Rhenium-186-Labeled Monoclonal Antibodies for Radioimmunotherapy: Preparation and Evaluation

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Rhenium-186 has been determined to be a leading radionuclide for radioimmunotherapy. However, the use of ^{186}Re has been limited due to the lack of a convenient and efficient method by which the radionuclide can be bound to monoclonal antibodies. We have developed a simple technique to label IgM, IgG, fragmented antibodies and tumor necrosis factor-α with ^{186}Re. This technique uses ascorbic acid (AA) for controlled reduction of antibody disulfide groups to sulfhydryls and $SnCl_2$ in citric acid for the reduction of $^{186}ReO_4^-$. The labeling yields as determined by instant thin-layer chromatography, molecular filtration and gel filtration were greater than 95% and the colloid formation was less than 5%. The labeled antibodies were stable when challenged with 100 and 250 molar excess of DTPA and HSA for 24 hr at 37°C. SDS-PAGE analysis and autoradiography of labeled IgM, IgG and $F(ab')_2$ monoclonal antibodies indicated uniform labeling and that no fragmentation of the monoclonal antibodies had taken place during the labeling procedure. Immunospecificity of ^{186}Re-labeled human neutrophil specific IgM, as determined by in vitro antigen excess assay, was comparable to that of indium-111-labeled c-DTPA-IgM and technetium-99m-labeled-IgM. A nuclear histone specific ^{186}Re-TNT-1-$F(ab')_2$ was evaluated in mice bearing experimental tumors. The tumor/muscle ratios at 4 and 24 hr were 5.9 ± 0.21 and 13.8 ± 6.7, respectively compared to that of 2.4 ± 0.3 at 4 hr p.i. with a nonspecific protein. The labeling technique is simple, reliable and has already been adapted to a single-vial kit preparation.

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Tumor-specific monoclonal antibodies (Mabs) are currently receiving much attention as vehicles for site-specific delivery of diagnostic and therapeutic radionuclides in malignant lesions. Rheunium-186 has been regarded as an ideal radionuclide for radioimmunotherapy (*1–4*) because of its physical half-life of 90 hr and beta emission of 1.07 MeV. The 137 keV (9%) gamma emission also allows simultaneous scintigraphic imaging.

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Several attempts have been made to label Mabs with ^{186}Re. Quadri and Wessels have reported 5%–18% yields using c-DTPA as a bifunctional chelating agent and stannous chloride as well as sodium dithionite as reducing agents for $^{186}ReO_4^-$ (*5*). Griffiths et al. (*6*) have labeled antibodies directly with ^{186}Re using $SnCl_2$ as a reducing agent, whereas Fritzberg et al. (*7–10*) have taken the indirect approach. The latter method is in routine use for clinical trials. The method calls for reduction of $^{186}ReO_4^-$ with stannous chloride in acidic media, chelation to a diaminodithiol ligand, and subsequent conjugation to an antibody. However the drawback to this technique is the multistep approach which is inconvenient for routine preparation in a hospital pharmacy. There has been considerable interest in the direct labeling of antibodies because of the possibilities of an instant kit formulation.

In our laboratory, antibodies have been labeled with ^{99m}Tc with high radiochemical purity using a direct labeling method (*11–13*). The similarities in the chemical properties of rhenium and technetium prompted us to examine the feasibility of labeling Mabs with ^{186}Re and to evaluate the stability of the tracer in vivo and in vitro.

MATERIALS AND METHODS

Human polyclonal IgG (HIgG), MCA-480 (IgM), monoclonal TNT-1 (IgG2a) and TNT-1-$F(ab')_2$ were obtained from Sandoz Pharmaceuticals, RhoMed Inc. and from Dr. A. Epstein of USCLA, respectively. Tumor necrosis factor (TNF) was obtained from Finn Tech Industries, New Jersey. The stock concentrations of all proteins used for radiolabeling ranged between 5 and 40 mg/ml in 0.9% saline. Upon receipt, the proteins were divided into 100 μg portions and kept frozen until ready for use. Rhenium-186, produced by neutron bombardment of ^{185}Re, was obtained as aluminum($^{186}ReO_4$)$_3$ in H_2O from Missouri Research Reactor Facility. Specific activity of ^{186}Re varied from 2 to 4 Ci/mg.

All other reagents were obtained from Sigma Chemicals. Centricon-30 molecular filtration devices were obtained from Amicon Inc., MA. The Mini Protean II electrophoresis unit was purchased from Biorad Labs., Richmond, CA. A Genevac SF 50 lyophilizer was obtained from Am. Genevac Inc., West Nyack, NY.

Radiolabeling of Antibodies

Sodium borohydride, stannous chloride and sodium dithionite were investigated as reducing agents for $^{186}ReO_4^-$. Trisodium methylenediphosphonate (MDP) and 2-hydroxyisobutyric acid were evaluated as transfer agents.

