
Immunoreactivity and Biodistribution of Indium-111-Labeled Monoclonal Antibody to a Human High Molecular Weight-Melanoma Associated Antigen

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The anti-human, high molecular weight-melanoma associated antigen (HMW-MAA) MoAb 225.28S was chelated with ^{111}In and then tested for its in vitro reactivity with cultured human melanoma cells and for its biodistribution in human melanoma bearing nude mice. In vitro studies showed that the radiolabeled antibody reacted specifically with cultured melanoma cells. However, binding of DTPA to the monoclonal antibody reduced its titer with cultured melanoma cells from 1:1024 to 1:512. Further labeling of the DTPA-antibody conjugate with ^{111}In caused an additional reduction of its titer to 1:128. Injection of the radiolabeled monoclonal antibody into nude mice resulted in the accumulation of significantly ($p < 0.001$) higher radioactivity in melanoma tissue than in nude mice injected with either [^{111}In]chloride or ^{111}In -labeled antibody to human acid phosphatase. The specificity of the distribution of the radiolabeled antibody in nude mice also was indicated by its poor localization in lesions other than melanoma (e.g., human prostate carcinoma and chronic abscess). The localization of antibody in liver and kidney was also high, although lower than that achieved in tumor. These results indicate that ^{111}In -labeled monoclonal antibodies to human tumor associated antigens may be useful for localizing malignant lesions. However, there is a need to improve labeling and/or purification of antibody in order to decrease renal and hepatic activity.

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Iodine-131- (^{131}I) labeled monoclonal antibodies to tumor associated antigens have been used for tumor detection and their use for tumor therapy has been advocated (1-4). However, the poor imaging characteristics of ^{131}I , in vivo deiodination, and possible inactivation of the antibody by attachment of the iodine at or near the antigen binding site have detracted from the value of these agents for tumor localization and therapy. For this reason, other methods for radiolabeling antibodies have been sought. One such method involves the

use of bifunctional chelates that are capable of covalently binding an antibody and also complexing with a metallic radionuclide (5-7). Indium-111- (^{111}In)labeled monoclonal antibodies to various tumor associated antigens have been used successfully for tumor imaging (8-13). Recently, monoclonal antibodies to a human high molecular weight-melanoma associated antigen (HMW-MAA) have been developed. This antigen has a restricted tissue distribution, being detected only in melanoma, nevi, and some skin tumors (14). This investigation was designed to determine the in vitro immunoreactivity and the biodistribution in human, melanoma-bearing nude mice of the anti-HMW-MAA monoclonal antibody (MoAb) 225.28S complexed with ^{111}In .

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MATERIALS AND METHODS

Tumor cell lines

Human melanoma cells Colo 38 and B lymphoid cells LG-2 were cultured in Roswell Park Memorial Institute 1640 medium with L-glutamine. Solid tumors were produced in athymic nude mice, Swiss/Webster, nu/nu* by injection of 10^7 melanoma cells in the flank. The tumors were allowed to reach a size of 4–5 mm in diam prior to experimentation. Human prostatic tumors[†] 1–2 mm in diam per slice, were implanted by trocar in the flank of athymic nude mice and allowed to reach a size of 4–5 mm prior to experimentation.

Monoclonal antibodies and conventional antisera

The anti-HMW-MAA 225.28S, an Ig2a, was prepared and characterized as previously described (15). The antibody was purified from ascites fluid by caprylic acid precipitation (16). A rabbit antihuman acid phosphatase antiserum was purchased.[‡]

Radiometallic labeling

Coupling of diethylenetriaminepentaacetic acid (DTPA) to antibody was accomplished using the cyclic anhydride method (17). The anhydride-to-protein ratio was 1:1. The reaction was carried out in HEPES-HCl buffer (pH 7.0). Complexing of the antibody-DTPA conjugate to carrier-free [¹¹¹In]chloride[§] was accomplished at pH 5–6. The radiometallic antibody was purified on a G-150 Sephadex column and eluted with HEPES-HCl buffer (pH 7.0). Characterization of the antibody was accomplished using trichloroacetic acid precipitation, polyacrylamide gel electrophoresis, paper chromatography (85% methanol and saline), and isoelectric focusing. The radiolabeling yield ranged between 35–45%. The specific activity on the ¹¹¹In-labeled monoclonal antibody was 5 $\mu\text{Ci}/\mu\text{g}$.

