Annals of Nuclear Medicine Vol. 5, No. 2, 69–75, 1991

# Variations in radioimmunoscintigraphic detection of tumor showed by five monoclonal antibodies to carcinoembryonic antigen

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Radioimmunoscintigraphy using mouse monoclonal antibodies to various parts of a carcinoembryonic antigen (CEA) molecule was performed. Four radiolabeled antibodies (F4-82, 28A, F3-30, F33-104) were injected into tumor transplanted nude mice to compare the accumulation of these antibodies in tumors. The four antibodies were accumulated selectively in CEA- producing tumors. The tumor visualization correlated with the tumor/blood radioactivity ratio, whereas the tumor/blood radioactivity ratio did not correlate with the *in vitro* percent binding to tumor cells or the *in vivo* percent injected dose in CEA-producing tumors. Among the four antibodies, F33-104 showed the highest tumor/blood radioactivity ratio and the best image quality in any CEA-producing tumor. These results suggest that the antibody which has a high tumor/blood ratio rather than high total tumor uptake may be useful for radioimmunoscintigraphy.

**Key words:** monoclonal anti-CEA antibody, radioimmunoscintigraphy, tumor/blood radioactivity ratio

## INTRODUCTION

SINCE GOLDENBERG et al[1] demonstrated in 1974 that infused radiolabeled antibody to carcinoembryonic antigen (CEA) accumulated in CEA-producing GW-39 human colonic carcinoma inoculated in hamsters, there have been numerous reports on the application of antibodies to cancer-associated antigens for tumor imaging. We have also reported the potential usefulness of tumor imaging with radiolabeled antibodies to CEA or α-fetoprotein,[2,3] but the tumor visualization and the positive rate of detection of the tumor were still unsatisfactory for clinical use.

Selection of an antibody with high specificity appears to be an essential factor for successful tumor imaging or targeting therapy for cancers. The cancer specificity of CEA remains obscure because of the presence of various CEA-related antigens in non-malignant tissues and in the circulation. Kuroki et al[4] found that the antigenic structure of CEA could be divided into five parts, that is, the non-specific cross-reacting antigen (NCA)-common part, the normal fecal cross-reacting antigen (NFCA)-common part, the normal fecal antigen-1 (NFA-1)-common part, a heterogeneous part which resides on a carbohydrate moiety of the CEA molecule and was previously referred to as an allotypic part,[5] and a CEA-distinctive part. Recently, they established a large number of monoclonal antibodies to CEA by means of hybridoma techniques and categorized them into five groups according to their reactivity with the five different antigenic parts of the CEA molecule.[6]

The present study was performed to determine the tumor accumulation of five selected monoclonal

Received January 17, 1991, revision accepted April 16, 1991.

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antibodies to the different antigenic parts of CEA by means of *in vitro* cell binding assays and *in vivo* experiments with tumor-inoculated nude mice.

## MATERIALS AND METHODS

*Monoclonal antibodies (MAbs) to CEA*

Five MAbs to CEA (F4-82, 28A, F3-30, F4-11, F33-104) used in this study were prepared by fusion of spleen cells[7] from BALB/c mice immunized with CEA purified from liver metastases of human colon carcinoma, and purified from ascites by means of a protein A column. The immunoreactivities of the five MAbs to CEA were previously described.[5] In brief, MAb F4-82 reacted with the NFCA-common part, 28A and F3-30 reacted with the NFA-1 common part, F4-11 reacted with the heterogenous part of the carbohydrate moiety, and F33-104 reacted with the CEA-distinctive part. The antigenic structure of CEA and the five related antigens, and the reaction specificities of the five MAbs are summarized in Fig. 1. All five MAbs to CEA belonged to the IgG 1 subclass. Their affinity constants (Ka) for the purified CEA molecule used for immunization were determined by the Farr assay as previously described[5] and calculated according to the method of Steward and Petty.[8] The immunoreactivity of the MAbs to CEA used for immunization was 80% or higher. MAb 28A and F4-82 revealed a high Ka ($2.50\times10^9\ M^{-1}$), and the Ka of F4-11 was $0.68\times10^9\ M^{-1}$, of F33-104 was $0.35\times10^9\ M^{-1}$, and of F3-30 was $0.24\times10^9\ M^{-1}$. The CEA non-reactive IgG 1 monoclonal was also used as a control.

