The Characteristics of Blood-Borne Radiolabels and the Effect of Anti-Mouse IgG Antibodies on Localization of Radiolabeled Monoclonal Antibody in Cancer Patients

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Quantitative and qualitative aspects of the blood survival of ^{131}I and ^{111}In-labeled monoclonal antibody 791T/36 have been examined in patients with colo-rectal carcinoma, ovarian carcinoma and osteogenic sarcoma who were receiving labeled antibody in diagnostic immunoscintigraphy trials. The blood clearance of intact antibody radiolabeled with either ^{131}I or ^{111}In was similar. A bi-phasic decline of both radiolabeled preparations was measured with initial half-lives 0.62 and 0.42 days for ^{131}I and ^{111}In labels and then with 1.85 and 1.40 day half-lives, respectively. The Fab fragment of the antibody was lost more rapidly (initial half-life 0.20 days and then 0.78 days). Blood-borne radioactivity was associated predominantly with plasma rather than cellular elements. Radioactivity was still attached to undegraded, uncomplexed, and immunologically active antibody as demonstrated by molecular filtration, immune precipitation, and antigen binding assays. However, anti-mouse-IgG antibody detected within 7 days of administration of radiolabeled antibody was present for at least 10 mo and has implications for the efficiency of repeated image studies.

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Imaging of primary and metastatic tumors following i.v. administration of radiolabeled antibodies against antigens associated with a number of human tumors has been reported (*1–15*). The localization of radiolabeled antibodies in tumor deposits is clearly dependent upon continued integrity and immunological reactivity of radiolabeled antibody in the circulation, at least until sufficient radioactivity has been accumulated to allow external imaging. However, little is known about the fate of radiolabeled monoclonal antibodies in the blood following intravenous administration. The mouse IgG2b 791T/36 monoclonal antibody has previously been effective in imaging of colo-rectal (*3,11,15*), mammary (*13*) and ovarian (*14*) carcinomas and osteogenic sarcomas (*8*). The present study was carried out to characterize blood-borne radioactivity in patients injected with iodine-131 (^{131}I) and indium-111- (^{111}In) labeled 791T/36 antibody, or ^{131}I-labeled Fab fragment during these diagnostic imaging trials (*3,8,11,14,15*). This has included an analysis of the blood clearance of the radiolabels with time, determination of whether radioactivity was associated with blood cells, and whether plasma radioactivity was still attached to the monoclonal antibody in an immunologically active form. In addition, the patients' development of anti-mouse IgG antibody was examined, and the influence of this on repeated imaging assessed.

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PATIENTS, MATERIALS, AND METHODS

Patients

Thirty-five patients had primary or recurrent colorectal carcinoma, two had osteogenic sarcomas and ten

had ovarian carcinomas. They each received a single injection of ^{131}I or ^{111}In-labeled 791T/36 antibody for the evaluation of its diagnostic potential. Patient imaging was carried out as described previously (*3,11,14,15*). Eleven patients, nine with ovarian carcinoma and two with colon carcinoma received labeled antibody for a second time between 3 wk and 17 mo after the initial injection. One of the patients with ovarian carcinoma received a third injection within 6 mo of the first administration.

Antibody, radiolabeling, and administration

The preparation, purification, ^{131}I and ^{111}In-labeling and clinical administration of the 791T/36 antibody and its Fab fragment have been described previously (*3,11,16,17*). Briefly iodine labeling was carried out using an iodogen technique to introduce ^{131}I directly into tyrosine residues of the antibody protein (*11*). Indium labeling was carried out by chelation to diethylenetriaminepentaacetic acid (DTPA) conjugated to the antibody by reaction with DTPA anhydride (*17*). Patients received 200 μg or 1 mg 791T/36 antibody or 200 μg Fab labeled with ^{131}I (70 MBq) or 1 mg labeled with ^{111}In (70 MBq) intravenously. Prior to i.v. administration all patients received a skin test with one-tenth of the antibody dose and were observed for 30 min. No patient showed reaction to the test dose. Aliquots of the administered preparations were retained for radioactivity counting.

Imaging

Imaging studies were carried out 48–72 hr after injection of radiolabeled antibody using a 40-cm field-of-view gamma camera fitted with a high-energy collimator (400 keV maximum) for studies of ^{131}I-labeled antibody and a medium energy collimator (300 keV maximum) for studies of ^{111}In-labeled antibody. Blood-pool subtraction was undertaken with studies using ^{131}I-labeled antibody using techetium-99m- (^{99m}Tc) labeled red blood cells and free pertechnetate (*18*).

Preparation of plasma and blood cells

Blood samples were taken immediately before antibody injection, within 5 min after, and subsequently at intervals of up to 10 mo. Samples were anti-coagulated with lithium heparin. Blood taken after 1–7 days was counted for radioactivity. Plasma was prepared from all blood samples by centrifugation and stored at −20°C.

In some cases blood was fractionated into plasma, mononuclear cells, granulocytes and red cells by density gradient centrifugation on Mono-Poly resolving medium.* With each blood sample duplicates of 4 ml of whole blood was layered onto 3 ml of medium in 13 × 100 mm conical based centrifuge tubes and centrifuged at 300 × *g* for 40 min. The upper plasma layer was drawn off with a Pasteur pipette. The two distinct white cell layers, the upper containing mononuclear cells and platelets and the lower granulocytes were each drawn off separately. These two cell suspensions, and the lower blood cell pellet, were each washed three times by centrifugation in phosphate buffered saline (PBS). Washings were added to plasma, and the plasma, granulocyte and mononuclear cell preparations counted for radioactivity. Total input count rates of ^{131}I or ^{111}In in four analyses were 1.3×10^5, 1.2×10^5, 2.7×10^5, and 3.0×10^5 cpm. Total recovery of radioactivity in relation to the count rate in the initial blood was 94–98%. Counts in each fraction were expressed as a percentage of the total recovered counts.

Precipitation of radiolabeled antibody and plasma-borne radiolabels

The proportion of plasma-borne radiolabel associated with protein was determined by precipitation of total protein with an equal volume of 10% w/v trichloracetic acid (TCA) (*16*). The precipitates forming overnight at 4°C were washed three times in 10% TCA before counting.

The proportion of radiolabel associated with mouse IgG was determined by precipitation with a rabbit anti-mouse IgG antiserum (*16,17*). Briefly 100 μl plasma was mixed with 20 μl mouse serum and a range of volumes of antisera (50, 100, 200, 500 μl). Precipitates forming overnight at 4°C were sedimented by centrifugation and washed three times in PBS before counting. Results are given as maximum precipitation achieved at the peak of the titration.

Cell binding assays with radiolabeled antibody and plasma-borne radioactivity

The ability of radiolabeled antibody preparations, radiolabeled antibody added to patients' plasma, and plasma-borne radioactivity to bind to 791T/36 defined antigen was assessed in cell-binding assays against antigen positive 791T cells and antigen negative HCT-8 cells (*17*). Briefly, 2×10^5 cells in 0.5 ml Hanks Balanced Salt Solution with 2% calf serum were incubated with 0.5 ml/aliquots of tenfold serial dilutions of labeled antibody or twofold dilutions of plasma for 4 hr at 4°C with constant agitation. Cells were sedimented by centrifugation, 0.8 ml of supernatant removed and counted for radioactivity. The activity in the cell pellet and remaining 0.2 ml of supernatant was also counted. The proportion of total count rates in each sample bound to the cell pellet was calculated and results are given as maximum binding achieved in the plateau region of the titration.

