Melanoma Targeting with a Cocktail of Monoclonal Antibodies to Distinct Determinants of the Human HMW-MAA

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The monoclonal antibodies (MoAbs) 149.53, 225.28, and 763.74 which recognize distinct and spatially distant determinants of the human high molecular weight-melanoma associated antigen (HMW-MAA) do not influence the binding of each other to cultured human melanoma cells. In vitro incubation of melanoma cells with a combination of the three ^{125}I-labeled anti-HMW-MAA MoAbs results in a marked additive binding only when the MoAbs are used at saturating concentrations. Injection of the combination of the three ^{125}I-labeled MoAbs (up to 300 μg per mouse) into human melanoma-bearing nude mice does not increase the amount of radioactivity specifically localized in melanoma lesions above the level observed upon injection of corresponding doses of individual MoAbs. These results may reflect the low concentration of MoAbs which reaches tumor lesions in vivo. Therefore, administration of combinations of MoAbs to distinct determinants of HMW-MAA may not increase the sensitivity of immunoscintigraphy to visualize lesions in patients with melanoma.

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By utilizing monoclonal antibodies (MoAbs), two human melanoma associated antigens (MAA) have been identified that meet the criteria to be useful markers for immunoscintigraphy, i.e., the p97 MAA (*1*) and the high molecular weight-melanoma associated antigen (HMW-MAA) (*2*). Investigations performed in a large number of patients with melanoma have shown that immunoscintigraphy with radiolabeled anti-p97 MAA and anti-HMW-MAA MoAbs may provide clinically useful information, since it can identify lesions which had not been detected with other clinical investigations and laboratory tests (*1,3,4*). However, the clinical application of immunoscintigraphy with radiolabeled MoAbs has been hampered by its limited sensitivity, which has allowed the detection of ~70% of the lesions tested (*1,3,4*).

In vitro studies have shown recently that incubation of melanoma cell lines and melanoma cells isolated from surgically removed melanoma lesions with a cocktail of anti-p97 MAA and anti-HMW-MAA MoAbs increases the amount of bound MoAbs as compared to that bound following incubation with an equal amount of individual MoAbs (*5*). Furthermore, incubation of cultured melanoma cells and surgically removed melanoma lesions with a cocktail of MoAbs to distinct determinants of HMW-MAA enhances the intensity of staining without affecting its specificity (*6*). These results suggest that the use of a combination of MoAbs recognizing distinct determinants of HMW-MAA may increase the sensitivity of immunoscintigraphy. In the present investigation we have tested this possibility by comparing the amount of radioactivity accumulated in melanoma lesions when xenografted nude mice were injected with a cocktail of three MoAbs to distinct determinants of HMW-MAA and with an equivalent amount of individual MoAbs.

MATERIALS AND METHODS

Cell Lines

Human melanoma cell lines Colo 38 and MeWo were grown in medium RPMI 1640 supplemented with 10% fetal calf serum and 2 m*M* l-glutamine. Melanoma cells (5×10^6) were injected subcutaneously into the flank of 6–8-wk-old

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nude mice (BALB/c background, Zentralinstitut für Versuchstiere, Hannover, FRG). Animals were entered into the experiments when tumor diameters reached 10–15 mm. A moderate proportion of necrotic tissue was observed in xenografts >7–10 mm.

Monoclonal Antibodies

The anti-HMW-MAA MoAbs 149.53 (IgG_1), 225.28 (IgG_{2a}), and 763.74 (IgG_1) have been developed and characterized as previously described (*7*). Scatchard plot analysis with melanoma cells Colo 38 showed that the MoAbs 149.53, 225.28, and 763.74 display association constants of 1.2×10^9, 8.0×10^8, and 4.6×10^8 M^{-1}, respectively. The MoAb HOPC1 (IgG2a) has no known specificity and was used as a specificity control.

MoAbs were purified from ascites by sequential chromatography on Protein A Sepharose (Pharmacia, Freiburg, FRG) and Mono Q anion exchange columns (Pharmacia). Purity of MoAbs was monitored by sodium dodecylsulfate polyacrylamide gel electrophoresis (*8*). MoAbs were labeled with iodine-125 (^{125}I) or iodine-131 (^{131}I) using the IODO-GEN method (*9*) at a specific activity of 2–3 μCi (corresponding to 0.15–0.23 atoms of iodine per molecule of IgG) and 7–8 μCi per μg protein (corresponding to 0.52–0.60 atoms of iodine per molecule of IgG) in the laboratories of SM and SF, respectively. The immunoreactive fractions of various batches of radiolabeled MoAbs 149.53, 225.28, and 763.74 were measured by testing with melanoma cells Colo 38 as described (*10, 11*). Only batches displaying immunoreactive fractions of 55–59%, 69–80%, and 78–85% in laboratory SM, and 57%, 93%, and 93% in laboratory SF for MoAbs 149.53, 225.28, and 763.74, respectively, were used in the experiments shown.

