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The Effect of Antibody Protein Dose of Anti–Renal Cell Carcinoma Monoclonal Antibodies in Nude Mice with Renal Cell Carcinoma Xenografts

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BACKGROUND. Antibodies preferentially can direct radionuclides to solid tumors. However, antibody uptake in tumors is often highly heterogeneous. This heterogeneity may be overcome by increasing antibody protein dose.

METHODS. The biodistribution of increasing protein doses of radioiodinated anti-renal cell carcinoma (RCC) monoclonal antibodies (MoAbs) G250 and RC 38 was studied in mice with NU-12 or SK-RC-52 RCC xenografts. In addition, MoAb affinity constants and antigen densities (Scatchard analysis) and MoAb processing (internalization) were determined in vitro.

RESULTS. The relative uptake of G250 in NU-12 tumors was very high at low protein doses (125% injected dose/g [%ID/g]), but decreased at higher doses, suggesting tumor saturation. Indeed, saturation of G250 antigen occurred at 3 μg protein. In this model, 9200 G250 determinants per NU-12 cell could be targeted, which is only 6.1% of the 150,000 G250 determinants per NU-12 cell as determined in vitro. The RC 38 uptake in NU-12 tumors remained constant up to the 10 μg dose level (40% *ID*/g) and decreased at higher doses. RC 38 antigens were saturated at 25 μg of RC 38. With RC 38, 15% of the available RC 38 antigens per NU-12 tumor cell were targeted. In contrast, G250 uptake in SK-RC-52 tumors was very low at low antibody dose (4% ID/g at 1 μg) and increased with increasing protein dose. These differences in G250 biodistribution might be related to differences in the processing of G250 by the tumor cells.

CONCLUSIONS. Our studies show that some RCC tumors can be saturated with anti-RCC MoAbs at low (25 μg) to very low (3 μg) protein doses. At nonsaturated doses relatively high tumor uptake can be achieved. Surprisingly, in NU-12 tumors only 6.1% and 15% of the available antigenic sites were targeted at the saturating dose levels with G250 and RC 38, respectively. ***Cancer* 1997;80:2390–7.**

KEYWORDS: antigen saturation, protein dose escalation, monoclonal antibody, renal cell carcinoma, radioimmunotargeting, animal studies.

Monoclonal antibodies (MoAbs) against tumor-associated antigens have been used successfully to direct radionuclides preferentially to solid tumors.[1–3] However, in general intratumor antibody uptake is highly heterogeneous and not all tumor regions are targeted adequately. This heterogeneous uptake might hamper the therapeutic applications of MoAbs. A number of parameters have been identified that may explain this heterogeneity, e.g., heterogeneous blood supply, high interstitial pressure, heterogeneous antigen expression, local necrosis, and locally enhanced vascular permeability.[4] The heterogeneous delivery of antibody in tumors also may be explained by anti-

body retention as a result of the successful binding to antigen on the tumor cell surface near blood vessels (i.e., the hypothesized binding site barrier).[5]

The heterogeneous antibody delivery to tumors may be overcome by using antibody fragments.[4] Yokota et al. have shown that intact immunoglobulin (Ig) G is concentrated in the region of or immediately adjacent to tumor blood vessels, whereas single chain Fv was distributed more evenly throughout the tumor mass.[6] Secondly, in a mathematic model Fujimori et al. suggested that antibody penetration can be enhanced by lowering the affinity constant of the antibody or by increasing the antibody dose.[5] Autoradiographic studies have shown that at higher antibody protein dose or lower affinity, MoAbs distributed more evenly in tumors xenografted in nude mice.[7]

In studies in renal cell carcinoma (RCC) patients the uptake of the anti-RCC MoAb G250 differed dramatically within and between tumors.[8,9] To assess this variation in tumor uptake, the effects of antibody protein dose on tumor uptake in nude mice with RCC tumors were studied.

MATERIALS AND METHODS

Antibody Preparation and Radiolabeling

The anti-RCC MoAb G250 (IgG1) is directed against the RCC-associated antigen G250. This antigen is expressed in 75% of RCCs, whereas expression in normal tissues is restricted to gastric mucosal cells and cells of the larger bile ducts.[10] The G250 antigen is homologous with the MN antigen, a recently cloned human tumor-associated protein.[11] The anti-RCC MoAb RC 38 (IgG1) is directed against human aminopeptidase-A. This antigen is expressed in 95% of RCCs and in 60% of metastatic RCC lesions. The RC 38 antigen is expressed in adult kidney and in several other normal tissues.[12] The anticarcinoembryonic antigen MoAb MN-14 (IgG1), which is not reactive with RCC, was used as a control antibody.[13]

Antibodies were radioiodinated with iodine-125 (^{125}I) (Amersham International, Buckinghamshire, United Kingdom) or ^{131}I (Nordion Europe, Fleurus, Belgium) using the iodogen method.[14] In all preparations >95% of the radioactivity was associated with the antibodies as determined by instant thin-layer chromatography.

