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Monoclonal Antibodies to Human Lung Tumor Antigens Demonstrated by Immunofluorescence and Immunoprecipitation[1]

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ABSTRACT

Various studies have demonstrated the usefulness of monoclonal antibodies in recognizing discrete tumor antigenic determinants. The present study describes the tissue reactivity of monoclonal antibodies prepared against a squamous cell carcinoma of the lung. Antigens were purified from the tumor extract by anti-β_2 microglobulin affinity chromatography. These β_2-associated antigens demonstrated tumor specificity by the leukocyte adherence inhibition assay. Antibody-secreting hybridomas were generated by fusion of mouse myeloma cells with mouse spleen cells immunized with purified tumor antigens. Hybridomas were selected by a solid-phase tumor membrane-binding immunoassay.

The target specificity of the secreted monoclonal antibodies was ascertained by radioimmunoprecipitation analysis and indirect immunofluorescence on various human tumor and normal tissue sections. Monoclonal antibody-secreting hybridomas 48.4.8 and 48.4.2 secreted immunoglobulin that selectively immunoprecipitated polypeptide fragments from human lung tumor membrane antigens. Hybridoma 9.2.2 secreted antibody that was strongly positive by indirect immunofluorescence on all tested lung squamous cell carcinomas. Adjacent or intervening normal lung tissue did not display significant immunofluorescence. Adenocarcinomas of the lung were negative or focally positive when focal squamous cell differentiation was present. Oat cell carcinomas were negative. The secreted antibody did not significantly stain three extrapulmonary tumors or a variety of normal tissues.

INTRODUCTION

The rejection of tumors transplanted between syngeneic animals demonstrated unequivocally the presence of antigenic constituents unique to tumors (6). Inasmuch as the techniques of graft rejection were initially used to demonstrate the antigenic uniqueness of some experimental tumor systems, the antigens so defined were called tumor-specific transplantation antigens. However, the structural nature of the tumor antigens so defined was unknown. Recently, the structure of the components of the major histocompatibility locus has been more completely defined from both a serological and a structural point of view, and an antigenic and structural relationship between tumor antigens and histocompatibility antigens has been noted. Alien histocompatibility antigens that are unique to haplotypes other than those found on the tumor-bearing animal have been demonstrated in a variety of experimental antigen systems (2, 3, 7, 13, 15).

In our laboratory with affinity chromatography using antisera to β_2-microglobulin, the common low-molecular-weight subunit of human histocompatibility antigen molecules, we have isolated from human tumor cell membranes molecules which are structurally and antigenically similar to human histocompatibility antigen molecules and which demonstrate tumor antigen activity *in vitro* by the leukocyte adherence inhibition assay (16, 20, 21). Attempts to produce tumor-specific heteroantisera to molecules isolated in this fashion from human squamous cell cancer tissue were unsuccessful. The present studies describe the production of monoclonal antibodies to such antigens purified from lung tumor.

MATERIALS AND METHODS

Cells. The P3-NS-1Ag4/1 myeloma cell line was obtained from Flow Laboratories (Rockville, Md.). The U698M, Bristol-8, and CEM cell lines were kindly provided by Dr. A. Sullivan, McGill Cancer Center. The SK-MES-1 squamous cell lung carcinoma cell line was obtained from Dr. J. Fogh, Sloan-Kettering Institute for Cancer Research. The MCF-7 mammary tumor cell line was obtained from Dr. D. M. P. Thomson, McGill Cancer Center. The HCT-8R colon tumor cell line was obtained from Dr. W. B. Rawls, MacMaster University. All cell lines were grown in RPMI[4] Medium 1640 supplemented with 0.01 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, streptomycin (100 mcg/ml), penicillin (100 units/ml), 1.25 mM pyruvate, and 2 mM glutamine containing either 15% heat-inactivated agamma horse serum or fetal calf serum.

Animals. Six-week-old female BALB/c mice were obtained from the McIntyre Animal Center (McGill University, Montreal, Canada).

Purification of Antigens from Human Lung Tumor and Adjacent Normal Tissues. Lung squamous cell carcinoma with adjacent normal tissue was obtained postmortem from the lung and hepatic metastatic deposits of one patient. Lung tumor-specific antigens were prepared as described previously (16). Briefly, the papain-solubilized lung tumor membrane fragments were passed through a DE-52 column and chromatographed on a calibrated Sephadex G-150 column. The M_r 45,000 to 50,000 fraction was pooled, concentrated, and chromatographed on an anti-human β_2-microglobulin AH-Sepharose 4B affinity column. The bound fraction, eluted with 3 M KSCN, was dialyzed, lyophilized, and chromatographed on a calibrated Sephadex G-100 column. The M_r 45,000 to 50,000 fraction was pooled and concentrated. By identical methodology, normal tissue antigens were isolated from the adjacent normal areas. Tumor specificity was tested by blocking leukocyte adherence inhibition assays (20) performed in the laboratory of D. M. P. Thomson.

