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Distribution of Mixture of Anti-CEA and Anti-CA 19-9 $F(ab')_2$ Antibodies in Human Tumor Xenograft[1]

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= Abstract =The in vivo localization of a mixture of radioiodinated monoclonal antibody $F(ab')_2$ fragments of CEA and CA 19-9 was investigated in human colon cancer xenograft in nude mice. Scintigrams were taken 3 and 5 days after injection of ^{131}I-labeled fragments in mice bearing transplanted tumor. Mice were killed afterward and the radioactivity in each tissue was analysed. Whole body scintigraphy clearly demonstrated selective localization of radioactivity over transplanted tumor without background subtraction with tumor:nontumor ratio of upto 3:1. Though absolute count in tumor of day 5 was lower than that of day 3, tumor to nontumor tissue ratio of day 5 was higher than that of day 3. Fragment of both monoclonal antibodies preferentially localized in tumor tissue compared with normal mouse IgG, as determined by differential tissue counting of radioactivity. The tumor to blood ratio for specific antibodies was much greater than that for normal IgG after injection. It is concluded that a radioactively labeled mixture of anti-CEA and anti-CA 19-9 antibodies is accumulated by colon carcinoma and that radioimmunodetection using gamma camera seemed to be useful for the detection of human tumor.

Key words: ***Radioimmunodetection, CEA, CA 19-9, Monoclonal antibody $F(ab')_2$ fragment, Human colon cancer xenograft***

INTRODUCTION

The use of radiolabeled polyclonal antibody directed againt tumor-associated antigen has permitted scintigraphic detection of human tumors (Goldenberg *et al.* 1978). with the availability of a large variety of specific monoclonal antibody (MAb) against tumor antigen (Order *et al.* 1975; Koprowski *et al.* 1979; DeLand *et al.* 1980; Ghose *et al.* 1980; Kim *et al.* 1980) due to recent progress in hybridoma technique (Kohler and Milstein 1975), tumor scintigraphy might become a routine diagnostic procedure (Larson and Caraquillo 1984; Henze *et al.* 1985).

However, external scintigraphy is occasionally not successful due perhaps to the detection by a single labeled MAb of an insufficient percentage of tumor cells among this presumably heterogeneous population or to an insufficient number of radiolabeled monoclonal molecules bound per cell. A mixture of MAbs directed against different tumor-associated antigens might overcome these difficulties since they would be expected to label a greater percentage of different cells within the tumor or increase the density of radiolabeled MAbs on individual tumor cells. In either case, tumor radioimmunodetection would be improved (Munz *et al.* 1986).

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Although intact antibody is cleared relatively rapidly from the blood stream, significant background activity remains for several days after injection (Primus *et al.* 1973; Hoffer *et al.* 1974). Therefore, antibodies of high specificity and those which are cleared more rapidly from the circulation are thus desirable and the elimination of the Fc portion of antibody is of preferential benefit in that regard (Hopf *et al.* 1976; Buchegger *et al.* 1983). It was found tht $F(ab')_2$ fragments give better and more rapid specific tumor localization than intact antibody of Fab fragment (Wahl *et al.* 1983; Mach *et al.* 1983).

The human tumor xenograft in nude mouse has proved an invaluable and widely used model for comparing the specificity and pharmacokinetics of tumor localizing antibodies (Roger *et al.* 1986).

The present experiments were undertaken to investigate accumulation of radioactively labeled mixture of anti-CEA and anti-CA 19-9 antibodies by human colon carcinoma xenograft in nude mice and the feasibility of in vivo localization using scintigraphic technique.

MATERIALS AND METHODS

Human tumor cell line

SNU-C4, a human colon cancer cell line, was established and characterized by Dr. J. G. Park (Park *et al.* 1987) and is maintained in our laboratory. SNU-C4 is a poorly differentiated carcinoma cell line. It is cultured in RPMI 1640 supplemented with 5% heat inactivated fetal bovine serum and antibiotics and culture is maintained in humidified incubator at 37°C in an atmosphere of 5% CO_2 and 95% air. Population doubling time is 34 hours. It actively secretes CEA and CA 19-9 into supernatant fluid.

