Radiolabeled Chimeric Anti-CEA Monoclonal Antibody Compared with the Original Mouse Monoclonal Antibody for Surgically Treated Colorectal Carcinoma

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Biodistribution and tumor uptake of a chimeric human-mouse monoclonal antibody (MAb) and the original mouse MAb have been comparatively studied. **Methods:** Eighteen patients with suspected colorectal cancer scheduled for surgery underwent immunoscintigraphy with ^{123}I-labeled chimeric anti-CEA MAb. Iodine-125 and ^{131}I trace-labeled chimeric and original mouse MAb were simultaneously injected for biodistribution studies. **Results:** Similar serum kinetics and a low immunogenicity were observed for both antibodies. Mean binding capacity to CEA measured in PBS after radiolabeling was identical for both MAbs and it was slightly decreased when measured in serum 1–4 hr after injection. Radiochromatograms of patients sera showed immune complex formation related to the amount of circulating CEA. Postoperative ex vivo radioactivity counting in tissue samples revealed similar antibody distributions with notably similar antibody uptakes in tumors. High tumor uptakes (between 0.02 to 0.06% injected dose per g) were observed in 3 of 13 patients operated for primary or metastatic colorectal cancer. **Conclusion:** In this dual-label technique, the radioiodinated anti-CEA IgG_4 chimeric MAb and the original mouse IgG_1 MAb were shown to have very similar behavior in colorectal cancer patients.

Key Words: chimeric monoclonal antibody; carcinoembryonic antigen; tumor uptake; colorectal carcinoma

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The concept of using monoclonal antibodies (MAbs) as carriers to deliver cytotoxic drugs, radioisotopes or toxins more selectively into tumors continues to stimulate experimental and clinical research (*1–3*). While radiolabeled antibodies have been shown to be useful for therapy of human carcinomas in nude mice (*4–8*), and of more radiosensitive tumors such as B cell lymphomas in patients (*9,10*), they have the additional advantage that they can yield precise information concerning biodistribution and tumor dosimetry. These data can be useful for the selection of the most appropriate type of antibody for radioimmunoscintigraphy and radioimmunotherapy (*9–19*).

Monoclonal antibodies used for tumor targeting are generally of murine origin and can elicit human anti-mouse IgG antibodies (HAMA) in many patients (*20–22*). High titers of HAMA are frequently observed after repeated injections of MAbs or when they are coupled to other immunogenic proteins such as toxins or enzymes. Chimerization of antibodies represents a first step toward reducing the immunogenicity of MAbs for application in patients (*12,20,23,24*). Immunogenicity is, however, still a problem for certain chimeric antibodies (*25*). Further humanization by grafting only the complementary determinant regions (CDR) of the mouse MAb DNA into human IgG DNA as described for the reshaped MAbs (*26,27*) or production of human antibodies (*28–30*) might be necessary to further reduce immunogenicity.

Chimeric antibodies allow the comparison of the biological behavior of selected human IgG subclasses and verification of their potential for tumor targeting. They can also be used to study their effector functions and the possibility of obtaining fragments for immunoscintigraphy and radioimmunotherapy (*12,23,31,32*). We have used such chimeric anti-CEA MAbs of different IgG subclasses in experimental animal models and could show that both the intact IgG_2 MAb and its $F(ab')_2$ fragment demonstrated excellent tumor targeting and in vivo stability (*31*). The tumor localization capacity of the intact IgG_4 chimeric MAb has been shown to be identical to the original mouse MAb in tumor bearing nude mice (*23*). However, the in vivo behavior of chimeric IgG_4 $F(ab')_2$ fragments was unsatisfactory in mice (*32*) and this fragment was therefore not used in patients.

Here we compare the intact chimeric anti-CEA MAb

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(human IgG_4) with its original murine IgG_1 MAb by differential labeling, co-injection and ex vivo measurement of tumor and normal tissue radioactivity distributions in patients.

METHODS

Patients

Patients selected for the comparative biodistribution study of chimeric and mouse anti-CEA MAb (n = 18) were suspected of colorectal cancer. Surgery was planned 1 to 5 days after MAb injection. Immunoscintigraphy was performed with the aim of staging more precisely the disease. Definitive diagnosis in these patients was: primary colorectal adenocarcinoma in nine patients, one of whom had initial liver metastasis, one local recurrence and five liver metastases. One patient had an ovarian carcinoma, one patient a benign polyp and one patient with a suspicion of liver metastasis had no tumor at the time of surgery. For three of these patients, surgery was either performed too late after antibody injection or was cancelled. For determination of the serum half-life of chimeric MAb, nine additional patients were included who were only injected with chimeric MAb and had a follow-up time of 2 to 6 days. Finally, one patient included here had surgery for a liver metastasis 8 days after injection of 2 mg of the IgG_4 chimeric MAb together with 30 μg of a chimeric MAb with the same variable regions but with human IgG_2 constant domains (*32*).

Monoclonal Antibodies

The mouse-human chimeric monoclonal antibody used here is of human IgG_4 subclass and was derived from the murine MAb CE25/B7 (*6,23*) (CIBA GEIGY, Basel, Switzerland). This MAb is directed against the epitope Gold 4 of CEA (*33*). It has a high specificity for CEA (*34*) and does not crossreact with NCA-55 or NCA-95 (*35*) or other granulocyte glycoproteins. Theoretically, the murine and chimeric antibody subclasses both have minimal effector functions: they should not react with Fc receptors on monocytes and macrophages and should not activate the complement cascade (*36*). The original mouse MAb has been used in patients both for immunoscintigraphy (*14*) and in a first trial of radioimmunotherapy (*1*). Mouse MAb was prepared from ascites by ammonium sulfate precipitation and ion exchange chromatography (*6*). Chimeric MAb was produced in Sp2/0 cells transfected with a single vector containing both the chimeric heavy and light chain genes (*23*).

