Human IgM Monoclonal Antibody 16.88: Pharmacokinetics and Immunogenicity in Colorectal Cancer Patients

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Twenty colorectal cancer patients were given an intravenous injection of human IgM monoclonal antibody (MAb) 16.88 (8 mg) conjugated to ^{131}I for tumor localization. After a 2-week interval, a second injection with 200, 500, or 1000 mg of unlabeled antibody added was given to groups of five patients each. At the end of the 2-hour infusion, 66% of the radioactivity remained in the circulation. Blood clearance of the ^{131}I -labeled MAb 16.88 was biphasic with a mean half-life ($T_{1/2\alpha}$) of 12 hours and $T_{1/2\beta}$ of 45 hours. Clearance rate was 0.09 L/hour. More than 90% of the ^{131}I in serum was protein bound, with an immunoreactive fraction of 80% in the first 48 hours. Size exclusion chromatography indicated no degradation products other than ^{131}I in serum and urine. The urinary excretion rate of ^{131}I increased to 1.5% of the dose per hour at 24 hours, with 50% of the dose excreted in 34 hours. The pharmacokinetic profile of ^{131}I -labeled MAb 16.88 was neither influenced by the total protein dose of antibody administered nor affected by specific uptake in tumor tissue in individual patients, as determined on early immunoscintigrams. The larger antibody doses showed a slightly slower excretion of ^{131}I . The assays applied to determine immunogenicity were enzyme-linked immunosorbent assay, radioimmunoassay, and the dot-blot assay. They had sensitivities ranging from 5 ng/mL to 0.5 $\mu\text{g/mL}$ for goat or rabbit antihuman IgM. The assays did not reveal antihuman antibody responses. [J Natl Cancer Inst 83:1813-1819, 1991]

Clinical trials with murine monoclonal antibodies (MAbs) have demonstrated the preferential localization of antibody in tumor lesions, a prerequisite for MAb-guided therapy. A major problem associated with the use of such reagents in patients is the development of antimouse antibodies, which limits the reagents' application for therapy. Mouse-human chimeric antibodies composed of the variable region of the mouse antibody and the constant region of the human immunoglobulin are less immunogenic than the murine antibody, but antimouse antibodies may still be produced (1). Human MAbs should minimize the occurrence of antibody responses, allowing multiple injections of antibody.

Human IgM MAb 16.88 (2) was developed from spontaneously transformed peripheral blood lymphocytes of a colorectal

cancer patient participating in an active specific immunotherapy trial for treatment of residual disease (3). The antibody was selected for specificity, stability, and growth properties of the clone. The aim of this study was to determine the pharmacokinetics in relation to the protein dose of ^{131}I -labeled MAb 16.88 and whether MAb 16.88 elicits an antihuman antibody response. This analysis formed a major part of the assessment of tumor-localizing properties of MAb 16.88. Various antibody doses were administered in an attempt to improve the relative uptake of ^{131}I -labeled MAb 16.88 in tumor lesions (4). Results of radioimmunodetection have been presented in detail in a separate report (5).

Materials and Methods

Patients and Study Design

Formalin-fixed, paraffin-embedded tissue sections from patients with advanced colorectal cancer were stained with MAb 16.88 and scored at a concentration of 0.3 $\mu\text{g/mL}$, using the immunoperoxidase technique. Twenty patients with positive staining of tumor sections were entered in the study after they gave their informed consent. Thyroid uptake of ^{131}I was blocked by the administration of sodium perchlorate or potassium iodide. Prior to each dose of MAb 16.88, patients were given a skin test with 0.01 mg of antibody. No reactions were observed, and antibody was administered by a 2-hour intravenous infusion. Patients received 185 MBq (5 mCi) of ^{131}I -labeled MAb 16.88

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(specific activity, 37 MBq/mg, 1 mCi/mg), supplemented with unlabeled antibody, to a total protein dose of 8 mg. To groups of five patients each, a second infusion of ^{131}I -labeled MAb 16.88 was given 2 weeks after the first, and unlabeled antibody was added to a total dose of 200, 500, or 1000 mg protein.

