The Humoral Immune Response to Macrocyclic Chelating Agent DOTA Depends on the Carrier Molecule

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The chelating agent 1,4,7,10-tetraazacyclododecane-*N,N′, N″,N‴*-tetraacetic acid (DOTA) is used to label monoclonal antibodies (mAbs) and peptides with ^{90}Y. DOTA allows the generation of clinically useful stable metallic radioconjugates for the treatment of a variety of tumors, but its immunogenicity has remained controversial. In this study, we evaluated the immune response to DOTA in a preclinical mouse model and in patients entered in a clinical trial. **Methods:** Sera were obtained from BALB/c mice injected intraperitoneally or subcutaneously with different doses and formulations of syngeneic and xenogeneic mAbs or peptide (murine mAb Mov19 [mM19]; its chimeric version; murine V/human C ChiMov19 [cM19]; or Tyr^3-octreotide)-DOTA conjugates. Sera from patients with neuroendocrine tumors, enrolled in a protocol for somatostatin receptor–mediated radionuclide therapy with ^{90}Y-DOTA-D-Phe^1-Tyr^3-octreotide (DOTATOC), were also collected before and after each treatment. Levels and specificity of antibody response to relevant (Mov19, ChiMov19, or Tyr^3-octreotide) and nonrelevant (human serum albumin) DOTA targets were tested by enzyme-linked immunosorbent assay and competition assays. An anti-DOTA mAb (IgG1) derived from a ChiMov19-DOTA immunized mouse was used, in a competitive radioimmunoassay, to determine the efficiency of DOTA presentation on the different carriers. **Results:** Depending on the immunogenicity and dosage of the mAb, a specific anti-DOTA response was revealed in the preclinical system. However, DOTA–peptide conjugate induced no immune-detectable response against either chelator or carrier. DOTA was poorly presented on small peptides, as determined using the anti-DOTA mAb. **Conclusion:** A humoral response against DOTA is possible, but only as a consequence of the response elicited against the carrier. Octreotide was not immunogenic. Thus, ^{90}Y-DOTATOC can be considered a safe and useful tool for receptor-mediated radionuclide therapy of somatostatin receptor–overexpressing tumors.

Key Words: DOTA; immunogenicity; antimacrocyclic antibodies; receptor-mediated radionuclide therapy

J Nucl Med 2001; 42:1697–1703

Received Feb. 16, 2001; revision accepted Jul. 18, 2001.

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Radioimmunoscintigraphic and radioimmunotherapeutic efforts have exploited the specificity of monoclonal antibodies (mAbs) to an antigen to target therapeutic and diagnostic reagents to cancer cells. However, the immunogenicity of mAbs, because of the presence of the Fc region and their relatively large size, represents significant drawbacks and limitations. New tumor targeting strategies (*1*,*2*) have involved the use of peptides and a variety of antibody-derived molecules (i.e., Fab fragments, single-chain Fv, and fusion proteins). Such peptides and antibody fragments have relatively high binding affinity and, in general, more favorable pharmacokinetics and reduced immunogenicity (*3*). A suitable peptide for radioisotope targeting is represented by somatostatin (SST)-14 and SST-28 and their synthetic analogs, which bind with high affinity to SST receptor (SSTR). This receptor is overexpressed in neuroendocrine tumors but is also expressed in tumors of the central nervous system, breast, lung, and lymphatic tissue (*4*,*5*).

A variety of mAbs and peptides have been labeled with metallic radionuclides such as ^{99m}Tc,^{111}In, ^{90}Y, ^{67}Ga, and ^{64}Cu through chelating agents coupled to the active peptide. In particular, an energetic β-ray emitter such as ^{90}Y (maximum energy of the β-emissions = 2.3 MeV [100%]) requires enhanced thermodynamic and kinetic stability of the radionuclide complex to avoid in vivo release of high levels of free ^{90}Y and, in turn, bone marrow toxicity (*6*,*7*).