The immunoreactive fraction of ^{125}I-G250, ^{131}I-RC 38, and ^{131}I-MN-14 essentially was determined as described by Lindmo et al. using SK-RC-52 RCC cells, NU-12 RCC cells, and LS180 colon carcinoma cells, respectively.[15] The immunoreactive fraction of ^{125}I-G250 was ≥85%, the immunoreactive fraction of ^{131}I-RC 38 was ≥70%, and the immunoreactive fraction of ^{131}I-MN-14 was ≥95%.

RCC Xenografts and Cell Lines

The NU-12 RCC xenograft was established from a primary tumor by serial subcutaneous (S.C.) transplantation in nude mice.[16] From this established tumor NU-12 also was propagated in vitro. The RCC cell line SK-RC-52 was derived from a mediastinal metastasis of a primary RCC.[17]

Biodistribution Studies

RCC tumors were initiated in 6-week-old BALB/c *nu/nu* mice. NU-12 was transplanted serially by implanting viable tumor specimens (1–2 mm^3) sc in the flank of the mice. SK-RC-52 cells were trypsinized, washed in saline, and 2×10^6 cells were injected sc into the flank of the mice. Experiments began when tumors weighed 0.02–0.5 g. Each group contained 3–5 mice. To study the biodistribution at different antibody protein doses (0.3, 1, 3, 10, 30, or 100 μg/animal), increasing amounts of unlabeled antibody were added to the radioiodinated antibody preparation. ^{125}I-G250 and ^{131}I-MN-14 or ^{125}I-G250 and ^{131}I-RC 38 were coinjected intravenously and after 3 days the biodistribution of the radiolabeled antibodies was determined. The amount of activity in the whole tumor and tissues (blood, muscle, liver, kidney, and spleen) was determined by gamma counting together with an injection standard. The percentage of injected dose per g (%ID/g), the absolute amount of antibody bound per g of tumor, and the tumor-to-blood ratios were calculated. Statistical analysis was performed using one-way analysis of variance, with Bonferroni posttest correction for multiple comparisons.

Scatchard Analysis

The affinity constant for the G250 antigen and the number of binding sites per NU-12 or SK-RC-52 cell were determined by Scatchard analysis on single cell suspensions of cultured cell lines.[18] In addition, Scatchard analysis was performed for the MoAb RC 38 on NU-12 cells.

Immunohistochemistry

RCC tumors were snap frozen in isopentane. Cryostat sections (4 μm) were fixed for 10 minutes in acetone. G250 antigen expression was determined using mouse/human chimeric MoAb G250 (a gift from Dr. S. O. Warnaar, Centocor BV, Leiden, The Netherlands) followed by rabbit antihuman Fc-peroxidase (DAKO A/S, Glostrup, Denmark). RC 38 antigen expression was determined using the MoAb RC 38 and rabbit antimouse-peroxidase (DAKO A/S) sequentially. Peroxidase activity was revealed using 3-3′-diaminobenzidine/0.03% hydrogen peroxide (Fluka Chemie, Bornem, Belgium).

Retention and Processing of G250 In Vitro

The internalization and degradation rate of ^{125}I-G250 by NU-12 and SK-RC-52 cells was measured in vitro essentially as described by Kyriakos et al.[19] Briefly, confluent NU-12 or SK-RC-52 cells were incubated for 2 hours at 37 °C with 7.5 or 3.8 ng of ^{125}I-G250 in 200 μL tissue culture medium, respectively. To determine nonspecific binding, a large excess unlabeled G250 was added to control wells. After washing to remove unbound ^{125}I-G250, the cells were cultured from 0 to 24 hours in 200-μL culture medium at 37 °C. Supernatant fluids were collected at various timepoints and trichloroacetic acid- (TCA)-precipitable (large fragments of G250 or intact G250) and non-TCA-precipitable (internalized and subsequently processed G250) activity was determined. The cells were washed twice and collected by solubilization with 2 M NaOH. Solubilized cells, TCA-precipited culture medium, and TCA supernatant fluid were counted in a shielded well-type gamma counter.

RESULTS

Biodistribution of ^{125}I-G250 in Mice with NU-12 Tumors

The effect of increasing G250 antibody protein dose on tumor uptake and tumor-to-blood ratios was determined in nude mice with a NU-12 tumor. MN-14 was used as a control antibody. Injection of 0.3 or 1 μg of the MoAb G250 resulted in very high ^{125}I-G250 tumor uptake (approximately 125%ID/g) (Fig. 1A). However, at higher protein doses the tumor uptake gradually decreased and was significantly lower compared with the uptake at the 0.3- and 1-μg dose levels, suggesting antigen saturation in the tumor. The uptake of ^{131}I-MN-14 in the tumors was similar at each dose level investigated (2.79 ± 0.67 %ID/g). The tumor uptake of ^{125}I-G250 was significantly higher than ^{131}I-MN-14 at each dose level, indicating specific G250 tumor uptake. The amount of ^{125}I-G250 in the blood at the 0.3-μg injected dose was significantly lower compared with 10 and 30 μg of G250 (Table 1). The amount of ^{125}I-G250 in the muscle, liver, spleen, and kidney was not influenced by the G250 antibody dose (Table 1). Tumor-to-blood ratios >10 were obtained only at the 0.3- and 1-μg dose levels. At higher protein dose levels the tumor-to-blood ratios were significantly lower compared with those at the 0.3- and 1-μg dose levels (Table 1).

