Melanoma Localization in Nude Mice with Monoclonal Fab Against p97

Paul L. Beaumier, Kenneth A. Krohn, Jorges A. Carrasquillo, Janet Eary, Ingegerd Hellström, Karl Erik Hellström, Wil B. Nelp, and Steven M. Larson

University of Washington School of Medicine, Oncogen, Seattle, WA; and Department of Nuclear Medicine, National Institutes of Health, Bethesda, Maryland

The tumor targeting capacity of monoclonal antibody Fab fragments was explored in nude mice bearing human melanoma xenografts. Radioiodinated Fab 8.2 and 96.5, specific for melanoma-associated antigen p97, were tested in vitro for immunoreactivity and in vivo for tumor localization relative to a co-administered control, Fab 1.4. Fab was cleared rapidly from the blood with a $T_{1/2}$ of 3–3.5 hr and >90% of the injected radioactivity was excreted by 16 hr. The mean specific Fab in tumor reached a maximum of 3.5% injected dose/g at 4 hr and decreased to 1.5% at 16 hr. Over the same period, the ratio of specific/control Fab in tumor normalized to blood, the localization index, rose from 3 to 25 compared with ratios near unity for all other tissues. The concentration of specific Fab in tumor could be correlated to the amount of Fab protein administered as well as its immunoreactivity.

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Disseminated malignant melanoma is a disease for which there is presently no effective therapy. The use of radiolabeled monoclonal antibodies (MAb) to melanoma-associated antigens may improve this situation. An understanding of the kinetics and tumor localizing properties of monoclonal antibodies and fragments is paramount to the clinical application of these agents.

MAb have been made to three cell surface antigens which are strongly expressed by human melanoma (*1*). One of these antigens, an oncofetal glycoprotein, p97, has been detected on the cell surface of 66 of 80 (83%) human melanoma biopsy specimens studies by immunohistology. Several murine MAb have been produced against p97, including 8.2 and 96.5 (*2*). Clinically promising radiopharmaceuticals were developed for diagnosis and therapy using Fab fragments of these antibodies labeled with iodine-131 (^{131}I) (*3*).

Fab fragments were chosen for clinical use because of their rapid tissue distribution and clearance and reduced immunogenicity relative to whole antibody. In conjunction with clinical trials in patients with disseminated melanoma (*4,5*), we performed laboratory studies in nude mice implanted with a human melanoma line which expressed p97 to characterize certain parameters affecting the tumor localization of intravenously administered anti-p97 Fab.

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For reprints contact: Paul L. Beaumier, PhD, Oncogen, 3005 First Ave., Seattle, WA 98121.

MATERIALS AND METHODS

Monoclonal antibody Fab fragments

Three murine monoclonal antibodies 8.2 (IgG_1), specific for epitope c of p97 (*2*), 96.5, (IgG_{2a}), specific for epitope a of p97 (*2*), and a control, 1.4, (IgG_1), which is specific for murine leukemia virus antigen gp70 but is not cross-reactive with p97, were prepared from ascitic fluid by protein A affinity chromatography as described previously (*2*). Since the NS-1 myeloma line was used in the generation of the MAb 96.5 producing hybridoma, ~15% of the MAb 96.5 had a nonspecific kappa light chain. However, MAb 8.2 was developed using the SP 2/0 fusion partner and was completely specific (*2*).

Fab fragments were prepared by papain digestion of intact antibody as described (*6*). Protein concentrations were determined by uv spectroscopy using extinction coefficients for a 1% solution at 280 nm of 14.0 for intact MAb (mol wt 150 kdalton) and 15.3 for Fab fragment (mol wt 50 kdalton) (*7*). The affinity constants for Fab 8.2 and 96.5 have been estimated at 1×10^{10} 1/mole (*6*).

Radioiodination

MAb or Fab fragments were labeled using the chloramine-T method. Protein (50 μg) was labeled with 0.5–1 mCi (for distribution studies) or with 2 mCi (for imaging studies) of $Na^{125}I$ or $Na^{131}I$ and 10 μg of freshly dissolved chloramine-T in 0.4 ml of 0.1*M* phos-

