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Biodistribution Study of Murine Monoclonal Anti-GD_3 Antibody in Nude Mice Bearing Human Melanoma Xenografts for Development of Immunoscintigraphy

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Department of Pharmacy, Yamagata University Hospital, Yamagata 990-9585, [1]Cyclotron and Radioisotope Center, Tohoku University, Sendai 980-0845, and [2]Nissin Food Products Co., Ltd., Tokyo Pharmaceutical Research Laboratories, Tokorozawa 359-1152

OJIMA, F., IDO, T., KIJIMA-SUDA, I. and NAKAGAWA, Y. *Biodistribution Study of Murine Monoclonal Anti-GD₃ Antibody in Nude Mice Bearing Human Melanoma Xenografts for Development of Immunoscintigraphy*. Tohoku J. Exp. Med., 1998, **185** (2), 89–100 —— Reactivity of the monoclonal antibody with the tumor markers is known to be different between cultured cells in vitro and transplanted tumors in vivo. The monoclonal antibody should be investigated regarding its specific accumulation in tumor-bearing mice for immunodetection or immunotherapy. We studied the biodistribution of radiolabeled monoclonal anti-GD_3 antibody (IgM) in normal mice and nude mice bearing human melanoma xenografts. Tissue-to-blood distribution ratios of the antibody in the liver, spleen and kidney increased with time in both normal and melanoma-transplanted mice, but no significant changes were observed in other normal tissues up to 5 days after injection. Specific accumulation of the monoclonal anti-GD_3 antibody in the grafted human melanoma (HMV-II) was observed 4 and 5 days after injection. On the other hand, no specific accumulation of standard murine IgM in the tissue of HMV-II was observed in mice bearing the HMV-II xenograft 5 days after injection. Because the tissue-to-blood ratio of the distribution in the tissue of HMV-II became larger than that of other tissues 4 and 5 days after administration, 4 days after the administration of the monoclonal anti-GD_3 antibody were required for immunoscintigraphy. Accumulation of the monoclonal anti-GD_3 antibody in other human melanomas (HMV-I, HMY-1 and SK-MEL188) inoculated into mice was also observed 4 days after the antibody administration. The monoclonal anti-GD_3 antibody used in this study would be useful in immunodetection or immunotherapy. ———— melanoma; monoclonal anti-GD_3 antibody; immunoscintigraphy © 1998 Tohoku University Medical Press

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The anti-tumor marker monoclonal antibody (mAb) has been developed for immunodetection in human tumors (Taylor et al. 1988; Eary et al. 1989). The mAb is useful for in vivo immunodetection and the in vitro immunoassay system (Taylor et al. 1988; Eary et al. 1989; Reman et al. 1990; Thakur et al. 1996). Because the reactivity of mAb with the transplanted tumor cells was not equal to the reactivity strength with the cultured cells in vitro (Sakahara et al. 1988), the specific reactivity of the mAb in the tumor xenografts should be confirmed before developmental study of the newly established mAb.

Disialoganglioside GD_3 was reported to be associated on the cell surface of lymphoblastic leukemia and other tumor cells (Reman et al. 1990). The anti-GD_3 mAb reacts with human melanoma cells and some other human cells in vitro as analyzed by the fluoresein immunodetection system (Reman et al. 1990). Therefore, GD_3 is an important tumor marker associated with human melanoma. There are many differences in surrounding environment of the disialoganglioside GD_3 on the cell surface between in vivo and in vitro. It is necessary to investigate the tissue distribution of the anti-GD_3 mAb in nude mice bearing human tumor xenografts for immunoimaging and immunotherapeutics (Sakahara et al. 1988). We examined the tissue accumulation of the anti-GD_3 mAb in human melanoma-bearing nude mice in vivo and the timing necessary to detect human melanoma using immunoscintigraphy.

Materials and Methods

Anti-GD_3 mAb

Anti-GD_3 murine mAb (IgM) was purified from the ascitic fluid of male BALB/c mice (Funabashi Farm, Funabashi) previously inoculated with antibody-producing hybridoma. The mAb was partially purified by Sephacryl S-300 (Pharmacia, Uppsala, Sweden) gel exclusion chromatography, and its purity was determined by electrophoresis. The mAb recognizes GD_3 (disialolactosylceramide) (Nagai et al. 1992).

Tumor models

HMV-I, HMV-II, HMY-1 and SK-MEL188 human melanoma cells were obtained from Dr. Hidekazu Nishimura (Department of Bacteriology, Yamagata University School of Medicine). The mAb examined in this study has been confirmed to react with the 4 melanoma cells in vitro using enzyme linked immunofluorescein assay (data not shown). Human melanoma cells were cultured in RPMI 1640 medium containing 10% fetal calf serum and subcutaneously injected (10^7/100 μL/mouse) in the back of male BALB/c nu/nu mice (SLC Japan, Shizuoka). The mice were given 0.1% NaI solution 3 days before injection to reduce the specific uptake of iodine into the thyroid. Human melanoma-transplanted nude mice were used in this study at 3-4 weeks after transplantation. Three or four mice were used in each study.