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[4] The abbreviations used are: RPMI, Roswell Park Memorial Institute; PBS, phosphate-buffered saline (0.15 M NaCl:0.01 M sodium phosphate buffer, pH 7.2).

Elaboration of Hybridomas. A 6-week-old female BALB/c mouse was immunized and boosted 6 weeks later by i.p. inoculations of 25 μg of purified lung tumor antigens mixed with equal volumes of complete Freund's adjuvant. Three days after the final immunization, the mouse was sacrificed, and the splenocytes were fused with NS/1 myeloma cells (11). Briefly, splenocytes were mixed with NS/1 cells at a ratio of 10:1, and the cell mixture (after pelleting by centrifugation) was suspended in 0.5 ml of 35% (w/v) Polyethylene Glycol-1500 (BDH Chemicals, Ltd., Poole, England) in serum-free RPMI Medium 1640 for 2 min. The cells were then gradually resuspended in complete medium containing 0.1 mM hypoxanthine, 0.4 μM aminopterin, and 16 μM thymidine and then distributed into four 24-well culture plates (Costar, Cambridge, Mass.). After 10 days in this medium, cultures were fed with complete medium containing 0.1 mM hypoxanthine and 16 μM thymidine. Colonies positive for binding to tumor membranes were subcultured into separate wells and subsequently cloned in soft agar 2 consecutive times (4).

Monoclonal Antibody Production. Hybridoma culture supernatants were used undiluted or after γ-globulin fractionation by precipitation with 50% saturated $(NH_4)_2SO_4$ followed by dialysis against PBS.

Isotyping of Monoclonal Antibodies. Monospecific goat antisera to murine immunoglobulin isotypes (Cappel Laboratories, Cochranville, Pa.) were used to determine the isotype of monoclonal antibodies secreted by hybridomas by double diffusion in agar gel.

Purification of Lung Tumor Membranes. Lung tumor plasma membranes were purified from the crude membrane fraction (16) by ultracentrifugation (100,000 × *g*, 60 min) through 50% (w/w) sucrose containing 0.01 M Tris, pH 7.4. The plasma membrane-containing fraction at the interface was diluted, pelleted (100,000 × *g*, 60 min) washed, and repelleted. The final pellet was resuspended in PBS, and the protein concentration was determined (14). These purified membranes were used in binding assay testing for monoclonal antibodies.

Assay for Binding of Monoclonal Antibody to Lung Tumor Membranes. Hybridoma culture supernatants were assayed for reactive antibodies using a solid-phase membrane-binding assay (18). Membranes (8 μg) were added to each well of 96-well polyvinyl U-bottomed microtiter plates (Dynatech Laboratories, Inc., Alexandria, Va.) and allowed to dry overnight by incubation at 37°. The membranes were fixed in 0.1% glutaraldehyde in PBS for 5 min and washed 3 times with PBS containing 10% agamma horse serum. Undiluted hybridoma culture supernatant (50 μl) was added to wells, and, after incubation for 90 min at room temperature, unbound immunoglobulin was removed by 3 washes with PBS containing 10% agamma horse serum. Rabbit $F(ab)_2$ anti-mouse IgG (heavy- and light-chain specific) affinity purified on mouse IgG:Sepharose or goat $F(ab)_2$ anti-mouse IgM (μ chain specific) (Cappel Laboratories) was labeled by the chloramine-T technique (10). Buffer (50 μl) containing 1 × 10^5 cpm of the appropriate ^{125}I-labeled reagent was added to each well and allowed to incubate for 90 min at room temperature. The wells were then washed 4 times in buffer, individual wells were separated from the plate, and bound radioactivity was determined in a LKB 1270 Rackgamma II counter.

Immunoprecipitations and Analysis of Tumor Antigen. Monoclonal antibodies were tested for their ability to precipitate lung tumor antigens by radioimmunoprecipitation. Purified tumor and the corresponding normal tissue antigens were ^{125}I labeled by the Bolton-Hunter procedure (New England Nuclear, Boston, Mass.) (1). Aliquots of antigen (1 × 10^6 cpm) were precleared by incubation with 1 μg of murine IgM (Daymar Laboratories, Toronto, Canada) and goat anti-mouse IgM (μ chain specific) (Cappel Laboratories) for 2 hr followed by centrifugation (10,000 × *g*, 30 min). Aliquots of the precleared antigen supernatant were then mixed with culture supernatant γ-globulins and incubated at 4° for 2 hr followed by overnight incubation with goat anti-mouse IgM. After centrifugation, pellets were washed 3 times in buffer (0.15 M NaCl:50 mM Tris:5 mM EDTA:0.5% Nonidet P-40, pH 7.3) with careful resuspension of the pellets each time. Antigens were subsequently eluted with electrophoresis sample buffer. Sodium dodecyl sulfate:polyacrylamide gel electrophoresis was performed under reducing conditions by the method of Laemmli (12).