To increase the stability of radioconjugates, chelating agents such as 1,4,8,11-tetraazacyclotetradecane-*N,N′,N″,N‴*-tetraacetic acid (TETA), diethylenetriaminepentaacetic acid (DTPA), and 1,4,7,10-tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid (DOTA) have been introduced to link the radionuclide to the carrier molecule. In particular, DOTA has been used to label mAbs and peptides with ^{90}Y, generating clinically useful radioconjugates for the treatment of a variety of tumors (*8*,*9*). Chelating agents are small haptens that are probably not immunogenic per se. However, conjugation to carrier proteins can present these molecules in a different manner, potentially resulting in a new epitope structure. Indeed, the immunogenicity of DOTA, as well as

that of other chelating agents, is currently an open question (*10*,*11*).

In this study, we focused on DOTA and evaluated the immune response after its conjugation to different carriers in a preclinical model. In our mouse model, we used an entire chimeric mAb with human constant regions to mimic the clinical conditions in which xenogeneic murine mAbs are used; an entire murine mAb to check the carrier efficiency of a mAb of the same species; and a small peptide, that is, a synthetic SST analog (Tyr^3-octreotide [TOC]), with high sequence homology to murine and human SST. We also evaluated patients receiving ^{90}Y-DOTA-D-Phe^1-TOC (DOTATOC) for any serum response to the chelating agent.

MATERIALS AND METHODS

Carriers

Table 1 summarizes the characteristics of the carriers used in evaluating DOTA immunogenicity. Briefly, we used an IgG2a murine mAb, Mov19 (mM19), directed against the human α-folate receptor (*12*); the chimeric version of mM19, murine V/human C ChiMov19 (cM19) (*13*); an isotype-matched murine control mAb, MGR6 (IgG2a), directed against the p185HER2 extracellular domain (*14*); and the SST analog TOC, an octapeptide that binds with high affinity to the SSTR subtypes 2, 3, and 5 (*5*). mAbs were purified by affinity chromatography on protein G.

Carrier Derivatization. The bifunctional chelating agent DOTA contains a chelating moiety that binds radiometals, such as ^{90}Y, that have high stability under physiologic conditions and a functional group for conjugation to the carrier (*15*). Briefly, DOTA was dissolved in methylsulfoxide anhydrous (DMSO) at 80°C, and the solution was allowed to cool under an argon atmosphere. *N*-hydroxy-2,5-pyrrolidinedione (NHS) in DMSO was added dropwise to the solution as it was stirred, followed by dropwise addition of dicyclohexylcarbodiimide (DCC) in DMSO. The final DOTA/NHS/DCC molar ratio was 1:1.4:0.8. The mixture was stirred overnight and then filtered. DOTA-activated *N*-succinimidyl ester (300:1) was added to carrier in 0.1 mol/L phosphate buffer, pH 8.0, for 2 h at room temperature. Coupling efficiency was determined before purification by coupling with ^{111}In in 0.1 mol/L acetate buffer and measuring the percentage of labeled protein by fast protein liquid chromatography (FPLC) using a gel filtration column (Superdex 200HR 10/30; Pharmacia Biotech, Uppsala, Sweden) or by instant thin-layer chromatography (Gelman Sciences, Ann Arbor, MI).

TABLE 1
Characteristics of Carriers

Carrier	Type of molecule	Molecular weight (kDa)
TOC	Peptide	1.133
ChiMov19	Entire chimeric mAb	150
Mov19	Entire murine mAb	150
MGR6	Entire murine mAb	150
Human serum albumin	Protein	68

Table 2 gives the average number of DOTA groups per carrier molecule. Unconjugated DOTA was removed by dialysis against 0.1 mol/L acetate buffer, pH 5.5 (Spectra/Por, molecular weight cutoff of 50,000 Da; Spectrum Laboratories, Inc., Rancho Dominguez, CA), and the radiochemical purity of the recovered conjugated protein was checked by FPLC.

The SST analog (DOTATOC) was synthesized at the Institute of Radiological Chemistry of Basel University (Basel, Switzerland) and kindly provided by Prof. Helmut R. Mäcke (University Hospital of Basel, Basel, Switzerland).

