In Vitro Complex Formation and Biodistribution of Mouse Antitumor Monoclonal Antibody in Cancer Patients

Harumi Sakahara, James C. Reynolds, Jorge A. Carrasquillo, Margarita E. Lora, Patrick J. Maloney, Michael T. Lotze, Steven M. Larson, and Ronald D. Neumann

Nuclear Medicine Department, Clinical Center, Surgery Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

The serum clearance and biodistribution of a murine monoclonal antibody were compared to the in vitro complex formation of the antibody with patients' sera. Iodine-125-labeled 9.2.27, an anti-melanoma antibody, was incubated with sera from ten melanoma patients who had received 9.2.27 in an earlier study. Complexes were observed in all patients using size exclusion high performance liquid chromatography and complex formation was partially blocked by nonspecific murine antibody, suggesting the presence of human anti-murine antibody in serum. All patients subsequently underwent imaging studies with [^{131}I] 9.2.27 given intravenously. The serum levels of the antibody obtained after the second administration were inversely correlated with the level of in vitro complex formation. Patients whose serum formed high levels of complex showed a rapid serum clearance, high hepatic uptake, and accelerated whole body clearance and urinary excretion of ^{131}I. This suggests that in patients who receive repetitive administration of murine antibody the serum clearance rate and biodistribution of intravenously injected antibody are altered by antibody complex formation in the serum.

J Nucl Med 30:1311–1317, 1989

Murine monoclonal antibodies (MoAbs) against human tumor-associated antigens have been extensively used for in vitro diagnosis and therapy of cancer patients (*1–4*). Development of human anti-murine antibody (HAMA), however, is a frequent problem in the repetitive administration of mouse antibodies to patients (*5–8*). HAMA may form high molecular weight complexes with the injected MoAb resulting in rapid blood clearance, high hepatic and/or splenic uptake, and reduced tumor targeting of the MoAb (*4,5,9,10*). Circulating antigen may also form complexes with MoAb and alter its biodistribution (*11,12*).

In this study, we examined the in vitro complex formation of anti-melanoma MoAb 9.2.27 with sera from melanoma patients who had received the antibody in another study 2 wk earlier, and compared the turnover and distribution of subsequently administered iodine-131 (^{131}I) 9.2.27 in these patients to the level of complex formation. All patients underwent two studies with the antibody as part of a larger protocol designed to assess the role of interleukin-2 on antibody clearance. But in the studies reported here the patients did not receive interleukin-2.

Received Sept. 9, 1988; revision accepted Apr. 20, 1989.

For reprints contact: James C. Reynolds, MD, Nuclear Medicine Dept., National Institutes of Health, Bldg. 10, Room 1C401, 9000 Rockville Pike, Bethesda, MD 20892.

MATERIALS AND METHODS

Mouse Monoclonal Antibody

The monoclonal antibody 9.2.27 is a murine IgG_{2a} antibody, which recognizes a 250 kDalton chondroitin sulfate/proteoglycan antigen on human melanoma cells (*13–15*). The purified antibody (Hybritech, Inc., La Jolla, CA) was labeled with iodine-125 (^{125}I) for in vitro studies and ^{131}I for human use, both by the Chloramine-T method (*16*). The specific activity of ^{125}I and ^{131}I labeled antibodies was 1.2–9.2 and 3.9–9.0 mCi/mg protein, respectively.

Patients

Ten patients who had histologically proven metastatic melanoma were studied. All patients had stage III melanoma and with extensive metastatic involvement in most cases. Eight tumors had positive immunohistochemical staining with 9.2.27, while two tumors were heavily pigmented and perox-

idase staining could not be verified. As part of an immunotherapy protocol they were given intravenously 10 mCi and nm100 mg of [^{131}I] 9.2.27 twice with an intervening period of 2 wk. To prepare specific activity doses of 10 mCi per 100 mg of 9.2.27, unlabeled 9.2.27 was added to [^{131}I] 9.2.27. The antibody was infused over 1 hr. Serum taken immediately before the second infusion was used for the in vitro complex formation measurement, and the level of complex formation was then compared to the in vivo biodistribution of the antibody after the second infusion. The correlation coefficient was calculated from the data and the significance of correlation coefficients was determined using t statistics.

