Coexpression of Different Antigenic Markers on Moieties That Bear CA 125 Determinants


INTRODUCTION

CA 125 is an antigenic determinant defined by the OC 125 murine monoclonal antibody that was generated against an established human serous cystadenocarcinoma cell line, OCVA 433 (1). CA 125 is expressed by more than 80% of epithelial ovarian carcinomas (2). Because multiple CA 125 determinants are associated with circulating antigen, a radioimmunoassay could be developed with the OC 125 antibody allowing quantitation of CA 125 determinants in sera from patients with ovarian cancer (3, 4). To date the clinical application of this radioimmunoassay has been for monitoring patients with epithelial ovarian cancer (5).

Recent studies suggest that CA 125 can be elevated prior to the diagnosis of epithelial ovarian carcinoma (6–8), suggesting that CA 125 might be one component in a strategy to detect epithelial ovarian cancer in women before it has disseminated throughout the abdominal cavity. Elevated CA 125 levels can be observed in sera of some patients with nonmalignant conditions including peritonitis and liver disease (9). Elevated CA 125 levels have also been found in sera from pregnant women and in amniotic fluid (10). To estimate the specificity of CA 125 in an amniotic fluid (10). To estimate the specificity of CA 125 in a population of apparently healthy women, CA 125 was measured in sera from 915 Roman Catholic nuns (11). CA 125 was >65 units/ml in 0.8% of all individuals tested. When only those women older than 50 years of age were considered, 0.6% of CA 125 values exceeded 65 units/ml (11). Despite this remarkable specificity, if a single CA 125 determination were used to screen a population of apparently healthy women, a prohibitive number of false-positive values could be encountered. Greater specificity might be attained if CA 125 were monitored over time (8) or if a confirmatory test could be devised to evaluate a single serum sample.

In a previous study, a remarkable parallel was observed between the levels of CA 125 and CA 19–9 while monitoring a single ovarian cancer patient at weekly intervals during a 3-month period (12). Blocking of 125I-labeled antibody binding to antigen with nonlabeled antibodies demonstrated that CA 125 and CA 19–9 are immunologically distinct determinants (13). When material in culture supernatants from the OVCA 433 cell line was affinity purified on an OC 125 column, CA 19–9 copurified with CA 125 and appeared to be coexpressed with CA 125 on a high molecular weight moiety demonstrated by Western transfer. In subsequent studies, CA 125 was further purified under more rigorous conditions and an M, 220,000 glycoprotein was isolated which lacked CA 19–9 activity but expressed CA 125 (14). Whether or not CA 125 and CA 19–9 are linked covalently, the coexpression of different epitopes on the same circulating macromolecular complex might provide a more specific test for cancer, if each epitope were uniquely expressed as the result of a different underlying physiological condition. Utility as a marker for early ovarian cancer would also depend upon the sensitivity of the test for identifying sera from patients with early stage disease, which might not be comparable to that for each epitope measured independently.

The goal of the present study was to investigate systematically the coexpression of different epitopes on CA 125* macromolecular complexes to determine whether a test that identifies moieties which bear multiple epitopes would prove sufficiently sensitive to detect patients with ovarian cancer.

MATERIALS AND METHODS

Chemicals. Cyanogen bromide-activated Sepharose and protein A-Sepharose CL-4B were purchased from Sigma Chemical Co. (St. Louis, MO). Electrophoresis reagents were purchased from Bio-Rad Corp. (Rockville Center, MD). Gelatin was obtained from Norland Products, Inc. (North Brunswick, NJ). NP40 was purchased from Particle Data...
Laboratories, Ltd. (Elmhurst, IL). Nitrocellulose was obtained from Schleicher & Schuell (Keene, NH).

Clinical Materials. Serum and ascites samples were obtained from women with advanced epithelial ovarian cancer confirmed by histopathological examination of tissue at the time of original diagnosis or at second-look surgical surveillance procedures. CA 125 levels for each of these samples were >200 units/ml. Sera were also obtained from apparently healthy women under protocols approved by the Institutional Review Board of the Duke University Medical Center. All serum and ascites samples were aliquoted and stored at −70°C until assay.

