J. Clin. Chem. Clin. Biochem.
Vol. 27, 1989, pp. 643–652

Limited Improvement of Tumour Diagnosis by the Simultaneous Determination of Carcinoembryonic Antigen (CEA) and of a Tumour-Associated CEA-Related Antigen of M_r 128000 in Serum

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(Received April 6/July 18, 1989)

Summary: Two biotin-avidin based enzyme immunoassays were developed using three monoclonal anti-CEA antibodies with distinct epitope- and antigen-specificities. A broadly cross-reactive monoclonal anti-CEA antibody (T84.1) was immobilized on a solid support. Either monoclonal antibody T84.66 or CEA.11 was used as the second, biotin-labeled monoclonal antibody. Both antibodies do not cross-react with normal granulocytes or bile canaliculi. Monoclonal antibody CEA.11 binds to CEA and to an antigen with a relative molecular mass of 128000 present in extracts from solid carcinomas. Monoclonal antibody T84.66 does not cross-react with the antigen of M_r 128000. After adsorption of tumour extracts to a CEA-specific immunosorbent, residual activity was measurable by the CEA.11 assay, but not by the T84.66 assay. Serum samples (n = 726) from patients with malignant and non-malignant disease, as well as from healthy volunteers, were analysed by both immunoassays and by two commercial CEA immunoassays. In comparison with the T84.66 assay and the commercial assays, the CEA.11 assay did not significantly increase the sensitivity or specificity of tumour diagnosis.

Introduction

The carcinoembryonic antigen (CEA) is a marker of human colorectal carcinomas. CEA is a well defined glycoprotein with a relative molecular mass (M_r) of 180000 (for review see l.c. (1)). Antibodies induced against CEA may cross-react with a variety of antigens present in normal and cancerous tissues, body fluids, effusions, and faeces. As shown recently, these antigens are the products of at least three distinct genes: the gene encoding CEA (2, 3), the non-specific cross-reacting antigen (NCA) (4, 5), and the biliary glycoprotein I (6, 7). According to a recent study, the primary transcript of the biliary glycoprotein I gene is modified by alternative splicing (7). This finding suggests the existence of several biliary glycoprotein I-related antigens. Our own data indicate that normal plasma contains two different biliary glycoprotein I-related antigens (8, 9).

In previous studies, we described five monoclonal antibodies against CEA which bind to different epitopes of the antigen (10–12). Two of these antibodies preferentially bind to tumour tissues (10, 13). The antigen specificities of both antibodies are distinct. One monoclonal antibody (CEA.11), in addition to CEA, binds a tumour-associated antigen with a relative molecular mass of 128000. The second monoclonal antibody (T84.66) does not cross-react with the latter antigen (14). This antigen may be similar to or identical with CEA_{low} described by *Hammerström* and co-workers (15). Since it is not yet established whether the CEA-related antigen of M_r 128000 is a product of an as yet undescribed member of the CEA gene family, whether it is a product of alternatively spliced mRNA, or whether it is generated by posttranslational modifications, the antigen will be designated "CEA-related antigen of M_r 128000 (CRA-128)".

Despite of the fact that the nature of CRA-128 is not firmly established, the antigen specificity of monoclonal antibody CEA.11 has been confirmed by other investigators (16, 17). *Suzuki* et al. (16) described an immunoassay which measured an antigen similar to CRA-128 in addition to CEA. In some serum specimens from tumour patients, significantly elevated antigen concentrations remained after adsorption to a CEA-specific immunadsorbent. However, no information on the relative frequency of such specimens among sera from tumour patients was given.

Since CRA-128 occurs in a high percentage of tumour tissues (unpublished observation), we addressed the questions of whether the serum determination of CEA + CRA-128 can increase the diagnostic sensitivity of tumour diagnosis and whether CRA-128 may be associated with tumours at certain sites.

Materials and Methods

Monoclonal antibodies and CEA standard

The monoclonal anti-CEA antibodies were induced, characterized and purified as described (10, 11). The monoclonal anti-hepatitis B surface (Hbs) antigen antibody was kindly donated by Hoffmann-La Roche, Basel, Switzerland. All monoclonal antibodies used in this study were of subclass IgG_1. The CEA standard preparation, which was purified according to *Pritchard & Todd* (18), was supplied by Dr. *Shively*, Beckman Research Institute of the City of Hope, Duarte, CA, U.S.A.

Biotinylation procedure

Monoclonal antibodies were biotin-labelled as described (19, 20). As antibodies, the monoclonal antibodies T84.66 and CEA.11 were used. Molar ratios of N-hydrosuccinimidobiotin (M_r = 341.4) to IgG (M_r = 150000) in the range of 10–600 were tested, in order to find which ratio yields the highest sensitivity in the solid phase EIA. Using monoclonal antibody T84.66, a ratio of 60–70 was found to be optimal. This ratio was also adopted for monoclonal antibody CEA.11.

Preparation of $F(ab)_2$-fragments

The IgG_1 fraction of monoclonal antibody T84.1 was purified from ascitic fluid over protein-A sepharose (21). IgG_1 (6–12 g/l) was digested with pepsin as described (22). The cleavage products were characterized by gel permeation HPLC (TSK 3000, LKB, Gräfelfing, F.R. Germany) and SDS-PAGE under reducing and non-reducing conditions.

