[CANCER RESEARCH 47, 3847–3850, July 15, 1987]

Radioimmunodetection of Human Glioma Xenografts by Monoclonal Antibody to Epidermal Growth Factor Receptor[1]

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ABSTRACT

Murine IgG2a monoclonal antibody (MAb) 425 specifically detects epidermal growth factor receptor, which is expressed on human gliomas and tumors of other tissue origin but rarely on normal brain tissues, and not at all on bone marrow and peripheral blood cells. ^{131}I-labeled F(ab')$_2$ fragments of this MAb injected into nude mice grafted with U-87 MG glioma cells preferentially localized in tumor tissue compared to normal mouse tissues, as determined by differential tissue counting of radioactivity. The mean tumor-to-tissue ratios of radioactivity ranged between 8.2 (blood) and 55.8 (muscle) at 2 days after the injection of 15 μCi of ^{131}I-425 F(ab')$_2$/mouse. Radiolabeled fragments of an anti-hepatitis virus IgG2a MAb did not localize in tumors. The localization index derived from the ratios of specific antibody to indifferent antibody in tumor tissue relative to blood was 9.94 at 2 days following the MAb injection. The labeled MAb did not localize in a xenograft of colorectal cancer tumor, which does not express the epidermal growth factor receptor. Tumors could be located by whole-body γ-scintigraphy without background subtraction following the injection of 100 μCi of radiolabeled MAb 425 F(ab')$_2$ fragments. The data suggest that MAb 425 is a likely candidate for clinical diagnostic and radioimmunotherapy trials.

INTRODUCTION

The reactivity of MAbs[3] with human tumor cells may allow the precise localization and diagnosis of malignant tumors (1–3). A murine antiglioma MAb has been shown to localize human gliomas xenotransplanted s.c. and intracranially into athymic mice (4–6). Initial clinical trials with various MAbs to image brain tumors in patients have shown modest results (7–9).

We chose to evaluate MAb 425, which binds specifically to the EGF receptor (10), for its ability to localize human malignant glioma xenografts. Expression of EGF receptors in brain tumors of glial origin, but not in normal brain tissue, has been reported (11–13), and an association between expression of the receptor and amplification of v-*erb*-B oncogene expression has been observed (14).

The feasibility of tumor targeting by MAb to the EGF receptor has been suggested by Masui *et al.* (15) in an epidermoid carcinoma xenotransplant model.

MATERIALS AND METHODS

Human Tumor Cell Lines. The glioma (astrocytoma grade III) cell line U-87MG (16) (American Type Culture Collection, Rockville, MD) and colorectal carcinoma cell line SW-707 (17) (A. Leibovitz, Scott and White Clinic, Temple, TX) were maintained in L-15 medium containing 10% fetal bovine serum.

MAbs. The production and characterization of MAb 425 (IgG2a) derived from mice immunized with A431 epidermoid carcinoma cells has been described (10). The binding specificity of MAb 425 for the EGF receptor has also been demonstrated (10).

As controls, MAb A5C3 (IgG2a) reacting with hepatitis virus (kindly supplied by Centocor, Malvern, PA) and P3X63Ag8 myeloma antibody were used.

IP Assays. Fresh tumor and normal tissues obtained from excess surgical specimens were immediately frozen in liquid nitrogen and stored at −70°C. Some specimens were in addition treated with Bouin's fixative. All tissues were cut into 6-μm sections and tested for MAb binding using the Vectastain ABC Kit (Vector Laboratories, Inc., Burlingame, CA) with some previously described modifications (18, 19). Frozen tissues showed more intense staining than fixed cells with MAb 425, and results are shown for frozen tissues only.

Cytospin preparations of normal unfixed human bone marrow and peripheral blood cells were also examined.

Purification and Fragmentation of MAbs. MAbs were purified from ascitic fluid on protein A-Sepharose columns, and F(ab')$_2$ fragments were produced by pepsin digestion as previously described (20).

