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P. Wersäll · I. Ohlsson · P. Biberfeld · V. P. Collins
S. von Krusenstjerna · S. Larsson · H. Mellstedt
J. Boethius

Intratumoral infusion of the monoclonal antibody, mAb 425, against the epidermal-growth-factor receptor in patients with advanced malignant glioma

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Abstract Malignant glioblastoma may over-express the epidermal-growth-factor receptor (EGF-R). Normal brain cells show a low or no expression of EGF-R. A mouse monoclonal antibody (IgG2A) (mAb 425) (EMD55900) (Merck KGaA, Bernstadt, Germany) directed against EGF-R was produced for therapeutic use. Eight patients with primary or recurrent, EGF-R-positive glioblastomas entered the study, which was designed to evaluate the clinical effect of the mAb. In order to achieve a high tumor cell saturation, the mAb was injected intratumorally twice weekly through an implantable catheter. The total administered dose varied between 4 mg and 120 mg. In 3 patients with solid tumors, a massive tumor necrosis was noted, with infiltration of macrophages, granulocytes and T cells. A further 3 patients developed clinical and radiological signs of an intense, local, inflammatory reaction. There may be a relation between the mAb dosage and the antitumor effect, insofar as higher doses seemed to cause a more pronounced, inflammatory reaction. Of the 8 patients, 6 developed human, anti-(mouse Ig) antibodies. This anti-EGF-R mAb may induce an intense, inflammatory reaction and a considerable necrosis in glioblastoma. However, the planned schedule could not be completed, even after the dose level was re-adjusted, owing to inflammatory reactions, which were severe without prior tumor debulking.

Key words Epidermal growth factor · Murine monoclonal antibody · Glioma · Immunotherapy

P. Wersäll · H. Mellstedt (✉)
Department of Oncology, (Radiumhemmet), Karolinska Hospital, S-17176 Stockholm, Sweden
Fax: +47 8 31 15 85

I. Ohlsson · J. Boethius
Department of Neurosurgery, Karolinska Hospital, Stockholm, Sweden

P. Biberfeld · V.P. Collins
Department of Pathology, Karolinska Hospital, Stockholm, Sweden

S. von Krusenstjerna · S. Larsson
Department of Nuclear Medicine, Karolinska Hospital, Stockholm, Sweden

H. Mellstedt
Department of Experimental Oncology, Uppsala University, Uppsala, Sweden

Introduction

Human epithelial tumors may express high numbers of the epidermal growth-factor receptor (EGF-R). Increased levels of EGF-R are associated with a poor prognosis in bladder carcinoma [17], breast carcinoma [24], lung carcinoma [7] and glioblastoma [12, 13]. The gene for EGF-R (c-*erb-B1*) is often amplified in human malignant glioma and the receptor is over-expressed [13]. As the EGF-R is rarely expressed in normal brain cells [25] and not at all in bonemarrow cells [26], this growth-factor receptor may be used as a target for antibody therapy of glioblastoma [2]. When administered systemically, monoclonal antibodies (mAb) did not cross the intact, blood brain barrier. Even in malignant brain tumors, where the barrier was disrupted, the uptake in the tumor was low, approximately 0.001% of the injected dose [9]. Similar results have been noted for other solid tumors [4, 10]. Thus, local administration of mAb should be the optimal route to achieve tumor-cell saturation. Intratumoral administration was performed, using a radiolabelled (^{131}I) mAb, and three partial and three complete remissions were seen in 17 patients [21]. A bispecific [anti-CD3/anti-(glioma NE-150)] mAb, together with lymphokine-activated killer (LAK) cells, were given via an Ommaya reservoir to 10 patients. Four partial regressions were noted and four patients had radiological and histological evidence of glioma cell eradication [18].

Murine mAb 425 (IgG2A-κ) binds to an epitope close to the EGF-binding site. Interaction of the mAb with this epitope inhibits binding of EGF and down-regulates the receptor. The receptor, tyrosine kinase, is not activated, and proliferation is not induced [23]. In antibody-dependent,

cellular cytotoxicity, mAb 425 lysed EGF-R$^+$ tumor cells [22]. The antibody also inhibited growth-factor-dependent proliferation in a variety of carcinoma cell lines in vitro [23] and the growth of human glioma xenografts in nude mice [2].