Radiolabeling

Two to 4 mg of chimeric MAb were labeled by the iodogen method with 15 to 30 mCi of ^{123}I for immunoscintigraphy (final specific activities were 2 to 4.5 mCi per mg antibody and 4 to 18 mCi were injected per patient). Batches of 0.2 mg chimeric MAb and of original mouse MAb were separately labeled using 250 μCi of ^{125}I and of ^{131}I, respectively (final specific activities were 0.8 to 1.1 μCi per μg antibody for the trace labelings). For three patients, the trace labelings were reversed and the chimeric MAb was labeled with ^{131}I and the mouse MAb with ^{125}I. Using the paired labeling method, it is possible to analyze the biodistribution and tumor localization capacity of the two MAbs in the same patient and thus compare results obtained under identical biological conditions. The ^{123}I-labeled and the trace-labeled MAbs were pooled, diluted in 100 ml of 0.9% NaCl and perfused intravenously within 15 min. Total amount of injected antibody (mouse and chimeric MAb together or chimeric MAb alone) ranged from 2 to 4 mg in all patients. The 15-min perfusion time (used for safety reasons) does not significantly influence the pharmacokinetic analysis because both antibodies have been injected in the same perfusion and showed a very similar behavior in the hours following injection.

Tissue Samples

Patient serum was collected immediately following and 1, 4–6, 24 and 48 hr after injection; additionally blood sampling was performed during surgery (at the moment of tumor removal). Tumor samples of primary tumors or local recurrences were analyzed together with normal colon and fat. Normal colon tissue was dissected into the normal mucosa, known to contain CEA (*37*), and the rest of normal bowel wall. Liver metastases were analyzed together with liver tissue surrounding the tumor and a small biopsy of distant normal liver and fat. The tumor was macroscopically separated from normal tissue and from necrosis. Tissue samples were weighed and counted in a triple channel gamma counter together with a sample of the injected material that served as reference for the total injected dose. Samples still containing ^{123}I were counted again after complete decay of this isotope. Final radioactivity measurements were corrected for crossover of ^{131}I in the ^{125}I channel.

In Vitro Testing of Radiolabeled MAbs

The in vitro immunoreactive fraction of radiolabeled chimeric and original mouse MAb was determined in a binding assay on CEA insolubilized on CNBr-Sepharose (Pharmacia, Uppsala, Sweden). Ten to 50 nCi of radiolabeled MAb were incubated for 16 hr at 25°C in PBS buffer containing 1% normal mouse serum and 1% normal human serum with 5 μl packed CEA-Sepharose (containing about 2 μg purified CEA). After washing, bound radioactivity was determined as percent of input radioactivity. Similarly, patients, serum samples obtained 1 to 4 hr after injection (containing similar amounts of radioactivity) were diluted 1/3 in PBS buffer containing 1% normal mouse serum and were also incubated with 5 μl packed CEA-Sepharose. Nonspecific binding was measured by incubation with irrelevant protein also coupled to CNBr-Sepharose. It was always below 2% and was subtracted from CEA binding values. Trichloroacetic acid precipitation of radioactivity after labeling showed that more than 95% of the radioactivity was bound to protein for all preparations. Analytical size chromatography of the radiolabeled MAbs was done on a Sephadex G-200 column or on a Superdex 200 FPLC column (both Pharmacia). Immediately after labeling, a sharp peak was obtained for both MAbs without detectable amounts of aggregates and with only trace amounts of free iodine.

CEA and HAMA Assay

Circulating CEA was determined in a serum sample of each patient taken before injection of radiolabeled antibodies using a previously described solid phase enzyme immunoassay (*38*).

HAMA and anti-idiotype antibodies were measured in three sandwich assays. Briefly, for the three tests A, B and C, polystyrene balls were coated with A), irrelevant mouse IgG, B), mouse MAb CE 25 or C), chimeric MAb, respectively. These balls were incubated for 3 hr at 25°C with 10 μl patients serum (taken 5–6 wk after antibody injection) diluted in 300 μl PBS and peroxidase coupled mouse MAb CE25 (test A and B) or radiolabeled chimeric MAb in test C. A rabbit anti-mouse $F(ab')_2$ antiserum (10 μl serum diluted 1:10'000 in PBS) served as a positive control. Normal human sera served as negative controls. All patients sera tested before any MAb injection were also negative.

TABLE 1
Patients Injected with Chimeric and Original Mouse Anti-CEA MAbs and Major Clinical Parameters

Patient no.	Age	Sex	Diagnosis	Differentiation	Dukes	Surgery (day)
1	47	M	Primary rectum	Interm	B	2
2	71	F	Liver met. sigmoid	Interm	C	1
3	69	F	Primary col asc	Interm	B	2
4	78	F	Prim and met col asc	Interm	D	1
5	70	F	Ovarian adenoca.	Low		5
6	57	M	Hyperplastic Polyp			1
7	65	F	Local rec. sigmoid	Interm	C	2
8	79	M	Primary sigmoid	Interm-well	B	1
9	44	M	Primary sigmoid	Low	C	2
10	73	F	Primary sigmoid	Interm	B	—
11	87	M	Primary sigmoid	Int.-well	D	1
12	66	M	Primary col tr	Interm	B	—
13	64	M	Liver met sigmoid	na	B	—
14	63	M	Liver met rectum	Interm	C	2
15	74	M	Liver met colon	na	C	2
16	72	F	Liver met rectum	Interm	C	5
17	42	F	Suspected liver metastasis			1
18	58	F	Primary colon dr	Interm	B	2

na = not available.

Statistics

Pharmacokinetics were analyzed by modeling a time (hr)–radioactivity (cpm/ml) curve for each patient. Time 0 immediately after injection was taken as 100%. Using the SIPHAR program (Simed, Creteil, France) individual patient data were analyzed in a two-compartment model. A weighted least-squares method with weights being the reciprocal of the predicted radioactivity was used to estimate the parameters. A linear correlation analysis was used to calculate the correlation coefficient between the chimeric and the original mouse MAb in serum.