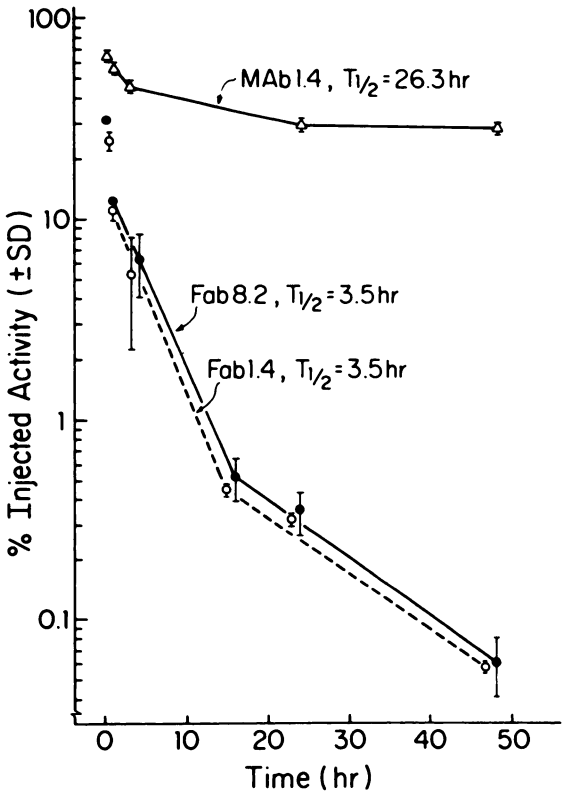

FIGURE 1
Mean percentages of injected activity in blood showing clearance of i.v. co-administered [^{125}I]Fab 8.2/[^{131}I]Fab 1.4 and of [^{131}I]MAb. For Fab, N = 3 at 0.25 hr and N = 5 at other time points. For MAb 1.4, N = 2 at all time points. Bars indicate range or ± s.d. Half-lives were determined over 1–16 hr for Fab and 1–24 hr for MAb using stripped data fitted by linear regression

phate buffer, pH 7.4, for 5 min on ice. The reaction was terminated by passage over a 5 ml Sephadex G-25 (superfine) column pretreated with 0.5 ml of heat-inactivated fetal calf serum (FCS) to minimize protein adsorption. The I/Fab molar ratio in product used for distribution studies was ~1:10 for both specific and control preparations.

Cell binding assay (CBA)

Freshly labeled and purified p97 specific Fab was tested for retention of immunoreactivity in vitro using a modification of a CBA described previously (*6*). Control Fab 1.4 showed only background binding. Adherent cultured SK MEL 28 cells were detached by trypsinization, suspended in Dulbecco's modified Eagle medium supplemented with 10% FCS, washed with phosphate buffered saline (PBS), and pelleted by centrifugation for 5 min at 800 *g*. About 2,000 cpm of labeled Fab (~1 ng) was diluted to 0.1 ml in FCS and mixed with 2 × 10^6 cells and incubated on ice for 15 min. The cells were washed in 5 ml of PBS, pelleted by centrifugation, and the cell-bound radioactivity was determined as a percentage of total added activity.

Animal model

Athymic nude mice, 16–22 g females, 1 mo old, were implanted subcutaneously with human melanoma, #2169, in 1 mm cubes in the right flank. This tumor line was derived from a nodule biopsied from a 65-yr-old woman with superficial, spreading, stage III melanoma and had been repetitively passaged in nude mice. The tumors were allowed to grow for 20 days to a weight of 50 to 200 mg and typically formed a solid white partially vascularized capsule. Two days before distribution studies were initiated, the mice were put on 0.5% (v/v) Lugol's iodine solution in the drinking water to reduce thyroid uptake.

Localization studies

The desired amounts of p97-specific Fab 8.2 or 96.5, labeled with ^{125}I, and control Fab 1.4, labeled with ^{131}I, were mixed to provide 1.5–6.5 μCi of each per injection aliquot. The protein doses of the co-administered Fab were adjusted and matched to the desired μg amount by the addition of unlabeled carrier Fab 8.2, Fab 96.5, or Fab 1.4. Nude mouse serum was sometimes added (10% v/v) as a protein carrier and the final volume was adjusted with PBS to give 100 or 200 μl per injection.

