Cancer Immunol Immunother (1998) 46: 201–212

ORIGINAL ARTICLE

Birgit C. Schultes · Richard P. Baum · Andreas Niesen
Antoine A. Noujaim · Ragupathy Madiyalakan

Anti-idiotype induction therapy: anti-CA125 antibodies (Ab_3) mediated tumor killing in patients treated with Ovarex mAb B43.13 (Ab_1)

Received: 14 October 1997 / Accepted: 9 January 1998

Abstract Intravenous injection of the murine monoclonal anti-CA125 antibody B43.13 (Ovarex: Ab_1) into ovarian cancer patients led to the induction of an idiotypic network. Of the 75 patients who received one to ten injections of a 2-mg dose of the antibody, 48 developed anti-(mAb B43.13) antibodies (Ab_2); 18 of these patients also had elevated levels of anti-[anti-(mAb B43.13)] antibodies (Ab_3; = anti-CA125 antibodies) compared to pre-injection values. Characterization of these antibodies revealed that the binding to CA125 could be inhibited by mAb B43.13 in most samples. Human anti-CA125 antibodies or Ab_3 purified from patient serum samples specifically recognized human ovarian tumor cells and tissues expressing CA125. In addition, these anti-CA125 antibodies were able to conduct F_c-mediated tumor cell killing (antibody-dependent cell-mediated cytotoxicity). This raises the possibility of using an Ab_1 for anti-idiotype induction immunotherapy of cancer.

Key words Anti-CA125 antibody · mAb B43.13 · Immunotherapy · Anti-idiotypic network · ADCC · Immune histochemistry

Introduction

Murine monoclonal antibodies (mAb) directed against tumor-associated antigens have been used for therapy of cancer in various protocols. Most clinical experience has been obtained with non-conjugated mAb [10, 20, 21, 28, 35, 45]. Various direct antitumor effector functions, such as antibody-dependent cell-mediated cytotoxicity (ADCC) [1, 38], complement-dependent cytotoxicity (CDC) [19] and apoptosis [48], have been attributed to the clinical outcome.

In addition to the direct effects, the injected antibody may also function as a vaccine. According to the Network hypothesis proposed by Niels Jerne [22], antibodies can elicit anti-idiotypic antibodies (Ab_2). Ab_2, in turn, can serve as a surrogate antigen to induce anti-(anti-idiotypic) antibodies (Ab_3), which recognize the original antigen of the Ab_1. Activation of Ab_3 has been correlated with cancer regression in animal models and cancer patients [46, 14, 15, 50, 39].

The monoclonal antibody B43.13 recognizes the human cancer-associated antigen CA125, an important target for immunotherapy of ovarian carcinomas [25, 37, 30]. mAb B43.13 was initially used for radio-immunodetection of recurrent ovarian carcinoma [37]. A retrospective study showed unexpected prolonged survival in some patients injected with mAb B43.13 [2, 3]. The improved clinical outcome was attributed to induction of the anti-idiotypic network by this Ab_1. Recent studies, further investigating this approach of idiotype induction immunotherapy for ovarian cancer, showed activation of both a humoral and a cellular CA125-specific response [2, 30]. mAb B43.13 forms the active ingredient of Ovarex, which is currently being investigated in phase II/III clinical trials in ovarian cancer patients.

In the present study, we analyzed sera of patients injected with mAb B43.13 for the presence of human anti-CA125 antibodies and their importance in killing ovarian carcinoma cells.

Materials and methods

Patients

Seventy-five patients with ovarian cancer (classified as FIGO I–IV, see Table 1) were injected i.v. one to ten times with 2 mg murine monoclonal antibody mAb B43.13, labeled with ^{99m}Tc (Ovarex) for immunoscintigraphic purposes at the Department for Nuclear Medicine at the Johann-Wolfgang-Goethe University in Frankfurt. Serum sam-

B.C. Schultes · A.A. Noujaim · R. Madiyalakan (✉)
AltaRex Corp., 300 Campus Tower, 8625 112 St. Edmonton, Alberta, T6G 1K8, Canada
Fax +1 403 436 0068

R.P. Baum · A. Niesen
Nuklearmedizin, Universitätsklinikum, Frankfurt, Germany

ples were drawn before and at various times after injection and stored at –80 °C until analysis.

mAb B43.13

mAb B43.13 was produced in ascites and purified under current good manufacturing practices. The antibody was reduced under UV light and prepared for radio-labeling with ^{99m}Tc as described previously [47].

Generation of chimeric mAb B43.13

The constant region of mAb B43.13 has been replaced by a human γ3 region by recombinant DNA techniques. The sequences of the light- and heavy-chain variable regions were obtained from the mRNA of the hybridoma, and cDNA of the variable regions was generated. The κ variable domain has been cloned into the κ KS+ shuttle vector. Following two rounds of site-directed mutagenesis, a good mutant has been cloned into the pSV2 expression vector for transfection. The γ variable domain was generated by the polymerase chain reaction (PCR) and cloned into the γ KS+ shuttle vector. Site-directed mutagenesis was performed, the mutants sequenced and a good ELVγ cloned into the expression vector. The two expression vectors were used to transfect the myeloma cell line NS-1 and screened for transfectoma secreting chimeric mAb B43.13 antibodies.

Generation of single-chain mAb B43.13 (scFv mAb B43.13)

The mAb B43.13 variable domain sequences were PCR-amplified using sequence-specific primers, and engineered into a cloning vector with a scFv orientation of V_l-linker-V_h. The DNA fragment coding for the scFv was then subcloned into a *Pichia pastoris* vector, pPIC-9, with αF secretion signals, resulting in the recombinant plasmid pPIC-B43.13 [29].

Generation of mAb BR11.1

The anti-idiotypic antibody BR11.1 against mAb B43.13 was generated by immunizing BALB/c mice with keyhole-limpet-hemocyanin-conjucated B43.13-F(ab′)$_2$ in the RIBI adjuvant system (RIBI ImmunoChem Research Inc.). Initial screening was performed on B43.13-F(ab′)$_2$-coated enzyme-linked immunosorbent assay (ELISA) plates. Promising clones were tested for inhibition of the binding to mAb B43.13 by CA125 and for the failure to bind to other mouse IgG1 antibodies. The clone BR11.1 gave the best results and was further characterized by generating Ab_3 in rats, which were able to bind CA125 and BR11.1 [44].

Cell culture

Ovarian cancer cells (NIH:OVCAR-3, Caov-4, SK-OV-3) were obtained from ATCC (Rockville, USA). OVCAR-NU3 cells were obtained from NIH:OVCAR-3 cells, passaged three times through nude-mouse tumors. All cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 1% insulin/transferrin/selenine and 5 ng/ml gentamicin (all from Gibco) at 7% CO_2 and 37 °C.

CA125 production

NIH:OVCAR-3 and Caov-4 cells were grown in triple-layer flasks (Nunc) in complete medium until confluent. Medium was removed, the cells were washed with 50 ml RPMI-1640 medium and supplemented with 100 ml RPMI-1640/flask. Cell supernatants were collected weekly until massive cell death started (after approximately 4 weeks). Supernatants were pooled and stored at –25 °C until purification. Batches of 2 l were purified by ammonium sulfate precipitation (30%–75% saturation) and gel filtration on Sephacryl S-500 HR. CA125-containing fractions (void volume) were pooled, concentrated 20-fold through an Amicon stir cell (YM-30 membrane) and frozen in aliquots at –80 °C. The antigen preparation was further purified by chromatography on wheat-germ-lectin-Sepharose (Sigma). Concentrated fractions from the gel filtration column were loaded in phosphate-buffered saline (PBS). CA125 was eluted with *N*-acetylglucosamine (200 mg/ml). CA125-containing fractions were pooled and dialyzed against PBS. Aliquots were stored frozen at –80 °C. The purity of the CA125 samples was checked by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS-PAGE), Western blotting and immune blotting after isoelectric focussing [43].

Detection of human anti-(mouse Ig) antibodies

Serum samples from patients before and after injection of mAb B43.13 were analyzed in the TruQuant human anti-(mouse Ig) antibody (HAMA) radioimmunoassay (Biomira Diagnostics Inc.) and the HAMA ELISA from Medac according to the manufacturer's instructions.