RESULTS

Eighteen patients were injected with ^{123}I-labeled chimeric anti-CEA MAb together with ^{125}I and ^{131}I trace-labeled chimeric and original mouse MAb (Table 1). Although immunoscintigraphy is not the objective of this study, two representative illustrations of a primary and a metastatic tumor immunoscintigraphy are shown in Figure 1. Serum CEA of these two patients was low (1.7 and 1.3 ng/ml) and immune complex formation was almost negative. Both immunoscintigraphs clearly show antibody uptake in the tumor 6 and 24 hr after injection. At these times, blood radioactivity was still high as it has been observed earlier after injection of radioiodinated intact MAbs.

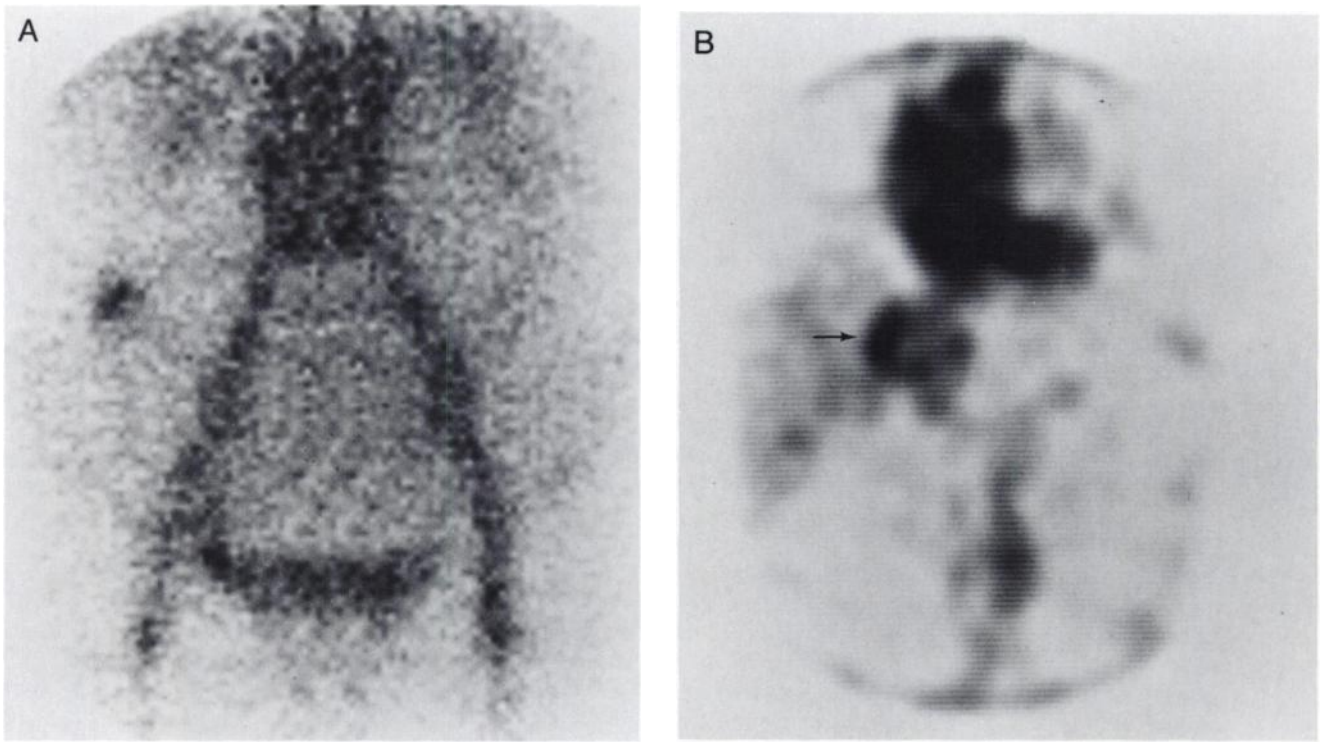

FIGURE 1. (A) Uptake of chimeric anti-CEA MAb in a primary tumor of the colon ascendens (Patient 3) and (B) in a liver metastasis of a colon carcinoma (Patient 19). In Panel A, a 6-g primary tumor of the colon ascendens is clearly visible on the planar view 6 hr after ^{123}I-MAb injection. Panel B shows a coronal section of the abdomen of a patient with an 8-g liver metastasis in the left liver lobe 24 hr after ^{123}I MAb injection. Uptake in the metastasis (arrow) is of similar intensity than that of heart and big vessels, with a hypoactive center corresponding to necrotic tissue. Activity in normal liver is low.

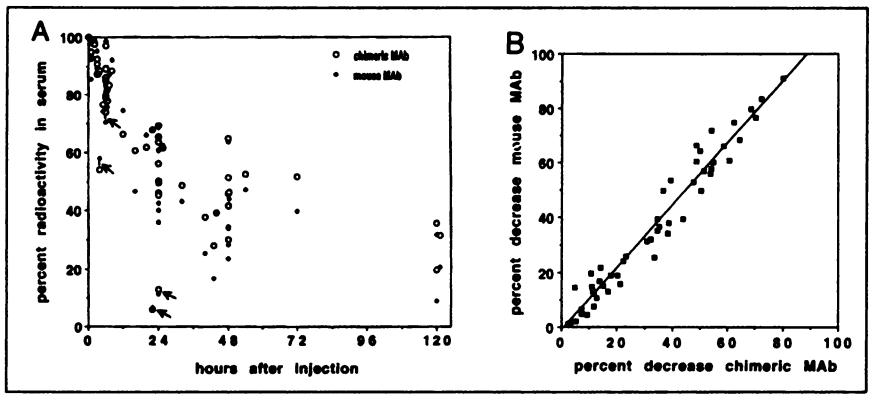

FIGURE 2. (A) Radioactivity concentrations of the chimeric MAb (○) and of the original mouse MAb (◆). Arrows indicate percent in sera of Patients 2 and 4 and show exceptionally short circulation times for both chimeric MAb and original mouse MAb. (B) Serum disappearance of mouse MAb is plotted against that of chimeric MAb of all serum samples in Panel A (Patients 2 and 4 are not included). The calculated regression straight line has the characteristics of:

$Y = -1.54 + 1.146x$;
correlation coefficient $r^2 = 0.95$.