CEA-immunoassays

Polystyrene beads were coated with the IgG_1 fraction or $F(ab)_2$-fragments of monoclonal antibody T84.1. Per 100 beads, 30 ml of a 20 mmol/l sodium acetate buffer, pH 5.5, containing 10 mg/l of antibody IgG or antibody fragments, respectively, was used. Coating was performed overnight at room temperature. Blocking of unspecific binding sites and storage of the beads was performed in phosphate buffered saline containing 20 mg/l bovine serum albumin. The CEA standard was dissolved in phosphate buffered saline containing 10 mg/l bovine serum albumin. Either monoclonal antibody T84.66 or CEA.11 was used as the second biotin-labelled antibody. The antibodies were dissolved in 0.1 mol/l potassium phosphate buffer, pH 7.3, containing foetal calf serum (200 ml/l), 9.2 ml/l Tween 20 and 30 ml/l normal mouse serum. The final concentration of biotin-labelled monoclonal antibody T84.66 was 2.7 mg/l. A final concentration of 5.7 mg/l was used for $F(ab)_2$-fragments from monoclonal antibody CEA.11.

Sample tubes contained the following solutions (volumes in brackets): serum (100 µl); phosphate buffered saline/bovine serum albumin (50 µl); solutions of biotinylated monoclonal antibodies (50 µl). Standard tubes were prepared as follows: normal serum from a serum pool with a CEA-concentration of < 1 µg/l (100 µl); CEA-standard in phosphate buffered saline/bovine serum albumin (50 µl); solutions of biotinylated monoclonal antibodies (50 µl). Subsequently, immobilized monoclonal antibody T84.1 was added. For the T84.66 EIA, undigested IgG_1 was used. The incubation time was 1 h (37 °C). For the CEA.11 EIA, immobilized $F(ab)_2$-fragments from monoclonal antibody T84.1 were incubated for 17 h at room temperature. After the appropriate incubation time, the beads were washed 3 times with 0.1 mol/l potassium phosphate buffer, pH 7.3. Subsequently, 200 µl of avidin-peroxidase conjugate (Sigma, Taufkirchen, F.R. Germany) diluted in 0.2 mol/l potassium phosphate buffer, pH 6.5, containing 200 ml/l biotin-free foetal calf serum, was added. The concentration of avidin-peroxidase conjugate was chosen as the highest concentration yielding negligible unspecific binding. After 30 min at room temperature, the beads were washed 3 times in 0.1 mol/l citrate buffer, pH 5.0, and 300 µl of substrate solution containing 6 mmol hydrogen peroxide and 40 mmol *o*-phenylenediamine (Sigma) per litre of 0.1 mol/l citrate buffer, pH 5.0, were added. After incubation for 30 min at room temperature in the dark, the reaction was stopped with 2 ml of a 1 mol/l HCl-solution. The absorbance was determined at 492 nm.

Immunoassays for anti-mouse IgG binding activity

In these assays, antibody T84.1 was replaced by a monoclonal anti-HBs antibody on the solid phase. Antibody CEA.11 or T84.66 was used as the second antibody. With the exception of these modifications, the single incubation step assays were performed as in the original CEA assays.

Extraction of tissues and Western blot

The extraction of tumour tissues, immunosorbent purification, and Western blots were performed as described (14).

Commercial CEA-immunoassays

Commercial CEA-immunoassays were obtained from Hoffmann-La Roche, Basel, Switzerland, and from Abbott, Wiesbaden, F. R. Germany. The assays were performed according to the instructions of the manufacturers.

Serum specimens

CEA determinations were performed in 726 serum samples using four different immunoassays (2 assays developed in our laboratory, 2 commercial assays). Serum samples were obtained from the following groups of persons or patients (number of samples in brackets):

Healthy blood donors, non-smokers (98);
healthy blood donors, smokers (42);
gastric cancer (21);
pancreatic cancer (10);
cervical cancer (13);
ovarian cancer (31);
mammary cancer (60);
lung cancer (67);
colorectal cancer (42);
other carcinomas (49);
ulcerative colitis (8);
Crohn's disease (8);
other non-malignant diseases of the gastrointestinal tract (52);
liver cirrhosis (46);
other non-malignant liver diseases (14);
pancreatitis (13);
non-malignant diseases of the gall bladder (9);
non-malignant lung disease (51);
non-malignant renal disease (60);
other non-malignant conditions (32).

Statistical procedures

The following non-parametric tests were used: *Spearman*'s coefficient of rank correlation, *Kolmogorov-Smirnov* test, and *Wilcoxon*'s matched pairs signed rank test.