The absolute amount of specifically accumulated G250 at the tumor site was calculated at every dose level (Fig. 2A). The specific tumor uptake was defined as the amount of G250 (μg) that accumulated per g of tumor at a particular protein dose, minus the amount of irrelevant MN-14 antibody present in the tumor at that protein dose. A linear increase of the absolute amount G250 targeted to the tumor was observed up to 3 μg of G250, after which point a plateau was reached. Thus, tumor saturation was achieved at 3-μg doses of the MoAb G250, because absolute amounts of G250 accumulating specifically in the tumor did not increase at higher dose levels. Approximately 2.3 μg of G250 can be targeted per g of NU-12 tumor at the saturating antibody dose level.

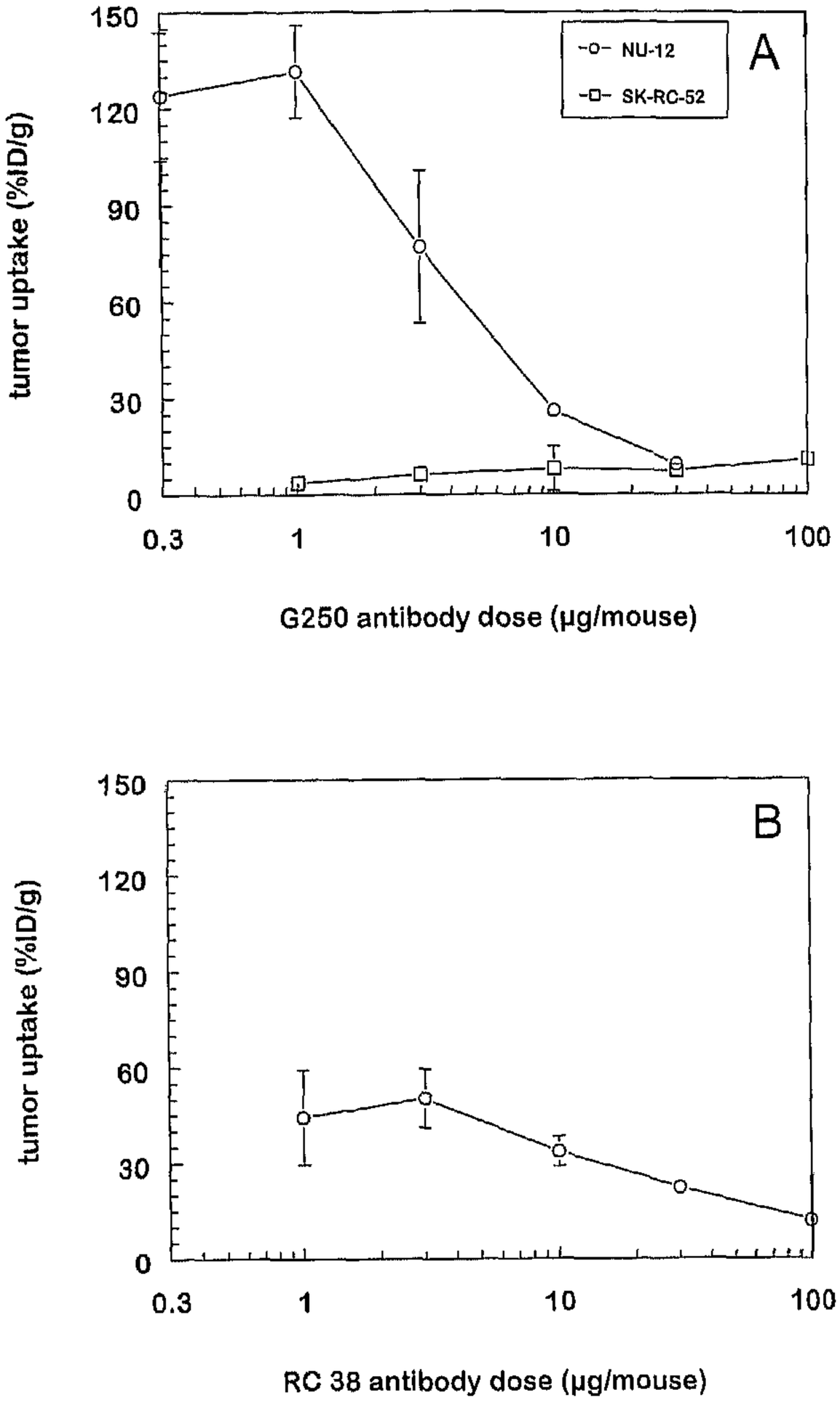

FIGURE 1. Tumor uptake of (A) iodine-125 (^{125}I)-G250 in NU-12 and SK-RC-52 tumors and tumor uptake of (B) ^{131}I-RC 38 in NU-12 tumors at protein doses varying from 0.3–100 μg/mouse. Error bars represent standard deviation (n = 3–5). ID/g: percent injected dose per g.

