Distribution of Radiolabelled *Anti*-CA125 Monoclonal Antibody OC125-F(ab')₂-Fragment Following Resection Guided by Antibodies (REGAJ) in Ovarian Cancer Patients

Martina M. Uttenreuther-Fischer,^{1,2} Herbert Feistel,³ Friedrich Wolf,³
and Wolfram Jäger^{2*}

¹Department of General Pediatrics, University Children's Hospital, Charité, and
Laboratory for Pediatric Molecular Biology, Berlin

²Department of Obstetrics and Gynecology, University of Erlangen, Erlangen, Germany

³Department of Nuclear Medicine,
University of Erlangen, Erlangen, Germany

Ovarian cancer is a highly malignant tumor of mainly postmenopausal women. The long-term prognosis of this malignancy is largely determined by micrometastasis present at the time of second-look surgery. In general, patients face a poor outcome. New radio-immunoscintigraphic methods to target tumor tissue specifically via antigen-antibody binding were developed. However, few studies so far investigated the pattern of in vivo distribution of radiolabelled mAbs and/or the specificity of antigen-antibody interaction. In this study we examined the immunological interaction and distribution of ¹³¹I-OC125-F(ab')₂-fragment, an anti-CA-125 mAb, in patients with CA-125 positive ovarian malignancies. Sixteen patients with primarily CA-125 positive gynecological tumors underwent REGAJ surgery. Biopsies of tumor tissue and not tumor infiltrated tissue, serum, and ascites were sampled during or prior to REGAJ surgery, respectively. After preparation of tissue cytosols, samples were assessed for CA-125 and radioactive uptake. By radiochromatography immunological analysis for presence of the target antigen CA-125, the mAb ¹³¹I-OC125-F(ab')₂-fragment, and immune complexes

was performed on different specimen. CA-125 concentrations were higher in serum samples, ascites, and malignant tissue biopsies of malignoma patients compared to those without signs of malignant disease. CA-125 was higher in the tissue cytosol than in the cell membrane fraction. Gel filtration revealed CA-125 with moieties of 75,000 to >600,000 d. Accumulation of radioactivity was more frequently associated with the presence of unbound ¹³¹I-OC125-F(ab')₂-fragment or high molecular weight immune complexes. Radioactive uptake, however, was not confined to tissue of high CA-125 expression. Moreover, both immune complex as well as ¹³¹I-OC125-F(ab')₂-fragment could be isolated from cytosols of tissue not infiltrated by tumor cells as well. Our study demonstrates that the majority of CA-125 is located intracellularly and thus inaccessible to ¹³¹I-OC125-F(ab')₂-fragment per se. The uptake of ¹³¹I-OC125-F(ab')₂-fragment into the cytosol of tumor-free and malignant tissue samples prompts us to speculate that certain mechanisms for antigen-specific and nonspecific cellular trafficking of mAbs do exist. We present a model to explain our observations. J. Clin. Lab. Anal. 11:94-103. © 1997 Wiley-Liss, Inc.

Key words: ovarian cancer; REGAJ; TAA CA-125; mAb OC125-F(ab')₂-fragment; antibody distribution

INTRODUCTION

Ovarian cancer is a highly malignant tumor that, in general, becomes clinically apparent around menopause. As early symptoms are mild, it is usually diagnosed at advanced stages of disease. Despite aggressive therapy-regimen, including debulging surgery and radiochemotherapy, long-term prognosis remains poor due to the highly metastatic potential of this malignancy [1–3].

The presence of micro- and macrometastases at second-look surgery dramatically increases the long-term risk of tumor relapse. Therefore, new diagnostic strategies such as

*Correspondence to: Wolfram Jäger, Dept. of Obstetrics and Gynecology, University of Erlangen, Universitätsstr. 20-21, D-91054 Erlangen, Germany.

