Bispecific Antibody and Bivalent Hapten Radioimmunotherapy in CEA-Producing Medullary Thyroid Cancer Xenograft

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The purpose of this study was to compare the toxicity and efficacy of two-step radioimmunotherapy using a bispecific anti-carcinoembryonic antigen (CEA)/anti-diethylenetriamine pentaacetic acid (DTPA) antibody (F6-734 bispecific monoclonal antibodies (BsMAbs) and an ^{131}I-di-DTPA-TL bivalent hapten with $F(ab')_2$ fragments of the same directly labeled anti-CEA ^{131}I-F6. **Methods:** Eight groups of nude mice subcutaneously grafted with the human TT medullary thyroid cancer cell line were injected once tumor volume reached about 200 mm^3. Two groups received 37 or 92.5 MBq (1 or 2 nmol) ^{131}I-di-DTPA-TL 48 h after injection of 2 or 4 nmol F6-734 BsMAb and two groups received 37 or 92.5 MBq (250 μg) ^{131}I-F6. Four control groups were treated respectively with (a) 92.5 MBq nonspecific ^{131}I-734 fragments, (b) 92.5 MBq ^{131}I-di-DTPA-TL 48 h after injection of a mixture of irrelevant F6-679 (anti-CEA/anti-histamine) and G7A5-734 (anti-melanoma/anti-DTPA) BsMAb, (c) 250 μg nonradiolabeled F6, and 250 μg F6-734 BsMAb and then 48 h later 1.25 nmol of nonradiolabeled hapten. A control group received no injections. Toxicity was evaluated by determining animal weight and the number of leukocytes and platelets, and efficacy by variation in tumor volume and thyrocalcitonin during a 90-d period. Histological analysis of tumors and statistical studies were performed. **Results:** The time required for the tumor to double in size was respectively 57 and 86 d with 37 and 92.5 MBq F6-734/^{131}I-di-DTPA-TL and 44 and 65 d with 37 and 92.5 MBq ^{131}I-F6. Changes in thyrocalcitonin levels were parallel to those in tumor volume. Weight loss was 5%, leukocyte nadirs respectively 1640 ± 838 and 1560 ± 1160/mm^3 and platelet nadirs 1.46 ± 0.52 10^6/mm^3 and 0.73 ± 0.38 10^6/mm^3 after injections of 37 and 92.5 MBq F6-734/^{131}I-di-DTPA-TL. Weight loss was respectively 8% and 16%, leukocyte nadirs 50 ± 100/mm^3 and 175 ± 50/mm^3 and platelet nadirs 0.71 ± 0.18 10^6/mm^3 and 0.48 ± 0.11 10^6/mm^3 after injections of 37 and 92.5 MBq ^{131}I-F6. **Conclusion:** Two-step radioimmunotherapy was as efficient as the one-step system and markedly less toxic.

Key Words: radioimmunotherapy; bispecific antibody; two-step targeting; medullary thyroid cancer

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Monoclonal antibodies (MAbs) directly labeled with ^{131}I have proved only modestly efficient for radioimmunotherapy (RIT) of solid tumors other than low-grade lymphomas. Uptake is generally below 0.01% for large tumors, and tumor-to-normal tissue ratios are moderate (usually <10) (*1,2*). The frequency of complete and even partial responses has been low, not attaining 5% in phase I/II studies including patients most often with large tumor masses (*3*). However, the affinity-enhancement system (AES), a two-step targeting technique using bispecific monoclonal antibody (BsMAb) and bivalent hapten, developed by Immunotech (Marseille, France), allows tumor-to-normal tissue ratios to be increased three- to five-fold in normal tissues by lowering radioactivity levels (*4,5*).

Medullary thyroid carcinoma (MTC), a neoplasm of the parafollicular cells, represents around 10% of thyroid cancers. The residual or metastatic forms of this neuroendocrine tumor, which is only slightly chemosensitive, constitute a potential application for RIT (*6*). It has been clearly established that its neoplastic cells express and secrete carcinoembryonic antigen (CEA) (*7*). A clinical immunoscintigraphic study performed with ^{111}I-labeled bivalent hapten has given encouraging results, visualizing occult metastatic sites biologically suspected after a rise in serum thyrocalcitonin (TCT) concentration (*8*). A clinical dosimetric study performed with ^{131}I-labeled bivalent hapten indicated that tumor doses of more than 100 cGy/mCi were probably tumoricidal in neoplasms weighing less than a few grams when injected activities were above 100 mCi (*9*).