In vitro binding assays

The binding assay with ¹¹¹In monoclonal antibodies was performed by incubating cultured human melanoma cells Colo 38 or B lymphoid cells LG-2 in triplicates for 1 hr at room temperature with the radiolabeled antibody. The cells were washed three times and the binding of antibody to cells determined in a gamma counter. The enzyme linked immunosorbent assay (ELISA) was performed in 96 well U bottomed plates[¶] precoated with Hanks balanced salt solution containing 1% bovine serum albumin (Hanks/BSA). Target cells (1×10^5) were incubated with 100 μl of antibody solution diluted with Hanks/BSA for 60 min at 4°C. Following three washings with Hanks/BSA, cells were incubated with 100 μl of an appropriate dilution of horse radish peroxidase conjugated anti-mouse Ig xenoantibodies. At the end of a 60-min incubation, cells were washed four times

and incubated with a freshly prepared substrate solution containing 0.05% *o*-phenylenediamine and 0.0075% hydrogen peroxide in McIlvain's buffer, pH 6. After a 30-min incubation at room temperature, absorbance of each test well was read at 405 nm. Positive and negative controls were performed utilizing anti-Class I HLA monoclonal antibodies and murine immunoglobulins, respectively.

Abscess formation

Cholesterol pellets were implanted s.c. in the flank of nude mice and induced (in ~ 3 wk) an abscess that reached a diameter of 4–5 mm at the time of experimentation.

EXPERIMENTAL DESIGN AND RESULTS

In vitro binding of radiolabeled anti-HMW-MAA MoAb 225.28S to melanoma cells

Figure 1 demonstrates that ¹¹¹In-anti-HMW-MAA MoAb 225.28S achieves significantly ($p < 0.001$) higher binding to melanoma Colo 38 cells (26.7% in the plateau region) than to control lymphoid B LG2 cells (3.1%). However, $\sim 75\%$ of the radiolabeled preparation did not bind to melanoma cells. To determine the cause of the inactivation of the ¹¹¹In MoAb 225.28S, the reactivity of the DTPA-MoAb 225.28S conjugate and the ¹¹¹In-labeled DTPA-MoAb 225.28S conjugate was tested with melanoma Colo 38 cells in ELISA and compared to the reactivity of unconjugated unlabeled control MoAb 225.28S. As demonstrated in Fig. 2, conjugation of DTPA to the MoAb 225.28S caused a reduction in its

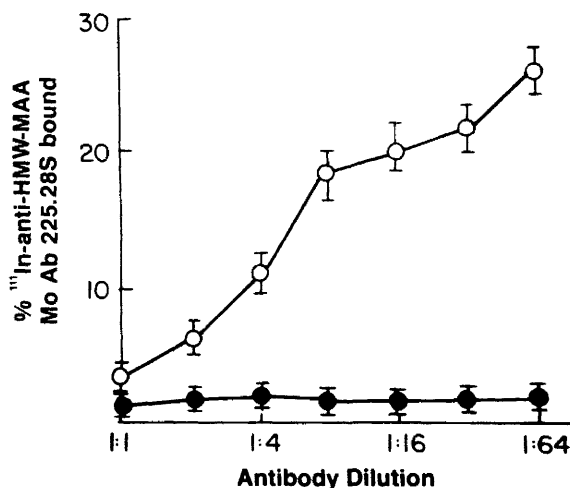


FIGURE 1
In vitro binding of ¹¹¹In anti-HMW-MAA MoAb 225.28S to cultured human melanoma Colo 38 cells (O) and to cultured human B lymphoid LG2 cells (●). Binding of ¹¹¹In-labeled MoAb to melanoma is superior ($p < 0.001$) to that achieved with lymphoid cells. Error bars depict ± 1 s.d.

