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A transition metal complex (Venus flytrap cluster) for radioimmunodetection and radioimmunotherapy

(carborane/radionuclides/colon cancer/carcinoembryonic antigen/monoclonal antibody)

RAYMOND J. PAXTON*†, BARBARA G. BEATTY‡, M. FREDERICK HAWTHORNE§, ARAVAMUTHAN VARADARAJAN§, LAWRENCE E. WILLIAMS¶, FREDERICK L. CURTIS*, CAROLYN B. KNOBLER§, J. DAVID BEATTY‡, AND JOHN E. SHIVELY*

*Division of Immunology, Beckman Research Institute of the City of Hope, Duarte, CA 91010; ‡Department of General Oncologic Surgery and ¶Division of Diagnostic Radiology, City of Hope National Medical Center, Duarte, CA 91010; and §Department of Chemistry and Biochemistry, University of California at Los Angeles, Los Angeles, CA 90024

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ABSTRACT **A novel transition metal complex, Venus flytrap cluster (VFC), is described for the preparation of radiolabeled antibodies. VFC contained ^{57}Co, which was held tightly between the faces of two covalently bridged carborane ligands by cluster bonding of the metal with appropriate ligand orbitals. Anti-carcinoembryonic antigen monoclonal antibody T84.66 was conjugated to ^{57}Co-VFC with full retention of immunological activity. Biodistribution studies in nude mice bearing carcinoembryonic antigen-producing tumors showed excellent tumor localization of ^{57}Co-VFC-T84.66. The accumulation of radionuclide in normal liver was low and independent of dose, which may reflect the stability of the radionuclide complex. These results presage the use of VFC systems for binding transition metals that are clinically useful for radioimmunodiagnosis and radioimmunotherapy.**

The use of monoclonal and engineered antibodies for the selective delivery of diagnostic and therapeutic radiometals to tumors requires suitable bifunctional reagents that efficiently form covalent bonds with antibody and stable radionuclide chelate complexes. With few exceptions, monoclonal antibody (mAb)–chelator conjugates have been prepared with bifunctional reagents that are members of the aminocarboxylate chelator family, with derivatives of diethylenetriaminepentaacetic acid (DTPA) and ethylenediaminetetraacetic acid (EDTA) being most popular (1–5). These conjugates form stable radiopharmaceuticals with ^{111}In that have been used for radioimmunodiagnosis of human tumors (6–8), although there is some concern about their uptake by normal tissues and possible metal release *in vivo*. Conjugates of this type, however, form less stable radiopharmaceuticals with ^{90}Y and ^{67}Cu (9, 10), which can be used for radioimmunotherapy. There is therefore considerable interest in the development of new bifunctional reagents that will form more stable chelate complexes with these and other clinically relevant radionuclides.

Radionuclides such as ^{67}Cu, ^{99m}Tc, ^{105}Rh, and ^{186}Re are transition metals. Thus, very efficient π-bonding ligands would be expected to form complexes of great stability with these radionuclides. Hawthorne and coworkers (11, 12) first described *commo*-bisdicarbollide transition metal complexes in which the metal ion is held between two π-bonding $[C_2B_9H_{11}]^{2-}$ dicarbollide ligands. With d^6 metal ions these complexes conform to both the 18-electron and cluster electron-counting rules and display extraordinary stability due to the cluster bonding of the transition metal with ligand orbitals of appropriate symmetry. Metal ions having other than six d electrons, such as d^3Cr^{3+} and d^8Cu^{3+}, also form stable bis-dicarbollide clusters, although in the case of electron-rich (d^7, d^8, etc.) species, the dicarbollide ligands undergo a "slip" distortion (12). The ability to produce these complexes in high yield in aqueous media, their expected stability to physiological conditions, and their expected resistance to enzyme degradation systems suggested their use in bifunctional reagents for transition metals. We have designed, synthesized, and characterized a prototype bridged *commo*-bisdicarbollide bifunctional reagent appropriate for mAb conjugates (13). This metal-containing reagent has been designated Venus flytrap cluster (VFC). The present report describes the preparation of a radiolabeled mAb using VFC and its initial *in vivo* evaluation.

MATERIALS AND METHODS

Antibody and Tumor Cell Line. Anti-carcinoembryonic antigen (CEA) mAb T84.66 (14, 15) was used for radiolabeling. T84.66 is a murine IgG1, is specific for CEA, does not crossreact with granulocytes or other normal tissues, and has an affinity constant for CEA of $>2 \times 10^{10}$ M^{-1}. DTPA-conjugated T84.66 was prepared as described (16) and labeled with [^{111}In]indium citrate (Hybritech). LS174T, a CEA-producing human colon cancer derived cell line, was grown in continuous culture in supplemented RPMI medium (17).