Assay of blood contents of each tissue

To clarify whether the radioactivities measured in the tissues were based on the specific accumulation of mAb or whether the counts based on the blood radioactivity were included in them, the blood content ratios were assayed with radioiodinated murine albumin. The murine albumin was partially purified from the ascitic fluid of male BALB/c mice (Funabashi Farm) with 40% ammonium sulfate followed by gel filtration and its purity determined with electrophoresis. The radioiodination was performed using ENZYMOBEADS (Radioiodination System, Bio-Rad, Anaheim, CA, USA). One hundred μL of murine albumin solution and 37 MBq of ^{131}I were added to 100 μL of ENZYMOBEADS suspension. The iodination was started by the addition of 1% β-D-glucose (25 μL), and then the reaction mixture was allowed to stand for 30 minutes at room temperature. After centrifugation, the supernatant was applied to a Sephadex G-25M column (PD-10, Pharmacia) to purify the labeled albumin with phosphate-buffered saline as the elution buffer. The void fraction was determined by monitoring the radioactivity and protein detection. The labeled albumin solution was filtered for sterilization before administration to the mice through the tail vein. The mice were sacrificed at 1, 3, 5 and 7 days after administration of one hundred μL of radioiodinated albumin solution. The blood, liver, heart, pancreas, spleen, small and large intestines, kidney, brain, muscle and bone were taken. The tissues were washed with 0.9% NaCl, wiped and weighed. The samples and labeled albumin solutions were counted using an auto-well gamma counter. The data were represented as the differential absorption ratio (DAR). The DAR stands for (counts of tissue/total injected counts) x (g body weight/g tissue weight). The tissue-to-blood ratio of DAR was also obtained to determine the content ratio of albumin in each tissue.

Radioiodination of anti-GD_3 mAb

The mAb was labeled with ^{131}I using ENZYMOBEADS as described for the radioiodination of murine albumin. Immunoreactivity of the labeled mAb was determined by comparison with the residual radioactivity in the wells coated with GD_3 or no GD_3 after incubation at 37°C for 120 minutes followed by three washings. The labeled mAb solution was filtered to sterilize it before administration to the mice through the tail vein (7.5–11.5 μg/100 μL/mouse).

Investigation of the tissue distribution of anti-GD_3 mAb in the mice bearing human melanoma

HMV-II-bearing nude mice were sacrificed on 3, 4 and 5 days after administration of labeled mAb through the tail vein. Blood, liver, heart, pancreas, spleen, small and large intestines, kidney, brain, muscle and bone were taken. The solid tissues were washed with 0.9% NaCl, wiped and weighed. The samples

and labeled mAb solution were counted using an auto-well gamma counter. Tissue distribution was expressed as the DAR for normalizing the body weight of each mouse. The tissue-to-blood ratio of DAR was also obtained to determine if accumulation would occur. The tissue distribution of anti-GD_3 mAb was also measured in the HMY-1 and SK-MEL188 human melanoma-bearing mice 4 days after the Ab administration.

Standard murine IgM was iodinated using the same method as mentioned above, and its tissue distribution ratio in HMV-II melanoma-bearing mice was examined at 4 days after administration of 100 μL of standard mouse IgM solution injection through the tail vein to ascertain the specificity of anti-GD_3 mAb accumulation to HMV-II.

Autoradiography

Regarding the HMV-I- and HMV-II-inoculated mice, the mice were sacrificed at 4 days after labeled mAb injection and frozen in hexane-dry ice to make a 3% sodium carboxymethyl cellulose block. Sliced samples were cut from the frozen whole body block with a −20°C cryotome and exposed to x-ray film for 2 days.

