Immunotherapy of Human Glioma Xenografts with Unlabeled, $^{131}$I-, or $^{125}$I-labeled Monoclonal Antibody 425 to Epidermal Growth Factor Receptor

Hans Bender, Hiroshi Takahashi, Koji Adachi, Paul Belser, Shaohong Liang, Marie Prewett, Martin Schrappe, Arne Sutter, Ulrich Rodeck, and Dorothee Herlyn


ABSTRACT

Monoclonal antibody (mAb) 425 (IgG2a) binds to the external domain of the epidermal growth factor receptor. This determinant is highly expressed by human glioma tissues but rarely by normal brain tissues, and is absent on peripheral blood lymphocytes and bone marrow cells. The mAb exerts variable cytotoxic effects against cultured human glioma cells in conjunction with human and murine effector cells. Inhibition of growth of s.c. glioma xenografts in nude mice by the mAb may be mediated by murine macrophages or may be related to the capacity of the mAb to antagonize growth stimulation of glioma cells by epidermal growth factor. In approaches to radioimmunotherapy of human glioma with mAb 425, the $^{125}$I-labeled mAb 425 exhibited more significant antitumor effects than the $^{131}$I-labeled mAb both in vitro and in vivo in xenotransplanted nude mice. These differences may be due to enhanced nuclear damage caused by $^{125}$I-labeled versus $^{131}$I-labeled fragments following their internalization into the glioma cells. Our studies provide the rationale for immunotherapy of glioma patients with either unlabeled or $^{125}$I-labeled anti-epidermal growth factor receptor mAb 425.

INTRODUCTION

Murine IgG2a mAb$^1$ 425 binds to an EGF-R protein determinant (1–4) that is expressed by most of the high grade (Grade III and IV) human glioma tissues, but rarely by normal brain tissues (5), and not at all by normal peripheral blood and bone marrow cells (5). mAb 425 inhibits the binding of EGF to its receptor without stimulating tyrosine kinase activity (2). After binding to the receptor, the mAb/receptor complex is rapidly internalized and a fraction of the mAb can be found in the nucleus as shown for colon carcinoma cells (4, 6). $^{125}$I-Labeled F(ab')$_2$ of mAb 425 specifically localizes in human malignant glioma xenografts in nude mice (5). $^{11}$In-Labeled mAb 425 IgG localizes in gliomas in patients (7, 8).

Radioimmunotherapy of gliomas in patients has been attempted with both $^{125}$I-labeled and $^{131}$I-labeled mAb to EGF-R (7–10). However, since these studies have been performed with mAb to different determinants on the EGF-R, possible differences in the immunotherapeutic effects of the two different radionuclides attached to anti-EGF-R mAb cannot be evaluated. The selection of the optimal nuclide for each mAb is crucial in approaches to cancer radioimmunotherapy.

$^{131}$I has medium-energy $\beta$ emission with a range greater than several cell diameters (11) and, therefore, the radiation emitted by $^{131}$I-labeled mAb bound to antigen-positive cells is potentially cytotoxic to neighboring antigen-negative (normal) cells. In addition, $^{131}$I emits high-energy $\gamma$- and X-rays that can be used for localization studies. The accompanying medium- and low-energy $\gamma$- and X-rays have a long tissue path and, therefore, exert high nonspecific tissue irradiation that limits the dose given at one time. However, the relatively short half-life (8.06 days) of $^{131}$I renders it an attractive nuclide for clinical application.

Until recently, $^{125}$I has not been considered for radioimmunotherapy of tumors with mAb because of its long half-life (60 days) and its extremely low energy emission (reviewed in Ref. 12). However, recently it has been demonstrated that $^{125}$I is highly effective if localized near or within the cell nucleus (13–22), suggesting the use of $^{125}$I-labeled mAb that are internalized into cells for radioimmunotherapy of cancer cells. After internalization the $^{125}$I-labeled mAb effectively destroyed the cells (15, 17, 19, 21, 22). This effect is most likely due to nuclear radiation by Auger electrons.

