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Radioimmunotherapy of Athymic Mice Bearing Human Colon Carcinomas with Monoclonal Antibody B72.3: Histological and Autoradiographic Study of Effects on Tumors and Normal Organs

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Abstract—*Monoclonal antibody (MAb) B72.3 has been linked successfully to several radionuclides forming stable complexes and analyzed* in vitro *and* in vivo *without significant loss of its immunoreactivity. Previous studies have demonstrated that radioiodinated B72.3 can selectively bind to human colorectal carcinomas grown in athymic mice. The same successful localization has been obtained more recently in clinical trials in patients with metastatic colorectal carcinomas. The high degree of selective binding of this MAb has led us to investigate its potential as a radioimmunotherapeutic agent. Athymic mice bearing human colon carcinoma xenografts were injected with either* 300 *or* 500 μCi *of* ^{131}I-*B72.3 IgG to assess the effect of the radiolabeled MAb on the tumor growth as well as potential toxic side effects in vital organs. In mice treated with the* ^{131}I-*B72.3 IgG, a marked inhibition of the growth of the human colon carcinoma xenografts was noticed in comparison with control mice injected with PBS or control mice that received unlabeled B72.3 IgG. The tumors from these control mice weighed* 2.7 *to* 3.7 *times more than the tumors from the treated mice at* 17 *days post-inoculation of the radiolabeled MAb. Autoradiographic studies demonstrated a heterogeneous distribution of radioactivity throughout the tumor mass at* 11 *days post-administration of MAb. With time, the periphery of the tumor contained significantly less radioactivity than the medial areas composed of predominantly non-viable tissue; these findings suggest that the more biologically active peripheral tumor zones, with higher mitotic rates, could have partially escaped the radiation effect of the single dose administered. The tumor cells could have continued dividing when the levels of circulating radiolabeled monoclonal antibody had decreased.*

Toxicity was readily evident in the mice injected with the high-dose regimen (500 μCi), *with confirmed bone marrow aplasia that proved lethal for* 2 *of* 10 *animals. The lower dose* (300 μCi) *resulted in a bone marrow suppression of approx.* 50% *of the cells, which proved to be non-lethal. The tumors in the treated mice showed extensive necrosis caused by the lethal dose of* ^{131}I-*B72.3 that irreversibly damaged the cells. Radiation-induced terminal differentiation of cells was also found as manifested by the drastically decreased mitotic count* (0–2 *vs.* 12–14 *per* 10 *high power fields seen in control tumors) in treated animals.*

INTRODUCTION

THE UTILITY of radiolabeled monoclonal antibodies specific for a variety of tumor-associated antigens for the detection of occult human malignancies has been extensively investigated [1–7]. The results of these studies have led to a better understanding of the parameters that play an important role in successful tumor localization. Much less information is available concerning the potential therapeutic value of radiolabeled MAb. Only a few studies performed in animal models [8–12] and more rarely in clinical trials [13–15] have been reported; very little is known, however, in terms of mechanisms of action, distribution, toxicities, dosimetry, etc., of high doses of radiolabeled MAb.

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B72.3 is a murine monoclonal antibody (MAb) with a high degree of specificity for carcinomas of the colon, breast ovary and lung [16–18]. B72.3 IgG has been successfully radiolabeled with ^{125}I or ^{131}I and used in model systems to localize human colon carcinoma xenografts in athymic mice [19, 20]. These studies demonstrated the specificity of the MAb binding, yielding tumor : liver, tumor : spleen and tumor : kidney ratios of approx. 20 : 1 at 7 days post-antibody administration. These studies also showed that approx. 20% of the injected dose/g was bound to the carcinomas for periods longer than 19 days [19], thus making this model an ideal system for studying the potential use of radiolabeled monoclonal antibodies as therapeutic agents. The utility of B72.3 IgG as a radiopharmaceutical for the detection of human malignancies has recently been demonstrated in patients with metastatic colorectal carcinomas [21–22].

In the present study, we injected ^{131}I labeled B72.3 at higher doses than those used for the localization studies into athymic mice bearing human colon carcinoma xenografts in order to investigate its therapeutic effect on the tumors, the mechanisms of the therapeutic effect at the microscopic level, and any potential side-effects on normal organs.

MATERIALS AND METHODS

Monoclonal antibody B72.3

MAb B72.3 was obtained by immunization of BALB/c mice with a membrane-enriched fraction of a human breast tumor metastasis to the liver. Its generation, characterization and reactivities have been described elsewhere [16–18]. Purification of the IgG was performed, as previously reported [19], from ascitic fluid by ammonium sulfate precipitation, ion-exchange and size-exclusion chromatography.

Iodination of MAb B72.3

B72.3 was labeled with $Na^{131}I$ using the Iodogen method [19]. The purified IgG (500 μg) was labeled with approx. 5 mCi of $Na^{131}I$ in a glass vial coated with 250 μg of Iodogen. After a 5 min incubation, the free ^{131}I was removed by gel filtration through a Sephadex G-25 column. The immunoreactivity of the labeled B72.3 was tested in a solid phase radioimmunoassay, using extracts of a human colon carcinoma xenograft [19].

Cell line

The LS-174T cell line was obtained from the American Type Culture Collection (CL 188, Rockville, MD) [23]. This cell line was established from a mucinous adenocarcinoma of the colon. The LS-174T was grown in Eagle's minimum essential medium supplemented with 1% non-essential amino acids (100 mM), including 1% glutamine (200 mM), 10% heat inactivated fetal calf serum and gentamycin (50 μg/μl). Cells were passaged weekly at a 1 : 10 dilution. They grew as a monolayer and were harvested using 0.1% trypsin in 0.5 mM EDTA. The cells were washed twice in serum-free minimal essential medium before inoculation.

Tumor growth in athymic mice

Female athymic mice were obtained from Charles River Inc. at 4–6 weeks of age. The animals were injected subcutaneously in the left flank with 4×10^5 cells (0.1 ml). Ten days after inoculation, the mice bearing tumors of approx. 0.5 cm dia. were selected and divided into 3 groups and given KI in their drinking water to minimize non-specific uptake of any unincorporated ^{131}I. Two groups of 10 mice each were injected intraperitoneally with 300 and 500 μCi respectively, of ^{13}I-B72.3 IgG. The tumor growth was measured in 2 diameters, twice weekly, using a precision caliper, until the mice were sacrificed by exsanguination 11 and 17 days post-inoculation. Tumor, liver, spleen, kidneys, lungs, vertebral column and blood were carefully weighed using an analytical balance and counted in a gamma counter; biodistribution studies were performed. Mice injected with PBS or unlabeled B72.3 were used as control group, and therefore, were also analyzed similarly.