This phase I study reports the antitumor effect, feasibility and side-effects, as well as the influence on immune functions, of unconjugated mAb 425 after intratumoral infusions into glioblastoma.

Materials and methods

Patient selection

Eight patients entered a single-center phase I trial (Table 1). The inclusion criteria were age above 18 years; tissue confirmation of anaplastic astrocytoma/glioblastoma with at least 20% EGF-R$^+$ tumor cells and adequate renal, liver and bone marrow functions. Furthermore, patients with serious cardiovascular disease, active infection, psychiatric illness or other concurrent antitumor therapy were excluded. All patients had to be able to give informed consent. Four were men and 4 women. The median age was 61 years (range 52–68). Four patients had inoperable glioblastoma and 4 recurrent disease. The tumors expressed EGF-R (Table 2). All patients with recurrent tumors had previously had surgery and patients 2, 5, 6 and 8 postoperative radiation therapy (52 Gy). One patient had also received adjuvant chemotherapy for a mammary carcinoma (patient 2). The Karnofsky index at the start of mAb therapy varied between 30% and 100% (mean 60%). The median time from diagnosis to the start of mAb therapy was 9 weeks (range 2–52 weeks).

Surgery and infusion technique

In order to implant the catheter for mAb infusion, the previous operating wound was re-opened, the bone flap removed and the dura opened. The brain was re-exposed and, when the inspection indicated tumor growth, visible tumor tissue was removed. Three or four shunt catheters were introduced for 1–2 cm into the tumor-bed tissue. The catheters were interlinked with Y-connectors to end as a single tube, which was attached to a subcutaneous reservoir (Port-a-Cath, Pharmacia, Uppsala, Sweden).

The infusion of the mAb started about 1 week later. Previous experience indicated that an infusion rate of 4 ml/h was well tolerated and produced an acceptable spread of the infusion solution in the brain tissue (data not shown).

Monoclonal antibodies

The production and the characterization of mAb 425 (EMD 55900, Merck KGaA) have been described earlier [16]. Before infusion, therapeutic antibody stock solution (mAb dissolved in sterile water, 5 mg/ml) was at doses below 20 mg, further diluted up to a final volume of 4 ml. The infusion rate was 4 ml/h. The infusion was made using an automatic syringe (syringe driver-type MS16A, Graseby Medical Ltd., Watford, England) connected to the Port-a-cath with a transcutaneous injection needle (Heubner).

Treatment schedule

The selected initial dose level was 10–20 times lower than the optimal doses we had used for the i.v. administration of mouse mAb in colorectal carcinoma patients [19]. The planned treatment schedule was 20 mg of mAb 425 (mouse IgG2A, EMD 55900, Merck KGaA) for about 60 min, repeated twice weekly for 1 month, and then once a week for a further 5 months until complete remission or progression. Before each mAb infusion, 1 mg mAb 425 was injected intradermally. The skin reaction was read after 20 min. In patients showing a positive skin reaction (>10 mm $\times$ >10 mm), the dose of mAb 425 was reduced and/or the infusion time doubled. As none of the patients could tolerate the dose schedule initially planned, the schedule was redesigned after the third patient enrolled. In the 5 subsequent patients (patients 4–8), an escalating dose schedule was used. The starting dose was 2 mg at each infusion during the first week, followed by 4 mg, 8 mg and 20 mg twice weekly for 1 month and then 20 mg once a week.

Clinical examinations

Before therapy, a careful clinical examination was made. The following laboratory tests were performed: hemoglobin concentration, white blood cell count, platelet count, liver-function tests, serum creatinine, serum electrolytes, serum protein electrophoresis including immunoglobulin quantification, and serum complement component (C3, C4 and C3d) concentration. Urine was analyzed for glucose and protein and was microscopically examined. An X-ray of the lungs was performed, as well as computerized tomography (CT) and positron emission tomography examination of the brain. For response evaluation, the relevant tests were repeated.

Serum specimens for mAb 425 and human anti-(mouse Ig) (HAMA) analyses were obtained from all patients 2 days before treatment, during the first infusion at 1, 2, 4, 8, 24 and 48 h after the start of the infusion and then before treatment at 0.5, 1, 2, 4 and 6 months.

Response evaluation

The response to the mAb was evaluated radiologically. In several cases, the radiological mass increased dramatically after the mAb infusion. CT also demonstrated a marked increase in peritumoral edema. For obvious reasons, it was impossible to state whether or not the increase in tumor mass was caused by tumor growth or an inflammatory reaction.