Immunofluorescence. Normal and neoplastic adult tissues were obtained at the time of either surgery or autopsy. Tissues were immediately frozen in isopentane and stored at −70°. Cryostat sections (6 μm) were incubated with a 1:10 dilution of monoclonal antibody or normal mouse serum control for 1 hr at 22° and 1 hr at 4°. The slides were then rinsed 3 times with PBS and incubated with a 1:10 dilution of fluorescein isothiocyanate-conjugated $F(ab)_2$ goat anti-mouse IgM (μ chain specific) (Cappel Laboratories) for 30 min at room temperature. The sections were then rinsed 3 times with PBS and mounted in glycerin:PBS (9:1). Adjacent sections of tissue were routinely stained with hematoxylin and eosin.

Paraffin sections were deparaffinized through successive changes of xylol and alcohol, rehydrated, and trypsinized for 1 hr at 37° [0.05% trypsin (w/v) (Type II; Sigma Chemical Co., St. Louis, Mo.):0.1% $CaCl_2$, pH 7.8] as described previously (9). Sections were rinsed and processed for immunofluorescence as described above.

Cultured human cells (5 × 10^5 to 1 × 10^6 cells) were pelleted by centrifugation at 300 × *g* for 5 min, resuspended in 50 μl of a 1:10 dilution of monoclonal antibody or serum control, and incubated on ice for 30 min. The cells were washed once with fetal calf serum and twice with RPMI Medium 1640 containing 5% fetal calf serum. The cells were resuspended in a 1:5 dilution of fluorescein isothiocyanate-conjugated goat $F(ab)_2$ anti-mouse IgM and incubated on ice for 30 min. The cells were washed 3 times as described above and mounted in one drop of fetal calf serum.

Fluorescence was visualized by incident illumination using a Leitz Orthoplan microscope equipped with an I_2 Ploem filter combination. The slides were read independently by 2 observers. Photography of test and control slides was performed using ASA 400 Kodak Tri-X films for constant time intervals of 3 min. Phase-contrast microscopy on the same slide was used to visualize the fluorescent and nonfluorescent underlying structures and delineate tumor islands.

RESULTS

Preparation of Lung Tumor-specific Antigen. Antigens purified from metastases of a moderately differentiated squamous cell carcinoma were demonstrated to be lung tumor specific in the leukocyte adherence inhibition assay (16, 20). Antigens isolated from adjacent normal liver and lung tissues by identical purification procedures showed no activity in the leukocyte adherence inhibition assay. The partially purified tumor antigen preparation was subsequently used as immunogen in the preparation of hybridomas.

Generation of Monoclonal Antibodies. Spleen cells from a mouse immunized with purified tumor antigen were fused with the murine NS/1 myeloma cell line. After fusion, 75 of 84 seeded culture wells showed growth of hypoxanthine:aminopterin:thymidine-resistant hybrid cells. Supernatant fluids from these cultures were screened for immunoglobulin production and for binding to lung tumor membranes. In the initial assay, 52 culture wells secreted antibodies reactive to lung tumor membranes (Chart 1). These cultures were expanded and frozen in liquid nitrogen. From these cultures, 5 (Wells 9, 25, 35, 47, and 48) were selected for further passage and cloning on the basis of their ability to maintain long-term and stable secretion of antibody. All positive subclones were further expanded and γ-globulin fractions of supernatants were prepared for use in subsequent binding and absorption studies.

Analysis by double immunodiffusion indicated that the isotypes of the selected hybrid clones secreted antibodies of the IgM subclass containing light chains. Therefore, reagents specific for the IgM subclass were used in subsequent analyses.