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immunoscintigraphic approaches using tumor-associated antigen (TAA)-specific, radiolabelled monoclonal antibodies (mAb) for tumor targeting, e.g., REGAJ (resection guided by antibodies) and RIGS (radioimmunoguided surgery), attempted intraoperatively to trace micrometastasis [4–8]. Although initial studies showed promising results, surveys of mAb-distribution in vivo demonstrated radioactive accumulation in ascites and benign tissue as well as in tumor tissue [7,9,10]. Jäger et al. [7] showed that although ¹³¹I-OC125-F(ab')₂ localized to ovarian cancer micrometastasis in vivo, radiolabelled mAb also was taken up by not tumor-infiltrated tissue. Other investigators detected high amounts of radioactivity in the peritoneal cavity and in ascites fluid of ovarian cancer patients after immunoscintigraphy [9,10]. Whether ¹³¹I-OC125-F(ab')₂ targeting was due to its specific binding to CA-125 (cancer antigen) or merely to nonspecific uptake was not clear. To investigate the specificity of radioimmunoscintigraphic targeting using anti-CA-125-mAb ¹³¹I-OC125-F(ab')₂, we analyzed not tumor-infiltrated and malignant tissue samples, serum, and ascites of 16 patients with CA-125 positive tumors undergoing primary, second-, or third-look REGAJ-surgery for presence of TAA CA-125, radioactivity, and ¹³¹I-OC125-F(ab')₂ and/or immune complexes.

MATERIAL AND METHODS

Patients

A total of 16 different patients with CA-125-positive tumors at the time of primary surgery were included in this study. Thirteen patients underwent second-look surgery and one patient (#16) third-look surgery for ovarian cancer. One patient, who had primary REGAJ surgery performed for endometriosis (#5), participated in this study. Another patient had REGAJ surgery done for preoperative assessment of disseminated ovarian cancer (#8). Patient #3 was assessed twice, prior to primary surgery (#3) and at the time of second-look surgery (#10); see Table 1.

Samples

Serum and ascites samples were drawn and then kept frozen at –20°C until analysis (for CA-125 assessment), or analyzed immediately (radioactive material for gel filtration). Tumor-free and malignant tissue biopsies were collected intraoperatively and split into halves. One piece was shock frozen at –70°C for cytosol preparation and consecutive column-chromatography. The other half was formalin-fixed for histological evaluation.

Sample classification and registration were performed according to the macroscopic aspect and radioactive emission into either tumor-free or malignant probes, according to the principle of radioimmuno-guided surgery. Highly radioactive

samples were initially recorded as malignant unless histological data upon final evaluation proved a different dignity. Thus all of our experiments were conducted as a blind study until histological data were available.

Intraoperative Tissue Sampling Utilizing REGAJ Surgery

Radioimmunoscintigraphy

Following preoperative radioimmunoscintigraphy using 1 mg of ¹³¹I-OC125-F(ab')₂, with a radioactivity of 70–110 MBq/mg mAb (CIS bio International, Dreieich, Germany), surgery was performed. After thawing, labeled ¹³¹I-OC125-F(ab')₂ was infused intravenously over 30 minutes. Prior to this, thyroid uptake of ¹³¹I was blocked by oral perchlorate therapy. On day 3 and day 5, immunoscintigraphic images of mAb ¹³¹I-OC125-F(ab')₂-distribution were taken with a rota-camera [5,7].

REGAJ

The REGAJ methodology has been described in more detail elsewhere [7]. Upon second-look surgery, radioactivity was traced intraoperatively using a handheld scintillation-probe (Stratec, Munich, Germany). The probe consisted of a 2.5 × 2.5 cm NaI-crystal, connected to a photomultiplier. The area for assessment of radioactivity was 44 mm in diameter. A collimator of 10 mm in diameter and 50 mm length could be connected. The scintillation probe was calibrated above the abdominal aorta. Areas showing 50% increased radioactivity compared to background were considered positive, excised, and histologically evaluated for presence of micrometastasis of ovarian cancer.

Upon laparotomy, ascites was collected instantaneously in 10 ml Sarstedt serum tubes. Thereafter several washings were performed to reduce nonspecific background activity prior to tissue sampling according to REGAJ-surgery.