Anti-idiotype assay (Ab_2)

Antibody concentrations were determined in an ELISA using mAb B43.13 chimeric antibody as coating agent (1.5 µg/ml, 100 µg/well) for Maxisorp plates (Nunc). Plates were blocked with 3% bovine serum albumin/2% sucrose in PBS for 30 min at room temperature (250 µl/well). Positive controls (Ab_2-positive serum pool) and serum samples were diluted 1/25 with newborn calf serum/1% normal mouse serum (diluent). Standards were prepared from a monoclonal anti-idiotypic antibody against mAb B43.13 (BR11.1) in the range 2.5–100 ng/ml. Standards, positive controls and samples (100 µl/well) were incubated for 1 h at room temperature under agitation. Plates were washed three times with PBS/0.1% Tween-20 and incubated with mAb B43.13 (0.2 µg/ml, 100 µl/well) for 1 h at room temperature. After three washes, horseradish-peroxidase (HRP)-conjugated goat anti-(mouse IgG1) (Southern Biotechnology Associates) was incubated for 1 h at room temperature under agitation. Wells were washed again as described and 2,2′-azino-bis-(3-ethylbenzthiazolinesulfonate (ABTS) substrate (Kierkegaard) was added for approximately 30 min. Color development was measured in an ELISA reader at 405/492 nm. Samples exceeding the range of the standard curve were re-assayed at a higher dilution.

Anti-CA125 assay

Nunclone microtiter plates (Nunc) were coated with purified CA125 (ammonium sulfate precipitation and gel filtration) at 4000 U/ml in PBS overnight at 4 °C (100 µl/well). Plates were blocked with 3% BSA/2% sucrose in PBS for 30 min at room temperature (250 µl/well). Serum samples were diluted 1/25 with newborn calf serum/1% normal mouse serum (diluent). Standards were prepared from chimeric B43.13 in the range 1.25–50 ng/ml. A positive control was obtained from a serum pool with high anti-CA125 antibody concentrations. Standards, control and samples (100 µl/well) were incubated for 2 h at room temperature under agitation. Plates were washed three times with PBS/0.1% Tween-20 and incubated with HRP-conjugated goat anti-(human IgG) (Southern Biotechnology Associates) for 1 h at room temperature under agitation. Wells were washed again as described and ABTS substrate (Kierkegaard) was added for approximately 30 min. Color development was measured in an ELISA reader at 405/492 nm. Samples exceeding the range of the standard curve were further diluted and assayed again. Positive samples were re-tested on plates coated with the CA125 purified on wheat-germ-lectin-Sepharose.

Table 1 Distribution of Ovarex injections administered to patients

No. injections	No. patients
1	29
2	22
3–5	42
>5	7

Rabbit Ab_2 assay for measurement of Ab_3

Anti-idiotypic antibodies against mAb B43.13 were generated in rabbits by subcutaneous immunization with mAb B43.13 in the RIBI adjuvant system (RIBI ImmunoChem Research Inc.). Serum from rabbits with positive concentrations was purified on protein A and AB_x anion-exchange HPLC. Isotypic rabbit anti-(mouse Ig) antibodies were removed by affinity chromatography on a non-specific mouse IgG1 column. The purified antibody was used to coat microtiter plates (Nunc, Maxisorp) at 2.5 µg/ml in PBS overnight at 4 °C (100 µl/well). Plates were blocked with 3% BSA/2% sucrose in PBS for 30 min at room temperature (250 µl/well). Ab_3-positive patient serum samples were diluted 1/25 with newborn calf serum/1% normal mouse serum (diluent). Standards were prepared from chimeric B43.13 in the range 1.25–50 ng/ml. Standards and samples (100 µl/well) were incubated for 2 h at room temperature under agitation. Plates were washed three times with PBS/0.1% Tween-20 and traced for bound human immunoglobulins as described in the anti-CA125 assay. Anti-idiotypic antibodies from rabbit serum instead of the murine monoclonal Ab_2 mAb BR11.1 were used for this assay in order to avoid cross-reactivity of HAMA.

Anti-CA125 inhibition assay

The assay was performed as described for the anti-CA125 assay. Serum samples were diluted 1/12.5, and 50 µl/well sample was incubated in the presence of 50 µl/well diluent or mAb B43.13 single chain in diluent (1 µg/ml) or CA125 (from wheat-germ-lectin-Sepharose purification) in diluent (10 000 U/ml). Inhibition was calculated in relation to the signal obtained with diluent only.

Purification of anti-CA125 antibodies from human serum

Anti-CA125 antibodies were purified by protein-A-Sepharose (Pharmacia) from sera containing high levels of these antibodies. Samples of 1–2 ml serum were applied to a 1-ml protein-I a column. The column was washed with 5 ml PBS and bound immunoglobulins were eluted with 0.1 M glycine/HCl, pH 3.0. The eluted antibodies were tested by Western blotting for their specificity to CA125. Samples bound to high-molecular-mass components (above 200 kDa) of the purified CA125, identical to chimeric mAb B43.13 (data not shown).

Histology

CA125-positive (NIH:OVCAR-3, Caov-4) and negative (SK-OV-3) tumor cell lines were grown in chambered tissue slides (Nunc) for 2 days and fixed in 0.1% glutaraldehyde/3.7% formaldehyde/PBS. Tumor tissues from NIH:OVCAR-3 tumor cells transplanted in nude mice or ovarian carcinoma from patients were snap-frozen and cut on a cryostat. Transplants from colon carcinoma cells were used as a control. Sections were fixed in 0.1% glutaraldehyde/3.7% formaldehyde/PBS. In all samples, endogenous peroxidase was blocked by incubation with 1.5% H_2O_2/0.1% NaN_3/5% normal human serum for 30 min at room temperature. Sections were incubated with chimeric mAb B43.13 (10 µg/ml), purified human immunoglobulins from patients with high anti-CA125 concentration (5 µg/ml) or normal human serum (10%) for 1 h at room temperature under slow agitation. After washing, bound human antibodies were detected by incubation with rabbit F(ab′)$_2$ anti-(human IgG), conjugated to HRP, for 1 h at room temperature. Color development was performed with 1 mg/ml diaminobenzidine in 0.1 M TRIS/HCl, pH 7.4/0.01% H_2O_2 for 5–10 min. Slides were washed in water, counter-stained with hematoxylin and mounted.

Chromium labeling of tumor cells

OVCAR-NU3 cells were harvested by trypsination and washed twice with complete medium. Approximately 1×10^6 cells were resuspended in 100 µl complete medium and 20 µl fetal bovine serum and 100 µCi [^{51}Cr]chromate (100 µl) were added. Cells were incubated at 37 °C for 1 h with agitation every 15 min. Cells were washed twice with 50 ml RPMI-1640 medium and resuspended in complete medium at a concentration of 1×10^5 cells/ml.

CDC

Chromium-labeled tumor cells (1×10^4 cells/well) were incubated with purified immunoglobulins from patients with high anti-CA125 antibody concentrations at concentrations ranging from 0 to 20 µg/ml for

Table 2 Analysis of sera from patients receiving Ovarex mAb-B43.13. Serum samples before and after Ovarex injections were analyzed in the human anti-(mouse Ig) antibody (HAMA), Ab_2 and anti-CA125 assay as described in Materials and methods. Patients who developed positive concentrations above 200, 50 or 200 ng/ml after receiving the Ovarex vaccine were considered to be positive. The cut-off values were determined as the mean + 3 standard deviations (SD), obtained from analysis of 100 normal serum samples. The mean values ± SD, the median, the range and the percentage of positive samples are given. The significance of the difference between pre- and post-injection levels was determined by Student's *t*-test

Parameter	Injection	Content in serum (ng/ml)			No. positive/ total	Positive (%)	*P* (significance pre/post Injection)
		Mean ± SD	Median	Range			
HAMA	Pre	34 ± 36.4	22	0–172	0/78	0.0	
	Post	33 085 ± 92 379	4304	10–588 890	90/100	90.0	0.00134
Ab_2	Pre	5.5 ± 15.3	0	0–67.5	2/48	4.2	
	Post	620 ± 1808	162.5	0–12 000	48/75	64.0	0.00412
Ab_3	Pre	58.3 ± 43.3	50	0–256	1/53	1.9	
	Post	190.8 ± 276.5	120	0–1603	18/75	24.0	0.00008

Table 3 Correlation between Ab_2 and Ab_3 induction. Patients were grouped into Ab_2 responders ($Ab_2 > 50$ ng/ml) and non-responders ($Ab_2 < 50$ ng/ml) and induction of Ab_3 was compared. The mean values ± SD, the percentage of positive samples and the significance of the difference between the two groups (Student's *t*-test) are given

Ab_2 (ng/ml)	Ab_3 mean ± SD (ng/ml)	No. positive/total (%)	Significance *P*
<50	84± 64.5	2/23 (8.9%)	
>50	239±326.25	15/50 (30%)	0.00209

2 h at 37 °C in round-bottom microtiter plates. Cells were spun down for 1 min at 30×*g*, the supernatant was removed, and 200 µl/well RPMI medium containing 15% fresh human serum was added for 4 h at 37 °C. Microtiter plates were centrifuged again for 1 min at 30×*g*, and 150 µl supernatant was removed for [^{51}Cr]chromate counting. The specific lysis was determined according to the formula:

$$\text{Lysis } (\%) = \frac{\text{specific release} - \text{spontaneous release}}{\text{total release} - \text{spontaneous release}} \times 100$$

The spontaneous release was determined by the release of [^{51}Cr]chromate in wells without antibody. The total release was obtained from wells incubated with 0.1% Triton X-100.

