

Evidence for Interaction of Human Anti-Idiotypic Antibodies with CA 125 Determination in a Patient after RadioimmunodetectionJochen Reinsberg,¹ Andreas Heydeweller,² Uwe Wagner,¹ Kathrin Pfell,² Peter Oehr,² and Dieter Krebs¹

Very high concentrations of CA 125 have been found in some ovarian cancer patients after repeated radioimmunodetection with anti-CA 125 antibodies [OC125-F(ab')₂]. In one patient we measured a CA 125 concentration of 135 000 kilo-arb. units/L, using an enzyme immunoassay involving OC125 antibodies. With an immunoradiometric assay involving use of two new anti-CA 125 antibodies (B43.13 and B27.1), the CA 125 concentration was 34 kilo-arb. units/L, indicating a discrepancy. The component responsible for the high result in the enzyme immunoassay could be purified by immunoaffinity chromatography on Protein A-Sepharose. Furthermore this component bound to anti-human IgG-Sepharose in the same manner as did the serum IgG fraction. Adsorption of human anti-mouse antibodies present in the serum did not decrease the CA-125-like material. Binding of whole OC125 antibodies to the purified CA-125-like material was inhibited completely in the presence of CA 125 antigen. We infer that the false-positive CA 125 activity is ascribable to a human IgG directed against an idiotope of the OC125 antibody, which was induced by repeated application of OC125 antibodies. To avoid falsely positive results in patients receiving OC125 antibodies, CA 125 should be measured by an assay in which other antibodies are used.

CA 125, a high-molecular-mass glycoprotein antigenic determinant, is expressed in more than 80% of nonmucinous ovarian adenocarcinomas (1). Bast et al. (2, 3) developed a murine monoclonal antibody (OC125) that can be used to measure CA 125 immunometrically in serum. The OC125 antibody also is used for radioimmunodetection in vivo. Radioimmunodetection in combination with the monitoring of CA 125 in serum seems to be a sensitive diagnostic tool for early detection of recurrence (4).

One side effect of in vivo administration of murine antibodies is the appearance of human antibodies to the applied murine immunoglobulin (5-9). Besides possible in vivo complications, such antibodies can interfere with assay of circulating antigen (5, 10).

In a previous investigation we found in some ovarian cancer patients, after radioimmunodetection, very high concentrations of CA 125 in serum but no appropriate clinical signs of disease (4). As reported here, we examined serum samples from one of these patients for interfering components responsible for the high concentration of CA 125. Our results suggest that human anti-idiotypic anti-

bodies in the patient's serum interfere with the routinely used CA 125 immunoassay, mimicking a redundant increase in CA 125.

Case Report

In April 1986, a 61-year-old white woman was referred to our hospital, after a peritoneal carcinomatosis was diagnosed laparoscopically in an outer hospital. We performed a laparotomy including tumor reduction. The histological examination confirmed a solid, papillary ovarian adenocarcinoma at stage III, according to the FIGO-system (11). After surgery the patient underwent chemotherapy consisting of two courses of mitomycin (12 mg/m²) intraperitoneally, followed by three courses of etoposide (150 mg/m² daily for three days) intravenously and another six courses with oral application (300 mg/m² daily for three days) at monthly intervals. CA 125 serum concentrations measured with the Abbott CA 125 EIA monoclonal decreased from a preoperative value of 283 kilo-arb. units/L into the normal range (11 kilo-arb. units/L) within three months after surgery, reflecting the effect of tumor removal and the disappearance of residual tumor under chemotherapy. In July 1987 the first immunoscintigraphy of the patient was performed, with application of 1 mg of ¹³¹I-labeled F(ab')₂ fragments of the OC125 antibody (Isotopen Diagnostik CIS, Dreieich, F.R.G.). No evidence of disease was found by radioimmunodetection, computed tomography, nuclear magnetic resonance tomography, or clinical examination. Also the CA 125 serum concentration remained within the normal range (12 kilo-arb. units/L). From August 1987 the patient received a low-dose oral cyclophosphamide chemotherapy (100 mg daily). In February 1988 the second immunoscintigraphy was performed, showing suspected findings in the para-aortic and subhepatic region and also in a part of the lung. Because CA 125 concentration was still normal (12 kilo-arb. units/L) and there were no other signs of progression at computed tomography and clinical examination, chemotherapy with cyclophosphamide continued. Four months after second immunoscintigraphy, an elevated CA 125 serum concentration of 1535 kilo-arb. units/L was measured, followed by a further increase up to a maximum value of 135 000 kilo-arb. units/L after the third immunoscintigraphy, performed in August 1988. Despite the increased CA 125 concentration in serum, no evidence of disease could be detected by radioimmunodetection, computed tomography, ultrasonography, or clinical examination. Chemotherapy with cyclophosphamide ended in February 1989. Except for increased CA 125 concentrations in serum, the patient has thus far shown no other signs of disease progression.

