Confirmation of a False-Positive Result in CA 125 Immunoradiometric Assay Caused by Human Anti-Idiotypic Immunoglobulin

Thomas L. Klug,1 Paul J. Green,2 Vincent R. Zurawski, Jr.,1 and Hugh M. Davis1

An immunoradiometric assay (IRMA) involving a monoclonal antibody (MAb OC125) to an ovarian carcinoma-associated antigenic determinant (CA 125) has been tested as one component in a strategy for early detection of epithelial ovarian cancer. We characterized one confirmed "false-positive" sample by murine antibody blocking studies, Western blotting, immunoaffinity, size-exclusion chromatography, and reactivity with polyclonal rabbit antisera to CA 125 antigen. The positive response of this serum in the CA 125 IRMA was due to a human IgM. The discrepant IgM was isolated from the serum by successive immunoaffinity steps with nonspecific murine MAb, MAb OC125, and goat antibodies to human IgM Fc. Purified IgM inhibited the binding of MAb OC125 to CA 125. Furthermore, rabbit antisera to CA 125 antigen competitively inhibited the binding of MAb OC125 to both CA 125 and the discrepant IgM. The discrepant activity thus appears to reflect binding of this human IgM to a idiotope of MAb OC125. Radioliodination of MAb OC125 by a different technique eliminated the discrepant activity and decreased the incidence of CA 125 positivity in an at-risk population of apparently healthy women, increasing the specificity of the IRMA to 99.8% in this group.

Additional Keyphrases: ovarian cancer • monoclonal antibodies • variation, source of • immunoaffinity purification • screening

The immunoradiometric assay (IRMA) of CA 125, based on the use of monoclonal antibody (MAb) OC125 (1,2), has demonstrated excellent sensitivity and predictive value as a serum marker assay, both for identifying patients with residual ovarian cancer after primary therapy (3), and for monitoring the clinical course of ovarian carcinomas (4).6 The excellent specificity of the CA 125 IRMA has led Einhorn et al. (5) to suggest the use of this test as a diagnostic adjunct for discriminating benign from malignant pelvic masses.

As currently constructed, however, the diagnostic utility of the CA 125 IRMA for screening apparently healthy women for ovarian cancer may be limited by the occurrence of "false positives." For example, in a study including 988 nonpregnant patients screened at a gynecologic clinic, Niloff et al. (6) reported that CA 125 concentrations were abnormally high (>65 kilo-arb.units/L) in 1.1% of patients, a prevalence that dropped to about 0.5% upon analysis of a second serum sample. Because most women in this group were premenopausal, some of these increases may have been due to the pronounced fluctuations in CA 125 occasionally associated with menstruation (7). In another study, however, involving 586 nonhospitalized women over 50 years old, Zurawski et al. (8) found a similar specificity for the test, 99.5% in the 50 years and older group and 99.2% overall, also based on the 65 kilo-arb.units/L cutoff value.

The specificity of the CA 125 IRMA might have been greater had women with pathophysiological conditions apparent at the time of testing been excluded from the study. However, a small portion of samples with above-normal CA 125 values would remain (2,6,8). Thus, a low frequency of false-positive samples would remain in the screened populations. Reducing the incidence of these false-positive results would undoubtedly enhance the diagnostic usefulness of the CA 125 IRMA.

Here we describe a healthy subject (B.K.) whose serum showed persistently increased CA 125 values even in the confirmed absence of CA 125 antigen. Biochemical analyses suggested that this false-positive activity was due to a highly specific human anti-idiotypic (anti-id) antibody to the murine MAb OC125. Use of a different radiolabeling technique decreases the incidence of such false positives and may increase the specificity of the CA 125 IRMA.

Materials and Methods

CA 125 Analysis and Immunoaffinity Techniques

All the immunoaffinity and antigen analysis techniques used here—including gel chromatography, Western blotting, and MAb OC125 affinity chromatography—have been described in detail elsewhere (9). Affinity chromatography for purifying the material in serum from B.K. that showed activity in the CA 125 IRMA was modified from that described (9) by using phosphate-buffered saline (PBS; PO₄³⁻/NaCl 150 mmol/L, pH 7.4, and MAb OC125 or anti-tetanus toxoid MAb coupled to Affi-gel 10 (Bio-Rad Laboratories, Richmond, CA), 3 mg per milliliter of gel. B.K. serum was passed through the anti-tetanus toxoid MAb column before OC125 MAb immunoaffinity. We prepared an anti-human IgM affinity column by coupling goat anti-human IgM Fc immunoglobulins (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) to CNBr-activated Sepharose (Pharmacia, Inc., Piscataway, NJ) at 1 mg of antibody per milliliter of gel, according to the manufacturer's instructions. We applied to this column the discrepant material from patient B.K. (affinity-purified with OC125 MAb), eluted it with 50 mmol/L diethylamine, pH 11.3, neutralized the eluate with Tris buffer (1 mol/L, pH 6.8), and diaxalted it against PBS. This purified fraction was concentrated 10-fold with a Centricon 10 microconcentrator (Amicon Corp., Danvers, MA).