ADCC

The experiment was performed as described for CDC. Chromium-labeled tumor cells (1×10^4 cells/well) were incubated with protein-A-purified immunoglobulins from patients with high anti-CA125 antibody concentrations, derived from a serum pool and also from individual patients. Instead of adding 15% fresh human serum, peripheral blood lymphocytes from healthy donors were added at a ratio of 1/25 (tumor/effector cells). Microtiter plates were centrifuged for 1 min at 30×*g* to bring cells into close contact and then incubated for 4 h at 37 °C.

Statistical analysis

Data were analyzed in Student's *t*-test (two-tailed, paired or non-paired, depending on application) to determine the significance of differences (*P* values) between the various groups.

Results

CA125 purification

CA125, produced under protein-free conditions in cell culture, could be purified to more than 95% purity via ammonium sulfate precipitation, gel filtration and chromatography on wheat germ lectin (no contaminants visible in SDS-PAGE with silver stain). CA125 purified by ammonium sulfate precipitation and gel filtration showed six bands in the SDS gel. Both preparations were tested in parallel by Western blot analysis using chimeric mAb B43.13 (positive control) and serum samples from patients with anti-CA125 antibodies. In all cases, only one band above 200 kDa was stained (data not shown). The preparations were also tested to determine whether they could coat microtiter plates. Highly pure CA125 after wheat germ lectin chromatography, and the cruder preparation without the affinity-chromatographic step, coat tissue-culture plates well. Anti-CA125-positive serum samples were tested by ELISA on plates coated with these two preparations and were positive in both assays. Those data show that no cross-reacting impurities are present in the cruder CA125 preparation. Owing to a much higher yield in the production of the antigen, we decided to use the less pure preparation in order to obtain large quantities of pure CA125 for clinical trial sample analysis.

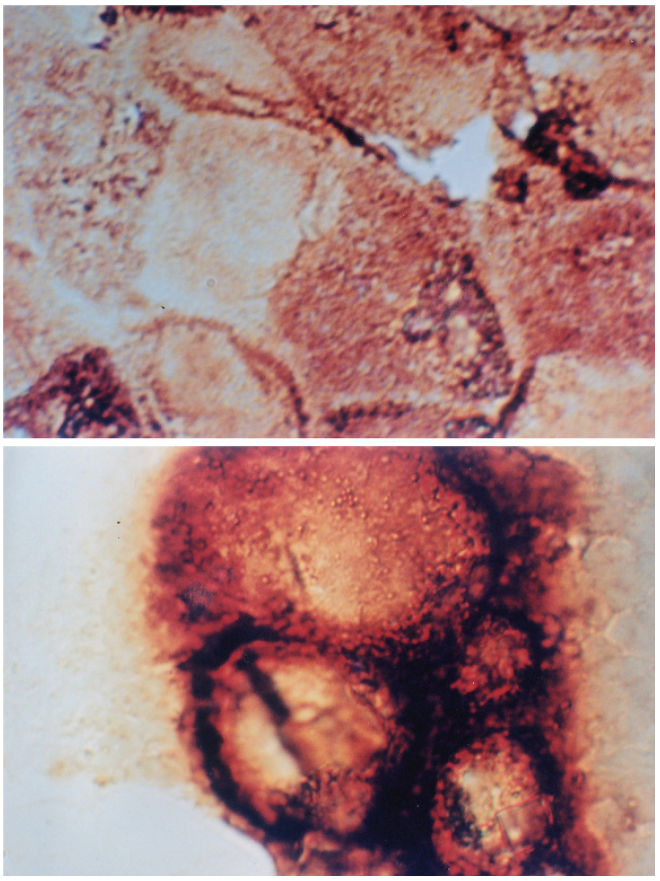

Fig. 1a,b Ab_3 staining of CA125-positive tumor cells. NIH:OVCAR-3 (**a**) and Caov-4 (**b**) cells were grown in chambered tissue slides, fixed and stained with purified Ab_3, horseradish-peroxidase (HRP)-conjugated anti-(human IgG) and diaminobenzidine

Anti-idiotype assay

The normal range of anti-idiotypic antibody levels was tested in 100 healthy blood donors. The cut-off level for the assay was set at 50 ng/ml, which reflects the mean + 3 SD of Ab_2 found in healthy blood donors.

A value for a positive control, prepared from patient sera with positive Ab_2 levels, was determined in 15 replicates. The mean±2 SD range for the control was 320±80 ng/ml. The control had to be within this range for all anti-idiotype assays to be valid. The assay was tested for interference with HAMA or circulating CA125 antigen. The use of

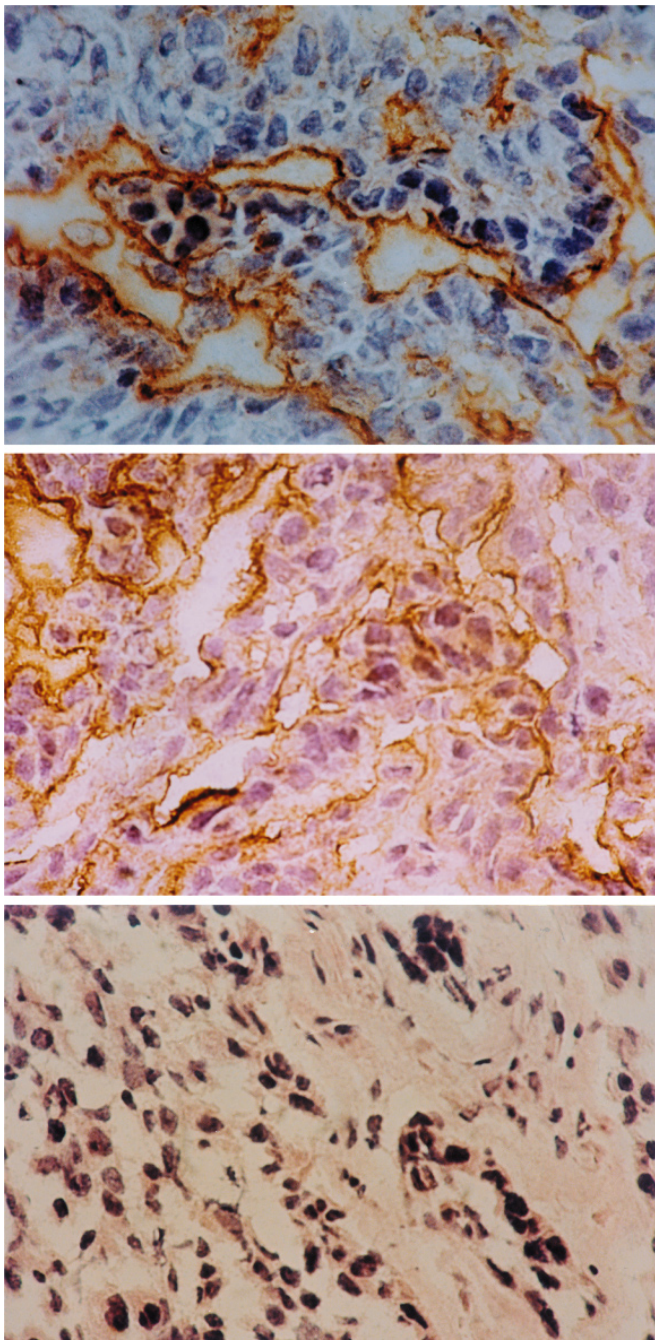

Fig. 2a–c Frozen sections of OVCAR-NU3 tumors from nude mice. The tumor sections were stained with chimeric mAb B43.13 (**a**), purified antibodies from patients with high anti-CA125 antibody concentrations (**b**) and normal human serum (**c**). Binding of antibodies was detected by HRP-conjugated anti-[human Ig (H+L)] and diaminobenzidine. Slides were counter-stained with hematoxylin

chimeric mAb B43.13 on the solid phase and the addition of normal mouse serum to the sample diluent could successfully block any HAMA interference up to 1 mg/ml. There was no binding of a control antibody to the patients' Ab_2 when this was tested during the validation procedure of the assay. Chimeric mAb B43.13 does not bind well to CA125 when coated on a solid surface [30]. Therefore, very high CA125 concentrations ($>100\,000$ U/ml) are necessary to inhibit the binding of Ab_2 to the chimeric antibody completely. A small amount of interference, with inhibition up to 10%, cannot be completely excluded for the CA125 levels present in the patients' samples (up to 2700 U/ml). Nevertheless, this potential interference did not prevent the detection of Ab_2 in those samples.