Gel filtration of radiolabeled antibody and plasma borne radioactivity

Radiolabeled antibody was added to normal human plasma and to plasma from patients prior to radiola-

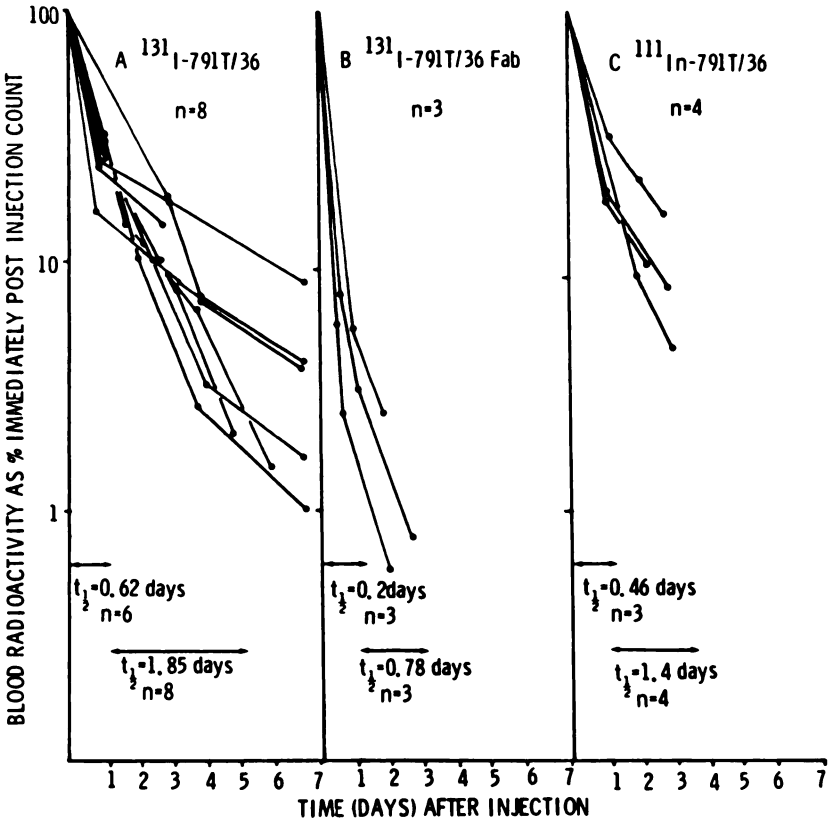

FIGURE 1
Blood survival of radiolabeled 791T/36 and its Fab fragment. Blood samples were taken within 5 min of injection of labeled antibody and subsequently at intervals shown. Patients had colo-rectal carcinoma

beled antibody injection at a concentration of 25 ng/ml and 1 ml passed through a Sephacryl S.300 column (90 × 1.5 m) eluted in PBS at a flow rate of 15 ml/hr (*16,17*). Optical density and radioactivity count rates were determined for 2 ml fractions. Plasma from patients having received radiolabeled antibody 2 to 3 days previously was similarly examined but without the addition of radiolabeled antibody.

Detection of anti-mouse IgG antibody

Iodine-131-labeled 791T/36 antibody was added to plasma (25 ng/ml) and 1 ml run on S300 as described above in either PBS or pH3 0.1*M* citrate phosphate buffer. The proportions of the total eluted radioactive count rates present in the three protein peaks of the plasma was calculated.

RESULTS

Blood levels of radiolabels

Blood samples were taken from eight colo-rectal carcinoma patients who had received 200 μg of ^{131}I-labeled antibody and counted for radioactivity. Immediately after administration 0.044 ± 0.003% of the administered radioactive dose was present/ml blood. Expressed as a proportion of the initial ^{131}I count rate the average level of blood radioactivity after 24 hr was 27% (n = 6), representing an initial blood half-life of 0.62 days (Fig. 1A). After this the blood levels declined more slowly, with an average half-life of 1.85 ± 0.30 days (n = 8). One patient given 1 mg of ^{131}I-labeled antibody had a blood level of 25% of the postinfusion value at 24 hr, and this then declined with a 2.0 day half-life. Patients who had received ^{131}I-Fab fragments had a 0.038 ± 0.009% of the dose/ml of blood immediately after injection but there was a rapid loss of ^{131}I, the initial drop having a 0.20 ± 0.05 (n = 3) day half-life, followed by a decline with a 0.78 ± 0.16 day half-life (Fig 1B). Patients with colo-rectal carcinoma receiving ^{111}In-labeled intact antibody showed blood levels of 0.040 ± 0.003% of the injected dose/ml blood immediately after injection. Blood levels then fell with a half-life of 0.46 ± 0.06 days with a subsequent decline with a 1.4 ± 0.23 day half-life (Fig 1C).

Patients images showed that the uptake of radioactivity into normal tissues relative to the blood was greater with ^{111}In-antibody than with ^{131}I-antibody. This resulted in a relative increased clearance of circulating ^{111}In-antibody as shown in Fig. 2A,B.

Characterization of blood-borne radioactivity

The proportion of blood-borne radioactivity associated with plasma and cellular elements was determined 2–3 days after ^{131}I or ^{111}In-labeled intact antibody infusion by separation of plasma, mononuclear cells, granulocytes and erythrocytes. Three patients, two with colo-rectal carcinomas and one with osteogenic