Overall, a similar serum disappearance kinetic of both antibody forms is apparent with a marginally shorter half-life for the mouse MAb.

Serum Kinetics

The serum half-life of the two MAb forms was very similar in all patients with a tendency of marginally longer retention of the chimeric MAb later after injection (after 24 to 48 hr, Fig. 2A).

Many of these patients were followed for only one to two days since serum collection was discontinued after surgery. Frequently, this short observation time did not permit us to obtain a clear result concerning serum alpha and beta half-lives. It appears, however, from the individual data shown in Figure 2A that there is only a minor difference in the circulation kinetics between the two antibodies. Thus, a high correlation (correlation coefficient $r^2 = 0.95$) was observed for the two radiolabeled antibody forms in the serum samples as shown in a double plot analysis (Fig. 2B).

In two patients (Patients 2 and 4), the serum half-lives of both the chimeric MAb and of the original mouse MAb were very short (5 and 7.1 hr) and only between 6 to 13% of radioactivity remained in circulation after one day for both antibody forms. In one of these two patients (Patient 4) the accelerated half-life was most likely due to binding of the antibodies to CEA, since no HAMA and no anti-idiotypes were detected in his serum. Indeed, he had a high amount of serum CEA (342 ng/ml) and 47% of the chimeric MAb and 43% of the mouse MAb were in aggregated form early after injection, as determined by size chromatography on Sephadex G200 (Fig. 3C). For the second patient (Patient 2), no obvious reason for the short half-life of the two antibody forms was found. CEA in serum was relatively low (24 ng/ml), and both antibody forms appeared to circulate at a high percent in monomeric form: the serum collected 4 hr after injection contained only about 4% of aggregates as determined by Sephadex G200 radiochromatography. No indication of increased dehalogenation in serum was found since only low amounts of free iodine (<2%) were detected and the remaining 94% of radioactivity eluted with an apparent molecular weight of 150 kDa. Still, it is possible that an increased rate of antibody degradation in this patient might have occurred in tissues (*39*) with later appearence of free iodine.

The alpha and beta half-lives have been calculated for the chimeric MAb from a group of 12 patients, 9 of whom had been injected with the chimeric MAb alone because no surgery was scheduled. In these patients with a follow-up time of 2 to 6 days (mean 3.3 days) (Fig. 4), the median alpha and beta half-lives were 7.2 hr (range 1.4 to 18.4 hr) and 91 hr (range 30 to 292 hr), respectively.

Immunoreactive Fraction and Immune Complexes

The immunoreactive fraction of both MAbs was measured in buffer immediately after radiolabeling and compared to binding in serum of patients collected 1 to 4 hr after injection (Table 2). For half of the patients, the immunoreactive fraction of radiolabeled chimeric and original mouse MAb in serum was similar to that observed before injection (less than 15% decrease in serum as compared to preinjection value). The remaining patients showed a more important decrease of binding activity for the two antibod-

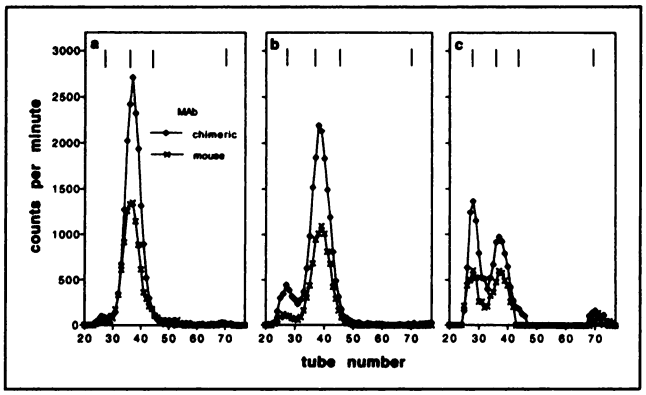

FIGURE 3. Sephadex G 200 radiochromatographs of three representative patients with low, medium and high amounts of serum CEA. Patient 8 (a) had 2.8 ng/ml of CEA and 2% of immunecomplexes for both MAbs were found. Patient 11 (b) had 26.7 ng/ml of CEA and 14% (chimeric MAb, ◆) and 8% (mouse MAb, x) of immune complexes were found. Patient 4 (c) had 342 ng/ml of CEA and 47% (chimeric MAb) and 43% (mouse MAb) of immune complexes were measured. Five to 6% of the radioactivity eluted as free iodine (or iodine bound to small peptides) in the last patient. Vertical lines indicate molecular weight standards. From left to right: IgM, IgG, albumin and peptides.