Isolation of CA 125 from OVCA 433 Tissue Culture Supernatant. The human ovarian carcinoma cell line OVCA 433 was maintained in Eagle's minimal essential medium supplemented with 2 mM glutamine, 1 mM sodium pyruvate, 1% nonessential amino acids, 100 units/ml penicillin, 100 μg/ml streptomycin, and 10% heat-inactivated fetal bovine serum. Culture supernatants were collected at 5- to 7-day intervals, centrifuged at 3000 × g to remove aggregates, concentrated to one-tenth their original volume by ultrafiltration on a PM-30 membrane (Amicon, Danvers, MA), and then filtered through a 0.22-μm membrane (Millipore, Bedford, MA). The 10× concentrate was loaded onto an HPLC® gel filtration column (TSK G3000; Altex) that had been equilibrated with PBS (pH 7.4). Material was eluted at a constant flow rate of 4 mL/min. The void fractions, containing all the CA 125 activity, as determined by the CA 125 immunoradiometric assay (3) were pooled, concentrated to 1/30 the original volume, and stored at −70°C. This material is referred to as OVCA433/HPLC.

Purification of CA 125 Antigen from OVCA433/HPLC and Ascites from Ovarian Cancer Patients. Monoclonal antibody OC 125 was coupled to CNBr-activated Sepharose 4B, according to the instructions of the manufacturer (Sigma). OVCA433/HPLC or patient ascites were diluted 1:10 with 0.1 M phosphate buffer, pH 6.8, and filtered through a 0.22-μm membrane before loading onto a 2-ml OC 125-Sepharose affinity column which had been equilibrated in the same phosphate buffer. After samples were recycled at least 5 times over 2-4 h, the column was successively washed with at least 10 column volumes each of 0.1 M phosphate buffer (pH 6.8), 0.2% NP40 in phosphate buffer, 0.5 M NaCl in phosphate buffer, and phosphate buffer alone. The completeness of each wash was confirmed by monitoring A280 to show that no additional material could be eluted from the column. Elution of CA 125 antigen was accomplished with 50 mM diethylenetriamine, pH 11.5 (14), and eluted fractions were pooled, neutralized to pH 7.0 with 1 M NaH2PO4, and dialyzed overnight against an excess of 0.1 M phosphate buffer, pH 6.8. Fractions were then concentrated to 1/30 the original volume by ultrafiltration on an Amicon PM-30 membrane. This affinity-purified antigen is referred to as OVCA433/AC or ascites/AC.

Monoclonal Antibodies. Monoclonal antibody OC 125 (1) was isolated from protein A-Sepharose chromatography (15) from ascites produced by hybridoma OC 125.2.1.1 grown in pristane-primed Balb/c mice in our laboratory. Murine monoclonal antibody A2C6 (IgG1), directed against hepatitis B surface antigen (16), was purified from murine ascites by ammonium sulfate precipitation and dialyzed with standard reagents (Southern Biotechnology Associates, Birmingham, AL) demonstrated that OC 3632 was an IgG2a. Unlike OC 125, the antibody failed to bind to one or more sections of normal pericardium, peritoneum, ovary fallopian tube, endometrium, or endocervix. OC 3632 did, however, react with the stroma of normal cervix and the epithelium of biliary ducts, renal tubules, and normal colonic mucosa. The hybridoma was maintained in our laboratory as ascites in pristane-primed Balb/c mice and IgG was purified from ascites by ammonium sulfate precipitation and DEAE-HPLC ion-exchange chromatography (DEAE 5PW, Waters Associates, Milford, MA). The determinants recognized by OC 3632 have been designated CA 3632.

Idionation of Monoclonal Antibodies. Monoclonal antibodies OC 3632 and Mov8 were labeled with 125I using a modification of the method described by Masuho et al. (21). Iodogen was reduced to 10 μg/reaction. SDS-PAGE analysis of the 125I-Mov8 and 125I-OC 3632 showed that all labeled protein migrated with the same mobility as authentic immunoglobulin heavy and light chain standards. SDS-PAGE. SDS-PAGE was performed by a modification of the method of Laemmli (22) using a 3.5% stacking gel and a 4% resolving gel. Sample buffer contained 6 μl urea-62.5 mM Tris-HCl, pH 6.8-10% glycerol, either with (reducing) or without (nonreducing) 5% β-mercaptoethanol. Samples equivalent to 800–1000 units of CA 125 were applied to each lane without prior heating. Omission of SDS from the sample buffer and avoidance of heating were necessary to preserve the immunoreactivity of the samples with OC 125. Protein standards included IgM (M, 900,000), thyroglobulin dimer (M, 669,000), and monomer (M, 335,000), as well as myosin (M, 200,000).