Biodistribution of ^{131}I-RC 38 in NU-12 Xenografts

To determine whether the G250 antigen saturation at a relatively low protein dose level in NU-12 tumors was an antibody specific phenomenon, the biodistribution of the RC 38 MoAb was determined in the NU-12 model at various protein doses. Uptake of ^{131}I-RC

TABLE 1
Biodistribution of G250 and RC 38 Anti-RCC Antibodies at Different Protein Doses in Different RCC Tumor Models

		Uptake (%ID/g) and TBR for different protein doses					
MoAb/Tumor	Organ	0.3 μg	1 μg	3 μg	10 μg	30 μg	100 μg
G250/NU-12	Blood	9.36 ± 1.07	11.73 ± 1.44	12.57 ± 1.9	13.33 ± 0.86	13.36 ± 0.49	
	Muscle	0.95 ± 0.32	1.09 ± 0.17	1.49 ± 0.48	0.86 ± 0.050	0.86 ± 0.12	
	Tumor	123.92 ± 20.00	131.57 ± 14.39	76.84 ± 23.84	25.86 ± 0.65	9.21 ± 0.60	
	Liver	3.07 ± 0.29	4.33 ± 0.32	4.68 ± 0.98	4.71 ± 0.79	4.46 ± 0.41	
	Kidneys	2.79 ± 0.58	3.65 ± 0.39	4.01 ± 0.58	4.08 ± 0.65	3.90 ± 0.21	
	Spleen	2.32 ± 0.29	2.87 ± 0.62	3.46 ± 0.74	3.21 ± 0.38	3.40 ± 0.46	
	TBR	13.19 ± 0.66	11.86 ± 2.63	6.25 ± 1.07	1.95 ± 0.15	0.69 ± 0.03	
RC 38/NU-12	Blood		9.74 ± 2.29	8.63 ± 0.78	10.58 ± 2.00	9.36 ± 1.33	11.14 ± 1.74
	Muscle		0.70 ± 0.17	0.72 ± 0.064	0.80 ± 0.13	0.68 ± 0.078	0.82 ± 0.23
	Tumor		44.42 ± 14.79	50.30 ± 9.14	33.67 ± 4.64	22.27 ± 2.09	11.91 ± 2.85
	Liver		2.43 ± 0.68	2.58 ± 0.81	3.13 ± 0.51	3.07 ± 0.75	3.43 ± 0.39
	Kidneys		2.52 ± 0.48	2.13 ± 0.30	3.09 ± 0.42	2.86 ± 0.42	3.25 ± 0.40
	Spleen		2.36 ± 0.94	2.20 ± 0.78	2.30 ± 0.44	2.06 ± 0.69	2.61 ± 0.35
	TBR		3.96 ± 2.24	5.91 ± 1.59	3.32 ± 0.94	2.43 ± 0.55	1.07 ± 0.18
G250/SK-RC-52	Blood		0.39 ± 0.17	1.42 ± 0.38	2.61 ± 2.49	5.03 ± 1.16	8.21 ± 0.68
	Muscle		0.08 ± 0.02	0.40 ± 0.16	0.32 ± 0.23	0.93 ± 0.69	0.60 ± 0.12
	Tumor		3.57 ± 2.05	6.08 ± 1.39	7.84 ± 6.93	7.23 ± 0.23	10.79 ± 1.67
	Liver		0.46 ± 0.28	1.61 ± 0.16	1.76 ± 1.51	1.83 ± 0.34	3.26 ± 0.53
	Kidneys		0.26 ± 0.04	0.54 ± 0.09	0.92 ± 0.76	1.70 ± 0.47	2.82 ± 0.28
	Spleen		0.18 ± 0.01	0.42 ± 0.02	0.75 ± 0.65	1.10 ± 0.34	1.65 ± 0.06
	TBR		11.29 ± 9.14	4.56 ± 1.73	8.86 ± 11.10	1.49 ± 0.35	1.32 ± 0.22

RCC: renal cell carcinoma; MoAb: monoclonal antibody; TBR: tumor-to-blood ratio; %ID/g: percent injected dose per g.

38 in the tumor was similar at 1, 3, and 10 μg of RC 38 (Fig. 1B). However, when 30 μg of RC 38 was injected the %ID/g lowered significantly, and at 100 μg of RC 38 the uptake was significantly lower compared with doses of 1, 3, and 10 μg, indicating saturation of the RC 38 antigen. Specific targeting of RC 38 in the tumor was demonstrated by the significant higher ^{131}I-RC 38 uptake compared with ^{131}I-MN-14 uptake. The relative amount of ^{131}I-RC 38 in the blood, muscle, liver, kidneys, and spleen remained constant over the entire dose range (Table 1).

Calculation of the absolute amounts of RC 38 accumulating in the tumors showed a binding curve with an apparent plateau at >100 μg of RC 38, indicating saturation of RC 38 antigen in the tumor (Fig. 2B). Extrapolation of the data indicated that RC 38 antigen saturation in the tumor occurred at a dose of approximately 25 μg of the RC 38 antibody. At this saturation level approximately 7.5 μg of RC 38 was bound specifically per g of NU-12 tumor.

