MONOCLONAL ANTIBODIES TO SMALL-CELL CARCINOMA OF THE HUMAN LUNG

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Small-cell carcinoma of the lung accounts for 20-25% of all cases of lung cancer in man [1, 5]. Courses of intensive chemotherapy for this form of cancer under protection of transplantation of autologous bone marrow are currently being used [5]. The problem of the effective diagnosis of bone marrow metastases accordingly arises. Cytological investigations have shown that small-cell carcinoma of the lung metastasizes to bone marrow in 20-30% of cases [3, 5, 12]. Recently much interest has been aroused by the obtaining of monoclonal antibodies to small-cell carcinoma of the lung and their possible use for immunodiagnosis and immunotherapy. The use of specific monoclonal antibodies has been shown to more than double the discovery rate of metastases in the bone marrow [4, 8].

The aim of this investigation was to obtain and characterize monoclonal antibodies to small-cell carcinoma of the human lung with a view to their possible use for immunodiagnosis and for synthesis of immunotoxins.

EXPERIMENTAL METHOD

Mice were immunized intraperitoneally with 10^6-10^7 H417 cells (small-cell carcinoma of the human lung). Spleen cells from an immune mouse were hybridized with cells of murine myeloma SP2.O[6]. Hybridoma clones were screened by the indirect immunofluorescence method on living H417 cells. The specificity of positive clones at the first stage of screening was investigated by the indirect immunofluorescence method on normal human bone marrow cells and on frozen sections of tumors from patients with small-cell carcinoma of the lung. A detailed study of the specificity of clones reacting positively with tumor preparations and not reacting with bone marrow cells, was carried out by the indirect fluorescence method on frozen sections of normal tissues and of various tumor tissues.

Selected primary clones were thrice cloned by the final dilutions method. To obtain ascites tumors, about 10^7 living hybridoma cells were injected intraperitoneally into mice.

For solubilization of membrane proteins, H417 cells were washed 3 times to remove growth medium with Na-phosphate buffer, pH 7.5, frozen in pellet form, and kept at –20°C. After thawing the cells were lysed in PBS containing 0.5% Triton X-100 for 15 min at room temperature.

The molecular weight of the antigens revealed by the antibodies thus obtained was determined by polyacrylamide gel electrophoresis of the membrane protein fraction of the H417 cells in the presence of SDS [7], and by immunoblotting [11].

Isotopes of the monoclonal antibodies were determined with the aid of a diagnostic kit from "ICN Immunobiologicals."