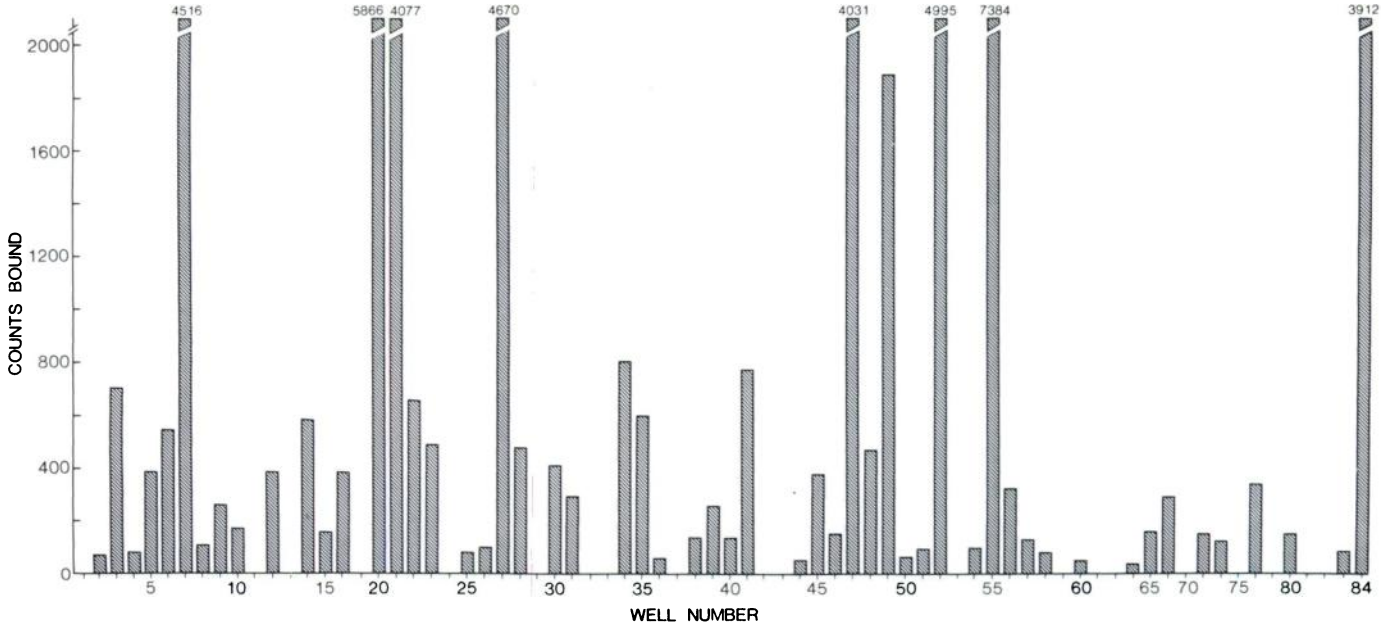

Chart 1. Initial screen against lung tumor membranes of undiluted supernatants from culture wells. Screening was done in duplicate by solid-phase indirect radioimmunoassay with ^{125}I-labeled rabbit anti-mouse IgG (heavy- and light-chain specific) as described in "Materials and Methods." NS-1 culture supernatant was used as a negative control, and bindings were corrected for this background binding. Binding greater than 100 cpm is at least 2 S.D. above background. *Numbers above bars*, counts bound in samples which are beyond the axis of the graph.

Identification of Antigenic Targets by Radioimmunoprecipitation. Purified papain-digested fragments of tumor and normal antigens derived as described above from metastases of squamous cell carcinoma and adjacent normal tissues were ^{125}I labeled and immunoprecipitated with secreted immunoglobulins from selected hybridoma clones. As demonstrated in Fig. 1*a* (*Lanes C* and *F*), secreted immunoglobulins from subclones 48.4.8 and 48.4.2 immunoprecipitated M_r 25,500 and 11,500 proteins from the purified tumor antigenic preparation specifically. In the corresponding normal antigenic preparation, Fig. 1*b* (*Lanes C* and *F*), no selective immunoprecipitation was observed. No specific bands in either the tumor or normal antigenic preparation were immunoprecipitated with secreted antibodies from clones 9.2.2 and 25.3.1 in Fig. 1 (*Lanes D* and *E*). An M_r 30,000 polypeptide was precipitated from both tumor and normal antigenic preparations by the tested hybridoma and culture supernatants as well as by the IgM control.

Immunohistological Characterization. Indirect immunofluorescence on cryostat sections was used to determine the target specificity of the monoclonal antibodies. In addition, some paraffin-embedded tissues were tested by immunofluorescence after dewaxing and trypsin digestion. Since the secreted immunoglobulin of clone 9.2.2 was strongly positive against tissue sections of squamous cell carcinoma, this antibody was used in subsequent studies. Antibodies secreted by clones 48.4.8 and 48.4.2 failed to give significant fluorescent staining of lung tumor sections.

Table 1 shows the results when the secreted antibody of clone 9.2.2 was tested against 11 lung tumors (9 surgical and 2 autopsy specimens). All tested squamous cell carcinomas were strongly positive. In all cases, substitution of normal mouse serum resulted in no significant fluorescence. Furthermore, when the secreted antibody of 9.2.2 was used, the adjacent or intervening nontumoral lung tissue revealed by concomitant phase-contrast microscopy did not display significant fluorescence. Adjacent sections stained with hematoxylin and eosin were used to identify the morphology of the positively and negatively stained areas of immunofluorescence. When squamous cell carcinomas were tested, it was observed that the positive staining was present diffusely in all tumor islands. At high magnification, cryostat sections showed positive tumor staining that was most marked at the plasma membrane, although cytoplasmic staining was also present.