Histopathology and immunohistochemistry

Biopsy material was fixed in 4% paraformaldehyde or Carnoy's fixative and processed for conventional paraffin embedding. A histopathological examination was made after hematoxylin/eosin (H&E) staining. Immunostaining using CD4 (Leu3A), CD8 (Leu2A), CD14 (LeuM3) (Becton Dickinson, San Jose, USA), CD45 (T29/33), CD45R0 (UCHL-1), CD68 (KP1) and neutrophil elastase (MP57), (Dakopatts, Glosstrup, Denmark) monoclonal antibodies was performed as previously described [14]. Sections were de-paraffinized, re-hydrated and incubated overnight with the antibody. Bound mAb was detected by a commercial avidin-biotin complex (ABC) detection kit, coupled to alkaline phosphate (Dako) or the Vectasin red kit as chromogen, according to the manufacturer's instruction (Vector, USA).

Formalin-fixed, paraffin-embedded, tumor tissue sections were immunohistochemically evaluated for EGF-R expression, using an indirect, avidin-biotin method. The section was first trypsinized and then incubated with the mouse mAb E30 (IgG1) (anti-EGF-R) (Merck KGaA) and Dako Duet (K492) avidin-biotin complex with peroxidase was used for detection with diaminobenzidine as a chromogen. A positive and negative control were run in parallel. The percentage of positive cells, the staining intensity and the degree of staining variability in different areas were recorded.

Mouse immunoglobulin and HAMA assays

Mouse immunoglobulin (mAb 425) and class-specific HAMA in serum were analyzed in the enzyme-linked immunosorbent assay (ELISA).

Table 1 Patient characteristics at start of mAb 425 therapy

Patient no.	Sex/age (years)	Primary/recurrent tumor	Tumor site	Tumor diameter[a] (cm)	Previous treatment	Karnovsky index (%)
1	M/68	Primary	Thalamic dx	3	None	50
2	F/64	Recurrent	Temporal dx	2	Surgery for primary tumor + radiation	90
3	F/63	Primary	Temporal dx	7	None	100
4	M/56	Primary	Central	3	None	50
5	M/52	Recurrent	Temporo parietally dx	3	Surgery for primary tumor + radiation	90
6	F/64	Recurrent	Temporo occipital dx	5	Surgery for primary tumor + radiation + 2 cycles of lomustine	30
7	M/68	Recurrent	Temporal sin	5	Surgery for primary tumor	100
8	F/52	Primary	Frontal dx	5	Subtotal surgery for primary tumor + radiation	100

[a] Computed tomography evaluation, dx = dexter

Table 2 Epidermal growth-factor receptor (EGF-R) expression of the tumor cells, amount of mAb 425 given and survival. + very weak, ++ weak, +++ moderate, ++++ strong, *NE* not evaluated

Patient no.	EGF-R+ cells (%)	Intensity of EGF-R expression	No. of infusions	Total amount mAb 425 (mg)	Survival from start of mAb therapy (weeks)	Survival from primary diagnosis (weeks)
No prior debulking surgery						
1	NE	NE	5	100	3	8
2	40	+	6	120	8	36
3	50	+++	1	20	2	12
4	>50	+++	9	36	5	8
Prior debulking surgery						
5	50	++	10	108	38	60
6	20	+	8	92	16	64
7	>50	++++	2	4	20	28
8	100	+++	2	4	56+	94+

Flat-bottomed, microtiter, ELISA plates (Costar) were coated at 4 °C overnight with goat anti-(mouse IgG) (Nordic, Tillburg, The Netherlands) for determination of mouse IgG (mAb 425) and with mAb 425 for detection of HAMA IgM and IgG respectively. After blocking with 1% bovine serum albumine in coating buffer for 1 h at 37 °C, diluted samples (1:4 and 1:40 for detection of mAb 425, 1:60 and 1:600 for HAMA IgM and 1:600 and 1:6000 for HAMA IgG) were added for 2 h at 37 °C. The plates were then reacted for 2 h at 37 °C with rabbit anti-(human IgG) and anti-(human IgM) alkaline phosphate conjugates (Sigma). After enzyme reaction for 20 min, using *p*-nitrophenyl phosphate (1 mg/ml; Sigma) in diethanolamine buffer, pH 9.8, the absorbance at 405 nm was measured using an automatic ELISA reader (Lab System, Multiscan PLUS, Lab Systems, Helsinki, Finland). The background values, determined by incubating antigen-coated wells with medium alone, were subtracted.

