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Immunology

Cell surface antigens of human ovarian and endometrial carcinoma defined by mouse monoclonal antibodies

(thyroid colloid antigen/epithelial differentiation antigens/heat-stable antigens)

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ABSTRACT **Mouse monoclonal antibodies to several cell surface antigens of human ovarian and endometrial carcinomas have been produced. The distribution of the antigens was determined by mixed hemagglutination assays on 153 normal and malignant cell cultures of various types and by immunoperoxidase staining of frozen sections of 27 normal adult and 24 fetal tissues. Five distinct antigens were characterized. MD144 antigen was detected on only a single ovarian carcinoma cell line and has the biochemical properties of a lipid. MH55 antigen is weakly expressed on ovarian and uterine cancer cell lines but not on other cells and tissues tested. MF61 antigen was detected on an ovarian carcinoma and some renal carcinoma cell lines but not on other cell lines tested. It was also detected by immunoperoxidase staining in the noncellular follicles of the thyroid and in uterine glandular epithelial cells. This antigen also has the properties of a lipid. MF116 antigen was detected on a proportion of ovarian, uterine, renal, and bladder carcinoma and neuroblastoma cell lines and on normal kidney epithelial cell cultures but not on other cell lines tested. It was not detected in sections of any normal tissue tested using the immunoperoxidase method. MF116 was readily detected in the spent culture medium but not in detergent-solubilized extracts of metabolically radiolabeled cells. This shed antigen is a glycoprotein of M_r 105,000 and isoelectric point lower than pH 4.0. MH94 antigen was detected on a proportion of ovarian, uterine, colon, breast, lung, cervical, and pancreatic carcinoma cell lines. In tissue sections it was detected in many but not all epithelia, predominantly in secretory epithelial cells. Antibody MH94 did not immunoprecipitate a detectable antigen.**

Mouse monoclonal antibodies to human tumor cell surface antigens have been produced in many laboratories (reviewed in ref. 1). The intention of these studies has frequently been to identify tumor-associated antigens that could be useful in tumor therapy or diagnosis. In practice, it was found that most or all of these antibodies recognized differentiation antigens. A few studies have described antibodies that detect very restricted antigens. Among these restricted antigens are the G_{D3} ganglioside antigen in melanoma/melanocytes (2, 3) and a novel ganglioside antigen that may provide a serum marker in colon, gastric, and pancreatic cancer patients (4). In the case of ovarian cancer, a mouse monoclonal antibody reactive with cryostat sections of ≈50% of ovarian carcinomas but not with normal tissues, except fallopian tube, endometrium, and endocervix, has been described (5, 6).

In this report, we describe studies on the production of mouse monoclonal antibodies to ovarian and endometrial cancer. The identification of five distinct cell surface antigens is described.

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MATERIALS AND METHODS

Target Cells. Cell lines used are listed in Table 1 and were cultured as described (7–9). Cultures of normal human fibroblasts, kidney epithelial cells, and melanocytes have also been described (7–9). Normal blood mononuclear cells were obtained by centrifuging heparinized blood onto a layer of Ficoll-Paque (Pharmacia). Total blood leukocytes were obtained by collecting the buffy coat after centrifugation for 10 min at 600 × *g* in 100-μl capillary tubes.

Production of Mouse Monoclonal Antibodies. BALB/c or (BALB/c × C57BL/6)F_1 mice were immunized with either the ovarian carcinoma SK-OV-3 or 2774 or the endometrial carcinoma SK-UT-1 cell lines. Intraperitoneal injections of ≈100 μl of packed cells were given two to five times at intervals of 2 weeks. Three days after the last injection, the fusion of immune spleen cells with mouse myeloma MOPC-21 NS/1 cells was performed as described (10). Culture supernatants were monitored for antibody activity by the anti-mouse Ig mixed hemagglutination assay (MHA) on a panel of cultured cells consisting of the immunizing cell line and other types of human tumor cells. Cloned hybridoma cells were injected subcutaneously into *nu/nu* mice. Sera from mice with progressive growing tumors were collected and used for serological and biochemical characterization. Antibody subclass was determined by double diffusion in agar with anti-Ig heavy chain-specific reagents (Bionetics, Kensington, MD).