Blood samples were obtained for pharmacokinetic and immunological studies immediately before infusion, within 5 minutes after completion of the infusion (time point = 0 hours), and subsequently at 0.5, 1, 2, 4, 24, 48, 120, and 168 hours. Serial urine specimens were also collected from each patient in 6-hour portions for the first 24 hours and in 24-hour portions thereafter, for up to 1 week after the infusion.

Antibody Labeling

Human IgM MAb 16.88 recognizes an antigen strongly expressed by colon cancer cells. The antibody was derived from peripheral blood B cells from a patient immunized with irradiated autologous colon cancer cells admixed with BCG (2). Another human IgM MAb reactive with colon cancer cells, 28A32 (2), was used as a control to determine the specificity of antihuman IgM antibody reactions.

Purified MAb 16.88 (6) was obtained from Biotechnology Research Institute. The iodogen method was used to label MAb 16.88 with ^{131}I . Unbound iodine was removed with a Sephadex column (Pharmacia, Uppsala, Sweden). The specific activity of ^{131}I was approximately 37 MBq/mg (1 mCi/mg) antibody. Precipitation with 10% trichloroacetic acid and thin-layer chromatography with 85% methanol indicated that more than 95% of the radioactivity was bound to protein in the final preparations. For in vitro studies, MAbs 16.88 and 28A32 were radiolabeled with ^{125}I in a similar fashion.

Immunoreactivity of iodinated MAb 16.88 was determined according to the procedure of Lindmo et al. (7), using WiDr human colon cancer cells harvested from tissue culture and fixed with 0.1% glutaraldehyde. Assays were standardized every month by incorporation of a reference ^{125}I -labeled MAb 16.88 preparation. The absolute immunoreactivity of the final preparation of ^{131}I -labeled MAb 16.88 was approximately 90%.

Pharmacokinetics

Clearance and excretion of ^{131}I were determined by counting 100- μL samples of serum and urine, which were compared with an appropriate standard. Clearance and half-lives were calculated according to the procedure of Tallarida and Murray (8). Patients' characteristics relevant to pharmacokinetics are listed in Table 1. Data were expressed as means $\pm$ SD and were tested for statistical significance using Student's *t* test for paired data with $P < .05$. The apparent volume of distribution (V_d) of ^{131}I -labeled MAb 16.88 for each infusion was calculated from the amount of radioactive antibody in the first serum samples. The proportion of radioactivity associated with protein in serum and urine samples was determined by precipitation with 10% (wt/vol) trichloroacetic acid. Concentrations of MAb 16.88 were calculated from acid-precipitable radioactivity in the serum. For detection of radiolabeled species other than MAb 16.88, gel filtration analysis of serum and urine samples was performed using a Superose 6 column (Pharmacia) with phosphate-buf-

Table 1. Patient characteristics

Characteristic	Group			
	I	II	III	IV
Mean age, y (range)	45.6 (23-67)	54.0 (49-57)	59.4 (49-68)	61.2 (41-68)
Sex				
Female*	2	3	0	1
Male*	3	2	5	4
Surface area, m^2 †	1.7 ± 0.2	1.8 ± 0.2	1.9 ± 0.2	2.0 ± 0.2
IgG, g/L†	10.3 ± 0.5	13.9 ± 1.7	14.5 ± 1.3	15.0 ± 1.8
IgM, g/L†	1.4 ± 0.3	1.7 ± 0.3	2.6 ± 0.3	2.4 ± 0.5
Plasma volume, L†	2.9 ± 1.3	2.8 ± 1.2	3.0 ± 1.3	3.2 ± 1.4
MAb 16.88				
First dose, mg	8	8	8	8
Second dose, mg		200	500	1000

*Values = No. of patients.

†Mean $\pm$ SD.

fered saline as eluent. Radioactivity and optical density at 280 nm (OD_{280}) of samples were recorded.

