Inhibition of Gallium-67 Uptake in Melanoma by an Anti-Human Transferrin Receptor Monoclonal Antibody

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The effect of an anti-human transferrin receptor (anti-TFR) monoclonal antibody (MoAb), designated B3/25, and an anti-melanoma antibody, designated 96.5, on the uptake of gallium-67 (^{67}Ga) by tumor was studied. Three groups of six athymic mice bearing a human melanoma were injected via tail vein with (a) 0.55 mg human serum albumin (HSA) (control group), (b) 0.5 mg MoAb B3/25 + 0.55 mg HSA, and (c) 0.5 mg MoAb 96.5 + 0.55 mg HSA, respectively. Twenty-four hours later, each mouse was given an intravenous dose of 5 μCi [^{67}Ga] citrate. Biodistribution of activity (percent injected dose per gram) determined 48 hr after injection of ^{67}Ga showed a 75% decrease in tumor uptake in the group of mice that received B3/25 (anti-TFR MoAb) compared with the control group. In contrast, MoAb 96.5 did not show any effect on melanoma uptake of ^{67}Ga. Histologic findings suggest that the decreased uptake was not due to cellular damage resulting from binding of B3/25 to TFR. The results of this study strongly suggest the involvement of TFR in the in vivo tumor uptake of ^{67}Ga.

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Uptake of gallium-67 (^{67}Ga) citrate by soft-tissue tumors was first reported by Edwards and Hayes in 1969 (*1*). Since that time, ^{67}Ga has been thoroughly investigated for use in the diagnosis and staging of many types of malignancies. It has proven useful in evaluation of a variety of tumors, including lymphoma, melanoma, and hepatoma. Despite its widespread clinical application, however, the exact mechanism of ^{67}Ga localization is still controversial.

It has been well established that ^{67}Ga binds to serum transferrin (TF) after intravenous injection of [^{67}Ga]citrate (*2*). However, the subsequent sequence of events that lead to the transfer of ^{67}Ga from TF to tumor are not clearly understood. Various mechanisms have been proposed for the preferential uptake of ^{67}Ga by soft-tissue tumors (*3*). One of the unsettled issues is the role of the transferrin receptor (TFR) in tumor uptake of ^{67}Ga (*4,5*). The present study was performed to clarify this issue by determining the effect of an anti-human transferrin receptor monoclonal antibody (anti-TFR MoAb) on tumor uptake of ^{67}Ga in a human melanoma nude mouse model. In addition, because it has been shown that the human melanoma-associated antigen p97 is structurally and functionally related to transferrin (*6*), it is conceivable that p97 may also bind ^{67}Ga. Hence, we also included anti-p97 MoAb in our study.

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MATERIALS AND METHODS

Monoclonal Antibodies and Radiopharmaceutical

Anti-human TFR MoAb (designated B3/25 MoAb) and anti-p97 MoAb (designated 96.5 MoAb) were kindly supplied by a commercial source.* Gallium-67 citrate was used as the radiopharmaceutical.†

Animals and Tumor

Female athymic mice (BALB/c-nu/nu)‡ were used as the host animals. The mice were ~3 wk old (approximately 11 g) at the time of tumor implantation. Groups of five mice were housed in plastic cages placed in laminar flow hood, and were fed pelleted food and water.

Human melanoma cells derived from a human tumor specimen were grown in tissue culture. The cells were passed through at least one generation of solid tumor in the nude mice hosts prior to study. Solid tumors were produced in the mice by implanting a tumor fragment in the flank using a 13-guage trocar. The tumors were allowed to grow to 103–333 mg (approximately 4 wk) prior to experimentation.

Effect of B3/25 and 96.5 MoAb on Biodistribution of [^{67}Ga]Citrate

Three groups of six melanoma-bearing mice were injected via tail vein with one of the following: (a) 0.55 mg human serum albumin (HSA) (control group); (b) 0.5 mg B3/25 MoAb + 0.55 mg HSA; and (c) 0.5 mg 96.5 MoAb + 0.55 mg HSA. Twenty-four hours later, each mouse was given an intravenous dose of 5 μCi (185 KBq) [^{67}Ga]citrate. Forty-eight hours after injection of [^{67}Ga]citrate, the mice were killed. Various tissues were removed, rinsed with saline, blotted dry, and weighed. The radioactivity associated with the tissues were counted in a Beckman gamma 8000 automatic gamma counter. The results were expressed as percent injected dose per gram of tissue (%ID/g), tissue-to-blood ratios, as well as tumor-to-tissue ratios.