In four of the ten patients the biodistribution data from the first study was used for comparison. The data from the other six patients' first study was excluded because they received interleukin-2 with the first infusion of 9.2.27, which altered the biodistribution of the antibody (*17*).

In Vitro Complex Formation

Twenty-five nanograms (5.8 μl) of [^{125}I] 9.2.27 was incubated with 50 μl of serum for 1 hr at 37°C, and applied to a high performance liquid chromatography (HPLC) system (Millipore Corp., Milford, MA) with TSK-2000 and TSK-4000 columns in series (Beckman Instrument Inc., San Ramon, CA). Incubation mixtures were eluted by 0.067 *M* phosphate buffered saline, 0.1 *M* KCl, pH 7.4 at flow rate of 0.5 ml/min (*18*). The radioactivity was monitored by an online detector (Beckman Instrument Inc.). The area of each peak of radioactivity was calculated by a chromatography data system (Nelson Analytical Inc., Paramus, NJ), and expressed as the percentage of total counts found in all peaks. The molecular weight of complexes was estimated by markers of goat IgM (900 kD), bovine thyroglobulin (669 kD), beta-galactosidase (570 kD), and goat IgG (150 kD).

To confirm that patients' sera have HAMA, an inhibition study with nonspecific mouse IgG was done. Fifty microliters of serum were incubated with 25 ng/2.5 μl of [^{125}I] 9.2.27, and 16.7 μg/3.3 μl of subclass matched irrelevant mouse MoAb 2-135. Antibody 2-135 (Damon Biotech Inc., Needham Heights, MA) is an anti-idiotype antibody against a human B cell lymphoma. Complex formation was analyzed again by size exclusion HPLC.

IgG free serum was prepared by adsorption with a Protein A affinity chromatography column (Pharmacia Fine Chemicals AB, Upsala, Sweden). For these preparations HPLC analysis with monitoring of absorbance at 280 nm showed no discernible IgG peak. To test whether the complexes formed were between circulating antigen and 9.2.27, 100 μl of IgG adsorbed serum (~1/5 of original serum concentration) was incubated with 5 ng/5 μl of [^{125}I] 9.2.27 and then analyzed with HPLC.

Serum Clearance of 9.2.27

Serum samples were obtained at 5 min, 30min, 1, 2, 24, 72, 96 hr and up to 7 days of postinfusion. Radioactive counts in serum as well as aliquots of the injected dose were measured by a gamma counter. The radioactivity in the protein-bound fraction was determined as the percentage of counts precipitable with trichloroacetic acid. In addition to measurement of radioactivity in the serum, mouse IgG levels were determined using an immunoradiometric assay. Affinity purified goat antibody against mouse IgG (Kirkegaard & Perry Lab. Inc., Gaithersburg, MD) was coupled to imidazolyl-carbamate activated agarose beads (Pierce Chemical Co., Rockford, IL). Fifty microliters of diluted serum, an aliquot of the injected dose of known 9.2.27 standards was incubated with 100 μl of the suspension of goat anti-mouse IgG coupled beads (GAM-beads) for 1 hr at room temperature. After washing and separation from supernatant by centrifugation, GAM-beads were incubated with 100 μl of ^{125}I-labeled goat anti-mouse IgG (Kirkegaard & Perry Lab. Inc.) for 1 hr at room temperature. The GAM-beads were then washed and centrifuged. The pellet was counted by a gamma counter and the concentration of mouse IgG was determined from a 9.2.27 standard curve.

Both the serum levels of protein-bound radioactivity and of mouse IgG were expressed as the percentage of the injected dose present in the total plasma. Plasma volume was estimated from the patients' body surface area using a standard nomogram (*19*).

Serum levels at 2 hr and 24 hr were selected as indices for the fast component of the serum clearance. The half-life after 24 hr in serum, as an index for slow component, was determined by a least squares analysis of the logarithm of serum concentration versus time.

Biodistribution Study

Gamma camera imaging was done on all patients. The data were stored in a computer and regions of interest were drawn over the heart, liver, spleen, and tumor. The values were expressed as the ratio of cpm per pixel divided by the injected counts determined from a dose standard that was imaged at the time of the patient imaging. This method corrected for both physical isotope decay and changes in camera performance. The urine was collected daily up to 96 hours postinfusion. Whole-body retention of the radioactivity was measured with a 5.0 × 5.0 cm sodium iodine detector, taking the immediate postinfusion value as 100%.

