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Improved radioimmunolocalization of human tumor xenografts following subcutaneous delivery of monoclonal antibodies

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Abstract. The localization of a radiolabeled murine monoclonal antibody reactive with choriocarcinomas to human choriocarcinoma xenografts following intravenous and subcutaneous injection was evaluated by gamma scanning and tissue sampling. Tumor xenografts were established in the popliteal node region of athymic nude mice after repeated innoculations of the hind foot pads with BEWO choriocarcinoma cells. In dual label specific antibody studies, tumor/non tumor uptake ratios following subcutaneous (resulting in considerable intralymphatic uptake) injection of ^{131}I-5F9.3 were significantly higher than those achieved post simultaneous intravenous injection of ^{125}I-5F9.3. Double label experiments with ^{131}I-5F9.3 and a nonspecific antibody, ^{125}I-UPC-10, following subcutaneous injection, demonstrated that the high localization to popliteal region tumors was largely due to antibody specificity. Gamma scans following subcutaneous antibody administration of specific antibody to tumor bearing animals showed tumors soon after subcutaneous injection, at times earlier than those typically seen following intravenous delivery. Similar subcutaneous injections showed little normal nodal uptake in BALB/c control animals on gamma scans. No correlation was seen between tumor localization by specific antibody between the intravenous and intralymphatic routes, implying a difference in the mechanisms of tumor uptake of antibody by these two routes. The subcutaneous approach to antibody delivery offers advantages over intravenous delivery in tumors of human origin, including higher tumor/non tumor ratios and earlier imaging times. This was true even though these tumors were many times larger than normal lymph nodes. This subcutaneous delivery advantage should be exploitable in imaging nodal metastases of human tumors.

Key words: Monoclonal antibody – Radioimmunodetection – Choriocarcinoma – Lymph node – Lymphoscintigraphy

The compelling concept of specific delivery of radionuclides to tumors for purposes of diagnosis and therapy has neared reality with the availability of monoclonal antibodies reactive with tumor associated antigens. While antibody localization to tumors following intravenous injection of radioantibody has been high enough in some instances to be useful diagnostically, relatively low tumor/non tumor ratios have often necessitated the use of background subtraction techniques (Goldberg et al. 1978) or antibody fragmentation (Wahl et al. 1983). While these techniques are useful, higher tumor/non tumor ratios are desirable.

We have faced these problems both clinically and in laboratory animals in radioimmunodetection studies of choriocarcinomas of testicular and trophoblastic origin. In studies using ^{131}I murine monoclonal antibody 5F9.3, reactive with choriocarcinomas and testicular carcinomas, tumor to background ratios higher than those of nonspecific antibody are achieved, but are still relatively low (Khazaeli et al. 1983; Wahl et al. 1987). While we have recently enhanced tumor visualization in patients through the use of a computer aided cinematic background display system, higher tumor/non tumor ratios would further enhance diagnostic accuracy (Wahl et al. 1986).

In the mid and late 1970's, radiolabeled polyclonal antibodies were used for tumor detection in patients following subcutaneous delivery (DeLand et al. 1980; Order et al. 1975). These studies showed potential, but concern was raised regarding the tumor shedding antigen, possibly resulting in false positive scan results (DeLand et al. 1980). More recently, Weinstein and coworkers demonstrated the value of subcutaneous antibody delivery, showing preferential uptake of specific antibody to tumor metastases in regional lymph nodes draining the site of the labeled antibody injection, due largely to antibody specificity (Weinstein et al. 1983). Improvement was demonstrated over the intravenous delivery route, however the tumor system studied used was not of human origin (Weinstein et al. 1983).

A murine monoclonal antibody reactive with BEWO choriocarcinoma as well as with non seminomatous testicular tumors has been developed (5F9.3) and shown capable of specific localization, (when radiolabeled), to xenografts of this tumor following intravenous administration in animals and humans (Khazaeli et al. 1983, 1987; Patillo et al. 1968, 1984). In this study we evaluated by dual label tissue distribution studies and external gamma scanning, whether subcutaneously administered (presumedly coursing largely intra-lymphatically) monoclonal antibodies reactive with a human choriocarcinoma tumor, would preferentially localize to tumors of human histology, produced by introducing single cell suspensions of this choriocarcinoma cell line (BEWO) into the foot pad region of nude mice resulting in the growth of such tumors in the popliteal fossa nodal region. If improved antibody localization to this tumor could be demonstrated following subcutaneous injection,

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then this delivery approach may have clinical relevance in humans, particularly since nodal metastases are common in testicular tumors.