Pathology Study

Two tumors from each of the three groups of mice were studied histologically for determination of possible cellular damage that may have resulted from binding of MoAb to cell surface antigen. Each tumor nodule was fixed in 10% buffered formalin, processed in a Fisher histomatic tissue processor (266 MP) for paraffin embedding, and stained with hemotoxylin and eosin. Sections were cut at 6 microns.

Data Analysis

Statistical analysis was performed using Student's t-test.

RESULTS

Effect of B3/25 and 96.5 MoAb on Biodistribution of [^{67}Ga]Citrate

The biodistribution of ^{67}Ga for the various groups of mice, expressed as percent injected dose per gram of tissue (%ID/g), are summarized in Table 1. The tissue-to-blood and tumor-to-tissue ratios for the various tissues studied are summarized in Table 2 and Table 3, respectively.

In terms of percent injected dose per gram of tumor, the mice injected with B3/25 MoAb showed an approximately 75% decrease in ^{67}Ga uptake compared with both the control group and the group injected with 96.5 MoAb. The spleen and the kidney were the only other tissues in the B3/25 group that showed significant differences in ^{67}Ga uptake to the same tissues in the control group. The experiment was repeated once with similar results (data not shown).

TABLE 2
Effect of B3/25 and 96.5 MoAb on Biodistribution of [^{67}Ga]Citrate in Nude Mice Bearing a Human Melanoma*

Tissue	HSA Group I	B3/25 + HSA Group II	96.5 + HSA Group III
Tumor	25.68 ± 4.63	5.20 ± 0.64	23.99 ± 3.59
Heart	1.44 ± 0.27	1.30 ± 0.20	1.28 ± 0.27
Lung	2.91 ± 0.29†	2.91 ± 0.29†	3.29 ± 1.12
Liver	10.71 ± 1.34	9.42 ± 0.89	10.08 ± 1.66
Spleen	4.92 ± 0.69	5.08 ± 0.40	4.44 ± 0.97
Kidney	12.08 ± 1.69	7.12 ± 0.80	11.53 ± 2.44
Intestine	5.46 ± 1.21	5.47 ± 1.63	4.64 ± 0.58
Muscle	0.61 ± 1.21	0.56 ± 0.20	0.52 ± 0.14
Bone	11.22 ± 2.91	9.93 ± 2.58	9.90 ± 2.16
Skin	3.26 ± 0.33	2.93 ± 0.38	2.98 ± 0.98

* Results are expressed as tissue-to-blood ratios ± s.d. Values are expressed as the mean of the individual tissue-to-blood ratios and not by dividing the mean % ID/g of tissue by the mean % ID/g of blood.
† These values are correct and not typographical errors.

Pathology Study

All of the tumors removed from test animals (Figs. 1 and 2) examined showed <25% necrosis, and one control animal had 35% necrosis (Fig. 3). In the control animals the region of minor necrosis was in the approximate center of the tumor nodule. The melanoma cells were essentially amelanotic by light microscopy and had uniform cytologic features (Figs. 1–3). Large

TABLE 1
Effect of B3/25 and 96.5 MoAb on Biodistribution of [^{67}Ga]Citrate in Nude Mice Bearing a Human Melanoma*