Serological Assays

The binding assay was performed by mixing melanoma cells Colo 38 (1×10^5/50 μl phosphate-buffered saline containing 1% bovine serum albumin [PBS-BSA]) with 50 μl of PBS-BSA containing a total amount of either 10 ng or 100 ng of radiolabeled MoAbs. Following a 90-min incubation at 4°C on a rotator, cells were centrifuged for 3 min. The supernatant was then carefully aspirated and bound radioactivity was measured in a gamma counter. Results are expressed as ng of radiolabeled MoAb bound.

The inhibition binding assay was performed by incubating melanoma cells Colo 38 (2×10^4 per well) with radiolabeled MoAb (20,000 cpm, 7–8 ng/50 μl per well) in the presence of 2.5, 25, and 250 ng of cold MoAb. At the end of a 2-hr incubation at 37°C, cells were washed twice and radioactivity was counted. Results are expressed as % of radiolabeled MoAb bound.

Distribution of Radiolabeled Anti-HMW-MAA MoAbs in Nude Mice with Melanoma Xenografts

The paired label assay was performed as described (*12,13*). Briefly, animals received a single or a triple dose of ^{125}I-labeled anti-HMW-MAA MoAb, or a cocktail of the three ^{125}I-labeled anti-HMW-MAA MoAbs together with ^{131}I-labeled MoAb HOPC 1. After 48 hr, animals were placed in a whole-body counter to determine residual radioactivity as a measure of the stability of radioiodinated MoAbs. The value in the experiments of the present investigation was in the range of 44–53% of the injected radioactivity. Then animals were killed and dissected, and the wet weight of tissue samples was determined immediately thereafter. MoAb accumulation was expressed as % of the injected dose per g tissue (wet weight); tumor:tissue ratios were calculated from these values. Coefficients of variation were in the order of 10%. The statistical significance of differences in tumor:tissue ratios recorded with groups receiving different MoAb preparations were calculated by the Mann Whitney U-test. Specificity indices (*11*) were calculated according to the formula

$$SI = \frac{cts/g(tumor)_{anti\text{-}HMW\text{-}MAA}: cts/g(organ)_{anti\text{-}HMW\text{-}MAA}}{cts/g(tumor)_{Control.MoAb}: cts/g(organ)_{Control.MoAb}}.$$

Semiquantitative autoradiography was performed according to the following procedure. Animals with MeWo transplants received 30 μCi (10 μg) of ^{125}I-labeled MoAb 225.28 either alone or in admixture with 100 μg or 300 μg of cold MoAb 225.28. After 48 hr, mice were killed. Excised tumors were embedded in methylcellulose and 20-μm cryotome sections were prepared. They were placed on a Kodak X-omat AR film after lyophilization. Exposed films were subjected to scanning densitometry at 50 μm resolution. Digitized film density distribution was transformed into radioactivity distribution (arbitrary units proportional to radioactivity per pixel) on the basis of a calibration procedure using brain paste standards (*14*). The method is referred to as "semiquantitative" because no attempt has been made to relate calculated radioactivity levels per pixel to % injected dose per unit volume.

RESULTS

Inhibition binding assays with melanoma cells Colo 38 confirmed that the anti-HMW-MAA MoAbs 149.53, 225.28, and 763.74 recognize distinct and spatially distant antigenic determinants. Furthermore, the three MoAbs were shown not to interfere with each other, since coating of melanoma cells with one MoAb inhibited binding of the homologous MoAb in a dose-dependent manner, but did not affect that of the other two MoAbs (Fig. 1). Therefore, the three MoAbs were utilized to test the effect of combinations of MoAbs on the specific binding of radioactivity to cultured melanoma cells. Figure 2 shows a representative experiment. The combination of two or three MoAbs induced a two- to threefold increase in the amount of radioactivity bound to melanoma cells, when each MoAb was used at saturating concentrations. On the other hand, only a slight increase was detected at nonsaturating MoAb concentrations.