In the present study, the anti-glioma effects of both $^{131}$I- and $^{125}$I-labeled mAb 425 to EGF-R were compared to preclinical studies in vitro and in vivo in xenotransplanted nude mice. In addition, the effects of radiolabeled mAb 425 were compared to those of unlabeled mAb. Studies with the unlabeled mAb are important for considerations of immunotherapy of gliomas in children in whom the use of unlabeled mAb often is preferred.

MATERIALS AND METHODS

Tumor Cells. The human glioma (astrocytoma Grade III and IV) cells A1207, U-87MG, U-373MG, and F39 have been described (23). Colorectal carcinoma cells SW707 and the medulloblastoma cells TE671 were obtained from the American Type Culture Collection, Rockville, MD. However, recently (after the completion of our studies) it has been suggested that TE671 cells are of rhabdomyosarcoma origin (24). All cells were maintained in L-15 medium supplemented with 10% fetal calf serum.

mAb. Murine mAb 425 to EGF-R (IgG2a) has been described (1–6). mAb H4G4B (IgG2a) binds to a 40 kDa protein on glioma cells (23). Anti-hepatitis virus mAb A5C3 (IgG2a, kindly supplied by Centocor, Inc., Malvern, PA) and anti-influenza virus mAb H24B5 (IgG2a, kindly provided by W. Gerhard, The Wistar Institute) were used as controls.

Purification and Fragmentation of mAb. mAb were purified from ascitic fluid with the use of protein A-agarose columns, and F(ab')$_2$ fragments were produced by pepsin digestion of IgG as previously described (25).

Radio labeling of mAb. F(ab')$_2$ fragments were labeled with either $^{131}$I or $^{125}$I (Amersham, Arlington Heights, IL) to a specific activity of 3–9 $\mu$Ci/$\mu$g of antibody by using the Iodo-Gen method (26).

Antibody Binding Assay. Immunoreactivities of radiolabeled mAb against tumor cells were determined by radioimmunoassay as previously described (25), and ranged between 30 and 80% for the various cell lines, i.e., 30–80% of the input counts maximally bound to various cell lines. mAb binding affinity and density on tumor cell surfaces were determined by RIA according to the method of Scatchard (27).

Antibody-dependent Cell-mediated Cytotoxicity. ADCC reactivity of mAb 425 against tumor cells was determined in either a 6-h $^{51}$Cr-release test (28), using human peripheral blood lymphocytes (depleted of...
monocytes) as effector cells, or a 3-day [3H]thymidine release assay (29), using thioglycollate-elicited murine peritoneal macrophages as effector cells. ADCC reactivity of murine microglia cells was determined in a 2-day [3H]thymidine release assay. Briefly, microglia cells were harvested from 2-week-old glial cell cultures derived from central nervous system cortices of newborn BALB/c mice as described previously (30). [3H]Thymidine-pulsed target cells were incubated for 48 h with mAb and microglia cells in the presence of 50% astrocyte-conditioned medium and unlabeled thymidine (10⁻³ μM).

In all assays, spontaneous release of radioactivity by the cells in the presence of mAb 425, but in the absence of effector cells, was <1% of maximal release per h.

Growth Assays with Unlabeled mAb 425. Growth assays were performed as described previously (31). Murine EGF (Collaborative Research, Bedford, MA; 5 ng/ml, 1 nM) and protein A-agarose purified mAb 425 (5 μg/ml, 32 nM) were added to the cells. Cell counts were determined by using a Coulter Counter at days 1 and 7 after seeding.

Intracellular Uptake of mAb 425. mAb internalization was determined as described (32). All values were corrected for nonspecific binding of the mAb by subtracting the activity in the presence of >200-fold excess of nonlabeled mAb. The internalization rate constant (kᵢ) was calculated from the values obtained during the initial 30 min of internalization as described by Wiley and Cunningham (33).