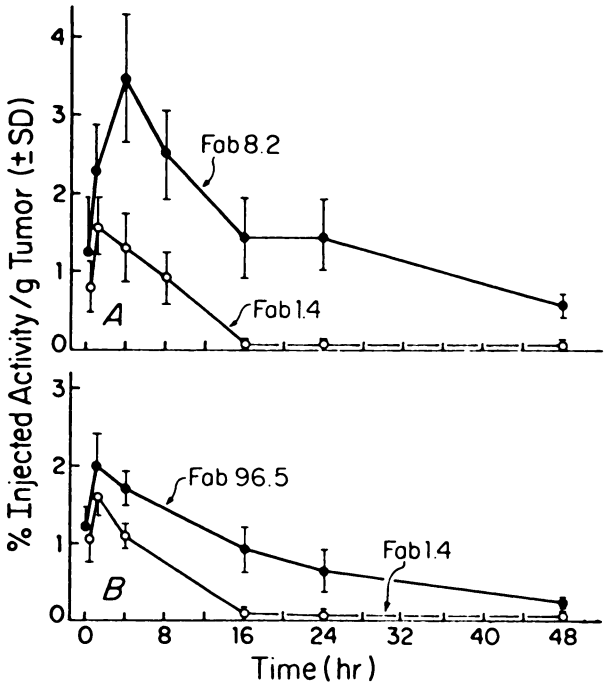

FIGURE 2
Comparison of means of injected activity/g in tumor for p97 specific Fab 8.2 versus co-administered control Fab 1.4 (A) and p97 specific Fab 96.5 compared with co-administered control Fab 1.4 (B). For A, N = 3 at 0.25 hr, N = 2 at 8 hr and N = 5 for all other time points. For B, N = 2 at 0.25 hr and N = 5 at all other time points. Bars indicate range or ± s.d. Mean specific Fab protein dose was 3.8 ± 0.4 μg/g body weight (~ 80 μg Fab 8.2/mouse) and 0.2 μg/g body weight (~ 5 μg Fab 96.5/mouse)

Replicate 10 μl aliquots of the injectant were prepared and counted as standards for the calculation of the total injected radioactivity.

The mice were lightly anesthetized with ether and intravenously injected through the tail vein, weighing the syringe before and after injection to determine the amount administered. At designated times after injection groups of mice (group size = N) were anesthetized, weighed, bled as completely as possible through the retro-orbital plexus, and killed by cervical dislocation. The mice were completely dissected and the following organs and tissues were isolated, rinsed with saline and blotted when appropriate: melanoma xenograft, blood, liver, kidney, spleen, heart, lungs, intestine, brain, stomach, lymph nodes (cervical, axial, and inguinal), pelvic fat, skin, tail, legs, and carcass/skull.

Samples were weighed then counted in a dual channel gamma counter. The sample weights and the corresponding cpm in the ^{125}I and ^{131}I windows were analyzed using a computer program (*8*). Data treatment included background cpm subtraction, calculation of activity in total blood assuming a blood volume of 8% of the body weight (*8*), correction for the ^{131}I Compton scatter into the ^{125}I window (about 11%), sample grouping, and statistical analysis. The activity retained per animal was determined from the sum of the cpm in all tissues and corrected blood. The difference between the retained activity and the injected dose (calculated from standards) determined the amount excreted.

Radioactivity excreted in the urine was analyzed by trichloroacetic acid (TCA) protein precipitation to determine the fraction that was protein bound. To 10 μl of urine was added 30 μl of carrier protein (FCS) and 1 ml of 10% TCA. The sample was mixed, incubated for 10 min at room temperature, and centrifuged at 8,800 *g* for 5 min. The percentage of protein-bound radioactivity (cpm in pellet × 100/cpm in sample) was calculated.

TABLE 1 A
Biodistribution of Co-administered Fab Fragments Expressed as Mean Percent Injected Dose per g Specific [^{125}I]Fab 8.2^{125}I*

	Time (hr)						
	0.25	1	4	8	16	24	48
	No. mice (N)						
Tissue	3	5	5	2	5	5	5
Tumor	0.7	2.2	3.5	2.3	1.3	1.5	0.5
s.d.	0.5	0.6	0.8	0.7	0.7	0.5	0.1
Blood	20.4	6.7	3.7	2.6	0.3	0.2	0.0
s.d.	1.6	0.4	1.8	0.5	0.1	0.0	0.0
Liver	4.3	2.2	1.6	1.2	0.2	0.2	0.0
s.d.	0.4	0.4	0.6	0.2	0.0	0.0	0.0
Kidneys	94.5	75.6	21.0	22.3	3.4	0.8	0.2
s.d.	9.5	23.5	6.7	0.8	2.1	0.1	0.3
Spleen	2.6	2.2	1.5	1.2	0.2	0.2	0.0
s.d.	0.7	0.7	0.2	0.3	0.1	0.1	0.0
Heart	4.4	2.8	1.5	0.5	0.1	0.1	0.0
s.d.	0.9	0.3	0.5	0.1	0.1	0.1	0.0
Lungs	5.6	4.0	2.6	1.3	0.2	0.2	0.0
s.d.	0.3	1.1	0.9	0.1	0.1	0.1	0.0
Stomach	1.9	8.5	11.1	13.8	0.5	0.9	0.1
s.d.	0.8	4.9	4.4	0.7	0.3	0.6	0.1
Lymph nodes	1.2	3.1	2.1	1.0	0.2	0.1	0.0
s.d.	1.1	0.7	0.5	0.7	0.1	0.1	0.0
Intestine	1.8	1.7	1.4	1.8	0.2	0.1	0.0
s.d.	0.1	0.4	0.2	1.2	0.1	0.1	0.0
Legs	1.3	1.3	1.1	0.9	0.1	0.1	0.0
s.d.	0.4	0.4	0.1	0.2	0.0	0.1	0.0
Carcass	2.1	1.5	1.4	2.3	0.5	0.6	0.0
s.d.	0.3	0.3	0.2	0.1	0.2	0.5	0.0
%D excreted	5.2	24.1	50.5	39.0	82.6	87.0	92.4
s.d.	9.1	8.8	4.9	6.6	4.4	4.2	0.2
Tumor/blood	0.0	0.3	1.1	0.9	4.5	7.5	18.3
s.d.	0.0	0.1	0.6	0.1	2.1	1.9	4.5

* Mean mouse body weight: 22.1 ± 2.5 g; mean tumor weight: 97.5 ± 62.6 g; Fab protein: 3.8 ± 0.4 μg/g body weight (~80 μg/mouse).