The reduction of the disulfide groups in the proteins was achieved by incubation of 100 μg of protein with 3500 molar excess of ascorbic acid (pH = 6.5) for 1 hr at 22°C (*11–13*). In the sodium borohydride reduction method, $^{186}ReO_4^-$ was incubated with 2–9 mg $NaBH_4$ at 22°C for 1 hr at pH 9 in the presence of 2 mg MDP as a transfer ligand. Sodium borohydride was then decomposed by heating at 90°C for 2 min and then brought to room temperature by rapid cooling in an ice bath. This reaction mixture was then allowed to incubate with ascorbic-acid-reduced antibody for 30 min. Experiments were also carried out with 2 mg 2-hydroxyisobutyric acid as a transfer agent (*14*).

In the $SnCl_2$ reduction method, a solution of 400 μg of $SnCl_2$ and 4 mg citric acid in 200 μl H_2O (pH = 3) was added to AA-reduced HIgG followed by the addition of $^{186}ReO_4^-$. In the case of all other proteins (MCA-480, TNT-1-IgG and TNT-1-F(ab')$_2$), the pH of $SnCl_2$/citric acid was raised to 5 with sodium citrate. The vial was then capped, the mixture vortexed and incubated for a predetermined period of time at 37°C or at 22°C. Following incubation, the pH was then raised to 7 with the addition of 300 μl 0.1 *M* sodium bicarbonate buffer, pH = 10. In both radiolabeling procedures, any radioactivity that may have remained unbound was eliminated by using a Centricon-30 molecular filtration device. The percentage of radioactivity bound to the protein was then determined.

Kit Preparation

In a typical kit preparation, 1 mg of HIgG was incubated with 4 mg ascorbic acid for 1 hr at 22°C. 400 μg $SnCl_2$ and 4 mg citric acid in 200 μl of H_2O were then added to this solution. In the case of other antibodies, the pH of $SnCl_2$/citric acid was raised to 5 with sodium citrate. Inositol and glycine (2 mg each in 50 μl H_2O) were also added as stabilizing agents and the contents were then lyophilized. The kits were then reconstituted with $^{186}ReO_4^-$ in saline and incubated at 37°C for the appropriate amount of time. After incubation, the pH was always raised to 7 with 0.1 *M* sodium bicarbonate buffer.

Quality Control

The labeled proteins were analyzed by instant thin-layer chromatography (ITLC) using silica gel impregnated glass fiber sheets (Gelman Sciences, Ann Arbor, MI) and with 2 *M* urea as the eluant. Radioactivity bound to antibody remained at the origin whereas free perrhenate migrated with the solvent front. About 1–2 μl of the labeled antibody was also spotted on ITLC sheets impregnated with HSA (*13,15*). These were then developed in ethanol:water:ammonium hydroxide (2:5:1). Radioactivity bound to antibody migrated with the solvent front whereas colloid remained at the origin.

Sephadex G-50 chromatography was also performed to determine the amount of ^{186}Re activity associated with the protein. A 0.3 × 15 cm Sephadex column was thoroughly washed with 5 mg of albumin solution followed by 0.1 *M* $NaHCO_3$ in 0.15 *M* NaCl. The radiolabeled antibodies were loaded on the column and eluted with 0.1 *M* $NaHCO_3$ in 0.15 *M* NaCl. Fractions, 200 μl in volume, were collected and monitored spectrophotometrically at 280 nm for the elution of protein. Concomitant radioactivity was measured using a Packard 5500 automatic gamma counter. These tests were performed on labeled proteins before and after Centricon-30 filtrations.

Stability of the tracer

The stability of the ^{186}Re-labeled antibody was tested by incubation at 37°C for 24 hr with 100- and 250-fold excess of DTPA, HSA and cysteine. Rhenium-186 activity that remained associated with the protein was determined by ITLC at periodic intervals.

SDS-PAGE Analysis

SDS-PAGE analyses were performed using 4%–20% gradient polyacrylamide gels purchased from Biorad. In a typical electrophoretic run, equal quantities of native antibody, ascorbic acid reduced antibody and ^{186}Re-labeled antibody were loaded in separate wells. Samples of the above antibodies treated with DTT and all appropriate protein standards were also run simultaneously. Tris base-glycine (pH = 8.3) was used as the running buffer and the electrophoresis was performed for 45 min at a constant voltage of 160 V. The gels were then autoradiographed, stained, photographed and finally cut and counted for quantification of radioactivity in each protein zone.

Immunospecificity of Labeled Proteins

Immunospecificity of the labeled protein was determined by its ability to interact with specific antigens. MCA-480 is specific for the human neutrophil surface antigen lacto-N-fucopentoase with a concentration of 5.1 × 10^5 per cell (*16*). Neutrophils (PMNs) obtained from a healthy human volunteer served as the source of antigens. Less than 1 μg of ^{186}Re-MCA-480 was added to two test tubes, each containing 10^7 PMNs in 0.5 ml plasma. After a 30 min incubation at 22°C, cells were centrifuged and washed with plasma. The radioactivity associated with PMNs and the supernatant was determined.

Animal Biodistribution

BALB/c mice, 18–24 gm (Taconic, MA) were used for in vivo stability testing of two types of labeled proteins. Tumors were induced in the right thigh of each animal by intramuscular administraiton of approximately 10^7 (NF-1) embryonal carcinoma cells and allowed to grow for 8–10 days to a size of approximately 1 cm in diameter.

