Interaction of Monoclonal Antibodies with Cell Surface Antigens of Human Ovarian Carcinomas

Y. Masuho, M. Zalutsky, R. C. Knapp, and R. C. Bast, Jr.

ABSTRACT

Two monoclonal antibodies, OC 125 and OC 133, bind to distinct determinants on the surface of human epithelial ovarian carcinoma cell lines. OC 125 and OC 133 recognize determinants on molecules with molecular weights greater than 200,000 and 80,000, respectively. When binding to four different cell lines was compared, apparent affinity constants for OC 125 ranged from $3.1 \times 10^9$ to $6.0 \times 10^7$ M$^{-1}$, whereas those for OC 133 ranged from $1.6 \times 10^7$ to $8.5 \times 10^6$ M$^{-1}$. An estimate of the number of antigenic determinants per cell ranged from $1.0 \times 10^7$ to $2.8 \times 10^6$ for OC 125 and from $4.0 \times 10^5$ to $3.4 \times 10^4$ for OC 133. Antigenic determinants recognized by OC 125 and OC 133 could be detected in spent culture medium. When radiolabeled OC 125 was incubated with each of four ovarian tumor cell lines, approximately 90% of the antibody remained bound to the tumor cell surfaces for more than 20 hr. Similar binding of OC 133 was observed with three of the four ovarian tumor cell lines. By contrast, >70% of OC 133 antibody was either shed or endocytosed after binding to OVCA 433 cells over the same period. Antigenic modulation was not induced by either antibody interacting with any of the four cell lines. These data suggest that antigen may be lost from the surface of human ovarian carcinoma cells by several different mechanisms and that antigen release is not inconsistent with binding of radiolabeled antibody to the tumor cell surface for prolonged periods.

INTRODUCTION

Development of monoclonal reagents has facilitated studies of the interaction of antibodies with tumor-associated antigens. Earlier studies with polyclonal reagents have demonstrated that antigen may be shed from the cell surface at a molecular level or in association with fragments of tumor cell surface membrane (5). Binding of monoclonal immunoglobulin to the tumor cell surface can induce antigenic modulation with shedding or endocytosis of antigen-antibody complexes (6, 16). Not all antigens, however, undergo modulation from the cell surface in the presence of antibody. Cell surface antigens that do not modulate should be appropriate targets for serotherapy and radionuclide imaging. Conversely, antigens that are shed from the cell surface may prove useful as markers for monitoring tumor progression or regression.

Recently, we have described 2 monoclonal antibodies, OC 125 (2) and OC 133 (4), which react with human epithelial ovarian carcinomas. OC 125 binds to more than 80% of nonmucinous epithelial ovarian carcinomas, including neoplasms of serous, endometrioid, and clear cell subtypes, as well as a fraction of undifferentiated ovarian tumors (9). Using a highly sensitive biotin avidin immunoperoxidase technique, traces of the antigen can be found during embryonic development in the coelomic epithelium, müllerian duct, and amnion (8). In the adult, antigen can also be detected in the epithelium of the fallopian tube, endometrium, and endocervix, which are derived from the müllerian duct. The antigen, however, is not expressed in normal ovarian tissue, either in the adult or in the fetus (2, 8). In studies to date, OC 133 has bound only to serous ovarian neoplasms (4). Among different adult tissues tested, this antibody has reacted with epithelial components of the endometrium and endocervix, but not the ovary or fallopian tube. Given the specificity of OC 125 and OC 133, we have investigated the interaction of these antibodies with several different human ovarian carcinoma cell lines. Although antigenic determinants are found in spent culture medium from each of these lines, substantial heterogeneity has been observed in the interaction of monoclonal reagents with cell surface determinants.

MATERIALS AND METHODS

Cell Lines. Four cell lines were established from different ovarian carcinomas. Each of the cell lines exhibited a distinctive epithelial morphology, karyotype, and pattern of growth in vitro. An additional cell line with epithelial morphology was established from a nonmalignant ovary. All cell lines were grown in Eagle’s minimum essential medium supplemented with 10% fetal calf serum, nonessential amino acids (0.1 µM each), 2 mM L-glutamine, 1 mM sodium pyruvate, penicillin G (50 units/ml), and streptomycin (50 µg/ml). Epstein-Barr-transformed B-lymphocyte lines were established from the peripheral blood leukocytes of the patients from whom tumor cell lines had been obtained.