Results

Establishment of immunoassays, elimination of anti-mouse IgG activity in sera

Two additive solid-phase enzyme immunoassays were established using three monoclonal anti-CEA antibodies with different epitope- and antigen-specificities. For both immunoassays, the broadly cross-reactive, high affinity antibody T84.1 was adsorbed to a solid support. Either monoclonal antibody T84.66 or monoclonal antibody CEA.11 was used as the second antibody. For simplicity, the immunoassay with monoclonal antibody T84.66 as second antibody will be designated "T84.66 assay", and the immunoassay with monoclonal antibody CEA.11 as second antibody will be designated "CEA.11 assay". In addition to the above immunoassays, an immunoassay for the detection of anti-mouse IgG binding activity in sera was developed. Instead of monoclonal antibody T84.1, a monoclonal anti-HBs antibody was immobilized on a solid support. Either monoclonal antibody CEA.11 or T84.66 was used as the second antibody. This immunoassay, in which no CEA-related antigens are measured, will be designated "interference assay". All immunoassays were based on the biotin-avidin system. Avidin-peroxidase conjugate was added to the biotin-labelled second antibody bound to the antigen. The colour reaction was developed with H_2O_2/*o*-phenylenediamine as substrate.

Initially, both the T84.66 and CEA.11 immunoassay versions, were established with native IgG_1 of monoclonal antibody T84.1 adsorbed to the solid support. In both initial versions, normal mouse serum was not added. When normal sera were investigated by the initial version of the CEA.11 immunoassay, CEA-values exceeding 10 µg/l were occasionally observed. This effect was absent in the T84.66 immunoassay. A preliminary comparison of the CEA-concentrations determined by the T84.66 and CEA.11 immunoassays with the values obtained from a commercial assay (Abbott) revealed significant differences in a serum sample from a patient who had received therapeutic doses of a murine monoclonal antibody.

In order to eliminate the unspecific interferences, serum specimens from the above patient and from an apparently healthy person were further analysed. After addition of 10 ml/l mouse serum (final concentration) to the T84.66 and CEA.11 immunoassay, respectively, the CEA-concentrations in the patient's sample decreased to concentrations comparable with those measured in a commercial assay (tab. 1). The addition of normal mouse serum did not affect the elevated CEA level measured in the normal serum sample by the CEA.11 immunoassay (tab. 1). The interfering serum samples were further analysed by an interference assay in which the anti-CEA monoclonal antibody adsorbed to the solid phase was replaced by a monoclonal antibody of identical subclass (IgG_1) with a specificity unrelated to CEA. As shown in figure 1, the normal serum interfered only after a prolonged incubation time, irrespective of which anti-CEA monoclonal antibody was used as second antibody. In contrast, the response with tumour serum approached a maximum after an incubation time of only 1 h. The interfering effect of the normal serum in the CEA.11 immunoassay could not be suppressed by the addition of non-immune mouse IgG at concentrations of up to 1 mg/reaction volume (not shown). In order to eliminate the unspecific interference of the normal serum in the CEA.11 immunoassay, $F(ab)_2$-fragments from monoclonal antibody T84.1 were prepared. As shown in table 1, the measured CEA value decreased to 1.0 µg/l when $F(ab)_2$ instead of native IgG fragments were adsorbed to the solid support. With the revised version of the CEA.11

Tab. 1. CEA concentrations [µg/l] determined in two serum samples by different solid phase immunoassays.

Sample	Immunoassay				
	T84.1/T84.66		T84.1/CEA. 11		T84.1, F(ab)$_2$/CEA. 11
	Without mouse serum	With[a])	Without mouse serum	With[a])	Without mouse serum
Patient	55.5	26.4	55.5	21.4	16.2
Normal	0.9	0.1	17.7	20.8	1.0

[a]) mouse serum added: 10 ml/l

Tab. 2. Assay characteristics of two double monoclonal, biotin-avidin based enzyme immunoassays for CEA.

Assay parameter	Immunoassays	
	T84.66	CEA. 11
Sensitivity[a])		
CEA [µg/l]	0.55	0.63
Measuring range	0.55–64.0	0.63–64.0
Recovery		
CEA added [µg/l]	4.8	4.8
CEA found [µg/l]	5.7	4.5
Recovery [%]	119	94
CEA added [µg/l]	19.6	19.6
CEA found [µg/l]	19.3	16.6
Recovery [%]	99	84
Between run precision		
Mean CEA [µg/l]	4.8	5.2
SD	0.55	0.80
CV [%]	11.5	15.3
n	8	15
Mean CEA [µg/l]	17.2	18.5
SD	1.50	2.50
CV [%]	8.7	13.4
n	8	15
Upper limit, normal range		
95% percentile [µg CEA/l]	4.2	6.5

[a]) concentration corresponding to the zero standard + 3 SD

Tab. 3. CEA concentrations in tumour extracts measured at different dilutions.