Transplantation of human colon carcinoma in nude mice

Four 3-week-old male nude mice (nu/nu Balb/c) were injected subcutaneously with $2.0\text{-}3.0 \times 10^7$ human colon cells in both subscapular area. Two weeks after implantation of tumor, 8 tumors weighing 1.0 to 1.9g were obtained.

Mouse monoclonal antibodies

A mixture of ^{131}I labeled MAbs anti-CEA $F(ab')_2$ and anti-CA 19-9 $F(ab')_2$ fragments, which is commercially available (IMACIS1*, International CIS), was used. It is composed of 1 mg of anti-CEA $F(ab')_2$ and 1 mg of anti-CA 19-9 $F(ab')_2$ in 2 ml of phosphate buffered saline (NaCl, 0.15M) and is labeled with 3 mCi of ^{131}I.

For negative control antibodies, ^{125}I-normal mouse IgG (19.3 μCi/μg) was injected to each mouse simultaneously with the ^{131}I-labeled MAbs.

Tumor localization of MAbs

Two weeks after tumor cell implantation, 300-320 μCi of ^{131}I-MAb $F(ab')_2$ fragments and 60-100 μCi of ^{125}I normal IgG were injected.

^{131}I images were obtained 3 and 5 days after injection using a gamma camera (Pho/Gamma HP) equipped with a 4 mm high-energy pinhole collimator and interfaced to a computer. Each image was stored on a disk with 64 × 64 byte mode for later analysis and display and recorded on Polaroid film simultaneously.

For quantitative analysis of digital computer images, specific regions of interest were selected to integrate the counts present in tumors and in nontumor tissue. The region of interest of nontumor tissue was made over the abdomen of each mouse, in which liver, spleen, kidney and intestine were included. From those counts, mean count of two tumor sites in each mouse and tumor to nontumor tissue ratio (T/NT) was calculated.

In vivo distribution of MAbs

Seven days after injection each mouse was exanguinated by cardiac puncture and dissected. Tumor and various organs (liver, spleen, kidney, lung, heart, intestine) were removed, weighed and radioactivity was counted in each and also in 1 ml of blood using a gamma-scintillation counter (Packard multiprias, United Technologies Packard, USA).

Using appropriately diluted injection mixture standards, the percentage of injected dose found per gram of tissue (% ID/g tissue) and tumor to tissue ratio for each mouse were calculated for each radioisotope. In addition, a localization ratio (L.R.) was derived using following equation:

$$\text{L.R.} = \frac{[^{131}\text{I MAb } F(ab')_2/\text{normal IgG}] \text{ recovered in tissue}}{^{131}\text{I MAb } F(ab')_2/\text{normal IgG}] \text{ injected}}$$

Statistics

The statistical significances of differences were determined using Student's t-test.