Results

Feasibility

Eight patients (Table 1) with primary or recurrent glioblastomas were treated with intratumoral infusions of mAb 425. The first three patients received the planned treatment schedule with 20 mg mAb 425 twice weekly during the first month and then once weekly without prior de-bulking surgery (Table 2). As none of the patients could tolerate the dose schedule initially planned, the 5 subsequent patients (patients 4–8) received a starting dose of 2 mg at each infusion during the first week, followed by a dose escalation up to 20 mg during the first month. None of the patients could complete more than ten infusions on this dose-reduced schedule. In 2 cases (patients 2 and 3) emergency operations were performed and the changed tumor mass was removed. A third patient (patient 6) was also operated on with a resection of the tumor mass after mAb treatment. These tissues were evaluated histopathologically as described below.

Antitumor response

Tumor necrosis could be evaluated by open surgery and histopathology in 3 patients. In 2 cases (patients 2 and 3) there was a massive tumor necrosis surrounding the catheter, reaching more than 1 cm out of the tumor tissue (Fig. 1). No radiological regression of tumor tissue was recorded. In several cases, a local edema was induced, verified by CT. A representative CT scan showing the local reaction is pre-

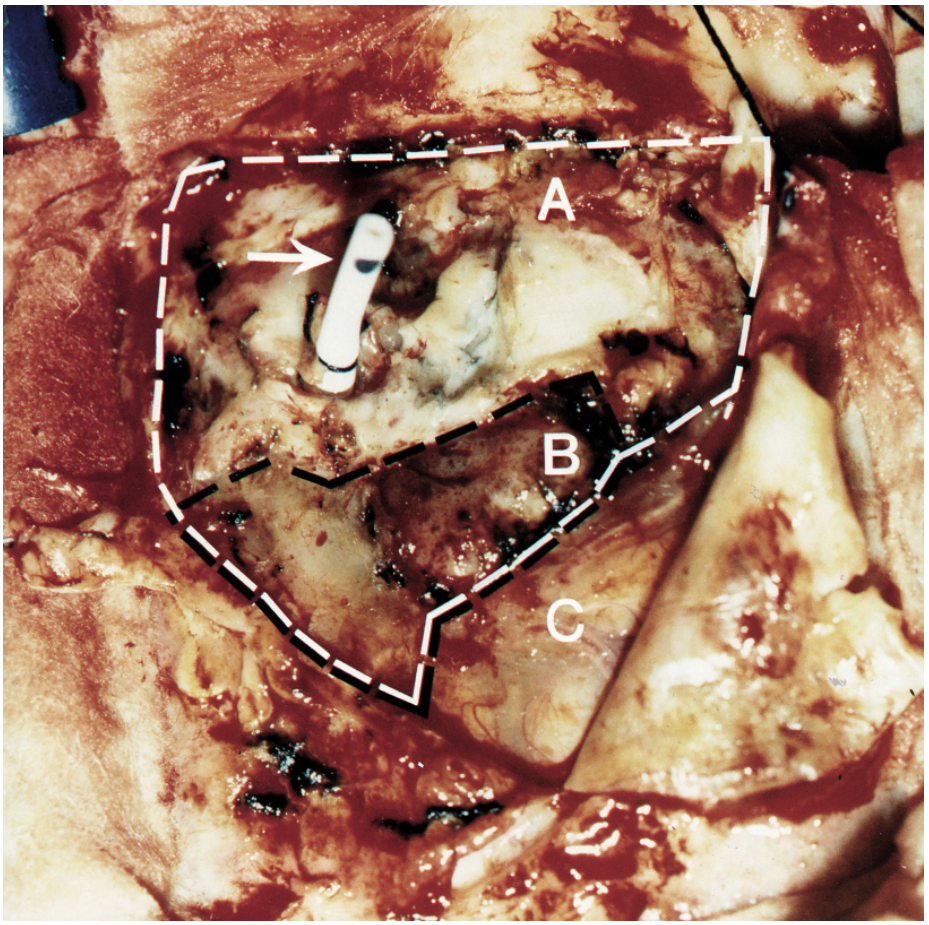

Fig. 1 Tumor area in patient 2 after treatment with mAb 425. The area within the white marks outlines the tumor margins. The *Arrow* the implanted infusion catheter. Three different areas can be identified: **A** necrotic tumor, **B** viable tumor and *C* normal brain tissue