Tumour	Reciprocal dilution	CEA-concentration [mg/l]	
		T84.66	CEA. 11
Rectal carcinoma	128×10^4	30.6×10^3	71.8×10^3
	256×10^4	27.4×10^3	86.3×10^3
	512×10^4	29.0×10^3	80.9×10^3
Gastric carcinoma	5×10^3	106.3	246.6
	10×10^3	114.9	260.0
	20×10^3	100.3	243.6
	40×10^3	112.4	259.8
Lung carcinoma	20	0.8	0.7
	40	0.8	0.7
	80	0.8	0.7

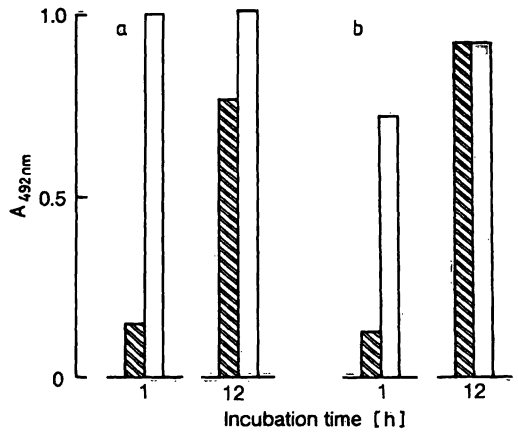

Fig. 1. Effect of incubation time on the absorbance in the CEA. 11 and T84.66 interference assays (anti-HBs antibody on the solid phase) using a normal serum sample (▨) and a serum sample from a patient treated with murine monoclonal antibodies (□). Labelled monoclonal antibodies:
a) CEA. 11;
b) T84.66.

immunoassay, CEA concentrations exceeding 10 µg/l were not observed in sera from healthy blood donors or in sera from healthy smokers.

Characterization of immunoassays

The characteristics of the final assay versions of the immunoassays (sensitivity, measuring range, recovery, precision) are shown in table 2. In dilution experiments, the dose-response curves of tumour extracts ran parallel with the standard curves (tab. 3).

Specificities of immunoassays

The specificities of the two immunoassays were characterized by the use of tumour extracts in which high amounts of the tumour-associated CEA variant of M_r 128 000 could be demonstrated by Western blots. Figure 2 shows a Western blot of an extract from a primary rectal carcinoma eluted from a T84.1 immunosorbent. Monoclonal antibody T84.66 (lane 1)

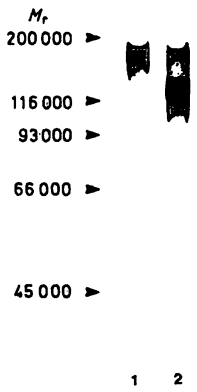

Fig. 2. Western blots of CEA and CRA-128 isolated from a primary rectal carcinoma. The rectal carcinoma extract was purified over a monoclonal antibody with broad cross-reactivity (T84.1) prior to Western blot analysis. Lane 1: monoclonal antibody T84.11; lane 2: monoclonal antibody CEA. 11
For methodological details see l. c. (12).

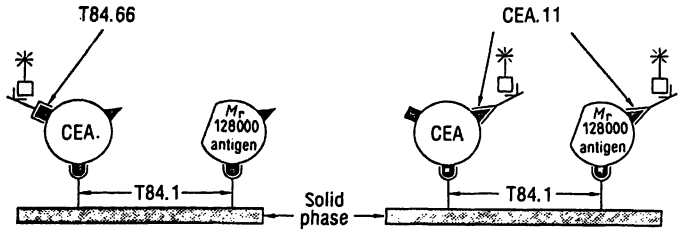

Fig. 3. Schematic presentation of the antigen specificities of the T84.1/T84.66- and T84.1/CEA. 11-immunoassays.
> biotin; □ avidin

exclusively binds to an antigen with a M_r of only 177 000. According to M_r and immunoreactivity, e. g., binding of each fo the five monoclonal anti-CEA antibodies with different epitope specificities, this antigen is probably identical with CEA. In addition to CEA, monoclonal antibody CEA. 11 binds an additional antigen with a M_r of 128 000 (lane 2). Antigen concentrations were determined in extracts from the rectal and from a gastric carcinoma prior to and after adsorption to a T84.66 immunosorbent. In both tumour extracts, the CEA concentrations determined by the CEA. 11 immunoassay were higher than those measured by the T84.66 immunoassay (tab. 4). After adsorption to a T84.66 immunosorbent, no CEA could be measured by the T84.66 immunoassay. In the CEA.11 immunoassay, a significant CEA-activity remained after immunoadsorption. Figure 3 gives a schematic presentation of the specificities of both immunoassays.

Tab. 4. CEA-concentrations in tissue extracts before and after adsorption to a T84.66 immunosorbent, determined by two different immunoassays.

Tissue	CEA. 11 assay		T84.66 assay	
	before	after	before	after
Rectal carcinoma [g/l]	79.8	55.4	29.0	<0.001
Gastric carcinoma [mg/l]	252.5	126.6	108.5	<0.001

Determination of CEA in serum samples

CEA was determined in a total of 726 serum samples from healthy persons and from patients with non-malignant and malignant disease, using four different immunoassays. Table 5 shows the distribution of measured CEA-concentrations in sera from healthy blood donors. In the commercial assays, the absorbance values of significant fractions of samples were below the absorbance values of the zero standards. This finding affects the values obtained for the 95%-percentiles.

Tab. 5. Percentiles of CEA-concentrations [µg/l] in healthy non-smokers (n = 98) as determined by four different CEA immunoassays.