Histologic studies

The tumor, spleen and vertebral column (previously decalcified for 30 min in D-Calcifier, Lerner Lab, New Haven, CT) from each animal, and selected tissues such as lung, kidney and liver from 2 animals per group, were fixed in 10% buffered-formalin and embedded in paraffin. Three- to five-micron sections were obtained from the paraffin blocks and stained with hematoxylin and eosin (H&E) for light microscopy studies.

Autoradiographic studies

Five-micron sections from selected tissues were used for autoradiographic studies. The sections deparaffinized and hydrated by passage through xylene and alcohols, respectively. The sections were then dried and coated with photographic emulsion (Ilford K 5) by immersion for 5 sec. After incubation periods of 2 and 4 weeks at −70° C in dehydrated light-tight boxes, the slides were developed using Kodak photographic developer (D-19) for 6 min. The process was stopped by immersing the slides in 1% acetic acid and then fixed using a photographic fixer. The tissues were then counterstained with hematoxylin and the sections

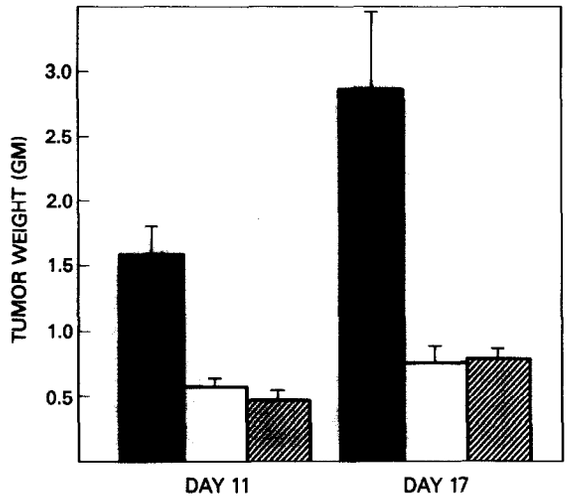


Fig. 1. Effect of ^{131}I-B72.3 therapy on the growth of human colon carcinomas (LS-174T) hosted in athymic mice. Mice injected with 300 μCi (□), 500 μCi (□), *and control mice (injected with PBS or unlabeled B72.3)* (■) *were sacrificed 11 and 17 days post-inoculation. The average weight* (g) *of the tumors of each group of 7–8 animals is represented on the* y *axis.*

were examined to determine the distribution and specific location of the radiolabeled MAb as shown by the silver grains.

RESULTS

Effect of ^{131}I-B72.3 IgG on tumor growth

Athymic mice were injected subcutaneously with the human colon carcinoma cell line (LS-174T). Approximately 10 days later, when the tumors measured approx. 0.5 cm in maximal dimension, the mice were injected with 300 or 500 μCi of ^{131}I-B72.3 IgG. The tumors were measured twice weekly until the animals were sacrificed at either 11 and 17 days post-inoculation of the ^{131}I-B72.3 IgG. The growth rate of the tumors in mice injected with 500 μCi was minimal during the first 11 days with tumors increasing in size from 0.5 × 0.6 cm to 0.6 × 0.7 cm maximal diameters. Tumors had shown some growth by the time the mice were sacrificed on day 17 post-injection. Tumors from the control mice, injected with PBS or unlabeled B72.3, experienced a rapid growth, doubling in size by day 11 post-injection and becoming approx. 3 times larger by day 17 post-injection. Figure 1 shows the tumor growth inhibition of different groups of mice by comparing the average weight (g) of the tumors from treated mice vs. untreated animals. The control tumors weighed 3.4 and 3.7 times more than the tumors from the mice injected with 500 μCi, at 11 and 17 days post-injection respectively. Similarly, the tumors from animals injected with 300 μCi had also a significant growth inhibition, with control tumors weights 2.7 and 3.7 times heavier than those of the treated mice. These differences are probably much more significant if one considers the fact that a large percentage of the tumor mass in treated animals was necrotic although still included in the measurement and weight of the tumors.

Morphologic changes in treated tumors

The LS-174T human colon carcinoma cell line forms moderately differentiated adenocarcinomas when implanted subcutaneously into athymic mice. Histologically, areas of predominantly solid growth alternate with areas of readily identifiable glandular structures with mucin in their lumens. The mitotic activity is high with 10–14 mitoses per 10 high power fields (hpf) and tripolar and other abnormal mitotic figures are common (Fig. 2A). The control tumor doubles in size from 0.5 cm in average dimension in approx. 2 weeks, at which point, central necrosis begins to appear. The morphologic modifications seen in the tumors treated with 300–500 μCi dose ^{131}I-B72.3 are those consistent with radiation effects [24]. Architecturally, the tumors lose their glandular pattern, adopting a loosely cohesive arrangement without recognizable glandular structures. Cytologically, there is extensive vacuolization of cytoplasm and nuclei, multinucleation, aberrant nuclei and prominent nucleoli. Mitotic counts decrease to 0–2 mitoses per 10 hpf, reflecting the terminal differentiation induced by the radiation (Fig. 2B).

The amount of necrosis present in these tumors is more extensive than that present in control tumors of approximately the same size. Figure 3A shows a tumor treated with the 500 μCi dose of radiolabeled MAb; it contains necrosis that accounts for approx. 90% of the total volume of the tumor. The remaining viable tissue, as can be appreciated in the microphotograph, is located in the periphery of the tumor (Fig. 3B), and more rarely surrounding large vessels (Fig. 3C). In contrast, a tumor of similar size from a control animal is depicted in Fig. 3D; notice the central necrosis that accounts for less than 20% of the total tumor volume and is surrounded by tumor with the usual histologic appearance. The histologic changes seen in the tumors from mice treated with 300 μCi can be considered similar to those described above, although not as extensive. The typical glandular architecture of the tumor has been replaced by an uniform and generally aglandular tissue where radiation changes can be seen in many cells. In contrast to the tumors treated with the 500 μCi dose regimen, one can see cells that appear normal mixed with cells with obvious radiation-induced changes. Also, the amount of necrosis is not as abundant as that present in the tumors treated with 500 μCi.