Results

Content ratios of blood in the tissues

The time course of ^{131}I-labeled murine albumin tissue distribution is re-

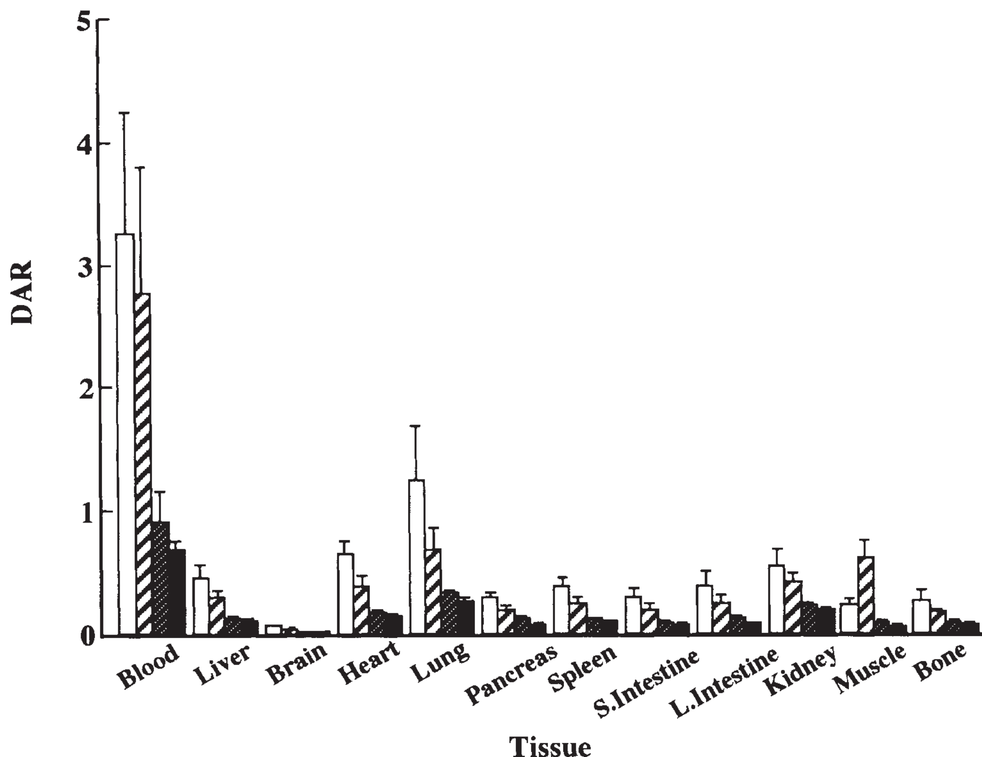

Fig. 1. Time course of tissue distribution of ^{131}I murine albumin in normal mice. □, 1 day; ▨, 3 days; ▩, 5 days; ■, 7 days.

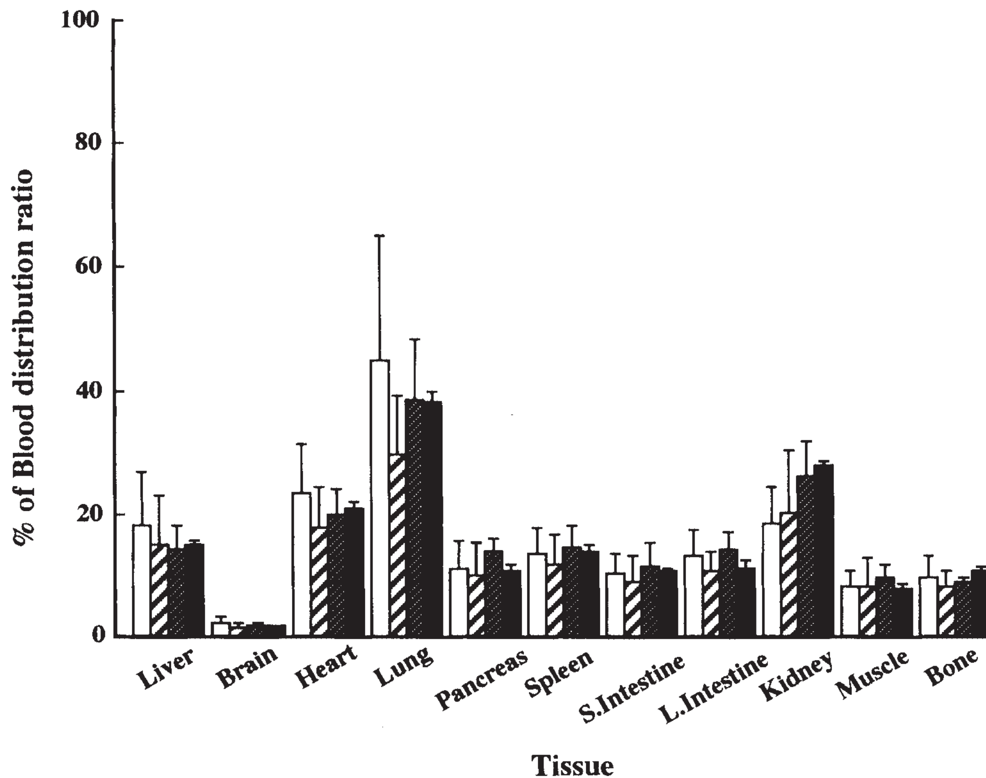

Fig. 2. Time course of tissue distribution ratio of ^{131}I murine albumin compared to the blood radioactivity in normal mice.
□, 1 day; ▨, 3 days; ▩, 5 days; ■, 7 days.

presented in Fig. 1. The radioactivity in all the tissues decreased with time, but the tissue-to-blood ratios of the radioactivity did not change with time except for the kidney where the excretion of free iodine occurred (Fig. 2). This indicates no specific accumulation in any tissue. Therefore, the ratios of ^{131}I-labeled murine albumin to the blood radioactivity indicated the content ratio of blood in each tissue. Although the tissue-to-blood ratio of the lung was large compared to the other tissues, the blood content of the lung in the live body was presumed to be less than 30% of the ratio obtained in this study, because the tissue density of the lung was low in the body.

Time course of biodistribution of labeled anti-GD_3 mAb in the normal mice

Tissue biodistribution of labeled anti-GD_3 mAb in normal mice was investigated at 1, 2, 3 and 4 days after injection. The radioactivities in all the tissues decreased with time (Fig. 3). However, the tissue-to-blood radioactivity ratios were at the same level as those with ^{131}I murine albumin in normal mice except for the liver, spleen and kidney (Fig. 4). The tissue-to-blood radioactivity ratios of the liver, spleen and kidney increased with time (Fig. 4).

Time course of biodistribution of labeled mAb in the nude mice bearing HMV-II human melanoma xenografts

The results of the biodistribution study of the labeled mAb are shown in Fig.