Biodistribution of ^{125}I-G250 in SK-RC-52 Xenografts

To investigate whether the G250 antigen saturation observed in the NU-12 model was unique for this particular tumor, a similar G250 protein dose escalation study was performed in the SK-RC-52 tumor model. In this model the biodistribution of ^{125}I-G250 was completely different compared with the NU-12 model. In contrast to the high G250 uptake in the NU-12 tumors, very low G250 tumor uptake was observed at low protein dose levels in the SK-RC-52 model (Fig. 1A). Tumor uptake increased to 10.79 %ID/g at 100-μg injected dose. The uptake of ^{131}I-MN-14 in the tumor remained constant (2.65 ± 0.82 %ID/g) over the entire dose range. At the 1-μg dose the %ID/g of ^{125}I-G250 and ^{131}I-MN-14 were not significantly different in the tumor, whereas at higher dose levels the amount of ^{125}I-G250 in the tumor was significantly higher. In addition, ^{125}I-G250 blood levels were extremely low (0.39 ± 0.17 %ID/g) at the lowest protein dose investigated (1 μg) (Table 1). The amount in the blood increased to 8.21 ± 0.68 %ID/g at the 100-μg dose level.

Scatchard Analysis

Scatchard analysis of G250 binding revealed that the number of antigenic sites per cell varied for both cell lines. SK-RC-52 cells contained 600,000 sites per cell and NU-12 contained 150,000, with a similar affinity constant (K_a) (0.96 nM^{-1} vs. 1.2 nM^{-1}) (Fig. 3). The number of RC 38 binding sites on NU-12 cells was 200,000, which was similar to the number of G250

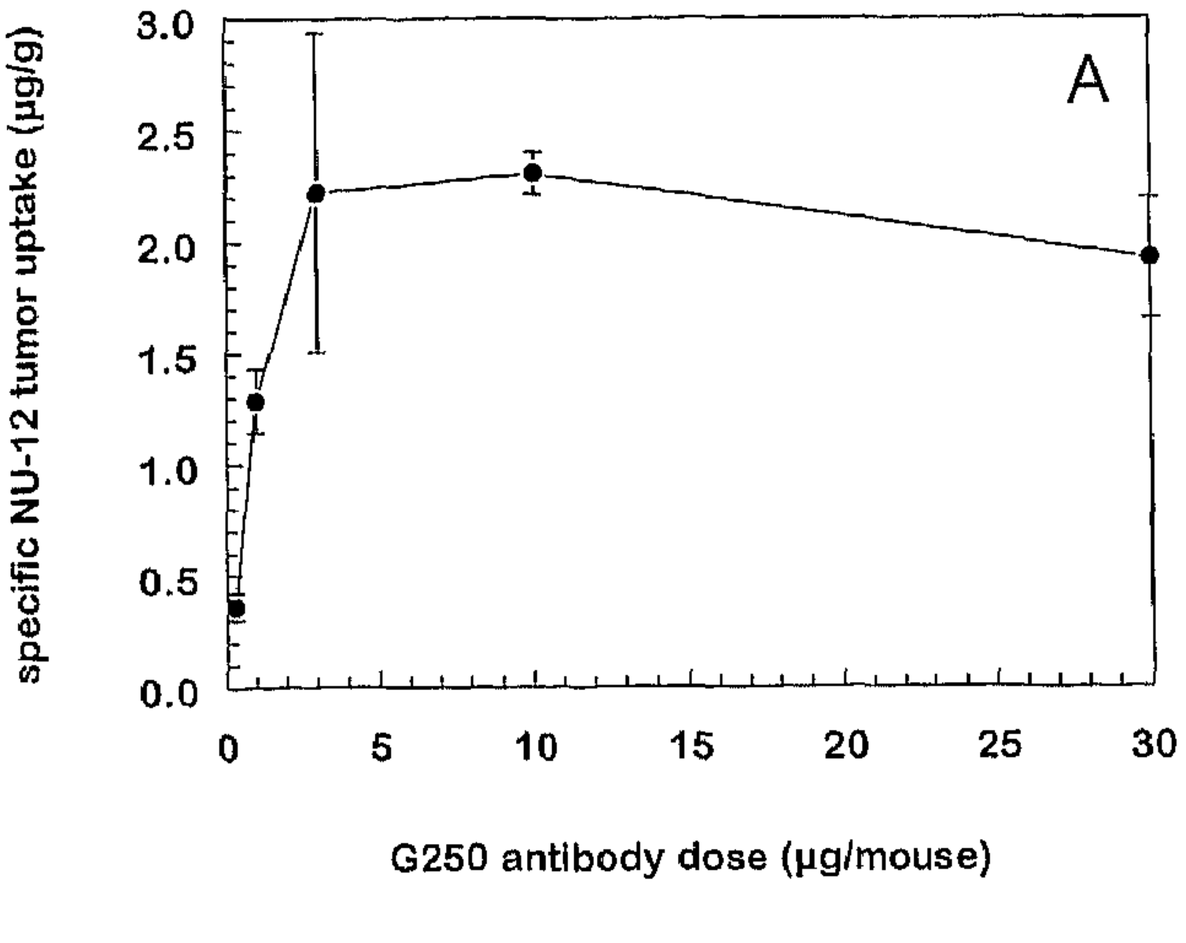

FIGURE 2. The absolute amount of (A) iodine-125 (^{125}I)-G250 and (B) ^{131}I-RC 38 specifically accumulating in NU-12 tumors. Error bars represent standard deviation (n = 5).

binding sites on these cells. The K_a of RC 38 (0.19 nM^{-1}) was 6.3-fold lower than the K_a of G250 (Fig. 3).