Effect of treatment in normal organs

The spleen and bone marrow from every treated and control animal were studied by light microscopy in order to evaluate the treatment-induced toxic side-effects. At 17 days post-injection of the 500 μCi dose, almost complete marrow aplasia with less than 5% of the marrow cellularity remaining was observed. The bone marrow from the vertebral column appears collapsed with rare lymphoid cell precursors present (most of the cells seen represent red blood cells extravasated into the marrow spaces when the vertebral column was obtained) (Fig. 4A). A similar picture is seen in the spleen, the other prominent hematopoietic organ of the mouse, where erythroid, myeloid, lymphoid and megakaryocytic precursors are rarely found. Due to the massive cell depletion, the spleen has become retracted and is much smaller (Fig. 4B). This observation was also made when the weights of the spleens were compared with those of the control animals. The bone marrow aplasia was considered responsible for the death of 2 of the 10 animals. In a parallel experiment where the animals were similarly injected with 500 μCi and sacrificed at 30 days post-injection, the marrow of all the survivors had experienced a total recovery with cellularity approaching 100% and with the erythroid, myeloid, lymphocytic and megakaryocytic cell lines present.

The lower dose of radiolabeled MAb (300 μCi) produced intermediate effects in the marrow and spleen with approx. 30–50% cellularity present by day 11 post-injection (Figs. 4C, D) and 70–80% by day 17. There were no deaths in this group. Figures E and F show the bone marrow and spleen, respectively, of the control animals for comparison. The cellularity of the marrow is close to 100% and the splenic parenchyma contains numerous and prominent lymphoid follicles. The other major organs were studied, with only the liver showing alterations. Focal necrosis was observed, but was also seen in the liver of the control animals and therefore considered to be unrelated to radiation toxicity.

Autoradiographic studies

Representative tumors from each group of treated animals were selected for studying the penetration of the radiolabeled MAb. The tumors from mice sacrificed 11 days post-inoculation exhibited an irregular distribution of the activity (Fig. 5A). The amount of radionuclide–monoclonal antibody complex appears to be equally intense in the center of the tumor as in the periphery. Denser areas of radioactivity can be appreciated in perivascular areas as well as in the mucin-filled glandular lumens. An interesting change in the localization of the radioactivity was observed in the tumors 17 days post-inoculation; the heterogeneous distribution of radioactivity evenly throughout the tumor, seen at 11 days, was replaced by a distribution pattern showing a concentration of radioactivity in the medial portions of the tumors, where necrosis was predominant. The peripheral zones had less activity, mostly concentrated in the pools of mucin (Fig. 5B, C). The reasons for the distribution of activity may have been due to either (a) the non-specific accumulation of the radiolabeled MAb in necrotic areas, as has been postulated, or (b) the result of radiolabeled MAb complex entrapment in previously viable areas that have become necrotic as the result of the therapeutic effect. Necrosis and subsequent partial architectural collapse would produce a further concentration of the activity in those areas. Mice bearing tumors of approx. 0.5–0.8 cm in dia. were injected with non-therapeutic doses of ^{131}I-B72.3 (7 μCi) in order to determine if the radiolabeled MAb is trapped in necrotic areas. As can be seen in Fig. 5D, the distribution of the radiolabeled MAb in the viable tumor is very similar to that seen in the tumors of the treated mice: there is an even and heterogeneous distribution with higher amounts deposited in perivascular and mucin-filled glandular spaces. No activity was present in the necrotic portion of the tumor, thus showing that the monoclonal antibodies are not non-specifically entrapped in the necrotic areas of the tumors. The necrosis found in the tumors of the mice treated with 500 μCi was, therefore, the result of the therapeutic effect of the radiolabeled MAb, and not simply due to the non-specific accumulation of the MAb in necrotic areas.

DISCUSSION

The immunological approach to cancer treatment has received a great deal of interest since hybridoma technology has permitted the production of large amounts of monoclonal antibodies that recognize tumor-associated antigens. Although many of these MAbs have minimal or no tumoricidal effect by themselves, the possibility of linking them to radionuclides or toxic substances provides a potential therapeutic agent.

We have previously shown in animal models that MAb B72.3 can not only be conjugated to radioiodides [19, 20] and ^{111}In (manuscript submitted) without significant loss of reactivity, but that the selective binding of the complex to human colon carcinoma xenografts in athymic mice was approx. 20 times higher than in normal tissues [19]. This selective targeting permitted the detection of the tumors by external gamma scanning in animal models [19, 20]. Recent studies in patients with metastatic colon carcinomas have demonstrated similar localization in the tumors [21, 22].

Athymic mice bearing human colon carcinoma xenografts (LS-174T) were chosen as an animal