Western Blotting. After PAGE the CA 125 antigen was electrophoretically transferred to nitrocellulose (23, 24) in a transblot apparatus (Bio-Rad, Rockville Centre, NY) at 200 mA for 18–20 h. Molecular weight standard lanes were cut off, stained with 0.1% amido black in 45% methanol/10% acetic acid for 20 min, and destained with 45% methanol/10% acetic acid. The electrophoretic blots were washed 3 times with PBS, pH 7.4, and blocked in 1% gelatin in PBS for 1 h at 23°C. Blots were then probed with the designated 125I-labeled monoclonal antibody (50,000 cpm/ml buffer) in PBS 1% gelatin and 0.2% NP40 for 4 h at 23°C. For competition assays, blots were incubated with nonlabeled monoclonal antibodies (0.001–10 μg/ml) for 2 h at 23°C, washed 4 times with PBS, and then probed with 125I-labeled antibodies. Finally, blots were washed 4 times with PBS, air dried, and autoradiographed on Kodak X-Omat AR film for 2–7 days at −70°C using Dupont Cronex “lightening” enhancing screens.

Solid Phase Radioimmunoassays. Double determinant radioimmunoassays to determine levels of antigenic determinants were performed according to the instructions of the manufacturer using CA 125 (3, 4)
and CA 19–9 (25) assay kits obtained from Centocor. Tag 72 levels were determined with the solid phase radioimmunoassay described by Klug et al. (26) using the B72.3 antibody. To test for the simultaneous presence of CA 125 and a second antigenic determinant, double determinant radioimmunoassays were performed. Serum or ascites samples (100 µl) diluted in buffer (100 µl PBS or 100 mM sodium citrate buffer, pH 5.9) were incubated in parallel for 6 h at 37°C with polystyrene beads containing either immobilized OC 125 or immobilized A2C6 (10 µg/bead). The beads were washed 3 times with water and incubated with 125I-labeled antibody (NS 19–9, MOV-8, B72.3 or 3632) at 23°C for 16 h. Finally, beads were washed 3 additional times with water and counted in a Packard gamma counter. Each data point was the mean of duplicate samples, and cross-reactivity was defined by the ratio:

\[
\frac{\text{cpm associated with the OC 125 bead}}{\text{cpm associated with the A2C6 bead}}
\]

RESULTS

Purification of the CA 125 Antigen. CA 125* molecules were isolated from the OVCA 433 tissue culture supernatant in a two-step procedure. First, a 10-fold concentrated culture supernatant was subjected to HPLC gel filtration on a TSK G3000 column. As shown in Fig. 1, all detectable CA 125 and CA 19–9 activity was recovered in the void fraction (M, >250,000) which represented <25% of the material that absorbed light at 280 nm (A280). Approximately 90% of the CA 125 activity applied to these columns could be recovered, suggesting a 3- to 4-fold purification. The HPLC void fractions were then further purified by affinity chromatography on OC 125-Sepharose columns. The final recovery of CA 125-bearing molecules was in the range of 60–70%. The pooled affinity-purified fractions typically contained CA 125 levels of 1000–4000 units/ml. Coomassie-stained SDS-PAGE of the OVCA433/AC were characterized by a single diffuse, lightly stained band at an apparent M, >300,000.

Immunoblot Analysis of OVCA433/HPLC and OVCA 433/AC. Standard methods (23, 24) were used, and OVCA433/HPLC and OVCA433/AC were transferred to nitrocellulose and immunoblotted with 125I-labeled OC 125 (Fig. 2). Analysis of OVCA433/HPLC under nonreducing conditions distinguished at least 5 different CA 125* complexes by apparent molecular weight using the OC 125 probe: 2 bands in the stacking gel (apparent M, >1000,000) and 3 bands in the separating gel at apparent M, 900,000, 600,000, and 400,000–450,000, respectively (Fig. 2, lane 1). OVCA433/AC contained the same bands that were found in the less pure material, with the exception of a band at apparent M, 400,000–450,000 (Fig. 2, lane 2). Under reducing conditions, overall immunoreactivity of each sample was decreased, suggesting that the antigen had been partially denatured. A new band at apparent M, 160,000, not seen in the unreduced samples, was observed for OVCA433/HPLC with reduction (Fig. 2, lane 3). This band was not seen, however, with OVCA433/AC under reducing conditions (Fig. 2, lane 4), and its position corresponded to the position of an overloaded band of protein in the OVCA433/HPLC sample as detected by amido black staining of a parallel lane from the blot (data not shown). Such an overloaded band might bind a small amount of probe nonspecifically. Finally, heating samples at 45°C for 30 min in the presence of 6 M urea (14) prior to electrophoresis caused no substantial change in the overall pattern of the blot (data not shown). Because even