Radiolabeling of F(ab')$_2$ Fragments. F(ab')$_2$ fragments were labeled with ^{131}I [425-F(ab')$_2$] or ^{125}I [A5C3-F(ab')$_2$] using the Iodogen method (21). The specific activities of ^{131}I-labeled 425 F(ab')$_2$ and ^{125}I-labeled A5C3 F(ab')$_2$ fragments ranged in different experiments between 2.7 and 8.3 and 2.0 and 2.4 μCi/μg of protein, respectively.

Antibody Binding Assay. Immunoreactivity and binding specificity of radiolabeled MAbs was determined *in vitro* prior to their use in tumor localization studies as described in detail elsewhere (20).

Mice and Xenografts. Four-to-6-week-old nude mice (*nu/nu* BALB/c background) were given injections s.c. of either 1.5×10^7 human glioma cells (U-87MG) or 5×10^6 colorectal carcinoma cells (SW-707) in the upper dorsal region. All mice received 0.1% (v/v) potassium iodide in their drinking water throughout the experiment beginning 48 h before radiolabeled antibody administration in order to block uptake of free radioiodine by the thyroid gland.

***In Vivo* Tissue Distribution of Radiolabeled MAbs.** Since the imaging characteristics of fragments are reportedly superior to those of intact antibody (20, 22–25), F(ab')$_2$ fragments of MAb 425 were used in all tumor localization studies. Twelve days after tumor implantation when the tumors weighed 200 to 500 mg, 15 μCi (1.8 to 5.5 μg) of ^{131}I-labeled 425 F(ab')$_2$ and 15 μCi of ^{125}I-labeled A5C3 control F(ab')$_2$ were simultaneously injected i.p. Two and 4 days later, mice were sacrificed and dissected. Tumor, blood, visceral organs, and muscle samples were weighed and assayed for radioactivity.

The results are expressed as (*a*) ratios of specific activity of antibody in tumor to normal mouse tissues [(cpm/g in tumor)/(cpm/g in normal tissue)], (*b*) percentage of injected radioactivity per g of tissue, and (*c*) localization index, *i.e.*, the ratio of specific ^{131}I to unrelated ^{125}I activity in tumors and organs divided by the same ratio in the blood (26).

Radioimaging of Human Tumor Xenografts. For γ-scintigraphy, mice xenografted with tumors 12 days before were given injections i.p. of 100 μCi (12.0 to 36.7 μg) of ^{131}I-labeled MAb 425 F(ab')$_2$ fragments, and images were obtained daily for up to 4 days after injection. Mice were anesthetized and images were obtained in lateral views as described previously (20).

Statistics. The statistical significance of differences was determined using Student's *t* test.

Received 12/1/86; revised 4/15/87; accepted 4/20/87.

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[1] This work was supported by the Förderer Fund for Excellence from Children's Hospital of Philadelphia, Kenneth Silverberg Memorial Fund from the Wistar Institute, and grants CA-25874 and CA-20815 from NIH.

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[3] The abbreviations used are: MAb, monoclonal antibody; EGF, epidermal growth factor; IP, immunoperoxidase.

RESULTS

Binding Specificity of MAb 425 to Various Tumor and Normal Tissues in IP Assays. Table 1 summarizes the reactivity of MAb 425 with frozen sections of various tumor and normal tissues as assessed in IP assay. MAb 425 bound to most astrocytomas, 3 of 10 medulloblastomas, and one of 2 meningiomas. It also bound to the one ovarian carcinoma and one lung carcinoma studied. MAb 425 did not bind to the colon carcinoma, melanoma, pheochromocytoma, or neurofibromas tissues tested.

The MAb showed weak binding to two of nine normal brain tissue specimens and one of three specimens of cervical spinal cord. It also bound to kidney tubules, hepatocytes, squamous cells, and sweat glands of the skin. MAb 425 showed no reactivity with hematogenous cells obtained from either bone marrow or peripheral blood and tissues from lung and femoral nerve.