Materials and methods

Cell line. BEWO choriocarcinoma cells were a generous gift of Dr. R. Patillo and were maintained in tissue culture at 37° C in the presence of 5% CO_2 in RPMI 1640 with 10% fetal calf serum and 2 mm 2 ME. Cells for animal inoculations were removed with 0.5% trypsin (Patillo et al. 1968).

Monoclonal antibody. The production of the monoclonal antibody 5F9.3 has been described elsewhere (Khazaeli et al. 1983, 1987; Patillo et al. 1984). In brief, this murine IgG antibody was raised against embryonal cell carinoma of the testis (following immunizations with fresh and frozen patient cancer cells) and shares reactivity with an antigen expressed on several choriocarcinoma cell lines including the BEWO line. The antibody was purified from ascites grown in BALB/c mice by staphylococcal protein A chromatography. Purity was confirmed by 7.5% SDS PAGE gels (Laemmli 1970). The control monoclonal antibody, UPC-10, was purchased from Litton Bionetics.

Animal model. Three-to 6-week-old female nu/nu BALB/c mice (Charles River) were inoculated using a sterile technique in each of the hind foot pads with 5–10 million viable BEWO choriocarcinoma cells in 100 µl or less of RPMI 1640. Repeat inoculations were given each two to four weeks. Animals were followed closely for signs of tumors, especially in the popliteal node region and selected for experiments when such tumors were palpable. Control BALB/c mice (standard white mice) were purchased from Charles River at ages three to five weeks and received no tumor cell injections.

Antibody labeling. Was conducted using the iodobead method (Pierce), using 2 beads with 1 mCi of ^{131}I or ^{125}I (New England Nuclear) and 50 µg of either antibody (Markwell 1982). Labeling efficiencies were in the 45%–80% range, with separation of labeled antibody from free iodine using biogel P60 chromatography. Labeled and unlabeled antibody were compared for binding efficacy to BEWO target cells using the radiolabeled staphylococcal protein-A binding technique (LoBuglio et al. 1983).

Tissue localization and imaging studies. Animals were selected for imaging and radiolocalization studies when their tumors became palpable. Animals with these tumors were then injected with five to ten µCi of ^{131}I-5F9.3 in the foot pad ipsilateral to the popliteal fossa with tumor, and simultaneously with five to ten µCi of ^{125}I-5F9.3 intravenously by tail vein. This represented a protein dose of approximately 1 µg by each route. Animals were imaged 4, 24, 48 and 72 h postinjection, using a large field of view gamma camera with a 20% window centered at the ^{131}I/364 KeV photopeak. The camera was equipped with a 5 mm pinhole collimator. Images were obtained for approximately 15000 counts, for a known period of time. Pentobarbital (1–2 mg IP) was used as the anesthetic. Image data was collected into a dedicated nuclear medicine computer (MDS A^2) using a 64×64 word matrix, and stored on magnetic tape. Analogue images were also acquired. In other experiments, ^{131}I-5F9.3 and a nonspecific antibody, ^{125}I-UPC-10, were simultaneously injected into the foot pad with the above approach to imaging undertaken.

Animals were killed at 72–96 h postinjection by asphyxiation. Tissues were removed for analysis of radioactive antibody uptake, weighed after blotting, dual energy counted in a gamma camera (at ^{125}I and ^{131}I windows), and injected doses/g of tumor and normal tissue (normalized to an animal weight of 1 kg) were determined after correction for spill over from ^{131}I to ^{125}I and physical decay rates (Wahl et al. 1983, 1984). Statistical analysis was by the students-t, paired-t, and analysis of variance methods.

Results

Animal model

The frequency of tumor induction following injections of the BEWO tumors via the foot pad was much lower than when a comparable number of cells was given subcutaneously in the shoulder region. In the latter case, virtually all of the animals innoculated with ten million cells developed tumors. By the foot pad route, even after repeated injections (2–4 times), only about 30% of the animals developed tumors in the popliteal fossa region. In no case did the animals develop tumors at the injection site in the footpad. The tumors from the footpad injections, when allowed to grow for some time, would eventually begin to invade the leg above and below the popliteal fossa region. The pathology of these tumors was choriocarcinoma. The detection of the tumors was superior by palpation than by visual examination, but was difficult if they were less than 100 mg in size. Even in a relatively small popliteal region tumor, resected when it had just become palpable, definite lymphoid elements were seen (implying it began in a lymph node), however no residual recognizable nodal architecture was seen (Fig. 1). The mean tumor size in our experiments was 200 mg. By contrast, the mean size of popliteal lymph nodes in normal BALB/c mice is approximately 1 mg (based on 6 determination).