Carrier Biotinylation. Carriers and targets were biotinylated as previously described (*16*) to ensure homogeneous coating of enzyme-linked immunosorbent assay (ELISA) plates. mM19-DOTA, cM19-DOTA, human serum albumin (HSA)-DOTA, and HSA were biotinylated by BIOSPA (Milan, Italy) to a final biotin-to-protein molar ratio ranging from 2.66 to 4.12 (Table 2). cM19 was dialyzed overnight at 4°C against 50 mmol/L $NaHCO_3$, incubated with biotin ester (1 mg/mL in DMSO) for 4 h at room temperature with shaking, and further dialyzed overnight at 4°C against phosphate-buffered saline (PBS).

Mice and Immunization

Six-week-old female BALB/c mice (Charles River, Calco, Lecco, Italy) were injected subcutaneously or intraperitoneally (4–5 mice per group) with the different carriers derivatized or not derivatized with DOTA (Table 2) in a total volume of 200 μL on days 0, 14, and 28 and boosted on day 49. Conjugates were injected at a low dose (carrier moiety: 0.4 mg/kg, 2.7×10^{-9} mol, which is in the same range as that used in clinical trials with similar radiolabeled reagents on a mol/kg-of-body-mass basis) and a high dose (carrier moiety: 4 mg/kg, 2.7×10^{-8} mol). In the case of DOTATOC, injection doses were also calculated on the basis of the MAb conjugate doses (low-dose DOTATOC was similar to the high-dose MAb conjugate). When administered subcutaneously, the immunogens were injected in 2 separate sites in association with an adjuvant (monophosphoryl lipid A as a 2.5% squalene-in-water emulsion [MPL-SE; RIBI ImmunoChem Research Inc., Hamilton, MT]). Control mice were injected subcutaneously with adjuvant alone. Sera were collected on days 7, 21, 35, and 56. Control sera were obtained 1 wk before the first injection.

Patient Treatment

Eleven patients with histologically confirmed neuroendocrine malignant tumors were enrolled in a protocol for receptor-mediated radionuclide therapy with ^{90}Y-DOTATOC. The protocol consisted of 3 serial administrations of ^{90}Y-DOTATOC (30 μg DOTATOC labeled with 1.1 GBq ^{90}Y) over a period of 6 mo (*4*). Five months later, the patients received 2 additional cycles of therapy 3 mo apart at a higher dose (60 μg DOTATOC labeled with 2.2 GBq ^{90}Y). Sera were collected before the initial administration and 30–60 d after each DOTATOC treatment and evaluated for a possible antibody response to DOTA.

Serologic Assays

ELISA for Mouse Anti-DOTA Antibodies. Biotinylated cM19-DOTA, HSA-DOTA, cM19, or HSA, at 1.3 μg/mL, was added to each well (100 μL per well) of streptavidin-coated 96-well microtiter plates (Pierce Chemical Co., Rockford, IL) for 2 h at room temperature. After being washed, the plates were incubated for 1 h at room temperature with 100 μL PBS and 0.03% bovine serum albumin (BSA) containing serial dilutions of pre- or postimmunization sera (starting from 1:200). Antibody binding was detected

TABLE 2
Average Number of DOTA-Biotin Groups per Carrier Molecule and Conditions of Immunization

Carrier	Groups per molecule (*n*)	Biotin/protein (molar ratio)	Dose of injection (mol × 10^{-9}/kg)	Type of injection
TOC	1.0	*	42 (L)/420 (H)	i.p.
ChiMov19	6.0	2.66	2.7 (L)/27 (H)	i.p./i.p. or s.c.
Mov19	5.0	4.12	2.7 (L)/27 (H)	i.p./i.p. or s.c.
	†	*	27 (H)	i.p.
MGR6	†	*	27 (H)	i.p.
Human serum albumin	3.0	3.10	‡	‡

*Not biotinylated, because not used as target for enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA).
†Not derivatized with DOTA; used to verify immunogenicity of carrier.
‡Not injected; used as target for ELISA and RIA.
L = low dose; H = high dose; i.p. = intraperitoneal; s.c. = subcutaneous.
Data for TOC are as reported by Paganelli et al. (*4*). TOC was used also in clinical study, from 50 to 84 mol × 10^{-9}/kg.

by further incubation for 45 min at room temperature with horseradish peroxidase-conjugated sheep antimouse immunoglobulin (Amersham, Little Chalfont, U.K.) diluted 1:1,000 in PBS and 0.03% BSA. Negative control wells contained only horseradish peroxidase-conjugated sheep antimouse immunoglobulin. Selected sera were tested after preabsorption on streptavidin-conjugated Dynabeads M-280 (Dynal Biotech, Oslo, Norway) coated with different biotinylated DOTA carriers.