The purpose of this study in the nude mouse grafted subcutaneously with a human MTC line was to compare the toxicity and efficacy of therapeutic injections of anti-CEA/anti-diethylenetriamine pentaacetic acid (DTPA) BsMAb and ^{131}I-di-DTPA-TL hapten with those of the $F(ab')_2$ fragment of the same antibody directly labeled with ^{131}I. The activities of the injected reagents (37 and 92.5 MBq) and the prelocalization period (48 h) of the two-step system were chosen according to the pharmacokinetic and dosimetric results obtained during preliminary studies in the animal (*10,11*).

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MATERIALS AND METHODS

Cell Line

The TT cell line of human MTC obtained from the American Type Culture Collection (Rockville, MD) for use in this study expresses CEA on its cell membrane and secretes TCT. It was cultured in adherent-cell monolayers in RPMI 1640 medium (Gibco BRL, Cergy-Pontoise, France) to which was added 10% fetal calf serum (FCS; Gibco BRL), 1% glutamine (L-glutamine 200 mm; Gibco BRL) and 1% antibiotic (penicillin 100 U/mL, streptomycin 100 U/mL; Gibco BRL; Life Technologies, Cergy-Pontoise, France).

Animal Model

Nude mice over 10 wk of age were grafted subcutaneously in the right flank with 10^6 TT cells in 0.3 mL of sterile physiological serum. The animals were housed in aseptic conditions and used once the tumors reached a size of approximately 200 mm^3, about 6 wk after injection. Lugol's solution 0.1% was added to drinking water (1 mL/100 mL) 1 wk before and 2 wk after injection of the radioiodinated reagent.

Antibody and Hapten

The reference antibody was an anti-CEA $F(ab')_2$ fragment designated F6. This mouse IgG_1 antibody, used in $F(ab')_2$-fragment form (*12*), was provided by CIS Bio International (Gif-sur-Yvette, France). The F6-734 BsMAb, obtained by chemical coupling of the Fab′ fragment of F6 antibody with the Fab′ fragment of 734 antibody (anti-DTPA-In IgG_1), was developed and provided by Immunotech. The $F(ab')_2$ fragment of nonspecific 734 antibody and the irrelevant BsMAb used as controls were obtained respectively by coupling the Fab′ fragment of F6 antibody to the the Fab′ fragment of 679 antibody (antihistamine murine IgG_1) and the Fab′ fragment of G7A5 antibody (antimelanoma murine IgG_1) to the Fab′ fragment of 734 antibody. These antibodies were developed and provided by Immunotech, together with bivalent hapten, Nα-(diethylenetriamine-N,N,N′,N″-tetraacetic acid-N″-acetyl)-tyrosyl-Nε-(diethylenetriamine-N,N,N′,N″-tetraacetic acid-N″-acetyl)-lysine (di-DTPA-TL) (*5*).

Labeling and Controls of the F6 Fragment

Direct Labeling of F6 Fragment with ^{131}I. The antibodies were labeled with iodogen method technic as described by Farker and Speck (*13*).

Measurement of the Specific Activity of ^{131}I-F6. The activity of labeled antibody was measured in an ionization chamber (Medi-202; Medisystème, Guyancourt, France), which gave values ranging from 185 to 555 MBq/mg for the different groups.

Measurement of the Radiochemical Purity of ^{131}I-F6. After purification, 2 ml of the ^{131}I-F6 solution were deposited on a 10 × 2 cm strip of ITLC-SG chromatographic paper (Gelman, Ann Arbor, MI). After drying, the strip was placed in a chromatographic tank containing trichloracetic acid 10%. After development of thin-layer (around 8 cm) chromatography, the strip, protected by a plastic film, was exposed on a phosphorus screen for 5 min. The screen was then scanned, and the chromatogram obtained was analyzed with IPLAB-Gel software (Signal Analytics, Vienna, VA). Radiochemical purity (the ratio of the number of pixels of the chromatographic deposit to the total number of pixels of the chromatogram) was always above 98%.

Measurement of the Immunoreactivity of ^{131}I-F6. Ten μL of an ^{131}I-F6 solution diluted 1:1000 were deposited in tubes coated with anti-idiotype antibody (IgG 44.12.13), provided by Immunotech, which contained 250 ml phosphate buffer 0.1 mol/L, pH 7, plus 0.5% bovine serum albumin (BSA). The total activity was measured after 1 h of incubation at room temperature with slow stirring. The tubes were then washed three times with phosphate buffer 0.1 mol/L, pH 7, plus 0.01% Tween 20, and the bound activity was measured. The immunoreactivity (the ratio of bound activity in the tubes after washing to total activity) was 85%–96%.