The five MAbs and the control mouse IgG 1 monoclonal were labeled with iodine-125 ($^{125}I$) by the Iodogen method.[9] The specific radioactivities were approximately 370 KBq/$\mu$g. The immunoreactivity of the MAbs was not changed with iodination as reported previously.[2] The radiolabeled anti-CEA antibodies were sterilized by filtration through 0.22 $\mu$m Millipore filters.

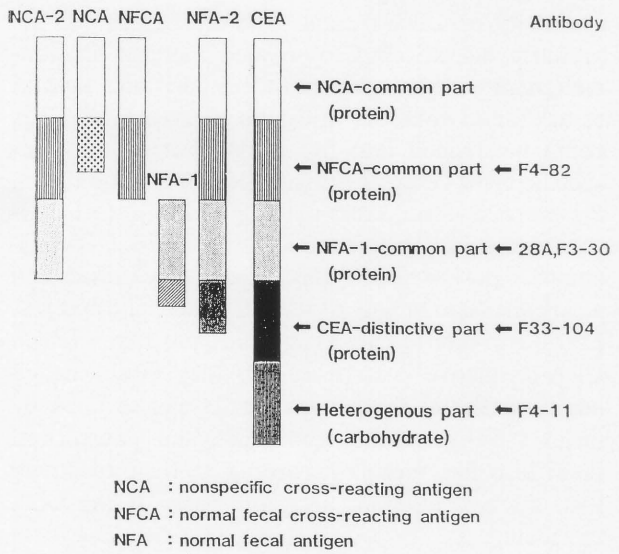


**Fig. 1** Schematic representation of CEA epitopes recognized by monoclonal antibodies to colonic tumor CEA.

*Tumor cell lines*

Four CEA-producing human tumor cell lines (BM314, FCC-1, M7609 KNS-62) and one CEA non-producing tumor cell line (RPMI) were used in this study. BM314, FCC-1, M7609, and RPMI were derived from a colorectal carcinoma. KNS-62 was derived from a lung carcinoma. These cell lines were maintained in RPMI 1640 supplemented with 10% fetal bovine serum and penicillin-streptomycin.

*In vitro binding of MAbs to tumor cells*

A direct binding study was performed to determine the percent binding of $^{125}I$-labeled MAbs to various human cell lines. Tumor cells ($5\times10^6$/100 $\mu l$) were incubated with 100 $\mu l$ of $^{125}I$-labeled MAb or control mouse IgG 1 monoclonal (30,000 cpm) in culture tubes at 37°C for 3 hours. After incubation, the cells were washed three times with Hank's solution and the radioactivity of the cell pellets was counted in a gamma counter.

*Radioimmunoscintigraphy in animals*

BALB/c nude mice (four-week-old) were subcutaneously inoculated into the left thigh with $5\times10^6$ cells of one of the human tumor cell lines. The number of mice used in each group was five. When the tumors became 0.5–1.0 cm in diameter, serum CEA levels were measured by means of RIA kits with polyclonal anti-CEA antibody (CEA-RIA-kit; Daiichi Radioisotope Laboratory, Tokyo, Japan) and 10 $\mu$g of each $^{125}I$-labeled MAb was injected intravenously via tail veins into each tumor-bearing mouse. Images were obtained at 3 and 7 days after injection. The mice were anesthetized and placed prone on the collimator face, and images were obtained with a medium-energy parallel-hole collimator and a scintillation camera (ALOKA REV-207) at 35.5 keV with a 20% window. The plasma disappearance rate for each antibody was determined by measuring the radioactivity of blood samples drawn at 10 min, 6, 24, 48, 72, 96, 144, 168 and 192 hours after injection. The mice were sacrificed 8 days after injection of antibodies. The spleen, liver, kidney, lung, heart, intestine, muscle and tumor were removed, washed in 0.9% NaCl solution and wet-weighed on an analytical balance. Radioactivities of tissues and blood were determined in a gamma scintillation counter. Results were ex-

pressed as tissue (cpm/g)/blood (cpm/g) radioactivity ratios or percent injected doses.

Plasma samples from mice drawn at 24, 48 and 96 hours after injection were applied to preparative high performance liquid chromatography (HPLC) to detect circulating CEA-radioactive anti-CEA antibody immune complexes.

## RESULTS

*In vitro binding MAbs to tumor cells*

The binding of $^{125}$I-labeled MAbs was studied in four CEA-producing cell lines (BM314, M7609, FCC-1, and KNS-62). As shown in Fig. 2, MAbs 28A had the highest percent binding to each, of the four cell lines and F4-82 had the second highest percent binding. No significant binding of F4-11 to any of the four cell lines was observed.

