Development of Two New Monoclonal Antibodies Reactive to a Surface Antigen Present on Human Ovarian Epithelial Cancer Cells

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ABSTRACT

Monoclonal antibodies which bind selectively to cancer cells are currently used for tumor localization and for targeting cytotoxic reagents. The success of these approaches depends on the specificity of the antibody and its reactivity to a majority of the tumor samples. Frequently, monoclonal antibodies are generated by immunizing mice with antigenic preparations from a single tumor cell line. Antibodies generated under these conditions often react to a narrow range of tumors. In the present study, mice were immunized with multiple ovarian cancer cell lines in a sequential manner to amplify the immune response against common antigenic determinants expressed in these cell lines. Spleen cells from the immunized mice were then fused with NS-1 myeloma cells to establish hybridomas. Two cell lines were selected on the basis of their selective reactivity to ovarian cancer cells after extensive screening. Monoclonal antibodies OVX1 and OVX2 bound to all 5 ovarian carcinoma cell lines tested and did not bind to normal fibroblast cells. These antibodies recognized a unique antigenic determinant present in ovarian and breast cancer cells. Cross-blocking studies showed that the binding of OVX1 and OVX2 is not displaced by 10 other previously described anti-ovarian antibodies including OC125. In immunocytochemical studies, OVX1 reacted to a majority of ovarian cancer tissues (17 of 20) and did not bind to normal ovarian tissues. Preliminary results indicate that OVX1 and OVX2 antibodies are directed to a high molecular weight antigen. These antibodies could be used in the preparation of cytotoxic conjugates.

INTRODUCTION

Mabs which react selectively to tumor cells have been produced by somatic cell hybridization (1). Because of the development of techniques to obtain a large amount of pure murine immunoglobulin from these hybridomas, it is possible to use these antibodies for therapeutic purposes including tumor localization and targeting. For successful targeting, the relevant tumor antigen should be present on the cell surface in abundance and should be efficiently internalized. The targeting efficiency is greatly affected by antigenic modulation and by the presence of free antigens in circulation. Tumor cells secrete many antigens in large quantities which are used as tumor markers. For example, the Mab OC125 which reacts to a high molecular weight glycoprotein complex, CA125 (2), has been useful as a marker for monitoring response to treatment in approximately 80% of nonmucinous epithelial ovarian carcinomas (3).

Some of the antigens present on ovarian cancer cells are also found on breast cancer cells. Frankel et al. (4) established 72 different hybridoma cell lines by immunizing mice with human breast cancer cells. Although the antigens recognized by many of these antibodies have not been characterized completely, some of them were reactive to antigens such as the TR and the protein encoded by the protooncogene, c-erbB2. Basically, five groups of antigens were defined by these antibodies: (a) high molecular weight glycoproteins, (b) mucins, (c) p180, (d) p66, and (e) p43. Mab 260F9 which reacts to the p66 antigen has been extensively studied in targeting toxin polypeptides (5). Recombinant ricin A chain chemically linked to this antibody (ImmunoToxins) was cytotoxic to breast and ovarian cancer cells, while it did not inhibit the proliferation of normal cells such as fibroblasts (5). Phase I clinical trials with this conjugate, however, showed some neurological problems in the treated patients. The neurotoxicity was subsequently related to the cross-reactivity of the antibody with neuronal components (6). Similarly, another antibody, OV3 directed to ovarian cancer cells formed effective cytotoxic conjugates when linked to Pseudomonas exotoxin (7). This antibody also had some cross-reactivity problems to basal ganglion and, therefore, was found not to be suitable for further clinical applications (8). Therefore, it is necessary to generate additional antibodies which would selectively bind to ovarian cancer cells. In the present study, we used a serial immunization protocol to boost the immune response against common antigenic determinants present on ovarian cancer cells. From the hybridomas, two antibodies were selected for strong binding to ovarian cancer cells with minimal reactivity to normal cells and ovarian tissues. The antibodies recognize a high molecular weight antigen present in large quantities in ovarian and breast cancer cells.