Serological Procedures. The MHA, on adherent and nonadherent (11) target cells using rabbit anti-mouse Ig and goat anti-mouse IgM, has been described (8, 12). Monoclonal sera were titrated starting at 10^{-3}. To confirm the specificity of antibodies, absorption tests were performed with the immunizing cell line and three melanomas (SK-MEL-28, SK-MEL-37, and MeWo), three astrocytomas (SK-MG-1, SK-MG-3, and U373 MG), three carcinomas (SK-BR-3, SK-LC-6, and Scaber), one T-cell leukemia (MOLT-4), one B-cell leukemia (Raji), and human erythrocytes. Absorption procedures have been described (7).

To test heat stability of antigens, cells were heated 5 min at 100°C before performing absorption tests. To test the hydrophobic nature of antigens, cell pellets were extracted with 20 vol of chloroform/methanol, 2:1. Solubilized material was dried and resuspended by sonication in Dulbecco's phosphate-buffered saline (GIBCO)/0.5% bovine albumin (fraction V, Sigma) to a volume equal to the original packed cell volume. This suspension was assayed for inhibitory activity of the appropriate antibody.

Immunoperoxidase staining of sections employed 5-μm cryostat sections. Air-dried sections were fixed for 10 min at room temperature with 2.0% buffered formaldehyde (13). A triple sandwich was used routinely, which consisted of

Abbreviations: MHA, mixed hemagglutination assay; Con A, concanavalin A.

Table 1. Reactivity of mouse monoclonal antibodies with cultured cells and cell lines

Cells	Antibody MD144	MF61	MF116	MH94
Ovarian carcinoma				
2774, SK-OV-6, SW 626, SK-OV-3	●000	●000	●000	0●●0
SK-OV-4, Colo 316, A7, A10	0000	0000	0000	0000
SW 626	0	0	0	0
Endometrial carcinoma				
SK-UT-1	0	0	●	●
Renal carcinoma				
SK-RC-1, -2, -7, -8	0000	●000	●●●●	0000
SK-RC-9, -10, -12, -17	0 00	0●●●	● ●00	00
SK-RC-35, Caki-2, SK-RC-4, -6	0000	●●00	0000	0000
SK-RC-16, -28, Caki-1	000	000	000	000
Bladder carcinoma				
253J, SCABER, RT4, VM-CUB-1	0000	0000	●000	0000
VM-CUB-2, 5637, 639-V, J82	0000	0000	0000	0000
486-P, JCCSUP	00	00	00	00
Colon carcinoma				
SK-CO-10, SW403, SW480, SK-CO-1	0000	0000	0000	●●●0
SW620, SW1222, HT-29	000	000	000	000
Breast carcinoma				
CAMA, SK-BR-3, -5, BT-20	0000	0000	0000	●000
BT-474, MCF-7, AlAb, ZR-75-1	0000	0000	0000	0000
DA-MB-361, MDA-MG-231	00	00	00	00
Lung carcinoma				
SK-LC-9, -15, -1, -2	0000	0000	0000	●●00
SK-LC-3, -4, -5, -6	0000	0000	0000	0000
SK-LC-7, -8, -10, -13	0000	0000	0000	0000
SK-LC-14, -16, Calu-1, SK-LC-LL	0000	0000	0000	0000
SK-LC-12	0	0	0	0
Cervical carcinoma				
ME 180	0	0	0	◒
Pancreatic carcinoma				
CAPAN-1, -2, ASPC-1	000	000	000	0●0
Neuroblastoma				
LA-N-1S, SK-N-SH, -MC, BE(2)-C	0000	0000	●000	0000
SH-EP1, SK-N-BE(2)	00	00	00	00
Melanoma				
SK-MEL-13, -19, -23, -26	0000	0000	000	0000
SK-MEL-28, -29, -31, -37	0000	0000	0000	0000
SK-MEL-75, -93-2, -93-3, -127	0000	0000	0000	0000
SK-MEL-130, -153, MeWo	000	000	000	000
Astrocytoma				
SK-MG-1, -2, -3, -5	0000	0000	0000	0000
SK-MG-6, -8, -11, -14	0000	0000	0000	0000
SK-MG-15, U138MG, U251MG, U373MG	0000	0000	0000	0000
Other carcinoma				
SK-HEP-1 (hepatoma), GCC-SV(c)	00	00	00	00
T-cell lymphoma and leukemia				
MOLT4, CCRF-HSB-2, CCRF-CEM, 45	0000	0000	0000	0000
Peer, P12/Ichikawa, HPB-ALL	000	000	000	000
B-cell lymphoma and leukemia				
SK-LY-16, -18, Daudi, Ball-1	0000	0000	0000	0000
SK-DLH-2, ARA-10, Raji	000	000	000	000
Null-cell leukemia				
NALM-1, -16	00	00	00	00
Myeloid leukemia				
HL-60, K562, KG-1	000	000	000	000
Myeloma				
SK-MY-1, LICR-Lon-HMy-2	00	00	00	00
Monocytic leukemia				
U937	0	0	0	0
Normal				
Fibroblasts (six), melanocytes	00	00	00	00
Kidney epithelia, 1, 2	00	00	◒◒	00
Non-human				
Vero, CHO	00	00	00	00