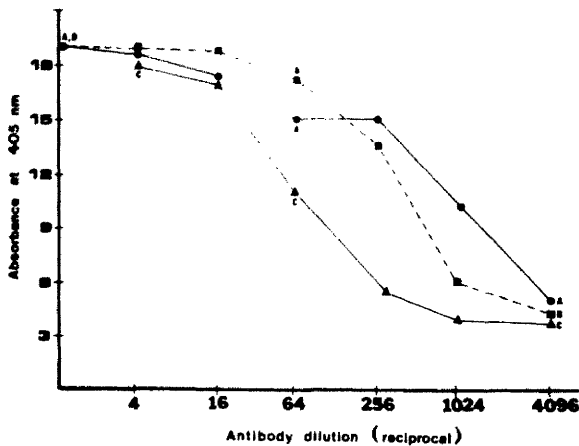


FIGURE 2
Effect of conjugation with DTPA (■—■) and with In-111 DTPA (▲—▲) on the reactivity of MoAb 225.28S with cultured human melanoma Colo 38 cells in ELISA assay. (●—●) Indicates reactivity of untreated monoclonal antibody with target cells

titer from 1:1024 to 1:512; further labeling with ¹¹¹In caused an additional reduction of the titer to ~1:128.

Distribution of radiolabeled anti-HMW-MAA MoAb 225.28S in human melanoma bearing nude mice

Athymic nude mice bearing Colo 38 human melanoma tumor were injected i.v. with 10 μCi of one of the following agents: [¹¹¹In]chloride, [¹¹¹In]DTPA, ¹¹¹In-anti-HMW-MAA MoAb 225.28S, and ¹¹¹In rabbit anti-human acid phosphatase (AP) antibody. In these and subsequent biodistribution studies, mice (six/group) were imaged with a gamma camera 4 days after radiotracer administration and immediately thereafter killed for determination of tissue radioactivity. This time

was chosen because blood levels of radioactivity were found to be considerably higher at earlier times. The percent injected dose (% ID) per gram tissue was determined in a well-type scintillation counter. As demonstrated in Fig. 3, the tumor concentration of ¹¹¹In-anti-HMW-MAA MoAb 225.28S was significantly ($p < 0.001$) higher (16.1% ID/g) than achieved in tumor-bearing control animals injected with [¹¹¹In]chloride (5.2%), [¹¹¹In]DTPA (0.05%), or nonspecific anti-AP antibody (2.9%). In addition, the ratio of tumor activity to other tissues (other than blood) was highest following the administration of ¹¹¹In-anti-HMW-MAA MoAb 225.28S. Figure 4 demonstrates the scintigraphic visualization of human Colo 38 melanoma (5 mm in diam) implanted in the flank of a nude mouse following administration of ¹¹¹In-HMW-MAA MoAb 225.28S. The image also shows high concentration of radioactivity in liver and kidneys.

Distribution of radiolabeled anti-HMW-MAA MoAb 225.28S in nude mice bearing lesions other than melanoma

The biodistribution of ¹¹¹In-anti-HMW-MAA MoAb 225.28S also was studied in athymic nude mice bearing a human derived prostate tumor and in athymic mice in whom chronic abscess had been induced. As demonstrated in Table 1, the concentration of radioactivity in these nonmelanoma lesions 4 days after tracer administration was low (2.3 and 2.8%, respectively).

