Radiolocalization of Human Pancreatic Tumors in Athymic Mice by Monoclonal Antibody DU-PAN 1

Andrew J. Worlock, Michael R. Zalutsky, and Richard S. Metzgar

Departments of Microbiology and Immunology [A. J. W., R. S. M.] and Radiology [M. R. Z.], Duke University Medical Center, Durham, North Carolina 27710

ABSTRACT

Monoclonal antibodies that selectively bind to pancreatic tumors may be useful in the therapy and diagnosis of pancreatic carcinoma. In this study we have examined the tumor localization of radiolabeled DU-PAN 1, a mouse monoclonal antibody that is selective for a human pancreatic cancer-associated antigen. After radiolabeling, both DU-PAN 1 intact monoclonal antibody and F(ab′)2 fragments retained immunoreactivity and showed high affinity for the pancreatic tumor cell line CA13 in vitro. Paired-label biodistribution studies in nude mice bearing CA13 s.c. xenografts were performed. Mice received both 131I-labeled DU-PAN 1 immunoglobulin G2a or F(ab′)2 fragment and 125I-labeled mouse myeloma immunoglobulin G2a or F(ab′)2 fragment. Tumor uptake for 5-µg doses of DU-PAN 1 immunoglobulin ranged from 4.8 to 11.83% injected dose/g. Tumor uptake values for mice given 5-µg doses of DU-PAN 1 F(ab′)2 ranged from 3.9 to 6.9% injected dose/g. Tumor uptakes of the respective myeloma controls were lower in all cases when compared with the DU-PAN 1 preparations. Tumor localization indices for 5-µg doses of DU-PAN 1 immunoglobulin were 3.0 at 24 h and 2.9 at 48 h. For 5-µg doses of DU-PAN 1 F(ab′)2, tumor localization indices were 29.9 at 24 h and 90.0 at 48 h. In most cases, tumor:normal tissue ratios were greater than 3 at all time points, indicative of tumor selectivity for both DU-PAN 1 preparations, but the ratios were considerably higher using the DU-PAN 1 F(ab′)2. The F(ab′)2 fragment thus displays better tumor localization characteristics when compared with the intact immunoglobulin. Protein doses of DU-PAN 1 F(ab′)2 of between 5 and 10 µg gave the best localization, although protein doses of up to 100 µg could be administered before apparent tumor saturation was seen.

INTRODUCTION

MoAbs1 that specifically localize to tumors when conjugated to radionuclides or cytotoxic drugs are potentially valuable in the diagnosis and therapy of cancer. The tumor selectivity of such agents could be used to direct treatment to the tumor site or to detect disease in cases of tumors for which no reliable test is yet available. Before any new conjugate can be used in the treatment of human disease, it is necessary to assess its ability to localize to human tumors grown as xenografts. Such studies have been used to evaluate MoAbs to colonic carcinoma (1–7), colorectal carcinoma (8), glioma (9, 10), mammary carcinoma (11), leukemia (12), melanoma (13), small cell carcinoma (14), and insulinoma (15).

The prognosis of pancreatic adenocarcinoma remains poor, with less than 2% of all patients surviving for 5 years (16). Since there are currently no reliable diagnostic tests for detecting pancreatic carcinoma at a treatable stage, targeted MoAbs may be useful in improving the diagnosis or therapy of the disease. Several studies using MoAbs to antigens expressed by pancreatic tumors have examined the ability of such agents to detect the tumor in vivo. In nude mice with human pancreatic cancer xenografts, Klapdor et al. (17–19) and Goldrosen et al. (20) investigated the localization of radiolabeled intact anti-CEA and anti-CA19-9 MoAbs. In the same model Klapdor et al. (18) and Senekowitsch et al. (21) used radiolabeled anti-CEA and anti-CA19-9 F(ab′)2 fragments for localization studies. BW 494/32 (22, 23), DU-PAN 2 (24), and B72.3 (25) MoAbs have also been used in nude mice with human pancreatic cancer xenografts. Radiolabeled anti-CEA, anti CA19-9, and BW 494/32 MoAbs have also been tested clinically and found to localize to some degree (24, 26–29). Although some success has been achieved with the antibodies used to date, many of the studies have utilized MoAbs raised against secreted tumor antigens such as CEA and DU-PAN 2; it would seem that better localization could be achieved if antibodies to membrane-bound tumor antigens were used. Also, since it is unlikely that any one MoAb will bind to every cell in a heterogeneous tumor, MoAb “cocktails” may be required for optimal tumor detection.

