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Characterization of a monoclonal antibody recognizing a 138 kDa glioblastoma-associated antigen

Gary M Klaich and Peter M Kanter

James T Grace Jr Cancer Drug Center, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263, USA. Tel: (+1) 716 845 3426; Fax: (+1) 716 845 4481.

A monoclonal antibody (A7) was produced which recognizes an oncofetal antigen expressed on glioblastoma multiforme cells, fetal brain, fetal kidney, but not adult tissues. Radioimmunoprecipitates of Zwittergent 3-14 solubilized glioblastoma cells identified a single band at 138 kDa on 8% polyacrylamide sodium dodecylsulfate gels. Distribution studies of A7 in mice demonstrated a tri-phasic serum clearance of $t_{1/2\alpha} = 2.1$ h, $t_{1/2\beta} = 16.7$ h and $t_{1/2\gamma} = 151.1$ h. Tumor localization studies using the U-87 MG xenograft demonstrated the ability of A7 to localize with a tumor:blood ratio of 1.294 ± 0.094 as compared with 0.293 ± 0.051 for control antibody AC. A7 does not damage cell membranes and is not internalized when bound to reactive tumor cells.

Key words: Glioblastoma multiforme, monoclonal antibody.

Introduction

Glioblastoma multiforme continues to have a poor prognosis due in part to its anatomic location and the rapid emergence of cells resistant to radiation and chemotherapy. Monoclonal antibodies have identified tumor associated antigens which have assisted in the classification and imaging of tumors of the central nervous system.[1–3] Unlike other malignant diseases, glioblastoma multiforme rarely metastasizes outside the confines of the central nervous system[4] and may be amenable to treatment with antibody-based immunoconjugates.

In this paper we describe the development and characterization of a monoclonal antibody reactive with an oncofetal antigen expressed on glioblastoma cells.

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Correspondence to PM Kanter

Materials and methods

Tissue culture

The glioblastoma cell lines OB, HWM, GLP, MHM, JJC and JLS were originated in this laboratory from biopsy material histologically diagnosed as glioblastoma multiforme, GO-G-CCM and GO-G-UVW were obtained from the European Collection of Animal Cell Cultures (Wiltshire, UK), and U-87 MG was obtained from the American Type Culture Collection (Rockville, MD). The ovarian cell lines A90, A121, NIH ovarian carcinoma and CADV3; the endometrial cell line HECLA; the renal cell line SE; the myeloma cell line HMY-2; and the mouse plasmacytoma cell line Sp2/0-Ag 14 were obtained from Dr Gary Crogham of this institute. The colon carcinoma cell lines Colo 201, Colo 205 and WiDr were obtained from Dr Harry Slocum of this institute. The neuroblastoma cell lines SH-SY5Y and IMR-32 were obtained from Dr Ralph Bernacki of this institute. The melanoma cell line CaCl 7436 was obtained from Dr Martin Goldrosen of this institute. Hybridoma AC, producing an IgG2a immunoglobulin reactive with the bacterium *Neisseria meningitidis*, was obtained from Dr Anthony Campagnari, State University of New York at Buffalo. All cells were adapted to grow in RPMI 1640 (Gibco BRL, Grand Island, NY) supplemented with 20% fetal calf serum (Gibco BRL) and 100 units/ml penicillin and 100 µg/ml streptomycin (Gibco BRL). The glioblastoma cell lines OB, HWM, GLP and U-87 MG were adapted to serum free culture conditions using ULTRASER G (IBF Biotechnics, Savage, MD) supplemented RPMI 1640 medium.

Hybridoma development

Female BALB/c mice (10 weeks old), obtained from the West Seneca breeding colony of this institute,

were injected intraperitoneally with brain tumor homogenate mixed with an equal volume of complete Freunds adjuvant, 400 μg total protein in 0.5 ml, on day 0. On day 28, animals were injected i.p. with brain tumor homogenate, 400 μg total protein in 0.5 ml total volume.

Hybridomas were produced by fusing day 31 spleen cells with Sp2/O-Ag 14 mouse plasmacytoma cells as originally described by Kohler and Milstein[5] and modified by Bankert *et al.*[6]

Tumor reactivity was determined by use of a solid-phase ELISA using 0.25% glutaraldehyde-fixed glioblastoma cells.