TABLE 1 B
Biodistribution of Co-Administered Fab Fragments Expressed as Mean Percent Injected Dose per g Control [^{131}I]Fab 1.4*

	Time (hr)						
	0.25	1	4	8	16	24	48
	No. mice (N)						
Tissue	3	5	5	2	5	5	5
Tumor	0.8	1.6	1.3	1.0	0.1	0.1	0.0
s.d.	0.3	0.4	0.4	0.3	0.0	0.0	0.0
Blood	16.1	6.1	3.2	2.4	0.2	0.2	0.0
s.d.	2.2	0.4	2.3	0.7	0.1	0.0	0.0
Liver	3.8	2.3	1.4	1.0	0.1	0.1	0.0
s.d.	0.3	0.3	1.1	0.3	0.0	0.0	0.0
Kidneys	135.3	74.1	13.4	6.9	0.8	0.4	0.1
s.d.	14.1	27.5	8.2	0.0	0.6	0.1	0.0
Spleen	2.6	2.1	1.2	1.3	0.1	0.1	0.0
s.d.	0.8	0.5	0.5	0.2	0.0	0.0	0.0
Heart	4.1	2.4	1.2	1.0	0.1	0.1	0.0
s.d.	0.8	0.2	0.6	0.2	0.1	0.0	0.0
Lungs	5.0	3.7	2.3	1.7	0.1	0.0	0.0
s.d.	0.1	0.7	1.3	0.3	0.1	0.0	0.0
Stomach	1.7	13.5	14.0	14.0	0.4	0.6	0.1
s.d.	0.4	7.2	4.5	0.7	0.3	0.6	0.1
Lymph nodes	2.9	3.1	1.9	1.5	1.3	0.1	0.0
s.d.	1.6	0.6	0.8	0.2	2.6	0.1	0.0
Intestine	1.6	1.9	1.3	1.8	0.1	0.1	0.0
s.d.	1.3	0.4	0.4	1.2	0.0	0.0	0.0
Legs	1.2	1.5	1.1	0.8	0.1	0.1	0.0
s.d.	0.3	0.5	0.3	0.2	0.0	0.1	0.0
Carcass	1.8	1.7	1.5	2.6	0.5	0.7	0.0
s.d.	0.2	0.4	0.4	0.1	0.2	0.7	0.0
%D excreted	2.8	20.8	57.2	53.9	93.8	92.9	99.4
s.d.	4.8	9.9	9.7	6.7	1.5	6.0	0.2
Tumor/blood	0.0	0.3	0.5	0.4	0.4	0.3	1.2
s.d.	0.0	0.1	0.2	0.0	0.0	0.2	1.0

* Mean mouse body weight: 22.1 ± 2.5 g; mean tumor weight: 97.5 ± 62.6 mg; Fab protein dose: 3.8 ± 0.4 μg/g body weight (~80 μg/mouse.)

The concentration of both specific and control iodinated Fab in tissues were calculated as a percentage of the injected activity per gram tissue or per organ and mean ± s.d. determined. The disappearance of Fab and MAb from blood was analyzed using linear regression and a curve stripping method that subtracted the slow later clearance phase from the initial early phase (*9*). Specific compared with control Fab uptake in tumor and the tumor to blood ratio for co-administered Fabs were determined from tissue values for each mouse and then averaged. Tumor specific localization was evaluated using a localization index (LI) (*10*), defined as the ratio:

$$LI = \frac{\dfrac{\%\text{ injected specific Fab/g tumor}}{\%\text{ injected specific Fab/g blood}}}{\dfrac{\%\text{ injected control Fab/g tumor}}{\%\text{ injected control Fab/g blood}}}$$

Fab protein dose-dependent studies

Fab accumulation in tumor was examined over a dose range of 0.01–16 μg each Fab/g animal body weight (0.1–320 μg/mouse). Further studies with Fab 8.2 only were conducted at 4 hr. The amount of administered protein was normalized to mouse body weight and expressed as μg Fab protein/g body weight.