DU-PAN 1 is a murine MoAb of the IgG2a isotype produced against the human pancreatic tumor cell line HPAF (30, 31). Although the molecular properties of the DU-PAN 1 antigen are still poorly defined, the antigen is a glycoprotein and is present in more than 50% of pancreatic tumors and pancreatic tumor cell lines (30–32). The DU-PAN 1 MoAb shows only limited reactivity with some normal secretory epithelial cells (32) and has not been detected in spent tissue culture media from antigen-expressing tumor cell lines (unpublished data). The purpose of these studies is to determine, in a preclinical model, the potential usefulness of the DU-PAN 1 MoAb in diagnosis and therapy of pancreatic cancer. Because of their potential pharmacological advantage, F(ab′)2 fragments of DU-PAN 1 were also studied. This report describes paired-label biodistribution studies of radiolabeled DU-PAN 1 IgG2 and F(ab′)2 fragments in nude mice with CA13 pancreatic tumor xenografts. Specific tumor uptake for both DU-PAN 1 intact immunoglobulin and F(ab′)2 fragment was demonstrated, with the F(ab′)2 fragment providing better tumor:normal tissue uptake ratios.

MATERIALS AND METHODS

Antibody Production and Purification. The in vitro culture methods used in this study for the murine hybridomas have been described previously (30). Ascites were made using the DU-PAN 1 hybridoma. Nude mice (nu/nu strain; Charles River, Wilmington, MA) were primed i.p. with 0.5 ml of 2,6,10,14-tetramethylpentadecane (Sigma, St. Louis, MO). Fourteen days later, 106 hybridoma cells were injected s.c. The ascitic fluid was harvested and the MoAb DU-PAN 1 was purified by high-pressure liquid chromatography using a Bakerbond Abx column (Phillipsburg, NJ). The separated antibody was dialyzed against phosphate-buffered saline (pH 7.2, Na2HPO4, 1.1 g/liter; NaH2PO4, H2O, 0.32 g/liter; NaCl, 8.5 g/liter) concentrated to 1 mg/ml and isotyped as IgG2a. A purified IgG2a mouse myeloma antibody (Sigma) was used as a control to assess nonspecific binding.

DU-PAN 1 and Myeloma F(ab′)2 Fragment Production. DU-PAN 1 and IgG2a mouse myeloma antibody were dialyzed against 0.1 M...
potassium phosphate (pH 4.5) and then incubated with pepsin (6000 units/mg of antibody), immobilized on agarose beads, overnight at 37°C with constant agitation. The beads were removed by centrifugation and the supernatant fluid was dialyzed against phosphate-buffered saline (pH 7.2). The Fc fragments and undigested antibody were removed from the solution by passage through a protein A-Sepharose CL 4B column (Pharmacia, Piscataway, NJ).

Radiolabeling of Intact DU-PAN 1 Immunoglobulin and F(ab')2 Fragments. Radiolabeling of DU-PAN 1 immunoglobulin and F(ab')2 fragments with 125I and of myeloma immunoglobulin and F(ab')2 fragments with 131I was performed using the Iodogen method. Intact immunoglobulin or F(ab')2 fragment (100 to 200 μg) and sufficient isotope to allow a specific activity of 0.6 to 1.9 μCi/μg were combined in a glass vial coated with 10 μg of Iodogen (Pierce, Rockford, IL). The iodination reaction was allowed to proceed for 10 min and was terminated by removal of the solution from the reaction vessel. Any unincorporated iodine was removed from the solution using a Sephadex G-25 column (Pharmacia). Purity of the radiolabeled immunoglobulin and F(ab')2 fragments was assessed using 7.5% sodium dodecyl sulfate gel electrophoresis under nonreducing conditions (Fig. 1).