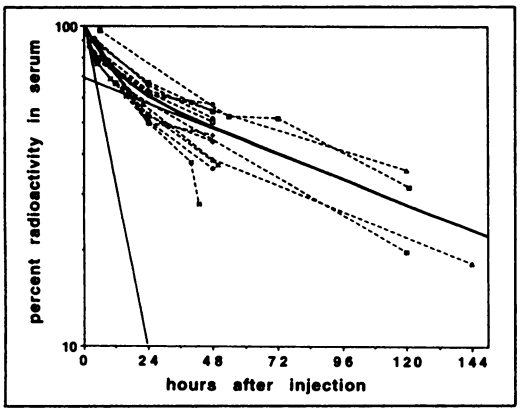

FIGURE 4. Serum radioactivity of chimeric MAb for 12 patients followed for 2 to 6 days (dotted lines). The thick curve represents the median half-life calculated for a two-compartment model with the characteristics of:

median $T_{1/2}\,\alpha = 7.2$ hr and median $T_{1/2}\,\beta = 91.1$ hr,

leading to:

$$Y = (100/V) \cdot (0.685 \cdot e^{-\lambda_1 t} + 0.315 \cdot e^{-\lambda_2 t}),$$

where $V = 0.99$, $\lambda_1 = 0.0076$ and $\lambda_2 = 0.097$. In addition, the two thin straight lines represent the calculated median alpha and beta half-lives (the beta half-life straight line is fused with the median half-life after 48 hr).

ies. There was no direct correlation between the decrease of antibody binding to CEA and the percentage of antibody aggregates. Interestingly, except for Patient 6, all other patients had a similar decrease of CEA binding for the chimeric and mouse MAb. Overall, binding capacity in serum was decreased as compared to binding after labeling by about 22% for both the chimeric MAb and the original mouse MAb. The mean binding of chimeric MAb in buffer and in serum was 71.9 ± 12.0 and 50 ± 21.3, respectively. The corresponding figures for mouse MAb are 71.6 ± 9.2 and 50.1 ± 19.9.

Analytic size chromatography was performed on the freshly labeled MAbs and on the early serum samples, which allowed the % of in vivo aggregated antibody to be calculated. The formation of immune complexes correlated with the amount of circulating CEA. Taking arbitrarily 30 ng of circulating CEA/ml as the limit, 12 of 17 patients had lower serum CEA levels and the percentage of aggregates was 3% to 14%. In contrast, all 5 remaining patients with CEA levels higher than 30 ng/ml had more than 15% of aggregates for both the chimeric MAb and the mouse MAb. In one patient with 342 ng/ml of circulating CEA (Patient 4), 47% and 43% of injected chimeric and mouse antibody, respectively, were found as immune complexes. At the time of antibody injection, none of these 5 patients had significant HAMA titers. In one of them (Patient 10) a low titer of anti-mouse IgG antibodies (test A + B) was found five weeks after injection, but no reactivity against the chimeric MAb appeared. Figure 3 shows the radiochromatograms of three representative patients with low (2.8 ng/ml), medium (26.7 ng/ml) and high (342 ng/ml) amounts

TABLE 2
Percent Binding of the Two Radiolabeled MAbs Before and After Injection and Serum CEA Levels

Patient no.	Percent binding				CEA in serum (ng/ml)
	Chimeric MAb		Mouse MAb		
	in vitro	in serum	in vitro	in serum	
1	50	52	61	52	25.2
2	82*	80	50	60	23.8
3	76	73	80	75	1.7
4	53	27	76	35	342
5	53	32	76	35	0.3
6	53	48	76	52	1.3
7	75	28	76	30	137
8	79	43	56	31	2.8
9	80*	35	70	36	16.7
10	81	61	72	56	33
11	81	18	79	20	26.7
12	81	16	79	19	9.6
13	76	42	76	46	33
14	84	75	82	84	1.9
15	83	81	81	80	90
16	77*	63	67	63	2.0
17	68	74	71	69	0.9
18	63	51	60	59	n.d.

*In three patients, trace labeling of chimeric MAb and original mouse MAb was with ^{131}I and ^{125}I, respectively, instead of the normally used ^{125}I-labeled chimeric MAb and ^{131}I-labeled original mouse MAb (reversion of isotopes).
nd = not done

of circulating CEA in whom about 2%, 10% and 45% of MAb aggregates were found. Note the appearance of significant amounts of free iodine (5% to 6%) only in the serum of Patient 4 (Fig. 3C) who had more than 40% of immune complexes.

HAMA were tested in 9 patients 5 to 6 weeks after injection of chimeric and mouse MAb. One patient developed anti-idiotype antibodies reacting with the chimeric molecule as well as HAMA to irrelevant mouse immunglobulin. An additional patient (mentioned above) had HAMA reacting only with the mouse immunglobulin but no reactivity against the chimeric MAb was detected.

Antibody Biodistribution in Tumor and Normal Tissues

Fifteen of the 18 patients injected with both antibody forms underwent surgery 1 to 5 days after injection (Table 1). In one patient, a suspected malignant recurrence turned out to be negative (Patient 17) and in another patient a benign polyp was removed at surgery while a first polyp showing malignant transformation had been completely removed during endoscopy before injection of the antibody (Patient 6). In a third patient with suspected adenocarcinoma of the rectum, the final diagnosis was a non-CEA producing ovarian carcinoma infiltrating the rectum (Patient 5).

Primary adenocarcinoma of colorectal origin or a locoregional recurrence were surgically removed from eight patients including both patients with short serum $T_{1/2}$ of the

TABLE 3
Comparison of Tumor Uptake of Radiolabeled Chimeric and Original Mouse MAb to Normal Bowel Wall Stripped from Normal Mucosa*

Patient number	Tumor		Normal bowel wall	
	Chimeric MAb	Mouse MAb	Chimeric MAb	Mouse MAb
1	9.8†	10.5	1.5 (6.5)‡	1.5 (7.0)
3	7.9	7.9	3.4 (2.3)	3.1 (2.5)
4	2.0	3.1	1.0 (2.0)	0.9 (3.4)
7	10.1	11.0	4.7 (2.1)	3.1 (3.5)
8	14.2	9.4	2.1 (6.8)	1.7 (5.5)
9	8.0	8.2	1.6 (5.0)	1.7 (5.0)
11	16.9	17.8	2.3 (7.3)	1.7 (10.4)
18	52.6	55.7	3.6 (14.6)	2.2 (25.3)

*Measured separately as shown in Figures 5–7.