MATERIALS AND METHODS

Cell Lines. Human ovarian epithelial cell lines OVCAR-3, OVCA 420, OVCA 429, OVCA 432, OVCA 433, and DOV-13 and four breast cancer cell lines BT-20, CAMA-1, MCF-7, and SK-BR3 were maintained as described before (9). Normal human foreskin fibroblast cell line Huff was obtained from Dr. K. Singer, Duke University Medical Center, Durham, NC, and cultured in Dulbecco's minimal essential medium supplemented with 2 mM L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and 10% heat-inactivated FBS (Hyclone Inc.). NS-1, a mouse myeloma cell line, was maintained in a similar medium except that it had 15% FBS and 1 mM sodium pyruvate, 1% (v/v) nonessential amino acid mixture, and 2% (v/v) 8-azaguanine (Sigma Chemical Co.) additionally.

Monoclonal Antibodies to Human Ovarian Cancer Cells. Mouse Mabs 317G5, 260F9, 2G3, and 520C9 which bind to breast/ovarian epithelial cancer cells and a Mab raised against human TR (445A12) were obtained from Cetus Corporation (4). OVB3 was a gift from Dr. D. FitzGerald, National Cancer Institute, Bethesda, MD. Mab MOV2 and MOV8 reactive with ovarian cancer (10) were obtained from Dr. Maria Colnaghi (Istituto Nazionale Umori, Milan, Italy). Mab OC125 which binds with the tumor marker, CA125 (2), was provided by Dr. R. C. Bast, Jr. (Duke University Medical Center). In addition, Mab 19.9 which was originally raised against gastrointestinal cancer but cross-reacts with human ovarian cancers (11) was a gift from Dr. Kaprowski
(Wistar Institute, Philadelphia, PA). Control Mab 31E6 (IgG1) which reacts with Thy 1.1 antigen was provided by Dr. Nowinski, and 3B4F11, a mouse Mab (IgM) which binds to opioid-binding cell adhesion molecule (12), was provided by Dr. Sabita Roy, University of Minnesota, Minneapolis, MN. Radiiodinated OC125 antibody was purchased from Centocor Corp.

Immunization and Production of Hybridomas. Somatic cell hybrids were generated by a modified procedure (11) of the original methods developed by Kohler and Milstein (1). Ovarian cancer cells were thoroughly washed with serum-free medium. Ten-week-old female BALB/c mice were immunized serially with ovarian cancer cell lines. Primary immunization was carried out by injecting 5 x 10^6 OVCA 420 cells i.p. Two weeks later, a similar number of OVCA 432 cells were injected. Finally, mice were immunized with five million OVCAR-3 cells i.p. Animals were sacrificed 3 days after the final immunization and their spleens were removed. A spleen cell suspension was prepared by teasing and fused with murine myeloma cells, NS-1 at a ratio of 5:1, by polyethylene glycol (Boehringer Mannheim). Fused cells were gently plated into two-24 well plates in the presence of hypoxanthine/aminopterin/thymidine medium (Sigma). Wells showing positive growth were identified after 2-3 weeks. An aliquot of the culture supernatants was removed and screened for antibodies reactive with ovarian cancer cells in an enzyme-linked immunosorbent assay. Briefly, 5 x 10^5 indicator cells (OVCA 420) were plated in a 96-well plate and cultured for 16-24 h. Cells were then fixed with 0.25% glutaraldehyde for 10 min and washed with PBS. Non-specific binding was minimized by blocking the wells with 2% BSA and 2% horse serum in PBS for 30 min at room temperature. The cells were rinsed with PBS, 100 μl of hybridoma culture supernatants was added. Incubation was continued for 60 min at room temperature. After the unbound fraction was removed, wells were developed with a Vector ABC-AP kit. 3-5 Nitrophenyl phosphate was used as the substrate (2 mg/ml in 100 mM sodium bicarbonate buffer, pH 9.5; 10 mM magnesium chloride). Positive wells were identified by measuring absorbance at 405 nm in an an enzyme-linked immunosorbent assay reader (SLT-Labinstruments, Salzburg, Austria). Positive wells were cloned by limiting dilution, and the screening process was repeated. In parallel, reactivity to normal cells was determined by using Huf cells grown in 96-well plates. Hybridomas secreting antibodies reactive to ovarian cancer cells but not to fibroblasts were selected and cloned. The cloning process was repeated until all the wells showing positive growth were positive for antibody production.