The symbols listed under the antibodies refer to the titer against the cell line in the corresponding position in the left-hand side of the table. The titer of the antibody was defined as the highest dilution producing at least 50% rosetting in the MHA assay. ●, 1×10^{-3}–1×10^{-6}; ◒, positive reaction but with <50% rosetting at 10^{-3} dilution of antibody; 0, no reactivity at antibody dilution of 10^{-3}.

monoclonal antibody (*nu/nu* mouse serum at 1:500), biotinylated horse anti-mouse Ig, and complexes of avidin and biotinylated horseradish peroxidase (Vectastain reagents, Vector Laboratories, Burlingame, CA), following procedures recommended by the manufacturer. For particular tissues that had excessive background with this procedure—namely, the kidney, liver, and pancreas—a double sandwich was used, which comprised monoclonal sera at 1:200 and peroxidase-conjugated anti-mouse Ig (DAKO P161) at 1:50. To ensure that fixation did not destroy the antigen investigated, each antibody was first tested on sections of tissue culture cells frozen in 10% dimethyl sulfoxide at 50% packed cell volume/volume. All antibodies tested were positive in this assay, when the immunizing cell line was used as the target.

Immunofluorescent staining of blood leukocytes in suspension was performed as described (14) with fluorescein-conjugated goat anti-mouse Ig (Cappel Laboratories, Cochranville, PA) at 1:40 and monoclonal sera at 1:50. Lymphocytes and granulocytes were distinguished by morphology.

Immunoprecipitation Procedures. Each antibody was tested for its ability to precipitate an antigen from detergent-solubilized extracts of the immunizing cell after labeling by three methods: metabolic incorporation of [^{3}H]glucosamine (15), metabolic incorporation of [^{35}S]methionine (10), or Chloramine-T ^{125}I labeling of solubilized cell membranes (16). Nonidet P-40 solubilization of labeled cells and concanavalin A (Con A)-Sepharose fractionation of labeled extracts, used in some experiments, have been described (10, 15, 16), as have immunoprecipitation procedures for ^{125}I-labeled samples, using *Staphylococcus aureus* (16). Aliquots of 2×10^6 ^{35}S cpm from unfractionated cell extracts were handled similarly, except that preclearing was omitted. From the Con A eluate fraction of ^{35}S-labeled extracts and for ^{3}H-labeled extracts, aliquots of 2×10^5 cpm and different washing buffers (17) were used. Precipitated molecules were extracted with 60 μl of 0.01 M Tris·HCl, pH 7.2/2.0% $NaDodSO_4$/12.0 mg of dithiothreitol per ml/15% (wt/vol) sucrose/0.01% pyronin Y by heating 5 min at 100°C and

were analyzed by polyacrylamide gel electrophoresis (10, 18), using 9% gels and by two-dimensional electrophoresis (15, 19). For unreduced samples, dithiothreitol was omitted and 14.0 mg of iodacetamide per ml was added to samples.