Reactivity with human tissues and cell lines

Human adult and fetal tissue obtained at autopsy were either frozen at −70°C or formalin fixed, embedded in paraffin and cut to a thickness of 5 μm.

Ficoll-Paque purified white blood cells (WBCs)[7] and whole blood were applied to poly-L-lysine coated Lab-Tek chamber-slides (Miles Scientific, Naperville, IL) and fixed with 10% phosphate buffered formalin (Sigma, St Louis, MO).

Tumor cell lines were cultured in Lab-Tek chamber-slides and fixed with 0.25% glutaraldehyde (grade I) (Sigma).

Reactivity with human tissue and tumor cell lines was determined by immunoperoxidase staining using the 'ABC' avidin–biotin immunoperoxidase staining system (Vector Laboratories, Burlingame, CA) as described by Hsu *et al.*[8]

Fetal tissue reactivity was determined by dot blot analysis using 10% (w/v) fetal tissue homogenates in 0.1 M Na_2HPO_4, 0.9% NaCl, pH 7.4 (PBS) applied to nitrocellulose membranes. The membranes were blocked with 3% bovine serum albumin in PBS, incubated with conditioned medium for 1 h, followed by washing in PBS. Peroxidase labeled goat anti-mouse serum was added for 1 h, the membranes washed and the peroxidase signal developed with 0.6 mg/ml DAB + 0.01% H_2O_2 in 50 mM Tris–HCl, pH 7.6.

Isotype determination of monoclonal antibody A7

The immunoglobulin isotype of A7 was determined by solid phase ELISA using a mouse hybridoma subtyping kit (Boehringer Mannheim, Indianapolis, IN).

Protein A–Sepharose purification of monoclonal antibody A7

Monoclonal antibody A7 was purified by Protein A–Sepharose (Pharmacia LKB Biotechnology, Piscataway, NJ) chromatography as described by Ey *et al.*[9]

^{14}C-labeling of monoclonal antibody A7

Reduced amino acid RPMI 1640 medium was formulated with the Gibco 'Select-Amine' kit (Gibco BRL) in order to obtain a 50% reduction in amino acids. ^{14}C-L-amino acid mixture (40 μCi, 1.89 mCi/mg in 0.01 N HCl) (ICN, Irving, CA) and 2×10^6 logarithmically growing hybridoma cells were added to 10 ml of reduced amino acid HAT medium supplemented with 1% Nutridoma SP (Boehringer Mannheim) and incubated for 5 days. ^{14}C-labeled monoclonal antibody A7 was purified by Protein A–Sepharose 4L-CB chromatography.

Radioimmunoprecipitation

Glioblastoma cells were solubilized with 0.5 ml of 1% Zwittergent 3-14 (CalBiochem, LaJolla, CA) in 10 mM Tris–HCl (pH 7.2), 4 mM phenylmethylsulfonyl fluoride, 5 mM iodoacetic acid and 25 mM ε-amino-*n*-caproic acid; and disrupted with a Branson Sonifier Cell Disrupter model 185 (Vernitron Medical Products, Carlstadt, NJ). The detergent–cell mixture was stirred for 18 h at 4°C followed by centrifugation (25 000 *g*, 60 min, 4°C) to remove insoluble material. Solubilized tumor extract was combined with 600 μCi $Na^{125}I$ (17 Ci/mg, > 350 mCi/ml) (New England Nuclear, Boston, MA) in an Iodogen (Pierce Chemical, Rockford, IL) coated Erlenmeyer flask and incubated on ice for 15 min. Free ^{125}I was removed from the labeled protein by gel filtration through Sephadex G-25.

The immunoprecipitation procedures of Kessler *et al.*[10] and Caincross *et al.*[11] were used and monoclonal antibody AC, an IgG2a monoclonal antibody reactive with the bacterium *N. meningitidis*, was included as a negative control. The immunoprecipitates were resolved using a Laemmli 8% polyacrylamide gel[12] electrophoresed at 25 mA constant current.

Distribution of ^{14}C-labeled monoclonal antibody A7 in mice

Six week old female, Swiss (HA/ICR) mice obtained from the breeding colony of the Grace Cancer Drug Center, Roswell Park Cancer Institute, were injected i.v. with 2×10^5 c.p.m. ^{14}C-labeled monoclonal antibody A7 (12 000 c.p.m./μg)/20 g body weight. At varying time points, three mice were selected, anesthetized with ether and blood collected. The serum was separated from the clotted blood by centrifugation, mixed with Soluscint A (National Diagnostics, Mannville, NJ) and counted.