"High-Performance" Liquid Chromatography (HPLC)

Immunoaffinity-purified B.K. discrepant material (250 μL) was applied to a 300 × 7.5 mm TSK 4000 size-exclusion column (Phenomenex, Rancho Palos Verdes, CA) equilibrat-

1 Centocor, 244 Great Valley Parkway, Malvern, PA 19355.
2 Department of Pathology and Cell Biology, Jefferson Medical College, Philadelphia, PA 19107.
3 Nonstandard abbreviations: MAb, monoclonal antibody; IRMA, immunoradiometric assay; CA 125, the antigenic determinant recognized by MAb OC125; CA 125 antigen, the molecule expressing CA 125; anti-id, anti-idiotypic; PBS, phosphate-buffered saline; RF, rhumatoid factor(s); and BSA, bovine serum albumin.

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ed with 0.2 mol/L phosphate buffer, pH 6.8; 0.5-mL fractions were collected and assayed for the presence of apparent CA 125 activity with the CA 125 IRMA. The molecular mass of the active material was then determined by comparison of its relative retention time with that of several molecular-

mass standards.

immunoassays

The CA 125 IRMA kits (Centocor, Malvern, PA) were used according to the manufacturer’s instructions. In the CA 125 IRMA, the same murine IgG1 MA 0C125 is used as both capture and tracer antibody in a simultaneous “sandwich” format (2), made possible by the multivalent or aggregate nature of the CA 125 antigen (9). In the CA 125 IRMA, MA 0C125 is immobilized on the 0.4-inch diameter polystyrene-

bead solid phase; 5 to 10 ng of radiiodinated 0C125 is used as the tracer. One adds 100 μL of serum to the bead in a tray, then adds tracer MA 125-labeled 0C125 (130 000 dpm), in 100 μL of a buffer containing a nonspecific murine MA 0C. Bacteroides concentration is expressed in arbitrary units: kilo-arb.units/L. To radiiodinate MA 0C125 for use in the IRMA, we used either the Bolton–Hunter reagent (8–13 Ci/g) (10) or Iodogen (1,3,4,6-tetrachloro-3,6-diphenylglycol-

uril, 15–20 Ci/g) (11) techniques, predominantly the former (i.e., unless otherwise stated). For some IRMAs, we used Bolton–Hunter radiolabeled 0C125 Fab(′)2 of similar specific activity and tracer concentrations to demonstrate that the anti-globulin interactions observed were not mediated through 0C125 IgG Fc regions. All other kit components were equivalent. In blocking studies we used protein A-purified murine MA IgG1 (OC125), an IgG1–IgG2b combination murine MA (2B82B3 anti-tetanus toxoid) (12), normal mouse serum, or protein A-purified polyclonal IgG obtained from BALB/c normal mouse serum, or these same purified IgGs aggregated by heating. Heating a 2 mg/mL solution of IgG in PBS for 20 min at 60 °C resulted in aggregation of 15 to 18% of the IgG. Nephelometric assays for rheumatoid factor (RF) were done with commercially available reagent and system (Immunochemistry System, Beckman Instruments).

Inhibition Assays for Presence of Anti-Id

We used two separate methods to confirm the presence of putative anti-Id immunoglobulin to MA 0C125: inhibition of binding of MA 0C125 to CA 125 antigen by the agent, and inhibition of the patient-B.K. discrepant activity by antibody to CA 125 antigen generated in rabbits. Partly purified CA 125 antigen OVCA 433/PCA/4B (9) was coated onto a 96-well polystyrene microtiter plate (Dynatech, Alexandria, VA) at 400 kilo-arb.units/L per 50 μL of CA 125 per well, washed, and blocked with a 50 g/L solution of BSA in isotonic saline. We then mixed B.K. serum or affinity-
purified B.K. discrepant material with radiiodinated MA 0C125 (5000 dpm per 50 μL), and applied 50 μL of the mixture to wells of the plate coated with CA 125 antigen. The plates were washed, cut, and the radioactivity was counted after incubation for 12 h at 4 °C. We used normal human serum and human polyclonal immunoglobulins in identical buffers as negative controls. Three rabbits were initially immunized with affinity-purified CA 125 antigen (9) followed by injections of partly purified CA 125 antigen over a six-month period. One rabbit eventually developed a low-titer antibody that competitively inhibited binding of MA 0C125 to CA 125 antigen in the CA 125 IRMA. We tested the ability of various dilutions of pre- and postimmu-
nization antisera from this rabbit to inhibit B.K. discrepant related binding of 125I-labeled OC125 tracer in the CA 125 IRMA.