Distribution

Radioimmunoscintigraphy was carried out within 2 hours after MAb administration (day 0) and at days 2, 5, and 7. Scintigrams were obtained by standardized techniques, using a large-field-of-view gamma camera fitted with a high-energy collimator. Anterior and posterior views of the chest and the abdomen-pelvis, respectively, were obtained using the 364-keV photopeak of ^{131}I and a 20% window. For distribution of ^{131}I -labeled MAb 16.88, immunoscintigrams obtained at day 0 were used. Regions of interest were manually drawn on target organs, excluding other overlapping organs. The geometrical mean count rates (counts per cm^2/MBq) were then calculated from the aligned anterior and posterior views.

Antibody Response

Serum samples obtained during the first 48 hours were analyzed for ^{131}I -labeled MAb 16.88 immunoreactivity as described above with the use of 50 μL of serum and 5×10^6 glutaraldehyde-fixed WiDr cells. Antibody responses against MAb 16.88 were assayed in sera obtained prior to each infusion and 1 week after the second infusion, and later samples were collected 5-13 weeks after tumor localization studies. Three assays were used: enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and a dot-blot assay.

For the ELISA, microtiter plates were coated with MAb 16.88, MAb 28A32, or control human IgM (Sigma Chemical Co., St. Louis, Mo.) antibody at 1 μg per well in phosphate-buffered saline. After 1 hour incubation at 37 $^\circ\text{C}$, the antibody solution was replaced with phosphate-buffered saline containing 1% bovine serum albumin, 0.05% Tween 20 (Sigma Chemical Co.), and 1% nonfat dry milk (block buffer) to minimize nonspecific absorption. After 0.5-1 hour, plates were rinsed with 1% glycerol and 0.05% Tween 20. To check each assay for performance, we coated control wells with tetanus toxoid (Lederle Laboratories, Pearl River, N.Y.). Sera were diluted 1:10, 1:100, and 1:1000 in block buffer, and 50 μL was added to triplicate

wells. Serum from a person with antitetanus toxoid antibody was added to tetanus toxoid wells. After 45 minutes of incubation at 37 °C, plates were washed, and peroxidase-conjugated goat antihuman IgG (K.P.L., Gaithersburg, Md.) was added for an additional incubation period of 45 minutes. Plates were washed, and substrate (3,3',5,5'-tetramethylbenzidine) was added. After the color reaction developed, 50 μ L H₂SO₄ was added to the wells and the absorbance was recorded at 450 nm. The sensitivity of the assay was determined with rabbit anti-human IgM and swine antirabbit immunoglobulin peroxidase conjugate (Dakopatts, Glostrup, Denmark).

The RIA was set up using MAb 16.88-coated wells, which were incubated simultaneously with patients' serum samples and ¹²⁵I-labeled MAb 16.88. Antibody-coated plates were prepared as for the ELISA assay. Plates coated with block buffer were used for determination of nonspecific binding. ¹²⁵I-labeled MAb 16.88 in block buffer (10 ng/mL, 50 μ L) and standard dilutions of goat antihuman IgM (K.P.L.) or 1:10 and 1:100 dilutions of the patient sera in block buffer (50 μ L) were added in duplicate. After incubation for 2 hours at 37 °C, unbound material was removed by washing with a solution of 1% glycerol and 0.05% Tween 20, and the bound radioactivity was detected by counting the individual wells in a gamma counter.

A dot-blot assay was performed as follows. Serum samples (50 μ L diluted 1:10 in block buffer) or goat antihuman IgM or WiDr cells were immobilized on nitrocellulose (0.45 μ m; Schleicher & Schuell, Inc., Keene, N.H.). Protein-binding sites were saturated with block buffer (1 hour, 37 °C), and the nitrocellulose sheets were incubated with ¹²⁵I-labeled MAb 16.88 or MAb 28A32 for 2 hours at room temperature. After being washed three times with phosphate-buffered saline containing 0.05% Tween 20, the sheets were covered with x-ray film (Kodak X-omat-S) and exposed for 14 hours at -70 °C. The nature of the reactions was examined on sodium dodecyl sulfate-polyacrylamide gels (7.5%) according to the procedure of Laemmli (9).

Results

Patients

No significant differences were present among the four groups, with respect to body surface area, serum IgG and IgM concentrations, or plasma volume (see Table 1).