Assay	Percentiles [%]					
	10	25	50	75	90	95
Abbott	0.0	0.0	0.4	0.9	1.9	2.6
Roche	0.0	0.3	0.6	1.1	1.6	1.9
T84.66	0.1	0.8	1.7	2.7	3.7	4.2
CEA. 11	1.4	1.8	2.5	3.7	5.3	6.5

The correlation of CEA-concentrations in serum samples from patients with malignant disease is demonstrated in figure 4 and table 6. The T84.66 immunoassay shows a good correlation with two commercial assays. The CEA.11 immunoassay shows a lower correlation to the T84.66 assay and to the commercial assays.

Figure 5 demonstrates the distributions of CEA concentrations in different malignant and non-malignant diseases. In colorectal cancer, the median of the CEA concentrations measured by the CEA. 11 assay is higher than the corresponding median of the T84.66 assay. This difference, however, is not statistically significant. In mammary cancer, the CEA-concentrations obtained by the T84.66 assay were significantly higher than the corresponding concentrations determined by the CEA. 11 assay. It is interesting to note that in the group of patients with non-malignant kidney disease, including a high percentage of patients with chronic renal failure, the CEA. 11 assay measured significantly higher concentrations than the T84.66 immunoassay.

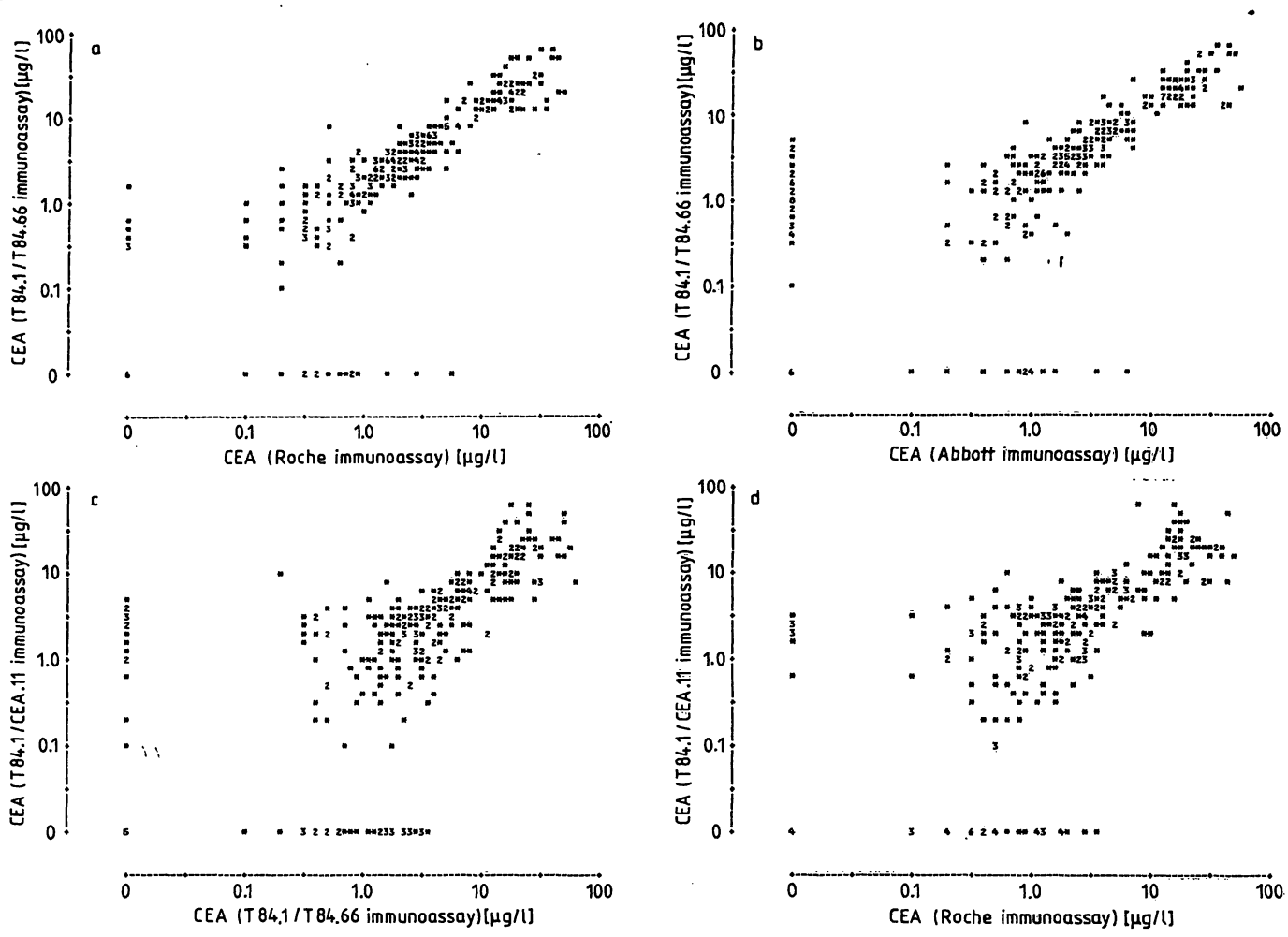

Fig. 4a—d. Correlation of measured CEA-concentrations in serum specimens from 293 patients with malignant disease as determined by different CEA immunoassays. In the case of CEA-concentrations above the upper limit of the measuring range, the concentration in the diluted samples are shown.