Preparation of ^{57}Co-VFC-Labeled Anti-CEA mAb T84.66. ^{57}Co (5.48 mCi, 7 mCi/μg of Co; 1 Ci = 37 GBq) as $CoCl_2$ in 47 μl of 0.1 M HCl (ICN) was added to 2.5 μmol of VFC ligand (13) in 100 μl of 2 M NaOH. The solution was "vortexed" in the dark at 50°C for 15 hr, cooled to 0°C, and carefully neutralized with 75 μl of 4 M HCl. ^{57}Co-VFC was isolated by diethyl ether extraction (3 × 200 μl) with a radiochemical yield of 3.22 mCi (59%). Sulfosuccinimido-^{57}Co-VFC was prepared by adding *N*-hydroxysulfosuccinimide [Pierce; 6 μmol in 20 μl of 0.2 M pyridine/HCl (pH 5.2)] and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride [Pierce; 6 μmol in 20 μl of 0.2 M pyridine/HCl (pH 5.2)] to ^{57}Co-VFC in 80 μl of HPLC-grade acetonitrile (Fisher). The reaction tube was vortexed continuously at room temperature for 1 hr. The product was purified by reversed-phase HPLC on a 2.1 × 30-mm RP-8 column (Brownlee Lab) using a linear gradient of 100% solvent A to 100% solvent B in 20 min at 0.2 ml/min. Solvent A was 0.1% trifluoroacetic acid/99.9% H_2O (vol/vol) and solvent B was 0.1% trifluoroacetic acid/9.9% H_2O/90% acetonitrile (vol/

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Abbreviations: VFC, Venus flytrap cluster; CEA, carcinoembryonic antigen; mAb, monoclonal antibody; DTPA, diethylenetriaminepentaacetic acid; ID, injected dose.

†To whom reprint requests should be addressed at: Division of Immunology, Beckman Research Institute of the City of Hope, 1450 East Duarte Road, Duarte, CA 91010.

vol). Prior to conjugation, T84.66 (produced by Damon Biotechnology, Needham, MA) was chromatographed on a 1 × 60-cm Superose 12 gel filtration column (Pharmacia) equilibrated with 0.1 M Hepes (pH 8.5) at 0.2 ml/min. Sulfosuccinimido-^{57}Co-VFC was added to 5 mg of T84.66 in 1 ml of 0.1 M Hepes (pH 8.5) and the reaction mixture was stirred at room temperature for 1 hr. The ^{57}Co-VFC conjugate was purified by chromatography on a 1 × 30-cm Superose 12 column equilibrated with 0.05 M Na_2HPO_4/0.15 M NaCl, pH 8.0, at 0.2 ml/min. The radiochemical yield of purified ^{57}Co-VFC-T84.66 was 0.52 mCi (26%). Radioactivity was measured using a Capintec CRC-10R dose calibrator.

***In Vivo* Evaluation of ^{57}Co-VFC-Labeled Anti-CEA mAb T84.66.** Four groups of 6- to 8-week-old athymic (nude) female mice (Simonsen Laboratories, Gilroy, CA) were injected subcutaneously in the left flank with 10^6 LS174T human colon cancer cells in 0.2 ml of phosphate-buffered saline. Fourteen days later the animals were injected intraperitoneally with either ^{57}Co-VFC-T84.66 or ^{111}In-DTPA-T84.66. Each mAb conjugate was administered at two dose levels. Animals were euthanatized 48 hr after antibody injection, and tissues were removed and weighed before measurement of radioactivity with a γ well counter (TM Analytic, Elk Grove Village, IL). An aliquot of the injected dose (ID) was "counted" with the tissues to correct for radionuclide decay. Uptake of radiolabel was expressed as a percentage of the ID per gram of tissue (%ID/g, mean ± SE). Statistical analysis was performed using Student's *t* test. The *t* test that does not assume equal standard deviation (SD) values was used for comparison of groups with sufficiently different SD values. Scintiscans were performed with a Siemens PHO/Gamma V nuclear imaging camera with a 5-mm pinhole collimator at 9.9 cm from the prone subject. A total of 25,000 counts was collected at each time point, and images were stored on a DEC Gamma-11 computer system.