TNT-1-F(ab')$_2$ is a murine antibody specific for nuclear histones (*17*). Approximately 20 μg TNT-1-F(ab')$_2$, labeled with 30–50 μCi of ^{186}Re (using the $SnCl_2$/citric acid method), was administered to tumor bearing mice through a lateral tail vein. The doses were weighed and measured for radioactivity, before and after injections. Suitable standard solutions were also prepared. The animals were killed at 4 and 24 hr postinjection by inhalation of halothane and then imaged with a gamma camera equipped with a pinhole collimator and a microdot imager. Technetium-99m human serum albumin served as a nonspecific protein. Tissues were then dissected, weighed and counted for the determination of radioactivity. Percent injected dose/gm of tissues and tumor-to-muscle ratios were calculated.

TNF is a protein with a molecular weight of 17 KD and has been shown to be cytotoxic for a variety of human cell lines (*18,19*). About 20 μg of TNF labeled with 30–50 μCi of ^{186}Re were administered to mice bearing tumors. The animals were sacrificed 4 hr postinjection and the % injected dose/gm of tissue were determined. Technetium-99m-TNF, prepared by an established direct labeling method (*11*), was used for comparison.

Determination of the Involvement of Disulfide Groups for ^{99m}Tc Binding

Our established direct labeling method of antibodies with ^{99m}Tc (*11–13*) was followed in this set of experiments. In the first control experiment, 1 mg HIgG was treated with 3500 molar excess of AA for 1 hr and then labeled with ^{99m}Tc reduced with sodium dithionite. The first study experiment involved treatment of 1 mg HIgG with 3500 molar excess of AA followed by incubation with 1500 molar excess of sodium iodoacetate (pH raises to 8 with triethylamine) at 22°C for 2 hr. The protein was then labeled with reduced ^{99m}Tc. In the second control experiment, excess AA was eliminated using Centricon-30 before labeling HIgG with reduced ^{99m}Tc. In the second study experiment, both excess AA and excess sodium iodoacetate were eliminated before labeling with ^{99m}Tc. In all these experiments, the unbound ^{99m}Tc was removed by molecular filtration and the labeling yields were thus determined. ITLCs were also performed on all samples.

RESULTS

Labeling yields ranging between 20% and 60% were obtained using the $NaBH_4$ reduction method with MDP as a suitable transfer ligand. When 2-hydroxyiosbutyric acid was added as a transfer ligand, transchelation to the protein was inefficient and gave varying yields.

Table 1 shows the results of radiolabeling various antibodies with ^{186}Re using the $SnCl_2$/citric acid method. From the table, it is evident that good labeling yields were obtained in an incubation time of 3–4 hr at 37°C for the various antibodies tested. In the case of HIgG, the pH of the reaction mixture had to be at 3 during incubation. However for MCA-480, TNT-IgG and TNT-F(ab')$_2$, good labeling yields were obtained at a pH of 5. The yields for TNT-F(ab')$_2$ were 80% and varied with the specific activity of $^{186}ReO_4^-$. The protype one-vial kit preparations also gave greater than 95% labeling yields and less than 5% colloid formation as confirmed by ITLC results with pre- and post-Centricon-30 molecular filtration. This was further confirmed by Sephadex G-50 chromatogram (Fig. 1), which shows that greater than 95% of the ^{186}Re activity is associated with the protein. The recovery of the radioactivity from this column was approximately 95%. The Sephadex G-50 chromatograms were similar for samples obtained before and after molecular filtration.

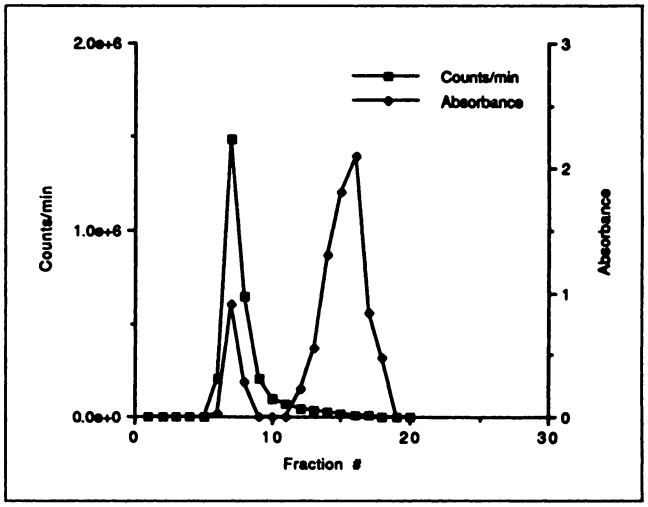

FIGURE 1. Sephadex G-50 chromatogram of ^{186}Re-HIgG showing that >95% of the radioactivity is associated with the protein. Radiolabeled HIgG is eluted from the column first, followed by low molecular weight ascorbic acid and citric acid contained in the preparation.