Preparation of Tissue Cytosols and Membrane Lysates

Cytosol samples and membrane lysates were obtained after tissue homogenization according to standard procedure [11–14]. Cytosol samples and membrane lysates were obtained after tissue homogenization [15–17]. All procedures were performed on ice to prevent antigen internalization. Briefly, after removal of blood contaminations from shock frozen tissue, stored at –70°C, tissue samples weighing 0.25–0.8 g were sliced and put into 2.5 ml of ice-cold phosphate-buffered saline (PBS), pH 7.5, containing 1% monothiolglycerate. The tissue was minced with an ultraturax at 24,000 rpm for 3 × 3 seconds. In between runs, tissue samples were kept on ice. After addition of another 500 µl PBS, ultracentrifugation was performed at 155,000 g for 60 minutes at 4°C. The supernatant was harvested and set aside as cytosol fraction. To solubilize cell membranes, the remaining pellets were incubated

TABLE 1. Patient Summary

No.	Histology of primary tumor/at time of relapse	Primary surgery with REGAJ	Prior to primary REGAJ surgery	Second look with REGAJ surgery	Third look with REGAJ surgery	Endometriosis with REGAJ surgery
1	serous cystadenocarcinoma of the ovary			X		
2	ovarian cancer			X		
3	invasive, papillary, serous cystadenocarcinoma of the ovary	X				
4	serous cystadenocarcinoma of the ovary			X		
5	endometriosis uteri externa					X
6	adenocarcinoma of the ovary			X		
7	ovarian cancer			X		
8	papillary, undifferentiated adenocarcinoma of the ovary		X			
9	ovarian cancer			X		
10	ovarian cancer			X		
11	papillary, differentiated cystadenocarcinoma of the ovary			X		
12	primary peritoneal cancer			X		
13	undifferentiated, epithelial ovarian cancer			X		
14	undifferentiated adenocarcinoma of the ovary			X		
15	ovarian cancer			X		
16	ovarian cancer				X	
17	serous, papillary adenocarcinoma of the ovary			X		
Σ		1	1	13	1	1

with 300 µl of Triton X-100 1% at 4°C for 15 minutes [14,17]. Thereafter PBS was added to a final dilution of 0.2% Triton X-100. Ultracentrifugation at 120,000 g for 60 minutes followed. Solubilized membrane bound antigen was contained in the supernatant fraction.

Evaluation of Patient Samples

The concentration of TAA CA-125 in serum samples was assessed by standard IRMA (immunoradiometric assay) technique first described by Klug et al. [18], using a commercial assay kit, CA-125 ELSA (ID CIS, Dreieich, Germany). This same IRMA was used to measure the concentrations of CA-125 in cytosol and ascites samples (19–26). Inter- and intra-assay variation was below 10%. Calibration curves proved a measurement range for this assay between 6.5 and 500 U/ml. ¹³¹I-OC125-F(ab')₂ was assessed with a γ-counter (Beckman). ¹³¹I-decay in serum samples, tissue cytosols, ascites, membranes lysates, and chromatography fractions was measured for 2 min/sample. Sample volumes were kept at ≤2.5 ml/sample to guarantee maximum accuracy upon assessment of radioactive decay.

Gel Filtration Analysis

Calibration of chromatography columns

A chromatography column, length 100 cm, diameter 2.6 cm, was filled with degassed Sephadex G 150 or G 200 (Pharmacia, LKB, Sweden), respectively, for gel filtration of patient samples. Prior to analytical runs, equilibration with citrate buffer containing 0.1 M trisodiumcitrate-dihydrate and 0.01% merthiolate (thiomersal), pH 5.9, was performed [18,27]. Buffer flow and velocity for chromatographic procedures were constantly between 16–17 ml/h. To obtain high resolution elution profiles, the volumes of collected fractions were kept low at 2.2–2.5 ml/fraction. The total gel bed volume V_t ($r^2\pi \times l_{\text{gel}}$) for different Sephadex-fills ranged between 453.7 and 509.4 ml. Void volumes V_o , assessed by filtration of Dextrane Blue 2000 ranged between 145 and 219 ml, respectively. Elution volumes for high and low molecular weight protein peaks were determined by spectrophotometry at A_{280} nm.