Immunoreactivity. Microtiter plates coated with CEA (10 μg/ml) were incubated with serial dilutions of T84.66 and ^{57}Co-VFC-T84.66. A double sandwich enzyme immunoassay was then performed, and the resulting binding curves were compared. Anti-CEA mAb was quantified as described (18).

SDS/Polyacrylamide Gel Electrophoresis (SDS/PAGE). T84.66 and ^{57}Co-VFC-T84.66 were electrophoresed in 10% (wt/vol) polyacrylamide gels by the method of Laemmli (19). Samples were heated at 100°C in 62.5 mM Tris, pH 7.5/20% (vol/vol) glycerol/2% (wt/vol) SDS with 5% (vol/vol) 2-mercaptoethanol (reducing conditions) or without 2-mercaptoethanol (nonreducing conditions) prior to electrophoresis. Gels were stained with Coomassie blue R-250 or exposed to X-Omat film (Kodak) for autoradiography.

RESULTS

The synthesis of the VFC ligand and the preparation and characterization of Co-VFC have been described (13). The structure of Co-VFC as determined by x-ray diffraction is shown in Fig. 1. The preparation of ^{57}Co-VFC followed a similar scheme, but on a much smaller scale. Briefly, $^{57}CoCl_2$ was incubated with a large excess of the VFC ligand in 2 M NaOH. The basic conditions removed B-H-B bridge hydrogen atoms present on each $[7,9\text{-}C_2B_9H_{11}]^{1-}$ ligand, allowing *in situ* incorporation of $^{57}Co^{3+}$ into the VFC ligand. These conditions also removed the protecting group on the pyrazole carboxyl function, which was subsequently used for ^{57}Co-VFC conjugation to antibody. Acidification and diethyl ether extraction of the reaction mixture afforded ^{57}Co-VFC with a radiochemical yield of 59%. Studies using nonradiolabeled $CoCl_2$ have shown that the ether-extracted product is essentially pure, because excess ligand and reaction by-products are insoluble in diethyl ether. ^{57}Co-VFC was converted to its sulfosuccinimido active ester and purified by reversed-phase HPLC with a radiochemical yield of 62%. The active ester was then reacted with anti-CEA mAb T84.66 (≈0.17 molecule of active ester per antibody molecule). The conjugate was purified by gel filtration chromatography with a radiochemical yield of 26%, corresponding to ≈0.044 molecule of ^{57}Co-VFC per antibody molecule.

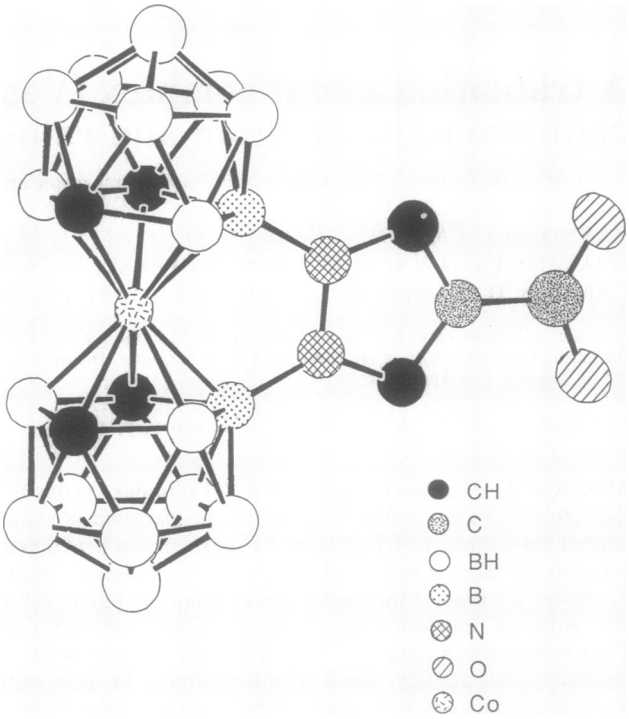

FIG. 1. Structure of *meso*-Co-VFC. The structure was determined by single-crystal x-ray diffraction (13). In this ORTEP (Oak Ridge thermal ellipsoid plotting) representation the hydrogen atoms have been removed for clarity.

SDS/PAGE with Coomassie blue staining showed that ^{57}Co-VFC-T84.66 was identical to unconjugated T84.66 under reducing (Fig. 2, lanes 1 and 2) and nonreducing (lanes 4 and 5) conditions. The molecular weight of >200,000 for this antibody under nonreducing conditions (lanes 4 and 5) resulted from the samples being heated at 100°C before electrophoresis. If this antibody is not heated before electrophoresis, a molecular weight closer to 150,000 is seen under nonreducing conditions (data not shown). Autoradiography of ^{57}Co showed that individual heavy and light chains (lane 3) and whole antibody (lane 6) were radiolabeled. These results and gel filtration chromatography suggested that the conjugation reaction did not lead to antibody crosslinking or aggregate formation.