Tissue	HSA Group 1	B3/25 + HSA Group II	p (II versus I)	96.5 + HSA Group III	p (III versus I)
Blood	0.85 ± 0.15	1.00 ± 0.18	0.16	0.95 ± 0.32	0.53
Tumor	21.42 ± 2.32	5.12 ± 0.61	0.00†	22.67 ± 7.63	0.71
Heart	1.20 ± 0.03	1.28 ± 0.17	0.29	1.15 ± 0.20	0.58
Lung	2.45 ± 0.28	2.90 ± 0.53	0.10	3.20 ± 1.86	0.37
Liver	9.01 ± 1.03	9.34 ± 1.55	0.67	9.23 ± 2.41	0.84
Spleen	4.13 ± 0.43	5.05 ± 0.81	0.03	3.98 ± 0.72	0.67
Kidney	10.30 ± 2.44	7.11 ± 1.49	0.01	10.69 ± 3.73	0.82
Intestine	4.54 ± 0.63	5.28 ± 1.17	0.21	4.26 ± 1.04	0.58
Muscle	0.51 ± 0.11	0.53 ± 0.10	0.80	0.47 ± 0.14	0.55
Bone	9.25 ± 1.25	9.62 ± 1.15	0.61	8.91 ± 1.94	0.73
Skin	2.75 ± 0.32	2.88 ± 0.30	0.49	2.61 ± 0.58	0.31
Number of animals	6	6		6	

* Results are expressed as mean percent of injected dose per gram of tissue ±s.d.
† 0.00 represents $p < 0.01$

TABLE 3
Effect of B3/25 and 96.5 MoAb on Biodistribution of [^{67}Ga]Citrate in Nude Mice Bearing a Human Melanoma*

Tissue	HSA Group I	B3/25 + HSA Group II	96.5 + HSA Group III
Blood	25.68 ± 4.63	5.20 ± 0.64	23.99 ± 3.59
Heart	17.91 ± 1.93	4.01 ± 0.18	19.34 ± 4.32
Lung	8.82 ± 1.23	1.79 ± 0.22	7.74 ± 2.07
Liver	2.39 ± 0.24	0.55 ± 0.04	2.45 ± 0.61
Spleen	5.20 ± 0.43	1.03 ± 0.16	5.58 ± 1.23
Kidney	2.14 ± 0.35	0.73 ± 0.10	2.18 ± 0.71
Intestine	4.79 ± 0.83	1.03 ± 0.36	5.24 ± 1.02
Muscle	42.81 ± 8.13	10.07 ± 2.76	48.90 ± 14.06
Bone	2.34 ± 0.31	0.54 ± 0.07	2.52 ± 0.60
Skin	7.82 ± 0.75	1.78 ± 0.14	8.98 ± 3.63

* Results are expressed as tumor-to-tissue ratios ± s.d. Values are expressed as the mean of the individual tumor-to-tissue ratios and not by dividing the mean % ID/g of tumor by the mean % ID/g of tissue.

pleiomorphic tumor cells, as commonly seen in secondary tumor sites in human patients, were not seen in any field. The cytomorphology was not appreciably altered by administration of the anti-TFR antibody.

DISCUSSION

Although it has been well established that, after intravenous administration, ^{67}Ga binds to serum TF (*2*), there is a lack of consensus on the subsequent events that lead to the transfer of ^{67}Ga from TF to tumor. Hayes (*5*) emphasized the role of ionic Ga and proposed hyperpermeability of tumor cell membrane as being mainly responsible for tumor uptake of ^{67}Ga. Sephton and Harris (*7*) demonstrated that TF stimulated an increase in uptake of ^{67}Ga in cultured tumor cells. Larson et al. subsequently have studied the role of TF in ^{67}Ga uptake and proposed an uptake mechanism that involves a specific TFR (*4*); Ga-labeled TF is believed to bind to TFR and the resulting Ga–TF–TFR complex is internalized by endocytosis. Though this uptake process seems plausible because it is essentially the same as that for iron (Fe) (*8–11*), other investigators have expressed reservations on the endocytic aspect of this proposed mechanism. Wong et al. (*12*) suggest that the Ga–TF complex, upon binding to TFR, simply releases the metal ion, which is then transported into tumor cells by a mechanism that is not clearly understood. Support for this alternate uptake mechanism also has been described for the uptake of Fe by rat hepatocytes (*13*).