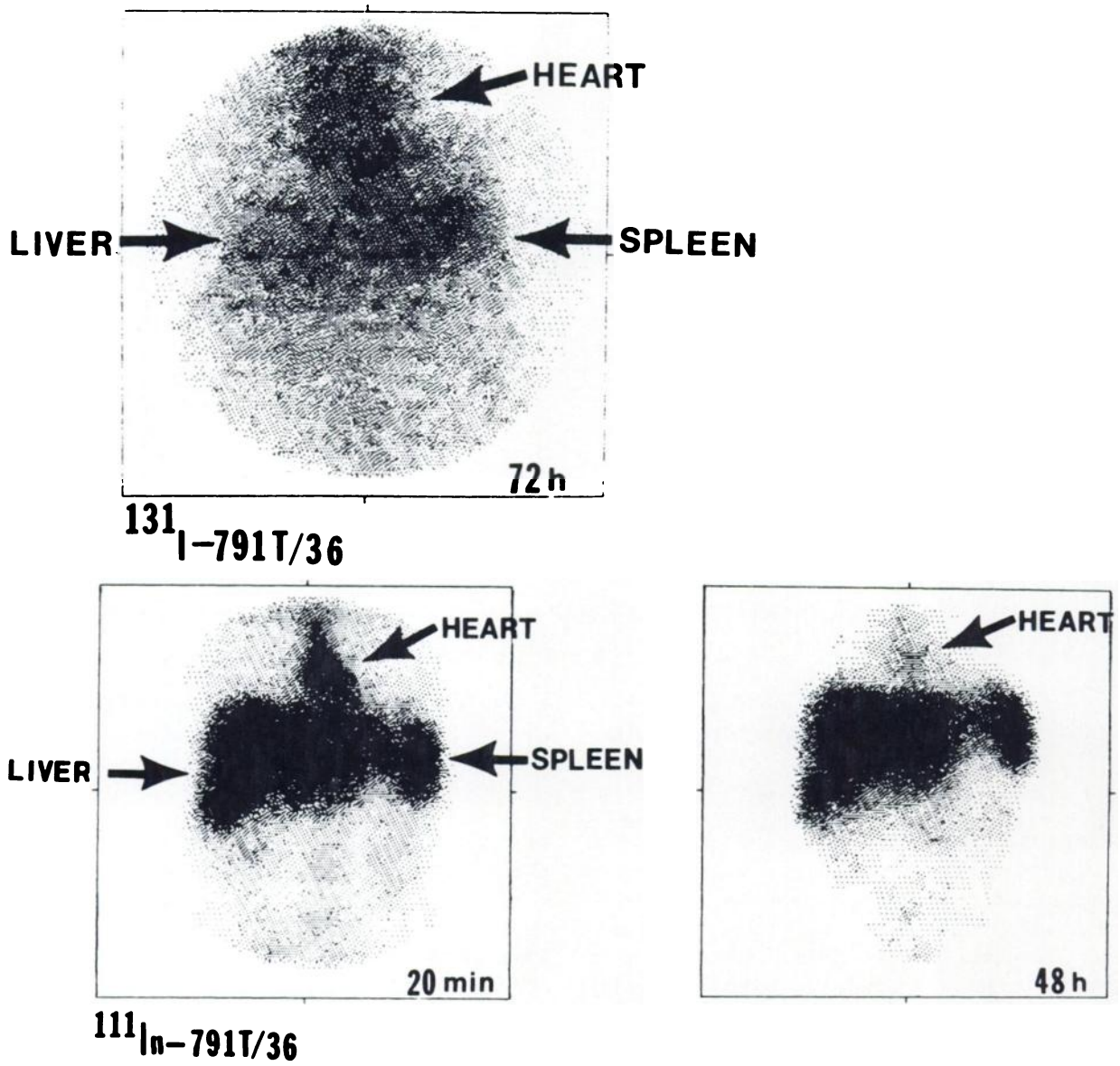

FIGURE 2
Upper: Anterior view of upper abdomen and thorax of 75-yr-old female patient with primary carcinoma of colon, 72 hr after administration of ^{131}I-791T/36. Liver was found to be free of disease at time of investigation. High levels of circulating activity can be seen in heart region together with activity throughout liver and spleen. Tumor uptake cannot be seen without blood pool subtraction. Lower: Anterior view of upper abdomen and thorax of 61-yr-old female patient with suspicion of recurrent colon cancer, 20 min and 48 hr after injection of ^{111}In-791T/36. Liver was found to be free of disease at time of investigation and tumor is not shown. Liver and spleen show high uptake. Relative clearance of circulating activity on delayed view can be seen in heart region

sarcoma had received a first injection of labeled antibody. One patient with colo-rectal carcinoma had received a second imaging dose of antibody 17 mo after his first. In all patients the majority (mean 88.2%, n = 3) of radioactivity was associated with the plasma, the mononuclear cells and granulocytes having means of 0.9 and 0.4%, respectively (Fig. 3). Erythrocytes had a mean of 10.1% of radiolabel (Fig. 3). This erythrocyte radioactivity was not removed by repeated washing in PBS or in pH3 citrate phosphate buffer.

Gel filtration of plasma radioactivity

Sephacryl S300 gel filtration of ^{131}I or ^{111}In-labeled intact antibody mixed with normal human plasma showed the majority of radioactivity in the second protein peak (Fig. 4). Plasma taken from patients with colo-rectal carcinoma 2 days after injection of each of these radio-labeled antibody preparations showed profiles virtually identical to antibody added to normal plasma (Fig. 4). Examination of plasma from six further patients with colo-rectal carcinoma and one pa-

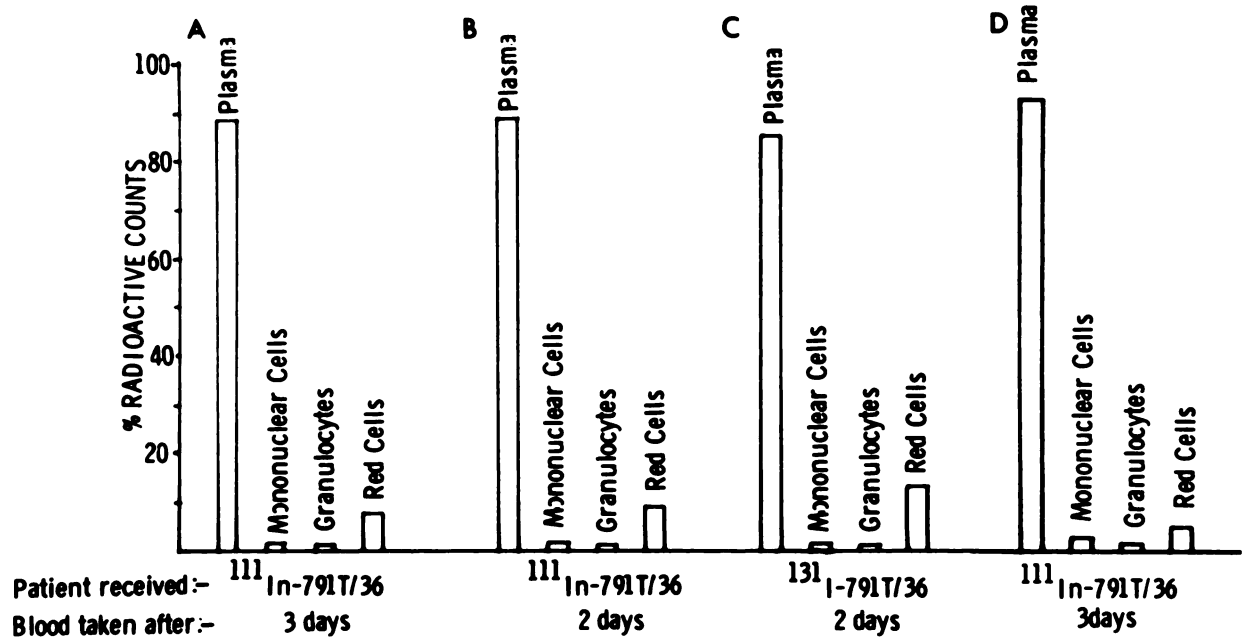

FIGURE 3
Blood distribution of radioactivity in four individual patients receiving radiolabeled 791T/36. Whole blood was fractionated as described in Patients, Materials, and Methods and radioactivity in each of the fractions expressed as proportion of recovered counts. Patients A, B, C had colo-rectal carcinoma. Patient 4 had osteogenic sarcoma. Patient B had received initial dose of antibody 17 mo previously

tient with ovarian carcinoma receiving ^{131}I-antibody, and four patients with colo-rectal carcinoma and one with osteogenic sarcoma given ^{111}In-antibody showed similar profiles, with no radioactivity present in higher or lower molecular weight forms than that of IgG.

Precipitation of plasma radioactivity

Precipitation with 5% trichloracetic acid showed 91 ± 4.7% (n = 9) of the plasma-borne radiolabel to be protein bound with blood samples taken 1–7 days after ^{131}I-antibody infusion (total nine samples, from three

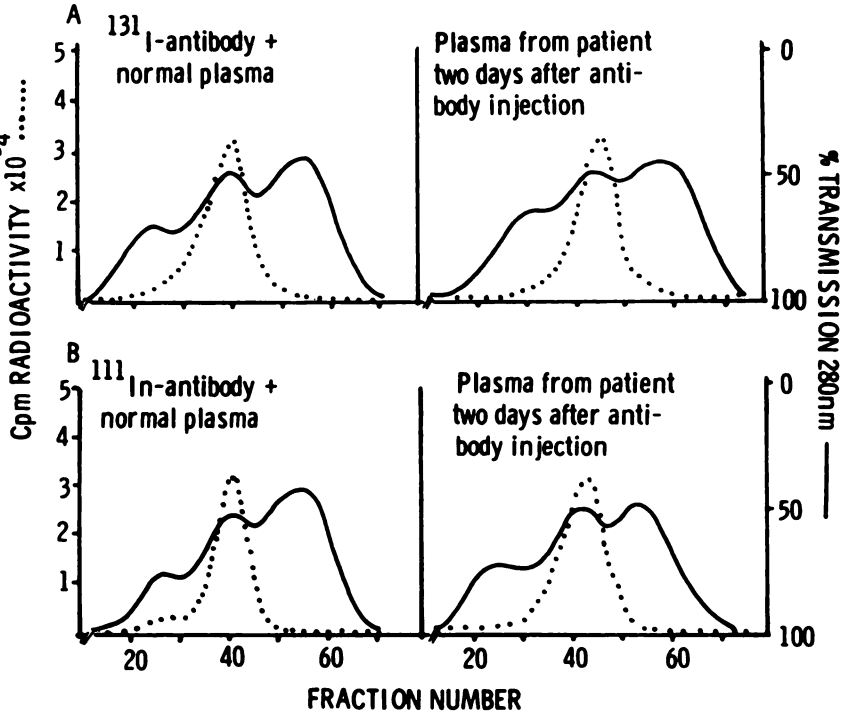

FIGURE 4
Sephacryl S300 gel filtration of radiolabeled 791T/36 and plasma-borne radiolabels. Labeled antibody was added to normal human plasma (25 ng/ml) and in addition plasma was collected from colo-rectal carcinoma patients injected 2 days previously with same labeled preparations