RESULTS

Patient characteristics and data from the in vitro complex formation studies are summarized in Table 1. Complexes with a molecular weight of greater than 150 kD were found in all patients. Three different molecular weight complexes were observed. The first was larger than 900 kD, the second was between 650K to 750 kD, and the third was 450 kD. Typical chromatograms of patients numbered 2, 3, and 8 are shown in Figure 1. The addition of nonspecific antibody 2-135 to serum resulted in a shift of ^{125}I activity from larger complexes to either smaller complexes or IgG, an effect that was observed in all patients. These results indicate that all patients had HAMA that recognize common antigenic determinants on mouse IgG_{2a} and that at least a portion of the complexes formed were between 9.2.27 and HAMA. Complexes which were not inhibited by excess 2-135 could represent either HAMA/9.2.27 complexes where HAMA was specific for 9.2.27 or antigen/9.2.27 complexes. Patients' sera from which human IgG was removed by Protein-A absorption still formed com-

TABLE 1
Patient Characteristics and Summary of In Vitro Complex Formation

Patient no.	Age (yr)	Sex	Main tumor	%Complex* >900KD	%Complex* 650–750KD	%Complex* 450KD	Complex formation after absorption of IgG
1	49	M	Skin	0 (0)†	4 (2)	0 (0)	N.D.‡
2	39	F	Skin	8 (0)	21 (25)	0 (0)	(+)
3	33	F	Ovary	81 (0)	0 (62)	0 (0)	N.D.
4	51	M	Lymph nodes	16 (0)	13 (10)	0 (0)	(+)
5	51	M	Lymph nodes	7 (0)	24 (25)	0 (0)	(+)
6	40	M	Lymph nodes	2 (0)	25 (18)	0 (0)	(+)
7	50	M	Lymph nodes	2 (0)	12 (10)	0 (0)	(+)
8	31	M	Lymph nodes	8 (0)	20 (5)	68 (0)	(+)
9	61	M	Skin	0 (0)	11 (2)	0 (0)	N.D.
10	42	F	Lymph nodes	0 (0)	6 (3)	0 (0)	N.D.

* Percentage of radioactivity in complexes formed by in vitro incubation.
† %Complex after addition of 2-135.
‡ Not done.

plexes (Table 1), suggesting the presence of antigen/9.2.27 complexes. Furthermore, the complexes formed by adsorbed serum and 9.2.27 were of a size of less than 900 kD (660 k–880kD), that could be formed with antigen/9.2.27 reactions.

The serum clearance rates defined as the percent injected dose remaining in plasma at 24 hr, or as the half-life of the slow component were then compared to the percentage of complex formed in vitro. When the total percentage of radioactivity present in three molecular weight complexes were taken into account, the correlation coefficient between percent complex and serum radioactivity levels at 24 hr was -0.539 ($p < 0.1$). In Patient 8, almost all [^{125}I] 9.2.27 was found as complexes but the serum clearance was not very fast. Plasma retention of protein-bound radioactivity was 35% at 24 hr in Patient 8, whereas it was 5% in Patient 3, whose serum also formed a high level of complexes. Most complexes formed by serum from Patient 8 were 450 kDaltons. The other nine patients did not show the smallest size complexes. Competition studies with 2-135 suggested that these complexes were HAMA/9.2.27 forms. To test whether these smallest molecular weight complexes were caused by a large excess of HAMA, unlabeled 9.2.27 was added in a range between 25 ng and 1575 ng. The peak of radioactivity shifted to free IgG rather than larger complexes (data not shown); indicating that the formation of the small complexes in