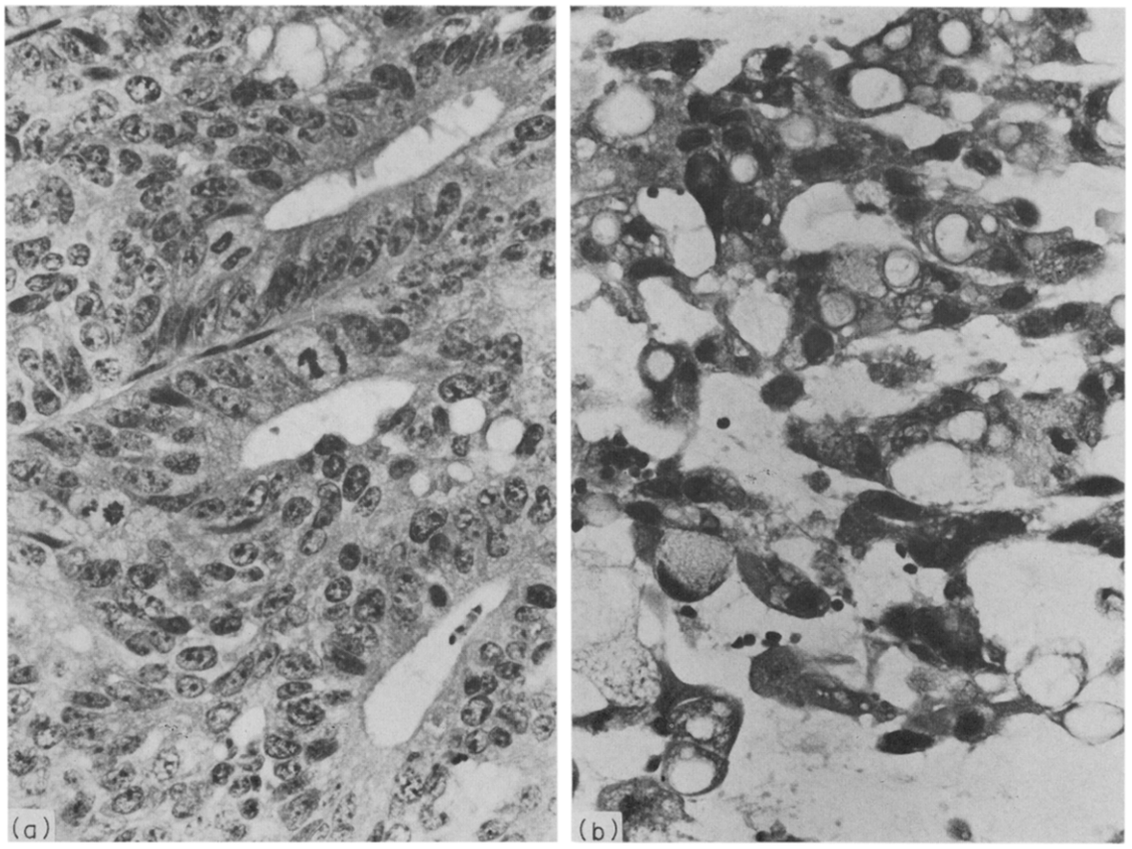


Fig. 2. Microphotographs of H & E stained sections of LS-174T human colon carcinoma hosted in athymic mice. (a) Control tumor from untreated animal. Notice the glandular pattern and numerous mitoses present in the field (× 190). *(b) Tumor from mouse treated with* 300 μCi *of* ^{131}I-*B72.3 IgG and sacrificed* 17 *days post-inoculation. The general architecture has been disrupted, many cells show the typical radiation changes and the absence of mitotic figures* (× 380).

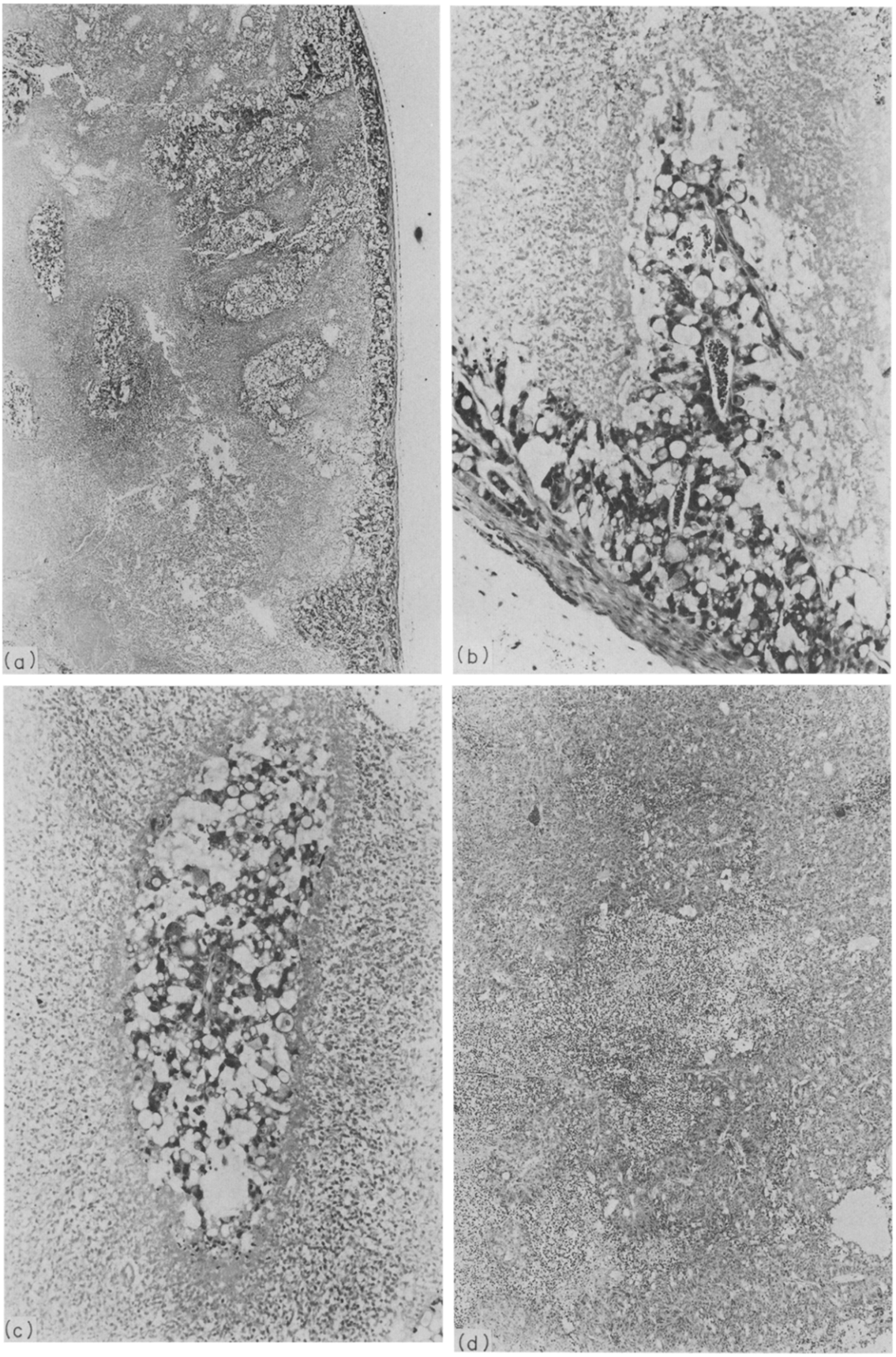


Fig. 3. Tumors from mice treated with 500 μ*Ci of* ^{131}I-*B*72.3 *IgG sacrificed* 17 *days post-inoculation.* (*a*) *Extensive necrosis secondary to treatment. Notice the scant viable tissue located in the periphery and in isolated foci surrounding blood vessels* (× 19). *High power views of peripheral* (*b*) *and perivascular* (*c*) *viable tumor showing radiation effect* (× 95). (*d*) *Untreated control tumor of similar size showing minimal central necrosis, representing less than* 20% *of the total tumor volume* (× 19).