Subjects

The subject (B.K.), who was a false positive in the CA 125 IRMA, was first discovered by Green et al. (13) during a study of approximately 500 healthy blood donors at Thomas Jefferson University Hospital, Philadelphia, PA. Apparent CA 125 concentrations in serum from B.K. have ranged from 150 to more than 450 kilo-arb.units/L. The subject is a healthy woman in her mid-twenties, with no known history of either benign or malignant disease.

In addition, we selected samples from a panel of sera collected from 1082 women over 40 years of age, 11 (1.0%) of whom had increased CA 125 concentrations (>65 kilo-

arb.units/L) in their serum on at least one occasion. These women are involved in an ongoing collaborative study to evaluate the use of the CA 125 IRMA as one component in a strategy for early detection of ovarian cancer (14). Twenty sera from women with advanced ovarian cancer were selected from a Centocor (Malvern, PA) serum bank and run in CA 125 IRMAs to compare the effect of radiolabeling technique on apparent (i.e., measured) CA 125 concentrations. Sera from patients with rheumatoid arthritis, anti-nuclear antibodies, and infectious mononucleosis were also from the Centocor serum bank.

Results

Confirmation of the False-Positive Nature of B.K.’s Serum Sample

Upon receipt of the discrepant sample from B.K., we sought to establish whether it indeed contained increased CA 125 concentrations or was a false-positive specimen. Initial tests demonstrated that the agent responsible for apparently increased CA 125 IRMA values did not resemble the CA 125 antigen in its biochemical behavior: (a) its molecular mass (870 kDa) as assessed by HPLC chromatography is less than that reported for serum CA 125 antigen (9) (Figure 1); (b) B.K. serum did not contain any CA 125 antigen detectable by sodium dodecyl sulfate/polyacryla-

mide gel electrophoresis and Western blotting with radiola-

beled MA OC125 under conditions in which confirmed CA 125-containing samples (CA 125 >65 kilo-arb.units/L) always did (9, Figure 2).

Specificity of B.K. Anti-Murine Immunoglobulin Response

We performed CA 125 assays on B.K. discrepant serum in the presence of increasing concentrations of both monoclonal and polyclonal BALB/c-derived murine immunoglobulins. Similar experiments were performed with heat-aggre-
gated murine immunoglobulins. Concentrations of normal mouse serum as high as 400 mL/L, final IgG concentration 1–2 mg/mL, and murine MA IgG1, IgG2 concentrations as great as 0.8 mg/mL, inhibited CA 125 IRMA values for the B.K. discrepant serum only slightly. The use of heat-

aggregated MA at similar concentrations also had little effect (data not shown). In contrast, the addition of 50 ng of purified MA 0C125 neutralized half of the discrepant activity in 0.1 mL of B.K. serum in the CA 125 IRMA. Three to four times more unlabeled MA OC125 antibody was required to neutralize B.K. discrepant activity than an equivalent activity of CA 125 antigen (Figure 3). A rate-
nephelometric assay for RF factor indicated that B.K.'s serum contained no RF-like activity. We also performed CA 125 IRMA on six serum samples known to have high titers of RF, six samples with anti-nuclear antibodies, and six samples with high IgM concentrations (infectious mononucleosis). One sample with an RF titer >1:10 000 had an apparent CA 125 concentration of 35 kilo-ARB units/L, whereas none of the other 17 samples had apparent CA 125 concentrations exceeding 18 kilo-ARB units/L (data not shown).