Cell Survival Assay with Radiolabeled mAb. The cell survival assay was performed as described (21). Briefly, cells were incubated with various mAb for 2 days and then plated in fresh growth media for 12 days. The cell survival fraction was determined as described (21).

mAb Treatment of Tumor-bearing Nude Mice. Six- to 8-week-old nude mice (nu/nu, BALB/c background) were given injections s.c. of 5–8 x 10⁵ glioma cells. For immunotherapy of the tumors with unlabeled mAb, 200 μg of mAb 425 IgG or control mAb IgG were injected i.p. daily for 5 days starting immediately, or 7 days after tumor inoculation.

In our initial radioimmunotherapy studies, both [125I]-425-IgG and [131I]-425-F(ab')₂ were administered to nude mice at various concentrations (100–300 μCi/animal) on the day of tumor inoculation and 7 days thereafter. Since in those studies, and in agreement with studies described by others (34), the anti-tumor effects were more significant (compared to radiolabeled control mAb) for the radiolabeled fragments than for the IgG; all later studies were performed with radiolabeled fragments only (see "Results"). In these preexperiments, the optimal dose of labeled fragments inducing maximal specific (compared to [125I]-labeled control F(ab')₂) antitumor effects without producing toxicity was 150 μCi/animal. In later experiments (see "Results"), this dose was administered to animals with established tumors, i.e., on days 4 and 11 after tumor cell inoculation.

In all experiments tumor volumes were determined 2 times weekly as described (35).

Statistical Analysis. Significances of differences between experimental and control values were analyzed by Student’s t test.

### RESULTS

Cytotoxicity of Unlabeled mAb 425 against Glioma Cells in Vitro. mAb 425 mediated specific ADCC against human glioma cells with either murine macrophages (A1207 cells only), murine microglia cells (A1207, U-87MG, and U-373MG cells), or human lymphocytes (A1207 and U-87MG cells) as effector cells (Table 1). Of the three effector cells tested, microglia cells were the most effective, based on the number of cell lines lysed and the optimal concentration of effector cells and antibody required for target cell lysis.

The ADCC reactivity of murine macrophages and human lymphocytes seemed to correlate with mAb/EGF-R density on the tumor cell surface, but not with the affinity of mAb binding (Table 1).

### Antagonistic Effects of mAb 425 on Growth Stimulation of Glioma Cells by EGF. EGF (5 ng/ml) stimulated proliferation of all 3 glioma cell lines (U-87MG, A1207, F39) tested. After 7 days in culture, stimulation was 2.5-fold for U-87MG, 2.1-fold for A1207, and 1.6-fold for F39 cells when compared to controls maintained in medium free of exogenous EGF. The mitogenic effect of EGF was inhibited specifically by mAb 425 (32 nM; 5 μg/ml) for A1207 cells (90%) and F39 cells (95%), but only marginally (20%; P > 0.05) for U-87MG cells (results not shown). Insulin-mediated growth stimulation of glioma cells was not affected by mAb 425 which demonstrates specificity of the effect of the mAb on EGF-induced growth stimulation.

In Vivo Cytotoxicity of Unlabeled mAb 425 in Glioma-xenografted Nude Mice. mAb 425 administered simultaneously with A1207 (Fig. 1) or F39 (Fig. 2) glioma cells significantly (P < 0.05) reduced the growth of glioma cells in nude mice. The mAb was effective even when it was first administered 7 days following inoculation of F39 cells (Fig. 2B). However, as demonstrated for A1207 cells (Fig. 1), all animals developed F39 tumors (Fig. 2) during mAb treatment. mAb 425 was unable to inhibit growth of U-87MG transplants (not shown).

Internalization of mAb 425 into Glioma Cells. mAb 425 is internalized into glioma cells U-87MG and A1207, and medulloblastoma/rhabdomyosarcoma cells TE671 (Fig. 3). The internalization rate constant, kᵢ, and the uptake of the mAb after 6 h was approximately 3 times higher for U-87MG cells as compared to A1207 and TE671 cells. A correlation does not seem to exist between either EGF-R density on tumor cell surfaces or mAb 425 binding affinity (Table 1) and internalization rate or intracellular uptake of mAb 425 (Fig. 3) into these cells.