Determination of molecular weight (MW)

Following calibration with standard low and high molecular weight calibration kits (Pharmacia, Freiburg) [28,29], molecular weights of unknown proteins were determined by calculation of K_{av} . K_{av} , an equilibration constant routinely used, defines the rehydrated gel mass as the stationary matrix of gel filtration and was determined by $K_{av} = (V_e - V_o) / (V_t - V_o)$ [30–33].

Radiochromatography

Radioactive emission was measured for identification of labeled proteins, i.e., ¹³¹I-OC125-F(ab')₂, ¹³¹I-OC125-Fab and

immune complexes. Assuming stable conjugation of ¹³¹I to the proteins examined, peak radioactive emission was used to (1) compute K_{av} , (2) determine molecular weight, (3) calibrate for certain known proteins, and (4) identify unknown substrates, e.g., immune complexes [12,34,35].

Chromatographic determination of CA-125 peaks

TAA CA-125, a glycoprotein of largely variant MW (10,000–1,500,000) [36–38] cannot be detected by radiochromatography. Therefore, elution fractions were screened for presence of CA-125 using the CA-125 ELSA. The elution volume V_e and K_{av} for CA-125 were assessed as described above.

Evaluation of chromatographic elution profiles

Upon column calibration with CA-125 standard solution from CA-125 ELSA and CA-125-containing patient samples, the glycoprotein was eluted with different moieties of 75,000 to >600,000 d. For data evaluation following assumptions were made: (1) high molecular weight compounds, >600,000 d, emitting radioactivity were classified as immune complexes, (2) compounds eluting within the V_e of ¹³¹I-OC125-F(ab')₂, as determined before, were classified as such—immunoreactivity of respective fractions had been assessed with an ELISA system (Enzygnost mIgG micro, Behringwerke, Marburg, Germany), (data not shown), and (3) radioactive substrates eluting at ½ MW ¹³¹I-OC125-F(ab')₂ were classified as Fab-fragments, accordingly to the time-dependent mAb-breakdown observed in patient #5 (data not shown).

Statistical Analysis

A Student's t-test was used for statistical analysis whenever appropriate.

RESULTS

Distribution of TAA CA-125

Serum samples

CA-125 concentrations were elevated above the cutoff value of 25 U/ml in sera of 4/14 patients (patient #10 included) undergoing second- or third-look surgery, and in two patients who were just diagnosed with ovarian cancer, prior to primary operative intervention (patients #3, #8). Patient #5 with endometriosis had a normal CA-125 serum concentration. Only 4/10 patients with histologically proven malignancy showed elevated CA-125-levels >25 U/ml (Fig. 1a).

Ascites samples

CA-125 concentrations were assessed in ascites samples and endometriosis fluid of 10/15 patients. Of the above, 4/5 patients with malignant disease and 3/4 patients with histologically benign tumors showed elevated CA-125 >25 U/ml

in the peritoneal fluid. However, the median CA-125 concentration of 224 U/ml in patients with malignant disease was considerably higher compared to a median concentration of 29 U/ml CA-125 in patients without apparent malignoma. Endometriosis fluid showed a slightly elevated CA-125 concentration of 27 U/ml (Fig. 1a).