Imaging studies

Two mice, pretreated with Lugol's iodine solution, were injected intravenously with ~0.5 mCi, 25 μg/mouse of [^{125}I]Fab. One mouse received Fab 96.5, (CBA = 75%) and a second mouse received control Fab 1.4. At 24 and 48 hr, the mice were anesthetized with 1:20 Nembutal IP, immobilized, and imaged with a gamma camera fitted with a pinhole collimator.

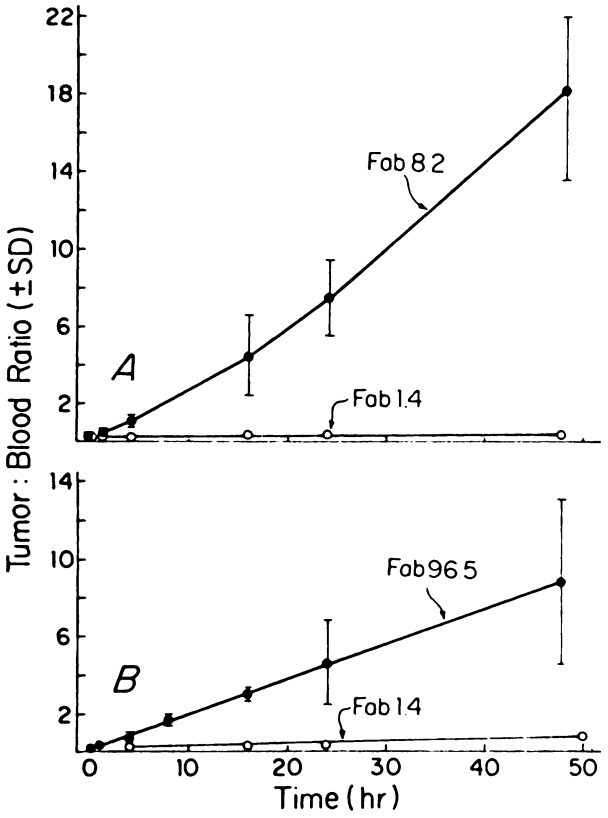

FIGURE 3
Tumor/blood ratio for Fab 8.2/1.4 (A) and Fab 96.5/1.4 (B). For A, N = 3 at 0.25 hr, N = 2 at 8 hr and N = 5 for all other time points. For B, N = 2 at 0.25 hr and N = 5 at all other time points. Bars indicate range or ± s.d

RESULTS

In vitro test of immunoreactivity

Fourteen Fab preparations (eight Fab 8.2 and six Fab 96.5) were tested for immunoreactivity by the CBA prior to their use in localization studies. The CBA % ranged from 56-83% and were essentially the same for Fab 8.2 (68 ± 7%) and Fab 96.5 (64 ± 7%). The CBA percentage for control Fab 1.4, was <3%.

In vivo kinetics of iodinated Fab

Figure 1 illustrates the contrast in blood clearance between Fab 8.2/1.4 and whole MAb 1.4. The blood clearance half-lives over 1-16 hr for co-administered Fab 8.2/1.4 were both 3.5 hr compared with a much longer clearance half-life over 1-24 hr for whole MAb 1.4 of 26.3 hr. Fab 96.5 showed a similar blood clearance half-life of 3.0 hr (data not shown). The biphasic clearance for the Fab seen in Fig. 1 consisted of a fast (1-16 hr) and a slow (16-48 hr) component which accounted for 11.9 and 1.6%, respectively, of the injected Fab 8.2, and 10.3% and 1.3%, respectively, of the co-administered Fab 1.4. For Fab 96.5, the fast and slow disposition components accounted for 9.3% and 0.7%, respectively, of the injected dose.

Biodistribution data for co-administered [^{125}I]Fab 8.2 and [^{131}I]Fab 1.4 over a 48-hr period were compiled in Table 1. Mean uptake as percent injected dose/g and s.d. for pertinent organs and tissues as well as injected dose excreted and tumor/blood ratios were tabulated for specific and control Fab. The data of Table 1 is presented graphically in part in Figs. 1-6.

Whole-body elimination of iodinated Fab occurred rapidly with >90% of the injected activity excreted by 16 hr. In one study, TCA precipitated urine samples from triplicate groups of mice which had received [^{125}I]Fab 8.2 contained 95 ± 2%, 22 ± 3% and 6 ± 0% protein-bound activity at 0.25, 1 and 4 hr after injection, respectively.

Tumor specific localization

The percent injected activity/g in tumor of antigen-specific Fab 8.2 and 96.5 were each compared with co-administered control Fab 1.4 (Fig. 2, panels A and B). Peak uptake was attained early between 1 and 4 hr. The antigen-specific fragments reached higher concentrations and exhibited longer retention in tumor compared to the control fragment. In other tissues for example liver or muscle (see Table 1) specific and control Fab concentrations declined in parallel and reached baseline between 4 and 16 hr. The tumor to blood ratio (T/B) for specific Fab increased linearly with time whereas the same ratio for control Fab remained <1 (Fig. 3).