Isotyping and Purification of Mabs. To determine the isotype, ovarian cancer cells were prepared as described before and first incubated with Mab (culture supernatant). After the wells were washed with PBS, isotype-specific anti-mouse antibodies linked to alkaline phosphatase conjugates (SBA Clonotyping system I) were added. Wells were finally developed by adding phosphatase substrate (Sigma).

For laboratory scale production of Mabs, BALB/c mice (12 weeks old) were primed with 0.5 ml of Pristane (Sigma) 2 weeks prior to the injection of hybridoma cells (1 x 10^5). Antibodies were purified from the ascites fluid. Clarified ascites fluid was precipitated with ammonium sulfate (60% saturation) and dialyzed against 10 mM sodium phosphate buffer, pH 7.4, containing 150 mM NaCl. The dialysate was concentrated by ultrafiltration using an Amicon YM-10 membrane and subsequently purified by a HPLC gel filtration preparative column (TSK 4000 SW, Spherogel; Beckman). Purity of the antibody was determined by SDS-polyacrylamide gel electrophoresis under reducing and nonreducing conditions (13).

Radiodiocination and Binding Studies. Mabs were labeled with Na 125I using the Iodogen method as described elsewhere (9). The efficiency of iodination ranged between 50 and 80% (percentage of protein bound radioactivity). The specific activity of the iodinated antibodies was less than 10 and 20 mCi/mg protein. A live cell radioimmunoassay was used to determine the binding of Mabs to target cells as described before. Cells were trypsinized and seeded at a density of 1 x 10^4/well in to 96-well removewell plates (Costar). After overnight incubation, monolayers were washed, and various amounts of labeled Mabs were added in a volume of 40 μl and incubated on ice for 4 h. In cross-blocking experiments, competing antibodies were added first and incubated for 1 h prior to the addition of labeled antibodies. Unbound antibodies were removed by washing the wells with ice-cold medium containing 5% FBS at least four times. Individual wells were then detached, and the radioactivity was determined in a Packard Gamma counter. The EBD program developed by Dr. McPherson was used to calculate the number of binding sites. (14).

Binding sites/cell =

\[
\text{Maximum binding (mol)} \times 6.23 \times 10^{23} \times \text{volume (liter)}
\]

Cell number

Immunofluorescence Studies. Logarithmically growing OVCAR-3 cells were scraped from the culture flasks, and a single-cell suspension was prepared by repeated pipetting. Cells (5 x 10^6) were washed with PBS containing 1% FBS and incubated in microtube tubes with 5 μg/ml of FITC-labeled Mab in a volume of 100 μl. Incubation was carried out on ice for 1 h, and the unbound antibodies were removed by washing twice with PBS. Cells were finally resuspended in PBS after excess conjugate was removed and analyzed in a Flow cytometer (FACStar Plus, Becton Dickinson). For labeling, a 20-fold molar excess of FITC was reacted with OVX1 and OVX2 antibodies in 0.1 M sodium bicarbonate buffer, pH 9.0, at 4°C overnight. The unreacted FITC was removed by gel filtration on a PD-10 column.

Confocal Microscopy. Freshly harvested ovarian cancer cells (OVCAR 433) were seeded at a density of 1 x 10^5 on poly-l-lysine-coated coverslips (12.3 μM poly-l-lysine in 25 mM sodium bicarbonate buffer, pH 8.5) and cultured overnight. The coverslips were washed with cold tissue culture medium. In parallel, ovarian tumors and normal ovaries removed for therapeutic purposes were obtained from patients and quickly frozen in liquid nitrogen before storing at —70°C. Frozen tissues were cut to 5- to 8-μm sections using a cryostat at —15°C. The sections were fixed for 10 min by acetone and treated with 2% BSA and 2% horse serum to block non-specific binding sites. Incubations with FITC-labeled OVX antibodies were carried out for 1 h on ice. After the unbound antibodies were removed by repeated washings, cells were fixed with 0.5% paraformaldehyde solution made in isotonic saline for 5 min. A drop of anti-fading agent, p-phenylenediamine was added and placed on microscope slides. The preparations were analyzed by using a MRC 600 (Bio-Rad, Boston, MA) confocal microscope. Cells were positioned by the epifluorescence accessory (Olympus, Lake Success, NY). The relative amounts of fluorescence on the cells were computer generated and printed.