Cell Lines. A clone of the human pancreatic tumor cell line HPAF, designated CA13, which expresses the DU-PAN 1 antigen (33), was used as a reactive target in vitro and in vivo. The cell line HUFF, a diploid human foreskin fibroblast obtained from the American Type Culture Collection (Rockville, MD), was used to measure nonspecific binding in immunoreactivity and affinity determinations (34).

DU-PAN 1 Immunoreactive Fraction and Affinity. After radiolabeling, the fraction of MoAb preparations reacting with the target cell line (immunoreactive fraction) and the affinity of the preparations for the target cell line were determined in vitro. The immunoreactive fraction was determined using the method of Lindmo et al. (35). Cell line CA13 was used as the positive target, and the HUFF cell line was used to determine nonspecific binding. Cells at concentrations between 6.6 × 10⁴/ml and 3.5 × 10⁵/ml were incubated overnight at 4°C with 15 ng of labeled MoAb preparation. Cells were washed 3 times and radioactivity in the pellet was determined using a gamma counter. Specific binding to the target cell was determined and immunoreactivity was calculated from a computer-generated linear regression plot of total applied/specifically bound cpm against the inverse of cell concentration.

Affinity of radiolabeled preparations was determined by growing 50,000 cells/well in 96-well plates (Dynatech, Chantilly, VA). Cells were incubated overnight at 4°C with 200 μl of labeled MoAb over a concentration range of 1.2 to 2500 ng and were washed 3 times, and the bound activity was determined using a gamma counter. DU-PAN 1 IgG and F(ab')2 affinities were determined from computer-generated Scatchard plots of specifically bound MoAb divided by free MoAb against antibody concentration.

Tumor Formation in Nude Mice. CA13 pancreatic tumor cells growing as monolayer cultures were removed from flasks by trypsinization. The cells were washed with phosphate-buffered saline (pH 7.2), and 2 × 10⁶ cells in 0.2 ml of sterile phosphate-buffered saline were injected s.c. into 6-week-old nude mice of both sexes (BALB/c nu/nu; Charles River). The tumors were then allowed to grow for 21 days (giving an average tumor weight of 85 mg) before in vivo localization studies commenced.

Time-dependent Biodistribution of DU-PAN 1, IgG2a. For this paired-label protocol, mice were arranged in 4 groups of 6 animals. Each mouse received an i.v. injection of 5 μg (6.5 μCi) of 125I DU-PAN 1 combined with 5 μg (9.7 μCi) of 131I mouse myeloma IgG2a. At 1, 2, 3, 4, and 7 days after MoAb injection, blood and urine were collected and selected tissues were removed from each mouse. The samples were weighed and their 131I and 125I activity was measured using a dual channel gamma counter with application of appropriate cross-over corrections for 125I activity in the 125I counting window. The percentage of the injected dose of DU-PAN 1 and myeloma immunoglobulins that localized in each tissue was calculated by comparison with standards of appropriate count rate.

Since the tissue distribution experiments were performed in paired-label format, a direct comparison of the uptake of DU-PAN 1 and myeloma, with each animal serving as its own control, was possible. Data were analyzed by using a paired t test (36). Only a probability of <0.05 has been considered to be statistically significant.

Time-dependent Biodistribution of DU-PAN 1 F(ab')2. Mice were arranged in 4 groups of 6 animals. Each mouse received an i.v. injection of 5 μg (5.9 μCi) of 131I-labeled DU-PAN 1 F(ab')2, combined with 5 μg (4.3 μCi) of 125I-labeled mouse myeloma F(ab')2. At 16, 24, 36, and 48 h after injection, blood, urine, and tissue samples were collected and the 131I-labeled DU-PAN 1 F(ab')2 and 125I-labeled myeloma F(ab')2 tissue uptakes were determined by the same method used for intact immunoglobulins.