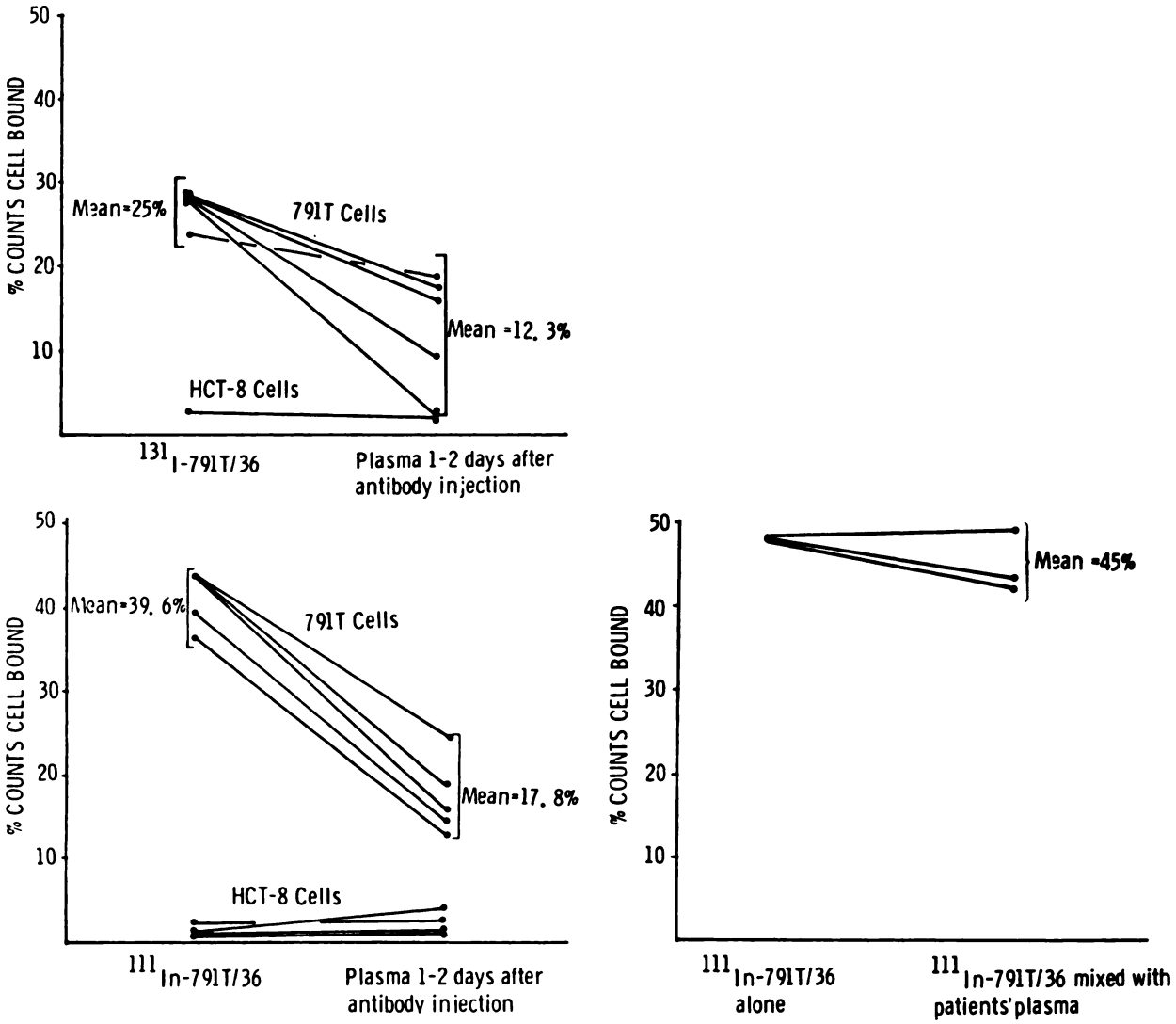

FIGURE 5
Binding of radiolabeled 791T/36 antibody to antigen positive 791T cells and antigen negative HCT-8 cells. Upper: Five individual ^{131}I-791T/36 preparations and plasma from five colo-rectal carcinoma patients injected 1 to 2 days previously with 200μg of these preparations. Lower left: Three individual ^{111}In-791T/36 preparations and plasma from five patients injected with them. Three of these plasma samples came from the same patients shown lower right. Lower right: One ^{111}In-791T/36 preparation alone or mixed at 50 ng/ml with plasma from three patients who were to be injected with it

colo-rectal carcinoma patients). Rabbit anti-mouse IgG antiserum precipitated 89.3 ± 5.0% (n = 6) of radioactive counts in plasma of colo-rectal carcinoma patients who had received ^{131}I-labeled-791T/36 1-4 days beforehand, and 88.6 ± 2.0% (n = 3) of radioactive counts in plasma of patients who had received ^{111}In-labeled antibody 1-2 days previously. These precipitation figures are comparable to those achieved with labeled preparations before injection (*16,17*).

Antigen binding activity of plasma-borne radiolabels

Iodine-131 and ^{111}In-labeled antibody prepared for clinical administration showed binding to 791T target cells but virtually no binding to antigen negative control cells (Fig 5A, B). Overall, the efficiency of binding with [^{111}In]antibody (mean 39.6%) was greater than that of the ^{131}I-labeled preparations (mean 25%). Radiolabels present in the plasma of patients with colo-rectal carcinoma, injected with these same preparations also showed binding activity against 791T cells (Fig 5A,B) although with reduced binding activity. In a further test, plasma taken from three of these patients before the injection of [^{111}In]antibody were added to [^{111}In]-antibody at a ratio of 50 ng of antibody/ml of plasma and tested for cell binding activity. Here there was virtually no reduction in binding activity (Fig 5C).

Detection of anti-mouse IgG antibody response

The development of anti-mouse IgG antibodies in patients was assessed by the degree of immune complex

