous effusion samples from cases of mesothelioma, lung carcinoma, and benign disease. The antibodies used were anti-carcinoembryonic (CEA) antigen, Ca, and anti-human milk fat globule membrane antigen. Antibodies to the Ca antigen and human milk fat globule membrane antigen stained 75% and 83% of mesothelioma and 75% of cases of lung carcinoma, respectively. The anti-CEA antibody stained most cases of lung carcinoma strongly but was negative on 11 of 12 cases of mesothelioma and showed weak staining on one case. Benign cases were negative with all three antibodies. These three antibodies may be useful in distinguishing benign and malignant mesothelial cells and lung carcinoma in serous effusions.

Patients with mesothelioma often develop pleural and peritoneal effusions, and as malignant cells may exfoliate into the effusions a cytological diagnosis can be made. Several authors have discussed the cytological features of mesotheliomas and have suggested that a high degree of accuracy can be obtained by light and electron microscopy.¹⁻³ The differentiation between a mesothelioma and a reactive mesothelial cell, however, is sometimes very difficult. Some adenocarcinomas, particularly of papillary type, may also be difficult to distinguish from mesothelioma. In these cases the estimation of hyaluronic acid can be of value as acid mucopolysaccharide is considerably increased in malignant mesotheliomas. Other useful criteria may include those derived from morphometric studies.⁴

Immunocytochemistry using antibodies to various cells and tissue antigens offers an objective means of identifying cell type. In previous publications we reported the potential value of using a panel of monoclonal antibodies to identify carcinoma cells in serous effusions.^{5,6} In this study we applied a panel of monoclonal antibodies to a series of effusion samples from

patients with suspected mesothelioma to determine if the staining patterns could be of use in the differential diagnosis of mesothelioma.

Material and methods

The samples in this study were from two groups of patients. The first group comprised four specimens of pleural or peritoneal fluid which had been sent to the cytology laboratory at the Churchill Hospital, Oxford. These samples were from patients in whom mesothelioma had been diagnosed cytologically. Histological confirmation was made in three cases. Air dried smears of the cell pellet were made as described previously.⁵

The second group comprised specimens sent to Dr Butler at St Mary's Hospital, Manchester. This group included cases of mesothelioma, carcinoma, and benign conditions that were sent as possible mesothelioma cases. The cases are grouped according to the final diagnosis (table 1). This was histological (biopsy or necropsy) in malignant disease and clinical, sometimes confirmed by necropsy, in benign disease.

Cytospin slides of effusion cells were made from the Manchester group of patients and sent to Oxford where the immunocytochemical staining was per-

Table 1 Diagnosis and immunocytochemical staining results

Diagnosis			Results of immunalkaline phosphatase staining		
Clinical	Cytological light microscopy	Transmission electron microscopy	Ca1/2	CEA	E29/HMFG2
Group 1					
Mesothelioma:					
Case No 1	Mesothelioma	NT	+	-/+	+
2	Mesothelioma	NT	+	-	+
3	Mesothelioma	NT	+	-	+
4	Mesothelioma	NT	+	-	+
Group 2					
Mesothelioma:					
5	Mesothelioma	Mesothelioma	-	-	+
6	Squamous	? Squamous	+	-	+
7	Mesothelioma	? Mesothelioma	+	-	+
8	Mesothelioma	Mesothelioma	+	-	+
9	Mesothelioma	Mesothelioma	+	-	±
10	Mesothelioma	NT	+	-	-
11	Mesothelioma	Mesothelioma	-	-	-
12	Mesothelioma	Unsatisfactory	+	-	+
Lung carcinoma:					
Adenocarcinoma					
13	Adenocarcinoma	Adenocarcinoma	+	+	+
14	Adenocarcinoma	Adenocarcinoma	+	+	+
15	Adenocarcinoma	Adenocarcinoma	+	+	+
Undifferentiated					
16	Negative	NT	+	+	+
17	Red cells and lymphocytes	Red cells	-	-	-
Squamous					
18	Squamous	Adenocarcinoma	+	+	+
Oat cell					
19	Oat cell	Oat cell	-	+	-
20	Oat cell	Unsatisfactory	-	-	-
Benign					
21	Pneumonia	Negative	-	-	-
22	Heart failure	Negative	-	-	-
23	Fractured femur	Negative	-	-	-
24	Rheumatoid	Negative	-	-	-
25	Rheumatoid: alive	? Lymphoma	-	-	-

+, positive; -/+, very weak staining; -, negative; NT = not tested.

formed. Air dried smears and cytospin slides were stored unfixed at -20°C for subsequent staining by the immunoalkaline phosphatase technique.

MONOCLONAL ANTIBODIES

Table 2 details the monoclonal antibodies used in this

Table 2 Details of monoclonal antibodies used for the immunocytological analysis of mesothelioma

Antibody	Specificity	Reference
11.285.14	Carcinoembryonic antigen (CEA)	Gatter <i>et al</i> ⁷
Ca1	Cancer associated glycoprotein	Ashall <i>et al</i> ⁸
Ca2	Human milk fat globule membrane antigen	Bramwell <i>et al</i> ⁹
E29		Cordell <i>et al</i> ¹⁰
HMFG2		Taylor-Papadimitriou <i>et al</i> ¹¹

study. The carcinoembryonic antigen antibody was obtained from Lilly Research Centre Limited, Ca1/2 from Professor H Harris, E29 from DY Mason and HMFG2 from Unipath. Sheep antiserum against mouse immunoglobulin was prepared by standard immunisation schedule. Monoclonal antialkaline phosphatase-alkaline phosphatase was prepared as described previously.¹²

Smears were fixed in acetone or acetone-methanol (50:50 v/v) for 10 minutes and then stained by an immunoalkaline phosphatase staining procedure as described previously.^{5,12} Briefly, this entailed incubation in turn with mouse monoclonal antibody, sheep antimouse immunoglobulin, and mouse monoclonal antialkaline phosphatase-alkaline phosphatase (APAAP) immune complexes. Each incubation step was followed by washing in Tris buffered saline.