Characterization of the Cell Surface Antigen. Ovarian cancer cell lines OVCAR-3 and OVCA 420 and the normal fibroblast cell line Huf were grown in 100-mm dishes and harvested by scraping the surface. After the cells were washed with Hanks' buffer to remove serum proteins, cells were solubilized with 20 μM O-GP (Calbiochem) in 50 mM Tris-HCl buffer (pH 7.4) for 1 h at room temperature on a rocking platform (15). The extracts were dialyzed at 4°C against Tris-HCl buffer (pH 7.4) with four changes and the soluble proteins were electrophoresed on a 6.5% SDS gel under nonreducing conditions. After electrophoresis, proteins were transferred onto Immobilon-P membranes (Millipore) using a transblot apparatus (Bio-Rad) at 30 mA for 18–20 h at 4°C. The blots were treated with 10% dry milk solution containing 1% BSA, 0.1% sodium azide, and 0.1% Tween-20 prepared in PBS. Membranes were then probed with radiiodinated Mab (0.05 μCi/ml in the blocking buffer) for 4 h at room temperature. After the membranes were washed four times with PBS containing 0.05% Tween-20, the blots were air dried and autoradiographed using a Kodak X-Omat AR film for 1-7 days at —70°C in the presence of Dupont Cronex enhancing screens.

## RESULTS

BALB/c mice were immunized with three different epithelial ovarian cancer cell lines in a sequential manner. It would be possible by using this strategy to elicit an immune response.

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against common antigenic determinants. Spleen cells from mice were then fused with NS-1 cells, and the resultant hybridomas were selected for growth and antibody production. In seven different fusions, about 70% of the wells showing growth had antibodies reactive to the immunogen. From these, two clones, OVX1 and OVX2, were identified to produce antibodies that reacted to ovarian cancer cell lines without any cross-reaction.

OVX1 and OVX2 antibodies were selected for growth and antibody production. In seven fusions, about 70% of the wells showing growth had antibodies reactive to the immunogen. From these, two clones, OVX1 and OVX2, were identified to produce antibodies that reacted to ovarian cancer cell lines without any cross-reaction.

Both OVX1 and OVX2 were of IgM (k) isotype.

To characterize the Mabs, ascites fluid was collected from pristane-primed mice that were injected with the hybridoma cells. After ammonium sulfate fractionation, a preparative HPLC column was used to separate IgM from contaminating proteins. From the five major peaks were analyzed on SDS-PAGE. A, fractions corresponding to the indicated retention times (C) were run on a 10% gel (lanes 1-6). Protein peak II (retention time, 27.46 min.) was pooled and concentrated. B, SDS-PAGE analysis of protein peak II of OVX1 and OVX2 antibodies under nonreducing (lanes 1 and 2) and reducing conditions (lanes 3 and 4). C, elution profile of proteins from TSK 4000 SW column. A and B: left ordinate, molecular weight in thousands; C: 280 nm absorbance.

Binding Characteristics of OVX1 and OVX2. Reactivity of the antibodies to various tumor cell lines was determined in a live cell radioimmunoassay. In these studies, 5 different ovarian epithelial cancer cell lines were used in parallel with four epithelial breast cancer cell lines and a control fibroblast cell line. Radioiodinated antibodies showed differential binding to the tumor cell lines. Both antibodies bound to all the ovarian cell lines, and among the breast cancer cell lines, no binding was detected on BT-20 cells. Neither of the antibodies reacted with normal fibroblasts. In general, OVX1 Mab bound to tumor cells better than OVX2. Among the ovarian cancer cell lines, OVCA 420 showed higher binding followed by OVCA 429 (Table 1). Interestingly, binding of OVX1 and OVX2 was much stronger to the breast cancer cell line, MCF-7.