When radiolabeled HIgG was incubated at 37°C for 24 hr (Table 2) with 100- and 250-fold molar excess of HSA and DTPA, >95% of the activity remained bound to the protein. However, with 100- and 250-fold excess of cysteine, only about 25% of the activity remained associated with the protein after 24 hr incubation at 37°C.

SDS-PAGE and subsequent autoradiography analyses of ^{186}Re-labeled MCA-480, HIgG and TNT-F(ab')$_2$ are shown in Figures 2, 3 and 4. The apparent molecular weights and amount of radioactivity associated with it are tabulated in Table 3.

Immunospecificity was determined by the ability of ^{186}Re-MCA-480 to bind to PMNs. The results (Table 4) are consistent with ^{111}In-c-DTPA-labeled MCA-480 and ^{99m}Tc-MCA-480.

The biodistribution of ^{186}Re-TNF and ^{99m}Tc-TNF is shown in Table 5. The liver activity with ^{186}Re-TNF is considerably lower than that of ^{99m}Tc-TNF, whereas the kidney uptake is higher. The tumors were visualized with

TABLE 1
Rhenium-186 Labeling of Antibodies with 400 μg $SnCl_2$/4 mg Citric Acid

Antibody	Incubation pH	Time (hr)	Temp. (°C)	Yield
HIgG	3	3	37	96
HIgG	3	24	22	95
HIgG	4	3	37	7
TNT-IgG	5	4	37	95
MCA-480	5	3	37	95
TNT-F(ab)$_2$	5	3	37	80

n = 10 for all the experiments.

TABLE 2
In Vitro Stability Studies of Rhenium-186-Labeled HIgG at 37°C for 24 hr

Challenge agent	1 hr	2 hr	6 hr	24 hr
1:100 DTPA	100	100	100	95
1:250 DTPA	100	100	100	95
1:100 HSA	100	100	100	90
1:250 HSA	100	100	100	90
1:100 Cys	93	67	46	26
1:250 Cys	80	53	36	21

n = 3 for each experiment.
Numbers denote % ^{186}Re activity associated with the protein.

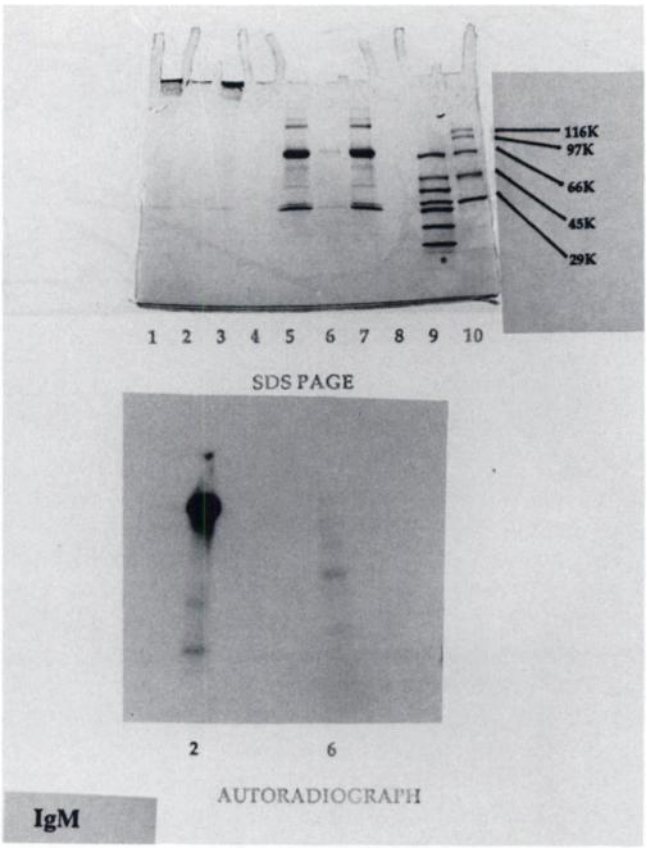

FIGURE 2. A typical SDS-PAGE analysis and autoradiograph of AA-treated IgM, ^{186}Re-labeled IgM and native IgM loaded on lanes 1–3. Lanes 5–7 represent SDS-DTT-treated antibodies in the same order. Low and high molecular weight standards were loaded on lanes 9 and 10. The figure indicates that the protein is not fragmented following AA reduction and radiolabeling procedure, and that the radioactivity is uniformly bound to the protein.