Anti-CA125 assay

The normal range of anti-CA125 antibody levels was tested in 100 healthy blood donors. Some binding to the coated CA125 was observed in all blood donor samples, independent of the purity of the CA125 used (CA125 with or without purification on wheat-germ-lectin-Sepharose). This interference is probably due to the presence of anti-carbohydrate antibodies in normal serum. The cut-off level for the assay was set at 200 ng/ml, which reflects the mean + 3 SD of anti-CA125 antibodies found in healthy blood donors.

A positive control was prepared from patient sera with positive anti-CA125 levels and assayed in 15 replicates. The mean $\pm$ 2 SD range for the control was 550 ± 65 ng/ml. The control had to be within this range in order for all anti-CA125 assays to be valid. Several samples with high anti-CA125 antibody values were re-tested on plates coated with CA125 purified on wheat-germ-lectin-Sepharose. All samples were positive again, confirming that the CA125 preparation obtained from the gel filtration column does not cause false positive results in this assay. The specificity of the assay was also demonstrated by inhibition with pure CA125 and B43.13 scFv (see Table 4). The presence of circulating CA125 in serum did not prevent the detection of Ab_3 within the range of antigen observed in our patient samples (2741 U/ml maximum). However, some interference was observed at high CA125 levels with an approximately 50% inhibition of Ab_3 at 5000 U/ml CA125 in the serum.

Induction of the idiotypic network in patients after mAb B43.13 injection

Ovarian cancer patients received one to ten injections of ^{99m}Tc-labeled mAb B43.13. Serum samples before and at various times after injection were tested for human anti-(mouse Ig) antibodies (HAMA), anti-idiotypic antibodies against mAb B43.13 (Ab_2) and anti-CA125 antibodies (Ab_3) as described in Materials and methods. Samples from 75 patients could be included for Ab_2 and Ab_3 analysis where results before and after injection were available. Altogether, 90% of the patients developed antibodies against mouse IgG (HAMA) during the follow-up of Ovarex injections. Anti-idiotypic antibodies were shown in 64% of the patients, and 18 patients generated positive concentrations against CA125 after injection of mAb B43.13 (24%) (Table 2). A statistical analysis was performed for comparison of antibody concentrations before and

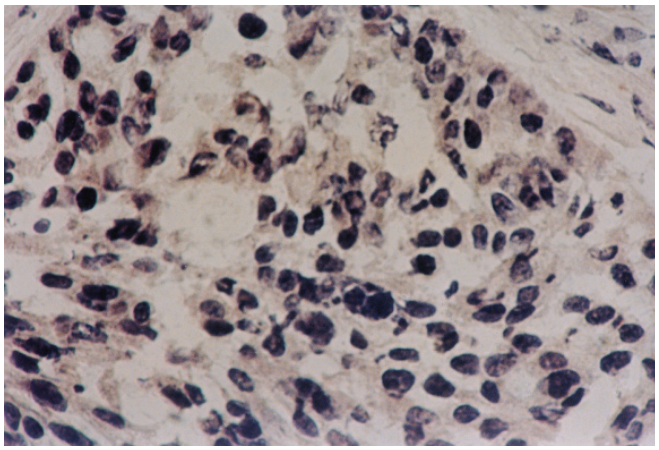

Fig. 3 Frozen sections of Colo205 tumors from nude mice. The tumor sections were stained with purified antibodies from patients with high anti-CA125 antibody concentrations as described in Fig. 2

after injection of mAb B43.13. In all three parameters tested, the two groups were significantly different (P values between 0.00008 and 0.004, Table 2). This confirms the induction of HAMA, Ab_2 and Ab_3 in response to mAb B43.13 injection. It could also be demonstrated that the generation of Ab_3 in these patients was strongly correlated with the presence of Ab_2 ($P = 0.002$) and that induction of the idiotypic network is required for formation of Ab_3 (Table 3).

Histology

Partially purified antibodies from a pool of anti-CA125-positive serum samples were used for histological staining of tumor cells and frozen sections and compared to chimeric mAb B43.13.

The two CA125-positive human ovarian cancer cell lines, NIH:OVCAR-3 and Caov-4, were positive for staining with purified immunoglobulins from patients with high anti-CA125 antibody concentrations (Fig. 1a,b) and gave similar results to those obtained with chimeric mAb B43.13 (data not shown). Human anti-CA125 antibodies did not bind to the CA125 negative cell line SK-OV-3 (data not shown). NIH:OVCAR-3 tumors, grown in nude mice, showed positive staining for chimeric mAb B43.13 and purified immunoglobulins from patients with high anti-CA125 antibody concentrations (Fig. 2a,b). The staining pattern of human anti-CA125 antibodies was similar to the one obtained with chimeric mAb B43.13. Both preparations bound focally to tumor areas. The antigen was strongly present in lumina and also on cell surfaces, but only a little was found within the cells. The polyclonal antibodies derived from patients resulted in somewhat higher background staining than that shown by chimeric mAb B43.13, lacking some of the specificity of the monoclonal antibody. Normal human serum did not produce positive staining of this tumor (Fig. 2c). A colon tumor grown in nude mice (CA125-negative, carcinoembryonic-antigen- and CA19.9-positive) did not reveal any specific binding of human anti-CA125 antibodies (Fig. 3).

Staining of human tumors resulted in a much higher background than immune histochemistry with murine tumors, because of the reaction of the anti-(human IgG) horseradish-peroxidase conjugate with human immunoglobulins present in the tissues and vessels. However, chimeric mAb B43.13 and patients' anti-CA125 antibodies revealed positive areas around the tumor cells, distinguishable from the background, but results were not as clear as with murine tumors (data not shown).

Characterization of human anti-CA125 antibodies

Anti-CA125 antibodies generated through the idiotypic network (Ab_3) should have the same binding properties as the original antibody Ab_1. In order to characterize the nature of anti-CA125 antibodies found in patients' sera after Ovarex injection, serum samples with positive anti-CA125 values were analyzed in an inhibition assay using CA125 (positive control), buffer (negative control) or single-chain mAb B43.13 as inhibitors. The single-chain antibody was chosen to avoid the interference of HAMA, present in various amounts in the serum samples. Chimeric mAb B43.13 served as a positive control for all inhibition assays. The results are summarized in Table 4.

All except two samples from one patient were inhibitable with CA125 antigen, indicating the specificity of the assay. Inhibition with single-chain mAb B43.13 varied from sample to sample but seemed to be similar within a patient. Out of 27 post-injection samples, 23 (10 out of 13 patients) could be inhibited with scFv mAb B43.13. Three patients (4 samples) did not show inhibition with scFv mAb B43.13.

Ab_3-positive samples were also tested for their binding to a polyclonal rabbit Ab_2 preparation. All samples that showed high inhibition with scFv B43.13 also recognized rabbit Ab_2, indicating the presence of true Ab_3. Samples that showed no or low inhibition with scFv B43.13 or had low Ab_3 concentrations in the CA125 assay did not bind to the rabbit Ab_2 (12 out of 27 samples, Table 4).

F_c-mediated tumor cell killing of mAb B43.13 and human anti-CA125 antibodies

To evaluate the possibility that the improved survival rate of patients injected with mAb B43.13 could be attributed to CDC and/or ADCC action of the injected antibody or the human anti-CA125 antibodies formed, chromium-release assays were performed, using mAb B43.13, chimeric mAb B43.13 and Ab_3 as mediators. SW1116 and mAb B25.10 (IgM against CA19.9) were used as a positive control for CDC assays.