†Tumor and normal bowel wall radioactivity uptake is expressed in %ID/g tissue × 10^{-3}.

‡Tumor-to-normal bowel wall radioactivity ratios are given in parentheses.

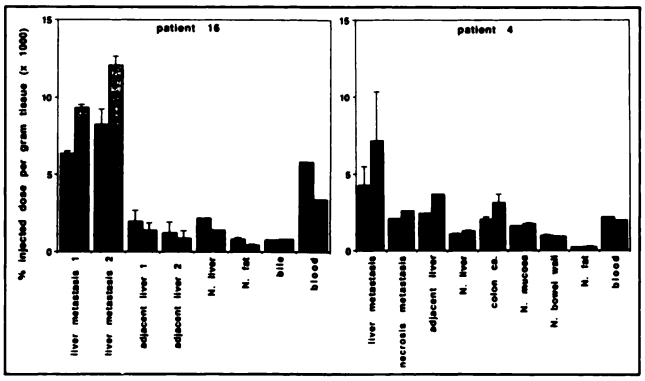

FIGURE 6. Tumor and normal tissue biodistribution of chimeric anti-CEA MAb IgG_4 (dark shading) and original mouse MAb (light shading) in two patients who had surgery for liver metastases of the colon and rectal carcinoma, respectively. Vertical bars indicate the range for two or more pieces. Patient 4 had surgery for a primary tumor and a liver metastasis. Uptake of both MAbs in the latter exceeded that of the primary tumor, whereas uptake in necrotic parts of the liver metastasis was not higher than that of adjacent normal liver.

antibodies. In these eight patients the mean % ID/g tumor was identical for both MAb with 0.015% and a large scatter, while the percentages in the normal bowel wall, stripped of the CEA producing mucosa, were 0.0025% and 0.002% for the chimeric and the mouse MAb, respectively (Table 3). Figure 5 shows one patient with a rectum carcinoma (Patient 1) with a typical median antibody biodistribution and in comparison the result of the patient with an ovarian carcinoma (Patient 5). In this second patient, the antibody concentrations in the ovarian malignancy (without evidence for production of CEA) was much less than in blood and also less than in the normal bowel wall. Figure 6 shows an additional patient with a colon carcinoma (Patient 4) having a very low uptake in its primary tumor (probably related to high amounts of CEA in the circulation and immuncomplex formation), while Figure 7 shows a patient with a primary colon carcinoma and a very high antibody uptake (Patient 18).

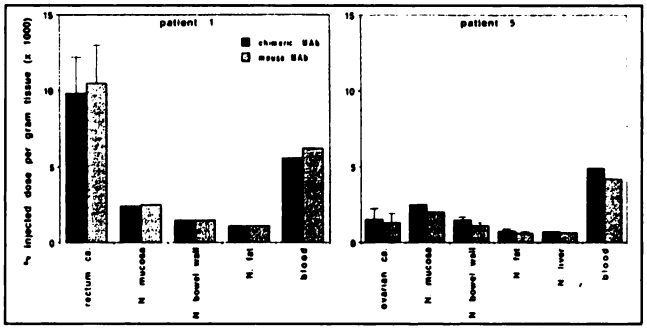

FIGURE 5. Tumor and normal tissue biodistribution of chimeric anti-CEA MAb IgG_4 (dark shading) and original mouse MAb (light shading) in two patients (one with rectal and one with ovarian carcinoma). Vertical bars indicate the range for tissues where two or more pieces were available. Clear uptake is shown in the rectal carcinoma of Patient 1, whereas no uptake is demonstrated in the ovarian carcinoma of Patient 5.

Seven liver metastases were surgically removed and counted from patients after injection of both the chimeric MAb IgG_4 and the original mouse MAb. One patient presented with a primary tumor and a liver metastasis (Dukes D, Patient 4), Patient 2 had a large liver metastasis and a micrometastasis and Patient 16 had two liver metastases that were surgically removed and analyzed.

In the seven metastases, for the chimeric and the mouse MAb, the mean % ID/g tumor was 0.015 and 0.014%, respectively, with a large scatter, while the percentages in normal distant liver were 0.0017% and 0.0023%, respectively (Table 4). Figure 6 shows one patient (Patient 16) who had two liver metastases from a colon carcinoma with

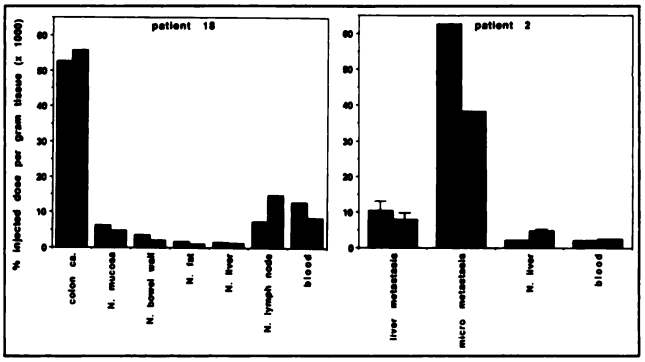

FIGURE 7. Tumor and normal tissue biodistribution of chimeric anti-CEA MAb IgG_4 (dark shading) and original mouse MAb (light shading) in two patients who had surgery for primary colon carcinoma and liver metastasis. Vertical bars indicate the range for two or more pieces. In Patient 18, a high uptake of both antibodies was observed in the primary tumor. The lymph node was found tumor-free by histologic examination. In Patient 2, a high concentration of both antibodies was found in a 60-mg micrometastasis, while antibody uptake in a 76-g metastasis was much less. The micrometastasis showed five to six times higher antibody uptake than the larger tumor.