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**Fig. 1.** HPLC gel filtration analysis of OVCA 433 tissue culture supernatant. Top, 10-fold concentrate of OVCA 433 cell culture supernatant loaded on a gel filtration column (TSK G3000) and analyzed in an HPLC system (Waters); middle, CA 125 activity of HPLC fractions; bottom, CA 19–9 activity of HPLC fractions.

**Fig. 2.** Analysis of OVCA433/HPLC and OVCA433/AC. CA 125: 800 units/lane. The Western blot was overlaid with 125I-OC 125 (50 Kcpm/ml). Nonreducing conditions: lane 1, OVCA433/HPLC; lane 2, OVCA433/AC. Reducing conditions: lane 3, OVCA433/HPLC; lane 4, OVCA433/AC. Ordinate, M, in thousands.
the affinity-purified preparation exhibited several diffuse bands, we thought it important to exclude binding artifacts. The bands of reactivity observed on Western transfers did not sharpen with dilution of the CA 125 (Fig. 3), suggesting that the apparent heterogeneity was not due to overloading of the lanes. Moreover, $^{125}$I-OC 125 binding could be blocked by OC 125 but not by an irrelevant isotype matched antibody, MOPC-21 (Fig. 4). Importantly, the other antibodies used in this study failed to block $^{125}$I-OC 125 binding to affinity-purified CA 125$^+$ material (Fig. 5), with the possible exception of 3632 (lane 6).

**Fig. 3.** Binding of $^{125}$I-OC 125 to different amounts of affinity-purified CA 125 antigen from B6 ascites. Western capillary transfers were performed after PAGE of 1000 units (lane 1), 750 units (lane 2), 500 units (lane 3), and 250 units (lane 4) of CA 125 antigen. The Western blot was overlaid with $^{125}$I-OC 125 (50 Kcpm/ml). Ordinate, M, in thousands.

**Fig. 4.** Blocking of $^{125}$I-OC 125 binding to CA 125 with specific and nonspecific antibodies. Western blots were prepared with affinity-purified CA 125 antigen from B6 ascites before and after OC 125 affinity purification. The Western blot was overlaid with $^{125}$I-OC 125 (50 Kcpm/ml). Ordinate, M, in thousands.

**Fig. 5.** Blocking of $^{125}$I-OC 125 binding to CA 125 (1000 units/lane) with diluent (lane 1) or 10 $\mu$g/ml OC 125 (lane 2), 19–9 (lane 3), MoV8 (lane 4), B72.3 (lane 5), OC 3632 (lane 6), and DF3 (lane 7). After washing, this blot was overlaid with $^{125}$I-OC 125 (50 Kcpm/ml). Ordinate, M, in thousands.

**Fig. 6.** Binding of different monoclonal antibodies to CA 125$^+$ moieties (800 units/lane) before and after OC 125 affinity purification. Lanes 1 and 3, OCVA433/HPLC; lanes 2 and 4, OCVA433/AC; lane 5, B6 ascites; lane 6, B6/AC; lane 7, B57 ascites; lane 8, B57/AC. The Western blot was overlaid with $^{125}$I-19-9 (lanes 1 and 2), $^{125}$I-MoV8 (lanes 3 and 4), $^{125}$I-B72 (lanes 5 and 6) (50 Kcpm/ml), and $^{125}$I-DF3 (lanes 7 and 8). Ordinate, M, in thousands.

Coexpression of Different Epitopes on CA 125$^+$ Antigen. CA 125$^+$ antigen was purified by OC 125 affinity chromatography from culture supernatants of the OVCA 433 cell line and from ascites collected from 6 epithelial ovarian cancer patients. Purified CA 125$^+$ material was then immunoblotted with $^{125}$I-labeled OC 125, NS 19–9, MoV8, B72.3, DF3, and OC 3632 monoclonal antibodies to detect coexpression of a second epitope on CA 125-bearing molecules.