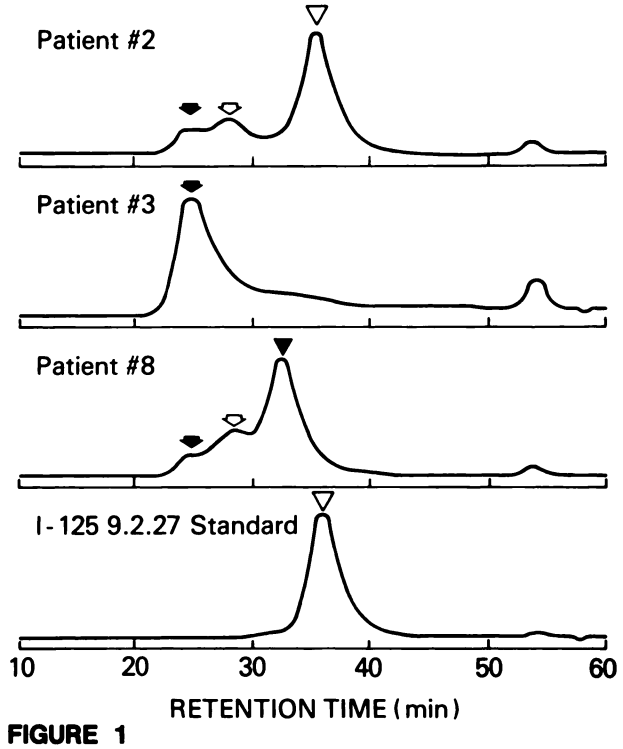

FIGURE 1
HPLC chromatograms of [^{125}I] 9.2.27 after the incubation with patients' sera and control [^{125}I] 9.2.27 without serum. Vertical axis is arbitrary scale of counts. Three different molecular weight complexes were observed. One was greater than 900 kD (↓), one was 650–750 kD (↓), and the other was 450 kD (▼). Open arrow head (▽) shows IgG peak (150 kD). In Patients 3 and 8, IgG peak was not detectable.

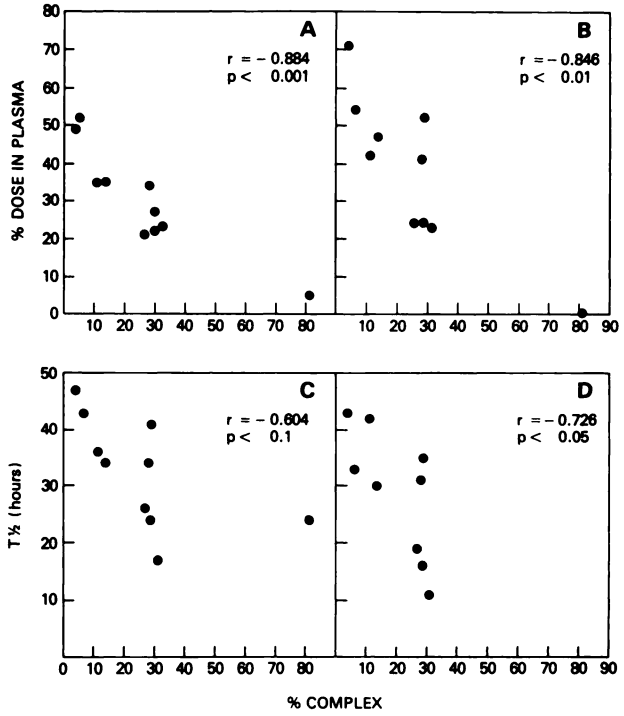

FIGURE 2
Relationship between the serum clearance of [^{131}I] 9.2.27 and the level of complex formation in vitro. The level of complex formation was expressed as the total percentage of counts found in the two larger molecular weight complexes. A: Serum levels of protein-bound radioactivity at 24 hr and percent complex; $r = -0.884$, $p < 0.001$. B: Serum levels of mouse IgG at 24 hr and percent complex; $r = -0.846$, $p < 0.01$. C: Half-life of protein-bound radioactivity in serum after 24 hr and percent complex; $r = -0.604$, $p < 0.1$. D: Half-life of mouse IgG in serum after 24 hr and percent complex. $r = -0.726$, $p < 0.05$. T1/2 of mouse IgG in Patient 3 was not calculated because serum levels later than 24 hr postinfusion could not be measured.

vitro was not the result of HAMA excess. When the smallest complexes were excluded from the analysis, the correlation coefficient between the total percentage of the two larger complexes and serum levels of protein-bound radioactivity at 24 hr was increased to −0.884 ($p < 0.001$, Fig. 2A). These findings suggest that larger molecular weight complexes have a more important role in altering the serum clearance of the antibody. Thus, the total of the percentage of counts found in the two larger molecular weight complexes were calculated and this value was used for further correlations.