Tab. 6. *Spearman*'s coefficient of rank correlation of different CEA-immunoassays in malignant disease (n = 293).

	Roche	T84.66	CEA.11
Abbott	0.90	0.86	0.77
Roche	—	0.91	0.76
T84.66	0.91	—	0.74

The discrimination by the T84.66 and CEA.11 immunoassays between sera from normal persons and patients with non-malignant disease on the one hand and with malignant disease on the other hand were analysed by the *Kolmogorov-Smirnov*-test (fig. 6). When sera from patients with colorectal cancer are compared with sera from patients with non-malignant gastrointestinal and liver disease, a higher level of significance is obtained for the CEA.11 assay than for the T84.66 assay. In contrast, the T84.66 assay shows a higher level of significance for carcinomas of the lung vs. benign lung disease and for mammary cancer vs. normal blood donors.

Discussion

Monoclonal anti-CEA antibodies bind to different human carcinomas such as colorectal, mammary, and lung carcinomas. According to their reactivity with normal tissues, two major subgroups can be distinguished. One subgroup is characterized by antibody-binding to normal granulocytes and/or bile canaliculi, whereas monoclonal antibodies of the second subgroup do not significantly cross-react with these normal tissues (11, 13). In previous studies, we described five monoclonal anti-CEA antibodies which bind to different epitopes on CEA (10—12). Three of these antibodies cross-react with granulocytes and/or bile canaliculi. One of three cross-reactive antibodies (T84.1) binds to products of each of the three CEA-gene families described so far e.g. CEA, the non-specific cross-reacting antigen, and the biliary glycoprotein I (6, 8, 14). Monoclonal antibody T84.1 binds CEA with high affinity (10, 22). The anti-CEA monoclonal antibodies, which show no significant cross-reactivity with granulocytes and bile canaliculi, show a distinct antigen specificity. In addition to binding