The results are demonstrated in Fig. 4. SW1116 cells could be lysed by mAb B25.10 and complement in a concentration-dependent manner (up to 70%). Neither mAb B43.13 nor chimeric mAb B43.13 could lyse the CA125-positive [^{51}Cr]chromate-labeled ovarian carcinoma

Table 4 Characterization of anti-CA125 antibodies in patients injected with Ovarex mAb-B43.13. Anti-CA125-positive samples were tested for inhibition of their binding to CA125 (solid phase) by CA125 (purified on wheat-germ-lectin-Sepharose) or scFv mAb B43.13. Also binding of the samples to rabbit-Ab_2 was analyzed in an enzyme-linked immunosorbent assay. The sample code is composed of the injection number and the time (days) after the last injection (i.e. 1D0 = before the first injection, 4D144 = 144 days after the 4th injection). *ND* not done, *NA* not available, – no inhibition or no binding, + binding

Patient	Sample code	Anti-CA125 Ab levels (ng/ml)	Binding to rabbit Ab_2	Inhibition (%)[a]	
				CA125 (10000 U/ml)	B43.13 scFv[b] (1 µg/ml)
B43.13c	Positive control	200	+	94.1	97.4
1	1D0	87.5	ND	ND	ND
	3D0	370	+	62.3	42.6
2	1D0	NA	NA	NA	NA
	1D185	238	–	21.6	–
3	1D0	115	ND	ND	ND
	3D86	635	+	80.2	84.4
	3D207	1218	+	91.4	94.0
	4D144	1993	+	77.1	93.0
	4D270	773	+	79.2	83.0
	4D309	418	+	77.0	83.0
	5D134	1603	+	89.1	83.3
4	1D0	115	ND	ND	ND
	2D15	590	–	62.3	–
	2D41	540	–	56.9	20.2
	2D76	578	–	63.6	29.3
	3D28	278	–	24.2	14.7
5	1D0	182.5	ND	ND	ND
	1D16	388	+	74.8	78.3
6	1D0	NA	NA	NA	NA
	3D0	258	+	54.0	60.2
7	1D0	373	–	29.7	–
	1D7	1478	+	77.1	87.1
	1D17	1173	+	78.4	86.5
8	1D0	65	ND	ND	ND
	3D112	230	–	–	16.0
	3D166	213	–	–	42.5
9	1D0	50	ND	ND	ND
	3D0	1038	+	30.8	39.2
10	1D0	37.5	ND	ND	ND
	5D134	220	–	19.0	24.4
	6D134	218	+	18.0	39.0
	9D26	335	–	54.5	19.3
	9D65	333	–	56.1	24.4
	10D40	235	+	61.4	37.0
11	1D0	160	ND	ND	ND
	2D14	265	–	24.5	–
12	1D0	112.5	ND	ND	ND
	1D15	288	+	30.8	47.4
13	1D0	0	ND	ND	ND
	2D17	253	–	30.3	–

[a] To be considered as significant, inhibition has to be at least 10%

[b] Single-chain mAb-B43.13 was used in the inhibition studies to avoid non-specific inhibition of the F_c portion of the antibody and cross-reactivity due to HAMA

cells (OVCAR-NU3). Lysis mediated by purified immunoglobulins from patients with high anti-CA125 antibody concentrations was slightly higher than the negative control (up to 9%) but lysis was not significantly higher than the control serum ($P = 0.1836$).

For ADCC testing, labeled OVCAR-NU3 cells were incubated with mAb B43.13, chimeric mAb B43.13, and MOPC 21 as well as human IgG (negative controls) at concentrations ranging from 0 to 25 µg/ml. The cells were also incubated with protein-A-purified immunoglobulins

Table 5 Correlation between idiotype induction and survival. Patients were grouped into Ab_2 or Ab_3 responders/non-responders and analyzed for their survival spans after diagnosis of the disease. The significance of the difference between responders and non-responders was determined by Student's *t*-test

Parameter (post-injection)	Survival (months)		
	Mean ± SD	Median	Significance *P*
Ab_2 <250 ng/ml	36.00±20.19	30.0	
Ab_2 >250 ng/ml	49.05±28.21	44.0	0.0475
Ab_3 increase <3×pre-injection value	37.36±18.15	38.0	
Ab_3 increase >3×pre-injection value	69.91±43.26	49.0	0.0029

from patients with high anti-CA125 antibody concentrations (serum pool and individual patients).

mAb B43.13 and MOPC 21 did not lyse the ovarian tumor cells (data not shown). Human IgG3 also does not lyse OVCAR-NU3 cells. On the other hand, chimeric B43.13 and especially purified antibodies from a serum pool with high Ab_3 concentrations (protein A chromatography) were able to lyse ovarian tumor cells, as demonstrated in Fig. 5a. OVCAR-NU3 cell lysis by the human anti-CA125 preparation was significantly higher than by the control human IgG ($P = 0.00194$). Similar results were obtained with immunoglobulins obtained from individual patients with high Ab_3 concentrations (Fig. 5b). All samples showed statistically significant differences in the lysis of OVCAR-NU3 cells compared to the control IgG (patient 3: $P = 0.0014$, patient 4: $P = 0.0054$, patient 7: $P = 0.0026$, patient 10: $P = 0.0013$, patient 12: $P = 0.0329$). Chimeric mAb B43.13 lysed ovarian cancer cells up to 15%. Purified antibodies from a high Ab_3 serum pool mediated the maximum lysis of 29%. The same preparation did not lyse CA125-negative tumor cells (SW1116 cells, data not shown). The anti-CA125 antibodies from individual patients generated maximum lysis between 5% and 16%.

Correlation of idiotype induction and survival of the patients

Where available, the survival data of patients treated with Ovarex mAb B43.13 were correlated with those for the induction of anti-idiotypic and anti-(anti-idiotypic) antibodies. The mean±standard deviation and median survival were calculated in responders and non-responders. The significance of the differences between the two groups was analyzed by Student's *t*-test. For this study, only patients with ovarian cancer classified as FIGO III and IV were included in the analysis in order to reduce the influence of a better survival prognosis in an earlier cancer stage.

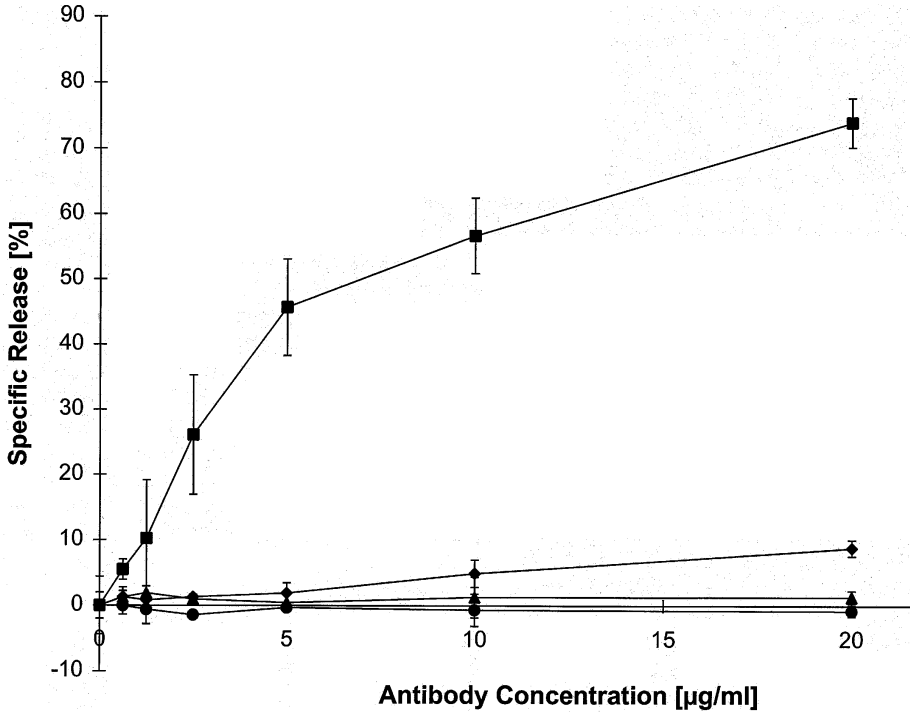

Fig. 4 Complement-dependent cytotoxicity. OVCAR-NU3 or SW1116 cells were labeled with ^{51}Cr as described in Materials and methods. The cells were incubated with different antibodies for 2 h at 37 °C and washed once. Complement-containing fresh human serum (15%) was added, the assay was incubated for 4 h at 37 °C and 150 µl supernatant was removed for gamma counting. The percentage lysis was calculated in relation to the maximum release, obtained from samples incubated with 1% Triton X-100. ■ SW1116/B25.10, ▲ OVCAR-NU3/mAb B43.13, ◆ OVCAR-NU3/Ab_3, ● OVCAR-NU3/chimeric mAb B43.13