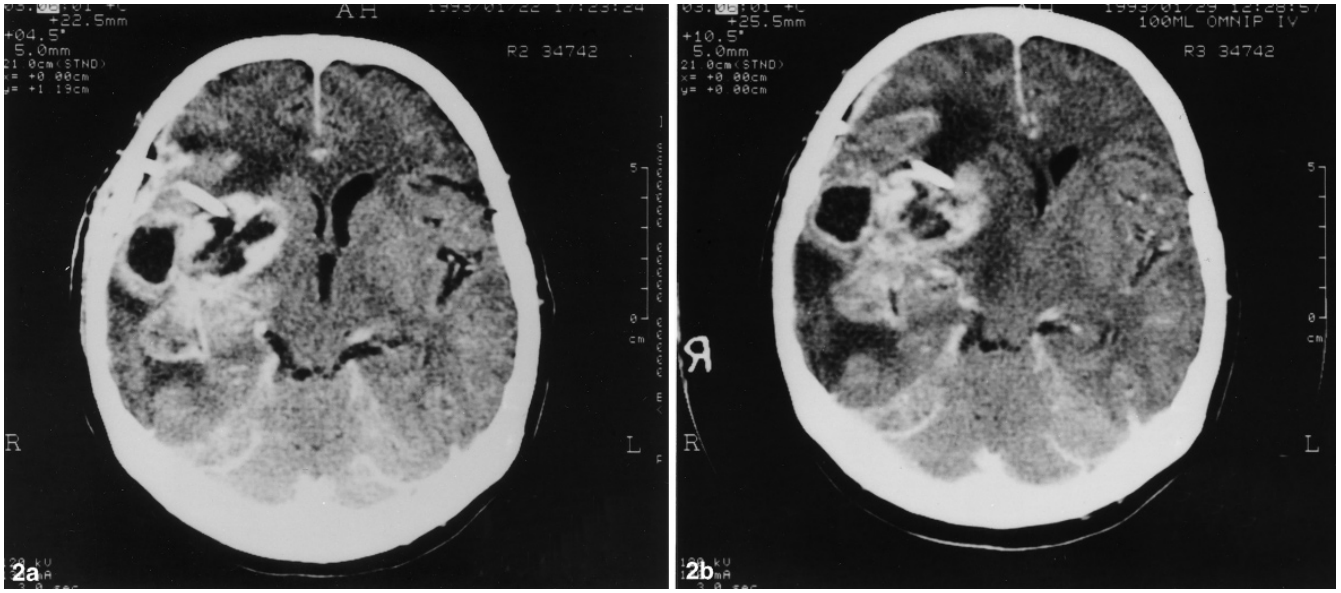

Fig. 2 A, B Computed tomography (CT) scan 5 days before (**A**) and 2 days after (**B**) an intratumoral infusion of 20 mg mAb 425 in patient 3. The catheter is visible in the frontal part of the tumor. Prior to mAb infusion the tumor was large (**A**) but the midline shift was moderate. The clinical condition of the patient was satisfactory. The day after the mAb infusion the patient started to deteriorate. A CT scan (**B**) performed 2 days after the infusion showed a pronounced increase in the midline shift that appeared to be caused by an increase in tumor volume as well as edema. (Both CT scans are at the same level)

Table 3 Evaluation of the clinical antitumor effects of intratumorally administered mAb 425 in patients with advanced malignant glioma