RESULTS

Selection of Hybridomas. Hybrids were initially selected for cloning on the basis of reactivity with the immunizing cell line and lack of reactivity with three melanomas and three astrocytomas. Monoclonal antibodies MD144, MF61, MF116, ME195, and ME46 were obtained after immunization with ovarian carcinoma cell line 2774, and antibodies MH55 and MH94 were obtained after immunization with endometrial carcinoma cell line SK-UT-1. The heavy chain subclasses of the seven antibodies are: MD144, γ_1; MH55, μ; MF61, μ; MF116, γ_{2a}; ME46, γ_1; ME195, γ_1; and MH94, γ_1. Two other antibodies from these fusions that detected more widespread antigens are described elsewhere (20). None of these antibodies reacted with glycoproteins carrying A, B, H, Le^a, Le^b, X, Y, or I blood group structures.

MD144. Antibody MD144 reacted with only a single ovarian carcinoma cell line, 2774, with a titer of 10^{-4} by immune rosetting; all 152 other cell lines tested were negative (Table 1). The antigen was not detected in sections of normal tissues (Table 2). Absorption experiments also did not detect the antigen on any cell type except 2774; in this assay, 1–3 μl of packed cells was required for nearly complete absorption. The antigen was not destroyed by heating at 100°C for 5 min, and it was present in the chloroform/methanol extract of 2774 cells. In immunoprecipitation experiments using cell extracts labeled with [^{3}H]glucosamine but not with [^{35}S]-methionine, radioactivity was precipitated that migrated at the dye front in both 9% and 12.5% acrylamide gels (Fig. 1). These properties all strongly suggest that the antigen is a lipid.

MH55. Antibody MH55 is an IgM antibody that reacts weakly with 4/8 ovarian carcinomas (2774, SK-OV-6, A10, and A7) and 1/1 uterine carcinoma (SK-UT-1) with a titer of 10^{-3} or lower; all 148 other cell types tested were negative. Varying the temperature of incubation with antibody, the density of the target cells, and the time interval between target cell plating and testing did not improve the titer. Therefore, we have not done absorption experiments. Antibody MH55 did not react with any tissue sections examined but it did react with sections of frozen pellets of A10 ovarian carcinoma cells.

MF61. Antibody MF61 reacted with 1/8 ovarian carcinomas and 6/16 renal carcinoma cell lines (Table 1); the other 146 cell types tested were negative. Absorption experiments revealed no additional positive cell types. Blood leukocytes were negative by immunofluorescence. Absorption tests were unusually sensitive, in that 0.1 μl of packed cells absorbed nearly completely; therefore, negative absorption under our standard conditions indicates expression of the antigen that is 1/300th or less than on the immunizing cell line. MF61 antigen, like the MD144 antigen, was heat-stable and soluble in chloroform/methanol. The chloroform/methanol extracts were as active as intact cells in absorption experiments. Also, antibody MF61 immunoprecipitated radioactivity from [^{3}H]glucosamine-labeled cell extracts that migrated at the dye front in both 9% and 12.5% acrylamide gels (Fig. 1). It was concluded that this antigen may be a lipid.

In tissue sections, antibody MF61 reacted with two normal tissues: glandular epithelial cells of the adult and fetal uterus and the noncellular follicles of the thyroid (Fig. 2*A*). It also reacted with the follicles of a pig thyroid (not shown).