When crude OVCA 433 supernatant or crude patient ascites was analyzed, several different epitopes were associated with a broad band of high molecular weight material similar to that of CA 125 (Fig. 6). Fig. 6 also shows that OC 125 affinity purification of the supernatant or ascites removed most of the cross-reacting molecules that bound the $^{125}$I-labeled monoclonal reagents. After affinity purification, however, each sample still contained a small population of CA 125$^+$ moieties that strongly bound one or more $^{125}$I-labeled second antibodies, suggesting coexpression or additional epitopes on CA 125-bearing molecules or complexes. Antigens that expressed CA 19–9 (lane 2),
Mov8 (lane 4), and TAG 72 (lane 6) determinants comigrated with CA 125* complexes of the highest molecular weight, whereas DF3 (lane 8) comigrated with the M, 900,000 form of CA 125* material.

CA 125* affinity-purified antigen from the 7 different sources bound 125I-labeled OC 125 (data not shown) and 125I-labeled OC 3632 (Fig. 7). Marked heterogeneity was observed in the apparent coexpression of determinants that reacted with MOv8, B72.3, DF3, or NS 19–9 (Table 1). No two specimens exhibited the same phenotype. Among the 6 ascites samples, CA 125 was expressed by 6 of 6 samples, CA 3632 by 6 of 6 samples, MOv8 by 5 of 6 samples, CA 19–9 by 3 of 6 samples, TAG 72 by 3 of 6 samples, and DF3 by 3 of 6 samples.

Double Determinant Radioimmunoassays. To quantitate coexpression of different epitopes in clinical samples, double determinant assays were constructed using OC 125 immobilized on a solid phase to bind antigen. Each of the other 125I-labeled antibodies was used as a probe to detect different epitopes on antigen that had been bound. Controls were also performed in which the A2C6 antibody directed against hepatitis B surface antigen was immobilized on the solid phase and each of the same 125I-labeled antibodies was used as a probe. Counts associated with antigen bound by OC 125 were compared to counts associated with antigen bound by A2C6. Results from 37 ascites samples and 3 pleural fluid samples obtained from patients with documented ovarian cancer are plotted in Fig. 8. The most impressive ratios were observed with NS 19–9, B72.3, and Mov8. If a ratio of 2.0 is arbitrarily chosen as a reference ratio for 40 specimens, coexpression of CA 125 was observed in 98% of cases with CA 72, in 53% with CA 19–9, in 65% with MOv8, in 28% with DF3, and in only 5% with CA 3632.

Given the coexpression of epitopes in ascites specimens, it remained to be determined whether coexpression of epitopes in serum could provide a test that was more sensitive or specific than the standard CA 125 assay for detecting ovarian carcinoma. When OC 125 was used to bind antigen and B72.3 used as a probe, a ratio of 2 excluded each of 50 control sera but identified 19% of 47 sera from different ovarian cancer patients (Fig. 9A). When the individual CA 125 and TAG 72 assays were used, results were somewhat different. Among sera from healthy individuals, only 1 of 50 had a serum CA 125 level ≥35 units/ml CA 125 (Fig. 9B) and only 4 of 50 (8%) had a TAG 72 level ≥6 units/ml (Fig. 6), whereas all 47 (100%) sera from ovarian cancer patients had elevated (≥35 units/ml) CA 125 levels (Fig. 9B) and 32 of 47 (68%) had elevated (≥6 units/ml) TAG 72 (Fig. 9C).

In a CA 125/CA 19–9 double determinant assay, a ratio of 2 excluded 108 of 109 (>99%) control sera and identified 13 of 50 (26%) sera from ovarian cancer patients (Fig. 10). Similar studies have been performed with the OC 3632 antibody that was raised against partially purified CA 125* antigen but which does not compete with OC 125 for binding to CA 125 determinants. Although the mean ratio was slightly higher for a group of 47 sera from ovarian cancer patients than for 50 sera from apparently healthy donors, the difference was not statistically significant. Moreover, a ratio of 2 was exceeded by only 3 sera from each group.