The distribution of radioactivity in human melanoma-bearing nude mice was analyzed first at a low dose level of radiolabeled MoAb. A standard dose of 10 μg of individual ^{125}I-labeled anti-HMW-MAA MoAbs was compared with a triple dose (30 μg) of individual MoAbs or with a total dose of 30 μg of the combination of the three anti-HMW-MAA MoAbs (10 μg of each). Results of a representative experiment performed in nude mice transplanted with human mel-

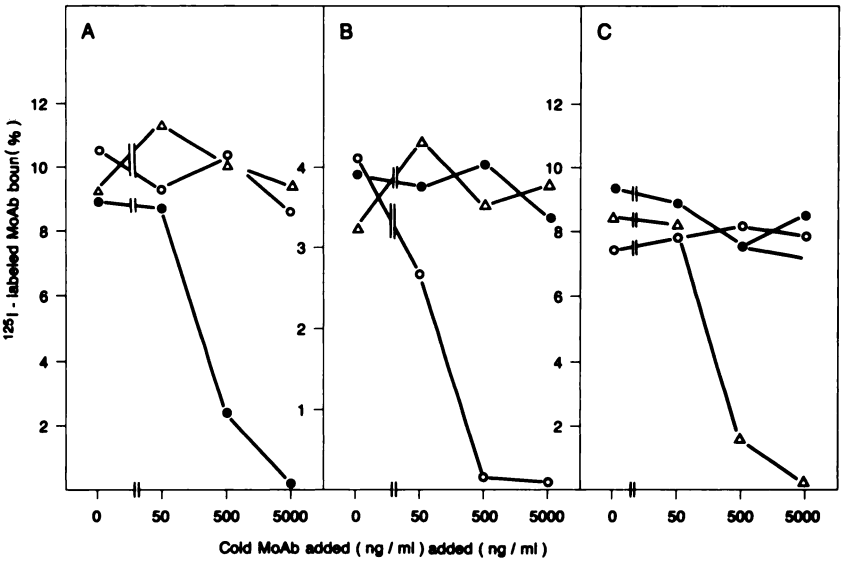

FIGURE 1
Mapping of the determinants recognized by MoAbs 149.53, 225.28 and 763.74 on HMW-MAA. Cultured melanoma cells Colo 38 were sequentially incubated with increasing amounts of cold MoAb 149.53 (●), 225.28 (△) or 763.74 (○) and with ^{125}I-labeled MoAb 149.53 (panel A), 763.74 (panel B) and 225.28 (panel C). At the end of incubation radioactivity bound to cells was measured in a gamma counter.

anoma cells Colo 38 are given in Table 1. The increase of the amount of individual MoAbs injected as well as the use of a cocktail of MoAbs 149.53, 225.28, and 763.74 did not enhance the amount of radioactivity accumulated in melanoma tumors. It should be noted that accumulation of radioactivity in the tumors was low in spite of the high in vitro binding of the three anti-HMW-MAA MoAbs to melanoma cells Colo 38. This discrepancy may reflect either reduced expression of HMW-MAA on melanoma cells Colo 38 transplanted in nude mice (results not shown), or differential accessibility of melanoma cells in the solid tumor nodule. A parallel experiment performed utilizing nude mice transplanted with human melanoma cells MeWo showed essentially similar data, although at a higher uptake level (not included). Since these results suggested that the amounts of MoAbs accumulated in melanoma lesions were insufficient to achieve saturation of binding sites, dose escalation experiments were performed, using xenografts of MeWo cells.

Xenografts of MeWo cells from nude mice injected with 10 μg, 110 μg, and 310 μg of MoAb 225.28 were subjected to semiquantitative autoradiography. By transforming x-ray film density into a parameter proportional to radioactivity per pixel, a highly heterogeneous distribution was recorded at low doses of MoAb. On the other hand, the distribution became fairly homogeneous at high doses of MoAb (Fig. 3). This observation suggests that the increase in tumor uptake associated with the increase in the dose of MoAb injected