The alkaline phosphatase reaction product was visualised with naphthol ASMX phosphate and fast

red as substrates. Slides were counterstained with haematoxylin and mounted in an aqueous mountant.

Results

The clinical, cytological, and immunocytochemical staining reactions are detailed in table 1.

GROUP 1

The patients with cytological evidence of mesothelioma all reacted positively with E29 or HMFG2 antibodies, or in combination with Cal/Ca2. A strong positive reaction of the malignant cells was observed with these antibodies. One case gave a very weak speckling with anti-carcinoembryonic antigen; the other cases were negative.

GROUP 2

Of the eight patients with histological, electron microscopic, or cytological confirmation of mesothelioma, cells from five gave positive reactions with E29/HMFG2 and Cal/Ca2 and two with E29/HMFG2 alone. One patient from whom two samples were obtained was consistently negative with all three antibodies, although the effusion samples seemed to contain cells with morphological characteristics of mesothelioma. Anti-carcinoembryonic antigen was negative on all mesothelioma specimens.

Patients with adenocarcinoma, squamous cell, and undifferentiated lung carcinoma gave positive staining reactions with E29/HMFG2, Cal/Ca2, and carcinoembryonic antigen. One case of small undifferentiated oat cell carcinoma was positive with carcinoembryonic antigen alone and another case was negative with all three antibodies. On these cytospin slides, however, there were only a few carcinoma cells. Patients with benign effusions were negative with all three antibodies except for the occasional positive plasma cell.

Discussion

Immunocytochemical staining reactions clearly distinguished between malignant and benign mesothelial cells. The former group were positive for the Ca antigen and human milk fat globule membrane antigen in 75% and 83% of cases, respectively, and 11 of 12 were negative for carcinoembryonic antigen, while benign mesothelial cells were negative with all three antibodies. Although this series included only a few benign cases, the staining reactions of these antibodies on benign cases has been documented previously.⁵ Human milk fat globule membrane antigen antibodies occasionally give a weak reaction on benign mesothelial cells but this can easily be dis-

tinguished from the strong staining reaction observed on malignant mesothelial cells. Ca antigen positive benign mesothelial cells have been observed in a small number of cases but the staining reaction is weak.^{5 9 13}

There have been few published reports on the antigenic profile of malignant mesothelial cells in effusions. Walts *et al* examined the expression of cytokeratin and carcinoembryonic antigen in exfoliated cells of fine needle aspirates and body fluids.¹⁴ They detected cytokeratin in normal and reactive mesothelial cells and in 56% of adenocarcinomas. On the other hand, 69% of adenocarcinomas were positive for carcinoembryonic antigen while reactive mesothelial cells were negative or only weakly positive. This profile of strong cytokeratin staining and weak or absent carcinoembryonic antigen staining of reactive and malignant mesothelial cells has been observed by other workers in studies of histological sections¹⁵⁻¹⁸ while lung carcinomas may be carcinoembryonic antigen positive or negative for carcinoembryonic antigen according to histological type.¹⁹

In a previous study of serous effusions a monoclonal anti-cytokeratin antibody showed strong positive staining of benign and malignant mesothelial cells and adenocarcinoma cells.⁵ Carcinoembryonic antigen was strongly expressed in lung carcinomas and was absent from benign mesothelial cells, and a weak reaction was seen in one mesothelioma case.

The expression of human milk fat globule membrane antigen and Ca antigen have been studied extensively on carcinoma cells in effusions. These studies showed that lung adenocarcinoma expressed these two antigens.^{5 6 9 13 20} In these previous studies, however, malignant effusion samples from patients with mesothelioma were rarely included. In a recent study on histological sections of pleura using the same antisera as that in the present study HMFG2, E29, and Cal/2 reacted with benign mesothelium in three of seven cases and with mesothelioma.¹⁸ We concluded that these antisera could not be used to distinguish between benign and malignant mesothelium. The reason for the difference observed between staining of histological sections and exfoliated cells is unclear. When cells exfoliate the surface properties may change, leading to phenotypic changes. Marshall *et al*²¹ also studied the staining of HMFG2 on histological sections of benign mesothelium and they found that few cases were positive. Their findings are more consistent with this present study.

Although none of the antibodies used in this study is specific for mesothelioma, the staining reactions of a panel could aid in the diagnosis of problem cases. A positive reaction with HMFG2 and Cal/2 and a negative reaction with carcinoembryonic antigen, although supporting a diagnosis of mesothelioma,

does not exclude carcinoma, especially from extrapulmonary sites. Pulmonary squamous and undifferentiated carcinomas are less often positive than adenocarcinomas for carcinoembryonic antigen, and ovarian and breast carcinomas have a much lower percentage of positivity. When the differential diagnosis between mesothelioma and other lung cancers is difficult, a positive immunocytochemical reaction for carcinoembryonic antigen strongly suggests that the tumour is of bronchial epithelial origin.

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