*Serum CEA concentrations in tumor-inoculated nude mice*

Serum levels of CEA were 250±59 ng/m*l* (mean±SD in 5 mice) in mice with the BM314 tumor, 18±5 ng/m*l* in M7609, 47±16 ng/m*l* in FCC-1, 93±83 ng/m*l* in KNS-62 and less than 2 ng/m*l* in RPMI.

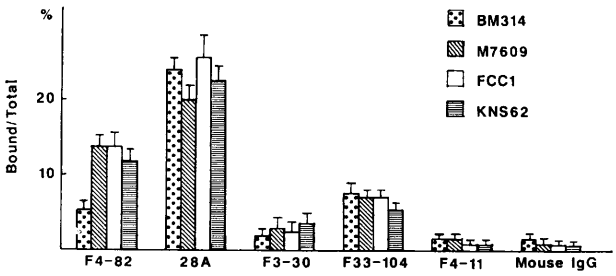


**Fig. 2** *In vitro* accumulation rate of $^{125}$I-labeled monoclonal anti-CEA antibodies. The accumulation rate is expressed as percentage of bound radioactivity (mean± SD of the results obtained from 5 samples).

**Table 1** Results of total body scintigraphy in nude mice inoculated with BM314 or M7609*

| Antibody | BM314 | | | | M7609 | | | |
|---|---|---|---|---|---|---|---|---|
| | − | ± | + | ++ | − | ± | + | ++ |
| F4-82 | | 1** | 2 | 1 | | 1 | 3 | |
| 28A | | | 1 | 3 | | 2 | 1 | 1 |
| F3-30 | | | 1 | 3 | | | 3 | 1 |
| F33-104 | | | | 4 | | | 1 | 3 |
| Mouse IgG | 3 | 1 | | | 1 | 1 | | |

*Scintigraphy was performed 7 days after injection of labeled MAbs.

**Numbers of mice showing the indicated intensity of tumor image.

++: well contrasted +: moderately contrasted

±: doubtful −: negative

*Radioimmunoscintigraphy of tumor*

Four MAbs (F4-82, 28A, F3-30, F33-104) which revealed significant *in vitro* binding to CEA-producing cells were injected into tumor-inoculated nude mice. In all tumor-inoculated nude mice injected with four MAbs to CEA, clear tumor images were observed on the 3rd and 7th days, but were not seen in nude mice injected with control mouse IgG 1 monoclonal.

The quality ratings of the tumor images judged by three radiologists are summarized in Table 1. Images were classified as "well contrasted" (++), "moderately contrasted" (+) or "doubtful" (±). Fig. 3 displays (−)—(++) tumor images in this study. Because the contrast of the "doubtful" images was not sufficient to indicate tumor localization, "±" images were scored as negative. The best image quality was obtained with MAb F33-104 among the four MAbs. The images were clearer on the 7th day than on the 3rd day because of decreased background radioactivity.

*In vivo distribution of radiolabeled MAbs*

The percent injected doses of the radiolabeled MAbs or control mouse IgG 1 monoclonal in one gram of the tumor are shown in the Table 2. The percent injected doses of the radiolabeled MAbs in the CEA-producing tumor were significantly higher than those of other tissues and the CEA non-producing tumor, whereas control mouse IgG 1 monoclonal did not show a significant accumulation in CEA-producing tumor.

*Tumor/blood radioactivity ratio*

The tumor/blood radioactivity ratios (T/B ratio) on the 8th day after injection of antibodies are shown in Table 3. The four MAbs had significantly higher T/B ratios than control mouse IgG 1 monoclonal. Among the four MAbs, the highest T/B ratios were always found in mice injected with radiolabeled MAb F33-104 regardless of the kind of CEA-producing tumor. T/B ratios of the four MAbs did not correlate with the *in vitro* percent binding to the tumor cells or the *in vivo* percent injected dose in CEA-producing tumors.