Cytosols and membrane lysates

Tissue biopsies of 14 patients were screened for their CA-125 content. Cytosol fractions and membrane lysates were assayed separately. CA-125 concentrations as determined by IRMA in general were higher in malignant than in benign tissue cytosols. This holds true when contrasting CA-125-values for cytosols of each individual patient (Fig. 1b) as well as upon comparison of mean CA-125-values for all cytosols of malignant and not tumor-infiltrated tissue samples examined. The latter is statistically significant at $P = 0.038$ (Fig. 1c). High concentrations of CA-125 were not restricted to tissue of malignant histology. Although 8/10 patients with malignant disease showed elevation of mean CA-125-values in malignant cytosols, 4/11 patients demonstrated elevated

Fig. 1. (a) CA-125 concentrations in serum samples (filled bars) and ascites samples (empty bars) of patients without (1) and with (2) proof of malignant disease are presented. (b) CA-125 concentrations in cytosols of benign (●) and malignant samples (○) and membrane lysates of benign (▲) and malignant (△) biopsies are presented. (c) Mean CA-125 concentrations of all benign and malignant tissue cytosols and membrane fractions demonstrate that the mean CA-125 concentration of malignant cytosols is significantly higher than the mean CA-125 concentration of benign cytosols ($P = 0.038$).

mean CA-125-values in tumor-free samples as well. Two of the latter four patients had malignant disease, however (Fig. 1b). CA-125 concentrations assessed in lysates of solubilized membranes were far below those of respective cytosols, but high CA-125 concentrations in membrane fractions always were paralleled by very high concentrations in tissue cytosols (Fig. 1b). In summary, CA-125 concentrations in serum and ascites, cytosols and membrane lysates of patients with malignant disease were higher than in patients with benign disease and/or not tumor-infiltrated tissue samples respectively.

Radioactive Uptake of ^{131}I in Malignant and Not Tumor-infiltrated Tissue Samples

Following radioimmunoscintigraphy and REGAJ surgery, tissue samples were assessed for radioactive uptake. ^{131}I -decay in cpm was determined in identical sample volumes of cytosols and membrane lysates prior to radiochromatography. Comparison of ^{131}I -uptake between different patients was limited by: (1) the divergent time between RIS and REGAJ, when the samples were obtained, and (2) the short half-life of ^{131}I . Mean radioactivity due to ^{131}I -uptake in benign and ma-

Fig. 2. (a) Mean radioactivity of benign (1) and malignant (2) tissue cytosols. (1) Hatched bars show the mean radioactivity in cytosols of patients without malignant disease at time of REGAJ surgery; empty bars show the radioactivity in benign cytosols of patients with malignant disease at the time of REGAJ. (b) Mean radioactivity of membrane

fractions of benign (1) and malignant (2) tissue biopsies. (1) Hatched bars show the mean radioactivity in membranes of patients without malignant disease at time of REGAJ surgery, whereas empty bars show the radioactivity in membrane fractions of benign biopsies of patients with malignant disease at the time of REGAJ.

lignant cytosols are presented in Figure 2a/b. Figure 2a is suggestive of accumulation of radioactivity in malignant versus cytosols of tumor-free samples. The latter can be demonstrated definitely for patients #4, #6, and #10–14. This difference cannot be demonstrated when comparing the radioactivity of membrane lysates from tumor-free and malignant biopsies (Fig. 2b).

Gel Filtration Analysis

Altogether, 12 serum, 5 ascites, 11 cytosol samples, and one endometriosis fluid were analyzed by gel filtration and screened for presence of ¹³¹I-OC125-F(ab')₂, CA-125-¹³¹I-OC125-F(ab')₂-immune complexes, and/or lower molecular weight fragments of ¹³¹I-OC125-F(ab')₂. A summary of all chromatography data is presented in Table 2 a/b.

Upon radiochromatographic analysis: (1) a nonradioactive substance, which could be traced in the CA-125-RIA, eluted with different moieties between 75,000 and >600,000 d, the latter being most likely consistent with TAA CA-125, (2) accumulation of radioactivity in native samples was frequently associated with elution peaks in the range of ¹³¹I-OC125-F(ab')₂ or high molecular weight compounds suggestive of immune-complexes (Fig. 3), (3) increased radioactivity in malignant cytosols was due to accumulation of ¹³¹I-OC125-F(ab')₂, which in most cases was not bound to CA-125 in an immune complex formation, (4) chromatographic profiles of malignant and cytosols of not tumor-infiltrated cells proved intracellular presence of high molecular weight immune complexes and free anti-CA-125 mAb ¹³¹I-OC125-F(ab')₂, and (5) moreover, elution of free ¹³¹I-OC125-F(ab')₂ and ¹³¹I-OC125-F(ab')₂, bound in immune complexes, was not restricted to tissue of increased TAA CA-125 expression. Evidence of the accumulation of an immune complex was demonstrated in 1/3 tumor-free and 1/8 malignant tissue cy-