TABLE 4
Comparison of Radiolabeled Chimeric and Original Mouse MAb Uptake in Liver Metastases and Normal Liver Tissue

Patient no.	Liver metastasis		Normal liver	
	Chimeric MAb	Mouse MAb	Chimeric MAb	Mouse MAb
2	10.4*	7.8	2.1 (5.0)†	4.8 (1.6)
2	62.6‡	38.1	2.1 (29.8)	4.8 (7.9)
4	4.3	7.3	1.2 (2.7)	2.1 (3.5)
14	8.5	17.6	1.3 (6.5)	1.3 (13.5)
15	4.6	3.8	1.8 (2.6)	1.7 (2.2)
16	6.4	9.3	2.1 (3.0)	1.4 (6.6)
16	8.2	12.1	2.1 (3.9)	1.4 (8.6)

*Radioactivity uptake in the liver metastasis and normal liver is expressed in %ID/g tissue × 10^{-3}.

†Ratios comparing liver metastasis radioactivity to that in normal liver are given in parentheses.

‡Antibody uptake in a micrometastasis of 40 mg found during dissection of normal liver tissue adjacent to the large metastasis.

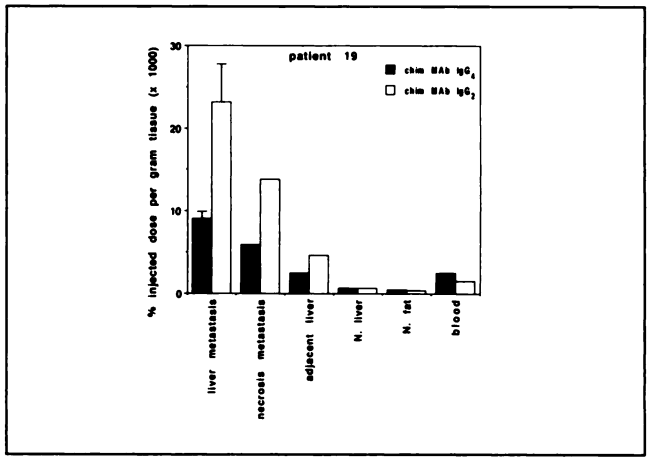

FIGURE 8. Comparison of tumor uptake of chimeric IgG_4 MAb (dark shading) with that of IgG_2 subclass (open bars) that has identical specificity (because it was deduced from the same original mouse MAb) in a patient with liver metastasis from colon cancer. The liver metastasis was surgically resected 8 days after injection of the two trace-labeled antibodies. Vertical bars indicate the range for two or more pieces.

a typical mean antibody uptake in tumor. A second patient shown in Figure 6 (Patient 4) presented with a colon carcinoma and with an initial liver metastasis. While antibody uptake in both tumor sites was low, uptake in the liver metastasis was about two times higher than in the primary tumor. This patient had the highest amount of circulating CEA and aggregated antibodies in the serum (43% and 47%) and a very short half-life of both antibody forms that might explain the low uptake of antibodies in the tumors. Patient 2 shown in Figure 7 was operated for a liver metastasis 1 day after antibody injection. While the large tumor (75 g) had a mean uptake of about 0.010% ID/g, a micrometastasis of 40 mg, found at dissection of the adjacent normal liver tissue, had an uptake of 0.062% and 0.038% injected dose per g for the chimeric and the mouse MAb, respectively, in other words, five to six times higher than in the large metastasis.

A final patient is presented in Figure 8 who was operated for a liver metastasis 8 days after injection of the IgG_4 chimeric MAb labeled with ^{123}I, together with a small amount (30 μg) of the IgG_2 chimeric MAb, labeled with ^{131}I. In this patient, 3.5 to 4.5 times higher tumor-to-blood and tumor-to-liver ratios were obtained with the chimeric MAb of IgG_2 subclass than with the IgG_4 chimeric MAb. Both antibodies injected in this patient (having the same variable domains) showed high binding to CEA after radiolabeling (79% for the IgG_4 chimeric MAb and 88% for the IgG_2 chimeric MAb) that was only marginally decreased in serum. If confirmed the surprisingly higher tumor uptake of the IgG_2 chimeric MAb would suggest its use in patients instead of the IgG_4 chimeric MAb.

DISCUSSION

In this study eighteen patients were injected with a chimeric MAb of human IgG_4 subclass together with the original mouse IgG_1 MAb. Both are directed against the same epitope of CEA and have an identical affinity (*23,32*).

The capacity of antibodies to localize in tumors depends on multiple parameters, among others on the immunoreactive fraction after radiolabeling and injection. Tumor size, the interstitial fluid pressure (*40*), vascularization and vascular permeability (*41*) tight junctions in well-differentiated tumors and the so called binding-site barrier (*42*) (absorption of high-affinity antibodies to antigen on tumor surface) can further modulate antibody uptake. The comparison of two antibodies using the double-labeling technique, whereby both antibody forms encounter identical biological parameters in each patient, allows a meaningful comparative analysis in a limited number of patients. Since the overall total amount of antibody injected in this study (4 mg) was low, tumor antigen was in excess and no major competition for antigenic sites should have occurred.

Overall, tumor uptakes and normal tissue radioactivity distributions showed a similar behavior for both MAb forms. Some variation was observed in individual patients that can be attributed to a certain degree of variability in the different batches of MAb preparations and in iodine quality and labeling. The in vitro quality controls reflect part of these variations while others might be apparent only in vivo as suggested by other studies (*32,39*).

In serum, the chimeric MAb had a marginally longer half-life than the original mouse MAb. This was observed whether patients had high or low amounts of circulating CEA. Since both antibodies have an identical affinity for antigen (*23,32*), the percentage of immune complexes for both was very similar in individual patients related to serum CEA. Apparently, the elimination of these immune complexes by the RES was also similar. The measured half-life of the IgG_4 chimeric MAb was shorter than expected. It is possible that even small amounts of circulating CEA induced formation of immune complexes that influenced the beta half-life. Additionally, the observation time

is relatively short in many patients and might have influenced evaluation of this half-life in some patients.