*Plasma disappearance rates of radiolabeled monoclonal antibodies*

Figure 4 shows plasma disappearance curves of $^{125}$I-labeled MAbs to CEA in BM314-inoculated nude mice. MAb F33-104 and F4-82 disappeared rapidly from the circulation, compared to MAb 28A and F3-30. The percent radioactivity of MAb F33-104 and F4-82 in the blood on the 8th day after injection was 1.9±0.3% and 2.1±0.3%, respectively. The percent radioactivity of MAb 28A and F3-30 on the 8th day

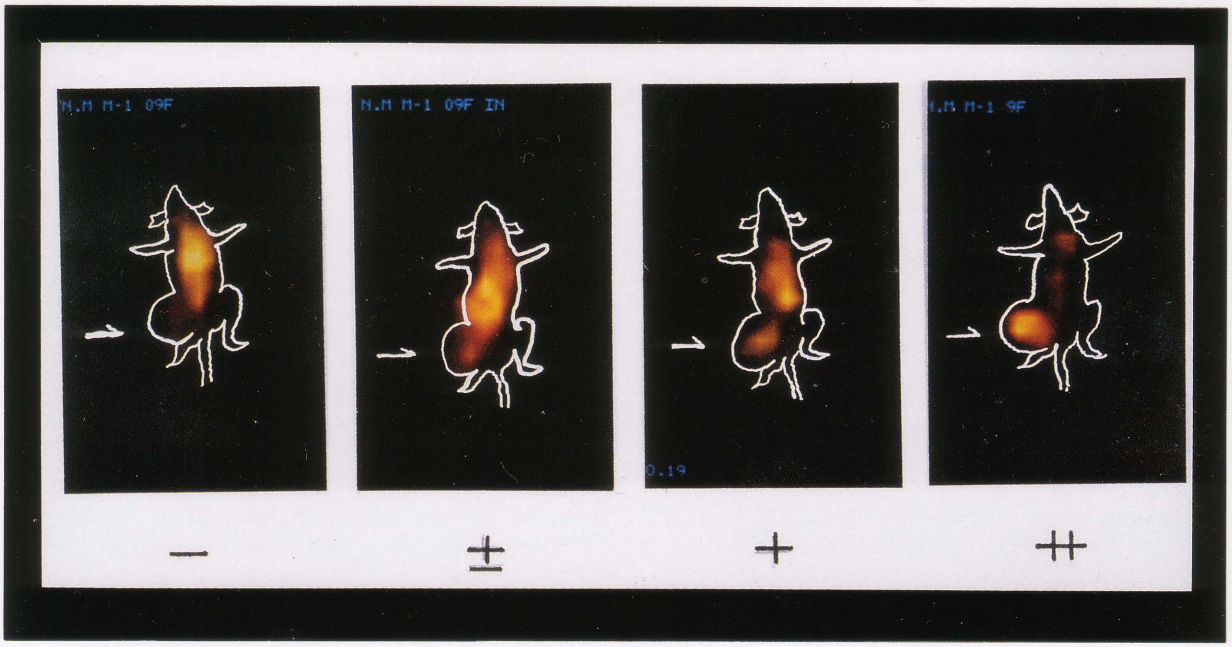


**Fig. 3** The representative tumor images obtained 7 days after injection of $^{125}$I-labeled MAbs.

**Table 2** Organ distribution of $^{125}$I-labeled monoclonal antibodies in nude mice bearing human carcinoma*

| | | F4-82 | 28A | F3-30 | F33-104 | IgG |
|---|---|---|---|---|---|---|
| *Organ* | *Cell* | | | | | |
| Tumor | BM314 | 0.7±0.10** | 3.2±1.1 | 3.5±0.4 | 2.2±0.2 | 0.96±0.35 |
| | M7609 | 1.5±0.2 | 3.2±0.4 | 3.3±0.03 | 4.3±0.8 | 0.6±0.14 |
| | FCC-1 | 2.2±0.1 | 3.9±1.1 | 5.75±0.5 | 2.92±0.8 | 0.65±0.17 |
| | KNS | 4.46±1.73 | 2.5±0.4 | 4.5±1.0 | 1.57±0.3 | 0.53±0.19 |
| | RPMI | | 0.4±0.08 | 0.46±0.07 | 0.57±0.06 | 0.6±0.56 |
| Liver | BM314 | 0.16±0.03 | 0.3±0.2 | 0.47±0.09 | 0.19±0.01 | 0.22±0.06 |
| | M7609 | 0.13±0.01 | 0.3±0.06 | 0.21±0.05 | 0.25±0.11 | 0.26±0.07 |
| | FCC-1 | 0.09±0.03 | 0.38±0.07 | 0.58±0.08 | 0.22±0.06 | 0.45±0.14 |
| | KNS | 0.24±0.1 | 0.22±0.07 | 0.84±0.25 | 0.09±0.03 | 0.2±0.08 |
| | RPMI | | 0.18±0.05 | 0.32±0.14 | 0.16±0.03 | 0.22±0.06 |
| Spleen | BM314 | 0.11±0.04 | 0.17±0.05 | 0.41±0.03 | 0.13±0.02 | 0.21±0.07 |
| | M7609 | 0.10±0.02 | 0.29±0.07 | 0.17±0.05 | 0.2±0.06 | 0.26±0.08 |
| | FCC-1 | 0.08±0.02 | 0.32±0.06 | 0.65±0.15 | 0.18±0.05 | 0.6±0.16 |
| | KNS | 0.23±0.1 | 0.22±0.07 | 0.59±0.12 | 0.07±0.03 | 0.23±0.07 |
| | RPMI | | 0.14±0.03 | 0.22±0.04 | 0.29±0.06 | 0.21±0.05 |
| Muscle | BM314 | 0.07±0.03 | 0.12±0.04 | 0.23±0.04 | 0.14±0.02 | 0.08±0.03 |
| | M7609 | 0.05±0.03 | 0.18±0.03 | 0.08±0.02 | 0.06±0.03 | 0.13±0.05 |
| | FCC-1 | 0.04±0.02 | 0.14±0.01 | 0.19±0.03 | 0.05±0.04 | 0.26±0.08 |
| | KNS | 0.09±0.06 | 0.1±0.02 | 0.31±0.07 | 0.03±0.02 | 0.1±0.04 |
| | RPMI | | 0.08±0.03 | 0.11±0.05 | 0.18±0.06 | 0.12±0.04 |