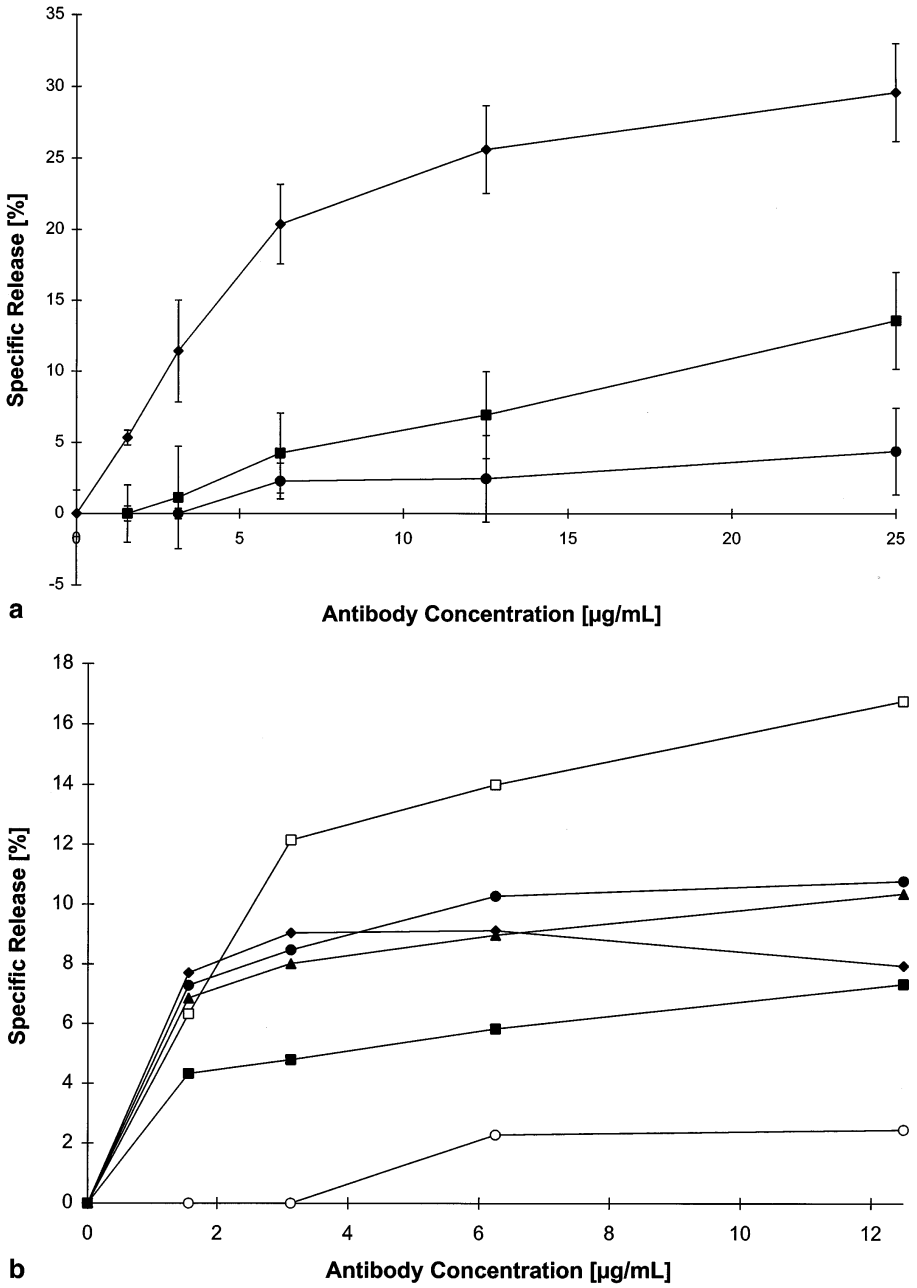

Fig. 5a,b Antibody-dependent cell-mediated cytotoxicity. OVCAR-NU3 cells were labeled with ^{51}Cr as described in Materials and methods. The cells were incubated with different antibodies for 2 h at 37 °C and washed once. Fresh human peripheral blood mononuclear cells from a healthy donor were added at a ratio of 1 to 25 (tumor to effector cells), the assay was incubated for 4 h at 37 °C and 150 µl supernatant was removed for gamma counting. The percentage lysis was calculated in relation to the maximum release, obtained from samples incubated with 1% Triton X-100. **a** Cells were incubated with chimeric mAb B43.13 (■), human IgG_3 (●), purified Ab_3 (◆). **b** Cells were incubated with purified human anti-CA125 antibodies from individual patients (patients: ◆ 3, ▲ 4, □ 7, ● 10, ■ 12) and human IgG_3 as a control (○)

If patients were grouped into Ab_2 responders and non-responders, the mean and median survival was higher in the Ab_2-positive group ($Ab_2 > 250$ ng/ml). Patients with no or low Ab_2 (< 250 ng/ml) showed a mean survival of 36 ± 20 months (median = 30 months) whereas patients with a high Ab_2 concentration lived for an average of 49 ± 28 months (median = 44 months) from the date of diagnosis of their cancer (Table 5). A concentration of at least 250 ng/ml was necessary to show a significant difference ($P = 0.0475$) in their survival.

The increase in the Ab_3 concentrations was used to analyze the survival data. If patients whose anti-CA125 antibody concentration increased by more than a factor of three after Ovarex mAb B43.13 injection were compared to patients who did not show such an increase, the difference between those two groups was highly significant ($P = 0.0029$). The mean survival times were 37 ± 18 months (median 38 months) in the group with no or low Ab_3 increase and 70 ± 43 months (median 49 month) in the group showing a threefold or more increase in the anti-CA125 concentration. No statistically significant difference was observed when patients with Ab_3 values above and below 200 ng/ml (cut-off value) were compared in regard to their survival patterns.

Discussion

Murine monoclonal antibodies directed against tumor-associated antigens have been used for various approaches in the therapy of cancer. The antibodies have been used as vehicles to bring tumor-destroying drugs and/or radioisotopes to the tumor or as a vaccine to stimulate the immune system to fight cancer. Various antitumor mechanisms have been attributed to these antibodies, such as antibody-dependent cellular cytotoxicity [1], complement-dependent cytotoxicity [17], apoptosis [48], idiotype network induction (for review see [5]) or stimulation of T cells [27].

Induction of Ab_3, binding to the original tumor antigen, via the idiotypic network, was first described by Herlyn et al. [18]. Anti-idiotypic antibodies (Ab_2) themselves have been used to induce immunity against viruses [23] and cancer. In many studies, various tumor-associated antigens have been used as targets for gastro-intestinal tumors [4, 11, 19], breast cancer [7, 42], ovarian cancer [41], lymphoma [40], melanoma [8, 36], lung cancer [53] or sarcoma [51] and tested in animal models. Induction of tumor-binding antibodies after injection of an Ab_1 has also been shown for two antibodies: 17-1A [12, 13, 16] and OC125 [43, 50].

In this paper we have described the induction of ovarian-cancer-binding antibodies after injection of mAb B43.13 in patients and discussed the possible effect of these antibodies on tumor reduction and survival benefit.

The actual mechanism by which mAb B43.13 mediates an improvement of the survival rate in ovarian cancer patients after multiple injections of the antibody is not yet completely understood. The role of the antibody in induction of the idiotypic network was studied by measuring the anti-idiotypic and anti-(anti-idiotypic) antibodies in the sera of patients treated with the mAb B43.13.

The study of the immune response in cancer patients who received mAb B43.13 clearly demonstrated the induction of the idiotypic network in 64% of the patients, as indicated by the presence of Ab_2 levels in their serum [30]. In 24% of the cases, where Ab_2 concentrations were high, anti-CA125 antibodies could also be detected. Those antibodies were purified from a serum pool and from individual patients and were demonstrated to bind to CA125-positive cell lines as well as to ovarian tumors.

We investigated whether those anti-CA125 antibodies (so-called Ab_3) were developed through the idiotypic network cascade by measuring inhibition with the injected Ab_1 mAb B43.13. In order to minimize effects of HAMA present in the serum samples, single-chain fragments of the antibody were used. Out of 23 samples from 10 patients, the majority (85%) showed inhibition with mAb B43.13.