Isolation of the B.K. Discrepant Material

Because the activity of B.K.'s sera in the CA 125 IRMA could be neutralized with MAB OC125, we attempted to bind it to MAB OC125 coupled to a solid matrix. The material bound to the MAB OC125 Aff-Gel 10 affinity column very rapidly, and could be eluted with 50 mmol/L diethylamine, with 85% recovery of activity. Less than 10% of the apparent CA 125 activity was in the column flow-through. The molecular mass (870 kDa, Figure 1) of the discrepant material as determined by HPLC suggested that it was an IgM. Therefore, we prepared and used an anti-human IgM affinity column. More than 85% of the B.K. discrepant IgM was bound to this column, and it could be eluted with 50 mmol/L diethylamine, with 80% recovery of activity (data not shown). These experiments were not consistent with the hypothesis that we were observing CA 125 antigen in immune complexes, because these would have been much larger and more heterogeneous than observed for B.K. discrepant activity (Figure 1).

Anti-idiotype Nature of the B.K. Discrepant Immunoglobulin

The immunoaffinity-purified and concentrated IgM from patient B.K. very substantially inhibited binding of radiolabeled MAB OC125 to CA 125 antigen as compared with controls. Concentrations of affinity-purified B.K. discrepant IgM five- and 10-fold that found in B.K. serum (about 4 μg/mL) inhibited binding of MAB OC125 to antigen by 50% and 62%, respectively, as compared with control human polyclonal immunoglobulin at matched concentrations. Moreover, it appeared that B.K.'s IgM could not completely inhibit binding of radiolabeled MAB OC125 to CA 125 antigen (Figure 4A).

The putative anti-idiotype nature of B.K.'s IgM was corroborated by experiments that showed that polyclonal rabbit antisera to CA 125 antigen could neutralize both CA 125 antigen and B.K. IgM activities in the CA 125 IRMA. Increasing concentrations of immune rabbit serum neutralized equivalent activities of CA 125 antigen and B.K. IgM identically, whereas pre-immune serum from this rabbit

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Fig. 1. HPLC elution profile of assayable CA 125 activity in B.K. discrepant sample: absorbance at 214 nm vs CA 125 activity 250 μL of an affinity-purified fraction from B.K.'s serum containing 400 kilo-ARB units of CA 125 activity per liter was applied to a TSK 4000 HPLC column, and 0.5-mL fractions (0.5 min per fraction) were collected for assay. The elution time for apparent CA 125 activity is shown relative to the elution times of the voided peak (A), thyroglobulin (B, 670 kDa), IgG (C, 160 kDa), and ovalbumin (D, 45 kDa). CA 125 antigen was eluted with the void volume (A).

Fig. 2. Western blot of serum samples run on a 3% to 12% gradient of sodium dodecyl sulfate/polyacrylamide gel; CA 125 antigen activity was detected with Bolton–Hunter 125I-labeled MAB OC125. Shown are sera (10 μL per lane) from B.K. (lane 1) three patients with epithelial ovarian carcinoma (lanes 2, 3, 4), and a healthy woman (lane 5), all as measured by CA 125 rax assay. Measured CA 125 activities in these sera were 260, 110, >500, 420, and 18 kilo-ARB units/L, respectively.

Fig. 3. Inhibition of CA 125 antigen activity (—) and B.K. discrepant activity (——) in the CA 125 rax assay by the addition of purified unlabeled OC125 monoclonal antibody of increasing concentrations. We assayed 100 μL of a sample containing 250 kilo-ARB units of CA 125 per liter, prepared from rax kit standard or patient B.K.'s serum, using the unmodified CA 125 rax in the presence of 0 to 200 ng of OC125 per assay. Points represent the mean and standard error of the mean of duplicate determinations.
inhibited only if high concentrations of the serum were used (Figure 4B).

Effect of Radiolabeling Method on Discrepant Activity

During a recent study of the effect of different radiolabeling techniques upon the performance characteristics of the CA 125 immunoassay, we noted that the method of radiolabeling the MAb OC125 had profound effects upon the apparent CA 125 concentration in B.K. discrepant serum. A serum sample from B.K. that measured about 260 kilo-ARB. units/L in the CA 125 immunoassay involving MAb OC125 IgG or F(ab')2 radiolabeled by the Bolton–Hunter method measured only 30 kilo-ARB. units/L when tracers labeled with Iodogen were used (Table 1). The B.K. sample did not give discrepant results when other Bolton–Hunter-labeled IgG1s were substituted as tracer in the CA 125 immunoassay (data not shown).