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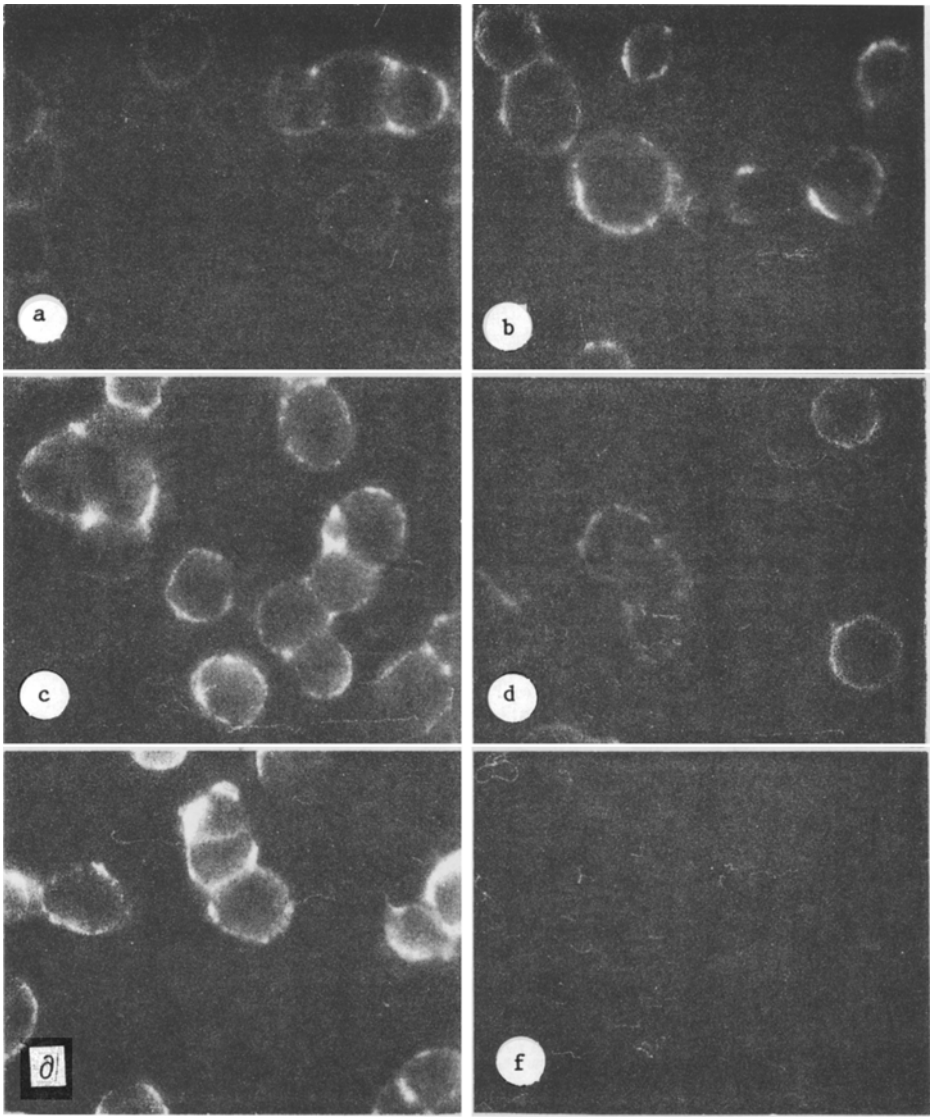

Fig. 1. Interaction of monoclonal antibodies to small-cell carcinoma of the human lung with cells of the H417 line. The cells were adsorbed on poly L-lysine and stained with hybridoma supernatants: a) H417.1, b) H417.3, c) H417.10, d) H417.17, e) H417.21. As secondary antibodies, FITC-labeled antibodies to mouse immunogloblins (from Sigma, USA) were used. f) Negative control (without primary antibodies). 1500×.

EXPERIMENTAL RESULTS

After primary screening of the hybridomas 38 clones reacting positively with H417 cells were selected At the first stage of investigation of their specificity five primary clones not reacting with normal bone marrow cells but reacting positively with tumor preparations, were selected (Figs. 1 and 2). These antibodies belong to immunoglobulin subclasses IgG1 (H417.3 and H417.21), IgG2a (H417.1), IgG3 (H417.17), and IgM (H417.10).

The results of a more detailed investigation of the specificity of the five selected clones are given in Table 1.

The specificity of the antibodies thus obtained relative to cell surface protein-antigens of H417 cells was determined by the immunoblotting method. Antibody H417.3 revealed a polypeptide with mol. wt. of 47-50 kDa in the composition of the membrane proteins of H417.2 cells. The results of immunoblotting are given in Fig 3.

TABLE 1. Results of Immunohistochemical Investigation of Human Normal Organs and Tumors