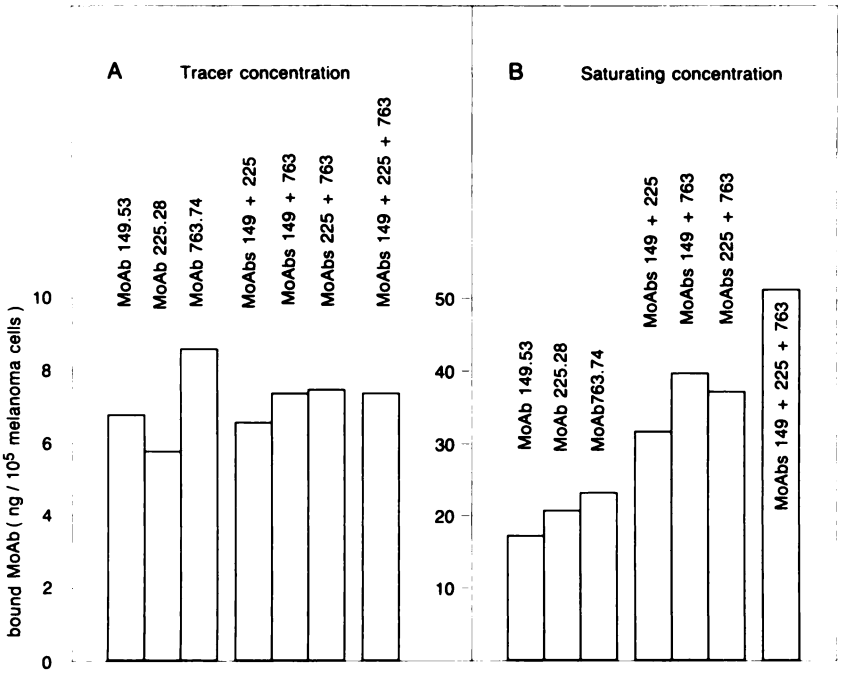

FIGURE 2
MoAb concentrations dependent increase of the radioactivity bound to cultured melanoma cells Colo 38 incubated with combinations of 125I-labeled MoAbs 149.53, 225.28 and 763.74. Cultured melanoma cells were incubated with a total amount of either 10 ng (panel A) or 100 ng (panel B) of individual ^{125}I-MoAbs or their combinations. At the end of the incubation radioactivity bound to cells was measured in a gamma counter.

TABLE 1
Effect of the Combination of Three Anti-HMW-MAA Monoclonal Antibodies on the Tissue Distribution of Radioactivity in Human Colo 38 Melanoma Lesion-Bearing Nude Mice: Low Dose Level

MoAb[†]	Dose	% Injected dose per gram tissue[*] Tumor	Blood	Spleen	Kidney	Liver	Muscle
763.74	10 μg	4.84	9.68	2.36	2.39	3.55	0.77
763.74	30 μg	5.21	11.16	2.13	3.27	3.40	1.05
225.28	10 μg	2.94	10.40	2.79	2.75	3.01	1.08
225.28	30 μg	2.64	7.31	2.88	2.30	3.30	1.29
149.53	10 μg	4.34	11.67	2.94	3.03	3.75	0.85
149.53	30 μg	3.44	12.06	3.56	2.64	3.96	0.98
Cocktail[‡]	3 × 10 μg	4.42	9.56	2.66	2.64	3.28	1.14

[*] Four mice were used for each MoAb; measurements were performed 48h following MoAb injection.
[†] MoAbs were labeled with ^{125}I (dose/mouse: 2 μCi, 10 μg or 6 μCi, 30 μg).
[‡] It contains MoAbs 225.28, 149.53, and 763.74 (dose/mouse 3 × 2 μCi, 3 × 10 μg).

results from enhanced MoAb binding in the central parts of the xenografts, thus leading to increased homogeneity of binding and eventually to saturation of binding sites. Concordantly, dissection of nude mice with xenografts of MeWo cells 48 hr after injection of [^{125}I]MoAb 225.28 mixed with escalating doses of cold MoAb 225.28 revealed a reduction of the fractional accumulation of radioactivity in lesions: 10.86% and 7.02% of injected dose per gram were recorded in the presence and absence, respectively, of 300 μg per mouse of cold MoAb ($p < 0.01$; Fig. 4). This difference was greater than that observed with the irrelevant MoAb HOPC 1 at the respective doses (3.26% vs. 2.95%, not significant).