Fig. 1 shows positive IP staining of astrocytoma and negative staining of normal brain, respectively, with MAb 425.

***In Vitro* Binding Specificity of ^{131}I-labeled MAb 425 F(ab')$_2$ Fragments.** Binding specificity of radiolabeled MAbs was determined *in vitro* as described in detail elsewhere (20). ^{131}I-labeled MAb 425 F(ab')$_2$ fragments demonstrated high binding reactivity to U-87MG target cells (40 to 60% of the labeled fragments maximally bound to the cells), but did not bind significantly to control SW-707 colorectal carcinoma cells (less than 5% maximum binding). ^{125}I-labeled F(ab')$_2$ fragments of monoclonal anti-hepatitis virus antibody did not bind significantly to U-87MG cells (less than 2% maximum binding).

***In Vivo* Tissue Distribution of ^{131}I-labeled MAb 425 F(ab')$_2$ Fragments.** Nude mice bearing U-87MG glioma xenografts were given injections of 15 μCi of ^{131}I-labeled F(ab')$_2$ fragments of MAb 425. The distribution of the F(ab')$_2$ fragments in various tissues of xenografted mice is shown in Table 2. F(ab')$_2$ fragments of MAb 425 demonstrated preferential localization in gliomas as reflected by high tumor-to-tissue ratios of radioactivity (ratios ranged between 8.2 and 55.8 on day 2 and between 7.04 and 31.9 on day 4) as well as high specific activities in tumor tissues (1.78% of the injected antibody dose/g tumor tissue) as compared to normal mouse tissues (maximum 0.15% for kidney tissue). In mice xenografted with SW-707 colorectal carcinoma cells (Table 2), and given injections of MAb 425, specific activity ratios in tumors compared to normal mouse tissue were strikingly lower than in glioma-bearing mice on both days tested ($P < 0.001$ to $P < 0.05$ for various tissues on days 2 and 4; t test). Comparison of the tumor and the normal tissue distribution of ^{131}I-labeled MAb 425 F(ab')$_2$ fragments and of ^{125}I-labeled F(ab')$_2$ fragments of anti-hepatitis virus MAb A5C3 revealed localization indices (relevant antibody per unrelated antibody ratios in tumor tissue relative to blood) between 9.94 (day 2) and 7.92 (day 4) as compared with indices between 0.24 and 1.30 in all normal mouse tissues tested. Thus, accumulation of MAb 425 in tumor tissue was significantly greater compared to accumulation of control antibody. These differences were more pronounced at 2 days following antibody injection.

Table 1 *Summary of MAb 425 reactivity with various frozen tissues by immunoperoxidase staining*

Tissue	No. of positive specimens/total no. tested
Tumor	
Astrocytoma	12/17
Medulloblastoma	3/10
Meningioma	1/2
Ovary carcinoma	1/1
Lung carcinoma	1/1
Normal	
Cerebrum	1/4
Cerebellum	1/5
Cervical spinal cord	1/3
Kidney tubules	1/2
Liver hepatocytes	1/1
Skin (squamous cells, sweat glands)	1/2