Intralymphatic imaging in control animals

To determine the scintigraphic and quantitative biodistribution of ^{131}I-5F9.3 injected into the subcutaneous tissues of the foot pad, healthy BALB/c mice were injected with 7.5 µCi of ^{131}I-5F9.3 via the foot pad and imaged to 72 h. These animals were also injected with ^{125}I-5F9.3 via the tail vein at the beginning of the study. Animals were killed and tissue distributions determined using standard dual window counting techniques (Wahl et al. 1983, 1984). Figure 2 (a, 24 h postinjection; b, 60 h postinjection) demonstrates the typical scan appearance of antibody clearance following injection of the hind foot pad of these healthy mice with ^{131}I-5F9.3 antibody. Activity is initially concentrated at the injection site in the foot, and gradually moves up the leg into the rest of the body. By 60 h after injection activity is seen only in the animal's body. No focal areas of intense uptake are seen to suggest high level sequestration by normal nodes in the popliteal fossa (*arrows*). In the animals killed 72 h postinjection, the blood and the foot pad where the injection was made retained the most activity (Table 1). The foot pad injected is, not unexpectedly, signifi-

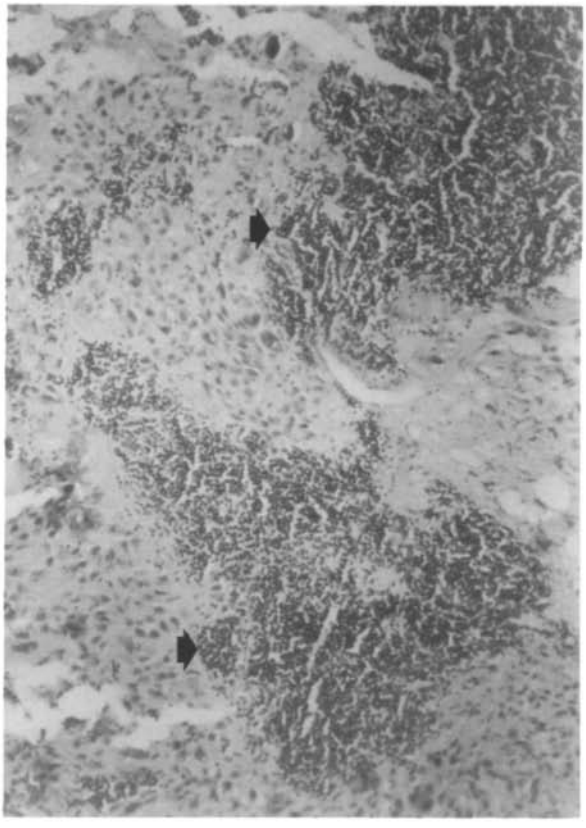

Fig. 1. Foci of lymphocytes (*arrow*) are seen in this choriocarcinoma tumor removed from the popliteal fossa. No normal nodal architecture is seen, despite this tumor focus being situated in the popliteal fossa