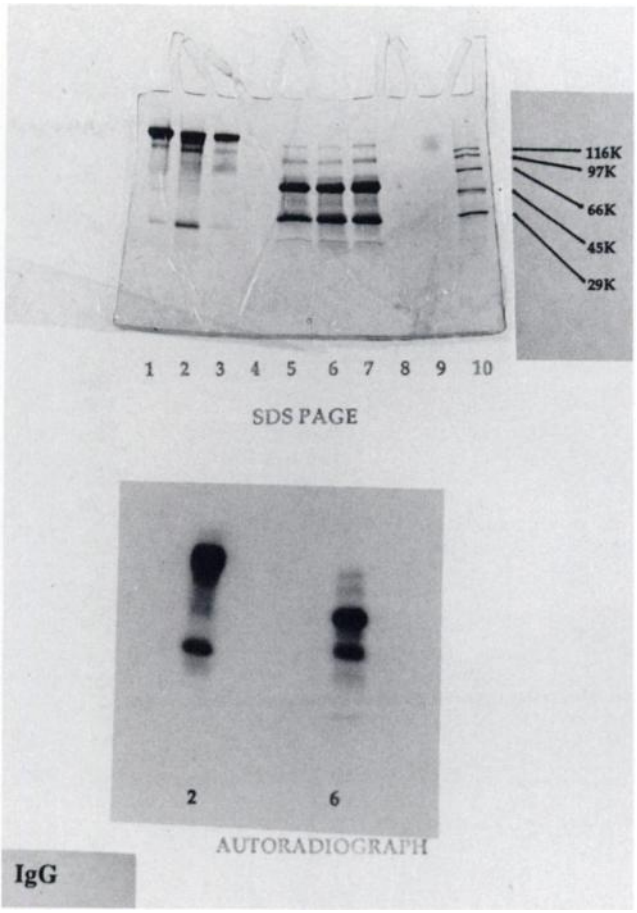

FIGURE 3. Same as Figure 2 except that the protein is HIgG.

both agents (Fig. 5) but the absolute tumor uptake with ^{186}Re-TNF was higher than that of ^{99m}Tc-TNF. The radioactivity in the remaining tissues was similar. The tissue distribution of ^{186}Re-TNT-F(ab′)$_2$ is shown in Table 6. Tumors were visualized at 4 and 24 hr postinjection and tumor/muscle ratios of 5.9 ± 0.2 and 13.8 ± 6.7, respectively were obtained. The 4-hr tumor/muscle ratio with ^{99m}Tc-HSA was only 2.4 ± 0.3. The images obtained with ^{99m}Tc-HSA as a nonspecific protein and ^{186}Re-TNT-F(ab′)$_2$ at 4 and 24 hr are shown in Figure 6.

The labeling yields for ^{99m}Tc-HIgG decreased from 90% to 34% when reduced antibodies were treated with sodium iodoacetate. Similarly, labeling yields decreased from 76% to 41% when reduced antibodies were treated with sodium iodoacetate and all excess reagents were eliminated before the addition of reduced pertechnetate.

DISCUSSION

Radiolabeling of Mabs is accomplished by three methods: (1) indirect labeling through a chelating group attached to the antibody; (2) labeling with a preformed ^{99m}Tc or ^{186}Re chelate subsequently bound to the antibody; and (3) direct labeling. In direct labeling methods, stannous chloride (*20*), 2-mercaptoethanol (*21,22*), dithiothreitol (*23*) and dithioerythritol (*24*) have been used as agents for reducing antibody disulfide groups to sulfhydryls. Recently, we investigated ascorbic acid (*11–13*) as a disulfide reducing agent and have labeled IgG, IgM, and F(ab′)$_2$ with ^{99m}Tc with very high labeling efficiency. In the present investigation, our aim was to apply this approach to label Mabs with ^{186}Re. Although the chemical properties of Re and Tc are similar, the reduction potential for $TcO_4^- + 4H^+ + 3e^- \rightarrow TcO_2 + 2H_2O$ is 0.738 V, whereas for $ReO_4^- + 4H^+ + 3e^- \rightarrow ReO_2 + 2H_2O$ is 0.51 V (*25*). This difference in redox potential of about 220 mV shows that Re complexes are thermodynamically more stable in their higher oxidation states than the corresponding Tc analogs. This difference in chemical properties of Re and Tc along with the difficulty in obtaining ^{186}Re in high specific activity makes the preparation of ^{186}Re radiopharmaceuticals difficult.

During the use of $NaBH_4$ as a reducing agent for ^{186}Re, with MDP as a transfer ligand, formation of a gray precipitate was noted. Since we used the Amerscan MDP kit as a source for MDP, the gray precipitate might have been due to the formation of metallic Sn from reaction with $NaBH_4$ of $SnCl_2$ contained in the kit. Reduction of

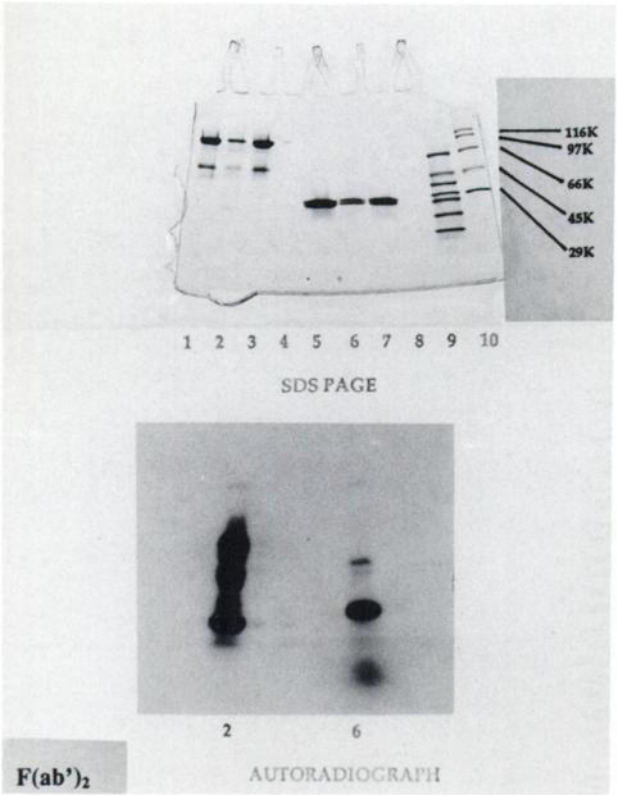

FIGURE 4. Same as Figure 2 except that the protein is TNT-F(ab')$_2$.