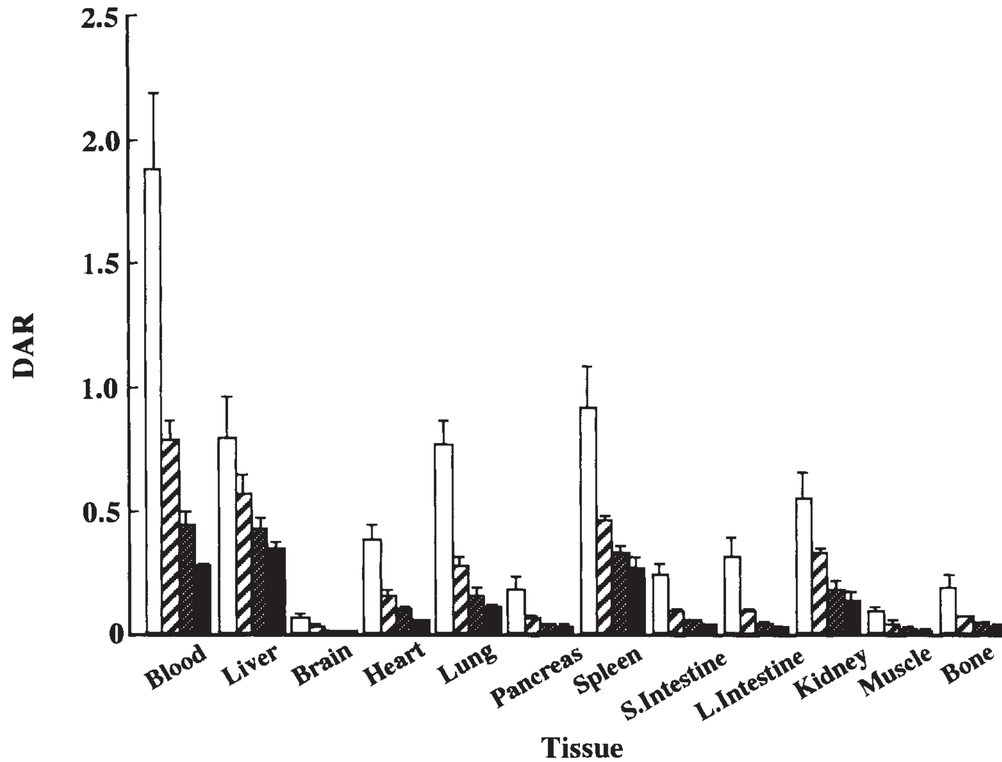

Fig. 3. Time course of tissue distribution of anti-GD_3 ^{131}I mAb in normal mice.
□, 1 day; ▨, 2 days; ▩, 3 days; ■, 4 days.

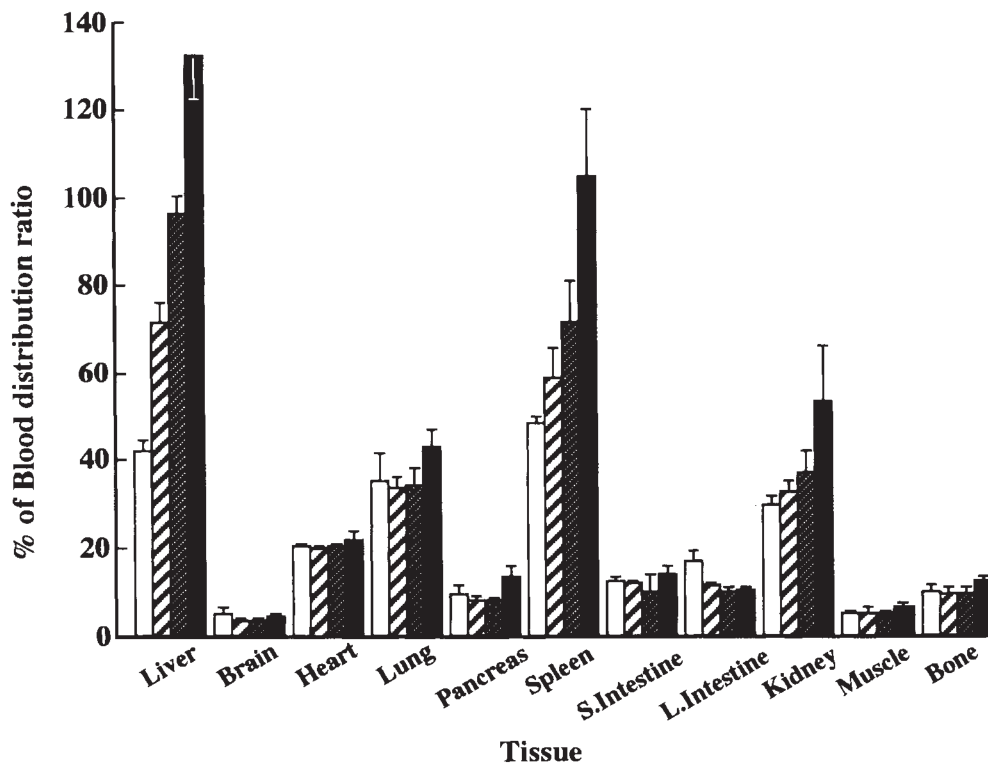

Fig. 4. Time course of tissue distribution ratio of ^{131}I anti-GD_3 mAb compared to the blood radioactivity in normal mice.
□, 1 day; ▨, 2 days; ▩, 3 days; ■, 4 days.