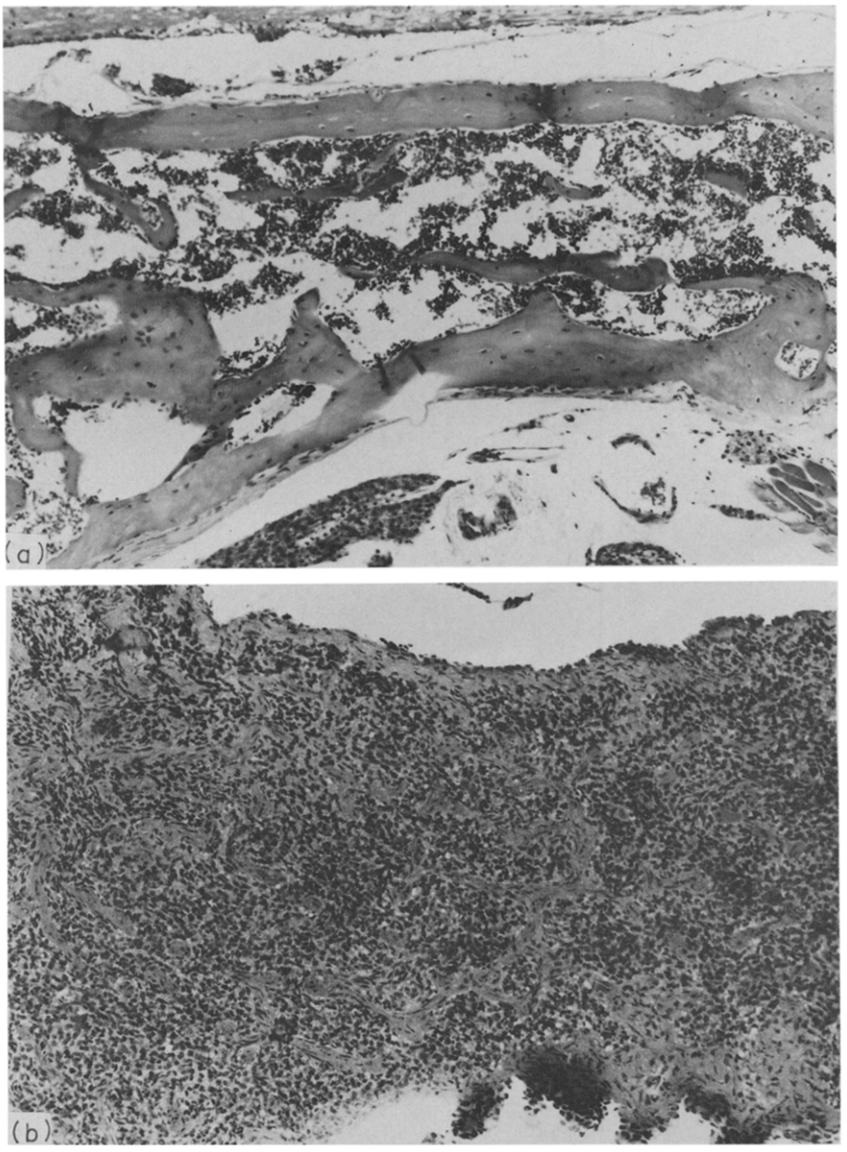
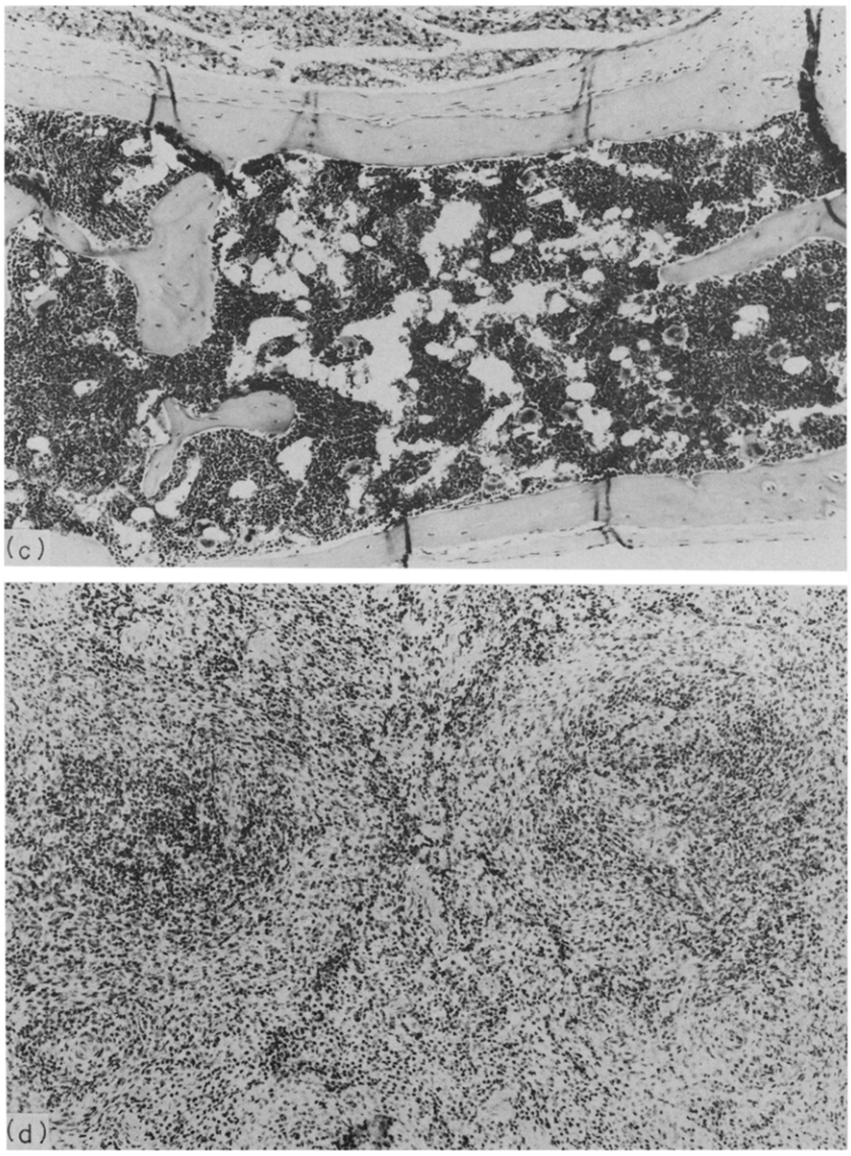


Fig. 4. Hematopoetic organs (bone marrow and spleen) from animals treated with ^{131}I-*B72.3. (a and b) Animal treated with* 500 μCi *showing* < 5% *of the normal hematopoietic cellularity (most of the cells seen in the bone marrow are mature erythrocytes from contaminating hemorrhage). No megakaryocytic precursors are present, only a few erythroid and lymphoid cells remained. Also note the smaller size of the marrow and spleen when compared to that of the control animals treated with PBS or unlabeled MAb (e, f), due to parenchymal collapse secondary to cellular depletion. Bone marrow (c) and spleen (d) 11 days post-inoculation of the low dose* 300 μCi *showing approx.* 60% *celullarity* (× 95).