### Table 1 ADCC and binding reactivity of mAb 425 against glioma cells

<table>
<thead>
<tr>
<th>Human glioma cell lines</th>
<th>Murine macrophages</th>
<th>Murine microglia</th>
<th>Human lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1207</td>
<td>16–26</td>
<td>31–75</td>
<td>41</td>
</tr>
<tr>
<td>U-87MG</td>
<td>1–2</td>
<td>42–80</td>
<td>36–45</td>
</tr>
<tr>
<td>F39</td>
<td>0–1</td>
<td>NT</td>
<td>0–5</td>
</tr>
<tr>
<td>U-373MG</td>
<td>4–5</td>
<td>34–75</td>
<td>0–7</td>
</tr>
</tbody>
</table>

* At optimal effector:target ratio (microglia: 20; macrophages: 50; lymphocytes: 100) and optimal antibody concentration (microglia: 0.015 μg/ml; macrophages and lymphocytes: 50 μg/ml); range in 1–5 experiments.

* Means of two experiments.

* Values differ significantly (>3×) from control values.

* Low/high affinity binding sites.

* NT, not tested.
1. Antibody Targeting of EGF-R on Gliomas

Radioimmunotherapy of Glioma Xenografts in Nude Mice with Radiolabeled F(ab')2 of mAb 425. In two independent experiments, either 125I-425-F(ab')2 (Fig. 5A) or 123I-425-F(ab')2 (Fig. 5B) was administered to nude mice with established glioma xenografts. Delayed administration of 123I-425-F(ab')2, significantly (P < 0.05) inhibited tumor growth as compared to either 123I-ASC3-F(ab')2 treatment or no treatment (Fig. 5A). At the end of the observation period of 42 days, 6 of 6 control mice had tumors as compared to 4 of 6 mice in the experimental group. The experiment had to be terminated because of high mortality in the control group. Delayed administration of 125I-425-F(ab')2 also caused significant (P < 0.05—0.001) antitumor effects as compared to 125I-A5C3-F(ab')2 (Fig. 5B). These differences were more pronounced than those observed following administration of 123I-

In Vitro Cytotoxicity of Radiolabeled mAb 425. As can be seen from the results shown in Fig. 4, both 125I- and 123I-labeled 425-F(ab')2, significantly (see legend to Fig. 4) inhibited colony formation of U-87MG cells as compared to control anti-influenza virus mAb. However, the effect of 123I-labeled 425-F(ab')2 was significantly (see legend to Fig. 4) greater than the effect of 125I-labeled 425-F(ab')2. Unrelated anti-glioma mAb HG4G8, when labeled with 123I, but not 125I, significantly (see legend to Fig. 4) inhibited cell growth as compared to radiolabeled anti-influenza virus control antibody. However, the anti-tumor effect of 123I-HG4G8 (which is not internalized into glioma cells) was much less (see legend to Fig. 4) pronounced than the effect of radiolabeled 123I or 125I mAb 425. Thus, of all radiolabeled mAb used in this assay, mAb 425 labeled with 125I showed the strongest tumoricidal effects.

Radioimmunotherapy of Glioma Xenografts in Nude Mice with Radiolabeled F(ab')2 of mAb 425. In two independent experiments, either 123I-425-F(ab')2 (Fig. 5A) or 125I-425-F(ab')2 (Fig. 5B) was administered to nude mice with established glioma xenografts. Delayed administration of 123I-425-F(ab')2, significantly (P < 0.05) inhibited tumor growth as compared to either 123I-ASC3-F(ab')2 treatment or no treatment (Fig. 5A). At the end of the observation period of 42 days, 6 of 6 control mice had tumors as compared to 4 of 6 mice in the experimental group. The experiment had to be terminated because of high mortality in the control group. Delayed administration of 125I-425-F(ab')2 also caused significant (P < 0.05—0.001) antitumor effects as compared to 125I-A5C3-F(ab')2 (Fig. 5B). These differences were more pronounced than those observed following administration of 123I-

Fig. 1. Inhibition of growth of A1207 glioma cells in nude mice by unlabeled mAb 425-IgG. Nude mice (6/group) were inoculated with 8 x 10⁶ A1207 cells s.c. and treated daily with 200 µg of either mAb 425 or normal (nl) mouse IgG i.p. starting on the day of tumor cell inoculation. Tumor volumes are measured weekly. At the end of the experiment on day 30, all animals in the experimental and control groups had developed tumors. Points, mean of 6 animals/group; bars, SD. *, values differ significantly (P < 0.05) from each other.