ELISA for Human Anti-DOTA Antibodies. ELISA on HSA-DOTA-biotin and HSA-biotin was performed using the same protocol as for mouse anti-DOTA sera. Human sera were diluted 1:5 and 1:20, and antibody binding was detected using horseradish peroxidase-conjugated goat antihuman immunoglobulin (Amersham).

Derivation of Anti-DOTA mAbs

Hybridomas were produced using standard methods (*17*). Spleen cells of a BALB/c mouse immunized with compound cM19-DOTA-biotin (Table 2) were mixed with NS0 mouse myeloma cells at a 5:1 ratio and seeded in 384 microtiter wells. Individual wells were continuously monitored for cell growth, and supernatants were tested by ELISA as described above. Positive wells were maintained and expanded, and supernatants from hybridoma cell lines 4/B2, 4/F11, and 3/B3 were collected for mAb purification. Three anti-DOTA mAbs (MDOTA-1, MDOTA-2, and MDOTA-3 from clones 4/B2, 4/F11, and 3/B3, respectively) were purified by G protein agarose FPLC chromatography. The functionality of mAbs was determined by ELISA, and purity by SDS-PAGE.

mAb isotype was determined by fluorescence-activated cell sorter analysis of the hybridoma-producing cell lines. Briefly, cell suspensions were permeated with ethanol, incubated with subclass-specific secondary biotinylated goat antimouse IgM, IgG1, IgG2a, and IgG2b mAbs (Amersham) followed by a second incubation with fluorescein isothiocyanate–labeled streptavidin (Amersham), and analyzed by flow cytometry (FACScan; Becton Dickinson, San Jose, CA). All 3 selected hybridomas produced IgG1 mAbs.

Inhibition Assay

mAb MDOTA-1 was used for labeling and competition experiments. The mAb was labeled with ^{125}I by the lactoperoxidase method (*18*) (specific activity, 162.8–262.7 kBq/μg) and tested for functionality by direct radioimmunoassay on streptavidin-coated plates treated with biotinylated target (HSA-DOTA and HSA). For the inhibition assay, biotinylated target was incubated with ^{125}I-labeled MDOTA-1 (2.5×10^5 cpm; 50 μL per well), alone or mixed with serial dilutions of sera from mice immunized with cM19- or mM19-DOTA-biotin, immunogen (DOTATOC, cM19-DOTA-biotin, mM19-DOTA-biotin, and MGR6), or target (HSA-DOTA-biotin) for 3 h at 37°C. Binding reactivity was evaluated using a γ-counter (Crystal II multidetector radioimmunoassay system; Packard BioScience Co., Meriden, CT).

RESULTS

Preclinical Evaluation of DOTA Immunogenicity

All mice survived the experiment with no evidence of altered vital signs. A specific antibody response to DOTA was measured by quantitative ELISA using cM19-DOTA-biotin or HSA-DOTA-biotin. Targets without DOTA were used as a negative control. ELISA revealed specific anti-DOTA antibody production after the first (subcutaneous) and second (intraperitoneal) injection of mAb-DOTA and no intragroup variability in humoral response level. As an example, the time course for the development of antibody response after injection of cM19-DOTA-biotin is shown in Figure 1. Treatment with adjuvant alone did not induce DOTA reactivity.

An antichelator response was observed when DOTA was conjugated to a mAb (cM19 or mM19), whereas sera from mice receiving DOTATOC showed no reactivity (Fig. 2A); reactivity was specifically directed against DOTA because no binding was detected on HSA-biotin (Fig. 2B). Furthermore, sera from mice injected with carrier-DOTA cM19 (high dose)- or mM19 (high dose and low dose)-DOTA-biotin (Table 2) also showed reactivity against the cM19 carrier. Anti-DOTA and anticarrier responses were confirmed by absorption on streptavidin-coated Dynabeads M-280 plus cM19-DOTA-biotin or HSA-DOTA-biotin and mAb generation.