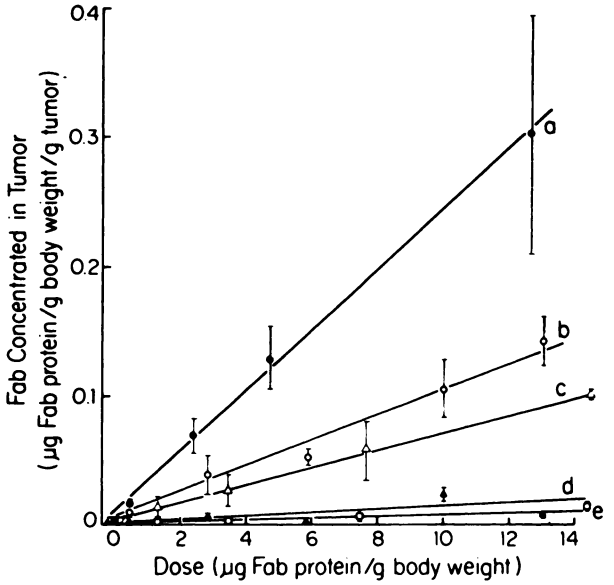

FIGURE 4
Dose dependency studies at 4 hr using Fab 8.2 alone (a) and at 16 hr using matched doses of Fab 8.2 and 1.4 (b and d, respectively) and Fab 96.5/1.4 (c and e, respectively). In order of increasing dose N = 4,5,9,9 and 7 (a), N = 6,4,11,2,3 and 2 (b and d) and N = 2,10,6,6,2 and 2 (c and e). Bars indicate range or ± s.d. Dose per mouse ranged from 0.01 to 320 μg Fab

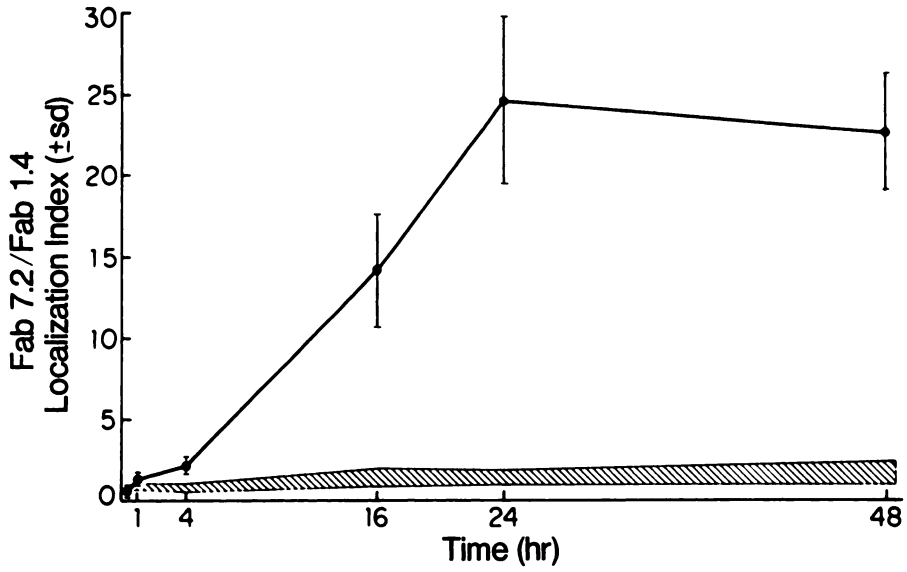

FIGURE 5
Mean LIs for Fab 8.2/1.4 versus time. Bars indicate ± s.d. N = 3 at 0.25 hr and N = 5 at other times. The range of the mean LIs calculated for a panel of nonantigen expressing tissues: liver, kidneys, spleen, heart, lungs, stomach, intestine, legs, selected lymph nodes, and carcass fall within cross-hatched area

Fab uptake in tumor correlated with protein dose

A direct correlation was noted between the fraction of specific Fab accumulated in tumor and the amount of administered protein at 4 hr (Fig. 4a) and at 16 hr (b and c). Since a plateau had not been reached at the highest protein dose tested (320 μg/mouse), saturation of accessible antigen binding sites was not attained. By comparison, tumor uptake with control Fab co-administered at matched protein doses increased only slightly with increasing dose (Fig. 4, d and e).

Localization index

Immunospecific localization relative to blood in the melanoma graft and 10 other organs and tissues has been summarized in Fig. 5. The mean LI for Fab 8.2/1.4 reached 24.6 ± 5.2 (N = 5) at 24 hr while the range of the mean LIs for liver, kidney, spleen, heart, lung, stomach, intestine, legs, lymph nodes, and carcass shown in the cross-hatched area (Fig. 5) varied from 0.5 to 2.5. A mean LI of 18.9 ± 3.2 (N = 5) was attained with Fab 96.5/1.4 at 24 hr (data not shown).