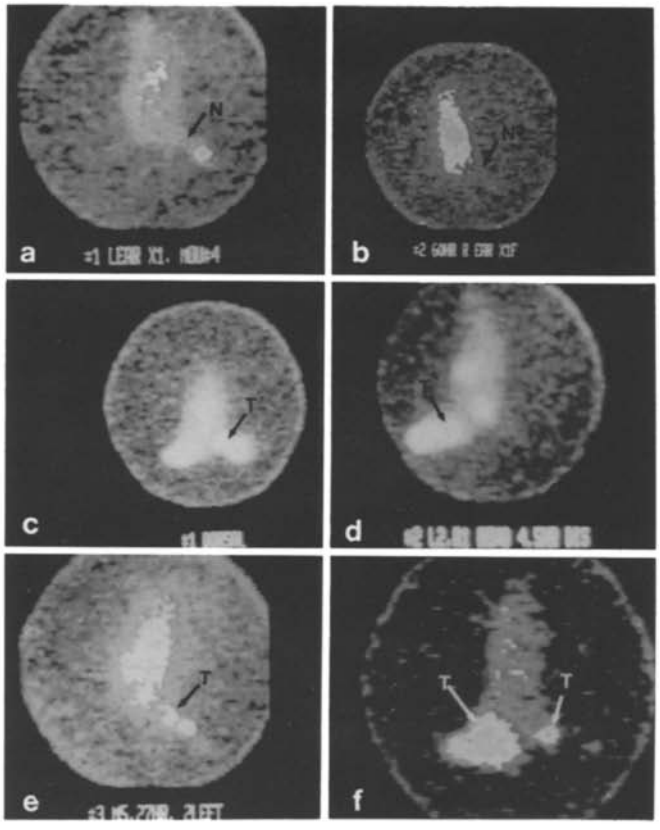

Fig. 2. a Prone view of BALB/c control animal 24 h postinjection subcutaneously of ^{131}I-5F9.3 into right foot pad shows foot pad injection site clearly as well as whole body activity. Note the lack of uptake in the normal popliteal fossa (*N-arrow*). **b** Sixty hours postinjection subcutaneously of ^{131}I-5F9.3 to this normal BALB/c animals right foot pad is nearly devoid of activity although considerable systemic activity is present. Again note the lack of uptake in the normal popliteal fossa (*N-arrow*). **c** In this dorsal view of a BeWo tumor bearing nude mouse injected subcutaneously with ^{131}I-5F9.3 in both foot pads, note activity in the foot pads, as well as the right popliteal fossa region tumor (*T-arrow*) on a view 4 h postinjection. **d** Uptake to a left popliteal node region BeWo tumor (*T-arrow*) is seen just 5 h postinjection ^{131}I-5F9.3 subcutaneously to the foot pad. Free iodine activity is seen in the stomach and bladder of this animal imaged in the prone position. **e** In another nude mouse less striking uptake is noted into a popliteal fossa BeWo tumor 27 h postinjection subcutaneously into the ipsilateral foot pad shows the BeWo tumor focus activity (*T-arrow*), foot pad and whole body. Contrast this appearance to Figs. 2a and b. **f** In the same animal as **c**, note 48 h postinjection, the intense uptake in the right leg (*T-arrow*) (a tumor focus was identified weighing 140 mg) as well as in a large tumor (*T-arrow*) in the left leg seen as a cold defect 4 h post antibody injection. This larger tumor weighed 1.1 g when the animal was killed

Table 1. Mean uptakes % kg dose/g ± SEM

	S.Q. (intralymphatic) administration	I.V. administration
Blood	0.096 ± 0.027	0.148 ± 0.024
Liver	0.016 ± 0.001	0.027 ± 0.003[a]
Muscle	0.02 ± 0.006	0.02 ± 0.005
Right foot pad (inj)	0.134 ± 0.051[b]	0.028 ± 0.001[a]
Left foot pad (non inj)	0.021 ± 0.008	0.036 ± 0.014[a]

Mean uptakes ± SEM in group ($n=5$) of BALB/C (standard white laboratory) mice given 7.5 μCi ^{131}I-5F9.3 via foot pad (intralymphatic) and 7.5 μCi ^{125}I-5F9.3 intravenously via tail vein. Animals were killed 72 h postinjection

[a] Tissue activity following intravenous administration is significantly different ($P<0.05$) than corresponding activity following intralymphatic injection

[b] Significantly higher than left foot pad following intralymphatic injection, $P<0.01$

cantly more radioactive than the contralateral uninjected foot pad ($P<0.01$). The 5F9.3 antibody given intravenously resulted in significantly higher ($P<0.05$) liver uptake than the same quantity of the antibody given intralymphatically. Blood levels in the intravenously injected group also tended to be higher at this time ($0.05<P<0.1$).

Liver/blood ratios were comparable between ^{125}I and ^{131}I labeled 5F9.3, suggesting that the antibodies has similar biologic behaviors, once they reached the circulation, with a mean liver/blood ratio of 0.174 by intralymphatic delivery and 0.184 by intravenous delivery ($P=$ NS).