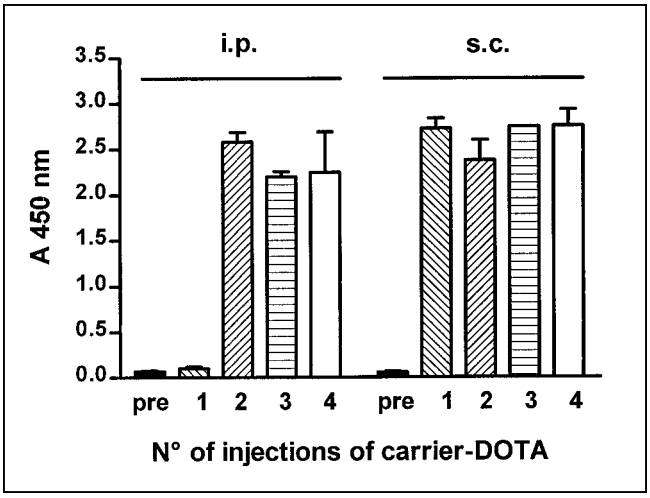

FIGURE 1. Number of injections needed to evoke humoral anti-DOTA response in mice receiving low-dose cM19-DOTA-biotin. Antibody binding was detected by quantitative ELISA on HSA-DOTA-biotin. Sera from 4 mice, obtained 1 wk after each injection, were pooled, diluted 1:200, and tested in duplicate. Data are mean ± SD from 2 independent experiments. A = absorbance; i.p. = intraperitoneal injection; pre = before injection; s.c. = subcutaneous injection.

Anti-DOTA MAb Generation

High-level antibody response against both DOTA and carrier (Fig. 2) identified a mouse injected with a high dose of cM19-DOTA-biotin as suitable for mAb generation. After cell fusion for hybridoma production, 36% of growing clones (38/106) were positive in ELISA and 74% (28/38) were directed to DOTA, whereas the remaining clones (10/38) were positive for carrier cM19. Three clones (MDOTA-1, -2, and -3) were maintained and expanded for further analysis and antibody purification. Inhibition assay of ^{125}I-labeled MDOTA-1 binding on HSA-DOTA-biotin showed that all 3 mAbs were directed against the same epitope.

Sera from mice receiving carrier (cM19 or mM19)-DOTA-biotin were used to further evaluate the specifics of the antibody response. Sera inhibited the binding of ^{125}I-labeled MDOTA-1 to HSA-DOTA-biotin (Fig. 3), and the inhibition was comparable with the binding activity on the same target (Fig. 2A).

Clinical Evaluation of DOTATOC Immunogenicity

Forty-seven sera from 11 patients with SSTR-positive tumors treated with ^{90}Y-DOTATOC were evaluated by ELISA on HSA-DOTA-biotin or HSA-biotin. Even at a final dilution of 1:5, sera showed only background reactivity with HSA-DOTA-biotin level, that is, superimposable on that found with HSA, indicating the absence of a specific humoral response against the chelator.

Efficiency of DOTA Presentation by Different Carriers

The efficiency of DOTA presentation by different carriers (cM19-DOTA, mM19-DOTA, and DOTATOC), and as control by the target HSA-DOTA, was evaluated by binding