Correlation of LI with CBA%

Using linear regression, the quality of tumor localization (expressed as LI) was correlated with the in vitro cell binding percentage (Fig. 6). The data considered was restricted to a protein dose range of 0.1–2 μg specific Fab protein/g body weight (0.01–40 μg/mouse) at the 16 hr time point. The correlation coefficients were 0.48 ($p < 0.025$) for Fab 8.2/1.4 and 0.61 ($p < 0.005$) for Fab 96.5/1.4.

Imaging studies

Images were acquired of each mouse for equivalent counts at one and two days after injection. Viewed from above, head up tail down, the tumor (indicated by the arrow) was apparent in the mouse which received the p97-specific Fab 96.5 (Fig. 7, panel B and D). A tumor of comparable size located in the same position (marked by the arrow) in the other mouse which received the control fragment Fab 1.4 was not localized (Fig. 7, panel A and C). Activity also accumulated in the thyroid (top midline of A–D), the heart (upper

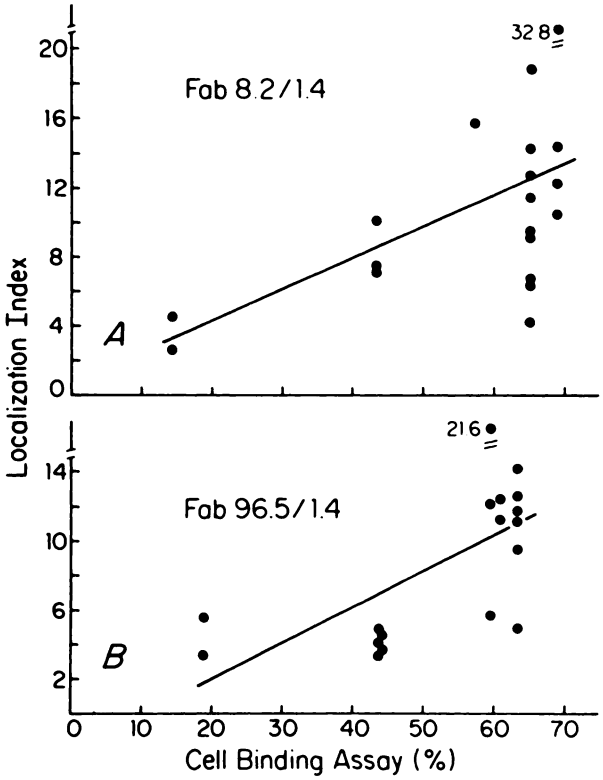

FIGURE 6
Correlation of LI and CBA percentage using linear regression for Fab 8.2/1.4 (A), N = 19, r = 0.48 and for Fab 96.5/1.4 (B), N = 18, r = 0.61. Data is restricted to 0.01–2 μg Fab/g body weight at 16 hr (0.2–40 μg/mouse)

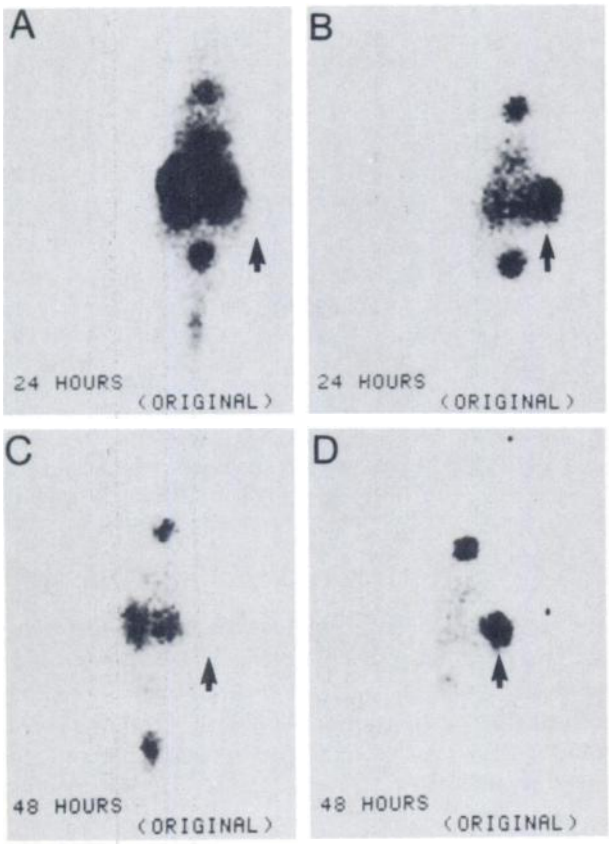

FIGURE 7
Anterior gamma camera pinhole images of mice receiving 500 μCi of control fragment [^{125}I]Fab 1.4 (A and C) or p97-specific fragment [^{125}I]Fab 96.5 (B and D) at 24 and 48 hr. Arrows indicate location of s.c. melanoma xenograft

midline in A and B), the kidneys (central midline in A–C), the bladder (lower midline in A–C), and some remained at the injection site [bottom midline (A and B) but immunospecific retention in tumor predominated at 48 hr (D)].