tosols. Another malignant sample was not clearly evaluable in this regard. Accumulation of free ¹³¹I-OC125-F(ab')₂, however, was shown in 1/3 tumor-free samples and 5/8 malignant tissue cytosols, respectively.

DISCUSSION

Since the initial development of monoclonal antibody technology [39], numerous methods to trace microscopic metastatic disease in vivo have been invented. Mostly radionuclides bound to mAb and mAb-Fragments were used to visualize metastasis prior to and during surgical interventions, i.e., radioimmunoscintigraphy (RIS) and radioimmunoguided surgery (RIGS, REGAJ) [5,7,40,41]. Although the latter technologies are frequently used for diagnosis and therapy in the field of modern oncology, the validity of the general immunological principle behind these methods has not been completely analyzed. Earlier investigations on a CA-125 and ¹³¹I-OC125-F(ab')₂-interaction [7] tempted us to speculate that immunotargeting of TAA by mAbs and mAb-uptake is not restricted to malignant tissue of high surface antigen expression. In other words, although immune recognition may play a significant role in tumor targeting, other mechanisms may be involved as well.

Our present study proved that CA-125 is highly expressed by ovarian cancer tissue. However, the majority of CA-125, which supposedly is a surface glycoprotein, seems to be localized intracellularly and thus is primarily inaccessible to circulating mAb ¹³¹I-OC125-F(ab')₂. The latter has been indicated by Fleuren et al. [42].

Analyzing the radioactive emission of tissue of different dignity, we could demonstrate higher radioactive uptake by malignant tissue versus not tumor-infiltrated samples. This seems to support the general theoretical principle of immune recognition as the basis for immunotargeting. However, we

TABLE 2. Results of Radiochromatography

Serum #	Patient #	High molecular weight, radioactive complex	¹³¹ I-OC125-F(ab) ₂	¹³¹ I-OC125-Fab	Low molecular weight mAb fragments	CA125 contents (U/ml)
(a) In benign disease						
1	1	—	(+)	+	+	<25
2	2	+	—	—	+	<25
3	5	—	+	+	—	<25
4	5	—	+	+	—	<25
5	9	+	+	—	—	<25
Ascites #						
1	2	+	—	+	+	520
Endometriosis fluid						
1	5	(+) (130,000d)	—	—	+	27
Cytosol #						
1	2	—	—	—	—	<25
2	2	—	—	—	—	<25
3	9	+	+	—	—	<25
(b) In malignant disease						
1	4	—	+	+	—	<25
2	8	+	+	—	—	2442
3	8	+	+	+	—	2442
4	10	+	+	—	—	<25
5	11	+	+	+	—	<25
6	12	+	+ (160,000d)	+	—	<25
7	13	+	+	—	—	1661
Ascites #						
1	4	—	+ (110,000d)	+	—	<25
2	6	+	+	—	—	109
3	8	+	—	+	—	>>500
4	17	+	+	—	—	433
Cytosol #						
1	8	+	+	—	—	1365
2	8	+ (low activity)	— (low activity)	+ (low activity)	—	1239
3	8	—	+ (140,000d)	+	—	1239
4	10	—	—	—	—	<25
5	11	—	+	+	—	>>500
6	12	—	—	—	—	2073
7	13	—	+	—	+ (90,000d)	67
8	13	—	+	—	—	67

Fig. 3. Radiochromatography of a serum sample of patient #8 on day 0, the day of ^{131}I -OC125-F(ab')₂ infusion (○) and on day 10–11 after infusion (□). High molecular weight immune complex, ^{131}I -OC125-F(ab')₂, and on day 10–11 low molecular weight ^{131}I -OC125-fragments can be identified.