Table 1 Coexpression of epitopes defined by monoclonal antibodies on molecules shed by epithelial ovarian cancers and purified on OC 125 affinity columns

<table>
<thead>
<tr>
<th>Antigen/source</th>
<th>OC 125</th>
<th>19.9</th>
<th>MOv8</th>
<th>B72.3</th>
<th>CA 3632</th>
<th>DF3</th>
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<tr>
<td>OVCA 433 supernatant</td>
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<td>D21/ascites</td>
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<td>D26/ascites</td>
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<td>B57/ascites</td>
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<td>B78/ascites</td>
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Fig. 7. Binding of 125I-3632 to affinity-purified CA 125 antigen (1000 units/lane) from OVCA 433 supernatant and different ascites. Lane 1, OVCA 433/AC; lane 2, D9/AC; lane 3, D21/AC; lane 4, D26/AC; lane 5, B6/AC; lane 6, B57/AC; lane 7, B78/AC. Ordinate, M, in thousands.

Fig. 8. Double determinant assays of ovarian cancer ascites (•) and pleural fluids (A). On left, CA 125 activity of samples was determined by CA 125 radioimmunoassay; on right, coexpression of epitopes was measured by the designated double determinant assays. Antigen was bound by immobilized OC 125 or A2C6 (control). Additional epitopes were detected with 125I-19.9, 125I-B72, 125I-MOV8, 125I-DF3, and 125I-3632. Point, ratio of cpm associated with immobilized OC 125 to cpm associated with immobilized A2C6.
DISCUSSION

The human ovarian carcinoma-associated determinant CA 125 is recognized and defined by the murine monoclonal antibody OC 125 (1). Our data indicate that CA 125+ moieties from the OVCA 433 supernatant and patient ascites exist as high molecular weight glycoprotein complexes that migrate as 3 or 4 disperse bands with apparent \( M_r \) between 400,000 and 1,000,000 on SDS-PAGE, in agreement with the previous studies of CA 125 from OVCA 433 supernatants and human serum (14). Whether these moieties represent oligomers of a smaller subunit, different glycoproteins bearing the same epitope, or macromolecular complexes of distinct glycoproteins cannot be determined from the present study.

Several epitopes can be coexpressed with CA 125 including TAG 72, CA 19-9, MOv8, DF3, and CA 3632. Coexpression of different determinants with CA 125 could reflect the presence of antigenically distinct peptides (27, 28) or carbohydrates (29–31). The latter could result from the aberrant patterns of glycosylation observed in many neoplasms. Among the epitopes that were coexpressed with CA 125 in the present study, CA 19–9 has been best defined chemically. CA 19–9 is a sialylated lacto-N-fucopentaose II carbohydrate determinant related to the Lewis blood group A (30, 31) that can be expressed on a high molecular weight mucin and on glycolipids. When CA 125+ material was isolated by immunoaffinity chromatography, moieties were obtained that expressed both CA 125 and CA 19–9. CA 125 and CA 19–9 determinants resisted disaggregation with high salt concentrations as well as ionic and nonionic detergents. In previous studies, treatment of similar material with 6 M urea and heating (45°C, 30 min) followed by gel filtration in the presence of 6 M urea and 0.1% SDS led to the isolation of immunoreactive moieties of apparent \( M_r \), 220,000, which were thought to be subunits of the complex (14). This material no longer reacted with NS 19–9, suggesting that CA 125 and CA 19–9 shared the same high molecular weight glycoprotein complex but were not present on the same glycoprotein molecule.

In the present study, gel filtration and antibody affinity chromatography often permitted recovery of up to 60 or 70% of the total CA 125 activity present in the starting material. When second antigens including TAG 72 and CA 19–9 were detected, these determinants were clearly bound to CA 125 in a complex sufficiently tight to resist disaggregation with high salt concentrations and nonionic detergent under conditions much more stringent than those utilized in processing samples for a routine clinical assay. Consequently, these operationally
defined CA 125* complexes could be sufficiently stable to provide targets for a potentially useful clinical test.

Imunoaffinity purification was undertaken to isolate CA 125 antigen from the OVCA 433 supernatant and from ascites samples from 6 ovarian cancer patients. Antigen from the various sources was systematically tested by immunoblot for reactivity with the OC 125, MOv8, NS 19–9, B72.3, and DF3 monoclonal antibodies. Material reactive with each of these antibodies has been detected in serum or ascites from patients with ovarian carcinoma (4, 12, 32–34). Samples were also tested for reactivity with OC 3632, a murine monoclonal antibody raised in our laboratory and selected for the ability to bind CA 125* complexes at a site that does not completely block binding of OC 125.