Table 1

Immunofluorescent staining of human lung tumor cells with monoclonal antibody 9.2.2

Patient	Detailed histological description	Immunofluorescent staining and distribution
W. S.	Well-differentiated squamous cell carcinoma	3+, diffuse
A. S.	Moderately differentiated squamous cell carcinoma	3+, diffuse
L. D.	Moderately to poorly differentiated squamous cell carcinoma[a]	3+, diffuse
N. B.	Adenocystic carcinoma	3+, focal
F. M.	Adenocarcinoma with minor focal squamous and bronchioloalveolar carcinoma	1+, focal
H. J.	Moderately differentiated adenocarcinoma	Negative
D. M.	Moderately to poorly differentiated adenocarcinoma	Negative
L. Y.	Well-differentiated adenocarcinoma	Negative
J. D.	Poorly differentiated adenocarcinoma[b]	Negative
J. M.	Oat cell carcinoma	Negative
D. K.	Oat cell carcinoma	Negative

[a] Metastatic to mediastinal lymph node.
[b] Metastatic to skin.

The indirect immunofluorescence by 9.2.2 on a squamous cell carcinoma tissue section is illustrated in Fig. 2. Hematoxylin and eosin staining shows tumor islands surrounded by stromal tissue (Fig. 2*A*). Only the tumor islands display significant fluorescent staining.

The positivity observed in one case of adenocarcinoma (Patient F. M.) was focal within the tumor nests. This latter tumor was not homogeneous, as revealed by routine hematoxylin and

eosin staining, and contained focal areas of squamous differentiation.

Oat cell carcinomas were negative.

Twenty-two normal tissues, including 12 from the lung and 10 from other sites, showed no significant fluorescence (Table 2). The extrapulmonary carcinomas tested were also negative.

DISCUSSION

Monoclonal antibodies against determinants on human lung tumor were produced using a purified lung tumor preparation as immunogen in conjunction with hybridoma technology. Hybrid clones were originally selected for the ability of their secreted antibodies to bind to human lung tumor membranes. Although this solid-phase immunoassay was useful for the initial screening and selection of clones, a nonspecific binding component due to the high affinity of mouse IgM (17) for polyvinyl plastic precluded the use of this assay for the determination of absolute tissue specificity. The use of immunofluorescence and radioimmunoprecipitation was used to ascertain the target specificity of the antibodies.

Monoclonal antibodies secreted from clones 48.4.8 and 48.4.2 immunoprecipitated specific polypeptides of molecular weights of 25,500 and 11,500 from a purified papain-digested extract of tumor membrane. Current studies are focusing on defining the membrane proteins corresponding to these papain-solubilized moieties. Monoclonal antibody secreted from clone 9.2.2 failed to selectively immunoprecipitate polypeptides from the immunizing tumor antigen preparation. This is not surprising, since monoclonal antibodies sometimes demonstrate apparently anomalous behavior (8). The complexes of solubilized tumor antigen and antibody from clone 9.2.2 may not cross-link sufficiently for immunoprecipitation. Alternatively, the Bolton-Hunter radioiodination technique may fail to label the relevant tumor antigen or may damage the epitopic site. Future studies will use Western blot analysis in an effort to identify the antigen.

From the experiments performed on cryostat sections from tumor and nontumor tissue, it is apparent that hybridoma 9.2.2 produced an antibody which proved to be sensitive in detecting squamous cell carcinoma of the lung. Although not absolute, its specificity with respect to this histological type of lung tumor can be characterized as high in view of the negative staining in other histological types of lung cancer.

In particular, the absence of positive staining in the case of oat cell carcinoma suggests that this antibody detects different antigenic determinants as compared with the monoclonal antibodies against oat cell carcinoma described by Cuttitta *et al.* (5). This negative immunoreactivity in oat cell carcinomas is consistent with their presumed neuroectodermal origin in contrast with the endodermal derivation of squamous cell carcinomas of the lung.

Four of 5 cases of adenocarcinoma of the lung were negative. Interestingly, the one case of adenocarcinoma that was focally positive contained focal areas of squamous cell differentiation. Occasionally, lung tumors contain both adenocarcinoma and squamous cell differentiation (19).

The absence of reactivity with the cell line derived from a case of squamous cell carcinoma of the lung can be explained by the marked phenotypic and behavioral differences which often characterize cells maintained in tissue culture. Although analyzing cell lines is a much more convenient method to screen antibodies, it would appear that variability associated with epithelial tissue culture occasionally fails to reflect the reactivity of native tissue.

The antigenic determinant is different from carcinoembryonic antigen as evidenced by the absence of staining in the case of colonic carcinoma. Furthermore, carcinoembryonic antigen was not detected by immunodiffusion and radioimmunoprecipitation in the tumor extract used for immunization (data not shown).

The preferential staining of squamous differentiation of lung tumor could be due to a normal component of squamous cells undergoing amplification with tumor growth. This possibility should be clarified after extensive testing of the antibody in other squamous lesions.