Comparison of simultaneous intralymphatic and intravenous specific antibody delivery to popliteal regional tumors

Ten animals with popliteal fossa tumors were selected for injection of ^{131}I-5F9.3 to the foot pad ipsilateral to the popliteal fossa with tumor. These same animals were simul-

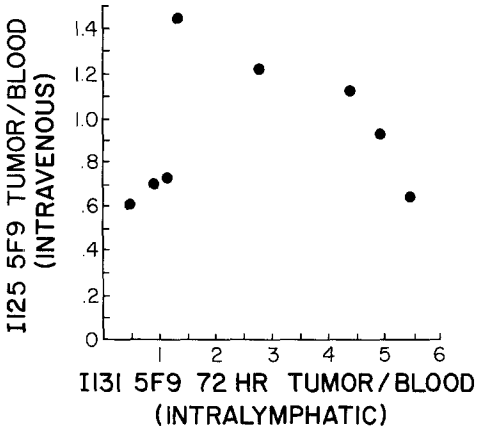

Fig. 3. Demonstrates tumor/blood ratios for subcutaneously administered 5F9.3 (^{131}I label) vs intravenously administered 5F9.3 (^{125}I label). The mean tumor/blood ratio by subcutaneous (intralymphatic) injection is 2.69 ± 0.71 and the mean tumor/blood by intravenous is 0.92 ± 0.11. These are significantly different at $P < 0.025$. Note the lack of correlation between tumor/blood ratios achieved by the two routes

taneously injected intravenously with ^{125}I-5F9.3 in the tail vein. These animals were imaged at the ^{131}I photopeak at several time points (generally 4, 24, 48 and 72 h postinjection), and were killed and dual-energy tissue counted at 72 h postinjection. In one animal, an injection of ^{131}I-5F9.3 was made to each foot along with the intravenous injection, as one examiner clinically suspected tumor in both popliteal fossae. Eight of the animals survived to 72 h postinjection. One died at 48 h and one at 5 h post antibody injection as a result of anesthesia complications. Seventy two hour intralymphatic and intravenous delivery mediated tumor/blood ratios are shown for each animal in Fig. 3. The mean intralymphatic/intravenous route specific localization ratio normalized to blood (tumor IL/blood IL)/(tumor IV/blood IV) is 3.09 ± 0.96 (SEM). The intralymphatic/intravenous route specific localization ratio normalized to liver (tumor IL/liver IL)/(tumor IV/liver IV) ratios is 2.87 ± 0.98 (SEM) (data not shown). Both numbers are significantly greater than 1 ($P < 0.05$) indicating that the intralymphatic route of delivery was superior to the intravenous route. Figure 3 shows the tumor/blood ratios for individual animals achieved by the intralymphatic route as compared to the intravenous route in the same animals. These plots are of particular interest as there is no correlation seen between tumor/blood or tumor/liver (data not shown) ratios achieved by the intravenous route and those achieved by the intralymphatic route. Thus, an animal with a high tumor/blood ratio by intralymphatic delivery might have a relatively low tumor/blood ratio by intravenous delivery. Mean tissue uptakes (% kg injected dose/g) for ^{131}I-5F9.3 given by the intralymphatic route and intravenously are shown in Table 2. Of note is that tumor uptake of ^{131}I-5F9.3 by the intralymphatic route was 0.177 ± 0.081 vs. 0.099 ± 0.017 for ^{125}I-5F9.3 by the intravenous route ($P =$ NS). Much as was the case in the control animals, these tumor bearing animals had significantly lower uptake of the 5F9.3 antibody in liver and blood by the intralymphatic route than the intravenous route which accounted for the superior tumor/non tumor ratios in the intralymphatic group.

Table 2. Organ uptakes postintralymphatic ^{131}I-5F9.3 injection and simultaneous intravenous ^{125}I-5F9.3 injection

	^{131}I-5F9.3 (IL)	^{125}I-5F9.3 (IV)
Tumor	0.177 ± 0.081	0.099 ± 0.017
Blood	0.056 ± 0.012	0.116 ± 0.02[a]
Liver	0.013 ± 0.002	0.027 ± 0.002[b]

Mean % kg injected dose/g $\pm$ SEM ($n = 8$)

[a] Tissue activity following intravenous injection is significantly greater than intralymphatic injection at $P < 0.005$

[b] Tissue activity following intravenous injection is significantly greater than intralymphatic injection at $P < 0.0005$

^{131}I and ^{125}I-5F9.3 have comparable binding in vitro to BEWO (chorio-carcinoma) target cells

The intralymphatic delivery route showed clear superiority over the intravenous route in tumor/non tumor ratios despite there being some variability among animals. Also observed was variability of uptake within individual tumors as in 1 case with a large tumor (approximately 1 g) a central necrotic region had a tumor/liver = 13.3/1 while its more viable center had a tumor/liver of 25.6/1.