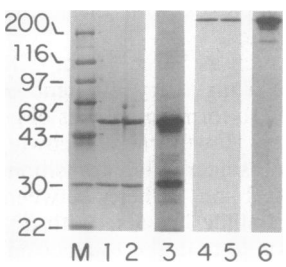

FIG. 2. SDS/PAGE of ^{57}Co-VFC-T84.66. Approximately 1 μg of unconjugated T84.66 (lanes 1 and 4) or 1 μg of ^{57}Co-VFC-T84.66 (lanes 2, 3, 5, and 6) was electrophoresed under reducing (lanes 1–3) or nonreducing conditions (lanes 4–6). Lanes 1, 2, 4, and 5 were stained with Coomassie blue. Lanes 3 and 6 were dried without staining and exposed to X-Omat film (Kodak) for 6 and 3 hr, respectively. Lane M, molecular mass markers (expressed as kilodaltons at left).

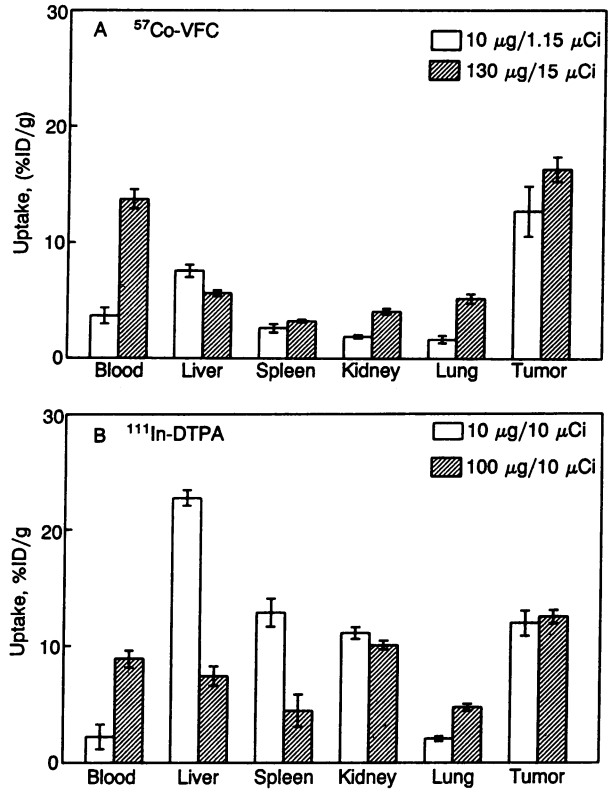

FIG. 3. Biodistribution of ^{57}Co-VFC-T84.66 and ^{111}In-DTPA-T84.66 in tumor-bearing nude mice. Tumor-bearing animals were injected with the indicated amounts of either ^{57}Co-VFC-conjugated T84.66 (*Upper*) or ^{111}In-DTPA-conjugated T84.66 (*Lower*). Animals were euthanatized 48 hr postinjection, and tissue radioactivity was determined as described in *Materials and Methods* and expressed as the mean %ID/g ± SE for each group. The average tumor weights (mean ± SE) of the four dosage groups were as follows: 10-μg ^{57}Co-VFC (n = 4), 1.53 ± 0.16 g; 130-μg ^{57}Co-VFC (n = 5), 1.18 ± 0.18 g; 10-μg ^{111}In-DTPA (n = 5), 1.77 ± 0.12 g; 100-μg ^{111}In-DTPA (n = 4), 0.84 ± 0.10 g.

SDS/PAGE of ^{57}Co-VFC-T84.66 under reducing conditions (Fig. 2, lane 3) showed only a trace of radioactivity at the electrophoretic front (*ca.* 22 kDa). This demonstrated that ^{57}Co-VFC-T84.66 was stable to the sample preparation procedure—i.e., 5 min at 100°C in 2% SDS/5% 2-mercaptoethanol—and confirmed the robust nature of the transition metal complex. These conditions completely dissociate ^{111}In from DTPA-conjugated antibodies (data not shown). Enzyme immunoassay of ^{57}Co-VFC-T84.66 and unconjugated T84.66 on CEA-coated microtiter plates showed equivalent reactivities, demonstrating retention of immunoreactivity for the radiolabeled mAb.