Human tumor xenograft localization

HI/CR female nude mice, obtained from Radiation Biology Department, Massachusetts General Hospital (Boston, MA), were transplanted with the U-87 MG tumor by s.c. injection of 1×10^7 cells over the left scapula. When tumor volume attained 32 mm^3, three animals per group were injected i.v. with 2×10^5 c.p.m./20 g body weight of either ^{14}C-labeled monoclonal antibody A7 (12 000 c.p.m./μg protein) or ^{14}C-labeled monoclonal antibody AC (14 000 c.p.m./μg protein). At 72 h after injection, the mice were anesthetized with ether, blood collected, the body organs removed, weighed and solubilized with 1 ml of Solusol (National Diagnostics). The digested tissue samples were mixed with Soluscint A and counted. An internal standard consisting of 2×10^4 c.p.m. of ^{14}C-labeled amino acid mixture was added to the previously counted samples, the vials recounted and a quench coefficient calculated:[13,14]

$$\text{quench coefficient} = \frac{(\text{c.p.m.}_{\text{int std}})}{(\text{c.p.m.}_{\text{sample+int std}}) - (\text{c.p.m.}_{\text{sample}})}$$

The corrected ^{14}C activity of the sample was determined as follows:

$$\text{Corrected } ^{14}\text{C-c.p.m.} = (\text{c.p.m.}_{\text{sample}}) \times (\text{quench coefficient})$$

[^{3}H]Nicotinamide permeability assay[15,16]

Glioblastoma cells (8×10^5 cells/ml) were suspended in culture medium containing 2 μCi [^{3}H]nicotinamide/ml (3.4 Ci/mmol, 5 μCi/50 μl; New England Nuclear) with 50 μl of the cell suspension distributed to the wells of a 96-well plate and incubated overnight. Unincorporated isotope was removed and 25 μl of either neat, A7 conditioned medium or 10-fold diluted rabbit anti-brain serum were added to triplicate wells and incubated at 37°C for 30 min. Twenty-five microlitres of either RPMI 1640 or 4-fold diluted rabbit complement (Pel-Freez Biologicals, Brown Deer, WI) were added followed by a 30 min incubation. Released ^{3}H activity was determined by scintillation counting. Specific release was calculated by:

$$\%\ \text{Specific release} = \frac{\text{expt c.p.m.} - \text{spont release c.p.m.}}{\text{max release c.p.m.} - \text{spont release c.p.m.}}$$

Binding and internalization of monoclonal antibody A7[17]

Glioblastoma cells in 24-well plates were treated with 1×10^5 c.p.m./well of ^{14}C-labeled antibody A7 for 60 min at 4°C, washed three times, followed by the addition of 0.5 ml growth medium. At varying times, two wells from each antibody treatment group were selected and the growth medium containing released antibody aspirated and saved. Membrane bound antibody was released by addition of 0.5 ml of 0.125% porcine trypsin and 0.01% EDTA followed by incubation at 37°C for 5 min. The trypsinized cells were centrifuged (650 *g*, 15 min, 4°C), the supernatant containing membrane associated antibody saved and the cell pellet containing internalized antibody solubilized by addition of 0.5 ml Solusol. Samples were mixed with 10 ml of Soluscint A with ^{14}C activity determined by liquid scintillation counting.

Results

Reactivity pattern of monoclonal antibody A7

Monoclonal antibody A7 is an IgG2a immunoglobulin produced by the fusion of spleen cells from mice immunized with glioblastoma tissue from patient OB. Reactivity with formalin fixed human tissues evaluated by immunoperoxidase staining is listed in Table 1. Formalin fixed, paraffin embedded OB tumor cells retained reactivity, confirming the stability of the epitope with regard to the preservation and sectioning procedures. No reactivity

Table 1. Reactivity of monoclonal antibody A7 with normal tissues

Brain	–
Spleen	–
Pancreas	–
Kidney	–
Liver	–
Lung	–
Heart	–
Adrenal	–
WBC	–
RBC A	–
RBC B	–
RBC O	–
Fetal brain	+
Fetal kidney	+

was observed with the normal adult tissues used in this survey. Dot blot analysis of fetal brain and kidney both demonstrated strong reactivity indicating the oncofetal nature of this antigen.