Fig. 2. Inhibition of growth of F39 glioma cells in nude mice by unlabeled mAb 425-IgG. Nude mice (4—5/group) were given injections s.c. of 5 x 10⁶ tumor cells and were treated daily for 5 days with 200 µg of either mAb 425 or normal (nl) mouse IgG i.p. starting either on the day of tumor inoculation (A) or 7 days thereafter (B). Tumor volumes were measured weekly. Points, mean of 4—5 animals/group; bars, SD. *, values differ significantly (P < 0.05) from each other.

Fig. 3. Intracellular uptake of 125I-labeled mAb 425. Glioma cells U-87MG, A1207, and TE671 were incubated with 40 ng/ml of 125I-425-IgG at 37°C. Intracellular uptake of radioactivity was determined at various time points and internalization constant, kₓ, was calculated as described in "Materials and Methods."

Fig. 4. In vitro cytotoxicity of radiolabeled mAb 425-F(ab')2 against U-87MG glioma cells. Cells were incubated with various concentrations of either 125I- and 123I-labeled F(ab')2 of antiglioma mAb 425 or control anti-influenza virus mAb H24B5 for 48 h at 37°C. After mAb was removed the cells were incubated for an additional 12 days and cell colonies were counted. The cell survival fraction was calculated as described in "Materials and Methods." Points, mean of colonies of 6 plates/group; bars, SD. Significances of differences: 123I-425-F(ab')2 versus 125I-H24B5-F(ab')2, P < 0.05—0.0001 at the various antibody concentrations; 123I-425-F(ab')2 versus 123I-H24B5-F(ab')2, P < 0.05—0.001; 123I-425-F(ab')2 versus 123I-HG4G8, P < 0.02—0.0001; 123I-HG4G8 versus 123I-H24B8, P < 0.05—0.001; 123I-HG4G8 versus either 123I- or 125I-425-F(ab')2, P < 0.05—0.01; all other differences were not significant (P > 0.05).
labeled 425-F(ab')$_2$ (see above). However, the fraction of mice exhibiting tumor growth (4 of 6) was the same in both experiments. Two mice remained tumor free for 6 and 8 months, respectively, until they died for unknown reasons. In neither experiment did the radionuclide control F(ab')$_2$ exert significant (P > 0.05) effects on tumor growth as compared to no treatment. Thus, as shown in the in vitro cell survival assay (see Fig. 4), 125I-425-F(ab')$_2$ was superior to 131I-425-F(ab')$_2$ in inhibiting growth of glioma xenotransplants in nude mice, although a direct comparison of the data obtained in the two different experiments performed independently is problematic.

Therefore, the tumoricidal effects of 125I- and 131I-labeled 425-F(ab')$_2$ were compared in the same experiment (Fig. 6). In that experiment, 4 groups of mice were treated with 125I- or 131I-labeled F(ab')$_2$ of either mAb 425 or control mAb H24B5, and one group was left untreated. Again, both mAb 425 preparations significantly (see below) inhibited tumor growth as compared to the control mAb. Although the tumor volumes in the mice treated with either 131I-labeled or 125I-labeled 425-F(ab')$_2$ did not differ significantly (P > 0.05), the effect of the 125I-425-F(ab')$_2$ was more pronounced (P < 0.001) than that of 131I-425-F(ab')$_2$ (P < 0.05) when compared to the corresponding control mice treated with either 125I-labeled or 131I-labeled control H24B5-F(ab')$_2$ (Fig. 6). This may be explained by the slight (but insignificant, P > 0.05) growth-inhibitory effect of the 125I-control F(ab')$_2$, but not 131I-control F(ab')$_2$ as compared to no treatment. One of the eight mice in the 125I-425-F(ab')$_2$-treated group, but none of the eight mice in the 131I-425-F(ab')$_2$-treated group, was tumor free at the end of the observation period on day 39 when the experiment had to be terminated because of the high mortality rate in the control group. The mouse remained tumor free for 5 months until it died for unknown reasons.