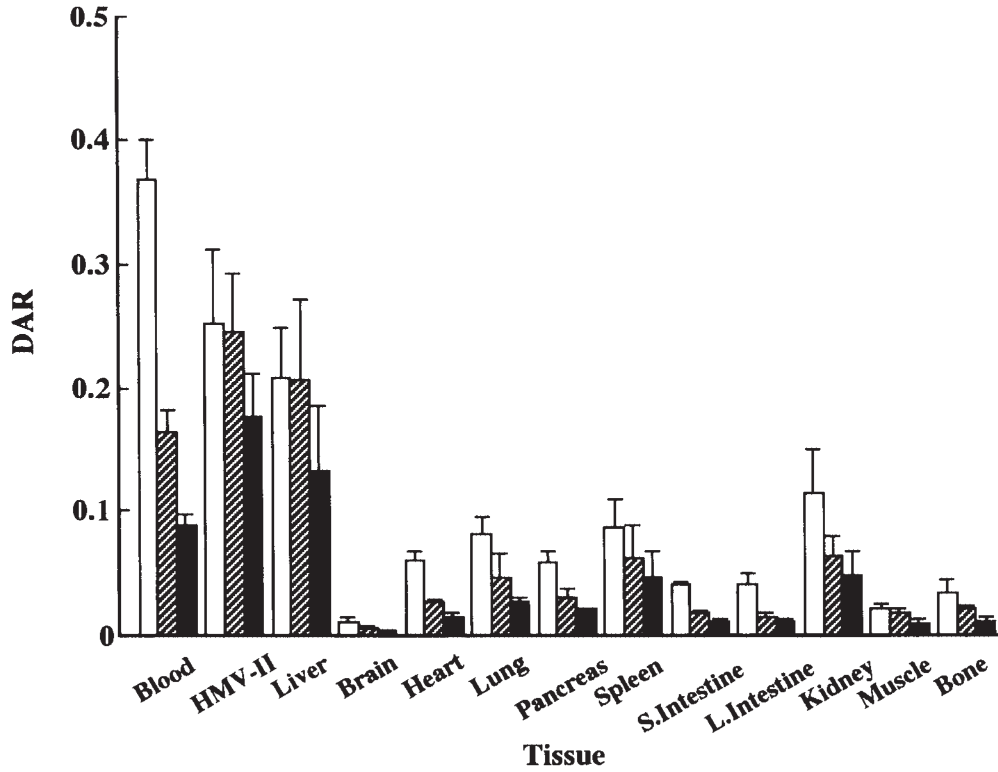

Fig. 5. Time course of tissue distribution of anti-GD_3 mAb labeled with ^{131}I in HMV-II-bearing mice.
□, 3 days; ▨, 4 days; ■, 5 days.

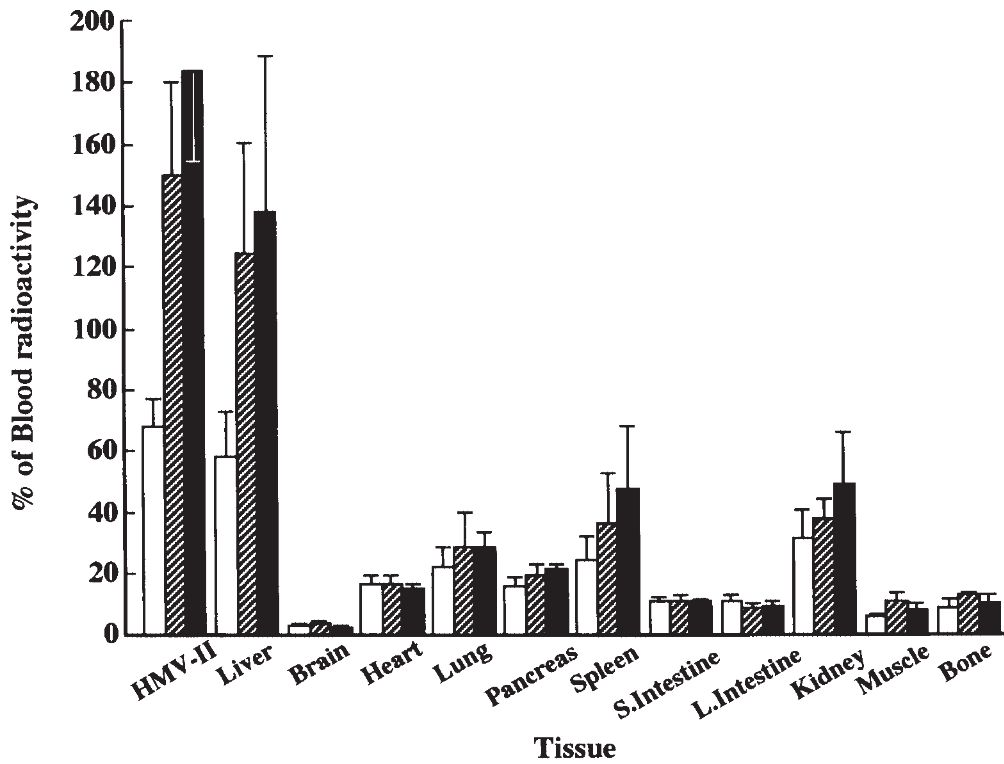

Fig. 6. Time course of tissue distribution ratio of anti-GD_3 mAb labeled with ^{131}I compared to the blood in HMV-II-bearing mice.
□, 3 days; ▨, 4 days; ■, 5 days.