Dose-dependent Biodistribution of DU-PAN 1 F(ab')2. Mice were divided into 7 groups of 6 animals. Each mouse received a MoAb dose of 1, 5, 10, 50, 100, 250, or 500 μg. Groups of mice received 125I DU-PAN 1 F(ab')2 in doses of 1 μg (0.6 μCi) or 5 μg (3.0 μCi). To avoid complications by irradiating the mice with high doses of 131I, mice that received injections of 10 to 500 μg of DU-PAN 1 F(ab')2 received 5 μg (3.0 μCi) of 125I-labeled DU-PAN 1 F(ab')2 and the remainder of the dose as unlabeled DU-PAN 1 F(ab')2. All mice received 5 μg (2.1 μCi) of 125I-labeled mouse myeloma F(ab')2, as a paired-label control for nonspecific binding. Twenty-four h after MoAb fragment injection, blood, urine, and tissue samples were collected and the tissue distribution of 125I and 131I was determined by the same method used for the labeled intact immunoglobulins.

RESULTS

Characterization of Labeled DU-PAN 1 Preparations. After radiolabeling, the in vitro immunoreactivity and affinity of DU-PAN 1 immunoglobulin and F(ab')2 fragments were tested against the pancreatic tumor cell line CA13. Intact DU-PAN 1 immunoglobulin used to determine time-dependent biodistribution was found to have an immunoreactivity of 100% and a mean affinity of (4.2 ± 1.2 [SE]) × 10⁶ M⁻¹. DU-PAN 1 F(ab')2 fragments used to determine time-dependent biodistribution...
were found to have an immunoreactivity of 65% and a mean affinity of \((7.8 \pm 1.1 \text{ [SE]}) \times 10^6 \text{ M}^{-1}\). DU-PAN 1 F(ab')2 fragments used in dose-dependent biodistribution studies were found to have an immunoreactivity of 70% and a mean affinity of \((3.7 \pm 0.8 \times 10^9 \text{ M}^{-1})\). Thus, it would seem that the affinity of DU-PAN 1 immunoglobulin is not affected by the enzymatic fragmentation. The high immunoreactive fraction of radiolabeled DU-PAN 1 immunoglobulin and F(ab')2 fragments suggests that the purity of the preparations was good. Purity was confirmed by the lack of contaminating bands when the DU-PAN 1 and IgG2a myeloma immunoglobulin and F(ab')2 fragments were analyzed by nonreducing sodium dodecyl sulfate gel electrophoresis (Fig. 1).

Biodistribution of Radiolabeled DU-PAN 1 Immunoglobulin and F(ab')2 Fragment. Biodistribution of 5-μg doses of radiolabeled DU-PAN 1 immunoglobulin and DU-PAN 1 F(ab')2 fragments was examined in nude mice with CA13 pancreatic tumor xenografts at various time points. In order to determine the in vivo binding of each labeled MoAb preparation to the tumor xenograft, tumor uptakes, expressed as percentage of antibody dose per g of tumor, were calculated from the biodistribution results. Tumor xenograft uptake values of DU-PAN 1 immunoglobulin were consistently higher than those for the mouse myeloma IgG2a nonspecific binding control over the 7-day evaluation period (Fig. 2). Maximum tumor uptake occurred at 48 h and was found to be \(11.8 \pm 7.0\% \text{ ID/g for DU-PAN 1 and 7.3} \pm 5.1\% \text{ ID/g for the mouse myeloma immunoglobulin. Tumor uptake differences were statistically significant on days 1, 2, 3, and 7 (P < 0.05). Tumor uptake of DU-PAN 1 F(ab')2 fragments was also significantly higher (P < 0.005) than that of the F(ab')2 mouse myeloma control (Fig. 3) over the 48-h evaluation period. Maximum tumor uptake was seen at 16 h and was found to be 6.6 \pm 1.9\% \text{ ID/g for DU-PAN 1 and 2.5 \pm 0.5}\% \text{ ID/g for the mouse myeloma F(ab')2 fragments. At 24 and 48 h postinjection, tumor uptake of DU-PAN 1 F(ab')2 was slightly reduced over that of intact DU-PAN 1 antibody (Figs. 2 and 3). At 24 and 48 h, tumor uptake of the control mouse myeloma F(ab')2 fragment was less than 0.5\% \text{ ID/g; the levels seen with the intact myeloma control immunoglobulin were 1.5 to 7.3} \% \text{ injected dose/g}."