To determine the number of binding sites/cell, saturation analysis was performed. Various concentrations of labeled antibody were added to two different ovarian cancer cell lines to saturate all the available binding sites. From the specifically bound radioactivity, the number of binding sites was calculated using a computer program developed by Dr. McPherson. Between the two cell lines, OVCA 420 expressed a higher number of binding sites for both OVX1 and OVX2 antibodies. OVCAR-3 cells had 4.41 ± 0.39 (SD) × 10⁶ sites for OVX1 and 1.86 ± 0.28 × 10⁶ sites for OVX2 Mab. OVCA 420 cells had approximately 2 × 10⁷ and 6.28 ± 1.95 × 10⁶ binding sites/cell for OVX1 and OVX2 antibodies, respectively.

To further characterize these antibodies, immunofluorescence studies were carried out with FITC-labeled OVX antibody. Two methods were used to determine the binding: (a) flow cytometric analysis and (b) confocal microscopy. In the former method, OVCA-3 cells were removed from culture dishes by scraping and treated with FITC-labeled antibodies. As a control, a monoclonal antibody reactive to mouse Thy 1.1 antigen was used. To compare the relative abundance of the OVX antigen on the cell surface, an antibody reactive to human transferrin receptor (S9-C11-FITC) was used. Flow cytometric analysis showed that OVX1 and OVX2 antibodies bound very well with ovarian cancer cells (Fig. 2, B and C). Reactivity of these antibodies was much higher when compared to the binding characteristics of the anti-transferrin receptor antibody (Fig. 2A). In order to determine the cellular localization of the target antigen, cells were stained with fluoresceininated OVX1 antibody and analyzed by a confocal microscope. A representative picture of OVX1 binding is shown in Fig. 2D. Intense staining was seen on the cell surface.

Immunohistochemical Localization. Binding of OVX1 and OVX2 to human ovarian cancer tissues and normal ovarian tissues was determined by immunofluorescence staining. Cryostat sections of 6 normal ovaries and 20 different ovarian cancer tissues were treated with OVX1 and OVX2 antibodies. The tumor tissues belonged to the following groups: (a) papillary serous adenocarcinomas (n = 8), (b) adenocarcinomas (n = 7), (c) clear cell adenocarcinoma (n = 1), (d) mixed mesodermal carcinoma (n = 1), (e) desgerminoma (n = 1), and (f) mucinous cystadenocarcinoma (n = 2). Staining characteristics indicate that OVX1 binding was much stronger than OVX2. Furthermore, the range of tumor tissues recognized by OVX1 was