Crude OVCA supernatants and patient ascites frequently bound several of the panel of second antibodies by immunoblot analysis. OC 125 immunoaffinity purification of CA 125 antigen from each source removed the bulk of this reactivity, consistent with the possibility that the predominant molecular species that expressed CA 19–9, MOv8, TAG 72, or DF3 did not coexpress CA 125. In each case, however, the purified antigen retained a moderate or strong immunoblot reaction with at least one of the second antibodies. This suggests that it is not unusual for CA 125* complexes to have tightly bound additional determinants resistant to dissociation by detergent and high salt concentration. These doubly positive complexes migrated at an apparent $M_r >900,000$, equivalent to the higher molecular weight forms of CA 125* antigen. This may reflect the fact that only the higher molecular weight forms are sufficiently multivalent to remain bound to the affinity column under the stringent washing conditions.

Marked heterogeneity was observed in the coexpression of different epitopes. Each of the ascites samples exhibited a quantitatively distinct phenotype. Heterogeneity might relate to the expression of novel proteins by neoplastic cells or to aberrant patterns of glycosylation. Alternatively, coexpression of different epitopes could be polymorphic traits expressed by both benign and malignant cells from different donors. In one recent report, expression of 11 different antigen families has been studied in benign and malignant ovarian epithelial cells using monoclonal antibodies and biotin-avidin immunoperoxidase staining of frozen tissue sections (35). Each of 14 ovarian carcinomas expressed a distinctive combination of antigens. Comparable heterogeneity was observed in the expression of different epitopes by benign epithelium, compatible with the possibility that the monoclonal antibodies recognized polymorphic determinants.

Lack of a consistent pattern of coexpression for different epitopes on CA 125* moieties may limit the utility of this approach for detecting epithelial ovarian cancer. Only monoclonal antibody OC 3632 reacted with all antigen preparations, confirming the conditions of its selection. In every case, the immunoblot reaction of OC 3632 was weaker than the corresponding reaction with OC 125. Fewer copies of the CA 3632 determinant may be present on CA 125* molecules, the molecules may be more susceptible to denaturation during SDS-PAGE and blotting, or OC 3632 may have lower binding affinity for CA 3632 than does OC 125 for CA 125.

Because immunoblotting is technically difficult, labor intensive, and only semiquantitative, we attempted to develop rapid double determinant assays in which OC 125 was bound to a solid phase and different $^{125}\text{I}$-labeled second antibodies were used as probes to detect coexpression of distinct epitopes in clinical specimens. Using double determinant assays, we evaluated a panel of 37 ascites and 3 pleural fluid samples from ovarian cancer patients. When a ratio of specific/nonspecific binding $>2$ was considered positive, a majority of ascites contained CA125*CA19–9*, CA125*TAG72*, and CA125–MOv8* moieties and a minority contained CA125/DF3* and CA125*/3632* components.

The most promising double determinant assay (CA 125/TAG 72) was applied to a panel of 50 normal and 47 ovarian cancer patient sera. While the double determinant assay was able to exclude all the healthy control sera, it detected only 19% of the cancer patient sera as positive. The sensitivity of each immunoassay used alone was substantially better. A CA 125 level $>35$ units/ml identified 100% of cancer patients, whereas a CA 72 level $>6$ units/ml identified 68%. Thus, the loss in sensitivity easily outweighed any gain in specificity that could be anticipated for the double determinant assay. Similar double determinant assays using $^{125}\text{I}$-NS 19–9 and $^{125}\text{I}$-OC 3632 monoclonal antibodies as probes also lacked sensitivity. This lack of sensitivity may relate to the frequency with which epitopes are coexpressed. Alternatively, the conditions utilized for the double determinant assays may not have permitted optimal binding of different antibodies or may have facilitated dissociation of noncovalently bound complexes.

Coordinate elevation of distinct moieties may provide a more specific test for ovarian carcinoma. When CA 125 was evaluated in combination with CA 15–3 and TAG 72, the coordinate elevation of CA 15–3 or TAG 72 with CA 125 identified a majority of patients with ovarian carcinoma and excluded 95% of patients with benign disease (33). Subsequent studies have demonstrated that coordinate elevation of the 3 markers may also be of value in distinguishing benign from malignant adnexal masses (36, 37). Consequently, coordinate elevation of different serum markers may prove more valuable than coordinate expression of different epitopes on the same moieties as markers for epithelial ovarian cancer.

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REFERENCES

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