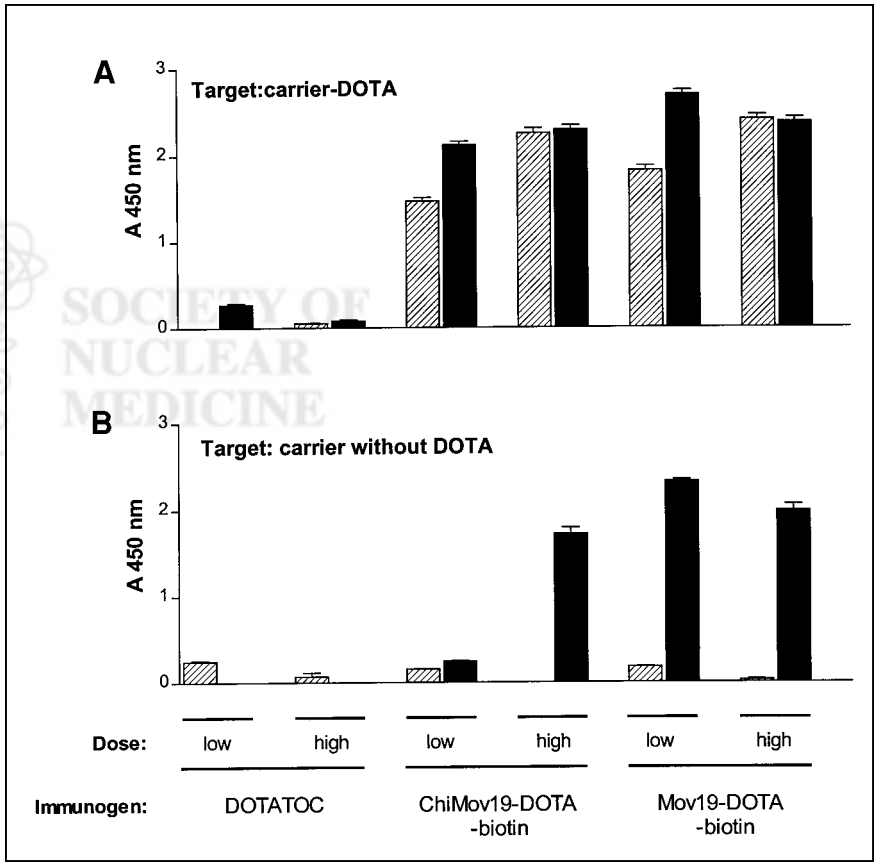

FIGURE 2. Specificity of binding reactivity of sera from mice immunized with different DOTA carriers. Binding activity was assessed on biotinylated HSA (hatched bars) or cM19 (solid bars), conjugated (A) or not conjugated (B) to DOTA, by quantitative ELISA. Sera (diluted 1:200 and tested in duplicate) were obtained after third injection (day 35) of immunogens, as described in Table 2. Data are mean ± SD of 2 replicates. A = absorbance.

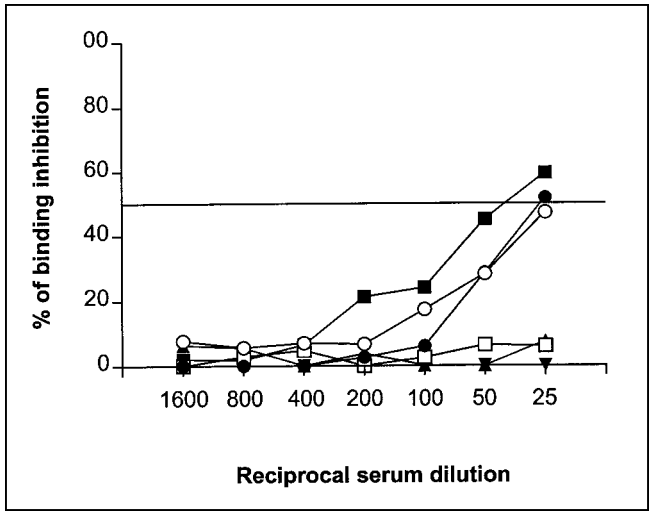

FIGURE 3. Inhibition of mAb MDOTA-1 binding by sera of DOTA carrier-immunized mice. ^{125}I-labeled MDOTA-1 and serially diluted sera (starting from 1:25) from mice immunized with low-dose TOC (▲), high-dose TOC (▼), low-dose cM19 (□), high-dose cM19 (■), low-dose mM19 (○), and high-dose mM19 (●) were incubated on HSA-DOTA-biotin. Samples were tested in duplicate; data are mean of 2 replicates (SD is not indicated because it was smaller than symbol).

inhibition of ^{125}I-labeled MDOTA-1 on HSA-DOTA on a DOTA molar basis. The inhibition induced by the 2 mAb-DOTAs (5 or 6 DOTA groups per carrier molecule) was comparable with that of HSA-DOTA (3 DOTA groups per carrier molecule) but higher than that of DOTATOC (1 DOTA group per carrier molecule) (Fig. 4). These data suggest a limited availability of DOTA to anti-DOTA mAb binding when presented by TOC.