Pharmacokinetics

The pharmacokinetics of the radionuclide in the serum is shown in Fig. 1. Analysis of the serum clearance curves provided the mean values for half-life ($T_{1/2}$), V_d , percent of the dose remaining at the end of infusion (%D T_0), and clearance (Cl) as listed in Table 2. The clearance of ¹³¹I-labeled MAb 16.88 was best described by a two-compartment model with a mean $T_{1/2\alpha}$ of 12 hours and a mean $T_{1/2\beta}$ of 45 hours. The average V_d was 4.7 L, which was 1.6 times the mean estimated plasma volume (3.0 L). At the end of the 2-hour infusion, 65.9% of the dose remained in the circulation, resulting in apparent antibody serum levels of 1.7 μ g/mL (8 mg), 54.8 μ g/mL (200 mg), 101.6 μ g/mL (500 mg), and 223.5 μ g/mL (1000 mg). Patients in group

Fig. 1. A) Pharmacokinetics of ¹³¹I in serum of patients after a 2-hour infusion of ¹³¹I-labeled 16.88. B) Urinary clearance of ¹³¹I in patients after a 2-hour infusion of ¹³¹I-labeled MAb 16.88. ○ = 8 mg; ● = 200 mg; △ = 500 mg; ▲ = 1000 mg.

II, who received sodium perchlorate instead of potassium iodide to block thyroid uptake of ¹³¹I, showed a significantly shorter $T_{1/2\alpha}$ at the 8-mg dose level and a relatively small V_d compared with those of the other groups. With higher doses of antibody in group II (200 mg), the $T_{1/2\alpha}$ and V_d were similar to those of the other groups, even though these patients received potassium perchlorate again to prevent thyroid uptake of ¹³¹I.

The urinary excretion rate of ¹³¹I increased from 0.9% $\pm$ 0.5% of the injected dose per hour at 6 hours to 1.5% $\pm$ 0.1% of the injected dose per hour at 24 hours and decreased thereafter. After administration of 8 mg MAb 16.88, half of the ¹³¹I was excreted in the urine within 30.2 $\pm$ 0.2 hour. Excretion was significantly slower in those patients who received either 500 mg or 1000 mg of antibody (Table 3). The other pharmacological data studied were not correlated with the patients' characteristics or with the protein dose administered.

Immunoreactivity of Circulating ¹³¹I-Labeled MAb 16.88

More than 90% of the circulating ¹³¹I remained bound to protein at all times after administration, regardless of the dose administered. Approximately 80% of the circulating ¹³¹I in the serum samples obtained during the first 48 hours was bound to immunoreactive MAb 16.88, as determined by a cell-binding assay. The immunoreactivity of the antibody in the serum varied among patients, but there was no correlation with the antibody dose or the immunoreactivity of the injected antibody preparation. Chromatography of serum and urine samples showed no evidence of aggregates or degradation products other than ¹³¹I.

Table 2. Analysis of serum clearance curves of ^{131}I -labeled MAb 16.88

Patient group	$T_{1/2\alpha}$, h*		$T_{1/2\beta}$, h*		V_d , L*		%D T_0 *		16.88 at T_0 , $\mu\text{g/mL}$ *		Cl, L/h*	
	8 mg	High dose†	8 mg	High dose†	8 mg	High dose†	8 mg	High dose†	8 mg	High dose†	8 mg	High dose†
I	10.2 $\pm$ 2.8		42.8 $\pm$ 4.1		5.7 $\pm$ 1.6		50.8 $\pm$ 11.9		1.4 $\pm$ 0.3		0.08 $\pm$ 0.01	
II	6.1 $\pm$ 0.2	12.5 $\pm$ 0.6	40.2 $\pm$ 4.4	49.7 $\pm$ 2.2	3.3 $\pm$ 0.1	3.6 $\pm$ 0.2	85.8 $\pm$ 9.1	76.7 $\pm$ 3.2	2.5 $\pm$ 0.1	54.8 $\pm$ 2.6	0.10 $\pm$ 0.01	0.11 $\pm$ 0.00
III	13.7 $\pm$ 2.0	11.6 $\pm$ 1.7	42.0 $\pm$ 3.2	39.7 $\pm$ 5.1	5.6 $\pm$ 0.8	4.9 $\pm$ 0.7	52.5 $\pm$ 14.3	61.0 $\pm$ 8.5	1.4 $\pm$ 0.2	101.6 $\pm$ 14.9	0.08 $\pm$ 0.01	0.08 $\pm$ 0.01
IV	13.5 $\pm$ 3.5	14.8 $\pm$ 1.1	47.9 $\pm$ 4.7	53.8 $\pm$ 2.4	5.3 $\pm$ 1.4	4.4 $\pm$ 0.3	60.0 $\pm$ 5.4	74.7 $\pm$ 7.7	1.5 $\pm$ 0.4	223.5 $\pm$ 16.6	0.08 $\pm$ 0.01	0.09 $\pm$ 0.00