In order to determine the specificity of tumor uptake, localization indices (the ratio of DU-PAN 1 or F(ab')2 fragment in tissue to nonspecific control MoAb or fragment in tissue normalized to simultaneous levels in blood) were calculated. Tumor localization indices of both intact DU-PAN 1 IgG2a and F(ab')2 fragments are shown in Table 1. Using DU-PAN 1, immunoglobulin, the mean localization index was 3.0 \pm 1.0 at 24 h and it increased to 7.9 \pm 6.3 at 168 h. Localization indices were significantly greater than unit at all time points except 4 days. For DU-PAN 1 F(ab')2, the mean localization index was 16.3 \pm 4.9 at 16 h; this increased to a maximum at 48 h. At 24 and 48 h after MoAb injection, localization indices were significantly higher (P < 0.01) using DU-PAN 1 F(ab')2 than using the intact immunoglobulin. In contrast, as shown in Table 2, the localization index for normal mouse tissues at 24 h for both DU-PAN 1 and its F(ab')2 fragment approaches 1, indicating a lack of specificity in normal tissue binding. Localization indices for normal mouse tissues at other time points showed a similar pattern (data not shown, but available upon request). The selectivity of DU-PAN 1 binding to the tumor in vivo was...
determined by calculating the tumor:normal tissue ratios (the percentage of injected dose of labeled DU-PAN 1 per g of tumor divided by the percentage of injected dose of labeled DU-PAN 1 per g of tissue). The tumor:normal tissue ratios at 24 and 48 h after MoAb or F(ab')2 injection are given in Table 3. Using DU-PAN 1 immunoglobulin at 24 h after injection, the lowest tumor:normal tissue ratio was seen with blood (1.2), and the highest was seen with muscle (13.3). At 48 h using DU-PAN 1 immunoglobulin, the tumor:blood ratio was slightly higher, but it remains the lowest at 1.5; the tumor: muscle ratio again was the highest at 15.3. All ratios, except that for the heart, were increased at 48 h over those for the same tissue at 24 h. Using the DU-PAN 1 F(ab')2 fragment at 24 h after injection, the lowest ratio was the tumor: blood ratio (9.3), while the tumor: muscle ratio was the highest (45.5). With DU-PAN 1 F(ab')2, at 48 h, the tumor: kidney ratio was the lowest (36.1), while muscle remained the highest (198.9). Tumor:normal - pancreas ratios at 24 h were 8.0 ± 0.5 and 36.8 ± 8.8 for DU-PAN 1 IgG2a and F(ab')2, respectively, compared to tumor: pancreas ratios of 2.5 ± 1.0 and 1.3 ± 0.4 for the corresponding mouse myeloma proteins. All tumor:tissue ratios at 48 h were increased over those obtained at 24 h. Tumor:normal - tissue ratios were consistently higher using the F(ab')2 fragment compared to the intact immunoglobulin at the same time points. In addition, the tumor selectivity advantage of DU-PAN 1 compared to myeloma control was much more apparent for the F(ab')2 fragment.