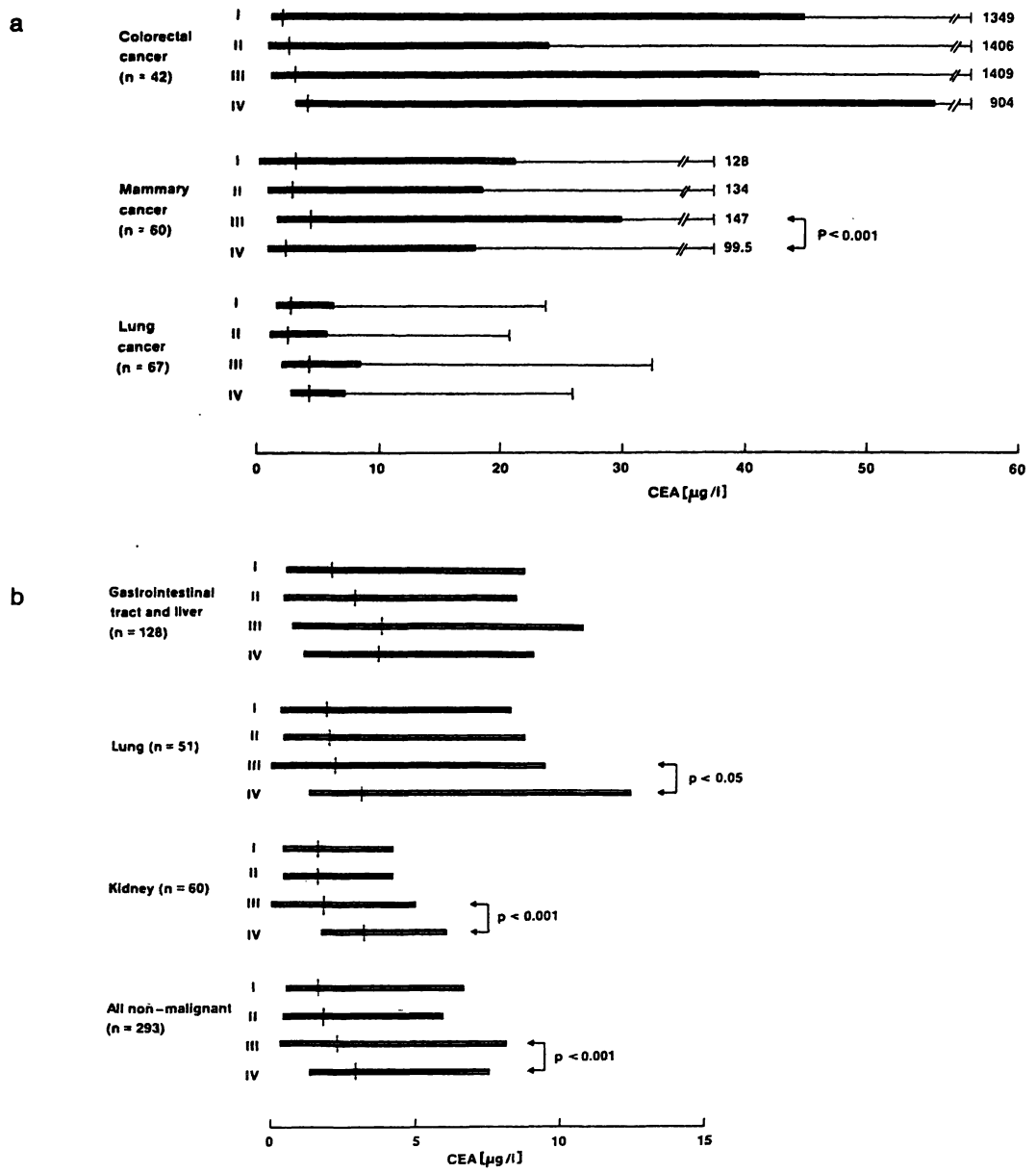

Fig. 5. Distribution of CEA-concentrations as measured by 4 different immunoassays in malignant and non-malignant diseases. (I) Abbott-assay; (II) Roche-Assay; (III) T84.1/T84.66 assay; (IV) T84.1/CEA.11 assay.
The *Wilcoxon* test was applied only to assays III and IV, because identical CEA-standard preparations were used in both assay versions. This avoids the introduction of bias, which would arise from different specific activities of standard preparations.
(a) malignant disease: horizontal bars indicate the concentration range between the 25% and 75% percentiles. The numbers refer to CEA-concentrations corresponding to the 90% percentiles;
(b) non-malignant disease: horizontal bars indicate the concentrations between the 10% and 90% percentiles.

to CEA, monoclonal antibody CEA.11 binds to a tumour-associated antigen with an M_r of 128000 (CRA-128). Monoclonal antibody T84.66 does not cross-react with CRA-128 (14). Monoclonal antibodies with binding properties similar to monoclonal antibody CEA.11 have been described by *Suzuki* et al. (16) and *Schwarz* et al. (17).

The relationship of CRA-128 to the different products of the CEA gene family has not been established. The relative molecular mass of the antigen is similar to the M_r of CEA_{low} (125000) described by *Hammerström* and co-workers (15). Structural studies suggest a close relationship to CEA (*J. Shively*, personal communication). Since CRA-128 does not bind monoclonal antibodies against the non-specific cross-reacting antigen (unpublished observation), it is different from antigens related to the non-specific cross-reacting antigen. According to *Barnett* et al. (7), the M_r of CRA-128 would be compatible with the product of the unspliced transcript of the biliary glycoprotein I gene published recently (6).

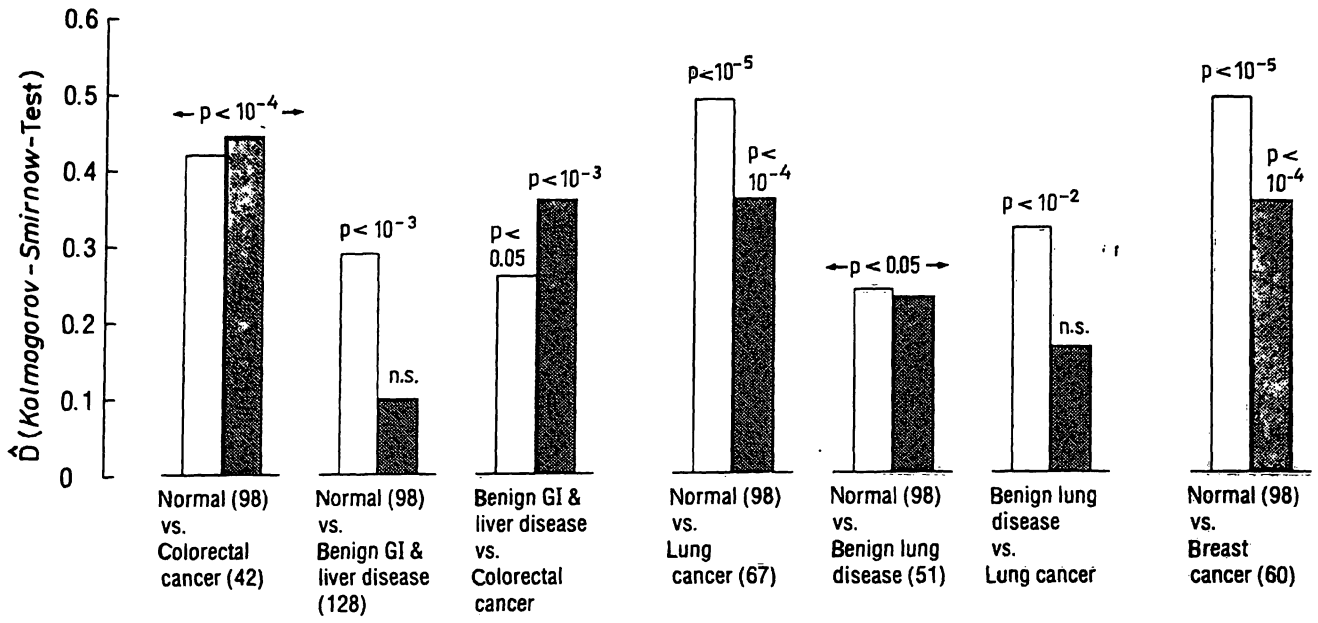

Fig. 6. Discrimination between malignant and non-malignant conditions by two different CEA-immunoassays. Results of *Kolmogorov-Smirnov*-tests.
□, T84.1/T84.66 immunoassay; ▨, T84.1/CEA 11 immunoassay.