Reactivity with various human tumor cell lines is listed in Table 2. A7 reacted with six glioblastoma cell lines isolated in this laboratory at various passage number ranging from 3 to 76; three glioblastoma cell lines obtained from cell repositories; melanoma cell line CaCl 7436; renal cell carcinoma line SE; and two ovarian cell lines, A90 and A121. In addition, glioblastoma cell lines OB, HWM, GLP and U-87 MG, adapted to culture conditions using the serum replacement supplement ULTRASER G, retained reactivity with A7. No reactivity was observed with the endometrial cell line HECLA, the colon carcinoma cell lines Colo 201, Colo 205 and WiDr, the neuroblastoma cell lines SH-SY5Y and IMR-32, the ovarian cell lines CADV3 and NIH ovarian carcinoma, and the myeloma cell line HMY-2 when evaluated under the same conditions.

Table 2. Reactivity of monoclonal antibody A7 with human tumor cell lines

Glioblastoma	
OB-62	+
HWM-76	+
GLP-29	+
U87MG-180	+
JLS-21	+
MHM-22	+
GO-G-UVW-13	+
GO-G-CCM-12	+
Melanoma	
CaCl 7436	+
Renal cell	
SE	–
Ovarian	
A90	+
A121	+
NIH ov carc	–
CADV3	–
Colon carcinoma	
Colo 205	–
Colo 201	–
WIDR	–
Neuroblastoma	
SH-SY5Y	–
Endometrial	
HECLA	–
Myeloma	
HMY-2	–

Antigen characterization

Attempts at the electrophoretic separation of glioblastoma tumor cell proteins with transfer onto nitrocellulose membrane and probing with monoclonal antibody A7 were negative, suggesting the denaturation and loss of immunological recognition of the reactive epitope. Nonidet P-40 was unsuccessful in solubilizing the reactive antigen necessitating the use of the zwitterion detergent, Zwittergent 3-14. Figure 1 is the autoradiograph from the immunoprecipitation experiment. Lane 1 is monoclonal antibody A7 precipitated protein and is present as a single band migrating with a M_r of 138 000. Lane 2 represents the results of the negative control precipitation using monoclonal antibody AC which is unreactive with glioblastoma cells.

Serum distribution of monoclonal antibody A7

The serum distribution of ^{14}C-labeled monoclonal antibody A7 in non-tumor bearing mice is tri-phasic with $t_{1/2\alpha} = 2.1$ h, $t_{1/2\beta} = 16.4$ h and $t_{1/2\gamma} = 151.1$ h, and is listed in Figure 2.

Tumor localization of monoclonal antibody A7

^{14}C-labeled monoclonal antibody A7 or control monoclonal antibody AC were i.v. injected into U-87 MG tumor bearing nude mice and allowed to circulate for 72 h. Figure 3 represents the results of the experiment in which the ^{14}C values have been