Test object	Monoclonal antibodies				
	H417. 1	H417. 3	H417. 10	H417. 17	H417. 21
Bone marrow	0	0	0	0	0
Lung	0	0	0	0	0
Spleen	0	0	0	0	0
Liver	0	0	0	0	0
Stomach	0	0	0	2	2
Intestine	3	3	3	2	2
Kidney	3	2	2	2	2
Striated muscles	2	0	2	2	2
Thyroid gland	2	3	3	2	0—1
Arteries	0	0	0	0	0
Carcinoma of the lung	3	3	3	2	2
small-cell	4	4	3	2	1
	4	4	4	3	3
squamous-cell	0	2—3	0	1—2	1—2
	0	0	0	0	0
	1	2	2	2	1
Adenocarcinoma of the lung	0	0	0	0	0
	3	3—4	2—4	2	2
Glandular-squamous-cell carcinoma of the ovary	0—2	0	0—2	0	0—2
Glandular-squamous-cell carcinoma of the stomach	0	0	0	0	0
Signet-ring-cell carcinoma of the stomach	1	1	1	2	3
Adenocarcinoma of the stomach	2—3	2—3	2—3	2—3	2—4
	0—3	0—3	0—3	0	0
Leiomyoma of the esophagus	0	0	0	0	0
Pleural fibroma	0	0	0	1	1
Infiltrative duct carcinoma of the breast	0	0	0	0	1
	0	0	0	0	0
Medullary carcinoma of the thyroid gland	3	2	2	1	1
	2	2	1	1	1
Papillary carcinoma of the thyroid gland	0	0	0	0	0
Squamous-cell carcinoma of the larynx	0—2	0—2	0—2	0—2	0—2
	1—2	1—2	1—3	1—3	0—2
	0	0	0	0	0
Squamous-cell carcinoma of the mouth	0	0	0	0	0
	0—2	0—2	0—2	0	0—2
T-cell lymphosarcoma	3—4	3	2—3	2—3	1
Melanoma	0	0	0	1	1
	0	1	1	0	1
	0	0	0	0	0
	0	0	0	0	0
Lymphogranulomatosis of the spleen	0	2	0	0	2
Adenocarcinoma of the large intestine	0	0	0	0	0
Giant-cell osteosarcoma	0	0	0	0	0

Legend. 0) No reaction; 1) small excess over background; 2) considerable excess over background; 3) large excess over background; 4) very large excess over background.