DISCUSSION

Numerous institutions are now actively investigating the use of radiolabeled monoclonal antibodies for tumor localization and therapy. Although initial results are encouraging, much remains to be done to insure the

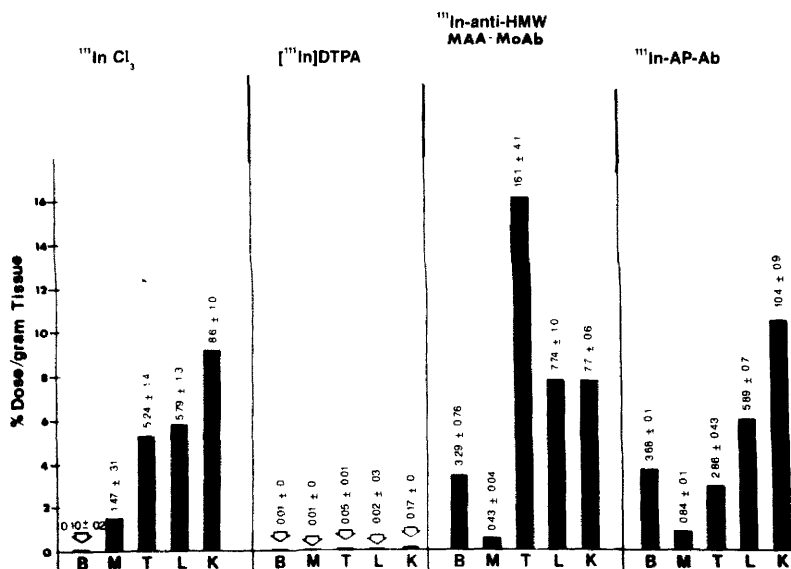


FIGURE 3
Biodistribution of ¹¹¹In-anti-HMW-MAA MoAb 225.28S compared to other radiolabels in human melanoma-bearing athymic nude mice. In vivo affinity of ¹¹¹In-anti-HMW-MAA MoAb 225.28S for human Colo 38 melanoma is significantly ($p < 0.001$) higher than that achieved in control melanoma-bearing mice injected with [¹¹¹In]chloride, [¹¹¹In]DTPA, or ¹¹¹In-labeled nonspecific antibody (anti-AP antibody). (B = Blood; M = Muscle; T = Tumor; L = Liver; K = Kidney)

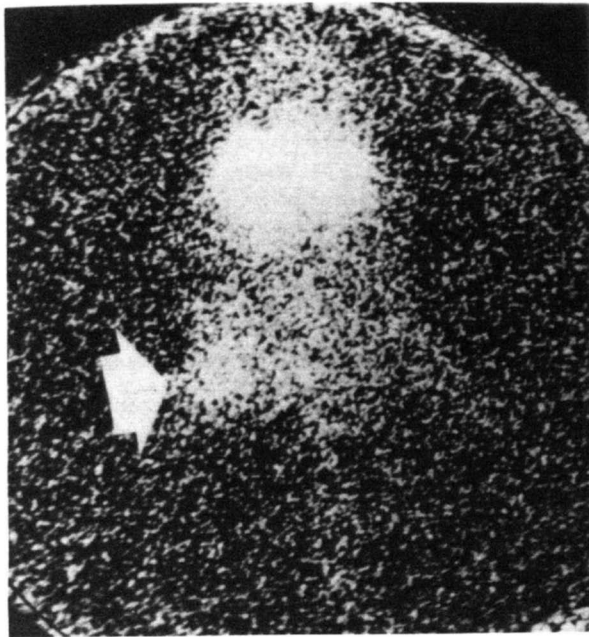


FIGURE 4

Representative scintigraphic image obtained in an athymic nude mouse bearing melanoma s.c. implanted in left flank and injected i.v. 4 days earlier with ^{111}In -anti-HMW-MAA MoAb 225.28S. Note increased activity in tumor which measures 5 mm in diam (arrow). There is also increased activity in liver and kidney

clinical usefulness of this approach. Of prime importance is the need to develop improved labeling techniques since the standard method of antibody labeling with iodine-131 has certain limitations (18, 19). The recent introduction of a method of antibody labeling with bifunctional chelation led us to examine the *in vitro* and *in vivo* reactivity with melanoma cells of anti-HMW-MAA MoAb 225.28S when chelated to ^{111}In .