In the present comparison of mouse and chimeric MAb, we measured the immunoreactive fraction before and after injection. While the sera of certain patients had little or no influence on the binding capacity of the antibodies, in other patients the binding capacity of both of them was drastically reduced after injection. Reduced binding was found in patients with large amounts of immune complexes due to circulating CEA, as in Patients 4, 7 and 13. In some cases, however, reduced antibody binding after injection was found in patients with low serum CEA, as in Patients 5 and 8. In these patients, the diminished binding after injection might be due to a non-specific influence of serum on antigen-antibody association: ionic strength has been shown to influence the affinity of antibodies against a closely related epitope of CEA by a factor of up to 100 (*43*). Our assay that measures binding of radiolabeled antibody to a limited excess of antigen might be particularly sensitive to such non-specific binding inhibition in serum.

Our results concerning HAMA and anti-idiotype antibody formation in patients show that both the radiolabeled chimeric and original mouse MAb have a low immunogenicity after a single injection. Several other reports have shown a higher immunogenicity of mouse MAbs, especially after repeated injections, but also low immunogenicity after single injections of MAb have been reported (*20–22*). Our results are in line with our previous observations where after a single injection of radioiodinated mouse MAbs for immunoscintigraphy detectable HAMA titers were only rarely found. Such a first MAb injection might, however, stimulate formation of memory cells in many patients. This is suggested by the fact that a second MAb injection was followed frequently by a rapid appearance of important HAMA titers already after 2 wk (unpublished observation).

For chimeric MAbs, a weak immunogenicity has been described more frequently (*12,24*). However, one study with the chimeric human IgG_4 MAb B72.3 showed that it had a high immunogenicity (*25*): after a single injection of 3.4 to 6.9 mg, 7 of 12 patients developed measurable HAMA titers against this antibody (*25*). This MAb had a longer serum beta half-life (224 ± 66 hr) as compared to our chimeric anti-CEA MAb (91 hr median beta half-life) and is directed against an antigen that is less frequently elevated in patient's serum. Since the two MAbs have different human IgG_4 constant domains, it remains unclear whether the higher immunogenicity of the B72.3 IgG_4 chimeric MAb is due to its different mouse variable domains or to the different allotypic epitopes on the human constant domains. Interestingly, the formation of immune complexes in our patients does not appear to promote HAMA formation. Immune complexes are preferentially bound by the low affinity IgG Fc receptors on macrophages (*44*) and have been used in immunization protocols.

Of interest are a few data concerning high tumor uptake of radiolabeled MAbs: Figure 7 shows a very good localization for both the chimeric MAb and the original mouse MAb in a primary tumor operated two days after antibody injection (Patient 18). Similar high antibody uptake in primary tumors has been observed in a series of six patients who were injected with higher amounts of the same chimeric anti-CEA antibody (10 mg per patient) trace labeled with radioiodine and coinjected with the identical antibody coupled to fluorescein for the purpose of immunophotodetection (*45*). Figure 7 shows another patient operated for a liver metastasis where on dissection of the adjacent normal liver tissue, a micrometastasis of 40 mg was found (Patient 2). Here, the micrometastasis showed, on a per gram basis, a five to six times higher antibody uptake than the large metastasis. This patient had a very short circulation half-life for both antibody forms that remains unexplained. Patient 19, who had surgery eight days after antibody injection, had a relatively high tumor uptake as compared to normal tissues: 0.01% of the injected dose per g tumor was found for the IgG_4 chimeric MAb, and more than 0.02% of the coinjected IgG_2 chimeric MAb labeled with ^{131}I. The two chimeric MAbs of different human IgG subclasses are derived from the same anti-CEA mouse MAb and have a similar affinity for CEA (*32*). Interestingly, the chimeric IgG_2 MAb injected in trace amounts (30 μg) gave 3.5 to 4.5 times higher tumor-to-liver and tumor-to-blood ratios than the IgG_4 chimeric MAb. While the limited amount of antibody injected might explain the faster disappearance from blood for the chimeric IgG_2 MAb, its higher tumor uptake certainly merits further investigation.

The three patients mentioned above with particularly high antibody uptake in tumor stimulate some reflections concerning dosimetry for a potential radioimmunotherapy. Considering radioimmunotherapy in a postoperative adjuvant setting where occult micrometastases would be the target, such micrometastases might accumulate higher antibody doses than large tumor masses as it is suggested by the results from Patient 2 and by other reports (*7,17,46*). Thus, a tumor uptake in the range of 0.06% ID/g could be obtained more frequently in small tumors as compared to larger ones. Assuming homogenous irradiation of such small nodules with a radionuclide emitting beta-radiation of medium energy (e.g., ^{131}I or ^{67}Cu), the MIRD formula would predict an irradiation of 2100 rad for an injection of 100 mCi ^{131}I and a 60-hr effective half-life in the tumor. For a repeated injection of 2 × 100 mCi, the total tumor dose would reach 4200 rad. Such radiation doses in micronodules that are optimally oxigenated and radiosensitive would have a good chance to be efficient. The effective tumor radiation dose could, however, be modulated by parameters such as the percent of absorbed energy in a given micronodule and for a given radiation type (*47*), the microheterogeneity of tumor irradiation due to uneven distribution of antibody (*48*) and the low dose rate of irradiation that is generally obtained in radioimmunotherapy (*49*). The extrapolation of our results and those from other groups as shown above should, however, encourage future experi-

mental and clinical radioimmunotherapy studies of minimal residual disease.

Altogether, the results obtained with the chimeric anti-CEA IgG_4 MAb showed a tumor localization capacity that is comparable to that of the original mouse MAb which had been selected for high affinity and antigen binding. The tumor uptake of these two MAbs was similar or even slightly superior to that observed with anti-B cell MAbs (*9,10*) which have been successfully used for radioimmunotherapy of lymphomas.

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