(c)
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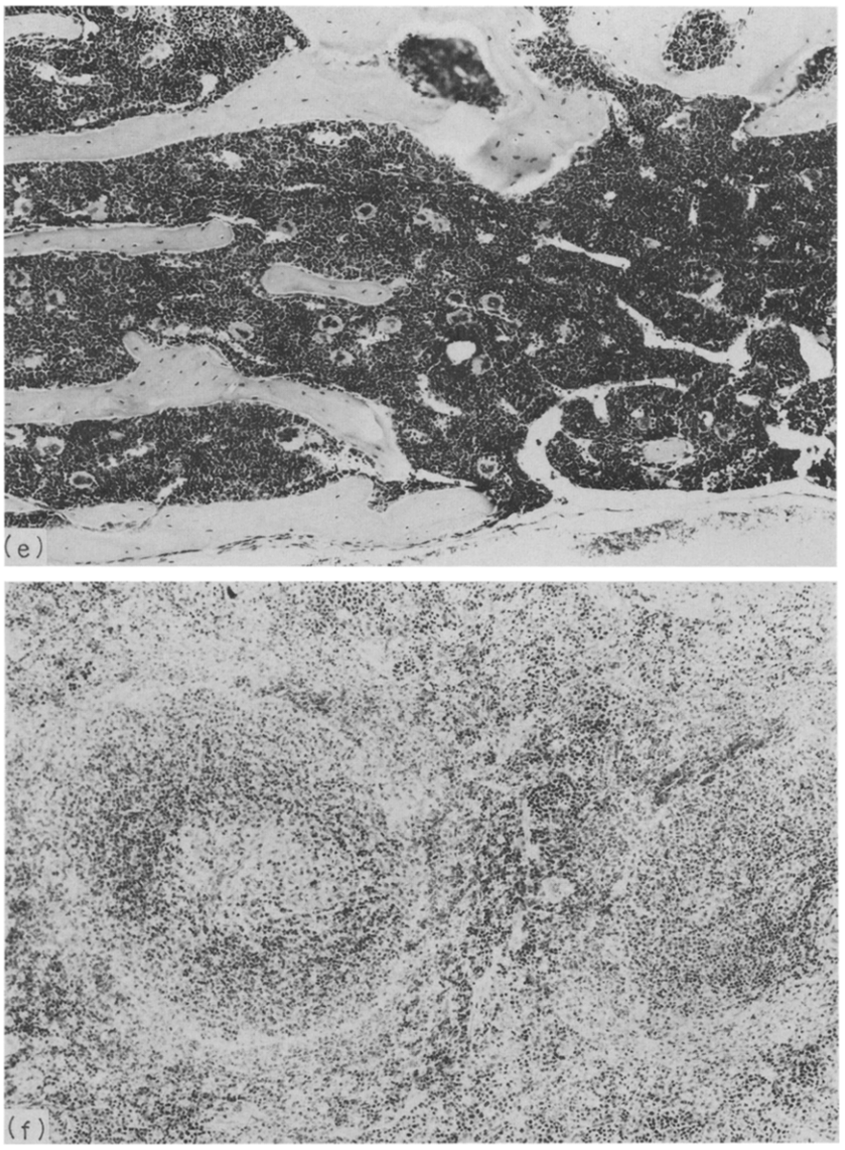
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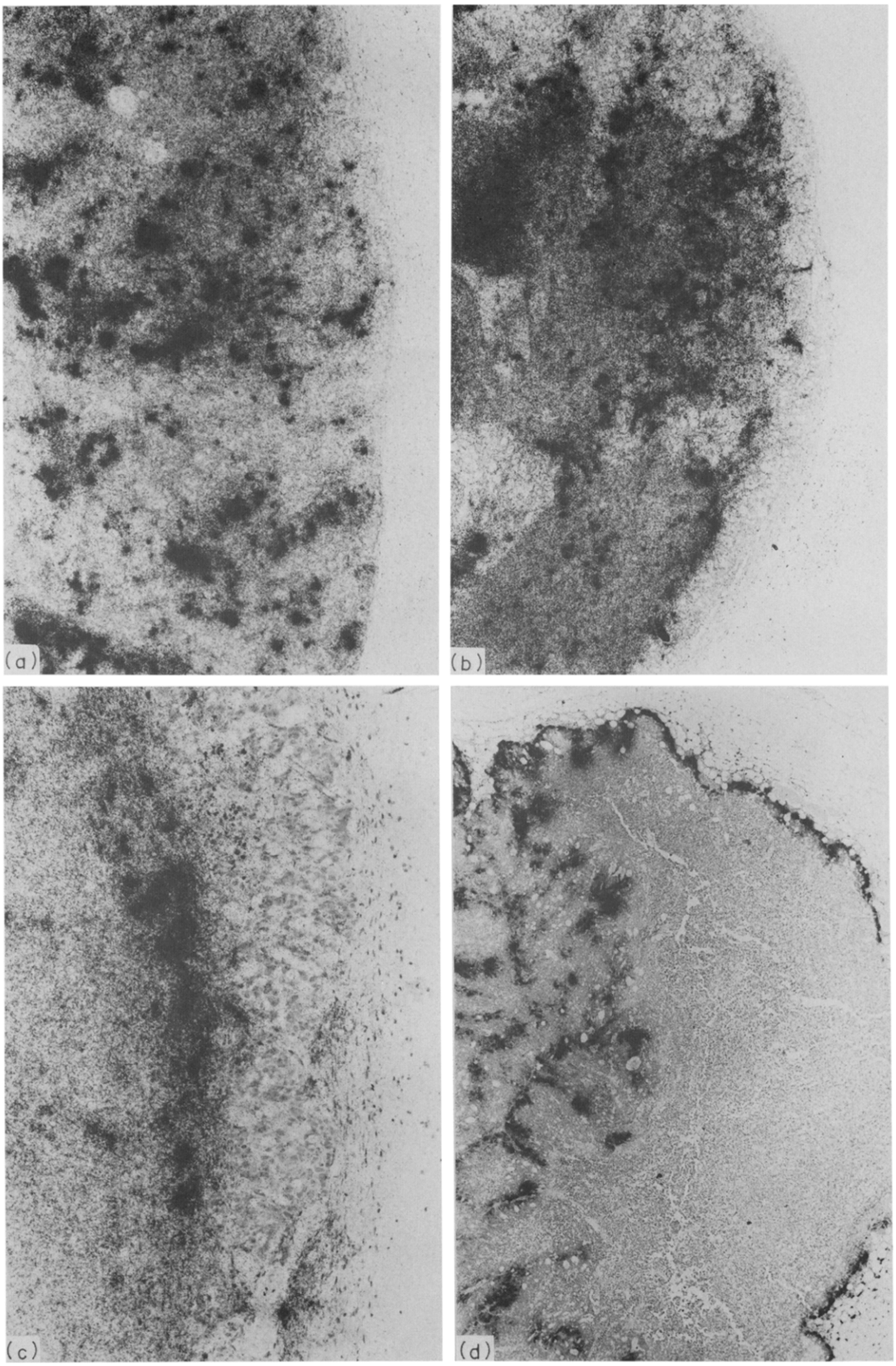