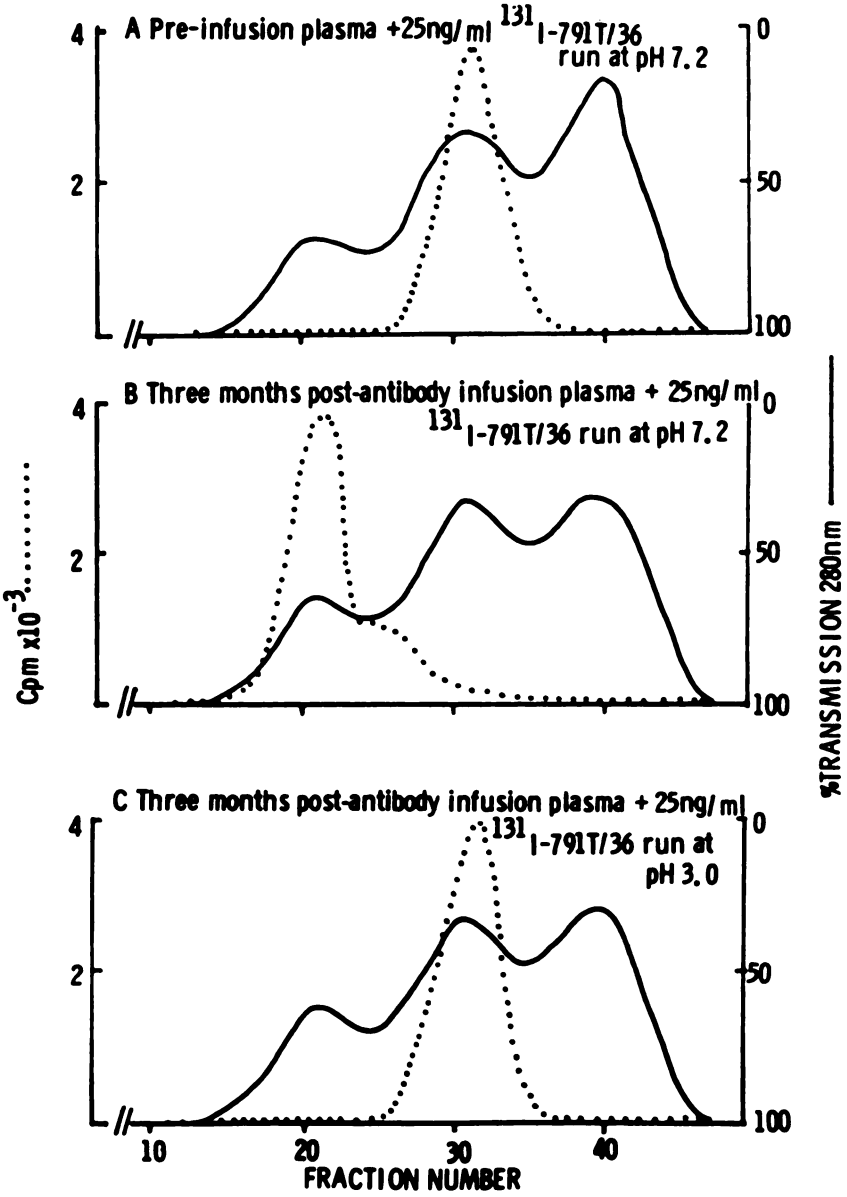

FIGURE 6
Development of anti-mouse IgG antibody in colo-rectal carcinoma patient infused with radiolabeled 791T/36. A: Patients pre-infusion sample mixed with labeled antibody. B: Plasma 3 mo after antibody injection mixed with labeled antibody. Note that radiolabeled antibody is now in higher molecular weight form. C: As B but column run in pH 3 citrate phosphate buffer. Complex formation is prevented

formation following addition of [^{131}I]antibody to plasma samples. Fig. 6 shows a typical result. ^{131}I-791T/36 added to the plasma of patients with colo-rectal carcinoma collected prior to injection of antibody and run on Sephacryl S300 showed radioactivity only in the second protein peak (Fig 6A). Labeled antibody added to plasma taken 3 mo after injection of labeled antibody showed radioactivity predominantly in the first protein peak, i.e., in a molecular weight higher than that of IgG (Fig. 6B). When the same mixture of labeled antibody and plasma was run at pH 3, radioactivity was again seen only in the second protein peak. Similar findings with post-antibody injection plasma run together with [^{131}I]antibody at both pH 7.2 and pH 3 were obtained in a further three patients.

Figure 7 shows a time course study of the levels of anti-mouse IgG antibodies in a further eight patients, seven with colo-rectal carcinoma and one with osteogenic sarcoma. Here the results are expressed as the proportion of the ^{131}I-791T/36 counts added to plasma which ran as complexes on Sephacryl S300. Anti-mouse IgG was detected by 7–10 days after 791T/36 antibody administration, continued to increase and was maintained for at least 120 days, the limit of observation. In most patients observed for such a long time period virtually all of the 25 ng/ml ^{131}I-791T/36 antibody added to plasma was in complex form on S300 filtration.

In one patient with colo-rectal carcinoma, a second injection of ^{131}I-antibody was administered for immunoscintigraphy 10 mo after the first. In this patient, a 10 mo postantibody infusion plasma sample complexed ^{131}I-791T/36 antibody added to it (Fig. 8B). Plasma from blood taken 2 hr after the second administration

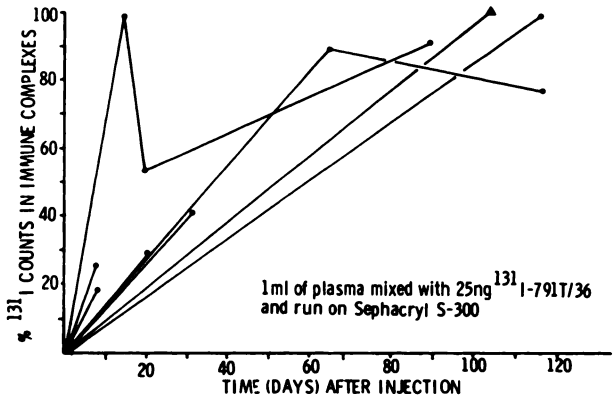

FIGURE 7
Time course of anti-mouse IgG antibody response in eight individual patients. Plasma was mixed with ^{131}I-791T/36 (25 ng/ml) and run on S300 as in Fig. 5. Proportion of radiolabel in complex form was calculated from the S300 protein and radioactivity count profiles. (●) Colo-rectal carcinoma patients; (▲) Osteogenic sarcoma patient

of ^{131}I-791T/36 antibody also showed virtually complete complex formation (Fig. 8C). In this particular case (a 63-yr-old female patient) the repeat imaging investigation successfully detected the site of a recurrent colon tumor in spite of the formation of these antibody-antibody complexes. However, other studies showed a reduction in image quality with serial investigations. For example, in one instance an endometrial carcinoma was successfuly localized in a 68-yr-old patient prior to surgery but two further investigations within a 6-mo period showed progressive uptake in the spleen which on the final occasion was so intense no other site of activity could be demonstrated (Fig. 9). This patient's plasma, after the third investigation, fully complexed ^{131}I-791T/36 added to it (25 ng antibody/ml plasma) as assessed by the Sephacryl S300 filtration technique. Figures 10 and 11 show the results of imaging studies of a patient with suspicion of recurrent mucinous adenocarcinoma of the right ovary. The first study (Fig. 10) demonstrated positive uptake in the pelvis indicating the presence of tumor. A follow-up study of this patient 3 mo later, to assess further the presence of recurrent disease, demonstrated increased uptake in the spleen with a reduction in the count rate from other areas of the body rendering the study technically unsatisfactory. Technically unsatisfactory images were obtained from three of seven other gynecological cancer patients having a second injection of labeled antibody.

DISCUSSION

Since effective tumor localization of radiolabeled antibody for diagnostic immunoscintigraphy depends upon the integrity of blood-borne antibody it is important to determine the efficiency of retention of active antibody in the circulation. Furthermore, since antibodies used for immunoscintigraphy are of rat or mouse origin, it is very likely that these will evoke antibody responses in patients, and this needs to be appreciated in the design of repeated or sequential imaging protocols.