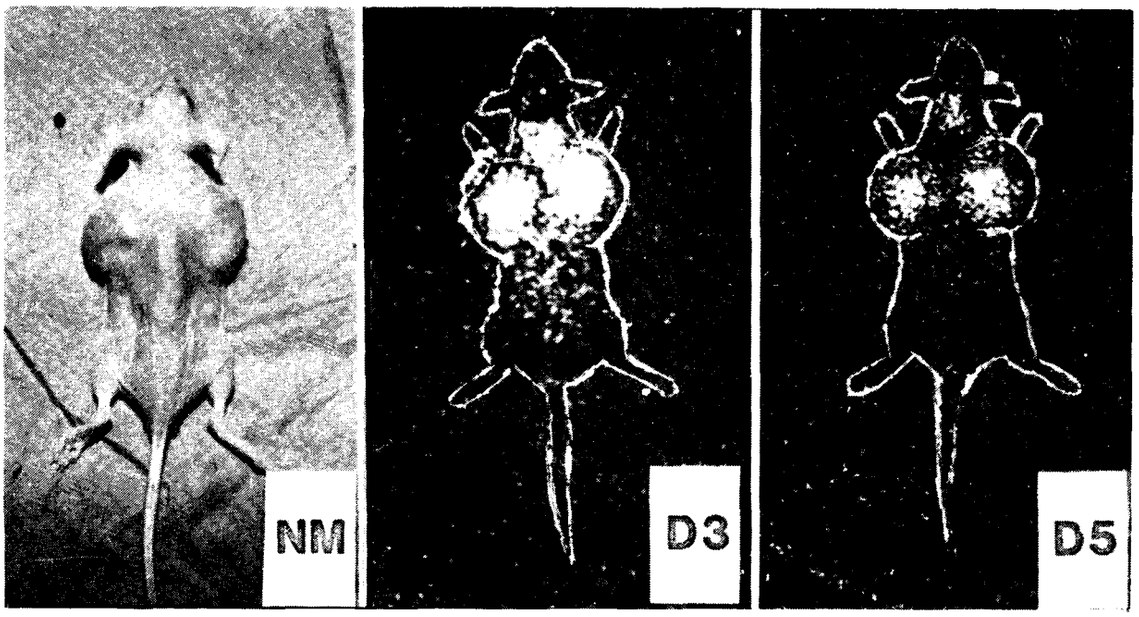

Fig. 1. Localization of ^{131}I-labeled anti-CEA F(ab')$_2$ and anti-CA 19-9 F(ab')$_2$ fragments in human colon cancer-bearing nude mouse. (NM) Tumor-bearing Balb/c nude mouse. (D3) Images obtained at 3 days after injection of ^{131}I-labeled MAb F(ab')$_2$ fragments showed clear definition of the tumors with some background radioactivity. (D5) On images obtained at day 5, tumor contrast improved.

Table 1. Mean Radioactivity of Tumor and Nontumor Tissues and Tumor to Nontumor Tissue (T/NT) Ratio quantitated by Analysing Digital Computer Image

Day	Radioactivity(cpm/pixel)*		T/NT ratio*
	Tumor	Nontumor	
Day 3	2.70	1.38	1.88
Day5	1.68	0.54	3.09

*mean value of four nude mice.

Table 2. Percentage of Injected Dose Found per Gram of Tissue (% ID/g tissue) of ^{131}I-labeled Anti-CEA F(ab')$_2$ and Anti-CA 19-9 F(ab')$_2$ and ^{125}-labeled Normal Mouse IgG

Tissue	% ID/g tissue	
	^{131}I-MAb F(ab')$_2$	^{125}I-IgG
Tumor	0.111±0.015*	2.334±0.160*
Liver	0.044±0.029	2.321±1.268
Spleen	0.069±0.032	2.797±1.300
Kidney	0.048±0.017	1.999±0.405
Lung	0.071±0.039	3.641±0.934
Heart	0.026±0.009	1.927±0.554
Intestine	0.015±0.002	0.661±0.094
Blood	0.042±0.013	5.047±1.411

*Mean±s.d. of four nude mice.

RESULTS

Tumor localization of MAbs

Images obtained at 3 days after injection showed clear visualization of the tumor with some background radioactivity. Though radioactivity decreased, tumor contrast improved on images obtained 5 days after injection (Fig. 1).

Mean cpm/pixel in tumor of day 3 was 2.07 and that of day 5 was 1.68 but tumor to nontumor tissue ratios calculated from digital computer images of day 3 and day 5 were 1.88 and 3.09 respectively (Table 1).

In vivo distribution of MAb

Table 2 and 3 list the % ID/g tissue and T/NT ratios of each organ for two isotopes. F(ab')^2 fragments of both MAbs demonstrated preferential localization in tumors as reflected by significantly higher T/NT ratio as well as higher % ID/g tissue in tumors as compared with nontumor tissues ($p<0.01$). There was no significant difference in ^{125}I-normal IgG distribution between tumor and nontumor tissue. Lung uptake was high with both isotopes.

Table 3. Tumor to Nontumor Tissue Ratio of ^{131}I-labeled Anti-CEA F(ab')$_2$ and Anti-CA 19-9 F(ab')$_2$ and ^{125}I-labeled Normal Mouse IgG

Tissue	Tumor to Nontumor Tissue Ratio	
	^{131}I-MAb F(ab')$_2$	^{125}I-IgG
Tumor	1.0	1.0
Liver	3.475±1.624*	1.168±0.453*
Spleen	1.932±1.123	0.972±0.448
Kidney	2.447±0.796	1.188±0.151
Lung	1.949±1.090	0.661±0.114
Heart	4.618±1.644	1.270±0.314
Intestine	7.287±1.193	3.554±0.267
Blood	2.749±0.613	0.481±0.104

*Mean±s.d. of four nude mice.