Peripheral blood leukocyte counts of the nude mice remained normal during treatment of the mice with radiolabeled mAb (not shown), indicating minimal or absent systemic toxicity of radioimmunotherapy.

**DISCUSSION**

The preclinical studies described here demonstrate the potential usefulness of anti-EGF-R mAb 425 as an immunotherapeutic reagent for glioma patients. The antitumor effects of radiiodinated mAb 425 emphasize the potential of this mAb for radioimmunotherapy of gliomas which are highly sensitive to irradiation. However, this form of therapy is problematic in childhood gliomas, given the increased sensitivity of normal tissues to radiotoxic effects in children as compared to adults.

Since unlabeled mAb 425 shows tumoricidal activity against glioma cells in culture and inhibits growth of these cells in nude mice, this unlabeled form of immunotherapy may have potential in the management of childhood gliomas.

Our in vitro studies have demonstrated that the antiglioma effects of unlabeled mAb 425 are dependent on the interaction of the mAb with effector cells in ADCC (Table 1), but complement plays no role (not shown). The demonstrated lymphocytic infiltration of patients' glioma tissues (36) emphasizes the potential usefulness of unlabeled mAb 425 in the treatment of these tumors. Similar to our previous studies with carcinoma cells (3), mAb 425 shows ADCC reactivity against glioma cells in conjunction with murine macrophages and human lymphocytes. Here we demonstrate that murine microglia cells are most effective in lysing human glioma cells followed by human
lymphocytes and murine macrophages. The high ADCC reactivity of microglia cells may be explained by the effects of cytokines present in the astrocyte-conditioned media used in this test system, although this has not been directly tested. In agreement with our previous studies with carcinoma and melanoma cells (3, 37), lysis of various glioma cells by mAb 425 and murine macrophages in vitro seemed to correlate with EGF-R density on the target cells.

Unlabeled mAb 425 inhibited growth of glioma xenotransplants A1207 and F39, but not U-87MG, in nude mice. This tumoricidal effect of the mAb against A1207 cells may be mediated by macrophages, as we have previously demonstrated for other mAb of IgG2a isotype (29). Alternatively, inhibition of glioma growth by mAb 425 in nude mice may be related to the capacity of the mAb to act as an EGF antagonist. Indeed, those glioma cell lines (A1207, F39) that were growth inhibited by mAb 425 in vitro also showed antagonistic effects of the mAb on EGF-mediated stimulation of the cells in vitro, and absence of in vivo antitumor effects of the mAb in U-87MG cells correlated with its inability to antagonize EGF stimulation of these cells. Since mAb 425 antagonized growth-stimulatory effects of human EGF on glioma cells in vitro and murine EGF interacts with the human EGF-R (38), it is possible that in the xenografted nude mice, the mAb inhibited tumor growth by antagonizing growth stimulation of the tumors by murine EGF.

Our studies clearly demonstrate enhanced cytotoxicity of $^{125}$I-labeled versus $^{131}$I-labeled mAb 425 when identical doses of the two radiolabeled mAb preparations were compared. These differences were more striking in vitro than in vivo. Both $^{125}$I and $^{131}$I already have been used in experimental radioimmunotherapy of glioma patients with mAb to EGF-R (7–10), although a direct comparison of the effects of the two radionuclides was not possible in those studies because the mAb bind to different determinants on the EGF-R. Our data suggest that $^{125}$I-425 should be preferred over $^{131}$I-425 for clinical trials. The radiation dose [estimated from our previously published data (6) according to the standard MIRD procedure] delivered to the tumor was much lower for $^{125}$I-425-F(ab')$_2$ (19 cGy/300 $\mu$Ci injected dose) as compared to $^{131}$I-425-F(ab')$_2$ (159 cGy/300 $\mu$Ci injected dose; 6 to 0.5% of the injected dose/g tumor, immediately to 4 days after injection) in spite of the higher in vivo antitumor effects of the $^{125}$I-425-F(ab')$_2$.