Tumor localization with ^{57}Co-VFC-T84.66 was demonstrated in nude mice bearing single xenografts of LS174T human colon cancer cells. A dose of 10 μg of ^{57}Co-VFC-T84.66 resulted in 12.74 ± 0.31 %ID/g in tumor, 7.59 ± 0.53 %ID/g in liver, and <4 %ID/g in the other normal tissues (Fig. 3 *Upper*). Increasing the dose of antibody to 130 μg did not alter the biodistribution of radiolabel significantly in tumor (16.36 ± 1.05 %ID/g) or normal tissues with the exception of blood, which increased from 3.76 ± 0.70 %ID/g (10-μg dose) to 13.75 ± 0.83 %ID/g (130-μg dose).

Administration of two similar doses of ^{111}In-DTPA-T84.66 to tumor-bearing mice resulted in tumor uptakes of 11.96 ± 1.06 %ID/g and 12.51 ± 0.59 %ID/g for 10-μg and 100-μg doses, respectively (Fig. 3 *Lower*). These values were comparable to those seen with ^{57}Co-VFC-T84.66. However, the uptake of radiolabel in liver (22.74 ± 0.69 %ID/g) and spleen (12.88 ± 1.20 %ID/g) for the 10-μg dose of ^{111}In-DTPA-T84.66 was significantly higher than for either dose of ^{57}Co-VFC-T84.66 ($P < 0.0002$). Increasing the dose of ^{111}In-DTPA-T84.66 to 100 μg reduced the amount of radiolabel in the liver (7.42 ± 0.82 %ID/g) and spleen (4.45 ± 0.31 %ID/g) to values similar to those seen for the VFC conjugate. Animals with similar tumor masses were used in these comparisons to minimize the effects of tumor size on the biodistribution (20).

Comparison of the biodistributions of the ^{57}Co-VFC and ^{111}In-DTPA conjugates (Fig. 3) indicated that with the exception of blood, there was an overall lower retention of radiolabel by normal tissue in the mice injected with ^{57}Co-VFC-T84.66. This difference was most marked at the 10-μg dose.

Tumor/liver (T/L) and tumor/blood (T/B) ratios were used as a quantitative measure of the effectiveness of antibody localization (Table 1). At the 10-μg antibody dose, the T/B ratio was higher with ^{111}In-DTPA-T84.66 (5.99 ± 0.53) than with ^{57}Co-VFC-T84.66 (3.57 ± 0.41), but at the high antibody doses, the T/B ratios for the two conjugates were decreased to comparable values. The decrease in the T/B ratios at the higher doses reflects an increased amount of antibody in the blood, because tumor levels remained nearly the same. The T/L ratio at the 10-μg dose for ^{57}Co-VFC-T84.66 was almost 3 times higher than for ^{111}In-DTPA-T84.66. At the high antibody doses, the T/L ratio for ^{57}Co-VFC-T84.66 was unchanged, whereas the T/L ratio for ^{111}In-DTPA-T84.66 increased from 0.58 ± 0.06 to 1.75 ± 0.17. The ^{57}Co-VFC-T84.66 conjugate gave T/L ratios that were significantly higher at both the low dose ($P < 0.05$) and the high dose ($P < 0.002$) of antibody relative to comparable doses of ^{111}In-DTPA-T84.66. Overall, the 10-μg dose of ^{57}Co-VFC-T84.66 gave the most favorable combination of T/L and T/B ratios.

Tumor localization was also demonstrated by immunoscintigraphy of a single animal at four time points after injection of the 130-μg dose of ^{57}Co-VFC-T84.66. As shown in Fig. 4, tumor uptake increased relative to that of normal tissue over the period from 24 hr to 96 hr. Biodistribution data at 168 hr of mice injected with the same dose also reflected this observation, giving T/L and T/B ratios of 3.48 ± 0.32 and 1.40 ± 0.12, respectively, which were slightly higher than those observed at 48 hr (Table 1).

Table 1. Tumor/liver and tumor/blood radioactivity ratios at 48 hr for tumor-bearing nude mice injected with ^{57}Co-VFC-T84.66 and ^{111}In-DTPA-T84.66

	Ratio			
Tissues	^{57}Co-VFC (10 μg/1.15 μCi)	^{111}In-DTPA (10 μg/10 μCi)	^{57}Co-VFC (130 μg/15 μCi)	^{111}In-DTPA (100 μg/10 μCi)
Tumor/liver	1.70 ± 0.28	0.58 ± 0.06	2.88 ± 0.13	1.75 ± 0.17
Tumor/blood	3.57 ± 0.41	5.99 ± 0.53	1.21 ± 0.10	1.44 ± 0.12

Tumor-bearing animals were injected with either ^{57}Co-VFC- or ^{111}In-DTPA-conjugated T84.66 as described in Fig. 3. Ratios were calculated from the %ID/g values for the respective tissues for individual animals and expressed as the mean ± SE for each group.