Effect of DU-PAN 1 F(ab')2 Dose of in Vivo Localization. In order to determine the dose dependence of DU-PAN 1 F(ab')2 localization, groups of mice with mean tumor weights ranging from 60 to 100 mg for the various groups were given MoAb doses ranging from 1 to 500 µg. Tumor uptake of DU-PAN 1 F(ab')2 doses at 24 h is shown in Fig. 4. The uptake per g of tumor decreased as the protein dose increased; however, it remained above the mouse myeloma F(ab')2 control over the dose range used. Localization indices for tumor and 3 normal mouse tissues at the various DU-PAN 1 F(ab')2 doses are shown in Table 4. Tumor localization indices remained higher than the localization indices observed for normal tissue throughout the dose range used. Tumor localization indices show significant decreases at doses greater than 100 µg.

Tumor:normal tissue ratios were highest at the 10-µg dose but were not significantly different from those obtained at 1, 5, and 50 µg. A rapid decrease in tumor:normal tissue ratio was observed at higher doses. For example, the tumor: pancreas ratios at 10, 50, 100, 250, and 500 µg of DU-PAN 1 F(ab')2 were 38 ± 3, 30 ± 19, 20 ± 11, 10 ± 3, and 5 ± 3, respectively. Lower tumor:normal tissue ratios at higher protein doses were due not only to lower tumor uptake but, at doses of 250 and 500 µg, also to greater retention of activity in normal organs. For example, uptake of DU-PAN 1 F(ab')2 in normal pancreas was 0.24 ± 0.11% ID/g to 0.40 ± 0.08% ID/g at 1–100 µg and increased to 0.59 ± 0.17% ID/g at 250 µg and 0.81 ± 0.19% ID/g at 500 µg.

### DISCUSSION

Improving the potential for effectively treating pancreatic cancer is critically dependent on the development of methods for the early detection of this disease. Although the sophistication of the conventional diagnostic armamentarium continues to improve, the reliable identification of lesions with diameters of less than 1–2 cm using ultrasound or computerized tomography remains problematic (37). Since clinical studies using a variety of radiolabeled monoclonal antibodies have demonstrated occult disease in patients with lymphoma, melanoma, and colon carcinoma (38), the use of radioimmunoscintigraphy for pancreatic cancer, both as a diagnostic test and as a precursor to therapy, is of considerable interest.

Unfortunately, it has not been possible to reliably identify pancreatic tumors by external imaging with labeled MoAbs. In
patients with pancreatic carcinoma receiving a cocktail of $^{131}$I-labeled F(ab')$_2$ fragments of anti-CA19-9 and anti-CEA, distribution of radioactivity in the abdomen generally was diffuse, with questionable focal uptake in regions corresponding to primary tumors. A subsequent study using the BW 494/32 MoAb labeled with $^{111}$In and its F(ab')$_2$ fragment labeled with $^{131}$I also reported that both primary and metastatic pancreatic carcinoma were detected poorly by planar scintigraphy (29). Although single photon tomographic imaging offered some improvement, tumor:visceral uptake ratios with $^{111}$In-labeled MoAb calculated from the SPECT images were only about 0.2–0.4:1.

The disappointing clinical results obtained using radiouimmunoscintigraphy to detect pancreatic carcinoma could be related to a number of factors, not the least of which is the fact that the MoAbs that have been studied lack true specificity for human pancreatic cancers. An additional problem is that most of the MoAbs studied both clinically and in human pancreatic carcinoma xenograft models react with antigens present in the circulation. Considering the anatomic proximity of the pancreas to the liver and spleen, the formation of labeled immune complexes followed by their deposition in the organs of the reticuloendothelial system could create a significant level of background activity that could obscure and compete with uptake of labeled MoAb in tumor sites. In the present study, we have investigated the tissue distribution of $^{131}$I-labeled DU-PAN 1 MoAb and its F(ab')$_2$ fragment in an athymic mouse model of human pancreatic carcinoma. This MoAb was selected because it reacts with a majority of pancreatic tumors and tumor cell lines (30–32) and because its antigen does not appear to be shed from antigen-expressing tumor cells. Our results indicate that radioidinated DU-PAN 1 MoAb and F(ab')$_2$ fragments localize specifically and selectively in CA13 pancreatic tumor xenografts to a degree that compares favorably with previously reported biodistribution data using other MoAbs and fragments (17–24).