Both types of dose escalation experiments suggest that injection of doses of MoAb higher than 100 μg/mouse results in a reduction of its relative uptake by melanoma xenografts, probably because of saturation of antigenic sites. Therefore, the experiment with the combination of the three MoAbs was repeated utilizing either the dose of 300 μg/mouse of MoAb 225.28 or the dose of 100 μg/mouse of each of the three MoAbs. When the dose of 300 μg/mouse of MoAb 225.28 was injected, the moderate reduction in the relative accu-

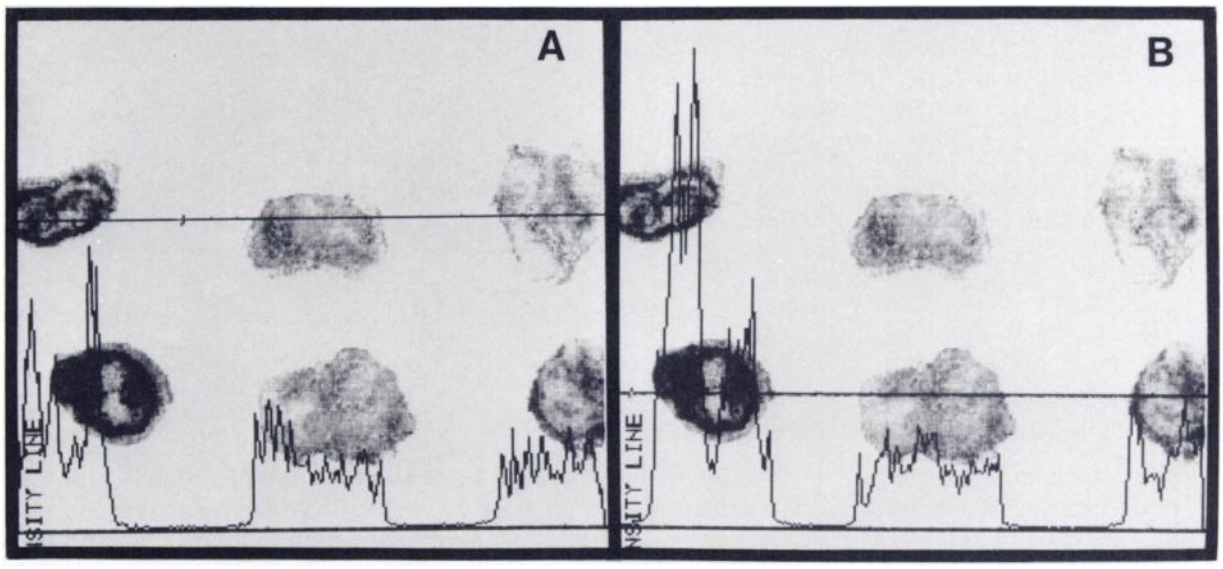

FIGURE 3
Radioactivity distribution in MeWo melanoma transplants 48 hr after injection of ^{125}I-MoAb 225.28 at three dose levels. Tumors (diameter 10–15 mm) were obtained from nude mice injected with 30 μCi (10 μg) of ^{125}I-MoAb 225.28 alone (left pair of sections derived from two different tumors) or with 30 μCi of ^{125}I-MoAb 225.28 mixed with 100 μg (middle pair of sections) or 300 μg (right pair of sections) of cold MoAb 225.28. The Figure shows the radioactivity distribution in tumor sections as calculated from the density distribution on the autoradiographic film by using a density-to-radioactivity calibration curve. Radioactivity profiles were placed through the upper row (panel A) and the lower row of sections (panel B) at positions marked by the horizontal lines. The ordinate is scaled in arbitrary units proportional to the amount of radioactivity per pixel. Note that radioactivity levels in the middle and right hand pairs of sections are lower than in the left hand pair of sections due to the co-injection of labeled and cold MoAb.