Materials and Methods

Serum samples were obtained from the patient six weeks

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after the third immunoscintigraphy. Serum was also sampled from a patient with ovarian cancer metastases, who served as a control.

Determination of CA 125: CA 125 was measured with the Abbott CA 125 EIA monoclonal (Abbott Diagnostic, Wiesbaden-Delkenheim, F.R.G.) and the IRMA Truquant OV2 (Biomira Inc., Edmonton, Alberta, Canada). Both of these are solid-phase immunometric assays for CA 125. In the Abbott CA 125 EIA, the OC125 antibody is used as both the immobilized and the labeled antibody. In contrast, in the Truquant OV2, two newly developed murine monoclonal antibodies (Truquant B43.13 and Truquant B27.1) are used, which recognize separate epitopes near the sites recognized by OC125 antibodies (12).

Determination of human anti-mouse antibodies (HAMA): These were determined with the ImmuSTRIP HAMA-EIA (Immunomedics Inc., Newark, NJ). A microtiter plate coated with mouse IgG is incubated with enzyme-conjugated mouse IgG and the patient's serum. The HAMA molecules bind to both the IgG of the solid phase and the enzyme-conjugated mouse IgG (bridge formation). The amount of HAMA is measured after incubation with enzyme substrate and subsequent termination of the reaction by acidification with sulfuric acid.

Determination of human serum IgG: Human serum IgG was determined by radial immunodiffusion on LC-Partigen IgG plates (Behring, Marburg, F.R.G.).

Affinity chromatography: For affinity chromatography of the serum samples on Protein A-Sepharose, we used the Immopure IgG Purification Kit (Pierce Chemical Co., Rockford, IL) according to the manufacturer's instructions.

For separation of IgG from the serum we also prepared an anti-human IgG affinity column by coupling goat anti-human IgG antibodies (Biogenesis, Bournemouth, England) to CNBr-activated Sepharose 4B (Pharmacia, Freiburg, F.R.G.). One milliliter of Sepharose gel was mixed with 1 mg of antibody in 3 mL of coupling buffer (0.1 mol of bicarbonate and 0.5 mol of sodium chloride per liter, pH 8.3) in an end-over-end mixer for 2 h at room temperature. Then the gel was transferred to 8 mL of blocking buffer (0.2 mol/L glycine, pH 8.0) and incubated for 2 h at room temperature to block the remaining active groups. The gel was then washed alternately five times with acetate buffer (0.1 mol of sodium acetate and 0.5 mol of sodium chloride per liter, pH 4.0) and coupling buffer. For chromatography the gel was packed into a disposable polystyrene column (Pierce).

We applied 0.1 mL of serum samples diluted 10-fold with Tris buffer (0.1 mol of Tris HCl and 0.5 mol of sodium chloride per liter, pH 8.0) to the 1-mL column equilibrated with Tris buffer. After a 10-min incubation at room temperature, the column was washed with 15 mL of Tris buffer to elute the unadsorbed fraction. The bound fraction was then eluted with 0.1 mol/L glycine HCl buffer, pH 2.8, and immediately neutralized with phosphate buffer (1 mol/L, pH 8.0).

To remove the HAMA, we coupled mouse IgG (Biogenesis) to Sepharose B4, 2 mg of IgG per milliliter of gel, as described above. We applied 0.1-mL serum samples, diluted 10-fold with Tris buffer, to 1-mL columns equilibrated with Tris buffer. After 60 min at room temperature the columns were washed with 15 mL of Tris buffer to elute the unadsorbed fraction.