With 791T/36 antibody the levels of blood radioactivity of ^{131}I or ^{111}In-labeled antibody and the Fab fragment were virtually identical immediately after a first injection, as would be expected. The blood levels of intact antibody fell sharply over the first 24 hr, presumably reflecting partly the loss by degradation and excretion of damaged antibody but mainly the extravasation of the remaining labeled antibody. Subsequently the average blood half-lives of the ^{131}I and ^{111}In radiolabels originally attached to intact antibody were 1.85 and 1.40 days. The majority of patients receiving ^{131}I-labeled antibody had 200 μg of antibody, while those given the ^{111}In labeled preparation received 1 mg. Discretion should be used when comparing the biodistribution of different doses of monoclonal antibody, since there is increasing evidence of a mass effect on tumor detection with radiolabeled antibodies (*19*). However, the one patient given 1 mg of ^{131}I-labeled antibody showed blood levels and kinetics virtually identical to those given 200 μg and this indicates that mass effects were not detectable within these dose ranges. Additionally, work with other radiolabeled antibodies administered in doses of 1–20 mg have shown that the plasma half life was not antibody dose dependent (*19*). Extrapolation from data on blood levels and rate of blood clearance (*20*) after the first 24-hr period with both ^{111}In and ^{131}I-labeled antibody, indicates that ~43% of the surviving antibody remaining is in the intravascular compartment and this is comparable to finding in man with normal human IgG (*20*). There is little information on kinetics of blood survival of other radiolabeled monoclonal antibodies, although with an anti-melanoma antibody Larson et al.(*7*) reported an average blood half-life of 31 hr, and Epenetos et al. (*4*) showed an anti-milk fat globule antibody to have a half-life range of 13–21 hr. With the 791T/36 Fab fragment there was a more rapid loss from the blood, as expected, since Fab fragments will extravasate more quickly than intact antibody and undergo rapid urinary excretion (*21*). From the limited data with the fragment, the initial half-life was calculated to be 0.20 days and subsequently as 0.78 days, but Larson et al. (*5*) reported a blood half-life as low as 83 min with an anti-melanoma antibody Fab fragment. Extrapolation from the limited amount of data on the 791T/36 Fab fragment indicates intravascular retention of 18% of the surviving material. The efficiency of tumor imaging with labeled 791T/36 Fab fragment has been poor compared with intact antibody (*15*). This could be due partly to its rapid clearance. However other antibody Fab fragments have

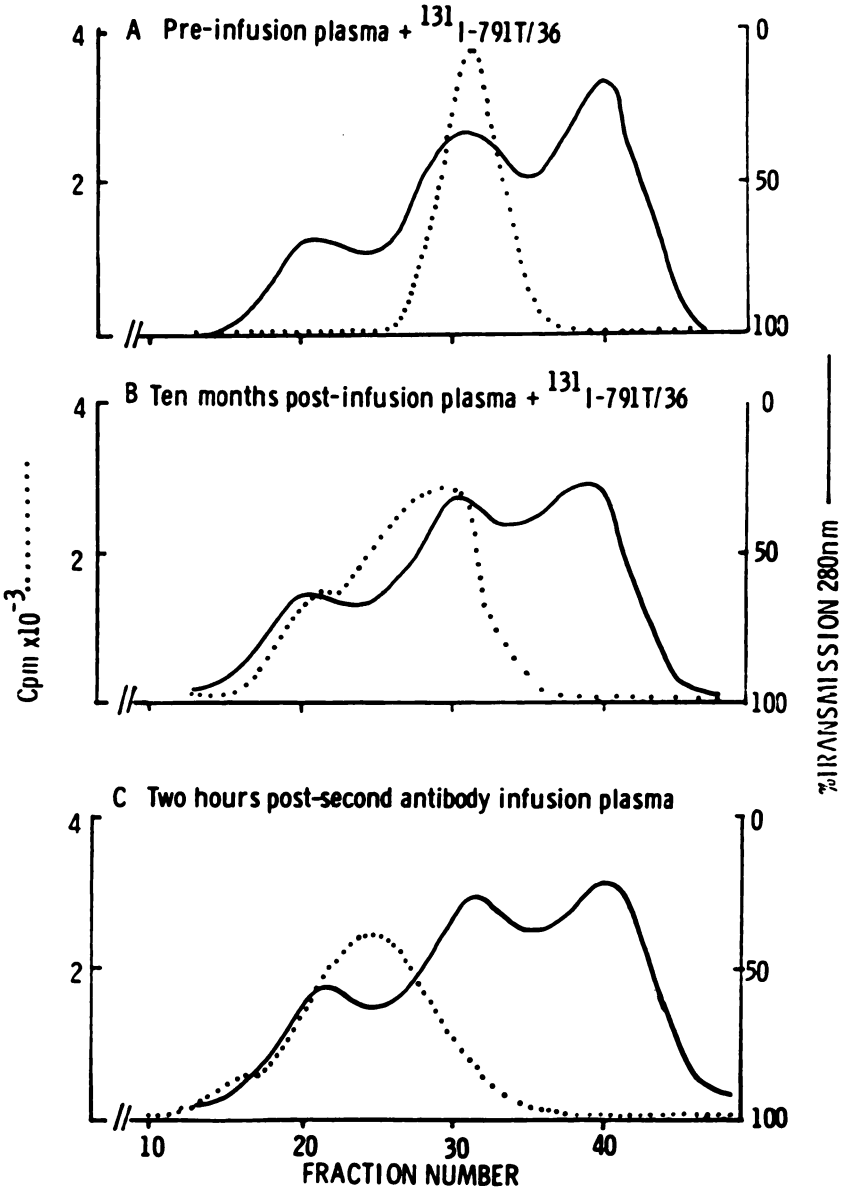

FIGURE 8
Formation of anti-mouse IgG-791T/36 complexes in blood of colo-rectal carcinoma patient given ^{131}I-791T/36 for a second time. A: Pre-first infusion plasma mixed with ^{131}I-791T/36. B: 10 mo postinfusion plasma mixed with same ^{131}I-791T/36 preparation showing complex formation. C: Plasma 2 hr after second i.v. administration of same ^{131}I-791T/36 antibody preparation as B, showing plasma-borne radiolabel in complex form

been efficient for imaging (*5*) and their rapid blood and body clearance improved discrimination between tumor and normal tissues.

With intact antibody, although the absolute clearance of both ^{131}I and ^{111}In-radiolabeled antibody were similar, patient images showed that the relative uptake of radioactivity into normal tissues was greater with [^{111}In]antibody. This is similar to previous results from imaging and dissection analysis of tumor xenografts in mice (*17*). Clinically this results in a relative increased clearance of ^{111}In-antibody and enables image interpretation without the use of blood-pool subtraction techniques.

Blood samples from patients receiving a first, or second injection of 791T/36 antibody showed that the circulating radiolabel was associated predominantly with plasma rather than cellular elements. There was no significant binding to mononuclear cells or granulocytes. Other monoclonal antibodies, especially those to some CEA epitopes which are shared with granulocyte glycoproteins, have shown granulocyte binding (*22*) and clearly this may be clinically detrimental, as well as influencing the efficiency of imaging. The low binding to red cells seen in the present study is not in keeping with earlier studies which showed no binding of 791T/36 to erythrocytes in vitro (*23*). However, it is unlikely that this in vivo binding reflects antigen-antibody interaction since bound antibody was not eluted at low pH.

Plasma-borne radiolabel was associated predominantly with mouse antibody after the first injection of labeled antibody as demonstrated by gel filtration and precipitation assays. Significantly there were no readily detectable antigen-antibody complexes in the plasma obtained following the first injection of antibody. Thus,