Immunohistochemistry

The intratumoral distribution of the G250 antigen was determined in NU-12 and SK-RC-52 xenografts. Membranous G250 antigen expression was observed in >70% of the cells in both xenografts. The RC 38 antigen was expressed on the membrane in approximately 80% of the cells in the NU-12 tumor.

Retention and Processing of G250 Bound to Tissue Culture Cells

In Figure 4 the retention and processing of G250 by RCC cells is shown. At 24 hours most of the ^{125}I-G250 was cell-associated for both cell lines. Processing of G250 was not very rapid because <20% of G250 was processed by the

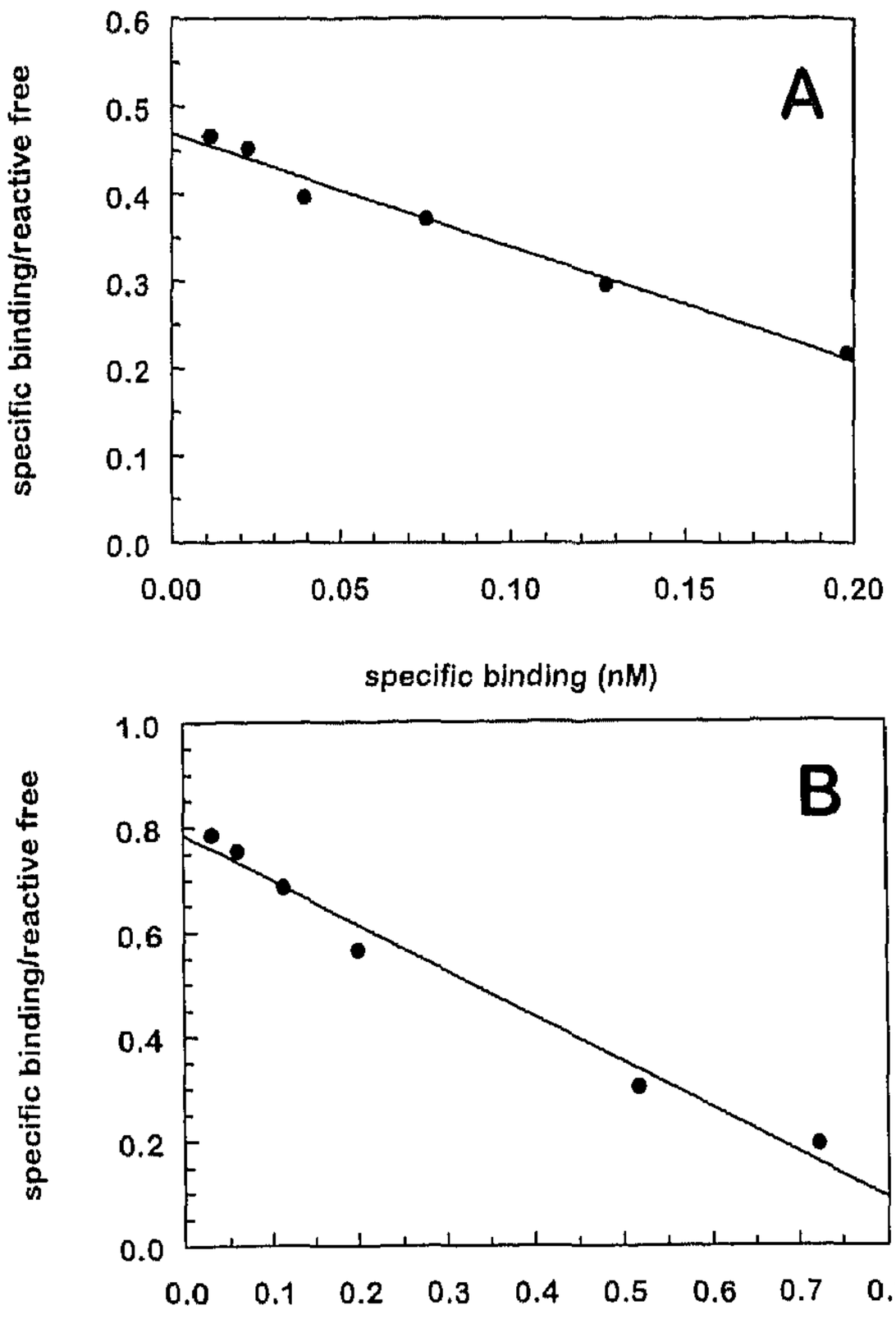

FIGURE 3. Scatchard analysis of (A) iodine-125 (^{125}I)-G250 binding to NU-12 cells, (B) ^{125}I-G250 binding to SK-RC-52 cells, and (C) ^{131}I-RC 38 binding to NU-12 cells.

cells at 24 hours. However, processing of G250 (i.e., internalization and degradation of the antibody and subsequent excretion of the radiolabel) by SK-RC-52 cells was faster than processing by NU-12 cells (17% vs. 8% non-TCA-precipitable activity at 24 hours).