The results of our study strongly suggest that TFR is involved in uptake of ^{67}Ga in melanoma in vivo. The inhibition of ^{67}Ga uptake observed in this study appears to be due to interference of TF binding to TFR by B3/25 rather than by a nonspecific mechanism, because histologic findings showed no greater cellular damage in tumors from the experimental groups compared with those from the control group. Alternatively, such inhibition may have resulted from reduction of TFR on tumor cell surface due to antigenic modulation of TFR upon binding by the anti-TFR MoAb. Down-regulation of TFR by B3/25 has been described (*14*). Our study assumes cross species interaction between mouse TF (to which the ^{67}Ga presumably binds) and the human TFR present in the tumor. Such cross species interaction has been documented previously (*15,16*). While human and mouse TFR differ in carbohydrate content (*17*) there may be cross reactivity between B3/25 and native mouse TFR, which is present in some normal tissues (*15*). Such cross reactivity, however, would not significantly affect the observed results.

These results are rather surprising in light of previous work by Trowbridge et al., suggesting that B3/25 MoAb, although it is a TFR binding antibody, does not inhibit binding of transferrin by the TFR (*18*). Indeed, the

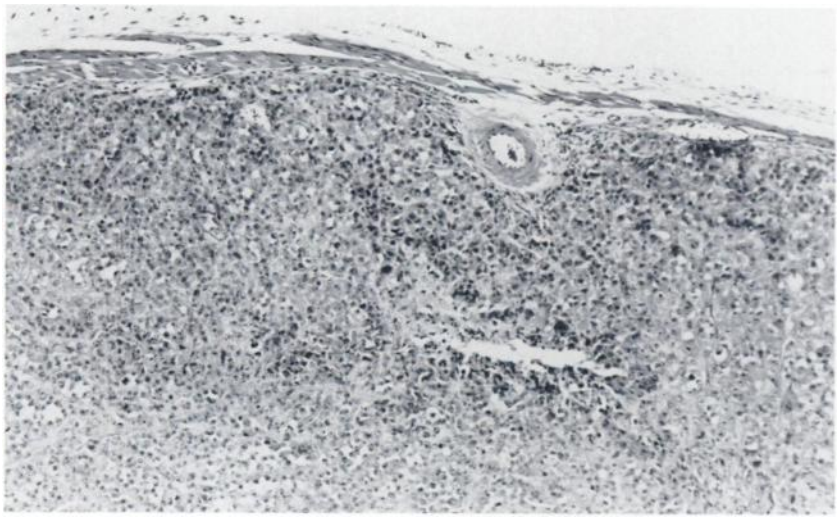

FIGURE 1
Photomicrograph of the melanoma nodule removed from an animal injected with anti-human transferrin receptor monoclonal antibody (B3/25 MoAb). The nodule is impinging on skeletal muscle at the top. The majority of the tumor cells are non-necrotic. Hematoxylin and eosin × 125.

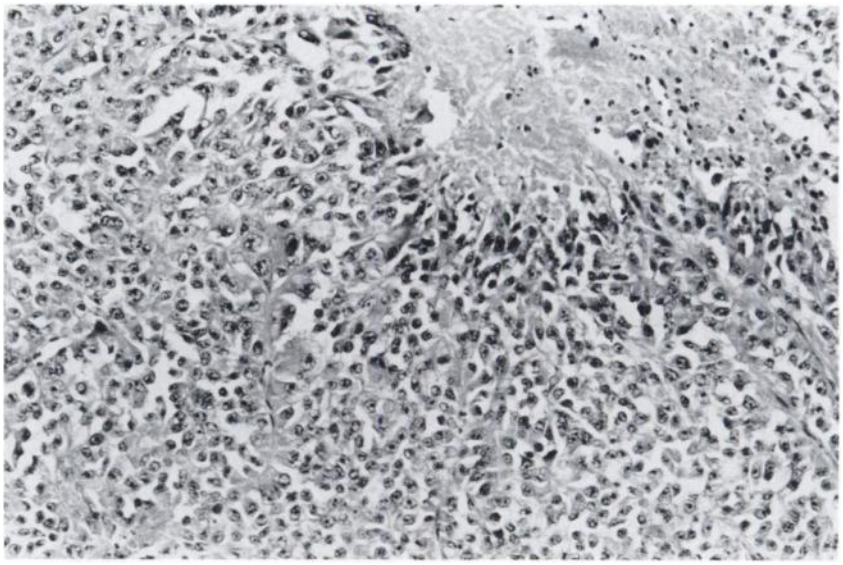

FIGURE 2
Human melanoma cells in a mouse injected with MoAb 96.5 that recognizes a 97 kD surface protein. The tumor cells appear uniformly epithelioid without visible melanin pigment. Minor necrosis is seen near the top center. Hematoxylin and eosin × 250.