### Table 1 Reactivity of OVX1 and OVX2 antibodies to various cell lines

<table>
<thead>
<tr>
<th>Cell type</th>
<th>125I-OVX1 (fmol/well)</th>
<th>125I-OVX2 (fmol/well)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OVCA-3</td>
<td>257.4</td>
<td>186.8</td>
</tr>
<tr>
<td>OVCA 420</td>
<td>877.8</td>
<td>957.0</td>
</tr>
<tr>
<td>OVCA 429</td>
<td>444.0</td>
<td>425.7</td>
</tr>
<tr>
<td>OVCA 432</td>
<td>11.2</td>
<td>12.5</td>
</tr>
<tr>
<td>OVCA 433</td>
<td>247.5</td>
<td>223.0</td>
</tr>
<tr>
<td>Breast cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BT-20</td>
<td>ND*</td>
<td>ND*</td>
</tr>
<tr>
<td>CAMA-1</td>
<td>211.2</td>
<td>167.0</td>
</tr>
<tr>
<td>MCF-7</td>
<td>1174.0</td>
<td>798.6</td>
</tr>
<tr>
<td>SK-BR3</td>
<td>386.0</td>
<td>181.5</td>
</tr>
<tr>
<td>Normal fibroblast</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Huff</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* ND, not detectable under the experimental conditions used.
Fig. 2. Flow cytometric analysis of OVX1/OVX2 binding. Purified antibodies linked to FITC were used to study the reactivity of OVX antibodies to ovarian cancer cells. OVCAR-3 cells (1 × 10⁶) were incubated with 5 µg/ml of fluoresceinated antibodies for 1 h on ice. The cells were washed 3 times with ice-cold tissue culture medium and 3 times with ice-cold PBS (pH 7.4). Finally, surface-bound immunoglobulins were fixed with 0.5% paraformaldehyde solution made in PBS and analyzed on a FACStar plus Flow cytometer. About 10,000 cells were analyzed from each sample. FITC-conjugated anti-mouse Thy 1.1 monoclonal antibody (31E6) was used in parallel to determine nonspecific binding (dotted line). Solid lines, binding of anti-transferrin receptor antibody, SE9-C11-FITC (A), OVX1-FITC (B), and OVX2-FITC (C). The percentage of cells positive were 71, 93, and 97, respectively. D, representative picture of OVX1-FITC binding to another ovarian cancer cell line, OVCA 433, as observed in confocal microscope. Scale: 1 inch = 25 mm; color-coded bar (inset), intensity of staining. Image of fluorescence was processed by a computer program.

higher than OVX2. OVX1 bound to 17 of 20 ovarian tumor tissues, whereas OVX2 was positive for 14 of them. The number of tumor samples studied is small, and therefore, it is difficult to conclude the association between the expression of OVX antigenic determinants and the tumor type. These studies, however, indicate that there are variations in the expression of respective antigenic determinants among the different tumors. Representative immunofluorescence localization (confocal mi-
MABS REACTIVE TO A SURFACE ANTGEN

croscopy) of antibodies on two papillary serous cystadenocarcinoma of the ovary is shown in Fig. 3. Fluorescence images were processed by a computer and presented. All the sections were identically treated, and the results indicate that OVX1 antibody did not bind to normal intestine (Fig. 3D), stomach (Fig. 3E), and liver (Fig. 3F). Further investigations are in progress to determine the cross-reactivity of OVX antibodies to other normal tissues. Both the antibodies were negative for 5 of 6 normal ovarian tissues tested thus far. One of the ovarian tissues was weakly stained at the glandular region (Fig. 3C). For a negative control a nonspecific IgM Mab (3B4F11) was used under similar conditions. This antibody did not show any reactivity to either normal or ovarian tumor tissues. As a positive control MOV2 (IgM isotype), reactive to ovarian cancer cells, was used. MOV2 bound about half of the tumor tissues (10 of 20) in this study and was not reactive to 5 of the 6 normal ovaries. Similar to OVX1, MOV2 also bound weakly to one of the normal ovaries at an identical location.

Cross-Reaction with Other Mabs Reactive to Human Ovarian Cancer Cells. A list of previously described Mabs with selective binding to ovarian cancer cells is presented in Table 2. These antibodies were generated in different laboratories using different cell lines as immunogens. They recognize mucins, high molecular weight glycoproteins, well-defined small molecular weight proteins, and glycolipids. In a live cell radioimmunoassay, saturating concentrations of these antibodies were used to determine whether the binding of OVX1 and OVX2 could be cross-blocked by any of these antibodies. Two representative ovarian cancer cell lines were studied. Competing antibodies were first added to block the available binding sites. To prevent antigenic modulation, incubations were carried out at 4°C. After the preincubation step, radioiodinated OVX1 and OVX2 antibodies were added. Fig. 4 shows a competitive inhibition profile obtained with the homologous combination and with a panel of other antibodies. Pretreatment of OVCAR-3 and OVCA 420 cells with the 10 antibodies up to a concentration of 20 ng/ml did not prevent the binding of either OVX1 or OVX2. Unla-

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**Table 2. Characteristics of anti-ovarian antibodies used in cross-blocking studies**