To investigate the generality of this phenomenon, we tested sera from 11 women with apparently increased CA 125, selected from a prescreened group of 1082 normal females over 40 years of age, using the Iodogen-radiolabeled OC125 tracer. Serum from at least three of the six women in this group demonstrated the same behavior as that from subject B.K.; i.e., they were positive in the CA 125 immunoassay in which Bolton–Hunter-labeled OC125 IgG and F(ab')2 tracer were used, but negative with Iodogen tracer (Table 1). There are also some significant differences in CA 125 concentrations between the Bolton–Hunter-labeled IgG and F(ab')2 tracers. To determine the effect of radiolabeling techniques upon CA 125 concentrations in true positives, we did similar studies with serum samples from 20 patients with ovarian cancer. Bolton–Hunter- and Iodogen-labeled tracers gave very similar results in the CA 125 immunoassay (Table 2).

Discussion

There are at least three hypotheses, not necessarily mutually exclusive, that could explain the B.K. discrepant result: (a) the presence of a human anti-mouse antibody, or rheumatoid factor-like crosslinking MAb OC125 on the solid phase to MAb radiolabeled OC125 tracer; (b) the presence of other soluble factors that might interact nonspecifically with mouse immunoglobulin MAb OC125; and (c) expression of the CA 125 antigenic epitope on a molecule biochemically very different from CA 125 antigen. Previous investigations such as that of Schröff et al. (15) and Courtenay-Luck et al. (16) found significant concentrations of human anti-mouse IgG Fc in sera of normal individuals. For this reason, nonspecific murine immunoglobulins are in-

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Table 1. Effect of Radiolabeling Method on CA 125 Measured in Patient B.K.'s Discrepant Serum and 11 Other Sera

<table>
<thead>
<tr>
<th>Sample</th>
<th>Diagnosis</th>
<th>Serum CA 125 value, kilo-ARB. units/L</th>
<th>Bolton-Hunter tracer</th>
<th>Iodogen tracer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>IgG</td>
<td>F(ab')2 IgG</td>
</tr>
<tr>
<td>B.K.</td>
<td>Healthy</td>
<td>360.0</td>
<td>221.5</td>
<td>30</td>
</tr>
<tr>
<td>83-3</td>
<td>Healthy</td>
<td>163.8</td>
<td>185.1</td>
<td>15.2</td>
</tr>
<tr>
<td>52-10</td>
<td>Healthy</td>
<td>93.0</td>
<td>69.8</td>
<td>26.7</td>
</tr>
<tr>
<td>68-07</td>
<td>Healthy</td>
<td>101.2</td>
<td>105.6</td>
<td>102.4</td>
</tr>
<tr>
<td>68-05</td>
<td>Healthy</td>
<td>198.1</td>
<td>259.5</td>
<td>238.7</td>
</tr>
<tr>
<td>10-03</td>
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<td>77.6</td>
<td>153.3</td>
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<tr>
<td>29-9</td>
<td>Healthy</td>
<td>79.5</td>
<td>85.0</td>
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<tr>
<td>72-9</td>
<td>Endometriosis</td>
<td>100.3</td>
<td>61.8</td>
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<td>64-08</td>
<td>Uterine myoma</td>
<td>125.3</td>
<td>88.3</td>
<td>148.3</td>
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<tr>
<td>65-06</td>
<td>Menorrhagia</td>
<td>88.5</td>
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<td>83.3</td>
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<tr>
<td>42-09</td>
<td>Ulcerative colitis</td>
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<td>391.2</td>
<td>256.6</td>
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<tr>
<td>70-09</td>
<td>Hernorrhoids</td>
<td>99.4</td>
<td>100.1</td>
<td>95.0</td>
</tr>
</tbody>
</table>

* Samples from women over 40 years of age with apparently increased CA 125 (CA 125 > 50 kilo-ARB. units/L). These 11 samples represented 1% of the screened population (n = 1082).

**Diagnoses made from medical histories and pelvic examinations at time of sampling. Menstrual status not recorded.

*“False-positive” of the B.K-type.

*Shown at least one previous or subsequent increase (three- to six-month sampling intervals)

Table 2. Effect of Radiolabeling Method on CA 125 Measurement in Serum Samples from 20 Patients with Ovarian Carcinoma