*Values = means $\pm$ SD.

†Group II: 200 mg. Group III: 500 mg. Group IV: 1000 mg.

Distribution of ^{131}I -Labeled MAb 16.88

The count rates for different organs at 2 hours after infusion are given in Fig. 2. There were no statistically significant differences (Student's *t* test, paired data $P < .05$) between the count rates for the 8-mg protein dose and the 200-mg, 500-mg, or 1000-mg dose of MAb 16.88. Organs with high blood content (heart, liver, spleen, and kidney) and organs close to large blood vessels (stomach and thyroid) showed higher count rates as compared with background rates in the abdomen. In tumors outside the liver, count rates were not significantly different from background rates. Liver metastases, however, showed higher count rates than background activity, but these rates were lower than those of normal parenchyma. Interpatient variability could not be related to the effect of total tumor volume on distribution of ^{131}I -labeled MAb 16.88.

Human Anti-16.88 Response

Three assays were employed to determine whether MAb 16.88 induced an antibody response. Apart from MAb 16.88, another human IgM MAb, 28A32, was used to determine the specificity of the antibody reactions (Table 4). In the ELISA system, antibodies bound to immobilized MAb 16.88 or human IgM were detected with a peroxidase-conjugated goat anti-human IgG. IgM antibodies could not be detected with this assay, because such a conjugate would bind to MAb 16.88 or human IgM coated on the assay plate. We used immobilized tetanus toxoid with immune human serum as a control for the reactivity of the antihuman IgG conjugate because an appropriate human antihuman IgM antibody was not available. Reactions were scored as positive if the OD₄₅₀ value was at least 50% of that of the tetanus toxoid controls. With rabbit anti-human IgM antibody and swine antirabbit immunoglobulin, a

sensitivity of 5 ng/mL rabbit antihuman IgM was obtained (Fig. 3, A). No reactivity of the sera was observed with MAb 16.88-coated wells. Three patients apparently had low-titer (1:10-1:100) antibodies against MAb 28A32, which did not change after administration of the 16.88 antibody. With control IgM, no reactivity was observed at serum dilutions of 1:10.

The RIA was set up with immobilized 16.88 and 28A32 antibodies simultaneously incubated with ^{125}I -labeled MAb 16.88 or 28A32 and serum or goat antihuman IgM. This assay proved to be very reproducible, with a sensitivity of 0.5 $\mu\text{g/mL}$ for goat antihuman IgM (Fig. 3, B). Although the sensitivity of this assay was much lower than that of the ELISA, we chose to perform the assay because it would detect both IgG and IgM responses. Sera from three patients reacted with MAb 16.88 at a dilution of 1:10, and sera from eight patients reacted with MAb 28A32 at a dilution of 1:10 or 1:100. None of the patients showed an increase in anti-16.88 antibody titer after administration of MAb 16.88.