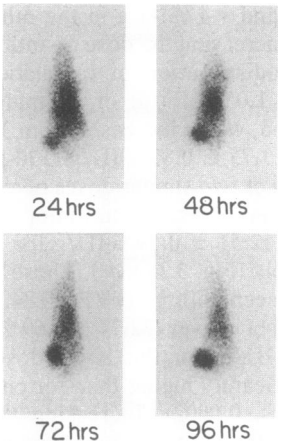

FIG. 4. Tumor imaging of a human colon cancer xenograft with ^{57}Co-VFC-T84.66. A female nude mouse was injected subcutaneously in the left flank with 10^6 LS174T tumor cells. Fourteen days later the animal was injected intraperitoneally with 130 μg of T84.66 mAb conjugated with 15 μCi of ^{57}Co-VFC, and scintiscans were performed 24, 48, 72, and 96 hr after injection.

DISCUSSION

Anti-CEA mAb T84.66 has previously been conjugated with DTPA (16), and the ^{111}In-labeled conjugate has been studied extensively in nude mice bearing LS174T human colon cancer xenografts (20–25) and in colon tumor patients (26–29). In the present report, we describe the preparation and subsequent characterization of radiolabeled T84.66, using a bifunctional reagent (VFC) designed specifically for transition metal radionuclides. ^{57}Co ($t_{1/2}$, 271 days; γ emission, 122 keV), although not used in clinical imaging because of its long half-life, was used as the transition metal radionuclide because of its availability, purity, high specific activity, and suitable γ emission for immunoscintigraphy and tissue biodistribution. ^{57}Co-VFC was prepared in good yield and conjugated to T84.66 via its hydroxysuccinimido active ester. The resulting conjugate was indistinguishable from nonconjugated T84.66 by gel filtration chromatography, SDS/PAGE, and enzyme immunoassay. Excellent chemical stability of the ^{57}Co-VFC conjugate was demonstrated by SDS/PAGE.

Unlike ^{111}In-DTPA-mAb conjugates in which DTPA is attached to the mAb prior to radiolabeling, VFC requires chemical preassembly of the radionuclide and ligand prior to conjugation with antibody. Although the VFC conjugation method is less convenient than the DTPA method, the chemical stability of VFC minimizes any possible release of radionuclide from the chelate, thereby making VFC very attractive for *in vivo* applications. Chelate stability is especially important in the preparation of radiolabeled mAbs for therapy, because radionuclide accumulation in normal tissues is dose-limiting.

Biodistribution results with ^{57}Co-VFC-T84.66 showed excellent tumor uptake, whereas radionuclide accumulation in normal tissues, especially liver, was low. This is in contrast to ^{111}In-labeled mAbs, which often show high levels of radionuclide in liver. The mechanisms of radionuclide accumulation in liver and other normal tissues are not fully understood, although they are thought to be dependent on both the uptake of radiolabeled mAb by normal tissues and its subsequent processing (30). We have previously shown that the high uptake of ^{111}In-DTPA-T84.66 in the liver is in part related to the formation of antigen–antibody complexes (23–25). The decrease in liver uptake of ^{111}In-DTPA-T84.66 shown here at the higher antibody dose was in agreement with our earlier studies in which liver uptake was strongly dependent on antibody dose (23). The low liver uptake and independence of antibody dose demonstrated by ^{57}Co-VFC-T84.66 could therefore be due to a decrease in the formation of these complexes; however, the retention of full immunoreactivity for ^{57}Co-VFC-T84.66 and its targeting to tumor suggest that the mAb was fully capable of forming antigen–antibody complexes. A more probable explanation for the low liver accumulation of ^{57}Co-VFC-T84.66 is that the processing of the conjugate and the subsequent excretion of ^{57}Co-VFC from the liver are accelerated relative to the ^{111}In-DTPA conjugate.