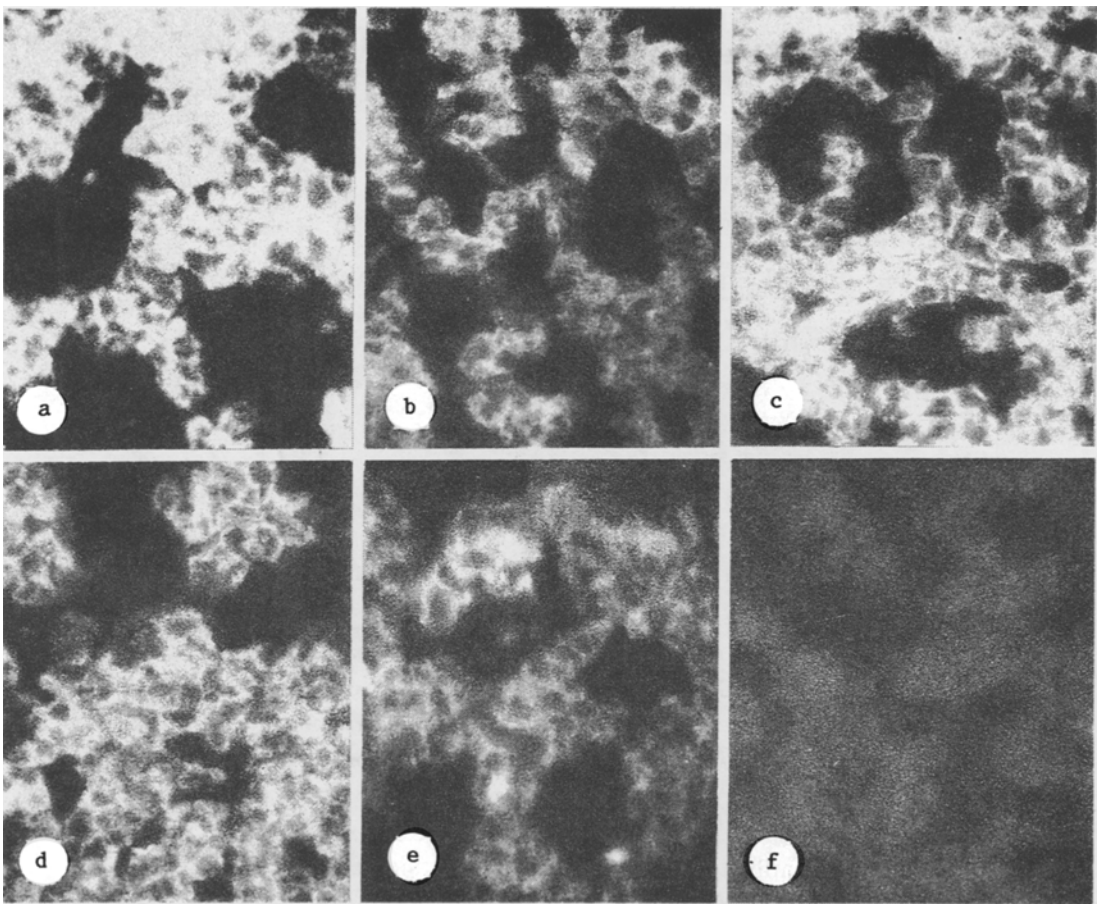

Fig. 2. Immunofluorescence staining of frozen sections of lung tumor from patient with small-cell carcinoma of the lung with the aid of monoclonal antibodies to small-cell carcinoma of the human lung. Sections 6 nm thick were stained with hybridoma supernatants: a) H417.1, b) H417.3, c) H417.10, d) H417.17, e) H417.21. As secondary antibodies, FITC-labeled antibodies to mouse immunoglobulins ("Sigma," USA) were used; f) negative control (without primary antibodies). 940×.

To obtain monoclonal antibodies to cells of a small-cell carcinoma of the human lung, preparations of isolated known antigens [2], tumor cells from patients with small-cell carcinoma of the lung, and cell lines can be used [8, 10]. In this investigation we used cell line H417 of small-cell carcinoma of the lung.

Antibodies reacting only with cells of a small-cell carcinoma of the lung and not reacting with cells of other tumors have been described [13]. Such antibodies can be used for the effective differential diagnosis of small-cell and other types of lung carcinoma, in bronchoscopic investigations, for example [9].

Since the antibodies we obtained reacted most intensively with small-cell carcinoma of the lung and did not react with some of the normal tissues tested and, in particular, with normal bone marrow cells, it seems likely that these antibodies may be used for the immunodiagnosis of metastases of small-cell carcinoma of the lung to the bone marrow. Comparative cytological and immunological investigation of the patients' bone marrow are currently in progress in order to study this possibility. Meanwhile, the specificity of the selected antibodies is not restricted purely to cells of a small-cell lung carcinoma. As will be clear from Table 1, these antibodies also react with some of the normal tissues and some of the tumors studied, including from patients with carcinoma of the lung other than the small-cell type. This crossed reactivity does not allow the antibodies we obtained to be used for the effective differential diagnosis between small-cell and non-small-cell carcinoma of the lung. It is intended in the future to synthesize immunotoxins on the basis of these antibodies and to analyze the possibility of their use for the elimination of tumor cells from the bone marrow of patients with small-cell carcinoma of the lung treated by autografting.

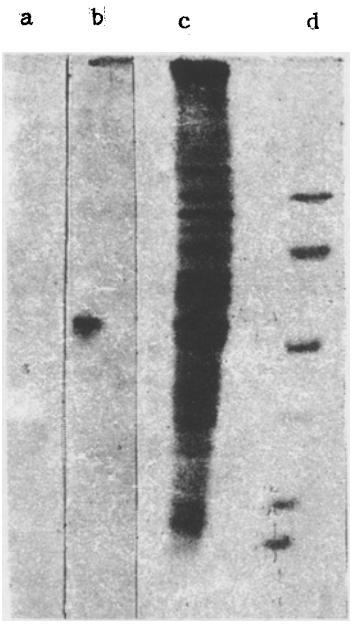

Fig. 3. Interaction of monoclonal antibodies to small cell carcinoma of the human lung with membrane proteins of H417 cell line, fractionated by polyacrylamide gel electrophoresis. a and b) Immunoblotting, c and d) stained with Coomassie R-250. a, b, and c) Membrane polypeptides of H417 cells; d) mixture of marker proteins with molecular weights of 97.4, 66.2, 42.7, 31.0, 21.5, and 14.4 kDa. b) Nitrocellulose filter was treated with H417.3 antibodies and then with goat antibodies to mouse immunoglobulins conjugated with horseradish peroxidase, and developed with 4-chloro-1-naphthol. a) Negative control (without primary antibodies).

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