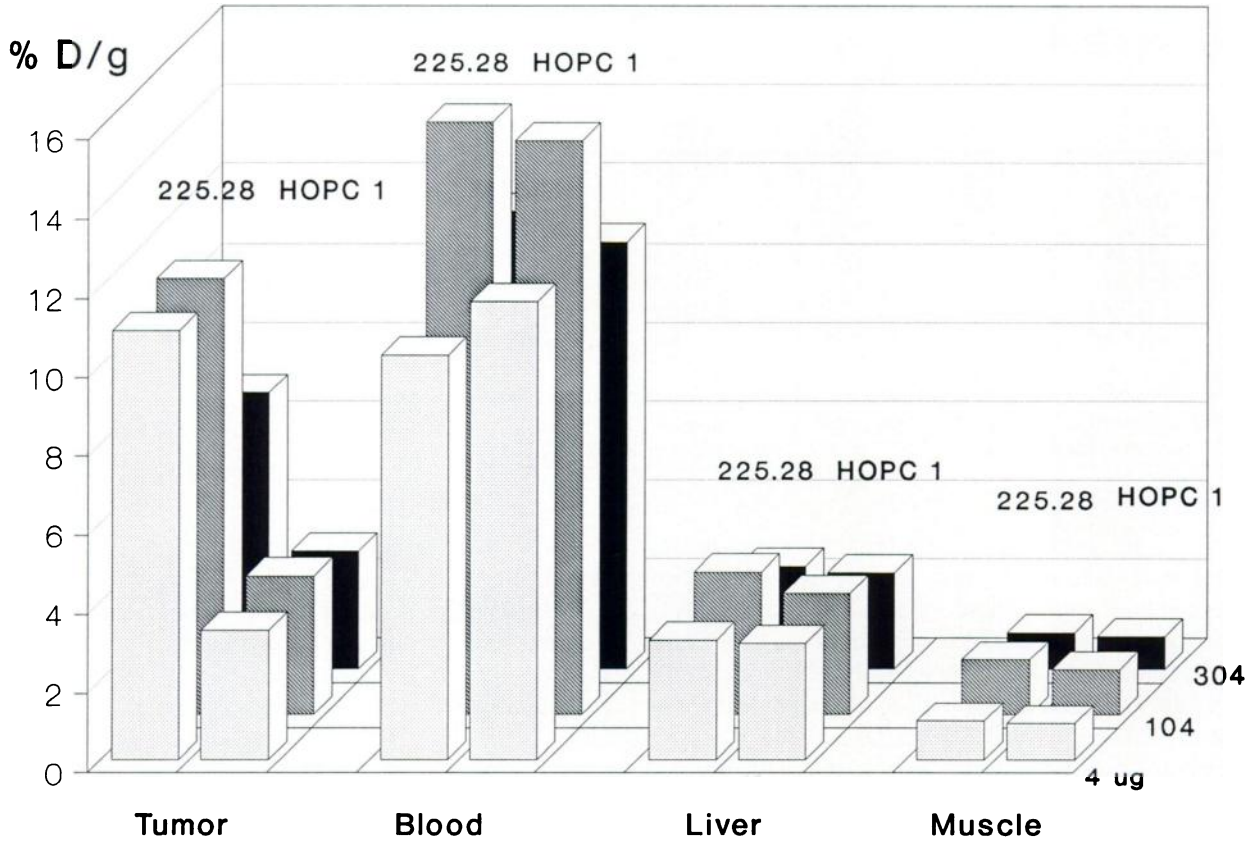

FIGURE 4
Effect of dose escalation on the biodistribution of ^{125}I-MoAb 225.28 in nude mice transplanted with human melanoma cells MeWo. Melanoma-bearing nude mice were injected with a mixture of ^{125}I-MoAb 225.28 (6 μCi) and ^{131}I-labeled control MoAb HOPC 1 (5 μCi) at three dose levels: stippled columns (front row), 3 μg MoAb 225.28 and 5 μg MoAb HOPC 1; hatched columns (middle row), 103 μg MoAb 225.28 and 105 μg MoAb HOPC 1; solid columns (back row), 303 μg MoAb 225.28 and 305 μg MoAb HOPC 1. Animals were killed 48 hr after injection. The ordinate gives the mean values of % injected dose per gram (D/g) from five animals.

mulation of MoAb 225.28 resulted in specificity indices which were lower than those obtained when the dose of 100 μg/mouse of MoAb 225.28 was injected. But the specificity indices were not improved by injecting a combination of 100 μg of the three MoAbs per mouse (Table 2).

DISCUSSION

Despite considerable progress in the specificity of MoAbs recognizing human MAA and in targeting methodology, the amount of radioactivity which accumulates specifically in melanoma lesions following injection of radiolabeled anti-MAA MoAbs is well below initial expectations. Strategies to improve this critical parameter are being investigated to increase the efficacy of immunoscintigraphy with radiolabeled MoAbs. The use of a combination of MoAbs to distinct MAA or to distinct determinants of a given MAA has found considerable interest, since it may also overcome the antigenic heterogeneity of melanoma cells. The latter represents a major limitation in the development of immunodiagnostic and immunotherapeutic approaches to melanoma. The present study, therefore, has tested the effect of a combination of three MoAbs to distinct determinants of HMW-MAA on accumulation of radioactivity in melanoma lesions. The HMW-MAA was selected, since it has already been shown to be a suitable marker for immunoscintigraphy (*2,4*). Furthermore, a number of determinants have been identified with murine MoAbs (*6,15*). Lastly, this molecule is located to a considerable extent in the outer cell coat and therefore the determinants are not likely to be inaccessible because of intra-membranous or intra-cytoplasmic localization. The MoAbs 149.53, 225.28, and 763.74 were selected from our large library of anti-HMW-MAA MoAbs, since they do not interfere with the binding of each other (*15*). Furthermore, they do not differ markedly from each other in terms of binding affinity and/or immunoreactivity following radiolabeling.