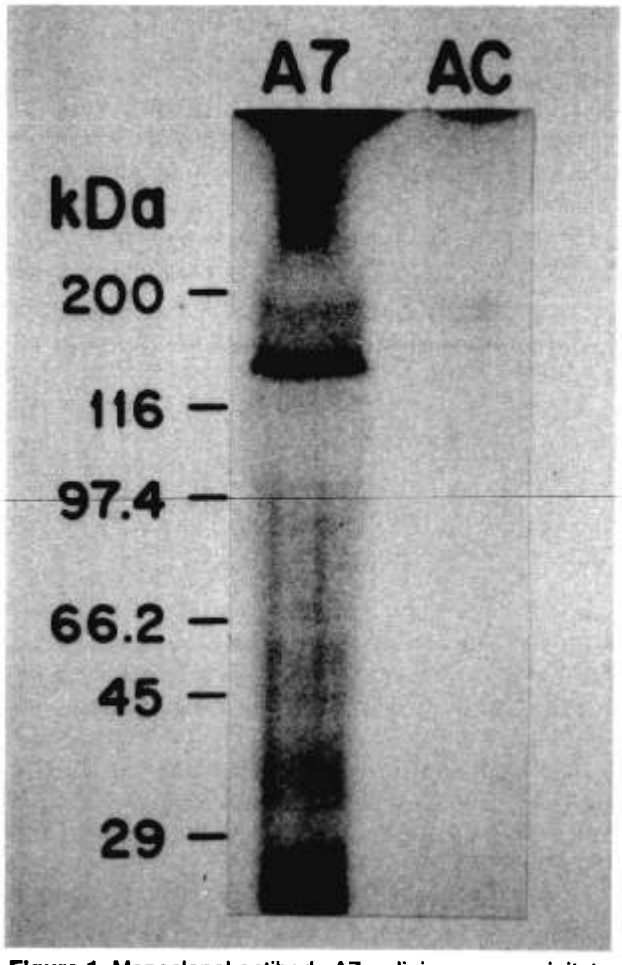

Figure 1. Monoclonal antibody A7 radioimmunoprecipitate of Zwittergent 3-14 solubilized glioblastoma cells. AC = negative control.

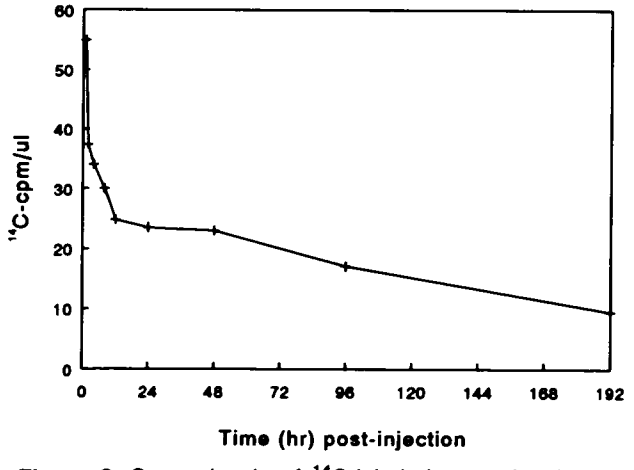

Figure 2. Serum levels of ^{14}C-labeled monoclonal antibody A7 versus time in normal mice.

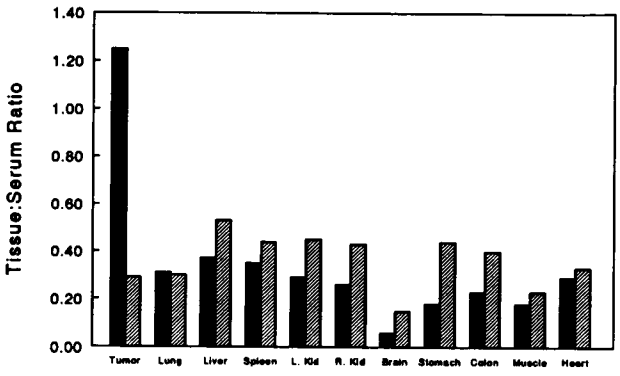

Figure 3. Tissue localization of ^{14}C-labeled monoclonal antibodies A7 (■) and AC (▨) in nude mice transplanted with the U-87 MG xenograft.

standardized by comparing the tissue with serum ratios. Monoclonal antibody A7 demonstrated the ability to localize at the tumor site with a tumor:serum ratio of 1.254 ± 0.094. No accumulation in normal tissues was observed with tissue:serum ratios of: lung = 0.306 ± 0.019, liver = 0.372 ± 0.028, spleen = 0.347 ± 0.043, left kidney = 0.295 ± 0.039, right kidney = 0.262 ± 0.016, brain = 0.062 ± 0.038, stomach = 0.179 ± 0.037, colon = 0.229 ± 0.045, muscle = 0.178 ± 0.009 and heart = 0.289 ± 0.045. In comparison, negative control monoclonal antibody AC did not demonstrate any accumulation in either tumor or normal tissues with tissue: serum ratios of: tumor = 0.293 ± 0.051, lung = 0.302 ± 0.019, liver = 0.532 ± 0.054, spleen = 0.439 ± 0.026, left kidney = 0.446 ± 0.041, right kidney = 0.432 ± 0.043, brain = 0.147 ± 0.032, stomach = 0.442 ± 0.024, colon = 0.397 ± 0.037, muscle = 0.234 ± 0.052 and heart = 0.326 ± 0.056.