Labeling and Controls of di-DTPA-TL Hapten

Labeling of di-DTPA-TL-In Hapten with ^{131}I. Di-DTPA-TL hapten was provided in the form of a 1.11 mmol/L solution in citrate-acetate buffer (10–100 mmol/L), pH 5. In a sterile 2-ml plastic tube were deposited successively 25 mL of di-DTPA-TL-In (25 nmol), 25 mL of phosphate buffer 0.3 mol/L (pH 6), 50 mL of a chloramine-T solution at 1 mg/mL in phosphate buffer 0.3 mol/L (pH 6) and 100 mL of a ^{131}I solution at 16.650 GBq/mL in sodium bicarbonate 0.1 mol/L (pH 8) (^{131}I-S3B, CIS Bio International). After 10 min incubation at room temperature, the reaction was stopped by addition of sodium metabisulfite at 1 mg/mL in phosphate buffer 0.3 mol/L, pH 6. The pH of the solution was brought to between 5 and 6 by addition of 750 ml N(2-hydroxyethyl) piperazine-N′ 2-ethane sulfonic acid 1 mol/L. The resulting solution was purified on a C18-grafted silica column (Sepack-C18; Millipore, St. Quentin Yuelines, France). Free iodine was eluted with 5 mL phosphate buffer 0.1 mol/L, pH 7, and the radiolabeled hapten by 5 mL phosphate buffer 0.1 mol/L (pH 7)-ethanol mixture (3:2).

Measurement of the Specific Activity of ^{131}I-di-DTPA-TL. The ethanolic solution activity corresponding to ^{131}I bound to the hapten was measured in an ionization chamber. The specific activity was 59.2–70.3 MBq/nmol.

Measurement of the Radiochemical Purity of ^{131}I-di-DTPA-TL. Ten microliters of a ^{131}I-di-DTPA-TL solution diluted 1:1000 were deposited in tubes coated with a 734 antibody provided by Immunotech, which contained 250 ml phosphate buffer 0.1 mol/L, pH 7, 0.5% BSA. After 1 h incubation at room temperature with slow stirring, total activity was measured. The tubes were then washed three times with phosphate buffer 0.1 mol/L, pH 7, 0.01% Tween 20, and the bound activity was measured. Radiochemical purity (the ratio of bound activity in the tubes after washing to total activity) was above 90%.

Radioimmunotherapy

Eight groups of 6–8 mice each were injected in the lateral tail vein with a 0.2 mL solution of diluted immunoconjugate or hapten in sterile physiological serum. Two groups with initial tumor volumes of 180 ± 88 and 245 ± 123 mm^3 were injected respectively with 37 and 92.5 MBq (250 μg) ^{131}I-F6 fragment. Two other groups with initial tumor volumes of 302 ± 50 and 170 ± 55 mm^3 were injected respectively with 2 and 4 nmol (200 and 400 μg) BsMAb F6-734, and then 48 h later with 37 and 92.5 MBq (1 and 2 nmol) ^{131}I-di-DTPA-TL hapten. Preliminary studies determined that 400 μg BsMAb represented an amount less than that required to saturate the tumor (unpublished results) and that a ratio of 0.5 at injection between the hapten and BsMAb was most favorable for targeting (*11*). Four control groups were treated respectively with (a) 92.5 MBq nonspecific ^{131}I-734 fragment, (b) 92.5 MBq ^{131}I-di-DTPA-TL hapten administered 48 h after the administration of a mixture of 4 nmol irrelevant F6-679 BsMAb and 4 nmol irrelevant G7A5-734 BsMAb, (c) 250 μg nonradiolabeled F6 fragment and (d) 2.5 nmol F6-734 BsMAb followed 48 h later by 1.25 nmol of nonradiolabeled hapten. The initial tumor volumes of

these four groups were respectively 113 ± 55, 167 ± 50, 228 ± 94 and 192 ± 125 mm^3. Finally, a control group of 12 mice with an initial tumor volume of 228 ± 94 mm^3 received no injections.

The length (L), width (w) and thickness (t) of tumors were measured with a sliding caliper twice a week for 90 d. Tumor volume (V) was calculated according to the formula: $V = \pi/6 \times L \times w \times t$ (*14*). All animals were weighed on the day of injection and then twice a week for 90 d. Biological monitoring was performed on a blood sample drawn from the inner border of the eye. The parameters used to evaluate the toxicity of each type of treatment were maximal weight loss and the variation in the number of leukocytes and platelets measured on days 0, 7, 15, 21, 30 and 60. These assays were performed on 4 mice from each group. The parameters used to evaluate the efficacy of each type of treatment were the minimal relative volume (ratio of the smallest tumor volume observed to the initial size before treatment), the growth delay (time required for the tumor to double in size after measurement on the day of treatment) and the variation in serum TCT concentration measured by radioimmunoassay on days 0, 15, 30, 45 and 60 (calcitonin immunoradiometric assay, CIS Bio International Co.). The detection limit was 50 pg/mL. These counts were performed on 2 mice from each group.