Although the percent dose of mouse IgG in the serum was slightly higher than that of protein-bound radioactivity, these two species were closely correlated except in Patient 3. In this patient mouse IgG levels were very low and could not be measured in serum obtained later than 24-hr postinfusion. Serum levels of mouse IgG at 24 hr were also inversely correlated with percent complex formed in vitro ($r = -0.846$, $p < 0.01$, Fig. 2B). In an earlier time point of 2 hr, a similar inverse correlation was observed between the levels of complex and serum antibody levels ($r = -0.852$, $p < 0.01$ for radioactivity, and $r = -0.901$, $p < 0.001$ for mouse IgG). Correlation between the half-life and the percentage of complex was weaker ($r = -0.604$, $p < 0.1$ for radioactivity and $r = -0.726$, $p < 0.05$ for mouse IgG, respectively, Fig. 2C and D).

The liver-to-heart count ratio at 2 hr was also related to percent complex formed in vitro ($r = 0.874$, $p < 0.001$, Fig. 3). However, there was not a significant relationship between splenic uptake and percent complex ($r = -0.123$). Whole-body retention of the radioactivity at 24 hr and percent dose of urinary excretion by 24 hr showed an inverse and direct relationship with percent complex formed in vitro ($r = -0.671$, $N = 9$, $p < 0.05$ and $r = 0.600$, $N = 8$, $p < 0.1$, respectively).

HAMA in patients' sera before the first infusion was tested in all patients. Sera from seven of 10 patients formed complexes with [^{125}I] 9.2.27 in vitro and in one of these patients (Patient 1), complex formation was inhibited by the addition of nonspecific murine antibody. This finding suggests that one of 10 patients had pre-existing HAMA. Figure 4 shows the serum time-activity curves in four patients who were studied twice.

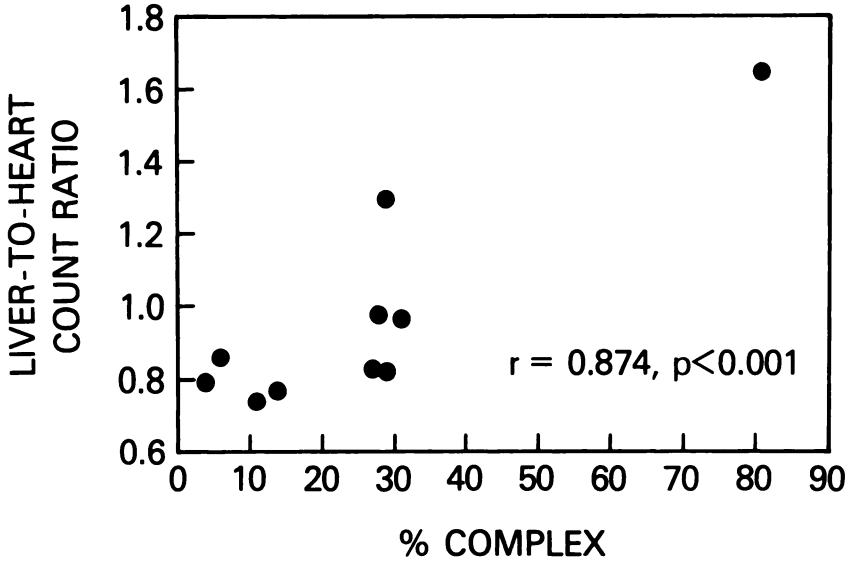

FIGURE 3
Relationship between liver to heart count ratio at 2 hr postinfusion and the level of complex formation in vitro. r = 0.874, p < 0.001.

The level of complex formation in the first study was 25% in Patient 1, 0% in Patients 2 and 4, and 6% in Patient 3. The molecular weight of complex formed in Patient 1 was 670 kD. In Patients 2, 3, and 4 the serum clearance was faster and whole-body clearance and urinary excretion was accelerated after the second infusion, when in vitro complex formation by the patients' serum was greater (Table 1). In these three patients, whole-body clearance and urinary excretion were also accelerated in the second study compared to the first study. For example, in Patient 4 whole-body retention at 2 days was 56% in the first study and 30% in the second study. Cumulative urinary excretion by 2 days was 38% and 76% of the injected dose, respectively. Liver, heart, and tumor region of interest data from Patient 3's scintigraphic studies is depicted in Figure 5. There is a striking difference between the first and second studies. In the first study tumor uptake increased over the first 2 days with activity clearing at later times. Hepatic activity gradually diminished. In the second study, when the patient's serum formed a high level of complexes with 9.2.27 by in vitro measurement, there was much higher initial accumulation of radioactivity in the liver, followed by a rapid clearance from the liver. In this study cardiac blood-pool activity was decreased and tumor activity was very low. Furthermore, target-to-background ratio was lower in the second study (4.9 in the first study and 2.9 in the second study 3 days postinfusion). A similar time activity curve for the liver, that is, an initial high uptake followed by a rapid clearance was seen in the second study of Patients 2, 4, and 5.