Evaluation of monoclonal antibody A7 for complement mediated cytotoxicity

The ability of A7 to damage tumor cell membranes was assessed using the [^{3}H]nicotinamide release assay and the results listed in Table 3. Monoclonal antibody A7 by itself or in the presence of rabbit complement does not damage U-87 MG cells as evidenced by lack of significant release of [^{3}H]nicotinamide or change in morphology of target cells. In comparison, rabbit anti-brain serum in the presence of rabbit complement results in the release of 84.8% of the maximum releasable [^{3}H]nicotinamide with a concomitant rounding up and detachment of the target cells from the culture surface.

Table 3. [^{3}H]NAD release from U-87 MG cells treated with monoclonal antibody A7 and complement

	Released NAD (c.p.m.)	% Specific release[b]	Microscopic morphology
Media + media	679	–	no change
Media + complement	960	–	no change
mAb A7 + media	575	0	no change
mAb A7 + complement	1028	1.23	no change
RAB[a] + media	1260	10.06	no change
RAB[a] + complement	5621	84.84	rounded up/floating
SDS (max release)	6454	100.00	no intact cells

[a]RAB = rabbit anti-brain.
[b]see text.

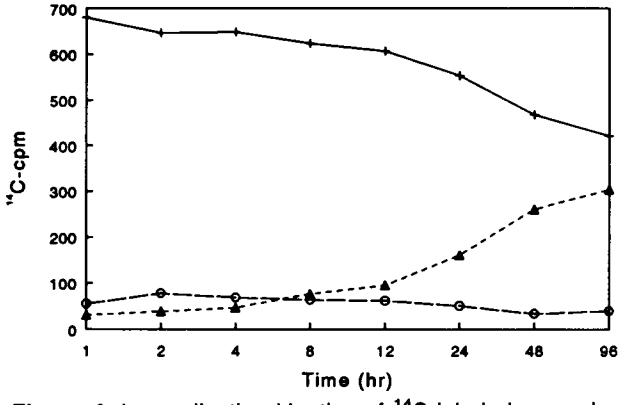

Figure 4. Internalization kinetics of ^{14}C-labeled monoclonal antibody A7. Culture medium (▲), trypsin sensitive (+) and trypsin resistant (○).

Monoclonal antibody A7 internalization kinetics

The results of the ^{14}C-labeled monoclonal antibody A7 internalization are presented in Figure 4. Initially, all of the ^{14}C activity was cell associated but still susceptible to removal by trypsin treatment. Over time, increasing ^{14}C activity was found in the culture media suggesting that free monoclonal antibody A7 or monoclonal antibody A7–antigen complex were being released from the cell. At no time was there any evidence that monoclonal antibody A7 was actively being internalized as evident by the low level of trypsin-resistant, cell-associated ^{14}C activity throughout the 96 h experiment.

Discussion

Our objective was to produce a glioblastoma multiforme reactive monoclonal antibody which may be of use as a therapeutic adjunct to surgery in the treatment of this highly fatal disease. Monoclonal antibodies that are 'cross-reactive' with fetal brain and various tumor cells may be therapeutically efficacious in a particular patient with a single tumor type, provided that they do not react extensively with normal adult tissues.[1] Monoclonal antibody A7, produced by immunizing mice with glioblastoma multiforme tumor tissue, reacts with glioblastoma cells but does not react with normal adult tissues. Dot blot analysis revealed reactivity with human fetal brain and kidney, indicating the oncofetal nature of the reactive antigen. A7 reacts with a common glioblastoma antigen expressed on all glioblastoma cell lines tested including six cell lines isolated in this laboratory, the U-87 MG cell line obtained from the American Type Culture Collection, and the GO-G-UVW and GO-G-CCM cell lines obtained from the European Collection of Animal Cell Cultures. The expression of the antigen in culture is stable with continued A7 reactivity being demonstrated up to passage number 180. OB, HWM, GLP and U-87 MG glioblastoma cell lines, when cultured using the serum replacement factor ULTRASER G, retain A7 reactivity indicating that serum components adhering to the cell surface are not responsible. Monoclonal antibody A7 reacts with melanoma, renal cell and some ovarian cell lines, but not with colon, endometrial, neuroblastoma and myeloma cell lines. The tumor reactivity pattern was predictable considering the fetal tissue reactivity and the shared embryological origin of the reactive tumors.