Inhibition of OC125 antibody binding: For characterization of binding of OC125 antibodies to the false-positive

CA-125-like material, microtiter wells were coated with the CA-125-like material (purified by affinity chromatography with Protein A-Sepharose) and diluted with phosphate-buffered saline (PBS; per liter, 10 mmol of phosphate, pH 7.4, and 150 mmol of NaCl) to give a concentration analogous to 800 kilo-*arb.* units of CA 125 per liter (0.05 mL per well). After incubation (18 h, 4 °C), the surface of the wells was blocked by further incubation with a 50 g/L solution of bovine serum albumin in PBS (0.3 mL per well; 2 h, 4 °C). Then the wells were washed three times with 0.3-mL portions of PBS. The immobilized CA-125-like material was incubated (20 h, 4 °C) with 50 μ L of 125 I-labeled whole OC125 antibodies (CA 125 RIA, Abbott) diluted 10-fold with PBS and 25 μ L of increasing concentrations of CA 125 (0–58 000 kilo-*arb.* units/L) in a serum matrix (provided by Baxter Deutschland, Unterschleisheim, F.R.G.). After incubation, the wells were washed three times with 0.3-mL portions of PBS and the bound radioactivity was measured. The nonspecific binding of 125 I-labeled OC125 antibodies, determined in microtiter wells pretreated only with bovine serum albumin, was zero.

Results

Serum from the patient, obtained after repeated immunoscintigraphy, was measured with the two distinct CA 125 immunometric assays involving different monoclonal antibodies, to establish whether it indeed contained high concentrations of CA 125 or was a false-positive specimen. With the CA 125 EIA an apparent CA 125 concentration of 135 000 kilo-*arb.* units/L was measured, whereas with the Truquant OV2 immunoradiometric assay the result was only 35 kilo-*arb.* units/L, indicating false-positive CA 125 values in the EIA. In the control serum, the measured CA 125 concentrations were more consistent: 12 090 and 13 740 kilo-*arb.* units/L by the CA 125 EIA and the Truquant assay, respectively.

The falsely positive CA-125-like material could be purified by affinity chromatography with Protein A-Sepharose. It was quantitatively bound by the Protein A-Sepharose column and was accounted for in the subsequent eluate. In contrast, about 52% of the CA 125 activity of the control sample passed directly through the column, and no CA 125 activity was found in the subsequent eluate.

The CA-125-like material also was bound by anti-human-IgG Sepharose. All CA-125-like activity was adsorbed, but only 32% of the applied CA-125-like material could be accounted for in the subsequent eluate. Similarly, serum IgG was also adsorbed by the anti-human IgG-Sepharose, and 38% of the applied IgG could be accounted for in the eluate. The CA 125 activity of the control serum passed through the column completely, whereas all the serum IgG was absorbed (Table 1).

Because false-positive results in immunometric assays can be due to HAMAs present in the tested specimen, we determined the HAMA titer in serum from the patient. Before the chromatographic treatment, we measured an increased HAMA titer of 7.65 mg/L. After chromatography on a column containing immobilized mouse IgG, the HAMA titer was not detectable, but the CA-125-like activity decreased only slightly.

To verify putative anti-idiotypic nature of the CA-125-like material, we studied the binding of the purified CA-125-like material to whole OC125 antibodies in the presence of increasing concentrations of CA 125. The binding of 125 I-labeled OC125 antibodies to the CA-125-like material

Table 1. CA-125-like Activity^a and Serum IgG Accounted for in the Fractions of Anti-Human IgG Affinity Chromatography

	Control		Patient	
	CA 125, arb. units	IgG, μ g	CA 125, arb. units	IgG, μ g
Applied to column	128	123	1230	129
Wash fraction	134	n.d.	n.d.	n.d.
Recovery, %	105	—	—	—
Euate	n.d.	71	396	50
Recovery, %	—	58	32	38

^a Determined with the CA-125 EIA.
n.d., none detected.

coated on microtiter wells was inhibited by CA 125 antigen in a concentration-dependent manner. At 19 000 kilo-arb. units/L, CA 125 binding was inhibited completely (Figure 1).

Discussion

The data presented confirm that the apparent high concentration of CA 125 measured in the serum of this patient after repeated immunoscintigraphy was not real. The repeated immunoscintigraphy seems to have produced some agent that interferes with the CA 125 determination with OC125 antibodies, producing a falsely high result with the CA 125 EIA but not with the Truquant OV2 immunoradiometric assay. Thus one could suppose that the CA-125-like material binds to the OC125 antibody but not to the B43.13 or the B27.1 antibodies. Krantz et al. (12) showed that monoclonal antibodies B43.13 and B27.1 inhibit the binding of OC125 antibodies to the CA 125 antigen and concluded that B43.13 and B27.1 recognize sites sterically near the sites recognized by OC125. Furthermore, co-expression of the epitopes recognized by B43.13, B27.1, and OC125 seems to be conserved on mucins secreted by different individual cancer patients. So the lack of a high value with the Truquant OV2 assay indicates that the agent that caused the positive result in the CA 125 EIA is not actually CA 125.