Imaging studies reflected the higher tumor/non tumor ratios achieved following subcutaneous injection. In Fig. 2d, uptake is seen into a popliteal region tumor (*arrow*) just 4.5 h after antibody injection. In another animal shown in Fig. 2c and 2f, uptake is seen in both feet soon after injection (4 h) as well as in the popliteal lymph node region on the right (*arrows* – Fig. 2c) (animals are imaged prone). By 48 h (Fig. 2f) uptake is seen clearly in the right popliteal node region (*arrow*), as well as now being seen in a large tumor focus in the left leg (*arrows*). The tumor on the left in this animal weighed 1.1 g, while the tumor on the right weighed only 140 mg. Of note is that the normal popliteal lymph nodes in BALB/c mice weigh only 1 mg, (mean of excisions of 6 nodes from healthy animals). Figure 2e shows less striking uptake of antibody into tumor (*arrow*) 27 h postinjection into the left foot pad of another mouse with a popliteal fossa tumor. Note the activity in the mid leg representing tumor, as well as the bladder and whole body activity. This in contrast to the lack of midleg activity in the control mice in Fig. 2a and b.

Localization following foot pad antibody injection in tumor-bearing mice (specific vs non-specific antibody)

Five nude mice bearing BEWO tumors in their popliteal fossae received a double label injection mixture composed of 5 μCi ^{131}I-5F9.3 and 5 μCi ^{125}I-UPC-10 nonspecific antibody (approximately 1 μg of each antibody) in the foot pad of the leg ipsilateral to the politeal fossa tumor. The animals were killed 96 h postinjection. Tumor/non tumor ratios were calculated for the specific and nonspecific antibodies. As can be seen in Table 3, tumor uptake of specific antibody given by the intralymphatic route is significantly greater ($P < 0.025$) than tumor uptake of nonspecific antibody administered by the same route. This high tumor uptake of specific antibody is in spite of uptake in other tissues showing the nonspecific antibody to have achieved significantly higher systemic levels of activity ($P < 0.01$). Tumor/non tumor ratios are shown graphically in Fig. 4 and indicate the considerable improvement in tumor accretion due

Table 3. Organ uptakes post dual label subcutaneous administration

	^{131}I-5F9.3	UPC-10
Tumor	0.22 ±0.05[a]	0.08 ±0.01
Blood	0.121±0.02[b]	0.162±0.025
Liver	0.023±0.005[c]	0.029±0.006

Mean % kg injected dose/g±SEM ($n=5$)
Significantly different from UPC-10 at $P<0.025$[a], $P<0.005$[b], or $P<0.01$[c]

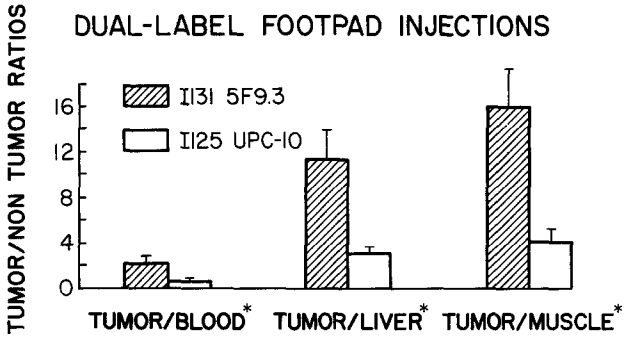

Fig. 4. Mean±S.E.M. tumor/non tumor ratios for specific 5F9.3 and nonspecific UPC-10 antibody following subcutaneous foot pad (intralymphatic) administration with the animal killed 96 h later. Tumor/non tumor ratios for specific antibody are in all instances superior to those for nonspecific antibody ($P<0.025$)

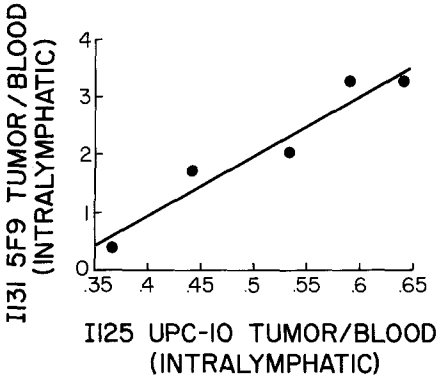

Fig. 5. Tumor/blood ratios of specific (5F9.3) and nonspecific (UPC-10) antibodies following subcutaneous foot pad (intralymphatic) injection to BEWO tumor bearing animals. Note strong correlation between tumor/blood ratios for specific (5F9.3) and non specific (UPC-10) antibodies given subcutaneously (intralymphatic). Y=10.41 X-3.242; R-squared: 0.93