Statistical Analysis

Owing to the limited number of animals, the means for the quantitative variables of the different groups were compared using nonparametric tests (the Mann-Whitney *U* test for comparison of two groups and the Kruskal-Wallis test for comparison of more than two groups). *P* values ≤ 0.05 were considered significant. BMDP Statistical Software, Version 7.0 (Cork, Ireland), was used for the analysis.

Histological Study

A histological analysis of tumors treated with specific radioiodinated reagents was performed at the time of minimum tumor volume and after resumption of growth. Untreated tumors were also subjected to histological analysis. The fragments were fixed in a 10% formol solution, embedded in paraffin, cut into 4-μm sections and stained with hemalum-eosin-safran. The degree of cellular pleomorphism was studied quantitatively by cell size and density, the nucleocytoplasmic ratio, the appearance of chromatin and the size of nucleoli. The proliferation index was estimated from the number of mitoses per field at high magnification (×400) and by an immunoperoxidase technique using MiB1 antibody directed against Ki67 antigen, the nuclear protein expressed in all active phases of the cell cycle (G_1, S, G_2 and M) but absent in G_0 and early G_1 (*15*). Cellular reactivity with anti-CEA and anti-TCT MAbs was studied by an indirect immunoperoxidase technique.

RESULTS

Radioimmunotherapy Toxicity

The mean maximal weight losses observed during the 2 wk after treatment were respectively 8% and 5% after injections of 37 MBq ^{131}I-F6 and F6-734/^{131}I-di-DTPA-TL ($P = 0.12$), and 16% and 5% after 92.5 MBq ^{131}I-F6 and F6-734/^{131}I-di-DTPA-TL ($P = 0.004$) (Table 1).

Blood cell counts are indicated in Table 2. In untreated controls, the mean leukocyte concentration was 2700/mm^3 (range 800–7000) and that of platelets 1.4 10^6/mm^3 (range 0.57–2.7 10^6). There was a significant difference relative to toxicity on leukocytes (expressed as a percentage of variation between the nadir at day 15 and the basal value at day 0) between the group treated with 37 MBq ^{131}I-F6 (−98% ± 3%) and that treated with 37 MBq F6-734/^{131}I-di-DTPA-TL (+11% ± 80%) ($P = 0.01$), and between the group treated with 92.5 MBq ^{131}I-F6 (−89% ± 8%) and that

TABLE 1
Tumor Effect and Toxicity of Radioimmunotherapy

Groups	Minimal relative tumor volume (%)	Growth delay (d)	Maximal weight loss	No. of mice that died	p'
^{131}I-F6 37 MBq	63 ± 14	44 ± 21*	8%* (14–5)	0	–
F6-734/^{131}I-di-DTPA-TL 37 MBq	65 ± 28	57 ± 09*	5% (10–1)	2 (D36, D67)	0.60
^{131}I-F6 92.5 MBq	42 ± 27	65 ± 11*	16%* (25–5)	2 (D28, D75)	0.18
F6-734/^{131}I-di-DTPA-TL 92.5 MBq	42 ± 18	86 ± 22*	5% (11–0)	2 (D52, D75)	0.03
No injection	100 ± 0†	12 ± 04	3% (7–2)	0	–
Nonradiolabeled F6	100 ± 0†	09 ± 02	2% (5–0)	0	–
Nonradiolabeled F6-734/di-DTPA-TL	100 ± 0†	23 ± 10	4% (11–1)	0	–
^{131}I-734	100 ± 0†	19 ± 04	9%* (14–7)	2 (D20, D52)	–
Irrelevant BsMAb/^{131}I-di-DTPA-TL	100 ± 0†	12 ± 04	4% (7–0)	1 (D60)	–

*$P \leq 0.05$ compared to the noninjected control group.
†No tumor shrinkage occurred in these mice.
d = day of death; p' = comparison of growth delay to reference treatment (^{131}I-F6 37 MBq); DTPA = diethylenetriamine pentaacetic acid.