$^{186}ReO_4^-$ with 9 mg of $NaBH_4$ and 2 mg of fresh MDP as a transfer agent also gave varying yields even though the formation of gray precipitate did not occur. This showed that the reduction of $^{186}ReO_4^-$ with $NaBH_4$ in alkaline pH is inconsistent.

A strong reducing agent like $SnCl_2$ in acidic pH is therefore necessary for the efficient reduction of $^{186}ReO_4^-$. The addition of a polyhydroxy carboxylic acid-like citric acid probably prompts the formation of an intermediate Re^{IV} or Re^{V} citrate which is then presumably transchelated to AA-reduced antibody. The incubation of AA-reduced antibody and $SnCl_2$/citric acid with perrhenate was necessary for high labeling yields. The labeling yields were much lower when $SnCl_2$/citric acid or AA alone were used as reducing agents for both Mab and perrhenate. The use of higher amounts of $SnCl_2$ were avoided to eliminate the possibility of reducing excessive number of disulfide groups. AA used along with $SnCl_2$ presented no such problems. In the case of HIgG, the pH of the reaction mixture during incubation had to be 3 for high labeling yields. However, the pH was always brought to 7 after incubation. A similar low pH effect was observed by Pettit et al. (*26*) for labeling proteins with ^{99m}Tc. This low pH is not required for labeling other IgG antibodies such as TNT-IgG or IgM antibodies. These were labeled at pH 5 and >95% labeling yields were obtained within 4 hr at 37°C. The reasons for the requirement of lower pH for efficient labeling of HIgG compared to the other antibodies tested are not yet clear. It should be pointed out that among the five antibodies tested, only HIgG is a polyclonal antibody. The inaccessibility of the reduced Re ions to the SH groups may be due to the way this protein folds at a higher pH (*27*). The validation of this hypothesis may not be an easy task.

TABLE 3
SDS-PAGE Analysis of Rhenium-186-Labeled Antibodies

Monoclonal antibody	SDS		SDS-DTT	
	mol wt	% ^{186}Re	mol wt	% ^{186}Re
IgM	900,000	97	100,000	31
	29,000	2	65,000	40
	14,000	1	24,000	29
IgG	150,000	72	100,000	12
	50,000	10	50,000	55
	25,000	18	27,000	26
			25,000	7
F(ab')$_2$	100,000	42	66,000	10
	50,000	19	20,000	90
	20,000	39		

Numbers denote % ^{186}Re associated with proteins of different mol wt.

TABLE 4
Immunospecificity of Rhenium-186-MCA-480 Preparations as Compared with Those of Indium-111-c-DTPA-MCA-480 and Technetium-99m-MCA-480

Method	Immunospecificity (% PMN bound)
^{111}In-c-DTPA	75 ± 2
^{99m}Tc-AA	84 ± 1
^{186}Re-AA	78 ± 2

n = 4.

TABLE 5
Tissue Distribution at 4 hr in BALB/c Mice Bearing Experimental Tumor

Organ	^{186}Re-TNF (% ad. dose/g)	^{99m}Tc-TNF (% ad. dose/g)
Urine	156.6 ± 42.0	34.5
Blood	3.1 ± 0.65	2.8 ± 0.58
Kidneys*	19.6 ± 3.1	7.0 ± 0.83
Intestine	1.8 ± 0.26	0.8 ± 0.27
Muscle	0.4 ± 0.08	0.2 ± 0.05
Tumor*	3.0 ± 1.3	1.1 ± 0.32
Spleen*	21.6 ± 3.5	19.3 ± 3.9
Lungs	4.0 ± 1.7	2.3 ± 1.1
Liver*	24.6 ± 3.2	45.9 ± 8.3
Heart	1.7 ± 0.5	1.0 ± 0.12
Tumor/Muscle	7.9 ± 4.7	4.7 ± 0.65
n	5	4

* p values: kidneys = 0.002, tumor = 0.07, spleen = 0.94, liver = 0.03.