<table>
<thead>
<tr>
<th>Monoclonal antibody</th>
<th>Immunogen</th>
<th>Isotype</th>
<th>Antigen</th>
<th>Ref.</th>
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</thead>
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<tr>
<td>317G5</td>
<td>Breast cancer cell line</td>
<td>IgG1</td>
<td>M, 47,000</td>
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<tr>
<td>260F9</td>
<td>Breast cancer cell line</td>
<td>IgG1</td>
<td>M, 55,000</td>
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<tr>
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<td>Breast cancer cell line</td>
<td>IgG1</td>
<td>Mucin</td>
<td>4</td>
</tr>
<tr>
<td>520C9</td>
<td>Fresh breast tumor BCIA</td>
<td>IgG1</td>
<td>c-erb B2</td>
<td>4</td>
</tr>
<tr>
<td>454A12</td>
<td>Fresh breast tumor BCIA</td>
<td>IgG1</td>
<td>TR</td>
<td>4</td>
</tr>
<tr>
<td>OV83</td>
<td>Ovarian cancer cell line,</td>
<td>IgG2b</td>
<td>NA*</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>OVCAR-3</td>
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<td></td>
<td></td>
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<tr>
<td>OC125</td>
<td>Ovarian cancer cell line,</td>
<td>IgG1</td>
<td>M, &gt;900,000</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>OVCA 433</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>MOV2</td>
<td>Membrane of mucinous</td>
<td>IgM</td>
<td>High molecular weight</td>
<td>20</td>
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<tr>
<td></td>
<td>ovarian cystadenocarcinoma</td>
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<td>MOV8</td>
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<td>IgG1</td>
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<td></td>
<td>ovarian cystadenocarcinoma</td>
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<tr>
<td>19-9</td>
<td>Colorectal carcinoma cell</td>
<td>IgG1</td>
<td>High molecular weight</td>
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<td>line</td>
<td></td>
<td></td>
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</table>

* Dr. D. Ring, personal communication.

* NA, information not available.

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Fig. 3. Reactivity of OVX1 antibody to human ovarian tumors. Frozen sections from two representative ovarian tumor samples (papillary serous cystadenocarcinomas) and some normal tissues were reacted with 5 μg/ml of OVX1-FITC conjugate. Bound antibody was visualized in confocal microscopy, and the images were processed by a computer to determine the relative fluorescence on tissue sections. A and B, OVX1-FITC binding to tumor sections; C, normal ovary; D, intestine; E, stomach; F, liver.

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Fig. 4. Cross-blocking studies. To determine whether OVX antibodies recognize any of the previously characterized determinants on ovarian cancer cells, blocking studies were carried out. Indicated concentrations of antibodies listed in Table 2 were preincubated with OVCAR-3 cells prior to the addition of radiolabeled OVX1 (A) and OVX2 (B) antibodies. Incubations were carried out at 4°C and the cell-bound radioactivity was determined after extensive washings with cold tissue culture medium. Point, mean of quadruplicate determinations. To confirm the unique epitope recognized by OVX antibodies, reverse combinations of competitive inhibition assays were carried out. In these experiments, radiiodinated OC125 and 2G3 antibodies were used as tracers (C and D, respectively).

do not block the binding of either 125I-OC125 or 125I-2G3 to the target antigens.

Characterization of the Antigen. Surface antigens of ovarian cancer cell lines, OVCAR-3, OVCA 420, and a control cell line, Huff, were solubilized with O-GP, a nonionic detergent. O-GP solubilizes membrane-bound proteins in their native state and can be easily removed by dialysis (15). Solubilized proteins were then separated in quadruplicate sets on a SDS gel and transblotted. After the membrane was blocked, it was cut and probed with radiiodinated antibodies separately. Both OVX1 and OVX2 showed strong binding to a high molecular weight complex (about Mr 900,000) in the extracts of OVCAR-3 and OVCA 420 (Fig. 5). Binding of OVX2 to OVCAR-3 cell extract was relatively weak, which can be explained by the lower antigen density on this cell line. The control antibody, 3B4F11, did not show any binding to the solubilized proteins from the ovarian or the control cell line.