TABLE 2
Blood Cells at Different Days After Therapeutic Injection

Groups	Day 0	Day 7	Day 15	Day 21	Day 30	Day 60
	Leukocytes (/mm³)					
37 MBq						
^{131}I-F6	2480 ± 454	ND	50 ± 100	3375 ± 1830	2075 ± 1021	2425 ± 531
F6-734/^{131}I di-DTPA-TL	1900 ± 806	2040 ± 699	1640 ± 838	3200 ± 2000	2840 ± 1290	3220 ± 988
92.5 MBq						
^{131}I-F6	2525 ± 2483	375 ± 171	175 ± 50	800 ± 294	1550 ± 759	1700 ± 816
F6-734/^{131}I di-DTPA-TL	2200 ± 535	2020 ± 998	1560 ± 1160	3000 ± 1920	1580 ± 1100	2450 ± 900
Controls						
^{131}I-734	2400 ± 570	1000 ± 524	625 ± 206	2675 ± 567	ND	2033 ± 1167
Irrelevant BsMAb/^{131}I-di-DTPA-TL	3360 ± 795	2300 ± 681	1380 ± 637	3020 ± 584	2420 ± 890	3050 ± 981
	Platelets (10^6/mm³)					
37 MBq						
^{131}I-F6	1.70 ± 0.55	ND	0.71 ± 0.18	1.52 ± 0.68	ND	1.01 ± 0.26
F6-734/^{131}I-di-DTPA-TL	1.18 ± 0.53	1.70 ± 0.44	1.46 ± 0.52	2.00 ± 0.80	2.42 ± 0.89	1.50 ± 0.20
92.5 MBq						
^{131}I-F6	1.47 ± 0.25	0.95 ± 0.46	0.48 ± 0.11	0.88 ± 0.16	1.23 ± 0.65	1.28 ± 0.65
F6-734/^{131}I-di-DTPA-TL	1.12 ± 0.58	0.88 ± 0.44	0.73 ± 0.38	1.92 ± 0.44	ND	1.80 ± 0.50
Controls						
^{131}I-734	1.35 ± 0.21	0.80 ± 0.19	1.04 ± 0.45	0.88 ± 0.31	ND	0.97 ± 0.32
Irrelevant BsMAb/^{131}I-di-DTPA-TL	1.17 ± 0.20	1.59 ± 0.62	1.35 ± 0.25	1.35 ± 0.74	1.23 ± 0.51	1.37 ± 0.28

ND = Not done.

treated with 92.5 MBq F6-734/^{131}I-di-DTPA-TL (−34% ± 41%) ($P = 0.04$). For platelet toxicity, no significant difference was found between the group treated with 37 MBq ^{131}I-F6 (−50% ± 26%) and that treated with 37 MBq F6-734/^{131}I-di-DTPA-TL (+45% ± 64%) ($P = 0.08$). Conversely, the difference between the group treated with 92.5 MBq ^{131}I-F6% (−66% ± 10%) and that treated with 92.5 MBq F6-734/^{131}I-di-DTPA-TL (−39% ± 24%) was significant ($P = 0.04$). After reaching the nadir point at day 14, hematopoiesis was restored spontaneously.

Nine animals died during this monitoring period (Table 1). The deaths occurring 28 d after injection of 92.5 MBq ^{131}I-F6 and 20 d after injections of 92.5 MBq ^{131}I-734 were related to leukopenia complicating the treatments. The other deaths that occurred later, after therapy, were related to infection (as verified by histological study at autopsy).

Efficacy of ^{131}I-F6 and F6-734/^{131}I-di-DTPA-TL

After injections of ^{131}I-F6 and F6-734 BsMAb/^{131}I-di-DTPA-TL hapten, all tumors decreased in size (Fig. 1A–D). The minimal mean relative tumor volumes of each group are indicated in Table 1. A resumption of tumor growth was noted in all animals. The growth delays were respectively 44 ± 21 and 65 ± 11 d with 37 and 92.5 MBq ^{131}I-F6, and 57 ± 9 and 86 ± 22 d with 37 and 92.5 MBq F6-734/^{131}I-di-DTPA-TL (Table 1). When treatment with 37 MBq ^{131}I-F6 was used as reference, only the growth delay after injection of 92.5 MBq F6-734/^{131}I-di-DTPA-TL was significantly longer (Table 1). The changes in the TCT concentrations of the 2 mice tested from each group (Fig. 2A and B) were parallel to those in tumor volume. Minimal serum concentrations were respectively 168 pg/mL at day 15 and <50 pg/mL at day 30 after injections of 37 and 92.5 MBq ^{131}I-F6, and 142.5 and <50 pg/mL at day 30 after 37 and 92.5 MBq F6-734/^{131}I-di-DTPA-TL. Biological responses lasted 15 d in 2 mice and 30 d in the other 2 with ^{131}I-F6, and 30 d in 2 mice and 45 d in the other 2 with F6-734/^{131}I-di-DTPA-TL.

Efficacy of Treatments in Control Groups

In the five control groups, rapid tumor growth was associated with a rise in serum TCT concentration (Figs. 1E–G and 2C and D). Growth delays were not significantly different between these groups (Table 1).