Further studies are in progress to test the specificities of the antibodies on a broader tumor spectrum and to determine biochemically the nature of the recognized tumor antigens. Our studies have defined a squamous cell antigen in lung cancer while Cuttitta *et al.* (5) have characterized antibodies that identify oat cell tumor markers. The collection of a battery of such monoclonal reagents directed against human lung tumor will permit identification of tumor-associated antigens, provide precise probes for their further characterization and purification, and be of potential use in clinical diagnosis, prognosis, and therapy.

Table 2

Reactivity of monoclonal antibody 9.2.2 on normal and tumor tissues

	Reactivity (Positive/total)
Lung tumors	
Squamous cell carcinoma	3/3
Adenocarcinoma	1/5
Adenocystic carcinoma	1/1
Oat cell carcinoma	0/2
Other tumors	
Carcinoma of the colon	0/1
Breast carcinoma	0/2
Melanoma	0/1
Normal lung (parenchyma, bronchi, trachea)	0/12
Normal tissues (colon, breast, liver, kidney, peripheral blood lymphocytes)	0/10
Human cell lines	
SK-MES 1 (squamous cell carcinoma of the lung)	Negative
MCF-7 (breast carcinoma)	Negative
HCT-8R (carcinoma of the colon)	+[a]
CEM (acute T-cell lymphoblastic leukemia)	Negative
Bristol 8 (lymphoblastoid B-cell line)	Negative
U698M (μ-secreting B-cell lymphoma)	Negative

[a] Positive in 5 to 10% of the cells.

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REFERENCES

1. Bolton, A. E., and Hunter, W. M. The labelling of proteins to high specific radioactivities by conjugation to a ^{125}I-containing acylating agent. Biochem. J., *133:* 529–539, 1973.
2. Bonavida, B., and Roman, J. M. Expression of alien histocompatibility antigens on SJL/J tumors detected by cell-mediated and serological anal-

yses. Cancer Immunol. Immunother., *11:* 115–123, 1981.
3. Clemetson, K. J., Bertschmann, M., and Luscher, E. F. Tumor-associated antigens of the mouse P-815 mastocytoma cell: evidence for an antigen with H-2 like characteristics. Mol. Immunol., *18:* 135–141, 1981.
4. Cotton, R. G. H., Secher, D. S., and Milstein, C. Somatic mutation and the origin of antibody diversity. Clonal variability of the immunoglobulin produced by MOPC 21 in culture. Eur. J. Immunol., *3:* 135–140, 1973.
5. Cuttitta, F., Rosen, S., Gazdar, A. F., and Minna, J. D. Monoclonal antibodies that demonstrate specificity for several types of human lung cancer. Proc. Natl. Acad. Sci. U. S. A., *78:* 4591–4595, 1981.
6. Foley, E. Antigenic properties of methylcholanthrene-induced tumors in mice of the strain of origin. Cancer Res., *13:* 835–837, 1953.
7. Gipson, T. G., Imamura, M., Conliffe, M. A., and Martin, W. J. Lung tumor-associated derepressed alloantigen coded for by the K region of the H-2 major histocompatibility complex. J. Exp. Med., *147:* 1363–1373, 1978.
8. Goding, J. W. Antibody production by hybridomas. J. Immunol. Methods, *39:* 285–308, 1980.
9. Huang, S. N., Minassian, H., and More, J. D. Application of immunofluorescent staining on paraffin sections improved by trypsin digestion. Lab. Invest., *35:* 383–390, 1976.
10. Hunter, W. M. Radioimmunoassay. *In:* D. M. Weir (ed.), Handbook of Experimental Immunology, Vol. 1, pp. 14.1–14.40. Oxford, England: Blackwell Scientific Publications, 1978.
11. Kohler, G., and Milstein, C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature (Lond.), *256:* 495–497, 1975.
12. Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature (Lond.), *227:* 680–685, 1970.
13. Lindahl, K. F. Anomalous H-2 antigens on tumour cells. Nature (Lond.), *280:* 105–106, 1979.
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. Protein measurement with the Folin phenol reagent. J. Biol. Chem., *193:* 265–275, 1951.
15. Parmiani, G., Carbone, G., Invernizzi, G., Pierotti, M. A., Sensi, M. L., Rogers, M. J., and Appella, E. Alien histocompatibility antigens on tumor cells. Immunogenetics, *9:* 1–24, 1979.
16. Rauch, J. E., Shuster, J., Thomson, D. M. P., and Gold, P. Isolation of HLA and tumor antigens by means of affinity chromatography employing anti-β_2 microglobulin antiserum. Cancer (Phila.), *42:* 1601–1615, 1978.
17. Salonen, E. M., and Vaheri, A. Immunobilization of viral and *Mycoplasma* antigens and of immunoglobulins on polystyrene surface for immunoassays. J. Immunol. Methods, *30:* 209–218, 1979.
18. Simrell, C. R., and Klein, P. A. Antibody responses of tumor-bearing mice to their own tumors captured and perpetuated as hybridomas. J. Immunol., *123:* 2386–2394, 1979.
19. Spencer, H. Carcinoma of the lung. *In:* Pathology of the Lung, pp. 773–859. Elmsford, N. Y.: Pergamon Press, Inc., 1977.
20. Thomson, D. M. P., Tataryn, D. N., O'Connor, R., Rauch, J., Friedlander, P., Gold, P., and Shuster J. Evidence for the expression of human tumor-specific antigens associated with β_2-microglobulin in human cancer and in some colon adenomas and benign breast lesions. Cancer Res., *39:* 604–611, 1979.
21. Thomson, D. M. P., Tataryn, D. N., Weatherhead, J. C., Friedlander, P., Rauch, J., Schwartz, R., Gold, P., and Shuster, J. A human colon antigen associated with β_2-microglobulin and isolated from solid tumour, serum, and urine is unrelated to carcinoembryonic antigen. Eur. J. Cancer, *16:* 539–551, 1980.