Initial attempts at characterizing the reactive antigen using Western blot analysis were unsuccessful, suggesting that the conformation of the reactive epitope was being destroyed by the electrophoresis conditions. Immunoprecipitation using Zwittergent 3-14 solubilized tumor cell extract identified the relative molecular weight of the tumor associated antigen to be M_r 138 000. Zwittergent 3-14 was utilized since NP-40 either denatured or was unable to solubilize the molecule of interest. Zwittergent 3-

14, a zwitterionic membrane solubilizing detergent, apparently stabilizes the native structure of the solubized molecules[18] with a greater likelihood of retaining epitope conformation and immunological recognition.

Serum distribution studies conducted in non-tumor bearing mice injected i.v. with ^{14}C-labeled monoclonal antibody A7 demonstrated a triphasic serum distribution with $t_{1/2\alpha}$ = 2.1 h, $t_{1/2\beta}$ = 16.7 h and $t_{1/2\gamma}$ = 151.1 h. This is in agreement with the observations of Ward[19] of $t_{1/2\alpha} < 14$ h and $t_{1/2\beta}$ = 176 h for the anti-ovarian monoclonal antibody HMFG2, and with Fahey[20] of $t_{1/2}$ in the range of 129.6–148.8 h for polyclonal mouse IgG2a.

Tumor localization studies were conducted in nude mice xenografted with the human glioblastoma cell line U-87 MG. ^{14}C-labeled monoclonal antibody A7 localized at the tumor site with a tumor : serum level of 1.24 ± 0.094 as compared with 0.29 ± 0.051 for negative control monoclonal antibody AC, confirming that specific tumor binding and not trapping of A7 by tumor vasculature was responsible for the observed localization. Neither A7 nor AC accumulated in any of the normal tissues sampled. Although an intracranial tumor model may appear more appropriate, the sole purpose of using the U-87 MG subcutaneous xenograft model was to determine the ability of monoclonal antibody A7 to discriminate between tumor cells and non-antigen expressing normal tissues. Since glioblastoma multiforme rarely metastasizes outside of the central nervous system, optimal therapeutic advantage of monoclonal antibody based therapies would be best realized by the direct instillation into the tumor cavity, thereby eliminating any obstacles presented by the blood–brain barrier when administered systemically.

Several advantages are conferred by using monoclonal antibodies labeled with ^{14}C for distribution studies. Since the exterior of the molecule is not chemically modified as with iodination procedures, antigen binding and behavior in the systemic circulation should be identical to the unlabeled molecule. *In vivo* studies have demonstrated the lack of non-specific accumulation of the isotope within the liver and kidneys which is common with ^{125}I-labeled monoclonal antibodies due to dehalogenation.[21,22] The use of reduced amino acid medium and serum-free conditions optimizes the incorporation of ^{14}C-labeled amino acids and, when combined with Protein A–Sepharose chromatography, results in the production of large quantities of stable, radioactively-labeled monoclonal antibodies.

Monoclonal antibody A7 does not damage the membranes of reactive tumor cells as measured by the [^{3}H]Nicotinamide permeability assay. [^{3}H]Nicotinamide readily traverses the cell membrane, and is incorporated into NAD and NADP,[15] which are retained by the cell membrane due to the addition of polar phosphate and quaternary amino groups. Membrane permeability changes mediated by binding of antibody or antibody–complement complex results in a rapid efflux of ^{3}H-labeled NAD and NADP out of the cell, which can be readily quantitated. In support of this observation was the lack of change in microscopic morphology of cells treated with monoclonal antibody A7 by itself or with complement as compared with rabbit anti-brain serum and complement treated cells, which round up and lift off the culture surface.

Monoclonal antibody A7–ricin immunoconjugates were synthesized but did not produce significant cell killing (data not presented), suggesting that the immunoconjugate was not being internalized by the tumor cell. Internalization studies using trypsin to strip bound, ^{14}C-labeled monoclonal antibody A7 from the cell surface indicated that the labeled antibody remains membrane bound for 48 h followed by slow release from the cell surface with no evidence of internalization.

Conclusion

We have reported the development of monoclonal antibody A7 which reacts with a 138 kDa glioblastoma associated antigen present in both fetal brain and kidney tissues, but not expressed by normal adult tissues. We have demonstrated its ability to discern antigen expressing tumor cells from non-antigen expressing tissues, that it is non-cytotoxic and is not internalized upon binding to its membrane associated antigen. Future efforts will be to develop monoclonal antibody A7 based immunoconjugates composed of isotopes and membrane active cytotoxic agents and to develop a model system in which the immunoconjugate is directly instilled into the tumor cavity thereby negating the blood–brain barrier.