5. The distribution ratio of the radioactivity declined with time in all tissues except for HMV-II and liver. The tissue-to-blood ratio is shown in Fig. 6. In the HMV-II-bearing mice, the tissue-to-blood ratio of the liver, spleen and kidney increased with time, but no changes were observed in any of the other tissues at 3, 4 and 5 days after injection.

Autoradiography

The accumulation of the anti-GD_3 mAb was observed in nude mice bearing

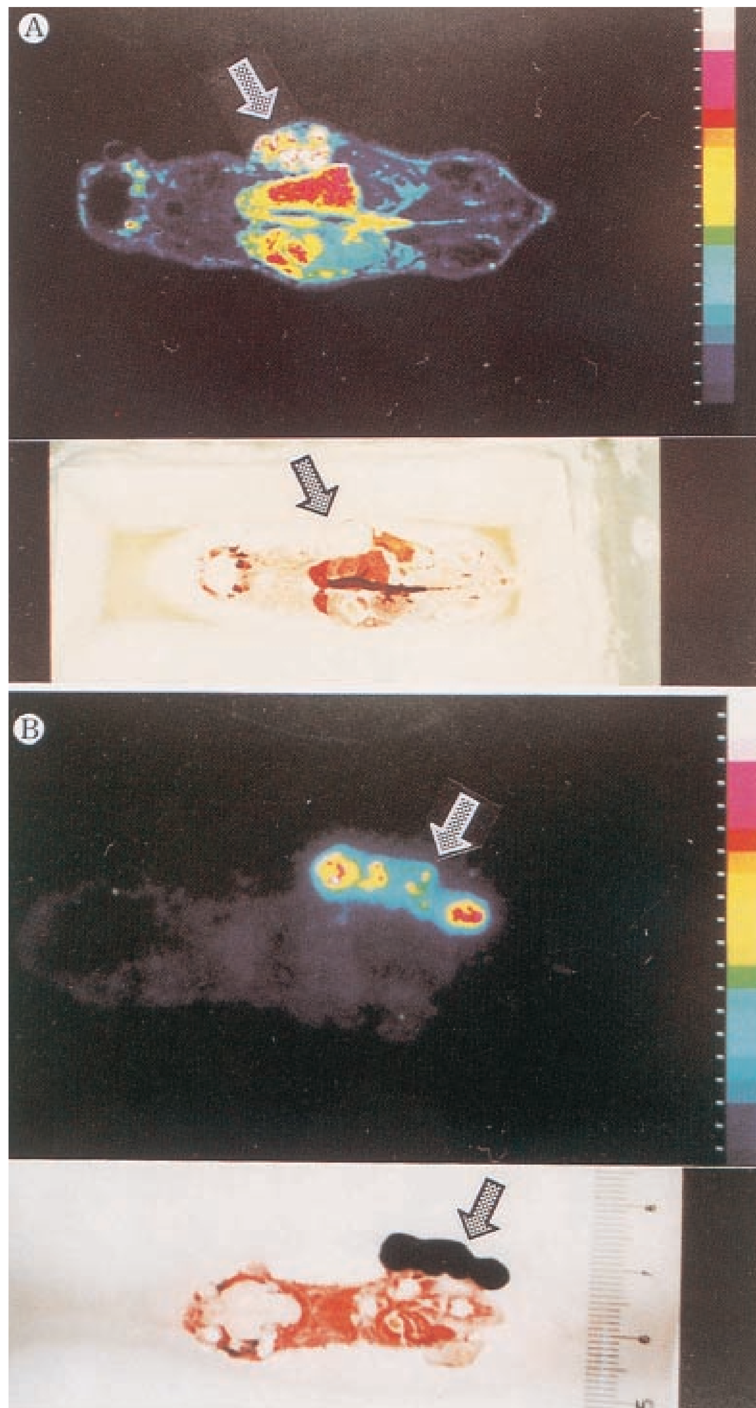

Fig. 7. Autoradiograms of whole body 4 days after ^{131}I anti-GD_3 mAb injection in HMV-I (A) and HMV-II (B)-bearing mice. Arrows indicate the melanoma.

both HMV-I and HMV-II human melanoma 4 days after mAb administration. Fig. 7 shows the whole body autoradiogram. A nonuniform uptake was observed in the melanoma tissues, whereas the accumulation was uniform in the other normal tissues. The radioactivity ratios of the other tissues to liver in HMV-I-bearing mice are shown in Table 1. In the HMV-I, the mean melanoma-to-liver ratio was 1.1 and the maximum ratio was 3.9. The mean melanoma-to-liver ratio was 7.9, and the maximum distribution ratio to the liver was more than 10 in the HMV-II-bearing mice (Table 1).