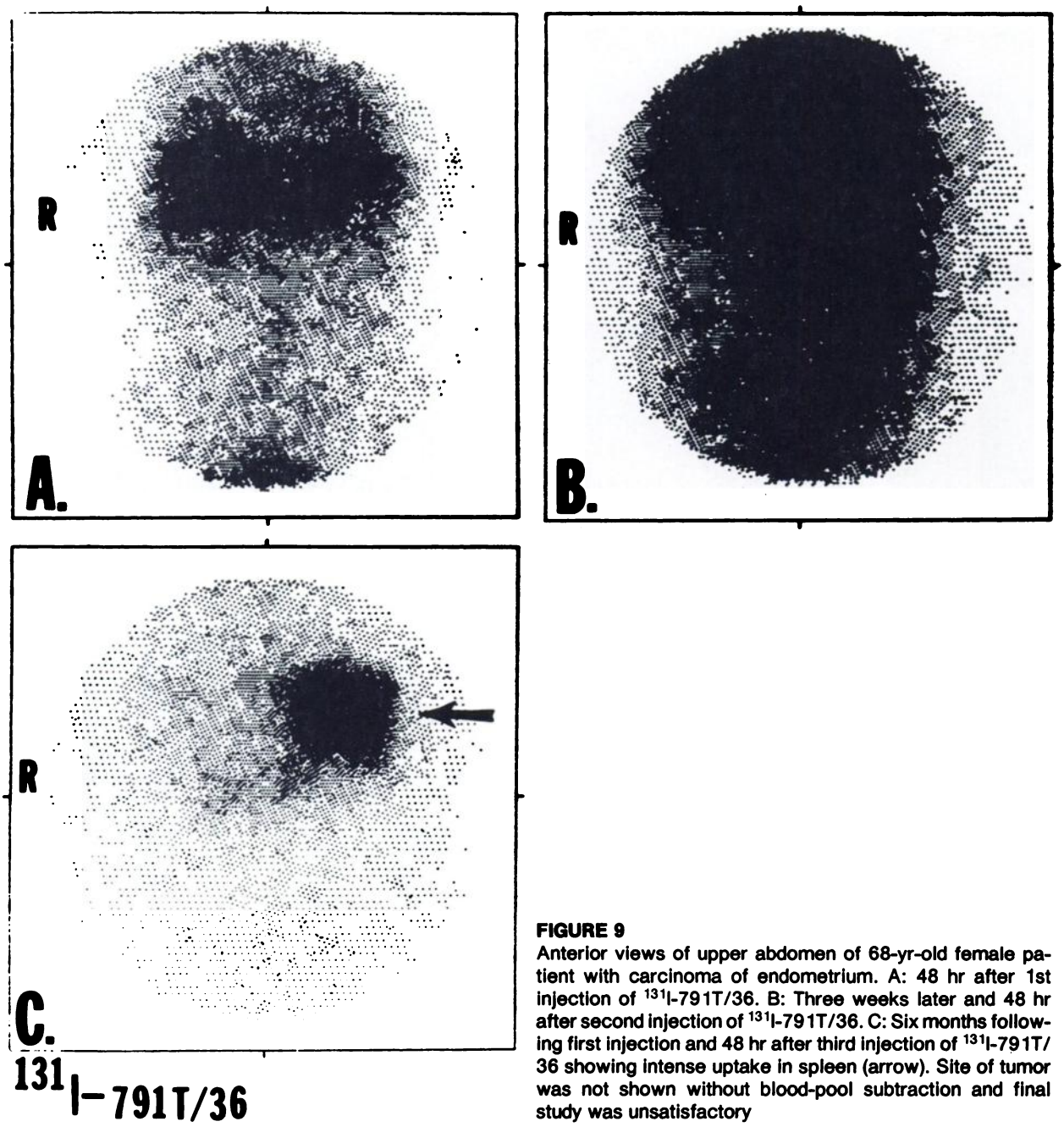

FIGURE 9
Anterior views of upper abdomen of 68-yr-old female patient with carcinoma of endometrium. A: 48 hr after 1st injection of ^{131}I-791T/36. B: Three weeks later and 48 hr after second injection of ^{131}I-791T/36. C: Six months following first injection and 48 hr after third injection of ^{131}I-791T/36 showing intense uptake in spleen (arrow). Site of tumor was not shown without blood-pool subtraction and final study was unsatisfactory

gel filtration showed plasma radioactivity only in the IgG containing protein peak, 2 to 3 days after injection, with no higher molecular weight components. However if some complexes had formed immediately after injection they might have cleared at this time and circulating complexes have been reported in other studies, particularly with heterologous antibodies against CEA (*24*), but there are little or no other data with monoclonal antibodies to either CEA or other tumor associated antigens.

Cell binding assays also failed to detect plasmaborne 791T/36 defined antigen. Thus labeled antibody added to patients' plasma in the concentration typically present 1–2 days after clinical administration bound as efficiently to target cells as antibody alone. Plasmaborne radiolabel also bound to target cells expressing the 791T/36 defined antigen, but not to antigen negative cells. However, this binding showed reduced efficiency and since this is probably not due to neutralization by circulating antigen, indicates that radiolabeled antibody, although retaining its structural integrity, is undergoing some loss of immunological reactivity. Another point to emerge from these binding assays is the level of immunological activity of the ^{111}In the ^{131}I-labeled antibody. Iodine-131 preparations showed overall only 25% binding, and this figure is comparable

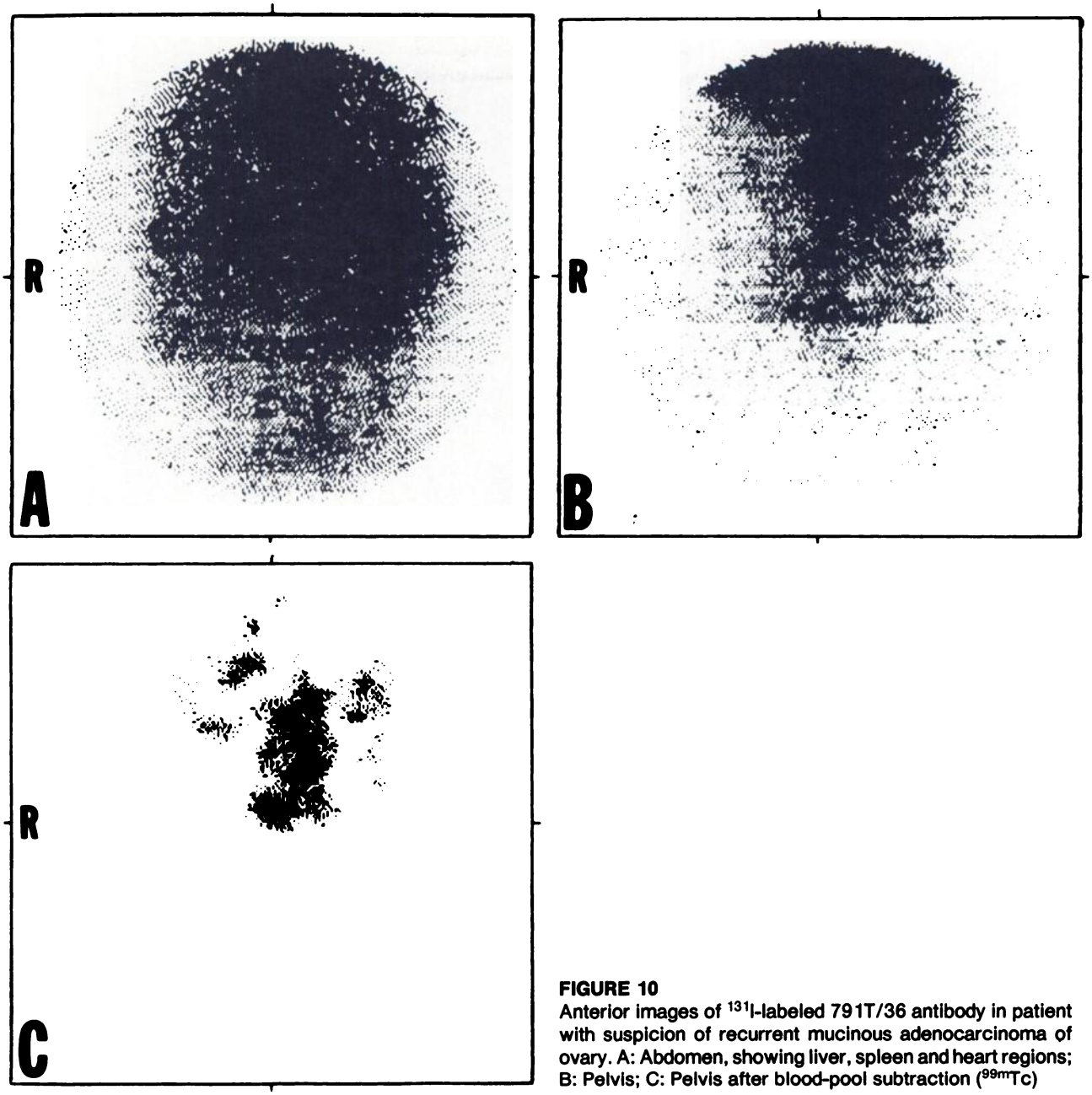

FIGURE 10
Anterior images of ^{131}I-labeled 791T/36 antibody in patient with suspicion of recurrent mucinous adenocarcinoma of ovary. A: Abdomen, showing liver, spleen and heart regions; B: Pelvis; C: Pelvis after blood-pool subtraction (^{99m}Tc)