In vitro studies demonstrated that ^{111}In -HMW-MAA MoAb 225.28S maintained its specific reactivity with melanoma cells. However, binding of DTPA to the MoAb caused marked reduction in its immunoreactivity. Additional reduction in the activity of MoAb occurred following labeling with ^{111}In . These findings are at variance from the scanty information in the literature about the effect of ^{111}In labeling on the *in vitro* immunoreactivity of antibodies. Khaw et al. reported that covalent attachment of DTPA to Fab fragments of rabbit anti-dog myosin antibodies did not markedly damage their antigen binding properties (20). Bernhard et al. reported a 30% reduction in the biologic activity of a murine monoclonal antibody to hepatocarcinoma following labeling with ^{111}In (10). The different results, if not technical in nature, may reflect the differential sensitivity of the various antibodies used to the denaturing conditions during DTPA conjugation and radiolabeling. Our *in vitro* studies emphasize the need to im-

prove the methodology to radiolabel antibodies with ^{111}In and/or to remove inactive antibodies from radiolabeled antibody preparations.

Since [^{111}In]chloride *per se* is known to have good tumor localizing properties, it was essential to compare its binding for human melanoma to that achieved with ^{111}In anti-HMW-MAA MoAb 225.28S. Such control studies comparing the biodistribution of the radiometal with the metal bound antibody have not been previously performed routinely and their omission can become a source of considerable confusion. Our *in vivo* results demonstrate the superior tumor localizing property of ^{111}In -anti-HMW-MAA MoAb 225.28S compared to that obtained with [^{111}In]chloride both in terms of concentration in tumor and with respect to the ratio of tumor activity relative to most other tissues. Two additional lines of evidence showed the specificity of the accumulation of radioactivity in melanoma lesions. First, low radioactivity accumulated in malignant lesions other than melanoma and in chronic inflammatory lesions. Second, low radioactivity accumulated in melanoma lesions when mice were injected with ^{111}In bound to anti-AP antibody. The latter was preferred as a specificity control to a monoclonal antibody to an irrelevant antigen since monoclonal antibodies, even of the same Ig class and subclass, may significantly differ in their properties. Conventional antisera are more likely to contain antibody populations with properties similar to those of the monoclonal antibodies being tested.

Although the specific binding of human melanoma for ^{111}In -HMW-MAA MoAb 225.28S was high, the relatively high concentration of radioactivity in liver and kidneys is disturbing because these areas are frequently investigated for malignancy. Possible explanations for these nonspecific accumulations of radioactivity are: (a) presence of inactivated antibody, immunoglobulins without antibody activity, or antibody aggregates in the

TABLE 1
Accumulation of ^{111}In -Anti-HMW-MAA MoAb 225.28S in Melanoma and Other Lesions*

Lesion	n	^{111}In activity		
		% dose/g	p_1^\dagger	p_2^\ddagger
Human melanoma (Colo 38)	6	16.09 ± 4.11	—	—
Human prostatic tumor	6	2.28 ± 0.15	<0.001	—
Chronic abscess (s.c. cholesterol pellets)	6	2.80 ± 0.33	—	<0.001

* Percent injected dose per g 96 hr postinjection.

$^\dagger p_1$ is significance level of difference between melanoma group and control with human prostate tumor.

$^\ddagger p_2$ is significance level of difference between melanoma group and control animals with abscess.

radiolabeled antibody preparation; (b) formation of immunocomplexes between the injected antibodies and antigens shed from the tumor; and (c) in vivo dissociation of the radioactive metal from the antibody. Thus, although these initial results are encouraging, they also indicate the need for improved labeling techniques and/or improved purification of antibody in order to reduce hepatic and renal activity and therefore improve the clinical usefulness of this test in tumor detection.

FOOTNOTES

* Memorial Sloan-Kettering Center, New York, NY.

† Courtesy of Dr. Lola Reid, Albert Einstein School of Medicine, Bronx, NY.

‡ New England Associates, Boston, MA.

§ Diagnostic Isotopes, Inc., Bloomfield, NJ.

¶ Falcon 3911, Becton Dickinson Labware, Oxnard, CA.

** Titertek Multiscan plate reader (Flow Laboratories, Inc., McLean, VA).

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