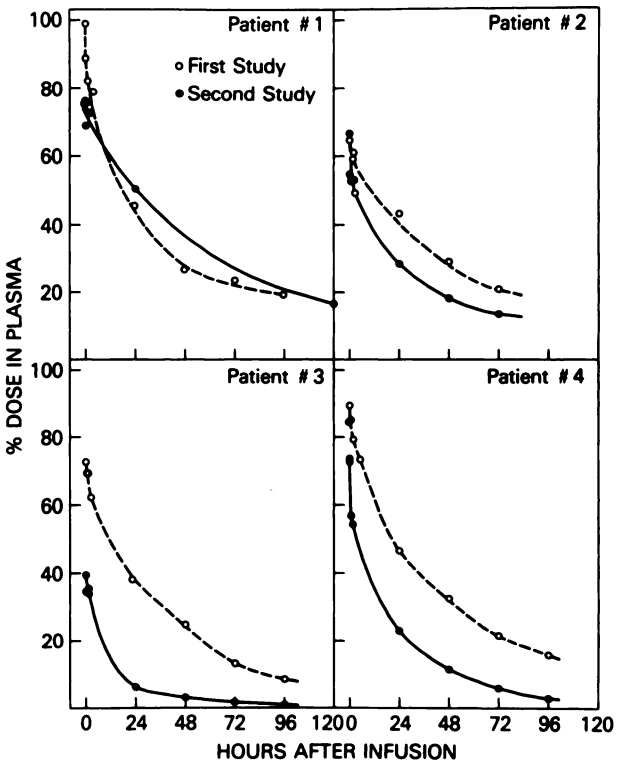

FIGURE 4
Serum clearance of protein-bound radioactivity from four patients. The percentage of the injected dose retained in plasma after the first (O) and second study (●) was plotted. Patient 1 received the first infusion of [^{131}I] 9.2.27 as a bolus rather than 1-hr infusion.

DISCUSSION

In this study, sera from patients who received prior injections of murine antibody formed complexes with 9.2.27 in vitro. These complexes consisted of HAMA/9.2.27 forms, which were from determinants common to IgG_{2a}, and other complexes that could not be blocked by a nonspecific murine IgG_{2a}. It is likely that some of these latter complexes were the result of antigen/antibody interaction as they were found when Protein A

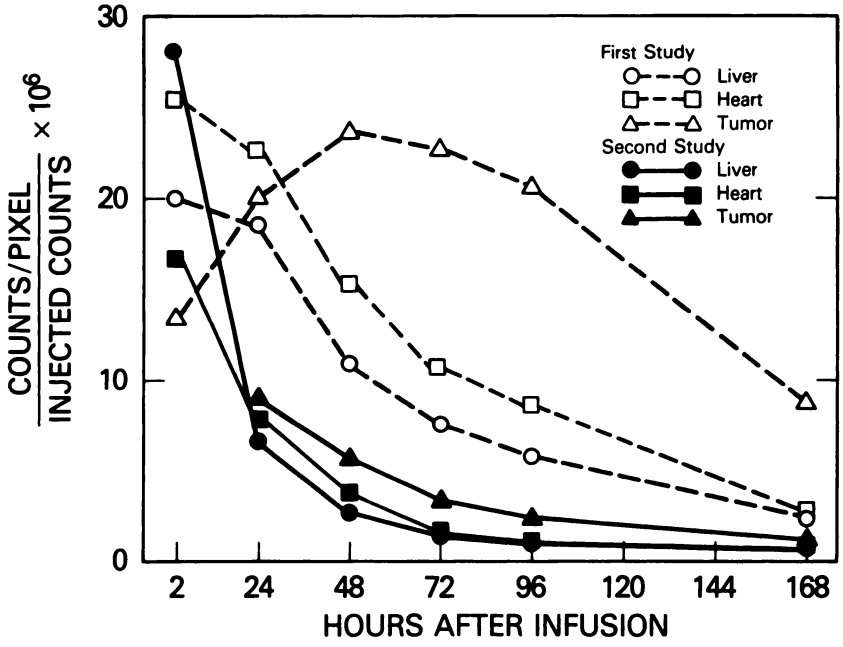

FIGURE 5
The time-activity curves in the liver, heart, and tumor of Patient 3 following the initial and second injection.