Monoclonal Antibodies. The development of monoclonal antibodies with specificity for human ovarian carcinoma has been described (2, 4). OC 125 was raised against cell line OVCA 433 (2), whereas OC 133 was raised against cell line OVCA 432 (4). The hybridomas were cloned by limiting dilution and expanded as ascites in mice previously primed with pristane. Both OC 125 and OC 133 were of the IgG1 isotype, determined by immunoprecipitation with isotype-specific anti-murine immunoglobulins (Meloy Laboratories, Springfield, VA). For the present study, the IgG1 antibodies were purified from ascites fluid. Each immunoglobulin was precipitated by the addition of solid ammonium sulfate to produce 50% saturation. The precipitate was dissolved in 2 ml of 5 mM sodium phosphate buffer, pH 7.8, and dialyzed against the same buffer. The crude fraction was applied to a DE52 column of 10-ml bed volume equilibrated with the same phosphate buffer. The protein concentration of eluted fractions was determined by measuring the absorbance at 280 nm. Binding of antibody to tumor cells was measured by radioimmunoassay using $^{125}$I-labeled sheep anti-mouse IgG as described below.

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2 Visiting Investigator from Teijen, Ltd.

3 Scholar of the Leukemia Society of America, Inc. To whom requests for reprints should be addressed, at Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115. Received December 2, 1983; accepted March 30, 1984.

4 H. Lazarus et al., unpublished data.
OC 125 did not bind to the column equilibrated with 5 mM sodium phosphate buffer, pH 7.8. OC 133 was eluted with 10 mM sodium phosphate buffer at the same pH. Purified preparations contained one protein band of approximately 160 kilodaltons when analyzed on SDS-PAGE. Each ml of OC 125 ascites fluid yielded 2 mg of protein, whereas each ml of OC 133 ascites yielded 10 mg of protein.

**Indirect Immunoglobulin Binding Assay.** Ovarian tumor cells were grown to confluent monolayers in 75-cm² culture flasks (Falcon Plastics Division, Becton, Dickenson and Co., Cockeysville, MD), detached with trypsin-versene (M. A. Bioproducts, Walkersville, MD), washed once with culture medium, and seeded into 96-well flat-bottomed tissue culture plates (Falcon Plastics) at a cell density of 2 × 10⁴ well. Before use, cells were incubated at 37°C for 3 to 5 days in an incubator with 5% CO₂-95% humidified air. The cell monolayers were washed 3 times with 150 μl of culture medium, and a 100-μl portion of medium containing OC 125 or OC 133 was added to each well. After incubation at 37°C for different periods, the plates were placed on an ice bath and washed 3 times with Eagle’s minimum essential medium containing 20 mM 4(2-hydroxyethyl)-1-piperazineethanesulfonic acid (M. A. Bioproducts) and 2% fetal calf serum. To measure antibody binding to the cell surface, 40 μl of appropriately diluted ¹²⁵I-labeled (Fab)² fragment of sheep anti-mouse IgG (5 to 10 CI/g; New England Nuclear; Billerica, MA) were added. After incubation on ice for 60 min, monolayers were washed 3 additional times, and cells were solubilized with 200 μl of 0.1 M NaOH. Radioactivity was measured in a γ-counter. Values were expressed as mean ± S.D. of triplicate samples.

**Direct Immunoglobulin Binding Assay.** For the direct binding assay, purified OC 125 and OC 133 were labeled with Na¹²⁵I using Iodogen (Pierce Chemicals, Rockford, IL). Iodogen was dissolved in methylene chloride to 50 mg/ml, and 100 μl were added to a glass test tube. The solvent was allowed to evaporate under a stream of nitrogen. One hundred μg of the antibody in 100 μl of phosphate-buffered saline were added to the iodogen tube, and then 1 ml of Na¹⁸I (New England Nuclear) was added. After a 10-min incubation at room temperature, the reaction mixture was passed through a Sephadex G-25 fine column (1 × 15 cm) to remove unreacted ¹²⁵I Iodide. The iodination protocol yielded ¹²⁵I-labeled OC 125 and ¹²⁵I-labeled OC 133 with specific activities of 4.5 × 10⁶ and 5.0 × 10⁵ cpm/μg, respectively. This is equivalent to approximately 0.2 iodine atoms/molecule for both OC 125 and OC 133.