In the dot-blot assay, immobilized serum was reacted with ^{125}I -labeled MAb 16.88 or 28A32. Goat antihuman IgM and WiDr cells (as a source of antigen) were used as controls. The sensitivity of this assay was 0.5 $\mu\text{g/mL}$ for goat antihuman IgM and the equivalent of 10^4 cells for antigen. Five patients showed reactivity with MAb 16.88, and three patients showed reactivity with MAb 28A32. As in this assay, antibodies against MAb 16.88 or 28A32 and antigen will react positively; sera were run on sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels to determine the nature of the reactions. No reactivity was observed with molecules with apparent molecular masses of 150

Table 3. Analysis of urinary excretion curves of ^{131}I

Patient group	Means $\pm$ SD			
	Initial excretion rate*		50% excretion, h	
	8 mg	High dose†	8 mg	High dose†
I	1.5 $\pm$ 0.4		34.6 $\pm$ 6.2	
II	1.7 $\pm$ 0.1	1.5 $\pm$ 0.3	29.8 $\pm$ 2	35.4 $\pm$ 7.1
III	1.7 $\pm$ 0.2	1.4 $\pm$ 0.1	31.1 $\pm$ 4.2	38.4 $\pm$ 3.5
IV	1.8 $\pm$ 0.5	1.2 $\pm$ 0.2	27.0 $\pm$ 5.7	42.0 $\pm$ 5.5

*% injected dose excreted per hour.

†Group II: 200 mg. Group III: 500 mg. Group IV: 1000 mg.

Fig. 2. Count rates (geometrical mean acquired from anterior and posterior views) for selected organs, 2 hours after the end of the 2-hour infusion with ^{131}I -labeled MAb 16.88. Open bars: 8-mg dose; closed bars: 200-mg, 500-mg, and 1000-mg dose.

Table 4. Anti-16.88 and anti-28A32 antibody activity in sera of colon cancer patients prior to and following administration of human MAb*

Patient	Dose, mg	Anti-MAb 16.88						Anti-MAb 28A32					
		ELISA†		RIA‡		Dot-blot assay§		ELISA†		RIA‡		Dot-blot assay§	
		Prior	Late	Prior	Late	Prior	Late	Prior	Late	Prior	Late	Prior	Late
1	8	-	-	-	-	+	-	-	-	-	-	+	-
2	8	-	-	-	-	-	-	-	-	-	-	-	-
3	8	-	-	-	-	+	+	100	10	-	-	-	-
4	8	-	-	-	-	-	-	-	-	100	100	-	-
5	8	-	-	-	-	-	-	-	-	-	-	+	-
6	8	-	-	-	-	-	-	-	-	-	-	-	-
7	200	-	-	-	-	-	-	-	-	-	-	-	-
	8	-	-	-	-	-	-	-	-	-	-	-	-
8	200	-	-	-	-	-	-	-	-	-	-	-	-
	8	-	-	-	-	-	-	-	-	-	-	-	-
9	200	-	-	-	-	-	-	-	-	-	-	-	-
	8	-	-	-	-	-	-	-	-	-	-	-	-
10	200	-	-	-	-	-	-	-	-	-	100	-	-
	8	NA	-	NA	-	NA	-	NA	-	NA	-	NA	-
11	200	-	-	-	-	-	-	-	-	-	-	-	-
	8	-	-	-	-	+	-	100	-	100	-	-	-
12	500	-	-	-	-	-	+	-	100	-	-	-	-
	8	-	-	-	-	-	-	-	-	-	-	-	-
13	500	-	-	-	-	-	-	-	-	-	10	-	-
	8	-	-	-	-	-	-	-	-	-	-	-	-
14	500	-	-	10	-	-	+	-	-	100	10	-	-
	8	-	-	NA	-	NA	-	-	-	NA	-	NA	-
15	500	-	-	10	-	-	-	10	10	100	-	-	-
	8	-	-	-	-	-	-	-	-	-	-	-	-
16	500	-	-	-	-	-	-	-	-	-	-	+	-
	8	-	-	-	-	-	-	-	-	-	-	-	-
17	1000	-	-	-	-	-	-	-	-	100	-	-	-
	8	-	-	-	-	+	-	-	-	-	-	-	-
18	1000	-	-	-	10	-	-	-	-	-	10	-	-
	8	-	-	-	-	-	-	-	-	-	-	-	-
19	1000	-	-	-	-	-	-	-	-	-	-	-	-
	8	-	-	-	-	-	-	-	-	-	-	-	-
20	1000	-	-	-	-	-	-	-	-	-	100	-	-
	8	-	-	-	-	-	-	-	-	-	-	-	-
	1000	-	-	-	-	-	-	-	-	-	100	-	-
	8	-	-	-	-	-	-	-	-	-	-	-	-

*Human antibody responses against MAb 16.88 were measured at day 7, day 14 (before the second infusion), and at later time points ranging from 5 to 13 wk. Results of late samples measured after the second infusion reflect the highest positive result in any of the samples analyzed. NA = not available.