*Animals were sacrificed 8 days after the injection of labeled MAbs
**Expressed as % injected dose/g (mean±SD for 5 animals)

Table 3 Tumor/blood radioactivity ratio 8 days after injection

| | F4-82 | 28A | F3-30 | F33-104 | IgG |
|---|---|---|---|---|---|
| BM314 | 1.85±0.43*,[a,b] | 2.6±0.62[a,b] | 2.12±0.98[a,b] | 4.67±0.67[b] | 0.34±0.05 |
| M7609 | 3.67±1.22[a,b] | 2.4±0.91[a,b] | 5.5±2.1[a,b] | 10.3±5.2[b] | 0.45±0.06 |
| FCC-1 | 5.33±0.83[b] | 3.03±1.21[a,b] | 4.06±1.02[a,b] | 7.63±3.0[b] | 0.45±0.06 |
| KNS | 2.43±0.62[a,b] | 2.0±0.94[a,b] | 2.0±0.8[a,b] | 4.55±2.18[b] | 0.65±0.08 |
| RPMI | | 0.33±0.09 | 0.25±0.06[c] | 0.50±0.12 | 0.41±0.11 |

*Data represents the mean±SD for 5 animals.
[a]$p<0.05$ vs. F33-104, [b]$p<0.01$ vs. control IgG, [c]$p<0.05$ vs. control IgG.

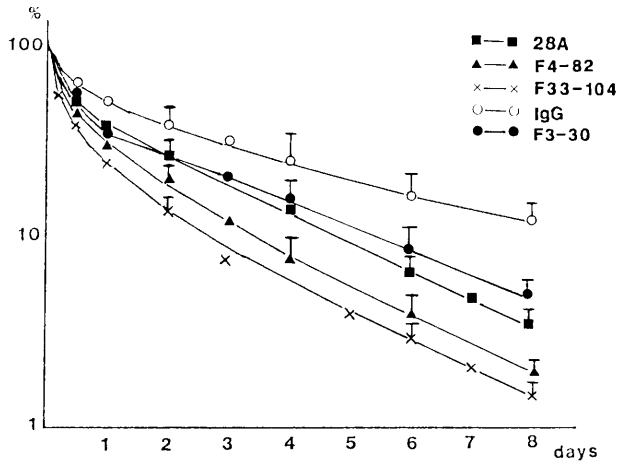


**Fig. 4** Plasma disappearance rates of $^{125}$I-labeled monoclonal antibodies in BM314-inoculated nude mice. Disappearance rates for each antibody were determined by the radioactivity of blood samples drawn after injection. The radioactivity of blood samples was expressed as a percent of those drawn 10 min after injection and values shown are the mean±SD obtained from 5 samples.

was 4.0±0.5% and 5.2±0.8%, respectively, while the plasma disappearance rate of control mouse IgG 1 monoclonal was lower than those of the MAbs. Similar results were also observed in mice inoculated with other CEA-producing tumor cell lines.

*Immune complexes*

Analysis of the plasma obtained 24 hours after injection of the radiolabeled MAb F33-104 into the BM314-inoculated mouse showed only a discrete peak that coincided with radiolabeled free antibody. Radiolabeled immune complexes could not be detected in the plasma of mice injected with any of the radiolabeled MAbs.