Biodistribution of labeled murine standard IgM antibody in the nude mice bearing HMV-II xenografts

The tissue-to-blood distribution ratios of labeled murine standard IgM and

TABLE 1. *Ratio of tissue radioactivity intensity compared to liver in HMV-I and HMV-II-bearing mice in autoradiograms*[a]

Mouse	Tissue	Radioactivity intensity ratio to liver
HMV-I bearing	HMV-I	Mean (whole): 1.1, Region of highest accumulation: 3.9
	Other Tissue	Blood : 0.93, Lung: 0.64, Spleen: 0.59, Kidney: 0.59
HMV-II bearing	HMV-II	Mean (whole): 7.9, Region of highest accumulation: >10

[a] Autoradiograms are shown in Fig. 7.

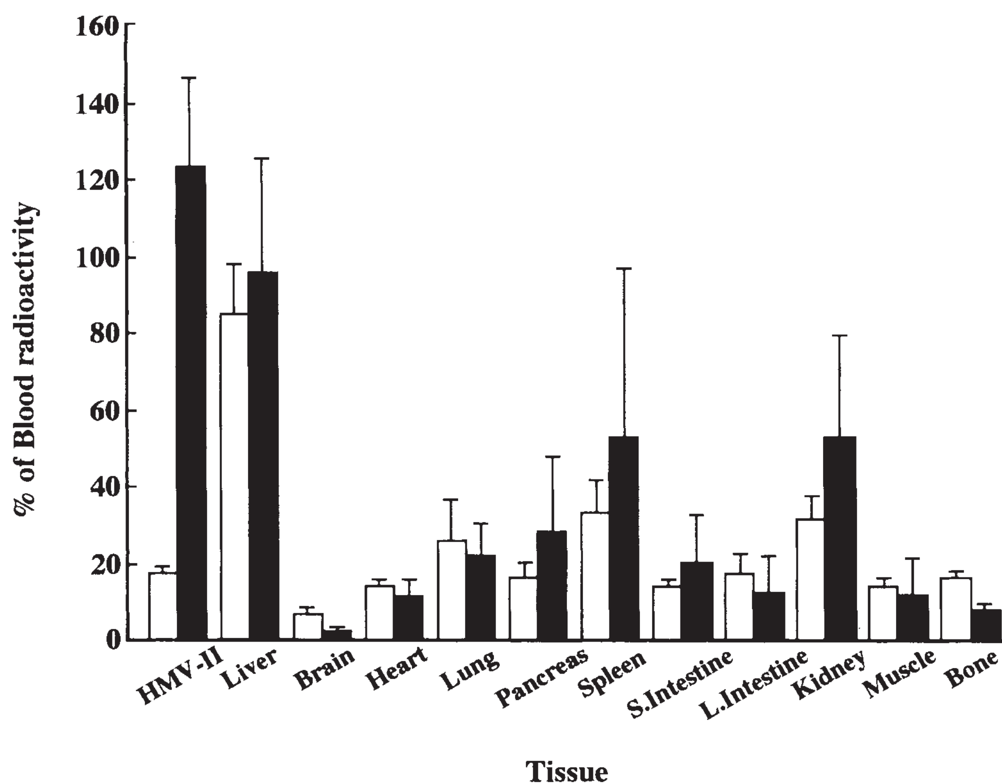

Fig. 8. Tissue distribution ratio of standard murine IgM and anti-GD_3 mAb compared to blood 4 days after injection in HMV-II-bearing mice.
□, Standard IgM; ■, Anti-GD_3 mAb.

anti-GD_3 Ab in HMV-II-bearing nude mice 4 days after antibody injection are represented in Fig. 8. For the standard IgM antibody, no specific accumulation was observed in HMV-II tissues compared to the anti-GD_3 mAb distribution ratio.

Distribution ratio of anti-GD_3 mAb in HMY-1 and SK-MEL188

The tissue-to-blood distribution ratios of anti-GD_3 mAb in HMY-1 and SK-MEL188 human melanoma-bearing nude mice 4 days after mAb injection are represented in Fig. 9. The mean distribution ratio of mAb in HMY-1 tissue showed the same radioactivity level as in the blood, whereas specific accumulation of mAb was seen in the SK-MEL188 tissue.

Discussion

The tissue distribution of an antibody is of significant importance in order to develop the Ab for delivering radionuclei or drugs to objective tumors. The immunoscintigraphy and/or immunotherapy of tumors would depend on the ratio of specific radioactivity delivered to tumors versus normal tissues. The murine monoclonal anti-GD_3 Ab was investigated for its biodistribution in the normal mice and nude mice bearing some human melanoma xenografts in this study.