to specific antibody binding. The mean specific localization ratio was 3.6±0.82 (SEM). Again, the specific localization ratio is significantly greater than one in these animals. The fraction of the injected dose reaching the tumor was always higher for the specific antibody than for the nonspecific antibody. This mirrors previous results following intravenous injection (Khazaeli et al. 1983). Note in Fig. 5 that as the tumor/blood ratios for specific antibody increased by the intralymphatic route, there was a significant parallel increase in the uptake of nonspecific antibody in a linear fashion ($r=0.93$). This is in contrast to the lack of correlation between specific uptake to tumors following simultaneous (intralymphatic) and intravenous injections of ^{131}I and ^{125}I specific antibody (Fig. 3).

Discussion

The subcutaneous injection route was effective in demonstrating, by gamma camera scintigraphy, human derived choriocarcinoma tumors in the popliteal fossa ipsilateral to the subcutaneous injection site in the foot pad in our nude mouse model. Overall, a significant improvement in tumor/non tumor ratio was seen for the subcutaneous route of antibody delivery, as compared to the tumor/non tumor ratio achieved by intravenous antibody delivery. This improvement is not just due to nonspecific trapping of protein in the lymph nodes or tumors, as was shown in double label coinjection experiments of specific and nonspecific antibody by the foot pad route. In these latter experiments there was a strong correlation between tumor accumulation of specific and nonspecific antibody, implying that similar factors may affect their nodal retention but indicating that the retention of specific antibody was invariably higher than that of nonspecific antibody. Images of high quality were obtained at earlier time points than has been seen by intravenous delivery of specific antibody (Khazaeli et al. 1983). Of additional importance is the fact that the improvement seen is in a tumor system of human origin using a monoclonal antibody shown capable of localizing human tumors in man (Wahl et al. 1987).

While some of these results are not totally unexpected, given the previous reports by Order, DeLand and Weinstein (Order et al. 1975; DeLand et al. 1980; Weinstein et al. 1983), limited histology even on the smallest tumors in our series showed only lymphoid elements, but no discrete nodal architecture. In fact, the average tumor in our series was 200fold larger than normal mouse popliteal lymph nodes. Thus, much of the node was totally replaced with tumor, yet improved localization was still seen by the subcutaneous route. This route preferentially delivers antibody to the lymphatics due to their lack of a basement membrane (Weinstein et al. 1983). Thus, this delivery method appears to hold promise, even if nodal metastases are fairly substantial in size. This may be of particular clinical relevance to following enlarged lymph nodes posttherapy that are stable in size and thought to represent fibrosis, or even during therapy (Heiken et al. 1984). There was no clear relationship between tumor weight and tumor/blood ratios in this study. Certainly, some tumors must eventually become large enough that nodal lymph flow is impaired, but this may only occur with extremely large nodes. Our experience in lymphoscintigraphy of melanoma and that of others suggests this nodal obstruction is an infrequent occurrence (Eberbach et al. 1987). Certainly nodal obstruction can occur and this can affect the results of colloidal lymphoscintigrams (Ege 1983). It is possible that the mechanism of nodal uptake may vary between colloidal and noncolloidal agents (Wahl et al. 1986).

Also of interest was the apparent dissociation between the tumor/blood ratios achieved by the intralymphatic and intravenous routes in the same tumors (Fig. 3). There was no correlation between specific antibody tumor/blood ratios following subcutaneous delivery and specific antibody tumor/blood ratios following intravenous administration in the coadministration studies. There was, however, a strong relationship between nodal uptake of specific antibody given by the subcutaneous route and nonspecific antibody given intralymphatically in this study ($r=0.93$). This suggests a basic difference in the mechanism of antibody uptake

for the two delivery routes. We know that both routes depend heavily on antibody specificity for uptake, but there appear to be some as yet unrecognized characteristics of certain tumors (such as a good lymphatic supply, but poor vascular supply or vice versa), that enhance delivery by one route over the other. Although the number of animals studied is small, due to the relative difficulty of establishing the tumors in the popliteal fossa, the numbers were clearly adequate to delineate this strong relationship between the uptake ratios of specific and nonspecific antibody administered by the subcutaneous route.