Cell monolayers in 96-well plates were prepared in the same manner as in the indirect binding assay. After washing, monolayers were incubated with 50 μl of diluted ¹²⁵I-labeled antibodies for different periods. After 3 additional washes with 150 μl of ice-cold wash medium, cells were dissolved with 200 μl of 0.1 N NaOH. Radioactivity was measured in a γ-counter and expressed as mean of triplicate samples. To determine affinity constants and to estimate the number of antigenic determinants per cell, monolayers were incubated with limited concentrations of ¹²⁵I-labeled antibodies at 37°C for 8 hr. A relatively long incubation was required to assure optimal binding.

**Detection of Shed Antigen.** The ovarian carcinoma tumor cell lines and the LAZ 434 cell line were grown in the medium described above. When monolayers had become confluent in T-25 flasks (Falcon Plastics), cells were harvested by scraping the monolayers with a rubber policeman. For analysis of the lymphocytes, cells were allowed to stand at room temperature for 15 min. The reaction mixture was transferred to a centrifuge tube, and the cells were washed 3 times with PBS containing 20 mM potassium iodide. Final cell pellet was solubilized on ice with 0.01 M Tris-HCl containing 2% (w/v) Nonidet P-40 and 1 mg/10⁴ polyethylene sulfonate fluoride (Sigma Chemical Co., St. Louis, MO) at pH 7.4 and centrifuged in an Eppendorf microtube for 15 min at 4°C.

For immunoprecipitation, 0.2 mg of purified OC 125 or normal mouse IgG was mixed with 0.8 mg of rabbit anti-mouse IgG (Cappel Laboratory, West Chester, PA), allowed to stand at room temperature for 1 hr, and was then placed at 4°C over night. The precipitate was washed 3 times with 0.01 M Tris-HCl containing 0.2% (w/v) Nonidet P-40 and 1 mg/ml of antibody in 100 μl of PBS, and cells were solubilized with 200 μl of 0.1 N NaOH. Radioactivity was measured in a γ-counter. Values were expressed as mean ± S.D. of triplicate samples.

**Radioiodination, Immunoprecipitation, and Polysacrylamide Gel Electrophoresis of Cell Surface Proteins.** In some experiments, confluent monolayers of ovarian tumor cells were detached from the flasks with trypsin-versene and washed once with culture medium, and seeded into 96-well flat-bottomed tissue culture plates (Falcon Plastics) at a cell density of 2 × 10⁴ well. Before use, cells were incubated at 37°C for 3 to 5 days in an incubator with 5% CO₂-95% humidified air. The cell monolayers were washed 3 times with 100 μl of culture medium, and a 100-μl portion of medium containing OC 125 or OC 133 was added to each well. After incubation at 37°C for different periods, the plates were placed on an ice bath and washed 3 times with Eagle’s minimum essential medium containing 20 mM 4(2-hydroxyethyl)-1-piperazineethanesulfonic acid (M. A. Bioproducts) and 2% fetal calf serum. To measure antibody binding to the cell surface, 40 μl of appropriately diluted ¹²⁵I-labeled (Fab)² fragment of sheep anti-mouse IgG (5 to 10 CI/g; New England Nuclear; Billerica, MA) were added. After incubation on ice for 60 min, monolayers were washed 3 additional times, and cells were solubilized with 200 μl of 0.1 M NaOH. Radioactivity was measured in a γ-counter. Values were expressed as mean ± S.D. of triplicate samples.

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phenylmethyl sulfonyl fluoride, pH 7.4, and resuspended in 100 μl of the same solution. The immunoprecipitate that had been formed with normal IgG was added to the radiiodinated cell lysate, and the mixture agitated for 4 hr at 4°. The precipitate was removed by centrifugation at 10,000 x g for 10 min at 4°, and the supernatant was divided into 2 halves. One-half was mixed with the immune precipitate that had been formed with OC 125, and the other with OC 133. After additional agitation at 4° for 18 hr, the precipitates were collected by centrifugation at 4° for 10 min and washed 3 times with 100 μl of 0.2% Nonidet P-40.