<table>
<thead>
<tr>
<th>Bolton-Hunter tracer</th>
<th>Iodogen tracer</th>
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<tr>
<td>IgG</td>
<td>IgG</td>
</tr>
<tr>
<td>Serum CA 125, kilo-ARB. units/L</td>
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<tr>
<td>55.0</td>
<td>56.0</td>
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<tr>
<td>84.5</td>
<td>78.5</td>
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<td>38.8</td>
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<td>11200.0</td>
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<td>4137.0</td>
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<td>448.2</td>
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<td>2371.0</td>
<td>2257.0</td>
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<td>30.6</td>
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<td>14520.0</td>
<td>4042.0</td>
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<td>402.7</td>
</tr>
<tr>
<td>738.3</td>
<td>748.4</td>
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</table>
cluded in the CA 125 IRMA and most other monoclonal antibody assays, and have been shown (2, 17) usually to block the activity of anti-murine immunoglobulin and polyclonal RF in human serum. In previous studies, however, no naturally occurring human antibody entirely specific for the Fab or F(ab')2 portion of a murine monoclonal antibody was found in normal individuals. Anti-id antibodies to tumor-associated antigen murine MAb have been reported, but only in patients who were actively immunized by injection of the antibody (18).

We have found that in at least one case the discrepant or false-positive activity in the CA 125 IRMA was ascribable to the presence of a highly specific component of human serum that simultaneously bound to murine MAB OC125 on the solid phase and to radioiodinated MAB OC125 tracer. Many facts support the hypothesis that this component is a human IgM that binds to a MAB OC125 idiotyp: (a) it is biochemically unlike CA 125 antigen; (b) its relative molecular mass and reactivity with specific polyclonal antibodies imply that it is a human IgM; (c) it inhibits the binding of MAB OC125 to CA 125 antigen; (d) its binding to MAB OC125 is inhibited by polyclonal rabbit antiserum to CA 125 antigen; and (e) it reacts only with OC125 IgG and F(ab')2, but this reactivity is decreased after a specific chemical modification of MAB OC125.

Jerne (18) has proposed that the immune system expresses a network of idiotypic and anti-idiotypic antibodies that are involved in the regulation of immune response. As a consequence, an antibody that is complementary to an antigen may also find its complement in an anti-idiotypic antibody. The latter might then possess an "internal image" of the antigen; i.e., an antigen and anti-id antibody may share related epitopes (19). The CA 125 antigen and putative anti-id antibody (B.K.'s IgM) may therefore share a related epitope recognized by MAB OC125. This hypothesis was supported by studies with rabbit antiserum to CA 125 antigen; polyclonal antibodies to CA 125 antigen competitively inhibited binding of MAB OC125 to both B.K.'s IgM and CA 125 antigen.

Radioiodination of MAB OC125 with Iodogen instead of Bolton–Hunter reagent in some way altered part of the structure of MAB OC125 and decreased the affinity of interaction with the B.K.-type human IgM. The only apparent difference between the radiolabeling methods we used in the present study is that the Bolton–Hunter reagent reacts with lysine residues whereas Iodogen oxidatively couples iodo to tyrosine residues. Dickerman et al. (20) have shown that chemical modification of a single tyrosyl residue of an individual idiotyp on an murine MAB by diazotization can lead to complete loss of reactivity with another MAB recognizing the unmodified idiotyp. Because Iodogen labeling of MAB OC125 tracer results in modified tyrosyl residues, our finding that B.K.'s discrepant IgM did not react with this modified tracer may have a similar explanation. The Iodogen procedure may also oxidatively modify other parts of the MAB OC125 molecule. Modification of MAB OC125 by Iodogen, however, did not affect MAB OC125 reactivity with the CA 125 antigenic determinant and, therefore, the related epitopes shared by CA 125 antigen and B.K.'s discrepant IgM may not be identical. The results are also consistent with the hypothesis that modification of the MAB OC125 idiotyp by Bolton–Hunter reagent increased its affinity of interaction with B.K.-type discrepant IgM relative to that with unlabeled or Iodogen-labeled MAB OC125.

While the relevance of the above results to the phenomenon of false positives in general is as yet uncertain, preliminary experiments with 11 serum samples with apparently increased CA 125 concentrations identified upon screening 1082 women suggested that serum from at least three women (i.e., 0.3% of the screened population) demonstrated B.K.-like behavior. Elimination of this type of false positive may increase the specificity of the current test in healthy women, particularly if the increases in CA 125 that accompany menstruation (7) are taken into account. Preliminary results with sera from 20 patients with ovarian cancer ("true positives") indicated that B.K.-type immunoglobulins are not commonly present in these patients. Consequently, human antibodies to MAB OC125 are not likely to interfere with the clinical usefulness of the CA 125 IRMA in women already diagnosed with ovarian cancer. Use of an Iodogen-labeled MAB OC125 tracer or OC125 tracers modified by similar or different chemical methods may markedly improve the diagnostic screening potential of the CA 125 IRMA.

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References