identification of B3/25 MoAb as a TFR antibody was dependent on the recognition that B3/25 MoAb bound the TF–TFR conjugate (*17*). A number of explanations for this apparent disparity exist. First, it is possible that B3/25 MoAb may not inhibit binding of TF by TFR on some cells such as the human leukemic cell line CCRF-CEM used by previous investigators (*19*) to investigate inhibition of binding, yet does interfere with binding in other cells such as our melanoma line. Second, B3/25 MoAb may not inhibit TF–TFR conjugation but may interfere with intracellular incorporation of the conjugate. Third, there may be two populations of TFR, both reactive to B3/25 MoAb but only one of which is inhibited by the antibody. Finally, it is possible, although unlikely, that we are observing a nonspecific cytotoxic effect on cellular metabolism produced by the B3/25 MoAb–tumor TFR interaction.

Trowbridge et al. (*19*) have developed another TFR antibody, designated 42/6, which does inhibit TF binding to TFR. It would be intriguing to determine any quantitative differences in inhibition of ^{67}Ga uptake produced by 42/6 MoAb versus B3/25 MoAb.

Our current study lends further support to two concepts regarding ^{67}Ga uptake in tumor. First, ^{67}Ga acts as an analog of ferric ion. Second, the TFR plays a major role in intracellular incorporation of ^{67}Ga. Recently investigation has begun into the use of TFR inhibitory antibodies for treatment of tumors (*17*). Our results suggest that ^{67}Ga imaging may be useful in determining which tumors might be suitable for such treatment and in monitoring the effectiveness of therapy.

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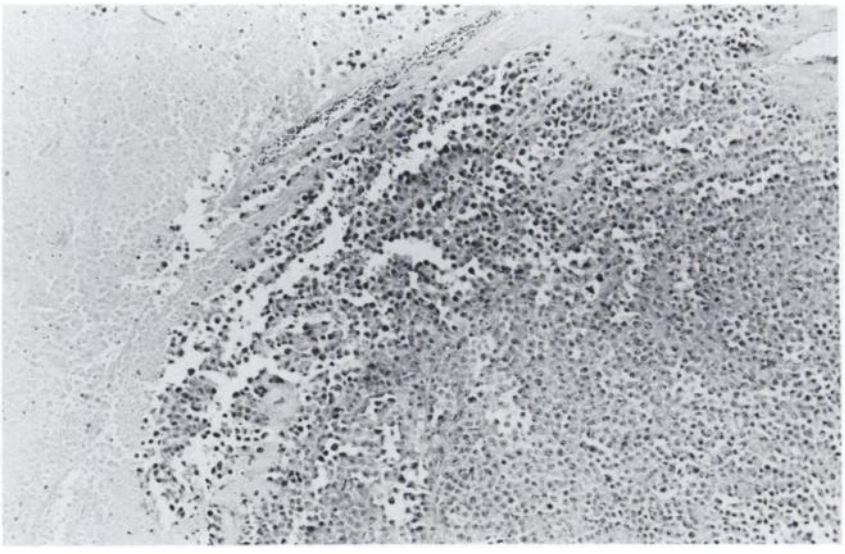

FIGURE 3
Melanoma nodule in a control mouse receiving only HSA in saline. Necrosis is visible at the left, which was near the center of the tumor nodule. The cytology appears similar in all tumors from treated (Figs. 1 & 2) and control (Fig. 3) animals. Hematoxylin and eosin × 125.

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NOTES

* Hybritech Inc., San Diego, CA.
† DuPont Company, North Billerica, MA.
‡ National Institutes of Health, Bethesda, MD.

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