Patient 1	The disease was stable at 3 weeks after mAb therapy (CT verified), when the patient died of septicemia. No other antitumor effect was recorded. The patients received concomitant steroid medication
Patient 2	Massive tumor necrosis evaluated by open surgery and histopathology (Fig. 1, Table 5). Radiologically the tumor volume increased dramatically immediately after the therapy, as did the surrounding edema
Patient 3	Massive tumor necrosis evaluated by open surgery and histopathology (Table 5). This patient showed the same radiological picture as patient 2
Patient 4	An intense, local, inflammatory reaction was diagnosed on CT. No surgery was performed. The inflammatory reaction was interrupted by steroid medication
In view of the previous experience, the strategy was changed, insofar as recurrent tumors were first removed surgically and then the mAb was infused into the tumor bed	
Patient 5	Five months after primary surgery, followed by post-operative radiation, a major surgical debulking of a recurrent tumor was done. Local mAb infusions were given. There was a radiologically obvious inflammatory reaction in the tumor bed. After a further 8 months, the tumor again relapsed at the same site but also at a distant site
Patient 6	A local edema was induced, verified by CT. Resected, post-treatment, tumor tissue showed a varying degree of necrosis with a strong inflammatory reaction and partly a prominent angiogenic reaction
Patient 7	This patient developed a nephropathy and had to be excluded from the study. Steroid medication was given. No detectable antitumor effect
Patient 8	This patient received two intratumoral infusions of the mAb. During the last infusion, she developed clinical and radiological signs of an intense, inflammatory reaction. Later, she developed a strong skin reaction against the mAb. The attempt to treat her further locally was therefore curtailed. The patient was then given 200 mg lomustine orally every 6th week. The patient is still alive and in a good general condition without any signs of tumor on CT 56+ weeks after the start of mAb therapy

Table 4 Number of patients with non-hematological side-effects

Side-effect	Toxicity incidence				
	0	1[b]	2	3	4
Allergic					
Fever			1	1	
Shivering		1		1	
Erythema		1		1	
Neurological					
Headache		1	1	4	
Confusion		1	1	3	
Hemiparesis			2	1	
Ataxia			1		
Memory dysfunction			1	1	
Nausea/vomiting				2	
Nephrological					
Proteinuria[a]				1	
Sodium leakage[a]				1	

[a] Patient 7 was diagnosed as having a nephropathy 1 week after the mAb infusions were terminated. The causal relationship between antibody infusion and nephropathy is remote

[b] ECOG grading

sented in Fig. 2. A detailed description of the antitumor effects is given in Table 3.

Side-effects

Immediate side-effects (within 2 h) were mild fever, shivering and skin erythema, which disappeared within 5 h. No anaphylactic reactions were noted (Table 4).

Late side-effects (within 5–48 h) were headache and confusion, declining slowly during the subsequent 24 h in most patients.

On three occasions, the side-effects were severe and were present for several weeks after discontinuation of therapy. In patient 2, a permanent, left-sided hemiparesis, requiring prompt surgical de-bulking, was recorded. In 2 other cases (patients 3 and 6), the hemiparesis receded after mAb therapy was terminated. The memory function was impaired in these 3 patients. In all cases, the treatment had to be terminated before the planned dose was attained, because of the side-effects or, in 1 case because of intercurrent infection (Table 3).

Cellular infiltration in the tumor after mAb therapy

An intense, inflammatory reaction developed with a massive necrosis and edema at the tumor site in all patients (patients 2, 3 and 6) from whom post-treatment tumor tissue was obtained. The reaction occurred after the sixth, first and eighth mAb infusions respectively. The total administered dose of mAb 425 was 120, 20 and 92 mg respectively. The reactions could not be satisfactorily controlled by steroid medication, so the patients had to be operated on with resection of the inflammatory tissue.

In all three cases, a marked infiltration of $CD45^+$ cells, macrophages and granulocytes was obtained. Patients 2 and 3 also showed the presence of $CD4^+$ and $CD8^+$ T cells. Moreover, an intense infiltration of plasma cells was seen. Biopsies from surrounding, normal, brain tissue showed only a weak infiltration of inflammatory cells (Table 5).

mAb 425 in serum and HAMA

mAb 425 could be detected in the blood of the majority of the tested patients (Fig. 3). The high concentration in patient 2 is probably explained by a changed position of the catheter, which was verified by CT, showing contrast leakage into the ventricular system. In 6 out of 8 patients,