In previous studies, we have shown in animals injected with ^{111}In-DTPA-T84.66 that the radionuclide is retained in the liver in the form of low molecular weight components likely to be catabolites of the radiolabeled mAb (24, 25). Using the same methods, we have not detected ^{57}Co-labeled low molecular weight components in animals receiving ^{57}Co-VFC-T84.66 (31). Further, the amount of radioactivity in feces is greater for the ^{57}Co-VFC conjugate than for the ^{111}In-DTPA conjugate (unpublished data). The chemical stability of ^{57}Co-VFC, its unique structure and chemical properties compared with ^{111}In-DTPA, and its expected resistance to biological degradation may be important factors in this apparently accelerated excretion mechanism.

Several clinically useful transition metal radionuclides can potentially be accommodated by the VFC architecture. ^{67}Cu is very promising for radioimmunotherapy because of its half-life of 61.5 hr, abundant β particles, and γ emissions suitable for imaging. DTPA- and EDTA-conjugated mAbs form unstable complexes with ^{67}Cu (10), although stable complexes have been obtained using 6-*p*-bromoacetamidobenzyl-1,4,8,11-tetraazacyclotetradecane-*N,N',N'',N'''*-tetraacetate, a macrocyclic aminocarboxylate bifunctional reagent (10, 32, 33). The rigidity of the bifunctional VFC ligand described here prevents the formation of the "slipped" structure characteristic of Cu^{3+} bisdicarbollide clusters (12); however, this structure can most likely be achieved by introducing greater flexibility into the VFC ligand through the use of 3- or 4-atom bridges, rather than the 2-atom bridge provided by pyrazole. Based on our results with ^{57}Co-VFC-T84.66, we anticipate that mAb conjugates prepared with a VFC ligand suitable for ^{67}Cu would be extremely stable and would provide excellent tumor/normal tissue ratios.

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1. Krejcarek, G. E. & Tucker, K. L. (1977) *Biochem. Biophys. Res. Commun.* **77**, 581–585.
2. Hnatowich, D. J., Layne, W. W., Childs, R. L., Lanteigne, D., Davis, M. A., Griffin, T. W. & Doherty, P. W. (1983) *Science* **220**, 613–615.
3. Meares, C. F., McCall, M. J., Reardan, D. T., Goodwin, D. A., Diamanti, C. I. & McTigue, M. (1984) *Anal. Biochem.* **142**, 68–78.
4. Brechbiel, M. W., Gansow, O. A., Atcher, R. W., Schlom, J., Esteban, J., Simpson, D. E. & Colcher, D. (1986) *Inorg. Chem.* **25**, 2772–2781.
5. Westerberg, D. A., Carney, P. L., Rogers, P. E., Kline, S. J. & Johnson, D. K. (1989) *J. Med. Chem.* **32**, 236–243.
6. Murray, J. L., Rosenblum, M. G., Sobol, R. E., Bartholomew, R. M., Plager, C. E., Haynie, T. P., Jahns, M. F., Glenn, H. J., Lamki, L., Benjamin, R. S., Papadopoulos, N., Boddie, A. W., Frincke, J. M., David, G. S., Carlo, D. J. & Hersh, E. M. (1985) *Cancer Res.* **45**, 2376–2381.
7. Hnatowich, D. J., Griffin, T. W., Kosciuczyk, C., Rusck-