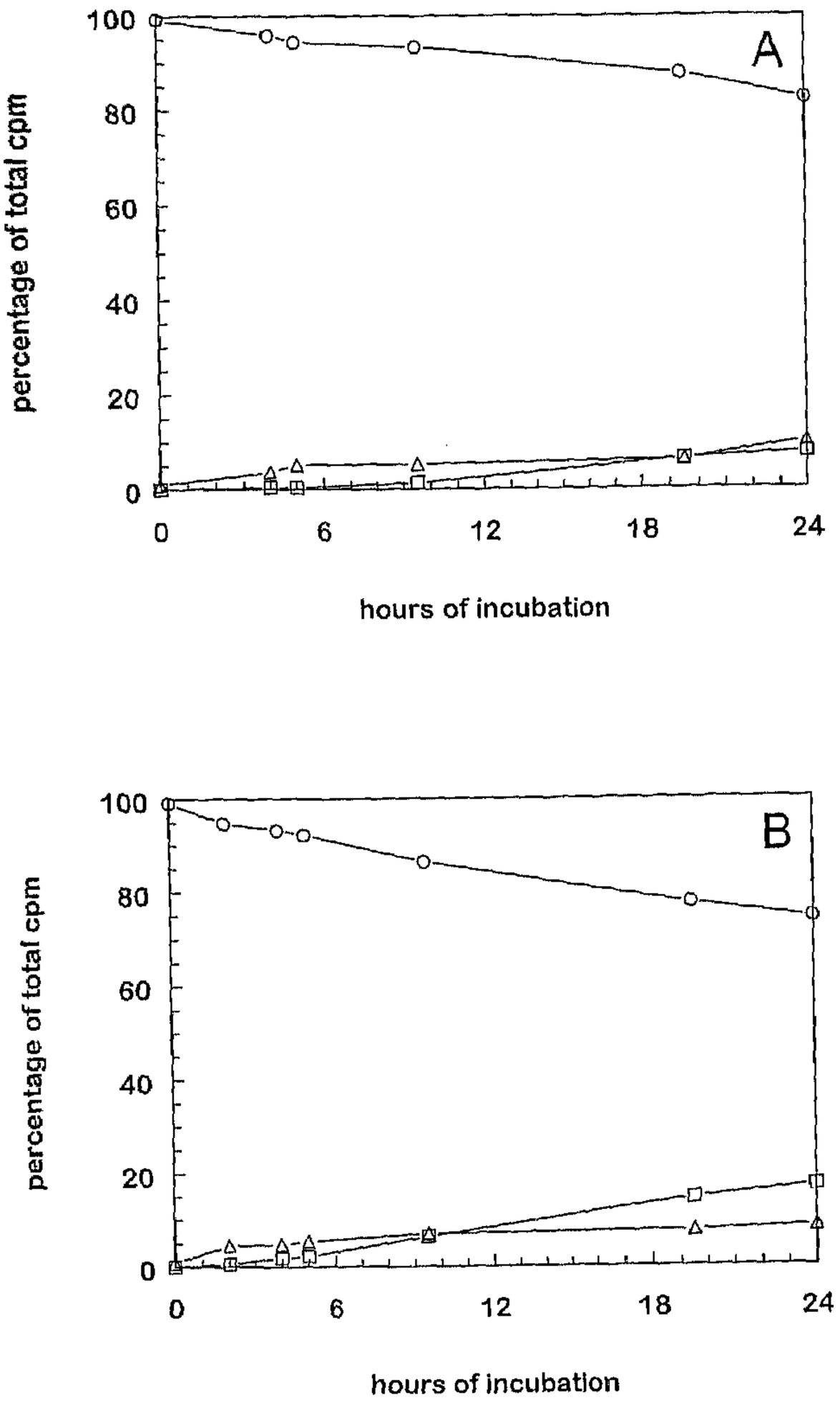

FIGURE 4. Retention and processing of iodine-125 (^{125}I)-G250 bound to (A) NU-12 cells, and (B) SK-RC-52 cells. Percentage of total counts per minute (cpm) retained by the cells (open circles), TCA-precipitable supernatant fluid (open triangles) and non-TCA-precipitable supernatant fluid (open squares) are displayed.

DISCUSSION

The MoAb G250 has been used successfully to direct ^{131}I to RCC tumors in RCC patients.[8,9] However, intratumor uptake was highly heterogeneous, which might hamper the therapeutic applications of the MoAb G250.[8] Heterogeneity in antibody distribution may be overcome by increasing the antibody dose.[5] In the current study the effect of antibody protein dose on the biodistribution of anti-RCC MoAbs in nude mice bearing RCC xenografts was investigated.