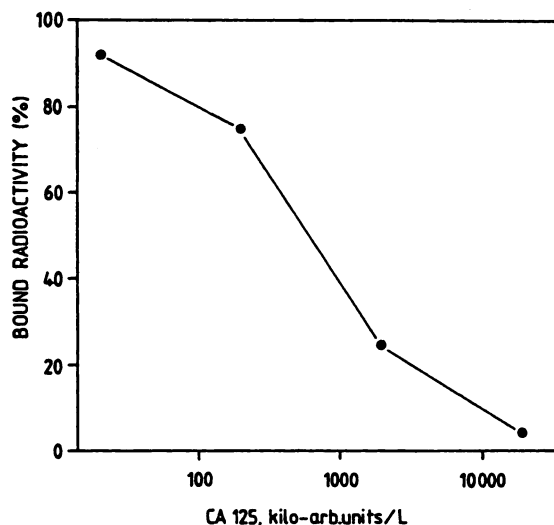


Fig. 1. Inhibition of binding of ¹²⁵I-labeled OC125 antibodies to CA-125-like material isolated from serum of the patient by increasing CA 125 concentrations

The component responsible for the false-positive result is absorbed by Protein A, a bacterial cell-wall protein that specifically binds to the Fc portion of human IgG. Furthermore, it also binds to anti-human-IgG antibodies in the same manner as the serum IgG fraction. These results suggest that the CA-125-like material is a human immunoglobulin G induced by injection of OC125 antibodies.

Appearance of human anti-mouse antibodies after repeated administration of murine antibodies has been reported in several studies (5-9). Two types of antibodies have been identified: nonspecific human anti-mouse antibodies, which reacted with all mouse IgG (HAMA), and anti-idiotypic antibodies, which were directed against the hypervariable region of the applied antibody.

Interference by HAMAs, which cross-link labeled antibody with the antibody on the solid phase, has been reported for several immunometric assays (5, 10, 13-16). Although we found a high concentration of HAMAs in the patient's serum, only a small part of the false-positive value studied here can be ascribed to interference by HAMAs: after HAMAs were removed by affinity chromatography on a column containing mouse IgG, the apparent CA 125 concentration was only slightly smaller—presumably because the CA 125 EIA we used in this study, as with most immunoassays, included nonspecific murine immunoglobulins to block the activity of HAMAs (10, 17).

Interferences by anti-idiotypic antibodies also have been reported for the CA 125 immunoradiometric assay involving the OC125 antibody (18). Because, in the assay, the OC125 antibodies are used as both the immobilized and the labeled antibody, the anti-idiotypic antibodies binding to an idiotope of the OC125 can cross-link both antibodies, resulting in falsely high values for CA 125. The hypothesis that the CA-125-like immunoglobulins studied here are anti-idiotypic antibodies is supported by the following: the CA-125-like immunoglobulins reacted only with monoclonal murine OC125 antibodies but not with the monoclonal murine antibodies used in the Truquant OV2 assay. These data indicate that the specificity of the CA-125-like immunoglobulins is not directed against determinants common to mouse immunoglobulins. Furthermore, the binding of the CA-125-like immunoglobulins to OC125 antibodies could be completely inhibited by the antigen CA 125. According to Jerne et al. (19), anti-idiotypic antibodies might carry an internal image of the antigen that the first antibody is directed against, and so might mimic the presence of the antigen. Therefore, inhibition of binding of CA-125-like immunoglobulins to OC125 antibodies by the CA 125 antigen can be interpreted as a competition between the CA 125 antigen and an anti-idiotypic antibody with an internal image of the CA 125 determinant for the CA 125 binding sites on the OC125 antibodies.

Our results suggest that the false-positive CA 125 values measured in the serum of this patient after repeated application of OC125 antibodies are attributable to human anti-idiotypic IgG that can cross-link OC125 antibodies used in most commercially available CA 125 test kits. Monitoring of CA 125 in patients who are receiving OC125 antibodies therefore should be performed with an assay that involves other antibodies.

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