owski, M., Childs, R. L., Mattis, J. A., Shealy, D. & Doherty, P. W. (1985) *J. Nucl. Med.* **26,** 849–858.
8. Halpern, S. E., Haindl, W., Beauregard, J., Hagan, P., Clutter, M., Amox, D., Merchant, B., Unger, M., Mongovi, C., Bartholomew, R., Jue, R., Carlo, D. & Dillman, R. (1988) *Radiology* **168,** 529–536.
9. Hnatowich, D. J., Virzi, F. & Doherty, P. W. (1985) *J. Nucl. Med.* **26,** 503–509.
10. Cole, W. C., DeNardo, S. J., Meares, C. F., McCall, M. J., DeNardo, G. L., Epstein, A. L., O'Brien, H. A. & Moi, M. K. (1987) *J. Nucl. Med.* **28,** 83–90.
11. Hawthorne, M. F., Young, D. C., Andrews, T. D., Howe, D. V., Pilling, R. L., Pitts, A. D., Reintjes, M., Warren, L. F. & Wegner, P. A. (1968) *J. Am. Chem. Soc.* **90,** 879–896.
12. Hawthorne, M. F. & Dunks, G. B. (1972) *Science* **178,** 462–471.
13. Hawthorne, M. F., Varadarajan, A., Knobler, C. B., Chakrabarti, S., Paxton, R. J., Beatty, B. G. & Curtis, F. L. (1990) *J. Am. Chem. Soc.* **112,** 5365–5366.
14. Wagener, C., Yang, Y. H. J., Crawford, F. G. & Shively, J. E. (1983) *J. Immunol.* **130,** 2308–2315.
15. Wagener, C., Clark, B. R., Rickard, K. J. & Shively, J. E. (1983) *J. Immunol.* **130,** 2302–2307.
16. Paxton, R. J., Jakowatz, J. G., Beatty, J. D., Beatty, B. G., Vlahos, W. G., Williams, L. E., Clark, B. R. & Shively, J. E. (1985) *Cancer Res.* **45,** 5694–5699.
17. Baldwin, H. T., Rutzky, L. P., Jakstys, M. M., Oyasu, R., Kaye, C. I. & Kahan, B. D. (1976) *In Vitro* **12,** 180–191.
18. Beatty, J. D., Beatty, B. G. & Vlahos, W. G. (1987) *J. Immunol. Methods* **100,** 161–172.
19. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
20. Williams, L. E., Duda, R. B., Proffitt, R. T., Beatty, B. G., Beatty, J. D., Wong, J. Y. C., Shively, J. E. & Paxton, R. J. (1988) *J. Nucl. Med.* **29,** 103–109.
21. Jakowatz, J. G., Beatty, B. G., Vlahos, W. G., Porudominsky, D., Philben, V. J., Williams, L. E., Paxton, R. J., Shively, J. E. & Beatty, J. D. (1985) *Cancer Res.* **45,** 5700–5706.
22. Philben, V. J., Jakowatz, J. G., Beatty, B. G., Vlahos, W. G., Paxton, R. J., Williams, L. E., Shively, J. E. & Beatty, J. D. (1986) *Cancer* **57,** 571–576.
23. Beatty, B. G., Beatty, J. D., Williams, L. E., Paxton, R. J., Shively, J. E. & O'Conner-Tressel, M. (1989) *Cancer Res.* **49,** 1587–1594.
24. Beatty, J. D., Beatty, B. G., O'Conner-Tressel, M., Do, T. & Paxton, R. J. (1990) *Cancer Res. Suppl.* **50,** 840s–845s.
25. Beatty, B. G., O'Conner-Tressel, M., Do, T., Paxton, R. J. & Beatty, J. D. (1990) *Cancer Res. Suppl.* **50,** 846s–851s.
26. Duda, R. B., Beatty, J. D., Sheibani, K., Williams, L. E., Paxton, R. J., Beatty, B. G., Shively, J. E., Vlahos, W. G., Werner, J. L., Kemeny, M., Kokal, W. A., Riihimaki, D. U., Wagman, L. D. & Terz, J. J. (1986) *Arch. Surg.* **121,** 1315–1319.
27. Beatty, J. D., Duda, R. B., Williams, L. E., Sheibani, K., Paxton, R. J., Beatty, B. G., Philben, V. J., Werner, J. L., Shively, J. E., Vlahos, W. G., Kokal, W. A., Riihimaki, D. U., Terz, J. J. & Wagman, L. D. (1986) *Cancer Res.* **46,** 6494–6502.
28. Beatty, J. D., Philben, V. J., Beatty, B. G., Williams, L. E., Paxton, R. J., Shively, J. E., Duda, R. B., Vlahos, W. G., Werner, J. L., Sheibani, K., Kemeny, M., Kokal, W. A., Riihimaki, D. U. & Terz, J. J. (1987) *J. Surg. Oncol.* **36,** 98–104.
29. Beatty, J. D., Hyams, D. M., Morton, B. A., Beatty, B. G., Williams, L. E., Yamauchi, D., Merchant, B., Paxton, R. J. & Shively, J. E. (1989) *Am. J. Surg.* **157,** 13–19.
30. Sands, H. & Jones, P. L. (1987) *J. Nucl. Med.* **28,** 390–398.
31. Beatty, B. G., Paxton, R. J., Hawthorne, M. F., Beatty, J. D., Varadarajan, J., Do, T. & Lewis, M. (1991) *Antibody Immunoconjugates Radiopharm.* **4,** 14 (abstr.).
32. Moi, M. K., Meares, C. F., McCall, M. J., Cole, W. C. & DeNardo, S. J. (1985) *Anal. Biochem.* **148,** 249–253.
33. Deshpande, S. V., DeNardo, S. J., Meares, C. F., McCall, M. J., Adams, G. P., Moi, M. K. & DeNardo, G. L. (1988) *J. Nucl. Med.* **29,** 217–225.