Similarly, the radiation dose delivered to normal mouse organs was lower for $^{125}$I-425-F(ab')$_2$ (0.3–6 cGy/300 $\mu$Ci injected dose) as compared to $^{131}$I-425-F(ab')$_2$ (2.6–40 cGy/300 $\mu$Ci injected dose; 0.8–0.02% of injected dose/g for various normal tissues immediately to 4 days after injection). These data further emphasize the advantage of $^{125}$I versus $^{131}$I in our studies.

The increased cytotoxicity of $^{125}$I-425 is most likely due to the enhanced nuclear toxicity exerted by $^{125}$I versus $^{131}$I (13, 14) following the internalization of the mAb-radionuclide complex. Internalization and intranuclear localization of mAb 425 have been demonstrated for colon carcinoma cells (4, 6) and most likely also occur in glioma cells, although this has not been directly demonstrated. However, only a small fraction of the mAb may have been internalized to the nucleus, whereas the majority of the mAb/EGF-R complexes, similar to EGF/EGF-R complexes (33, 39, 40), may have been degraded by intracytoplasmic lysosomal enzymes and the products of degradation released to the surrounding extra cellular fluid by exocytosis. It is postulated that, following mAb 425 internalization, the Auger electron-mediated effects of $^{125}$I (13, 14) are considerably more lethal than the medium-energy electrons released by $^{131}$I, although, until definitive studies are done with higher doses and longer incubations, we cannot exclude an additional effect of medium-energy electrons (15) or the 35 KeV photons emitted by $^{125}$I (14, 15).

The degree of mAb 425 internalization by different glioma cells in vitro seems to be independent of the EGF-R affinity and density on the surfaces of these cells. It is possible that, in addition to the density of EGF-R on cells, other factors, such as receptor cycling and degradation, plays a role in the internalization of $^{125}$I-425 (33).

In our xenograft model, the therapeutic effects of radiolabeled fragments of mAb 425 were more significant than those of radiolabeled intact mAb in spite of the fragments delivering an assumed lower dose of radioactivity to the tumor (25, 41, 42). Significant amounts of radioactivity were found in the xenografts as long as 6 weeks after the mice were treated with $^{125}$I-labeled 425-F(ab')$_2$ (not shown). The advantage of the fragments for radioimmunotherapy of tumors is that they quickly penetrate the tumor and are quickly cleared from the blood and organs of the mice (25, 41, 42). This may explain the absence of host radiotoxicity from the radioiodinated fragments. Similarly, Blumenthal et al. (34) have achieved increased antitumor effects with $^{131}$I-labeled F(ab')$_2$ as compared to $^{131}$I-labeled IgG of an anti-colon carcinoma mAb in xenografted nude mice, and the antibody fragments were significantly less toxic to the host than the intact antibody. These and our own data imply the advantage of radioiodinated fragments versus IgG in the syngeneic animal. These studies may directly relate to the situation in cancer patients treated with xenogeneic murine mAb. In patients, enhanced clearance from blood and tissue of radiolabeled mAb F(ab')$_2$ versus IgG has been reported (43, 44).

Numerous mAbs to different determinants on glioma cells have been generated in various laboratories (reviewed in Ref. 45). Some of these mAbs are potentially useful for the treatment of glioma patients. Among all the antiangioma mAbs described thus far, mAb to EGF-R are unique since they are internalized into the cells. This offers the possibility for highly effective radioimmunotherapy with the use of $^{125}$I to enhance nuclear radiation of the cells.

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