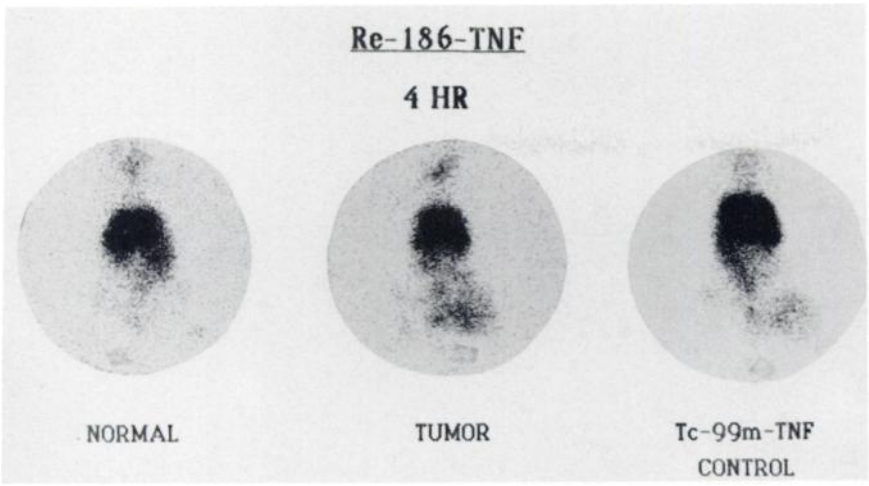

FIGURE 5. Four-hour posterior images of three separate mice bearing embryonal carcinoma in the right thigh. The animal in the center of the figure received ^{186}Re-TNF, which delineated the tumor conspicuously as did the animal in the far right, which received ^{99m}Tc-TNF. Animal at the far left received ^{99m}Tc-HSA, which was not taken up by the tumor in any appreciable quantity.

Rhenium-186-labeled HIgG antibodies were stable when challenged with DTPA and HSA. Furthermore, the fact that there was no appreciable loss of activity during incubation with DTPA showed the absence of binding to low affinity sites in the proteins (*28*). Our work in progress indicates that less than 5% nonspecific binding occurs under the conditions specified (John E, Wilder S, Thakur ML, *unpublished data*). In direct labeling methods, reduced Tc or Re is bound to sulfhydryl groups (*29*) produced by the reduction of disulfide bonds in the proteins. This is confirmed by the fact that the labeling yields of HIgG with ^{99m}Tc were considerably reduced (*30*) when sodium iodoacetate known to block SH groups ($-CH_2SH + ICH_2COOH \rightarrow HI + -CH_2S-CH_2-COOH$) is allowed to react with AA-reduced antibody prior to adding reduced pertechnetate. This is further ascertained by the fact that only about 25% of the activity remained bound to the protein on incubation with 100- and 250-fold excess of cysteine for 24 hr at 37°C. This is expected due to the large excess of cysteine with its sulfhydryl groups (*31*). The stability of the tracer is not expected to be any different for the other four antibodies tested as the coordination around Re would remain the same for all antibodies under similar labeling conditions. It has been demonstrated in our laboratory with ^{99m}Tc-labeled antibodies that the stability of the Tc-S bond does not vary with the antibodies used (*11–13*).

TABLE 6
Tissue Distribution of Rhenium-186-TNT-F(ab')$_2$ in Tumor-Bearing BALB/c Mice

	(% Administered dose/g)	
Organ	4 hr	24 hr
Muscle	0.20 ± 0.04	0.041 ± 0.03
Intestine	4.0 ± 4.9	0.27 ± 0.22
Urine	110	3.0
Heart	2.3 ± 0.19	0.35 ± 0.21
Lungs	5.6 ± 4.5	0.63 ± 0.43
Blood	0.98 ± 0.11	0.11 ± 0.05
Spleen	9.0 ± 2.7	3.1 ± 1.2
Kidneys	10.2 ± 0.45	2.1 ± 0.42
Liver	11.2 ± 1.6	2.5 ± 0.69
Tumor	1.2 ± 0.27	0.46 ± 0.13
Tumor/muscle	5.9 ± 0.21	13.8 ± 6.7
Tumor/blood	1.3 ± 0.31	4.5 ± 0.78
n	3	3

SDS-PAGE and autoradiography of labeled HIgG, MCA-480 and TNT-F(ab')$_2$ showed that in all cases there was excellent correlation between native antibody, AA-reduced antibody and ^{186}Re-labeled antibody in both reducing and nonreducing conditions. This indicated that the labeling process did not cause any detectable fragmentation of the Mab molecules. The autoradiography of the labeled antibodies indicated as expected that the binding of ^{186}Re with the antibody was random. The appearance of low molecular weight bands (under nonreducing conditions) in the case of labeled HIgG and TNT-F(ab')$_2$ is not an indication that these antibodies have been fragmented during our labeling procedure. In the SDS-PAGE protocol, antibodies were heated at 100°C with SDS and so under these conditions even the presence of the slightest amount of reducing agent (like AA) can fragment the antibody as observed during the SDS-DTT procedure. Similar SDS-PAGE results have been obtained with other directly labeled antibodies (*32*). The SDS-PAGE of labeled IgM is consistent with the behavior of an antibody with a molecular weight of 900 KD in a 4%–20% gradient gel and does not indicate the presence of colloids. This was confirmed when this IgM preparation was digested with DTT and analyzed similarly. In this instance, there was a negligible amount of radioactivity at the top of the gel, thereby indicating the absence of colloids.