Immunoprecipitates with OC 125 and OC 133 were analyzed by SDS-PAGE. Electrophoresis was conducted on a 5 to 15% gradient polyacrylamide slab gel as described by Laemmli (11). 125I-Labeled proteins were visualized by radioautography of the dried gels. Molecular weight standards were run for each experiment and included myosin (M, 200,000), phosphorylase b (M, 94,000), bovine serum albumin (M, 67,000), ovalbumin (M, 47,000), trypsin inhibitor (M, 20,500), and α-lactalbumin (M, 14,400).

RESULTS

Specificity of OC 125 and OC 133 Binding to Ovarian Carcinoma Cell Lines. The reactivity of OC 125 and OC 133 with various cell lines was measured by indirect immunofluorescence. Both antibodies bound strongly to all 4 ovarian tumor cell lines, but neither antibody bound to the LAZ 434 cell line which had been derived from normal ovarian tissue. Neither OC 125 nor OC 133 bound to B-lymphocyte lines derived from the donors of ovarian cancer cell lines OVCA 433 and OVCA 432. As the 2 monoclonals exhibited a similar pattern of reactivity (2, 4), we examined whether the 2 different antibodies would compete in binding to determinants associated with the ovarian tumor cell surface (Chart 1). The binding of 125I-labeled OC 125 or 125I-labeled OC 133 to OVCA 433 cells was measured in the presence or absence of excess nonlabeled antibody. The addition of excess OC 133 did not inhibit the binding of 125I-labeled OC 125 (Chart 1A), and the addition of OC 125 did not affect binding of 125I-labeled OC 133 (Chart 1B). Thus, the 2 antibodies appeared to recognize 2 distinct antigenic determinants.

Quantitation of OC 125 and OC 133 Binding to Ovarian Carcinoma Cell Lines. Affinity constants were estimated, and the antigenic determinants were enumerated by measuring the binding of 125I-labeled OC 125 and 125I-labeled IC 133 to 4 ovarian
tumor cell lines. Linear double-reciprocal plots were obtained for all combinations of antibodies and cell lines (Chart 2). From these plots, affinity constants and the number of antigen determinants per cell were calculated (1). The affinity constants of the 4 cell lines for OC 125 range from $3.1 \times 10^9$ to $6.0 \times 10^9$ M$^{-1}$, and the values for OC 133 range from $1.6 \times 10^8$ to $8.5 \times 10^8$ M$^{-1}$ (Table 1). Great heterogeneity was observed in binding of OC 125 to different cell lines, which suggests that the antigen structure recognized by OC 125 and/or its microenvironment on the cell surface are heterogeneous. By contrast, the affinity of OC 133 binding varied little between cell lines. The numbers of antigenic determinants per cell range from $1.0 \times 10^7$ to $2.8 \times 10^5$ for OC 125, and from $4.0 \times 10^5$ to $3.4 \times 10^4$ for OC 133.

Detection of Shed Antigen in Spent Culture Medium. The amount of antigen activity in spent culture medium was determined for each of the ovarian tumor cell lines and for the LAZ 434 line derived from a normal ovary. Judged by the inhibition of antibody binding, antigenic activity was detected in supernatants from all 4 ovarian carcinomas. Binding of OC 125 was strongly inhibited by all supernatants. The order of inhibitory potency was OVCA 432 > OVCA 433 > OVCA 429 > OVCA 420 (Chart 3A). A similar order was observed in the number of cell surface determinants among the 4 cell lines (Table 1). Culture supernatants inhibited binding of OC 133 to a lesser degree than binding of OC 125. Once again, the greatest inhibition of antibody binding was observed with OVCA 432 with less binding inhibition produced by OVCA 433, OVCA 429, and OVCA 420 (Chart 3B). When cell-free specimens of spent media were sedimented at 100,000 x g for 1 hr, greater than 90% of the antibody-binding inhibitory activity was associated with the supernatant. These data are most consistent with shedding of antigen at a molecular rather than at a supramolecular level. Heating of supernatants for 45 min at 100° destroyed the inhibitory activity for both OC 125 and OC 133 (Chart 3). When supernatants were analyzed by gel filtration on Sephadex G-200, inhibitory activity for OC 125 eluted with the void volume, whereas the inhibitory activity for OC 133 eluted between immunoglobulin and serum albumin (Chart 4). Fractions which inhibited binding of OC 125 did not inhibit binding of OC 133. Conversely, fractions which inhibited OC 133 binding did not inhibit OC 125.