Fig. 5. Autoradiographs of tumors from animals treated with 500 μCi *of* ^{131}I-*B72.3. (A) Heterogeneous distribution of the activity at* 11 *days post-inoculation. There is a high concentration of the conjugate in perivascular areas and within the glandular mucin* (× 40). (*b and c*) *Tumors at* 17 *days post-injection with predominant location of the silver granules in the medial areas containing extensive necrosis; less activity is present on the mostly viable peripheral rim of tumor* (*b*, × 38; *c*, × 95). (*d*) *Tumor from a mouse injected with non-therapeutic dose showing a lack of deposition of the MAb within the necrotic areas* (× 19).

model to study which effects, if any, higher doses of ^{131}I-B72.3 IgG would have on tumor growth. Our primary interest centered on the morphologic changes in the histologic appearance of the tumor, the penetration and distribution of the radionuclide–monoclonal conjugate throughout the tumor over time, and the potential toxic effects on the different organs. The 2 dose regimens selected, 300 and 500 μCi of ^{131}I-B72.3 IgG, were based on previous studies [25] with the same experimental model where 300 μCi of ^{131}I-B72.3 inhibited the growth rate of the tumors, but had no visible toxic effect in the mice. A 500 μCi dose, however, showed increased inhibition of tumor growth, but produced toxic effects in the mice as manifested by the premature demise of several animals.

The effect of both dose regimens on the histologic appearance of the tumors was obvious at 11 days post-inoculation of the conjugate. The necrosis observed in the treated tumors was more extensive than that in the controls, representing 90–95% of the total tumor volume in many of the tumors in mice receiving the 500 μCi dose regime. This extensive necrosis can be explained by 2 different mechanisms: (a) lethal dose of radiation resulting in immediate cell death, and (b) sublethal dose causing a terminal differentiation of the cells with definitive arrest of their mitotic activity leading to eventual death [24]. The first mechanism is probably responsible for most of the cell death observed during the initial post-inoculation period when the highest concentration of the radiolabeled MAb is present. The second mechanism can be demonstrated by the mitotic counts on the tumors which dropped from 12–14 mitoses/10 hpf seen in control tumors to 0–2 mitoses/10 hpf found in the treated tumors; this is indicative of terminal differentiation in the majority of the cell population [24]. The same low mitotic count persisted in the tumors of the animals that were sacrificed at 17 days post-injection, reflecting the lack of cellular recovery during that period of time. Other cytotoxic mechanisms such as complement- and/or macrophage-mediated cytotoxicity may also play a role in the cytolytic activity, but this would represent only a minute part as demonstrated by the lack of tumor growth inhibition obtained when the unlabeled MAb was injected.

The only viable cells present in the majority of the treated tumors were located in the periphery, forming a ring around the necrotic material. More rarely, small areas of tumor cells, embracing large sized vascular channels located medially, were also seen. The periphery of the tumors were biologically the most active areas with mitotic counts slightly higher than in the medial portions, and a continuous process of expansion and angioneogenesis occurs which could explain the higher survival of the tumor cells in that location. Autoradiographic studies showed a decreased activity precisely on those viable peripheral areas when compared with the mostly necrotic central portions. Since necrotic tissue does not entrap the radionuclide–monoclonal antibody complex in a non-specific manner (Fig. 5D), we can hypothesize that the 500 μCi dose of radiation administered was not sufficient to affect all the cells in the peripheral region of more active tumor cell replication. Due to the increased mitotic rate, these cells continued to expand over time while the radioactivity available progressively decreased due to the radiological $T_{1/2}$ of the radionuclide, the biological $T_{1/2}$ of the monoclonal antibody, and possibly due to dehalogenation. This observation may prove to be of critical importance at the time of planning a therapeutic course: a fractionated dosing schedule could be used to deliver additional radiation 7–10 days after the first inoculation when the radioactivity in circulation has started to decrease, and thus provide a lethal dose to the peripheral rim of viable tissue.

Explanation for the survival of tumor cells around medium-sized vessels in more medial portions of the tumors is difficult to account for. Autoradiography shows that most of the radioactivity remains in perivascular areas, at least during the first few days post-injection, and then diffuses out into the interstitial spaces. Therefore, these perivascular regions should receive the highest dose delivered to the tumor. The same autoradiographic studies have shown also a great deal of antigenic heterogeneity as manifested by the irregular distribution of the radioactivity throughout the tumor with areas densely grained alternating with others of minimal activity. It is possible that some of these antigen-negative pockets of cells, located at a sufficient distance from antigen-positive cells that bind the ^{131}I-B72.3, could escape the radiation effect and eventually expand. If this hypothesis proves to be true, it may be necessary to use 'cocktails' of antibodies to improve the distribution of the radionuclide throughout the tumor.