References

1. Fischer D, Chen T, Narayan R. Immunological and biochemical strategies for the identification of brain tumor-associated antigens. *J Neurosurg* 1988; **68**: 165–80.
2. Kemshead J, Coakham H. The use of monoclonal antibodies for the diagnosis of intracranial malignancies and

the small round cell tumors of childhood. *J Pathol* 1983; **141**: 249–57.
3. Coakham H, Garson G, Brownell B, *et al.* Monoclonal antibodies as brain tumor diagnosis: a review. *J R Soc Med* 1984; **77**: 780–7.
4. Morley T. The recovery of tumor cells from venous blood draining cerebral gliomas, a preliminary report. *Can J Surg* 1959; **2**: 363–5.
5. Kohler G, Milstein C. Continuous culture of fused cells secreting antibody of predefined specificity. *Nature* 1975; **256**: 495–7.
6. Bankert R, Mazzaferro D, Mayers G. Hybridomas producing hemolytic plaques used to study the relationship between monoclonal antibody affinity and the efficiency of plaque inhibition with increasing concentration of antigen. *Hybridoma* 1981; **1**: 47–58.
7. Boyum A. Separation of white blood cells. *Nature* 1964; **204**: 793–4.
8. Hsu S, Raine L, Fanger H. Comparative study of the peroxidase–antiperoxidase method and an avidin–biotin complex method for studying polypeptide hormones with radioimmunoassay antibodies. *Am J Clin Pathol* 1981; **75**: 734–8.
9. Ey P, Prowse C, Jenkin C. Isolation of pure IgG1, IgG2a and IgG2b immunoglobulins from mouse serum using protein-A sepharose. *Immunochemistry* 1978; **15**: 429–36.
10. Kessler, S. Cell membrane antigen isolation with the staphylococcal protein-A adsorbent. *J Immunol* 1976; **117**: 1482–90.
11. Caincross J, Mattes M, Beresford H, *et al.* Surface antigens of human astrocytoma defined by mouse monoclonal antibodies: identification of astrocytoma subsets. *Proc Natl Acad Sci USA* 1982; **79**: 5641–5.
12. Laemmli U. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; **227**: 680–5.
13. Hansen D, Bush E. Improved solubilization procedures for liquid scintillation counting of biological materials. *Anal Biochem* 1967; **18**: 320–32.
14. Kobayashi Y, Maudsley D. Practical aspects of sample preparation. In: Kobayashi Y and Maudsley D, eds. *Biological applications of liquid scintillation counting*. New York: Academic Press 1974: 153–69.
15. Kurth R, Medley G. A membrane permeability test for the detection of cell surface antigens. *Immunology* 1975; **29**: 803–11.
16. Wikstrand C, Bigner D. Surface antigens of human glioma cells shared with normal adult and fetal brain. *Cancer Res* 1979; **39**: 3235–43.
17. Hashimoto Y, Sugawara M, Kamiya T, *et al.* Basic approach to application of chemoimmunoliposomes for cancer therapy. *NCI Monogr* 1987; **3**: 89–94.
18. Navarrete R, Serrano R. Solubilization of yeast plasma membranes and mitochondria by different types of non-denaturing detergents. *Biochim Biophys Acta* 1983; **728**: 403–8.
19. Ward B, Wallace K. Localization of the monoclonal antibody HMFG2 after intravenous and intraperitoneal injection into nude mice bearing subcutaneous and intraperitoneal human ovarian cancer xenografts. *Cancer Res* 1987; **47**: 4714–8.
20. Fahey J, Sell S. The immunoglobulins of mice V: the metabolic (catabolic) properties of five immunoglobulin classes. *J Exp Med* 1965; **122**: 41–58.
21. Hagan P, Halpern S, Chen A, *et al. In vivo* kinetics of radiolabeled monoclonal anti-CEA antibodies in animal models. *J Nucl Med* 1985; **26**: 1418–23.
22. Stern P, Hagan P, Chen A, *et al.* The effect of the radiolabel on the kinetics of monoclonal anti-CEA in a nude mouse-human colon tumor model. *Prog Cancer Res Ther* 1982; **21**: 245–54.

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