The immunoreactivity of the ^{186}Re-labeled MCA-480 was in the same range as that of ^{111}In-DTPA-MCA-480 and ^{99m}Tc-MCA-480. Extensive work to determine the immunoreactivity of MCA-480 labeled with ^{111}In and ^{99m}Tc has been done in our laboratory. This antibody labeled with ^{99m}Tc by the AA method is currently being evaluated in patients for imaging abscesses (*33*). Immunoreactivity has been previously assayed by determining

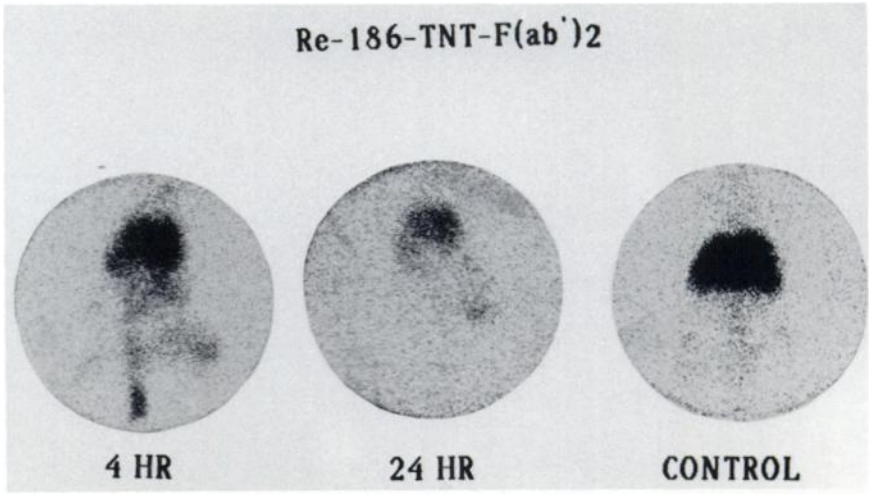

FIGURE 6. Gamma camera images of mice bearing embryonal carcinoma. Images were obtained at 4 and 24 hr postinjection of ^{186}Re-TNT-F(ab')$_2$. At 24 hr, clearance of radioactivity from all organs can be seen. However, tumor to muscle ratio increased from 5.9 ± 0.21 at 4 hr to 13.8 ± 6.7 at 24 hr. The control animal bearing a similar tumor received ^{99m}Tc-HSA as a nonspecific protein and was imaged at 4 hr postinjection. Tumor was not detectable with this agent.

the binding of serial dilution of the antibody as well as by scatchard plot analysis (*16*). This type of antigen excess assay for determination of immunoreactivity has also been recommended by Steward and Steensgaard (*34*). The current protocol was then developed for speed and simplicity of the test. Therefore, in our present work we have compared the immunoreactivity of ^{186}Re-labeled antibody with ^{111}In- and ^{99m}Tc-labeled MCA-480. Results show that the labeling conditions do not alter the antigen affinities of these antibodies. This is consistent with the use of AA and other mild chemical conditions used here. MCA-480 is specific only for human PMNs and not for the seven common laboratory animal PMNs (*16*). This prevented us from examining ^{186}Re-MCA-480 in animals.

Tumors were easily visualized with ^{186}Re-TNF as well as ^{99m}Tc-TNF. The biodistribution of ^{186}Re-TNT-F(ab')$_2$ in tumor bearing mice for a period of 24 hr showed that there was substantial clearance from all organs. However the tumor/muscle ratio at 24-hr postinjection increased from 5.9 ± 0.2 at 4-hr postinjection to 13.8 ± 6.7. The fast blood clearance and relatively high kidney uptake (% ID = 10.2) of this agent are consistent with the clearance of fragmented antibodies. Our present work (*35*) with metabolites of ^{186}Re-labeled antibodies have shown that 90% of the radioactivity in the blood is still associated with the antibody at 4-hr postinjection and thereby indicates the in vivo stability of the tracer. The liver uptake of ^{186}Re- and ^{99m}Tc-labeled antibodies are quite comparable at 4-hr postinjection and appear to be high. However, the liver activity cleared subsequently and fell in the range observed by other investigators (*36*). Also, liver uptake of radiolabeled antibodies in tumor bearing mice can be high due to the presence of circulating antigens (*37*). TNT-1-F(ab')$_2$ is an antinuclear antibody; the low tumor uptake (%ID/g = 1.2) is probably due to the lack of a large number of necrotic cells in the small tumors.

Our main goal with the present work was to establish a procedure to label antibodies efficiently with ^{186}Re. The results of our preliminary investigation have shown that we have been able to do so. The only other published work with directly labeled ^{188}Re antibodies is by Griffiths et al. (*5*); our results are quite comparable to theirs. However, contrary to their observation, we have not observed any instability of ^{186}Re-labeled antibodies in saline for up to 72 hr. The relatively long incubation time (3–4 hr at 37°C or 24 hr at 22°C) for quantitative yields does not cause a significant inconvenience, since the half-life of ^{186}Re is long and therapeutic treatments are generally planned well ahead of time. No postlabeling purification was needed with this procedure and AA does not have to be eliminated before injection of humans. This labeling technique is simple and convenient, and has already been adapted to a kit labeling procedure.

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