In spite of preferential localization of F(ab')$_2$ in tumor, absolute value of % ID/g tumor tissue of F(ab')$_2$ was much lower than that of normal IgG, mainly due to more rapid clearance of F(ab')$_2$ from the circulation.

Localization ratio is shown in Figure 2. Localization ratio (mean±s.d.) of tumor was 0.047±0.005 and that of liver was 0.017±0.003, spleen 0.025±0.007, kidney 0.024±0.004, lung 0.020±0.012, heart 0.013±0.001, intestine 0.023±0.003 and blood 0.008±0.001 respectively. The ratio obtained in the tumor was significantly greater ($p<0.01$) than those in nontumor tissues.

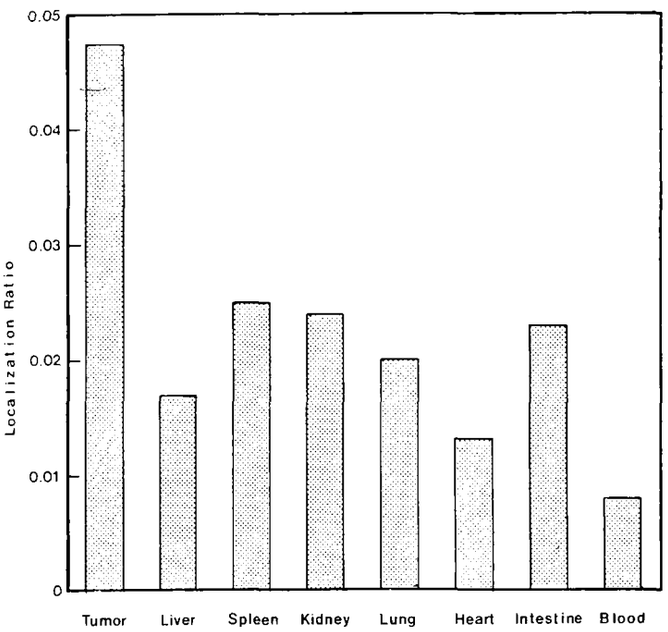

Fig. 2. Localization ratio of tumor and nontumor tissues at 7 days after simultaneous injection of 300-320 μCi of ^{131}I-labeled anti-CEA F(ab')$_2$ and anti-CA 19-9 F(ab')$_2$ and ^{125}I-labeled normal mouse IgG. The ratio obtained in the tumor was significantly greater than that in nontumor tissues.

DISCUSSION

This experimental model was chosen because human colon cancer transplanted in nude mouse retains the same histological morphology as the primary human tumor (Povlsen and Rygaard 1971) and synthesizes and releases CEA and CA 19-9, as observed in patients (Mach *et al.* 1974; Buchegger *et al.* 1983).

CEA is present on normal and malignant colonic tissue marker as well as other cancers. It is both a tumor tissue and a serum marker. It is actively produced and secreted by colon carcinoma cell lines, although levels vary widely (Primus *et al.* 1973).

CA 19-9 is a sialylated lacto-N-fucopentaose II, an oligosaccharide related to Lewis blood group substances. Its level elevates in the sera of patient with colonic and other gastrointestinal cancer (Magnani *et al.* 1981; Sears *et al.* 1982).

The specificity of antibody accumulation was demonstrated by using the paired labeling method (Pressman *et al.* 1975), in which a control mouse IgG or its fragment labeled with ^{125}I is injected simultaneously with the ^{131}I-labeled MAb.

In the present study, the tumor contrast of digital computer images improved on the day 5 though the absolute value of tumor uptake tended to decrease. In agreement with present study, Herlyn *et al.* (1983) and Chatal *et al.* (1984) reported that the tumor images with the sharpest contrast were obtained 4 to 5 days after F(ab')$_2$ fragment injection. The good scintigraphic contrast at late time intervals is the result of a faster radioactivity clearance from normal tissue than from the tumor and makes it generally unnecessary to resort to computerized subtraction.

In the present study, a mixture of MAb F(ab')$_2$ fragments of CEA and CA 19-9 was used because it is well known that the combination use of several antibodies which recognize different

antigens is a reasonable approach toward increasing the chances of tumor detection (Chatal *et al.* 1984).