Analysis of Cell Surface Antigens on SDS-PAGE. When cells were harvested by enzymatic techniques, the immunoprecipitates formed with OC 125 exhibited bands with molecular weights of 180,000 and <20,000 on SDS-PAGE. OC 133, however, showed only a single band (Mr 80,000). Similar bands were observed with antigen isolated from OVCA 432 and OVCA 433. When cells were harvested by mechanical techniques, similar results were obtained for OC 133. Immunoprecipitates with OC 125, on the other hand, were excluded from the stacking gel. Consequently, the results with the tumor cells isolated by mechanical means were consistent with a molecular weight in excess of 200,000 similar to that observed by gel filtration of culture supernatants on Sephadex G-200.

Immunoglobulin Binding after Prolonged Incubation of Ovarian Tumor Cells with Monoclonal Antibodies. In other systems, prolonged incubation of tumor cells with antibody has sometimes been required to observe antigenic modulation or endocytosis of antigen-antibody complexes. Consequently, different ovarian tumor cell lines were cultured in the presence of nonlabeled OC 125 or OC 133 for periods ranging up to 24 hr. Excess monoclonal antibody was washed from the system, and the amount of murine immunoglobulin bound to the cell surface was measured by the addition of 125I-labeled sheep anti-mouse IgG (Chart 5). The amount of OC 125 which bound to each of the cell lines did not change after initial equilibration. The amount of OC 133 bound to OVCA 433 diminished significantly during incubation, although the amount of antibody which bound to other cell lines did not decrease over 24 hr. In subsequent experiments, OVCA 433 cells were used to compare the activity of the 2 antibodies.

When different concentrations of OC 125 were incubated with OVCA 433 cells, the amount of immunoglobulin bound to the tumor cell surface did not change after initial equilibration in the presence of 0.2 to 5.0 µg of OC 125 per ml (Chart 6A). Incubation of OVCA 433 with lower concentrations of OC 133 (0.0062 to

![Graph](chart3.png)

**Chart 3.** Inhibition of OC 125 (A) and OC 133 (B) binding to tumor cells by culture supernatants. Percentage of inhibition has been plotted as a function of relative supernatant concentrations. The confluent cell monolayers of OVCA 433 (C), OVCA 432 (D), OVCA 420 (E), OVCA 429 (F), and LAZ 434 (G) were cultured for 4 days, and supernatant media were collected. A portion of the OVCA 433 supernatant was heated in a boiling water bath for 45 min (H).
0.062 μg/ml resulted in a gradual and persistent decrease in binding of immunoglobulin to the cell surface over 24 hr (Chart 6B). After incubation with higher concentrations of OC 133 (0.62 to 6.2 μg/ml), no significant decrease in immunoglobulin binding was observed.

Two explanations might be offered for the progressive reduction in binding of OC 133 during prolonged incubation with the OVCA 433 cell line. Antigen might disappear altogether from the tumor cell surface, undergoing modulation comparable to that observed with the common acute lymphoblastic leukemia antigen (12, 16). Alternatively, with limiting dilutions of the monoclonal reagent, antigen might be reexpressed at the cell surface following shedding or endocytosis, but sufficient antibody might not be available in the supernatant to produce a detectable interaction with the newly synthesized antigen. To decide between these possibilities, OVCA 433 cells were incubated with different concentrations of OC 133 for 24 hr. The amount of immunoglobulin bound to the surface of tumor cells was measured by incubation with 125I-labeled sheep anti-mouse IgG, either with or without the addition of saturating concentrations of fresh antibody (Chart 7). From these data, it appears that free antigenic determinants were indeed present at the tumor cell surface after 24-hr incubation with concentrations of OC 133 less than 1 μg/ml. The total amount of immunoglobulin bound in the presence of saturating amounts of OC 133 remained relatively constant, despite pretreatment with concentrations of the antibody ranging from 0.01 to 10 μg/ml. Consequently, antigenic modulation did not occur under conditions where bound antibody was eliminated from the surface of the tumor (Chart 6). This raised the possibility that bound antibody might be endocytosed and that new antigenic determinants might be reexpressed during 24-hr incubation.