DISCUSSION

A possible limitation that should always be considered in the design and development of new therapeutic antibody- or peptide-based agents is their immunogenicity, either intrinsic or consequent to chemical modification. Macrocyclic compounds such as DOTA and TETA have been introduced to the process of linking radiometals to antibodies or peptides to obviate the problem of in vivo instability of the direct labeling (*8,9*). Although stable metallic radioconjugates are produced, the use of such macrocyclic compounds raises the possibility of modification of protein conformation, resulting in new epitopes. Immunogenicity refers to the ability of a molecule to induce an immune response, whereas the antigenicity of a molecule relates to its recognition by preformed antibodies, defining the single epitopes of the same antigen. Thus, it appears that immunogenicity, and not antigenicity, is important in the clinical setting (*2*).

We focused on the chelating agent DOTA because, at present, it is the most widely used therapeutically on account of its ability to bind yttrium with a high degree of kinetic inertness in vivo. We tested its immunogenicity both in a preclinical in vivo model using different immunization protocols with DOTA conjugated to different carriers and in sera of patients undergoing receptor-mediated radionuclide therapy with ^{90}Y-DOTATOC. Together, our data indicate that DOTA induces a humoral response only when presented by an immunogenic carrier (xenogeneic and syngeneic mAbs) in a dose-dependent manner. TOC is not immunogenic, and thus, no anti-DOTA response was elicited in the treated patients.

Despite the slightly different timing in response induction (detectable after 1 subcutaneous injection in the presence of an adjuvant vs. 2 intraperitoneal administrations), the final level of the anti-DOTA response, when elicited, was comparable. As expected, the chimeric antibody cM19 xenogeneic carrier in the mouse system evoked a rapid and robust humoral response against itself and, as a consequence, against the chelator moiety. The specificity of the response allowed the generation of anti-DOTA–specific mAbs, all of which appeared to be directed against the same epitope, probably as a consequence of the simple structure of the molecule. In the only other report of mAbs against DOTA, the mAbs derived were specific for the metal–chelate complex (*19*).

A sizeable immune response against both the carrier and the hapten, comparable with that achieved with the xenogeneic carrier, was also detected when DOTA was pre-

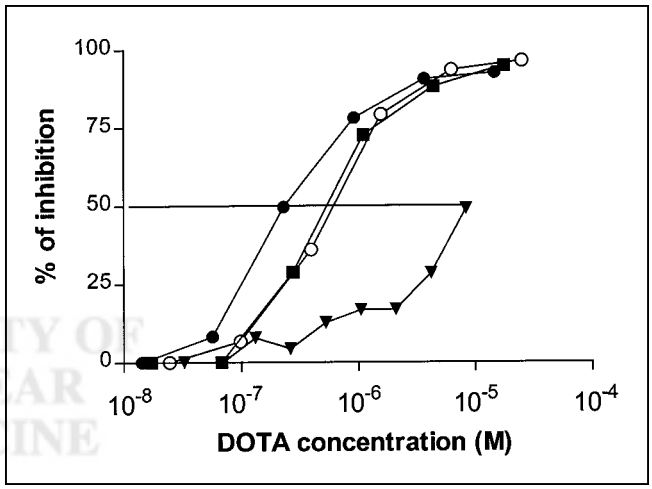

FIGURE 4. Efficiency of DOTA presentation by different carriers as tested by binding inhibition of anti-DOTA mAb MDOTA-1. Mixture of ^{125}I-labeled MDOTA-1 (2.5×10^5 cpm; 50 μL per well) and serial dilutions of DOTATOC (▼), cM19-DOTA-biotin (■), mM19-DOTA-biotin (●), or HSA-DOTA-biotin (○) were incubated on HSA-DOTA-biotin. Each sample was tested in duplicate; data are mean of 2 replicates (SD is not indicated because it was smaller than symbol). Inhibitory concentrations of 50% (related to DOTA molarity) were 2.8×10^{-7} mol/L (cM19-DOTA-biotin), 4.4×10^{-7} mol/L (mM19-DOTA-biotin), 6×10^{-7} mol/L (HSA-DOTA-biotin), and 8×10^{-6} mol/L (DOTA-TOC). Molar excess, related to target (HSA-DOTA-biotin) molarity (2×10^{-9} mol/L), was ×138 (cM19-DOTA-biotin), ×220 (mM19-DOTA-biotin), ×300 (HSA-DOTA-biotin), and ×3,000 (DOTATOC). M = mol/L.