could demonstrate that intracellular trafficking of ^{131}I -OC125-F(ab')₂ was far more complex and less specific than primarily expected. By gel filtration analysis, we showed that intracytosolic accumulation of radioactivity was always associated with either detection of ^{131}I -OC125-F(ab')₂ or immune complex. However, increased radioactivity was not confined to malignant tissue but also demonstrated in tumor-free tissue cytosols. The reason for this observation may be the ubiquitous distribution of TAA CA-125, which also can be heavily expressed on benign tissue originating from the Mullerian duct, and upon inflammatory processes respectively [38,43]. This is not contradictory to our primary principle of immune recognition. More difficult to explain are our find-

ings that (1) although intracellular accumulation of radioactivity was always due to presence of ^{131}I -OC125-F(ab')₂, the latter was not immune-complex bound in the majority of all cases and (2) neither isolation of ^{131}I -OC125-F(ab')₂ nor of immune complex was restricted to tissue of increased CA-125 expression. Moreover, we could isolate a radioactive, high molecular weight immune complex as well as free ^{131}I -OC125-F(ab')₂ from 1/3 not tumor-infiltrated and 1/8 malignant and from 1/3 tumor-free and 5/8 malignant cytosols, respectively.

Several biological features of tumors such as size, localization, and heterogeneity of antigen expression may determine the extent of mAb-uptake [44–47]. This in turn influences circulating mAb levels and antibody pharmacokinetics [48,49]. Boerman et al. [50], using a radiolabelled anti-CEA-mAb in a nude mouse xenograft model showed that tumor mass had a significant impact on uptake, pharmacokinetics, and biodistribution of mAbs [50]. In contrast, earlier studies by Dueswell et al. [51] and Blumenthal et al. [52] had demonstrated increased radioactive uptake of smaller tumors compared to larger ones. The reasons for this biological behavior of small tumors may be their better vasculature, or fewer necrotic areas. Smaller tumor nodules secrete lower amounts of TAA; therefore immune-complex-formation in the circulation, increasing mAb clearance, is minimized, i.e., more mAb can localize to cell membrane-bound TAA [51,52]. Additionally nonspecific uptake of ^{131}I -OC125-F(ab')₂ by tissue not infiltrated by tumor cells may play a role.

Our chromatographic findings are explained by a model of antigen-specific and not-antigen-specific cellular antibody uptake (Fig. 4). Whereas Figure 4A shows a potentially CA-125-specific uptake of ^{131}I -OC125-F(ab')₂-fragment and intracellular trafficking of the latter [50,53–56], Figure 4B demonstrates nonspecific immunological interaction of ^{131}I -OC125-F(ab')₂ and a cross-reacting antigen, e.g., transferrin, which is highly expressed on the surface of various tumor cells [57–60] and benign tissue [61]. Recently, processes as described in Figure 4A have been demonstrated for antigen-antibody interaction in vitro [56,62]. Those postulated in Figure 4B, however, remain speculative.

Taken together, our results suggest that the specific immune recognition between CA-125 and ^{131}I -OC125-F(ab')₂ plays a role in immunoscintigraphy. However, the importance of nonspecific processes leading to increased uptake of radiolabelled mAb in benign as well as malignant tissue may have been underestimated so far.

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Fig. 4. Endocytosis model of antigen specific (A) and nonspecific (B) intracellular uptake of ^{131}I -OC125-F(ab')₂, and intracellular mAb trafficking. A shows a potentially CA-125-specific uptake of ^{131}I -OC125-F(ab')₂-fragment and respective radiochromatographic profiles (1–5). After the immune-complex is internalized (1), mAb and antigen dissociate (2), and the antigen is recycled (3), whereas the mAb is broken down into lower molecular weight substrates (4,5). B demonstrates nonspecific immunological interaction of ^{131}I -OC125-F(ab')₂ and a cross-reacting antigen, e.g., transferrin, which is highly expressed on the surface of various tumor cells.

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