Disappearance of Antibody from the Cell Surface of OVCA 433. To determine how long antibody was retained at the cell surface, OVCA 433 cells were incubated with OC 125 or OC 133 for 30 min. After washing, cells were incubated in medium without antibodies. At different times thereafter, the amount of immunoglobulin still bound to the tumor cell surface was determined by incubation with 125I-labeled sheep anti-mouse IgG. A striking difference was observed in the behavior of OC 125 and OC 133 over the next 23 hr (Chart 8). The amount of OC 125 bound to the cell surface decreased by 10% over 3 hr, but no additional decrease was observed over the next 20 hr. Thus, determinants recognized by OC 125 appeared to remain fixed at cell surface and were not shed or endocytosed following interaction with antibody. By contrast, the amount of OC 133 bound by OVCA 433 cells continued to decrease over the entire period of incubation, falling to 24% of the initial value after 23 hr. Simple dissociation of OC 133 from the cell surface seemed unlikely, as OC 133 exhibited a higher affinity for OVCA 433 than did OC 125 (Table 1).

Uptake of OC 133 by OVCA 433 Cells. Disappearance of OC 133 from the tumor cell surface could occur either by release into the culture medium with shed antigen or by endocytosis into...
and the amount of immunoglobulin remaining at the cell surface measured with \( \text{O} \). After washing, cells were cultured at 37° for different periods without antibody, incubated at 37° for 30 min with OC 125 (•) (0.25 \( \mu \)g/ml) or OC 133 (0.05 \( \mu \)g/ml) \( ^{125}\text{I}-\text{labeled sheep anti-mouse IgG. Bars, S.D.} \)

Charts. Persistence of antibody at the cell surface. OVCA 433 cells were incubated at 37° for 30 min with \( ^{125}\text{I}-\text{labeled OC 125 (0.25 \( \mu \)g/ml) (•) or OC 133 (0.05 \( \mu \)g/ml) (O). After washing, cells were incubated at 37° for different periods without antibody, and the radioactivity associated with cells was measured. Bars, S.D.

Chart 9. Persistence of antibody in the cytoplasm and at the cell surface. OVCA cells were incubated at 37° for 30 min with \( ^{125}\text{I}-\text{labeled OC 125 (0.25 \( \mu \)g/ml) (•) or \( ^{125}\text{I}-\text{labeled OC 133 (0.05 \( \mu \)g/ml) (O). After washing, cells were incubated at 37° for different periods without antibody, and the radioactivity associated with cells was measured. Bars, S.D.

Chart 10. Accumulation of OC 125 and OC 133 in cells. OVCA 433 cells were cultured for various periods with \( ^{125}\text{I}-\text{labeled OC 125 or \( ^{129}\text{I}-\text{labeled OC 133, leaving the monoclonal antibodies in contact with the tumor cells. Although OC 125 showed no change after equilibration at any of the concentrations tested, uptake of \( ^{129}\text{I}-\text{labeled OC 133 continued to increase over 23 hr (Chart 10).} \)

DISCUSSION

Several lines of evidence suggest that the monoclonal antibodies OC 125 and OC 133 recognize distinct antigenic determinants which are associated with human ovarian carcinomas. In earlier studies, OC 125 bound to more than 80% of nonmucinous ovarian carcinomas including tumors of serous, endometrioid, clear cell, and undifferentiated histologies (9). Traces of the CA 125 antigen defined by OC 125 could be detected in epithelium derived from the müllerian duct including the mucosa of the fallopian tube, endometrium, and endocervix (8). By contrast, OC 133 bound exclusively to tumors of serous histology and to normal endometrium and endocervix, but not to the mucosal epithelium of the fallopian tube (4). In the present study, OC 125 and OC 133 did not compete for binding to antigenic sites associated with an epithelial ovarian carcinoma cell line. Moreover, the antigens recognized by these antibodies exhibited distinct molecular weights on gel filtration and polyacrylamide gel electrophoresis. When care was taken to avoid proteolysis by trypsin, OC 125 bound to a heat-labile \( M, >200,000 \) moiety, whereas OC 133 bound to a \( M, 80,000 \) moiety under reducing and nonreducing conditions.