DISCUSSION

Tumor cells arise as variants from normal cells. They differ from normal cells in morphological and biochemical characteristics. Recently, a variety of surface components have been identified by using monoclonal antibodies as probes. These include structural proteins, mucins, and products of certain protooncogenes. Antibodies directed to these determinants have been evaluated for diagnostic and therapeutic values. Epithelial ovarian carcinomas arise from the single layer of epithelial cells covering the ovaries. Ovarian cancer cells have been found to express unique antigens and growth factors. These include the overexpression of epidermal growth factor receptor (16), protooncogene Her-2/neu which has been shown to have some prognostic value (17). In addition, epithelial ovarian cancer cells are found to secrete growth factors such as macrophage colony-stimulating factor (9), and in a selective group, the receptor for macrophage colony-stimulating factor which is a protooncogene, c-fms, is also coexpressed (18). Overexpression of these surface components offers a unique opportunity to target respective monoclonal antibodies to localize and deliver cytotoxic reagents.

A number of Mabs reactive to specific antigens on ovarian cancer cells have been made in the past. Some of them (OC125,
B72.3, MOV2, DF3, HMFG1, HMFG2, 2G3) recognize high molecular weight mucin-like glycoproteins (2, 4, 10, 19–23). Other antigens expressed on ovarian cancer cells are an M, 230,000 protein recognized by the antibody 41B4 (24), an M, 200,000 protein (c-erbB2 protooncogene product) recognized by the antibody 520C9, an M, 66,000 protein (4), an M, 48,000 protein (25), and an M, 43,000 protein recognized by the antibody 317G5 (4). In addition to these moieties, a unique glycolipid antigen is also found on ovarian cancer cells (26). While the majority of these antibodies are selective in binding to ovarian cancer tissues, some of them cross-react to neuronal components and other normal tissues. Such cross-reactions restrict the therapeutic application of these antibodies.

In the present study, two new monoclonal antibodies, OVX1 and OVX2, were generated. OVX1 and OVX2 are IgM, κ isotype, and bind very strongly to ovarian cancer cells when compared to some of the previously generated Mabs. Between the two antibodies OVX1 bound to a wider range of ovarian tumors than OVX2. The binding characteristics were better than the other anti-ovarian antibodies such as MOV2, which reacted with 10 of the 20 ovarian tumor samples only. It is possible that individual tumors may have variations in the expression of these antigens. Cross-blocking studies indicate that OVX1 and OVX2 recognize a novel class of antigenic determinant. However, it is difficult to conclude from the current data that they bind to a totally different antigen. None of the 10 antibodies tested competitively inhibited the binding of OVX1 and OVX2. Furthermore, OVX1 seems to inhibit the binding of OVX2 better than the reciprocal combination. This could possibly be explained by affinity differences or recognition of overlapping determinants. Current evidence is insufficient to support either of the possibilities. It is interesting, however, that a fraction of ovarian tumor tissues negative for OVX2 determinants are still positive for OVX1.

Binding characteristics show that OVX1 and OVX2 antigens are not only present on ovarian cancer cells but are also found on human breast cancer cells at a higher density. In fact, the recognition of OVX antibodies compared to some of the previously generated Mabs. Between the two antibodies OVX1 bound to a wider range of ovarian tumors than OVX2. The binding characteristics were better than the other anti-ovarian antibodies such as MOV2, which reacted with 10 of the 20 ovarian tumor samples only. It is possible that individual tumors may have variations in the expression of these antigens. Cross-blocking studies indicate that OVX1 and OVX2 recognize a novel class of antigenic determinant. However, it is difficult to conclude from the current data that they bind to a totally different antigen. None of the 10 antibodies tested competitively inhibited the binding of OVX1 and OVX2. Furthermore, OVX1 seems to inhibit the binding of OVX2 better than the reciprocal combination. This could possibly be explained by affinity differences or recognition of overlapping determinants. Current evidence is insufficient to support either of the possibilities. It is interesting, however, that a fraction of ovarian tumor tissues negative for OVX2 determinants are still positive for OVX1.

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REFERENCES


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