Histological Study

Tumor proliferation was microscopically comparable in the different samples obtained from growing tumors, consisting of a dense growth of large cells with a high nucleocytoplasmic ratio. The large nuclei displayed regular borders, fine chromatin and small nucleoli. Proliferation took the form of rows and lobules separated by a thin fibrovascular stroma.

Control tumor obtained from untreated mice was characterized by an absence of necrosis and a mitotic index of 50 mitoses per 10 high-power fields (one HPF = 0.312 mm²). MiB1 labeled 300 nuclei per 10 HPF, and 100% of the cells expressed CEA and TCT.

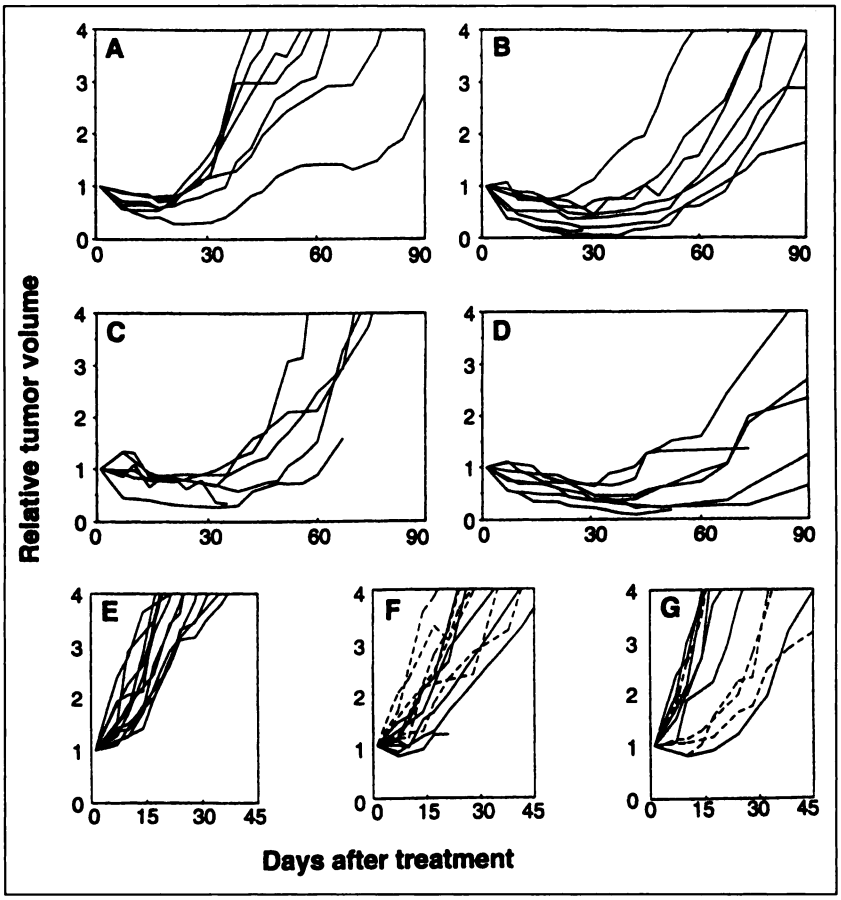

FIGURE 1. Variation of tumor size in treated mice and controls. (A) ^{131}I-F6 37 MBq, (B) ^{131}I-F6 92.5 MBq, (C) F6-734/^{131}I-di-DTPA-TL 37 MBq, (D) F6-734/^{131}I-di-DTPA-TL 92.5 MBq, (E) no injection, (F) nonradiolabeled F6 (—) and nonradiolabeled F6-734/di-DTPA-TL (---) and (G) ^{131}I-734 (—) and irrelevant BsMAb/^{131}I-di-DTPA-TL (---).

The tumor fragments removed at the time of the smallest relative volume showed 50%–60% fibrosis. Mitoses were rare (<10 per 10 HPF). Antibody labeled 100 nuclei per 10 HPF, and 100% of the cells expressed CEA and TCT.

However, recurring tumors showed a varying number of necrotic areas: fewer than 10% after injection of 92.5 MBq ^{131}I-F6 and 30%–50% after injection of 92.5 MBq F6-734/^{131}I-di-DTPA-TL. The mitotic index was 100–120 mitoses per 10 HPF. MiB1 antibody labeled 1500–2000 nuclei per 10 HPF, and 100% of the cells expressed CEA and TCT.