When OC 125 and OC 133 were incubated with different epithelial ovarian carcinoma cell lines, marked heterogeneity was observed in the apparent affinity of the antibodies. Recent articles have, however, pointed to the limitations of kinetical analysis in defining the binding of monoclonal antibodies to cell surface antigens (14, 17). Although the antibodies used in the present study were homogeneous on polyacrylamide gel electrophoresis without excess heavy or light chains, the whole divalent IgG1 antibody was radiolabeled and used to define binding characteristics. Studies were carried out at 37° which could have permitted translocation, modulation, internalization, or shedding of the antigen. Shedding or internalization of antigen was not observed, however, when OC 125 was incubated with each of the 4 ovarian tumor lines tested or when OC 133 was incubated with 3 of the same 4 cell lines. Antigenic modulation was not produced by either of the monoclonals in any of the cell lines, but translocation of the antigen, permitting bridging of determinants, could not be ruled out. When antigens recognized by OC 133 or OC 125 were immunoprecipitated after labeling cell surface determinants with \( ^{129}\text{I}, \) similar bands with molecular weights of 80,000 or >200,000 were detected on polyacrylamide gel electrophoresis of material from each of the 4 cell lines tested. Despite this immunochemical homogeneity, substantial variation was observed in the amount of OC 133 or OC 125 bound to individual cells within as well as
between different cell lines. Consequently, substantial heterogeneity was encountered in the targets for antibody binding. Given these limitations, a precise estimate of binding affinity could not be obtained, but assays performed on different cell lines under similar conditions did demonstrate substantially greater heterogeneity in binding OC 125 than in binding OC 133. To the extent that the tumor cell lines still resemble the ovarian cancers from which they were derived (7), these data suggest that a similar heterogeneity might be encountered between patients during the clinical application of OC 125 as a carrier to radionuclide imaging or drug delivery. In clinical studies with anti-carcinoembryonic antigen antibody, affinity appeared to be one critical determinant of effective localization (13).

Shed antigen could be detected in culture supernatants judged by inhibition of antibody binding to tumor cells. Recently, a more sensitive sandwich assay has been developed for determinants recognized by OC 125 in serum and ascites fluid (10). Elevated antigen levels could be detected in serum from more than 80% of patients with surgically demonstrable epithelial ovarian carcinoma, and increases or decreases in antigen levels correlated with progression or regression of disease in >90% of instances studied (3). Supernatants from cultures of OVCA 433 cells have provided a convenient source of antigen for the standardization of the sandwich assay. Despite the presence of antigen in spent culture medium, radiolabeled 125I-labeled OC 125 remained bound to each of the 4 ovarian carcinoma cell lines for up to 23 hr without significant modulation, internalization, or shedding of antigen from the cell surface membrane. Antigen may have appeared in culture supernatants through active secretion or by inhibition of antibody binding. Soluble antigen was detected in supernatants from all 4 cell lines for up to 23 hr. Whether qualitative differences can be detected between shed and membrane-bound antigen remains to be determined.

In 3 of the 4 epithelial ovarian cell lines, most OC 133 remained associated with the tumor cell surface for 23 hr. In a fourth cell line, however, a significant amount of antibody was either shed or internalized. In each of the 4 cell lines, OC 133 could immunoprecipitate a M, 80,000 moiety. Thus, similar molecules bearing determinants recognized by a single monoclonal antibody appeared to behave quite differently when associated with different epithelial ovarian carcinoma cell lines. This degree of heterogeneity in the disposition of cell surface antigen raises a note of caution with regard to conclusions obtained with only a single cell line and may reflect a similar heterogeneity among ovarian tumors in vivo.

Interestingly, antigenic modulation was not induced by either of the monoclonals on any of the 4 cell lines. Although antigenic modulation has been induced with monoclonal antibodies against common acute lymphoblastic leukemic antigen (12, 16) or T3 (6), the phenomenon evidently does not occur with many antigens recognized by monoclonal reagents. Since antigenic modulation may be one of the factors which limits effective serotherapy in vivo (15, 16), the choice of antigenic targets that do not modulate may be important. Our own data are consistent with the possibility that antigenic modulation may be the exception rather than a general phenomenon that would be observed with all cell surface determinants.

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