It is well known that $F(ab')_2$ fragments provide better and more rapid specific tumor localization that intact MAb or Fab fragments (Wahl *et al.* 1983; Henze *et al.* 1987). However, one of the drawbacks reported for localization using $F(ab')_2$ fragments is radioactivity fades from the tumor faster than that of the intact antibody, mainly due to the faster blood clearance of the fragments. In our study, % ID/ml blood of $F(ab')_2$ was much lower than that of normal IgG reflecting faster blood clearance of $F(ab')_2$ fragments. As the decline in tumor radioactivity is mainly dependent upon an equilibrium between antibody or fragment in tumor and the circulating antibody or fragment in blood (Wahl *et al.* 1983), the % ID/g of tumor of $F(ab')_2$ was much lower than that of normal IgG in spite of the preferential accumulation of $F(ab')_2$ fragments in tumor tissue.

In light of the heterogeneity in the expression of antigen determinants and lower absolute concentration of $F(ab')_2$ fragments in the tumor, application of mixture of MAbs directed against different antigens might result in both enhanced tumor contrast and detection of more tumor sites (Chatal *et al.* 1984; Munz *et al.* 1986). MAb mixture consisting of more than two components might yield even better results, and is preferable to the use of polyclonal reagents in light of the better tumor selectivity and specificity as well as reproducibility of MAb preparation.

In conclusion, our result in the use of a mixture of MAb $F(ab')_2$ fragments to CEA and CA 19-9 are quite encouraging and can serve as an obvious basis for using such MAbs for clinical diagnostic and therapeutic trials in cancer patients.

REFERENCES

Buchegger F, Haskell CM, Schreyer M, Scazziger BR, Randin S, Canel S, Mach JP. Radiolabeled fragments of monoclonal antibodies against carcinoembryonic antigen for localization of human colon carcinoma grafted into nude mouse. J. Exp. Med. 1983, 158:413-427

Chatal JF, Saccavini JC, Fumoleau P, Douillard JY, Curtet C, Kremer M, Mevel BL, Koprowski H. Immunoscintigraphy of colon carcinoma. J. Nucl. Med. 1984, 25:307-314

DeLand FH, Kim EE, Goldenberg DM. Lymphoscintigraphy with radionuclide-labeled antibodies to carcinoembryonic antigen. Cancer Res. 1980, 40:2997-3000

Ghose T, Norvell ST, Aquino J. Localization of ^{131}I-labeled antibodies in mouse hepatoma and correlation with tumor detection by photoscanning. Cancer Res. 1980, 40:3018-3031

Goldenberg DM, DeLand F, Kim EE. Use of radiolabeled antibodies to carcincembryonic antigen for the detection and localization of diverse cancer by external photoscanning. N. Engl. J. Med. 1978, 298:1384-1388

Henze E, Kubel r, Waitzinger J, Buchler M, Adam WE, Beger HG. Tumor scintigraphy with ^{131}I anti CEA monoclonal antibodies and $F(ab')_2$ in colorectal cancer. Eur. J. Nucl. Med. 1987, 13:125-129

Herlyn D, Powe J, Alavi A. Radioimmunodetection of human tumor xenografts by monoclonal antibodies. Cancer Res. 1983, 43:2731-2735

Hoffer PB, Lathrop K, Bekerman C. Ue of ^{131}I-CEA antibody as tumor scanning agent. J.Nucl. Med. 1974, 15:323-327

Hopf U, Zum Buschenfelde KHM, Dierich MP. Demonstration of binding sites for IgG Fc and the third complement component(C3) on isolated hepatocytes. J. Immunol. 1976, 117:639-645

Kim EE, DeLand FH, Nelson MO. Radioimmunodetection of cancer with radiolabelled antibodies to alpha-fetoprotein. Cancer Res. 1980, 40:3008-3012

Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of proven defined specificity. Nature 1975, 256:495-497

Koprowski H. Steplewski Z, Mitchell K. Colorectal carcinoma antigens detected by hybridoma antibodies. Somatic Cell Genet. 1979, 5:957-971

Larson SM, Caraswuillo JA. Nuclear oncology. Seminars Nucl. Med. 1984, 14:268-276