The hematopoietic system, as represented in the mice by the bone marrow and the spleen, was the only system that exhibited any radiation-induced toxic effects at the light microscopic level. Doses of 300 μCi of radiolabeled MAb produced a partial marrow suppression with approx. 50% cellularity remaining at 11 days post-injection. The 500 μCi dose caused marrow aplasia that persisted at 17 days post-injection and presumably was responsible for the death of 2 of 10 mice. When the mice surviving the high dose were allowed to live up to 30 days post-injection, they experienced a total recovery of the bone marrow. The marrow toxicity was probably due to the gamma emission radiating from the circulating MAb-radionuclide conjugate,

since the mouse is relatively very small and the radionuclide, ^{131}I, is a strong beta and gamma emitter. Therefore, larger animal models may be more appropriate for toxicity studies once the therapeutic potential of the MAb, as well as dosing schedules, are determined.

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REFERENCES

1. Farrands PA, Pimm MV, Embleton MJ, *et al.* Radioimmunodetection of human colorectal cancers by an anti-tumor monoclonal antibody. *Lancet* 1982, **2**, 397–400.
2. Mach J-P, Chatall J-F, Lumbroso J-D, *et al.* Tumor localization in patients by radiolabeled monoclonal antibodies against colon carcinoma. *Cancer Res* 1983, **43**, 5593–5600.
3. Moldofsky PJ, Powe J, Mulhern CB Jr, *et al.* Metastatic colon carcinoma detected with radiolabeled $F(ab')_2$ monoclonal antibody fragments. *Radiology* 1983, **149**, 549–555.
4. Chatal J-F, Saccavini J-C, Fumoleau P, *et al.* Immunoscintigraphy of colon carcinoma. *J Nucl Med* 1984, **25**, 307–314.
5. Epenetos AA, Mather S, Granowska M, *et al.* Targeting of iodine-123-labelled tumor-associated monoclonal antibodies to ovarian, breast, and gastrointestinal tumours. *Lancet* 1982, **2**, 999–1005.
6. Armitage NC, Perkins AC, Pimm MV, Farrands PA, Baldwin RW, Hardcastle JD. The localization of an anti-tumour monclonal antibody (791T/36) in gastrointestinal tumours. *J Surg* 1984, **71**, 407–412.
7. DeLand FH, Goldenberg DM. Diagnosis and treatment of neoplasms with radionuclide-labeled antibodies. *Seminars in Nucl Med* 1985, **15**, 2–11.
8. Zalcberg JR, Thompson CH, Lichtienstein M, McKenzie FC. Tumor immunotherapy in the mouse with the use of ^{131}I-labeled monoclonal antibodies. *JNCI* 1984, **72**, 697–704.
9. Capone PM, Lawrence D, Papsidero LD, Croghan GA, Ming Chu T. Experimental tumoricidal effects of monoclonal antibody against solid breast tumors. *Proc Natl Acad Sci U.S.A.* 1983, **80**, 7328–732.
10. Jones DH, Goldman A, Gordon I, Pritchard J, Gregory BJ, Kenshead JT. Therapeutic application of a radiolabelled monoclonal antibody in nude mice xenografted with human neuroblastoma: tumoricidal effects and distribution studies. *Int J Cancer* 1985, **35**, 715–720.
11. Goldenberg DM, Gaffar SA, Bennet J, Beach L. Experimental radioimmunotherapy of a xenografted human colonic tumor (GW-39) producing carcinoembryonic antigen. *Cancer Res* 1981, **41**, 4354–4360.
12. Wakabayashi S, Okamoto S, Taniguchi M. Anti-tumor effects of radiolabeled syngeneic monoclonal anti-melanoma antibodies. *Gann* 1984, **75**, 707–713.
13. Courtenar-Luck N, Epenetos AS, Halnan KE, *et al.* Antibody-guided irradiation of malignant lesions: three cases illustrating a new method of treatment. *Lancet* 1984, **2**, 1441–1443.
14. Epenetos AA, Courtenay-Luck N, Pickering D, Hooker G, Lavender JP, McKenzie CG. Antibody-guided irradiation of brain glioma by arterial infusion of radioactive monoclonal antibody against epidermal growth factor receptor and blood group A antigen. *Br Med J* 1985, **290**, 1463–1466.
15. Carrasquillo JA, Krohn KA, Beaumier P, *et al.* Diagnosis of, and therapy for, solid tumors with radiolabeled antibodies and immune fragments. *Cancer Treat Rep* 1984, **68**, 317–328.
16. Colcher D, Hand P, Nuti M, Schlom J. A spectrum of monoclonal antibodies reactive with human mammary tumor cells. *Proc Natl Acad Sci U.S.A.* 1981, **78**, 3199–3203.
17. Stramignoni D, Bowen R, Atkinson BF, Schlom J. Differential reactivity of monoclonal antibodies with human colon adenocarcinomas and adenomas. *Int J Cancer* 1982, **31**, 543–552.
18. Nuti M, Teramoto YA, Mariani-Constantini R, Horan Hand P, Colcher D, Schlom J. A monoclonal antibody (B72.3) defines patterns of distribution of a novel tumor-associated antigen in human mammary carcinoma cell populations. *Int J Cancer* 1982, **29**, 539–545.
19. Colcher D, Keenan AM, Larson SM, Schlom J. Prolonged binding of a radiolabeled monoclonal antibody (B72.3) used for the *in situ* radioimmunodetection of human colon carcinoma xenografts. *Cancer Res* 1984, **44**, 5744–5751.
20. Keenan AM, Colcher D, Larson SM, Schlom J. Radioimmunoscintigraphy of human colon cancer xenografts in mice with radioiodinated monoclonal antibody B72.3. *J Nucl Med* 1984, **25**, 1197–1203.
21. Colcher D, Carrasquillo JA, Sugarbaker P, *et al.* Biodistribution of monoclonal antibody N72.3 (I-131) in colon cancer patients. *J Nucl Med 1985*, **26**, P15.
22. Colcher D, Esteban JM, Carrasquillo JA, *et al.* Comparison of route of administration of radiolabeled monoclonal antibodies in patients with colorectal cancer. *J Nucl Med* 1986, **27**, 902.
23. Tom BH, Rutzky LP, Jakstys MM. Human colonic adenocarcinoma cells. I. Establishment and description of a new line. *In Vitro* 1976, **12**, 180–191.
24. Hall EJ. *Radiobiology for the Radiologist.* Philadelphia, Harper & Row, 1978.

25. Colcher D, Mornex F, Esteban J, Minelli MF, Schlom J. Radioimmunotherapy of human colon carcinoma xenografts using monoclonal antibody B72.3. *J Nucl Med* 1986, **27**, 1019.