MF116. Three antibodies were obtained from two fusions that react with the same antigen, of which the prototype antibody is MF116. The other two antibodies, ME46 and ME195, are IgG1, whereas antibody MF116 is IgG2a. Antibody MF116 reacted with 1/8 ovarian carcinomas, 1/1 endometrial carcinoma, 6/16 renal carcinomas, 1/10 bladder carcinomas, and 1/6 neuroblastoma cell lines. It also reacted with 2/2 normal kidney epithelial cell cultures (Table 1). The other 141 cell types tested were negative. Absorption experiments revealed no additional positive cells; 10 μl of packed cells was required for nearly complete absorption; this result is consistent with a low expression of antigen on the cell surface. Blood leukocytes were negative by immunofluorescence. By immunoperoxidase, MF116 was not detected in any normal tissues examined, including normal kidney, ovary, and uterus.

MF116 antigen was immunoprecipitated from [^{3}H]glucosamine- or [^{35}S]methionine-labeled spent medium from ovarian carcinoma 2774. No antigen was detected in solubilized cell extracts labeled with [^{3}H]glucosamine, [^{35}S]methionine, or ^{125}I. This antigen is preferentially shed or secreted in the medium, although it must be present on the cell surface because it is detected in rosetting assays. The M_r is 105,000, as estimated by polyacrylamide gel electrophoresis (Fig. 1). If the antigen was not reduced, it migrated slightly faster, indicating some intrachain disulfide bonds (not shown). The iso-

Table 2. Summary of reactivities of monoclonal antibodies

Antibody	On tissue culture cells*	On normal adult tissue sections†	On normal fetal tissue sections‡
MD144	Ovarian carcinoma (1/8)	Negative	NT
MH55	Ovarian (4/8) and uterine (1/1) carcinoma	Negative	NT
MF61	Ovarian (1/8) and renal (6/16) carcinoma	Positive: uterine epithelial cells and thyroid colloid	Positive: uterine epithelial cells
MF116	Normal kidney epithelial cells, ovarian (1/8), uterine (1/1), renal (6/16), bladder (1/10) carcinoma and neuroblastoma (1/6)	Negative	Negative
MH94	Ovarian (2/8), uterine (1/1), colon (3/7), breast (1/10), lung (2/18), and cervical (1/1) carcinoma	Positive: pancreas, ureter, breast, prostate, cervix, urinary bladder epithelial cells, sweat and sebaceous glands of skin	Positive: stomach, intestine, pancreas, ureter, urinary bladder, uterus and cervix epithelial cells

NT, not tested.

*Antibodies were tested on 153 cell lines of various types (Table 1). All tests except those listed were negative.

†Antibodies were tested on sections of 27 normal adult tissues: lung, heart, liver, spleen, gall bladder, esophagus, stomach, small intestine, colon, pancreas, kidney, ureter, urinary bladder, adrenal, thyroid, breast, prostate, testes, ovary, fallopian tube, uterus, cervix, placenta, skin, brain, lymph nodes, and muscle. Tissues not listed were negative.

‡Antibodies were tested on 24 normal fetal tissues: lung, heart, spleen, thymus, liver, gall bladder, esophagus, stomach, small intestine, colon, pancreas, kidney, ureter, urinary bladder, adrenal, testes, ovary, fallopian tube, uterus, cervix, skin, brain, lymph nodes, and muscle. Tissues not listed were negative.

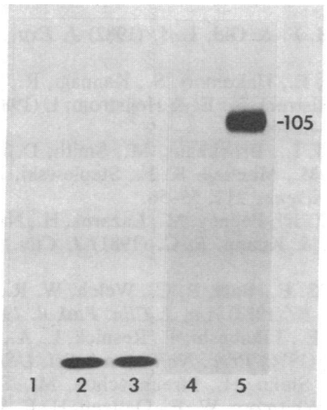

FIG. 1. Fluorograms of immunoprecipitates of [^{3}H]glucosamine-labeled extracts from ovarian carcinoma 2774 analyzed by polyacrylamide gel electrophoresis. Lanes 1–3, the antigen was a Nonidet P-40-solubilized cell extract and the gels were 12.5% acrylamide; lanes 4–5, the antigen was spent culture medium and the gels were 9% acrylamide. The precipitating antibodies were 0.1 μl of normal *nu/nu* mouse serum (lanes 1 and 4), MD144 (lane 2), MF61 (lane 3), or ME195 (lane 5). ME195 and MF116 react with the same antigen. The molecular weight ($\times 10^{-3}$) of the ME195 antigen is indicated. Five to 21 days of exposure.