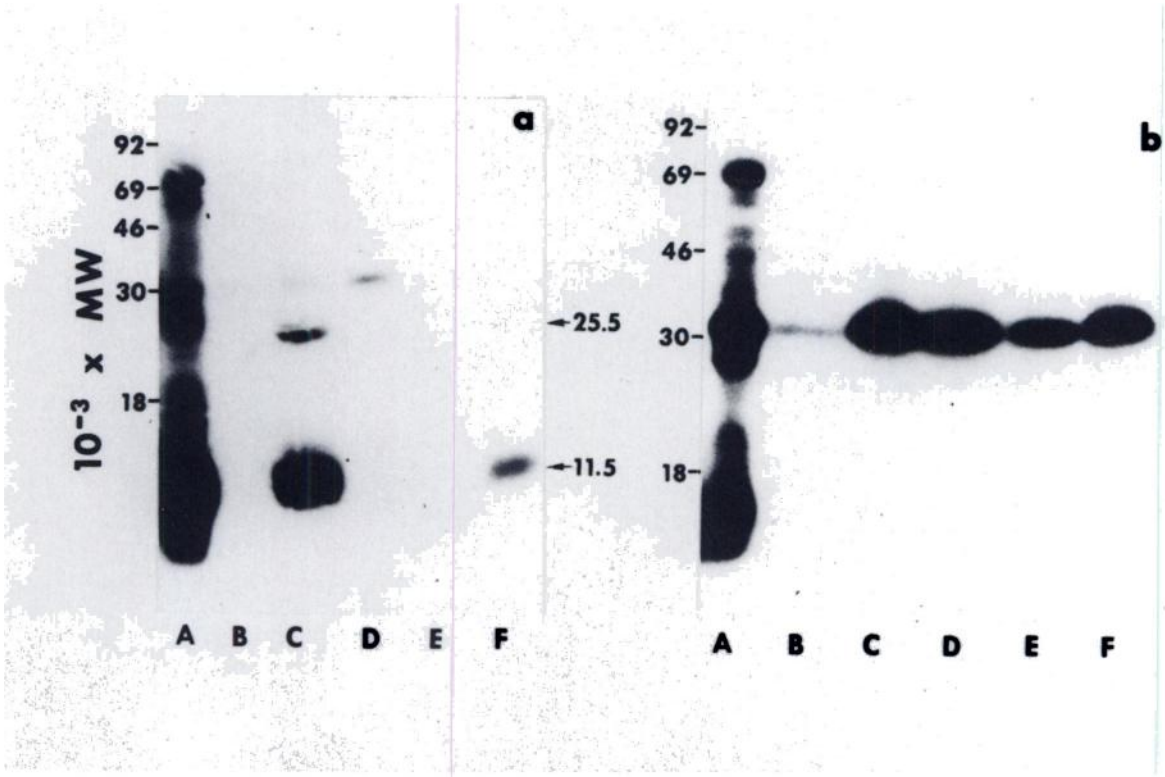

Fig. 1. Radioimmunoprecipitation of ^{125}I-labeled antigens by monoclonal antibodies. Immunoprecipitations were done as described in "Materials and Methods" using sodium dodecyl sulfate electrophoresis in 12.5% gels using phosphorylase B (*M*, 92,500), albumin (*M*, 69,000), ovalbumin (*M*, 46,000), carbonic anhydrase (*M*, 30,000), and lactoglobulin A (*M*, 18,367) as molecular weight standards. *a*, immunoprecipitation of ^{125}I-labeled tumor antigen (*Lane A*) by control murine IgM (*Lane B*) and monoclonal antibodies 48.4.8 (*Lane C*), 9.2.2 (*Lane D*), 25.3.1 (*Lane E*), and 48.4.2 (*Lane F*). *b*, immunoprecipitation of ^{125}I-labeled normal antigen (*Lane A*) by control murine IgM (*Lane B*), monoclonal antibodies 48.4.8 (*Lane C*), 9.2.2 (*Lane D*), 25.3.1 (*Lane E*), and 48.4.2 (*Lane F*). *MW*, molecular weight.