†Dilution of serum giving >50% of the OD₄₅₀ from antitetanus toxoid serum reactive with tetanus toxoid in the same assay. Sensitivity was 5 ng/mL with hrp-conjugated swine antirabbit immunoglobulin and rabbit antihuman IgM.

‡Dilution of serum giving greater than three times background values. Sensitivity was 0.5 µg/mL with goat antihuman IgM.

§Reaction was scored as either positive (+) or negative (-). Sensitivity was 0.5 µg/mL for goat antihuman IgM and the equivalent of 10⁴ WiDr cells for antigen.

kd or 900 kd, thus reducing the possibility of a specific anti-16.88 IgM response.

Discussion

Twenty patients received a 2-hour infusion with 8 mg ¹³¹I-labeled human IgM MAb 16.88 (185 MBq, 5 mCi). Groups of five patients each received a second injection with the labeled antibody admixed with 200, 500, or 1000 mg of unlabeled antibody. Analysis of serum showed that ¹³¹I remained associated with immunoreactive MAb 16.88. ¹³¹I-labeled MAb 16.88 was cleared biexponentially from the serum with a T_{1/2α} of 12 hours and a T_{1/2β} of 45 hours. The antibody rapidly diffused from the circulation, as indicated by the apparent volume of distribution, which was 1.6 times the plasma volume. The distribution of ¹³¹I-labeled MAb 16.88, determined on scintigrams obtained at 2 hours after completion of the infusion, showed preferential localization in well-vascularized normal organs. Elimination of the radioactivity was via the urine, with 50% of the dose ex-

creted within 34 hours. The antibody dose did not affect the pharmacological data or distribution, with the exception of ¹³¹I excretion, which was slightly slower at higher protein doses. No antibody response could be detected in the sera obtained from patients up to 13 weeks after the last injection.

Thus far, only a few studies have been carried out with radiolabeled human MAbs in cancer patients. Steis et al. (6) reported on the use of MAbs 16.88 and 28A32 in colorectal cancer patients, whereas Ryan et al. (10) described the application of human IgM MAbs YBB-190, YBM-209, and YBY-088 in breast cancer patients. In these studies, tumor sites were successfully detected in 21 of 28 and five of seven patients, respectively. We found that immunoscintigraphy with ¹³¹I-labeled MAb 16.88 was positive in 16 (80%) of 20 patients in at least one organ site with known disease (5). In general, early immunoscintigrams of tumors showed relatively "cold" areas because of adjacent organs with high radioactivity, such as the liver. Optimal visualization of disease was obtained at days 5 and 7 after clearance of MAb 16.88 from nontarget tissues (5).

Fig. 3. A) Standard curve for ELISA with rabbit antihuman IgM and horseradish peroxidase-conjugated sheep antirabbit IgG. Δ = MAb 16.88; $\bullet$ = MAb 28A32; $\square$ = IgM. B) Standard curve for RIA with goat antihuman IgM and ^{125}I -labeled MAb 16.88 (Δ) or ^{125}I -labeled MAb 28A32 ($\bullet$).