The present study has shown that in vitro reaction of melanoma cells with a cocktail of the three anti-HMW-

TABLE 2
Effect of the Combination of Three Anti-HMW-MAA Monoclonal Antibodies on the Tissue Distribution of Radioactivity in Human MeWo Melanoma Lesion-Bearing Nude Mice: High Dose Level

MoAb	Dose	% Appl. dose per gram tissue* Tumor	Blood	Spleen	Kidney	Liver	Muscle	Bone
225.28	100 μg	10.40	10.78	2.48	3.25	3.08	0.83	1.04
149.53	100 μg	6.60	13.00	2.32	3.91	3.56	1.18	1.62
763.74	100 μg	11.00	14.26	2.40	3.84	4.08	1.06	1.01
225.28	300 μg	10.37	13.76	3.25	3.53	3.88	1.16	1.51
Cocktail†	3 × 100 μg	10.05	13.53	2.71	3.53	3.61	1.02	1.26
HOPC 1	100 μg	3.26	14.05	1.91	3.41	3.60	0.91	1.00
HOPC 1	100 μg	4.25	16.00	1.99	3.10	4.12	1.06	1.31
HOPC 1	100 μg	3.88	16.10	2.15	3.42	3.70	0.99	1.15
HOPC 1	300 μg	3.22	17.68	2.41	4.43	4.21	1.30	1.60
HOPC 1	300 μg	4.17	17.12	2.51	4.15	4.71	1.09	1.28
					Specificity indices‡			
225.28	100 μg		4.17	2.45	3.37	3.71	3.50	3.07
149.53	100 μg		2.83	2.12	1.80	2.43	2.25	2.02
763.74	100 μg		3.21	2.76	2.86	3.03	2.71	3.34
225.28	300 μg		2.88	1.50	2.15	2.59	2.23	2.12
Cocktail	3 × 100 μg		3.08	2.06	2.52	2.46	2.49	2.37

* Six mice were used for each MoAb; measurements were preformed 48 hr postinjection. Anti-HMW-MAA MoAbs were labeled with ^{125}I (dose/mouse: 5 μCi, 100 μg, or 5 μCi, 300 μg). Control MoAb HOPC 1 was labeled with ^{131}I (dose/mouse: 5 μCi, 100 μg, or 5 μCi, 300 μg).

† It contains MoAbs 225.28, 149.53, and 763.74 (dose mouse 3 × 5 μCi, 3 × 100 μg).

‡ These were calculated as indicated in the "Materials and Methods" section.

MAA MoAbs results in an increase of the cell bound radioactivity only when cells are incubated with saturating concentrations of each MoAb. On the other hand, no increase in the radioactivity accumulated in melanoma lesions was detected following injection of a cocktail of low doses of anti-HMW-MAA MoAbs 149.53, 225.28, and 763.74 into nude mice as compared to those injected with a comparable amount of individual MoAbs. This conclusion was valid irrespective of whether % injected dose per g, tumor-to-tissue ratios, or specificity indices were used as parameters for the comparison. Escalation of injected doses up to 300 μg per mouse, which corresponds to ~1 g of MoAb per patient, resulted in a moderate reduction in the relative uptake. In addition, semiquantitative autoradiography of melanoma xenografts directly visualized increasing uniformity of the radioactivity distribution pattern, i.e., the more central parts of the xenografts, which were not stained at low doses of MoAb, were found to be stained at high doses. Both observations suggested saturation of antigenic sites to occur when this high dose of MoAb was used. Nevertheless, the injection of the cocktail of MoAbs resulted in no significant increase in the amount of radioactivity accumulated in melanoma lesions.