electric point was determined to be less than pH 4.0, because the antigen migrated at or off the acidic end of the isoelectric focusing gel. This antigen bound to Con A-Sepharose and was eluted with methyl-α-D-mannoside. The antigen was destroyed by heating at 100°C, as determined in absorption experiments. MF116 was not detected by immunoprecipitation in the spent medium of two other cell lines (SK-UT-1 and SK-RC-1) that were positive by rosetting, so the release of relatively large amounts of the antigen occurs only with certain cell lines.

MH94. MH94 antigen was detected on various carcinoma cell lines, being detected on 2/8 ovarian carcinomas, 1/1 endometrial carcinoma, 3/7 colon carcinomas, 1/10 breast carcinomas, 2/18 lung carcinomas, 1/1 cervical carcinoma, and 1/3 pancreatic carcinoma cell lines (Table 1). All 142 other cell types tested were negative. Absorption experiments did not reveal additional positive cells; 3 μl of packed SK-UT-1 cells gave nearly complete absorption. Blood leukocytes were negative by immunofluorescence. By immunoperoxidase, MH94 was detected in the acinar and duct-lining cells of the pancreas, the epithelial cells of the ureter (Fig. 2*B*), breast, pancreas, cervix, and urinary bladder, and the sweat and sebaceous glands of the skin. It was also found in fetal stomach, intestine, pancreas, ureter, urinary bladder, endometrium, and endocervix.

The MH94 antigen was not destroyed by heating to 100°C but was not detected in a chloroform/methanol extract of cells. It was not precipitated under any conditions tested, which included labeling two cell lines with three isotopes. Therefore, its biochemical nature is uncertain.

DISCUSSION

These studies describe five antibodies detecting highly restricted antigens that are of considerable interest for the analysis of ovarian and uterine tumors (Table 2). More broadly reactive antibodies derived from the same fusions, recognizing glycoprotein antigens, were described previously (20). These antibodies generally had higher titers than the ones described here, perhaps reflecting the characteristics of the antigens recognized as discussed below.

Both MD144 and MF61 antigens have properties of lipids or hydrophobic proteins. A substantial fraction of very restricted mouse monoclonal antibodies, produced in several

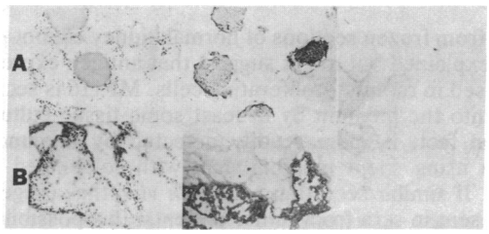

FIG. 2. Photographs of 5-μm cryostat sections of normal human thyroid (*A*) and normal human ureter (*B*). For each tissue type, *Left* is of a section stained with hematoxylin, to show the morphology, *Center* is of a section stained by immunoperoxidase with MF61 (*A*) or MH94 (*B*), and *Right* is of a section stained by immunoperoxidase with either a negative monoclonal serum or normal *nu/nu* mouse serum, using the same microscope and camera settings as in the preceding photographs. In *A*, only the colloid is stained. In *B*, only the epithelium is stained.

laboratories, have recognized glycolipids (2–4), a result that was quite unexpected on the basis of previous work using xenoantisera to human tumor cells. Our characterization of these two antigens is incomplete. Resistance to heating at 100°C, which is one of their characteristics, could be a property of lipids, carbohydrate determinants on glycoproteins, or exceptional protein determinants. MD144 and MF61 antigens are soluble in chloroform/methanol, but this does not distinguish between lipids and hydrophobic proteins (21). Likewise, the immunoprecipitation by antibodies MD144 and MF61 of radioactivity running at the dye front in acrylamide gel electrophoresis, after labeling with [^{3}H]glucosamine, does not prove the antigens are glycolipids, because small glycoproteins would have similar properties. Also, we have evidence that some hydrophobic interactions are not completely disrupted in the presence of the detergents used to solubilize cells, so glycolipids might be coprecipitated by antibodies to hydrophobic proteins or to a nonglycosylated lipid.