With intact DU-PAN 1 IgG, uptake of $^{131}$I in CA13 xenografts was about 10% injected dose/g or higher during the first 48 h after injection. Determination of the specificity of tumor localization required the co-injection of an isotype-matched nonspecific MoAb or fragment in order to calculate the localization index. Localization indices in tumor were between 2.9 and 7.9 at all time points, indicating that tumor uptake of DU-PAN 1 was specific. In comparison, Senekowitsch et al. (21) reported that the uptake of radioiodinated anti-CEA MoAb was only about 3% injected dose/g over the same time period. Since a nonspecific control MoAb was not used, the specificity of anti-CEA localization in pancreatic tumor xenografts cannot be ascertained.

Since F(ab')$_2$ fragments are cleared more rapidly from the blood than are their intact equivalents, use of these fragments, compared to 3.0 for intact MoAb at 24 h, the tumor:normal-tissue ratios are as much as 20 times higher, and the magnitude of tumor uptake is only slightly lower; thus, the F(ab')$_2$ fragment appears to be the more promising form of DU-PAN 1 for diagnostic and therapeutic applications.

Radioimmunotherapeutic trials using labeled MoAb in animal models of human pancreatic cancer have been uniformly unsuccessful. After i.v. injection, tumor doses of only about 500–1000 rad per mCi of $^{131}$I-labeled MoAb could be achieved (17, 19). Since the tumor localization observed for $^{131}$I-labeled DU-PAN 1 F(ab')$_2$ was considerably higher than that reported for the MoAbs and fragments used in these studies, investigation of the radioimmunotherapeutic potential of $^{131}$I-labeled DU-PAN 1 F(ab')$_2$ (and possibly intact MoAb) appears to be warranted. If DU-PAN 1 F(ab')$_2$ is to be used in radiotherapy, it is important to maximize the absolute level of tumor uptake of radioactivity which can be achieved. One approach for increasing the radiation dose to tumor is to raise the specific activity of the labeled MoAb. However, Colapinto et al. (42), using $^{131}$I-labeled Mel-14 F(ab')$_2$, fragment, reported that immunoreactivity and affinity were reduced at specific activities greater than 13 μCi/μg. The specific activity limitation for DU-PAN 1 F(ab')$_2$ has not been determined and, indeed, may be different from that of Mel-14 F(ab')$_2$.

Increasing the amount of MoAb protein administered also could raise the quantity of radioactivity delivered to tumor. This strategy is limited, however, by the need to keep circulating, unbound MoAb concentrations to acceptable levels. Therefore, it is necessary to determine the maximum MoAb protein dose that can be administered before tumor versus normal-tissue localization is compromised by MoAb saturation of the tumor. The dose range used in this study was 1 to 500 μg of protein. Tumor localization 24 h after MoAb injection was optimal at doses of 5 to 10 μg, but satisfactory tumor uptake values, localization indices, and tumor:normal tissue ratios were also obtained at doses of up to 100 μg. Therefore, we believe that it will be feasible to study the therapeutic efficacy of DU-PAN 1 F(ab')$_2$ in athymic mice with pancreatic tumor xenografts. These studies could form the impetus for a clinical trial of the toxicity and therapeutic efficacy of radiolabeled DU-PAN 1 F(ab')$_2$ for treatment of patients with pancreatic carcinoma.

ACKNOWLEDGMENTS

The authors would like to thank Susan Slade and Cary Harrison for excellent technical help and Ann Tamariz for editorial assistance.

REFERENCES


Radiolocalization of Human Pancreatic Tumors in Athymic Mice by Monoclonal Antibody DU-PAN 1

Andrew J. Worlock, Michael R. Zalutsky and Richard S. Metzgar