In our study, we found a $T_{1/2\alpha}$ of 12 hours, which is quite similar to the $T_{1/2\alpha}$ of 16 hours found by Steis et al. (6), using the same antibody. The α -half-life is believed to represent the equilibrium of antibody with the extravascular and intravascular spaces. The similar α -half-life found for IgM, as compared with IgG (11), suggests that both types of antibody extravasate at the same rate and could therefore have similar tumor-targeting properties. The terminal serum half-life $T_{1/2\beta}$ of ^{131}I -labeled MAb 16.88 of 34 hours in our patients compares well with the terminal serum half-life of approximately 40 hours found for ^{111}In -labeled human IgM MAbs YBM-209 and YBY-088 in breast cancer patients (10). This terminal half-life is considerably shorter than that of the ^{131}I -labeled human myeloma IgM antibody, which supposedly has a half-life of about 5 days in humans (12). The reason for the relatively short half-life of the human IgM MAbs is unclear. Clonal variation in carbohydrate composition of the MAbs produced in tissue culture or labeling and purification methods may accelerate the metabolism of the antibodies. Alternatively, in vivo binding to peripheral white blood cells could reduce the circulation half-life time. As with others (10), we could not detect, however, substantial binding of the IgM MAbs to leukocytes.

The volume of distribution was approximately 1.6 times the estimated plasma volume. The antibody may have diffused out of the vasculature or could have been metabolized. In case of dehalogenation, the iodine would have been rapidly excreted. This process appeared less likely in the time course of the in-

fusion because the iodine excretion rate was slow in the first 6 hours. The high count rates in the stomach and thyroid at 2 hours after infusion have to be explained by the activity in the vascularization in adjacent sites. Accordingly, the relatively high volume of distribution values most likely reflected the proportion of ^{131}I -labeled MAb 16.88 diffused from the circulation.

The lack of immunogenicity of the human IgM antibody after repeated doses is an important finding. Steis et al. (6) determined the occurrence of an antihuman IgM antibody response in patients receiving either 16.88 or 28A32 antibody. These investigators used a latex bead agglutination assay with a sensitivity of approximately 0.1 $\mu\text{g/mL}$. After repeated doses of 8-200 mg of 16.88 antibody (for a total dose of 800 mg), none of the nine patients developed an antibody response. In the same study, four of 14 patients who received MAb 28A32, another human IgM antibody, had a low titer (1:10 dilution) of anti-28A32 antibodies. Low-titer anti-28A32 antibodies were also found in five of 12 patients who received control sera and four of nine patients who received MAb 16.88. Our patients who received protein doses up to 1000 mg had no detectable antibody response to MAb 16.88, as measured by assays with sensitivities ranging from 0.5 $\mu\text{g/mL}$ to 5 ng/mL. We observed apparent anti-28A32 antibodies in four of 20 patients by ELISA assay, seven of 20 patients by RIA, and none of the 20 patients by dot-blot assay. The difference in response to MAbs 16.88 and 28A32 might be explained by their derivation; MAb 16.88 was produced by transformed human lymphocytes, whereas MAb 28A32 was derived from a murine-human heterohybridoma. Steis et al. (6) suggested that the antibody produced by the heterohybridoma might contain murine immunoglobulin, and the anti-28A32 reactivity seen might be from naturally occurring antimurine antibodies.

The administration of a dose of 200-1000 mg of antibody 2 weeks after the initial infusion resulted in comparable serum pharmacokinetics. Therefore, it appears that no saturation of extravascular binding sites occurred, even at the highest dose of antibody. Moreover, the lack of alteration in the pharmacokinetics and distribution at the second infusion suggests no substantial antibody response that would be expected to shorten the antibody clearance rate and to increase the uptake of antibody in liver and spleen.

The excretion of ^{131}I in the urine was slightly slower at antibody doses of 500-1000 mg, despite similar pharmacokinetics in the serum. This finding may be the result of saturated metabolism at peripheral sites, causing slower dehalogenation at the higher MAb 16.88 doses. As a consequence, patients given high doses of antibody received higher radiation doses than patients given lower amounts of protein. Future studies with larger groups of patients should elucidate the significance of this observation.

We conclude that human IgM MAb 16.88 does not induce a specific antibody response after repeated infusions of up to 1000 mg of antibody. Weakly immunogenic or nonimmunogenic antibodies should be preferred for clinical studies of MAb-guided tumor detection or therapy. Repeated injections over a long period of time are important for therapy. Totally human MAbs that are not immunogenic would permit such a treatment.

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