In the time course study of the tissue distribution of radioactive tracers, the radioactivity contents in the tissues changed with time where no specific accumulation would occur, because each tissue included the blood and radioactivity in the blood that changed with time during metabolism and excretion. Therefore,

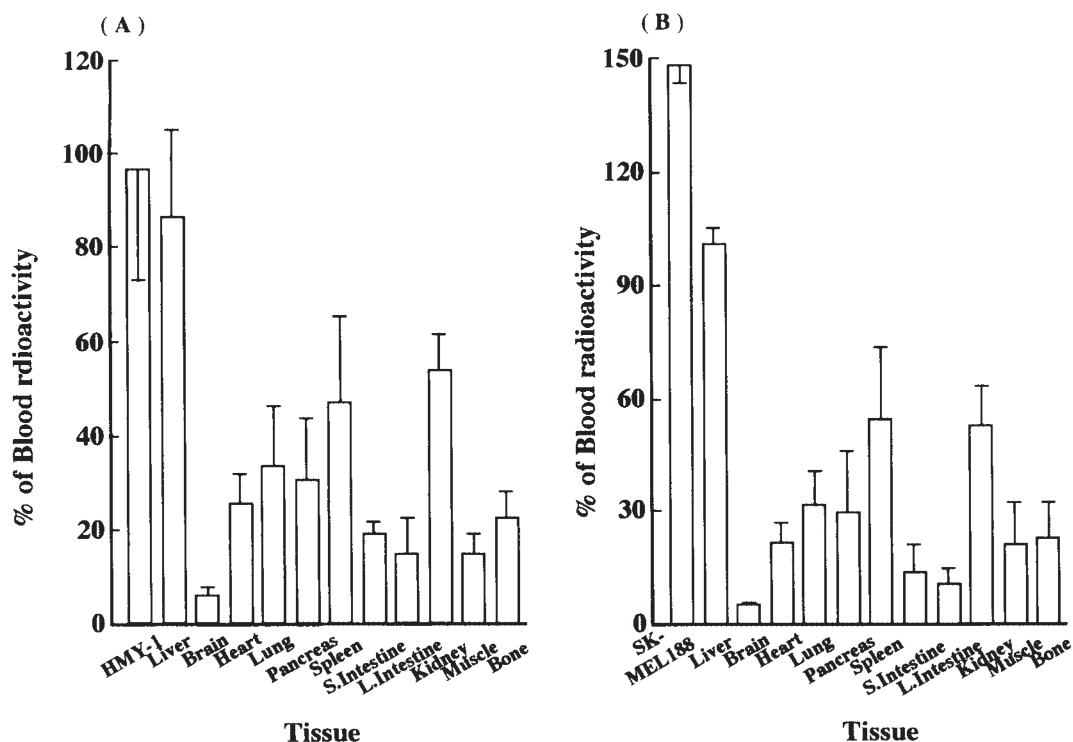

Fig. 9. Tissue distribution ratio of ^{131}I anti-GD_3 mAb compared to blood radioactivity in HMY-1 (A) and SK-MEL188 (B) bearing mice 4 days after injection.

it is necessary to obtain the blood content ratios of each tissue. Because the tissue-to-blood ratios of murine albumin in each tissue had almost no variation with time, it denotes the blood content ratio in each tissue.

In the HMV-II study, the accumulation of labeled Ab seemed to occur 3 days after injection. No specific accumulation was seen in standard murine IgM in the HMV-II-bearing nude mice; therefore, the accumulation of Ab in HMV-II was specific for anti-GD_3 Ab. Because the concentration of radioactivity in the blood was higher than that of HMV-II, it was not suitable for immunoscintigraphy till 3 days after administration. Compared to all other tissues, the highest accumulation of Ab in HMV-II was observed at 4 and 5 days after injection. The tissue accumulation dose was higher at 4 days after Ab injection than that at 5 days after Ab injection. Therefore, it was suitable for the immunodetection of melanoma in this IgM subclass murine anti-GD_3 monoclonal Ab at 4 days after administration. An autoradiography and tissue distribution study were performed 4 days after Ab administration. Concerning the mean values, because the distribution ratios of the HMV-I and HMY-1 melanoma tissues were same as those in the liver, the melanoma tissues seemed not to be detectable with this Ab. However, as shown in the autoradiograms and Table 1, nonuniform accumulation was observed in the melanoma tissues. This suggested that the regions of highest accumulation in the melanoma tissue were detectable with immunoscintigraphy. These results indicated that the melanoma region would be detectable with this anti-GD_3 mAb even in the liver, because the radioactivity of the region of highest accumulation in the melanoma was larger than that of the liver, although the exact shape and volume of the melanoma could not be obtained. The nonuniform accumulation in the melanoma was based on the capillaries in the tumor tissue and the affinity of Ab and cell surface antigen. For example, a high affinity Ab is difficult to separate from the antigen near the capillary and to diffuse into the inner region of the tumor tissues.

The accumulation ratio of this mAb was approximately 1% of the injected dose per gram of wet tissue 4 days after injection. A larger accumulation ratio was reported when using the IgG subclass Ab (Buchegger et al. 1983; Halpern et al. 1983). The specificity of this mAb was sufficient for immunodetection, but a greater accumulation was necessary for immunotherapeutics. It is difficult for the IgM subclass Ab used in this study to pass through the capillaries in the melanoma tissues, because of its high molecular weight. It is necessary to study the biodistribution of Fab or F(ab') obtained from enzyme digestion in the melanoma-bearing nude mice and other radiolabeling methods (Wahl et al. 1983; Beaumier et al. 1985; Sakahara et al. 1985; Eary et al. 1989; Koizumi et al. 1989) for immunotherapy.

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