DISCUSSION

Several studies in animals have clearly shown that therapeutic injection of directly labeled MAbs generally

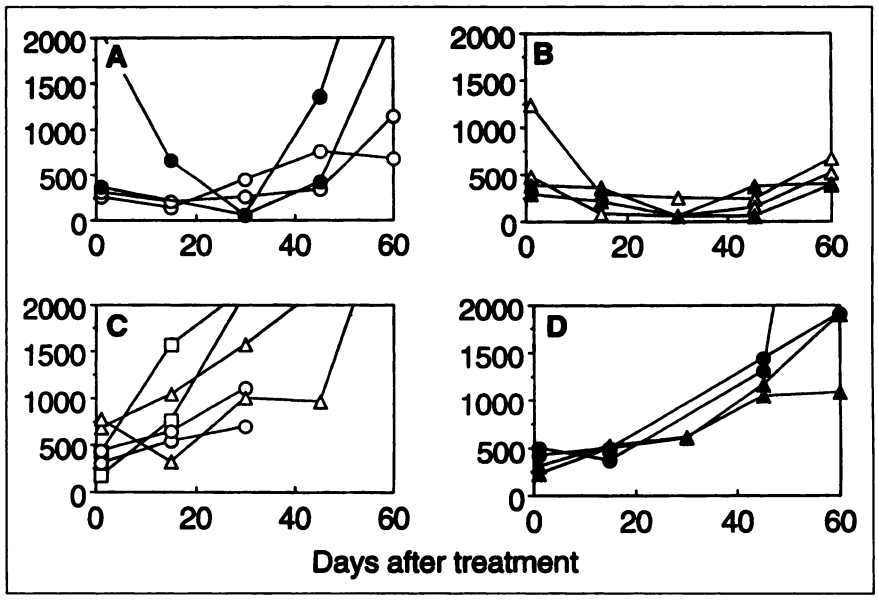

FIGURE 2. Variation of thyrocalcitonin concentration (pg/mL) in treated mice and controls (2 mice/group). (A) ^{131}I-F6 37 MBq (○) and 92.5 MBq (●), (B) F6-734/^{131}I-di-DTPA-TL 37 MBq (△) and 92.5 MBq (▲), (C) no injection (□), nonradiolabeled F6 (○) and nonradiolabeled F6-734/di-DTPA-TL (△) and (D) ^{131}I-734 (●) and irrelevant BsMAb/^{131}I-di-DTPA-TL (▲).

results in elevated hematotoxicity related to the persistence of high circulating radioactivity (*14,16*). Two-step targeting techniques are particularly promising approaches to reduce this hematotoxicity. In fact, the targeting by small molecules of antibodies prelocalized on the surface of tumor cells has improved the selectivity of uptake and reduced radioactivity in normal tissues (*4,5*). A reduction in medullary toxicity has been obtained with ^{90}Y-dodecane tetraacetic acid (DOTA)-biotin prelocalized by the MAb coupled to streptavidin as compared to the same MAb labeled conventionally with ^{90}Y-DOTA (*17*).

AES uses a bispecific antibody composed of two chemically linked Fab′ fragments and a bivalent hapten. A study compared the biodistribution of F6-734 BsMAb/^{125}I-di-DTPA-TL hapten with that of ^{125}I-F6 MAb in a TT xenograft model of MTC. The results showed that tumor-to-blood, tumor-to-liver and tumor-to-kidney ratios at 24 h were respectively 20.5, 4.2 and 2.9 times greater with the two-step than the one-step system for an equivalent level of tumor uptake (*10*). In this study, a prelocalization interval of 48 h was chosen because it allowed satisfactory tumor uptake to be obtained while minimizing activity in the blood compartment and normal tissues (*11*).

Treatments with the two activities of the ^{131}I-F6 fragment produced severe leukopenia (50 and 175/mm^3) and a drop in platelets which did not lead to hemorrhagic events. In comparison with the one-step technique, a statistically significant reduction in leukocyte and platelet toxicity was noted with the AES. Human bone marrow is two to three times as sensitive to ionizing radiation as murine marrow (*16*). When there is no neoplastic infiltration of bone marrow, the AES apparently produces markedly decreased medullary irradiation, not inducing severe pancytopenia in patients who would require platelet transfusion, G-CSF growth factor and bone-marrow autograft. The management of cancers at a metastatic stage generally requires the association of several potentially synergistic treatments. The AES appears to be ideal for combination therapy with, for example, chemotherapeutic agents which can also induce myelosuppression.