adsorbed sera were used for incubation. Alternatively these complexes could have been due to the interaction of HAMA with sites specific to 9.2.27, such as the variable region. None of the patients included in this study had known prior exposure to murine antibodies. However, the analysis of pre-study serum before the first injection suggested that Patient 1 had pre-existing HAMA.

Studies with high concentration of nonspecific antibody show that antibody-antibody complexes can be of several sizes. Complexes of 900 kD or larger could be composed of MoAb 9.2.27 reacting with IgM HAMA or polymeric forms of IgG HAMA. Complexes of 650–750 kD likely represent HAMA IgG and specific IgG polymers. On the other hand, complexes of size 450 kD could be related to two molecules of HAMA IgGs interacting with one molecule of 9.2.27, or conversely two 9.2.27 with one HAMA IgG. It was noted that the percentage of the lower molecular weight complex forms did not correlate strongly to the turnover rates of 9.2.27. These findings are analogous to those for anti-insulin antibodies in which smaller immune complexes did not shorten the circulation of the insulin (*20*).

There was an inverse correlation between the percentage of complexes formed in vitro and the rate of antibody clearance from the circulation. This was true whether the antibody concentration was determined as protein-bound radioactive counts or as IgG. Patients with a high level of complexes in vitro, showed a low serum level at 2 and 24 hr, and an elevated liver-to-heart count ratio at 2 hr, which suggests that the murine antibody formed complexes immediately after the infusion and these complexes were then cleared rapidly by the liver. Acceleration of the slow component of the serum clearance could result from a secondary response of HAMA following the booster immunization. Deiodination of radioiodinated antibody in vivo is a well-known phenomenon (*21*). Because the patients received ^{131}I-labeled antibody, ^{131}I could be released from antibody or complexes that accumulated in the liver. Released ^{131}I could be excreted through the kidney. In the second study the clearance rate of ^{131}I from the liver after the initial high uptake was faster than the clearance from the liver observed following the first injection. Deiodination of complexes may be faster than that of free ^{131}I antibody in the liver. An inverse relationship between in vitro complex formation and whole-body retention, and a direct correlation with urinary excretion are in accordance with the changes in catabolism of ^{131}I antibody after the complex formation. A dramatic decrease of tumor targeting was seen in one patient after the second infusion, when the serum formed high level of complexes in vitro. The interference of tumor targeting may be the result of a faster serum clearance with a lower time-activity product. In addition, other factors such as a reduced capillary permeability for large complexes or blocking of the antigen binding site of 9.2.27 by antigen or anti-idiotype HAMA could have an effect.

Although this is a small study, the results suggest that in patients who have repeat administration of murine antibody, the serum clearance rate and biodistribution of intravenously injected antibody are related to the ability of serum to form complexes with the antibody, and monitoring of forming complexes in serum prior to administration may predict the handling of antibody after subsequent diagnosis and treatment. There are several characteristics of the altered biodistribution syn-

drome. In patients whose serum formed high levels of complexes, plasma clearance of antibody is accelerated with increased hepatic uptake. When patients receive radioiodinated antibody, radioactivity in the liver is rapidly decreased after the initial high accumulation and whole-body clearance and urinary excretion of radioiodine is fast. Finally, tumor targeting is reduced with both lower total accumulated activity and a lower tumor-to-background activity ratio.