DISCUSSION

We have explored some of the parameters affecting immunospecific tumor localization in vivo using two Fabs recognizing p97, a cell surface antigen of human melanoma. The behavior of Fab in vivo is principally determined by its immunoreactivity and its low mol wt. A number of considerations are relevant to a discussion of Fab immunoreactivity. Compared to whole antibody, monovalent Fab prepared from high affinity MAb (> 10^{10} 1/mole) has reduced but still sufficient affinity for localization. The CBA was helpful in predicting the quality of in vivo tumor localization in that labeled preparations with a poor binding percentage (< 45%) generally produced poorer localization compared to preparations with a good binding percentage (> 65%) (Fig. 6). Scatter among the LIs using high CBA% preparations (Fig. 6) is likely related to other biological factors such as the viability of tumor cells and the structure and degree of vascularization of the tumor.

The low mol wt (50 kdalton) of the Fab fragment as well as other factors discussed below accounted for its rapid clearance from blood ($T_{1/2}$ = 3.5 hr) compared to whole MAb (150 kdalton, $T_{1/2}$ = 26.3 hr). Fab lacks the Fc effector region which keeps antibody in circulation and mediates interaction with broadly distributed tissue receptors. Fab's smaller size permits a more rapid rate of passive diffusion into the extravascular compartment. Also, its low mol wt approximates the glomerular threshhold (*11*), allowing a significant fraction of i.v. administered Fab (~ 50% by 4 hr) to be cleared by urinary excretion. Similar findings with iodinated polyclonal Fab (99% excreted by 18 hr) have been reported (*12*). Rapid clearance is advantageous in clinical imaging since it may facilitate the delineation of tumor sites by reducing the whole body background and improving the target/nontarget ratio.

Antigen-specific tumor targeting is best evaluated relative to a co-administered control Fab which shares physical properties with specific Fab but lacks the Ag recognition capacity. By comparing the biodistribution of paired specific and control Fab fragments, immunospecific tumor localization was critically determined. A comparison of specific compared with control Fab concentrations in tumor (Fig. 2) demonstrated peak uptake was attained shortly after injection (1–4 hr) but the maximal difference in specific compared with control Fab occurred later, at 16 hr. The tumor/blood ratio continued to increase over time for specific Fabs (panel A and B of Fig. 3) relative to control ratios which showed only a slight increase. This linear increase resulted from a combination of specific Fab retention in tumor and the opposed but more rapid process, its clearance from the blood. Colcher et al., using Fab′ fragments in nude mice bearing human mammary tumors, found the tumor/blood ratio increased from 8–24 hr but declined slightly by 32 hr after injection (*13*).

The amount of protein administered also affected tumor accumulation of specific Fab. Fig. 4 shows that an increase in the amount of administered Fab produced a proportional increase in the fraction of the injected dose localized in the tumor. Similar findings were reported by Baldwin and Pimm (*14*) who described a linear correlation between antibody dose and tumor uptake over a narrow dose range of 2–50 μg in mice implanted with an osteogenic sarcoma. We found this proportionality held at both 4 and 16 hr for both specific Fabs. This implies that, to some extent, high protein dose could be exploited to increase the amount of Fab deposited in the tumor target.

Maximal localization occurred at 24 hr with a LI of 24 for Fab 8.2/1.4 and 19 for Fab 96.5/1.4. This is considerably better than the ratios observed clinically in human melanoma patients, LI = 5 (*5*). However,

human tissues, especially the liver, contain low levels of p97 resulting in the clearance of a significant fraction of the administered dose.

In this model system, p97 is present exclusively in the human tumor graft. This is supported by the wide disparity between antibody concentrations in tumor compared with a panel of other mouse tissues (Fig. 5). Good localization could be anticipated. Other factors further remove this animal model from the situation in man. Nude mice lack an immune system, are injected with murine antibody and clear and metabolize iodinated Fab more quickly than man (*5*). Nevertheless, the nude mouse model offers an in vivo testing system useful for pre-clinical screening of monoclonal antibody-based radiopharmaceuticals. In combination with in vitro immunoreactivity assays and immunohistochemical evaluation of antigen expression in tumor sections, localization studies constitute a minimal work-up prerequisite to the clinical use of MAb. These studies also form a basis for comparing various types of antibody fragments, labeling methods and different antigen systems as targets for testing new monoclonal antibody-based approaches for the diagnosis and treatment of melanoma.

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