G250 antibody uptake in NU-12 tumors was very high at the lowest injected doses (approximately 125% ID/g). However, at doses exceeding 1 μg the %ID/g gradually decreased with apparent tumor saturation occuring at a dose of 3 μg. High tumor-to-blood ratios were achieved at the lowest protein doses, mainly due to the high tumor uptake at these dose levels. Although tumor saturation has been described, saturation at this low antibody dose level is uncommon. Boerman et al. and Fenwick et al. observed saturation of antigens at doses >100 μg using anticolon carcinoma antibodies in animals with a GW-39 colon carcinoma xenograft.[20,21] The animal data obtained with the NU-12 model are strikingly in line with the human data obtained in a Phase I antibody dose escalation study using radioiodinated chimerized MoAb G250 in RCC patients; at lower protein doses relatively high tumor uptake was observed, whereas at doses >10 mg, 10-fold lower tumor uptake was observed, suggesting that saturation of available G250 epitopes occurred when relatively low G250 protein doses were administered intravenously to RCC patients.[8]

To determine whether the observed G250 tumor saturation was unique for the G250 antigen, we tested another anti-RCC MoAb. For the RC 38 MoAb tumor saturation also was observed. However, the level of antigen saturation was different for both antibodies (3 μg for G250 vs. 25 μg for RC 38). The maximum amount of G250 bound in the tumor at the saturation dose level was 3-fold lower than the maximum amount of RC 38 bound in the tumor (2.3 vs. 7.5 μg/g). This might be explained by the somewhat lower number of G250 antigenic sites per NU-12 cell and/or the somewhat higher K_a of G250.

Using the anti-RCC MoAb A6H, Sands et al.[22] and Palme et al.[23] also found high tumor uptake in RCC nude mouse models, which was attributed to the increased tumor permeability in RCC compared with non-RCC.[22] In our study we observed high tumor uptake with G250 and moderately high uptake with RC 38 in the same NU-12 model, indicating that other factors also play a role in RCC tumor uptake of MoAbs.

The biodistribution of G250 also was investigated in another RCC model to determine whether the observed G250 saturation was specific for the particular model used. In the SK-RC-52 model the biodistribution of G250 was completely different. Three days after injection of low protein doses extremely low blood and low tumor levels were found. In contrast, in nontumor-bearing mice ^{125}I-G250 blood levels were independent from G250 dose (approximately 10.7 %ID/g), indicating that the SK-RC-52 tumor had a major effect on the biodistribution of G250. Despite low tumor uptake, high tumor-to-blood ratios were obtained at the lowest protein dose levels due to the extremely low G250 blood levels. Tumor saturation was not observed as a result of the rapid clearance of G250 in SK-RC-52 tumor-bearing mice.

What might explain the extreme differences in the biodistribution of G250 in these different tumor models? Internalization of G250 by the tumor cells may cause rapid processing and subsequent excretion of radioiodinated G250 in the SK-RC-52 model. G250 was degraded slightly faster by SK-RC-52 cells compared with NU-12 cells as determined by the antibody processing assay. It is possible that in vivo this difference in antibody processing is more pronounced, thereby explaining the very low amounts of G250 in the tumor and in the circulation in mice with SK-RC-52 tumors. Vascular permeability, which can be determined using an irrelevant antibody,[22] may determine the absolute antibody tumor uptake. Because the biodistribution of the control IgG in the models studied was similar, it is unlikely that the different biodistribution of G250 can be attributed to vascular permeability differences. Different antigen distribution also does not appear to play a role; approximately 70–80% of the tumor cells expressed G250 antigen in both tumors. Another explanation might be that G250 antigen density drives the G250 MoAb targeting. However, no relation between the number of G250 sites per cell and tumor uptake was obvious. SK-RC-52 contained a fourfold higher number of antigenic sites per cell, whereas in vivo very low tumor uptake was observed at low antibody doses. Alternatively, shedding of G250 antigen might be more prominent in SK-RC-52 tumors, causing differences in tumor targeting. However, in tissue culture supernatant fluid from NU-12 and SK-RC-52 cells no G250 antigen could be detected (data not shown).

From the amount of G250 specifically bound per g of NU-12 tumor at the saturation dose the number of antibody molecules targeted per tumor cell were calculated. Assuming 10^9 cells per g of tumor, 2.3 μg of G250 specifically bound per g of tumor represents 9200 antibody molecules per cell. In contrast, Scatchard analysis on cultured cells demonstrated the presence of 150,000 antigenic sites per NU-12 cell. Provided that expression levels in vivo are similar, this would indicate that only 6.1% of the antigenic determinants present were accessible for G250 antibody binding in the xenografted tumor. With RC 38 only 30,000 of 200,000 of the RC 38 sites (15%) were targeted at maximum tumor uptake.

It is interesting to note that tumor saturation also was observed at relatively low G250 protein dose levels (≥25 mg) in G250-treated RCC patients. Injection at saturated G250 levels (25 or 50 mg) resulted in a tumor uptake of 0.0015 %ID/g. This would mean that 1500–3000 G250 epitopes per cell were targeted in these patients. These numbers are strikingly similar to the numbers calculated for our NU-12 model.

These studies indicate that some RCC tumors can be saturated at relatively low antibody protein doses. At nonsaturated dose levels, very high tumor uptake and very high tumor-to-blood ratios can be achieved. At maximum tumor uptake only a minority (6.1 and 15%, respectively) of the antigenic determinants in the tumor were targeted in the experimental NU-12 RCC tumor.

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