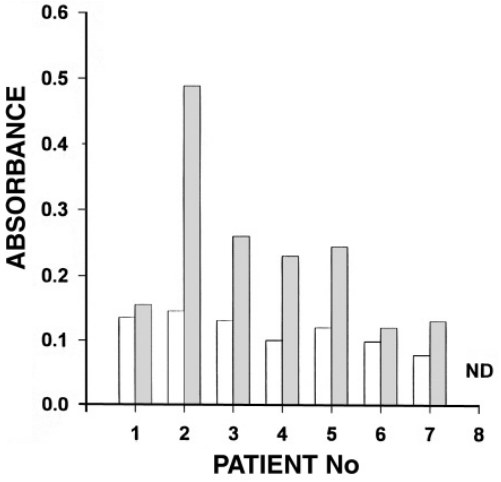

Fig. 3 mAb 425 concentration (absorbance) in serum before (□) and 4–48 h after (□) an intratumoral infusion of mAb 425

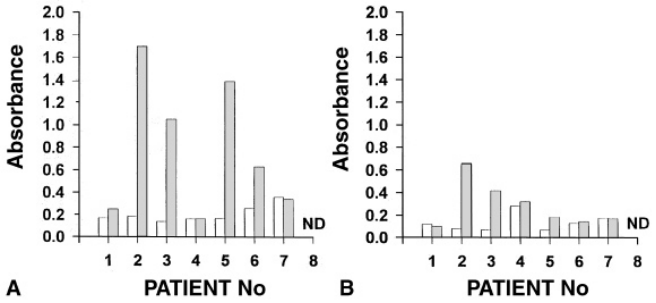

Fig. 4A, B Human, anti-(mouse IgG) (**A**) and anti-(mouse IgM) (**B**) antibodies in the blood before (□) and the maximum value after (□) infusions of mAb 425. Absorbances of negative controls (mean ± SEM) for IgG: 0.29 ± 0.04 (n = 8) and for IgM: 0.11 ± 0.01 (n = 8)

HAMA IgG were induced and, in 5 patients, HAMA IgM (Fig. 4A, B).

Discussion

The dismal prognosis for patients with high-grade malignant glioma, despite aggressive treatment with radio- and chemotherapy, has prompted the search for new treatment modalities [8]. Malignant gliomas may express relatively high levels of EGF-R, as compared with normal brain cells, which do not usually express this receptor. Monoclonal antibodies against the receptor may, after binding, activate various immune functions, which may destroy the tumor cells [2, 22, 23]. Thus, anti-EGF-R mAb may be an option for the therapy of malignant glioma.

In 3 out of 8 patients with advanced malignant glioblastoma, mAb 425 induced an intense, local, inflammatory reaction, including tumor necrosis, verified by immunohistochemistry. In a further 3 patients, an intense, local, inflammatory reaction was diagnosed by CT.

One mechanism for tumor cell destruction is antibody-dependent cellular cytotoxicity mediated by macrophages and granulocytes [1]. These cells were numerous in the tumor lesion after mAb administration. T cells may also contribute to tumor cell death. Specific T cells, induced through the idiotypic network and recognizing the nominal antigen, have been shown to be correlated with the clinical outcome [5]. Other T cell subsets recognizing epitopes on the infused mAb may also kill tumor cells that have processed and presented mAb peptides in the MHC molecules [11]. An increase in CD4 and CD8 T cells in the tumor lesion in mAb therapy has also been shown to be favorably correlated with the outcome [6, 27]. CD4 T cells were abundantly seen in the tumors of the mAb-treated patients and CD8 T cells were also increased. The selectivity of the mononuclear cell infiltrate is strengthened by the weak inflammatory reaction seen in the surrounding, normal, brain tissue.

To obtain a local, therapeutic, inflammatory response, it is probably important to achieve a high saturation of the tumor cell surface with the mAb. The most pronounced reaction was noted when the dose exceeded 20 mg mAb/injection. In one study, 10 mg of ^{125}I-labelled mAb 425 was given intra-arterially, a mode of administration that gives a lower bio-availability. Only 1 of the 25 patients showed an inflammatory reaction at the tumor site, demonstrated by CT scan [3]. In another study, a bispecific, mouse monoclonal antibody, together with LAK cells, was injected intratumorally into patients with glioblastoma [18]. No marked inflammatory reaction was seen, in spite of repeated dosing for several weeks. A 200 times lower dose of the mAb (100 μg injection) with a maximum total dose of 0.6 mg was given.