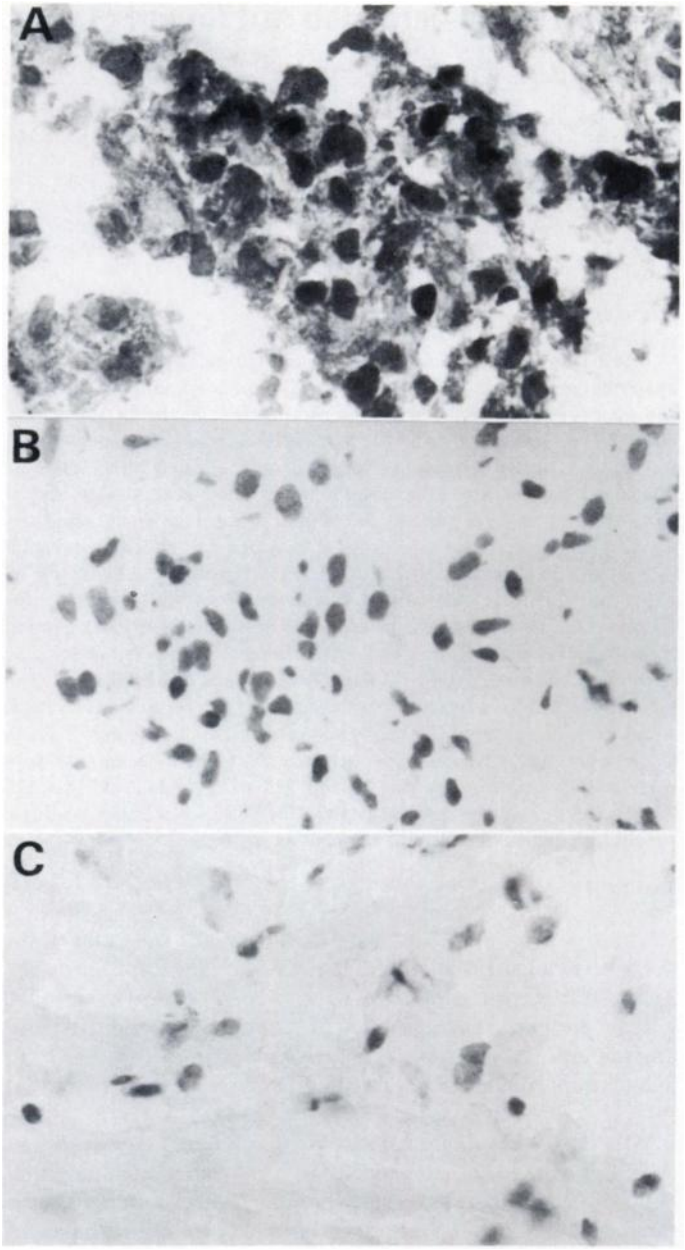

Fig. 1. IP staining of frozen sections of astrocytoma reacted with (*A*) anti-EGF receptor MAb 425, where staining of malignant cells is seen as dark areas, and (*B*) negative control myeloma antibody P3X63Ag8, where no staining is observed. IP staining of frozen section of normal brain reacted with MAb 425 (*C*) demonstrates no reactivity. Avidin-biotin reaction counterstained with hematoxylin, × 640.

Tumor Localization of ^{131}I-labeled MAb 425 F(ab')$_2$ Fragments by Radioimmunoimaging.

γ-Scintigraphy was performed in three mice xenografted with U-87MG cells and given injections of 100 μCi of ^{131}I-labeled MAb 425 F(ab')$_2$ fragments. All three mice xenografted with glioma cells showed clear tumor localization 36 h after antibody administration (Fig. 2). It was possible to localize the tumor

Table 2 *Distribution of ^{131}I-labeled MAb 425 F(ab')$_2$ fragments in tissues of human tumor-grafted nude mice*