to that seen with other ^{131}I monoclonal antibodies for clinical imaging, for example, 25–30% binding with anti-melanoma body (*7*), 15–50% with its Fab fragment (*5*) and 16–28% with anti-colon carcinoma antibody (*9*). These low levels of binding activity may reflect co-purification of IgG without antibody activity from ascites fluid and/or inactivation of antibody, during purification, storage or radiolabelling. In the latter context, ^{111}In-labeled 791T/36 was considerably more efficient at cell binding than ^{131}I-labeled antibody, indicating that iodination of antibody, even at the low specific activities used (about 200 MBq/mg), does significantly reduce its activity. Similar findings have been reported with other antibodies (*10*) and emphasize the superiority of ^{111}In rather than ^{131}I as a radiolabel for immunoscintigraphy, in addition to its advantageous physical characteristics (*17*) except for detection of liver deposits where there will be elevated levels of ^{111}In. The 40–50% antigen binding activity of ^{111}In-labeled 791T/36 is comparable to that achieved with other monoclonal antibodies labeled with metallic radionuclides by way of chelating agents (*25*).

A number of studies have reported the development of anti-mouse IgG antibodies in patients receiving monoclonal antibodies for diagnostic or therapeutic purposes, although generally this has been examined in patients receiving repeated and/or large doses of antibody (*7,26,27*). One effect of these anti-mouse IgG

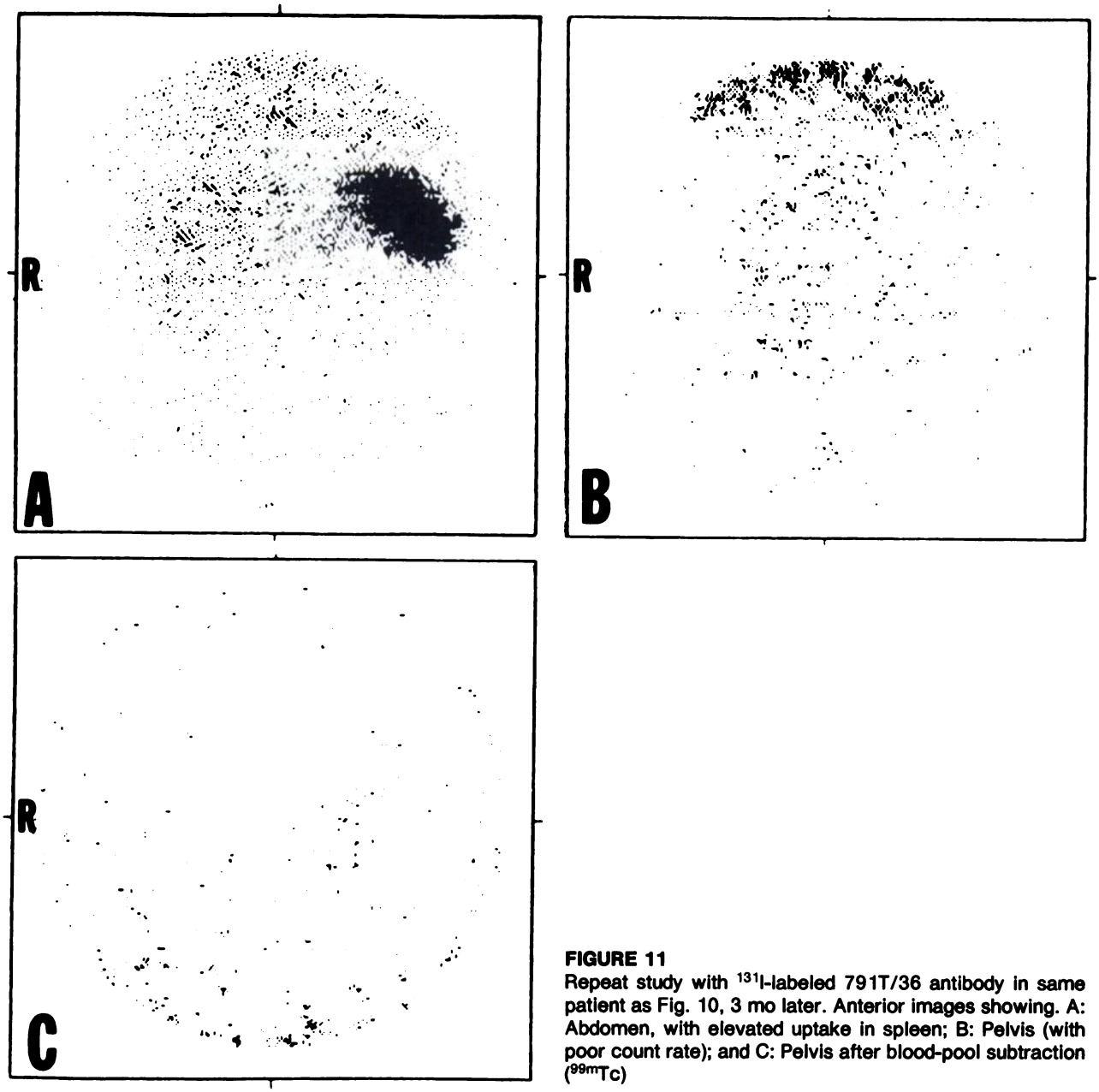

FIGURE 11
Repeat study with ^{131}I-labeled 791T/36 antibody in same patient as Fig. 10, 3 mo later. Anterior images showing. A: Abdomen, with elevated uptake in spleen; B: Pelvis (with poor count rate); and C: Pelvis after blood-pool subtraction (^{99m}Tc)

antibodies can be rapid clearance of a second administration of the same radiolabeled antibody for imaging (*26*). The present study was designed to detect the formation of mouse-IgG-anti-mouse IgG complexes in patients' plasma containing monoclonal antibody at concentrations likely to be attained following administration of labeled antibody for imaging. In these circumstances in vitro analysis showed the formation of complexes when radiolabeled antibody was added to plasma of patients after they had received an imaging dose of labeled antibody. These complexes were sensitive to cleavage at low pH and were not formed with plasma from the same patients before the administration of 791T/36 antibody indicating that they are almost certainly antibody-antibody complexes, although the precise size of the complexes has not yet been determined, nor the class of human antibody involved. In some studies anti-antibody production has been reported to be only transient, soon becoming undetectable (*27*), but in the present study anti-mouse antibody was detected for at least 10 mo after a single injection of radiolabeled 791T/36 antibody. It is possible that in vivo formation of antibody-antibody complexes could interfere with sequential imaging studies (*7*) even when carried out several months after the initial investigation. Such in vivo formation of complexes was seen in one patient examined 10 mo after the first injection of antibody. Although imaging of tumor was successful in this one particular patient, repeated imaging studies in other patients, with gynecological tumors, have demon-

strated reduced image quality (Fig. 11). Overall the implication is that the anti-mouse antibody response may be a serious limitation for repeat diagnostic imaging.

FOOTNOTE

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