When discussing the results of the present study in light of the information available in the literature, one should distinguish between investigations performed with a cocktail of MoAbs recognizing distinct tumor associated antigens (TAA) and of those recognizing distinct determinants of a given TAA, as well as between investigations in tumor-bearing patients and in nude mice transplanted with human tumors. The approach used with MoAbs to distinct TAA aims at overcoming the heterogeneity in the antigenic profile of tumor cells. On the other hand, the approach used with MoAbs to distinct determinants of a TAA aims at increasing the number of antibody molecules targeted to a tumor cell and will therefore be effective only when MoAbs are used at saturating concentrations. Furthermore, in tumor-bearing patients anti-TAA MoAbs react not only with malignant lesions, but also with normal tissues expressing the same or crossreacting antigens. In nude mice transplanted with human tumors the reactivity of anti-TAA MoAbs with normal tissues is virtually absent. Therefore the effect of MoAb dose escalation on the distribution of radioactivity in tumor and nontumor compartments, which leads to shifts in contrast, can be analyzed in tumor bearing patients, but not in nude mice xenografted with human tumors. But the effect of combinations of anti-TAA MoAbs on the uptake of radioactivity by tumor cells can be evaluated more

effectively in human tumor-bearing nude mice than in patients, since in the latter binding of radiolabeled anti-TAA MoAbs to normal tissues may confuse the interpretation of the results. In this regard, Wahl et al. (*16*) have shown that an increase in the dose of radiolabeled anti-HMW-MAA MoAb 225.28 injected into human melanoma-bearing nude mice from 6.25 to 1,875 μg did not markedly change the percentage of radioactivity uptake in melanoma lesions. Rodgers et al. (*17*) have obtained similar results with the anti-CEA MoAb 1H12: an increase of the dose of radiolabeled MoAb from 16 to 500 μg/mouse resulted in a linear increase in the absolute concentration of radioactivity found in the transplanted tumor with no detectable change in the percentage of radioactivity uptake. Similarly Pimm and Baldwin (*18*) found that an increase in the amount of radioactivity localized in a human osteosarcoma xenograft was associated with an increase in the dose of radiolabeled MoAb 791T/36 injected up to 500 μg per nude mouse. When the dose was greater than 500 μg/mouse, a reduction in the fractional accumulation of radioactivity in transplanted lesions was observed. Hence, evidences obtained with different tumor models and different TAA systems uniformly demonstrate that even with the great fractional accumulation of MoAbs obtained in nude mouse xenografts, saturation of binding sites in transplanted tumors occurs only when very high doses of radiolabeled MoAbs are injected. In clinical studies, dose escalation of radiolabeled anti-TAA MoAb has been reported to result in no difference in biodistribution or tumor imaging (*19,20*), or in an increase in detection sensitivity of malignant lesions (*3, 20–26*) together with a prolongation in blood-pool clearance (*3,22,24–27*) and a decrease in fractional accumulation of radioactivity in some nontumor tissues (*24–27*). It has been speculated that the beneficial effect of dose escalation with some human tumors may be based on the saturation of "antibody sinks" in nontumor tissues expressing low levels of the target antigen (*26,27,29*), although increasing uniformity of MoAb retention may also play a role.

As far as the approach with combinations of anti-TAA MoAbs is concerned, we are aware only of investigations performed with mixtures of MoAbs recognizing distinct TAA. Muntz et al. (*29*) reported a significantly enhanced tumor contrast in nude mice bearing human colon carcinoma xenografts when a mixture of $F(ab')_2$ fragments of MoAbs reacting with human tumors of the gastrointestinal tract was utilized. But Kawabata et al. (*30*), though relying on a similar model system, found no significantly greater uptake of a mixture of $F(ab')_2$ fragments. According to Chatal et al. (*31*), an increase in the sensitivity of immunoscintigraphy in patients with colon carcinoma may be obtained by injecting MoAbs to two tumor associated antigens.

Although the results of the experiments with the cocktail of anti-HMW-MAA MoAbs have been negative in human melanoma tumor-bearing nude mice, the use of a combination of anti-HMW-MAA MoAbs may have an advantage over individual MoAbs in patients with melanoma because of the heterogeneity in the expression of distinct determinants of HMW-MAA on melanoma cells within a lesion and among lesions in different anatomic sites (*6,32*). Furthermore, combinations of MoAbs to distinct determinants of HMW-MAA may be useful as carriers of distinct components of a therapeutic system (e.g., two chains of a toxin) which have to be delivered in close proximity at the cell surface, so that they may assemble and function in selected areas of high antigen expression.

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