Tissue	Tumor/tissue ratios of radioactivity [(cpm/g tumor)/(cpm/g tissue)][a]				Specific tissue activity in mice bearing glioma U-87MG cells (% antibody dose injected/g of tissue)[a]		Localization index in mice bearing glioma U-87MG cells [(cpm ^{131}I/g tissue)/(cpm ^{125}I/g tissue)] / [(cpm ^{131}I/g blood)/(cpm ^{125}I/g blood)][b]	
	Glioma U-87 MG		Colon carcinoma SW-707					
	Day 2	Day 4	Day 2	Day 4	Day 2	Day 4	Day 2	Day 4
Tumor	1.0	1.0	1.0	1.0	1.78 ± 0.40	0.52 ± 0.36	9.94 ± 4.78	7.92 ± 0.02
Blood	8.24 ± 0.88	7.04 ± 4.05	0.45 ± 0.10	0.40 ± 0.08	0.24 ± 0.04	0.07 ± 0.01	1.0	1.0
Small intestine	22.86 ± 6.09	16.47 ± 10.44	1.33 ± 0.15	1.00 ± 0.08	0.08 ± 0.02	0.03 ± 0.01	0.52 ± 0.27	0.98 ± 0.19
Colon	44.22 ± 8.99	21.00 ± 9.46	2.25 ± 1.55	1.48 ± 0.31	0.04 ± 0.01	0.02 ± 0.01	0.40 ± 0.12	0.74 ± 0.11
Stomach	16.38 ± 4.49	16.70 ± 7.81	0.38 ± 0.05	0.50 ± 0.12	0.11 ± 0.03	0.03 ± 0.01	0.68 ± 4.49	0.76 ± 0.29
Spleen	25.82 ± 4.36	14.66 ± 12.46	1.18 ± 0.17	0.78 ± 0.13	0.07 ± 0.01	0.04 ± 0.01	0.24 ± 0.05	0.24 ± 0.05
Kidney	11.52 ± 1.03	8.56 ± 3.93	0.60 ± 0.20	0.53 ± 0.13	0.15 ± 0.03	0.06 ± 0.01	0.28 ± 0.04	0.32 ± 0.04
Liver	27.24 ± 4.38	17.16 ± 12.28	1.35 ± 0.39	0.63 ± 0.19	0.06 ± 0.01	0.03 ± 0.01	0.28 ± 0.04	0.28 ± 0.04
Lung	13.12 ± 2.57	10.98 ± 8.02	0.68 ± 0.15	0.58 ± 0.10	0.14 ± 0.02	0.05 ± 0.01	0.58 ± 0.04	0.60 ± 0.10
Heart	27.66 ± 3.91	17.44 ± 9.90	1.58 ± 0.50	1.38 ± 0.38	0.06 ± 0.01	0.03 ± 0.01	0.92 ± 0.04	1.16 ± 0.15
Muscle	55.76 ± 17.05	31.90 ± 17.49	1.80 ± 0.73	2.20 ± 0.63	0.03 ± 0.01	0.02 ± 0.01	0.78 ± 0.11	1.30 ± 0.30

[a] Mean ± SD of 4 to 5 mice at 2 and 4 days after the injection of 15 μCi of ^{131}I-labeled 425 F(ab')$_2$.
[b] Mean ± SD of 5 mice at 2 and 4 days after the simultaneous injection of 15 μCi each of ^{131}I-labeled 425 F(ab')$_2$ and ^{125}I-labeled A5C3 F(ab')$_2$.

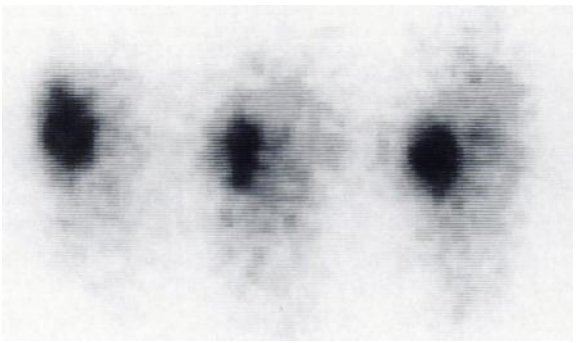

Fig. 2. γ-Scintigraphy in U-87MG tumor-bearing mice given injections of 100 μCi of ^{131}I-labeled 425 F(ab')$_2$ 36 h before. Each mouse had a tumor weighing between 270 and 430 mg in the upper dorsal region. Right lateral view images were obtained. The images clearly demonstrate the tumor xenografts. These images are not contrast enhanced.

without background subtraction. The total counts within the entire tumor region were determined from the computerized images and compared to an equal sized region of interest over the contralateral site. The ratios were 2.5, 1.8, and 2.1 for the three mice, respectively. Similar results were obtained in another γ-scintigraphy experiment including four glioma-grafted nude mice (not shown).