Four samples (3 patients) could not be inhibited with mAb B43.13. These CA125-binding antibodies were thought to be developed through the antigen itself. Since none of these patients produced anti-CA125 antibodies before injection of Ovarex, injection of mAb B43.13 appears to have other effects in augmenting the immune response to CA125, besides the induction of anti-(anti-idiotypic) antibodies. The hypothesis is suggested that anti-CA125 antibodies can also be generated through complex formation with mAb B43.13, thus rendering the antigen more immunogenic [34]. Several papers have demonstrated that antibodies specific for an antigen can enhance the immune response to this antigen via augmented antigen presentation by macrophages and B cells [6, 9, 26, 52].

Nevertheless, binding of most of the samples to CA125 could be inhibited with mAb B43.13 (85%). Most of these samples also bound to rabbit Ab_2. Induction of Ab_3 was highly correlated with the presence of Ab_2 ($P = 0.0021$). This demonstrates the important role of the idiotypic network for induction of cancer-binding antibodies (Ab_3). On the other hand, we also observed that the Ab_2 response was favored when patients were expressing the CA125 antigen in their serum, an observation that could be explained by a protective effect of CA125 for the mAb B43.13-binding region. Manca et al. [32, 33] showed that antibodies can preserve the sequence of the epitope they are binding to during antigen processing. This can also be valid for the antibody-binding region through protection by bound antigen.

Traditionally, mAb were thought to be acting as magic bullets, damaging the cells they bind to either through CDC or ADCC. These mechanisms have been described as less relevant and effective for several antibodies in terms of tumor cell killing [24]. In addition, mAb B43.13 by itself was not able to activate CDC or ADCC. Further, the injection of only 2 mg/patient produces only a very low concentration of mAb B43.13 in the patient's serum, probably too low to mediate CDC or ADCC efficiently. Therefore, other mechanisms of antitumor activity must be involved for the observed clinical responses induced by Ovarex. The tumor-binding Ab_3 described were efficient in mediating those F_c-dependent immunological responses, especially ADCC. The patient's own antibodies against the tumor may have a much greater potential than the injected mAb B43.13 or a chimeric mAb B43.13 because of their constant supply by the patient's immune system. Thus, Ab_3-mediated tumor killing may be a factor of the Ovarex action contributing to the patients' survival benefit. The statistical data on survival times showed a highly significant difference between patients who developed an increase in their anti-CA125 concentration and patients in whom the anti-CA125 concentration did not increase ($P = 0.0029$). There was also a survival benefit for patients who developed anti-idiotypic antibodies compared to patients who did not respond with Ab_2 formation. However, the difference between these two groups was less significant ($P = 0.0475$).

These data show the important effect of anti-CA125 antibody induction through the idiotypic network. On the other hand, the relatively low percentage of anti-CA125-positive patients (24%) suggests that Ab_3 alone may not account for the observed clinical outcome and that other mechanisms play a role, especially in terms of cell-mediated immunity. We have already reported that elevated interferon γ levels (and interleukin-2 levels) were detectable in 36% of the patients injected with mAb B43.13 [31], indicating the involvement of T cells in the response to the

antibody. Cytokines have well-known immunoregulatory effects [27] and can also mediate bystander effects on tumor cell killing. The analysis of cellular responses in patients injected with mAb B43.13 will be the subject of further studies.

Acknowledgements We would like to thank S. Dharampaul, M. Kuzma, K. Agopsowicz and E. Frank for excellent technical assistance.

References

1. Adams DO, Hall T, Steplewski Z, Koprowski H (1984) Tumor undergoing rejection induced by monoclonal antibodies of the IgG2a Isotype containing increased numbers of macrophages activated for distinctive form of antibody dependent cytolysis. Proc Natl Acad Sci USA 81:3506
2. Baum RP, Noujaim AA, Nanci A, Moebus V, Hertel A, Niesen A, Donnerstag B, Sykes T, Boniface G, Hör G (1993) Clinical course of ovarian cancer patients under repeated stimulation of HAMA using mAb OC125 and B43.13. Hybridoma 12:583
3. Baum RP, Niesen A, Hertel A, Nanci A, Hess H, Donnerstag B, Sykes TR, Sykes CJ, Suresh MR, Noujaim AA, Hör G (1994) Activating anti-idiotypic human anti-mouse antibodies for immunotherapy of ovarian cancer. Cancer 73:1121
4. Bhattacharya-Chatterjee M, Mukerjee S, Biddle W, Foon KA, Köhler H (1990) Murine monoclonal anti-idiotype antibody as a potential network antigen for human carcinoembryonic antigen. J Immunol 145:2758
5. Bhattercharya-Chatterjee M, Foon KA, Kohler H (1994) Idiotype antibody immunotherapy of cancer (review). Cancer Immunol Immunother 38:75
6. Celis E, Chang TW (1984) Antibodies to hepatitis B surface antigen potentiate the response of human T lymphocyte clones to the same antigen. Science 224:297
7. Chakraborty M, Mukerjee S, Foon KA, Köhler H, Ceriani RL, Bhattacharya-Chatterjee M (1995) Induction of human breast cancer-specific antibody responses in cynomolgus monkeys by a murine monoclonal anti-idiotype antibody. Cancer Res 55:1525
8. Chattopadhyay P, Starkey J, Morrow WJ, Raychaudhuri S (1992) Murine monoclonal anti-idiotype antibody breaks unresponsiveness and induces a specific antibody response to human melanoma-associated proteoglucan antigen in cynomolgus monkeys. Proc Natl Acad Sci USA 89:2684
9. Chestnut R, Grey HM (1981) Studies on the capacity of B cells to serve as antigen-presenting cells. J Immunol 126:1075
10. Cheung NKV, Lazarus H, Miraldi FD, Abramowsky CR, Kallick S, Saarinen UM, Spitzer T, Stransjord SE, Coccia PF, Berger NA (1987) Ganglioside GD2 specific monoclonal antibody 3F8: a phase I study in patients with neuroblastoma and malignant melanoma. J Clin Oncol 5:1430
11. Durrant LG, Denton GWL, Jacobs E, Mee M, Moss R, Austin EB, Baldwin RW, Hardcastle JD, Robins RA (1992) An idiotypic replica of carcinoembryonic antigen inducing cellular and humoral responses directed against human colorectal tumours. Int J Cancer 50:811
12. Fagerberg J, Frödin J-E, Wigzell H, Mellstedt H (1993) Induction of an immune network cascade in cancer patients treated with monoclonal antibodies (ab1). I. May induction of ab1-reactive T cells and anti-anti-idiotypic antibodies (ab3) lead to tumor regression after mAb therapy? Cancer Immunol Immunother 37:264
13. Fagerberg J, Frödin J-E, Ragnhammar P, Steinitz M, Wigzell H, Mellstedt H (1994) Induction of an immune network cascade in cancer patients treated with monoclonal antibodies (ab1). II. Is induction of anti-idiotype reactive T cells (T3) of importance for tumor response to mAb therapy? Cancer Immunol Immunother 38:149
14. Fagerberg J, Hjelm AL, Ragnhammer P, Frödin JE, Wigzell H, Mellstedt H (1995) Tumor regression in monoclonal antibody-treated patients correlates with the presence of anti-idiotype-reactive T lymphocytes. Cancer Res 55:1824
15. Ferrone S (1993) Human tumor-associated antigen mimicry by anti-idiotypic antibodies. immunogenicity and clinical trials in patients with solid tumors. Ann NY Acad Sci 690:214
16. Frödin J-E, Faxas M-E, Hagström B, Lefvert A-K, Masucci G, Nilsson B, Steinitz M, Unger P, Mellstedt H (1991) Induction of anti-idiotypic (ab2) and anti-anti-idiotypic (ab3) antibodies in patients treated with the mouse monoclonal antibody 17-1A (ab1). Relation to the clinical outcome - an important antitumoral effector function? Hybridoma 10:421
17. Herlyn, DM, Koprowski H (1981) Monoclonal anticolon carcinoma antibodies in complement-dependent cytotoxicity. Int J Cancer 27:769
18. Herlyn D, Ross AH and Koprowski H (1986) Anti-idiotypic antibodies bear the internal image of a human tumor antigen. Science 232:1300
19. Herlyn D, Ross AH, Iliopoulos D, Koprowski H (1987) Induction of specific immunity to human colon carcinoma by anti-idiotypic antibodies to monoclonal antibody CO17-1A. Eur J Immunol 17:1649
20. Houghton AN, Mintzner D, Cordon-Cardo C, Welt S, Fliegel F, Vadham S, Carswell E, Melamed MR, Oettgen HF, Old LY (1985) Mouse monoclonal IgG3 antibody detecting GD3 ganglioside: a phase I trial in patients with malignant melanoma. Proc Natl Acad Sci USA 82:1242
21. Irie RF, Morton DL (1986) Regression of cutaneous metastatic melanoma by intralesional injection with human monoclonal antibody to ganglioside GD2. Proc Natl Acad Sci USA 83:8694
22. Jerne NK (1974) Towards a network theory of the immune system. Ann Inst Pasteur 125C:373
23. Kennedy RC, Dreesman GR (1984) Enhancement of the immune response to hepatitis B surface antigen: in vivo administration of anti-idiotype induces anti-HBS that expresses a similar epitope. J Exp Med 159:655
24. Kosmas C, Epenetos AA, Courtenay-Luck NS (1994) Activation of cellular immunity after intracavitary monoclonal antibody therapy of ovarian cancer. Cancer 73:3000
25. Krantz MJ, MacLean G, Longenecker BM, Suresh MR (1988) A radioimmunoassay for CA125 employing two new monoclonal antibodies. J Cell Biochem Suppl 12E:139
26. Lanzavecchia A (1985) Antigen-specific interactions between T and B cells. Nature 314:537
27. Lanzavecchia A, Abrignani S, Scheidegger D, Obrist R, Dörken B, Moldenhauer G (1988) Antibodies as antigens. The use of mouse monoclonal antibodies to focus human T cells against selected targets. J Exp Med 167:345
28. LoBuglio AF, Saleh MN, Lee J, Khazaeci MB, Carrano R, Holden H, Wheeler RH (1988) Phase I trial of multiple large doses of murine monoclonal antibody CA17-1A. Clinical Aspects. J Natl Cancer Inst 80:932
29. Luo D, Geng M, Noujaim AA, Madiyalakan R (1997) An engineered bivalent single-chain antibody fragment that increases antigen binding activity. J Biochem (Tokyo) 121:831
30. Madiyalakan R, Sykes TR, Dharampaul S, Sykes CJ, Baum RP, Hör G, Noujaim AA (1995) Anti-idiotype induction therapy: evidence for the induction of immune response through the idiotypic network in patients with ovarian cancer after administration of anti-CA125 murine monoclonal antibody B43.13. Hybridoma 14:199
31. Madiyalakan R, Yang R, Schultes BC, Baum RP, Noujaim AA (1997) Ovarex mAb B43.13: IFN-γ could improve the ovarian tumor cell sensitivity to CA125-specific allogenic cytotoxic T cells. Hybridoma 16:41
32. Manca F, Fenoglio D, Kunkl A, Cambiaggi C, Sasso M, Celada F (1988) Differential activation of T cell clones stimulated by macrophages exposed to antigen complexed with monoclonal antibodies. J Immunol 140:2893