sented by the syngeneic mM19. Consistent with the potential immunogenicity of the mAb idiotypic region in the recipient (*2*), our preliminary analysis of the anti-mM19 response indicated that it is directed against the variable region of the mAb. Moreover, the humoral response against both carrier (mAb) and hapten (DOTA) was elicited in a dose-dependent manner, indicative of efficient activation of the immune system.

By contrast, when DOTA was carried by the synthetic SST analog TOC, which has high sequence homology with both the human and the murine peptides, no binding reactivity on DOTA was detected, even using doses 10 times higher than those used in clinical trials. Furthermore, no anti-DOTA antibodies were detected in 47 sera from 11 patients with solid neuroendocrine tumors treated with ^{90}Y-DOTATOC. Use of an anti-DOTA–specific mAb served to refine the determination of serum activity of immunized mice and of the efficiency of DOTA presentation on the different carriers. The data indicated that, even when the number of DOTA groups per carrier molecule was considered (1 for DOTATOC and 3–6 for HSA and antibodies; Table 2), the interaction of the mAb with the hapten is partially constrained when it is bound to a small peptide.

Our findings may help to reconcile the disparate observations on the immunogenicity of chelating agents. In particular, a study reported a specific antibody response against DOTA in patients with different types of solid tumors receiving ^{90}Y-DOTA-entire murine mAb (HMFG1 or H17E2; molecular weight, 150 kDa) or entire humanized mAb directed against polymorphic epithelial mucin or placental alkaline phosphatase (*10*,*20*). Similarly, a response against a different chelating agent, (*S*)-4-{2,3-bis[bis(carboxymethyl)aminopropyl]isothiocyanate} of DTPA (CITC-DTPA), was detected after treatment with ^{90}Y-CITC-DTPA-entire murine mAb HMFG1 (*21*). Therefore, any chelating agent, regardless of its structural rigidity, can act as a hapten and elicit an immune response when coupled to an appropriate carrier molecule. By contrast, DeNardo et al. (*11*) showed that macrocyclic compounds are not immunogenic in lymphoma patients treated with ^{90}Y-DOTA-entire murine mAb (Lym-1; molecular weight, 150 kDa) directed against an antigen associated with B-cell lymphomas. DeNardo et al. also alluded to similar observations in unpublished studies. Despite differences in the parameters and protocols in those studies (i.e., different routes of administration of the radioimmunoconjugates, the presence or absence of protein aggregates in the preparation, and different antibodies and patient populations), the main determinant in defining the development of a response against a chelating agent was the concomitant absence or presence of a human antimouse antibody or antiidiotypic response. All the mentioned studies, as well as our current report, dealt with the entire mAb molecule, whose immunogenicity depends not only on the species of origin but also on the presence of an efficient Fc region and on total molecular mass (*22*). Together, these data suggest that in the case of a chemically defined structure such as that of the macrocyclic chelator DOTA, an active carrier is necessary in the recruitment of the host immune system.

CONCLUSION

Our data provide experimental proof that the most important factor in determining the anti-DOTA response is the intrinsic immunogenicity of the carrier, which, in turn, is related to its molecular nature and mass. These findings, consistent with previous clinical observations, suggest that the macrocyclic chelator DOTA or molecules with a similar structure can safely be used to generate stable radiolabeled therapeutic agents, provided that the immunogenicity of the carrier guiding the therapy is absent or minimized.

ACKNOWLEDGMENTS

The authors thank Alessandra Cipollina and Eleonora Del Fabbro for secretarial assistance. This study was partially supported by Associazione Italiana Ricerca sul Cancro and Fondazione Italiana Ricerca sul Cancro.

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