## DISCUSSION

The positive rate of radioimmunoscintigraphy for detection of CEA-producing tumors has been quite variable. Primary or metastatic human CEA-producing tumors were detected with radiolabeled polyclonal anti-CEA antibodies in 90% of patients reported by Goldenberg et al.,[10,11] 42% by Mach et al.[12] and 50% by Ishii et al.[2,3] These differences may have been due to differences in methods which were used or to the antibodies which were employed. Recently, radiolabeled mouse MAbs to CEA have been used for radioimmunoscintigraphy of CEA-producing tumors in patients and have been reported to be positive in about 50% of patients.[13]

From the analysis of the molecular structure of CEA, Primus et al.[14,15] identified an epitope that appeared specific for a subpopulation of the CEA molecule that was not present in NCA or the meconium antigen. They also suggested that the expression of the CEA-specific epitope was associated with the functional differentiation of colon cancers.

Five monoclonal antibodies reacting with different parts of the CEA molecule were employed in the present study. Among the antibodies, the *in vitro* percent binding to CEA-producing cells was the highest in 28A, moderate in F4-82, and relatively low in F33-104 and F3-30. These results correlated with the affinity constant (Ka) of each antibody for the purified CEA molecule, although F4-11 which reacts to the heterogenous part of the CEA molecule did not show any significant binding to the CEA-producing cells used in this study.

In the animal experiments, positive tumor images corresponding to subcutaneously transplanted tumor in nude mice were obtained with four MAbs. Although large tumors with low tumor/blood radioactivity ratios sometimes appeared to have clear tumor images in scintigraphy, the image quality of the tumor, at least, correlated with the tumor/blood radioactivity ratio, whereas the tumor/blood radioactivity ratios of the four antibodies did not correlate with the *in vitro* percent binding to tumor cells or the *in vivo* percent injected doses in CEA-producing tumors. The dissociation of the results for *in vitro* and *in vivo* experiments is of interest. The localization and the polarity of CEA molecules are different in normal tissues from those in tumor tissues,[16-18] and also different in tumor cells in culture from those

*in vivo*.[19,20] Furthermore, recent studies demonstrated that CEA molecules function as an accessory adhesion molecule.[21,22] It is possible that the conformation of CEA molecules *in vivo* may be different from that in culture, or some parts of CEA molecules *in vivo* may be modified by some factors, resulting in the difference between the affinity of anti-CEA antibody for CEA in *in vitro* and *in vivo* experiments. Sharkey et al.[23] also reported that the tumor targeting ability of anti-CEA antibodies did not depend on their immunoreactivity to CEA in patients with a CEA-producing tumor. Among the four antibodies, F33-104 showed the highest tumor/blood radioactivity ratios and the best image quality of any CEA-producing tumors. Plasma disappearance rates among the MAbs differed, although all of the MAbs used in this study belonged to the IgG 1 subclass. MAb F33-104 disappeared more rapidly from the circulation than the other MAbs in any of the CEA-producing tumor transplanted mice. The differences in the plasma disappearance rates among MAbs appears to be one of the most important factors in immunoscintigraphy and may be affected by various conditions, for example, methods of purification of MAbs, clearance rates of immune complexes from circulation, degradation of MAbs within tumor cells, etc.

The circulating immune complexes were not detected by HPLC at 24, 48 and 96 hr after the injection of labeled MAbs. The immune complexes may be present within 24 hr and circulating immune complexes may be cleared rapidly by the endotheliar system within 24 hr. However, in the present study, the influence of immune complexes on the plasma disappearance rates of MAbs from serum remains unsettled.

Since tumors themselves may catabolize large amounts of anti-CEA antibodies without an immune complex formation in the circulation as reported previously[24], these results also suggest that the catabolic rates of MAbs in the tumors may differ among the MAbs employed. There is, as yet, no definitive explanation, but it does not appear to have been due to the difference in tumor size or the type of CEA-producing cells.

Accola et al.[25] emphasized possible applications of MAbs to CEA as a tool for radioimmunoscintigraphy and antibody-directed therapy of tumors. In this study, MAb F33-104 which reacts with the CEA-distinctive part gave more satisfactory results than MAbs which react with other parts of the CEA molecule. Although it is not yet known whether the MAb reacting with the CEA distinctive part is suitable for radioimmunoscintigraphy, it is clear from this study that the antibody which has a high tumor/blood ratio rather than total tumor uptake may be useful for radioimmunoscintigraphy.

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