33. Manca F, Fenoglio D, Pira GL, Kunkl A, Celada F (1991) Effect of antigen/antibody ratio on macrophage uptake, processing, and presentation to T Cells of antigen complexed with polyclonal antibodies. J Exp Med 173:37
34. Marusic-Galesic S, Marusic M, Pokric B (1992) Cellular immune response to the antigen administered as an immune complex in vivo. Immunology 75:325
35. Mellstedt H, Frödin JE, Masucci G, Ragnhammer P, Fagerberg J, Hjelm AL, Shetye J, Wersäll P, Österborg A (1991) The therapeutic use of monoclonal antibodies in colorectal carcinoma. Semin Oncol 18:462
36. Mittelman A, Chen ZJ, Yang H, Wong G, Ferrone S (1992) Human high molecular weight melanoma-associated antigen (HMW-MAA) mimicry by mouse anti-idiotype monoclonal antibody MK2-23: induction of humoral anti-HMW-MAA immunity and prolongation of survival in patients with stage IV melanoma. Proc Natl Acad Sci USA 89:466
37. Noujaim AA, Baum RP, Sykes TR, Madiyalakan R, Sykes CJ, Hertel A, Niesen A, Hör G (1994) Monoclonal antibody B43.13 for immunoscintigraphy and immunotherapy of ovarian cancer: In: Klapdor R (ed) Current tumor diagnosis: applications, clinical relevance, trends. Zuckschwerdt, München, p 823
38. Ortaldo JR, Woodhouse C, Morgan AC, Herberman RB, Cheresh DA, Reisfeld R (1987) Analysis of effector cells in human antibody-dependent cellular cytotoxicity with murine monoclonal antibodies. J Immunol 138:3566
39. Pervin S, Chakraborty M, Bhattacharya-Chatterjee M, Zeytin H, Foon KA, Chatterjee S (1997) Induction of antitumor immunity by an anti-idiotype antibody mimicking carcinoembryonic antigen. Cancer Res 57:728
40. Pohl C, Renner C, Schwonzen M, Schobert I, Liebenberg V, Jung W, Wolf J, Pfreundschuh M, Diehl V (1993) CD30-specific Ab1-Ab2-Ab3 internal image antibody network: potential use as anti-idiotypic vaccine against Hodgkin's lymphoma. Int J Cancer 54:418
41. Schlebusch H, Wagner U, Grünn U, Schultes B (1995) A monoclonal antiidiotypic antibody ACA 125 mimicking the tumor-associated antigen CA 125 for immunotherapy of ovarian cancer. Hybridoma 14:167
42. Schmitt H, Khazaeli MB, Rodenburg CM, Kaul S, Bastert G (1994) Production of mouse monoclonal anti-idiotypic antibodies specific to epitopes of tumor-associated mucin TAG-12. Hybridoma 13:389
43. Schultes BC, Reinsberg J, Schlebusch H, Oehr P, Biersack HJ, Krebs D, Wagner U (1993) Idiotypic cascades after injection of the monoclonal antibody OC125: a study in a mouse model. Eur J Clin Chem Clin Biochem 31:427
44. Schultes BC, Qi W, Yang R, Kuzma M, Liu D, Noujaim AA, Madiyalakan R (1997) CA125 mimicking anti-idiotypic antibody mAb-BR11.1 elicits humoral and cellular response specific for the nominal antigen. Tumor Biol 18 S2:16
45. Sears HF, Atkinson B, Mattis J, Ernst C, Herlyn D, Steplewski Z, Häyry P, Koprowski H (1982) Phase-I clinical trial of monoclonal antibody in treatment of gastrointestinal tumours. Lancet I:762
46. Somasundaram R, Zaloudik J, Jacob L, Benden A, Sperlagh M, Hart E, Marks G, Kana M, Mastrangelo M, Herlyn D (1995) Induction of antigen-specific T and B cell immunity in colon cancer carcinoma patients by anti-idiotypic antibody. J Immunol 155:325
47. Sykes TR, Woo TK, Baum RP, Qi P, Noujaim AA (1995) Direct labeling of monoclonal antibodies with technetium-99m by photoactivation. J Nucl Med 36:1913
48. Trauth BC, Klas C, Peters AMJ, Matzku S, Möller P, Falk W, Debatin KM, Krammer PH (1989) Monoclonal Antibody mediated tumor regression by induction of apoptosis. Science 245:301
49. Wagner U (1993) Antitumor antibodies for immunotherapy of ovarian carcinomas. Hybridoma 12:521
50. Wagner U, Reinsberg J, Oehr P, Briele B, Schmidt S, Werner A, Krebs D, Biersack H-J (1990) Clinical courses of patients with ovarian carcinomas after induction of anti-idiotypic antibodies against a tumor-associated antigen. Tumordiagn Ther 11:1
51. Warren RQ, Tsang KY (1990) Induction of immunity to a human osteosarcoma-associated antigen in mice using anti-idiotypic antibodies. Clin Immunol Immunopathol 56:334
52. Wiermsa EJ (1992) Enhancement of the antibody response to protein antigens by specific IgG under different experimental conditions. Scand J Immunol 36:193
53. Zwicky C, Stahel RA, Jaksche H, Waibel R, Lehmann HP, Loibner H (1991) Polyclonal anti-idiotypic antibodies mimicking the small cell lung carcinoma antigen cluster-5A interact with a panel of antibodies and induce specific immune response in animals. Br J Cancer 14:67