Mach JP, Carrel S, Merenda C, Sordat B, Cerottini JC. In vivo localization of radiolabeled antibodies to carcinoembryonic antigen in human colon carcinoma grafted into nude mice. Nature 1974, 248:704-709

Mach JP, Chatal JF, Lumbroso JD. Tumor localization in patients by radiolabeled monoclonal antibodies against colon carcinoma. Cancer Res. 1983, 43:5593-5600

Magnani JL, Brockaus M, Smith DF. A monoganglioside is a monoclonal antibody-defined antigen of colon carcinoma. Science 1981, 212:55-56

Munz DL, Alavi A, Koprowski H, Herlyn D. Improved radioimmunoimaging of human tumor xenografts by a mixture of monoclonal antibody $F(ab')_2$ fragments. J. Nucl. Med. 1986, 27:1739-1745

Order SE, Bloomer WB, Jones AG, Kaplan WB, Davis MA, Adelstein SJ, Hellman S. Radionuclide

immunoglobulin lymphoscintigraphy: A case report. Cancer 1975, 35:1487-1492

Park JG, Oie HK, Sugarbaker PH, Henslee JG, Chen TR, Johnson BE, Gazdar A. Characteristics of cell lines established from human colorectal carcinoma. Cancer Res. 1987, 47:6710-6718

Povlser CO, Rygaard J. Heterotransplantation of human adenocarcinomas of the colon and rectum to the mouse mutant nude. A study of nine consecutive transplantations. Acta. Pathol. Microbiol. Scand. 1971, 79:159-167

Pressman D, Day ED, Blan M. The use of paired labeling in the determination of tumor-localizing antibodies. Cancer Res. 1975, 17:845-854

Primus FJ, Wang RM, Goldenber4g DM, Hansen HJ. Localization of human GW-39 tumors in hamsters by radiolabeled heterospecific antibody to carcinoembryonic antigen. Cancer Res. 1973, 33:2977-2982

Roger GT, Pedley RB, Boden J, Harwood PJ, Bagshake KD. Effect of dose escalation of a monoclonal anti-CEA IgG on tumor localization and tissue distribution in nude mice xenografted with human colon carcinoma. Cancer Immunol. Immunother. 1986, 23:107-112

Sears HF, Atkinson B, Mattis J, Herlyn D. Phase-I clinical trial of monoclonal antibody in treatment of gastrointestinal tumors. Lancet 1982, 1:762-765

Wahl RL, Parker CW, Philpott GW. Improved radioimaging and tumor localization with monoclonal $F(ab')_2$. J. Nucl. Med. 1983, 24:316-325

= 국문초록 =

누드 마우스에 이종이식된 인체종양에서 항 CEA 및 항 CA 19-9 $F(ab')_2$ 단세포군 항체의 분포

서울대학교 의과대학 내과학교실, 외과학교실* 및 서울대학교병원 중앙연구실**

이명철 · 이명혜 · 박재갑* · 조보연 · 고창순 · 정재민**

인공적으로 인체 대장암을 이식받은 누드마우스에서 I-131로 표지된 CEA와 CA 19-9에 대한 단세포군항체 $F(ab')_2$를 이용하여 종양을 관찰하고, 체내에서의 분포를 연구하였다. 항체 투여 후 3일과 5일째에 스캔을 시행한후 실험동물을 희생시켜 각 장기의 방사능치를 측정하였다. 실험동물의 전신스캔상, CEA와 CA 19-9에 대한 단세포군항체 $F(ab')_2$는 I-125로 표지된 정상 mouse IgG와 비교해 볼때 종양에 선택적인 집적을 보여 타조직의 방사능치에 대한 종양의 방사능치의 비가 5일째 스캔에서는 3 : 1에 이르렀다. 혈액에 대한 종양의 비는 단세포군항체에서 정상 IgG에 비해 월등히 높았다. 그러므로 방사성동위원소로 표지된 CEA와 CA 19-9에 대한 단세포군항체는 대장암에 특이적으로 집적됨을 알 수 있었고, 감마카메라를 이용한 방사면역학적 진단은 인체 대장암의 진단에 있어서 유용한 방법이 될 수 있을 것으로 사료된다.