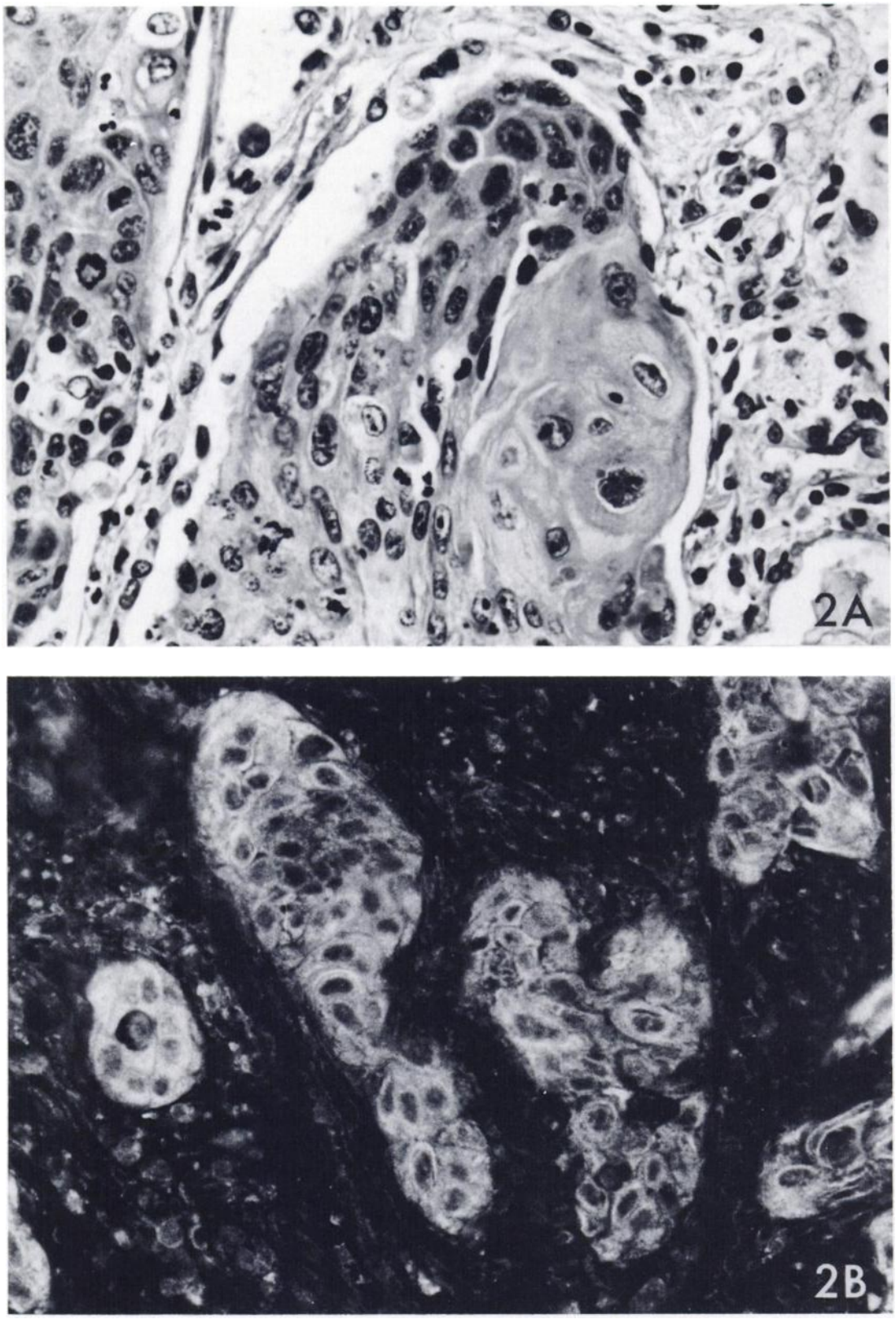

Fig. 2. Photomicrography demonstrating indirect immunofluorescence on squamous cell carcinoma (W. S.) tissue sections by monoclonal antibody 9.2.2. *A*, tumor islands and adjacent stromal tissue. H & E, × 480. *B*, immunofluorescent staining of tumor islands by monoclonal antibody 9.2.2 followed by fluorescein isothiocyanate goat anti-mouse IgM. Significant fluorescent staining is restricted to tumor islands within section, × 480.