As a control, one tumor-bearing nude mouse was given an injection of 100 μCi of ^{131}I-labeled irrelevant MAb [A5C3 F(ab')$_2$]. The tumor could not be localized 36 h after antibody administration (not shown). The ratio of counts within the tumor to the contralateral site was 0.9.

DISCUSSION

We have demonstrated here that a MAb reacting with human EGF receptor selectively binds to brain tumors, particularly astrocytomas. F(ab')$_2$ fragments of this MAb specifically localized in xenografts of human malignant gliomas in mice. Imaging of tumors was successful without the use of subtraction methods, and the optimal imaging time appears to occur around the second day. Of the tumors of other tissue origins tested, only ovarian and lung carcinomas showed some antibody reactivity. Hematogenous cells were nonreactive, and normal human brain tissues only rarely showed reactivity. While the EGF receptor was detected at low density in a few normal tissues, *i.e.*, kidney tubules, hepatocytes, squamous cells, and sweat glands, this is unlikely to preclude the potential usefulness of MAb 425 as an imaging and/or radioimmunotherapeutic agent because of the relatively low concentrations of this antigen in normal tissues. For example, MAb 17-1A, with binding reactivity to both tumors of the gastrointestinal tract and normal epithelial tissues (27) localized in cancer patients in tumor tissues but not in normal tissues (1–3). Furthermore, in immunotherapy trials with unlabeled MAb 17-1A, no adverse effects have been reported (28).

From the biodistribution data, the use of 1.0 mCi of ^{131}I-labeled 425 F(ab')$_2$ in patients would result in a maximal estimated radiation dose of 174 mrads to whole body and 193, 176, and 193 mrads to the bone marrow, testes, and ovaries, respectively. These radiation dosimetry data are well within acceptable limits. The use of ^{131}I to label MAb 425 F(ab')$_2$ in this study was based on our previous radioimaging studies which indicated best localization results using ^{131}I as compared with ^{123}I and ^{111}In (29). The immunoreactivity of ^{123}I-labeled fragments was variable in different labeling experiments and only occasionally comparable to the immunoreactivity of ^{131}I-labeled fragments (29).

If it is the case that the presence of a brain tumor implies a compromised blood-brain barrier only in the lesional area, then MAb 425 may preferentially localize in tumor tissues following its i.v. injection into cancer patients. However, if MAb is unable to penetrate the blood-brain barrier to the tumor, the use of mannitol to open this barrier (30) may circumvent this problem. The specific localization of radiolabeled MAb 425 in brain tumors of patients might open approaches to the radiotherapy of these tumors considering their high radiosensitivity. In that case, the use of intact rather than F(ab')$_2$ fragments of the MAb for radiotherapy may be preferable since higher radiation doses are delivered by intact antibodies as compared to fragments (31); in fact, our experiments (Table 2) show that specific activities of ^{131}I-MAb 425 F(ab')$_2$ in tumor tissues dropped dramatically between days 2 and 4.

One patient reportedly benefitted transiently from administration of ^{131}I-labeled MAb reacting with a carbohydrate determinant (identical with that of blood group A) of the EGF receptor (9). MAb 425, which binds to a protein determinant on the external domain of the EGF receptor (10), may be more

suitable for treatment of patients since its binding is independent of blood group type.

ACKNOWLEDGMENTS

We are very grateful to Drs. Luis Schut, Leslie Sutton (Children's Hospital of Philadelphia), Thomas Novak, Anil Nanda (Hahnemann University Hospital), and Stanley Stellar (St. Barnabas Medical Center) for providing the tumor specimens. We thank Marie Prewett, Roseann Kowalski, and Marina Aquino for technical assistance, and Marina Hoffman for editorial assistance.

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