Images obtained following subcutaneous delivery were generally considerably superior to those that have been seen previously in this tumor system by the intravenous route (Wahl et al. 1986). These improved tumor images were also seen at earlier time points by the subcutaneous delivery route than intravenously. This suggests that the optimum imaging time may be earlier for subcutaneously administered antibodies than for intravenously administered intact monoclonal antibodies. This, if verified in humans, will be useful in establishing a more prompt diagnostic study than the intravenous route of antibody delivery offers.

In this series, the accretion of antibody to tumors following subcutaneous administration was specific, as shown in double label experiments. Of interest was the fact that in the double label specific antibody studies, although there was a trend for higher quantities of the administered dose of antibody given subcutaneously to reach the tumor sites than for the same dose given intravenously, this did not achieve statistical significance. The improved tumor/blood ratios by the subcutaneous route were a statistically significant phenomenon, however. Also, in the tumor bearing animals a lower proportion of the subcutaneously administered dose reached the blood, as compared with the intravenous route. These higher systemic antibody levels following intravenous as compared with subcutaneous injection achieved statistical significance in the control BALB/c mice in whom the liver uptake following intravenous injection was significantly greater than that following subcutaneous injection. These lower systemic levels may be related to catabolism of antibodies by the tumor, the node, or subcutaneous tissues, to a greater extent when given subcutaneously than when given intravenously. One would expect that sustained release of antibody from the injection site might result in higher levels systemically at later time points. This was not the case. Since we did not kill animals at multiple time points, the reason for lower systemic antibody levels following subcutaneous administration is not yet resolved.

The explanation for the higher tumor/non tumor ratios seen following subcutaneous administration is most likely related to a higher proportion of the injected dose reaching the tumor initially with an opportunity for improved first pass extraction. This is in contrast to intravenous delivery where many potential interactions of the antibody with non target antigens occur prior to the antibody reaching the tumor. If there is a high first pass clearance by tumor or normal nodes, then there would be a lower proportion of labeled antibody reaching the blood stream. This would account for a higher tumor/non tumor ratio and should also result in a higher total tumor uptake of radioantibody. Tumor uptakes tended to be higher by subcutaneous delivery, but did not achieve statistical significance in the group of animals studied by simultaneous intravenous and subcutaneous coinjection. Naturally, if catabolism or deiodination occurred at the tumor, node, subcutaneous tissues, or in the lymphatics, then total nodal uptake might be lower or drop with time. The image data suggests that the radiolabeled antibody reached the popliteal tumors via the lymphatic channels as in several cases the tumor were seen very early (e.g., Fig. 2c, d), prior to visualization of the rest of the animals blood pool without uptake seen in the intervening leg. Experiments recently performed with another IgG2a antibody given in the foot pad of normal rats has shown a less than two fold increase in activity in the muscle between the popliteal node and foot pad at four hours post injection, despite dramatically increased activity in the popliteal lymph node (Wahl et al. 1986). Thus, image data and tissue counting data do not lend support to the possibility that the phenomenon of increased uptake is due to diffusion of activity through muscle separate from lymphatic flow to tumors in the popliteal region. We have also performed preliminary direct inratumoral injection of labeled specific antibodies and have not seen the degree of increased tumor activity at delayed times seen by the subcutaneous, and presumedly intralymphatic, methods (R. Wahl, unpublished data). Thus, our data favors the lymphatics as the mode of delivering to the popliteal tumor much of the tumor imaging dose given subcutaneously.

For imaging purposes, the higher tumor/non tumor ratios seen following subcutaneous injection will be beneficial. For therapy of tumor in the nodes, the problem of skin dose at the injection site needs to be addressed. Even with three days to clear the injected dose, in control animals the injected foot's activity is comparable to blood levels, indicating a significant radiation dose to the injection site (Table 1).

In conclusion, the subcutaneous route of antibody delivery offers significant advantages over the intravenous route in cases where there are tumor foci in or replacing lymph nodes draining the injection site. Tumor foci far larger than normal lymph nodes in this study had specific uptakes that were superior by the subcutaneous delivery route as compared to the intravenous route and the uptake in these tumors was largely due to antibody specificity as shown in dual label studies. Of note is that tumors that take up specific antibody well by the subcutaneous route may not have similar superior uptake by the intravenous route and vice versa due to factors that are not yet fully defined, but most likely related to antibody access to tumor antigens. The subcutaneous delivery approach appears to be of value for human tumors including those that are larger than normal sized lymph nodes. This route of delivery warrants additional study in man.

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