Table 5 Infiltration of cells in the malignant glioblastoma tumor after infusion of the anti-EGF-R mAb. + very weak, ++ weak, +++ moderate, ++++ strong, *ND* not done, – negative

Patient no.	Tissue type	Leukocyte common antigen (CD45)	CD4 T cells	CD8 T cells	Macrophages (CD14/CD68	Granulocyes (elastase$^+$)	CD45RO$^+$ leukocytes
2	Necrotic tumor	+++	++	+	++++	+++	+++
	Normal brain	+	+	–	++	++	–
3	Necrotic tumor	ND	++	+	++++	++++	++
	Normal brain	ND	–	–	+	+	–
6	Necrotic tumor	+++	ND	ND	++++	+++	++
	Liquor	ND	ND	ND	ND	++++	ND
	Normal brain	ND	ND	ND	ND	ND	ND

Table 6 Summary of clinical phase I/II studies in brain tumors using mAb 425 (mouse IgG2A anti-EGF-R monoclonal antibody). *CR* complete response, *PR* partial response

Type of mAb	Total dose of mAb (mg)	Number of patients	Clinical diagnosis	Route of administration	Clinical response	Reference
Unconjugated	760–2400	16	Recurrent glioblastoma	Repeated i.v. infusions	No major response (CR/PR). 7 of 13 evaluated patients had stable disease for 1–4 months	Stragliotti et al. [25]
	4–120	8	Recurrent or primary inoperable glioblastoma	Repeated intratumoral infusions	6 patients developed signs of tumor necrosis/local inflammatory reaction	Present study
^{125}I-conjugated	4–15 mg (40–224 mCi)	25	Adjuvant treatment after primary debulking surgery + external radiotherapy (54–65 Gy)	Repeated i.v. or i.a. infusions	Median survival time 15.6 months	Brady et al. [3]

It may be adventageous to have few tumor cells to achieve an optimal clinical effect. In patients who had undergone prior debulking surgery (patients 5–8), the survival from the start of mAb therapy seemed to be longer. Although an antitumor effect was achieved, the overall survival of most patients was short. The total median survival from diagnosis was 39 weeks and from the start of mAb therapy 18.5 weeks. The expected median survival time in this patient category with inoperable or recurrent, malignant glioblastoma may be 24 weeks from the start of therapy [20].

In our application, side-effects were much more pronounced than those normally reported from mouse mAb therapy. In general, such therapy has been performed without any adverse reactions of clinical significance. In fact, we were not able to treat the patients with the total dose intended, even after re-adjusting the schedule. The side-effect profile is most likely due to the infusion of a foreign protein into the cerebral tissue and to the induction of an inflammatory reaction with tumor necrosis and subsequent release of various soluble factors. The side-effects may also be due to the induction of HAMA, since the highest HAMA responses were seen in patients with the most symptoms (patients 2, 3, 5 and 6). These observations favor the future use of chimeric or humanized antibodies. The kind of nephropathia observed in patient 7 has never previously been reported in mAb therapy. The reason for the reversible kidney damage is unclear, especially when the small amount of the mAb reaching the systemic circulation is considered. The dose level of each mAb used for intratumoral administration of cerebral lesions needs to be carefully explored in a phase I study prior to further work.

Unconjugated mAb 425 has been given intravenously to 16 patients with recurrent malignant glioma [25]. In 80% of the patients, no adverse events were noted and, in the remainder, the side-effects were mild, though repeated doses were given. Only 1 patient developed HAMA, compared with 6 out of 8 in the present study. No major response was noted, but stabilization of the disease for 1–4 months was observed in 7/13 (54%) evaluable patients. The discrepancy between our study and that of Stragliotto et al. [25] is most likely to be explained by the route of mAb administration. We probably achieved a much higher saturation of the tumor cell surface, inducing an intense, inflammatory reaction. Moreover, 12 of their patients were on steroid medication, which hampers inflammation, while only 2 of our patients were on steroid therapy. These 2 patients (patients 1 and 7) showed no evidence of an inflammatory reaction.

A summary of phase I–II studies using mAb 425 in brain tumors is given in Table 6.

mAb 425 induced a profound, local, inflammatory reaction and, in some cases, tumor necrosis. Part of the effect was probably mediated by the activation of various immune functions. However, owing to the severe side-effects, it was considered inappropriate to continue the study. Chimeric or humanized antibodies may be prefered to mouse antibodies, as these might more efficiently activate human effector cells [15] and are less immunogenic. Furthermore, adjuvant treatment may also be considered if one takes into account that the presence of so few tumor cells is able to cause a local inflammatory reaction.

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