The significance of MD144 antigen, which is found on only a single ovarian cancer cell line, is uncertain. This component might be a rare human allele, a rarely expressed gene product, or a mutant form of a normal cell component. Although unique antigens have been demonstrated on chemically induced animal tumors (22) and on human tumors (23), present data indicate that these antigens are proteins or glycoproteins (24–26) and therefore differ in this respect from MD144. MF61 has an unusual distribution in normal tissue, being present in the noncellular follicles of the thyroid and in uterine glandular epithelial cells. The dominant antigen of the thyroid colloid is thyroglobulin, but antibody MF61 was not reactive with human thyroglobulin. A second colloid antigen has been described (27) but has not been characterized biochemically. On tumor cells, MF61 is very restricted in its distribution, being detected only on one ovarian carcinoma line and six renal carcinoma cell lines.

MH94 was detected on a small fraction of carcinoma cell lines tested, including carcinomas of the ovary, uterus, colon, breast, lung, cervix, and pancreas. The fact that this antigen was detected on only 1/10 breast carcinomas and 2/20 lung carcinomas indicates the importance of testing many cell lines of each tumor type in determining the distribution of an antigen. In frozen sections, MH94 was detected in secretory epithelial cells of many normal tissues.

On tissue culture cells, MF116 was found on normal kidney cells as well as on some carcinomas of the ovary, uterus, kidney, and bladder and on one neuroblastoma. The most frequent tumor type that was positive was renal carcinoma, for which 6/16 cell lines were positive. MF116 was not detected in sections of any normal tissue. The presence of MF116 on normal kidney cells in tissue culture and its ab-

sence from frozen sections of normal kidney cannot presently be explained but might suggest that antigen expression is increased in rapidly proliferating cells. MF116 is secreted or shed into the medium by at least some tissue culture cells and, in fact, is more readily detected by immunoprecipitation using spent medium than with solubilized cell extracts. If similar secretion occurs *in vivo* this antigen might be present in sera from tumor patients; this possibility must be investigated.

Both MF116 and MF61 show patterns of distribution that seem to be related to the embryological origin of the tissues. Thus, these antigens were detected on tumor cell lines of the ovary, uterus, kidney, and bladder but not on cell lines from lung, colon, breast, and pancreatic tumors. The former tumors are all from mesoderm-derived epithelia, whereas the latter are endodermal or ectodermal in origin. The presence of the antigens in frozen sections of fresh tumor specimens of various types must be examined with the immunoperoxidase procedure. Preliminary results indicate that MF116, MF61, and MH94, but not MD144, are expressed on a proportion of ovarian carcinomas.

A number of ovarian tumor antigens have been detected by using xenogeneic polyclonal sera (reviewed in ref. 28) but none of them seems to be related to the antigens described above. Other laboratories (5, 6, 29) have also described monoclonal antibodies to human ovarian carcinoma, but again, these appear to be different in specificity from those described in this study.

The five antibodies described were selected from a large number of hybridoma antibodies produced to ovarian and endometrial cancer cell lines. One problem in attempting to produce antibodies to restricted antigens of epithelial tumors is a tendency to produce many antibodies to common, strongly antigenic components. This factor might be overcome by removing strong antigens from a solubilized cell extract, by the use of immunoadsorbents, before immunization. Another factor is that only a small fraction of ovarian carcinomas can be grown and maintained in tissue culture. Therefore, immunizations with fresh tumor cells may be needed to detect the whole spectrum of ovarian and endometrial tumor antigens.

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