REFERENCES

1. Larson SM, Carrasquillo JA, McGuffin RW, et al. Use of I-131 labeled, murine Fab against a high molecular weight antigen of human melanoma: preliminary experience. *Radiology* 1985; 155:487–492.
2. Carrasquillo JA, Bunn PA, Keenan AM, et al. Radioimmunodetection of cutaneous T-cell lymphoma with ^{111}In labeled T101 monoclonal antibody. *N Engl J Med* 1986; 315:673–678.
3. Carrasquillo JA, Krohn KA, Beaumier P, et al. Diagnosis of and therapy for solid tumors with radiolabeled antibodies and immune fragments. *Cancer Treat Rep* 1984; 68:317–328.
4. Zimmer AM, Rosen ST, Spies SM, et al. Radioimmunotherapy of patients with cutaneous T-cell lymphoma using an iodine-131-labeled monoclonal antibody: analysis of retreatment following plasmapheresis. *J Nucl Med* 1988; 29:174–180.
5. Goodman GE, Beaumier P, Hellstrom I, Fernyhough B, Hellstrom KE. Pilot trial of murine monoclonal antibodies in patients with advanced melanoma. *J Clin Oncol* 1985; 3:340–352.
5. Schroff RW, Foon KA, Beatty SM, Oldham RK, Morgan AC Jr. Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy. *Cancer Res* 1985; 45:879–885.
7. Shawler DL, Bartholomew RM, Smith LM, Dillman RO. Human immune response to multiple injections of murine monoclonal IgG. *J Immunol* 1985; 135:1530–1535.
8. Courtenay-Luck NS, Epenetos AA, Moore R, et al. Development of primary and secondary responses to mouse monoclonal antibodies used in the diagnosis and therapy of malignant neoplasms. *Cancer Res* 1986; 46:6489–6493.
9. Larson SM, Carrasquillo JA, Krohn KA, et al. Localization of ^{131}I-labeled p97-specific Fab fragments in human melanoma as a basis for radiotherapy. *J Clin Invest* 1983; 72:2101–2114.
10. Pimm MV, Perkins AC, Armitage NC, Baldwin RW. The characteristics of blood-borne radiolabels and the effect of anti-mouse IgG antibodies on localization of radiolabeled monoclonal antibody in cancer patients. *J Nucl Med* 1985; 26:1011–1023.
11. Hnatowich DJ, Griffin TW, Kosciuczyk C, et al. Pharmacokinetics of indium-111-labeled monoclonal antibody in cancer patients. *J Nucl Med* 1985; 26:849–858.
12. Hagan PL, Halpern SE, Chen A, et al. In vivo kinetics of radiolabeled monoclonal anti-CEA antibodies in animal models. *J Nucl Med* 1985; 26:1418–1423.
13. Morgan AC, Galloway DR, Reisfeld RA. Production and characterization of monoclonal antibody to a melanoma-specific glycoprotein. *Hybridoma* 1981; 1:27–36.
14. Bumol TF, Reisfeld RA. Unique glycoprotein-proteoglycan complex defined by monoclonal antibody on human melanoma cells. *Proc Natl Acad Sci* USA 1982; 79:1245–1249.
15. Carrasquillo JA, Abrams PG, Schroff RW, et al. Effect of antibody dose on the imaging and biodistribution of indium-111 9.2.27 anti-melanoma monoclonal antibody. *J Nucl Med* 1988; 29:39–47.
16. Reynolds JC, Maloney PJ, Rotman M, Fejka R, Farkas R, Larson SM. Radiohalogenation of antibodies—a review of labeling methods and their chemical consequences. In: Srivastava SC, Reba R, eds. *Radiolabeled monoclonal antibodies for imaging and therapy—potential, problems, and prospects.* New York: Plenum, 1988.
17. Sakahara H, Carrasquillo JA, Lotze MT, et al. Effect of interleukin-2 on biodistribution of I-131 labeled monoclonal antibody 9.2.27 [Abstract]. *J Nucl Med* 1988; 29:901.
18. Reynolds JC, Carrasquillo JA, Lora MA, et al. Measurement of human anti-murine antibodies in patients who received radiolabeled monoclonal antibodies. *Nuklearmedizin Suppl* 1987; 23:425–427.
19. International committee for standardization in haematology. Recommended methods for measurement of red-cell and plasma volume. *J Nucl Med* 1980; 21:793–800.
20. Sodoyez-Goffaux F, Sodoyez JC, Dozio N, Miceli A, De Vos CJ. Abnormalities of 123-I-insulin biodistribution and degradation in insulin immunized diabetic patients [Abstract]. *J Nucl Med* 1987; 28:583.
21. Sullivan DC, Silva JS, Cox CE, et al. Localization of I-131-labeled goat and primate anti-carcinoembryonic antigen (CEA) antibodies in patients with cancer. *Invest Radiol* 1982; 17:350–355.