Several groups have obtained partial or complete tumor remissions after RIT in animal models of xenografted solid tumors such as colorectal carcinomas, hepatomas or small-cell lung cancers (*14,16,18*). In our study, significantly longer growth delays than those for the untreated group were obtained with the two activities of ^{131}I-F6 and F6-734/^{131}I-di-DTPA-TL. There was no significant difference between the control groups and the untreated group, so that efficacy was not due to a biological activity specific to the antibodies themselves or to nonspecific irradiation. The AES targeting system appeared to be more efficient than the one-step system, particularly for the higher activity of 92.5 MBq. In fact, growth delays were longer. At day 45, a renewed rise in TCT serum level was noted in only 2 mice after injections of F6-734/^{131}I-di-DTPA-TL, but in four after injections of ^{131}I-F6. Tumor necrosis was markedly greater after injection of 92.5 MBq F6-734/^{131}I-di-DTPA-TL than 92.5 MBq ^{131}I-F6. A study performed in a multicell spheroid model of colon adenocarcinoma established that uptake kinetics were faster and retention half-life longer with the AES than the one-step system (*19*). The bivalent hapten forms a bridge between two BsMAb molecules bound to the surface of tumor cells, thereby stabilizing antigen-antibody binding (*5*). However, in the present study, relapses in tumors with the same endocrine differentiation were noted in all cases.

The histologic effects observed in treated tumors corresponded to previously described radioinduced lesions (*18*). An early effect of radiation was noted in dividing cells, leading to a reduction in proliferation indices. Subsequently, necrotic areas were observed within the xenografts. Tumors recurring after irradiation had higher proliferation indices.

Several hypotheses could account for resumed tumor growth after a reduction in size after RIT. Histological analysis of xenografts showed intense, homogeneous antigenic expression and indicated that recurrence was not due to a proliferation of nontargeted CEA-negative cells, since antigenic expression was not reduced in treated compared to untreated tumors. The dosimetric study (*10*) showed that injections of 37 and 92.5 MBq in the two systems allowed the delivery of tumor doses of around 60 and 150 Gy, respectively. This low dose-rate irradiation was apparently inadequate to eradicate this form of thyroid cancer with known relative radioresistance (*6*). Moreover, in many solid tumors of this size, accessibility to antigens and the presence of hypoxic cells can limit therapeutic efficacy (*20*). As RIT delivers an irradiation dose that decreases with the distance from blood vessels, these highly radioresistant hypoxic cells are less irradiated. This situation could correspond to that of the model studied. Autoradiography has shown a very heterogeneous distribution of ^{131}I-F6 and F6-734/^{131}I-diDTPA-TL MAbs in TT xenografts (*10*). Histological analysis revealed a low percentage of cells within tumor nodules still in the cell cycle after RIT. Cells not in the cycle were not destroyed by irradiation and could have led to recurrences.

Different approaches can be considered in order to improve therapeutic efficacy. The approach with AES can be regarded from two points of view: that of the selectivity of tumor targeting, for which the 48-h delay chosen in this study appears quite suitable; or that of an integral dose delivered to the tumor, for which a shorter interval would be preferable (*11*). In the latter case, tumor localization is maximal, but the tumor-to-blood ratio is low. Given the modest toxic effects observed in this study, the 48-h delay could be maintained and the amounts of F6-734 BsMAb and hapten with the same specific activity could be increased in the same proportions. The second approach, involving injection of the same activity of radioiodinated hapten but with a shorter prelocalization interval, would probably increase myelosuppression (*21*).

Moreover, as CEA expression by the tumor during regrowth is intense, reinjections of BsMAb/hapten could be

considered after the resumption of tumor growth. These reinjections would target tumors with a greater number of cells in the cell cycle. Furthermore, several teams have demonstrated the lesser toxicity of another therapeutic approach, which consists in fractionating the injections of immunoconjugates to allow the administration of higher cumulative activities. Concerning the AES, it would be necessary to fractionate the doses of BsMAb and hapten and maintain a constant prelocalization period and ratio when injecting them. If a single injection of BsMAb were performed, followed by several injections of hapten, the prelocalization period would increase and the amount of antibody in tumor would decrease because of the dissociation at each new administration of radiohapten, which would not be favorable for targeting (*11*). However, it is not certain that a fractionated RIT protocol would improve efficacy in this type of tumor, as it presumably would in more proliferative tumor models such as colon adenocarcinoma (*20,22*). Furthermore, the presence of human antimouse antibodies would probably limit reinjections of murine antibodies, therefore requiring the use of humanized forms (*22,23*).

Finally, in vitro and in vivo studies have clearly shown the benefit of certain chemotherapeutic drugs and new generations of bioreactive agents in potentiating the effect of RIT (*24*). This strategy would appear to be of particular interest for the model used here.

CONCLUSION

This study clearly demonstrates that the use of two-step targeting with anti-CEA/anti-DTPA BsMAb and ^{131}I-di-DTPA-TL hapten significantly reduces the myelotoxicity induced by RIT. Thus, the AES system would appear to be particularly favorable since the therapeutic application of radiolabeled MAbs in oncology would be performed in conjunction with other treatments such as chemotherapy, external radiotherapy and the use of radiosensitizing agents which can induce considerable myelosuppression.

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