According to recent Western blot studies, CRA-128 was found in 6/9 malignant tissues or effusions (unpublished observation). Furthermore, *Suzuki* et al. (16) provided evidence that a related antigen is present in serum samples from patients with malignant disease. These data indicate that the determination of CRA-128 in serum may be of value in tumour diagnosis.

In order to answer the question of whether CRA-128 may appear in the blood of tumour patients, two different additive solid phase immunoassays were established. In both assays, the broadly cross-reactive monoclonal antibody T84.1 was immobilized on a solid support. Either monoclonal antibody T84.66 or monoclonal antibody CEA. 11 was used as the second antibody. The specificities of these immunoassays were analysed by the use of tumour extracts, in which both CEA and CRA-128 had been shown to be present by Western blots. After immunoadsorption of the extracts to a T84.66 immunosorbent, no residual CEA activity could be detected in the T84.66 immunoassay. In contrast, residual antigen binding was demonstrated by the CEA. 11 immunoassay. These data show that the CEA. 11 immunoassay detects an antigen which does not interfere in the T84.66 assay.

Like other investigators (23—26) we found that anti-mouse IgG activity in sera of normal persons and tumour patients can give rise to falsely elevated antigen concentrations. In the T84.66 immunoassay, in which antigen and antibodies are incubated for 1 h, the unspecific interferences could be blocked by the addition of 10 ml/l mouse serum. Because of the longer incubation time of the CEA. 11 assay (17 h), this assay was more sensitive to interference by anti-mouse IgG binding substances of low affinity, which could not be eliminated by the addition of normal mouse IgG even at high concentration. The unspecific binding effects were eliminated by the use of $F(ab)_2$-fragments of monoclonal antibody T84.1 on the solid phase.

Serum samples from patients with malignant and non-malignant disease, as well as from apparently healthy persons, were analysed by the above immunoassays as well as by two commercial assays. The correlation between the T84.66 assay and the commercial assays was comparable with the correlation between the commercial assays. A poorer correlation was observed between the CEA. 11 assay and the remaining assays. This finding may be related either to the distinct antigen specificity of the CEA. 11 assay or to its higher variance. The higher variance of the CEA. 11 assay is probably due to the lower affinity of monoclonal antibody CEA. 11 in comparison with monoclonal antibody T84.66 (10).

The distributions of CEA concentrations in sera of patients with colorectal cancer vs. non-malignant gastrointestinal and liver disease were discriminated by the CEA. 11 assay at a higher level of significance than by the T84.66 assay (fig. 6). However, a direct comparison of the CEA concentrations determined by the CEA. 11 and T84.66 immunoassays by *Wilcoxon*'s matched pairs signed rank test revealed no

significant differences in patients with colorectal cancer or in patients with benign gastrointestinal and liver disease. In the diagnosis of mammary and lung cancer, the CEA.11 immunoassay yielded significantly lower CEA concentrations than the T84.66 assay. The diagnostic value of the CEA.11 assay is further limited by the finding that significantly higher CEA values were observed in several non-malignant conditions, notably in patients with non-malignant kidney disease, including a significant number of patients with chronic renal failure. It has been reported that the rate of false positives in chronic renal failure differs considerably in different CEA immunoassays. This finding may be explained by the epitope specificities of the antibodies.

The results reported here indicate that CRA-128, a CEA related tumour-associated antigen with a relative molecular mass of 128000, is not significantly elevated in the sera of patients with malignant disease. *Suzuki* et al. (16) described an immunoassay with an antigen specificity similar to the CEA.11 assay. In three serum specimens from tumour patients, significantly elevated antigen concentrations remained after adsorption to a CEA-specific immunosorbent. No information on the relative frequency of such serum specimens among sera from tumour patients were given. Though CRA-128 was detected in 6/9 extracts from malignant tissues or effusions in malignant diseases (unpublished observation), the release of CRA-128 into the circulation seems not to be significant. A possible explanation for this finding are the differences between the membrane anchors of CEA-related antigens. CEA is anchored to the cell membrane via phosphatidylinositol (27–29). It has been suggested that the cleavage of CEA from the membrane by endogenous phospholipase C may be critical for the release into the blood stream (28). In contrast to the CEA-gene, the gene coding for biliary glycoprotein I predicts a transmembraneous and a cytoplasmic domain. If the M_r 128000 antigen is anchored via the latter mechanism, as suggested by *Barnett* et al. (7), it would not be easily released into the circulation.

Acknowledgement

We thank Dr. *John Shively* for the supply of monoclonal antibodies and of purified CEA. This work was supported by grant Wa-473/4-1 from the Deutsche Forschungsgemeinschaft.

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